

Q127: 40-2

- Histology
- qPCR
- Rotarod

Histology

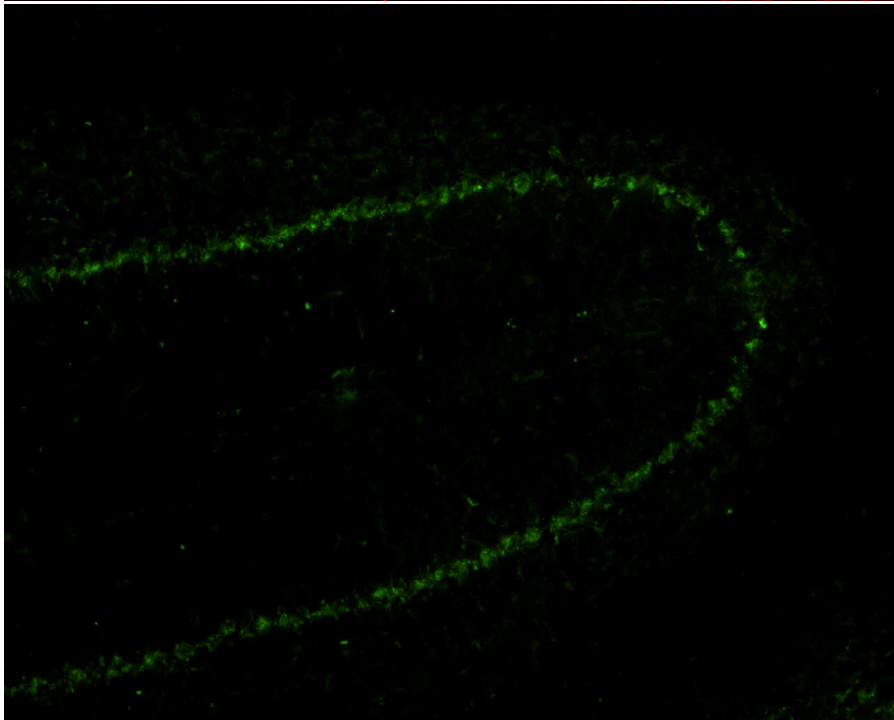
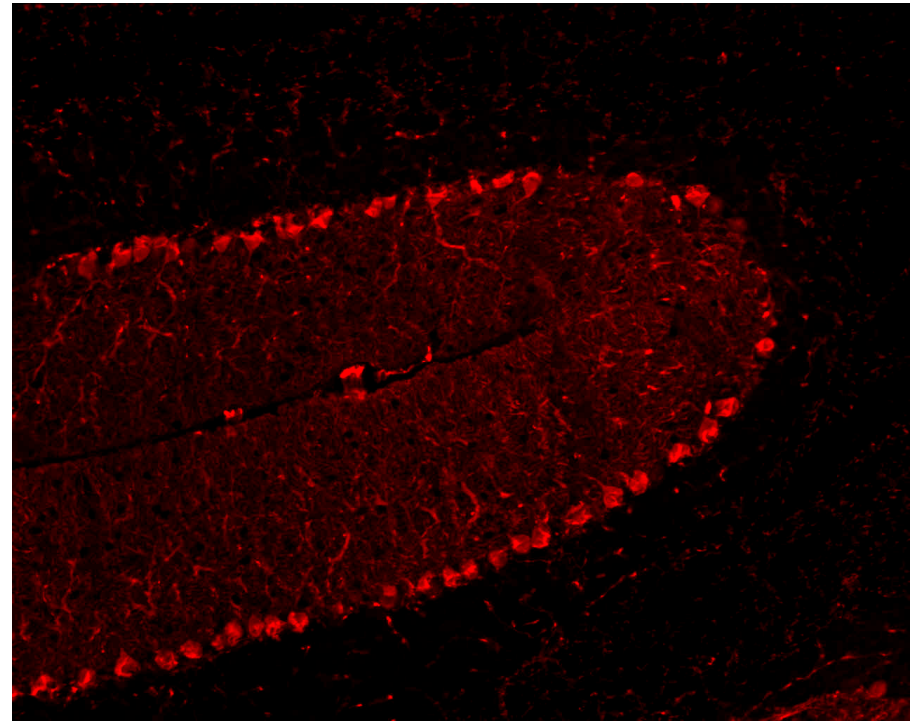
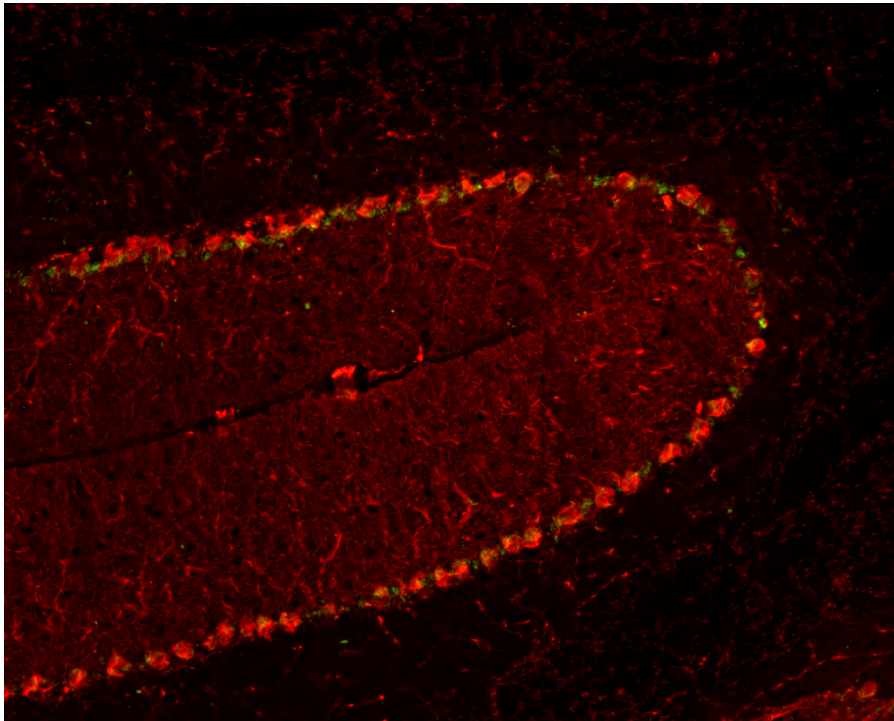
- Not all slides were stained together
 - Pictures give a sampling of histology across time
 - All tissue processed with same protocol
- All histology pictures made from the primary fissure

IHC Methods

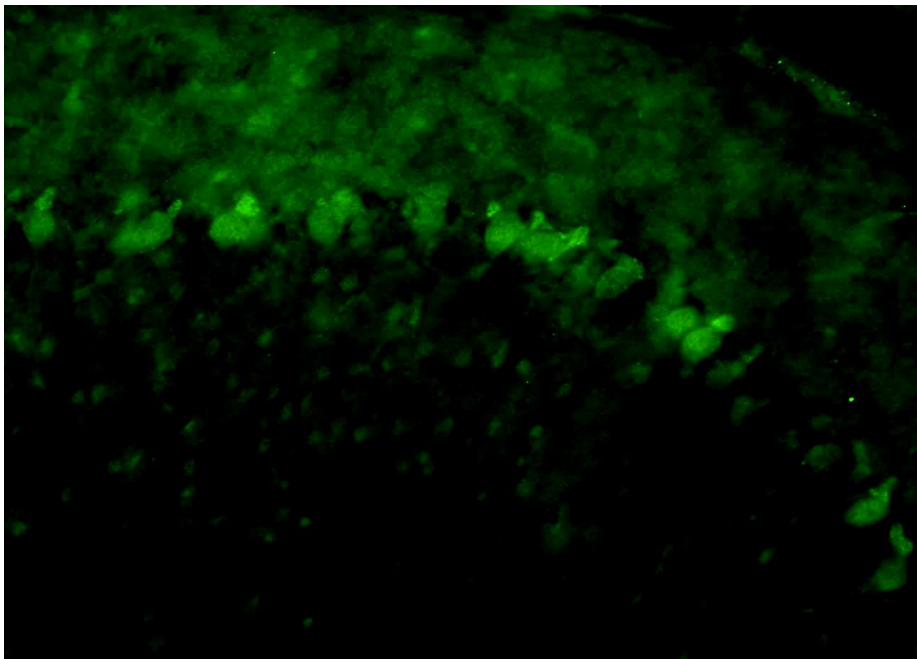
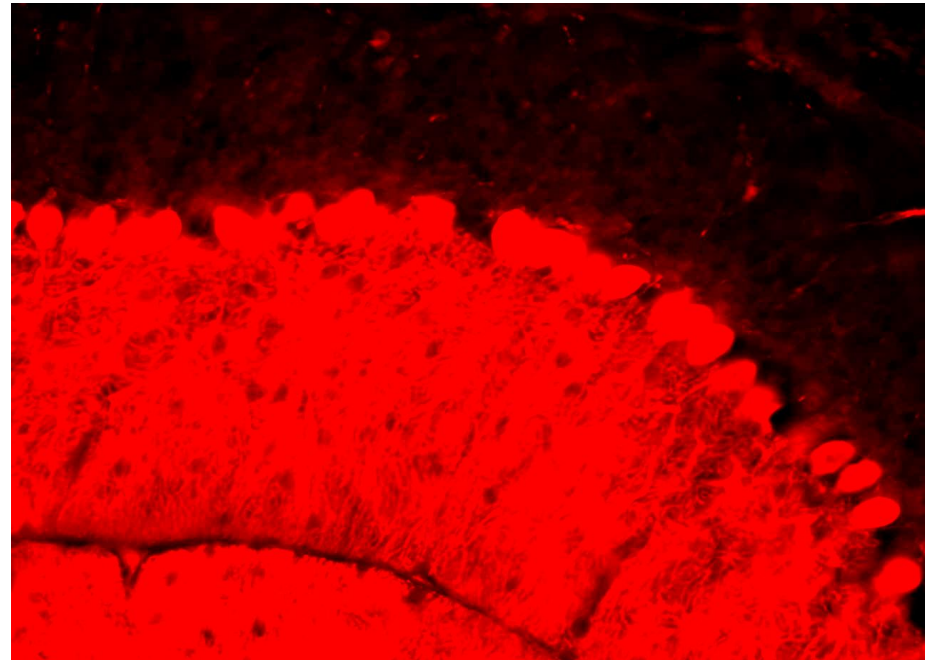
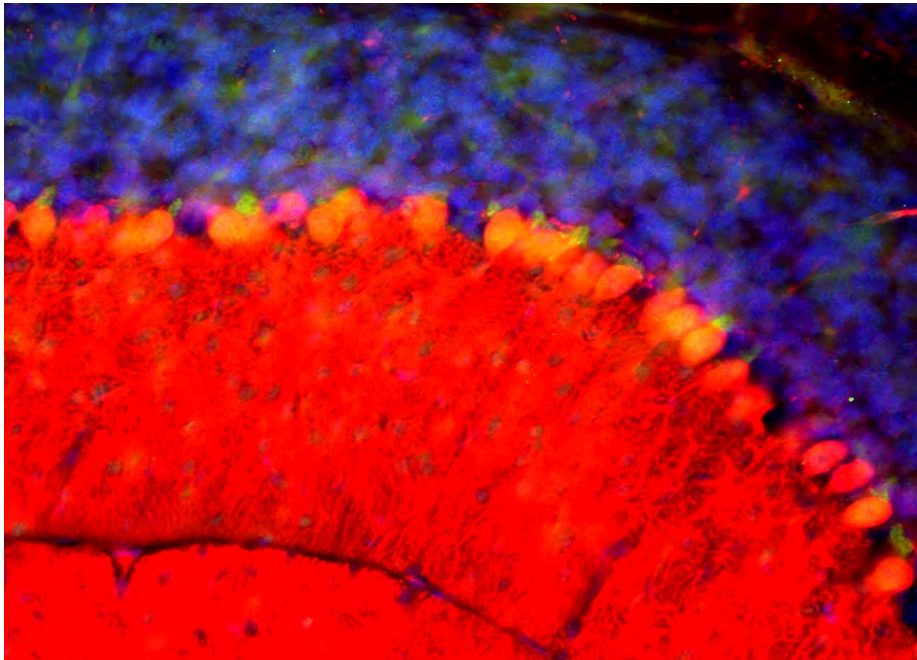
- 20 μ M brain sections
- 1x PBS: wash 3x @ 10min
- Permeabilize and block **4hrs** @ RT
- Primary antibody 24hrs @ 4c
- Wash 3x @ 15min with wash buffer
- Secondary antibody 2hrs @ RT
- Wash 3x @ 15min with wash buffer

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

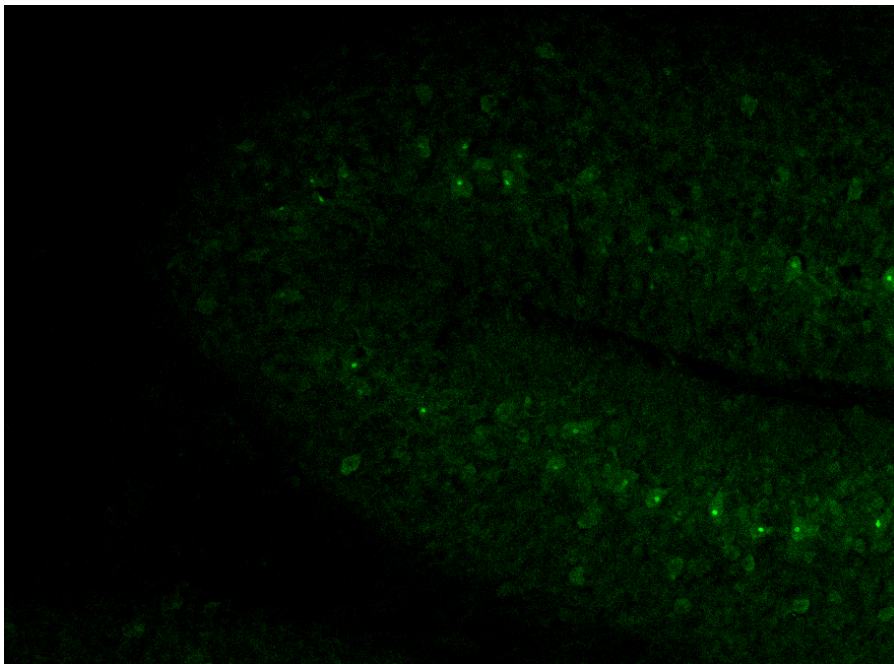
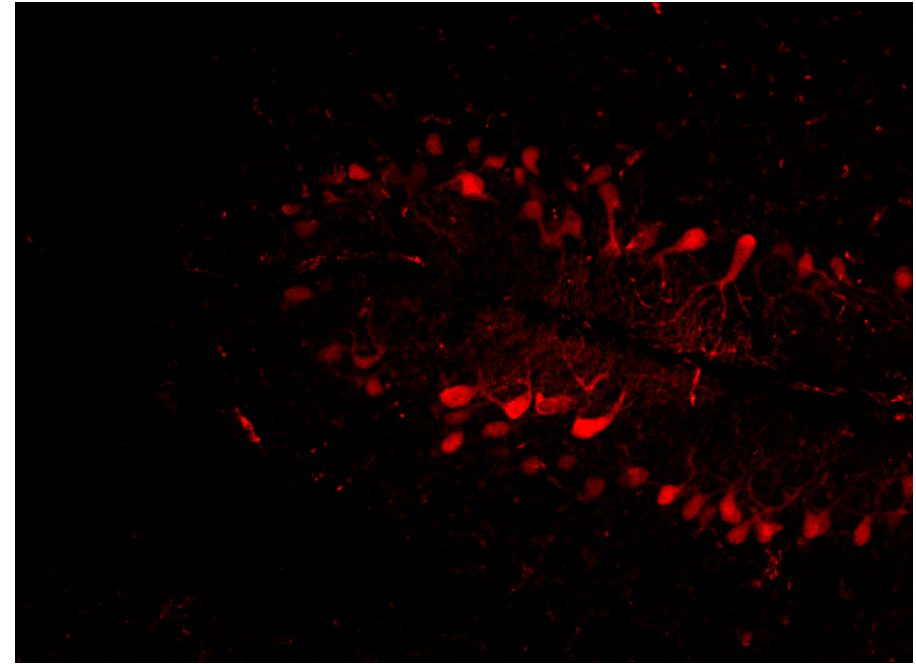
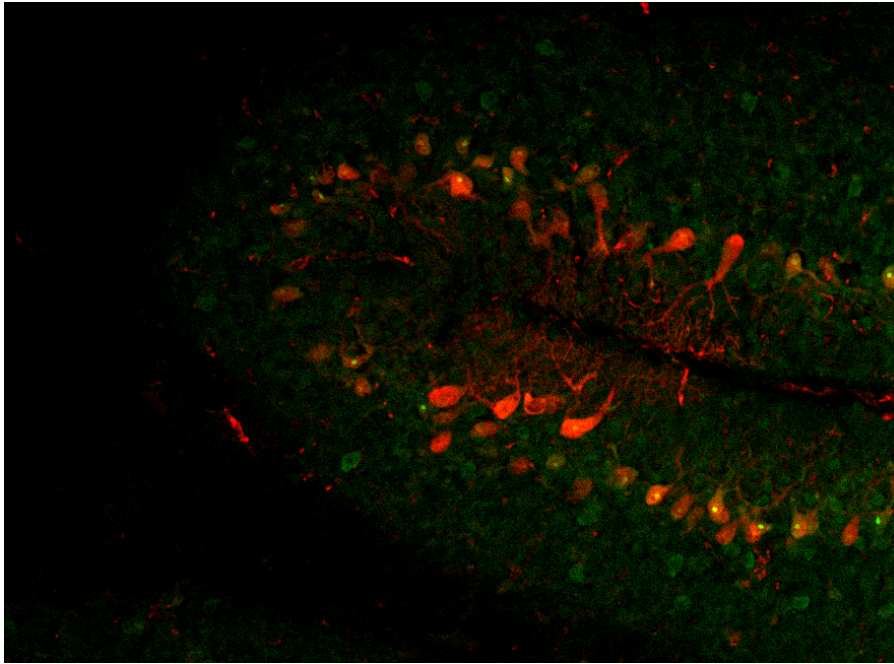


40-2: 105 (wt) 28 weeks



40-2: 215 (Tg)
 ♂ 9 weeks old
 20x

AB 280 (green)
 Calb (red)
 Dapi (blue)



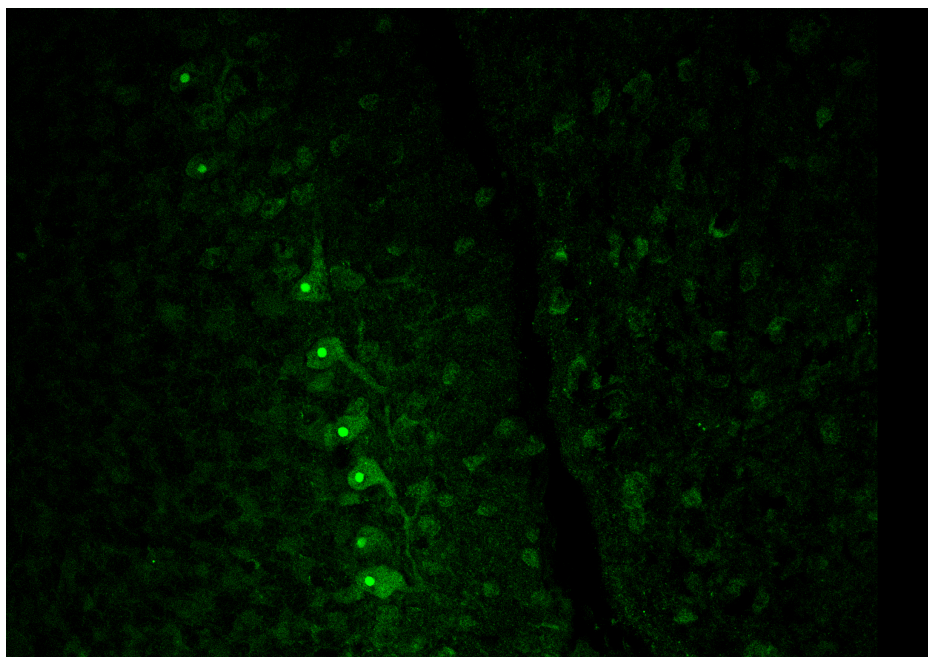
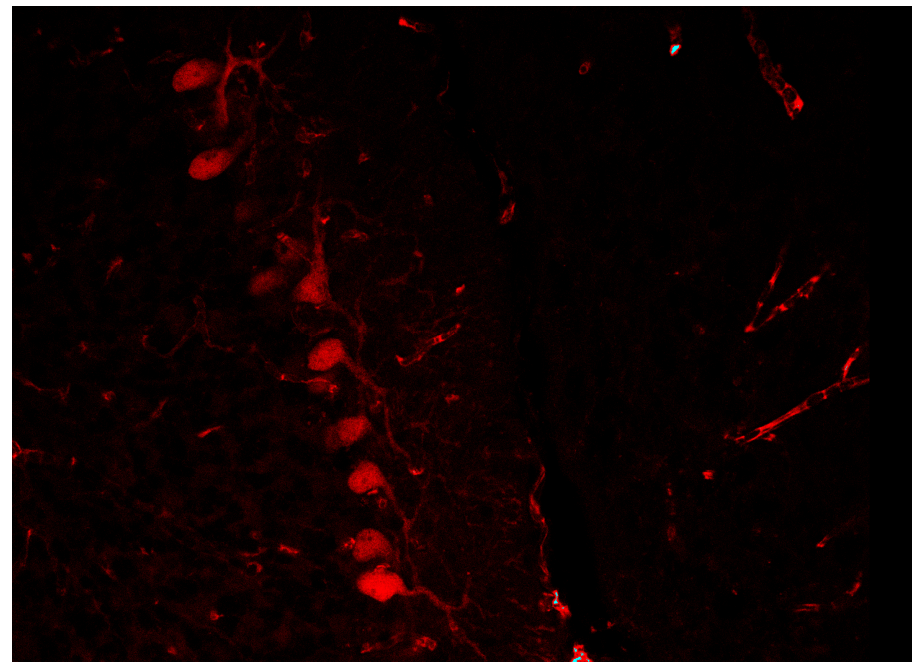
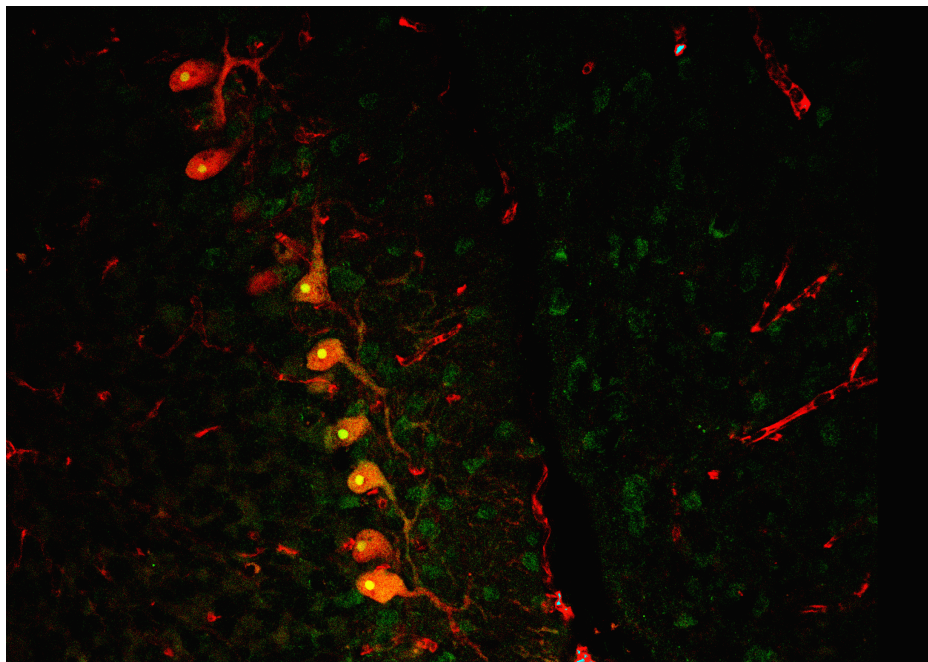
40-2: 143 (Tg)

♂ 24 weeks

20x

AB 280 (green)

Calb (red)



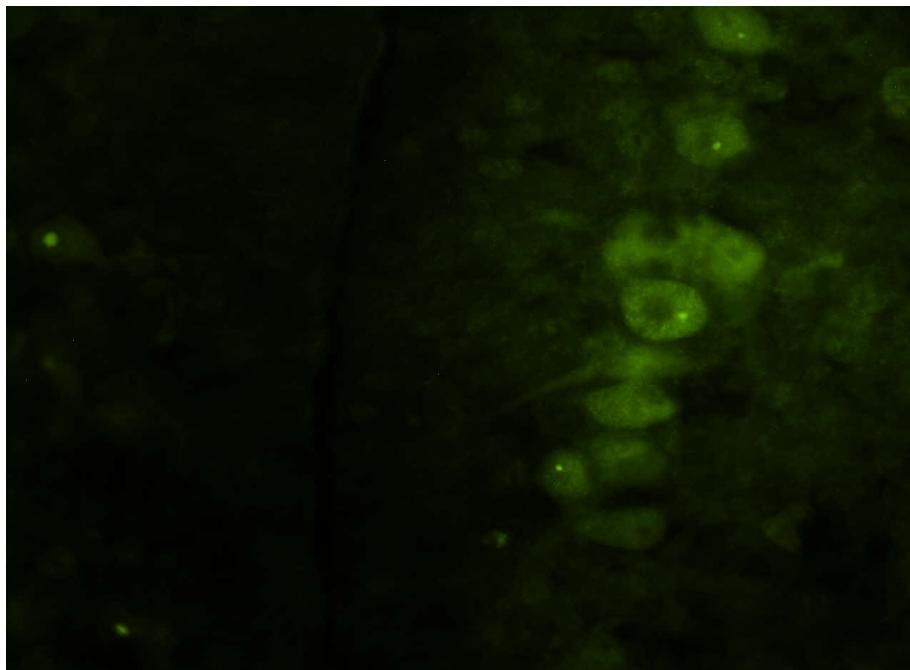
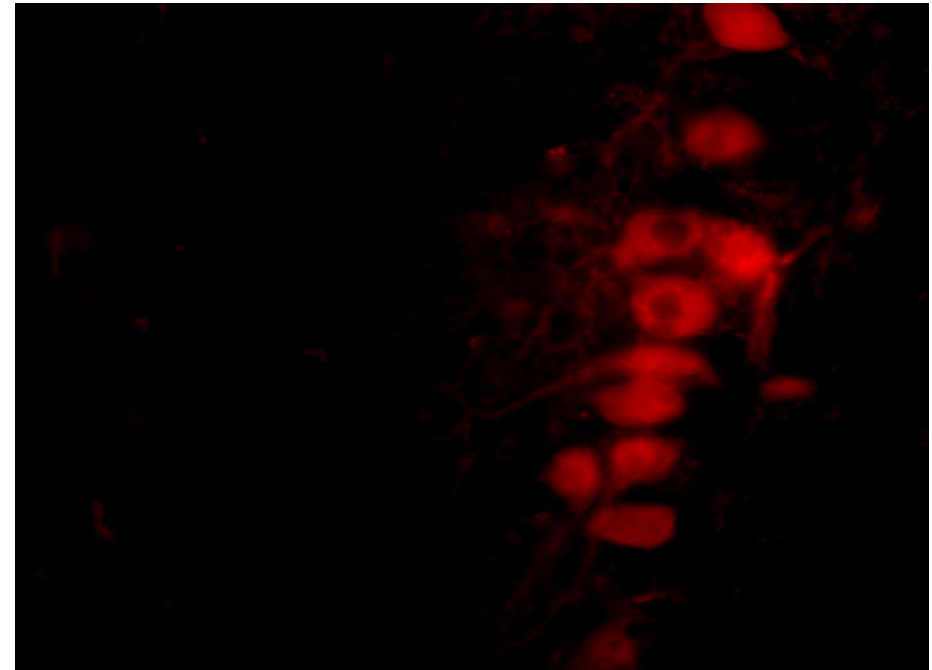
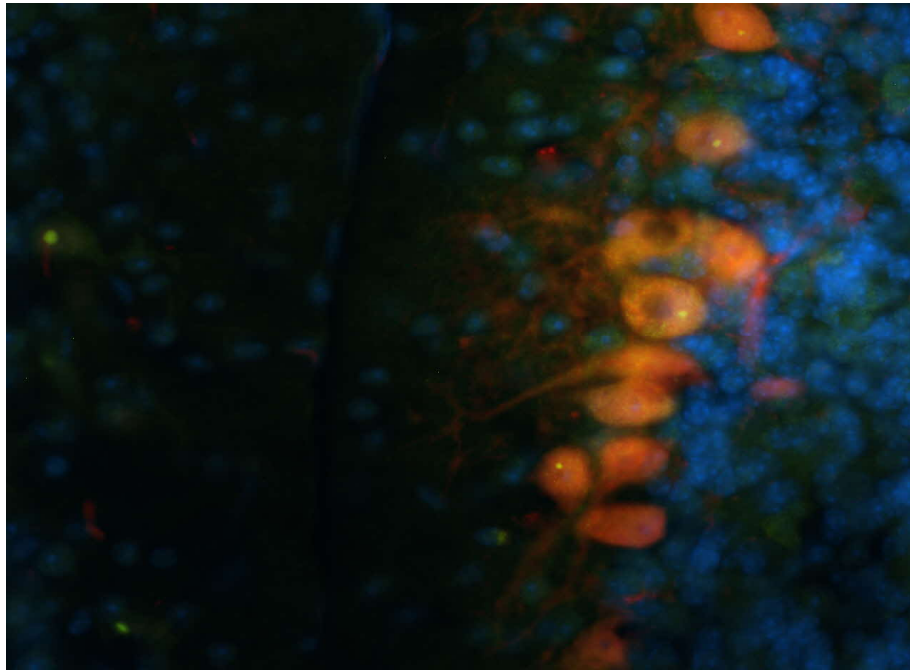
40-2: 143 (Tg)

♂ 24 weeks

20x

AB 280 (green)

Calb (red)



40-2: 160 (Tg)

♂ 36 weeks

40x

AB 280 (green)

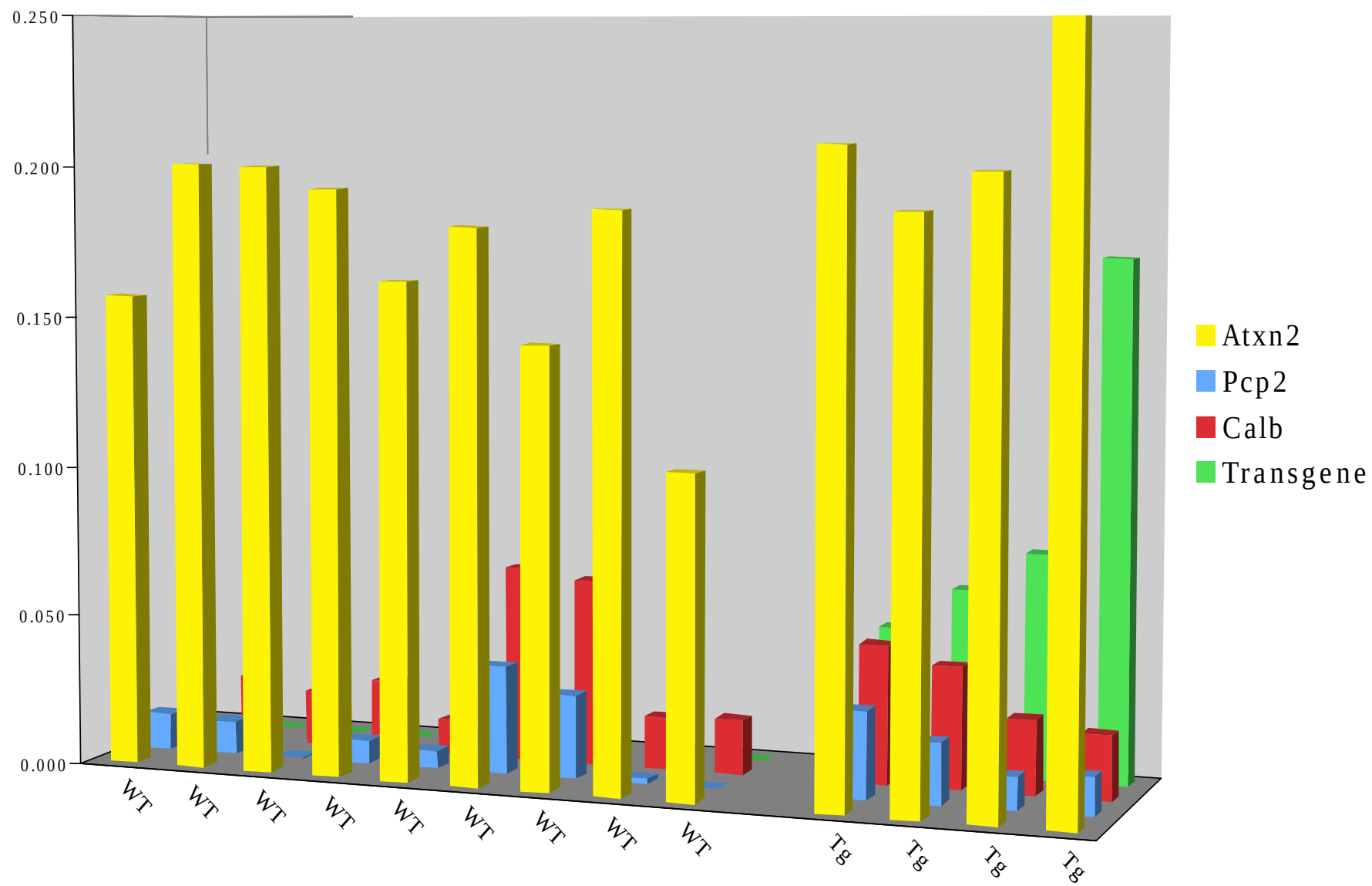
Calb (red)

Dapi (blue)

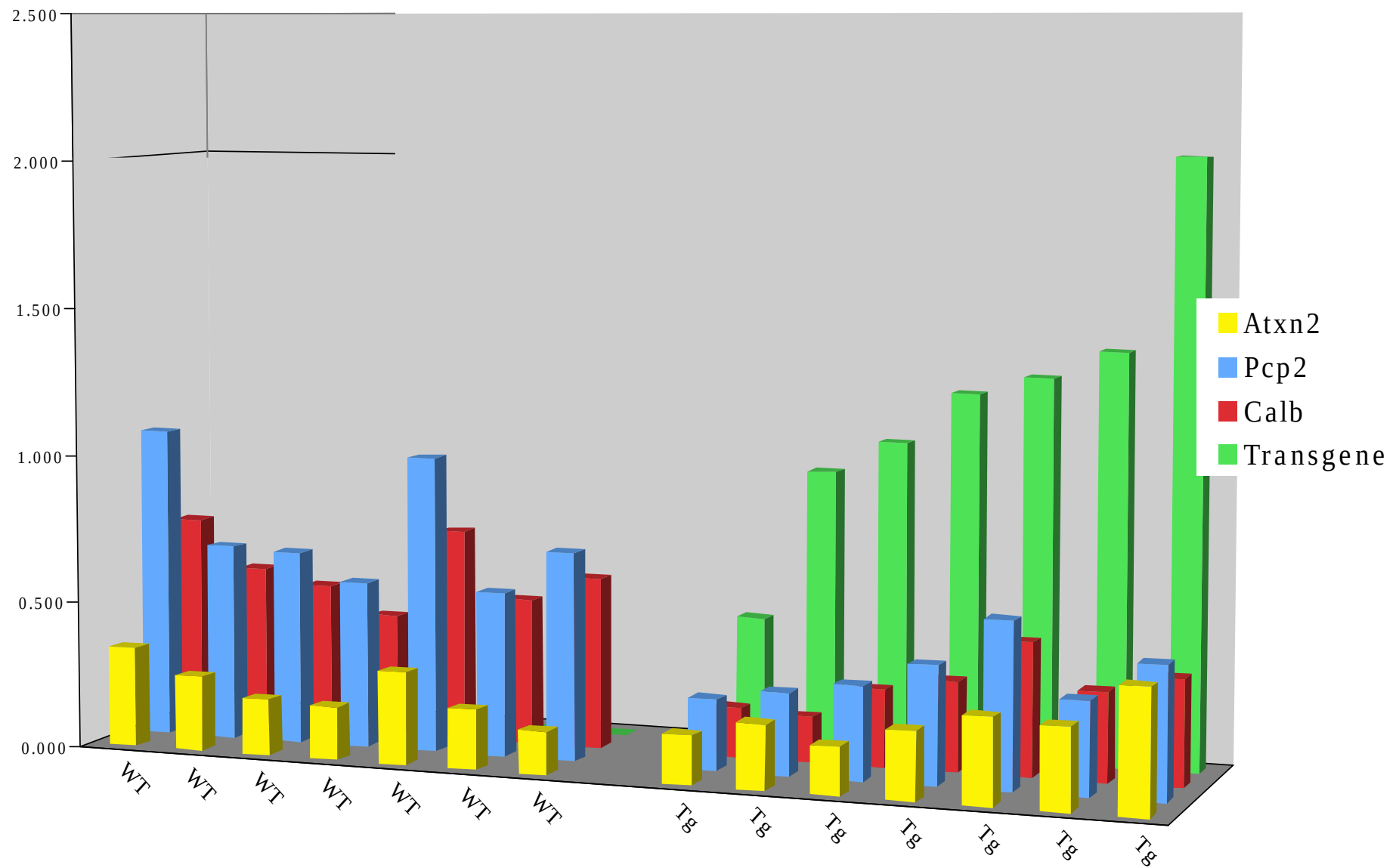
qPCR

- Samples grouped by age were processed together
- Relative expression of genes normalized to WASF-1
- Next four slides represent data from individual animals

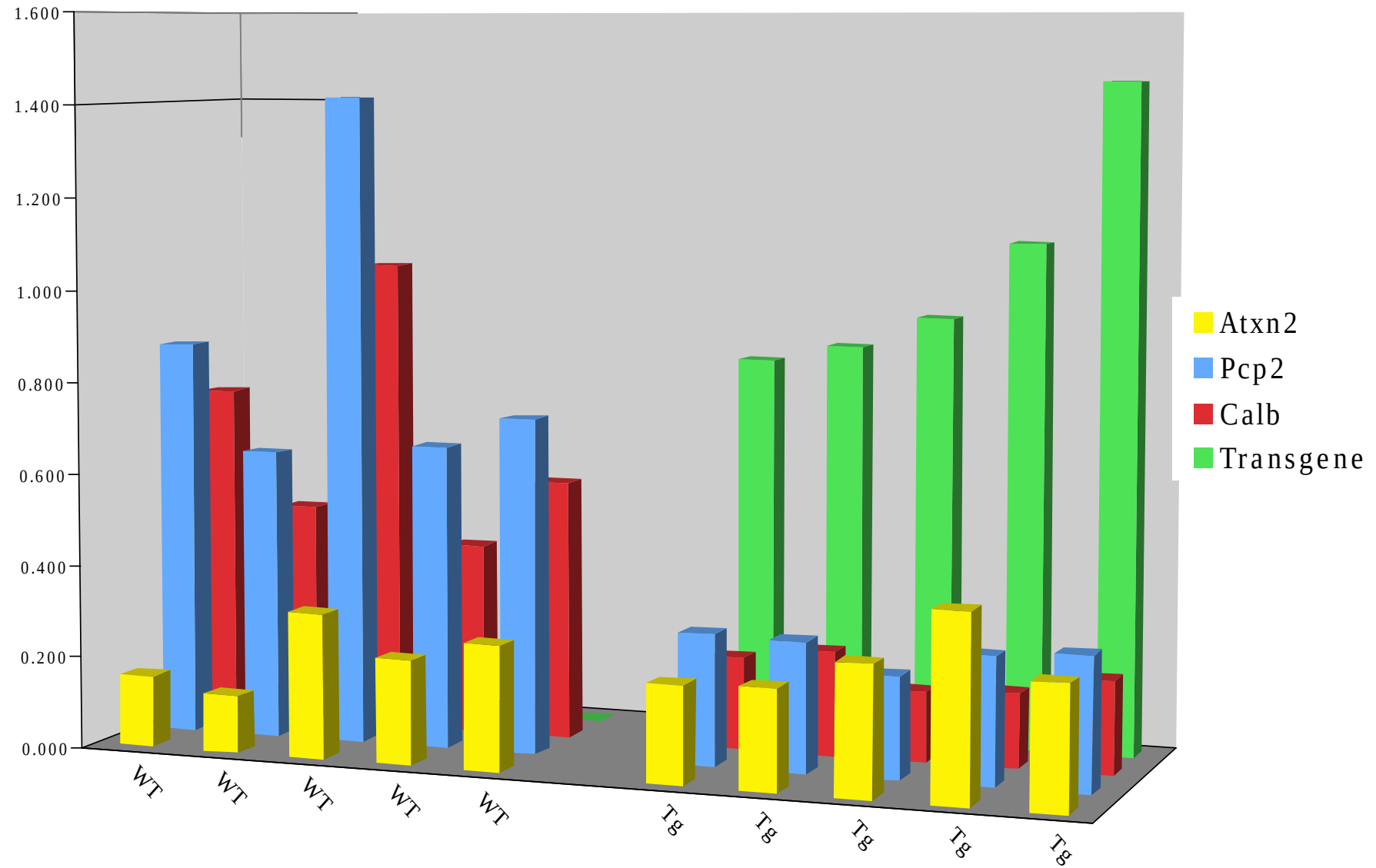
Line 40-2 at age 1 day - relative expression



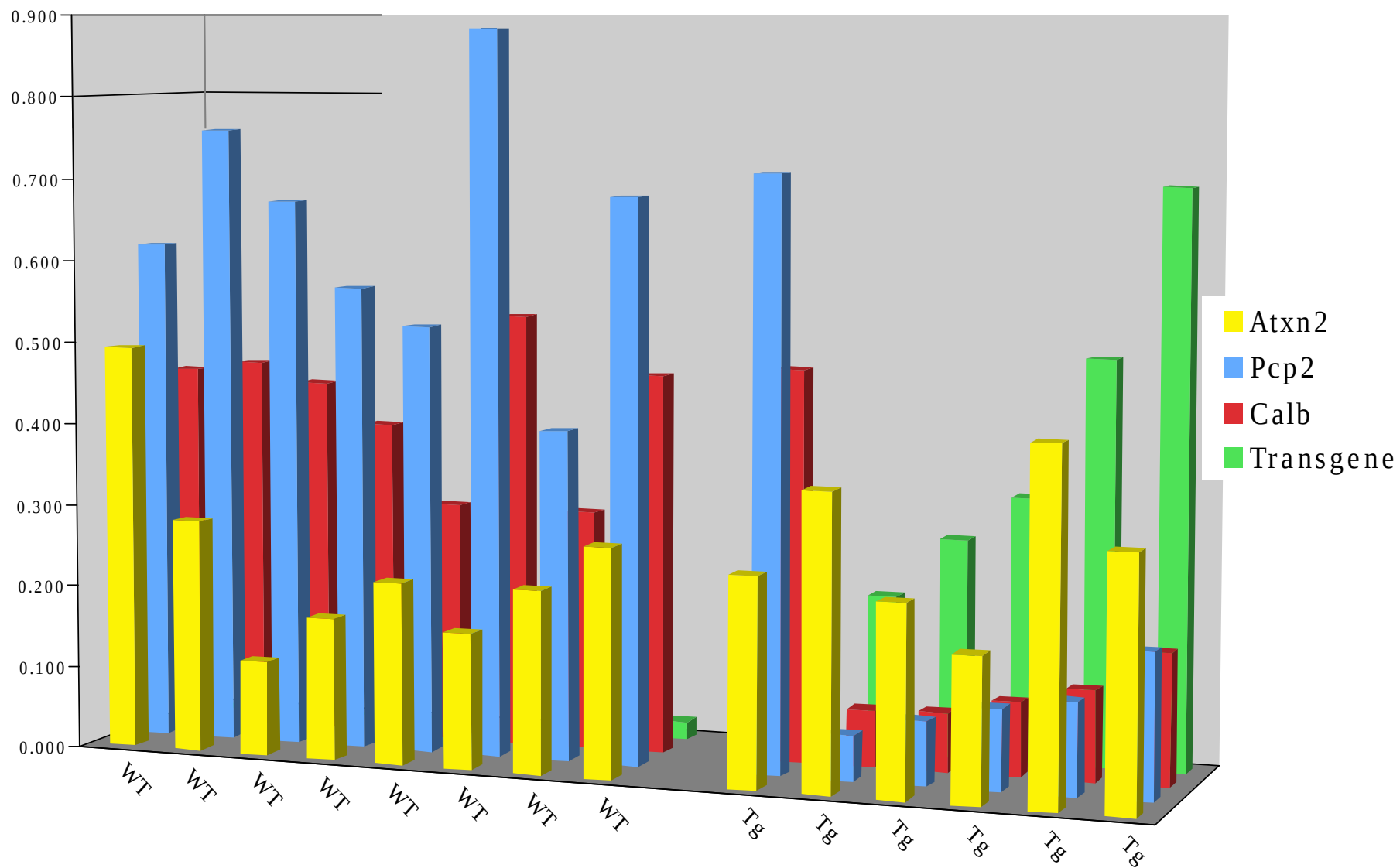
Line 40-2 at age 60 days - relative expression



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



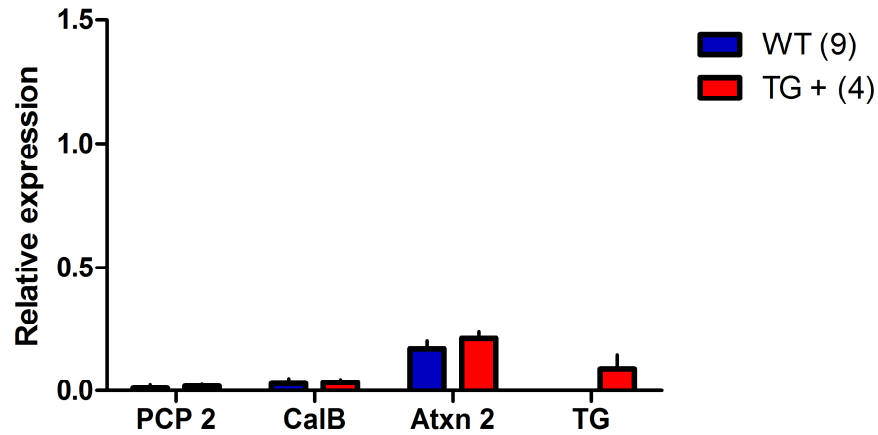
Line 40-2 at age 389/392 days - relative expression



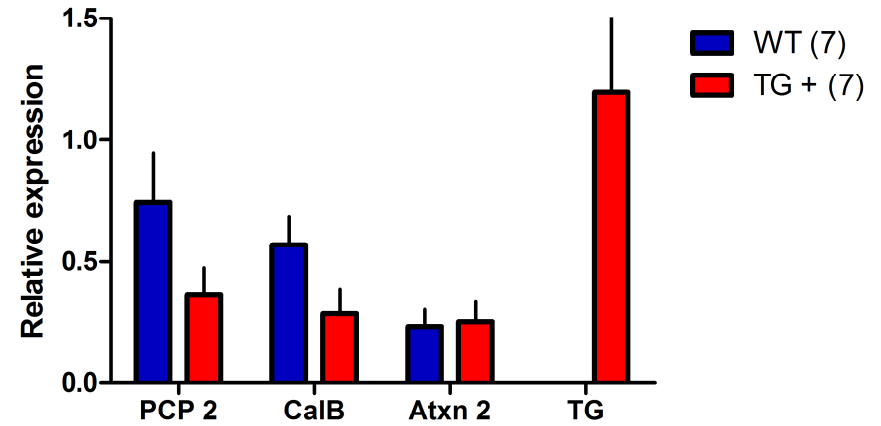
qPCR

- The final qPCR slide represents the collapsed data previous slides
 - Error bars represent standard-deviation
- Data represented in same scale

Day 1

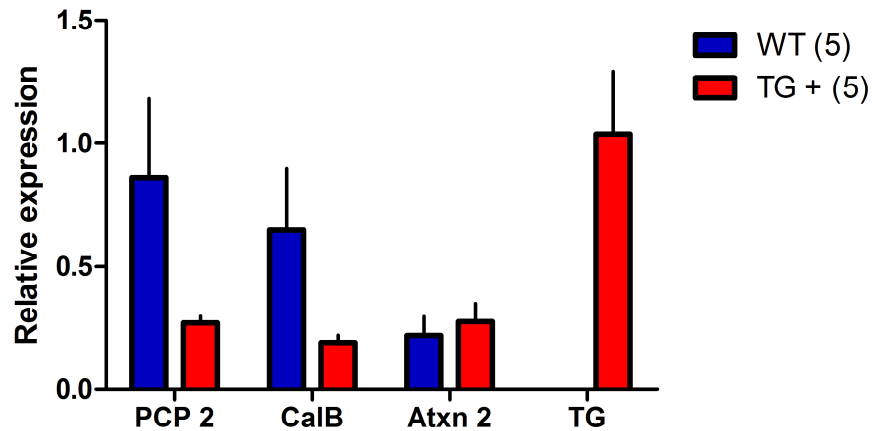


Day 60

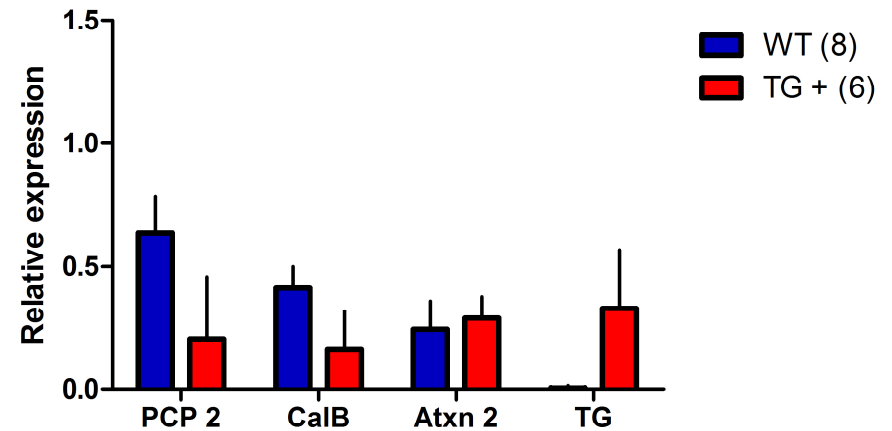


Collapsed qPCR data summary

Day 200

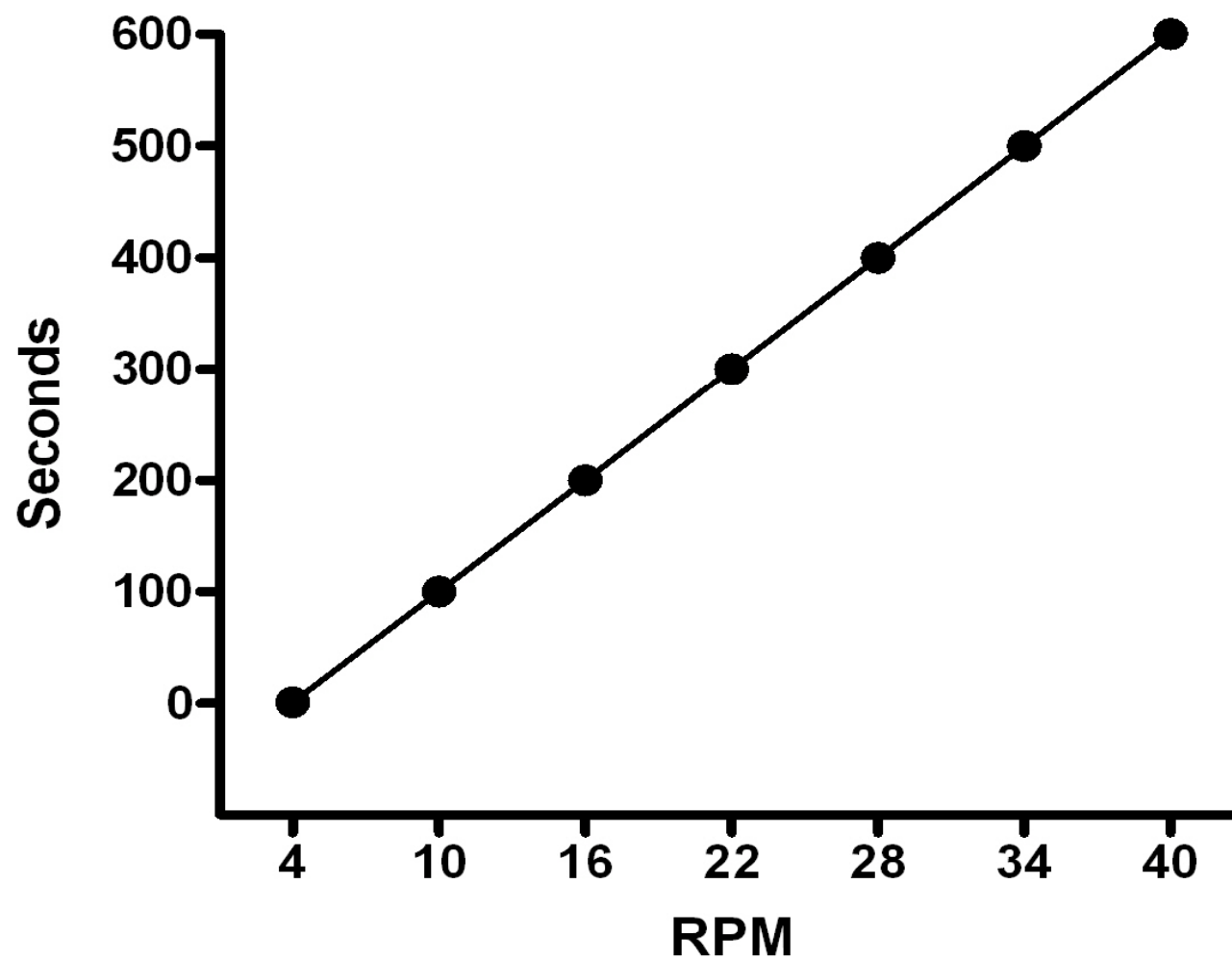


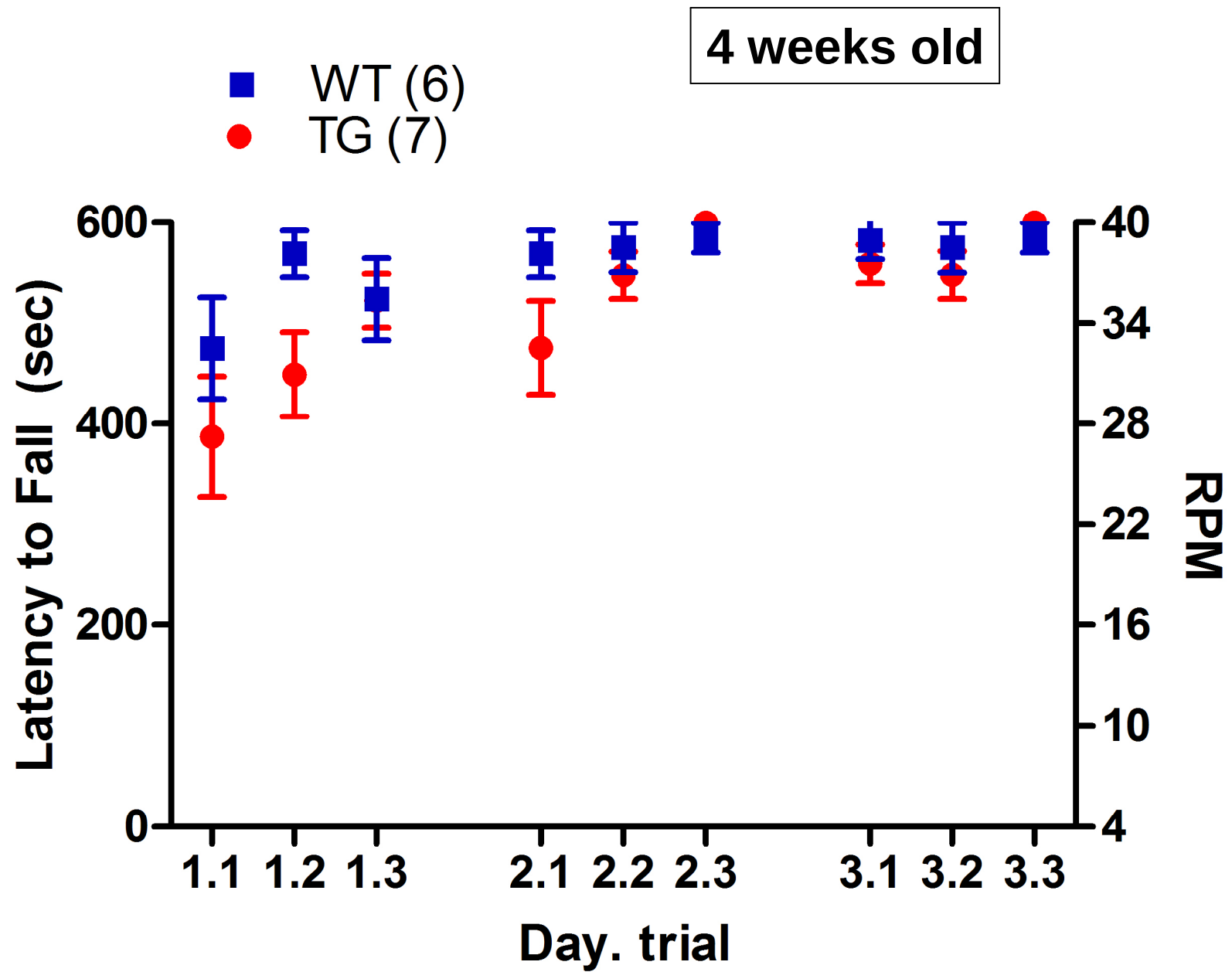
Day 360

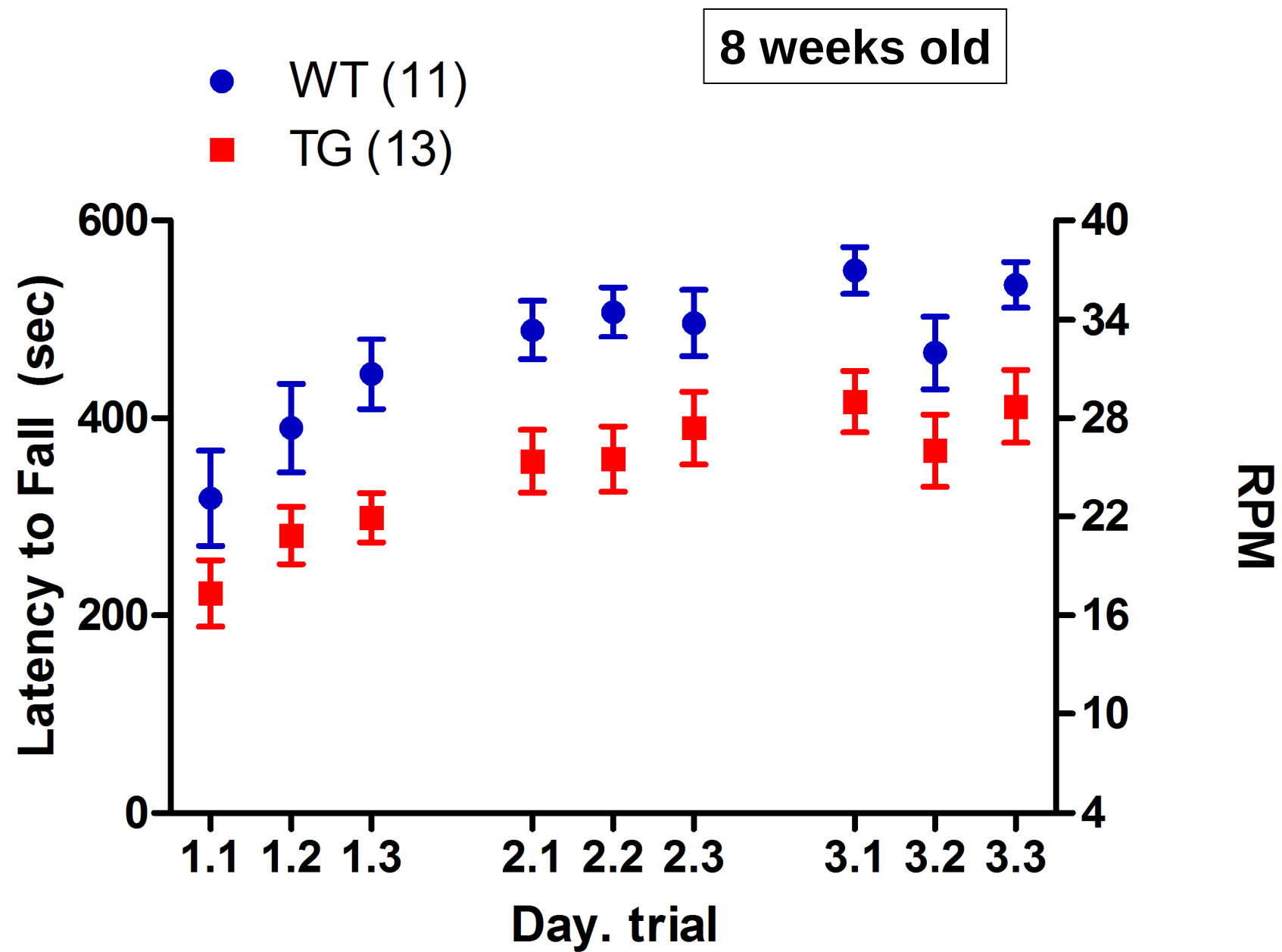


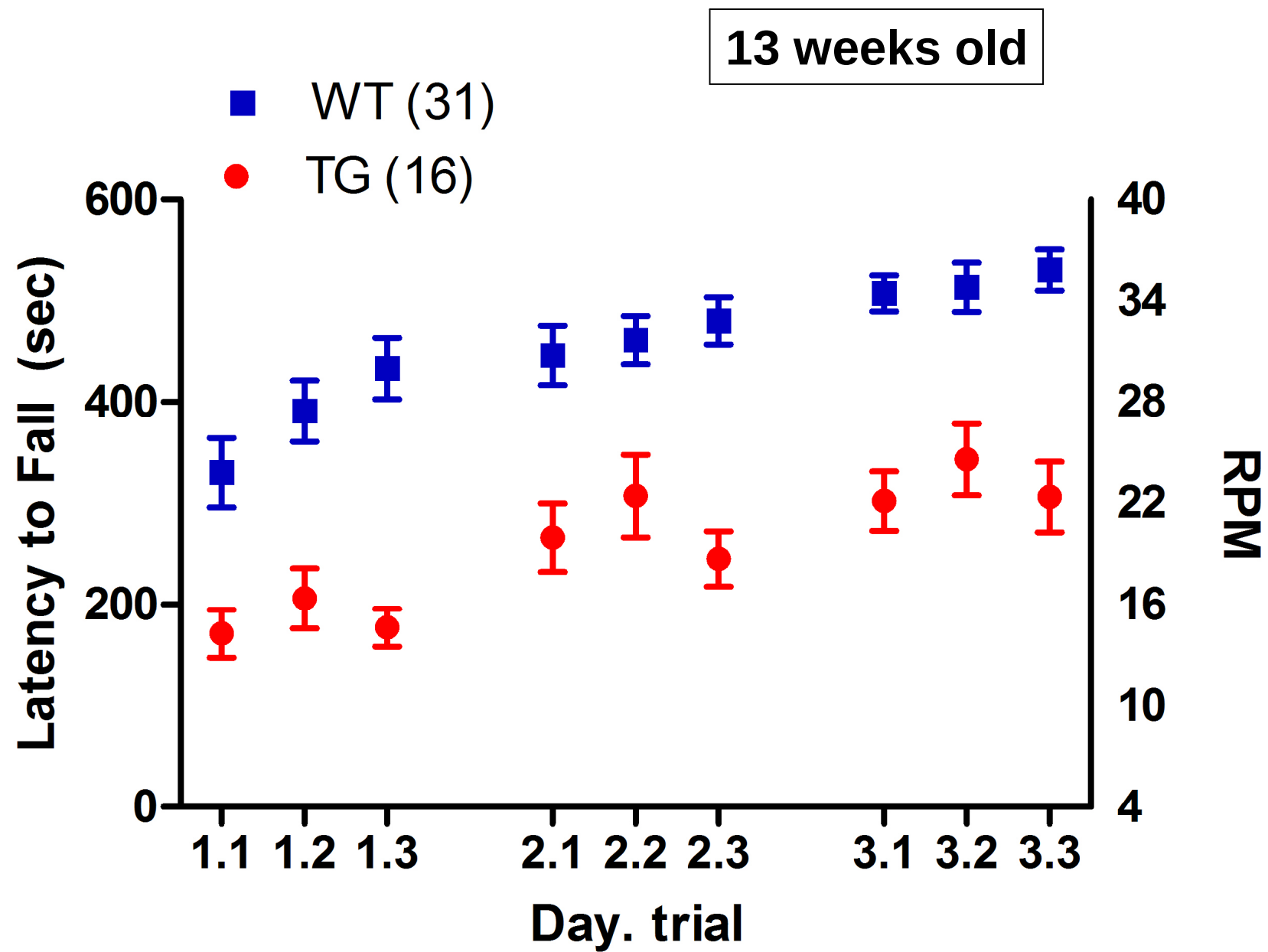
Expression normalized to WASF-1

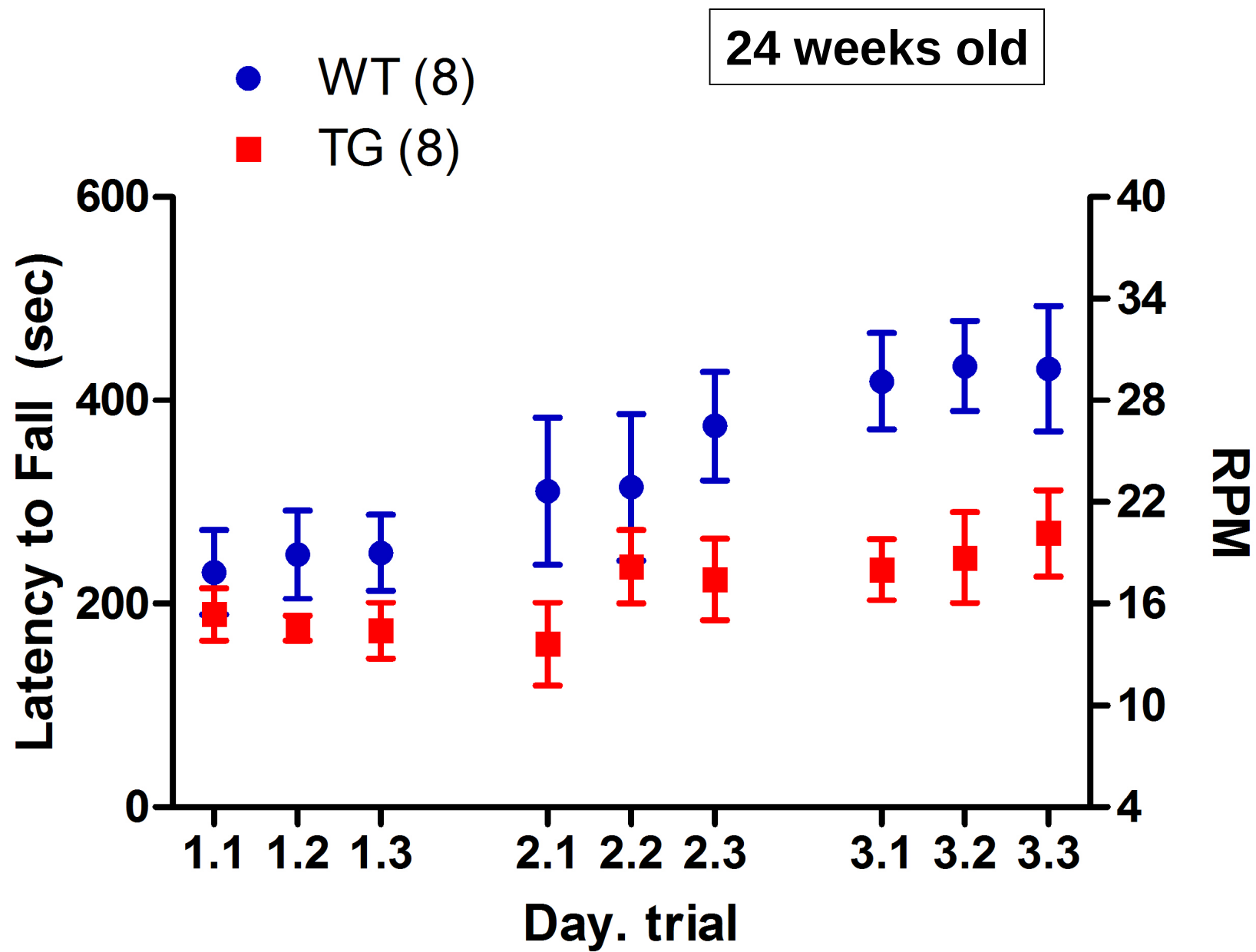
Accelerating Rotarod

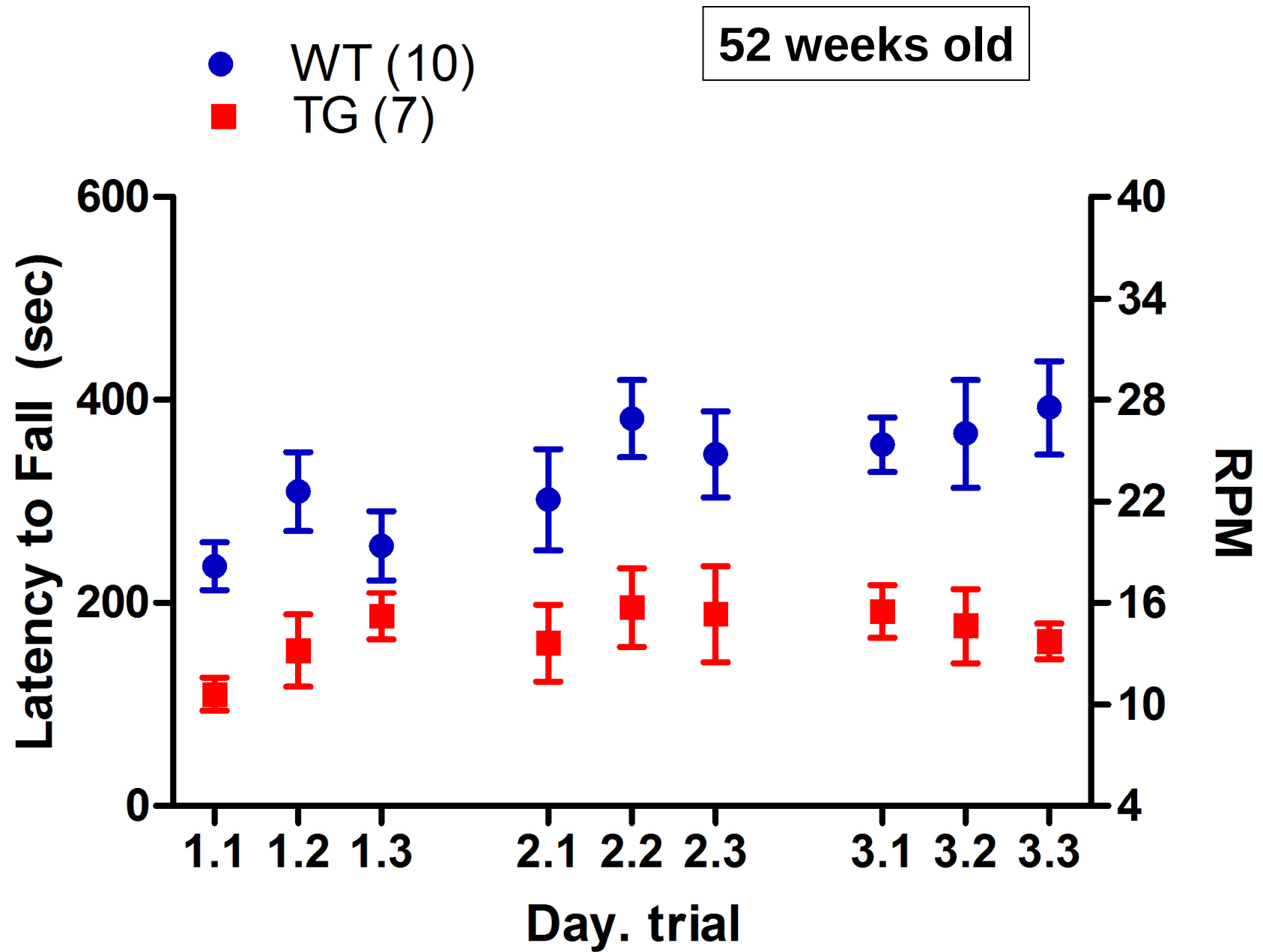






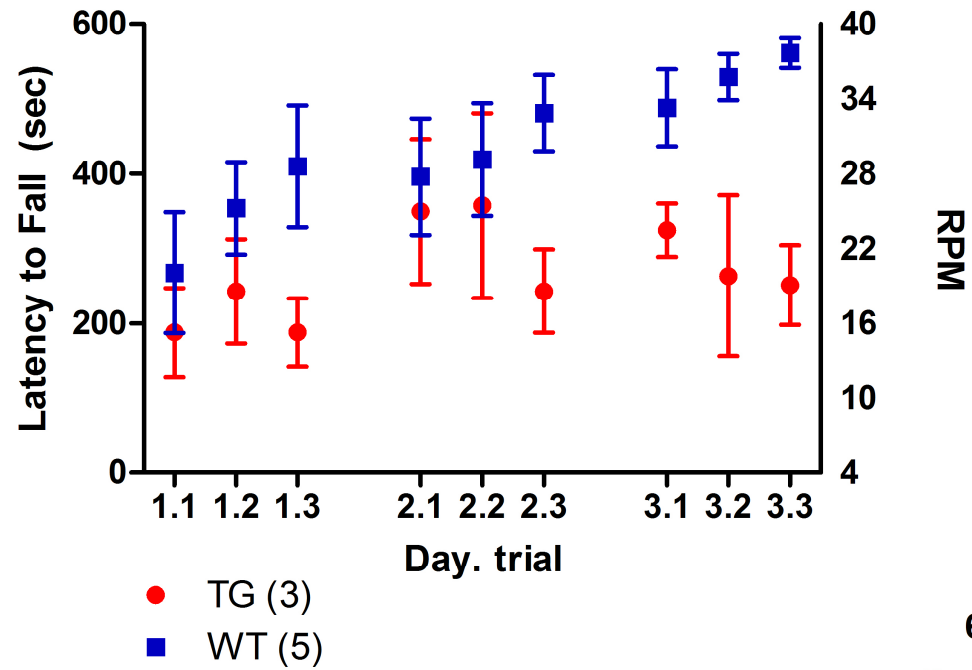




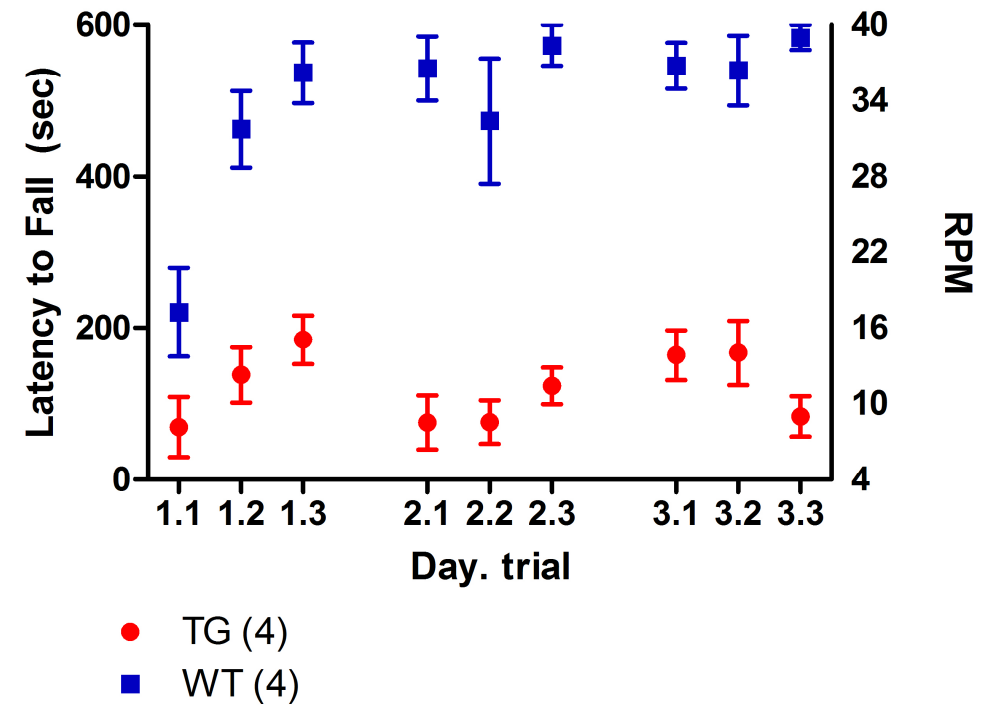


OTIS MICE

3 months

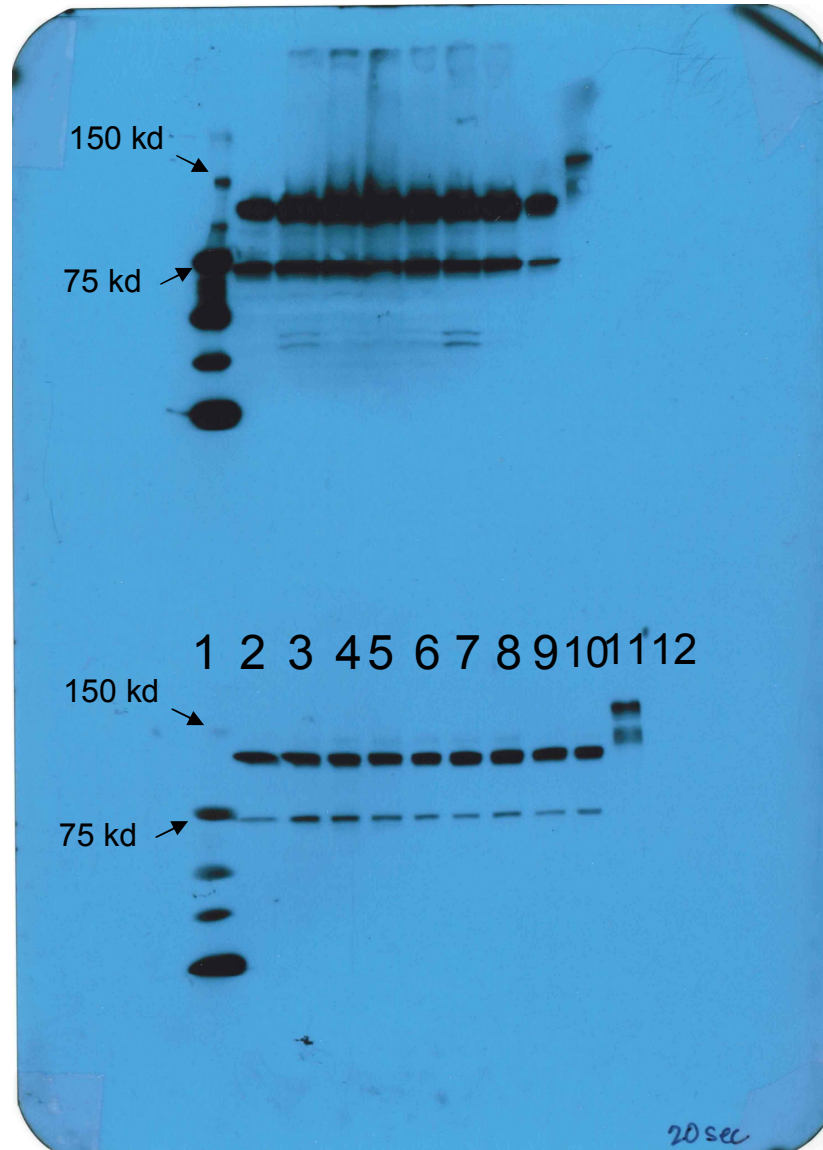


7 months



Extra

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