




# Central nervous system regeneration is driven by microglia necroptosis and repopulation

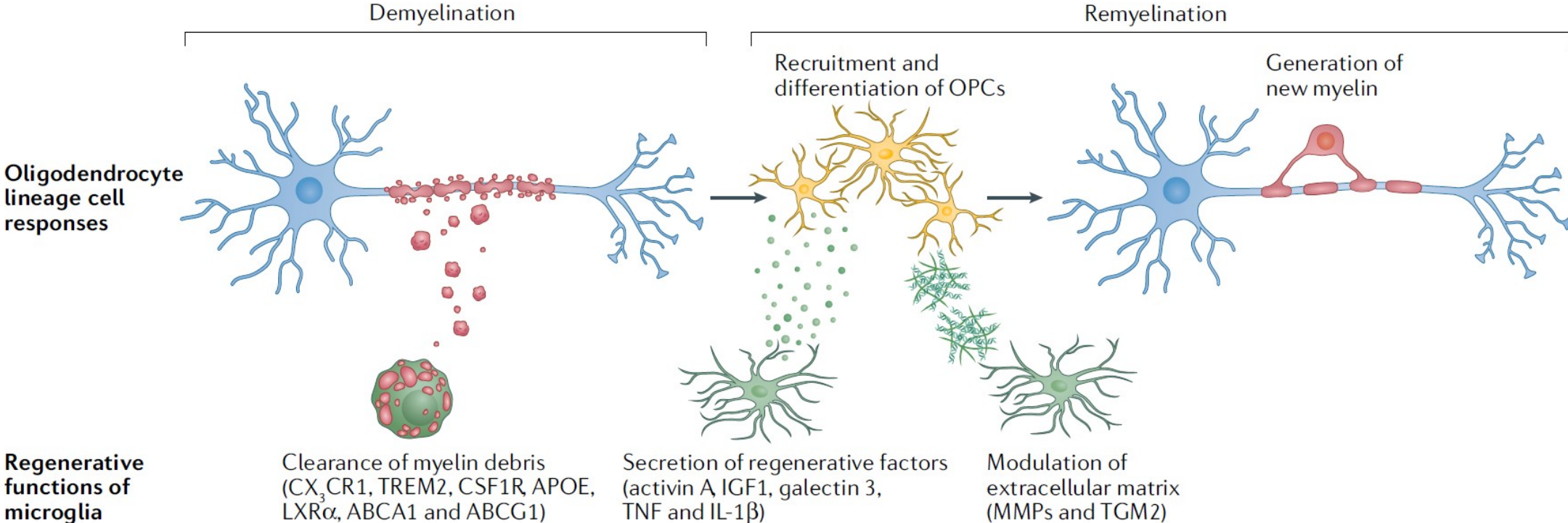
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by Michael Springer

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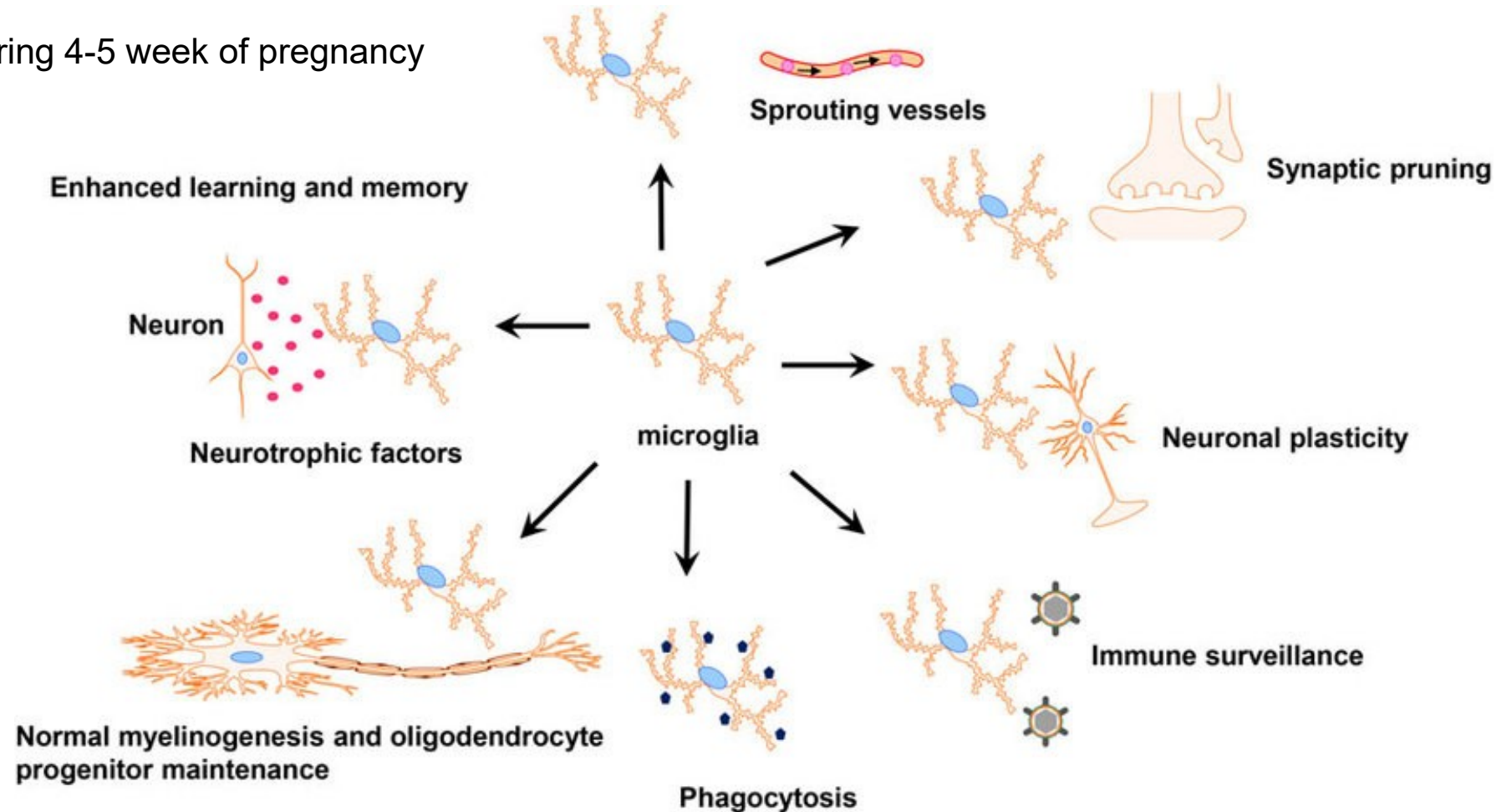
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# CNS regeneration

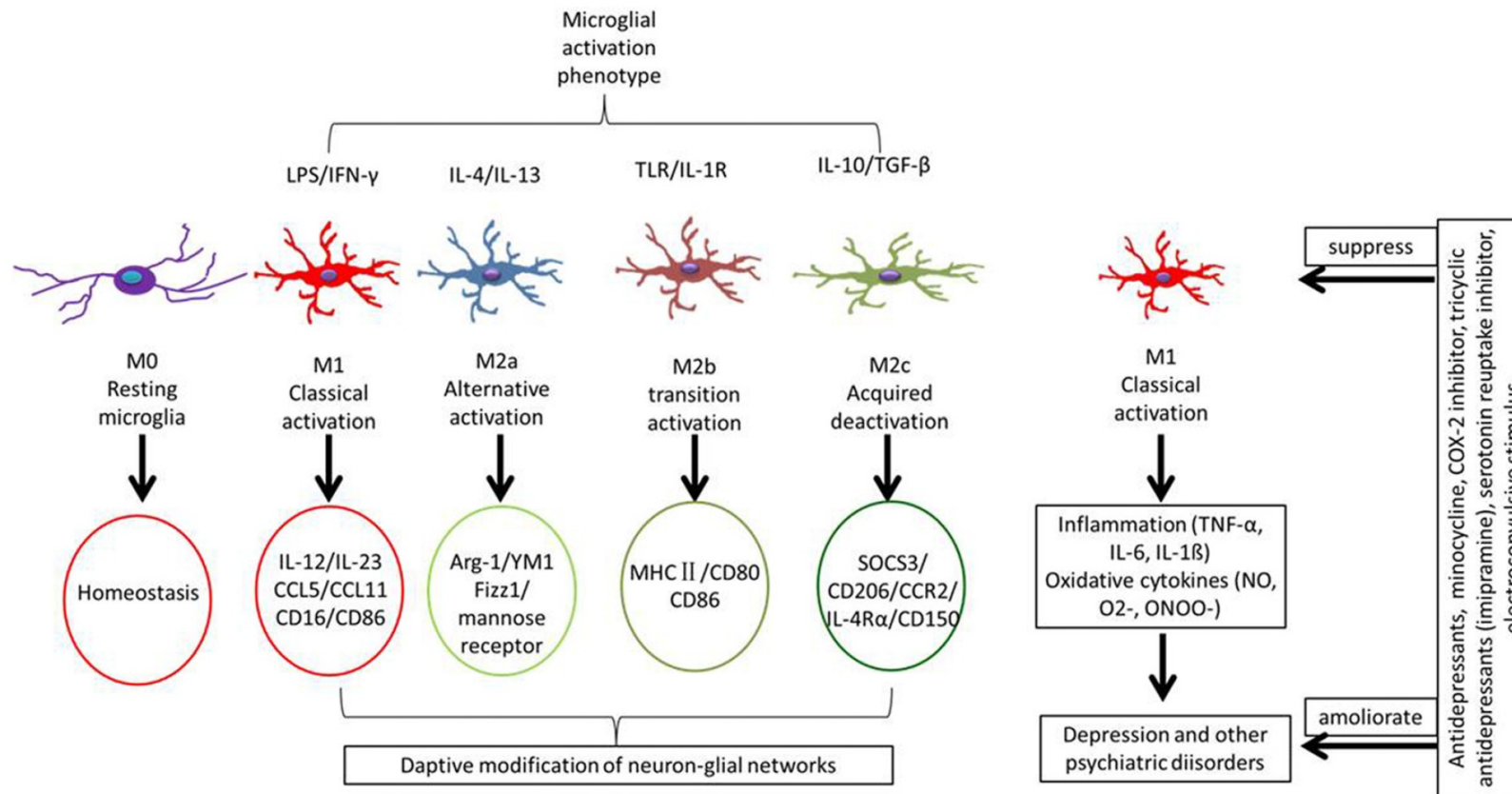


# Microglia

- Originate from yolk sac
- Emigrate towards brain during 4-5 week of pregnancy
- Multifunctional



# Microglia subtypes

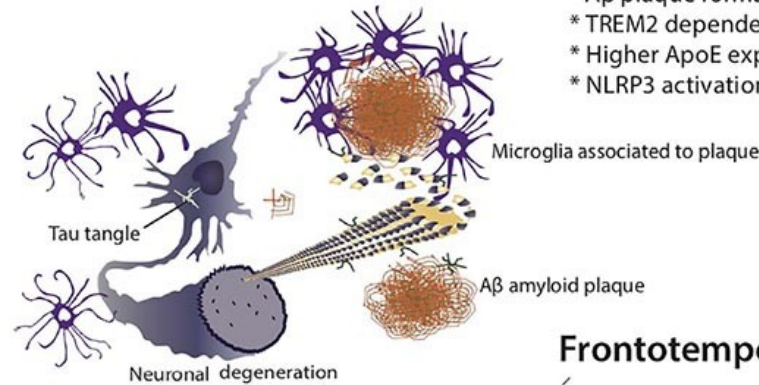


- M1: Proinflammatory
- M2: Pro-regenerative, immunosuppressive

# Neurological diseases

Extracellular A $\beta$  aggregates  
 Intracellular Tau tangle formation  
 Hippocampal and cortical neurodegeneration

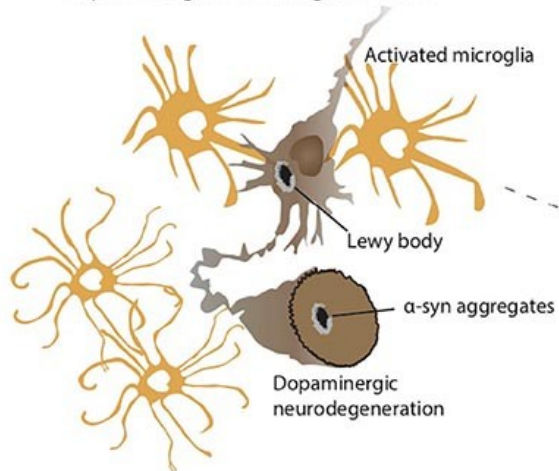
## Alzheimer's disease (AD)



- \* A $\beta$  plaque formation triggers microglial activation
- \* TREM2 dependent microglial activation
- \* Higher ApoE expression in activated microglia
- \* NLRP3 activation in microglia associated to plaque

## Parkinson's disease (PD)

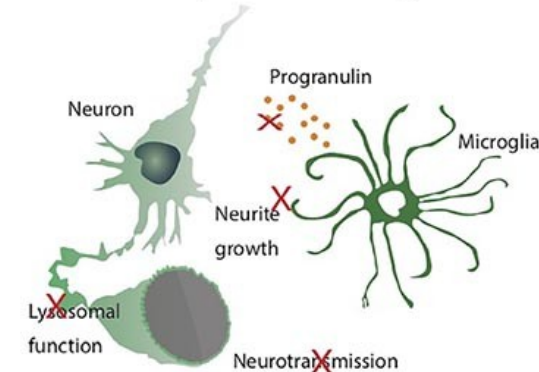
Intracellular  $\alpha$ -synuclein aggregates  
 Lewy body and Lewy neurites  
 Dopaminergic neurodegeneration



- \* Neuronal dysfunction leads to microglial activation
- \*  $\alpha$ -syn aggregates triggers microglial activation
- \* TLR2 and TLR4 activation conformation dependent
- \* MHCII upregulation in response to  $\alpha$ -syn

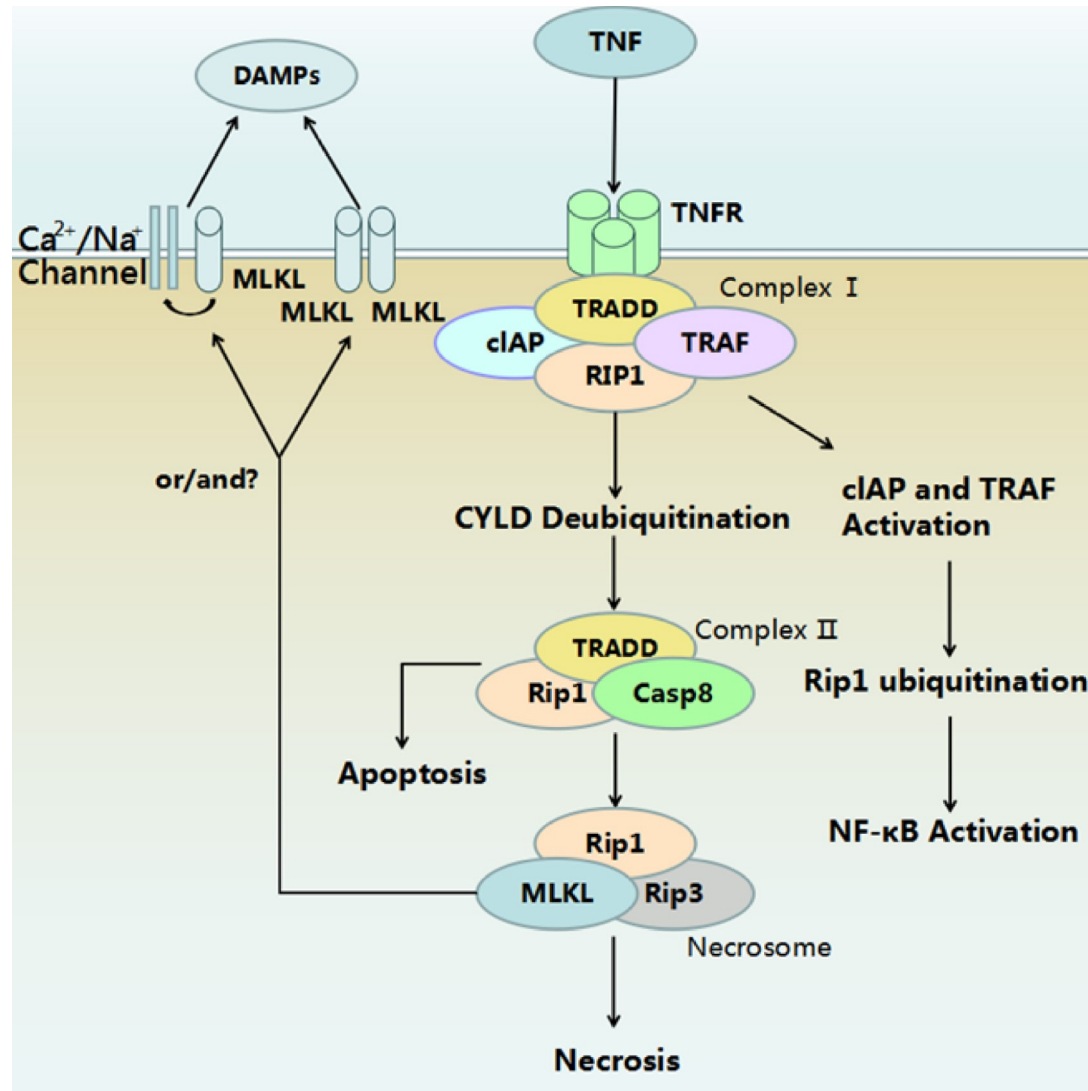
## Frontotemporal dementia (FTD)

FTLD-Tau (Tau inclusions)  
 FTLD-TDP (TDP43 inclusions)  
 FTLD-FUS (Tau and TDP43 negative inclusions)

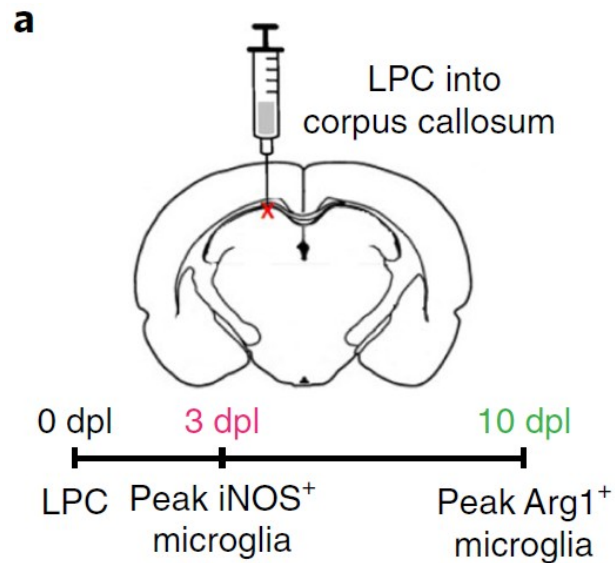


- \* Tau containing neurofibrillary-tangles triggers microglial response
- \* Microglial defective progranulin-release
- \* TREM2 related mutations linked to FTD
- \* Excessive complement system reaction

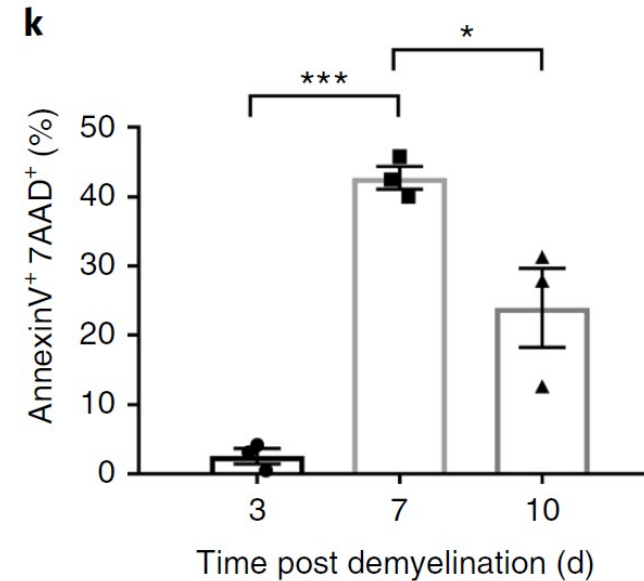
# Necroptosis



# Covering the basics



- iNOS: marker of M1 phenotype
- Arg1: marker of M2 phenotype
- Annexin-V: Cell death marker
- 7-AAD: Cell death marker



**g**

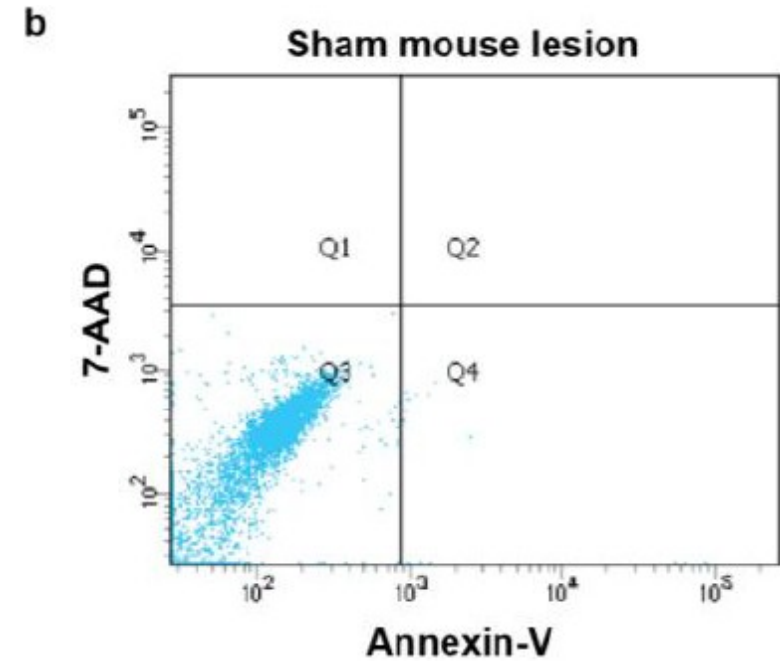
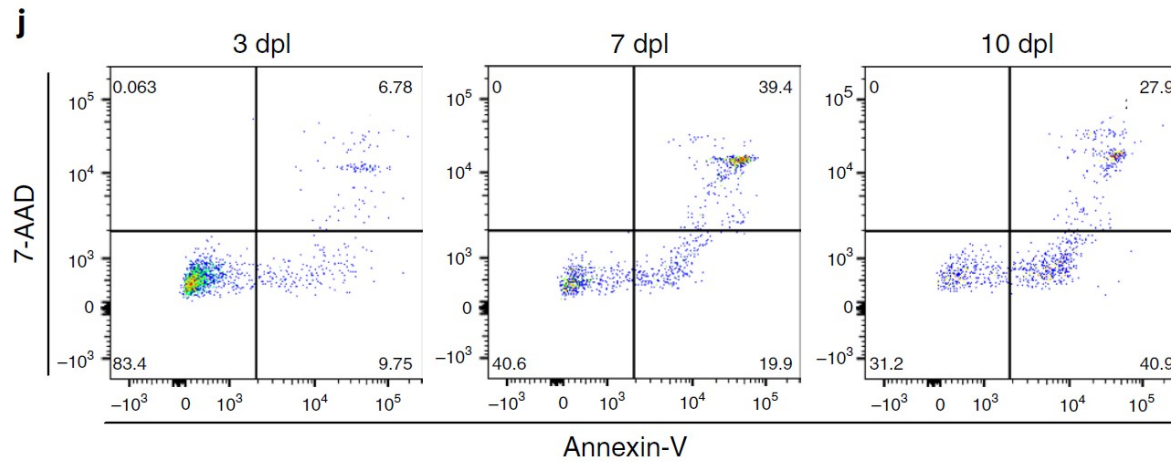
## Ingenuity pathway analysis

| Molecular & cellular functions  | No. of molecules | <i>P</i> value range |
|---------------------------------|------------------|----------------------|
| Cellular growth & proliferation | 80               | 0.0371–0.000203      |
| Cellular development            | 82               | 0.0371–0.000342      |
| Cellular function & maintenance | 97               | 0.0371–0.000452      |
| Cell morphology                 | 55               | 0.0371–0.00111       |
| Cell death & survival           | 37               | 0.0348–0.00121       |

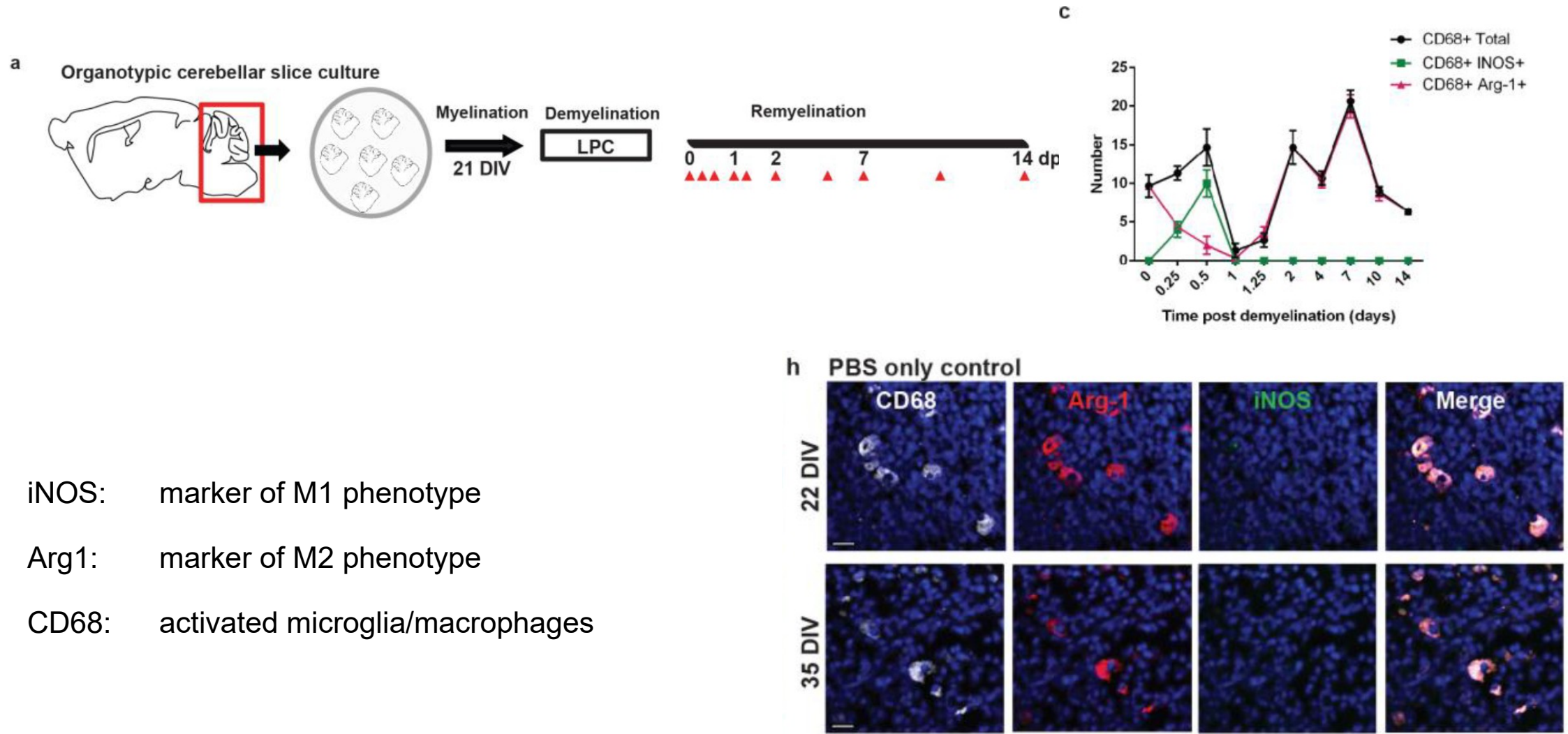


# Control with sham mouse lesion

Using flow cytometry



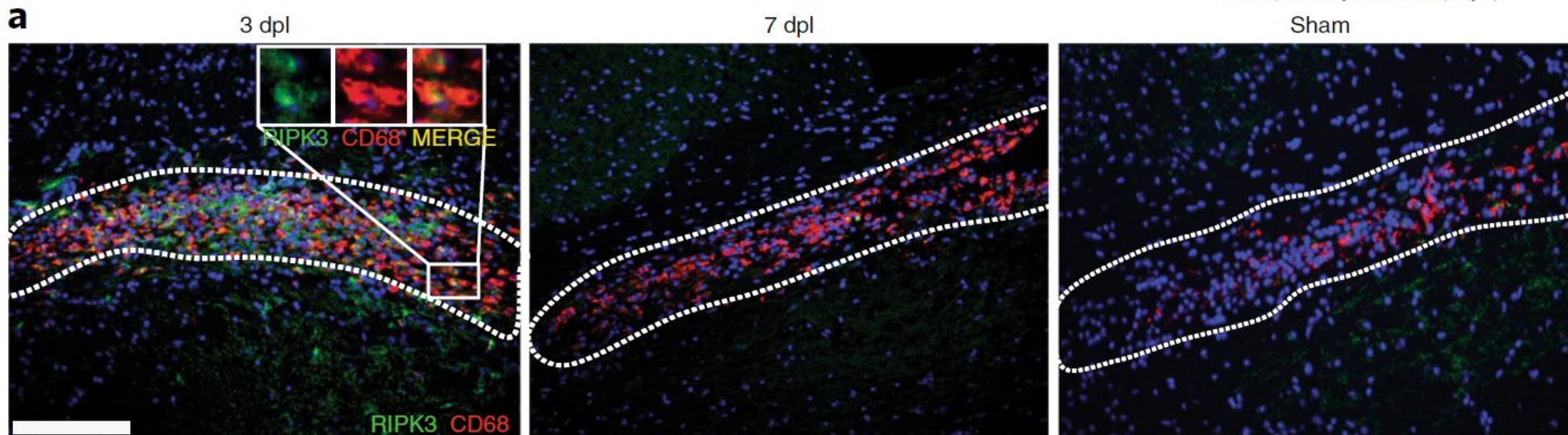
# Activated macrophage subtype population



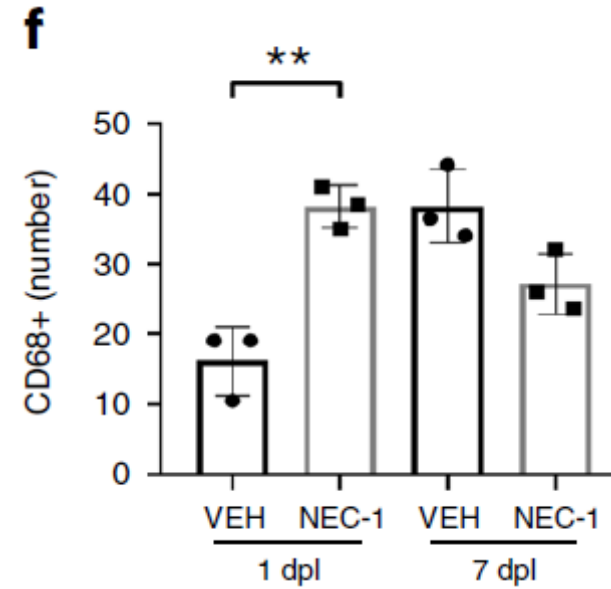
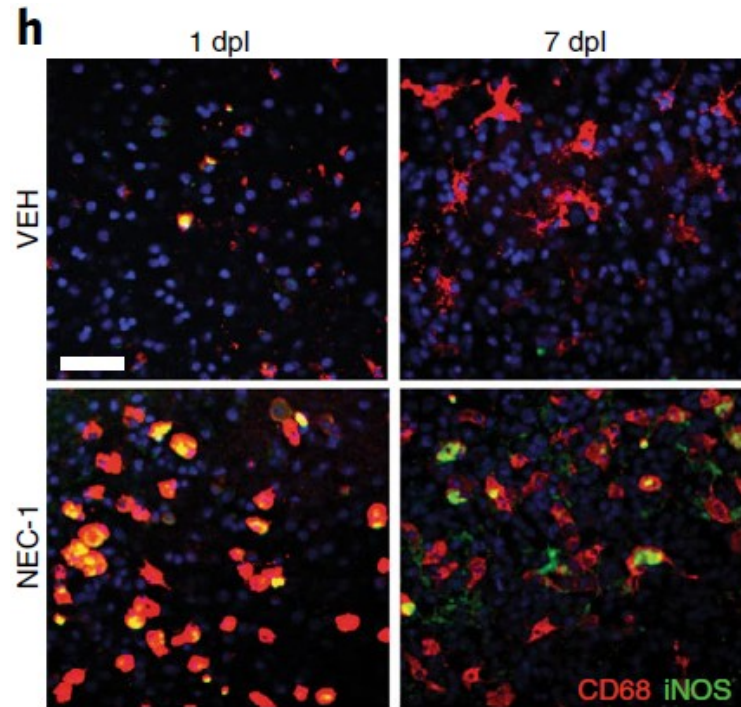
- iNOS: marker of M1 phenotype
- Arg1: marker of M2 phenotype
- CD68: activated microglia/macrophages

# How do these cells die?

- RIPK3: marker of necroptosis
- MLKL: marker of necroptosis
- CD68: activated microglia/macrophages

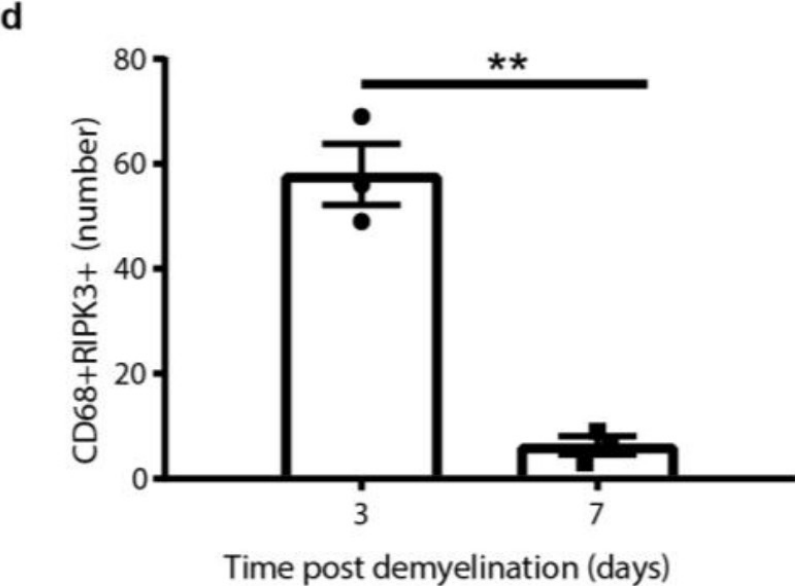
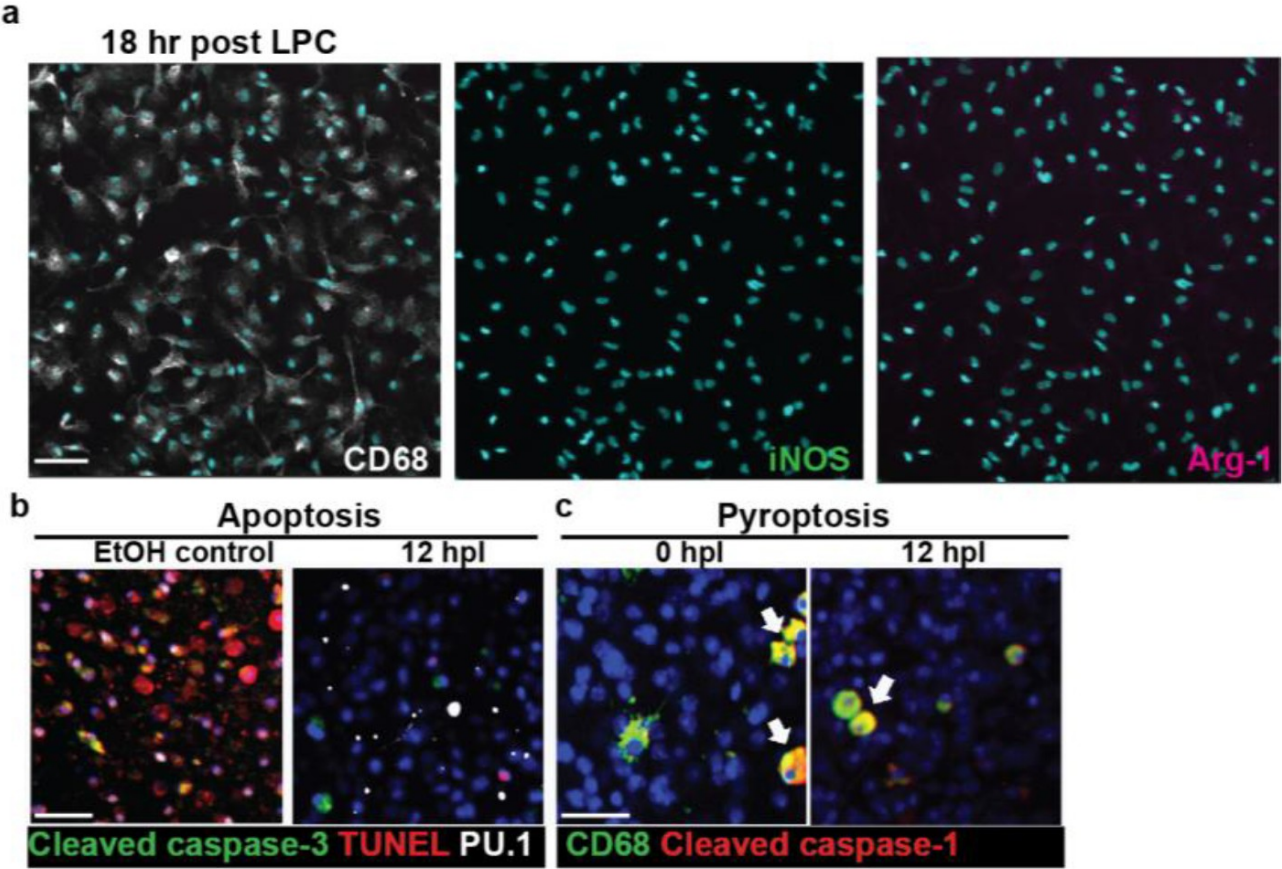


# Verify with necrostatin-1



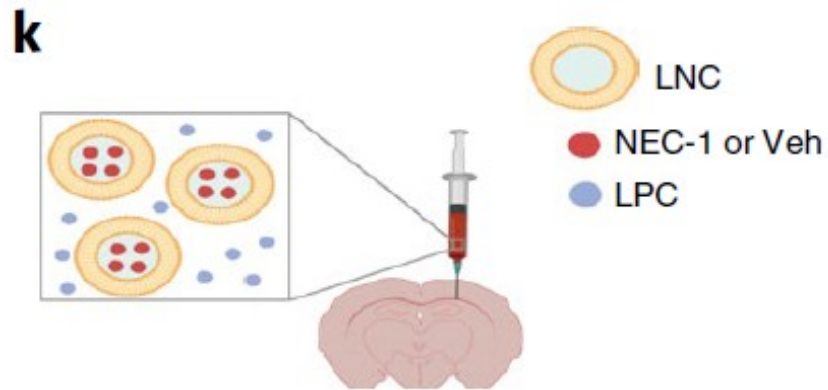
Necrostatin-1 prevents necroptosis

# Eliminate other causes of cell death

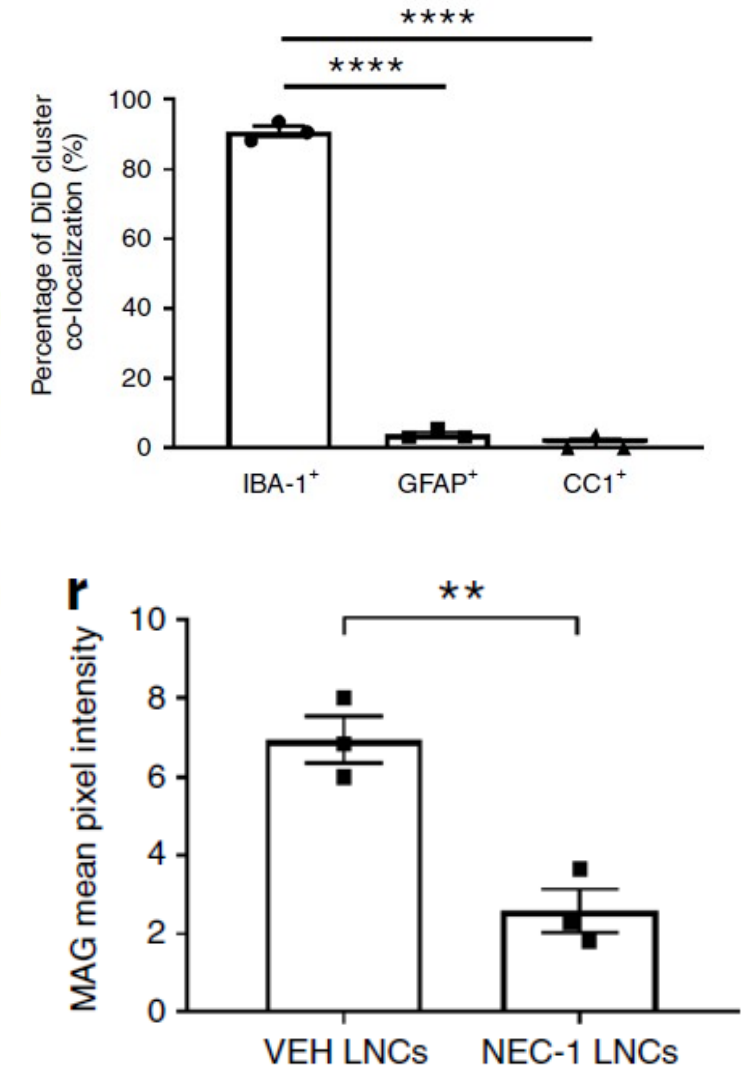
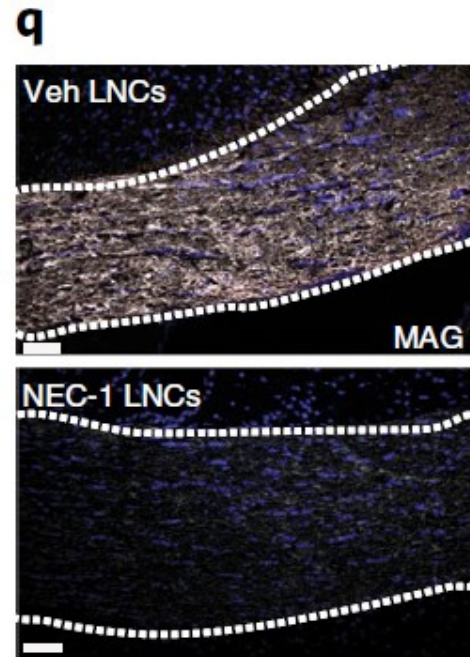


# Verifying THEY die

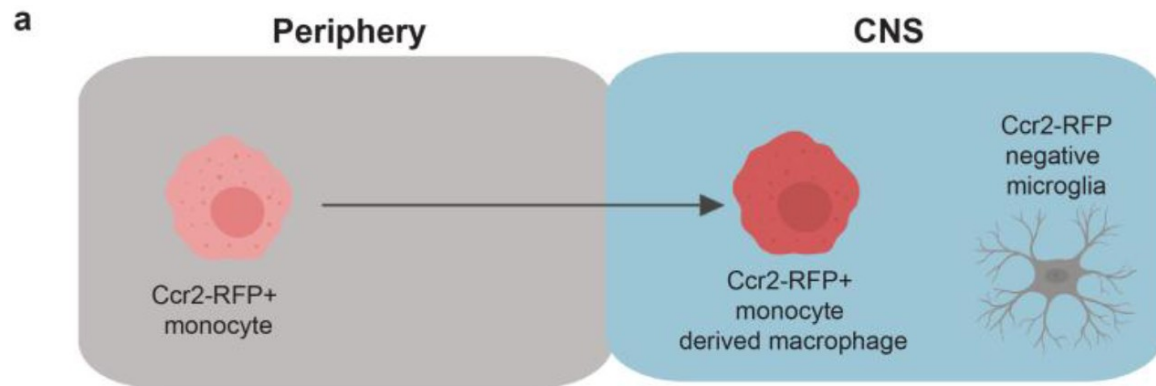
LNCs are taken up by macrophages



- MAG: early remyelination marker

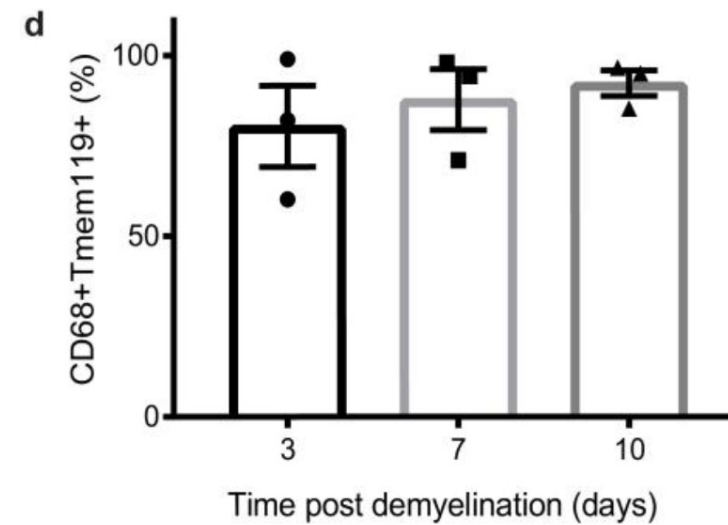
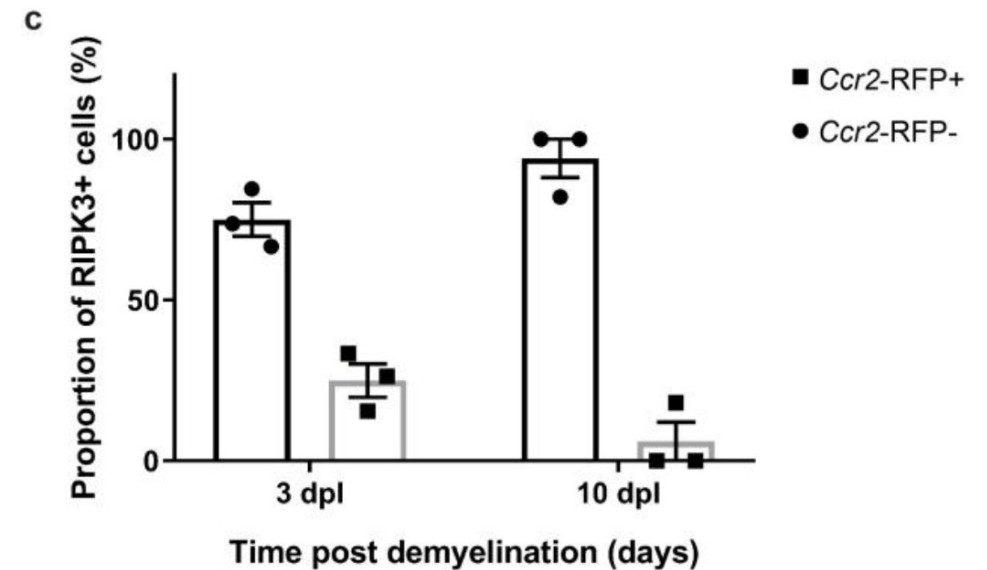


# Monocyte or microglia



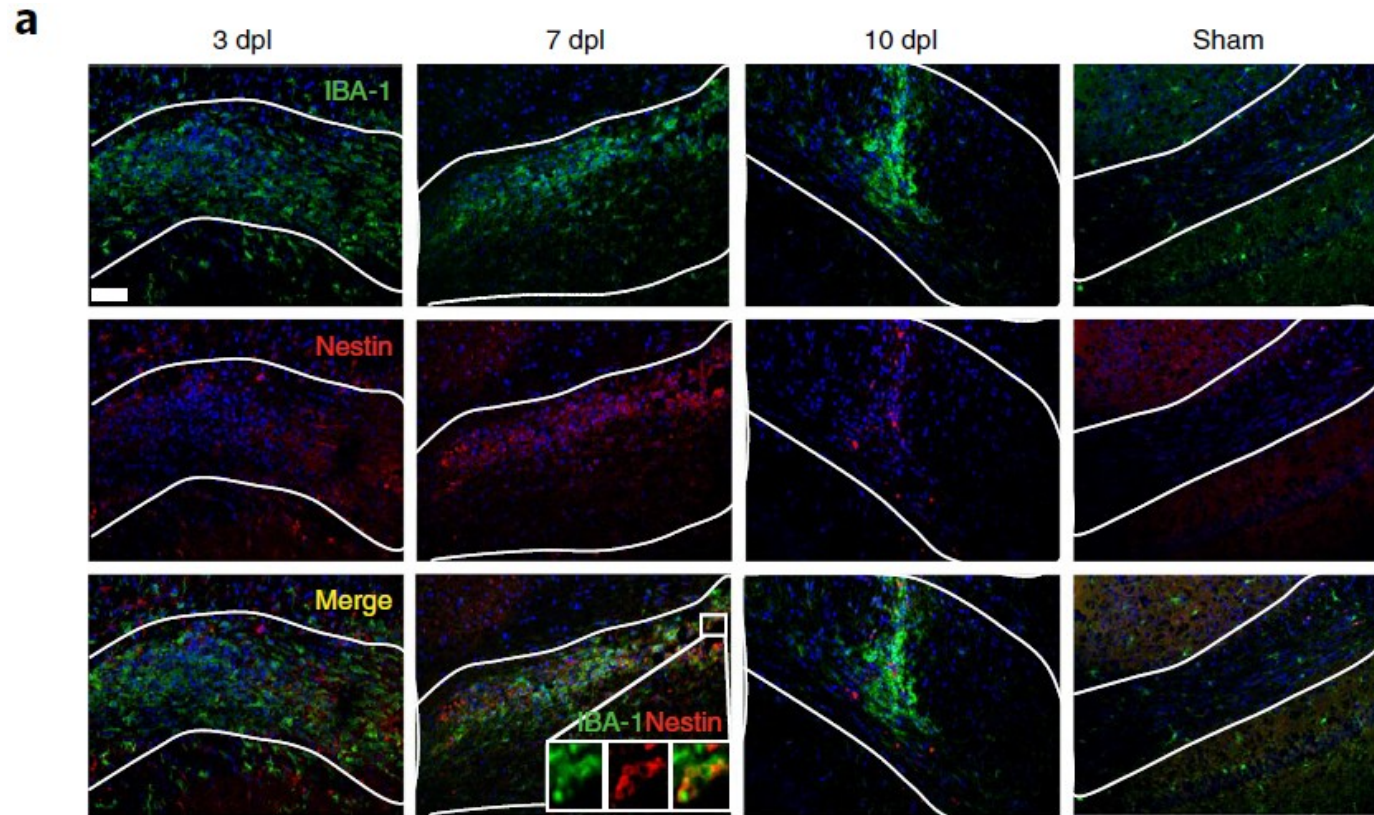
- RIPK3: marker of necroptosis
- Tmem119: microglia marker
- CD68: activated microglia/macrophages

→ Mainly microglia



# Repopulation of microglia

Test for nestin, used to identify repopulating microglia

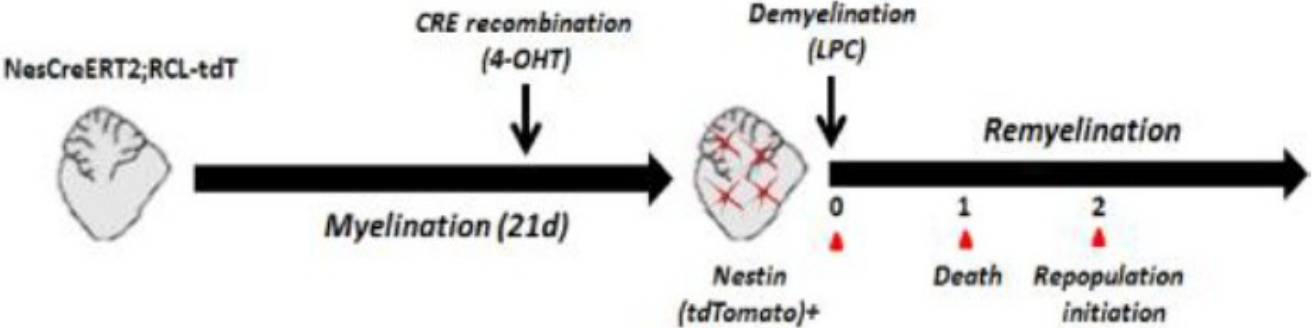


→ Originate from residual microglia

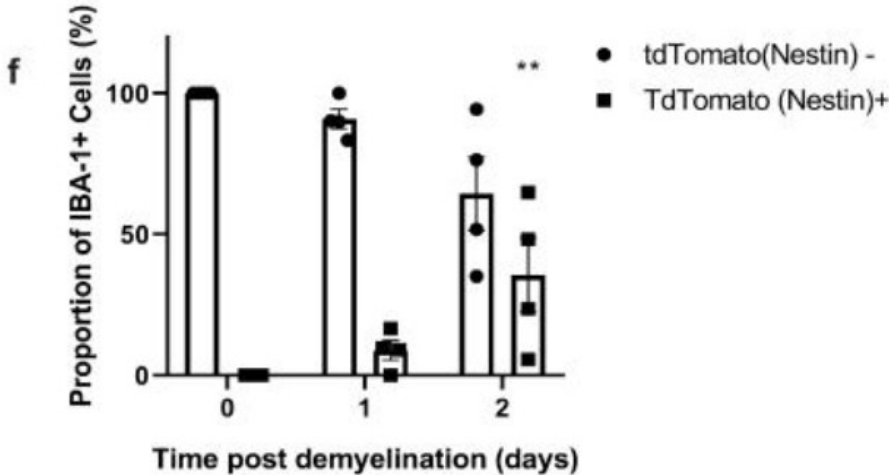
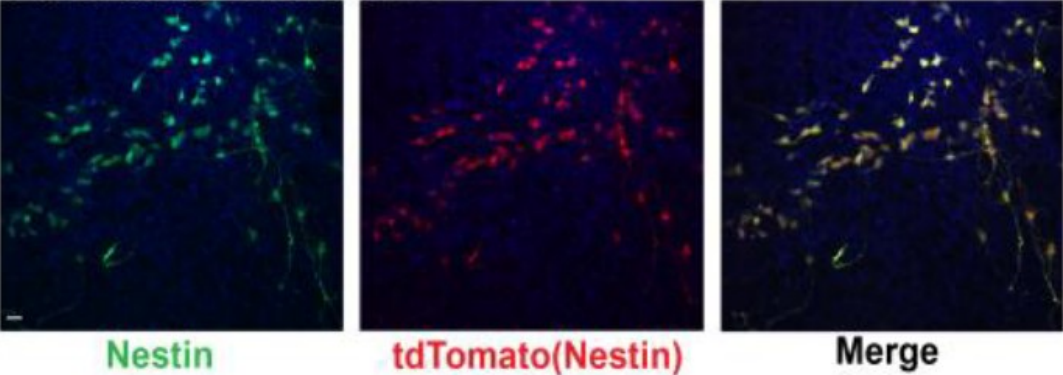


# Repopulation of microglia

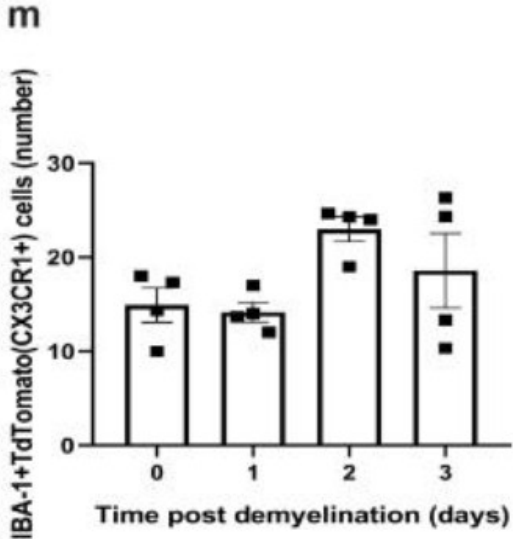
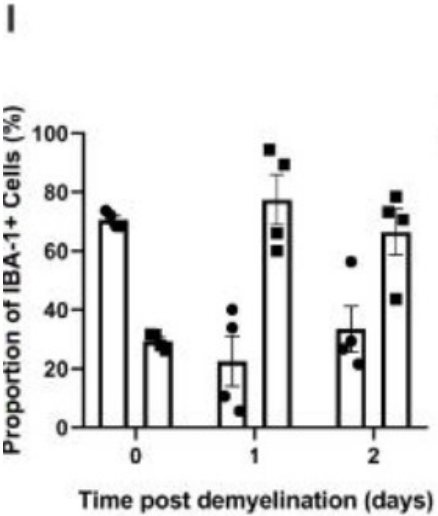
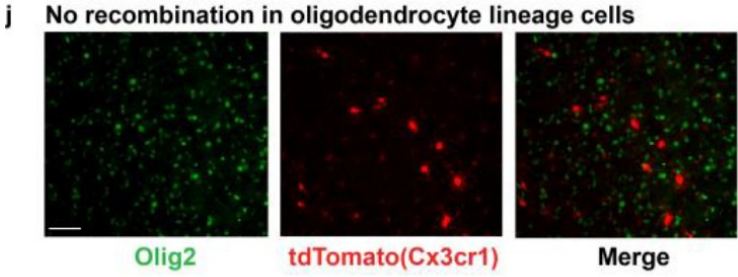
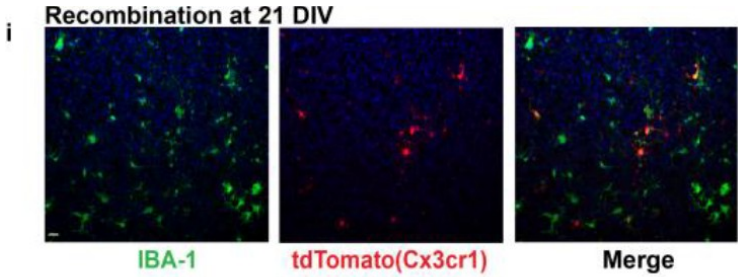
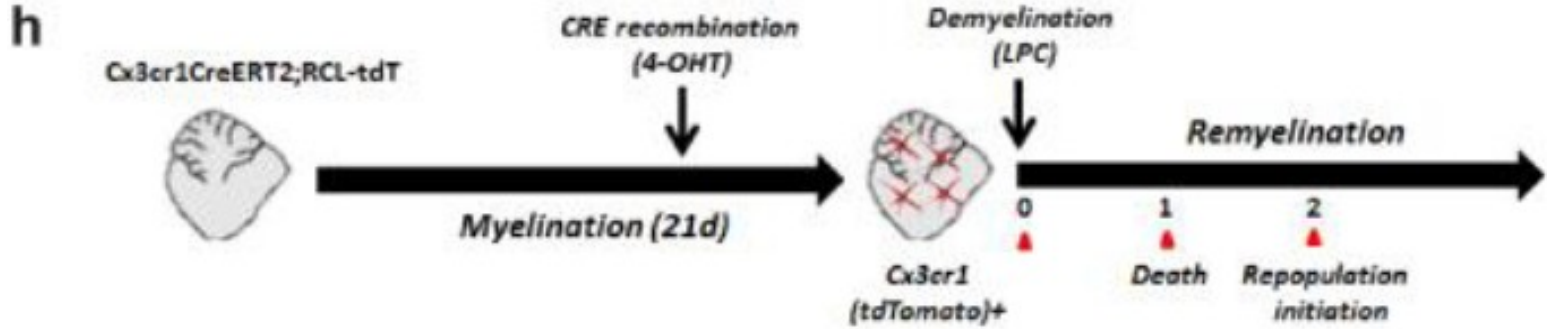
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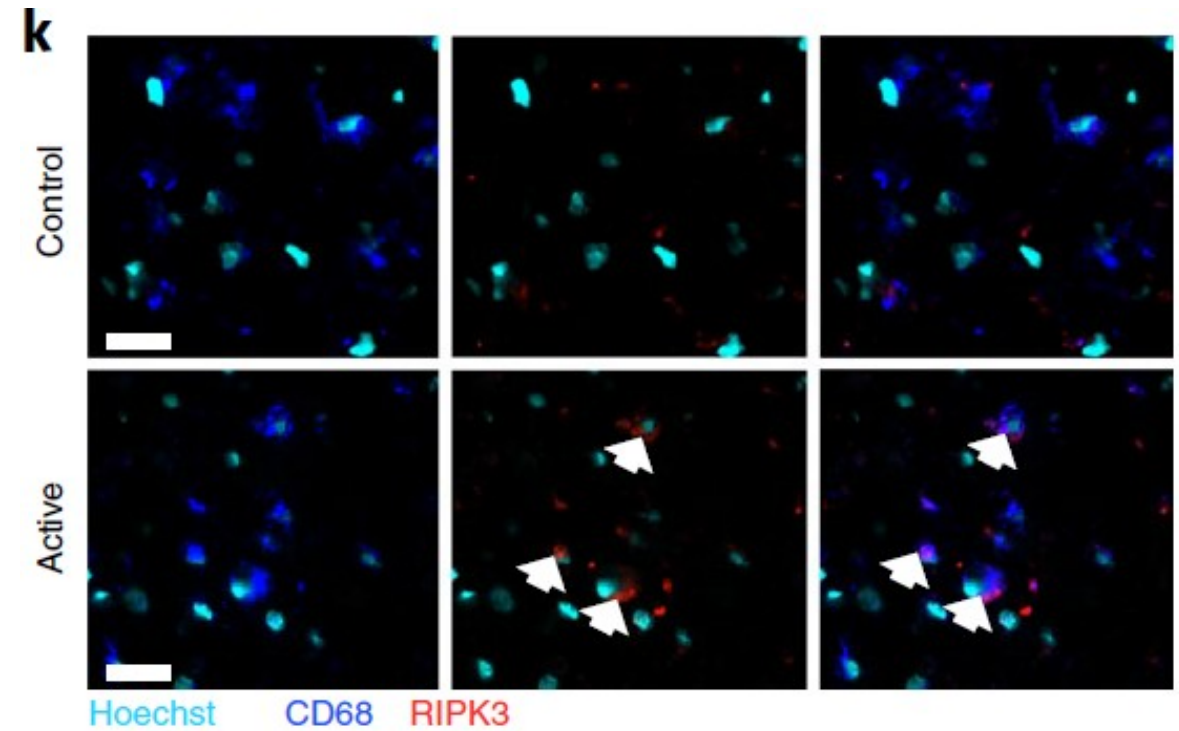
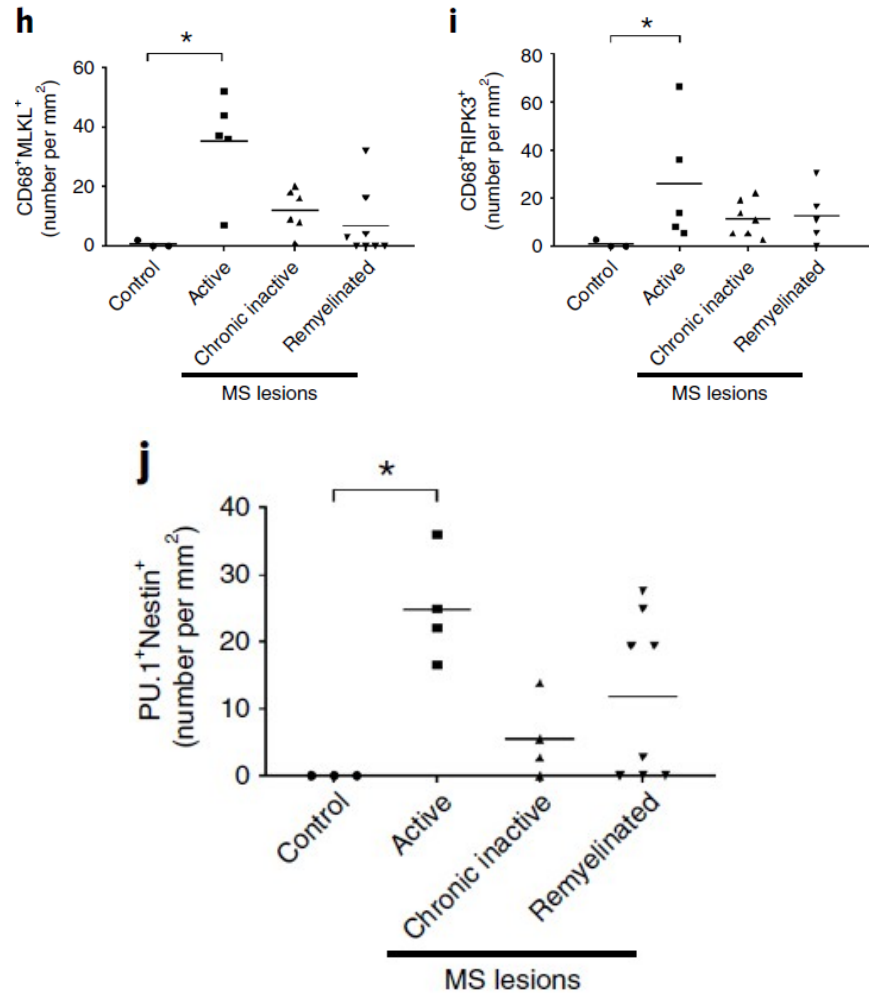
d Recombination at 21 DIV



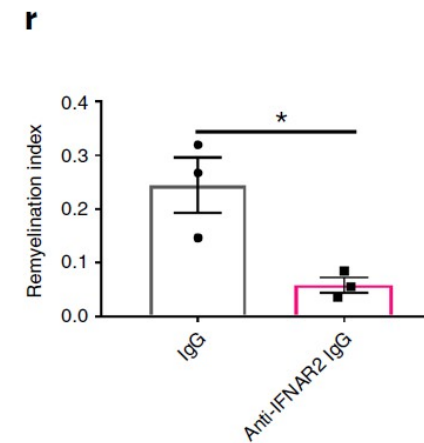
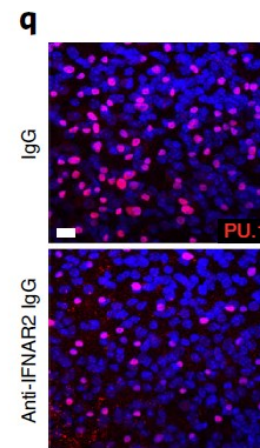
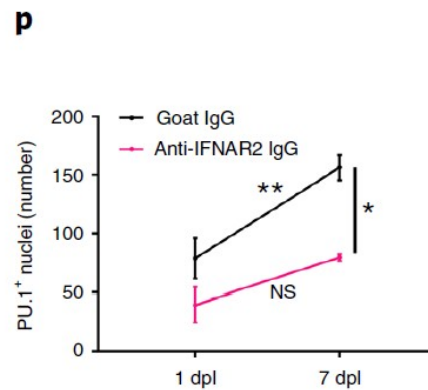
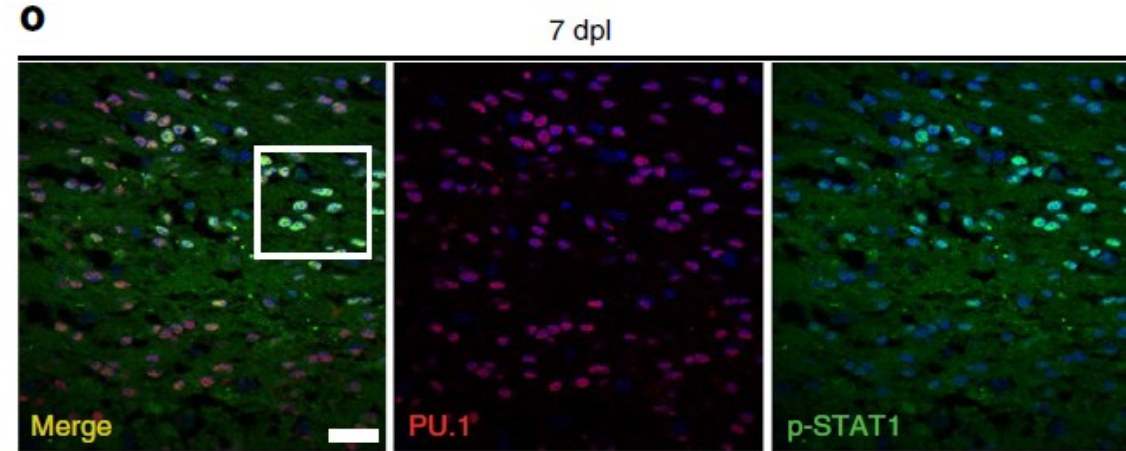
# Repopulation of microglia



# Necroptosis and repopulation in human white matter



# IFN-1



White matter remyelination is positively regulated by type-1 IFN signalling.

# Summary

- Proinflammatory microglia die via necroptosis
- Repopulation from resident microglia
- White matter remyelination is positively regulated by type-1 IFN signalling

# Discussion