Supplementary Materials: Expression of Carbonic Anhydrase I in Motor Neurons and Alterations in ALS

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Table S1. Samples used for immunohistochemical staining of paraffin sections in Figures 2 and 4.

Label	Sex	Age (Year/Day)	Race	Post Mortem Intervals (h)	Disorder	Cause of Death
A	Male	55/32	Caucasian	24	Control	Arteriosclerotic Cardiovascular Disease
B	Male	27/42	Caucasian	15	Control	Accident, multiple injuries
C	Male	26/253	Caucasian	18	Control	multiple injuries
D	Male	57	Caucasian	N/A	ALS	complication of disorder
E	Female	87/176	Caucasian	18	ALS	complication of disorder

N/A: not known.

Table S2. Samples used for the Western analysis in Figure 3.

Label	Sex	Age (Year/Day)	Race	Post Mortem Intervals (h)	Disorder	Cause of Death
Control_1	Male	15	African American	13	Control	Accident, multiple injuries
Control_2	Male	14/308	Caucasian	16	Control	Accident, multiple injuries
Control_3	Male	27/42	Caucasian	15	Control	Accident, multiple injuries
Control_4	Male	55/32	Caucasian	24	Control	Arteriosclerotic Cardiovascular Disease
Control_5	Male	26/253	Caucasian	18	Control	Multiple injuries
SALS_1	Female	73/231	Caucasian	20	ALS	complication of disorder
SALS_2	Male	69/53	Caucasian	22	ALS	complication of disorder
SALS_3	Female	46/263	Caucasian	3	ALS	complication of disorder
SALS_4	Female	57/70	Caucasian	14	ALS	complication of disorder
SALS_5	Male	45/344	Caucasian	15	ALS	complication of disorder
SALS_6	Female	56/17	Caucasian	6	ALS	complication of disorder
SALS_7	Female	87/176	Caucasian	18	ALS	complication of disorder
SALS_8	Male	49/39	Caucasian	9	ALS	complication of disorder

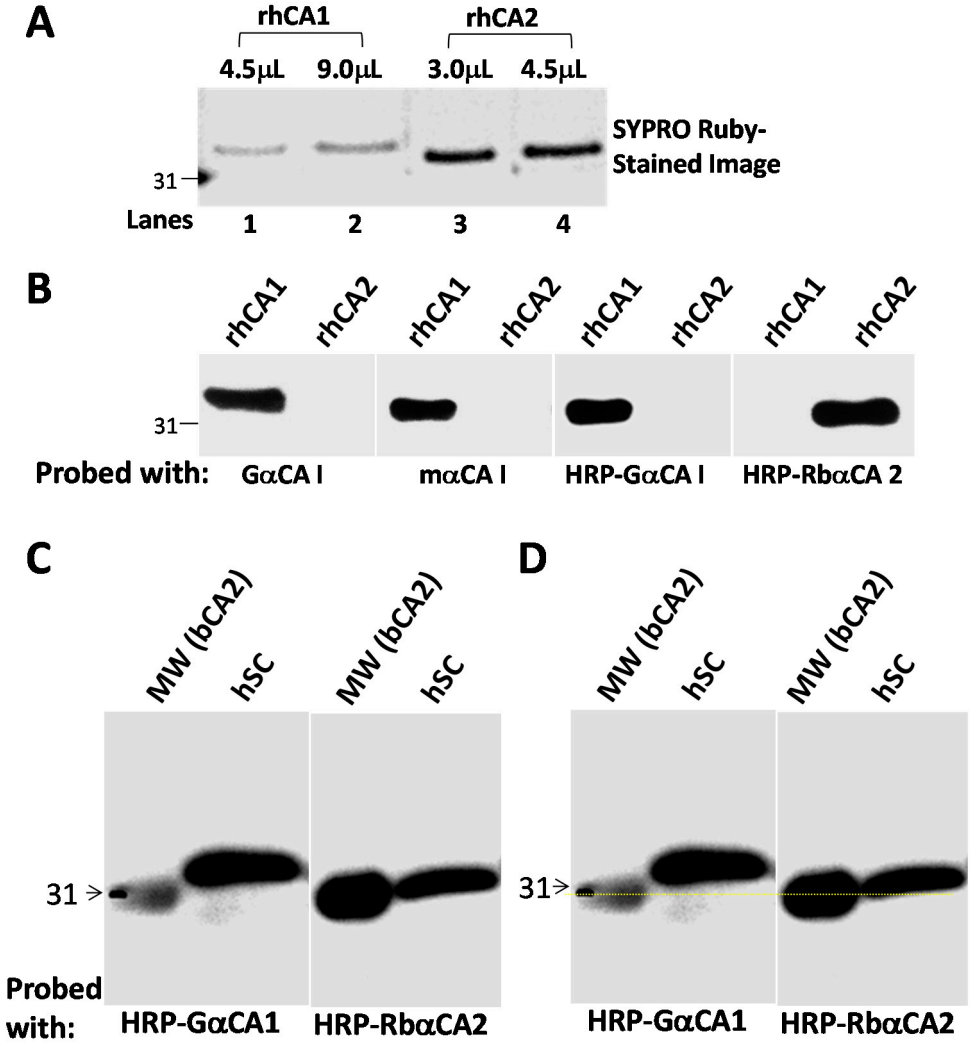

Figure S1. CA1 antibodies recognize human CA1 but not human CA2. (**A**,**B**) Commercially available recombinant His-tagged human CA1 and CA2 proteins were used for SYPRO-Ruby staining and Western analysis; (**A**) Two different amounts of CA1 (4.5 μL and 9.0 μL) and CA2 (3.0 μL and 4.5 μL) from the prepared protein samples were used and visualized by SYPRO Ruby-staining on SDS-PAGE; (**B**) Four identical Western strips with 9.5 μL of CA1 and CA2 proteins for each lane on each blot were probed with 3 different sources of CA1 antibodies (GαCA1, mαCA1, and HRP-GαCA1) and one CA2 antibody (HRP-RbαCA2); (**C**) Identical blots with proteins from the human spinal cord (20 μg/lane) together with the MW which contains the bovine CA2 (bCA2) were analyzed by Western blot using HRP-GαCA1and HRP-RbαCA2, respectively. The bCA2 shares 58% and 80.4% in amino acid sequence identity with human CA1 and CA2, respectively. The CA1 antibody recognized one hCA1 band and cross-reacted to a small degree with bCA2. The CA2 antibody recognized one hCA2 band and readily recognized bCA2; (**D**) The exact same data as (**C**) with a yellow-dotted line drawn across the center of bCA2 signal to illustrate the different positions of the CA1 and CA2 signals recognized by their respective antibodies using bCA2 as the reference. MW: Molecular Weight marker; hsc: human spinal cord.

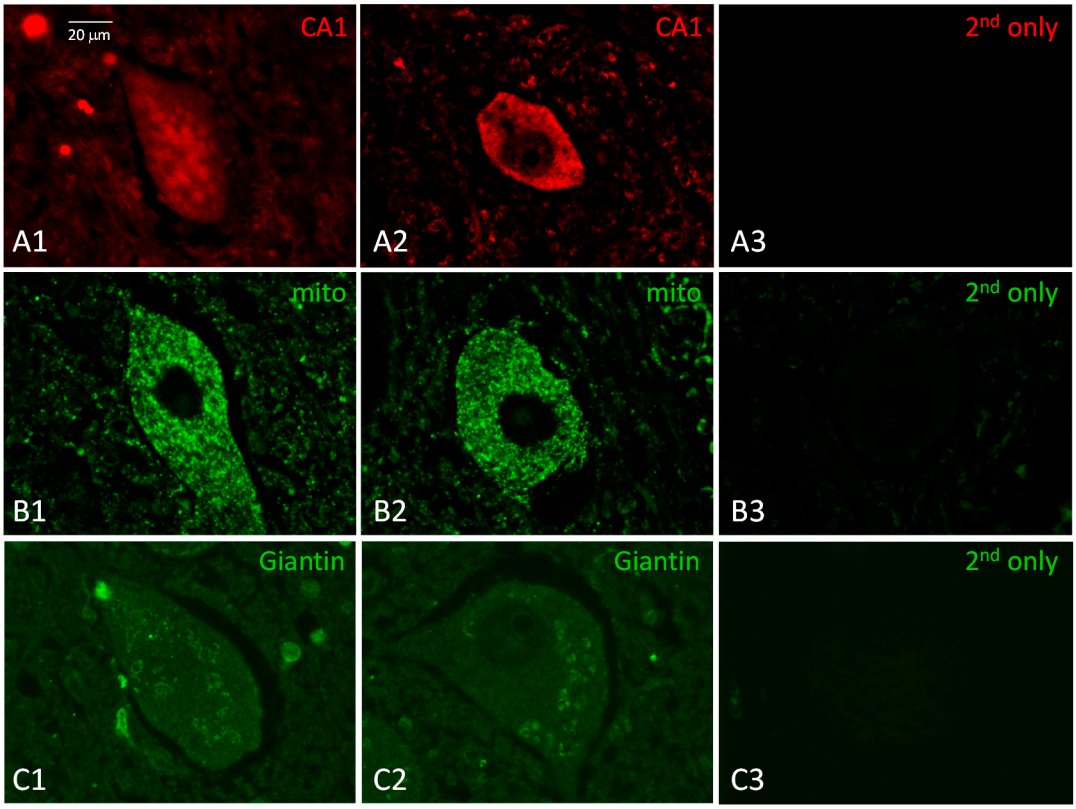

Figure S2. The pattern of CA1 immunoreactivity in human spinal cord motor neuron did not resemble those from mitochondria or Golgi. The control human spinal cord sections were immunofluorescently stained with antibodies against CA1 (GαCA1, **red**, **A1**,**A2**); and the molecular markers of subcellular organelles including mitochondria (**green**, mito, **B1**,**B2**); and Golgi (**green**, Giantin, **C1**,**C2**). The background staining images with the secondary antibodies only for each primary antibody were included on the most **right** column (2nd antibody only, **A3**, **B3**, and **C3**). The **white** scale bar indicates 20 μm.

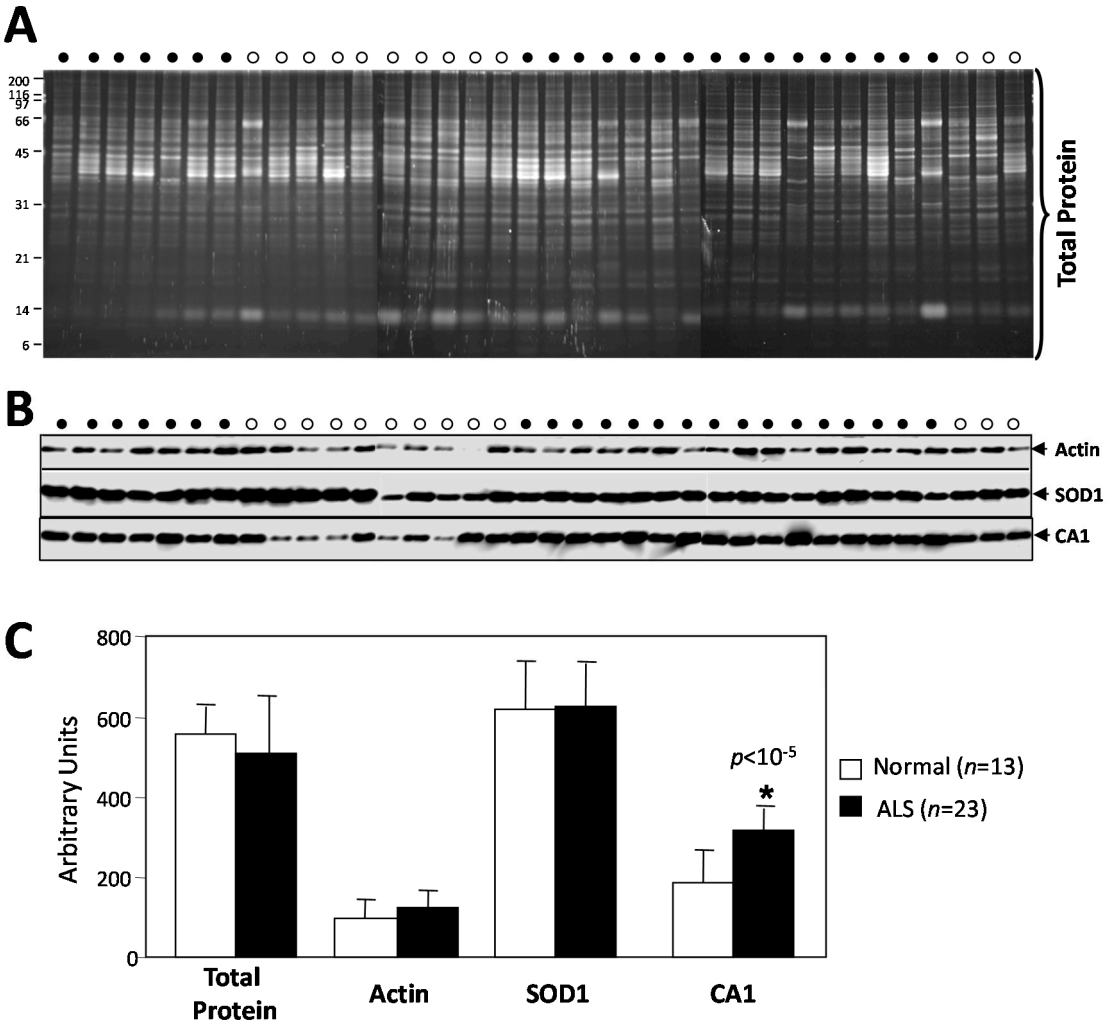

Figure S3. The levels of cytosolic CA1 proteins were increased in ALS spinal cords and cytosolic SOD1 protein levels behave similar to those of actin which can serve as the internal control for sample loading. Cytosolic proteins from both control (open circles) and ALS (filled circles) spinal cords were extracted as described and used for SYPRO-Ruby staining and the Western analysis. (**A**) An equal amount of 1.0 μg of proteins was loaded for each lane on 3 SDS-PAGE gels and stained with SYPRO-Ruby for visualization of the total proteins with the Broad Range Molecular Standards indicated on the most left; (**B**) An equal amount of 20 μg of proteins was loaded for each lane on 3 SDS-PAGE gels and processed for Western analysis with antibodies against actin and SOD1; (**C**) Quantitation of the intensities of the total protein in each lane (**A**) and immunoreactive signals of actin, SOD1 and CA1 (**B**). The intensity of the total protein for each sample was normalized to the same amount Broad Range Molecular Weight Standards on each gel (not shown) so that the intensities of all samples can be combined and compared. A common spinal cord protein sample (not shown) was used for each Western blot and the intensity of the immunoreactive band for actin, SOD1, and CA1 was normalized to the corresponding actin, SOD1, and CA1 signal in the common sample so that the intensities of all samples can be combined and compared. The data are expressed as "Mean ± SD". There were no significant differences in the total amount of proteins loaded in each lane, nor in the immunoreactive intensities of either actin or SOD1, between control and ALS groups. Therefore, both actin and SOD1 can serve as the internal control for sample loading.