SUPPLEMENTARY INFORMATION

Adaptation of the protein misfolding cyclic amplification (PMCA) technique for the screening of anti-prion compounds.

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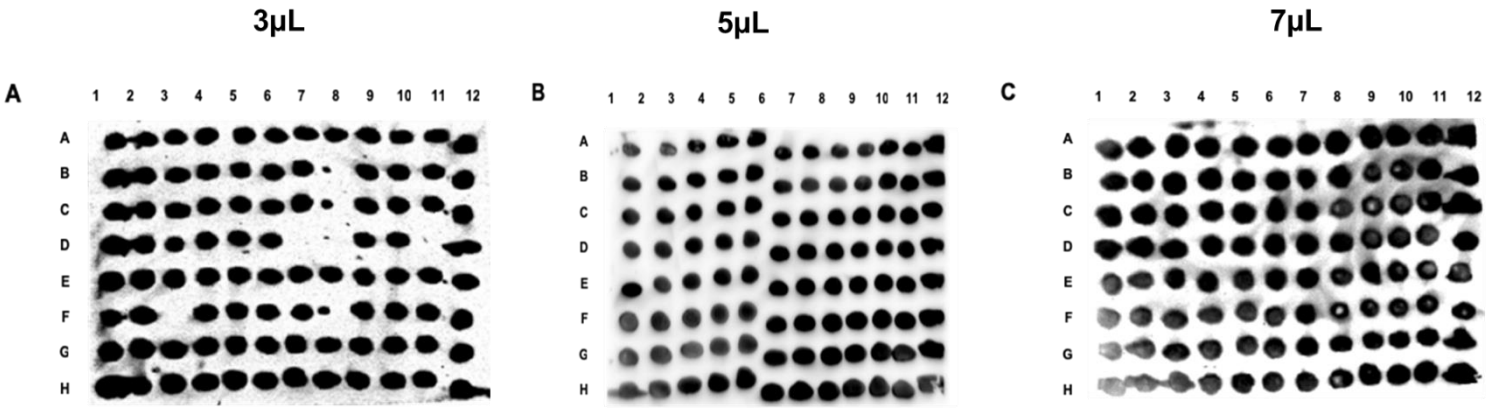
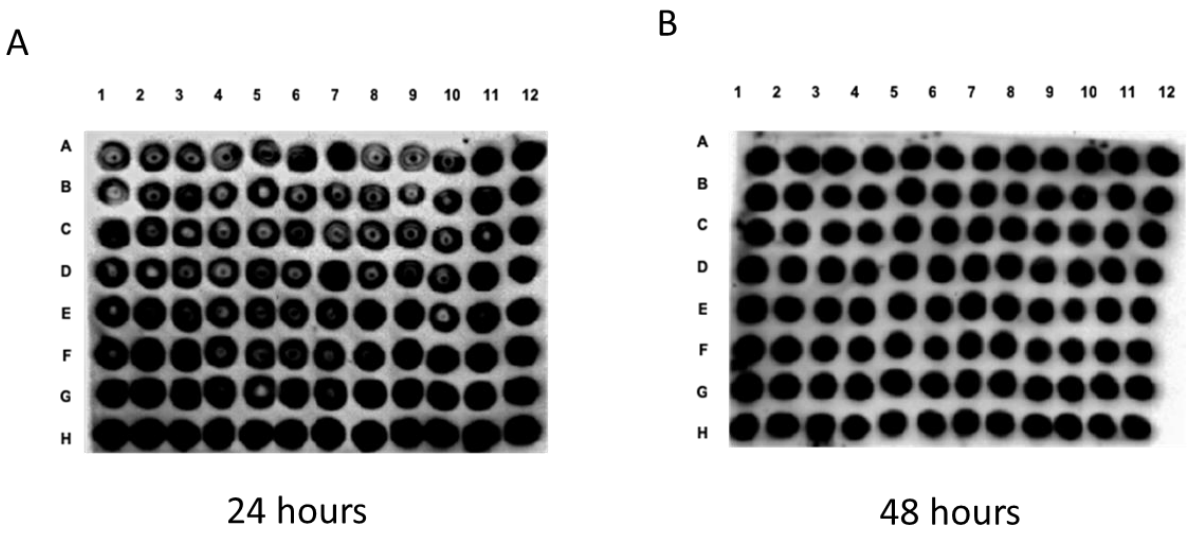
<u>Supplementary Table 1.</u> Previously reported active concentrations for some of the anti-prion and anti-amyloid compounds used in this study. The results provided in this table are based in previous reports (as referenced) and used in cell culture models of prion replication (or other amyloids), aggregation assays or bioassays.

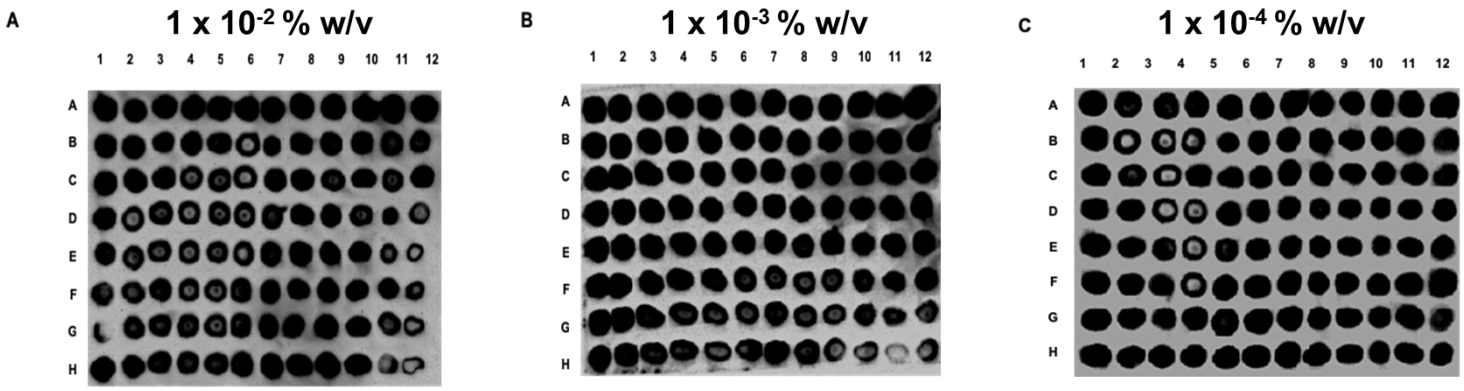
Compound	***Literature-reported active concentrations***
Tannic Acid	0.1 µM[1]
Astemizole	0.5-1 µM[1]
Congo Red	1-100 µM[2]
Curcumin	10 µM[1]
Quinacrine dihydrochloride	0.1-0.5 µM[1]
Imatinib	10 µM[3]
Reservatrol	1-200 µM[4]
Tetracycline HCl	100 µM- 1 µM [5]
Thioflavin T	122 µM (For Amyloid β)[6]
Tetrandrine	1-10 µM[1]
Azure A	0.108 µM (For tau)[7]
Azure B	2.5 µM (For Amyloid β)[8]
Azure C	0.09 µM (For Amyloid β)[6]
Quinacrine mustard	2.051 mM (For Amyloid β)[6]
Rhodanine	309 µM (For Amyloid β)[6]
Thionine acetate	0.098 µM (For Tau)[7]



Supplementary Figure 1. Effect of sample volume in the dot assay. Different volumes of sample including 3 **(A)**, 5 **(B)** and 7 **(C)** µL were loaded into the nitrocellulose membrane as described in Methods.

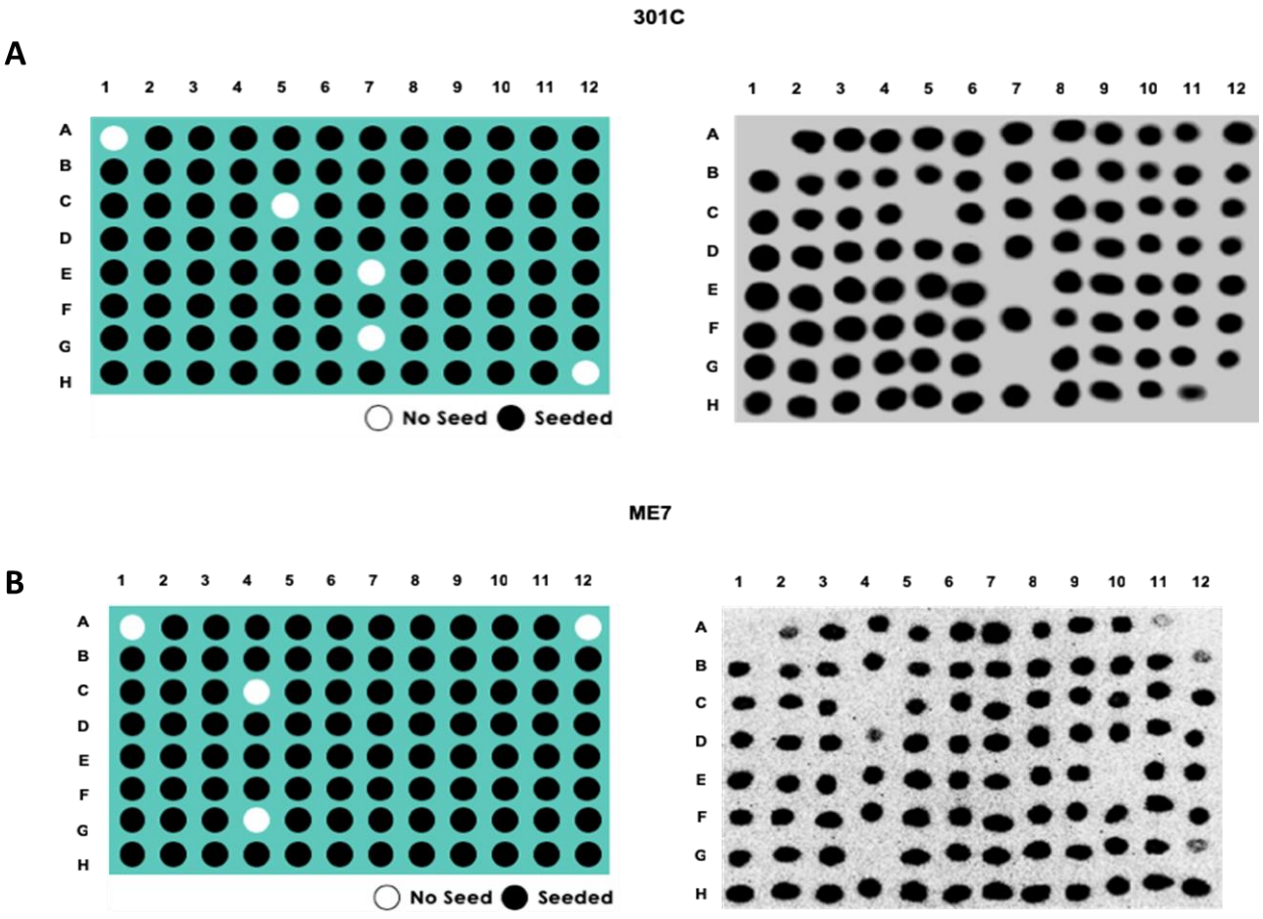


<u>Supplementary Figure 2.</u> Effect of the number of incubation/sonication cycles in PMCA replication using 96 well plates. Different incubation/sonication cycles extending for a total of 24 **(A)** or 78 **(B)** hours were tested using RML prions. All samples were treated with PK before loading them in the nitrocellulose membrane as described in Methods.

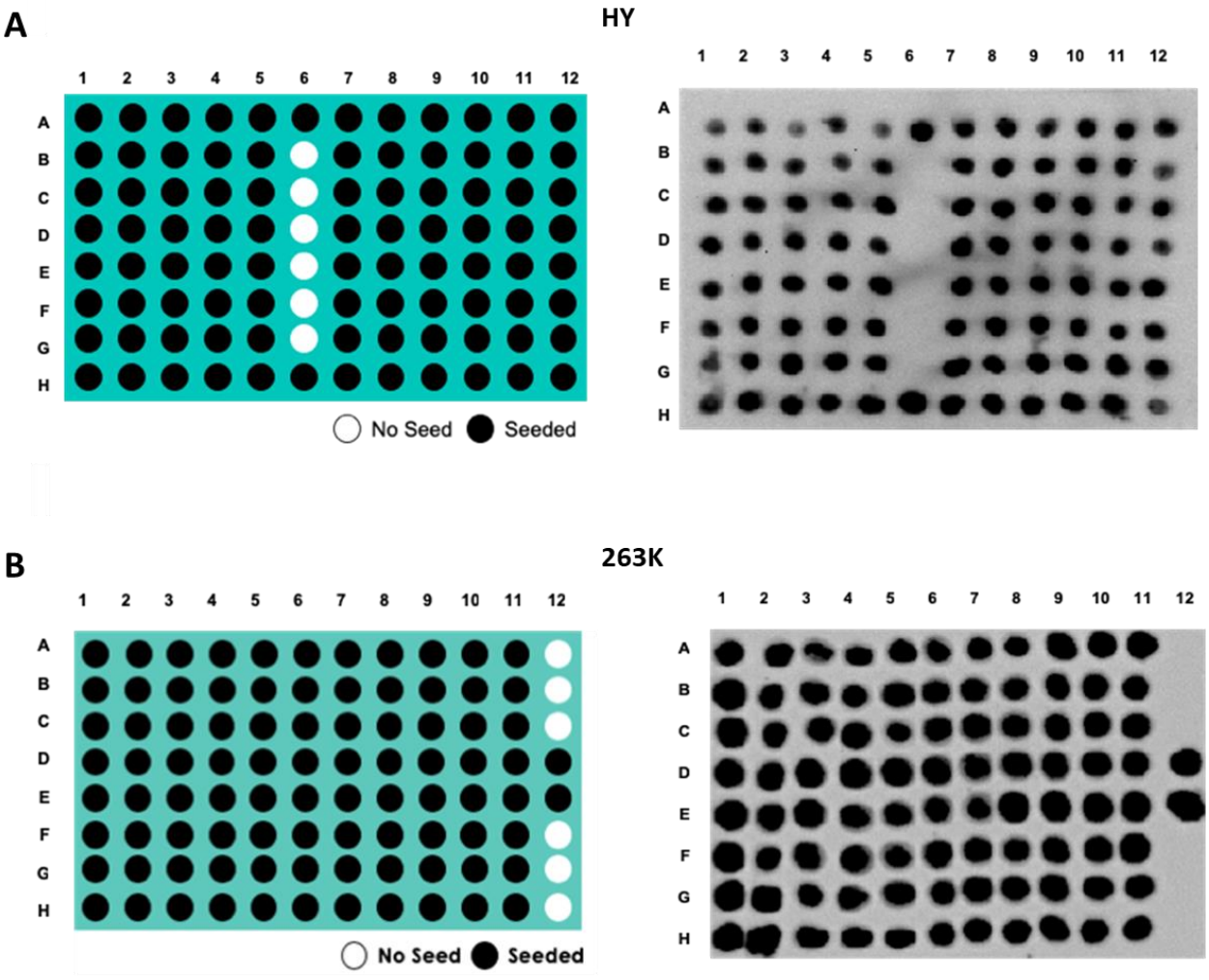


<u>Supplementary Figure 3.</u> Effects of varying PrP^{Sc} concentrations in the 96wp-PMCA assay.

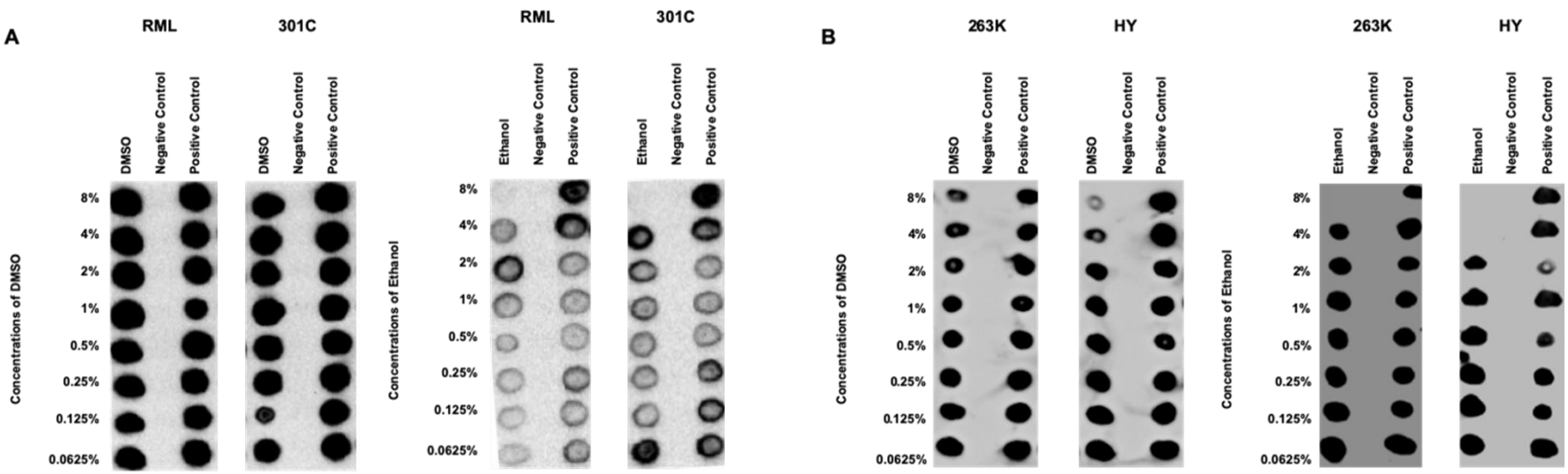
To explore the minimum concentration of prions allowing for uniform detection across a 96 well plate, several dilutions of a brain extract containing RML prions were used. These dilutions were as follows: 10^{-2} **(A)**, 10^{-3} **(B)**, and 10^{-4} **(C)**. All samples were treated with PK before been loaded in the nitrocellulose membrane as described in Methods.



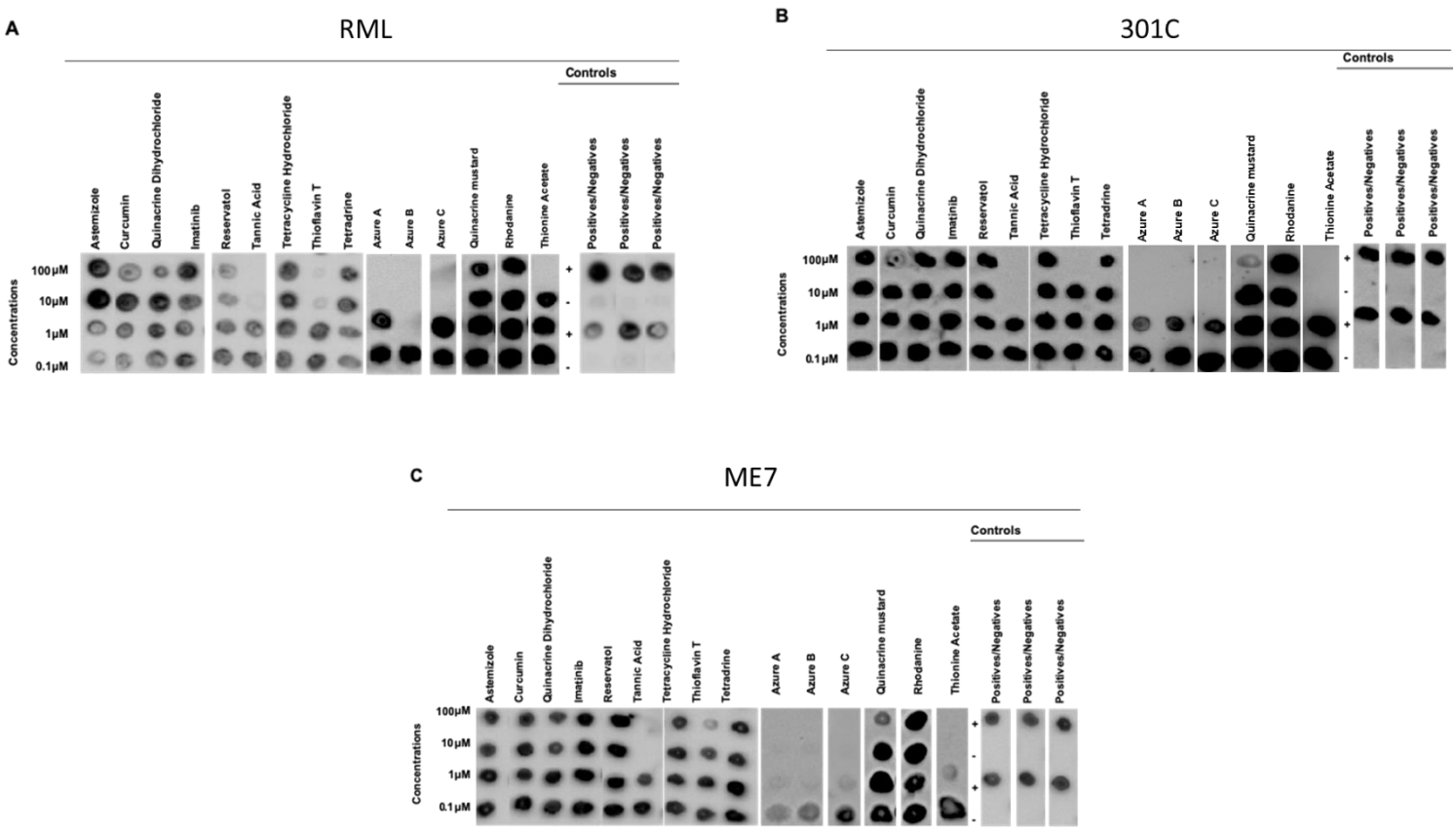
<u>Supplementary Figure 4.</u> Representative panels of optimized 96wp-PMCA assays for 301C and ME7 mouse prion strains. *In vitro* replication of 301C **(A)** or ME7 **(B)** prions. Left panels explain how seeded (black circles) and unseeded (white circles) reactions were positioned across the 96 well plate. Right panels demonstrate actual results. All samples shown in this experiment were PK treated before been loaded in the nitrocellulose membrane as described in Methods.



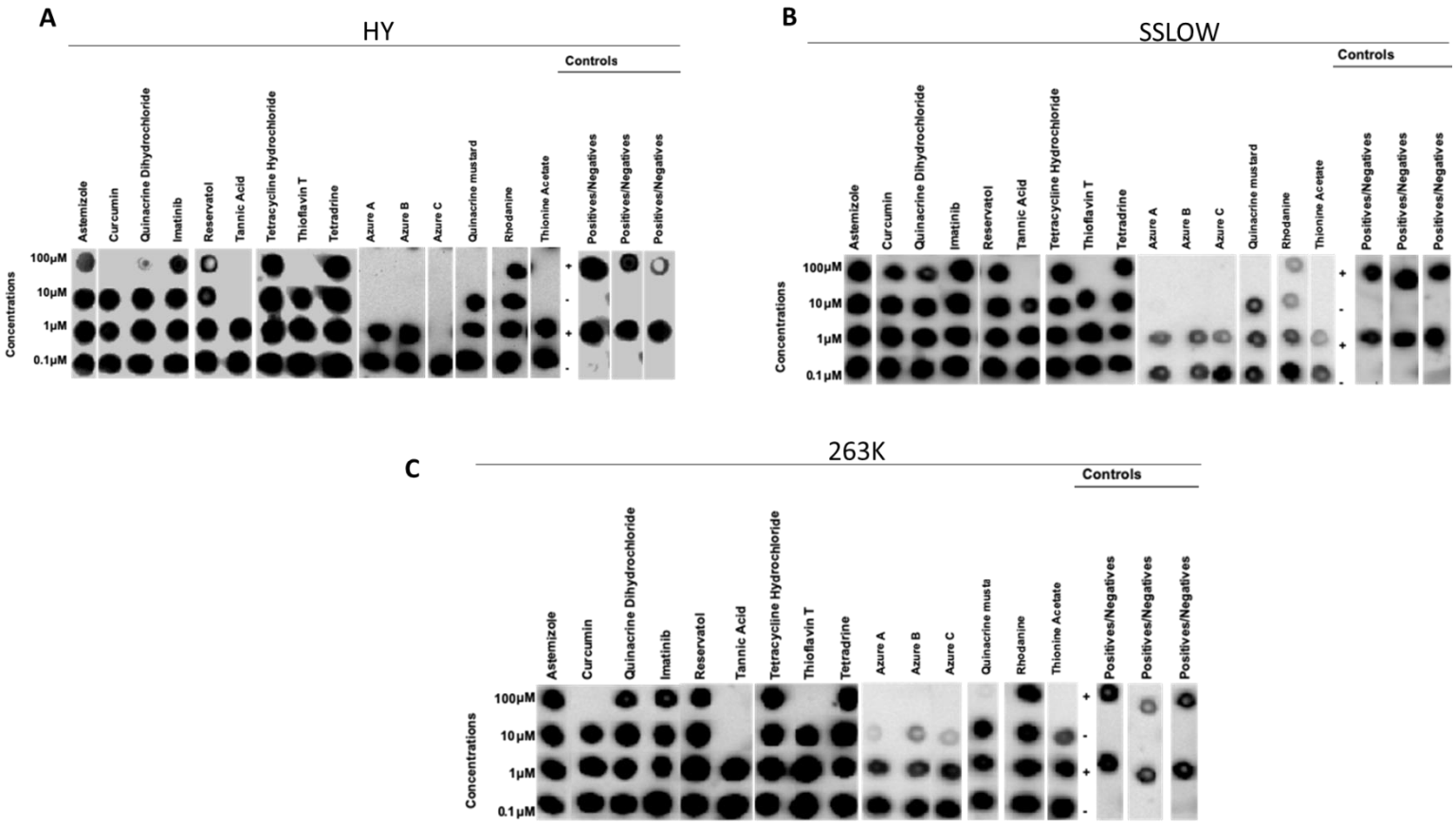
<u>Supplementary Figure 5.</u> Representative panels of optimized 96wp-PMCA assays for HY and 263K mouse prion strains. *In vitro* replication of HY **(A)** or 263K **(B)** prions. Left panels explain how seeded (black circles) and unseeded (white circles) reactions were positioned across the 96 well plate. Right panels demonstrate actual results. All samples shown in this experiment were PK treated before visualized in dot blots, as described in Methods.



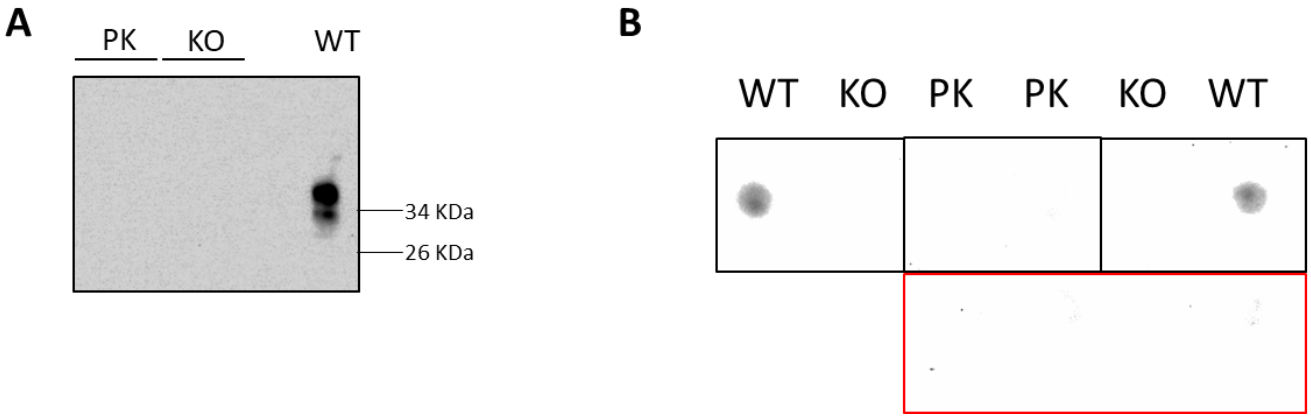
<u>Supplementary figure 6:</u> Effect of different solvents in 96wp-PMCA. Serial dilutions of either DMSO (left panels) or EtOH (right panels) (ranging from 8 to 0.0625%) were tested in two different mouse (RML and 301C **(A)**) and Syrian hamster (263K and HY **(B)**) prion strains.



<u>Supplementary Figure 7.</u> Specific activity of small molecules of known anti-prion and anti-amyloid activity in mouse prion strains (dissolved in EtOH). Fifteen small molecules with proven anti-prion or anti-amyloid activity (dissolved in EtOH) were tested at four different concentrations for their anti-prion activities against the RML **(A)**, 301C **(B)** and ME7 **(C)** prion strains using the 96wp-PMCA. Each molecule was tested in concentrations ranging from 0.1 to 100 µM. Controls for solvent representative for all strains, positive samples and negative samples without compounds were included to monitor the assay. Dot blots were modified for labeling. The panels shown in this figure are representative from three independent assays. Congo Red (previously tested in the DMSO condition) was not included in this analysis.



<u>Supplementary Figure 8.</u> Specific activity of small molecules of known anti-prion and anti-amyloid activity in Syrian hamster prion strains (dissolved in EtOH). Fifteen small molecules with proven anti-prion or anti-amyloid activity (dissolved in EtOH) were tested at four different concentrations for their anti-prion activities against the HY **(A)**, SSLOW **(B)** and 263K **(C)** prion strains using the 96wp-PMCA. Each molecule was tested in concentrations ranging from 100 µM to 0.1 µM. Controls for solvent representative for all strains, positive samples and negative samples without compounds were included to monitor the assay. Dot blots were modified for labeling. The panels shown in this figure are representative from three independent assays. Congo Red (previously tested in the DMSO condition) was not included in this analysis.



<u>Supplementary Figure 9.</u> Effect of PrP^C removal in the 96wp-PMCA reactions. **A)** Western blot of the different substrates used in this experiment, including a PK-treated (PK) wild-type mouse brain extract, and a brain homogenate from a PrP^C knock out (KO) mouse. A brain of an untreated wild type mouse (WT) was used as control. Numbers at the right represent molecular weight markers. **B)** 96-wp PMCA products were visualized by dot blots using the different substrates described in (A). The red square denotes four unseeded reactions using the WT substrate (negative controls).

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