Supplementary Information for:

Characterization of the activity, aggregation, and toxicity of heterodimers of WT and ALS-associated mutant Sod1

Brasil, A. A.[1*], Carvalho, M. D. C.[1*], Gerhardt, E.[2], Queiroz, D. D.[1], Pereira, M. D.[1], Outeiro, T. F.[2,3,4#], Eleutherio, E. C. A.[1**#]

[1] Institute of Chemistry, Federal University of Rio de Janeiro (UFRJ), 21941-909, Rio de Janeiro, RJ, Brazil.

[2] Department of Experimental Neurodegeneration, Center for Nanoscale Microscopy and Molecular Physiology of the Brain, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, 37073, Göttingen, Germany.

[3] Max Planck Institute for Experimental Medicine, Göttingen, Germany.

[4] Institute of Neuroscience, The Medical School, Newcastle University, Framlington Place, Newcastle Upon Tyne, NE2 4HH, UK.

* Equal contribution.

** Corresponding author:
eliscael@iq.ufrj.br

[#]Co-senior authors

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Supplementary text of Methodology

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Methods

SDS-PAGE and Immunoblotting

Cells were collected in lysis buffer (TBS with 0.5 % Triton X-100) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany), incubated on ice for 30 min. and cleared by centrifugation for 10 min at 5°C and 10,000 g. Total protein concentration was determined using the Bradford assay (BioRad Laboratories, CA, USA). The lysates were boiled at 95 °C for 5 min in protein sample buffer (PBS, 50 mM Tris-HCl pH 6.8; 2 % SDS; 10 % glycerol; 1 % β-mercaptoethanol; 0.02 % bromophenol blue), and resolved by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a 10 % gel. Proteins were transferred for 7 min (to 20V) with the iBLOT 2 Gel Transfer Device (Invitrogen, Thermo Fisher Scientific, Germany) on nitrocellulose membrane (Immobilon-FL-Membrane, Millipore, USA) and blocked for 1 h in TBS-Tween with 5 % bovine serum albumin, respectively. The membranes were incubated overnight at 4 °C with primary antibodies using the following dilutions: anti SOD1 (Santa Cruz Biotechnology, USA) and ɣ-Tubulin (T5192, Sigma) 1:1000. Membranes were washed with TBS-Tween and incubated for 1 h at room temperature with the corresponding HRP-labelled secondary antibodies (GE Healthcare, UK) 1:10,000. Immunoreactivity was visualized by chemiluminescence using an ECL detection system (Millipore, Billerica, MA, USA).

Size-Exclusion Chromatography and Dot Blot

Sod1-KD cells were collected 48 h after transfection in a phosphate buffer (TBS with 0.5 % Triton X-100) freshly supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany) and centrifuged for 10 min at 5°C and

10,000 g. 1.5 mg of total protein in a maximum volume of 500 µl was filtered using a 0.45-µm Spin-X centrifuge filter (Sigma, Hamburg, Germany) before loading onto a Superose 6 (Superose 6 10/300GL, GE Healthcare Life Science, Sweden) column and subsequent high-performance liquid chromatography (HPLC) (Äkta Purifier10, GE Healthcare Life Science, Sweden) in 50-mM ammonium acetate pH 7.4 buffer with a flow rate of 0.5 ml/min. The collected HPLC fractions of 500 µl were boiled at 95 °C for 10 min and centrifuged at 14000g, 5°C for 5 min. For the dot blot assay, the fractions were loaded on a nitrocellulose membrane, using a dot blot vacuum system. Membranes were further processed as described under Western blotting except that all steps were performed in PBS instead of TBS.

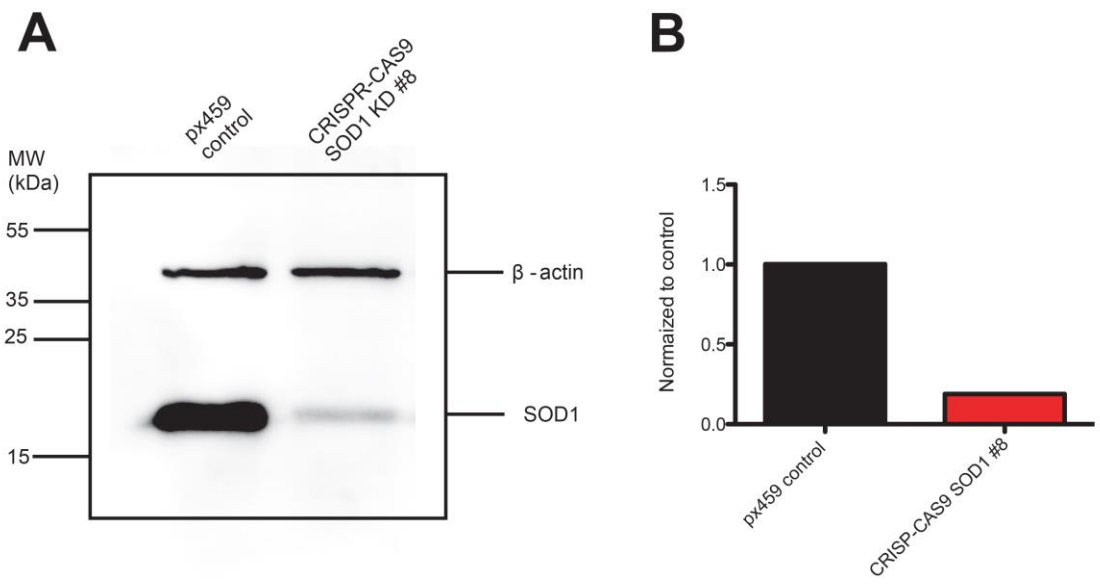


Figure S1. CRISPR-cas9 mediated knock-down of human Sod1 in HEK293 cells. (A) CRISPR-cas9 mediated knock-down of human Sod1 is confirmed by immunoblotting using an antibody against human Sod1 in comparison to control cells transfected with the empty vector PX459. (B) Quantification of the immunoblot signals using ImageJ.

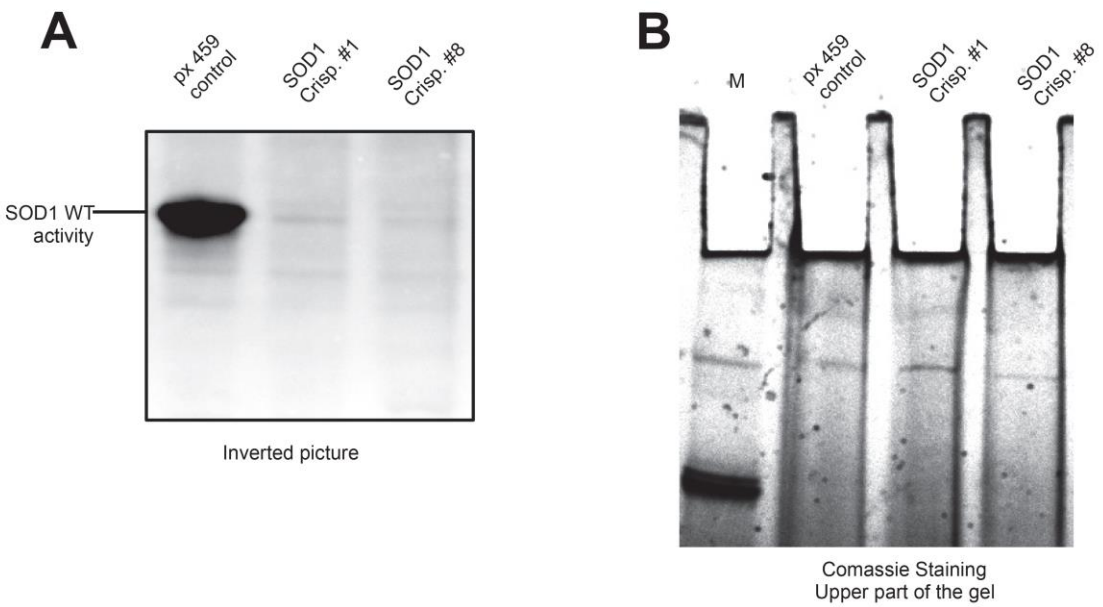


Figure S2. *In situ* Sod1 activity gel. Protein lysates of the control cell-line PX459 and of two different Sod1-KD cell-lines were separated by SDS PAGE and Sod1 activity was assayed within the gel. (A) Equal amounts of protein (30 µg/lane) were loaded and active Sod1 is indicated by white bands, shown here as an inverted picture. (B) The upper part of the SDS PAGE was stained using Coomassie as a loading control.

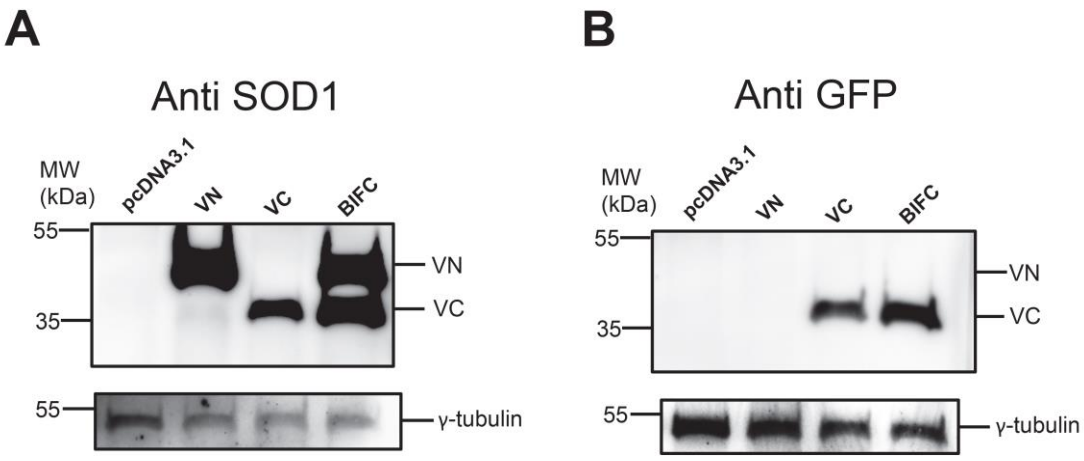
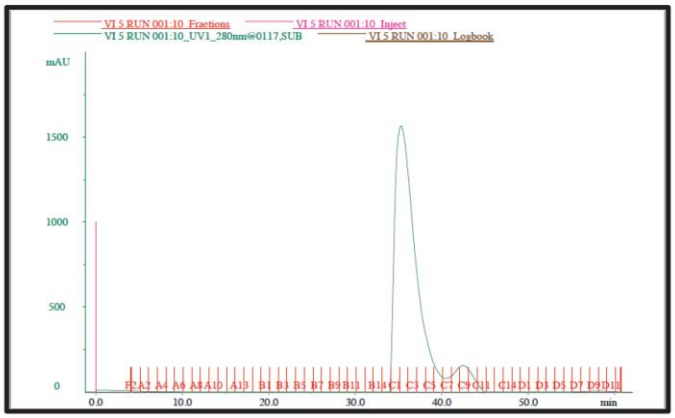


Figure S3. Detection of Sod1 BiFC using different antibodies.

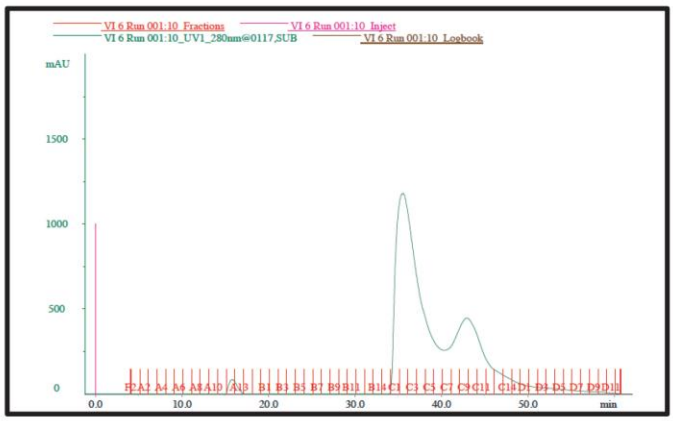
HEK293-Sod1-KN cells overexpressing VN-Sod1, Sod1-VC and Sod1-BIFC proteins can be detected by an immunoblotting analysis using antibodies against human Sod1 (A) or GFP (B). The GFP antibody only detects the VC fragment of Venus in the BiFC-assay.

A



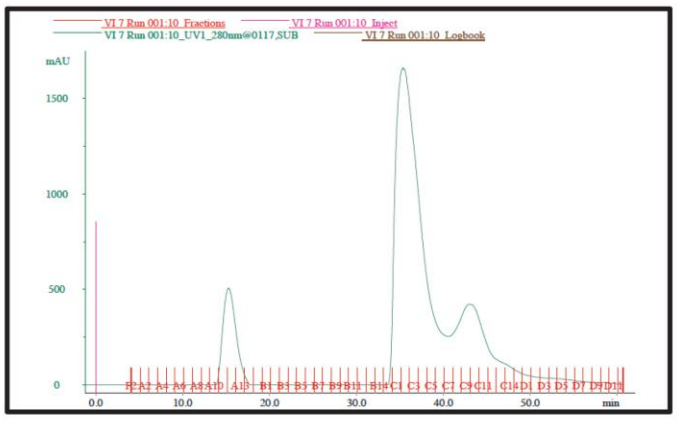
SOD1-KD pcDNA

B



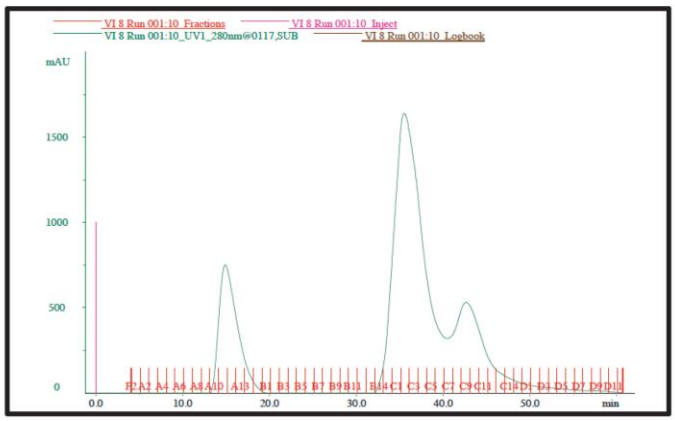
SOD1-KD WT-WT

C



SOD1-KD A4V-A4V

D



SOD1-KD WT-A4V

Figure S4. Chromatograms from SEC analysis of SOD1-KD cells expressing hSod1-BiFC. HEK293 Sod1-KD cells expressing the different Sod1 constructs (VN- and VC-fusions) have been separated by SEC. (A) SEC chromatograms from HEK293 SOD1-KD cells transfected with pcDNA3.1, (B) transfected with the Sod1 WT homodimer, (C) transfected with the Sod1 A4V homodimer, and (D) transfected with the Sod1 heterodimer Sod1 WT- A4V.