

# Supplemental Material

## ***In vitro* Characterization of the Regional Binding Distribution of Amyloid PET Tracer Florbetaben and the Glia Tracers Deprenyl and PK1195 in Autopsy Alzheimer's Brain Tissue**

### **Methods**

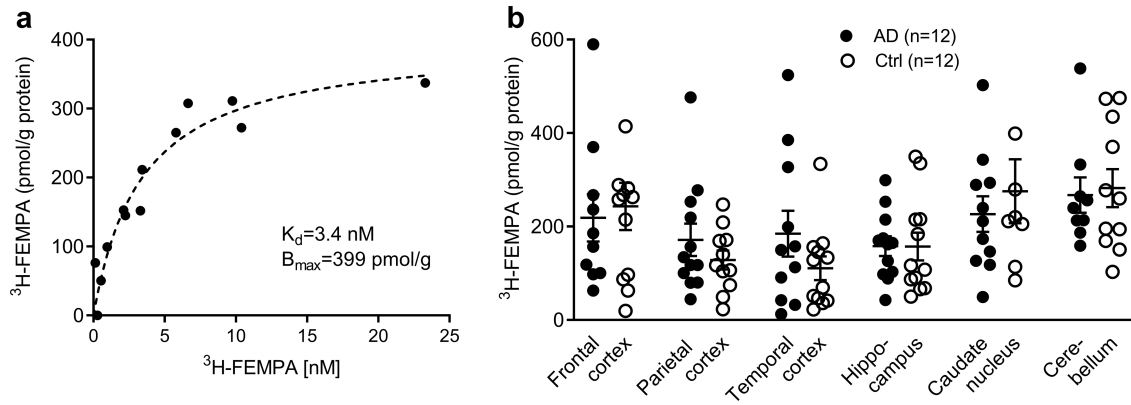
#### *Immunohistochemistry*

Sections from the frontal cortex and hippocampus of four AD and four control cases were double stained with A $\beta$  antibody 4G8 (A $\beta$ <sub>17-24</sub>, 1:200 Signet/Covance, Princeton, NJ, USA) in combination with anti-GFAP (1:500, Dako, Denmark) or with anti-ionized calcium-binding adaptor molecule 1 (Iba) (1:500, Wako, Osaka, Japan). Immuno-reactivity was visualized using AP and horseradish peroxidase (HRP) systems (Invitrogen, La Jolla, CA, USA) and evaluated at 4 x magnification using a light microscope (Leica, Wetzlar, Germany) with an attached ProgRes® video camera and ProgRes®Capture Pro2.8.8 image analysis system (Jenoptik, Jena, Germany). Quantification was performed using ImageJ software (NIH, Bethesda, MD, USA). Results were presented as immunopositive cells/ mm<sup>2</sup>.

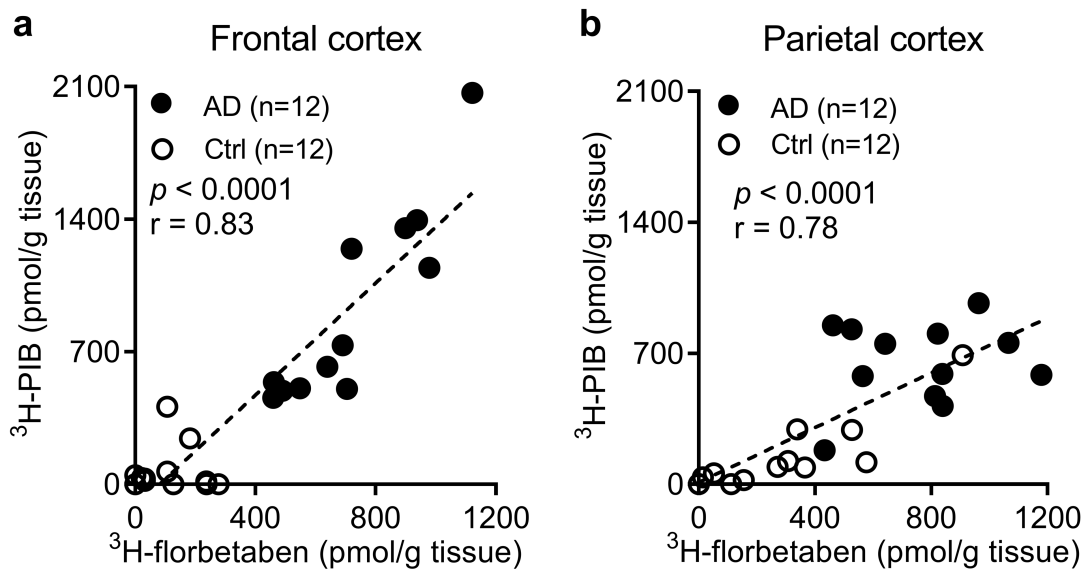
### **Results**

#### *Immunohistochemical analysis of A $\beta$ plaques, reactive astrocytes, and microglia in AD and control brain*

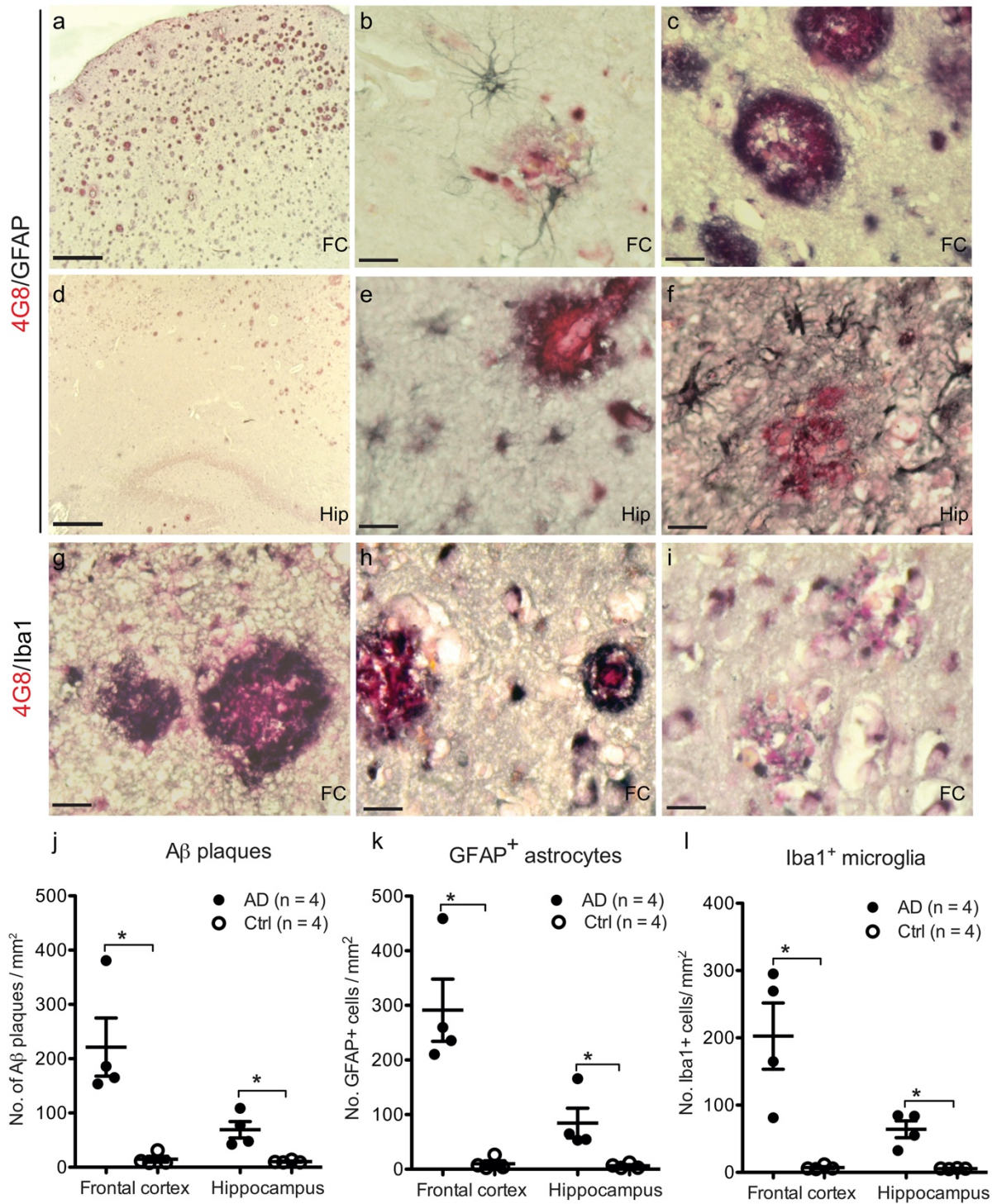
To understand the imaging tracer binding-histopathology relationship, double immunohistochemical staining using 4G8 (for A $\beta$ <sub>17-24</sub>) antibodies followed by anti-GFAP or anti-Iba1 antibodies were performed in brain slices from the frontal cortex and the hippocampus of four AD and four control cases. Supplementary Figure 3 shows representative staining of 4G8/GFAP and 4G8/Iba1 in the slices from the frontal cortex and the hippocampus of one AD case. Greater numbers of A $\beta$  deposits, A $\beta$ -associated GFAP<sup>+</sup> astrocytes, and A $\beta$ -associated Iba1<sup>+</sup> microglia were found in the frontal cortex ( $p = 0.0286$ ,  $p = 0.0286$ ,  $p = 0.0286$  respectively) and the hippocampus ( $p = 0.0286$ ,  $p = 0.0286$ ,  $p = 0.0286$  respectively) of AD (n = 4) compared to control cases (n = 4) (Supplementary Figure 3e-g).



**Supplementary Figure 1.** a) Saturation binding curve for 3H-FEMPA in increasing concentration up to 25 nM. b) Regional binding of 3H-FEMPA in 6 different brain regions from 12 AD and 12 controls .



**Supplementary Figure 2.** a) Positive correlation between  $^3\text{H-florbetaben}$  and  $^3\text{H-PIB}$  binding in frontal cortex from 12 AD and 12 control brains. b) Positive correlation between  $^3\text{H-florbetaben}$  and  $^3\text{H-PIB}$  binding in parietal cortex from 12 AD and 12 control brains.



**Supplementary Figure 3.** Representative staining of 4G8/GFAP and 4G8/Iba1 in the slices from the frontal cortex and the hippocampus of one AD case. Greater numbers of Aβ deposits, Aβ-associated GFAP<sup>+</sup> astrocytes and Aβ-associated Iba1<sup>+</sup> microglia were found in the frontal cortex ( $p = 0.0286$ ,  $p = 0.0286$ ,  $p = 0.0286$ , respectively) and the hippocampus ( $p = 0.0286$ ,  $p = 0.0286$ ,  $p = 0.0286$ , respectively) of AD ( $n = 4$ ) compared to control cases ( $n = 4$ ) (e-g).