Supplementary Material

Experimental mutations in superoxide dismutase 1 provide insight in potential mechanisms involved in aberrant aggregation in familial amyotrophic lateral sclerosis

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Figure S1. All rotamers of the D40 variant are predicted to destabilize local structure near the β-barrel plug. A-H, Loss of van der Wäals interactions relative to E40 are predicted to weaken the interaction between the 3-4 and 5-6 loop elements (compare to Figure 3B of main text). Introduction of unfavorable interactions (discs and arrows). Although the steric clashes in panels c) and e) are not as severe as in other panels, the contacts between the side chain oxygen atoms of D40 and the carbonyl oxygen atoms of D90 and A89, respectively, are strongly repulsive.
Figure S2. Example of images used to assess the frequency of inclusion formation by variants SOD1 encoding ALS mutations at position E133. Recombinant DNA vectors encoding SOD1 fused in-frame to YFP were transfected into CHO cells as described in Materials and Methods, and images were captured at 24 and 48 hours.
Figure S3. Mutation of E133 to D, G, L, or M does not induce aggregation. CHO cells were transfected with plasmids for each SOD1:YFP variant and after 24 or 48 hours the cells were fixed and immunostained as described in Materials and Methods. Each transfection was repeated at least 3 times and at least 3 fields of view were counted for cells expressing the YFP fusion protein. The experimental mutants tested produced less inclusions than fALS associated mutations (Table 1).
Figure S4. Immunoblot analysis of mutant SOD1:YFP expression in CHO cells. At 48 hours after transient transfection, cells were lysed as described in Materials and Methods, and cell lysates were analyzed by immunoblotting. A and C) Each lane was loaded with 10 µg of protein as described in Methods. B) Each lane was loaded with 4 µg of protein as described in Methods. The mutants expressed in each cell transfection are noted above each image. Below each image we provide the average percentage of cells expressing each mutant that develop inclusions. Each blot was probed with SOD1 rabbit polyclonal antibody described in Materials and Methods at 1:4000 overnight and then incubated with secondary antibodies for ECL imaging.