Supplementary material
Molecular Neurobiology

Cortical neurotoxic astrocytes with early ALS pathology and miR-146a deficit replicate gliosis markers of symptomatic SOD1G93A mouse model

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Supplementary Table 1. Purity of primary culture of astrocytes isolated from mSOD1 and WT mice

	WT	mSOD1
Iba-1	4.74 ± 0.99	3.44 ± 0.79
NG2	3.87 ± 1.12	4.22 ± 1.02
βIII-Tubulin	0.07 ± 0.07	1.04 ± 1.04

mSOD1, *SOD1*G93A mice; WT, wild type mice; Iba-1, Ionized calcium binding adaptor molecule 1, microglia marker; NG2, oligodendrocyte progenitors marker; βIII-Tubulin, class III β-tubulin, neurons marker. Results are represented as percentage of positive cells. Data are mean ± SEM from at least three independent experiments

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Supplementary Table 2. List of antibodies used in the present study

Primary antibody against:	Source	Specie	Dilution used in:		
			IHC	ICC	WB
SOD1	Cruz Biotechnology, sc-11407	Rabbit	1:50	1:250	1:500
GFAP	Novocastra, GFAP-GA5, Leica	Mouse	1:100	1:100	---
	Sigma-Aldrich, G9269	Rabbit	---	1:500	1:500
GLT-1/EAAT2	AbCam, ab41621	Rabbit	1:180	1:180	1:500
GLAST/EAAT1	AbCam, AB416	Rabbit	---	1:500	1:500
S100B	AbCam, ab52642	Rabbit	1:200	1:200	---
Ki-67	Santa Cruz Biotechnology, sc-7846	Goat	---	1:50	---
Iba-1	Wako 019-19741, Wako Pure Chemical Industries Ltd, Osaka, Japan	Rabbit	---	1:250	1:500
HMGB1	BioLegend, 651402	Mouse	---	---	1:200
NeuN	Millipore, MAB377	Mouse	---	---	1:200
PSD-95	Millipore, MAB1598	Mouse	---	---	1:1000
SYP	Millipore, MAB329-c	Mouse	---	---	1:500
phospho-eIF-2α	Cell Signaling, D9G8	Rabbit	---	---	1:800
ATF-4/CREB-2	Santa Cruz Biotechnology, sc-200	Rabbit	---	---	1:200
NG2	Millipore, AB5320	Rabbit	---	---	1:500
MBP	Millipore, AB980	Rabbit	---	---	1:800
RAGE	Chemicon, MAB5328	Mouse	---	---	1:500
Beclin-1	Millipore, MABC34	Mouse	---	---	1:200
LC3B	Cell Signaling, 2775S	Rabbit	---	---	1:1000
NF-kB p65	Santa Cruz Biotechnology,sc-372	Rabbit	---	1:100	1:500
NF-kB p65 phospho S536	Ab131109, Abcam	Rabbit	---	---	1:500
βIII-tubulin	Merck Millipore, MAB1637	Mouse	---	1:500	---
β-actin	Sigma, A5441	Mouse	---	---	1:5000

Secondary antibody	Source	Dilution		
Anti-rabbit HRP	Santa Cruz Biotechnology, sc-2004	---	---	1:5000
Anti-mouse HRP	Santa Cruz Biotechnology, sc-2005	---	---	1:5000
Anti-mouse AlexaFluor 594	Invitrogen Corporation, A-11005	1:200	1:1000	---
Anti-rabbit AlexaFluor 488	Invitrogen Corporation, A-11008	1:200	1:1000	---
Anti-goat AlexaFluor 594	Invitrogen Corporation, A-21468	---	1:1000	---

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Supplementary Table 3. List of primer sequences used in qPCR

Gene	Forward primer sequence	Reverse primer sequence
GFAP	5'-CCAAACTGGCTGATGTCTACC-3'	5'-GCTTCATCTGCCTCCTGTCTA-3'
S100B	5'-GAGAGAGGGTGACAAGCACAA-3'	5'-GGCCATAAACTCCTGGAAGTC-3'
Ki-67	5'-CAGTACTCGGAATGCAGCAA-3'	5'-CAGTCTTCAGGGGCTCTGTC-3'
Cx43	5'-ACAGCGGTTGAGTCAGCTTG-3'	5'-GAGAGATGGGGAAGGACTTGT-3'
HMGB1	5'-CTCAGAGAGGTGGAAGACCATGT-3'	5'-GGGATGTAGGTTTTCATTTCTCTTTC-3'
Cd11b	5'-CAGATCAACAATGTGACCGTATGGG-3'	5'-CATCATGTCCTTGTACTGCCGCTTG-3'
NG2	5'-GGGCTGTGCTGTCTGTTGA-3'	5'-TGATTCCCTTCAGGTAAGGCA-3'
IRAK1	5'-GAGACCCTTGCTGGTCAGAG-3'	5'-GCTACACCCACCCACAGAGT-3'
TRAF6	5´-AAAGCGAGAGATTCTTTCCCTG-3´	5'-ACTGGGGACAATTCACTAGAGC-3'
IL-6	5'-CCGGAGAGGAGACTTCACAG-3'	5'-GGAAATTGGGGTAGGAAGGA-3'
β-actin	5'-GCTCCGGCATGTGCAA-3'	5'-AGGATCTTCATGAGGTAGT-3'

miRNA	Target sequence
hsa-miR-146a-5p	5'-UGAGAACUGAAUUCCAUGGGUU-3'
hsa-miR-21-5p	5'-UAGCUUAUCAGACUGAUGUUGA-3'
hsa-miR-124-3p	5'-UAAGGCACGCGGUGAAUGCC-3'
mmu-miR-155-5p	5'-UUAAUGCUAAUUGUGAUAGGGGU-3'
hsa-miR-125b-5p	5'-UCCCUGAGACCCUAACUUGUGA-3'
SNORD110	Reference gene

GFAP, glial fibrillary acidic protein; S100B, S100 calcium binding protein B; Cx43, connexin 43; HMGB1, high-mobility-group-box-1 protein; IRAK1, Interleukin-1 receptor-associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6, IL-6, Interleukin 6; miR, microRNA.

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Supplementary Table 4. Similar cell viability profile was observed in cortical astrocytes isolated either from mSOD1 or WT mice pups

	WT	mSOD1
Viable cells	92.9 ± 1.1	91.9 ± 1.7
Early apoptotic cells	6.3 ± 0.9	8.7 ± 0.7
Late stages of apoptosis/necrosis	0.6 ± 0.1	0.9 ± 0.1
Debris	0.2 ± 0.1	0.3 ± 0.1

mSOD1, *SOD1*G93A mice; WT, wild type mice. Results are indicated as cell percentages. Data are mean ± SEM from at least four independent experiments. Two-tailed unpaired Student's *t-test* was used to assess differences between groups. No differences were noticed between WT and mSOD1 astrocytes.