



Q: What happens when a neurotransmitter falls in love with a receptor?

A: You get a binding relationship

# Background

- Model polyglutamine disease in mice
  - Anatomical and physiological effect of CAG repeat
  - Channelopathy: pathological Ataxin-2 effect on  $Ca^{++}$  channel conductance
  - Parkin dosage effect on SCA2 phenotype

# Q127

- 9 lines created
- 7 lines show expression of the TG
  - 4 show qPCR RNA expression
  - Only 1 shows behavioral phenotype
  - Only 1 shows anatomical pathology?

# 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 nitro then placed on dry ice: stored at -80 C for biochemical analysis
    - qPCR; WB
  - Histology half: Cryoprotection- brain submerged 24hrs in ice cold paraformaldehyde 4%. Followed by 24hrs in 20% then 24hrs in 30% sucrose solution all at 4 C.



# Immunohistochemistry

# IHC Methods

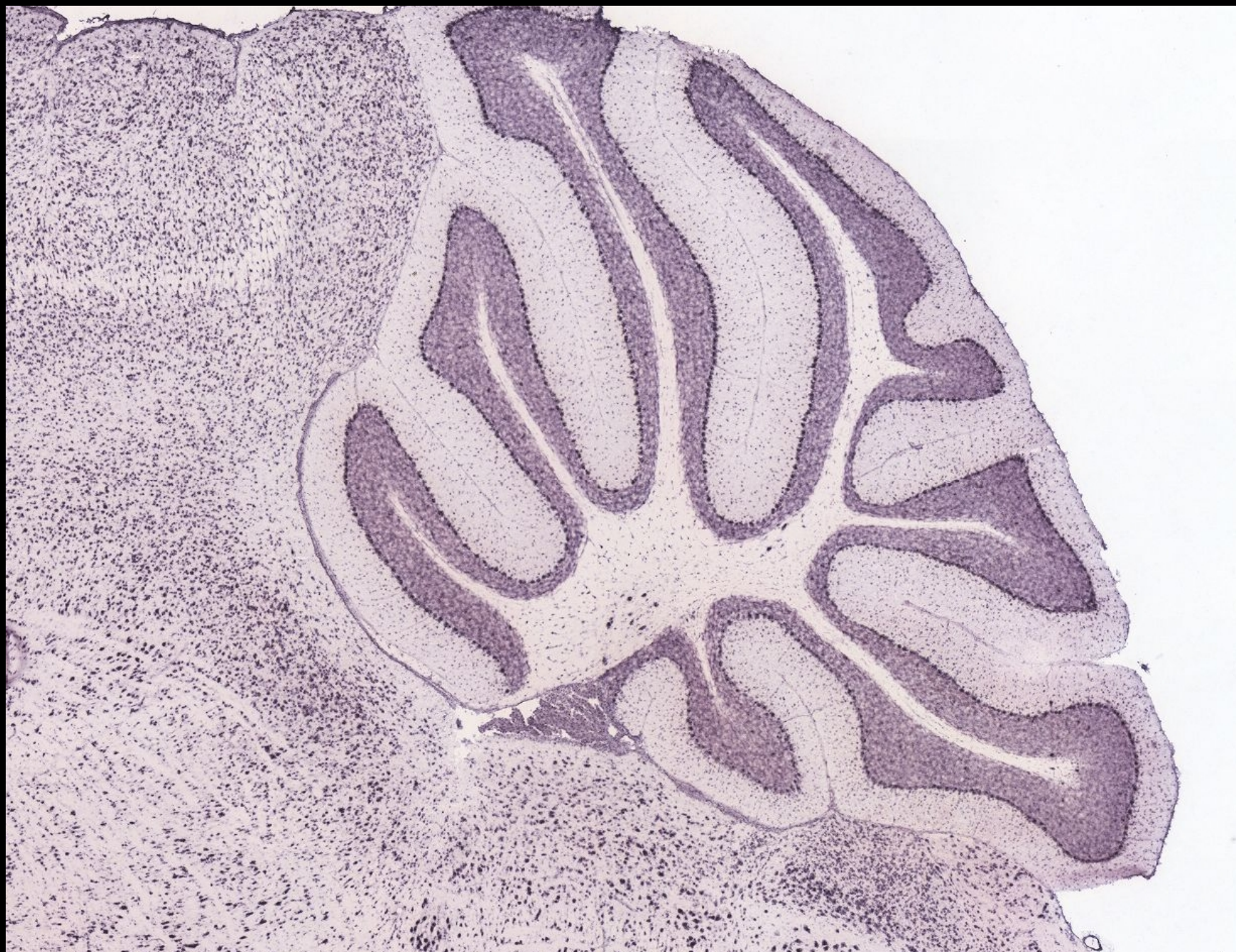
- 20  $\mu$ M brain sections
- 1x PBS: wash 3x @ 10min
- Permeabilize and block 4hrs @ RT
- Primary antibody 24hrs @ 4c
- Wash 3x @ 15min with wash buffer
- Secondary antibody 2hrs @ RT
- Wash 3x @ 15min with wash buffer

# Solutions:

- Permeabilization & blocking buffer: 0.3% Triton in 1x PBS with 5% skim milk; 0.01% Na-azide
- Wash buffer: 0.1% Triton in 1x PBS with 1% skim milk
- AB solution: 0.1% Triton in 1x PBS with 5% skim milk
- Slide fixative media: Prolong Gold

# Camera

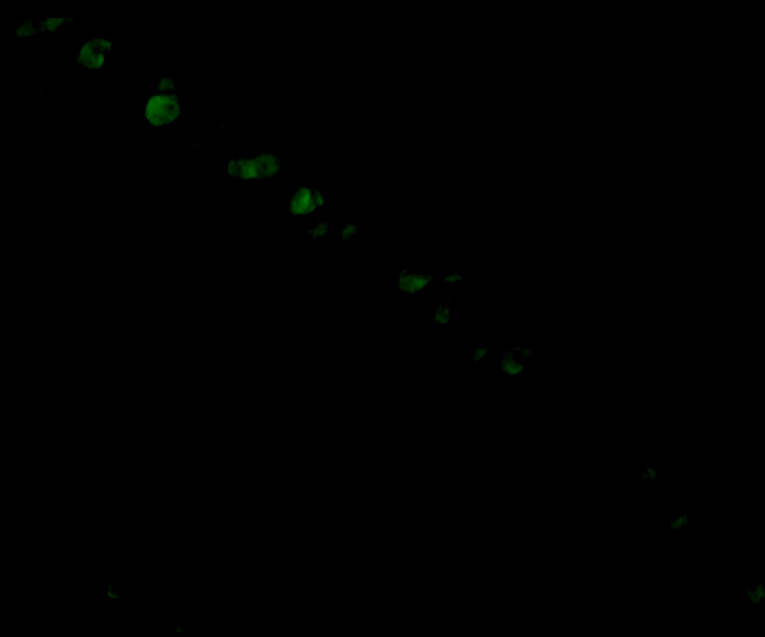
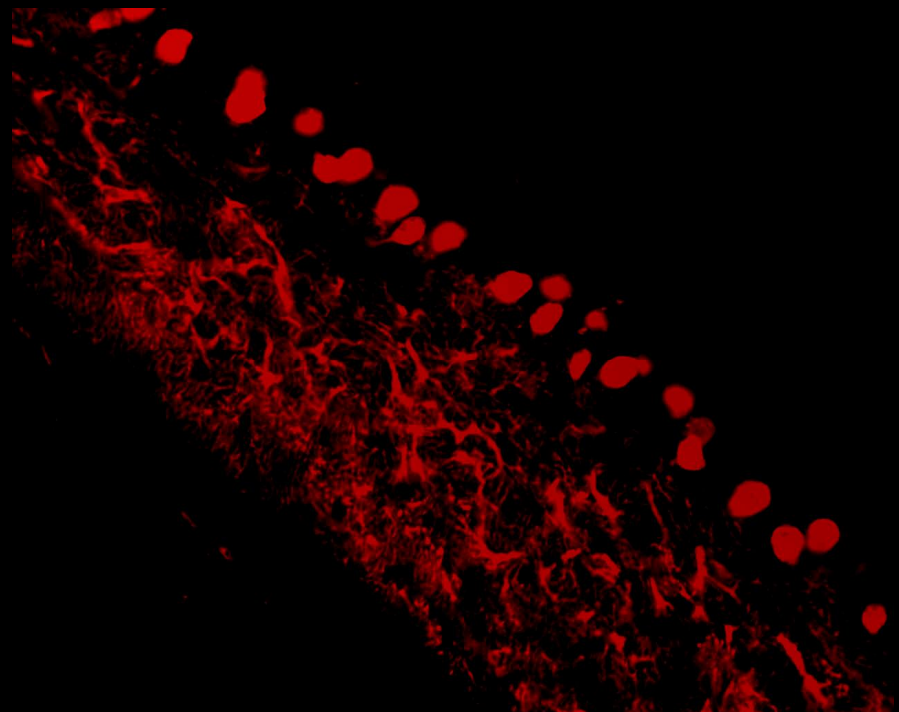
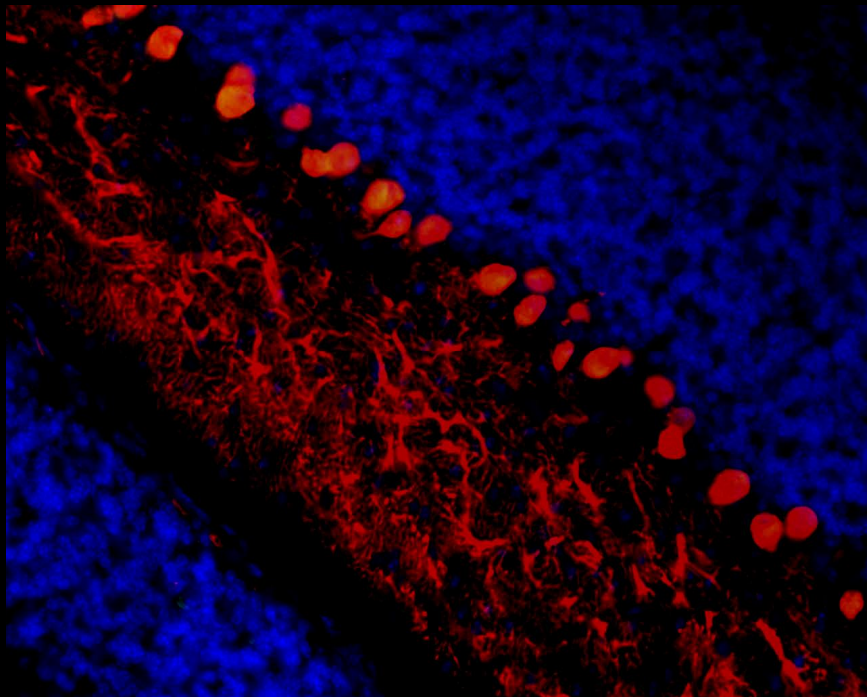
- Camera settings optimized
  - Control: secondary AB only
    - Background fluorescence subtracted from control
  - All settings remain constant across session



# 40-2

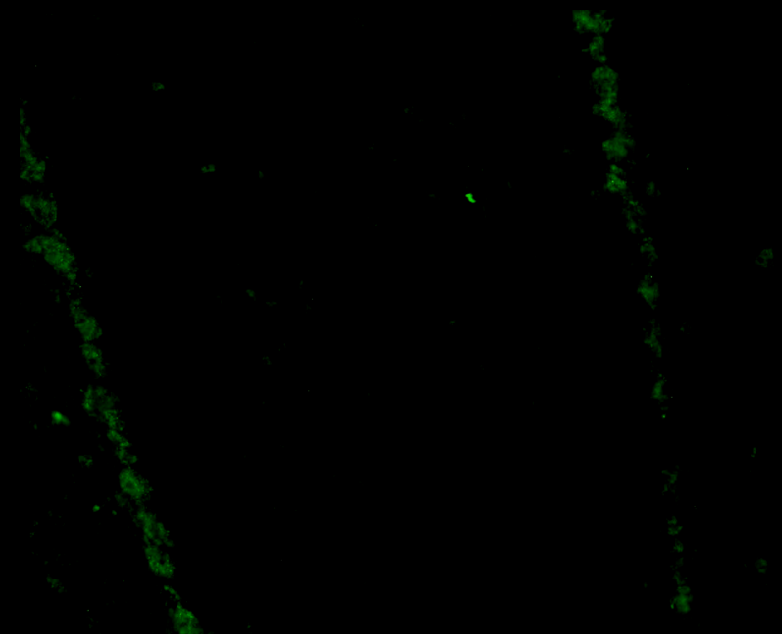
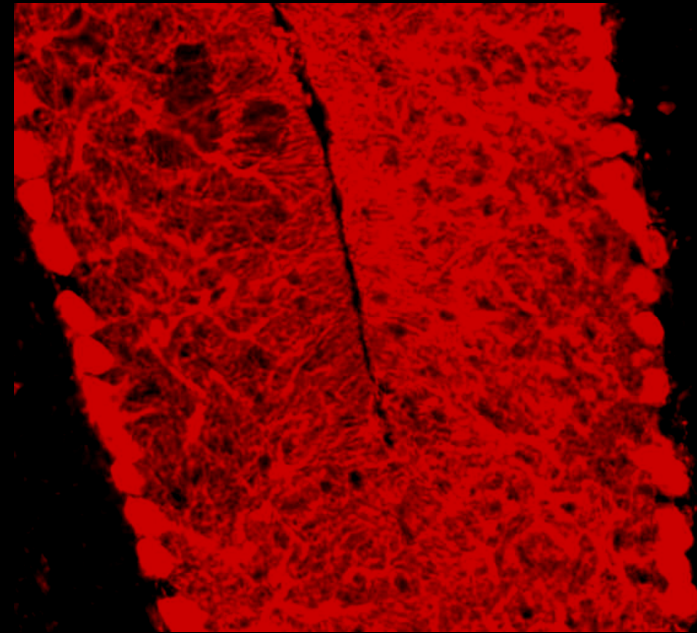
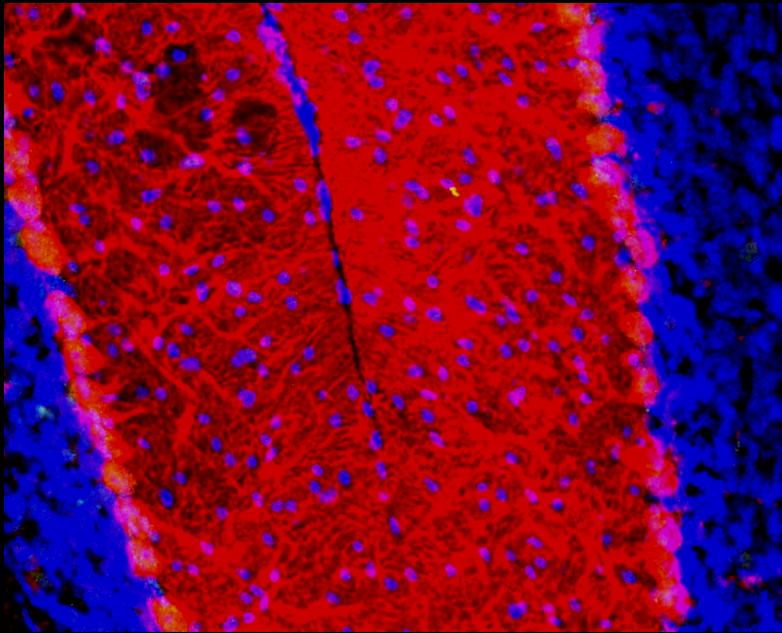
- Express ataxin
- Behavioral phenotype
- IHC phenotype
- RNA expression





Sca2ko 12 -/-  
Fluorescent 20x

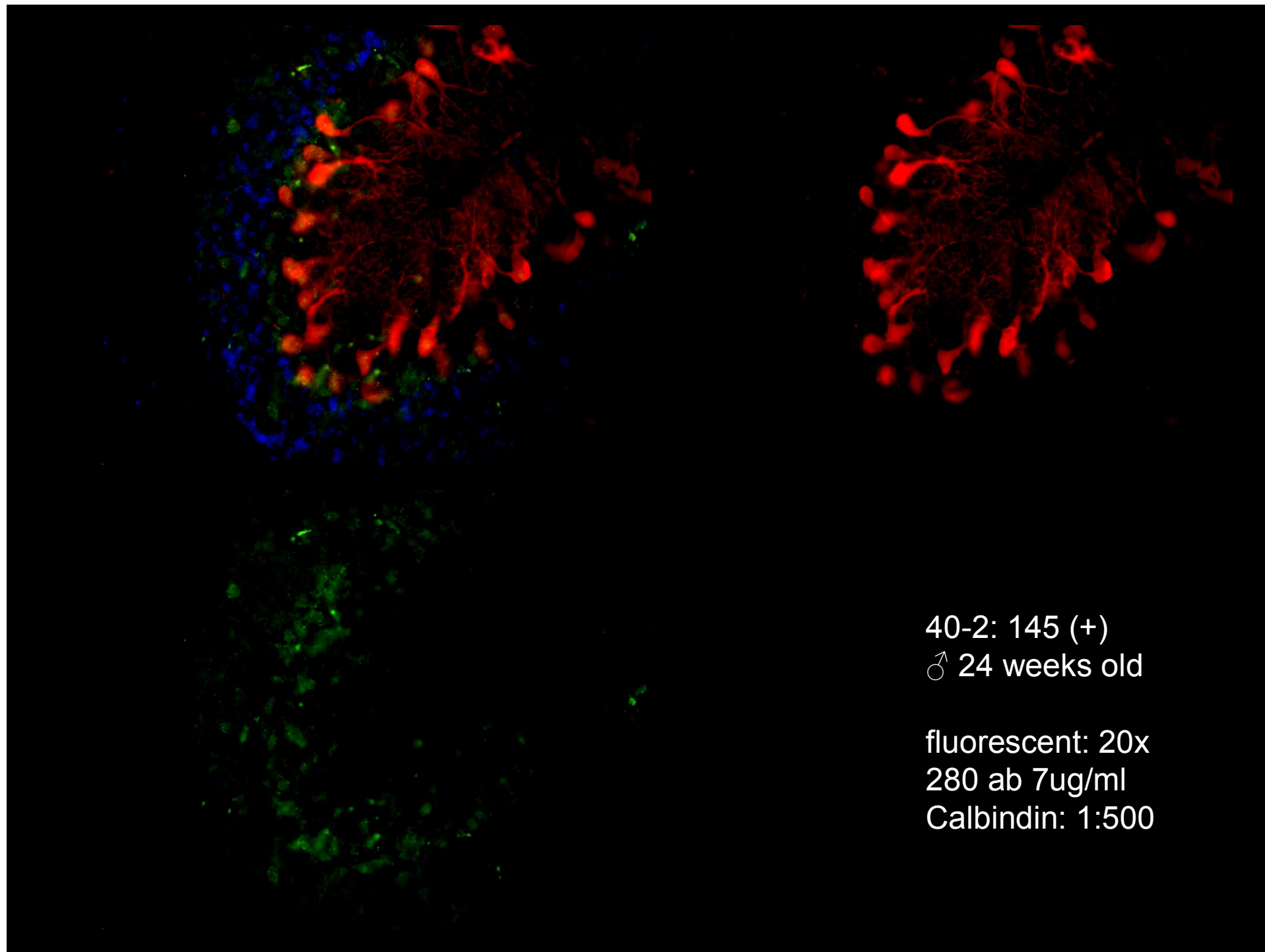
280 ab 7ug/ml  
Calbindin 1: 500



40-2: 147 (wt)  
♂ 24 weeks

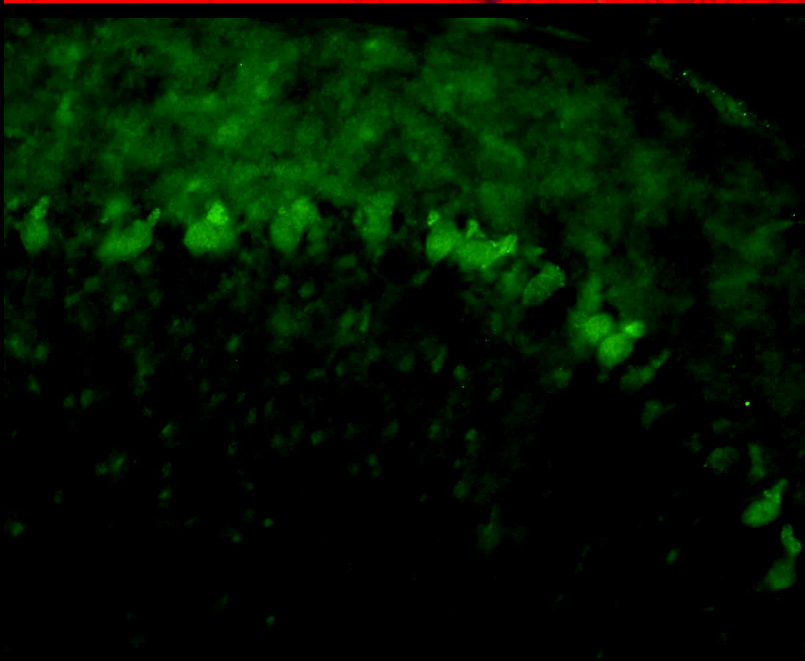
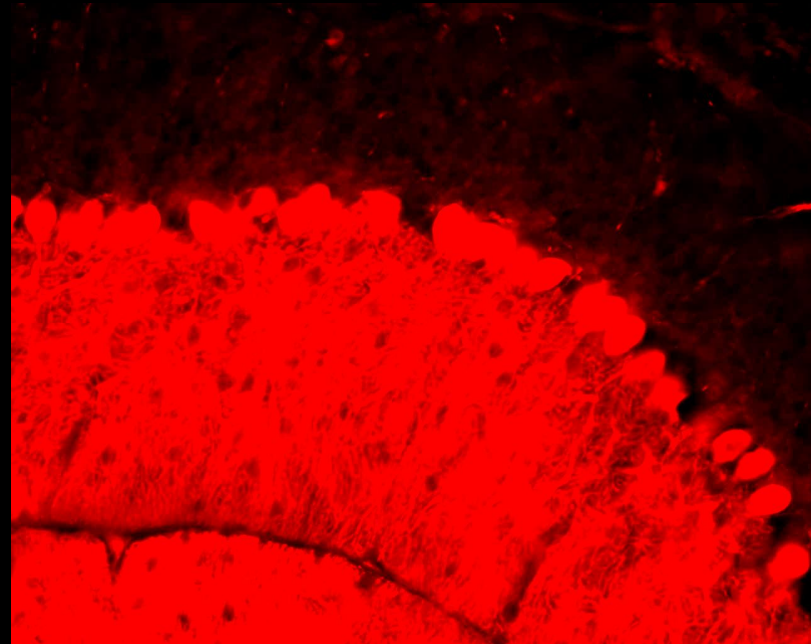
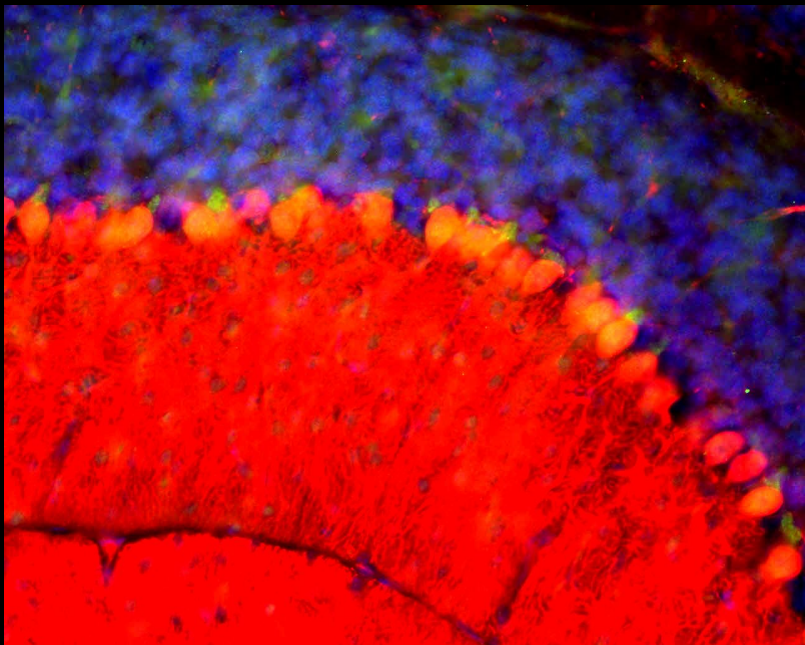
fluorescent: 20x  
280 ab 7ug/ml  
Calbindin: 1:500





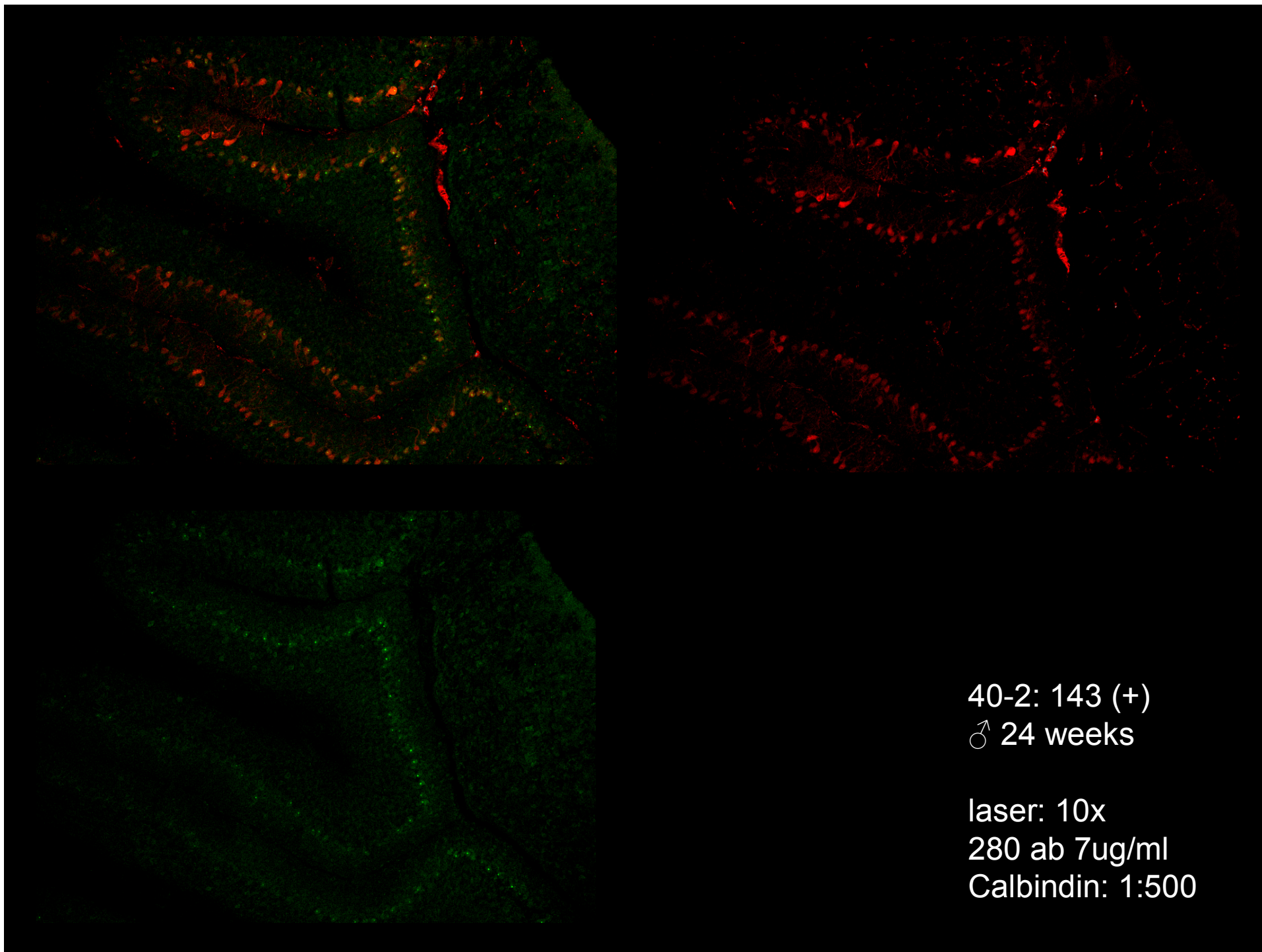
40-2: 145 (+)  
♂ 24 weeks old

fluorescent: 20x  
280 ab 7ug/ml  
Calbindin: 1:500

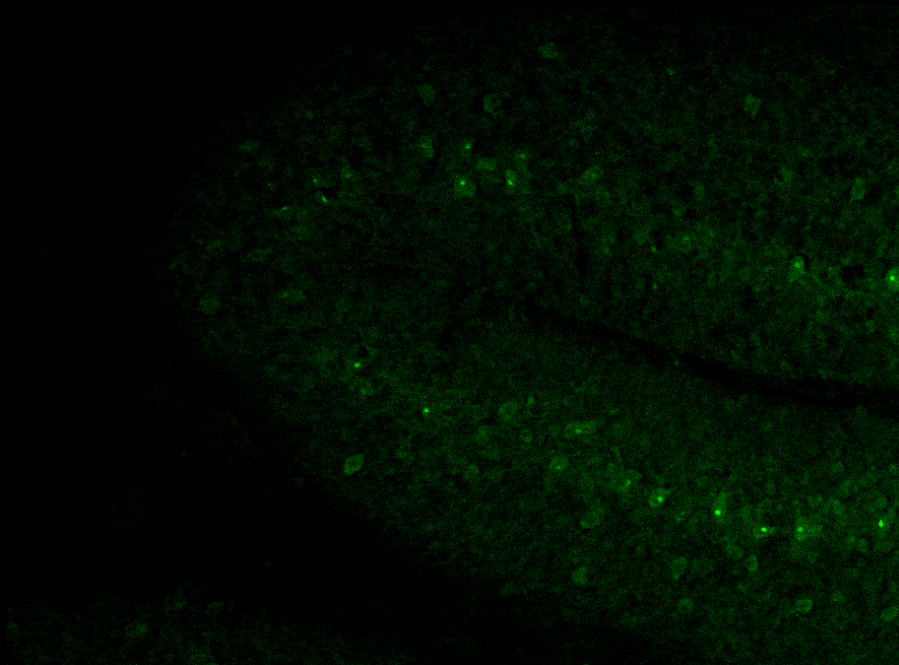
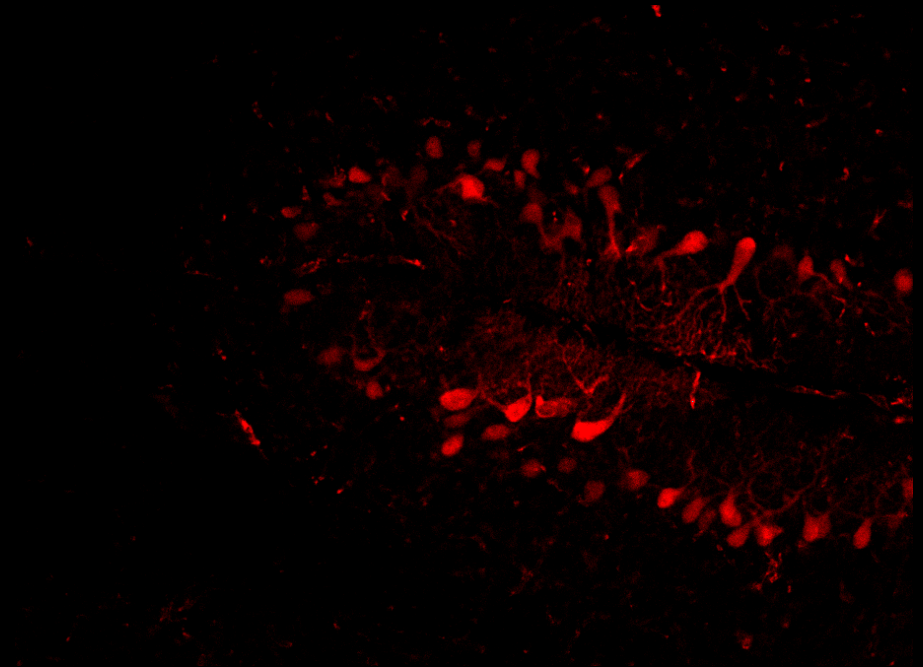
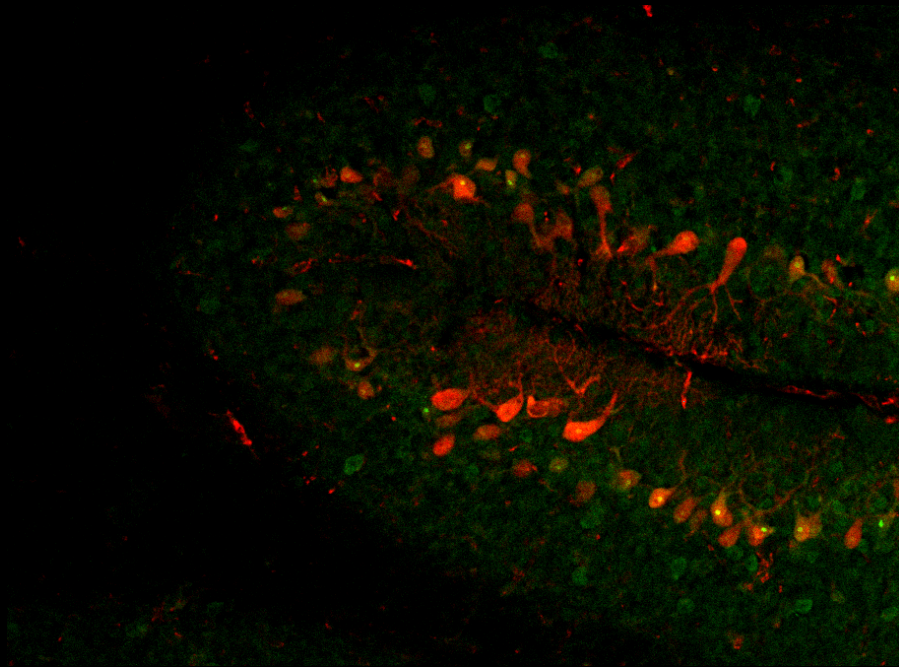


40-2: 215 (+)  
♂ 9 weeks old

fluorescent: 20x  
280 ab 7ug/ml  
Calbindin: 1:500

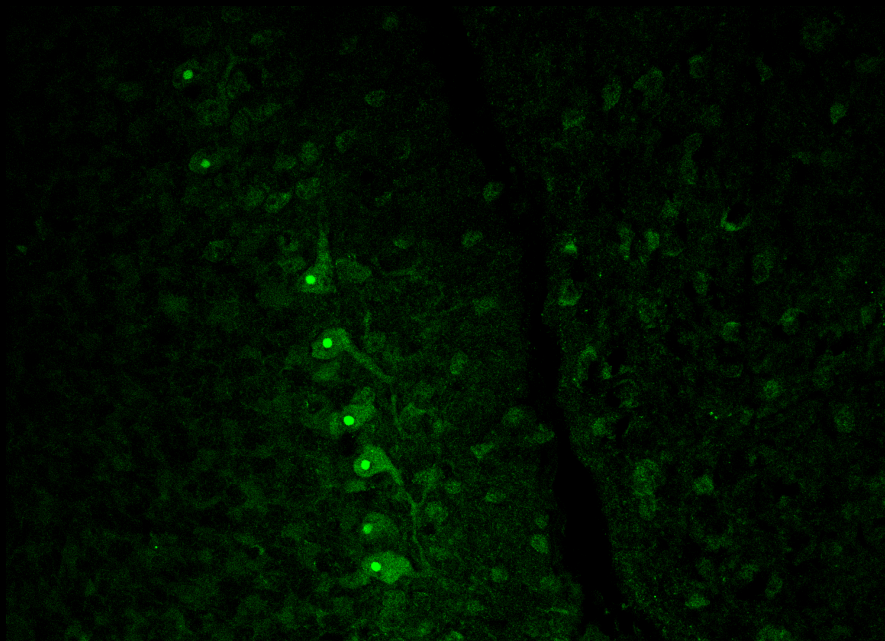
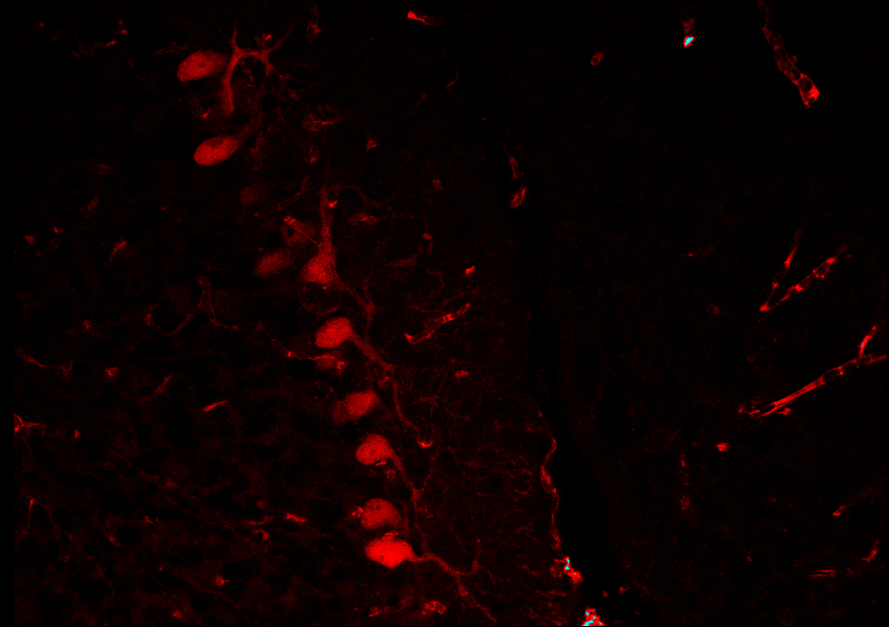
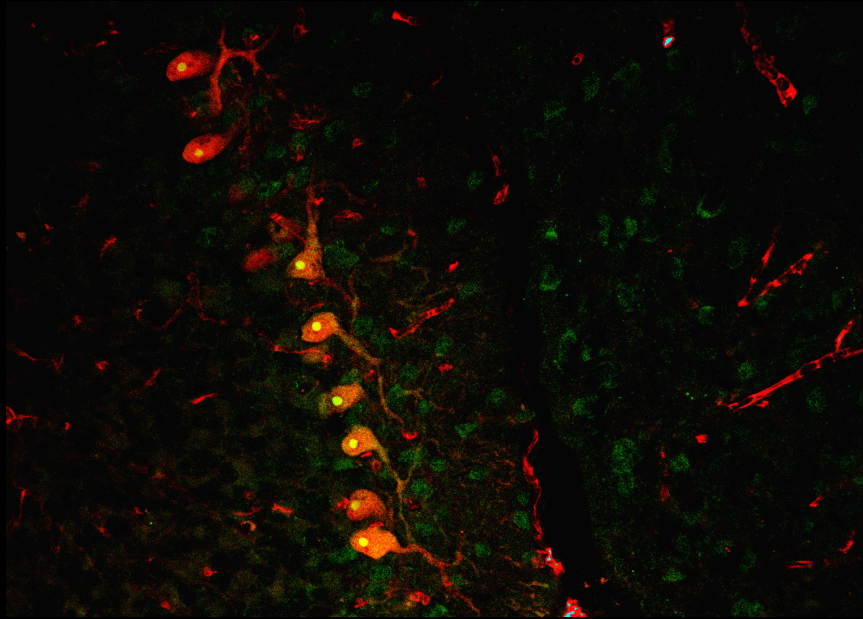






40-2: 143 (+)  
♂ 24 weeks

laser: 20x  
280 ab 7ug/ml  
Calbindin: 1:500



40-2: 143 (+)

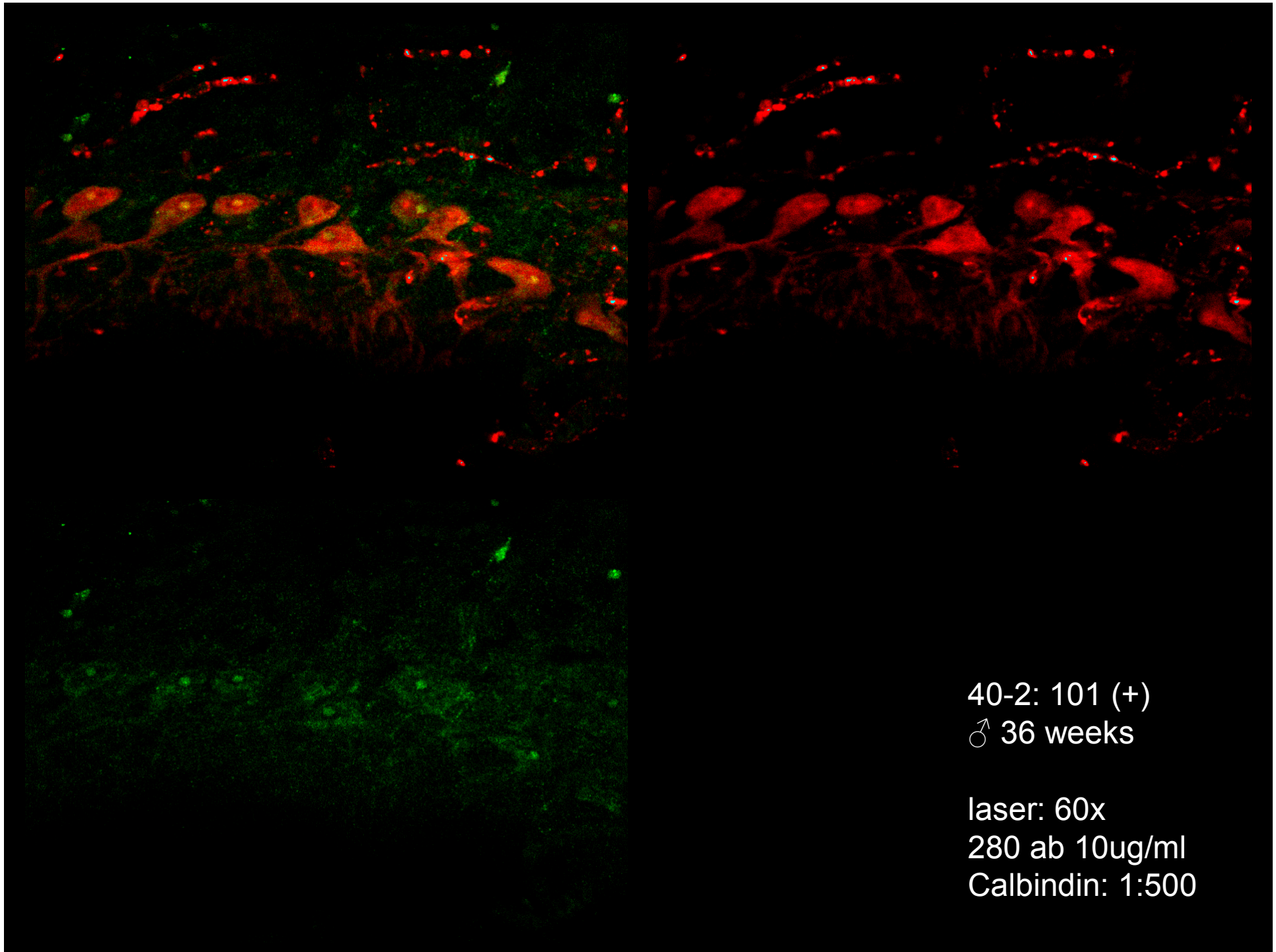
♂ 24 weeks

laser: 40x

280 ab 7ug/ml

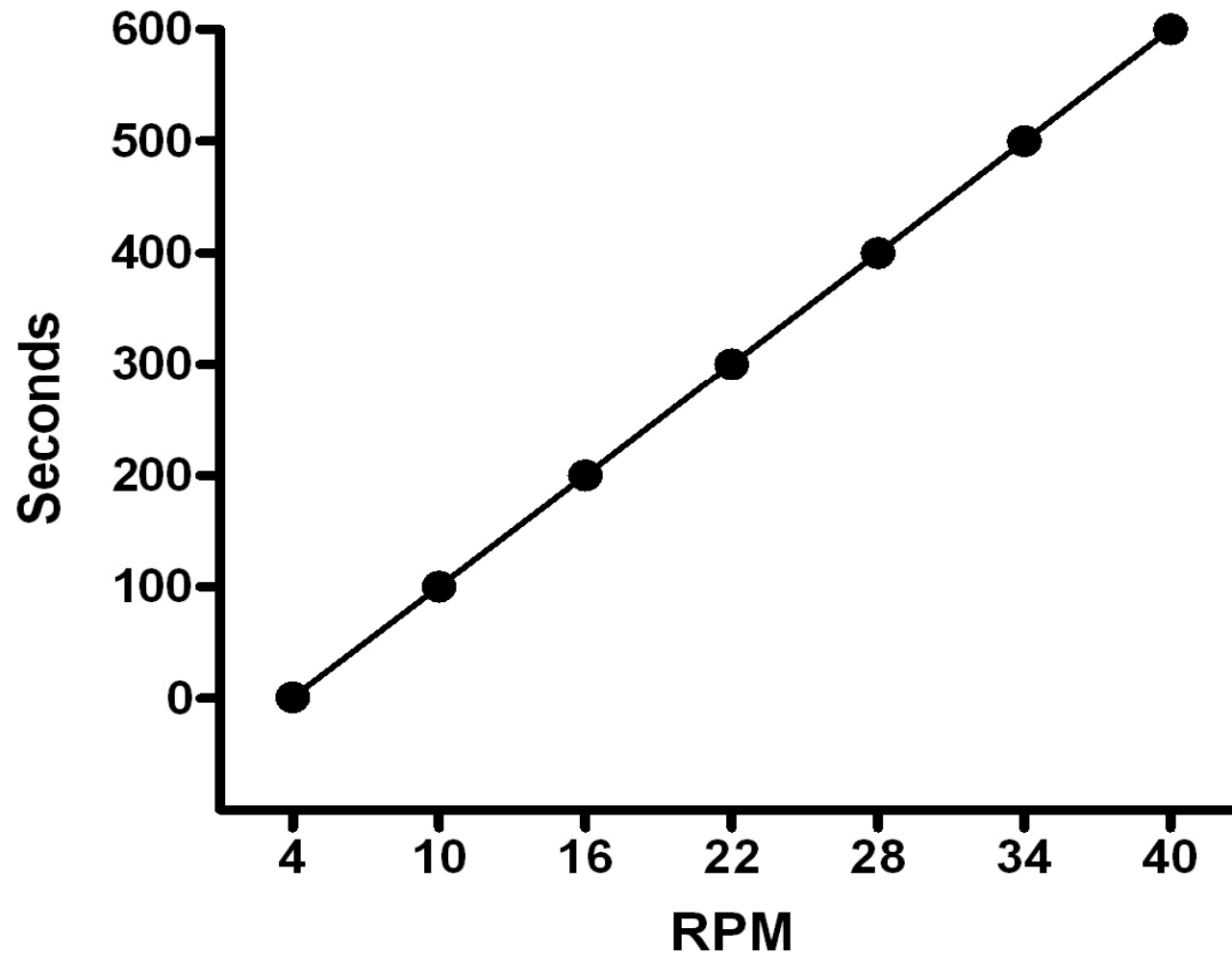
Calbindin: 1:500



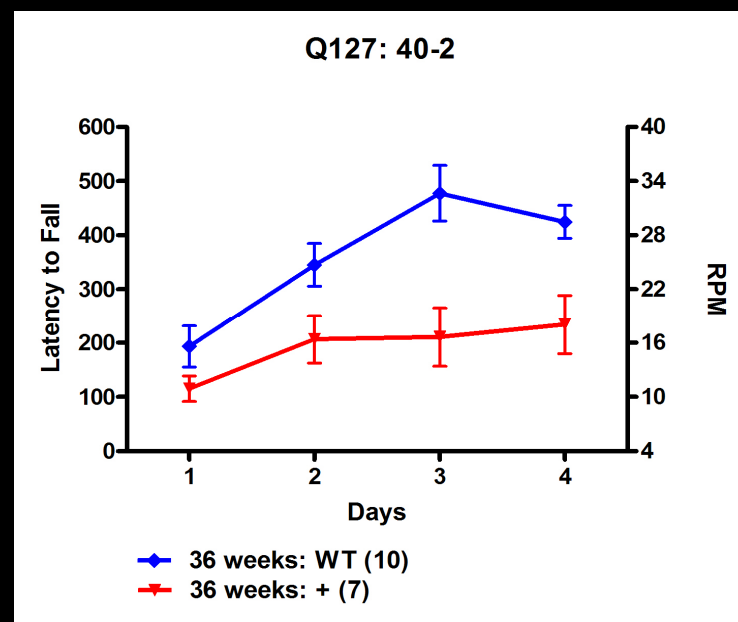
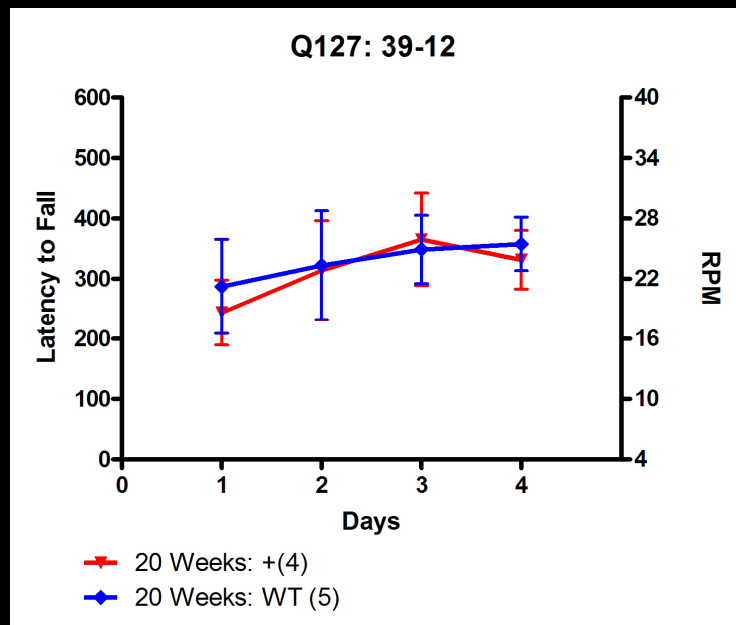
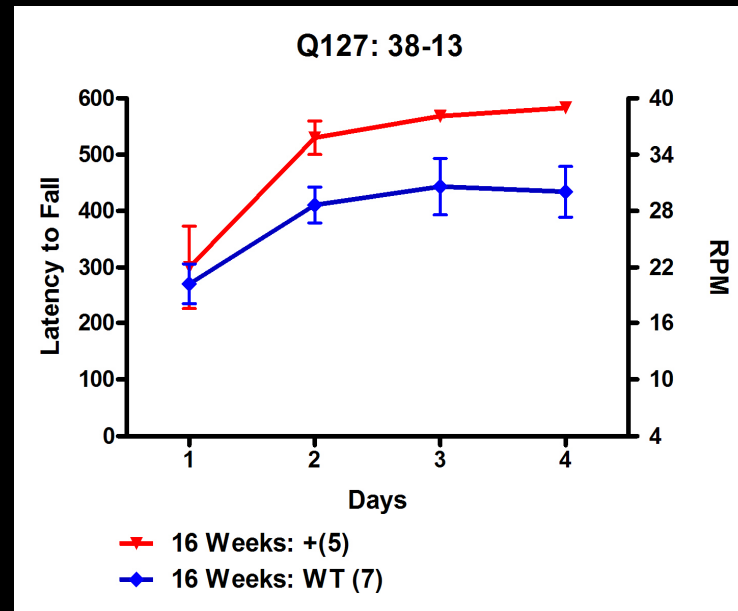
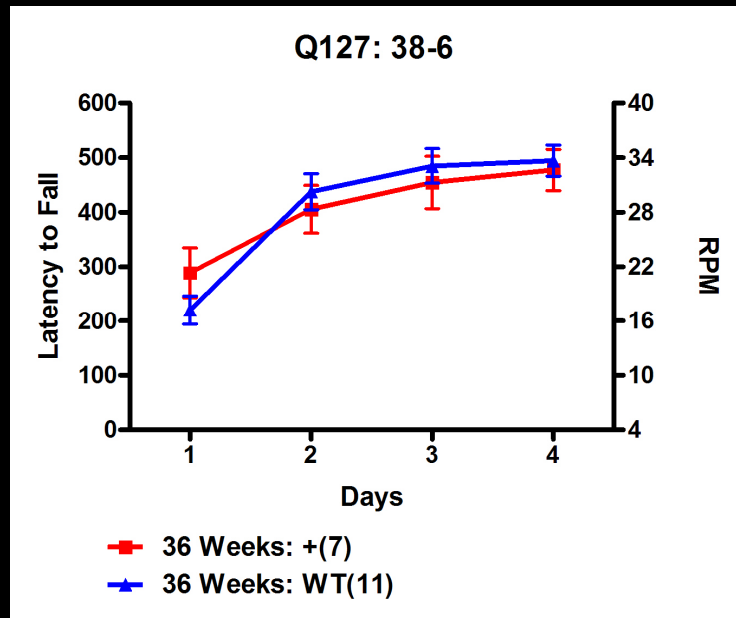


# Rotarod

Accelerating Rotarod

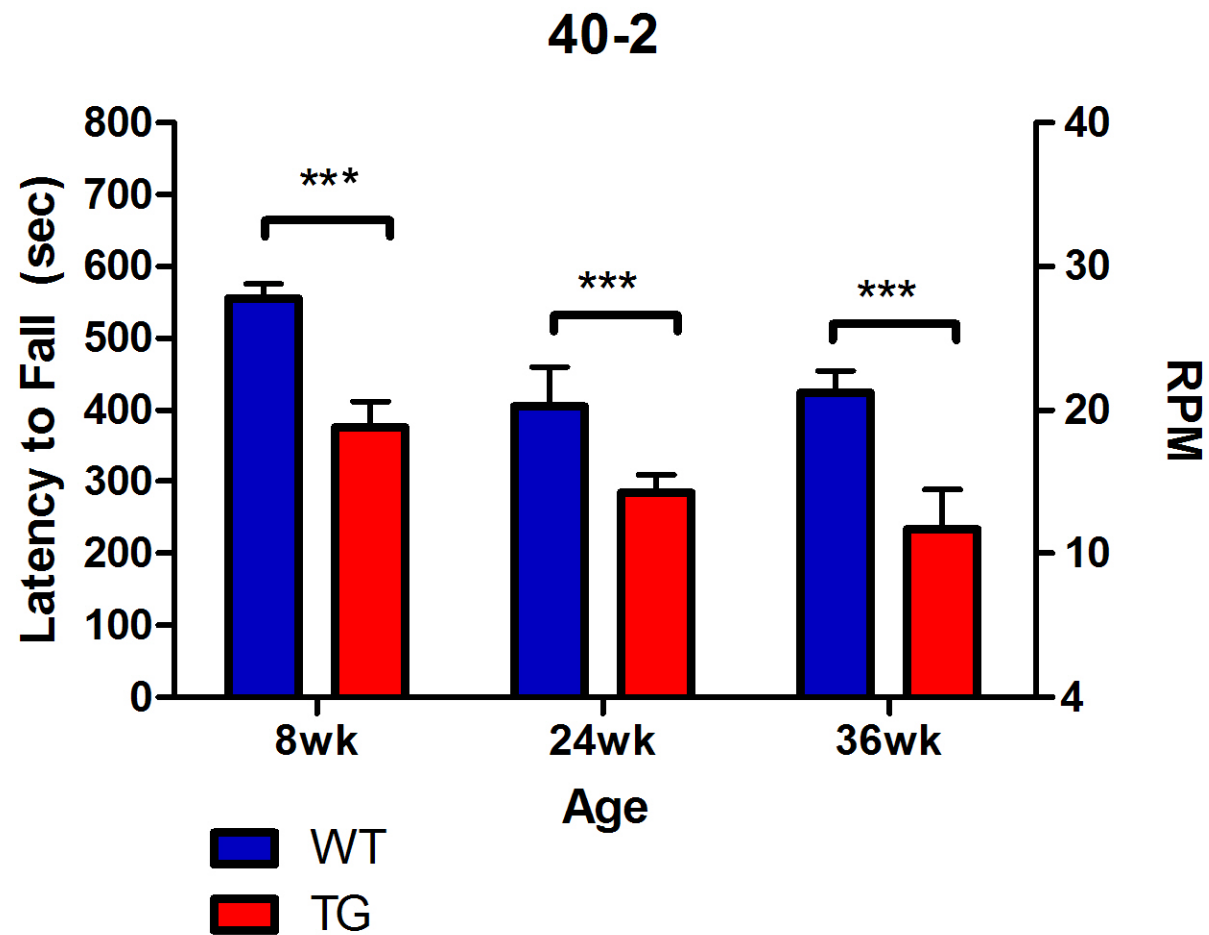


# Rotarod

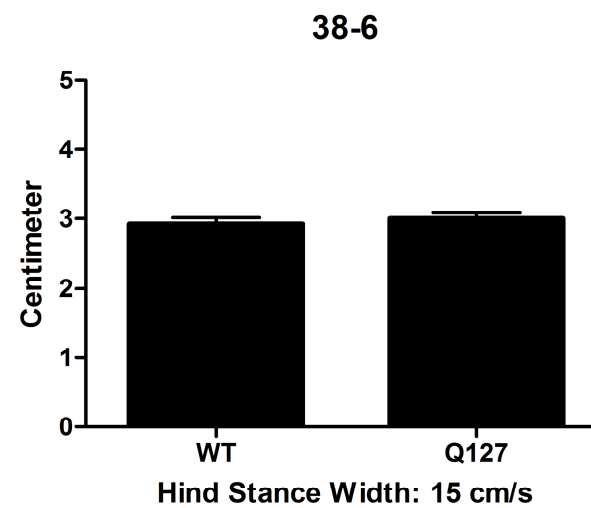
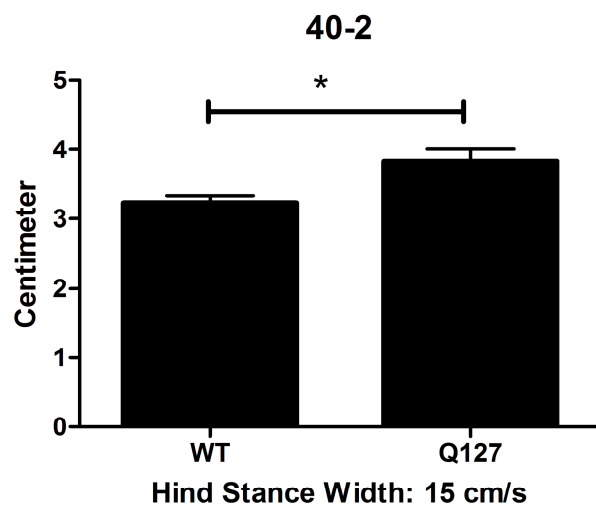
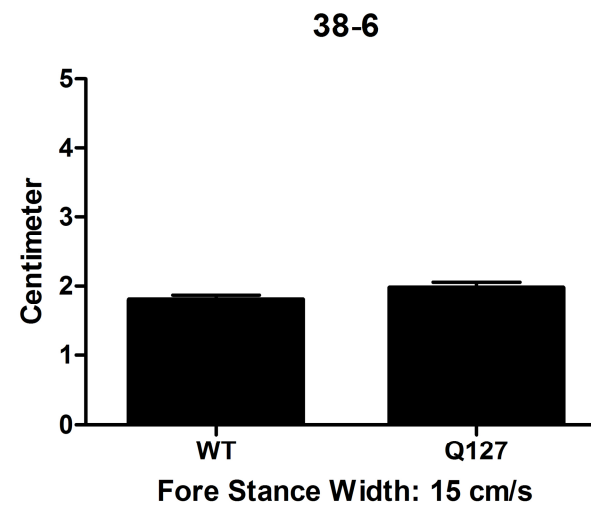
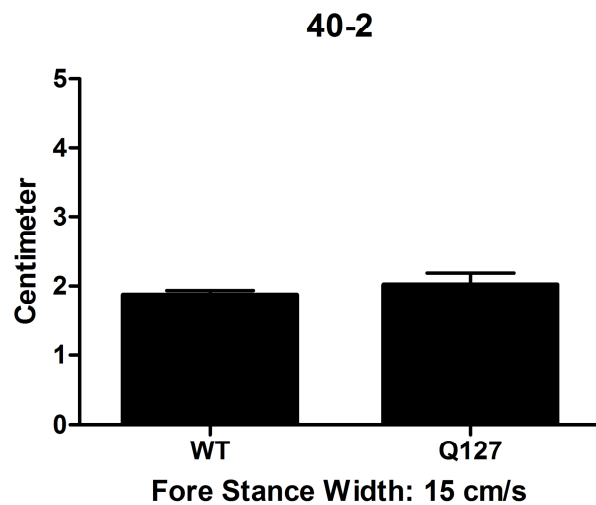




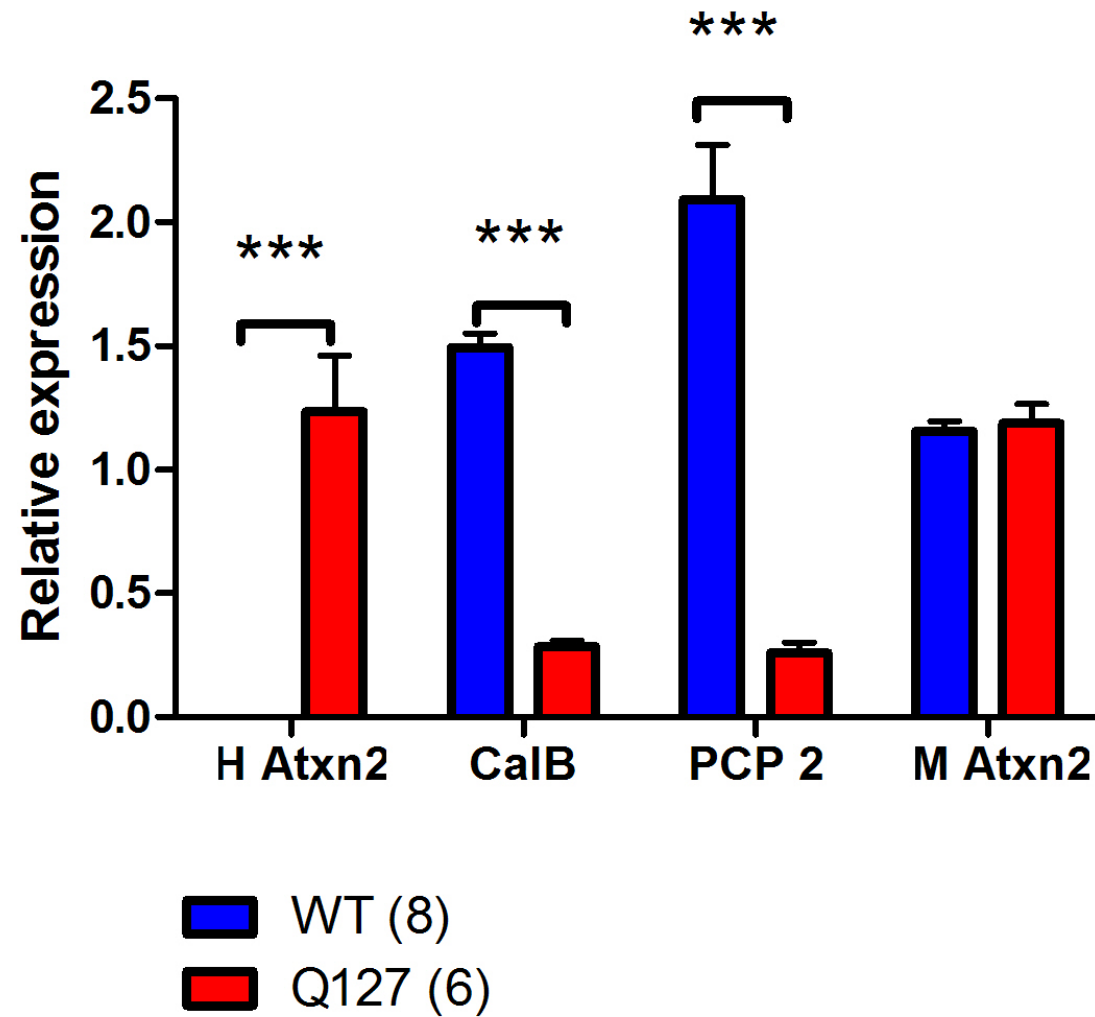
# Rotarod



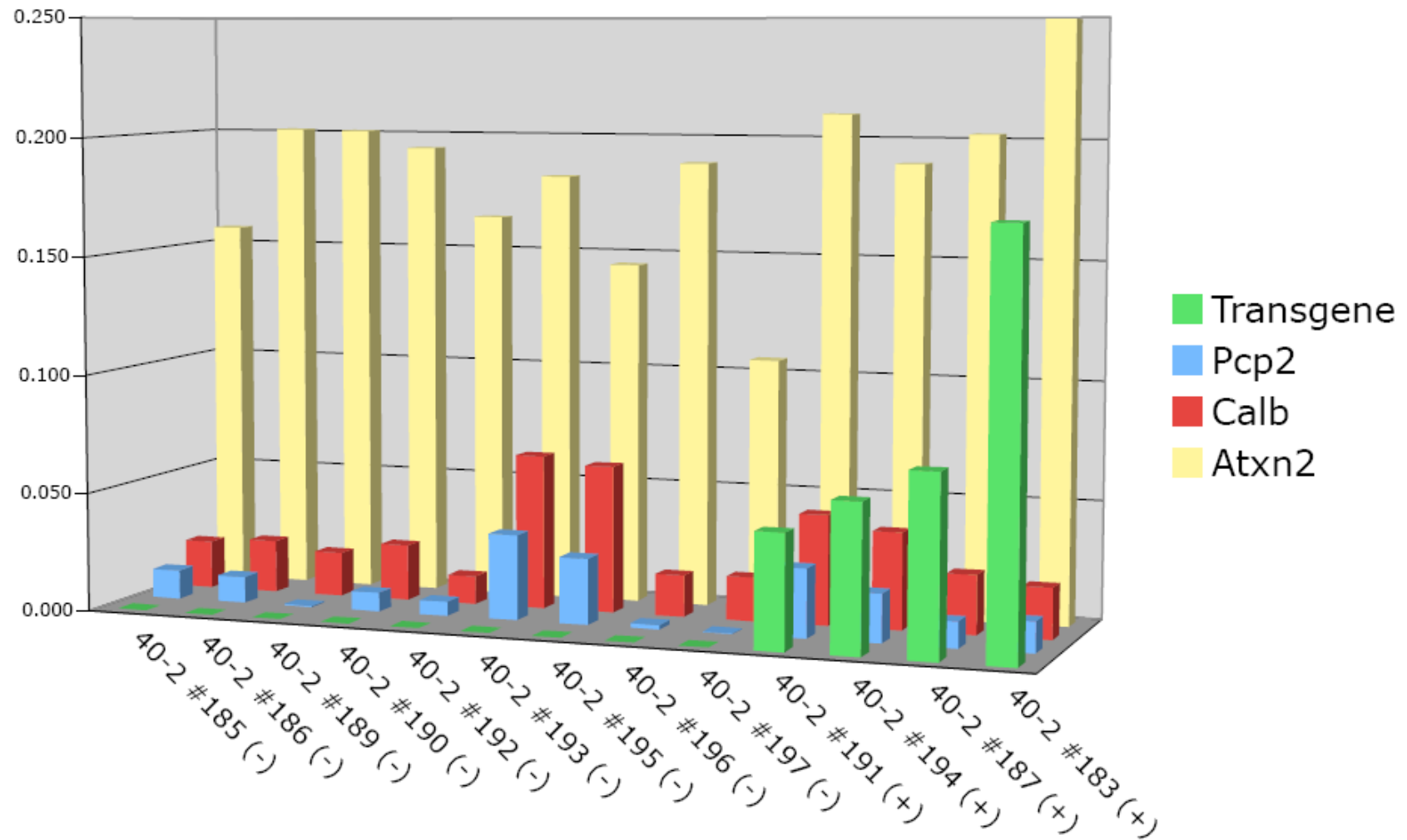
# Digigait



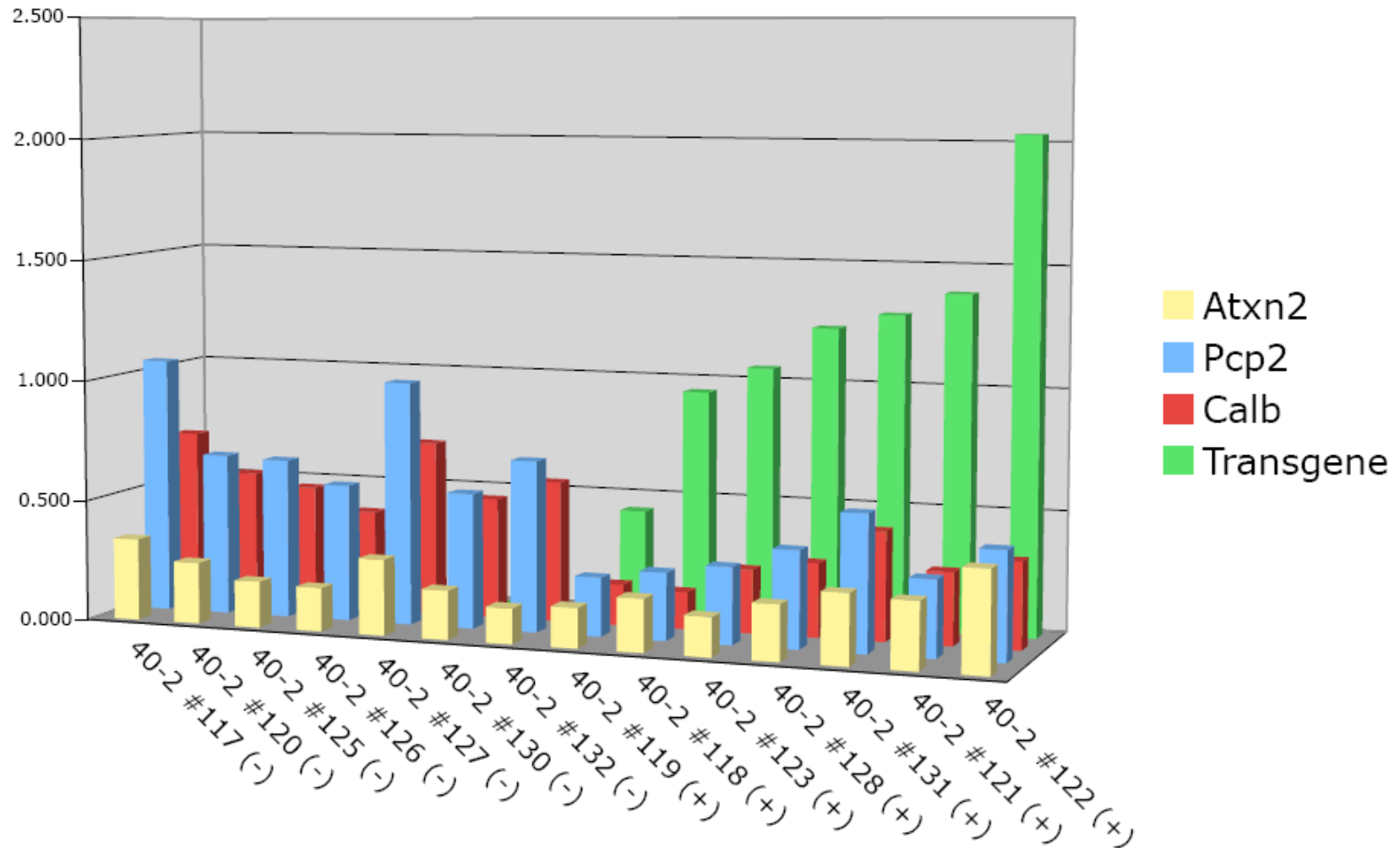
# qPCR



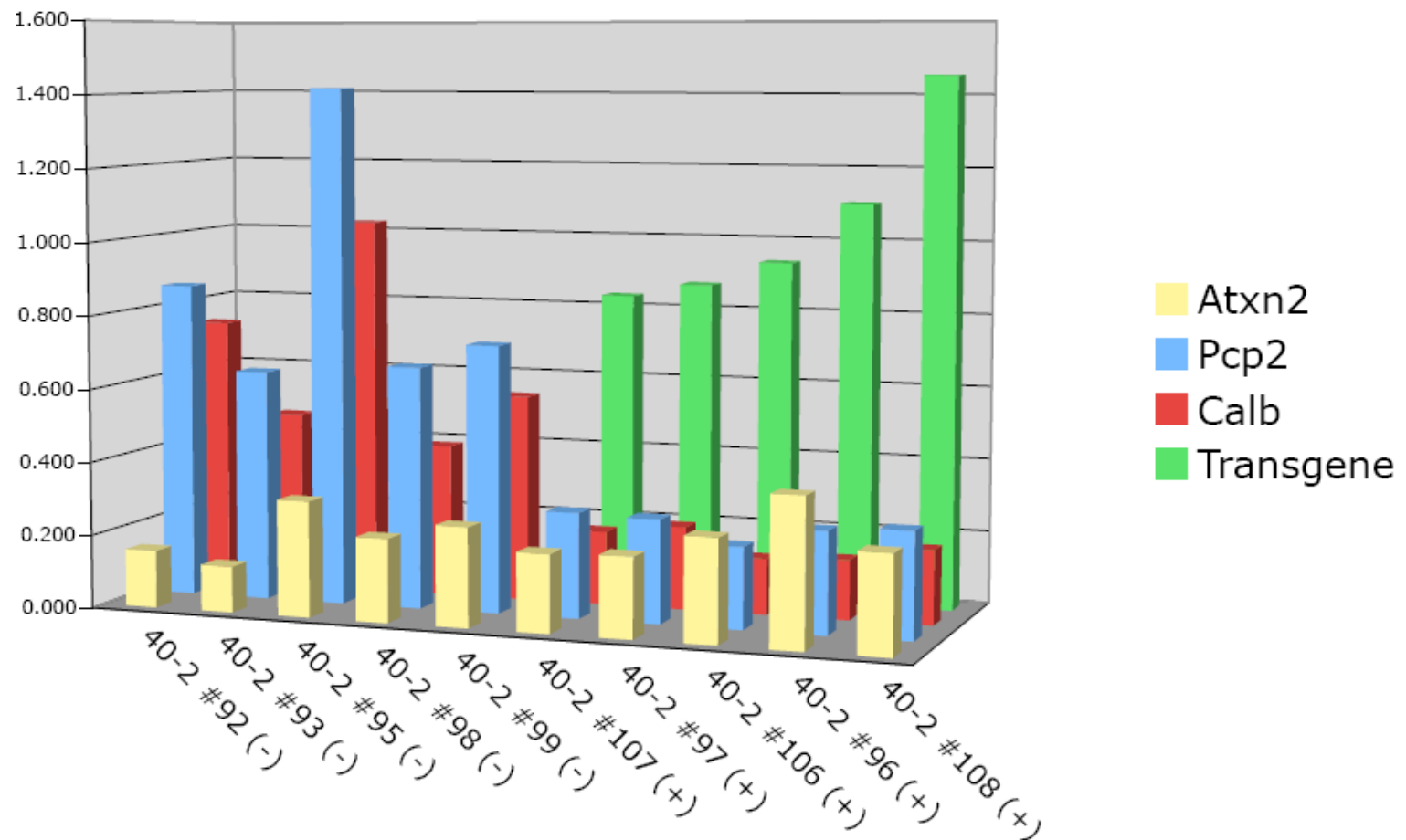
## Line 40-2 at age 1 day - relative expression



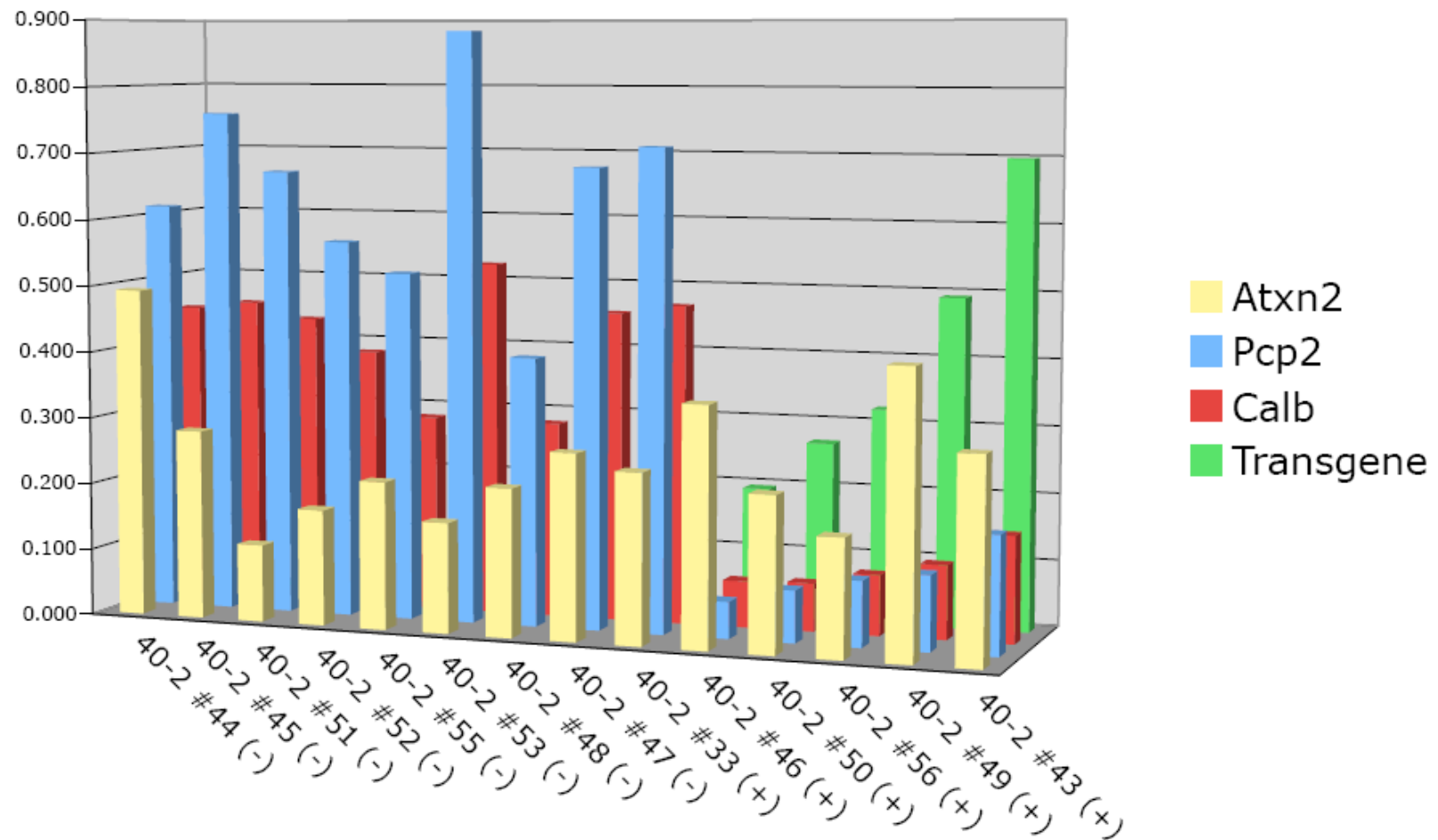
## Line 40-2 at age 60 days - relative expression



## Line 40-2 at age 194/205 days - relative expression

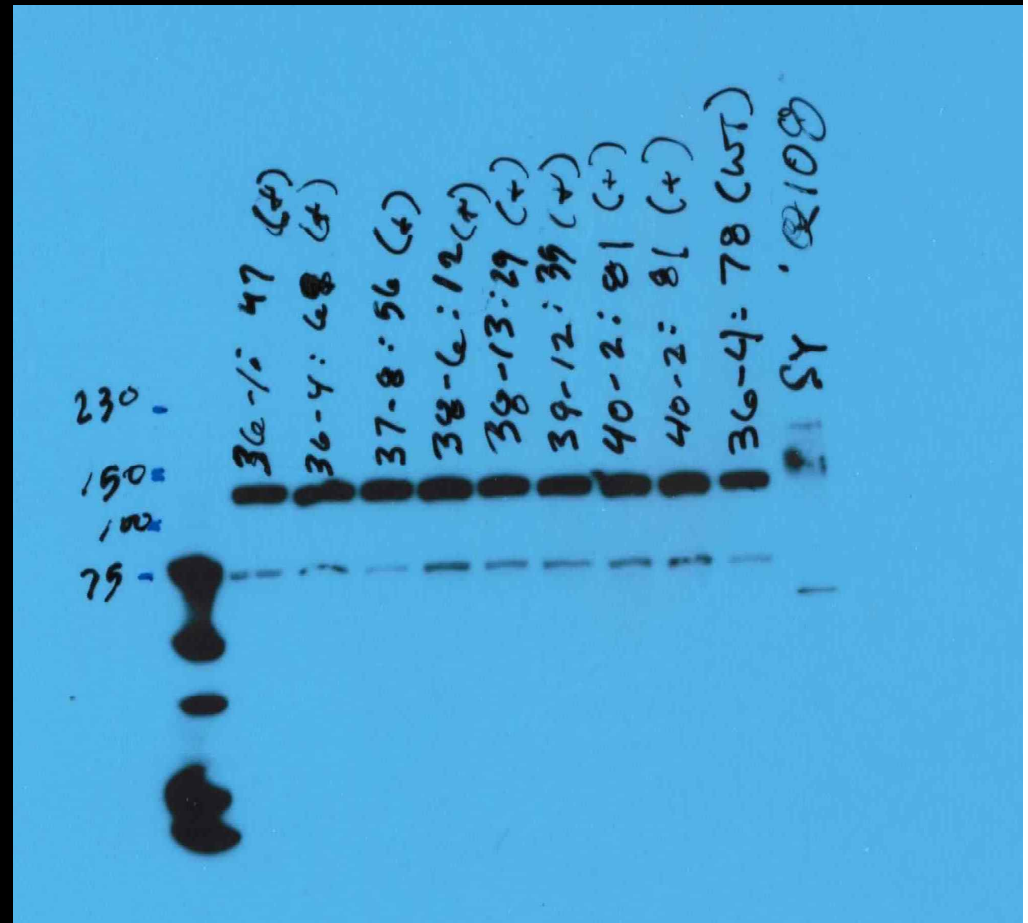


## Line 40-2 at age 389/392 days - relative expression



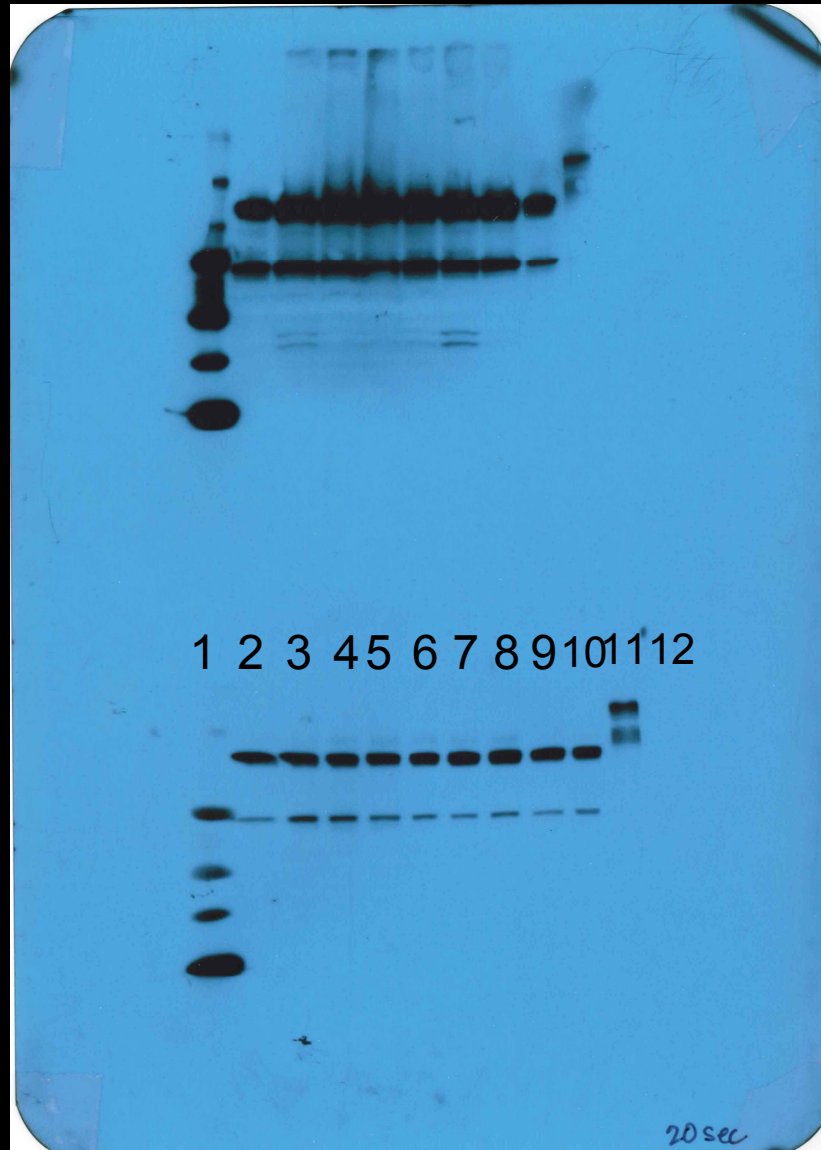
# Western blot: Q127

- 714 AB; 1:5k
- Q127 positive animal from each line
- All animals ~12 months
- SHSY q108 control





# Western blot: Q127



- 1- marker
  - 2- 40-2: 143 (+)
  - 3- 40-2: 145 (+)
  - 4- 40-2: 147 (wt)
  - 5- 40-2: 214 (wt)
  - 6- 40-2: 215 (+)
  - 7- 38-6: 52 (+)
  - 8- 38-13: 55 (+)
  - 9- 39-12: 40 (+)
  - 10- B6
  - 11- SHSY Q108
  - 12- 1x loading buffer
- 24 weeks old
- 8 weeks old

714 AB 1:5k  
20 sec exp

top:  $\frac{1}{4}$  cerebellum boiled only

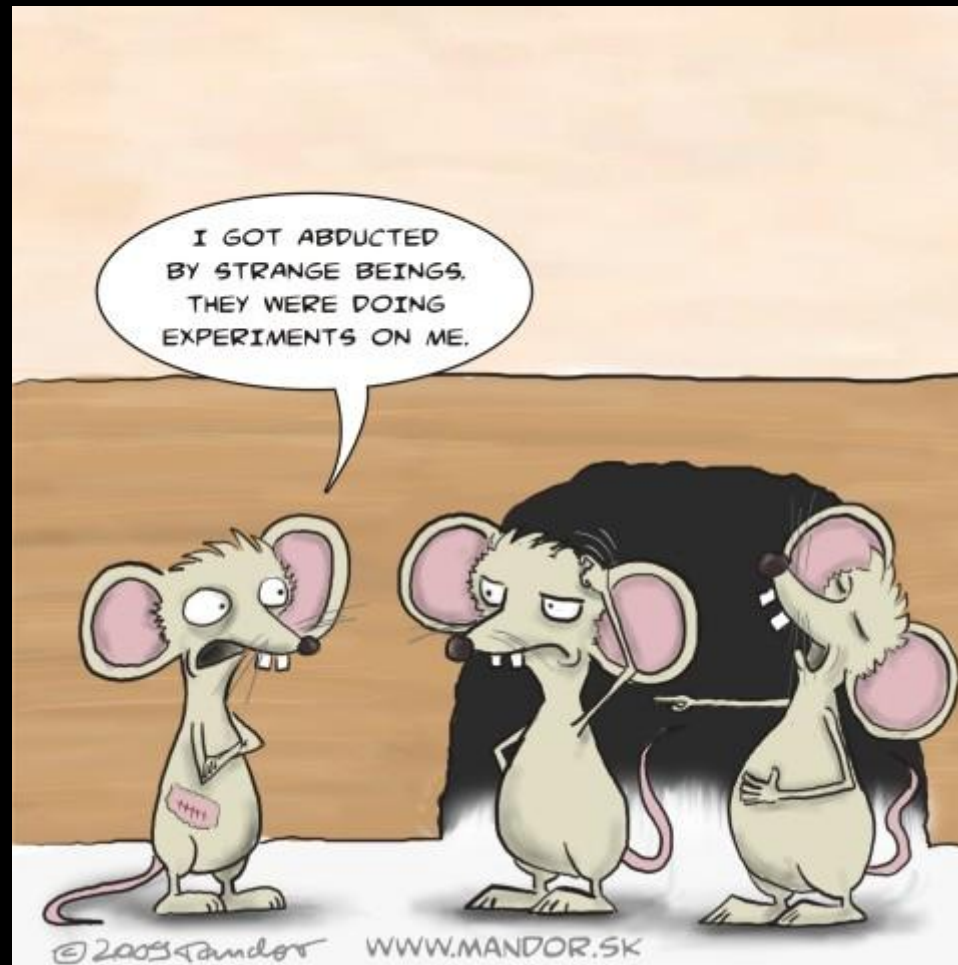
bottom:  $\frac{1}{4}$  cerebellum centrifuge  
purified

# 40-2

- 29 weeks old: 33 animals [19+; 14 wt]
  - Male: 21
  - Female: 12
- 11 weeks old: 47 animals [17+; 30 wt]
  - Male: 21
  - Female: 26

# Next

- Western blot: new technique; new AB?
- Northern blot – detect RNA



Luciferase

# Breeding

- 1<sup>st</sup> injection:
  - 235 eggs injected → 61 pups → 2 TG positive
    - Luc-2
      - Harem mated with 6 female B6 mice
      - 28 pups: 4-5 weeks old
      - Avg litter:
    - Luc-53
      - 23 pups 4-5 weeks old
      - Avg litter:

# Breeding

- 2nd injection:
  - 200 eggs injected → 29 pups → 5 TG positive
    - Transferred from TG core on 11/16/09
      - 4 weeks quarantine
    - Will receive animals on ~12/14/09
      - Approximately 8 weeks old
      - Transferred B6 breeders along with TG animals

# Future

- Luc-IHC: 6 ½ cerebellum ready for slicing
- Luc-WB: 6 ¼ cerebellum for WB
- Light Read Assay
- Biophotonic imaging (IVIS)
  - *In vivo* imaging for luciferase