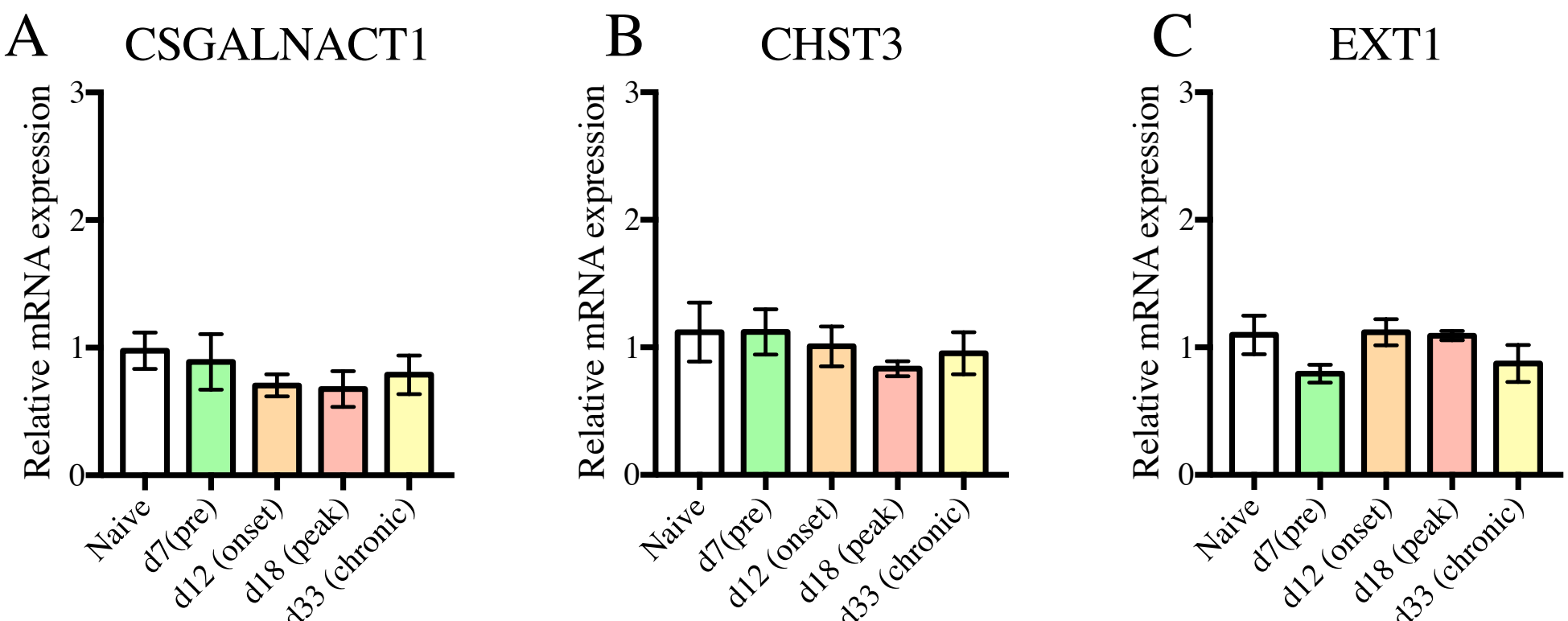
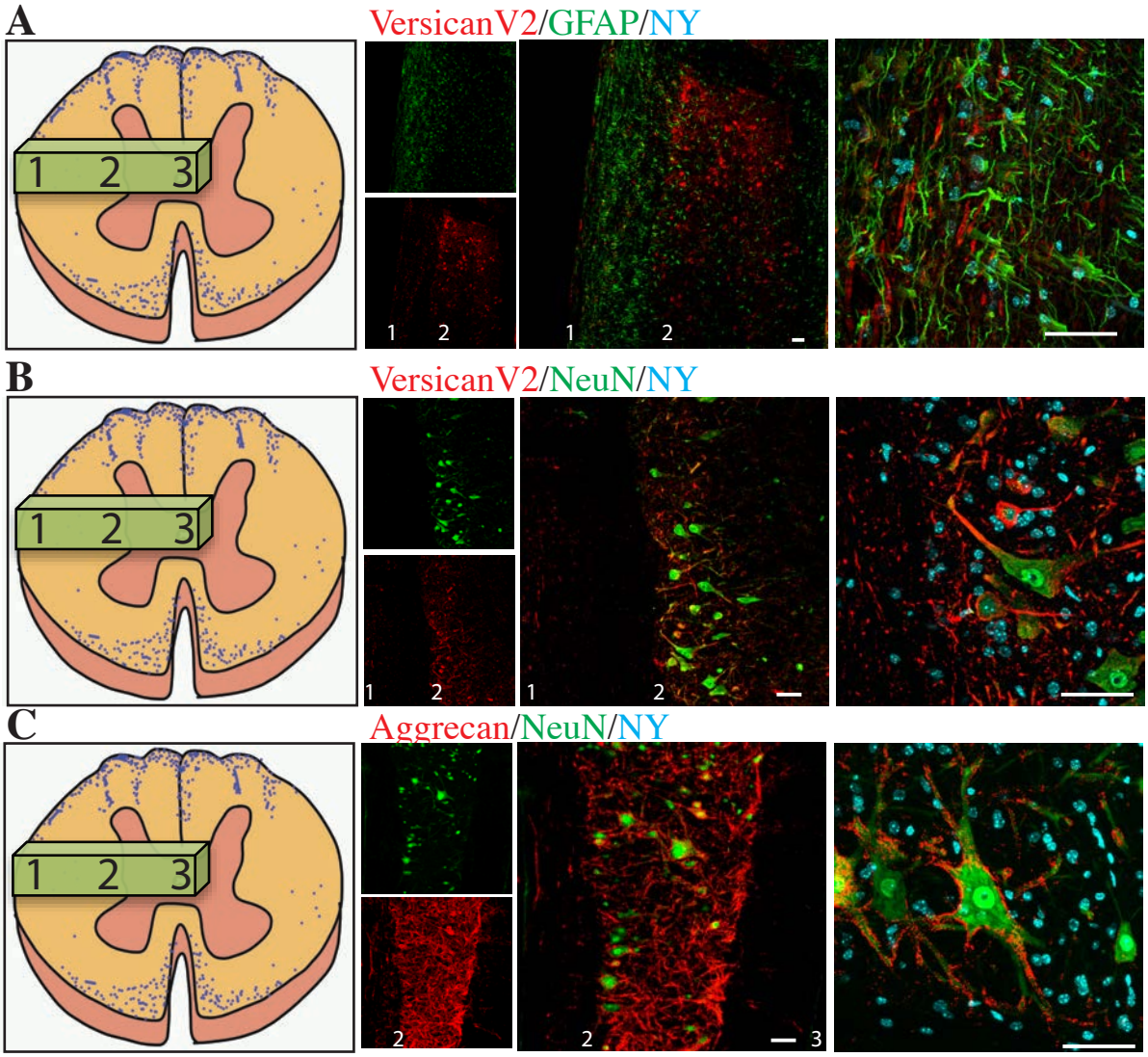
Supplementary Figure 1. Real-time PCR of selected enzymes involved in CSPG or HSPG synthesis. (A) Chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGALNACT1) is an enzyme involved in the first CSPG-specific synthesis step for GAG chains. (B) Chondroitin 6-O sulfotransferase 1 (CHST3) is an enzyme involved in posttranslational sulfation of GAGs. (C) Exostosin glycosyltransferase 1 (EXT1) is an enzyme involved in diverting synthesis towards heparan sulfate proteoglycans (HSPGs). N=5 mice per group. Data represent mean +/- SEM. Dunnett ANOVA was performed, and there was no significant difference between groups.

Supplementary Figure 1



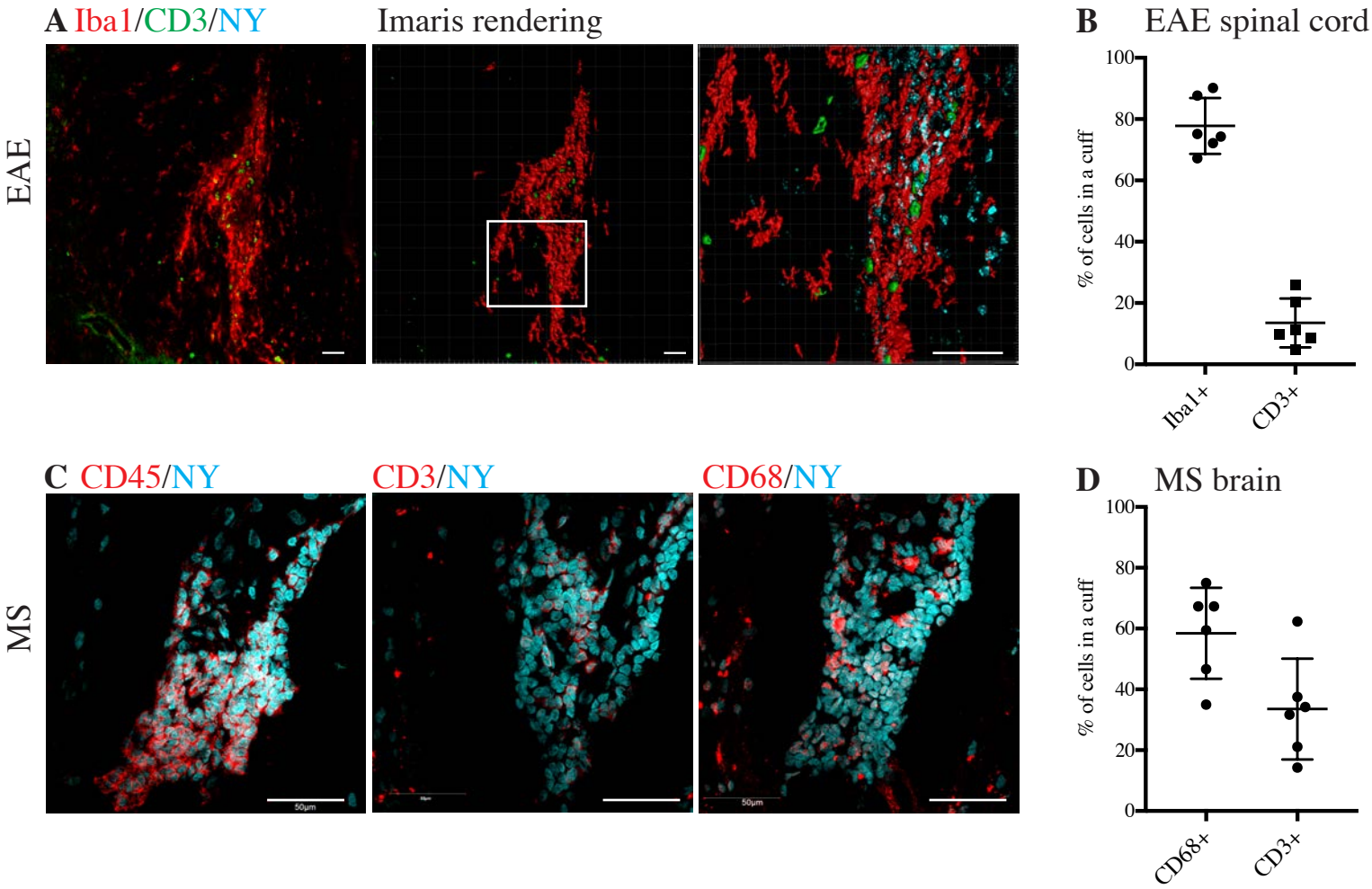
Supplementary Figure 2. Aggrecan and Versican V2 are expressed in perineuronal nets. (A) Versican V2 expression in both the white matter and gray matter of the spinal cord. The higher magnification image (far right) shows versican V2 expression in the white matter. (B) Versican V2 expression in perineuronal nets in the gray matter, encircling NeuN+ neurons. (C) Aggrecan expression in the gray matter, encircling numerous NeuN+ neurons. Spinal cord schematics on the far left show the slice orientation, and numbers that corresponds to pial region (1), gray matter boundary (2), and center of the spinal cord (3), that are labelled in the lower left panel of the immunohistochemistry images. Scale bars represent 50μm.

Supplementary Figure 2



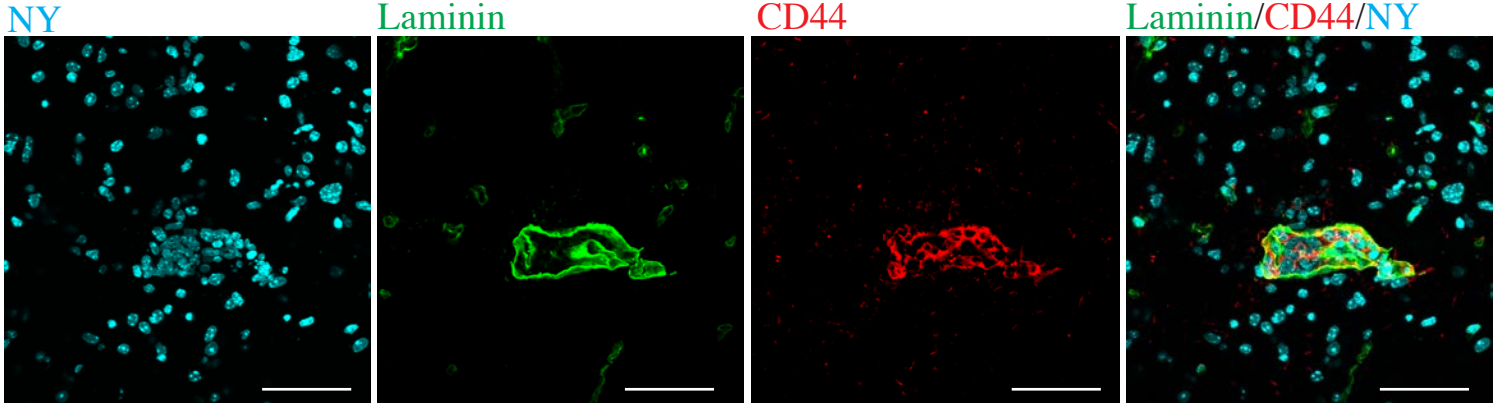
Supplementary Figure 3. Cell types in perivascular cuffs. (A) Left, perivascular cuff in EAE stained for Iba1 (red), CD3 (green), and nuclear yellow (NY, blue) and middle, Imaris rendering of Iba1 and CD3. Right, Imaris rendering showing the abundance of Iba1 versus CD3 positive cells in the perivascular space. (B) Percent of nuclear yellow-positive cells attributed to Iba1+ versus CD3+ cells in perivascular cuffs from 6 mice stained for Iba1, CD3, and nuclear yellow. (C) MS brain stained with nuclear yellow (NY, blue) and CD45 (red, left), CD3 (red, middle) or CD68 (red, right). (D) Quantification of number of CD68+ cells in a cuff and number of CD3+ cells in a cuff in MS. MS perivascular cuffs were stained separately for CD68 and CD3. Six cuffs from 3 MS patients were quantified. Scale bars represent 50μm.

Supplementary Figure 3



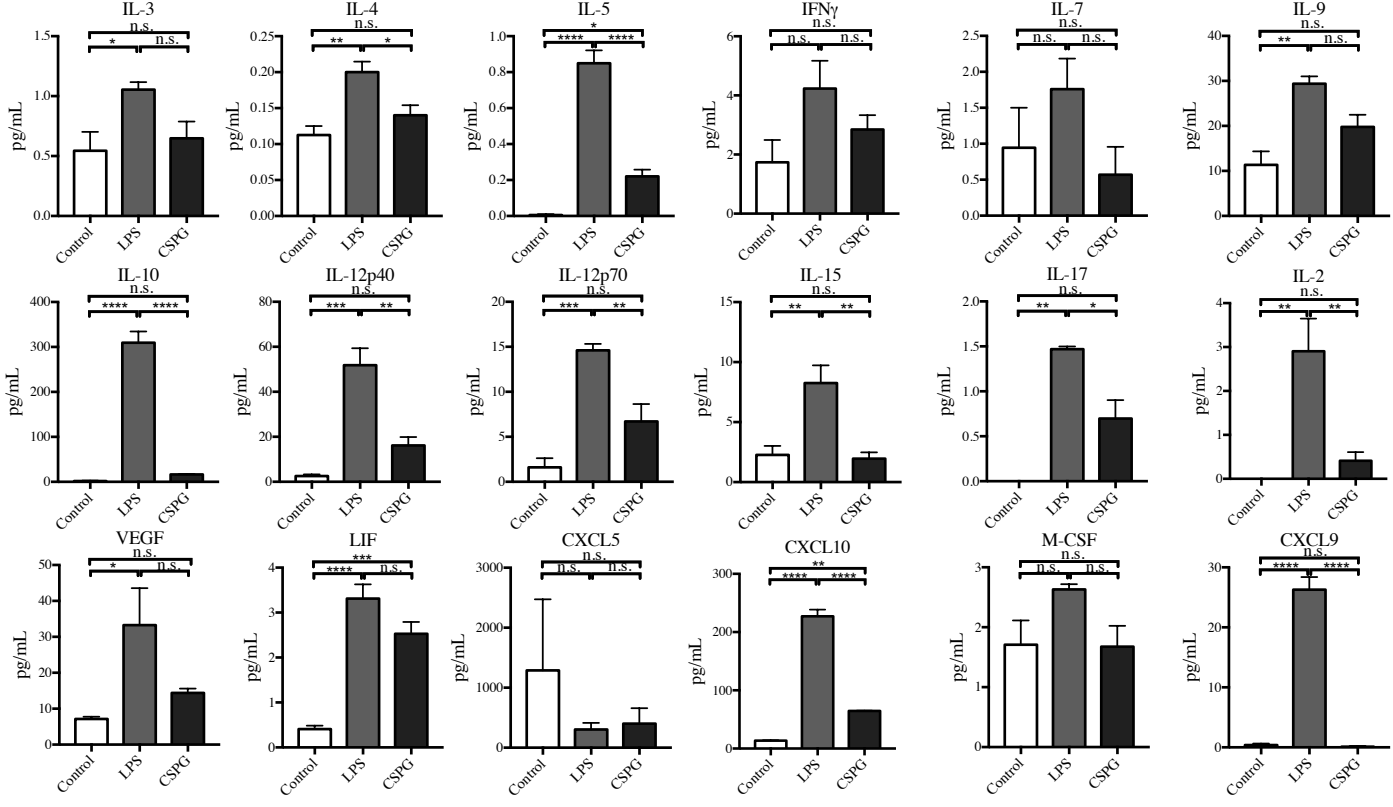
Supplementary Figure 4. EAE perivascular cuff stained for nuclear yellow (NY, blue), laminin (green), and CD44 (red).

Supplementary Figure 4



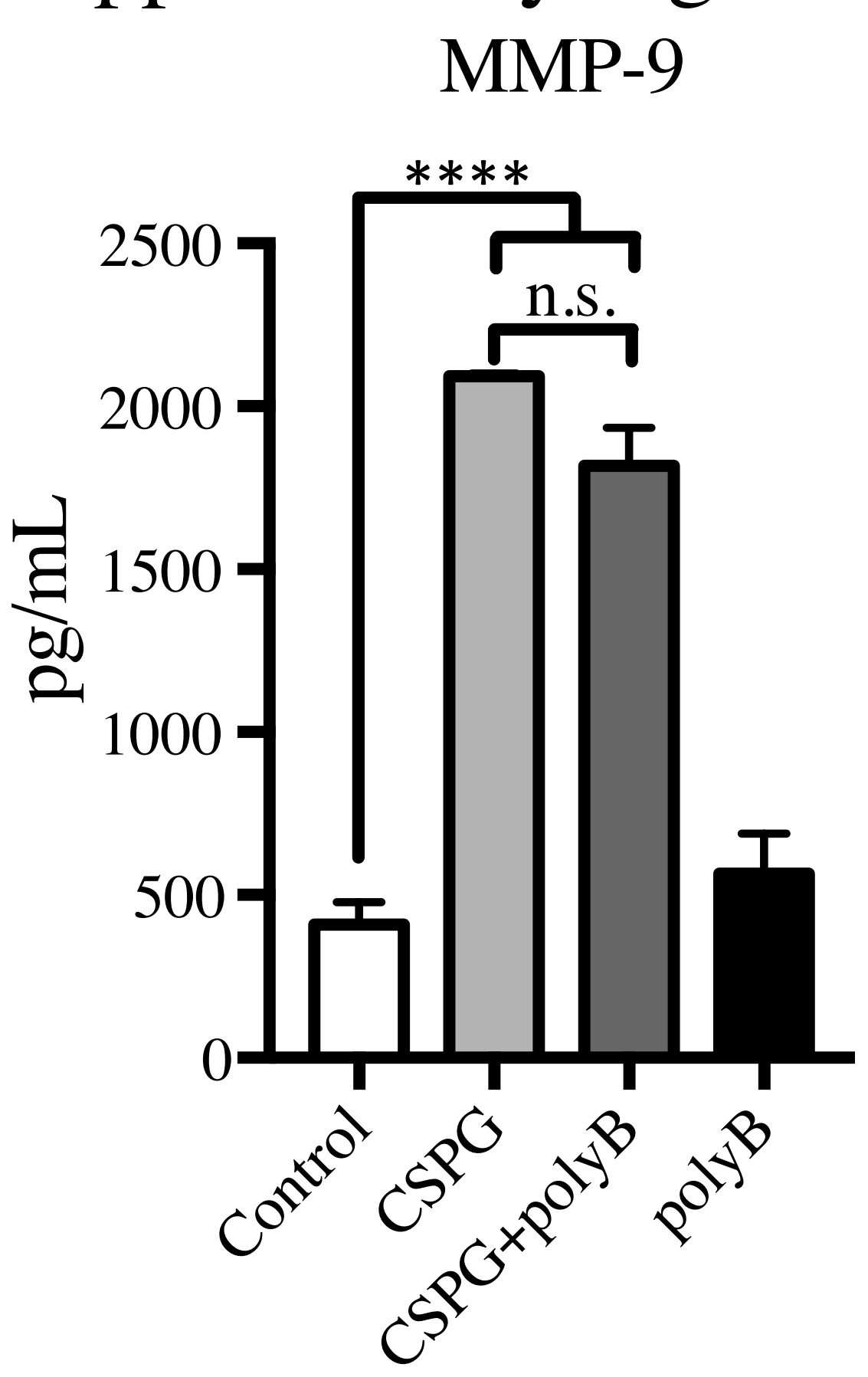
Supplementary Figure 5. Cytokine 31-plex of bone marrow-derived macrophages treated for 24 hours with 10μg/ml CSPGs, 100ng/ml LPS, and media only was used as a control. Tukey ANOVA was performed, comparing all treatments against each other, *$p<0.05$, **$p<0.01$, *** $p<0.001$. n.s. refers to non-significance.

Supplementary Figure 5



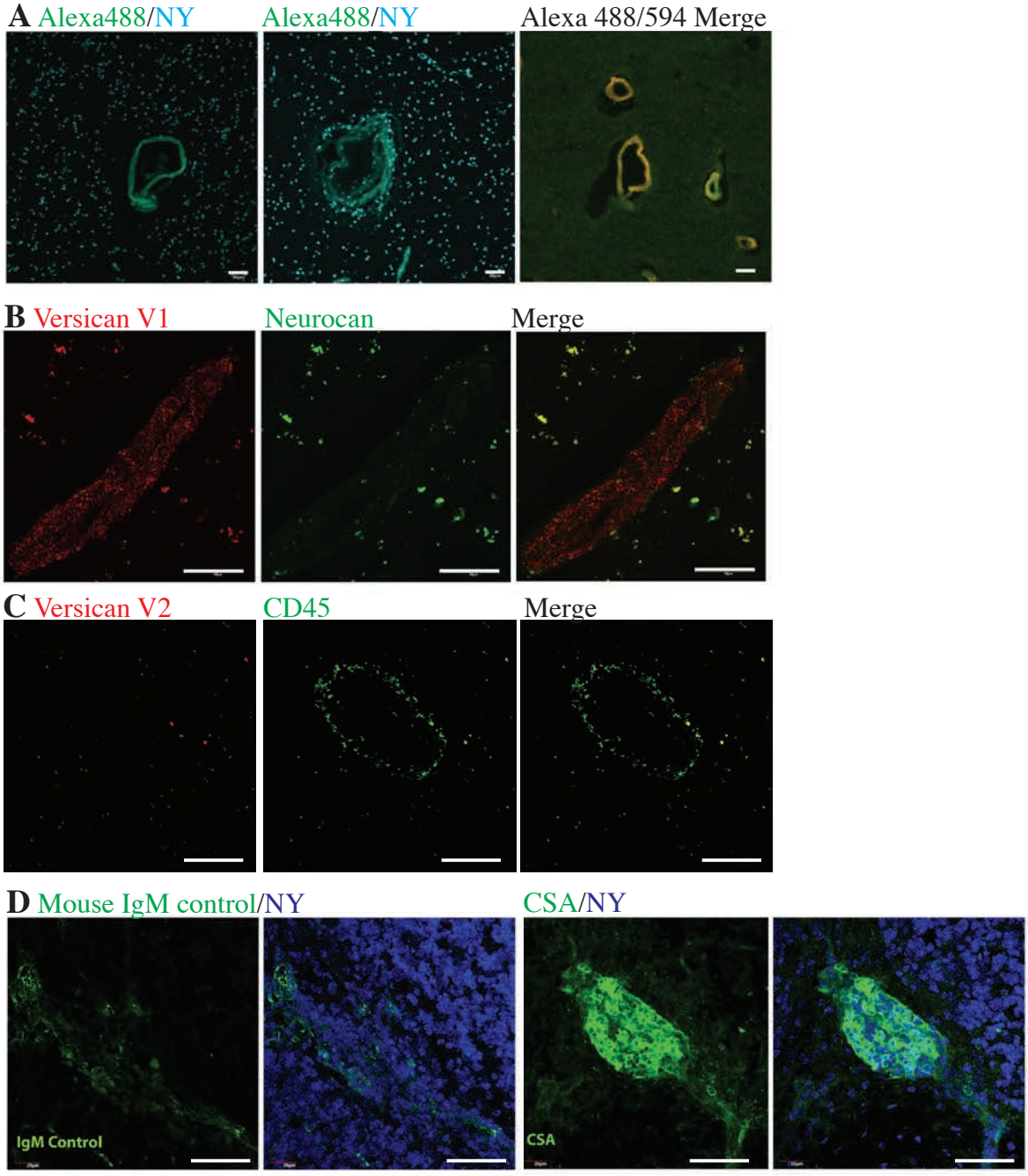
Supplementary Figure 6. CSPGs enhance MMP-9 production even in the presence of polymyxin B. Bone marrow-derived macrophages were incubated in the presence of CSPGs, polymyxin B ('polyB'), or both CSPGs and polymyxin B ('CSPG+polyB'). Polymyxin B is a known scavenger of LPS, preventing its endotoxin ability, and acted as a control to ensure the effects of CSPGs are not due to endotoxin contamination. Treatment with CSPG and polymyxin B was not significantly different than treatment with just CSPGs. Tukey ANOVA was performed, comparing all treatments against each other, *$p<0.05$, **$p<0.01$, *** $p<0.001$. n.s. refers to non-significance.

Supplementary Figure 6



Supplementary Figure 7. Immunohistochemistry controls. (A) Two images of perivascular cuff staining on human tissue with only secondary (Alexa488, far right) and nuclear yellow (NY, blue), and a composite image with only Alexa488 and Alexa594 secondary antibodies to show non-specific fluorescence in the perivascular cuff. (B) Versican V1 (red) and neurocan (green) staining on human MS brain, showing that only versican V1 is expressed in the perivascular cuff. (C). Versican V2 (red) with CD45+ cells (green) depicting a perivascular cuff, showing the absence of versican V2 in the perivascular cuff. (D) Example of a perivascular cuff showing background to a mouse IgM antibody, and the true staining of CSA. Two images on the left show staining on EAE mouse tissue with a mouse IgM labelled with an anti-mouse secondary antibody (green), and an overlay with nuclear yellow (NY, blue). The two images on the right show the primary antibody to chondroitin sulfate A (CSA, mouse anti-mouse IgM) labelled with the anti-mouse secondary antibody (green), and an overlay with nuclear yellow (NY, blue). Scale bars represent 50μm.

Supplementary Figure 7



Supplementary Table 1. CSPG antibodies used for immunohistochemistry and western blot

Antigen	Host, Antibody	Company	Catalogue#	Use
4-sulfated CS GAG	Mouse anti-mouse, IgM	Cosmo	NU-07-001	Immunohistochemistry/Western blot
4- and 6- sulfated CS GAGs (CS56)	Mouse anti-mouse,	Abcam	Ab11570	Immunohistochemistry/Western blot
4-sulfated and 6-sulfated CS GAG stubs	Mouse anti-mouse	Millipore	MAB2030	Western blot
Versican V1/V0 (beta domain)	Rabbit anti-mouse	Millipore	AB1033	Immunohistochemistry/Western blot
Versican V2/V0 (alpha domain)	Rabbit anti-mouse	Millipore	AB1032	Immunohistochemistry/Western blot
Aggrecan	Rabbit anti-mouse	Millipore	AB1031	Immunohistochemistry/Western blot
Brevican	Mouse anti-mouse	Millipore	MABN491	Western blot
CD45	Rat anti-mouse	BD Pharminogen	550539	Immunohistochemistry
Pan-laminin	Rabbit anti-mouse, anti-human	Gift from L. Sorokin	N/A	Immunohistochemistry
Versican V1	Rabbit anti-human	Thermo Fisher Scientific	PA1-1748A	Immunohistochemistry

Versican V2/V0	Rabbit anti-human	Thermo Fisher Scientific	PA3-119	Immunohistochemistry