

## Supporting Information

### Article Title

“Label-Free LC-MS/MS Proteomic Analysis of Cerebrospinal Fluid Identifies Protein/Pathway Alterations and Candidate Biomarkers for Amyotrophic Lateral Sclerosis”

### Authors

Mahlon A. Collins<sup>#¶</sup>, Jiyang An<sup>¶</sup>, Brian L. Hood<sup>§</sup>, Thomas P. Conrads<sup>§</sup>, and Robert P. Bowser<sup>¶\*</sup>

### Author Affiliations

<sup>#</sup> Department of Neurobiology, University of Pittsburgh, E1448 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA, USA 15261

<sup>¶</sup> Departments of Neurology and Neurobiology, Barrow Neurological Institute, NRC427, 350 West Thomas Road, Phoenix, AZ, USA 85013

<sup>§</sup> Women’s Health Integrated Research Center, 3289 Woodburn Road, Annandale, VA, USA 22003

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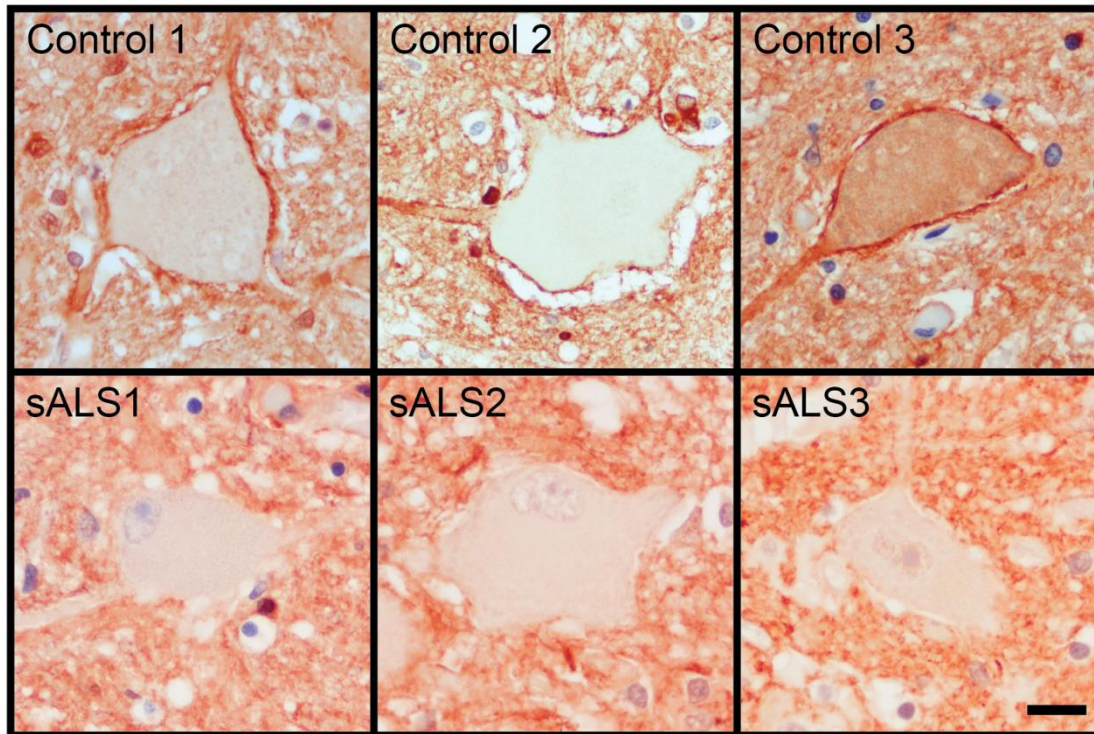
**Figure S-1.** Tenascin R Validation Data.

**Figure S-2.** eIF 4e Transporter (4e-T) Validation Data.

**Table S-1.** Global Results.

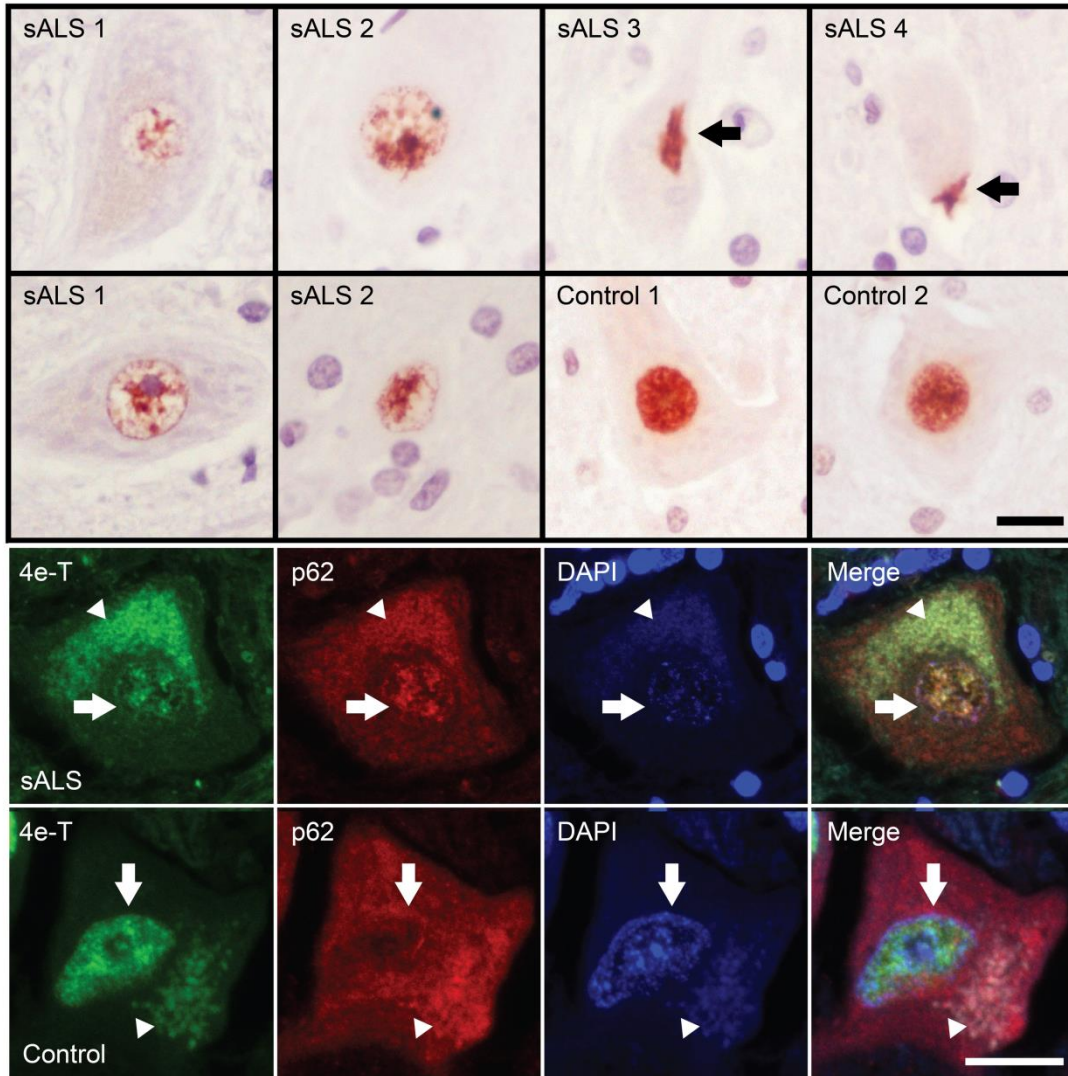
**Table S-2.** All Differentially Abundant Proteins.

**Figure S1**



**Figure S-1.** Tenascin R Validation Data. Lumbar spinal cord sections from sALS and HC patients were stained using a polyclonal anti-tenascin R antibody. Top Panel. Motor neurons from HC subjects show strong tenascin R immunoreactivity around the cell body. Bottom panel. Motor neurons from sALS cases show an absence of this staining pattern. All images were acquired with a 40x objective and the scale bar = 10  $\mu$ m.

**Figure S2**



**Figure S-2.** eIF 4e Transporter (4e-T) Validation Data. Lumbar spinal cord sections from sALS and HC patients were stained using a polyclonal 4e-T antibody. Top panels. Light microscopy was used to visualize 4e-T staining in the lumbar spinal cord of sALS and HC subjects. Staining in HC subjects is diffuse throughout the nucleus. In contrast, sALS cases show nuclear and cytoplasmic (arrows) 4e-T inclusions and a loss of diffuse staining. All images were acquired with a 40x objective and the scale bar = 10  $\mu$ m. Bottom panels. Immunofluorescence was used to further characterize 4e-T alterations in sALS. sALS 4e-T nuclear inclusion staining overlaps with p62 staining (arrows). Nuclear 4e-T staining in HC is diffuse and no nuclear p62 is observed (arrows). The presence of cytoplasmic autofluorescent lipofuscin is prominent in both cases and is indicated by arrowheads. All images were acquired with a 63x objective and the scale bar = 10  $\mu$ m.

**Table S-1.** Global Results. The raw data set comprising all spectral counts for all groups prior to normalization is shown. Protein name and Uniprot accession number is shown and protein isoform is indicated in the accession number. HC = healthy control, OND = other neurological disease, MS = multiple sclerosis, UMND = upper motor neuron disease, LMND = lower motor neuron disease, AD = Alzheimer's disease.

**Table S-2.** All Differentially Abundant Proteins. All proteins of significantly increased or decreased relative abundance in sALS samples are shown by  $q$  value rank (lowest to highest). Protein name, gene name, and Uniprot accession number is shown and protein isoform is indicated in the accession number. Log<sub>2</sub> fold difference (FD) for each two group comparison is shown.