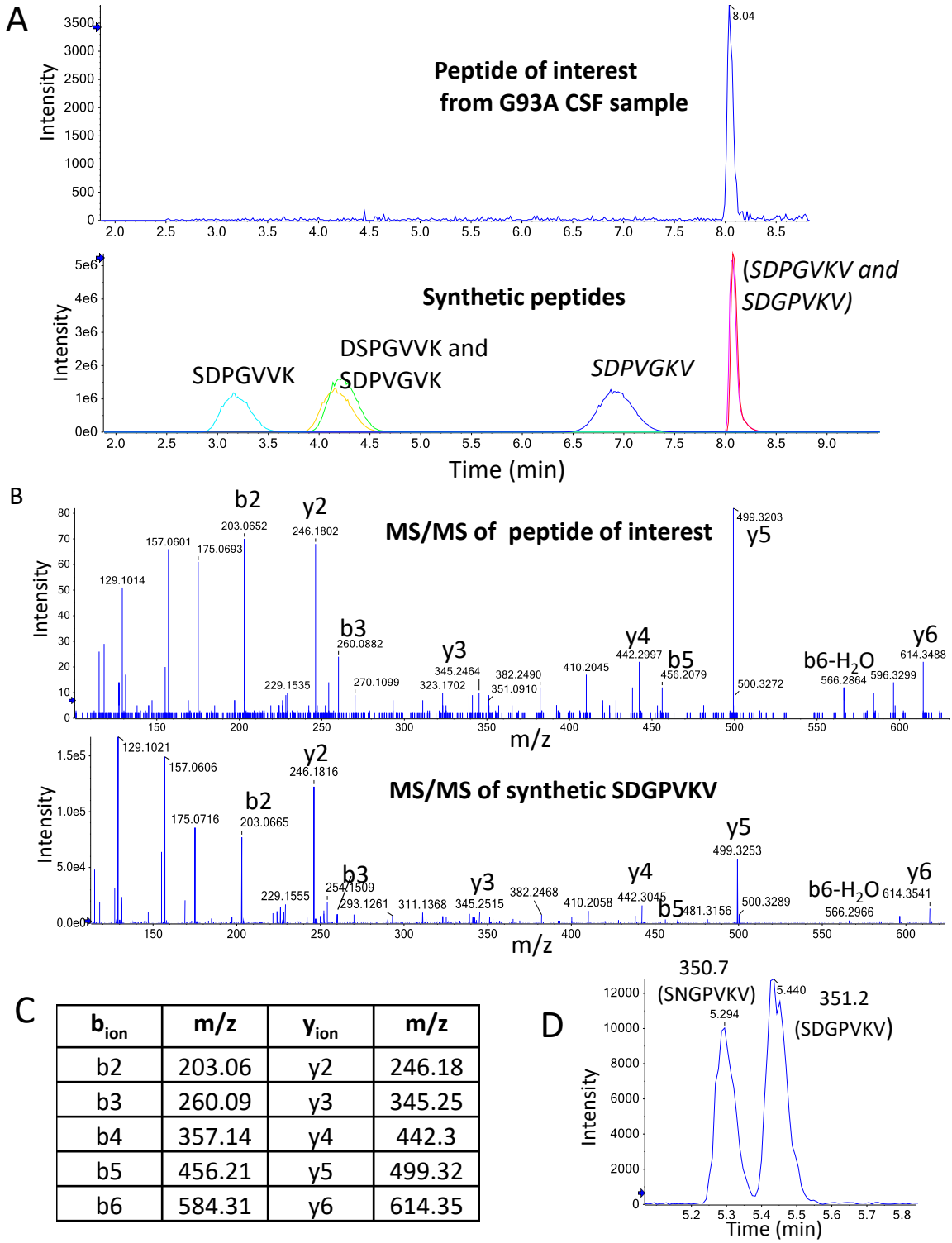
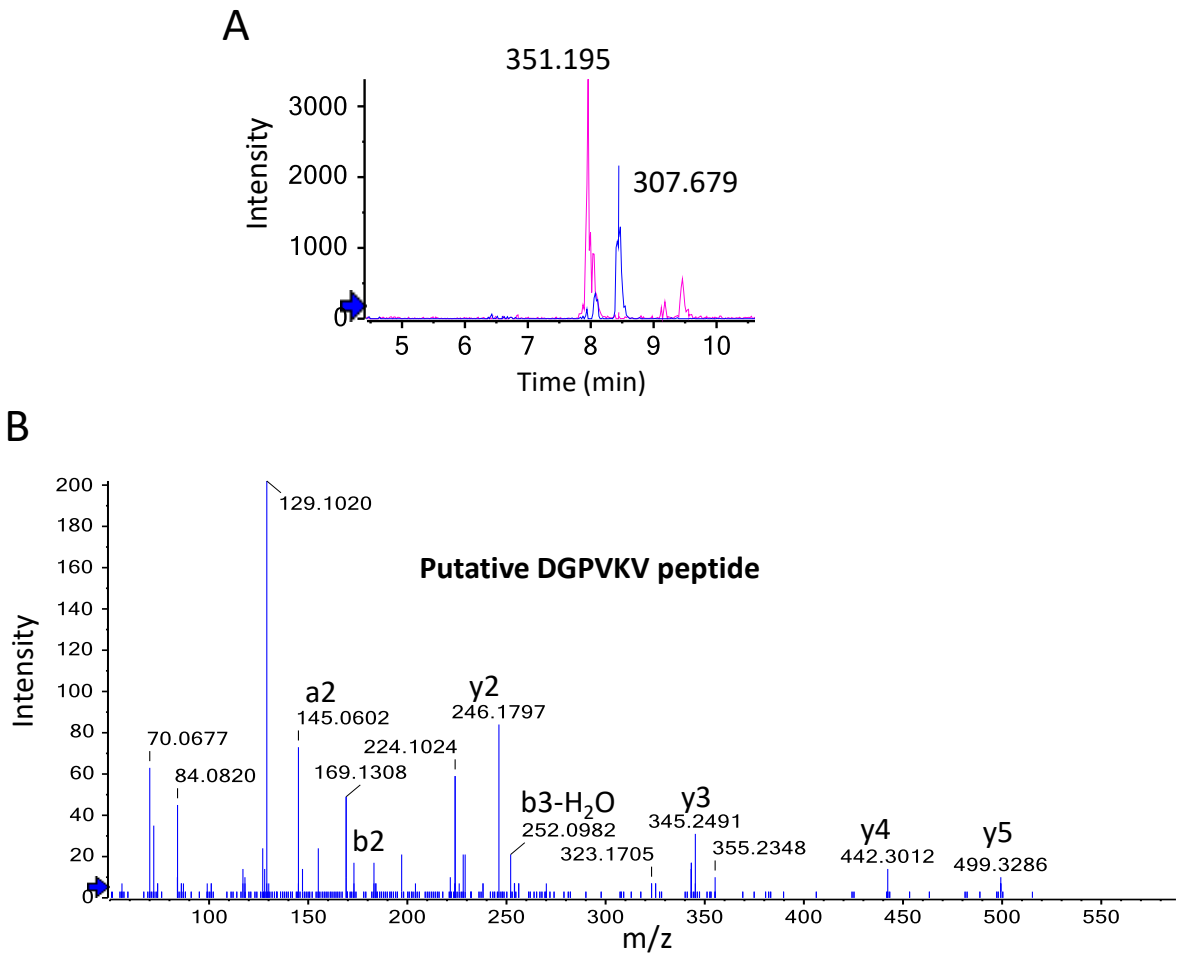
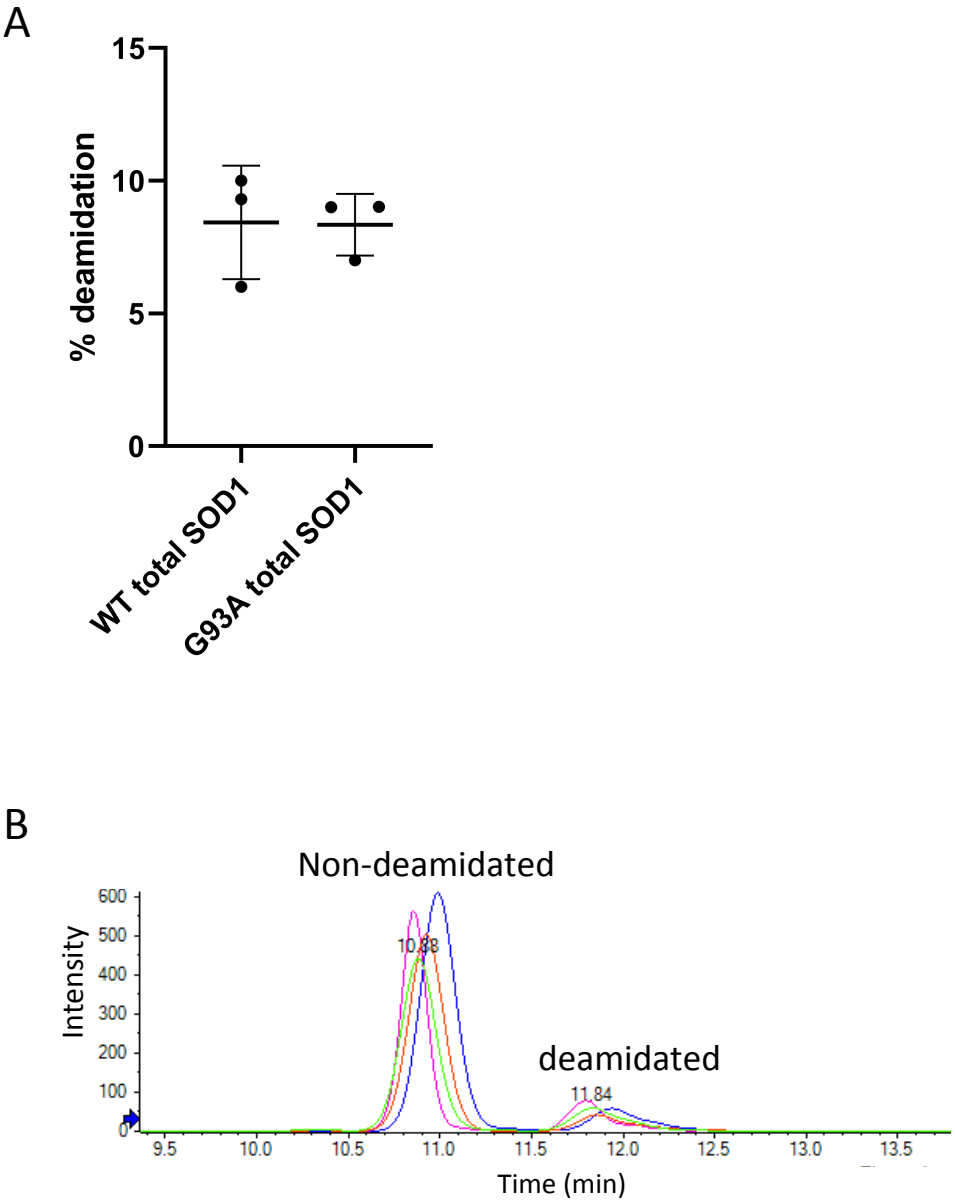


b_{ion}	m/z	y_{ion}	m/z
b2	203.06	y2	246.18
b3	260.09	y3	345.25
b4	357.14	y4	442.3
b5	456.21	y5	499.32
b6	584.31	y6	614.35

Supplemental Figure 1. (A) Peptide of interest (m/z 351.195) had multiple possible amino acid arrangements based on product ion scan. The mostly likely sequence arrangements were identified after matching data with in-silico fragmentation (Prospector software, UCSF). These peptides (6 in total, sequences shown in left panel) were custom synthesized and ran under similar chromatographic conditions. Two of these matched the elution times of the ion of interest, but only one matched the MS/MS pattern of that ion, SDGPVKV (B). (C) Product ion table listing the primary b and y ions for sequence SDGPVKV, many of them labeled in the spectra above. D) Custom synthesized peptide standards (5nM concentration) of both the non-deamidated (350.7) and deamidated (351.2) forms run together, demonstrating that the two forms would be resolved in our runs if both were present, however only the 351.2 ion was present in the CSF samples.



Supplemental Figure 2. (A) Two ions (351.195 and 307.679) of interest from untargeted metabolomics are resolved by approximately 0.5 minutes in the chromatographic run. MS/MS of 308 ion is shown in (B). The 351 ion was identified as described in supplemental figure 1, while the 308 ion has been putatively identified based on similarities and differences in product ions as compared to the 351 ion. The y ion series is identical from y2-y5, while the b ion series differs due to the lack of a serine in the N-terminal sequence of 308 (e.g. b2 ion of 173.06, b3-H2O of 252.1).

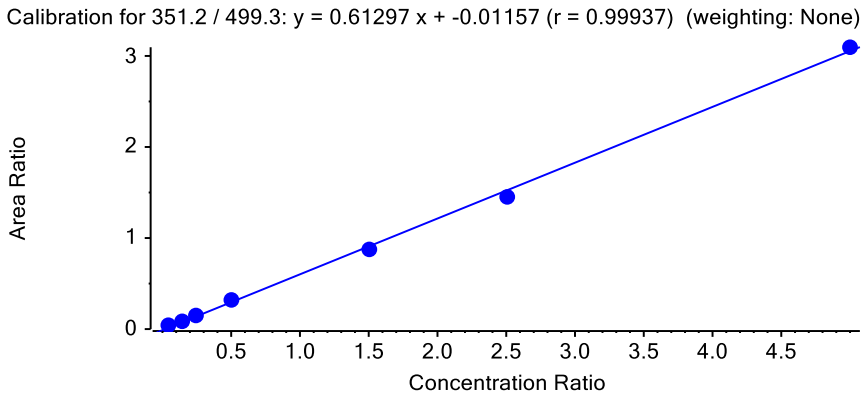


Supplemental Figure 3. Proteomic analysis of ASN 26 deamidation from intact SOD1 protein from rat spinal cord (G93A vs. WT). (A) Graph compares differences in deamidation of ASN 26 in native SOD1 protein following trypsinization and proteomic analysis (n=3 unique rats for each cohort). No stastical difference was observed ($p>0.5$, Mann-Whitney U-test). The tryptic fragment: ESNGPVKVWGSIK was used for analysis. (B) Resolution of the non-deamidated tryptic fragment m/z 700.88 (MH^{+2}) containing ASN 26, with the deamidated version (701.38 m/z). Several different runs are shown overlayed, with ratios of deamidated to total peptide plotted in A. The peaks were identified by MS/MS spectra using ProteinPilot software (AB Sciex), with over 99% confidence scores for each peak, and also confirmed by manual verification and comparison of MS/MS data from both peaks. Integration and relative quantitation was done using PeakView (AB Sciex).

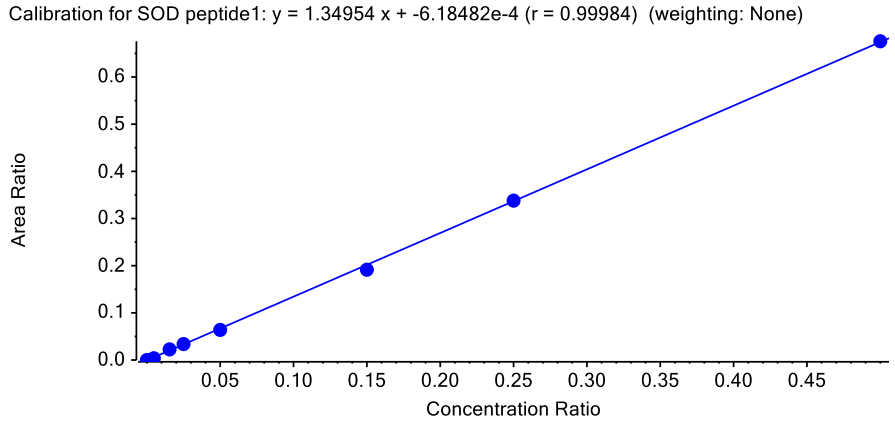
#	AA	Score	#	AA	Score	#	AA	Score	#	AA	Score	#	AA	Score
1	M	0.505684	34	G	0.150926	67	P	0.043573	100	I	0.645849	133	E	0.210111
2	A	0.110804	35	S	0.064932	68	L	0.051862	101	E	0.141497	134	E	0.56757
3	T	0.173248	36	I	0.837709	69	S	0.050371	102	D	0.937015	135	S	0.050957
4	K	0.426797	37	K	0.104637	70	R	0.987533	103	S	0.106818	136	T	0.167847
5	A	0.478373	38	G	0.31646	71	K	0.162607	104	V	0.185478	137	K	0.165182
6	V	0.876122	39	L	0.919276	72	H	0.477812	105	I	0.471112	138	T	0.254034
7	C	0.177545	40	T	0.139124	73	G	0.077171	106	S	0.232528	139	G	0.532905
8	V	0.348869	41	E	0.875287	74	G	0.035981	107	L	0.964047	140	N	0.323455
9	L	0.903601	42	G	0.567158	75	P	0.031112	108	S	0.204837	141	A	0.164159
10	K	0.227723	43	L	0.876731	76	K	0.026736	109	G	0.743814	142	G	0.218046
11	G	0.268609	44	H	0.237028	77	D	0.403791	110	D	0.602982	143	S	0.180716
12	D	0.456453	45	G	0.564109	78	E	0.701918	111	H	0.201822	144	R	0.462845
13	G	0.039266	46	F	0.81927	79	E	0.925094	112	C	0.291692	145	L	0.910944
14	P	0.040512	47	H	0.244368	80	R	0.529148	113	I	0.455584	146	A	0.778545
15	V	0.030931	48	V	0.595477	81	H	0.176573	114	I	0.138873	147	C	0.574702
16	Q	0.507692	49	H	0.881032	82	V	0.569926	115	G	0.782839	148	G	0.210221
17	G	0.788339	50	E	0.396603	83	G	0.337422	116	R	0.969492	149	V	0.659909
18	I	0.728263	51	F	0.76443	84	D	0.636777	117	T	0.138756	150	I	0.171045
19	I	0.677467	52	G	0.10623	85	L	0.418864	118	L	0.893028	151	G	0.233554
20	N	0.130187	53	D	0.749538	86	G	0.202844	119	V	0.852606	152	I	0.816083
21	F	0.900661	54	N	0.058141	87	N	0.109556	120	V	0.600204	153	A	0.311341
22	E	0.122029	55	T	0.554019	88	V	0.487887	121	H	0.216163			
23	Q	0.441377	56	A	0.164984	89	T	0.291166	122	E	0.597864			
24	K	0.12418	57	G	0.207297	90	A	0.150034	123	K	0.313125			
25	E	0.809031	58	C	0.200282	91	D	0.847418	124	A	0.338134			
26	S	0.414302	59	T	0.142514	92	K	0.079504	125	D	0.606239			
27	N	0.052033	60	S	0.524869	93	D	0.258967	126	D	0.137354			
28	G	0.064162	61	A	0.276596	94	G	0.040625	127	L	0.074145			
29	P	0.026578	62	G	0.528456	95	V	0.695556	128	G	0.112703			
30	V	0.027211	63	P	0.095363	96	A	0.104562	129	K	0.08959			
31	K	0.316204	64	H	0.035634	97	D	0.654672	130	G	0.107111			
32	V	0.927412	65	F	0.59049	98	V	0.501123	131	G	0.288043			
33	W	0.791248	66	N	0.521593	99	S	0.078284	132	N	0.0423			

Supplemental Figure 4. *In-silico* proteasomal fragmentation prediction of human SOD1 (full sequence) using Netchop 3.0. Scores are on a scale from 0 to 1, with those closer to 1 revealing a more likely amino acid cleavage site and vice versa, with values between 0.8 and 1.0 predicting a strong likelihood of proteasomal cleavage. Note, the cleavage probability on either end of the peptide fragment (color coded yellow) of interest is especially high (>0.8), as compared to scores within the peptide. Interestingly, the next highest score internally in this peptide is the cleavage of the N-terminal serine (0.41), which was the other peptide seen elevated from the SOD1 rat data.

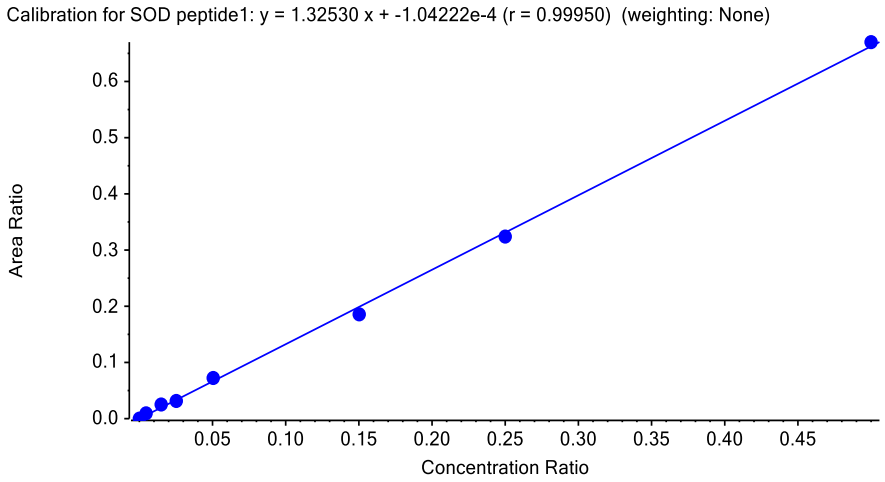
A



B



C



Supplemental Figure 5. Standard curves for the three major sample analysis days (all correlation coefficients >0.999). A) Data collected on an API 4000 LC-MS/MS (AB Sciex) with a standard curve ranging from 100pM to 5,000pM (X axis represents the concentration of unlabeled peptide (in pM) divided by the concentration of the stable isotope standard spiked into each extraction (2,000pM final after sample resuspension). The majority of SOD1-ALS samples were acquired on two different days (panels B and C) using an adjusted standard curve (ranging from 10pM to 1000pM), on a different LC-MS (API 4500 by AB Sciex). Three of ALS samples and control samples measured on day 1 (panel A) were below the lowest standard curve point, and so were normalized to data from B and C using several control samples that were rerun with the adjusted standard curve. All other ALS samples are presented in absolute concentration using 7-pt standard curves run within the same batch, with no further normalization. Identical MRM transitions were used throughout all analytical runs.

Supplementary methods

Proteomics methods

Sample preparation

Spinal cords from both $SOD1^{G93A}$ and $SOD1^{WT}$ rats were harvested as described previously (Vande Velde C, et al. referenced in manuscript). Tissue was frozen in liquid nitrogen and stored in -80°C until extracted. Prior to extraction, tissue was ground up in a liquid nitrogen containing mortar, using a frozen pestle while tissue was still immersed in liquid nitrogen. Powder was then transferred into pre-frozen Eppendorf tubes, flash frozen again and stored in -80°C until extracted. Weights of powder were between 10-20 milligrams for total SOD1 determination experiments and for folded SOD1 immunoprecipitation experiments. The exact amount of weight was not important to note, since we were only concerned with the ratio of deamidated to non-deamidated protein within each sample, not actual amount. For further processing of aliquots intended for measuring total SOD1, we used a modified version of a previously published method (Guttman et al, Proteomics, 2009). We added 20 µL of 30 mg/mL Rapigest™ surfactant (mass spec friendly detergent solution, by Waters Corp.) solution dissolved in 10X TEN (Tris, EDTA, NaCl) buffer pH 7.1, followed by 100 µl water. Samples were boiled for 5 minutes, followed by the addition of 200 µL of water and 15 µL of 35 mM TCEP. Samples were incubated at 37°C for 30 minutes, then 13 µL of 50 mM IAA solution was added and incubated at 37°C again for 30 minutes. 12 µL of 0.5 mg/mL trypsin (Promega, sequencing grade, cat# V5111) was added to each solution, and the pH was titrated to pH 7.0 w/ 1M tris pH 7.5 solution (checked with litmus paper). It was important to keep the pH at 7.0 or lower to limit artificial deamidation. Samples were trypsinized at 37°C for 5 hours. 40 µL of 1M HCL was added to each sample (pH 2.5-3.5) to cleave and remove Rapigest™, then incubated at 60°C for 1 hour. Samples were centrifuged at 4°C at 16kG's for 30 minutes, supernatant was then removed, and 3 µL of 28% ammonium hydroxide was added to each sample to get to a final pH of between

5.0 and 6.0. Additional ammonium hydroxide was added in samples where pH was below this point. The sample was then dried using a speed vac connected to a lyophilizer to maintain cold temperature and limit further deamidation. Dried samples were resuspended in 100 µL of 0.1% formic acid and salt exchanged using Aspire tips (Thermo Fisher). Samples were eluted from tips with 70% acetonitrile, dried, and then finally resuspended in 25 µL of 5% ACN/0.5% TFA. 5 µL were injected per run MS run on an AB Sciex 5600 Triple-TOF. Chromatography and instrument parameters are described below in MS section.

LC-MS methodology and deamidation calculations

All runs were performed with a nano-scale reversed-phase UPLC (Waters corporation nano ACQUITY) coupled to an AB Sciex 5600-Triple TOF. Capillaries were packed using a 20 cm-75 micron ID glass capillary packed with 2.5-µm C18 (130) CSH^{TM} beads (Waters corporation). Flow rates were 250nL/min. The mobile phase compositions were as follows: Buffer A-0.2% FA in water, Buffer B-100% ACN + 0.2% FA. The following gradient was used for each run: 1) 0.1 to 10 minutes (3% B to 10% B), 2) 10.0 to 15 minutes (10% B to 15% B), 3) 15 to 70.0 minutes (15% B to 30% B), 4) 70 minutes to 78 minutes (30% B to 85% B), 5) 78 to 82 minutes (85% B isocratic), 6) 82 to 82.1 minutes (85% B to 3% B), and 7) 82.1 to 100 minutes (3% B isocratic).

MS runs were processed using ProteinPilot software (AB Sciex) and searched against both rat and human genome databases. SOD1 protein was covered at greater than 75% with over 20 unique peptides identified at less than 1% false discovery rate. One of these peptides contained the sequence: ESNGPVKVWGSIK, with a m/z of 700.8. This peptide had the ASN 26 of interest, which was identified by protein pilot in two forms, the intact peptide, and the deamidated form with a m/z of 701.3 which eluted nearly 1 minute later, as shown in Suppl. fig. 3. Both peaks were integrated within each run using PeakView software (AB Sciex), and the

area ratios of the two were then compared between the different mutant and WT forms as also represented in Suppl. fig. 3.