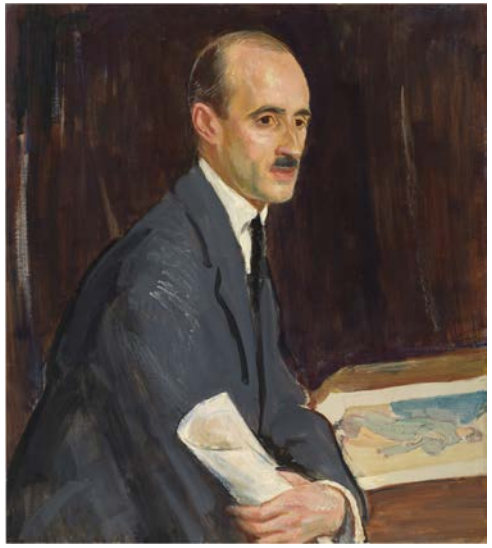
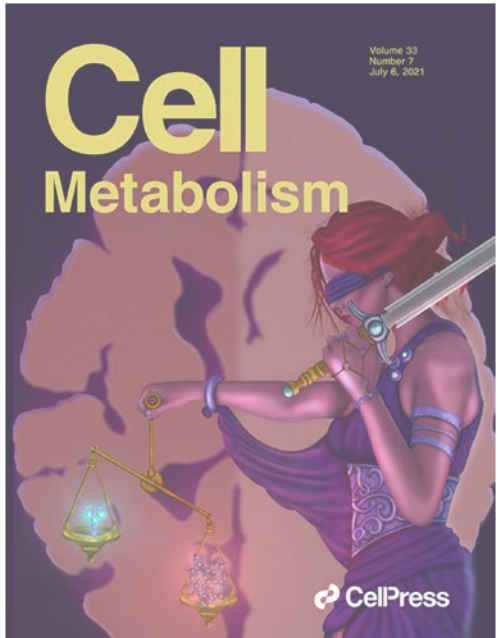


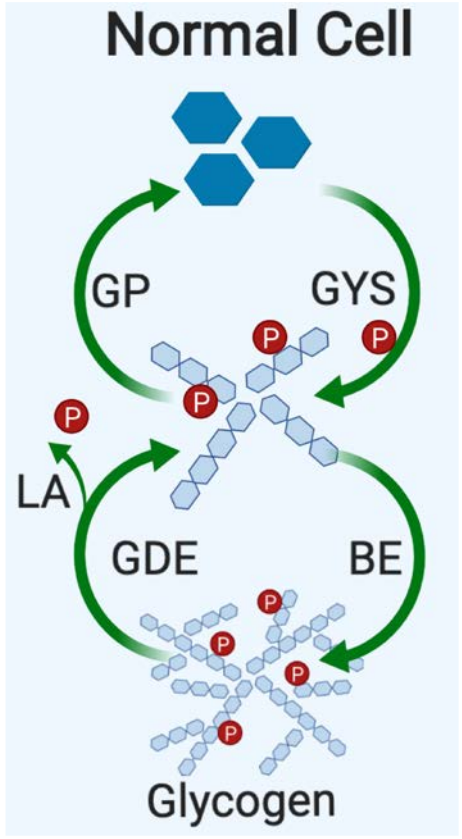
# Preclinical Therapies Developed for LD: where we are and future perspectives




## Lafora disease Science Symposium
## Bologna, Italy
## October 9-10, 2023

**Matthew S. Gentry, Ph.D.**
Professor & Chair, Biochemistry & Molecular Biology
University of Florida, College of Medicine
Director, Lafora Epilepsy Cure Initiative (LECI)

# Brain Glycogen Metabolism




**Glycogen synthesis:**

**GS - glycogen synthase**

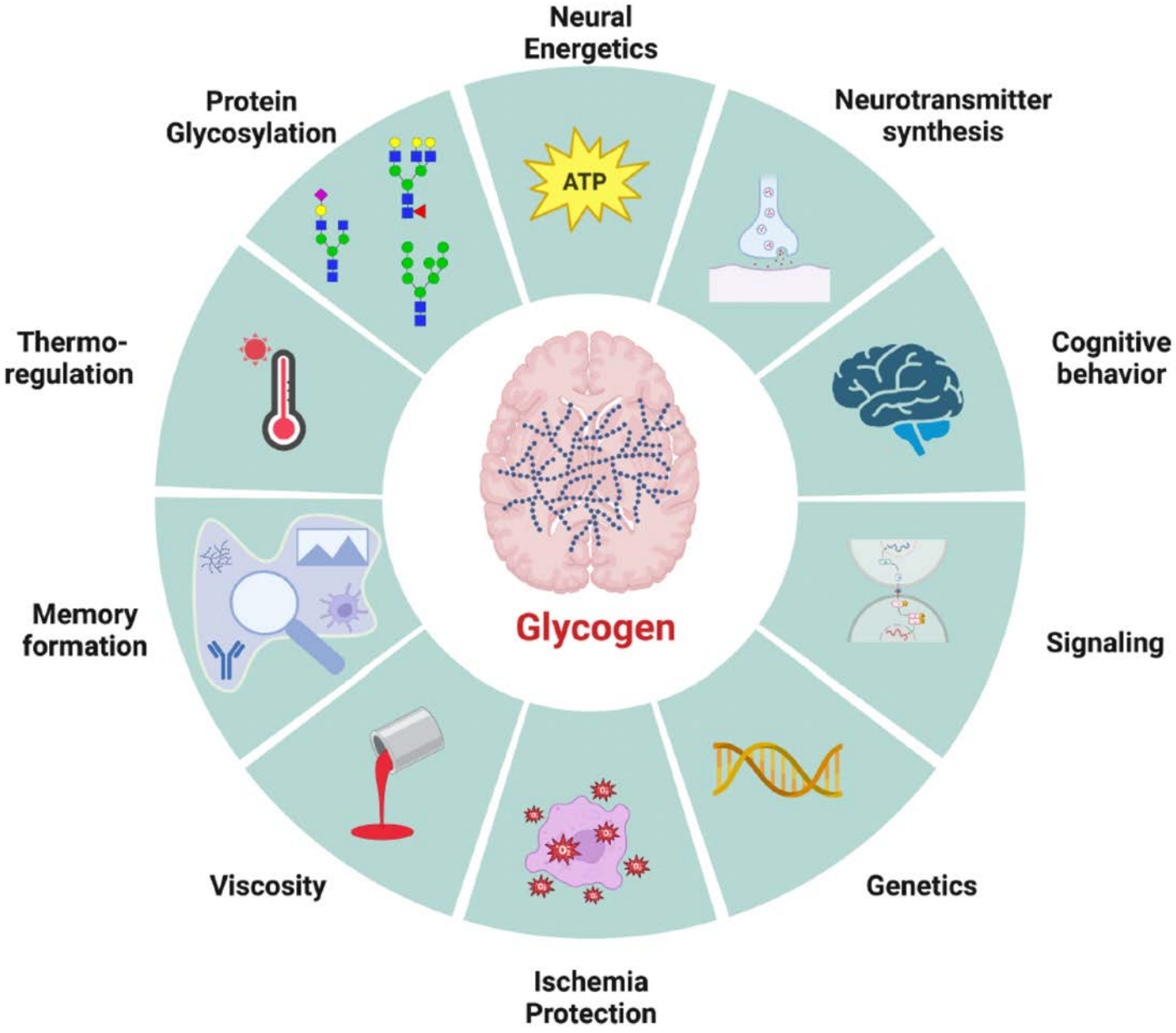
**GBE - glycogen branching enzyme**

**Glycogen breakdown:**

**GP - glycogen phosphorylase**

**GDE - glycogen debranching enzyme**

**LA - laforin (glycogen phosphatase)**



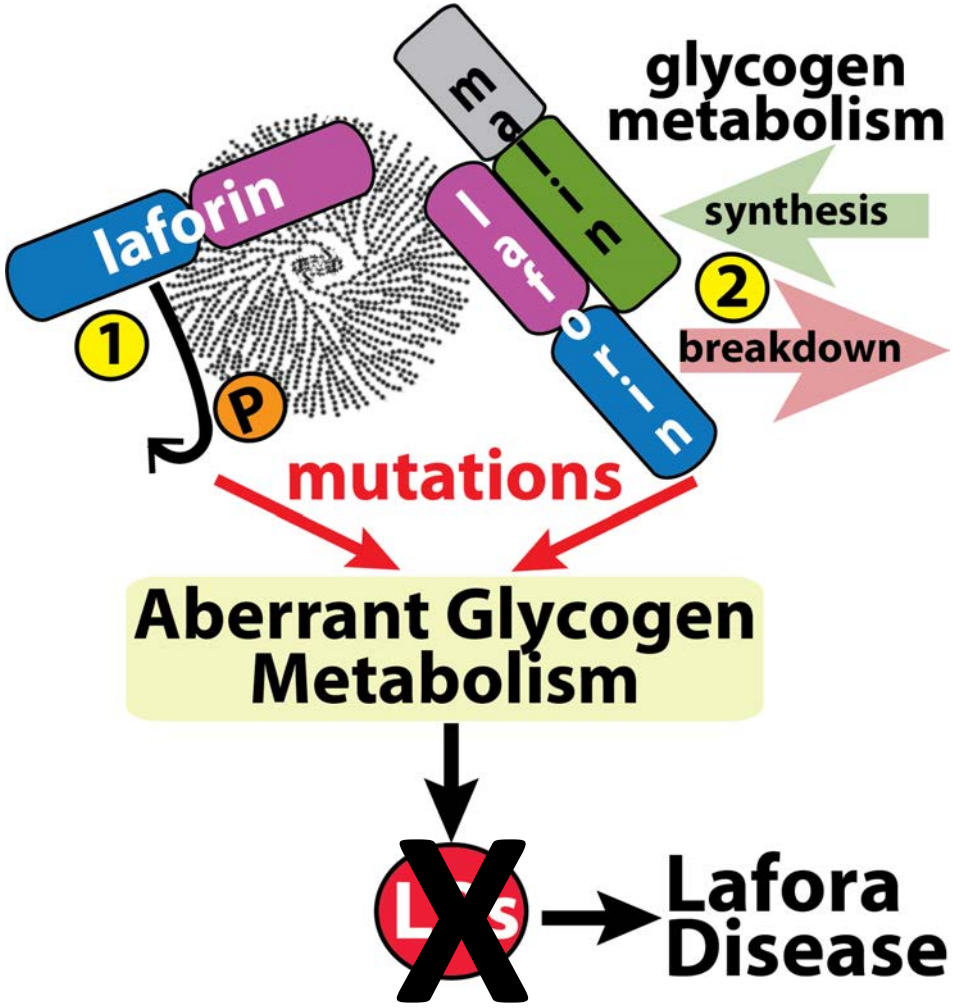

Markussen et al., 2023, *J Neurochemistry*

# Lafora bodies (LBs/PGBs) drive Lafora disease

## anti-Lafora body IHC

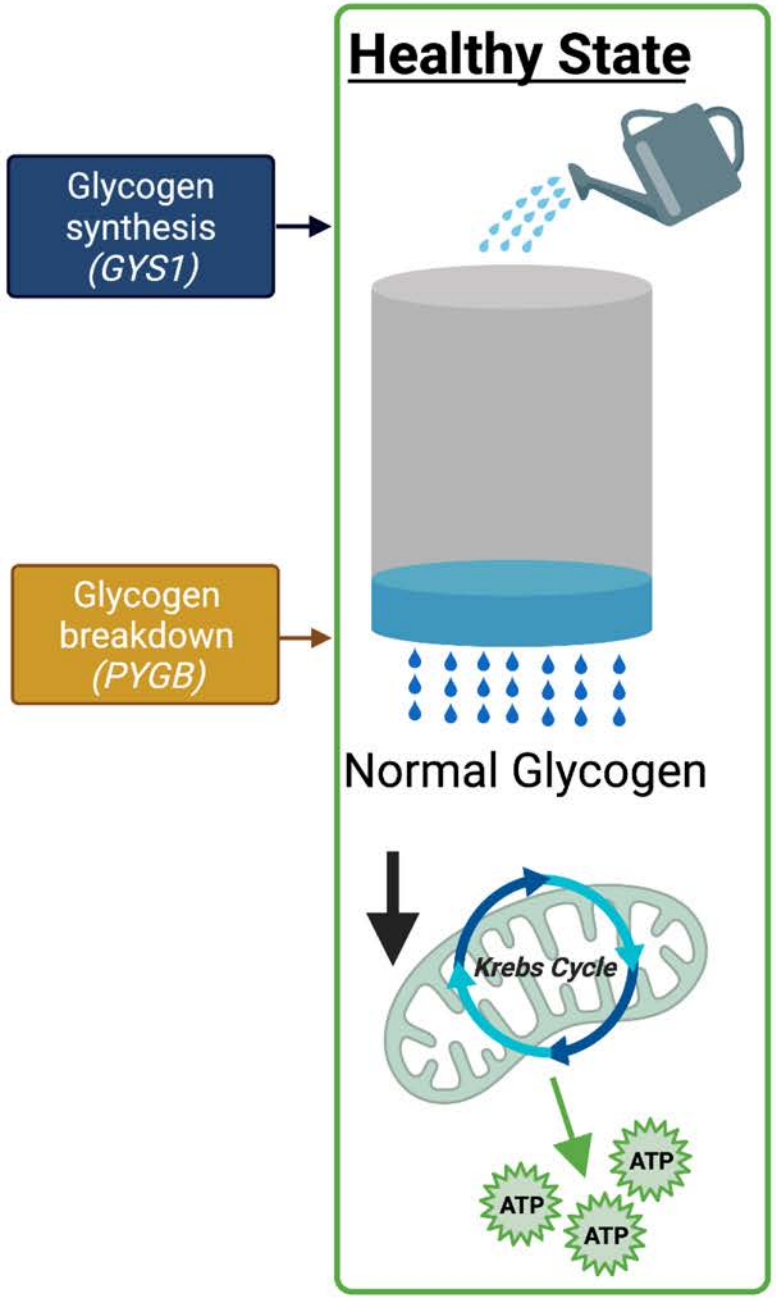
WT

KO




**50% reduction in glycogen synthesis eliminates LBs & greatly reduces epilepsy threshold in LD mice**

# Defined Disease Mechanism = Therapeutic Opportunities




Brain glycogen levels

# NIH P01: Lafora epilepsy – basic mechanisms to therapies

UNIVERSITY OF KENTUCKY College of Medicine

UT SOUTHWESTERN MEDICAL CENTER

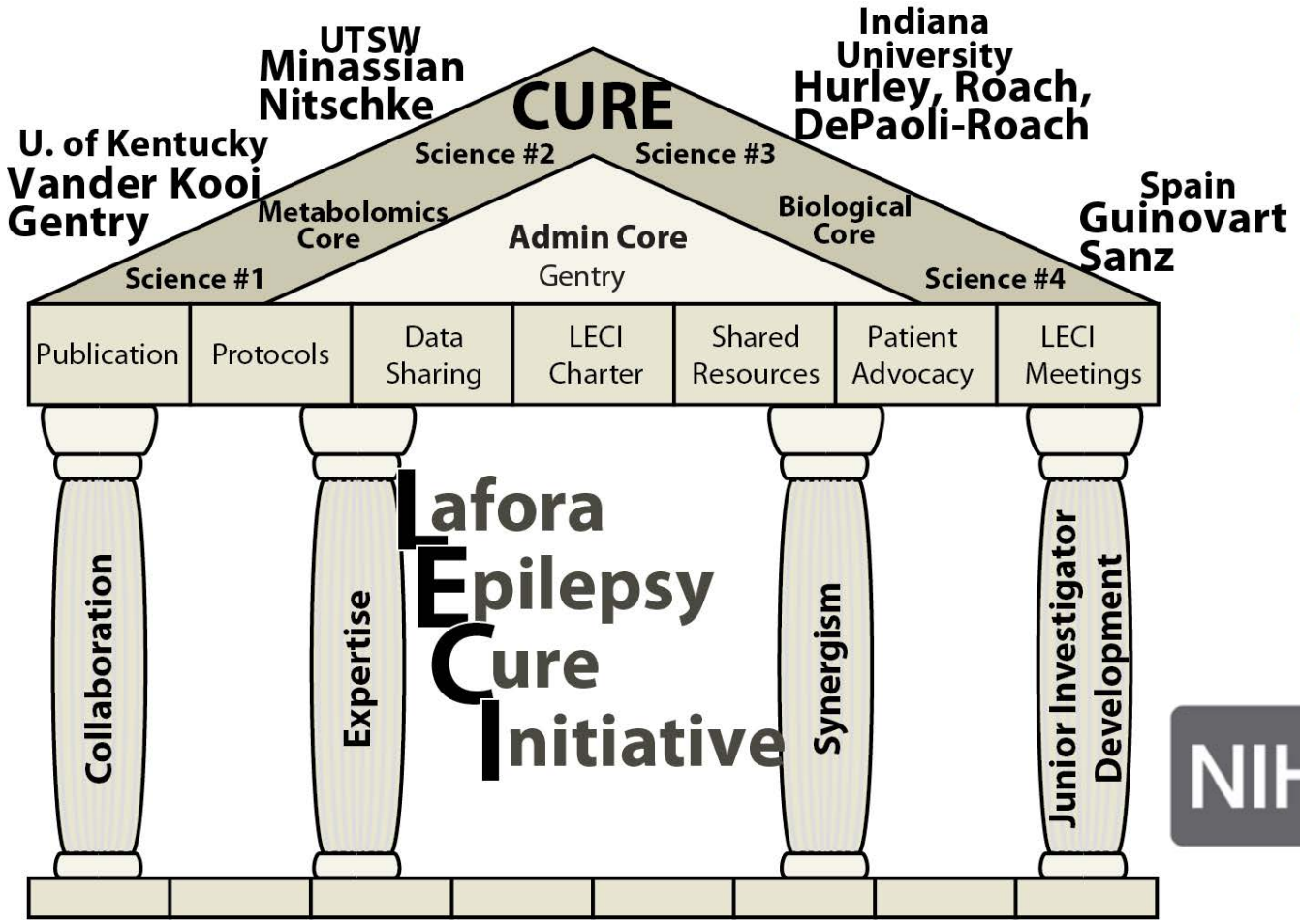
SCHOOL OF MEDICINE INDIANA UNIVERSITY

IRB BARCELONA

iis FJD INSTITUTO DE INVESTIGACIÓN SANITARIA FUNDACIÓN JIMÉNEZ DÍAZ

UC San Diego

ibv INSTITUTO DE BIOMEDICINA DE VALENCIA CSIC




**3 Cores:**

- **Admin – Gentry**
- **Medicinal Chem – Watt**
- **Biological – Madrid, Spain**

NIH National Institute of Neurological Disorders and Stroke

# NIH P01: Lafora Disease – Basic Mechanisms to Therapies

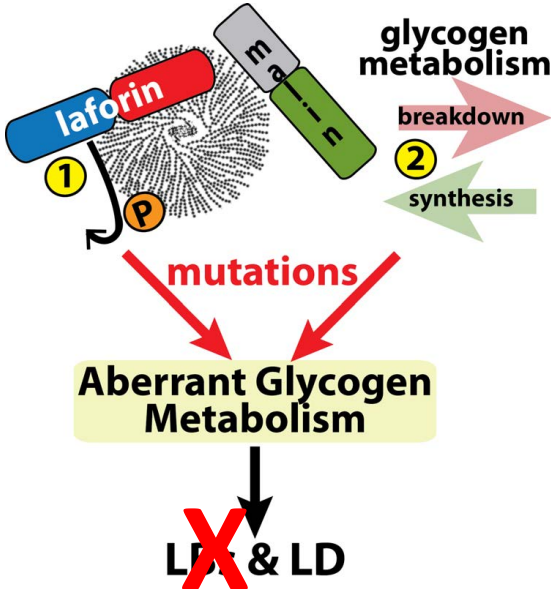
## Science Projects:

**Gentry/Vander Kooi/Sanz:** Personalized medicine, antibody-enzyme therapy, & repurposing.

**Roach/Depaoli-Roach/Hurley:** Small molecule inhibition of glycogen synthesis as LD therapy.

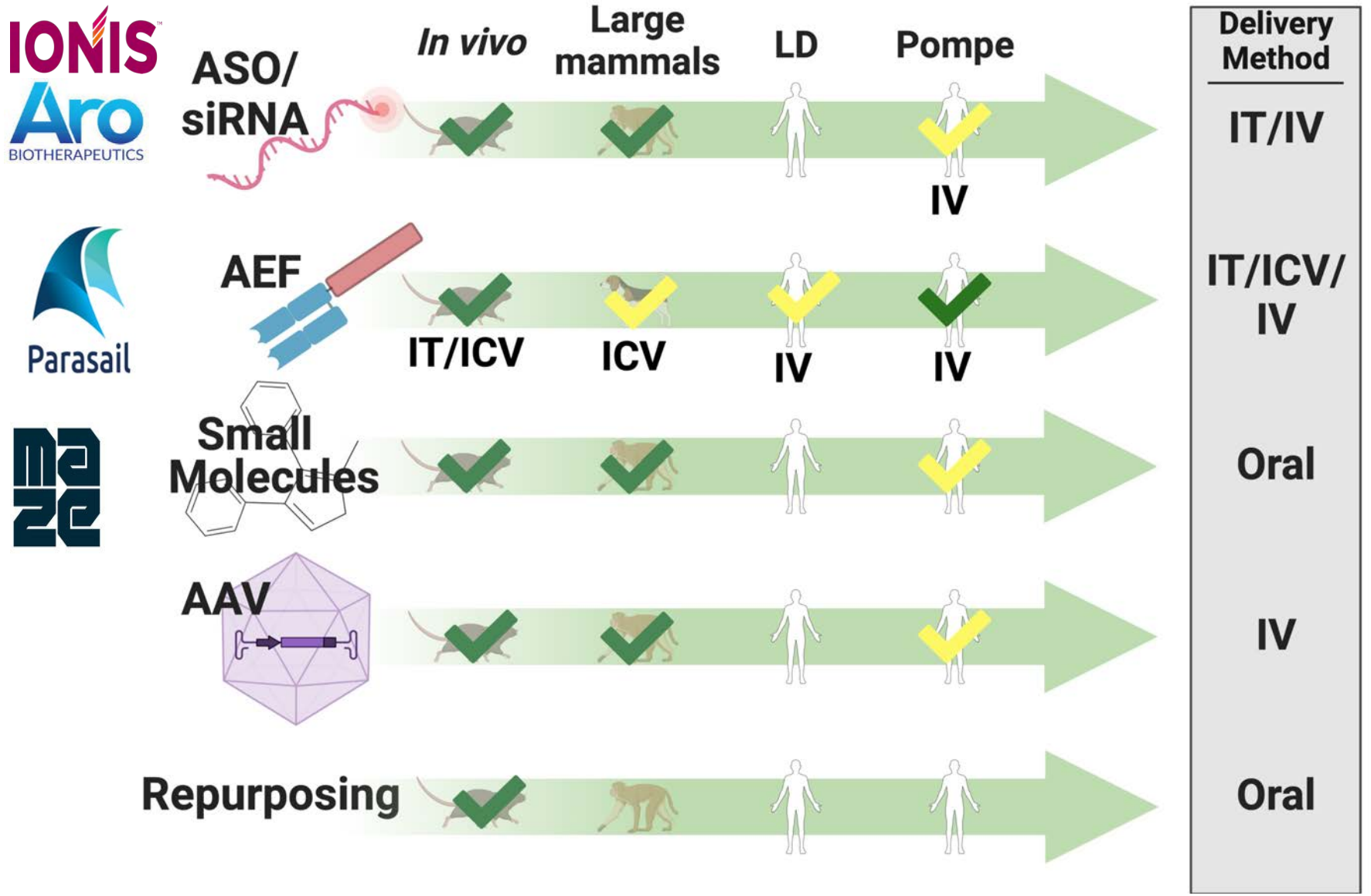
**Minassian:** Genome editing & ASO mRNA suppression as LD therapies.

**Guniovart/Duran:** Defining the therapeutic window for the treatment of LD.




Lafora Epilepsy Cure Initiative

# Defined Mechanism = Therapeutic Developments

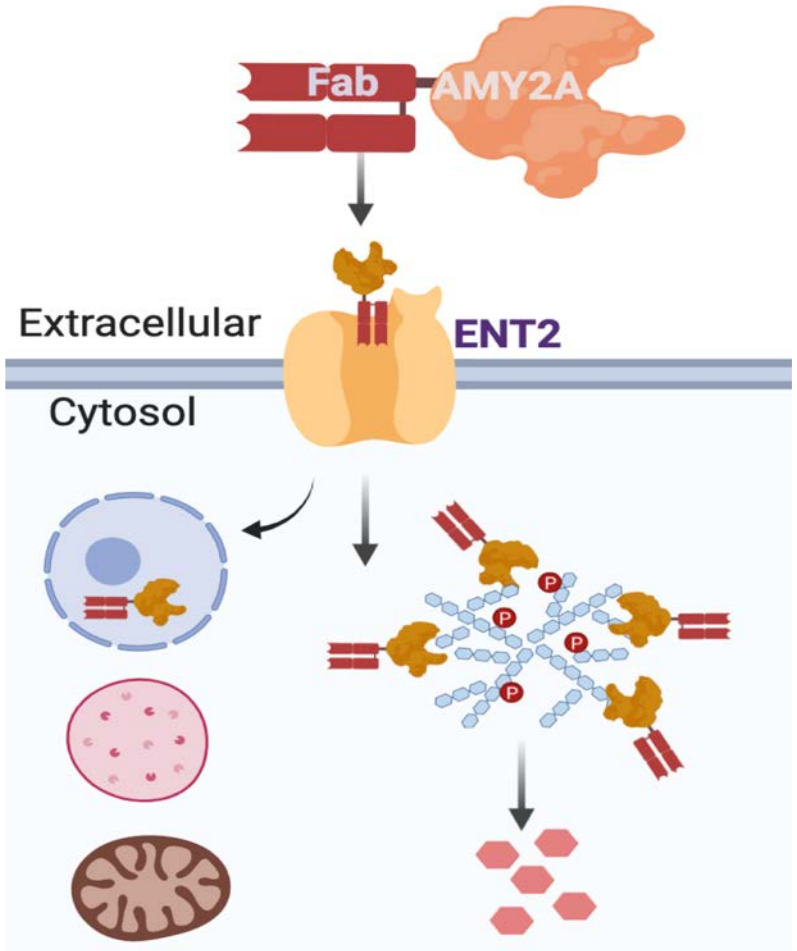



✓ = completed ✓ = ongoing/planned

Lafora Epilepsy Cure Initiative

# Project 1: Antibody-Enzyme Fusions

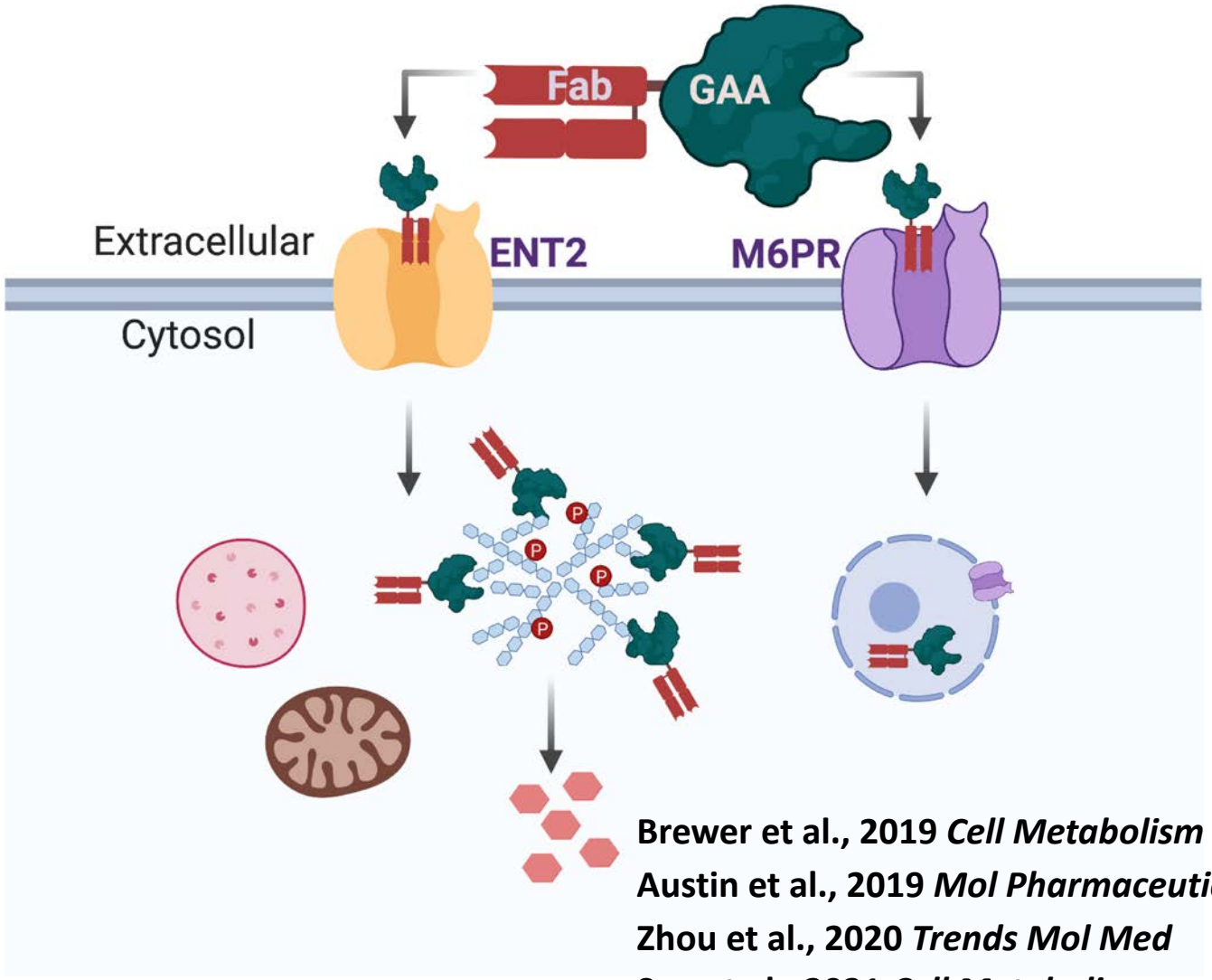
## VAL-0417




valerion

Parasail

## VAL-1221





Brewer et al., 2019 *Cell Metabolism*

Austin et al., 2019 *Mol Pharmaceutics*

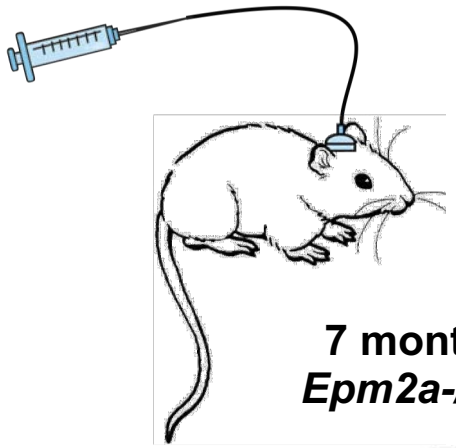
Zhou et al., 2020 *Trends Mol Med*

Sun et al., 2021 *Cell Metabolism*

Young et al., 2022 *EMBO Mol Med*

# VAL-0417 ICV administration ablates brain LBs *in vivo*

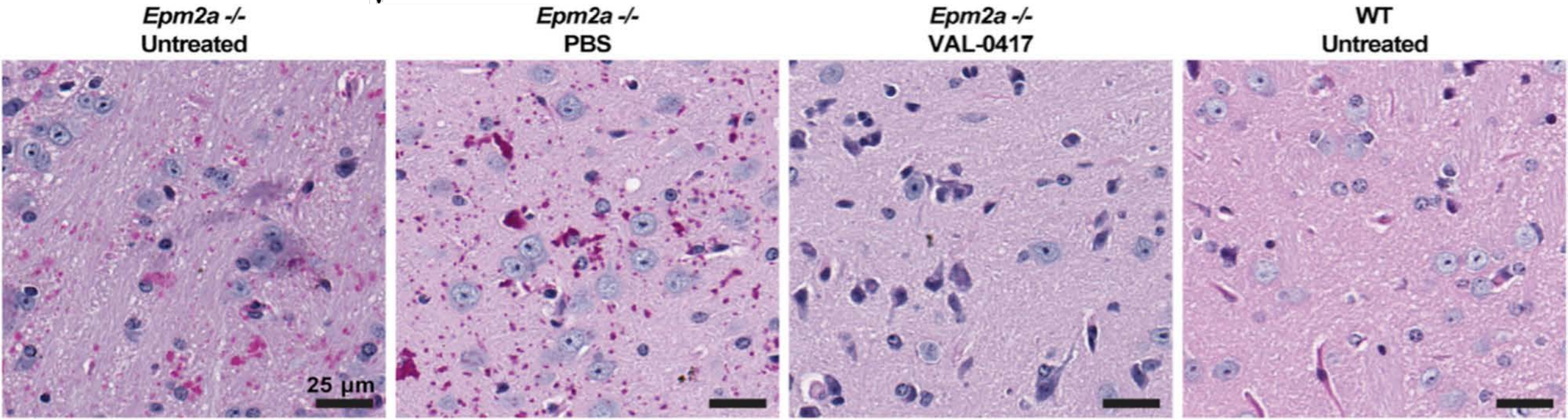
**Intracerebroventricular (ICV) administration**



7 month old *Epm2a-/-* mice

**Continuous infusion of VAL-0417 (0.08mg/day) or PBS for 7 days** → **Euthanized on 8th day** → **PAS staining**

**3-day continuous VAL-0417 infusion clears LBs**

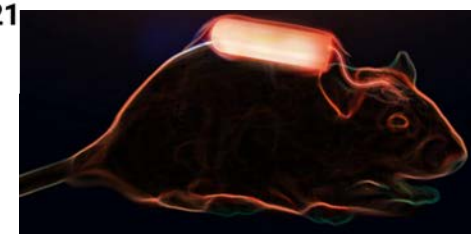



Brewer et al., 2019
*Cell Metabolism*

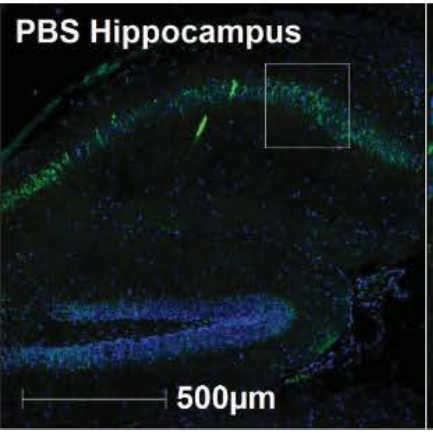
# Broad brain biodistribution of VAL-1221 via i.c.v

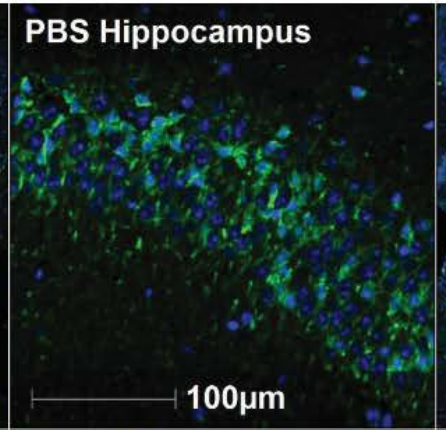

VAL-1221

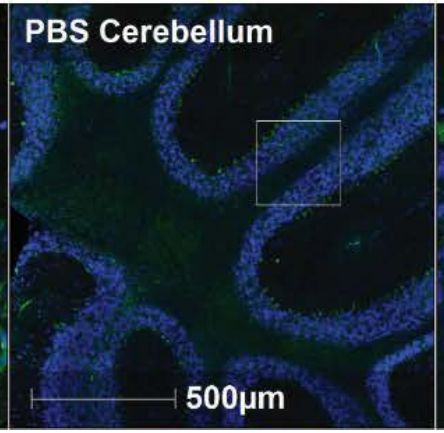
**Intracerebroventricular (i.c.v.) administration via Alzet pumps**

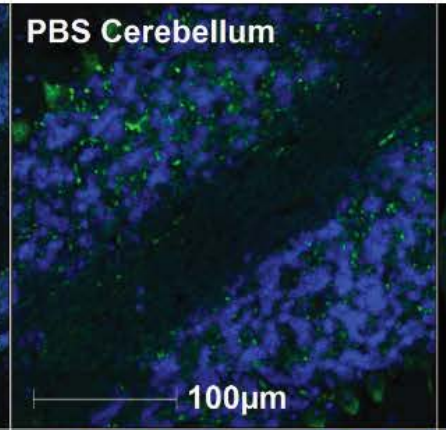


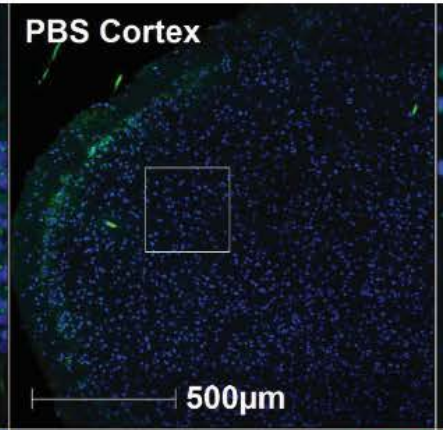
**Continuous infusion of VAL-1221 (0.03mg/day) or PBS for 7 days** → **Euthanized on 8th day** → **Biochemical, IHC, PAS, IF analysis of VAL-1221 delivery & LB load**

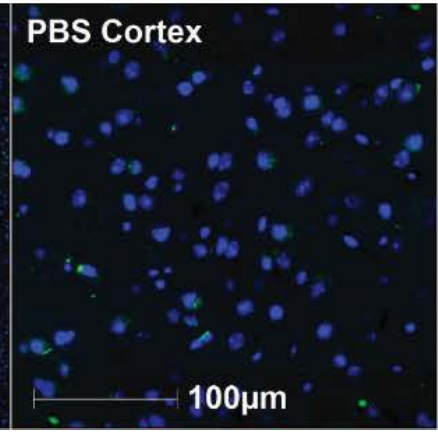


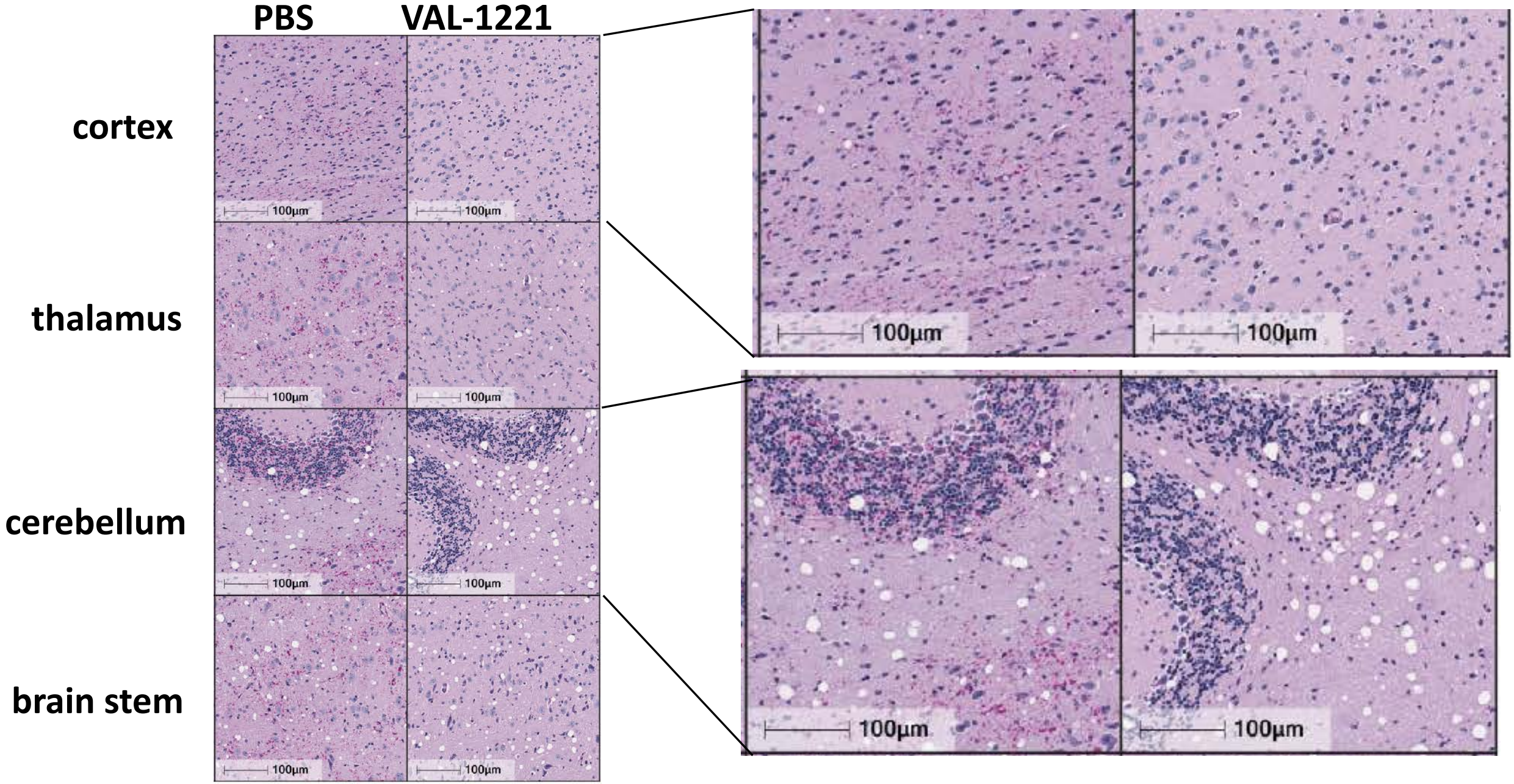



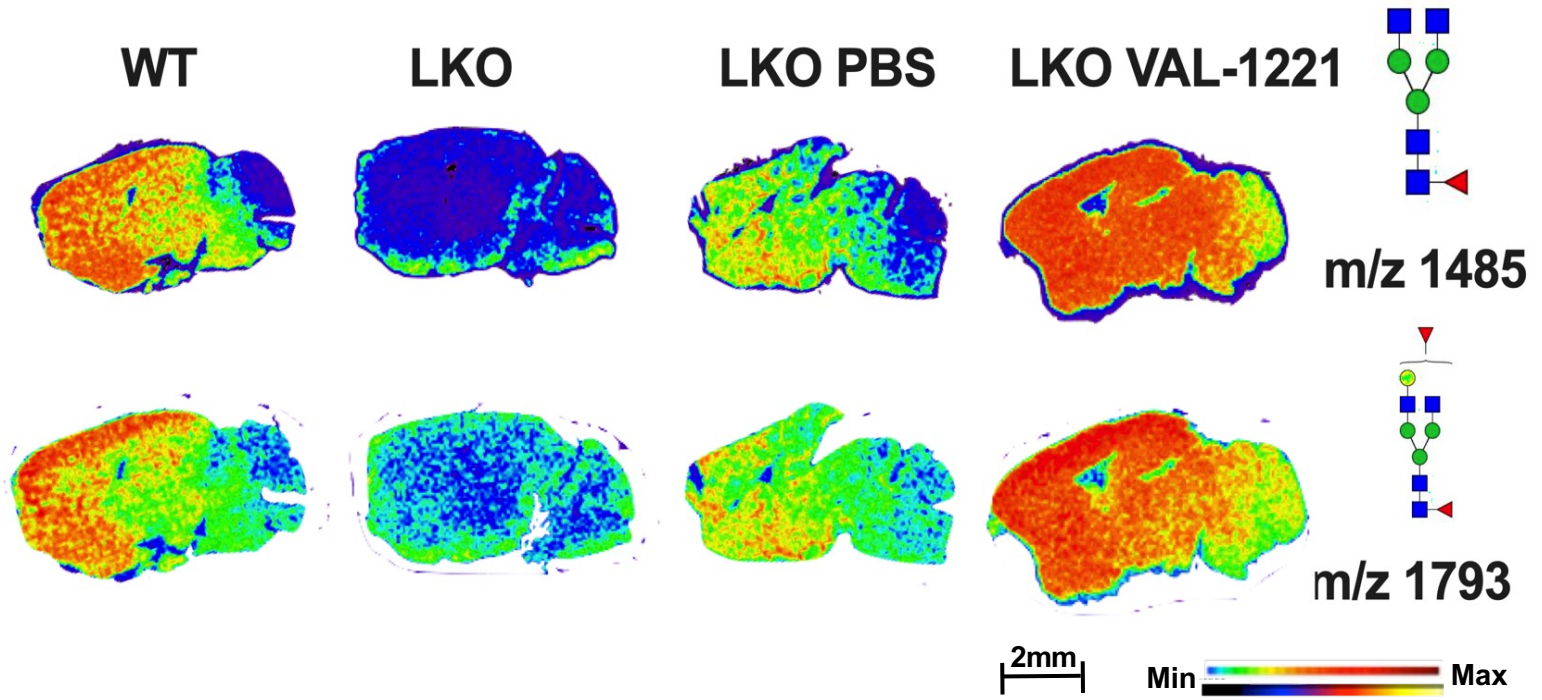



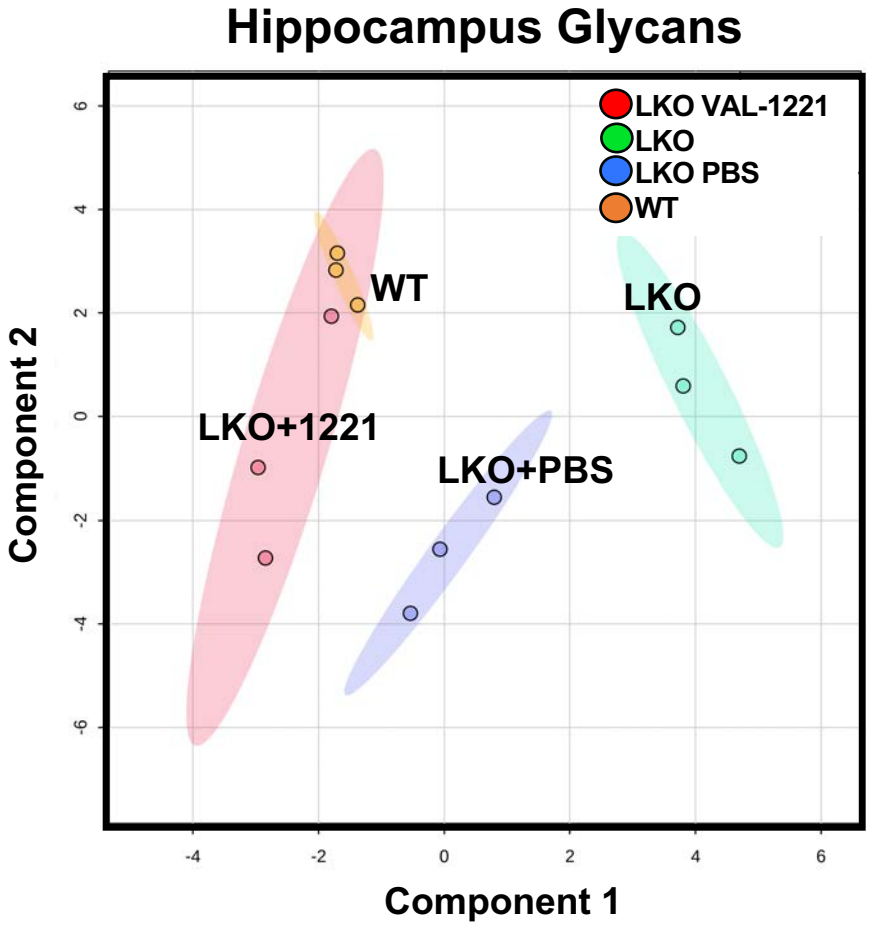



# VAL-1221 ablates LBs after 7-day i.c.v

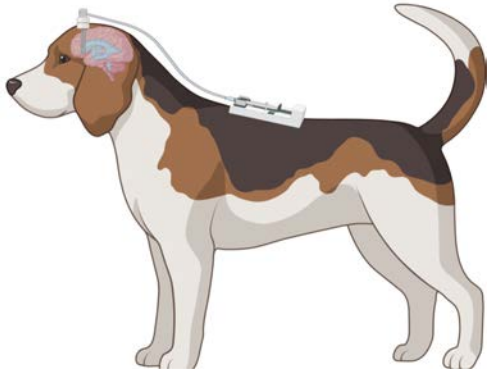


# VAL-1221 rescues LKO N-glycan deficiency



# Canine VAL-1221 i.c.v. infusion




cerebral therapeutics
CNS SCIENCE DELIVERED.

**1.8 ml infusion of 10 mg/ml VAL-1221 in right lateral ventricle**

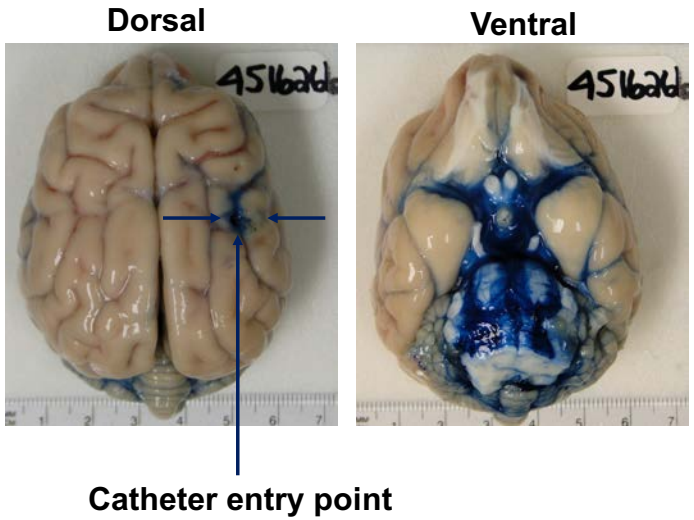
↓

**Euthanized 14 days after infusion**

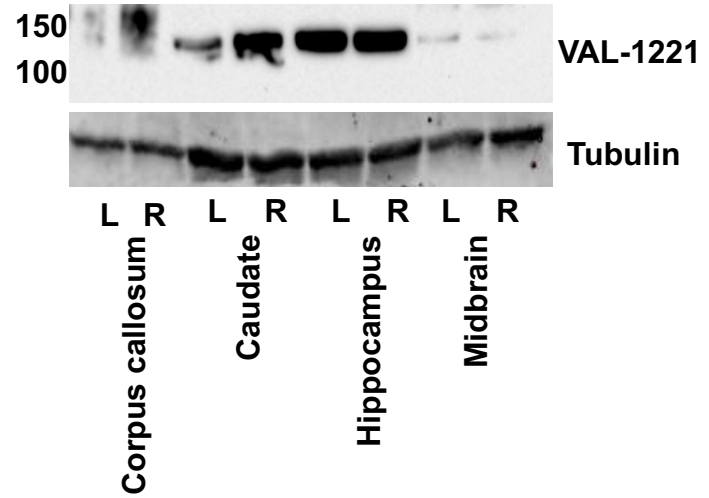
↓

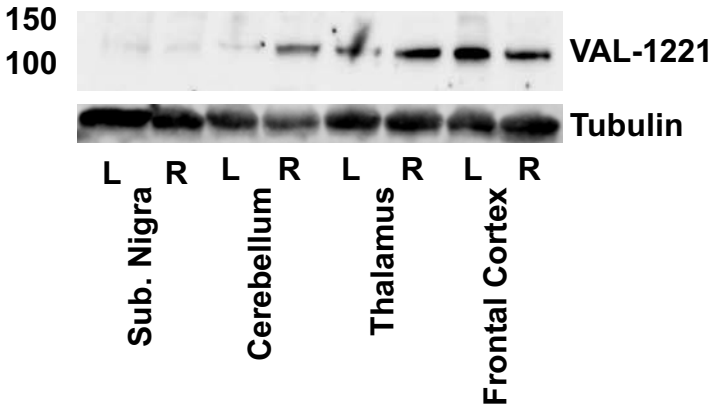
**Multiple tissues collected and assessed for GAA**

## Evans blue injection at necropsy

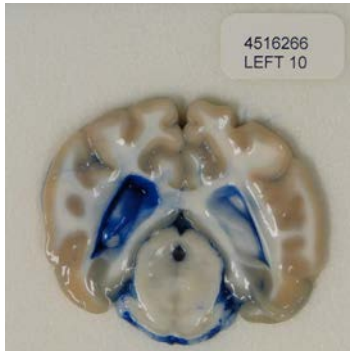


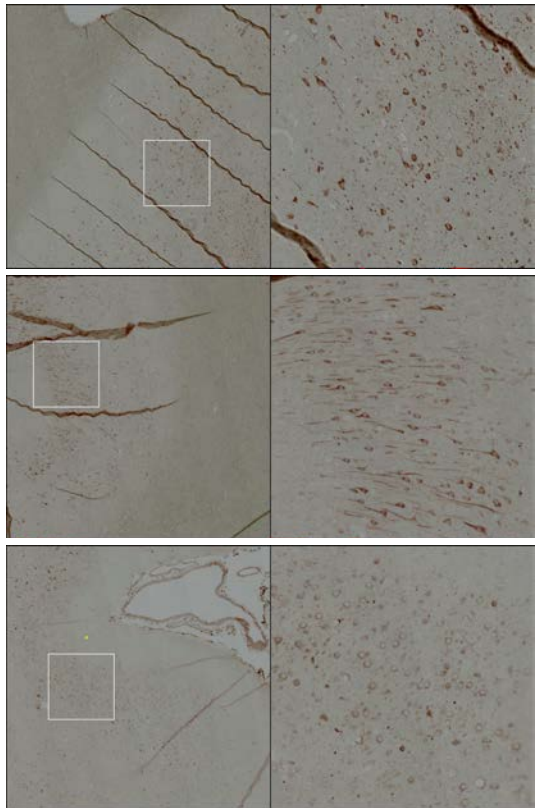

## VAL-1221 in brain homogenates

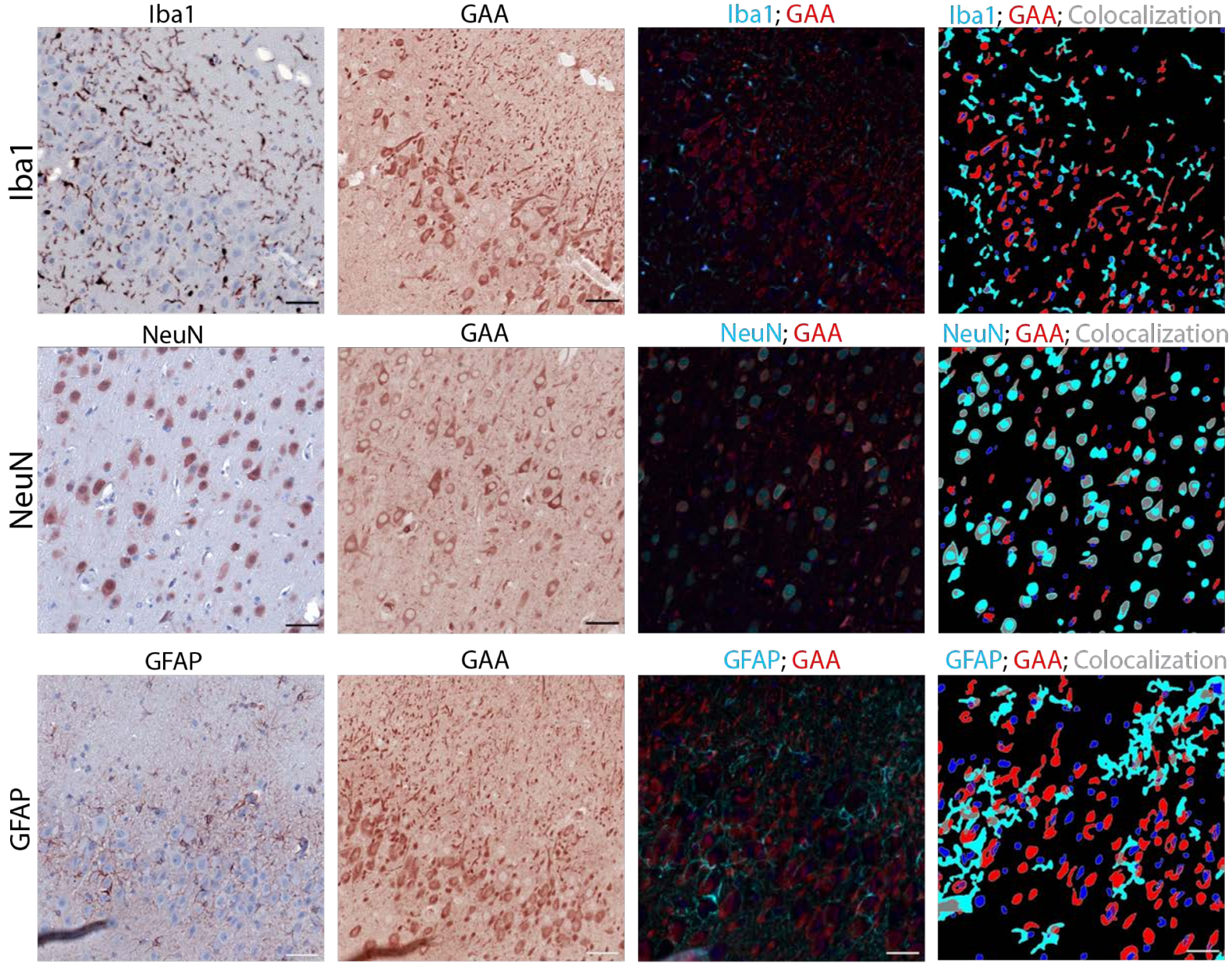


# VAL-1221 is enriched in neurons in hippocampus





# Summary of LD Antibody-Enzyme Fusion Data

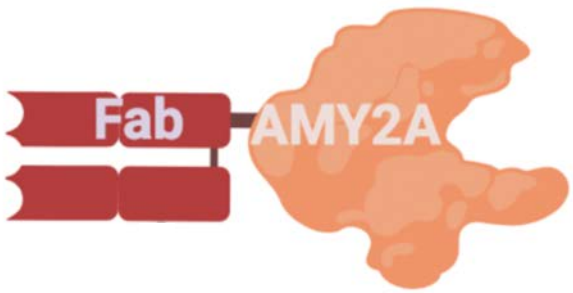
## We have tested >400 mice to date with VAL drugs

**Laforin KO**
**Malin KO**
**WT**

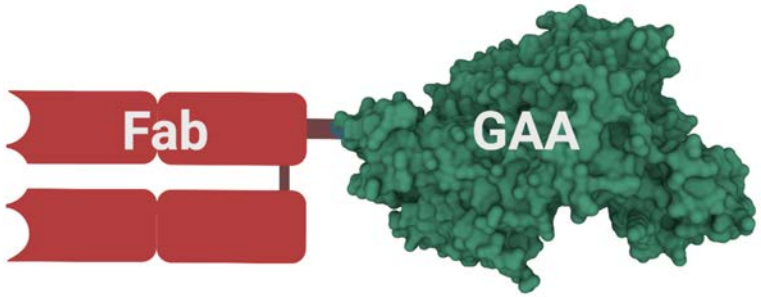
| Delivery Method | VAL-0417 (Fab AMY2A) | VAL-1221 (Fab GAA) |
|---|---|---|
| I.M. | ✓ | ✓ |
| I.V. systemic | ✓ | ✓ |
| I.C.V. | ✓ | ✓ |
| I.V. brain | Testing | Testing |
| NHP safety | - | I.V. NHP ✓ |
| Canine study | - | I.C.V. Testing |
| Clinical Trial | | I.V. / I.C.V. |


Brewer et al., 2019 *Cell Metabolism*
Sun et al., 2019 *Cell Metabolism*
Austin et al., 2019 *Mol Pharmaceutics*
Zhou et al., 2020 *Trends Mol Med*
Sun et al., 2021 *Cell Metabolism*
Young et al., 2022 *EMBO Mol Med*

# Current VAL-0417 & VAL-1221 efforts




VAL-0417

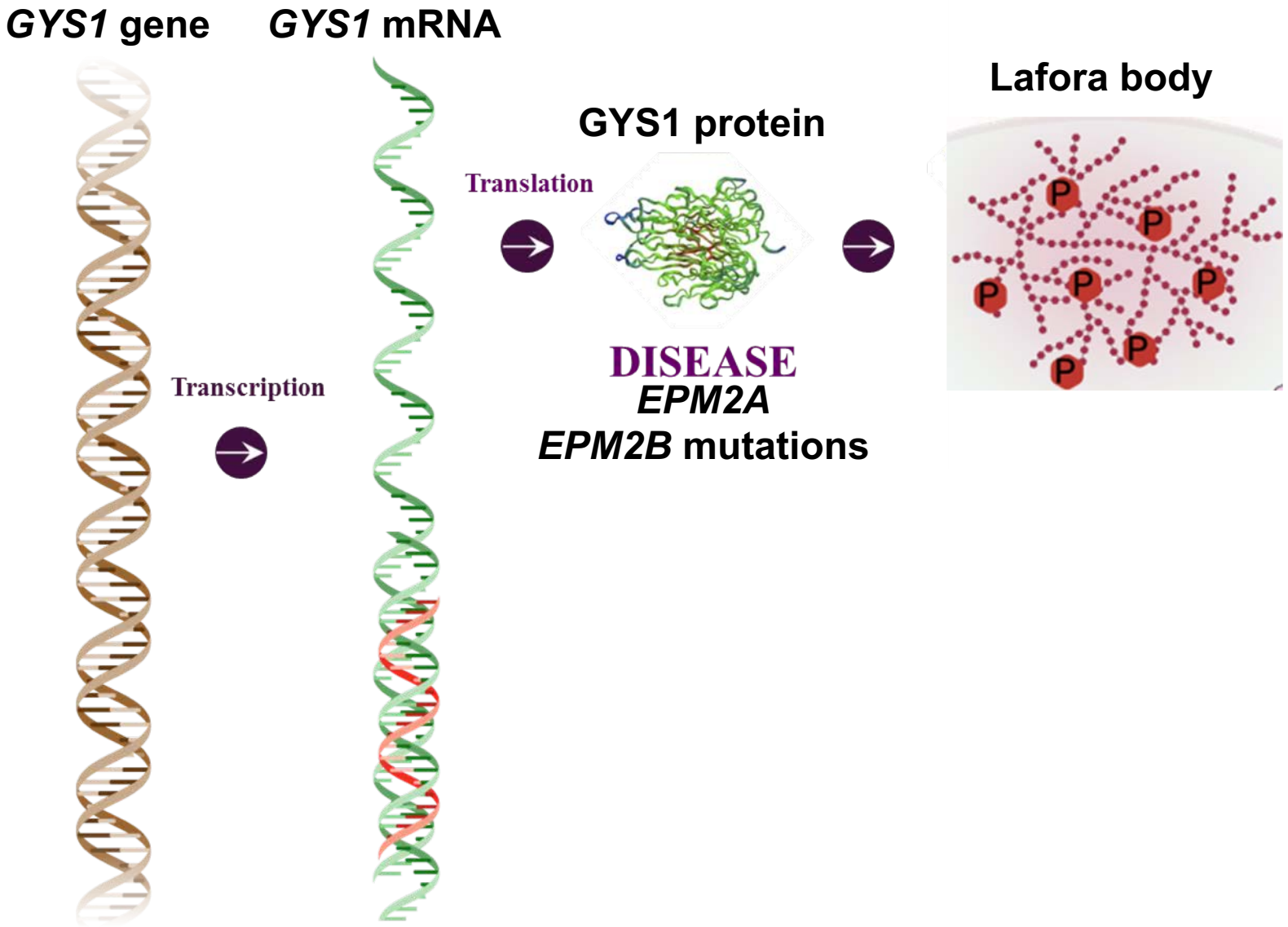



VAL-1221

## Gentry lab:

- Define minimum dose w/ maximal response
- Define LB re-accumulation rate
- Establishing human & mouse  biomarkers

# Project 2: Anti-Sense Oligonucleotide (ASO) Therapy

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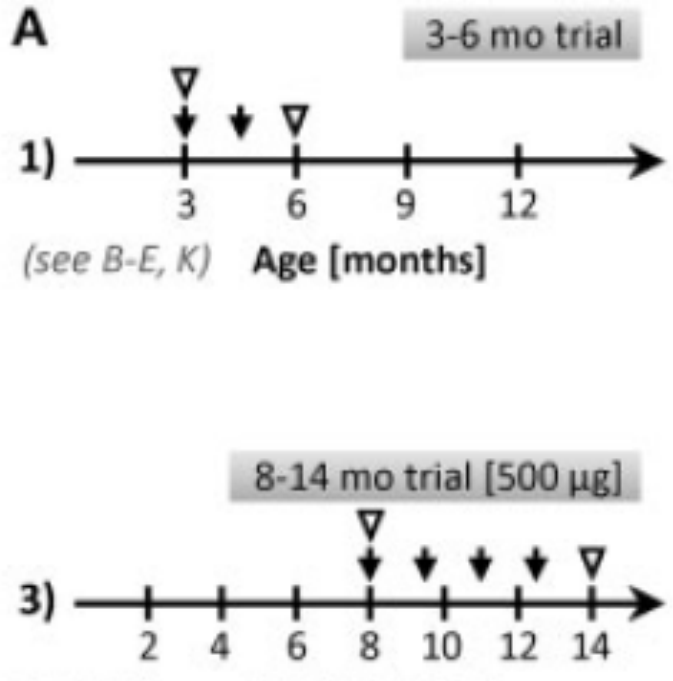
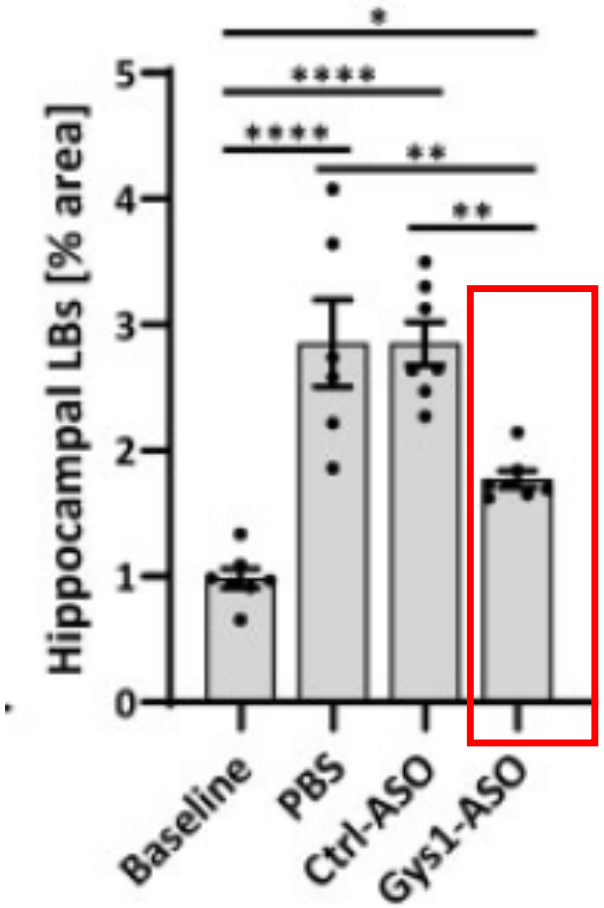
Donohue et al., 2023 *Neurotherapeutics*
Nitschke et al., 2022, *Brain*
Ahonen et al., 2021, *Brain*

# *GYS1* ASO Tx slows LBs in laforin KO mice

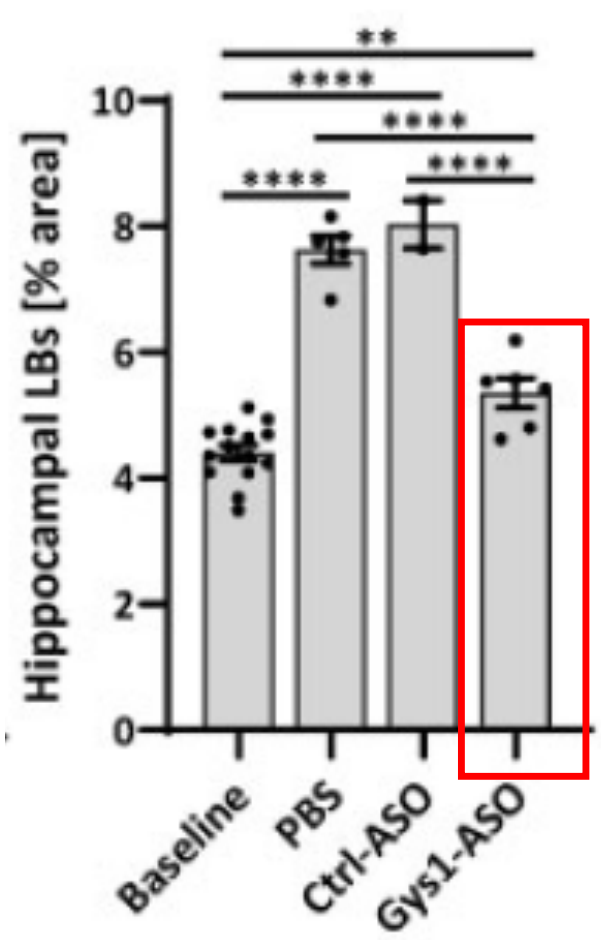
**ASO i.t. delivery after disease onset**




**E** **3-6m trial**

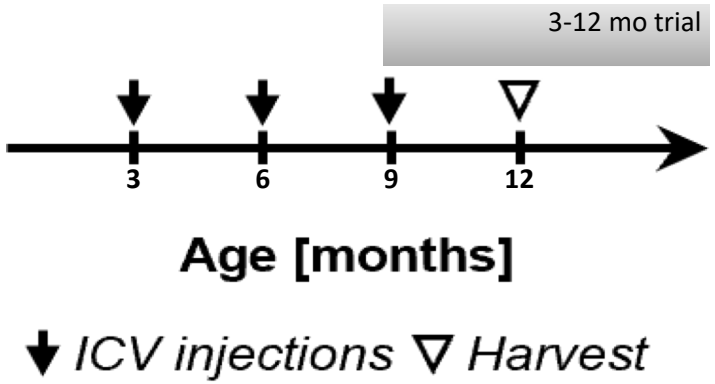


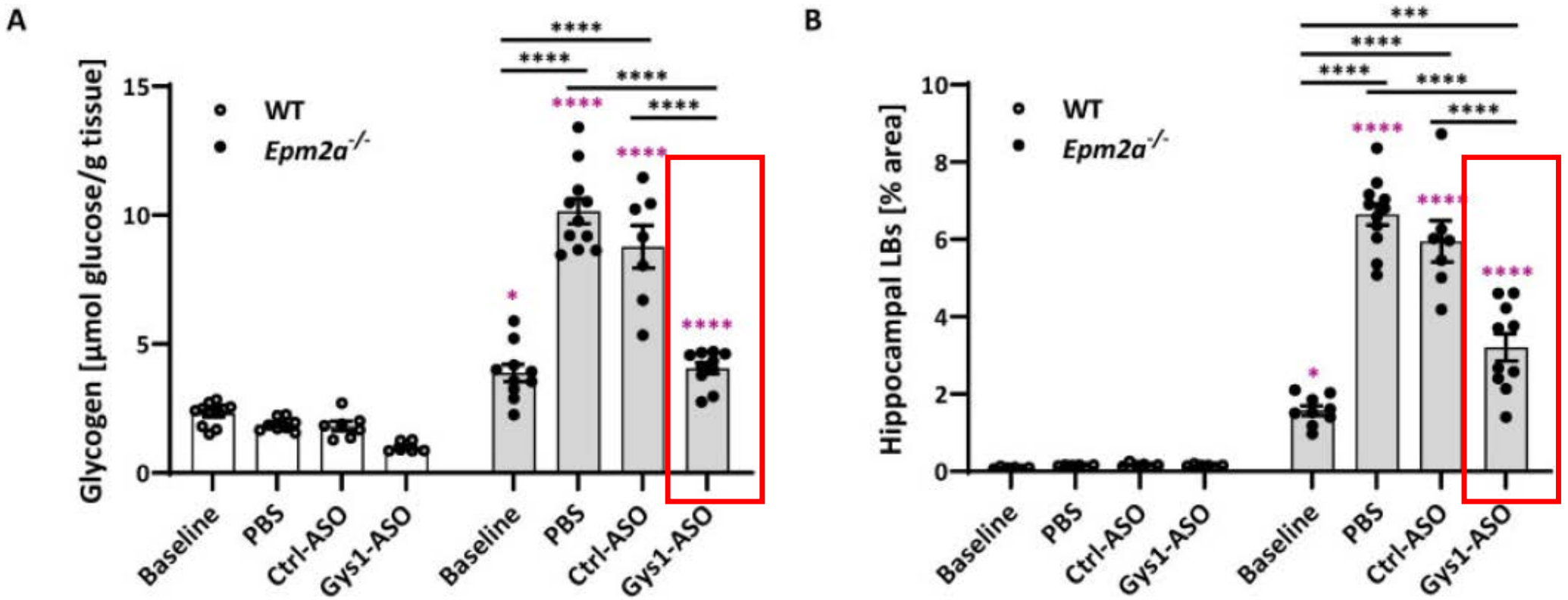

**J** **8-14m trial**




Ahonen et al., 2021, *Brain*

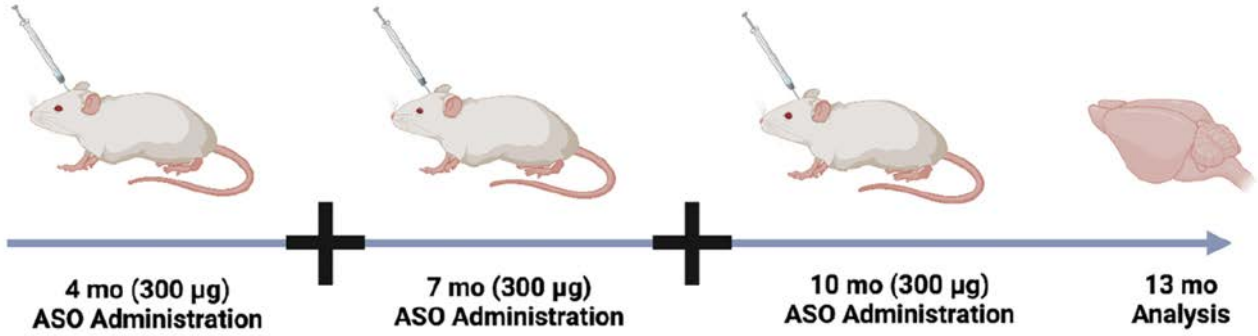
# Long-term ASO Tx prevents LBs in laforin KO mice

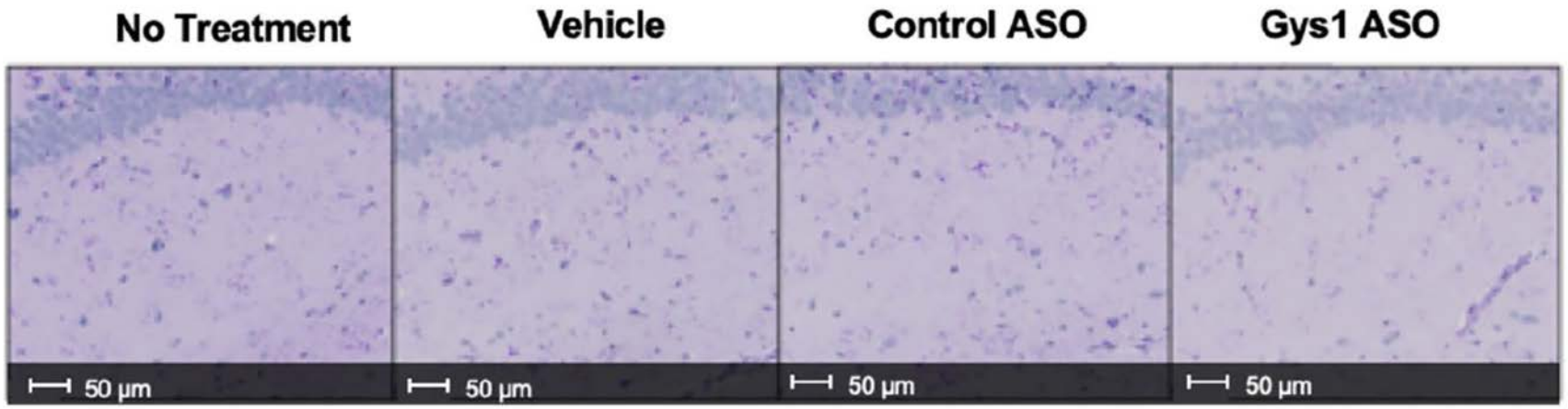


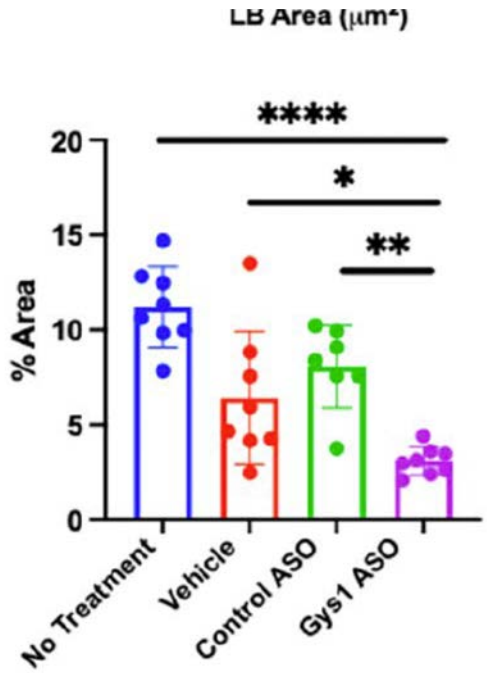




Ahonen et al., 2021, *Brain*

# *GYS1* ASO Tx slows LBs in malin KO mice

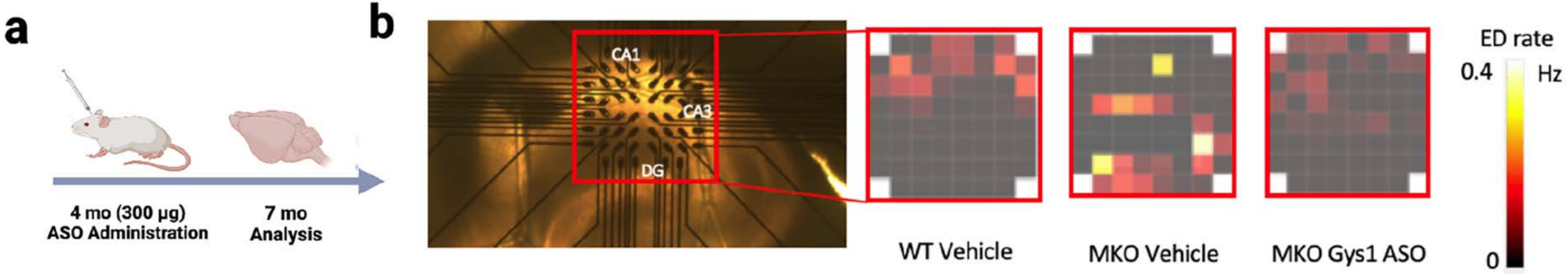


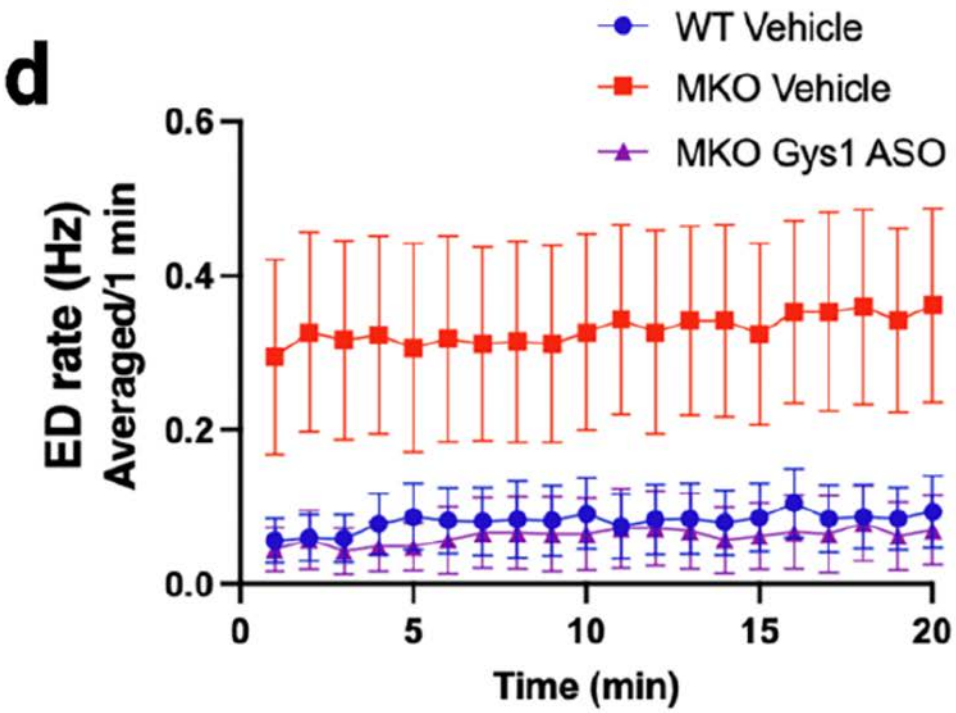



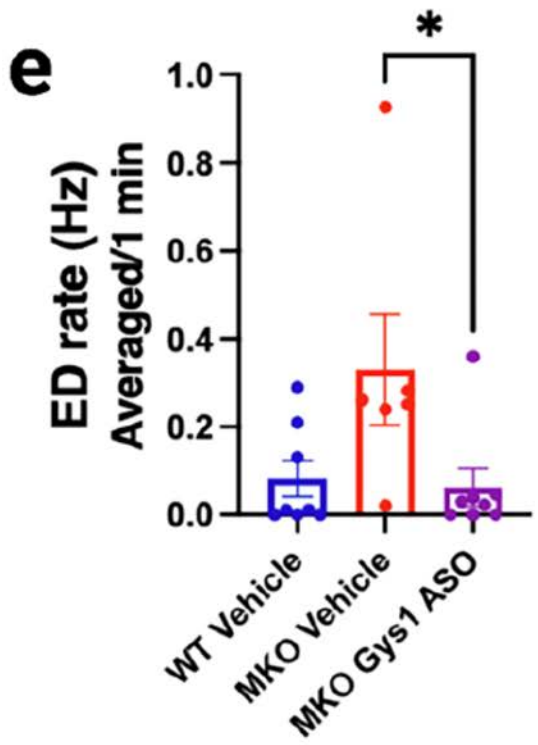




Donohue et al., 2023 *Neurotherapeutics*

# *GYS1* ASO Tx Decreases Epileptiform Discharges




Donohue et al., 2023 *Neurotherapeutics*

# Current *GYS1* ASO efforts

## Minassian & Gentry labs:

- Testing the ASO in other disease models

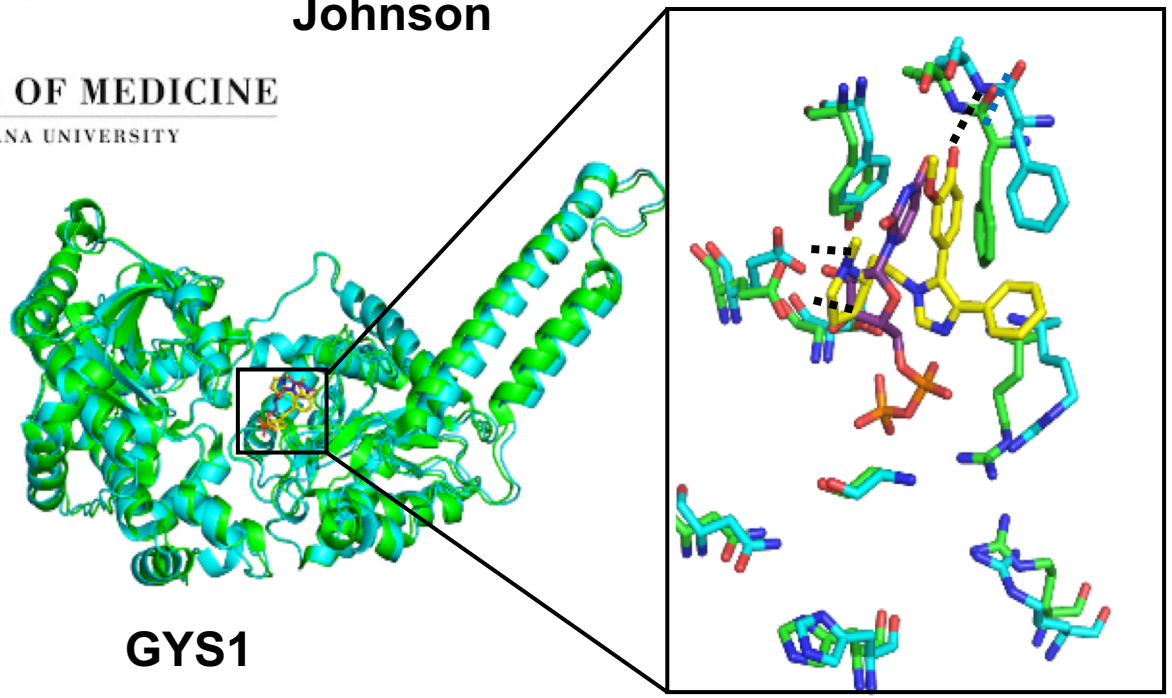
## Ionis Pharmaceuticals:

- Completing analysis of Natural History Study, NCT03876522
- Working and has worked with other companies for partnerships

# Project 3: Small Molecule Therapy

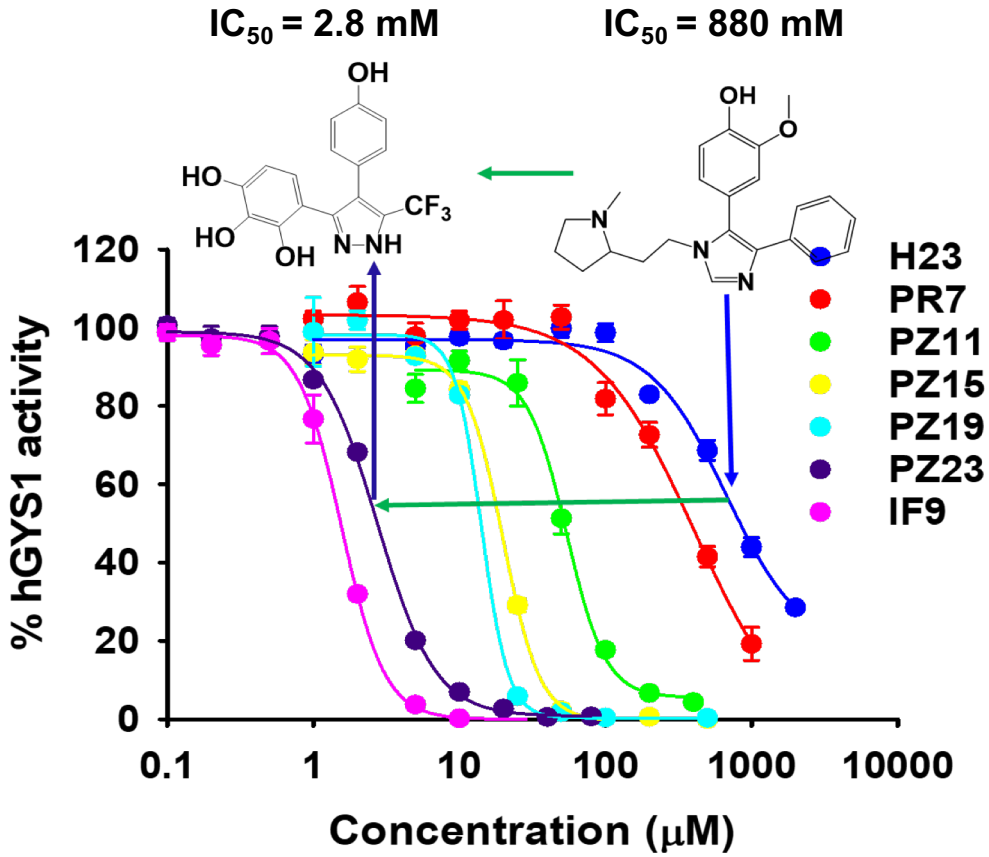
Roach
Depaoli-Roach
Hurley
Johnson

SCHOOL OF MEDICINE
INDIANA UNIVERSITY



GYS1

## Inhibiting GYS1

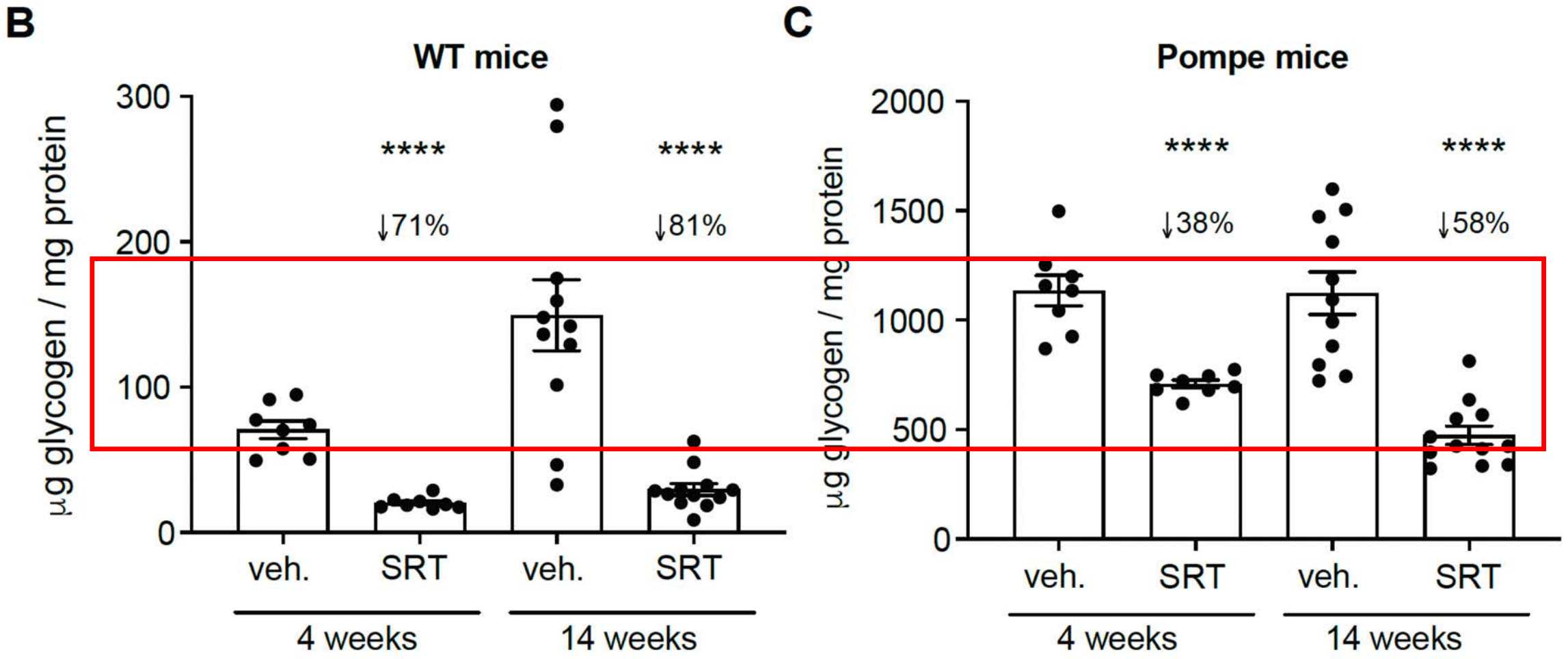



UK
UNIVERSITY OF KENTUCKY
College of Medicine

Dave Watt


Ulman et al., *Sci Trans Med* In Press
Tang et al., 2020, *J Med Chem*
Skurat et al., 2017, *Glycobiology*
Contreras et al., 2016, *Arch Biochem Biophys*

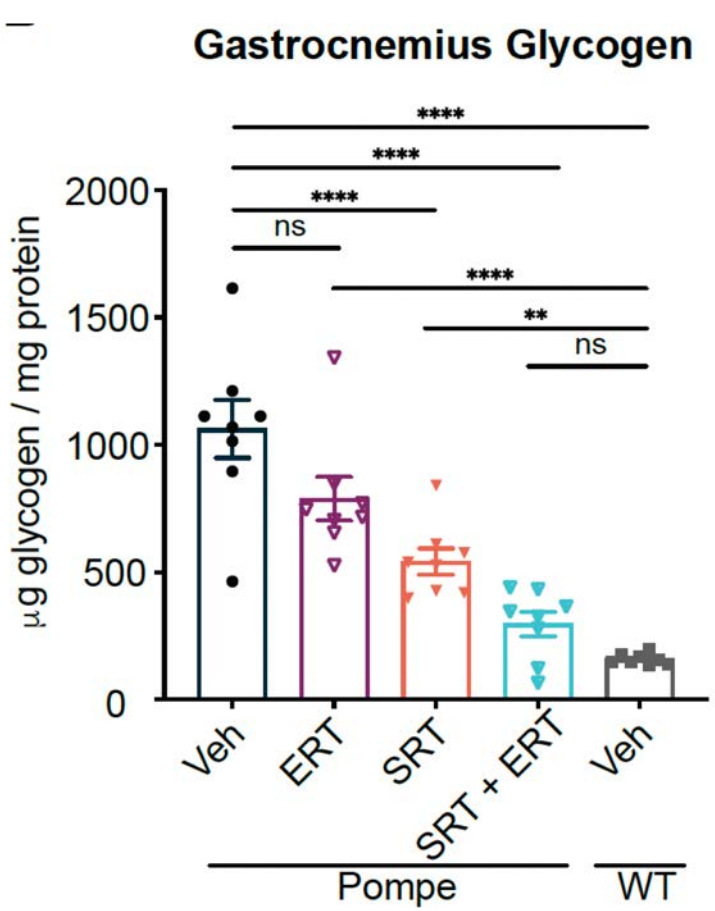
# MZE001 decreases glycogen in WT & Pompe mice

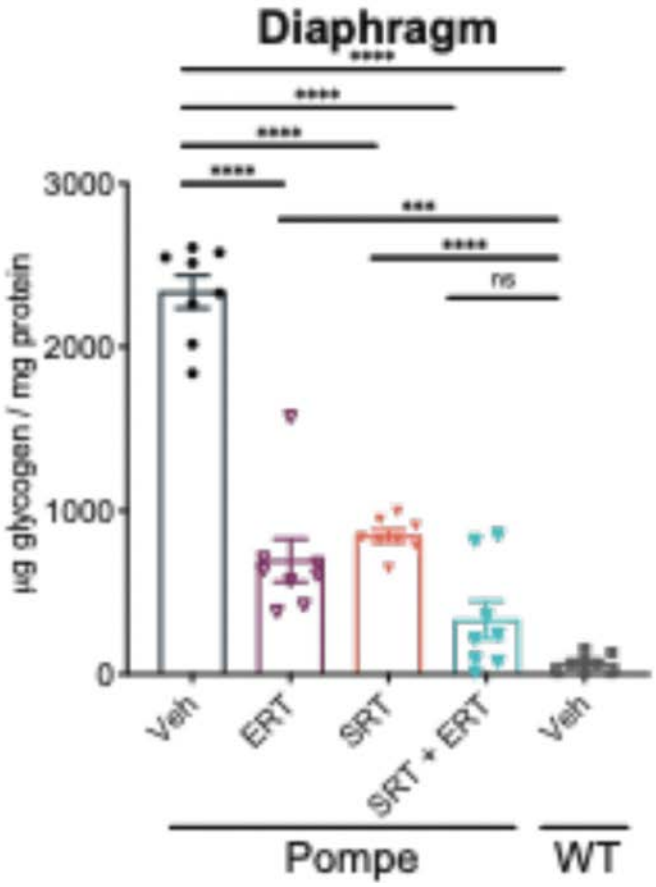


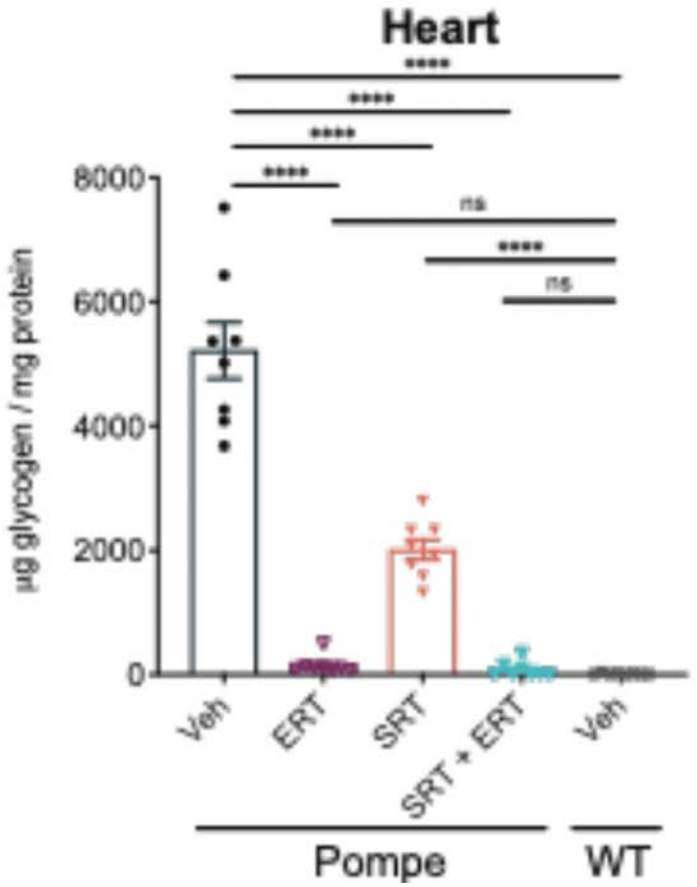

- **Pompe mice exhibit 10-fold higher glycogen**
- **MZE001 reduces the levels by 50%, i.e. 5-fold**

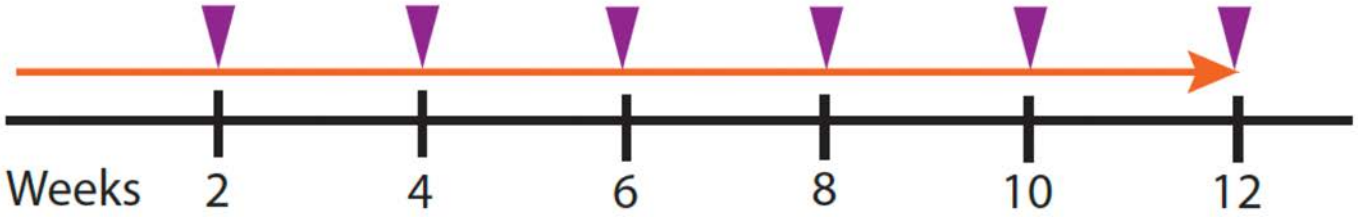
Ulman et al., *Sci Trans Med* In Press

# MZE001 + ERT normalizes glycogen in Pompe mice




**SRT** (MZE001)

**ERT** (Myozyme)

Ulman et al., *Sci Trans Med* In Press

# Current MZE001 efforts

## MAZE Therapeutics:

- Completed Phase I in 120 unaffected individuals
- Announced Phase II in Pompe patients for Q3 2023
- Presented data on possible use in muscular dystrophy patients
- Sold the entire program to Sanofi for $750M
- Sanofi is onboarding the program
- No data to suggest that MZE001 crosses BBB
- *Science Translational Medicine* paper in press

# Project 4: Window of Opportunity – Treatment Timing

MALIN$^{KO}$

Mouse model of Lafora disease

MALIN$^{OE}$

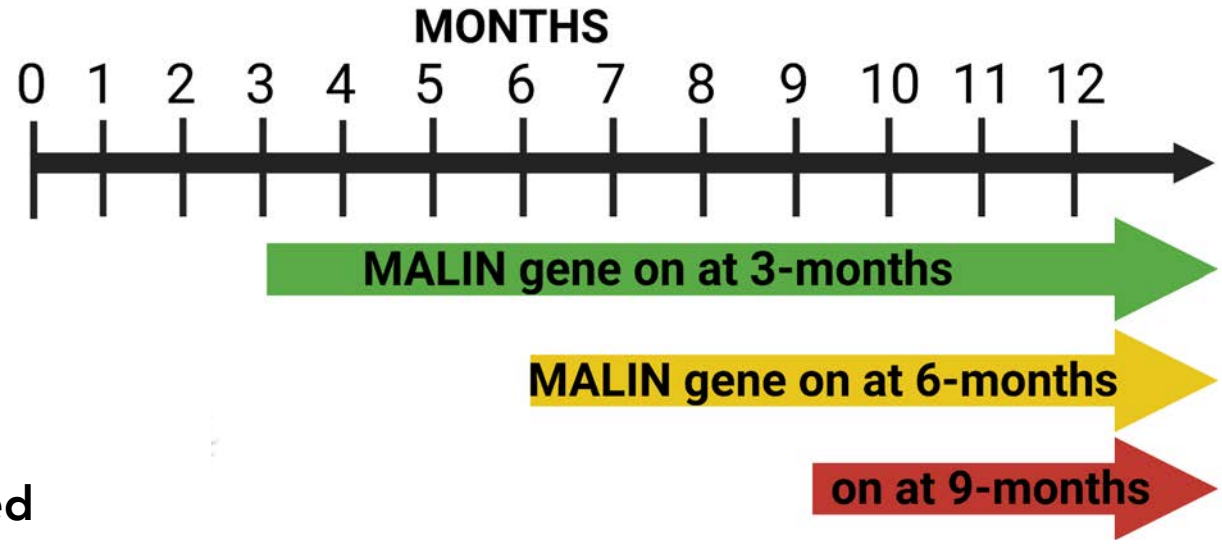
Inducible overexpression of malin

MALIN$^{KO}$ + MALIN$^{OE}$

Devoid of malin until re-expression is activated

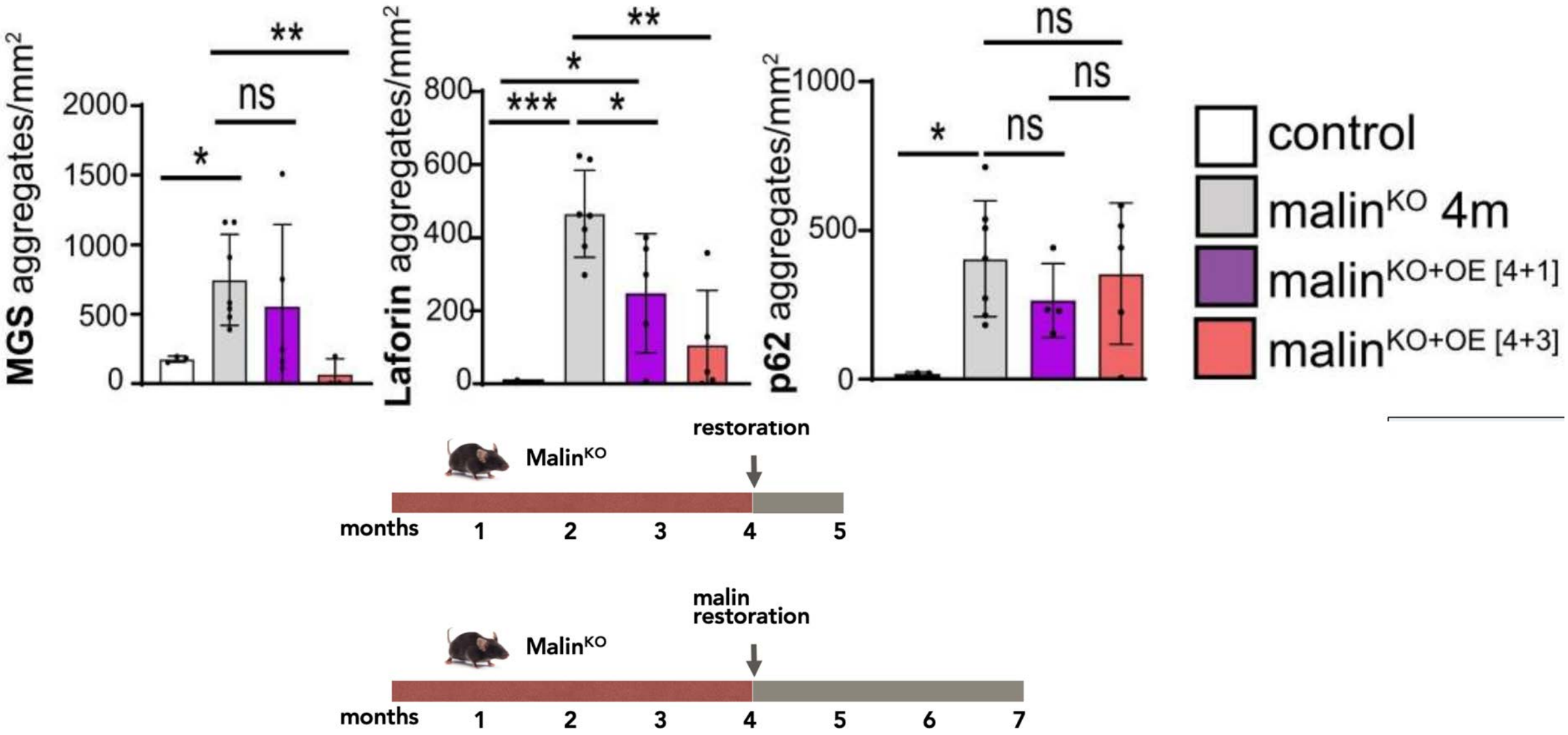
IRB BARCELONA

**Guinovart & Duran labs**



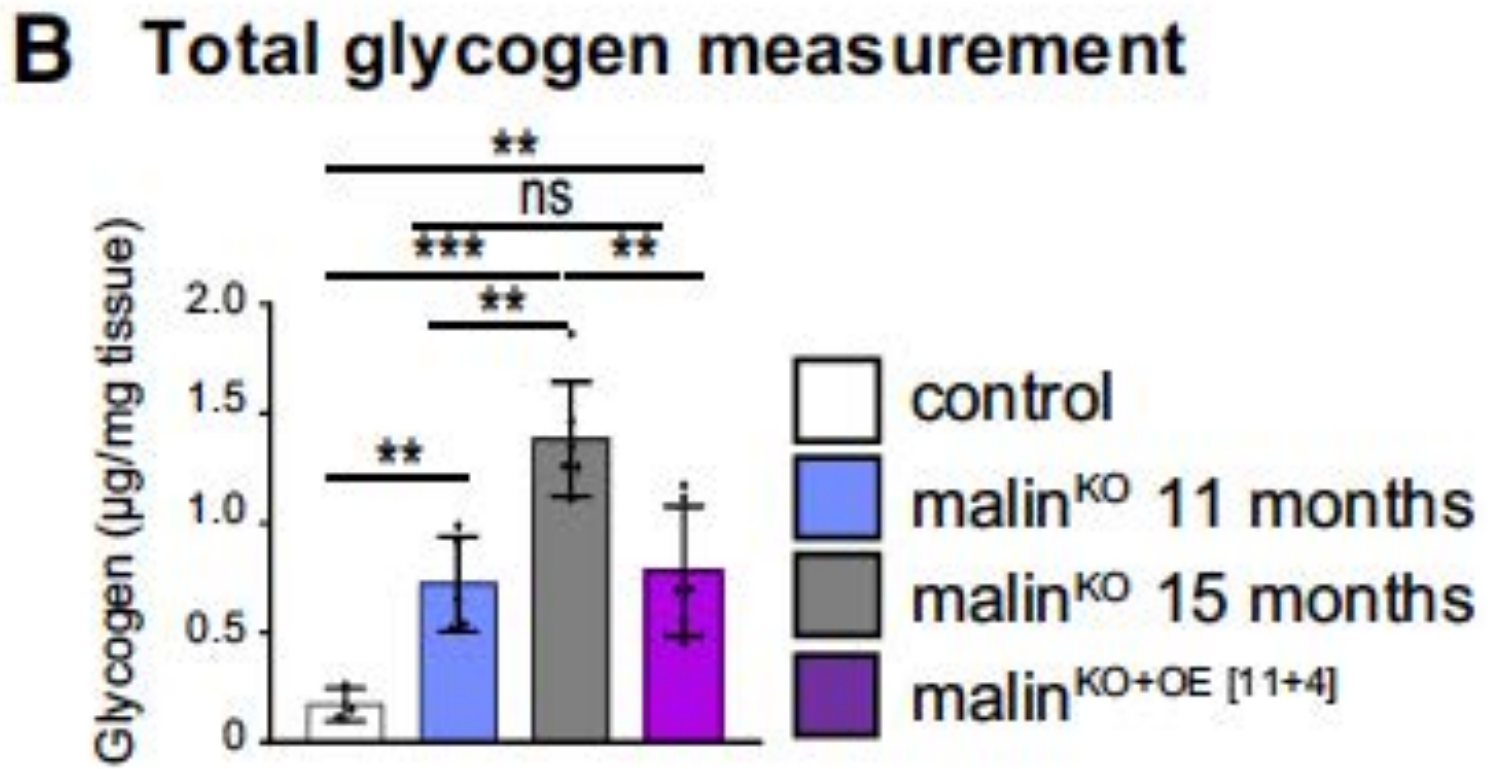

Varea et al., 2022, *Brain Communication*
Verea et al., 2021, *Neurobiology of Disease*
Duran et al., 2020, *Mol Neurobiol*
Auge et al., 2018, *Glia*

# Early re-expression of malin reverses LB aggregates




Varea et al., 2022, *Brain Communication*

# Late re-expression of malin stops LB progression


Varea et al., 2022, *Brain Communication*

# GSDs are ultra-rare alone, more common together

| Type | Alternate names or subtype | Affected Enzyme/Pathway | Gene | OMIM* Phenotype no. |
|---|---|---|---|---|
| 0 | 0a | Liver glycogen synthase | *GYS2* | 240600 |
| | 0b | Muscle glycogen synthase | *GYS1* | 611556 |
| I | Ia; von Gierke | Glucose-6-phosphatase α | *G6PC* | 232200 |
| | Ib; von Gierke | Glucose-6-phosphate transporter | *SLC37A4* | 232220 |
| II | Pompe | Acid α-glucosidase | *GAA* | 232300 |
| III | Cori/Forbes | Glycogen debranching enzyme | *AGL* | 232400 |
| IV | Andersen | Glycogen branching enzyme | *GBE1* | 232500 |
| V | McArdle | Muscle glycogen phosphorylase | *PYGM* | 232600 |
| VI | Hers | Liver glycogen phosphorylase | *PYGL* | 232700 |
| VII | Tarui | Muscle phosphofructose kinase | *PFKM* | 232800 |
| IX | IXa | Phosphorylase kinase (α2 subunit) | *PHKA2* | 306000 |
| | IXb | Phosphorylase kinase (β subunit) | *PHKB* | 261750 |
| | IXc | Phosphorylase kinase (γ subunit) | *PHKG2* | 613027 |
| | IXd | Phosphorylase kinase (α1 subunit) | *PHKA1* | 300559 |
| X | – | Muscle phosphoglycerate mutase | *PGAM2* | 261670 |
| XI# | Fanconi-Bickel | Glucose transporter 2 | *SLC2A2* | 227810 |
| XII | – | Aldolase A | *ALDOA* | 611881 |
| XIII | – | β-enolase | *ENO3* | 612932 |
| XV | – | Glycogenin-1 | *GYG1* | 603942 |

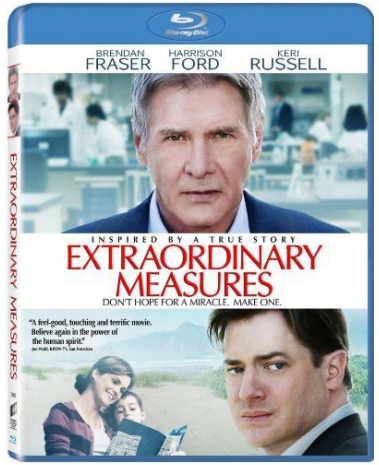
sanofi

Lumizyme®

Myozyme®

2006 FDA approved

**$1.1B in 2022**




Pompe Disease

**GSDs impact 1:~25,000**


DENALI THERAPEUTICS
Amicus Therapeutics
sanofi
AskBio
rocket pharma
Roche
AVROBIO
BIOMARIN
SAREPTA THERAPEUTICS
genzyme
oxyrane
IMMUSOFT
Actus Therapeutics
Selecta Biosciences
GREENOVATION Biopharmaceuticals
Spark THERAPEUTICS
AVIDITY BIOSCIENCES
AUDENTES
Aro BIOTHERAPEUTICS
Takeda
Lacerta therapeutics
astellas
valerion

J Neuropathol Exp Neurol. 2008 Aug;67(8):803-18. doi: 10.1097/NEN.0b013e3181815994.

## Temporal neuropathologic and behavioral phenotype of 6neo/6neo Pompe disease mice

Richard L Sidman [1], Tatyana Taksir, Jonathan Fidler, Michael Zhao, James C Dodge, Marco A Passini, Nina Raben, Beth L Thurberg, Seng H Cheng, Lamya S Shihabuddin

Proc Natl Acad Sci U S A. 2009 Jun 9;106(23):9419-24. doi: 10.1073/pnas.0902534106. Epub 2009 May 27.

## Neural deficits contribute to respiratory insufficiency in Pompe disease

Lara R DeRuisseau [1], David D Fuller, Kai Qiu, Keith C DeRuisseau, William H Donnelly Jr, Cathryn Mah, Paul J Reier, Barry J Byrne

ATM ANNALS OF TRANSLATIONAL MEDICINE

Ann Transl Med. 2019 Jul; 7(13): 289.
doi: 10.21037/atm.2019.04.49

PMCID: PMC6642933
PMID: 31392201

## An emerging phenotype of central nervous system involvement in Pompe disease: from bench to bedside and beyond

Aditi Korlimarla,# Jeong-A Lim,# Priya S. Kishnani, and Baodong Sun

Mol Ther. 2012 Jan;20(1):21-7. doi: 10.1038/mt.2011.214. Epub 2011 Oct 18.

## Spinal delivery of AAV vector restores enzyme activity and increases ventilation in Pompe mice

Kai Qiu [1], Darin J Falk, Paul J Reier, Barry J Byrne, David D Fuller

Review. Ann Transl Med. 2019 Jul;7(13):290. doi: 10.21037/atm.2019.05.56.

## Pompe disease gene therapy: neural manifestations require consideration of CNS directed therapy

Barry J Byrne [1], David D Fuller [2], Barbara K Smith [2], Nathalie Clement [1], Kirsten Coleman [1], Brian Cleaver [1], Lauren Vaught [1], Darin J Falk [3], Angela McCall [4], Manuela Corti [1]

J Neurophysiol. 2022 Nov 1;128(5):1133-1142. doi: 10.1152/jn.00026.2022. Epub 2022 Aug 17.

## Chemogenetic activation of hypoglossal motoneurons in a mouse model of Pompe disease

Michele L Singer [1 2 3 4], Sabhya Rana [2 3 4], Ethan S Benevides [1 2 3 4], Brian E Barral [3 4], Barry J Byrne [5 6], David D Fuller [1 2 3 4]

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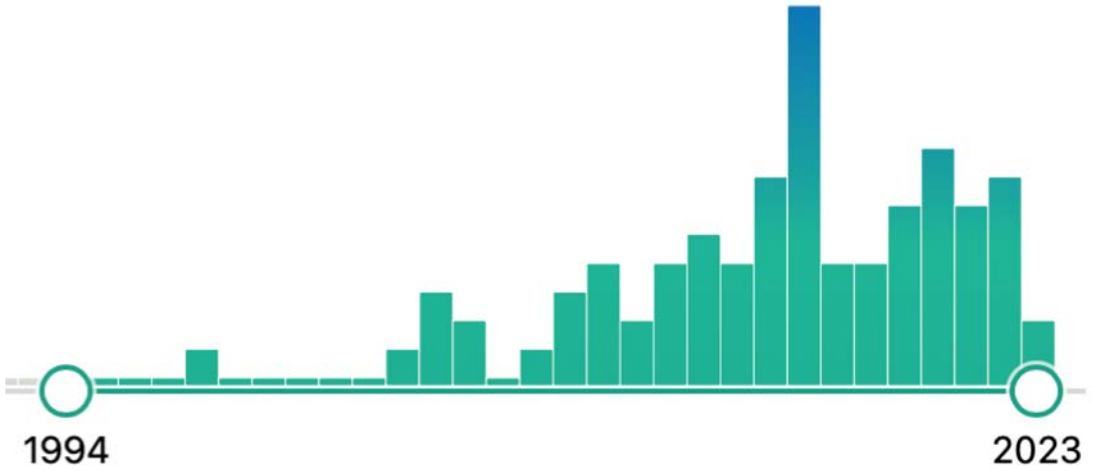
August 08, 2023; 101 (6) RESEARCH ARTICLE
OPEN ACCESS

## Neurofilament Light and Its Association With CNS Involvement in Patients With Classic Infantile Pompe Disease

Maarten J. Mackenbach, Eline A.J. Willemse, Jan J.A. van den Dorpel, Nadine A.M.E. van der Beek, Jordi Díaz-Manera, Dimitris Rizopoulos, Charlotte Teunissen, Ans T. van der Ploeg, Johanna M.P. van den Hout

First published June 19, 2023. DOI: https://doi.org/10.1212/WNL.0000000000207482

# Pompe disease patients have CNS & diaphragm issues




**75 papers**

> Front Rehabil Sci. 2023 Jul 31:4:1184031. doi: 10.3389/fresc.2023.1184031. eCollection 2023.

## Diaphragm pacing and independent breathing in individuals with severe Pompe disease

Cristina Liberati [1], Barry J Byrne [2], David D Fuller [3 4], Chasen Croft [5], Teresa Pitts [6 7], Jessica Ehrbar [3], Carmen Leon-Astudillo [2], Barbara K Smith [2 3 4]

Affiliations + expand

AAV-mediated delivery of secreted acid α-glucosidase with enhanced uptake corrects neuromuscular pathology in **Pompe** mice.

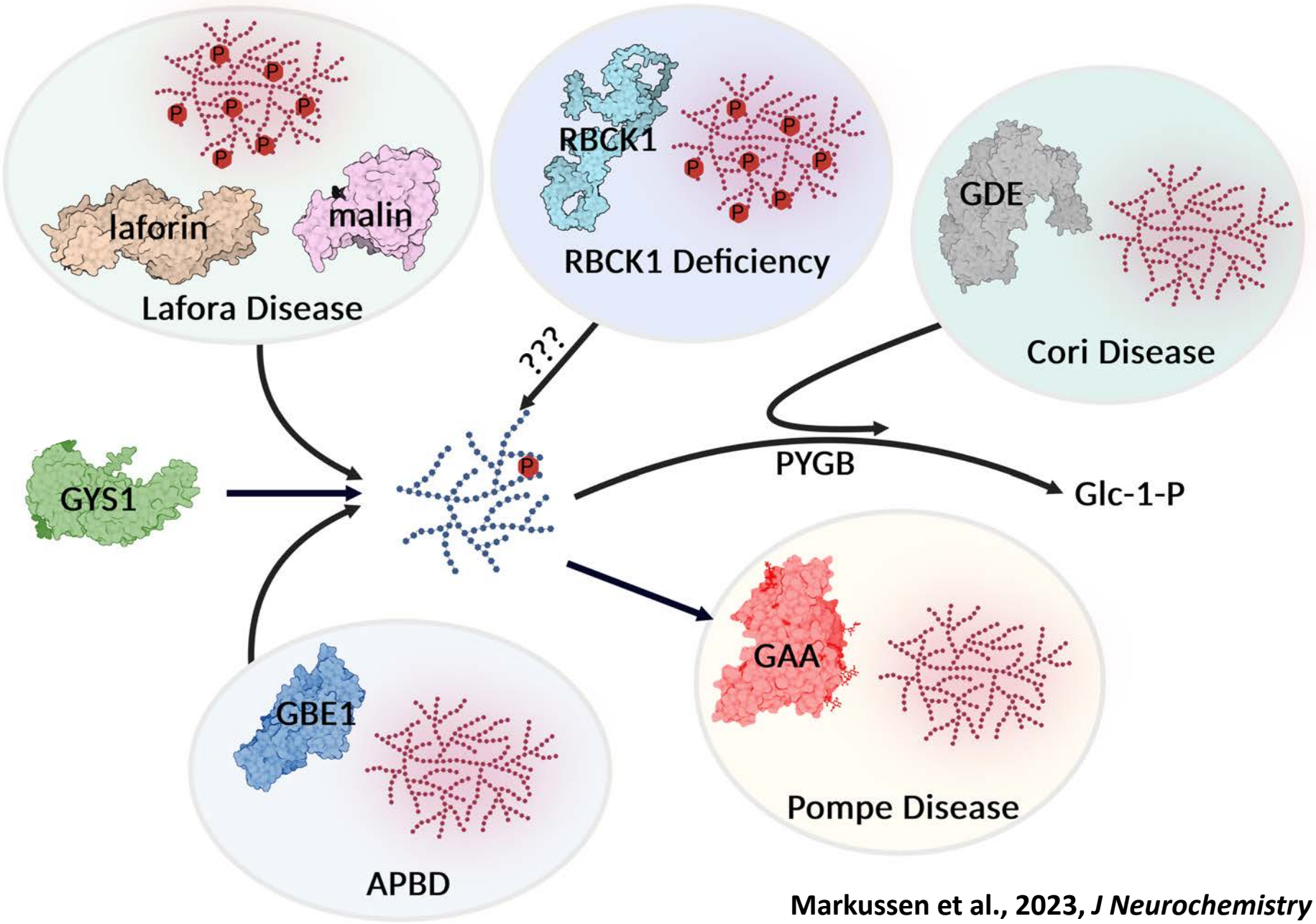
Meena NK, Randazzo D, Raben N, Puertollano R.

JCI Insight. 2023 Aug 22;8(16):e170199. doi: 10.1172/jci.insight.170199.

# Neurological GSDs (n-GSDs): One Drug – Multiple Diseases

**n-GSDs:**

**Lafora disease**
**APBD**
**Cori Disease**
**Pompe disease**
**RBCK1-deficiency**




Markussen et al., 2023, *J Neurochemistry*

# Ongoing & Future Pompe Disease Clinical Trials

**Sanofi/Maze:**

- MZE001 completed successful Phase I
  - Randomized with 120 unaffected individuals
  - NCT05249621, completed 21 Dec. 2022
- Sanofi acquired MZE001 for $750M
- Upcoming Phase II in Pompe patients

**Aro Biotherapeutics:**

- ABX1100 siRNA targeting *GYS1*
- FDA granted it Orphan Drug Status
- starting Phase I in Canada Q4 2023
- Unknown if it crosses the BBB

**AAV**

- 3 ongoing in clinicaltrials.gov
- NCT04093349, NCT05567627, NCT05793307

# Acknowledgments

Dr. Ramon Sun

Dr. Craig Vander Kooi


NIH National Institute of Neurological Disorders and Stroke

NIH National Cancer Institute

NIH National Institute on Aging

CURE EPILEPSY

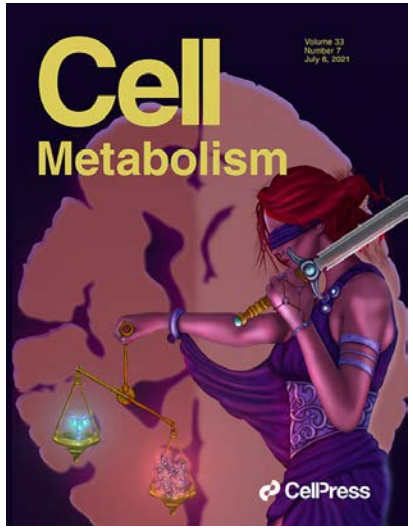
EPILEPSY FOUNDATION END EPILEPSY TOGETHER

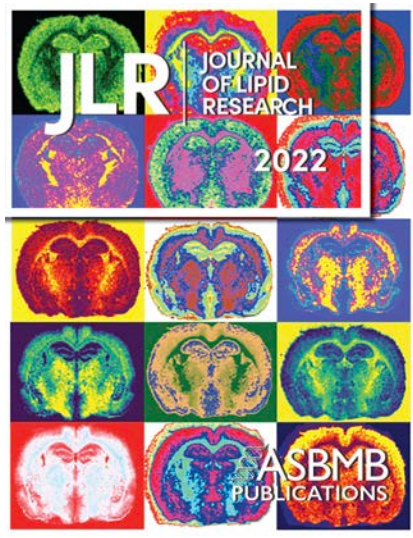
NSF

NIH R35 NS116824
NIH P01 NS097197
NIH R61 NS111081
NIH R01 CA266004
NSF MCB1252345

Epilepsy Foundation
Valerion Therapeutics
Ionis Pharmaceuticals
Maze Therapeutics





# Symptomatic treatment vs. Disease Modifying vs Curative

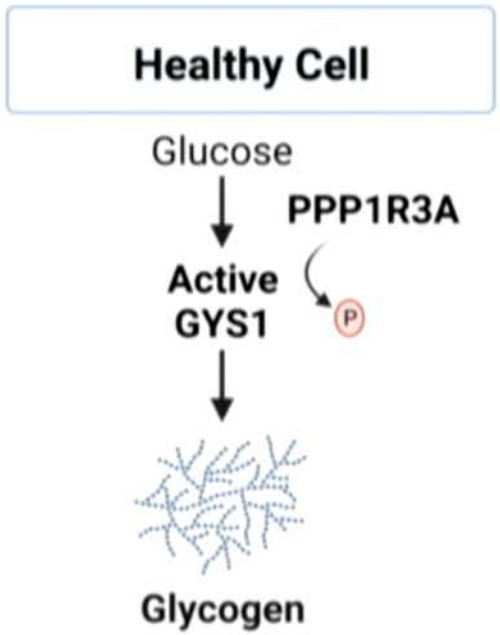
**Symptomatic treatment**, supportive care, or supportive therapy is any medical therapy of a disease that only affects its symptoms, not the underlying cause.

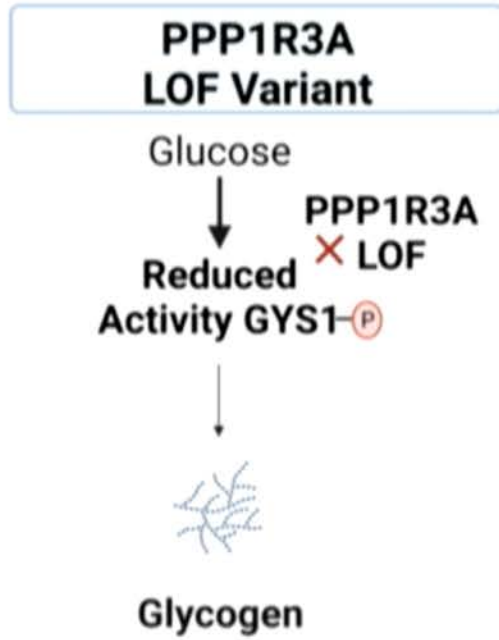
A **disease-modifying treatment, disease-modifying drug, or disease-modifying therapy** is a treatment that delays or slows the progression of a disease by targeting its underlying cause. They are distinguished from symptomatic treatments that treat the symptoms of a disease but do not address its underlying cause.

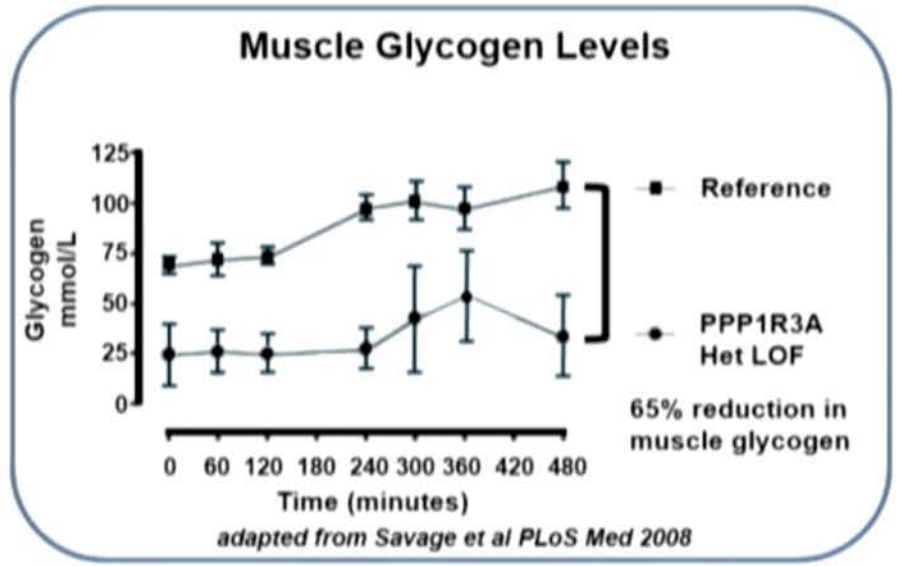
**Curative treatment** refers to treatment and therapies provided to a patient with the main intent of fully resolving an illness and the goal of bringing the patient – ideally - to their status of health before the illness presented itself.

Lafora Epilepsy Cure Initiative

# Human genetics suggests ~65% reduction in muscle glycogen is safe & well tolerated



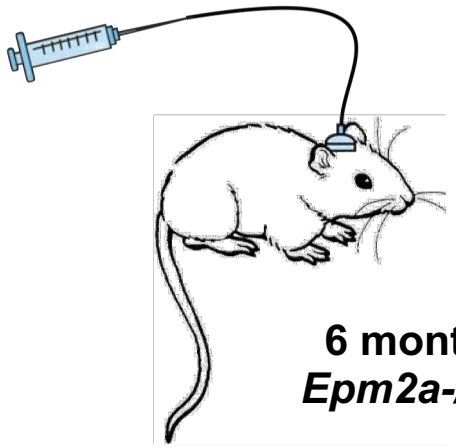

- Protein phosphatase 1 regulatory subunit 3A (PPP1R3A) is a key activator of GYS1. Loss of function (LOF) variant is found in ~1.1% of Caucasian individuals identified in UK Biobank

**Analysis of research conducted using data from UK Biobank human subjects with 65% less muscle glycogen reveals no significant association across hundreds of phenotypes including:**

- ***No difference in exercise capacity (exercise output or maximum exercise heart rate)***
- ***No impact on cardiac function (LVEF, LV wall thickness) nor correlation with heart failure***
- ***No change in serum glucose or correlation with Type II Diabetes***

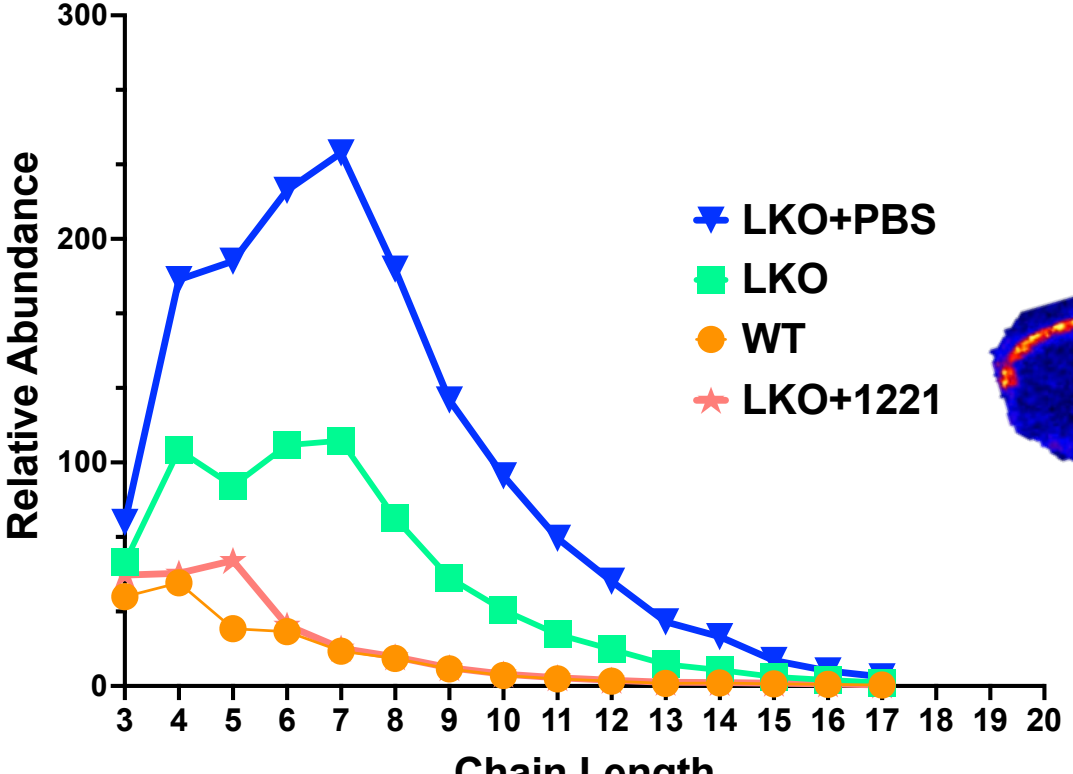
# VAL-1221 ICV administration ablates brain LBs *in vivo*

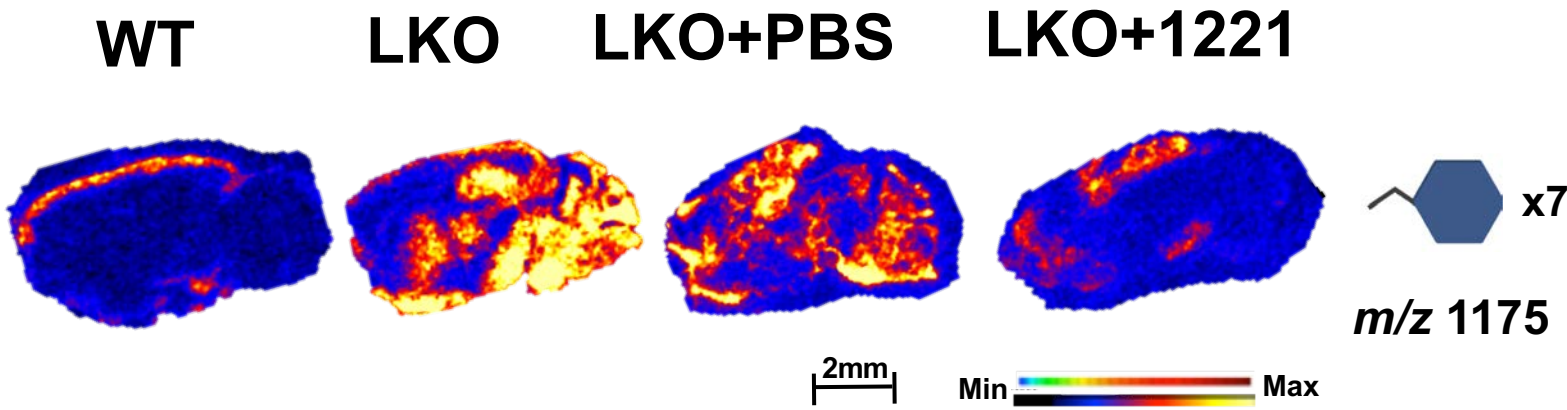
**Intracerebroventricular (ICV) administration**



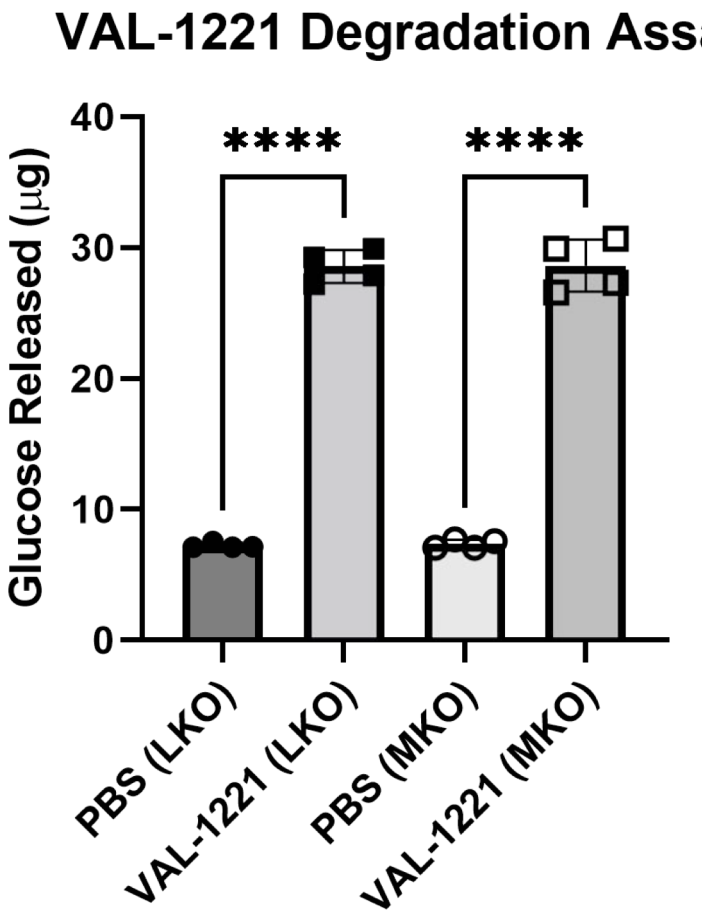
6 month old *Epm2a-/-* mice

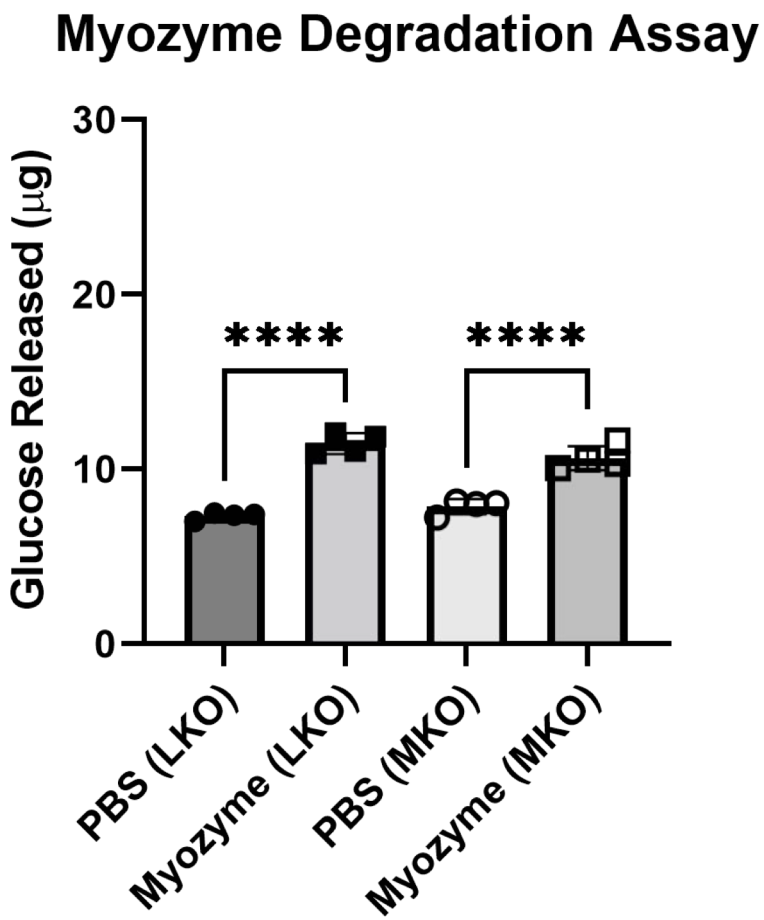
Continuous infusion of VAL-1221 or PBS for 7 days → Euthanized on 8th day → PAS staining



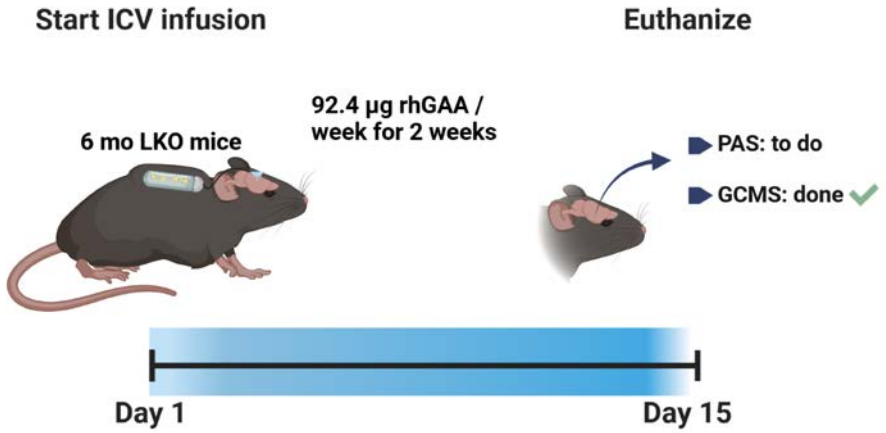
# VAL-1221 and Myozyme® degrade LBs *in vitro*

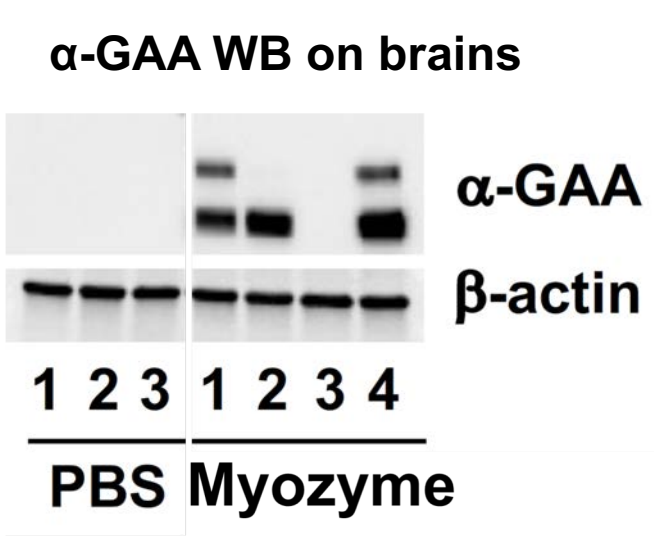


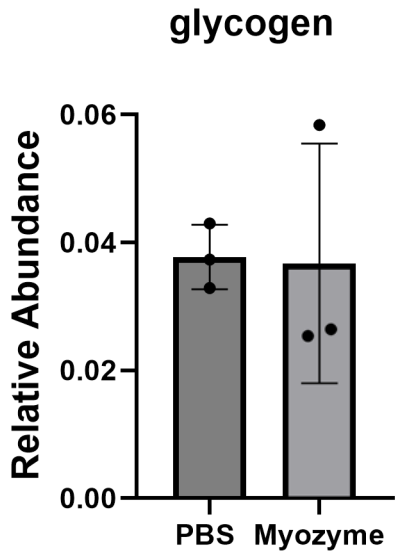




**10 µg VAL-1221 or Myozyme® incubated with 80 µg LBs at 37°C pH 7.5 for 3 days**

# Gentry lab Myozyme® ICV experiment



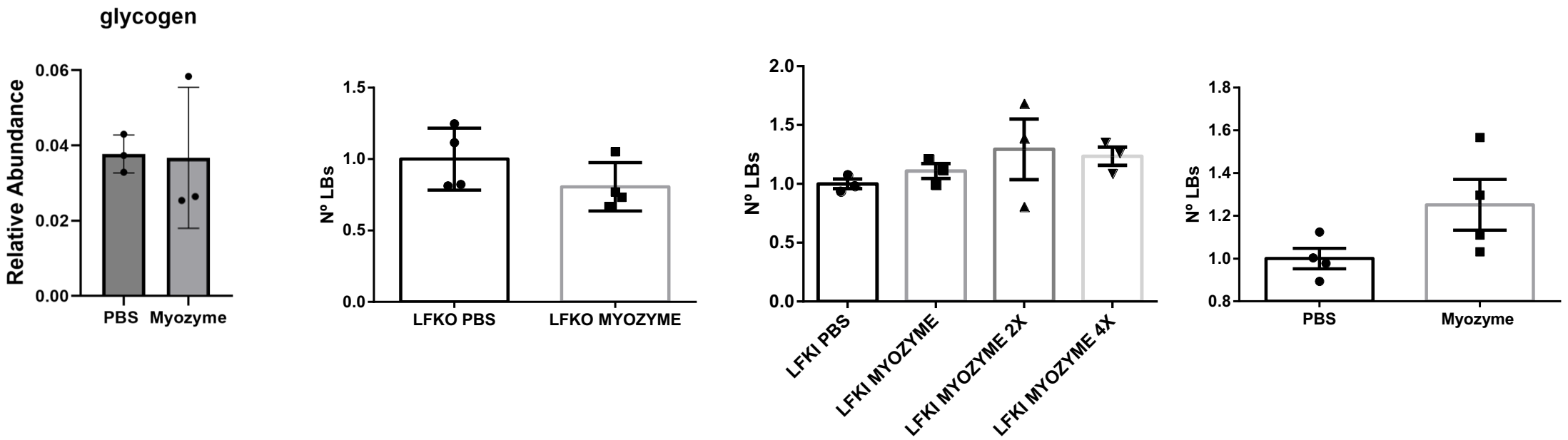




- ICV infusion for 2 weeks with PBS or rhGAA (0.185 mg total) in 6 mo LKO mice
- Distribution of Myozyme in the brains of 3 mice
- No significant change in glycogen content according to GCMS

# Summary on Myozyme ICV experiments

| Experiment | Genotype | Age | Number of mice treated with Myozyme® | Delivery method | Total amount of rhGAA | Duration of treatment | GAA infusion rate | Assay |
|---|---|---|---|---|---|---|---|---|
| Gentry | LKO | 6 months | 3 | Alzet osmotic pump | 185 µg | 2 weeks | 92.4 µg / week | GCMS |
| Serratosa 1 | LKO | 12 months | 4 | Punctual ICV | 120 µg | 1 week | n.a. | PAS |
| Serratosa 2 | Laforin R240X | 12 months | 9 | Punctual ICV | 120, 240, 480 µg | 1 week | n.a. | PAS |
| Serratosa 3 | LKO | 9 months | 4 | Alzet osmotic pump | 530 µg | 1 month | 120 µg / week | PAS + behavior |
| | | | **total = 20** | | | | | |

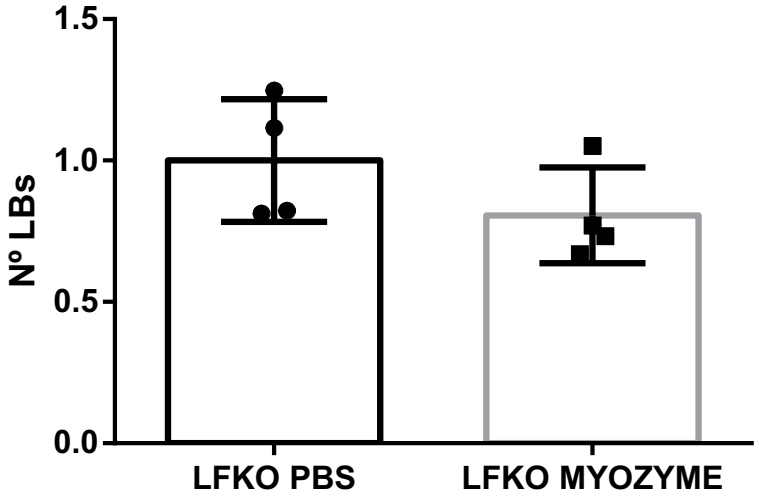


# Serratosa lab Myozyme® ICV results – Experiment 1

- **Genotype and age :** 12 mo LKO mice
- **Administration :** Punctual 0.73 mg Myozyme®/10 µl saline, 10ul ICV injection (coordinates: AP:-0.3; ML: 0.9; DV: -2.5)
- **Efficacy studies :** PAS-D staining at 7 days after injection

- **Animals analyzed :** 8 mice
  - 4 mice PBS
  - 4 mice Myozyme®

- **Results**

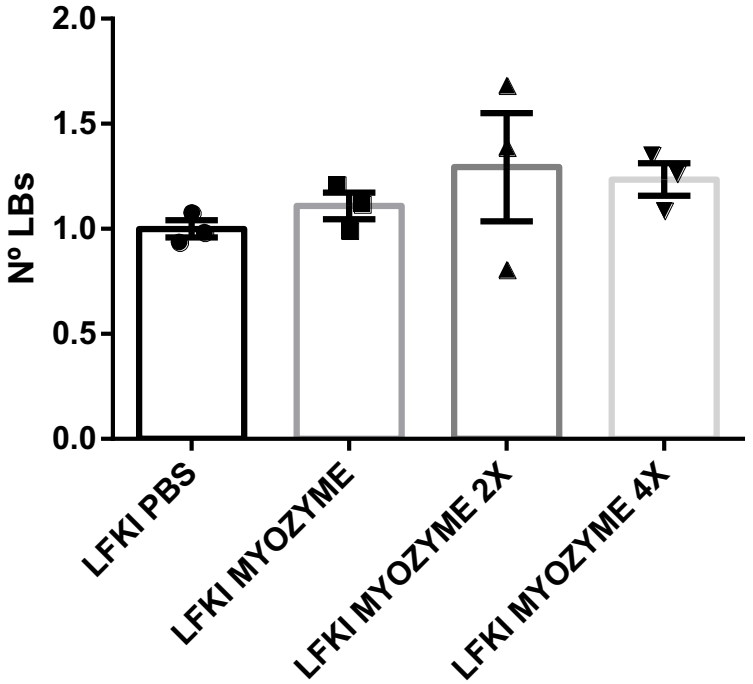


# Serratosa lab Myozyme® ICV results – Experiment 2

- **Genotype and age :** 12 mo Laforin knock-in R240X mice
- **Administration :** Punctual ICV injections (0.73 mg Myozyme®/10 µl saline; 1.46 mg Myozyme®/10 µl saline; 2.92 mg Myozyme®/10 µl saline ) (coordinates: AP:-0.3; ML: 0.9; DV: -2.5)
- **Efficacy studies :** PAS-D staining at 7 days after injection

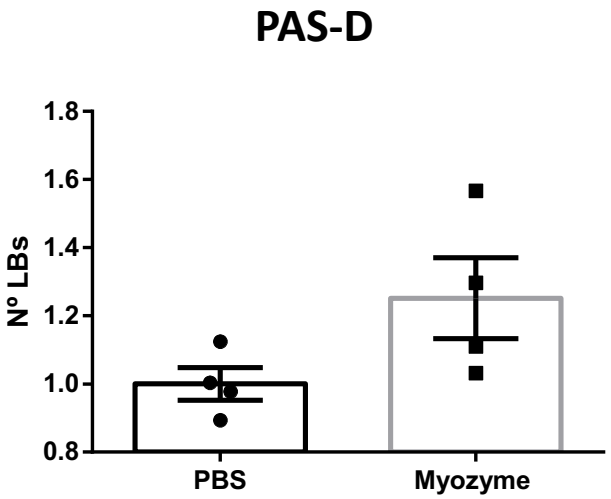
- **Animals :** 12 mice, 3 per group

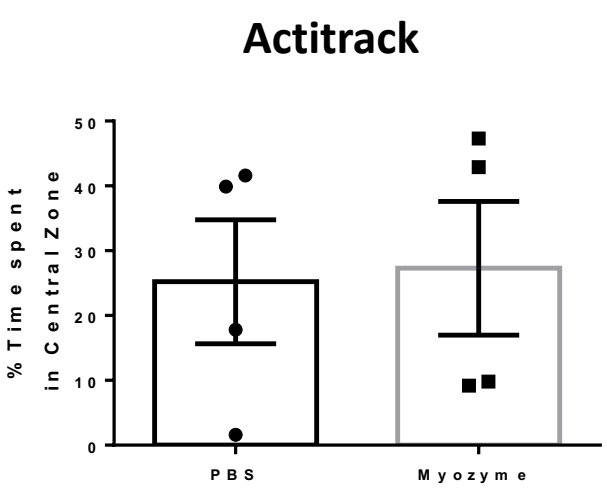
- **Results :**

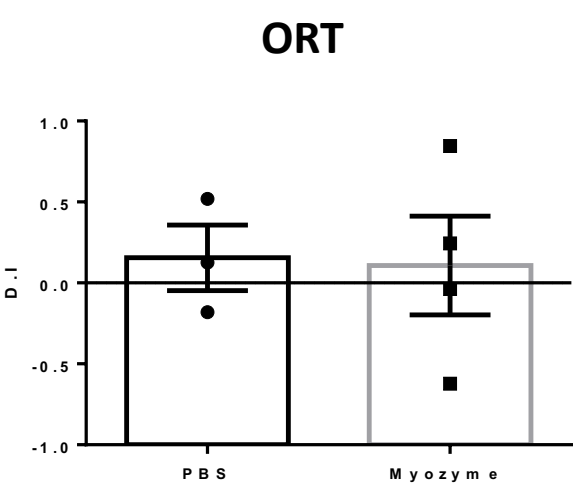


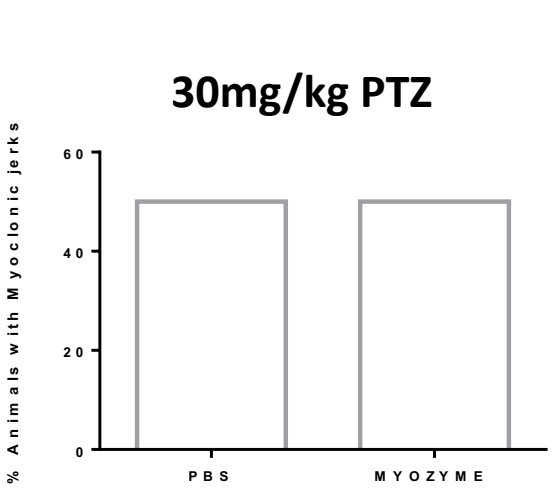
# Serratosa lab Myozyme® ICV results – Experiment 3

- **Genotype and age :** 9 mo LKO mice
- **Administration :** Alzet® Micro-Osmotic Pump Model 1004 + Cannula ICV (0.11µl/h) (Cannula in coordinates: AP:-0.3; ML: 0.9) (3.23 mg Myozyme®/100 ul saline)
- **Efficacy studies :** Behavioral analysis and PAS-D staining at 1 month after pump implantation
- **Animals analyzed :** 8 mice



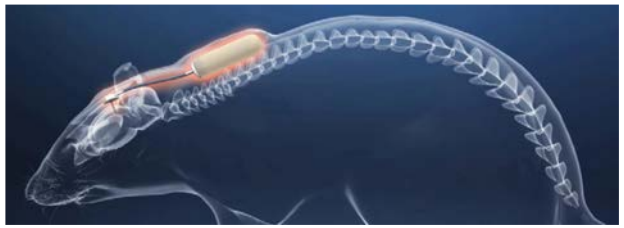



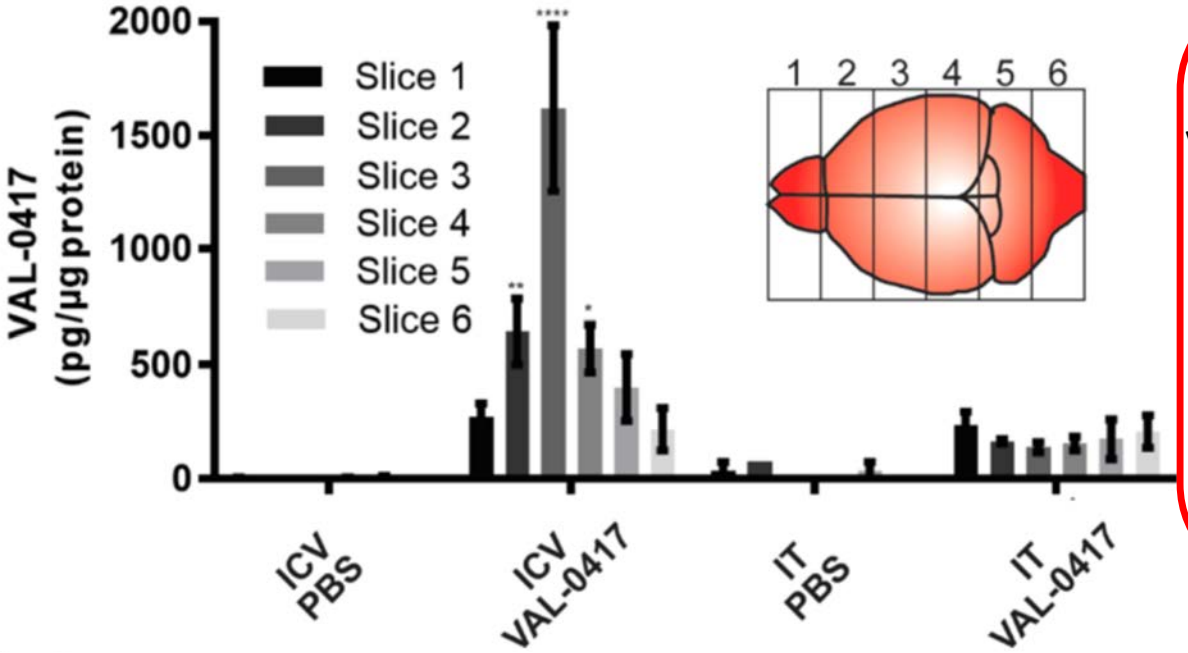



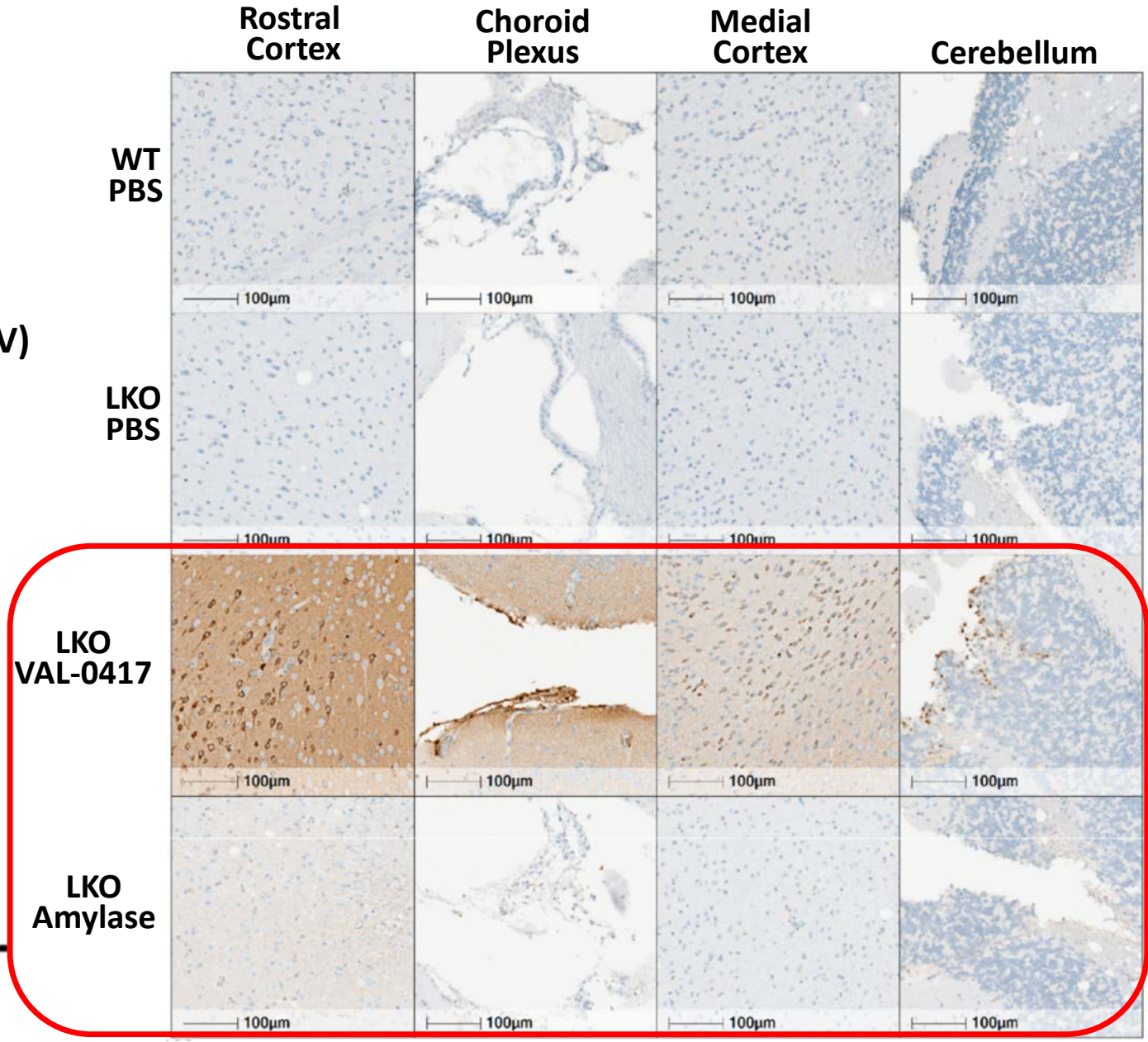
# IT versus ICV administration in WT mice

Intrathecal (IT)



Intracerebroventricular (ICV)

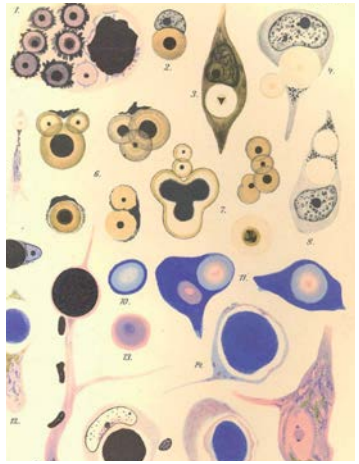



Austin et al., 2019
*Mol Pharmaceutics*

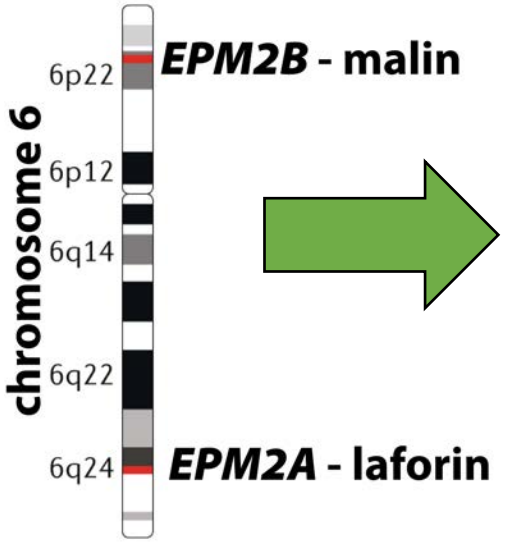
# 112 Year Summary of Lafora Disease (LD) Research

## Syndrome Described

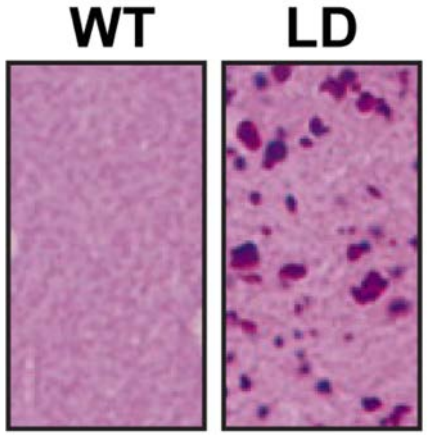


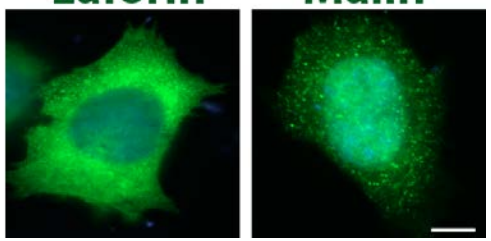
Lafora, *Virchows Arch Pathol Anat Physiol Klin Med* 1911

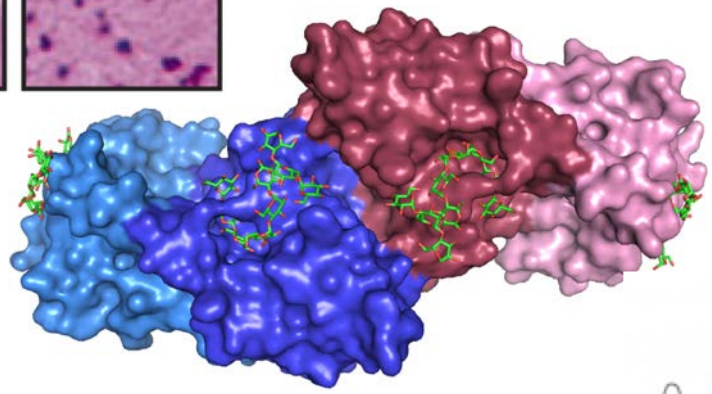
## Gene Identification



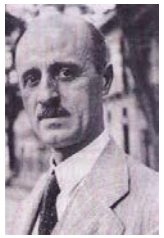

## Mouse models Biochemistry & Cell Biology



**1911**



Progressive myoclonus epilepsy with amyloid deposits characterized
*Gonzalo Rodriguez Lafora*

**1968-1970**
Lafora bodies are starch-like
*Yokoi et al.*
*Sakai et al.*

**1965**
Lafora disease named
*Gabriel Schwartz*

**1995**
Gene mapped to chrom. 6q
*Serratosa& Delgado-Escueta*

**1998**
EPM2A identified
*Delgado-Escueta, Minassian & Serratosa*

**2003**
NHLRC1/EPM2B identified
*Minassian, Delgado-Escueta & Scherer*

**2006**
laforin characterized as a glucan phosphatase
*Worby, Gentry & Dixon*

**2007**
laforin dephosphorylates glycogen
*Roach & DePaoli-Roach*
*Minassian & Delgado-Escueta*

**2013-2015**
LBs are mechanism
*Guinovart*
*Roach*
*DePaoli-Roach*
*Minassian*

**2015**
laforin crystal structure
*Gentry*

**2016**
NIH P01

**small molecule therapy**
**enzyme therapy**
**ASOs**
**repurposing drugs**
**gene therapy**
*- EPM2A (laforin)*
*- EPM2B (malin)*

**Gentry et al., 2020,**
***Epilepsy & Behavior***

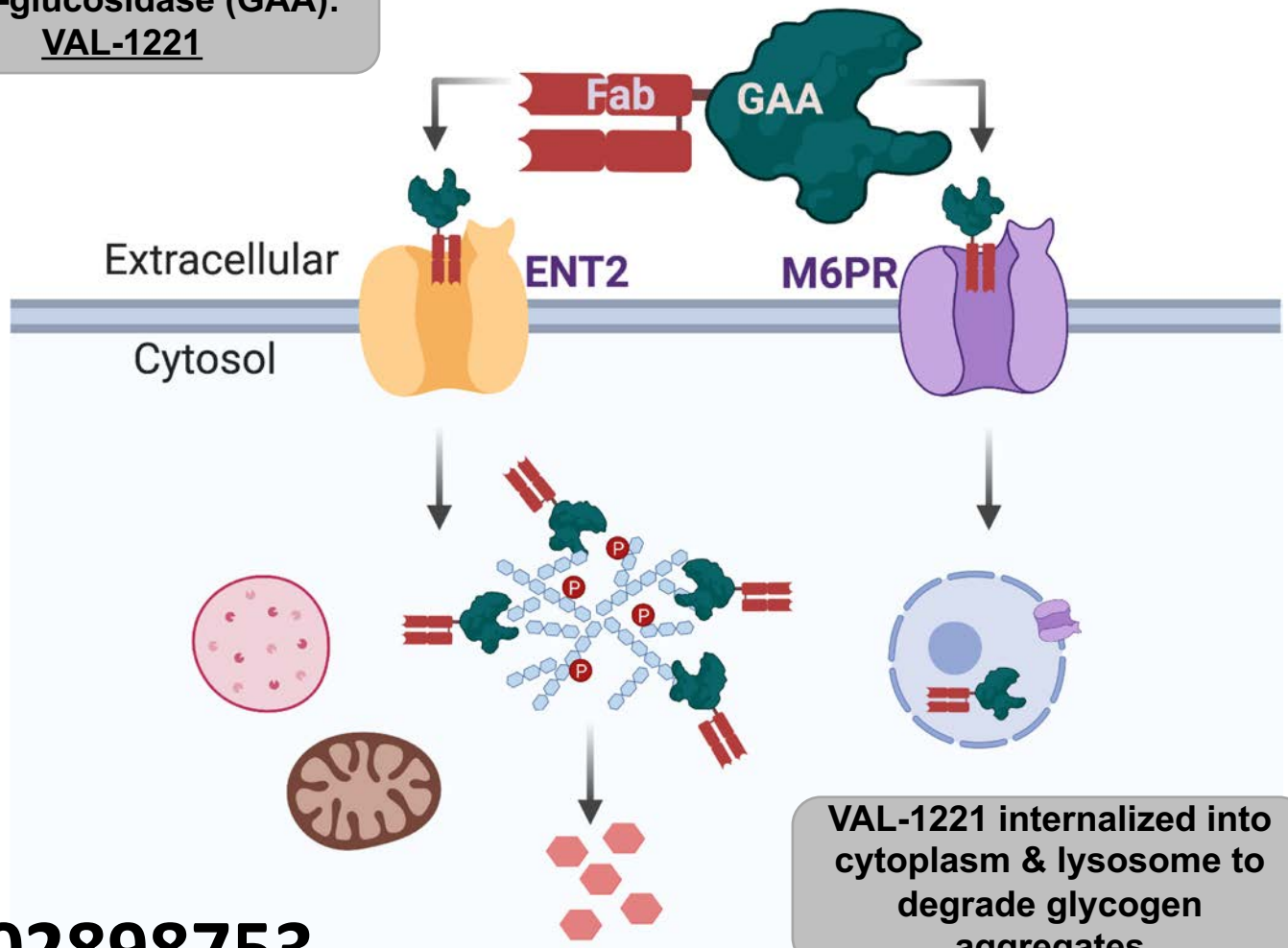
Lafora Epilepsy Cure Initiative

Chelsea's Hope
Lafora Children Research Fund

NIH National Institute of Neurological Disorders and Stroke

# VAL-1221 mechanisms of cellular entry

Fab fragment linked to alpha-glucosidase (GAA): <u>VAL-1221</u>




Fab recognizes ENT2 & GAA recognizes M6PR receptor and is transported across the membrane

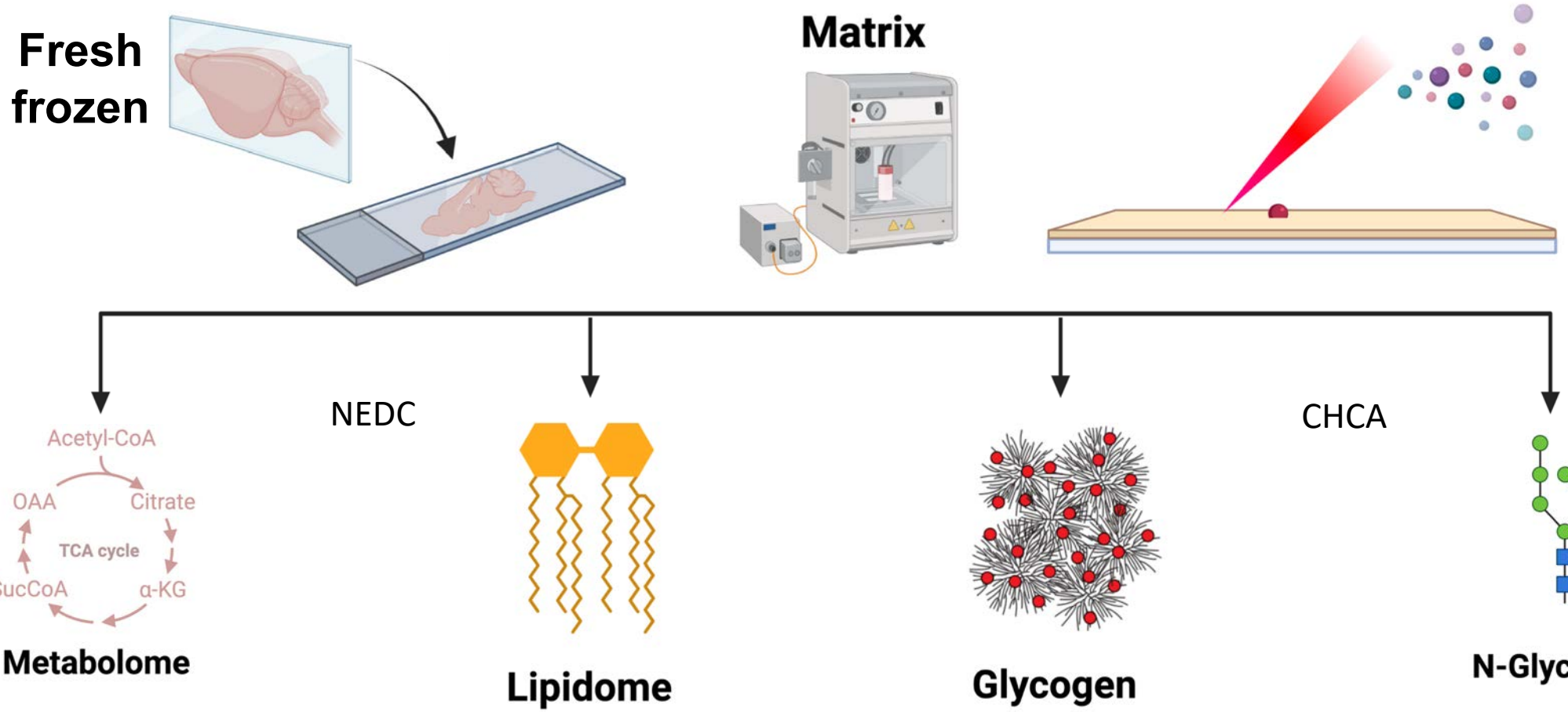
VAL-1221 internalized into cytoplasm & lysosome to degrade glycogen aggregates

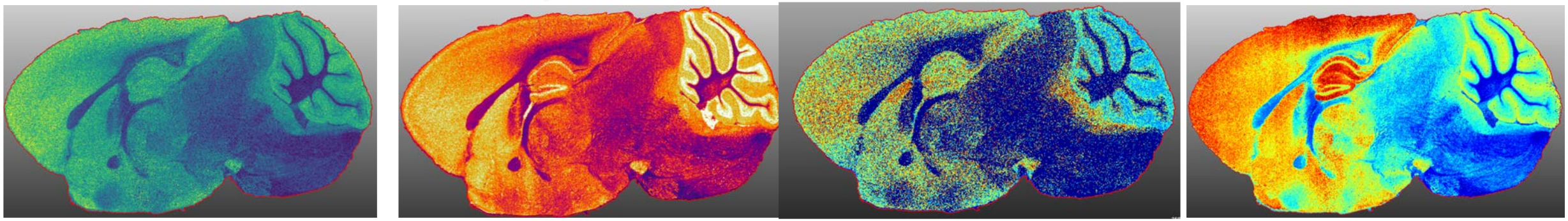
## Clinical Trial: NCT02898753

**Initiated: June 2017**

**Completed: June 2020**

# Spatial Metabolomics, Lipidomics, and Glycomics



# VAL-1221 and Myozyme – tail vein injection (IV)

**Tail vein (IV) injections**

**6 month old**
**WT and *Epm2a-/-* mice**

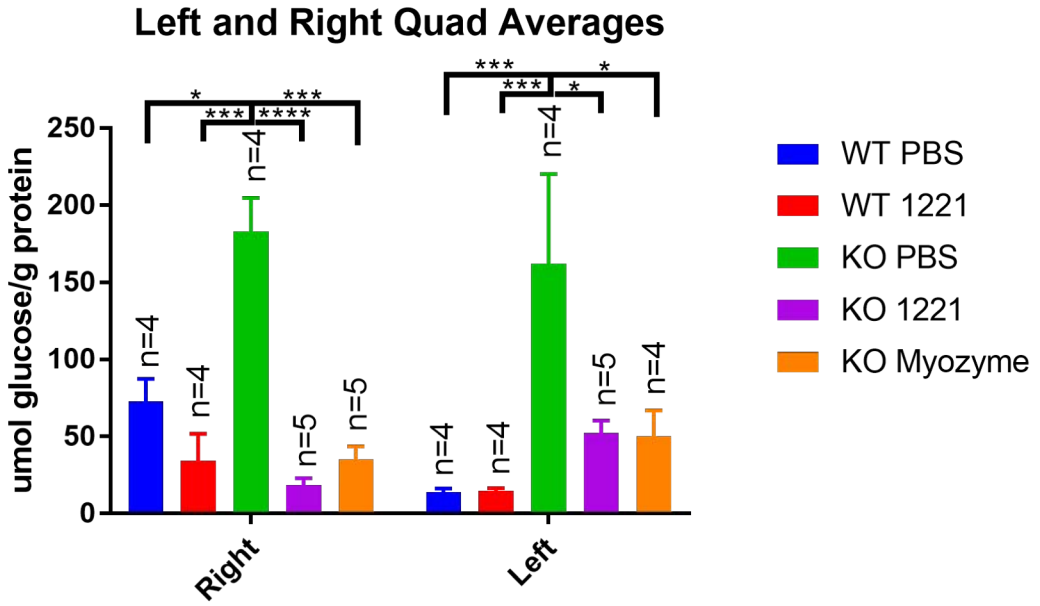
**4 injections of PBS, Myozyme, or VAL-1221 in tail vein over 14 days**

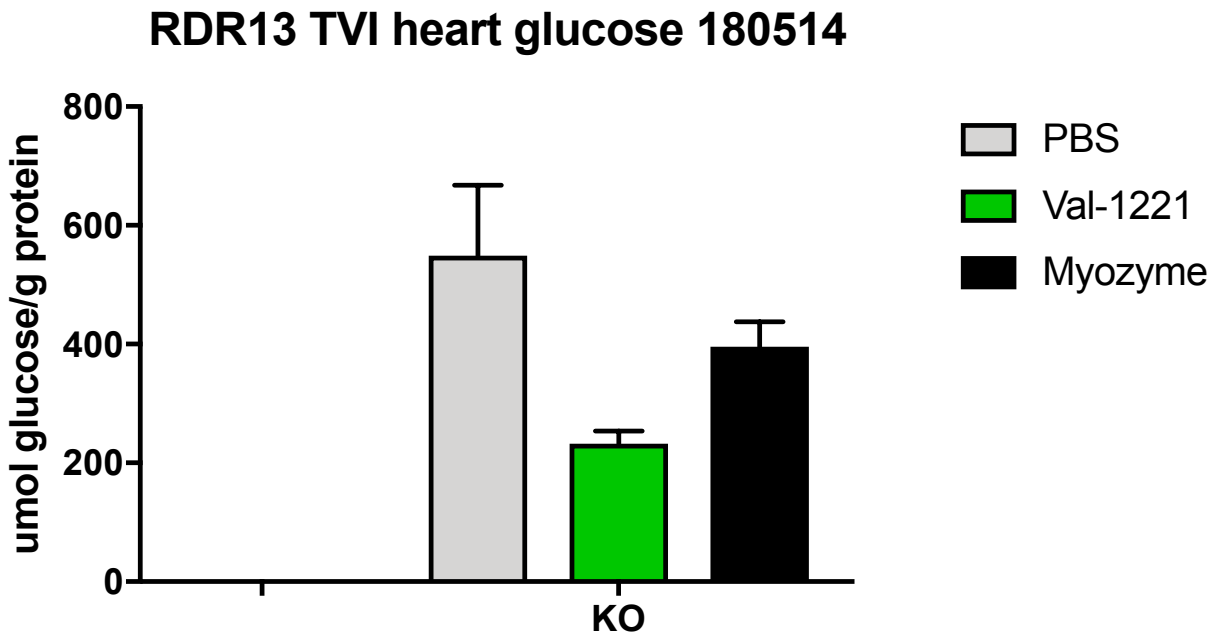
↓

**Euthanized on 18th day**
**Multiple tissues collected**

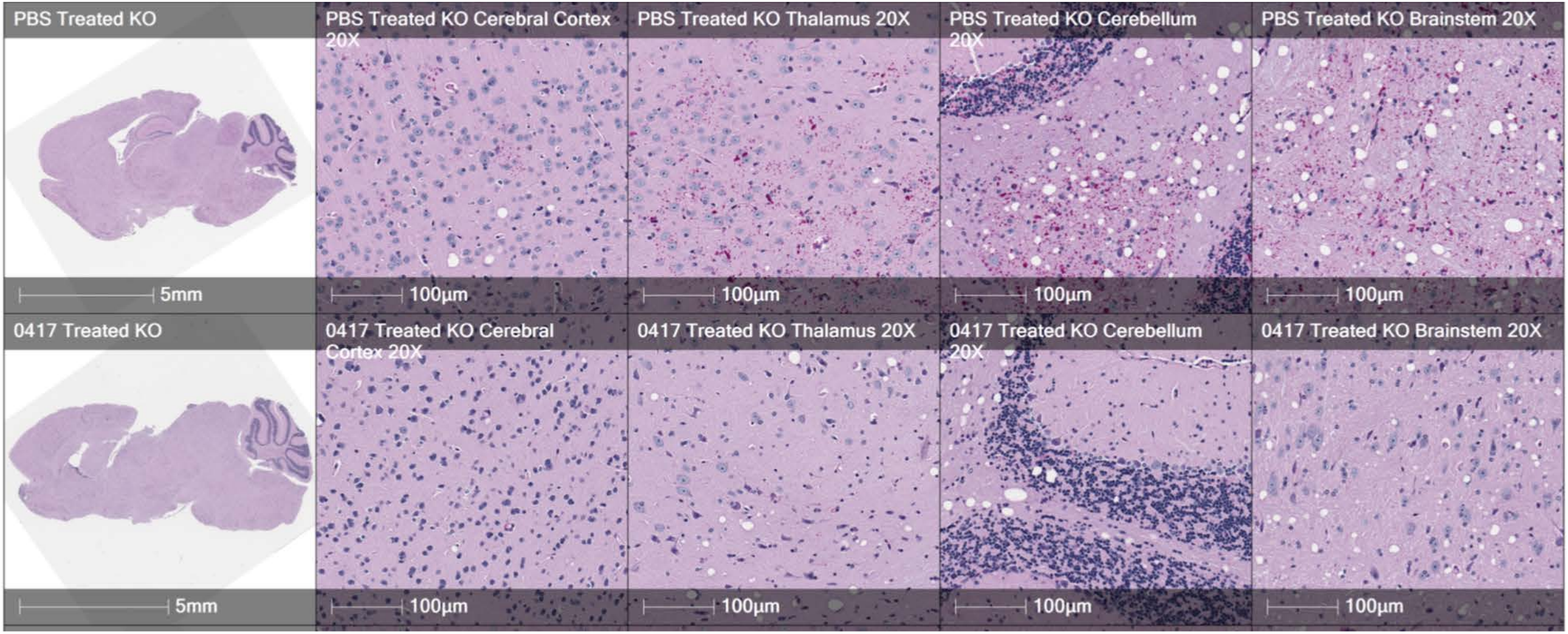
↙ ↘

**Polysaccharide content determined** | **PAS staining**



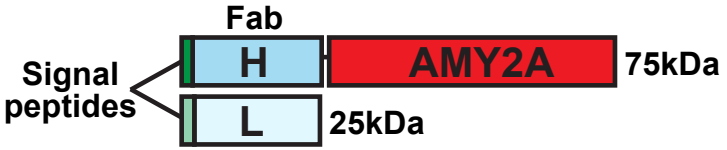



# VAL-0417 ICV administration ablates brain LBs *in vivo*

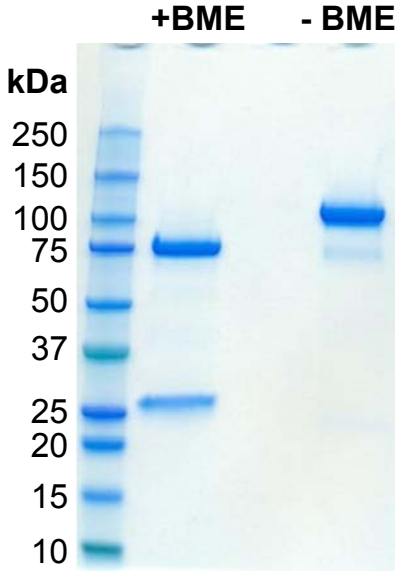


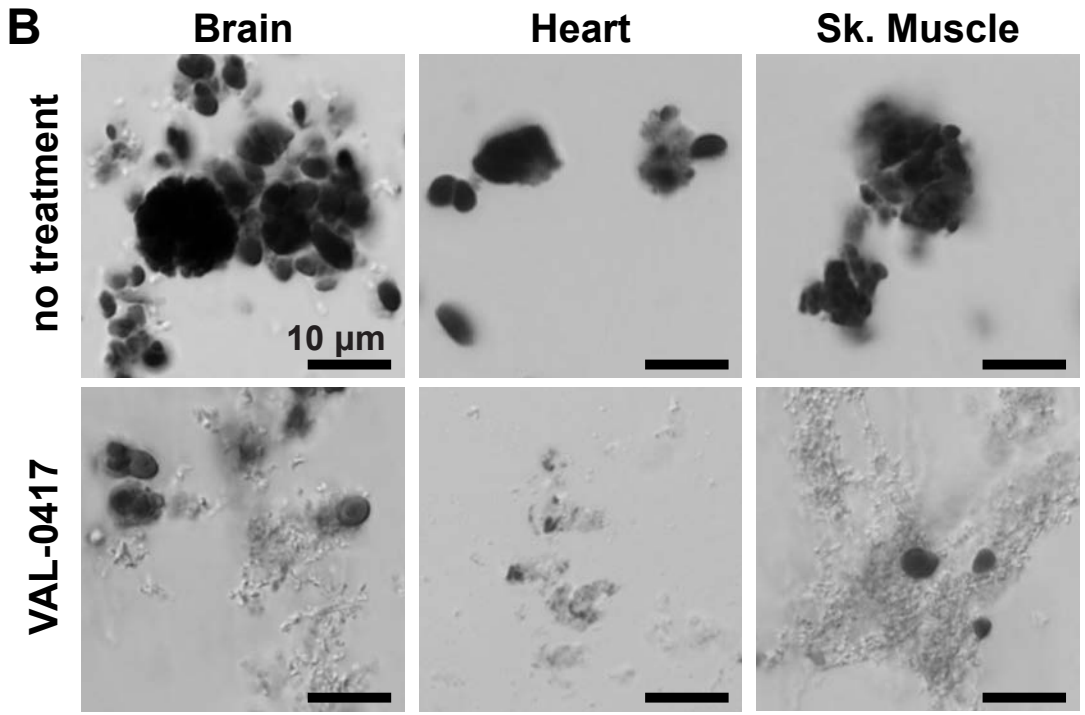
# Antibody-enzyme fusion (AEF) VAL-0417 degrades LBs

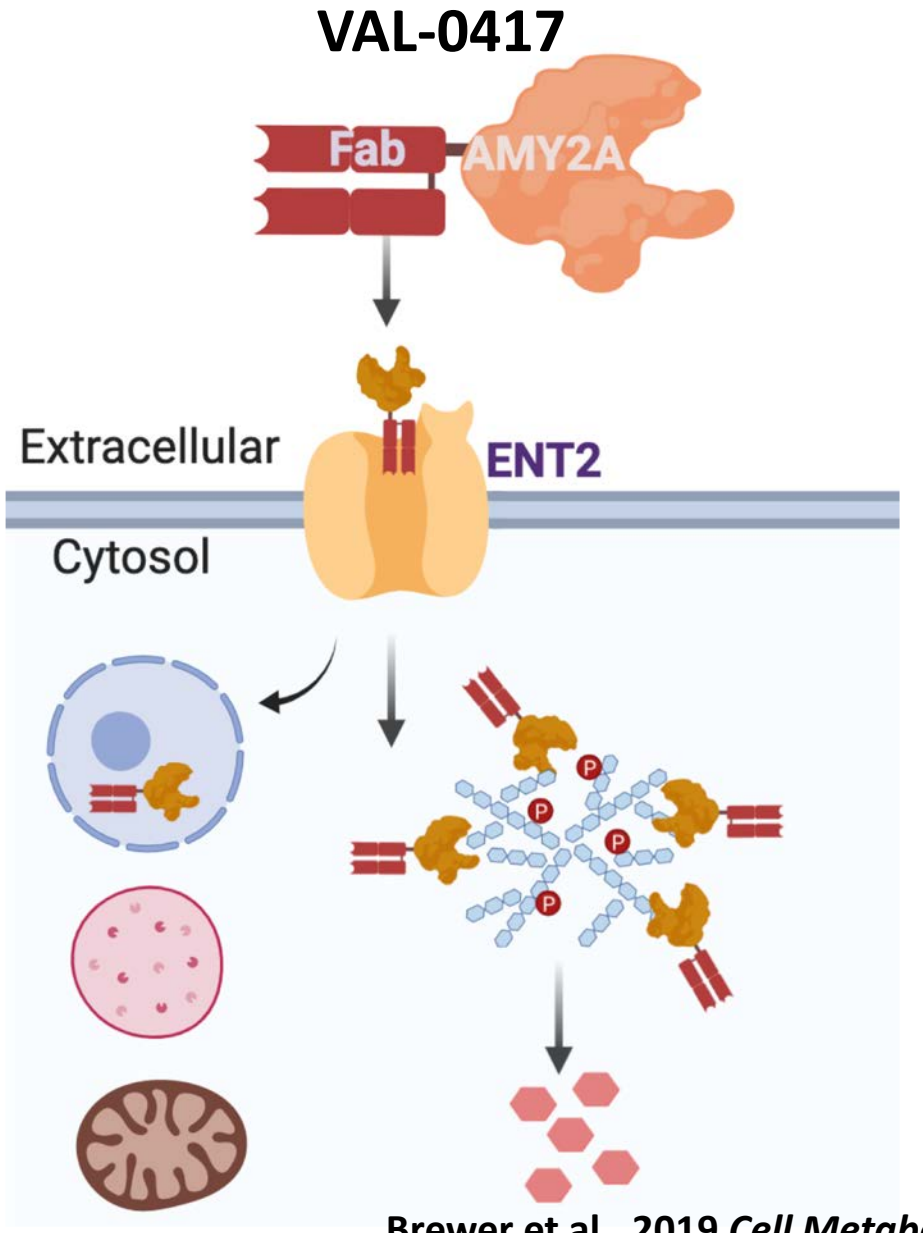
hFab + pancreatic amylase (AM2A)




**VAL-0417**



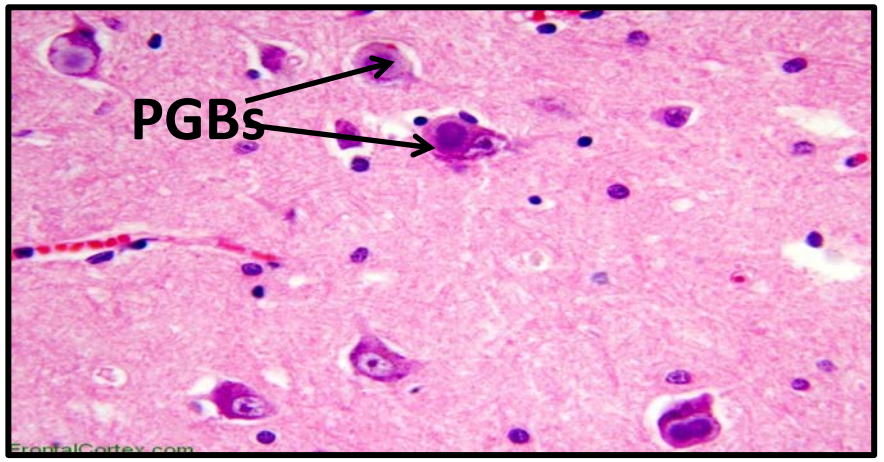



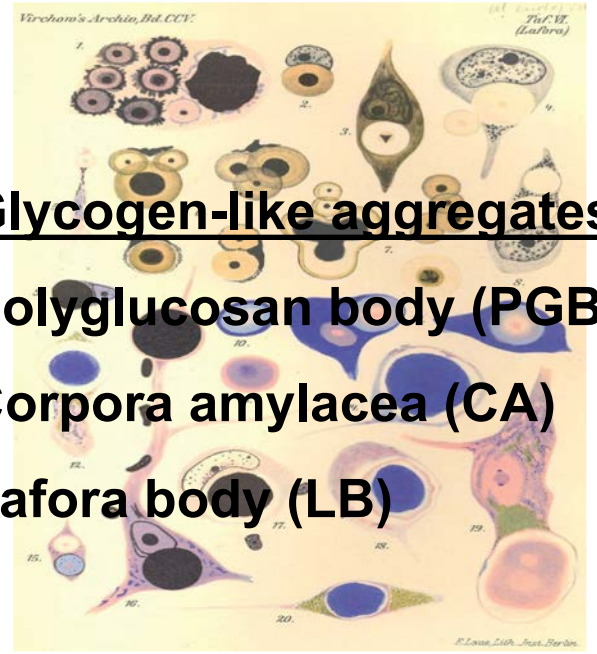

Brewer et al., 2019 *Cell Metabolism*

# Lafora Disease (LD) – Childhood Dementia

- LD was first described >100 years ago by Gonzalo Rodríguez Lafora
- fatal, autosomal recessive disorder – childhood dementia with horrendous epileptic episodes
- glucan/carbohydrate inclusions found in cytoplasm of cells from most tissues, (polyglucosan bodies, PGBs); glycogen storage disease (GSD)




Minassian, *Pediatr. Neurol.*, 2001



**Glycogen-like aggregates:**

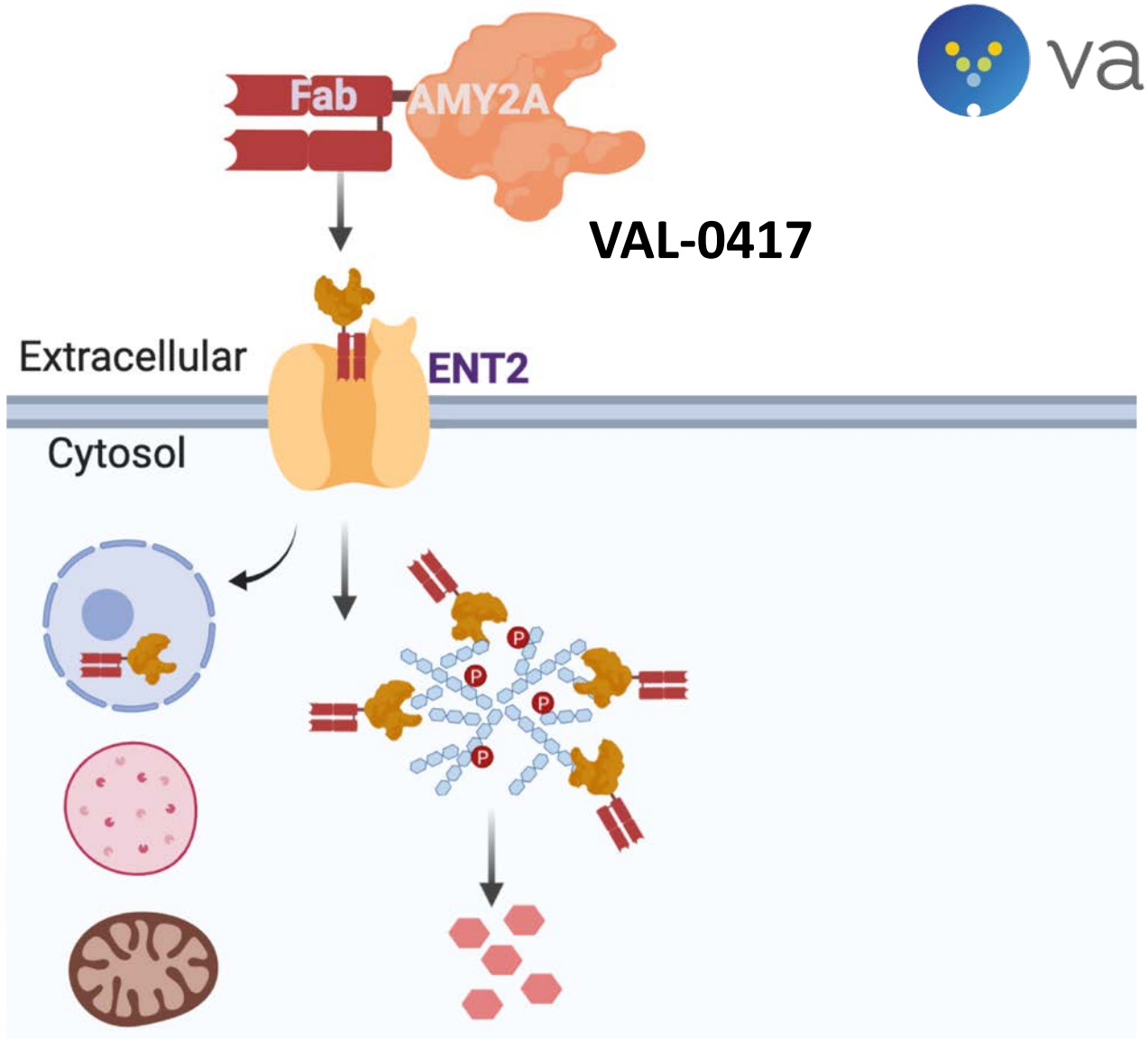
Polyglucosan body (PGB)

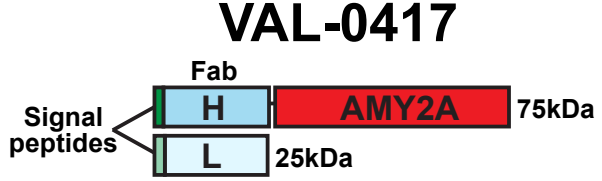
Corpora amylacea (CA)

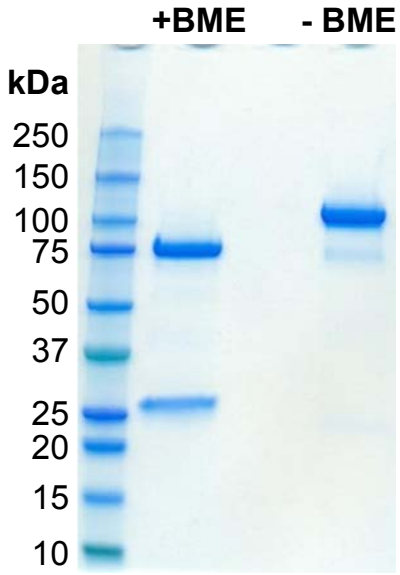
Lafora body (LB)

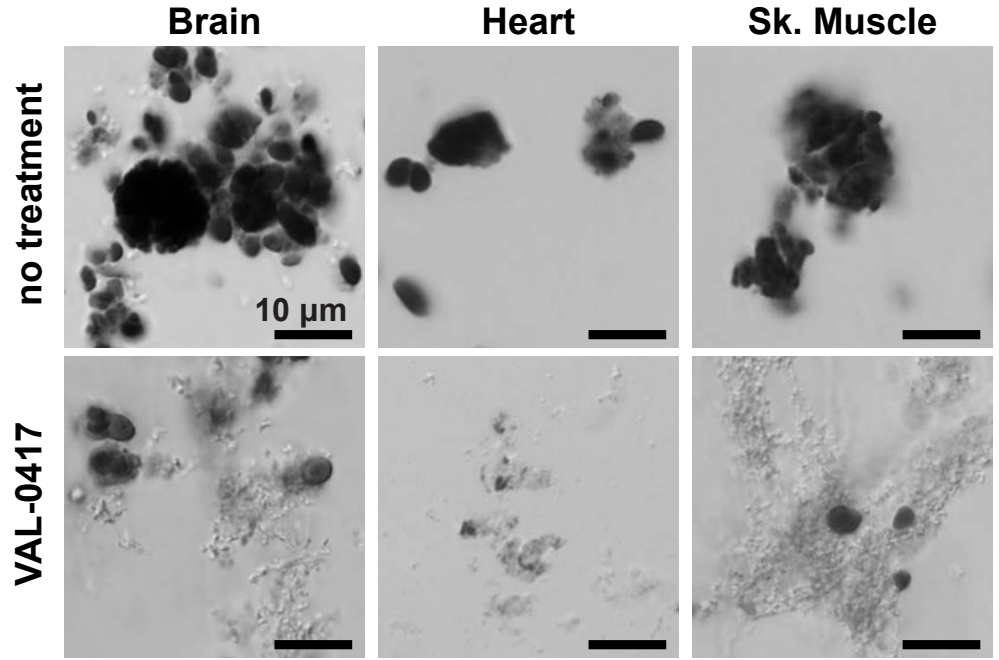
Lafora, *Virchows Arch Pathol Anat.*, 1911

# Antibody-enzyme fusion (AEF) platform



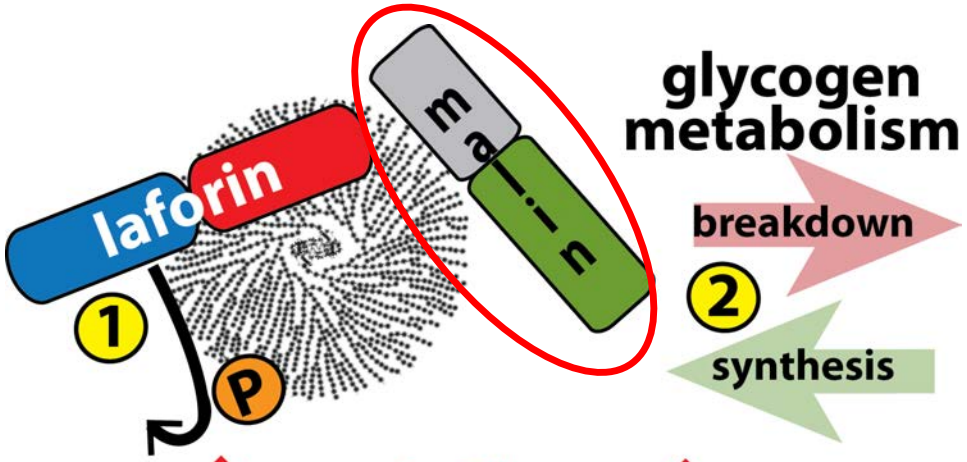





Weisbart et al., 1990 *J Immunology*
Hansen et al., 2007 *JBC*
Hansen et al., 2012 *Sci Trans Med*
Yi et al., 2016 *Human Mol Gen*

# Glycogen Architecture – Laforin & Malin

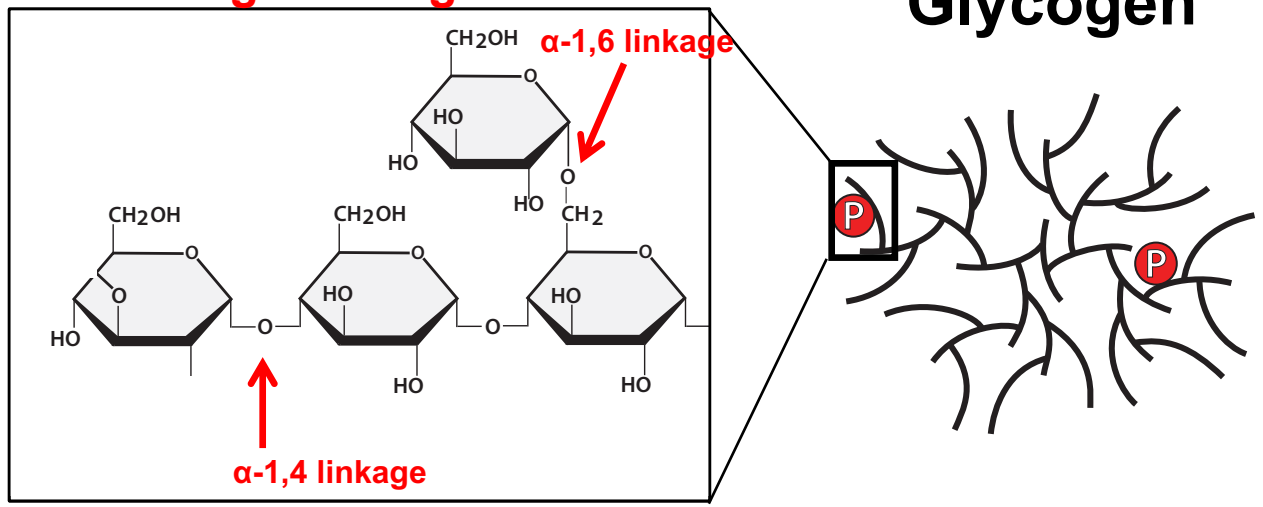



**Aberrant Glycogen Metabolism**

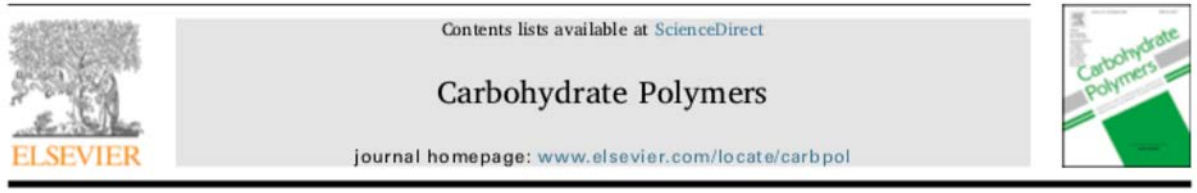
**Polyglucosan body (PGB) formation**

Gentry et al., 2005, *PNAS*
Worby/Gentry et al., *JBC*
Gentry et al., 2007, *JCB*
Gentry et al., 209 *TiBS*

**Chain length: ~13 glucose molecules**



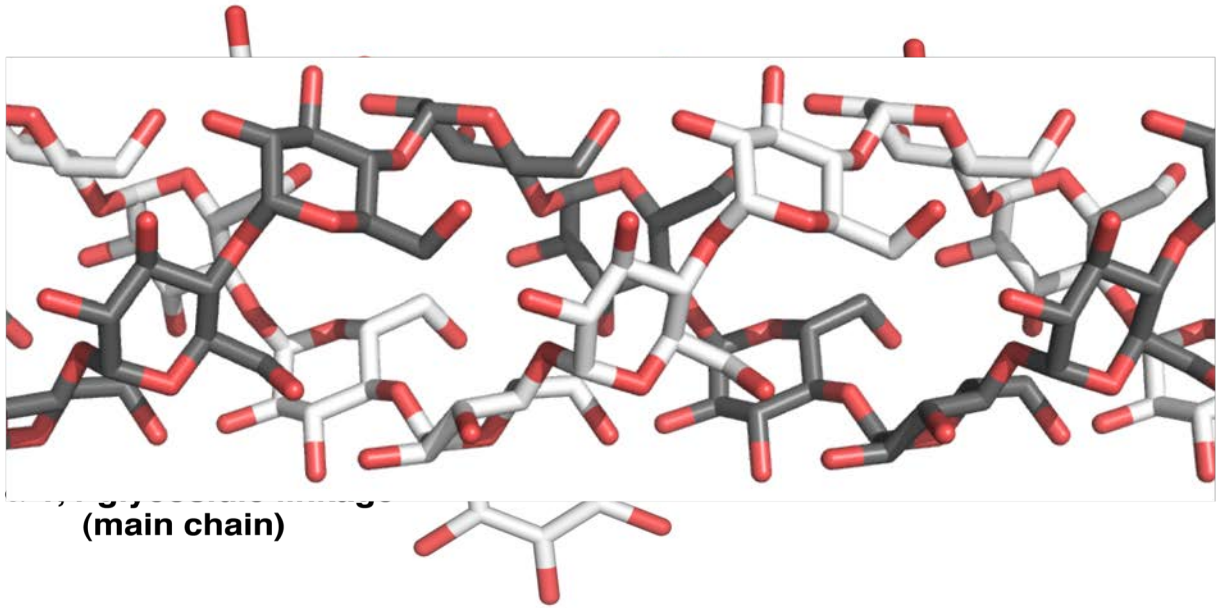

1. Degree of branching
2. Chain length distribution
3. Phosphate content (G2P, G3P and G6P)

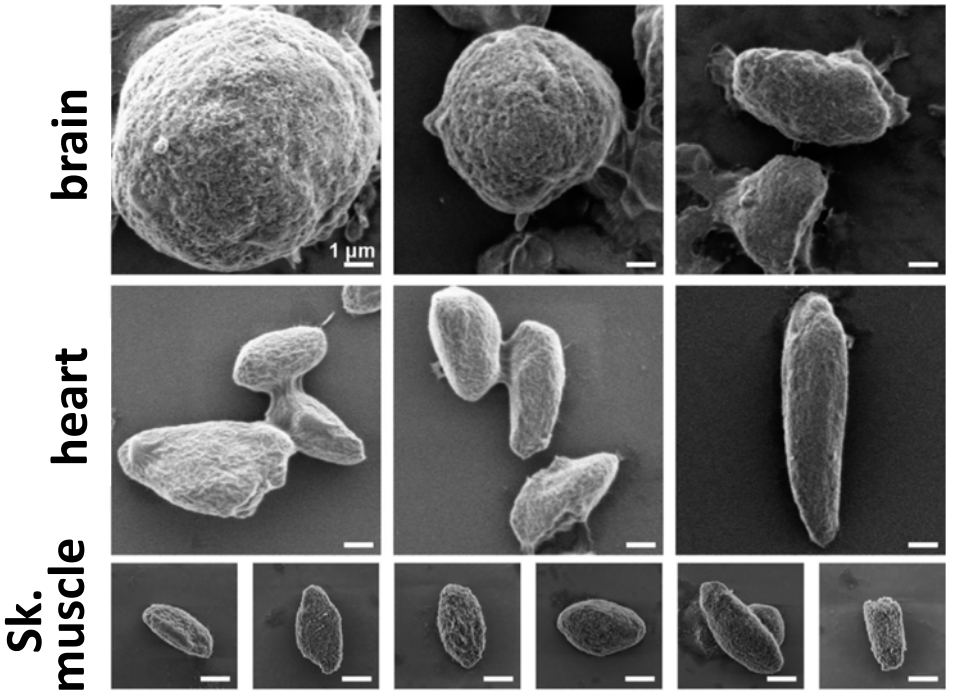


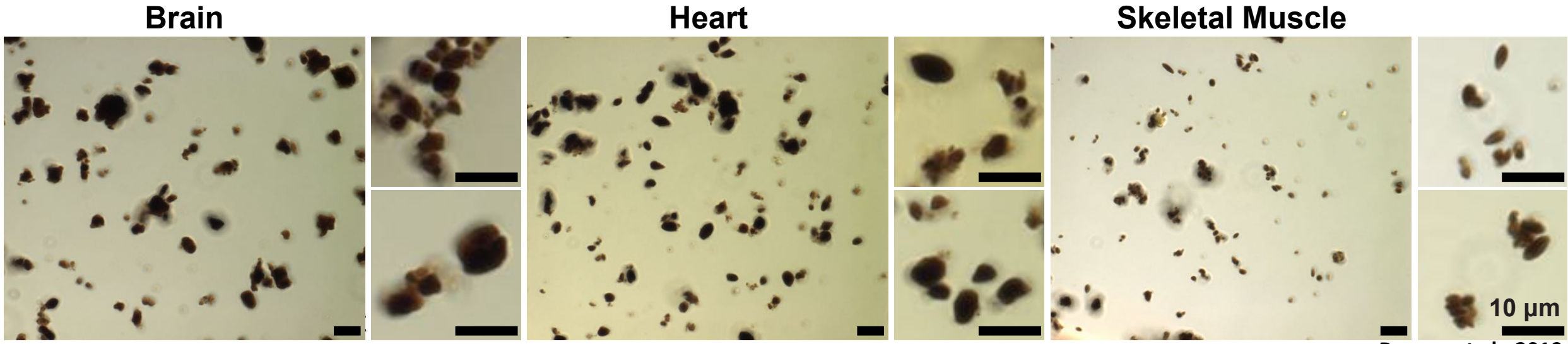

Accurate and sensitive quantitation of glucose and glucose phosphates derived from storage carbohydrates by mass spectrometry

Young et al., 2020
*Carb Polymers*

# Purification of native Lafora bodies (LBs)




Brewer et al., 2019
*Cell Metabolism*