



A gene therapy to slow down the progression of Amyotrophic Lateral Sclerosis

based on CRISPR/SaCas9 technology.

Giovanna Di Ruocco, Lorenza Mautone Mock project

Gene therapy and genome editing course, prof. ssa Isabella Saggio Tutor: dott. ssa Mattia La Torre

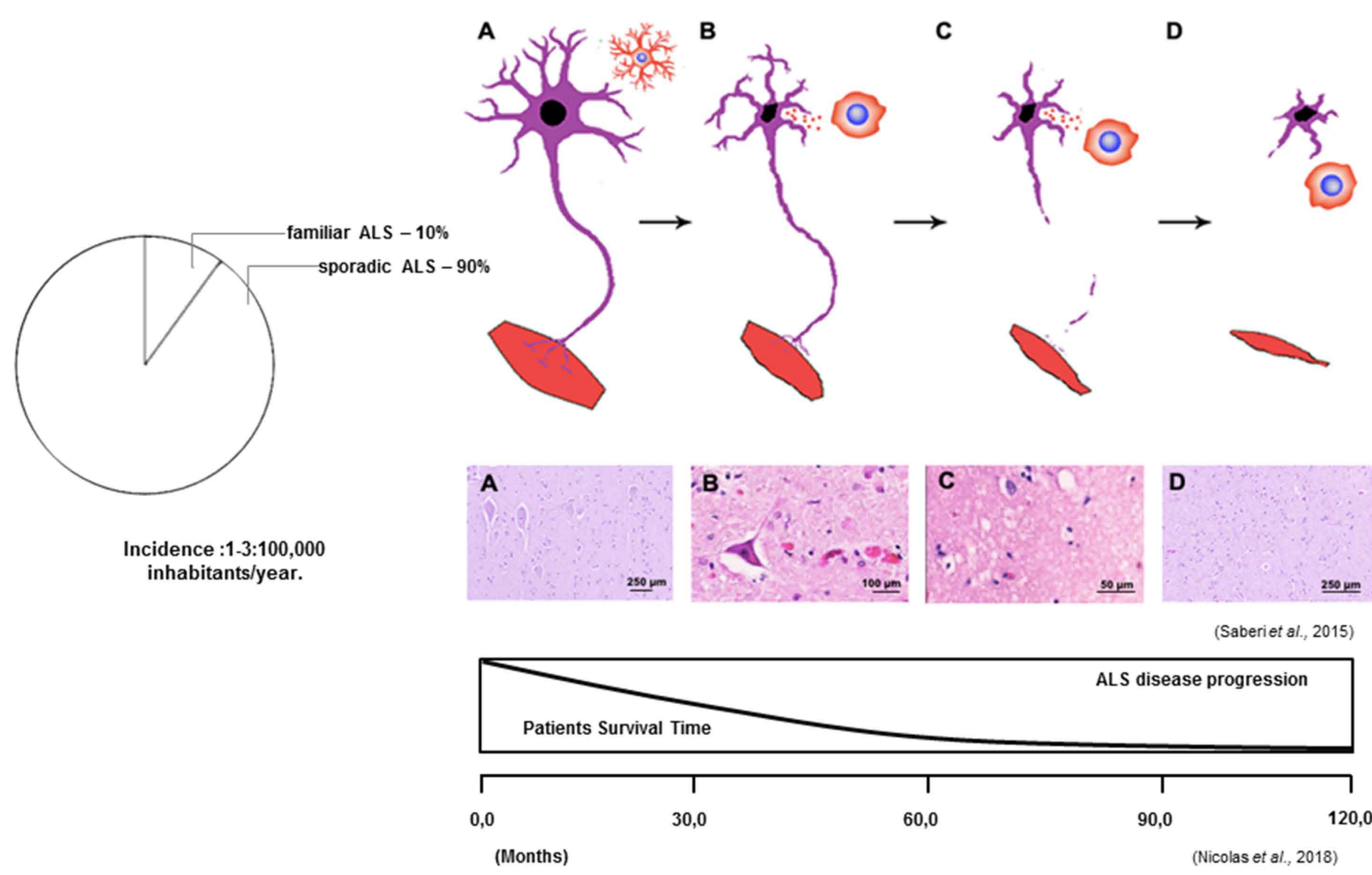
Master Stem cells and genome editing (u-stem) In memoriam of Paolo Bianco

Interfacoltà Scienze MM.FF.NN. Farmacia e Medicina

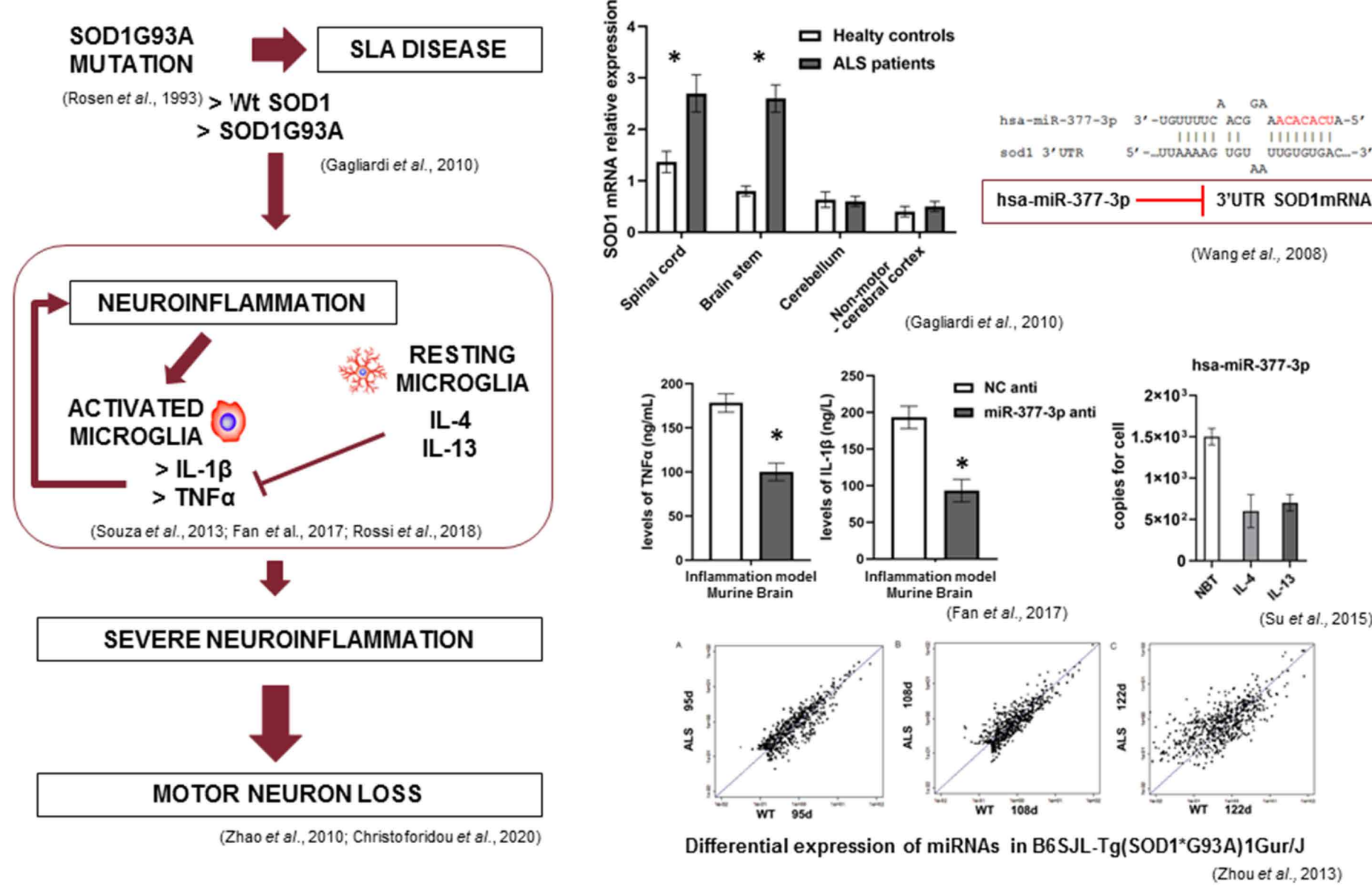
Anno Accademico 2019/2020

Amyotrophic Lateral Sclerosis (ALS)

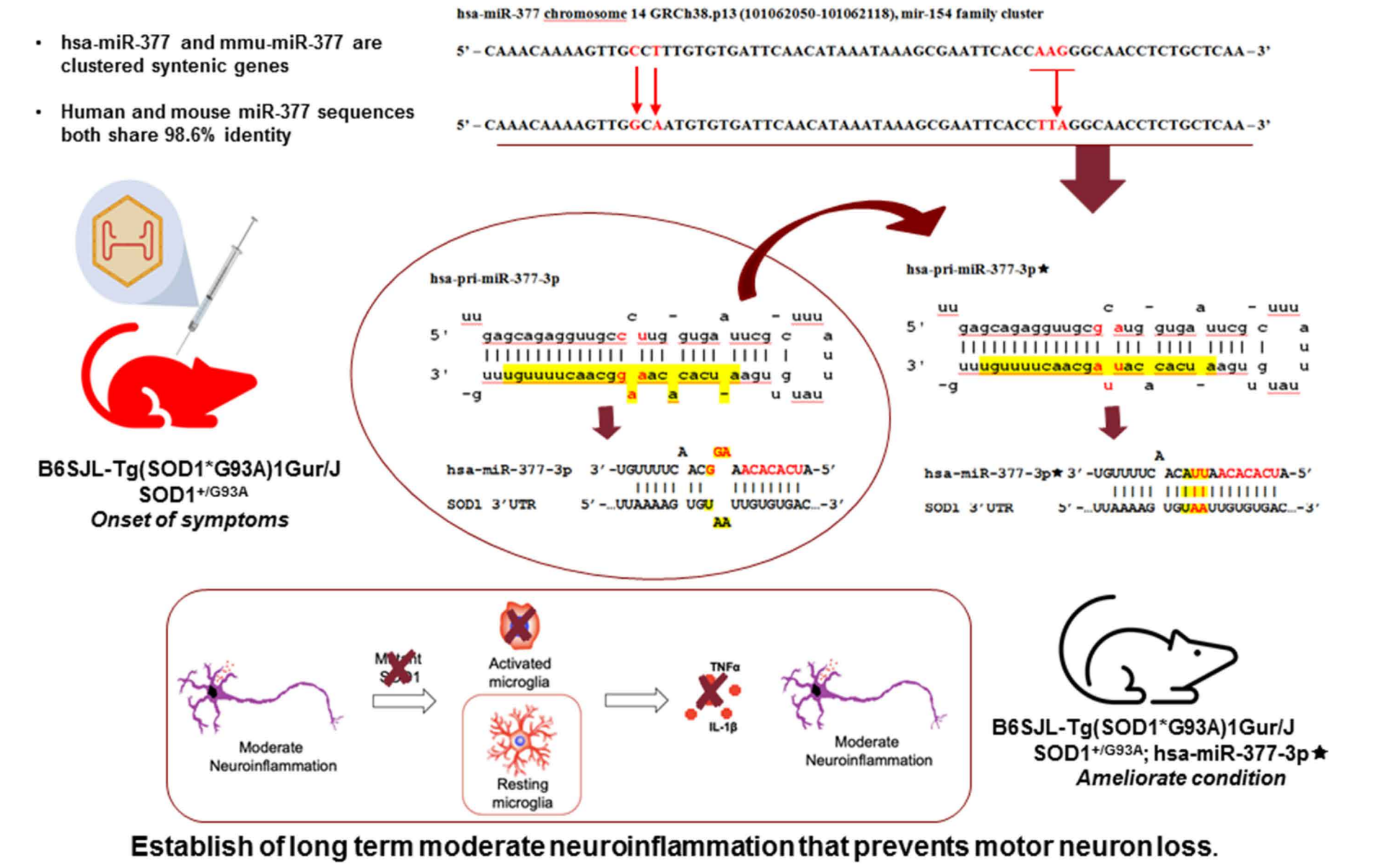
A neurodegenerative multifactorial disorder



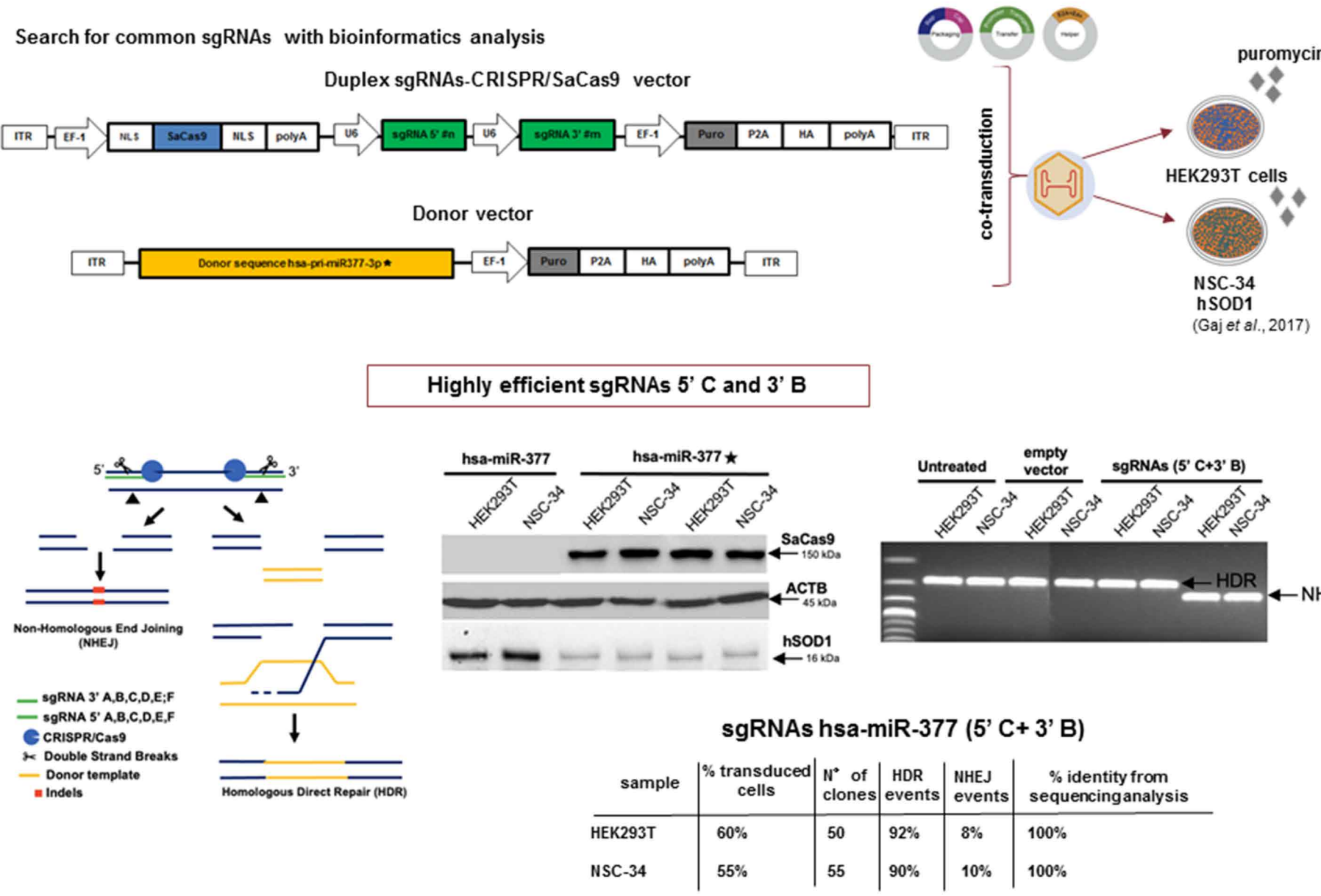
Microglia activation is a commonly pathological hallmark of neurodegenerative diseases



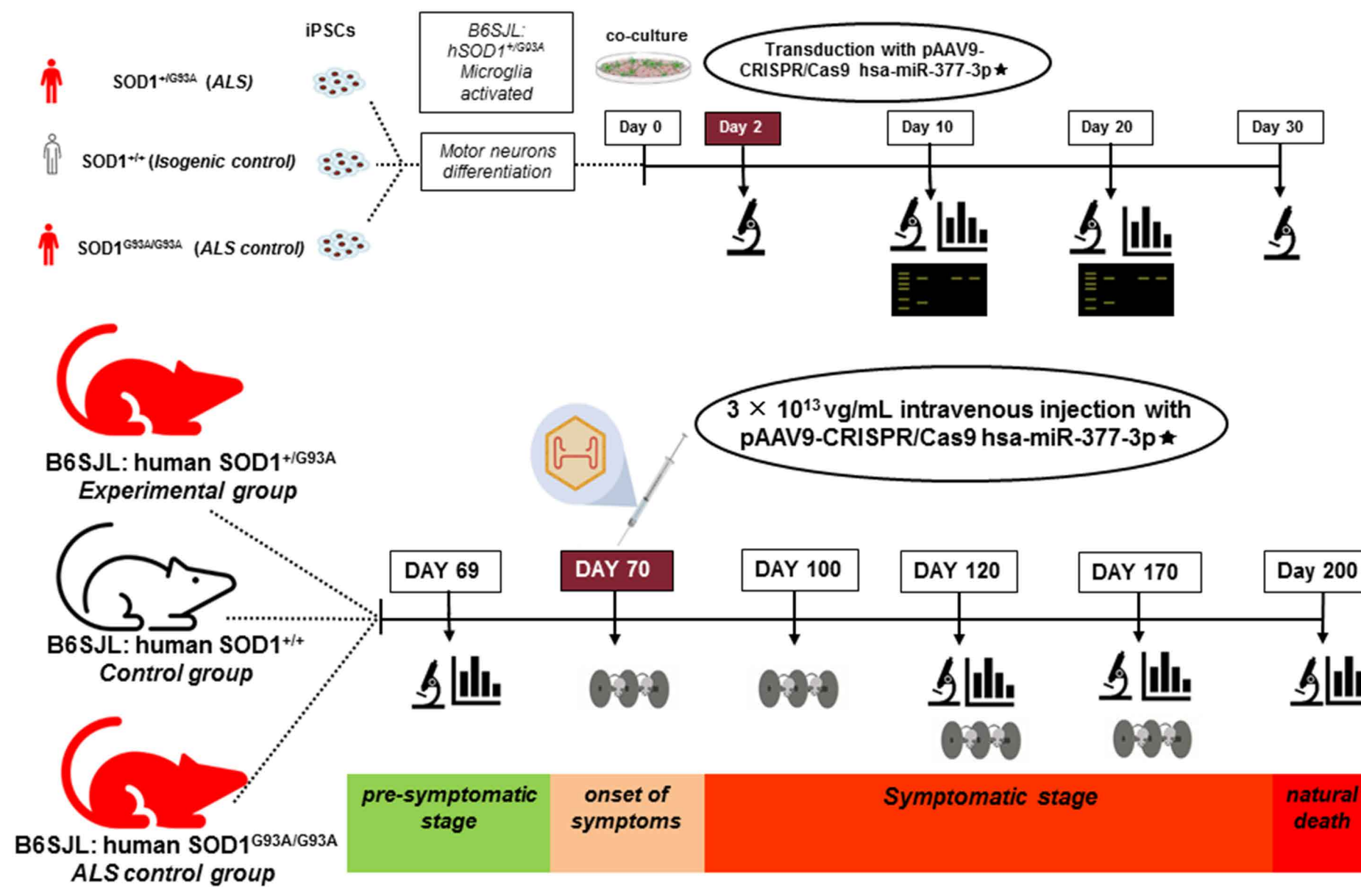
Aim of the project: slow down the progression of Amyotrophic Lateral Sclerosis preventing motor neurons loss



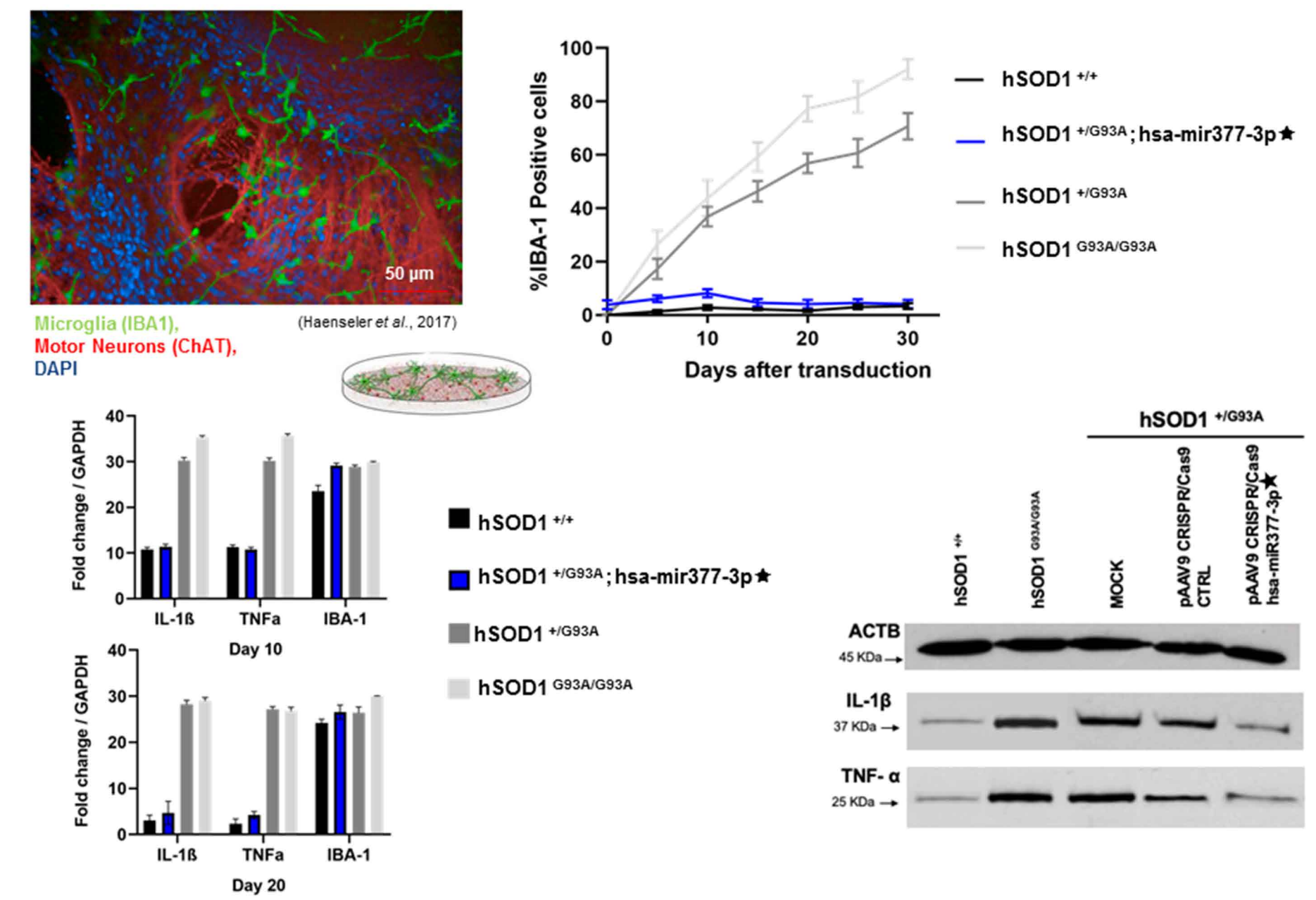
AAV9 CRISPR/SaCas9-based system



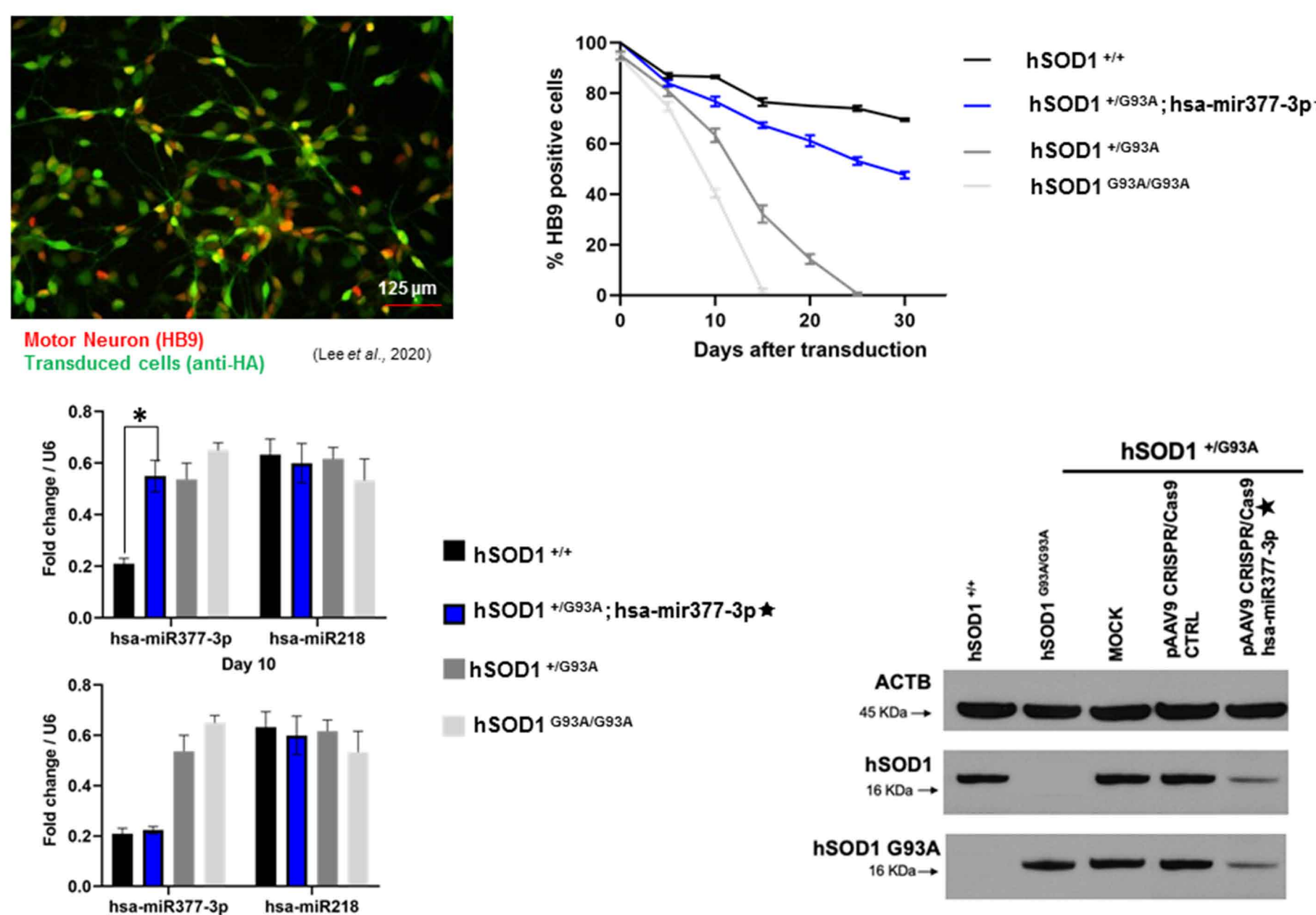
Experimental Plan



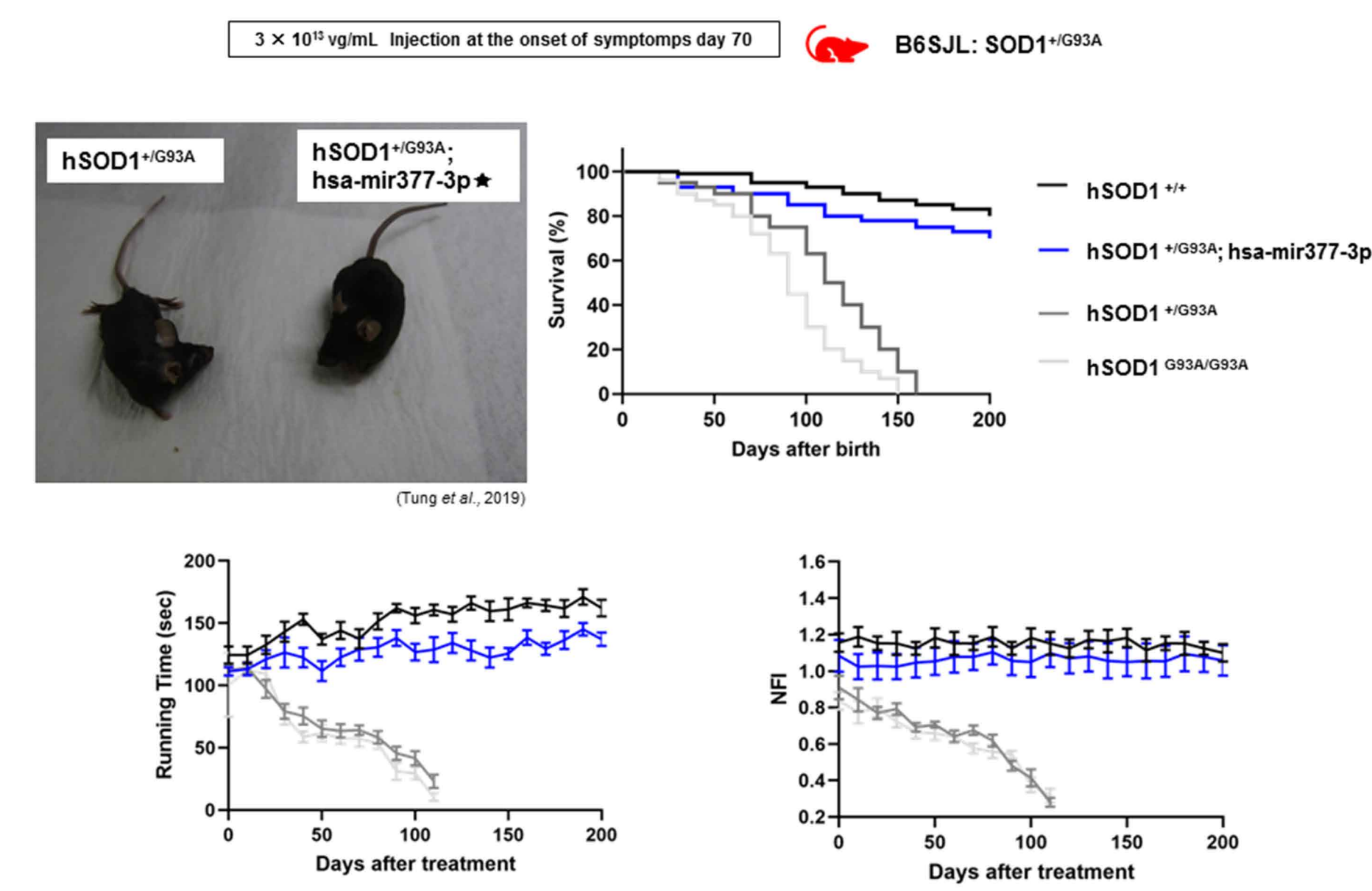
SOD1 downregulation reduces neuroinflammation in vitro



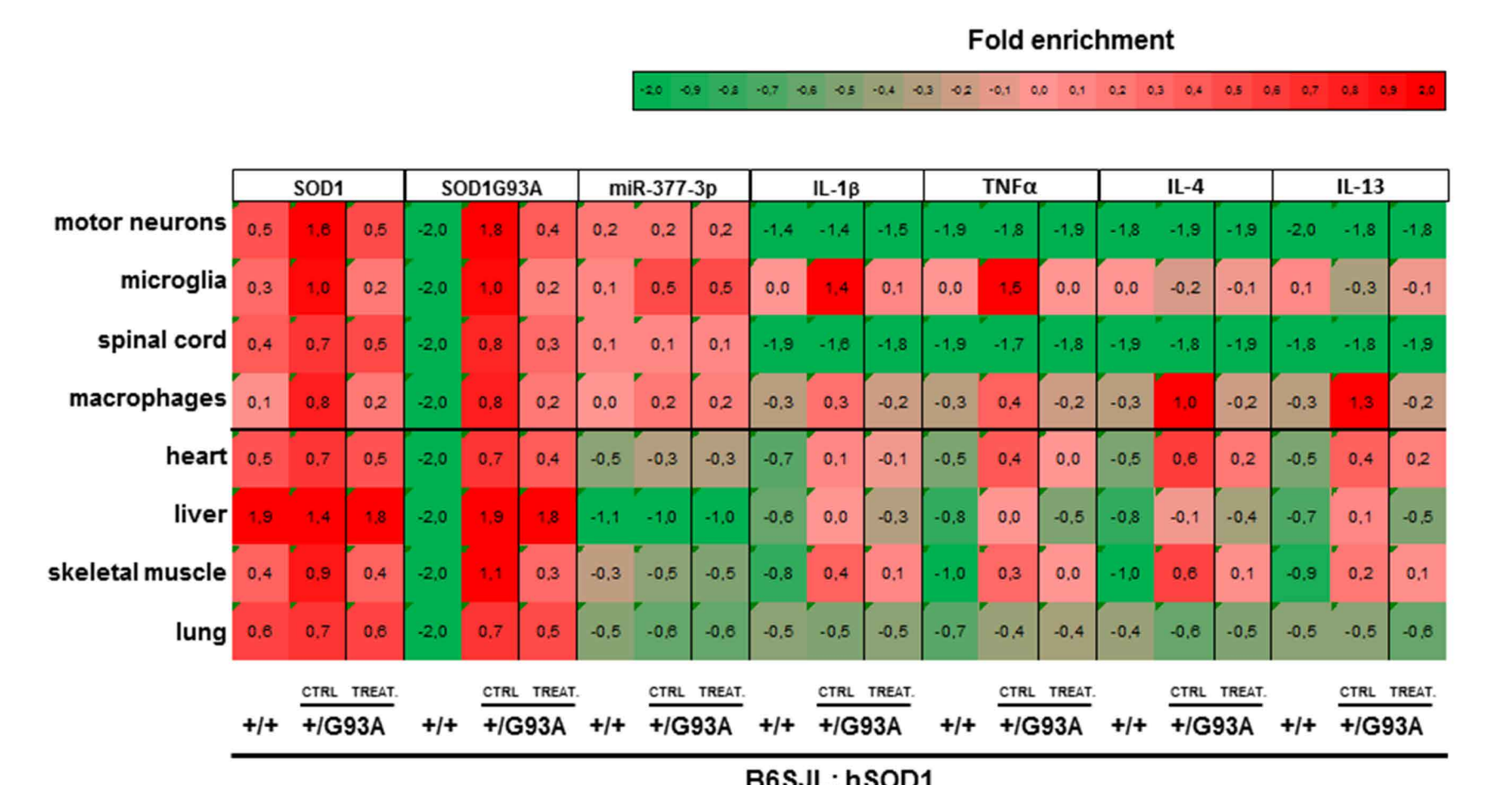
SOD1 downregulation promotes motor neurons lifespan in vitro



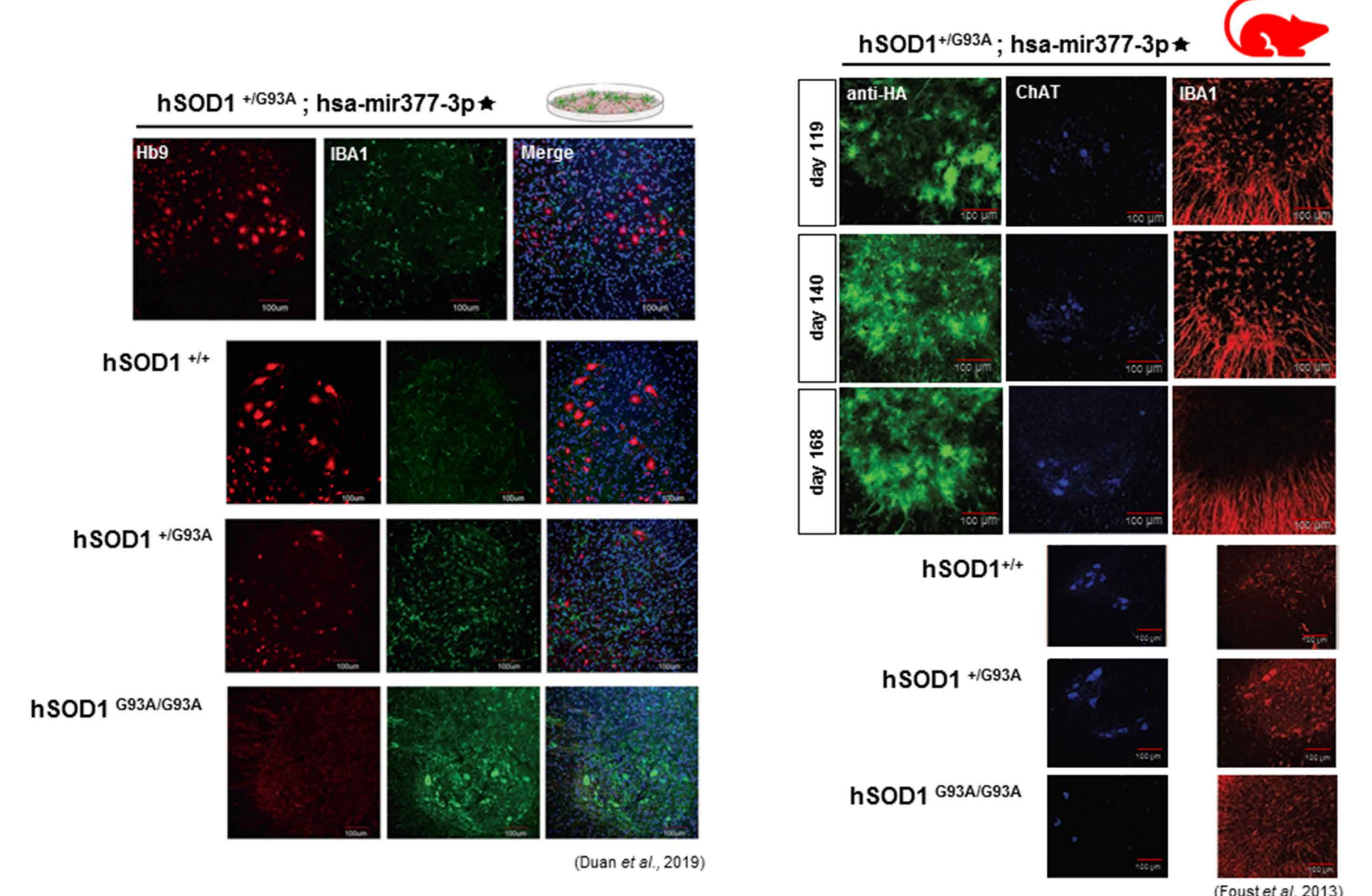
In vivo genome editing provides therapeutic benefit to SOD1^{+/G93A} mice



In vivo genome editing helps to equilibrate inflammation in CNS without altering miRNA pathway in other organs in SOD1^{+/G93A} mice



Our treatment prevents motor neuron loss reducing neuroinflammation in ALS in vitro and in vivo models



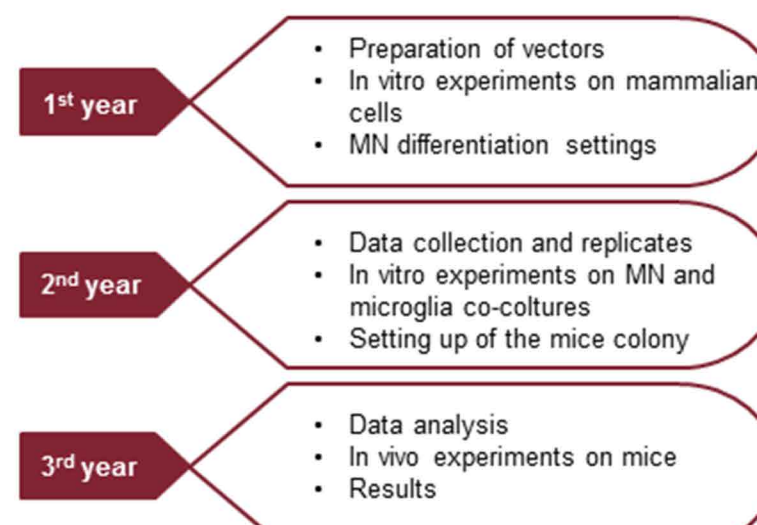
Pitfalls and solutions

- Lipofectamine can be used to improve therapy efficiency.
- Adeno associated viruses (AAVs) cause a very mild immune response, and serotype 9 is specific for the central nervous system. Furthermore, for our therapy, we use specific constitutive promoters specific for the CNS to avoid ectopic disruptions.
- There are bioethical issues linked to irreversible mutations caused by CRISPR/Cas9 technology.
- This gene therapy cures people who are about to die. A single injection acts against the differentiated cells of the CNS, the EF-1 promoter is silenced in the stem cells, allowing them to skip the effect of the therapy.
- Sequence mutation of a miRNA could be unhealthy for a patient.
- We induce 5 bp mutations on one pri-miR377 of a cluster to improve the miRNA machinery processing on specific a target, without altering any other process of other organs.

References

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Timeline



Materials and costs

	€
4 weeks B6SJL-Tg(SOD1 ^{G93A})Gur/J female and male (The Jackson Laboratory, Bar Harbor, Maine, Stati Uniti)	540 x 2
4 weeks B6SJL-Tg(SOD1 ^{+/+})Gur/J female and male (The Jackson Laboratory, Bar Harbor, Maine, Stati Uniti)	470 x 2
AAV9 production (Genemedi, Shanghai, China)	3,000
Misfolded SOD1 Mouse Monoclonal Antibody B8H10, MM-0070-P (Medimabs, Montreal (Quebec), Canada)	495 x 3
Antisuperoxide-dismutase 1 antibody, ab52950 (Abcam, Cambridge, Regno Unito)	355 x 3
IBA1 Polyclonal Antibody, PAS-27436 (Invitrogen, Carlsbad, California, Stati Uniti)	410 x 3
Recombinant Anti-Choline Acetyltransferase antibody, ab178850 (Abcam, Cambridge, Regno Unito)	435 x 3
DAPI (Merck KGaA, Darmstadt, Germany)	91 x 3
Anti-HB9/HLB9MNX1 antibody, ab221884 (Abcam, Cambridge, Regno Unito)	337 x 3
Anti-HA tag antibody, ab18181 (Abcam, Cambridge, Regno Unito)	430 x 3
Anti-CRISPR-cas9 antibody, ab203933 (Abcam, Cambridge, Regno Unito)	370 x 3
Recombinant Anti-IL-1 beta antibody, ab216995 (Abcam, Cambridge, Regno Unito)	350 x 3
Recombinant Anti-TNF alpha antibody, ab215188 (Abcam, Cambridge, Regno Unito)	370 x 3
RNA and miRNA extraction's kits from cells and tissue (Norgen, Thorold, ON, Canada)	1,500 x 6
cDNA synthesis kit, 1708891 (Biorad, Hercules, California, Stati Uniti)	528 x 6
Realtime kit, 1725121 (Biorad, Hercules, California, Stati Uniti)	293 x 6
Animal Facilities	9,000 x 3y
Lab equipments, reagents and service for Western Blot	2,500 x 3y
Lab equipments and service for quantitative Real Time PCR	1,500 x 3y
Lab equipments and service for immunofluorescence	3,000 x 3y
Lab equipments and reagents for cell lines cultures (STEMCELL Technologies, Vancouver, Canada)	5,000 x 3y
Salary for researchers	144,000
FINAL COST:	236,875
(for three years of research)	