

Supplemental materials

Preserving prion strain identity upon replication of prions *in vitro* using recombinant prion protein

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Supplemental Figure Legends

Figure S1. An attempt to produce Ha-rPrP^{Sc} with help of PolyA. (A) PolyA facilitates conversion of Ha-rPrP into self-propagating PK-resistant conformation in sPMCAb (left panels show three parallel reactions conducted in the presence of PolyA). As a reference, Ha-rPrP fibrils produced *in vitro* in the presence of 2 M GdnHCl and digested with PK were loaded onto the gels (F^{2M}). (B) Analysis of Syrian hamster and tg7 mouse brains from animals inoculated with Ha-rPrPres^{PolyA} produced in sPMCAb. Asterisks marks under-digested full-length PrP^C and C1 proteolytic fragment. (C) Serial transmission (2nd passage) of Ha-rPrPres^{PolyA} produced in sPMCAb and passaged in Tg7 mice. Small amounts of PrP^{Sc} is detected with SAF-84 and confirmed with 3F4 antibody.

Figure S2. Generation of rPrPres^{PE}. sPMCA reactions were seeded with 10³-fold diluted brain-derived Hyper (HY) or SSLOW, then subjected to four sPMCAb rounds in the presence of PE with 3-fold dilutions between rounds and analyzed by Western blot with 3F4 antibody. rPrPres^{PE} detected in the reactions seeded with SSLOW is indicated by arrow.

Figure S3. Analysis of conformational stability and electrophoretic mobility of SSLOW^{PE+PolyA}. (A) 1% BHs from the 1st and 2nd passages of SSLOW^{PE+PolyA}, SSLOW and 263K were incubated with increasing concentrations of GdnHCl from 0.4 to 4 M for 1 h, as indicated, then diluted out of GdnHCl, equilibrated for 1 h at room temperature and digested with 20 µg/ml PK. Undigested brain material exposed to 0.4 M GdnHCl is provided as a reference (-PK). Western blots were stained with 3F4 antibody. Dashed arrows point at blind spots on Western blots that arose due to transfer of PK. While all four groups showed similar conformational stability, the band pattern that appeared due to PK-related blind spot was different between 263K and the remaining three groups. (B) 1% BHs from the 2nd passages of SSLOW^{PE+PolyA}, SSLOW and

263K were treated with PNGase and PK, and analyzed by Western blot. Western blot was stained with 3F4 antibody.

Figure S1

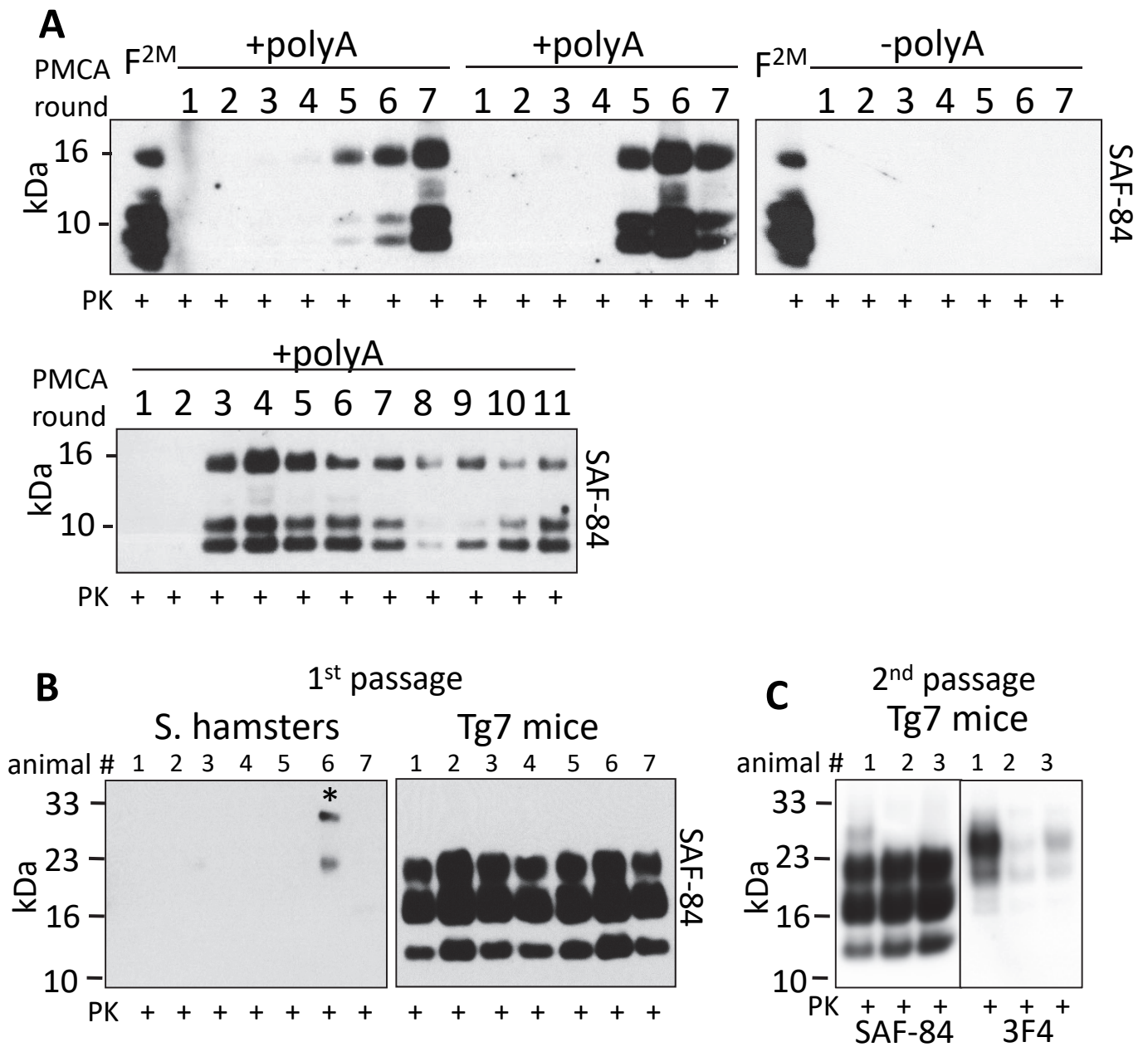


Figure S2

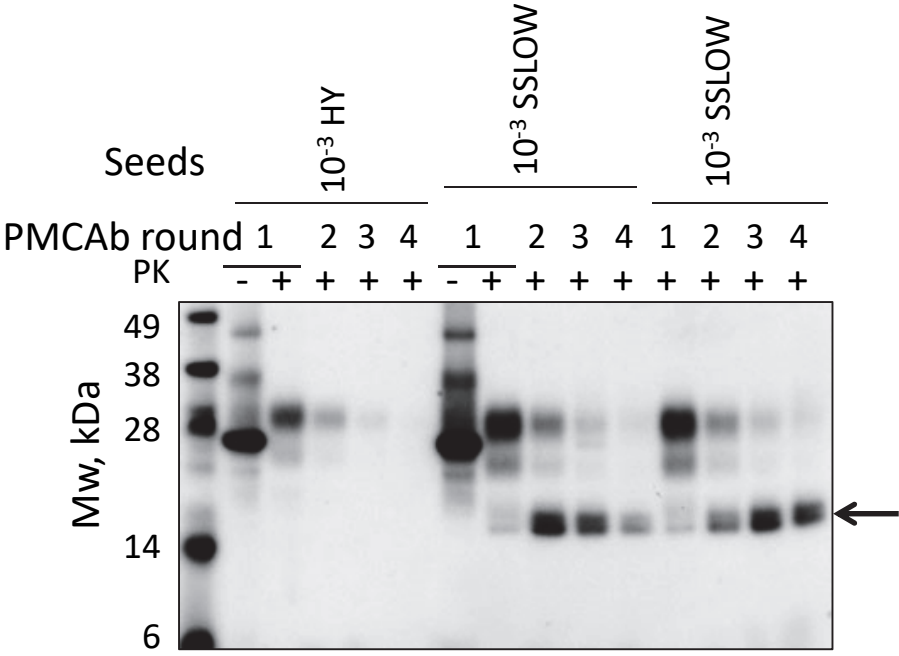


Figure S3

