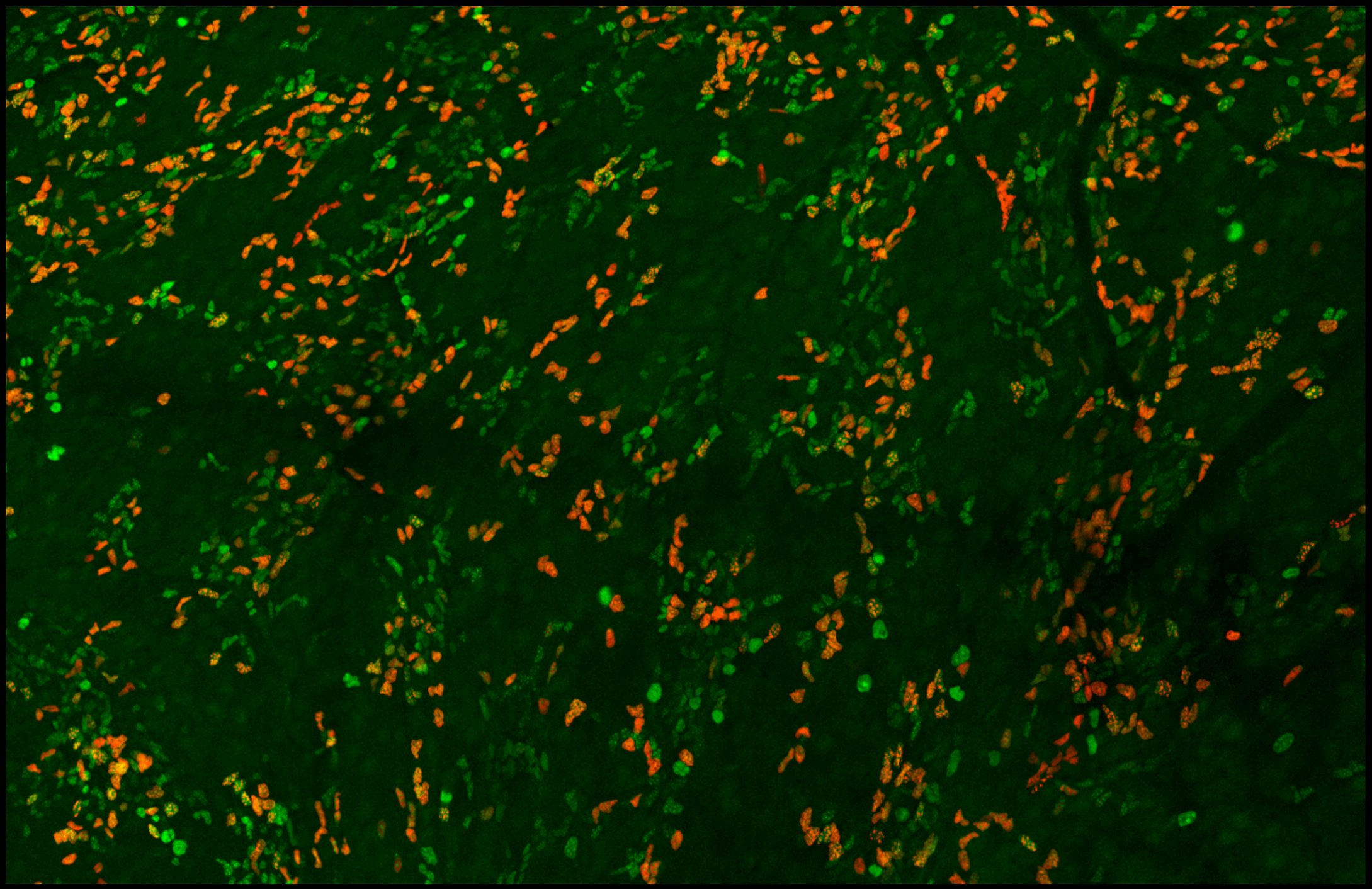
- Whole mount immunofluorescence

- Brains from mice perfused with 2% PLP fixative made with fresh formaldehyde dissolved from paraformaldehyde powder

Rat anti-KI-67 monoclonal antibody test
50 mg/kg EdU 40 min chase
KI-67 EdU



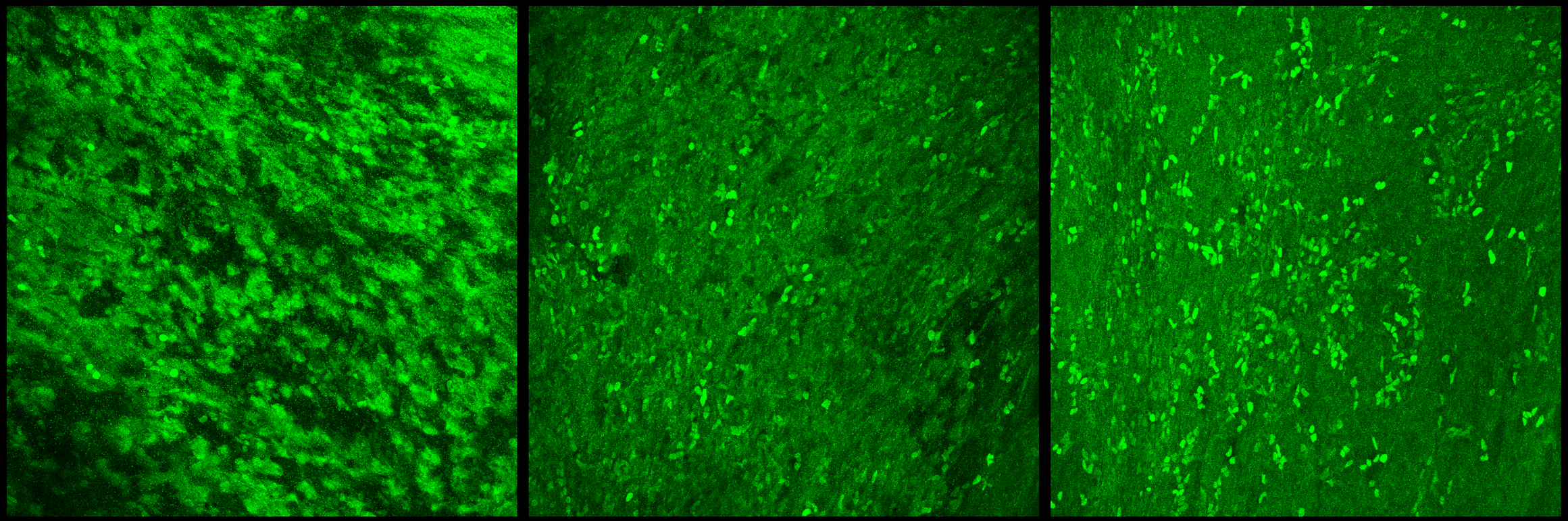
- Good signal
- Immunostaining worked fine, reproducibly, every time

- Also tried stabilized formaldehyde from EM Sciences
- Has methanol and other chemicals added
- Different fixation quality of the brains
- Less good immunostaining than fresh formaldehyde
- Needed optimization again

4% PLP o/n

2% PLP o/n

1% PLP o/n

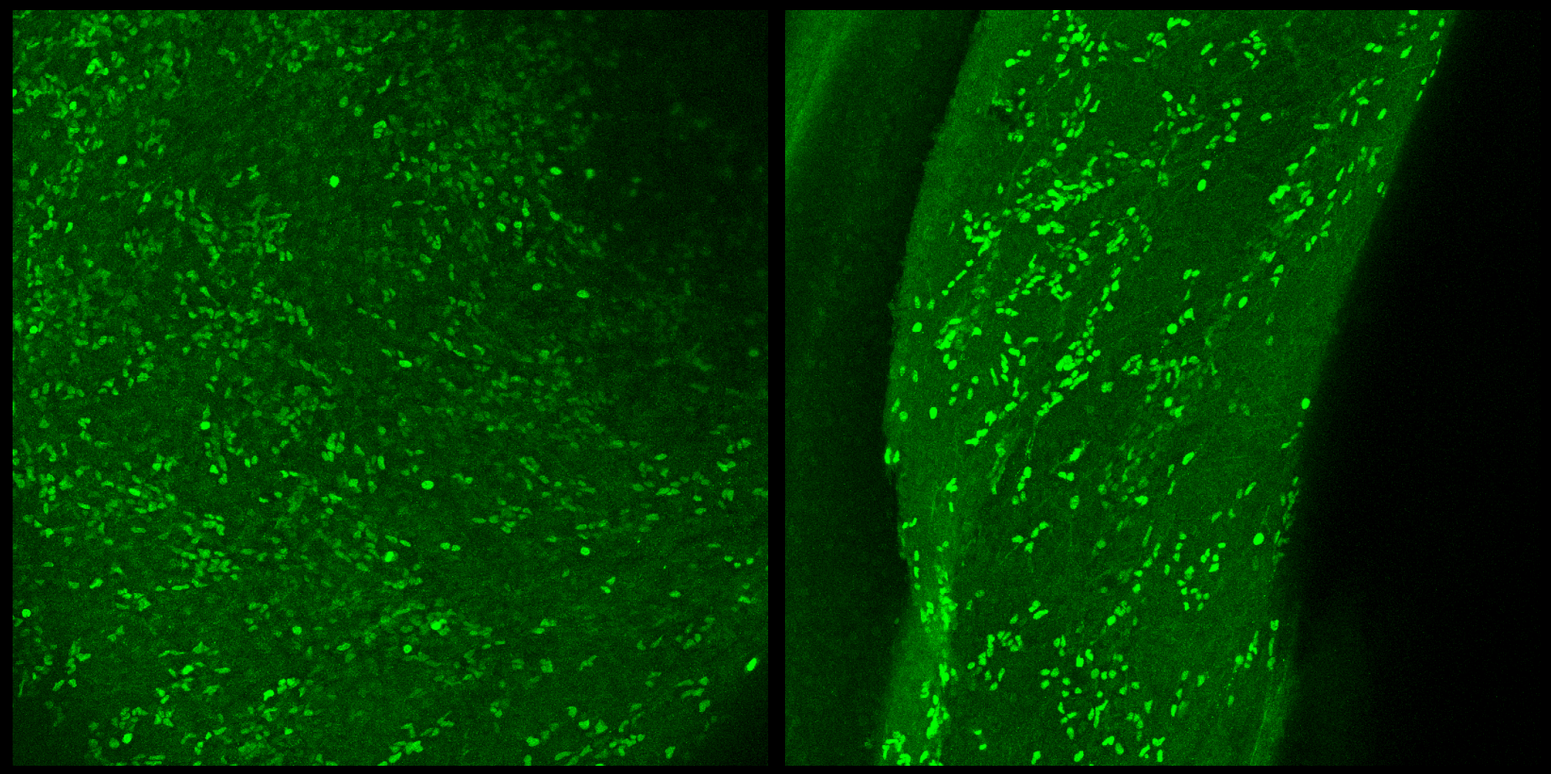


Rat anti-KI-67 signal

Higher/longer fix → higher background and less specific signal

2% PLP 2 h

1% PLP 2 h

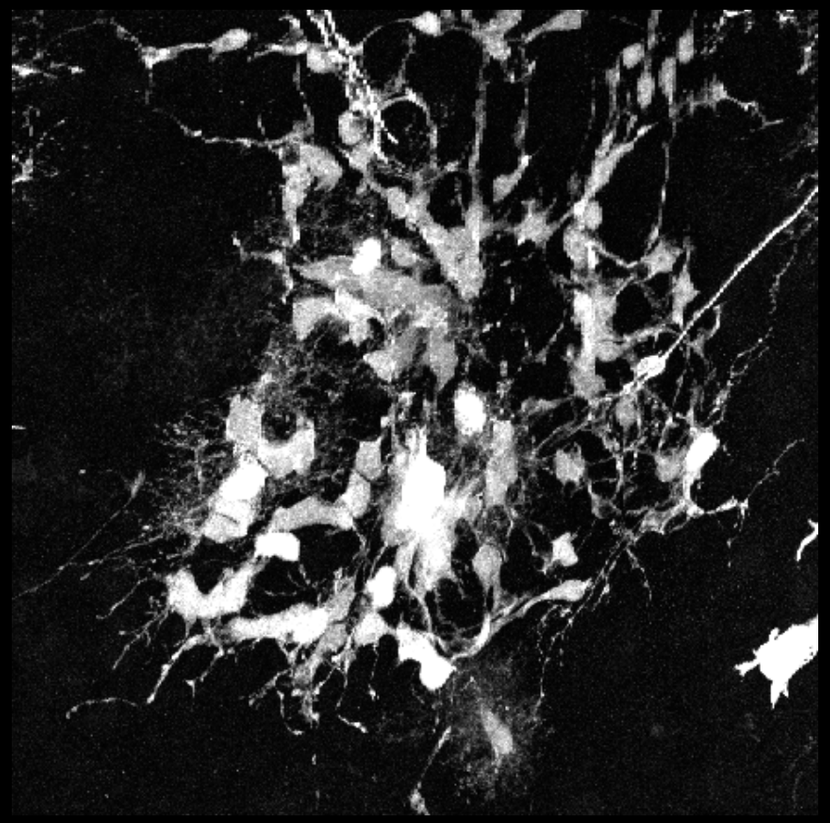


- In short, better signal with less fixation

- But the tissue probably not stable over long-term with less fixation, although I didn’t test this

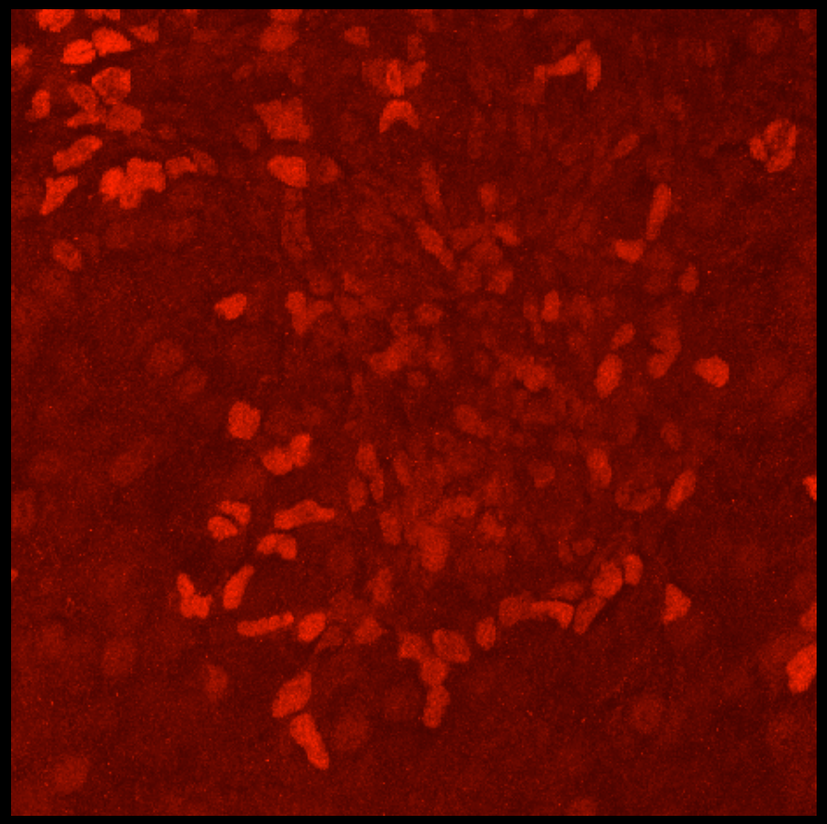
- Once optimized, immunostaining worked well again

RFP

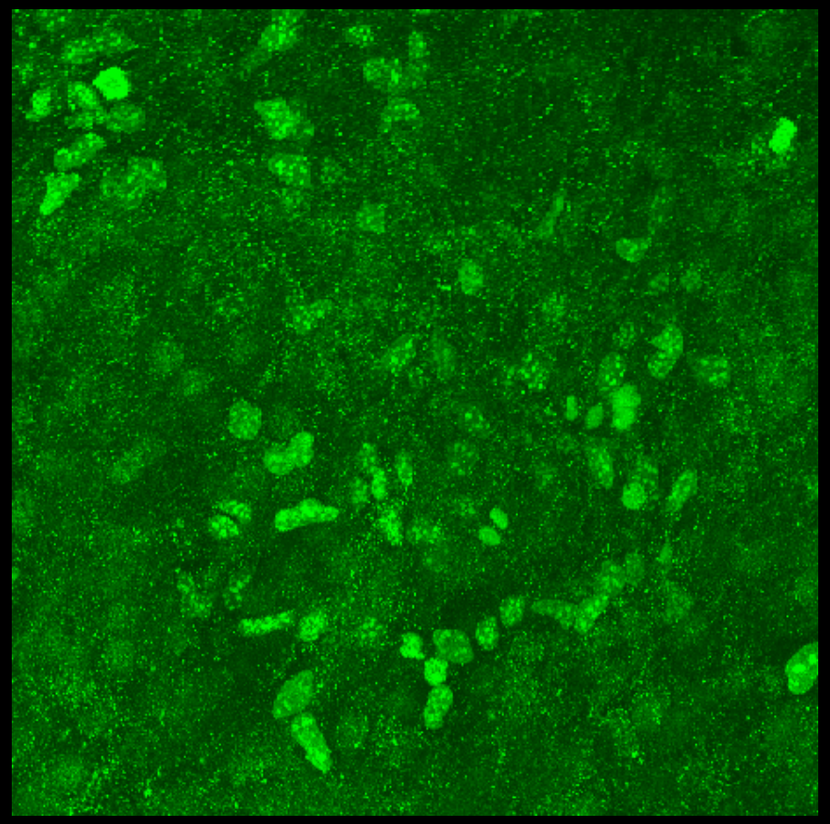


A large cluster in a mouse ~8 months after TMX

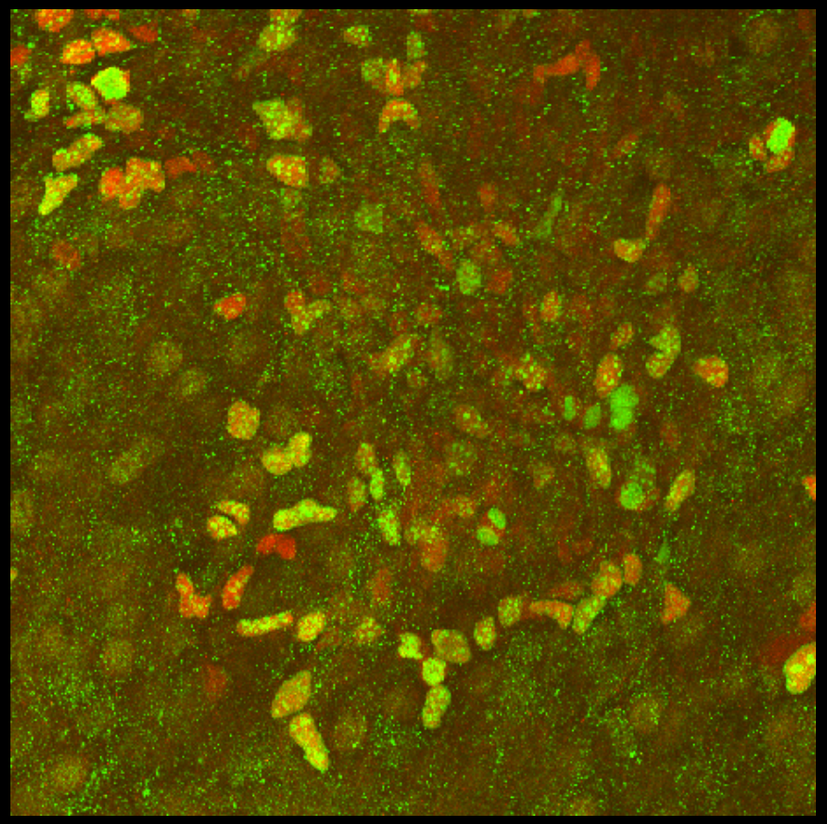
MCM2



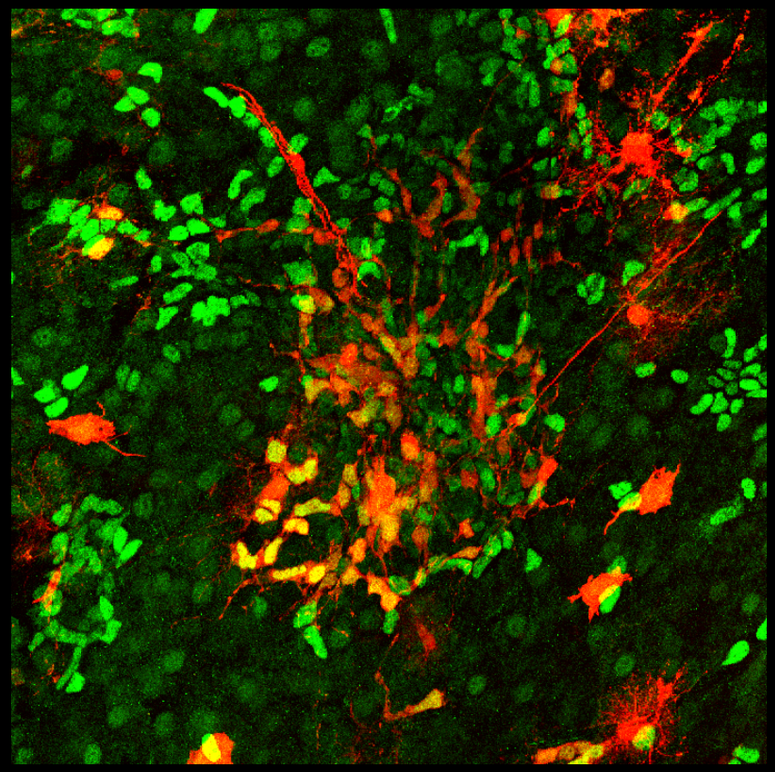
KI-67



MCM2 KI-67

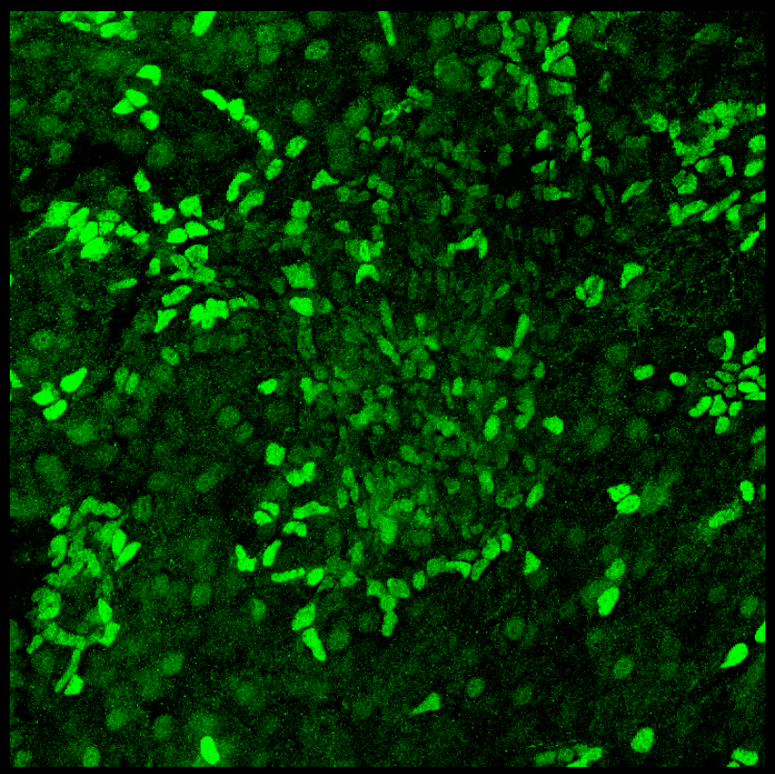


RFP MCM2

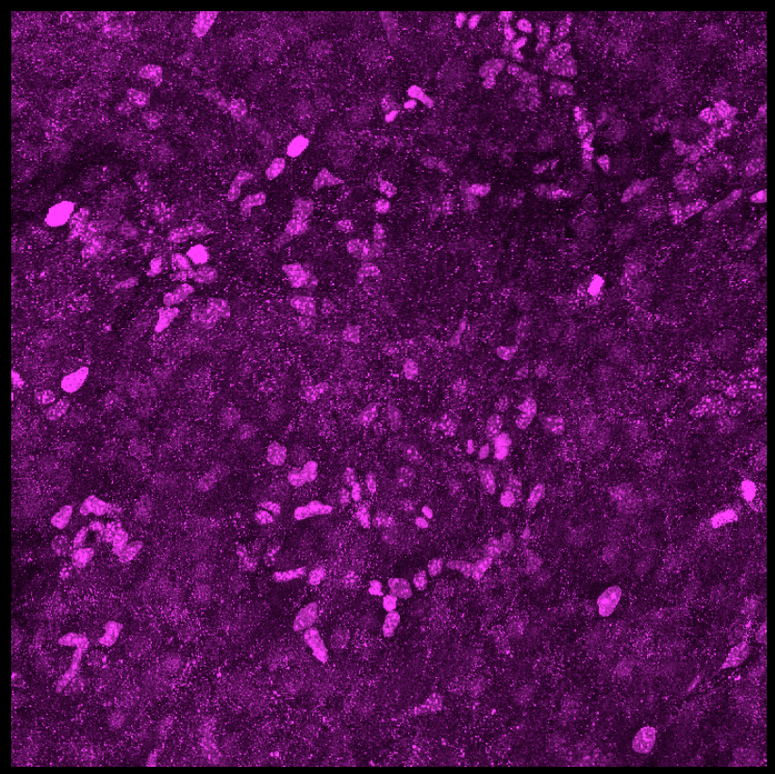


A large cluster in mouse ~8 months after TMX

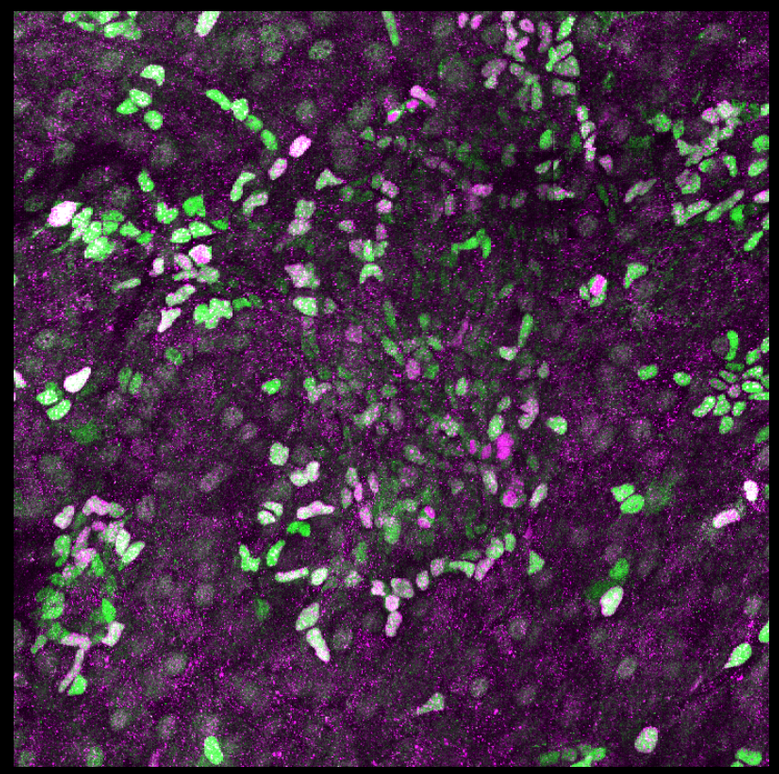
MCM2



KI-67



MCM2 KI-67



- As for the formaldehyde, in the end, I switched back to using fresh formaldehyde dissolved from powder
- 2% PLP made with fresh formaldehyde from powder
- Overnight fix with the 2% PLP worked fine, reproducibly
- What I use now for every whole mount immunostaining