SUPPLEMENTAL INFORMATION

Supplemental Material and Methods

Immunohistochemical localization of immuno and standard proteasomes in EAE cerebellum

Cerebella from acute EAE animals were fixed overnight in methacarn and then mounted in paraffin. Tissue was sectioned in the sagital plane (6-µm thick) and mounted on Vectabond™-treated slides (Vector Laboratories, Burlingame, CA, USA). Sections were deparafinized with xylenes and a graded alcohol series, and then rinsed with PBS for 10 min. Slices were collected, rinsed three times with PBS, blocked with 10% (v/v) normal goat serum and incubated with the mixture of two primary antibodies overnight at 4˚C, followed by incubation with the corresponding fluorescent secondary antibodies (Alexa Fluor® 488 and Alexa Fluor® 647, 1:100, Molecular Probes). After washing, the sections were cover slipped with anti-fade fluorescent mounting medium. Cells containing immunoproteasomes and standard proteasomes were identified by using rabbit antibodies against iβ2 (1:500; Enzo) and β5 (1:500; Enzo), respectively. The various cell types were detected by using antibodies against GFAP (1:500, mouse monoclonal; Sigma), Iba1 (1:250, mouse monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA), CD3 (1:100, mouse monoclonal, Santa Cruz), adenomatous polyposis coli protein C-terminal (1:125, mouse monoclonal, Chemicon, Temecula, CA) and NeuN (1:250, mouse monoclonal, Chemicon). Images were captured with a Zeiss 200m microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY) equipped with a Hamamatsu C4742-95 digital camera (Hamamatsu Corp., Bridgewater, NJ). Images were imported into Image J software to obtain merged pictures.

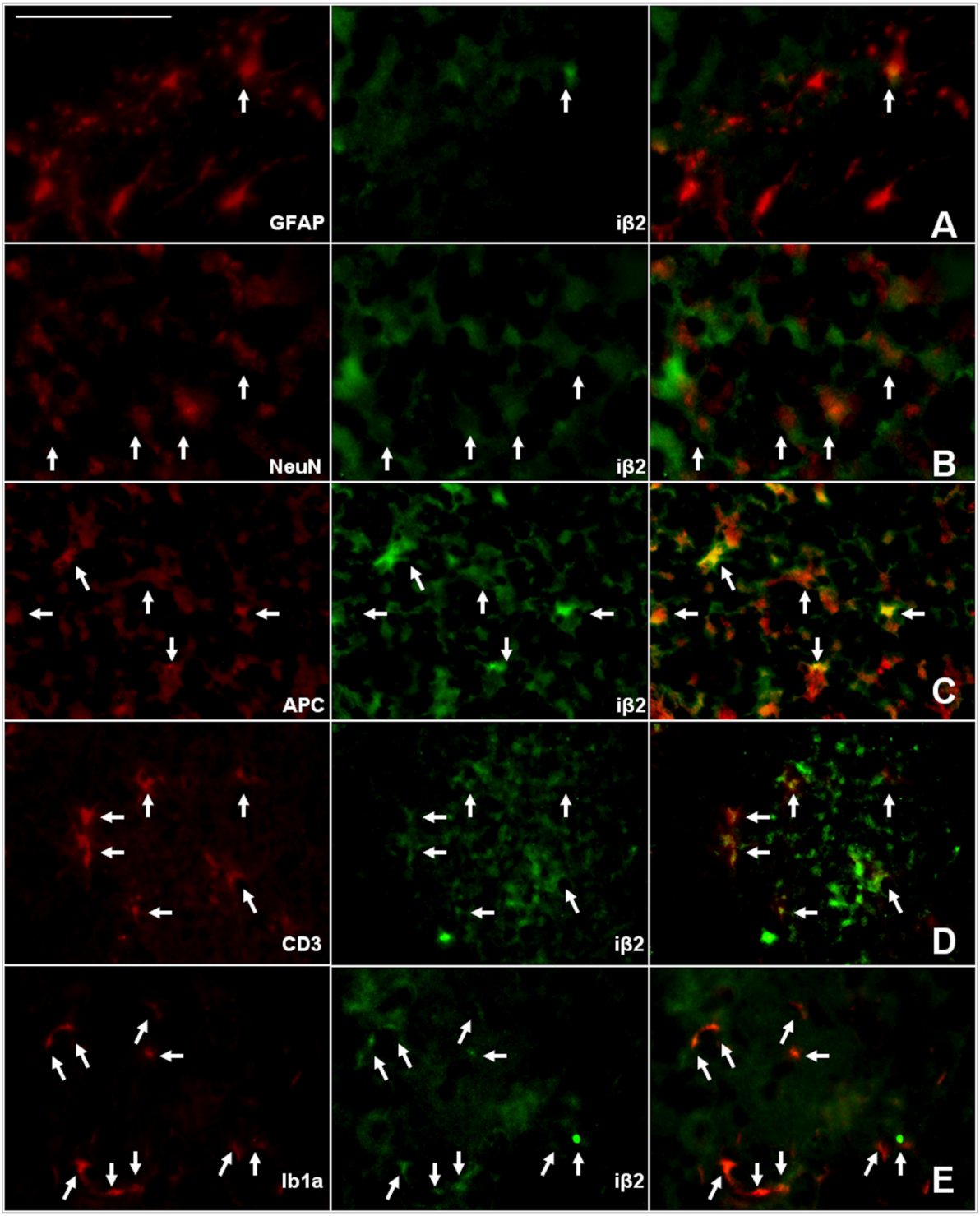

Fig. S1- Immunoproteasomes are expressed in all cells types in the cerebellum of acute EAE mice except astrocytes. Double immunofluorescence analysis was performed as described above. Red channel is for the various cell markers while green channel is for immunoproteasome detected by iβ2 subunit staining. Right panels show the merged images. Note that immunoproteasomes are present in neurons (NeuN; panel B), oligodendrocytes (APC; panel C), T cells (CD3; panel D) and activated microglia (Ib1a; panel E). In contrast, immunoproteasomes are mostly absent in astrocytes (GFAP; panel A). Bar= 200μm.

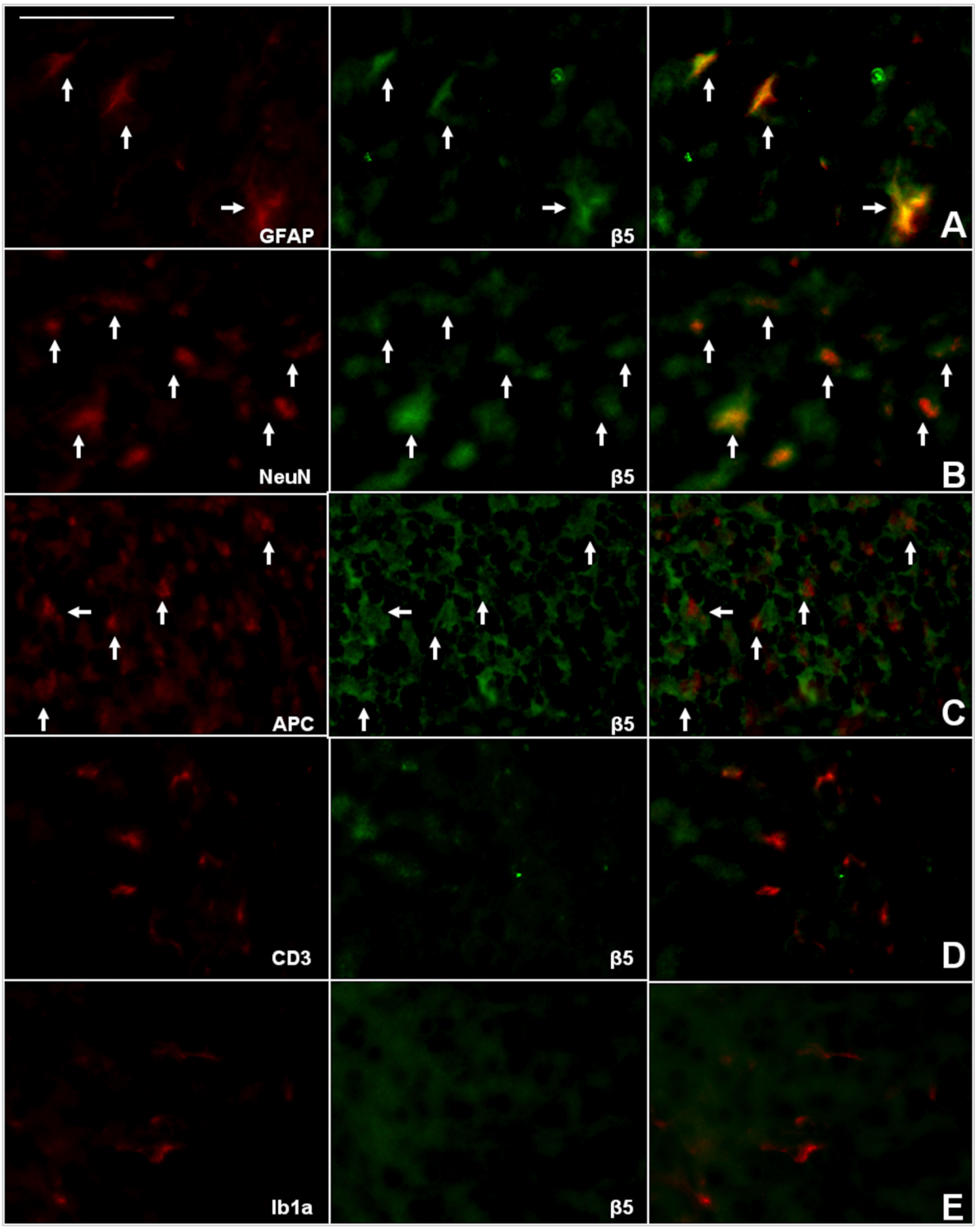

Fig. S2 - Standard proteasomes are expressed in the three major cell types of the cerebellum of acute EAE mice but not in T cells and microglia. Double immunofluorescence analysis was performed as described above. Red channel is for the various cell markers while green channel is for standard proteasome detected by β5 subunit staining. Right panels show the merged images. Note that standard proteasomes are present in astrocytes (GFAP; panel A), neurons (NeuN; panel B) and oligodendrocytes (APC; panel C) but not in T cells (CD3; panel D) and activated microglia (Ib1a; panel E). Bar= 200μm.