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Supporting Material

Ultramicroscopy Reveals Axonal Transport Impairments in Cortical Motor Neurons at Prion Disease

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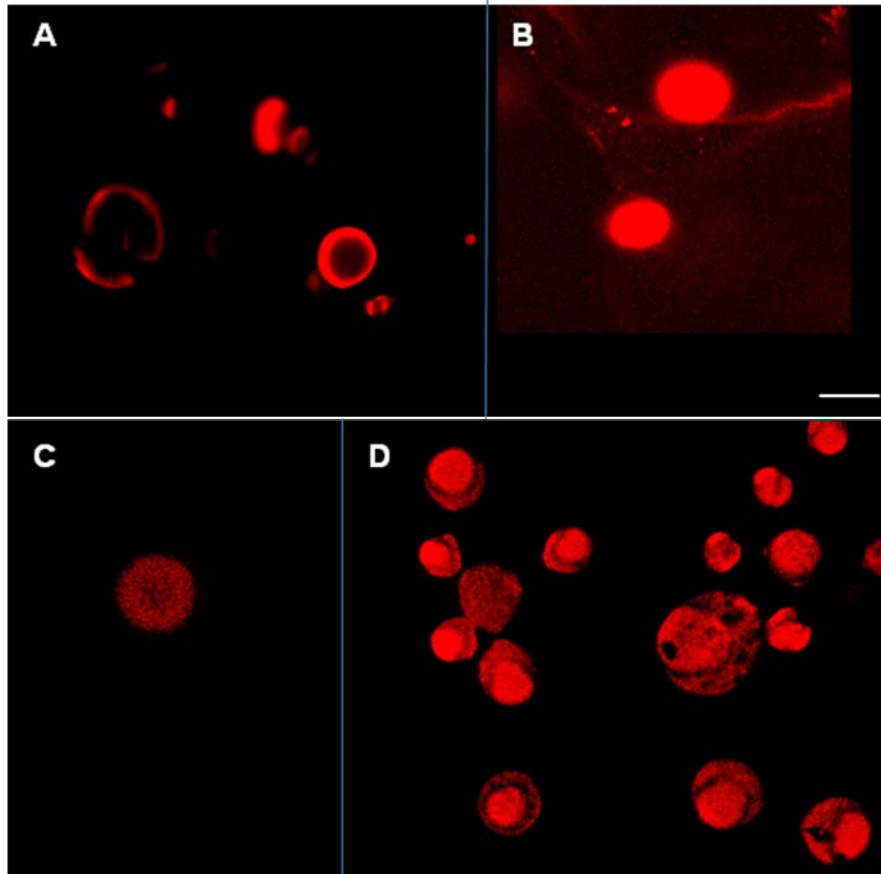
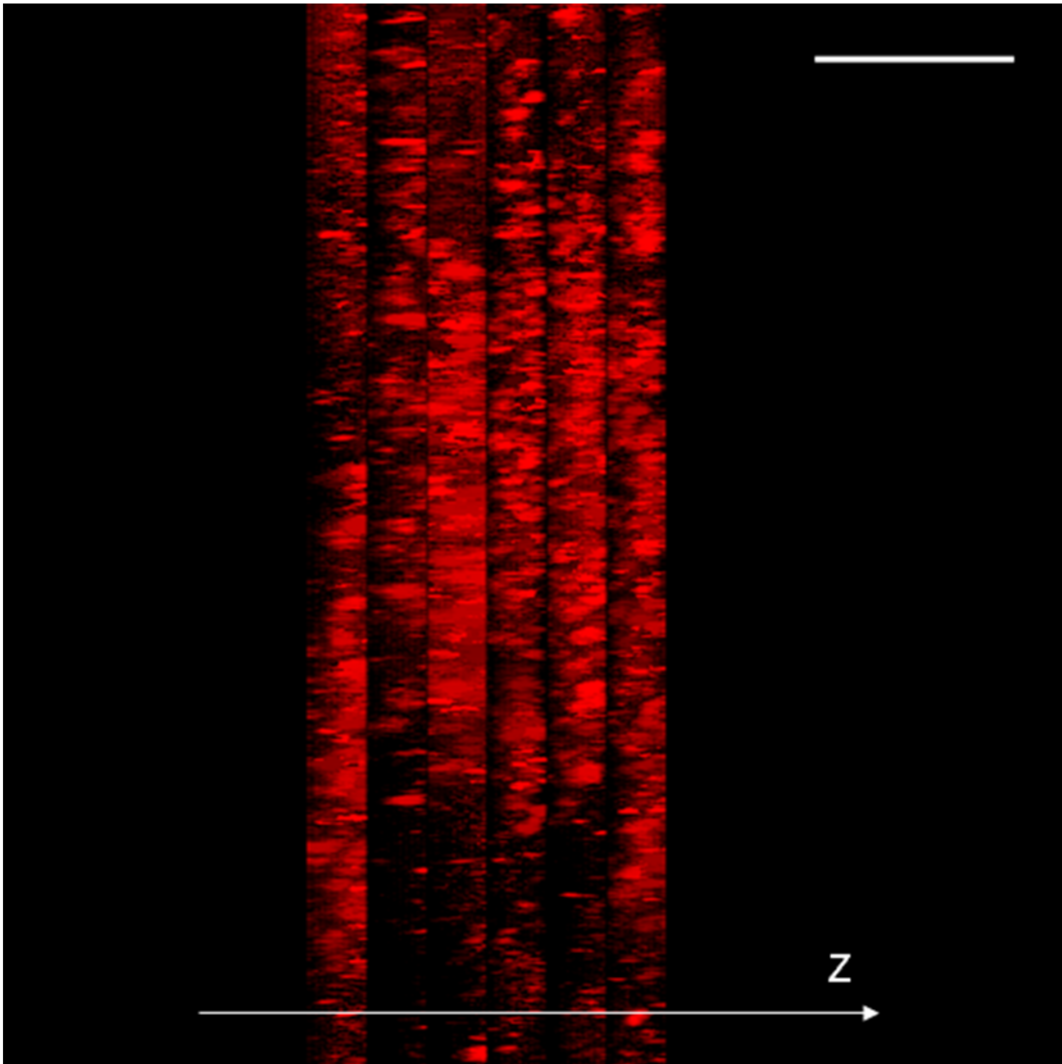
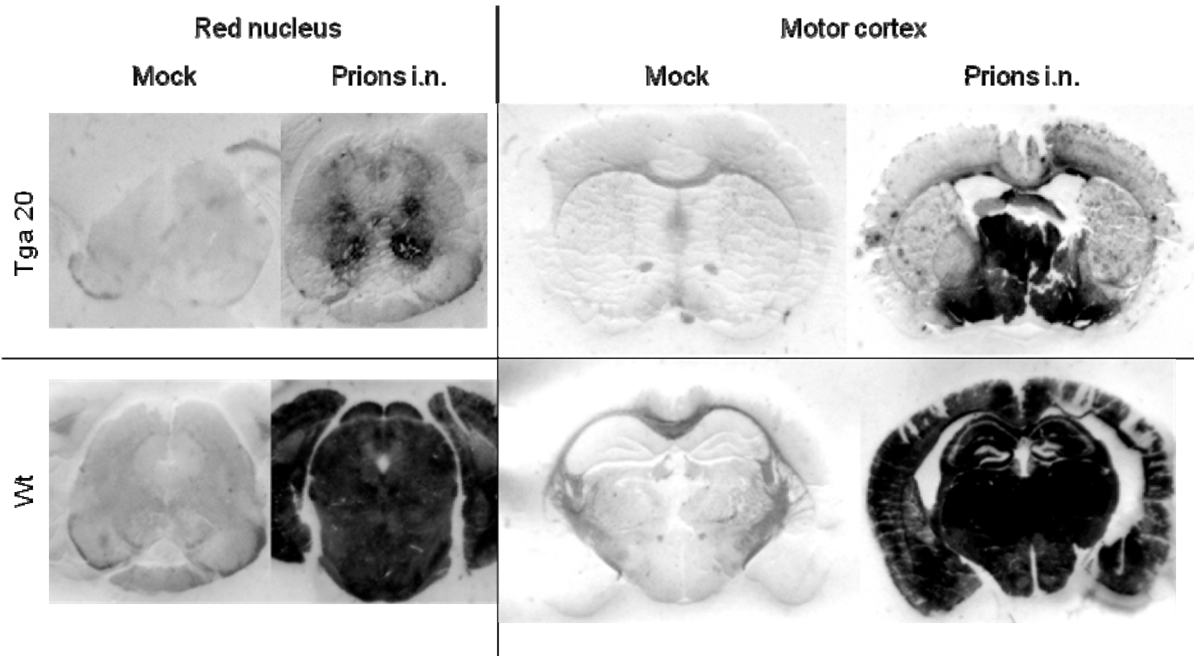


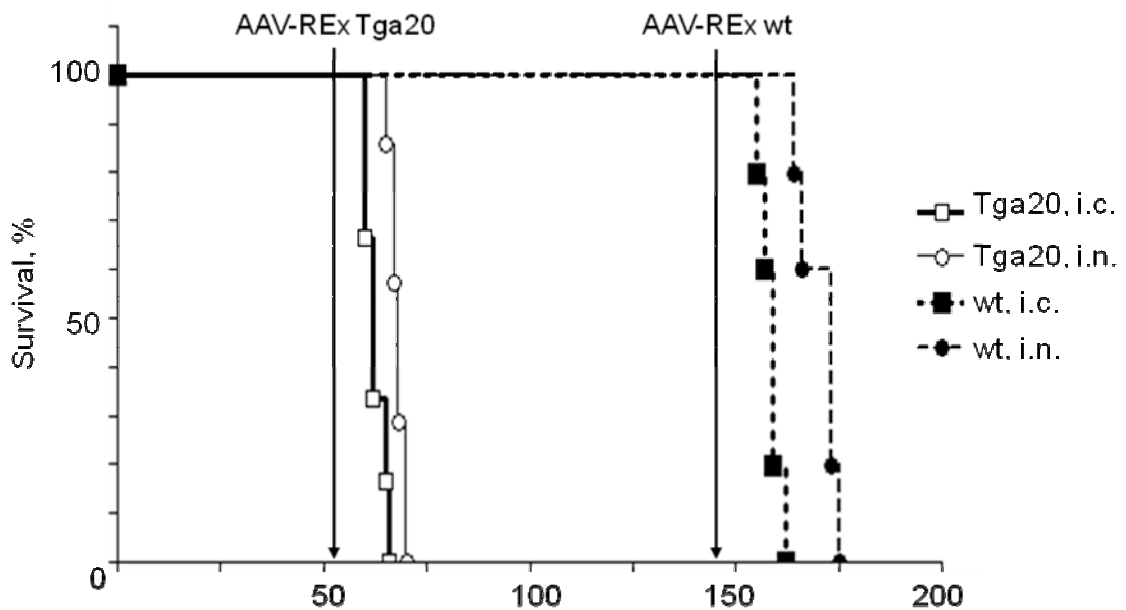
Supplemental Figure 1. Agarose beads coated with DsRed and imaged using ultramicroscopy (A), multiphoton imaging (B) and confocal microscopy, immobilized in agarose (C) or on the glass slide (D). The fluorescence intensity was the same for the confocal and multiphoton imaging, but 1.5-fold higher for ultramicroscopy. Scale bar: 50µm.



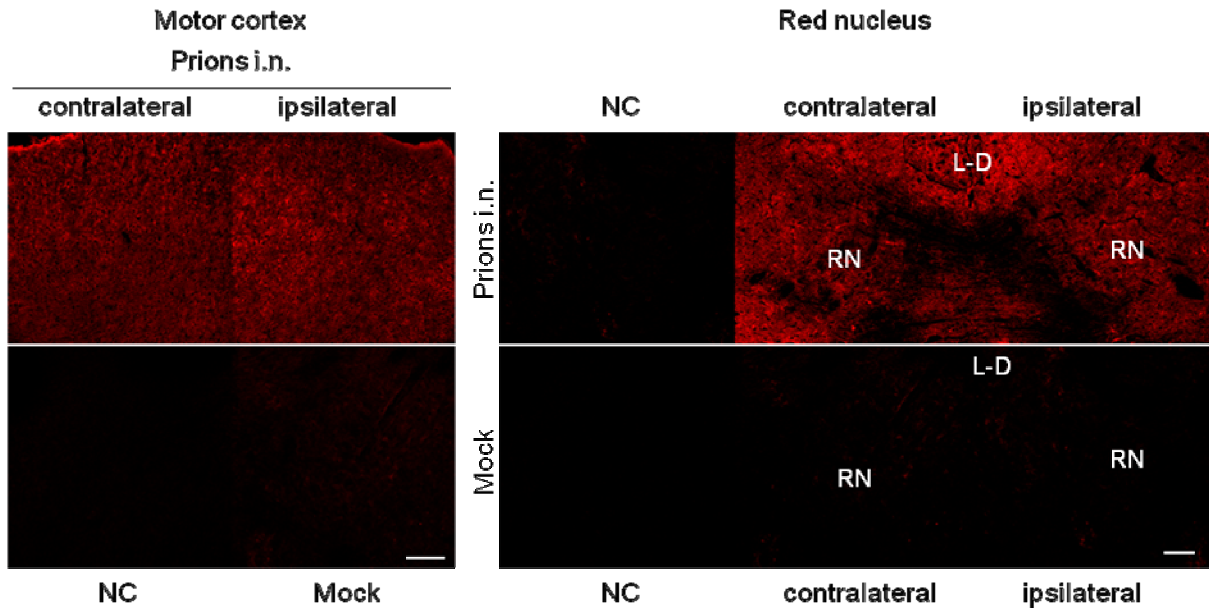
Supplemental Figure 2. Three-dimensional reconstruction done on six confocal stacks from 20-µm-thick cryo-sections using Volocity software package. Different cryo-sections demonstrate typical changes of imaging quality on the edges of each section. Since the sections are photographed independently, it is impossible to position all them to obtain continious stacks. These drawbacks seriously compromise possible 3D reconstructions.



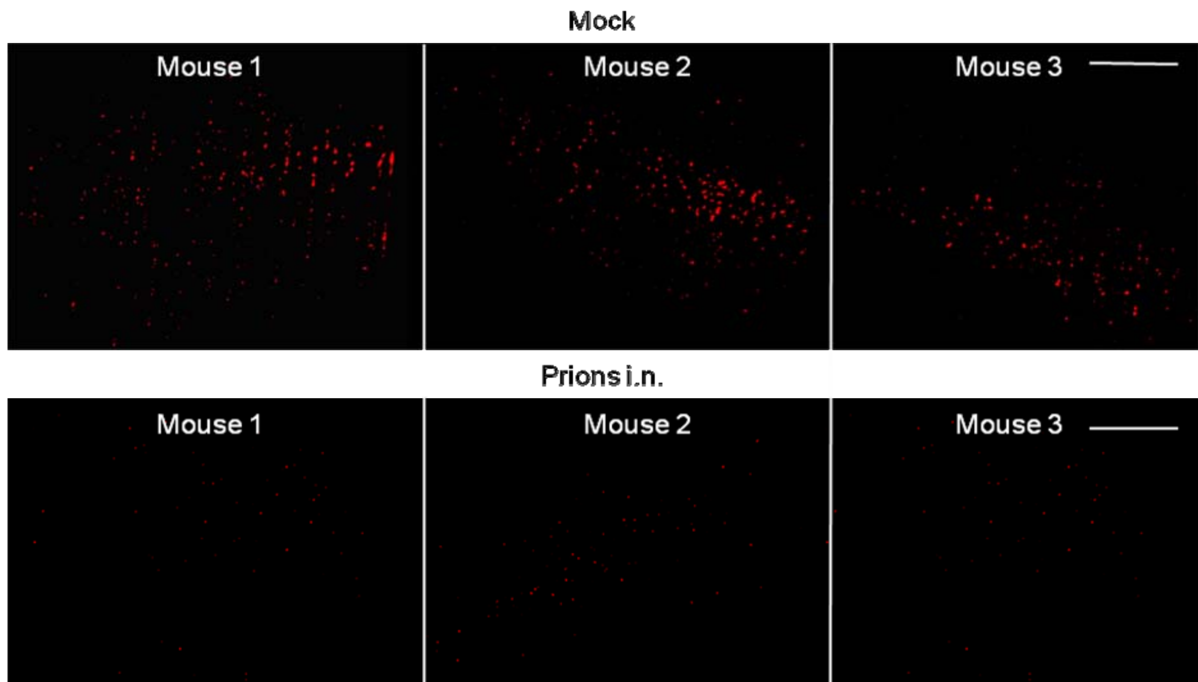
Supplemental Figure 3. Pet-Blot showing the PrP^{Sc} accumulation in proteinase-K treated brains of terminally ill Tga20 (at 67 days post infection, dpi) and wild-type (at 167 dpi) mice upon prion challenge into the sciatic nerve (prions i.n.). Mock controls were also treated with proteinase-K.



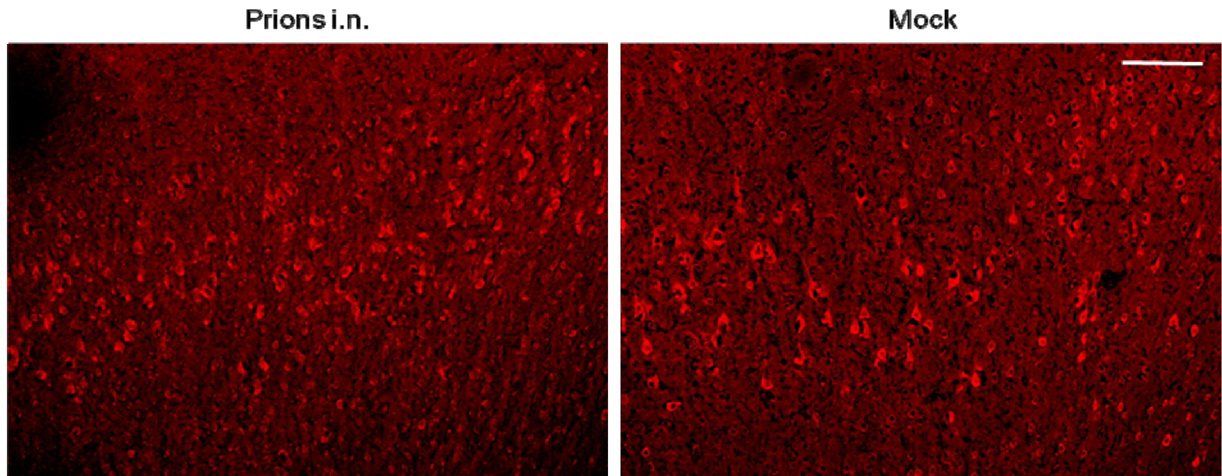
Supplemental Figure 4. Survival of Tga20 and wild-type mice upon prion inoculation into the sciatic nerve (i.n.) and, as a reference, intracerebrally (i.c.). The incubation times were: 67.9±1.8 dpi (Tga20, i.n., n/n0=7/7); 62.5±2.5 dpi (Tga20, i.c., n/n0=6/6); 168.8±5.5 dpi (wild-type, i.n., n/n0=6/6); 157.5±1.9 dpi (wild-type, i.c., n/n0=4/4). All values are: Mean±Standard Deviation. Arrows: time points of tracer application (54 dpi and 136 dpi for Tga20 and wild-type, respectively.



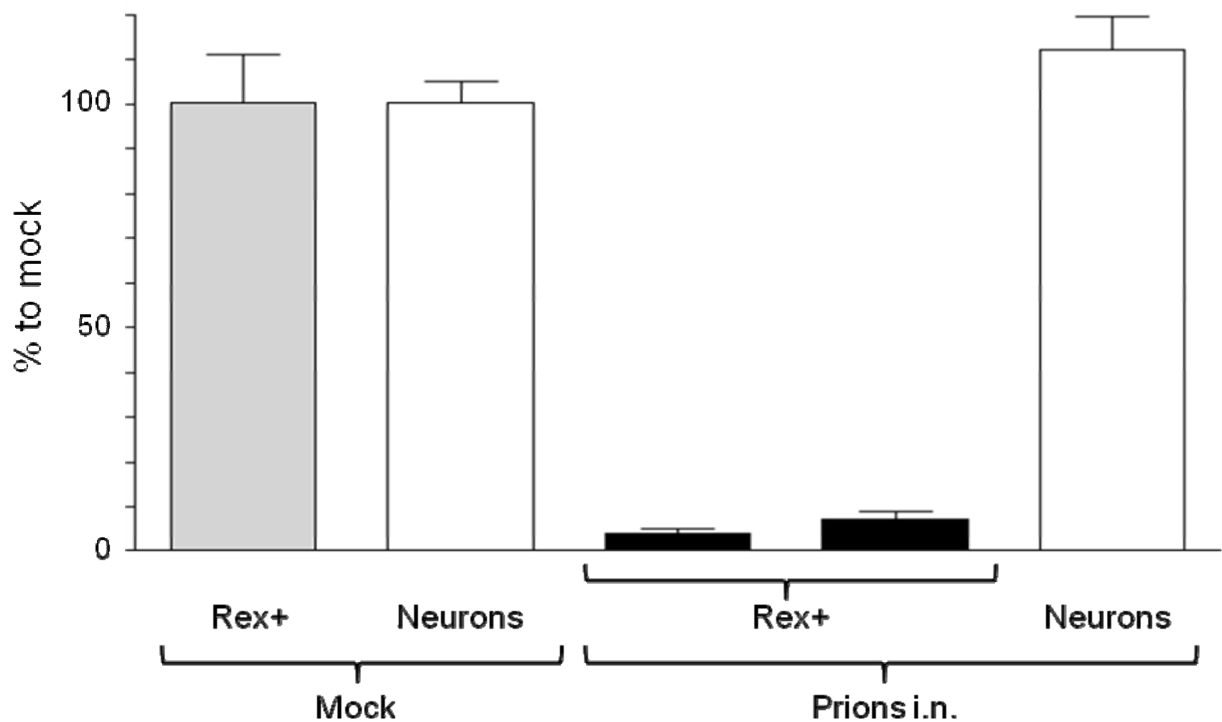
Supplemental Figure 5. Confocal microscopy shows PrP^{Sc} localization in the motor cortex (MC) and red nucleus (RN) from wild-type mice at 136 days post unilateral inoculation in the right sciatic nerve. The prions were visualized with R340 anti-PrP antibodies combined with formic acid treatment to remove the host PrP. NC – negative control without R340 antibody. Prions are localized not only in MC and RN, but also in other centers, for example lateral periaqueductal gray nuclei and nuclei of Darkschewitsch (L-D). Scale bar: 100μm.



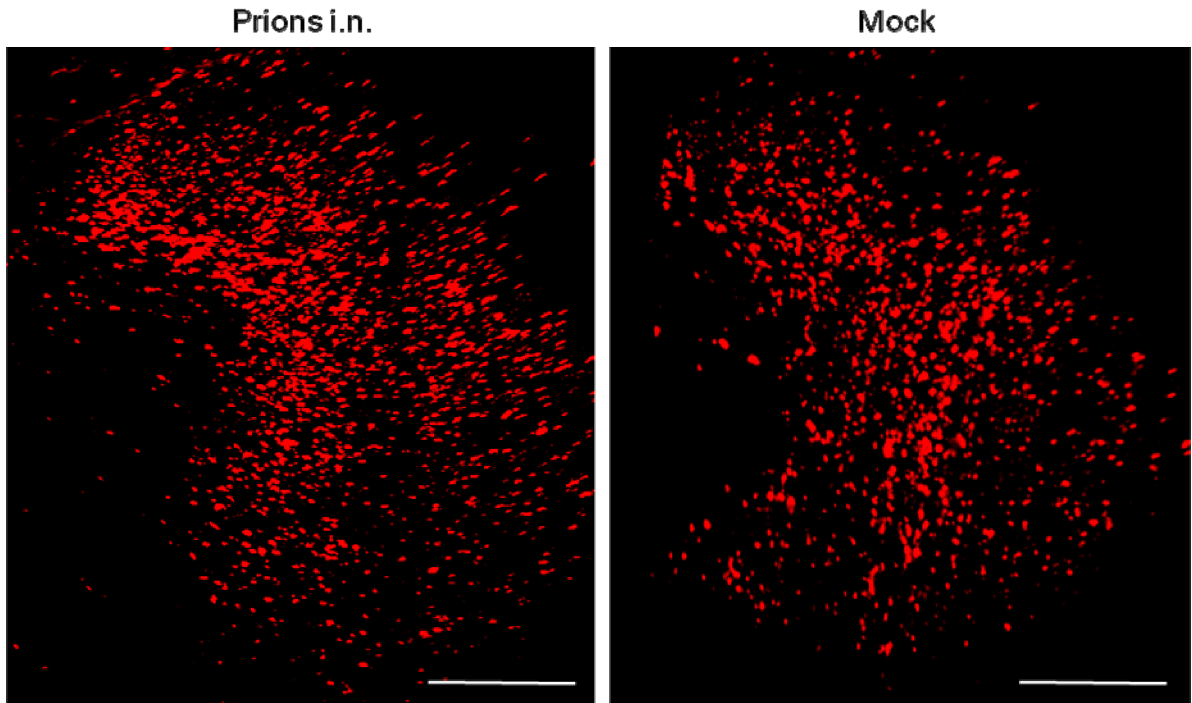
Supplemental Figure 6. Representative ultramicroscopy image from contralateral side of the motor cortex of wild-type mice taken from z-stacks used for the quantification of REx-positive cells. The images do not significantly differ within the experimental groups. The difference between mock and prion-inoculated mice is clearly seen. The signal-to-background ratio (2.7±0.5) and background deviation (1.3±0.1-fold) did not significantly differ between specimens from mock and prion-infected littermates. Scale bar: 100μm.



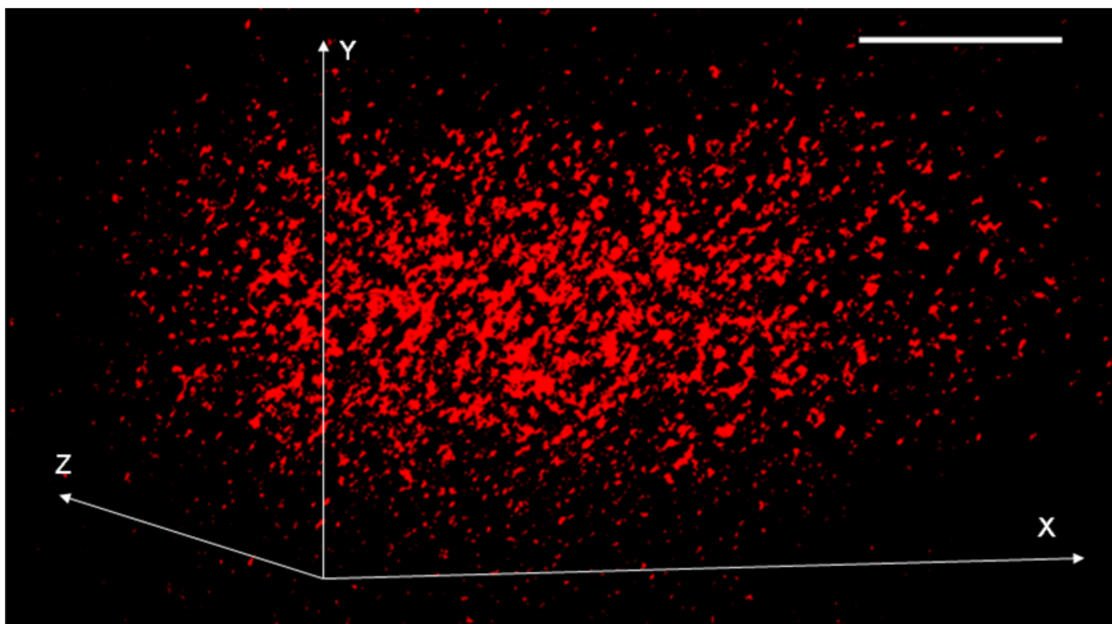
Supplemental Figure 7. The neurons in the motor cortex of wild-type mouse visualized with PGP9.5 neuronal marker at the onset of clinical prion disease. Scale bar: 100μm.



Supplemental Figure 8. REx-positive cells and neurons in the motor cortex. The numbers of neurons (Neurons) in the motor cortex region targeted with AAV-REx were quantified on the cryo-sections stained with anti-NeuN and -PgP9.5 neuronal markers (n=4 for mock and prion-inoculated mice). The neuron count does not differ significantly between mock controls and mice inoculated with prions in the sciatic nerve (Prions i.n.). The number of REx-positive cells(REx+), however, differed dramatically.



Supplemental Figure 9. The ultramicroscopy micrographs of cervical spinal cord of Tga20 mice. Stack analysis demonstrated no significant differences between mock and prion-inoculated mice at 54 days post inoculation. Scale bar: 100µm.



Supplemental Figure 10. 3D reconstruction after 0.5 mm-thick stacks done on multi-photon microscope demonstrates 0.5 mm penetration depth, however the background comparable to the confocal microscopy (signal-to-background ratio 2.0±0.1, background deviation 5.8±1.7). Imaging with multi-photon microscope for the red nucleus and spinal cord turned out to be impossible because of high background. Scale bar, 100µm