

## **Supplementary Materials**

**Supplementary File S1** Histopathological changes and PrP<sup>Sc</sup> accumulation in the brain of mice that succumbed to disease.

### **Materials and Methods**

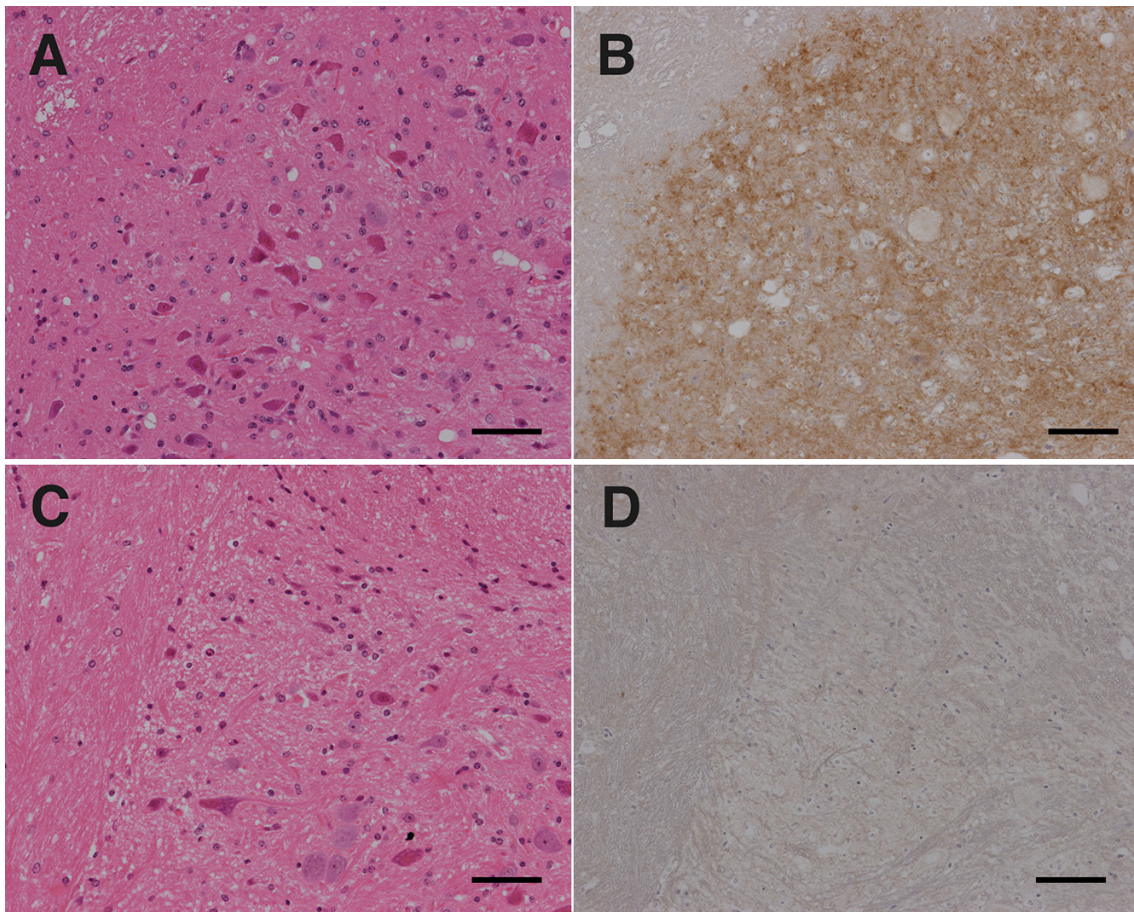
#### **Histopathology and Immunohistochemistry**

Formalin-fixed left hemispheres of the brain were immersed in 98% formic acid to reduce infectivity and embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin (H&E) to evaluate neuropathological changes. After epitope retrieval by autoclaving in 0.01 M citrate buffer (pH 6.0), immunocytochemistry of PrP<sup>Sc</sup> was performed with monoclonal anti-PrP antibody 2G11. Immunoreactions were developed by using anti-mouse universal immunoperoxidase polymer (Nichirei Histofine Simple Stain MAX-PO (M); Nichirei, Tokyo, Japan) as the secondary antibody, and visualized with 3,3'-diaminobenzidine tetrachloride as the chromogen.

### **Results**

#### **Accumulation of PrP<sup>Sc</sup> in the Brains of Mice that Succumbed to Disease**

Histopathological analysis showed the presence of mild to moderate spongiform changes in the grey matter of the brain stem and cerebellar nuclei of all mice that succumbed to disease (all 5 mice injected with PBS-treated scrapie prion, and 2 of 6 mice injected with CAC-717-treated scrapie prion). Immunohistochemistry with antibody 2G11 showed that PrP<sup>Sc</sup> accumulated in the cerebral cortex, grey matter of the brain stem, and cerebellar nuclei of all succumbed mice (again, all 5 mice injected with PBS-treated scrapie prion, and 2 of 6 mice injected with CAC-717-treated scrapie prion), whereas no such accumulation of PrP<sup>Sc</sup> was seen in mice that survived (4 of 6 mice injected with CAC-717-treated scrapie prion).



**Figure S1** Histopathological changes and PrP<sup>Sc</sup> accumulation in the brain of mice. (A) H&E staining showed mild spongiform changes in the cerebellar dentate nucleus of a succumbed mouse inoculated with PBS-treated scrapie prions. (B) Accumulation of PrP<sup>Sc</sup> was confirmed by immunohistochemistry in the nucleus of a succumbed mouse inoculated with PBS-treated scrapie prions. (C) H&E staining showed no change in the cerebellar dentate nucleus of a surviving mouse inoculated with CAC-717-treated scrapie prions. (D) Immunohistochemistry showed no PrP<sup>Sc</sup> accumulation in the nucleus of a surviving mouse inoculated with CAC-717-treated scrapie prions. Bars, 50 µm.