



Supplementary Information for

Large SOD1 aggregates, unlike trimeric SOD1, do not impact cell viability in a model of amyotrophic lateral sclerosis

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Figs. S1 to S6

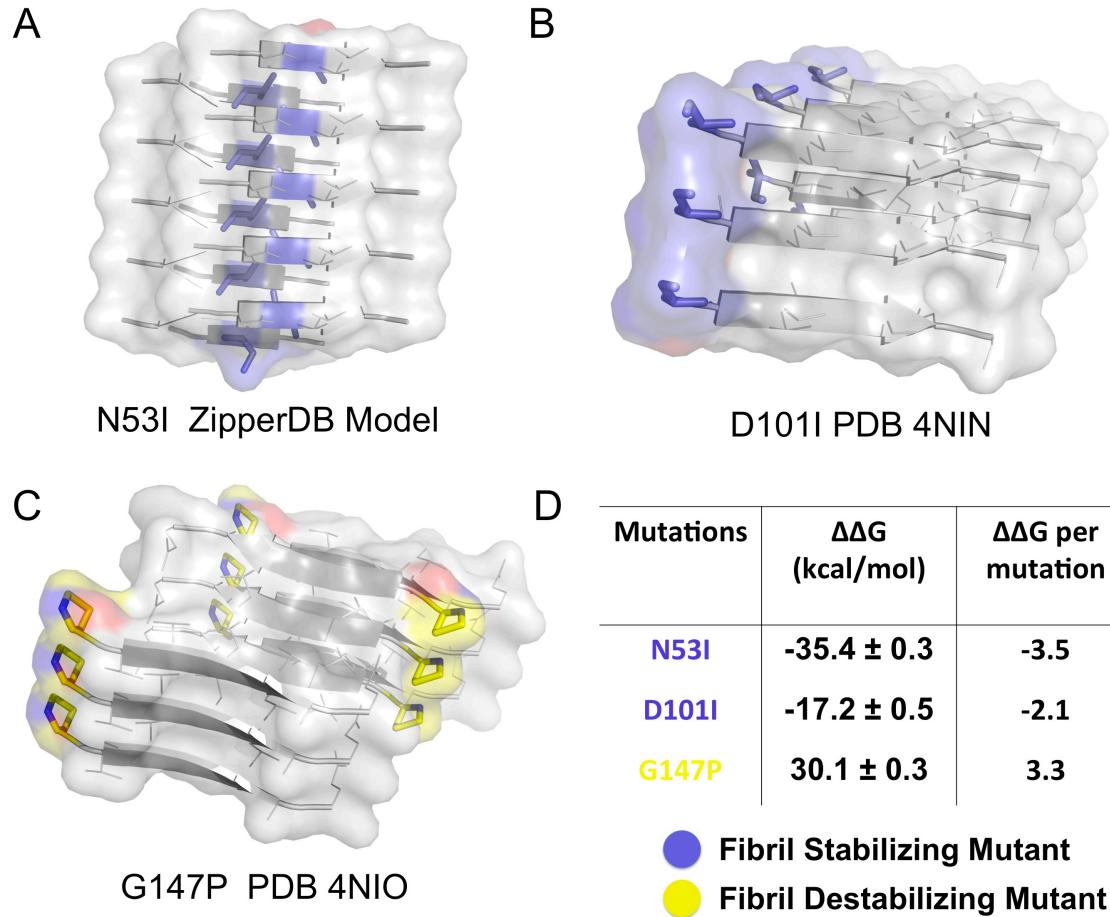


Fig. S1. Eris prediction on the change of fibrillar stability upon mutations.

$\Delta\Delta G = \Delta G_{\text{mutant}} - \Delta G_{\text{wild type}}$ (negative: stabilizing, positive: destabilizing). (A) - (C) The structural model of fibrils were shown with surfaces and the mutagenesis sites were shown as sticks (N53 and D101I, navy; G147, yellow). For D101I and G147P, crystal structures (4NIN 101-DSVISLS-107 4NIO, 147-GVTGIAQ-153) were used as input models for Eris; for N53I, computational model from ZipperDB (51-GDNTAG-56) were used. (D) Summary of Eris calculation (\pm means standard deviation).

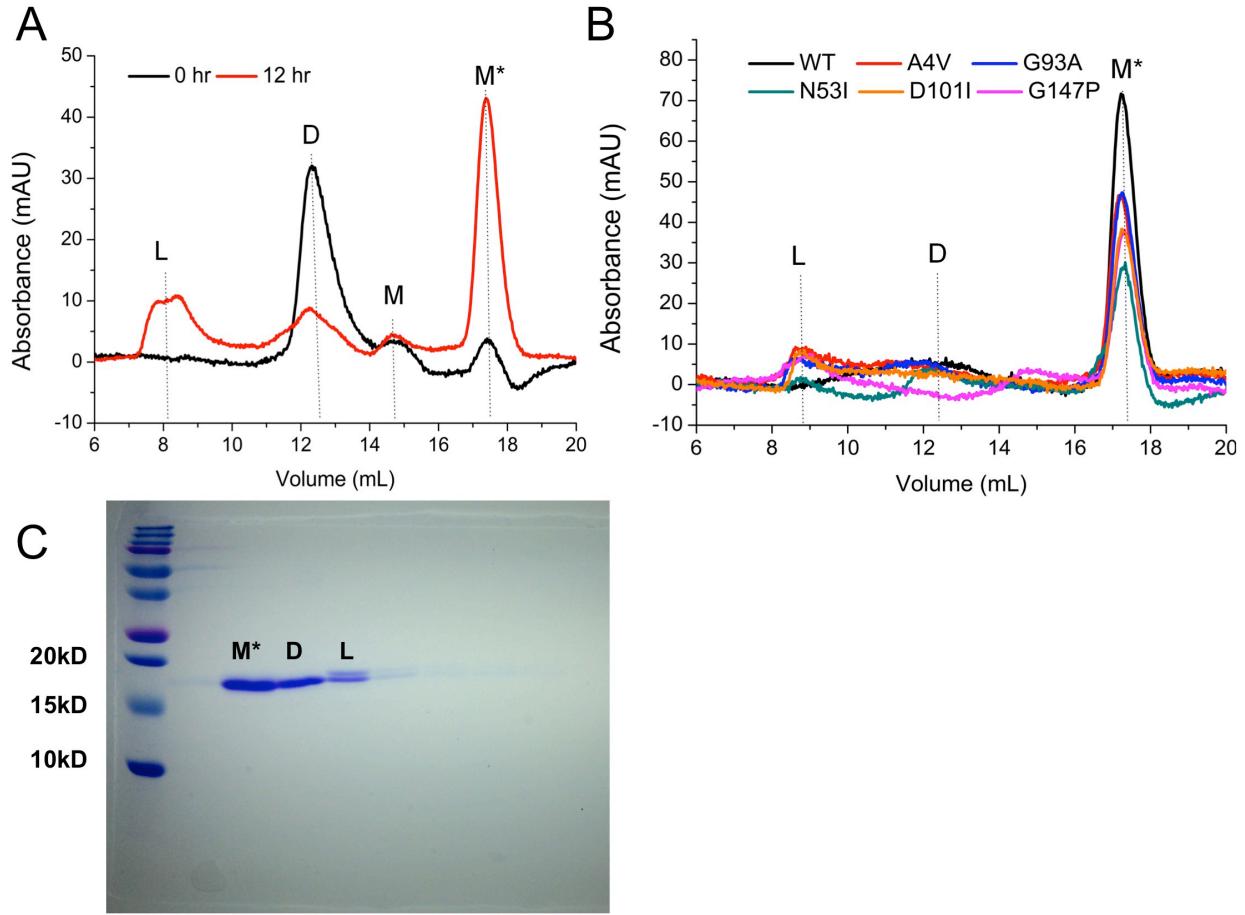
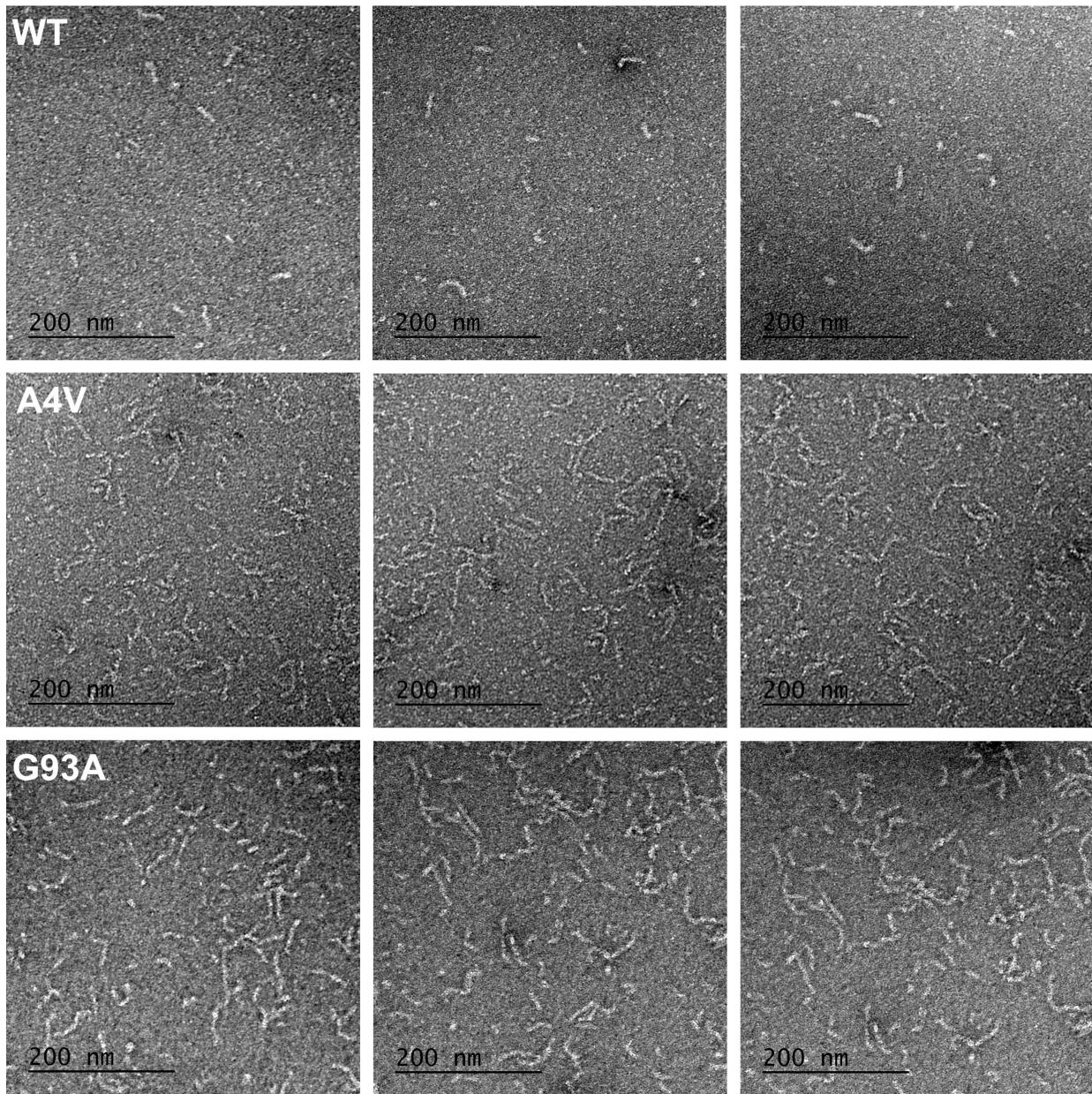


Fig. S2. Analytical size exclusion chromatograms reveal the distribution of different oligomeric states of WT-SOD1 (L, T, D, M and M* as defined in Fig. 1). The protein samples of each mutant were incubated at denaturing condition (50 mM acetate, 150 mM NaCl and 10 mM EDTA, pH 3.5) before applied to Superdex 75 10/300 GL column. (A) WT-SOD1 initially contained a large amount of dimers. The denaturing condition prompted the formation of misfolded monomers (M*) and large aggregates (L). (B) Upon two days incubation, M* and L became the dominating species. The misfolded monomer, as seen previously, is the precursor of non-native SOD1 oligomers and fibrils. (C) SDS-PAGE analysis of the eluted peaks in Fig. S1A (12 hr). The molecular weight of monomeric WT-SOD1 is 15.8 kD. The misfolding of monomers (M*) may lead to exposure of hydrophobic residues and non-specific interaction between M* species and the Superdex column, resulting in delayed elution time of M* peaks in comparison to that of M peaks.



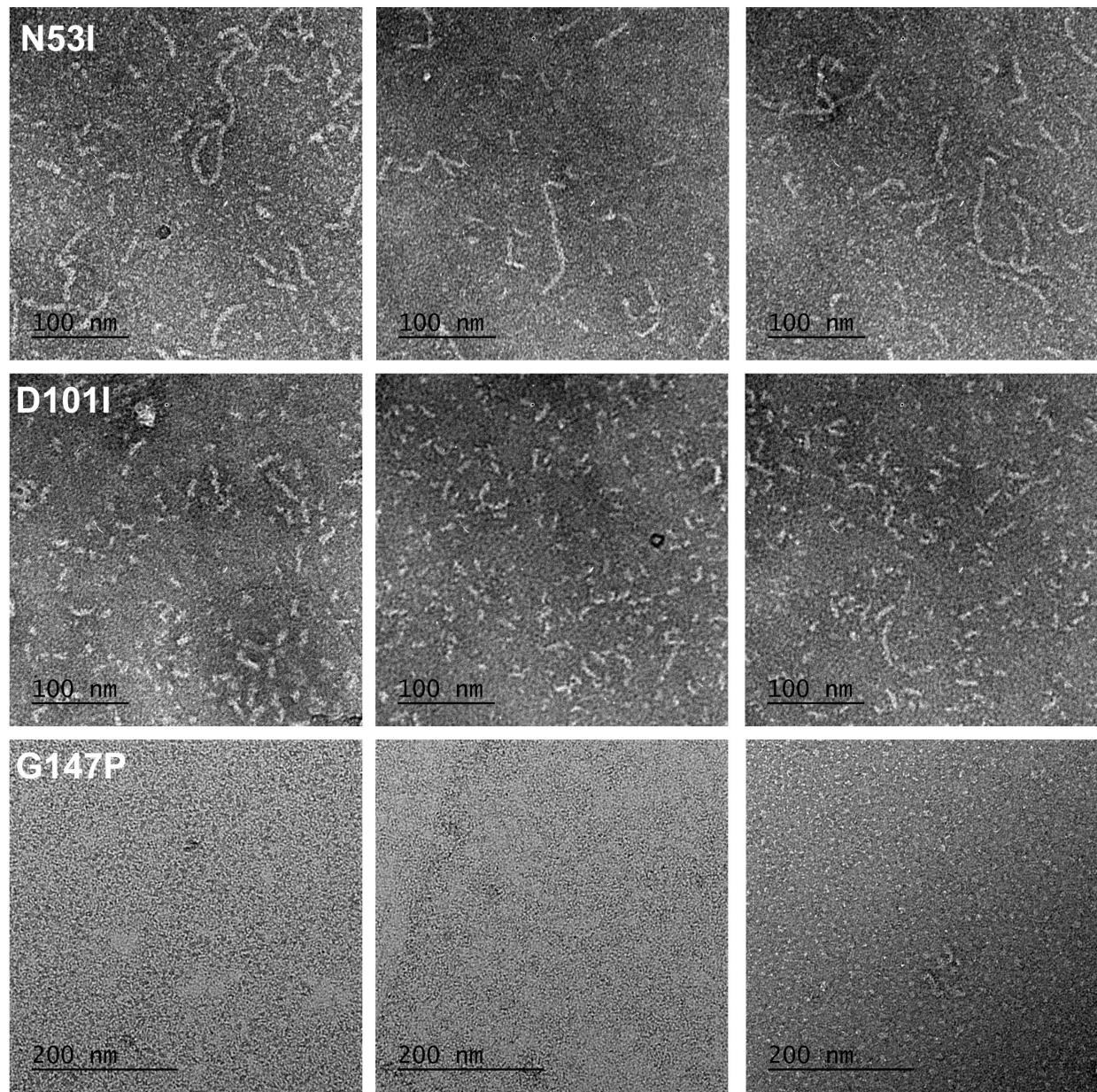
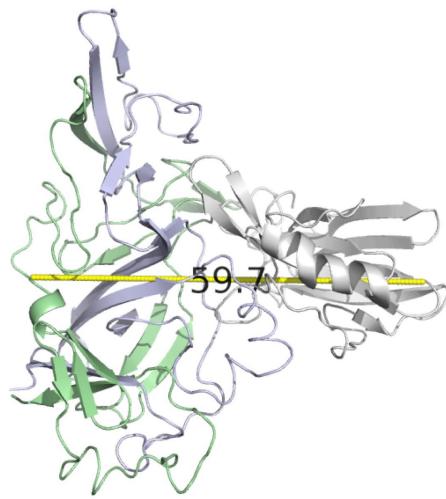
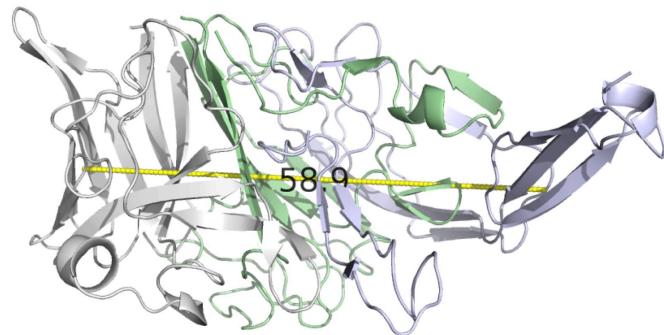
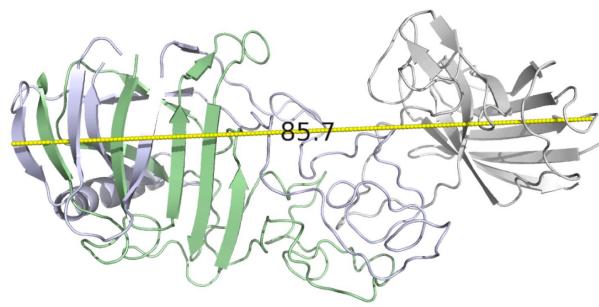
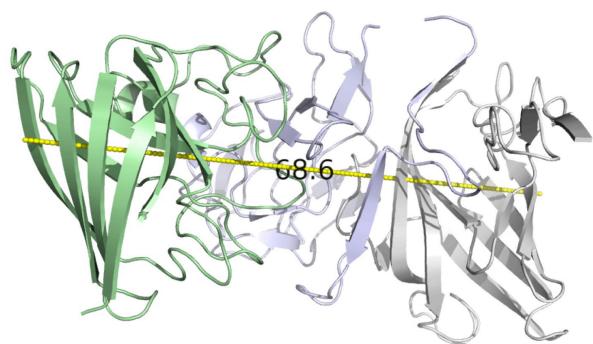
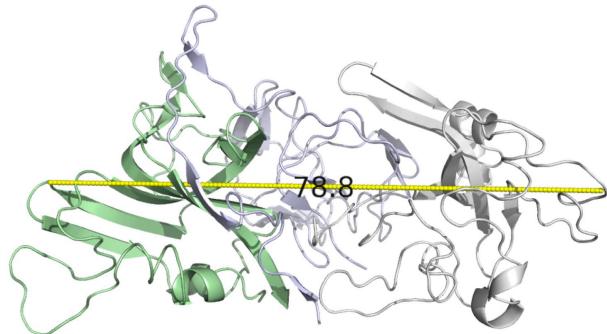
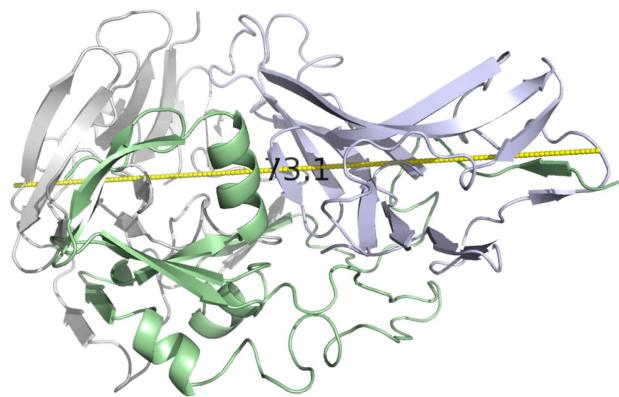


Fig. S3. Electron microscopy negative stain images. Three representative images were shown for WT-SOD1, A4V- and G93A-SOD1 (ALS-associated mutants), N53I- and D101I- SOD1 (fibril-stabilizing mutants), and G147P-SOD1 (trimer-stabilizing mutant)



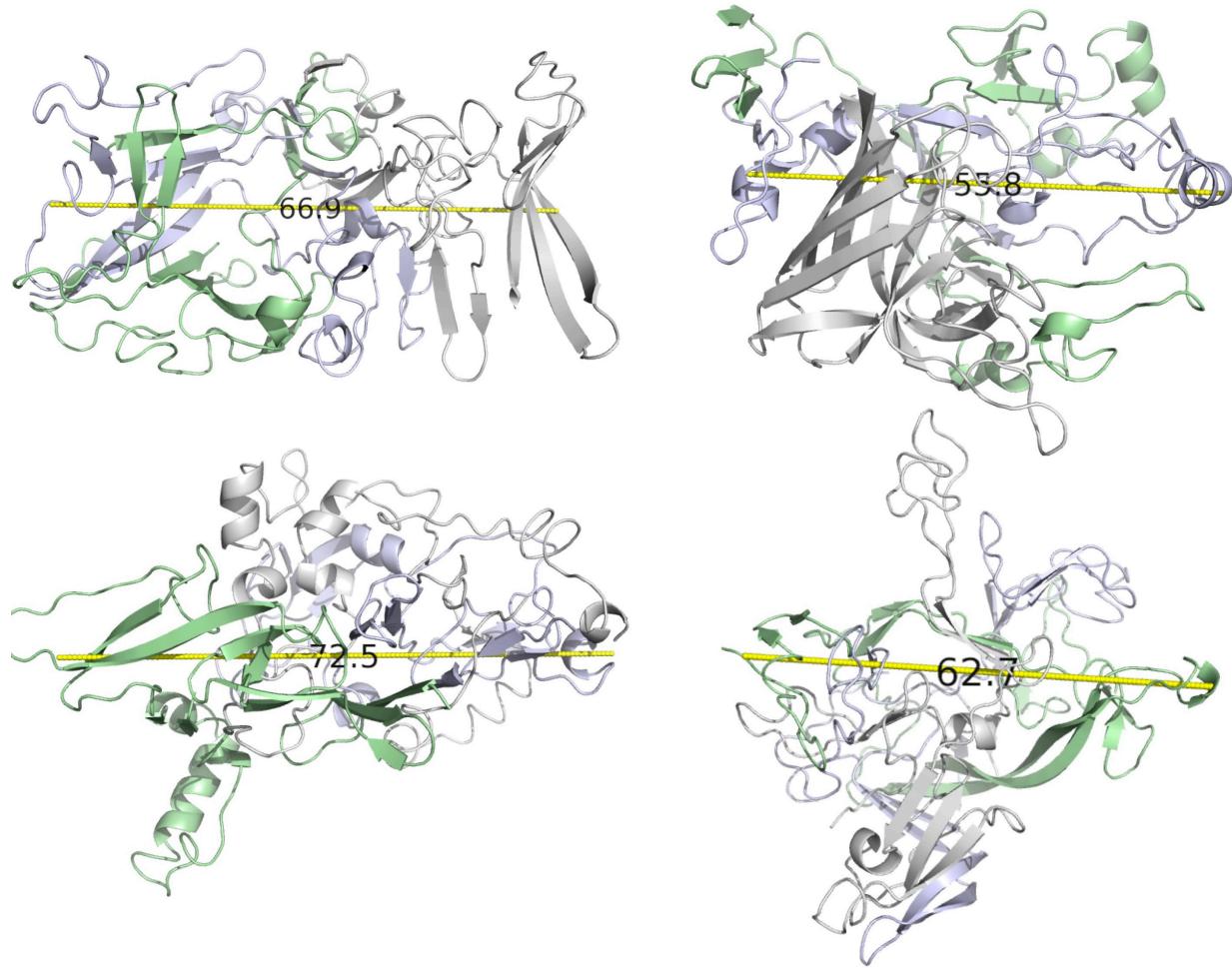


Fig. S4. Illustration of SOD1 trimer structural models and the measurement of SOD1 trimer diameters (yellow dashed line).

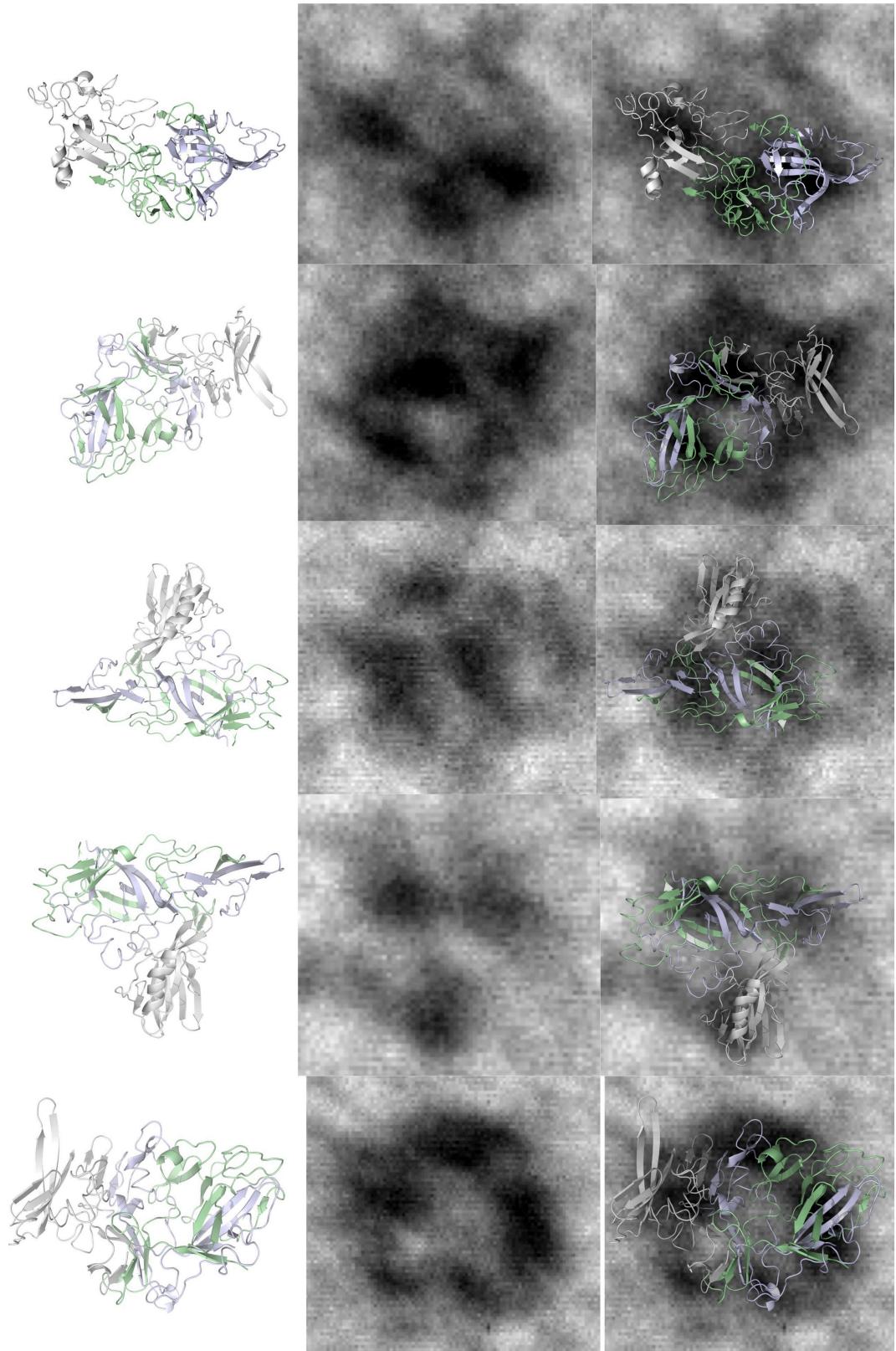


Fig. S5. Illustration of heterogeneity of SOD1 trimer structures.

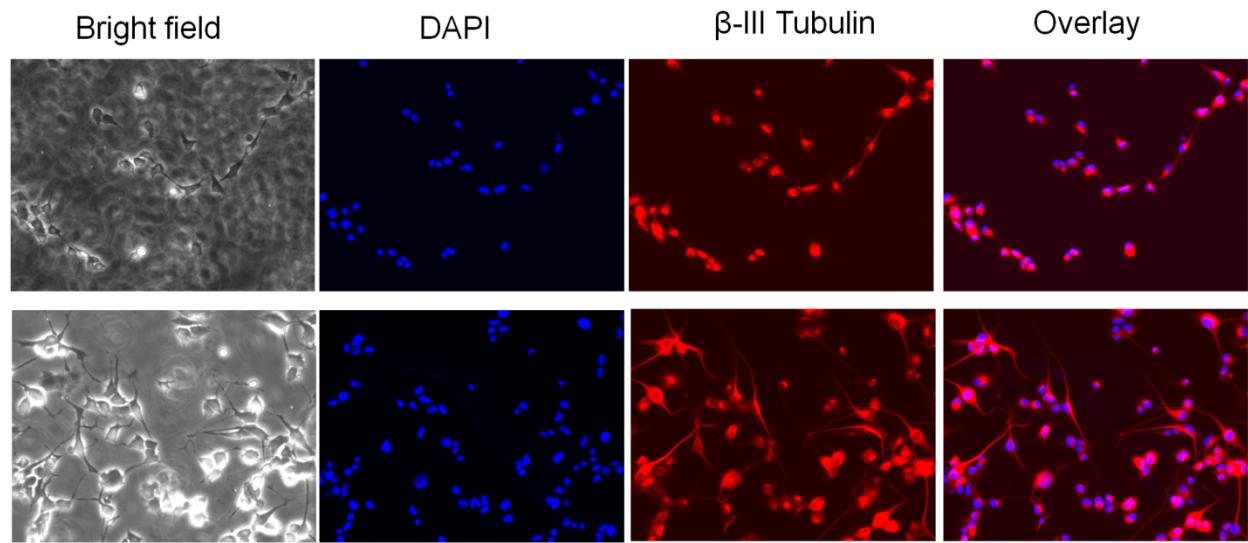


Fig. S6. Determination of NSC-34 differentiation conditions. Upper row: growth medium without retinoic acid. Lower row: differentiation medium with retinoic acid. The images were taken two-days after differentiation. The neuron-specific axons can only be visualized with the differentiation medium.