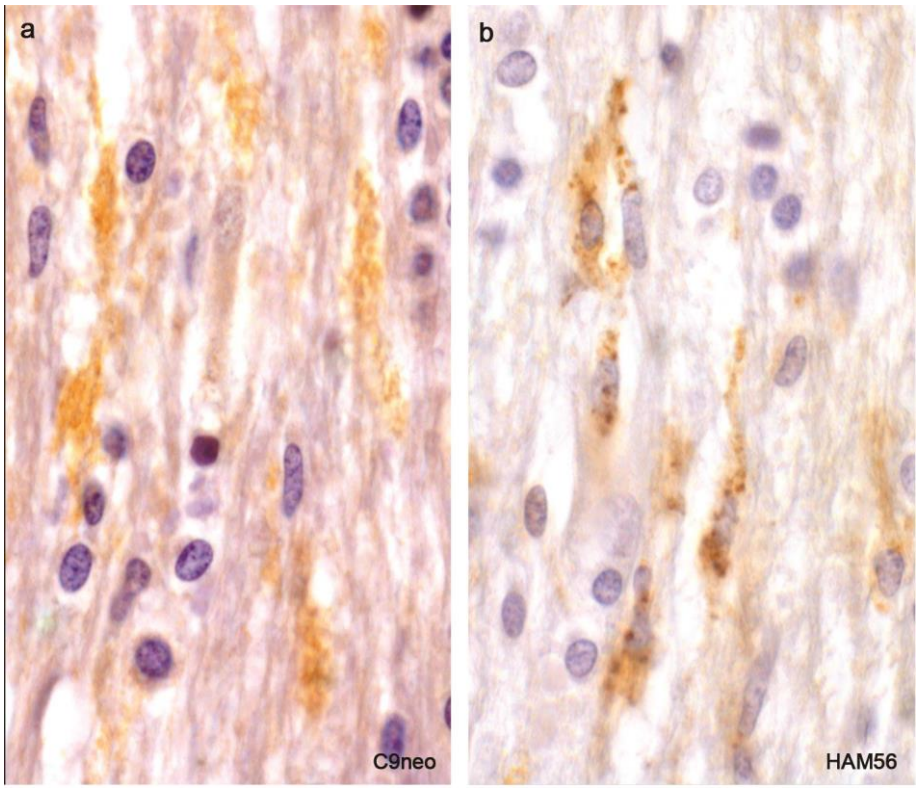
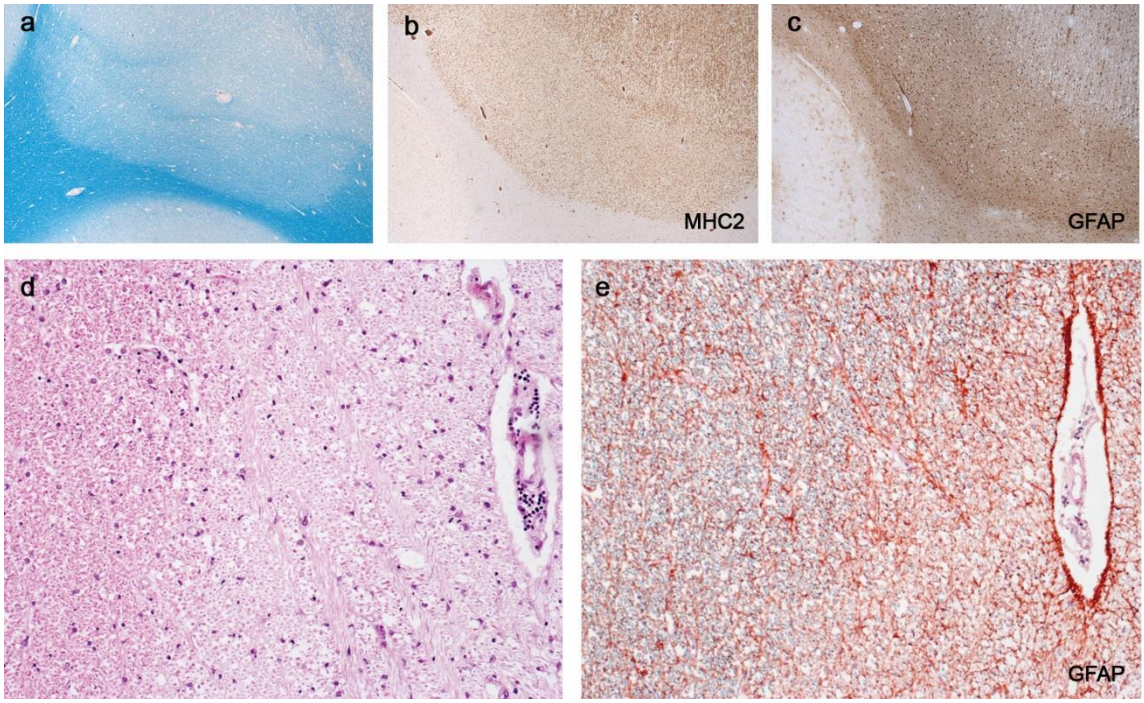
Supplementary Material

Fig S1



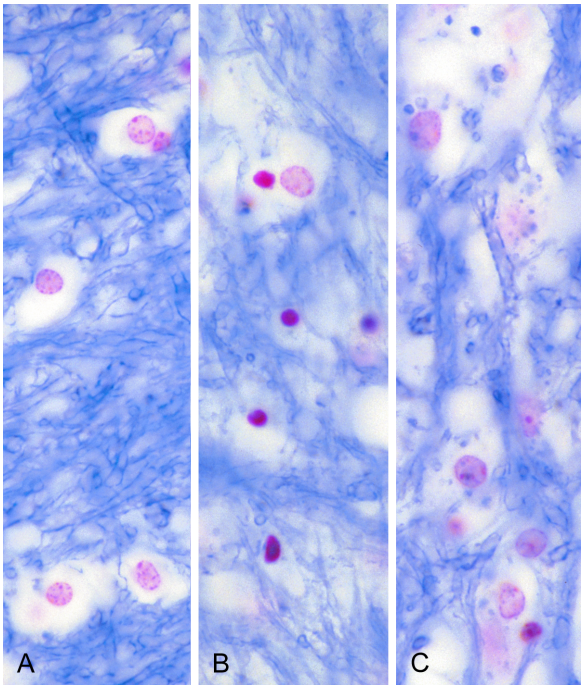
Supplementary Figure S1 Prephagocytosis - complement deposition. a: Intact myelin sheaths stain positively for membrane attack complex C9neo. There is a cell with a pyknotic nucleus in the center of the field – possibly a dead oligodendrocyte. The several cells present with small elongated nuclei are ramified microglia with activated morphology (enlarged nuclei). **b:** A skip serial section of the same lesion shows small microglia/ macrophages in the same area. There are no cells present with the morphology of monocytes. (Case 5- plaque1. **a** C9neo. **b** HAM56. **ab** x1000)

Fig S2



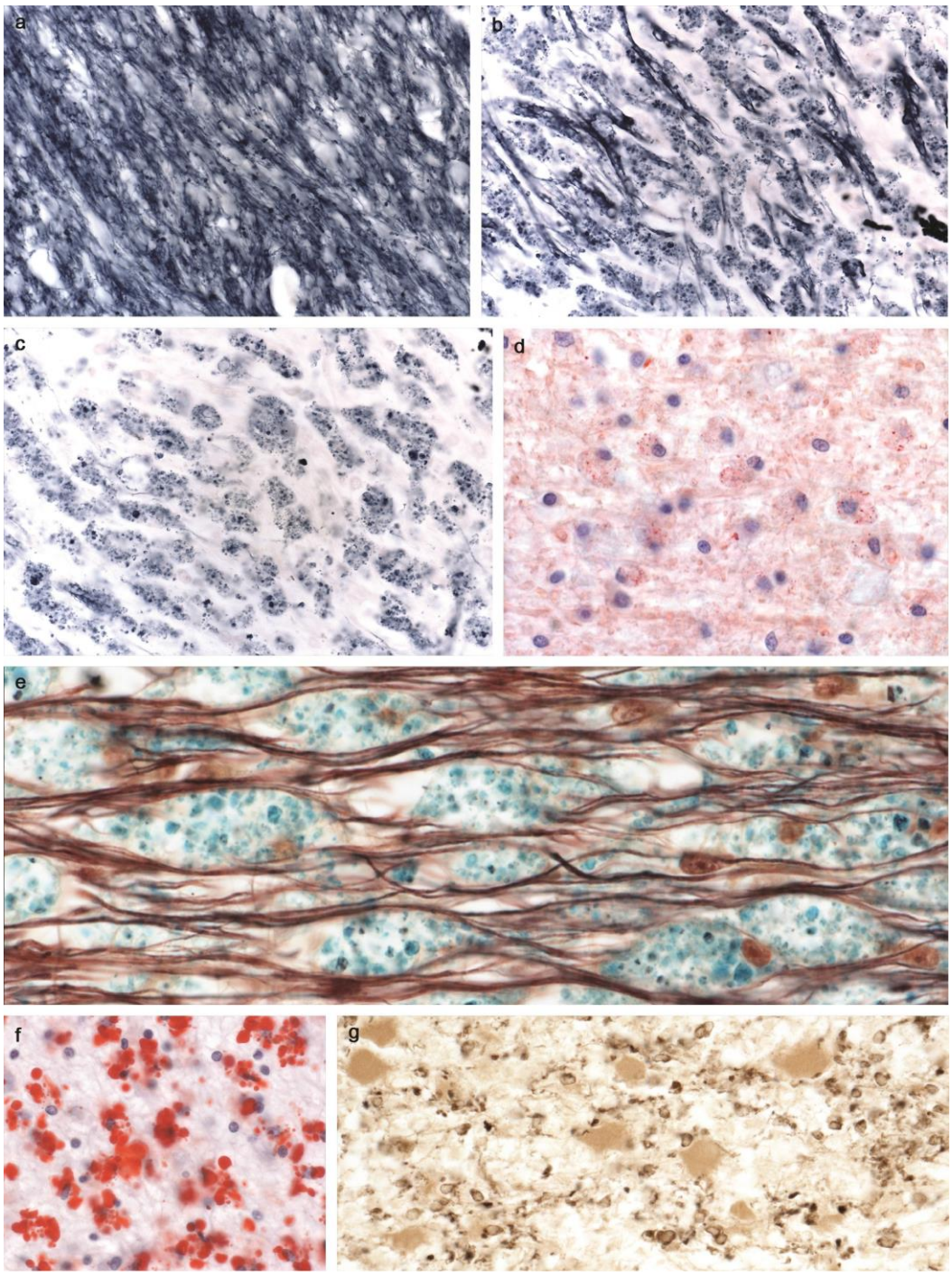
Supplementary Figure S2 Prephagocytosis. abc: The expanding edge of an active plaque in Case 2. Extension into the surrounding intact tissue is in the form of a band of pale myelin **(a**) that stains positively for MHC2+ ramified microglia (**b**) and enlarged GFAP+ astrocytes (**c**). **de:** The caudal end of a 14mm long fatal medullary lesion in Case 9 (Index Case 9, plaque 1) appears pale in a section stained using HE (**d**) and stains normally for GFAP (**e**). A previously reported study of this lesion noted a complete absence of oligodendrocytes in the lesion, with numerous pyknotic oligodendrocytes in the surrounding intact (prephagocytic) tissue (APD Henderson et al Ann Neurol 2009;66:739-753). **(abc** Case 2 **a** LFB, **b** MHC2, **c** GFAP. **abc** x9. de Case 9 d HE, **e** GFAP **de** x116).

Fig S3



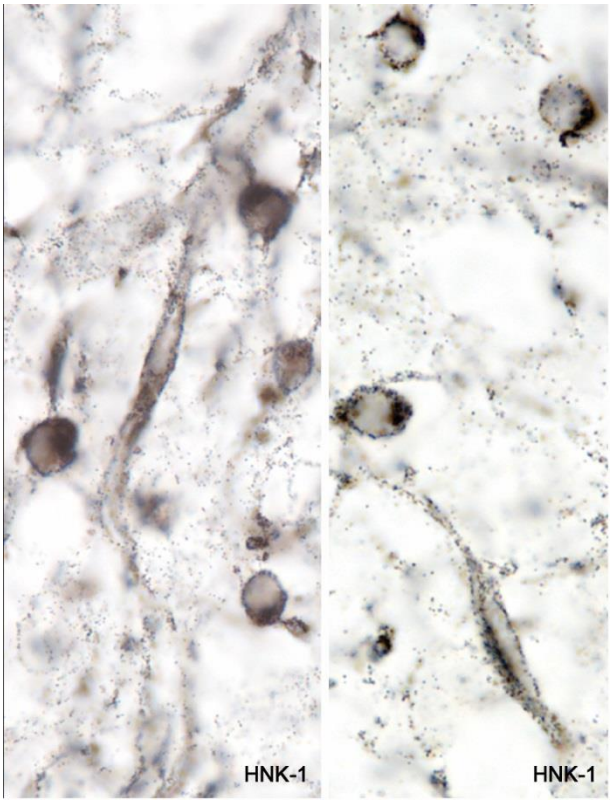
Supplementary Figure S3 Prephagocytosis - oligodendrocyte pyknosis. A stain that colours nuclear chromatin red has been used to identify nuclei in tissue where myelin is stained blue. A: Tissue remote from the plaque shows normal appearing oligodendrocyte nuclei. B: Oligodendrocytes with pyknotic nuclei located in intact myelinated tissue immediately bordering the plaque. C: Adjacent area of commencing myelin destruction. (Luxol fast blue periodic acid-Schiff with nuclear fast read counterstain x630)

Fig S4



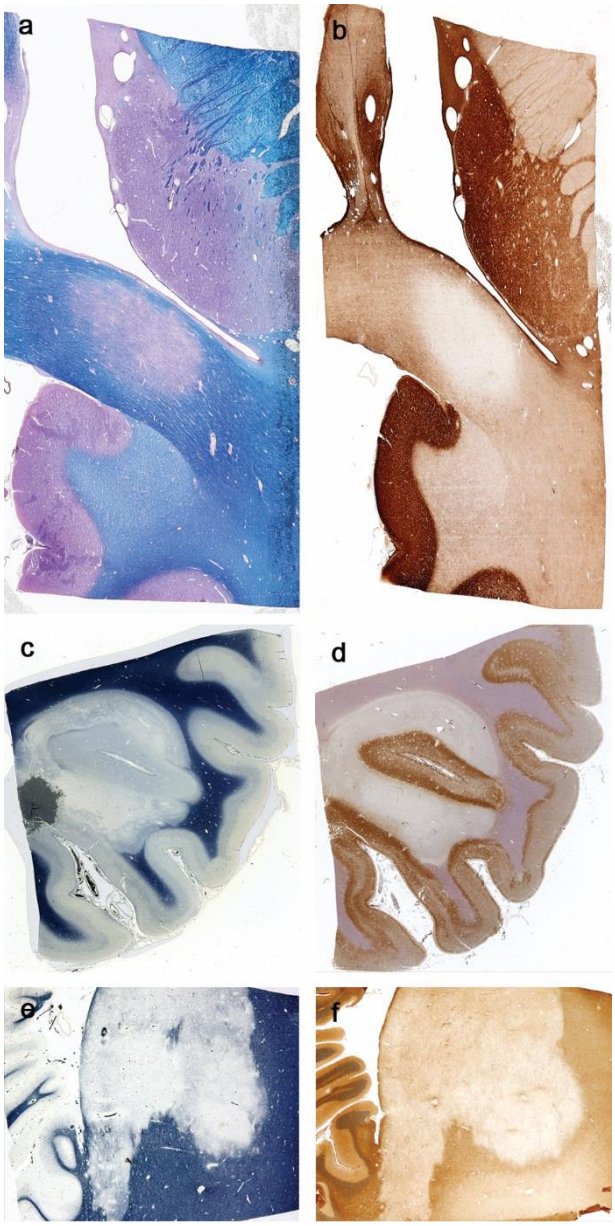
Supplementary Figure S4 Staging macrophage activity. **a**: Commencing myelin ball formation (prephagocytic). **b**: Active myelin breakdown (phagocytic). **c**: Immediate postphagocytic (myelin loss complete, macrophage contents LFB+, MBP+, MOG+). **d**: Immediate postphagocytic tissue stained for neutral lipids (phagocytosed myelin Oil Red O negative). **e**: Immediate postphagocytic tissue stained for myelin and axons. **f**: Classical hypercellular postphagocytic tissue stained using Oil Red O. **g**: Classical hypercellular postphagocytic tissue stained for HNK-1 (numerous unstained large- body astrocytes; numerous HNK-1+ immature oligodendrocytes). (**abc** Case 4 LFB x250. **d** Case 6 Oil Red O x500. **e** Case 5 Bodian/LFB x760. **f**: Case 17 Oil Red O x380. **g**: Case 14 HNK-1 (immunogold-silver) x300).

Fig S5



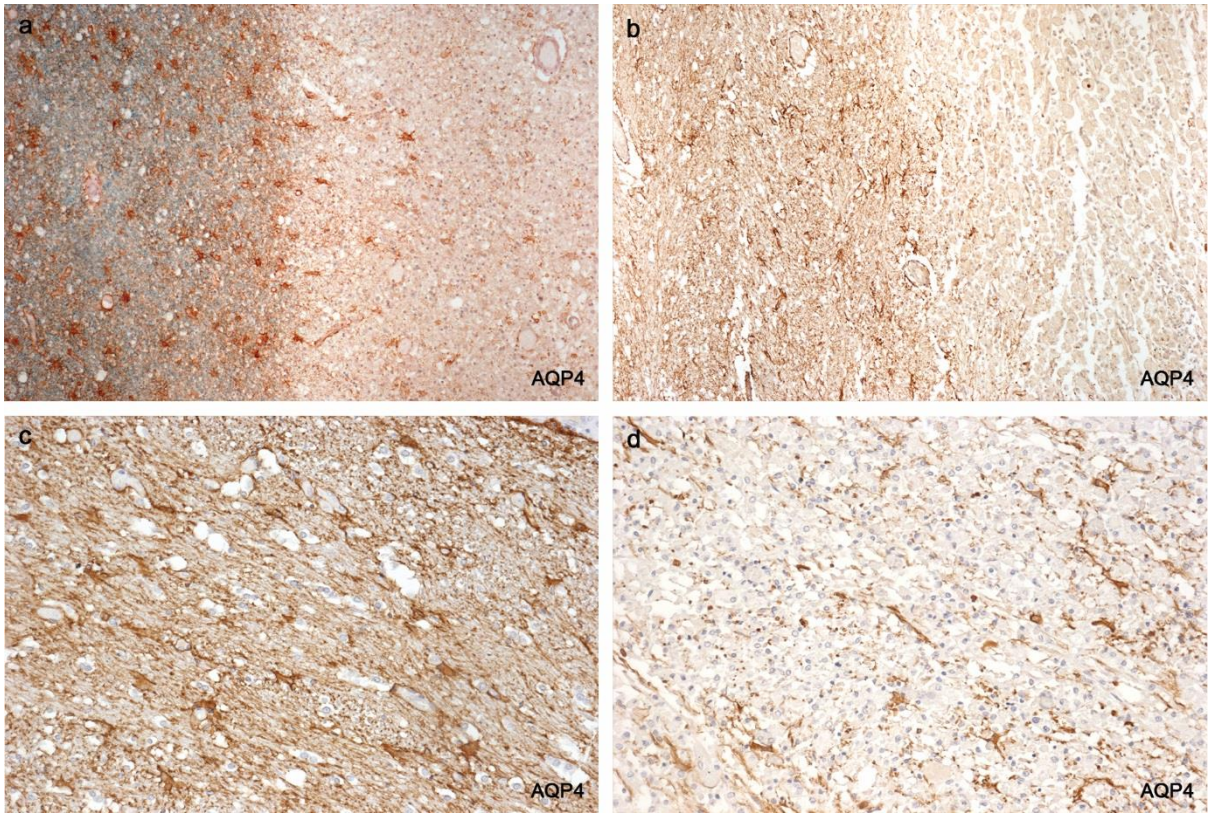
Supplementary Figure S5. HNK1+ precursor astrocytes and oligodendrocytes. **ab**: Recently demyelinated tissue. (**ab** Case 6, plaque 1 HNK-1 x740)

Fig S6



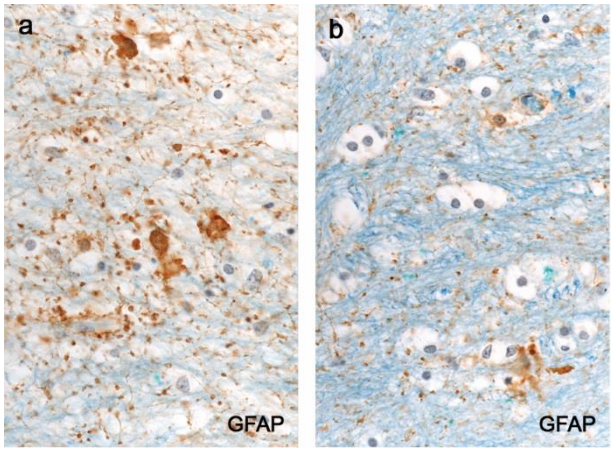
Supplementary Figure S6 AQP4 – negative actively demyelinates plaques. The loss of AQP4 immunoreactivity is restricted to demyelinated tissue. This is an important difference between NMO and MS early lesions. NMO lesions show AQP4 loss extending into still- myelinated tissue. (**ab** corpus callosum lesion Case 9**. a** LFB-PAS, **b** AQP4 **ab** x2.7; **cd** Case 6. **c** LFB. **d** AQP4. **cd** x1.4. **ef** Case 4, plaque 1. **e** LFB. **f** AQP4. **ef** x2.6).

Fig S7



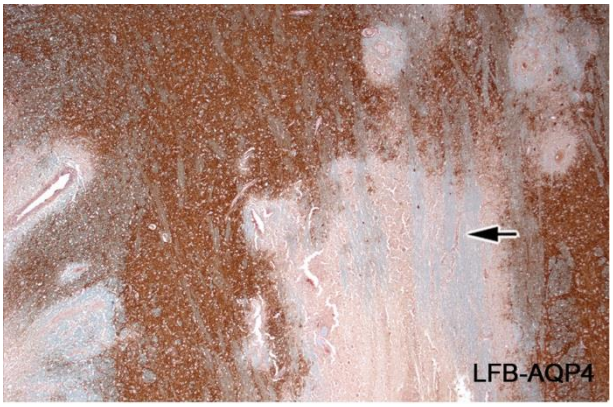
Supplementary Figure S7 Loss of AQP4-positive stellate astrocytes a: Case 6 plaque 1. **b**: Case 3, a second actively demyelinating plaque. **cd**: Intact border (**c**) and edge (**d**) of a recently active plaque in a 68 year old female patient with clinically active disease. (**a** Case 6 plaque 1 AQP4. **b** Case 3 plaque 1 AQP4**. cd** AQP4. **ab** x60 **cd** x100)

Fig S8



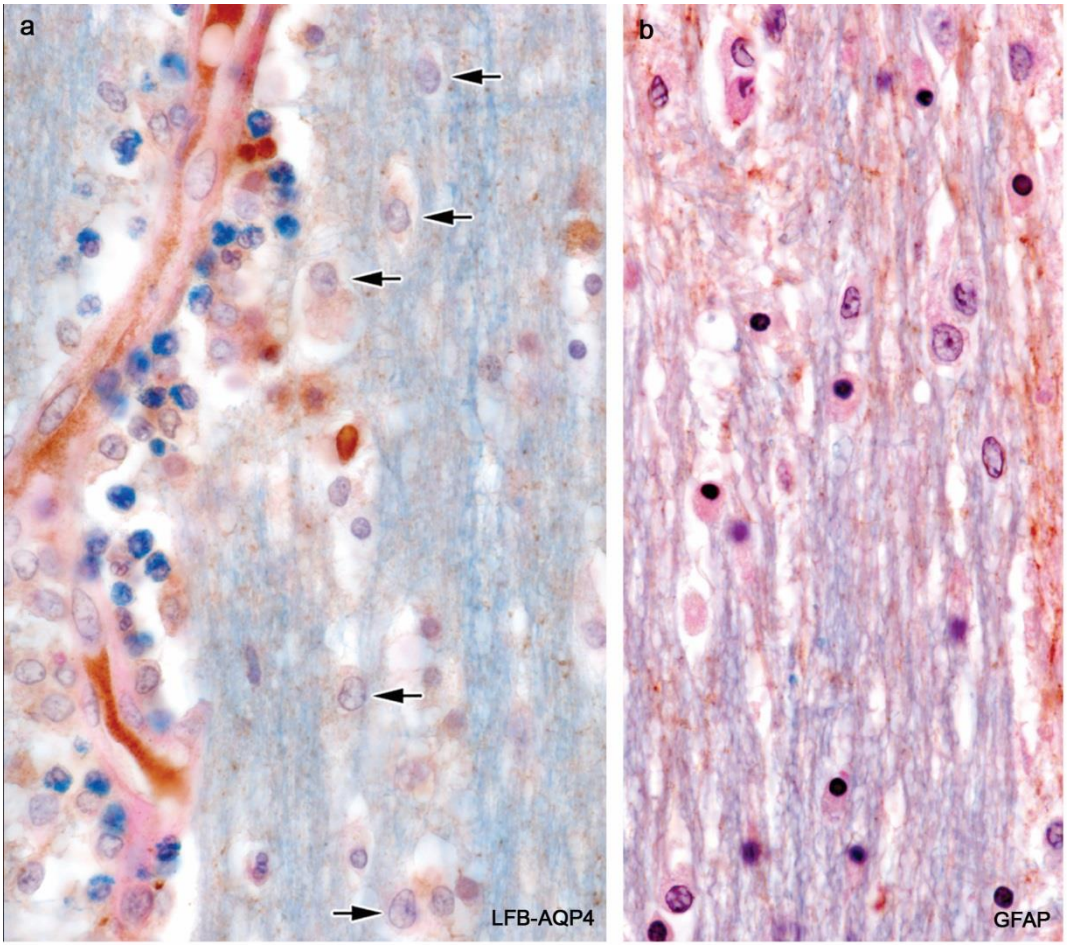
Supplementary Figure S8. Clasmatodendrosis ab: In otherwise normal tissue remote from any visible plaque, GFAP+ astrocyte cell bodies and fibrous processes have fragmented to form small nodules and dust-like particle. Myelin sheaths are intact and there is no apparent loss of oligodendrocytes. (**ab** Case 1 LFB/GFAP x180)

Fig S9



Supplementary Figure S9. Neuromyelitis optica. A non-necrotic still- myelinated plaque and surrounding still- myelinated perivascular lesions show a complete absence of AQP4 immunoreactivity. The vessel indicated by the arrow is shown at higher magnification in **Supplementary Figure S9.** (LFB-PAS/AQP4 **x16**).

Fig S10



Supplementary Figure S10. Neuromyelitis optica. a: A magnified view of the area indicated by the arrow in **Supplementary Figure S8**. Myelin sheaths are pale but intact, oligodendrocytes are difficult to identify and there are a number of macrophages amongst the myelinated fibers (**arrows**). There are no AQP4+ astrocytes present. The perivascular space is packed with eosinophils (eosinophils stain blue in LFB-PAS stained sections).**b:** Another still-myelinated early, non-necrotic NMO lesion, in this instance stained for GFAP. There are no GFAP+ astrocytes present. Macrophages of normal appearance are present amongst the myelinated fibers. Oligodendrocytes have pyknotic nuclei, compact eosinophilic cytoplasm, and lack the fluid – filled 'halo' normally seen around oligodendrocyte nuclei in tissue obtained at autopsy. (**a** LFB-PAS/AQP4. **b** HE/ GFAP. **ab** x680)