SUPPORTING INFORMATION TO:

Deleterious effects of glycogen accumulation in flies and mice

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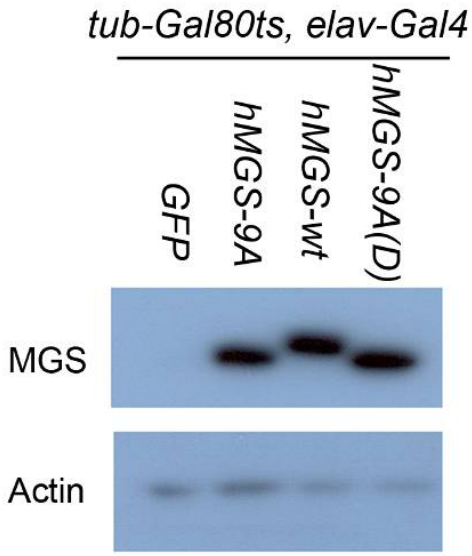
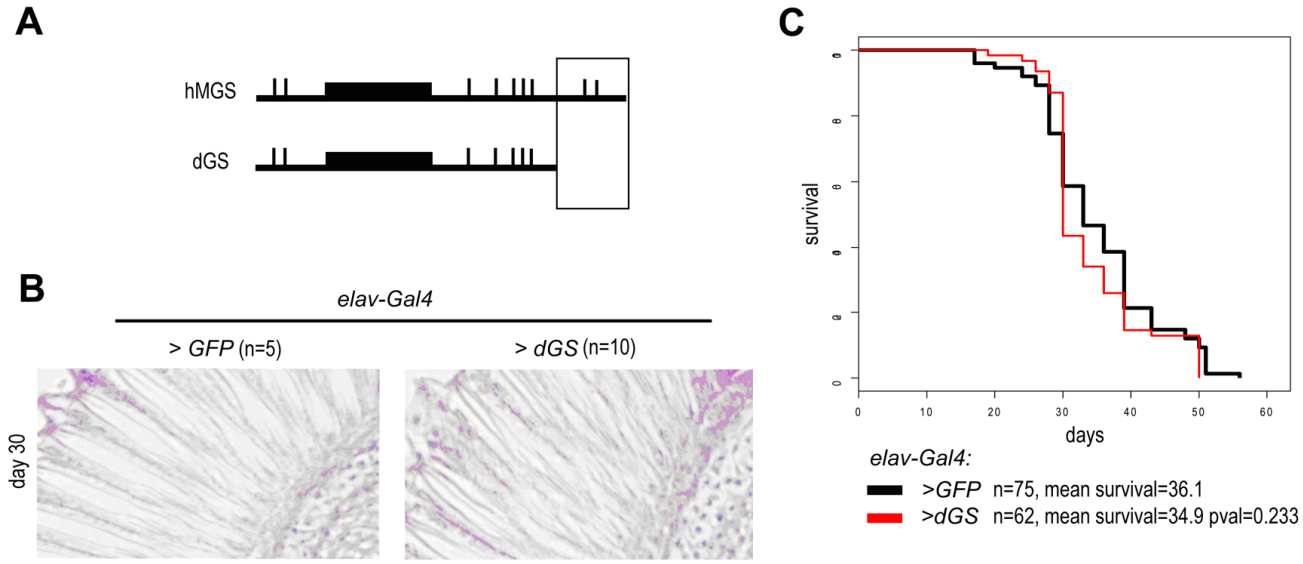
Table of contents:

SUPPORTING INFORMATION FIGURES



Supporting Information Figure 1.

Western blot analysis showing expression of *wild-type* and mutant forms of hMGS in heads of flies of the following genotypes: *tubGal80^{ts}, elav-Gal4; UAS-mCD8-GFP* (lane 1)*, tubGal80^{ts}, elav-Gal4; UAS- hMGS-9A* (lane 2)*, tubGal80^{ts}, elav-Gal4; UAS- hMGS-wt* (lane 3)*, tubGal80^{ts}, elav-Gal4; UAS- hMGS-9A(D)* (lane 4)*.* Upper panel: MGS. Lower panel: ACTIN.

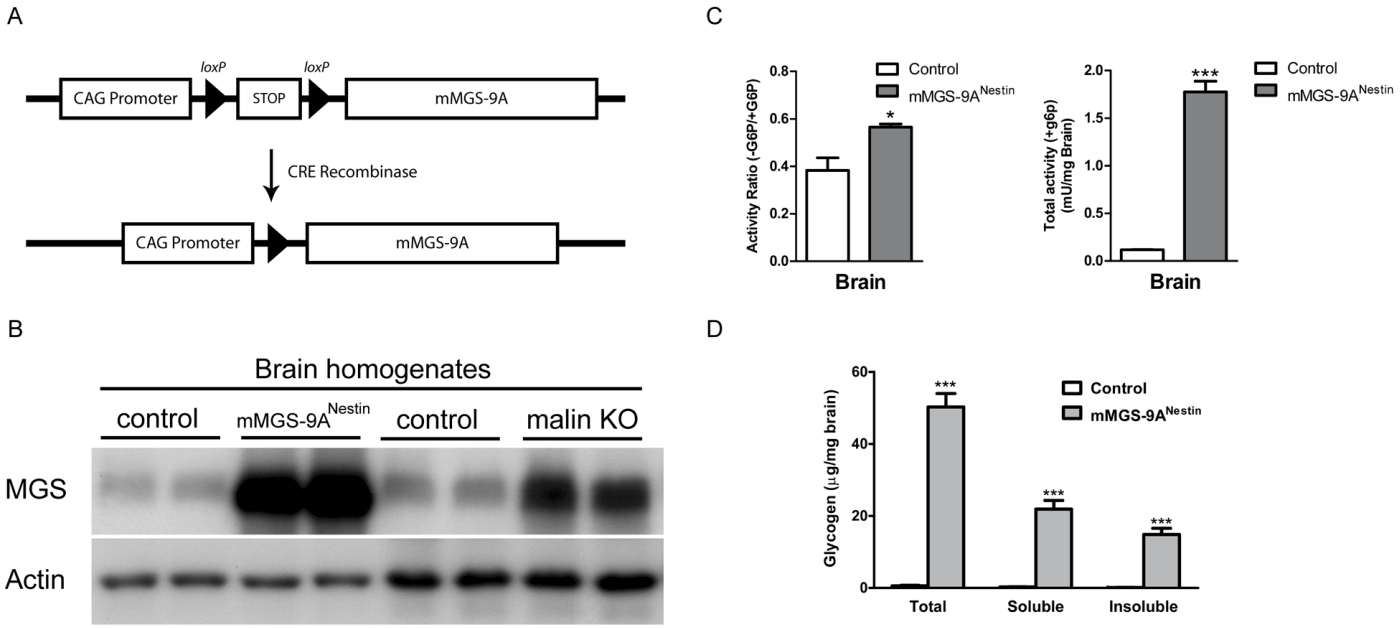


Supporting Information Figure 2.

A. Schematic representation of human (hMGS) and Drosophila (dGS) GS isoforms showing an extra domain (inbox) in hMGS containing two regulatory phosphorylation sites absent in dGS. Upright bars represent phoshorylation sites. Black box represents the core catalytic region. The overall protein sequence identity is 59%.

B. PAS (pink)/Hematoxilin (blue) staining of retina sections of elavGAL4 adult flies driving expression of *UAS-mCD8-GFP* (left), *UAS-dGS* (right) transgenes at 29ºC during 30 days. Note that no PAS positive staining was detected. The images are representative of staining performed in 4 to 5 individuals per condition.

C. Survival assay of elav-Gal4 adult flies driving expression of *UAS-mCD8-GFP* (black)(n=75, μx=36.1,max =56) and *UAS-dGS* (red)(n=62, μx=34.9 *p*=0.233, max=50). max=maximum lifespan (days), μx= mean survival (days) and *p*-value calculated by logrank test compared to GFP control.



Supporting Information Figure 3.

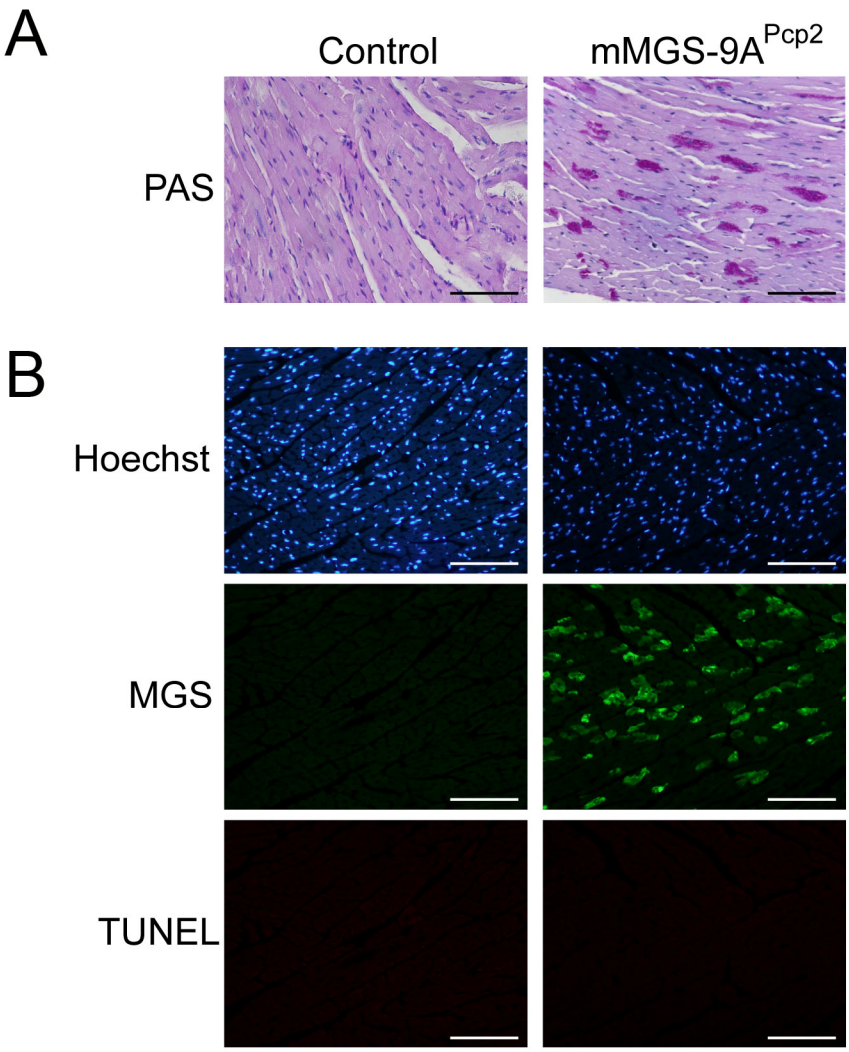
A. Conditional expression cassette based on the CRE-lox technology. Non-inactivatable mMGS cDNA is expressed under the control of the ubiquitous CAG promoter. A loxP-flanked transcription Stop cassette is included between the promoter and the transgene to allow CRE recombinase-dependent induction of gene expression.

B. Western blot analysis to quantify MGS protein expression in whole brain homogenates of *mMGS-9A*^{Nestin} mice and their control littermates, and from *Epm2b* KO mice (LD model) and their control littermates.

C. Quantification of total GS activity (right panel, measured in the presence of 6.6 mM G6P) and of GS activity ratio (-G6P/+G6P) in brain homogenates from *MGS-9A*^{Nestin} mice and from their control littermates (n=3 animals per group; *p*-values: (Activity ratio) *p*=0.029; (Total activity) *p*=0.00011).

D. Quantification of glycogen accumulated in the brains of *MGS-9A*^{Nestin} mice and from their control littermates. Glycogen was measured in whole homogenate (Total) and in the supernatant (Soluble) and pellet (Insoluble) fractions obtained after a low speed centrifugation (n=3 animals per group; *p*-values: (Total) *p*=8.5E-05; (Soluble) *p*=0.00038; (Insoluble) *p*=0.00048).

*p<0.05, **p<0.01, ***p<0.001



Supporting Information Figure 4.

Expression of *mMGS-9A* in cardiomyocytes does not induce apoptosis.

A. PAS (pink) and Hematoxilin (blue) staining of heart sections of p75 *mMGS-9A*^{Pcp2} and control animals. Note strong accumulation of glycogen mMGS-9A-expressing cardiomyocytes. Scale bars, 100 µm. The images are representative of staining performed in 3 individuals per condition.

B. Cardiomyocytes of p75 *mMGS-9A*^{Pcp2} animals labeled to visualize by TUNEL staining of apoptotic cells (red) and expression of MGS protein (green). Sections were also labeled with Hoechst (blue). Scale bars, 100 µm. The images are representative of staining performed in 3 individuals per condition.

SUPPORTING INFORMATION METHODS.

GS Activity Determination.

To measure GS activity, tissue samples (100 mg) were added to 1 ml of ice-cold homogenization buffer containing 10 mM Tris-HCl (pH 7), 150 mM KF, 15 mM EDTA, 15 mM 2-mercaptoethanol, 0.6 M sucrose, 25 nM okadaic acid, 1mM sodium orthovanadate, 10 μg/ml leupeptin, 10 ug/ml aprotinin, 10 μg/ml pepstatin, 1 mM benzamidine, and 1 mM phenylmethanesulfonyl fluoride and were then homogenized (Polytron) at 4 °C. GS activity was measured in whole homogenates (Thomas et al, 1968) in the absence or presence of 6.6 mM glucose 6-phosphate (G6P), representing active or total activity, respectively. The −/+ glucose 6-phosphate activity ratio is an estimation of the activation state of the enzyme (Guinovart et al, 1979).

Analysis of glycogen content.

Brain glycogen content was determined by an amyloglucosidase-based assay. Tissue samples (100mg) were added to 400uL of 30% KOH and homogenized with polytron. The extract was then boiled for 15 min. In order to know the amount of insoluble glycogen, tissue samples (100mg) were added to 400 μL of the buffer used for GS activity determination, described above. Then, samples were homogenized with polytron at 4ºC and centrifuged at 13000 xg for 15 min. 30% KOH was added to sediments and supernatants and they were boiled for 15 min. Glycogen from total homogenates, supernatant and sediments were measured as described (Chan & Exton, 1976).

Electrophoresis and Immunoblotting.

Flies were rapidly frozen in liquid nitrogen and vortexed to separate the heads from the bodies. Five heads per sample were used to prepare a total homogenate and 10μg of protein were loaded. In the case of mice, animals were anesthetized with tiobarbital and the brain was quickly dissected and frozen in liquid nitrogen. Each animal was treated separately and 30μg of total protein homogenates were loaded.

Immunoreactivity was determined by resolving homogenates by 10% SDS-PAGE. The protein was transferred onto Immobilon-P membrane (Millipore) and membrane was probed with MGS (Epitomics 1741 or Cell Signalling 3886) in a 1:1000 dilution. For protein loading control, anti-Actin (Clone AC-40 from Sigma)

antibodies were used in a 1:1000 dilution. Secondary antibodies conjugated to horseradish peroxidase against rabbit (GE Healthcare) or mouse immunoglobulins (Dako) were used in a 1:1000 dilution. Immunoreactive bands were visualized using an Immobilon Western Chemiluminiscent HRP Substrate (Millipore), following the manufacturer's instructions.

List of references

Chan TM, Exton JH (1976) A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem 71**:** 96-105

Guinovart JJ, Salavert A, Massague J, Ciudad CJ, Salsas E, Itarte E (1979) Glycogen synthase: a new activity ratio assay expressing a high sensitivity to the phosphorylation state. FEBS Lett 106**:** 284-288

Thomas JA, Schlender KK, Larner J (1968) A rapid filter paper assay for UDPglucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-14C-glucose. Anal Biochem 25**:** 486-499