Title:

Overexpression of ferroptosis defense enzyme Gpx4 retards motor neuron disease of SOD1G93A mice

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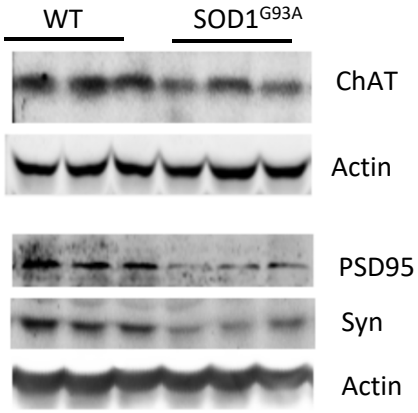
Supplementary Material

One supplemental Table and ten supplemental figures are provided to give additional information for the study.

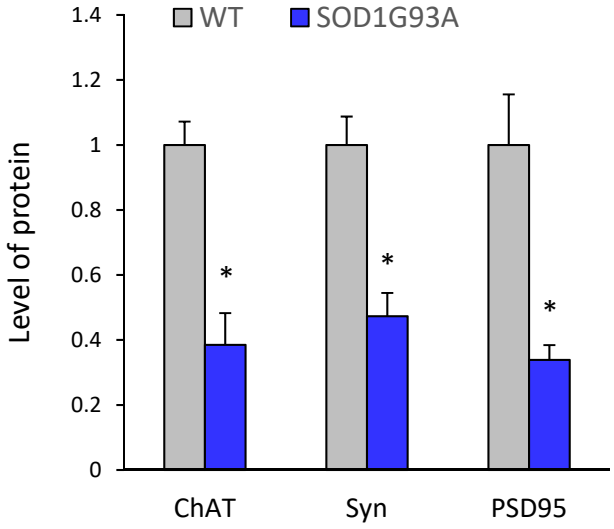
Case ID	Age (Year)	PMI (hour)	Diagnosis	Gender
A1	40	15.6	ALS	M
A2	54	29.1	ALS	M
A3	59	20.3	ALS	F
A4	61	7.5	ALS	F
A5	70	18.9	ALS	M
A6	76	8.6	ALS	F
A7	81	4	ALS	F
A8	81	17.2	ALS	M
A9	82	20.5	ALS	M
A10	82	12.1	ALS	F
C1	41	13	control	M
C2	41	20	control	M
C3	41	14	control	M
C4	42	27	control	F
C5	45	19	control	M
C6	49	26	control	F
C7	50	15	control	F
C8	50	24	control	M
C9	65	25	control	M
C10	74	18	control	M

Supplemental Table 1. Information of human lumber spinal cord tissues used in this study.

s1A

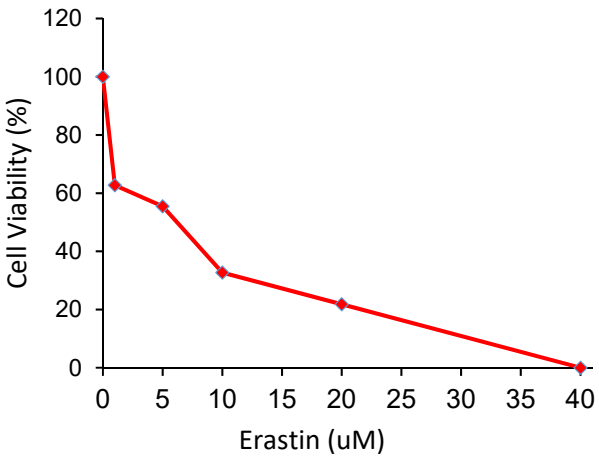


s1B

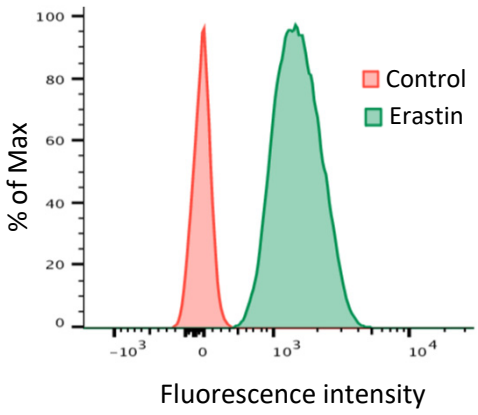


Supplemental Fig. 1. Loss of neuronal proteins in advanced symptomatic stage in SOD1^{G93A} mice. s1A. Graph of western blots showing levels of neuronal marker proteins in lumbar spinal cord tissues of WT and SOD1^{G93A} mice at 150 days of age. Syn: synaptophysin. **s1B**. Quantified results of western blots. n = 3. *: $p < 0.05$.

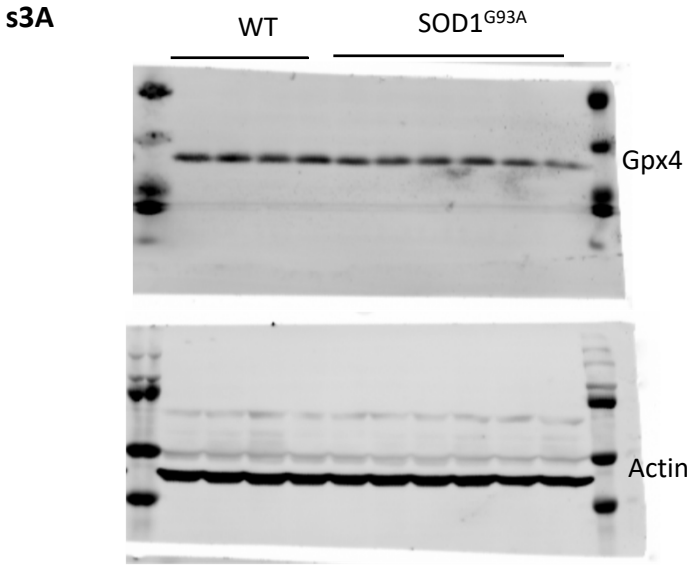
s2A

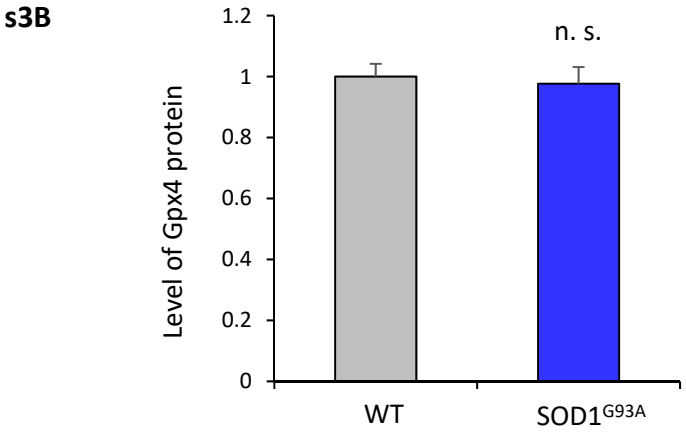


s2B

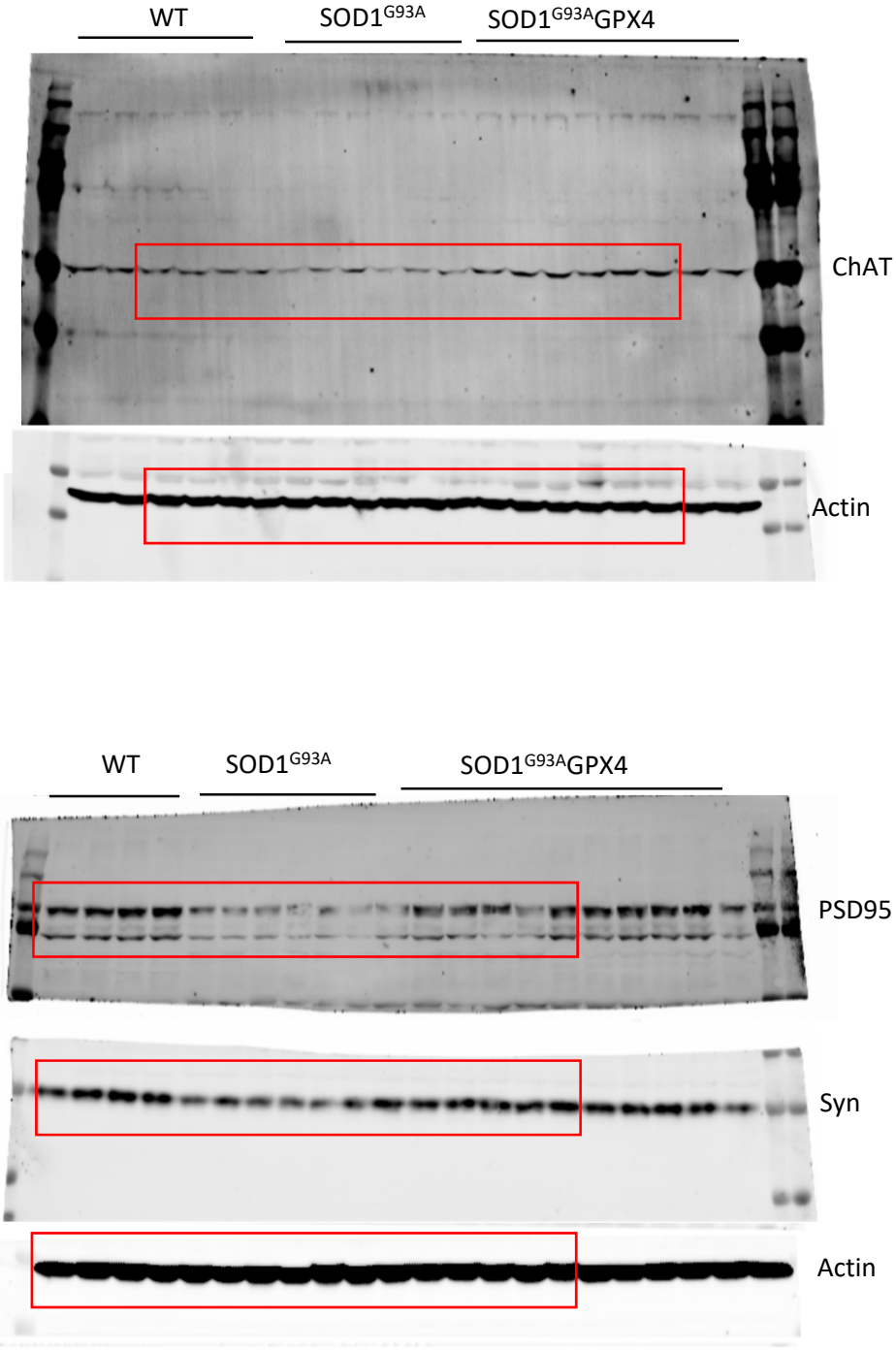


Supplemental Fig. 2. Erastin treatment induced cell death in NSC-34 cells as well as increased lipid ROS. s2A. Viabilities of NSC-34 cells exposed to Erastin. **s2B.** Graph of flow cytometry analysis showing oxidation of BODIPY581/591 C11 in NSC-34 cells exposed to 10 μM Erastin

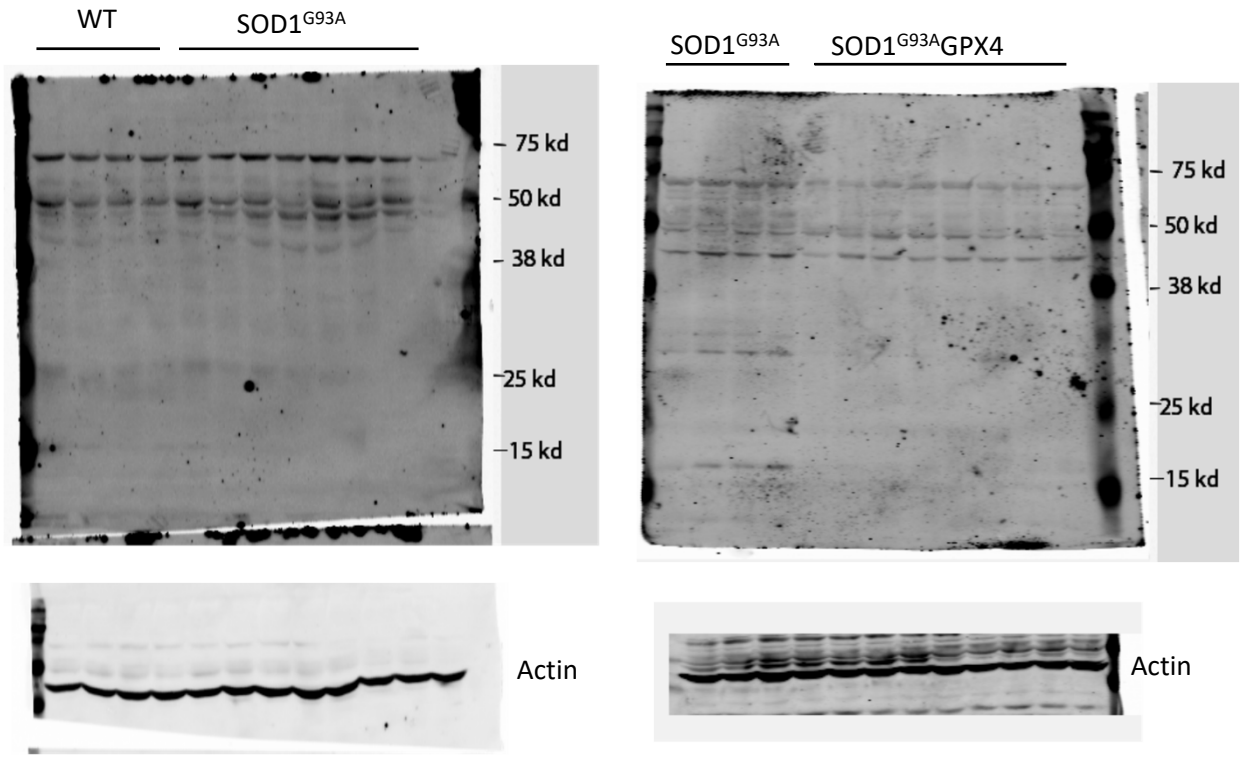




Supplemental Fig. 3. Level of Gpx4 protein in the spinal cords of asymptomatic SOD1^{G93A} mice. s3A. Graph of western blots showing levels of Gpx4 proteins in lumbar spinal cord tissues of WT and asymptomatic SOD1^{G93A} mice (60 days of age). **s3B**. Quantified results of western blots. n. s.: not statistically significant

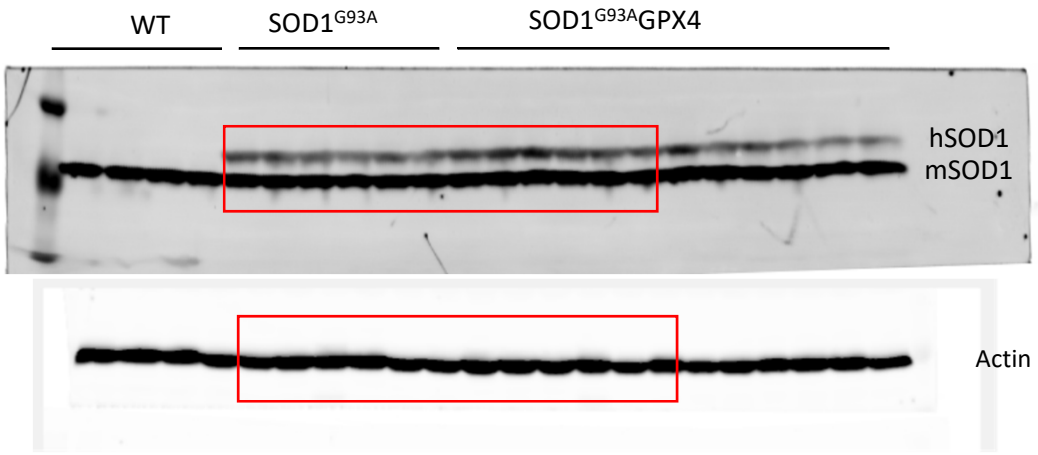


Supplemental Fig. 4. Full western blots of Fig 4A. Red boxes indicate regions of blots used**.**

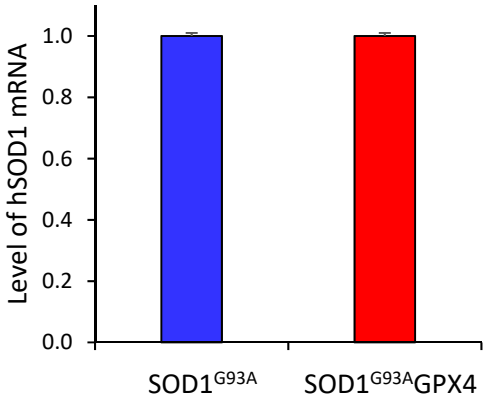


Supplemental Fig. 5. 4HNE blots for Fig. 4D

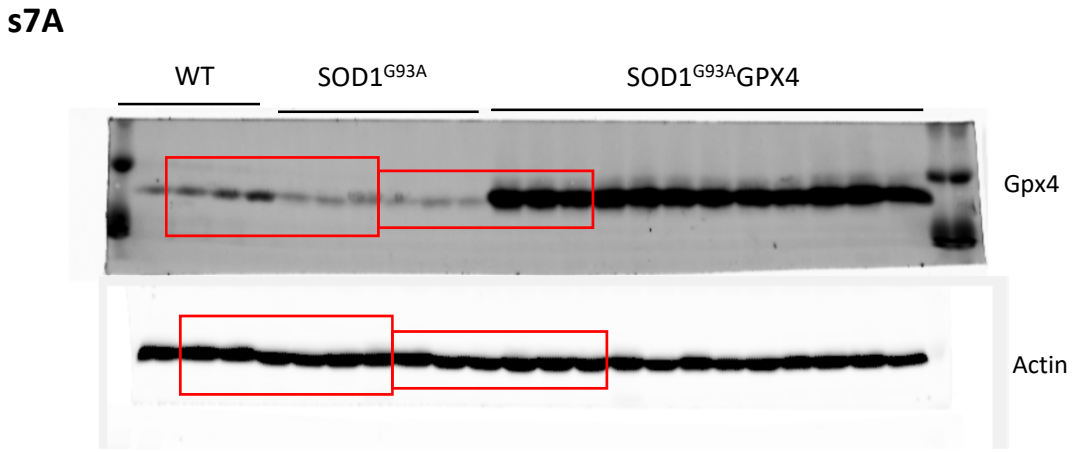
s6A

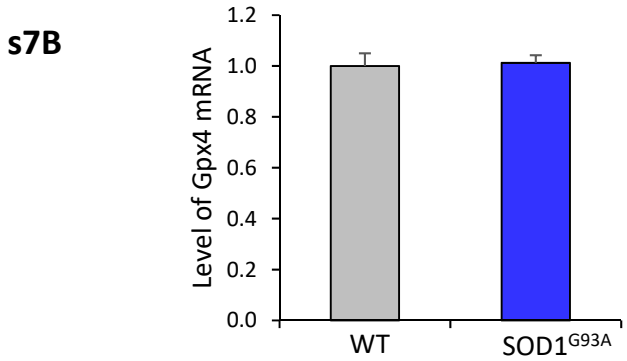


s6B

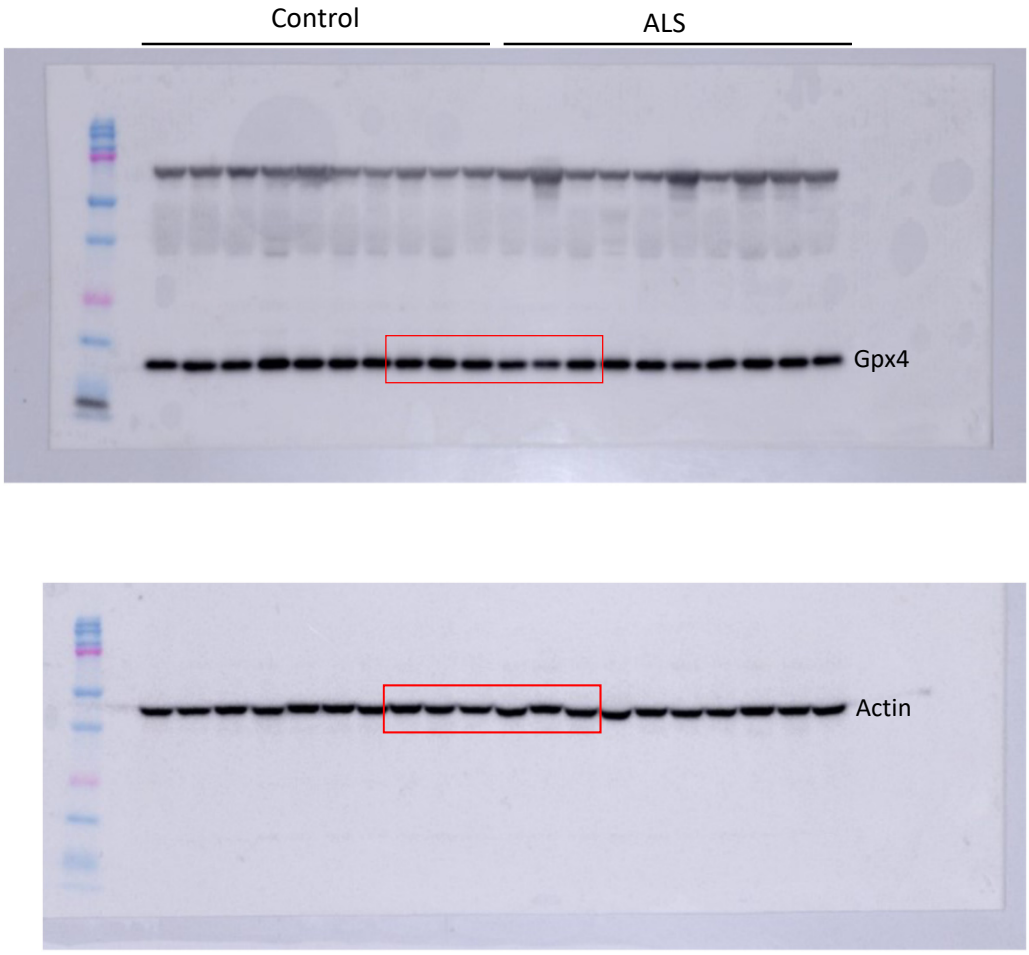


Supplemental Fig. 6. A. Full western blots of Fig 4E. Red boxes indicate regions of blots used**. B.** Levels of hSOD1 mRNA quantified by qRT-PCR. n = 4.

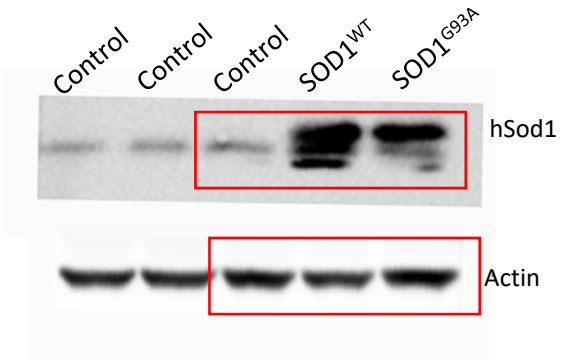


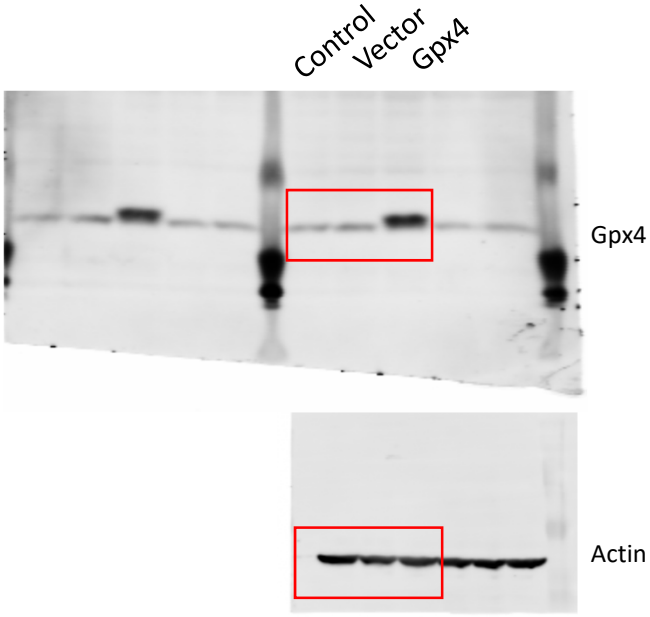


Supplemental Fig. 7. s7 A. Western blots showing levels of Gpx4 protein in spinal tissues of WT, symptomatic $SOD1^{G93A}$ mice and $SOD1^{G93A}$ mice (120 days of age). Small and big red boxes indicate regions of blots used in Fig. 1B and Fig. 6A, respectively. **s7B.** Levels of Gpx4 mRNA quantified by Real-Time qRT-PCR.

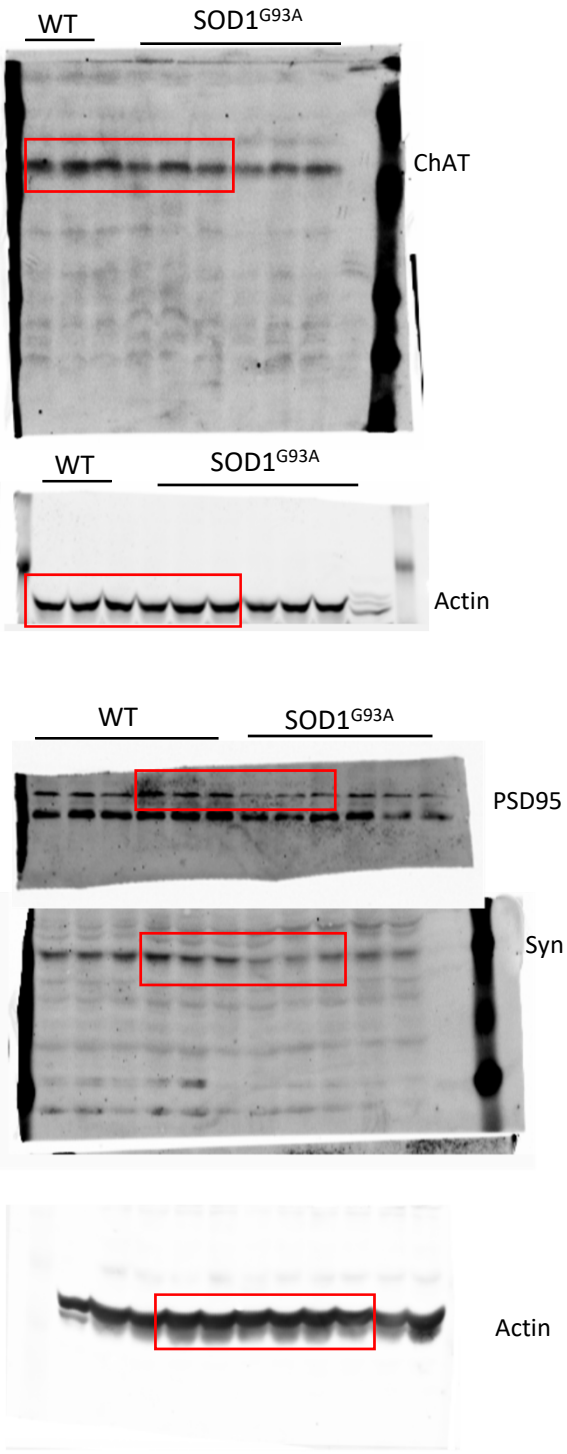


Supplemental Fig. 8. Western blots showing levels of Gpx4 protein in spinal tissues from ALS cases and Control (non-ALS) cases. Red boxes indicate regions of blots used in Fig. 6F.





Supplemental Fig. 9. Western blots of Fig. 5A and Fig. 5B. Red boxes indicate regions of blots used**.**



Supplemental Fig. 10. Western blots of Supplemental Fig. 1A. Red boxes indicate regions of blots used**.**