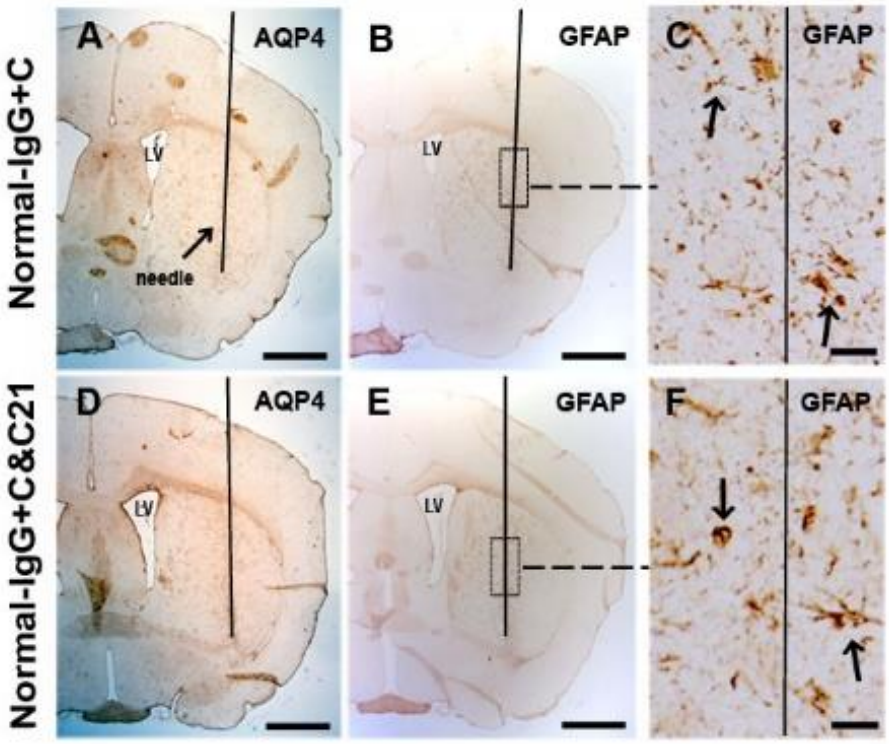
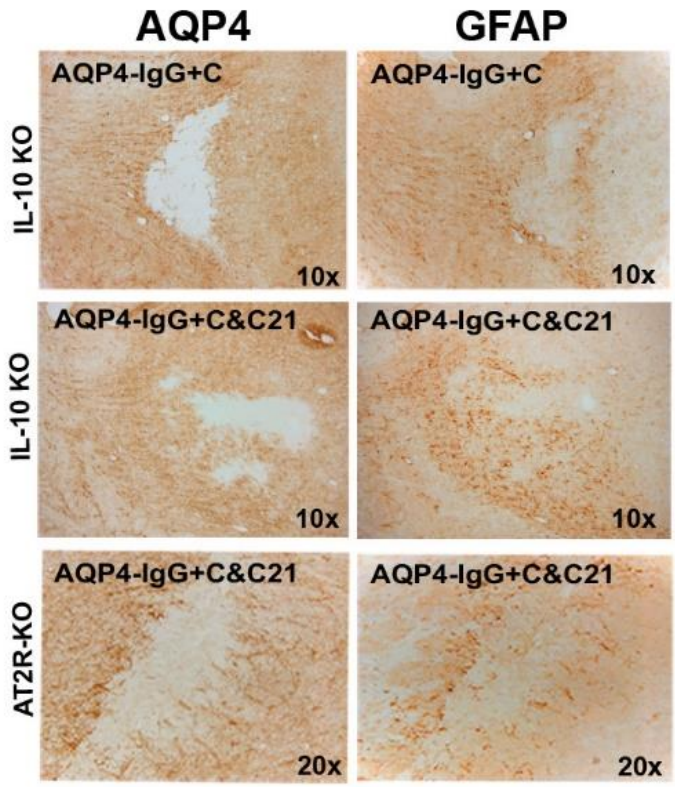
Supplementary materials:

Purification of IgG and C

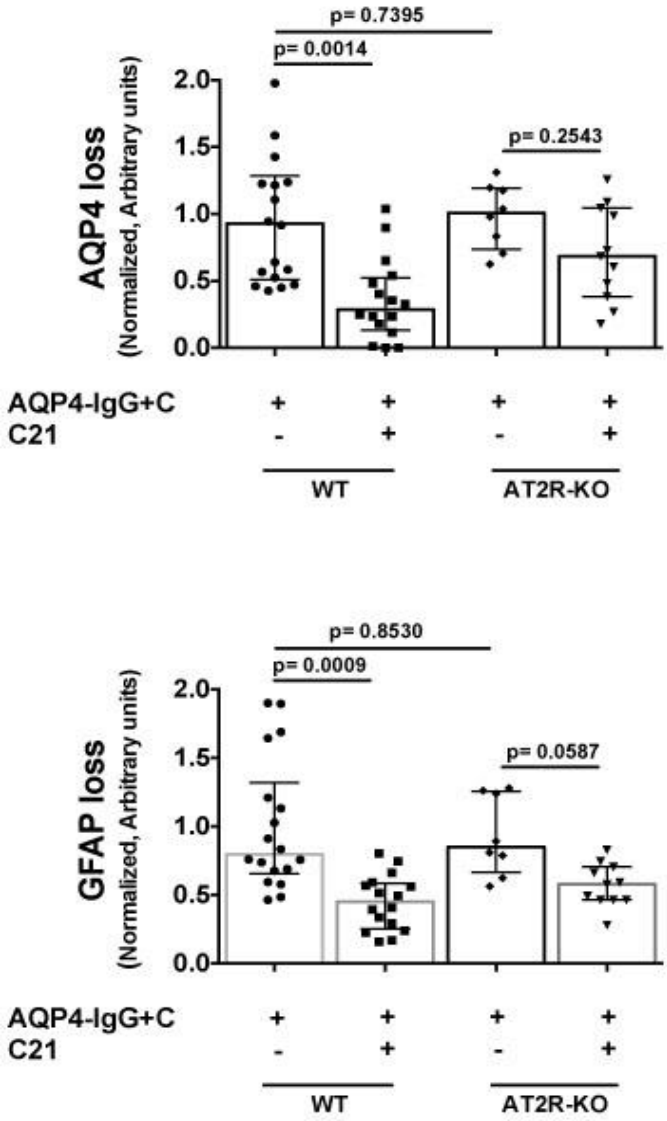
The use of human material, AQP4-IgG, Normal-IgG and C [25], in animals was approved by the Danish Ethical Committee (ref. no. S20080142). In brief, IgG from an NMO-patient and healthy controls was purified from plasma on Protein A columns, eluted with glycine followed by immediate neutralization in Tris, pooling, dialysis against Hartmann's solution, and finally concentrated by dialysis against polyethylene glycol [25]. C originated from a pool of healthy serum donors and the same batch was used in all experiments.



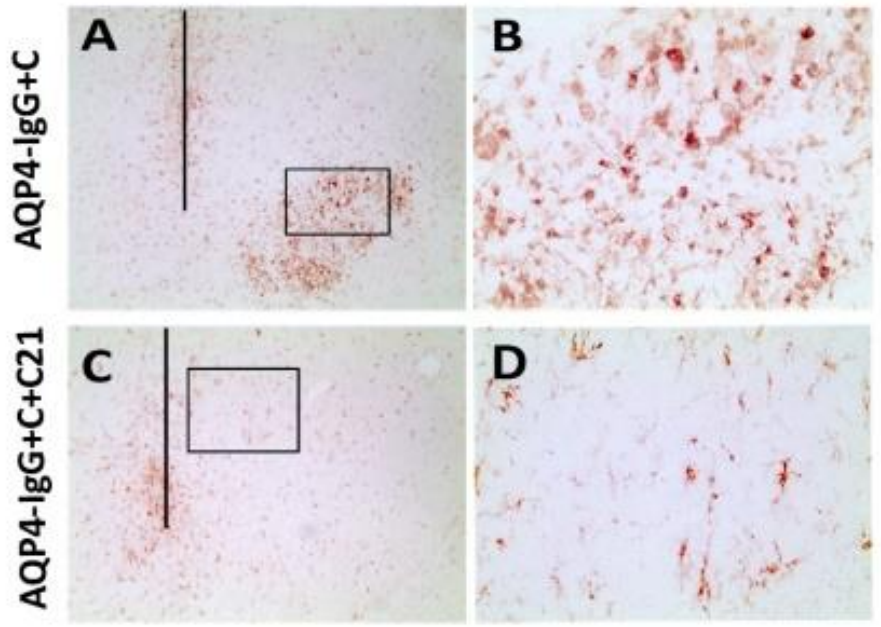
Supplementary Fig. 1. Normal-IgG + C did not induce pathology. A, B, D, E) Overview of sequential sections, stained with AQP4 and GFAP, from WT mice that received intrastriatal co-injection of Normal-IgG + C and C21 or vehicle (PBS) on day 0 followed by intrathecal injection of vehicle (A, B) or C21 (D, E) at day 2, and sacrificed at day 4. Lines approximate needle tracks. C, F) show magnification of corresponding areas marked by boxes in (B) and (E), respectively. Arrows indicate reactive astrocytes proximal to the needle track. Scale bars 500 µm (A, B, D, E) and 20 µm (C, F).



Supplementary Fig. 2. Representative sequential brain sections from different strains and treatments, stained for AQP4 (left) and GFAP (right), showing areas with pathology, without being outlined.



Supplementary Fig. 3. AT2R-KO and WT mice received AQP4-IgG + C with C21 or vehicle by stereotactic intrastriatal injection on day 0, followed by intrathecal administration of C21 or vehicle at day 2. Mice were sacrificed at day 4. Pathology was calculated as described in Materials and Methods. Graphs show that the loss of AQP4 and GFAP was not significantly different between AT2R-KO that received C21 (n=11) or vehicle (n=8) and WT mice that received AQP4-IgG + C. The degree of pathology was significantly reduced in WT mice treated with C21 (n=16) compared to mice treated with vehicle (n=18). Bar graphs show normalized AQP4 and GFAP loss in WT mice and in AT2R-KO mice treated with C21 or vehicle. Results are shown as medians with interquartile range.



Supplementary Fig. 4. Evaluation of microglial reactivity. Microglia were stained using anti-Iba1 antibody. A and C) Overview of a representative section stained with Iba1, from WT mice that received intrastriatal co-injection of AQP4-IgG + C and vehicle (A) or C21 (C) on day 0 followed by intrathecal injection of vehicle (A) or C21 (C) at day 2. Mice were sacrificed at day 4. Lines approximate needle tracks. Microglia became activated in mice that received AQP4-IgG +C with or without C21. Iba1 reactivity was observed at the needle track, reflecting the normal glial response to tissue trauma, as well as distal from the needle track. Intense Iba1-reactive cells with characteristic microglia/macrophage morphology were observed in the area (box in A) corresponding to the area of AQP4/GFAP loss. These areas with intense Iba1 reactivity were reduced or not observed in brains from C21-treated mice (box in C). B, D) show magnification of corresponding areas marked by boxes in (A) and (C), respectively. A and C (10x magnification) B and D (40x magnification).