

SCA 2 KO: blinded samples 1 – 12

- Pattie euthanized animals and submerged 1/3 of the cerebellum in 4% PFA
 - Overnight @ 4c
- 20% sucrose; overnight @ 4c
- 30% sucrose; overnight @ 4c
- Frozen in OCT @ -80c until slicing

Methods

- Slices from Cerebellum; no heart yet
- AB:
 - 280 @ 5ug/ mL
 - Atxin-2 @ 3ug/ mL

Methods

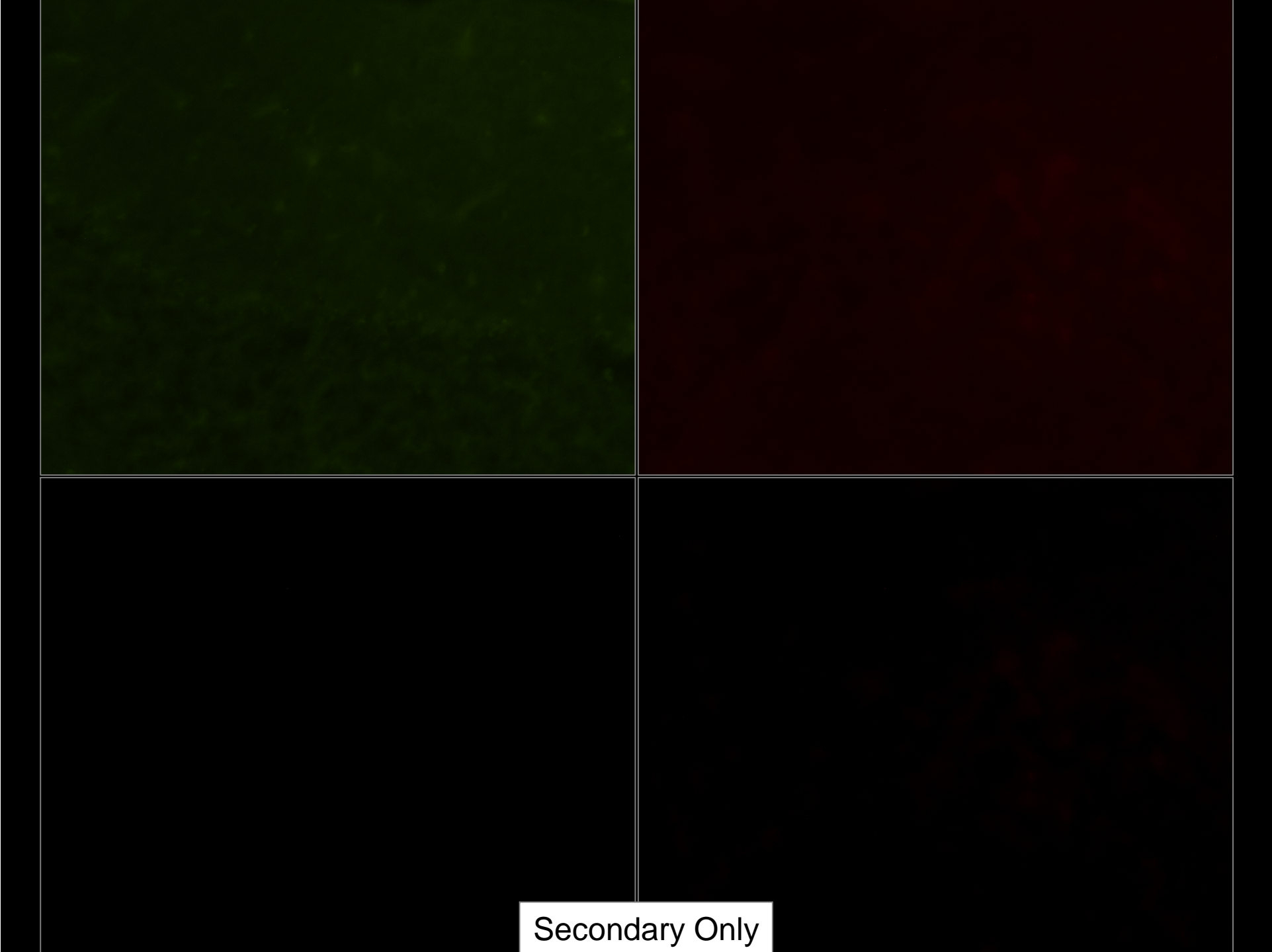
- 20 μ M brain sections
- 1x PBS: wash 3x @ 10min
- Permeabilize and block **6hrs** @ RT
- Primary antibody Overnight @ 4c
- Wash 3x @ 15min with wash buffer
- Secondary antibody 2hrs @ RT
- Wash 3x @ 15min with wash buffer

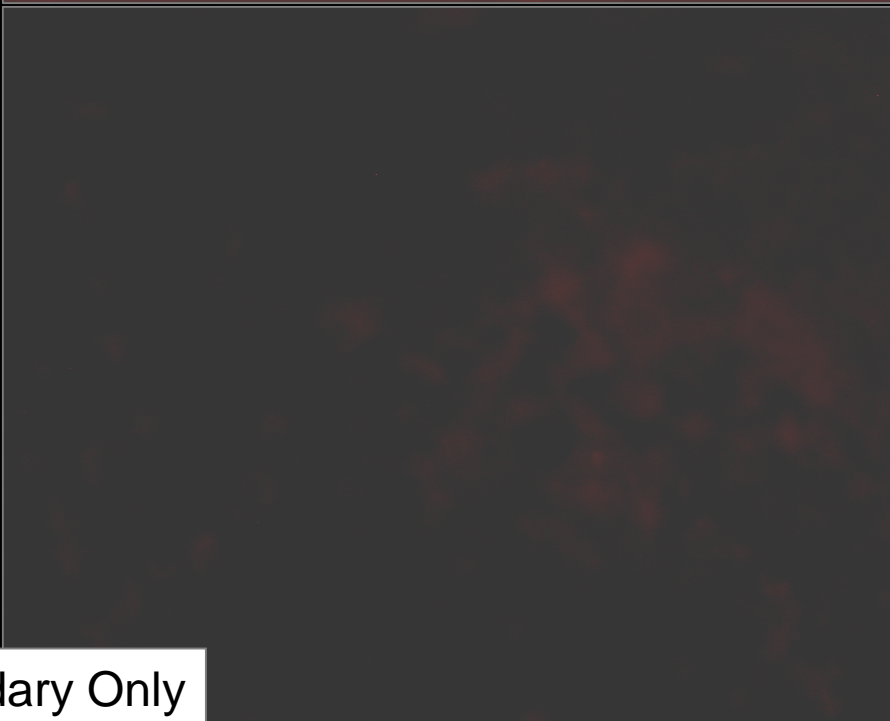
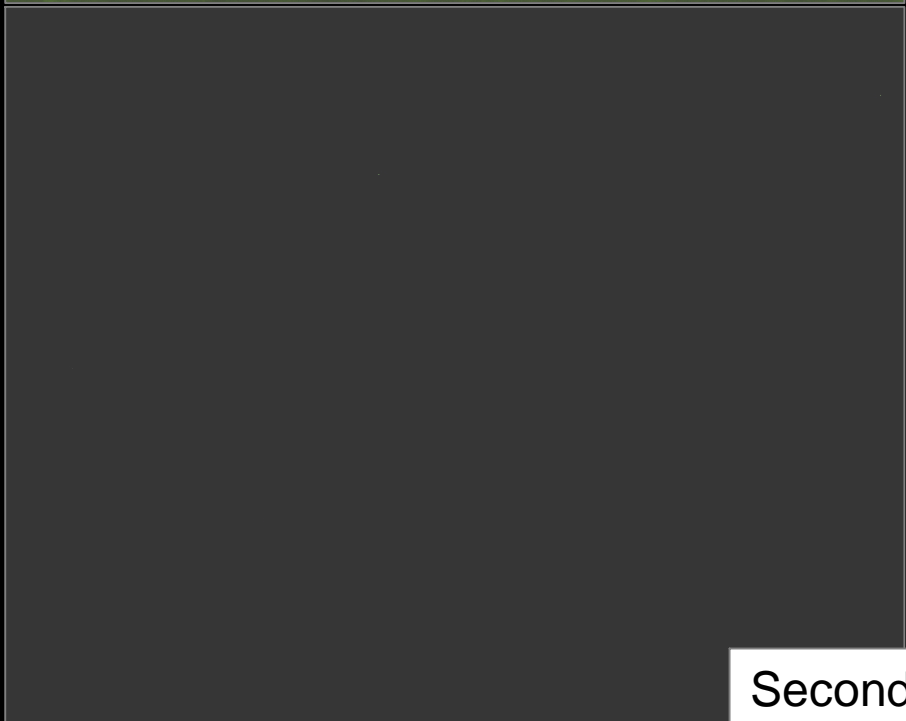
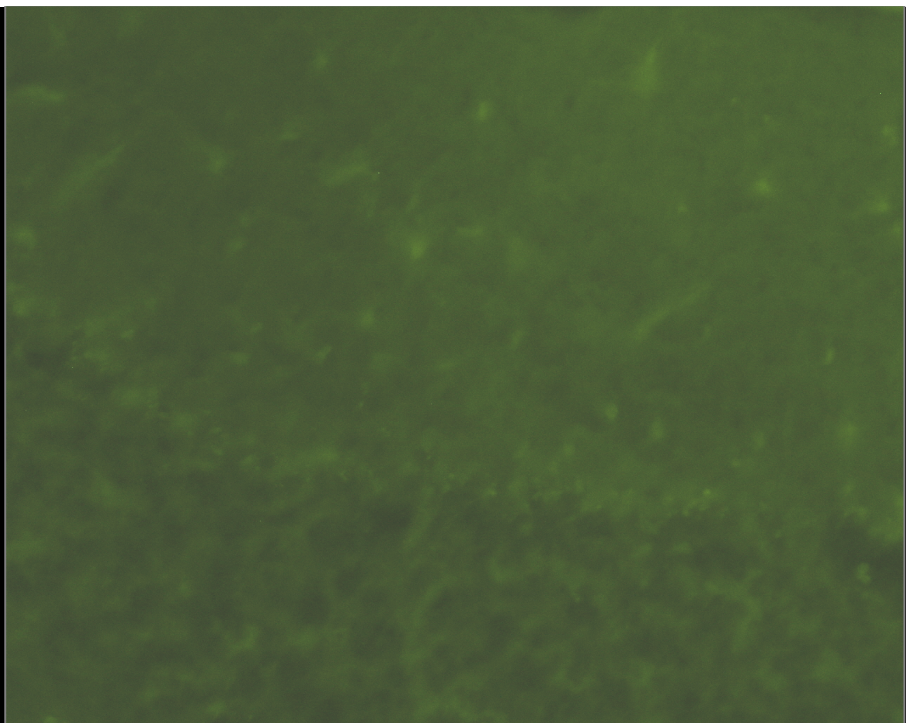
Solutions:

- Permeabilization & blocking buffer: 0.3% Triton in 1x PBS with 5% skim milk; 0.01% Na-azide
- Wash buffer: 0.05% Triton in 1x PBS with 1% skim milk
- AB solution: 0.05% Triton in 1x PBS with 5% skim milk

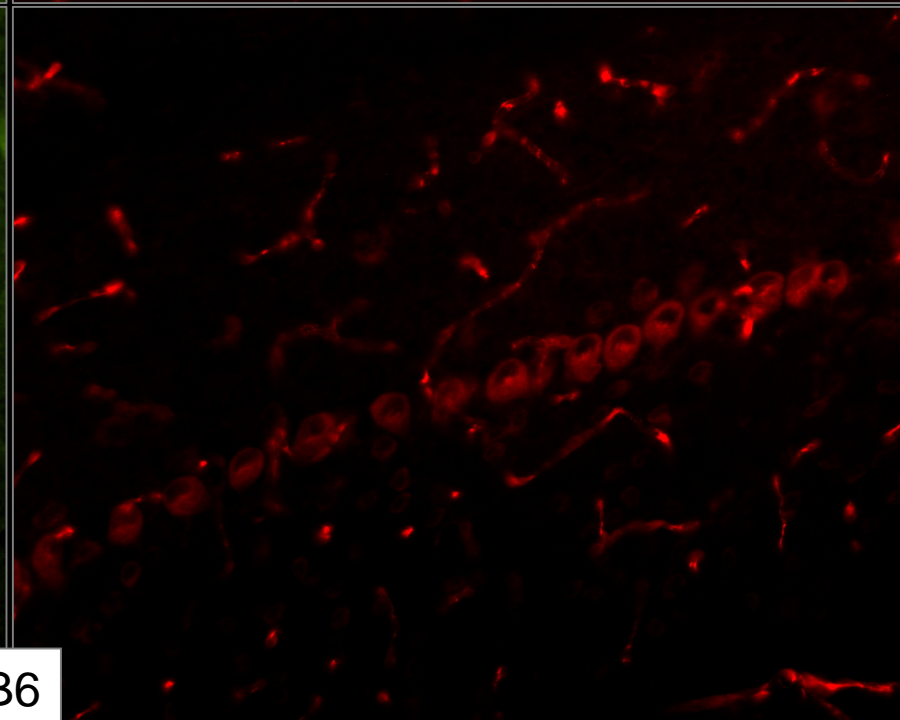
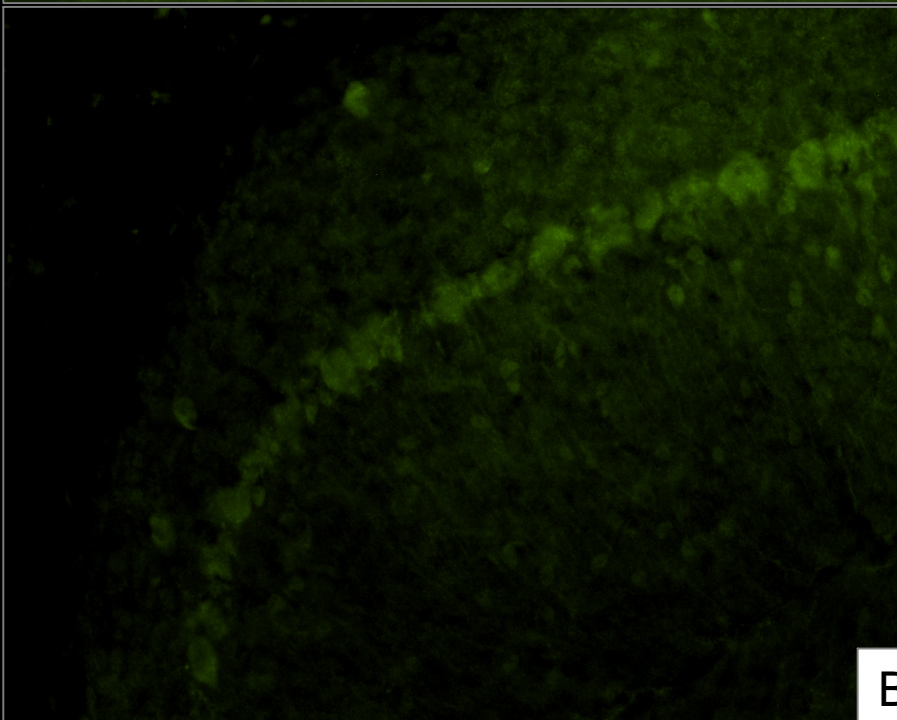
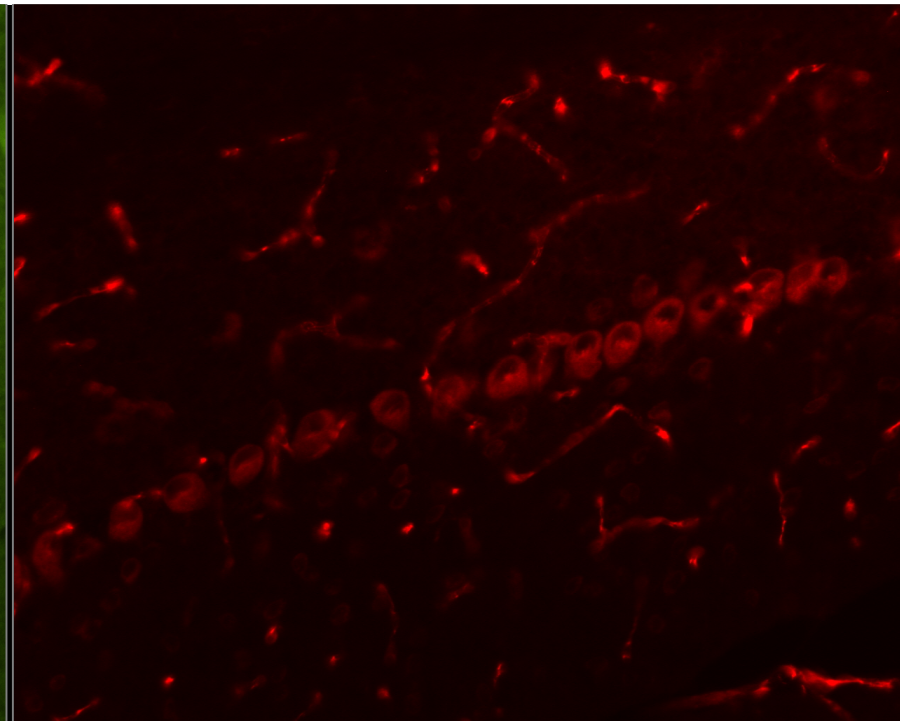
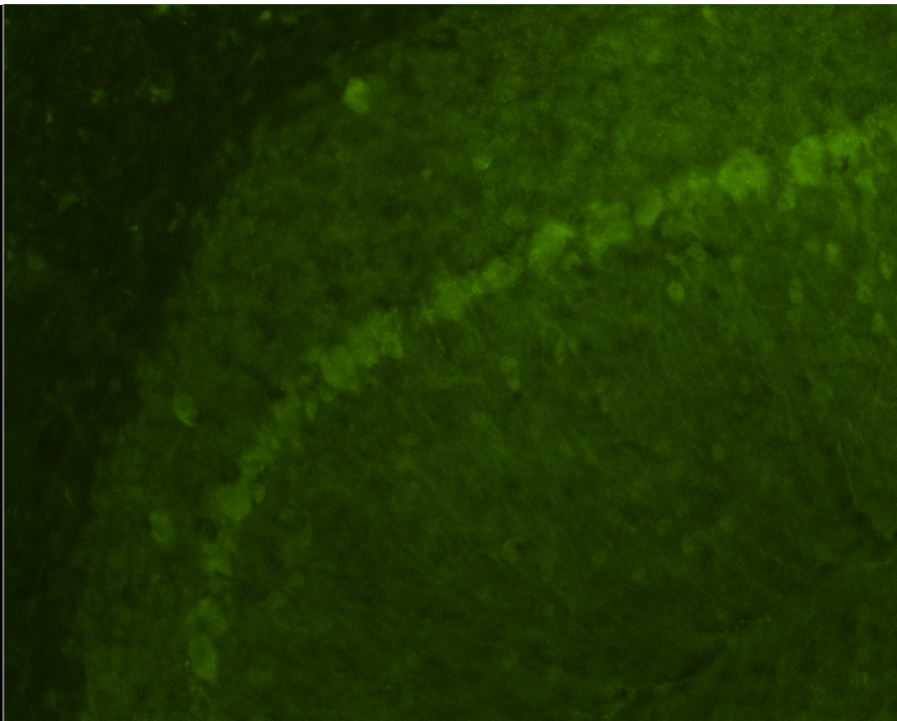
Camera

- Camera settings optimized
 - Negative Control: secondary AB only
 - Background florescence subtracted from control
 - Positive Control: B6 WT (euthanized in Dec but sliced and stained with KO's)

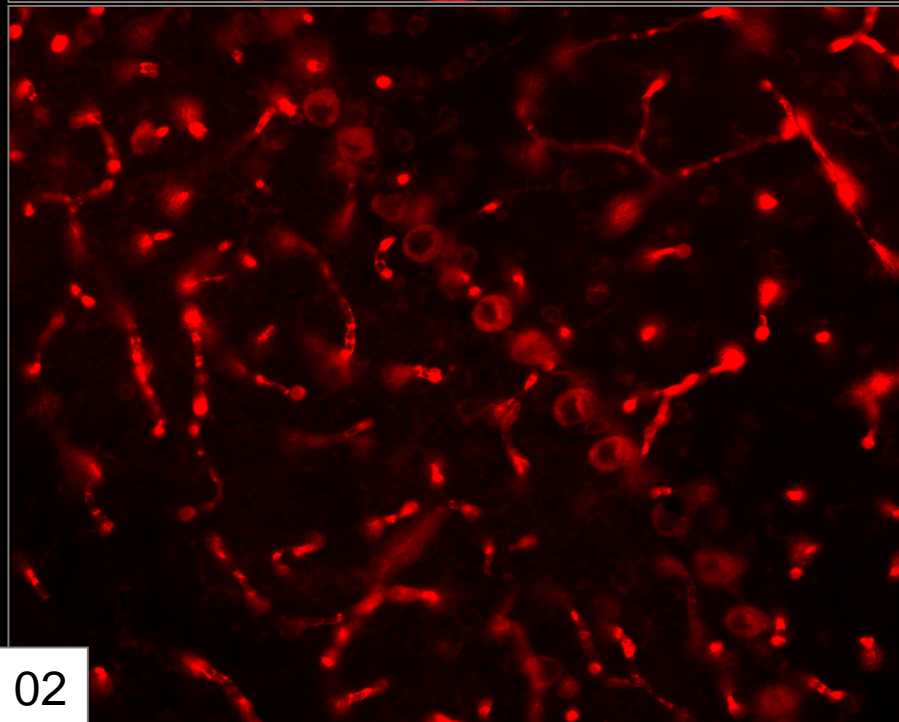
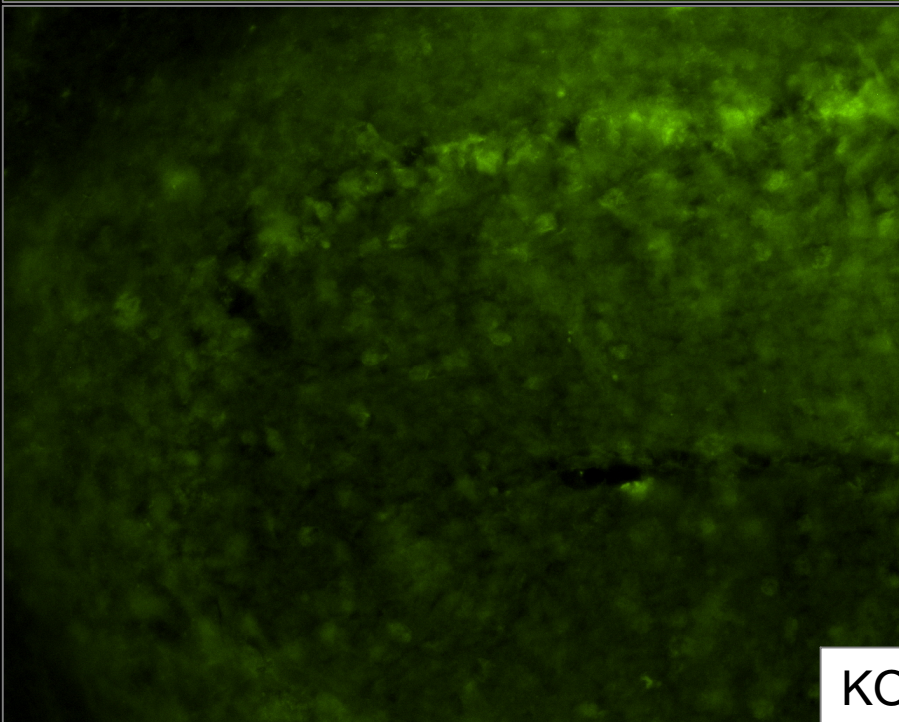
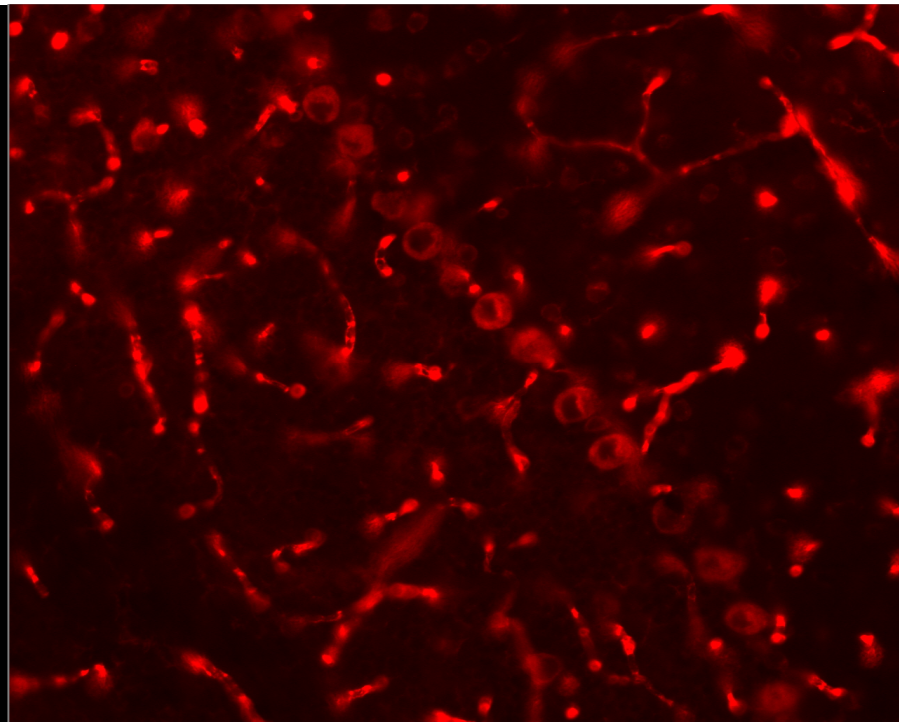
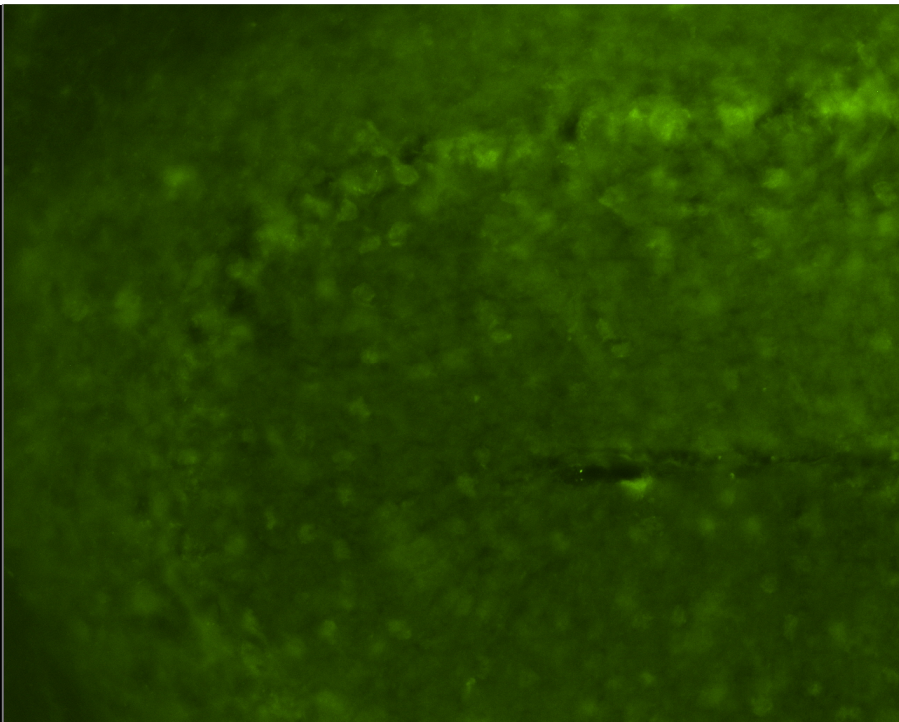




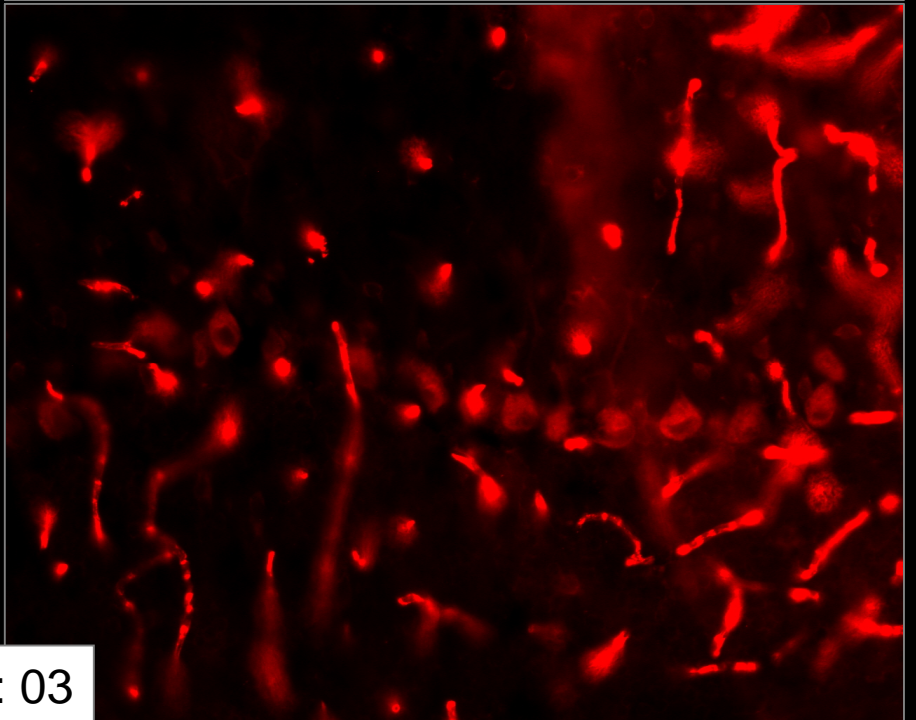
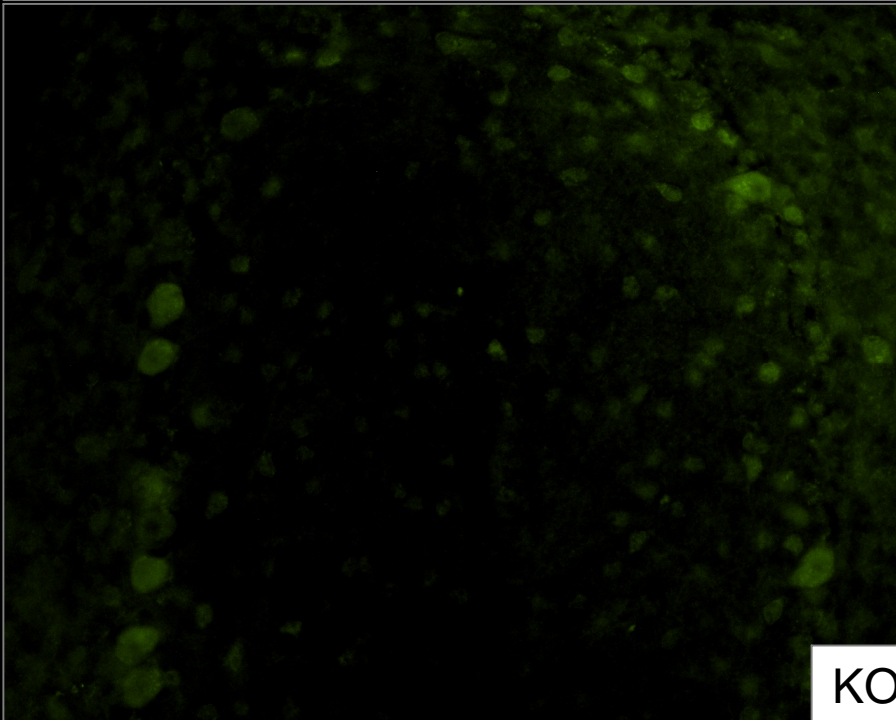
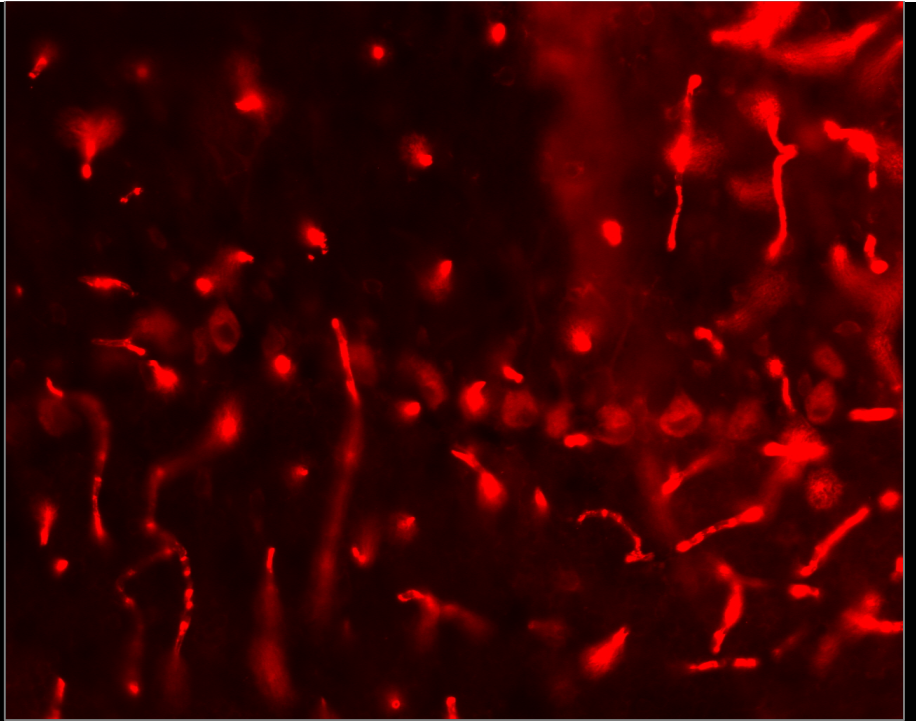
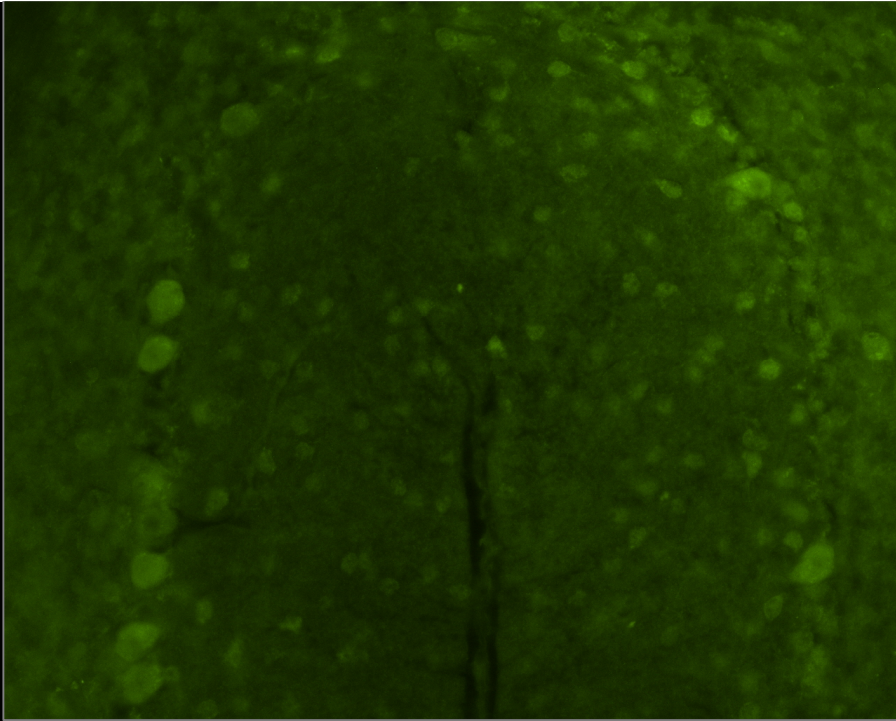
Secondary Only



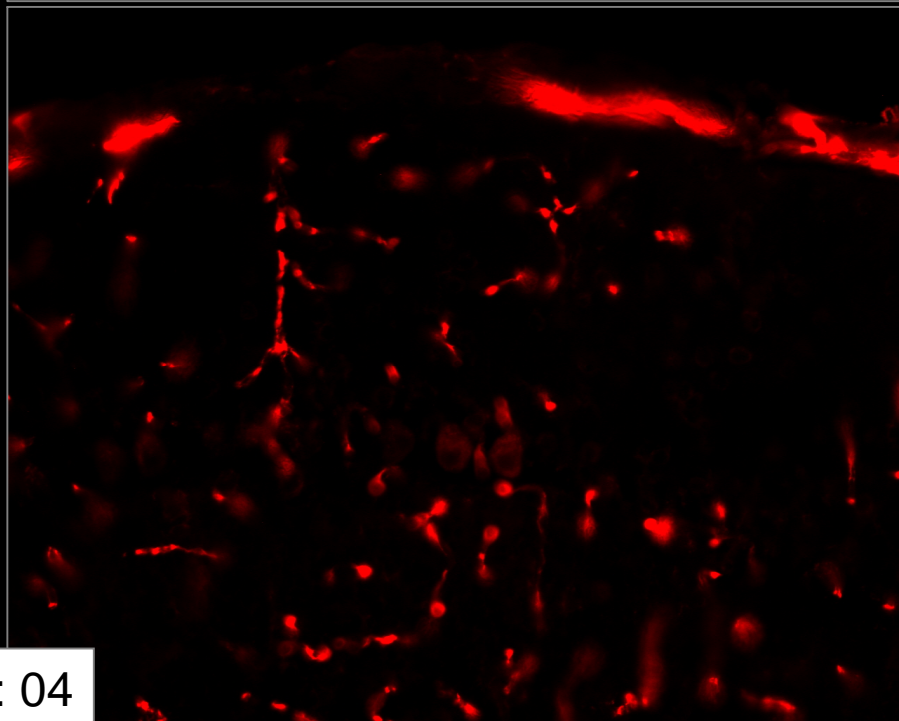
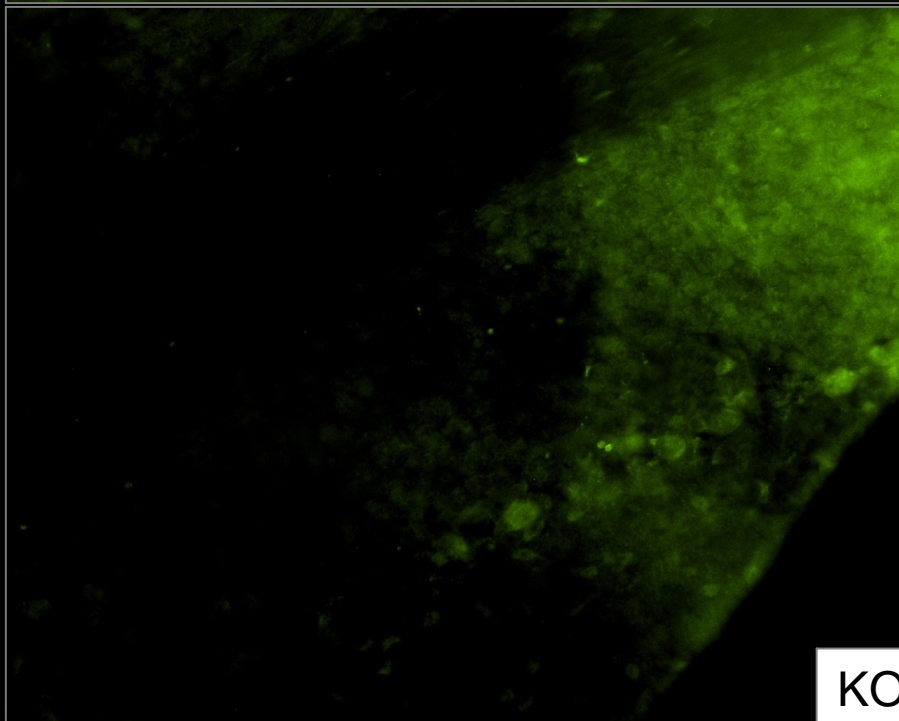
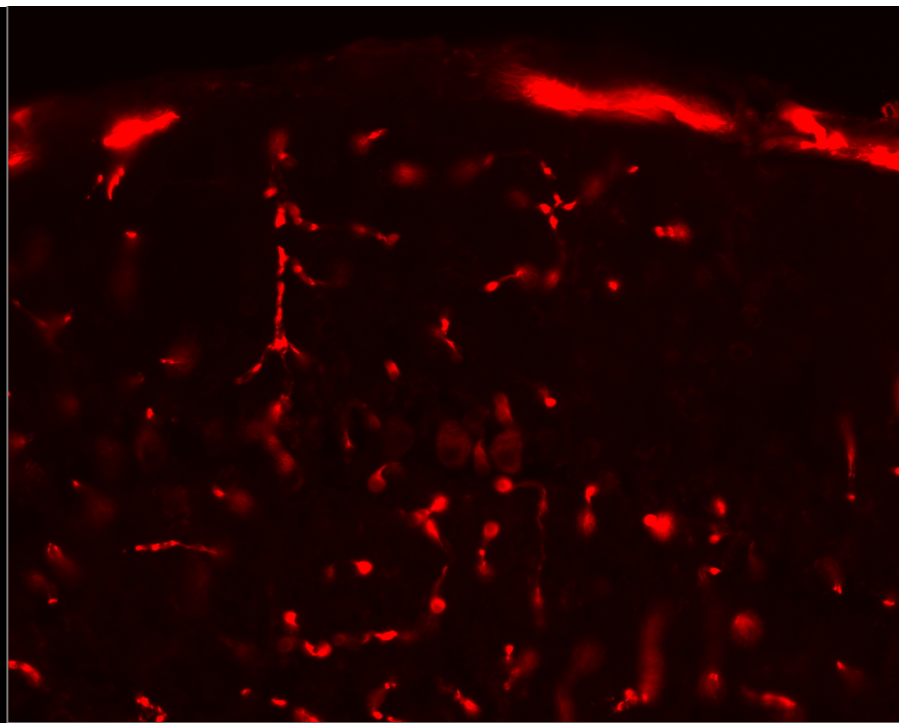
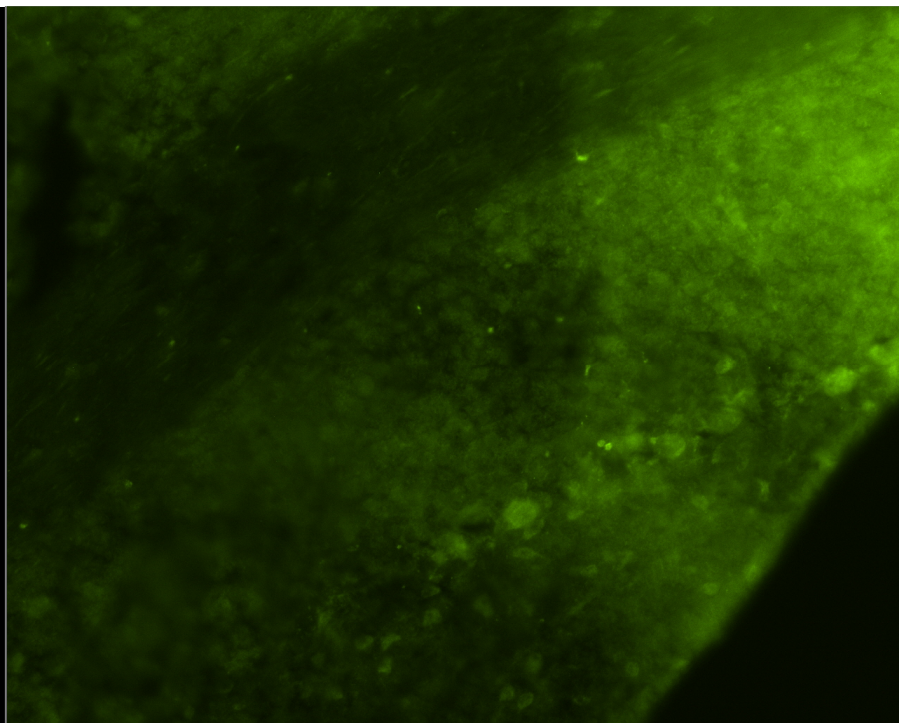
B6



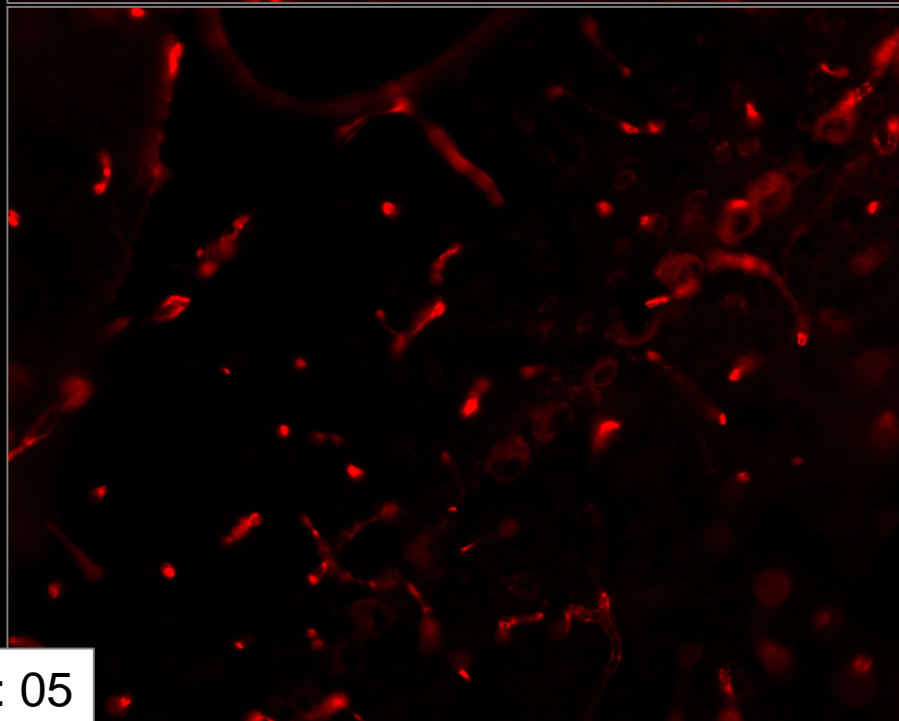
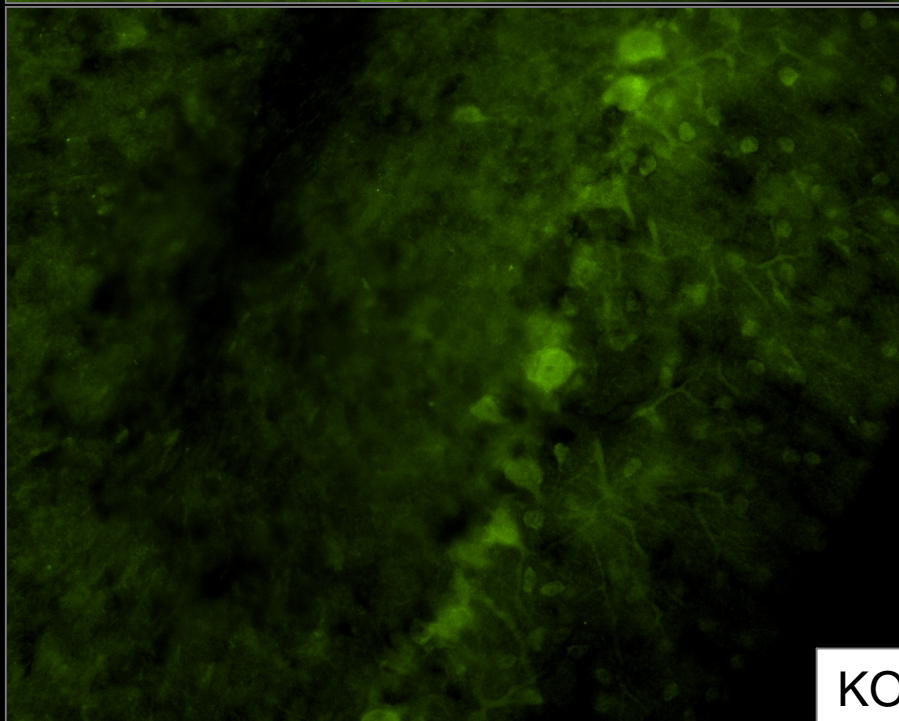
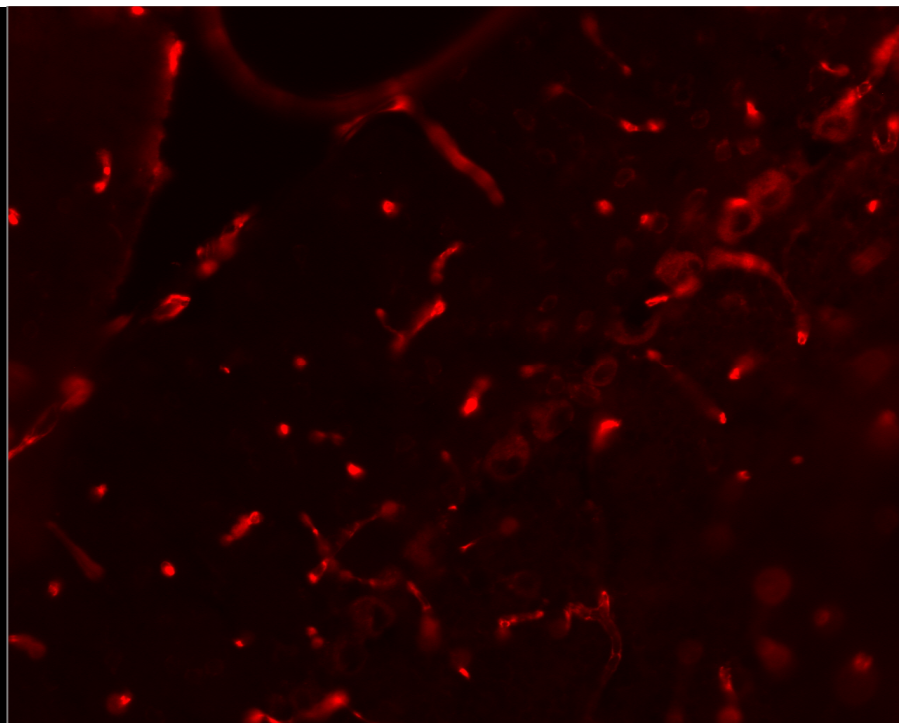
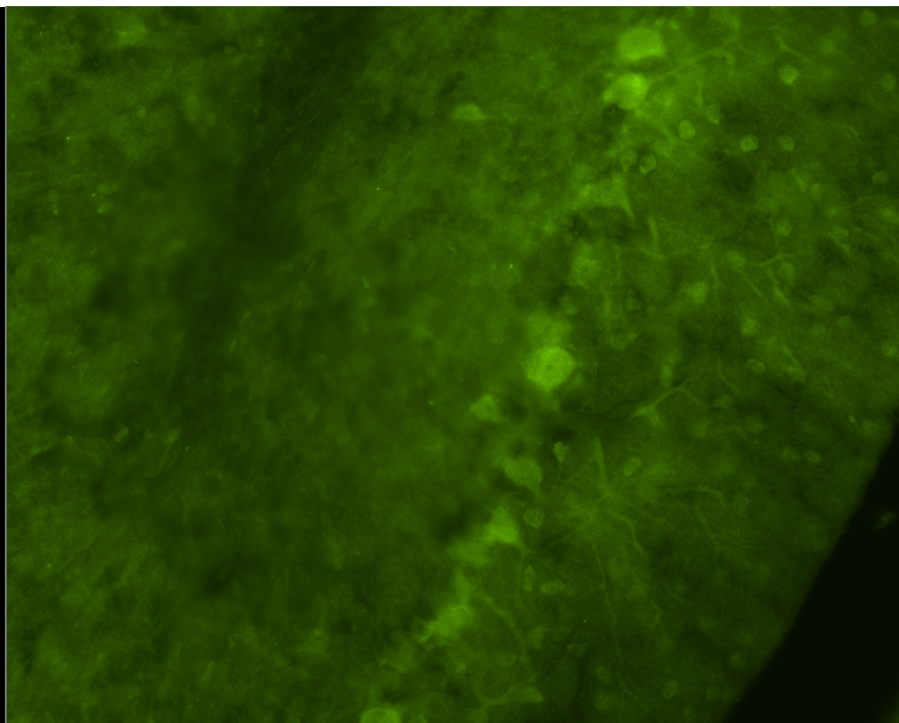
KO: 02



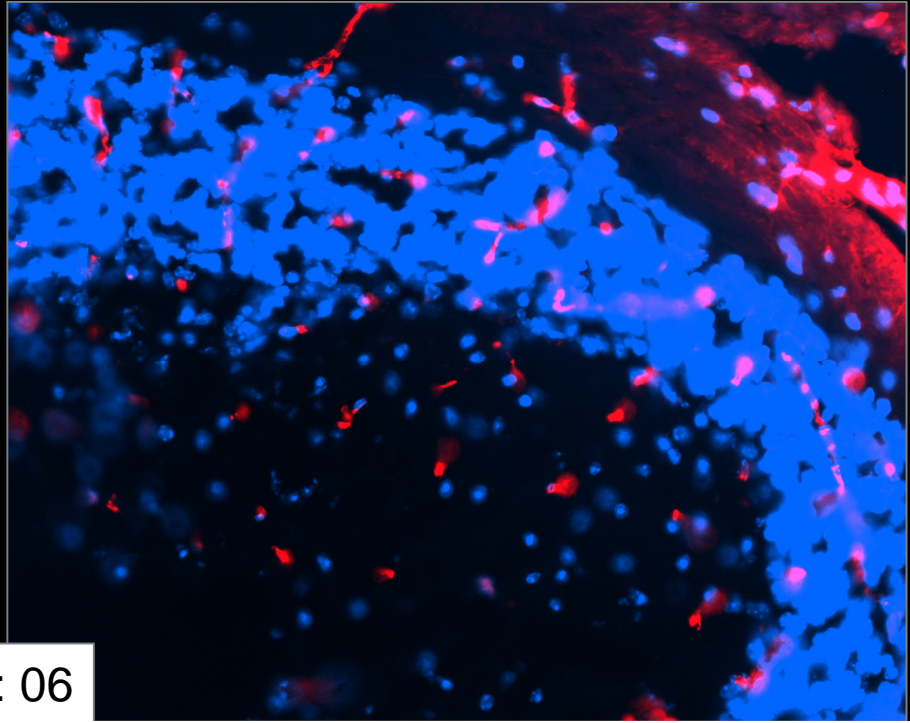
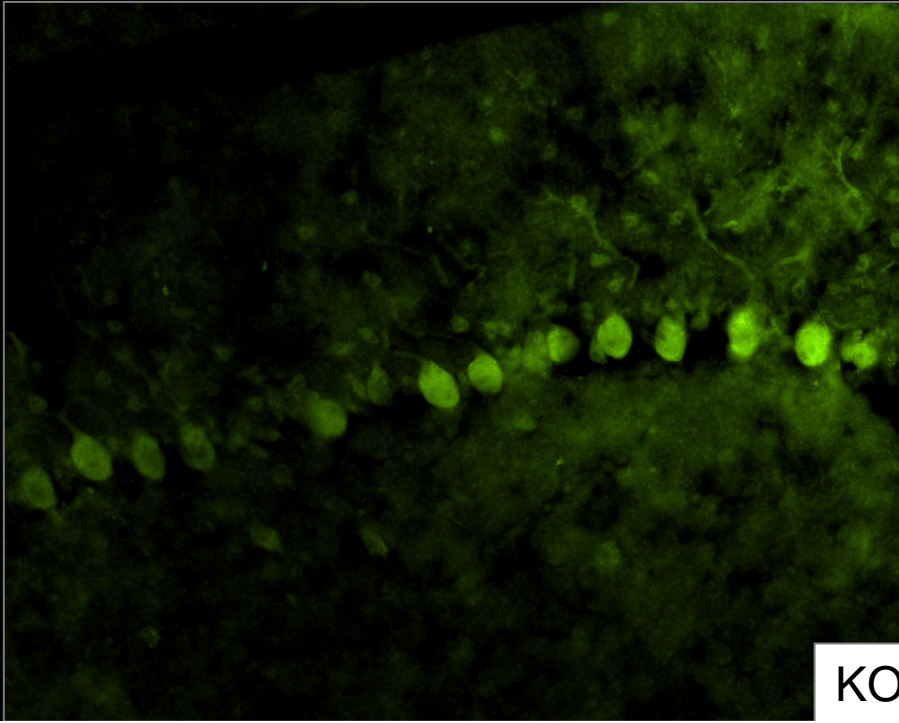
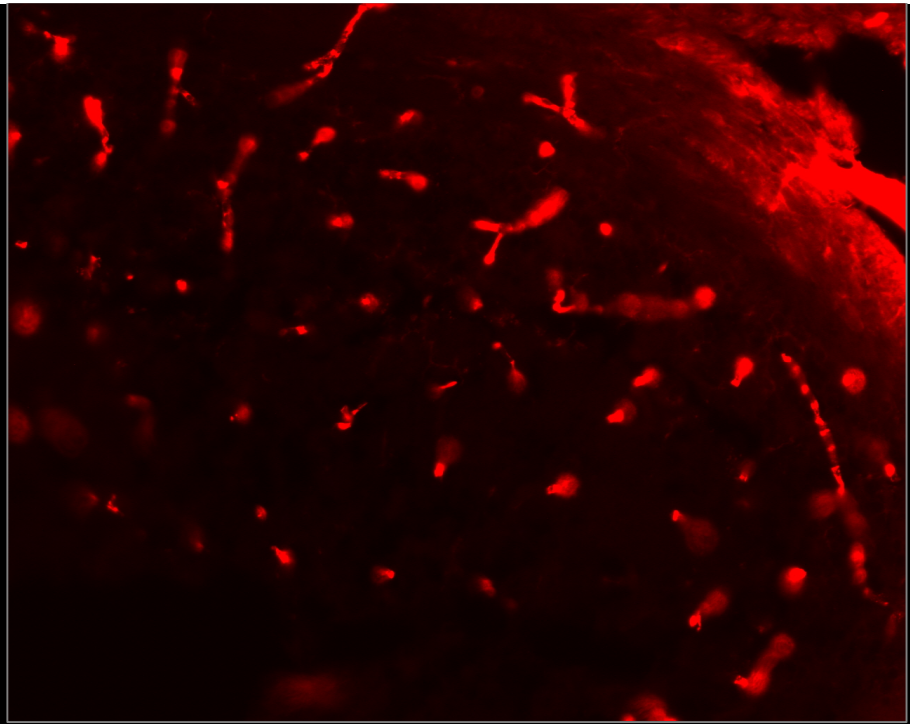
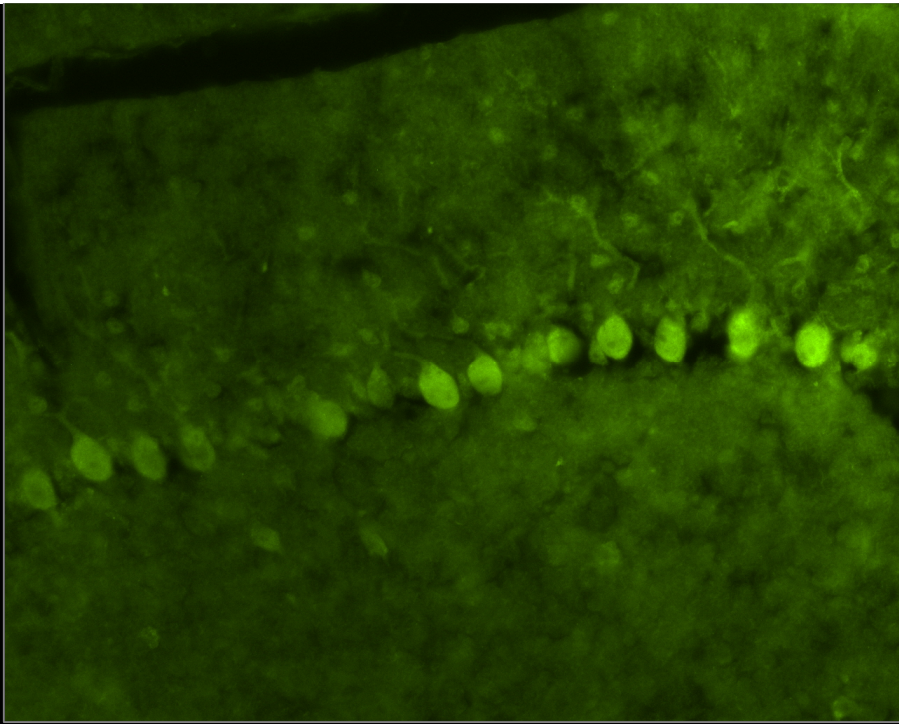
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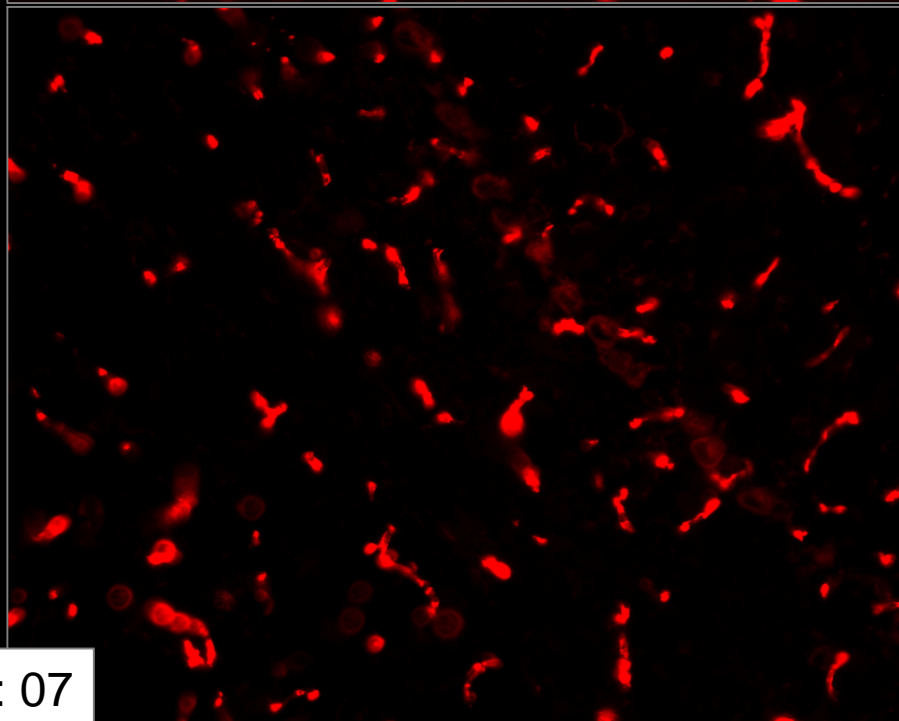
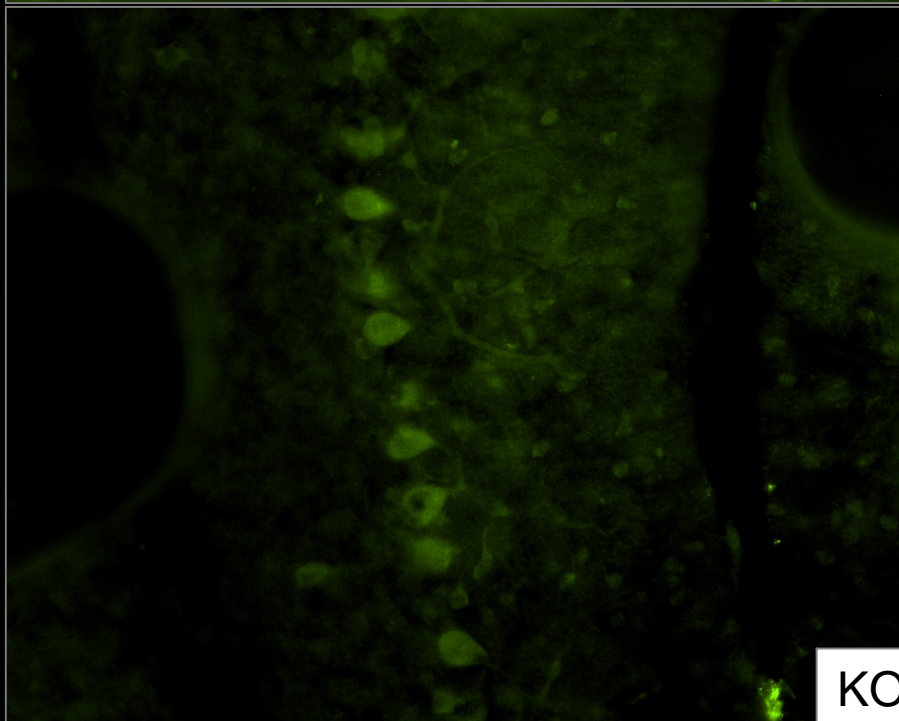
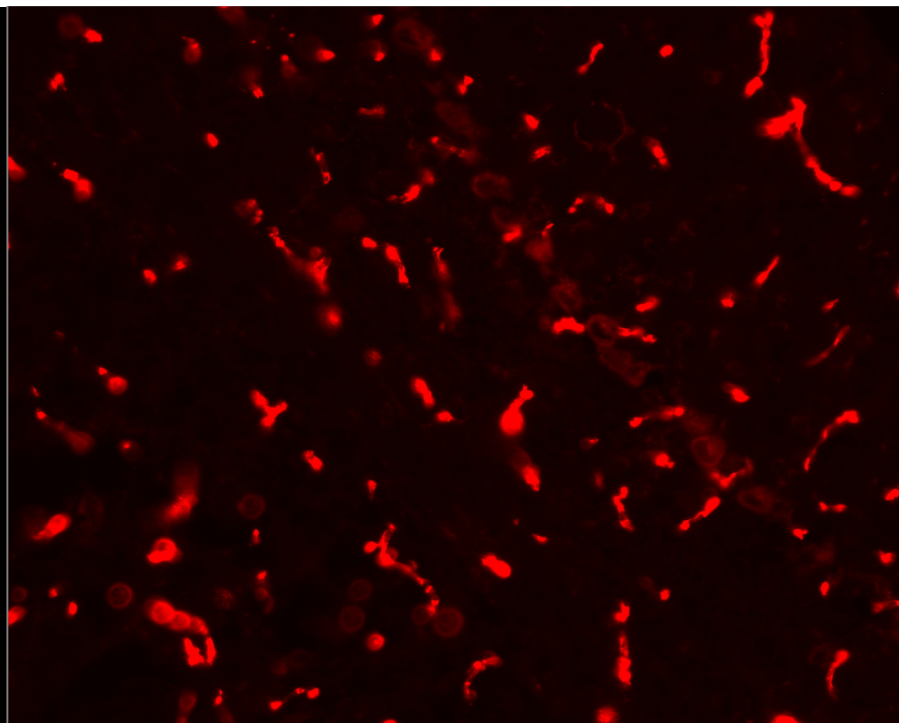
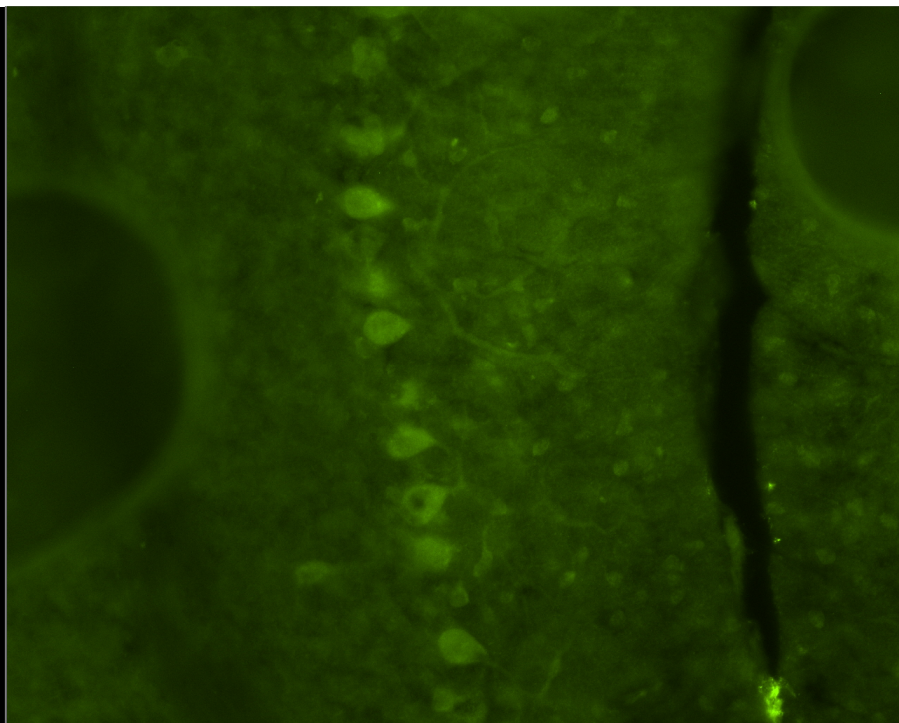
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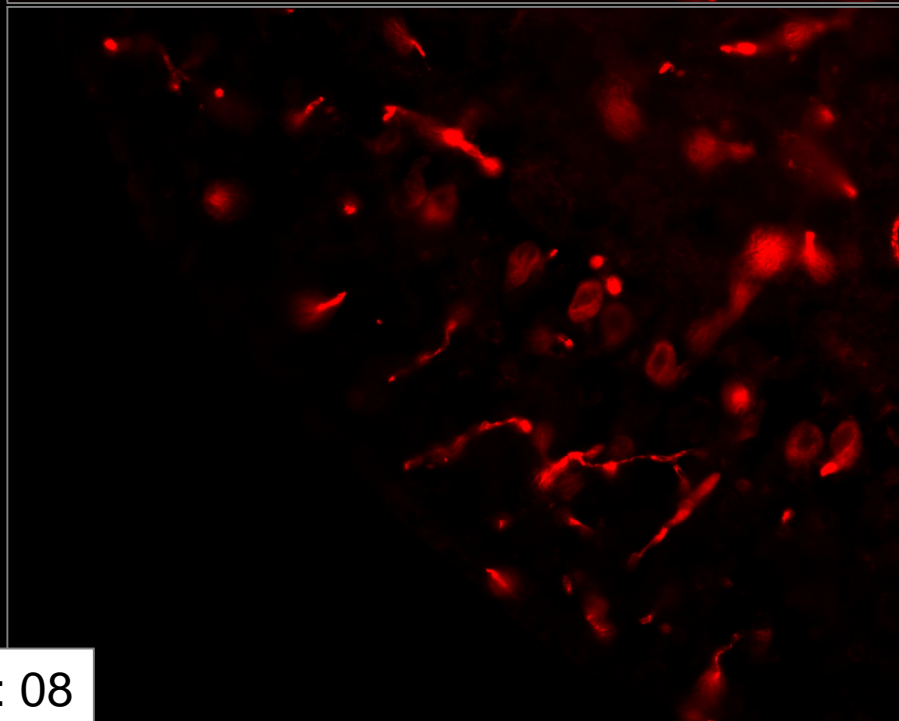
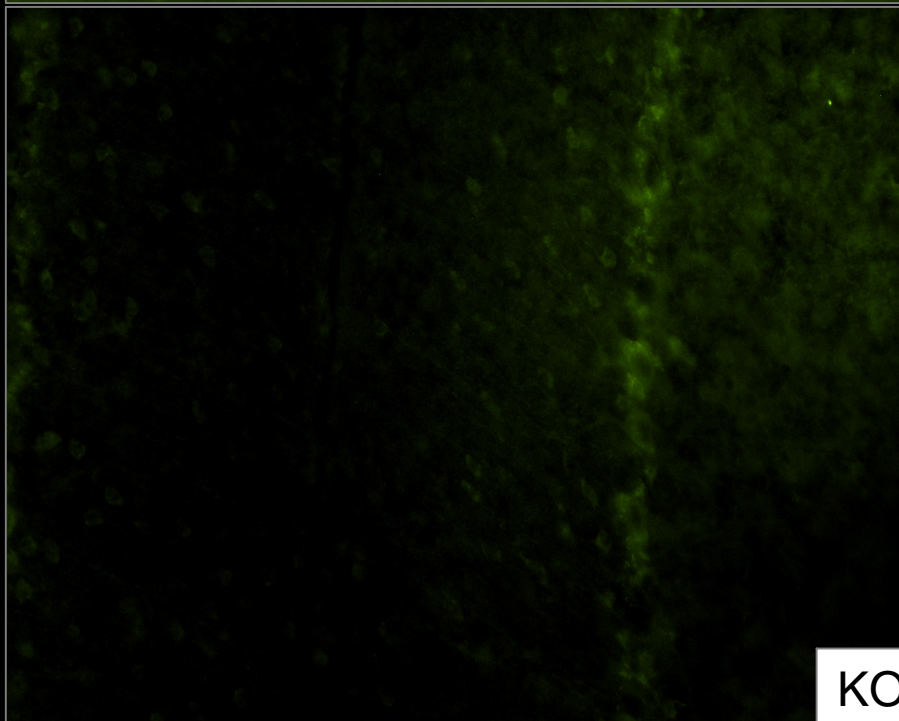
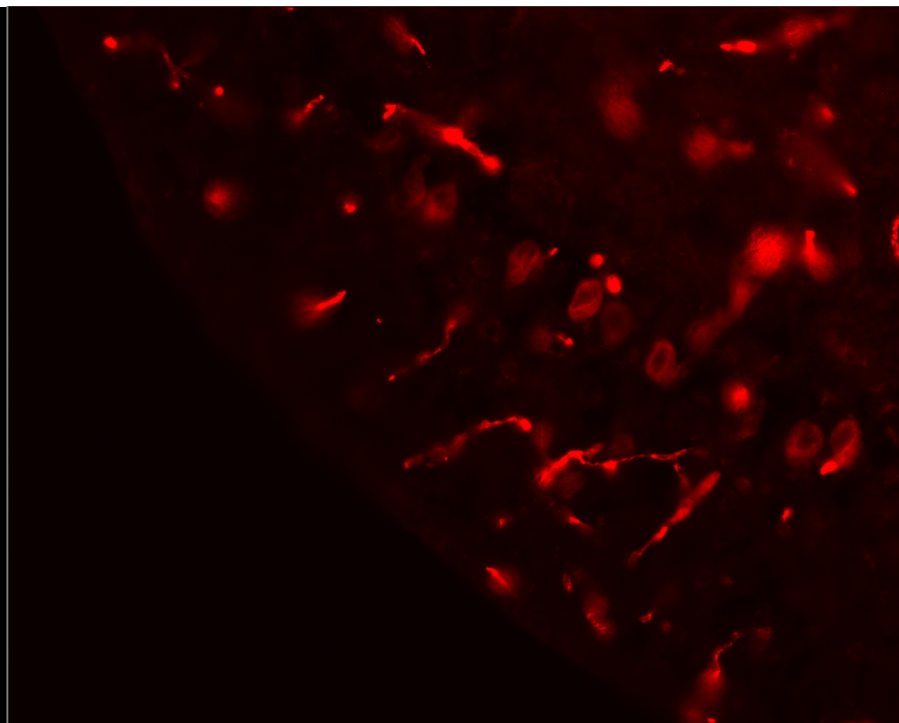
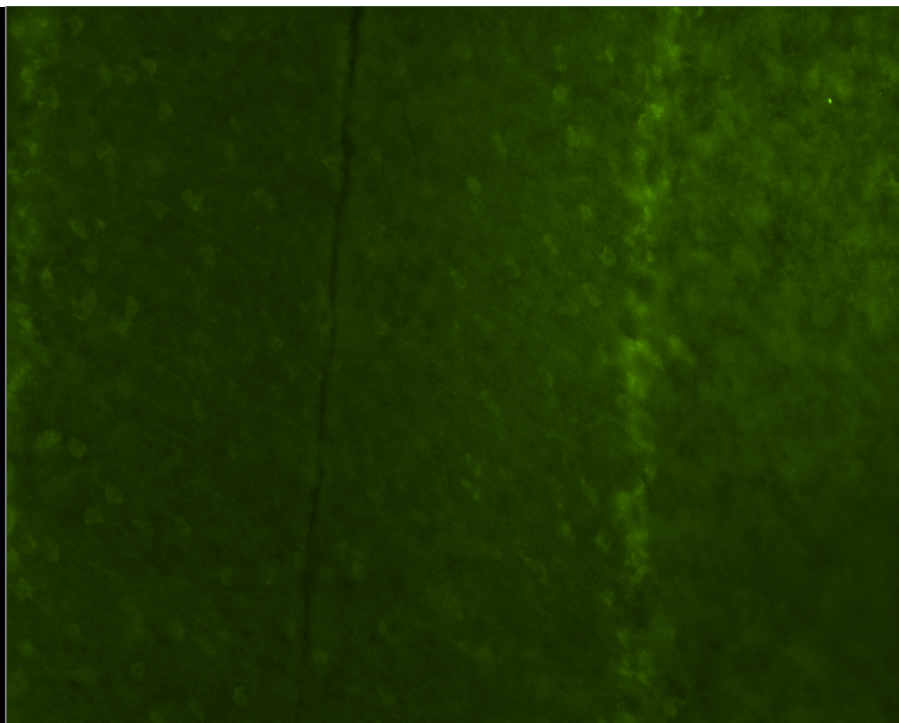
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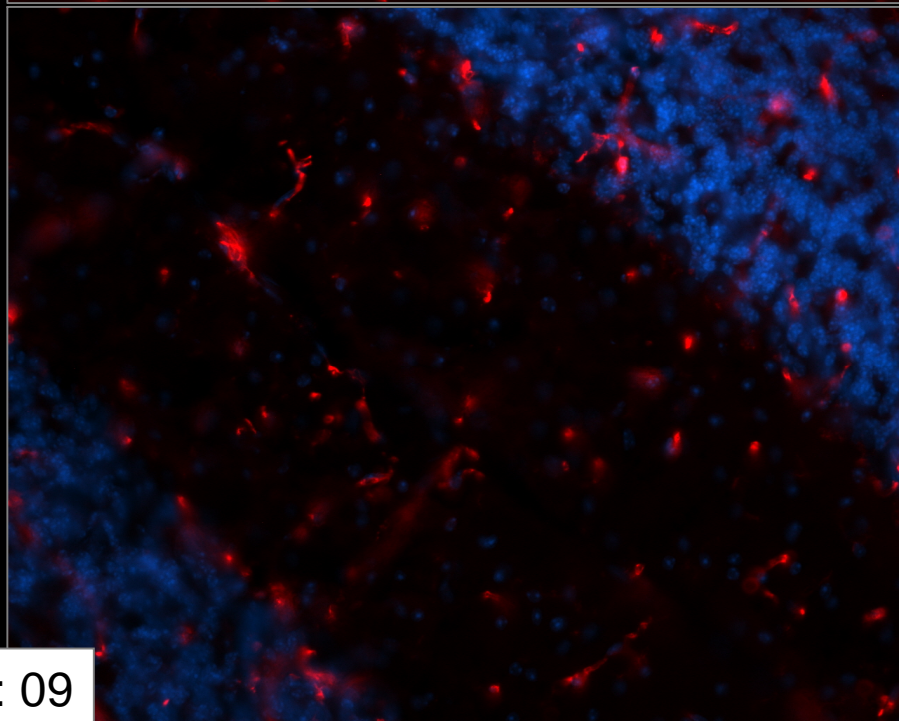
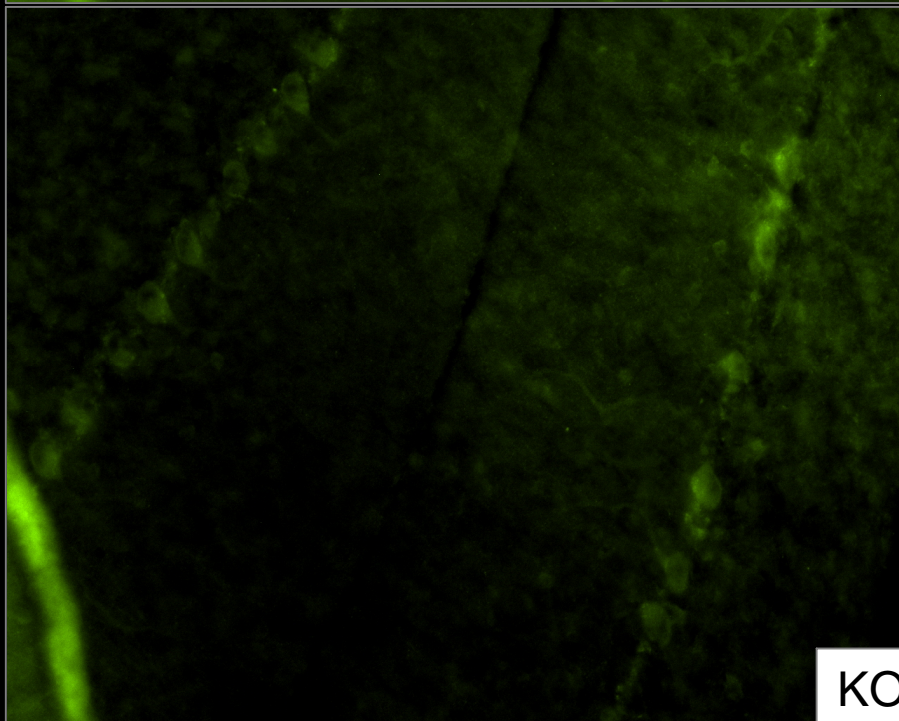
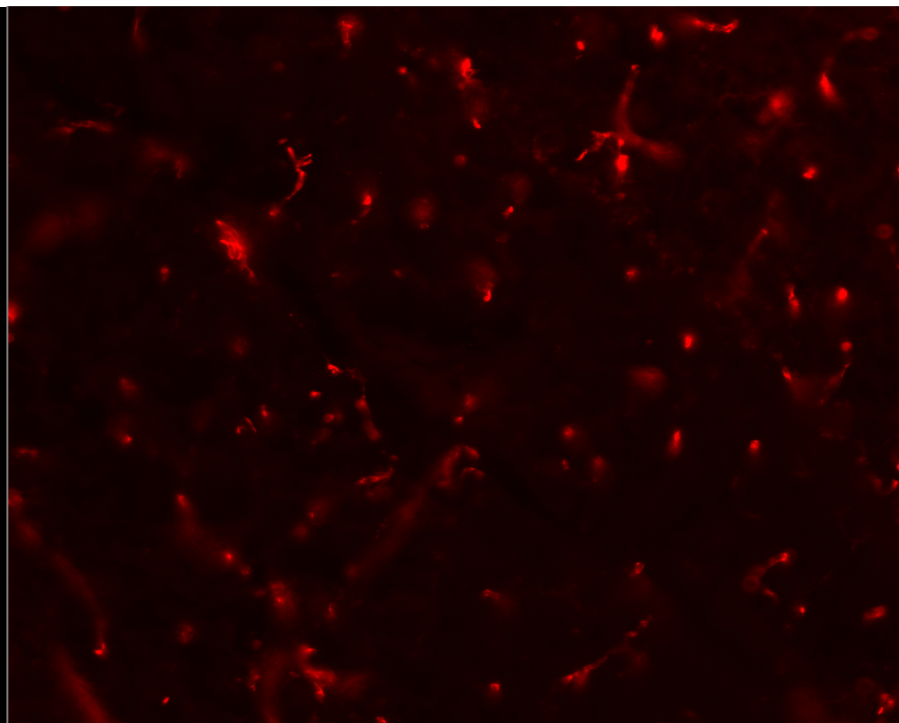
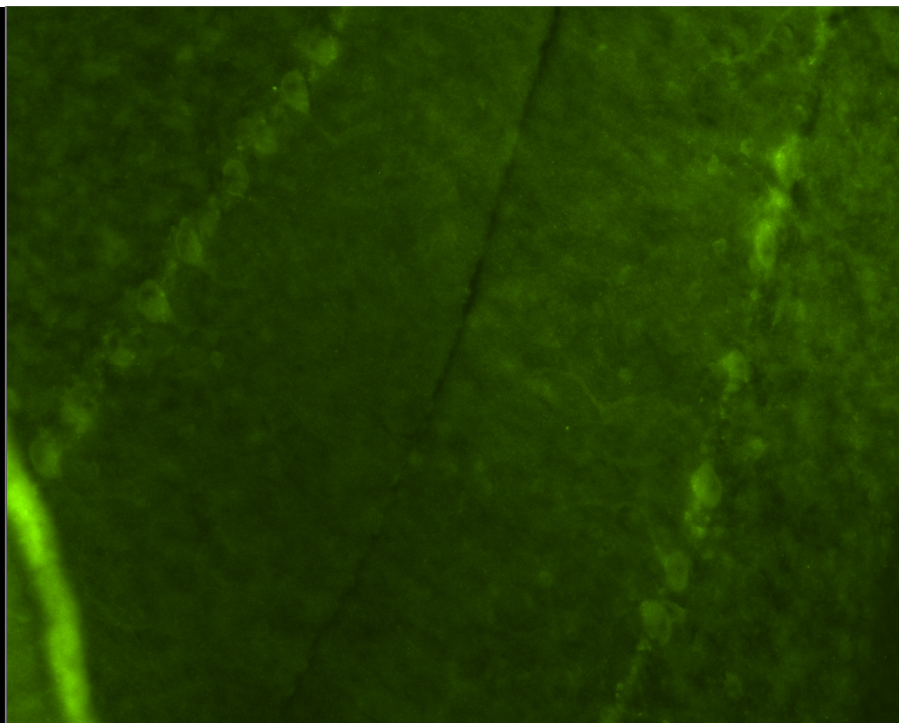
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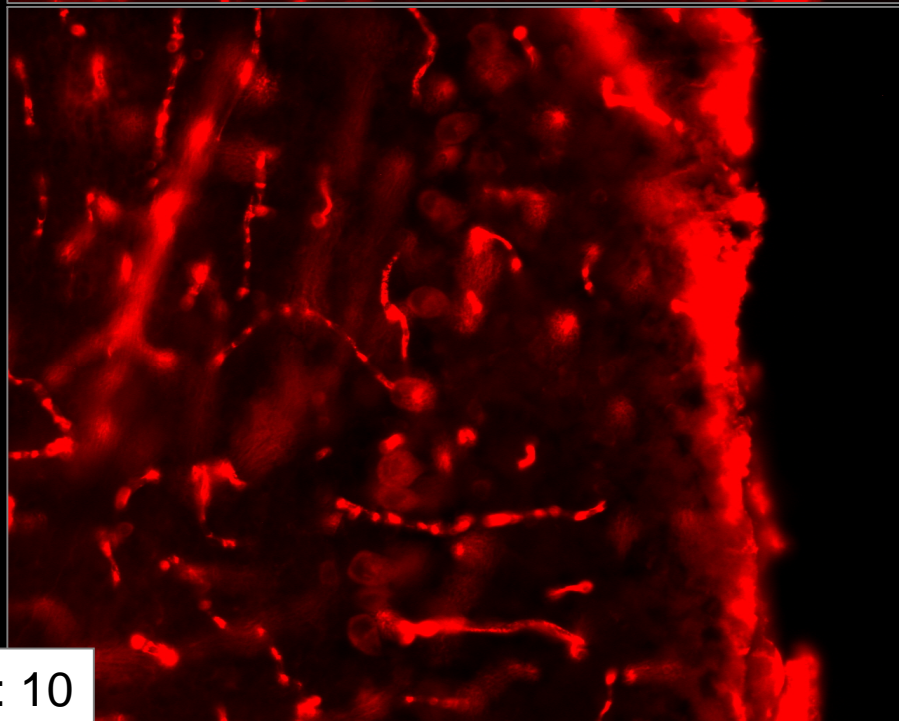
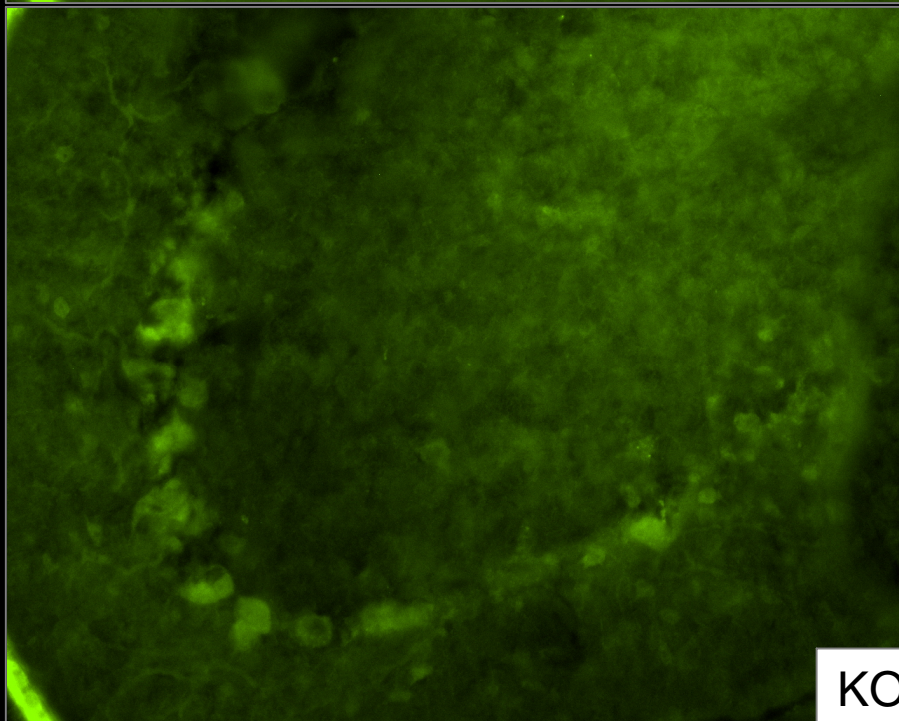
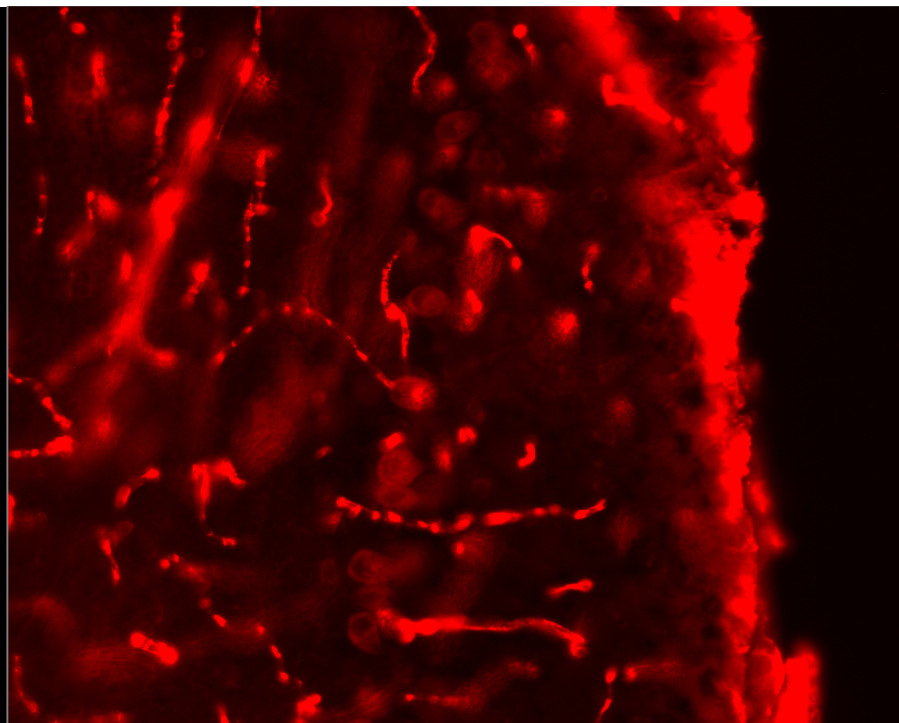
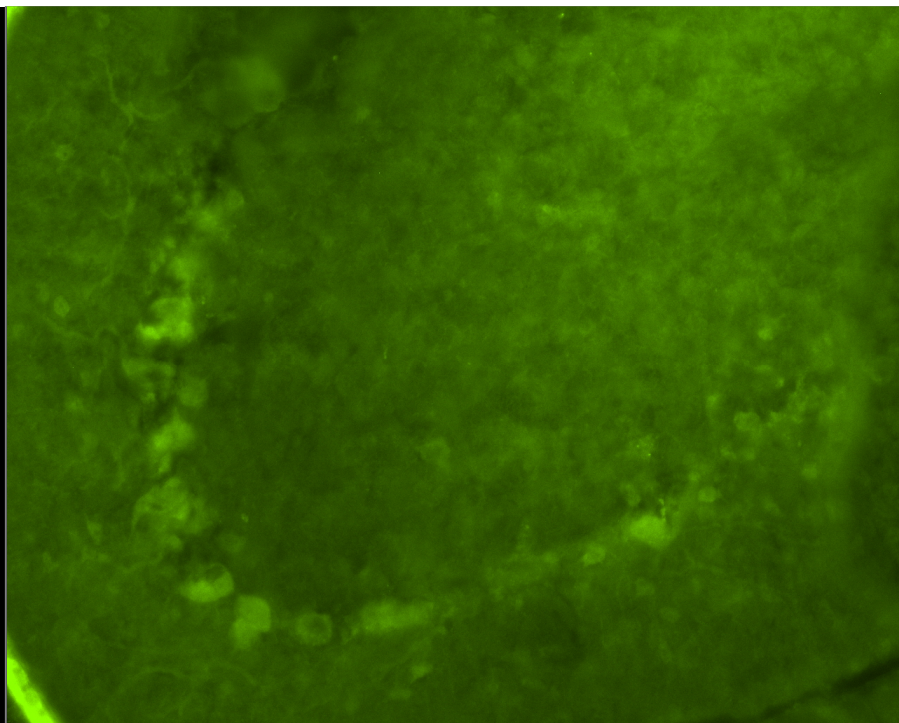
KO: 07



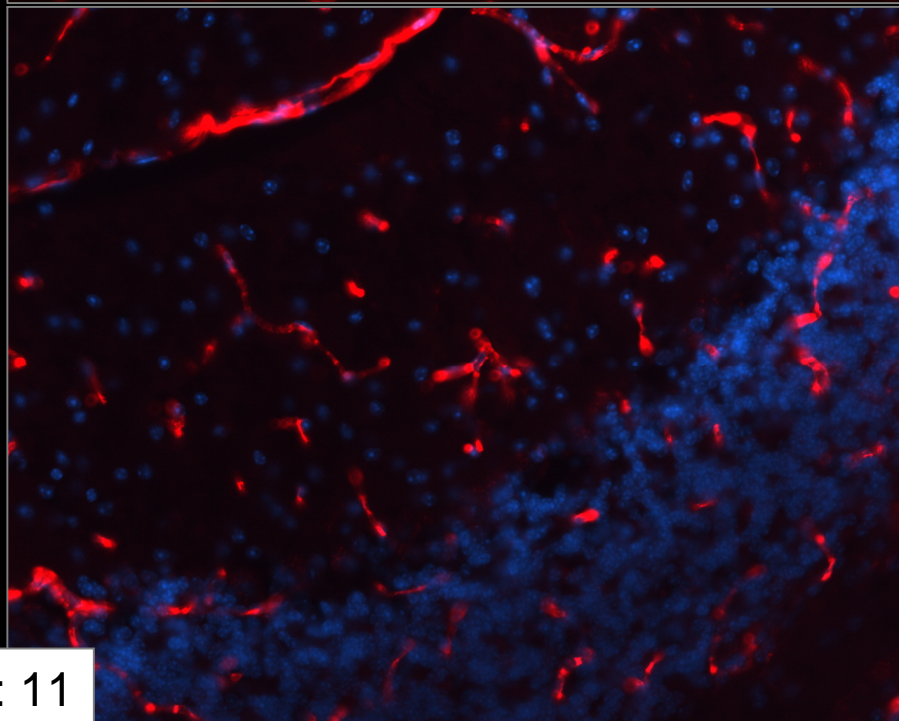
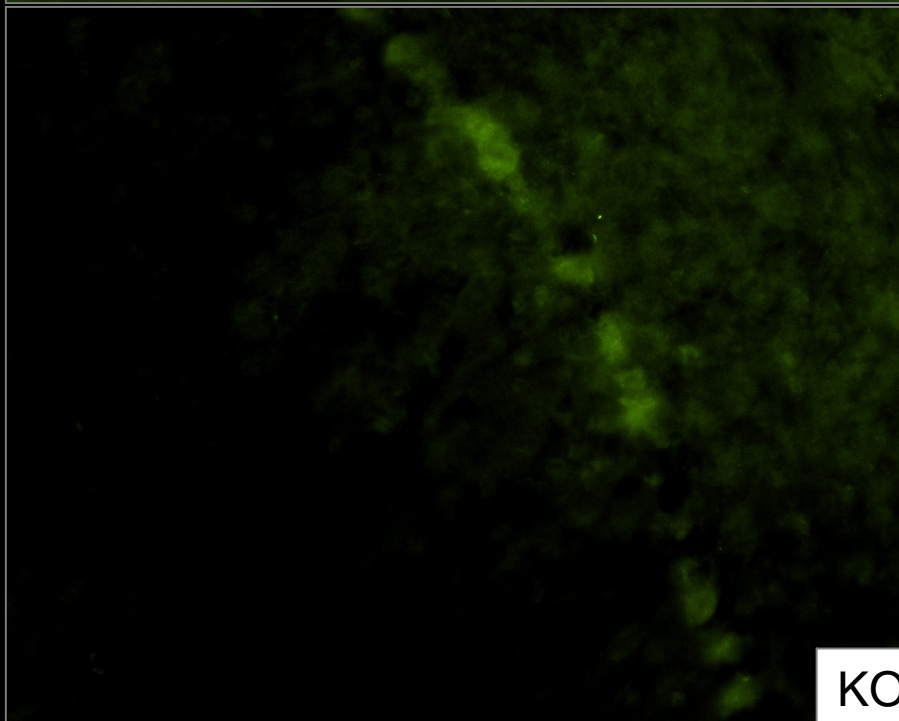
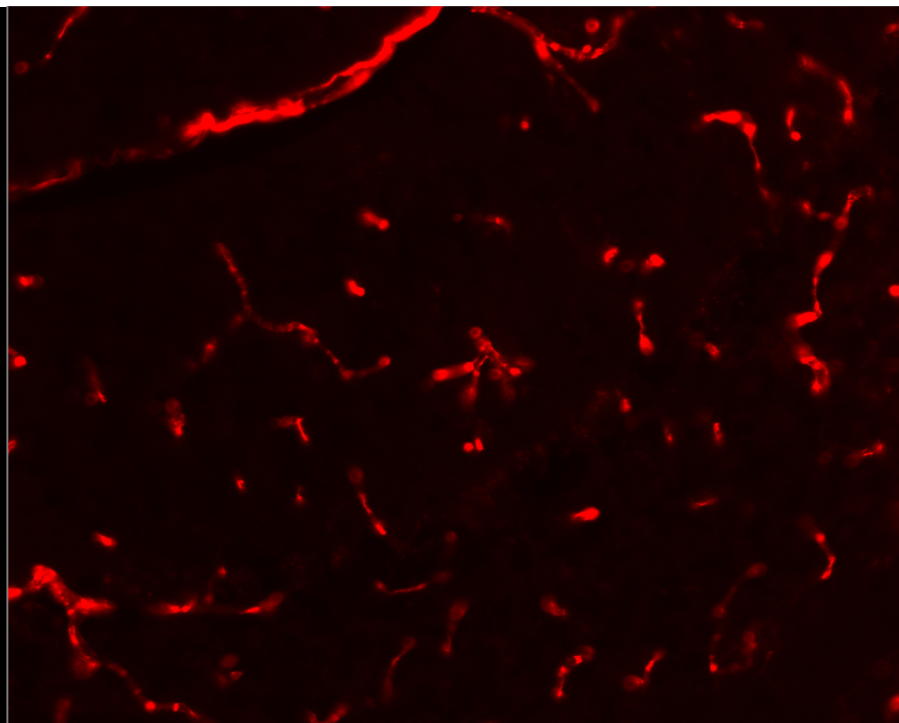
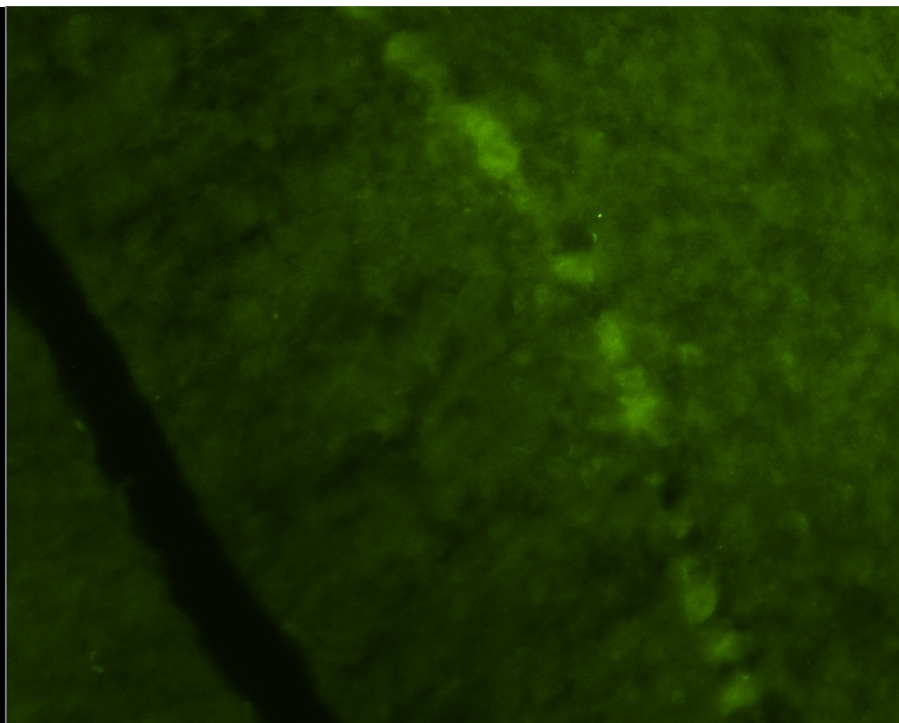
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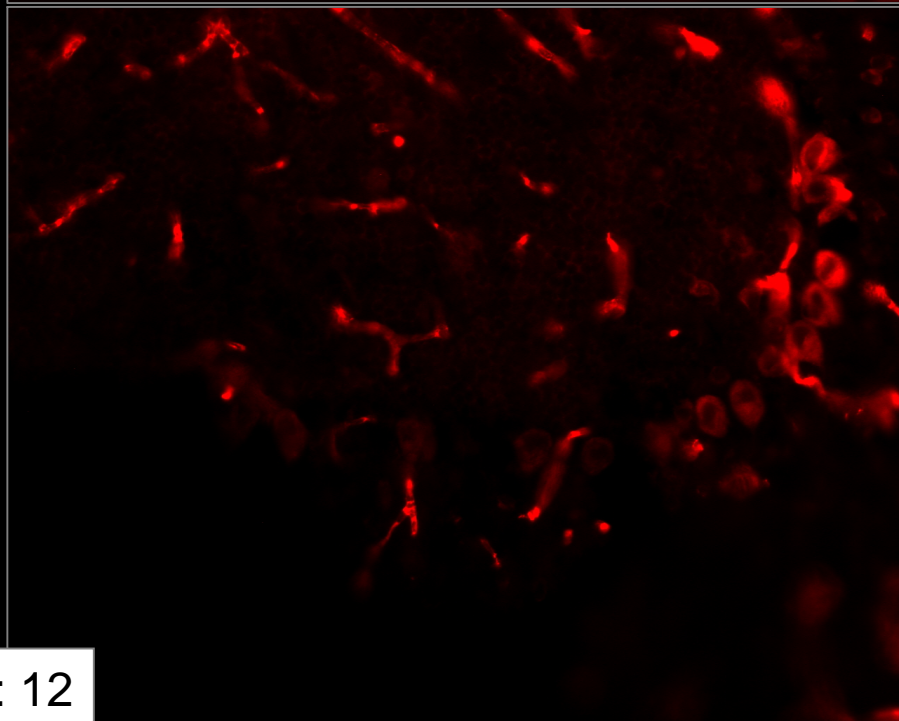
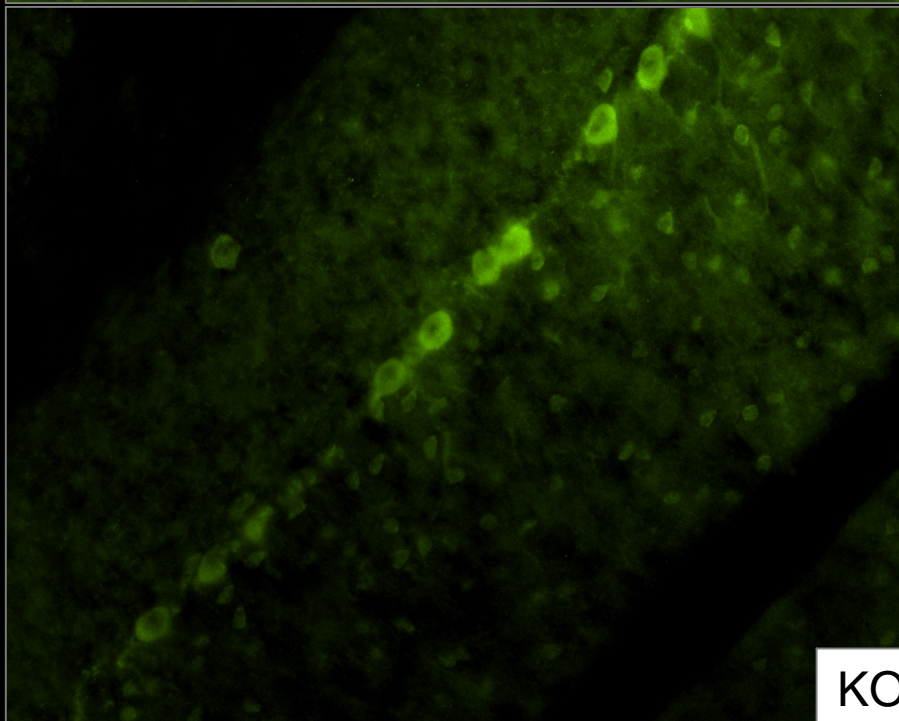
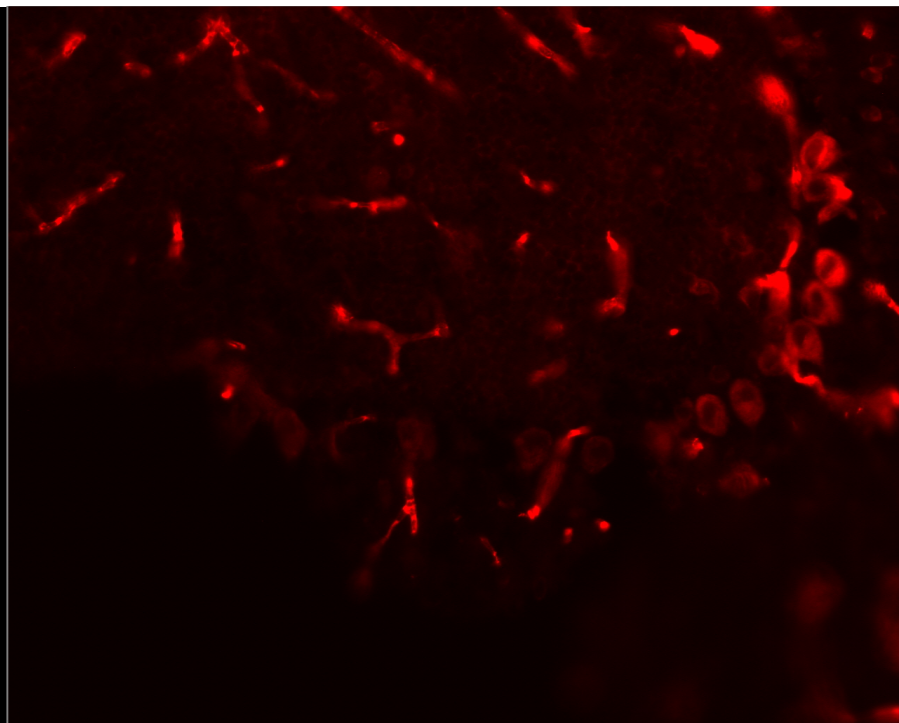
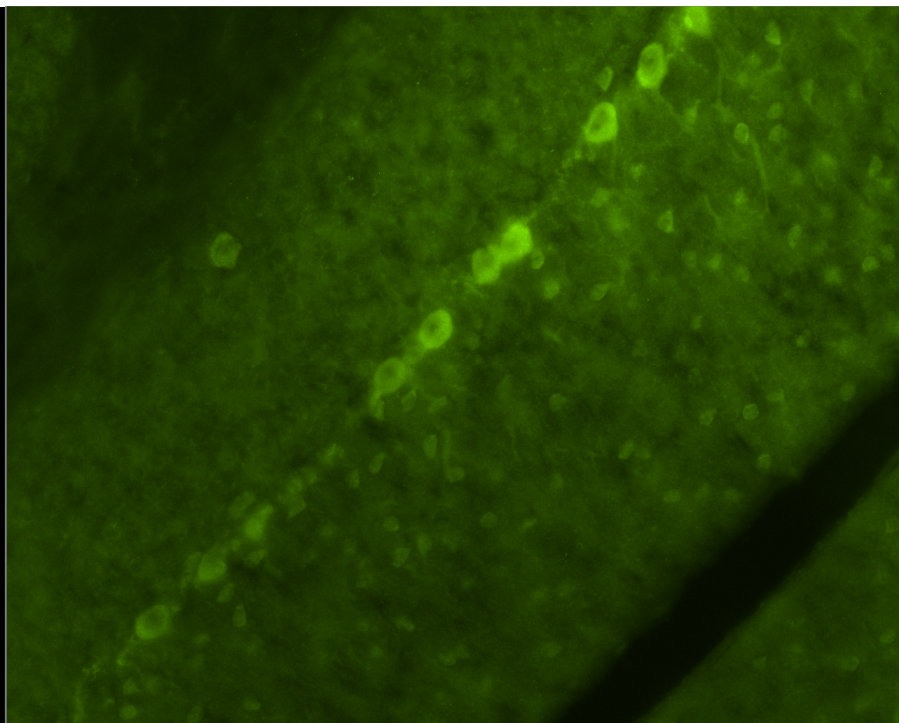
KO: 09



KO: 10



KO: 11



KO: 12