GemPharmatech™

Gys1 Cas9-CKO Strategy

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Design Date: 2024-1-12

Overview

Target Gene Name

- Gys1

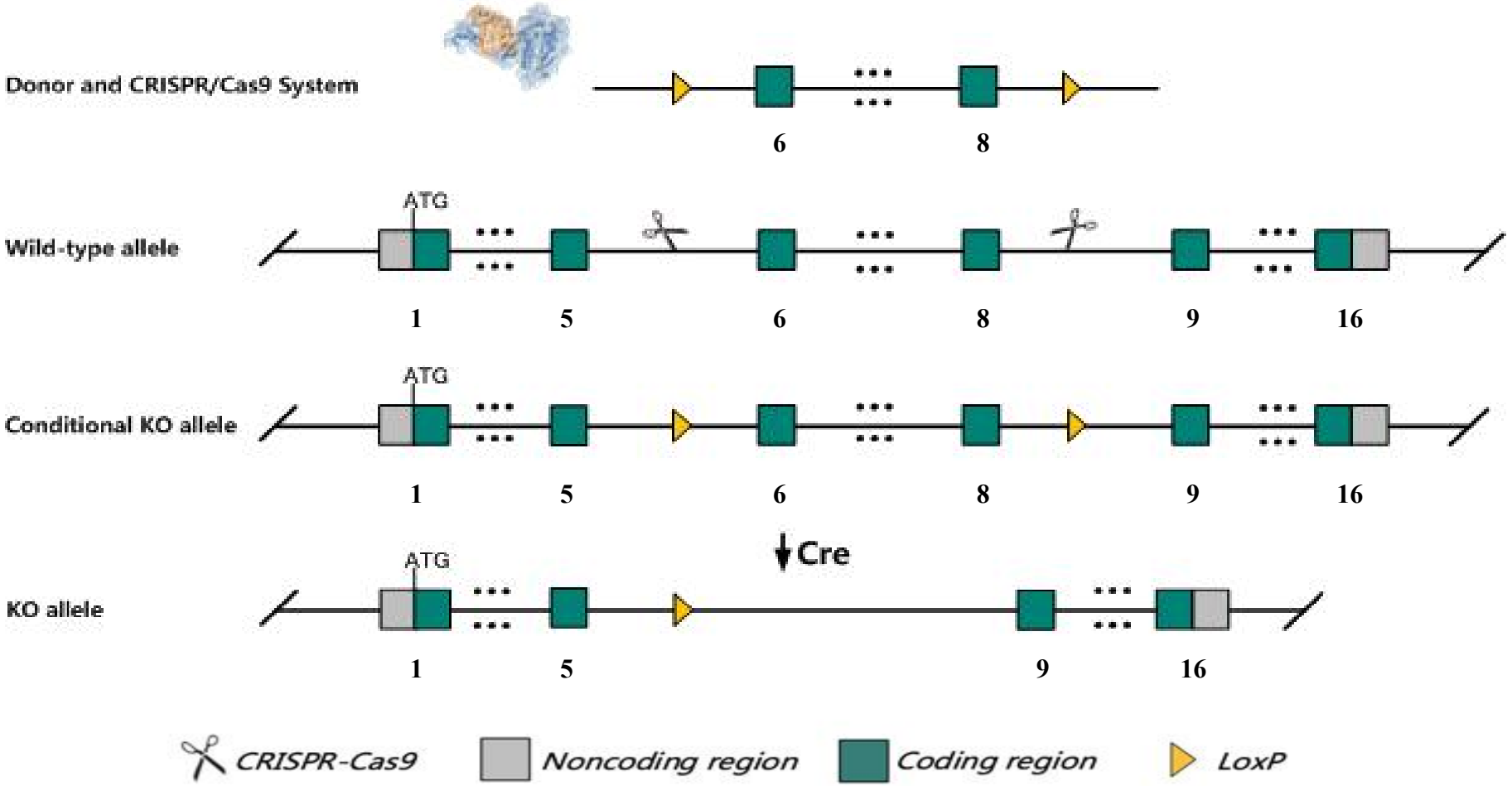
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Gys1* gene.

Technical Information

- The *Gys1* gene has 6 transcripts. According to the structure of *Gys1* gene, exon6-exon8 of *Gys1*-201 (ENSMUST00000003964.17) transcript is recommended as the knockout region. The region contains 346bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Gys1* gene. The brief process is as follows: CRISPR-Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Gene Information

Gys1 glycogen synthase 1, muscle [Mus musculus (house mouse)]

Gene ID: 14936, updated on 31-May-2023

Summary

Official Symbol	Gys1 provided by MGI
Official Full Name	glycogen synthase 1, muscle provided by MGI
Primary source	MGI:MGI:101805
See related	Ensembl:ENSMUSG00000003865
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gys3, MGS
Summary	Enables glycogen (starch) synthase activity. Acts upstream of or within glycogen biosynthetic process and heart development. Located in cytoplasm and inclusion body. Is expressed in several structures, including alimentary system; brain; genitourinary system; respiratory system; and sensory organ. Human ortholog(s) of this gene implicated in cardiovascular system disease. Orthologous to human GYS1 (glycogen synthase 1). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in mammary gland adult (RPKM 63.2), heart adult (RPKM 38.0) and 24 other tissues See more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

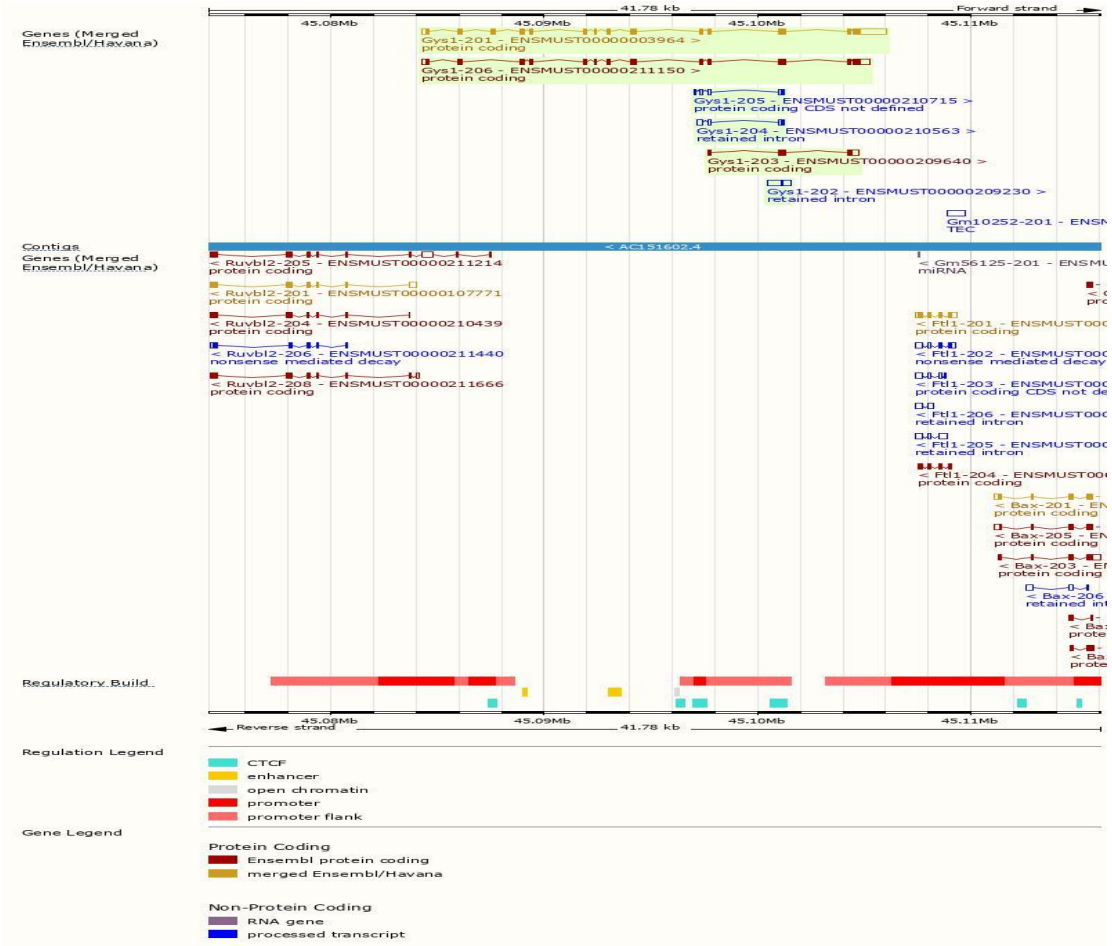
The gene has 6 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000210715.2	Gys1-205	480	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000209230.2	Gys1-202	1000	No protein	Retained intron		-	TSL:1
ENSMUST00000210563.2	Gys1-204	605	No protein	Retained intron		-	TSL:3
ENSMUST00000003964.17	Gys1-201	3678	738aa	Protein coding	CCDS21244	Q9Z1E4	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000211150.2	Gys1-206	2681	674aa	Protein coding		A0A1B0GT92	GENCODE basic TSL:5
ENSMUST00000209640.2	Gys1-203	904	177aa	Protein coding		A0A1B0GR90	TSL:3 CDS 5' incomplete

The strategy is based on the design of *Gys1*-201 transcript, the transcription is shown below:

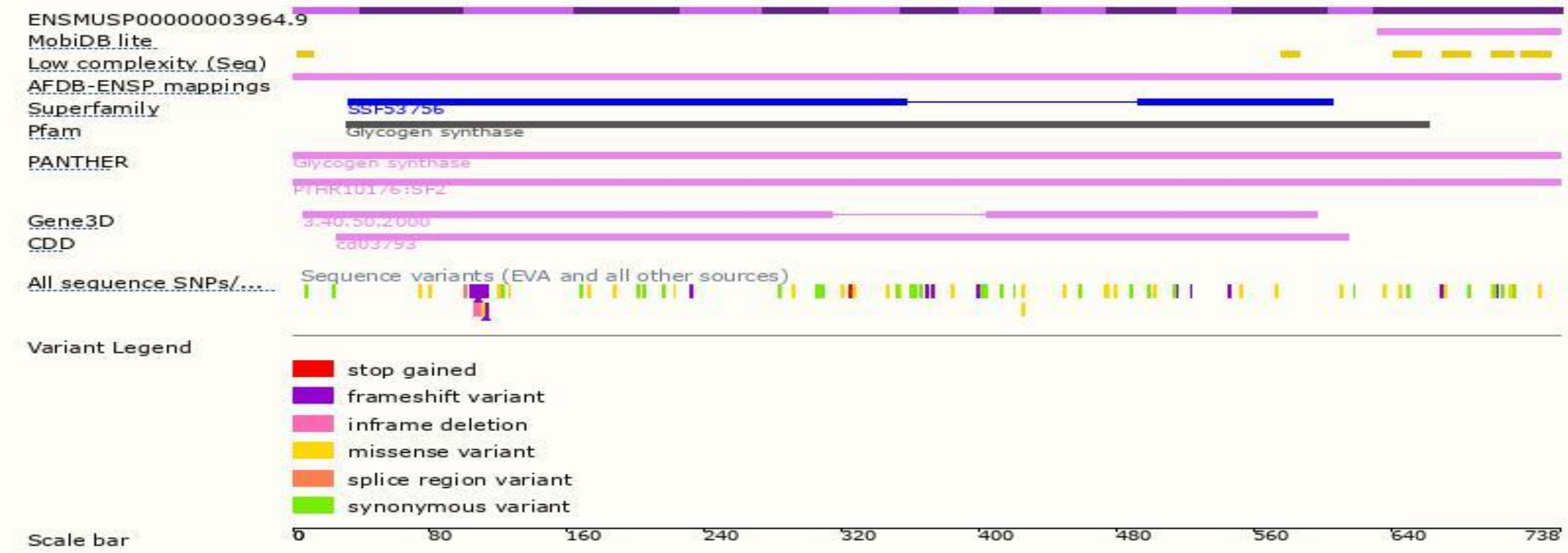
Source: https://www.ensembl.org

Genomic Information



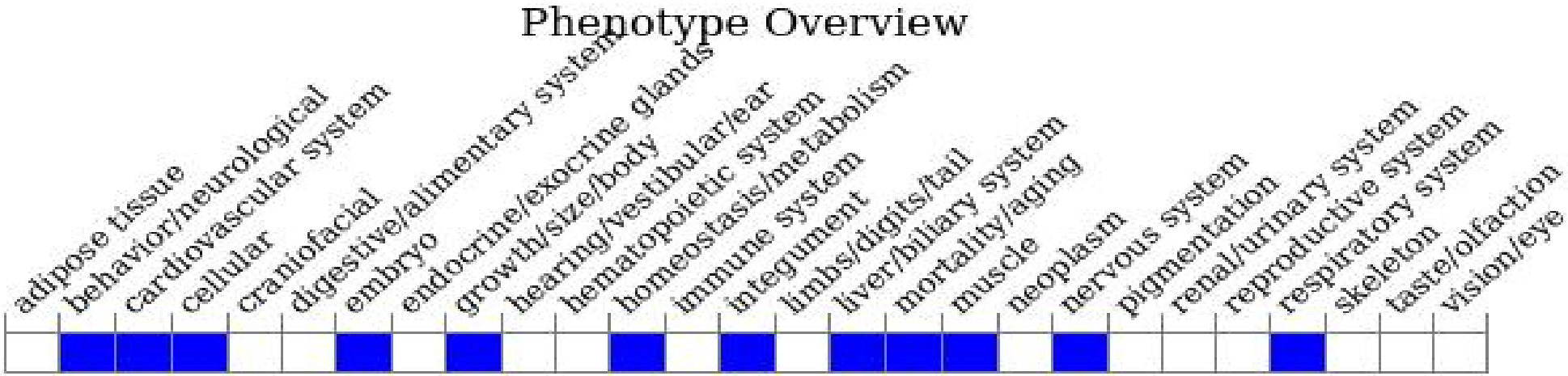
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygous null mice display neonatal lethality due to impaired cardiac function and exhibit reduced reduced ventricular chamber size, dilated atria, vascular congestion, and liver hemorrhage. Mice homozygous for a knock-in allele show altered glycogen homeostasis.

Source: https://www.informatics.jax.org

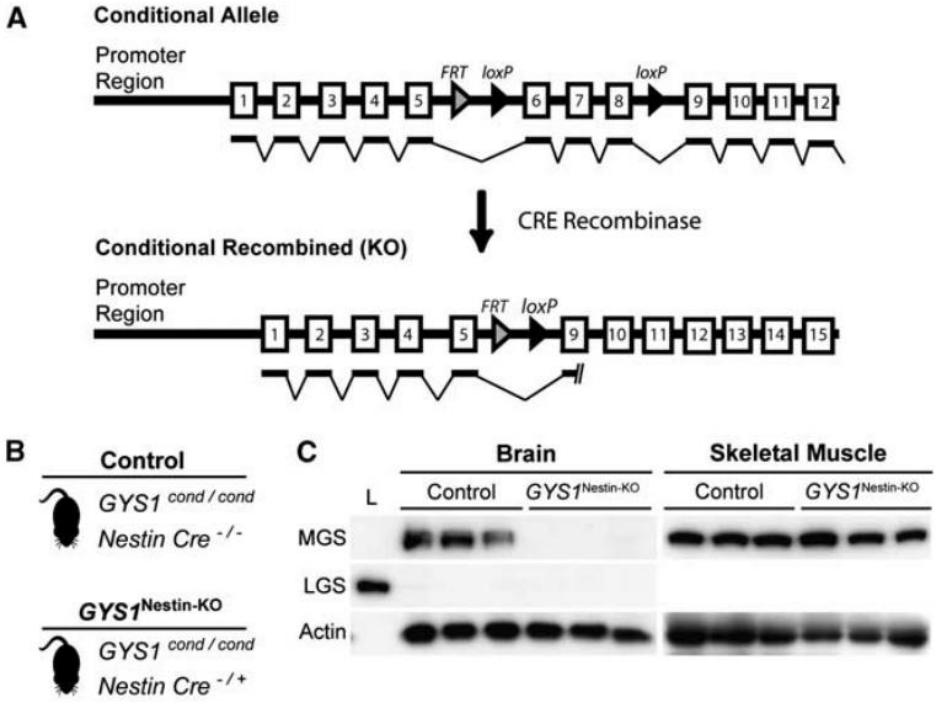
Important Information

- *Gys1* is located on Chr7. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- The effect of this strategy on transcript-202, 203,204,205 is unknown.
- The flox region is about 4kb away from *Ruvbl2* gene , this strategy may affect the function of *Ruvbl2* gene.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

References

Impairment in long-term memory formation and learning-dependent synaptic plasticity in mice lacking glycogen synthase in the brain

Jordi Duran[1,2,5], Isabel Saez[1,3,5], Agnès Gruart[4], Joan J Guinovart[1,2,3] and José M Delgado-García



Duran J, Saez I, Gruart A, Guinovart JJ, Delgado-García JM. Impairment in long-term memory formation and learning-dependent synaptic plasticity in mice lacking glycogen synthase in the brain. J Cereb Blood Flow Metab. 2013 Apr;33(4):550-6. doi: 10.1038/jcbfm.2012.200. Epub 2013 Jan 2. PMID: 23281428; PMCID: PMC3618391.