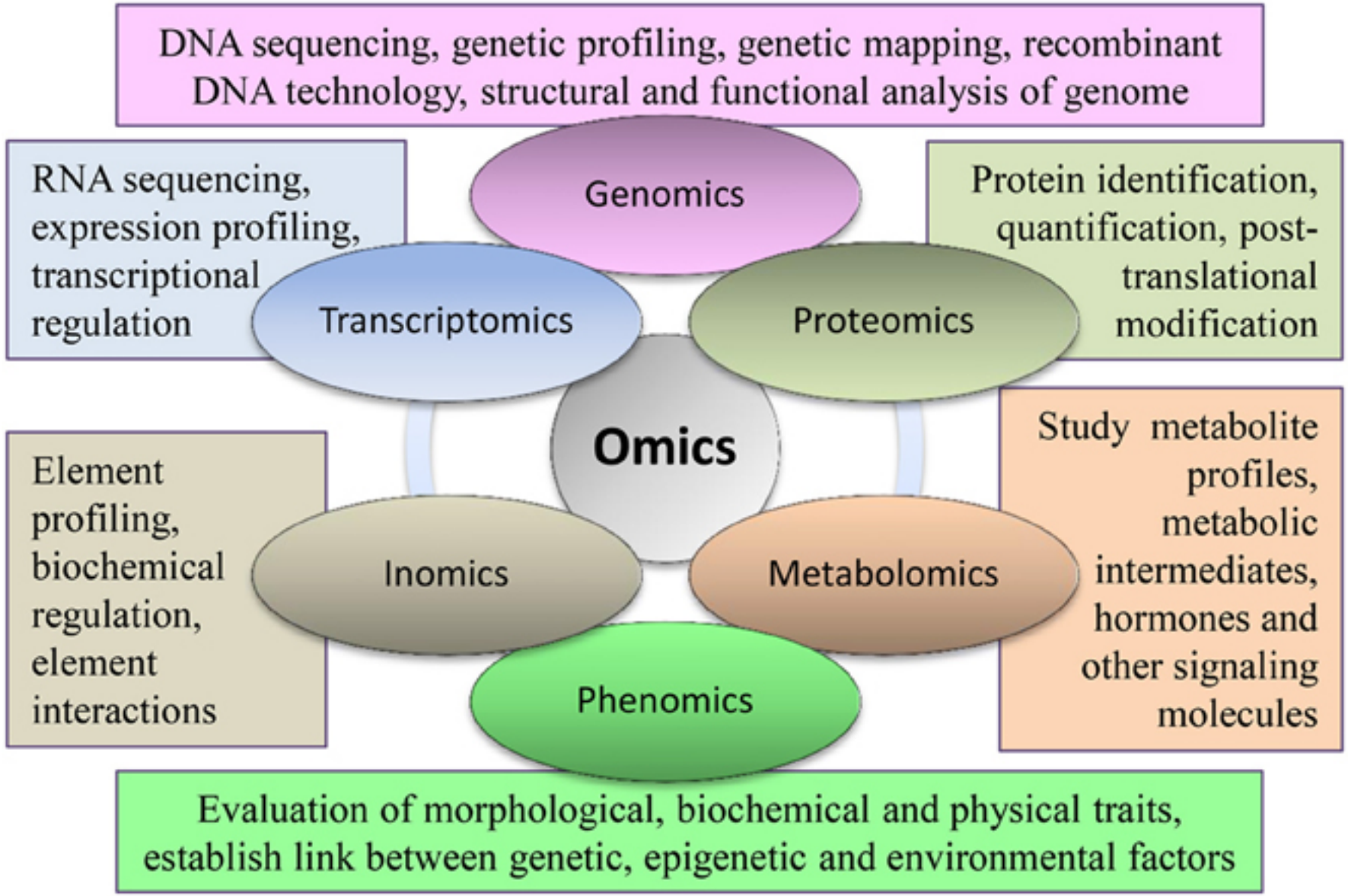
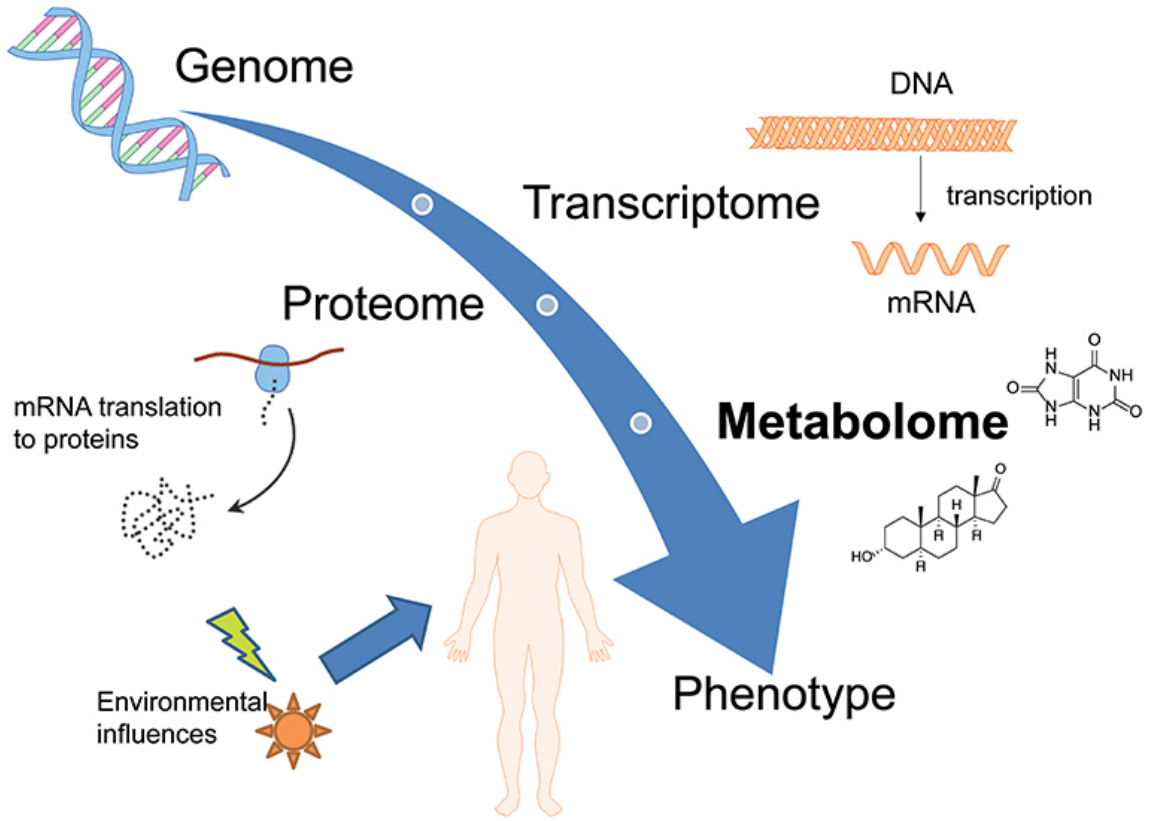
Metabolomics to investigate neurodegenerative diseases

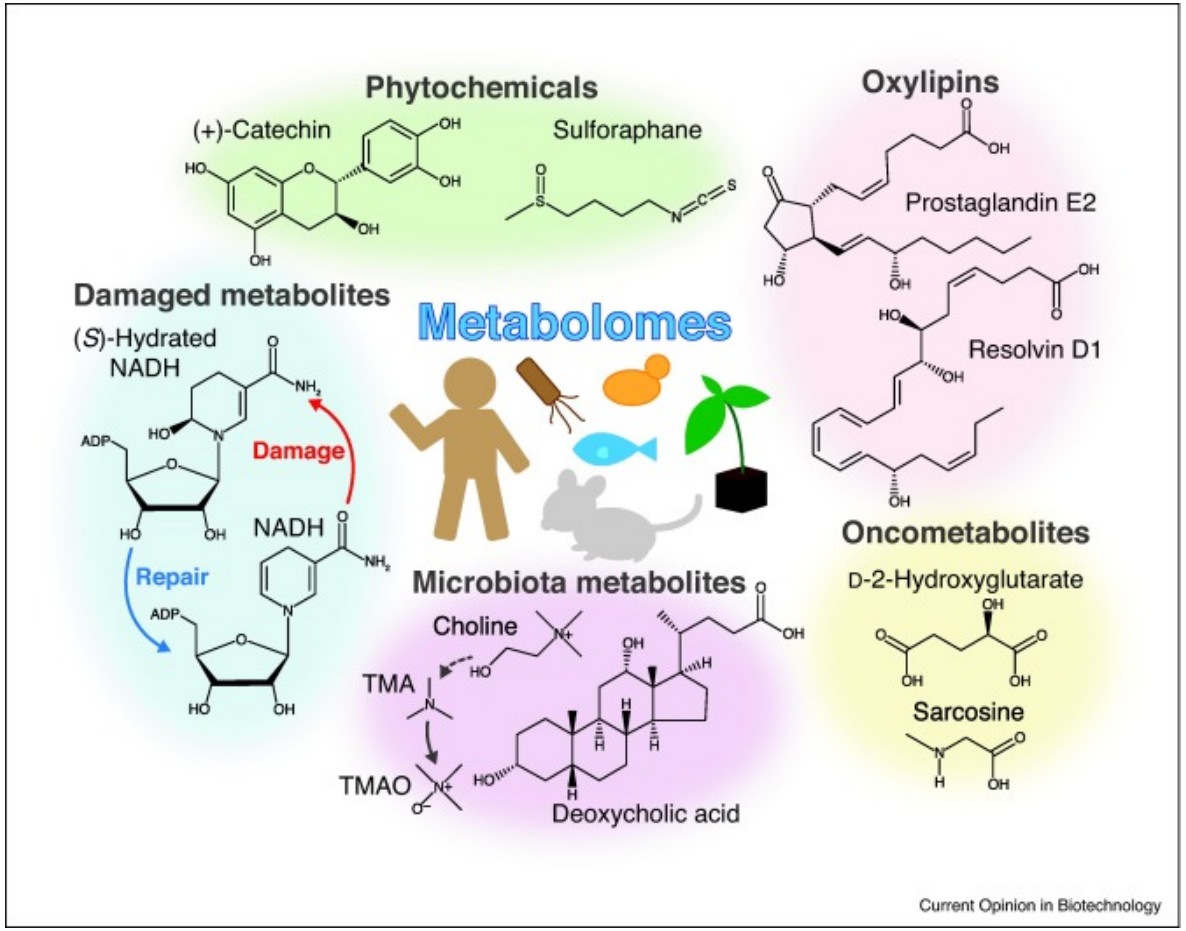
31.08.21

Elena De Cecco


DNA sequencing, genetic profiling, genetic mapping, recombinant DNA technology, structural and functional analysis of genome
RNA sequencing, expression profiling, transcriptional regulation
Genomics
Protein identification, quantification, post-translational modification
Transcriptomics
Proteomics
Omics
Element profiling, biochemical regulation, element interactions
Study metabolite profiles, metabolic intermediates, hormones and other signaling molecules
Inomics
Metabolomics
Phenomics
Evaluation of morphological, biochemical and physical traits, establish link between genetic, epigenetic and environmental factors

Metabolomics is the study of all the small molecules (<1 kDa), commonly known as metabolites, within cells, biofluids, tissues and organs
Substrates and products of metabolism are influenced by both genetic and environmental factors





Metabolomics

Large volumes (uL - mL)

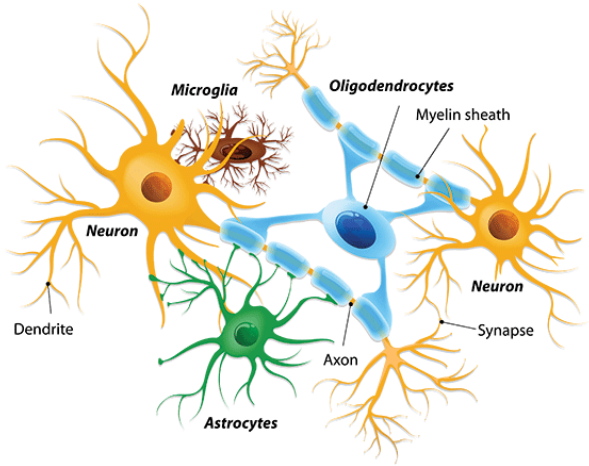
Small volumes (nL – pL)

Tissues

Body fluids (i.e. cerebrospinal fluid)

Single cells

Biomarkers discovery

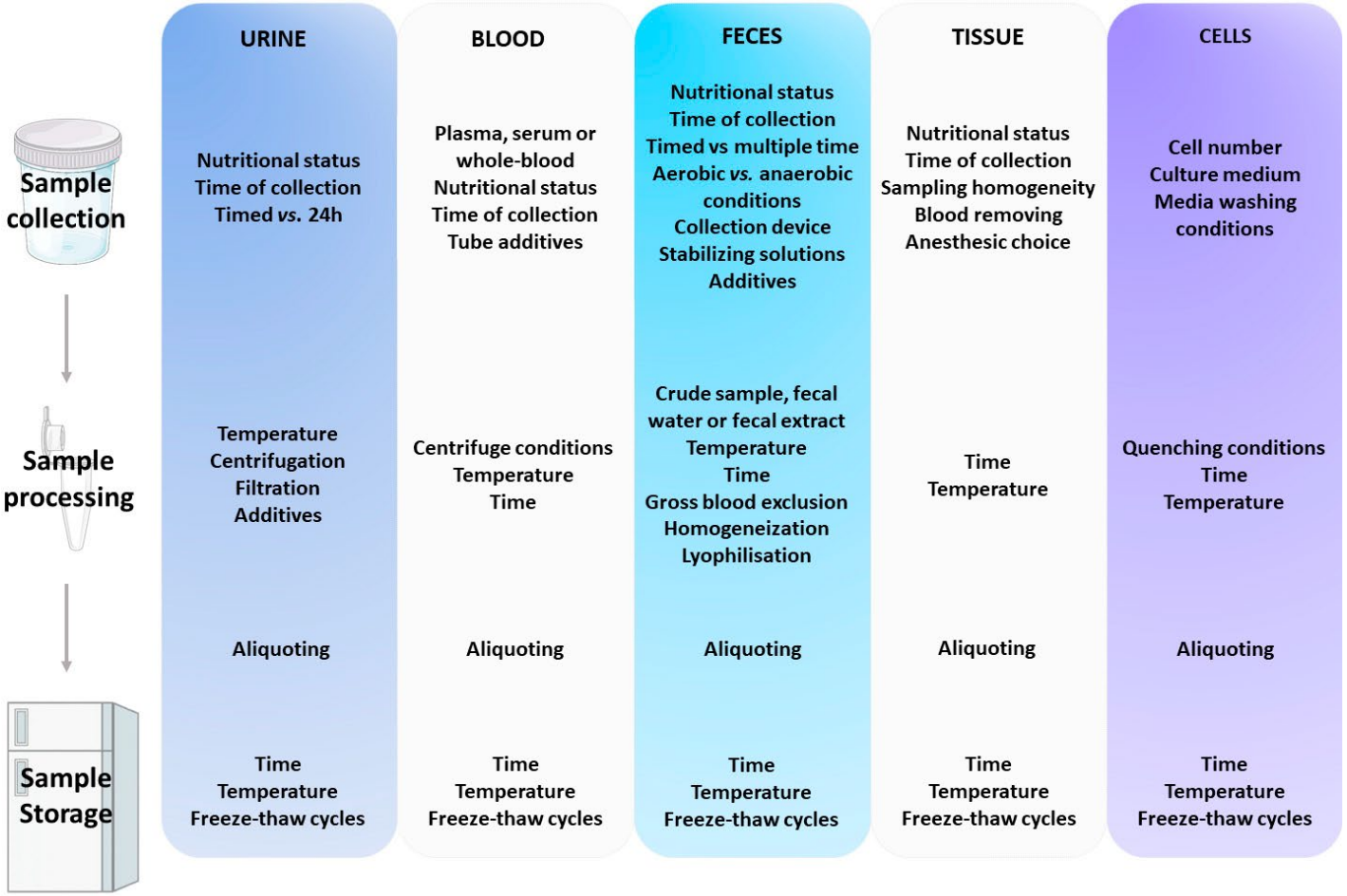


Investigate pathogenic changes in subsets of cells

Study the physyological state of cells and their pathwyas

Step 1: sampling

Bulk metabolomics makes use of easily collectable tissues and fluids



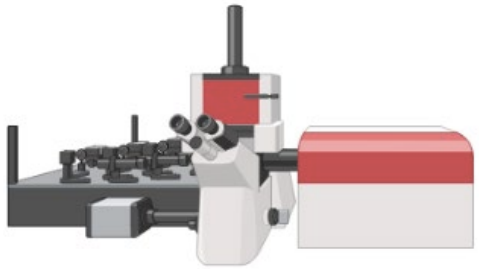
Smith et al., Important Considerations for Sample Collection in Metabolomics Studies with a Special Focus on Applications to Liver Functions. 2020

Step 1: sampling

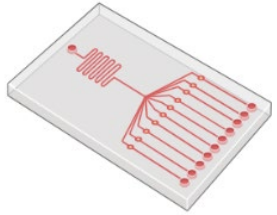
- Isolate only the selected individual cells
- Minimize the content of interfering material (i.e. extracellular matrix)
- Quench cellular metabolism immediately prior to the analysis (i.e. with organic solvents)

Manual sampling

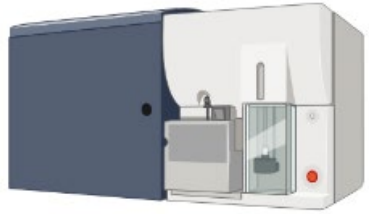
Micromanipulator

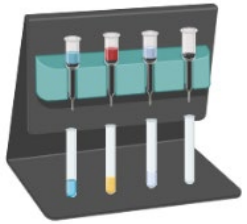


Laser capture microdissection
or optical trapping



Microfluidic devices





FACS and MACS

Step 2: analysis

Separation step

LC/GS or CE

⇩

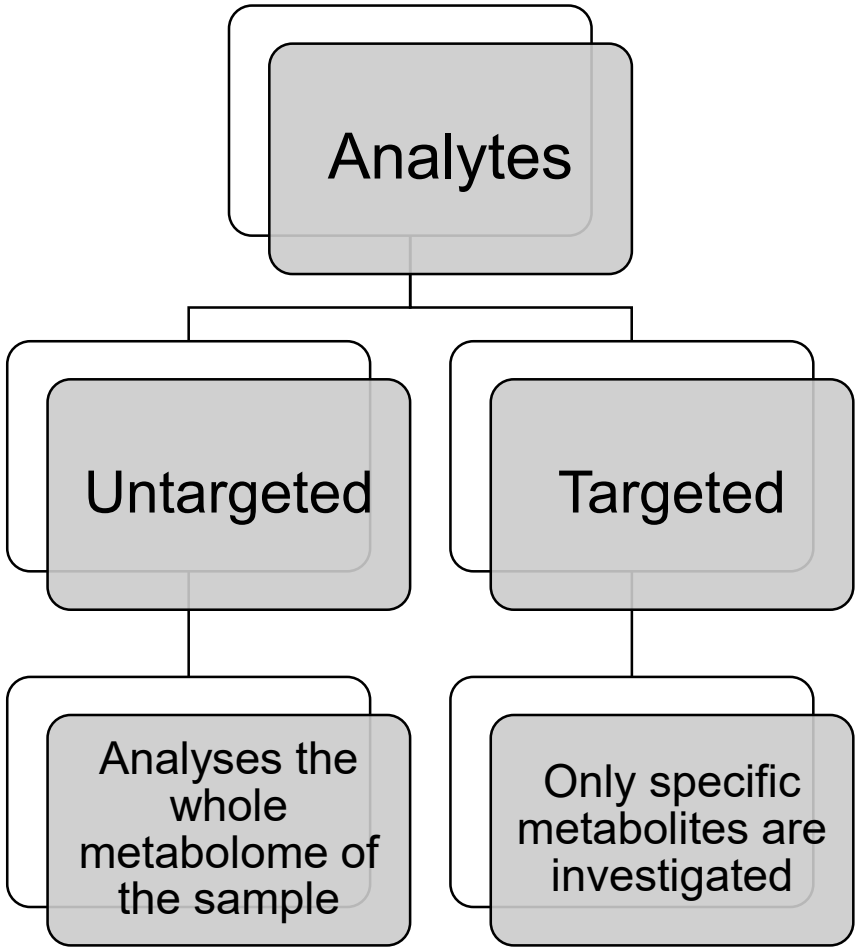
Analysis

Mass spectrometry

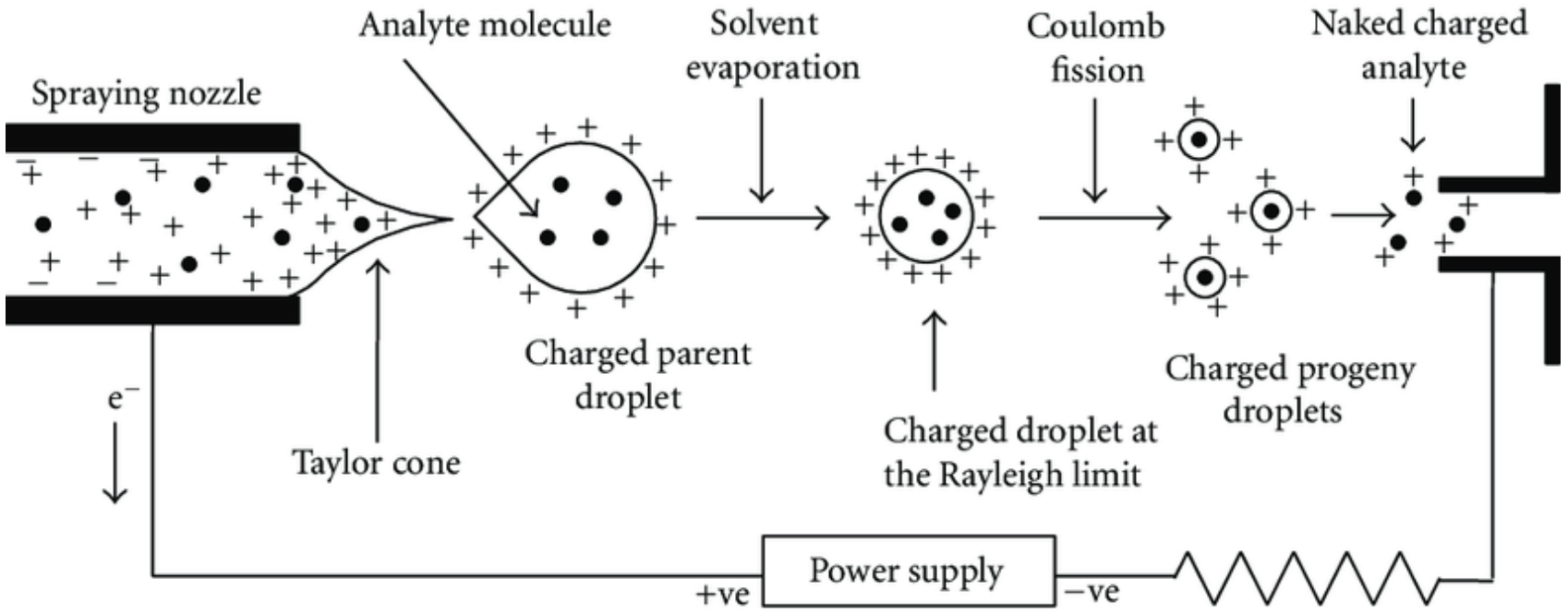
- Requires small volumes
- Used in both metabolomics approaches and mainly for single-cell metabolomics

NMR

- Requires large volumes
- Used in metabolomics of tissues and body fluids



Electrospray ionization (ESI)



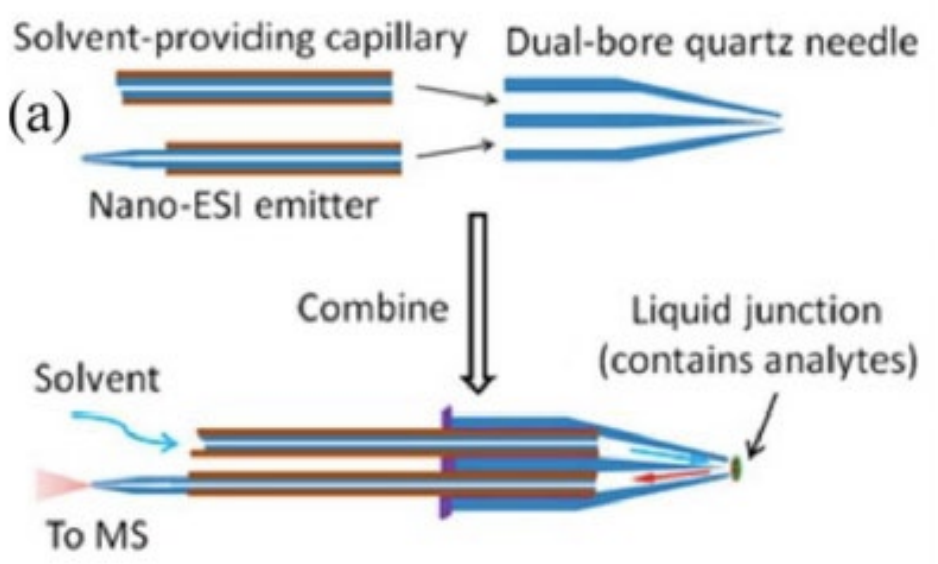
Metabolites in solution are transferred to the gas phase through either solvent or ion evaporation.
Analytes are separated by their electrophoretic mobility (CE) or by chromatography and are electrosprayed and analysed by MS.

ESI does not require a matrix and is therefore applicable to virtually all metabolites.

Nucleotides

Ions

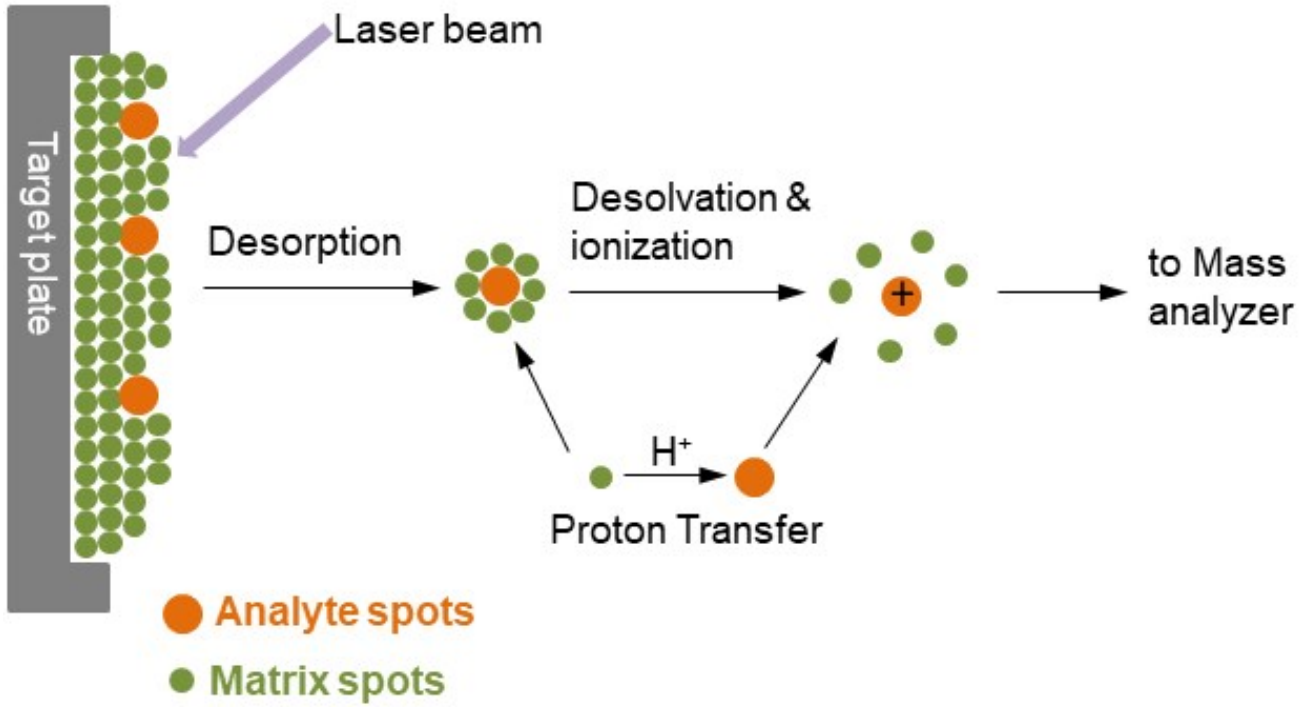
Neurotransmitters



Probe-based ESI

- A tungsten probe is inserted into single cells for metabolite enrichment
- Metabolites interact with the tip and are immediately electrosprayed and analysed

MALDI

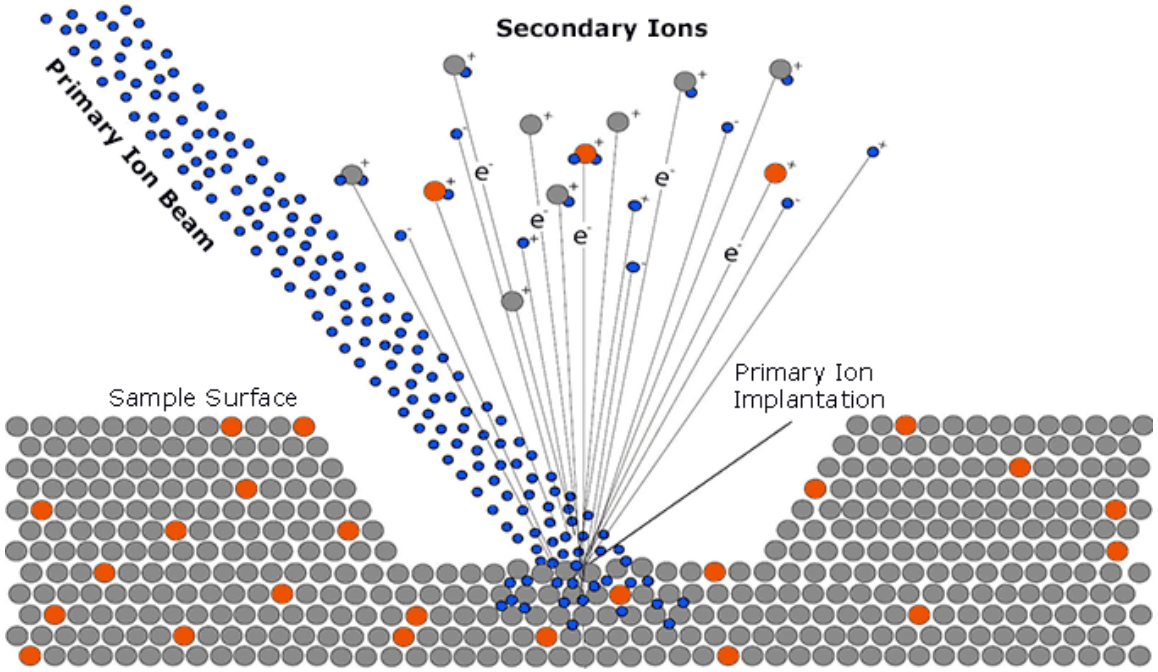


Metabolites are adsorbed on the matrix, which is then hit by a high-energy pulsed laser. The analytes are ionized and analysed with minimal fragmentation

MALDI MS is very sensitive (attomoles of analytes), but the matrix limits the range of possible analytes

→ Mainly used for peptides and lipids

Secondary Ion Mass Spectrometry (SIMS)

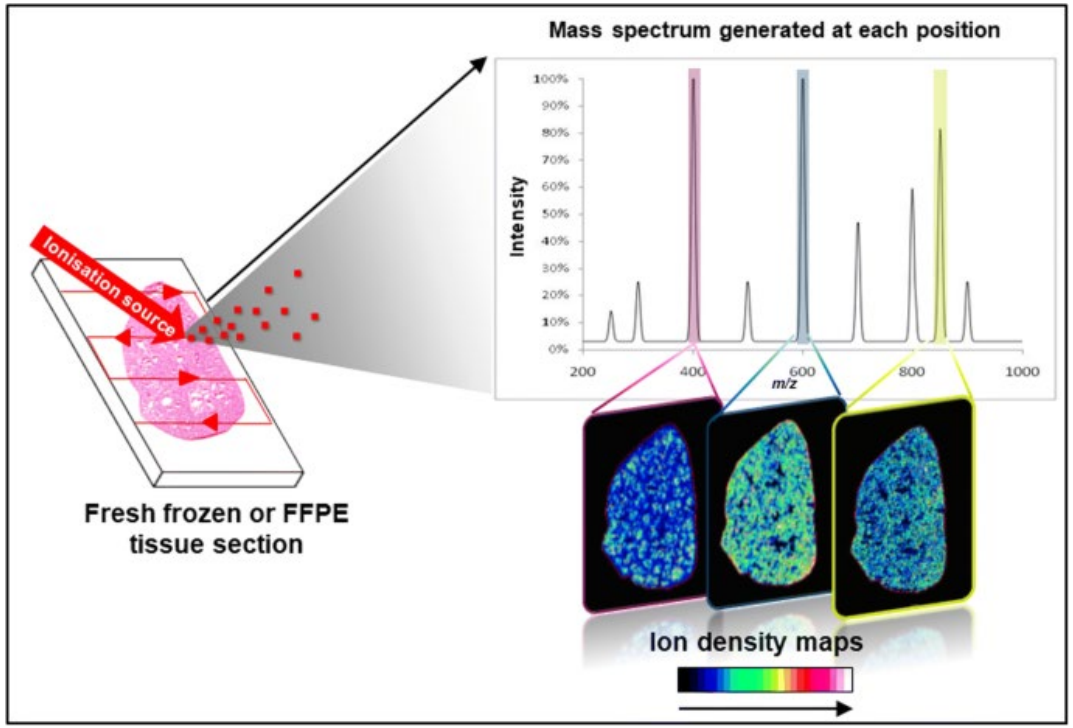


SIMS does not require a matrix.
A focused beam of primary ions bombards the sample surface and generates secondary ions from the sample. These ions are then analysed by MS

SIMS works well for small metabolites, while fragmentation of big molecules often occurs.

→ Mainly used for lipids and fatty acids

Mass Spectrometry Imaging (MSI)



MSI visualizes the position of analytes in a sample by measuring their masses and reconstructing a map of the scanned sample.

MSI scans the sample, collects a mass spectrum at one spot, then moves to another region. The stitch of all the mass spectra reconstructs the sample and shows the position of the analytes.

Only applicable to single-cell metabolomics!

Why studying metabolomics?

Understanding cellular heterogeneity

- Essential for organs functionality
- Explains why and how single cells and subpopulations respond to treatments

Complementing transcriptomics data

- More comprehensive insight on processes occurring in biological systems

Discovering pathways

- Changes in metabolites helps to uncover mechanisms of action leading to pathological statuses

Contents lists available at ScienceDirect

Redox Biology

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

Research paper

Sorting cells alters their redox state and cellular metabolome

Elizabeth M. Llufrio[a], Lingjue Wang[a], Fuad J. Naser[a], Gary J. Patti[a,b,*]

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Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Neuroprotective potential of Ayahuasca and untargeted metabolomics analyses: applicability to Parkinson's disease

Albert Katchborian-Neto[a], Wanderleya T. Santos[b], Karen J. Nicácio[a], José O.A. Corrêa[b], Michael Murgu[c], Thaís M.M. Martins[d], Dawidson A. Gomes[d], Alfredo M. Goes[d], Marisi G. Soares[a], Danielle F. Dias[a], Daniela A. Chagas-Paula[a,*], Ana C.C. Paula[b,**]

metabolites

Article

Altered Brain Metabolome Is Associated with Memory Impairment in the rTg4510 Mouse Model of Tauopathy

Mireia Tondo [1,2], Brandi Wasek [1], Joan Carles Escola-Gil [3], David de Gonzalo-Calvo [4], Clinton Harmon [1], Erland Arning [1] and Teodoro Bottiglieri [1,*]

ARTICLE OPEN ACCESS

Association of caffeine and related analytes with resistance to Parkinson disease among *LRRK2* mutation carriers

A metabolomic study

Grace F. Crotty, MD, Romeo Maciuca, PhD, Eric A. Macklin, PhD, Junhua Wang, PhD, Manuel Montalban, BS, Sonnet S. Davis, PhD, Jamal I. Alkabsh, BS, Rachit Bakshi, PhD, Xiqun Chen, MD, PhD, Alberto Ascherio, MD, DrPH, Giuseppe Astarita, PhD, Sarah Huntwork-Rodriguez, PhD, and Michael A. Schwarzschild, MD, PhD

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Neurology® 2020;95:e3428-e3437. doi:10.1212/WNL.0000000000010863

Cell Death & Differentiation (2018) 25:1408–1425
https://doi.org/10.1038/s41418-018-0148-x

Cell Death & Differentiation

ARTICLE

Alterations in neuronal metabolism contribute to the pathogenesis of prion disease

Julie-Myrtille Bourgognon[1] · Jereme G. Spiers[1] · Hannah Scheiblich[1] · Alexey Antonov[1] · Sophie J. Bradley[2] · Andrew B. Tobin[2] · Joern R. Steinert[1]

Received: 22 January 2018 / Revised: 14 May 2018 / Accepted: 4 June 2018 / Published online: 18 June 2018

Prion diseases induce changes in the brain

PLOS PATHOGENS

RESEARCH ARTICLE

Genome-wide transcriptomics identifies an early preclinical signature of prion infection

Silvia Sorce[1☯], Mario Nuvolone[1,2☯], Giancarlo Russo[3], Andra Chincisan[1], Daniel Heinzer[1], Merve Avar[1], Manuela Pfammatter[1], Petra Schwarz[1], Mirzet Delic[1], Micha Müller[4], Simone Hornemann[1], Despina Sanoudou[5], Claudia Scheckel[1]*, Adriano Aguzzi[1]*

Transcriptomic analysis revealed that prion diseases profoundly affect cell state and the overall physyology of the affected brain region

eLife

RESEARCH ARTICLE

Ribosomal profiling during prion disease uncovers progressive translational derangement in glia but not in neurons

Claudia Scheckel*, Marigona Imeri, Petra Schwarz, Adriano Aguzzi*

Institute of Neuropathology, University of Zurich, Zurich, Switzerland

No detailed metabolomic analysis of prion-infected brain regions has ever been described

Mouse model

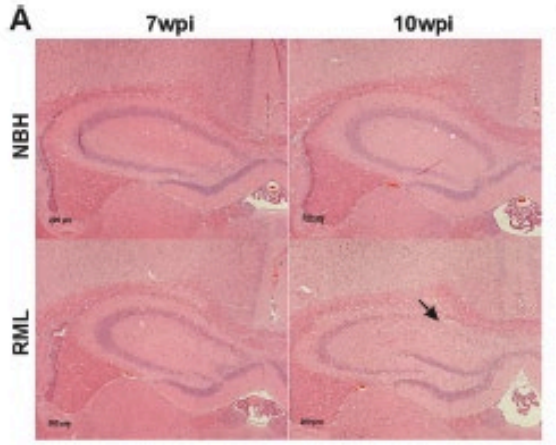
Inoculation with RML/NBH

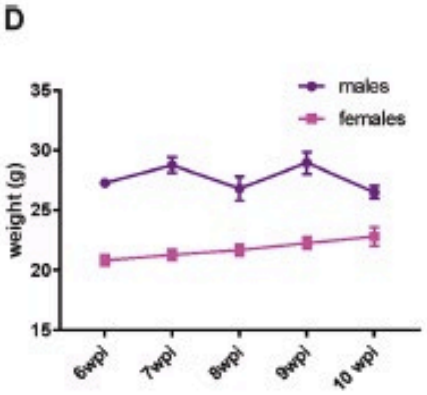
10 weeks p.i.

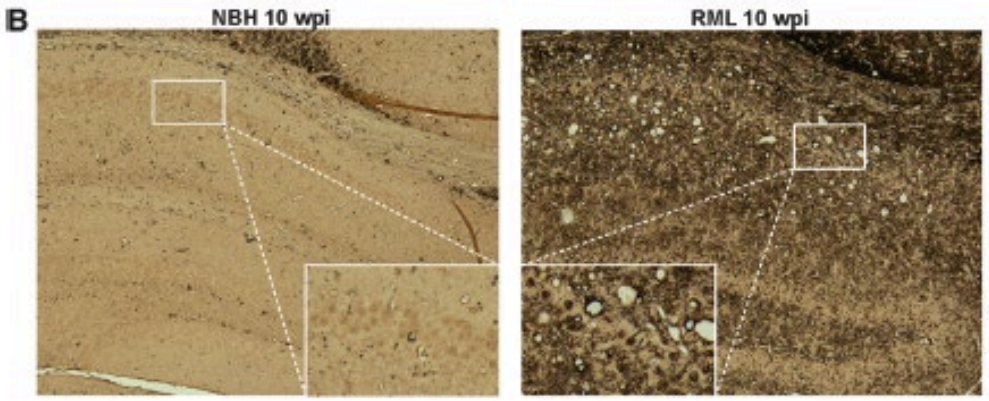
Brain extraction

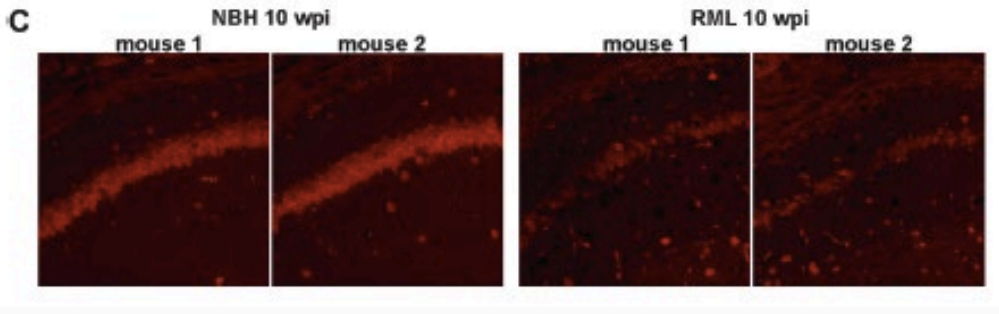
Cortex

Hippocampus







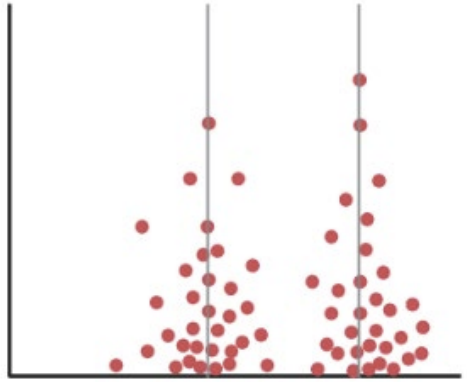


Mice at 10 weeks post inoculation present clear characteristics of the disease (neuronal loss and apoptosis) but lack other symptoms like ataxia and weight loss

Brain tissue

Ultra high-performance LC

ESI MS



Analyes segregated mainly based on:

- Brain region
- Treatment

Two-way ANOVA identified 141 metabolites as being significantly affected ($p<0.05$) by prion infection, and 236 metabolites that differed within the two brain regions

The majority of metabolites influenced by prion infection had the same trend in both brain regions, showing to be confirmed independently of the brain region affected

Cellular metabolism is heavily affected by prion diseases

Significantly altered biochemicals *Prion/control*	Total number of metabolites with $p \leq 0.05$	Metabolites upregulated / downregulated $p \leq 0.05$	Total number of metabolites with $0.05 < p < 0.10$	Metabolites *upregulated* / *downregulated* $0.05 < p < 0.10$
Hippocampus	101	79 / 22	37	27 / 10
Cortex	100	45 / 55	40	17 / 23

➡ **Significantly altered pathways:**

Neuropeptide homeostasis

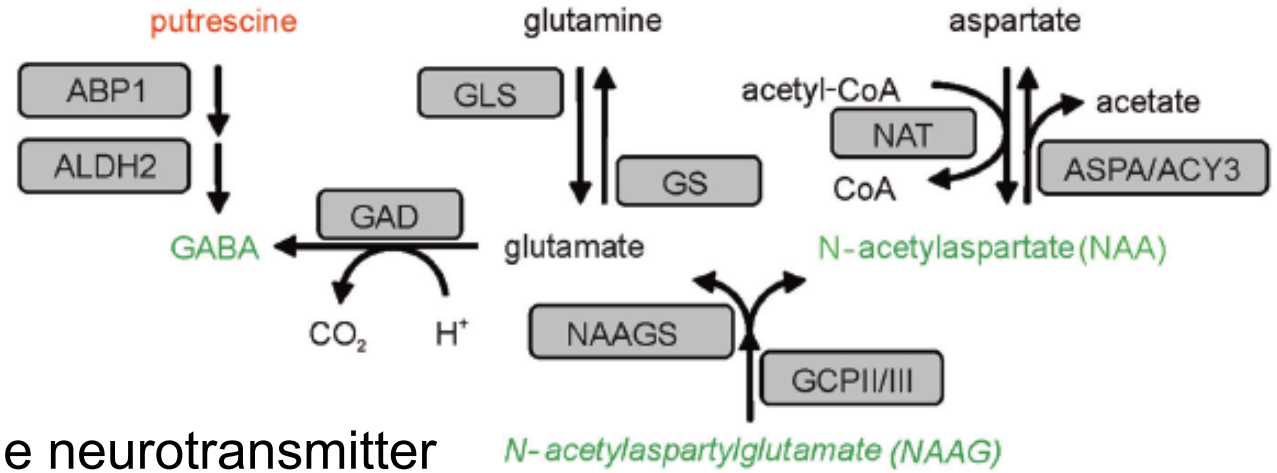
Sphingolipid signalling

Glucose utilisation

Prostaglandin production

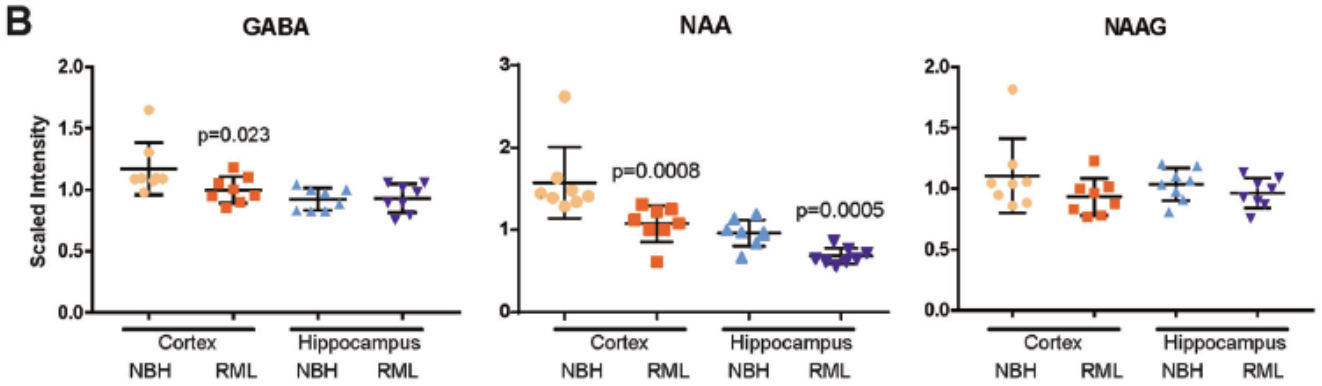
NO signalling

Neuropeptide synthesis is downregulated in prion diseases

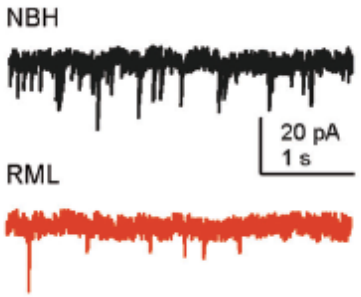


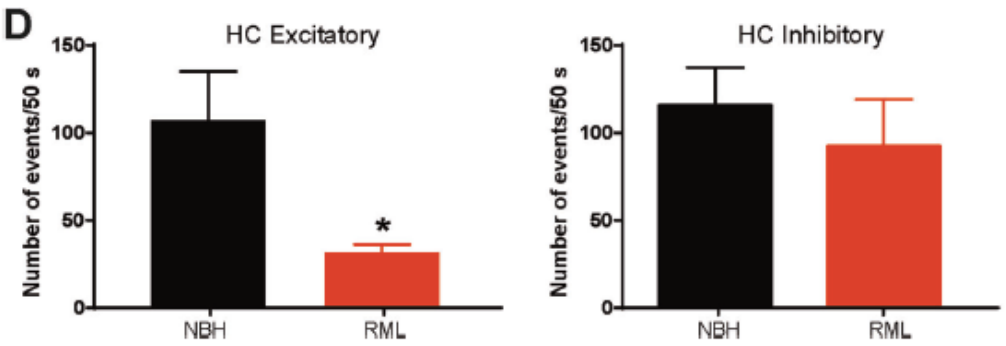
NAA is a neuronal precursor of the neurotransmitter NAAG and is downregulated in prion diseases in both brain regions

The neurotransmitter GABA is downregulated only in the cortex

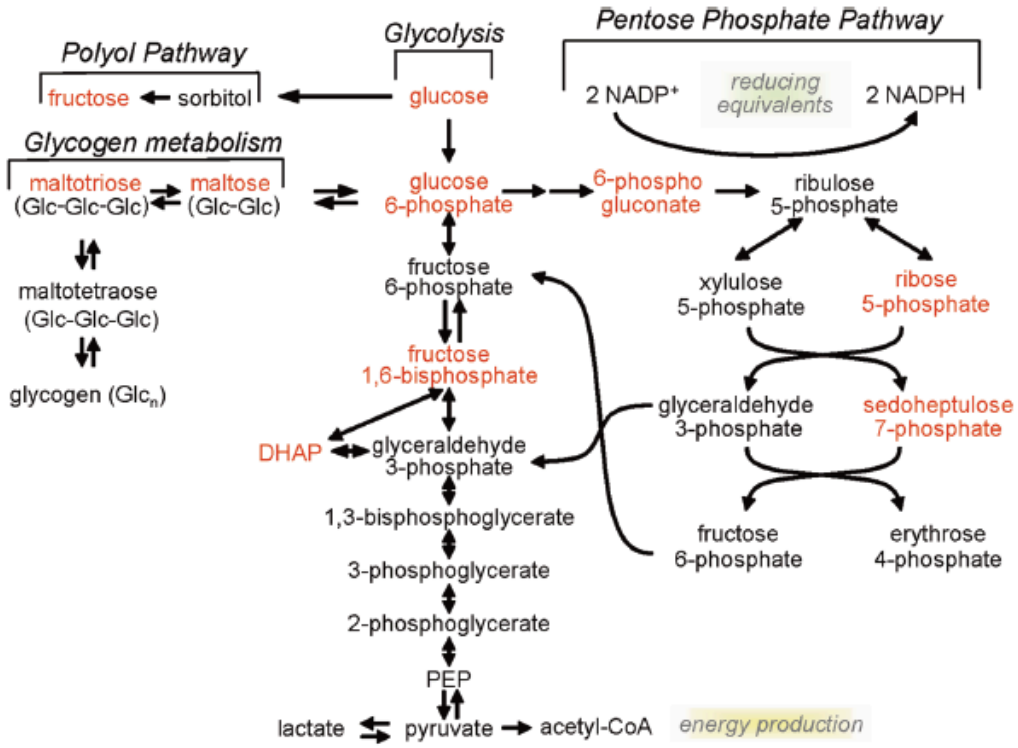


70% decrease in excitatory events





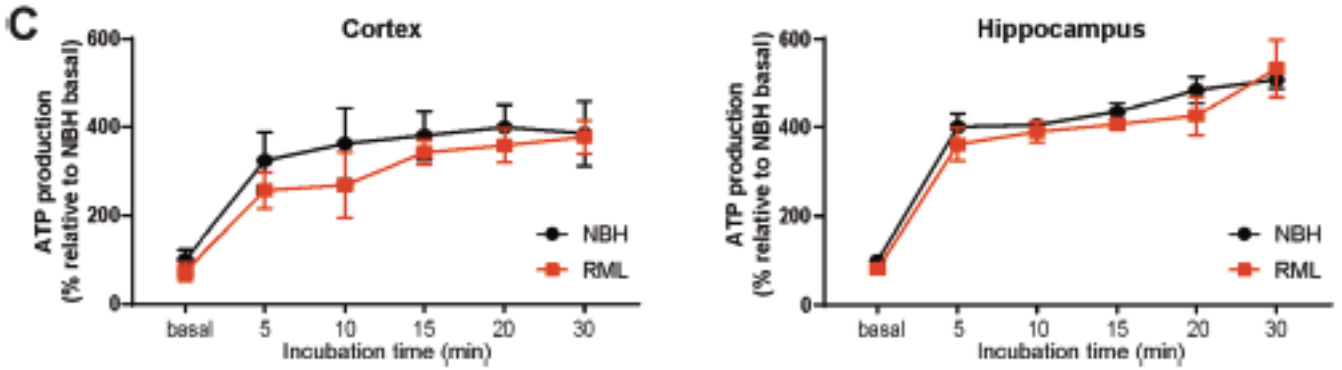
Glucose utilisation is reduced in prion diseases



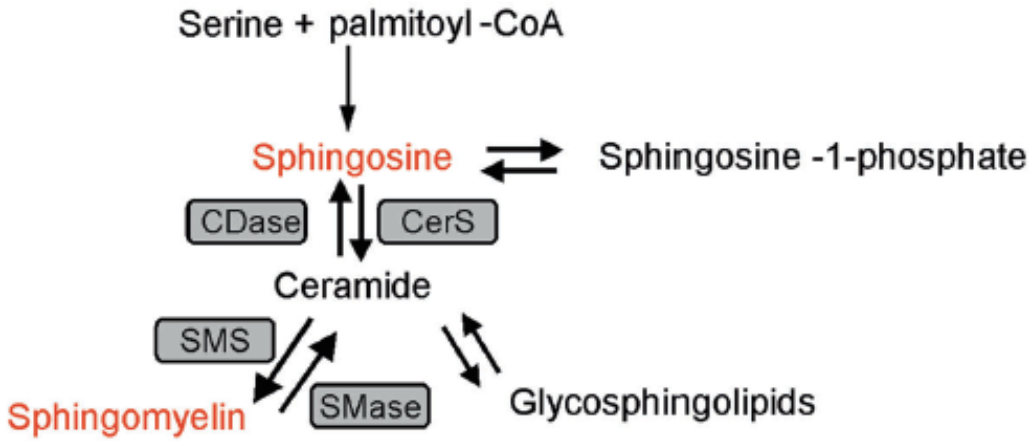
PrP^C positively regulates the expression of Glut1 and depletion of PrP^C leads to impaired glucose tolerance and reduced glycolysis.

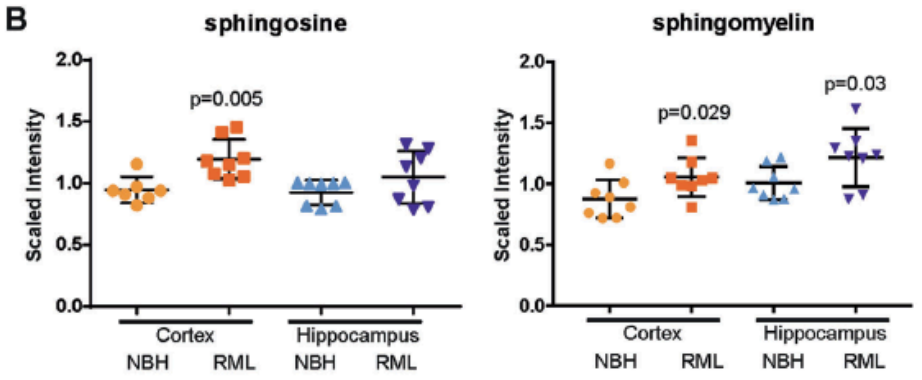
Glucose and several glycolytic pathway intermediates were increased in both hippocampus and cortex of prion-infected mice.

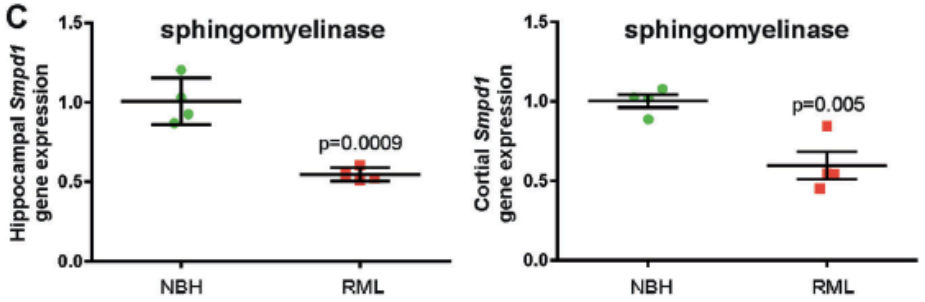
ATP production is maintained in prion-infected mice, probably by upregulation of glucose-related pathways



Sphingolipid singalling is modified in prion diseases







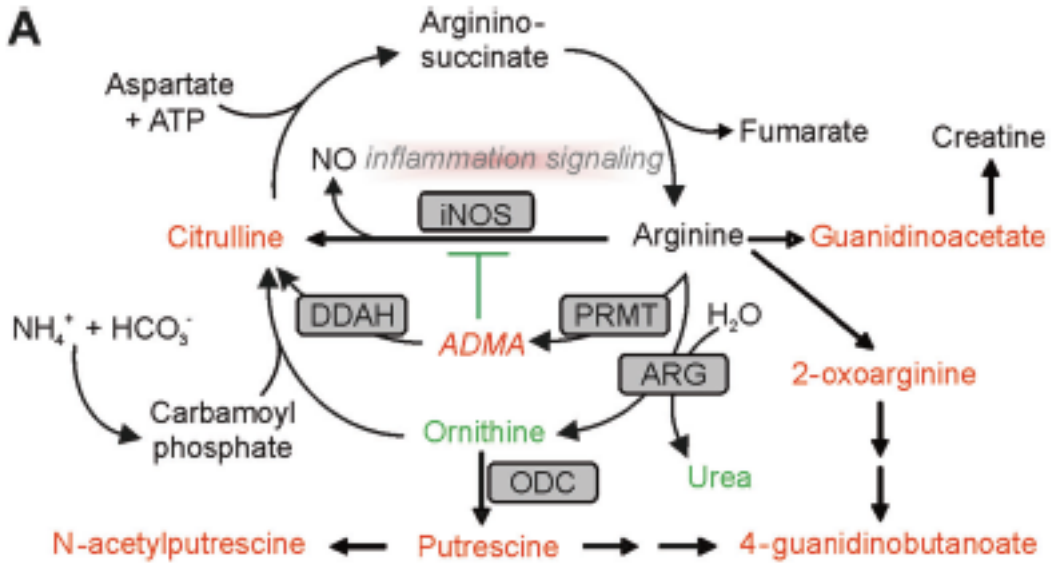
Sphingolipids play important roles in signal transmission and cell recognition.

Ceramide is the main constituent of the myelin sheath of axons.

Ceramids and sphingolipids have pro-apoptotic and antiproliferative properties

Sphingosine and sphinogmyelin are enriched in prion diseases, and the expression of the enzyme sphingomyelinase is downregulated

Arginine utilisation and NO signalling are affected in prion diseases

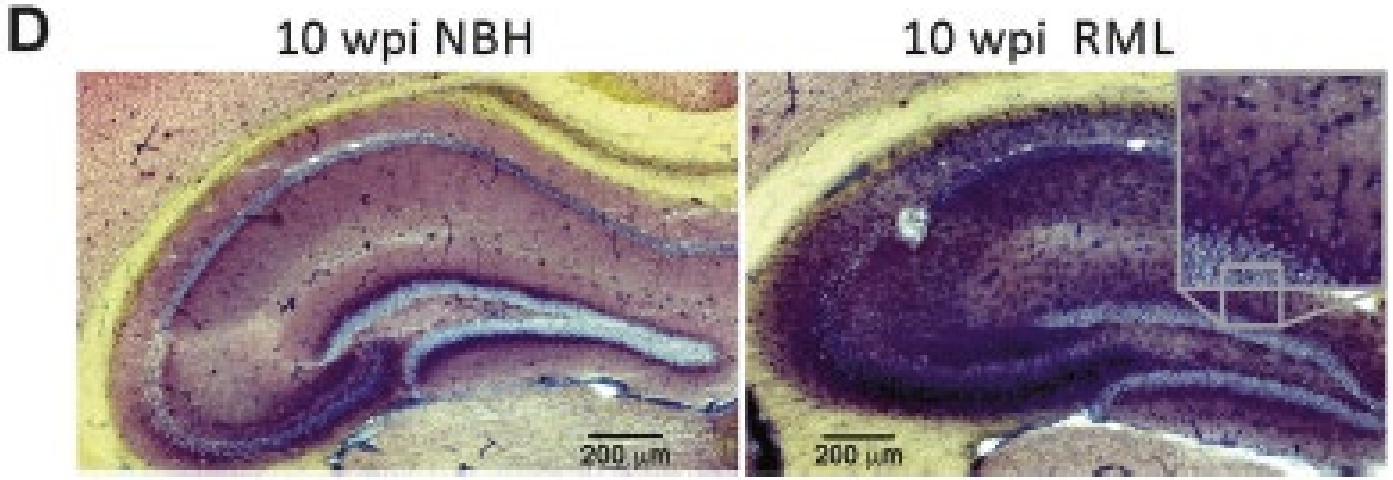


Arginine is a key metabolite in the nitric oxide (NO) signalling pathway, as it is converted into NO and citrulline in the arginase pathway

Many arginine-related metabolites are enriched in prion-infected mice, indicating a strong increase in NO signalling.

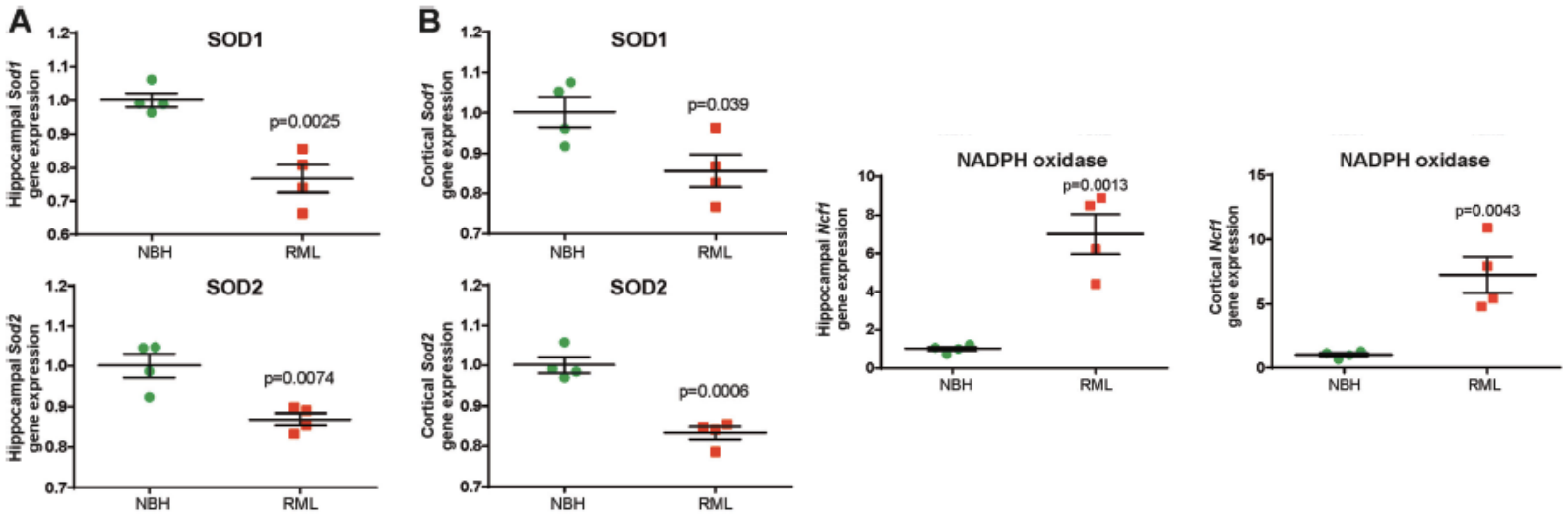
Putrescine and its N-acetylated derivative are enriched, probably due to changes in the ornithine decarboxylase activity. Putrescine can be converted into the neurotransmitter GABA

These findings point towards an enhanced neuroinflammatory activity, in which NOS are upregulated and lead to neurotoxicity.

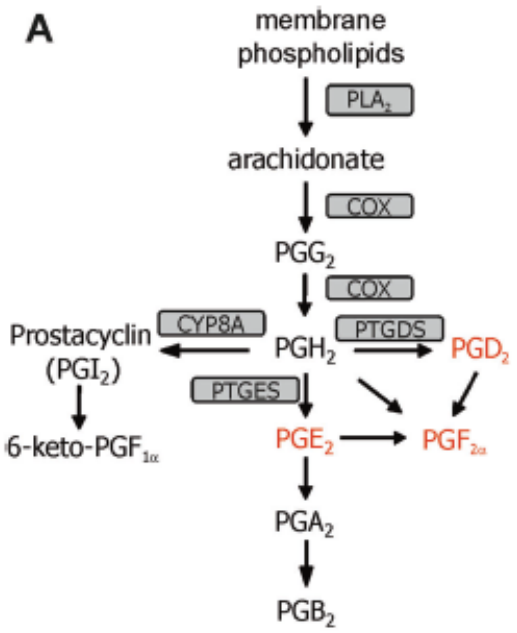


NADPH diaphorase assay showed strong signal in the hippocampus of prion-infected mice

Antioxidant genes (SOD1 and SOD2) are downregulated and pro-oxidant genes are upregulated in prion-infected mice



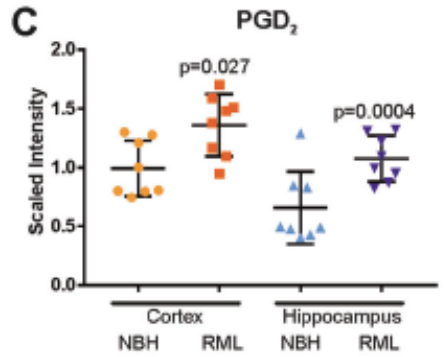
Prostaglandin production is upregulated in prion diseases

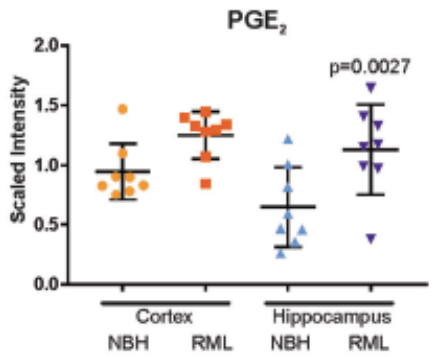


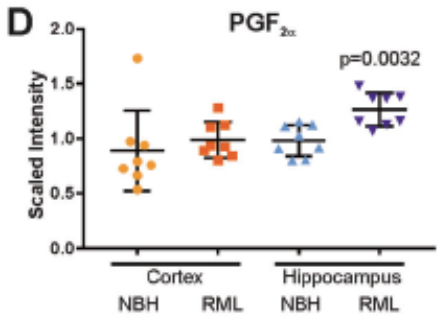
Prostaglandins are involved in inflammation signalling and modulation of neuronal communication.
The main signalling axis is the metabolism of arachidonic acid (AA) via COX1 and COX2 processing.

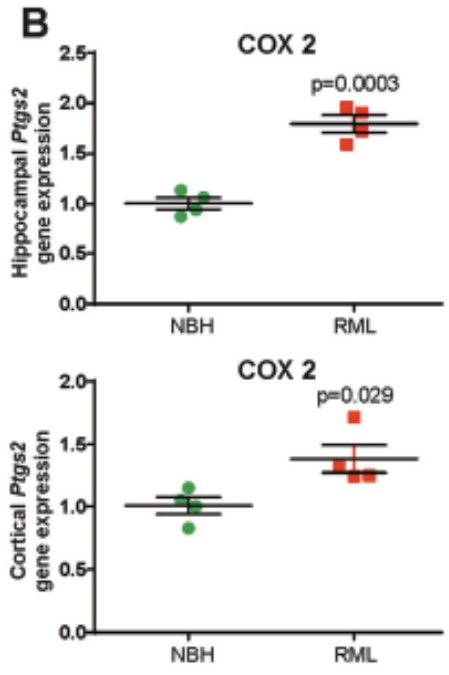
Prostaglandins E_2, D_2 and F_{2a} were increased in prion-infected mice, and COX genes were overexpressed

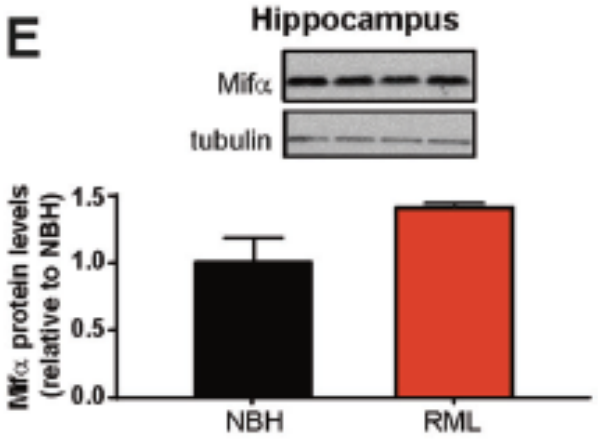
Similar findings were reported in the CSF of sCJD patiens



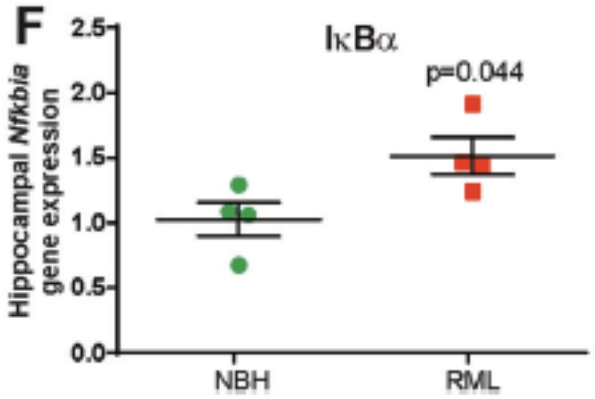


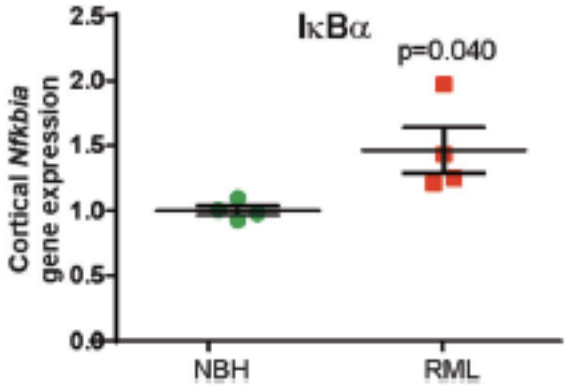






Macrophage migration inhibitory factor α (Mifα) is involved in monocyte recruitment and macrophage inhibition.
In prion disease samples, Mifα is increased of around 40%
→ to me, the bands look hydentical…





IkBα is also upregulated in prion samples.

These results suggest an activation of the anti-inflammatory system in RML samples due to a strong inflammatory condition

Conclusions

- **Altered glutamate neuropeptide homeostasis**: linked to neuronal and axonal loss and to memory deficits (in AD). Compensatory response to compensate for the reduced inhibitory signalling

- **Reduced glycolysis and energy production**: reduced utilisation of glucose as seen in AD and CJD patients. Defective mitochondrial function which may contribute to neuronal dysfunction and death

- **NO signalling augmented**: upregulation of metabolites in the arginine pathways as seen in post-mortem brain tissues.

- **Enhanced prostaglandin-mediated inflammation**: enhanced prostaglandin levels could contribute to neuroinflammation

Many metabolites identified in this study were altered also in AD and PD patients

Journal of Neurochemistry

JNC

Check for updates

JOURNAL OF NEUROCHEMISTRY | 2019 | 150 | 282–295

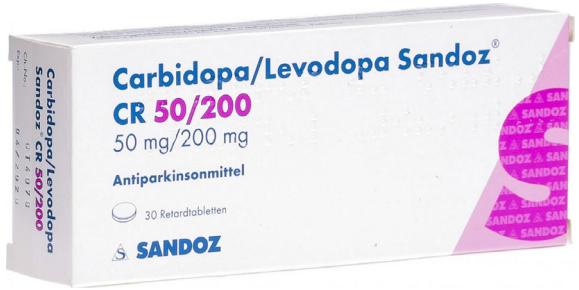
doi: 10.1111/jnc.14774

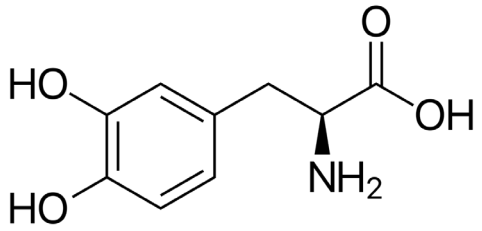
ORIGINAL ARTICLE

Metabolomics-driven identification of adenosine deaminase as therapeutic target in a mouse model of Parkinson's disease

Wanqiu Huang*†, Yazhou Xu†, Yuxin Zhang‡, Pei Zhang*§¶, Qianqian Zhang*†, Zunjian Zhang*† and Fengguo Xu*†

Looking for new therapeutic targets



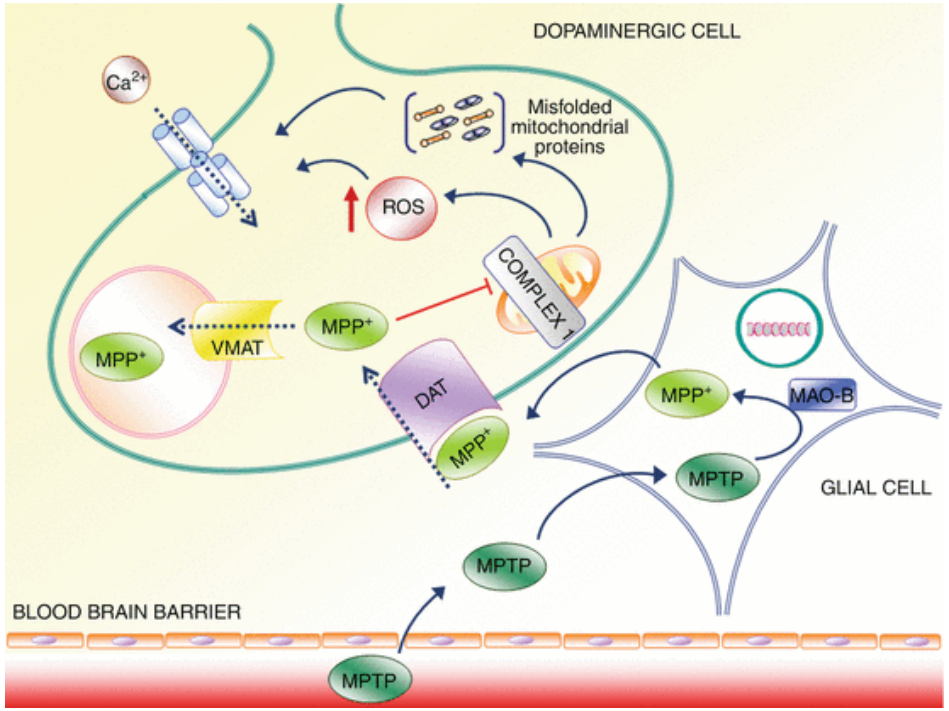


Levodopa is the first-line pharmacotherapy for PD treatment

- Only symptomatic relief – no slow down of pathology progression
- Incapable of alleviating nonmotor symptoms of PD
- Long-term complications (i.e. dyskinesia)

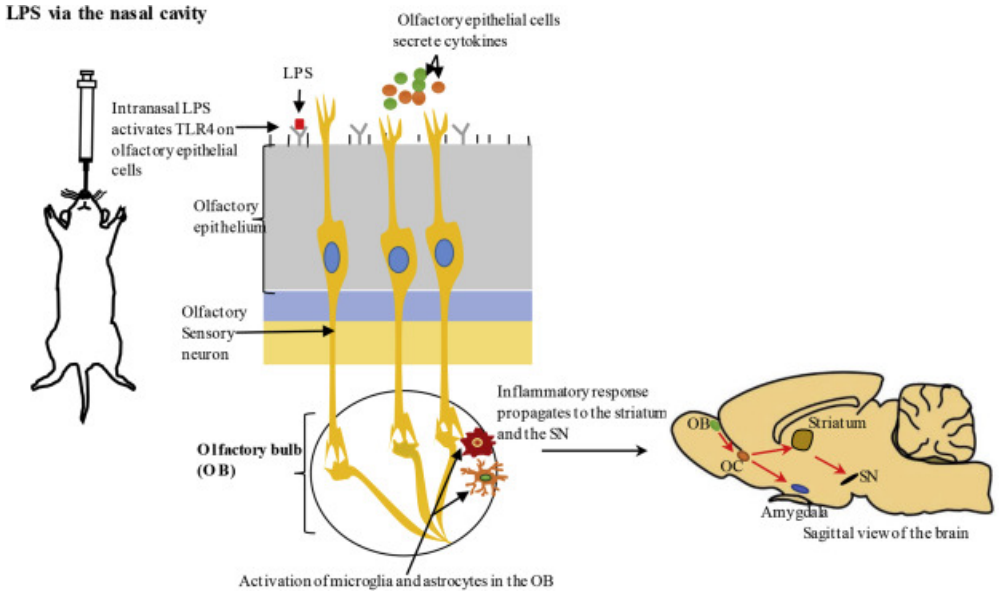
➡ Need for new therapeutics with neuroprotective action

Neuroinflammation is one of the driving forces in PD pathogenesis. Deciphering the inflammatory mechanisms might lead to the identification of novel therapeutic targets



MPTP is a neurotoxin that specifically destroys dopaminergic neurons of the substantia nigra.

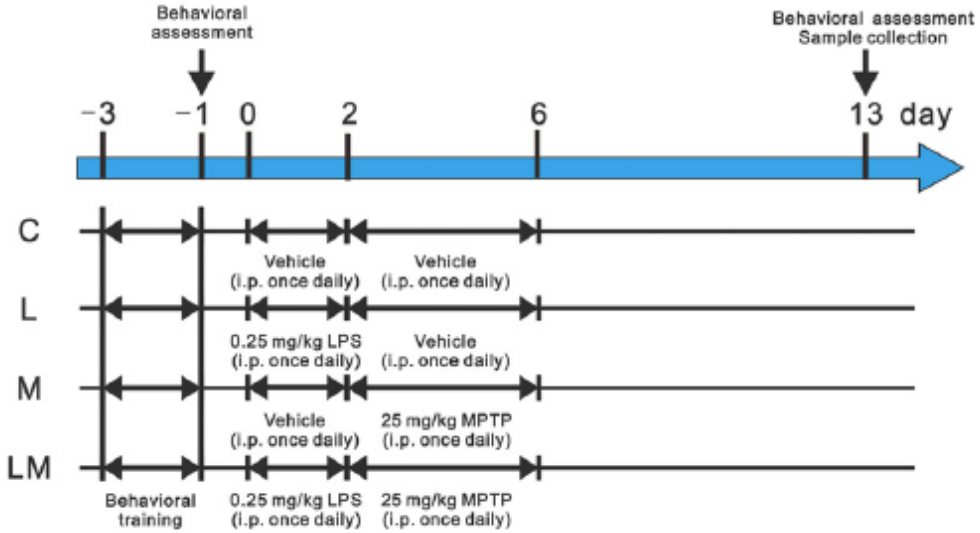
It causes permanent symptoms that recapitulate Parkinson's disease



LPS is a potent stimulator of microglia and is used to study the inflammatory process in the pathogenesis of PD

LPS + MPTP mouse model of Parkinson used in this study

Characterization of the model

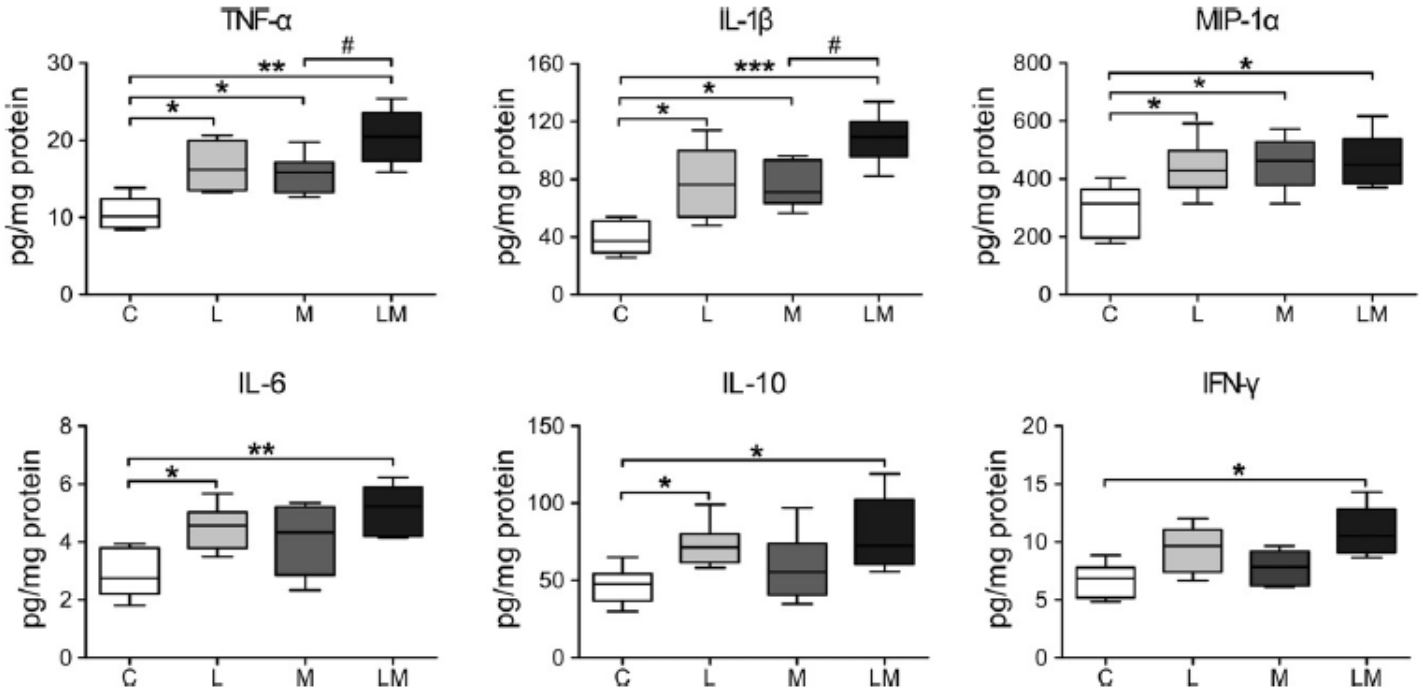


Three animal models:

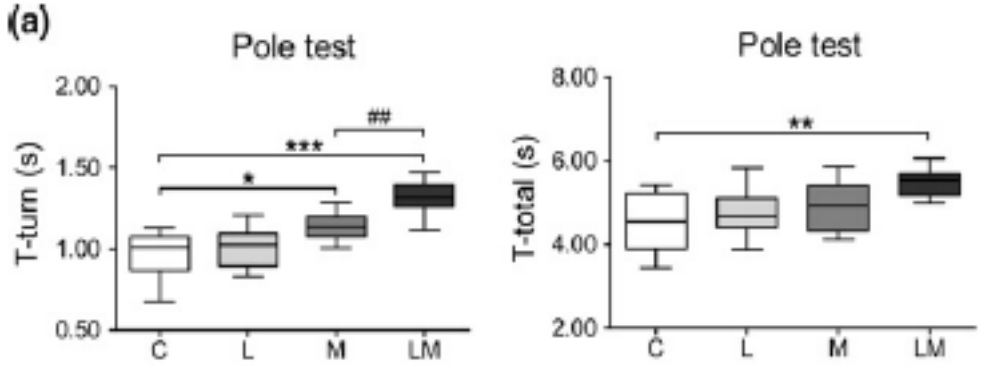
- LPS-treated (intraperitoneal injection)
- MPTP-treated
- LPS + MPTP-treated

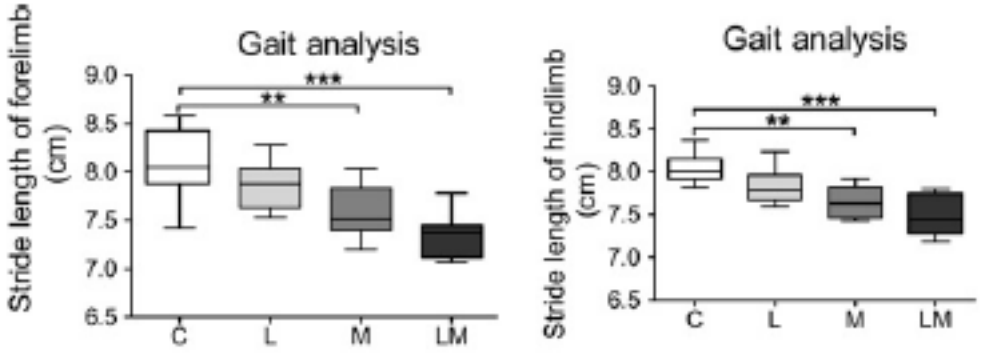
LPS-induced neuroinflammation is characterized by the release of pro-inflammatory cytokines.

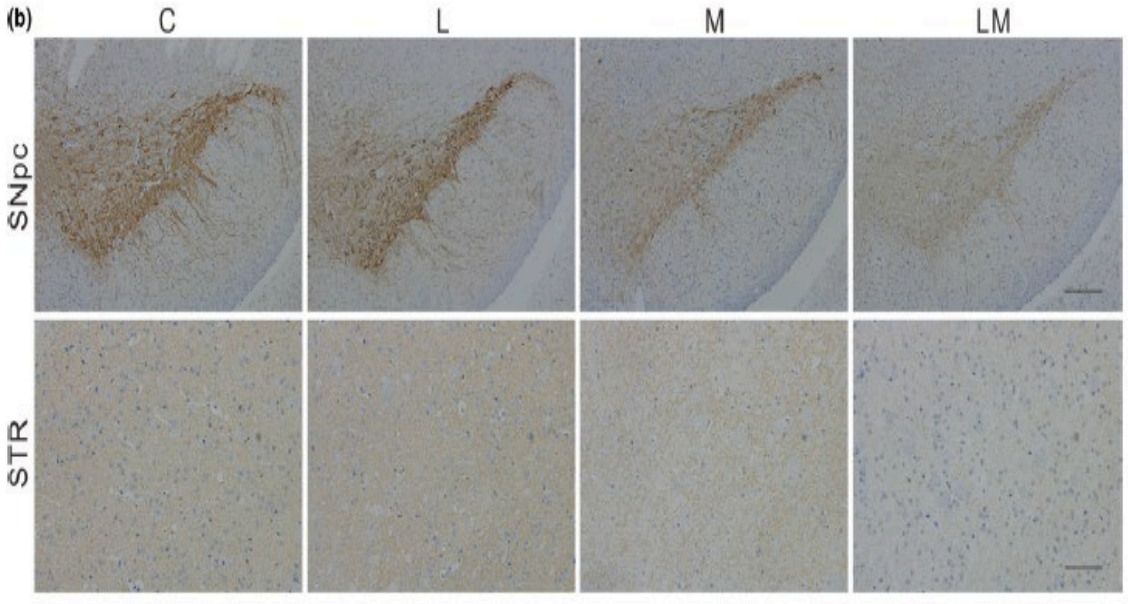
LPS injection exaggerates the MPTP-induced neuroinflammation (i.e. IL-6, IL1b, TNF-a, IL-10, IF-gamma)

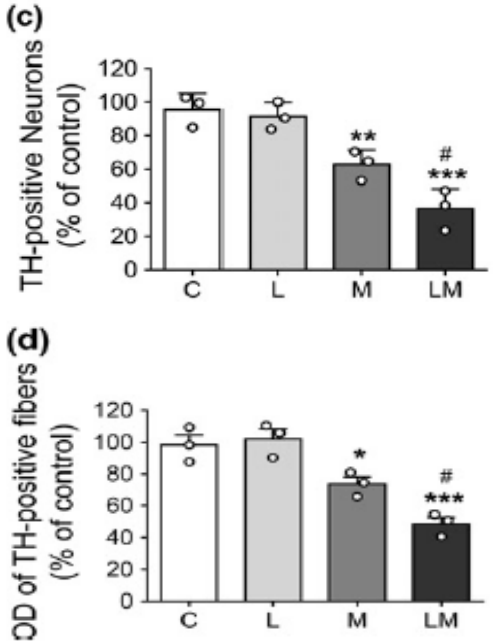


LPS exacerbated motor deficits induced by MPTP (increase in T-turn and decrease in the stride lenghts of fore and hindlimbs).



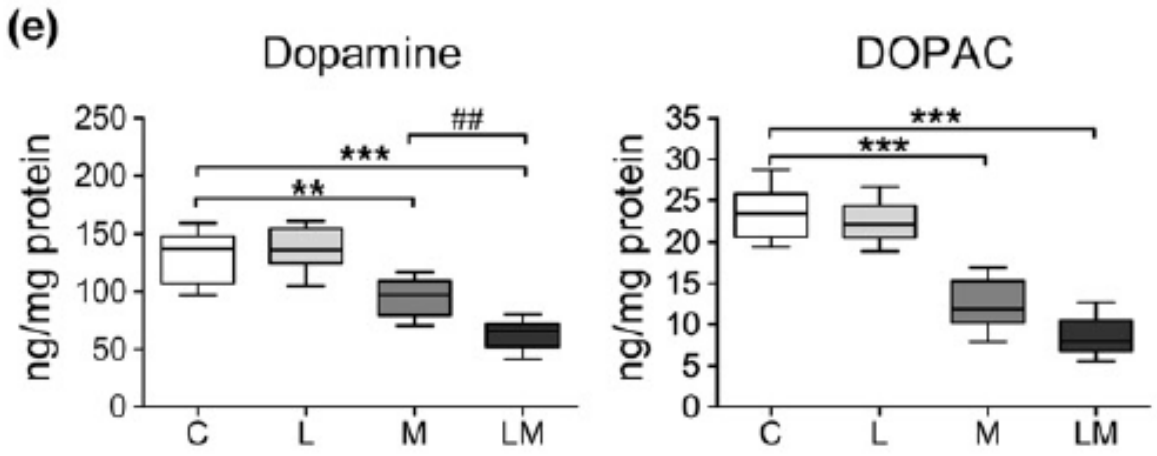






LPS did not cause loss of TH-cells.
MPTP reduced TH-cells of 38%.
Combined treatment of LPS and MPTP caused a strong decrement of TH cells (72%) and nerve terminals (48%)

Similarly, significant alterations in dopamine and DOPAC levels were found only in the MPTP mice challenged with LPS

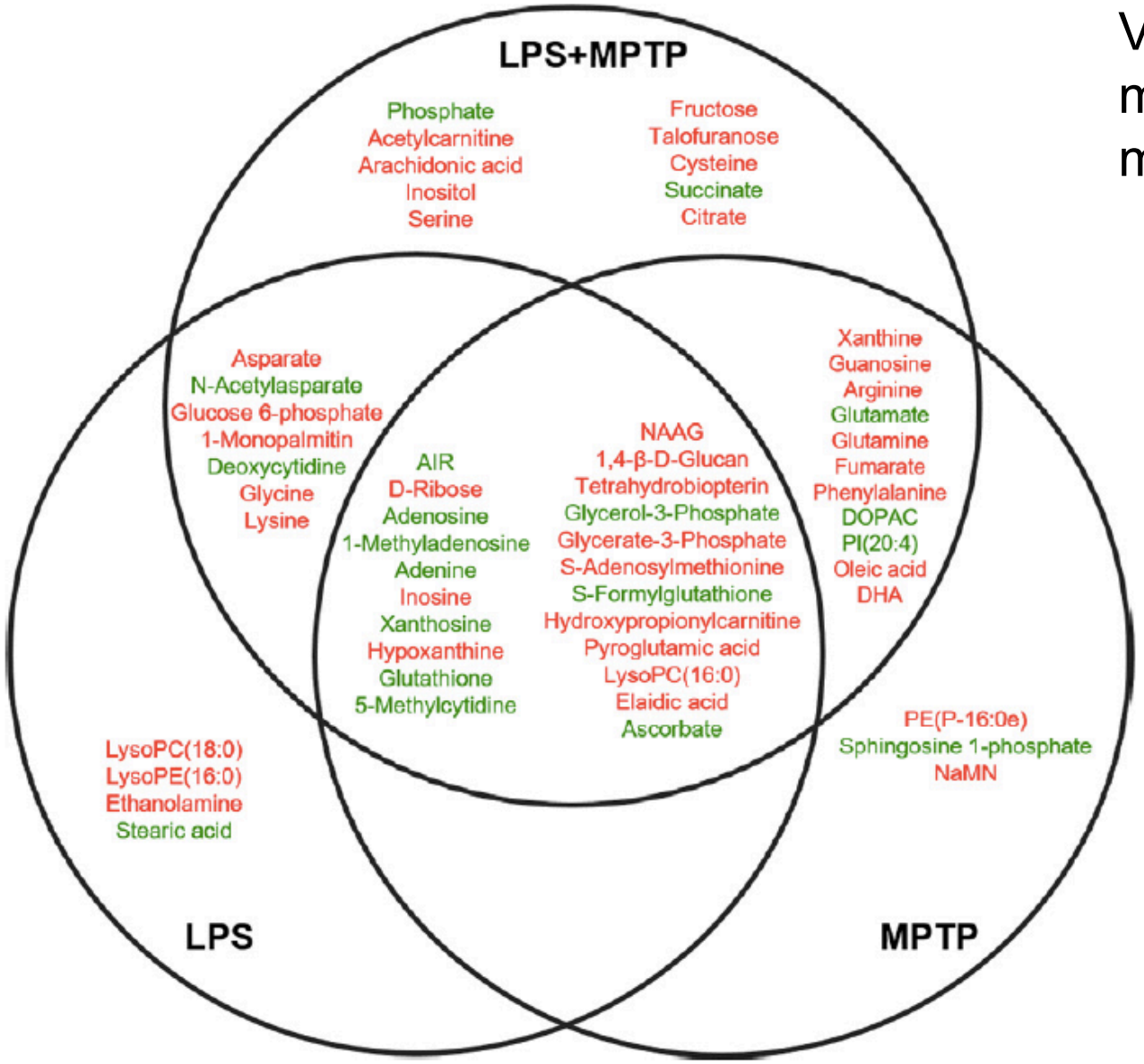


These results show that LPS injection increases the vulnerability of dopaminergic neurons of MPTP-treated mice, supporting the important role of neuroinflammation in PD

➡ Untargeted metabolomic analysis on the striatum of the three PD-mouse models

Striatum → Mass spectrometry → LC/MS, GC/MS → PCA → Metabolic pathway analysis

Metabolism blocked by addition of MeOH



Venn diagram representing the differential metabolites identified by untargeted metabolomics

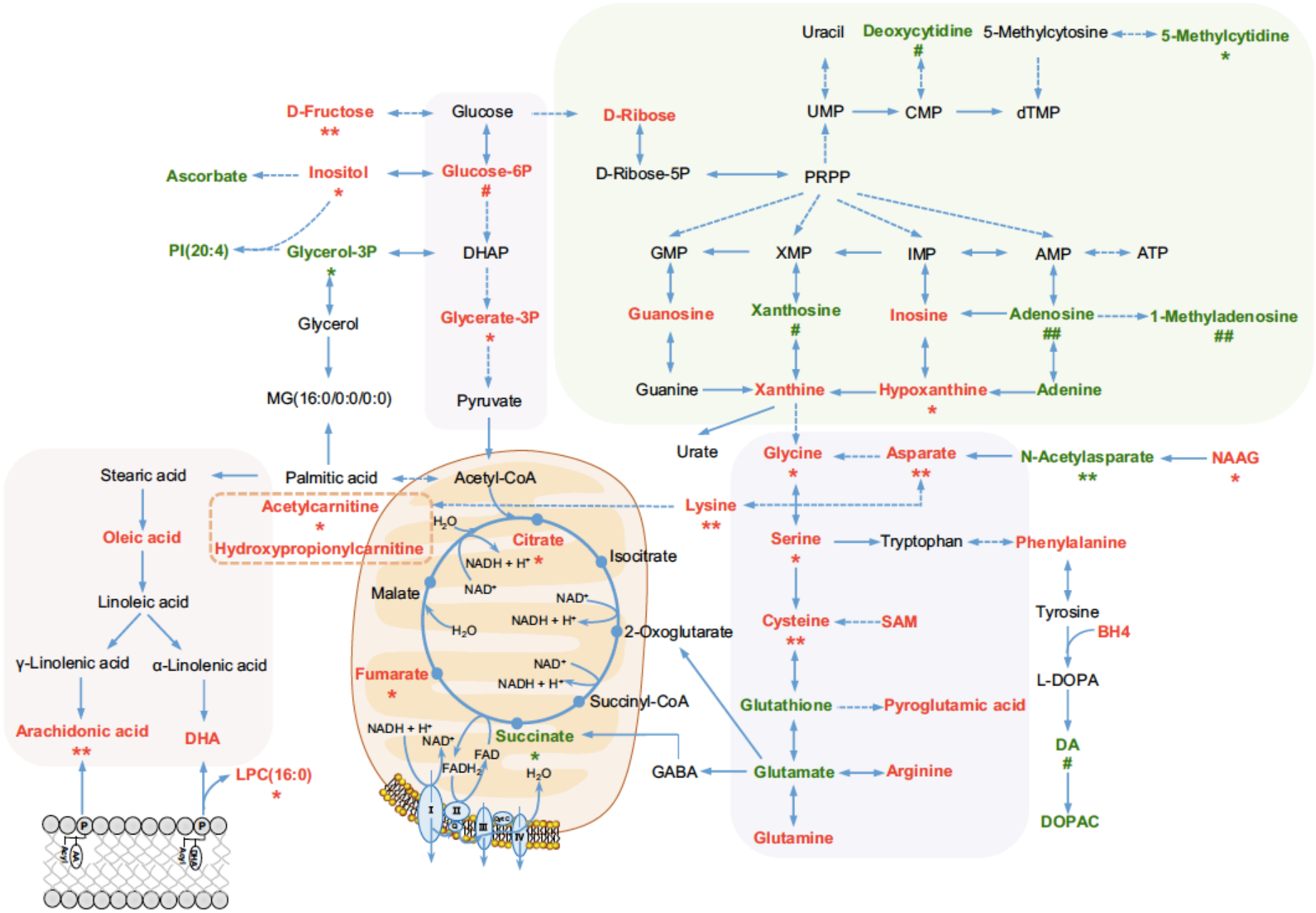
Red = upregulated

Green = downregulated

The LPS+MPTP group had the most significant metabolic alterations (17 additional metabolites due to the combined treatments)

Pathway analysis with MetabAnalyst

Metabolic pathway reconstruction → the purine metabolism pathway is the most significantly altered



Adenosine
1-methyladenosine
Xanthosine
Deoxycytidine
Glucose-6-phosphate

Significantly altered metabolites in LPS+MPTP group compared to MPTP only

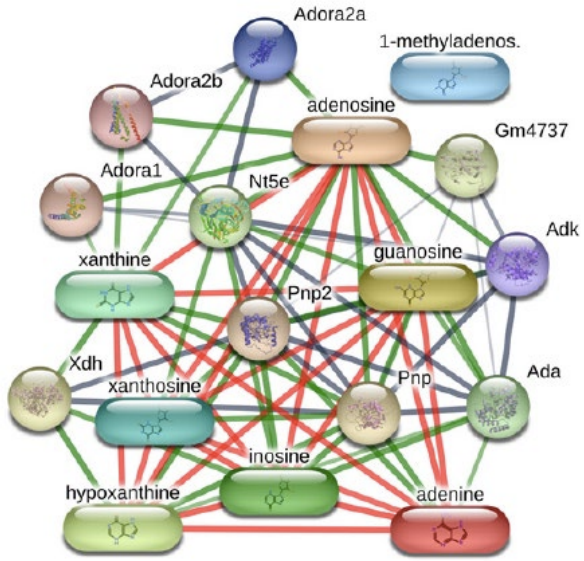
Essential metabolites in purine metabolism

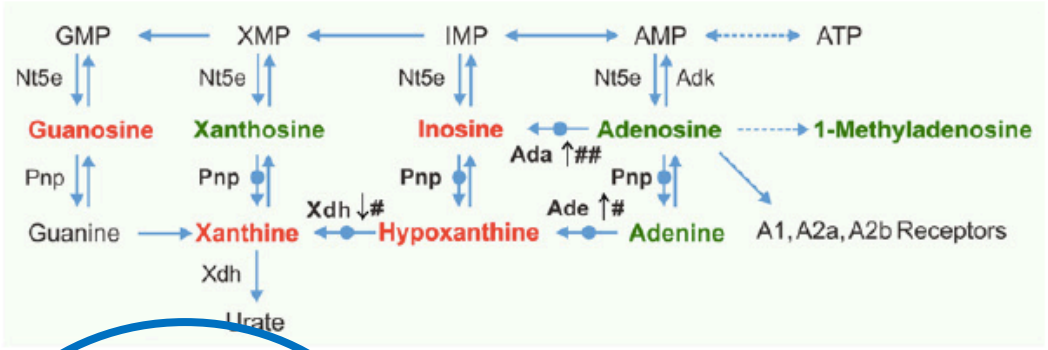
Closely related to purine metabolism

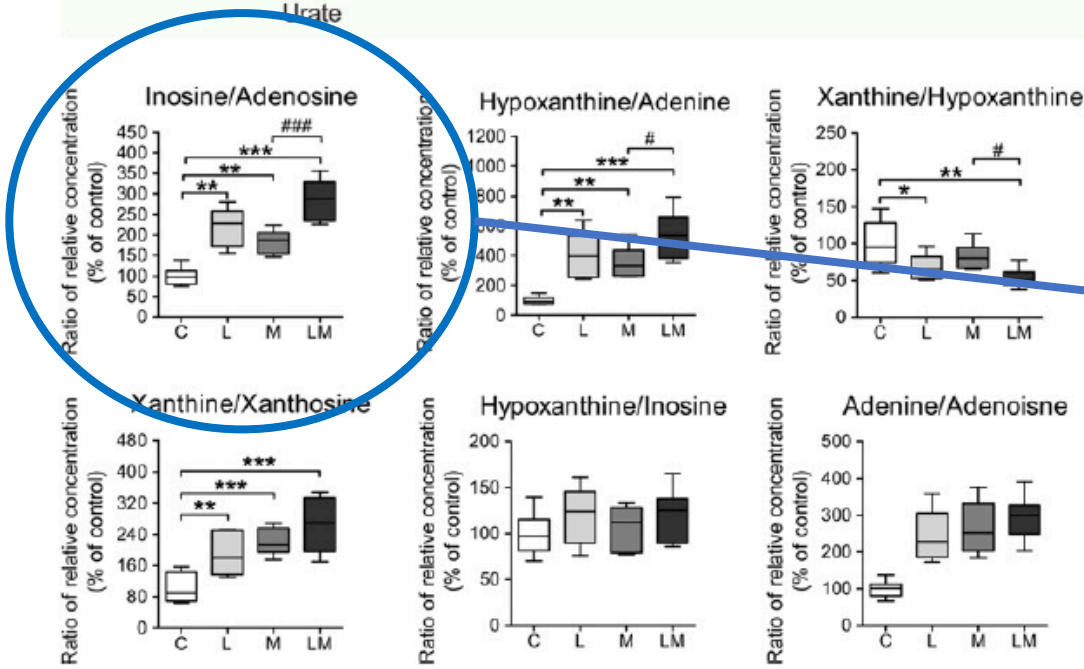
Metabolite-protein interaction analysis using the Search Tool for Interaction of Chemical database → analysis of the potential perturbations of functional proteins in the purine pathway

Two groups of functional proteins identified:

- Adenosine receptor signalling pathways (Adenosine receptors A_1 and A_{2B}, $A_{2A}R$)
- Adenosine metabolic processes (ADA and adenosine kinase)







Using the metabolomic data, they calculated the concentration ratios of the metabolites to see which enzyme was determinant in blocking the pathway

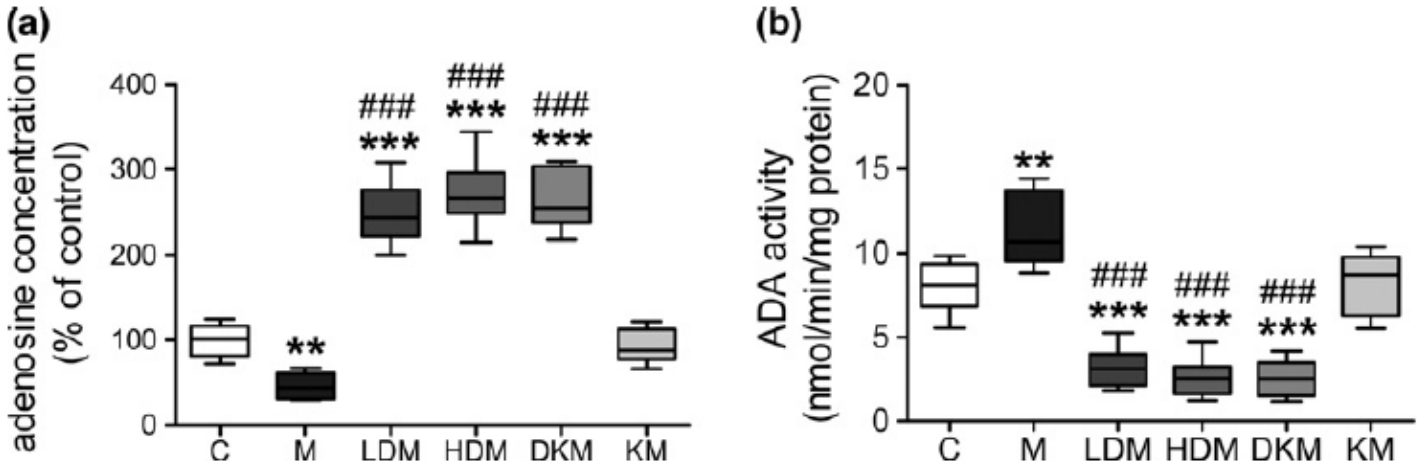
The ratio inosine:adenosine was the most significantly altered in the LPS+MPTP group

ADA is the enzyme responsible for the deamination of adenosine to inosine

An increase in the deaminase activity of ADA leads to higher levels of inosine and might contribute to the exacerbation of MPTP-mediated neurodegeneration

Validation of the neuroprotective roles of ADA inhibitors

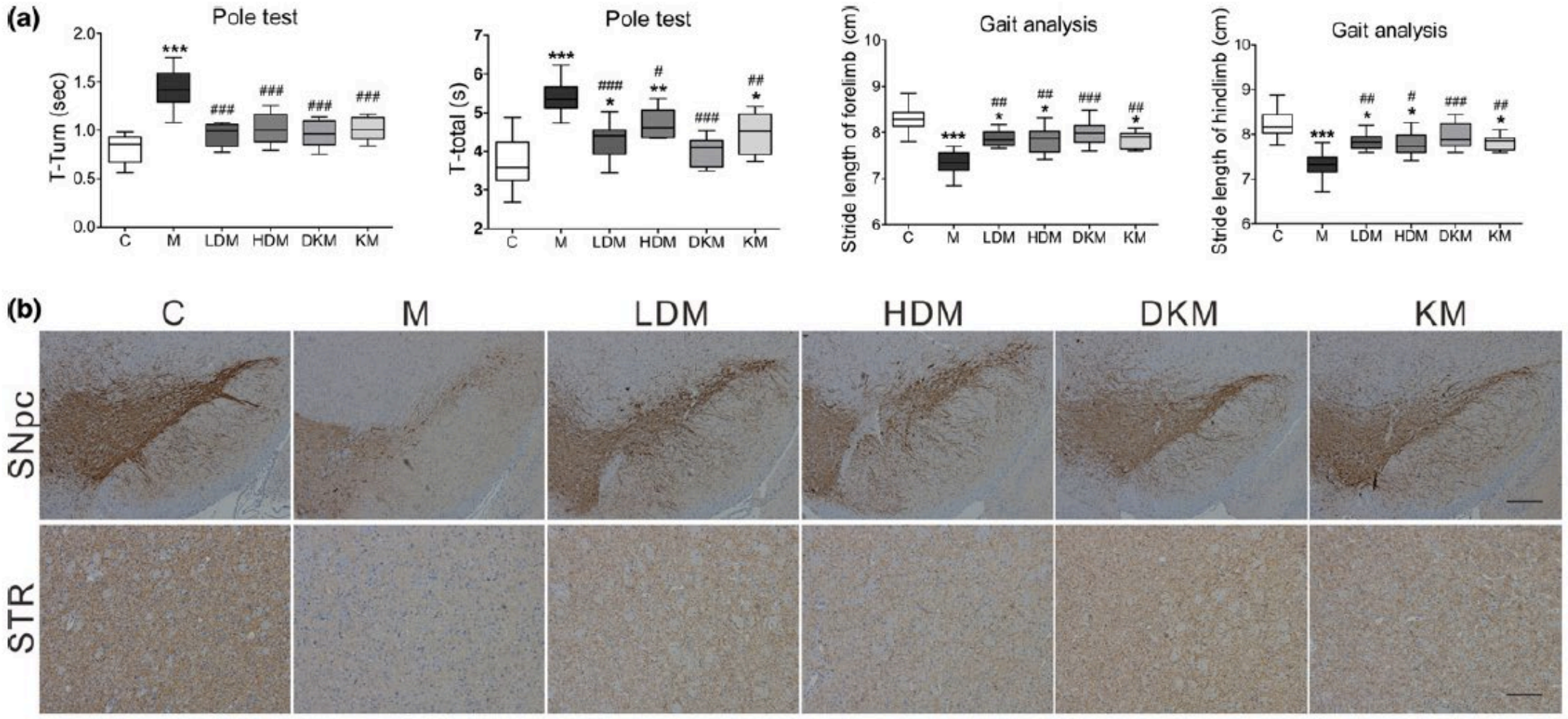
Analysis of the preventive role of ADA inhibitor (DCF) alone or in conjuntion with an $A_{2A}R$ antagonist (KW6002) already used in the treatment of PD.



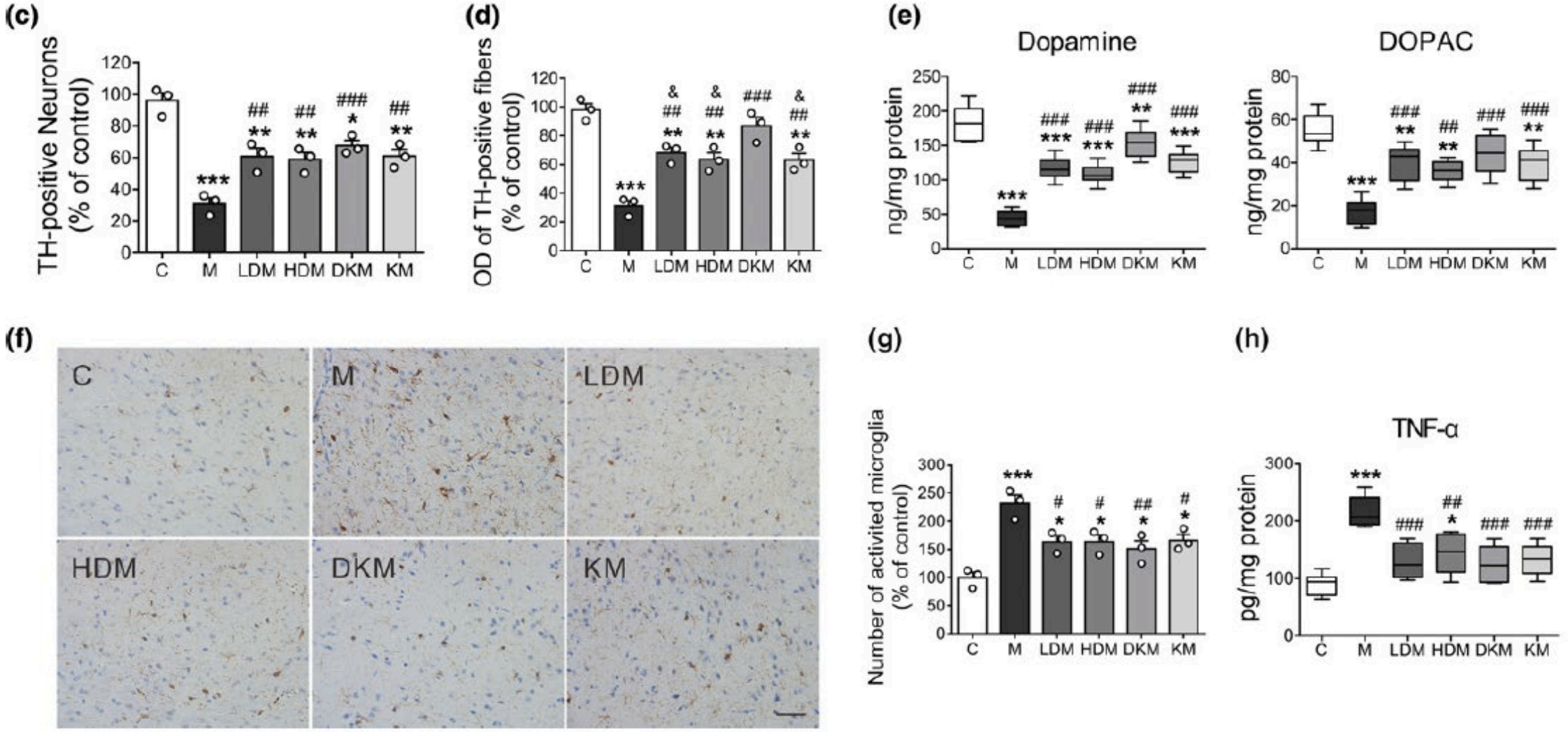
Adenosine concentration are low in a subacute MPTP-based model of PD. Both doses of DCF and DCF + antagonist increased adenosine levels

DCF exerts its effect by decreasing the activity of ADA, which is very high in MPTP mouse models. ADA deaminates adenosine into inosine

MPTP mice treated with DCF and DCF + antagonist show substantial improvements in both motor tests



Similarly, treatment with DCF and DCF + antagonist ameliorated MPTP-mediated TH immunoreactivity and increased the number of TH-positive fibers.



DCF and the antagonist fight PD-induced neuroinflammation by reducing the production of TNF-a and attentuating microglial activation

Conclusions

- Uncontrolled inflammation is involved in the exacerbation of PD progression
- MPTP treatment recapitulates some features of PD, mainly neuroinflammation, but it does not trigger Lewy body pathology. Concomitant use of LPS amplifies PD-related neuroinflammation and degeneration of dopaminergic neurons
- Untargeted metabolomics on MPTP+LPS-treated mice revealed that the purine metabolism pathway is the most affected, and similar metabolites were found altered in PD brains
- The ratio inosine:adenosine was increased in PD mouse models, due to a higher activity of ADA enzyme
- Chemical inhibitors of ADA enzyme show neuroprotective effects by inhibiting ADA activity and increasing the levels of adenosine.

- The effects of DCF on ADA were not dose dependent, and DCF was effective even at low doses.

- Prolonged irreversible inhibition of ADA can promote the accumulation of metabolites that exert toxicity on T-lymphocytes → need to test reversible inhibitors

- ADA inhibitors should be tested on models recapitulating all features of PD, especially the motor symptoms.

- Adenosine pathway is very complex, and a multi-target approach might be more effective → combination of DCF and $A_{2A}R$ antagonists attenuate the neuroinflammatory response

- The combination of both drugs resulted in addittive antiparkinsonian activity

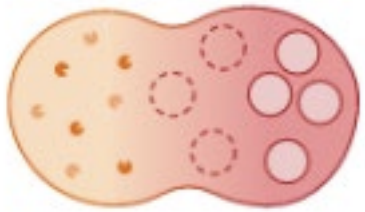
ARTICLES
https://doi.org/10.1038/s41592-021-01182-8

nature methods

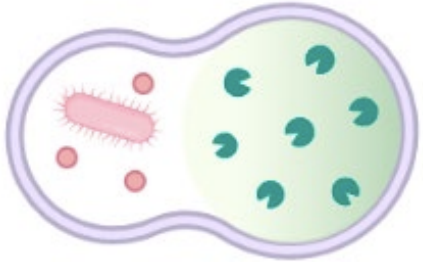
Metabolomic profiling of single enlarged lysosomes

Hongying Zhu[1,7], Qianqian Li[1,7], Tiepeng Liao[1,7], Xiang Yin[1], Qi Chen[1], Ziyi Wang[1], Meifang Dai[1], Lin Yi[1], Siyuan Ge[1], Chenjian Miao[1], Wenping Zeng[1], Lili Qu[1], Zhenyu Ju[2], Guangming Huang[3], Chunlei Cang[1,4] ✉ and Wei Xiong[1,5,6] ✉

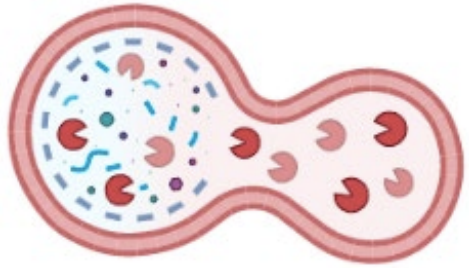
Lysosomes are very diverse in composition and function



Endolysosome



Phagolysosome



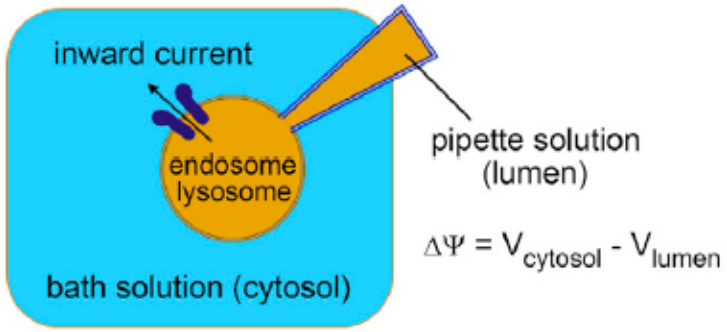
Autolysosome

Lysosome

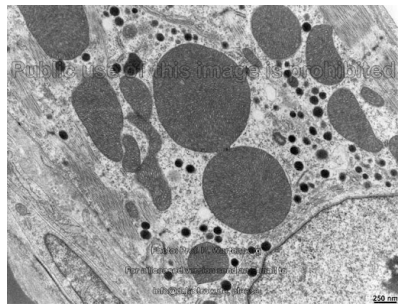
Lysosomes are present in hundreds inside cells and are involved in energy and metabolic homeostasis, signal transduction, organelle recycling.

Lysosomes are very heterogeneous and are organized in subclasses which play roles in distinct cellular processes and might contibute to the onset of pathologies (i.e. lysosomal storage disorders, neurodegenerative diseases).

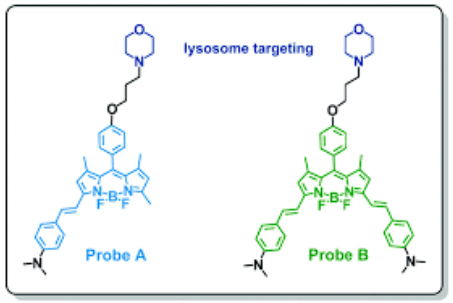
Single-lysosome technology is used to study different types of lysosomes and characterize their content and their functions.



Lysosomal patch-clamp



Electron microscopy



Lysosome-targeting probes

Electrophysyological properties

Dynamics

Function

Metabolic profiling of lysosomes

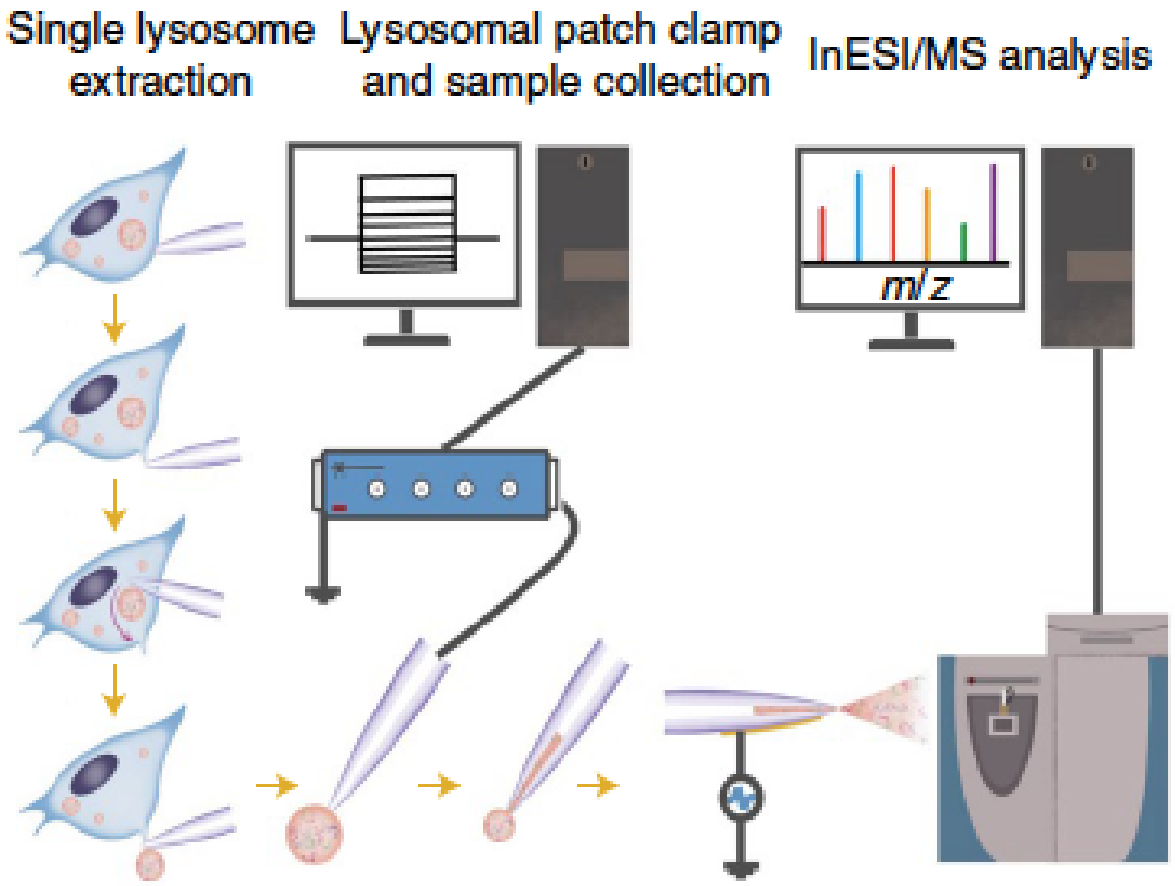
⇩

Lysosomal patch clamp

✚

nanoESI-Mass Spectrometry

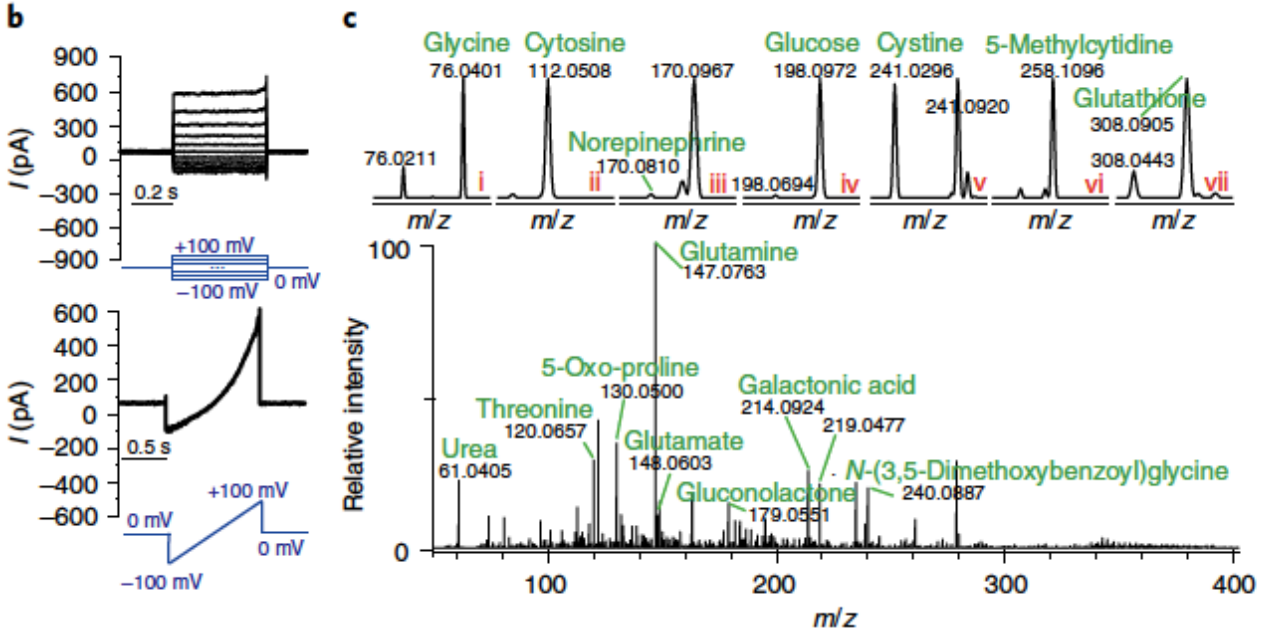
SLMS platform

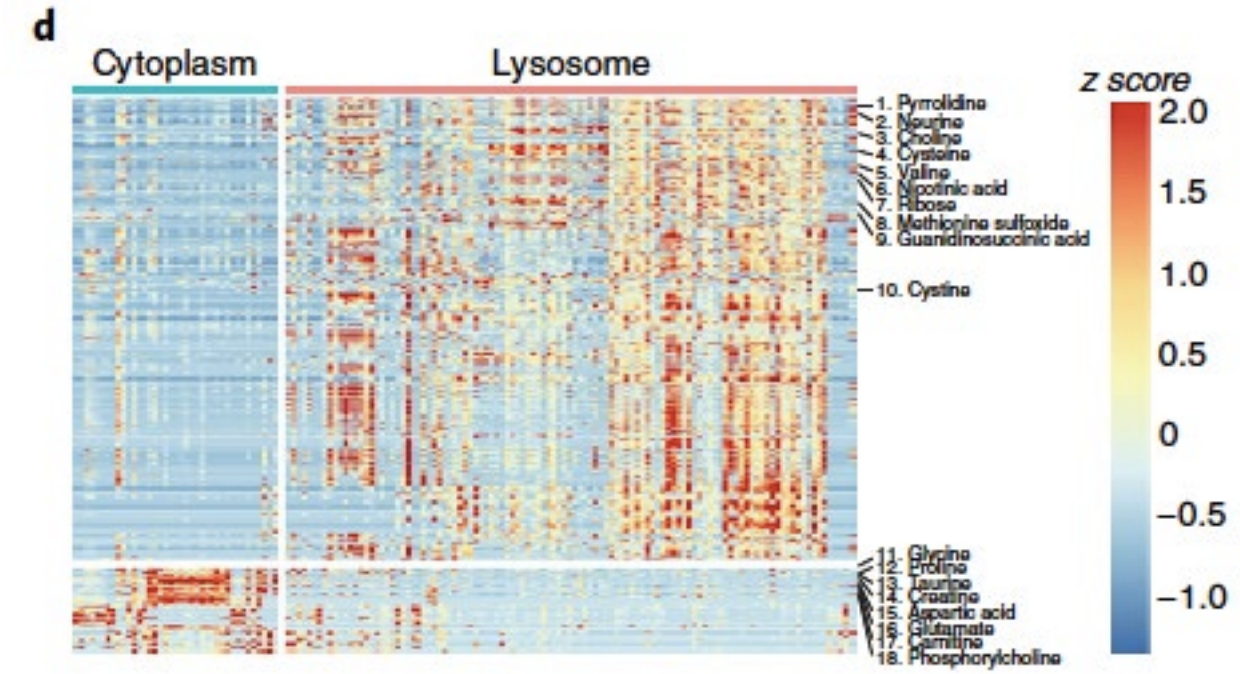


Extracellular solution was used as blank control for MS analysis

Most of the identified metabolites were specific to the lysosomes

As lysosomes are too tiny to be patched, homotypic fusion was triggered using vacuolin-1 treatment, that does not interfere with lysosomal metabolism

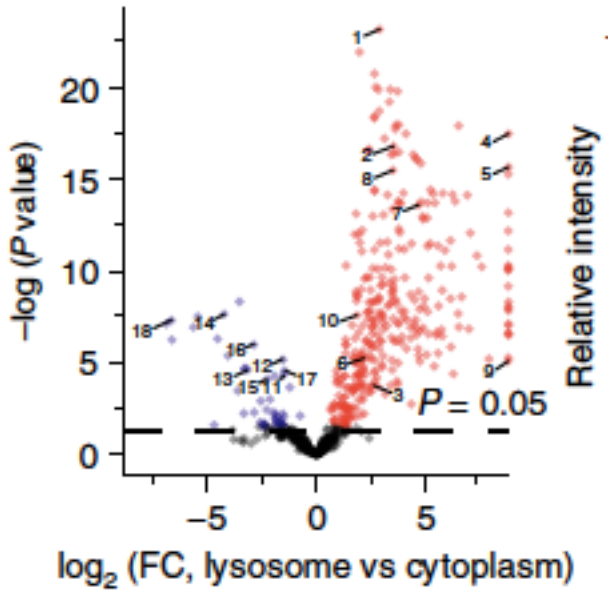




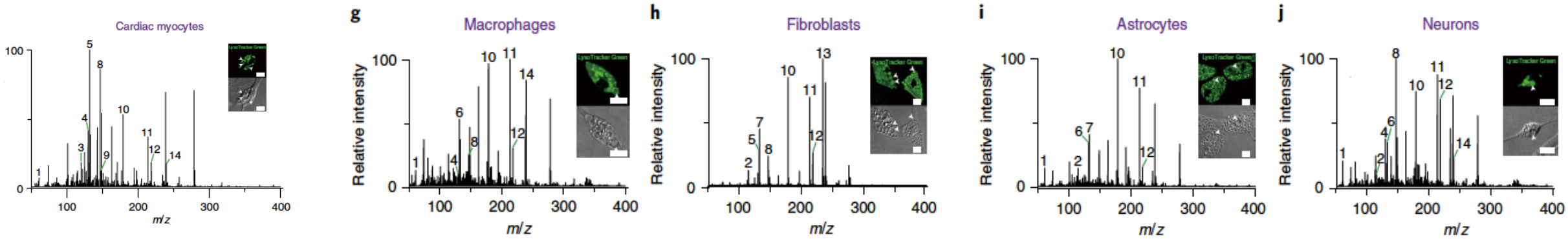
606 metabolites were detected, and 283 passed the quality controls and were assigned to the lysosomes

Concentrations of most metabolites were enriched in the lysosomes compared to the cytoplasm

SLMS is a valid technique for lysosomal metabolic profiling

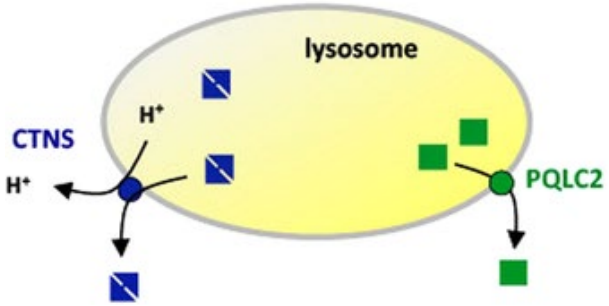


Electrophysyological and metabolic recordings of lysosomes of four different cell types



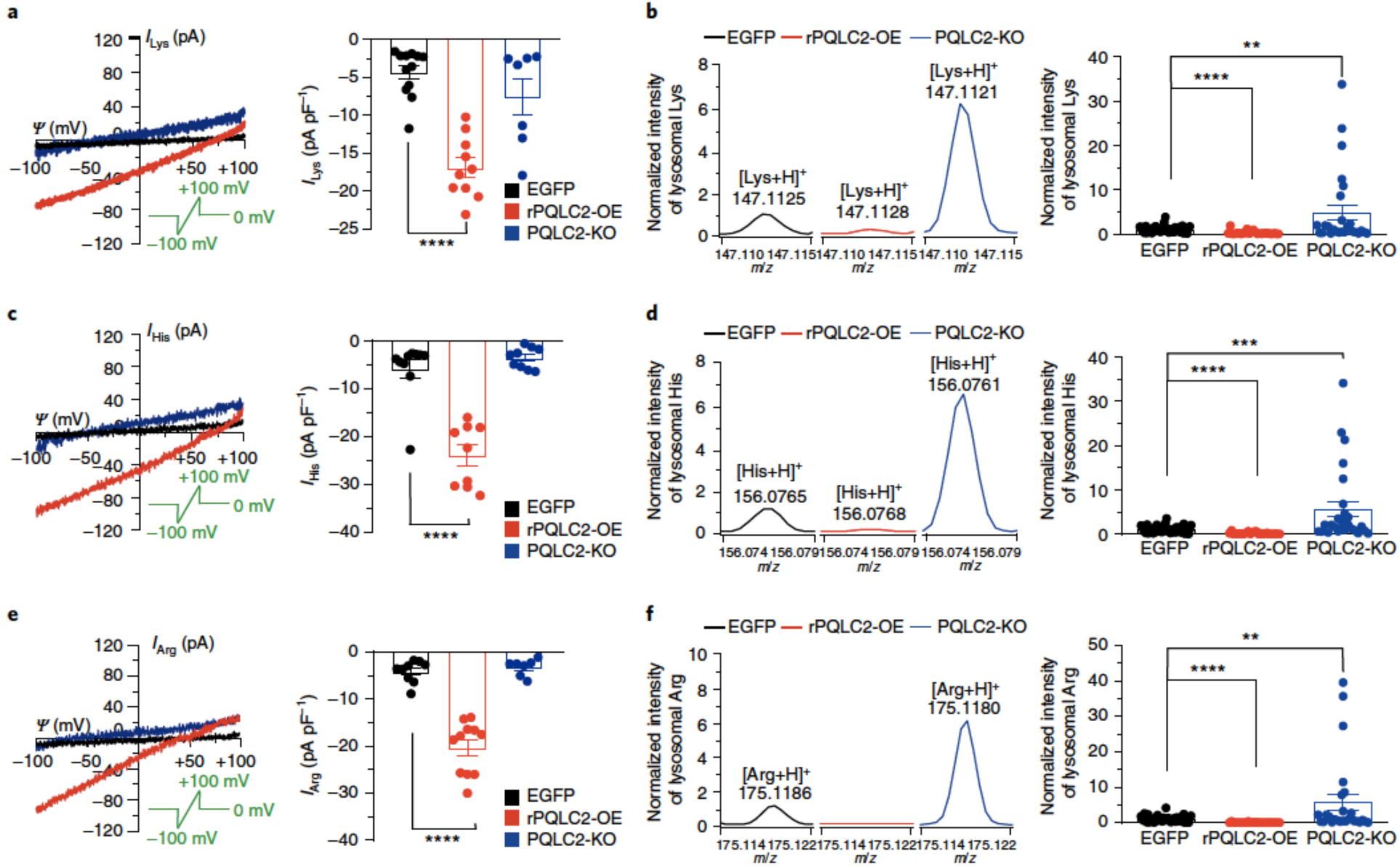
Successful!!

Functional verification #1: analysis of lysosomal amino acid transporters by SLMS



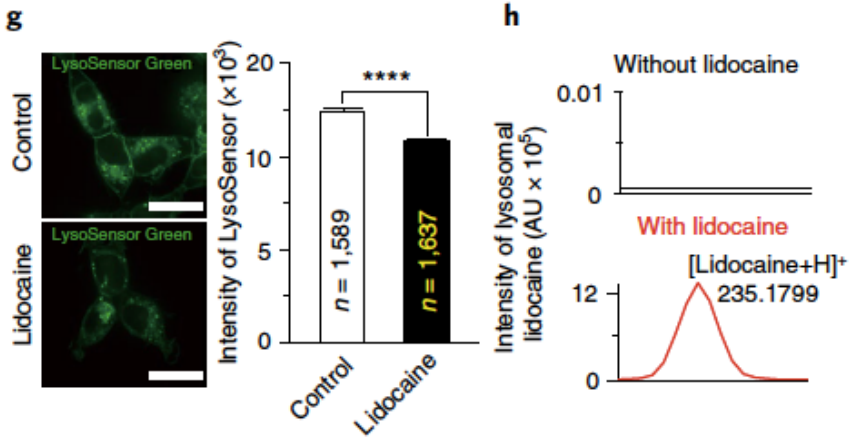
PQLC2 exports cationic amino acids from the lumen to the cytosol

SLMS on HEK cells overexpressing or KO for PQLC2 → larger currents and decrease in Arg and Lys levels in overexpressing cells.
Accumulation of Lys, Arg and His in KO cells.
No difference in lysosomal currents before and after treatment with vacuolin-1.


a
I_{Lys} (pA)
Ψ (mV)
+100 mV
0 mV
−100 mV
I_{Lys} (pA pF^{-1})
****
EGFP
rPQLC2-OE
PQLC2-KO
b
EGFP rPQLC2-OE PQLC2-KO
Normalized intensity of lysosomal Lys
[Lys+H]$^+$ 147.1125
[Lys+H]$^+$ 147.1128
[Lys+H]$^+$ 147.1121
m/z
**
****
c
I_{His} (pA)
I_{His} (pA pF^{-1})
d
Normalized intensity of lysosomal His
[His+H]$^+$ 156.0765
[His+H]$^+$ 156.0768
[His+H]$^+$ 156.0761
***
e
I_{Arg} (pA)
I_{Arg} (pA pF^{-1})
f
Normalized intensity of lysosomal Arg
[Arg+H]$^+$ 175.1186
[Arg+H]$^+$ 175.1180

Functional verification #2: trapping of drugs by the lysosomes

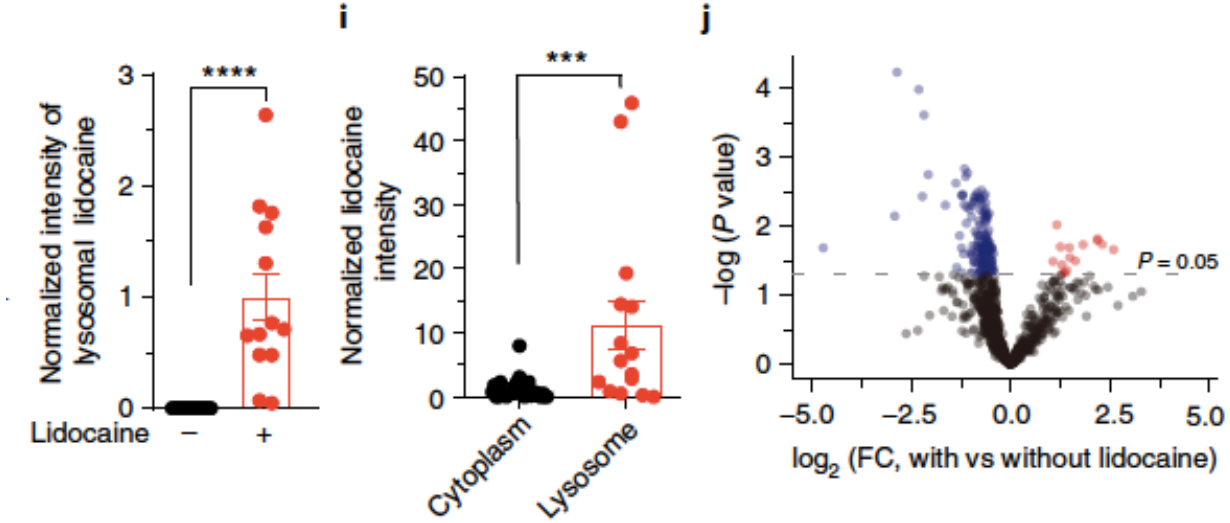
Lysosomotropic drugs that are weak bases can accumulate in acidic lysosomes, increase their pH and lead to lysosomal dysfunction



pH increases after addition of lidocaine. SLMS detected lidocaine inside lysosomes

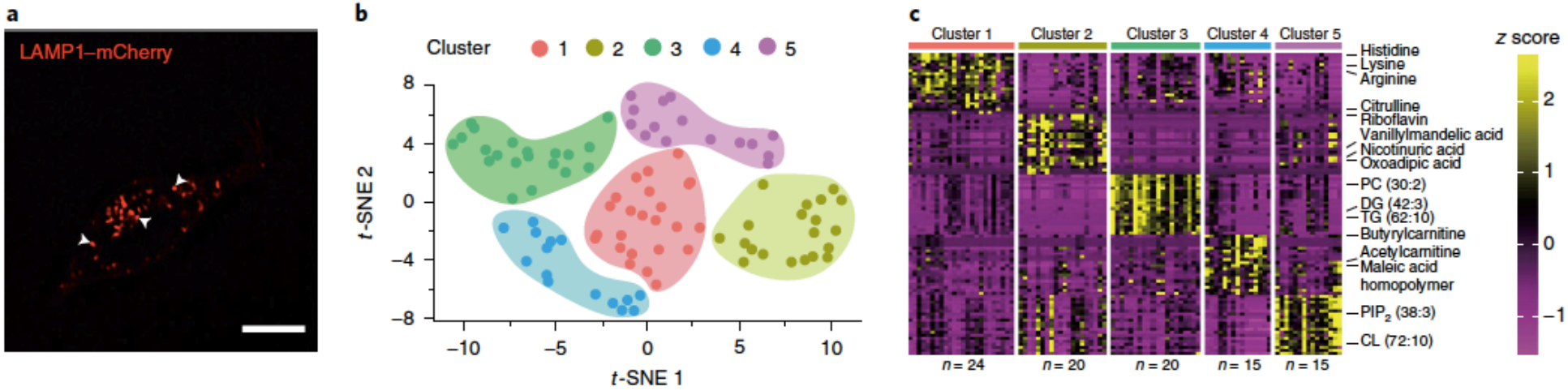
SLMS measured a tenfold higher concentration of lidocaine in lysosomes.

70.9% of all metabolites were decreased in lysosomes containing lidocaine, suggesting some dysfunction in catabolism



Metabolomic-based classification of lysosomes

SLMS was used to analyse the metabolome of HEK cells-derived lysosomes. Lysosomes were clustered into five subpopulations and subtype-specific metabolites were classified using a Wilcoxon rank-sum test.



Cluster 1
Arg, Lys, His

Cluster 2
Oxoadipic acid
Nicotinuric acid

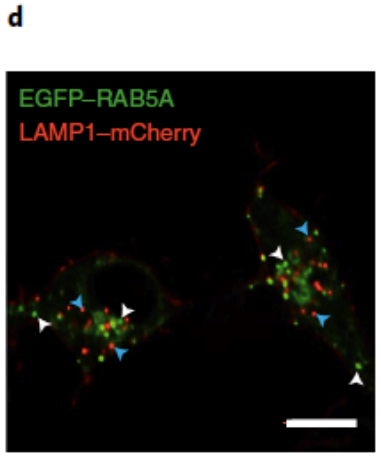
Cluster 3
Triglicerids
Phosphatidylcholine

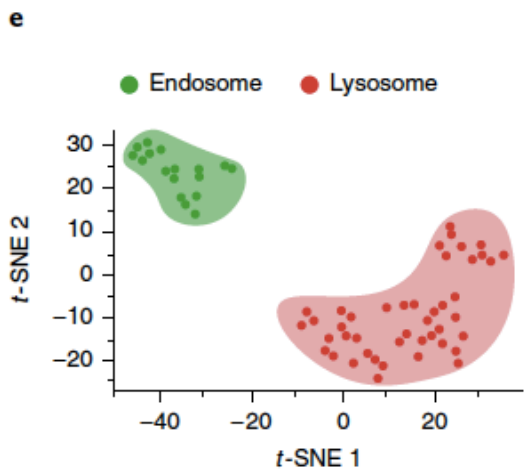
Cluster 4
Fatty acids

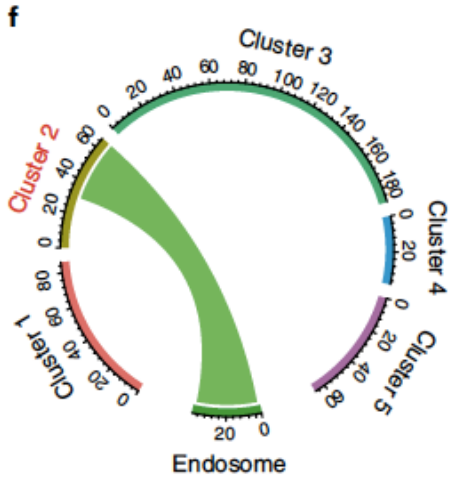
Cluster 5
Other lipids

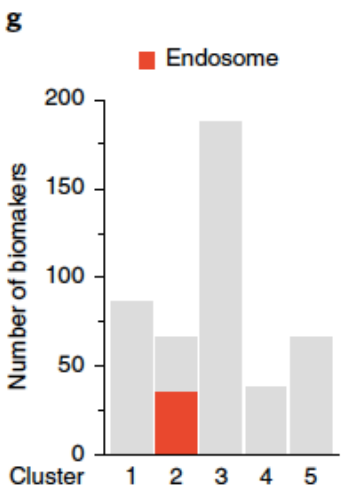
Discrimination between endosomes and lysosomes

Late endosomes fuse with lysosomes for the degradation of endocytic substrates







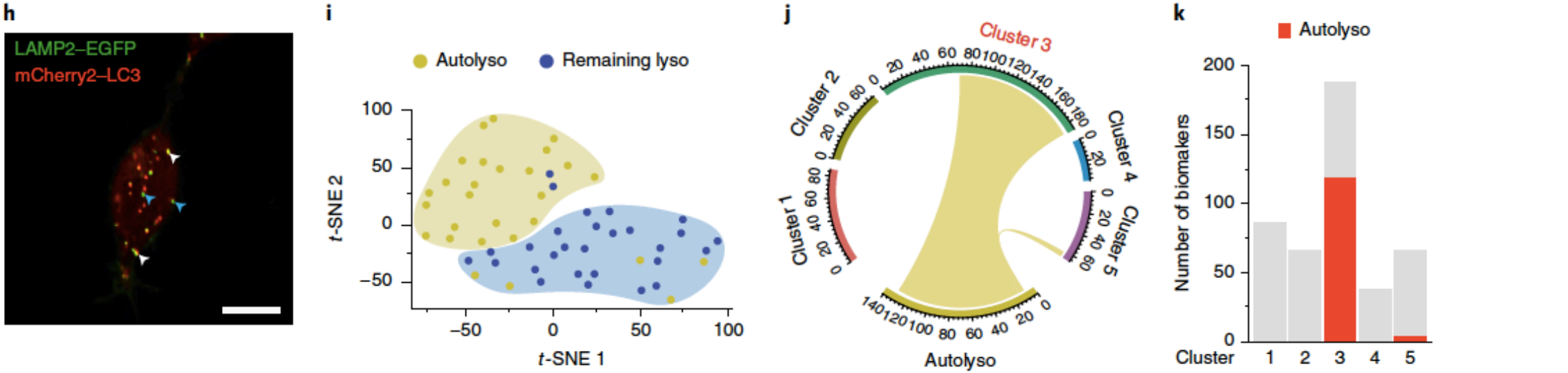


505 different metabolites
456 increased
49 decreased

Endosomes and lysosomes clustered separately, indicating a metabolic heterogeneity

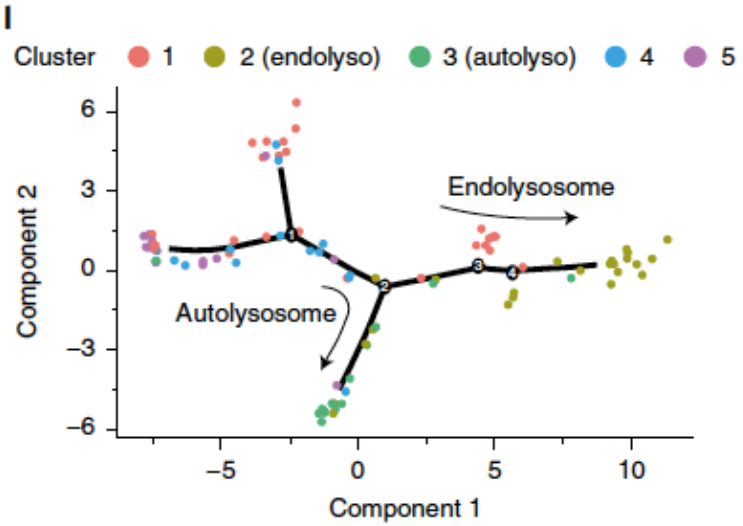
Metabolites found in endosomes show a strong and specific overlap with cluster 2, suggesting that it might represent endolysosomes

Autolysosomes (LC3-positive) are a subtype of lysosomes that degrade autophagic substrates

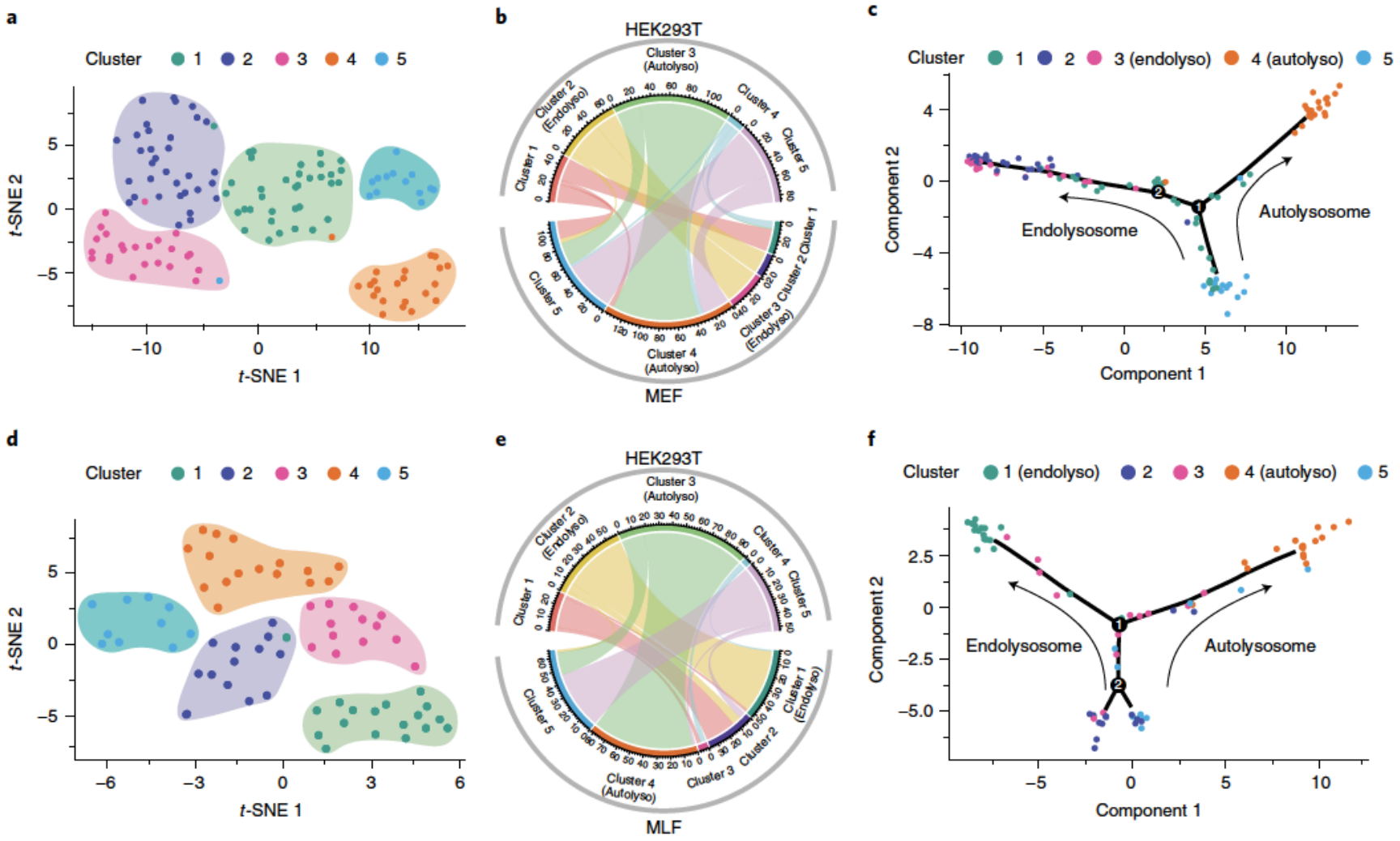


Autolysosomes show a characteristic metabolomic profile that distinguish them from the other lysosomes (192 increased and 73 decreased metabolites).

Autolysosomes profile closely match that of cluster 3



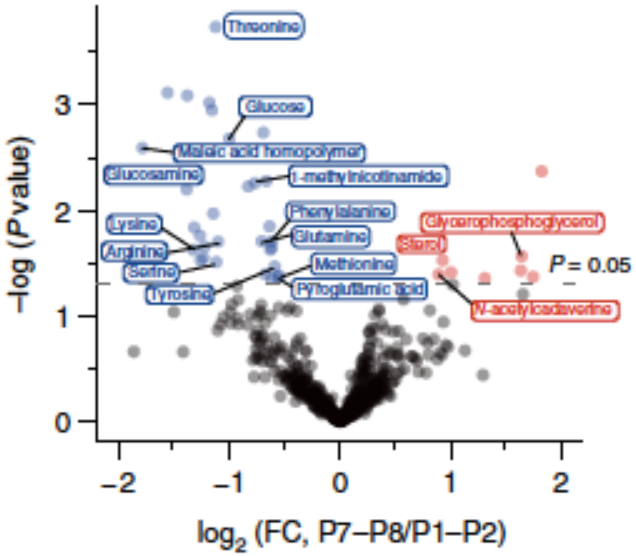
Lysosomal profiling of MEFs and MLFs

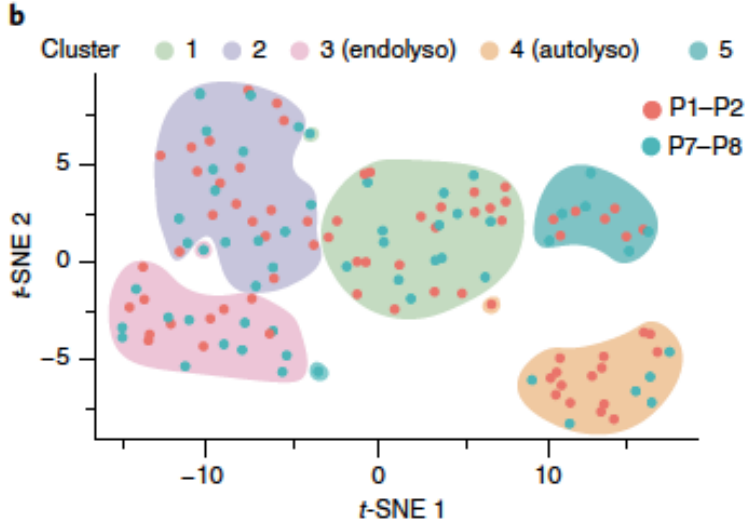


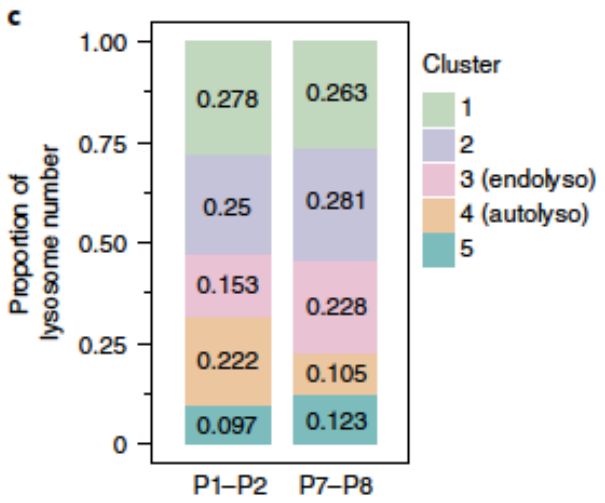
Lysosomes of mouse embryonic and lung fibroblasts clustered in 5 subtypes as already observed in HEK cells

Lysosomal metabolism during senescence

Lysosomes from MEFs at passages 1-2 and passages 7-8 (senescent cells) were compared by SLMS.

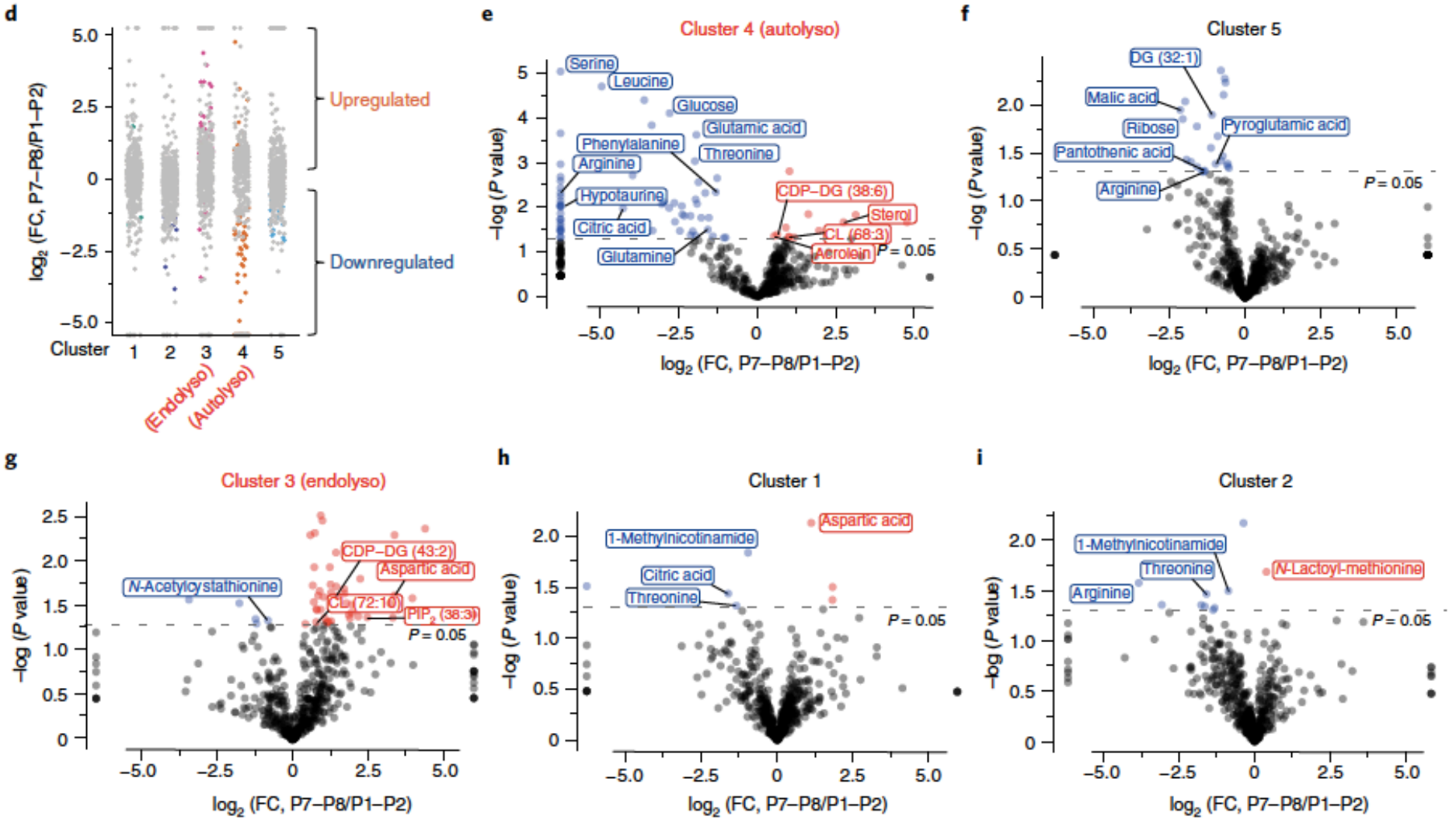






Decrease in amino acids content → defects in catabolism of large biomolecules

Decrease in the amount of autolysosomes (cluster 4) in senescent cells → consistent with reports of autophagy decreasing with age



Metabolic changes during senescence are specific to lysosomal subpopulations

Conclusions

- SLMS combines lysosomal patch-clamp (analysis of ion channels and transporters) and MS (metabolomic analysis of lysosomal content).
- To perform SLMS, cells were treated with vacuolin-1 to trigger the homotypic fusion of lysosomes.
- SLMS allowed the identification of five subpopulations of lysosomes in different cultured cell types.
- Two of the five clusters correspond to endolysosomes and autolysosomes, while the other three are either immature or newly discovered lysosomes.
- Metabolic analysis revealed important differences occurring during senescence and cancer.


Thank
You
Merci
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