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Supplemental Information

Glucagon Receptor Antagonism Improves Glucose Metabolism and Cardiac Function by Promoting AMP-Mediated Protein Kinase in Diabetic Mice

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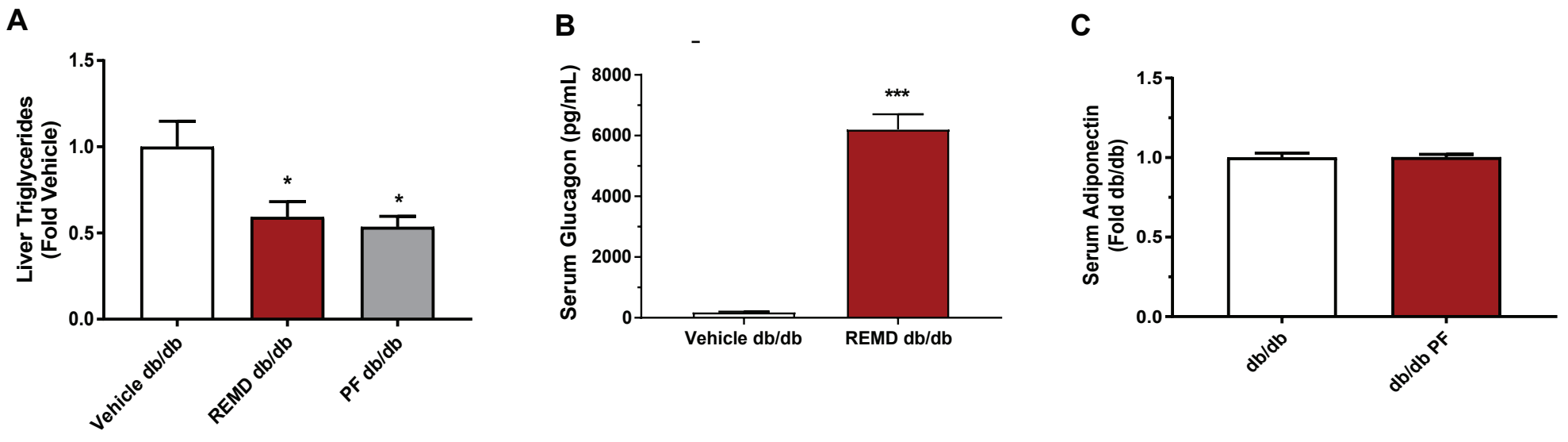
Inventory of Supplemental Materials

We have included 4 supplemental figures. Supplemental figure 1 provides data on the effects of REMD 2.59 on serum glucagon and pair-feeding on hepatic steatosis and circulating adiponectin. Supplemental figure 2 details glucose levels prior to and during hyperinsulinemic-euglycemic clamps and summarizes relevant clamp parameters. Supplemental figure 3 details the effects of REMD 2.59 on sphingolipids in serum and selected tissues in $Lep^{ob/ob}$ mice after antibody administration – specifically, it shows levels of individual serum ceramide species, liver glucosylceramide species, and liver/soleus 1-O-acylceramide species. Supplemental figure 4 details the hyperinsulinemic euglycemic-or-hypoglycemic clamp conducted on wildtype mice to show that hypoglycemia, which is often observed in REMD 2.59 treated mice, does not increase AMPK activation. Additionally, supplemental figure 4 provides data on individual cardiac ceramide derivative species and other sphingoids in LPL^{GPI} mice treated with REMD 2.59 or vehicle.

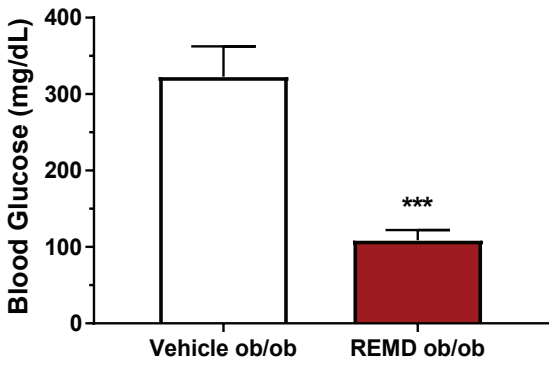
Supplemental Figure 1, Related to Figure 1



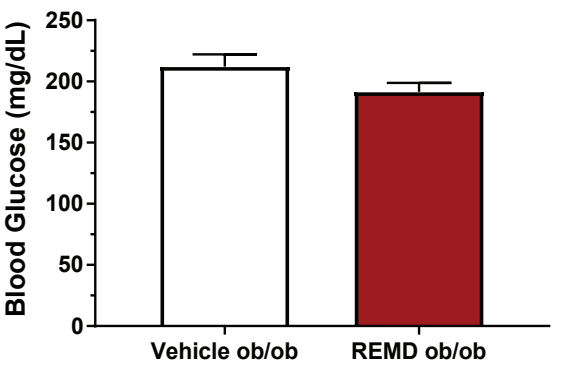
Supplemental 1. A) Liver triglycerides were assessed in $Lep^{db/db}$ mice after 10 days following REMD 2.59/Vehicle treatment or pair-feeding. **B)** Serum glucagon levels were assessed via ELISA in REMD 2.59 and vehicle treated $Lep^{db/db}$ mice on day 7 of the 10-day timecourse. **C)** Circulating adiponectin levels were measured via western blot using 0.25µL of serum from chow-fed and pair-fed $Lep^{db/db}$ mice, following 10 days of feeding. *P<0.05, **P<0.01, ***P<0.001 by student's *t* test. Data represented as mean ± SEM.

Supplemental Figure 2, Related to Figure 3

A



B

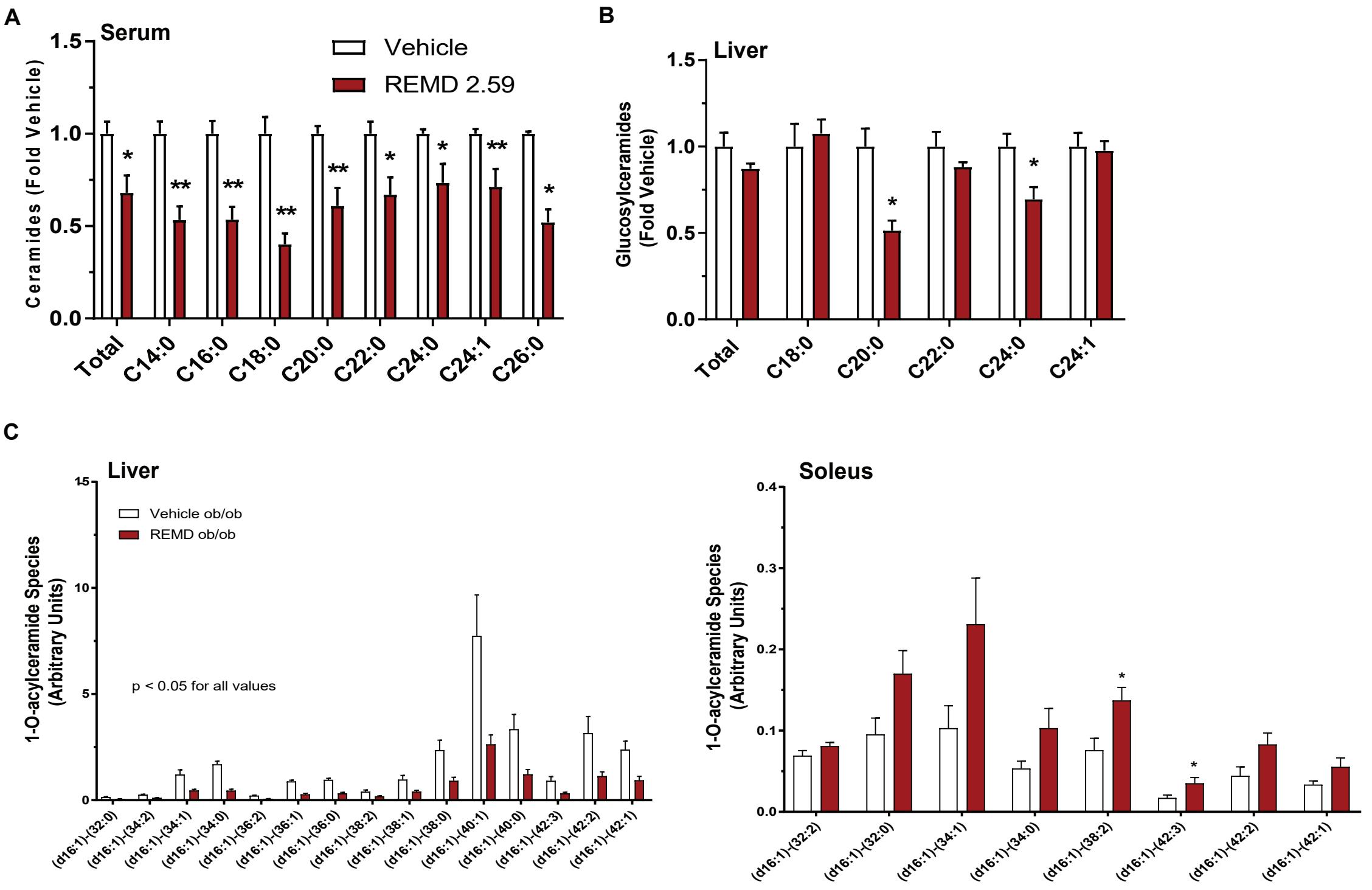


C

	Body Weight (g)	Basal				Clamped			
		Plasma Glucose (mg/dL)	Plasma Insulin (ng/mL)	Plasma NEFA (mmol/L)	Plasma Glycerol (mg/mL)	Plasma Glucose (mg/dL)	Plasma Insulin (ng/mL)	Plasma NEFA (mmol/L)	Plasma Glycerol (mg/mL)
Vehicle ob/ob	47.5 ± 1.3	322.8 ± 39.7	8.93 ± 3.65	0.84 ± 0.09	1.44 ± 0.12	212.1 ± 10.0	16.59 ±1.15	0.82 ± 0.09	1.82 ± 0.10
REMD ob/ob	49.9 ± 2.8	108.7 ± 13.3	8.69 ± 2.49	0.87 ± 0.17	1.50 ± 0.15	191.5 ± 7.4	14.74 ± 2.03	0.39 ± 0.05	1.73 ± 0.23

Supplemental 2. A) Circulating blood glucose levels were assessed following a 3-hour fast prior to hyperinsulinemic clamps. **B)** Circulating blood glucose levels were measured in the clamped state. **C)** Relevant metabolic parameters before and during hyperinsulinemic-euglycemic clamp. **P*<0.05, ***P*<0.01, ****P*<0.001 by Student's *t* test. Data represented as mean ± SEM.

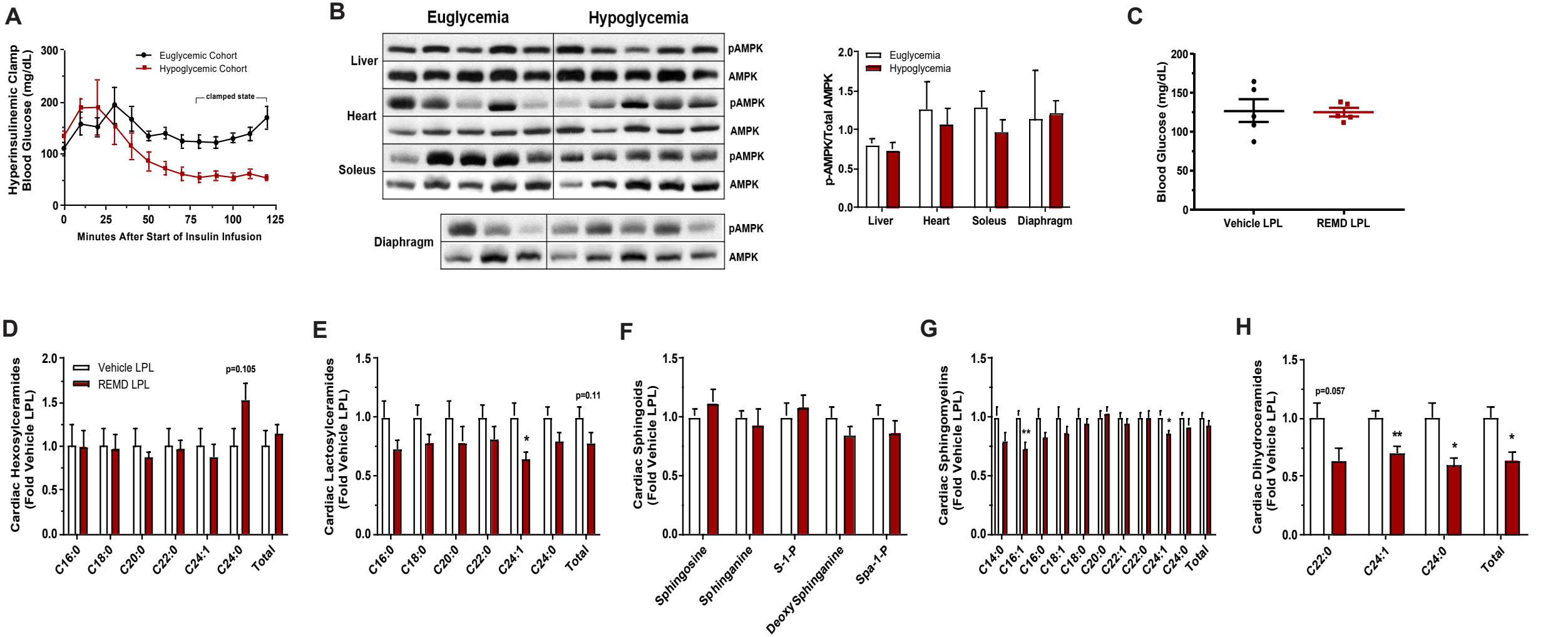
Supplemental Figure 3, Related to Figure 4



Supplemental 3. A-B) Ob/ob mice were treated with REMD 2.59 or vehicle (5 mg/kg subcutaneous injection) and sacrificed 5 days later and tissues were harvested. Serum ceramides and liver glucosylceramides were assayed via mass spectrometry. **C)** 1-*O*-acylceramide species were quantified in liver (left) and soleus (right) by LC-MS/MS.

**P*<0.05, ***P*<0.01, ****P*<0.001 by Student's *t* test. Data represented as mean ± SEM.

Supplemental Figure 4, Related to Figure 6



Supplemental 4. A-B) Hyperinsulinemic clamp studies were conducted on age-matched wildtype mice. Mice (n=5 per cohort) were clamped at either euglycemia (~130 mg/dL) or hypoglycemia (~70 mg/dL) for an hour, at which point mice were sacrificed and tissues were collected for protein analysis. **A)** Average blood glucose over the course of the clamp shows that mice were adequately maintained at either euglycemia or hypoglycemia. **B)** Representative immunoblot for activated AMPK and total AMPK in liver, heart, soleus, and diaphragm (left) and the ratio of activated AMPK to total AMPK was quantified via densitometry (right). **C)** Fed glucose was measured in LPL^{GPI} mice at 15 weeks of age. **D-H)** Cardiac hexosylceramides, lactosylceramides, dihydroceramides, sphingomyelins, and sphingoids were quantified via mass spectrometry. **P*<0.05, ***P*<0.01, ****P*<0.001 by Student's *t* test. Data represented as mean ± SEM.