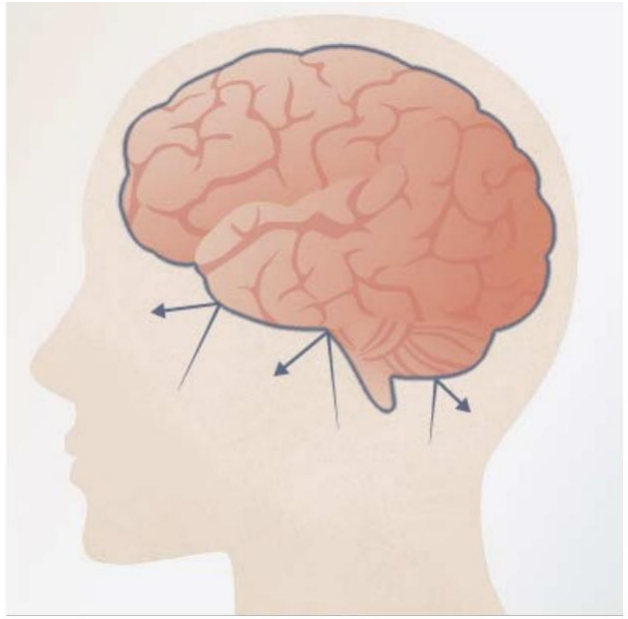
ETV:SGSH, a brain-penetrant enzyme transport vehicle for SGSH, corrects heparan sulfate accumulation, lysosomal lipid storage and inflammation in MPS IIIA mouse brain

Annie Arguello, PhD

Denali Therapeutics, Inc

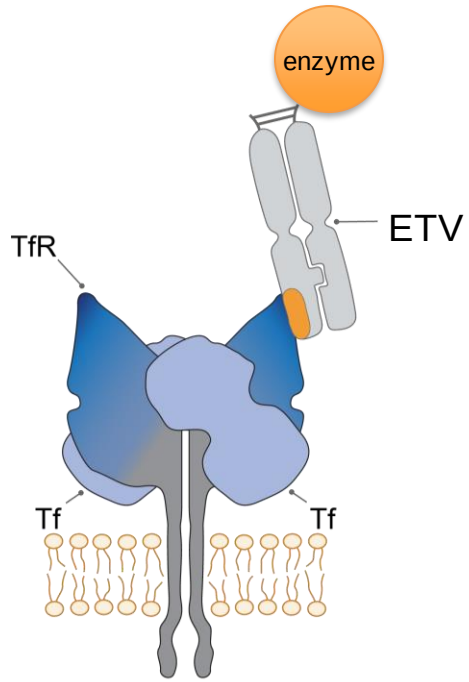
DENALI'S APPROACH TO ENZYME REPLACEMENT THERAPY

THE BLOOD-BRAIN BARRIER (BBB) CHALLENGE



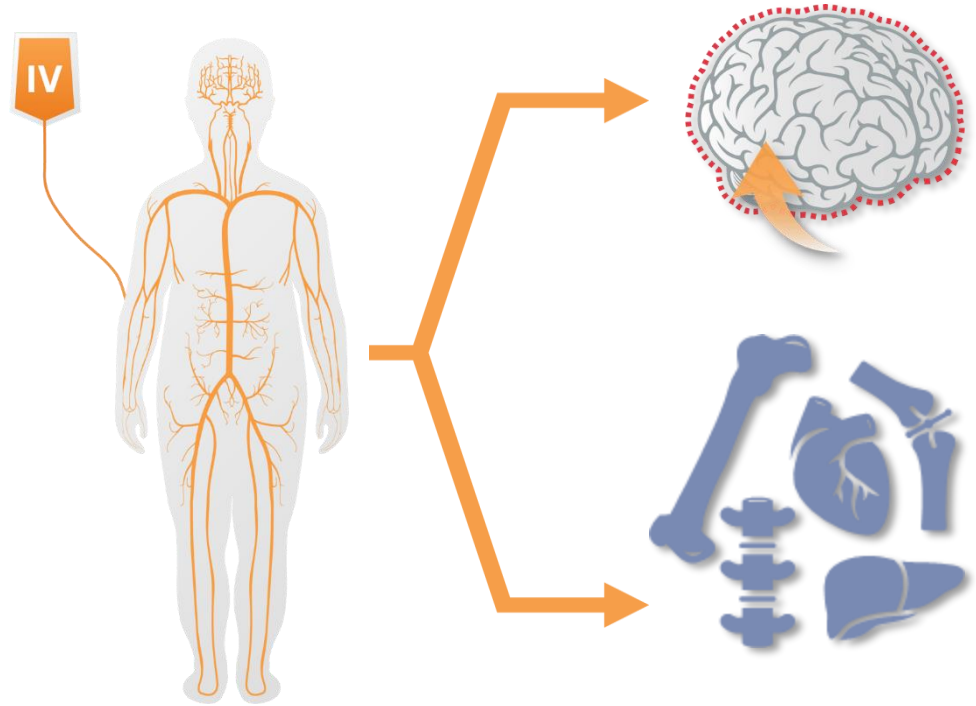
The BBB is a major obstacle for brain delivery of enzymes

ENZYME TRANSPORT VEHICLE (ETV)



- The ETV uses the Transferrin Receptor (TfR) to cross the BBB enzymes into the brain.
- The TfR is the body's mechanism for iron transport from blood into brain.

IV ADMINISTRATION AND BROAD BIODISTRIBUTION

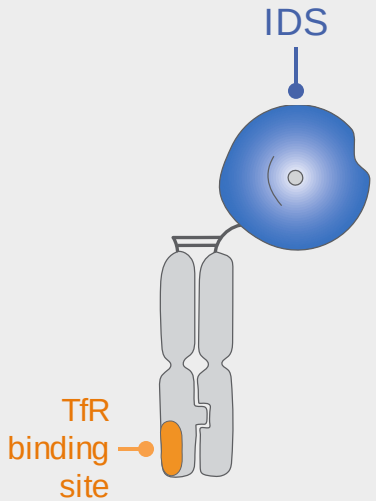


- There are many TfRs at the BBB, which enable transport of ETV and enzyme into the brain.
- TfRs may also help enzyme get into other tissues such as bone, cartilage, and the heart.

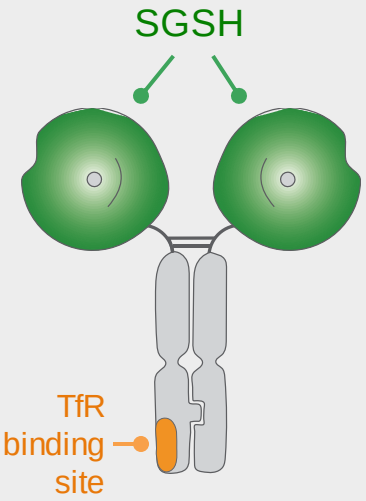
References: Jefferies WA, et al., 1984; Qian ZM, et al., 2002; Bakardjiev AI, 2021; Arguello A et al., 2021; Arguello A, Mahon CS et al., 2022; Ullman JC, et al., 2020; Wang S, et al., 2020; Gammella E, et al., 2017; Carlevaro MF, et al., 1997.

LEARNINGS FROM MPS II ADVANCE MPS IIIA EARLY EFFORTS

MPS II
DNL310 (ETV:IDS)



MPS IIIA
DNL126 (ETV:SGSH)

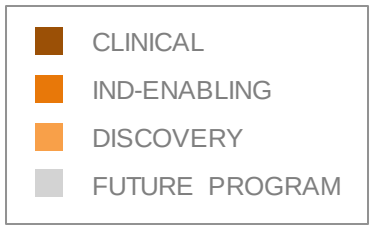


Both molecules utilize ETV platform designed for optimal brain delivery via TfR-mediated transcytosis

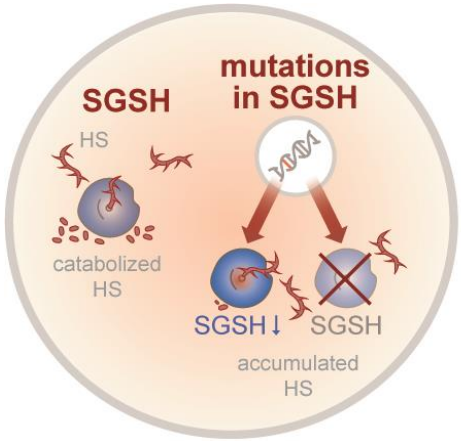
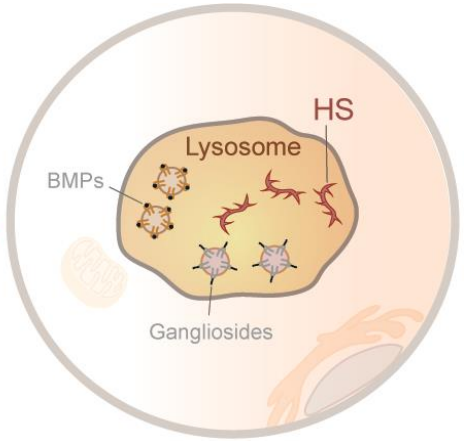
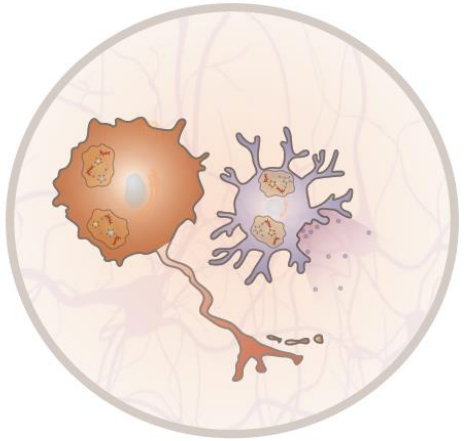
Path Forward

- Expanded portfolio of ETV programs
- Advance the development of DNL126 (ETV:SGSH), a novel brain-penetrant enzyme replacement therapy for MPS IIIA

ETV Portfolio



MPS IIIA PATHOGENESIS AND BIOMARKERS

TARGET	PATHWAY		CLINICAL
Genetic defect with loss of SGSH enzyme activity	**SGSH deficiency impairs lysosomal function**	**Lysosomal dysfunction causes inflammation and cell loss**	**Clinical Disease**
			
Accumulation of Heparan Sulfate (HS)	*Accumulation of lysosomal lipids (e.g. Gangliosides) and lysosomal markers (e.g. LAMP2)*	*Increased inflammatory markers (e.g. CD68, GFAP, YKL-40)*	*Developmental delay, decline in cognition and adaptive behavior*

Currently, there are no approved therapies for MPS IIIA, representing a high unmet medical need

PERIPHERAL ADMINISTRATION OF ETV:SGSH RESULTS IN DOSE DEPENDENT INCREASES IN SERUM AND BRAIN SGSH EXPOSURE

Mouse models

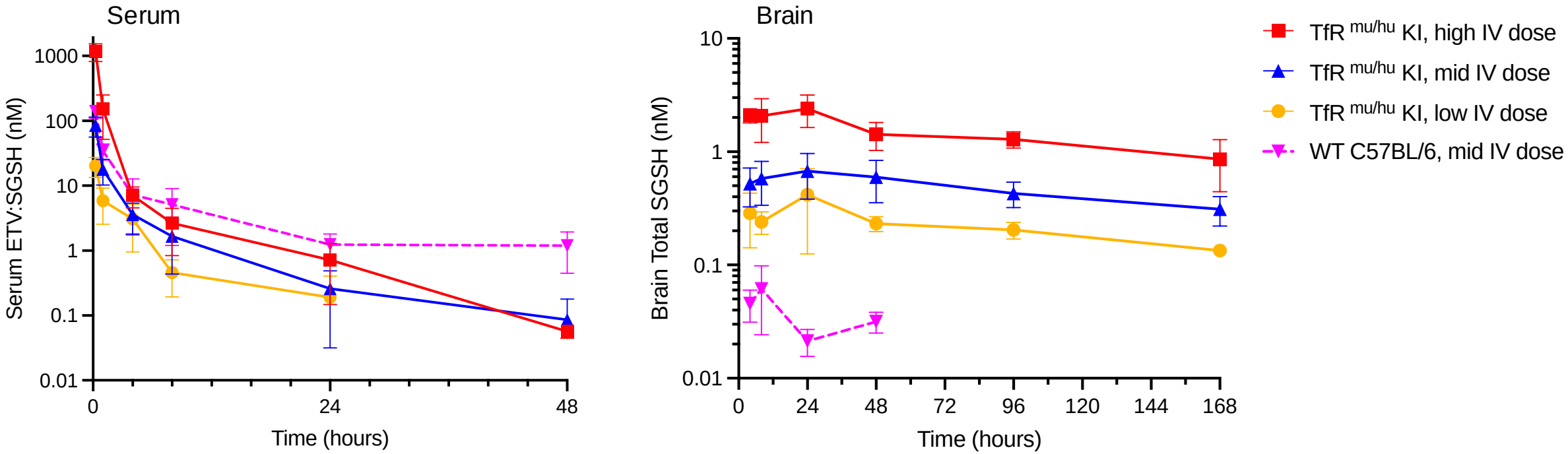
WT mice = wild-type C57Bl/6J

TfR$^{mu/hu}$ KI mice = harbors human TfR1 apical domain knocked into the mouse TfR1, resulting in a chimeric transferrin receptor

Study Design

Single IV dose of ETV:SGSH

Serum timepoints: 0.25, 1, 4, 8, 24, and 48 hours
Brain timepoints: 4, 8, 24, 48, 96, and 168 hours



Fusion of SGSH enzyme to the ETV improves delivery to the brain

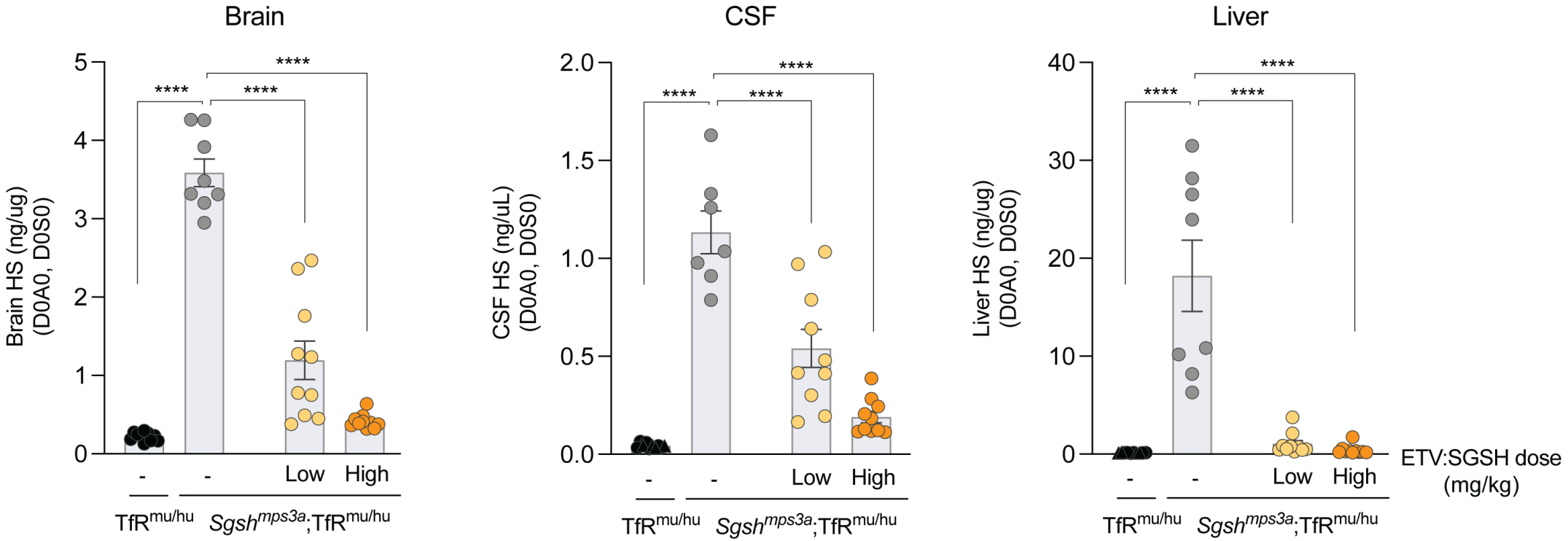
PERIPHERAL ADMINISTRATION OF ETV:SGSH REDUCES BRAIN, CSF, AND LIVER HS LEVELS IN A MPS IIIA MOUSE MODEL

Mouse models

$TfR^{mu/hu}$ = non-disease control

$Sgsh^{mps3a}$; $TfR^{mu/hu}$ = disease model of MPS IIIA; reduced sulfamidase activity (3-4% of WT levels) and harbors a chimeric transferrin receptor

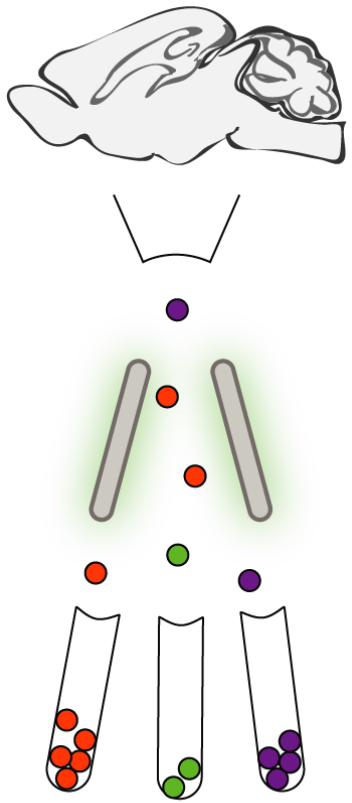
Study Design



ETV:SGSH delivers functional SGSH to the CNS and periphery

*Graphs display mean ± SEM and p values: one-way ANOVA with Tukey's multiple comparison test; ****p < 0.0001*

ETV:SGSH REDUCES HS LEVELS IN NEURONS, ASTROCYTES, AND MICROGLIA IN THE BRAIN PARENCHYMA OF MPS IIIA MICE



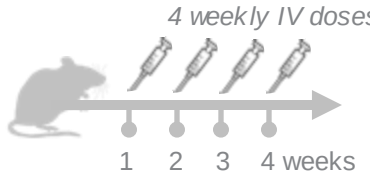
Whole brain digestion

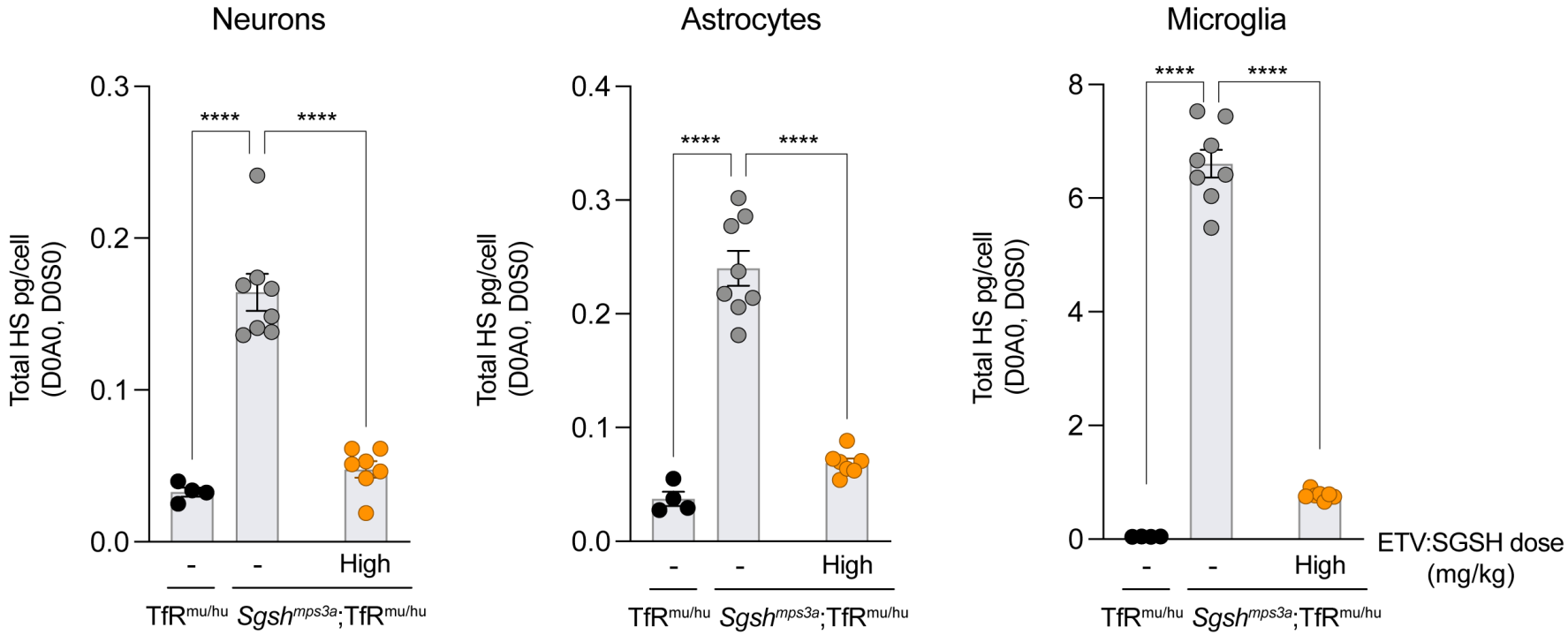
↓

Cell populations isolated by FACS

↓

HS analysis

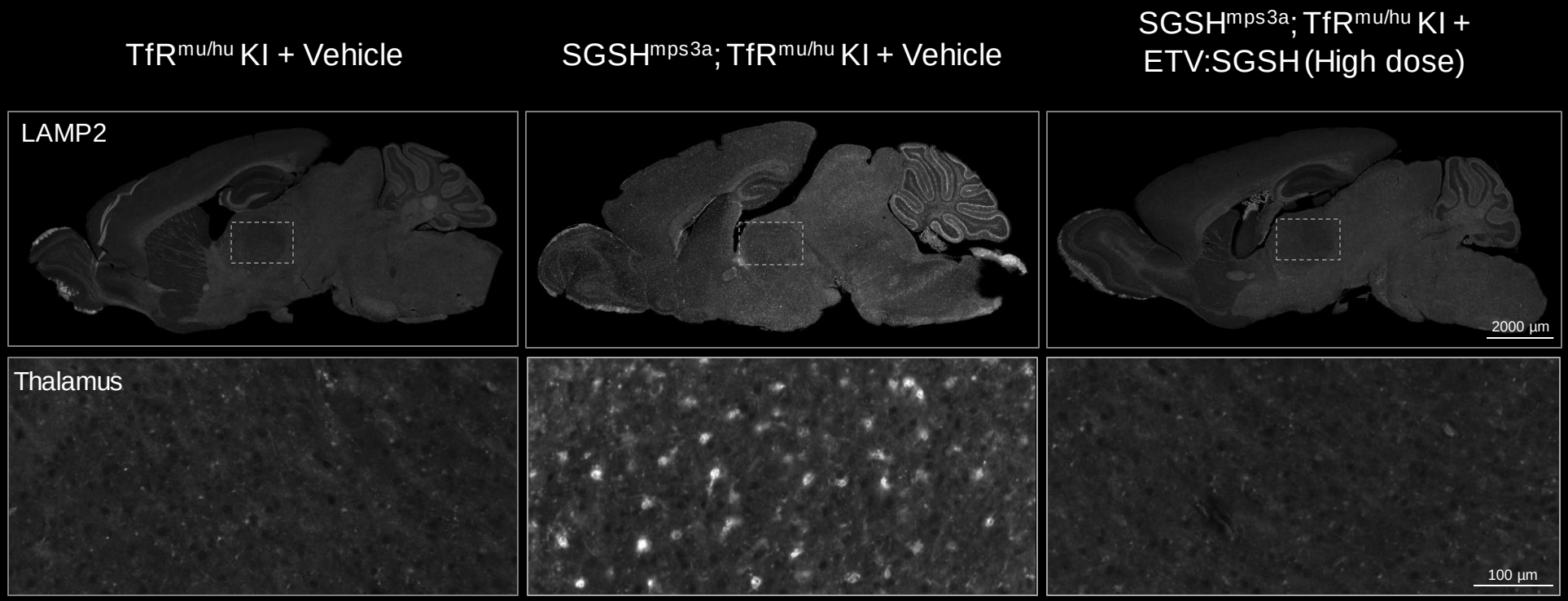


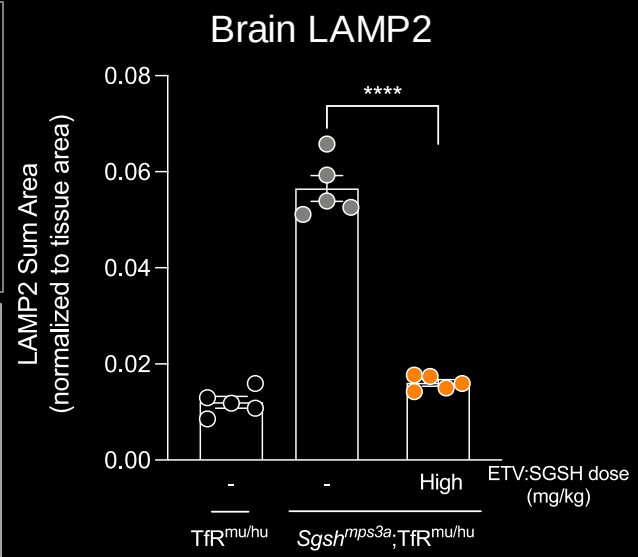


Peripheral administration of ETV:SGSH achieves broad distribution of functional enzyme to brain cells

*Graphs display mean ± SEM and p values: one-way ANOVA with Dunnett's multiple comparison test; ****p < 0.0001*

ETV:SGSH CORRECTS LAMP2 STAINING IN BRAIN OF MPS IIIA MICE

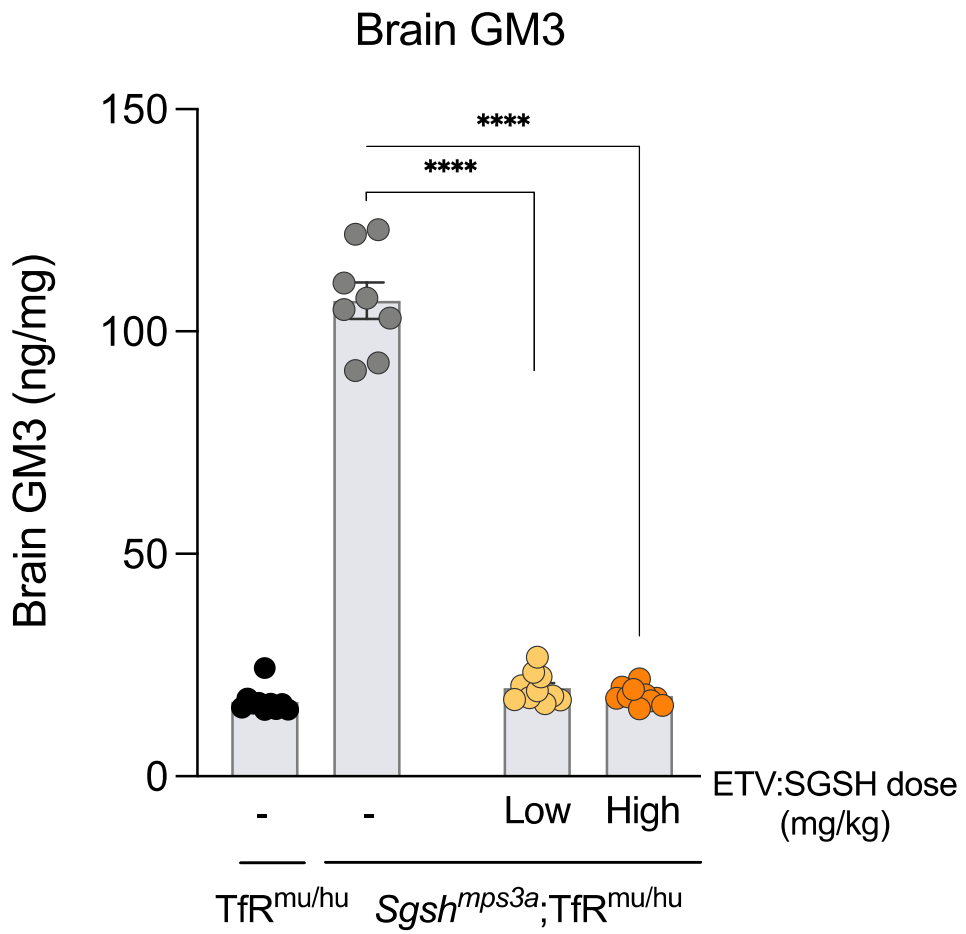




ETV:SGSH corrects lysosomal proteins in the brain, suggesting improved lysosome function

*Graphs display mean ± SEM and p values: one-way ANOVA with Tukey's multiple comparison test; ****p <0.0001*

ETV:SGSH CORRECTS GANGLIOSIDE GM3 LEVELS IN BRAIN OF MPS IIIA MICE



ETV:SGSH corrects lysosomal lipids in the brain, consistent with improved lysosome function

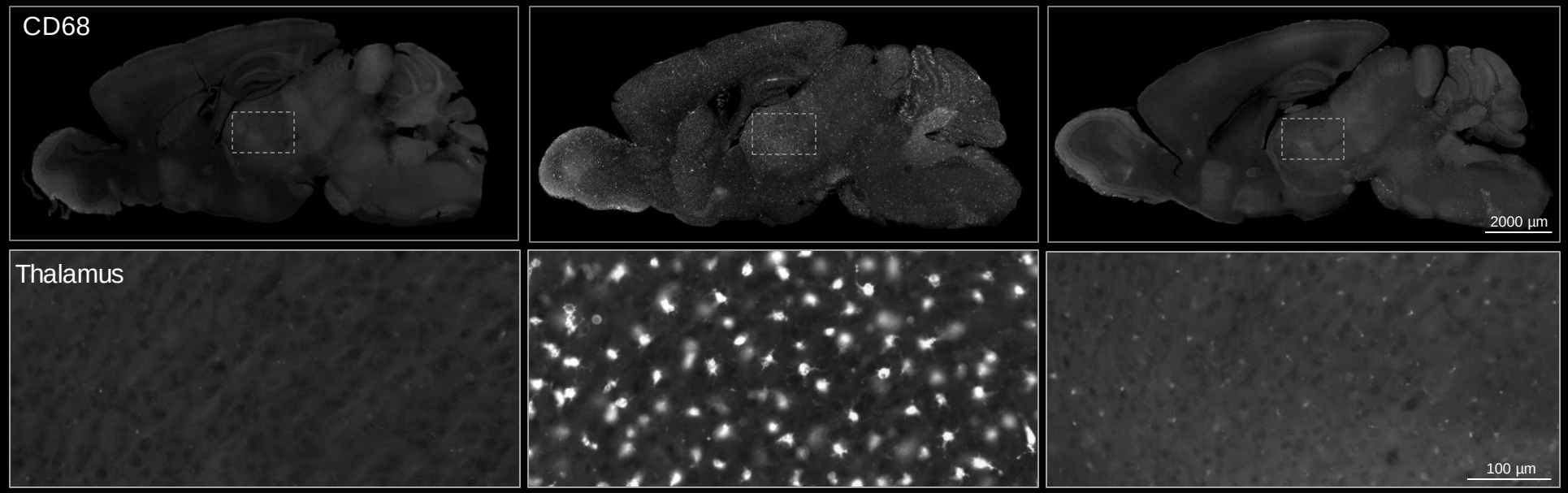
*Graphs display mean ± SEM and p values: one-way ANOVA with Tukey's multiple comparison test; ****p <0.0001*

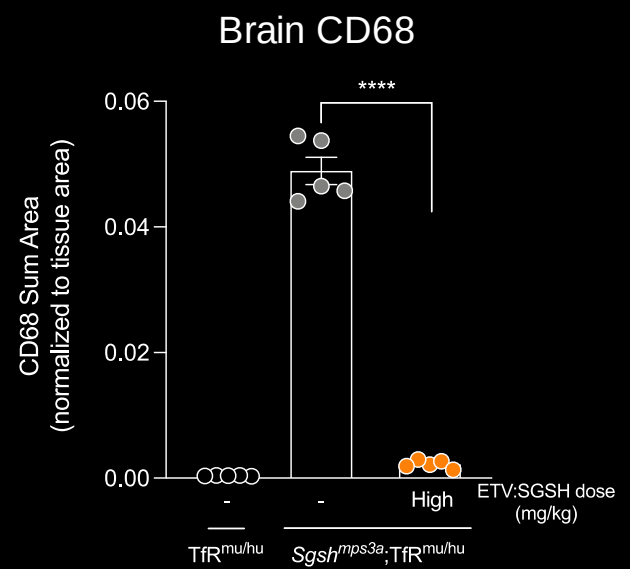
ETV:SGSH CORRECTS CD68 STAINING IN BRAIN OF MPS IIIA MICE

TfR$^{mu/hu}$ KI + Vehicle

SGSH^{mps3a}; TfR$^{mu/hu}$ KI + Vehicle

SGSH^{mps3a}; TfR$^{mu/hu}$ KI + ETV:SGSH (High dose)

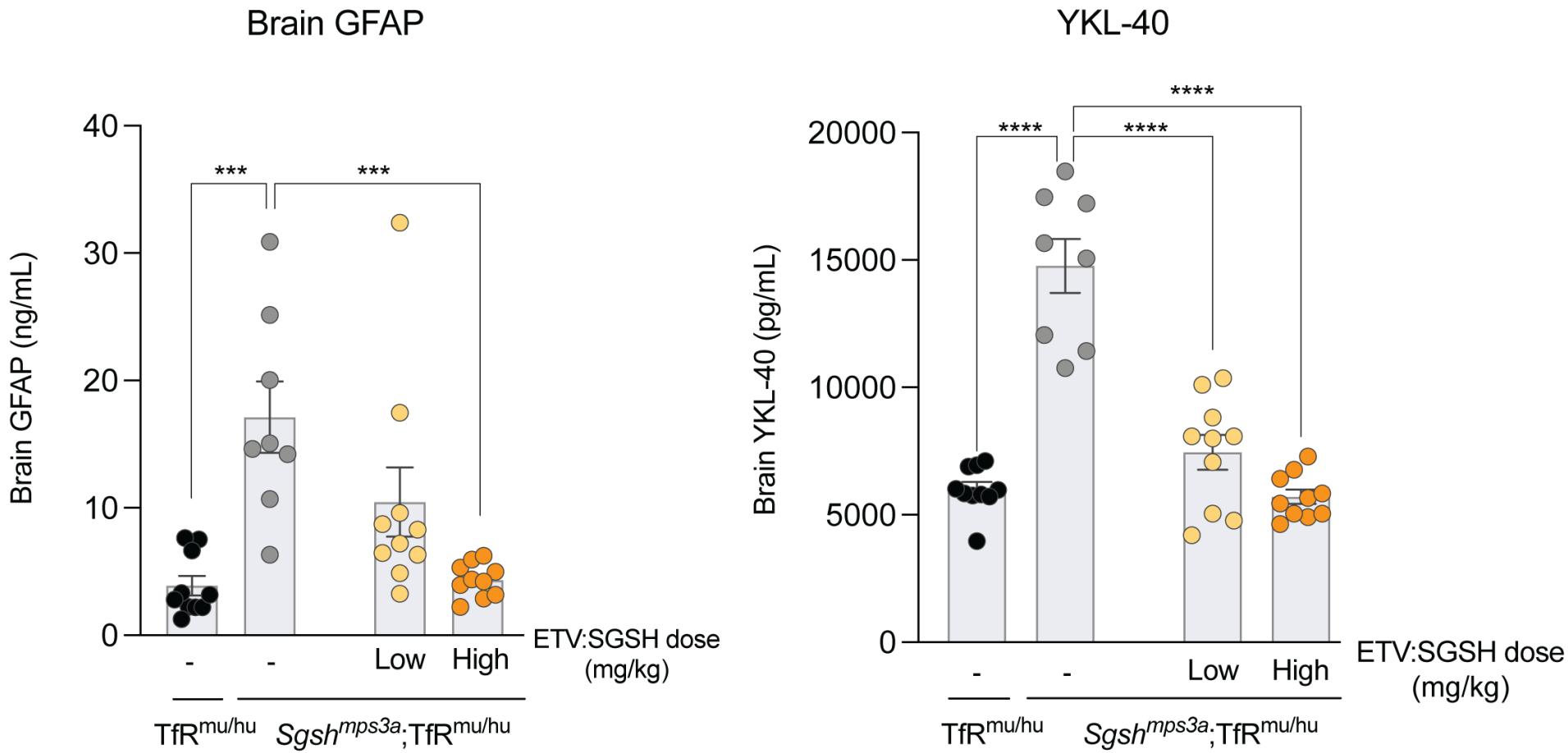




ETV:SGSH corrects neuroinflammation in the brain

*Graphs display mean ± SEM and p values: one-way ANOVA with Tukey's multiple comparison test; ****p <0.0001*

ETV:SGSH CORRECTS GFAP AND YKL-40 IN BRAIN OF MPS IIIA MICE

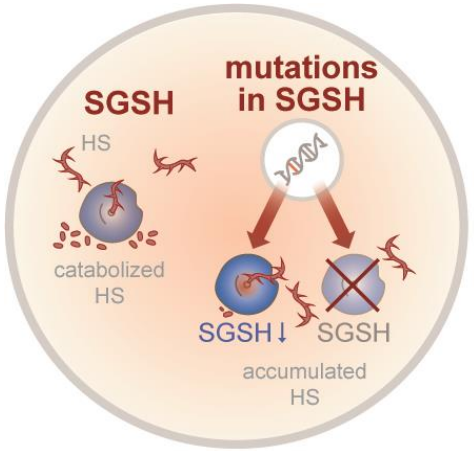


ETV:SGSH corrects neuroinflammation in the brain

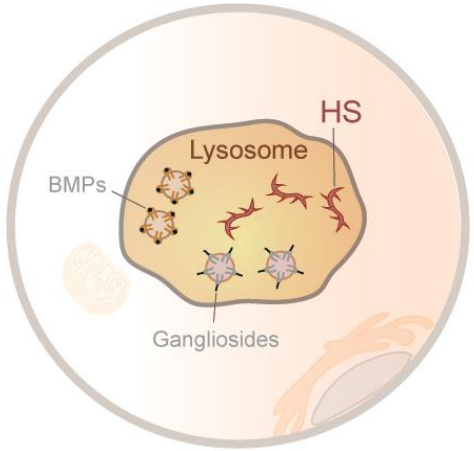
*Graphs display mean ± SEM and p values: one-way ANOVA with Tukey's multiple comparison test; ***p < 0.001 and ****p < 0.0001*

ETV:SGSH CORRECTS SUBSTRATE ACCUMULATION, LYSOSOMAL FUNCTION AND INFLAMMATORY MARKERS IN A MPS IIIA MOUSE MODEL

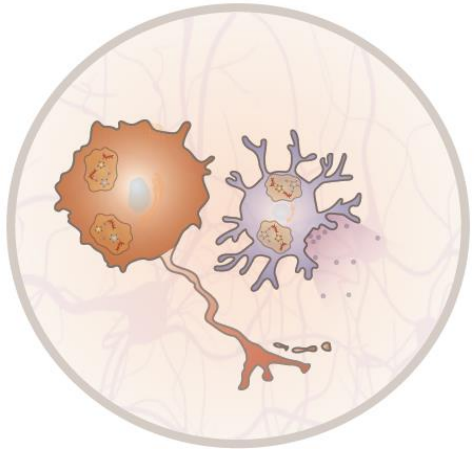
Genetic defect with loss of SGSH enzyme activity



SGSH deficiency impairs lysosomal function



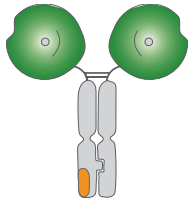
Lysosomal dysfunction causes inflammation and cell loss



MPS IIIA Disease

MPS IIIA mouse model

ETV:SGSH effects in MPS IIIA mice



SUBSTRATE ACCUMULATION	LYSOSOMAL FUNCTION		NEUROINFLAMMATION		
HS reduction in brain, CSF, liver and CNS cell types	Normalization of LAMP2 staining in brain	GM3 lipid normalization in brain	Normalization of CD68 staining in brain	GFAP normalization in brain	YKL-40 normalization in brain
✓	✓	✓	✓	✓	✓

ACKNOWLEDGEMENTS

DENALI THERAPEUTICS
and entire ETV:SGSH Project Team

Shababa T. Masoud, Mohammad Jafarnejad, Hoang N. Nguyen, Buyankhishig Tsogtbaatar, Alexander Seay, Elliot R. Thomsen, Kensuke Yamanokuchi, Oliver Davis, Katie Sokolowski, Lakshman Annamalai, Candice Herber, Aaron Chesterman, Meng Fang, Cathal Mahon, Jonathan Gal, Robert Thorne, Heather Hogg, Dolo Diaz, Anna Bakardjiev, Kirk R. Henne, Pascal E. Sanchez, Kimberly Scearce-Levie, Carole Ho, Ryan J. Watts

Denali Therapeutics Inc. has filed patent applications related to the subject matter
ETV:SGSH (DNL126) is an investigational treatment and is not approved by any Health Authority