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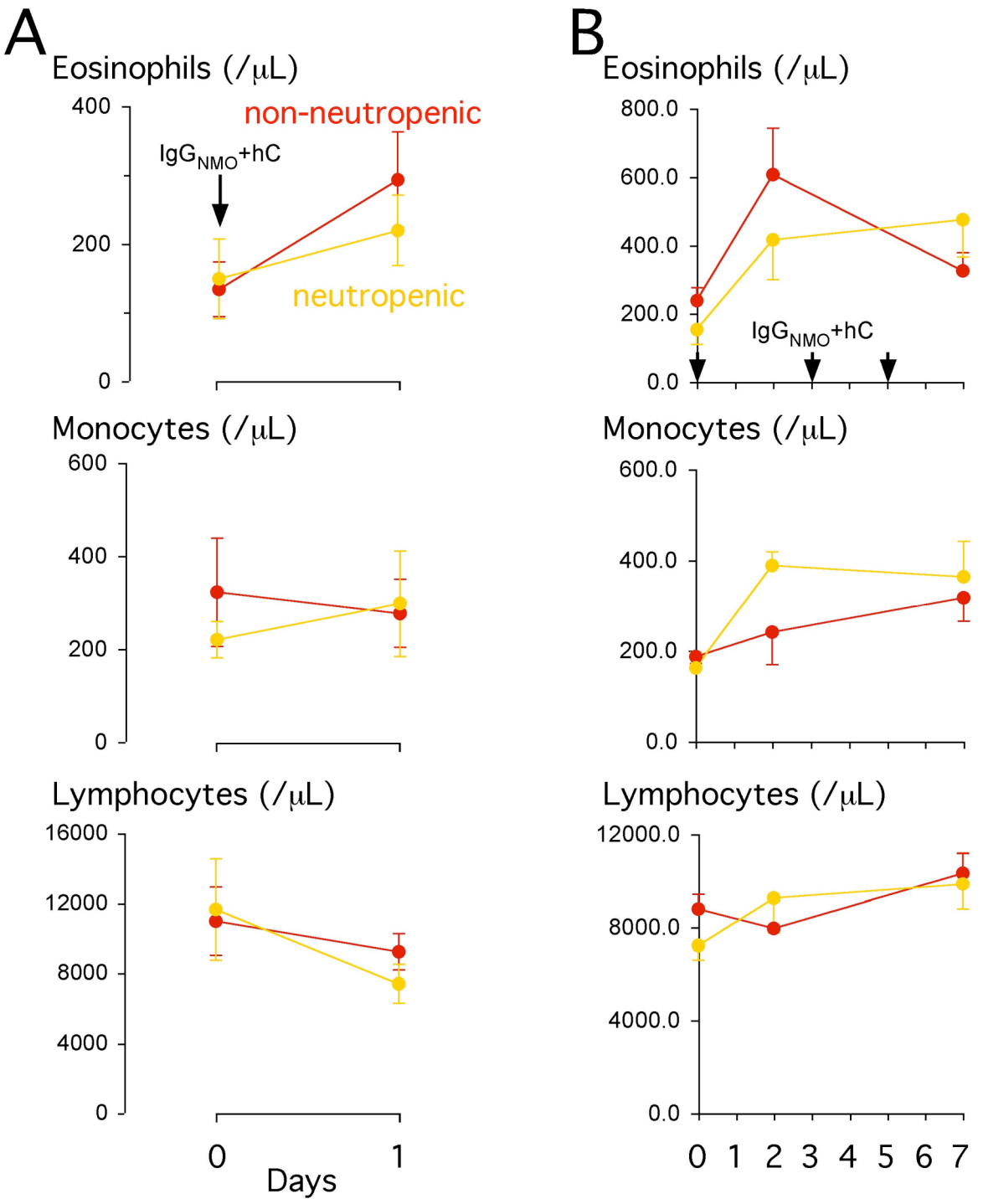
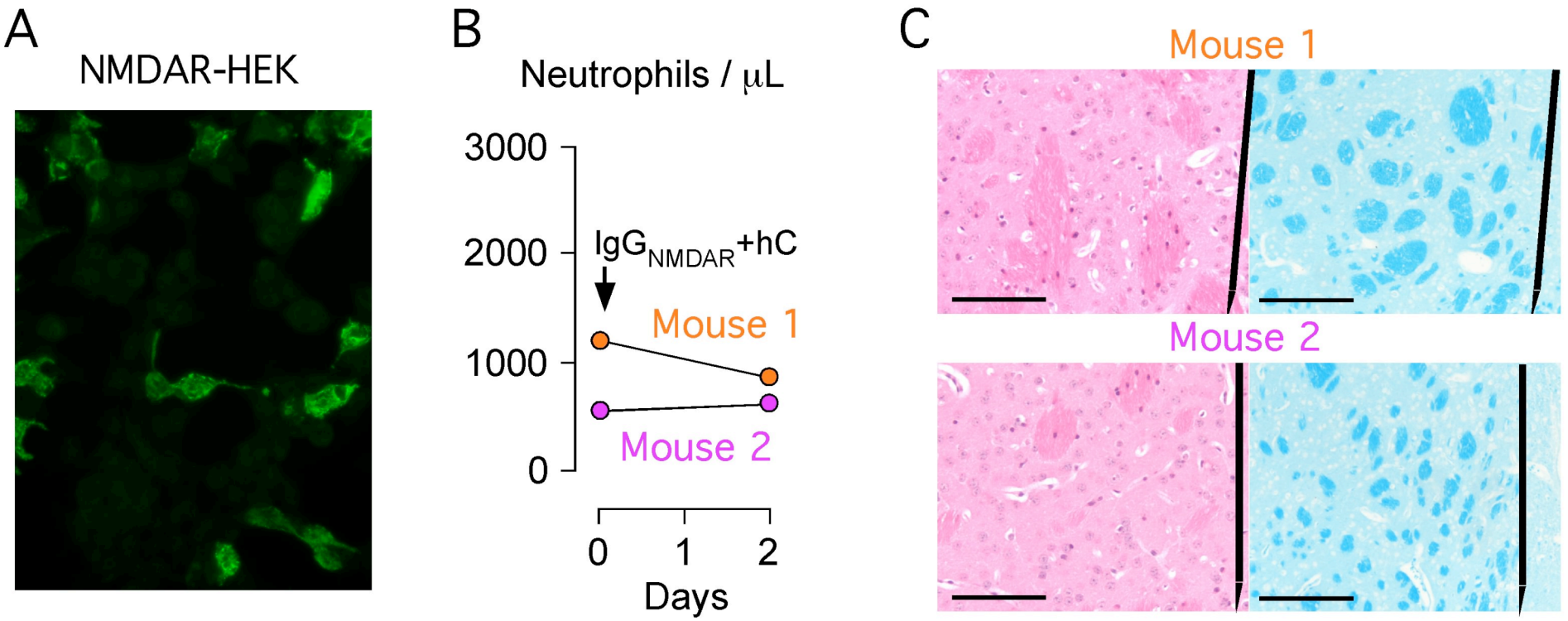
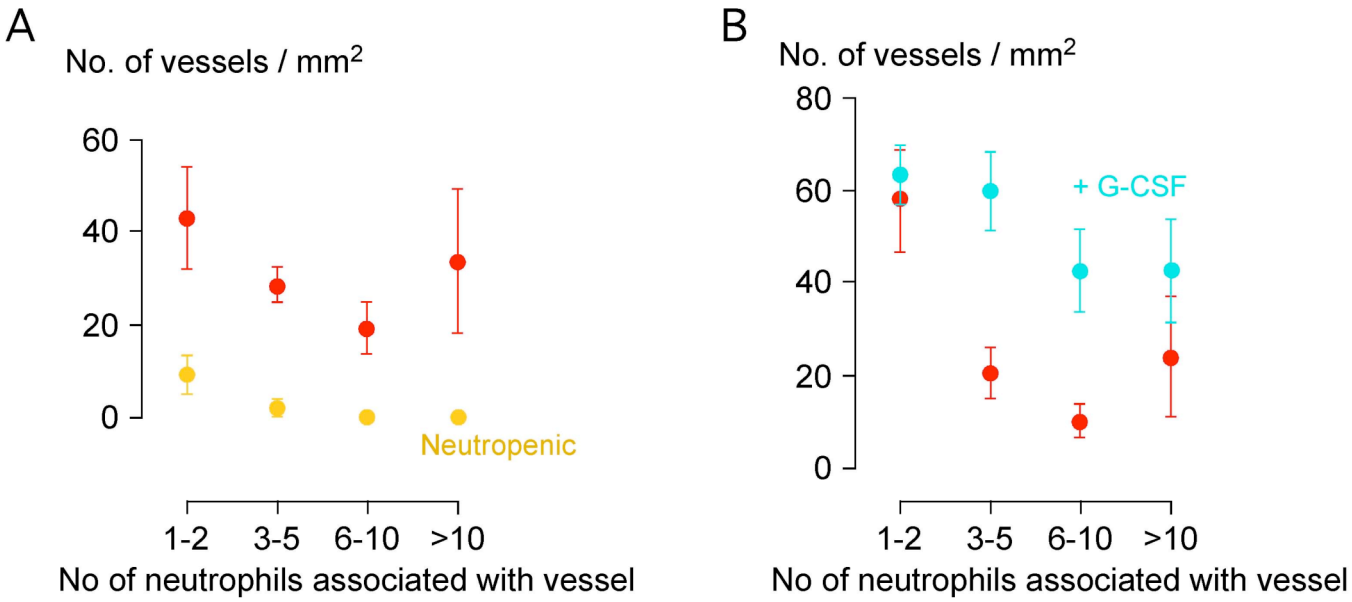
Filename: neutrophil protease inhibition - supplemental figures.pdf



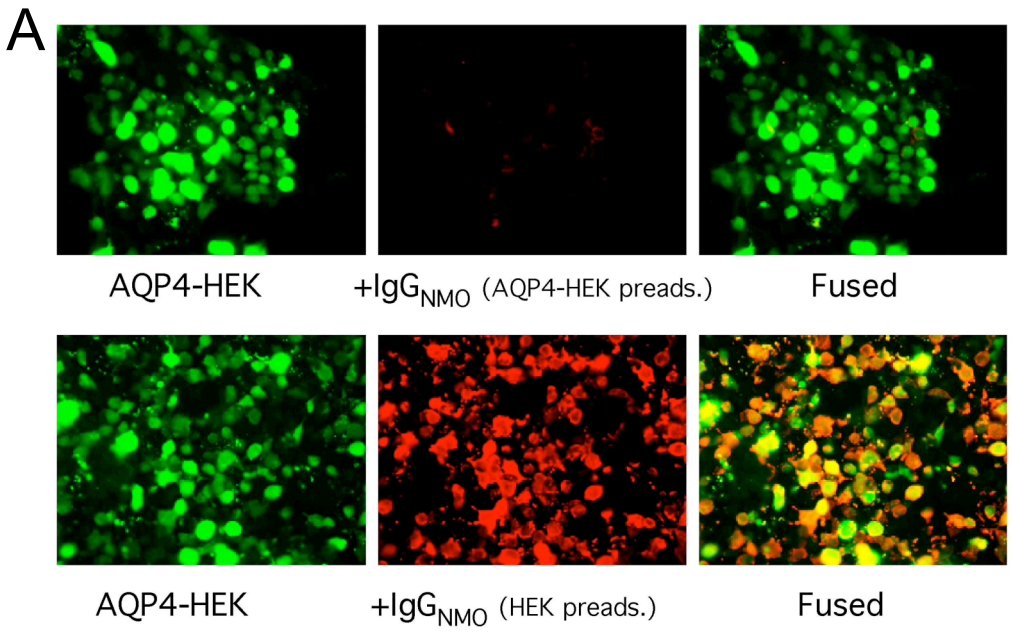
Supplementary Fig. i. Peripheral white cell counts in non-neutropenic and neutropenic mice. A. Mice were killed 24 h after intracerebral injection of IgG_{NMO} + hC. Neutrophil counts are shown in Fig. 1A. **B.** Mice were injected intracerebrally with IgG_{NMO} + hC on days 0, 3 and 5 and killed on day 7. Neutrophil counts are shown in Fig. 3A. Mean ± SEM. For each white cell type, the differences between non-neutropenic and neutropenic mice were not significant.

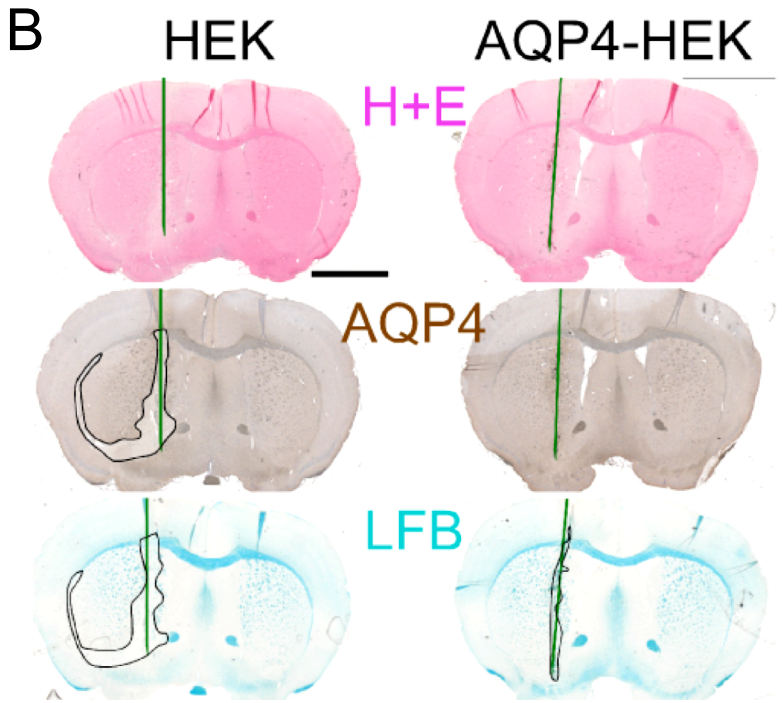


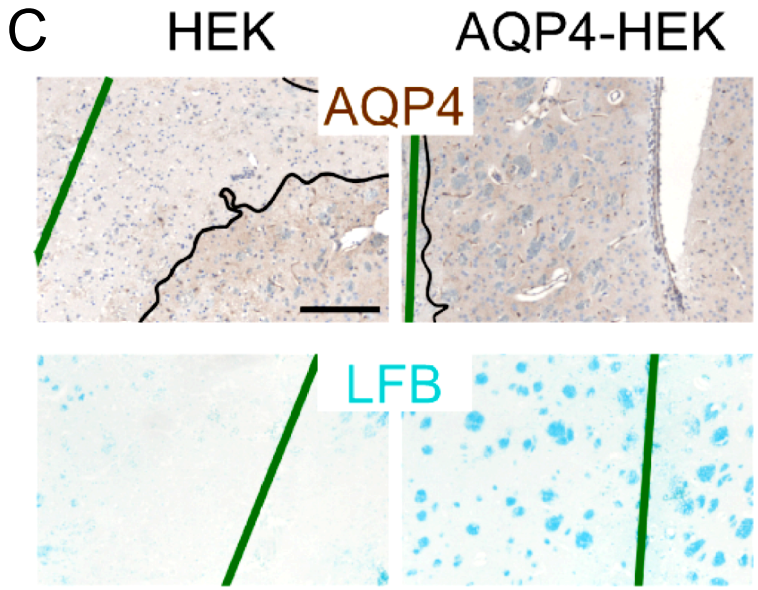
Supplementary Fig. ii. Inracerebral injection of IgG$_{NMDAR}$ + hC does not cause peripheral neutrophilia, brain inflammation or demyelination. IgG was isolated from the serum of two patients with NMDAR encephalitis who had high titers of anti-NMDAR IgG. Mouse 1 was injected intracerebrally with 16.8 μl IgG$_{NMDAR}$ from patient 1 + 11.2 μl hC and mouse 2 was injected with 16.8 μl IgG$_{NMDAR}$ from patient 2 + 11.2 μl hC as explained in the Methods section. Mice were killed at 48 h after injection. **A.** IgG from anti-NMDAR encephalitis patient binds to HEK cells transfected with NMDAR on EuroImmun slide. Bound IgG was visualized using green fluorescence. **B.** Peripheral neutrophil counts before and 48 h after the injection. **C.** *(Left)* H&E and *(right)* LFB staining of brain adjacent to the needle tract. Bar = 100 μm (H&E), 200 μm (LFB).



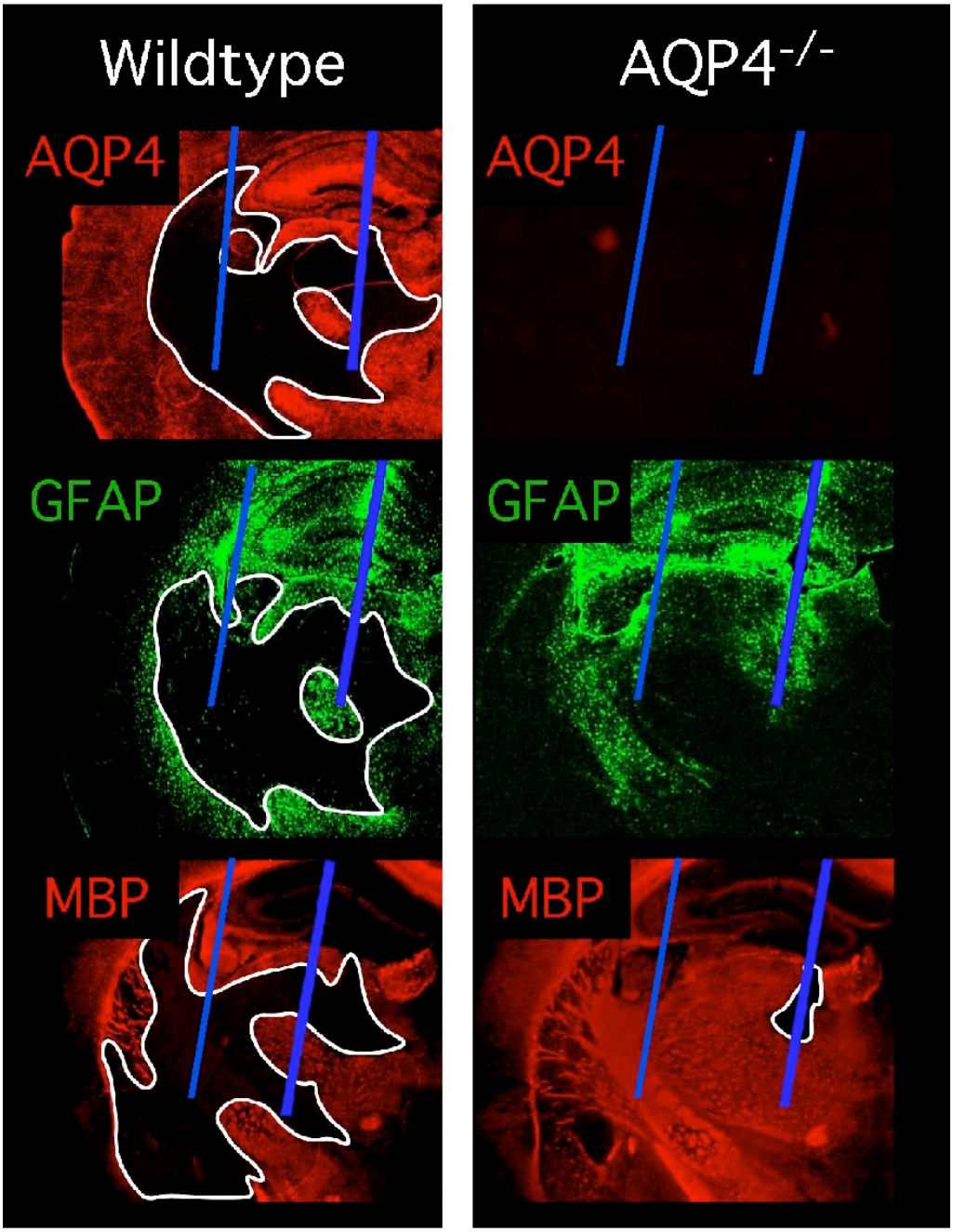
Supplementary Fig. iii. Quantification of brain neutrophil inflammation. We counted vessels as described in the Methods section. Inflamed vessels were classed into different groups according to the number (1-2, 3-5, 5-10, >10) of associated neutrophils. A neutrophil was considered to be associated with the vessel if it was adherent to the vessel wall intraluminally, in the vessel wall, or perivascularly within a 20 μm radius. **A.** These data correspond to Fig. 1C in the manuscript, comparing non-neutropenic (red) *vs.* neutropenic (yellow) mouse brains at 24 h after injecting IgG_{NMO} + hC. **B.** These data correspond to Fig. 2B in the manuscript, comparing non-neutropenic (red) *vs.* neutrophilic (light blue, +G-CSF) mouse brains at 24 h after injecting IgG_{NMO} + hC. Mean ± SEM. In A., non-neutropenic *vs.* neutropenic $P < 0.05$ (1-2, 6-10), $P < 0.0005$ (3-5), *NS* (>10). In B., non-neutropenic *vs.* +G-CSF $P < 0.05$ (3-5, 6-10), *NS* (1-2, >10).







Supplementary Fig. iv. Intracerebral injection of IgG$_{NMO}$ + hC after depleting the anti-AQP4 antibody does not produce NMO lesions. A. IgG$_{NMO}$ was incubated with HEK cells (HEK-preadsorbed) or AQP4-transfected HEK cells (AQP4-HEK preadsorbed). (*Left*) AQP4-expressing HEK cells (green fluorescence). (*Middle*) Binding (red fluorescence) of pre-adsorbed IgG$_{NMO}$ to AQP4-expressing HEK cells. (*Right*) Fused left and middle images. **B.** Mouse brain at 24 h after injecting 16.8 μL HEK-preadsorbed or AQP4-HEK-preadsorbed IgG$_{NMO}$ + 11.2 μL hC. H+E stain, AQP4 immunostain, LFB stain. Area with loss AQP4 expression and myelin loss outlined. **C.** Magnified view adjacent to the needle. In B. and C., the needle is green. Bar 2 mm (B), 0.2 mm (C).



Supplementary Fig. v. Intracerebral injection of recombinant monoclonal anti-AQP4 antibody + hC produces NMO lesions in mice. In these experiments 8 μL (0.8 μg) recombinant monoclonal anti-AQP4 IgG_1 antibody (rAb-53 [23], gift from Prof. J. Bennett) + 6 μL hC was injected intracerebrally, split equally between the following sites in mm from bregma (behind, right, deep): (1, 1, 3) and (1, 2, 3). Wildtype (left column) and $AQP4^{-/-}$ mice were used. The mice were killed 6 days later and coronal sections were immunostained for AQP4 (top, red), GFAP (middle, green) and myelin basic protein (MBP, bottom, red). Loss of immunreactivity is outlined. Injecting needles in blue.

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Supplementary Table i: Clinical details of the 5 NMO patients and their serum IgG samples used in this study. For detailed explanation of the different NMO-IgG assays (FIPA, CBA) see: Waters P and Vincent A. *Int MS J* 2008; 15: 99–105.

Patient	Sex	Ethnic Group	Age at onset	First symptom	Brain involvement (MRI)	Cord lesions (MRI)	Disease course	CSF OCB	CSF cells (per mm^3)	Treatment	IgG_{NMO}		
											Protein (mg/mL)	FIPA* (FU)	CBA**
1	F	Cau	51	ATM	None	C1-C7	Relapsing	-ve	10 lymph 26 neutr	Prednisolone	10.9	21,384	4
2	F	Asi	12	ON	Extensive demyelination	T5-T8	Relapsing	-ve	No	IVMP followed by prednisolone	6.0	26,103	4
3	F	Cau	56	ON	Small vessel lesions	T1-T8	Relapsing	+ve	48 lymph 12 neutr	Prednisolone and methotrexate	16.3	18,122	4
4	F	Cau	53	ATM	Small non-specific lesions	C2-C5	Relapsing	+ve	No	None	21.0	8,005	4
5	F	Cau	55	ATM	Brainstem / cervical lesion	C1-C7	Relapsing	+ve	10 lymph 4 neutr	None	38.0	14,010	4

Asi – Asian, ATM – Acute transverse myelitis, Cau – Caucasian, CBA – Cell based assay, F – Female, FIPA – Fluoro-immuno-precipitation assay, FU – Fluorescence Units, IVMP – intravenous methylprednisolone, lymph – lymphocytes, neutr – neutrophils, OCB – Oligoclonal bands, ON – Optic neuritis, SD – Standard deviation, * – FIPA cut-off is taken as 600 FU, which is 3 SD above the mean of 6 non-NMO controls, ** – In the CBA, the intensity of cell labeling is scored 0 – 4 by inspection

Supplementary Table ii. Lesion characteristics of the six human CNS samples used in the study.

Diagnosis	Patient	Lesion site	AQP4	GFAP	Myelin	Inflam	Foamy mϕ	Axons	Periv. Compl.
NMO	A	Cord	↓	↓↓	↓	↑↑	↑	↓	✓
NMO	B	Brain	↓	↓↓	↓	↑↑	↑	↓	✓
NMO	C	Brain	↓	↓	↓	↑v	↑	→	✓
MS	D	Brain	→	→	↓	↑↑	↑	→	×
MS	E	Brain	↑	↑	↓	↑↑	↑	↓	×
MS	F	Brain	→	→	↓	↑↑	↑	→	×

AQP4 immunostaining: ↓ extensive loss of staining, → mostly perivascular (normal), ↑ upregulated; GFAP immunostaining: ↓↓, no staining, ↓ reduced staining, → normal, ↑ upregulated (reactive astrocytes); Myelin (LFB) staining: → normal, ↓ partial axonal myelin loss, ↓↓ no myelin; Inflam (inflammation): → nil (normal), ↑v mostly perivascular, ↑p mostly parenchymal, ↑↑ mostly perivascular and parenhymal; Foamy mϕ: 0 (absent), ↑ present, ↑↑ abundant; Axons: → preserved, ↓ some axonal loss, ↓↓ prominent axonal loss; Perivascular deposition of activated complement (C9neo): ✓ (present), × (absent).