

# Supporting Online Material for

## **Lentivector-mediated RNAi efficiently suppresses PrP and prolongs survival of scrapie-infected mice**

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### **Supplemental Figure 1**

Analysis of LVsh512 infected cells.

(A) N2a cells were infected with a MOI of 10. Western blot showing passages 1, 3 and 5 of LVshscr or LVsh512 transduced cells. (B) Fluorescence imaging of primary murine cerebellar granule cells infected with LVsh512. (C) Western blot analysis of PrP<sup>Sc</sup> accumulation in ScN2a cells 7 days after infection with the indicated lentivectors. Control, uninfected cells.

### **Supplemental Figure 2**

Transduction of murine ES cells with LVsh512.

(A) Schematic representation of lentivectors used. The restriction site of *Bam*HI and the DNA probe used for Southern blot analysis are indicated. (B) Southern blot analysis of LVEGFP (GFP.x) and LVsh512 (512.x) transduced ES cell clones. Representative cell clones analyzed by Western Blot in Fig. 2A are indicated by arrows.

### **Supplemental Figure 3**

Analysis of EGFP expression in the CNS of chimeric mouse #1917.

(A) Sagittal overview of the mouse brain [Paxinos, 2001 #35] (left). Sections through cortex, hippocampus and cerebellum are indicated by 1, 2 and 3, respectively. Immunohistological analysis of the indicated sections of a transgenic (tg, 90% chimeric mouse #1917) and a wild-type (wt) brain using anti-EGFP antibodies (right). (B) Quantification of transgenic (EGFP+) cells in the posterior cerebrum (mean  $\pm$  SD) of a 60% chimeric mouse (#1936) determined by immunohistochemical stainings. Neurons were identified by staining with anti-NF200 antibodies (NF200+); NF200+/EGFP+, EGFP-positive neurons. (C) Number of transgenic cells in cerebrum and cerebellum of two chimeric mice (40% and 60%, judged by coat color

chimerism) measured by real-time duplex PCR. A transgenic animal (carrying the LVEGFP vector) was taken as control (100%).

**Figure 4: Relation between chimerism and survival time of scrapie infected mice.**

Survival times of RML scrapie infected mice (derived from LVsh512 or GFP3 transduced ES cells) are plotted against the degree of chimerism (percentage of agouti coat color).

**Supplemental Table 1**

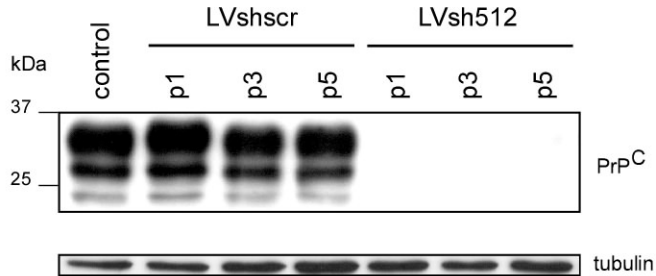
Sequences of shRNAs used for cloning into LVshPrPC

**Supplemental Table 2**

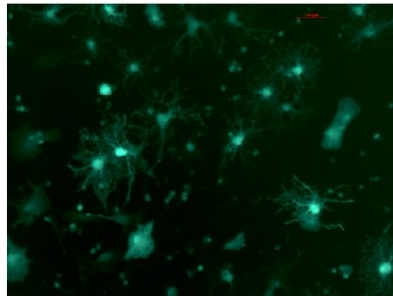
Chimerism, mean survival times and neuropathological changes in scrapie infected mice

Supplemental Figure 1, Pfeifer et al.

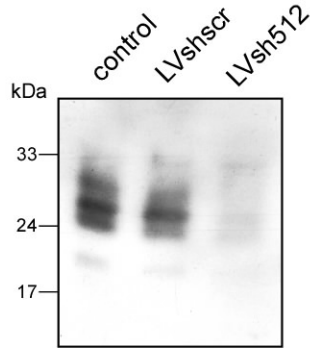
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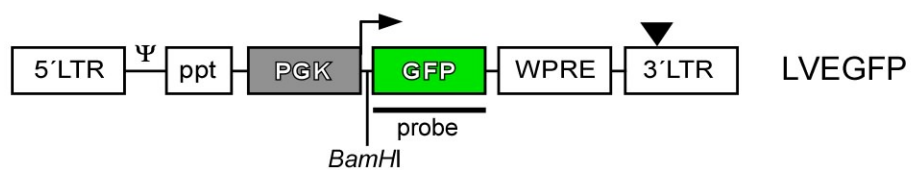
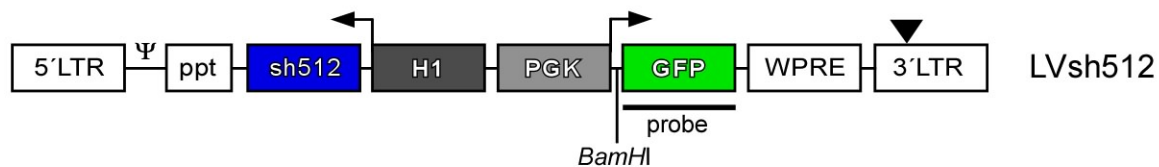
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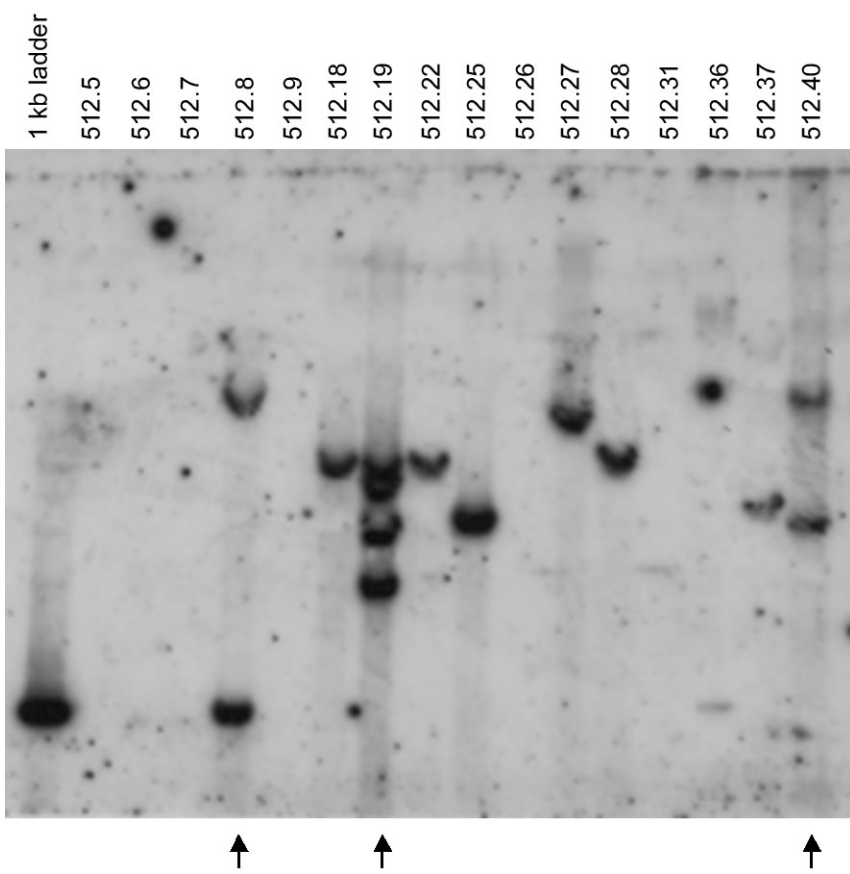
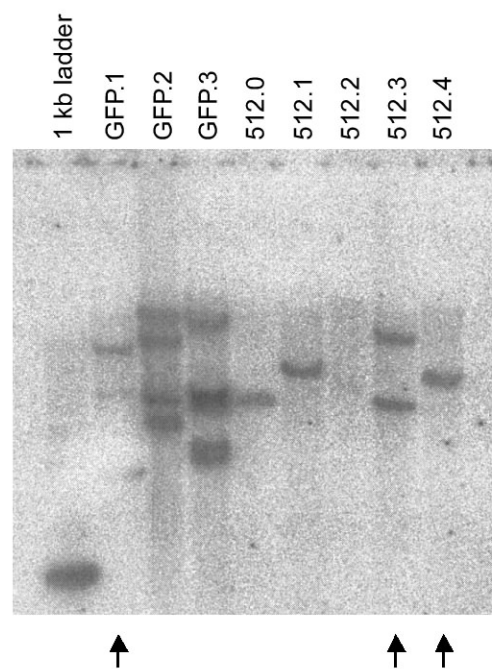
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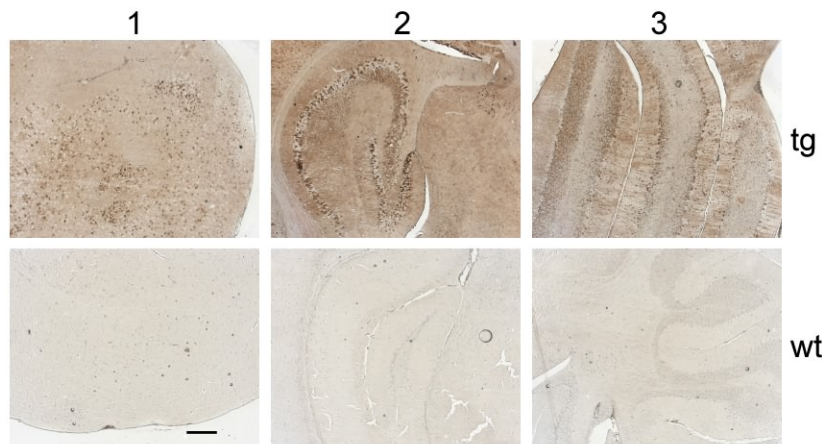
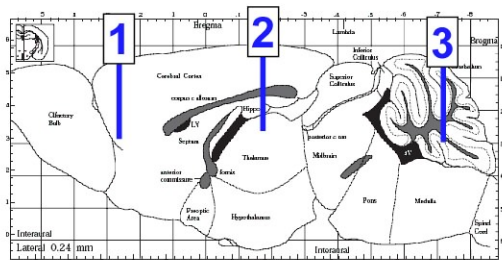
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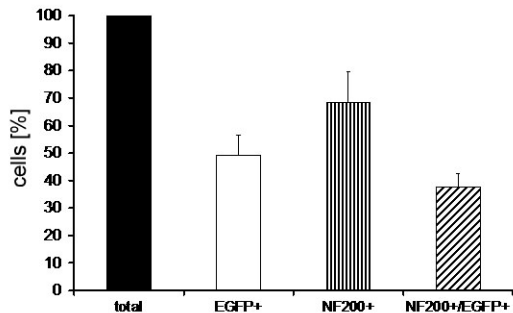
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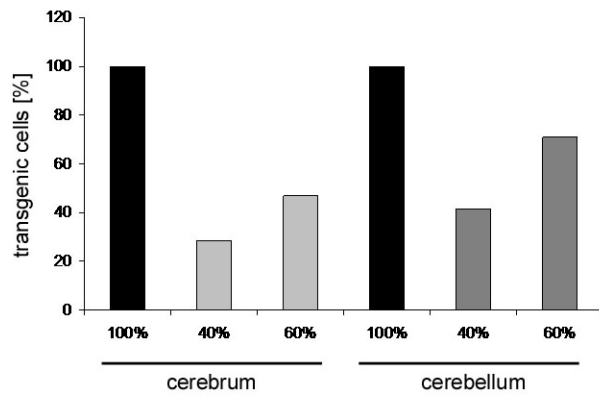
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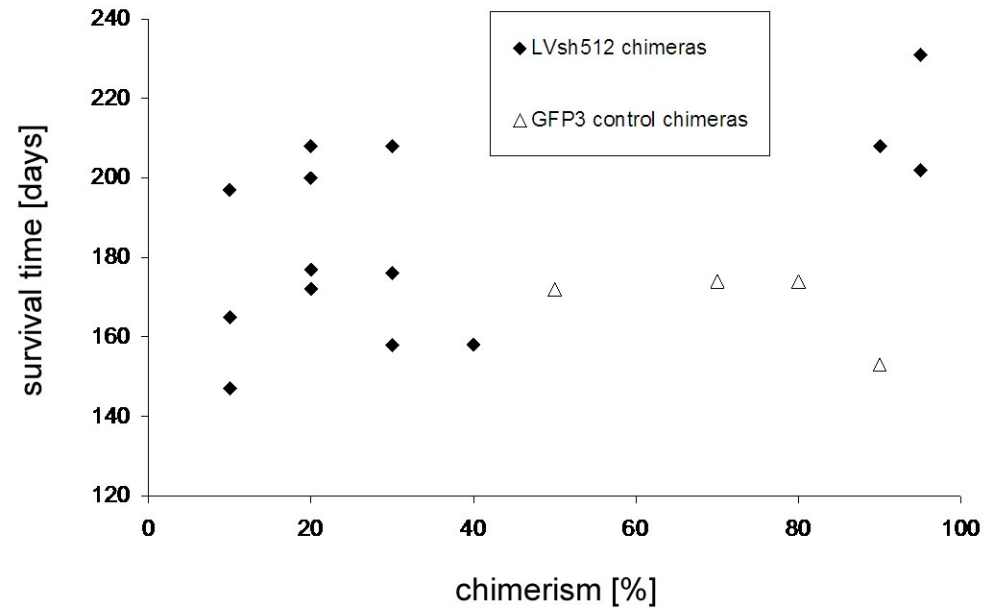
B



C



Supplemental Figure 4, Pfeifer et al.



**Supplemental Table 1**

Sequences of shRNAs used for cloning into LVshPrPC

shRNA	Oligonucleotide Sequence (5' → 3')
512	sense <u>loop</u> antisense <i>termination-signal</i> AGAACAACCTTCGTGCACGACTTTCAAGAGAAGTCGTGCACGAAGTTGTTCTTTTT
455	sense <u>loop</u> antisense <i>termination-signal</i> ACAAGCCCAGCAAACCAAATTCAAGAGATTTGGTTTGCTGGGCTTGTTTTT
482	sense <u>loop</u> antisense <i>termination-signal</i> ACTACAGGCCAGTGGATCAGTTTCAAGAGAACTGATCCACTGGCCTGTAGTTTTT
521	sense <u>loop</u> antisense <i>termination-signal</i> GGGCTTGGTGGCTACATGCTTTCAAGAGAAGCATGTAGCCACCAAGGCCCTTTTT
739	antisense <u>loop</u> sense <i>termination-signal</i> CATCGGTCTCGGTGAAGTTCTTTGATATCCGAGAACTTCACCGAGACCGATGTTTTT
796	sense <u>loop</u> antisense <i>termination-signal</i> TCACCCAGTACCAGAAGGAGTTTCAAGAGAACTCCTTCTGGTACTGGGTGATTTTT
scr	CGAACAATCAATGCGTGCCTATTCAAGAGATAGGCACGCATTGATTGTTTCGTTTTT



## Supplemental Table 2

Chimerism, mean survival times, and neuropathological changes in scrapie infected mice

Mouse type	Chimerism (%)	Mean survival time (dpi)	Scoring for gliosis (1, low - 3, high)					Scoring for spongiformity (1, low - 3, high)				
			Cerebellum	Brain stem	Basal Ganglia	Hippo-campus	Cortex	Cerebellum	Brain stem	Basal Ganglia	Hippo-campus	Cortex
129sv	-	165	2	2	2	2,5	1,5	3	3	2	1	2
129sv	-	167	2	1	2	3	2	3	3	2	1	3
129sv	-	169	3	3	n.d.	3	2	3	3	2	2	3
C57x129sv	-	166	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C57x129sv	-	161	3	3	2	3	1	3	3	2	3	2
C57x129sv	-	156	2	3	n.d.	3	2	3	3	n.d.	2	2
C57x129sv	-	169	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
GFP3	70	174	2	1	2	2,5	2	1	1	1	1	1
GFP3	90	153	2	2	3	3	3	2	3	1	1	2
GFP3	50	172	n.d.	n.d.	3	3	2,5	n.d.	n.d.	1	1	1
GFP3	80	174	2	2	1	2,5	1	1	1	1	1	1
512.19	10	147	1	n.d.	2	2	1	1	2	1	1	1
512.19	10	165	1	2	2	1,5	1	1	1	2	1	1
512.19	10	197	1,5	2	2	2	1,5	2	2	2	2	2
512.4	20	172	2	3	2	3	2	2	3	1	2	1
512.8	20	177	3	3	2,5	3	2,5	2	2	1	1	1
512.3	20	200	2	3	3	3	2	1	2	1	1	2
512.19	20	208	2,5	2	3	3	3	1	1	1	1	1
512.19	30	158	1,5	2,5	2	1	1,5	2	1	1	1	1
512.8	30	176	3	2	2,5	3	2	2	2	1	1	1
512.19	30	208	2	3	2	3	2,5	2	1	2	1	1
512.8	40	158	2	3	2,5	3	2	2	2	2	1	1
512.4	95	202	1	2	3	2	2	2	2	1	2	1
512.19	90	208	2,5	2,5	2	2	2	1	1	1	1	1
512.8	95	231	3	1,5	3	2,5	3	3	3	2	1	2