Supplementary information

Misfolded SOD1 pathology in sporadic Amyotrophic Lateral Sclerosis

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Supplementary Table 1: Summary of misSOD1 accumulation in human patient spinal cords by following standardized immunohistoschemistry

	4-20RA	24-39RA	57-72RA	C4F6	131-153RA
FALS-SOD1A4V	(1,3,5)	(1, 2, 4)	(1, 2, 5)	(1, 2, 4)	(1, 2, 5)
SALS02	(4, 5)	(1, 2, 4, 5)	ND	(1, 2, 3)	ND
SALS03	ND	(1, 2, 4, 5)	(3, 4, 5)	(1, 2, 3)	(1, 3, 4)
SALS04	ND	(1, 4, 5)	ND	(1, 5)	(1, 3, 4, 5)
SALS05	ND	(2, 5)	(3, 4, 5)	ND	(1, 4)
SALS06	(1, 3, 4, 5)	(1, 4, 5)	(1, 3, 4, 5)	(3)	(1, 3, 4, 5)
SALS07	(1, 4, 5)	(4, 5)	(3, 4, 5)	(1, 3, 5)	(2, 3, 4, 5)
SALS08	(4, 5)	(4, 5)	ND	(4)	(4, 5)
SALS09	(3, 4, 5)	(3, 4, 5)	(3, 4, 5)	ND	(3, 4, 5)
SALS10	(0)	(1, 2, 3, 4, 5)	(3, 4, 5)	(3)	(1, 2, 3, 4, 5)
SALS11	(4)	(1, 4)	(4, 5)	ND	(1, 4, 5)
SALS12	(0)	(2, 3, 5)	ND	(1,5)	ND
SALS13	(0)	(1, 5)	ND	(1, 4, 5)	(1, 4, 5)
SALS14	ND	ND	(1, 5)	(1, 3, 4)	(1, 3, 5)
CTRL21	(0)	(0)	ND	ND	(0)
CTRL22	(0)	ND	ND	(0)	(0)
CTRL23	(0)	(0)	(0)	(0)	ND
CTRL24	(0)	(0)	(0)	ND	ND

0) no detected immunoreactive signal, 1) misSOD1 cytoplasmic accumulation, 2) cytoplasmic misSOD1-positive deposits, 3) axonal misSOD1-positive accumulation, 4) ring-like misSOD1-positive accumulation, and 5) misSOD1-positive nuclear accumulation. ND: not determined

Supplementary Table 2: Detailed description of the proposed guidelines

	Guidelines	Complete description
1	To use more than one misSOD1 specific antibody before concluding any results	As not all conformational specific misSOD1 antibodies can equally detect different misfolded SOD1 species of WT SOD1, which can be attributed to antibody affinity or antibody mapping to different epitopes, using more than one misSOD1 antibody must be considered
2	To test and use optimal antibody working concentrations	Antibody affinity can be affected by many variables including temperature, pH and buffer constituents. Therefore, the optimal working concentration of each individual antibody must be determined for experimental conditions. Even though some commercial antibodies have recommended dilutions for various applications, optimization is often required to reduce signal to noise ratio and obtain the best staining with minimal background
3	To optimize antigen retrieval time for each antibody [1]	Antigen retrieval time is a crucial aspect in immunohistochemistry, as antigen may often by masked by a variety of chemical modifications, following tissue fixation and embedding, that can reduce the detectability of proteins. As protocols of cell sample preparation and fixation, used in cytopathology, are not standardized across laboratories, we recommend to always optimize the antigen retrieval procedure to enhance antigenicity for both IHC and immunofluorescence. Such optimizations will necessarily means the addition of positive and negative controls
4	To always use citrate-based instead of TRIS/EDTA-based buffers	Considering the denaturing effect of certain antigen retrieval buffer on the SOD1 native protein, the use of appropriate citrate-based buffer solution must be considered. For instance, EDTA-based buffer is to be proscribed as it is known to be a metal ions scavenger that may interfere with the proper folding of metalloprotein such as SOD1, and give raise to false positive signals [2]. Reducing agents such as dithiothreitol (DTT) and β-mercaptoethanol, known to alter the folding and precipitation capacity of the wild-type protein [3], must also be proscribed when studying misSOD1 regardless of the detection technique used [2,4–6]
5	To test different central nervous system regions (cervical, lumbar, thoracic spinal cord sections and other brain regions)	As site of onset and severity of the disease can be highly heterogenous from patient to patient, we recommend to always perform histopathological examination on different CNS regions including brain (as the motor cortex and frontal lobe) and spinal cord sections. Examination of spinal cord must also include at least cervical, lumbar and thoracic sections
6	To perform, in parallel, Hematoxylin/Eosin coloration on adjacent sections, as well as IHC control with no primary antibody	Performing unspecific HE coloration and control IHC without primary antibody will help in the interpretation of results confirming that the detected misSOD1-positive aggregates are not unspecific chromophilic structures

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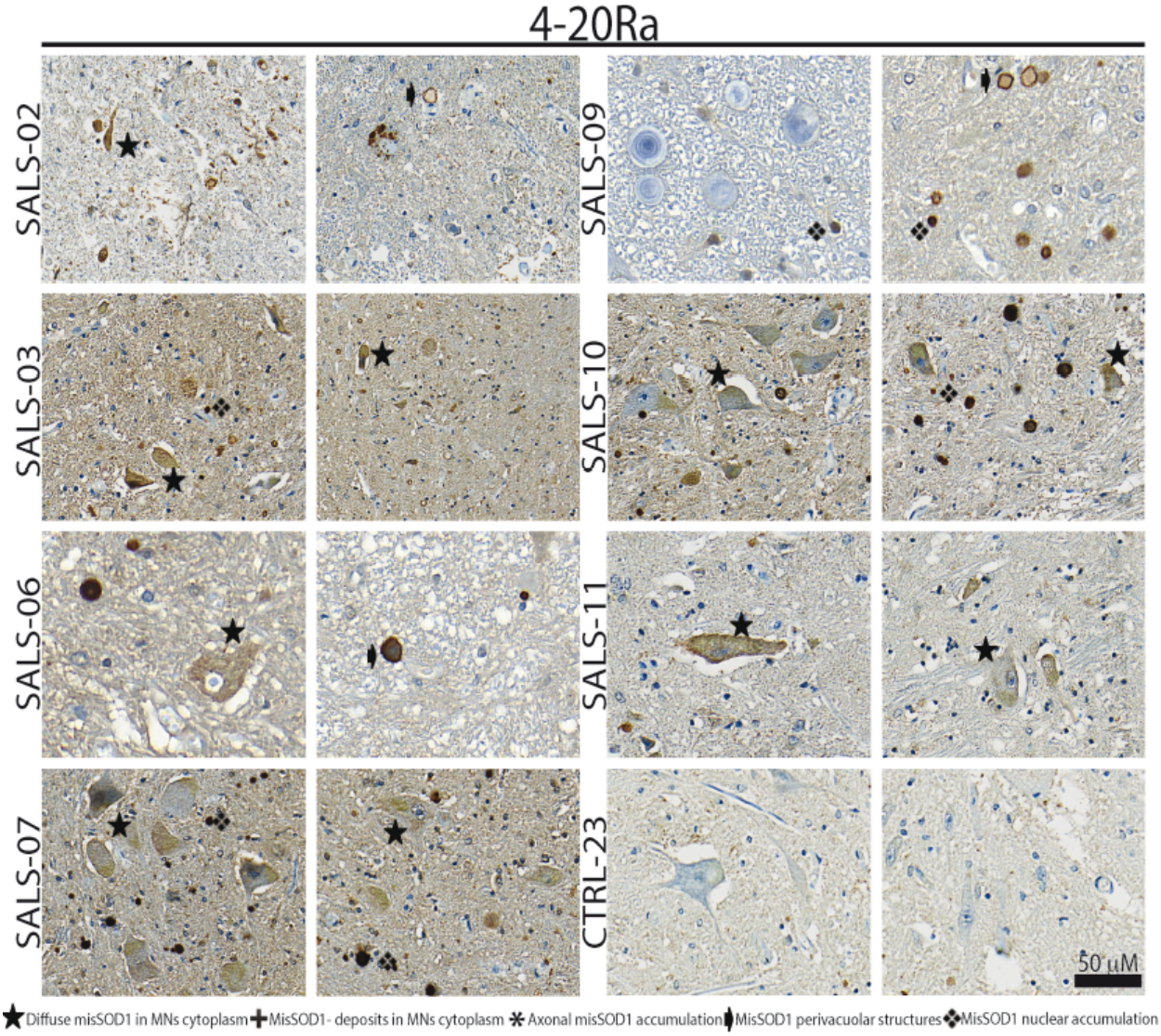
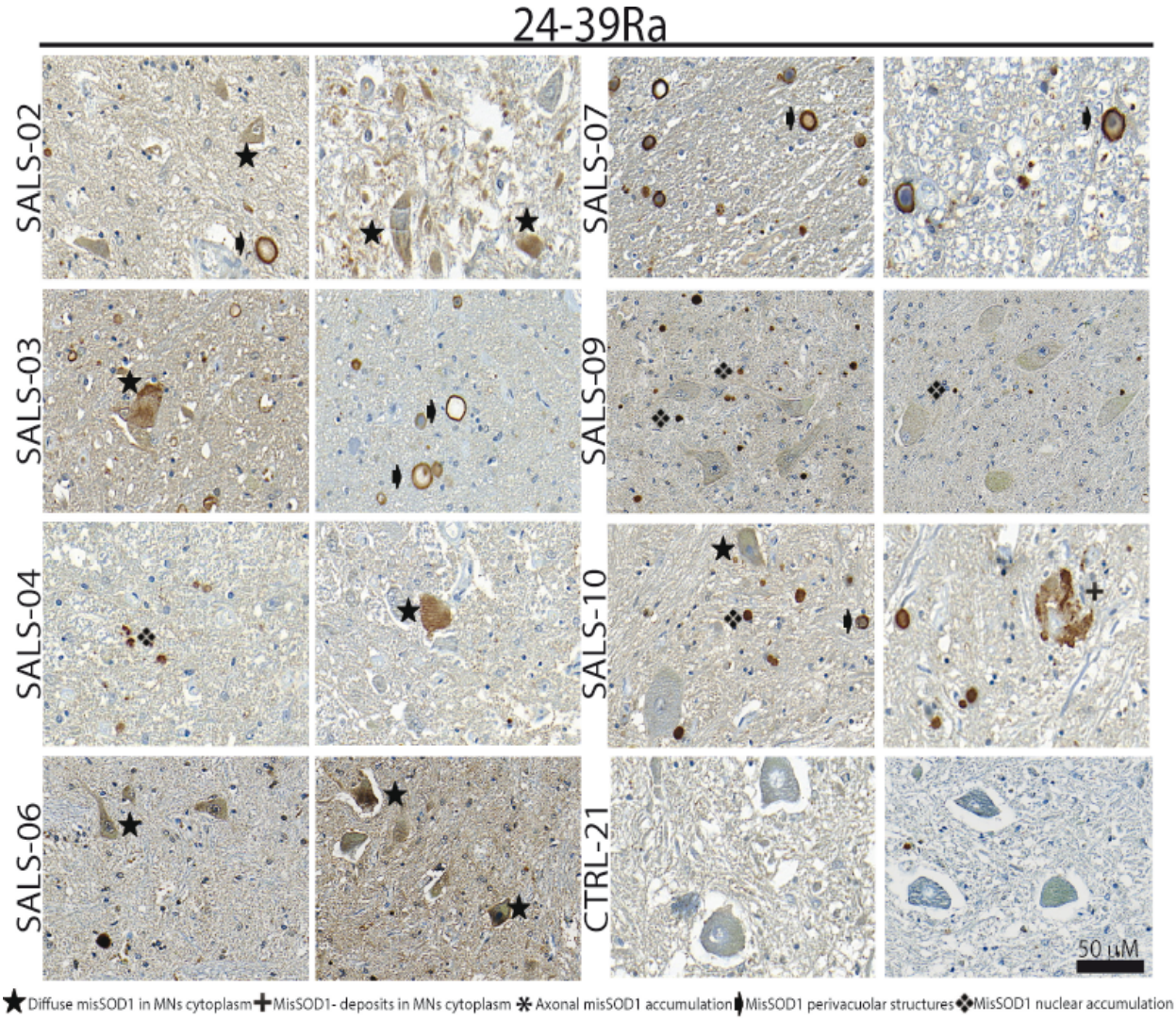


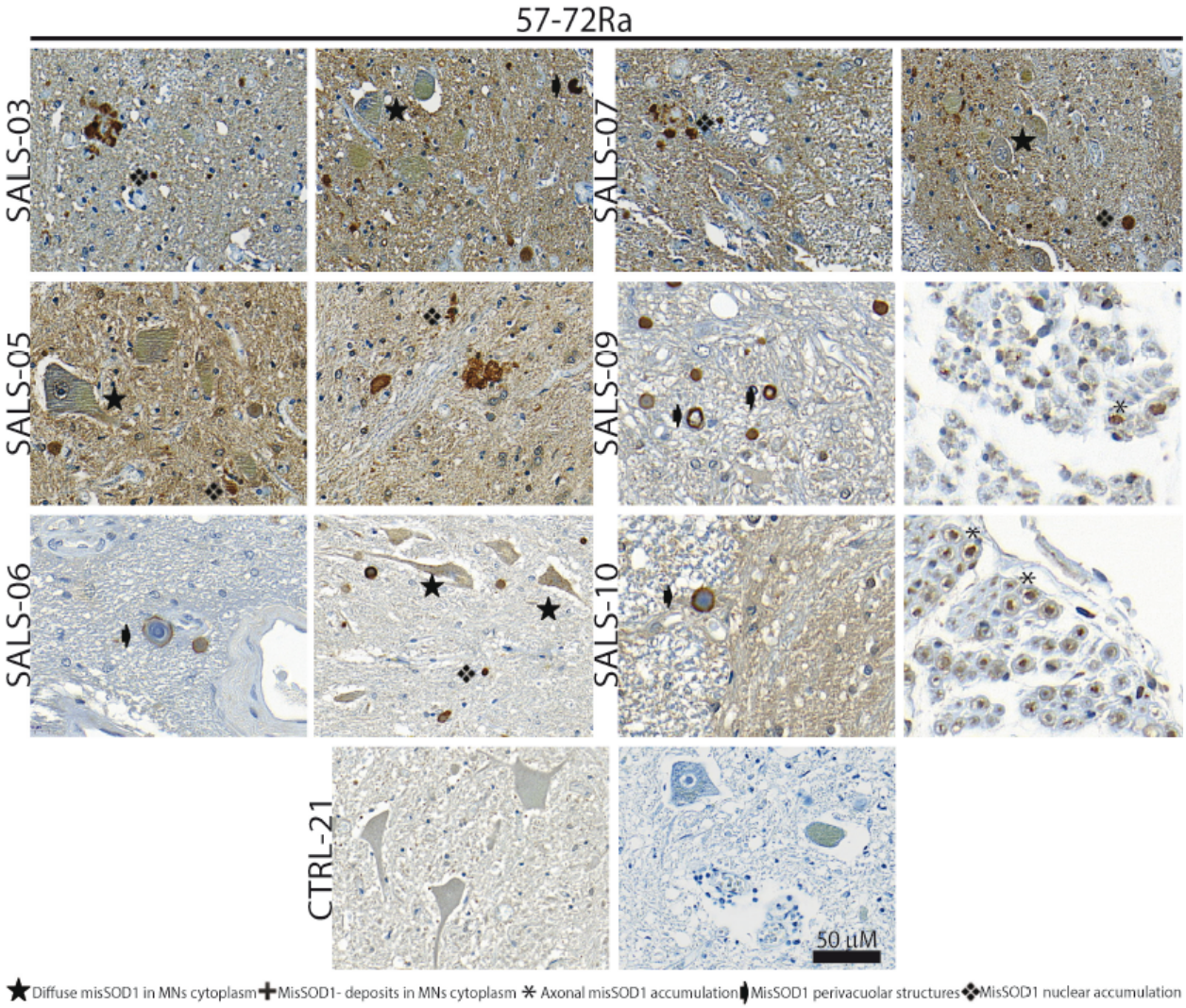
Supplementary Figure 1: Immunodetection of misfolded SOD1 species detected in post-mortem tissue sections from SALS patients by immunohistochimestry using the misfolded SOD1-specific 4-20Ra antibody

Spinal cord sections from SALS patients and non-ALS control individual immunostained using the 4-20 rabbit polyclonal misSOD1-specific antibody. Two representative pictures for each SALS patients and control are showed. Representative misSOD1 staining patterns are highlighted in each display panels and are labeled as followed: ★ diffuse misSOD1 staining in the cytoplasm of motor neurons; + misSOD1-positive deposits in the cytoplasm of motor neurons; * axonal misSOD1 accumulation; ➧ misSOD1-positive perivacuolar ring-like structures; ❖ shows misSOD1 nuclear accumulation. Scale bar: 50 µm.



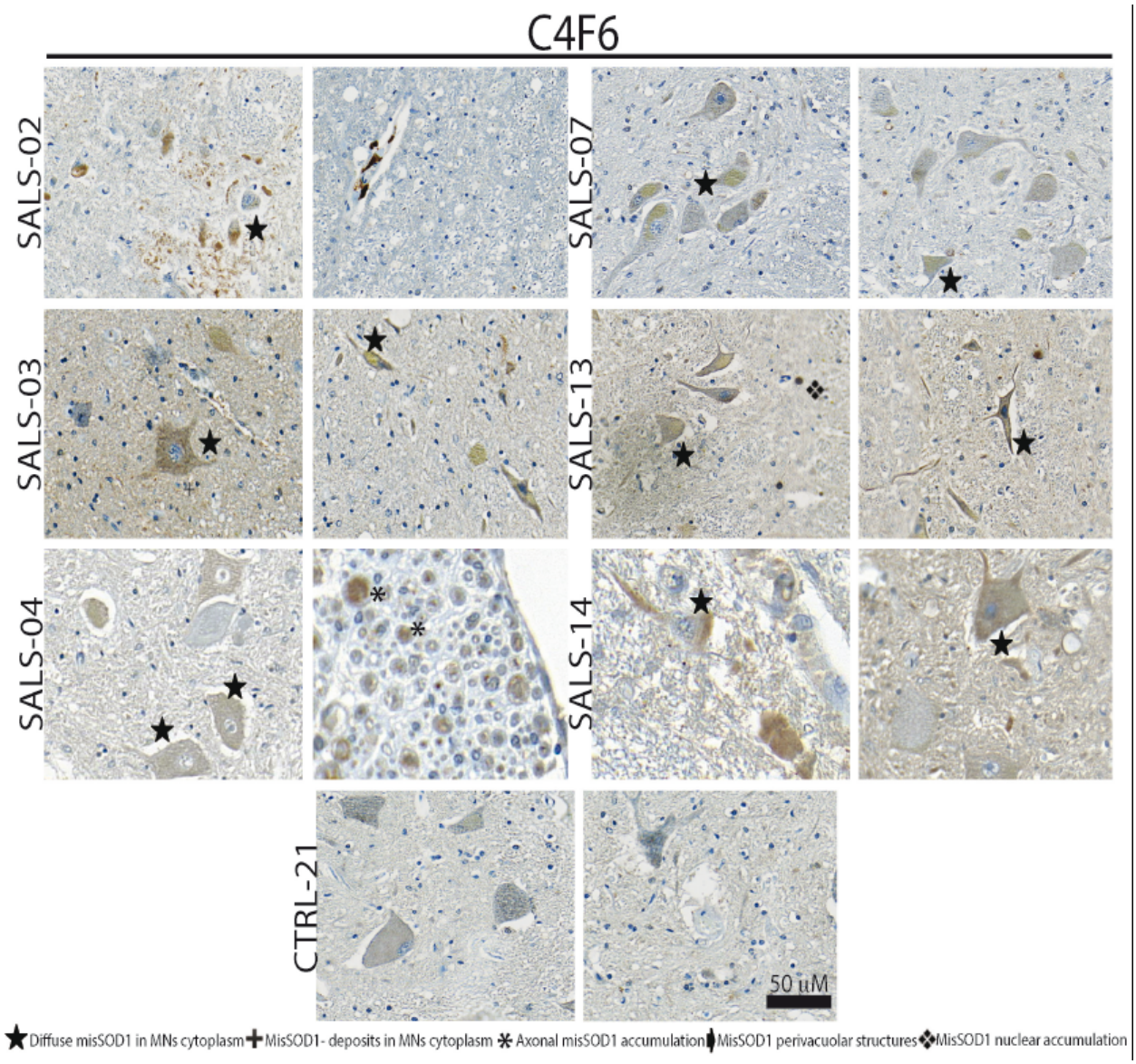
Supplementary Figure 2: Immunodetection of misfolded SOD1 species detected in post-mortem tissue sections from SALS patients by immunohistochimestry using the misfolded SOD1-specific 24-39Ra antibody

Spinal cord sections from SALS patients and non-ALS control individual immunostained using the 24-39 rabbit polyclonal misSOD1-specific antibody. Two representative pictures for each SALS patients and control are showed. Representative misSOD1 staining patterns are highlighted in each display panels and are labeled as followed: ★ diffuse misSOD1 staining in the cytoplasm of motor neurons; + misSOD1-positive deposits in the cytoplasm of motor neurons; * axonal misSOD1 accumulation; ➡ misSOD1-positive perivacuolar ring-like structures; ❖ shows misSOD1 nuclear accumulation. Scale bar: 50 µm.



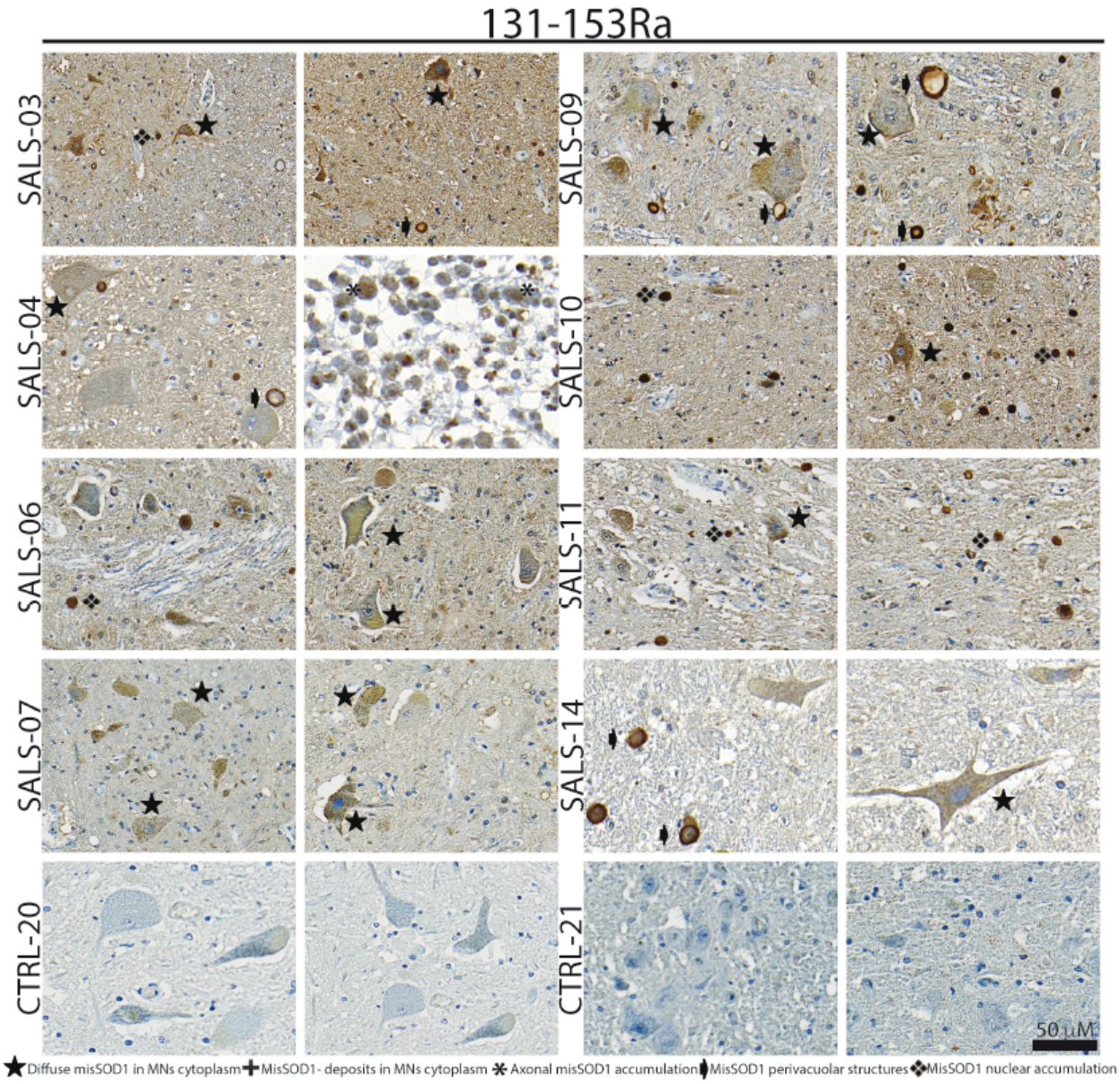
Supplementary Figure 3: Immunodetection of misfolded SOD1 species detected in post-mortem tissue sections from SALS patients by immunohistochimestry using the misfolded SOD1-specific 57-72Ra antibody

Spinal cord and ventral root sections from SALS patients and non-ALS control individuals immunostained using the 57-72 rabbit polyclonal misSOD1-specific antibody. Two representative pictures for each SALS patients and control are showed. Representative misSOD1 staining patterns are highlighted in each display panels and are labeled as followed: ★ diffuse misSOD1 staining in the cytoplasm of motor neurons; + misSOD1-positive deposits in the cytoplasm of motor neurons; * axonal misSOD1 accumulation; ➡ misSOD1-positive perivacuolar ring-like structures; ❖ shows misSOD1 nuclear accumulation. Scale bar: 50 µm.



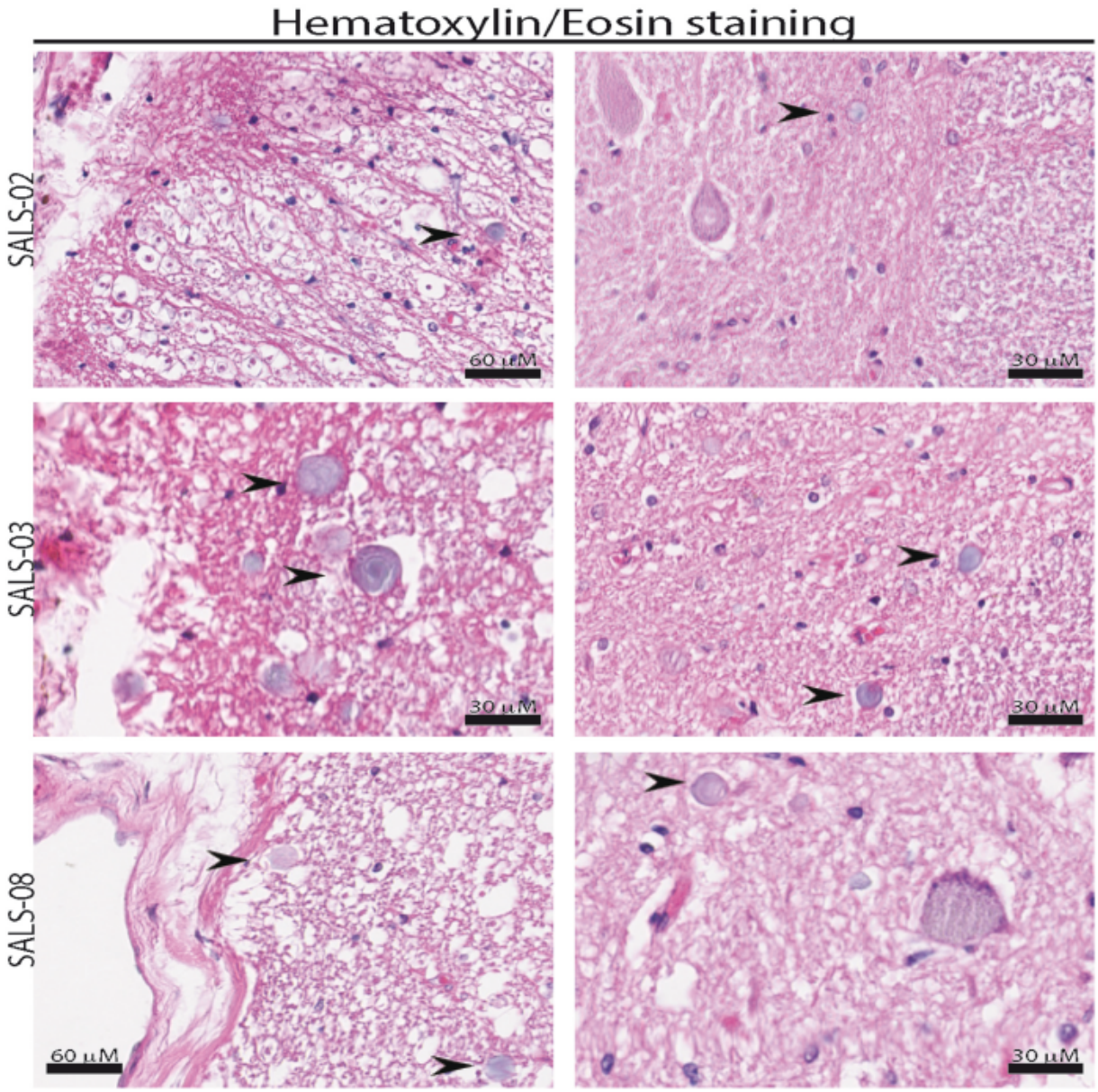
Supplementary Figure 4: Immunodetection of misfolded SOD1 species detected in post-mortem tissue sections from SALS patients by immunohistochimestry using the SOD1 conformation-specific C4F6 antibody

Spinal cord and ventral root sections from SALS patients and non-ALS control individuals immunostained using the C4F6 mouse monoclonal misSOD1-specific antibody. Two representative pictures for each SALS patients and control are showed. Representative misSOD1 staining patterns are highlighted in each display panels and are labeled as followed: ★ diffuse misSOD1 staining in the cytoplasm of motor neurons; + misSOD1-positive deposits in the cytoplasm of motor neurons; * axonal misSOD1 accumulation; ➡ misSOD1-positive perivacuolar ring-like structures; ❖ shows misSOD1 nuclear accumulation. Scale bar: 50 µm.



Supplementary Figure 5: Immunodetection of misfolded SOD1 species detected in post-mortem tissue sections from SALS patients by immunohistochimestry using the misfolded SOD1-specific 131-153Ra antibody

Spinal cord and ventral root sections from SALS patients and non-ALS control individuals immunostained using the 131-153 rabbit polyclonal misSOD1-specific antibody. Two representative pictures for each SALS patients and control are showed. Representative misSOD1 staining patterns are highlighted in each display panels and are labeled as followed: ★ diffuse misSOD1 staining in the cytoplasm of motor neurons; + misSOD1-positive deposits in the cytoplasm of motor neurons; * axonal misSOD1 accumulation; ➡ misSOD1-positive perivacuolar ring-like structures; ❖ shows misSOD1 nuclear accumulation. Scale bar: 50 µm.



Supplementary Figure 6: Hematoxylin & Eosin stainings of corpora amylacea in sporadic ALS patients spinal cord sections

Representative hematoxylin/Eosin colorations on post-mortem spinal cord tissue sections from sporadic ALS patients. Two pictures for each patient are displayed (left panel: spinal white matter; and right panel: spinal grey matter). Corpora amylacea were mainly detected at the spinal cord periphery and beneath the pia. CA lying in the white matter and beneath the pia matter were often found to be larger in size. CA lying in the grey matter are smaller in size. Arrowheads show corpora amylacea. Scale bars are indicated in the bottom right of each displayed panel.