Supplemental Material

Direct and indirect mechanisms for wild-type SOD1 to enhance the toxicity of mutant SOD1 in bigenic transgenic mice

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Supplemental Figures

Figure S1. Thioflavin S stained fibrillary structures in spinal and brainstem motor neurons from Cu-V103Z Line D-14 mice. The scale bar is 10 μm in all these figures, which were selected from a panel of different images from different animals captured at the same magnification.

Figure S2. A representative motor neuron filled with C4F6 immunoreactive fibers from Cu-V103Z Line E-17 mice. Similar structures are seen in mice from both line D-14 and E-17. Green - C4F6 (1:500), Blue- DAPI.

Figures S3 and S4. Original low power images that were used to extract the digitally enhanced images shown in Figure 3 of the main text.

Figure S5. Comparisons of Thioflavin-S and C4F6 immunostaining in bigenic mice expressing L126Z and WT-hSOD1:YFP, L126Z alone, and littermate controls. Thioflavin positive inclusions were detected in bigenic L126Z/SOD1:YFP mice (A) and mice expressing L126Z alone (B). Note that the processing of the tissue for thioflavin staining inactivates the YFP fluorescence. Only lipofuscin autofluorescence is detected in non-transgenic littermates after Thioflavin staining (C). Immunostaining using C4F6 detected similar structures in spinal cords of L126Z L45 x WT-SOD1:YFP mice (D), and L126Z Line 45 mice (E), but not the non-transgenic littermates (F).

Figure S6 and S7. Immunoblots of CHO cell lysates from cells transfected with vectors to express SOD1-gLuc fusion proteins. A representative immunoblot is shown in which we validated that the cell lysates used in our ex vivo split luciferase assays contained comparable amounts of each of the gLuc fusion proteins. The immunoblots were probed with wSOD1 antibody, which does not recognize the gLuc1 or gLuc2 proteins. The amount of lysate to use for ex vivo assays from cells expressing gLuc1 and gLuc2 was estimated by total protein content.

Figures S8A and S8B. Graphs of the raw data for luciferase activity in the various combinations of luciferase complementation assays. The tables provide the statistical analysis of selected comparisons.

Figure S9. Supplemental AviTag capture analysis data. A representative immunoblot of an AviTag capture assay is shown for cells co-expressing SOD1-AviTag with A4V and G85R hSOD1. The graph shows quantitative data in which we used a capture index to rate the relative efficiency of SOD1-AviTag binding to the co-expressed untagged SOD1 variant. The capture index was calculated by determining the ratio of SOD1- AviTag protein to target protein in whole lysates and then dividing this number by the ratio of the two proteins after avidin affinity capture.
**Figure S10.** Immunoblot analysis of SOD1 levels in G37R/WT-SOD1:YFP bigenic mice. Spinal cords were homogenized by sonication as follows: tissue was held on ice and sonicated 3 times for 10-15 seconds each in 1X TEN buffer (10 mM Tris, pH 7.5; 1mM EDTA, pH 8.0; 100 mM NaCl) containing 1:100 v/v protease inhibitor cocktail (Sigma, St. Louis, MO, USA), followed by centrifugation at 800g for 10 min. The pellet was discarded and the protein concentration of the supernatant was determined by bicinchoninic acid assay, as described by the manufacturer (Pierce Biotechnology). 10 µg of total protein from the supernatant was analyzed by SDS-PAGE and immunoblotting with an SOD1 antibody that recognizes mouse and human SOD1 (m/hSOD1 antibody). The membrane was then washed with PBS-T, incubated for 1 h at room temperature with a goat anti-rabbit IgG secondary antibody at 1:5000 in PBS-T and 5% milk before developing with enhanced chemiluminescence reagents (Thermo Scientific Inc., Rockford, IL, USA) and visualizing with a FluorChem E imager (Protein Simple, Santa Clara, CA, USA). The intensity of G37R SOD1 and mouse SOD1 immunoreactivity were quantified by software in the Protein Simple operating system. Lane 1, nontransgenic spinal cord. Lane 2, WT-SOD1:YFP spinal cord. Lanes 3-5, PrP.G37R-Line 110 spinal cords. Lanes 6-8, spinal cords from bigenic G37R/WT-SOD1:YFP mice. Using the levels of endogenous mouse SOD1 to normalize the data, the ratio of mouse (Mo) to human G37R-SOD1 in bigenic G37R/WT-SOD1:YFP mice is not statistically different from that of PrP.SOD1-G37R mice (p>0.08).

**Figure S11.** Representative images of C4F6 immunostaining compared to immunostaining with antibodies to YFP. Representative images of the ventral horn of the spinal cord from 3-5 different animals (at least 6 sections per animal) after immunostaining with either C4F6 or antibody (JL-8) that recognizes YFP.
Supplemental Fig. S3

Cu-V103Z (C4F6)   Cu-V103Z xGurWT (C4F6)
Supplemental Fig. S4

Cu-V103Z xGurWT (SEDI)  GurWT (SEDI)
**Supplemental Fig. S8A**

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T-test vs WT - WT
T-Test vs gLuc1 - gLuc2
Statistical Analyses

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T-test vs WT
T-test vs L126-Z
T-test vs L126-WT
T-test vs WT-L126
T-test vs CuV103Z-CuV103Z
T-test vs CuV103Z-WT
T-test vs WT-CuV103Z

Supplemental Fig. S8B
Supplemental Fig. S9

1) Cell lysate

2) Biotinylated/Avidin Purified

3) Avidin Purified – no biotinylation

Capture Index

- **WT**
- **A4V**
- **G37R**
- **G85R**
- **L126Z**
Supplemental Fig. S10

G37R x WT-SOD1:YFP

Ratio G37R-SOD1 to Mouse SOD1

NTg  WT-SOD1:YFP  G37R  G37R x WT-SOD1:YFP

1 2 3 4 5 6 7 8 9

2.6 2.1 1.8 1.4 1.6 1.8

20 kDa 30 kDa 40 kDa 50 kDa 60 kDa
Supplemental Fig. S11

C4F6 immunostain
G37R/WT-SOD1:YFP

A

YFP immunostain G37R/WT-SOD1:YFP

C

C4F6 immunostain
WT-SOD1:YFP

B

YFP immunostain WT-SOD1:YFP

D

YFP immunostain Non Tg

E