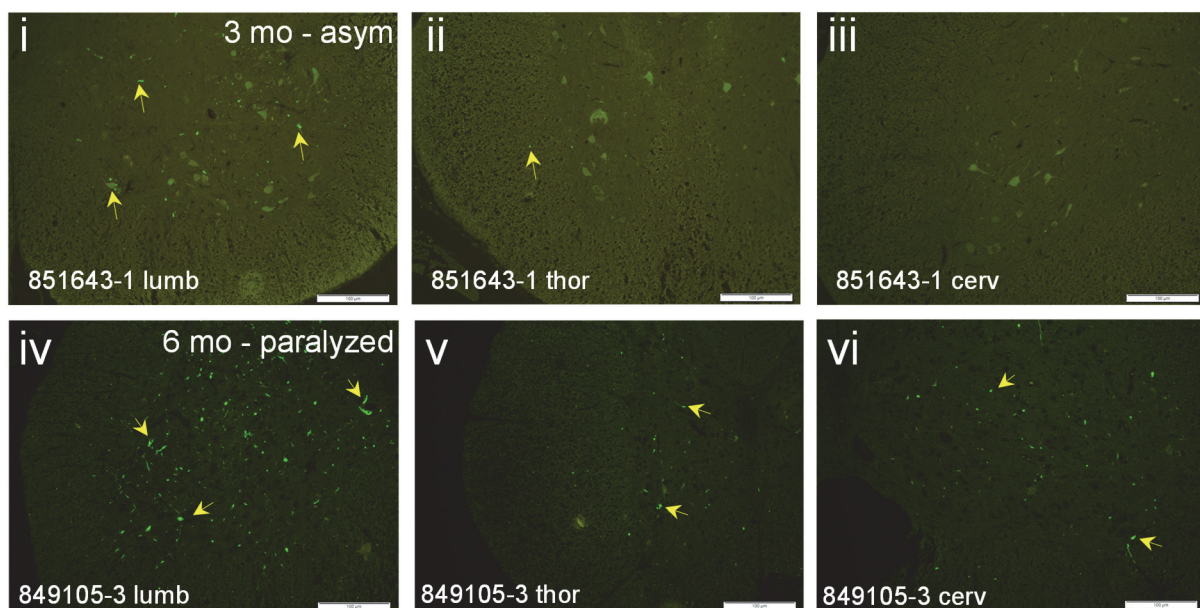


Supplementary Figures and Tables

Fast and slow strains of misfolded mutant superoxide dismutase 1 in familial amyotrophic lateral sclerosis

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A



B

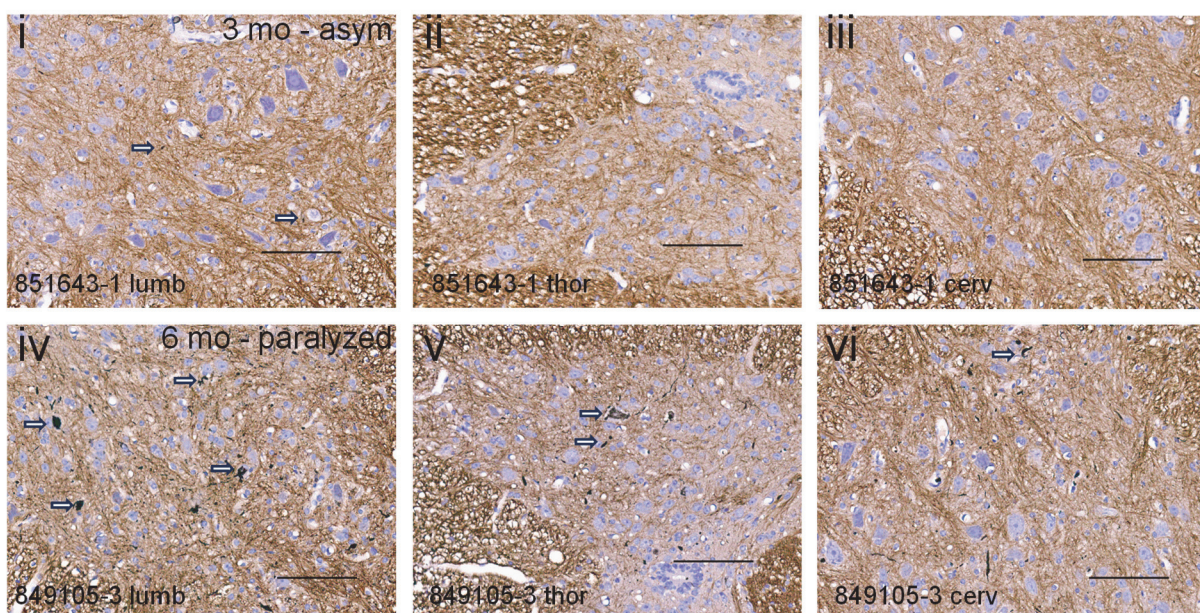


Fig S1. Time course of SOD1 inclusion pathology in sciatic nerve-injected G85R-SOD1: YFP mice. A) The level of inclusion pathology in G85R-SOD1:YFP mice was assessed by visual inspection of tissue sections. Six asymptomatic mice in cohort 2 were harvested at 3 months post-injection. Three of the six mice harvested at 3 months of age lacked inclusion pathology (not shown), whereas three mice exhibited sparse inclusion pathology in the lumbar spinal cord (A i-iii, arrows) (see Supplemental Table S3). (A iv-vi) Representative images from a paralyzed animal harvested at 6 months post-injection. Arrows mark examples of inclusions. B) Inclusion pathology was confirmed by Campbell-Switzer silver staining. (B i-iii) Representative images of three spinal levels in an asymptomatic animal harvested at 3 months post-injection. A few small puncta of silver deposition were visible (arrows). (B iv-vi) Representative images from a paralyzed animal harvested at 6 months post-injection. Lumbar spinal cords of paralyzed mice had the highest level of silver-stained inclusion pathology (arrows). Scale bar = 100 μ m.

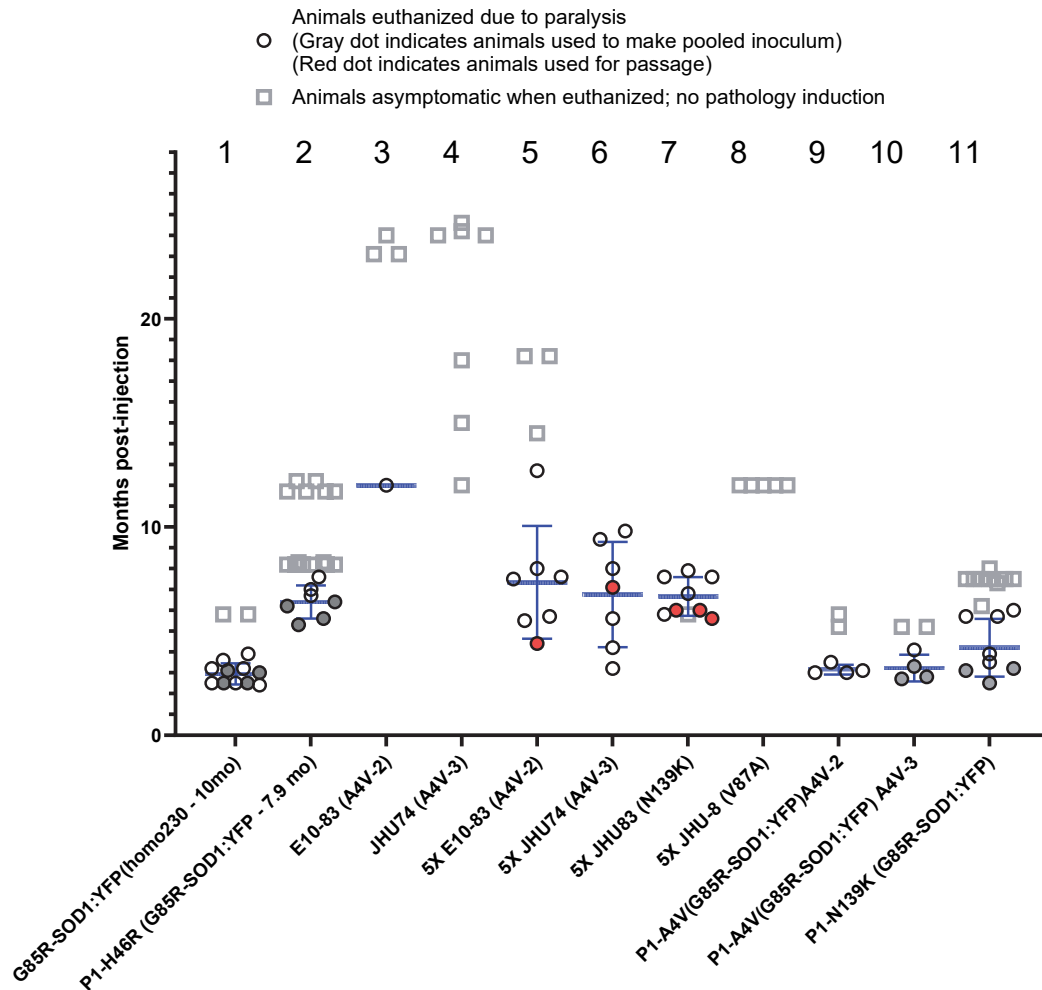


Fig S2. Passage and selection for generation of pooled seeding homogenates for the H46R, A4V, N139K, and G85R-SOD1:YFP-Line 230 variants. A graphic summary of the time to endpoint after injection for new isolates is shown. Newborn G85R-SOD1:YFP mice were intraspinally injected with 1 μ l of the homogenates from paralyzed sources. Homogenate from a paralyzed 10 month old homozygous G85R-SOD1:YFP – Line 230 animal served as a reference control (Column 1). The initial seeding homogenates with H46R homogenates from paralyzed transgenic rats were largely ineffective, but when insoluble components of these homogenates were concentrated 5-fold by sedimentation (abbreviated as 5X), we were able to induce paralysis in one G85R-SOD1:YFP animal by ~8 months post-injection [20]. We then prepared an inoculum from this animal for passage into G85R-SOD1:YFP mice. Although most of the animals failed to develop paralysis, there were 7 that became paralyzed between 5 and 8 months of age (Column 2). Spinal homogenates from 4 of these mice (gray dots) were prepared to produce a pooled P2 inoculum of H46R SOD1 conformers.

We obtained tissues from human decedents expressing the A4V, V87A, and N139K variants. Two cases with A4V mutations had durations of < 2 years. The V87A and N139K cases represent rare variants, which had durations of 5 and 6 years, respectively. Initial seeding attempts with the two A4V human tissue homogenates

produced paralysis in only 1 animal at 12 months post-injection; ten other animals were asymptomatic when euthanized (Columns 3 and 4). We therefore decided to try and concentrate the aggregates by centrifugation and sedimentation, finding that after 5-fold concentration we induced early paralysis in most of the mice injected with the two A4V cases (abbreviated as 5X) (Columns 5 and 6). Following these data, we injected G85-SOD1:YFP mice with the concentrated homogenates from the N139K and V87A cases (Columns 7 and 8). Incubation periods in the first passage from human to G85-SOD1:YFP mice were highly variable, but in the second passage, the time to paralysis became much more consistent (Columns 9 -11). Animals selected for passaging are noted by red symbols. The A4V and N139K seeds generally showed a 2-3 month incubation period. We harvested spinal tissues from 3 of the G85R-SOD1:YFP mice injected with A4V and N139K seeds following secondary passage to produce pooled inoculums (gray dots). None of the five G85R-SOD1:YFP mice injected with concentrated homogenates from the V87A case developed paralysis or showed any inclusion pathology by 12 months of age when they were euthanized (Column 8).

See Supplemental Table S5 for a complete list of all animals used in this study.

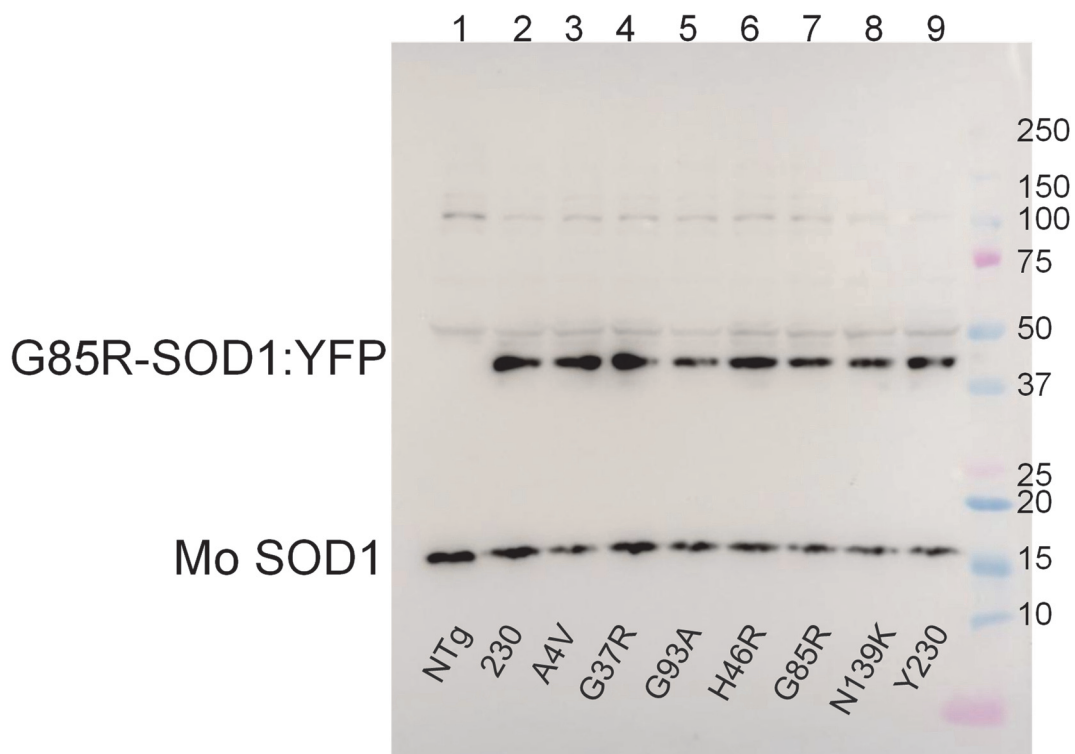


Fig S3. Immunoblot analysis of SOD1 proteins in pooled seeding homogenates. An aliquot of each pooled seeding homogenate was mixed with 4× Laemmli buffer, heated to 95 °C for 5 minutes, and separated on a 4–20% gradient SDS-PAGE gel. Proteins were transferred to nitrocellulose membranes and probed with a rabbit polyclonal antibody against full-length human SOD1. A volume of 4 µL per sample was loaded per lane. Comparable levels of total mutant SOD1 were observed across samples. Homogenate from a nontransgenic mouse provided a negative control.

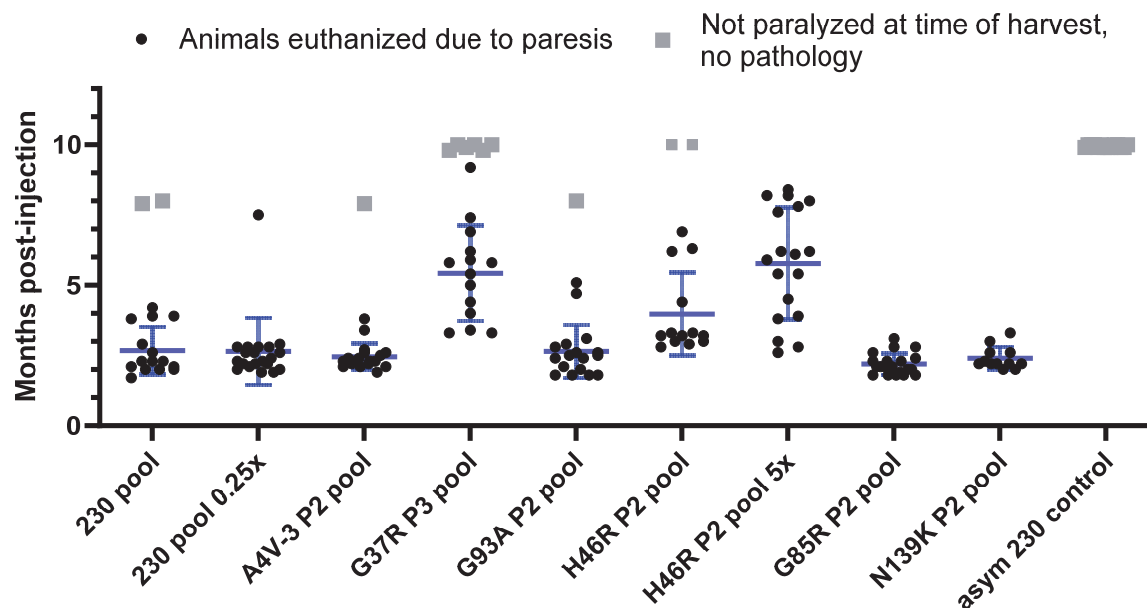


Fig S4. Graphic summary of the timing to euthanasia for all Line 230 mice injected with seeding lysates including asymptomatic mice. Newborn G85R-SOD1:YFP-Line 230 mice (homozygous) were injected intraspinally with pooled spinal homogenates from paralyzed G85R-SOD1:YFP mice that had been seeded with ALS prions derived from seven different sources, as noted in the legend to the x-axis. As a control, Line 230 mice were also injected with spinal homogenates from younger asymptomatic Line 230 mice. None of these animals were symptomatic or exhibited pathology when euthanized at 10 months of age (gray square). Data for mice that were paralyzed are the same as Figure 2. N = 11-20 per genotype, see Supplemental Table S3 for a complete list of all mice used in this study.

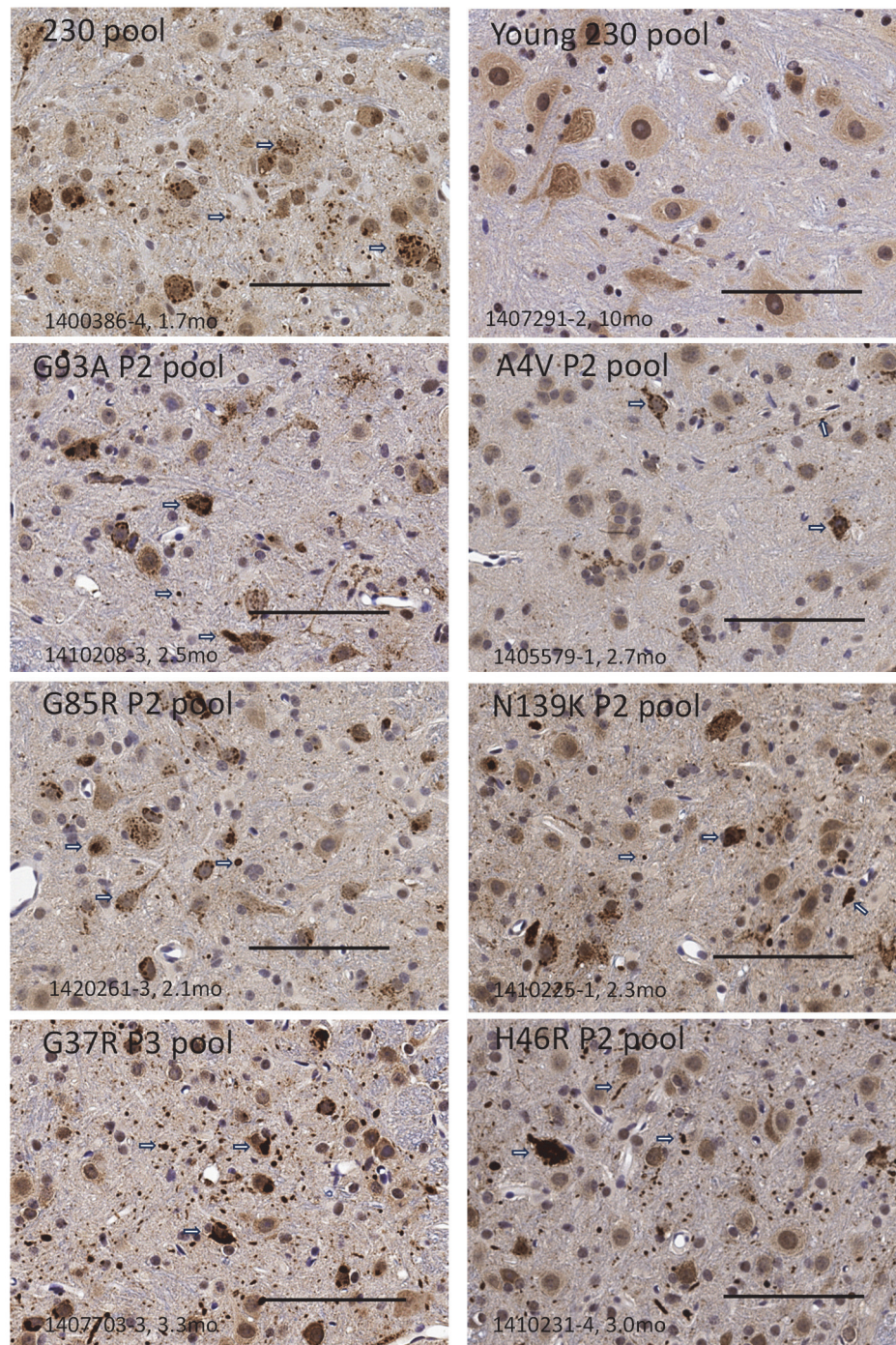


Fig S5. Ubiquitin immunostaining of lumbar spinal tissues from Line 230 mice inoculated with different seeding preparations. Tissues from the lumbar spinal cords of paralyzed mice were embedded in paraffin and sectioned. Fluorescence images were captured, and then the coverslips were removed, and sections were immunostained with antibodies to ubiquitin. Every paralyzed animal was pathologically validated. The images shown are representative of an analysis of 2-3 sections per animal. Scale bar = 100 μ m. N = 11-20 per genotype, see Supplemental Table S3.

Supplemental Table 1. Human demographic data

Dcedent	R/G	Yr of Death	age at DOD	age at onset	PATH ATPSY INTERV	Site of Onset	<u>approx.</u> duration in months	Diagnosis	SOD1
E10-83	F	2010	62	62	NA	Bulbar	5	fALS	A4V
JHU-8	WM	2003	58	54	8HRS	LE's	60	fALS	V87A
JHU-74	WM	2012	47	46	18.5HRS	Bulbar	15	fALS	A4V
JHU-83	BM	2012	50	44	4.5HRS	left leg	74	fALS	N139K

Supplemental Table S2. List of all transgenic mice analyzed in Figure 1

Cohort 1 G85R-SOD1:YFP sciatic nerve injection at ~2 months of age with G37R P1 inoculum

Mouse ID	Sex	Age at harvest (mo)	Symptoms	Post-injection interval to harvest (mo)	YFP aggregates	Silver Stain
0720152-1	F	8.3	Paralyzed	5.6	+++	++
0724367-2	F	8	Paralyzed	5.8	+++	+++
0725429-2	F	8.1	Paralyzed	6	+++	++
0720152-4	F	8.9	Paralyzed	6.1	++	+
0725429-4	F	8.3	Paralyzed	6.2	+++	+++
0720152-3	F	10.7	Paralyzed	8	+++	++
0724368-1	F	11	Paralyzed	8.7	+++	++
0720152-2	F	12.3	Paralyzed	9.5	+++	+++

Cohort 2 G85R-SOD1:YFP sciatic nerve injection at ~2 months of age with G37R P1 inoculum

Mouse ID	Sex	Age at harvest (mo)	Symptoms	Post-injection interval to harvest (mo)	YFP aggregates	Silver staining confirmation	Image used in figure
0851643-1	F	5.5	Healthy	3 (Timed harvest)	+	-	Figure S1
0853162-2	F	5.2	Healthy	3 (Timed harvest)	+/-	-	
0853162-3	F	5.2	Healthy	3 (Timed harvest)	+	-	
0853162-5	F	5.2	Healthy	3 (Timed harvest)	+/-	-	
0853165-2	F	5.3	Healthy	3 (Timed harvest)	-	-	
0853165-3	F	5.3	Healthy	3 (Timed harvest)	-	-	
0849104-5	F	7.5	Both hindlimbs paralyzed	4.8	+++	++	
0849109-1	F	7.5	Both hindlimbs paralyzed	4.8	+++	++	
0849109-2	F	7.5	Both hindlimbs paralyzed	4.8	++	++	
0849110-2	F	7.7	Both hindlimbs paralyzed	4.8	+++	++	

0851627-3	F	7.6	Left hindlimb paralyzed	5 (Timed harvest)	+	+	
0851627-4	F	7.6	Both hindlimbs paralyzed	5 (Timed harvest)	+++	++	
0851631-1	F	7.6	Both hindlimbs paralyzed	5 (Timed harvest)	++	++	
0851631-4	F	7.6	Both hindlimbs paralyzed	5 (Timed harvest)	+++	++	
0849105-5	F	8	Both hindlimbs paralyzed	5.4	+++	++	
0849104-3	F	8.8	Both hindlimbs paralyzed	6	+++	++	
0849105-3	F	8.7	Both hindlimbs paralyzed	6	+++	++	Figure S1

Cohort 3 G85R-SOD1:YFP sciatic nerve injection at ~2 months of age with pooled G37R P2 inoculum

Mouse ID	Sex	Age at harvest (mo)	Symptoms	Post-injection interval to harvest (mo)	YFP aggregates	Silver staining confirmation
1232435-1	F	8.1	Lt. hindlimb paralyzed; Rt. hindlimb very weak	5.9	++	+
1216776-1	F	8.5	Left hindlimb paralyzed; Right hindlimb weak	6	++	++
1234194-3	F	8.2	Lt. hindlimb paralyzed; Rt. hindlimb weak	6.2	+++	++
1216776-2	F	8.9	Both hindlimbs paralyzed	6.5	++	++
1216776-3	F	8.9	Both hindlimbs paralyzed	6.5	++	+
1217784-2	F	9.6	Left hindlimb paralyzed and right hindlimb very weak.	7.2	++	+
1234194-1	F	17.2	Healthy	15.2	-	-
1234194-2	F	17.2	Healthy	15.2	-	-
1217784-1	F	18.8	Healthy	16.3	-	-

Cohort 4 G85R-SOD1:YFP sciatic nerve injection at ~2 months of age with inoculum from asymptomatic nontransgenic mouse

Mouse ID	Sex	Age at harvest (mo)	Symptoms	Post-injection interval to harvest (mo)	YFP aggregates	Silver Stain
0414339-5	F	8.5	labored breath; thin; discovered tumor in chest cavity	6.2	-	-
0628358-3	F	15.5	Not paralyzed	12.5	-	-
0628358-4	F	15.5	Not paralyzed	12.5	-	-
0628358-5	F	15.5	Not paralyzed	12.5	-	-
0629117-2	F	15.3	Not paralyzed	12.5	-	+/-
0629117-3	F	15.3	Not paralyzed	12.5	-	+/-
0414339-1	F	14.9	No paralysis	12.6	-	-
0414339-3	F	14.9	No paralysis	12.6	-	-

0628359-1	F	15.5	Not paralyzed	12.6	-	-
0628359-2	F	15.5	Not paralyzed	12.6	-	-
0628363-2	F	15.6	Not paralyzed	12.6	-	-
0626717-1	F	15.9	Not paralyzed	12.7	-	-
0628364-1	F	15.7	Not paralyzed	12.7	-	-

Supplemental Table S3. List of all G85R-SOD1:YFP Line 230 newborn that received intraspinal injections of pooled inoculum in Figure 2

Inoculum paralyzed Line 230 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1400386-4	F	1.7	Left hindlimb paralyzed	++	+++	++	Figure 3, S5
1400386-2	F	2	Left hindlimb paralyzed	+	+	+	
1400386-3	F	2	Right hindlimb paralyzed	+	+	+	
1400702-3	F	2	Right hindlimb paralyzed	+	+	++	
1400388-4	M	2.1	Left hindlimb paralyzed	+	+	++	
1400388-5	M	2.1	Right hindlimb paralyzed	+	+	+	
1400702-2	F	2.3	Left hindlimb paralyzed	+	+	+	
1400703-4	M	2.3	Both hindlimbs paralyzed	+	+	++	
1400703-5	M	2.3	Left hindlimb paralyzed	+	+	++	
1400388-3	M	2.6	Right hindlimb & Right forelimb paralyzed, Left hindlimb & forelimb weak	+	+	++	
1400703-3	M	2.9	Both hindlimbs very weak	+	+		
1400703-2	M	3.8	Right hindlimb paralyzed	++	+++	++	
1400388-1	M	3.9	Right hindlimb paralyzed	+	+	++	
1400388-2	M	3.9	Left forelimb paralyzed	+	+	++	
1400703-1	M	4.2	right hindlimb paralyzed	+	+++	++	
1400702-1	F	7.9	Healthy	-	-		Excluded from statistical analysis
1400386-1	F	8	Healthy	-	-		Excluded from statistical analysis

Inoculum paralyzed Line 230 pool (0.25x)

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1
1445368-3	F	1.9	Both hindlimbs paralyzed	++	++	+++
1445368-4	F	1.9	Both hindlimbs paralyzed	++	++	+++
1445367-1	F	2	Both hindlimbs & LFL paralyzed	++	++	+++
1445368-2	F	2	Both hindlimbs paralyzed	++	++	+++
1445371-1	F	2.1	Both hindlimbs paralyzed	++	++	+++
1445371-2	F	2.2	Left hindlimb paralyzed	++	++	
1445371-3	F	2.2	Left hindlimb paralyzed	++	++	
1445371-4	F	2.2	Left hindlimb paralyzed	++	++	
1445367-2	F	2.3	Left forelimb paralyzed	++	++	

1445372-1	M	2.3	Left hindlimb paralyzed	++	+	
1445369-1	M	2.4	Both hindlimbs weak	++	+	
1445370-1	F	2.6	Both hindlimbs paralyzed	++	++	
1445370-2	F	2.6	Both hindlimbs paralyzed	++	++	
1445372-2	M	2.6	Both hindlimbs very weak	++	++	
1445369-2	M	2.8	Both hindlimbs paralyzed	++	++	
1445372-3	M	2.8	Left hindlimb paralyzed	++	+	
1445372-4	M	2.8	Left hindlimb paralyzed	++	++	
1445372-5	M	2.8	Right hindlimb paralyzed	++	++	
1445368-1	F	2.9	Left hindlimb paralyzed	++	++	
1445369-3	M	7.5	Both hindlimbs weak	++	+	

Inoculum paralyzed A4V-3 P2 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1404548-4	F	1.9	Left hindlimb paralyzed	++	+	++	
1404548-2	F	2.1	Both hindlimbs very weak	+	+	++	
1404548-3	F	2.1	Right hindlimb paralyzed	+	+	+	
1404550-4	M	2.1	Both hindlimbs very weak	+	+	+	
1405580-4	F	2.2	Left hindlimb paralyzed & Right Hindlimb weak	+	+	++	
1405581-4	M	2.2	RHO paralyzed & Left hindlimb very weak	+	+	++	
1405579-2	F	2.3	Left hindlimb paralyzed	++	+	+++	
1405580-3	F	2.3	Left hindlimb paralyzed	+	+	++	
1405581-2	M	2.3	Both hindlimbs very weak	+	+	+	
1405581-3	M	2.3	Right hindlimb paralyzed	++	+	+	
1404549-2	M	2.4	Both hindlimbs paralyzed	++	++	++	
1405580-2	F	2.4	BHL very weak	++	+	++	
1404550-2	M	2.5	Left hindlimb paralyzed & Right hindlimb very weak	+	+	+	
1404550-3	M	2.5	No tissue collected, but ALS symptomatic				Excluded from statistical analysis
1404550-1	M	2.6	Right hindlimb paralyzed	+	+	+	
1405580-1	F	2.6	Left hindlimb paralyzed (walk fast)	+	+	++	
1405579-1	F	2.7	All limbs weak, especially Right forelimb (eye glued)	++	+	++	Figure 3, S5
1404548-1	F	3.4	Right hindlimb paralyzed	++	++	++	
1405581-1	M	3.8	Both hindlimbs weak	+	++	++	
1404549-1	M	7.9	HEALTHY	-	-		Excluded from statistical analysis

Inoculum paralyzed G37R P3 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1407703-4	F	2.9	NOT ALS!				Excluded from statistical analysis
1407697-2	F	3	Found dead				Excluded from statistical analysis
1407703-3	F	3.3	all limbs weak	+++	+++	+++	Figure 3, S5
1424973-1	F	3.3	Both hindlimbs paralyzed	++	++	+++	
1424973-2	F	3.4	Both hindlimbs paralyzed	++	++	++	
1424973-5	F	4	Right forelimb paralyzed & all other limbs very weak	++	++	+++	
1424973-3	F	4.4	Both hindlimbs very weak	++	++	+++	
1407703-2	F	5	Both hindlimbs very weak	+++	++	+++	
1407701-1	F	5.4	Both hindlimbs paralyzed	+++	++	+++	
1407699-2	M	5.8	Left hindlimb very weak	++	++	+++	
1407703-1	F	5.8	Both hindlimbs very weak	+++	++	+++	
1424973-4	F	5.9	LFL very weak	+++	+++	+++	
1424974-3	M	6.2	No ALS	+++	+++		
1424974-2	M	6.9	Both hindlimbs weak	++	++	+++	
1407699-4	M	7.4	Right forelimb very weak and Both hindlimbs weak	+++	+++	+++	
1407699-1	M	9.2	Both eyes closed, Move slowly, All limbs starting to get weak (no paralysis)	++	+++	+++	
1407697-1	F	9.8	Healthy	-	-		Excluded from statistical analysis
1407699-3	M	9.8	Healthy	-	-		
1407701-2	F	9.9	Healthy	-	-		
1424974-1	M	10	Healthy	-	-		
1424981-1	F	10	Healthy	-	-		
1424981-2	F	10	Healthy	-	-		

Inoculum paralyzed G93A P2 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1410208-4	F	1.8	Right hindlimb almost completely paral. & Left hindlimb weak	++	+	++	
1410209-5	M	1.8	BHL very weak	++	+	+	
1424680-1	M	1.8	Left hindlimb paralyzed	++	+++	+++	
1424680-2	M	1.8	Left hindlimb almost paralyzed	++	++	++	
1424680-3	M	2	All limbs paralyzed & too weak to stand up	++	++	++	
1424680-4	M	2.1	Right hindlimb paralyzed	++	++	+++	

1410206-2	F	2.4	BFL very weak & BHL weak	++	++	++	
1424681-1	M	2.4	Both hindlimbs weak	+++	++	++	
1410208-3	F	2.5	Right forelimb paralyzed & LFL weak	++	+	++	Figure 3, S5
1410209-4	M	2.5	BHL paralyzed	++	++	++	
1410208-2	F	2.6	Thin & all limbs very weak	++	+	++	
1424681-2	M	2.6	Left hindlimb paralyzed	++	+	++	
1410209-3	M	2.8	Right forelimb & Left hindlimb weak	++	++	++	
1410206-1	F	2.9	Both hindlimbs very weak	++	++	++	
1410209-2	M	3.1	All limbs very weak	++	++	++	
1410209-1	M	4.7	All limbs very weak	+	+	++	
1424681-3	M	5.1	Right hindlimb paralyzed	++	+	++	
1410208-1	F	8	Healthy	-	-		Excluded from statistical analysis

Inoculum paralyzed H46R P2 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1409659-4	F	2.8	Right hindlimb paralyzed	+++	+++	+++	
1409659-3	F	2.9	Both hindlimbs very weak	+++	++	++	
1410231-4	F	3	Left forelimb paralyzed and all others weak	+++	+++	+++	Figure 3, S5
1410231-5	F	3	Left forelimb paralyzed and all others weak	+++	+++	+++	
1410230-1	F	3.2	Both hindlimbs very weak	+++	+++	+++	
1410230-2	F	3.2	Both hindlimbs very weak	+++	+++	+++	
1410231-3	F	3.2	Both hindlimbs very weak	+++	+++	+++	
1409659-2	F	3.3	Both hindlimbs very weak	+++	++	+++	
1409660-3	M	3.3	Right hindlimb paralyzed	+++	+++	+++	
1410231-2	F	4.4	Left forelimb and left hindlimb paralyzed	+++	+++	+++	
1410231-1	F	6.2	Both hindlimbs very weak	++	+++	+++	
1409659-1	F	6.3	Right forelimb paralyzed	+++	++	+++	
1410220-1	M	6.8	Injured, NO ALS	-	-		
1409660-2	M	6.9	Both hindlimbs very weak	++	++	+++	
1409660-1	M	9.8	OK but walk slowly	++	++		Excluded from statistical analysis
1410220-2	M	9.8	Healthy	-	-		

Inoculum paralyzed H46R P2 pool 5X

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Staining	Notes
1458555-3	M	1.7	Euthanized fighting			Excluded from statistical analysis
1461371-1	F	2.6	Both hindlimbs very weak	+++	+++	
1461371-2	F	2.8	Both hindlimbs paralyzed	+++	+++	

1461371-3	F	3	Both hindlimbs paralyzed	+++	+++	
1448510-3	F	3.7	Found dead			Excluded from statistical analysis
1458519-1	F	3.8	Both hindlimbs very stiff	+++	+++	
1448510-1	F	3.9	Right hindlimb weak	+++	+++	
1461372-1	M	4.5	Both hindlimbs and Right forelimb weak	+++	+++	
1461372-2	M	5.4	Both hindlimbs very weak and stiff still walking	+++	+++	
1461372-3	M	5.4	Both hindlimbs very weak and stiff still walking	++	+++	
1458519-2	F	5.9	All limbs weak & stiff	+++	+++	
1448510-2	F	6.1	Both hindlimbs very weak	+++	+++	
1448528-1	M	6.2	Both hindlimbs weak and stiff	+++	+++	
1458555-1	M	6.2	Both hindlimbs weak	++	++	
1448528-2	M	7.6	Both hindlimbs weak, stiff	++	+++	
1448524-1	M	7.8	Both hindlimbs very stiff	+++	+++	
1448528-3	M	8	All limbs weak & stiff	++	+++	
1448524-2	M	8.2	Both hindlimbs very stiff	+++	+++	
1448528-4	M	8.2	Both hindlimbs weak & stiff	++	+++	
1458555-2	M	8.4	BHL PARESIS, Perianal soiling, Porphyrin	+++	++	

Inoculum paralyzed G85R P2 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1420260-1	F	1.8	Both hindlimbs paralyzed	++	++	+++	
1420260-2	F	1.8	Both hindlimbs very weak	++	+	++	
1420261-1	F	1.8	Both hindlimbs paralyzed	++	+	++	
1420261-2	F	1.8	Both hindlimbs very weak	++	+	++	
1420283-1	F	1.8	Right hindlimb almost paralyzed	++	+	++	
1420283-2	F	1.9	Right hindlimb paralyzed	++	+	+++	
1420290-1	F	2	Left hindlimb paralyzed	++	+	+++	
1420290-2	F	2	Left hindlimb almost paralyzed	++	+	++	
1420261-3	F	2.1	Right hindlimb paralyzed	++	+	++	Figure 3, S5
1420284-1	M	2.1	Both hindlimbs very weak	++	+	++	
1420284-2	M	2.1	Both hindlimbs weak	++	+	++	
1420290-3	F	2.1	Both hindlimbs very weak	++	+	+++	
1420261-4	F	2.3	Right hindlimb paralyzed	++	+	+++	
1420266-2	M	2.3	Right hindlimb paralyzed & all others weak	++	+	++	
1420283-3	F	2.3	All limbs weak (lying on side, eyes closed)	++	+	+++	
1420284-3	M	2.4	Both hindlimbs weak	++	+	++	
1420266-1	M	2.6	All hindlimbs weak and stiff	++	+	++	
1420284-4	M	2.8	Both hindlimbs paralyzed	++	++	+++	
1420290-4	F	2.8	Both hindlimbs paralyzed	++	+	+++	
1420290-5	F	3.1	Both hindlimbs very weak	++	++	+++	

Inoculum paralyzed N139K P2 pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver stain	UBi-1	Notes
1410226-4	F	2	Found dead				Excluded
1410228-3	F	2	Right hindlimb paralyzed, otherwise very healthy	+	+	++	
1410228-4	F	2	Right hindlimb paralyzed & Left hindlimb very weak	++	+	++	
1410228-5	F	2	F.D. (ALS unknown)				Excluded
1410225-2	F	2.2	All limbs very weak	++	+	++	
1410226-1	F	2.2	Right hindlimb almost paralyzed & all limbs weak	++	+	++	
1410226-2	F	2.2	Right hindlimb almost paralyzed & all limbs weak	++	+	++	
1410226-3	F	2.2	All limbs very weak	++	+	++	
1410228-2	F	2.2	Left forelimb paralyzed	++	+	++	
1410225-1	F	2.3	LFL paralyzed & other limbs weak	++	+	++	Figure 3, S5
1410227-2	M	2.6	Right hindlimb paralyzed	++	+	+	
1410229-1	M	2.6	Right hindlimb paralyzed	++	+	++	
1410228-1	F	3	Right hindlimb paralyzed	++	+	++	
1410227-1	M	3.3	Left hindlimb paralyzed, but general good shape	++	++	++	

Inoculum Young 230 (Y230) pool

Mouse ID	Sex	Age	Symptoms	YFP aggregates	Silver Stain	UBi-1	Notes
1407291-4	M	4.9	Fight wounds				Excluded
1407291-1	M	9.9	Healthy	-		-	
1407291-2	M	9.9	Healthy	-	-	-	Figure 3, S5
1407291-3	M	9.9	Healthy	-		-	
1407292-1	F	9.9	Healthy	-		-	
1407292-2	F	9.9	Healthy	-	-	-	
1407292-3	F	9.9	Healthy	-	-	-	
1407292-4	F	9.9	Healthy	-	-	-	
1406584-1	M	10	Healthy	-		-	
1406584-2	M	10	Healthy	-		-	
1406584-3	M	10	Healthy	-	-		
1406585-1	F	10	Healthy	-		+/-	
1406585-2	F	10	Healthy	-		-	
1406585-3	F	10	Healthy	-		-	
1406585-4	F	10	Healthy	-		-	

Supplemental Table S4. Complete list of statistical comparisons for Figure 2.

Inoculum details	Geometric mean 1	Geometric mean 2	Ratio	SE of log (ratio)	Summary	Adjusted P Value
230 pool vs. 230 pool 0.25x	2.561	2.519	1.017	0.04476	ns	>0.9999

230 pool vs. A4V-3 P2 pool	2.561	2.420	1.058	0.03756	ns	>0.9999
230 pool vs. G37R P3 pool	2.561	5.186	0.4938	0.04950	****	<0.0001
230 pool vs. G93A P2 pool	2.561	2.535	1.010	0.04679	ns	>0.9999
230 pool vs. H46R P2 pool	2.561	3.769	0.6795	0.05152	ns	0.1157
230 pool vs. H46R P2 pool 5x	2.561	5.410	0.4734	0.05198	****	<0.0001
230 pool vs. G85R P2 pool	2.561	2.166	1.182	0.03697	ns	0.8445
230 pool vs. N139K P2 pool	2.561	2.388	1.072	0.03948	ns	>0.9999
230 pool vs. asym 230 control	2.561	9.950	0.2574	0.03331	****	<0.0001
230 pool 0.25x vs. A4V-3 P2 pool	2.519	2.420	1.041	0.03459	ns	>0.9999
230 pool 0.25x vs. G37R P3 pool	2.519	5.186	0.4857	0.04729	****	<0.0001
230 pool 0.25x vs. G93A P2 pool	2.519	2.535	0.9936	0.04444	ns	>0.9999
230 pool 0.25x vs. H46R P2 pool	2.519	3.769	0.6683	0.04940	ns	0.0627
230 pool 0.25x vs. H46R P2 pool 5x	2.519	5.410	0.4656	0.04988	****	<0.0001
230 pool 0.25x vs. G85R P2 pool	2.519	2.166	1.163	0.03395	ns	0.8754
230 pool 0.25x vs. N139K P2 pool	2.519	2.388	1.055	0.03667	ns	>0.9999
230 pool 0.25x vs. asym 230 control	2.519	9.950	0.2531	0.02992	****	<0.0001
A4V-3 P2 pool vs. G37R P3 pool	2.420	5.186	0.4666	0.04054	****	<0.0001
A4V-3 P2 pool vs. G93A P2 pool	2.420	2.535	0.9546	0.03718	ns	>0.9999
A4V-3 P2 pool vs. H46R P2 pool	2.420	3.769	0.6421	0.04298	*	0.0128
A4V-3 P2 pool vs. H46R P2 pool 5x	2.420	5.410	0.4473	0.04354	****	<0.0001
A4V-3 P2 pool vs. G85R P2 pool	2.420	2.166	1.117	0.02367	ns	0.8215
A4V-3 P2 pool vs. N139K P2 pool	2.420	2.388	1.013	0.02742	ns	>0.9999
A4V-3 P2 pool vs. asym 230 control	2.420	9.950	0.2432	0.01739	****	<0.0001
G37R P3 pool vs. G93A P2 pool	5.186	2.535	2.046	0.04921	****	<0.0001
G37R P3 pool vs. H46R P2 pool	5.186	3.769	1.376	0.05373	ns	0.4249
G37R P3 pool vs. H46R P2 pool 5x	5.186	5.410	0.9586	0.05418	ns	>0.9999
G37R P3 pool vs. G85R P2 pool	5.186	2.166	2.394	0.04000	****	<0.0001
G37R P3 pool vs. N139K P2 pool	5.186	2.388	2.171	0.04232	****	<0.0001
G37R P3 pool vs. asym 230 control	5.186	9.950	0.5212	0.03663	***	0.0001
G93A P2 pool vs. H46R P2 pool	2.535	3.769	0.6726	0.05124	ns	0.0919
G93A P2 pool vs. H46R P2 pool 5x	2.535	5.410	0.4686	0.05171	****	<0.0001
G93A P2 pool vs. G85R P2 pool	2.535	2.166	1.170	0.03659	ns	0.8988
G93A P2 pool vs. N139K P2 pool	2.535	2.388	1.061	0.03912	ns	>0.9999
G93A P2 pool vs. asym 230 control	2.535	9.950	0.2548	0.03287	****	<0.0001
H46R P2 pool vs. H46R P2 pool 5x	3.769	5.410	0.6966	0.05603	ns	0.2833
H46R P2 pool vs. G85R P2 pool	3.769	2.166	1.740	0.04247	**	0.0014
H46R P2 pool vs. N139K P2 pool	3.769	2.388	1.578	0.04467	*	0.0125
H46R P2 pool vs. asym 230 control	3.769	9.950	0.3788	0.03932	****	<0.0001
H46R P2 pool 5x vs. G85R P2 pool	5.410	2.166	2.498	0.04303	****	<0.0001
H46R P2 pool 5x vs. N139K P2 pool	5.410	2.388	2.265	0.04520	****	<0.0001
H46R P2 pool 5x vs. asym 230 control	5.410	9.950	0.5437	0.03992	***	0.0002
G85R P2 pool vs. N139K P2 pool	2.166	2.388	0.9068	0.02661	ns	0.9764
G85R P2 pool vs. asym 230 control	2.166	9.950	0.2177	0.01608	****	<0.0001
N139K P2 pool vs. asym 230 control	2.388	9.950	0.2400	0.02122	****	<0.0001

Supplemental Table S5. List of all G85R-SOD1:YFP mice in Supplemental Figure S2 that received intraspinal injections as newborns to propagate misfolded SOD1.

Column 1: Line 230 inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
1229027-1	F	2.4	Left hindlimb paralyzed	+	+		
1218525-3	F	2.5	Both hindlimbs paralyzed	+	+	+	Seeds for 230 pool (2024)
1218534-3	M	2.5	Right hindlimb paralyzed	+	+	+	Seeds for 230 pool (2024)
1234716-1	F	2.5	Both hindlimbs paralyzed	+	+	+	
1234716-2	F	2.5	Both hindlimbs paralyzed	+	+	+	
1218525-2	F	3	Both hindlimbs paralyzed	+	+	+	Seeds for 230 pool (2024)
1218534-1	M	3.1	Both hindlimbs paralyzed	+	+	+	Seeds for 230 pool (2024)
1229032-2	M	3.2	Both hindlimbs very weak	+	+	+	
1234715-1	M	3.2	Both hindlimbs paralyzed	+	+		
1234715-2	M	3.6	Right hindlimb paralyzed.	+		+	
1229032-1	M	3.9	Right hindlimb paralyzed	+	+/-		
1218525-1	F	5.8	not symptomatic				no tissue, excluded
1218534-2	M	5.8	not symptomatic				no tissue, excluded

Column 2: P1 H46R inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
1175410-1	M	5.3	Both hindlimbs paralyzed	+	+	+	Seeds for H46R pool (2024)
1175410-3	M	5.5	not symptomatic				No tissue, excluded
1185120-1	F	5.6	Both hindlimbs paralyzed	+	+	+	Seeds for H46R pool (2024)
1170718-1	M	6.2	Both hindlimbs paralyzed	+	+	+	Seeds for H46R pool (2024)
1175408-2	F	6.4	Both hindlimbs very weak.	+	+	+	Seeds for H46R pool (2024)
1175407-5	M	6.7	Both hindlimbs paralyzed	+	+	+	
1185119-1	F	7	Right hindlimb paralyzed	+	+		
1175407-2	M	7.6	Both hindlimbs very weak	+	+	+	
1185118-1	M	8.2	Healthy	-	-	-	
1185118-2	M	8.2	Healthy	-	+/-	+/-	
1185119-2	F	8.2	Healthy	-	+/-	+/-	
1185119-3	F	8.2	Healthy	-	-	-	
1185120-2	F	8.2	Healthy	-	-	-	
1185121-1	M	8.2	Healthy	-	-	-	
1185122-1	M	8.3	Healthy	-	-	-	
1185123-1	F	8.3	Healthy	-	-	-	

1175410-2	M	11.7	Healthy	-	-		
1175410-4	M	11.7	Healthy	-	-		
1175410-5	M	11.7	Healthy	-	-		
1175411-5	F	11.7	Healthy	-	-		
1171667-1	M	12.2	Healthy	-	-		
1171668-1	F	12.2	Healthy	-	-		

Column 3: E10-83 (A4V-2) inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Notes
0643460-1	F	23.1	No paralysis	-	-	
0643460-2	F	23.1	No paralysis	-	-	
0643461-1	F	2.6	Self-mutilation			Excluded
0654437-1	F	24.0	No paralysis	-		
0660564-1	F	12.0	Paralyzed	++	++	

Column 4: JHU-74 (A4V-3) inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain
0628369	F	2.1	eye problems	ND	ND
0628369-1	F	24.2	No paralysis	-	-
0628371-1	F	15.0	Tumor; No paralysis	-	-
0628371-2	F	18.0	Tumor; No paralysis	-	-
0628371-3	F	24.6	No paralysis	-	-
0632880-1	F	24.0	No paralysis	-	-
0632880-2	F	12.2	Self-mutilation	ND	-
0632880-3	F	24.0	No paralysis	-	-

Column 5: 5X - E10-83 (A4V-2) inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
0767085-1	M	3.7	Found dead - 7/21/17				Excluded
0767084-2	F	4.4	Forelimbs paralyzed; hindlimbs weak	++	+		
0755555-4	F	5.5	Paralyzed	++	++	+++	
0755555-2	F	5.7	Paralyzed	++	++	+++	
0755555-1	F	7.5	Paralyzed	++	++	+++	
0767085-4	M	7.6	Paralyzed	+++	++	+++	
0767085-3	M	8	Paralyzed	++	++	+++	
0755555-3	F	12.7	Paralyzed	++	+	+++	
0767085-2	M	14.5	open wound	-	-	-	
0767084-1	F	18.2	Sac for breathing difficulty	-	+/-	++	
0767084-3	F	18.2	Sac for breathing difficulty	-	+/-	+	

Column 6: 5X - JHU-74 (A4V-3) inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
0762920-1	F	3.2	Paralyzed	+	Yes	+++	
0762920-3	F	4.2	Paralyzed	+	Yes	++	
0762920-2	F	5.6	Paralyzed	+	Yes	++	
0762920-4	F	7.1	Paralyzed	++	Yes	+++	
0762921-1	M	8	Paralyzed	+	Yes	+++	
0762921-2	M	9.4	Paralyzed	+	Yes	+++	
0762920-5	F	9.8	Paralyzed Front L	+	Yes	+++	

Column 7: 5X - JHU-83 (N139K) inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
0851647-2	M	2.7	Found dead - 5/21/18				Excluded
0851644-1	M	5.6	Paralyzed (Both)	+	+	+++	
0865527-2	M	5.8	Per ACS- Fight Wounds	+	+		Used in Pool for Inoculum
0865527-4	M	5.8	Per ACS- Fight Wounds	+	-	+	
0851645-1	F	6	Paralyzed (Both)	++	++	+++	
0851646-1	F	6	Paralyzed (Both)	++	++	+++	
0865527-1	M	6.8	Paralyzed (P++→L P++→R) (SpC Homogenate Mix for Inj., No PIC)	++	++	+++	Used in Pool for Inoculum
0865527-3	M	7.6	Paralyzed (P++→L P+→R) (SpC Homogenate Mix for Inj., No PIC)	++	++	+++	
0865527-5	M	7.6	Paralyzed (P++→L P++→R) (SpC Homogenate Mix for Inj., No PIC)	++	+	+++	Used in Pool for Inoculum
0851647-1	M	7.9	Paralyzed (Both)	++	++	+++	

Column 8: 5X JHU-6 (V87A)

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain
877940-3	F	12.0	Healthy	-	-
877940-4	F	12.0	Healthy	-	-
877944-1	M	12.0	Healthy	-	-
877944-3	M	12.0	Healthy	-	-
877944-4	M	12.0	Healthy	-	-

Column 9: P1 A4V-2 inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
1225230-2	M	3	Right hindlimb paralyzed	+	+	+	
1225231-2	F	3	Left forelimb and left hindlimb paralyzed	+	+		
1178466-2	F	3.1	Lt. hindlimb paralyzed; Rt. hindlimb weak	+	+	+	

1178467-1	M	3.5	Both hindlimbs paralyzed	+	+	+	
1178466-1	F	5.2	not symptomatic				no tissue, excluded
1225230-1	M	5.8	not symptomatic				no tissue, excluded

Column 10: P1 A4V-3 inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
1179528-4	M	2.7	Both hindlimbs paralyzed	+	+	+	A4V-3 pool
1179528-3	M	2.8	Both hindlimbs paralyzed	+	+		A4V-3 pool
1179528-2	M	3.3	Lt. hindlimb paralyzed; Rt. hindlimb severe paresis	+	+	+	A4V-3 pool
<i>1179535-1</i>	<i>F</i>	<i>4.1</i>	<i>Both hindlimbs very weak; Right forelimb paralyzed</i>	+	+	+	
1179535-2	F	5.2	not symptomatic				No tissue, excluded

Column 11: P1 N139K inoculum

Mouse ID	Sex	Age (mo)	Symptoms	YFP aggregates	Silver Stain	Ubi-1	Notes
1203935-2	F	2.5	Both hindlimbs paralyzed	+	+	+	Seeds for N139K pool (2024)
1203934-1	M	3.1	Both hindlimbs paralyzed	+	+	+	Seeds for N139K pool (2024)
1186408-2	M	3.2	Both hindlimbs paralyzed (Bad perfusion)	+	+	+	Seeds for N139K pool (2024)
1185124-1	M	3.4	Both hindlimbs paralyzed	+	+	+	
1217377-2	M	3.9	Left hindlimb paralyzed	+	+		
1186408-1	M	5.7	Both hindlimbs paralyzed	+	+	+	
1203933-2	F	5.7	Right hindlimb paralyzed	+	+	+	
1186409-2	F	6	Left hindlimb paralyzed; Right hindlimb weak	+	+	+	
1217377-1	M	6.2	not symptomatic				no tissue, excluded
1203910-1	M	7.5	Healthy	-	-		
1203910-2	M	7.5	Healthy	+/-	-		
1203933-1	F	7.5	Healthy	++	-		
1203934-2	M	7.5	Healthy	+/-	-		
1203935-1	F	7.5	Healthy	-	-		
1203935-3	F	7.5	Healthy	-	-		
1186409-1	F	8	Healthy	-	-	-	