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## Host prion protein expression levels impact prion tropism for the spleen

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Short title: PrP<sup>C</sup> level features in prion lymphotropism

## 1 **Abstract**

2 Prions are pathogens formed from abnormal conformers (PrP<sup>Sc</sup>) of the host-encoded cellular  
3 prion protein (PrP<sup>C</sup>). PrP<sup>Sc</sup> conformation to disease phenotype relationships extensively vary  
4 among prion strains. In particular, prions exhibit a strain-specific tropism for lymphoid  
5 tissues. Prions can be composed of several substrain components. There is evidence that these  
6 substrains can propagate in distinct tissues (e.g. brain and spleen) of a single individual,  
7 providing an experimental paradigm to study the cause of prion tissue selectivity. Previously,  
8 we showed that PrP<sup>C</sup> expression levels govern prion substrain selection in the brain.  
9 Transmission of sheep scrapie isolates (termed LAN) to multiple lines of transgenic mice  
10 expressing varying levels of ovine PrP<sup>C</sup> in the brain resulted in the phenotypic expression of  
11 the dominant sheep substrain in mice expressing near physiological PrP<sup>C</sup> levels, whereas a  
12 minor substrain replicated preferentially on high-expressors. Considering that PrP<sup>C</sup> expression  
13 levels are markedly decreased in the spleen compared to the brain, we interrogate whether  
14 spleen PrP<sup>C</sup> dosage could drive prion selectivity. The outcome of the transmission of a large  
15 cohort of LAN-like scrapie isolates in the spleen from high-expressors correlated with the  
16 replication rate dependency on PrP<sup>C</sup> amount; There was a prominent spleen colonization by  
17 the substrain preferentially replicating on low-expressors and a relative incapacity of the  
18 substrain with higher-PrP<sup>C</sup> level need to propagate in the spleen. Early colonization of the  
19 spleen allowed neuropathological expression of the lymphoid substrain after intraperitoneal  
20 inoculation. In addition, a pair of substrain variants resulting from the adaptation of human  
21 prions to ovine high-expressors, and exhibiting differing brain versus spleen tropism, showed  
22 different tropism on transmission to low-expressors, with the lymphoid substrain colonizing  
23 the brain. Overall, these data suggest that PrP<sup>C</sup> expression levels are instrumental in prion  
24 substrain lymphotropism.

## 1 **Author summary**

2 The cause of prion phenotype variation among prion strains remains poorly understood. In  
3 particular prions replicate in a strain-dependent manner in the spleen. This can result in prion  
4 asymptomatic carriers. Based on our previous observations that dosage of the prion precursor  
5 (PrP) determined prion substrain selection in the brain, we examine whether PrP levels in the  
6 spleen could drive prion replication in this tissue, due to the low levels of the protein. We  
7 observe that the prion substrain with higher PrP need for replication did barely replicate in the  
8 spleen, while the component with low PrP need replicated efficiently. In addition, other  
9 human co-propagating prions with differing spleen and brain tropism showed different  
10 tropism on transmission to mice expressing low PrP levels, with the lymphoid substrain  
11 colonizing the brain. PrP<sup>C</sup> expression levels may thus be instrumental in prion tropism for the  
12 lymphoid tissue. From a diagnostic point of view, given the apparent complexity of prion  
13 diseases with respect to prion substrain composition, these data advocate to type extraneural  
14 tissues or fluids for a comprehensive identification of the circulating prions in susceptible  
15 mammals.

## 1 **Introduction**

2 Mammalian prions are proteinaceous pathogens causing fatal neurodegenerative  
3 diseases termed transmissible spongiform encephalopathies (TSE) in humans and animals.  
4 TSE include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle,  
5 chronic wasting disease (CWD) in cervid populations and Creutzfeldt-Jakob disease (CJD) in  
6 humans [1]. Prions are formed from abnormal,  $\beta$ -sheet enriched conformers ( $\text{PrP}^{\text{Sc}}$ ) of the  
7 host encoded cellular prion protein ( $\text{PrP}^{\text{C}}$ ). Prions replicate by templating the conversion and  
8 polymerization of  $\text{PrP}^{\text{C}}$  by an autocatalytic process [2, 3]. Multiple strains of prions are  
9 recognized phenotypically within the same host species. Strains are conformational variants  
10 of  $\text{PrP}^{\text{Sc}}$ , at the level of the tertiary and/or quaternary structure [4-6]. In the infected host,  
11 prion strains exhibit specific incubation periods, stereotyped clinical signs and  
12 neuropathology, and specific tropism for the central nervous system (CNS) and lymphoid  
13 tissues (for review [7, 8]). Thus, certain prions can replicate early and at fairly high levels in  
14 tissues of the lympho-reticular system such as the Peyer's patches, spleen and lymph nodes  
15 [9-11]. Lymphotropic prions are then transported from these early reservoirs of infectivity to  
16 the brain either by the enteric nervous system (Peyer's patches) or by the peripheral nervous  
17 system and the spinal cord (spleen, lymph nodes) [12-14]. After peripheral infection, the  
18 presence of differentiated follicular dendritic cells (FDC) is required for efficient prion  
19 replication in the lymphoid tissue and subsequent neuroinvasion [9, 15-21]. Yet, prions can  
20 differ in their capacity to neuroinvade. Certain prions can persist in the lymphoid tissue  
21 without accessing the CNS [22-25]. Of particular concern are BSE/variant CJD prions, which  
22 are suspected to accumulate in human lymphoid tissue without neuroinvasion. 1:2000  
23 exposed individuals in the UK may be silent carriers in the lymphoid tissue, causing risks of  
24 secondary transmission [26].

1 Although one dominant PrP<sup>Sc</sup> conformation is usually detected by conventional  
2 immunodetection methods, there is clear evidence that natural or experimental prion sources  
3 can be composed of several substrains, in variable proportions, and with differing PrP<sup>Sc</sup>  
4 conformations [27-30]. As a result, experimental prion transmission, in a homotypic context  
5 (i.e. host PrP<sup>C</sup> and prion PrP<sup>Sc</sup> share identical primary sequence) or not, can lead to the  
6 isolation of different substrains in the brain and spleen of a single transgenically modified  
7 mouse expressing PrP [22, 23, 28, 31]. The reasons for such substrain segregation between  
8 the brain and the spleen and more generally for the incapacity of certain strains to replicate in  
9 the spleen remain poorly understood. It has been proposed that tertiary or quaternary PrP<sup>Sc</sup>  
10 conformations impact peripheral prion replication [25, 31-33]. The aforementioned tissue  
11 segregative transmission provides a relevant experimental paradigm to study prion  
12 lymphotropism.

13 Intracerebral transmission of natural sheep scrapie isolates (referred to as LAN isolates [9,  
14 34]) to multiple lines of transgenic mice expressing ovine PrP<sup>C</sup> (VRQ allele at codons 136,  
15 154 and 171 of the PrP-encoding gene, where V, R, and Q stand for valine, arginine, and  
16 glutamine, respectively) and at variable levels revealed that the PrP<sup>C</sup> expression level in the  
17 brain critically determines prion substrain selection [29]. The so-called LA21K dominant  
18 component in the sheep brain (giving the strain signature of LAN isolates) was selected in the  
19 brain of mice expressing near physiological PrP<sup>C</sup> levels, while preferential selection of the so-  
20 called LA19K prion subcomponent occurred in the brain of ‘high-expressor’ mice.

21 PrP<sup>C</sup> levels are markedly lowered in the spleen, - approximately 20-fold compared to the  
22 brain-, in wild-type mice as in some high-expressor transgenic mouse models [22]. We thus  
23 interrogate whether PrP<sup>C</sup> expression levels could impact prion peripheralization. We compare  
24 the outcome of the transmission of a large cohort of sheep scrapie isolates, including the

1 LAN-like isolates, in the brain and spleen tissue of high-expressors. In accordance with their  
2 affinity for PrP<sup>C</sup> levels, we show preferential replication of LA21K and absence of replication  
3 of LA19K prions in the spleen. Consequently, varying the inoculation route allows the  
4 dominant expression of LA21K prions in the brain of high-expressors. Further, we show that  
5 a pair of strains resulting from the adaptation of cortical MM2 CJD subtype to ovine high-  
6 expressors, and exhibiting differing brain versus spleen tropism (T1<sup>Ov</sup> strain in spleen and  
7 T2<sup>Ov</sup> strain in brain [28]) showed opposite tropism in low-expressors. Collectively, these data  
8 raise the possibility that PrP<sup>C</sup> expression levels are instrumental in specific tropism for the  
9 lymphoid tissue.

10

## 11 **Methods**

### 12 **Ethics statement**

13 Animal care and experiments were conducted in strict compliance with ECC and EU  
14 directives 86/009 and 2010/63. They were reviewed and approved by the local ethics  
15 committee of the author's institution, (name: COMETHEA: Comité d'Ethique en  
16 Expérimentation Animale du Centre INRA de Jouy-en-Josas et AgroParisTech). The permit  
17 numbers delivered by the COMETHEA are 12/034 and 15/056.

### 18 **Transgenic mice**

19 As high-expressors, we used tg338 mice that overexpress the VRQ allele of ovine PrP. The  
20 transgene construct consists of a large DNA sequence derived from ovine BAC libraries,  
21 encompassing natural regulatory sequences of the PrP gene transcription unit [35]. The PrP<sup>C</sup>  
22 levels in the brain are ~8-fold higher than in the sheep brain [29]. The spleen-to-brain PrP<sup>C</sup>  
23 ratio (~1:20) in tg338 mice is comparable to that found in conventional mouse models [22].  
24 The strongest PrP<sup>C</sup> staining in tg338 spleens is FDC-associated [22]. Thus, there is no

1 aberrant expression of PrP<sup>C</sup> in the spleen of these mice, quantitatively or qualitatively. As  
2 low-expressor mice, we used tg335<sup>+/-</sup> (same transgene construct as the tg338 mice) and  
3 tg143<sup>+/-</sup> mice, which express 1.2-fold and 1.5-fold PrP<sup>C</sup> as compared to sheep brain,  
4 respectively [29, 35].

## 5 **TSE sources**

6 Brain and spleen from sheep terminally affected with natural scrapie (LAN404 isolate) were  
7 provided by the Institut National de la Recherche Agronomique (O. Andréoletti, Toulouse,  
8 France). Brain extracts from French, Dutch, Spanish, Irish, and English sheep scrapie isolates  
9 were provided by the Institut National de la Recherche Agronomique (O. Andréoletti,  
10 Toulouse, France), the Central Veterinary Institute (J.M. Langeveld, Wageningen, Lelystad,  
11 Netherlands), the French National TSE Reference Laboratory (T. Baron, Anses, Lyon,  
12 France), CISA-INIA (J.M. Torres, Madrid, Spain), the Central Veterinary Research  
13 Laboratory, (E. Monks, Dublin, Ireland) and the former European TSE Reference Laboratory  
14 (J. Spiropoulos, VLA, Addlestone, UK). The CH1641 sheep scrapie strain [36] was provided  
15 by the Institute for Animal Health (N. Hunter, Edinburgh, UK). The TSE isolates are detailed  
16 in [supplemental Table 1](#).

17 The T1<sup>Ov</sup> and T2<sup>Ov</sup> strains were isolated after serial transmission of a human sporadic CJD  
18 brain (MM2, rare cortical form) to ovine PrP tg338 mice [28]. As inoculum, a pool of brains  
19 from tg338 mice at the 4<sup>th</sup> serial passage of MM2-CJD and containing T1<sup>Ov</sup> and T2<sup>Ov</sup> prions,  
20 with T2<sup>Ov</sup> in higher proportion, was used. For comparison, pools of brains from tg338 mice  
21 inoculated with cloned T1<sup>Ov</sup> or bicloned T2<sup>Ov</sup> prions were used [28].



## 1 **Mouse transmission assays**

2 Sheep tissue extracts were prepared as 10% w/v homogenate in 5% w/v glucose with a  
3 Precellys rybolyzer (Ozyme, Montigny-le-Bretonneux, France). To avoid any cross-  
4 contamination, a strict protocol based on the use of disposable equipment and preparation of  
5 all inocula in a class II microbiological cabinet was followed. For intracerebral inoculations,  
6 twenty microliters were inoculated in the right hemisphere to groups of individually identified  
7 mice, at the level of the parietal cortex. For intraperitoneal inoculations, hundred microliters  
8 of a 2% w/v solution in 5% glucose were used. For subsequent passage, mouse brains and  
9 spleens were collected with dedicated, sterile, disposable tools, homogenized at 20% w/v in  
10 5% glucose; twenty microliters were reinoculated intracerebrally at 10% w/v. Animals were  
11 supervised daily for TSE development. Animals at terminal stage of disease or at end life  
12 were euthanized. To study the kinetics of PrP<sup>Sc</sup> accumulation in the spleen and in the brain  
13 after intraperitoneal or intracerebral infection, mice were euthanized healthy in triplicates at  
14 regular time-points post-inoculation, as indicated. For immunoblot analyses, brains and  
15 spleens were immediately frozen at -80°C until use. For histoblot analyses, the collected  
16 brains were frozen on dry ice before storage at -80°C.

## 17 **Immunoblot analyses**

18 Brains and spleens were analyzed for proteinase K (PK)-resistant PrP<sup>Sc</sup> (PrP<sup>res</sup>) content using  
19 a previously published protocol [28]. Briefly, PrP<sup>res</sup> was extracted from 20 % w/v tissue  
20 homogenates with the Bio-Rad TeSeE detection kit. Aliquots were digested with PK (200  
21 µg/ml final concentration) for 10 min at 37 °C before B buffer precipitation and centrifugation  
22 at 28,000 × g for 15 min. Pellets were resuspended in Laemmli sample buffer, denatured, run  
23 on 12 % Bis/Tris gels (Bio-Rad), electrotransferred onto nitrocellulose membranes, and  
24 probed with 0.1 µg/ml biotinylated anti-PrP monoclonal antibody Sha31 antibody (human PrP

1 epitope 145-152, [37]) or with 0.1 µg/ml anti-PrP 12B2 antibody (human PrP epitope 89-93,  
2 epitope, [38]) and followed by streptavidin conjugated to horseradish peroxidase (HRP) or by  
3 HRP conjugated to goat anti-mouse IgG1 antibody (1/20 000 final dilution), respectively.  
4 Immunoreactivity was visualized by chemiluminescence (GE Healthcare). The relative  
5 amounts of PrP<sup>res</sup> glycoforms were determined by the use of GeneTools software after  
6 acquisition of chemiluminescent signals with a GeneGnome digital imager (Syngene,  
7 Frederick, MD).

## 8 **Histoblot analyses**

9 Brain cryosections were cut at 8-10 µm, transferred onto Superfrost slides and kept at -20 °C  
10 until use. Histoblot analyses were performed as described [39], using the 12F10 anti-PrP  
11 antibody (human PrP epitope 142-160, [40]). Analysis was performed with a digital camera  
12 (Coolsnap, Photometrics) mounted on a binocular glass (SZX12, Olympus). The sections  
13 presented are representative of the analysis of three brains samples.

## 14 **Resistance to proteinase K digestion**

15 20% (w/v) brain homogenates from tg338 mice infected with 127S and LA19K prions were  
16 diluted at 5% in a solubilization buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 5 mM EDTA,  
17 1 mM DTT, 2% (w/v) dodecyl- β-D-maltoside (Sigma)) 2% N-lauryl sarcosine (Fluka), final  
18 concentrations) and digested for 2h at 37°C with increasing concentrations of PK (0 to  
19 10 000 µg/ml), as indicated. After digestion, the samples were diluted in equal volumes of  
20 Laemmli buffer, denatured and analyzed for PrP content by western blot, as above.

## 1 **PrP<sup>Sc</sup> degradation by primary cultured peritoneal macrophages**

2 To induce the multiplication of peritoneal macrophages, healthy tg338 mice were  
3 intraperitoneally injected with 3% Brewer thioglycolate broth (BD Biosciences). Three days  
4 after the injection, mice were euthanized by cervical column disruption. Peritoneal lavage was  
5 performed with 5 ml D-PBS (Gibco). The lavage fluid was mixed with an equivalent volume  
6 of 4°C Dulbecco's Modified Eagle Medium (DMEM, Lonza) supplemented with 10% fetal  
7 calf serum (FCS, Biowhitaker), streptomycin and penicillin (PS, Gibco) before centrifugation  
8 at 100 x g for 5 min. Red cells were lysed with hemolytic medium (155 mM ammonium  
9 chloride, 12 mM sodium carbonate, pH 7.4). The cells were resuspended in DMEM-10%  
10 FCS-PS and washed twice by centrifugation. After a live cell count, the cells were aliquoted  
11 in 6-well plates ( $6 \cdot 10^6$  cells / well) and incubated at 37°C. After 24h, non-adhering cells were  
12 discarded by washing the wells with D-PBS. Macrophages were exposed to brain  
13 homogenates from tg338 mice infected with 127S and LA19K prions (1% (w/v) dilution in  
14 DMEM-10% FCS-PS) for 24h at 37°C. After two washes in D-PBS, the cells were lysed or  
15 incubated for 9 days. At regular time points, the contents of the wells were lysed in lysis  
16 buffer (0.5% sodium deoxycholate, 0.5 Triton X-100, 50 mM Tris-HCl, pH 7,4) and  
17 centrifuged for 1 min at 500 x g. The supernatants were collected and analyzed for protein  
18 content (MicroBCA kit, Pierce). The equivalent of 250 µg of proteins was digested by 1µg of  
19 PK for 1h at 37°C. The samples were then methanol precipitated, resuspended in Laemmli  
20 buffer, denatured and analyzed for PrP<sup>res</sup> content by western blot.

21

## 1 **Results**

### 2 **Distinct PrP<sup>res</sup> types in the brain and spleen of high-expressor mice intracerebrally**

#### 3 **inoculated with LAN-like and CH1641-like sheep scrapie isolates**

4 The tg338 mice overexpress the VRQ allele of ovine PrP. The PrP<sup>C</sup> levels in the brain are ~8-  
5 fold higher than in the sheep brain [29]. The spleen-to-brain PrP<sup>C</sup> ratio is ~1:20 in tg338, as in  
6 wild-type mice [22]. We transmitted by intracerebral (IC) route 37 sheep scrapie isolates from  
7 various countries and genotypes (including VRQ) to tg338 mice (details in [supplemental](#)  
8 [Table 1](#)). The isolates were from the LAN group [29] or closely resembling to CH1641 sheep  
9 scrapie isolate ([41-44], [Figure S1](#)). Sheep brains from the LAN group exhibit a PrP<sup>res</sup>  
10 electrophoretic signature with unglycosylated fragments migrating around 21 kDa (21K-  
11 PrP<sup>res</sup>, [Figure 1A](#)). These cases are composed of two prion strain types termed LA21K and  
12 LA19K, which are preferentially selected in transgenic mice expressing low or high levels of  
13 PrP<sup>C</sup>, respectively [29]. The CH1641-like isolates exhibit a 19 kDa signature in the brain, as  
14 CH1641 (19K-PrP<sup>res</sup>, [Figure 1A](#), [Figure S1](#), [42, 43]). Initially, these isolates were found to  
15 exhibit a signature similar to BSE in sheep and were termed BSE-compatible isolates ([Figure](#)  
16 [S1](#), [41]). However, transmission of CH1641-like isolates to ovine PrP mice (ARQ or VRQ  
17 allele) led to isolation of prions with strain features similar to LA19K prions ([41-43] and this  
18 study). For comparison, the PG127 sheep scrapie isolate ([Figure 1A](#)), which led to the  
19 accumulation of the same prion strain type in tg338 brain and spleen was used [45].  
20 All the inoculated isolates induced a typical prion disease in tg338 mice. The PrP<sup>res</sup>  
21 electrophoretic signature was compared between the brain and spleen of the same animals at  
22 the terminal stage of disease, so as to determine which substrain component was preferentially  
23 replicated. The results are summarized in [Table 1](#) and a representative immunoblot is shown  
24 in [Figure 1A](#).

25

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1 **Table 1. PrP<sup>res</sup> electrophoretic pattern in the brain and spleen after intracerebral**  
2 **inoculation of sheep scrapie field isolates to tg338 mice**

3

TSE sources	Number <sup>a</sup>	PrP <sup>res</sup> type <sup>b</sup>		
		Sheep brain	tg338 brain (n/n <sub>0</sub> ) <sup>c</sup>	tg338 spleen (n/n <sub>0</sub> ) <sup>c</sup>
LAN-like	26	21K	19K (77/79)	21K (53/53)
CH1641-like	11	19K	19K (38/38)	21K (18/21) <sup>d</sup>
PG127	1	21K	21K	21K

4 <sup>a</sup>Number of isolates transmitted to tg338 mice

5 <sup>b</sup>As referred to the size of unglycosylated PrP<sup>res</sup> in immunoblots

6 <sup>c</sup>Number of mice with the PrP<sup>res</sup> type/number of mice tested

7 <sup>d</sup>3 spleens were PrP<sup>res</sup> negative

8 After IC inoculation of LAN- and CH1641-like isolates, all but two tg338 brains analyzed  
9 showed prominent accumulation of 19K-PrP<sup>res</sup>, as reported previously with the prototypal  
10 LAN404 isolate [29]. The resulting agent was termed LA19K because of this prominent 19K-  
11 PrP<sup>res</sup> signature in the brain [29]. In striking contrast, nearly all the spleens analyzed exhibited  
12 a 21K-PrP<sup>res</sup> signature. The distinct PrP<sup>res</sup> patterns in the brain and spleen from the same mice  
13 were observed over iterative passages of brain material by IC route, as illustrated with the  
14 LAN404 isolate (LA19K at the 6<sup>th</sup> passage, [Figure 1B](#)).  
15 Transmission of sheep scrapie isolates from the LAN group occasionally led to the emergence  
16 of a highly pathogenic ‘mutant’ designated LA21K *fast* in a minority of tg338 mice [29]. IC-  
17 inoculation of LAN-like isolates resulted in the emergence of LA21K *fast* in two out of the 79  
18 brains analyzed ([Table 1](#)).

19 As LA21K *fast* prions accumulate in tg338 mouse spleens ([Figure 1B](#) and [46]), we  
20 questioned whether the 21K-PrP<sup>res</sup> signature observed in the spleen on IC inoculation of  
21 LAN-like and CH1641-like isolates could be similar to the LA21K *fast* one. The strain-  
22 specific [47] glycoform profile from LA21K *fast* PrP<sup>res</sup> in the spleen was distinct from that of  
23 the 21K-PrP<sup>res</sup> found in the spleen on serial passage of LAN404, the latter being enriched in  
24 diglycosylated forms ([Figure 1B-C](#)). The prion types accumulating in the spleen on IC

1 inoculation of LAN-like and CH1641-like isolates are thus likely different from LA21K *fast*  
2 prions.

3 A time-course analysis of PrP<sup>res</sup> accumulation was performed in the brain and spleen tissue at  
4 the 5<sup>th</sup> passage of LAN404 in tg338 mice. As shown in [Figure 1D](#), 21K-PrP<sup>res</sup> accumulation  
5 plateaued in the spleen from day 60 post-infection onward (bottom panel).

6 In the brain, 21K-PrP<sup>res</sup> was detected at day 60. At day 100 and at terminal stage of the  
7 disease, 19K-PrP<sup>res</sup> was mostly detected (top panel). The use of the 21K-selective anti-PrP  
8 monoclonal antibody 12B2 [38] to reveal the immunoblots demonstrated that 21K-PrP<sup>res</sup> was  
9 still present in the brain at these time points (middle panel). This suggested co-propagation of  
10 the 19K-PrP<sup>res</sup> and 21K-PrP<sup>res</sup> components in the brain.

11 To summarize, while IC transmission of LAN-like and CH1641-like isolates to tg338 mice  
12 leads to the isolation of LA19K prions in the brain, a divergent, prominent 21K-PrP<sup>res</sup>  
13 signature was observed in the spleen. This 21K-PrP<sup>res</sup> signature, also subdominant in the  
14 brain, differed from that of LA21K *fast*, suggesting that LA21K prions replicated in the  
15 spleen of the infected mice.

16

### 17 **Co-propagation of LA19K and LA21K prion strain variants in the brain and spleen of** 18 **high-expressor mice**

19 To substantiate the view that the 21K-PrP<sup>res</sup> signature in the spleen is associated with LA21K  
20 replication and formally exclude LA21K *fast* replication or a tissue-specific proteolytic  
21 processing of the LA19K strain type [48], we transmitted (IC route) spleen and brain extracts  
22 from the same tg338 mouse infected with LA19K (3<sup>rd</sup> passage of LAN404) to reporter tg338  
23 mice. We also challenged intracerebrally low-expressor tg143<sup>+/-</sup> mice (expressing 1.5-fold  
24 PrP<sup>C</sup> compared to sheep brain [29]), as they allow the dominant propagation of LA21K prions  
25 in the brain [29]. The resulting disease phenotypes in the two mouse lines are summarized in

1 [Figure 2A](#). In tg338 mice, the mean incubation duration (ID) was >3-fold longer with the  
2 spleen than with the brain extract. The spleen ID was also 3-fold longer than LA21K *fast* ID  
3 at the limiting dilution [6, 29]. The PrP<sup>res</sup> pattern found after inoculation of spleen material  
4 was 21K in all the brains and spleens analyzed ([Figure 2A-B](#)). The neuroanatomical  
5 deposition pattern of PrP<sup>res</sup> strikingly differed after inoculation of brain and spleen. In  
6 particular, numerous plaque-like PrP<sup>res</sup> deposits were seen specifically after inoculation of the  
7 spleen extract ([Figure 2C](#)). This phenotype was reminiscent of LA21K prions [29]. Similar  
8 isolation of LA21K prions in tg338 mouse brains was observed on IC transmission of spleens  
9 from tg338 mice inoculated with other LAN- or CH1641-like isolates ([Figure S2](#)). In tg143<sup>+/-</sup>  
10 mice, the spleen and brain extracts induced a long incubation time, with a 21K-PrP<sup>res</sup> pattern.  
11 Such characteristics were reminiscent of LA21K prions in these mice ([Figures 2A-B](#), [29]).  
12 To consolidate the view that the 19K-PrP<sup>res</sup> and the 21K-PrP<sup>res</sup> components were independent  
13 replicative entities in tg338 mice, we investigated the brain versus spleen colonization  
14 following IC challenge with cloned LA19K prions (double biological cloning by limiting  
15 dilution at the 3<sup>rd</sup> iterative passage of LA404 [29]). 19K-PrP<sup>res</sup> was detected in the brain  
16 ([Figure 1B](#)), with no evidence for the co-presence of a minor 21K-PrP<sup>res</sup> signature, as assessed  
17 with 12B2 antibody ([Figure 1D](#)). All the spleens of the terminally-sick animals were PrP<sup>res</sup>-  
18 negative ([Figure 1B-1D](#)). Reporter tg338 mice were inoculated IC with these negative spleens  
19 to estimate the amount of LA19K infectivity by an incubation time bioassay. As shown in  
20 [Figure 2A](#), the spleen extracts induced disease in 2 out of 8 mice >300 days post-infection,  
21 with a 19K-PrP<sup>res</sup> signature in the brain. On the basis of such ID and the low attack rate [6],  
22 the spleen of biologically cloned LA19K prions harbored 10<sup>5</sup>-fold less infectivity than the  
23 brain.  
24 Collectively, these data indicate that IC inoculation of LAN- and CH1641-like isolates to  
25 tg338 mice results in the preferential replication of the LA19K and LA21K subcomponents in

1 the brain and spleen, respectively. In the brain, both LA19K and LA21K subcomponents  
2 coexist, with the LA19K subcomponent in higher proportion. In the spleen, LA21K prions  
3 dominate, LA19K prions being barely detectable. LA19K prions thus show a preferential  
4 tropism for tg338 mouse brain. Such tissue-dependent segregation is reminiscent of the  
5 diverging selection of LA19K and LA21K prions on transmission to transgenic mice  
6 expressing PrP<sup>C</sup> at varying levels [29].

7

### 8 **Expression of LA21K prions in high-expressor mouse brain on intraperitoneal** 9 **inoculation of the LAN-like and CH1641-like isolates**

10 Intraperitoneal (IP) inoculation primarily favors the neuroinvasion of prions that primo-  
11 replicate in the spleen, by transport through the peripheral nervous system and the spinal cord  
12 (review [49]). We thus asked whether extraneural inoculation of LAN-like and CH1641-like  
13 isolates would lead to the dominant expression of LA21K prions in the brain as LA21K  
14 prions rapidly colonized the spleen of high-expressor tg338 mice. A group of representative  
15 sheep scrapie sources was transmitted to tg338 mice by IP route. The dose injected was the  
16 same as for the IC route. Four isolates were from the LAN-like group (LAN404, ARQ16,  
17 378, 454) and 2 from the CH1641-like group (O100 and m48). The PG127 isolate and  
18 LA19K prions served for comparison. Mice were euthanized at regular time-points post-  
19 injection and at terminal stage of disease (or end life) to examine early spleen colonization  
20 and analyze the molecular PrP<sup>res</sup> profile in both the spleen and the brain. In conventional  
21 mouse models inoculated IP with splenotropic prions, PrP<sup>res</sup> is detected at early time-points  
22 and the mean IDs are ~1.3-fold prolonged compared to IC infections at similar dose [50-53].  
23 Similar features were found with the PG127 isolate with regard to early PrP<sup>res</sup> positivity in the  
24 spleen (from day 30) and ID prolongation ([Figures 3A-B; supplemental Table S2](#)). Similarly,  
25 the spleens were uniformly and early PrP<sup>res</sup>-positive following IP inoculation with the LAN-



1 like and CH1641-like sheep scrapie isolates (Figures 3A-B). Yet, the tempo and the clinical  
2 manifestation of the disease and the dominant strain type replicating in the brain were not  
3 uniform amongst the mice analyzed. The mean IDs were ~3-fold longer than in the IC  
4 inoculated animals, ranging from 520 to 560 days (Figure 3A). Twenty-four percent of the  
5 brains analyzed were PrP<sup>res</sup>-negative. Fifty-nine percent of the brains exhibited a 21K-PrP<sup>res</sup>  
6 pattern and sixteen percent a 19K-PrP<sup>res</sup> pattern (Figures 3A-B). The 21K- PrP<sup>res</sup> pattern  
7 dominated over the 19K- PrP<sup>res</sup> pattern for all but one isolate (ARQ16). We conclude that IP  
8 inoculation of LAN-like and CH1641-like isolates favors the neuropathological expression of  
9 the splenotropic LA21K prions at the expense of LA19K prions. The significant proportion of  
10 mice with no PrP<sup>res</sup> in the brain despite early, widespread accumulation of PrP<sup>res</sup> in the spleen  
11 suggests a delayed efficacy of LA21K prions to replicate at full attack rate in the brain  
12 following IP inoculation.

13 We studied whether changing the inoculation route similarly impacted the pathogenesis of  
14 LA19K prions. Two distinct passages were used, the 4<sup>th</sup> and the 6<sup>th</sup> IC-brain passage of  
15 LAN404. We detected an early accumulation of 21K-PrP<sup>res</sup> in all the spleen analyzed,  
16 independently of the number of passages (Figures 3A, 3C). In the brain, the proportion of  
17 21K-PrP<sup>res</sup> mice decreased with the number of passages, at the expense of the 19K-signature.  
18 71% and ~45% of the brains analyzed were 21K-positive at the 4<sup>th</sup> and 6<sup>th</sup> passage of  
19 LAN404, respectively (Figures 3A, 3C). These results were consistent with an enrichment in  
20 LA19K prions over serial IC passaging.

21 IP inoculation of bi-cloned LA19K prions resulted in a disease at near full attack-rate, with  
22 19K-PrP<sup>res</sup> accumulating in the positive brains, whereas all the spleens remained PrP<sup>res</sup>-  
23 negative (Figures 3A, 3C). These findings suggest that LA19K prions could directly  
24 neuroinvade from the peripheral sites of infection, a pathway previously observed for other  
25 prions in situation of impaired replication in the spleen [20, 21, 54]. However, the ID was still

1 ~2.6-fold longer than by IC route, approaching values obtained with primary sheep material  
2 or uncloned LA19K prions ([Figure 3A](#)), indicating a delayed efficacy of LA19K prions to  
3 neuroinvade from the periphery.

4

#### 5 **Preferential selection of LA21K prions on inoculation of high-expressor mice with spleen** 6 **material from LAN404 primary isolate**

7 We asked whether a similar distinctive splenotropism between LA21K and LA19K prions  
8 pre-existed in the original sheep isolate. A spleen extract from the LAN404 isolate was  
9 inoculated by IC and IP routes to tg338 mice. At variance with the IC-inoculation of LAN404  
10 brain material which resulted in dominant expression of LA19K prions in the brain and  
11 LA21K in the spleen ([29] and [Figure 4A](#)), IC-inoculation of LAN404 spleen material led to  
12 accumulation of prions with a 21K-PrP<sup>res</sup> signature in both brain and spleen in ~450 days  
13 ([Figure 4A-B](#)). After IP inoculation, the incubation time established at 660 days and all the  
14 brains and spleens analyzed exhibited a 21K-PrP<sup>res</sup> signature ([Figure 3A-B](#)).

15 We conclude that in the spleen of the natural host, the LA21K component predominates over  
16 the LA19K component to levels allowing molecular expression in the CNS and in the spleen  
17 on primary transmission to high-expressor mice.

18

#### 19 **Another pair of strains co-propagating in high-expressor mice and with differing** 20 **tropism for the brain and spleen shows divergent selection according to PrP<sup>C</sup> expression** 21 **level**

22 To further explore the possibility that prion capacity to replicate in the spleen is controlled by  
23 PrP<sup>C</sup> expression levels, we compared the replicative capacity in mice expressing low levels of  
24 PrP<sup>C</sup> of two pairs of strains with opposite splenotropism in high expressors. Previously, we

1 reported that a pair of prion strains, designated T1<sup>Ov</sup> and T2<sup>Ov</sup>, isolated through the adaptation  
2 of cortical MM2 sporadic CJD (MM2-sCJD) prions to tg338 mice exhibited differing tissue  
3 tropism. T2<sup>Ov</sup> replicated dominantly in the brain with T1<sup>Ov</sup> being present as a subcomponent,  
4 while T1<sup>Ov</sup> preferentially populated the spleen ([28] and [Figure 5A-B](#)). A brain extract from  
5 tg338-passaged MM2-sCJD prions (4<sup>th</sup> passage) was inoculated by IC route to tg335<sup>+/-</sup> mice,  
6 expressing 1.2-fold PrP<sup>C</sup> compared to sheep brain [29]. For comparison, brain extracts  
7 containing solely T2<sup>Ov</sup> prions (obtained by bicloning by limiting dilution [28]) or T1<sup>Ov</sup> prions  
8 (PMCA selection and single cloning [28]) were inoculated to tg335<sup>+/-</sup> mice. The transmission  
9 of tg338-passaged MM2-sCJD prions to tg335<sup>+/-</sup> mice led to the dominant expression of T1<sup>Ov</sup>  
10 prions, as based on the mean ID ([Figure 5A](#)), the PrP<sup>res</sup> signature in brain and spleen by  
11 western blot ([Figure 5B](#)) and the neuroanatomical distribution of PrP<sup>res</sup> by histoblotting  
12 ([Figure 5C](#)), which all showed the T1<sup>Ov</sup> characteristics observed after infection with cloned  
13 T1<sup>Ov</sup> prions. The apparent counter-selection of T2<sup>Ov</sup> prions in tg335<sup>+/-</sup> mice was not due to  
14 their incapacity to replicate on low-expressor mice as bicloned T2<sup>Ov</sup> prions elicited disease in  
15 these mice in ~330 days ([Figure 5A](#)) with a T2<sup>Ov</sup>-PrP<sup>res</sup> specific banding pattern in the brain  
16 ([Figure 5B](#)) and presence of low levels of PrP<sup>res</sup> by histoblotting ([Figure 5C](#)). Together, these  
17 data indicate that for another pair of co-propagating prion strains, there is a tight correlation  
18 between capacity to replicate in the spleen and capacity to be dominantly expressed on mice  
19 expressing low PrP<sup>C</sup>-levels in the brain.

20 Remarkably, the dominant expression of T1<sup>Ov</sup> prions on passage of tg338-passaged MM2-  
21 sCJD prions to tg335<sup>+/-</sup> mice occurred to the detriment of T2<sup>Ov</sup> prions. Over two serial back  
22 passages to tg338 mice, T2<sup>Ov</sup> prions were not phenotypically recovered. The T1<sup>Ov</sup> signature  
23 remained dominant in all brains and spleens from tg338 mice ([Figure 5A, 5C](#)). Such outcome  
24 was not observed on retro passage of LAN-like isolates from low- to high-expressor mice as  
25 LA19K prions were recovered after intermediate passage onto low-expressors [29].

- 1 Alternance of transmission in mice expressing variable levels of PrP<sup>C</sup> may thus result in a
- 2 strain evolution as strong as that observed in a heterotypic PrP transmission context.
- 3

## 1 **Discussion**

2 Building on our previous observations that the phenotypic expression of two prion  
3 subcomponents present in variable proportions in numerous sheep scrapie isolates is driven by  
4 PrP<sup>C</sup> expression levels in transgenic mice, we now show that the segregation between these  
5 two agents occurs between the brain and the spleen of the same infected high-expressor  
6 mouse, these tissues expressing different PrP<sup>C</sup> levels. Further, the replicative dominance in  
7 the brain of a pair of strains derived from sporadic CJD prions with opposite tropism for the  
8 brain and spleen appears tightly controlled by PrP<sup>C</sup> expression levels in transgenic mice  
9 expressing varying PrP<sup>C</sup> levels. Host PrP<sup>C</sup> levels may thus impact prion (in)capacity to  
10 replicate in the lymphoid tissue.

11  
12 LA19K prions predominated in high-PrP<sup>C</sup> expressor tg338 mouse brains inoculated IC with a  
13 large panel of natural sheep scrapie isolates composed in variable proportion of LA19K and  
14 LA21K prions (LAN-like, [29] and this study). Here, we show early, widespread and  
15 dominant accumulation of LA21K in the spleen of these mice, -as based on molecular strain  
16 typing, and phenotypic characterization on high- and low-PrP<sup>C</sup> expressor mice. The  
17 LA19K/LA21K segregation was also observed after IC inoculation of CH1641-like isolates in  
18 which the 19K subcomponent was originally dominant, indicating that the lymphoid tissue is  
19 a privileged site for LA21K prions replication. In contrast, cloned LA19K prions replicated  
20 with limited efficacy in the spleen, and thus were considered as mostly neurotropic.  
21 That co-propagating TSE substrains replicate preferentially in distinct tissues from the same  
22 host is not restricted to transgenic mice nor to the VRQ allele of ovine PrP. We show here a  
23 similar enrichment of LA21K prions in the spleen relative to brain in the LAN-infected sheep.  
24 A similarly distinct selection of PrP<sup>res</sup> conformers with 19K and 21K signature was observed  
25 in the brain and spleen tissue from transgenic mice expressing the ARQ allele of ovine PrP

1 inoculated with CH1641-like scrapie isolates [55]. A similar tissue-specific segregation of  
2 prion subcomponents due to distinct tropism was observed on transmission of a human vCJD  
3 case to transgenic mice overexpressing human PrP [23, 56], on transmission of cortical MM2-  
4 sporadic CJD to tg338 mice [28], directly in a sporadic CJD individual [57] and in CWD-  
5 infected elk [58].

6 These data advocate for strain typing in both brain and spleen tissues, including in high  
7 expressor mice. Blind strain typing of LAN-like isolates using tg338 mouse brain only would  
8 categorize them as CH1641 prions. However, examining the lymphoid tissue or changing the  
9 route of infection allows expression of the LA21K strain component, consistent with studies  
10 in transgenic mice expressing variable PrP levels. *In fine* transgenic mice in which PrP is  
11 introduced by additional transgenesis prove to be a valuable tool to reveal natural prion strain  
12 diversity present in TSE isolates, provided the PrP-encoding constructs allow correct PrP<sup>C</sup>  
13 expression in the lymphoid tissue.

14  
15 The host and structural requirements for prion replication in the lymphoid tissue remain  
16 mostly unknown. From the prion pathogen viewpoint, two broad hypotheses recently  
17 emerged, the sensitivity to degradation of the PrP<sup>Sc</sup> assemblies and their aggregation size. A  
18 study from Shikiya et al. suggested that absence of DY prions replication in hamster spleens  
19 was due to a particular sensitivity of DY PrP<sup>Sc</sup> to protease digestion [59], yet without  
20 providing *in vivo* or *ex vivo* evidence (e.g., clearance of PrP<sup>Sc</sup> by macrophages). Here, we  
21 firmly exclude this possibility as LA19K prions were not more sensitive than 127S prions to  
22 either PK digestion or clearance by peritoneal macrophages (Figure 6). Aguilar-Calvo et al.  
23 proposed that prion conformations could impact pathogenesis after peripheral infection, i.e.,  
24 highly fibrillar prion assemblies are poor neuroinvaders compared to subfibrillar prions but  
25 high replicators in the spleen [31]. PrP<sup>Sc</sup> assemblies' particulate profile may indeed impact

1 access to the FDC from the site of infection [60, 61]. LA21K prions and vCJD prions would  
2 follow this rule as they would be categorized as ‘highly fibrillar’, as based on their  
3 sedimentation velocity profile and the presence of plaque-like deposits in the brain (this study  
4 and [23, 62]). However, T1<sup>Ov</sup> and T2<sup>Ov</sup> prions would escape such a rule as they exhibit similar  
5 ‘subfibrillar’ aggregation size [63], despite distinct tropism for the spleen.  
6 From the host viewpoint, mature FDC are necessary [9, 15-21, 64, 65], being ontogenetically  
7 present or induced by chronic inflammation [66]. Co-factors have been proposed to be  
8 involved, including notably complement [67, 68], but the differential requirement amongst  
9 strains is not known. Here, LA19K / LA21K prions preferential targeting for brain and spleen  
10 correlated with PrP<sup>C</sup> expression levels in these tissues. The superimposable PrP<sup>C</sup>-dependent  
11 selection obtained with a second pair of prion strains isolated from MM2-sCJD in tg338 mice  
12 and showing differing tropism for the lymphoid versus the cerebral tissue, that is the most  
13 lymphotropic is preferentially replicated on low-PrP<sup>C</sup> expressor mice and the most  
14 neurotropic on high-PrP<sup>C</sup> expressor mice, further argues that PrP<sup>C</sup> expression levels may be a  
15 major driver of prion (sub)strain selection in different tissues of the infected host.  
16 Additionally, at the molecular level, PrP<sup>C</sup> conformational landscape is likely to differ between  
17 spleen and brain, due to different trimming [48], glycosylation [69] including sialylation [70],  
18 and thus may impact conversion by certain PrP<sup>Sc</sup> subspecies, as observed during cross-species  
19 prion transmission. A combination of both PrP<sup>C</sup> expression levels and variations in spleen  
20 PrP<sup>C</sup> conformations may well explain why intermediate passage of tg338-passaged-MM2-  
21 sCJD prions on low expressors resulted in the abrupt exclusion of T2<sup>Ov</sup> prions on back  
22 passage to tg338 mice, an outcome that was reminiscent of that observed during cross-species  
23 transmission events (for reviews [7, 8]). Such factors may be drivers of within-host prion  
24 diversification/evolution during homotypic transmissions.

25

1 It may be noted that PrP<sup>C</sup> expression levels are classically quantified in the whole spleen and  
2 in the whole brain, yet meaning that different variations in PrP<sup>C</sup> levels could occur locally in  
3 prion-replicating cells [71]. The exact contribution of FDC to spleen PrP<sup>C</sup> expression level is  
4 unknown due to the fact that these cells only account for 0.1-1% of the cells, making isolation  
5 and quantification of their PrP<sup>C</sup> expression levels a highly challenging issue. However, we  
6 previously reported by immunofluorescence that PrP<sup>C</sup> expression levels appeared low in  
7 tg338 FDC compared to neurons [22].

8  
9 As a tentative explanation for strain-dependent polymerization rate as a function of PrP<sup>C</sup> levels,  
10 we inferred by mathematical modelling that differences in the number of PrP<sup>C</sup> monomers  
11 integrated in the growing PrP<sup>Sc</sup> assemblies (different kinetic order of the templating process)  
12 could explain PrP<sup>C</sup>-dependent substrain selection [29]. LA19K growth would necessitate  
13 integration of more PrP<sup>C</sup> molecules than for LA21K prions. In theory, the strain necessitating  
14 more PrP<sup>C</sup> to replicate should also start accumulating in the spleen. However, the plateau of  
15 PrP<sup>Sc</sup> accumulation observed in the spleen quite early after infection [10, 23, 45], possibly due  
16 to a cessation of replication activity [72, 73] would prevent the chance that the kinetically-  
17 disadvantaged substrain replicates in this tissue.

18  
19 The IP route of inoculation, known to favor neuroinvasion of prions from the lymphoid tissue  
20 (review [49]) allowed dominant accumulation of LA21K PrP<sup>res</sup> in the brain of a proportion of  
21 high-expressor mice at the expense of LA19K PrP<sup>res</sup>. The incubation time lengthening when IP  
22 and IC routes of infection were compared for LAN-like isolates was overtly prolonged as  
23 compared to PG127 in tg338 mice or other experimental models and a number of IP-inoculated  
24 tg338 mice remained PrP<sup>res</sup>-negative in the brain while their spleen was strongly and early  
25 positive. This suggests an impaired capacity of LA21K prions to establish at full attack rate in



1 the brain, as previously observed with other prion types [23, 24]. Bicloned LA19K prions were  
2 able to establish in the brain after IP infection, despite absence of replication in the spleen, by  
3 a direct neuroinvasion process. However, direct neuroinvasion after IP infection usually results  
4 in ID similar to those observed after primo-replication in the spleen [20, 21]. This suggests that  
5 the IC versus IP ID was also aberrantly prolonged for bicloned LA19K prions. Comparison of  
6 ID on low- versus high-expressors after inoculation of LA21K *fast* versus LA19K prions show  
7 ‘abnormal’ prolongation of ID for the latter [29]. It is therefore likely that PrP<sup>C</sup>-limiting events  
8 are similarly occurring in high-expressor mice during prion neuroinvasion after IP inoculation.  
9 Of note, when LAN404 sheep spleen instead of brain was transmitted to tg338 mice, the IP  
10 versus IC injection delay observed (x1.47, [Figure 4](#)) was rather consistent with the ~1.3 value  
11 found in other experimental models [50-53] and with PG127 in tg338 mice ([Figure 3](#)). As the  
12 sheep spleen is less enriched than the brain in LA19K prions, these data would collectively  
13 suggest that LA19K prions give the disease tempo after IP infection even if LA21K PrP<sup>res</sup>  
14 molecular signature prions can be dominant.

15  
16 In conclusion, these data and our previous report [29] suggest an emerging contribution of PrP  
17 as a molecular driver/modulator of within-host prion selection. Events similar to the  
18 brain/spleen segregation observed here could also occur directly within the brain owing to the  
19 regional heterogeneity in PrP<sup>C</sup> levels and conformations in this tissue [74]. From a diagnostic  
20 point of view, given the apparent complexity of natural animal and human TSE isolates in terms  
21 of substrain heterogeneity [27, 75, 76], these data advocate to type extraneural tissues or fluids  
22 for a comprehensive identification of the TSE agents circulating in susceptible mammals.

23

24

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7 colleagues for kindly providing sheep TSE sources.

## 9 **Data Availability**

10 All relevant data are within the manuscript and its supporting information files. Data are fully  
11 available without restriction.

## 13 **Figure legends**

15 **Figure 1. PrP<sup>res</sup> electrophoretic pattern in brains and spleens of high-expressor tg338**  
16 **mice inoculated IC with LAN-like and CH1641-like isolates.**

17 A) Distinct PrP<sup>res</sup> electrophoretic pattern in the brain and spleen of tg338 high-expressor  
18 mice following IC challenge with representative LAN-like and CH1641-like isolates.  
19 The patterns observed after PG127 inoculation are shown as control. The PrP<sup>res</sup> profile  
20 in the sheep brain are shown for comparison on the left side of the western blot.  
21 B) PrP<sup>res</sup> detection and electrophoretic pattern in the brain and spleen of tg338 mice on  
22 serial transmission by IC route (6<sup>th</sup> passage) of LAN404 isolate (LA19K), cloned  
23 LA19K and LA21K *fast* prions. MM: molecular mass markers.

- 1 C) Ratio of diglycosylated *vs* monoglycosylated PrP<sup>res</sup> in the spleen of mice on serial  
2 transmission of LAN404 isolate (gray symbol), LA21K *fast* (green symbol) and PG127  
3 (orange symbol) ( $n=5$  spleens analyzed at the 6<sup>th</sup> passage, data plotted as mean  $\pm$  SEM).
- 4 D) Time course analysis of PrP<sup>res</sup> accumulation in the brain and spleen of tg338 mice on  
5 serial transmission (5<sup>th</sup> passage) of LAN404 isolate (LA19K). Three mice were  
6 analyzed at each time point. The immunoblots were revealed with Sha31 and 12B2 anti-  
7 PrP antibodies, as indicated. PrP<sup>res</sup> detection with these antibodies was also performed  
8 with tissues from terminally-sick mice after infection with cloned LA19K and LA21K  
9 *fast* prions. The amount of mg tissue loaded on the gels is indicated.

10

11

12 **Figure 2. Strain phenotype of prions replicating in tg338 mouse spleens on serial passage**  
13 **of LAN404 isolate.**

- 14 A) Summary of the transmissions by IC route of brain or spleen extracts from tg338 mice  
15 infected with 19K prions (3<sup>rd</sup> passage of LAN404) or with bicloned LA19K prions to  
16 reporter tg338 mice or to tg143<sup>+/-</sup> mice expressing ovine PrP<sup>C</sup> at near physiological  
17 levels. Transmission with brain or spleen extracts are indicated with black and red  
18 lines, respectively. The number of affected/inoculated mice (mice with TSE and  
19 positive for brain PrP<sup>res</sup> by immunoblot) and the mean survival times in days  $\pm$  SEM  
20 are indicated for each inoculated group. Segmented, doubled circles are used to  
21 indicate the proportion of mice with 19K PrP<sup>res</sup> signature (blue), 21K PrP<sup>res</sup> signature  
22 (grey) or absence of PrP<sup>res</sup> in the brain (inside of the circle, black lines) and the spleen  
23 (outside of the circle, red lines). The data shown are representative of 5 independent  
24 transmission experiments with different mice infected with LA19K or cloned LA19K  
25 prions. Data in *italic* are from [29].

- 1 B) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in brain and spleen  
2 from high-expressor tg338 mice and low-expressor tg143<sup>+/-</sup> mice, depending on  
3 whether tg338-passaged brain (Br) or spleen (Sp) material (3<sup>rd</sup> tg338-passage of  
4 LAN404) is used for IC inoculation.
- 5 C) Representative histoblots at the level of the striatum (left panel) and the brain stem  
6 (right panel) showing the distribution of PrP<sup>res</sup> deposits in the brains of high-expressor  
7 tg338 mice inoculated with brain or spleen extracts (3<sup>rd</sup> tg338-passage of LAN404).  
8 Scale bar, 1 mm.

9

10 **Figure 3. PrP<sup>res</sup> electrophoretic pattern in brains and spleens of high-expressor tg338**  
11 **mice inoculated IP with LAN-like and CH1641-like isolates.**

- 12 A) Comparison of the transmissions by IP versus IC route of PG127, LAN-like, CH1641-  
13 like sheep scrapie isolates and LA19K prions (4<sup>th</sup> and 6<sup>th</sup> IC-brain-passage (4p, 6p)  
14 and cloned agent). The mean  $\pm$ SEM incubation durations (ID) are indicated following  
15 challenge by IC route (green bar) and IP route (red bar). The ID<sub>IP</sub> / ID<sub>IC</sub> ratio are  
16 indicated. The grey vertical bar indicates the time at which PrP<sup>res</sup> was first detected in  
17 the spleen ([Supplemental Table S2](#)). Segmented, doubled circles are used to indicate  
18 the proportion of mice with 19K PrP<sup>res</sup> signature (blue), 21K PrP<sup>res</sup> signature (grey) or  
19 absence of PrP<sup>res</sup> in the brain (inside of the circle, black lines) and the spleen (outside  
20 of the circle, red lines).
- 21 B) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in brain and spleen  
22 from tg338 high-expressor mice inoculated IP with isolates from the LAN-like and  
23 CH1641-like groups, at the terminal stage of disease. The spleen and the brain from  
24 the same animal are presented, meaning that the brain can harbor a 21K or 19K PrP<sup>res</sup>  
25 signature whereas a unique 21K signature is detected in the spleen. The spleen PrP<sup>res</sup>

1 profiles are also shown at early time points post-inoculation (from 50 to 200 days  
2 depending on the isolate, [Supplemental Table S2](#)). Patterns after PG127 infection are  
3 shown for comparison.

4 C) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in brain and spleen  
5 from tg338 high-expressor mice inoculated IP with LA19K prions (4<sup>th</sup> IC-brain-  
6 passage (4p), 6<sup>th</sup> IC-brain-passage (6p) and cloned (cl)), at the terminal stage of  
7 disease. The spleen and the brain from the same animal are presented, meaning that  
8 the brain can harbor a 21K or 19K PrP<sup>res</sup> signature (with the 6<sup>th</sup> pass, a double 21/19K  
9 signature was observed) whereas a unique 21K signature is detected in the spleen. The  
10 spleen PrP<sup>res</sup> profiles are also shown at early time points post-inoculation (from 20 to  
11 50 days depending on the number of passages, [Supplemental Table S2](#)). Patterns after  
12 PG127 infection are shown for comparison.

13

14 **Figure 4. Strain phenotype of prions replicating in tg338 mouse spleen and brain on**  
15 **primary passage of LAN404 spleen.**

16 A) Summary of the transmissions by IC or IP route of brain or spleen extracts from  
17 LAN404 sheep scrapie isolate to tg338 mice. Legends are as in Figure 2. Data in italic  
18 are from [29].

19 B) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in (Br) and spleen  
20 (Sp) from high-expressor tg338 mice, upon inoculation with LAN404 spleen material  
21 by IP or IC route. PrP<sup>res</sup> electrophoretic pattern of LA21K *fast* and cloned LA19K in  
22 tg338 mouse brain is shown for comparison.

23

24 **Figure 5. PrP<sup>C</sup>-level dependent selection of prions with differing splenotropism in ovine**  
25 **PrP transgenic mice**

- 1        A) Summary of the transmissions by IC route of brain extracts from tg338 mice infected  
2            with MM2-sCJD prions (4<sup>th</sup> passage) or with cloned T1<sup>Ov</sup> and T2<sup>Ov</sup> prions to tg335<sup>+/-</sup>  
3            mice expressing ovine PrP<sup>C</sup> at near physiological levels, before back passage to tg338  
4            mice. The number of affected/inoculated mice (mice with TSE and positive for brain  
5            PrP<sup>res</sup> by immunoblot) and the mean survival time in days  $\pm$  SEM are indicated for  
6            each inoculated group. Segmented, doubled circles are used to indicate the proportion  
7            of mice with T2<sup>Ov</sup> (brown or clear brown depending on the accumulation levels) or  
8            T1<sup>Ov</sup> (yellow or clear yellow) PrP<sup>res</sup> signatures in their brains (inside of the circle,  
9            black lines) and spleens (outside of the circle, red lines). Data in italic are from [28].
- 10        B) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in brain and spleen  
11            from tg338 high-expressor mice and tg335<sup>+/-</sup> low-expressor mice infected with MM2-  
12            sCJD prions (4<sup>th</sup> passage) or with cloned T1<sup>Ov</sup> and T2<sup>Ov</sup> prions. The gels on the right  
13            side are portions of the gels (colored red and green lines) that are purposely  
14            overexposed to detect T2<sup>Ov</sup> PrP<sup>res</sup> in the spleen.
- 15        C) Representative immunoblot showing PrP<sup>res</sup> electrophoretic pattern in brain and spleen  
16            from tg338 high-expressor mice after one or two back passages from tg335<sup>+/-</sup> low-  
17            expressor mice infected with MM2-sCJD prions or with cloned T1<sup>Ov</sup> and T2<sup>Ov</sup> prions.  
18            PrP<sup>res</sup> signatures from T1<sup>Ov</sup> and T2<sup>Ov</sup> prions in tg338 mice are shown for comparison.
- 19        D) PrP<sup>res</sup> deposition pattern in the brain of tg335<sup>+/-</sup> low-expressor mice infected with  
20            tg338-passaged MM2-sCJD, T1<sup>Ov</sup> and T2<sup>Ov</sup> prions. Representative histoblots of  
21            antero-posterior coronal brain sections at the level of the septum (i), hippocampus (ii),  
22            midbrain (iii) and brainstem (iv). Scale bar, 1 mm.

23  
24        **Figure 6. PrP<sup>res</sup> electrophoretic pattern in brains and spleens of high-expressor tg338**  
25        **mice inoculated IC with LAN and CH1641-like isolates.**

- 1        A) Comparative PK digestion assay performed on brain material from tg338 inoculated  
2                with 127S or cloned LA19K prions. The homogenates were treated with increasing  
3                concentrations of PK, as indicated, and analyzed by western blotting.
- 4        B) Representative immunoblot showing PrP<sup>res</sup> degradation in tg338 peritoneal  
5                macrophages exposed to 127S and LA19K prion for the indicated period of time  
6                (duplicates analysis).
- 7

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16

## 17 **Supporting Information Legends**

18

### 19 **Figure S1. PrP<sup>res</sup> electrophoretic profile from CH1641-like isolates**

20 Electrophoretic pattern of PrP<sup>res</sup> in the brain from CH1641-like cases, compared with CH1641  
21 isolate [36], PG127 isolate [35] and sheep experimentally inoculated with BSE prions [77].  
22

23 **Figure S2. Strain phenotype of prions replicating in tg338 mouse spleens on serial passage**  
24 **of LAN-like or CH1641-like isolates.**

1 Transmissions by IC route of brain or spleen extracts from tg338 mice infected with the LAN-  
2 like isolate (99-378 isolate, 2<sup>nd</sup> passage) or the CH1641-like isolate (O100 isolate, 2<sup>nd</sup>  
3 passage) to reporter tg338 mice. Transmission with brain or spleen extracts are indicated with  
4 black and red lines, respectively. The number of affected/inoculated mice (mice with TSE and  
5 positive for brain PrP<sup>res</sup> by immunoblot) and the mean survival times in days  $\pm$  SEM are  
6 indicated for each inoculated group. Segmented, doubled circles are used to indicate the  
7 proportion of mice with 19K PrP<sup>res</sup> signature (blue) or 21K PrP<sup>res</sup> signature (grey) in the brain  
8 (inside of the circle, black lines) and the spleen (outside of the circle, red lines).

9

#### 10 **Supplementary Table 1**

11 TSE sources transmitted to tg338 mice.

12

#### 13 **Supplementary Table 2**

14 PrP<sup>res</sup> signature in the brain and spleen after intraperitoneal inoculation of LAN, CH1641-like  
15 isolates and LA19K prions to tg338 mice.

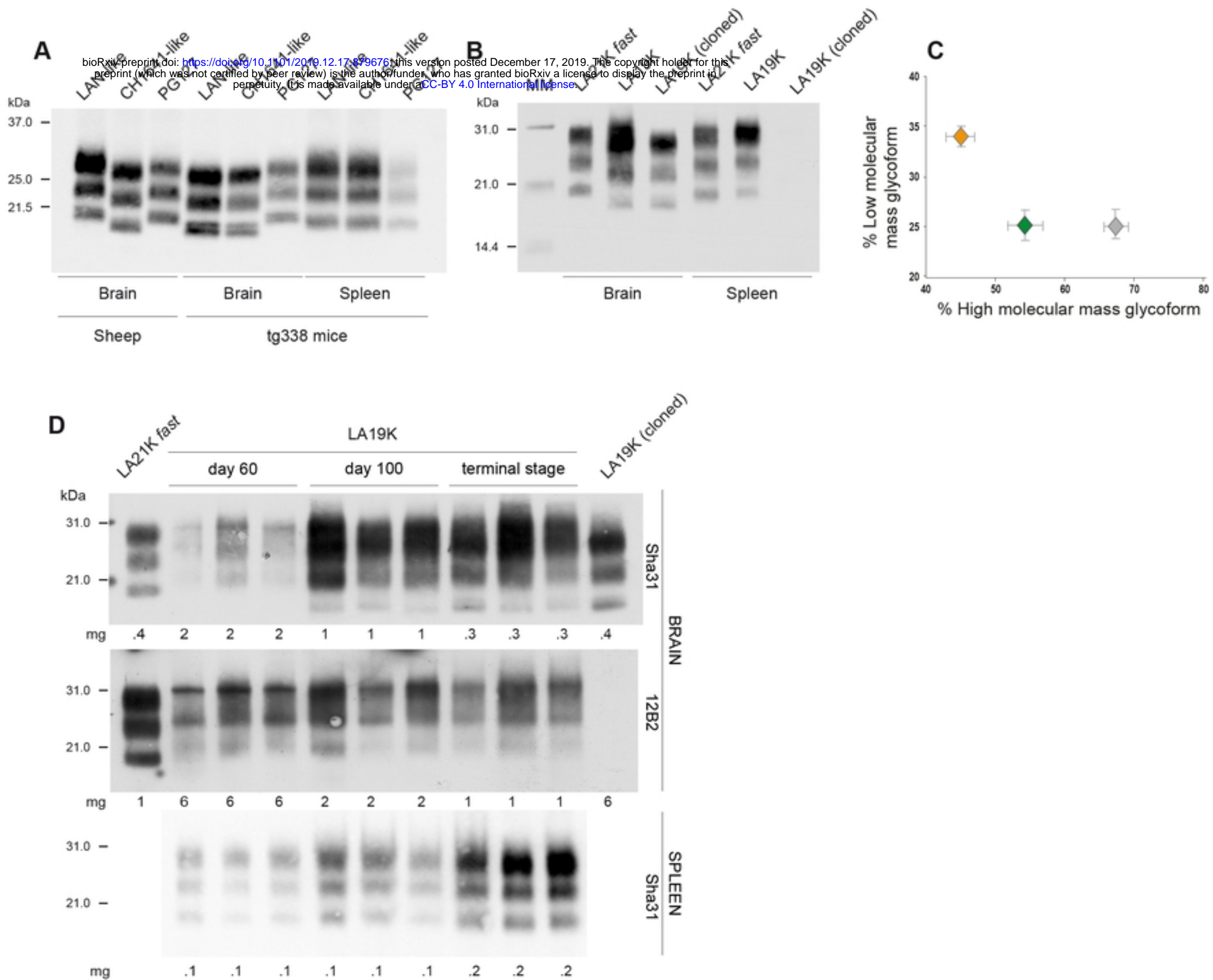
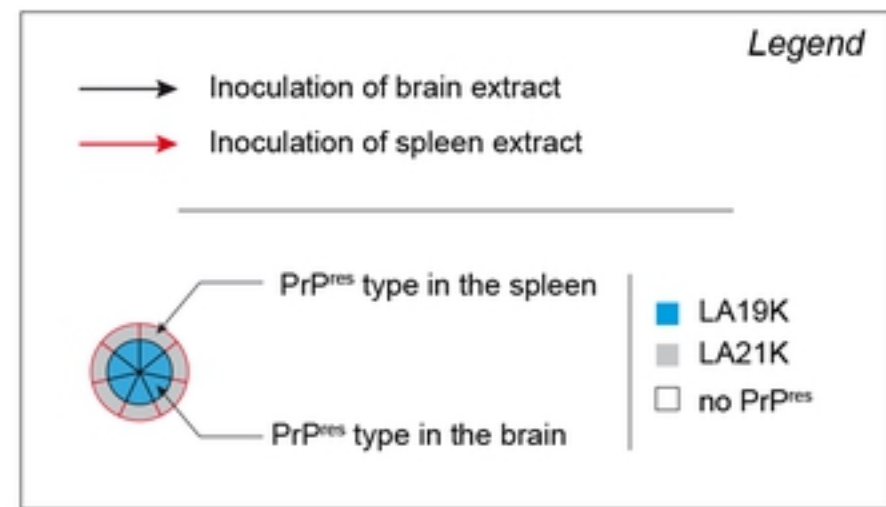
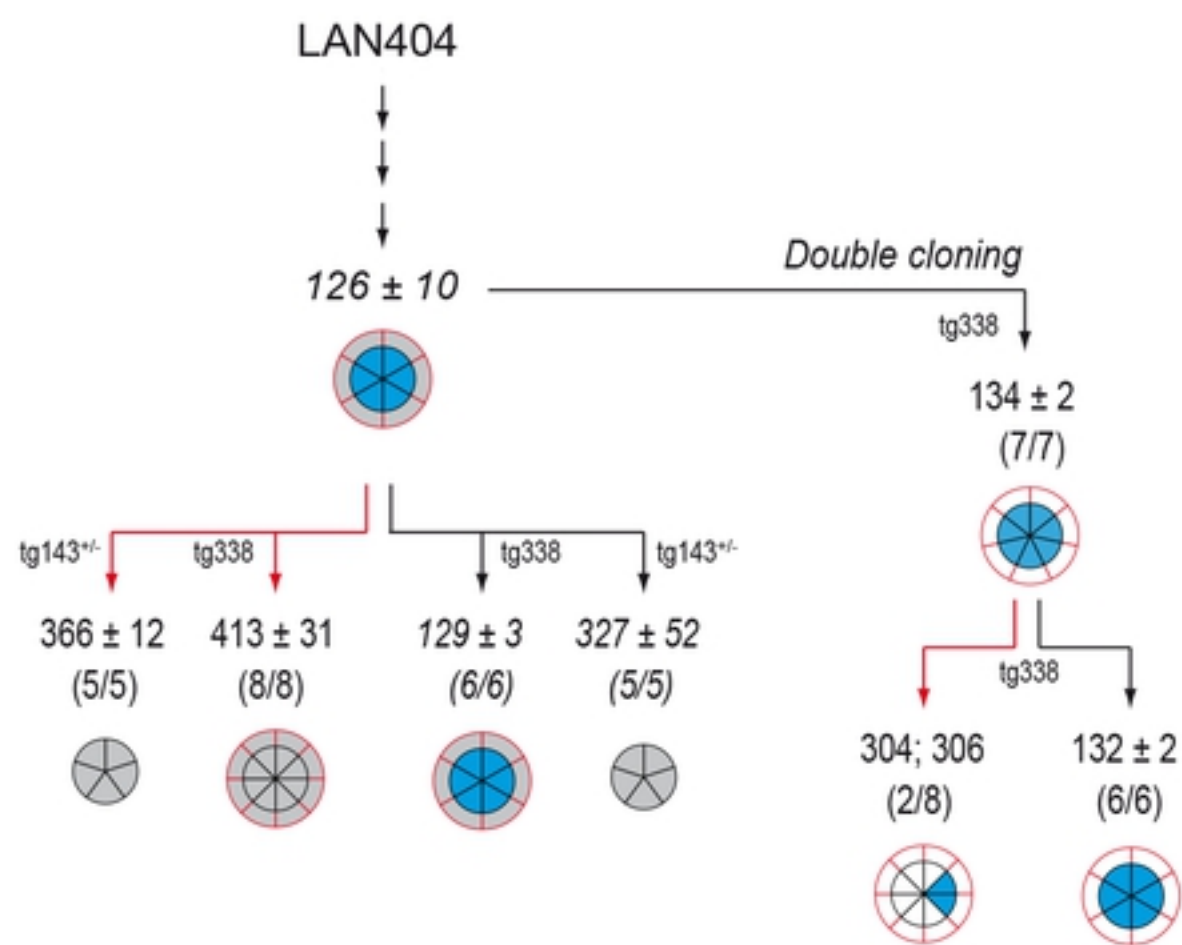


Figure 1

**A****B**

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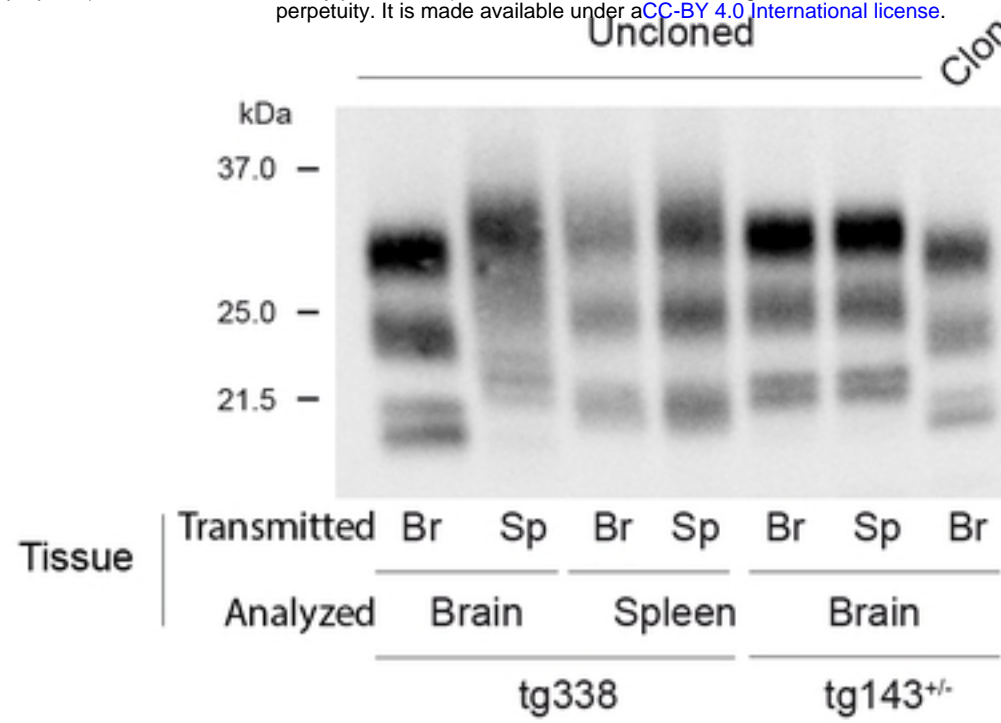
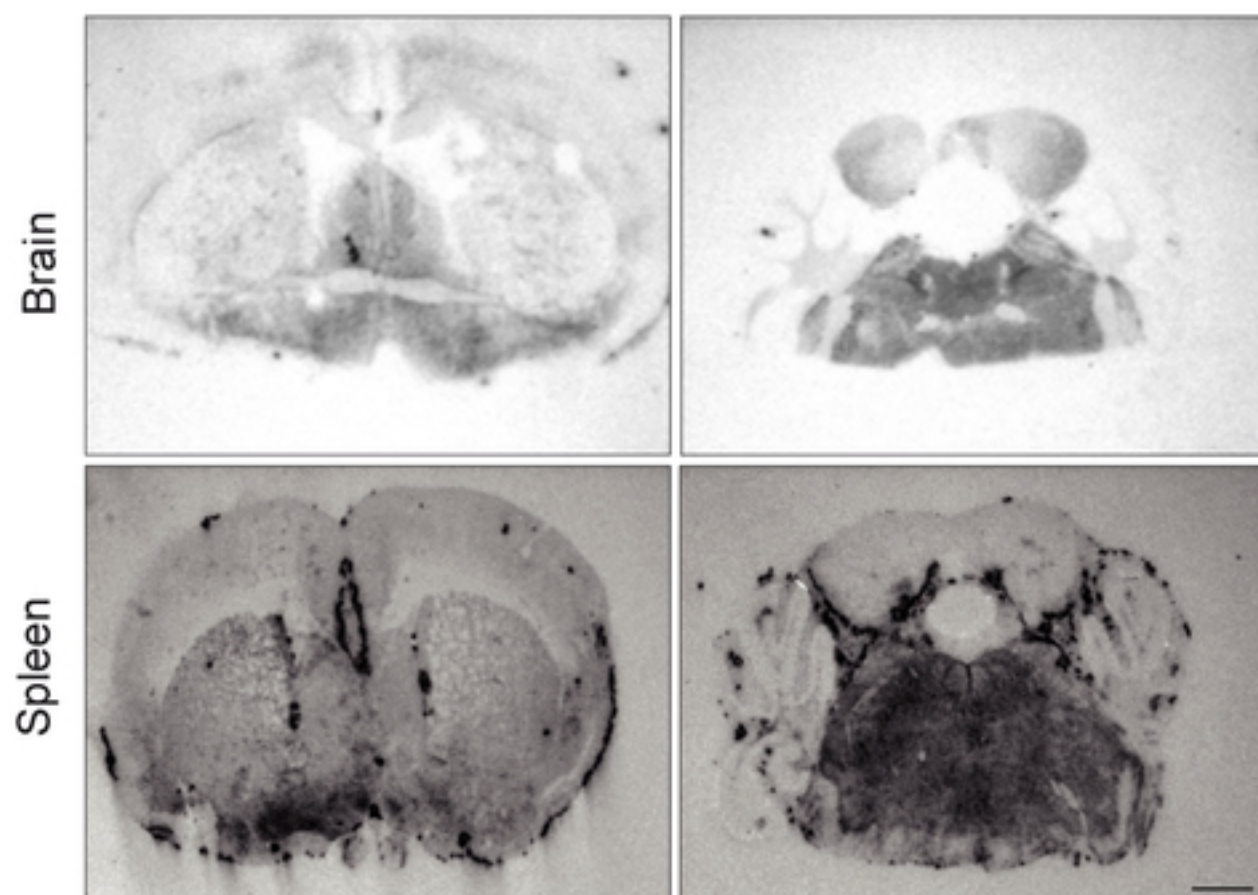
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Figure 2

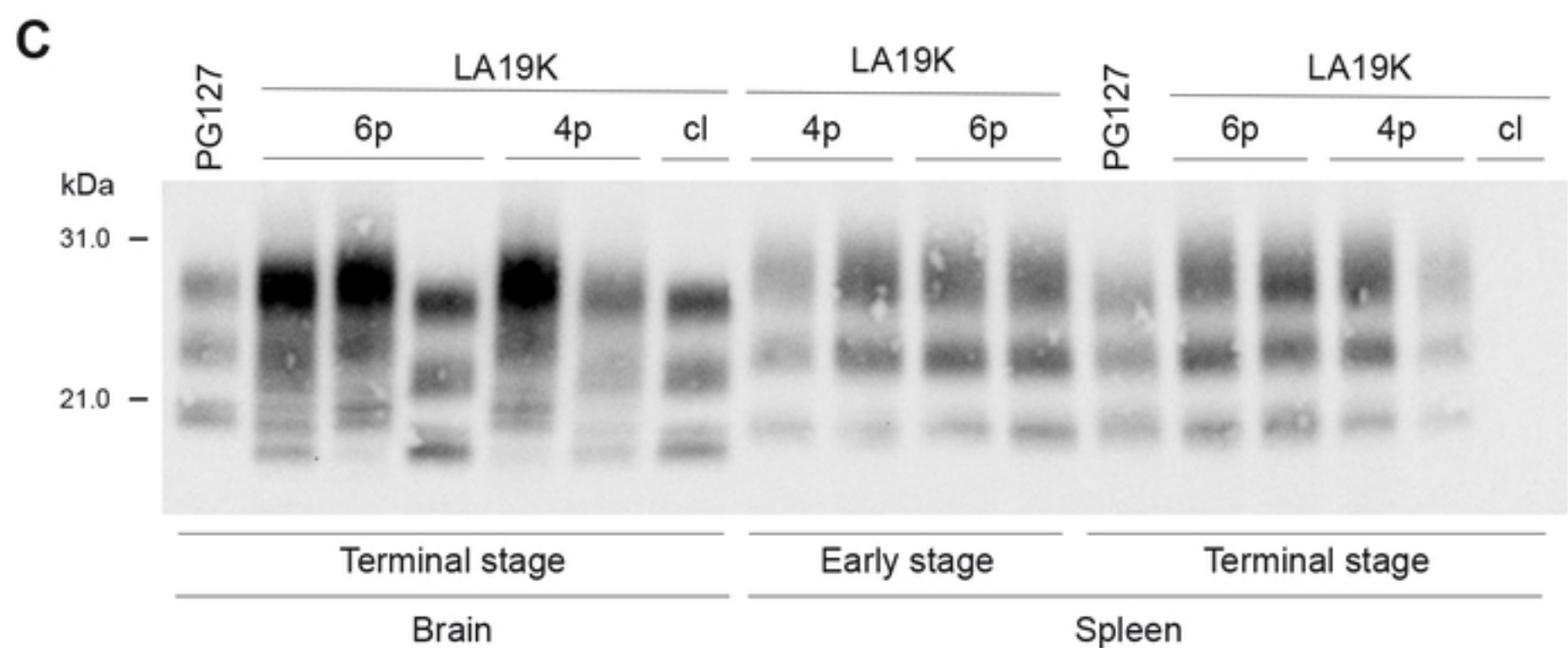
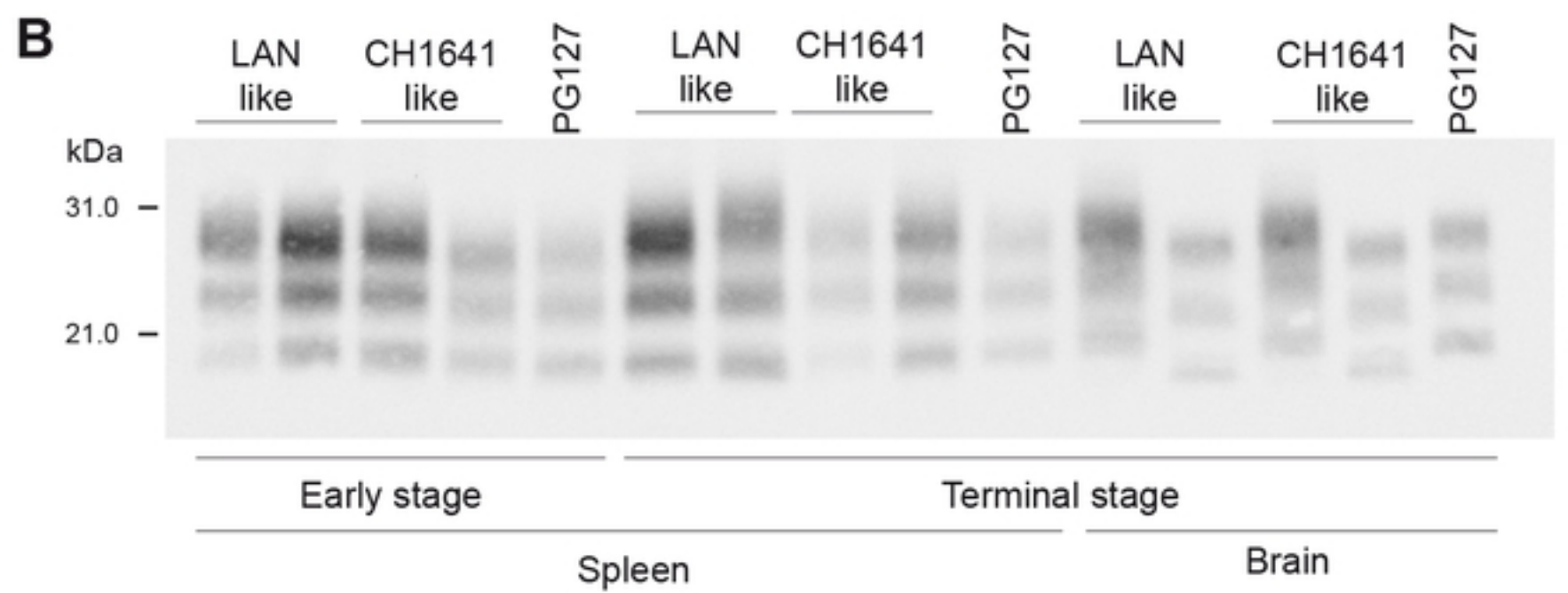
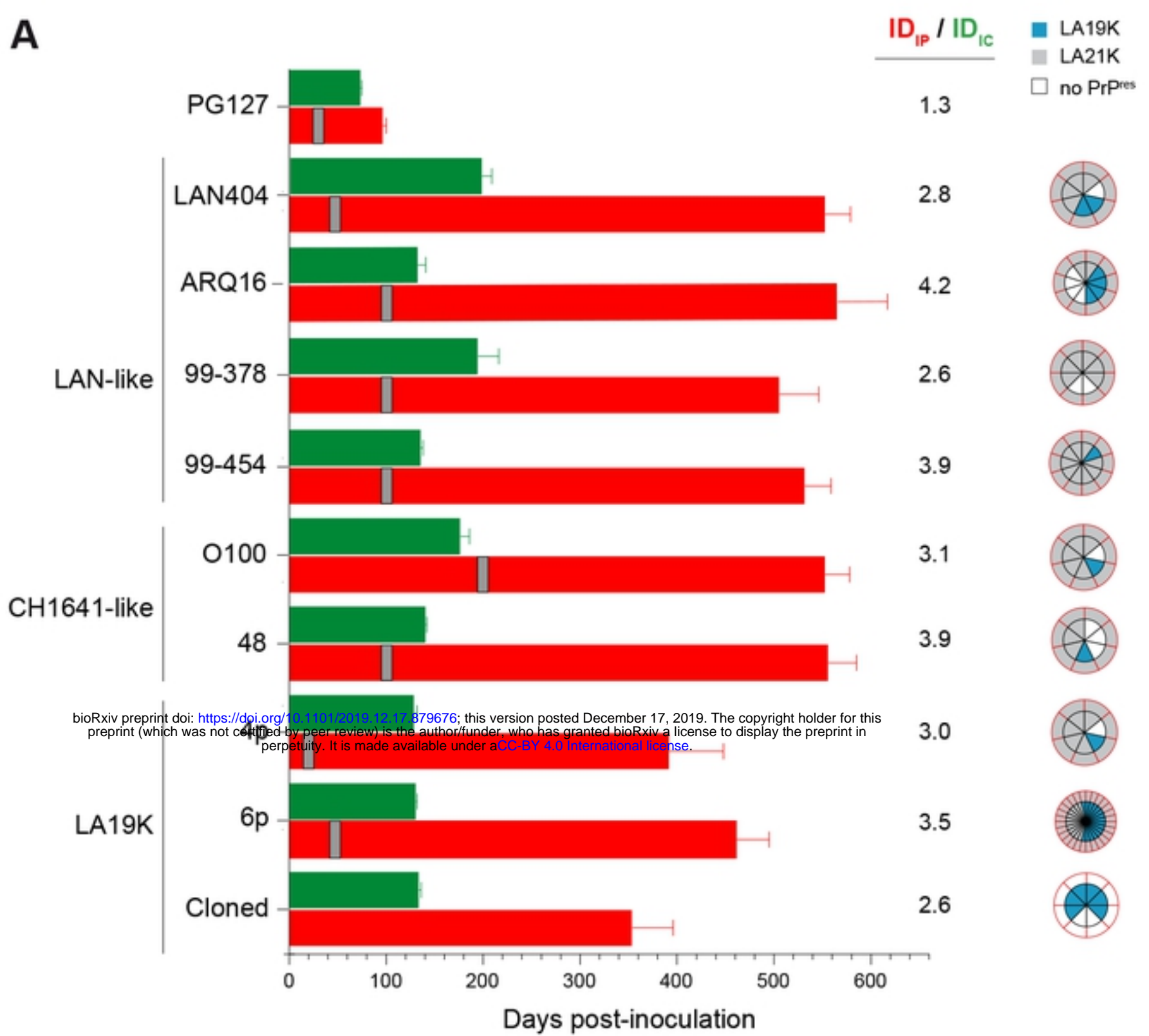


Figure 3

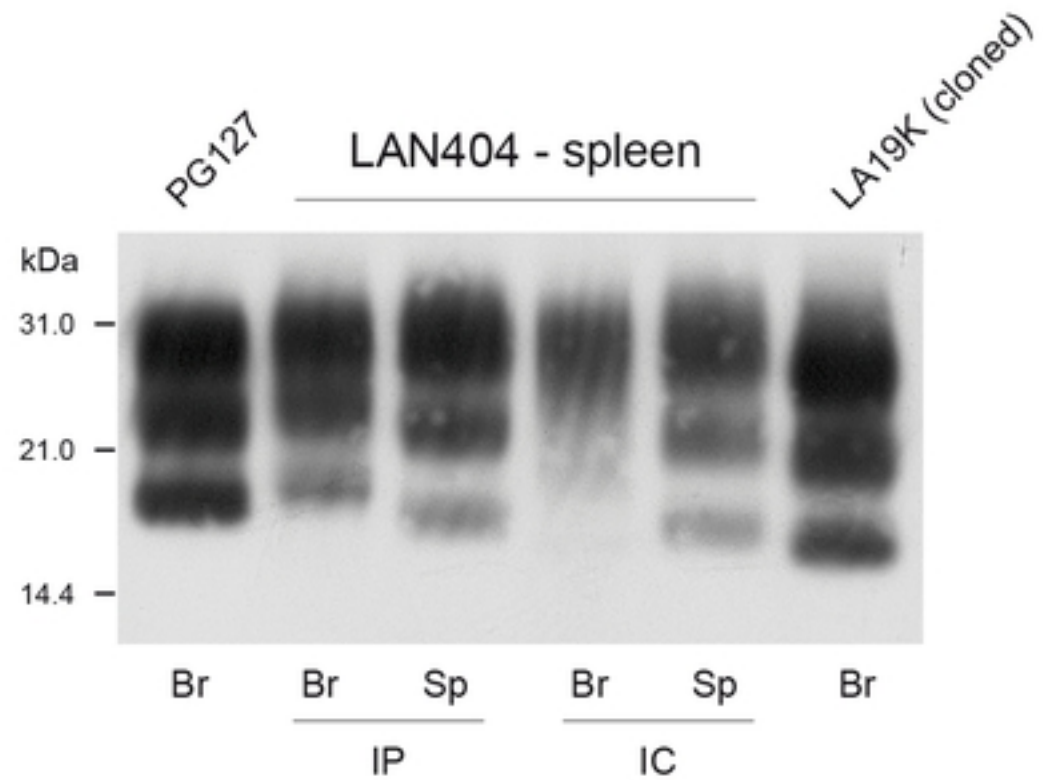
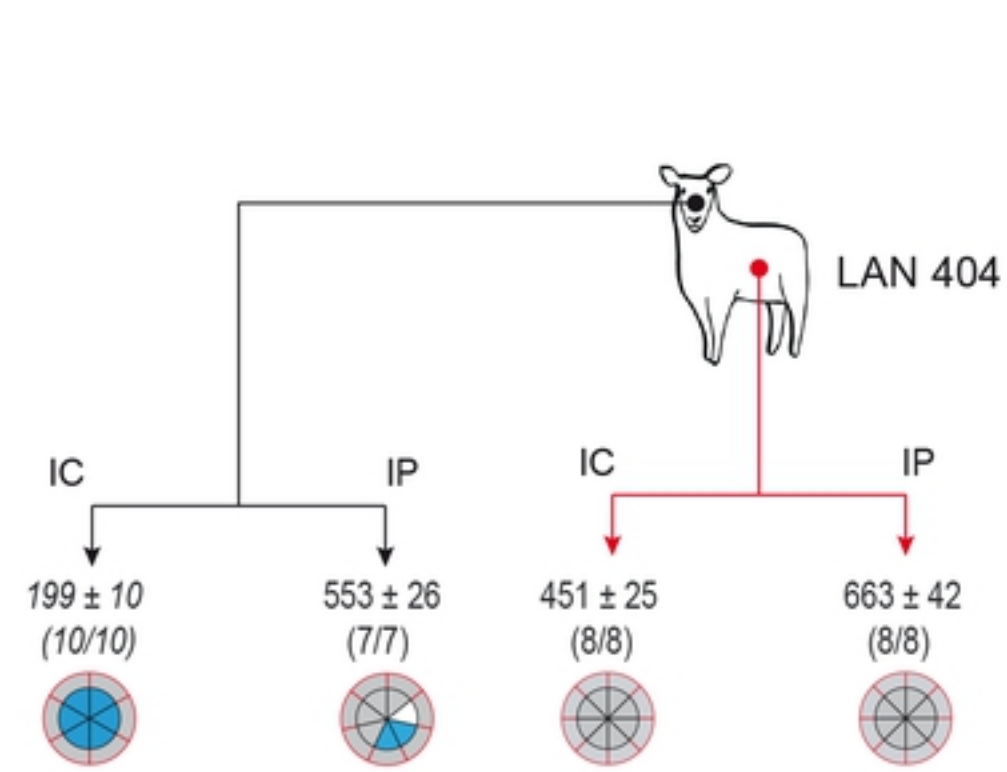


Figure 4



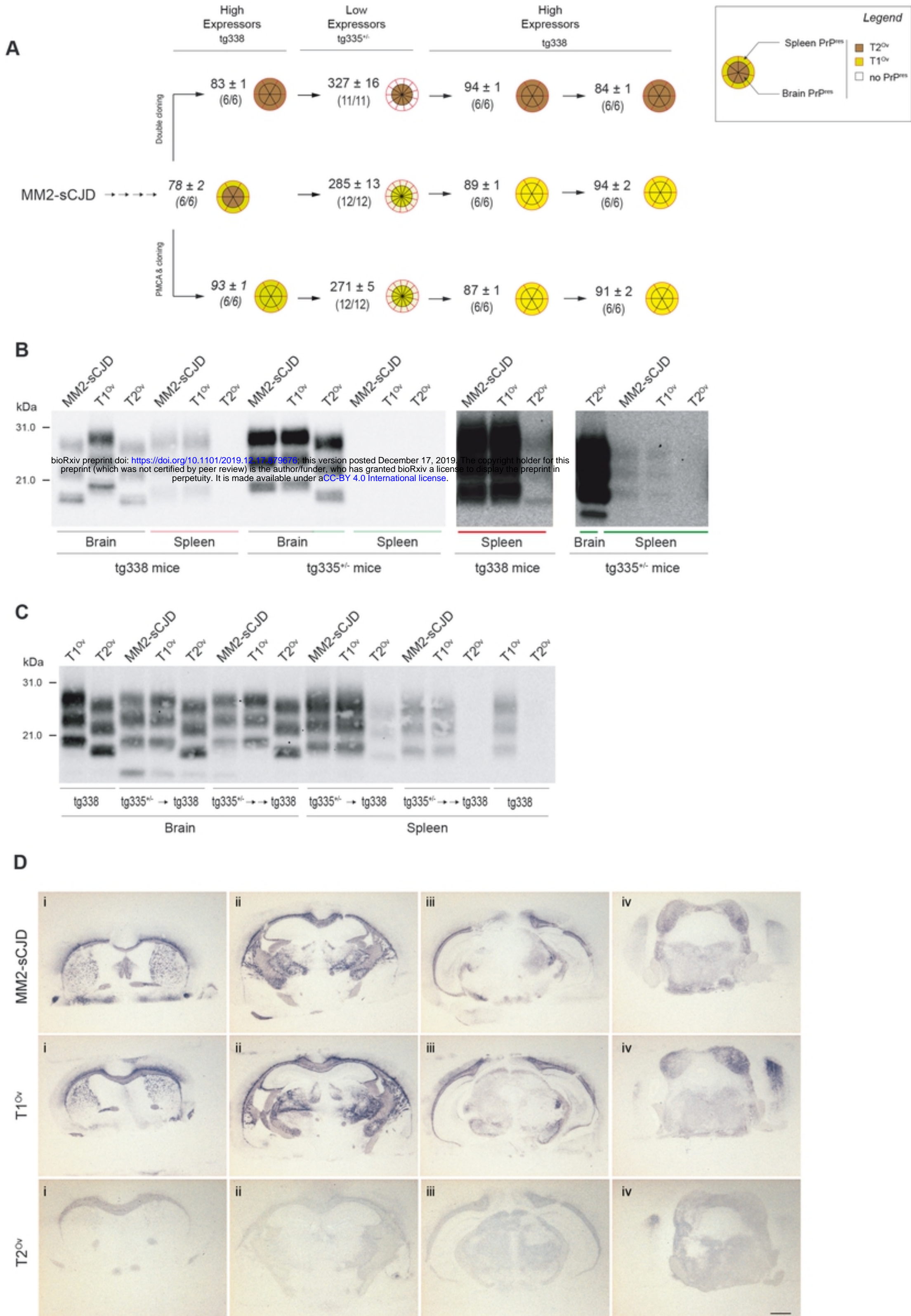


Figure 5

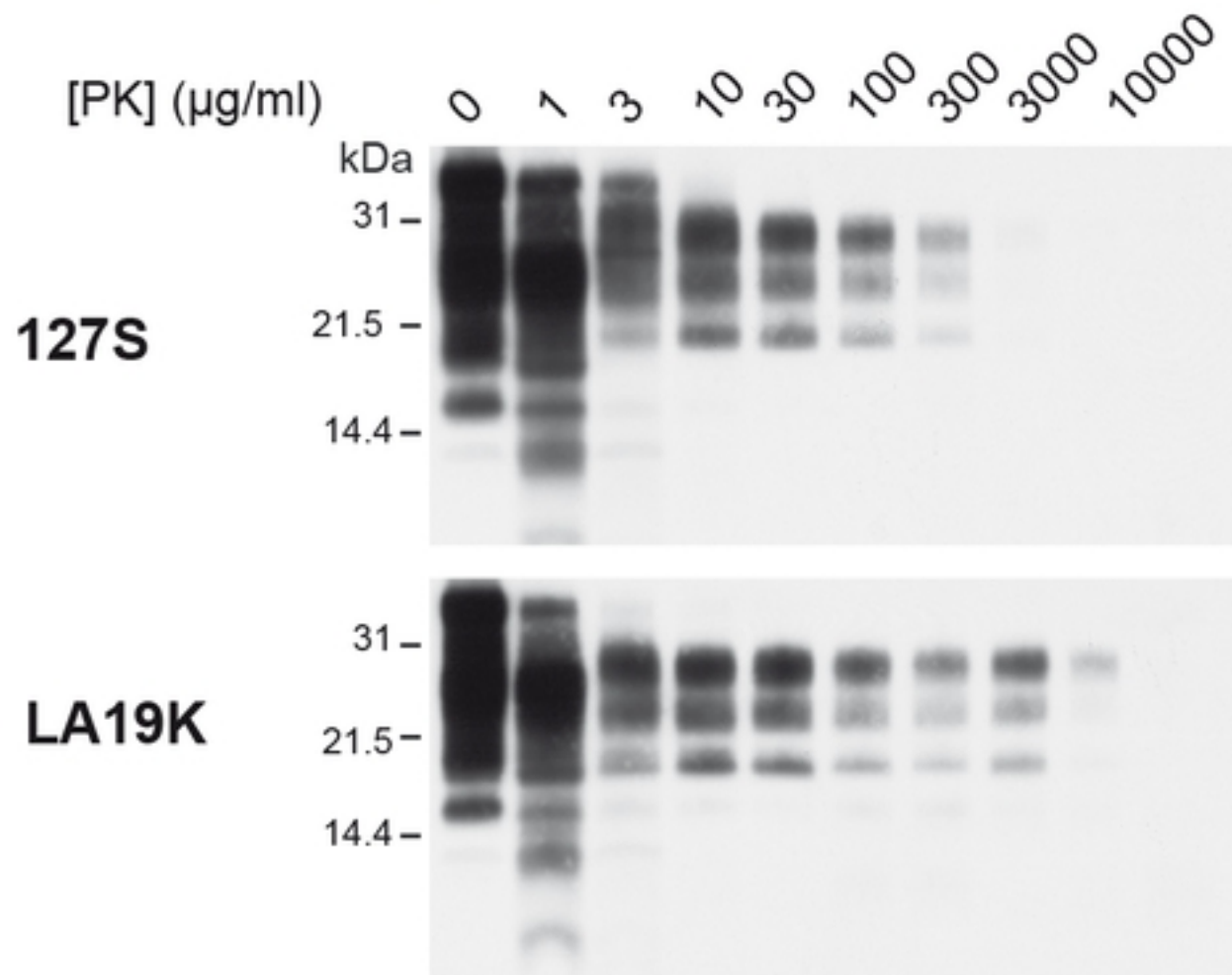
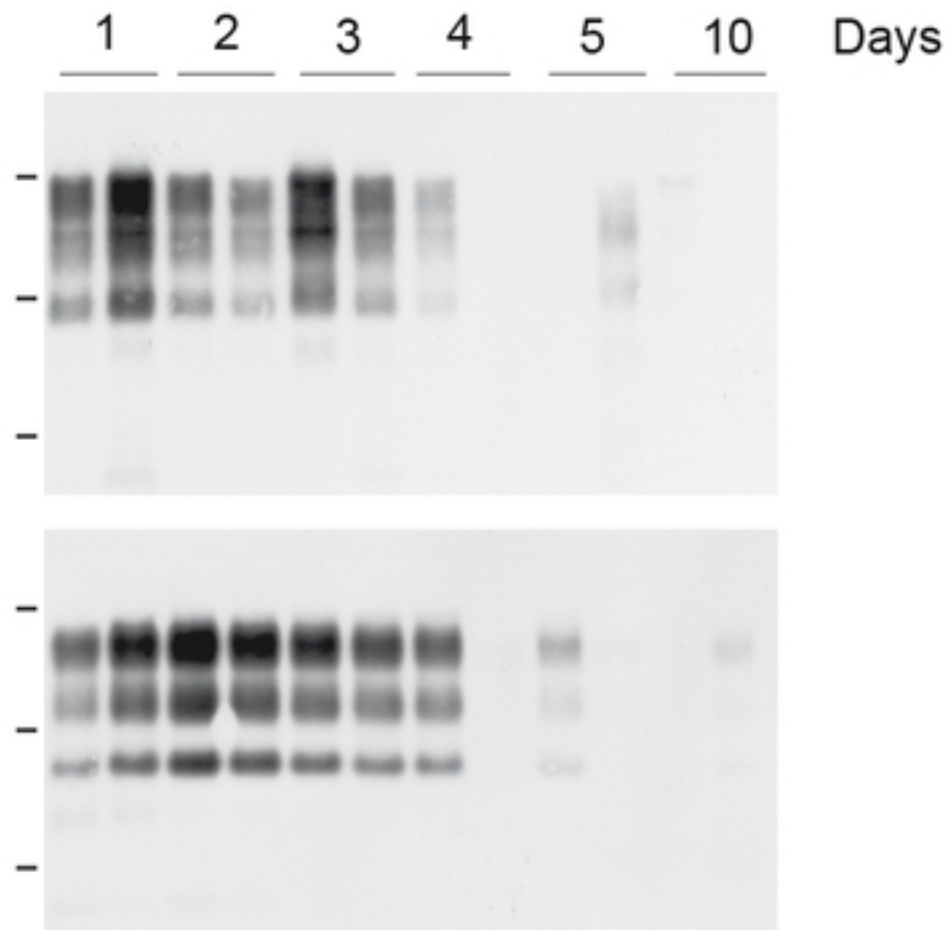
**A****B**

Figure 6