In vitro seeding activity of glycoform-deficient prions from variably protease-sensitive prionopathy and familial CJD associated with PrP^{V180I} mutation

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Table S1

Table S1 ANOVA analysis

Groups	Comparison	p values
Fig. 2b	Seeds	>0.05
	Substrates	>0.05
	Interaction	<0.0005
Fig. 2e	Seeds	>0.05
	Substrates	<0.0001
	Interaction	<0.0001
Fig. 3c	Seeds	<0.0005
	Substrates	<0.0001
	Interaction	<0.0001
Fig. 3g	Seeds	<0.0001
	Substrates	<0.0001
	Interaction	<0.0001

Fig. S1

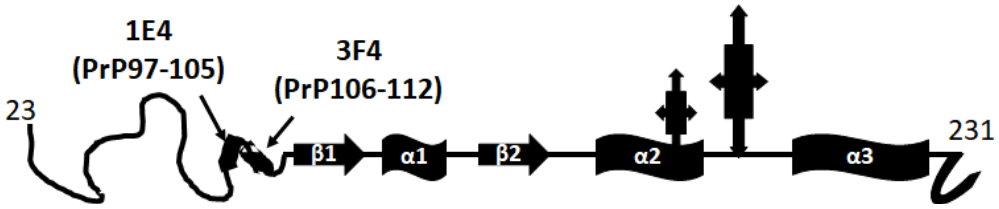

Fig. S1 Schematic diagram of locations of the 1E4 and 3F4 epitopes on PrP and structure of human PrP. The mature human PrP contains 209 amino acids from residues 23 to 231 with an unstructured N-terminal domain from residues 23 to 127 and a structured C-terminal domain from residues 128 to 231. The 1E4 and 3F4 epitopes are next to each other, localizing in the unstructured domain between residues 97 and 105 for the 1E4 epitope and between residues 106 and 112 for the 3F4 epitope. In the structured C-terminal domain, there are two short β-sheets (PrP128-131 for β1 and PrP161-164 for β2) and three α-helixes (PrP144-154 for α1, PrP173-194 for α2 and PrP200-228 for α3) [26]. There are two N-linked glycosylation sites at residues 181 and 197 (black quad arrow callouts) and a GPI anchor at the residue 231 (black circular arrow) [27, 28].

Fig. S2

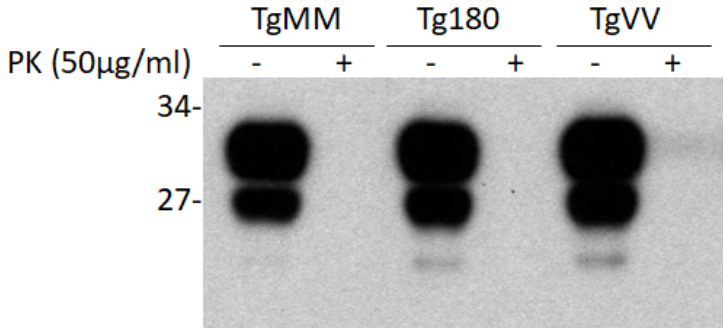

Fig. S2 Comparison of gel profile of the PrP molecule from brain homogenates of humanized TgMM, Tg180 and TgVV mice. Representative Western blotting of PrP from brain homogenates of humanized Tg mice expressing human wild-type PrP with 129MM polymorphism (TgMM), PrP^{V180I} mutation (Tg180), or wild-type PrP with 129VV (TgVV) treated with or without digestion of PK at 50 μg/ml. The blot was probed with 3F4. Molecular weight markers are shown in kDa on the left side of the blots.

Fig. S3

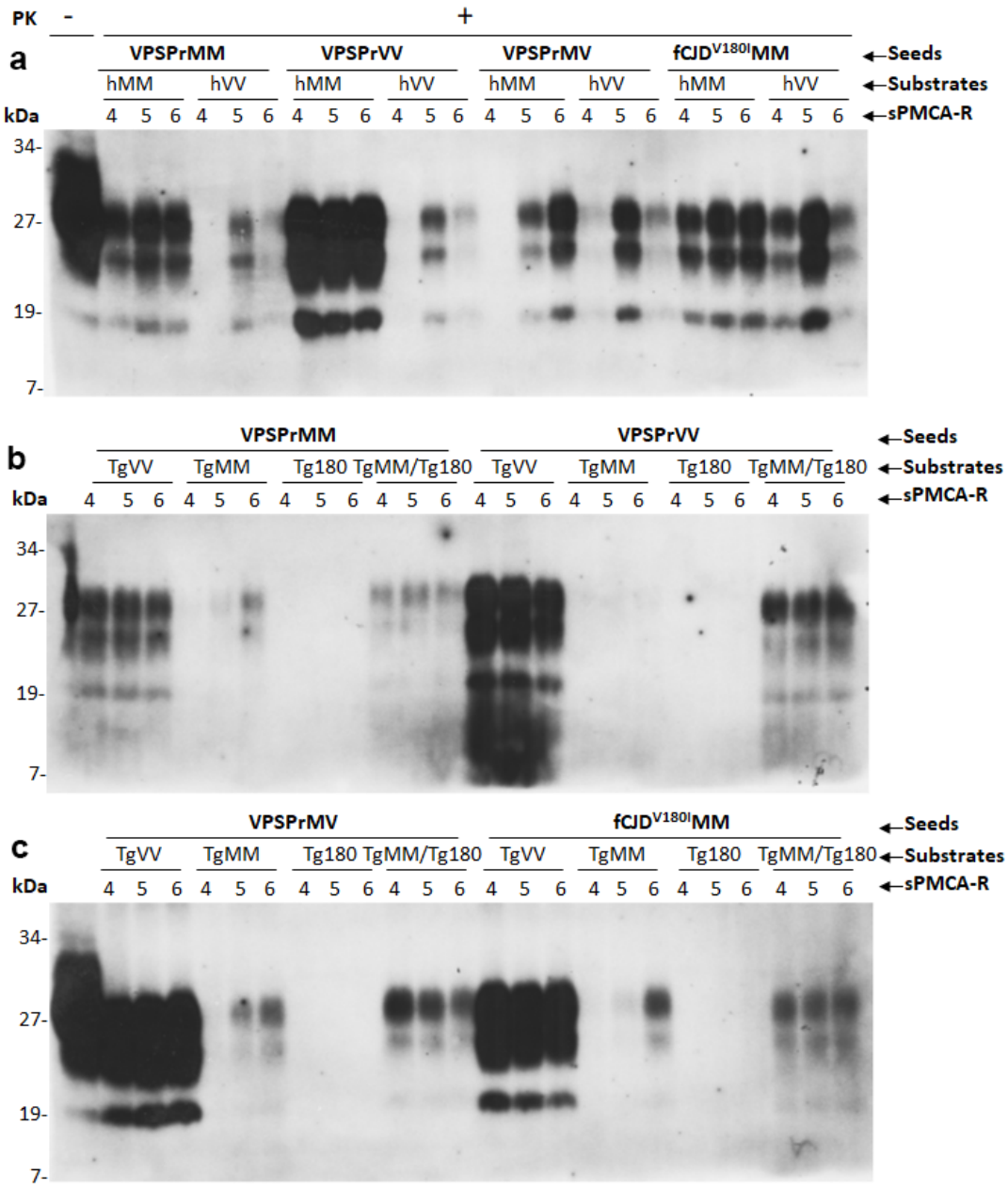

Fig. S3 Serial PMCA of PrP^{Sc} from VPSPr and $fCJD^{V180I}$ in human or Tg mouse brain homogenate substrate. **(a)** Representative Western blotting of PrP^{Sc} amplified with 4-6 rounds of sPMCA by seeding PrP^{Sc} from VPSPrMM, VPSPrVV, VPSPrMV, $fCJD^{V180I}$, and $fCJD^{T183A}$ in the hMM or hVV brain substrate. **(b, c)** Representative Western blotting of PrP^{Sc} amplified with 4-6 rounds of sPMCA by seeding PrP^{Sc} from VPSPrMM and VPSPrVV **(b)** as well as VPSPrMV and $fCJD^{V180I}$MM (**c**) in humanized transgenic mouse brain homogenates from TgVV, TgMM, Tg180, or TgMM + Tg180 mouse lines probed with the

Tohoku 2 antibody. sPMCA-R: sPMCA rounds. Molecular weight markers are shown in kDa on the left side of the blots.

Fig. S4

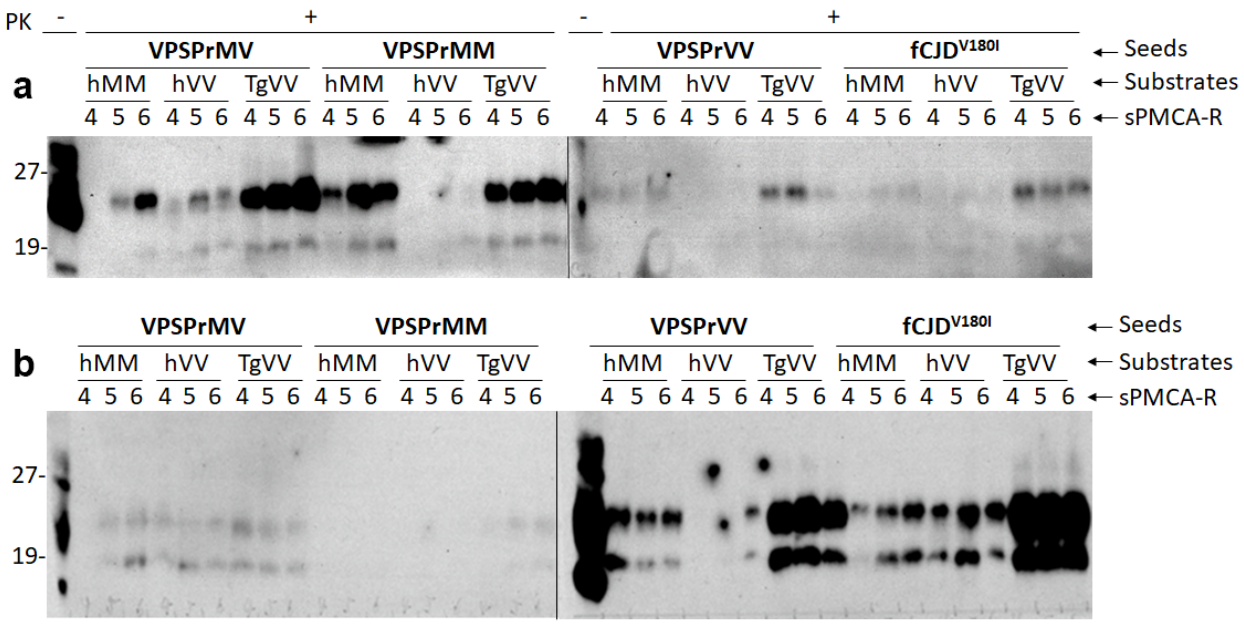

Fig. S4 Serial PMCA of PrP^{Sc} from VPSPr and $fCJD^{V180I}$ in human brain homogenates or humanized Tg mouse brain homogenates. Representative Western blotting of PrP^{Sc} from VPSPrMV, VPSPrMM, VPSPrVV, or $fCJD^{V180I}$ amplified with 4-6 rounds of sPMCA in human brain homogenates from non-CJD MM (hMM) or VV (hVV) or mouse brain homogenates from humanized TgVV mice probed with the Bar209 **(a)** or V14 (**b**) antibody. sPMCA-R: sPMCA rounds. Molecular weight markers are shown in kDa on the left side of the blots. PK: Proteinase K.