Supporting Information

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S1 Materials and Methods

Immunoprecipitation. Spinal cord, brain, or liver fractions (70 μg) or whole-cell extracts (100 μg) were solubilized in IP buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, plus 1x protease inhibitors) and incubated overnight with B8H10 (MédiMabs) antibodies previously cross-linked to Dynabeads protein G (Invitrogen) 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 by boiling in a 2x sample buffer.

SOD1 Aggregation Assay by Differential Extraction. The protocol for SOD1 aggregation by differential extraction was similar to that described previously (52). Spinal cords were homogenized with a homogenizer in 1:10 (wt/vol) 1 TEN (10 mM Tris, 1 mM EDTA, and 100 mM NaCl), then mixed with an equal volume of 2x extraction buffer 1 (10 mM Tris, 1 mM EDTA, 100 mM NaCl, 1% Nonidet P-40, and 1x protease inhibitor mixture) and homogenized as described above. The resulting lysate was centrifuged for 5 min at 100,000 × g to separate the nonionic detergent-insoluble pellet (P1) from the supernatant (S1). S1 was then decanted and stored for analysis. P1 was resuspended in 200 μL of 1x extraction buffer 2 (10 mM Tris, 1 mM EDTA, 100 mM NaCl, 0.5% Nonidet P-40, and 1x protease inhibitor mixture) and then sonicated to resuspend. The extract was then centrifuged for 5 min at 100,000 × g in a Beckman Airfuge to separate the pellet (P2) from the supernatant. The P2 fraction was then resuspended in buffer 3 (10 mM Tris, 1 mM EDTA, 100 mM NaCl, 0.5% Nonidet P-40, 0.25% SDS, 0.1 M HCl, pH 7.2) to reduce endogenous peroxidase activity. Slices were stained with DAPI (according to the manufacturer’s instructions). The slices were then washed three times in 0.1 M PBS, then incubated for 2 h at room temperature with a fluorescent-conjugated secondary goat anti-mouse (1:5,000, Alexa Fluor 405; Invitrogen), chicken anti-rabbit (1:5,000, Alexa Fluor 647; Invitrogen), or chicken anti-goat (1:5,000, Alexa Fluor 488; Invitrogen) antibodies, and with DAPI for nuclear staining. Images were acquired on a Nikon C2Plus laser unit docked to a Nikon Ti eclipse unit of a confocal microscope using 20x and 60x oil objectives. Scanning settings were reused across the samples.

Mitochondria and ER Purification. Mitochondria were purified as described previously (22). Spinal cords were homogenized on ice in 5 volumes of ice-cold homogenization buffer (HB), composed of 210 mM mannitol, 70 mM sucrose, 1 mM EDTA (Tris), and 10 mM Tris-HCl pH 7.2. Homogenates were centrifuged at 1,000 × g for 10 min. The supernatants were then recovered, and the pellets were washed with half-volume HB and centrifuged at 1,000 × g for 5 min. The supernatants were pooled and centrifuged at 17,000 × g for 15 min to yield a crude mitochondrial pellet. The supernatant was used to make cytosolic and ER fractions by further centrifugation at 100,000 × g for 1 h. The mitochondria were gently resuspended in a 12–14% OptiPrep density gradient medium diluted in HB and centrifuged at 17,000 × g for 15 min. Myelin from the spinal cords was removed, and mitochondria were collected from the pellet, washed once with HB to remove the OptiPrep, and centrifuged at 17,000 × g for 15 min. Then the supernatant was discarded, and the pellet was resuspended in a small volume of HB. The proteins were quantified and run in a 13% SDS/PAGE gel.

Immunoblotting. Proteins were separated on a 13% SDS/PAGE gel, transferred to nitrocellulose membranes, and probed with various antibodies, including goat anti-SOD1 (C-17; Santa Cruz Biotechnology), sheep anti-SOD1 (Calbiochem), monoclonal anti-VDAC/porin 31HL (Calbiochem), goat anti-MIF (N-18; Santa Cruz Biotechnology), rabbit anti-MIF (FL-115; Santa Cruz Biotechnology), rabbit anti-human SOD1 (ab52950; Abcam), and rabbit anti-VDAC (ab154856; Abcam). Horseradish peroxidase-conjugated anti-mouse, anti-rabbit, anti-sheep, or anti-goat IgG secondary antibodies (Jackson ImmunoResearch) were used and detected by ECL (GE Healthcare).

Immunofluorescence. Mice were anesthetized by inhalation of 1.5–3% isoflurane, followed by a transcardial perfusion of 250 mL of 0.1 M PBS, which was then switched to 4% paraformaldehyde in 0.1 M PBS. The spinal cords were dissected out and postfixed in 4% formaldehyde at 4 °C overnight, cryoprotected in 20% sucrose (48 h at 4 °C), and then stored at 4 °C with 0.02% sodium azide. Free-floating sections (35 μm thick) were blocked with a blocking peroxidase buffer (0.1 M PBS, 20% methanol, 0.2% Triton-X100, and 1.5% H2O2) to reduce endogenous peroxidase activity. Slices were stained following standard protocols. Sections were blocked for 1 h in a blocking solution (1x PBS, 5% free fatty acid BSA, and 0.3% Triton-X100), immunostained for 48 h at 4 °C with antibodies made in 1x PBS, 2% free fatty acid BSA with 0.3% Triton-X100, including mouse anti-neuronal nuclei antigen (1:500, NeuN; Millipore), monoclonal anti-misfolded SOD1 (1:100, B8H10; MédiMabs), or goat anti-MIF (1:100, N18; Santa Cruz Biotechnology).

After the first antibody incubation, sections were washed three times in 0.1 M PBS, then incubated for 2 h at room temperature with a fluorescent-conjugated secondary goat anti-mouse (1:5,000, Alexa Fluor 405; Invitrogen), chicken anti-rabbit (1:5,000, Alexa Fluor 647; Invitrogen), or chicken anti-goat (1:5,000, Alexa Fluor 488; Invitrogen) antibodies, and with DAPI for nuclear staining. Images were acquired on a Nikon C2Plus laser unit docked to a Nikon Ti eclipse unit of a confocal microscope using 20x and 60x oil objectives. Scanning settings were reused across the samples.

Cell Culture and Plasmids. To generate pCDNA-hMIF, the cDNA of the human MIF (obtained from Jurgen Bernhagen, University Hospital RWTH, Aachen, Germany) was amplified by PCR and inserted into pCDNA3.1(−) plasmid using the BamHI and XbaI sites. pEGFP-hSOD1WT, pEGFP-hSOD1G85A, and pEGFP-hSOD1G85R were kindly provided by Jean Pierre Julien (Laval University, Canada), and pCI-hSOD1WT, pCI-hSOD1G85A, and pCI-hSOD1G85R were generated by inserting human SOD1 constructs into the pCI-NEO vector (Promega), between the EcoRI and the NotI sites. NSC-34 and SH-SY5Y cells were grown at 37 °C and 5% CO2 in DMEM supplemented with 10% tetracycline-free FBS, 2 mM l-glutamine, and 100 U/mL penicillin/100 mg/mL streptomycin (all reagents from Biological Industries).

Transfection was performed using TurboFect reagents (Thermo Fisher Scientific) according to the manufacturer’s protocol. When cotransfections were performed, empty plasmids were always transfected as controls. At 48 h after transfection, the cells were stained with DAPI (according to the manufacturer’s protocol) and then analyzed with Operetta. Cell viability was analyzed using the CellTiter 96 AQueous One-Solution cell proliferation assay (Promega) and ELISA at 490 nm, according to the manufacturer’s protocol.

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**Fig. S1.** Characterization of MIF KO mice. Immunoblotting of brain extracts recovered by MIF+/- and MIF-/- mice and probed with anti-MIF or anti-SOD1 antibodies.

**Fig. S2.** Endogenous MIF colocalizes with SOD1 in the spinal cord of mutant SOD1<sup>G85R</sup> mice. Representative micrographs of lumbar spinal cord sections from SOD1<sup>G85R</sup> mice at the presymptomatic disease stage, processed for immunofluorescence using antibodies to MIF (A and D), antibodies to SOD1 (B and E), and the merge of both, including DAPI staining in blue (C and F). (Scale bar: 25 μm.)
**Spinal cord SOD1^{G85R}**

**A**

MIF  
NeuN  
MERGE

**B**

MIF  
NeuN  
MERGE

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**Fig. S3.** Endogenous MIF accumulates at very low levels in the spinal neurons of mutant SOD1^{G85R} mice. Shown are representative micrographs of lumbar spinal cord sections from SOD1^{G85R} mice at the presymptomatic stage of the disease, processed for immunofluorescence using antibodies to MIF (A and D), antibodies to NeuN (for the identification of neurons) (B and E), and the merge of both, including DAPI staining (C and F). (Scale bars: A–C, 100 μm; D–F, 20 μm.)

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**Fig. S4.** Deletion of endogenous MIF enhances the mutant SOD1-insoluble fraction in the spinal cords of mutant SOD1^{G85R} mice at disease onset. (A) Schematic drawing of a protocol for testing whether the deletion of endogenous MIF in SOD1^{G85R} mice enhances the accumulation of mutant SOD1 aggregates. (B) Spinal cord extracts were recovered from SOD1^{G85R}/MIF^{+/+} (WT) and SOD1^{G85R}/MIF^{-/-} (KO) mice at disease onset or in its symptomatic stage. The amount of SOD1 aggregation was determined by immunoblotting of the soluble and insoluble fractions isolated as described in A. Immunoblots were probed with an anti-SOD1 antibody.