SUPPLEMENTARY MATERIAL

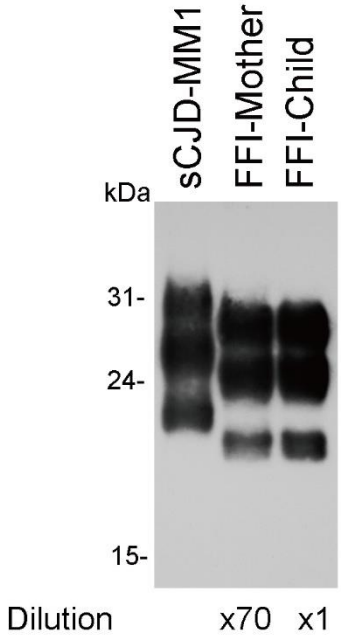
Supplemental method

Preparation of seeds or substrates and cell-PMCA

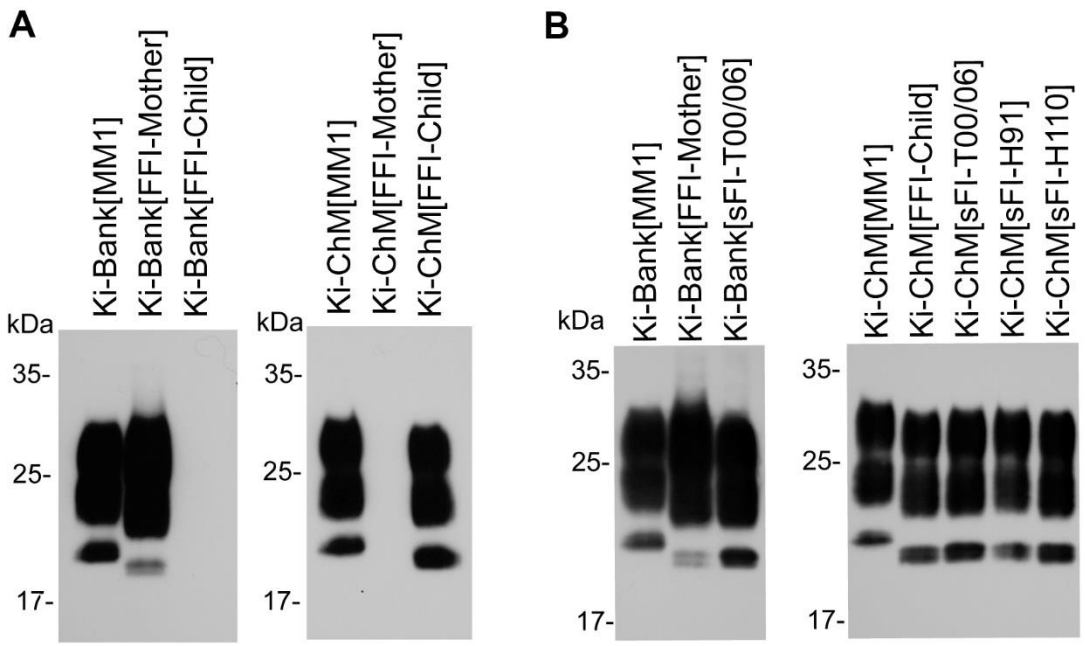
129 cases of sCJD (MM1: 90 cases; MM2C: 9 cases; sFI: 10 cases; MV1: 1 case; MV2: 7 cases; VV1: 1 case, and VV2: 1 case), FFI (4 cases) or non-CJD (6 cases) was used as a seed for cell PMCA. Ten % (w/v) sCJD, FFI or non-CJD patient brain homogenates were prepared in PMCA buffer (50 mM HEPES-NaOH (pH7.5), 2% (v/v) Nonidet-P40, 160 mM NaCl, 4mM EDTA and 5% (v/v) glycerol and 5% (w/v) trehalose) with a ceramic bead homogenizer (Precellys 24 homogenizer, Bertin Instruments, France) followed by centrifugation at 8,000g for 2 min to remove insoluble debris. Brain homogenates were stored at −80°C in small aliquots until use. Cell lysates as a substrate from FreeStyle 293F cells (InvitrogenTM) stably expressing human PrP^{C} with methionine at codon 129 were prepared at 20% (w/v) in PMCA buffer (50 mM HEPES-NaOH (pH7.5), 2% (v/v) Nonidet-P40, 160 mM NaCl, 5% (v/v) glycerol and 5% (w/v) trehalose) with probe sonication. 4 mM EDTA were added to the cell lysates after centrifugation at 8,000 g for 2 min to remove insoluble debris, and the cell lysates were stored at −80°C in small aliquots until use. For amplification of the CJD prions, brain homogenates were combined with the cell lysates, and 1% (w/v) Sodium Dextran Sulfate (Nacalai Tesque, Inc, Kyoto, Japan; Cat No. 10912-92) was added to the reaction mixture prior to the amplification in a 0.1-mL thin-walled polymerase chain reaction tube with a screw cap. Each PMCA round comprised 192 cycles of sonication (five pulses of 5 sec with 1 sec rest) and agitation (30

min at 37◦C) and was carried out using a fully automatic cross-ultrasonic protein activating apparatus (ELESTEIN 070-GOT, Elekon Science, Japan). Either before or after PMCA, samples were digested with 50 μg/ml proteinase K at 37ºC for 60 min. The digested samples were subjected to SDS-PAGE and western blot analysis. Anti-PrP monoclonal antibody 3F4 (Signet, Dedham, MA, USA) was used as the primary antibody. Anti-mouse EnVision+ (Dako) was used as the secondary antibody.

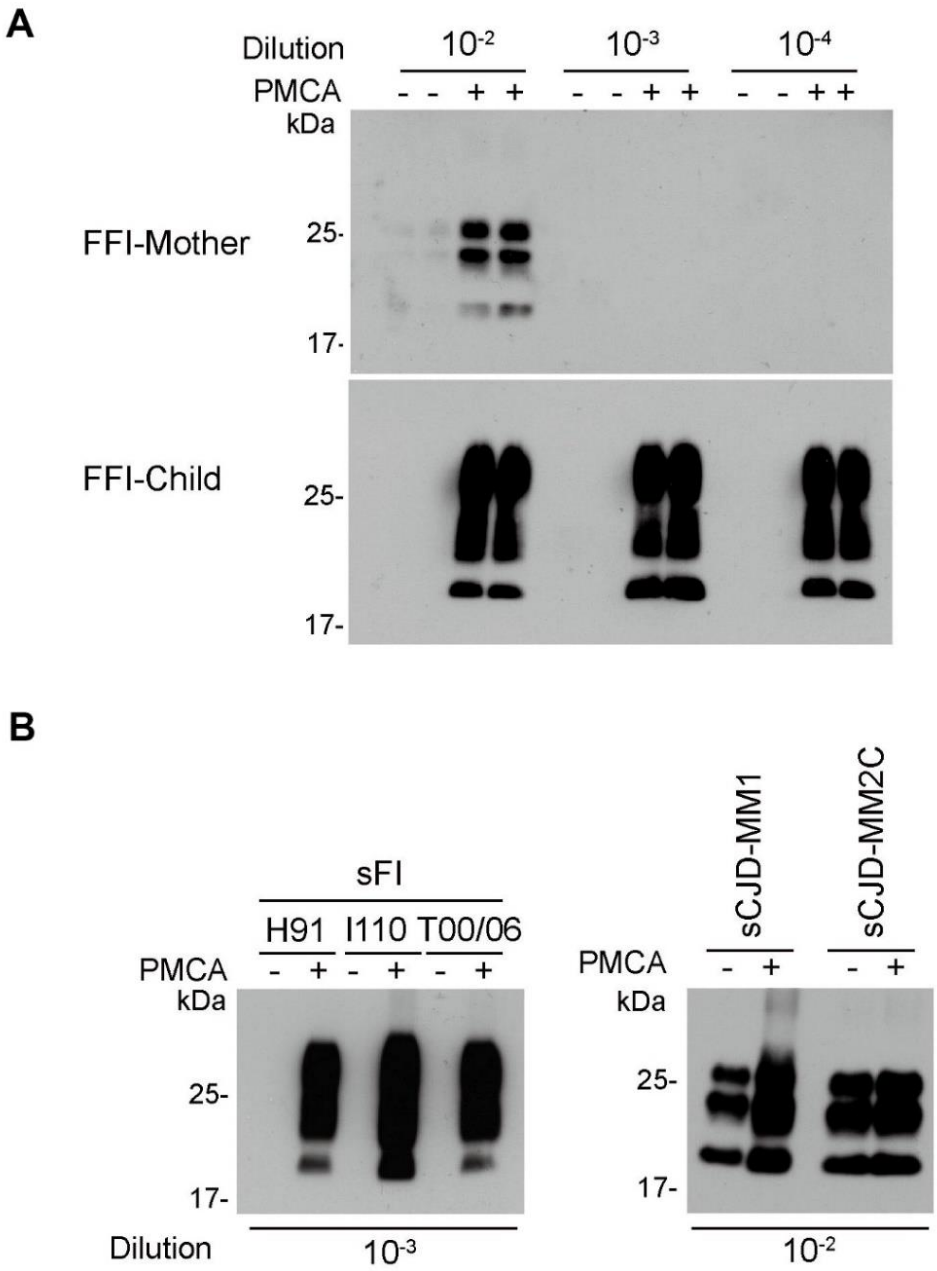
Supplemental figures



Supplementary figure 1. Western blotting of the brains from FFI kindred. Each sample was diluted to an appropriate concentration of PrP^{Sc} for signal detection. PrP^{Sc} of both FFI-Mother and FFI-Child in the brains showed type 2. Anti-PrP monoclonal antibodies 3F4 was used as the primary antibodies. Numbers indicate the molecular size standards (kDa).



Supplementary figure 2. Western blotting of the brains from the inoculated Ki-Bank mice or Ki-ChM mice with prions from the FFI kindred or sFI. (A) Positive immunoreactivities were observed in Ki-Bank mice inoculated with sCJD-MM1 (Ki-Bank [sCJD-MM1]) or FFI-Mother (Ki-Bank [FFI-Mother]) and in Ki-ChM mice inoculated with sCJD-MM1 (Ki-ChM [sCJD-MM1]) or FFI-Child (Ki-ChM [FFI-Child]). **(B)** Positive immunoreactivities were observed in Ki-Bank mice inoculated with sCJD-MM1 (Ki-Bank [sCJD-MM1]), FFI-Mother (Ki-Bank [FFI-Mother])or sFI-T00/06 (Ki-Bank [sFI-T00/06]) and in Ki-ChM mice inoculated with sCJD-MM1 (Ki-ChM [sCJD-MM1]), FFI-Child (Ki-ChM [FFI-Child]), sFI-T00/06 (Ki-ChM [sFI-T00/06]), sFI-H91 (Ki-ChM [sFI-H91]) or sFI-H110 (Ki-ChM [sFI-H110]). Anti-PrP monoclonal antibodies 3F4 for the Ki-ChM mice and SAF83 for the Ki-Bank mice were used as the primary antibodies. Numbers indicate the molecular size standards (kDa).



Supplementary figure 3. Western blotting of amplified products from the brains of the FFI kindred, sFI, or sCJD using cell-PMCA. (A) Brain homogenates from FFI-Mother or FFI-Child were amplified with the substrate lysates prepared from 293F cells stably expressing human 129MPrP^{C}. For the amplification of prions, brain homogenates were combined with the cell lysates and the mixtures were then subjected to 192 cycles

of PMCA up to a 10^{-4}-fold dilution (e.g. 100, 1,000, 10,000 times). Either before (-) or after (+), the PMCA samples were treated with proteinase K (50 μg/ml at 37ºC for 60 min) and subjected to western blotting using the anti-PrP monoclonal antibody 3F4. **(B)** Representative image of cell-PMCA analysis of sFI, sCJD-MM1 or -MM2C. Cell-PMCA of the brains of sFI and sCJD were performed at a 10^{-3} and a 10^{-2} fold dilution with cell lysates expressing human 129MPrP^{C}, respectively. Each of the results was typical of experiments performed at least three times. Numbers show the molecular size standards (kDa).