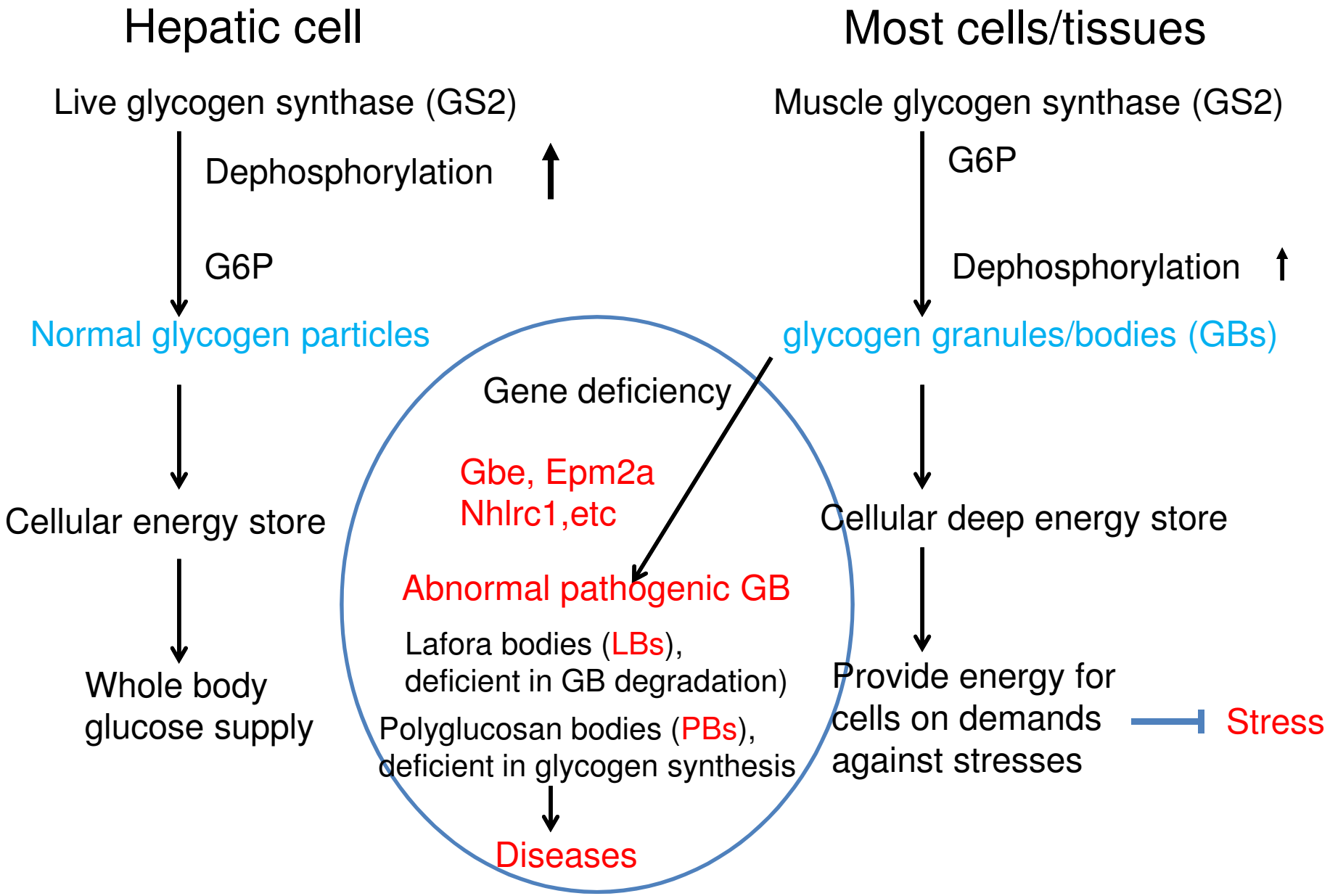
Glycogen body metabolism is required for neurons against stresses

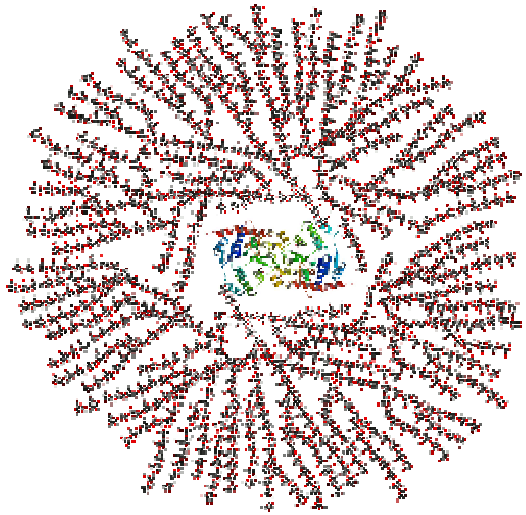
Yan Liu, PhD
Research Assistant professor

Center for Cancer and Immunology Research, Children's National Medical Center and George Washington University, Washington, DC 20010, USA.

Glycogen body (GB) metabolism in mammalian cells

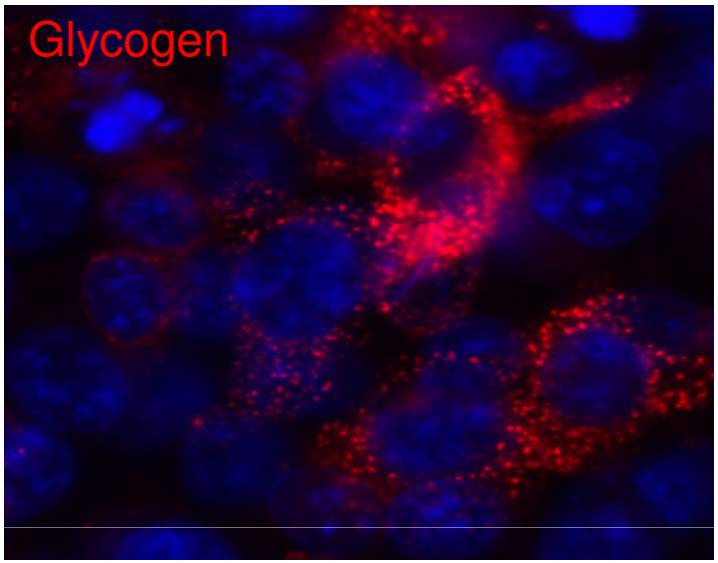


Difference between normal glycogens and GBs

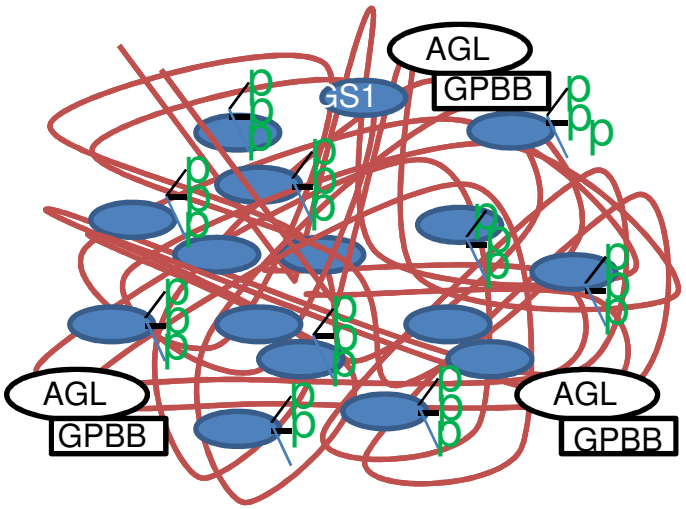


Soluble glycogen particle

Liver glycogen synthase (GS2)

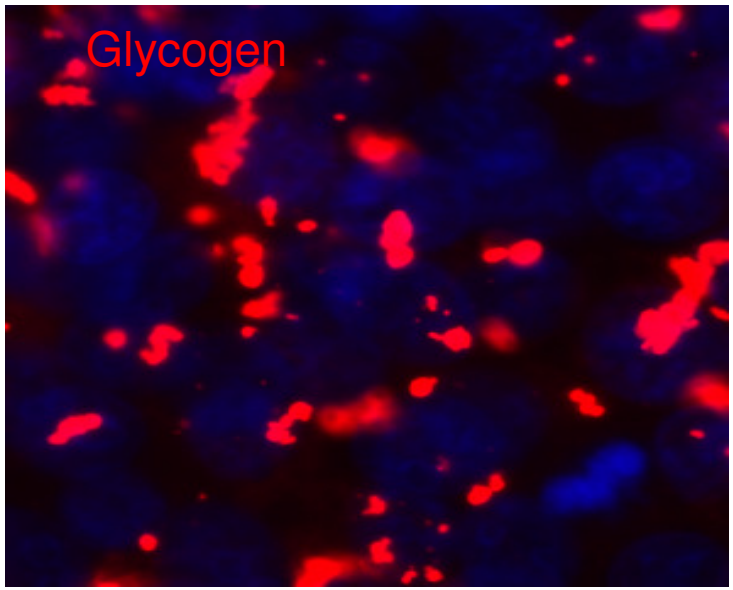


Normal glycogens



Insoluble glycogen body

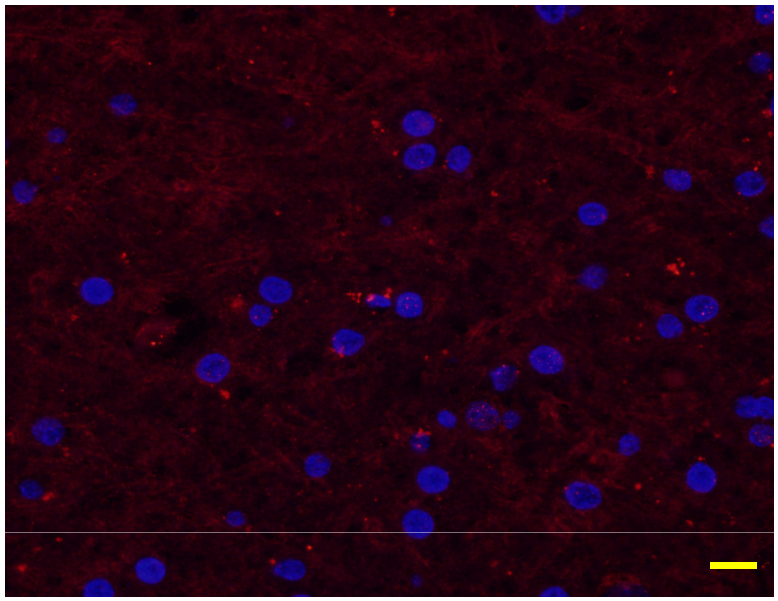
Muscle glycogen synthase (GS1)



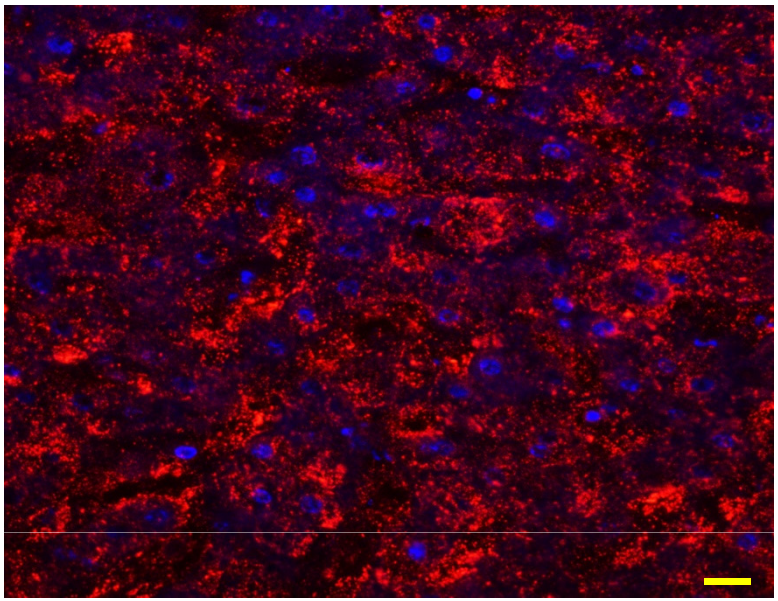
Glycogen bodies

Glycogen bodies exist in normal tissues of human

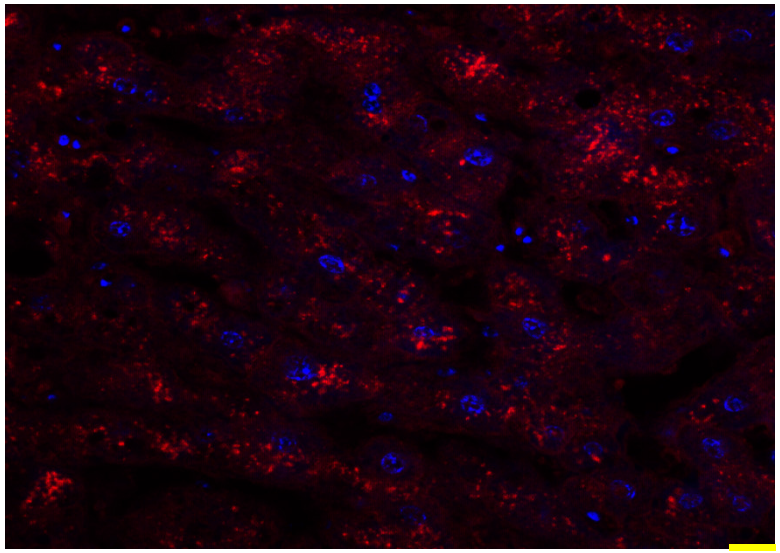
Normal brain of human/glycogen



Normal liver of human/glycogen



Normal liver of human/GPBB



The GPBB-binding glycogen bodies may act as a deep glycogen store to supply energy glucose under stress conditions that increase cellular AMP.

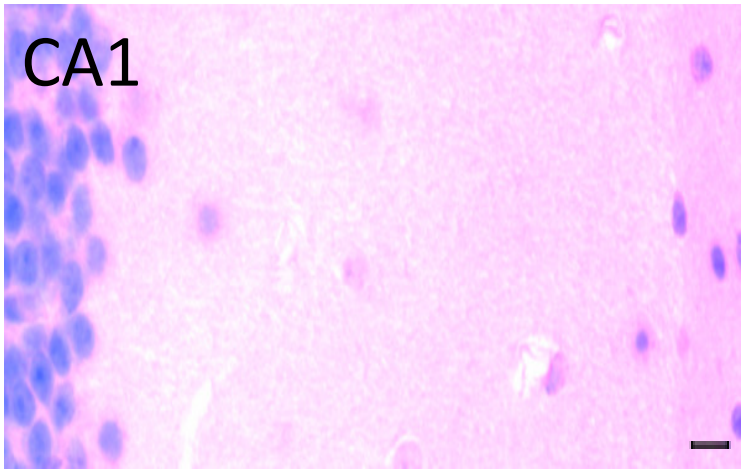
What does LB look like

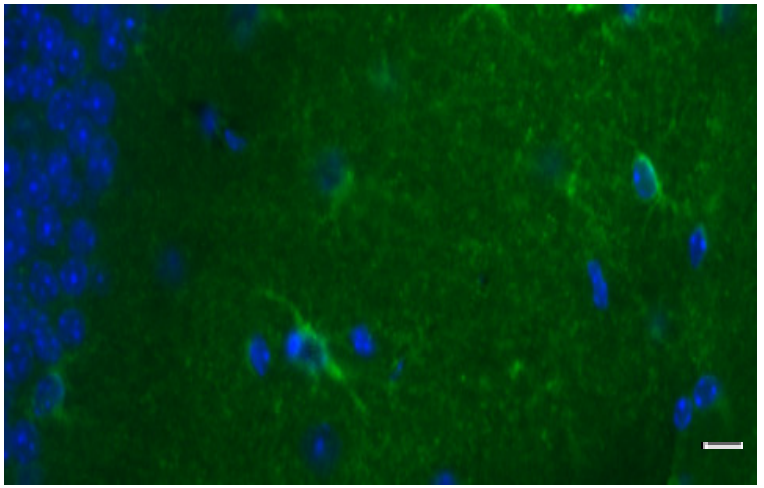
PAS (Periodic acid Schiff Stain)

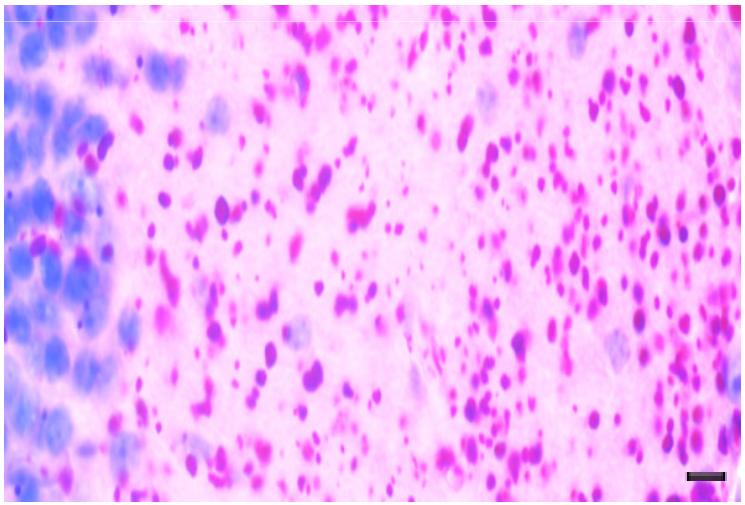
pS641/645 GS1

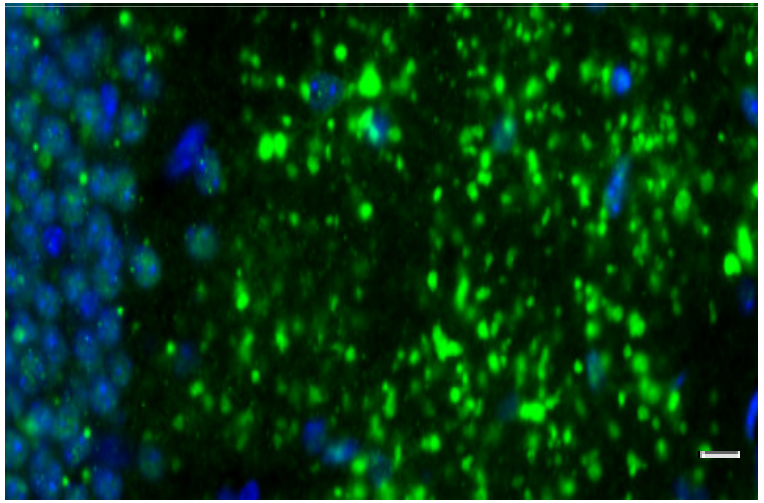
WT
(9 Mon)

Epm2a KO
(9 Mon)



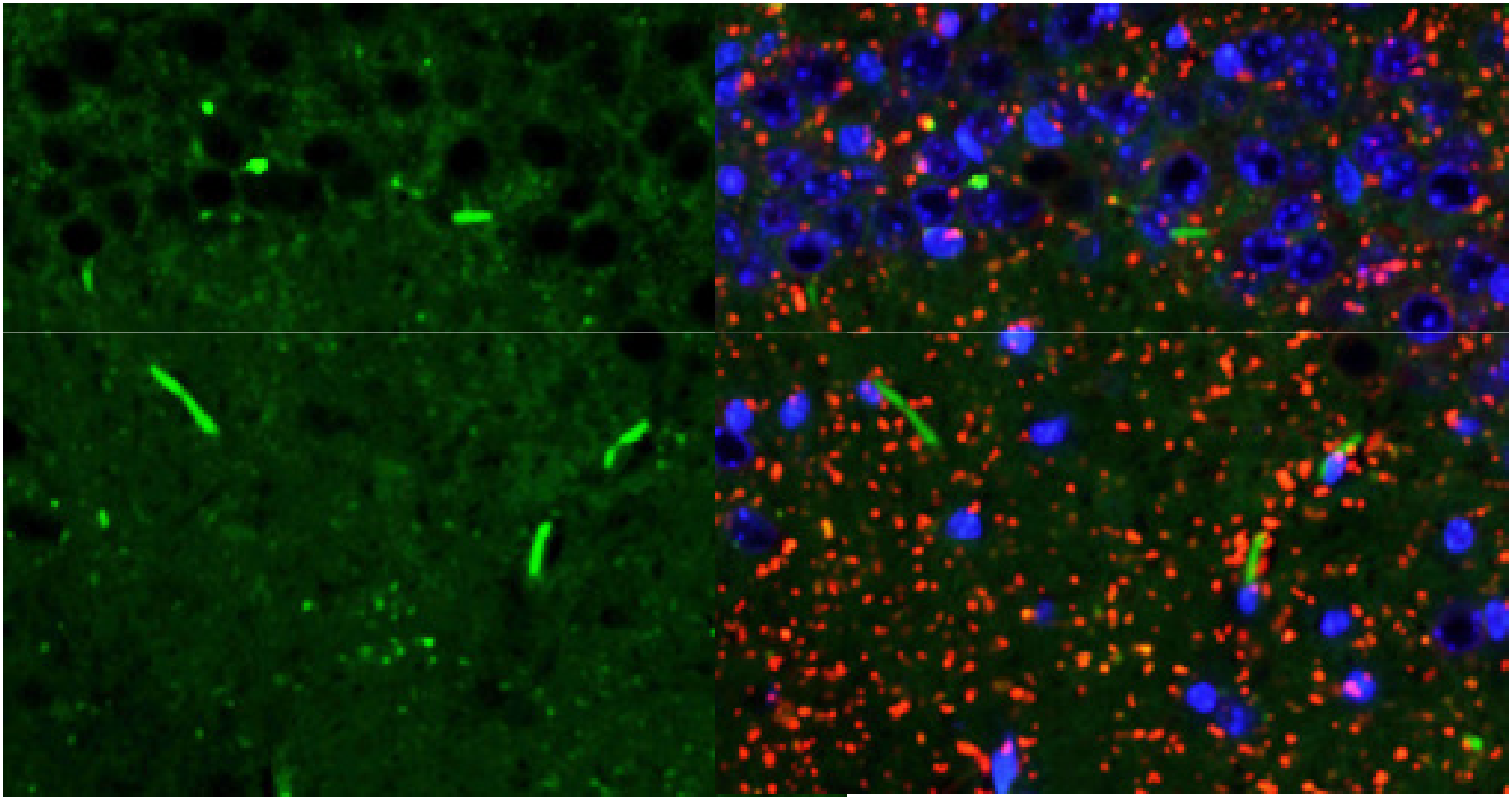






Polyglucosan bodies (PBs) of Gbe-deficient mice

Hippocampus/ CA3 pS641/645 GS1 PBs /pS641/645 GS1 / DAPI

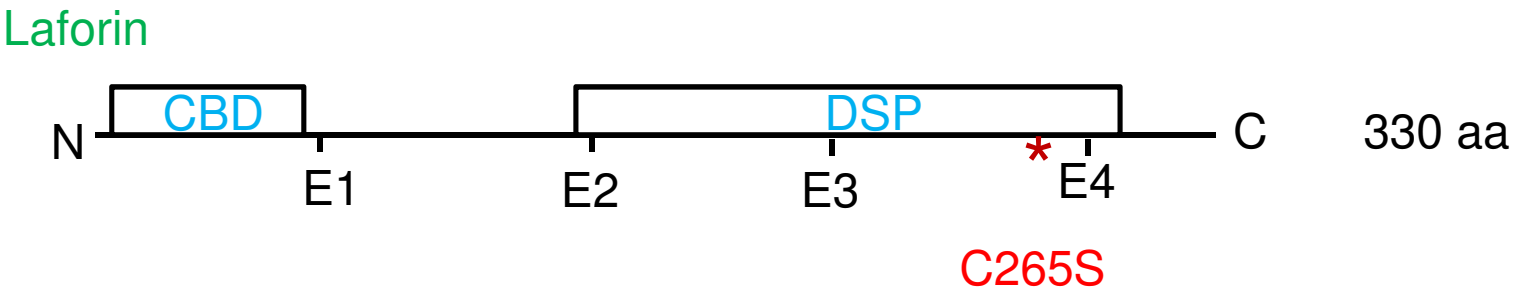


EPM2A and Laforin

EPM2A, Epilepsy, Progressive Myoclonus Type 2, encodes the dual specificity phosphatase (DSP) called Laforin.

EPM2A is located in chromosome 6q24 in human and 10 in mouse. Loss of heterozygosity (LOH) at 6q22-27 in human occurs at a very high frequency in cancers.

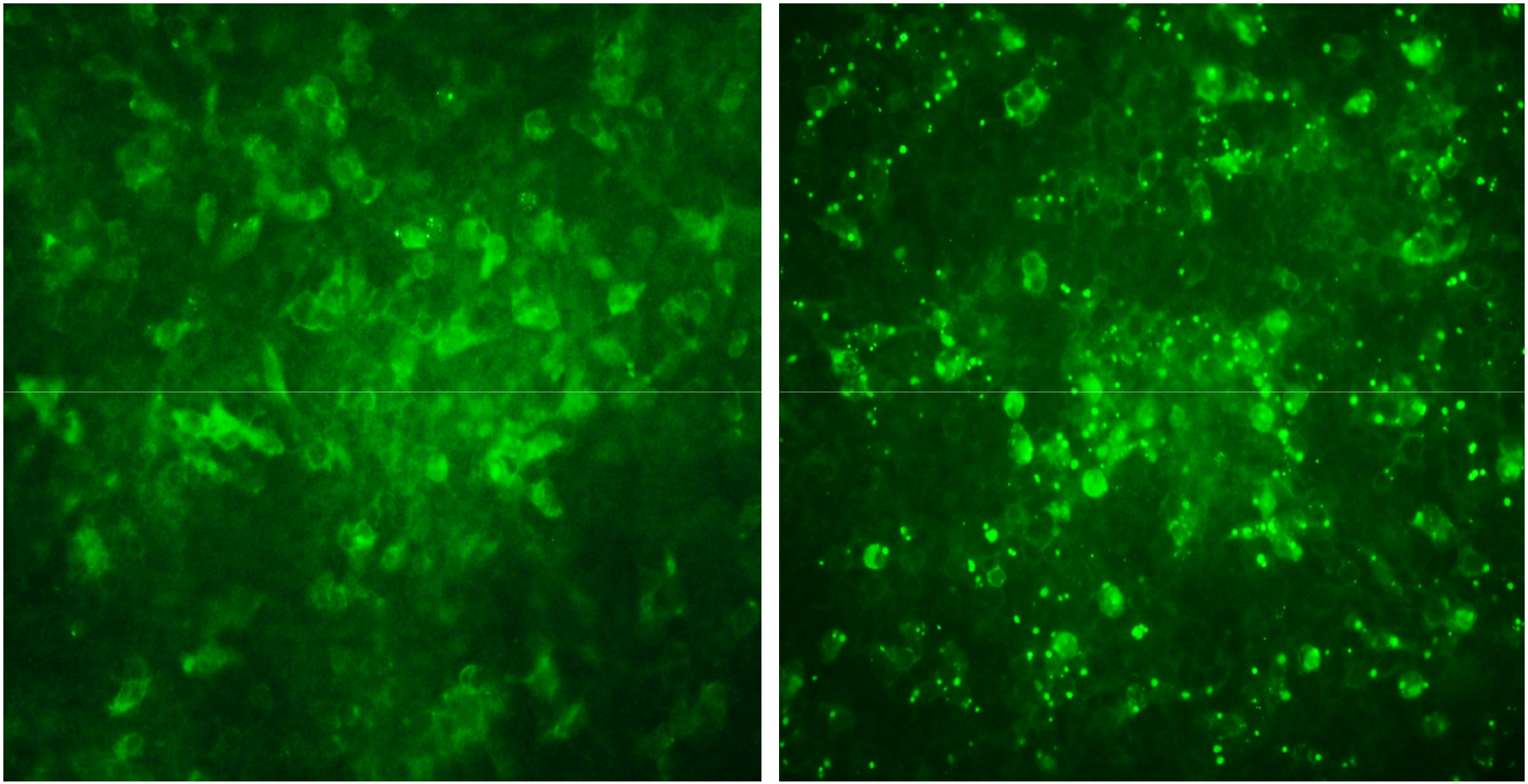
Laforin is comprised of carbohydrate binding domain (CBD) and DSP domain



Laforin is a phosphatase of GBs

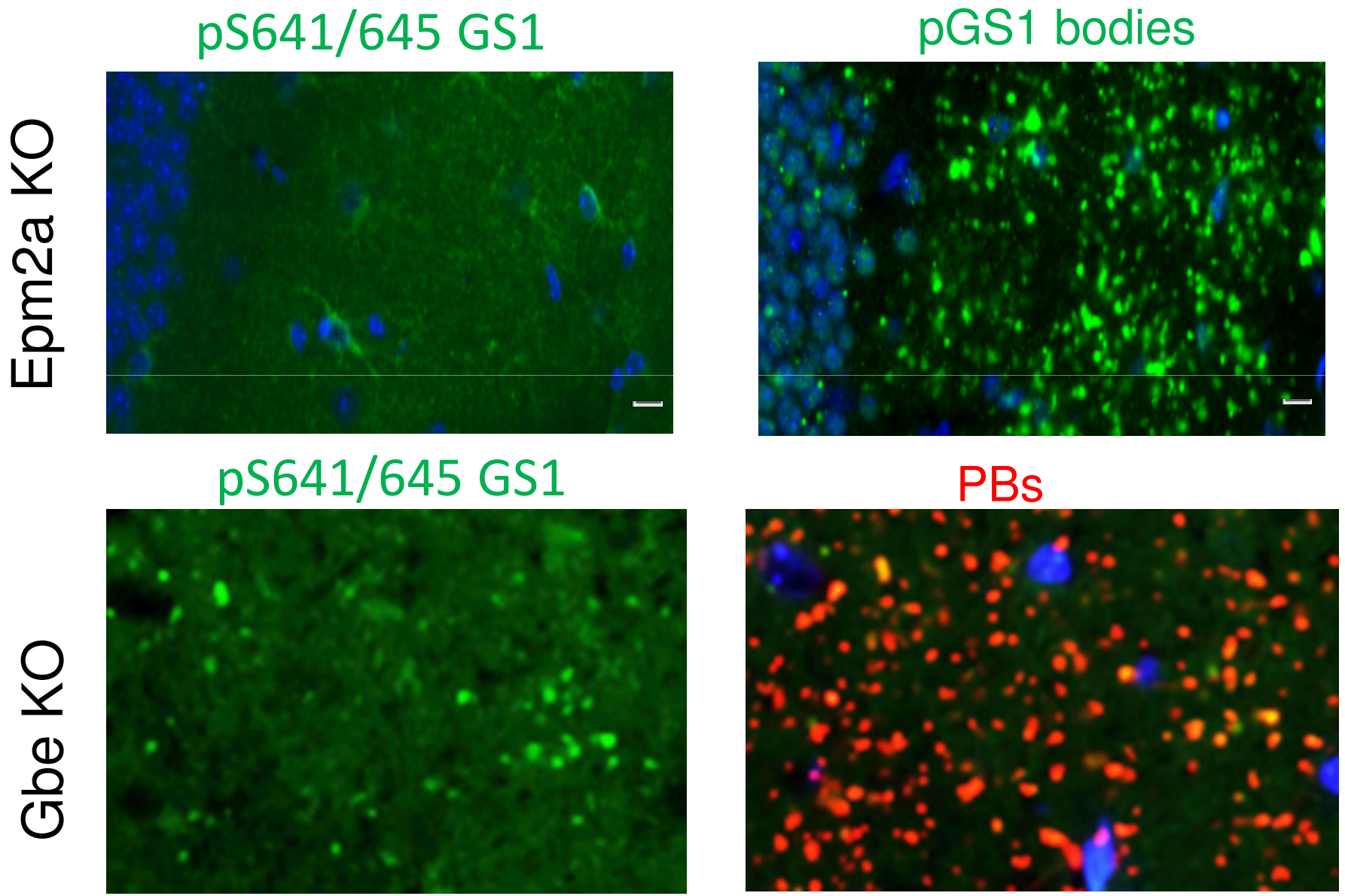
Laforin-HEK293, pS641GS1

C265S-HEK293, pS641GS1

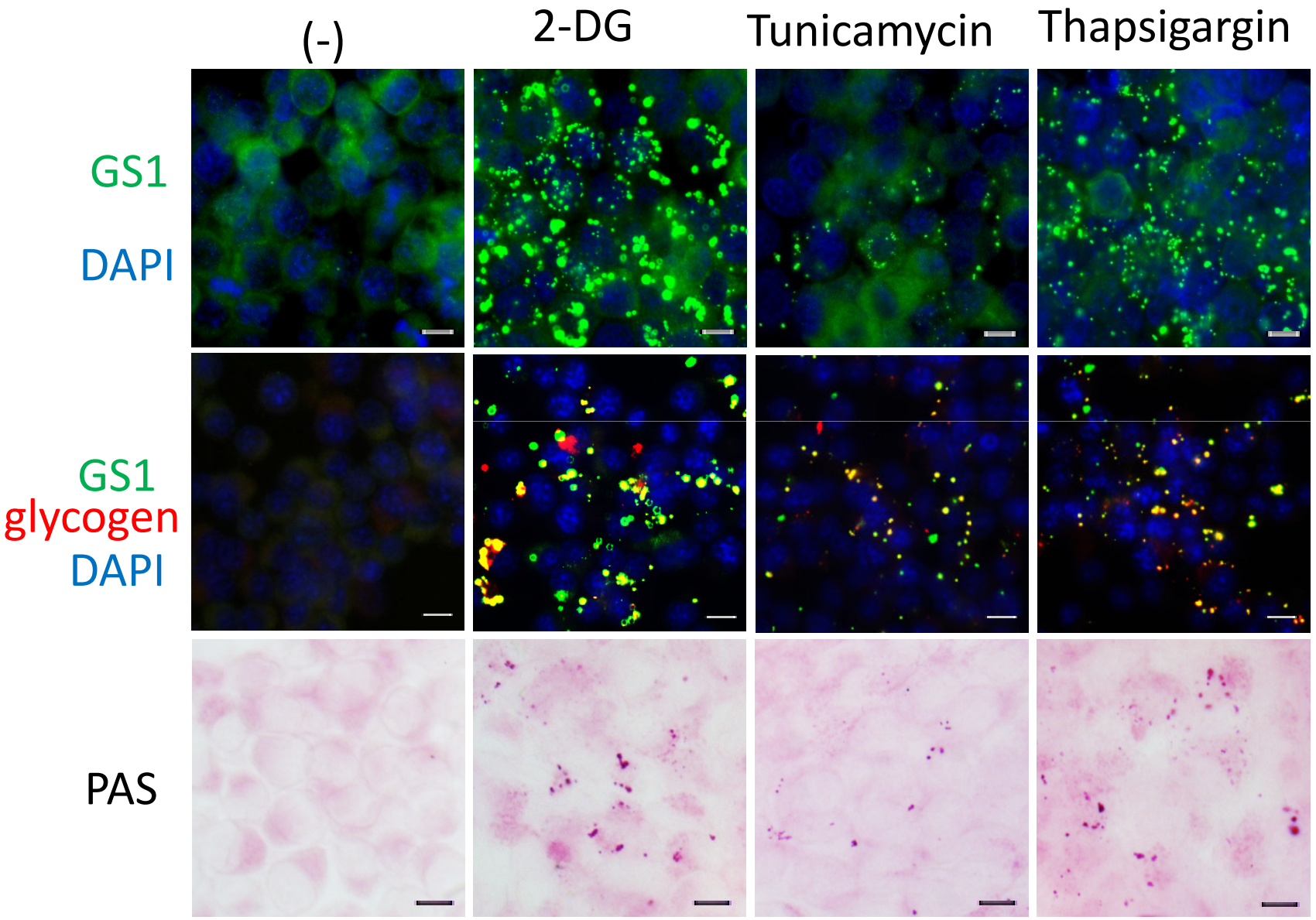


HEK293 cells were transfected with laforin or with its phosphatase-dead mutant C265S for 36h before being stained with antibody of phosphor-S641GS1.

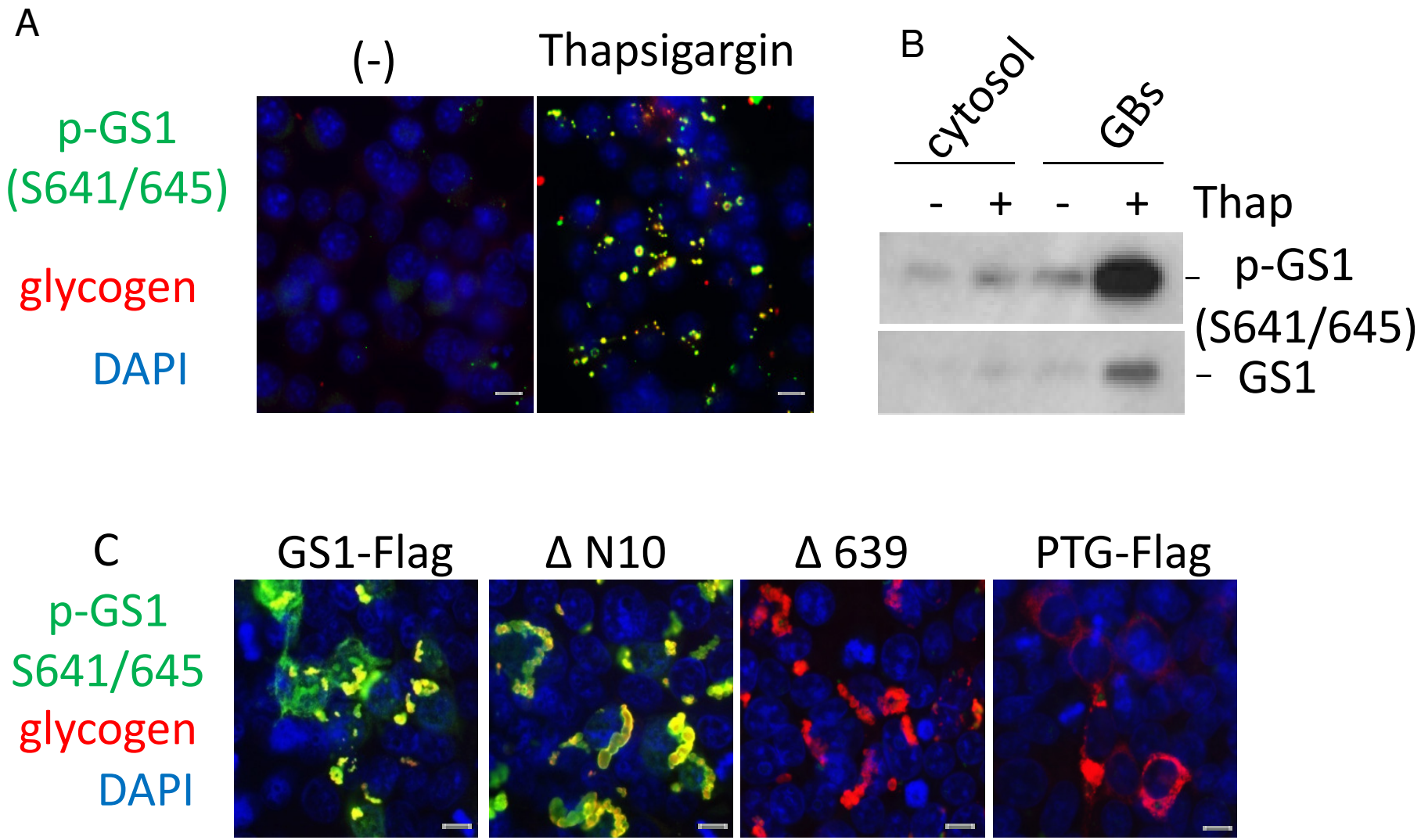
Epm2a knockout causes the accumulation of pS641GS1 bodies.



ER stress induces glycogen bodies in Neuro2a cells

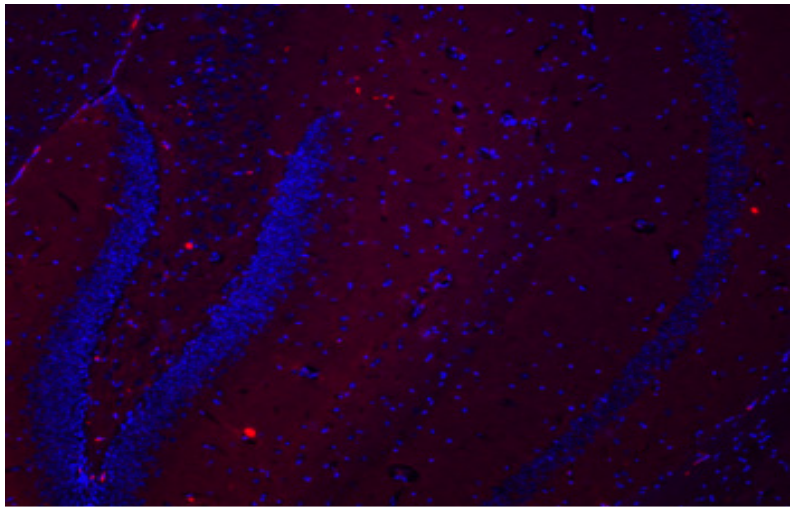


GS1 is phosphorylated at S641/645 by GSK3

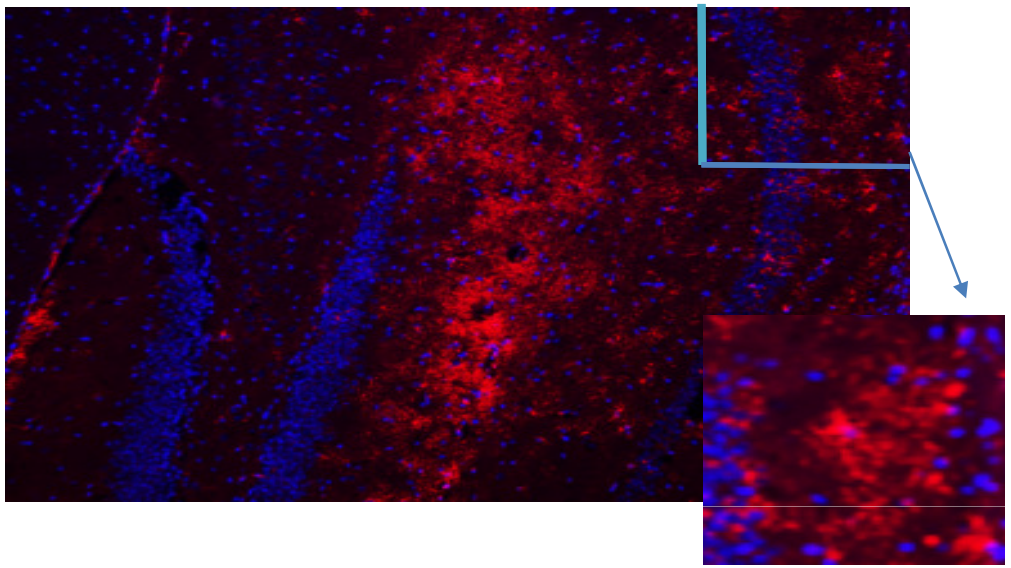


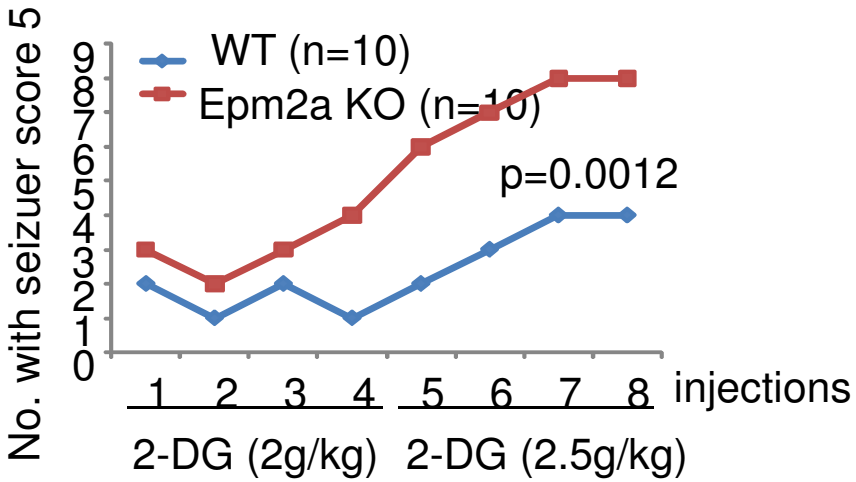
GBs are induced in WT neurons by energy deprivation stress

WT/ Glycogen / DAPI

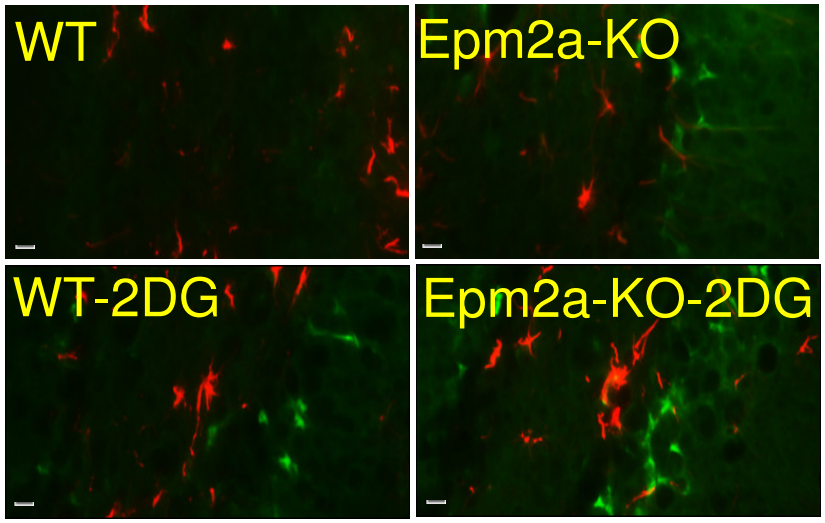


WT+2-DG/ Glycogen / DAPI





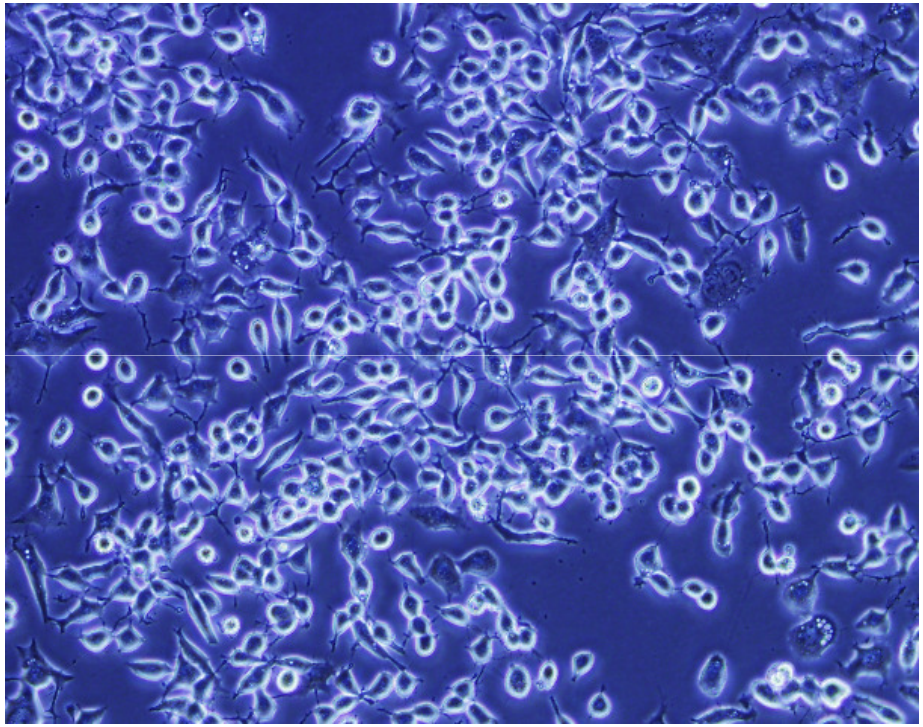
C



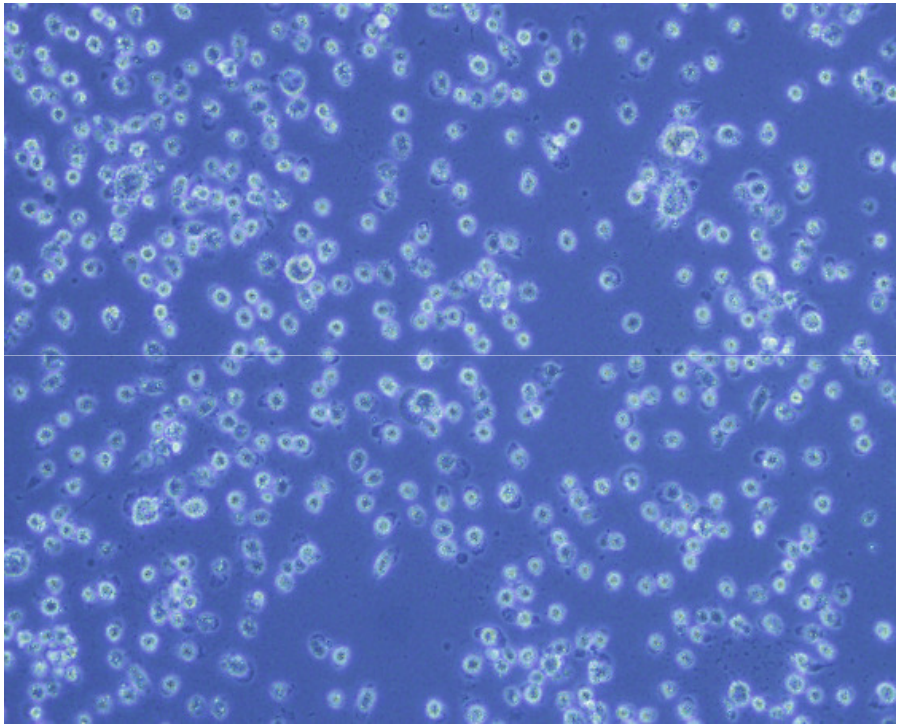
GFAP F-Jade-C

GPBB knocked out in neuronal cells causes their death/apoptosis under stresses such as low glucose, 2-DG treatment.

Control, low glucose (5mM) for 48h



GPBB (-/-), low glucose (5mM) for 48h



GPBB was knocked out in Neuro2a cells
by sgRNA-guided Cas9-directed genomic editing

The role of Laforin in the formation and accumulation of glycogen bodies (GBs) induced by stress

1. Stress induces GB synthesis via elevating G6P that allosterically activates GS1.

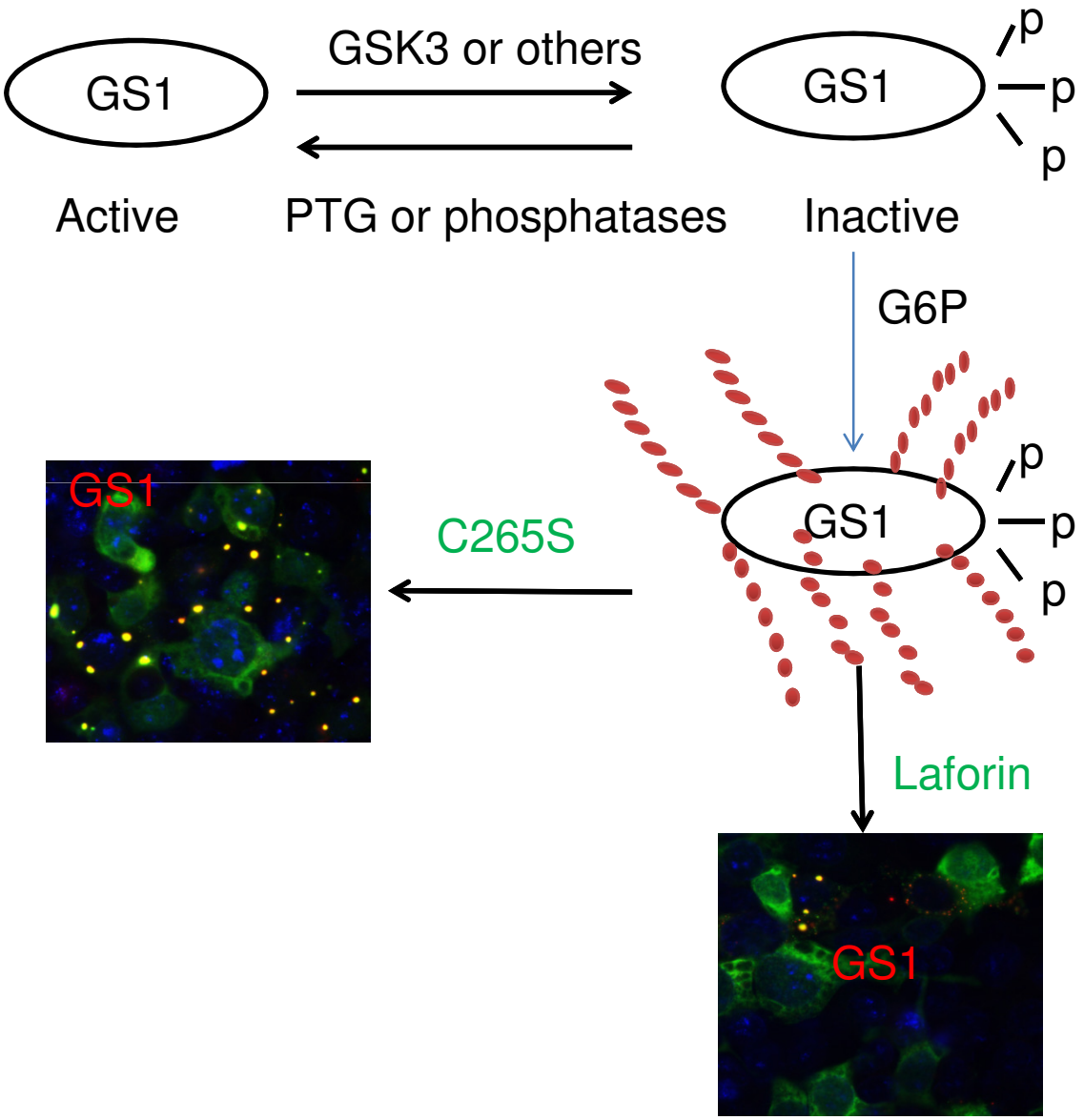
2. Synthesized GB mingles with GS1 that is phosphorylated by GSK3.

3. Laforin dephosphorylates GB's GS1 for their degradation against stress persistence.

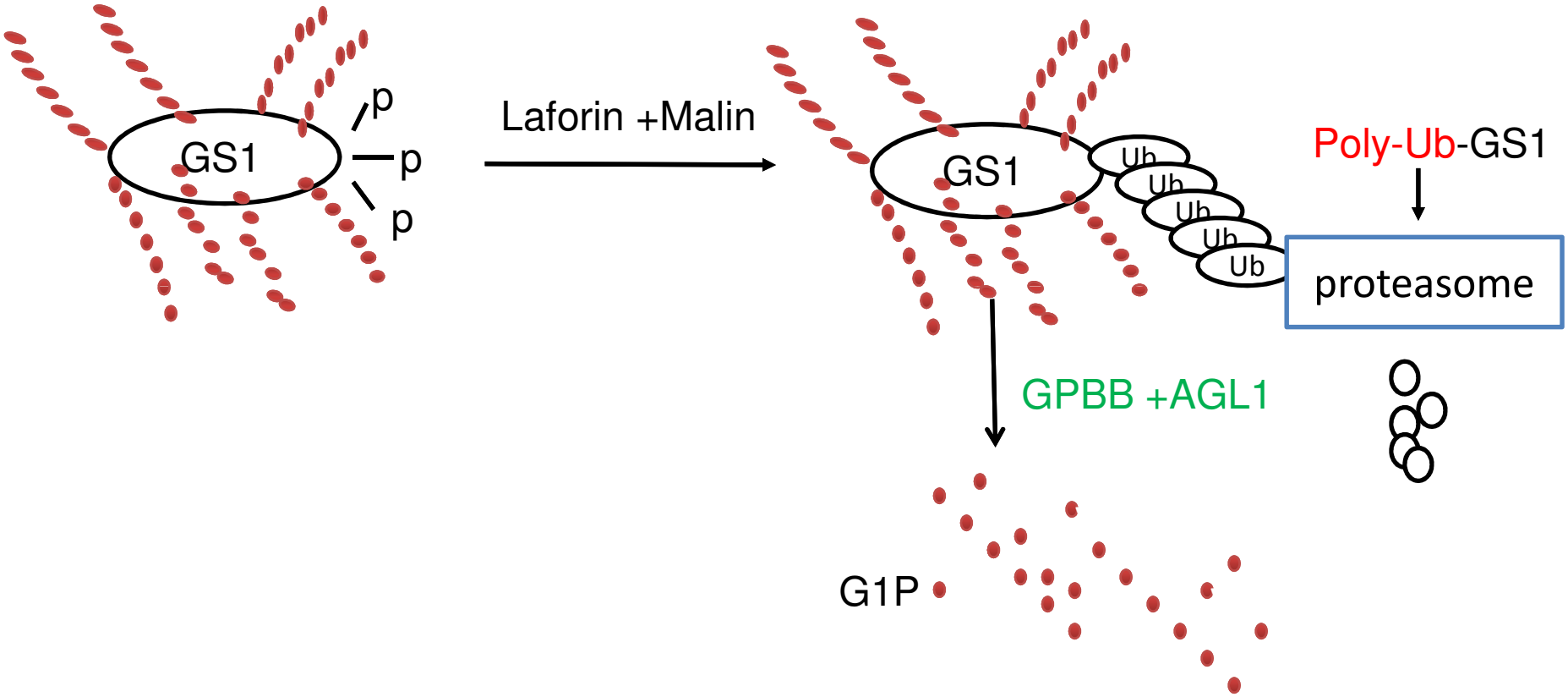
4. Deficiency of Laforin increases LB phosphorylation and accumulation, accelerates stress-induced LD progression.

5. Stress-induced GB formation and then degradation is a protective process of cells surviving from persistent stress.

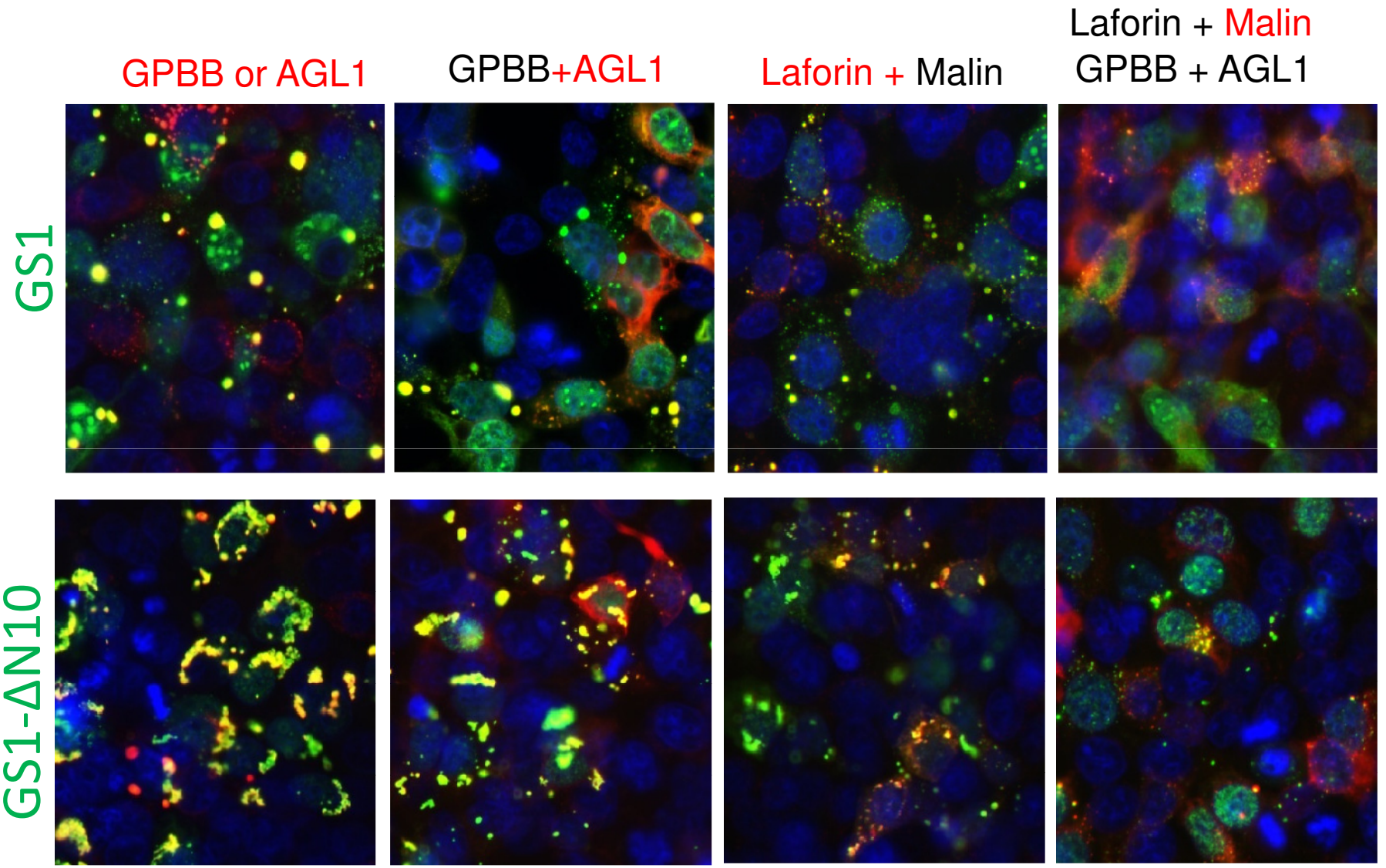
Laforin is a phosphatase of GBs and targets phosphorylated GS1



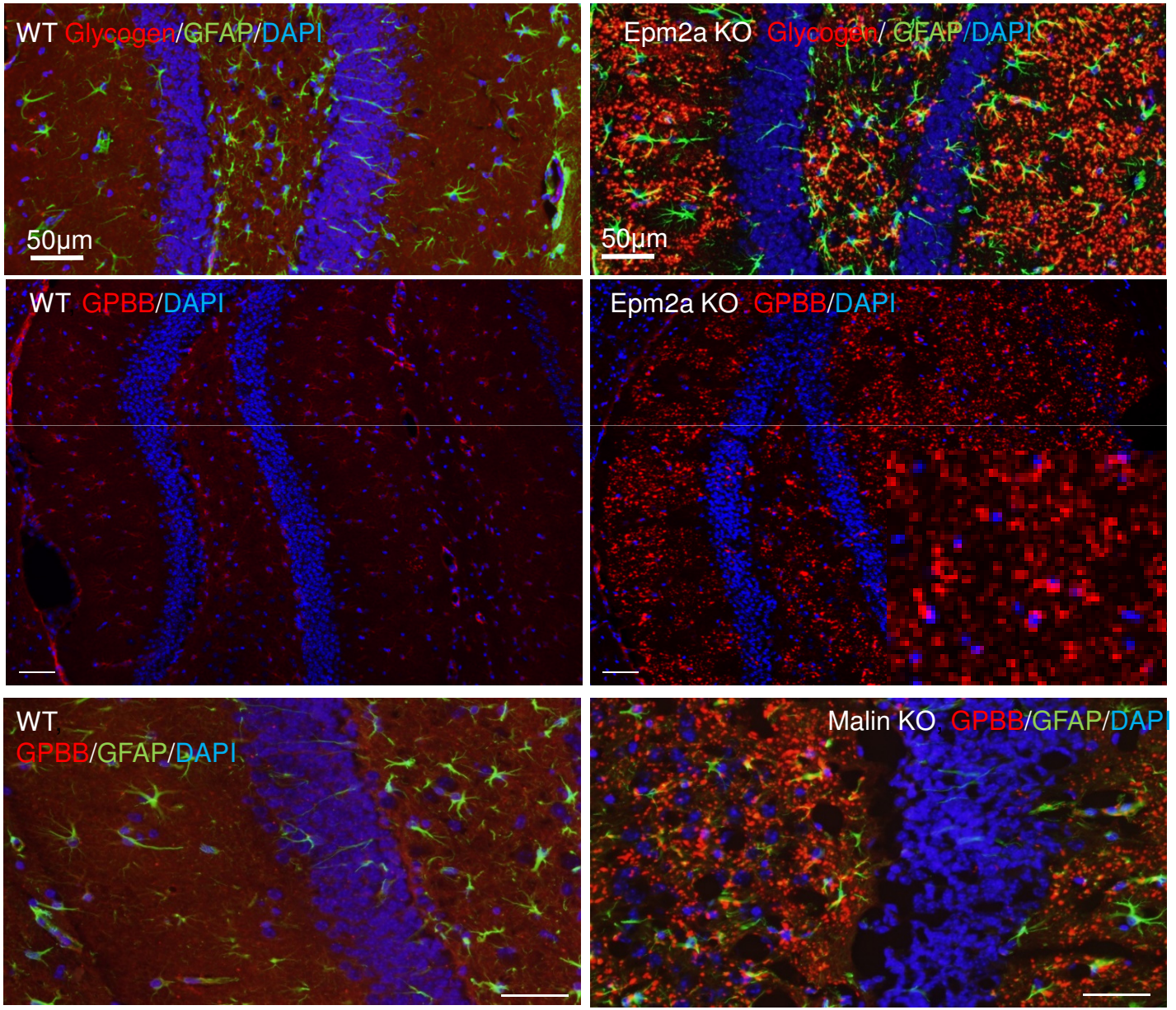
Proteans' complex machinery is required for the degradation of pGS1-GBs



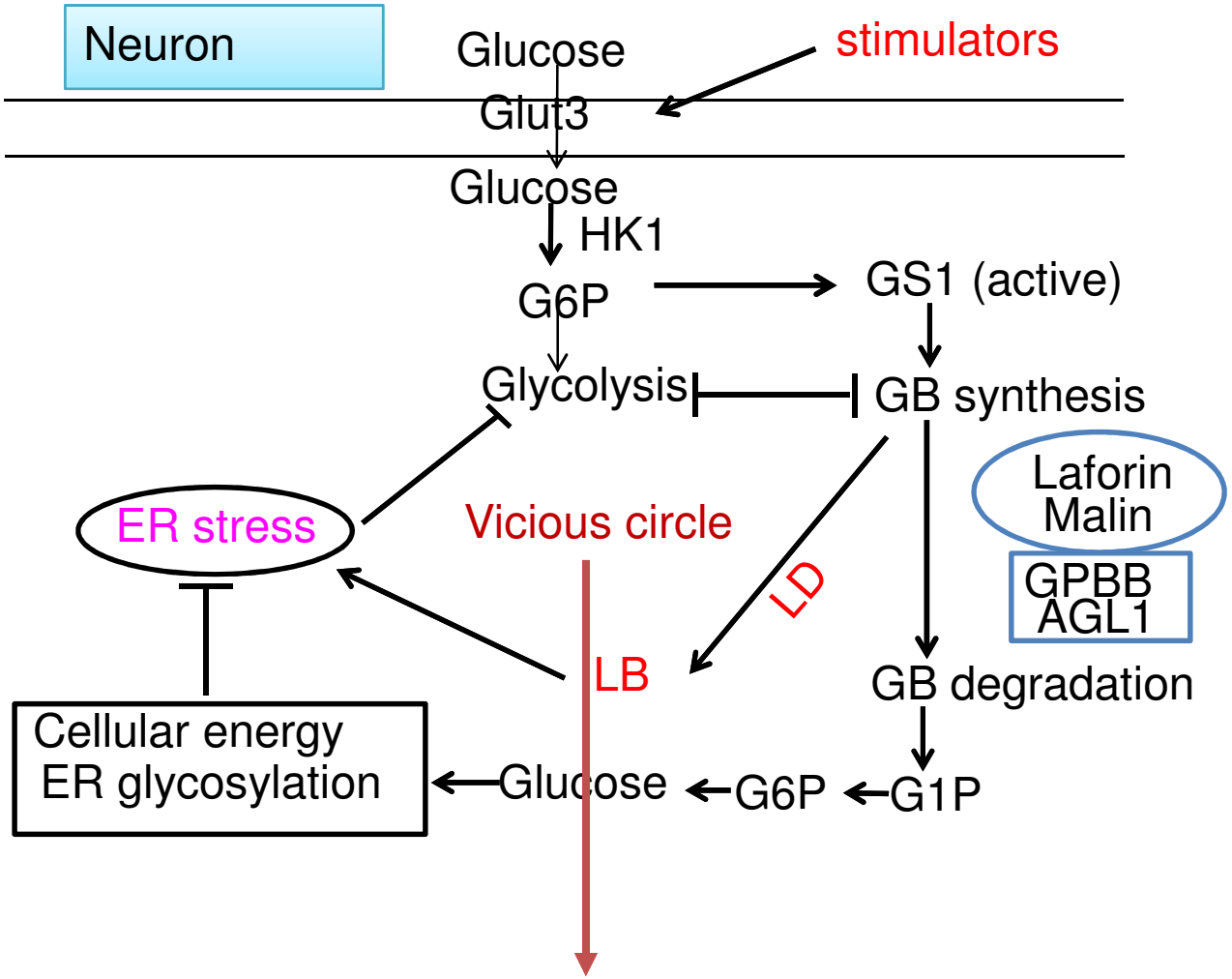
Laforin-Malin complex in concert with GPBB and AGL1 degrades GBs completely in vivo



GPBB accumulates in the brain LBs of Epm2a or Nhlrc1 KO mice



Molecular mechanism underlying Lafora disease



Progressive Myoclonus Epilepsy, Neuron degeneration, Demetria.

Acknowledgement:

Yin Wang
Keni Ma
Zeng Li
(General Surgery
The University of Michigan)

Helen Zhang
Jack M. Parent
(Neurology Department
The University of Michigan)

Pan Zheng
Yang Liu

(CCIR, Children's National Medical Center)

Peixiang Wang
Berge A. Minassian
(*The Hospital for Sick Children and University of Toronto, Canada*)