Supplementary Table 1 Patient Demographics for CSF samples used in lysolecithin model

Diagnosis	**PPMS**	**RRMS**	**SPMS**	**HC**
No. of patients (CSF samples)	8	7	6	3
Age at sample collection, mean (SD), years	56.3 (9.3)	38.9 (12.9)	60.0 (11.1)	50.3 (17.6)
Gender distribution	3 F; 5 M	7 F; 0 M	4 F; 2 M	1 F; 2 M
EDSS at sample collection, mean (SD)	6.13 (1.96)	1.93 (2.40)	5.70 (1.04)	N/A
Disease duration at sample collection, mean (SD), years	10.6 (7.1)	2.5 (1.7)	7.2 (3.9)	N/A
CSF cell count per mL, mean (SD)	1535 (1257)	3837 (3583)	1775 (2666)	N/A
CSF protein concentration, mean (SD), µg/mL	929.1 (253.1)	787.7 (167.7)	872.3 (255.5)	793.3 (221.0)

HC: healthy control; EDSS: Expanded Disability Status Scale

Supplementary Table 2 Patient Demographics for PPMS CSF samples used for filtration and IgG depletion

	5 kDa filtration	100 kDa filtration	IgG depletion
No. of patients (CSF samples)	4	4	3
Age at sample collection, mean (SD), years	44.5 (14.1)	40.0 (12.6)	40.0 (13.2)
Gender distribution	2 F; 2 M	2 F; 2 M	1 F; 2 M
EDSS at sample collection, mean (SD)	5.4 (3.2)	3.9 (3.2)	4.3 (2.9)
Disease duration at sample collection, mean (SD), years	11.8 (12.3)	8.6 (11.8)	7.0 (9.6)
CSF cell count per mL, mean (SD)	13024 (20664)	14868 (20033)	16797 (23561)
CSF protein concentration, mean (SD), µg/mL	781.0 (203.1)	854.8 (181.2)	830.1 (217.7)

EDSS: Expanded Disability Status Scale

Supplementary Table 3 Cell counts for CSF samples used to produce IgG1 rAbs for intrathecal injections

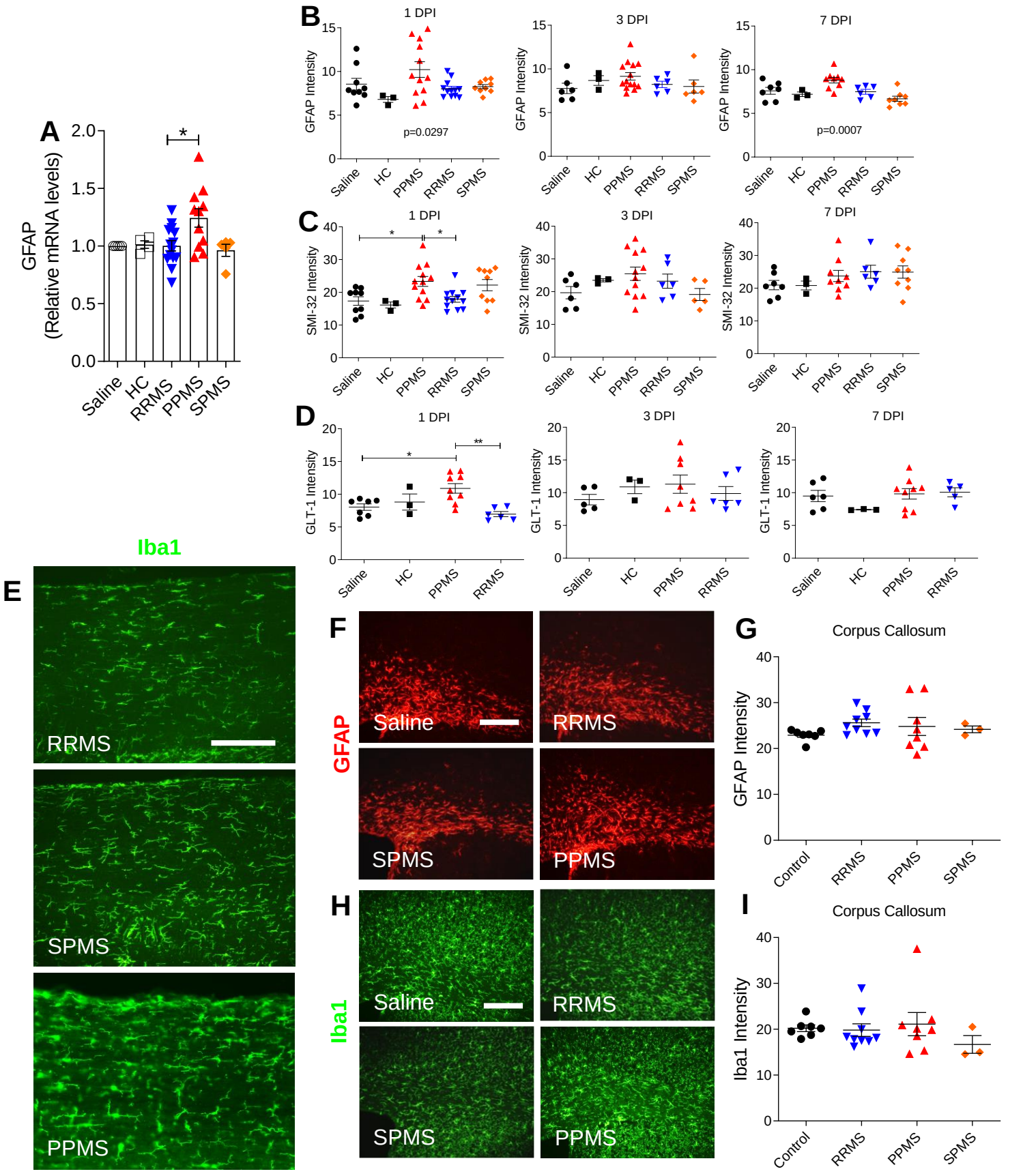
Patient CSF sample	No. of lymphocytes	No. of $CD19^{+}CD138^{-}$ B cells	No. of $CD19^{+}CD138^{+}$ plasmablasts	No. of $CD19^{-}CD138^{+}$ plasma cells	No. of expanded clones	Isotype of original antibody
PPMS 1	1640	10	0	12	0	IgG/IgM
PPMS 2	12746	84	32	4	2	IgG
PPMS 3	13271	59	71	15	5	IgG
PPMS 4	1701	10	9	3	0	IgG
PPMS 5	98390	2772	454	10	6	IgG
PPMS 6	51335	641	580	8	7	IgG
PPMS 7	65228	936	903	44	5	IgG
RRMS 1	6042	20	1	6	0	IgM
RRMS 2	4429	7	0	0	0	IgM
RRMS 3	59838	1981	84	6	3	IgG
RRMS 4	6205	39	7	14	1	IgG
SPMS 1	12442	204	58	6	1	IgA
SPMS 2	6017	146	304	18	7	IgG
SPMS 3	995	7	31	0	1	IgG
SPMS 4	8850	28	40	56	2	IgG
DC 1	16900	145	17	18	0	IgG
DC 2	1736	28	0	9	0	IgM
DC 3	5171	11	0	4	0	IgG

rAb: recombinant antibody; DC: disease control

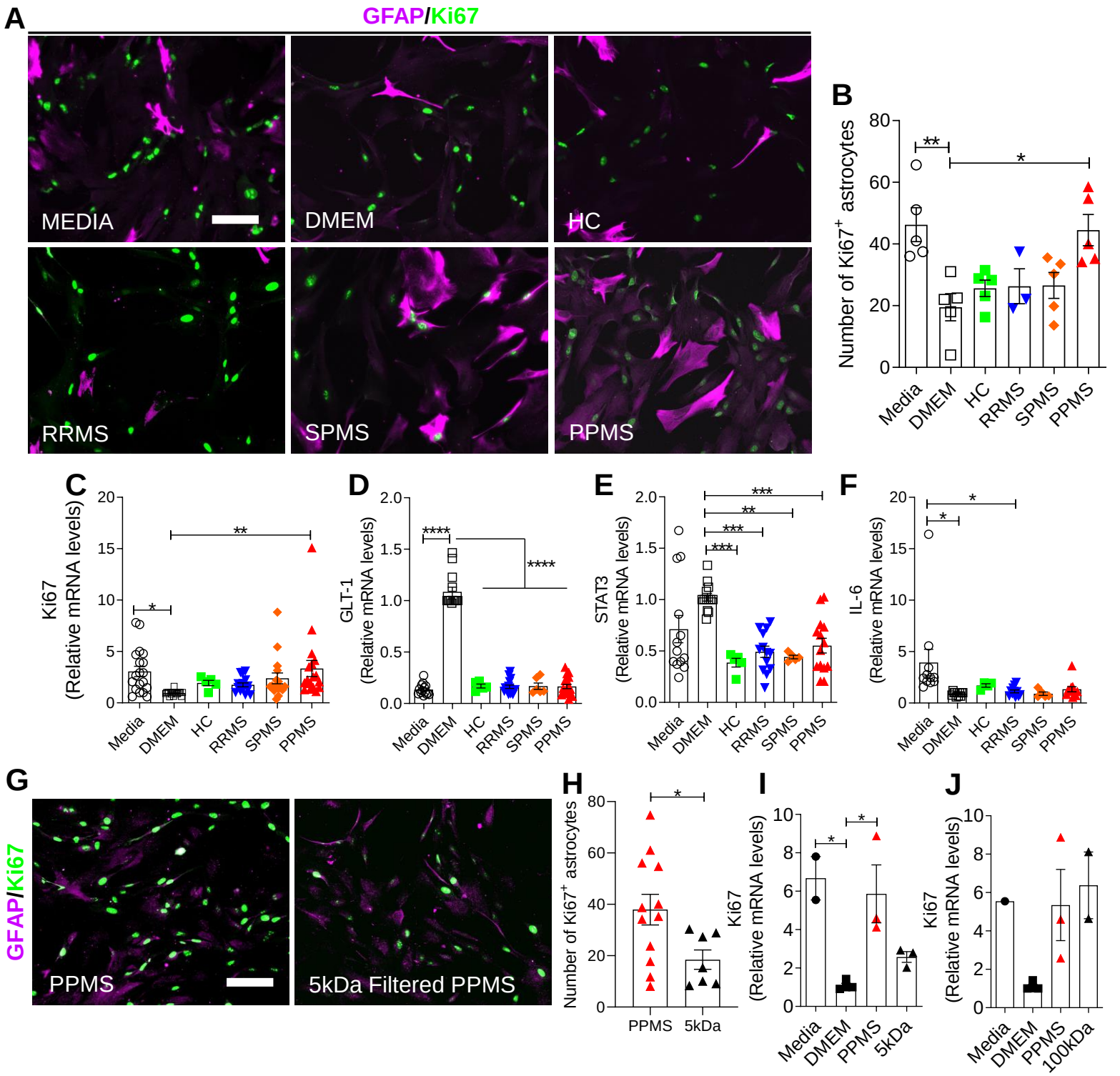
Supplementary Table 4 Pathogenic effects of IgG1 rAbs used for intrathecal injections

Patient	No. of rAbs injected	Percentage of mice with motor deficits (%)	Demyelinated Lesions?	Positive human IgG staining in mouse spinal cord?
PPMS 1	2	78 (7/9)	Yes (1/2 rAbs)	Yes
PPMS 2	2	50 (3/6)	Yes (1/2 rAbs)	N/A
PPMS 3	2	73 (11/15)	Yes (2/2 rAbs)	Yes
PPMS 4	1	100 (3/3)	No	Yes
PPMS 5	2	67 (2/3)	No	Yes
PPMS 6	3	75 (9/12)	Yes (1/3 rAbs)	Yes
PPMS 7	1	100 (3/3)	No	Yes
RRMS 1	1	17 (1/6)	No	No
RRMS 2	1	50 (1/2)	No	Yes
RRMS 3	1	50 (1/2)	No	Yes
RRMS 4	1	67 (2/3)	No	No
SPMS 1	1	0 (0/2)	No	No
SPMS 2	1	50 (1/2)	No	No
SPMS 3	1	33 (1/3)	No	No
SPMS 4	1	33 (1/3)	No	No
DC 1	1	33 (1/3)	No	N/A
DC 2	1	0 (0/3)	No	No
DC 3	1	0 (0/3)	No	N/A

rAb: recombinant antibody; N/A: not assessed; DC: disease control

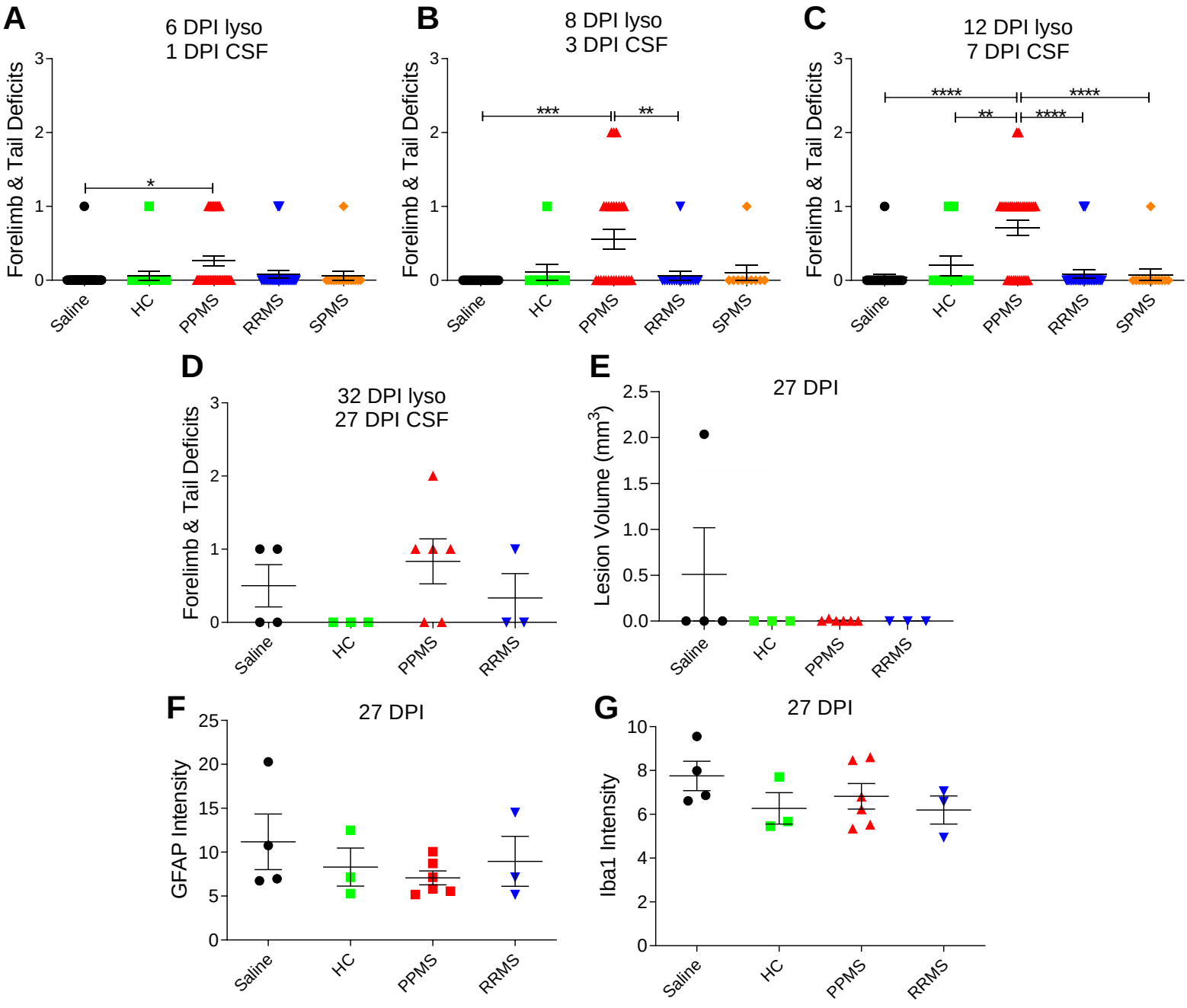


Supplementary Figure 1. PPMS CSF induces pathology in the spinal cord but not the brain. (**A**) GFAP mRNA levels are significantly elevated at 1 DPI in cervical spinal cords of PPMS CSF-injected mice. (**B-D**) Time course of protein expression of GFAP (**B**), SMI-32 (**C**), and GLT-1 (**D**) in the cervical spinal cord dorsal white matter at 1, 3 and 7 DPI. (**E**) Morphological changes in Iba1^{+} microglia reveal greater activation in PPMS CSF-injected mice. (**F-I**) Corpus callosum GFAP (**F** and **G**) and Iba1 (**H** and **I**) immunostaining reveal a lack of reactive astrogliosis and microglial activation at 1 DPI in the brains of PPMS CSF-injected mice. Data plotted as mean ± SEM. Each point represents an individual mouse. One-way ANOVA with Bonferroni's test. $^{**}P < 0.01$, $^{*}P < 0.05$. Scale bars, 100 µm.

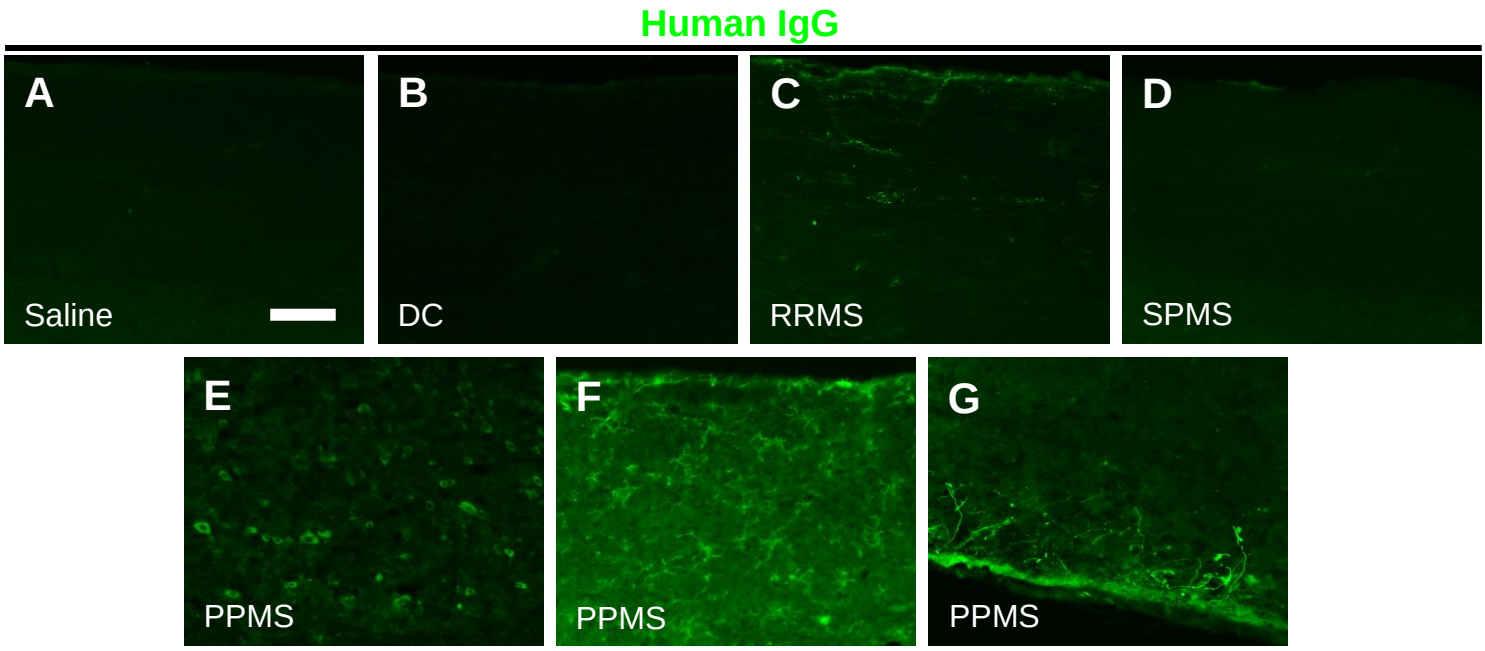


Supplementary Figure 2. PPMS CSF, but not RRMS or SPMS CSF, induces proliferation of human astrocytes. (**A**) Representative images of GFAP and Ki67 immunostaining of primary human astrocytes incubated in media, DMEM, or CSF obtained from healthy controls, or RRMS, SPMS, or PPMS patients for 24 hours. Scale bar, 100 µm. (**B**) Quantification of the number of Ki67$^+$ proliferating astrocytes following 24-hour exposure to 50% CSF. (**C-F**) Human astrocyte mRNA levels of Ki67 (**C**), GLT-1 (**D**), STAT3 (**E**), and IL-6 (**F**) following 24-hour exposure to 50% CSF, as measured by qPCR. (**G**) Representative images of GFAP and Ki67 immunostaining of primary human astrocytes incubated in 50% PPMS CSF or 5 kDa-filtered PPMS CSF for 24 hours. Scale bar, 100 µm. (**H**) Quantification of the number of Ki67$^+$ proliferating astrocytes following 24-hour exposure to 50% PPMS CSF or 5 kDa-filtered PPMS CSF. (**I** and **J**) Ki67 mRNA levels in human astrocytes following 24-hour exposure to 50% PPMS CSF, 5 kDa-filtered PPMS CSF (**I**) or 100 kDa-filtered PPMS CSF (**J**).

Data plotted as mean ± SEM. Each point represents an individual well. One-way ANOVA with Bonferroni's test (**B-F**, **I**, **J**). Two-tailed Student's t-test (**H**). ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.



Supplementary Figure 3. Time course of motor deficits and lesion pathology induced by PPMS CSF in lysolecithin-injected mice. (**A-D**) Motor deficit scores at 1 (**A**), 3 (**B**) 7 (**C**) and 27 days (**D**) post CSF injection in lysolecithin-injected mice. (**E-G**) Analysis of lesion volume (**E**), GFAP intensity (**F**) and Iba1 intensity (**G**) at 27 days post lysolecithin injection. Data plotted as mean ± SEM. Each point represents an individual mouse. One-way ANOVA with Bonferroni's test. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.



Supplementary Figure 4. Heterogeneous human IgG staining in PPMS rAb-injected mice. Representative images of human IgG immunostaining in cervical spinal cords of mice injected with saline (**A**), DC rAb (**B**), RRMS rAb (**C**), SPMS rAb (**D**), or PPMS rAbs (**E**-**G**) at 1 DPI. Patterns of human IgG staining in PPMS rAb-injected mice resemble neuronal morphology (**E**), microglial morphology (**F**), and axonal morphology (**G**). Scale bar, 100 μm.

Supplementary Materials and Methods

CSF Single Cell B-Cell FACS and Recombinant Antibody Production

Pelleting Cells

CSF obtained from lumbar puncture was transferred to a 15 mL polypropylene tube and immediately spun down at 170 x g for 15 min at room temperature. Cell-free CSF was removed except for a residual 300 µL used to resuspend the pelleted cells for hemocytometer cell count. After cell count and transfer to a 1.5 mL tube, the remaining cells were pelleted again by spinning at 150 x g in a tabletop microcentrifuge. The remaining CSF was removed, and the cell pellet resuspended in 250 µL PBS.

Immunostaining and FACS

Human BD Fc Block™ (BD Biosciences cat. 564219) was added to the resuspended cells. Anti-human CD19-APC (BD Biosciences cat. 555415) and anti-human CD138-FITC (BD Biosciences cat. 552723) were then added to stain for CD19 and CD138, respectively. After staining for 30 min on ice, FACS was performed on a BD FACSAria™ II. Single cells were sorted into 24-well PCR plates (USA Scientific cat 1402-0240) containing reverse transcription (RT) buffer, dNTPs, RT primers (see the primer table in the next section), and 1% NP-40. Plates were then spun down at 1000 x g for 10 min and frozen overnight at –40ºC or below.

Reverse Transcription (RT) and Immunoglobulin (Ig) Gene PCRs

Thawed plates were heated to 65ºC for 5 min. Superscript™ III (Thermo Fisher cat. 18080044) reverse transcriptase was added and RT was performed according to manufacturer's protocol. After RT, RNase H (NEB cat. M0297L) was added and incubated for 20 min at room temperature. Ig variable region nested PCR was performed using the following primers sets modified from Wang and Stoller[1]:

RT Primers			
CMI	5'-GCAGGAGACGAGGGGGA-3'		
CGI	5'-AGGGYGCCAGGGGGAA-3'		
CAI	5'-TGGACCAGGCAKGCGAYGAC-3'		
CKI	5'-AACAGAGGCAGTTCCAGA-3'		
CLI	5'-TGTGGCCTTGTTGGCTTG-3'		
Outer light chain primers		Outer heavy chain primers	
VKL-1	5'-GCTCAGCTCCTGGGGCTCCTG-3'	VHL-1	5'-TCACCATGGACTGSACCTGGA-3'
VKL-2	5'-CTGGGGCTGCTAATGCTCTGG-3'	VHL-2	5'-CCATGGACACACTTTGYTCCAC-3'
VKL-3	5'-TTCCTCCTGCTACTCTGGCTC-3'	VHL-3	5'-TCACCATGGAGTTTGGGCTGAGC-3'
VKL-4	5'-CAGACCCAGGTCTTCATTTCT-3'	VHL-4	5'-AGAACATGAAACAYCTGTGGTTCTT-3'
		VHL-5	5'-ATGGGGTCAACCGCCATCCT-3'
VLL-1	5'-CCTCTCCTCCTCACCCTCCT-3'	VHL-6	5'-ACAATGTCTGTCTCCTTCCTCAT-3'
VLL-2	5'-CTCCTCACTCAGGGCACA-3'		
VLL-3	5'-ATGGCCTGGAYCSCTCTCC-3'	CGII	5'-GCCAGGGGGAAGACSGATG-3'
		CMII	5'-CAGGAGACGAGGGGGAAAAG-3'
CKII	5'-TTTCAACTGCTCATCAGATGGCGG-3'	CAII	5'-YMGAGGCTCAGCGGGAAGAC-3'
CLII	5'-AGCTCCTCAGAGGAGGGYGG-3'		
Inner light chain primers		Inner heavy chain primers	
VKF-1	5'-CGMCATCCRGWTGACCCAGT-3'	VHF-1	5'-CAGGTSCAGCTGGTRCAGTC-3'
VKF-2	5'-CGATRTTGTGATGACYCAG-3'	VHF-2	5'-CAGRTCACCTTGAAGGAGTC-3'
VKF-3	5'-CGAAATWGTGWTGACRCAGTCT-3'	VHF-3	5'-SAGGTGCAGCTGGTGGAGTC-3'
VKF-4	5'-CGACATCGTGATGACCCAGT-3'	VHF-4	5'-CAGGTGCAGCTGCAGGAGTC-3'
		VHF-5	5'-GARGTGCAGCTGGTGCAGTC-3'
VLF-1	5'-CCAGTCTGTGCTGACTCAGC-3'	VHF-6	5'-CAGGTACAGCTGCAGCAGTC-3'
VLF-2	5'-CCAGTCTGCCCTGACTCAGC-3'		
VLF-3	5'-CTCCTATGAGCTGACWCAGC-3'	CGIII	5'-GACSGATGGGCCCTTGGTGGA-3'
		CMIII	5'-GAAAAGGGTTGGGGCGGATGC-3'
CKIII	5'-AAGATGAAGACAGATGGTGC-3'	CAIII	5'-GGAAGACCTTGGGGCTGGTC-3'
CLIII	5'-GYGGGAACAGAGTGACCG-3'		

After 2 rounds of PCR, products were visualized in agarose gels. Bands were excised, purified, and cloned into sequencing plasmids. After sequencing, light and heavy variable regions were analyzed for clonal expansion.

Sequence Analysis

Sequenced full length variable regions were aligned at IMGT.com using their IMGT/V-Quest alignment software. The third complementarity-determining region (CDR3) was used to identify clonally expanded cells. Cells with identical CDR3 heavy chain regions must have identical CDR3 light chain regions to be considered expanded. Expanded cells were defined as 2 or more cells with identical CDR3 heavy and light chain regions. Ig isotype can also be determined via the sequenced reverse primer (which primes in the CH1 constant region) and verified by the small remaining CH1 sequence amplified by the PCR. Kappa or lambda light chains were also identified similarly. Sequencing data are available on the Tisch MSRCNY website at www.tischms.org.

Recombinant Antibody Production

IgG1 constant region sequence and either lambda or kappa constant region sequence were added to the variable regions to obtain full heavy and light chain sequences, respectively. In addition, human signal sequence for protein secretion was added to both chains. Heavy chains were cloned into expression plasmid pOptiVEC™ (Thermo Fisher, cat. 12744017) and light chains were cloned into expression plasmid pcDNA3.3™ (Thermo Fisher, cat. K830001). Plasmids were transfected in HEK293-F cells (Thermo Fisher, cat. R79007) using the Freestyle™ MAX 293 Expression System (Thermo Fisher, cat. K900010). After transfection, antibodies were produced in a shake flask on an orbital platform (Thermo MaxQ 2000 CO_2 Plus, cat. 88881103) set to 225 rpm in 8% CO_2 at 37ºC for 5-7 days. Recombinant antibodies were purified by running through a Protein G agarose column (Thermo Fisher, cat. 20397). Purified proteins were eluted (Thermo Fisher cat. 21004) and dialyzed into PBS.

References

1. Wang X, Stollar BD. Human immunoglobulin variable region gene analysis by single cell RT-PCR. *J Immunol Methods.* 2000;244:217-225.