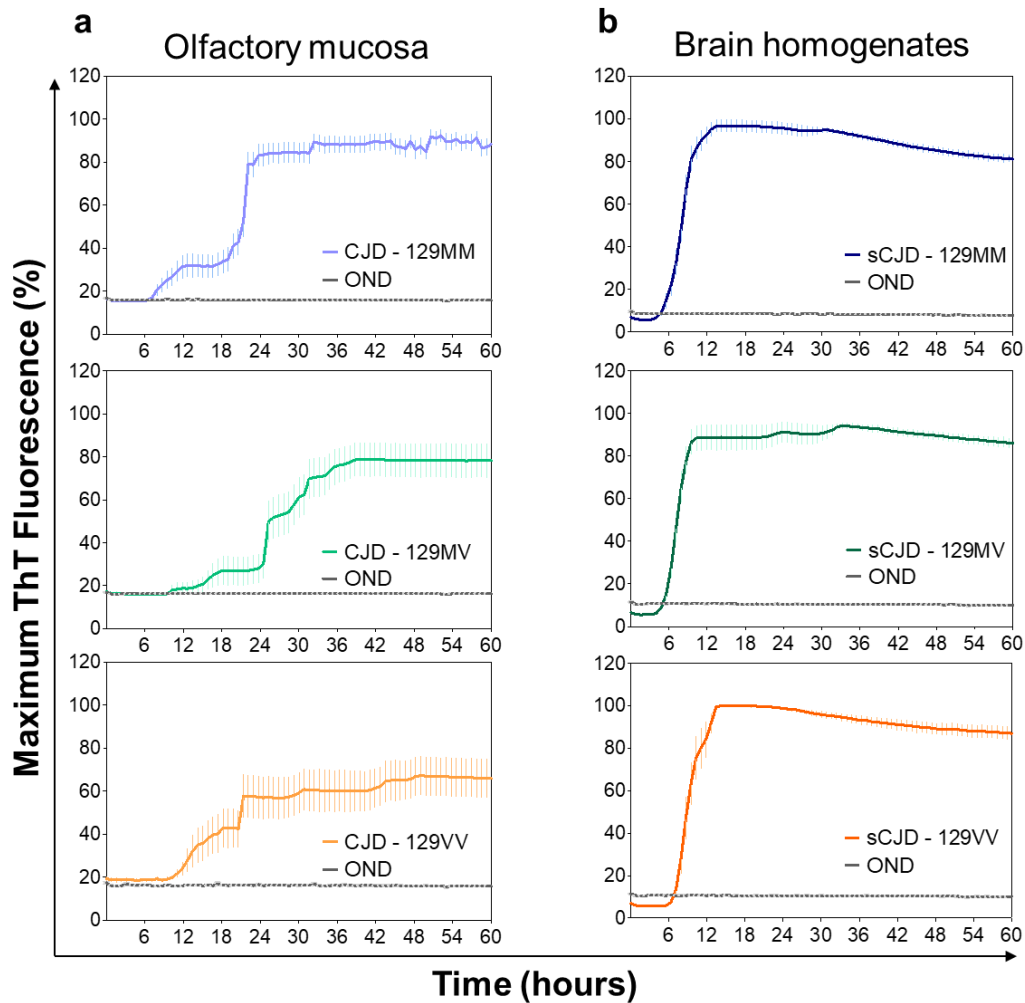
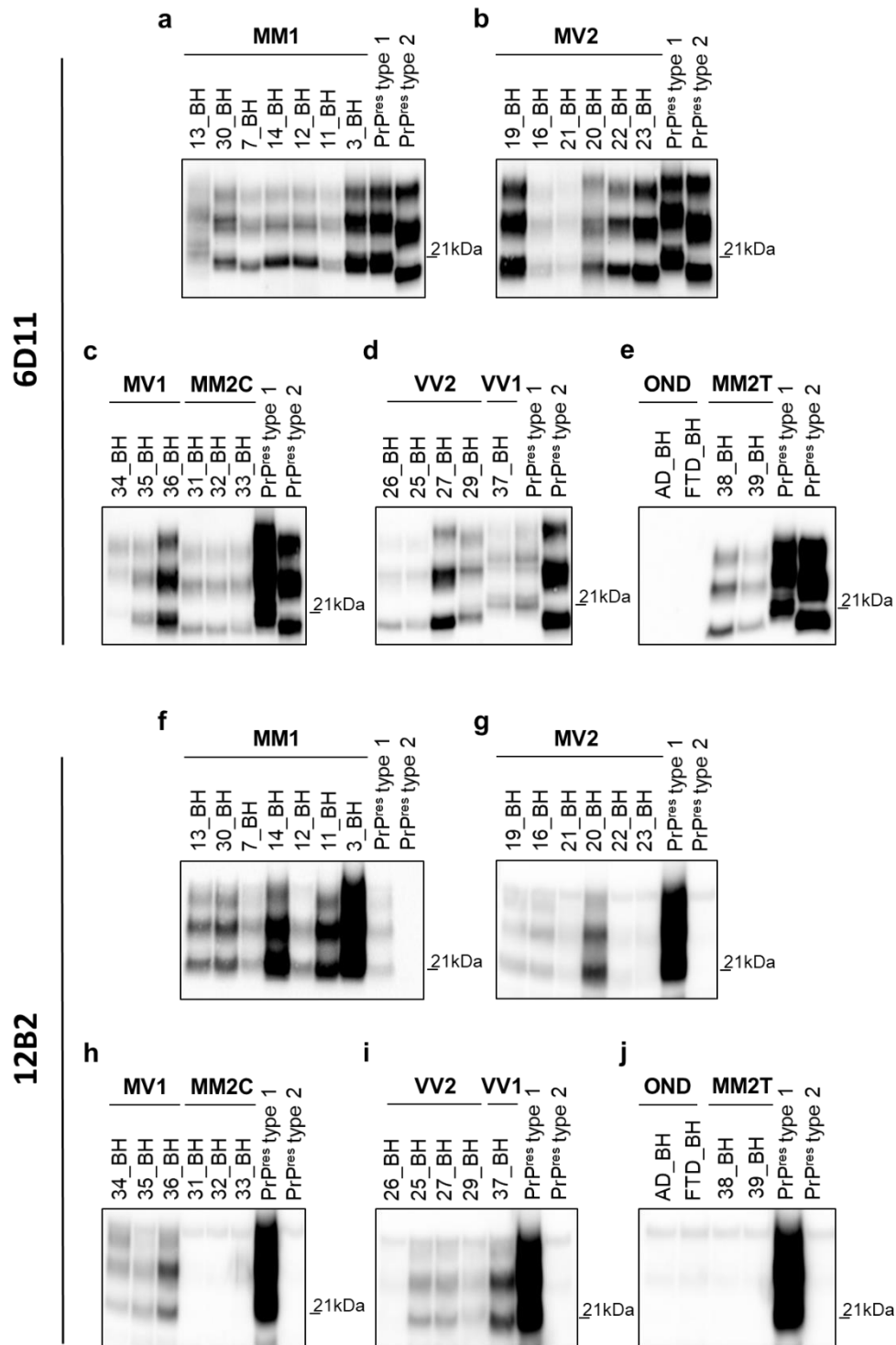


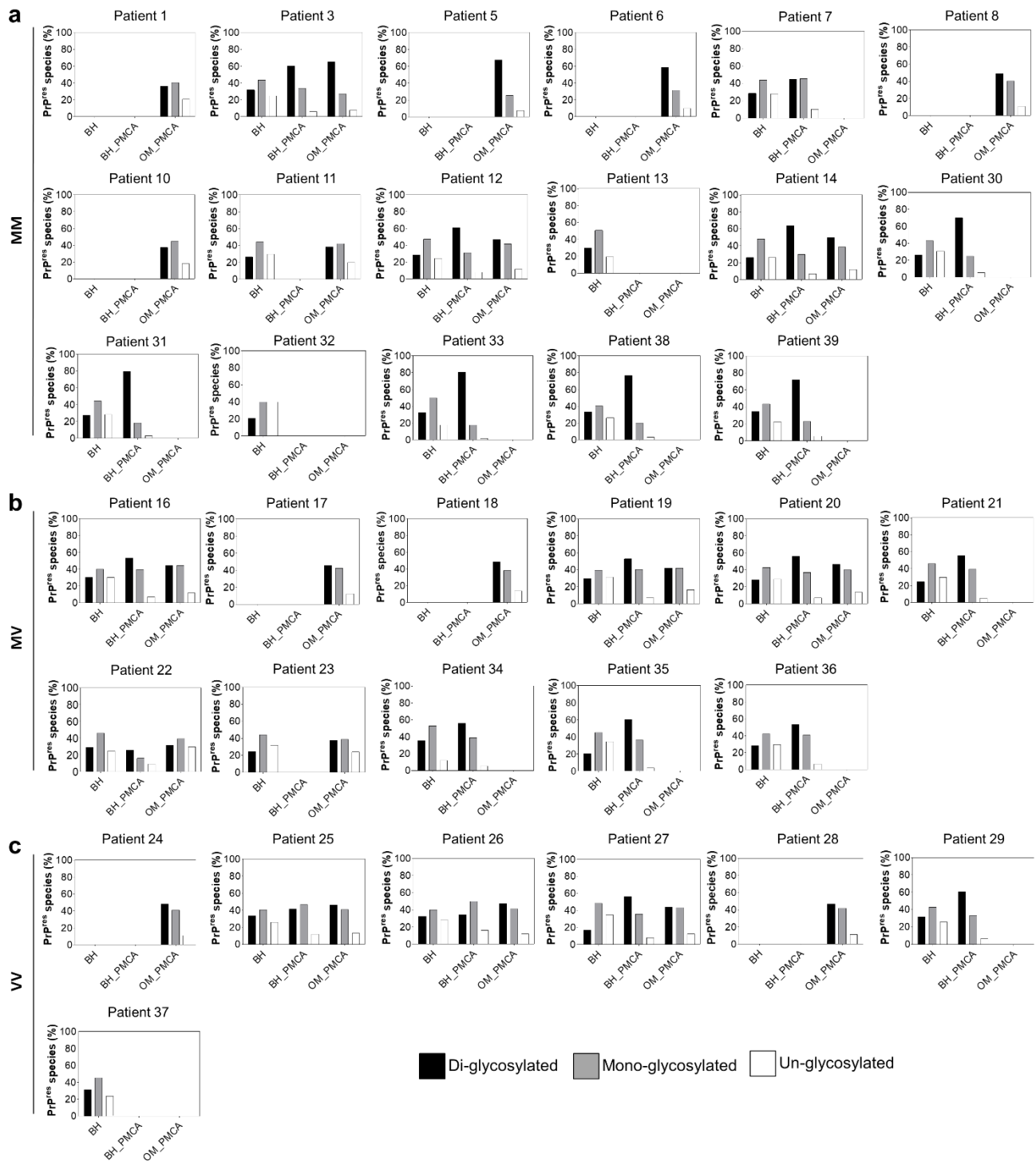
## Supplementary material



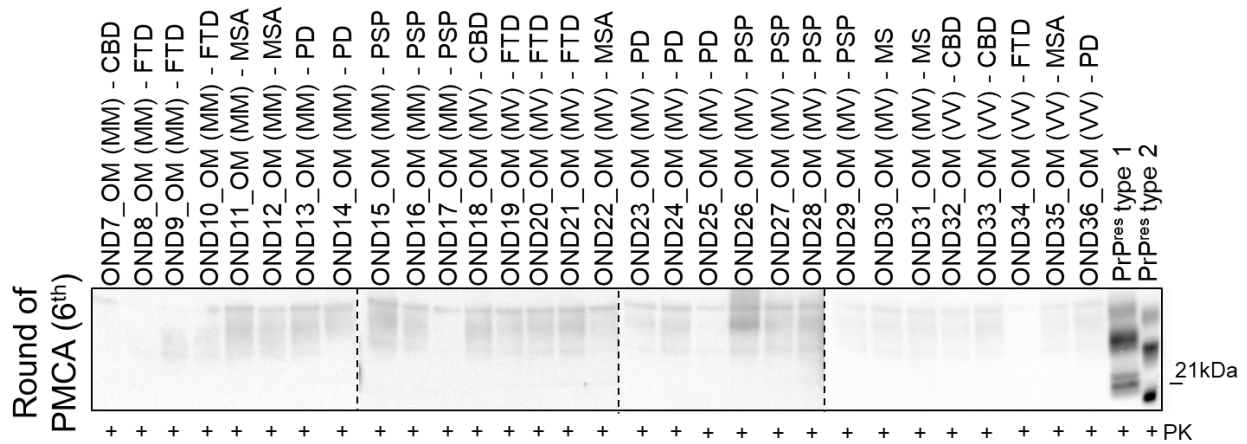
**Supplementary Fig. 1 RT-QuIC results of the OM and BHs samples included in the study. (a)** All OM samples collected from sCJD and gCJD patients (MM=14, light blue line; MV=9, light green line and VV=6, light orange line) induced an efficient RT-QuIC seeding activity, regardless of *PRNP* genotype/mutation, while all OM samples collected from OND (MM=14, MV=16 and VV=6) did not (light grey lines in each graph of group a). **(b)** All BHs collected from sCJD patients (MM=12, dark blue line; MV=9, dark green line; VV=5, dark orange line) induced a rapid RT-QuIC seeding activity while samples collected from OND did not (light grey lines in each graph of group b)



**Supplementary Fig. 2 Biochemical analysis of brain homogenates.** Western blot (Wb) analysis of BHs enabled to recognize the following sCJD subtypes: (**a-f**) MM1=7, (**b-g**) MV2=6, (**c-h**) MV1=3 and MM2-cortical=3, (**d-i**) VV2=4 and VV1=1, (**e-j**) MM2-thalamic=2 and OND=2. PrP<sup>res</sup> was detected in all sCJD BHs except for the brains of OND (AD and FTD). The 6D11 monoclonal antibody was used as a probe in a, b, c, d and e, while the 12B2 monoclonal antibody was used as a probe in f, g, h, i and j. The brain homogenates of sCJD patients with known PrP<sup>res</sup> typing (1 and 2) were used as controls of migration. Numbers in the right of each Wb indicate the molecular weight marker



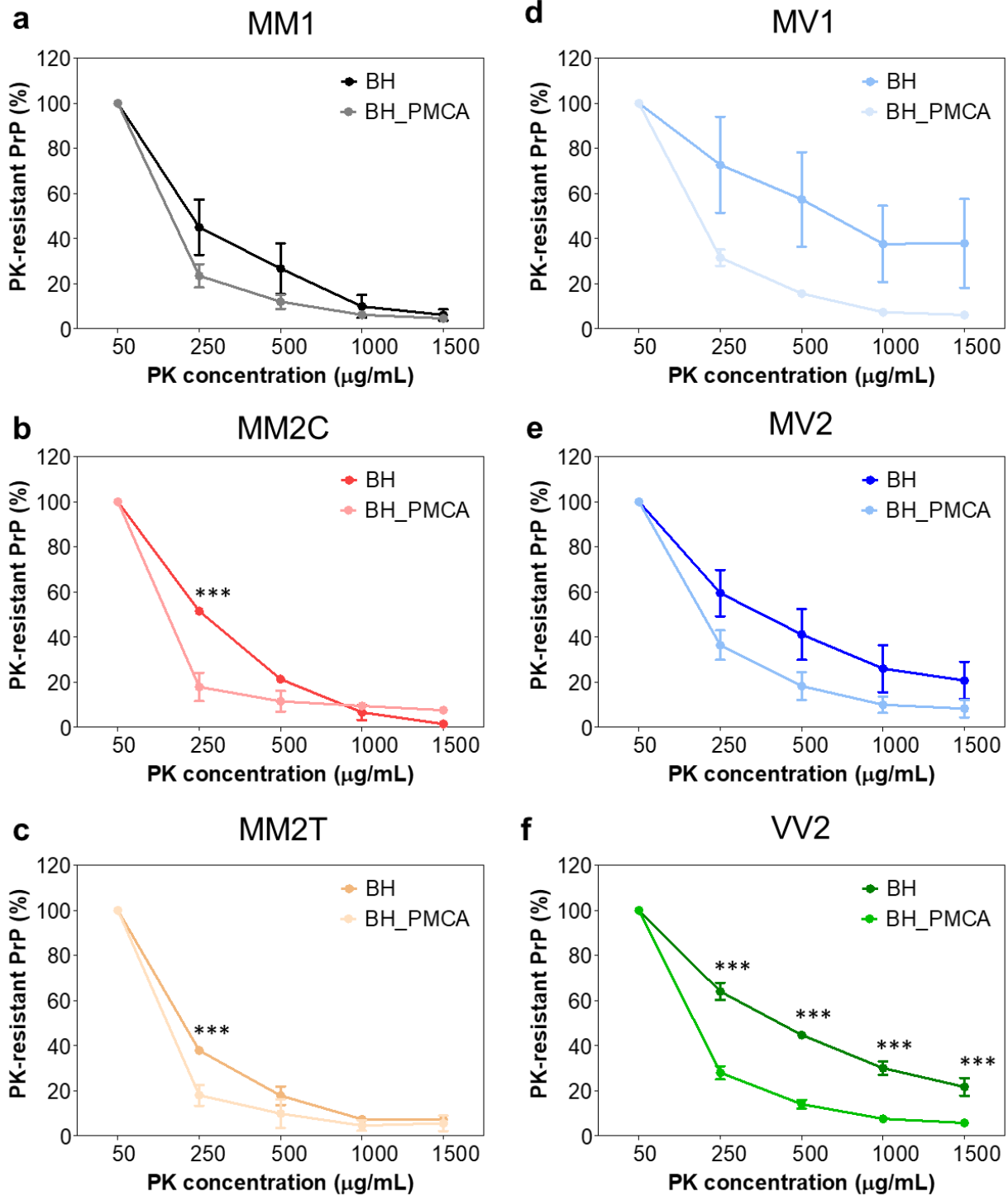
**Supplementary Fig. 3 Densitometric analysis of PrP<sup>res</sup> species of the original strains and their amplification products.** Histograms showing the signal intensity of each of PrP<sup>res</sup> band (di-, mono- and un-glycosylated) from BH and BH\_PMCA and/or OM\_PMCA of all sCJD patients included in the study (n=39). Samples were divided according to *PRNP129* (a) MM (n=17), (b) MV (n=11) and (c) VV (n=7). Genetic CJD patients (4 and 15) were not included in the analysis. Two sporadic CJD patients (2 and 9) were also not included since their BHs were not collected and the OM samples remained negative after PMCA



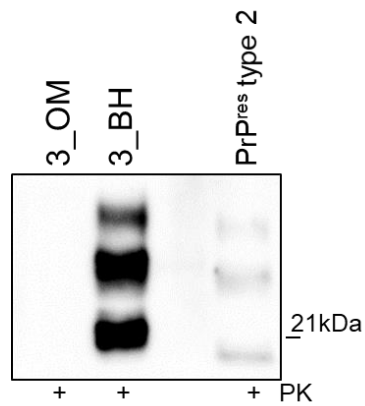
**Supplementary Fig. 4 Analysis of olfactory mucosa samples collected from OND patients by PMCA.** Wb of the 6<sup>th</sup> PMCA round is shown. No PrP<sup>res</sup> was found in the OM of the OND patients with MM (CBD: OND7; FTD: OND8, OND9 and OND10; MSA: OND11 and OND12; PD: OND13 and OND14; PSP: OND15, OND16 and OND17), MV (CBD: OND18; FTD: OND19, OND20 and OND21; MSA: OND22; PD: OND23, OND24 and OND25; PSP: OND26, OND27, OND28 and OND29; MS: OND30 and OND31) and VV (CBD: OND32 and OND33; FTD: OND34; MSA: OND35; PD: OND36) polymorphisms at *PRNP129*. Dashed lines indicate cropped images. Numbers in the right of each Wb indicate the molecular weight marker

		Raw brain homogenates (BHs)		Amplified brain homogenates (BH_PMCA)		Amplified olfactory mucosa samples (OM_PMCA)	
Patient	PRNP	Predominant PrP <sup>res</sup> species	PrP <sup>res</sup> typing	Predominant PrP <sup>res</sup> species	PrP <sup>res</sup> typing	Predominant PrP <sup>res</sup> species	PrP <sup>res</sup> typing
3	MM	Mono	1	Di	2	Di	2
7	MM	Mono	1	Di/Mono	1	-	-
11	MM	Mono	1	-	-	Di/Mono	1
12	MM	Mono	1	Di	2	Di/Mono	1
13	MM	Mono	1	-	-	-	-
14	MM	Mono	1	Di	2	Di/Mono	1
16	MV	Mono	2	Di	1	Di/Mono	1
19	MV	Mono	2	Di	1	Di/Mono	1
20	MV	Mono	2	Di	1	Di/Mono	1
21	MV	Mono	2	Di	1	-	-
22	MV	Mono	2	Di	1	Di/Mono	1
23	MV	Mono	2	-	-	Di/Mono	1
25	VV	Mono	2	Di/Mono	1	Di/Mono	1
26	VV	Mono	2	Di/Mono	1	Di/Mono	1
27	VV	Mono	2	Di	1	Di/Mono	1
29	VV	Mono	2	Di	1	-	-

**Table S1 Summary of the biochemical properties of PrP<sup>res</sup>.** The biochemical properties of PrP<sup>res</sup> present in the brains (BHs) or generated by PMCA from the brains (BH\_PMCA) and olfactory mucosa (OM\_PMCA) are shown. Di: prevalent di-glycosylated band; Mono: prevalent mono-glycosylated band; Di/Mono: equally representation of di- and mono-glycosylated bands



**Supplementary Fig. 5 Comparison of PK resistances between BH and their amplified products collected at the 6<sup>th</sup> PMCA round.** PK resistant profiles of BH vs BH\_PMCA of (a) MM1, (d) MV1 and (e) MV2 patients were different but not in a statistically significant manner. The PK resistant profiles of BH vs BH\_PMCA of (b) MM2C, (c) MM2T and (f) VV2 showed statistically significant differences. Statistical analyses: repeated measure analysis of variance (ANOVA); BH vs BH\_PMCA:\*\*\*p<0.001; error bars: ± standard error of the mean [SEM]



**Supplementary Fig. 6 Western blot analysis of OM and BH from a patient with sCJD (MM1).** Wb of BH (10% w/v) and OM (2  $\mu$ g) of patient 3. The 3\_OM sample was digested with PK, and no PrP<sup>res</sup> was found. In contrast, 3\_BH sample treated with PK shows a type 1 PrP<sup>res</sup>. The 6D11 monoclonal antibody was used. The brain homogenate of a sCJD patient with PrP<sup>res</sup> type 2 was used as a control of migration. Number in the right of Wb indicates the molecular weight marker

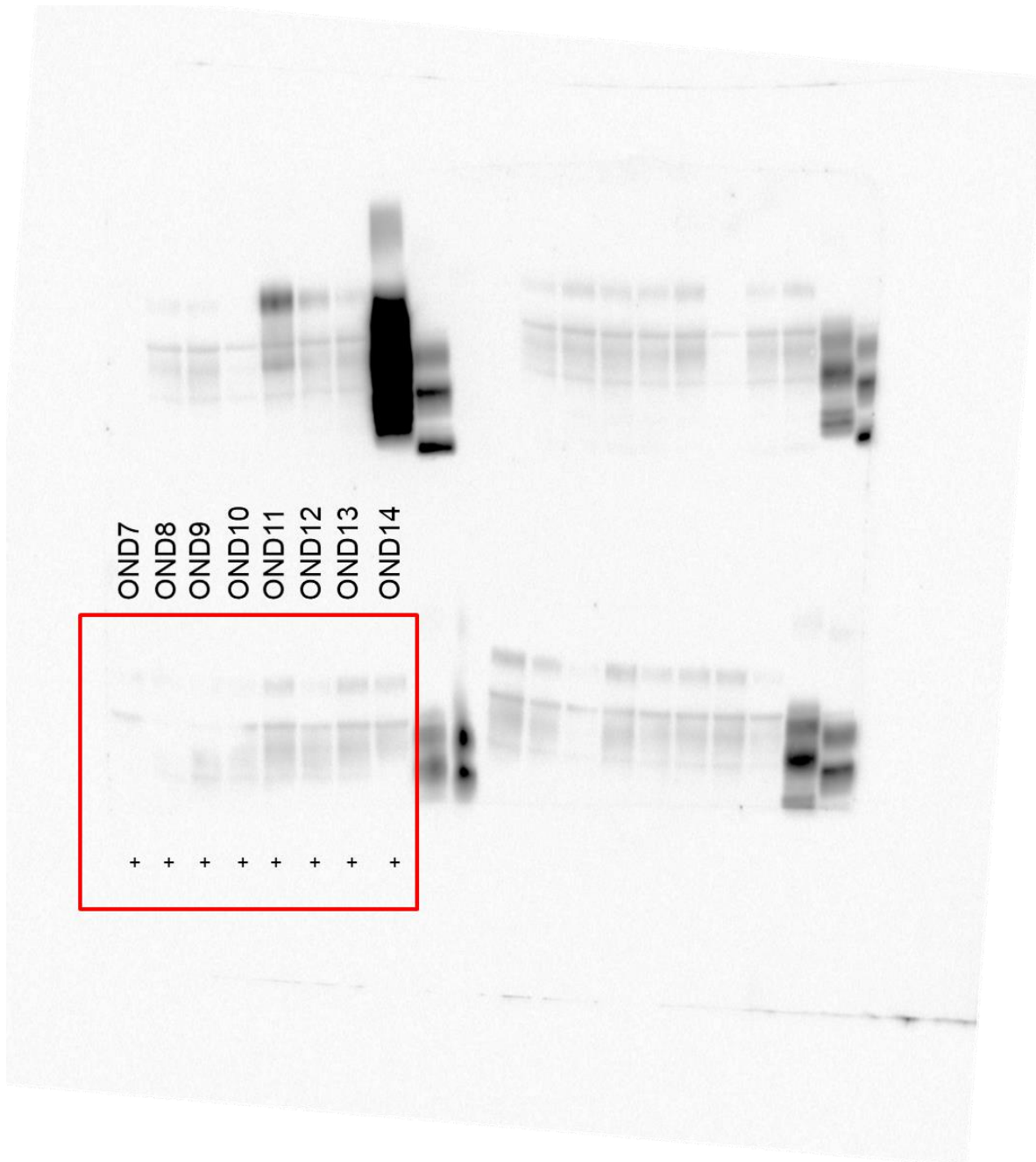
	<b><i>PRNP129</i></b>	<b>BH</b>	<b>OM</b>	<b>Ref. Figure</b>
Patient 3	MM1	8.42 ng	$8.42 \times 10^{-11}$ g	Fig. 5c
Patient 16	MV2	5.69 ng	$5.69 \times 10^{-20}$ g	Fig. 5d
Patient 19	MV2	10.96 ng	$1.096 \times 10^{-21}$ g	Fig. 5d
Patient 25	VV2	14.75 ng	$1.475 \times 10^{-21}$ g	Fig. 5e
Patient 26	VV2	18.62 ng	$1.862 \times 10^{-13}$ g	Fig. 5e

**Table S2 Comparison of PrP<sup>res</sup> concentration in BH and OM samples.** Estimation of PrP<sup>res</sup> concentration in BH and OM samples of MM1 (n=1), MV2 (n=2) and VV2 (n=2) patients obtained by qPMCA analysis.



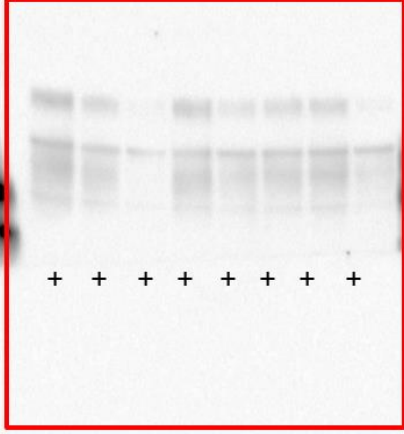
Cropped images of Western blots

Supplementary Fig. 4 Analysis of olfactory mucosa samples collected from OND patients by PMCA.

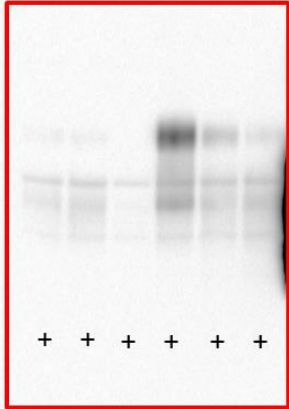


OND15  
OND16  
OND17  
OND18  
OND19  
OND20  
OND21  
OND22

+ + + + + + + +



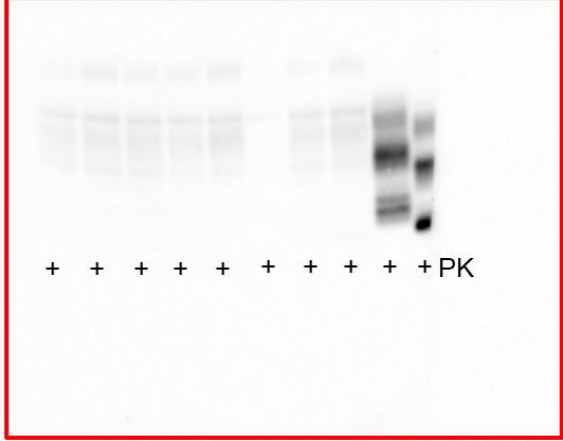
OND23  
OND24  
OND25  
OND26  
OND27  
OND28



+ + + + + +

OND29  
OND30  
OND31  
OND32  
OND33  
OND34  
OND35  
OND36

PrPres type 1  
PrPres type 2



+ + + + + + + + + +PK