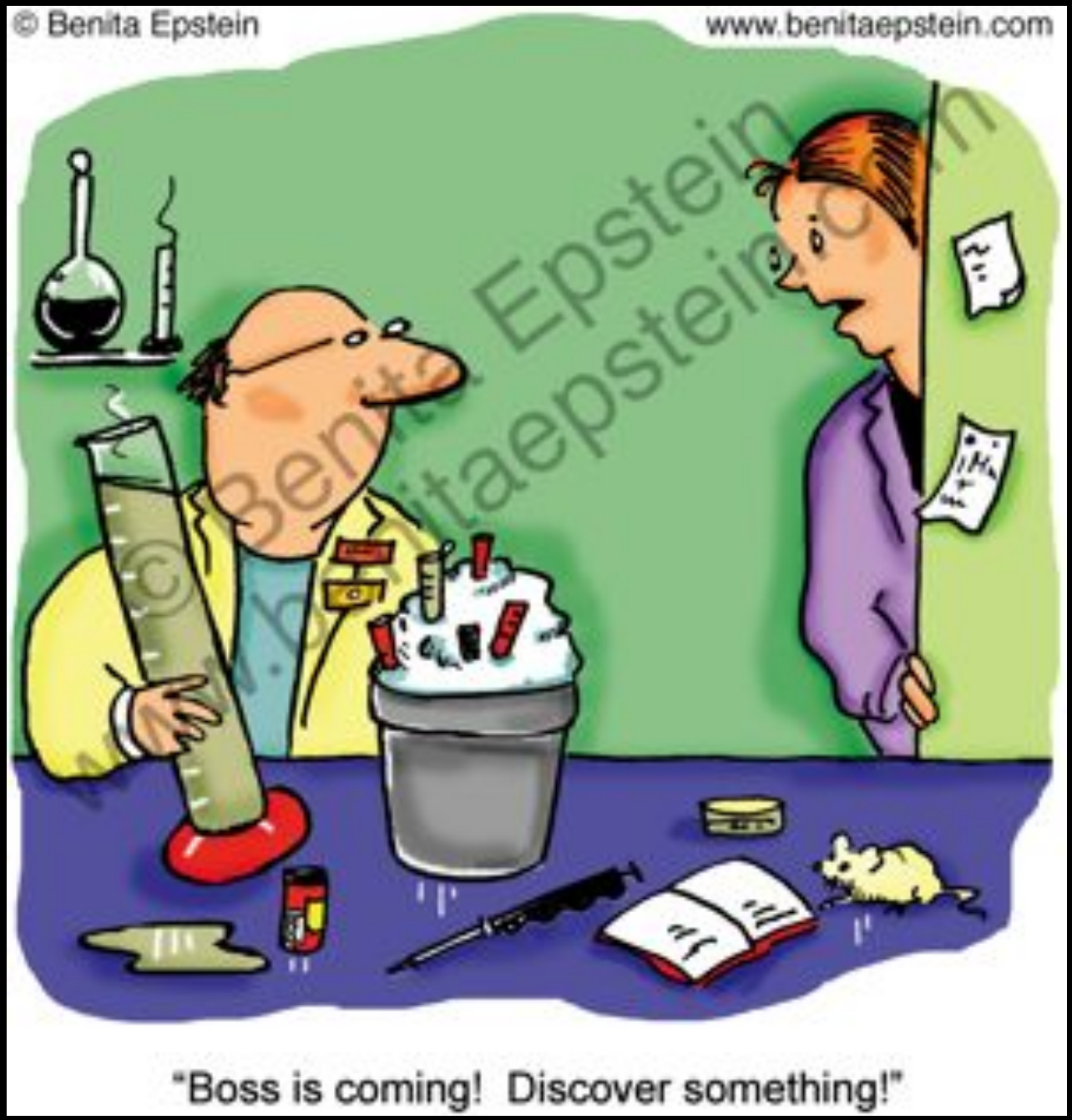
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"Boss is coming! Discover something!"

- Immunohistochemistry:
  - Problem: erratic detection of ataxin 2s
  - Solution:
    - Concentration response curve

      all AB
    - Longer blocking time
    - Change blocking agent
      - NGS→skim milk

Methods

- 20 uM brain sections

  38-13 and B6 sliced and stained together

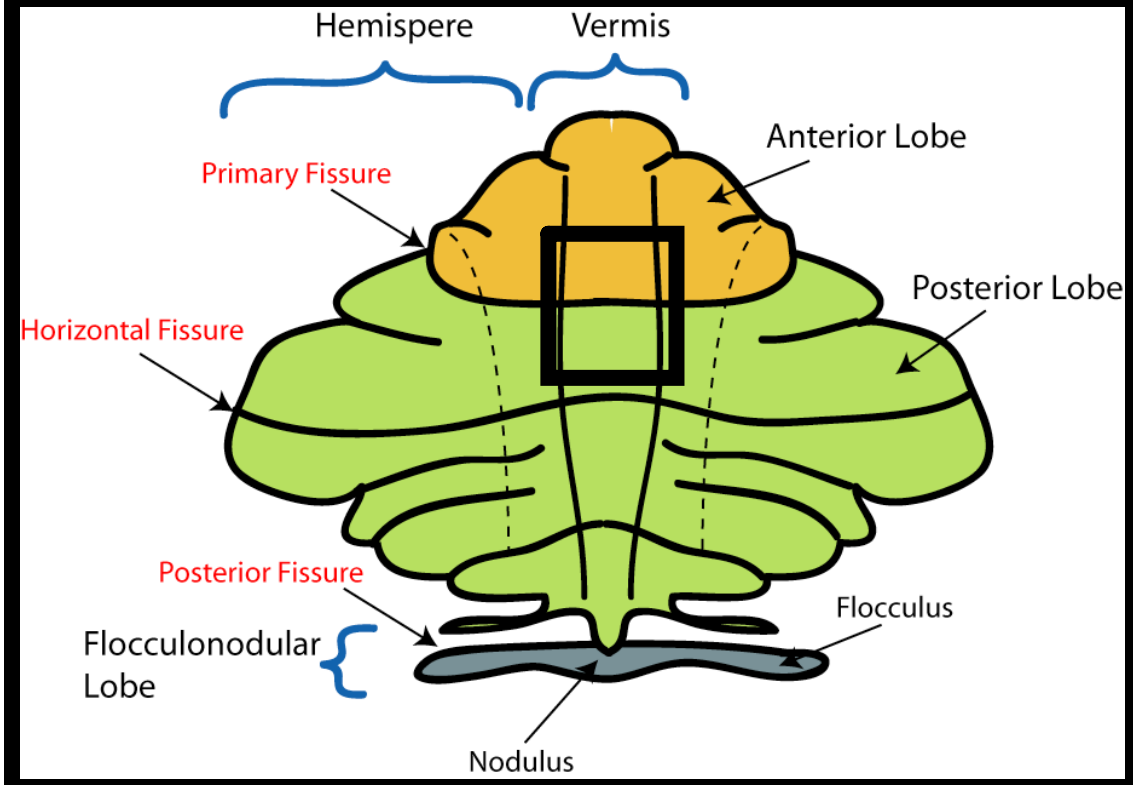
- 1x PBS: wash 3x @ 10min
- Permeabilize and block**overnight** @ RT
- Primary antibody 24hrs @ 4c
- Wash 3x @ 15min with wash buffer
- Secondary antibody 2hrs @ RT
- Wash 3x @ 15min with wash buffer

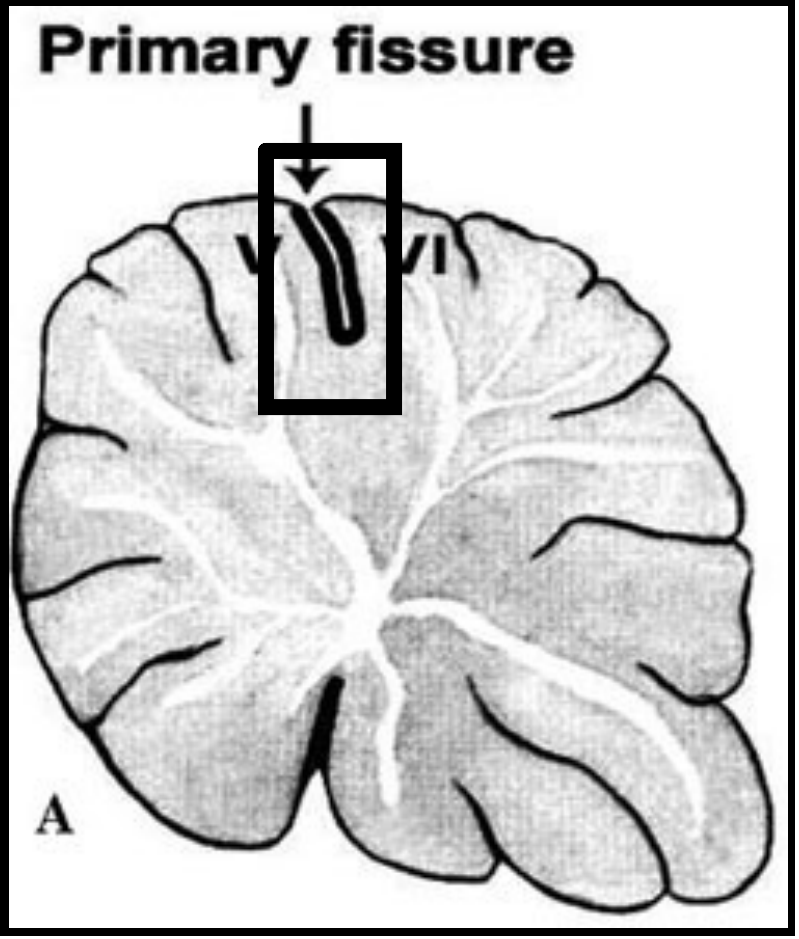
Solutions:

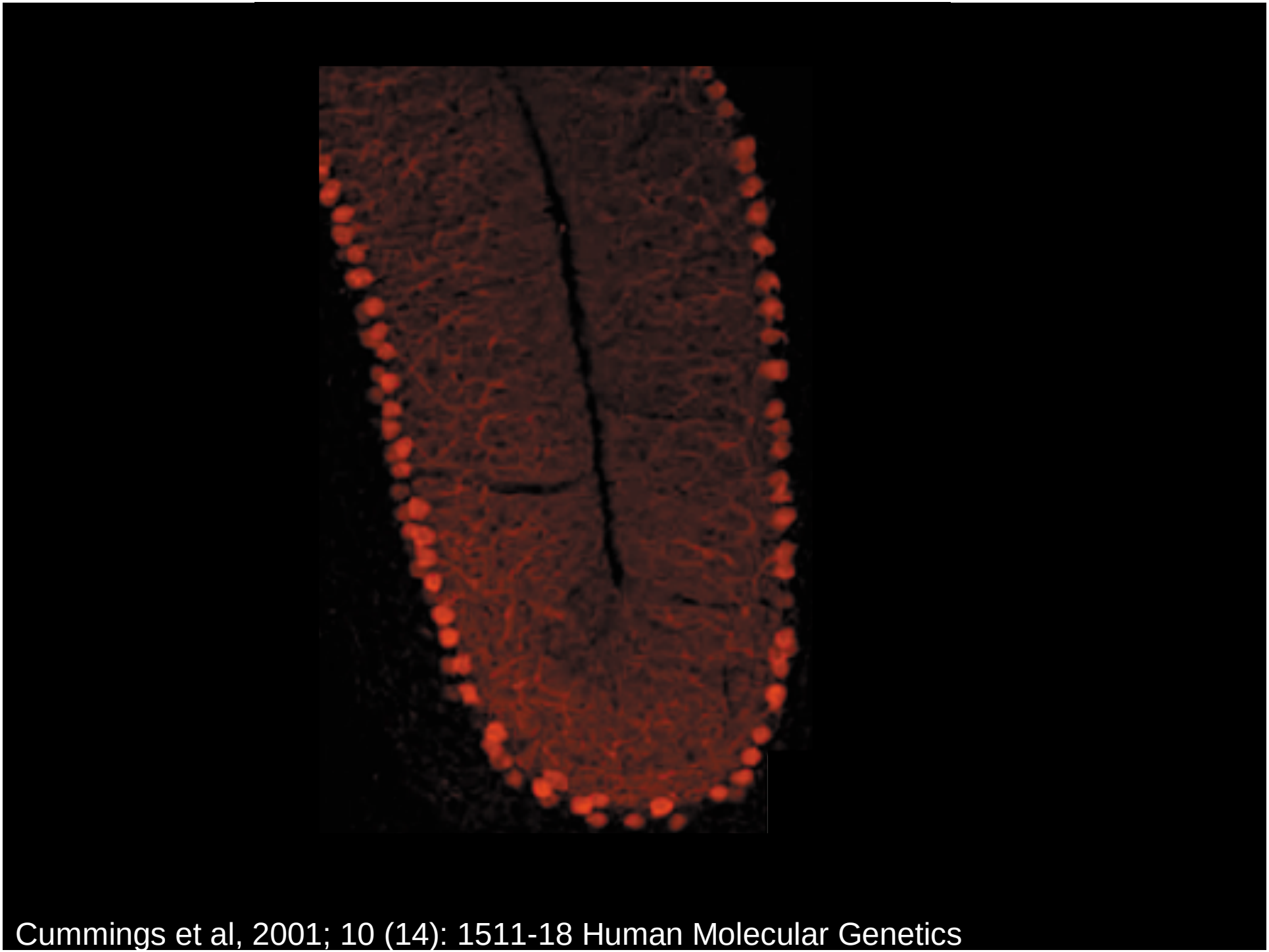
- Permeablization & blocking buffer: 0.3% Triton in 1x PBS with 5% skim milk
- Wash buffer: 0.1% Triton in 1x PBS with 1% skim milk
- AB solution: 0.1% Triton in 1x PBs with 5% skim milk
- Slide fixative media: Prolong Gold

Camera

- Camera settings optimized
  - Control: secondary AB only
  - All settings remain constant across session
    - Exposure time: 3s
    - Gain: 2.0
    - Black level: 335
    - Gamma level: 1.0
    - White level: 3350


Hemispere
Vermis
Anterior Lobe
Primary Fissure
Posterior Lobe
Horizontal Fissure
Posterior Fissure
Flocculus
Flocculonodular Lobe
Nodulus

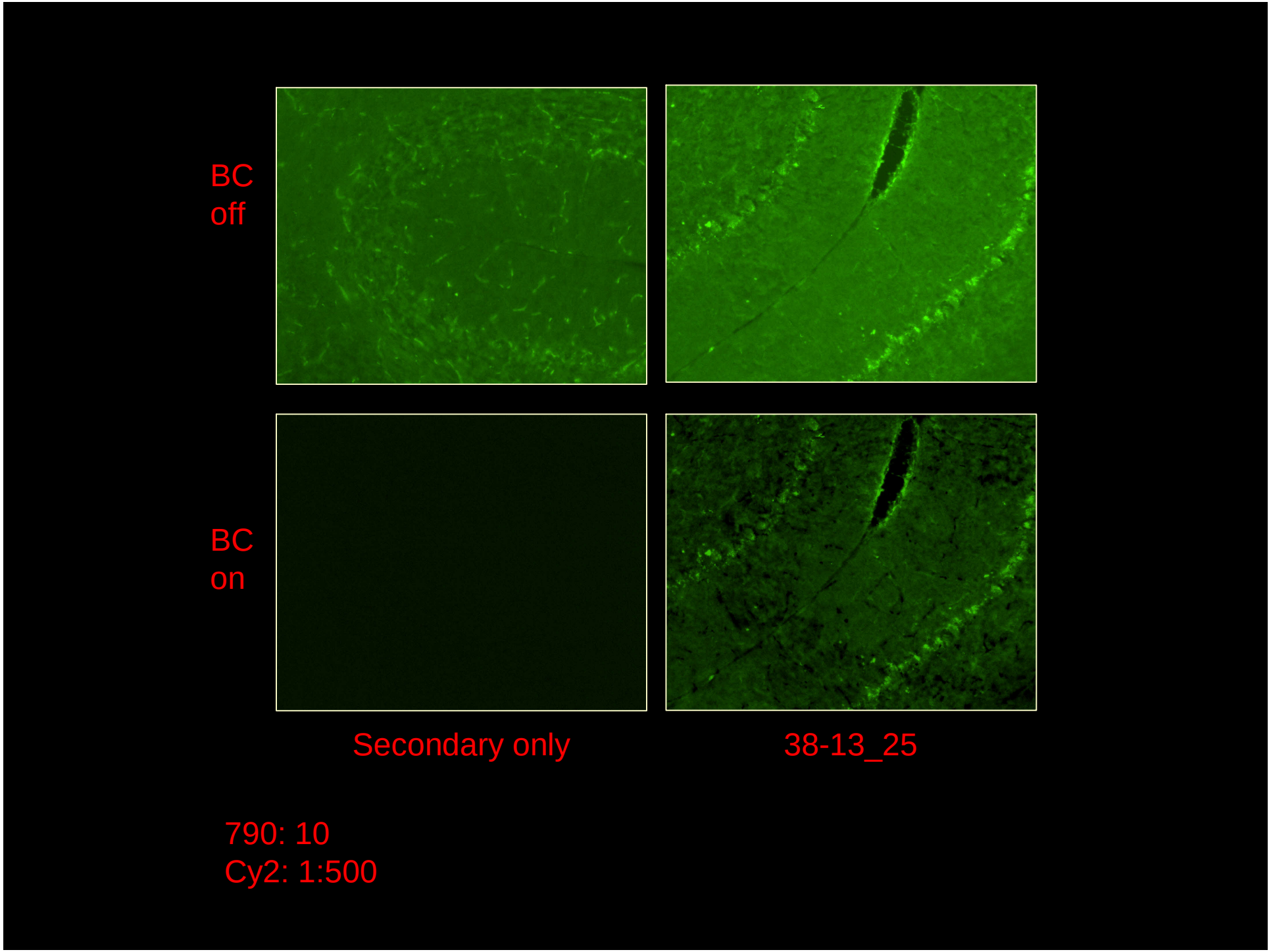

Primary fissure
A

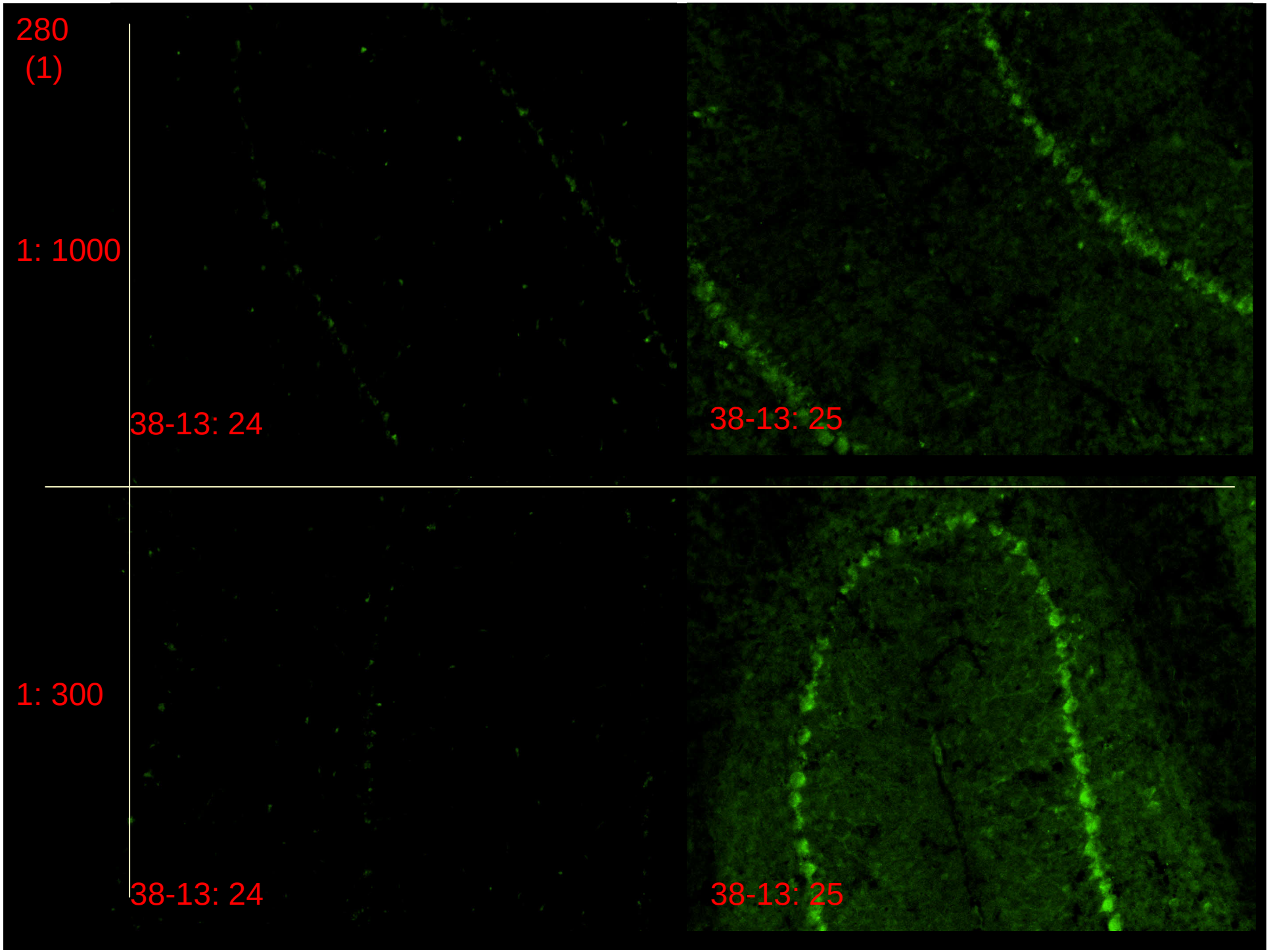


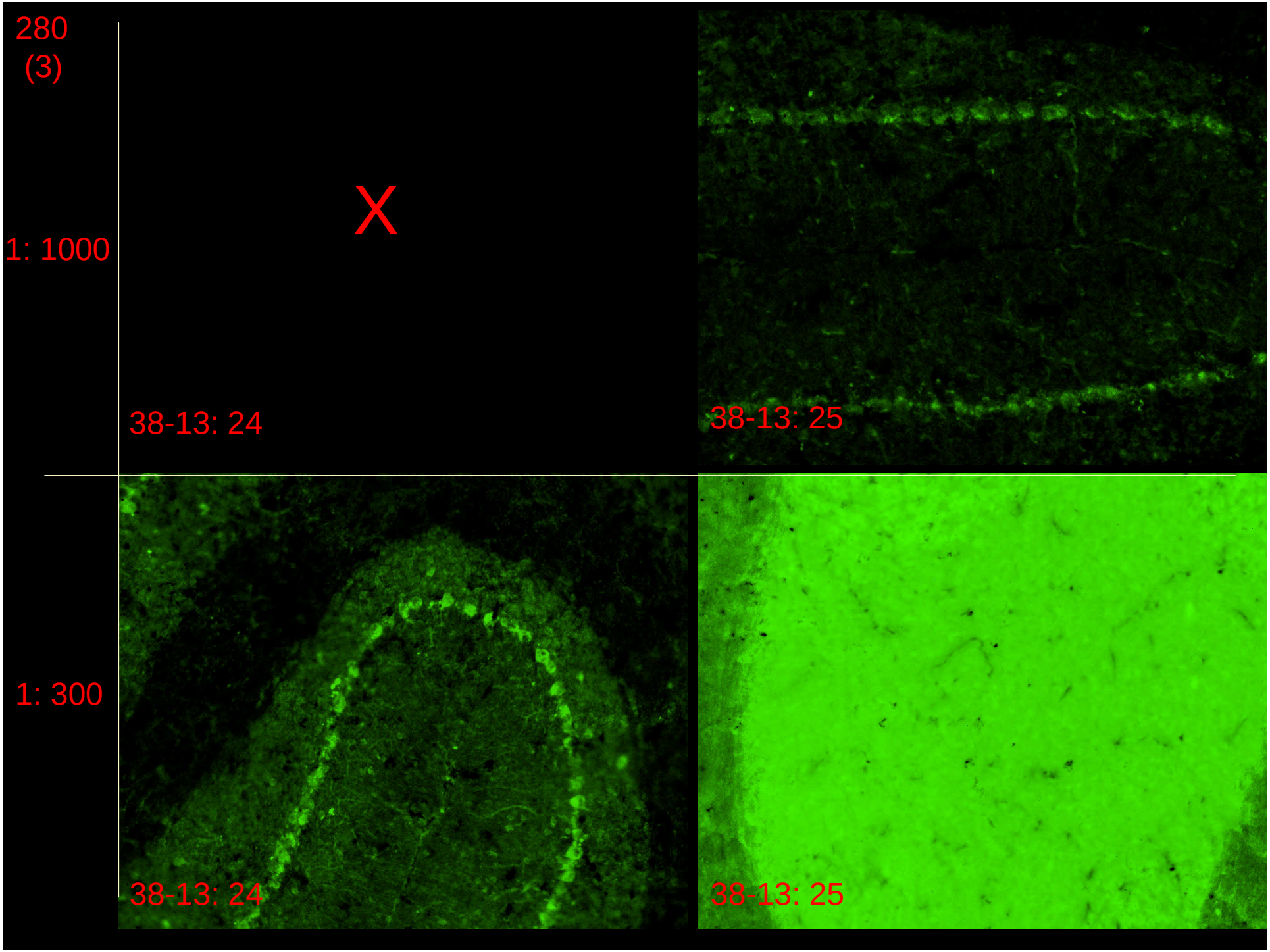
Cummings et al, 2001; 10 (14): 1511-18 Human Molecular Genetics

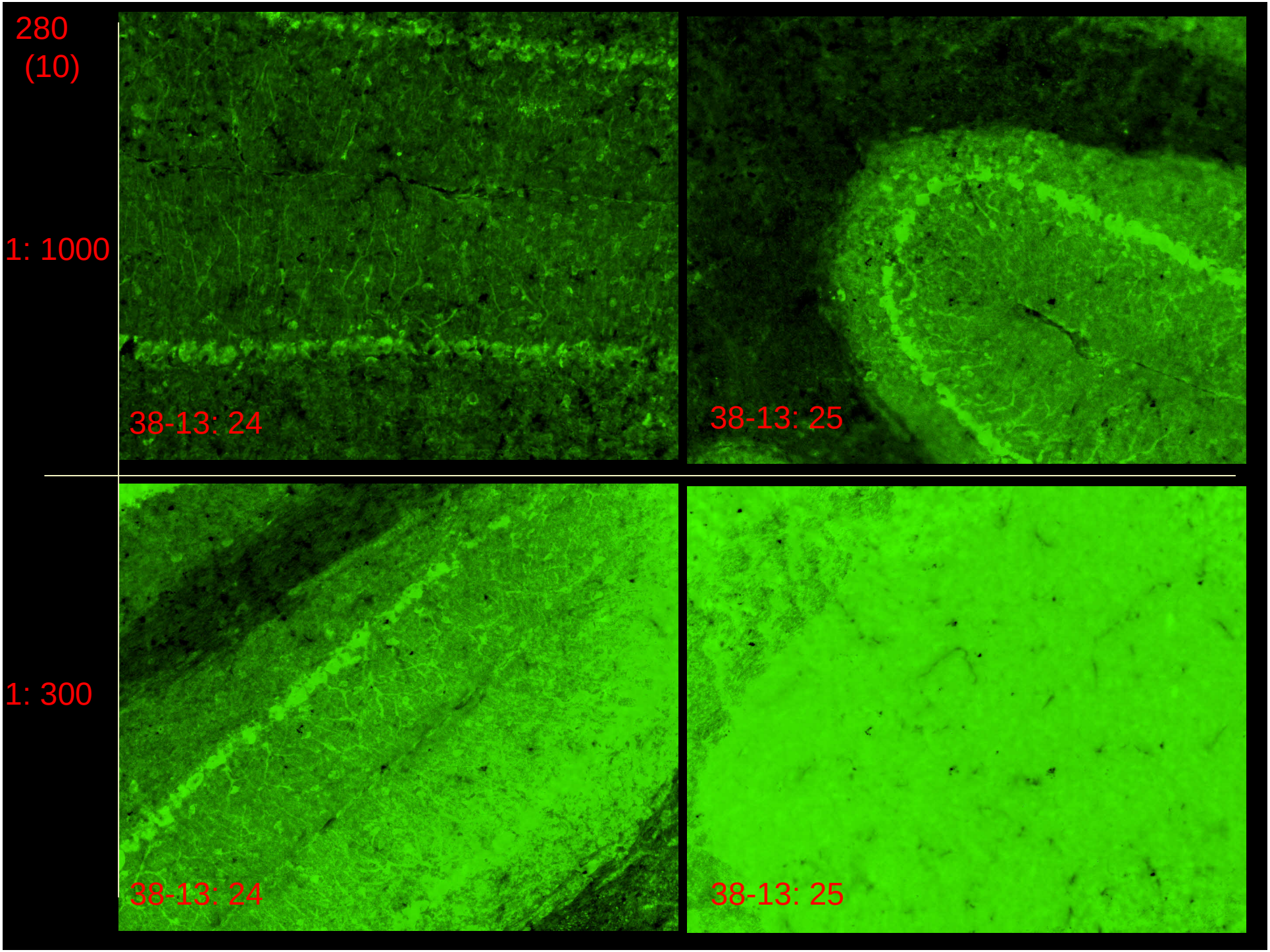
Design

- 3 primary antibodies tested:
  - 280, 714, 790
    - 3 concentrations: 1, 3, 10
  - Secondary AB tested at 1:300, 1: 1,000


BC
off
BC
on
Secondary only
38-13_25
790: 10
Cy2: 1:500


280
(1)
1: 1000
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


280
(3)
1: 1000
X
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


280
(10)
1: 1000
38-13: 24
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1: 300
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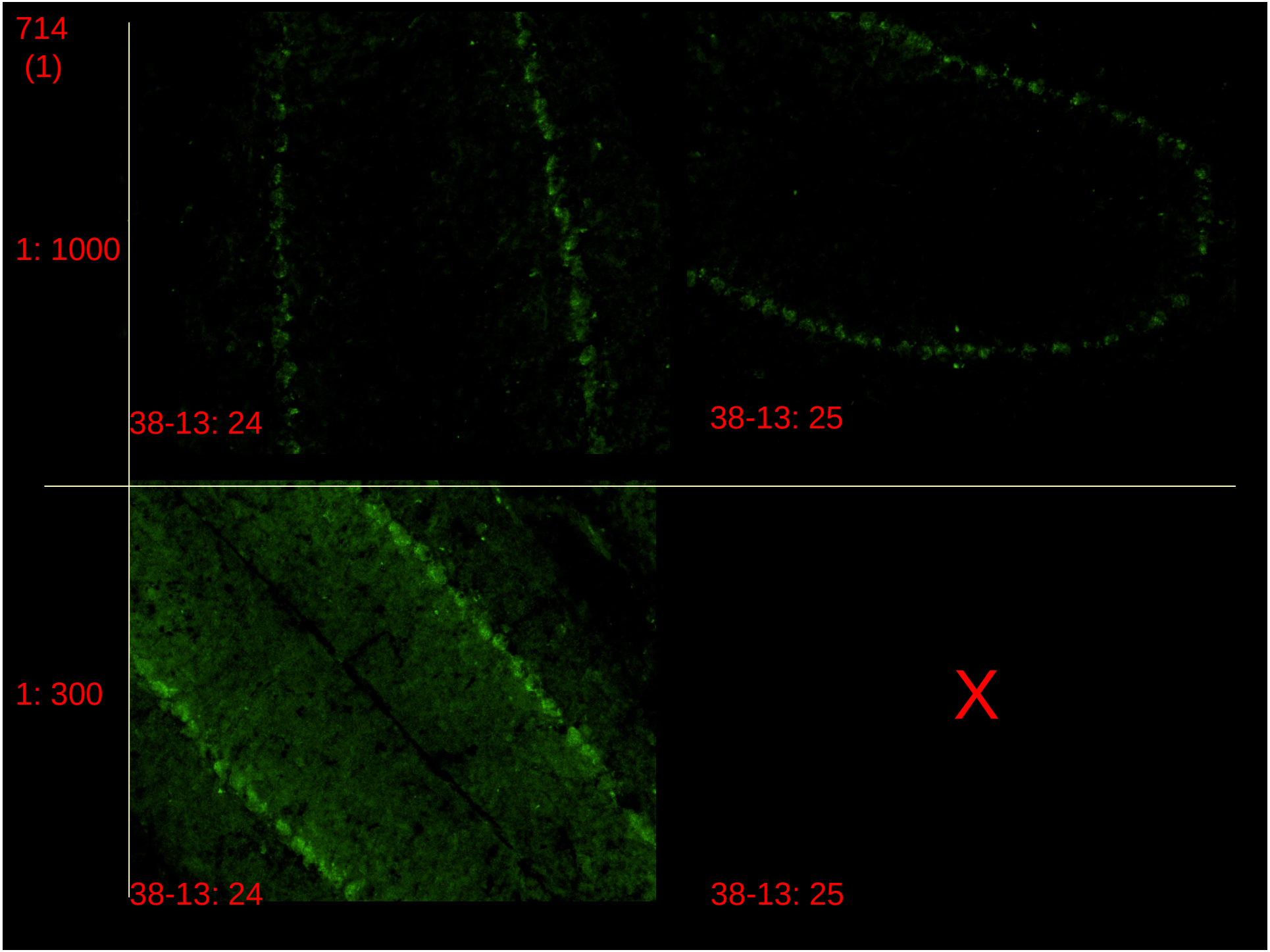
38-13: 25


714
(1)
1: 1000
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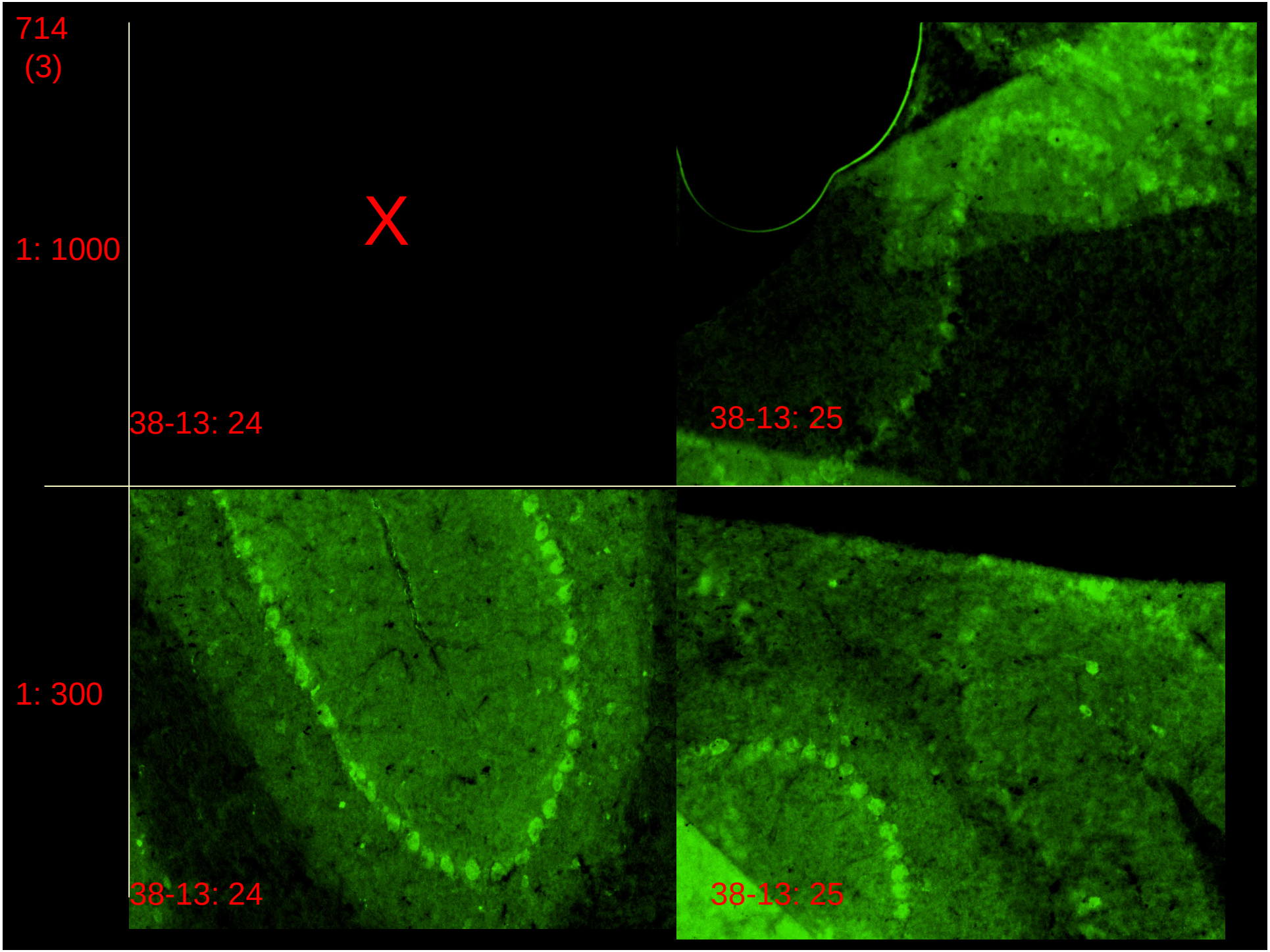
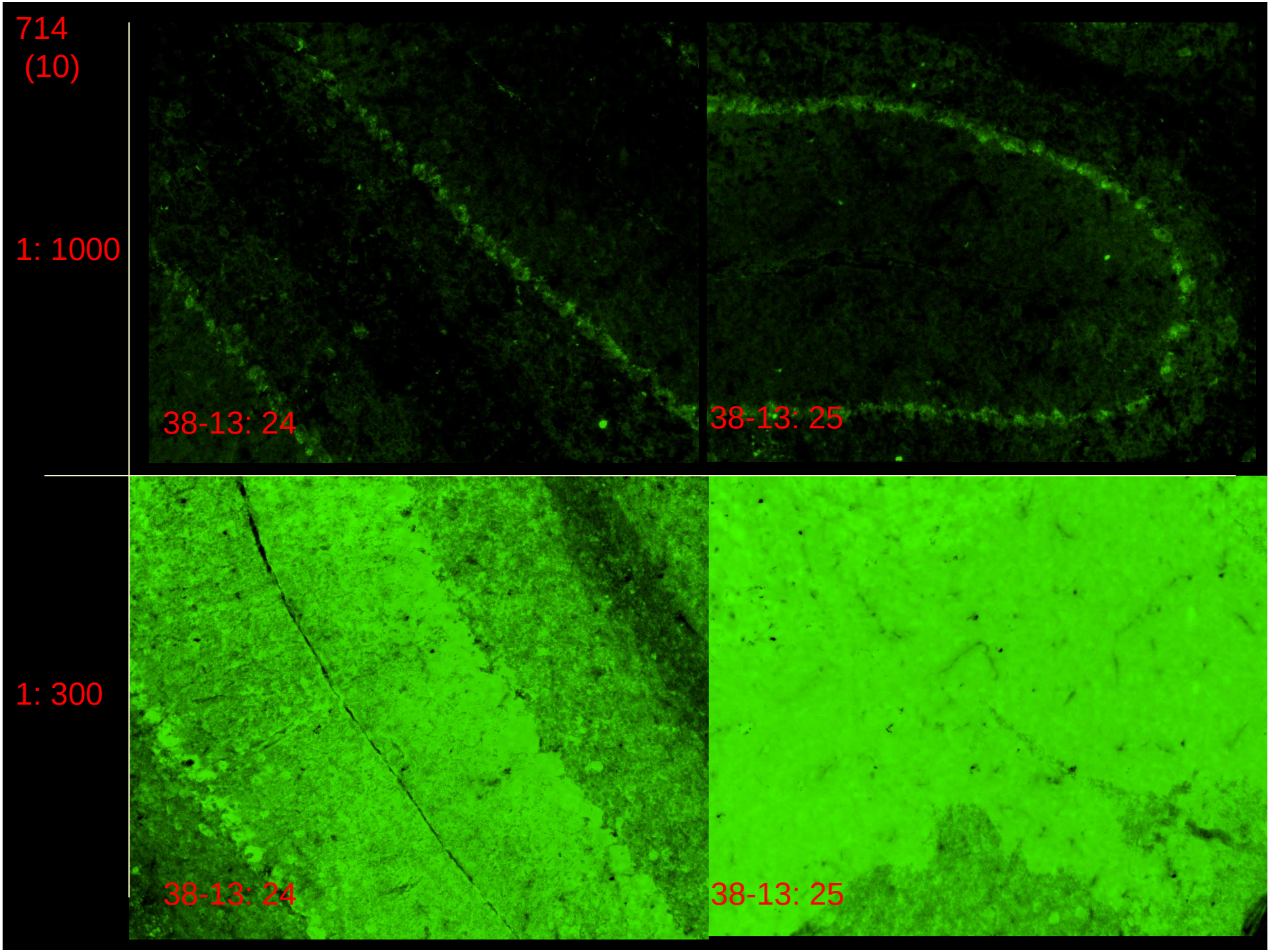
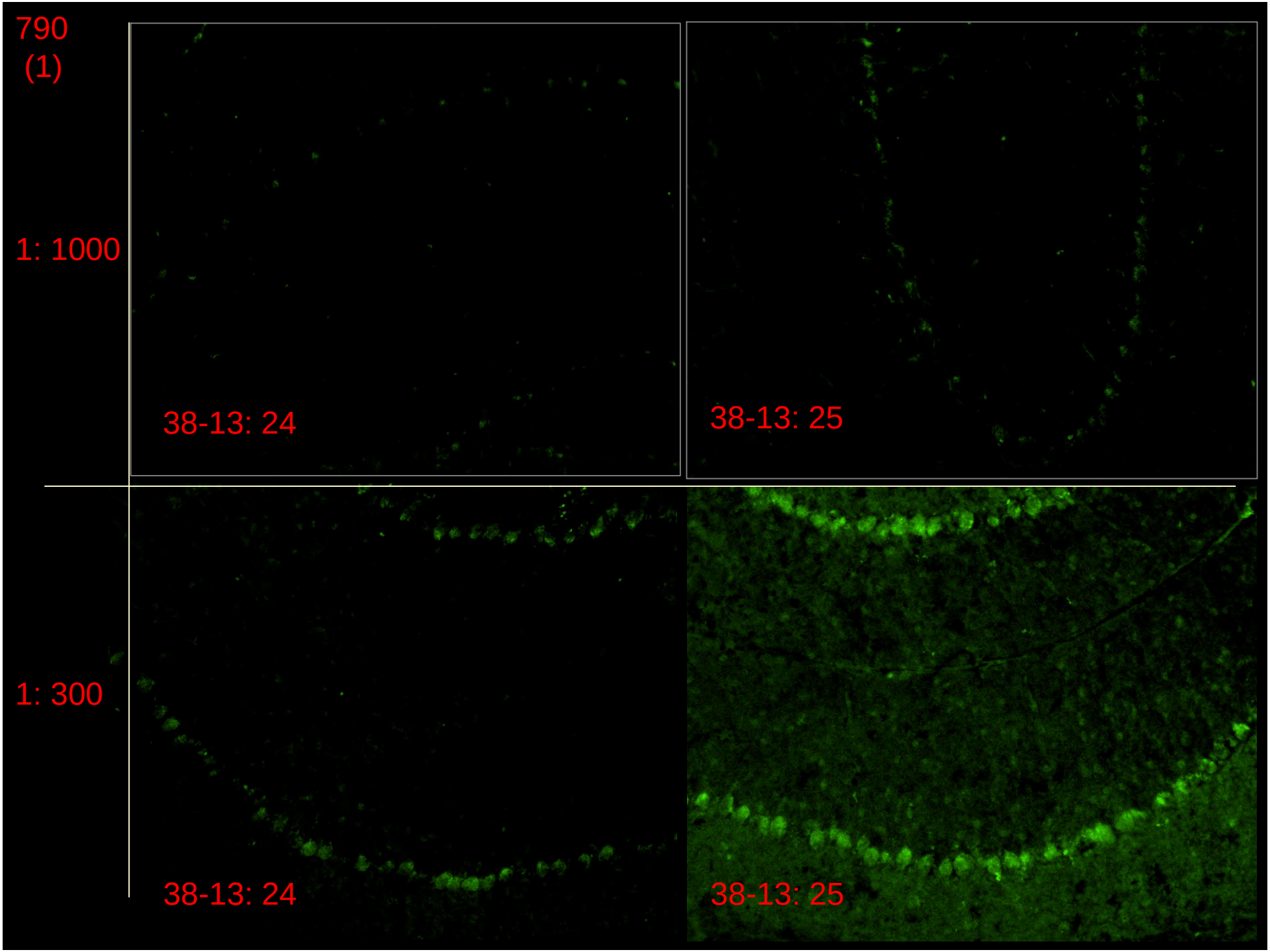
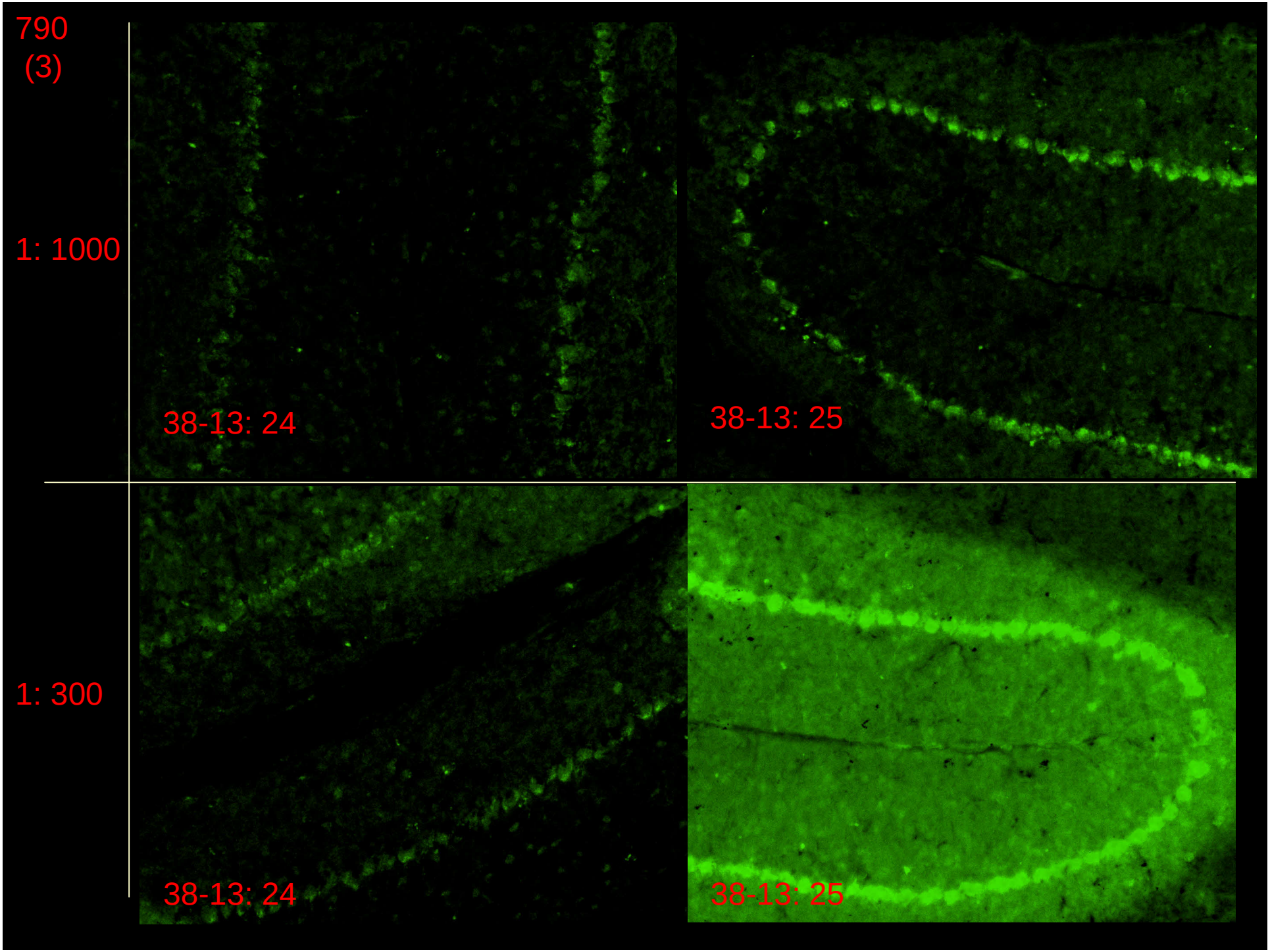
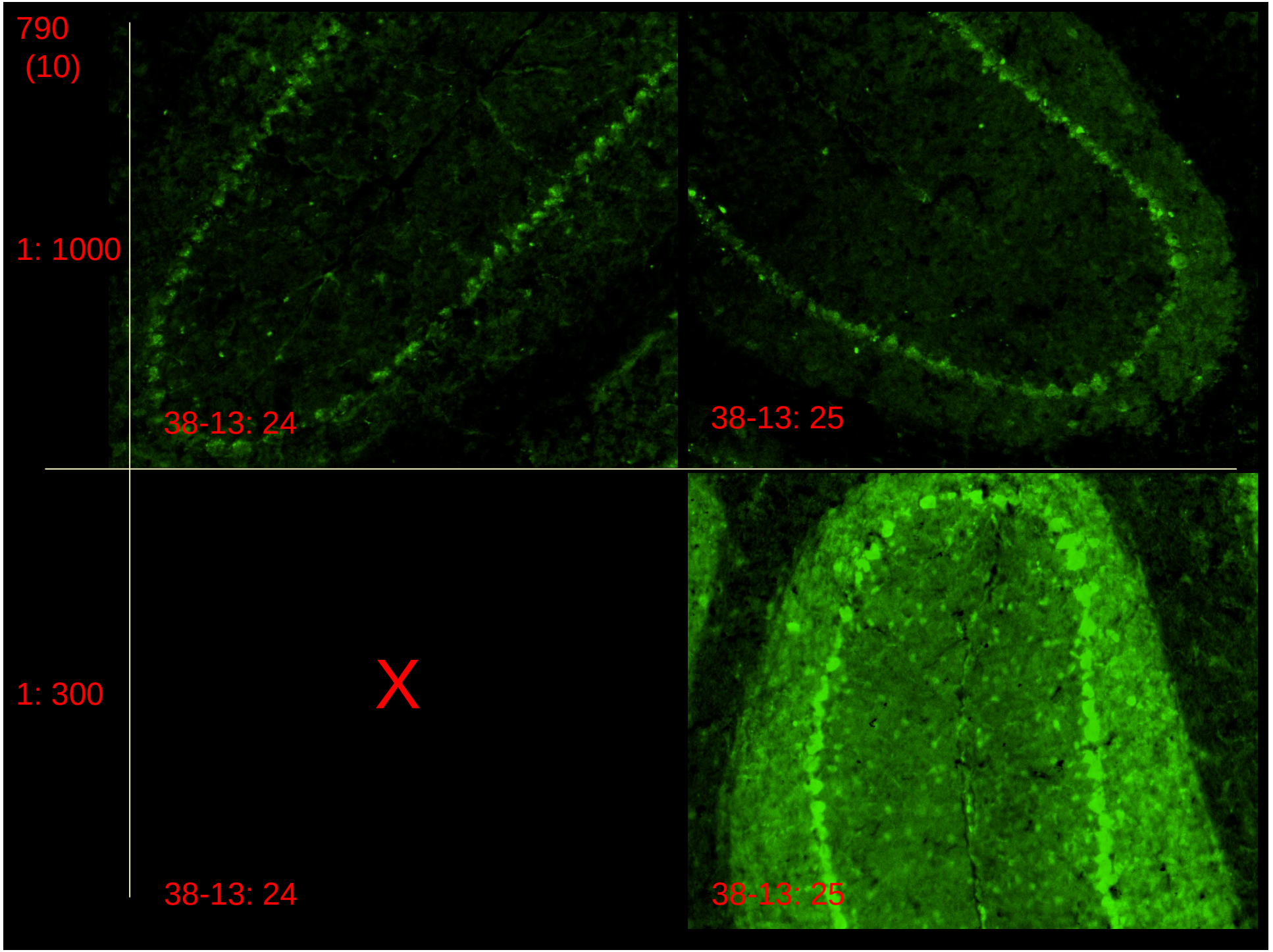
1: 300
38-13: 24
38-13: 25
X


714
(3)
1: 1000
X
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


714
(10)
1: 1000
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


790
(1)
1: 1000
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


790
(3)
1: 1000
38-13: 24
38-13: 25
1: 300
38-13: 24
38-13: 25


790
(10)
1: 1000
38-13: 24
38-13: 25
1: 300
X
38-13: 24
38-13: 25

Bench work

- Each cerebellum is halved: qPCR, WB, IHC
  - WB: comparing expression of ataxin protein between lines—more work to be done…
    - Thank you Sharan!

End