Exosomes from mesenchymal stem cells: experimental assessment on *in vitro* and *in vivo* models of ALS.

Roberta Bonafede
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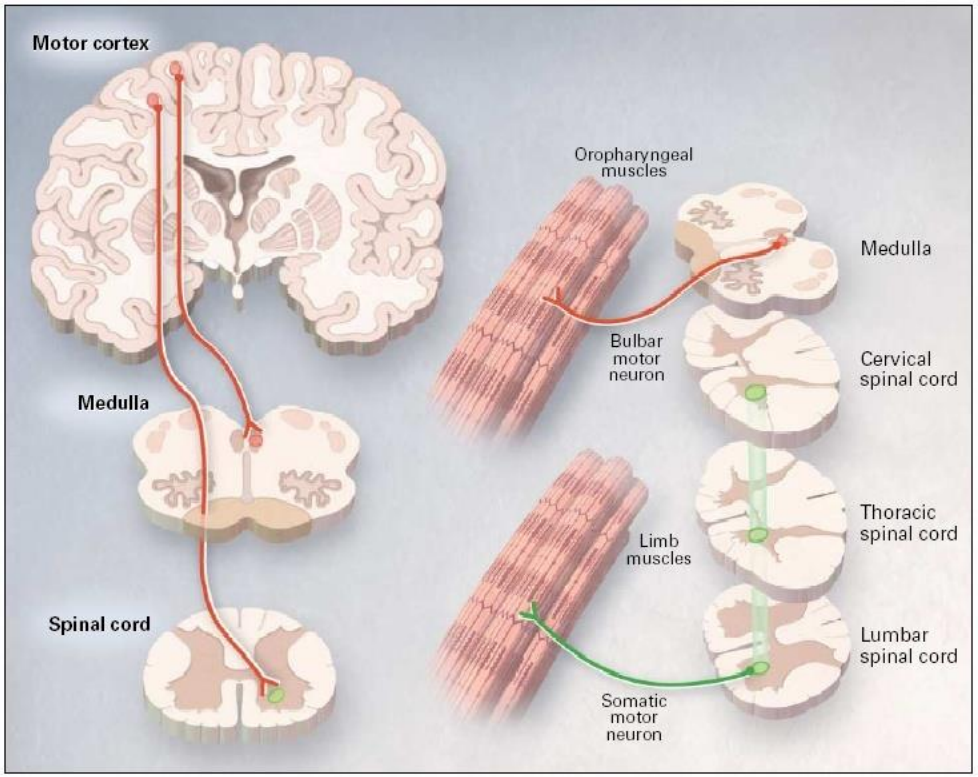
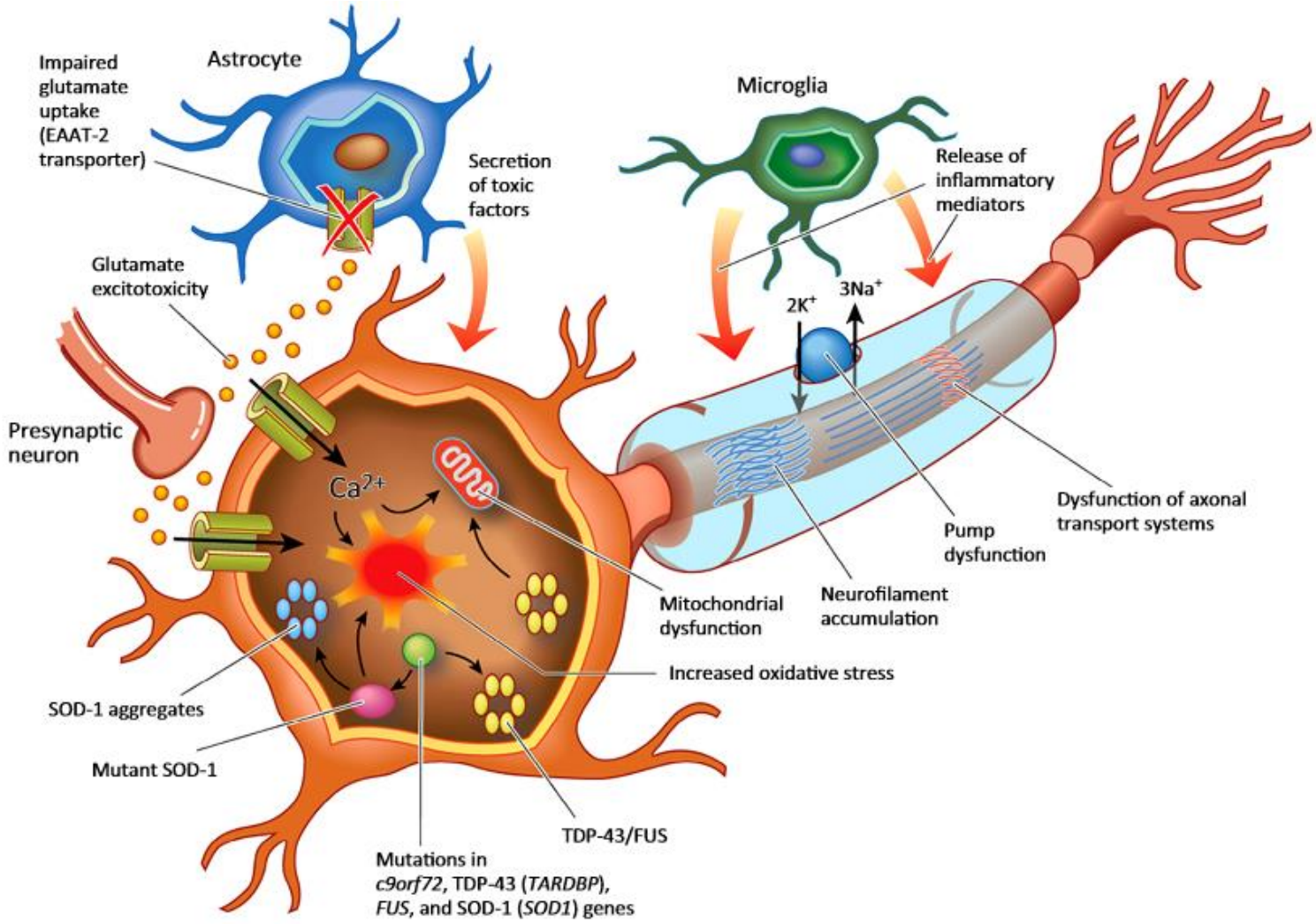
Amyotrophic lateral sclerosis (ALS)



- Neurodegenerative disorder
- Degeneration of motor neuron
- Adult disease onset
- 90% sporadic cases (SALS), 10% familial inheritance (FALS)
- 20% of FALS caused by mutations in superoxide dismutase 1 (SOD1) gene

ALS pathophysiology



Vucic *et al*., 2014

Mesenchymal stem cells (MSCs)

- Multipotent stem cells
- Self-renew and differentiation potential
- Isolated from a variety of fetal and adult tissues

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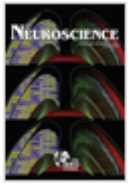
ASCs: Adipose Stem Cells

- Available in large amounts from liposuction
- Expansion *in vitro* is simple to do
- Less susceptible to malignant changes and genetic abnormalities
- Attracted to sites of tissue injury, where they increase production of trophic factors

The transplantation of adipose stem cells delays symptom progression in murine FALS

Systemic treatment with adipose-derived mesenchymal stem cells ameliorates clinical and pathological features in the amyotrophic lateral sclerosis murine model



S. Marconi[a], M. Bonaconsa[a], I. Scambi[a], G.M. Squintani[b], W. Rui[a], E. Turano[a], D. Ungaro[c], S. D'Agostino[a], F. Barbieri[a], S. Angiari[d], A. Farinazzo[a], G. Constantin[d], U. Del Carro[c], B. Bonetti[a], R. Mariotti[a].

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Homing of ASC:
low concentration in the spinal cord; beneficial effects probably due to the paracrine activity of ASC

Beneficial effect of MSCs due to paracrine activity

NIH Public Access
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Circ Res. Author manuscript; available in PMC 2009 November 21.

Paracrine mechanisms in adult stem cell signaling and therapy

Massimiliano Gnecchi[1,3,4,*], Zhiping Zhang[1,2,*], Aiguo Ni[1,2], and Victor J. Dzau[1,2]

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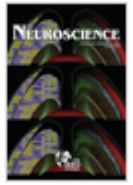
PLOS | ONE

Paracrine Effect of Mesenchymal Stem Cells Derived from Human Adipose Tissue in Bone Regeneration

Itali Linero[1], Orlando Chaparro[2]*

ELSEVIER

Systemic treatment with adipose-derived mesenchymal stem cells ameliorates clinical and pathological features in the amyotrophic lateral sclerosis murine model



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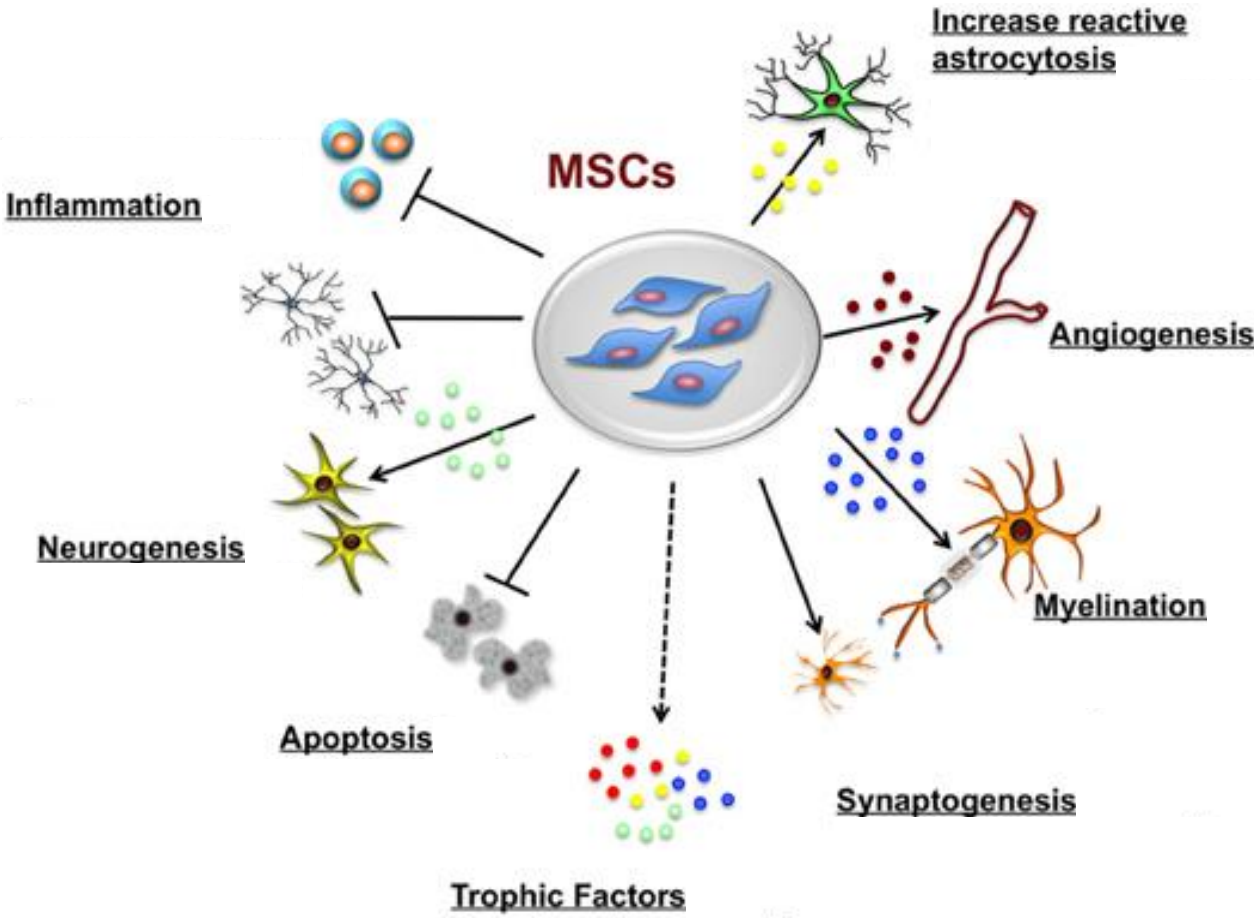
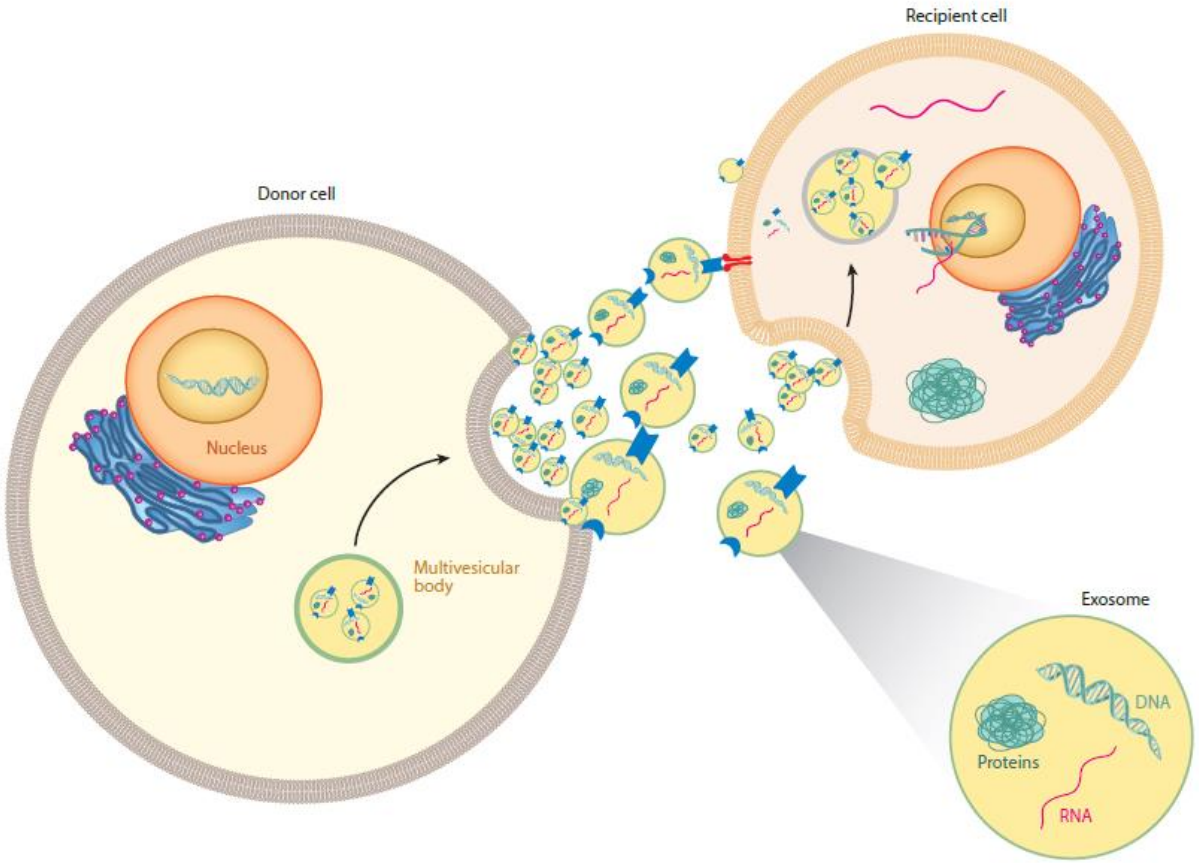
Paracrine activity of MSCs



Castillo-Melendez *et al*., 2013

ASCs exosomes (ASC-Exo)



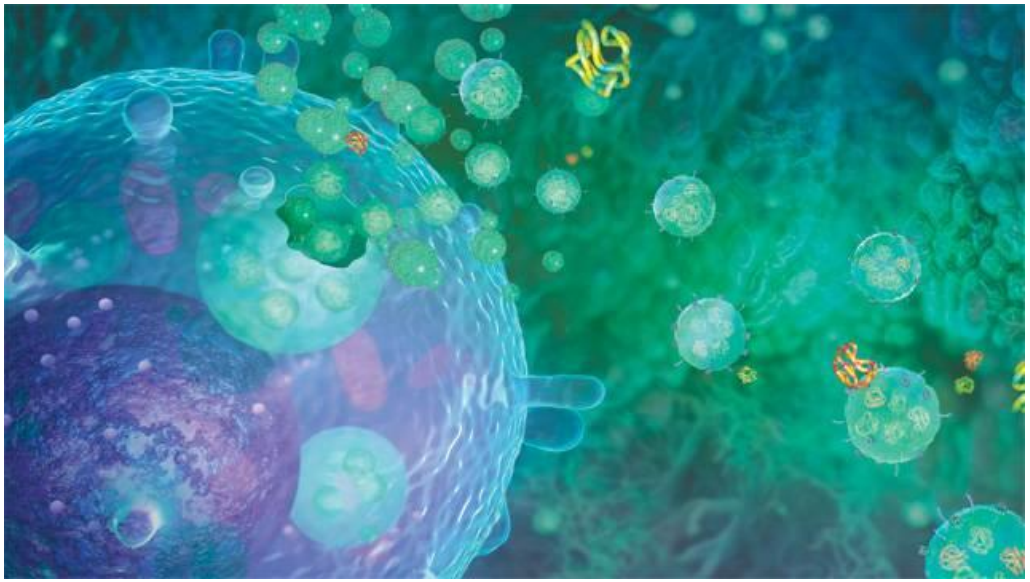
- 30-100 nm in diameter
- Cell-cell communication
- Proteins, lipids, DNA and RNA

Kourembanas, 2014

Novel cell-free therapy approaches

ASC-Exo:

- prolonged permanence in the tissues
- < immune response, risk of infection
- < cancer risk
- avoid mesenchymal differentiation

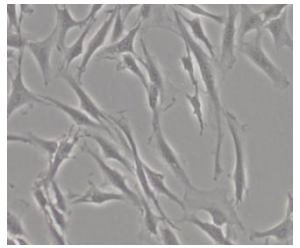


Research aims

- assess the neuroprotective effect of ASC-Exo *in vitro*
- assess the neuroprotective effect of ASC-Exo *in vivo*
- label exosomes with superparamagnetic iron oxide agent to identify their homing

Isolation of ASC-Exo

ASC cultures

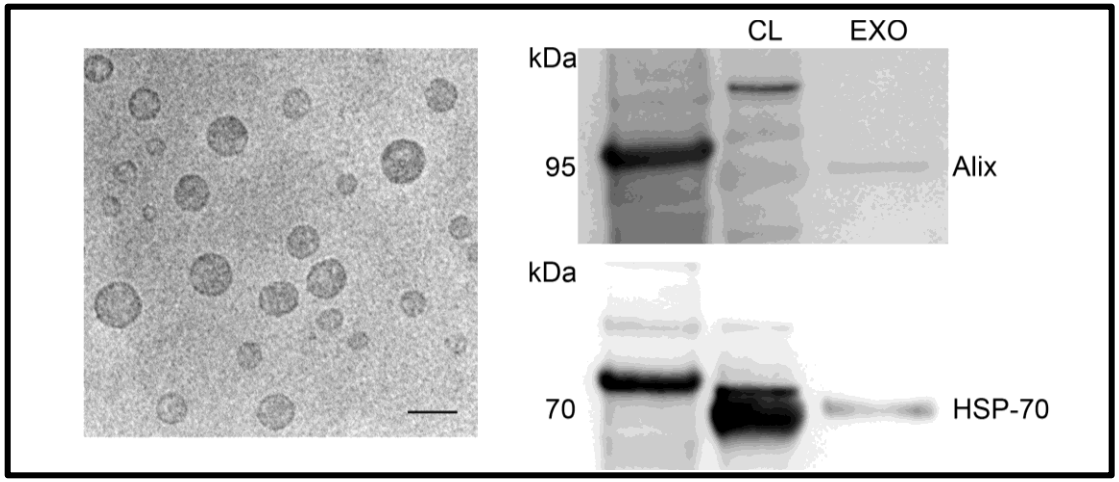


ASCs culture from inguinal adipose tissue of wild type mice

48h FBS deprivation and supernatant collection

Exosomes isolation

Characterization of Exo: electron microscopy and western blot



Neuroprotective effect of ASC-Exo *in vitro*

- NSC34 motor neuron-like cell line NAÏVE and TRANSIENTLY transfected with hSOD1(G93A) gene
- Oxidative insult (100 µM H_2O_2) with or without ASC-Exo (0.2 µg/ml)
- Cell death

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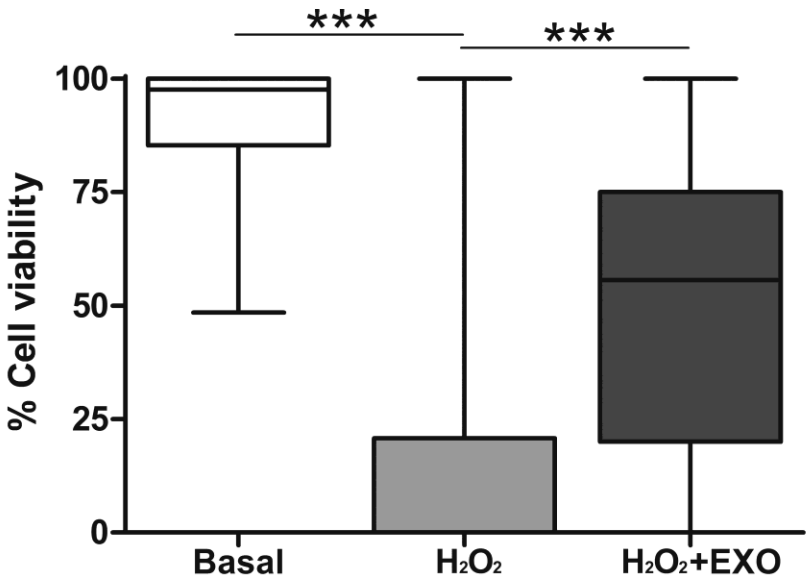
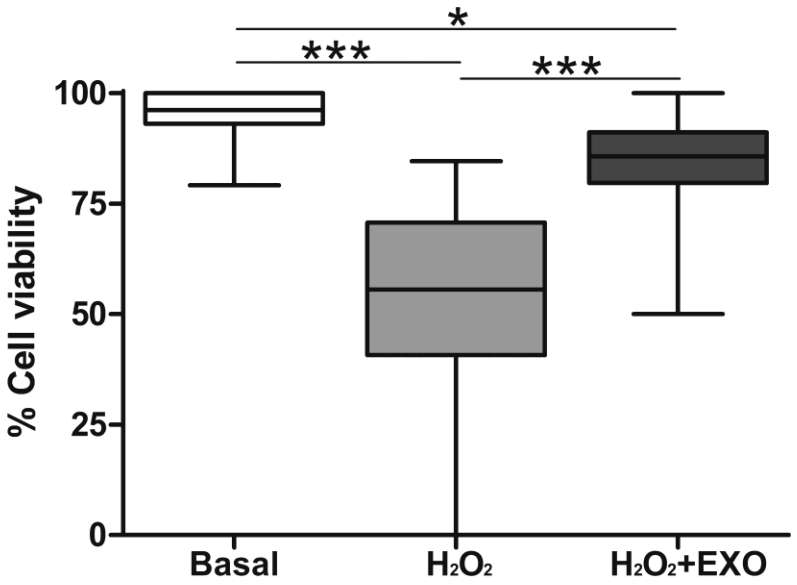
NSC34 naïve

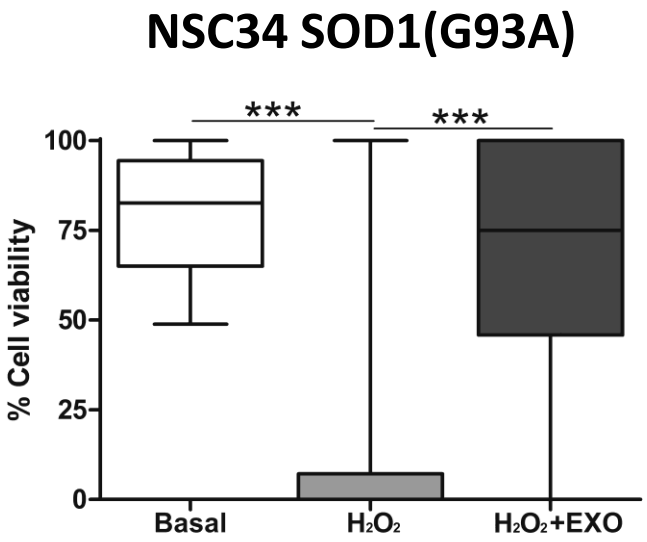


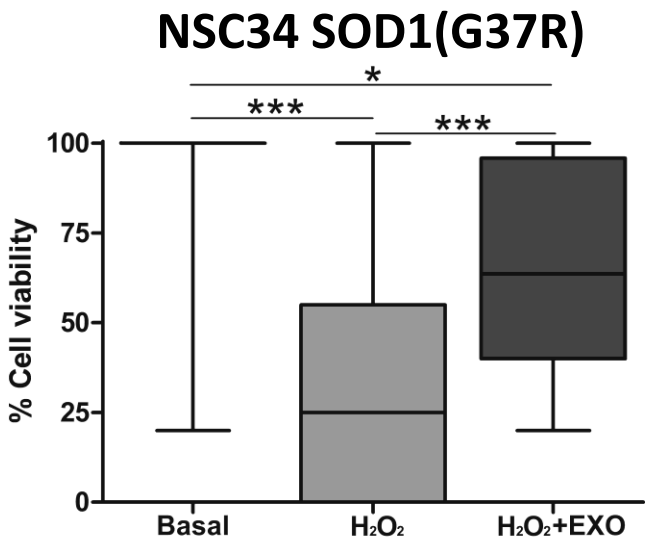
NSC34 SOD1(G93A)

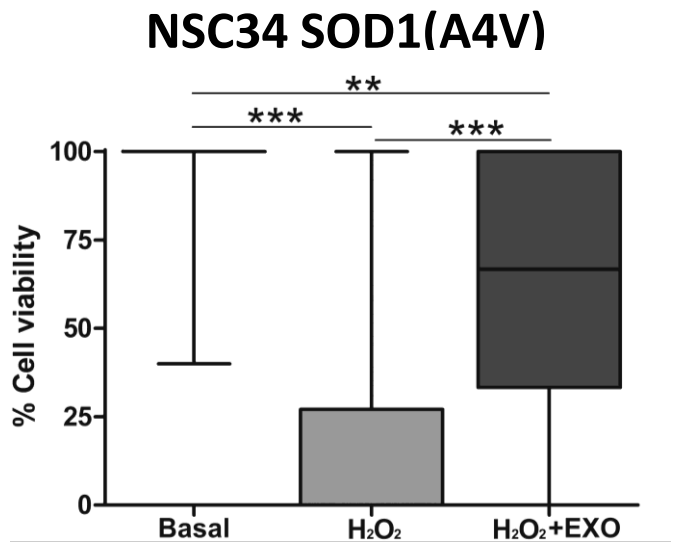


Bonafede *et al*., under revision

NSC34 STABLY transfected with hSOD1(G93A), hSOD1 (G37R) and hSOD1(A4V) gene







ASC-Exo have neuroprotective effect on naïve and transfected NSC34 cells

Bonafede *et al.*, under revision

- Exosomes exerted a neuroprotective effect on cell viability increasing their tolerance and survival to oxidative damage

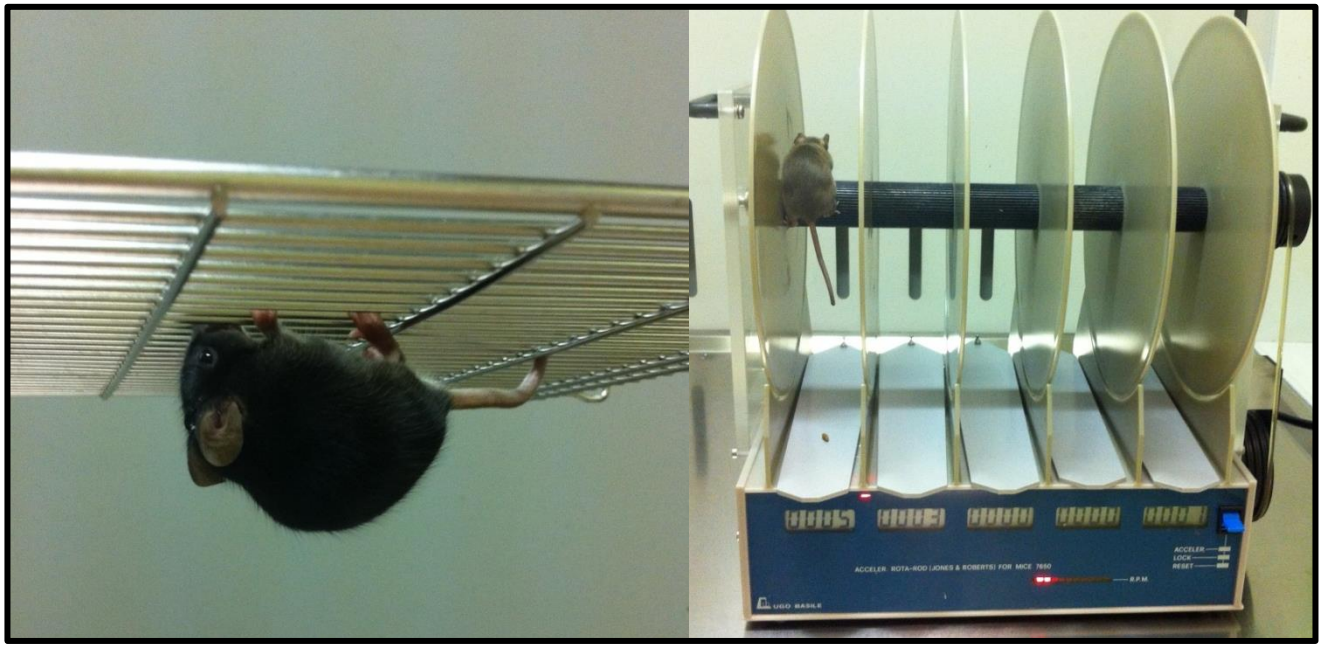
- Modulate the microenvironment and influence gene expression of recipient cells

- Next step: miRNA (e.g. miRNA21, miRNA222 and miRNAlet7a) with neuroprotective function?

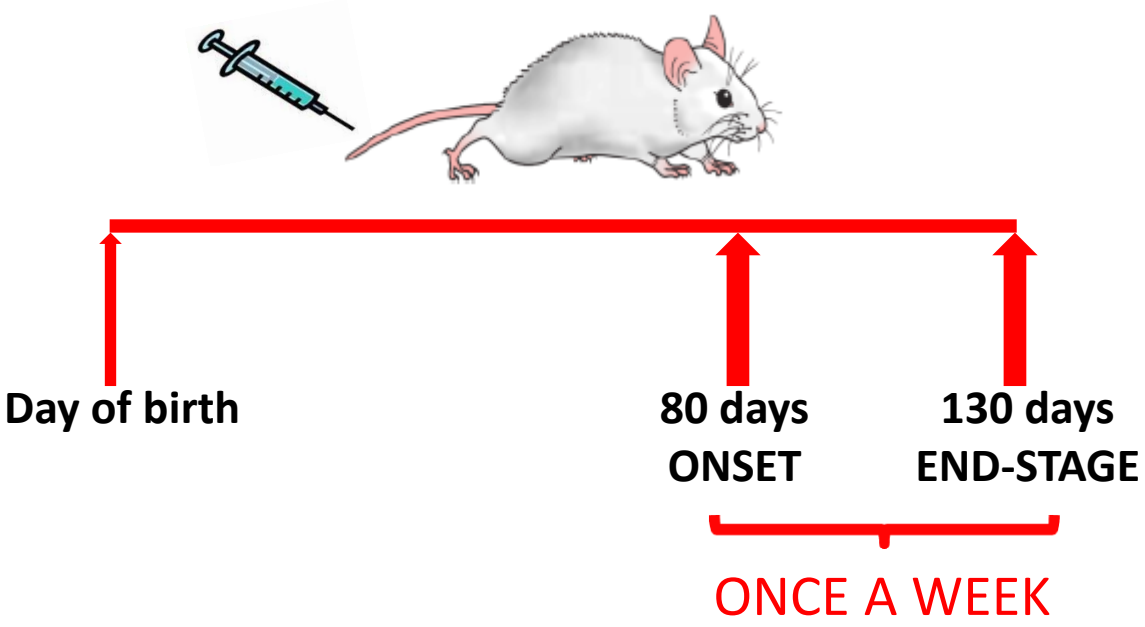
Neuroprotective effect of ASC-Exo *in vivo*

- Transgenic mice overexpressing human SOD1(G93A) gene
- The mostly used model for ALS: clinical and histopathological features of human ALS
- Transgenic progeny is genotyped by PCR
- Onset disease: 80-85 days; end-stage: 120-130 days

We assessed body weight, tremors and extension reflex, rotarod test and paw grip endurance to evaluate the disease progression and duration

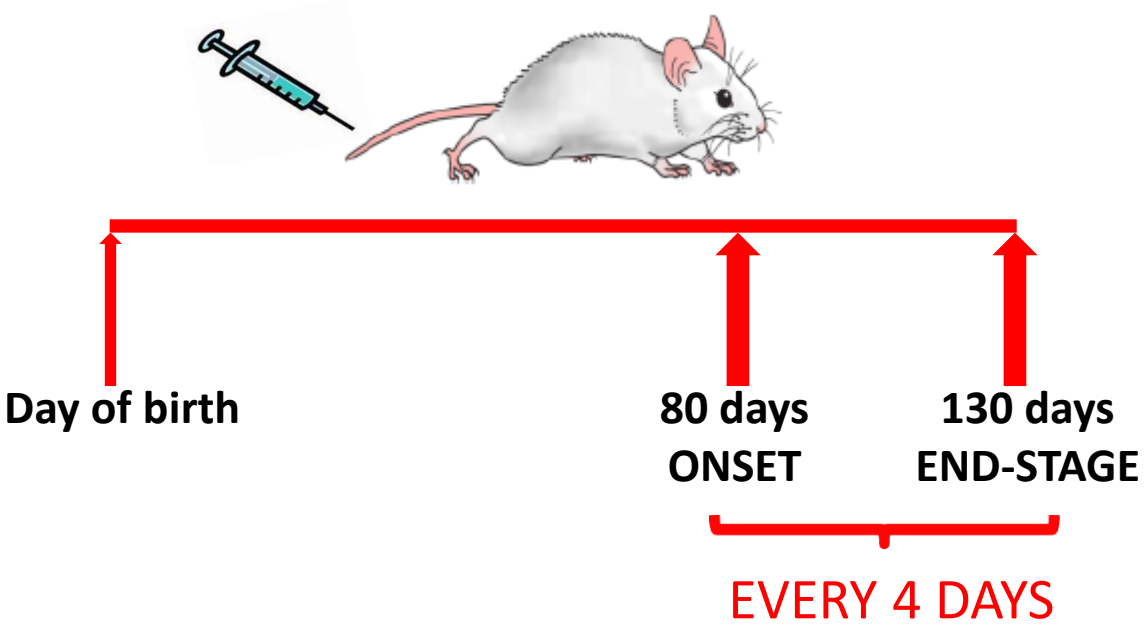


1st: ASC-Exo (from $8x10^6$ cells) or PBS, at clinical onset until end stage, once a week

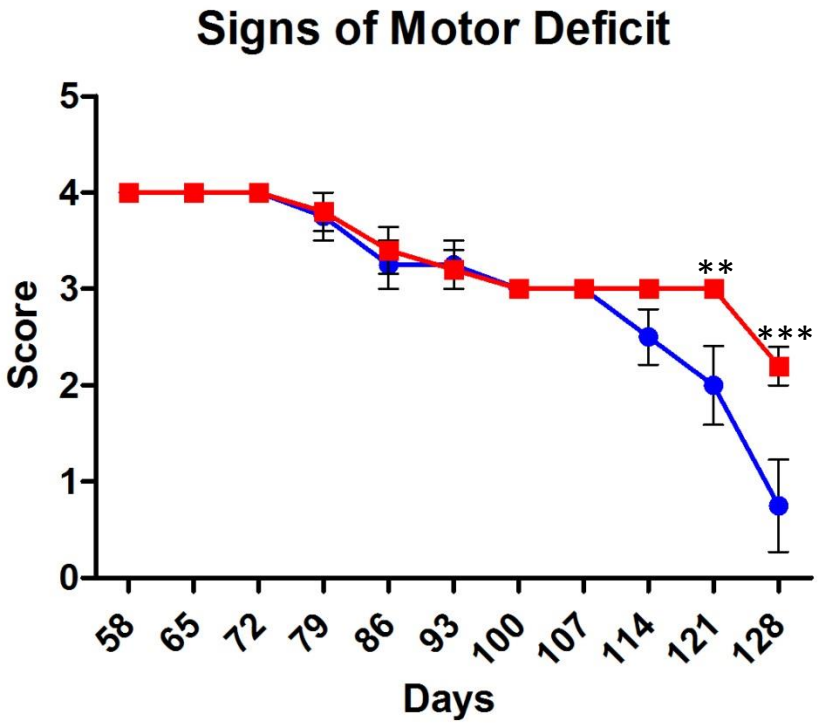


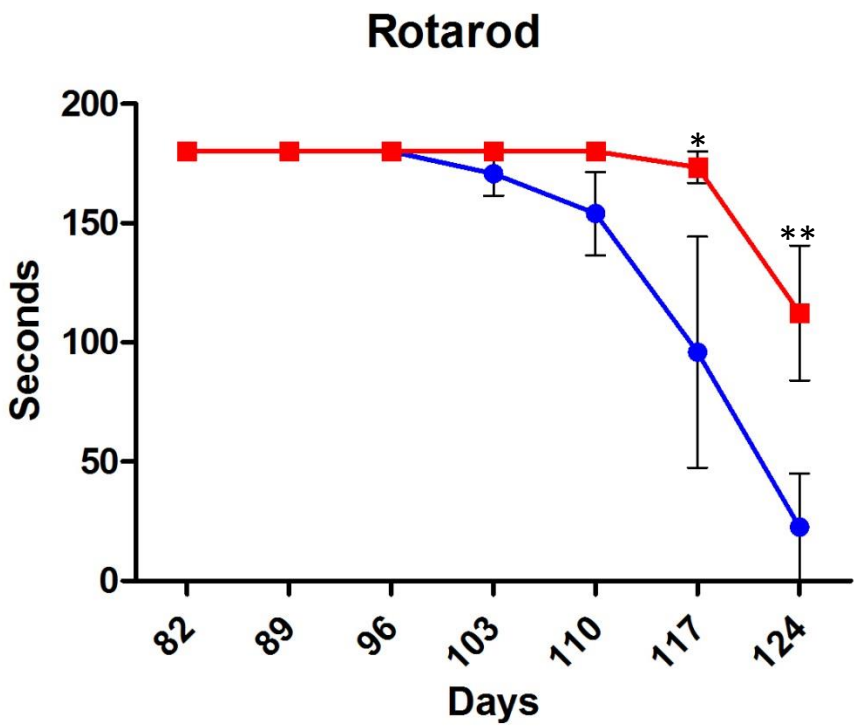
We did not find any differences in terms of motor performance and in lifespan between ASC-Exo and PBS treated animals

2nd: ASC-Exo (from 1,5x10^6 cells) or PBS, at clinical onset until end stage, every 4 days



Motor performance

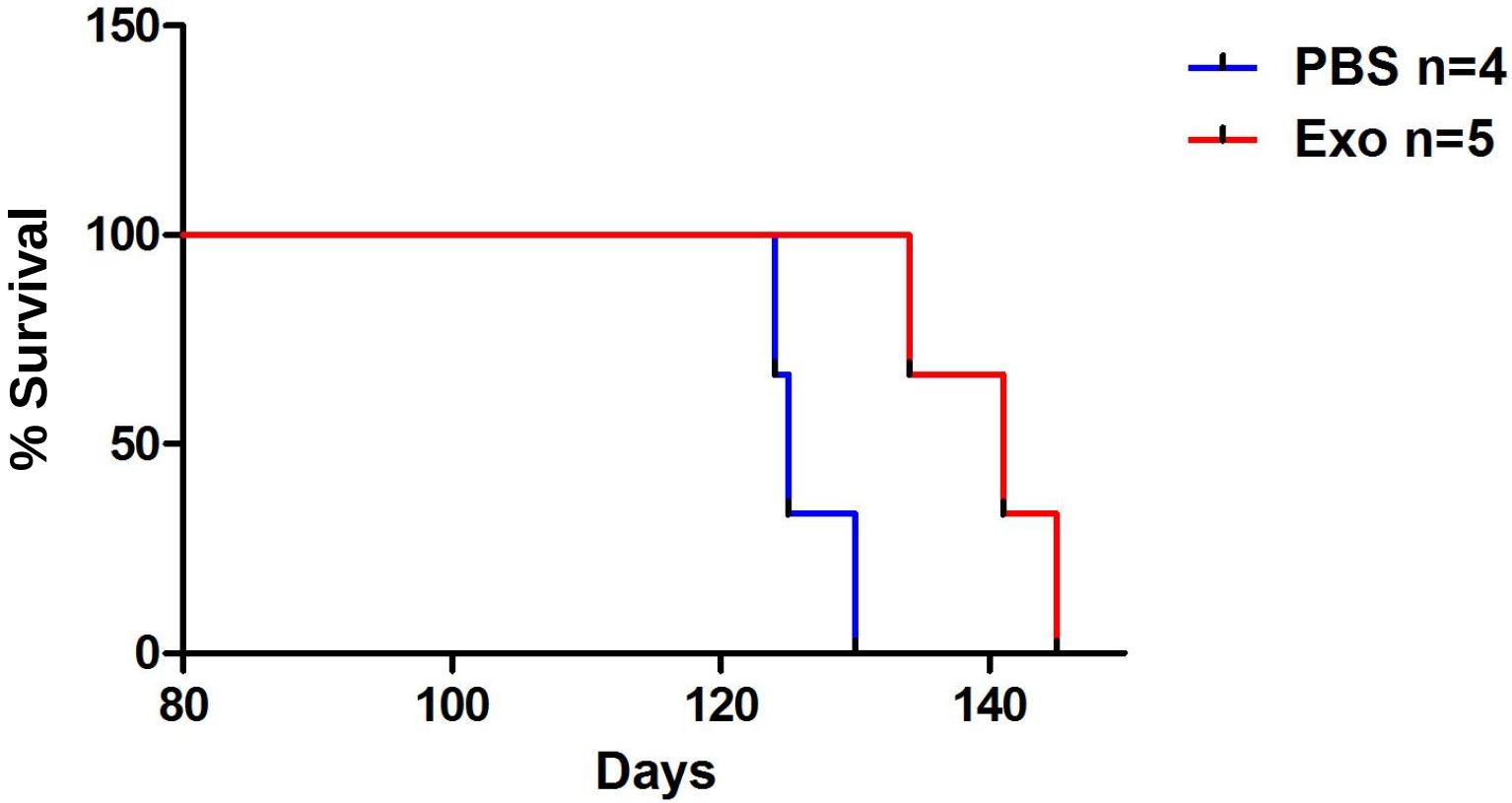




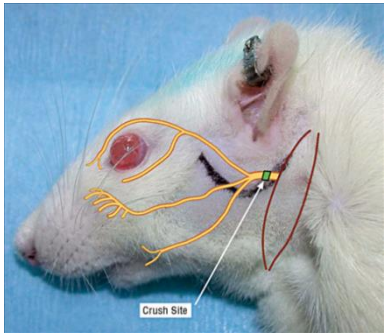
PBS n=4

Exo n=5

Survival



Day 1: **Facial nerve crush**



Day 2-6:

ASC-Exo (treated animals)

PBS (control animals)

Day 14:

Immunohistochemical investigation of the facial motor nucleus

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 22 May 2014
doi: 10.3389/fncel.2014.00117

Microglia centered pathogenesis in ALS: insights in cell interconnectivity

*Dora Brites[1,2] * and Ana R. Vaz[1,2]*

Neurology Research International

Hindawi Publishing Corporation
Neurology Research International
Volume 2011, Article ID 718987, 7 pages
doi:10.1155/2011/718987

Glial Cells in Amyotrophic Lateral Sclerosis

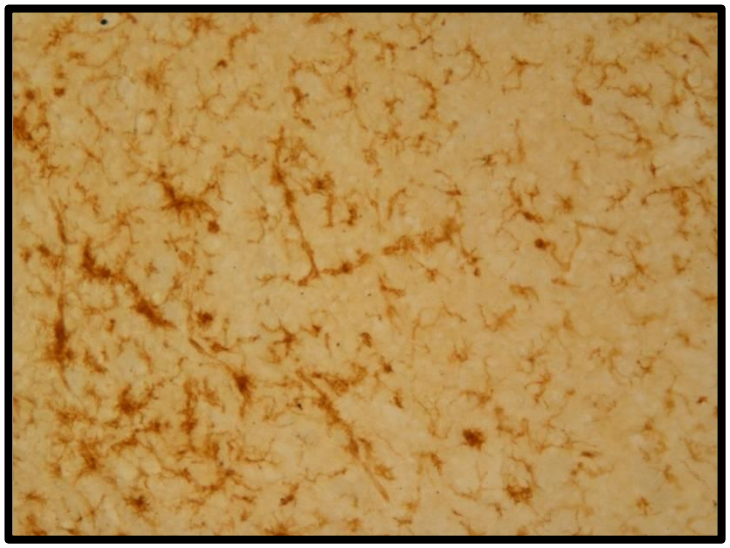
Jurate Lasiene[1] and Koji Yamanaka[1, 2, 3]

NIH Public Access
Author Manuscript
J Neurodegener Regen. Author manuscript; available in PMC 2014 March 24.

Delayed functional recovery in presymptomatic mSOD1^{G93A} mice following facial nerve crush axotomy

Nichole A. Mesnard, PhD[a,b,c,*], Melissa M. Haulcomb, PhD[b,c], Lisa Tanzer, MS[a,b], Virginia M. Sanders, PhD[d], and Kathryn J. Jones, PhD[b,c]

NO CRUSH PBS



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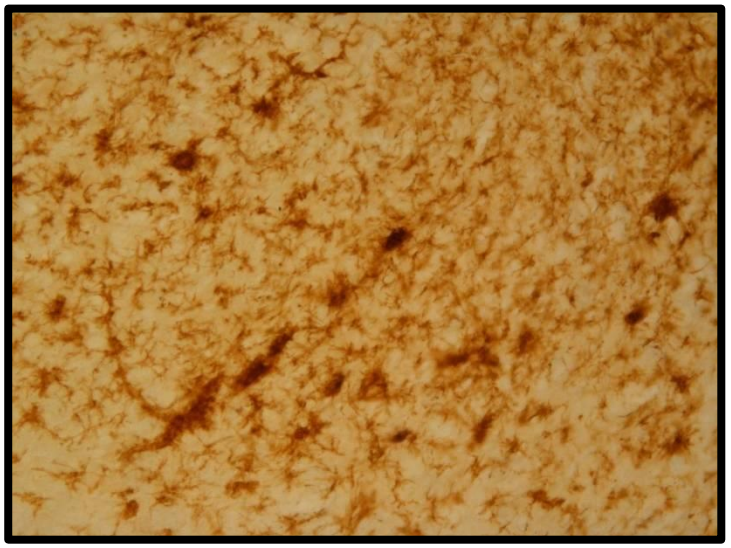

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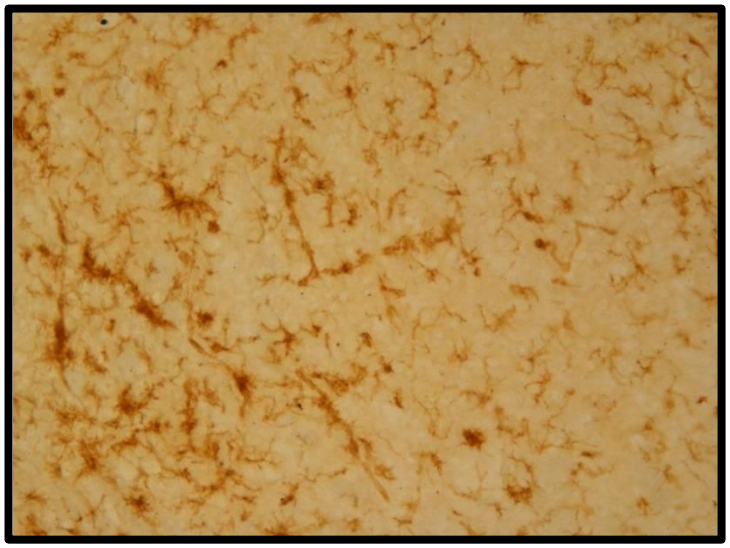

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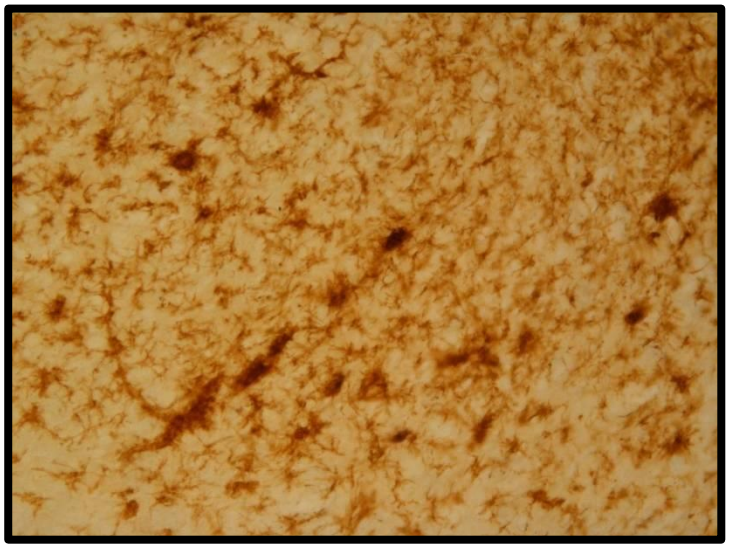

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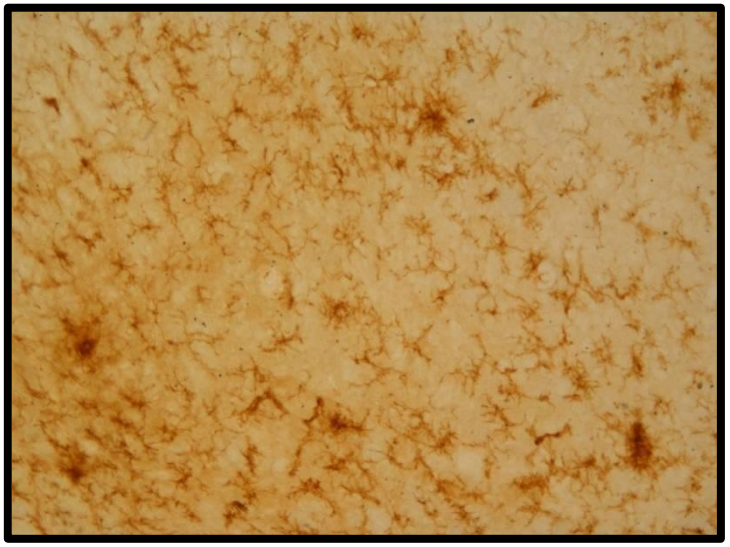
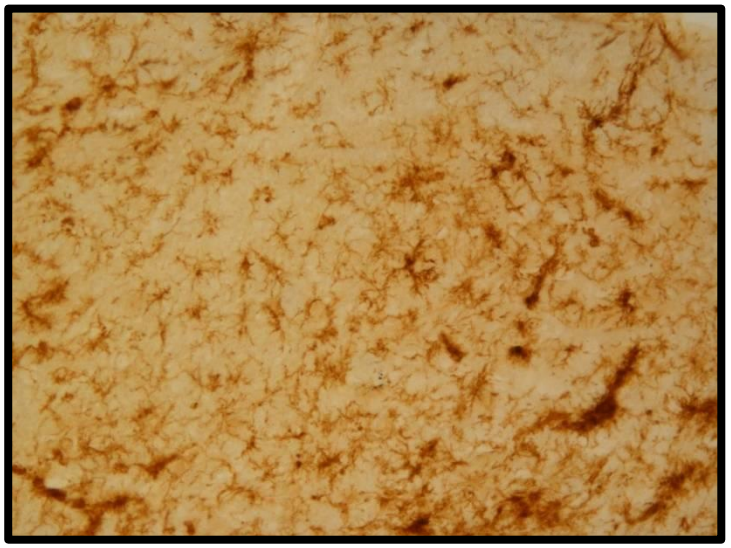
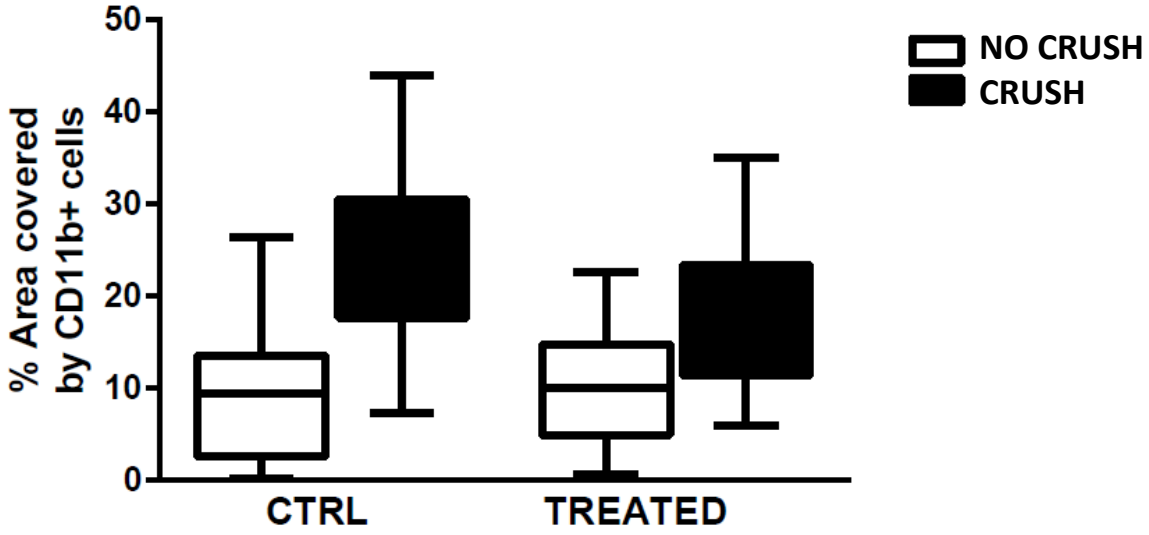


CRUSH EXO



Microglial activation



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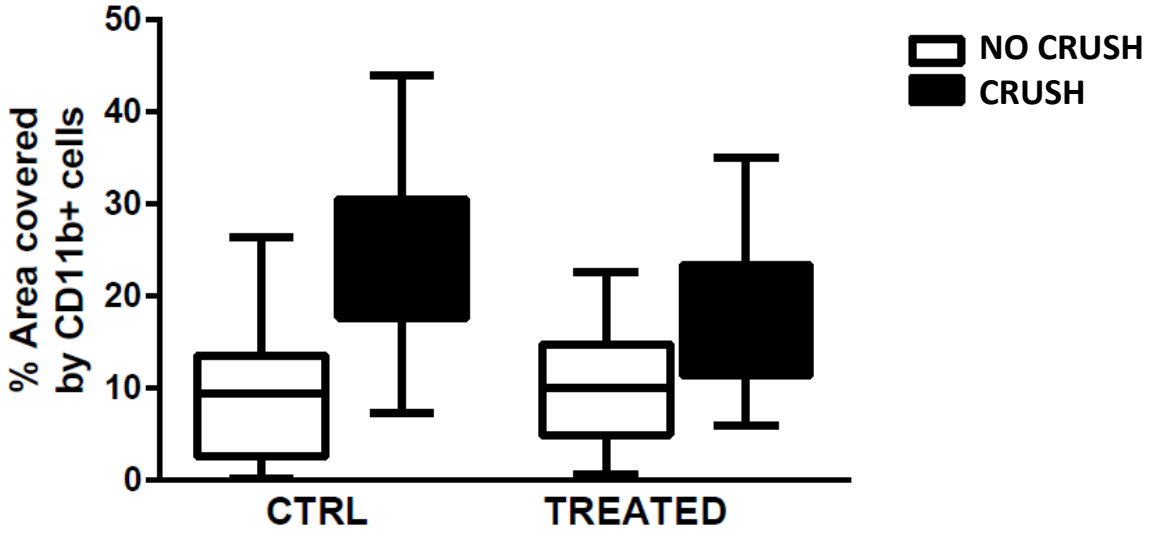
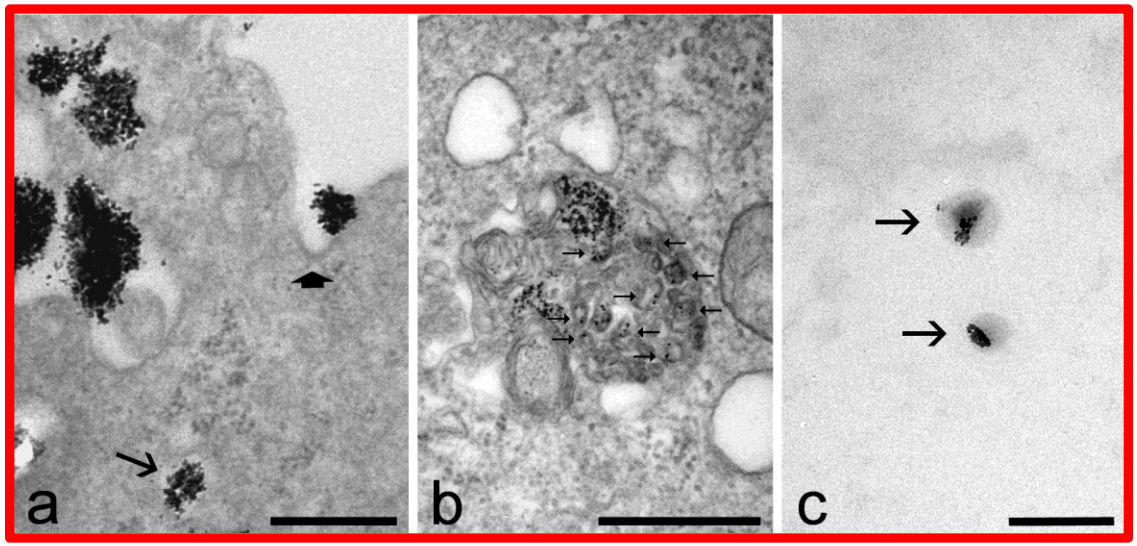


ASC-Exo decrease microglial response in damage area

ASC-Exo labeling protocol

Ultrasmall superparamagnetic iron oxide (USPIO)

- Particle size: 4-6 nm
- 24h cells incubation (200 µg/ml)
- No interference with cell viability
- Nanoparticles internalization (Prussian Blue staining)
- ASC-Exo labeled detected with MRI
- Intracellular nanoparticles internalization (TEM)



Conclusions (1)

- Identified a reproducible protocol for ASC-Exo isolation and characterization
- ASC-Exo have neuroprotective effect *in vitro*
- ASC-Exo have neuroprotective effect *in vivo*
- ASC-Exo decrease microglial response of facial motor nucleus after crush injury *in vivo*

ASC-Exo can represents a promising therapeutic approach in neurodegenerative disorders due to their ability to modulate pathological environments

Conclusions (2)

- Identified a protocol for ASC-Exo labeling with superparamagnetic iron oxide agents (Exo-USPIO)

Inject **labeled ASC-Exo** to verify
their **homing**

Acknowledgements

Dr. Raffaella Mariotti
Dr. Ilaria Scambi
Dr. Lorenzo Schiaffino
Dr. Alice Busato
Dr. Pietro Bontempi
Dr. Alessia Farinazzo
Prof. Bruno Bonetti

Department of Neurological and Movement Sciences
University of Verona

Prof. Pasquina Marzola

Department of Computer Science
University of Verona

Dr. Daniele Peroni
Prof. Alessandro Quattrone

CIBIO Centre for Integrative Biology
University of Trento