Supporting Information

Preparation of SOD1 calibration curve

An SOD1 dilution series was prepared from solutions of recombinantly purified H48Q and WT human SOD1 (protein concentrations were determined by absorbance at 280 nm). These solutions were analyzed with LC-ESI-MS using chromatographic and ESI-MS parameters that are identical to those used for LC-ESI-MS analyses of S1, P2, and P3 samples from transgenic mouse spinal cord. An SOD1 ion extract was performed on the most abundant molecular ion for each protein sample followed by a numerical integration of the ion extract. The resulting integral value was plotted against SOD1 concentration (in terms of micrograms loaded onto the LC column). SOD1 ion extractions from S1, P2 and P3 chromatograms were similarily performed and numerically integrated. The total amount of SOD1 loaded onto the column from each sample was determined mathematically from the calibration curve.

MS/MS identification of proteins contained in detergent-resistant pellets (DRPs)

10 uL aliquots from fractions separated during LC-ESI-MS were transferred into 1.5 ml microcentrifuge tubes. DTT (15 µL, 10 mM DTT in 50 mM ammonium bicarbonate; USB brand) was added and the mixture was incubated at 37 °C for 60 minutes. Iodoacetamide (15 µL, 55 mM in 50 mM ammonium bicarbonate; Sigma) was added and the mixture incubated for another hour at 37 °C. Lastly, 12.5 uL of 6ng/uL trypsin in 50mM ammonium bicarbonate was added and incubated at 37 °C for 3 hours. After incubation, samples were dried down by centrifugal evaporation.

Samples were analyzed by nano LC-MS/MS with data-dependent acquisition (Q STAR XL; Applied Biosystems, Foster City, CA) after dissolution in 10 µl of 0.1% formic acid, 5% acetonitrile (v/v) using either a split-flow HPLC (Ultimate, Dionex, CA) or a direct pumping HPLC (Eksigent, CA). Samples were loaded to a trapping column (5 uL/min) previously equilibrated for 20 min with 100% A (A, 0.1% formic acid, 5% acetonitrile in water; B, 0.1% formic acid in acetonitrile) using an independent pump (Switchos on Ultimate or micropump on Eksigent). Ten minutes after sample loading the flow was switched (10-port valve, Switchos or Eksigent) such that the trap was back flushed by the nano-HPLC system. An analytical capillary reverse-phase column (75 µm x 10 cm; C18 5 µm, 300 Å; Microtech, CA) was equilibrated for 20 min at 300 nL/min. with 100% A (A, 0.1% formic acid, 5% acetonitrile in water; B, 0.1% formic acid in acetonitrile) prior to the switching event and initiation of a compound linear gradient ramping to 80% A, 20% B at 8 min; 65% A, 35% B at 30 min; 25% A, 75% B at 40 min; and 100% A at 41 min. The analytical column flow was directed to a stainless steel nano-electrospray emitter (ES301; Proxeon, Odense, Denmark) at 2.5 kV for ionization without nebulizer gas. The mass spectrometer was operated in information-dependent acquisition mode with a survey scan (*m*/*z* 400–1500), data-dependent MS/MS on the two most abundant ions with exclusion after two MS/MS experiments. Individual sequencing experiments were matched to a global protein sequence database (MSDB) using Mascot software (Matrix Sciences, London, UK). The search was run under the "trypsin" mode to identify only tryptic peptides with fixed modification of Cys to carboxyamidomethylated form (+57 Da). Variable modification of methionine to sulfoxide (+16 Da) was included. The results of Mascot searches were manually scrutinized in the case of marginal peptide hits.

Preparation of anti-hSOD1 immunoaffinity media

The anti-hSOD1 immunoaffinity Sepharose media was prepared as followed. First, rabbit polyclonal antiserum was raised against hWT SOD1. Polyclonal antibodies were chosen over

monoclonal forms because a variety of SOD1 structures (i.e. native, unfolded, aggregated etc.) and a variety of mutant proteins can be expected to be bound by polyclonal antibodies. The hWT SOD1 used to generate the immunoglobulins was purified from recombinant yeast, as previously described (3). To purify anti-SOD1 immunoglobulins, the anti-serum was purified with immuno-affinity (IA) chromatography using a Sepharose matrix that contained immobilized hSOD1 protein. The immobilized-hSOD1 media was prepared following manufacturer instructions (Amersham Biosciences). Briefly, ~30 mg of hWT and E100K mutant SOD1 in 100 mM bicarbonate buffer, pH 10.0, was mixed with 2 g of wet CNBr-Sepharose beads and incubated on a rotary shaker at room temperature. The coupling reaction was monitored by the change in optical absorption (280 nm) of the mobile phase until maximum coupling was achieved. Polypeptides absorb at 280 nm, so as the protein covalently attaches to the media, the mobile phase will decrease in absorbance at 280 nm. The remaining active CNBr groups on the Sepharose media were blocked by treatment with 0.2 M glycine. The media was washed with 10 volumes of PBS (phosphate buffered saline) by repetitive centrifugation and resuspension in PBS. The SOD1-Sepharose matrix was then mixed with the anti-SOD1 rabbit serum, diluted 1:1 with PBS, and incubated overnight at 4 °C on a rotary shaker. The SOD1-Sepharose beads were packed into a plastic column (0.5 × 2.5 cm) and washed extensively with PBS until the absorbance of the effluent (280 nm) reached a baseline. The anti-SOD1 antibodies were eluted from the SOD1-Sepharose matrix with 0.1 M glycine-HCl buffer, pH 2.8; 0.7 mL fractions were collected directly into the microcentrifuge tubes containing 0.5 mL of 0.5 M phosphate buffer, pH 7.4, to immediately neutralize the pH of the eluent. The purified antibodies were concentrated and used to construct an anti-SOD1 antibody Sepharose matrix according to the same procedure that is described above for the generation of the SOD1-Sepharose matrix.

MS/MS identification of proteins co-eluting with SOD1 in chromatographic fractions

Tryptic digests were analyzed by μLC-MS/MS with data-dependent acquisition using an LCQ-DECA ion trap-quadrupole ESI-MS (ThermoFinnigan, San Jose, California). A reverse-phase column (200 μm x 10 cm; PLRP/S 5 μm, 300 Å; Michrom Biosciences, San Jose) was equilibrated for 10 minutes at 1.5 μL/min with 95% solution A (0.1% formic acid in water) and 5% B (0.1% formic acid in acetonitrile) prior to sample injection. Following sample injection, a linear gradient was initiated (1.5μL/min): time/%B; 0/5, 10/5, 50/50, 65/80. Column eluent was directed to a coated glass electrospray emitter (TaperTip, TT150-50-50-CE-5, New Objective) at 3.3 kV for ionization without nebulizer gas. The mass spectrometer was operated in 'triple-play' mode with a survey scan (400-1500 m/z), data-dependent zoom scan and MS/MS. Individual sequencing experiments were matched to a custom mouse protein database, downloaded from NCBI (www.ncbi.nlm.nih.gov) using Sequest™ software (ThermoFinnigan, San Jose). This database contained the sequence of mouse SOD1 and all pertinent ALS mutant SOD1 proteins. The search was conducted without specification of the protein cleavage reagent the 'no enzyme' mode to identify non-tryptic peptides. Attention was focused on identifications returned with X_{corr} values greater than 3.0.

1. Wang, J., Xu, G., Li, H., Gonzales, V., Fromholt, D., Karch, C., Copeland, N. G., Jenkins, N. A., and Borchelt, D. R. (2005) *Hum Mol Genet* **14**(16), 2335-2347
2. Wang, J., Slunt, H., Gonzales, V., Fromholt, D., Coonfield, M., Copeland, N. G., Jenkins, N. A., and Borchelt, D. R. (2003) *Hum Mol Genet* **12**(21), 2753-2764
3. Rodriguez, J. A., Valentine, J. S., Eggers, D. K., Roe, J. A., Tiwari, A., Brown, R. H., Jr., and Hayward, L. J. (2002) *J Biol Chem* **277**(18), 15932-15937

Supplemental Table 1. A complete list of proteins that sedimented with SOD1 in detergent resistant pellets organized by subcellular localization.

Identified Proteins (organized by subcellular localization)	H46R/ H48Q (1)[a]	G37R (1)	G93A (1)	hWT (1)	nTG (1)	Combined # of protein ms/ms ion instances[b]
Mitochondrial						
ATP synthase, H+ transporting, mitochondrial						1
mitochondrial import stimulation factor S1 chain	1/1					9
ubiquinol cytochrome c oxidoreductase						1
Ubiquinol-cytochrome c reductase core protein	1/1					2
NADH dehydrogenase (ubiquinone)	1/1	1/1				4
Dihydrolipoamide Acetyltransferase						8
dihydrolipoamide dehydrogenase						3
malate dehydrogenase	1/1					15
Glutamate oxaloacetate transaminase	1/1					4
Succinyl-CoA synthetase						2
Veph-B protein	1/1					2
Nuclear						
2',3'-cyclic-nucleotide 3'-phosphodiesterase (EC 3.1.4.37) 2, brain - mouse	1/1					25
ATP-dependent RNA helicase DDX3X						2
Glutamic acid-rich region profile/Arginine-rich region protein						2
matrin 3						2
Msx2-interacting protein		1/1				3
Setd2 protein	1/1					2
Vitamin D response element binding protein		1/1				3
Cytoplasm/ Metabolic						
aspartate transaminase	1/1					7
Dgkd protein		1/1				2
fructose-bisphosphate aldolase						2
malate dehydrogenase	1/1					15
Glutamate oxaloacetate transaminase	1/1					4
Glyceraldehyde-3-phosphate dehydrogenase	1/1					16
Glycogen phosphorylase	1/1					3
L-lactate dehydrogenase	1/1					11
Phosphoglycerate kinase 1	1/1					2
phosphopyruvate hydratase	1/1					16
Selenoprotein T	1/1					2
Tyrosine 3-monooxygenase/tryptophan 5 -monooxygenase activation protein	1/1					8
Cu, Zn Superoxide Dismutase	1/1	1/1	1/1			13
Heat Shock Proteins/ Chaperones						
dnaK-type molecular chaperone hsc7	1/1					13
heat shock protein 8						4
Heat shock protein 8.	1/1					13

heat shock protein 90kDa (HSP90)	1/1					1
Proteolysis inducing factor		1/1				4
Truncated hsp25						1
Structural/ Cytoskeletal						
Calcium/calmodulin-dependent protein kinase (CaM kinase)						2
microtubule-associated protein						2
Actin	1/1	1/1		1/1	1/1	21
alpha internexin					1/1	23
Annexin		1/1		1/1		5
Fodrin	1/1					14
Keratin[c]	1/1	1/1	1/1	1/1	1/1	1668[c]
myelin basic protein	1/1	1/1	1/1	1/1	1/1	44
neurofilament (H, L, and Medium chains)	1/1	1/1	1/1	1/1	1/1	61
Taxilin	1/1					2
Tubulin					1/1	67
Vimentin	1/1	1/1	1/1			9
glial fibrilary acidic protein	1/1	1/1		1/1	1/1	99
Membrane						
Calcium-activated potassium channel		1/1				2
Na+/K+ ATPase	1/1					9
Polycystic kidney disease 1 like 1	1/1					5
extracellular/ secreted						
Immunoglobulin Heavy Chain VHJ558-JH2 Region	1/1	1/1				2
matrix metalloproteinase 7	1/1					4
Endoplasmic Reticulum						
Cytochrome P450			1/1			2

[a]This number refers to the number of DRPs from each variant that were analyzed with LC-MS, fractionated and trypsinized and re-analyzed with LC-ESI-MS/MS. The fraction number represents the number of positive identifications for each protein per DRP sample; blank spaces indicate that a particular protein was not detected in DRPs from that specific variant.

[b]The "Combined # of protein ms/ms ion instances" refers to the number of peptide identifications for each protein, combined from analyses for all samples from ALS mutant, hWT and non-transgenic control mice. A peptide identification is defined by a result having a high scoring ms/ms spectra ($p < 0.05$). The total number of instances for any given protein may include instances derived from different tryptic peptides from that protein, each with a unique sequence. The total number, however, may also include the same tryptic peptide that was detected in different spinal cord samples, rendering multiple instances for the same tryptic peptide sequence. Due to the variation in tryptic peptide ionization potential throughout a protein sequence, the total number of instances more accurately expresses the relative abundance of a protein compared to percent sequence coverage.

[c]Keratin is a large contaminant protein commonly found in mass spectrometry preparations and the high abundance is most likely artifactual.

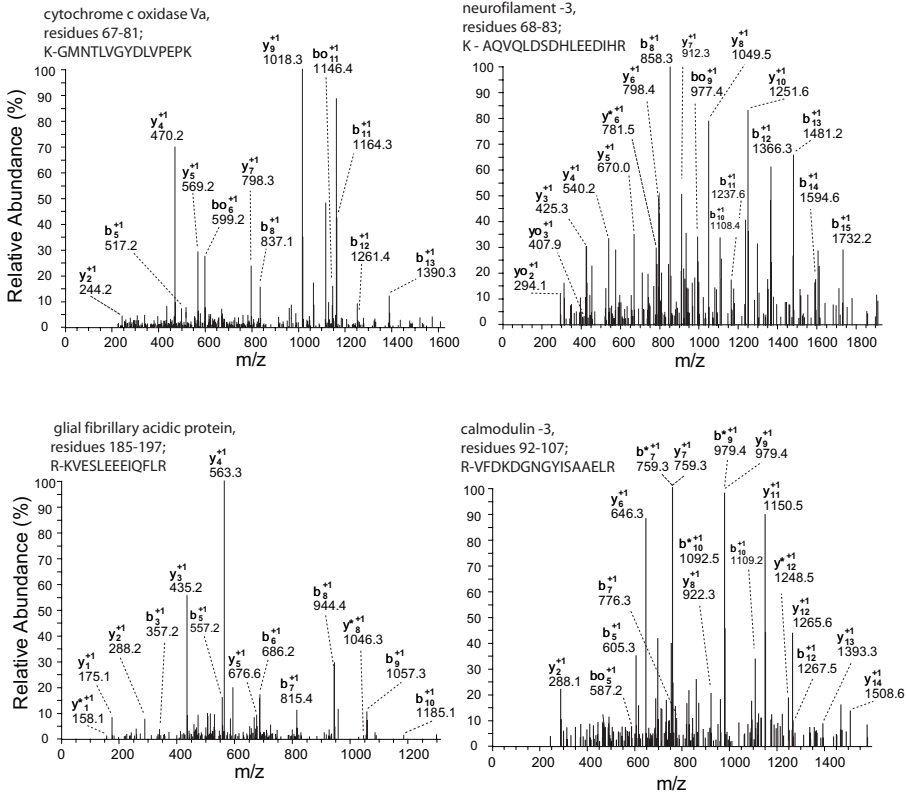
Supplemental Table 2. Proteins detected to co-elute with ALS variant SOD1 in SE/IA-LC separation. These proteins were only detected in the void volume fractions (> 70 kDa) from the SE column (Figure 4). No additional proteins (besides SOD1) were consistently detected in the resolving fractions (< 70 kDa) with MS/MS, suggesting that no proteins are complexed with SOD1 in < 70 kDa complexes.

Identified Proteins (organized by subcellular localization)	H46R /H48Q (7[a])	G37R (3)	hWT (2)	nTG (2)	[b]Combined # of protein ms/ms ion instances
mitochondrial					
cytochrome c oxidase, subunit Va [Mus musculus]	1/7	0/3	0/2	2/2	5
L-2-hydroxyglutarate dehydrogenase [prolactin-induced protein; prolactin-inducible	3/7	0/3	0/2	0/2	3
apolipoprotein D precursor [Homo sapiens]L-2-hydroxyglutarate dehydrogenase	3/7	0/3	0/2	0/2	3
RIKEN cDNA 0610033L03 [Mus musculus] Ubiquinonapolipoprotein D precursor [Homo sapiens]	1/7	2/3	0/2	0/2	3
RIKEN cDNA 0610033L03 [Mus musculus] Ubiquinon	3/7	0/3	0/2	0/2	3
Nuclear					
similar to PDZ domain containing guanine nucleotide exchange factor(GEneurofilament 3, medium; neurofilament, medium polypeptide [Mus musculus]	2/7	0/3	0/2	0/2	2
similar to PDZ domain containing guanine nucleotide exchange factor	2/7	0/3	0/2	0/2	2
transcription factor EB [Mus musculus]vimentin [Homo sapiens]	1/7	0/3	0/2	0/2	2
similar to hypothetical protein FLJ20047 [Homo sapiens] [Mus musculus] transcription factor EB [Mus musculus]	1/7	0/3	0/2	0/2	2
Ppar binding protein, isoform 2; TRAP 220 [Mus musculus]	2/7	0/3	0/2	0/2	2
MYB binding protein (P160) 1a; nuclear protein P160 [Mus musculus]myosin X [Homo sapiens]	1/7	0/3	0/2	0/2	2
Cytoplasm/ Metabolic					
SOD_HUMAN SUPEROXIDE DISMUTASE [CU-ZN]	6/7	3/3	2/2	2/2	126
calmodulin 3 [Mus musculus]	4/7	0/3	0/3	0/2	9
similar to glyceraldehyde-3-phosphate dehydrogenase [Mus musculus]superoxide dismutase 1, soluble; Cu/Zn superox	3/7	0/3	0/2	0/2	7
NICE-4 protein [Homo sapiens]similar to glyceraldehyde-3-phosphate dehydrogenase [Mus musculus]	3/7	0/3	0/2	0/2	6
cullin 2; RIKEN cDNA 1300003D18 gene [Mus musMYB binding protein (P160) 1a; nuclear protein P160 [Mus musculus]	2/7	0/3	0/2	0/2	2
Structural/ Cytoskeletal					
glial fibrillary acidic protein [Mus musculus]	3/7	0/3	1/2	0/2	5
actin, beta, cytoplasmic; A-X actin-like protein; melanoma X-actin [Mus	1/7	0/3	0/2	0/2	3
RIKEN cDNA 2810411G23 [Mus musculus] actin, beta, cytoplasmic; A-X actin-like protein; melanoma X-actin	1/7	0/3	0/2	0/2	3
neurofilament 3, medium; neurofilament, medium polypeptide [Mus musculus] RIKEN cDNA 2810411G23	2/7	0/3	0/2	0/2	2
vimentin [Homo sapiens]	2/7	0/3	0/2	0/2	2

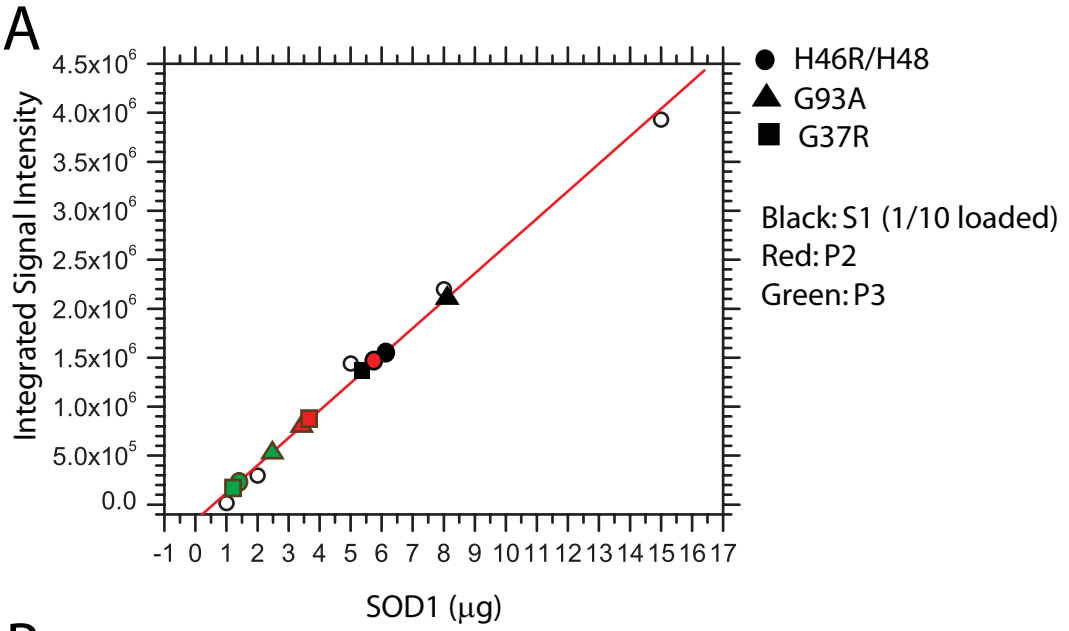
myosin X [Homo sapiens]similar to gasdermin [Mus musculus]	2/7	0/3	0/2	0/2	2
actin, alpha, cardiac [Mus musculus]ATPase, Na+/K+ transporting, beta 1 polypeptide; sodium/potassium ATPase	1/7	0/3	0/2	0/2	2

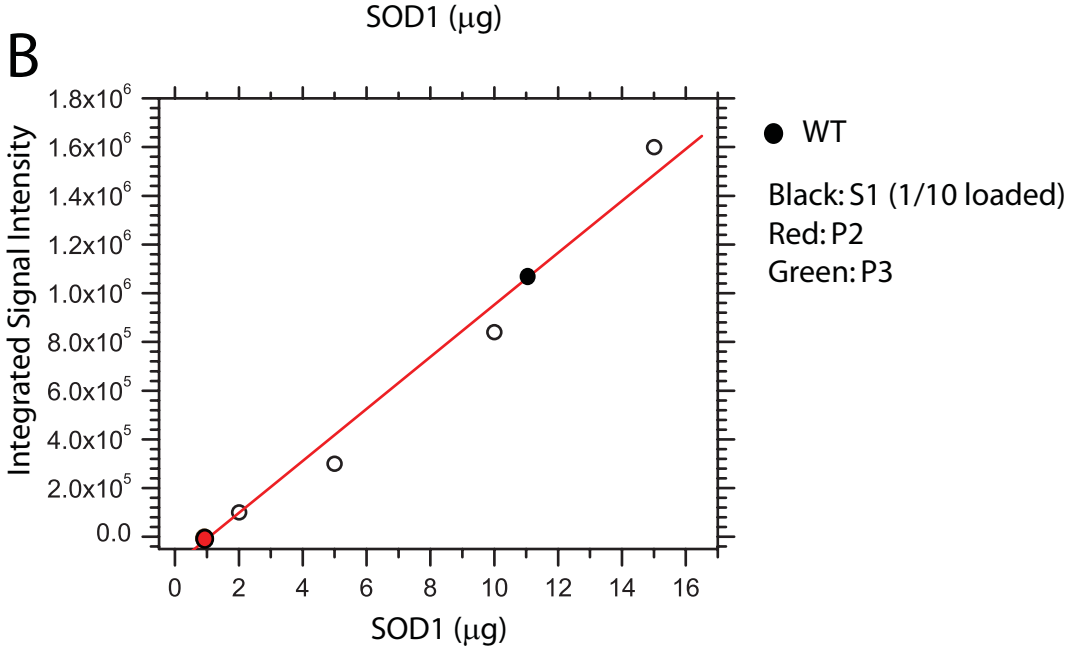
[a]This number refers to the number of separate SE-IA-LC preparations of SOD1 from each variant that were trypsinized and analyzed with LC-ESI-MS/MS. The fraction number represents the number of positive identifications for each protein per sample.

[b]The "Combined # of protein ms/ms ion instances" refers to the number of peptide identifications for each protein, combined from analyses for all samples from ALS mutant, hWT and non-transgenic control mice. A peptide identification is defined by a result having a high scoring ms/ms spectra (X-correlation > 3.0). The total number of instances for any given protein may include instances derived from different tryptic peptides from that protein, each with a unique sequence. The total number, however, may also include the same tryptic peptide that was detected in different spinal cord samples, rendering multiple instances for the same tryptic peptide sequence. Due to the variation in tryptic peptide ionization potential throughout a protein sequence, the total number of instances more accurately expresses the relative abundance of a protein compared to percent sequence coverage.



Supporting Figure 1. Selected MS/MS spectra of tryptic fragments from proteins that were inconsistently detected to co-elute with SOD1 during SE/IA-LC isolation. The spectra shown here were generated from the void volume (> 70 kDa) fractions of the G-75 column. Daughter ions (y and b) are assigned in each spectrum ("bo" or "yo" and "b*" or "y*" refers to a daughter ion that have undergone the loss of H_2O or NH_3 respectively).

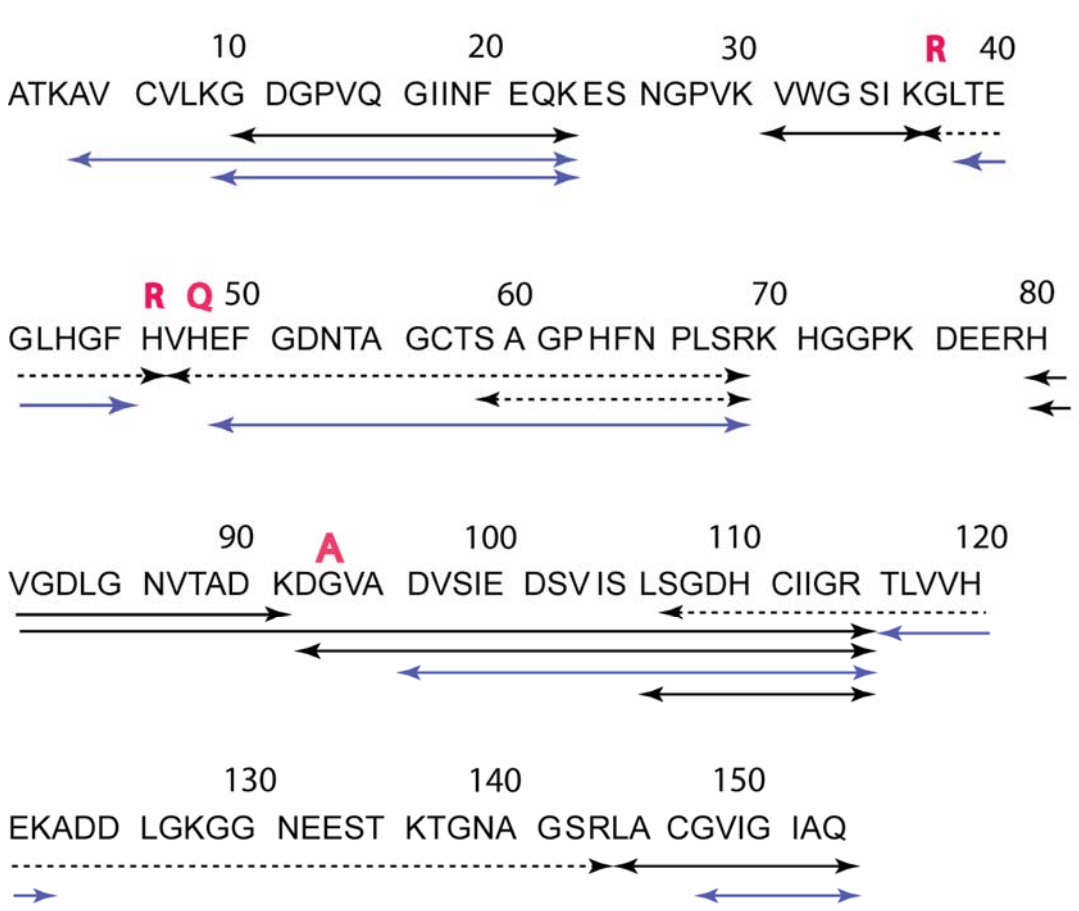




C

	S1 (µg)	P2 (µg)	P3 (µg)	Total (µg)	% Soluble	% Pellet
H46R/H48Q	60.8	5.7	1.4	66.5	91.4	9.6
G37R	53.8	3.8	1.2	57.6	93.4	6.4
G93A	79.6	3.6	2.5	83.2	95.6	4.4
hWT	108	0	0	108	100	0

Supporting Figure 2 Approximating the amount of SOD1 in supernatant (S1) and pelleted (P2 and P3) samples from symptomatic ALS mutant and hWT transgenic mouse spinal cord. Solutions containing recombinant H48Q (**A**) and hWT (**B**) SOD1 of known concentrations were analyzed with LC-ESI-MS and signal intensities were integrated in order to generate the calibration curve (open circles, red line). Solid black, red, and green symbols in A and B represent integrated intensity values from S1, P2 and P3 samples, respectively. Note: 1/10 of the S1 from ALS variant and hWT spinal cord was loaded onto column. Resulting S1 microgram values in A and B are multiplied by 10 to yield total SOD1 content in supernatant. P2 and P3 data points for hWT (B) are equivalent with only P2 being visible. **C**) Summary of the relative amounts of ALS mutant and hWT SOD1 in S1, P2 and P3 samples. Approximately 4-10% of ALS variant SOD1 exists in detergent resistant pellets.



Supporting Figure 3. Tryptic digest of hSOD1. Arrows indicate peptides that were detected with LC-MS/MS after a 24 hr trypsin incubation of ALS mutant SOD1 (purified from spinal cord with SE-IA chromatography). Amino acid substitutions G37R, H46R, and H48Q and G93A are indicated with red letters. The substitutions H46R and G37R, which result in additional sites of proteolysis, alter the pattern of proteolysis with trypsin at positions 37 and 46. Black arrows indicate tryptic fragments that were detected in both G37R and H46R/H48Q SOD1; blue arrows indicate tryptic peptides that were detected only in G37R SOD1 and dashed arrows indicated peptides that were detected only in H46R/H48Q SOD1.