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# Bringing Gene therapy based SOD1 silencing towards clinical trials

A highly efficacious, off-target free and  
biomarker supported strategy for familial ALS

Joseph Scarrott



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# Study aims

2

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1. Evaluate the *in vivo* efficacy of SOD1 silencing in the SOD1-G93A mouse model by a clinic ready vector.

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2. Measure CSF SOD1 protein levels as a biomarker of effective dosing and efficacy of SOD1 knockdown.
3. Investigate miRNA-like sequence specific off-target effects.

## RNA interference as a strategy for SOD1-fALS gene therapy.

Foust, K.D. et al., 2013. *Molecular Therapy*, 21(12), pp.2148–59.

Stoica, Lorelei et al. *Annals of neurology* 79.4 (2016): 687–700

Miller, T.M. et al., 2013. *The Lancet. Neurology*, 12(5), pp.435–42.

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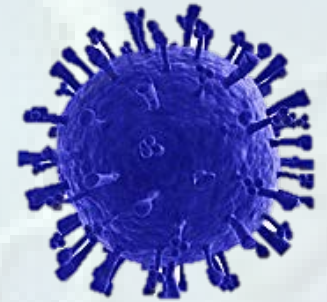
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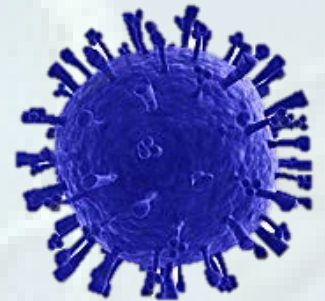
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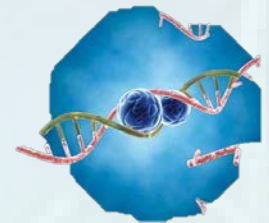
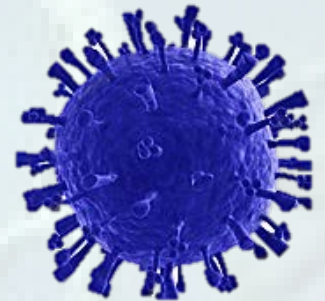
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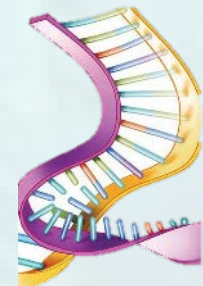
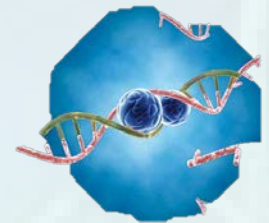
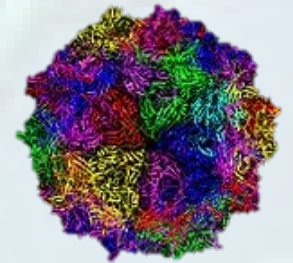
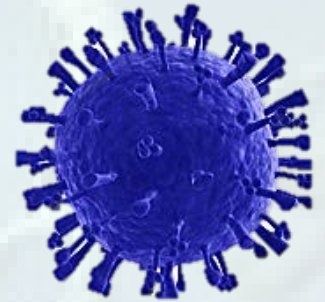
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- Lentivirus delivery of shRNA
- AAV delivery of shRNA
- AAV delivery of artificial miRNA
- Antisense oligonucleotide therapy



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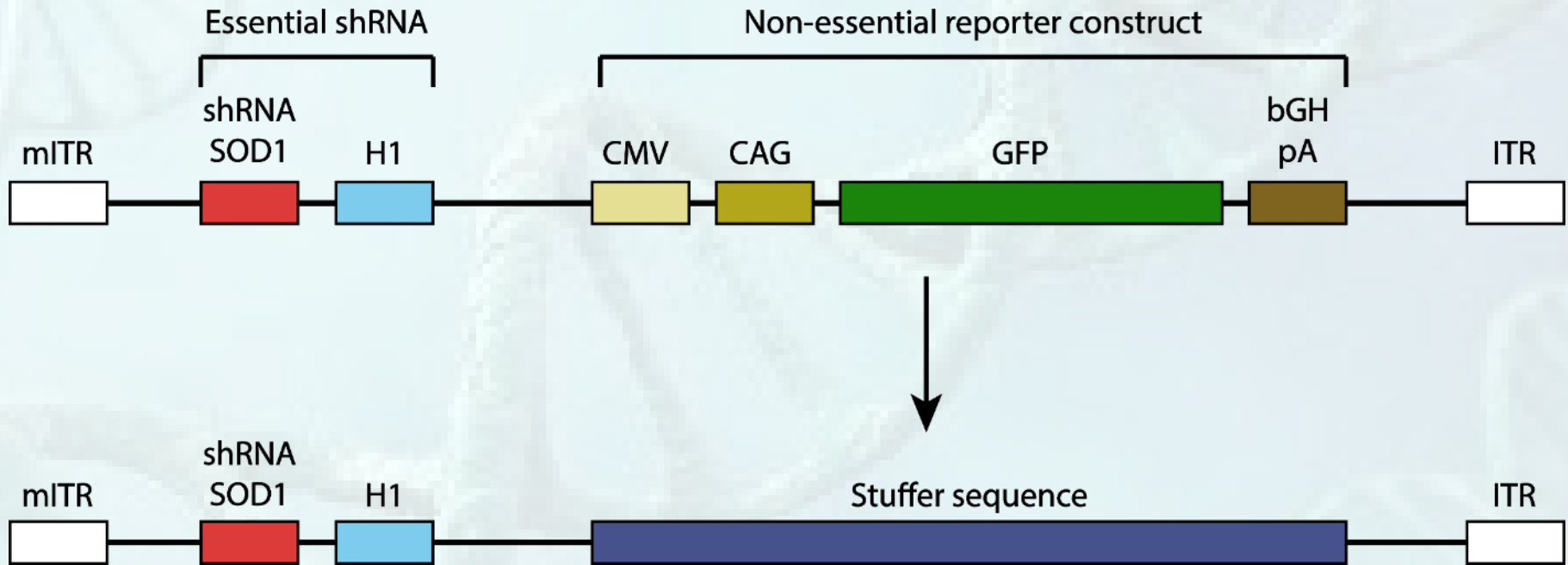
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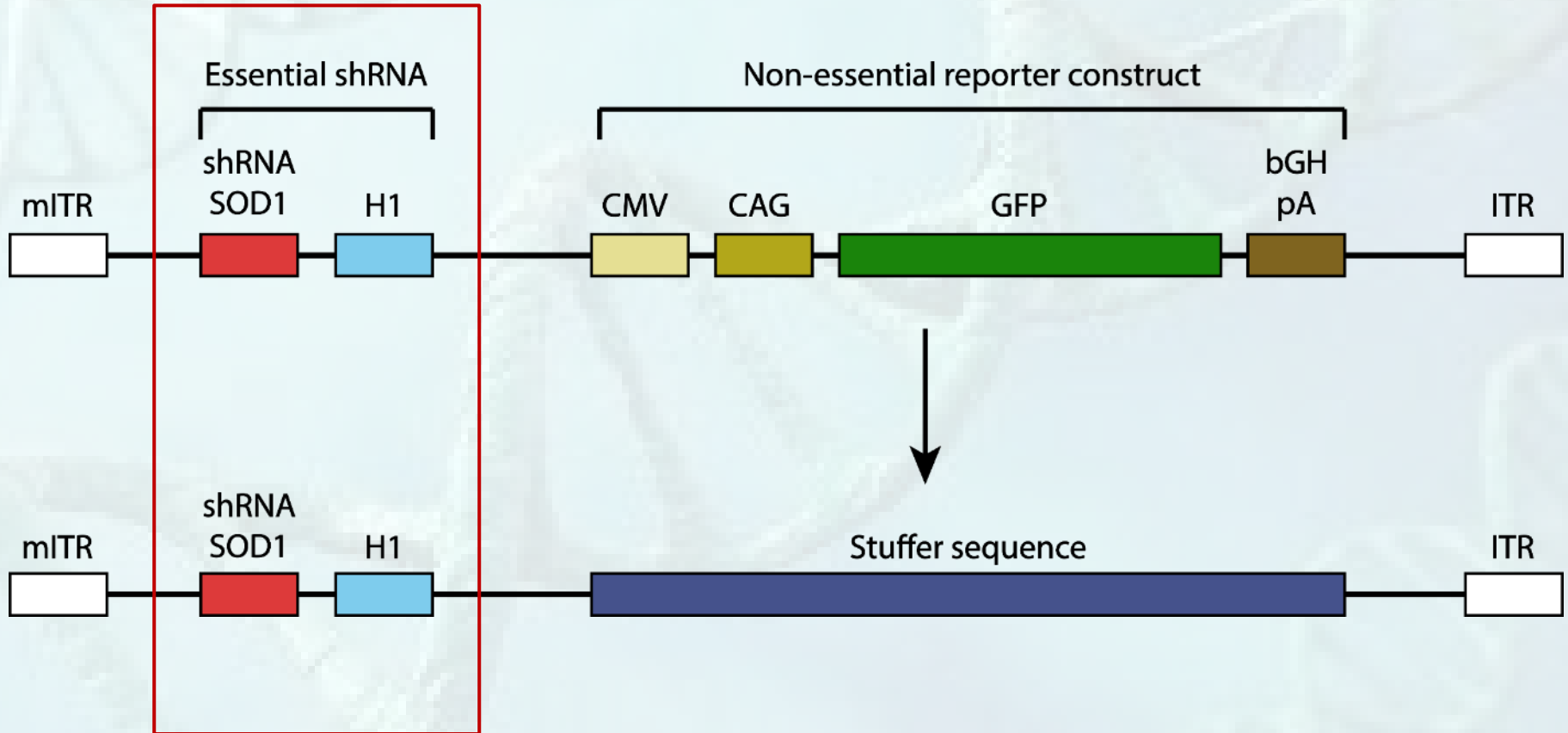


# Clinic-ready vector design





# Clinic-ready vector design





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# *In Vivo* Efficacy

# Route of injection

CB

Ctx

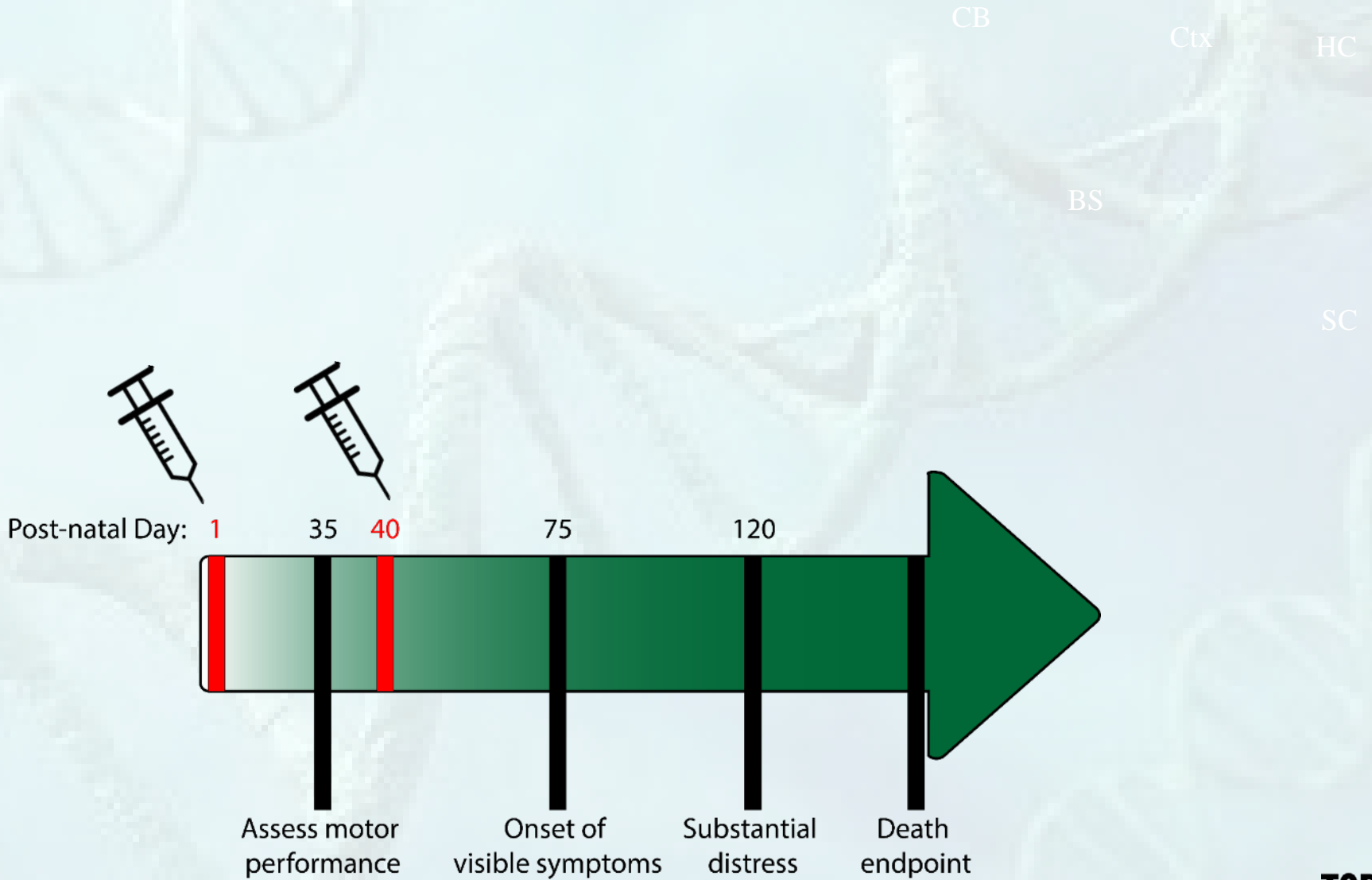
HC

BS

SC



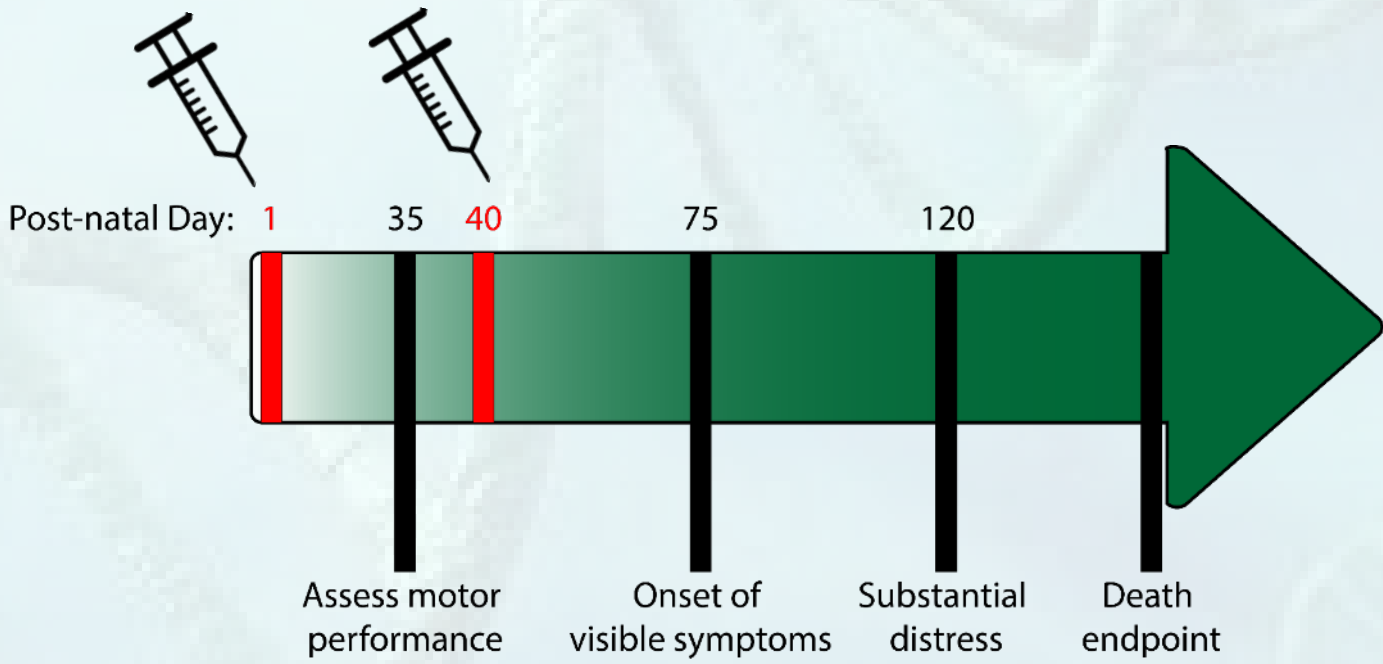
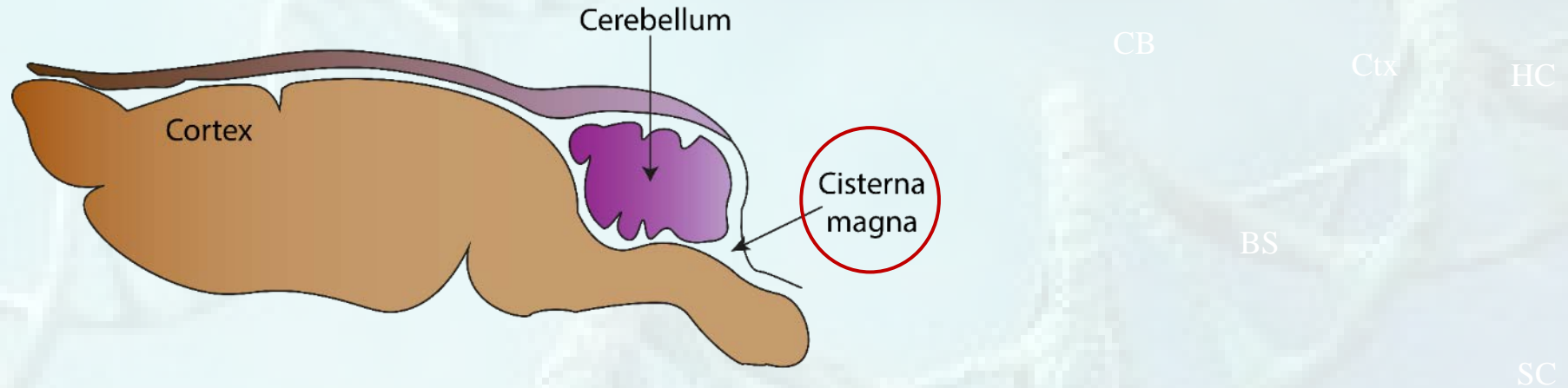
# Route of injection





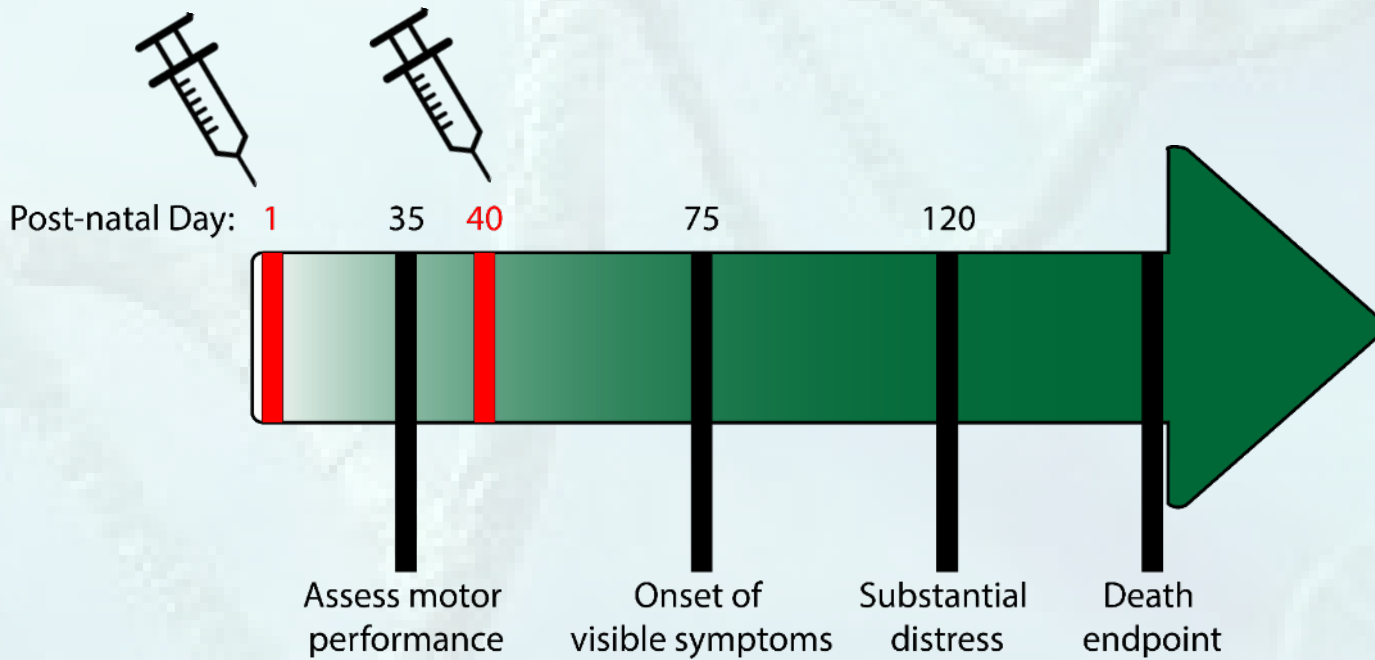
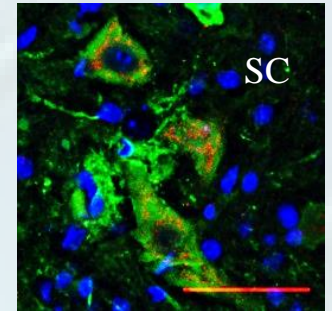
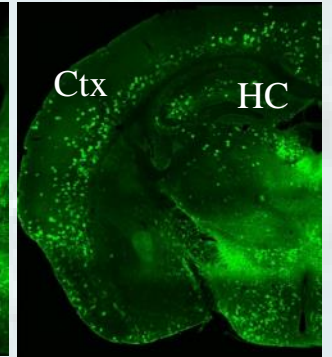
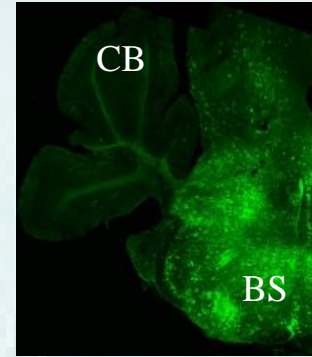
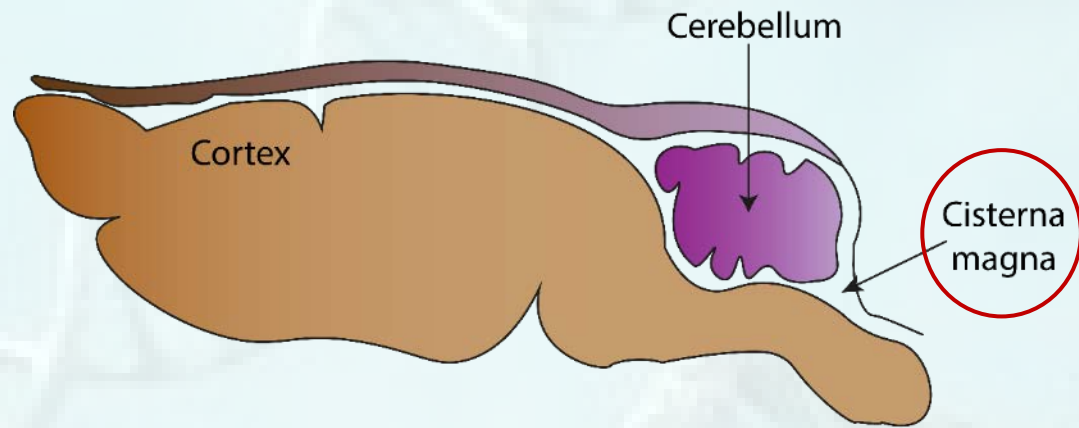


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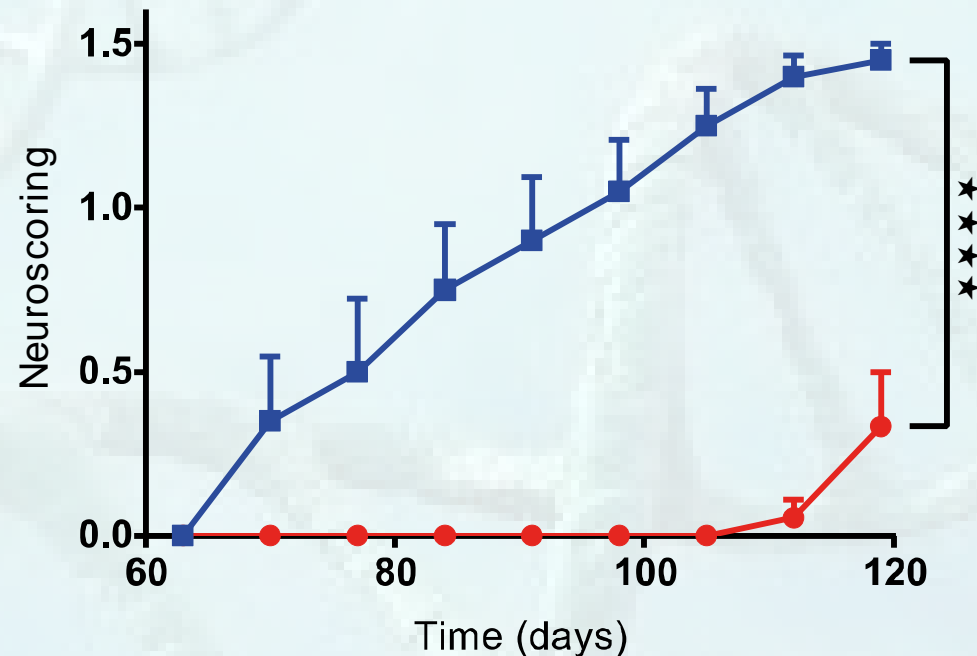


# P1 – neuroscoring and onset

<u>Neuroscoring Assessment</u>	
Onset	<b>0.5</b>
Abnormal gait	<b>1</b>
Severe “waddle”	<b>1.5</b>
Dragging one hind limb	<b>2</b>
Paralysis of one hind limb	<b>3</b>
Moribund	<b>4</b>

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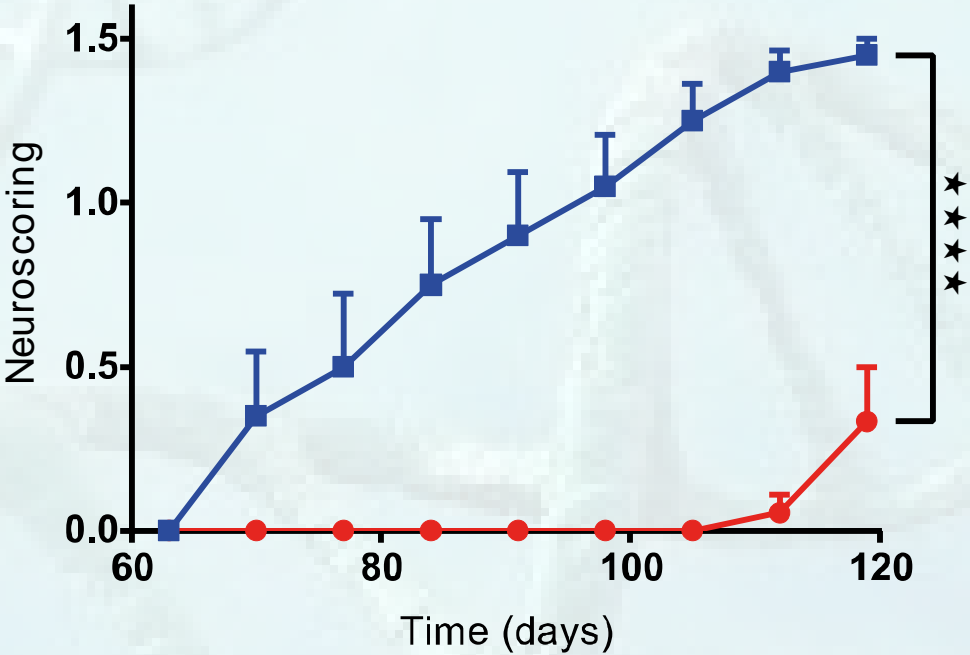
■ scAAV9-hSOD1ssi  
● scAAV9-hSOD1si



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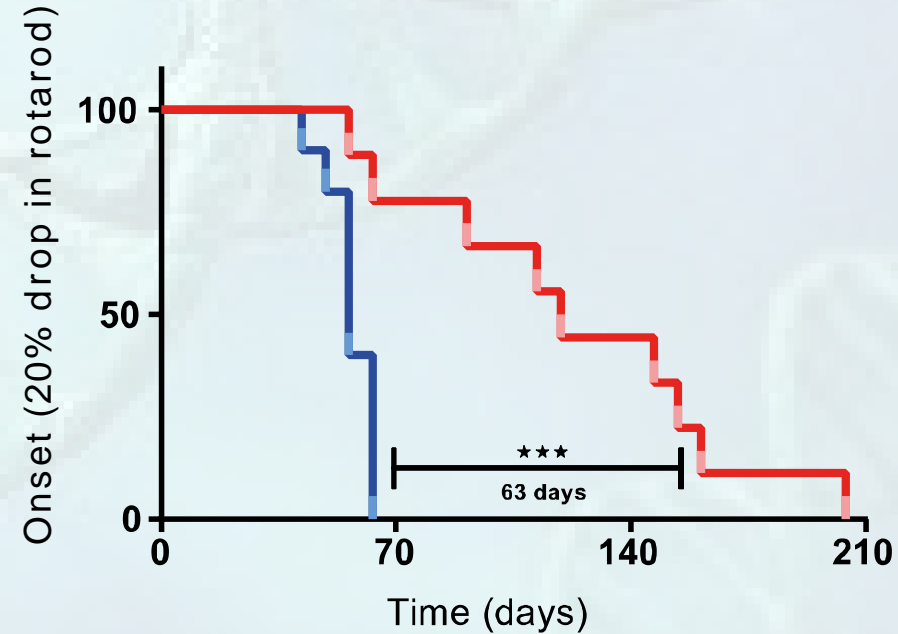
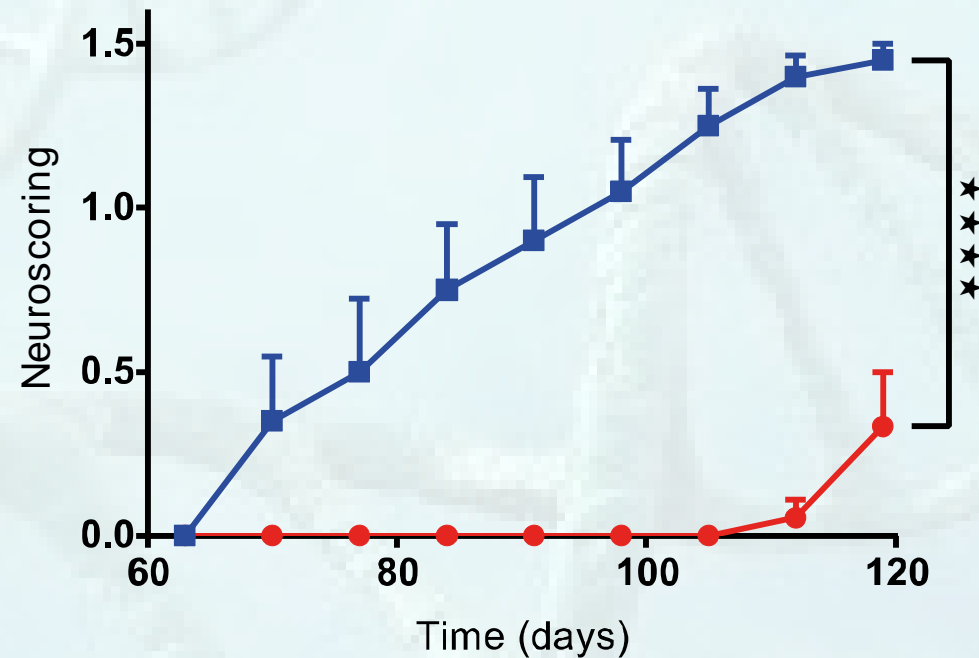
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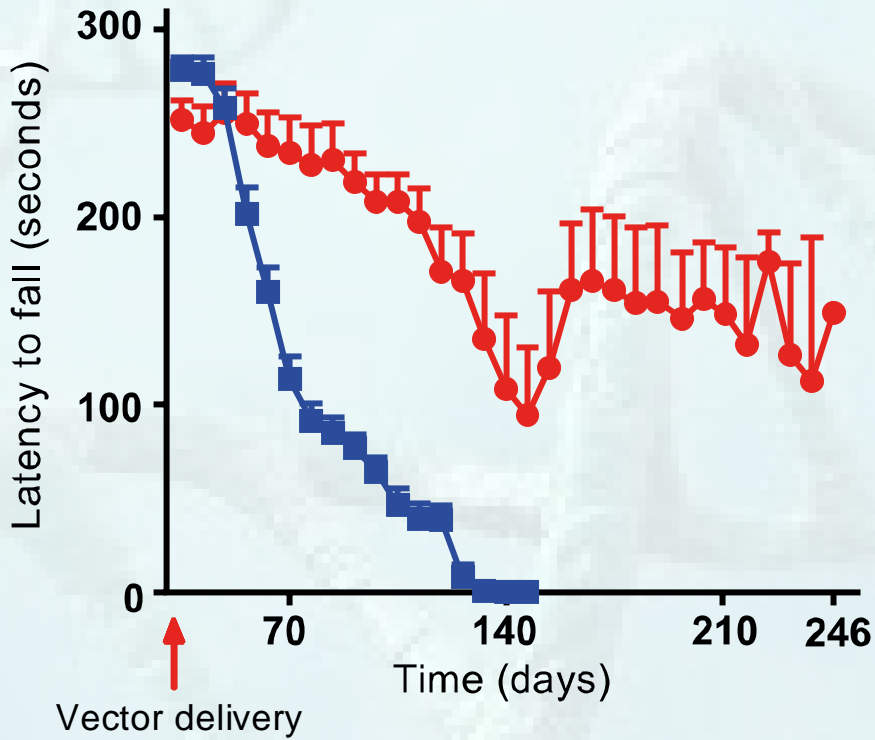


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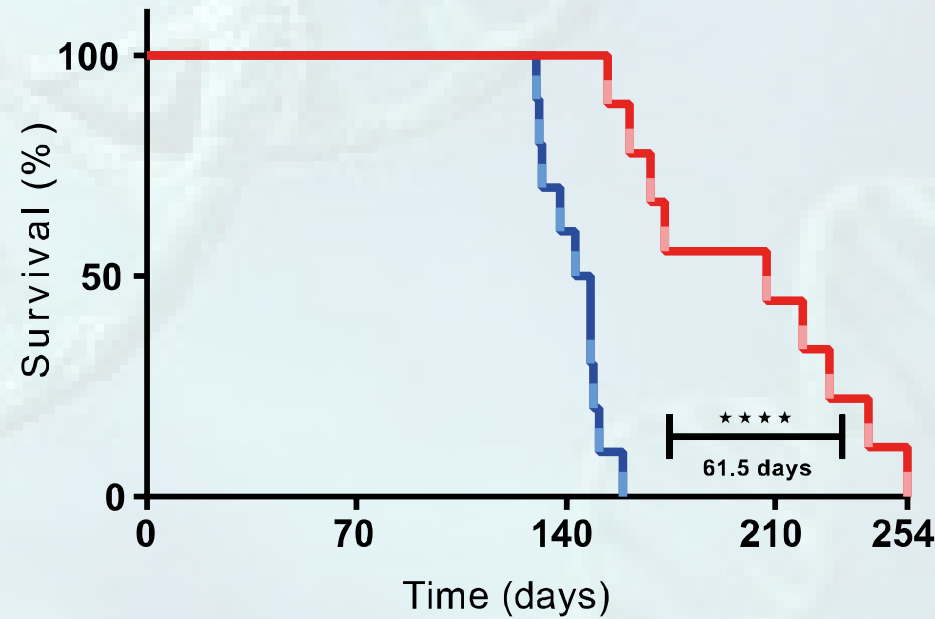
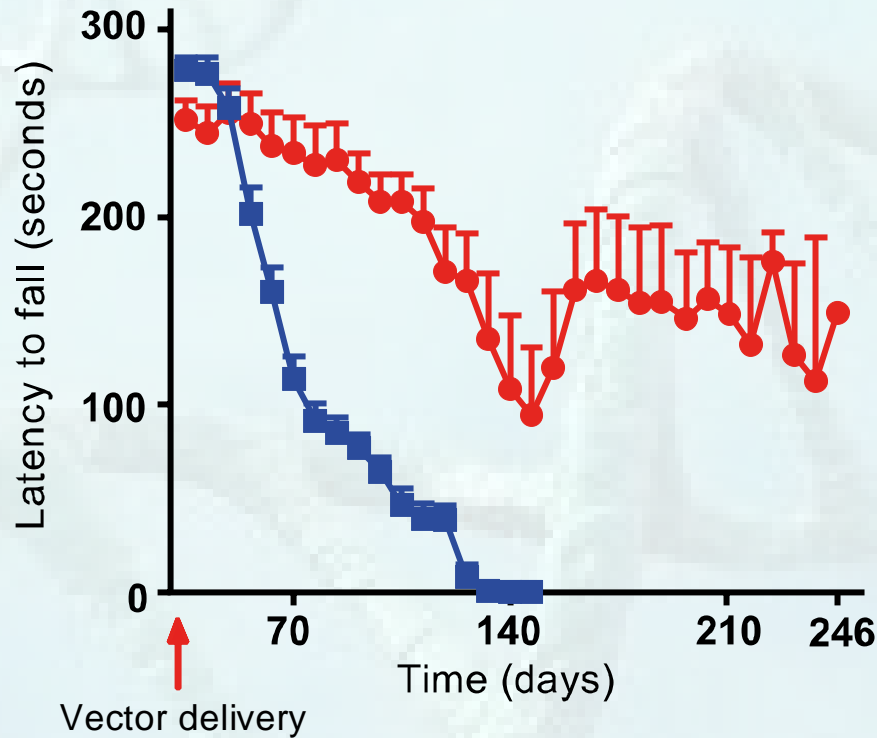




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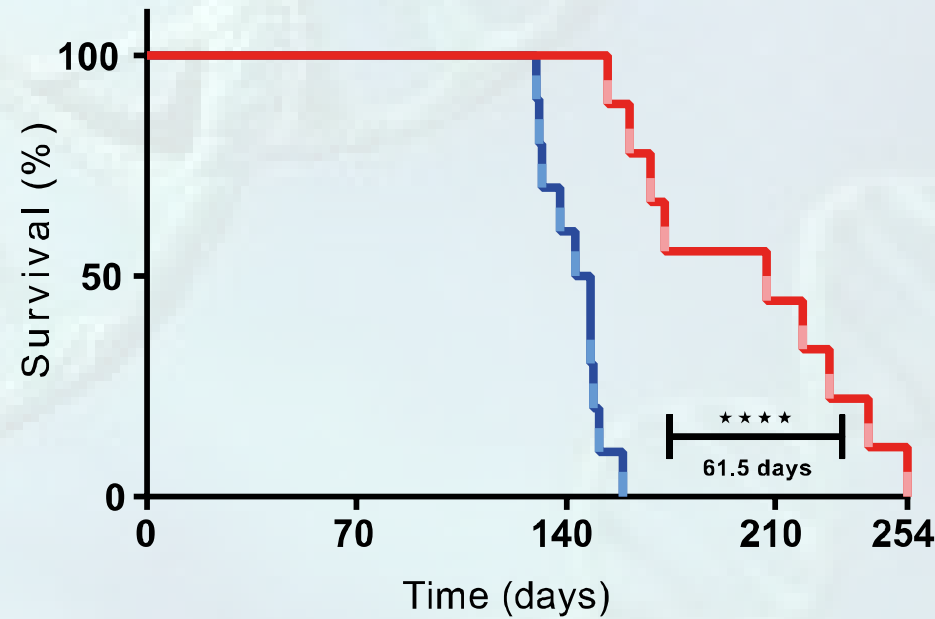
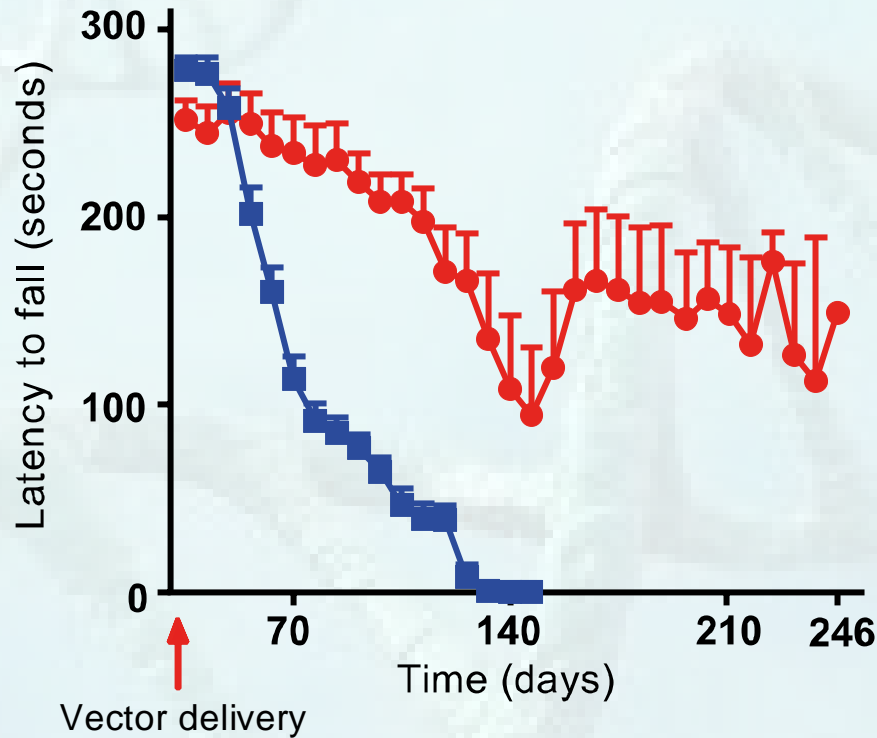




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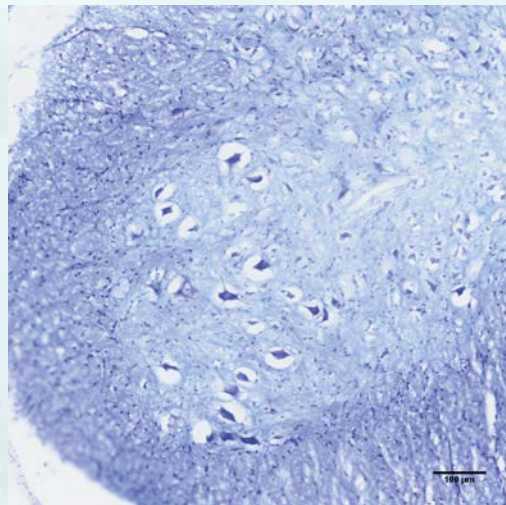
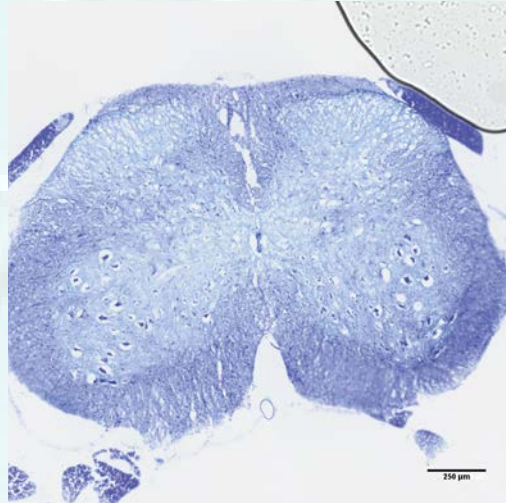
42% increase

# P 1 mice – 144 days old

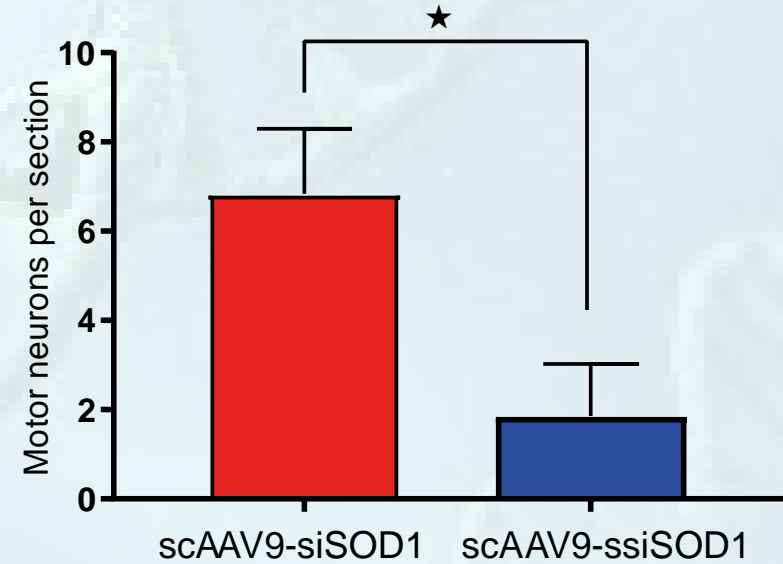
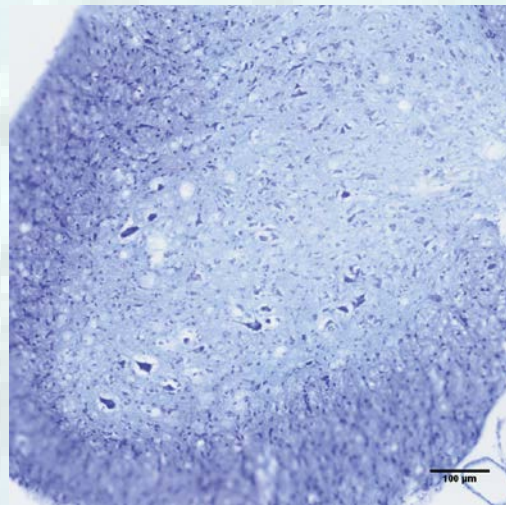
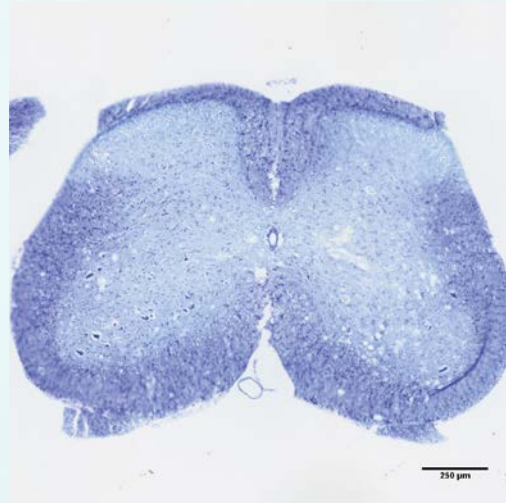


# Treatment rescues motor neurons

shRNA



Control

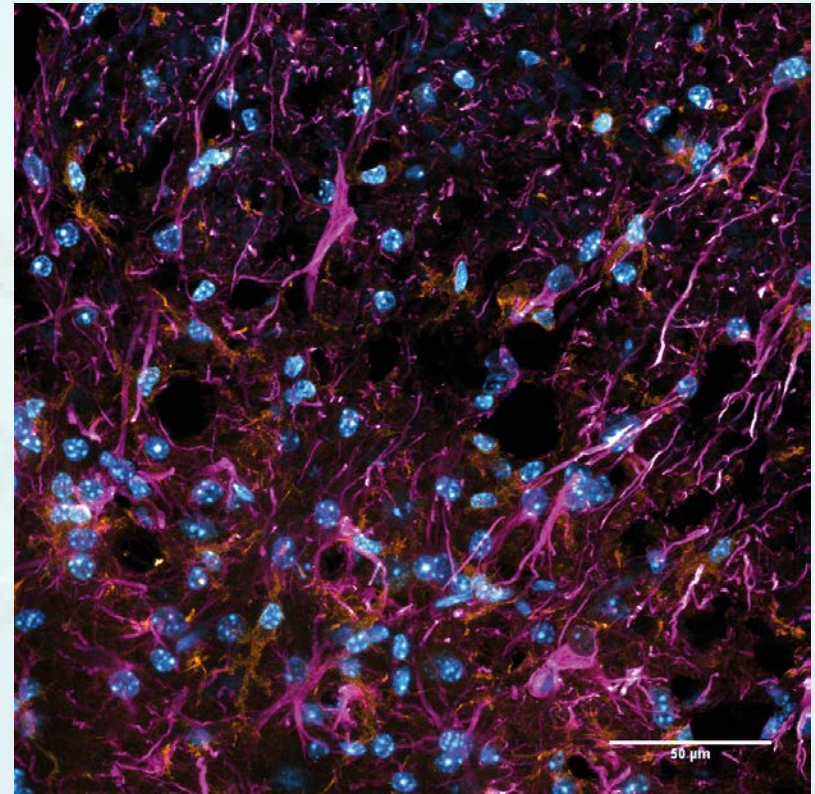
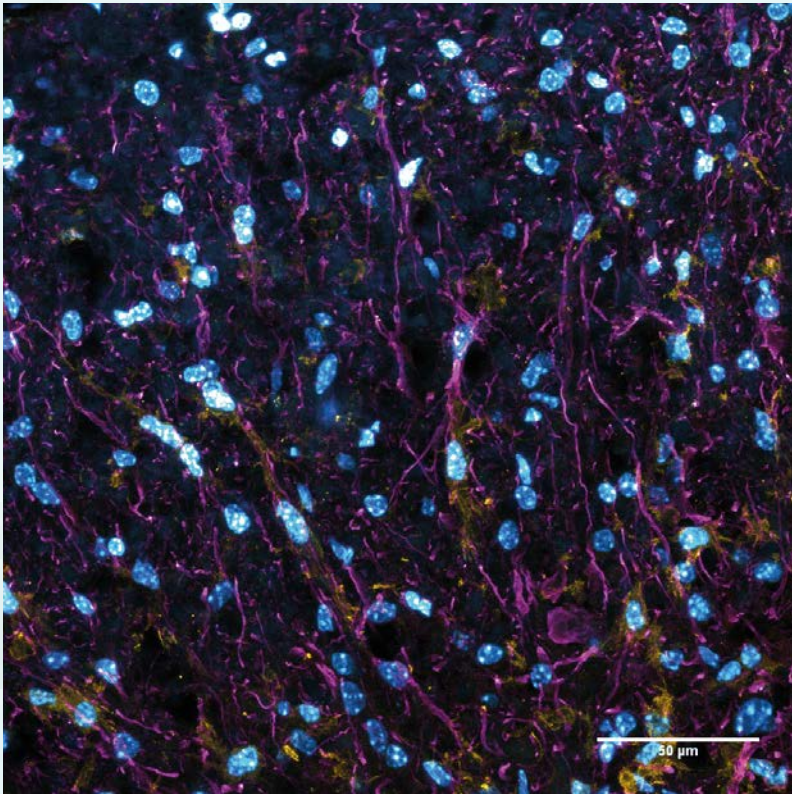




# Treatment reduces reactive gliosis <sup>11</sup>

shRNA

Control



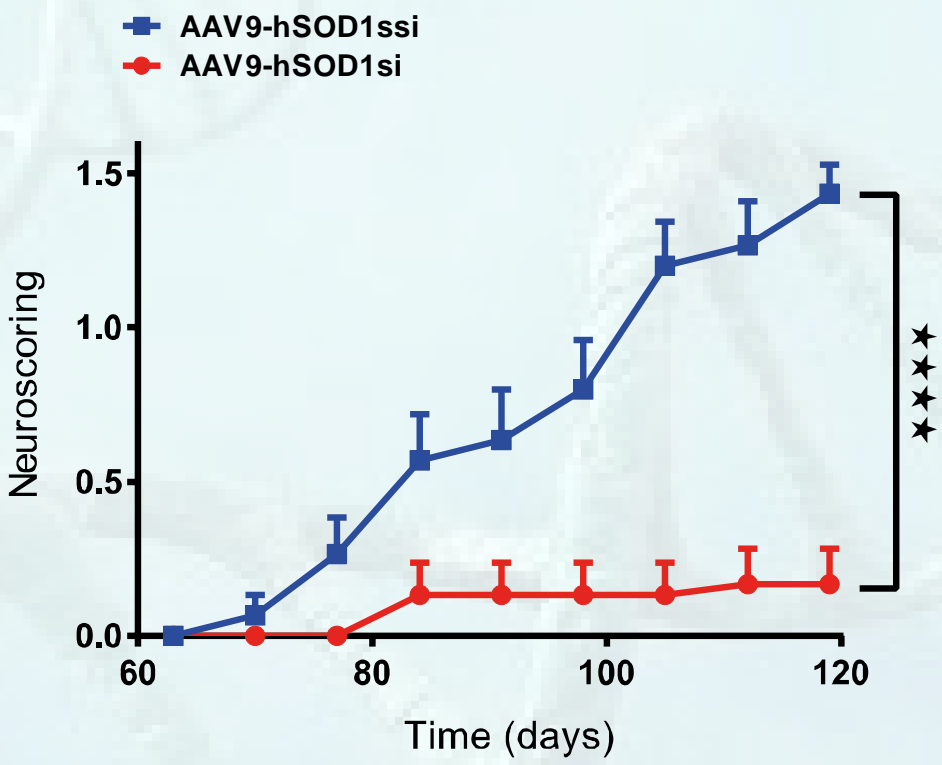
Purple = GFAP

Orange = Iba1

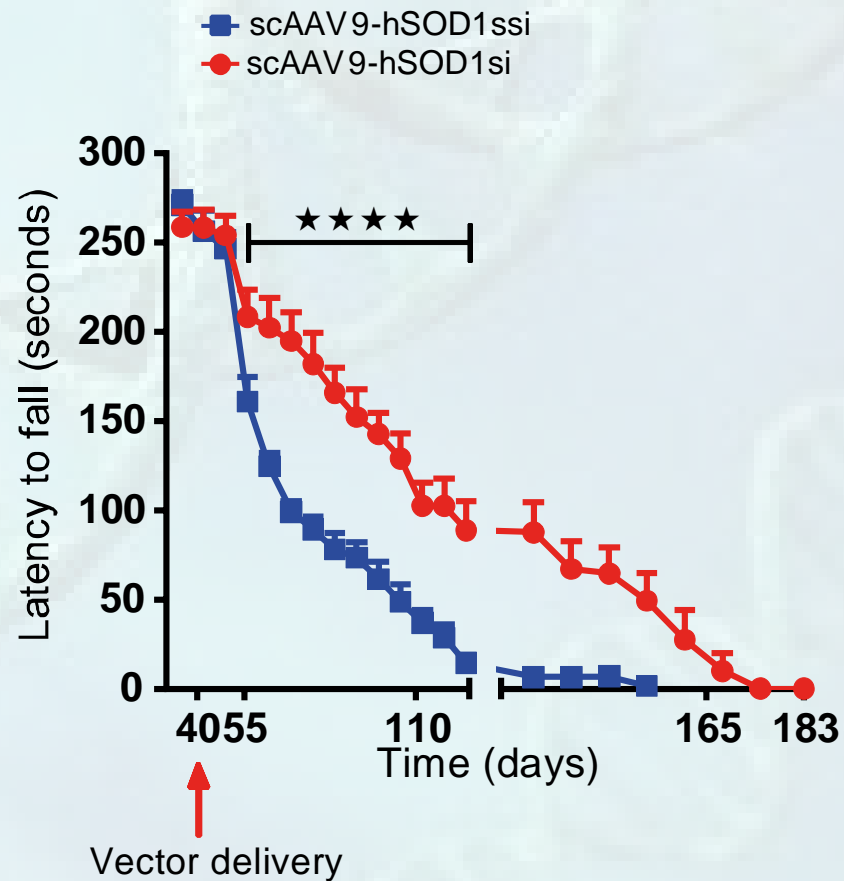
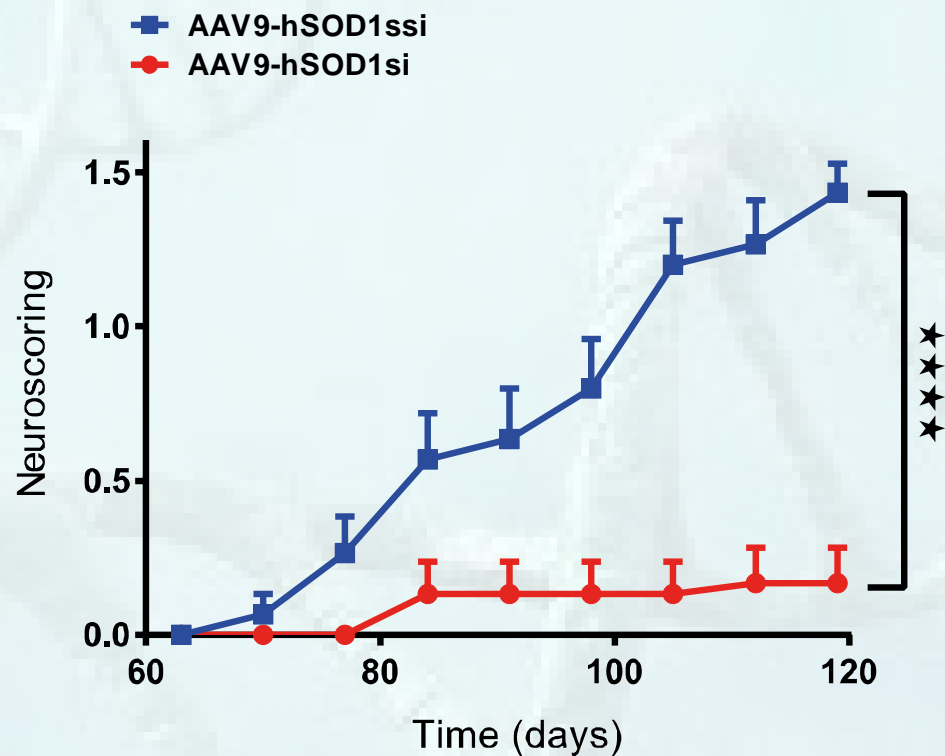


# P40 – neuroscoring and rotarod

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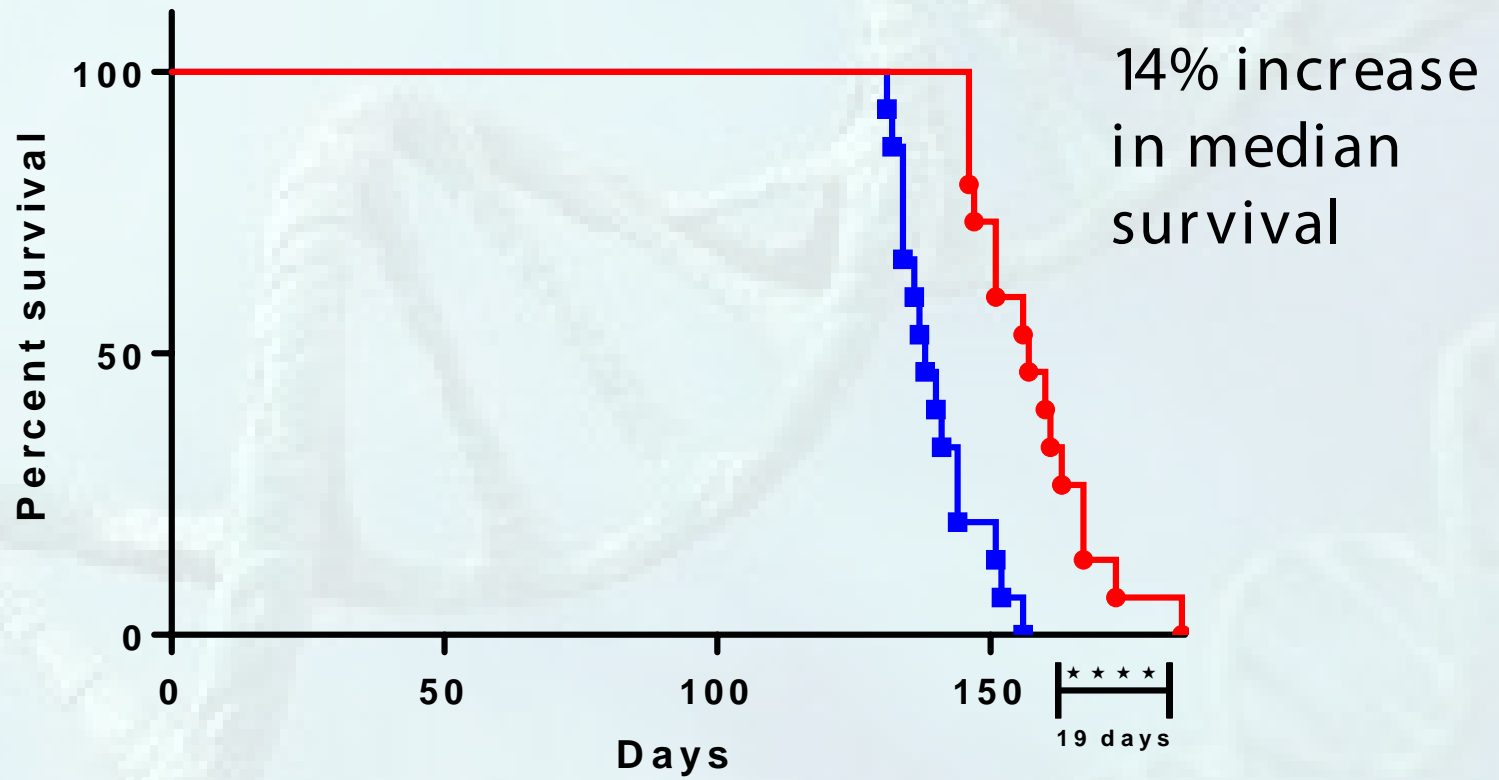
# P40 – neuroscoring and rotarod





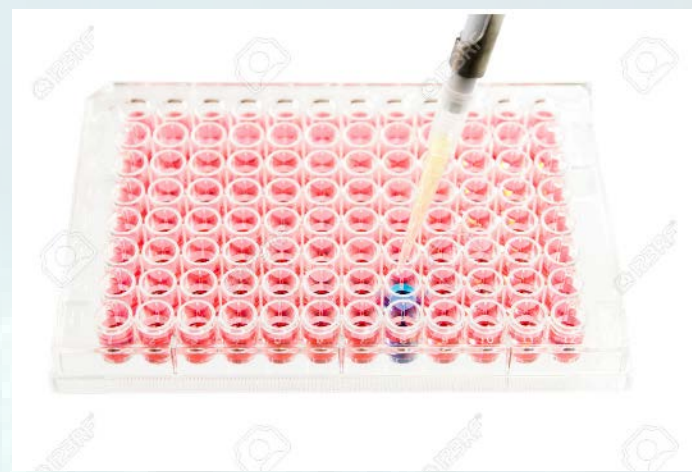


# P40 – survival

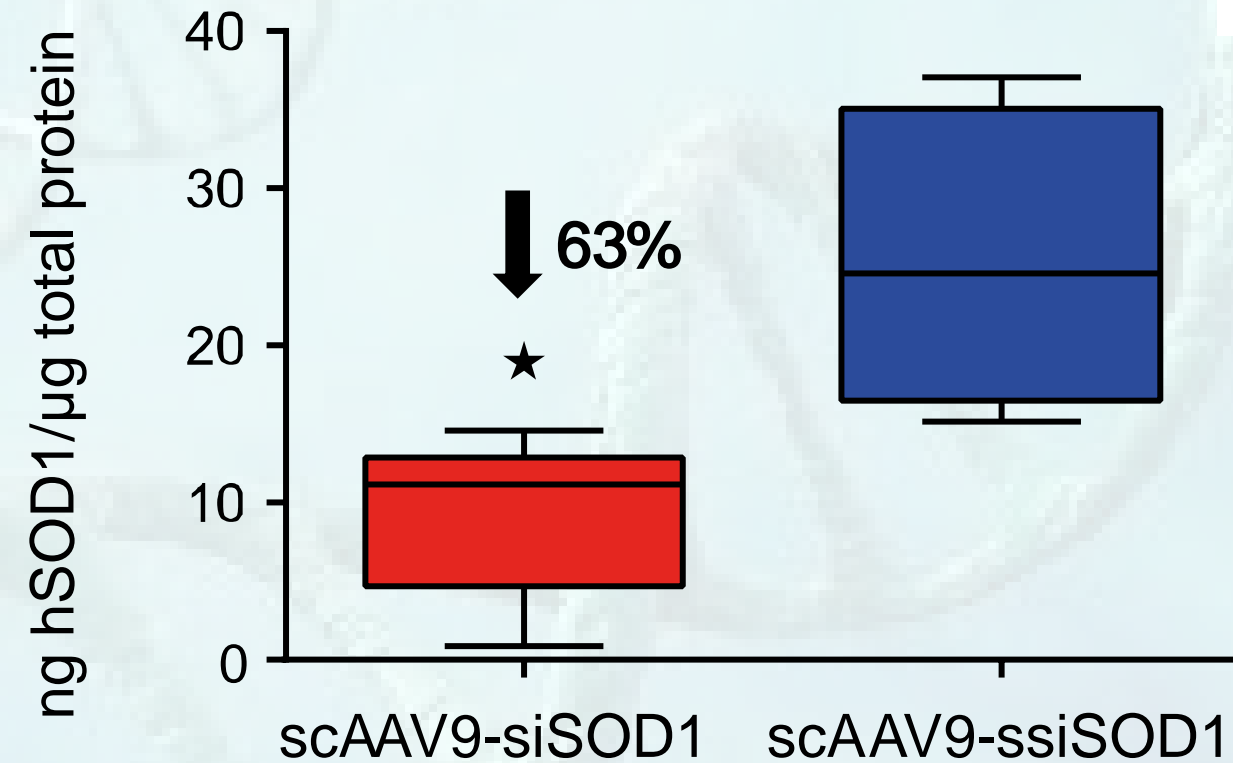




# Measurement of SOD1 depletion in the CSF



## Cerebrospinal Fluid

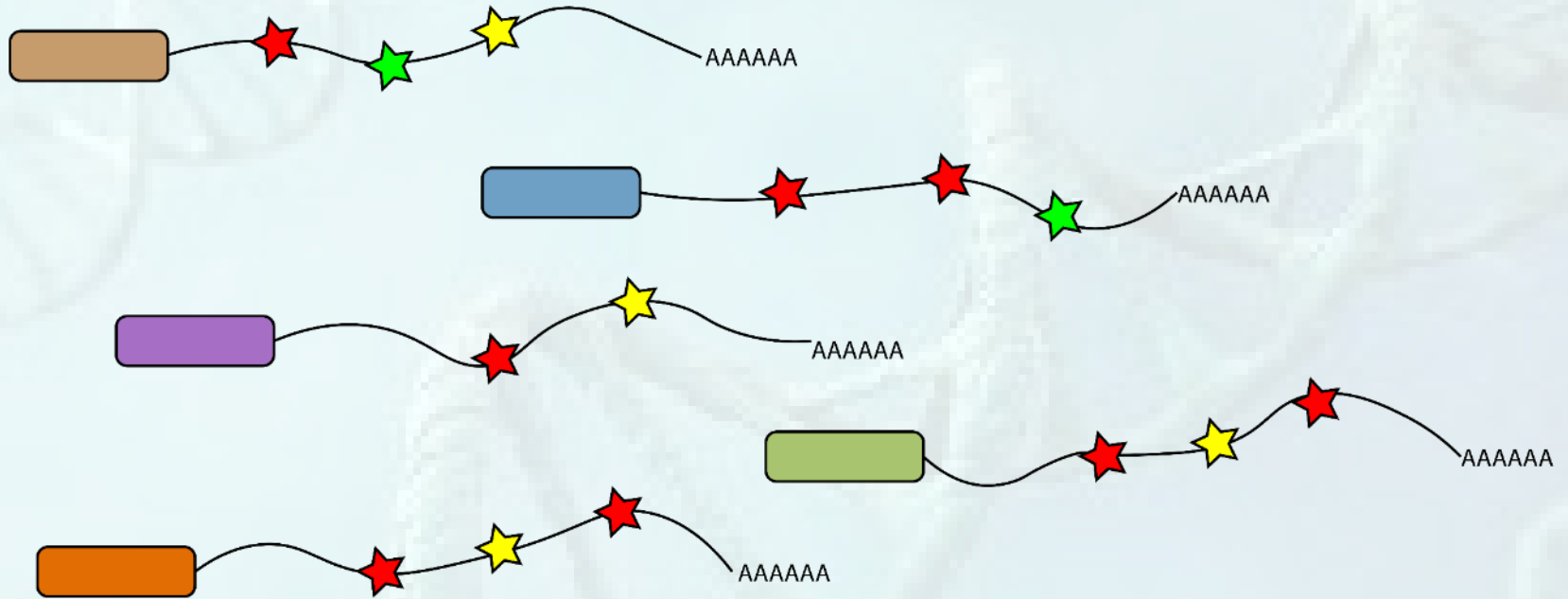




# *In vitro* investigation of off-target effects.



# Seed regions in 3'UTRs <sup>17</sup>



3' UTR Seed region frequency



High



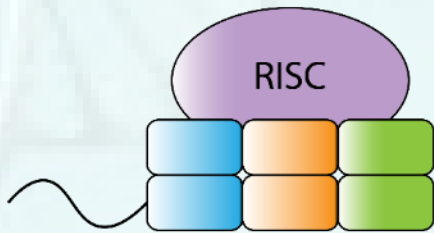
Medium



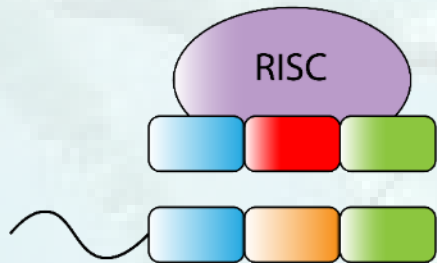
Low



## On-target mRNA



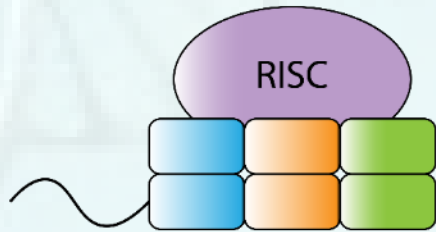
Knockdown  
due to  
perfect  
complementarity



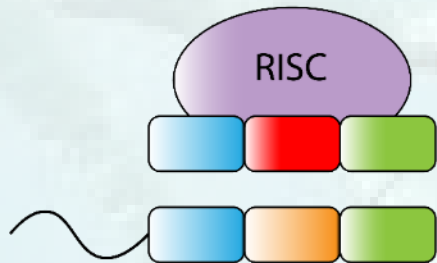
No knockdown  
due to  
sequence  
mismatch



## On-target mRNA

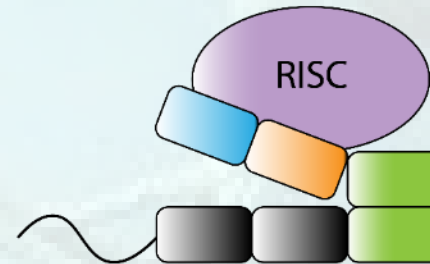


Knockdown due to perfect complementarity

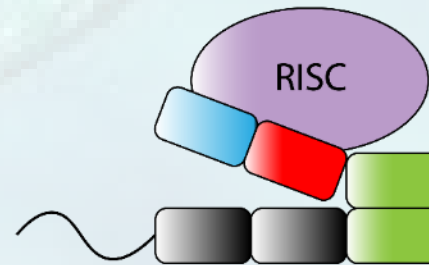


No knockdown due to sequence mismatch

## Off-target mRNA

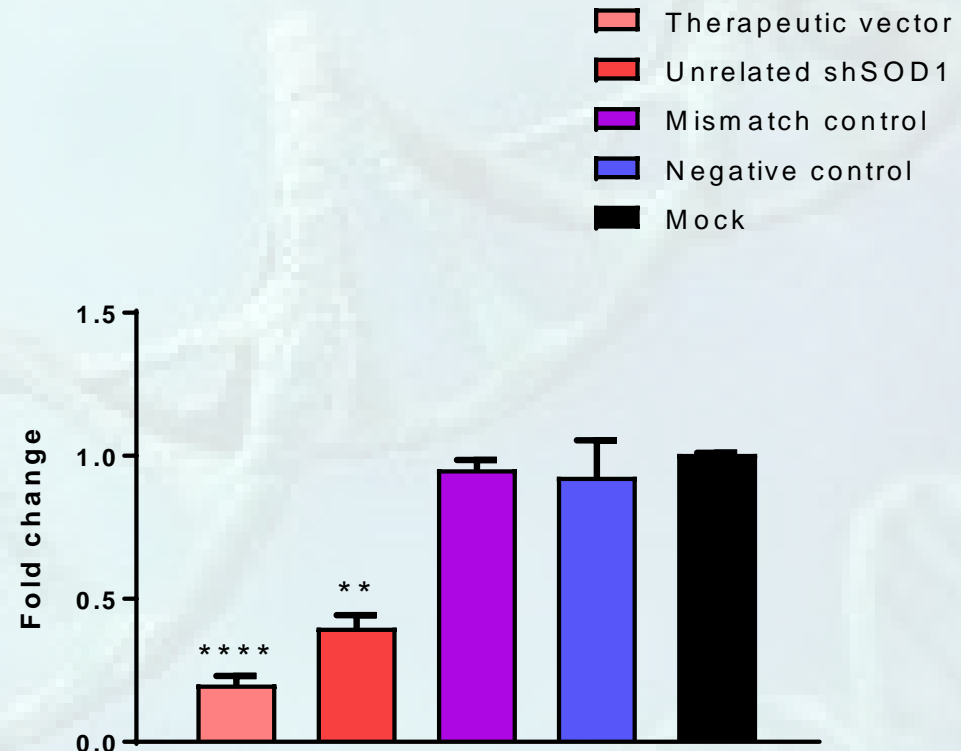


Repression of off-target mRNA due to seed region match



Repression of off-target mRNA due to seed region match despite central mismatch

<u>Construct</u>	<u>SOD1 Targeting?</u>	<u>Seed Region</u>
Therapeutic construct	YES	CATGAAC
Unrelated shSOD1	YES	CAGTCAG
Mismatch control	NO	CATGAAC
Negative control	NO	None



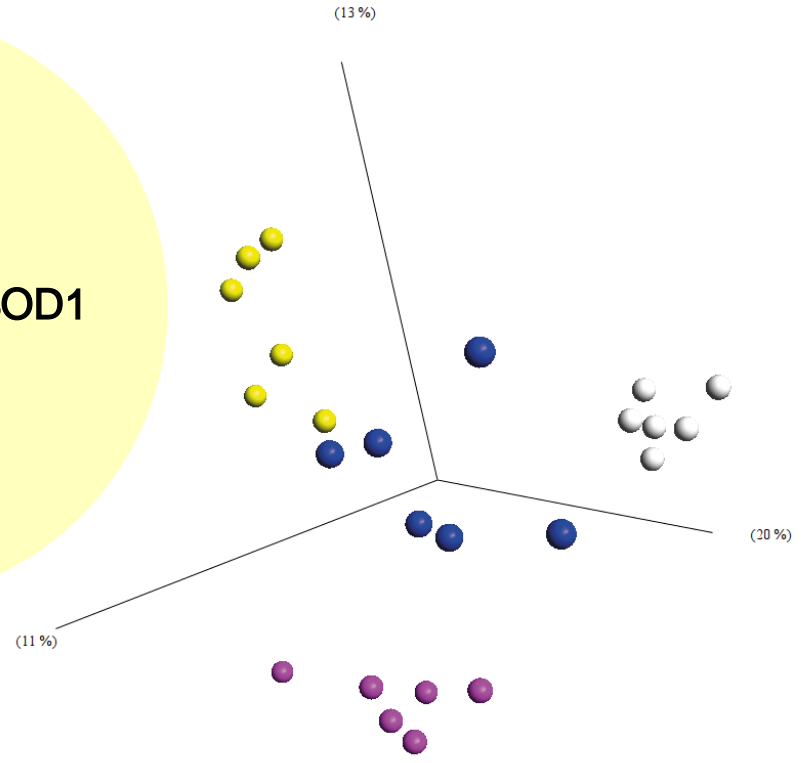
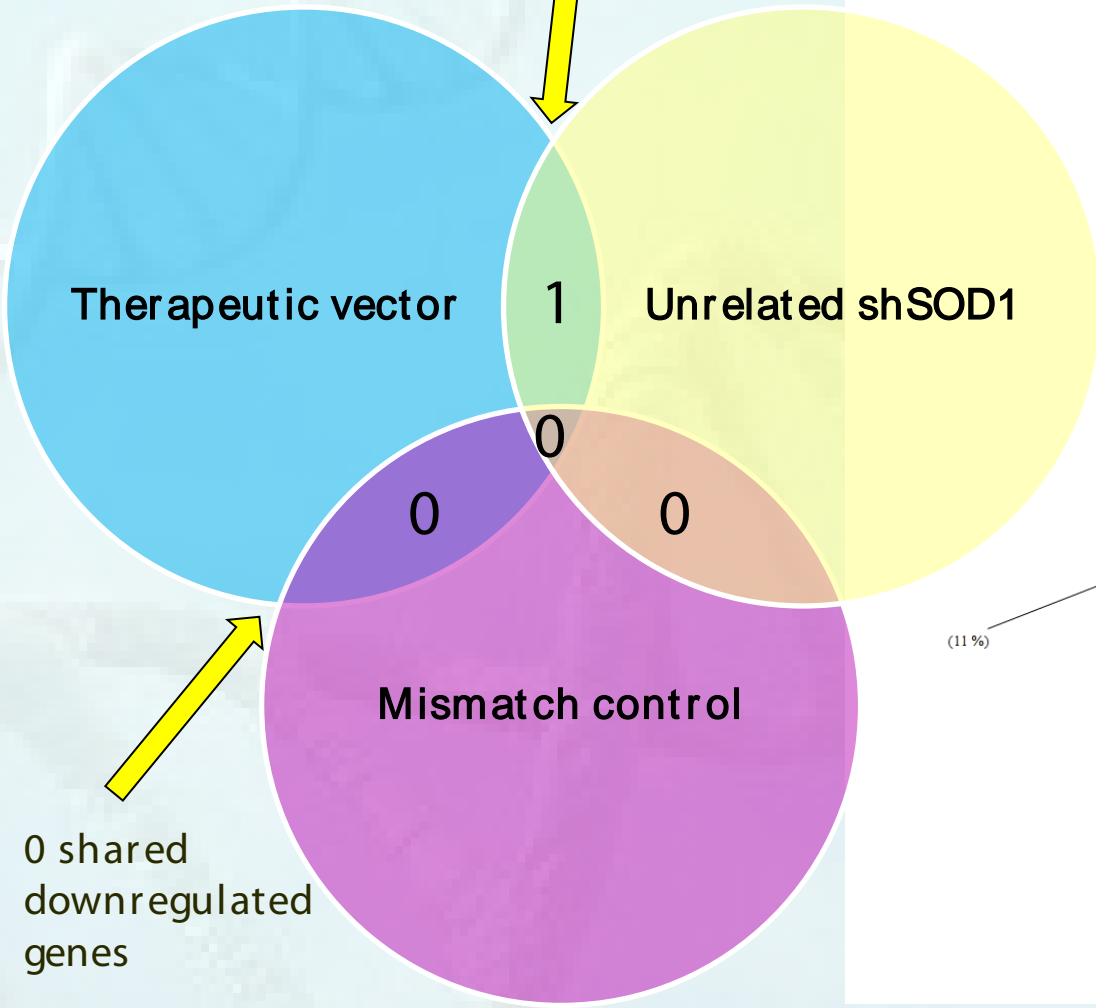




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1 shared  
downregulated  
gene: **SOD1**

# Genes differentially expressed<sup>20</sup> from Negative control



# Summary

- The clinic ready vector is effective at prolonging lifespan and ameliorating disease in a mouse model of ALS
- The therapeutic shRNA appears to be specific to SOD1 mRNA with no noticeable sequence-specific off-target effects
- Measurement of SOD1 depletion in the CSF of treated patients can be a simple and effective biomarker of treatment efficacy

# Acknowledgements

- Prof. Mimoun Azzouz
- Prof. Dame Pam Shaw
- Tommaso Iannitti
- Ian Coldicott
- Azzouz Lab Group
- Kaspar Lab Group





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Thank you for your  
attention!