Supplemental Figures

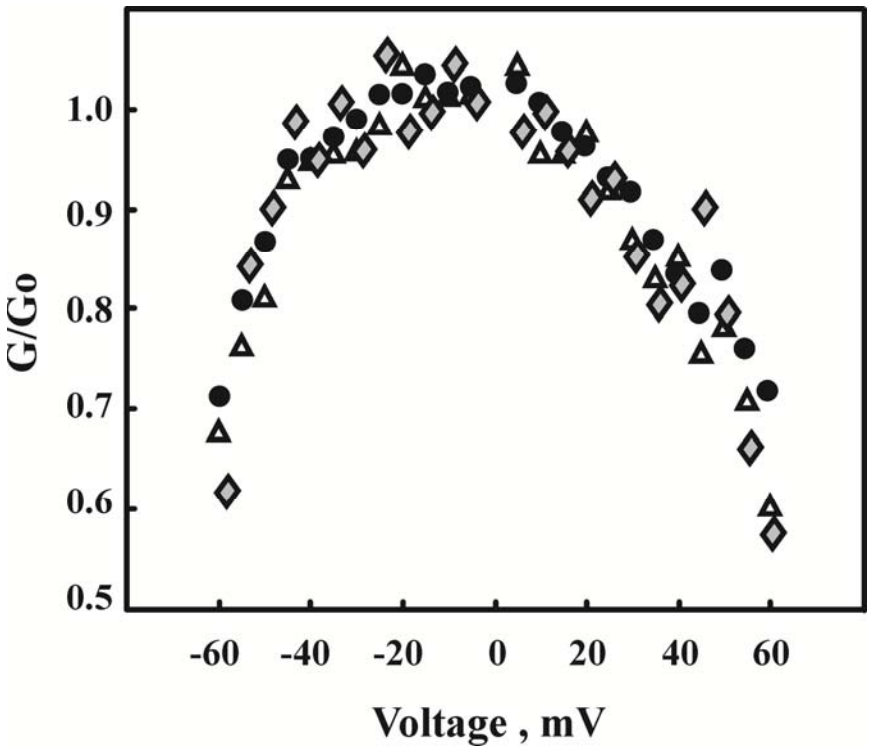

Fig. 1S. Alpha-synuclein does not affect conductance of bilayer-reconstituted VDAC. VDAC was reconstituted into a PLB and currents through VDAC in response to a voltage step from 60 to –60 mV were recorded before (●) and 30 min after the addition of 50 µg alpha-synuclein wild type (Δ), or mutant alpha-synuclein E46K (◊). Relative conductance was determined as the ratio of the conductance at a given voltage (G) to the maximal conductance (Go).

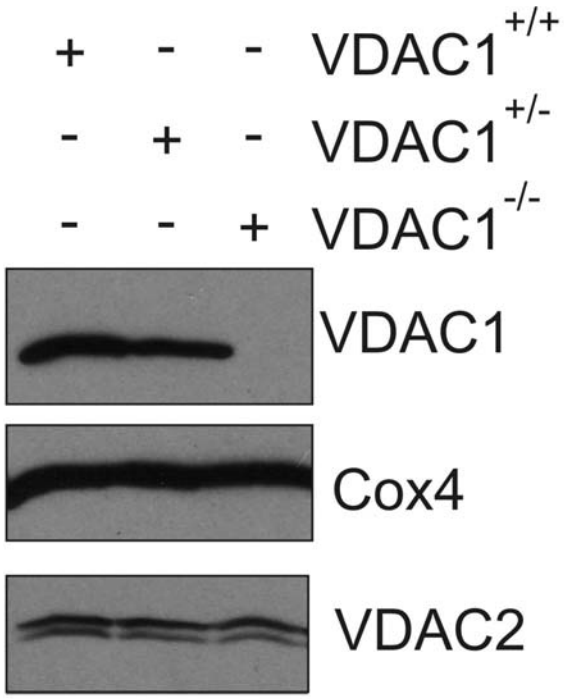

Fig. 2S. VDAC1 heterozygous mice accumulate about half the normal level of VDAC1. Spinal cord extracts from wild type, VDAC1$^{+/-}$ and VDAC1$^{-/-}$ mice were subjected to immunoblot using VDAC1, Cox4 or VDAC2 antibodies.

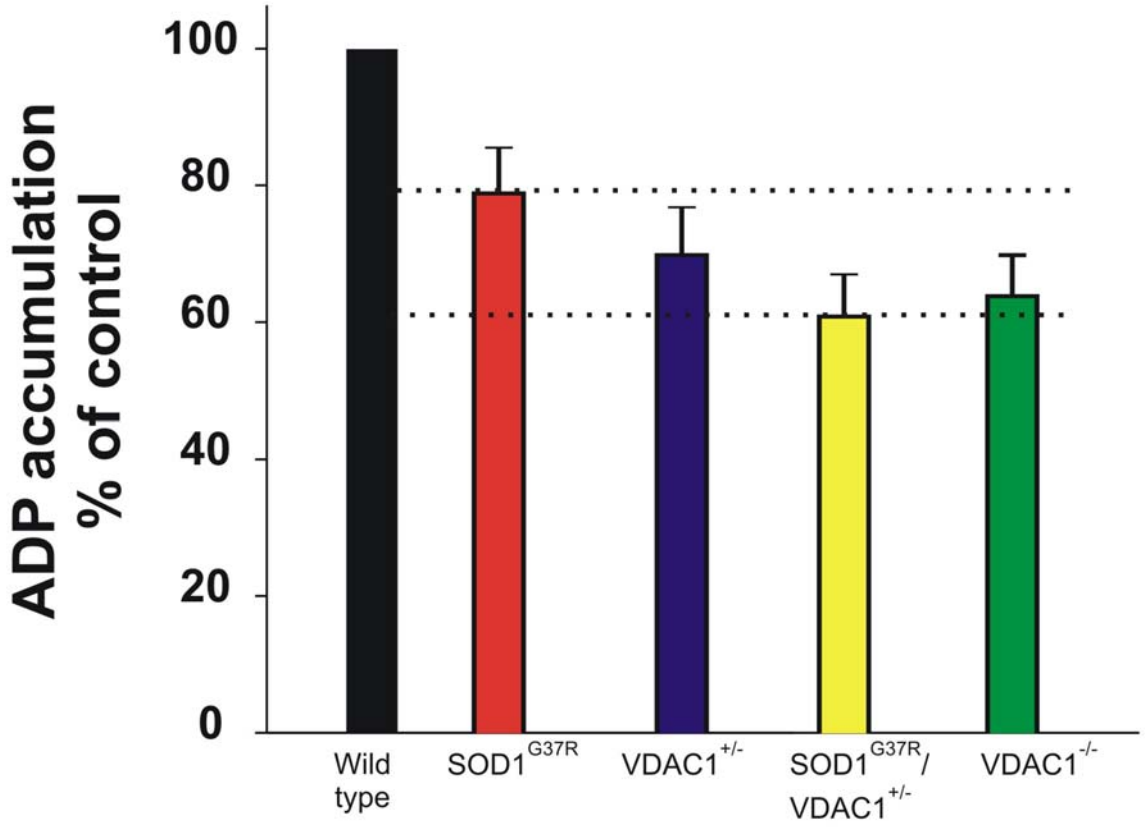

Fig. 3S. ADP transport across the outer mitochondrial membrane is reduced in mitochondria from SOD1^{G37R} ALS mice. ADP accumulation into isolated spinal cord mitochondria was measured using radiolabeled [^{3}H]ADP for wild type (black), SOD1^{G37R} (red), VDAC1$^{+/-}$ (blue), SOD1^{G37R}/VDAC1$^{+/-}$ (yellow) and VDAC1$^{-/-}$ (green) mice. ADP accumulation in spinal mitochondria from SOD1^{G37R}/VDAC1$^{+/-}$ mice is reduced to the level of VDAC1$^{-/-}$ animals

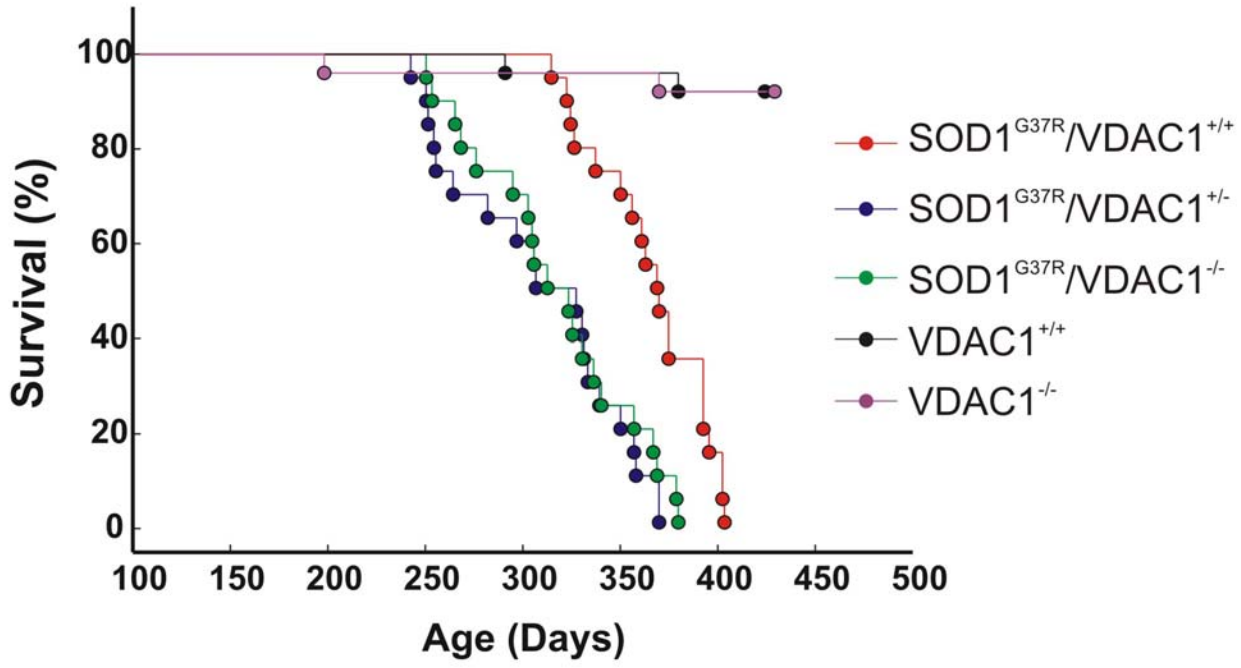

Fig. 4S. Reduction of VDAC1 levels diminishes survival in the hSOD1^{G37R} mouse model of ALS. Survival curves of wild type (black), VDAC1$^{-/-}$ (purple), SOD1^{G37R}/VDAC1$^{-/-}$ (green), SOD1^{G37R}/VDAC1$^{+/-}$ (blue) and SOD1^{G37R}/VDAC1$^{+/+}$ littermates (red).

Supplemental Experimental Procedures

Protein Purification

Baculovirus stocks expressing human wild type and mutants for SOD1 were provided by Dr. Lawrence Hayward (University of Massachusetts). Recombinant $hSOD1^{wt}$, $hSOD1^{G93A}$ and $hSOD1^{G85R}$ were expressed in sf-9 cells and purified using an Hydrophobic Interaction Chromatography (HIC) and Ion Exchange Chromatography (IEX), as described previously (Hayward et al., 2002).

VDAC was isolated from rat spinal cord mitochondria as described previously (Shoshan-Barmatz and Gincel, 2003). Isolated mitochondria (5 mg/ml) were incubated for 20 min at 4°C in a solution containing 10 mM Tris, pH 7.4, 3 % Triton X-100 and protease inhibitors. After centrifugation at 50,000 x g for 20 min, the supernatant was applied to and an hydroxyapatite/celite (2:1 w/w) column (1 g/mg protein) and eluted with a buffer containing 10 mM Tris, pH 7.4 and 3 % Triton X-100. The VDAC containing fractions were collected, diluted 3-fold with 10 mM Tris, pH 7.4 and loaded onto a reactive red agarose column (0.1 ml/mg initial protein), pre-equilibrated with 10 mM Tris, pH 7.4 and 0.3% Nonidet P40. The loaded column was washed with same buffer and VDAC was eluted at high concentration with same buffer containing 0.4 M NaCl (Shoshan-Barmatz and Gincel, 2003).

Immunoprecipitation

Isolated mitochondria (100 μg) were solubilized in immunoprecipitation (IP) buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40 plus protease inhibitors] and incubated overnight with antibodies previously crosslinked to Dynabeads protein G (Invitogen) with dimethyl pimelimidate (Pierce) according to the manufacturer's instructions.

The beads were magnetically isolated and washed three times with IP buffer. Samples were eluted with boiling in 2.5x sample buffer.

DSE2 Antibodies

DSE2 antibodies were kindly provided by Neil R. Cashman. Disease-specific epitopes (DSE) of SOD1 were as previously described (Vande Velde et al., 2008). The epitope recognized by the antibodies were predicted to be exposed and unstructured during misfolding or metal depletion (Rakhit et al., 2007). Two independent IgG monoclonal clones (3H1 and 8D1) were selected by reactivity to the DSE2 peptide (comprising the electrostatic loop of hSOD1; residues 125-142), to denatured and/or oxidized hSOD1 *in vitro*.

Immunostaining

For immunohistochemistry, mice were perfused with 4% paraformaldehyde and lumbar spinal cord cryosections were prepared as described before (Yamanaka et al., 2008). Floating sections were first incubated in a blocking solution containing PBS, 0.5% Tween-20, 1.5% BSA for 1h30 at room temperature and then in PBS, 0.3% Triton-X100 over night at room temperature with the following primary antibodies: monoclonal antibody against misfolded SOD1, DSE2 (8D1) (1/500), polyclonal rabbit antibody against TOM20 clone FL145 (1/100, Santa Cruz). Primary antibodies were washed with PBS and then detected using donkey anti-rabbit Cy5 (1/500) and donkey anti-mouse Cy3 (1/500) coupled secondary antibodies (Jackson ImmunoResearch, West Grove, PA). The secondary antibodies were washed with PBS and the spinal cord sections were further incubated with a monoclonal mouse antibody against neuronal nuclei marker, NeuN-Alexa488 (1/1,000, Chemicon) for 1h30 at room temperature. The sections

were washed with PBS and mounted. Analysis was performed on a Nikon Eclipse laser scanning confocal microscope.

Ca^{2+} and ADP Accumulation by Mitochondria

Ca^{2+} uptake by freshly isolated rat spinal or liver mitochondria was assayed for 1 to 20 min at 30°C in the presence of 220 mM mannitol, 68 mM sucrose, 0.5 mM nitrilotriacetic acid, 120 □M $CaCl_2$ (containing $^{45}Ca^{2+}$ $3x10^4$ cpm/nmol) free [Ca^{2+}]= 33.6 □M), 15 mM Tris/HCl, pH 7.2, 5 mM succinate, 0.1 mM Pi and 0.5 mg/ml mitochondria. Uptake was terminated by rapid Millipore filtration followed by wash with 5 ml of 0.15 M KCl. The maximal Ca^{2+} accumulated in rat spinal mitochondria was between 100 and 200 nmol/mg protein. It should be noted that using succinate as the energy source, and in the presence of Pi, Ca^{2+} accumulation became transient, reaching a maximal level and then rapidly decreasing to less than 40% of its maximal value (reflecting PTP opening). The time course for this transient Ca^{2+} accumulation was affected by the Ca^{2+} and Pi concentrations; thus for convenience, we chose to use 120 □M Ca^{2+} and 0.1 mM Pi.

ADP accumulation in freshly isolated rat spinal or liver mitochondria was assayed for 1 min at 30°C in the presence of 220 mM mannitol, 68 mM sucrose, 0.5 mM nitrilotriacetic acid, 16 □M ADP (containing ^3H[ADP] $7.5x10^7$ cpm/nmol), 15 mM Tris/HCl, pH 7.2, and 0.5 mg/ml mitochondria. Uptake was terminated by rapid Millipore filtration followed by wash with 5 ml of 0.15 M KCl.

Immunoblotting

Proteins were separated on SDS-PAGE and transferred to nitrocellulose membranes and probed with various antibodies as indicated. Antibodies to SOD1 have been described (Clement

et al., 2003; Howland et al., 2002). Commercial antibodies include goat anti-SOD1 (C-17; Santa Cruz Biotechnology); monoclonal anti-VDAC/porin 31HL (Calbiochem), goat anti-VDAC1 (N-18; Santa Cruz Biotechnology), goat anti-Hexokinase-I (Santa Cruz Biotechnology), goat anti-VDAC2 (Abcam), monoclonal anti-COX4 (Clontech), goat anti-TOM40 (Santa Cruz Biotechnology) and monoclonal anti cyclophilin-D (Mitosciences). Horseradish peroxidase-conjugated anti-mouse, anti-rabbit, or anti-goat IgG secondary antibodies (Jackson Immunochemicals) were used and detected by ECL (GE Biosciences).

Supplementary References

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