

Lab meeting 7/28/09



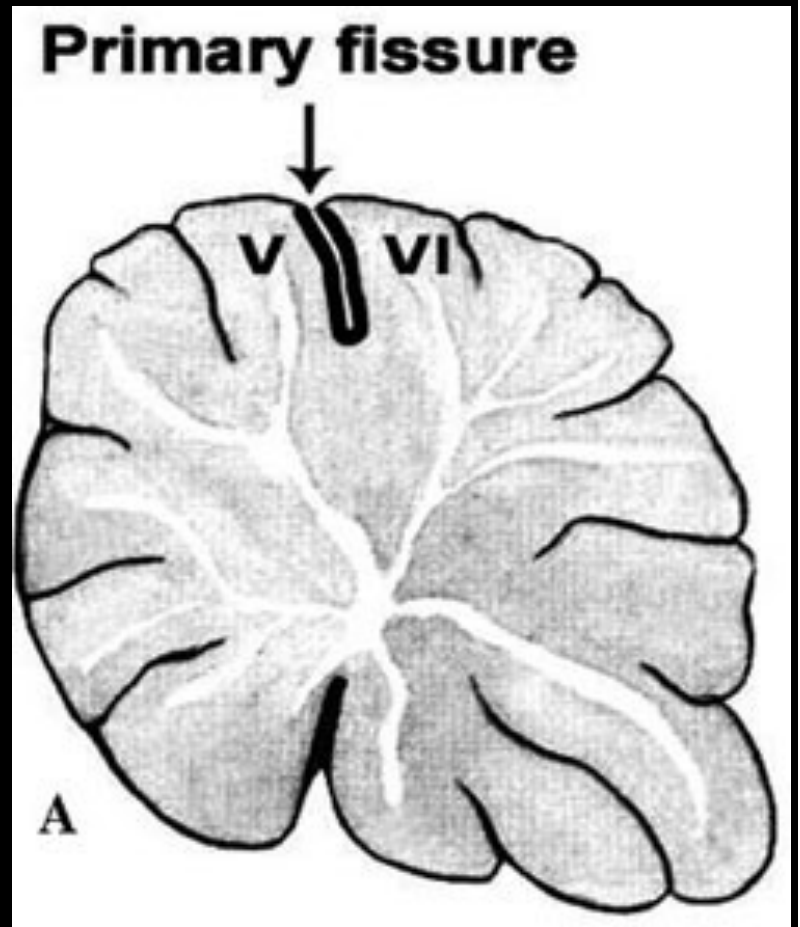
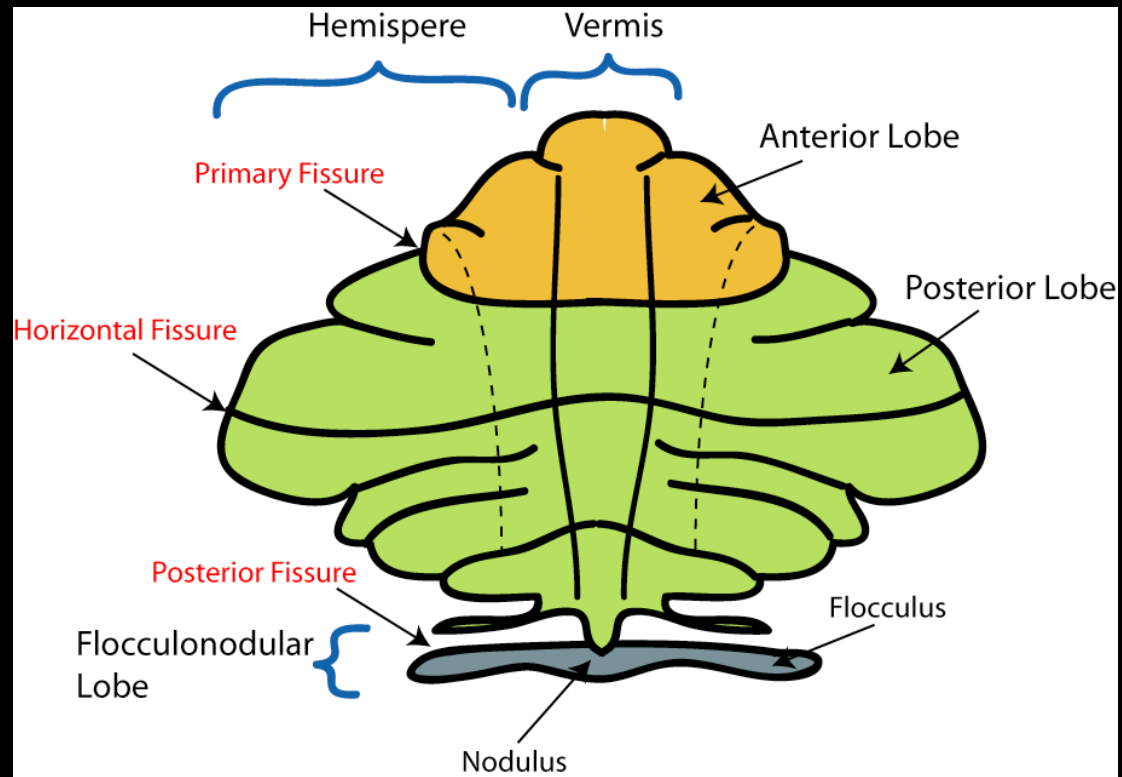
What did the hippocampus say during
its retirement speech?

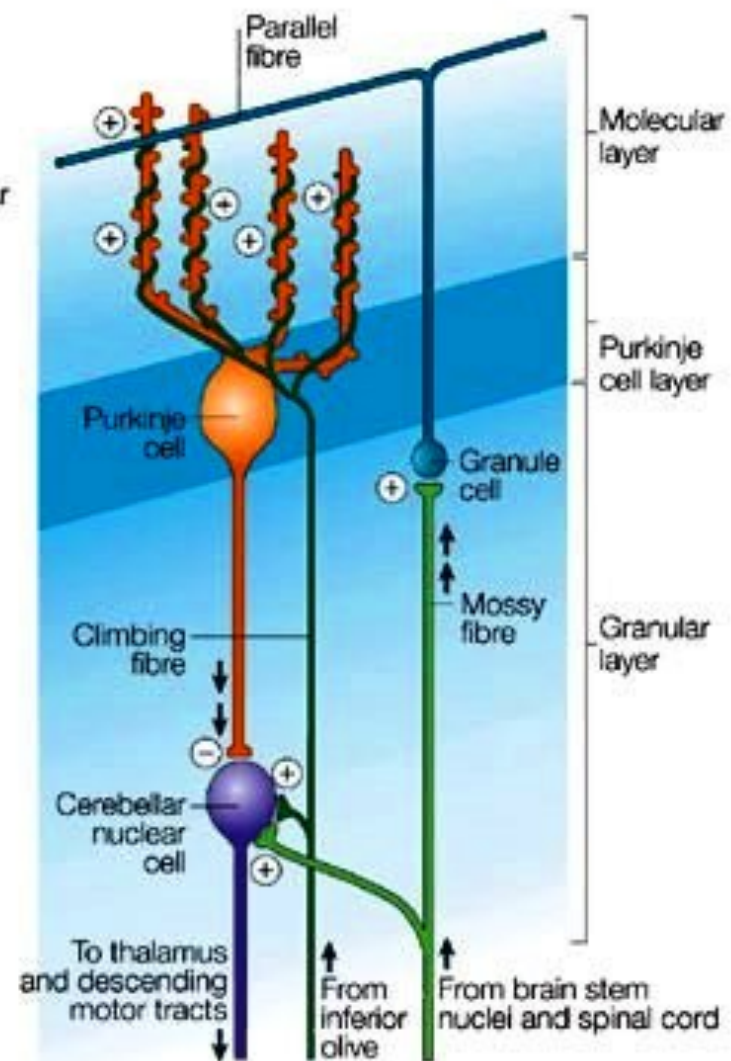
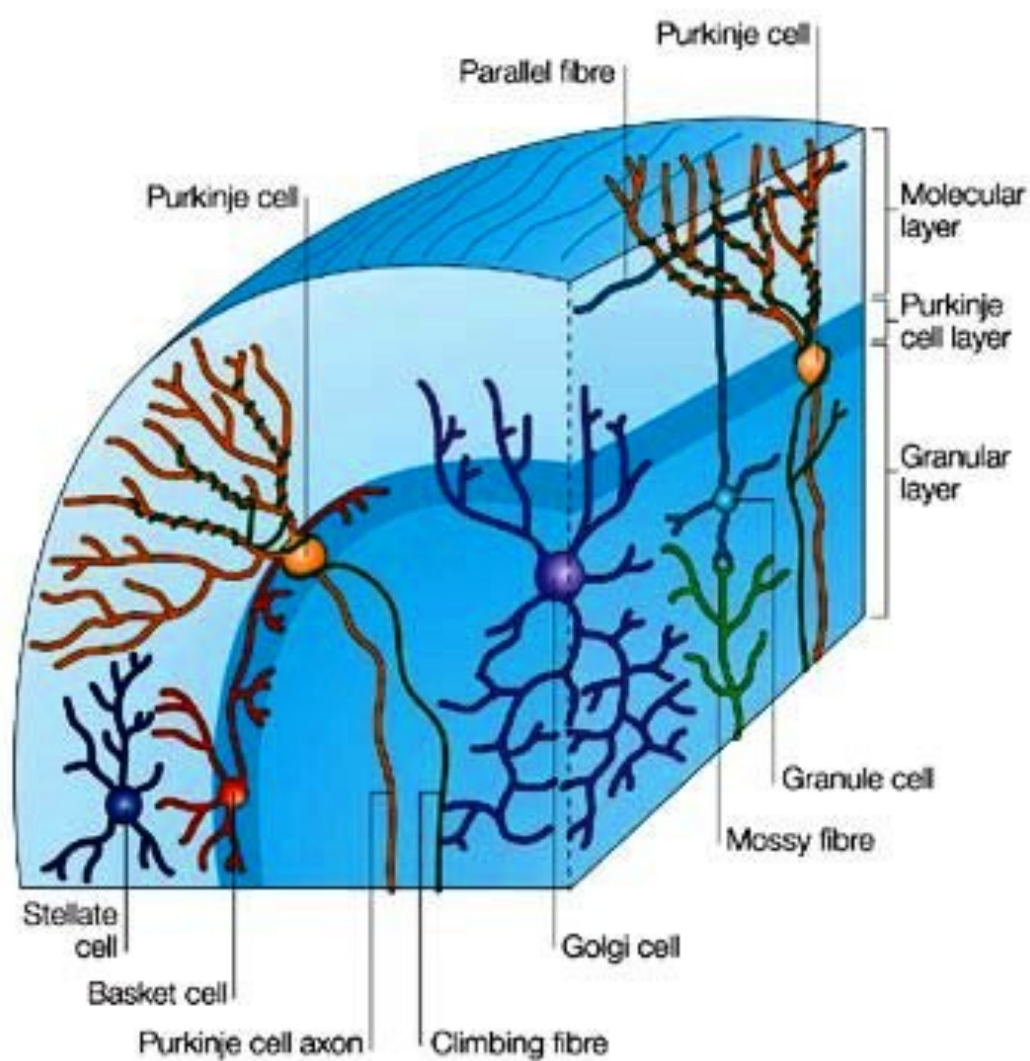
Thanks for the memories...

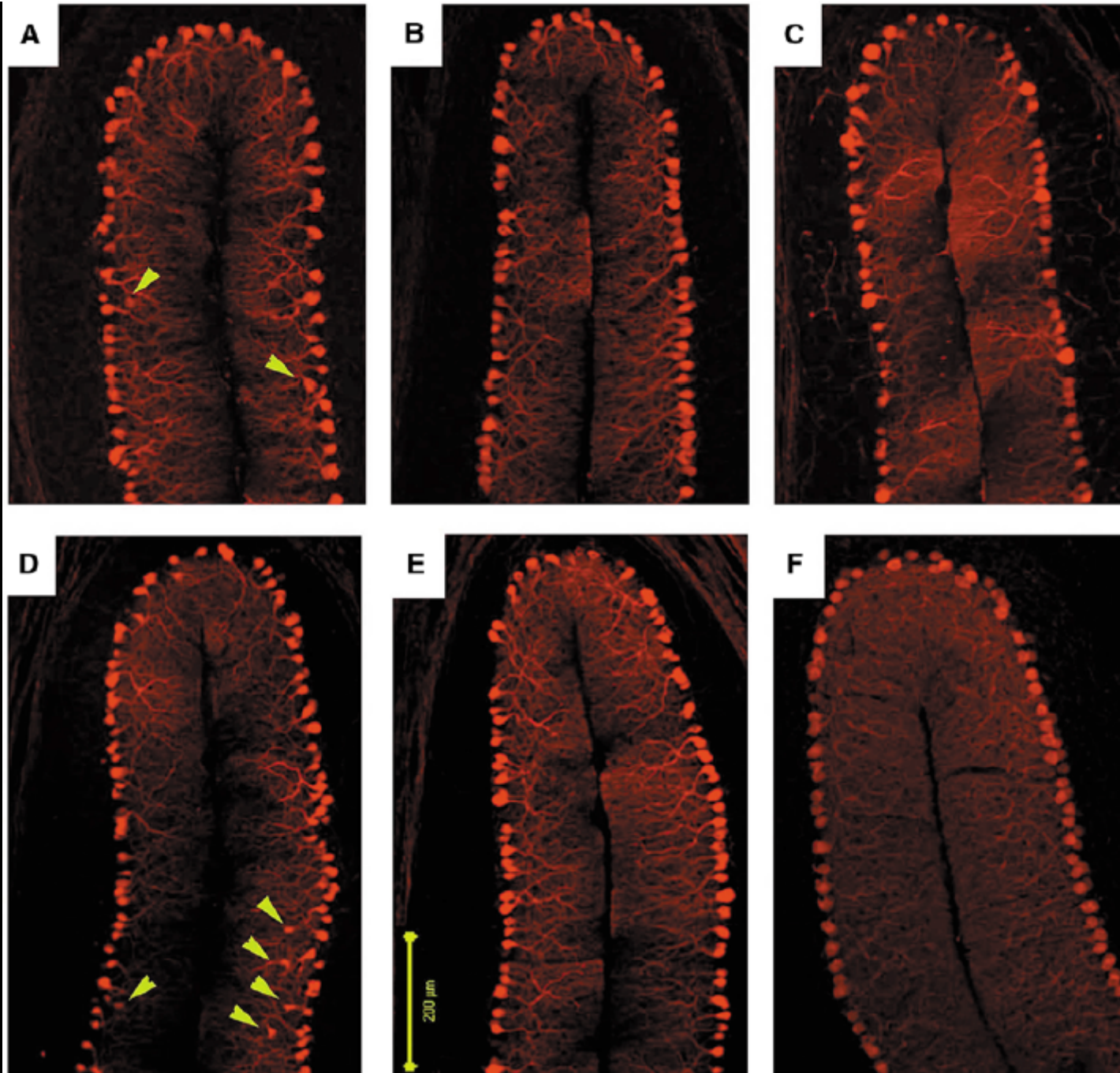
- Development of a longer yet stable CAG repeat in mouse model:
 - Modeling a more toxic human pathology
 - Earlier onset of symptoms

Q127

- Human SCA-2 driven by the PCP2 promoter
 - Transgene should be confined to the Purkinje Cell







Methods: brain preparation

- Brain perfused with ice-cold PBS
- Overnight in 4% PFA at 4c
- Cryoprotection: 20% → 30% sucrose
- Embedded within OCT medium
 - Brain positioned for sagittal slices of the vermis
- Kept at -20c until time of slicing

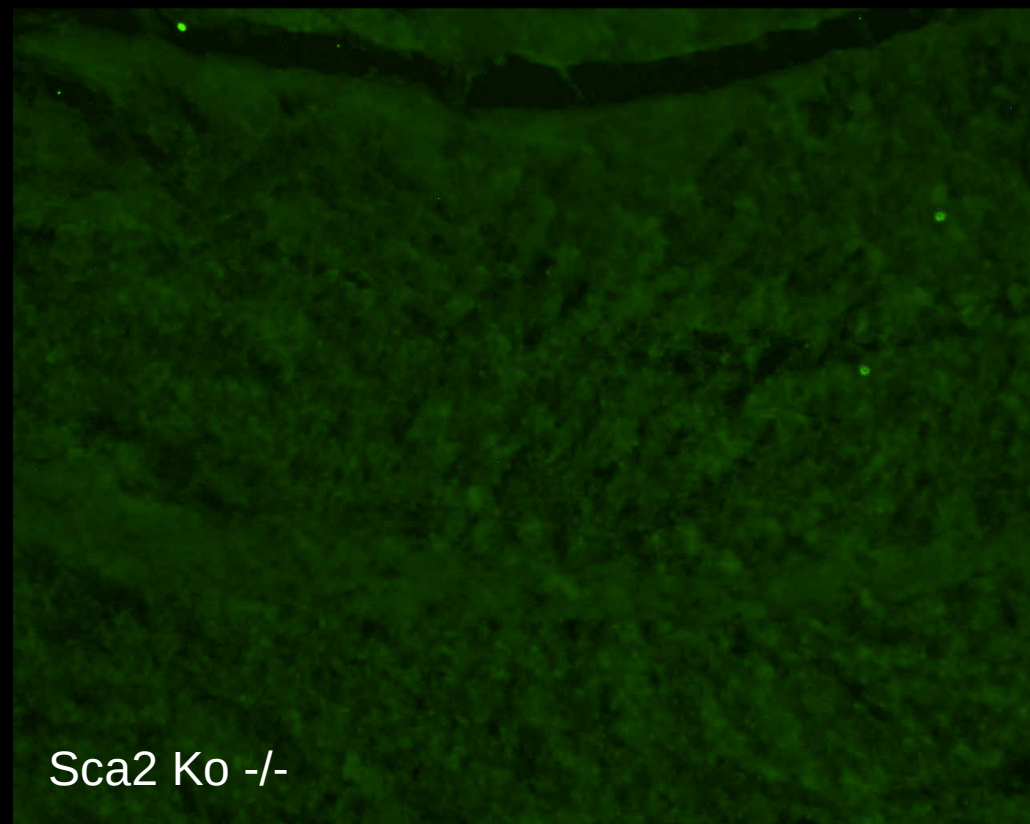
Methods: slice

- Cryostat set to -20c
- 10 μ M sections – slide mounted
- Stored at -80c until time of staining

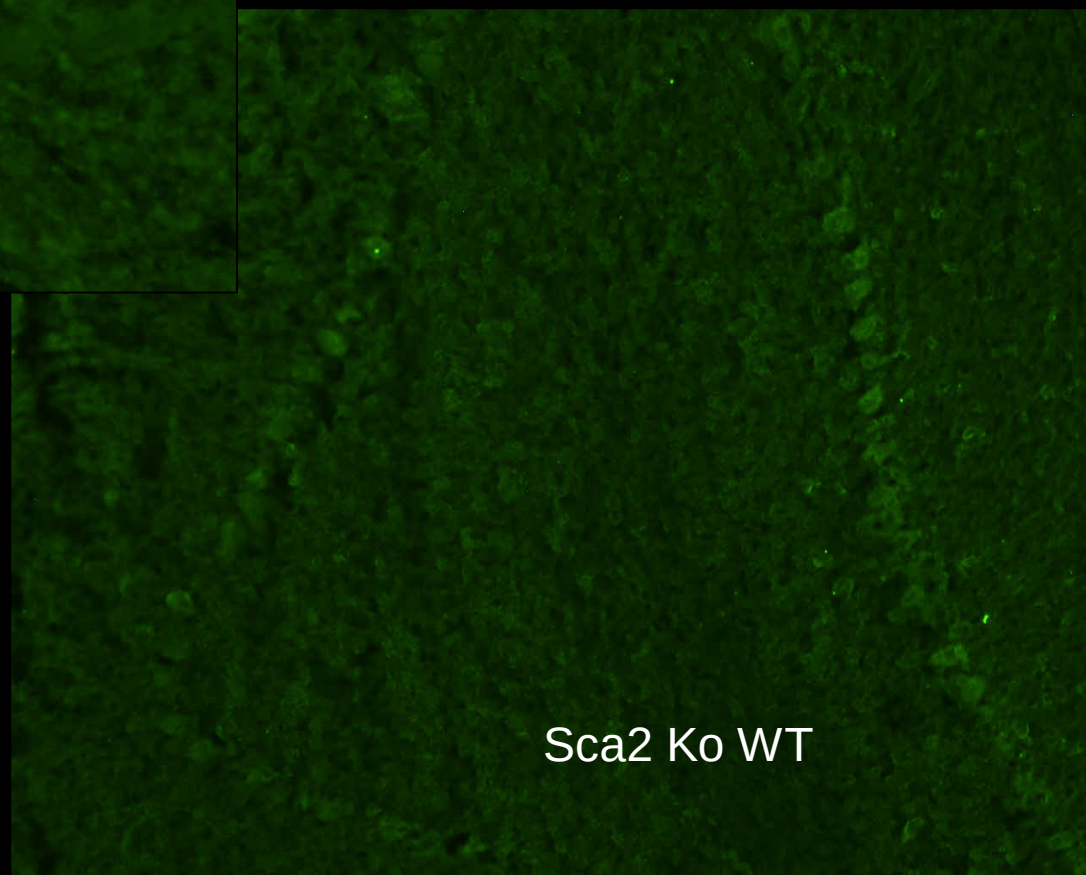
Methods: Stain

- 3 x 10min 1 x PBS wash
- Permeabilize (0.3% Triton) and block (10% NDS) in 1 x PBS @ room temp, 2hrs
- Primary antibody (714- 15 μ g/mL); Calbindin ab (1:300) in 0.1% Triton – 5%NDS in 1 x PBS overnight at 4c
- 3 x 15min 0.1% triton and 1% NDS in 1 x PBS
- Secondary ab: Cy3 (red; anti-mouse), Cy2 (green; anti-rabbit)
- 3 x 10min wash: 0.1% triton and 1% NDS in 1 x PBS (dapi 1:6k on final wash)
- 3 x 5min 1 x PBS
- Cover with slip with Prolong-Gold → dry overnight

All camera settings are held
constant across slides



Sca2 Ko -/-



Sca2 Ko WT

Control Sca2 KO

GL

PL

ML

Sca2Ko: 23 -/-

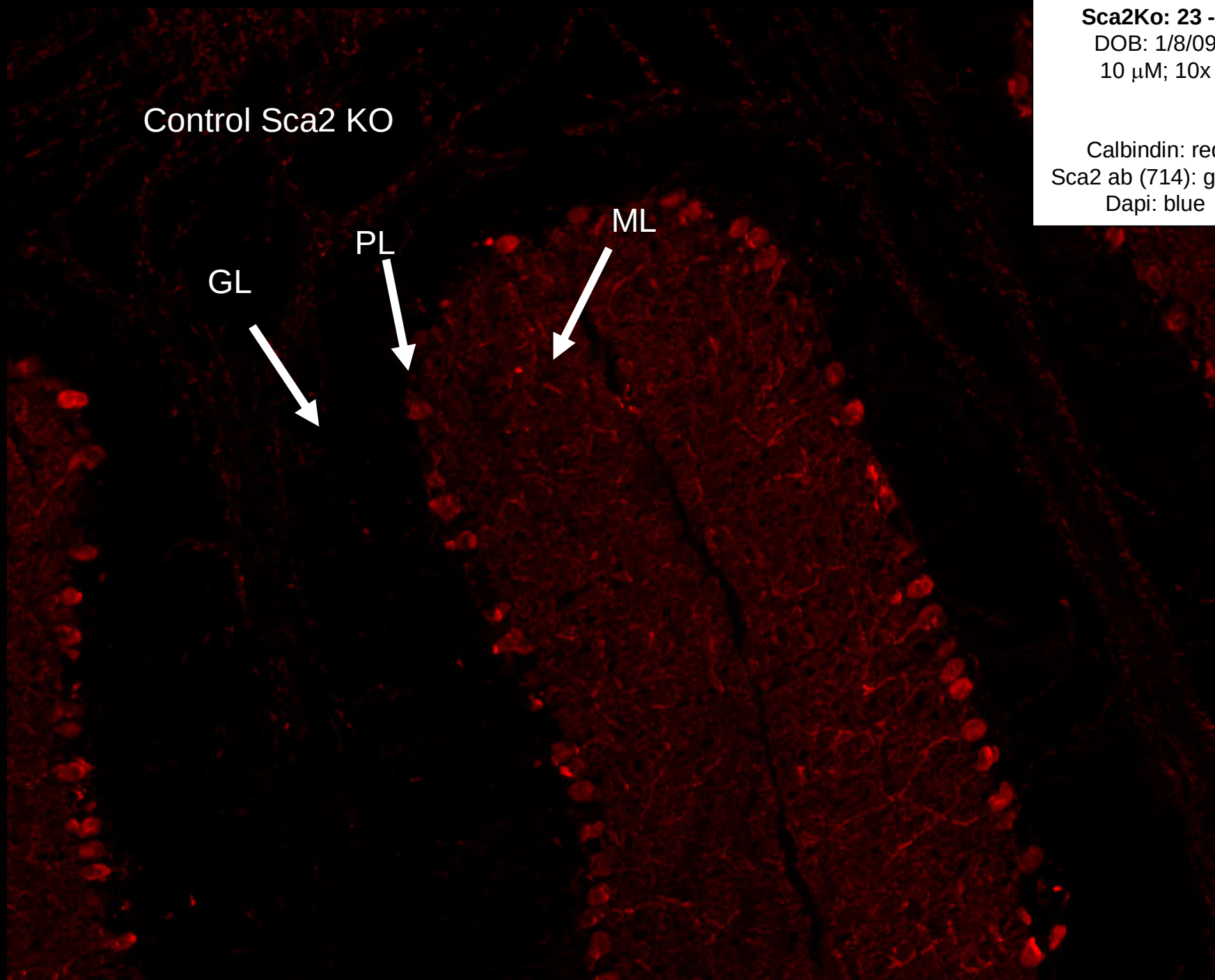
DOB: 1/8/09

10 μ M; 10x

Calbindin: red

Sca2 ab (714): green

Dapi: blue



Sca2Ko: 23 -/-

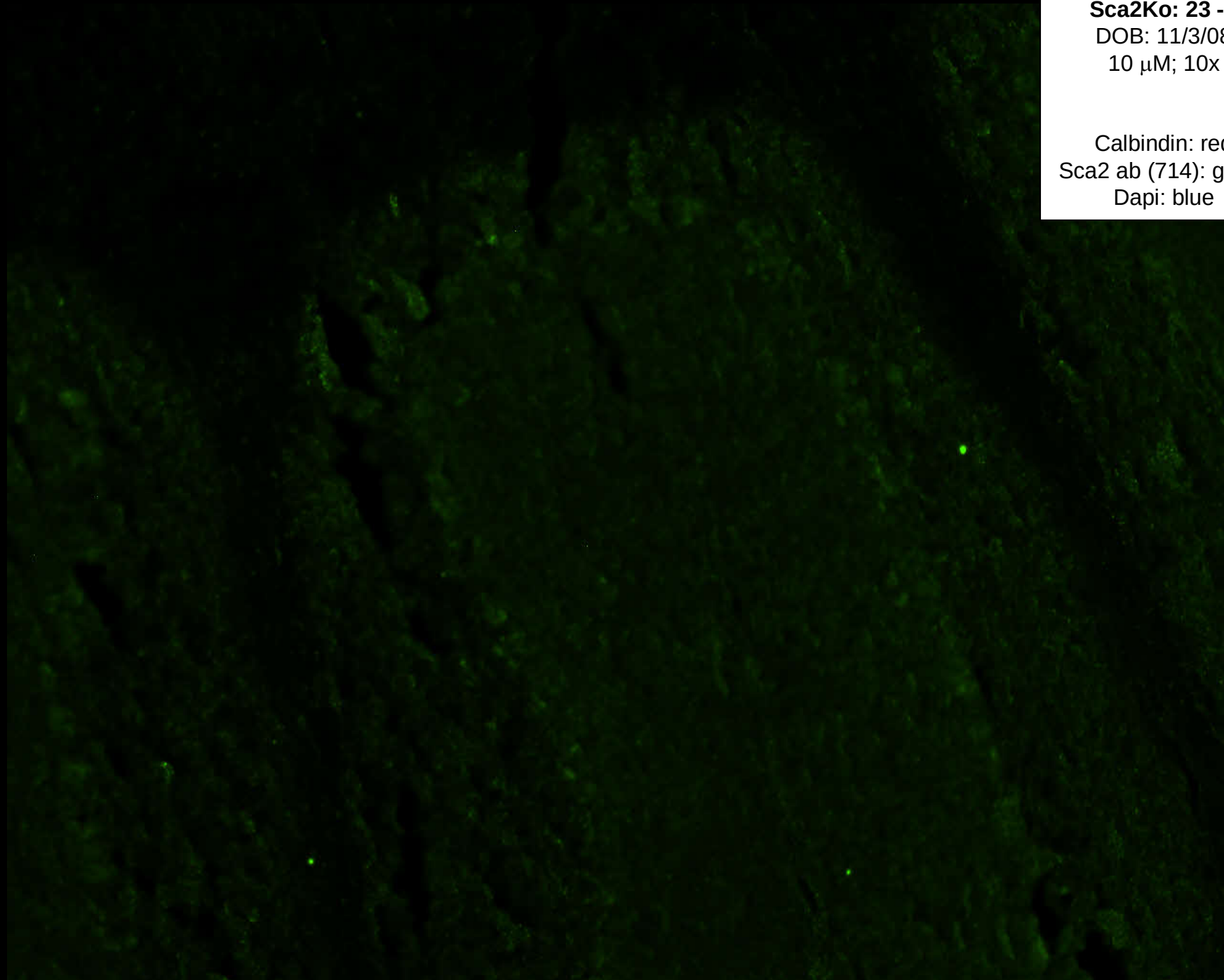
DOB: 11/3/08

10 μ M; 10x

Calbindin: red

Sca2 ab (714): green

Dapi: blue



Sca2Ko: 24 -/-

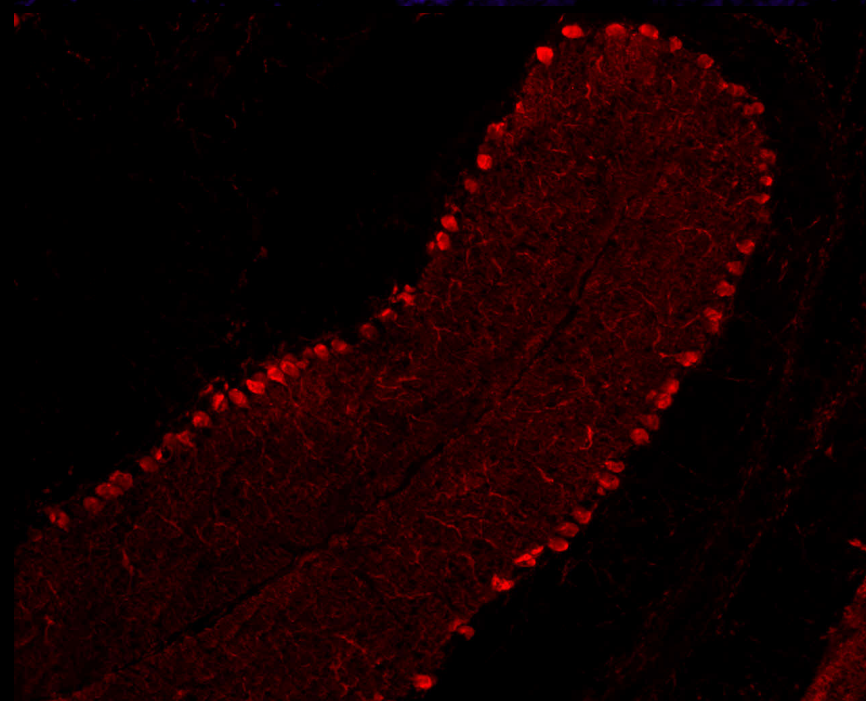
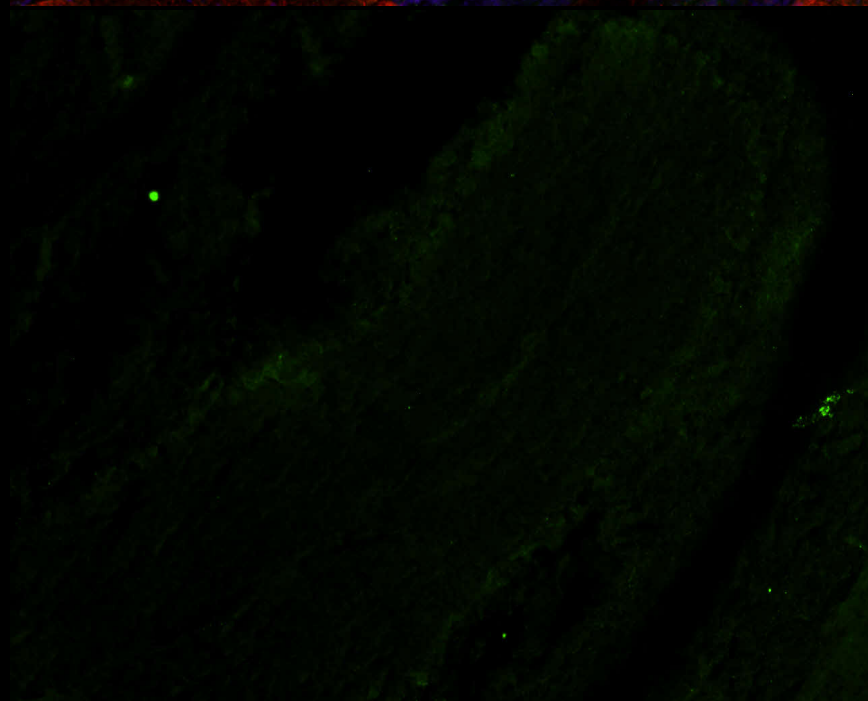
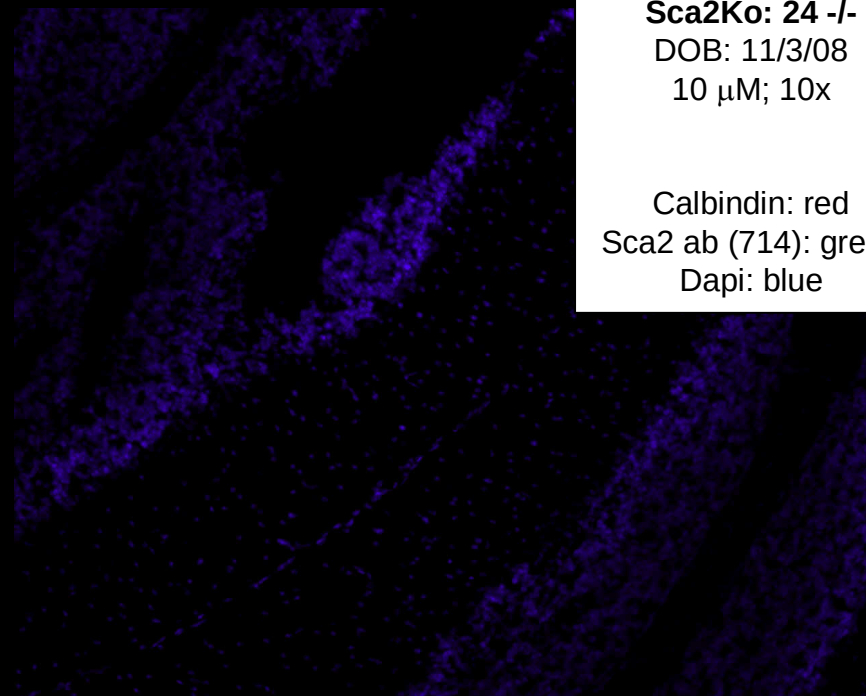
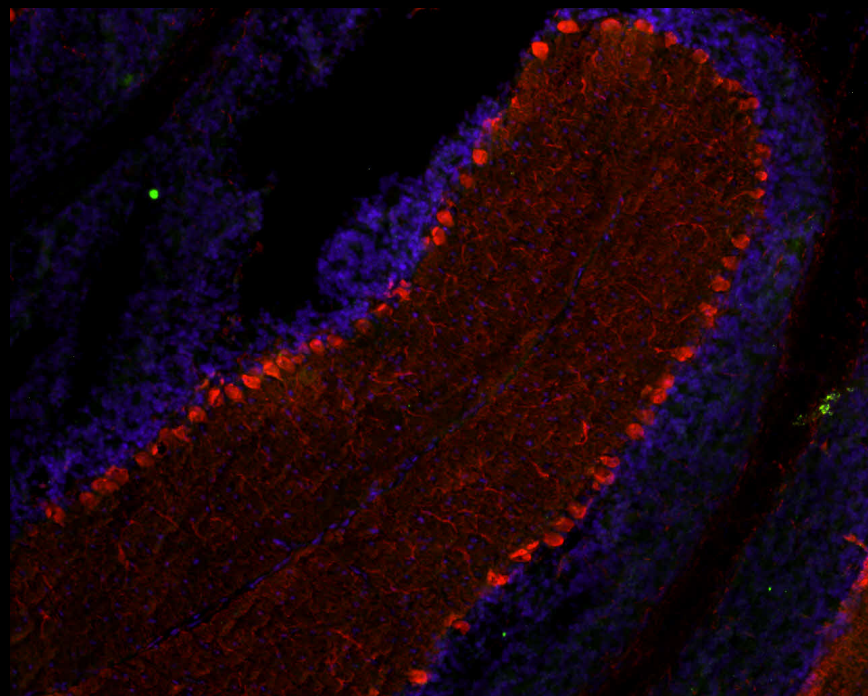
DOB: 11/3/08

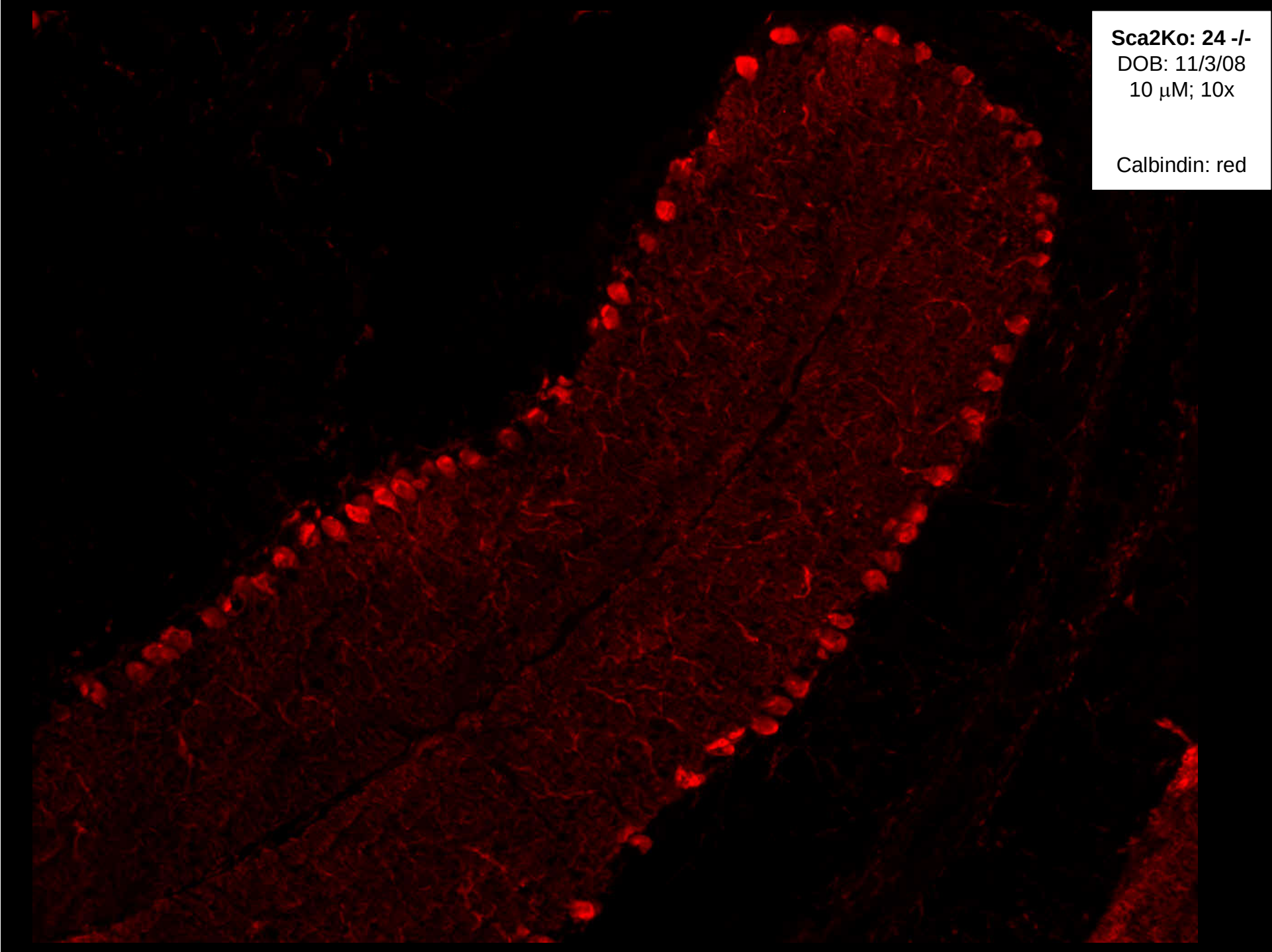
10 μ M; 10x

Calbindin: red

Sca2 ab (714): green

Dapi: blue





Sca2Ko: 24 -/-
DOB: 11/3/08
10 μ M; 10x

Calbindin: red

40-2: 6 months

Q127 40-2: 105 (wt)

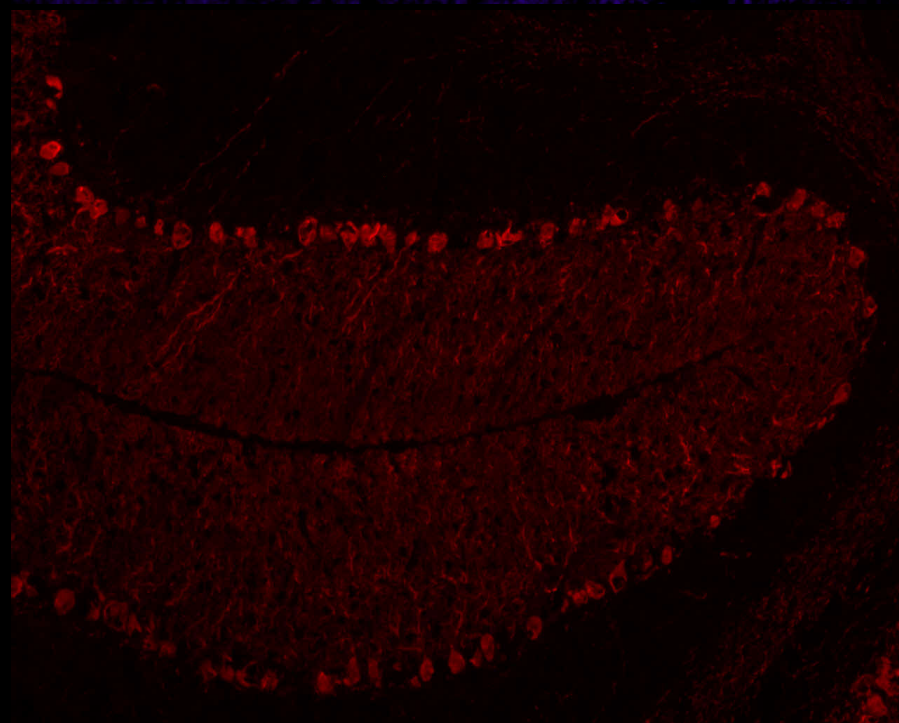
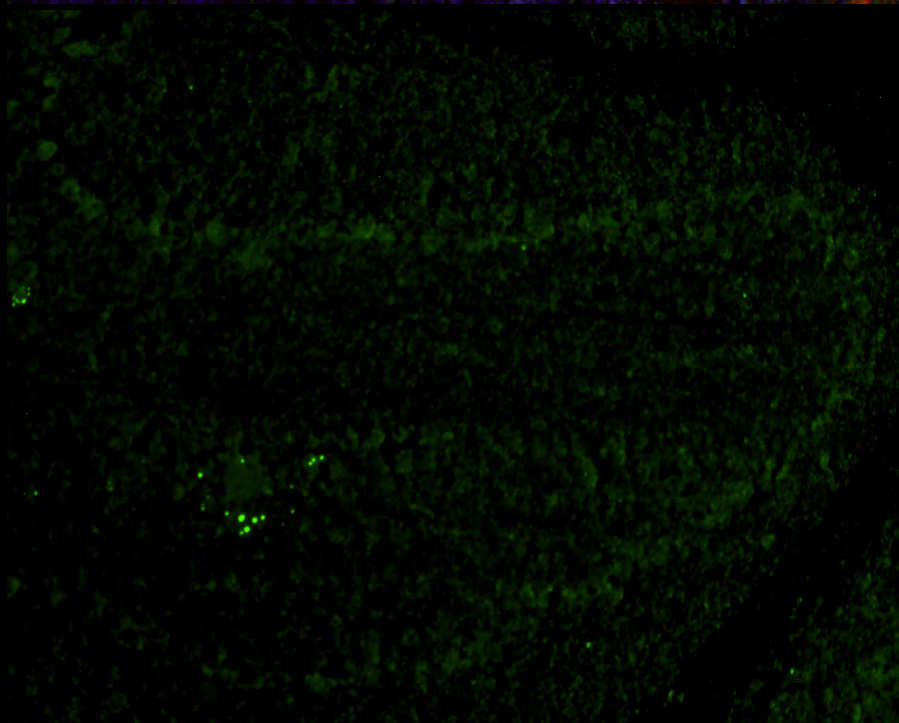
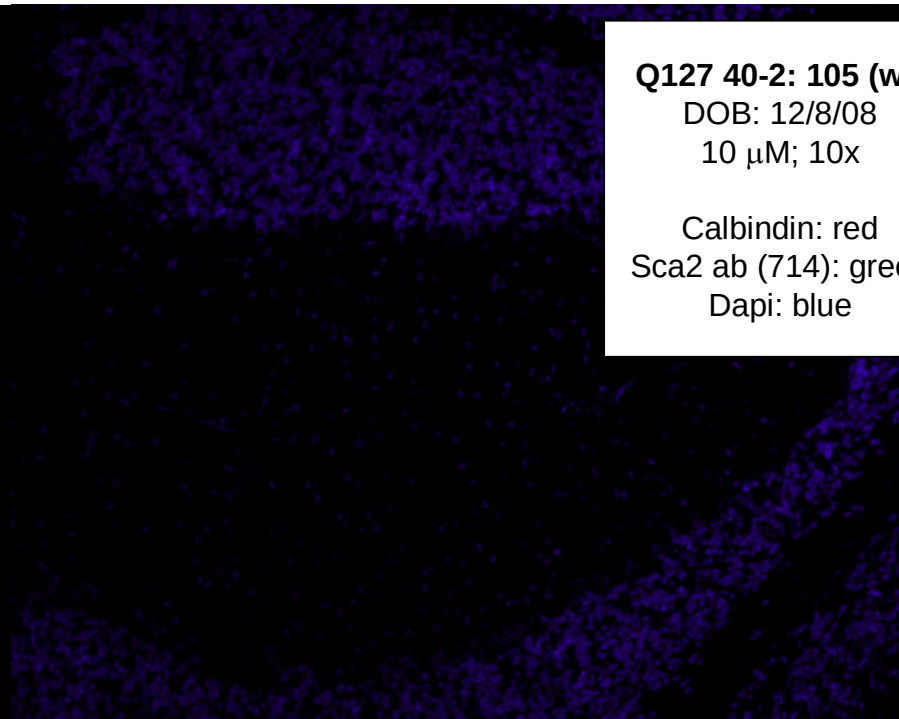
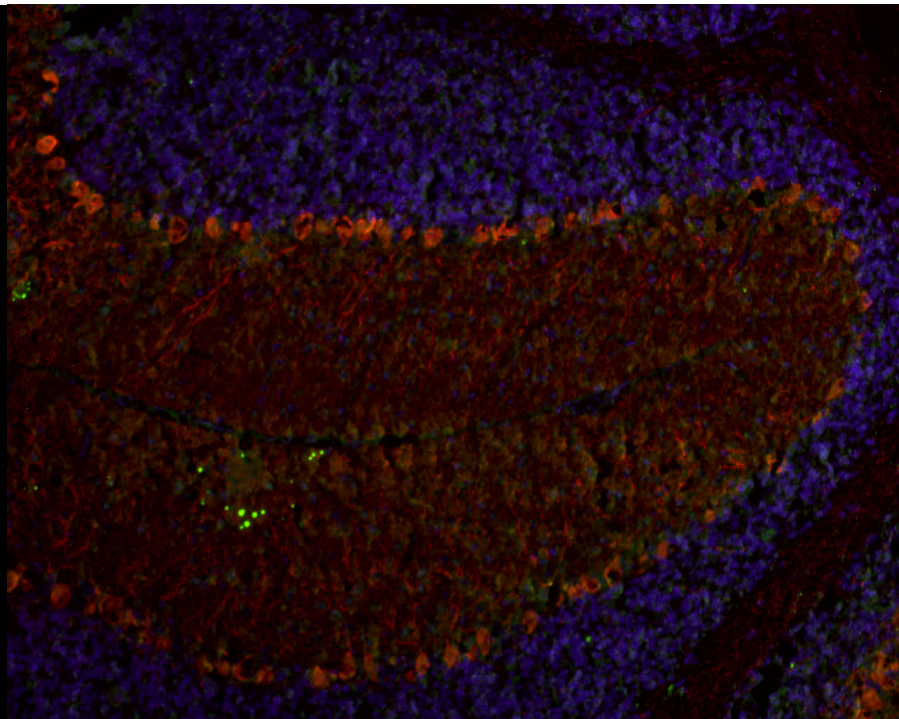
DOB: 12/8/08

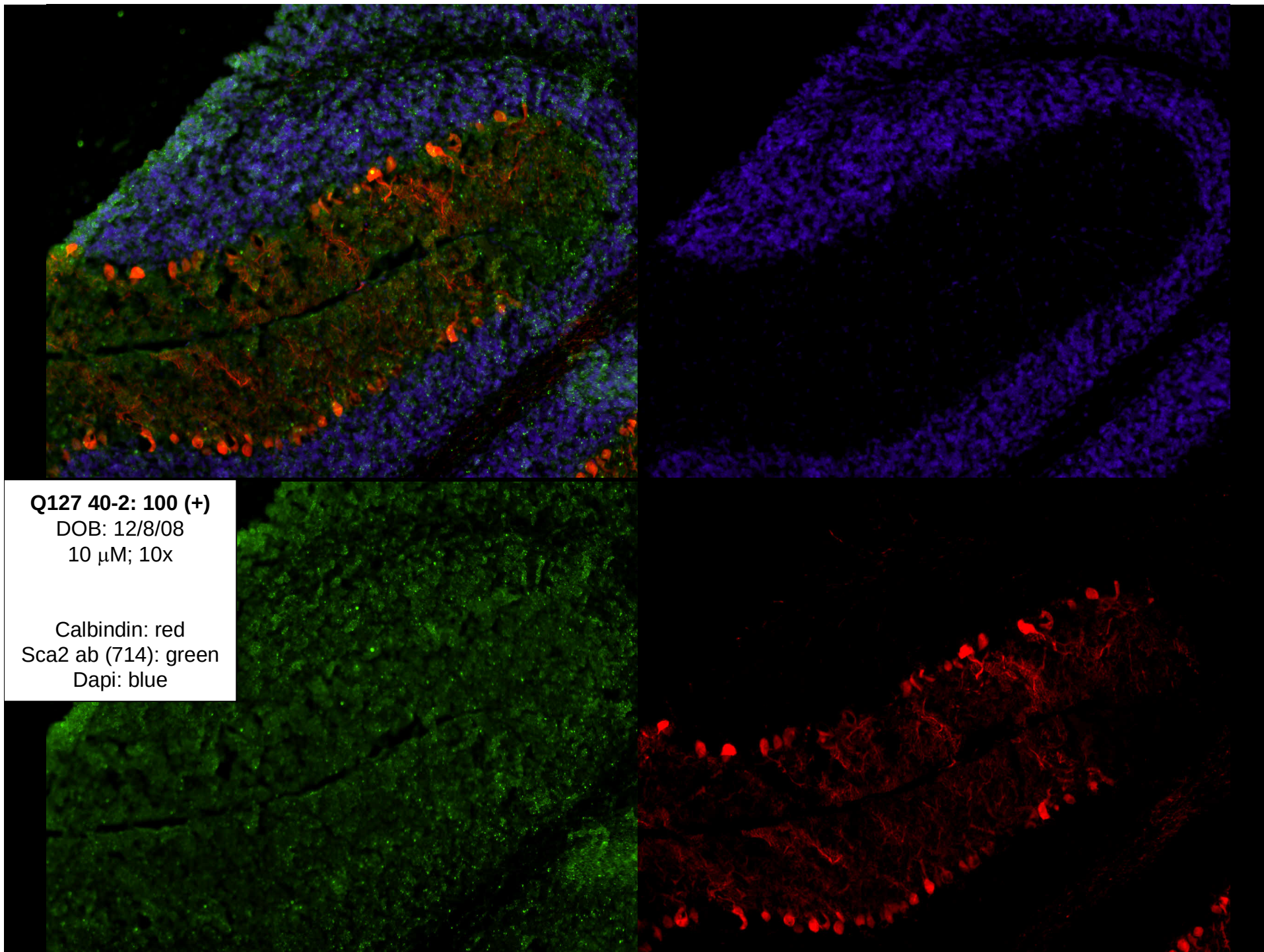
10 μ M; 10x

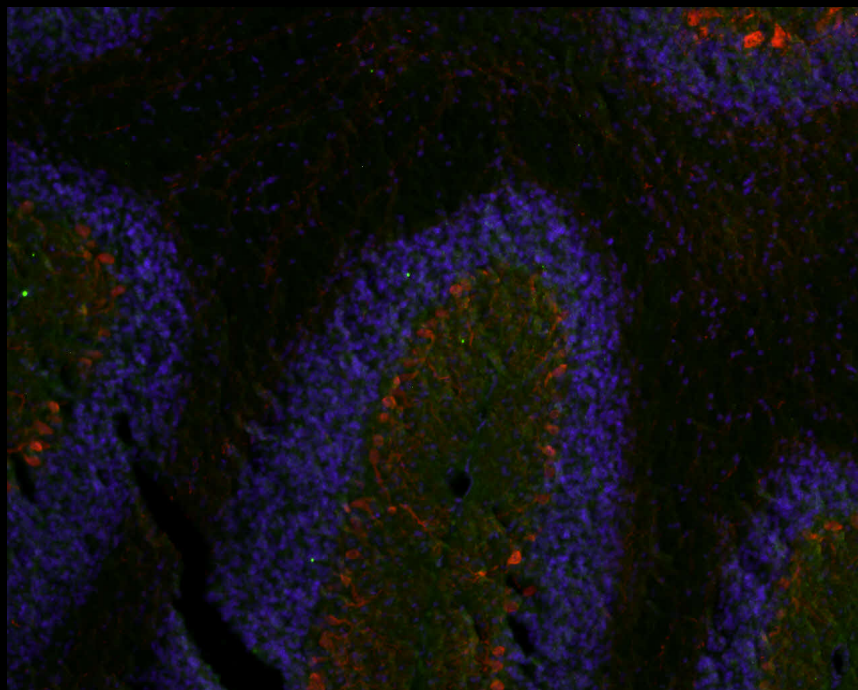
Calbindin: red

Sca2 ab (714): green

Dapi: blue







Q127 40-2: 100 (+)

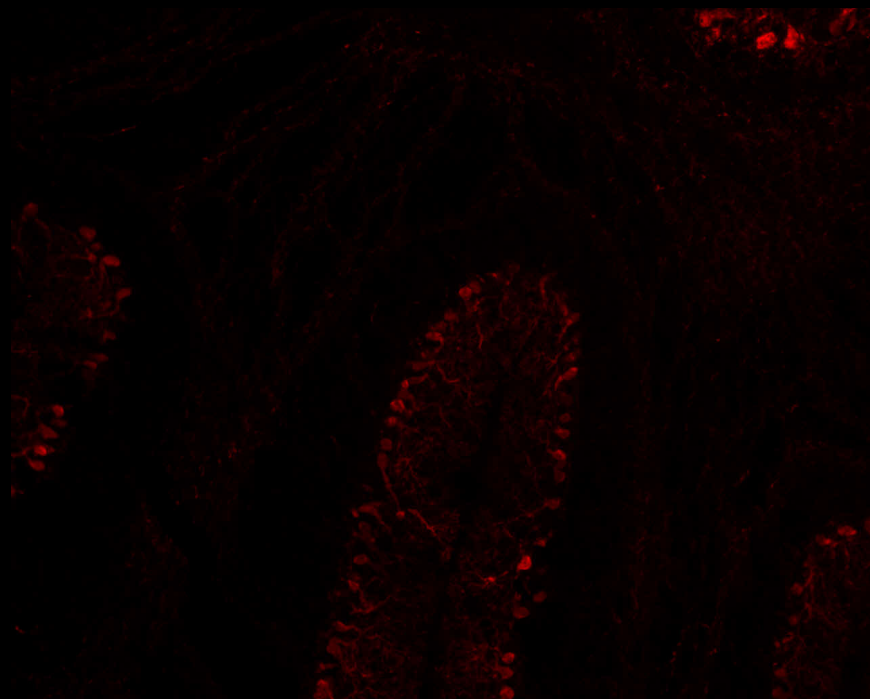
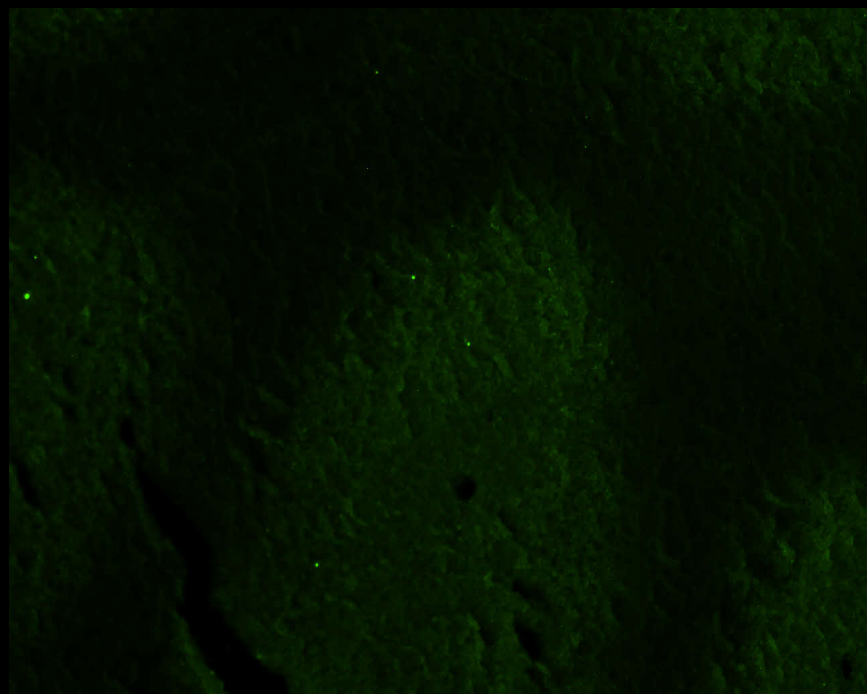
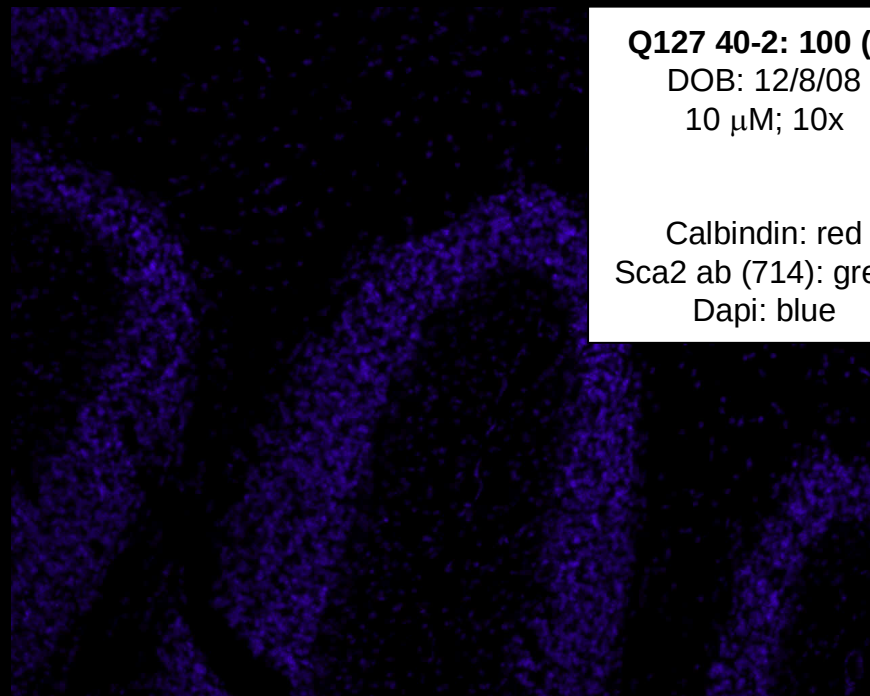
DOB: 12/8/08

10 μ M; 10x

Calbindin: red

Sca2 ab (714): green

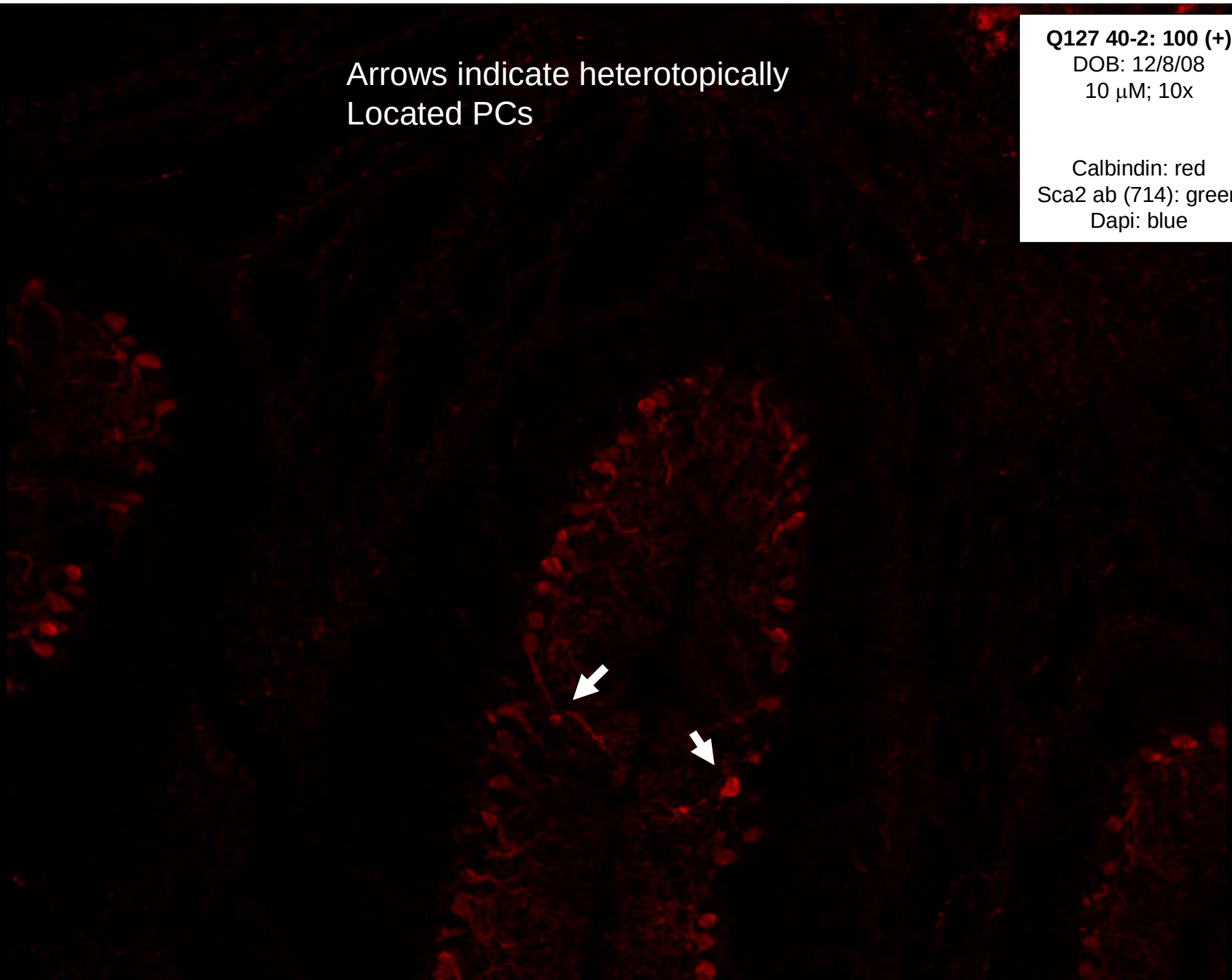
Dapi: blue



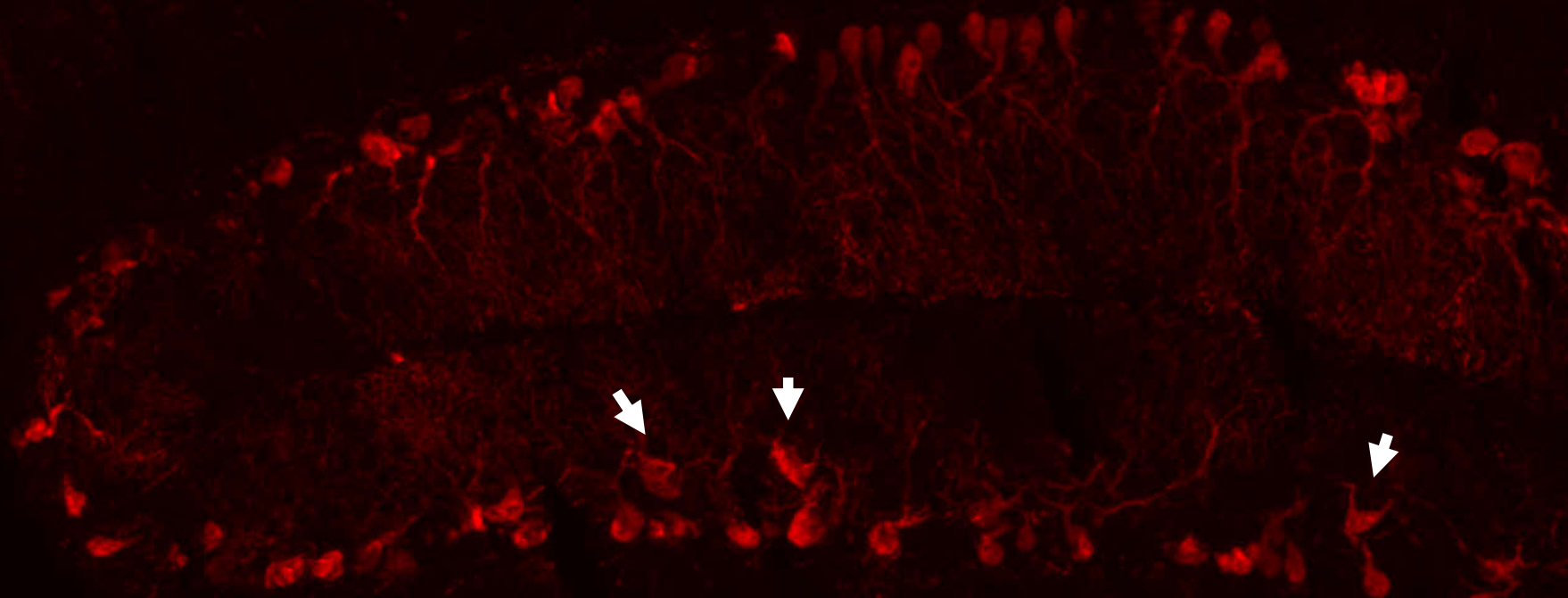
Arrows indicate heterotopically
Located PCs

Q127 40-2: 100 (+)
DOB: 12/8/08
10 μ M; 10x

Calbindin: red
Sca2 ab (714): green
Dapi: blue



Arrows indicate heterotopically
Located PCs



Q127 40-2: 100 (+)
DOB: 12/8/08
10 μ M; 10x

Calbindin: red
Sca2 ab (714): green
Dapi: blue

40-2: 8 weeks

- The following brain slices were not processed with the slides previously shown
- Calbindin stain only
- Coronal sectioning

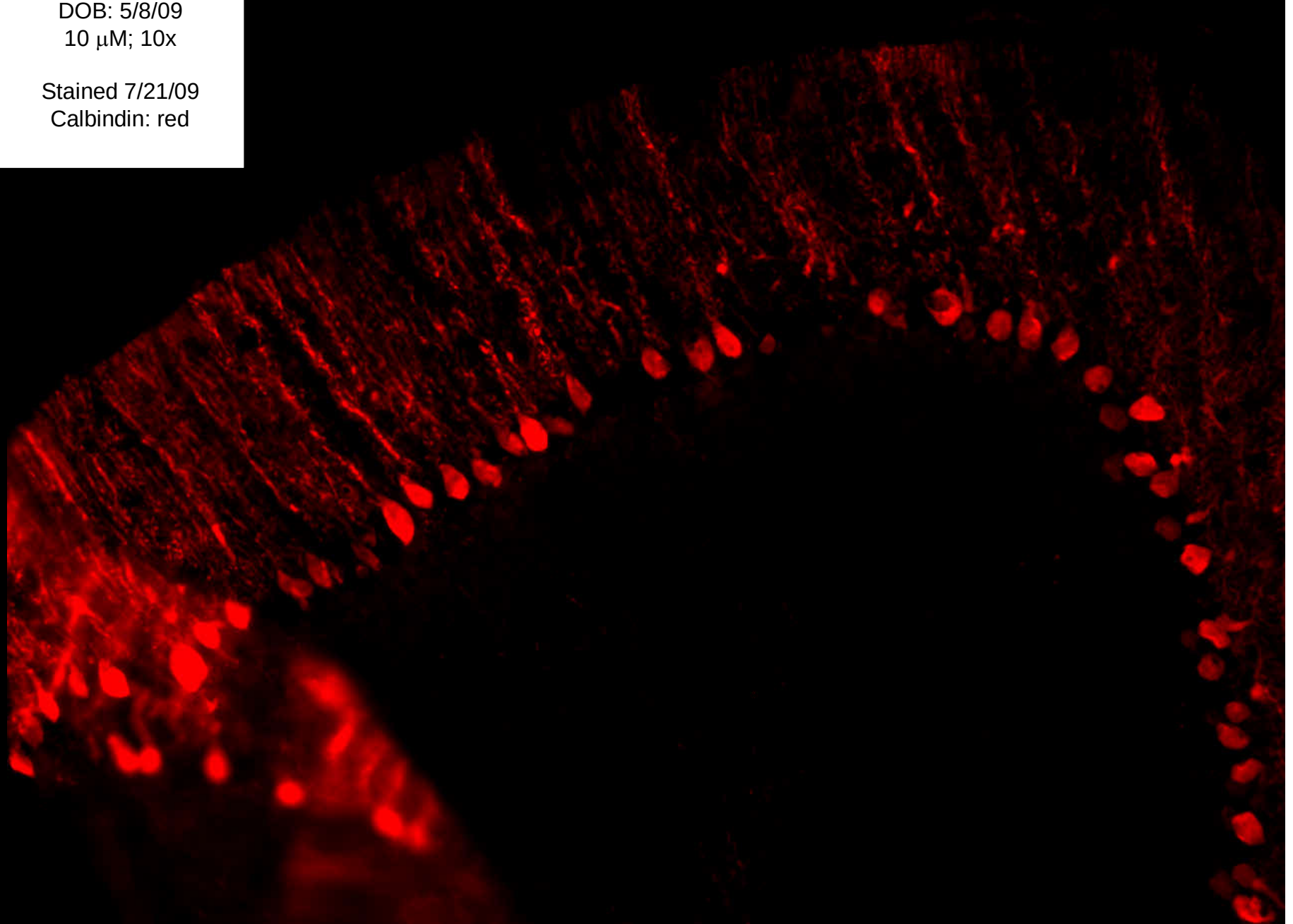
Q127 40-2: 139 (+)

DOB: 5/8/09

10 μ M; 10x

Stained 7/21/09

Calbindin: red



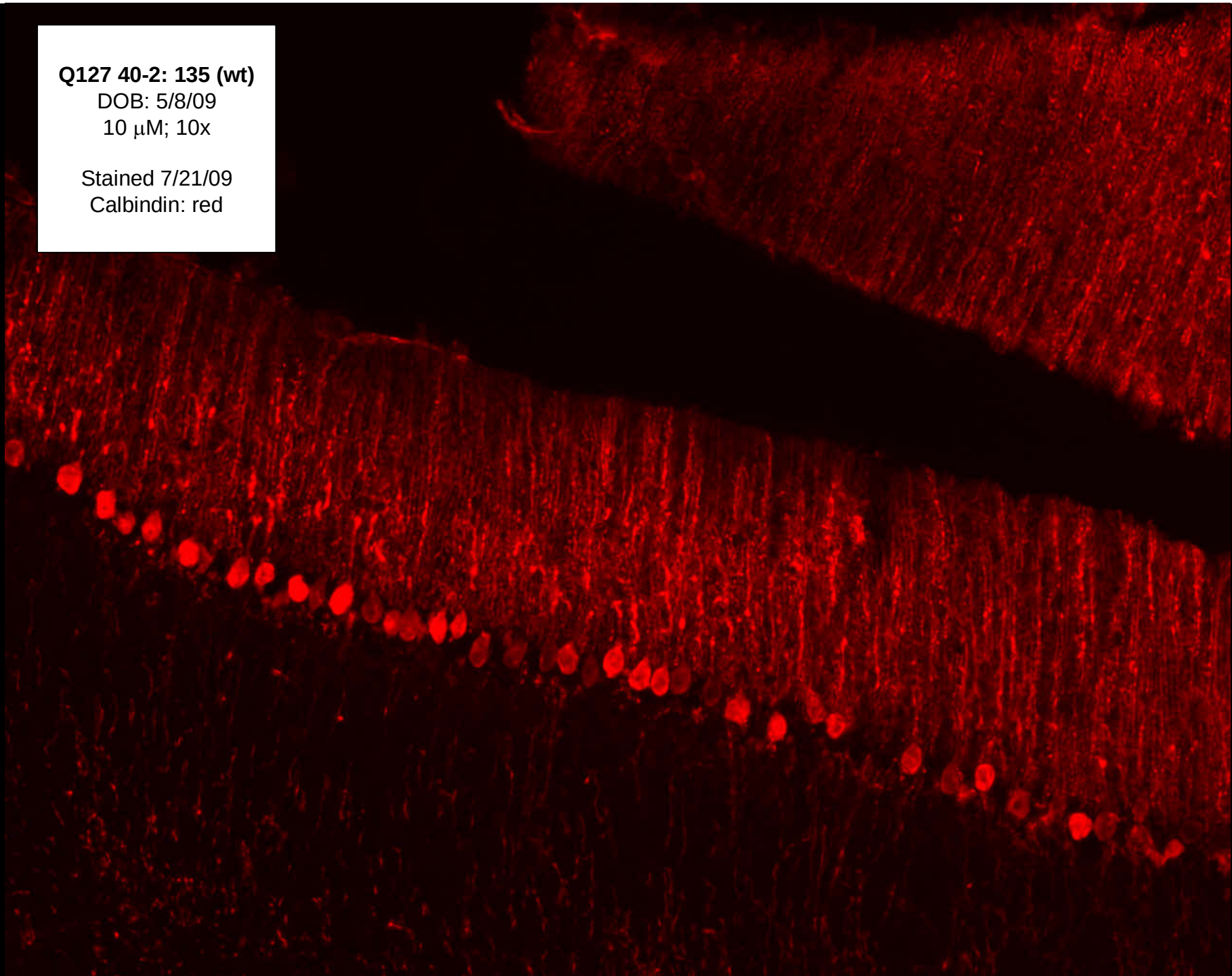
Q127 40-2: 135 (wt)

DOB: 5/8/09

10 μ M; 10x

Stained 7/21/09

Calbindin: red

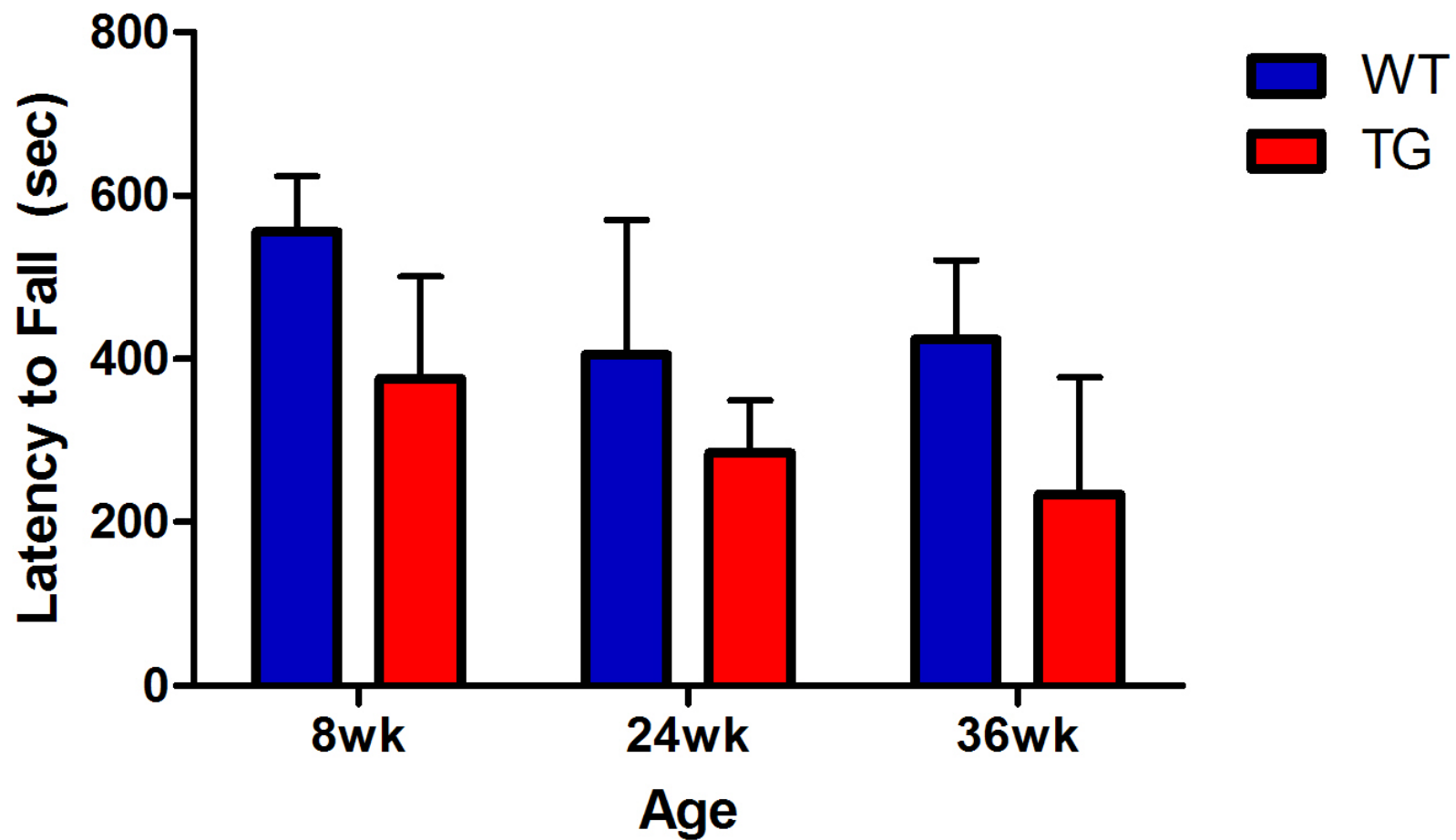


Future

- IHC profile across age & genotype
- WB and Q PCR of same
- Test
- Breed Q127 animals with existing Park KO animals
- Ca++ functionality

Extra slides

Q127: 40-2: rotarod



Q127 40-2: 105 (wt)

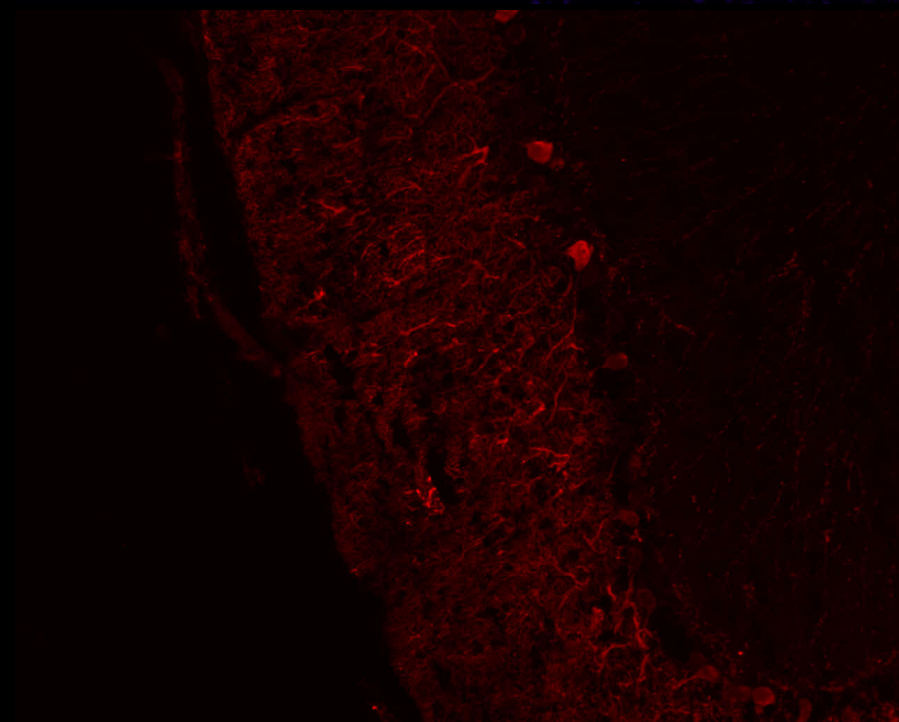
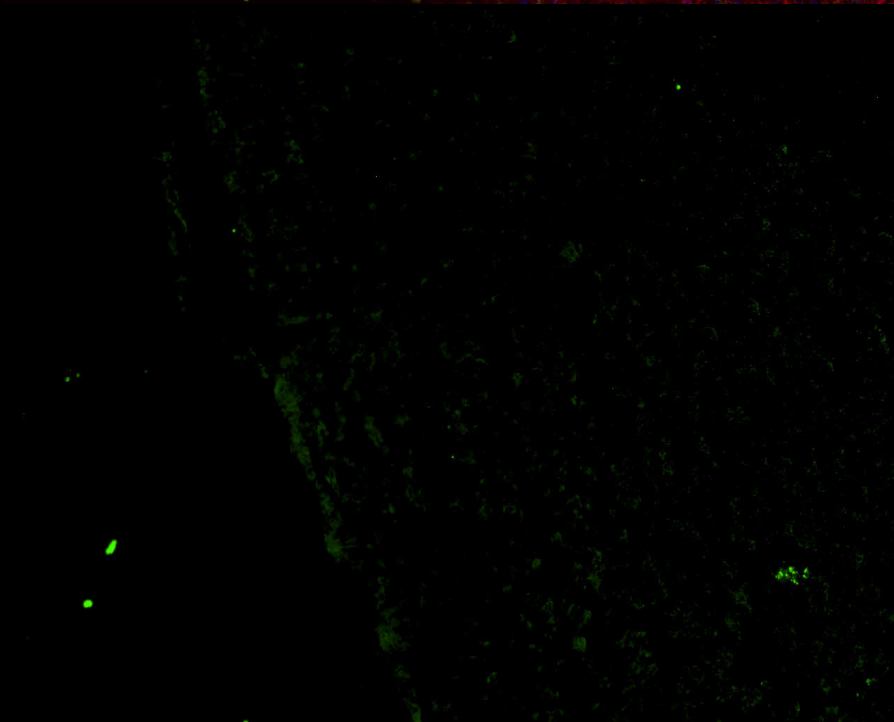
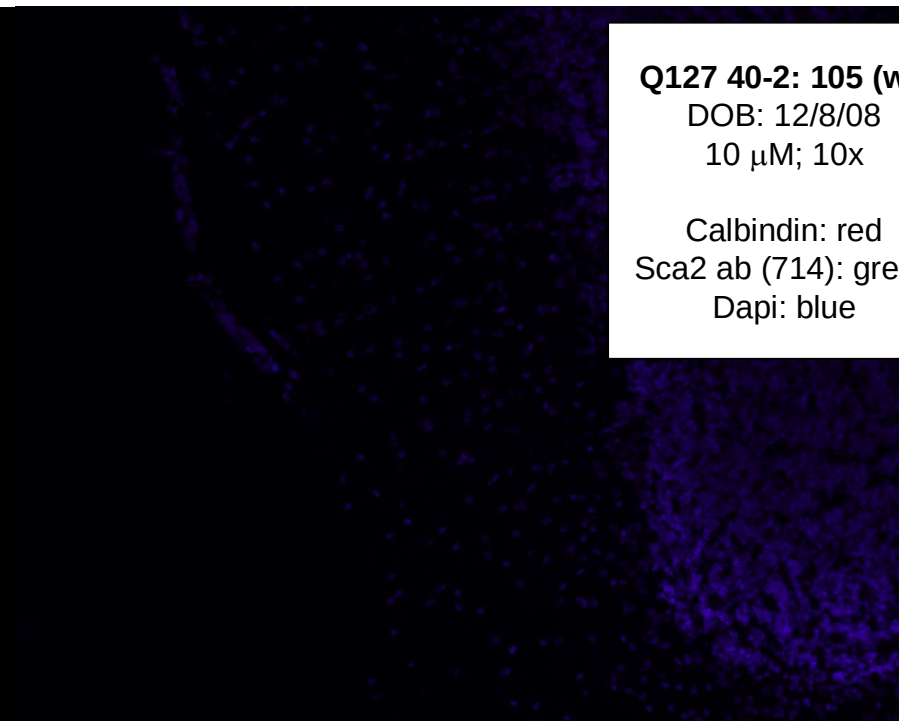
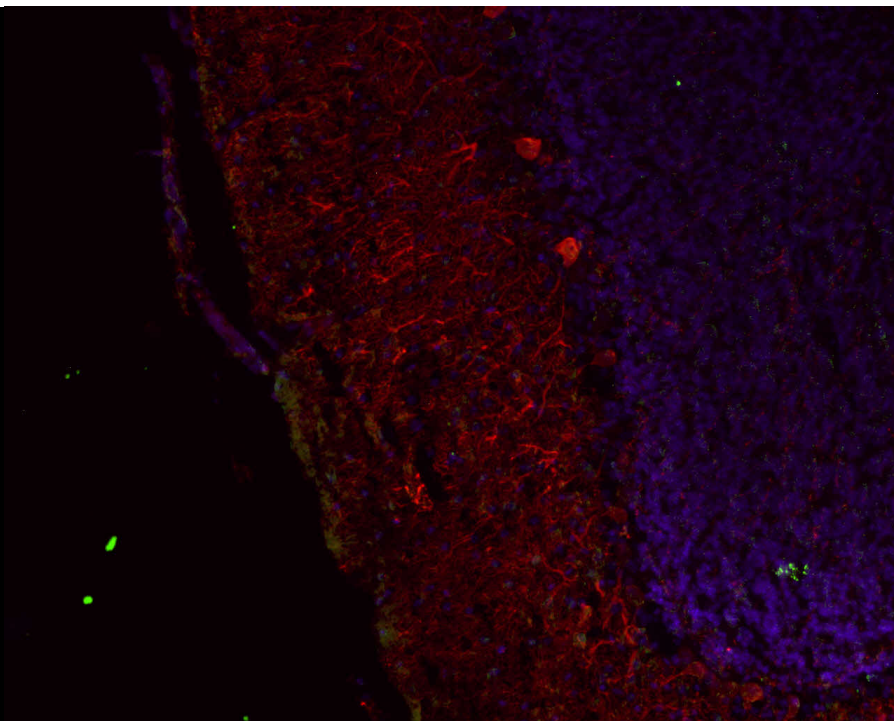
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Q127 40-2: 100 (+)

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