Supplemental Material

Two superoxide dismutase prion strains transmitting amyotrophic lateral sclerosis

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Supplemental Methods

Mice. In this study, we used hemizygous mice that express G85R mutant human hSOD1 ($hSOD1^{G85R}$) (1). The lifespan of this mouse line is 397 ± 49 days (n = 101) in our laboratory. There were no differences between the sexes: the lifespans of the females were 398 ± 60 days (n = 31) of the males 397 ± 44 days (n = 70). For preparation of strain B seeds $hSOD1^{D90A}$ mice were used (2). Both mouse strains were backcrossed >30 generations in C57BL/6 mice. The use and maintenance of the mice and the experimental protocol described in this article were approved by the Umeå Regional Ethics Committee for Animal Research.

Preparation of the hSOD1 strain A and B aggregate seeds. For strain A aggregates, spinal cords from terminally ill $hSOD1^{G85R}$ mice were used. For strain B, a $hSOD1^{D90A}$ mouse with short lifespan (359 d) was used. Both strain A and B aggregates form in $hSOD1^{D90A}$ mice, but the B-aggregates dominate in mice with short lifespans (3). Whole spinal cords from the mice were homogenized in 25 volumes of PBS containing 1% of the detergent NP40 and 1 M guanidinium chloride (GdmCl), using an Ultraturrax followed by sonication. The homogenates were centrifuged at 1000 x *g* for 10 min, and the resulting supernatants layered on top of a 13% iohexol density cushion (*d* = 1.074). The tubes were centrifuged at 175,000 x *g* for 1 h. The supernatants were carefully removed, and the pellets washed by sonication in a large volume of plain PBS followed by a second ultracentrifugation for 1 h. The resulting pellets were suspended by sonication in a small volume of PBS, 1 µl per mg of spinal cord used for the preparation. The final suspensions, which contained 5 ng/µl and 10 ng/µl strain A- and strain B-aggregated hSOD1, respectively, were aliquoted into several tubes and kept at -80°C until use for the inoculations. Control seeds were prepared similarly using spinal cords from non-transgenic C57BL/6 mice.

Inoculation of the seed into lumbar spinal cord. The mice were sedated with a combination of ketamine (78 mg/kg i.p.), xylazine (26 mg/kg i.p.), and buprenorphine (3 µg s.c.), making them unresponsive to pain for around an hour. The mouse to be inoculated was then fixed in a small animal stereotaxic frame (model 940; Kopf) and the eyes were covered with an eye ointment. The body temperature was regulated with a TCAT-2LV temperature controller (Physitemp) during the whole procedure. A longitudinal midline skin and fascia incision was made in the back. Two small bilateral, longitudinal cuts close to the spine were then made, making pockets for attachment of a stabilizing spine clamp. To reach the spinal cord, a 3–4 mm transversal cut through the muscles was made, a partial laminectomy was performed, and the meninges were punctured using a fine needle. The required depth of syringe insertion to reach the ventral horn was calculated from computer measurements on spinal cords from 18 C57BL/6 mice using histopathology slides from level L2–L3. The depth of insertion was controlled using a digital display console with 10 µm resolution (model 940-B, Kopf).

Injections of 1 µl hSOD1 strain A and B aggregate seeds or the control spinal cord preparation were carried out in the left spinal cord ventral horn at level L2–L3 using a Syringe model 75 RN Neuros with a sharp custom syringe (33 / 78 / 4)S 30˚ (Hamilton). The injection velocity was 0.125 µl/min controlled by an infusion pump (Legato 130, KD Scientific). The syringe was then slowly pulled out from the spinal cord and the fascia was sutured (Ethicon silk 6-0 non-absorbable thread, C-1 needle). The skin was closed with surgical staples (Reflex Autoclip System, 7 mm). 200 µl 0.9% NaCl was injected subcutaneously for rehydration and the mouse was then moved to a heating blanket for recovery from anesthesia. The duration of the surgical procedures was about 25 min, and in total the mice were under anaesthesia for about 1 h. Buprenorphine was injected 2–3 times at 12-h intervals until the mice were pain free. The staples were removed 2–3 weeks after surgery.

To make sure that the aggregate injection really reached the ventral horn after surgery, injections with 1 µl 4% Fluoro-Gold™ (hydroxystilbamidine bis, Sigma-Aldrich) were done in 4 control mice before the first injections of aggregate seeds. The mice were formalin-fixed by whole-animal perfusion 2–3 days after injection. The spinal cord was paraffin-embedded, cut with a microtome and the slides investigated unstained under a fluorescence microscope (Olympus, BX53, filter cube MWU-2). Satisfactory left lumbar spinal cord labelling was found.

Supervision of the mice. No obvious early urinary bladder problems appeared in the inoculated mice, and the sensory response in the hind legs appeared normal. The mice were

examined at least every third day. In the strain A- and B-inoculated mice, the onset was uniformly splaying of a hind leg. The mice were considered terminally ill when the hind legs were fully or almost fully paralyzed. In cases with more prominent foreleg symptoms (some control preparation-inoculated and non-inoculated mice) severe eye infection in combination with severe foreleg weakness was the criterion for terminal disease.

Excluded mice. Nineteen mice were excluded and not reported in the Results and Discussion section. Fifteen mice died during the anesthesia and surgical procedures. Two mice were euthanized because of wound infections during the first few days, and two because of late infections.

Tissue handling and histopathology. The mice were sacrificed by intraperitoneal injection of pentobarbital, decapitated and the spinal cords were removed by flushing with saline using a syringe inserted distally. The cords were then divided sagittally, and typically alternating right or left halves were immersion fixed for histopathology. The other half was divided into lumbar, thoracic, and cervical sections and snap-frozen in -80°C for subsequent analysis of amounts and structures of hSOD1 aggregates. The brains were dissected and divided into right and left hemispheres, cerebellum and brainstem and processed like the spinal cords. In some cases all the parts were frozen as above or the animal perfusion fixed. Fixed tissue was paraffin-embedded, and 4 µm thick sections were cut with a microtome (Microm HM400), and mounted on glass slides. Sections were routinely stained with hematoxylin/eosin.

For immunohistochemistry, sections were immunostained according to the manufacturer's recommendations using the Ventana Benchmark Ultra (Ventana Medical Systems Inc.). The primary anti-hSOD1 antibody used was raised to a peptide corresponding to amino acids 131–153 in the sequence (see below) and was used at the concentration 0.7 µg/ml. The primary antibodies were located with corresponding biotin-conjugated secondary antibodies coupled to a streptavidin-horseradish peroxidase conjugate and developed using 3,3´diaminobenzidine tetrahydrochloride as the precipitating enzyme product (brown color) (iView DAB detection kit, Ventana). Sections were counterstained with hematoxylin, washed, dehydrated and mounted with Pertex (DakoCytomation). Micrographs were taken in the same microscope (lamp set at fixed voltage, intensity adjusted by standard grey filters) equipped with a DP72 camera. Images were acquired using the Olympus CellSens program with standard settings and white balancing on out-of-sections areas.

In Supplemental Figure 10 typical results of the histopathological investigations are shown. Five onset and 11 terminally ill strain A-inoculated mice and 2 onset and 9 terminally ill strain B-inoculated mice were investigated. Five control preparation-inoculated mice with a mean age of 190 d were examined. Six non-inoculated $hSOD1^{G85R}$ control mice were investigated at terminal disease. In each mouse, sections from lumbar, thoracic, and cervical spinal cord, brain stem, cerebellum and brain were investigated.

Counting of neurons in the spinal cord ventral horns were performed on 8 strain A-inoculated, 8 strain B-inoculated and 4 control-inoculated mice (mean age 195 d). Formalin-fixed paraffin-embedded sections were used. The sections were stained for NeuN-antigen reactivity using a mouse anti-NeuN antibody (Chemicon; MAB377, 1:200). All of the histological samples were whole-slide digitalized using a scanning system (Pannoramic 250, Plan-Apochromat, CIS_VCC_F52U25CL). Computer graphic analysis was performed with Panoramic Viewer software (vers. 1.15.2 SP2), both from 3D Histotech Ltd., Budapest, Hungary. The ImageJ software (http://rsb.info.nih.gov/ij/docs/index.html) was used for morphological filtering. The ventral horn was selected for counting of cells and defined as the area within the grey-matter border and anterior to a frontal plane through the central canal. Cell profiles immunopositive for NeuN was identified using the ImageJ software and profiles larger than 150 μm^2 in this region were used in the analysis. The number of profiles for all sections analyzed per animal (usually 7–10 sections) were divided with the number of sections analyzed. Statistical analysis was performed by Mann–Whitney test using the Statistica™ 12 software (Statsoft Inc.).

Counting of neuropil threads and cytoplasmic aggregates were done on sections stained with the aa 131–153 anti-hSOD1 Ab as described above (Supplemental Table 2). The sections were then scanned using the scanning systems described above and randomly numbered. Three observers, blinded to the mouse type, independently counted neuropil threads and cells with cytoplasmic SOD1-immunoreactive aggregates at different anatomical locations. . The number of threads were counted in a box measuring 100 µm x 100 µm placed centrally in the ventral horn. The number of cells with cytoplasmic granular aggregates were counted in the grey matter of the ventral horn as defined above. Statistical analyses showed high concordance between the observers. In the final statistical evaluation the mean result of the three observations was used. Statistical evaluation used the non-parametric Kruskal-Wallis ANOVA and post-hoc testing was done using Bonferroni-corrected two-sided Mann-Whitney U-test. Testing was done using the Statistica™ 12 software (Statsoft Inc.)

Antibodies. Antibodies to peptides (corresponding to aa 4–20, 24–39, 43–57, 57–72, 80–96, 100–115, and 131–153 in hSOD1) that were coupled to keyhole limpet hemocyanin were raised in rabbits as previously described (4, 5). The antibodies were purified with Protein A-Sepharose (GE Healthcare, Uppsala, Sweden) followed by Sulfolink gel with the respective peptides coupled (Pierce, Rockford, IL). An antibody raised to the C-terminal end in G127insTGGG mutant hSOD1, amino acids 111–132 were prepared in the same way. Amino acids 128–132 represent a neosequence in the mutant, which is why the 111–132 antibody can only react with epitopes covering amino acids 111–127 in full-length hSOD1. It is therefore designated so in assays involving full-length hSOD1s. For specific analysis of murine SOD1 an antibody raised against aa 24-36 in the sequence was used. For muscle immunohistochemistry an antibody against slow myosin was used (clone NOQ 7,5,4D; Cat no M8421, Sigma).

Tissue homogenization and binary epitope-mapping assay for hSOD1 aggregate structure. A detailed description is found in (3). The method is based on 8 antibodies raised against short peptides that cover over 90% of the 153 amino acids (aa) long hSOD1 subunits (Figure 1). Since the configurational space of short peptides is very large, their randomly induced antibody-eliciting epitopes are unlikely to match defined ordered structure in proteins and in cores of protein fibrils. In reaction with fibrils/aggregates the binding of the anti-peptide antibodies is essentially "binary". There is no response to the ordered core of protein aggregates/fibril or to segments otherwise hidden. Unrecruited sequence elements, which have lost their native contacts and therefore are disordered, will react with the antibodies. Since the antipeptide antibodies are polyclonal there is no loss of reaction because of mutations in the tested segments, but some effects on binding intensity can be seen, c.f. difference in reactivity of the 80-96 antibody with strain B aggregates composed of $hSOD1^{D90A}$ (the seed) and $hSOD1^{G85R}$ (terminal spinal cord) in Figure 1B.

Usually lumbar, thoracic, and cervical spinal cord, brainstem, brain (dissected free from midbrain, pons, and medulla) and cerebellum were examined. In most cases, the left and right sides were analyzed separately. The dissected tissues were homogenized with an Ultraturrax apparatus (IKA, Staufen, Germany) for 30 s and by sonication for 1 min in 25 volumes of ice-cold PBS containing an antiproteolytic cocktail (Complete®, Roche Diagnostics, Basel, Switzerland). The tissue homogenates were added to 20 volumes of PBS containing 1% NP40, sonicated for 30 s, and then centrifuged at 200 x *g* for 10 min. The supernatants were diluted 1 + 1 step-wise in the PBS, and 100 µl were captured on 0.2 µm cellulose acetate

filters in a 96-well dot-blot apparatus (6) (Whatman GmbH, Dassel, Germany). Following washing of the wells, the filters were blocked with 5% dry milk and 0.1% Tween 20 for 1 h, and cut in slices which were incubated with the anti-hSOD1 peptide antibodies (0.01–0.02 μg/ml in blocking buffer) overnight at 4°C. After washing, the blots were developed with HRP-substituted goat anti-rabbit Ig antibodies and ECL Select (GE Healthcare), and recording was done with a ChemiDoc apparatus. Values below the mean plus 2 SD results of 8 non-transgenic control mice are considered as blank reactions (3).

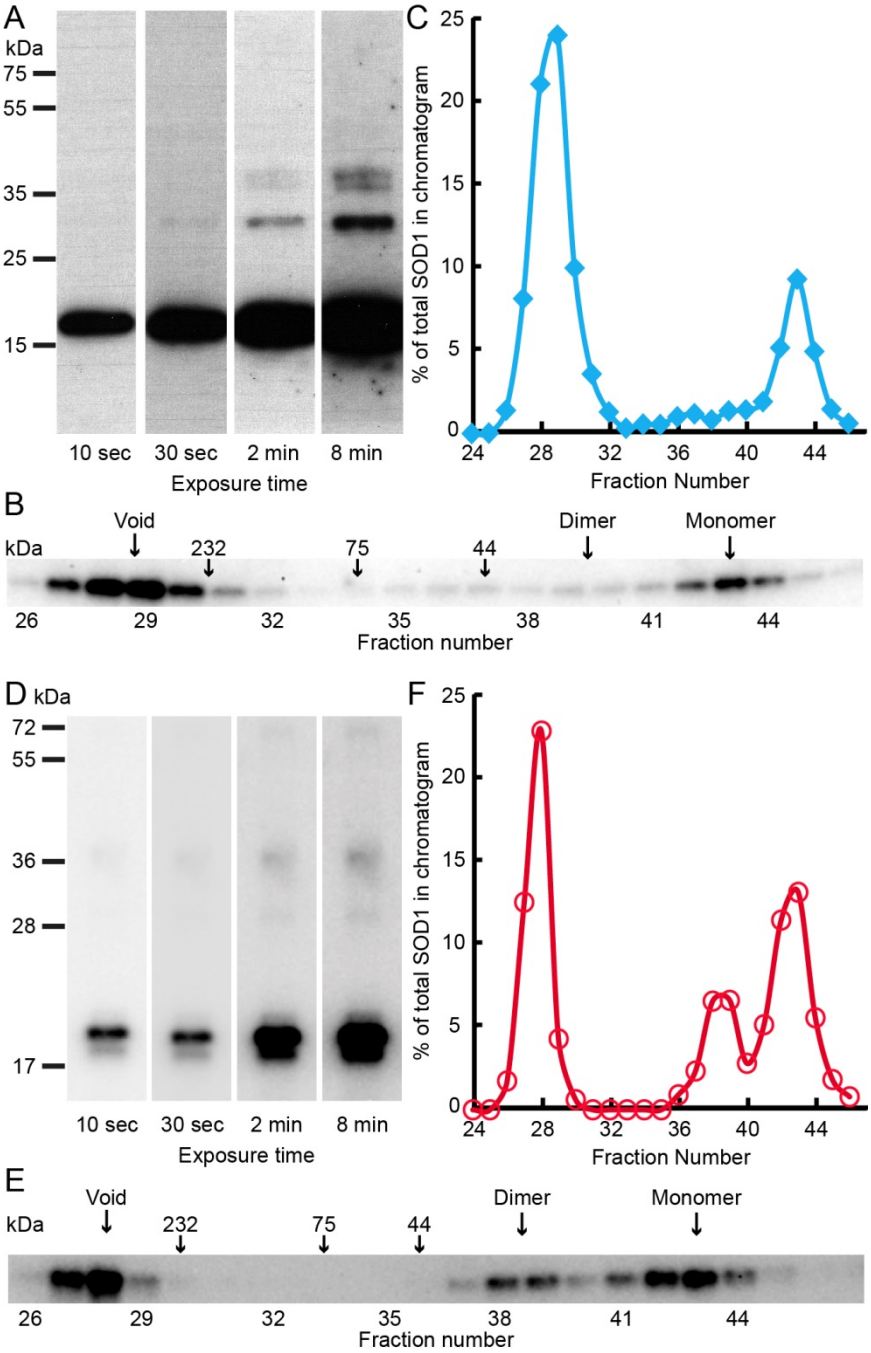
To allow comparison and quantification, one homogenate of a spinal cord from a terminally ill $hSOD1^{G93A}$ mouse was designated as a standard (set to 1) and kept in multiple aliquots at -80°C. Dilution series (1 + 1) of this standard were run in 1 or 2 lanes of all filters, and were stained with the 57–72 antibody. All blots of all homogenates with all antibodies were quantified against this standard. To facilitate comparison of staining patterns, in some cases the staining intensities of the 8 antibodies of individual homogenates were normalized against the staining of the homogenate with the 57–72 antibody (taken as 100%) for strain A aggregates. For strain B aggregates, the 111–127 antibody is optimal for pattern comparison purposes (3).

Quantification of detergent-resistant hSOD1 aggregates in spinal cord. Spinal cords were homogenized using an Ultraturrax followed by sonication in 25 volumes of PBS containing 1% NP40. The homogenates were then diluted 20-fold with the NP40-containing PBS and aliquots subjected to 1 h ultracentrifugation at 175,000 x g. The contents of hSOD1 in the pellets were determined with western blotting.

Analysis of the total protein content of the seeds. Aliquots of the seed suspensions and tissue homogenates were mixed with equal volumes of 2 x SDS-PAGE sample buffer without glycerol and bromophenol blue and were boiled for 10 min. The protein contents were analyzed with the BCA protein assay (Pierce) using bovine serum albumin boiled in 1x sample buffer as standard.

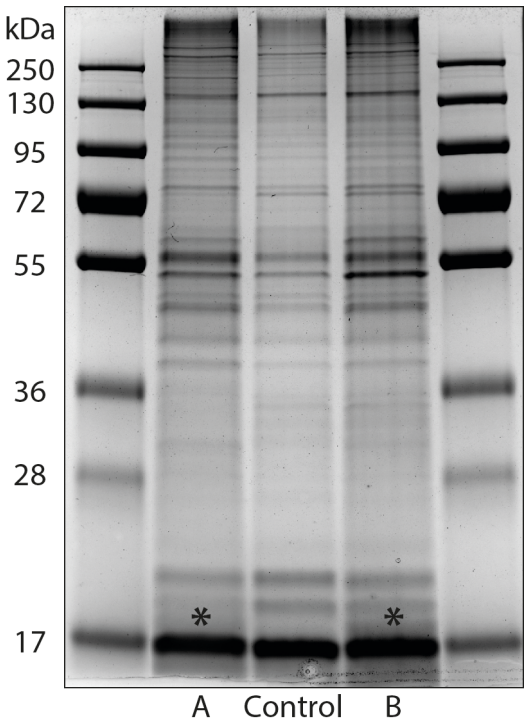
Immunoblots and quantifications. The western immunoblots were carried out as described (4) using antibodies raised in rabbits against peptides in the human or murine SOD1 sequences as indicated in the figure legends. Chemiluminescence of the blots was recorded in a ChemiDoc apparatus and analyzed with Quantity One software.

Supplemental Figures

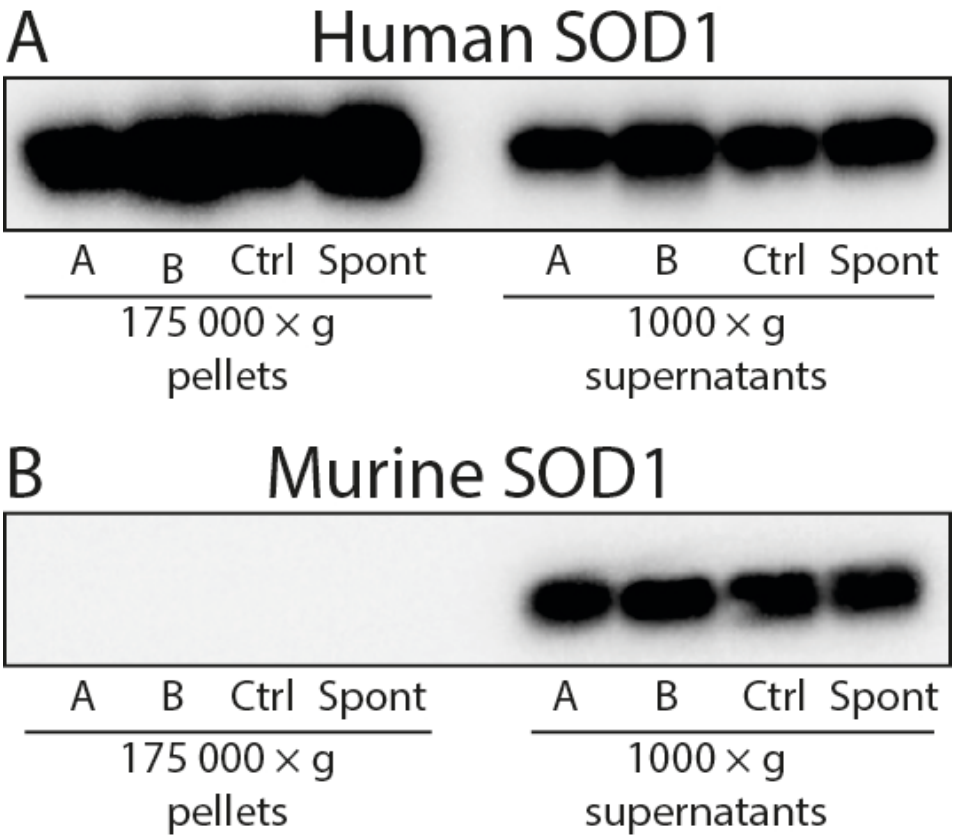


Supplemental Figure 1. Characterization of the A and B seeds. (**A** and **D**) Aliquoted strain A and B seed tubes were thawed and the content suspended by 1 min of vortexing in 25 volumes of PBS containing the Complete protease inhibitor cocktail. The whole seed suspensions were analyzed by western immunoblotting for hSOD1. In the strain A seed (**A**), virtually all the hSOD1 appeared to be full-length monomers; there was no evidence for any

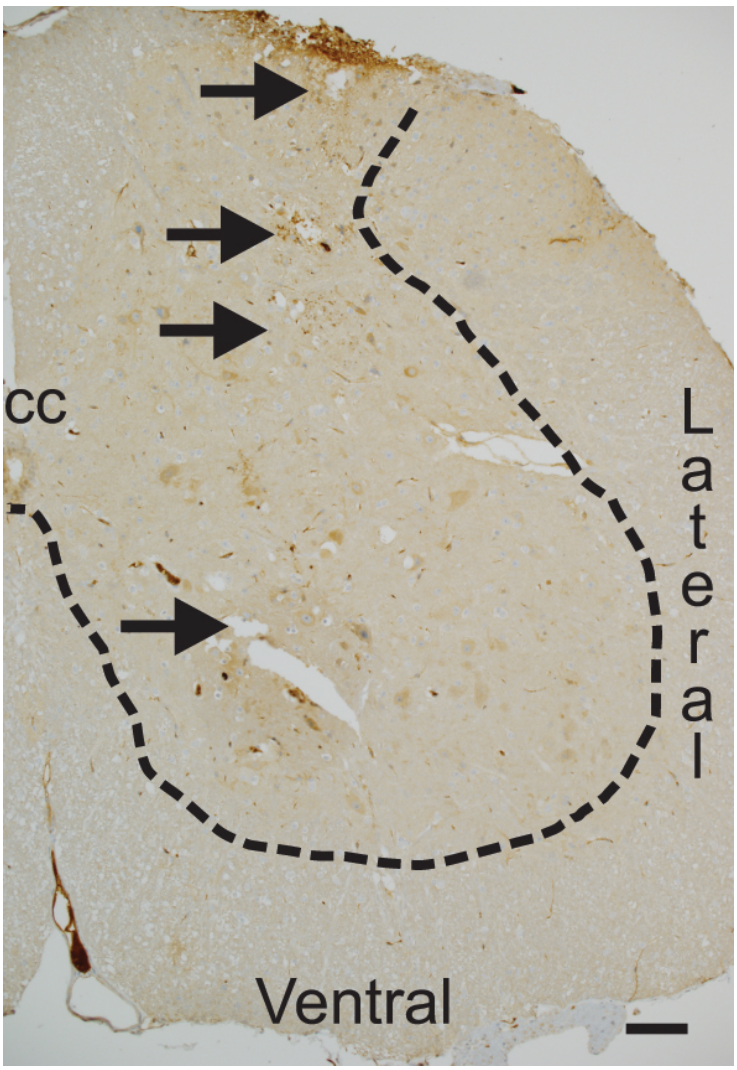
truncated species. Upon long exposure, minute high molecular-weight bands appeared, estimated to be <0.5% of the total hSOD1 in the A seed. In the B seed (**D**), 85% appeared to be full-length, 11% truncated and 4% high molecular-weight species. (**B, C, E** and **F**) The suspensions were then centrifuged at 20,000 x *g* for 15 min at 4°C. The supernatants were subjected to size exclusion chromatography (SEC) on molecular weight marker-calibrated (shown in kDa) Superdex 75 10/300 GL columns (GE Life Sciences). Different columns were used for A and B. The eluted fractions were analyzed by western immunoblotting for hSOD1 using the aa 57-72 Ab. In the A seed, the 20,000 x *g* pellet was found to contain 96% of the hSOD1 in the seed suspension, and in the B seed 91%. In the SEC of the supernatants, 80% of the hSOD1 from the A seed eluted at the void volume, apparently representing small fibrils (**B, C**). The rest, 20%, eluted at the position of folded prosthetic metal replete C6A/C57A/F50E/G51E mutant hSOD1 monomers. In the B seed supernatant, 42% eluted at the void volume, 18% at the position of a folded dimer and 40% at the position of a folded hSOD1 monomer (**E, F**). Thus, of all hSOD1 in the A seed, >99% appeared to exist in aggregates/fibrils. In the B seed about 95% of the hSOD1 appeared to exist in fibrils. There was no evidence for the presence of any “oligomeric” species. The mono and dimeric hSOD1 is likely to represent material dissociated from hSOD1 fibrils (7, 8). The larger levels in the B seed could be explained by the much greater fragility of B aggregates as compared to A aggregates (3).



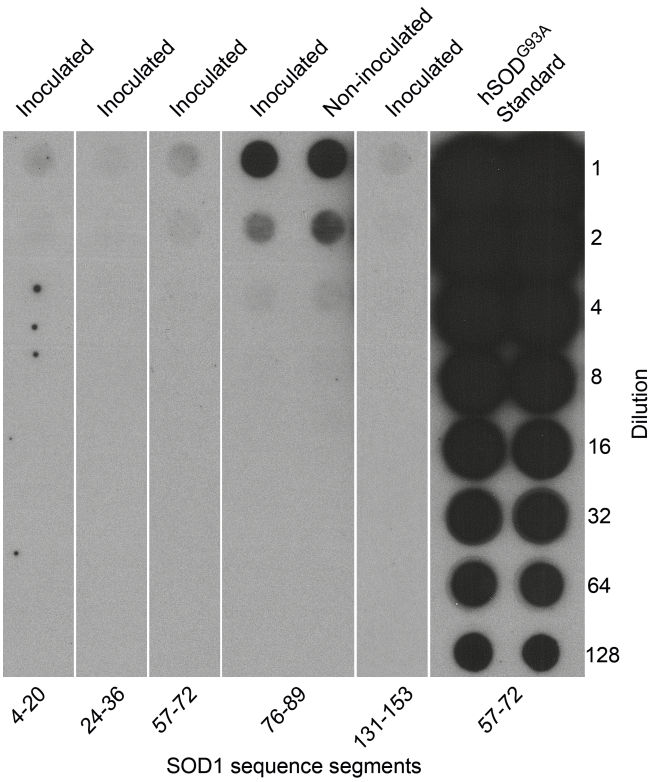
Supplemental Figure 2. Analysis of proteins present in the seeds by SDS-PAGE. Examples of strain A, B and control seeds separated by SDS-PAGE and stained with Coomassie Brilliant Blue. The total protein contents of the seeds vary between preparations and were for those depicted in the gel from left to right 462, 417 and 619 µg/ml, respectively. The electrophoretogram shows that multiple different proteins are present, without major differences between the aggregate and control seeds. ∗, the positions of G85R and D90A mutant hSOD1s in the A and B seeds as deduced from western blots. No distinct protein band can be discerned at that position. The strain A and B seeds contained 7 and 10.5 µg/ml hSOD1 respectively. The hSOD1 in the seeds thus accounted for around 1.5% of the total protein.



Supplemental Figure 3. No coaggregation of murine SOD1 with strain A or B hSOD1 aggregates. Spinal cords from end-stage hSOD1^{G85R} mice, which had been inoculated with the strain A, strain B and control seeds or had spontaneously developed disease, were homogenized in PBS containing 1% of the detergent NP40. The homogenates were first centrifuged at 1000 x g for 10 min. The supernatants were collected and centrifuged at 175000 x g for 1 h. The figure shows western blots of the 1000 x g supernatants and the 175000 x g pellets using human-specific (aa 24-39) (**A**) and murine-specific (aa 24-36) (**B**) anti-SOD1 antibodies.

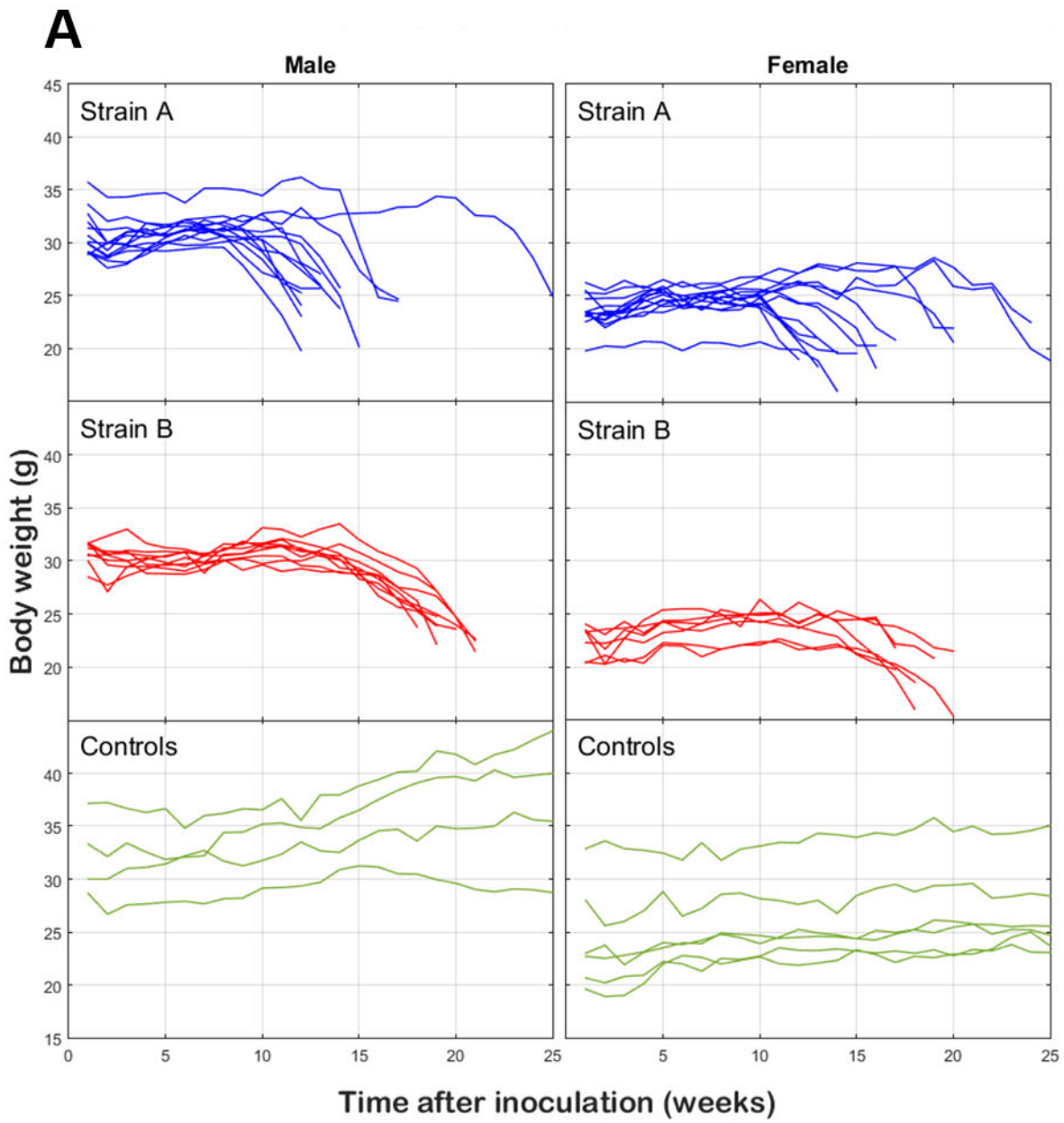


Supplemental Figure 4. Effect of syringe stick on histopathological appearance of lumbar ventral horn. A syringe was inserted into the lumbar ventral horn of a 100-day-old hSOD1^{G85R} mouse and 1 µl PBS injected, similar to the inoculation experiments. The mouse was sacrificed after 24 h. The section was stained for hSOD1 using the aa 131-153 Ab. Arrows indicate defects in the tissue induced by the stick, localizing its position. No staining is seen which is similar to the rounded patch which appeared after inoculation of the strain A seed in Figure 1C. Cc = central channel. The dotted line delineates the ventral horn. Bar = 50 µm.



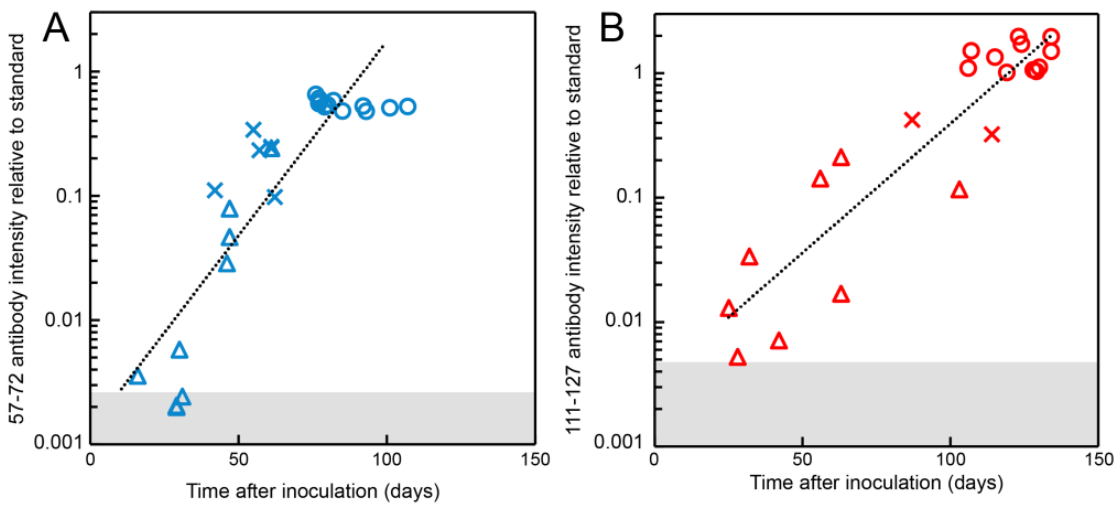
Supplemental Figure 5. Inoculation of the strain A seed in non-transgenic C57BL/6 mice: search for aggregation. For control purposes, 6 non-transgenic C57BL/6 mice were inoculated with the strain A seed. One mouse had to be sacrificed after 31 days owing to a wound in the back. Another mouse developed a flaccid paralysis involving the rear half of the body, accompanied by loss of pain response in the same area and a distended urinary bladder. This picture was different from the progressive spastic paralysis seen in the inoculated hSOD1^{G85R} mice and it was sacrificed after 176 days. Upon flushing the spinal cord out of the spinal channel, the lumbar segment was not recovered, suggesting it had been necrotic and autolytic. A homogenate made of the thoracic and cervical segments (1 + 520 volumes PBS) was analyzed with the epitope-mapping assay using anti SOD1 peptide antibodies: human aa 4-20, cross-reacts well with murine SOD1; murine aa 24-36; human aa 57-72, cross-reacts well with murine SOD1, aa 76-89, and aa 131-153, both segments equal in murine and human SOD1. The figure shows results for the inoculated mouse and a C57BL/6 mouse which had not been inoculated (non-inoculated). To the right results for a dilution series of the homogenate of spinal cord (1 + 520) from a terminally ill hSOD1^{G93A} mouse which is used as a standard in the epitope-mapping assay. No significant reactions were found, except for that with the aa 79-86 Ab which cross-reacted with both C57BL/6

homogenates close the level of the 1/128 dilution of the standard. Thus no murine SOD1 aggregates could be demonstrated. Hence, although the background to the pathology in this mouse was not revealed, the picture was different from the ALS-like phenotype of the other inoculated groups. The remaining 4 mice are currently 546, 546, 599, and 772 days old and without symptoms.

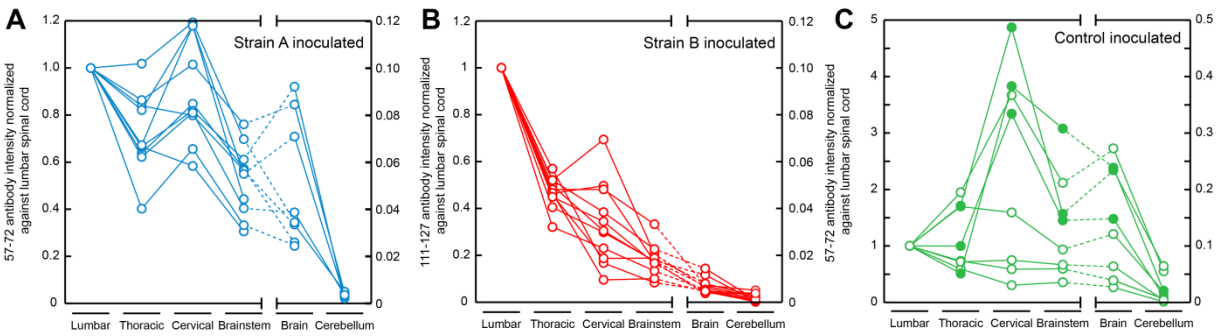

A
Male
Female
Strain A
Strain A
Strain B
Strain B
Controls
Controls
Body weight (g)
Time after inoculation (weeks)
B
C

Supplemental Figure 6. Weight loss in male and female mice inoculated with hSOD1 aggregates, and fiber type grouping in skeletal muscle.

(**A**) (—), (—), and (—) strain A, strain B, and control preparation-inoculated mice, respectively. Mice inoculated with both aggregate strains lost weight, suggesting denervation-induced muscle atrophy. The time from peak weights to the end stage differed significantly between the A and B groups (44.7 ± 15.8 d (n = 26) vs. 57.8 ± 13.2 d (n = 16), p = 0.003; Bonferroni-corrected two-sided Mann-Whitney U-test). The losses from maximal weights were not significantly different between strain A and B-inoculated mice; in males they were 25.4 ± 7.8% (n = 13) and 26.0 ± 5.1% (n = 9), respectively, and in females 23.1 ± 3.7% (n = 13) and 21.8 ± 9.6% (n = 7), respectively. (**B** and **C**) Micrographs of triceps surae muscle from (**C**) a terminally ill strain A-inoculated mouse and (**B**) an age-matched control C57BL/6 mouse. The muscles were stained with an antibody against slow myosin and the one from the terminally ill mouse shows fiber type grouping indicating denervation-induced atrophy.



Supplemental Figure 7. Human SOD1 aggregation growth in spinal cords of mice inoculated with strain A and B aggregates. (**A** and **B**) The aggregations show essentially exponential buildups (log antibody intensity vs. time: $R^2 = 0.762$, $t_{x2} = 10$ days; $R^2 = 0.836$, $t_{x2} = 15$ days; squared Pearson correlation coefficient), respectively, in hSOD1^{G85R} mice inoculated with strain A and strain B aggregates. (△, △) non-symptomatic, (X, X) early symptomatic, and (◯, ◯) terminally ill strain A and strain B-inoculated mice, respectively.

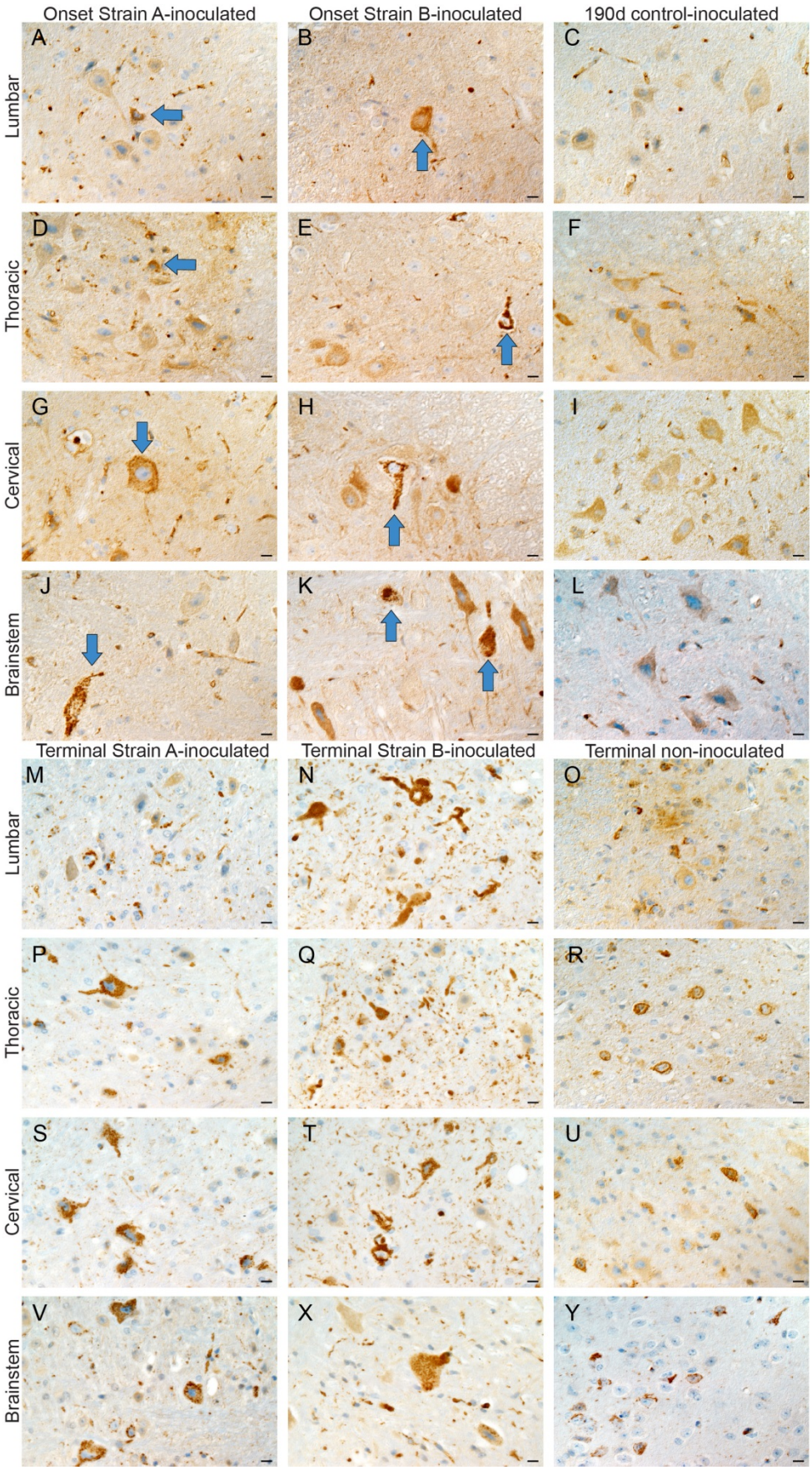


Supplemental Figure 8. Comparison of aggregate distributions along the neuraxis in terminally ill inoculated and non-inoculated mice. The aggregate levels in the segments of the individual end-stage mice were normalized against their levels in lumbar spinal cord. See Figure 3, B, C, and F for absolute values. For statistical analysis of differences between the groups for segments downstream of lumbar spinal cord, see Supplemental Table 1. The differences between strain B- and strain A- as well as control-inoculated mice are highly significant. There was no significant difference between the strain A- and control-inoculated mice. (●), control seed-inoculated hSOD1^{G85R} mice with foreleg onsets.

Supplemental Table 1. Comparison of aggregate distributions along the neuraxis

Neuroaxis segment/ ANOVA (*p*)	Comparison	Significance (*p*)	Corrected significance (*p*)
Thoracic sp. c.	Strain A - strain B	0.002	0.006
0.000	Strain A - control	0.248	0.744
	Strain B - control	0.001	0.003
Cervical sp. c.	Strain A - strain B	0.000	0.000
0.000	Strain A - control	0.248	0.744
	Strain B - control	0.002	0.006
Brain stem	Strain A - strain B	0.000	0.000
0.000	Strain A - control	0.016	0.048
	Strain B - control	0.000	0.000
Brain	Strain A - strain B	0.000	0.000
0.000	Strain A - control	0.036	0.108
	Strain B - control	0.000	0.000

The table shows comparison of aggregate distributions along the neuraxis in end-stage mice inoculated with the strain A-, B- and control seeds. The aggregate levels in the segments of the individual mice were normalized against their levels in lumbar spinal cord, c.f. Figure 3, B, and F and supplemental Figure 7. The statistical analyses were carried out with Kruskal-Wallis ANOVA, using Bonferroni-corrected two-sided Mann-Whitney *U*-test for post-hoc analyses.


Onset Strain A-inoculated
Onset Strain B-inoculated
190d control-inoculated
Lumbar
Thoracic
Cervical
Brainstem
A
B
C
D
E
F
G
H
I
J
K
L
Terminal Strain A-inoculated
Terminal Strain B-inoculated
Terminal non-inoculated
M
N
O
P
Q
R
S
T
U
V
X
Y

Supplemental Figure 9. Histopathology of mice at onset of symptoms and at terminal disease. The tissues were stained with a rabbit Ab against aa 131–153 in the hSOD1 sequence. The first column shows findings from the strain A-inoculated mice (**A,D,G,J** at symptom onset and **M,P,S,V** at terminal disease) while the second column shows findings from the strain B-inoculated mice (**B,E,H,K** at symptom onset and **N,Q,T,X** at terminal disease). The upper half of the last column (**C,F,I,L**) shows findings from age-matched (about 190 d) control preparation-inoculated mice and the lower half (**O,R,U,Y**) stage-matched terminally ill non-inoculated $hSOD1^{G85R}$ mice (these were about 200 d older than the terminal strain A or strain B-inoculated mice). Arrows indicate neurons containing condensed aggregates (shown only in the onset mice). The amounts of condensed aggregates in the neuropil are higher in the terminal stage (**A-L** vs. **M-Y**). Scale bars = 20 μm.

Supplemental Table 2. Counting of SOD1-positive aggregates and threads in the spinal cord.

		Strain A inoculated		Strain B inoculated		Terminal non-inoculated	
	ANOVA (*p*)	***n***	**Number**	***n***	**Number**	***n***	**Number**
Cervical ventral horn							
Neuropil threads	0.016	9	29 ± 14[A]	6	47 ± 15[A]	5	20 ± 21
Cells with cytoplasmic granular aggregates	n.s.	9	17 ± 5.7	6	17 ± 2.9	6	8.5 ± 6.0[C]
Thoracic ventral horn							
Neuropil threads	0.000	11	28 ± 13[A]	9	46 ± 8.6[A]	6	18 ± 13[C]
Cells with cytoplasmic granular aggregates	0.003	11	13 ± 6	9	11 ± 2.9	6	4.3 ± 2.9[B]
Lumbar ventral horn							
Neuropil threads	0.014	9	29 ± 12[A]	7	41 ± 11[A]	5	22 ± 31
Cells with cytoplasmic granular aggregates	0.006	9	11 ± 6.5	7	11 ± 4.4	5	2.3 ± 2.1[B]

Means ± SD are shown. (*n*) = number of mice counted. The statistical analyses were carried out with Kruskal-Wallis ANOVA, using Bonferroni-corrected two-sided Mann-Whitney *U*-test for post-hoc analyses. Symbols denote significantly differences between: [A], strain A and strain B inoculated mice; [B], terminal non-inoculated mice and both strain A and strain B inoculated mice; [C], terminal non-inoculated mice and strain B inoculated mice.

Ventral horn is here defined as the grey matter of the spinal cord in front of a frontal plane through the central canal and within the grey-white matter border. The number of threads were counted in a box measuring 100 µm x 100 µm placed centrally in the ventral horn. The number of cells with cytoplasmic granular aggregates were counted in the grey matter of the ventral horn.

Supplemental references

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