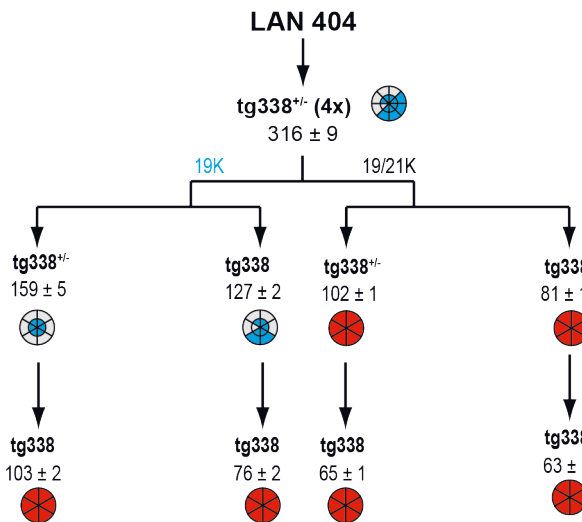


**Supplementary Figure 1. Transmission of diluted LAN404 sheep scrapie isolate to transgenic mice overexpressing ovine PrP.**

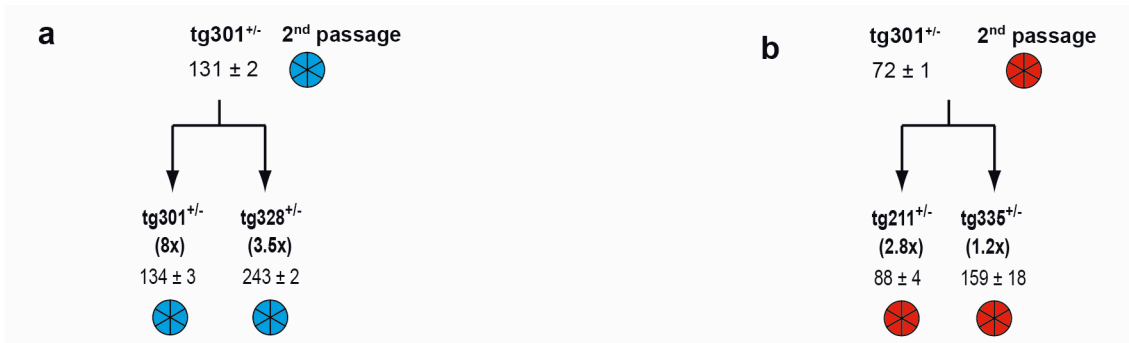
(a) Primary transmission of 1:1000 diluted LAN404 sheep scrapie isolate and subpassage to *tg338* mice (6 mice per brain inoculated). On primary transmission, all the mice showed a 21K PrP<sup>res</sup> signature in their brain. On secondary transmission, made with undiluted brain homogenate from mouse no. 4 and 7, divergent phenotypes were observed: either a short ID

typical of *LA21K fast* prions (red), or a longer ID together with 21K (grey) and mixed 21K/19K (grey + blue) PrP<sup>res</sup> profiles. (b) PrP<sup>res</sup> electrophoretic patterns at primary passage (P1) and secondary passage (P2). The 21K PrP<sup>res</sup> signatures (fragment size, glycoform ratio) are similar between P1 and P2 for mouse no.7, whereas for mouse no.4 the brains exhibit a *21K fast*-like signature at P2. Electrophoretic patterns of *LA21K fast* and *LA19K* prions serially passaged in *tg338* mice are shown as controls. (c) Representative histoblots (2<sup>nd</sup> passage) of antero-posterior coronal brain sections at the level of the septum (i), hippocampus (ii), midbrain (iii) and brainstem (iv). The pattern observed at P2 for mouse no.4 is specific of *LA21K fast*, with prominent PrP<sup>res</sup> deposition in the corpus callosum, lateral hypothalamic nuclei and raphe nuclei of the brain stem. The pattern observed at P2 for mouse no.7 is different, with plaque-like PrP<sup>res</sup> deposits scattered in the corpus callosum, the dorsal thalamus, the optic tract, the periaqueductal area, the tegmental nuclei and the cerebellum. Scale bar = 1 mm.



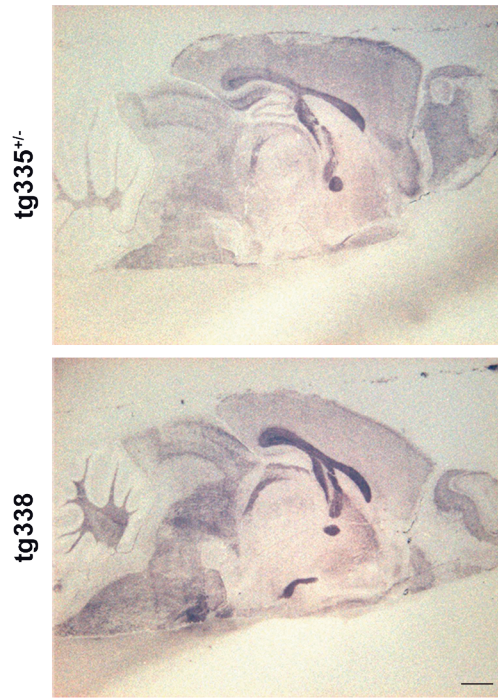
**Supplementary Figure 2. Serial transmission of LAN404 scrapie isolate to mice expressing ovine PrP at intermediate levels.**

Transmission to middle expresser  $tg338^{+/-}$  mice and subpassage to the same line or to high expresser mice ( $tg338$ ). Segmented circles are used to indicate the proportion of mice with  $LA19K$  (blue),  $LA21K$  (grey) or  $21K$  fast (red) PrP<sup>res</sup> profiles in their brains. A double circle is used to indicate the proportion of mice with mixed signature. Middle expresser mice allow silent propagation of  $LA19K$  agent for at least two subpassages.



**Supplementary Figure 3. Transmission of *LA19K* and *LA21K fast* prions to mice expressing ovine PrP at middle or low levels.**

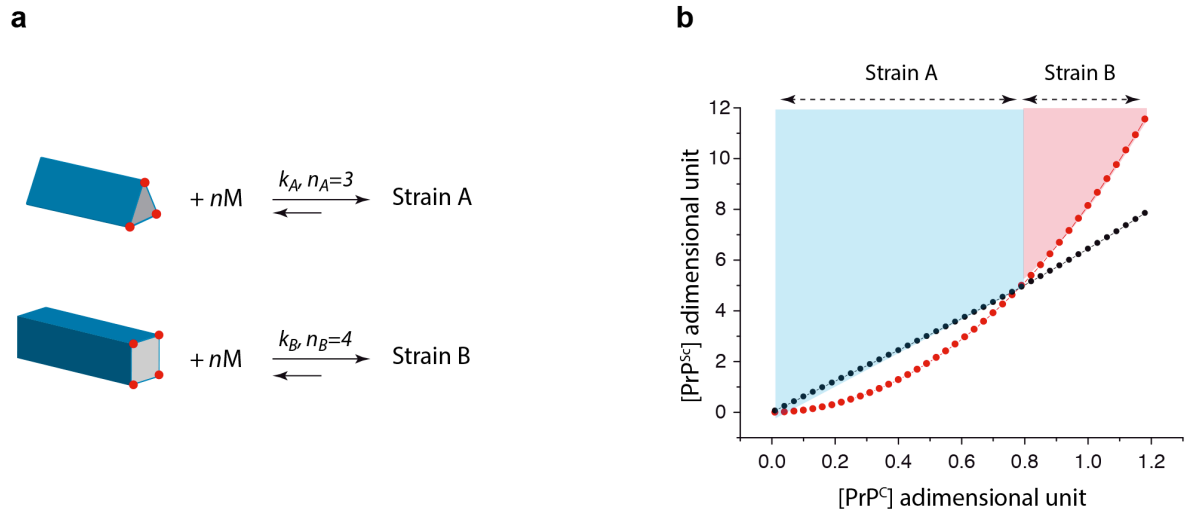
*LA19K* (blue) and *LA21K fast* (red) prions at the second passage on *tg301*<sup>+/-</sup> (see **Fig. 1a**) were further transmitted to lines expressing PrP<sup>C</sup> at lower levels, as indicated. Segmented circles are used to indicate the proportion of mice with *LA19K* (blue) or *LA21K fast* (red) PrP<sup>res</sup> profiles in their brains. Both *LA19K* and *LA21K fast* prions can be faithfully propagated in low or middle expresser mice.



**Supplementary Figure 4. PrP<sup>C</sup> expression pattern in low and high expresser PrP mice.**

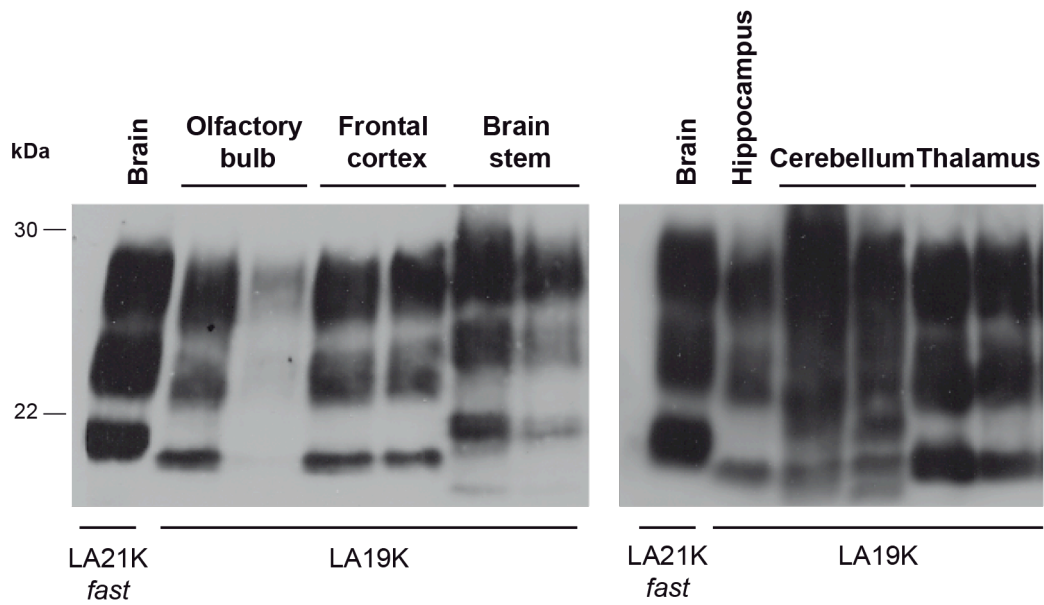
Histoblots of representative sagittal sections from *tg335<sup>+/-</sup>* mice and *tg338* mice.

Scale bar = 1 mm.



**Supplementary Figure 5. Modelling strain-dependent polymerization rate as a function of PrP<sup>C</sup> levels.**

**(a)** Kinetic scheme of the conversion process of substrains A and B with two different kinetic order ( $n_A = 3$  and  $n_B = 4$ ).  $k_A$  and  $k_B$  correspond to conversion kinetics rate constants. **(b)** The simulation of PrP<sup>Sc</sup> accumulation for substrains A and B as a function of PrP<sup>C</sup> concentration reveals that below certain amount of PrP<sup>C</sup>, strain A is the dominant population whereas at high PrP<sup>C</sup> concentration strain B is the dominant population. For more details see **Supplementary Note 1**.



**Supplementary Figure 6. PrP<sup>res</sup> glycopattern in different mouse brain regions of *tg338* mice inoculated with an uncloned LA19K prion source (3<sup>rd</sup> passage in *tg338* mice).**

The brain stem of the infected mice was enriched in PrP<sup>res</sup> species with a 21K signature, and prominent proportion of diglycosylated species as compared to *LA21K fast* (shown as control). A mixed 21K/19K profile was observed in the cerebellum.

**Supplementary Table 1: Duration of disease in mice expressing PrP<sup>C</sup> at variable levels, inoculated with LAN404 sheep brain or *tg338*-derived LAN404 prions (2<sup>nd</sup> passage, 19K PrP<sup>Sc</sup> signature).**

Transgenic mouse line	PrP <sup>C</sup> level*	Days to death		Difference
		<i>tg338</i> -LA19K	LAN404	
<i>tg338</i> <sup>+/-</sup>	4	175	316	-141
<i>tg328</i> <sup>+/-</sup>	3.5	242	314	-72
<i>tg211</i> <sup>+/-</sup>	2.8	258	341	-83
<i>tg143</i> <sup>+/-</sup>	1.5	327	351	-24
<i>tg335</i> <sup>+/-</sup>	1.2	444	424	+20

\*Expressed as x-fold that in sheep brain



## Supplementary Note 1. Kinetic simulation of substrain selection as a function of PrP<sup>C</sup> expression level.

In order to simulate the outcome of LAN404 isolate transmission to transgenic mice expressing PrP<sup>C</sup> at different, levels, we assumed the following:

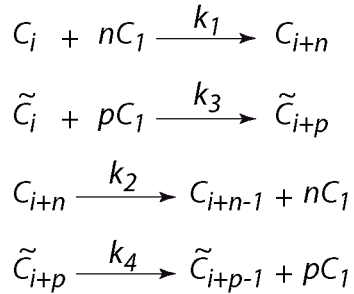
- 1- Two substrains A and B exist in the same isolate.
- 2- The kinetic order of the templating process for each substrain is different. The replication of substrain A occurs by the integration of three PrP<sup>C</sup> molecules (n=3) when for substrain B, four molecules are required (n=4). This replication mode contrasts with the generic model proposed by Weissman and colleagues for Sup35, for which the templating occurs through the integration/conversion of one monomer<sup>1</sup>. Recent developments in our group strongly suggest that mammalian prion assemblies are constituted of oligomeric, elementary bricks. As an example, for 263K hamster prions, this elementary brick would correspond to a trimer (Igel-Egalon et al., to be published).
- 3- The conversion rate remains constant during the evolution of the pathology
- 4- The fragmentation rate is not considered in order to have a constant conversion process.
- 5- PrP<sup>C</sup> expression level does not vary (overexpressing mice) or to a limited extent during the early phase of replication<sup>2</sup>.

Then we defined  $C_i$  and  $\bar{C}_i$  respectively as the concentration of substrain A and B.  $i$  defines the size of assemblies as was defined in the simulation as adimensional parameter.

Kinetics constants used for simulations are :

$$\begin{array}{lll} k1 = 100 \text{ M}^{-3}\text{s}^{-1} & k2 = 0.16 \text{ s}^{-1} & n=3 \\ k3 = 1.05 \text{ M}^{-4}\text{s}^{-1} & k4= 0.01 \text{ s}^{-1} & p=4 \end{array}$$

Simulations were run using ODE 15s from MATLAB routine for a concentration range of  $10^{-8}$  to  $10^{-6}$  as  $\mu\text{M}$ . Results were reported as additional variable. Phase space were analysed using Jacobin and Rout-Hurwitz criteria using a homemade MATHLAB code.



$$\frac{dC_{i+n}}{dt} = k_1 C_i C_1^n - k_2 C_{i+n}$$

$$\frac{d\tilde{C}_{i+p}}{dt} = k_3 \tilde{C}_i C_1^p - k_4 \tilde{C}_{i+p}$$

$$\frac{dC_1}{dt} = k_2 C_{i+n} + k_4 \tilde{C}_{i+p} - (k_1 C_i C_1^n + k_3 \tilde{C}_i C_1^p) + U$$

## **References**

- 1 Collins, S. R., Douglass, A., Vale, R. D. & Weissman, J. S. Mechanism of prion propagation: amyloid growth occurs by monomer addition. *PLoS Biol* **2**, e321, doi:10.1371/journal.pbio.0020321 (2004).
- 2 Mays, C. E. *et al.* Prion Infectivity Plateaus and Conversion to Symptomatic Disease Originate from Falling Precursor Levels and Increased Levels of Oligomeric PrPSc Species. *J Virol* **89**, 12418-12426, doi:10.1128/JVI.02142-15 (2015).