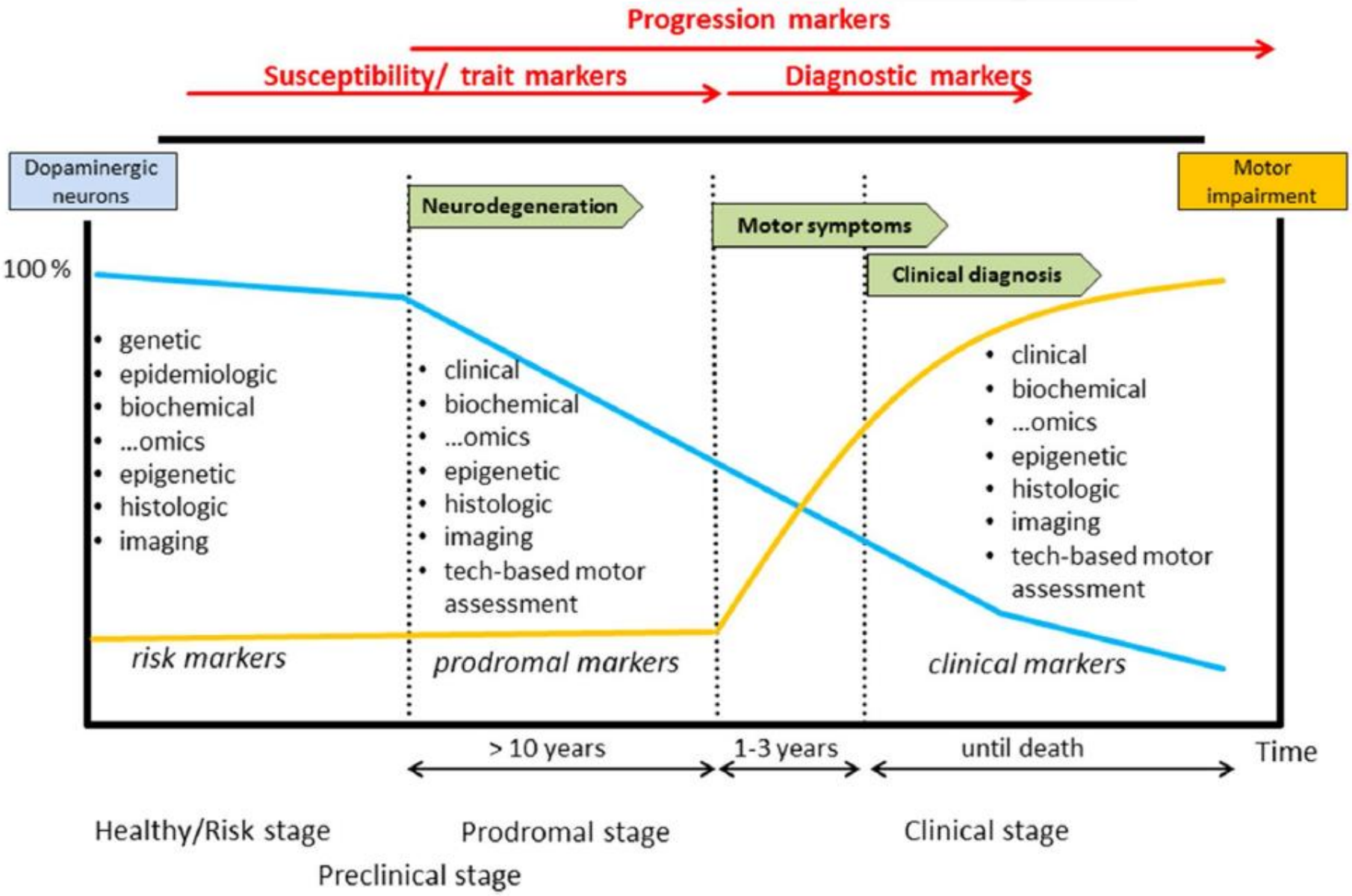
Parkinson's disease –

focus on proteotoxicity

(part 2)

PD2

Stages of PD and biomarkers



Yilmaz et al., J Neural Trasm 2019

Pathogenesis-driven biomarkers of PD

	Proposed pathogenesis	Genetic evidence	Biomarkers	Therapeutic implications
Increase in *SNCA* expression	Increase in α-synuclein protein leads to increased aggregation and cell death and dysfunction	Increased *SNCA* gene dose (duplications or triplications) causes Parkinson's disease; *SNCA* common variants probably lead to increased expression	α-Synuclein and phospho-synuclein measurement in blood and CSF	Decrease in *SNCA* transcription or translation (eg, with ASO therapy)
Increase in α-synuclein aggregation	Formation of oligomers and fibrils leads to cellular toxicity	Coding mutations in *SNCA* lead to increase in α-synuclein aggregation	rt-QUIC assays for aggregation based on CSF, skin biopsies, olfactory mucosal biopsies, and saliva	Antiaggregation therapies
Mitochondrial dysfunction	Reduced complex 1 activity, abnormal calcium homoeostasis, increased reactive oxygen species, and reduced mitochondrial ATP production	Multiple Parkinson's disease gene mutations lead to changes in mitochondrial function including *PRKN*, *PINK1*, and *LRRK2*	Magnetic resonance spectroscopy analysis of Pi to ATP ratios, measurement of ATP, and mitochondrial function in skin fibroblasts	Enhancing mitochondrial biogenesis and function
Altered endosomal-lysosomal trafficking	Activation of LRRK2 and VPS35 lead to phosphorylation of Rab proteins, which leads to decreased lysosomal function and altered response to membrane damage	Rare pathogenic variants in *LRRK2* (eg, Gly2019Ser) and *VPS35* lead to increased Rab phosphorylation	Measurement of Rab protein phosphorylation in cells from peripheral blood; measurement of urinary BMP phospholipids	Reducing LRRK2 protein levels, or kinase activity, or both, with ASO therapy or kinase inhibitors
Lysosomal dysfunction	Impaired α-synuclein degradation leads to increased cellular α-synuclein	*GBA1* mutations are associated with Parkinson's disease, and rare variants in other genes might be relevant	Measurement of GCase protein and enzyme activity; measurement of GSLs in blood and CSF; measurement of urinary BMP phospholipids	Modulators of GCase activity
Immune activation and neuroinflammation	Multiple factors (α-synuclein aggregates, mitochondrial antigens, and gut bacterial endotoxins) promote both innate and adaptive immune responses, culminating in increased neuroinflammation and neuronal toxicity	Association between HLA variants and Parkinson's disease; LRRK2, PRKN, and PINK1 are involved in inflammatory pathway	Measurement of C-reactive protein, interleukins, and PET imaging of activated microglia	Immunomodulatory or anti-inflammatory therapies
Cell-to-cell spread	Toxic forms of α-synuclein spread between anatomically contiguous cells, or over longer range, and might be contained in extracellular vesicles	NA	rt-QUIC assays for aggregation based on CSF, skin biopsies, olfactory mucosal biopsies, and saliva	Reduction in release, extracellular transit, or uptake by recipient cells using monoclonal antibody therapy or other therapies

ASO=antisense oligonucleotide. BMP=bis(monoacylglycerol)phosphate. CSF=cerebrospinal fluid. GCase=β-glucocerebrosidase. GSLs=glycosphingolipids. NA=not applicable. rt-QUIC=real time quaking-induced conversion.

Morris et al., www.thelancet.com Vol 403 January 20, 2024

Research News

Breaking News: Parkinson's Disease Biomarker Found

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

April 13, 2023

α-synuclein seeding amplification assay (αSyn-SAA)

detects pathology in CSF of PD patients and

in individuals at a **high risk of developing PD**

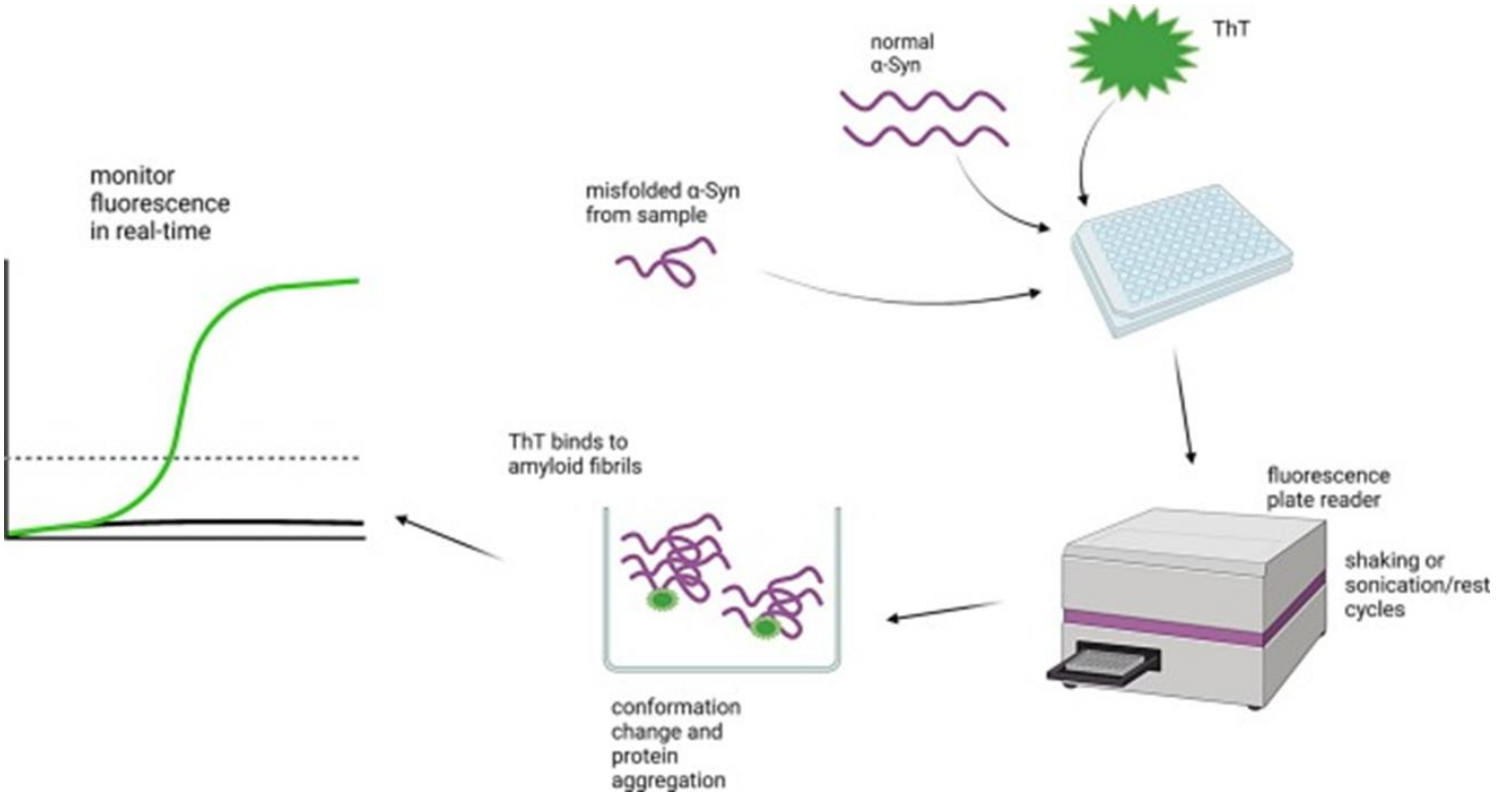
- 93% of PD patients have an abnormal test (93% sensitivity)

- abnormal test in < 5% of people without PD (> 95% specificity)

α-synuclein seeding amplification assay (αSyn-SAA)

The PMCA/RT-QuIC process

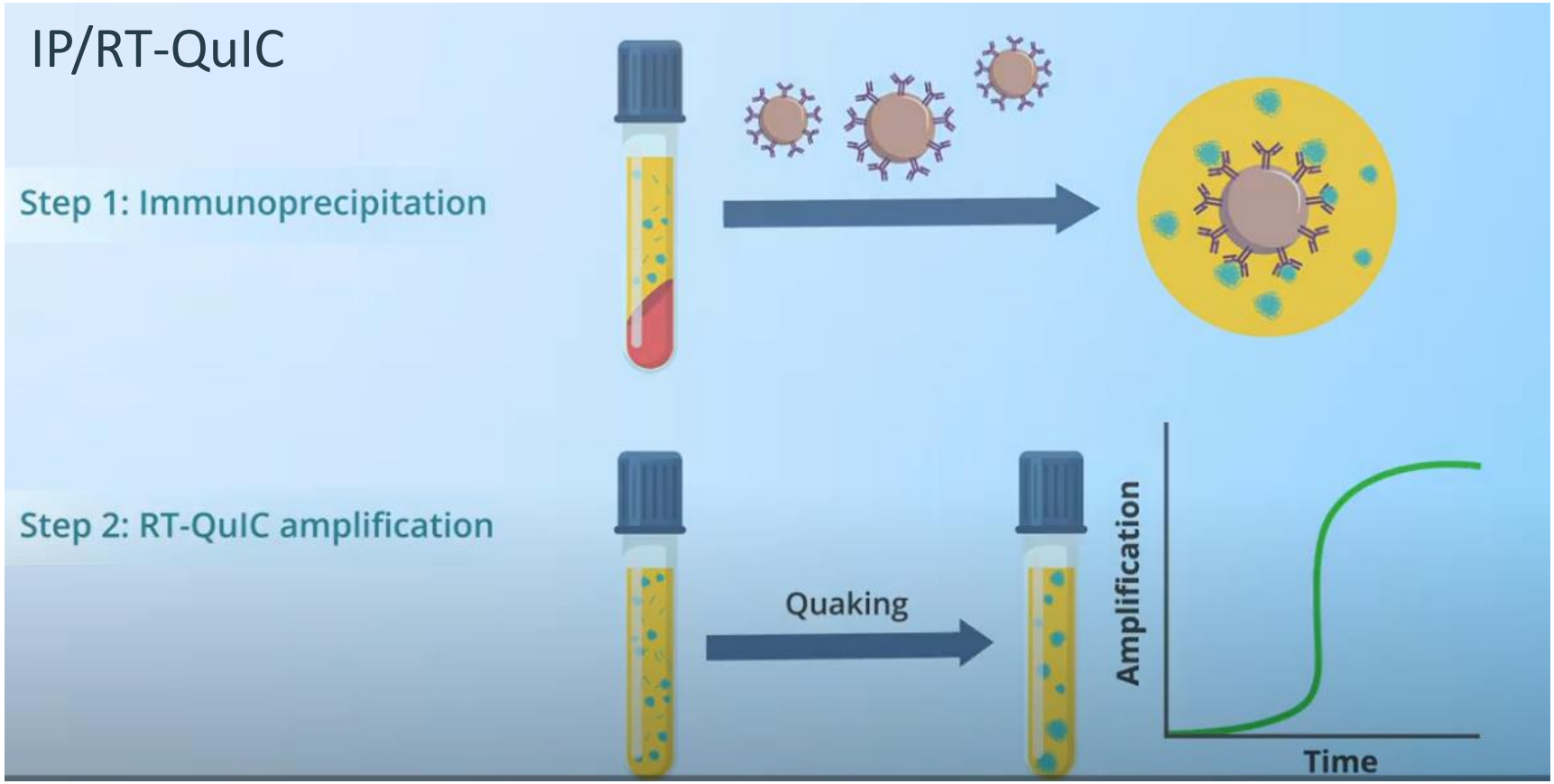
(PMCA=protein-misfolding cyclic amplification
RT-QuIC=real-time quaking-induced conversion)



1. pathogenic (misfolded) α-syn protein is combined with normal α-syn protein and ThT.
2. shaking (RT-QuIC) or sonication/rest (PMCA) cycles induce prion-like propagation and amyloid fibril formation, which is measured in real-time with ThT fluorescence.

αSyn-SAA can elicit a **binary response** — showing that abnormal synuclein is either present or not — there is tremendous promise in optimizing it, in order to measure the amount of alpha-synuclein present.

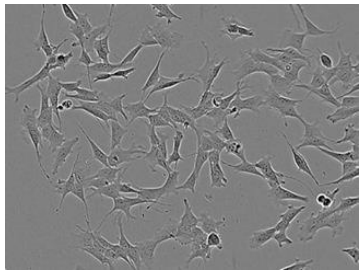
Optimized assays would also detect abnormal synuclein through blood draw (in serum with IP/RT-QuIC) or nasal swab.

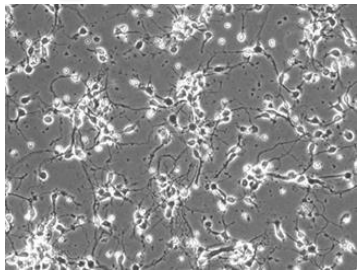


Aim 2: to study the molecular mechanisms involved in autophagy dysfunction in cellular models

PD-related toxins (rotenone, paraquat, …) or genetic alterations (A53T mutant α-synuclein, wt α-synuclein)

human neuroblastoma SH-SY5Y cells



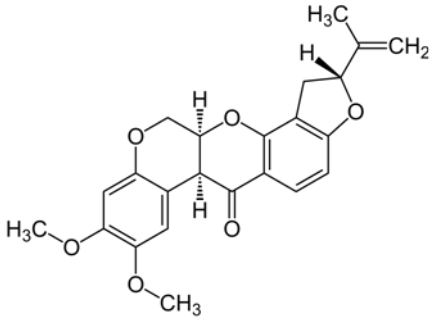


C57BL/6 mouse cortical neurons

(Dr. A.M. Colangelo, BtBs, UNIMIB)

Neuroprotective compounds

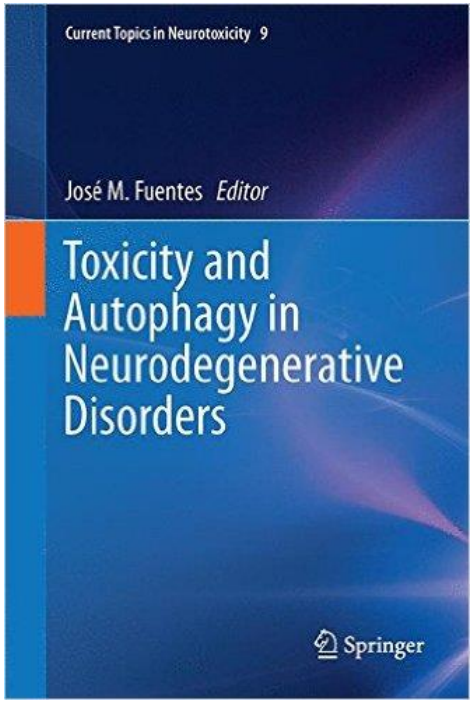
Better understanding of PD pathogenesis
Identification of new therapeutic targets



ROTENONE

- pesticide and mitochondrial complex I inhibitor

- used to generate PD animal models



Chapter 12
Exploring the Role of Autophagy in the Pathogenesis of Rotenone-induced Toxicity

Gessica Sala, Giovanni Stefanoni, Daniele Marinig and Carlo Ferrarese

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BioMed Research International
Volume 2013, Article ID 846725, 10 pages
http://dx.doi.org/10.1155/2013/846725

Research Article

Rotenone Upregulates Alpha-Synuclein and Myocyte Enhancer Factor 2D Independently from Lysosomal Degradation Inhibition

Gessica Sala,[1] Alessandro Arosio,[1,2] Giovanni Stefanoni,[1,3] Laura Melchionda,[1] Chiara Riva,[1] Daniele Marinig,[1] Laura Brighina,[3] and Carlo Ferrarese[1,3]

NeuroToxicology 54 (2016) 161–169

Contents lists available at ScienceDirect

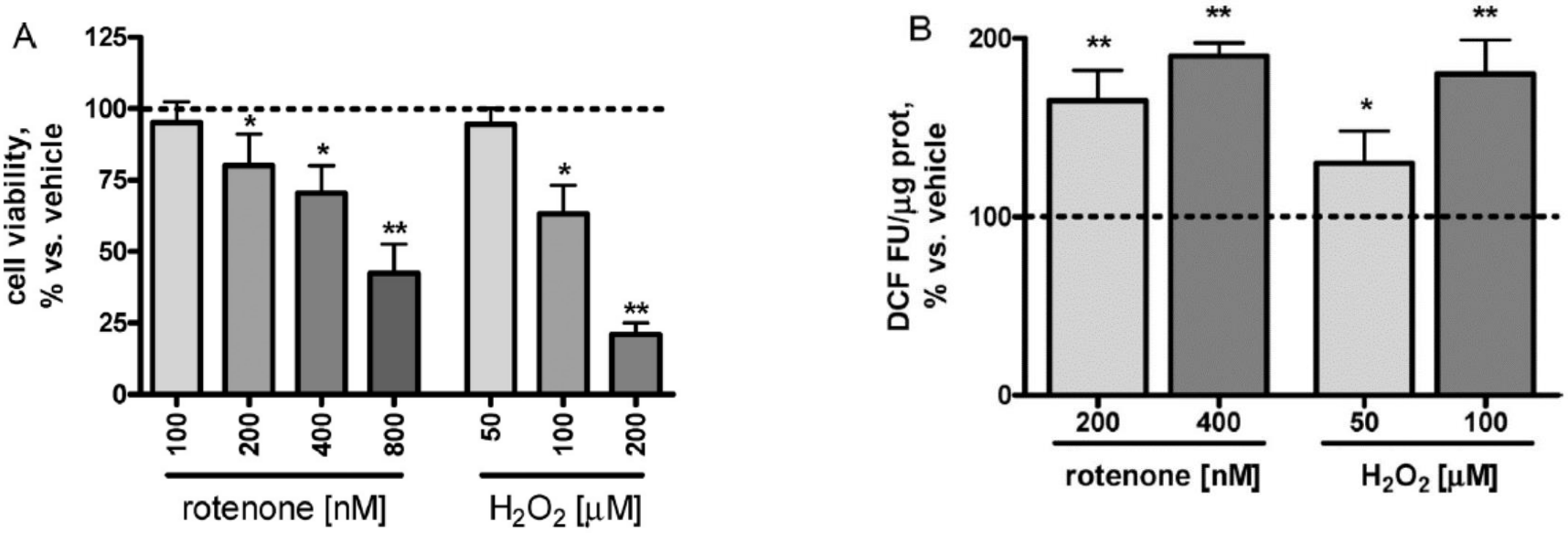
NeuroToxicology

Full length article

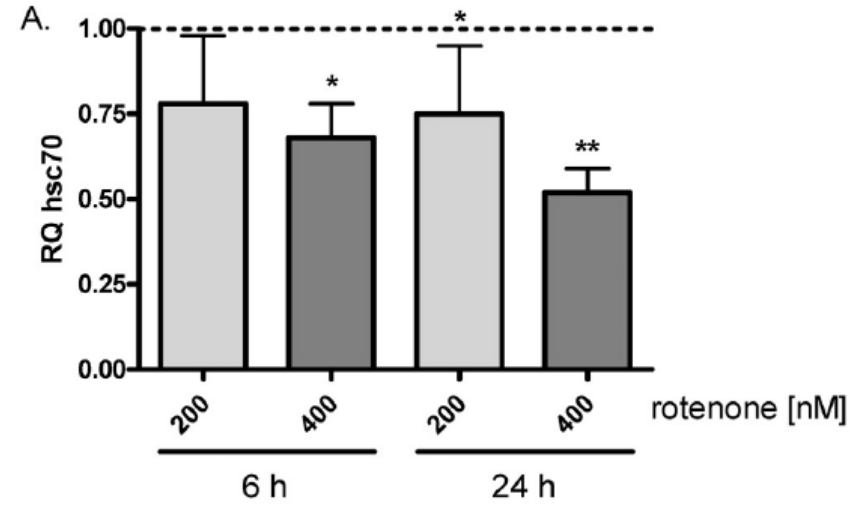
Rotenone down-regulates HSPA8/hsc70 chaperone protein *in vitro*: A new possible toxic mechanism contributing to Parkinson's disease

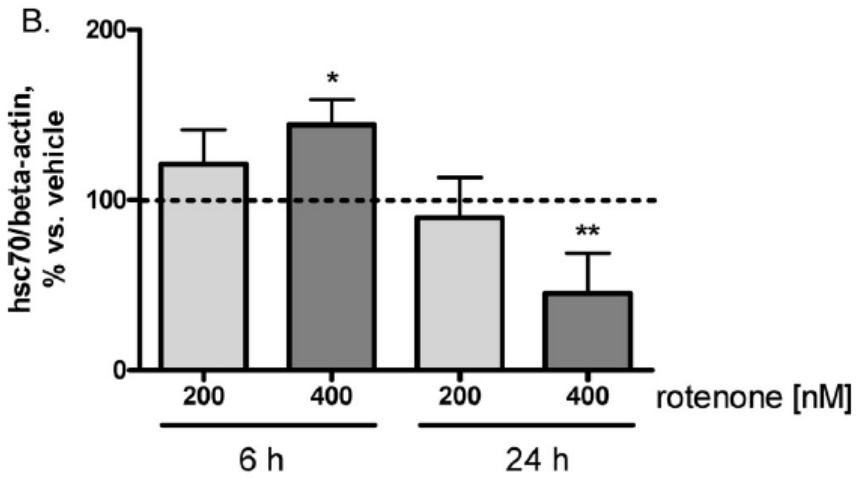
Gessica Sala[a,f,*], Daniele Marinig[a,b,f], Chiara Riva[a,f], Alessandro Arosio[a,f], Giovanni Stefanoni[a,c], Laura Brighina[c,f], Matteo Formenti[d,e], Lilia Alberghina[d,e,f], Anna Maria Colangelo[d,e,f], Carlo Ferrarese[a,c,f]

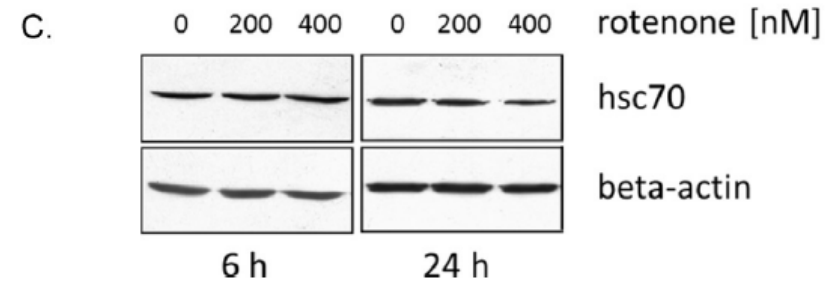
human neuroblastoma SH-SY5Y cells

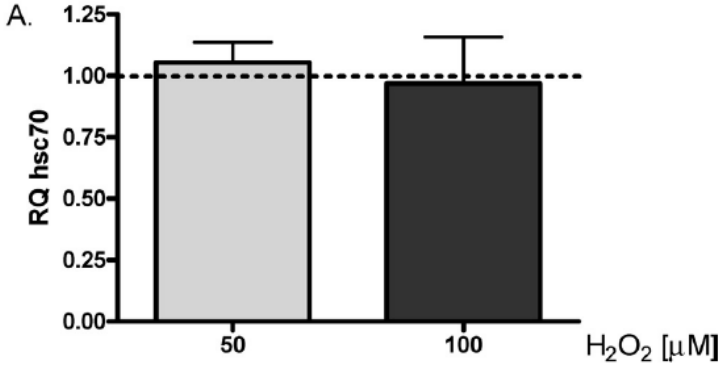


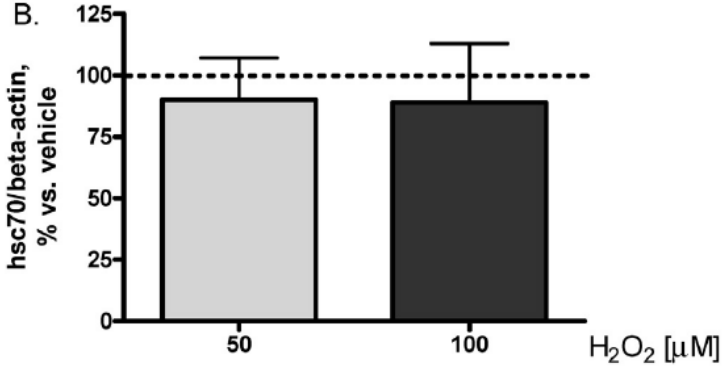
Sala et al., Neurotoxicology 2016

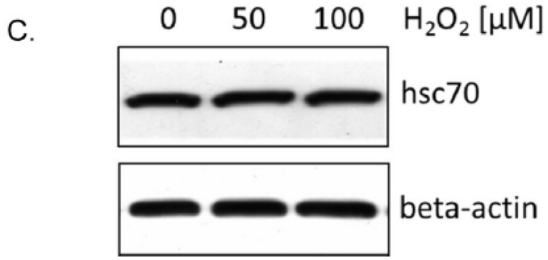




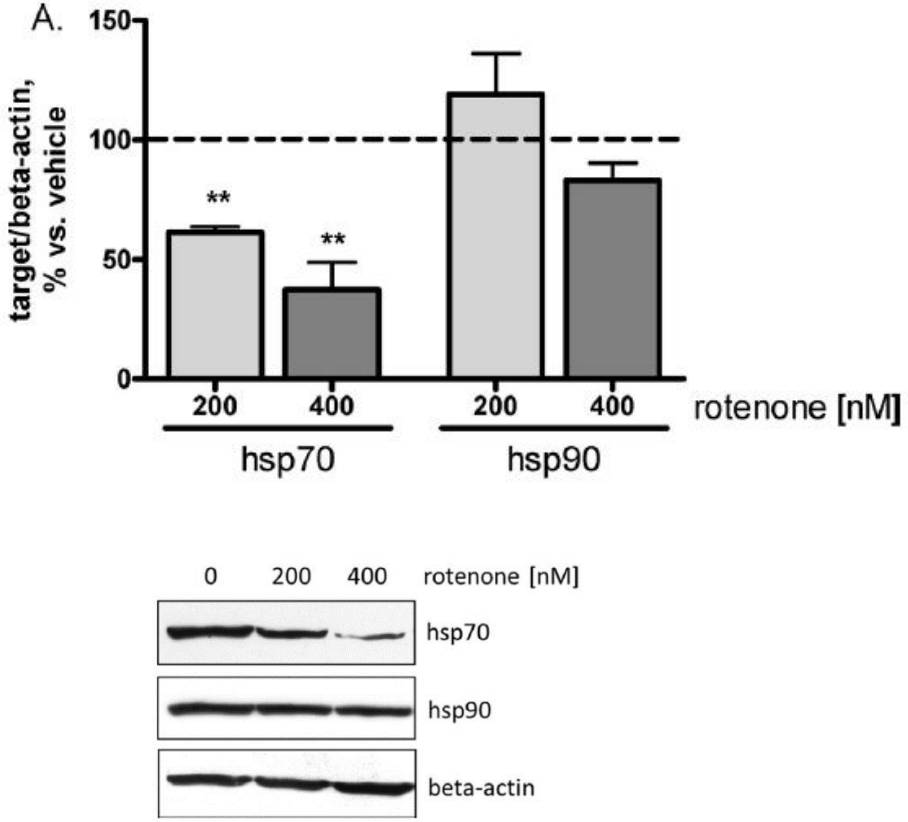




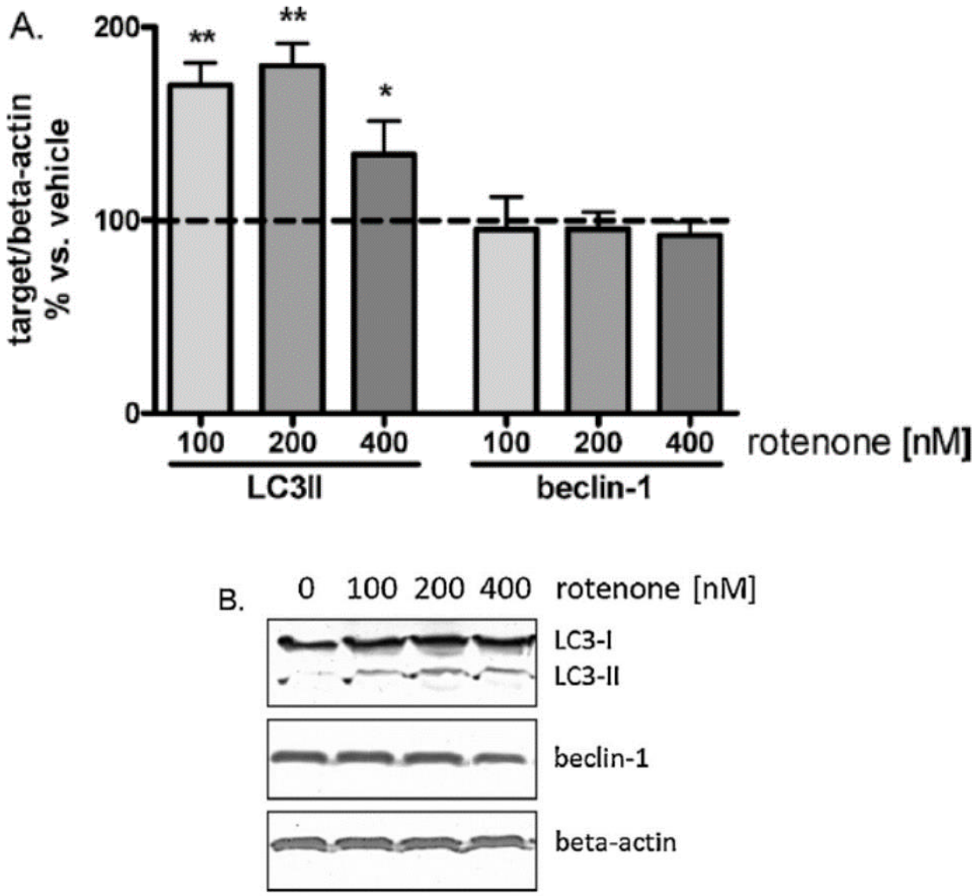




Sala et al., Neurotoxicology 2016



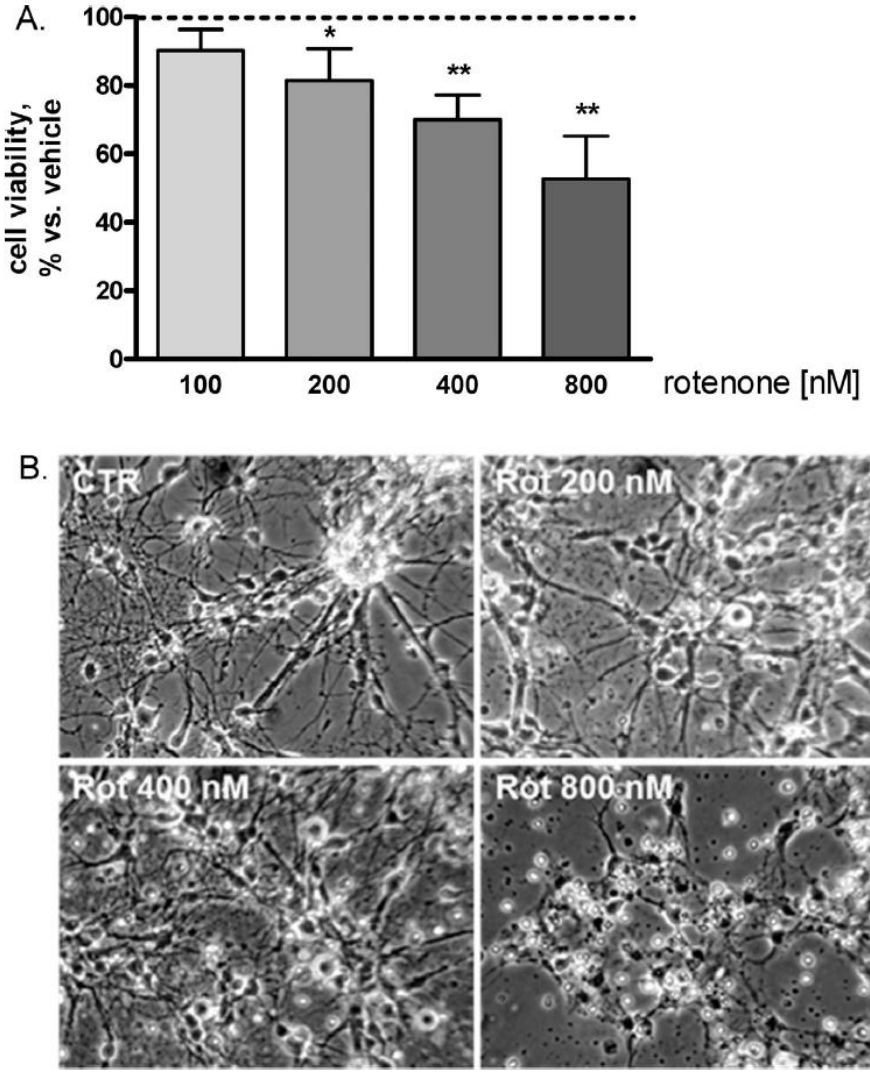
Sala et al., Neurotoxicology 2016

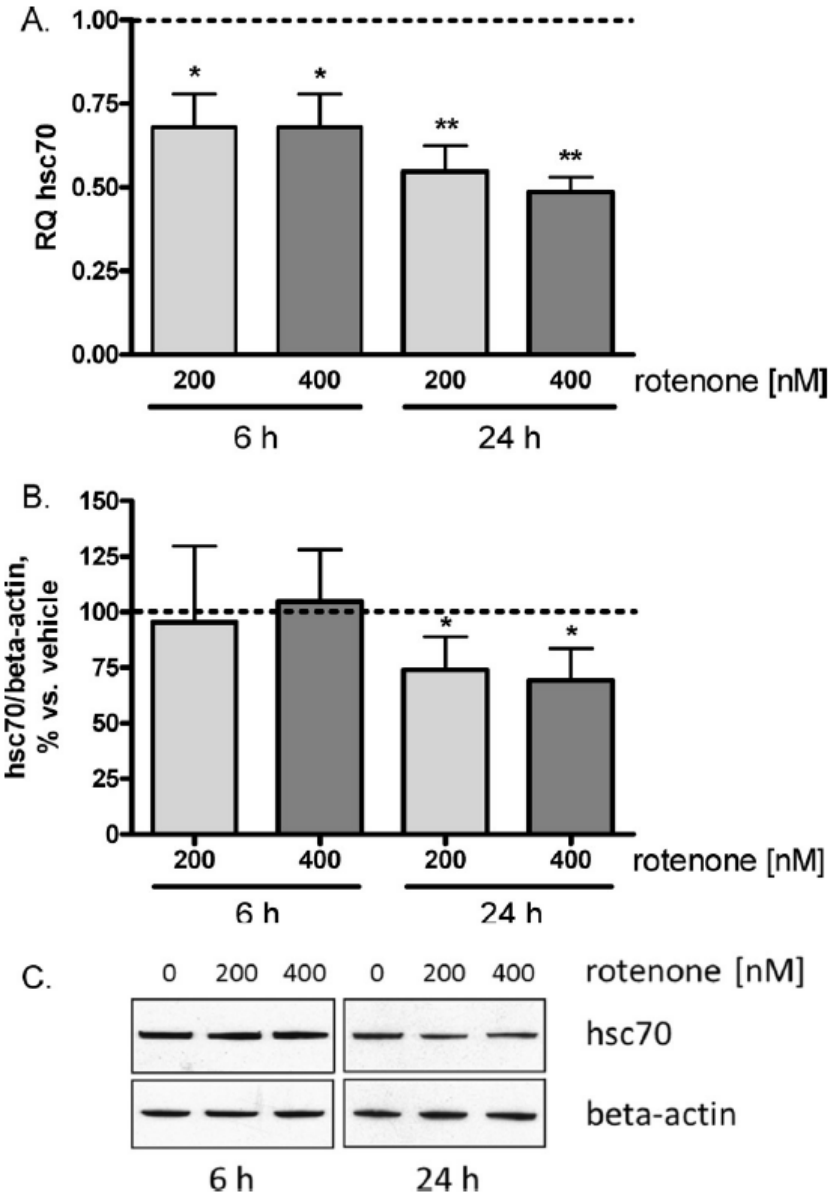


↑ LC3-II ➡ macroautophagy induction …or autophagosome accumulation

Sala et al., Neurotoxicology 2016

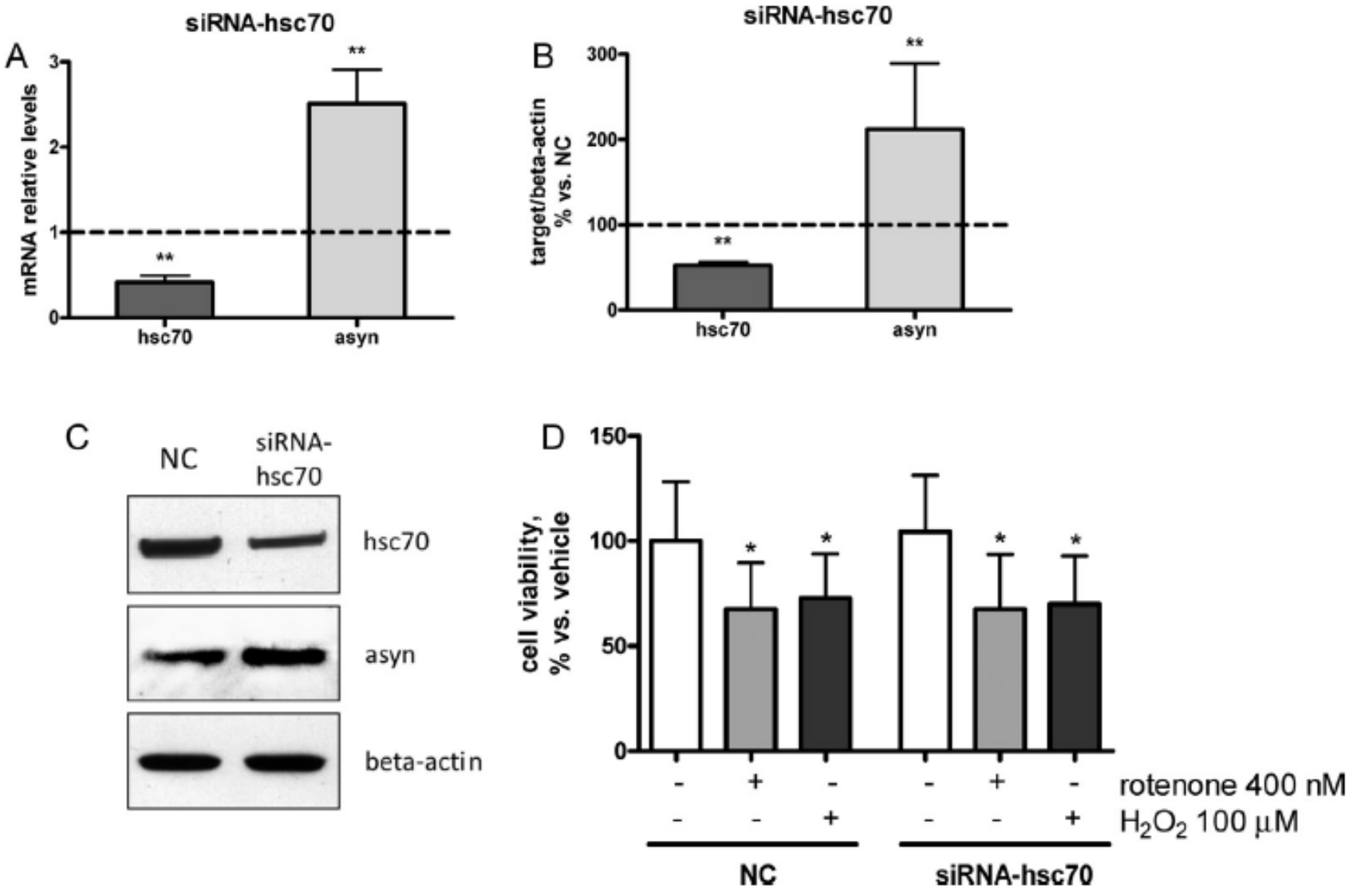
mouse cortical neurons *





* Prof. A. M. Colangelo

Sala et al., Neurotoxicology 2016



Sala et al., Neurotoxicology 2016

Neuroscience Letters 736 (2020) 135272

Contents lists available at ScienceDirect

Neuroscience Letters

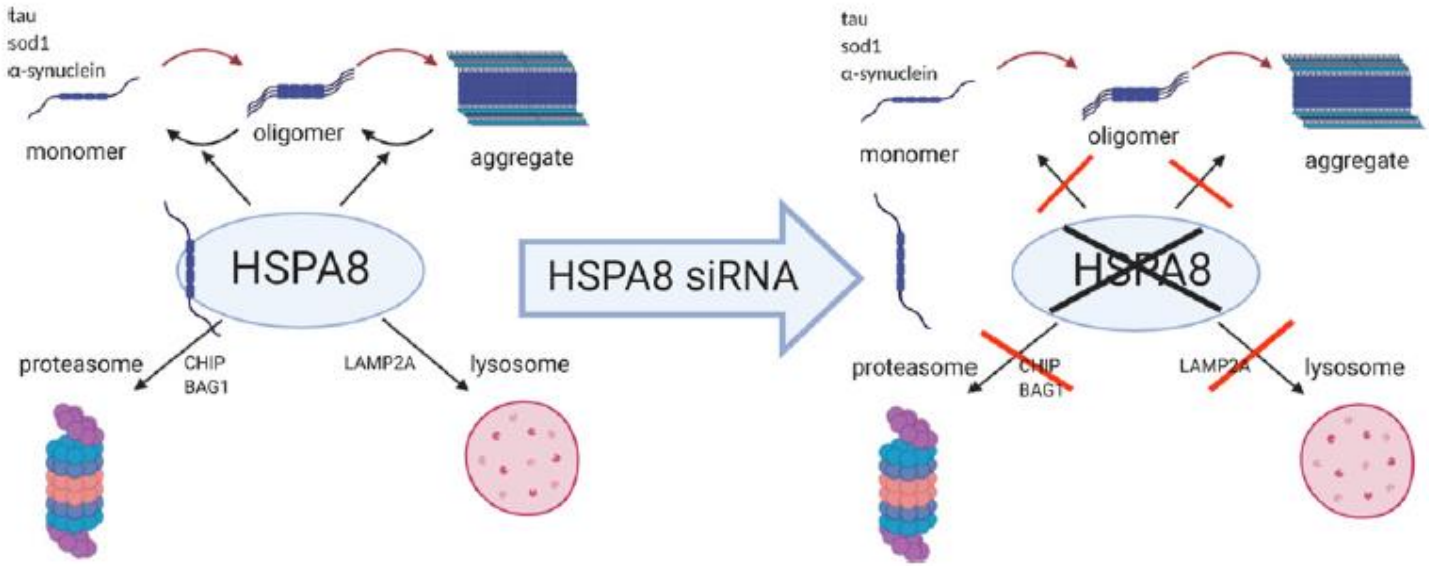
journal homepage: www.elsevier.com/locate/neulet

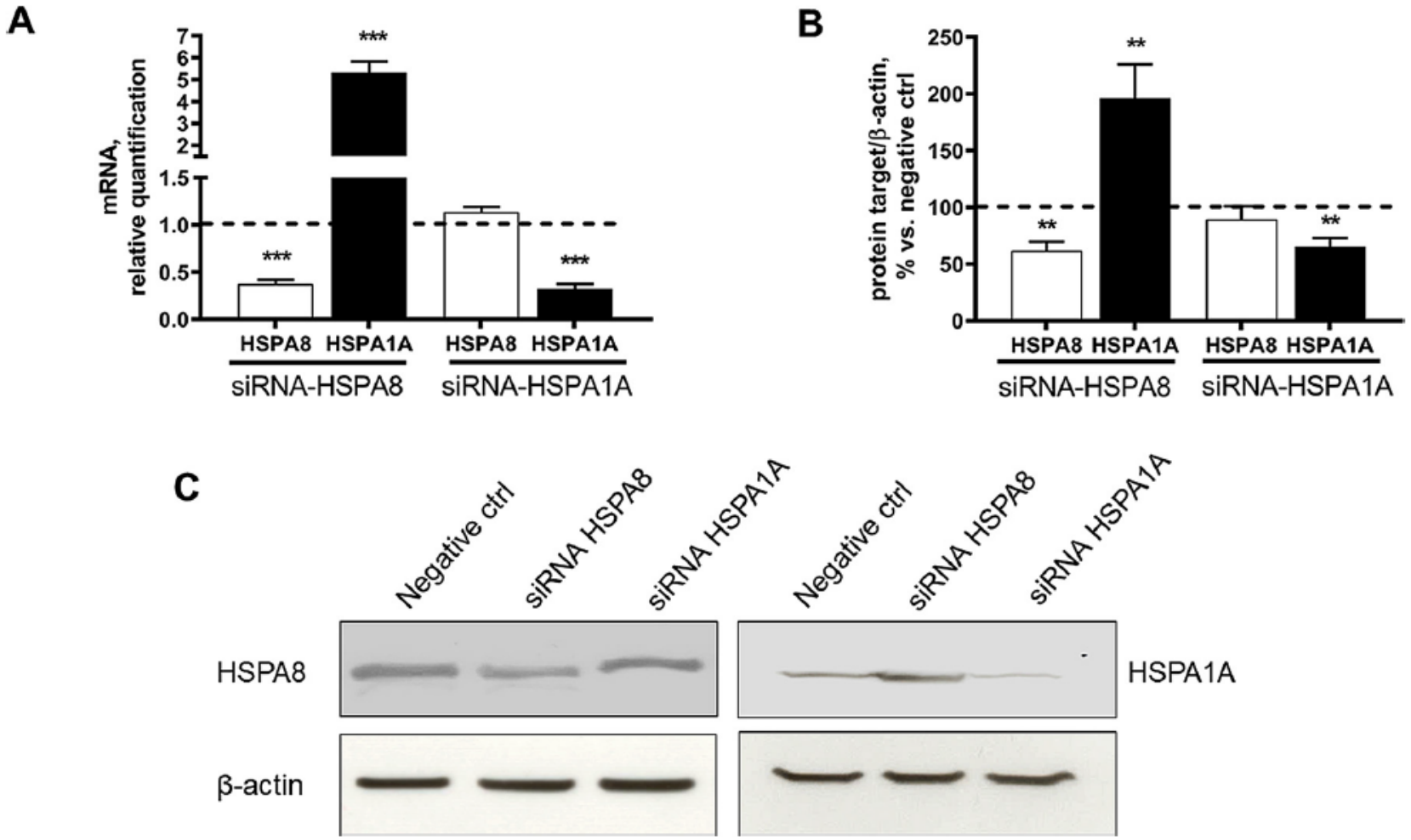
Research article

HSPA8 knock-down induces the accumulation of neurodegenerative disorder-associated proteins

Riccardo Sirtori[a,*], Chiara Riva[a], Carlo Ferrarese[a,b], Gessica Sala[a]

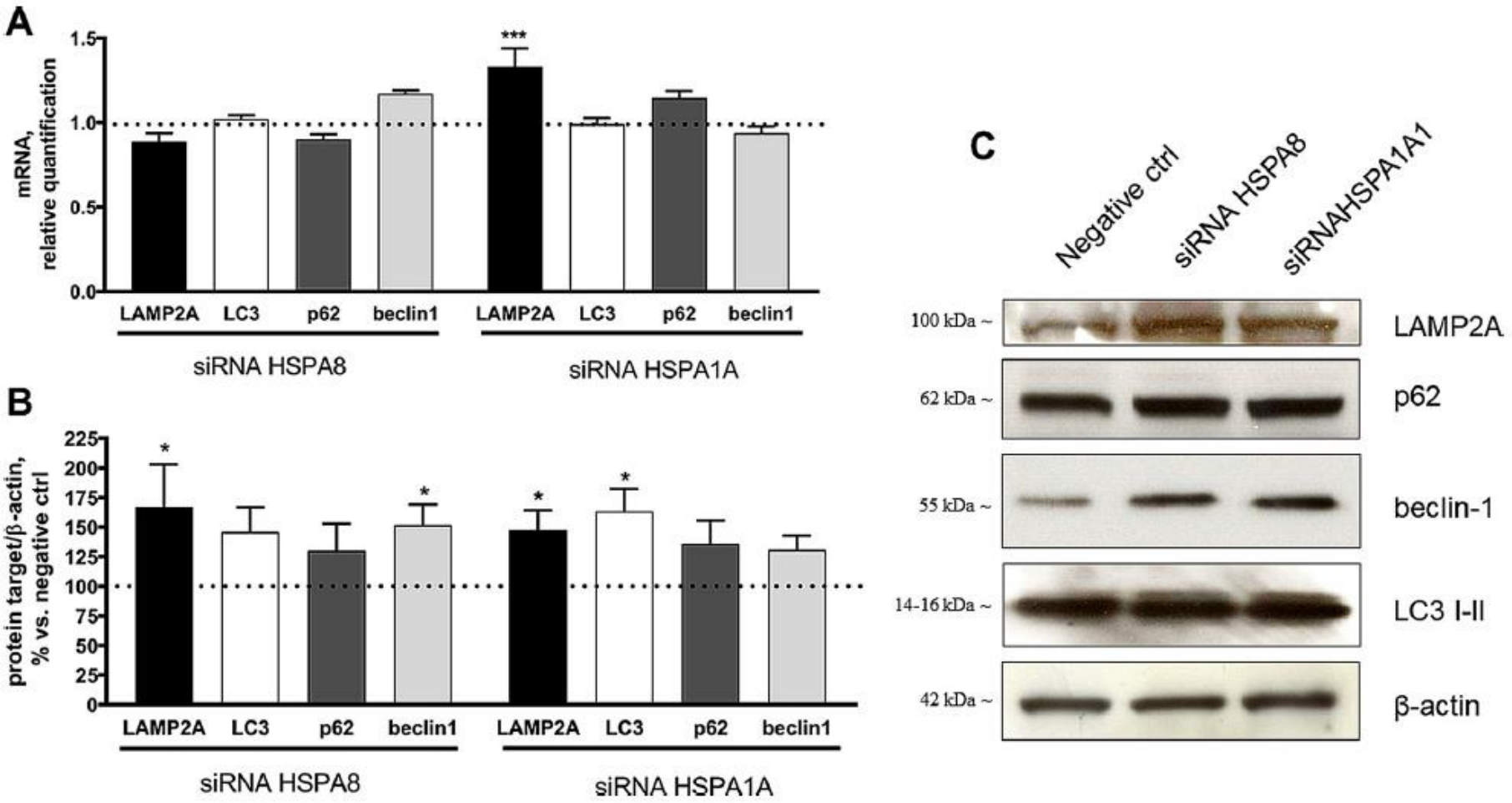
[a] School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza, Italy
[b] Department of Neurology, ASST-Monza, San Gerardo Hospital, Monza, Italy





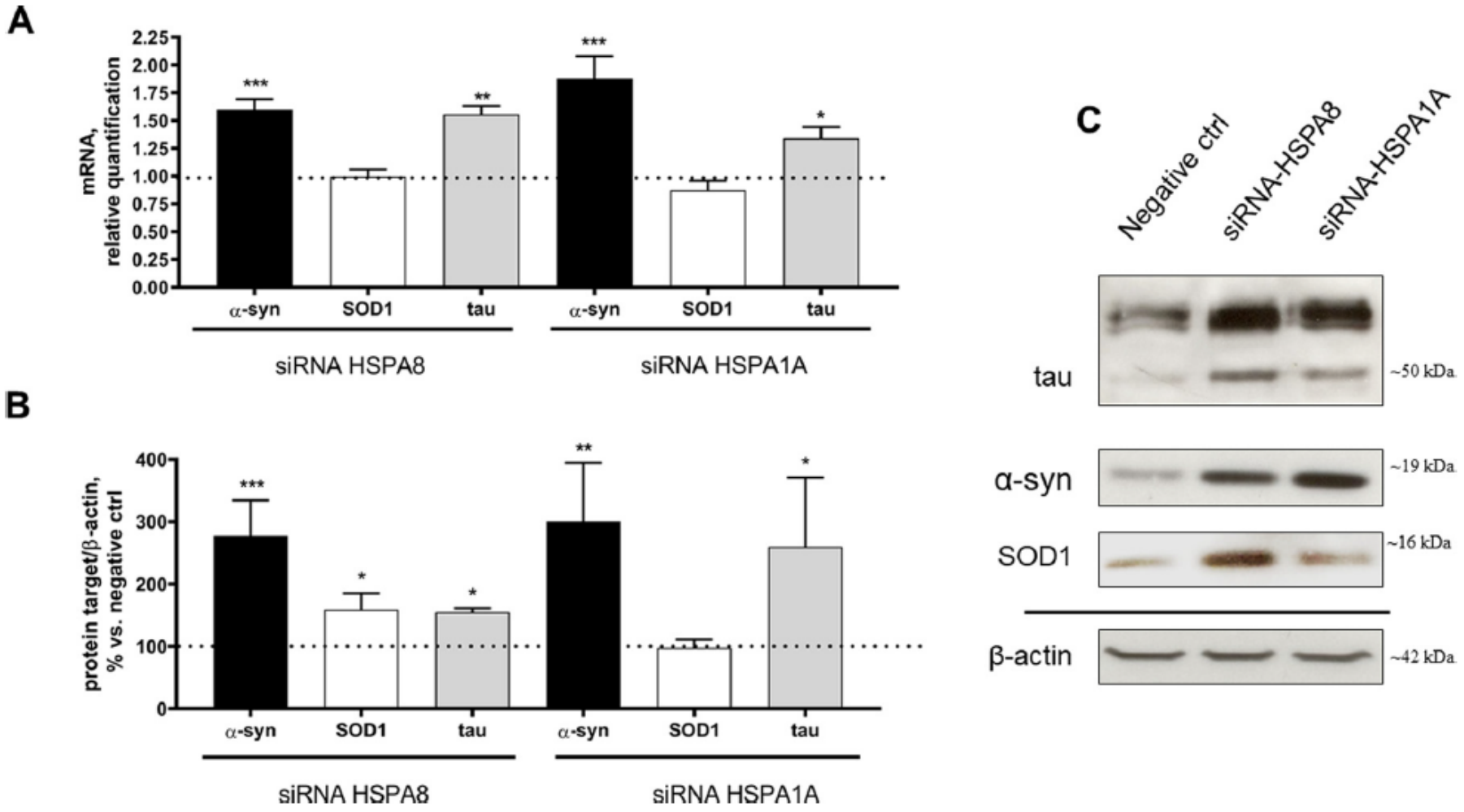
HSC70 (HSPA8) knock-down induces HSP70 (HSPA1A) overexpression

Sirtori et al., Neurosci Lett 2020



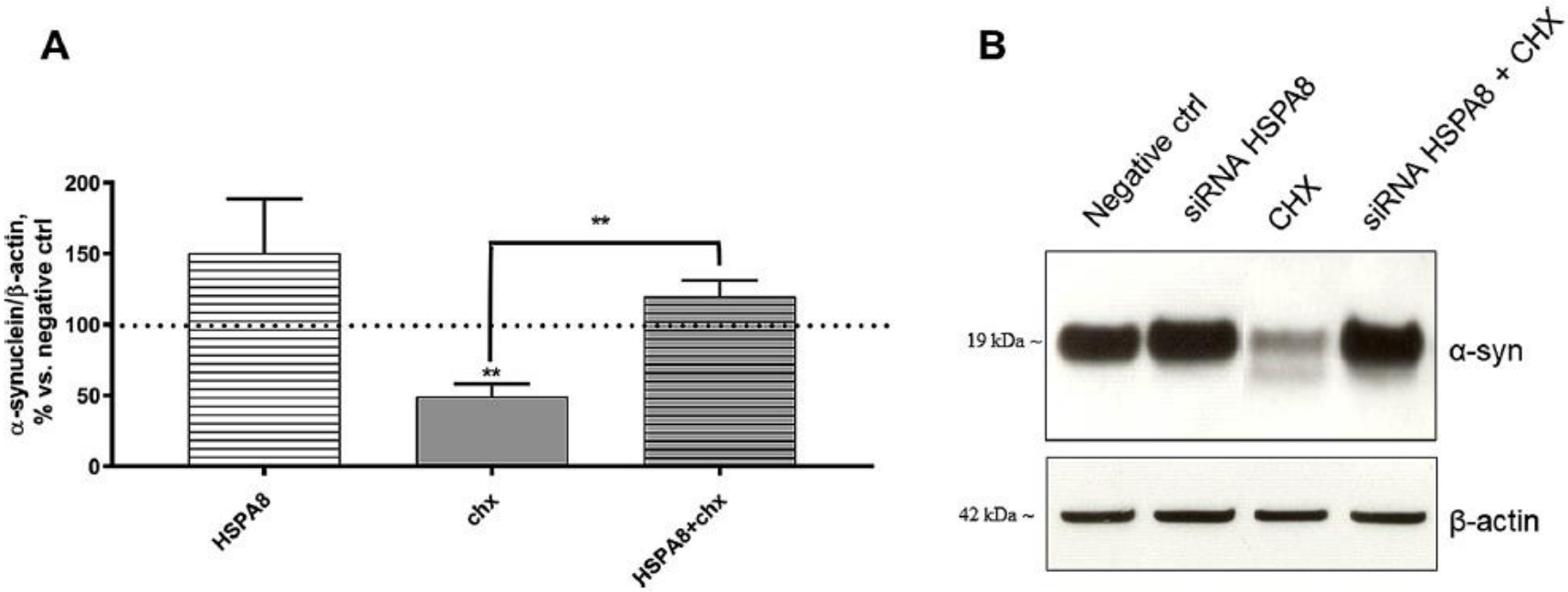
HSPA8 and HSPA1A knock-down induces the expression of proteins implied in autophagic processes

Sirtori et al., Neurosci Lett 2020



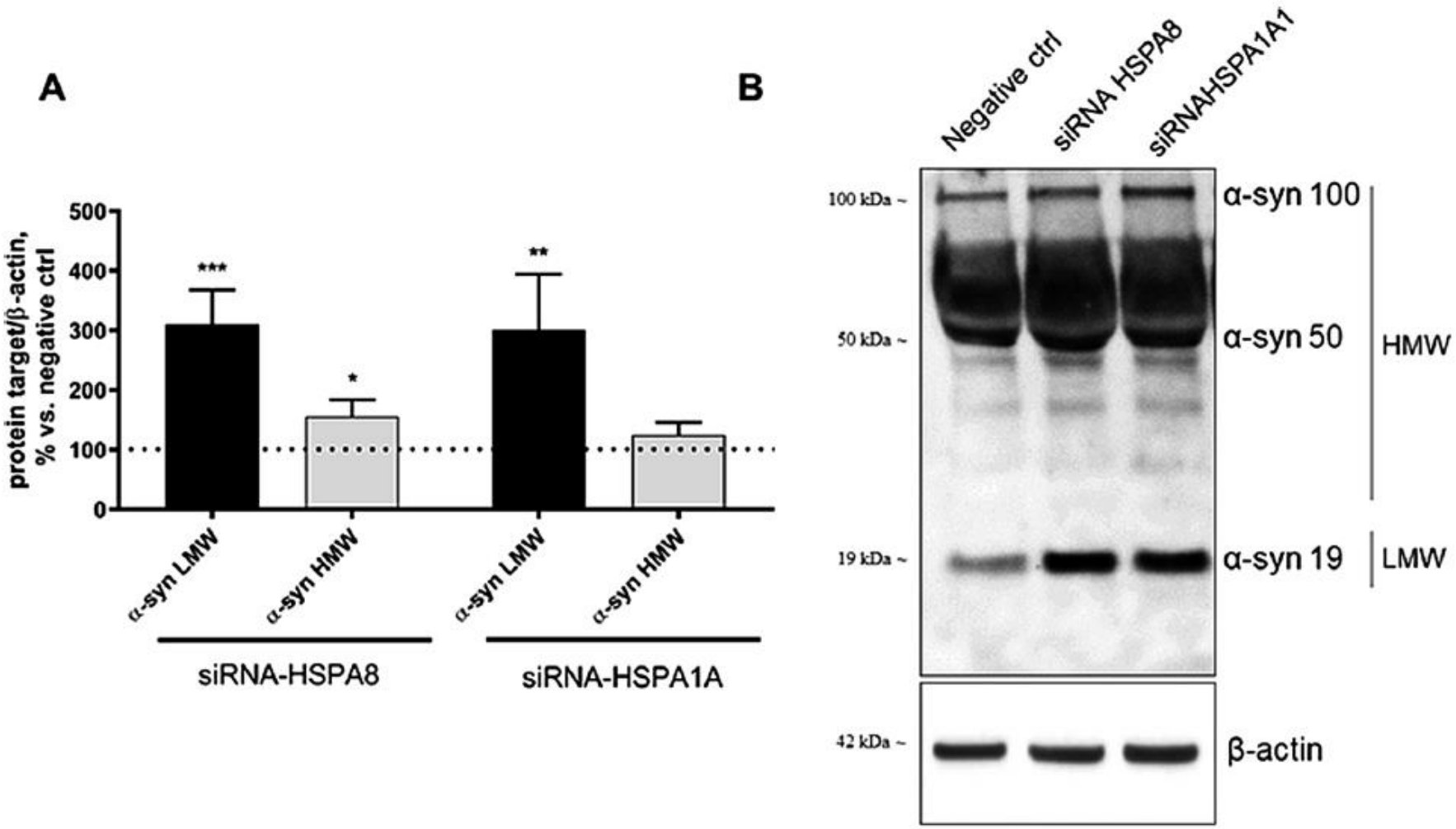
HSPA8 or HSPA1A knock-down increases the expression of different proteins related to neurodegeneration

Sirtori et al., Neurosci Lett 2020



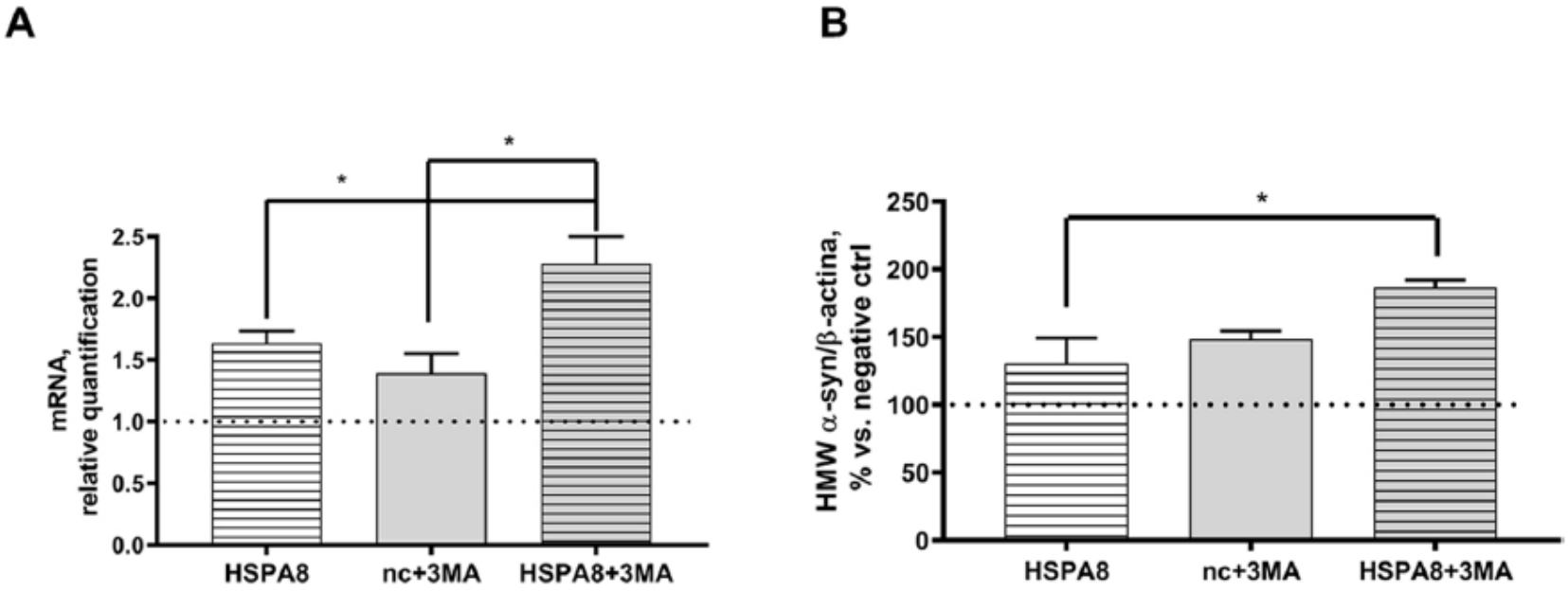
a-syn increase induced by HSPA8 or HSPA1A knock-down is due to a reduced degradation

Sirtori et al., Neurosci Lett 2020



HSPA8 and HSPA1A knock-down induces the expression of asyn monomeric and oligomeric forms

Sirtori et al., Neurosci Lett 2020



HSPA8 and macroautophagy inhibition synergistically increase the accumulation of HMW asyn

Sirtori et al., Neurosci Lett 2020

Neuroprotective compounds: 1. therapeutic use (drug repositioning)

AMBROXOL

eFarma

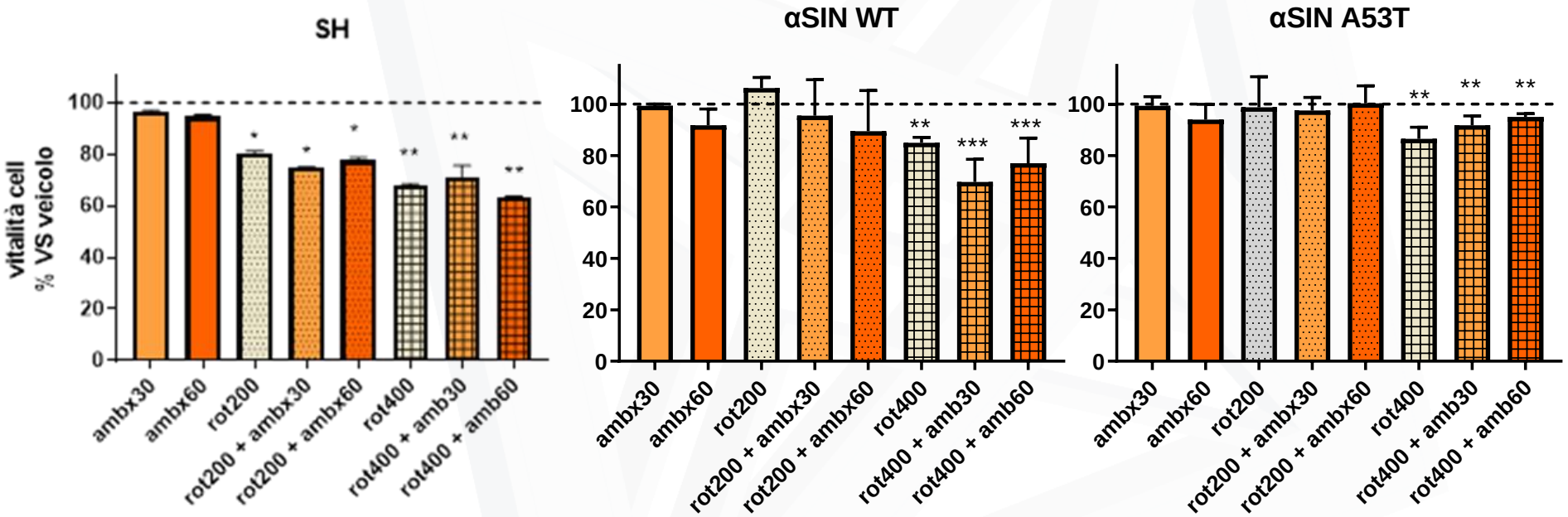
- expectorant and anti-inflammatory activity

- ↑ lysosomal biochemistry

potential disease-modifying treatment for PD

Effect of ambroxol on cell viability in rotenone-treated cells

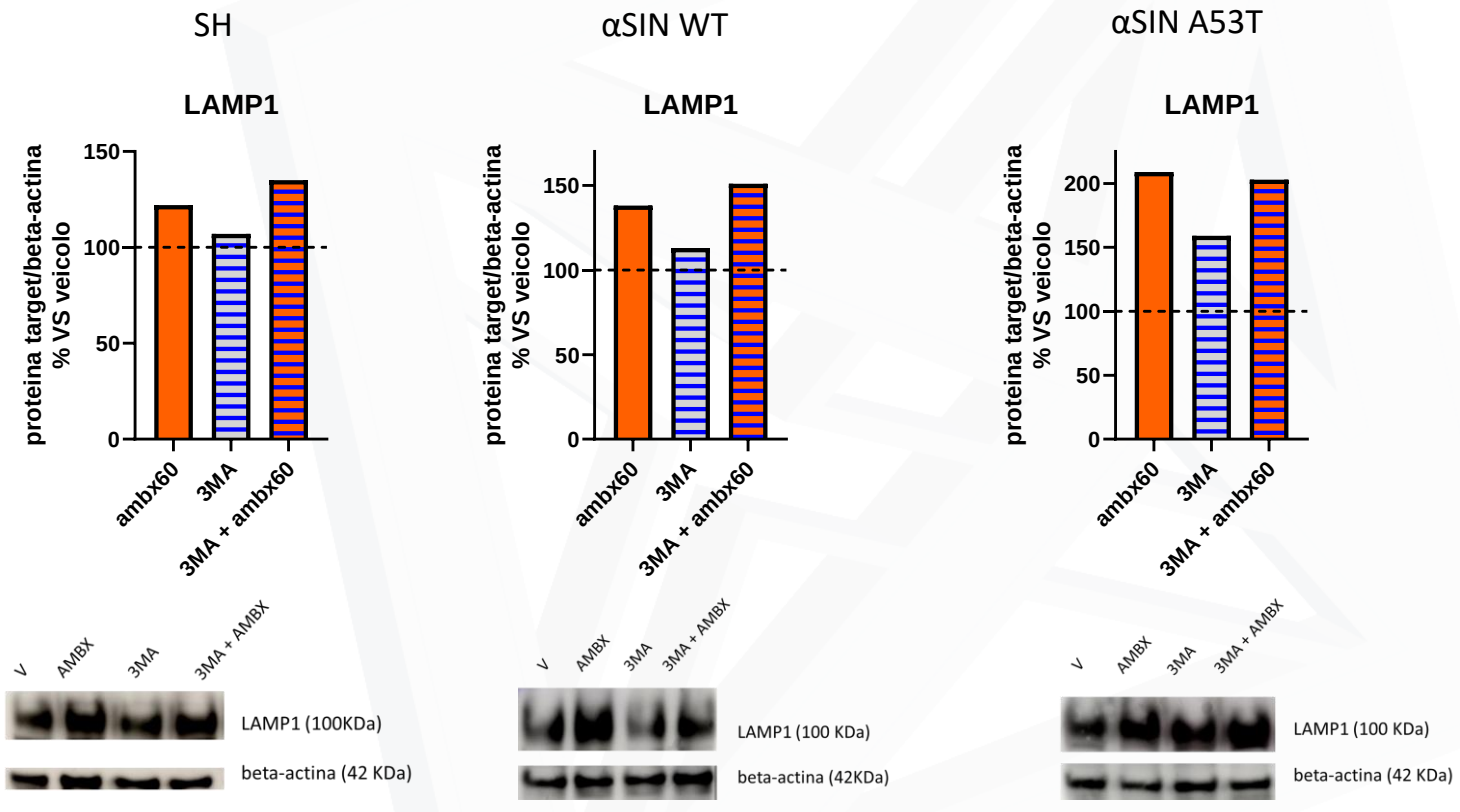
Cells pre-treated with ambroxol (30 or 60 µM for 48 h), then co-treated with ambroxol + rotenone (200 or 400 nM for 24 h)



mild citoprotective effect of ambroxol only in A53T asyn cells

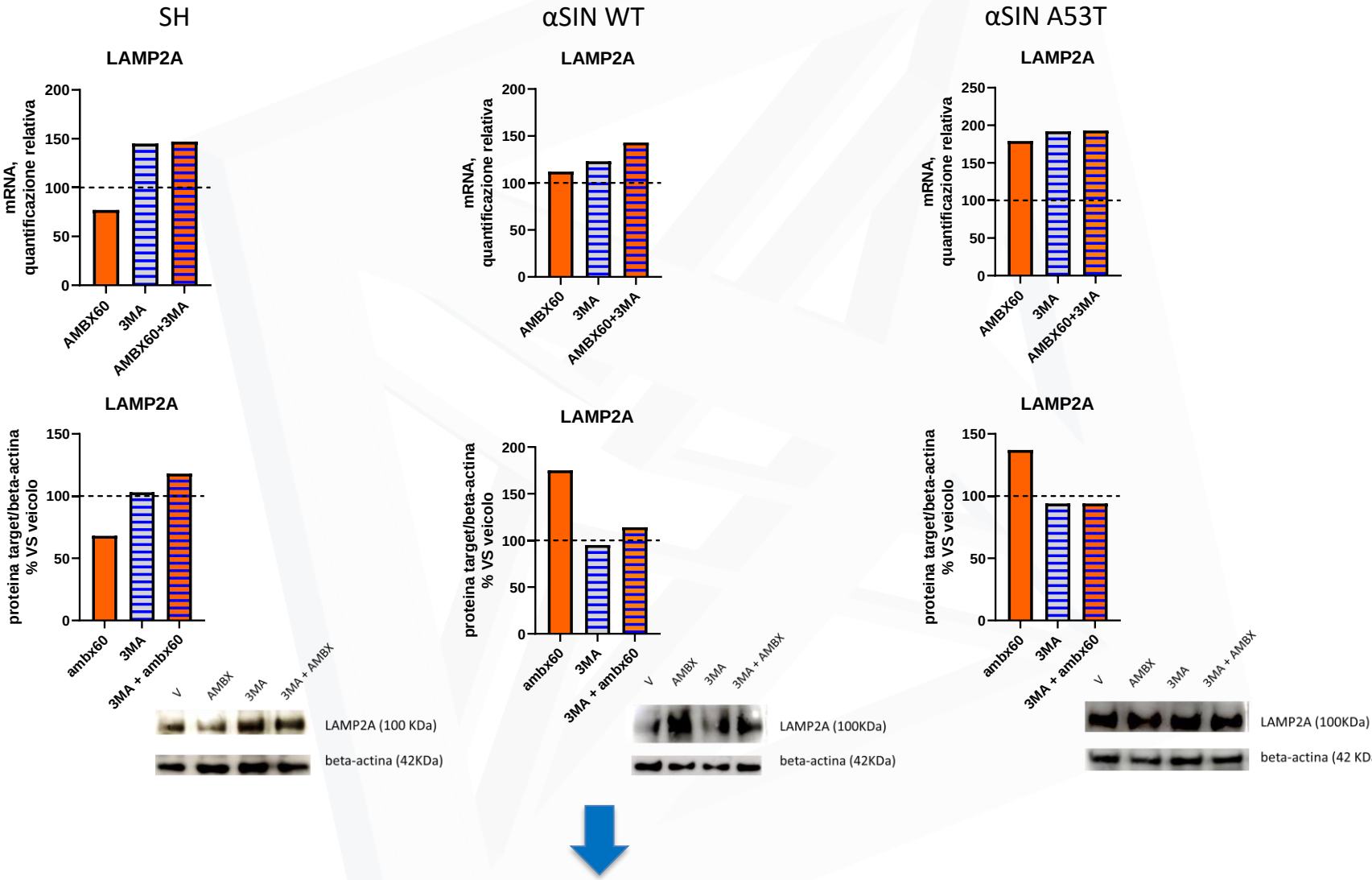
Effect of ambroxol on lysosomes

Cells pre-treated with ambroxol (60 µM/48 h), then co-treated with ambroxol + 3-MA (5 mM/24 h)



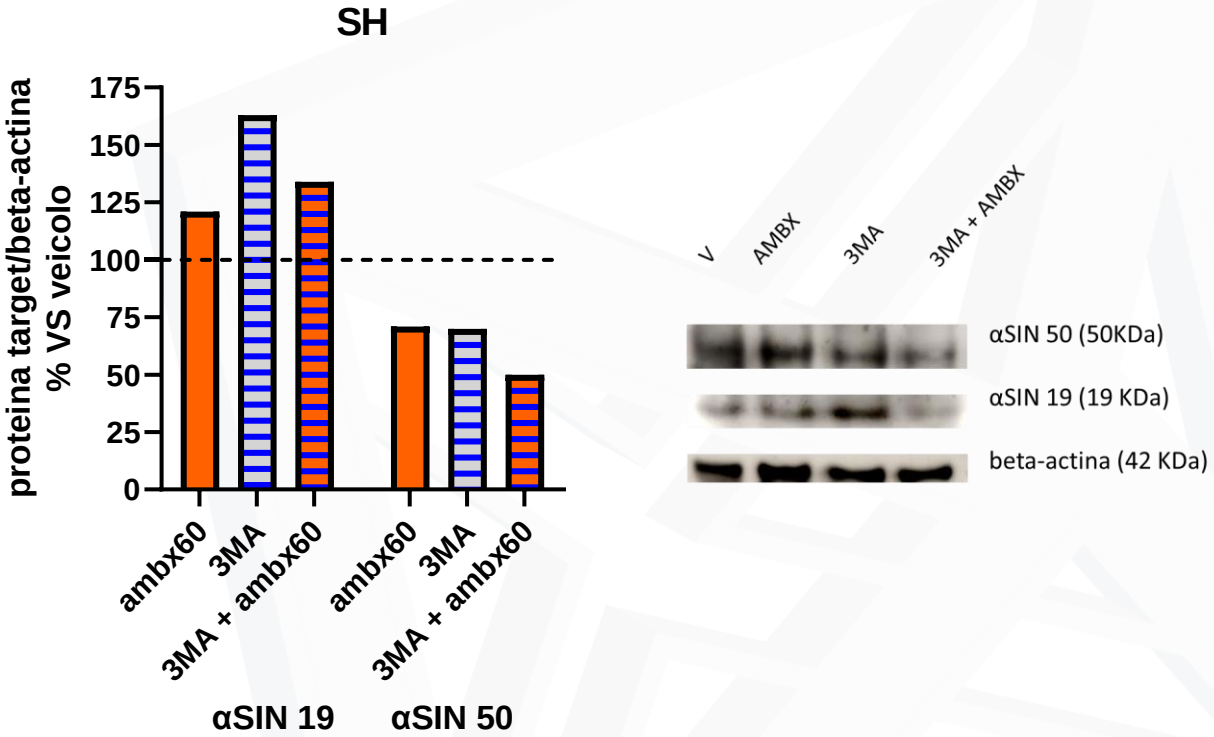
ambroxol induces the expression of a lysosomal marker

Effect of ambroxol on CMA



ambroxol induces LAMP2A expression only in transfected cells, but the effect of 3-MA on LAMP2A is prevalent

Effect of ambroxol on asyn



ambroxol partially counteracts the macroautophagy inhibition induced by 3-MA

Neuroprotective compounds: 2. preventive approach

COFFEE EXTRACTS

COCOA EXTRACTS

HOP EXTRACTS

CINNAMON EXTRACTS

collaboration with Prof. Cristina Airoldi, BioNMR Lab, BtBs, UNIMIB

Food Chemistry 252 (2018) 171–180

Contents lists available at ScienceDirect

Food Chemistry

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

NMR-driven identification of anti-amyloidogenic compounds in green and roasted coffee extracts

Carlotta Ciaramelli[a], Alessandro Palmioli[a], Ada De Luigi[b], Laura Colombo[b], Gessica Sala[c,d], Chiara Riva[c,d], Chiara Paola Zoia[c,d], Mario Salmona[b], Cristina Airoldi[a,d,*]

[a] Department of Biotechnologies and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milan, Italy
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Food Chemistry 341 (2021) 128249

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Food Chemistry

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

NMR-based *Lavado* cocoa chemical characterization and comparison with fermented cocoa varieties: Insights on cocoa's anti-amyloidogenic activity

Carlotta Ciaramelli[a,d], Alessandro Palmioli[a,d], Ada De Luigi[b], Laura Colombo[b], Gessica Sala[c,d], Mario Salmona[b], Cristina Airoldi[a,d,*]

[a] BioOrgNMR Lab, Department of Biotechnologies and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milan, Italy
[b] Department of Biochemistry and Molecular Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri" IRCCS, Via Mario Negri 2, 20156 Milano, Italy
[c] School of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900 Monza, Italy
[d] Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, P.zza dell'Ateneo Nuovo 1, 20126 Milano Italy

frontiers | Frontiers in Chemistry

ORIGINAL RESEARCH
published: 08 June 2022
doi: 10.3389/fchem.2022.896253

NMR-Driven Identification of Cinnamon Bud and Bark Components With Anti-Aβ Activity

Carlotta Ciaramelli[1,2†], *Alessandro Palmioli*[1,2†], *Irene Angotti*[1], *Laura Colombo*[3], *Ada De Luigi*[3], *Gessica Sala*[2,4], *Mario Salmona*[3] and *Cristina Airoldi*[1,2*]

[1]*BioOrgNMR Lab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy,* [2]*Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Milano, Italy,* [3]*Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri"- IRCCS, Milano, Italy,* [4]*School of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy*

ACS Chemical Neuroscience

pubs.acs.org/chemneuro

Research Article

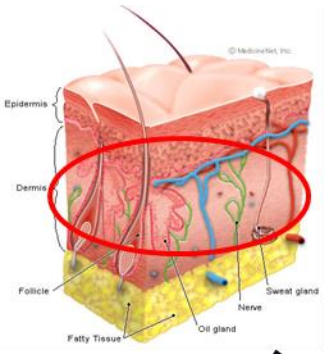
Alzheimer's Disease Prevention through Natural Compounds: Cell-Free, *In Vitro*, and *In Vivo* Dissection of Hop (*Humulus lupulus* L.) Multitarget Activity

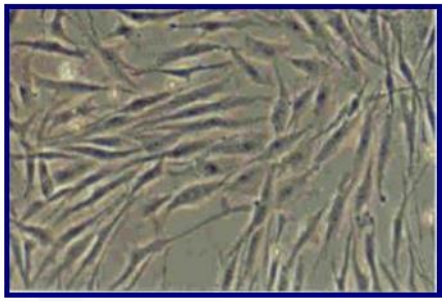
Alessandro Palmioli,* Valeria Mazzoni, Ada De Luigi, Chiara Bruzzone, Gessica Sala, Laura Colombo, Chiara Bazzini, Chiara Paola Zoia, Mariagiovanna Inserra, Mario Salmona, Ivano De Noni, Carlo Ferrarese, Luisa Diomede, and Cristina Airoldi*

Cite This: https://doi.org/10.1021/acschemneuro.2c00444

Accepted: October 12, 2022

Patient-derived fibroblast cell lines





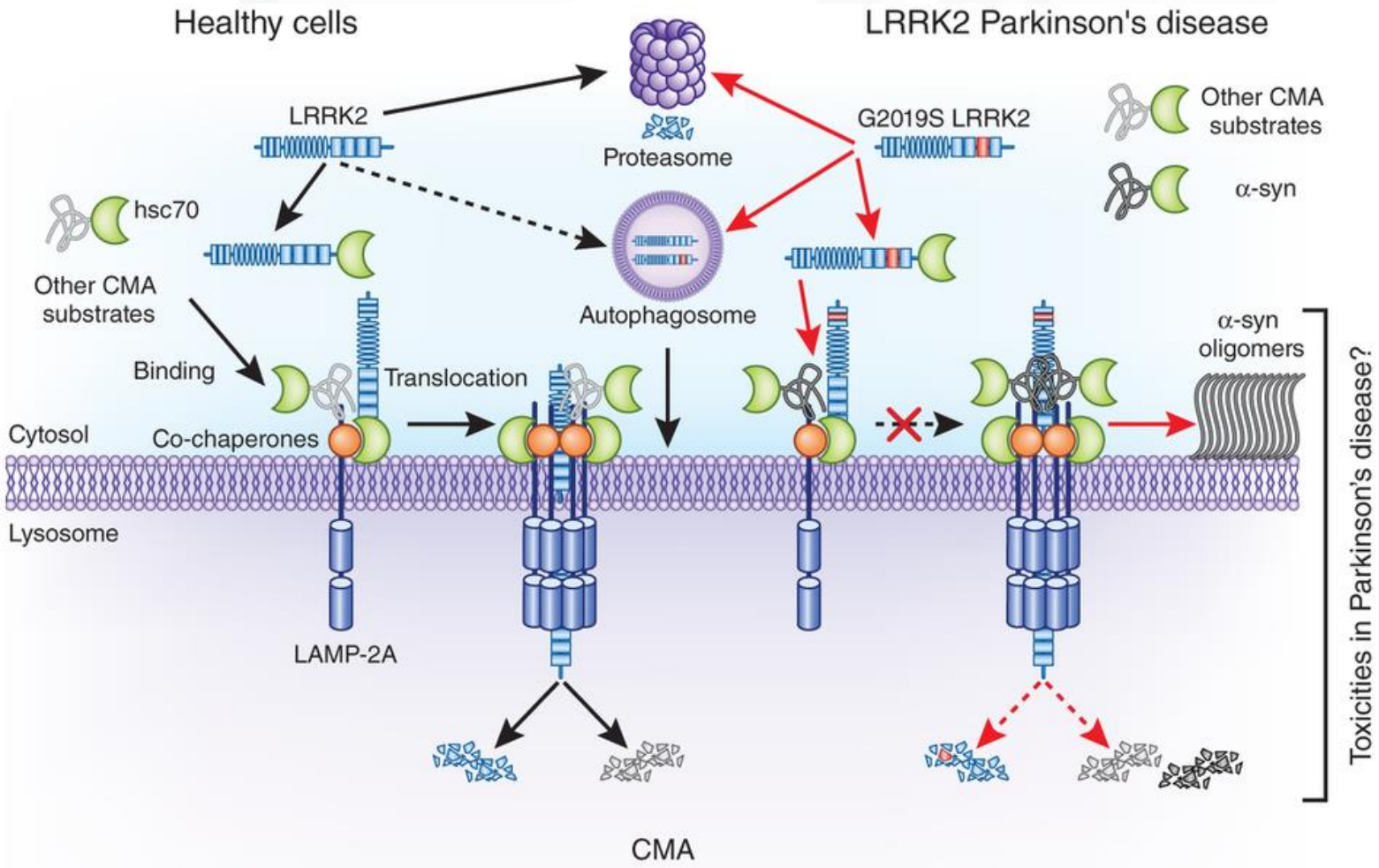
	Controls	*Sporadic PD*	*G2019S LRRK2* *
Number	5	6	3
Age (years)	63.2 ± 8.8	64.3 ± 8.9	68 ± 6.6
Sex (M/F)	2/3	2/4	1/2
Age at onset (years)	n.a.	62 ± 3.4	52 ± 6.6
Disease duration (years)	n.a.	6.5 ± 1.3	16 ± 1.7

* G2019S LRRK2 cell lines were provided by the “Cell line and DNA biobank from patients affected by genetic diseases” and “Parkinson Institute Biobank”

Marinig et al., *manuscript in preparation*

Mutant LRRK2 impairs autophagy

Interference of mutant LRRK2 with autophagosome maturation (Alegre-Abarrategui *et al*., 2009) and CMA (Orenstein *et al.*, 2013)



from: Yue and Yang, Nature Neuroscience 2013

Autophagy restoration:

a promising therapeutic approach for neurodegenerative diseases

1. Macroautophgy-enhancing agents

mTOR-dependent pathways

mTOR-independent pathways

Ok in preclinical models

(↓ cell death, asyn aggregation, ox stress, mitochondrial dysfunction)

clinical translation is problematic

(limited selectivity for autophagy)

selective targeting

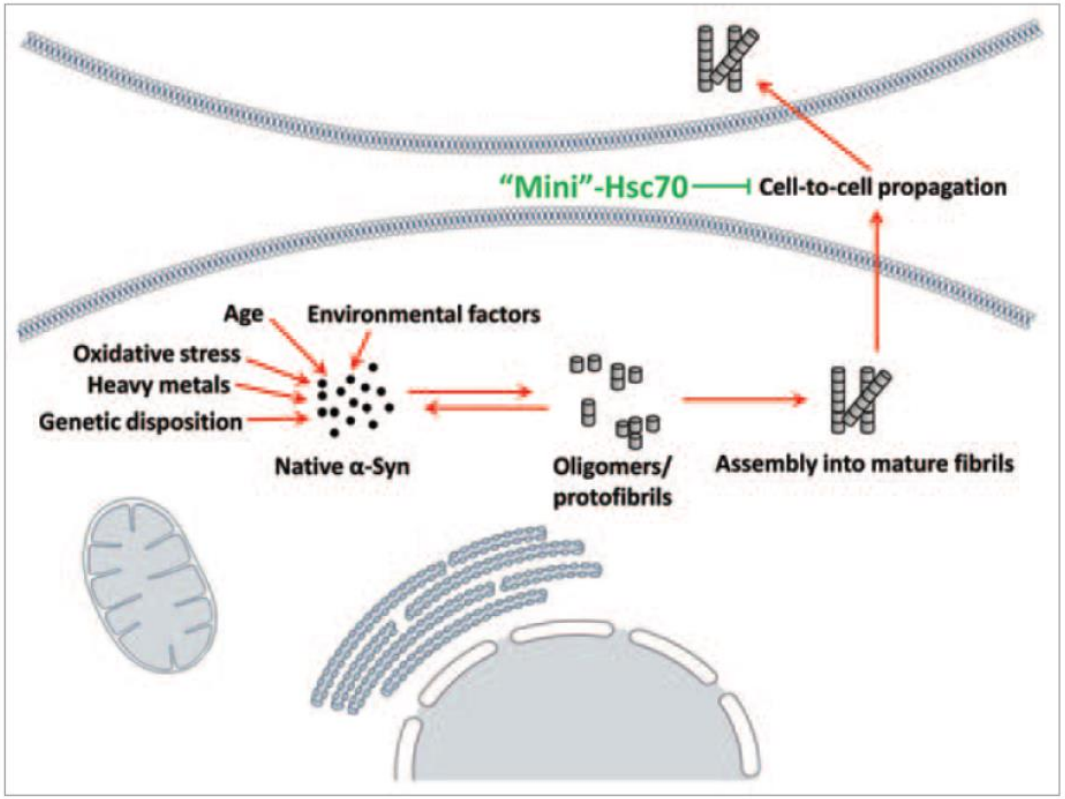
(beclin-1, lysosomes, mitophagy, micro-RNAs)

2. CMA-modulating approaches

1. lamp2A overexpression ↑ CMA activity and ↓ asyn turnover and neurotoxicity

2. targeted (rat SN) lamp2A overexpression ↓ asyn pathobiology

3. synthetic derivatives of RA → chemical CMA increase



-hsc70 binds to soluble asyn and slows down its assembly into fibrils

-hsc70 binds to fibrillar asyn 5-fold tighter than soluble asyn

4. engineering a 'minichaperone'-hsc70 useful against asyn assembly and propagation

New areas for investigation

1. **Glial autophagy**

2. **Autophagy and secretion of proteins**

   - **asyn** (↑ exosomal asyn release in PD)

   Danzer et al. Mol. Neurodegener. 2012; Ejlerskov et al. J. Biol. Chem. 2013; Lee et al. Exp. Mol. Med. 2013

Study of the cellular and molecular mechanisms of tDCS in an *in vitro* neuronal model: effects on levels and degradation of alpha-synuclein and implications for Parkinson's disease

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School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza

Tommaso Bocci
Alberto Priori

Aldo Ravelli Center for Neurotechnology and Experimental Brain therapeutics

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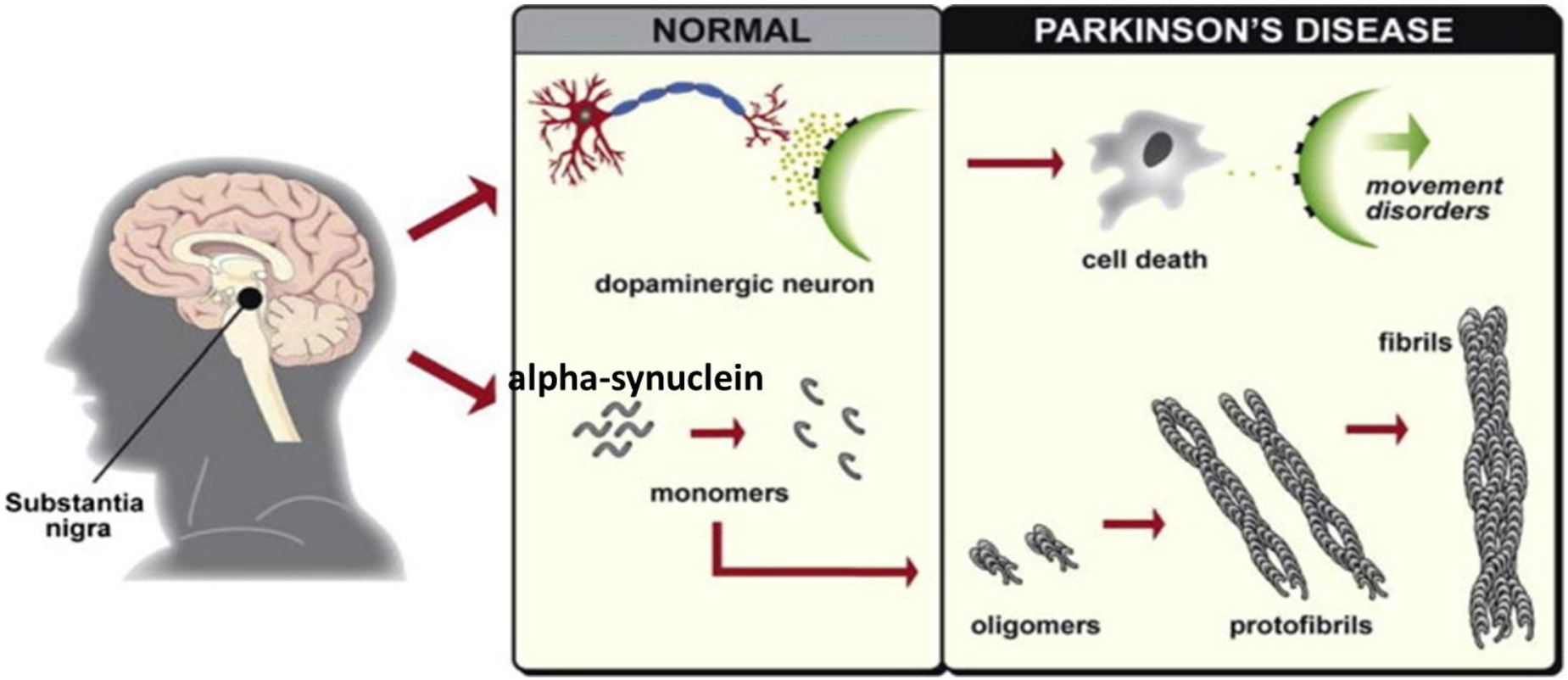
NeuroMI MILAN CENTER FOR NEUROSCIENCE

Parkinson's disease (PD) is a proteinopathy

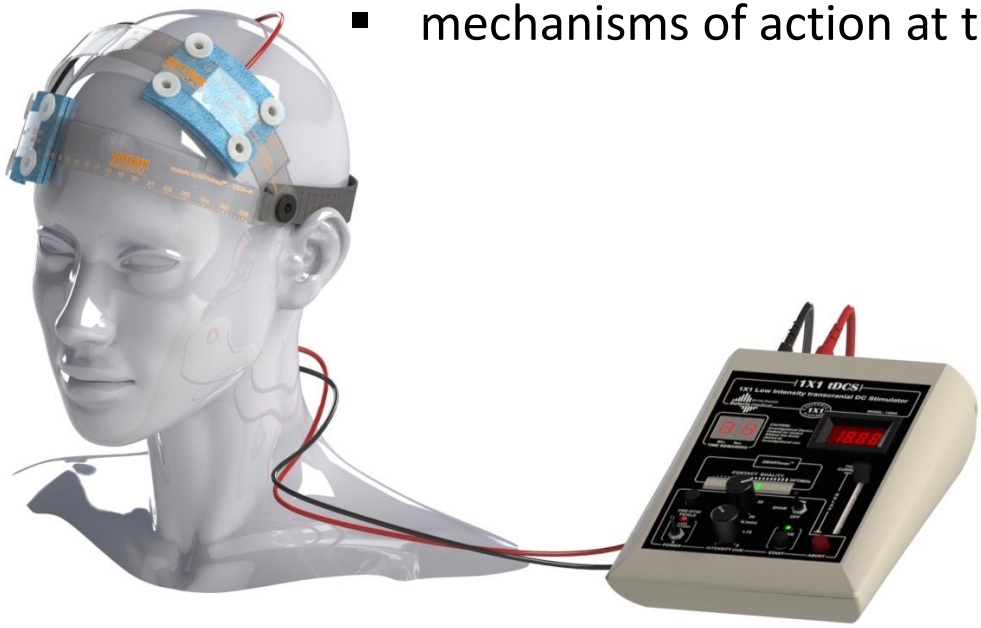


modified, from: Ruipérez et al., Prog Lipid Res. 2010

transcranial Direct Current Stimulation (tDCS) and PD

tDCS:

- non-invasive and safe tecnique to modulate neuronal excitability
- currents under the threshold (1 - 2.5 mA)
- improves motor and non-motor symptoms in PD patients
- mechanisms of action at the cellular and molecular level unknown



AIM

To elucidate the molecular effects of
Direct Current Stimulation (DCS)

on-line and *off-line* effects on:

- expression
- aggregation
- degradation

} alpha-synuclein

in human neuroblastoma SH-SY5Y cells

- **1. under basal conditions**
- **2. in presence of synucleinopathy**

EXPERIMENTAL PROTOCOL OF *IN VITRO* DCS

Human neuroblastoma SH-SY5Y cells

In collaboration with:
Prof. Alberto Priori
Dott. Tommaso Bocci

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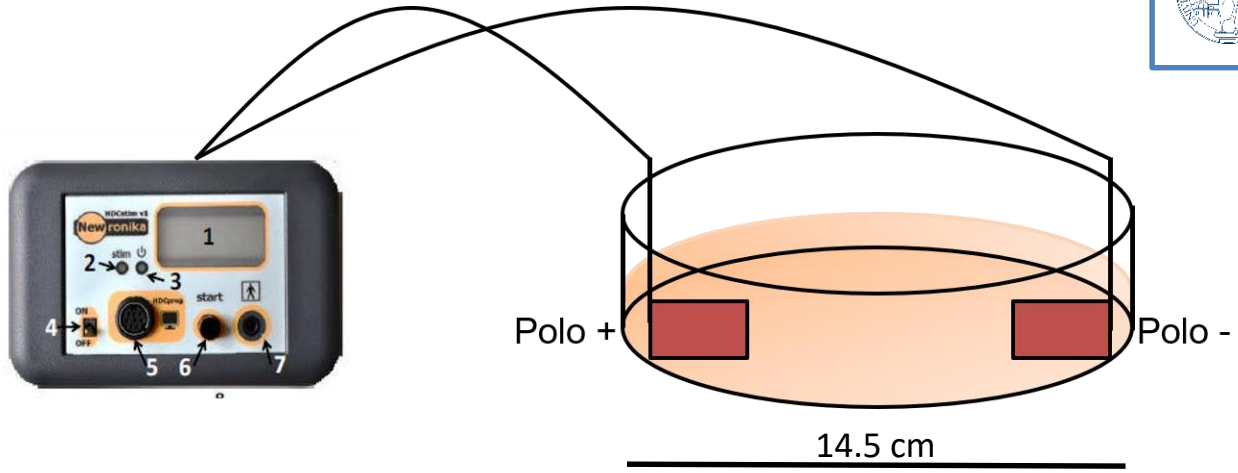
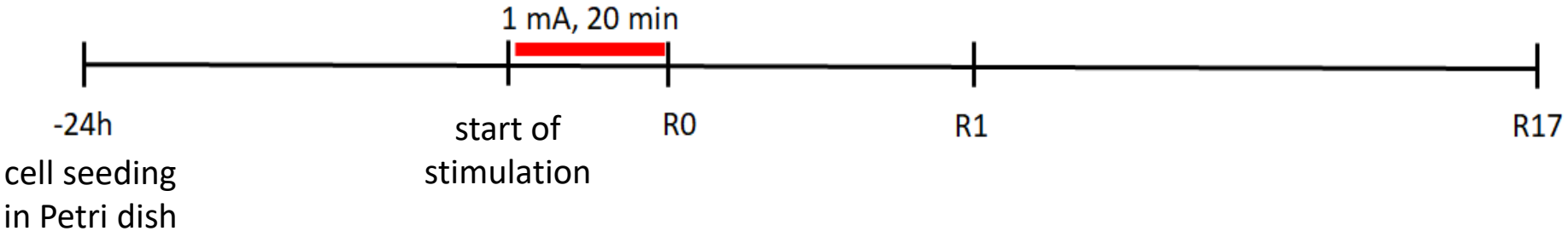
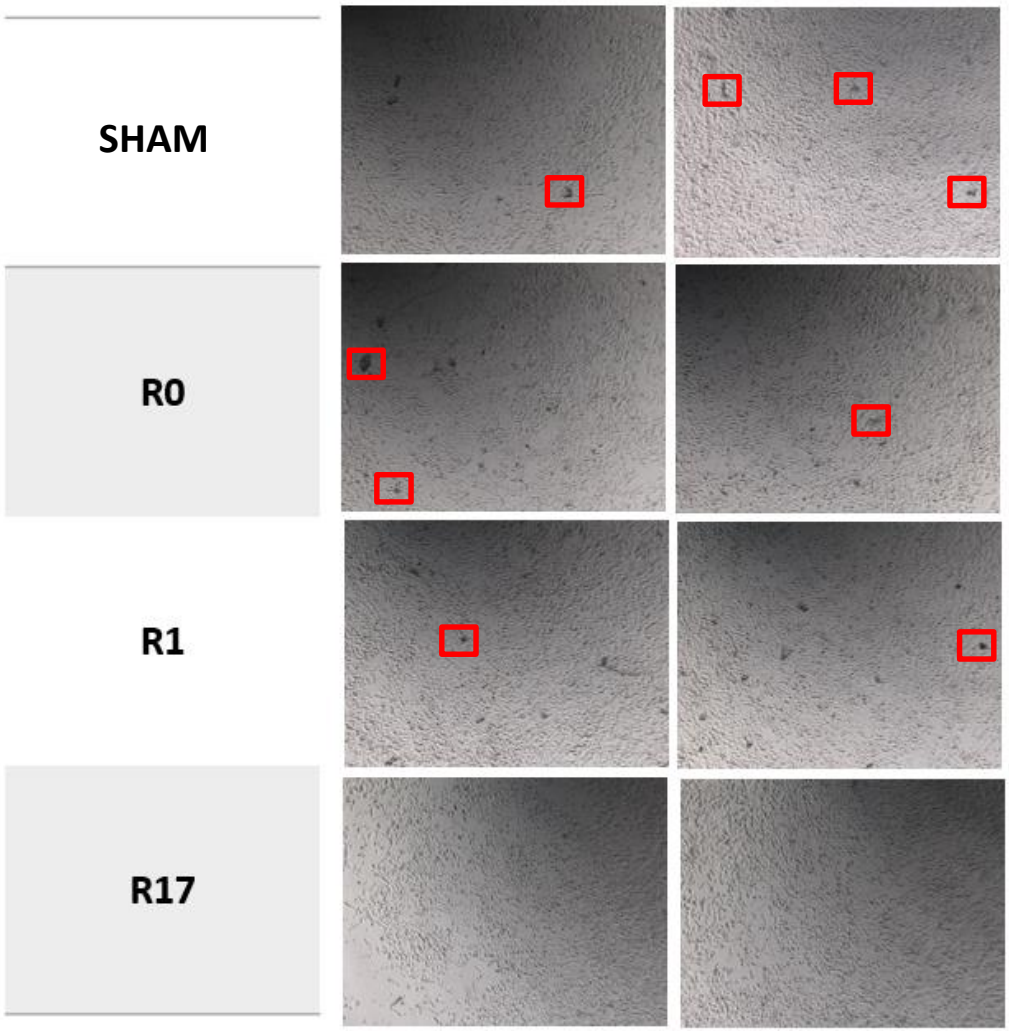
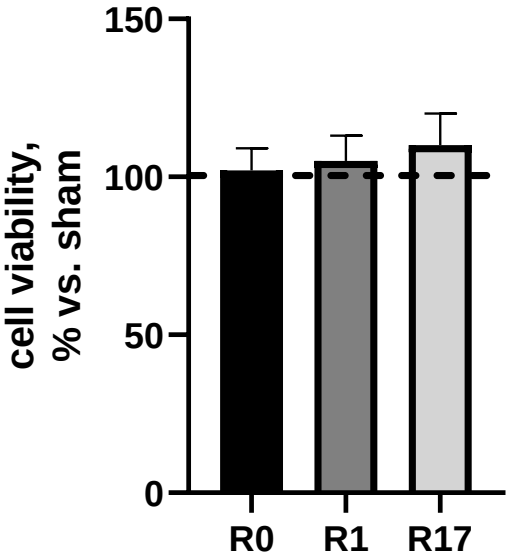




EFFECTS OF DCS ON CELL VIABILITY AND MORPHOLOGY





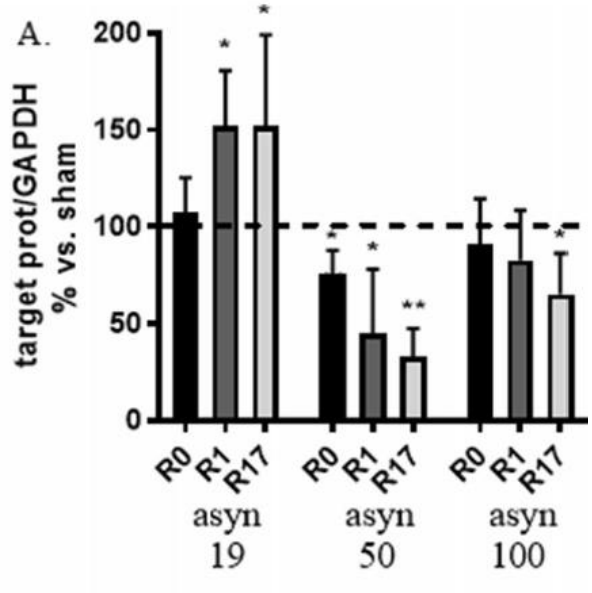
No cytotoxicity

Sala et al., Sci Rep. 2021

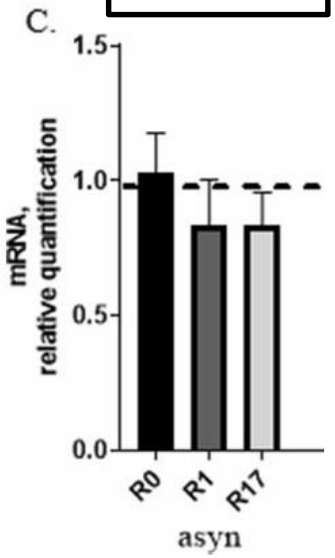
DCS - BASAL CONDITIONS

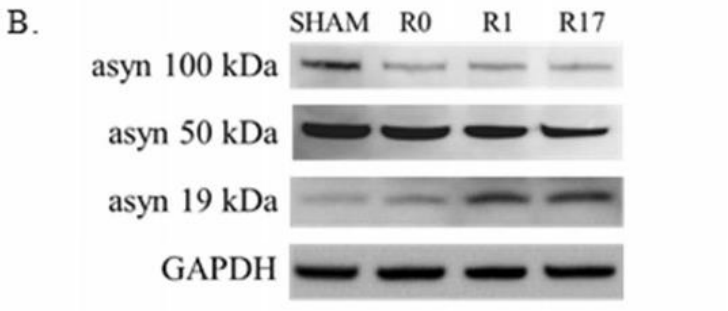
EFFECTS ON PROTEIN AND GENE LEVELS OF A-SYN

Protein levels



Gene levels





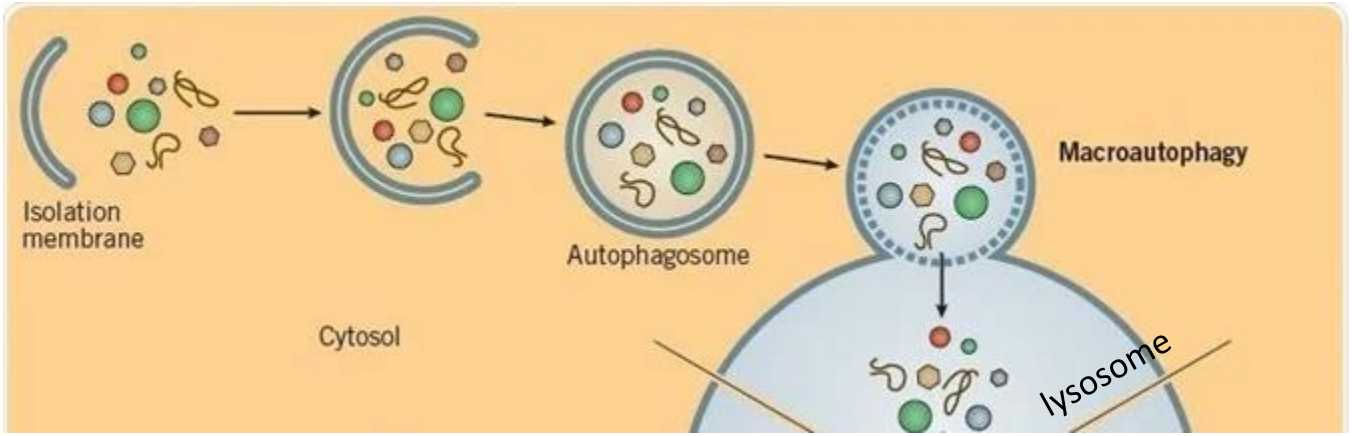
↓ oligomeric/aggregated a-syn forms

↑ monomeric a-syn

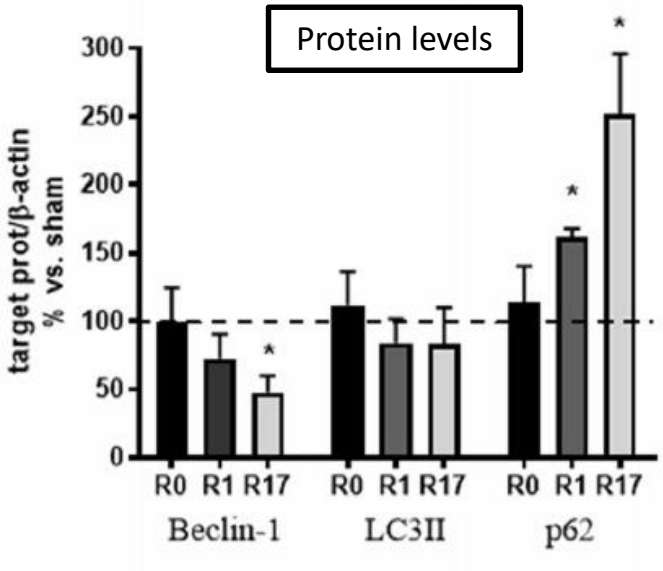
Sala et al., Sci Rep. 2021

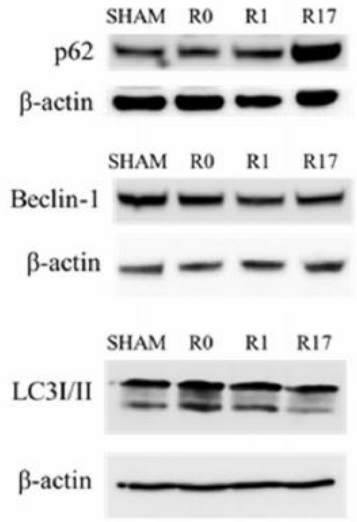
DCS - BASAL CONDITIONS

EFFECTS ON PROTEIN AND GENE LEVELS OF MACROAUTOPHAGY TARGETS

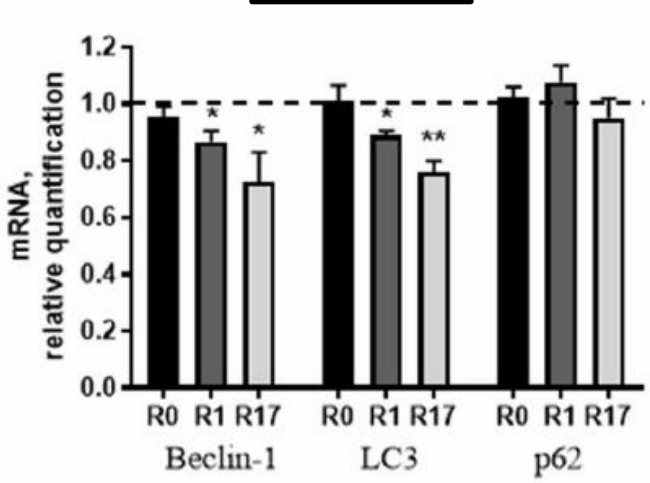


Protein levels





Gene levels

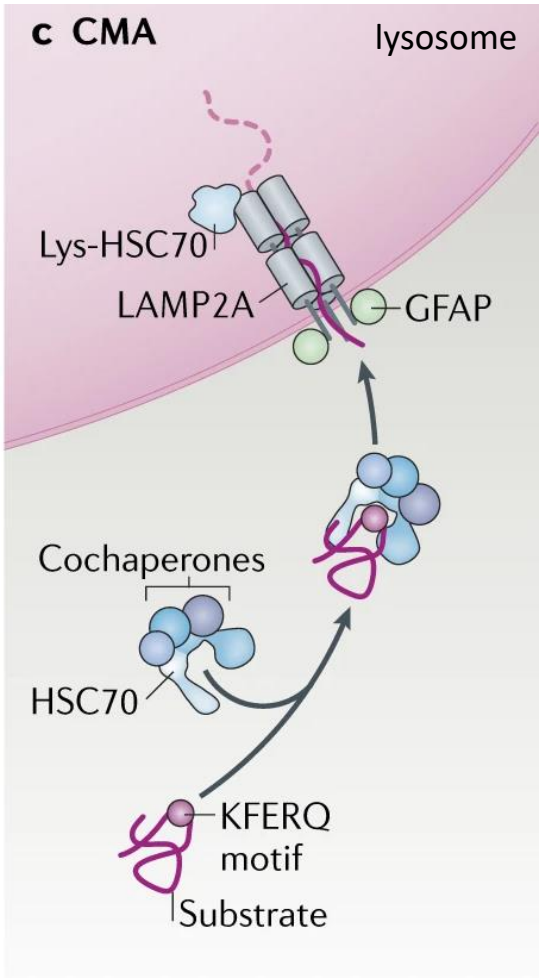


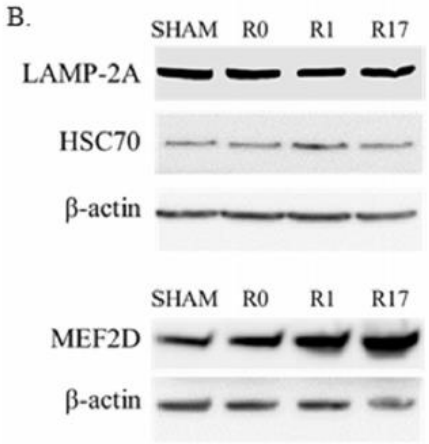
Down-regulation of macroautophagy

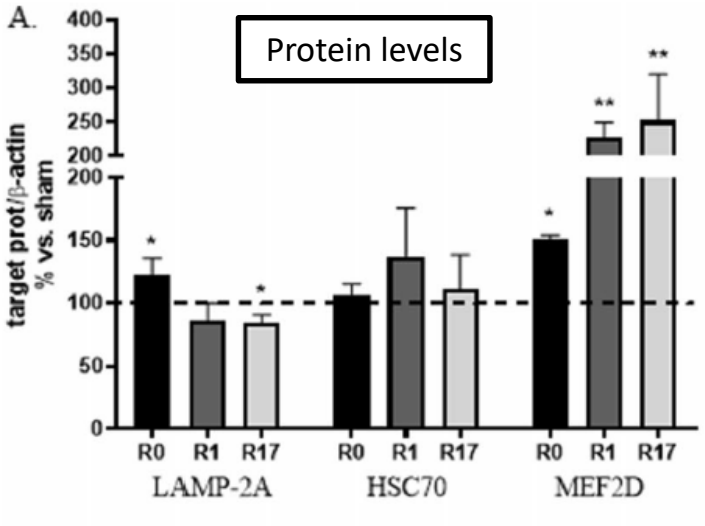
Sala et al., Sci Rep. 2021

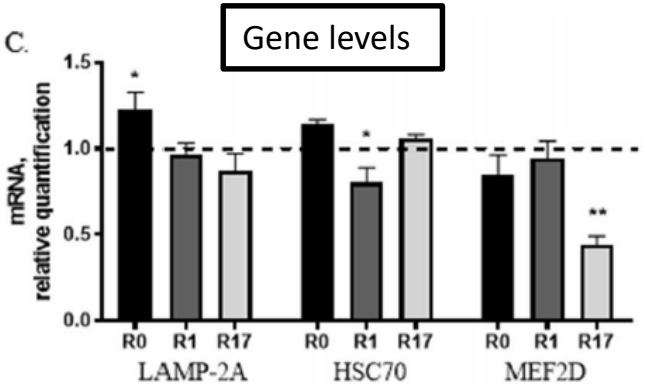
DCS - BASAL CONDITIONS

EFFECTS ON PROTEIN AND GENE LEVELS OF CMA TARGETS





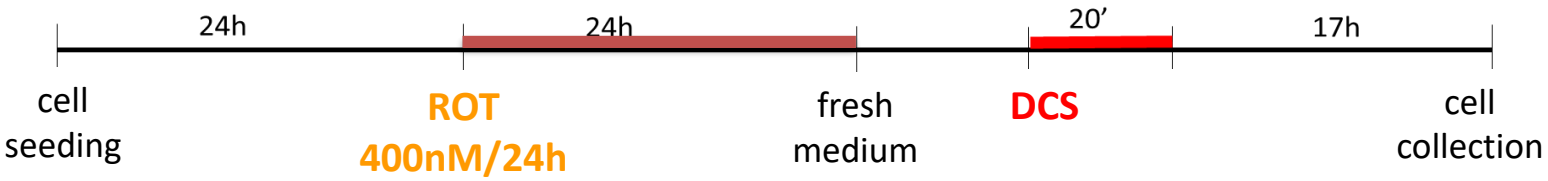




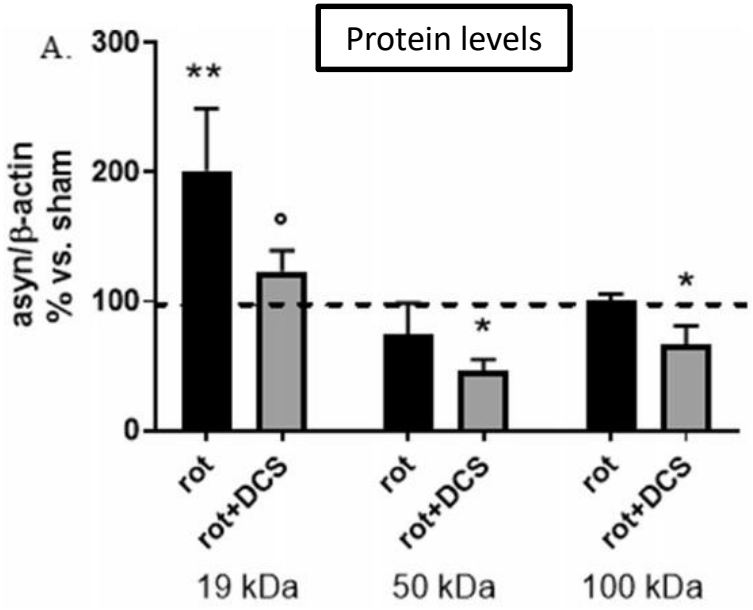
Down-regulation of Chaperone-Mediated Autophagy

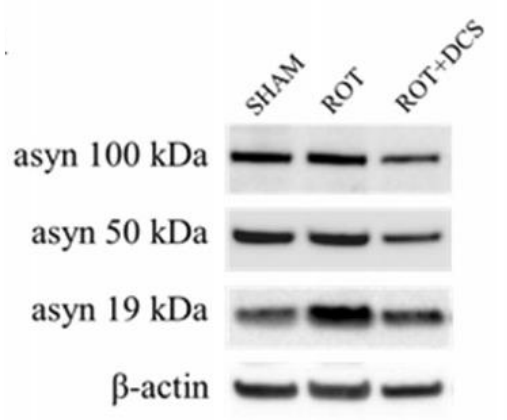
DCS – SYNUCLEINOPATHY (ROTENONE)

1. ROTENONE (mitochondrial complex I inhibitor)



EFFECTS ON PROTEIN AND GENE LEVELS OF ALPHA-SYNUCLEIN





No effect of DCS on asyn gene expression in rotenone-treated cells

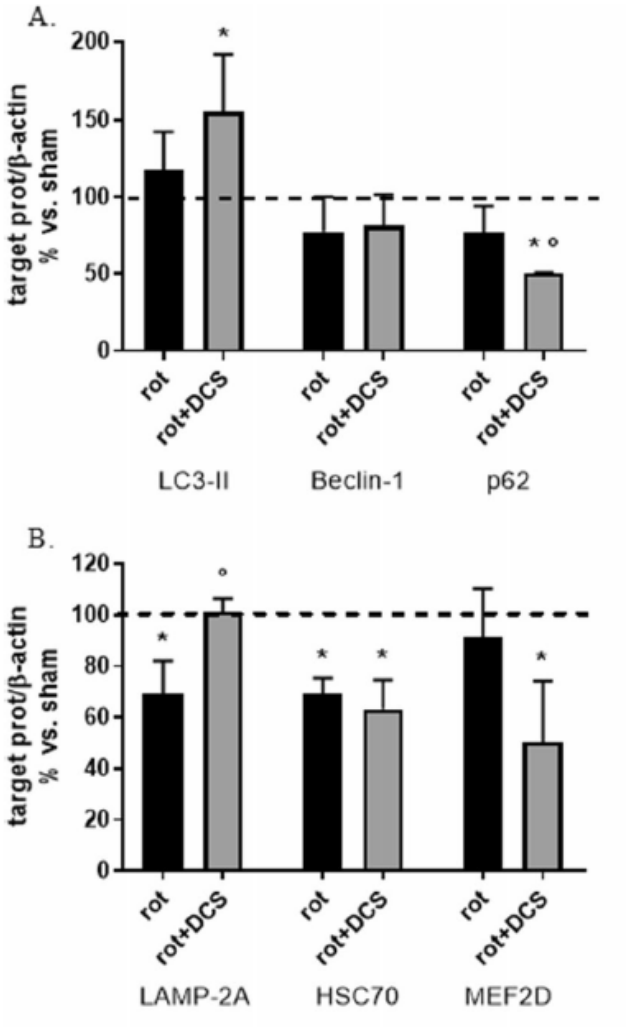
DCS counteracts the rotenone-induced asyn increase

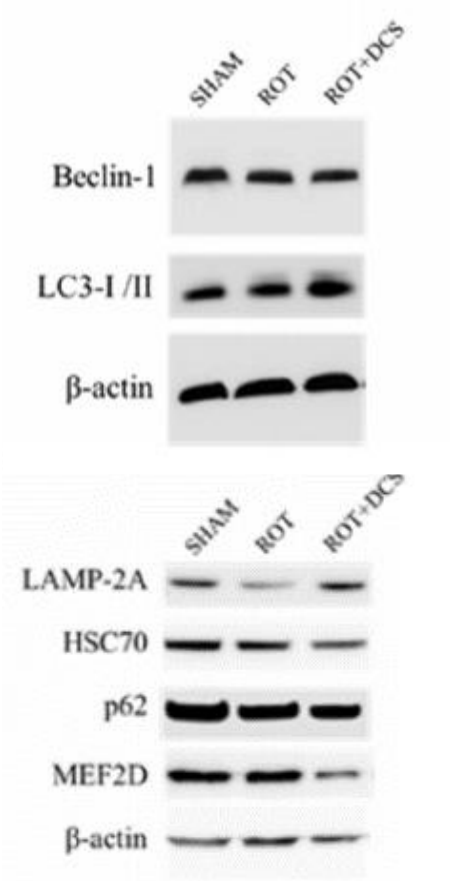
DCS – SYNUCLEINOPATHY (ROTENONE)

EFFECTS ON PROTEIN LEVELS OF MACROAUTOPHAGY AND CMA TARGETS

MACROAUTOPHAGY

CMA



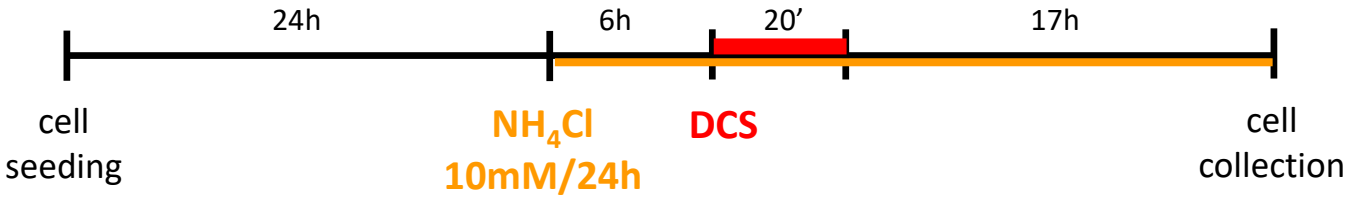


Up-regulation of macroautophagy and CMA

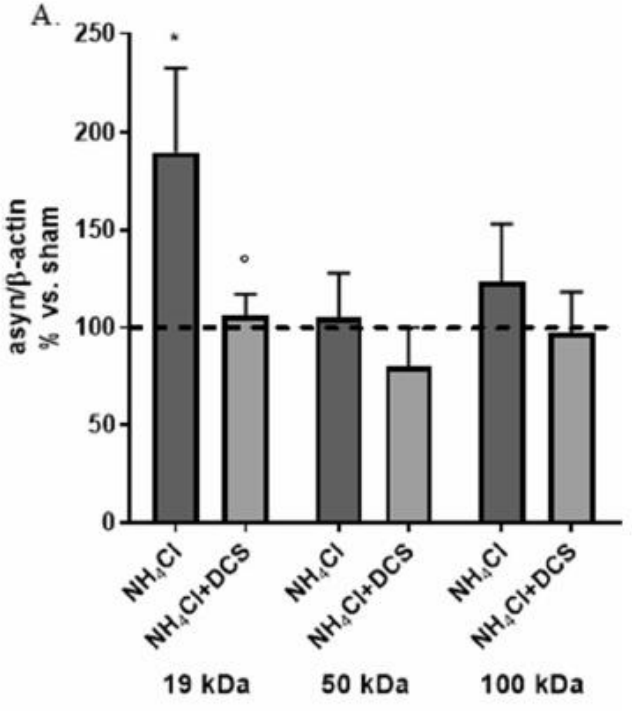
Sala et al., Sci Rep. 2021

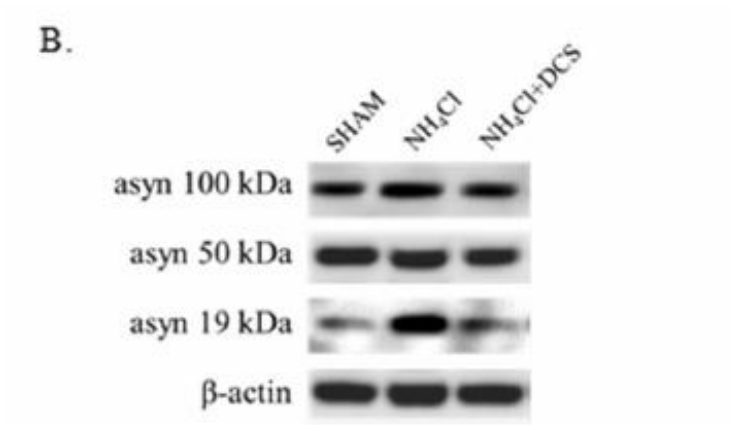
DCS – SYNUCLEINOPATHY (NH_4Cl)

2. NH_4Cl (lysosomal inhibitor)



EFFECTS ON PROTEIN LEVELS OF ALPHA-SYNUCLEIN

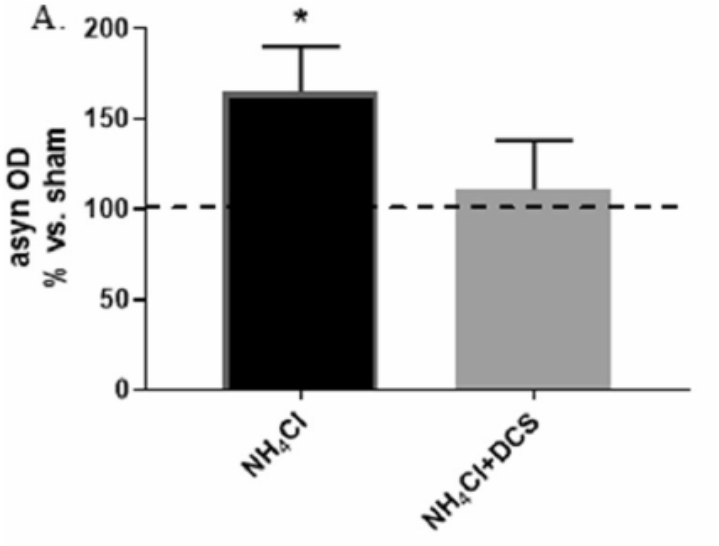


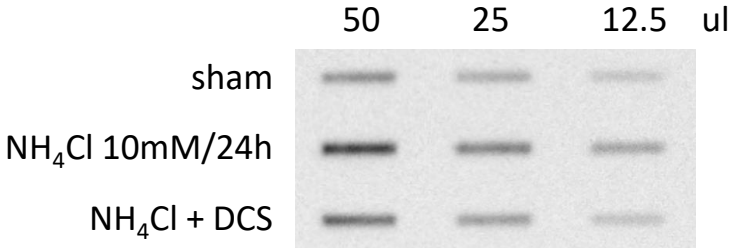


DCS counteracts the NH_4Cl-induced asyn increase

DCS – SYNUCLEINOPATHY (NH_4Cl)

EFFECTS ON EXTRACELLULAR LEVELS OF ALPHA-SYNUCLEIN





Dot-blot on cell media

DCS counteracts ↑ asyn extracellular levels induced by NH_4Cl

Sala et al., Sci Rep. 2021

CONCLUSIONS

DCS

- Under basal conditions:

affects asyn expression and aggregation
(↑ soluble, ↓ oligomeric)

improves cell homeostasis
(↓ macroautophagy and CMA)

- In presence of a synucleinopathy:

counteracts asyn accumulation by increasing autophagic degradation
(↑ macroautophagy and CMA)

- In presence of lysosomal inhibition:

↓ protein levels of different asyn forms (also independently from autophagic degradation) and ↓ asyn release

Therapeutic potential of DCS vs. toxicity associated with asyn aggregation