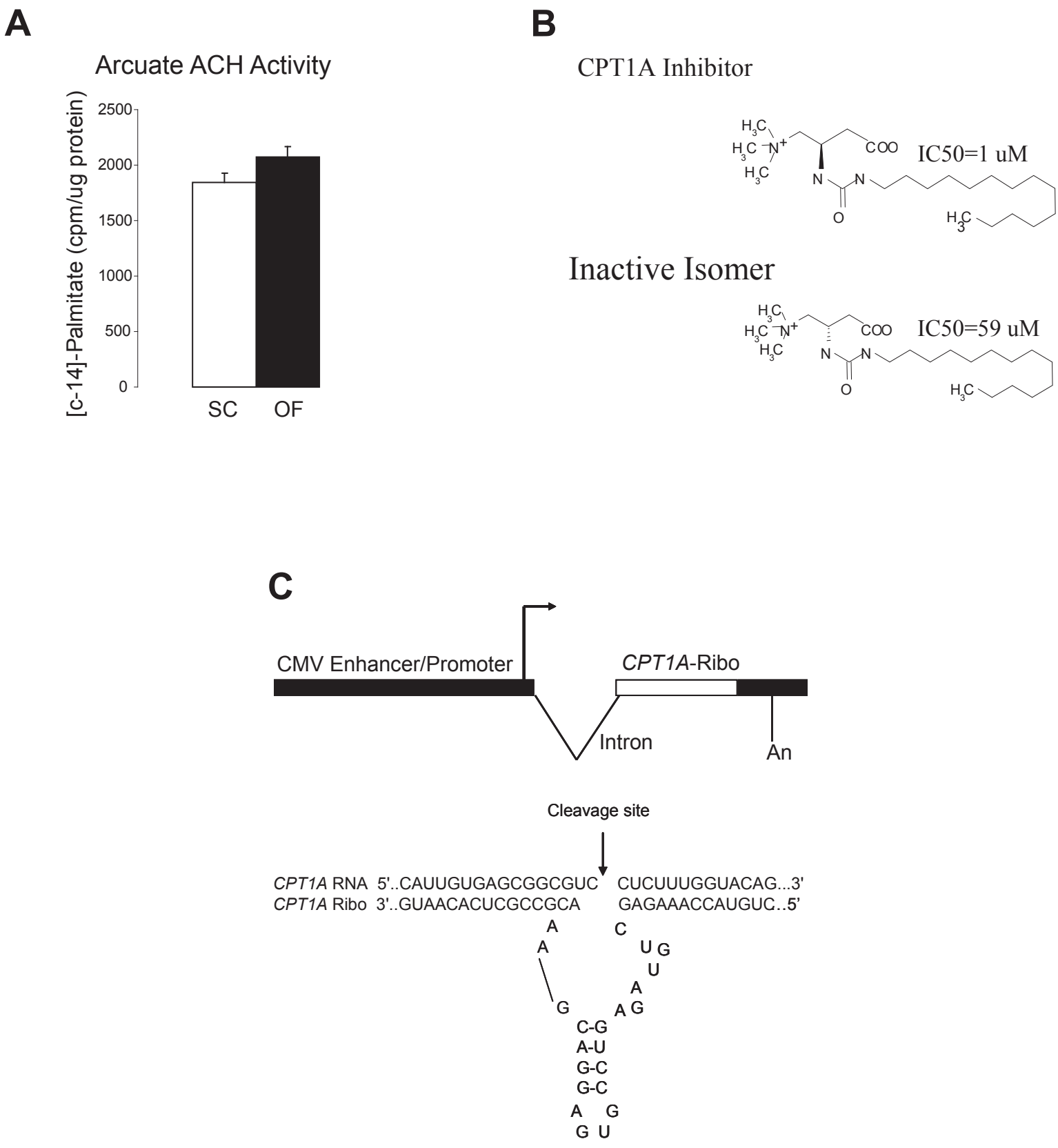
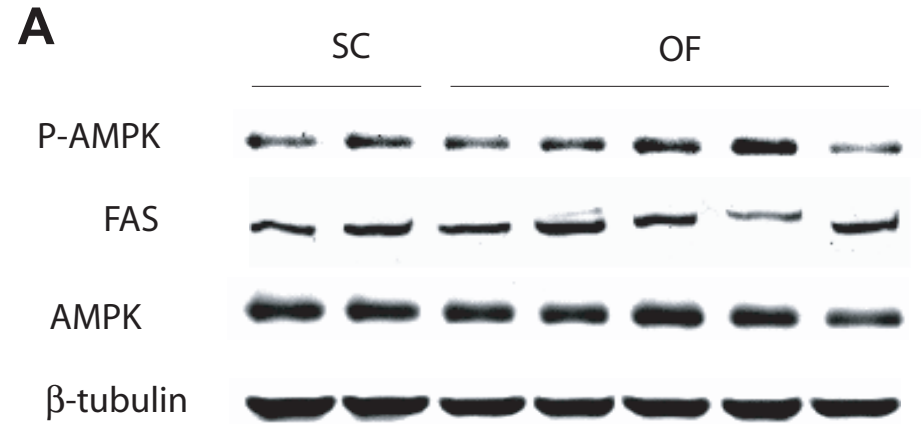
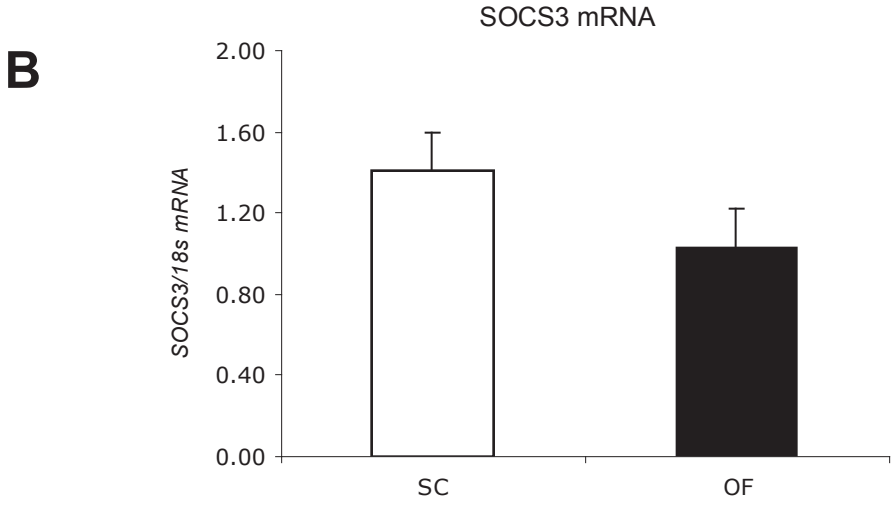
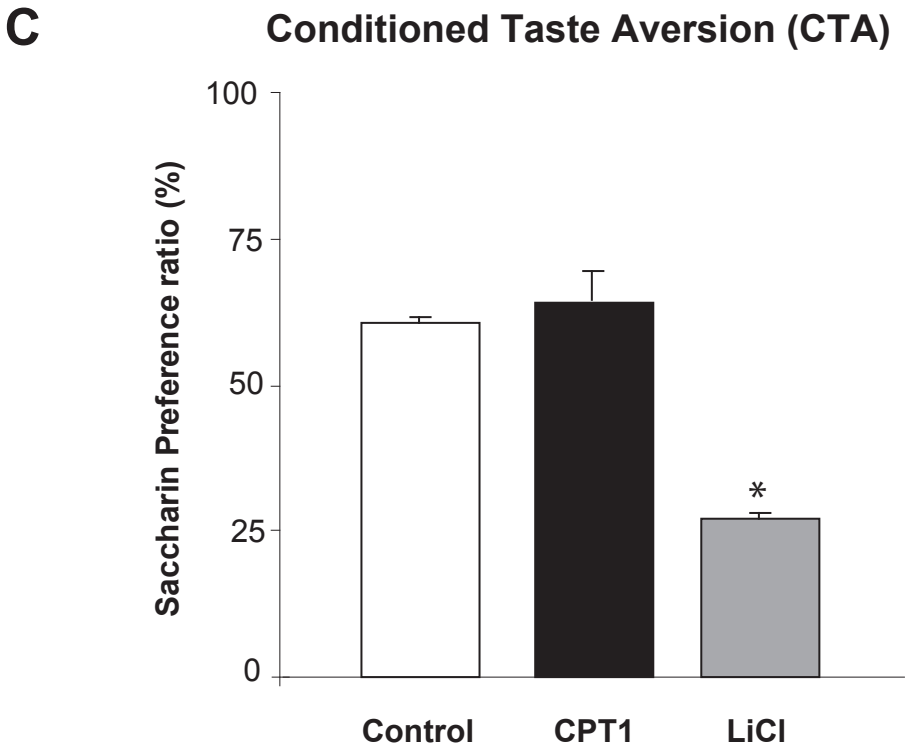


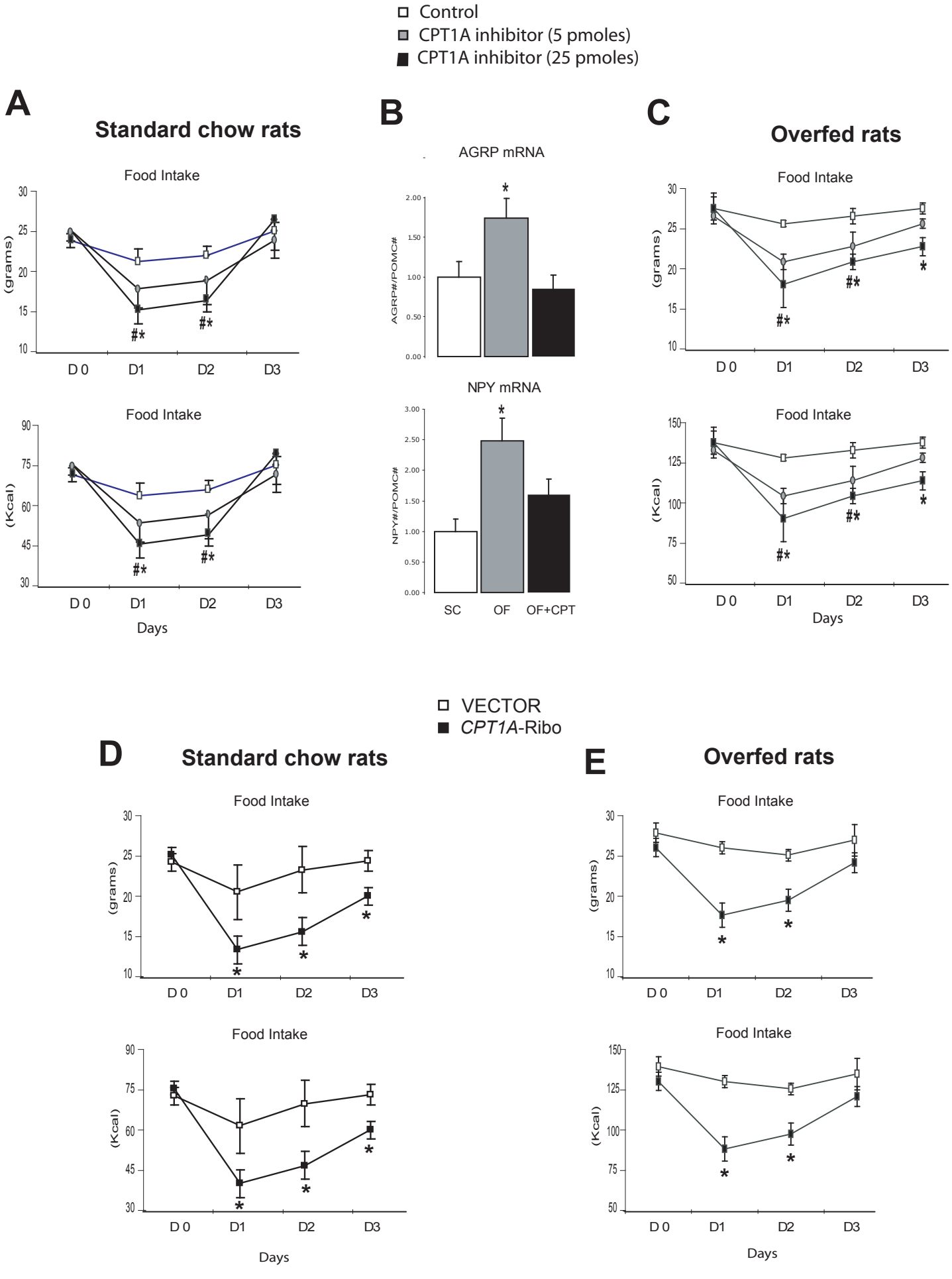

Suppl Figure 1



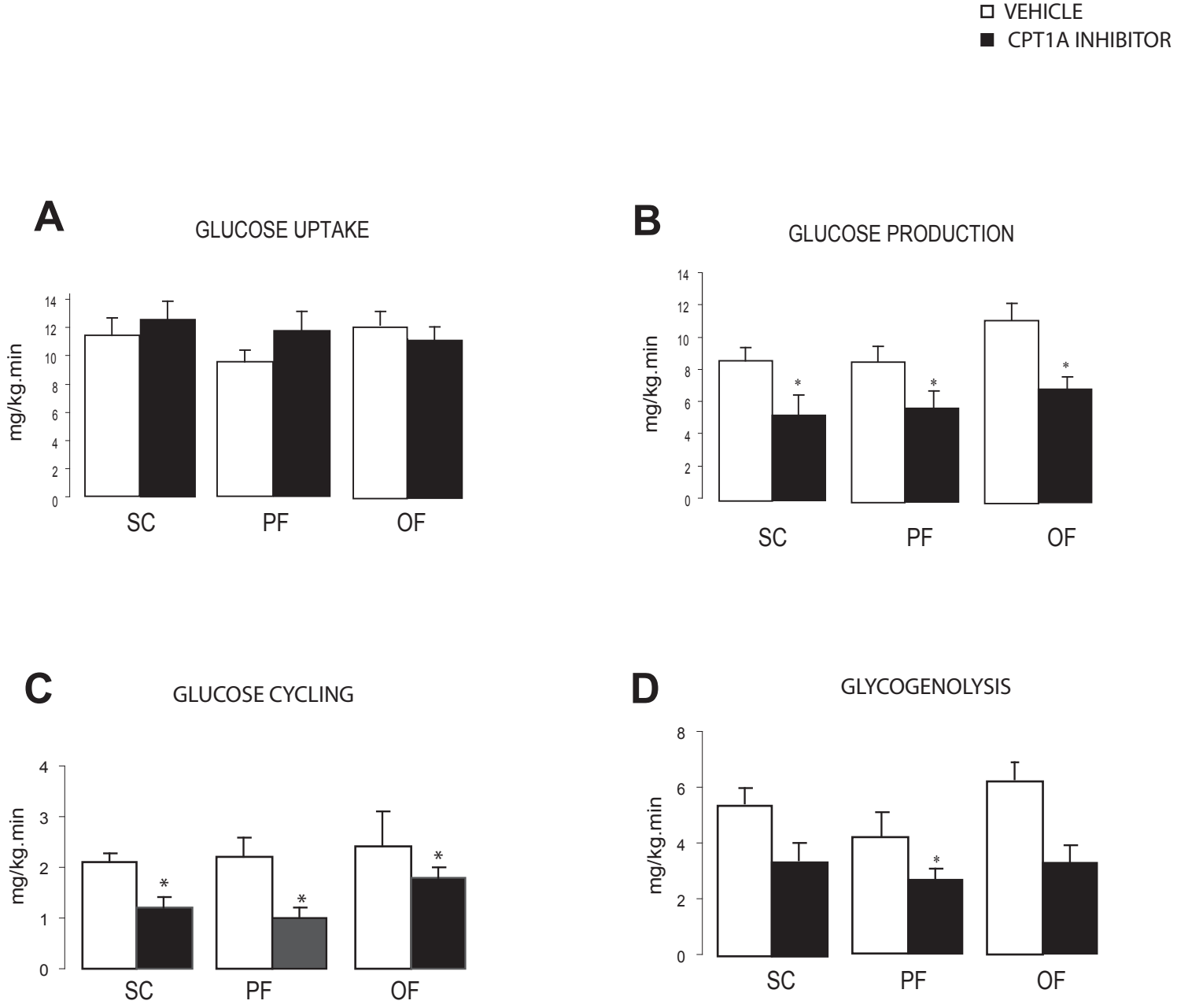



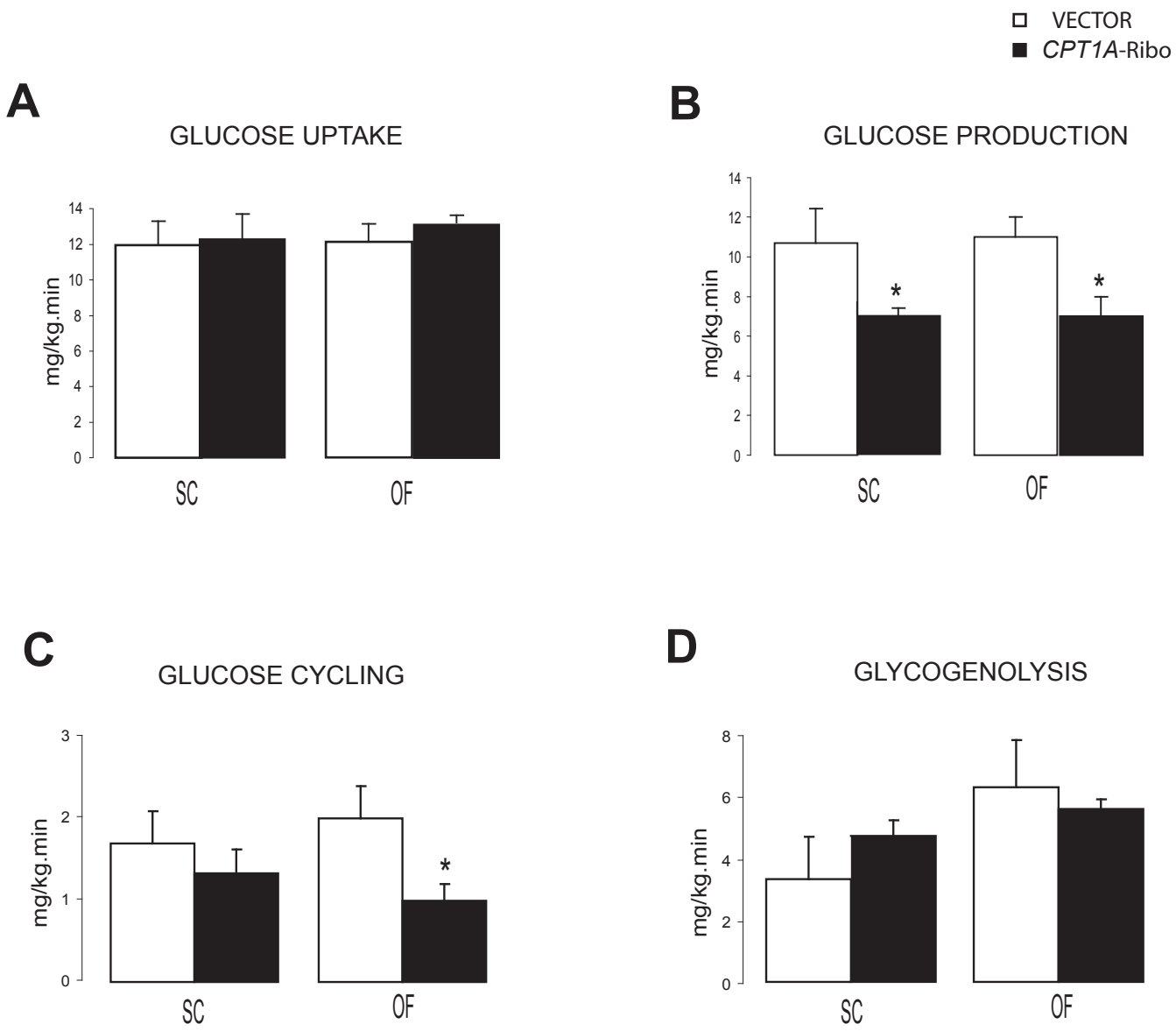

Suppl Figure 2




Suppl Figure 3




Suppl Figure 4




Suppl Figure 5

**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1**: A. Acyl CoA hydrolase (ACH) activity in arcuate nuclei obtained from standard chow (SC) and overfed (OF) rats. B. CPT1A Inhibitor and Inactive stereoisomer (Control). C. *CPT1A*-Ribozyme.

**Supplemental Figure 2:** A. Western blot analysis of MBH AMPK and FAS in SC and OF rats . B. Q-PCR of MBH SOCS3 mRNA in SC and OF rats. C. Preference ratio of saccharine intake over total fluid intake after a two-bottle choice at 24 h. Conditioned taste adversion (CTA) was induced by i.p. lithium chloride (LiCl) but not by ICV CPT1-inhibitor. *P<0.05 versus control

**Supplemental Figure 3**: (A,C) Daily food intake after a single ICV injection on day 0 of two different doses of the CPT1A inhibitor, or of its inactive stereoisomer (Control) in rats fed a standard chow diet (SC) or overfed (OF). (B) ICV administration of CPT1A inhibitor decreased expression of neuropeptide Y (NPY) and agouti-related protein in MBH. SC, standard show; OF, rats fed with high fat diet ad libitum for three days followed by one day in which they received the same amount of food eaten by the rats treated with CPT1A inhibitor (OF+CPT). (E,F) Effect of a single ICV injection of *CPT1A*-Ribo or control VECTOR on food intake in SC and OF rats.

**Supplemental Figure 4:** *Pharmacological inhibition of hypothalamic* CPT1A *improved hepatic glucose homeostasis in overfed rats*

Effect of central inhibition of CPT1A on (A) Glucose uptake and (B) Glucose production during pancreatic-insulin clamp in rats fed a standard chow diet (SC), pair-fed to the SC group with high fat diet (PF) or overfed (OF). CPT1A Inhibitor markedly suppressed glucose cycling (C), while glycogenolysis (D) was not modified. *$P$< 0.05 vs inactive stereoisomer.

**Supplemental Figure 5**: *Molecular inhibition of hypothalamic CPT1A improved hepatic glucose homeostasis in overfed rats.*

(A) Glucose uptake and (B) Glucose production in rats fed a standard chow diet (SC) or overfed (OF) treated with ICV administration of *CPT1A*- Ribo or VECTOR control two days before the clamp. *CPT1A*-Ribo markedly suppressed glucose cycling (C), while glycogenolysis (D) was not modified. **P*< 0.05 vs VECTOR treated rats.

**Suppl. Table 1.** Pharmacological inhibition of Hypothalamic CPTIA: specific activities of hepatic substrates used to calculate the "direct pathway" and the "indirect pathway"

| | SC | | PF | | OF | |
|---|---|---|---|---|---|---|
| | **Control** | **CPT1A i** | **Control** | **CPT1A i** | **Control** | **CPT1A i** |
| N | 5 | 7 | 5 | 5 | 6 | 4 |
| 3H-Glucose Plasma SA (dpm/nmol) | 44.5±1.1 | 52.7±4.5 | 35.0±4.8 | 41.4±5.8 | 50.5±4.1 | 45.4±8.3 |
| 3H-UDPglucose Liver SA (dpm/nmol) | 8.1±0.6 | 6.8±0.6 | 6.4±1.2 | 7.0±1.5 | 8.0±1.5 | 5.9±0.3 |
| % Direct | 18.1±1.2 | 12.1±0.9 | 18.4±3.6 | 17.4±3.6 | 16±2.9* | 21.2±1.6 |
| 14C-PEP (dpm/nmol) | 9.6±1.9 | 9.7±1.5 | 9.7±1.7 | 3.4±0.4 | 8.4±2.1 | 9.6±0.9 |
| 14C-UDP glucose (dpm/nmol) | 6.5±1.0 | 5.8±0.8 | 8.2±1.4 | 3.2±0.5 | 6.3±2.2 | 5.9±1.1 |
| % Indirect | 43.3±8.6 | 52.2±16.2 | 43.3±3.0 | 47.5±6.5 | 42.7±10.7 | 31.1±5.7 |

Data are means±SEM.*p<0.05 vs vector treated animals. SC, standard chow fat diet; PF, calories intake matched to the group receiving SC with high fat diet; OF, overfed. CPT1Ai, CPT1A-Inhibitor. *p<0.05 vs respective vehicle control.

**Suppl. Table 2.** Molecular Inhibition of Hypothalamic *CPT1A*: specific activities of hepatic substrates used to calculate the "direct pathway" and the "indirect pathway"

| | SC | | OF | |
|---|---|---|---|---|
| | **Pair-fed VECTOR** | ***CPT1A*-Ribo** | **Pair-fed VECTOR** | ***CPT1A*-Ribo** |
| N | 4 | 5 | 7 | 4 |
| 3H-Glucose Plasma SA (dpm/nmol) | 52.6±6.3 | 56.6±6.4 | 53.3±2.6 | 44.6±4.9 |
| 3H-UDPglucose Liver SA (dpm/nmol) | 7.0±0.7 | 8.0±1.6 | 7.1±2.4 | 4.4±0.8 |
| % Direct | 13.6±1.3 | 14.2±2.7 | 14.4±2.5 | 11.1±1.0 |
| 14C-PEP (dpm/nmol) | 8.6±0.6 | 10.7±0.8 | 9.4±1.2 | 11.65±1.8 |
| 14C-UDP glucose (dpm/nmol) | 8.1±1.0 | 6.1±1.2 | 7.9±1.6 | 5.8±1.0 |
| % Indirect | 48.1±6.9 | 29.8±6.9 | 39.0±8.2 | 25.2±2.9* |

Data are means±SEM.*$p<0.05$ vs vector treated animals. SC, standard chow diet; OF, overfed.

## Suppl. Table 3. DIET COMPOSITION

| **Calories provided** | **Rodent Diet (Standard Chow)** | **Rodent Diet with 10% Lard* (High Fat Diet)** |
|---|---|---|
| Carbohydrate (%) | 60 | 45 |
| Protein (%) | 28 | 22 |
| Fat (%)<br><br>Saturated<br>Monounsaturated<br>Polyunsaturated | 12<br><br>3.1<br>4.7<br>5.0 | 33<br><br>9.4<br>11.2<br>4.9 |
| Total Calorie Provided by Digestible Nutrients (Kcal/g) | 3.00 | 5.14 |

*Lard composition:2% myristic acid, 24% palmitic acid, 13% stearic acid, 46% oleic acid, 12% linoleic acid.