SUPPLEMENTAL TABLE

Table S1 Identification of the mass peaks observed in MALDI-TOF mass spectra (Fig. S3). Based upon observed m/z, peptides are identified using a PAWS program and listed together with the theoretical m/z. Confirmation of the m/z-based identification is performed by MS/MS analysis in some peptides shown in the list. Although four amino acids (Gly-Ser-His-Met) remain at the N-terminus even after removal of a tag with thrombin agarose, numbering of the amino acid residue is based upon an SOD1 sequence starting from Ala.

WT			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.9	yes
136-152	1587.9	1587.9	
17-30	1602.8	1602.9	
0-16	1673.9	1673.9	yes
134-153	1904	1904.1	
-3-16	1955	1955	yes
17-34 or 18-35	2032	2032.1	
17-37	2330.2	2330.3	yes
129-153	2390.2	2390.3	yes
127-153	2575.3	2575.4	yes
91-116	2686.3	2686.4	yes
91-117	2799.4	2799.5	yes
89-116	2872.4	2872.5	yes
88-116	2973.4	2973.6	yes
89-117	2985.5	2985.6	
87-116	3072.5	3072.6	yes
88-117	3086.5	3086.6	yes
91-120	3134.6	3134.7	
-1-28	3167.6	3167.7	
87-117	3185.6	3185.7	yes
-3-29	3410.7	3410.9	
-3-30	3538.8	3539	

A4V			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
137-153	1587.8	1587.8	yes
135-153	1816.9	1816.9	yes
134-153	1904	1904	
-3-16	1983	1983	yes
129-153	2390.2	2390.1	
127-153	2575.3	2575.2	

G37R			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.8	
137-153	1587.8	1587.8	yes
17-30	1602.8	1602.8	yes
127-143	1649.8	1649.7	
136-153	1715.9	1715.9	
135-153	1817	1816.9	yes
134-153	1904	1904	yes
-3-16	1955	1954.9	yes
129-153	2390.2	2390.1	yes
127-153	2575.3	2575.2	yes
-3-29	3410.7	3410.7	yes

G41D			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.8	yes
137-153	1587.8	1587.8	
0-16	1673.9	1673.9	yes
1-18	1769	1768.9	
135-153	1816.9	1817	
134-153	1904	1904	
-3-16	1955	1955	yes
15-35	2330.2	2330.1	
129-153	2390.2	2390.2	
127-153	2575.3	2575.3	
90-115	2799.4	2799.5	
24-49	2872.4	2872.5	
71-98	2973.4	2973.4	
88-116	3072.5	3072.5	
71-99	3086.5	3086.5	
-3-29	3410.7	3410.8	
-3-30	3538.8	3538.9	

G41S			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.8	
137-153	1587.8	1587.8	
0-16	1673.9	1673.9	
1-18	1769	1768.9	
135-153	1816.9	1817	
134-153	1904	1904	
-3-16	1955	1955	yes
107-125	2065	2065	
129-153	2390.2	2390.2	
127-153	2575.3	2575.3	
24-49	2872.4	2872.5	
71-98	2973.4	2973.4	
-3-29	3410.7	3410.8	
-3-30	3538.8	3538.9	

H46R			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.8	yes
137-153	1587.8	1587.8	
0-16	1673.9	1673.9	yes
134-153	1904	1904	
-3-16	1955	1955	yes
15-35	2330.2	2330.1	
129-153	2390.2	2390.2	
127-153	2575.3	2575.3	
90-115	2799.4	2799.5	
24-49	2872.4	2872.5	
71-98	2973.4	2973.4	
-3-29	3410.7	3410.8	

G93R			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.8	yes
137-153	1587.8	1587.8	yes
17-30	1602.8	1602.8	
0-16	1673.9	1673.9	yes
-3-16	1955	1955	yes
97-117 or 98-118	2214.1	2214.1	
93-114 or 94-115	2285.1	2285.1	
129-153	2390.2	2390.2	yes
94-117	2499.3	2499.2	yes
127-153	2575.3	2575.3	
94-119	2697.4	2697.4	
94-120	2834.5	2834.4	yes
113-141 or 117-145	2953.5	2953.6	
94-121	2963.5	2963.5	
88-115 or 89-116	2971.5	2971.4	
88-116	3072.5	3072.5	
89-117	3084.6	3084.6	
94-122	3091.6	3091.6	
88-117	3185.6	3185.6	
94-125 or 96-127	3392.7	3392.7	
-3-29	3410.7	3410.8	

G85R			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.7	
137-153	1587.8	1587.8	yes
14-30	1602.8	1602.8	yes
72-86	1678.8	1678.8	
118-133	1696.8	1696.8	
118-134	1783.8	1783.8	
117-133	1809.9	1809.8	yes
123-141	1820.8	1820.8	
21-37	1843	1842.9	
118-135	1884.9	1884.9	yes
117-134	1896.9	1896.9	yes
87-105	1919.9	1919.9	yes
-3-16	1955	1955	yes
117-135	1998	1997.9	yes
17-34 or 18-35	2032	2032	
97-116	2101	2101	yes
118-137	2114	2114	
117-136	2126.1	2126	
116-136 or 117-137	2227.1	2227.1	
68-87	2286.2	2286.2	
84-105 or 85-106	2303.2	2303.1	
94-116	2386.2	2386.2	
127-153	2575.3	2575.3	yes
91-116	2686.3	2686.3	yes
91-117	2799.4	2799.4	
89-116	2872.4	2872.4	yes
88-116	2973.4	2973.4	
87-116	3072.5	3072.5	yes
88-117	3086.5	3086.5	

L126X			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.9	yes
112-125	1565.9	1566	
17-30	1602.8	1603	
0-16	1673.9	1674	yes
-3-16	1955	1955.1	yes
106-125	2178.1	2178.3	yes
16-36 or 17-37 or 18-38	2330.2	2330.4	
94-116	2386.2	2386.4	
94-117 or 95-118	2499.3	2499.5	
87-111	2532.2	2532.4	
91-116	2686.3	2686.6	yes
91-117	2799.4	2799.7	
86-113 or 88-115 or 89-116	2872.4	2782.6	
88-116	2973.4	2973.7	yes
87-116	3072.5	3072.8	yes
88-117	3086.5	3086.8	
96-124 or 97-125	3107.6	3107.8	
87-117	3185.6	3185.9	yes
94-125	3392.7	3393	
-3-29	3410.7	3411.1	yes
-3-30	3538.8	3539.1	
91-125	3692.8	3693.2	yes
89-125	3878.9	3879.3	
88-125	3979.9	3980.4	yes

L144F			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
138-153	1520.8	1520.8	
1-16	1542.8	1542.9	yes
17-30	1602.8	1602.9	
137-153	1621.8	1621.8	yes
0-16	1673.9	1673.9	
-3-16	1955	1955	yes
97-116	2101.1	2101.1	yes
97-117 or 98-118	2214.1	2214.1	
16-36 or 17-37 or 18-38	2330.2	2330.3	
94-116	2386.2	2386.2	yes
128-152	2424.2	2424.2	
93-116	2443.2	2443.2	yes
94-117	2499.3	2499.3	yes
93-117	2556.3	2556.3	
91-116	2686.3	2686.3	yes
91-117	2799.4	2799.4	yes
89-116	2872.4	2872.4	yes
88-116	2973.4	2973.4	yes
89-117	2985.4	2985.5	yes
87-116	3072.5	3072.5	yes
88-117	3086.5	3086.5	yes
91-120	3134.6	3134.6	yes
-1-28	3167.6	3167.6	
87-117	3185.6	3185.6	yes
89-120	3320.7	3320.7	yes
91-122	3391.7	3391.7	
-3-29	3410.7	3410.7	yes
88-120	3421.7	3421.8	yes
87-120	3520.8	3520.8	yes
-3-30	3538.8	3538.8	
90-124 or 91-125	3692.8	3692.8	
87-122	3777.9	3778	

I149T			
Amino acid residues	Theoretical m/z	Observed m/z	MS/MS
1-16	1542.8	1542.9	yes
17-30	1602.8	1602.9	
0-16	1673.9	1673.9	yes
26-44	1949.1	1949.1	yes
-3-16	1955	1955	yes
17-34 or 18-35	2032	2032.1	
25-44	2036.1	2036.1	
24-44	2165.1	2165.2	
17-37	2330.2	2330.3	yes
21-44	2550.3	2550.4	
92-116	2558.2	2558.3	
91-116	2686.3	2686.4	
91-117	2799.4	2799.5	
86-113 or 88-115 or 89-116	2872.4	2872.5	
87-115	2971.5	2971.5	
89-117	2985.5	2985.5	
88-117	3086.5	3086.6	
91-120	3134.6	3134.7	
87-117 or 88-118	3185.6	3185.6	
94-125 or 96-127	3392.7	3392.9	
-3-29	3410.7	3410.8	
-3-30	3538.8	3538.9	

Figure S1

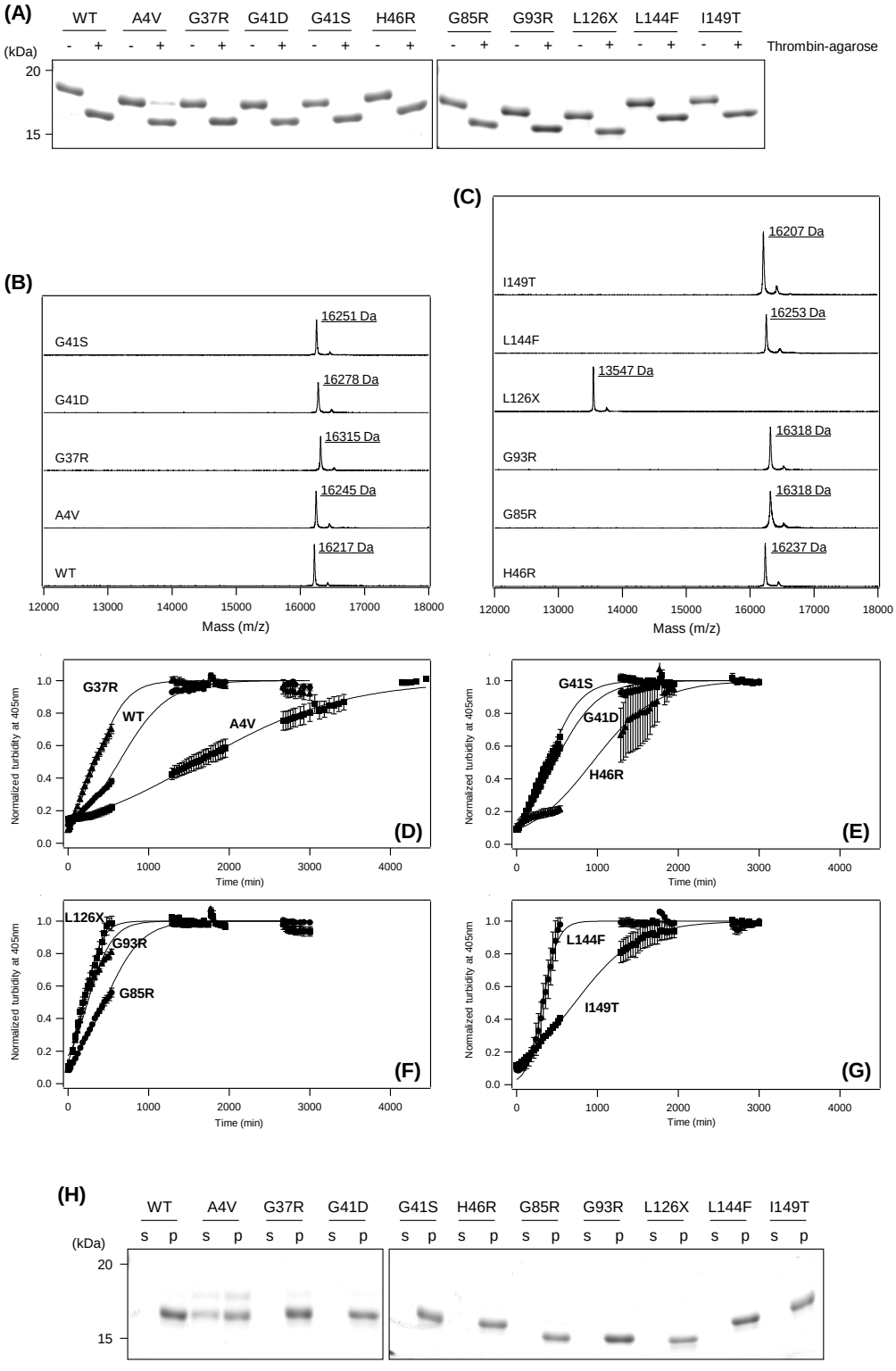

(A)
WT
A4V
G37R
G41D
G41S
H46R
G85R
G93R
L126X
L144F
I149T
(kDa)
Thrombin-agarose
20
15
(B)
G41S
16251 Da
G41D
16278 Da
G37R
16315 Da
A4V
16245 Da
WT
16217 Da
Mass (m/z)
(C)
I149T
16207 Da
L144F
16253 Da
L126X
13547 Da
G93R
16318 Da
G85R
16318 Da
H46R
16237 Da
Mass (m/z)
Normalized turbidity at 405nm
Time (min)
(D)
G37R
WT
A4V
(E)
G41S
G41D
H46R
(F)
L126X
G93R
G85R
(G)
L144F
I149T
(H)
s
p
(kDa)
20
15

Figure S2

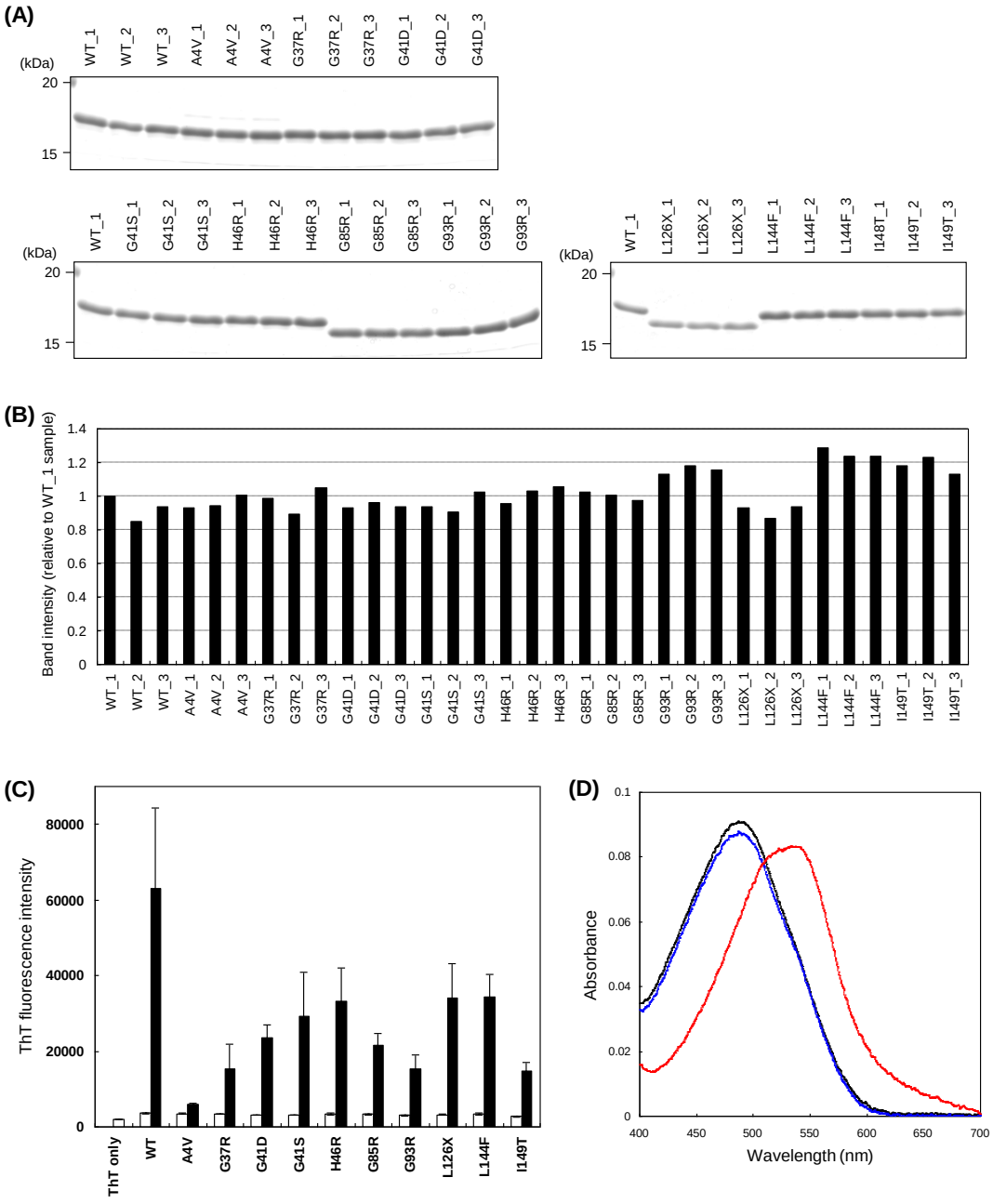

(A)
(kDa)
20
15
WT_1
WT_2
WT_3
A4V_1
A4V_2
A4V_3
G37R_1
G37R_2
G37R_3
G41D_1
G41D_2
G41D_3
G41S_1
G41S_2
G41S_3
H46R_1
H46R_2
H46R_3
G85R_1
G85R_2
G85R_3
G93R_1
G93R_2
G93R_3
L126X_1
L126X_2
L126X_3
L144F_1
L144F_2
L144F_3
I148T_1
I149T_2
I149T_3
(B)
Band intensity (relative to WT_1 sample)
(C)
ThT fluorescence intensity
ThT only
WT
A4V
G37R
G41D
G41S
H46R
G85R
G93R
L126X
L144F
I149T
(D)
Absorbance
Wavelength (nm)

Figure S3

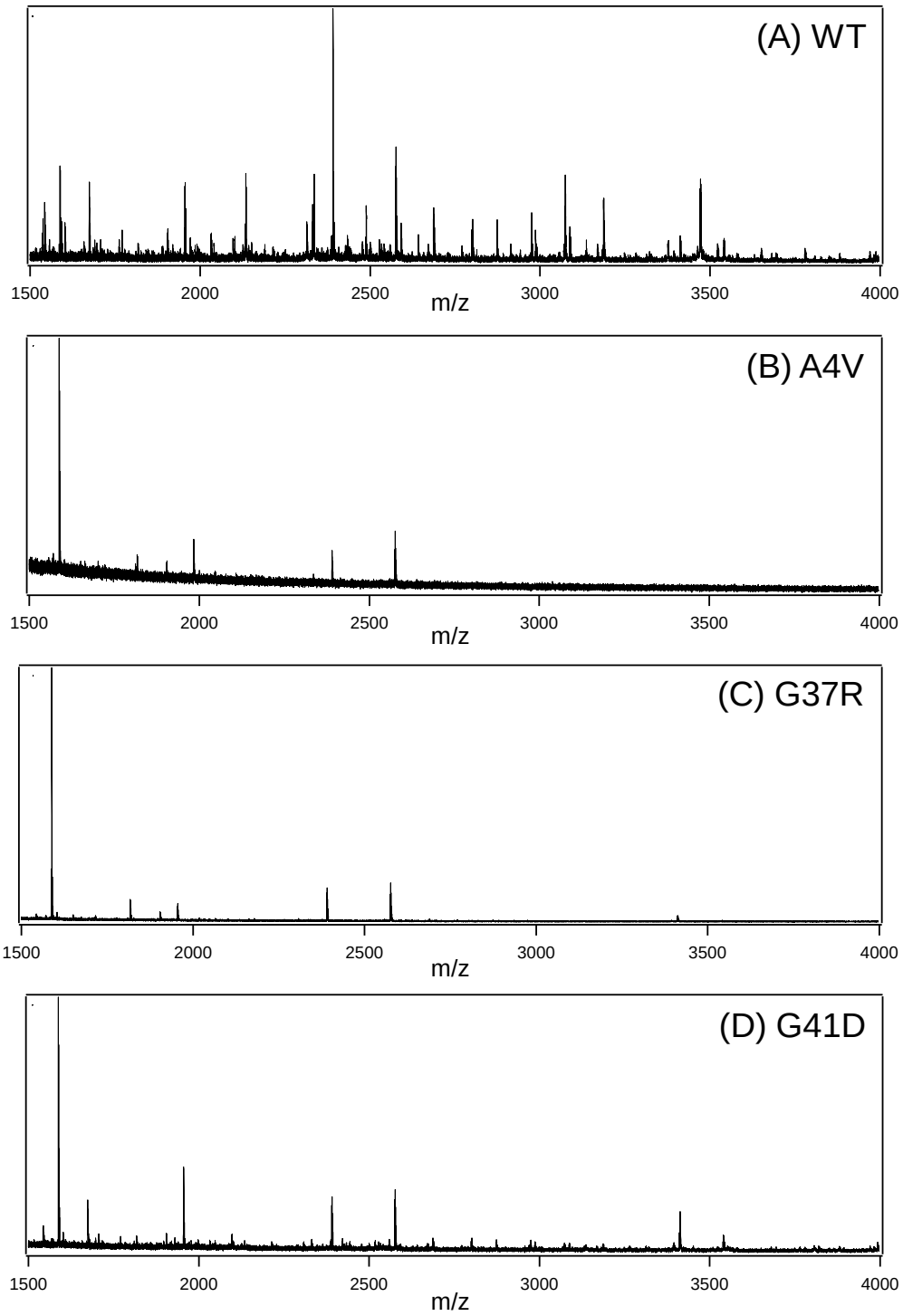

(A) WT
1500
2000
2500
3000
3500
4000
m/z
(B) A4V
1500
2000
2500
3000
3500
4000
m/z
(C) G37R
1500
2000
2500
3000
3500
4000
m/z
(D) G41D
1500
2000
2500
3000
3500
4000
m/z

Figure S3

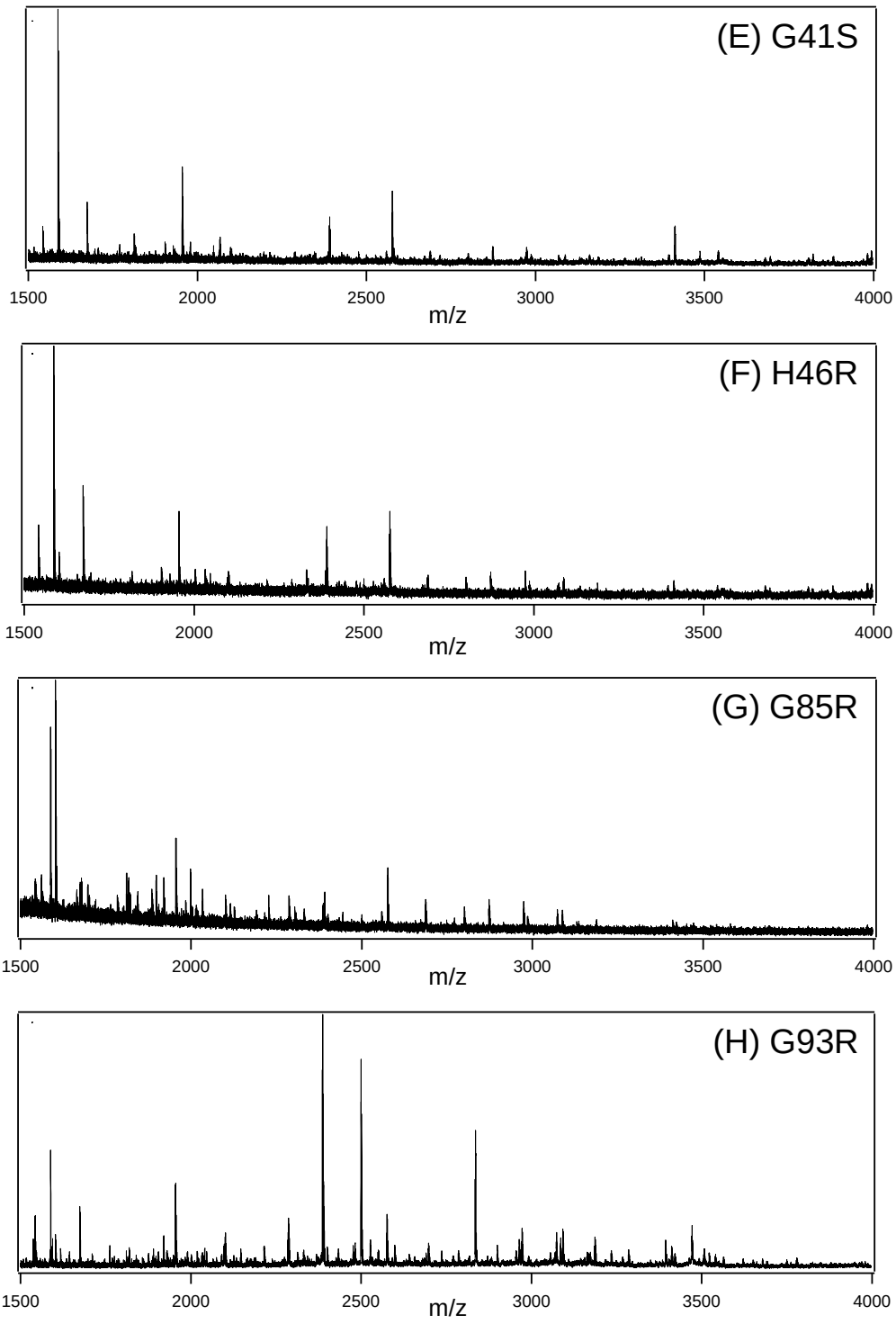

(E) G41S
1500
2000
2500
3000
3500
4000
m/z
(F) H46R
1500
2000
2500
3000
3500
4000
m/z
(G) G85R
1500
2000
2500
3000
3500
4000
m/z
(H) G93R
1500
2000
2500
3000
3500
4000
m/z

Figure S3

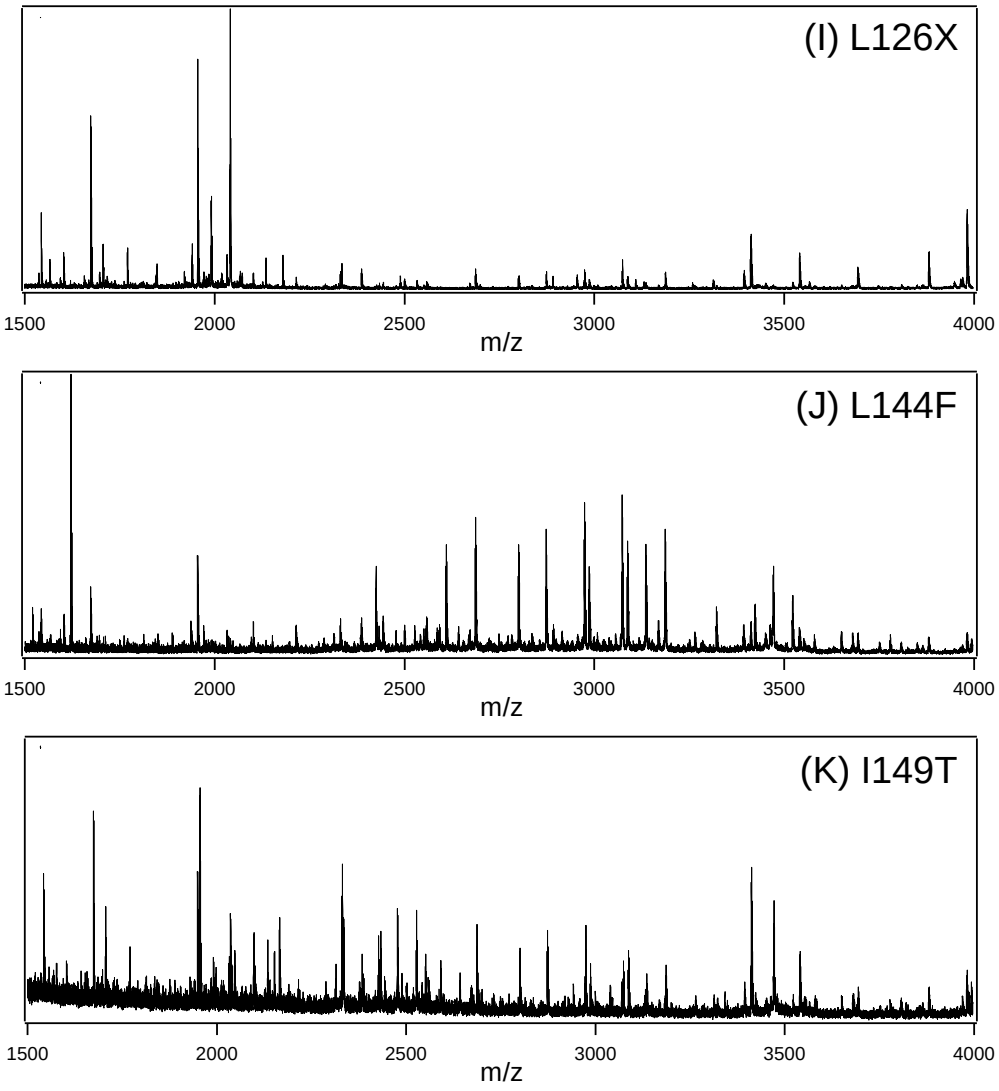

(I) L126X
1500
2000
2500
3000
3500
4000
m/z
(J) L144F
1500
2000
2500
3000
3500
4000
m/z
(K) I149T
1500
2000
2500
3000
3500
4000
m/z

Figure S3

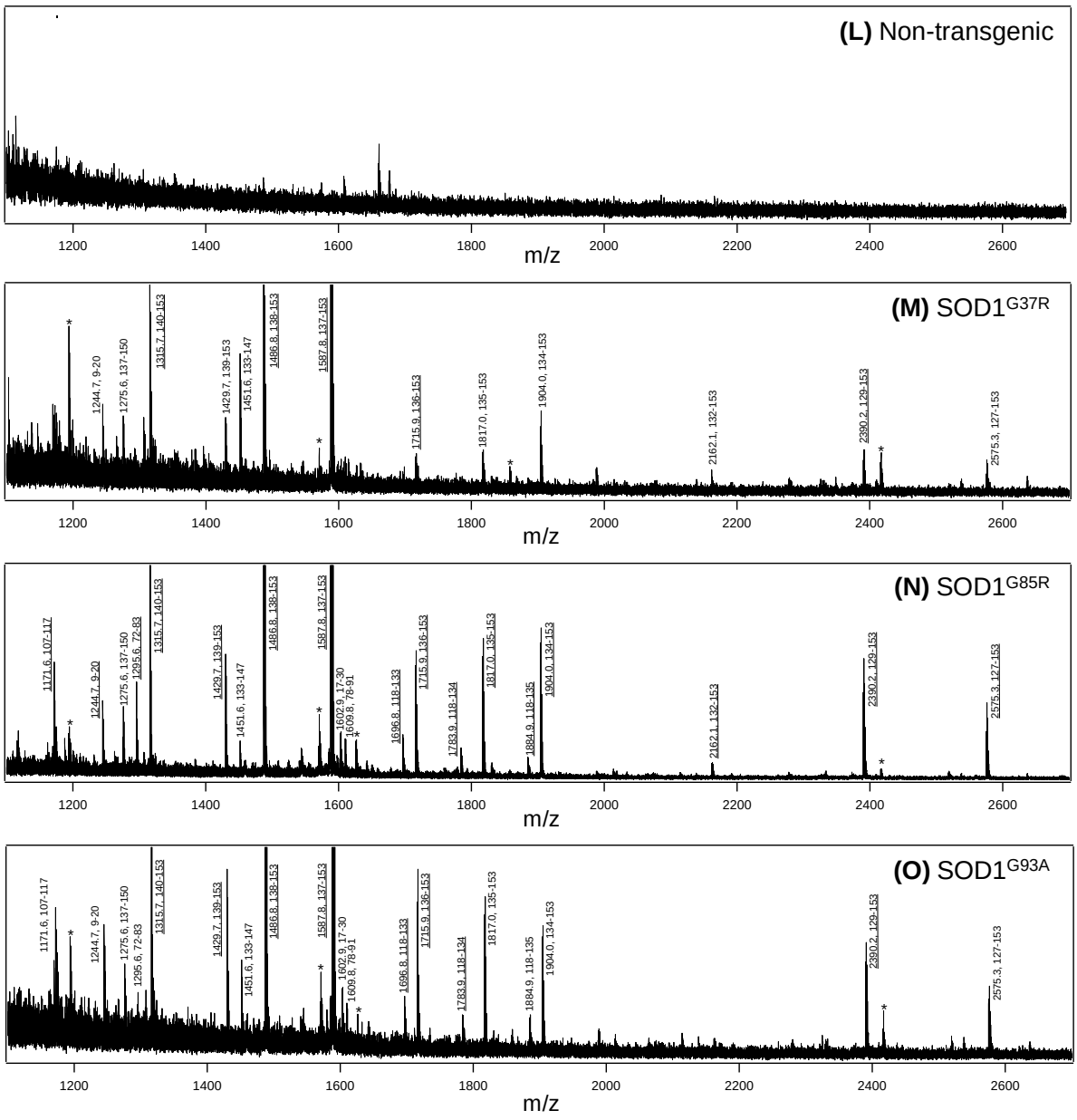
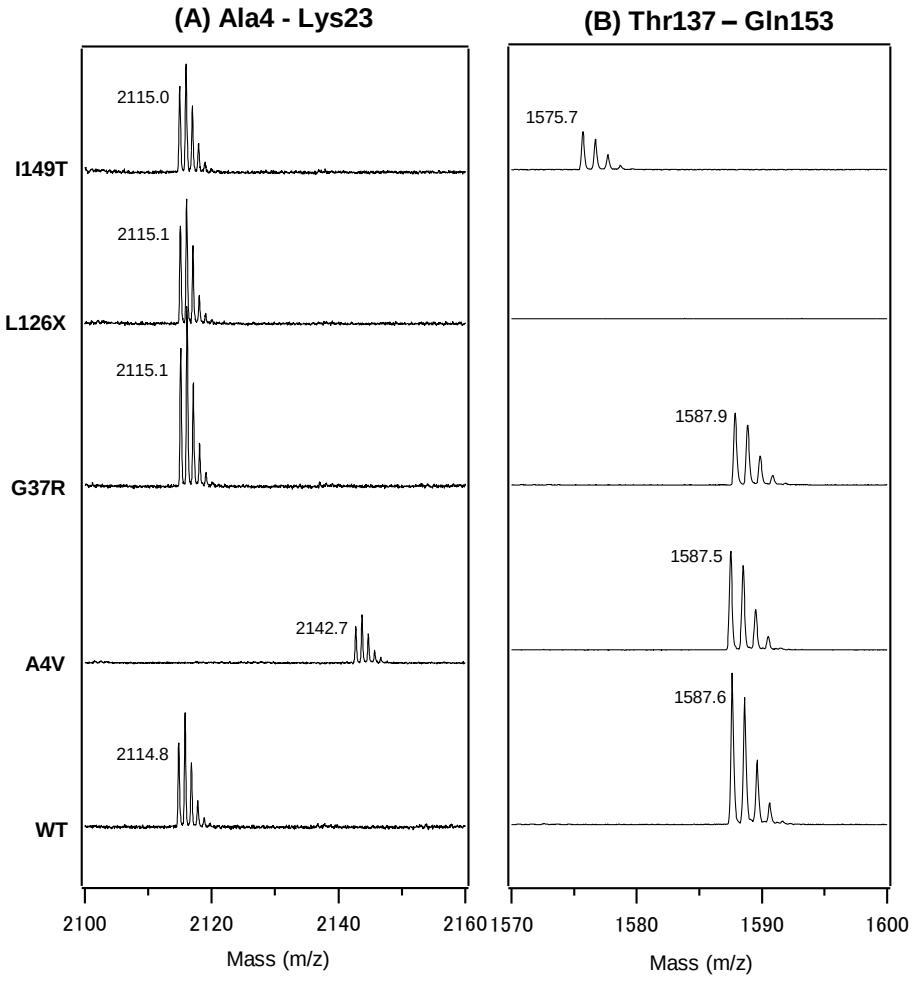

(L) Non-transgenic
(M) SOD1G37R
(N) SOD1G85R
(O) SOD1G93A
1244.7, 9-20
1275.6, 137-150
1315.7, 140-153
1429.7, 139-153
1451.6, 133-147
1486.8, 138-153
1587.8, 137-153
1715.9, 136-153
1817.0, 135-153
1904.0, 134-153
2162.1, 132-153
2390.2, 129-153
2575.3, 127-153
1171.6, 107-117
1295.6, 72-83
1602.9, 17-30
1608.8, 76-91
1696.8, 118-133
1783.9, 118-134
1884.9, 118-135
1200
1400
1600
1800
2000
2200
2400
2600
m/z

Figure S4


(A) Ala4 - Lys23
(B) Thr137 – Gln153
I149T
L126X
G37R
A4V
WT
2115.0
2115.1
2115.1
2142.7
2114.8
1575.7
1587.9
1587.5
1587.6
2100
2120
2140
2160
1570
1580
1590
1600
Mass (m/z)
Mass (m/z)

LEGENDS FOR SUPPLEMENTAL FIGURES

Fig. S1. Aggregation of SOD1 proteins after removal of an N-terminal His-tag in His-tagged SOD1 by thrombin agarose. **(A)** 2 μg of SOD1 before and after digestion with thrombin agarose was boiled in a Laemmli buffer containing 5 mM TCEP, loaded on a 12.5 % SDS-PAGE gel and stained with CBB. Mobility of the protein band increases after treatment with thrombin agarose, suggesting successful and efficient cleavage of a His-tag from SOD1. **(B and C)** Mass (m/z) of each SOD1 sample after cleaved with thrombin agarose was measured by MALDI-TOF mass spectrometry. Theoretical m/z of SOD1 without a His-tag is as follows; 16,217 in WT, 16,245 in A4V, 16,316 in G37R, G85R and G93R, 16,275 in G41D, 16,247 in G41S, 16,236 in H46R, 13,547 in L126X, 16,253 in L144F, and 16,205 in I149T. Observed m/z values indicated on each spectrum support successful removal of a His-tag sequence from tagged SOD1 proteins and confirm that SOD1 amino acid sequence remains intact. **(D-G)** Aggregation kinetics of SOD1 proteins monitored by turbidity changes at 405 nm: **(D)** WT, A4V and G37R, **(E)** G41D, G41S and H46R, **(F)** G85R, G93R and L126X, **(G)** L144F and I149T. Aggregation reaction was started by agitation of 100 μM apo-SOD1^{SH} at 1,200 rpm, 37 ^{o}C in an NNE buffer containing 5 mM DTT. Detailed experimental conditions were mentioned in Experimental procedures. Experiments were performed three times to estimate errors. **(H)** Formation of insoluble SOD1 pellets after aggregation reactions. After turbidity changes reached at a plateau (D-G), protein samples were ultracentrifuged at 110,000 x g for 30 min and fractionated into supernatant and pellets. Pellets were re-dissolved in the same volume of an NNE buffer containing 2 % SDS with that of supernatant. Then, equal volumes of supernatant (s) and pellets (p) were loaded on a 12.5 % SDS-PAGE gel and stained with CBB. Except A4V, all mutant and WT SOD1 proteins are found only in the pellet fraction, showing complete conversion of all SOD1 molecules into insoluble aggregates. In A4V, however, a supernatant fraction still contains SOD1, which is probably due to relatively sluggish kinetics of A4V aggregation (D).

Fig. S2. Amyloid-like tinctorial properties of SOD1 aggregates. **(A)** Quantification of SOD1 aggregates was performed by SDS-PAGE. Aliquots of SOD1 insoluble pellets re-suspended in water were boiled in a Laemmli buffer containing 5 mM TCEP, loaded on a 12.5 % SDS-PAGE gel and stained with CBB. Given that all of WT SOD1 molecules become insoluble pellets after aggregation reaction (Figs. S1D and H), we can reasonably assume the concentration of SOD1 molecules in the pellet fraction. Experiments were repeated three times (e.g. WT_1, WT_2 and WT_3 indicated on top of gels), and the sample, WT_1, was loaded as a loading control in different gels. **(B)** Densitometric analysis of protein bands in (A) was performed, and the band intensity relative to that of WT_1 sample was plotted. Based upon this analysis, a relative concentration of SOD1 protein in each aggregated sample was estimated. **(C)** Distinct properties of SOD1 aggregates probed with ThT fluorescence intensity. After preparation of SOD1 aggregates by ultracentrifugation, 3 μM SOD1 aggregates were mixed with 25 μM ThT in an NNE buffer, and fluorescence intensity was measured (black filled bars). As a control, fluorescence intensity of 25 μM ThT with 3 μM soluble apo-SOD1^{SH} was also examined (white open bars). Experiments were repeated three times to estimate errors. **(D)** An absorption spectrum of 10 μM Congo red in an NNE buffer (black curve) is not affected by addition of 10 μM apo-SOD1^{SH} (blue curve). In contrast, addition of 10 μM aggregated apo-SOD1^{SH} red-shifts the absorption peak of Congo red (red curve), which is a typical spectroscopic change upon binding of Congo red with amyloid-like fibrillar protein aggregates.

Fig. S3. Representative MALDI-TOF mass spectra of SOD1 aggregates treated with pronase. **(A-K)** aggregates prepared from recombinant SOD1 protein with an indicated mutation, **(L-O)** aggregates prepared from spinal cords of transgenic mice expressing SOD1 with an indicated mutation. As

mentioned in Experimental procedures, SOD1 aggregates were first digested with pronase, ultracentrifuged to purify the pronase-resistant insoluble peptides and then analyzed by a MALDI-TOF mass spectrometry. Identification of mass peaks shown in **(A-K)** was summarized in Table S1. In **(L-O)**, m/z values are indicated at the observed mass peaks, and peaks further confirmed by MS/MS analysis were underlined. Amino acid residues of the peptide corresponding to its mass peak are also indicated. Mass peaks with asterisks are not derived from SOD1 protein.

Fig. S4. Mass peaks of the "unmodified" peptides in Alexa555-modified SOD1 aggregates after digestion with lysyl endopeptidase. **(A)** Ala4 – Lys23 and **(B)** Thr137 – Gln153. As shown in (B), an unmodified Thr137 – Gln153 peptide was observed in WT, A4V and G37R aggregates; however, the Thr137-Gln153 peptide modified with Alexa555 was not observed (Fig. 3D, right). Similarly, an unmodified Ala4 – Lys23 peptide was observed in all five SOD1 aggregates (A), but the Alexa555-modified peptide was not detected (Fig. 3A, right). These results suggest that no observed mass peaks of Alexa555-modified Ala4 – Lys23 or Thr137 – Gln153 peaks (Figs. 3A and D) are due to less reactivity of each peptide toward Alexa555 but not due to inefficient digestion of the modified samples by pronase. Because L126X is a truncated SOD1 at Leu126, a peptide spanning from Thr137 to Gln153 (B) is not detected.