Antibody Responses Targeting Proteolipid Protein1 Anchored Membrane Domains in Multiple Sclerosis: Pathogenicity, Antigen Complexity and Prevalence.

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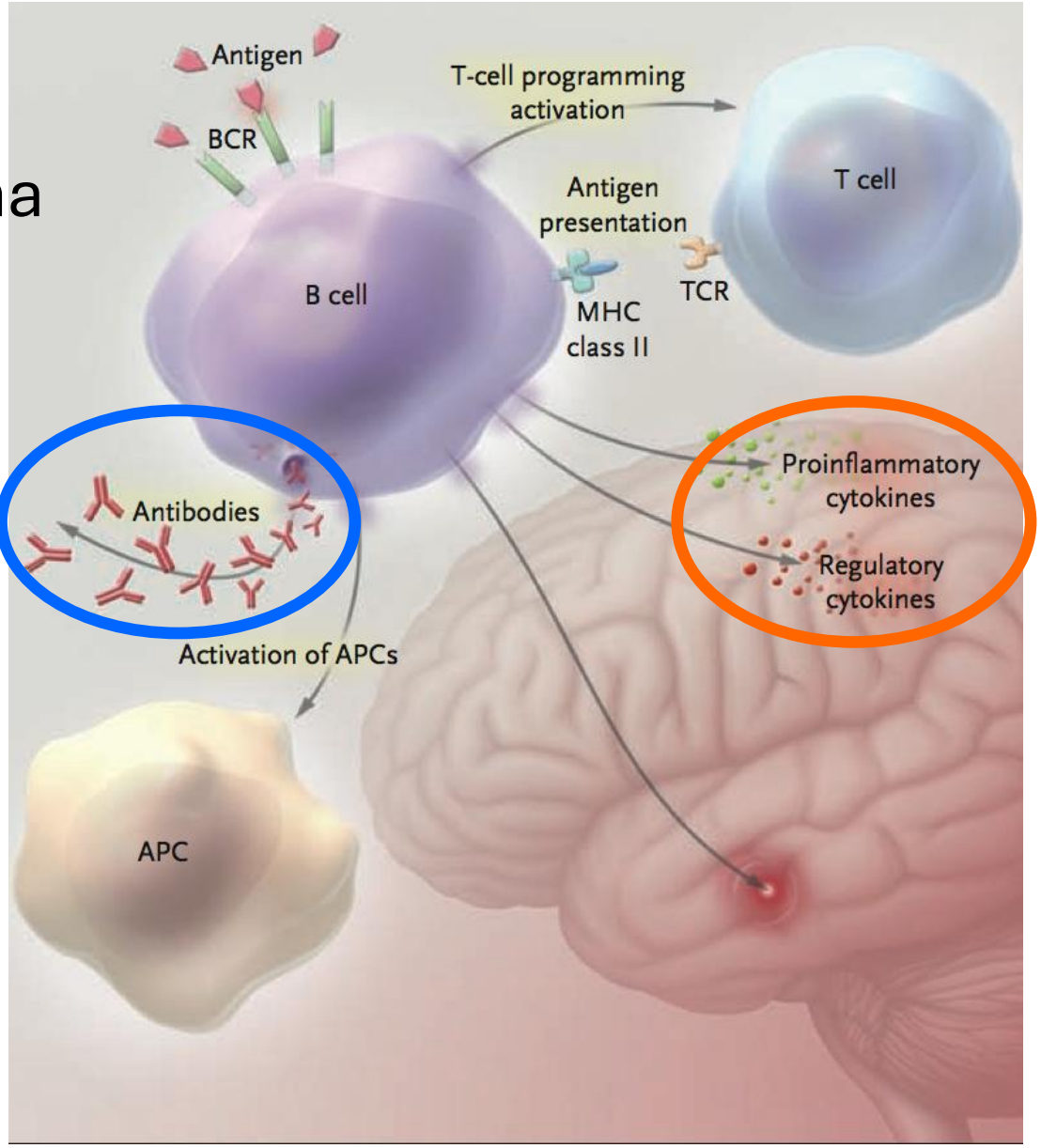
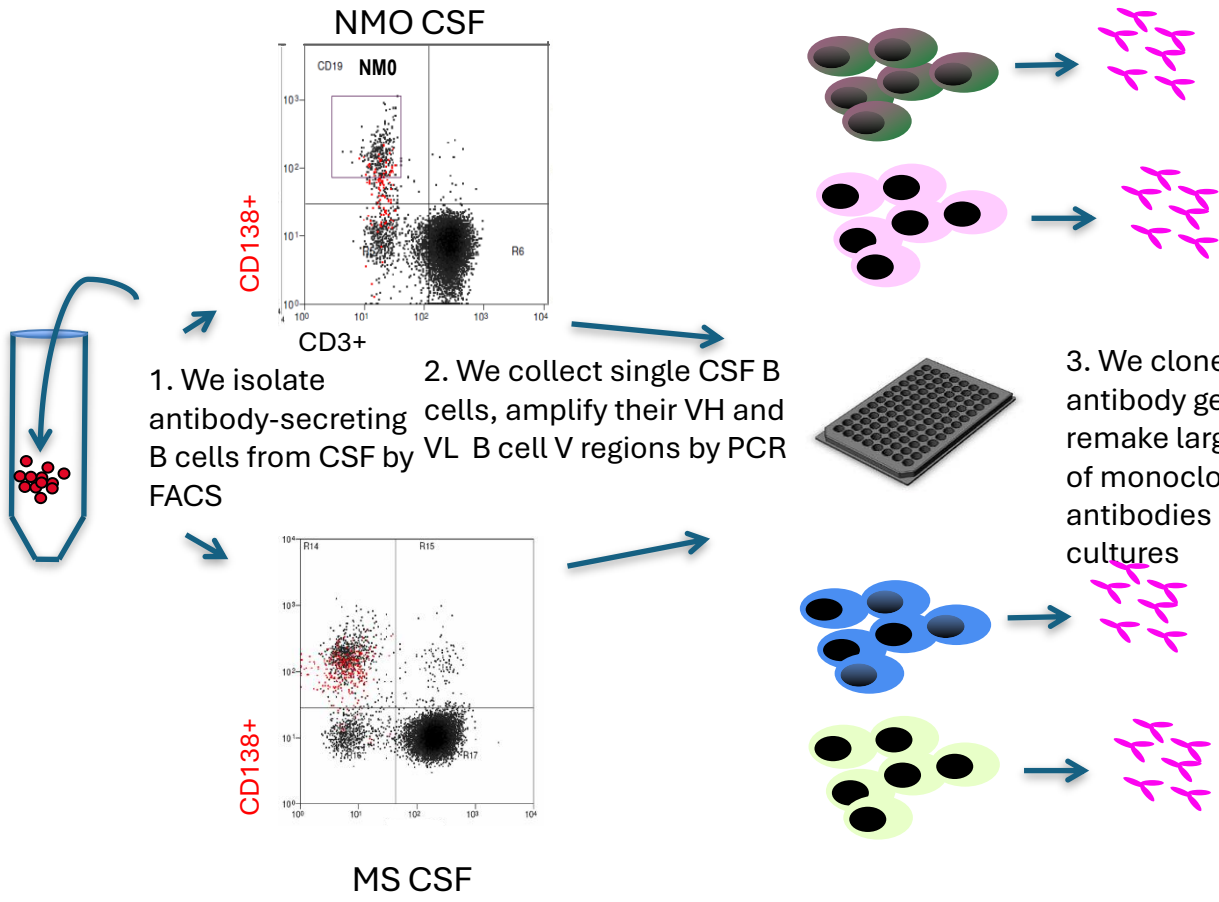
B Cells Play A Role in MS Pathology via Multiple Potential Mechanisms

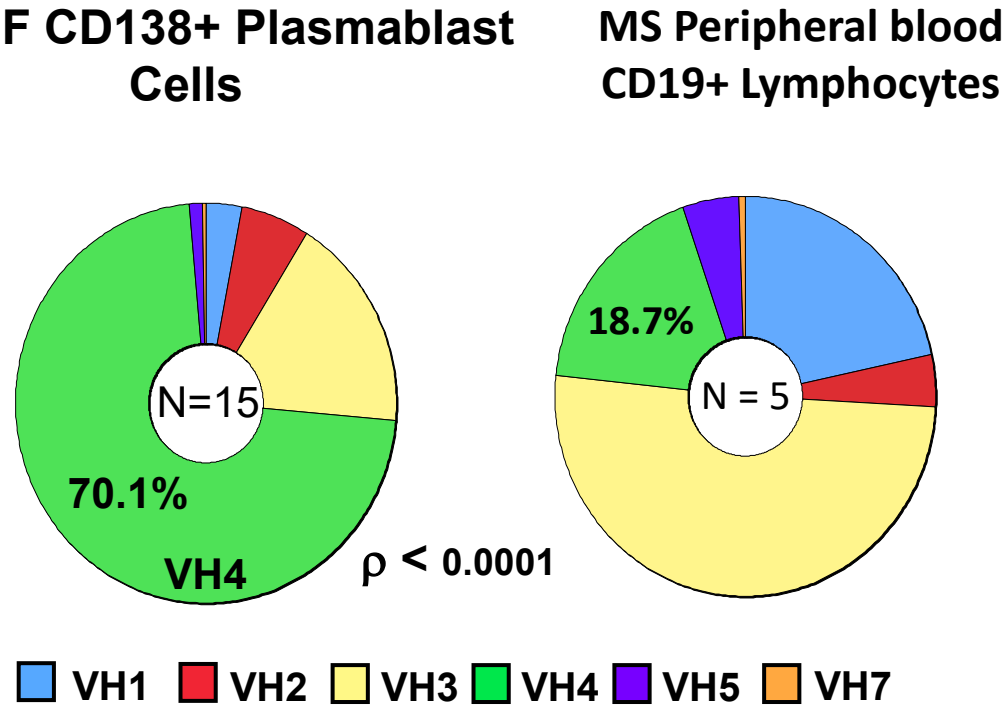
- Increased (5-10-fold) CSF B cells, plasma blasts and IgG oligoclonal bands
- Effective treatment with B cell depleting therapies
- Complement deposition in some active lesions

  - Pathogenic Antibodies
  - MHC Class II Antigen Presentation
  - Activate APCs
  - Regulatory Cytokine Functions



MS CSF PLASMA BLAST REPERTOIRES DISPLAY A UNIQUE DISEASE-SPECIFIC BIAS FOR RECOMBINED VH4 Ig GERMLINE SEGMENTS





Inexhaustible supply of MS patient derived monoclonal recombinant IgG 1 antibodies duplicating MS CSF plasma cells

Owens et al. (2007) J Immunol

Table S1. Clinical, MRI and CSF Features of MS Patients used for CSF monoclonal recombinant antibody production[a]

Patient	Age	Sex	Diagnosis	White Matter Lesions	CSF Cells/µl	IgG Index	OCBs	PLP1+ rAbs[b]	Disease Duration at Lumbar Puncture	Time of Lumbar Puncture from Onset of Last Exacerbation
MS03-1[c]	25	F	Relapsing-remitting MS	5	0	1.4	6		6 months	2 months
MS04-2	26	F	Relapsing-progressive MS	36	0	1.7	5	MS#30 MS#11	6 months	1 month
MS05-3	30	F	Relapsing-remitting MS	4	18	1.7	21		14 months	2 months
ON07-7[c]	18	F	Relapsing-remitting MS	11	26	1.33	16	ON#34 ON#49	2 months	2 months
MS02-19	49	F	Primary-progressive MS	multiple	10	1.2	6		3 years	Not applicable

[a] Patients were not receiving immunomodulatory therapy at the time of lumbar puncture.

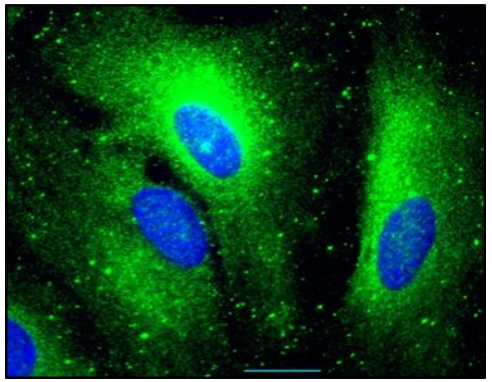
[b] Patient-derived rAbs from CSF plasmablast clones demonstrated to bind myelin and/or PLP1-transfected cells.

[c] CSF was collected and analyzed following their first clinical event. Patients subsequently developed relapsing-remitting MS within a 6-18 month period.

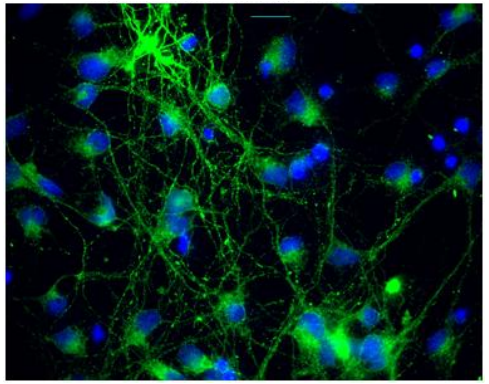
Patterns of CNS Cell and Tissue Immunoreactivity

Pattern 1 – Neuron and Astrocyte Reactive

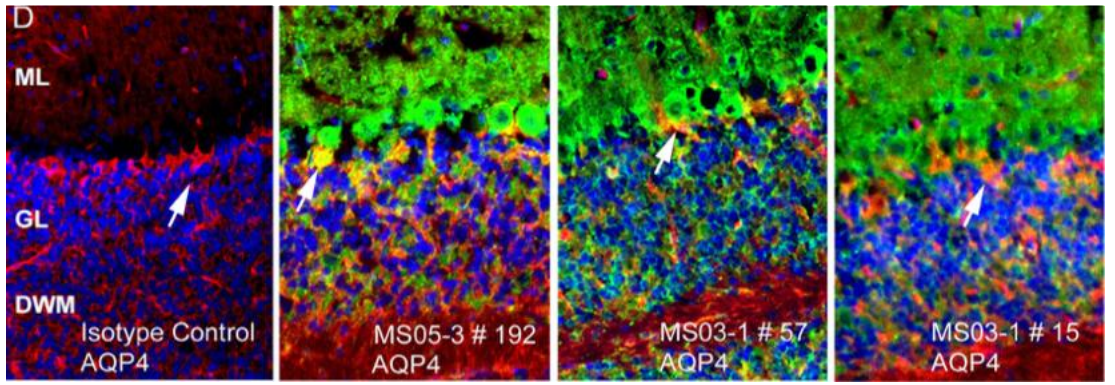
Human Astrocytes



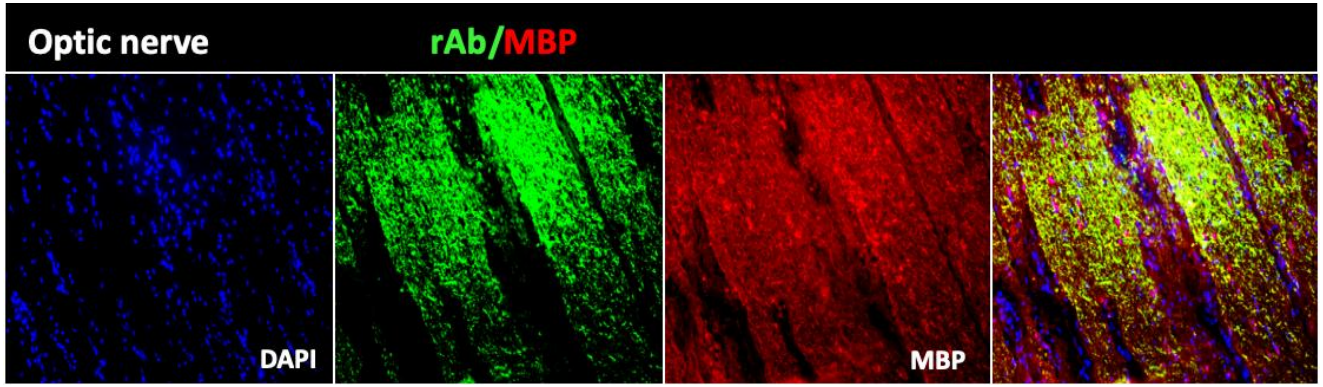
Human Neurons



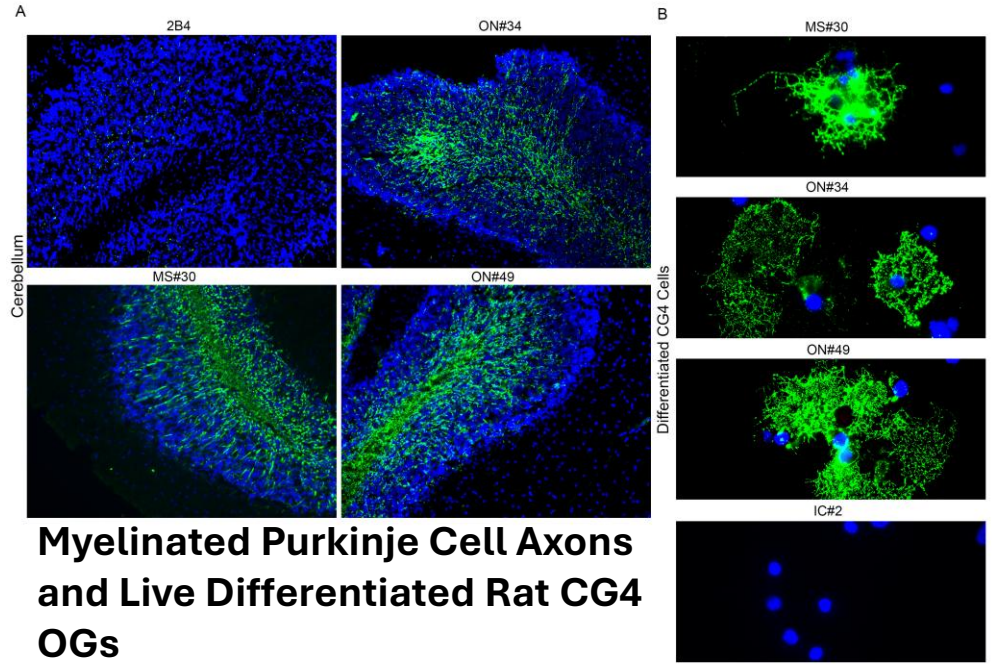
Mouse Cerebellum



Pattern 2 – Myelin-reactive Abs



Blauth et al. (2015). *Acta Neuropathologica* 130:765



Myelinated Purkinje Cell Axons and Live Differentiated Rat CG4 OGs

Models to Address Mechanisms of Pathology

MS rAbs

20 ug/ml rAb + 20% HC

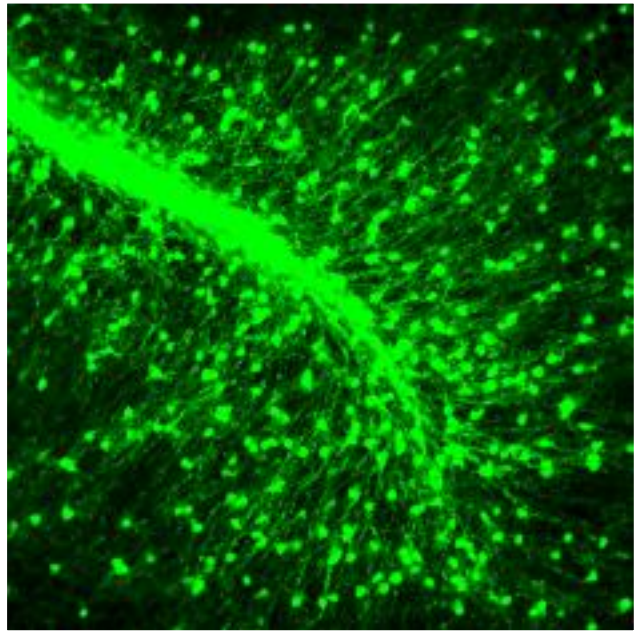
Mallon et al., 2002

PLP-eGFP mouse

20 ug/ml rAb + 20% HC

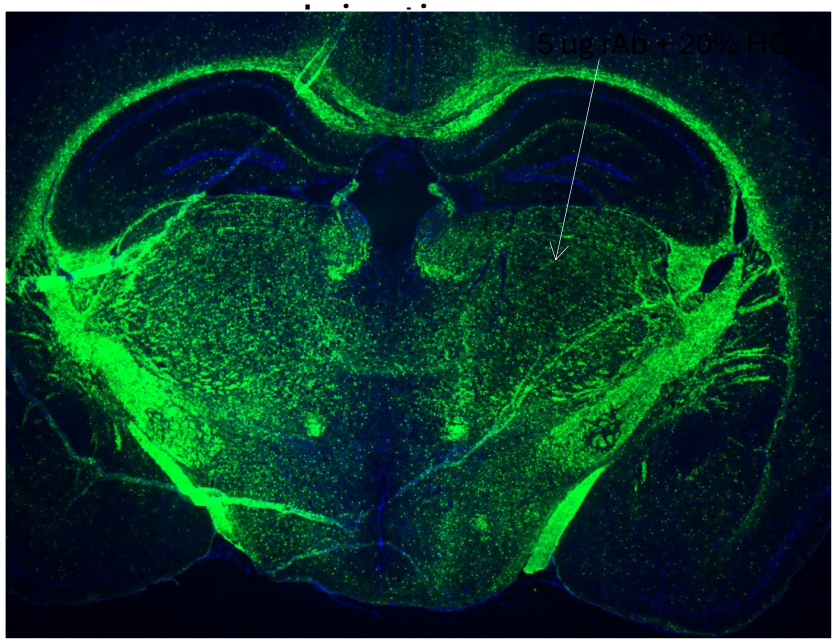
Ex Vivo Cerebellar Slice Cultures

Intracerebral and Spinal Cord



Questions

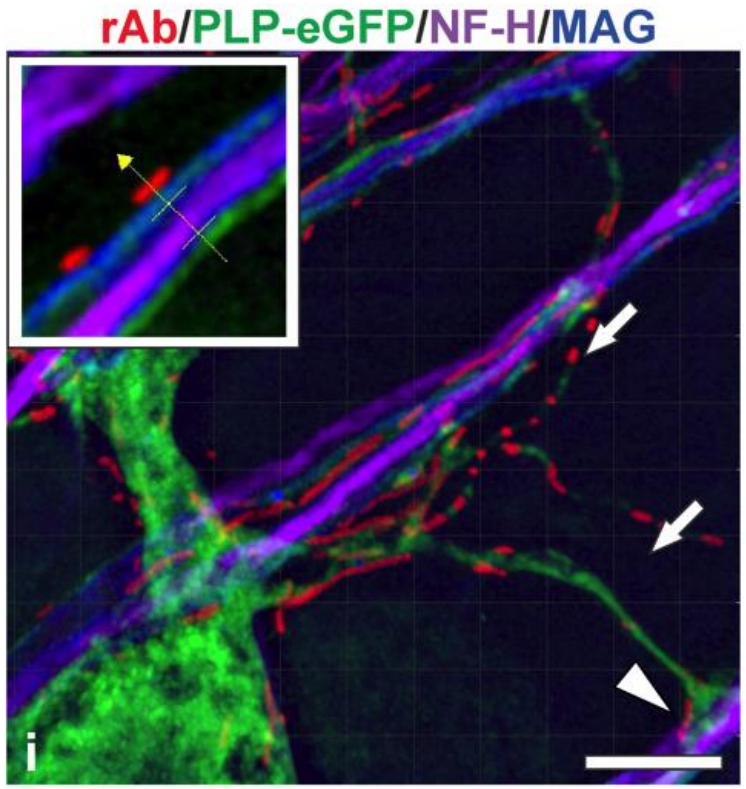
- OG Cell death
- Demyelination
- Repair

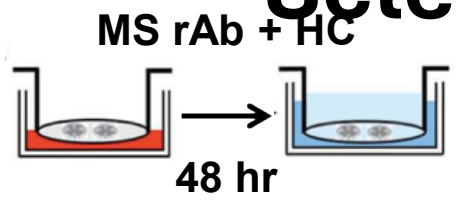


PLP-eGFP mouse labels oligodendrocytes and myelinated processes

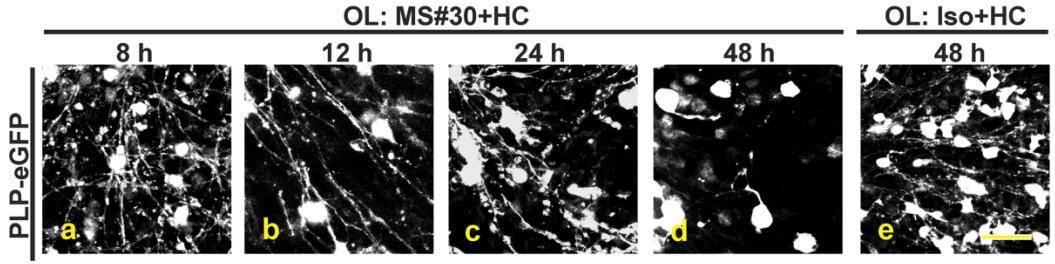
Antibody Pathology: Multiple Sclero

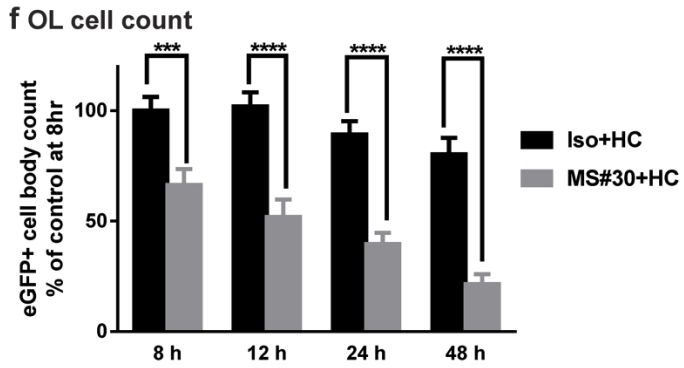
MS CSF: Anti-Myelin rAb

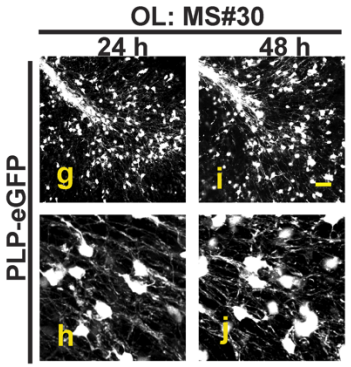


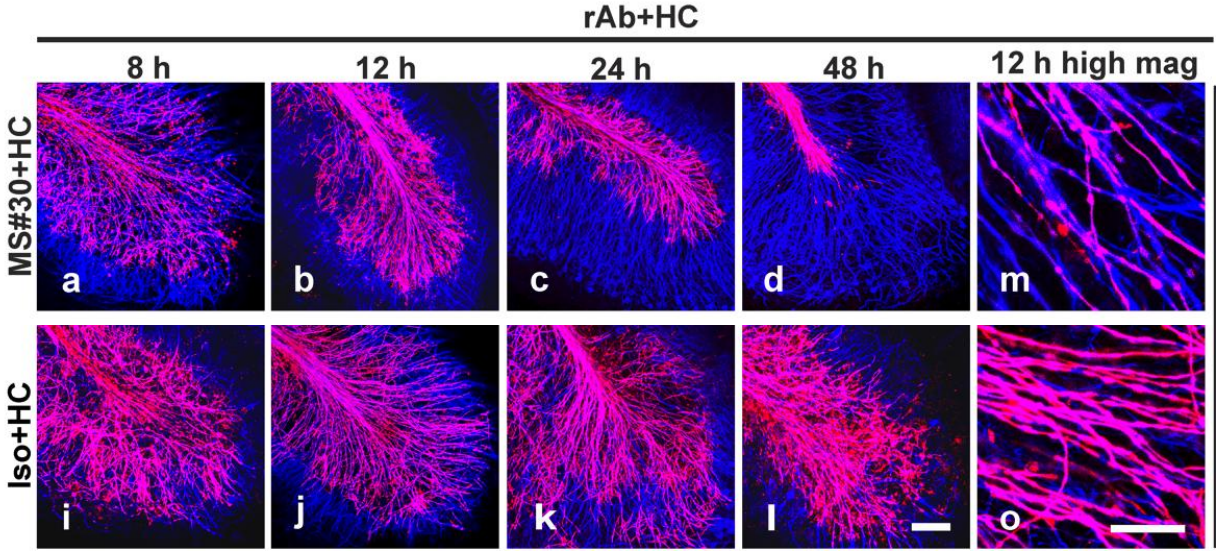


OG Cell Death and Demyelinati







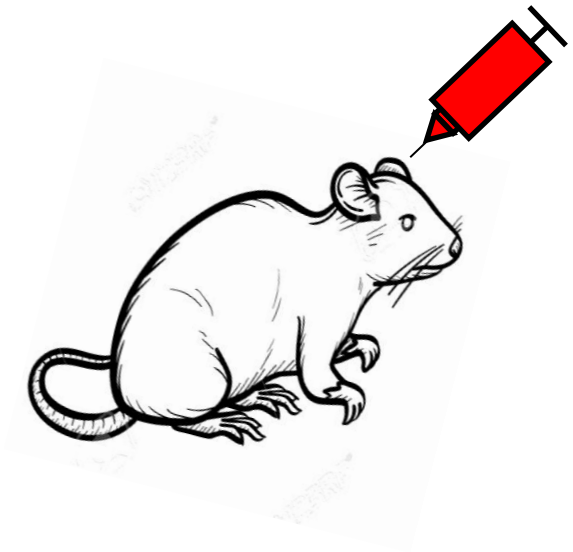


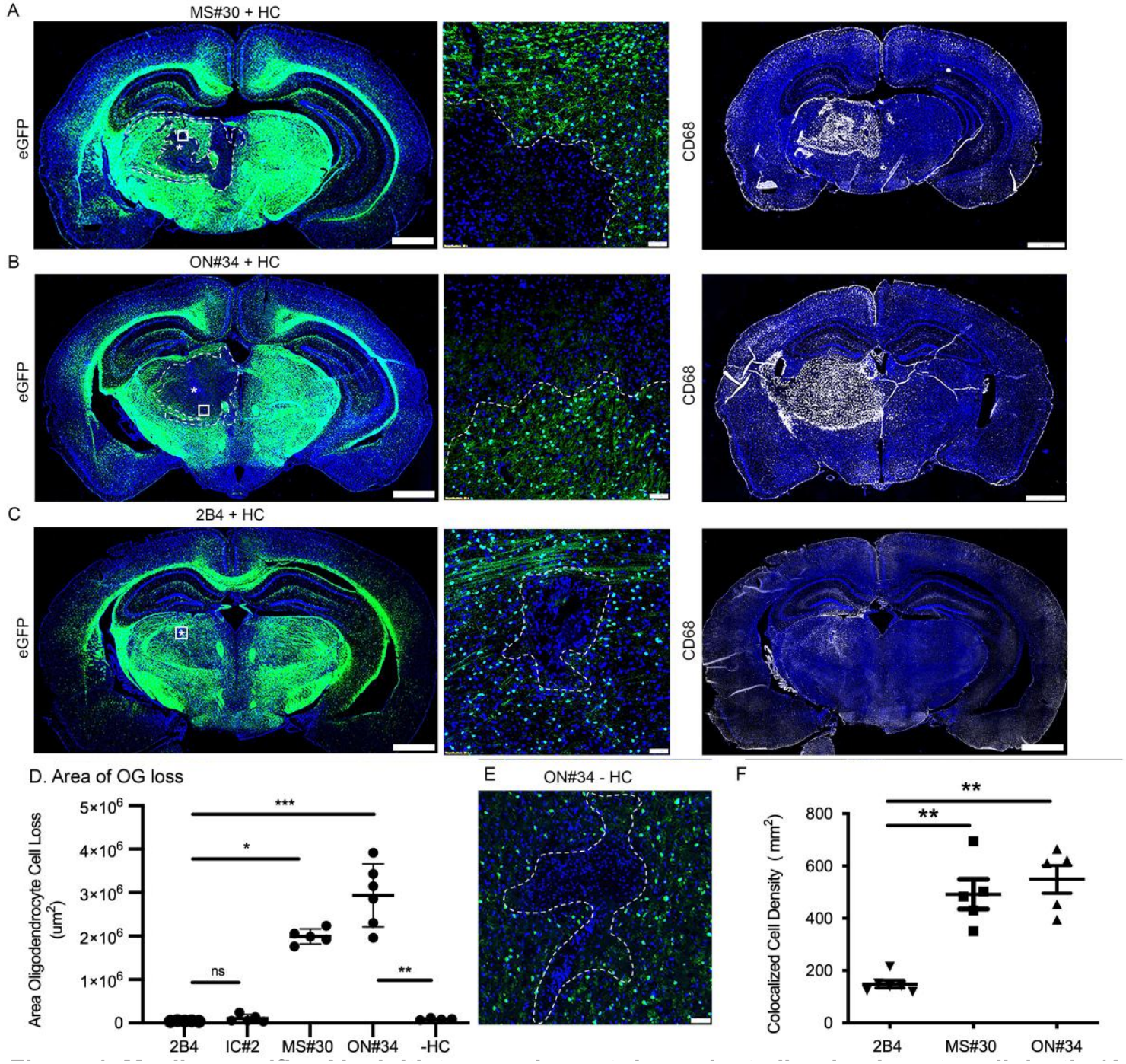
Binding to

- **Surface domains on myelinated axons**
- **Oligodendroglia processes**

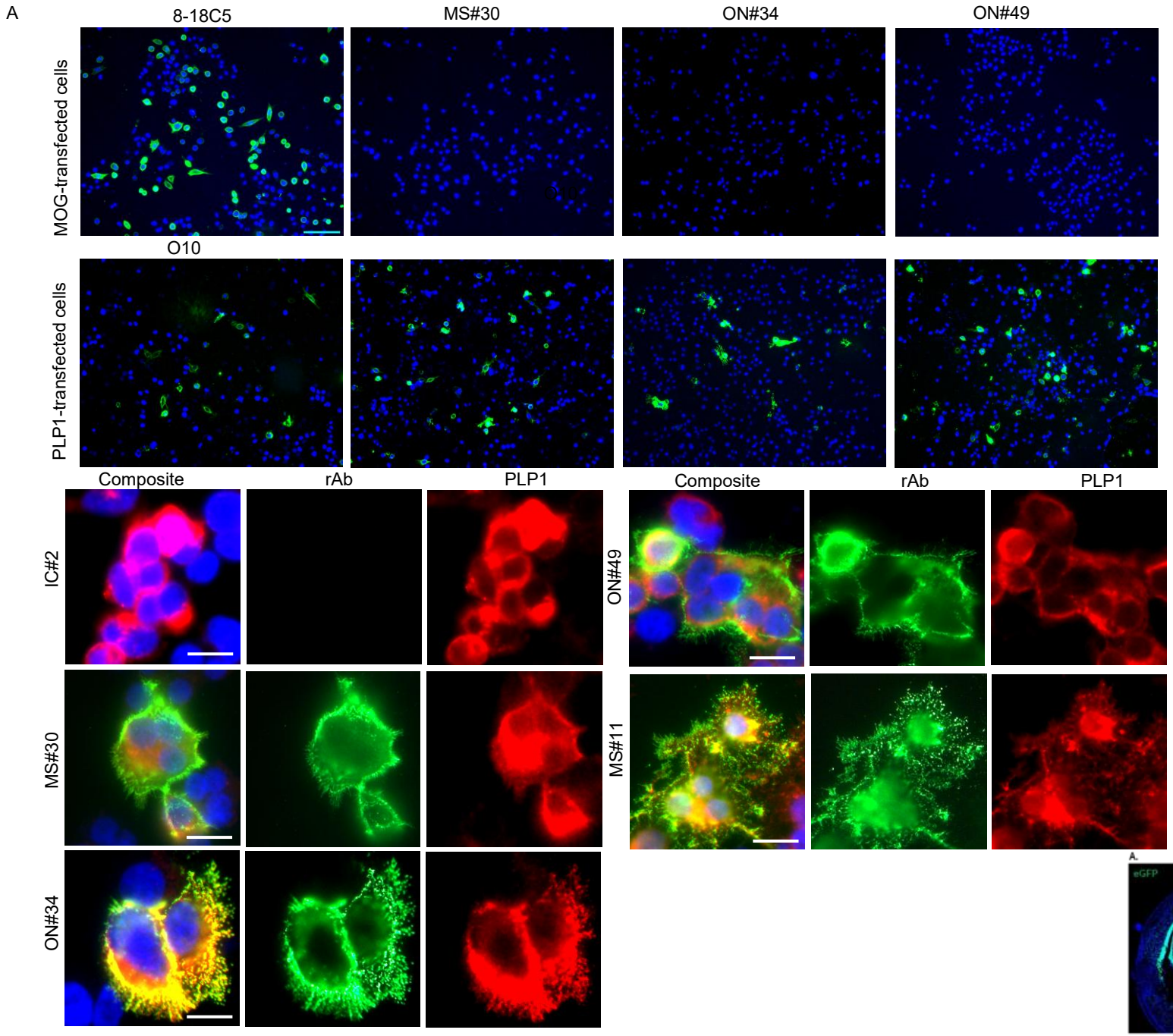
Liu et al. (2017) Acta Neuropathol Comm, 5:25.

Intracerebral injection
MS rAb + HC



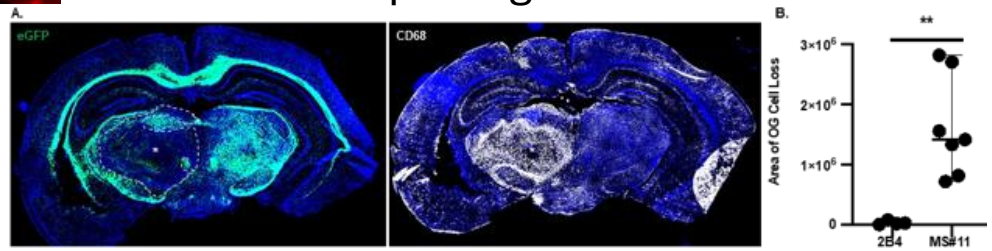


Owens et al, (2023) J Clin Invest, 133(19):

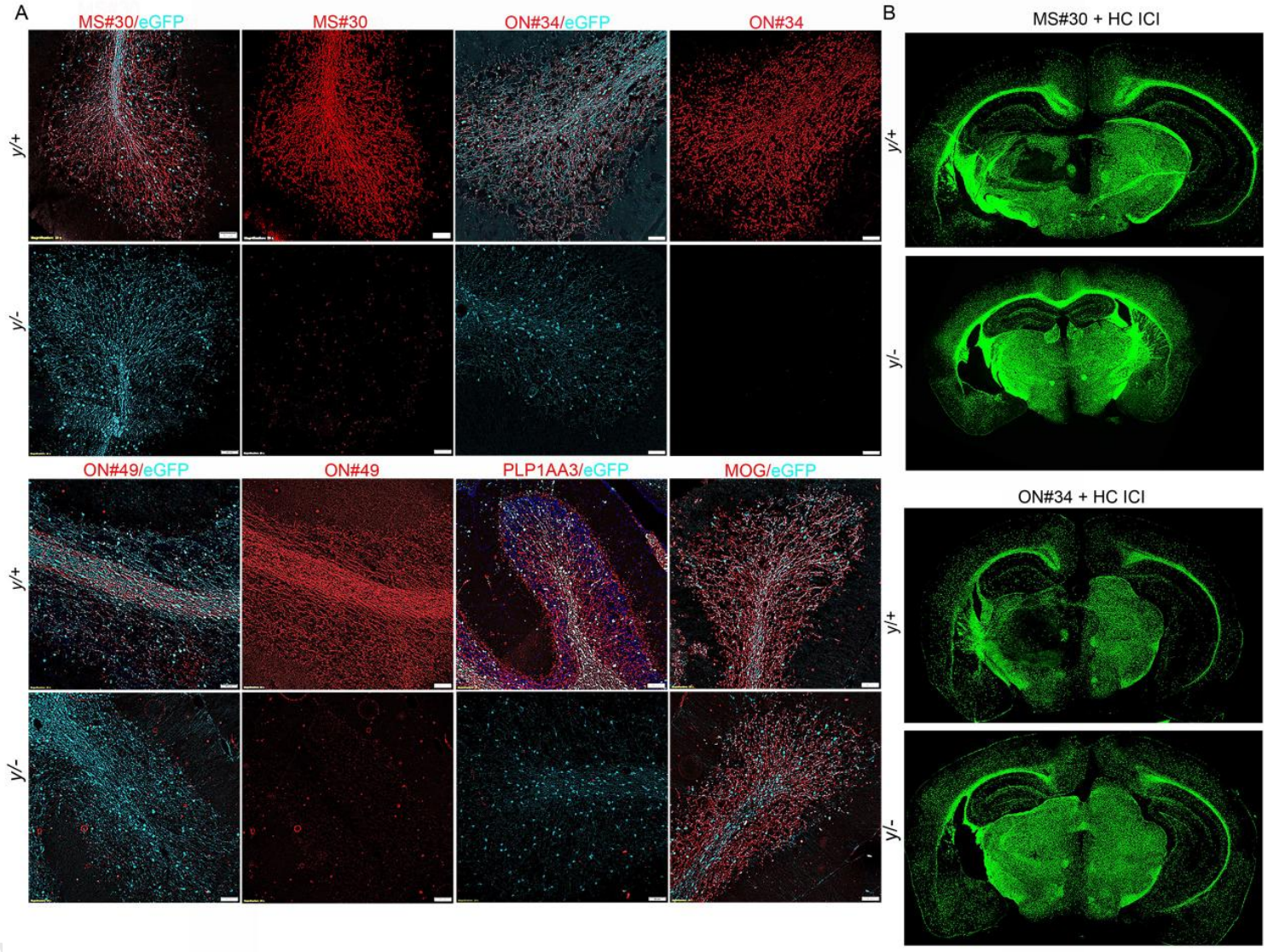


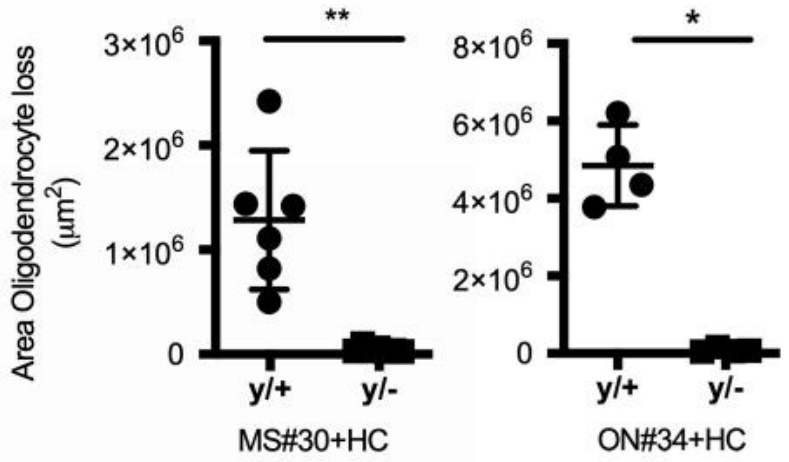
MS rAbs bind to the surface of PLP1-transfected cells.

MS11 is pathogenic



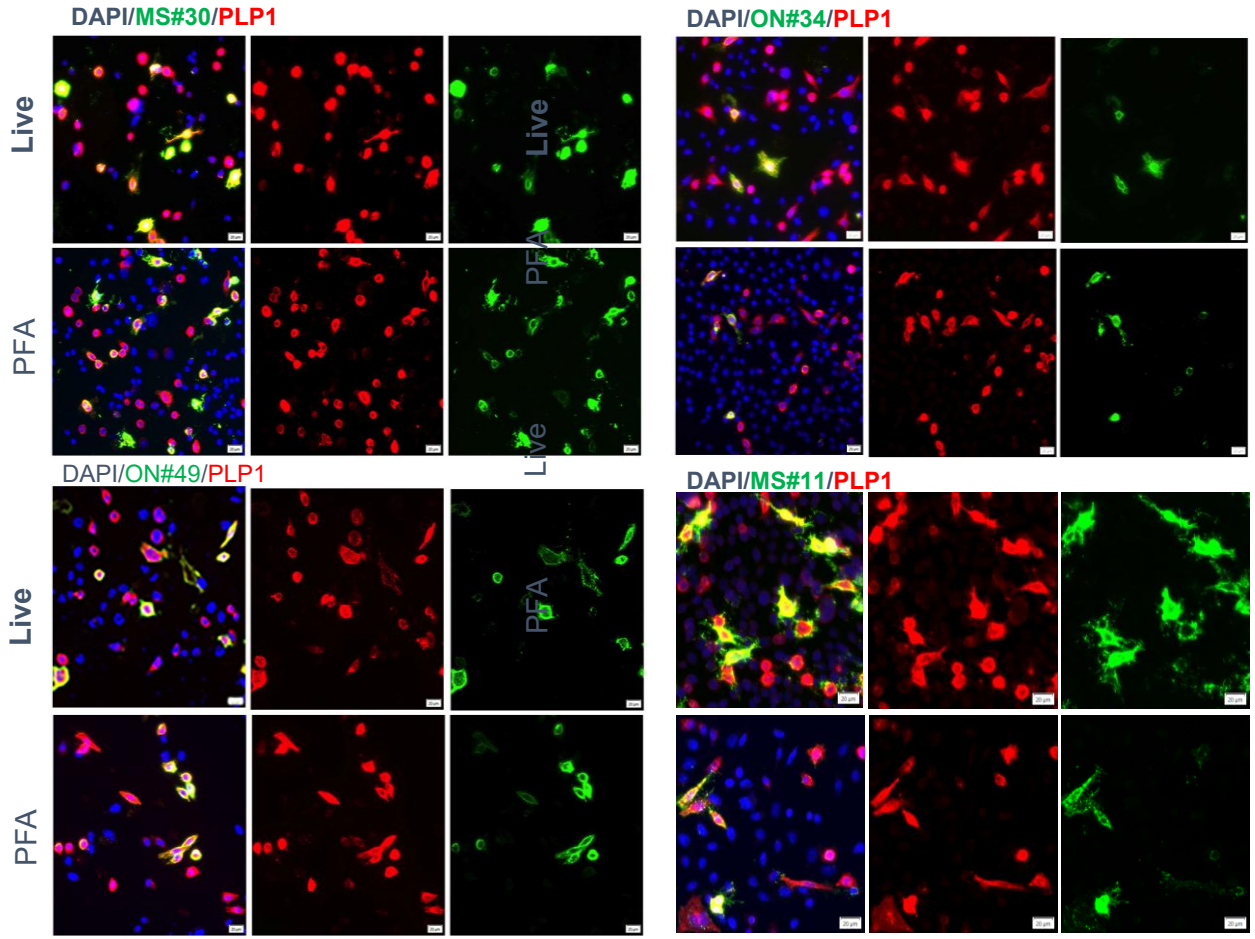
MS rAbs Do Not Bind Myelin or Mediate Demyelination in PLP1 Null Mice

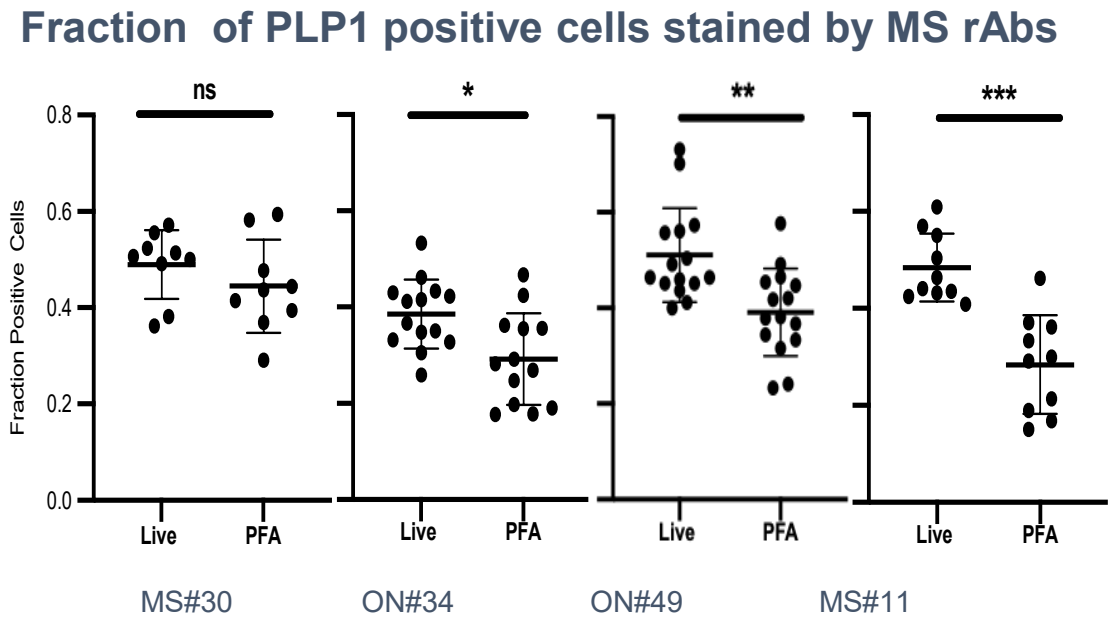




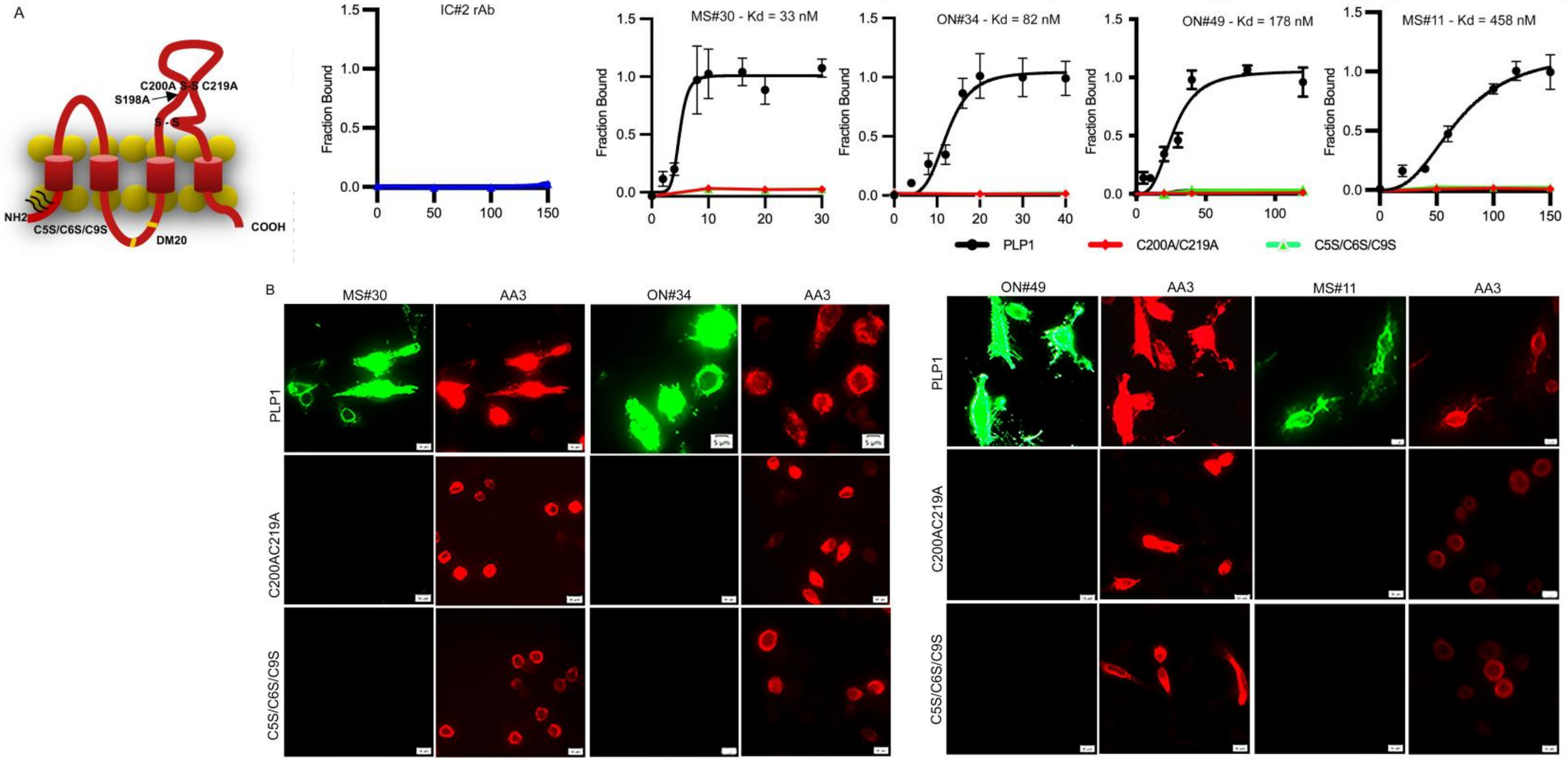
Owens et al, (2023) J Clin Invest, 133(19):e162731.

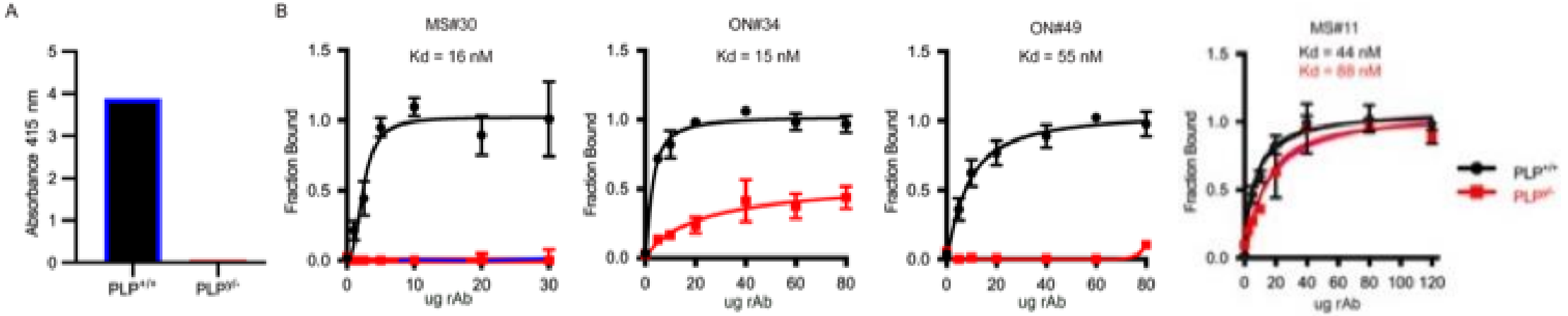
PLP1 Epitopes Appear Post-Translational





PLP1Antigen Recognition requires both Intracellular N-terminal Cysteines and Extracellular Disulfide Domains





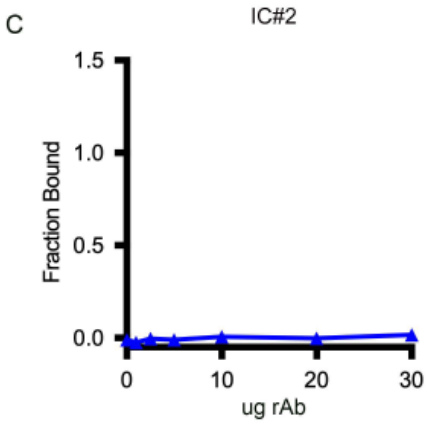
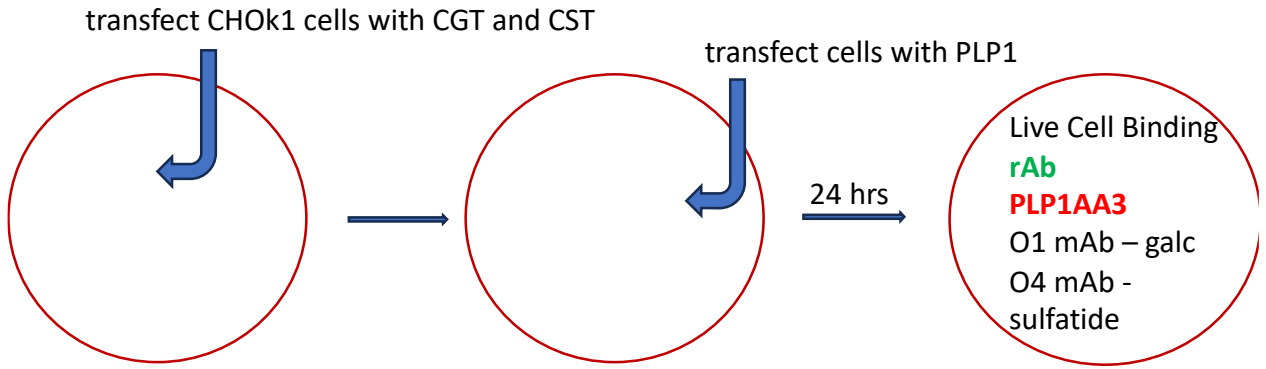


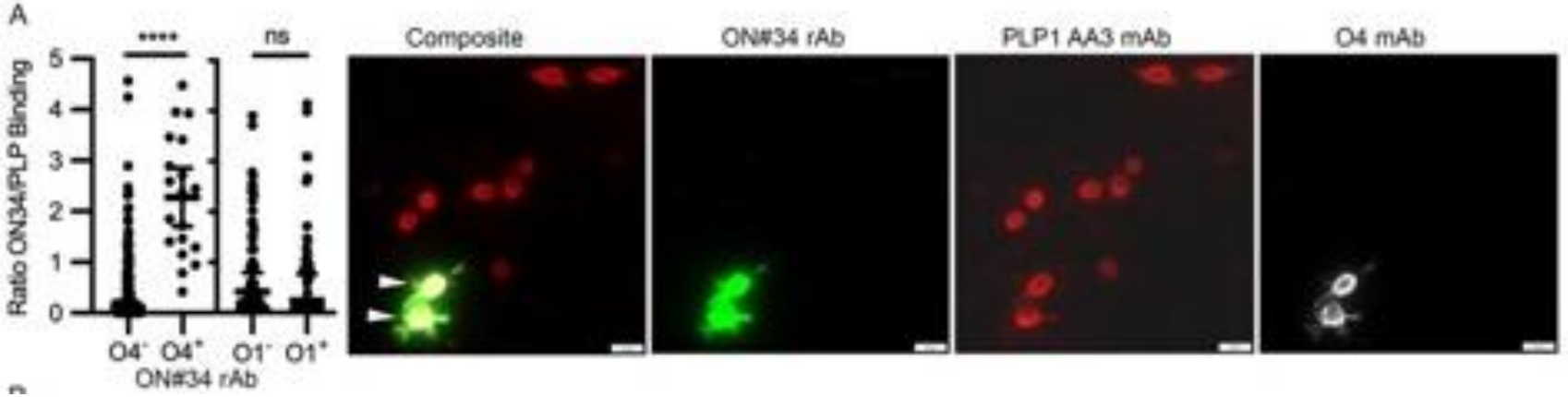
Table 1. Differential binding affinity of myelin-specific rAbs to PLP1-transfected cells and purified myelin

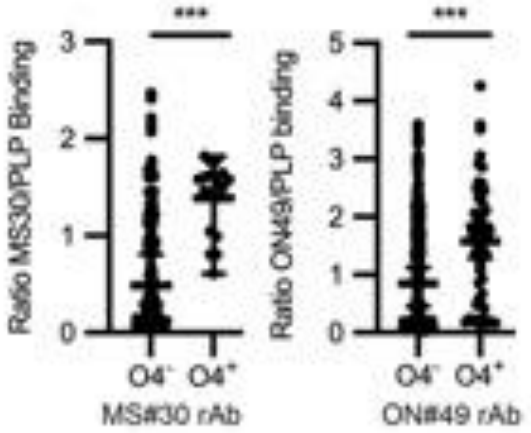
PLP1-specific MS rAb	Transfected CHOK1 Cells	Myelin	p value
MS#30	17 ± 16 nM	22 ± 15 nM	0.823
ON#34	80 ± 2 nM	13 ± 2 nM	<0.001
ON#49	198 ± 17 nM	49 ± 14 nM	<0.001
MS#11	358 ± 88	47 ± 16 nM	0.023

Values represent the average apparent binding affinity of myelin-specific MS rAbs to PLP1-expressing cells (CHOK1) or purified myelin. Values were obtained from 3 - 5 independent assays per antibody and statistical comparisons made using Welch's t-test.

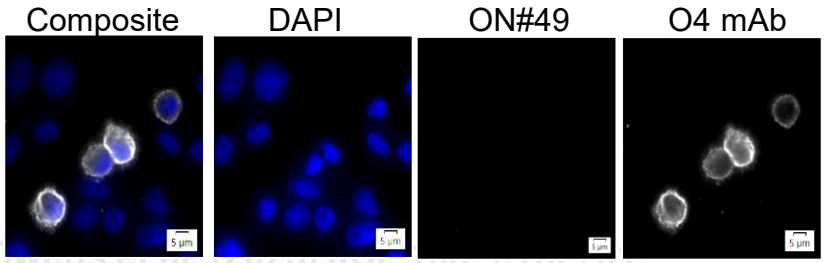
Sulfatide Enhances Binding of MS rAbs to PLP1-expressing Cells

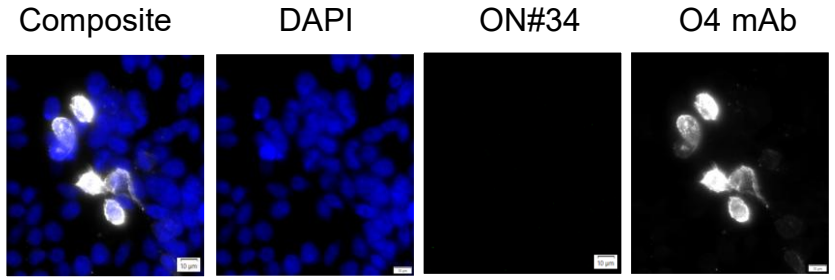




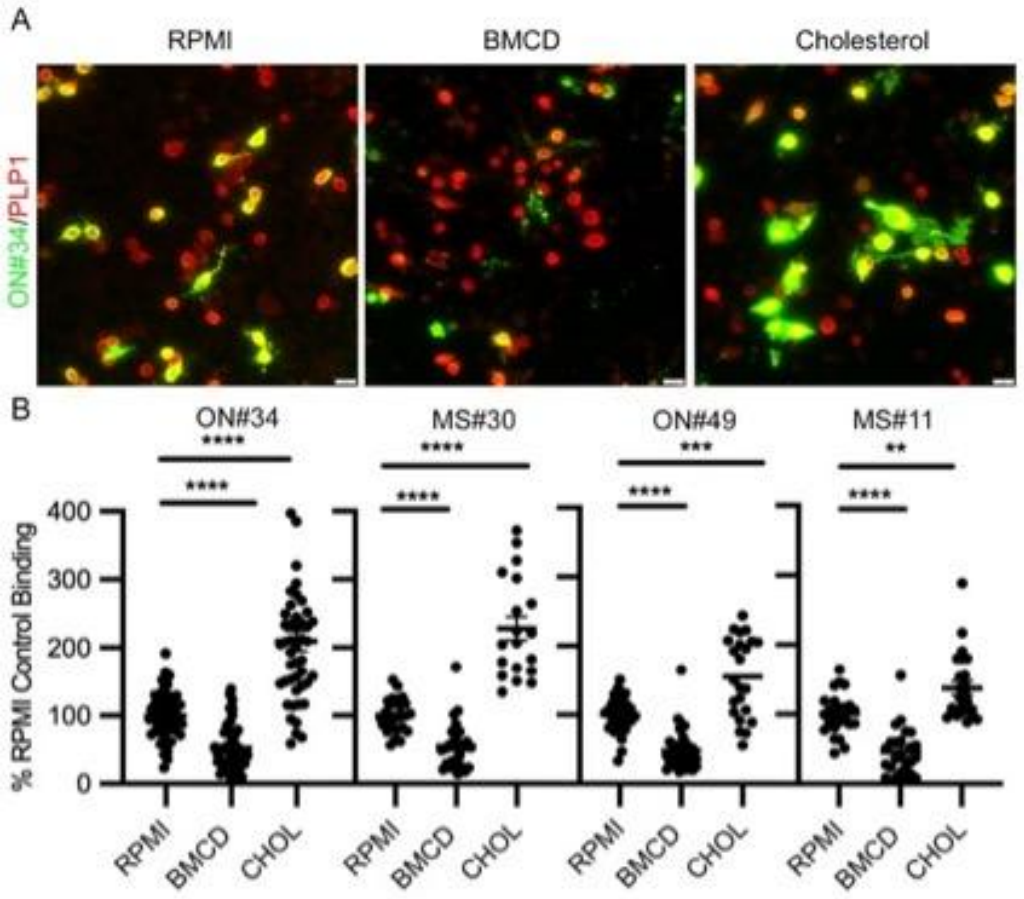


PLP-specific Abs do not bind glycolipids in the absence of PLP1





Cholesterol Affects Binding of MS rAbs to PLP1-expressing Cells



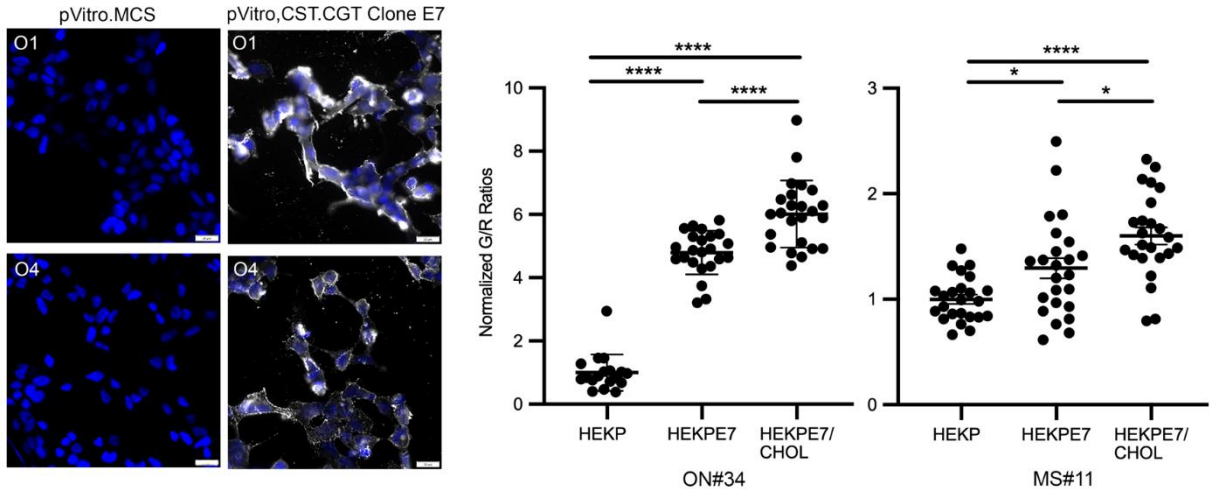
Rationale – PLP1 organized into lipid membrane domains is necessary for antigen recognition. Depletion or addition of cholesterol, an important structural component of membrane rafts and enriched in myelin will modulate MS rAb binding to PLP1.

β-methyclyclodextrin (BCMD): Depletes cholesterol

Cholesterol (CHOL): Cholesterol supplementation

Produced a stable cell line (HEKPE7) in parental HEK293 and Human oligodendroglioma cell lines (HEKP) constitutively expressing surface galc and sulfatide

Figure S7. Effects of sulfatide and cholesterol on antibody binding to PLP1-expressing HEK293 cells.



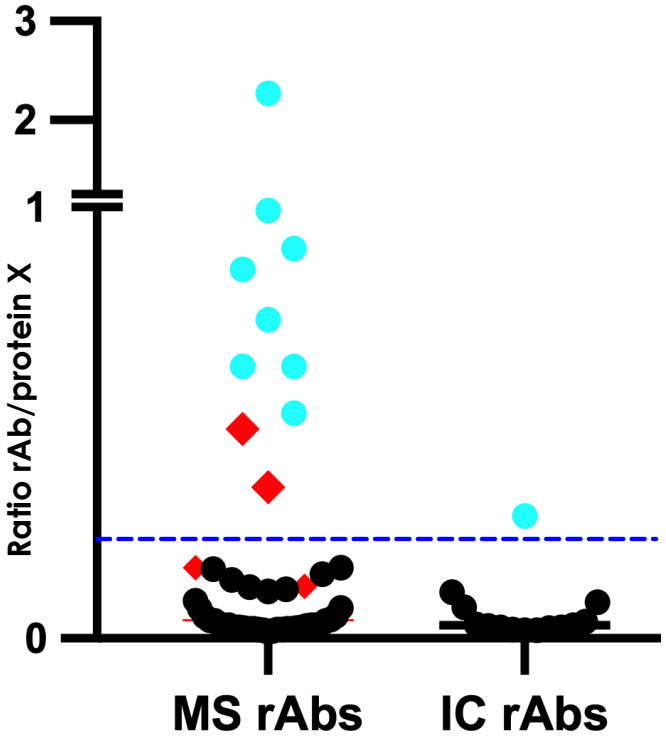
Owens et al. (2023) JCI, 133: e162731.

Working Hypothesis

Antibodies Differentially Recognize PLP1-Enriched Domains

- PLP1 is a tetraspanin, molecules involved in molecular organization
- Cholesterol
- Glycolipids
- Unknown surface protein partners

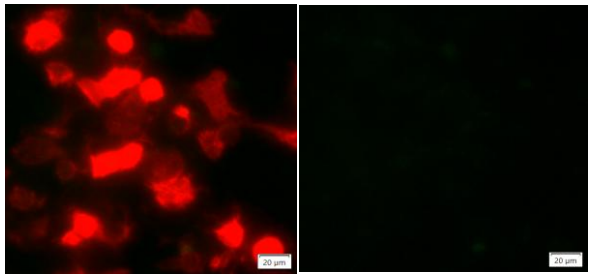
MS & IC rAb Binding to Putative PLP Binding Partner



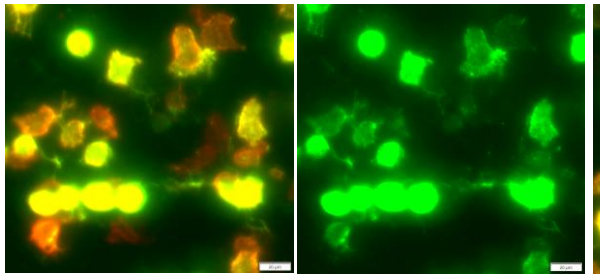
PLP1 BP positive rAbs

PLP1+ MS rAbs

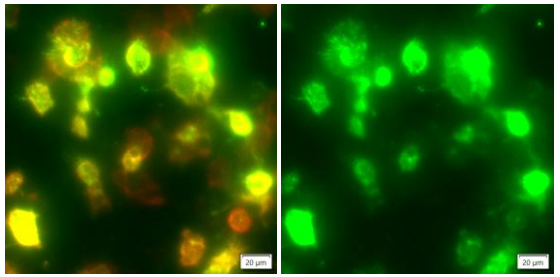
IC#2



MS05-3 #192



MS03-1 #3



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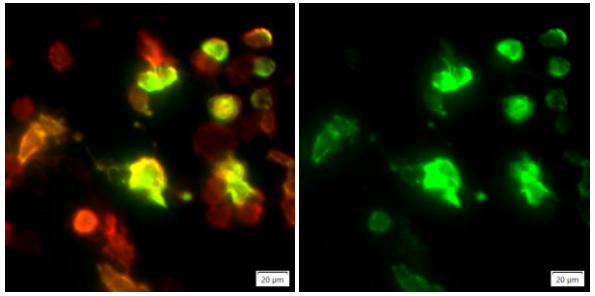
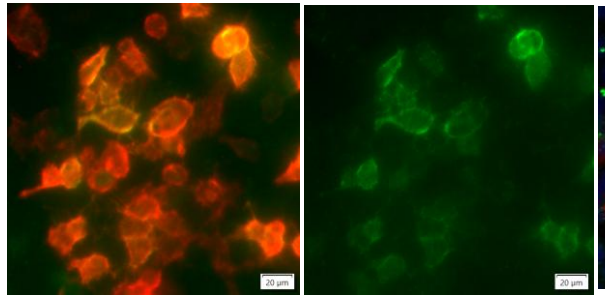
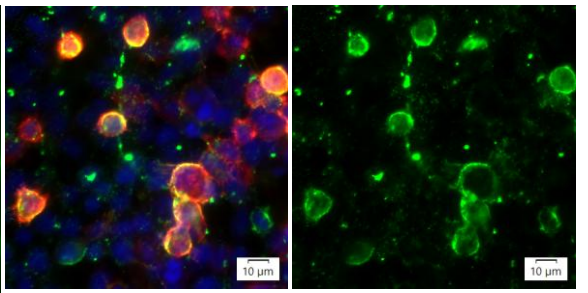


ONO7-7 #49



MS03-1#15



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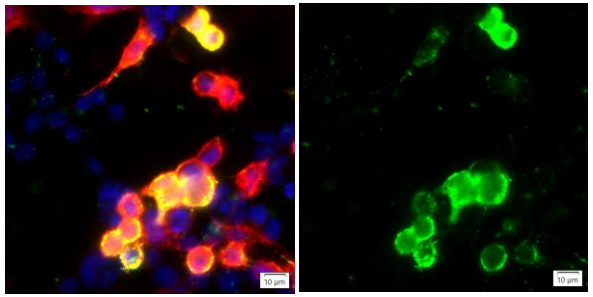

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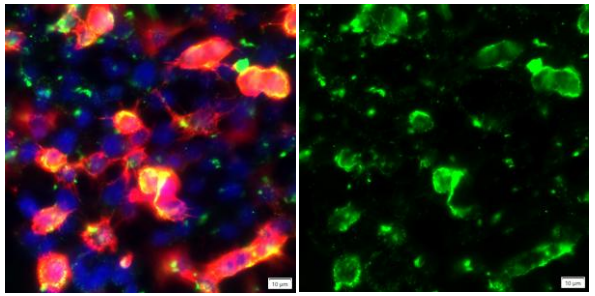
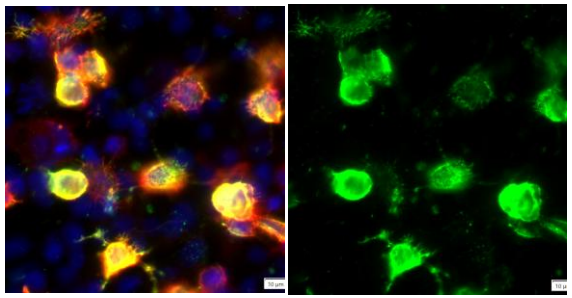
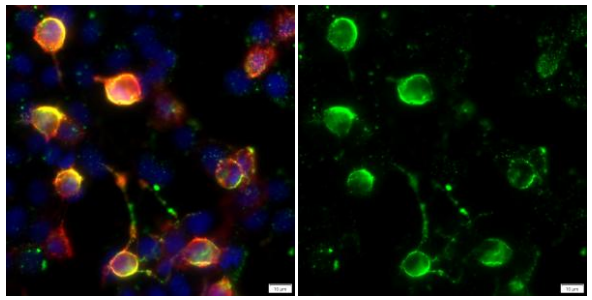


MS03-1 #69



ST#176(MS plaque)



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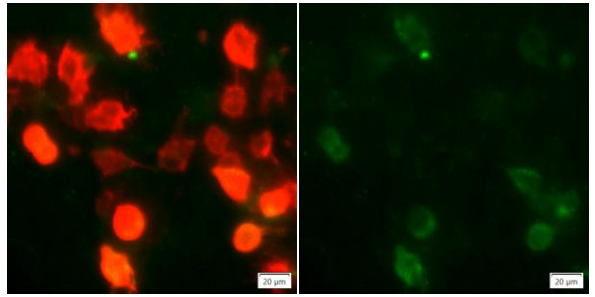
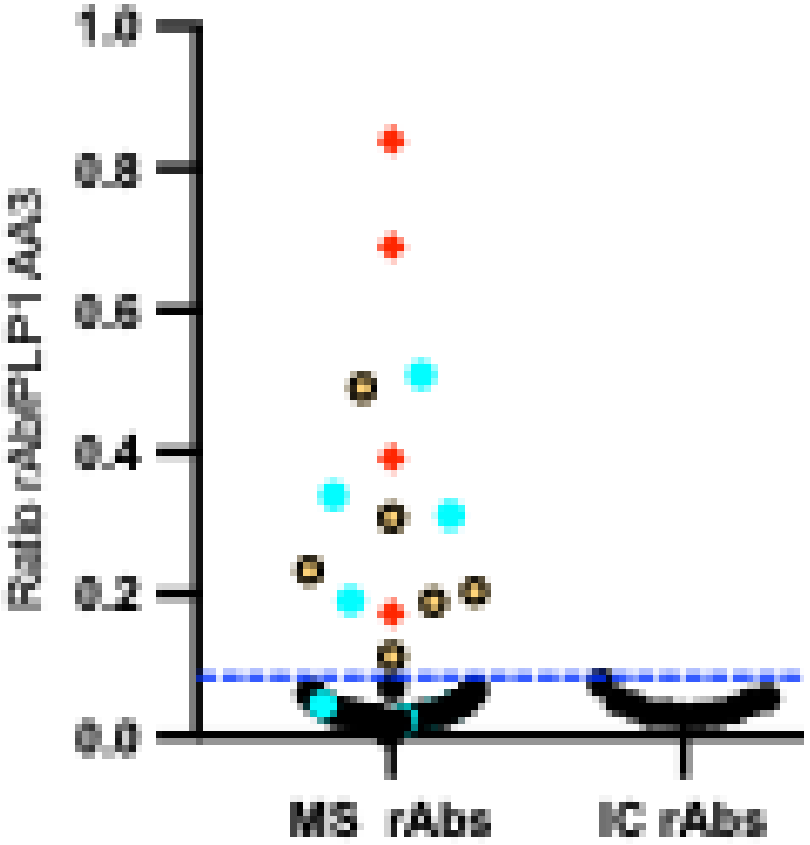
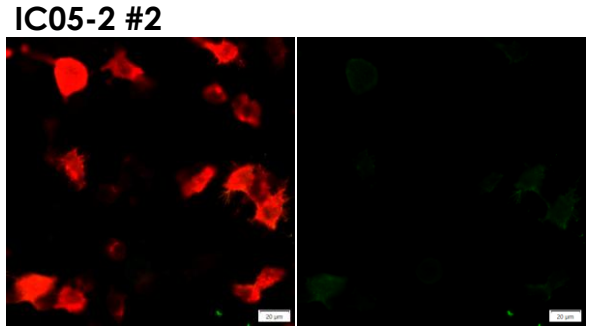
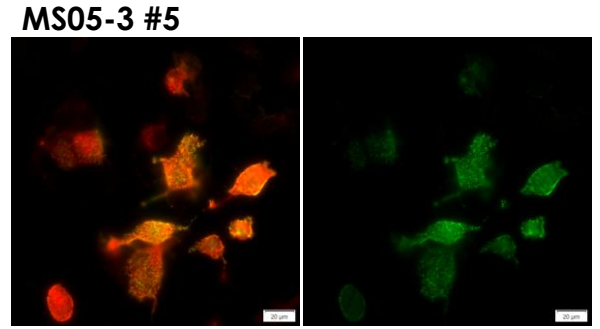


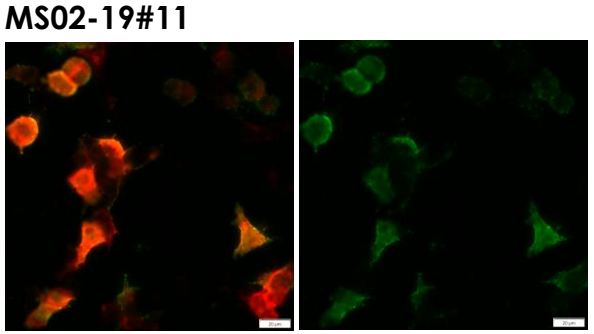
MS & IC rAb Binding to PLP1/PLP1Binding Partner Expressing Cells

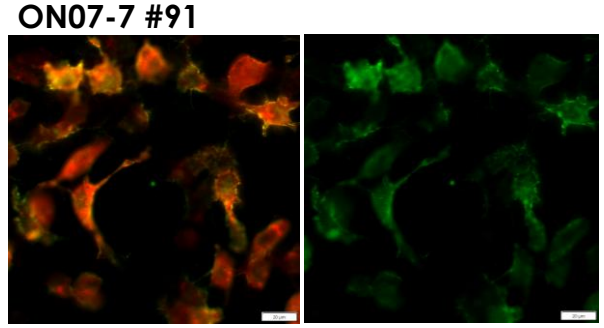


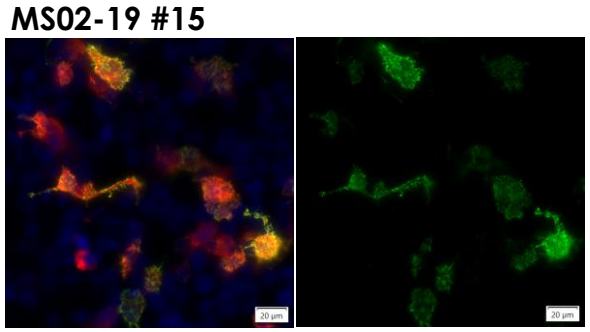
- pLP1 BP+ rAbs
- PLP1+ MS rAbs
- PLP1/BP dependent

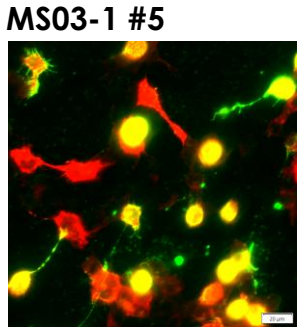


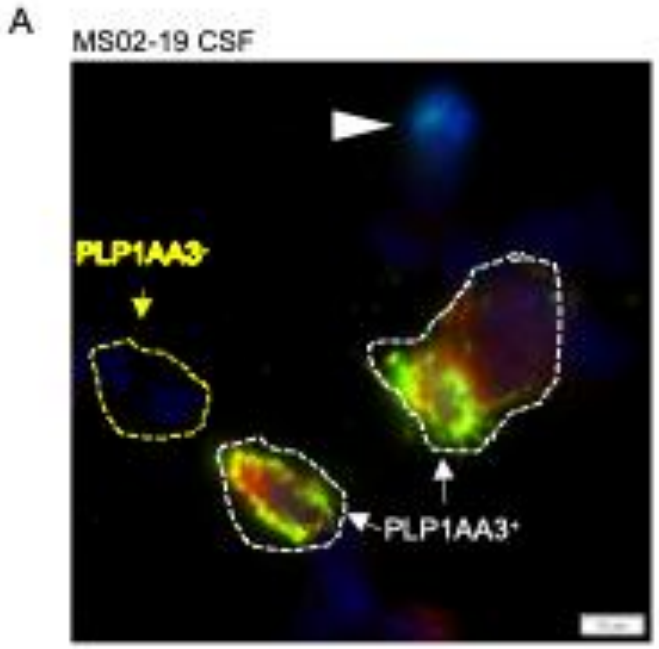






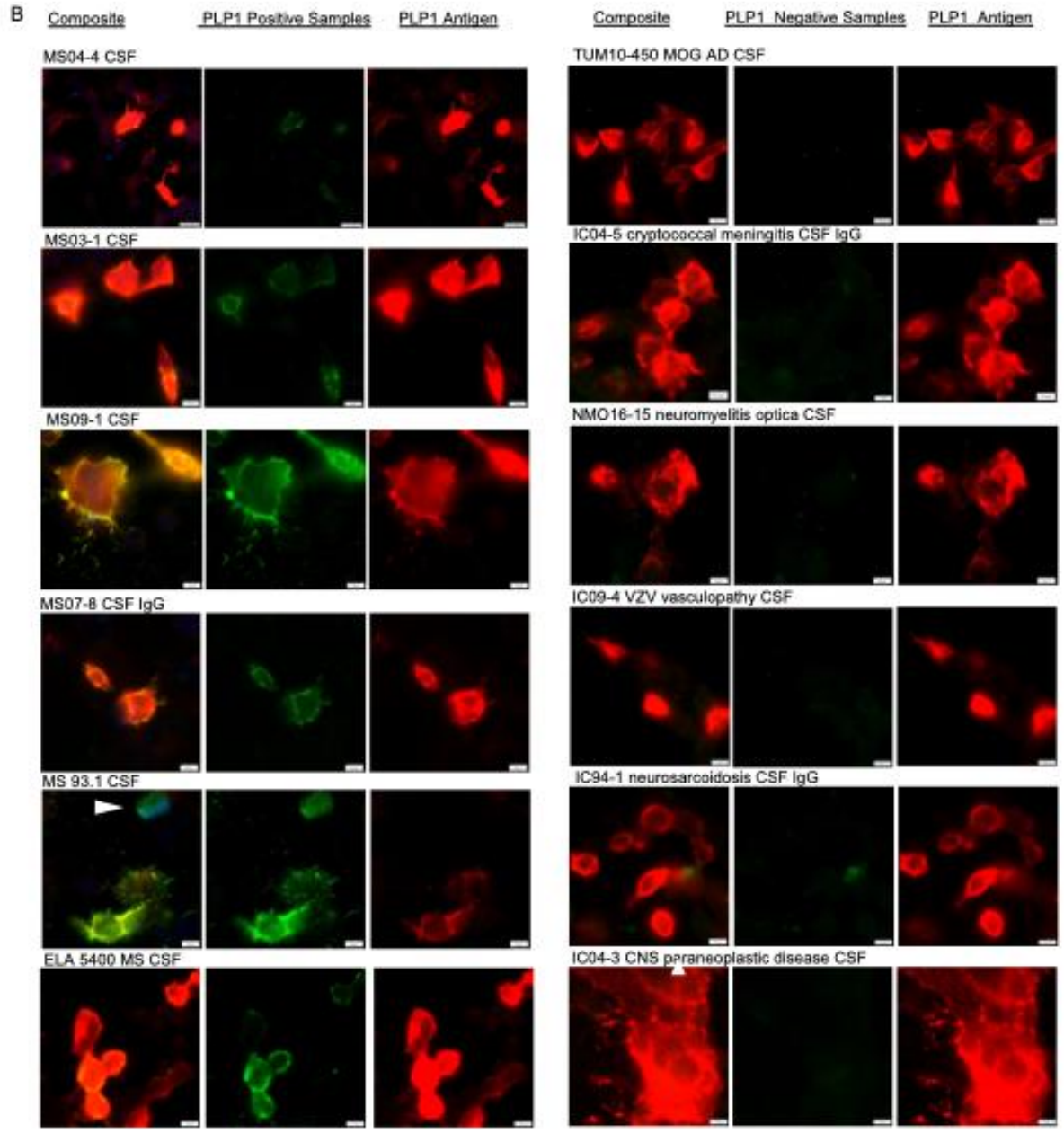




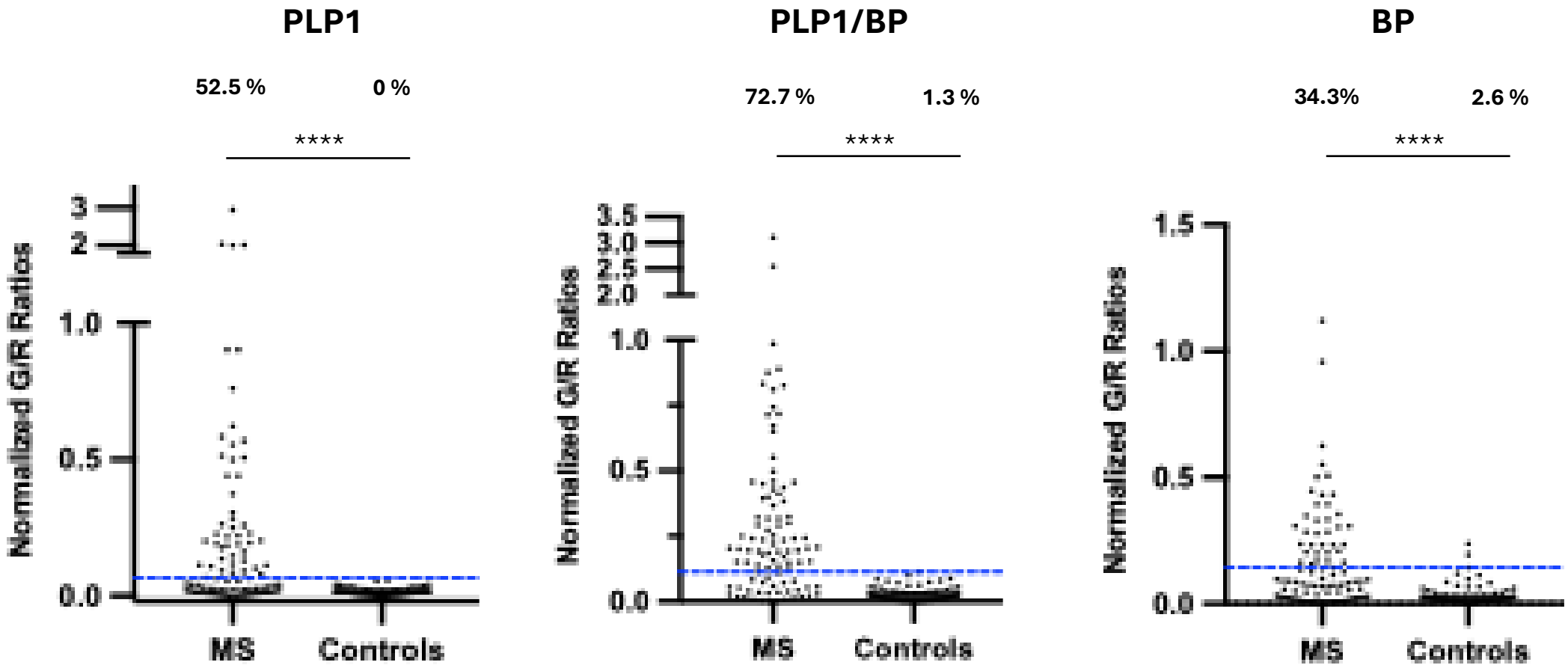


$$\text{Normalized G/R Ratio} = \frac{GF^{PLP1+} - GF^{PLP1-}}{RF^{PLP1+} - RF^{PLP1-}} \times \frac{\text{ON34 rAb G/R}^{Exp\ 1}}{\text{ON34 rAb G/R}^{Exp\ x}}$$

Semi-quantitative binding assay to screen neat MS and control CSF for PLP1c positive binding.



PLP1C Binding in CU MS Patient Cohort



Positive G/R ratio = 3 SD greater than mean of neurologic controls

CSF Binding Assays - CU Cohort

CSF Source	#	PLP1 Pos	% Pos.	3 x SD
Neurologic CTLs	n = 79	0	0%	> 0.052
MS/CIS	n = 101	53	52.5 %	

CSF Source	#	PLP1/ BP	% Pos.	3 x SD
Neurologic CTLs	n = 80	1	1.3 %	> 0.106
MS/CIS	n = 100	72	72.0 %	

CSF Source	#	BP Pos	% Pos.	3 x SD
Neurologic CTLS	n = 77	2	2.6 %	> 0.160
MS/CIS	n = 99	34	34.3 %	

Single Assay-specific Positives

ASSAY	PLP1	PLP1/IgSF8	BP
Neurologic CTLS	0	2	1
MS/CIS	6	13	2

CSF Source	#	All Assays	% Pos.
Neurologic CTLS	n = 80	3	3.7 %
MS/CIS	n = 101	81	80.2 %

Sensitivity [95% CI]

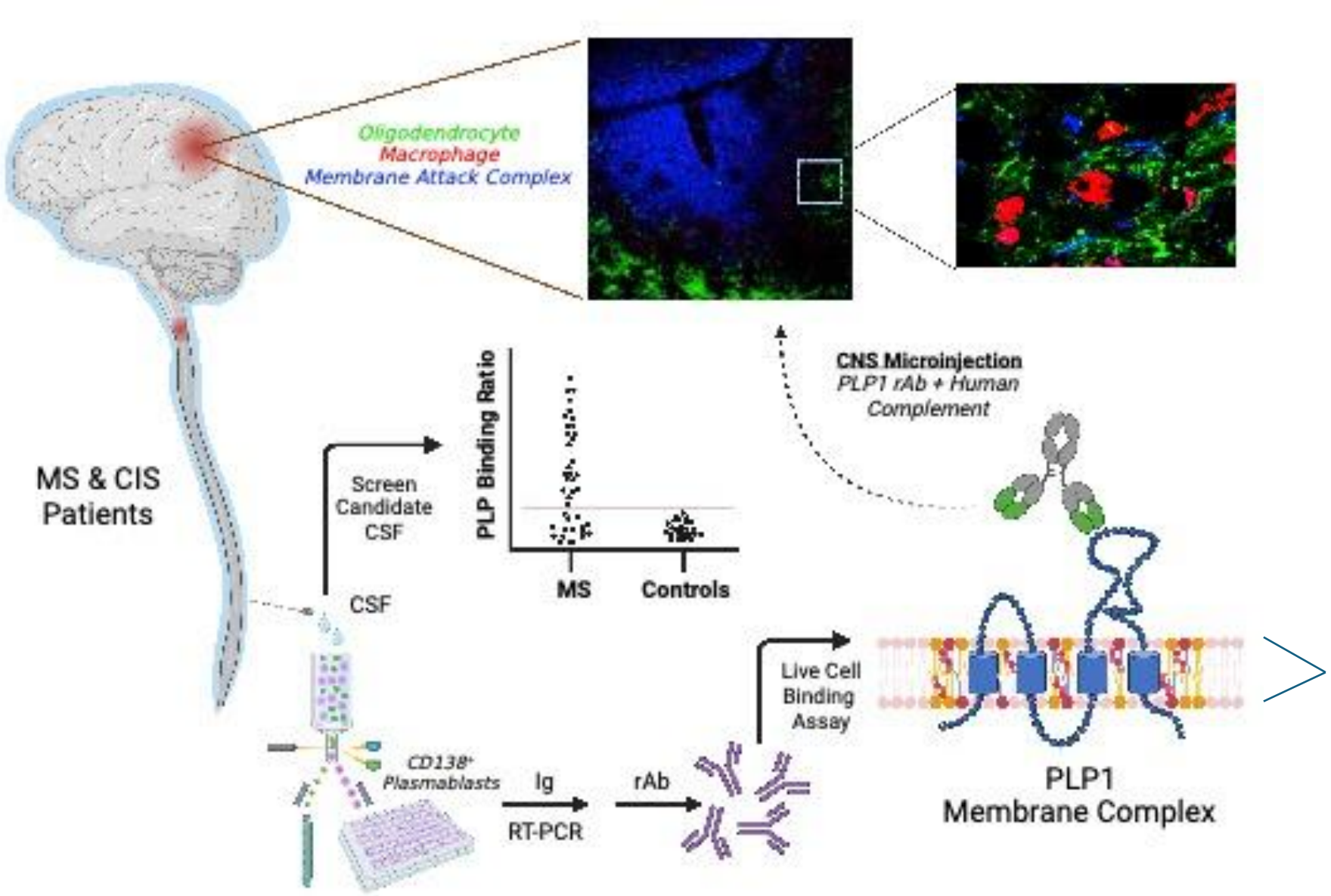
- PLP1: 52.5% [42.8 - 61.9]
- PLP1/IBP: 72.0% [62.5 - 79.9]
- BP: 34.4% [25.7- 44.1]

Specificity [95% CI]

- PLP1: 100% [95.3 - 100]
- PLP1/BP: 98.8% [93.2 - 99.9]
- BP: 97.4% [91.0 - 99.5]

MS CSF Cohorts	#	Positives	% Pos.
Pediatric MS – Johns Hopkins	n = 12	8	67%
Italy – Verona	n = 56	38	68%
OBOE Patient Trial – Wash U	n= 108	73	68%
Munich -TUM	n = 97	In progress	

SUMMARY


Oligodendrocyte
Macrophage
Membrane Attack Complex
MS & CIS Patients
Screen Candidate CSF
CSF
PLP Binding Ratio
MS
Controls
CNS Microinjection
PLP1 rAb + Human Complement
Live Cell Binding Assay
CD138+ Plasmablasts
Ig
RT-PCR
rAb
PLP1 Membrane Complex

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