Molecular Medicine

Supplemental Data

Intravenous Mesenchymal Stem Cells Improve Survival and Motor Function in Experimental Amyotrophic Lateral Sclerosis

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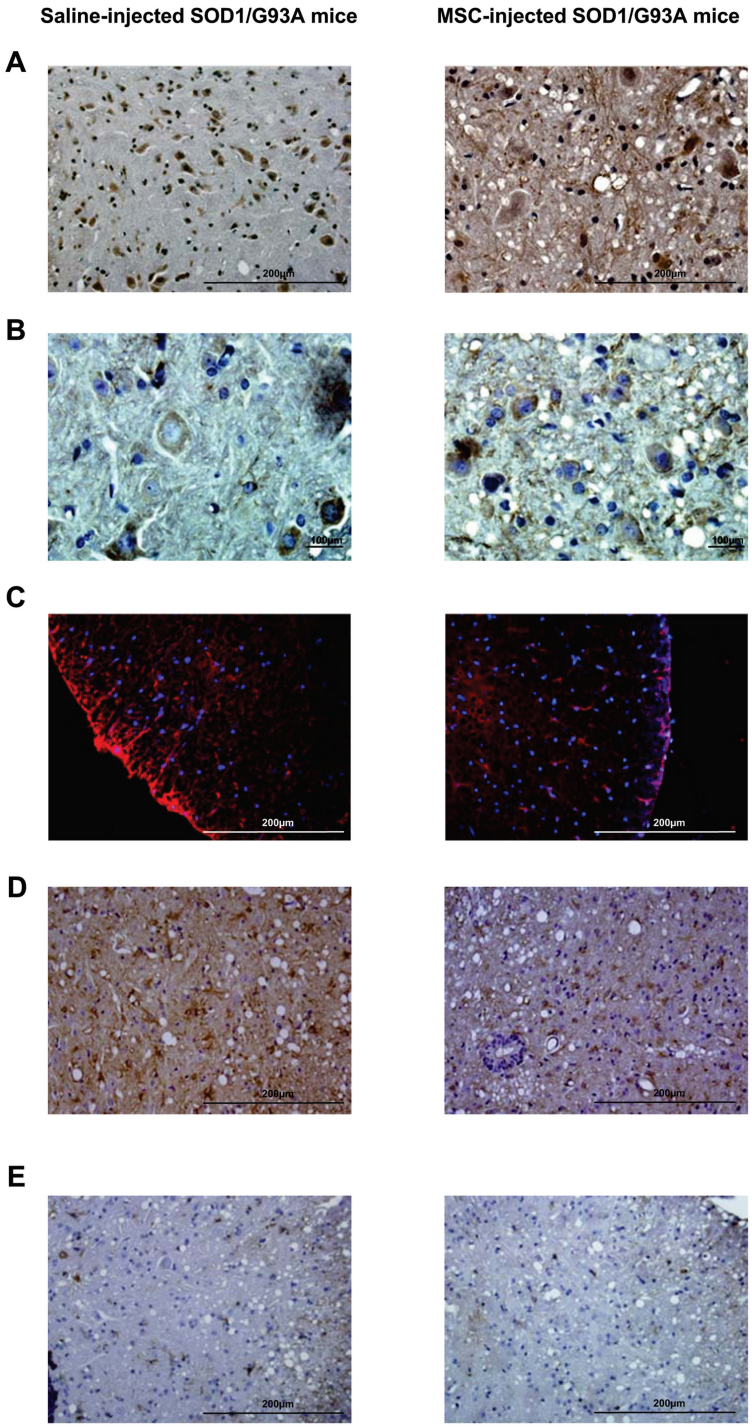
Supplementary Table S1. Parameters utilized to score hind limb extension reflex and gait, as indexes of pathology progression.

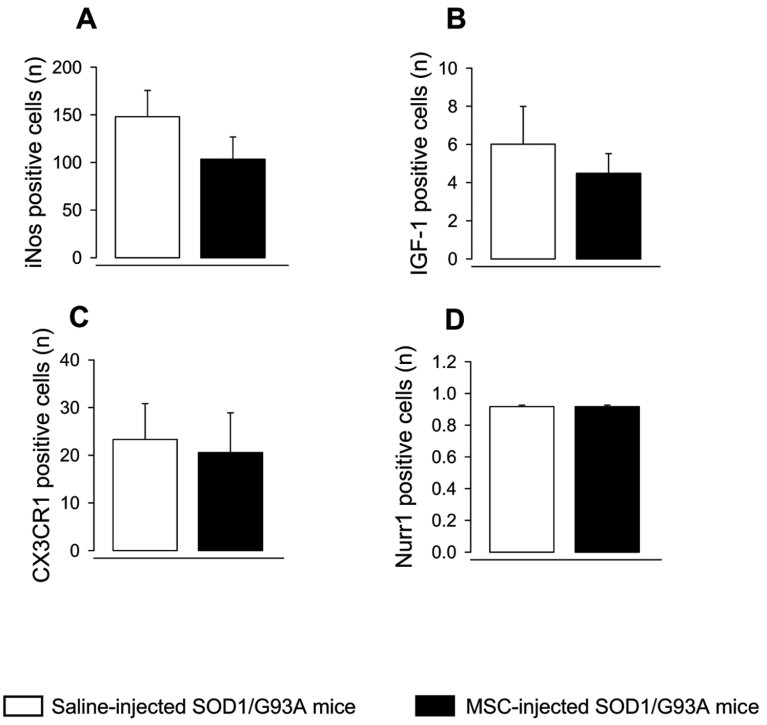
HIND LIMBS EXTENSION REFLEX (mice suspended by the tail)	
5	normal functions, no signs of disease
4.5	slight limb extension deficits
4	minor limb asymmetry; slight tremor
3.5	moderate extension asymmetry or bilateral extension deficit ; tremor
3	evident extension asymmetry or bilateral extension deficit; first signs of paralysis; severe tremor
2.5	pronounced extension incapability; partial paralysis of one or both limbs; first signs of arched spine
2	severe extension incapability or persistent limb elongation
1.5	nearly total paralysis with still autonomous movements; persistent limb elongation
1	modest limb movements after touch stimulation; accentuated arched spine
0.5	total paralysis of both limbs; no limb movements after touch stimulation
0	total paralysis (euthanasia)
GAIT (mice walking in open space)	
5	normal straight gait, no signs of disease
4.5	slight stagger gait
4	evident stagger gait
3.5	wobbling but straight gait; first signs of hind limb motor activity impairment
3	evident signs of one or both hind limbs motor activity impairment
2.5	severe tremor; walking on tiptoes
2	partial dragging gait using forelimbs; stiffness of one or both hind limbs
1.5	dragging gait mostly using forelimbs
1	dragging gait using forelimbs only because of total paralysis of both hind limbs; still straight gait direction
0.5	signs of forelimb impairment; turning around gait
0	the animal is unable to move (euthanasia)

Supplementary Table S2. Primers utilized for quantitative real-time RT-PCR experiments.

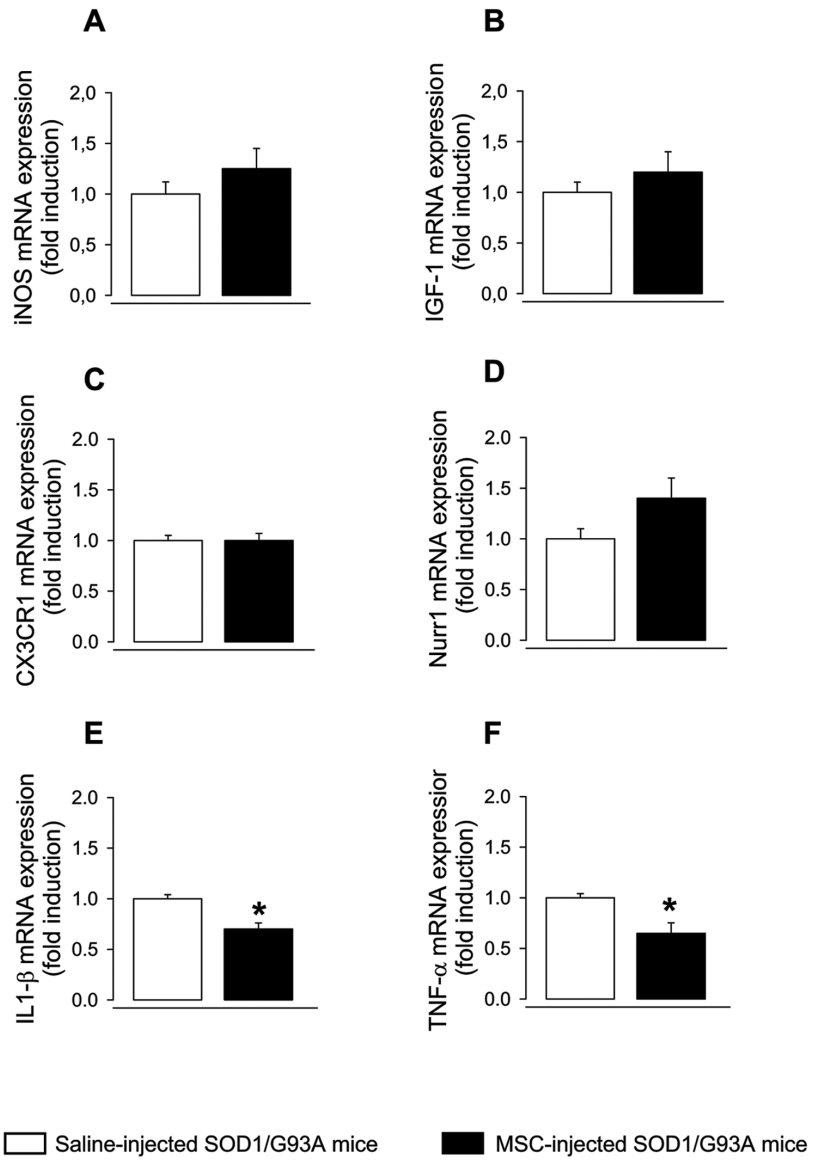
MT1 forward	5′-CTGCTCCACCGGCGG-3′
MT1 reverse	5′-GCCCTGGGCACATTTGG-3
MT2 forward	5′-TCCTGT GCCACAGATGGATC-3′
MT2 reverse	5′-GTCCGAAGCCTCTTTGCAGA-3
MT3 forward	5′-GGAGGAACCAAGCTACGGC-3′
MT3 reverse	5′-ACATAGGCTGTGTGGGAGGG-3′
iNOS forward	5′-TGAACTTGAGCGAGGAGCA-3
iINOS reverse	5′-TTCATGATAACGTTTCTGGCTCT-3′
CX3CR1 forward	5′-AAGTTCCCTTCCCATCTGCT-3′
CX3CR1 reverse	5′-CAAAATTCTCTAGATCCAGTTCAGG-3′
IGF-1 forward	5′-AGCAGCCTTCCAACTCAATTAT-3′
IGF-1 reverse	5′-GAAGACGACATGATGTGTATCTTTATC-3′
Nurr1 forward	5′-TCAGAGCCCACGTCGATT-3′
Nurr1 reverse	5′-TAGTCAGGGTTTGCCTGGAA-3′
GLT-1 forward	5′-CATCCTTGTCTGCTTATATGGC-3′
GLT-1 reverse	5′-GTTTCTGTTCCCTTCTCACC -3′
IL1β forward	5′-AGTTGACGGACCCCAAAAG-3′
IL1β reverse	5′-TTTGAAGCTGGATGCTCTCAT-3′
TNF forward	5′-TCTTCTCATTCCTGCTTGTGG-3′
TNF reverse	5′-GGTCTGGGCCATAGAACTGA-3′



Supplementary Figure S1. Spinal cord images representative of the data shown in Fig 4 and 5. The following proteins were stained: (A) ubiquitin, (B) ChAT, (C) GLT-1, (D) GFAP, (E) IB4. SOD1/G93A mice were treated with 10^6 MSCs at d 90 or saline and sacrificed at d 125 and transcardially perfused with 4% paraformaldehyde. The spinal cord was removed, post-fixed, and embedded in paraffin and 5 μm sections were stained for histological examination. The following antibodies were used: mouse anti-ubiquitin, diluted 1:250; rabbit anti-ChAT, diluted 1:500; guinea pig anti-GLT-1, diluted 1:100; rabbit anti-cow GFAP, diluted 1:100; isolectin IB4 conjugated antibody, diluted 1.100. Scale bars: 200 μm (A, C–D); 100 μm (B).



Supplementary Figure S2. Effects of MSC administration on the number of (A) iNOS-, (B) IGF-1-, (C) CX3CR1-, and (D) Nurr1-positive cells in the spinal cord of SOD1/G93A mice. SOD1/G93A mice were treated with 10^6 MSCs or saline at d 90 and sacrificed at d 125 and transcardially perfused with 4% paraformaldehyde. Spinal cord was removed, post-fixed, and embedded in paraffin. Five μm sections were stained for histological examination. The following antibodies were used: rabbit anti-iNOS, diluted 1:100; rabbit anti- IGF1, diluted 1:200; rabbit anti-CX3CR1, diluted 1:100; anti-human/mouse Nurr1, diluted 1:200. Quantification was performed on 6 sections per animal (7 animals per group) from three segments apart. Data (means ± SEM) represent the number of positive cells/mm^2.



Supplementary Figure S3. Effect of MSC administration on (A) iNOS, (B) IGF-1, (C) CX3CR1, (D) Nurr1, (E) IL1β and (F) TNFα mRNA expression in the spinal cord of SOD1/G93A mice. SOD1/G93A mice were treated with 10^6 MSCs or saline at d 90 and sacrificed at d 125. mRNAs were analyzed by RT-PCR. Six mice per group were used. Data (means ± SEM) are expressed as fold induction respect to saline-injected SOD1/G93A mice. (* $p < 0.01$, Mann-Whitney).