SUPPLEMENTARY MATERIAL

Fig.S1 Specificity of the binding between Bcl-2 and SOD1. (A) Spinal cords from SOD1-G93A mice (onset and end stage) were homogenized and immunoprecipitated with either the anti-loop Bcl-2, the anti-SOD1 antibody or equal amounts of their corresponding IgGs. Co-precipitates were analyzed by WB with anti-SOD1 and anti-Bcl-2 antibodies respectively. The mutSOD1/Bcl-2 complex is immunoprecipitated only by the precipitating antibodies and not by the corresponding IgGs, clearly indicating the specificity of SOD1/Bcl-2 binding. TL= total lysates **(B)** HEK293T cells which do not express detectable levels of Bcl-2 were transfected with either Bcl-2 or empty plasmid (mock) and immunoprecipitation with anti-SOD1 or IgG was performed, followed by WB for Bcl-2 or SOD1 in order to determine the specificity of the binding between SOD1 and Bcl-2. As shown, the anti-SOD1 antibody co-precipitates Bcl-2 only in transfected, but not in naïve, mock transfected cells. Control IgGs failed to precipitate Bcl-2 even in cells transfected with Bcl-2. In mock transfected cells, control IgGs failed to aspecifically precipitate other proteins in the 25-30 KDa range. TL=total lysates. **(C)** HEK293T cells are a suitable system to study mutSOD1-toxicity in the absence of Bcl-2. Cells were transfected with Bcl-2, fixed and stained with anti Bcl-2 (blue) and the mitochondrial marker mitotracker (300 nM, red). There is a negative staining with the anti-Bcl-2 antibody in non-transfected cells confirming that HEK293T cells do not express detectable levels of endogenous Bcl-2 (scale bar 20 μm). WB shows expression levels of Bcl-2 prior and after transfection.

Figure S2. Schematic representation of Bcl-2 and the antibodies used in IP/WB experiments. (A) Linear representation of Bcl-2 with its BH (1-4) domains and the epitopes of the antibodies used in our experiments. **(B)** FACS analysis of

conformational changes in Bcl-2 induced by mutSOD1**.** Exposure of the BH3 domain in SH-SY5Y was also assessed by FACS analysis using the conformation-specific αBcl-2/BH3 antibody. The shift in fluorescence in presence of mutSOD1s indicates an increased exposure of the BH3 domain.

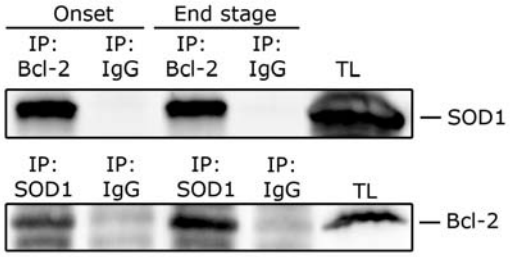
Figure S3. Lack of SOD1-G93A toxicity in H4 cells expressing nuclear but not mitochondrial Bcl-2. (A) H4 cells (ATCC cat. # HTB-148) were stained with DAPI (as nuclear marker) and for Bcl-2 and Cytochrome C (as mitochondrial marker). Bcl-2 co-localizes with DAPI (nuclear localization) but not with Cytochrome C (mitochondria localization). The bottom panel represents expression of Bcl-2 in the total lysate (TL), nuclei (Nuc), mitochondria (Mito) and cytoplasm (Cyto) (scale bar 20 μm). **(B)** In the absence of mitochondrial Bcl-2, SOD1-G93A does not induce Cytochrome C release. H4 cells were co-transfected with SOD1-G93A-EGFP, immunostained for Bcl-2 (blue) and Cytochrome C (red) to assess mitochondrial integrity. In the absence of mitochondrial Bcl-2, mutSOD1-expressing (green) H4 cells show puntate Cytochrome C staining indicating mitochondrial integrity (scale bar 20 μm).

SUPPLEMENTARY METHODS

*FACS analysis***:** SH-SY5Y cells were grown in DMEM media, 5% FBS with antibiotics. 2 days before transfection, cells were plated at a concentration of 100,000/well in 12-well plates and transfected with 500 ng of SOD1-EGFP constructs using Lipofectamine 2000 following manufacturer's instructions. After 24 hrs, cells were detached, washed twice in PBS, incubated for 1 hr at RT with PBS 5% goat serum and then fixed and permeabilized in Cytofix/Cytoperm (BD, Franklin Lakes, NJ, USA). Following permeabilization, cells were incubated with rabbit α-Bcl2-BH3 domain

(Abgent, San Diego, CA, USA) 1: 250 in Cytoperm/Cytowash 5% goat serum for 1 hr and subsequently in anti-rabbit Alexa Fluor 546 (Carlsbad, CA, USA) 1:400 in Cytoperm/Cytowash 5% goat serum for 1 hr. Cells were then analyzed using FACScalibur (BD, USA).

A


Onset
End stage
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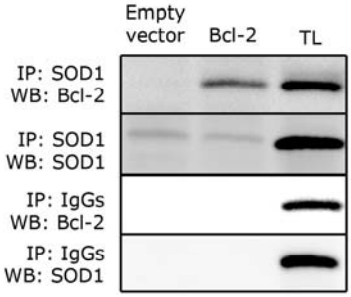
IP: Bcl-2
IP: IgG
TL
SOD1
IP: SOD1
IP: IgG
IP: SOD1
IP: IgG
TL
Bcl-2

B


Empty vector
Bcl-2
TL
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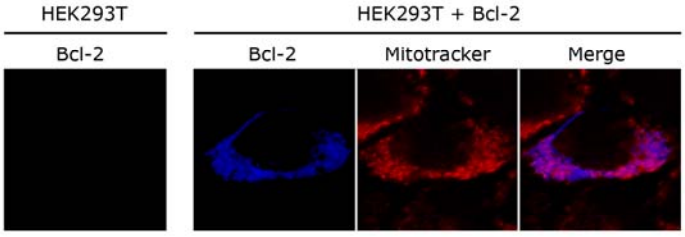
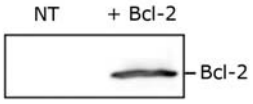
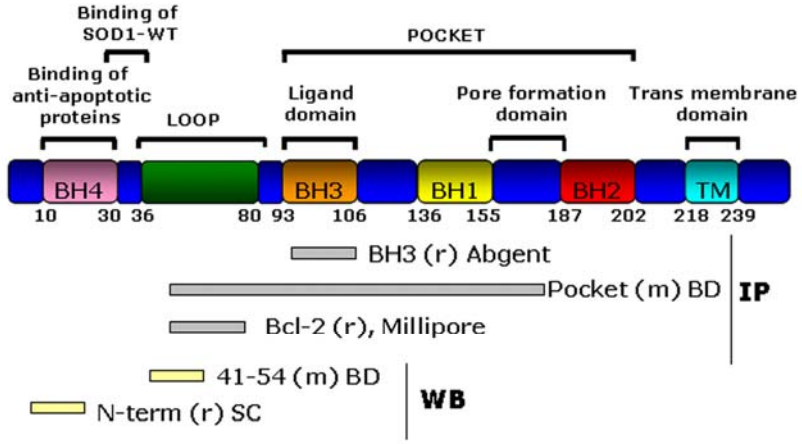
WB: Bcl-2
IP: SOD1
WB: SOD1
IP: IgGs
WB: Bcl-2
IP: IgGs
WB: SOD1

C

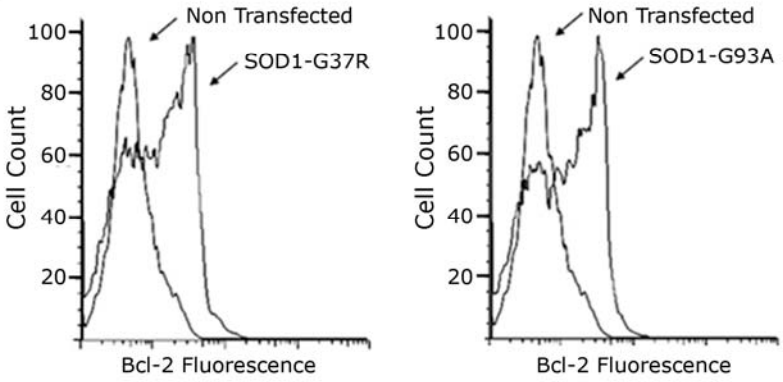

HEK293T
HEK293T + Bcl-2
Bcl-2
Bcl-2
Mitotracker
Merge


NT
+ Bcl-2
Bcl-2

A


Binding of SOD1-WT
POCKET
Binding of anti-apoptotic proteins
LOOP
Ligand domain
Pore formation domain
Trans membrane domain
BH4
BH3
BH1
BH2
TM
10 30 36 80 93 106 136 155 187 202 218 239
BH3 (r) Abgent
Pocket (m) BD
IP
Bcl-2 (r), Millipore
41-54 (m) BD
N-term (r) SC
WB

B


Non Transfected
SOD1-G37R
Cell Count
100
80
60
40
20
Bcl-2 Fluorescence
Non Transfected
SOD1-G93A
Cell Count
100
80
60
40
20
Bcl-2 Fluorescence

Pedrini et al, Supplementary Figure 3