



**S3 Fig: Deepest titration of Mo protein-only recPrP<sup>Sc</sup> into BV BH observed.** Western blots probed with anti-PrP mAb 27/33 showing three-round BH sPMCA reactions with either Mo BH (top row) or BV BH (bottom row) substrates. Reactions were seeded with 10-fold serial dilutions of Mo protein-only recPrP<sup>Sc</sup>. The  $10^{-1}$  reaction was seeded with 6  $\mu\text{g/mL}$  of Mo protein-only recPrP<sup>Sc</sup> for a final reaction concentration of 0.6  $\mu\text{g/mL}$  of seed. This figure is a replicate of the experiment shown in Fig. 2A, showing the highest level of sensitivity ( $10^{-5}$  dilution) that we observed for BV BH substrate seeded with protein-only recPrP<sup>Sc</sup>.