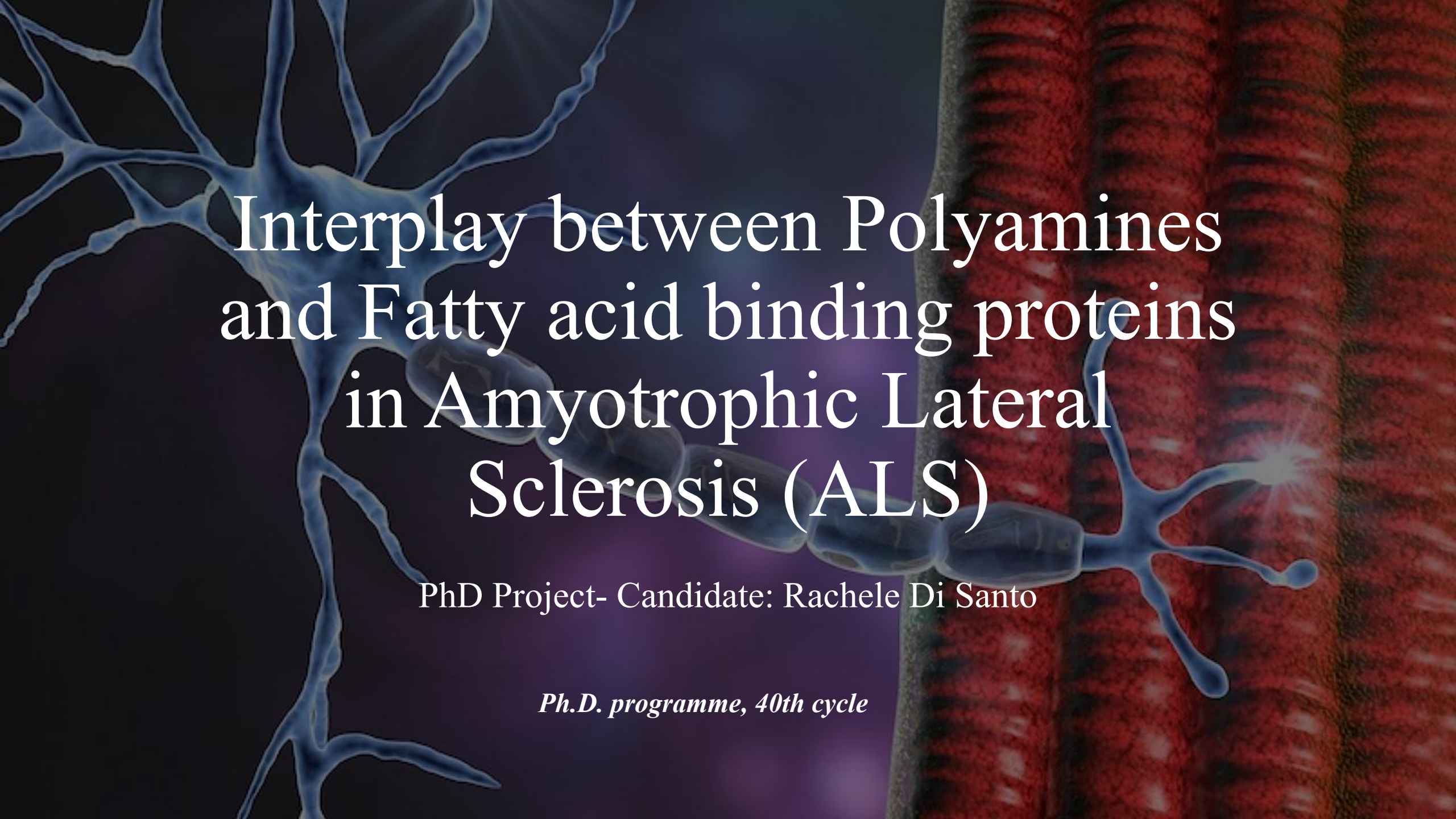




Interplay between Polyamines and Fatty acid binding proteins in Amyotrophic Lateral Sclerosis (ALS)

PhD Project- Candidate: Rachele Di Santo

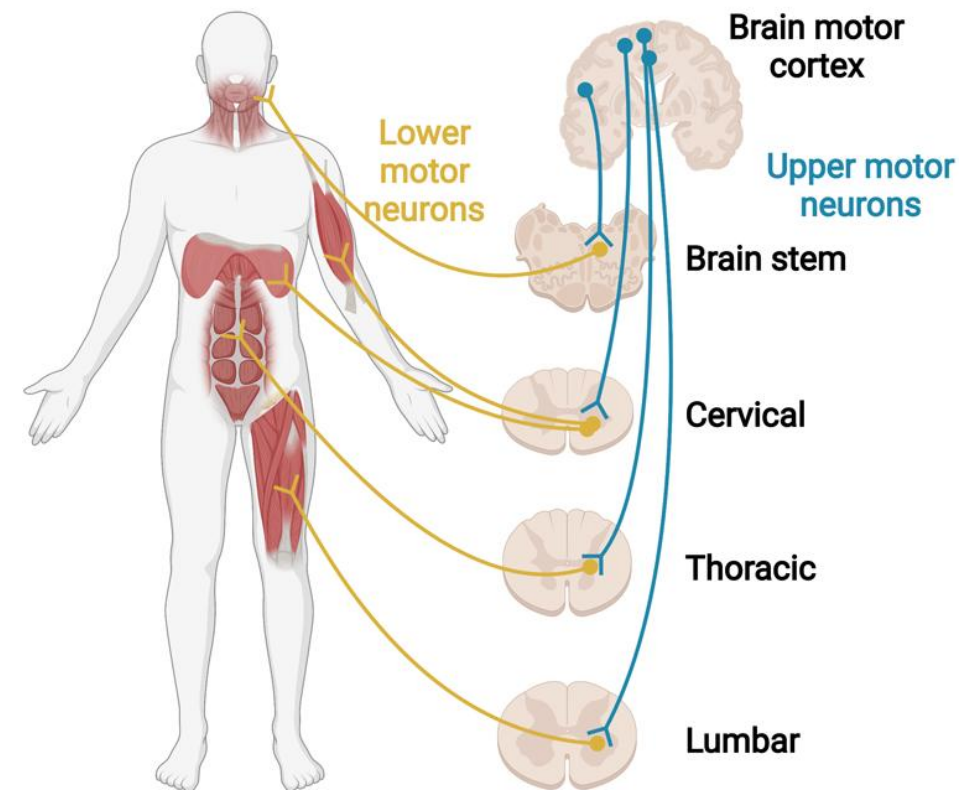
Ph.D. programme, 40th cycle

Amyotrophic Lateral Sclerosis (ALS)

Fatal neurodegenerative pathology that primarily affects the **cortico-spinal motor circuit**

ALS can be classified as either **familial** (15%) or **sporadic** (85%)

Over 40 ALS-associated genes have been identified ➡ **superoxide dismutase 1 (SOD1)**



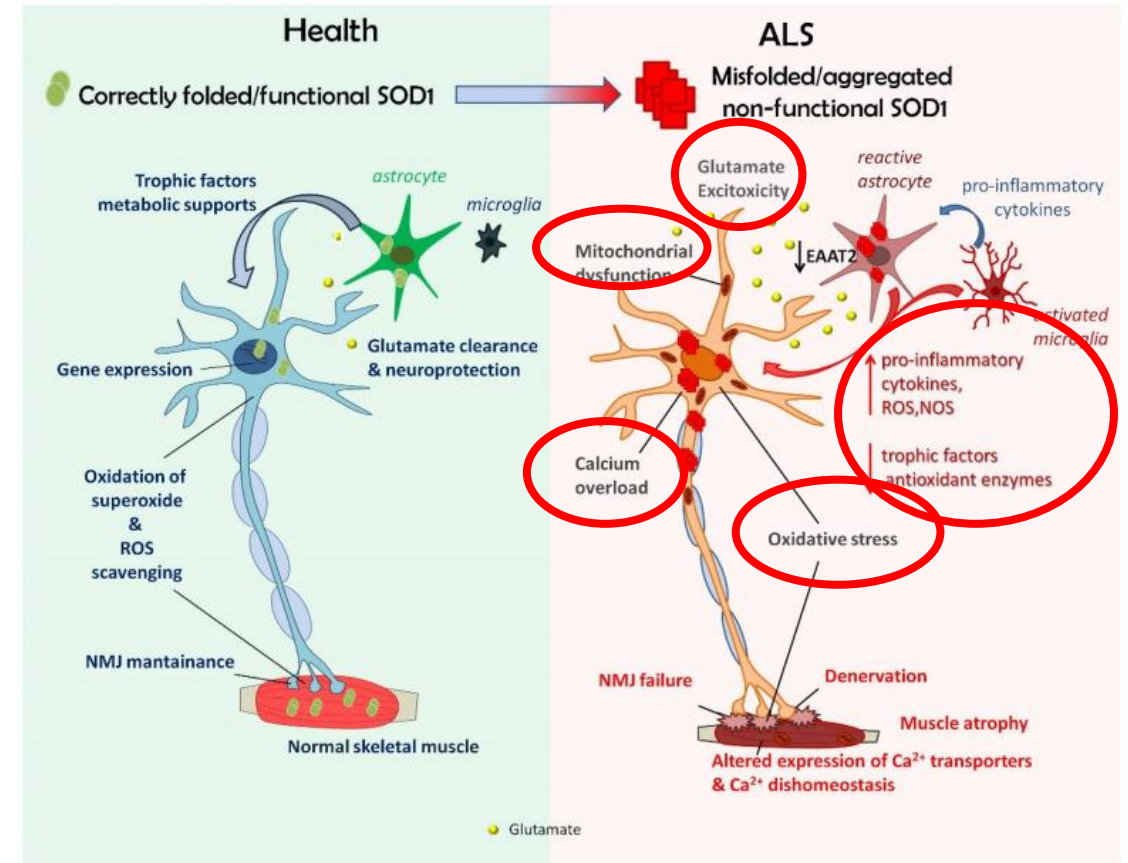
Amyotrophic Lateral Sclerosis (ALS)



SOD1(G93A) ALS mouse model

Transgenic model, which expresses large amounts of mutant human SOD1 that contains a point mutation at amino acid position 93 (G->A).

It is the most used animal model for ALS due to its similarity in disease progression to humans.



Exploring the effect of Spd administration as ALS treatment

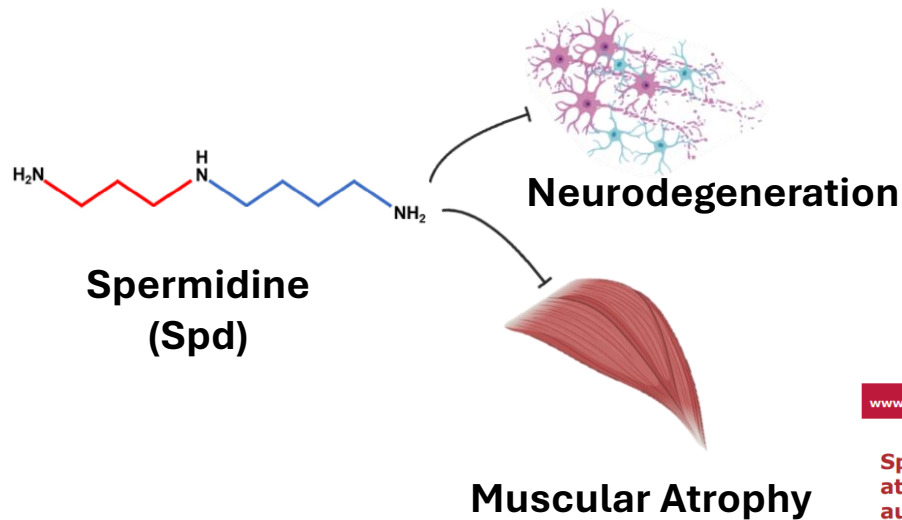


- Slow down ALS progression
- Different side effects



Find new ALS therapies

Exploring the effect of Spd administration as ALS treatment



RESEARCH

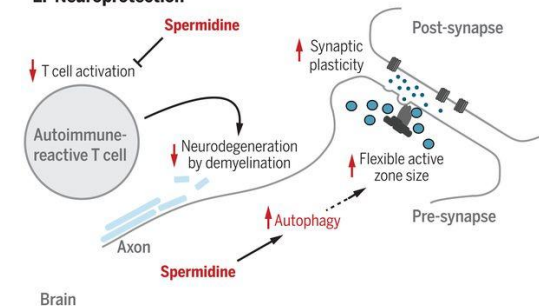
REVIEW SUMMARY

AGING

Spermidine in health and disease

Frank Madeo,^{*,†} Tobias Eisenberg,[†] Federico Pietrocola, Guido Kroemer^{*}

E. Neuroprotection



www.impactjournals.com/oncotarget/

Oncotarget, 2017, Vol. 8, (No. 11), pp: 17475-17490

Research Paper: Gerotarget (Focus on Aging)

Spermidine coupled with exercise rescues skeletal muscle atrophy from D-gal-induced aging rats through enhanced autophagy and reduced apoptosis via AMPK-FOXO3a signal pathway

Jingjing Fan¹, Xiaoqi Yang², Jie Li², Ziyang Shu², Jun Dai², Xingran Liu², Biao Li², Shaohui Jia¹, Xianjuan Kou¹, Yi Yang¹ and Ning Chen¹

¹Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal 576104, India

²School of Pharmacy and Pharmacology, MHIQ, QUM Network, Griffith University, Gold Coast, Queensland, Australia

Neuropeptides 83 (2020) 102083

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Neuropeptides

journal homepage: www.elsevier.com/locate/npep



News and Reviews

Spermidine, an autophagy inducer, as a therapeutic strategy in neurological disorders

Indrani Ghosh^a, Runali Sankhe^a, Jayesh Mudgal^a, Devinder Arora^{a,b}, Madhavan Nampoothiri^{a,*}

^aDepartment of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal 576104, India

^bSchool of Pharmacy and Pharmacology, MHIQ, QUM Network, Griffith University, Gold Coast, Queensland, Australia

ARTICLES

nature
cell biology

Induction of autophagy by spermidine promotes longevity

Tobias Eisenberg¹, Heide Knauer¹, Alexandra Schauer¹, Sabrina Büttner¹, Christoph Ruckenstein¹, Didac Carmona-Gutierrez², Julia Ring¹, Sabrina Schroeder¹, Christoph Magnes¹, Lucia Antonacci³, Heike Füssi¹, Luiza Deszcz^{2,4}, Regina Hart^{1,6}, Elisabeth Schraml¹, Alfredo Criollo^{2,7}, Evgenia Megalou¹, Daniela Weiskopf^{1,8}, Peter Laun^{1,1}, Gino Heeren¹, Michael Breitenbach^{1,1}, Beatrix Grubeck-Loebenstein^{1,9}, Eva Herker^{1,1}, Birthe Fahrenkrog^{1,1}, Kai-Uwe Fröhlich¹, Frank Sinner¹, Nektarios Tavernarakis¹, Nadege Minois^{1,1,1}, Guido Kroemer^{1,1,1} and Frank Madeo^{1,1}

Journal of Biomedical Science (2022) 29:106
<https://doi.org/10.1186/s12929-022-00890-3>



Journal of Biomedical Science

RESEARCH

Open Access

Modulation of histone H3K4 dimethylation by spermidine ameliorates motor neuron survival and neuropathology in a mouse model of ALS

Seung-Gun Choi^{1,†}, Ali Yousefian-Jazi^{1,†}, Seung Jae Hyeon¹, Phuong Thi Thanh Nguyen^{1,2}, Jie Chen^{1,3}, Sojung Kim¹, Sungho Lee¹, Hannah L. Ryu⁴, Neil W. Kowall^{4,5}, Hoon Bwu^{1,2}, and Sanghee Lee^{4,5*}

¹Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

²Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

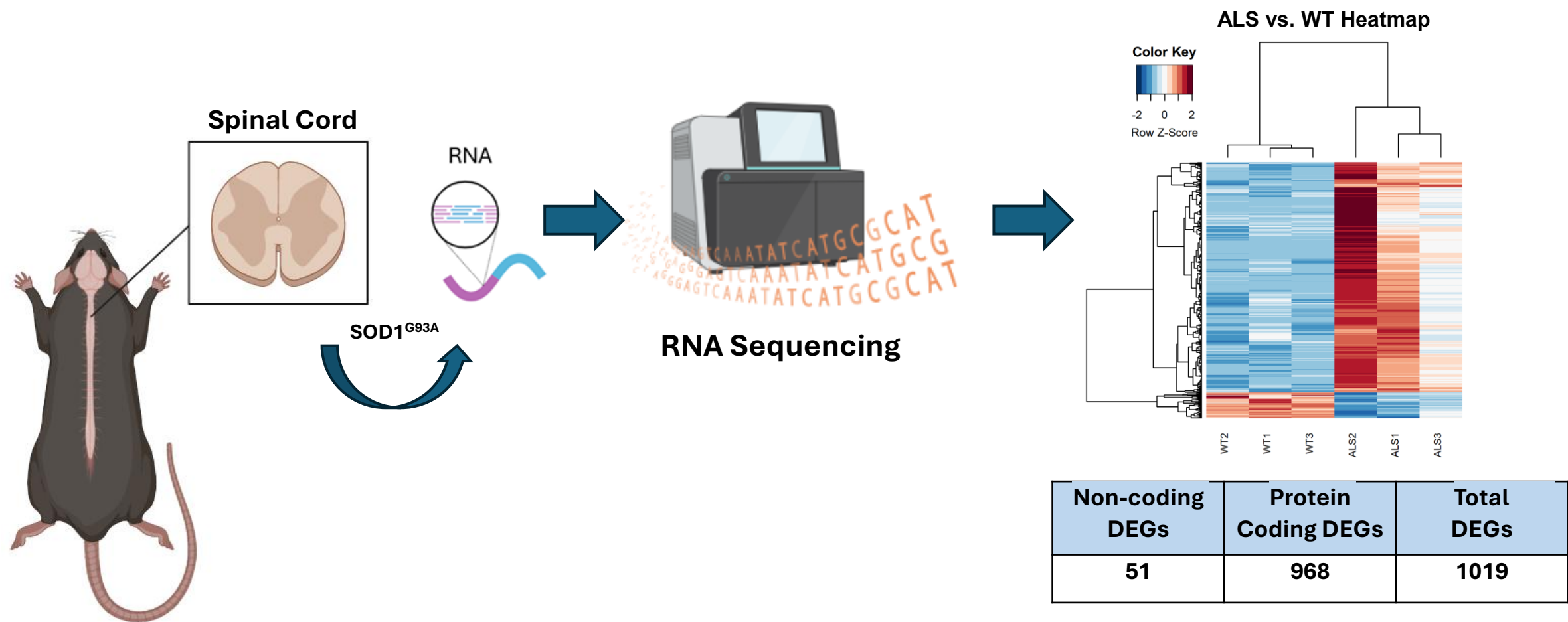
³Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

⁴Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

⁵Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

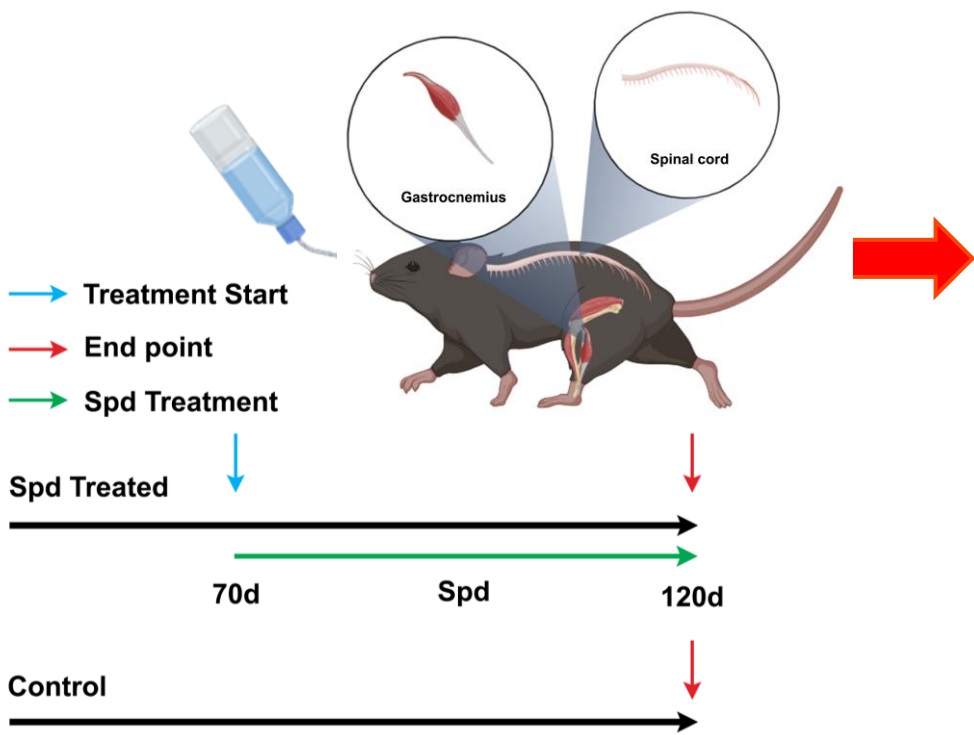
Preliminary Data

Bulk RNA-seq Analysis of both Wt and G93A mice, treated or not with Spd



Preliminary Data

Bulk RNA-seq Analysis of both Wt and G93A mice, treated or not with Spd



ALS vs. CTR Log ₂ (FC)	Gene Symbol	ALS SPD vs. ALS Log ₂ (FC)
3.63	Ly9	-1.62
1.71	S100a1	-0.62
1.13	Fabp7	-0.93
0.9	Gm26586	-0.88
0.68	Spp1	-0.75
NA	Gm44190	0.9
NA	Gm15344	-0.51
NA	1810014B01Rik	-0.51

Key regulator of lipid metabolism and energy homeostasis

One of the main differentially expressed gene in ALS mouse spinal cord meta-analysis



Article
A Transcriptomic Meta-Analysis Shows Lipid Metabolism Dysregulation as an Early Pathological Mechanism in the Spinal Cord of SOD1 Mice

Luis C. Fernández-Beltrán¹, Juan Miguel Godoy-Corchuelo¹, Maria Losa-Fontangordo¹, Debbie Williams², Jorge Matias-Guiu¹ and Silvia Corrochano^{1,*}

¹ Neurological Disorders Group, Hospital Clínico San Carlos, IdISSC, 28040 Madrid, Spain; luisferb18@hotmail.com (L.C.F.-B.); juanmiguelgodoycorchuelo@gmail.com (J.M.G.-C.); marlosa@ucm.es (M.L.-F.); jorge.matiassguir@salud.madrid.org (J.M.-G.)
² Mammalian Genetics Unit, MRC Harwell Institute, Oxfordshire OX11 0RD, UK; d.williams@har.mrc.ac.uk
* Correspondence: silvia.corrochano@salud.madrid.org; Tel.: +34-913303000

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2013; 14: 177–189

informa
healthcare

ORIGINAL ARTICLE

Meta-analysis of gene expression profiling in amyotrophic lateral sclerosis: A comparison between transgenic mouse models and human patients

CHRISTIAAN G. J. SARIS, EWOUT J. N. GROEN, JOHAN A. F. KOEKKOEK, JAN H. VELDINK & LEONARD H. VAN DEN BERG

Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht

Fatty acid-binding proteins (Fabps) in ALS

ALS → Mitochondrial dysfunction → β -oxidation → Worst ALS prognosis

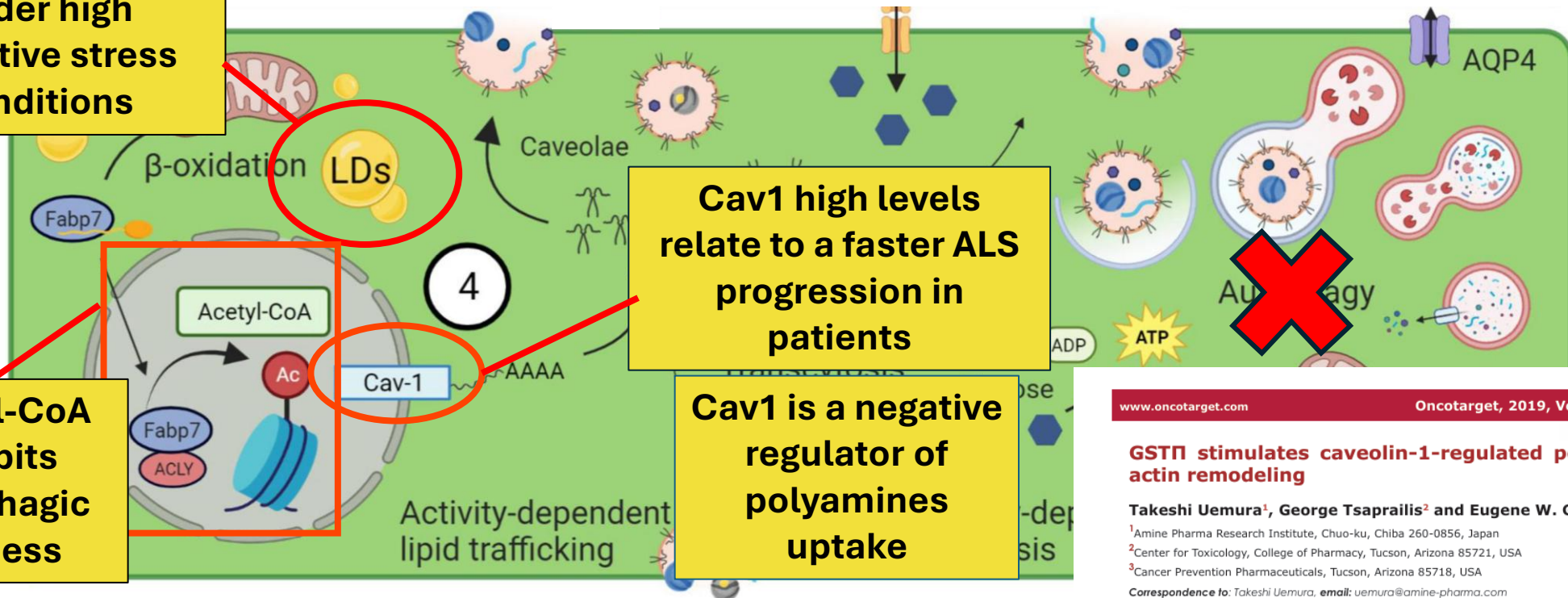
Fabps regulate FAs uptake and transport

Lipid Droplets (LDs) are formed under high oxidative stress conditions

Acetyl-CoA inhibits autophagic process

Cav1 high levels relate to a faster ALS progression in patients

Cav1 is a negative regulator of polyamines uptake



www.oncotarget.com

Oncotarget, 2019, Vol. 10, (No. 55), pp: 5713-5723

Research Paper

GSTN stimulates caveolin-1-regulated polyamine uptake via actin remodeling

Takeshi Uemura¹, George Tsapralis² and Eugene W. Gerner³

¹Amine Pharma Research Institute, Chuo-ku, Chiba 260-0856, Japan

²Center for Toxicology, College of Pharmacy, Tucson, Arizona 85721, USA

³Cancer Prevention Pharmaceuticals, Tucson, Arizona 85718, USA

Correspondence to: Takeshi Uemura, email: uemura@amine-pharma.com

Keywords: polyamine; caveolin; GSTN; transport; actin

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Published: October 01, 2019

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Aims of the Project



**ALS there is a
RNA processing
impairment**



Analyse in ALS vs. WT and ALS Spd vs. ALS RNAseq

- ncRNAs
- alternative splicing

1° year

**Spd promotes autophagy
and downregulation of
Fabp7**



Study in ALS cell models
the Fabps role in autophagy

2° year

**LDs are marker of
neurodegeneration**



Analyse in ALS cell models
Spd effect in LDs formation

**Fabps target Cav-1 is a
negative regulator of
PAs uptake**



Analyse in ALS cell models
PAs uptake in response to
Spd treatment and caveolins inhibition

3° year

1^o year of PhD

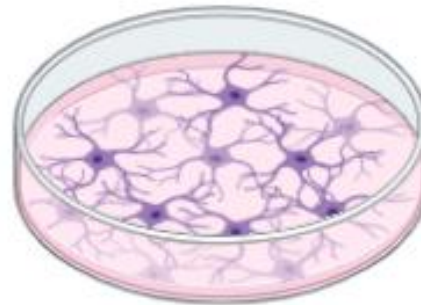
AIM 1 : Set up cellular systems

- Transfect with vectors to overexpress SOD1(WT) and SOD1(G93A)



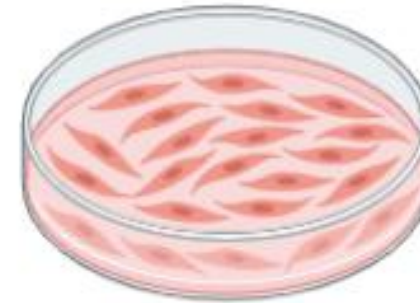
NSC34 cells

Motor neurons



C8-D1A cells

Astrocytes



C2C12 cells

Skeletal muscle cells

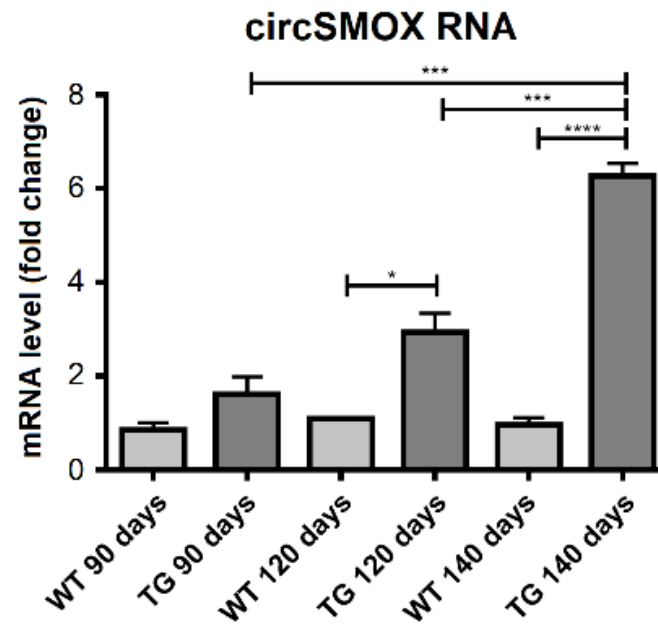
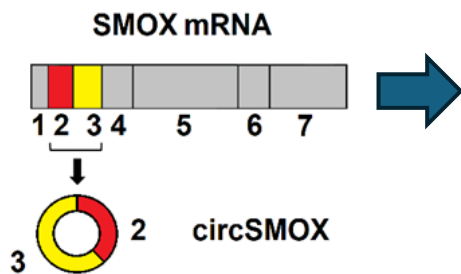
AIM 2 : Analysis of the non-coding transcriptome and alternative splicing

- By bioinformatic analysis and qPCR

1^o year of PhD

AIM 3 : Investigate the role of circSMOX and mir-340-5p in ALS cellular models

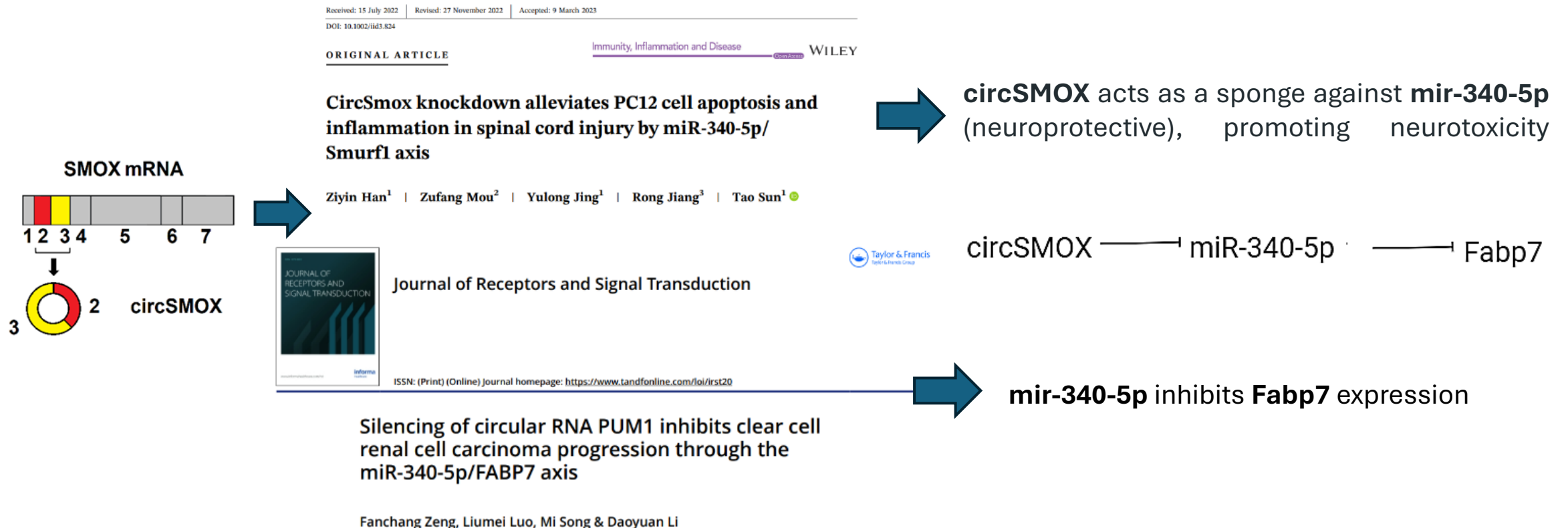
- Demonstrate the presence of circSMOX-miR-340-5p-Fabp7 in the cellular systems by qPCR, Western Blotting, RNA-Immunoprecipitation Assay and Pull-down Assay



1^o year of PhD

AIM 3 : Investigate the role of circSMOX and mir-340-5p in ALS cellular models

- Demonstrate the presence of circSMOX-miR-340-5p-Fabp7 in the cellular systems by qPCR, Western Blotting, RNA-Immunoprecipitation Assay and Pull-down Assay

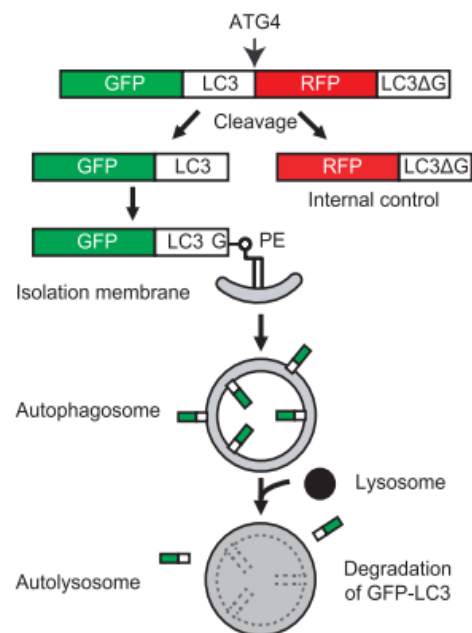


2^o year of PhD

AIM 1 : Investigate the role of Fabps in regulation of autophagy in ALS models

- Transfect cell lines with autophagic vector and evaluate autophagic flux by fluorescent microscopy

GFP-LC3-RFP-LC3ΔG system



Molecular Cell Technology

An Autophagic Flux Probe that Releases an Internal Control

Takeshi Kaizuka,^{1,2} Hideaki Morishita,^{1,2} Yutaro Hama,¹ Satoshi Tsukamoto,¹ Takahide Matsui,¹ Yuichiro Toyota,^{1,2} Akihiko Kodama,¹ Tomoaki Ishihara,¹ Tohru Mizushima,¹ and Noboru Mizushima^{1,2}

¹Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

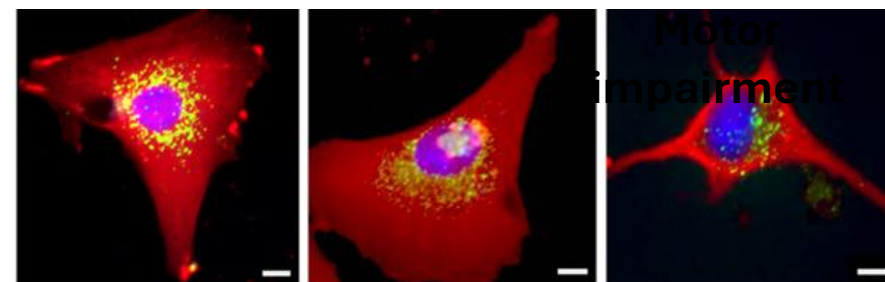


Exp Group 1	Exp Group 2	Exp Group 3
GFP-LC3-RFP-(LC3ΔG)	GFP-LC3-RFP-(LC3ΔG)	GFP-LC3-RFP-(LC3ΔG)
	+ Spd	+ Spd + Fabp7(Wt)

Choksomngam, et al., 2021. *Obesity* rReinoso-Sánchez et al., 2020. *International journal of molecular sciences*, 21(21), 8227.

BODIPY™ 493/503

FABP7, BODIPY, DAPI



Islam et al., 2019. *Molecular neurobiology*, 56(8),

Exp Group 1	Exp Group 2	Exp Group 3
Ctrl	+ Spd	+ Fabp7 Inhibitor

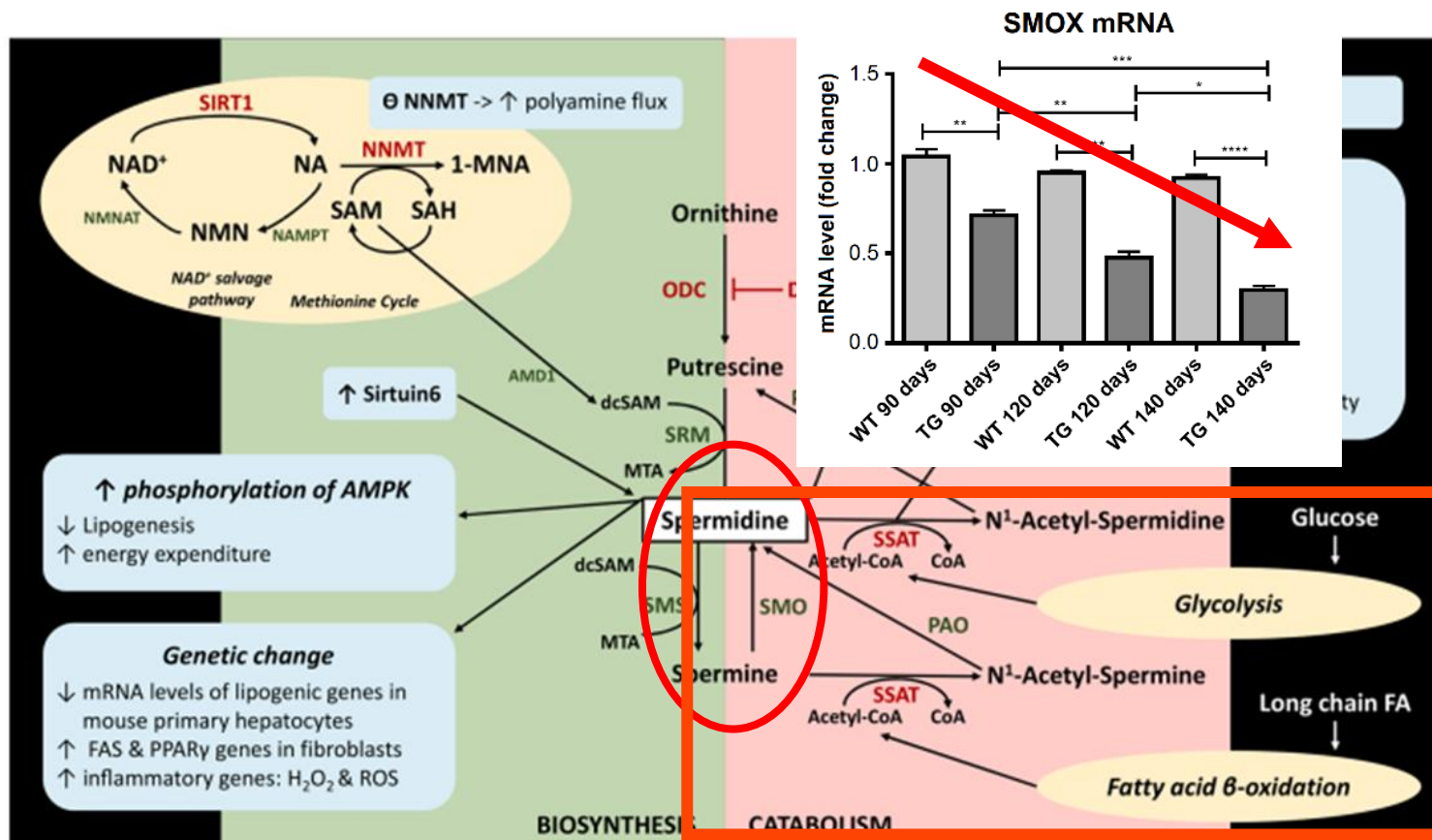
In SOD1(G93A) cells

D'Ercole et al., 2022. *Cell reports*, 41(12), 111861.

2^o year of PhD

AIM 3 : Analysis of PAs metabolism

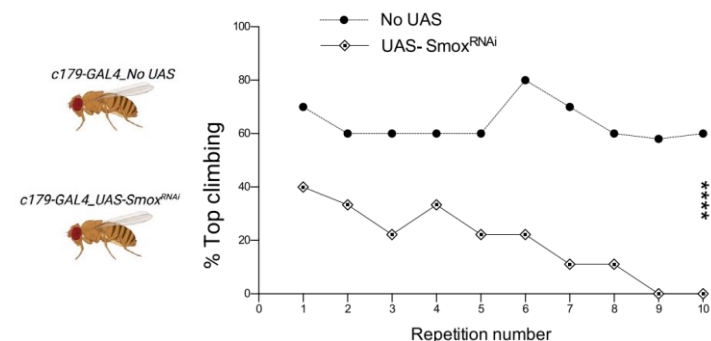
- Analyse gene expression levels by qPCR and SAT1 activity by high-throughput colorimetric assay



Fabp7 is involved in Acetyl-CoA production and FAs β -oxidation



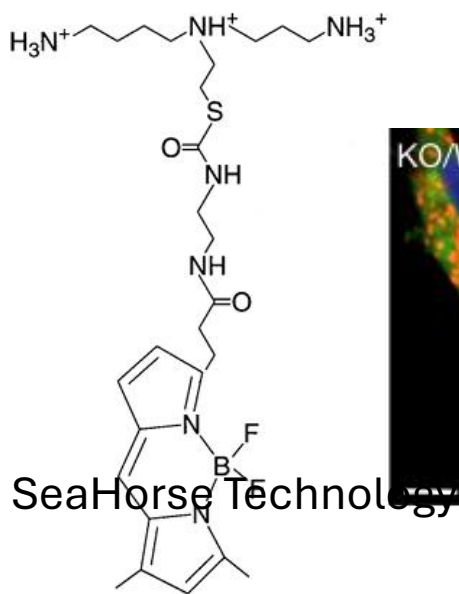
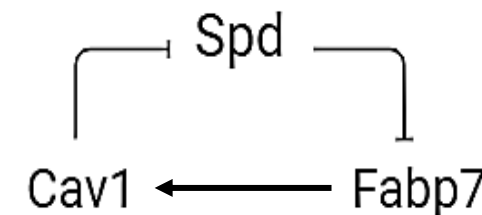
SAT1 uses Acetyl-CoA as a substrate to acetylate PAs



3^o year of PhD

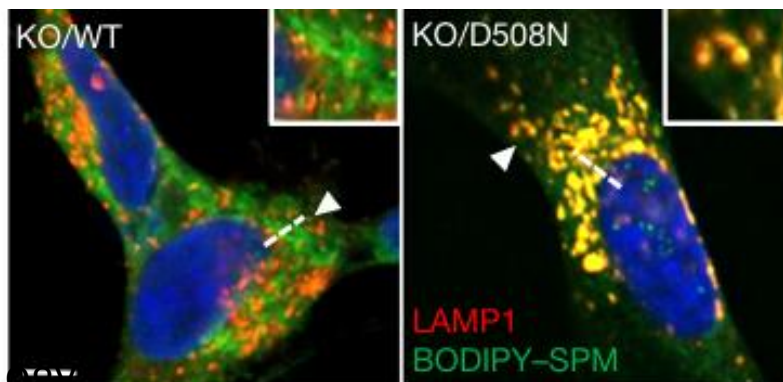
AIM 1: Study the role of Spd-Fabps-caveolin axis in the regulation of polyamines uptake

- Measure PAs intracellular levels by HPLC
- Analyse PAs uptake by Spd-C₂-BODIPY probe



SeaHorse Technology

Spd-C₂-BODIPY



van Veen et al., 2020. *Nature*, 578(7795), 419–424.

nature

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Article | Published: 29 January 2020

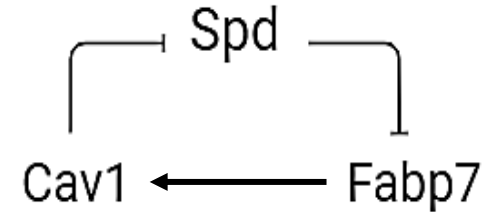
ATP13A2 deficiency disrupts lysosomal polyamine export

Exp Group 1	Exp Group 2	Exp Group 3
Ctrl	+ Spd-C ₂ -BODIPY	+ Spd-C ₂ -BODIPY + Cav Inhibitor

3^o year of PhD

AIM 1: Study the role of Spd-Fabps-caveolin axis in the regulation of polyamines uptake

- Measure PAs intracellular levels by HPLC



SeaHorse Technology



**Measure of ATP
production**



**Effect on ALS phenotype
of Spd-Caveolins inhibitor
co-treatment in our ALS
cell models**

Exp Group 1	Exp Group 2	Exp Group 3
Ctrl	+Spd	+Spd + Cav Inhibitor

Conclusions

- Elucidate the alternative splicing and ncRNAs mechanisms in ALS pathology
- Identify a possible network between **circSMOX** - **miR-340-5p** - **FABP7** in ALS pathology
- Investigate the contribution of FABP7 in Spd autophagy induction
- Analyse the effect of Spd treatment on LDs formation in ALS cellular models
- Investigate the effects of **Spd - FABP7- caveolins** in polyamines uptake
- Investigate the effect of the combined treatment of Spd and caveolin inhibitors in ALS phenotype

**a more comprehensive depiction
of pathology**



**progress in the search for
ALS therapy**



Thank you for your attention!