

## Supplemental data

### Anti-diabetic effect and mode of action of cytopiloyne

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**Supplemental Table 1.** Survival rate of db/db mice during long-term treatments.

Treatment	Survival rate (%)			
	6	10	20	22 (week)
Vehicle (n=4)	100	100	75	75
GLM 2.5 mg/kg (n=4)	100	100	100	75
CP 0.5 mg/kg (n=7)	100	100	100	100
CP 2.5 mg/kg (n=7)	100	100	100	100

Each group of db/db mice aged at 6 to 8 weeks were tube-fed with vehicle, cytopiloyne (0.5 or 2.5 mg/kg/day) or glimepiride (2.5 mg/kg/day) once a day. Survival rate was monitored for the indicated time. The number of mice used is indicated in parentheses in the first column.

20 **Supplemental Figure Legends**

21 **Supplemental Figure 1. GF109203X can inhibit PKC $\alpha$  phosphorylation in  $\beta$**   
22 **cells.** RIN-m5F cells were pre-treated with GF109203X (GF), a pharmacological  
23 agent, for 24 h and stimulated with PMA for 5 min. The cells were lysed and  
24 underwent Western blot with anti-phospho-PKC $\alpha$  and anti-actin antibodies.

25

26 **Supplemental Figure 2. Prophylactic effect of cytopiloyne in db/db mice.** (A)  
27 Two groups of diabetes-free db/db mice aged 4 weeks were daily tube-fed with  
28 vehicle (1  $\mu$ l DMSO per 1 ml of PBS) or cytopiloyne (CP, 0.5 mg/kg), for 4  
29 weeks. Postprandial blood glucose levels in these mice were measured. (B)  
30 Blood insulin levels from the above mice (A) were measured. Results from 3  
31 independent experiments are expressed as mean  $\pm$  SEM, and  $P$  (\*) < 0.05 was  
32 considered to be statistically significant. The mouse number (n) is indicated in  
33 parentheses.

34

35 **Supplemental Figure 3. Cytopiloyne dose-dependently enhances insulin**  
36 **release in the medium containing different doses of glucose.** Rat pancreatic  
37 islets were incubated with KRB buffer containing vehicle, glimepiride (GLM, 10  
38  $\mu$ M) or cytopiloyne at 1.5, 3, 7, 14, 28 and 42  $\mu$ M in the presence of 16.7 mM  
39 glucose (HG, upper row) or 3.3 mM glucose (LG, middle row). Similarly, rat  
40 pancreatic islets were incubated with glucose-free KRB buffer containing vehicle  
41 or cytopiloyne at 1.5, 3, 7, 14, 28 and 42  $\mu$ M (lower row). The insulin levels  
42 were determined using an insulin ELISA kit. The data are presented as mean  $\pm$   
43 SEM of 3 independent experiments. All the results from 3 independent  
44 experiments are expressed as mean  $\pm$  SEM, and  $P$  (\*) < 0.05 was considered to  
45 be statistically significant.

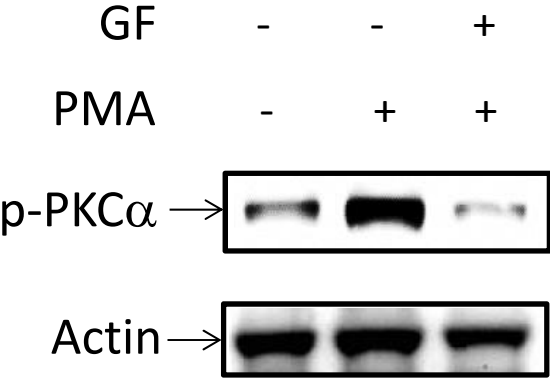
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47 **Supplemental Figure 4. Cytopiloyne-mediated insulin release depends on**  
48 **calcium and potassium channels.** Rat pancreatic islets were incubated with  
49 KRB buffer containing vehicle or cytopiloyne (CP, 42  $\mu$ M) in the presence of  
50 calcium channel inhibitors (10  $\mu$ M EGTA and 1  $\mu$ M nimodipine (Nimo)) and  
51 potassium channel opener (100  $\mu$ M diazoxide (Diaz)) for 30 min. Insulin levels  
52 in KRB buffer were determined. All the data are presented as mean  $\pm$  SEM of 3  
53 independent experiments and  $P$  (\*) < 0.05 was considered to be statistically  
54 significant.

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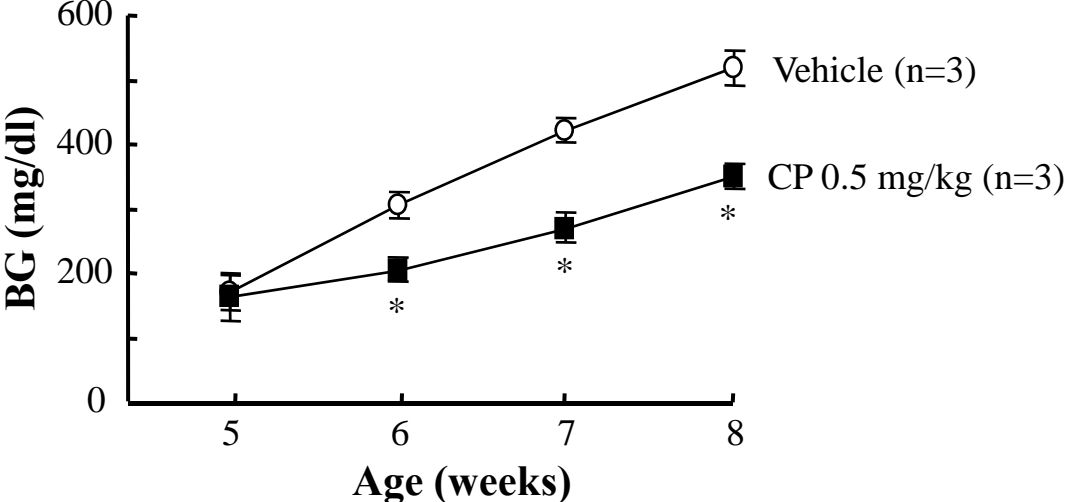
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# Supplemental Figure 1

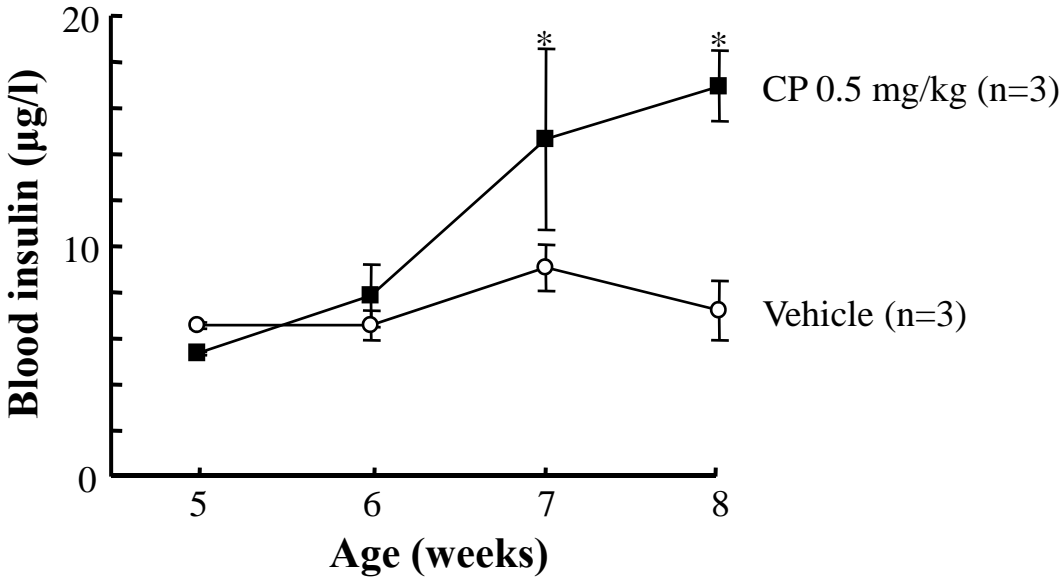


# Supplemental Figure 2

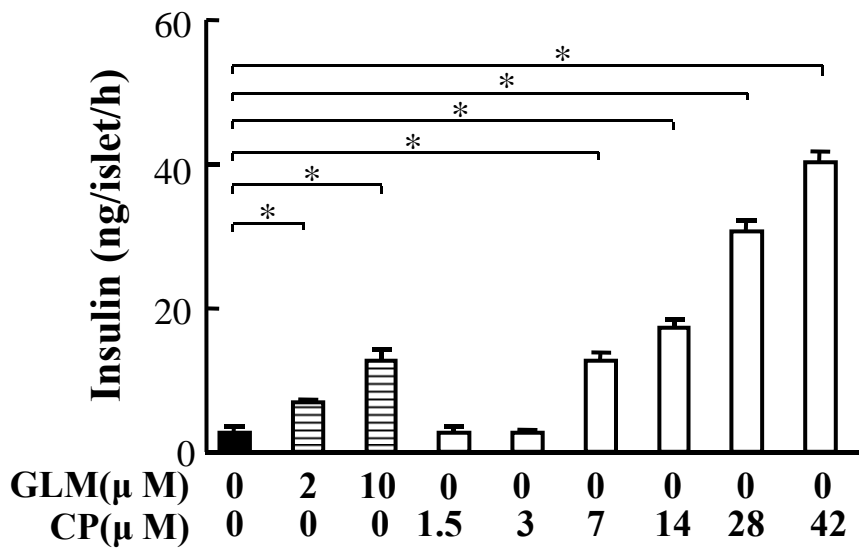
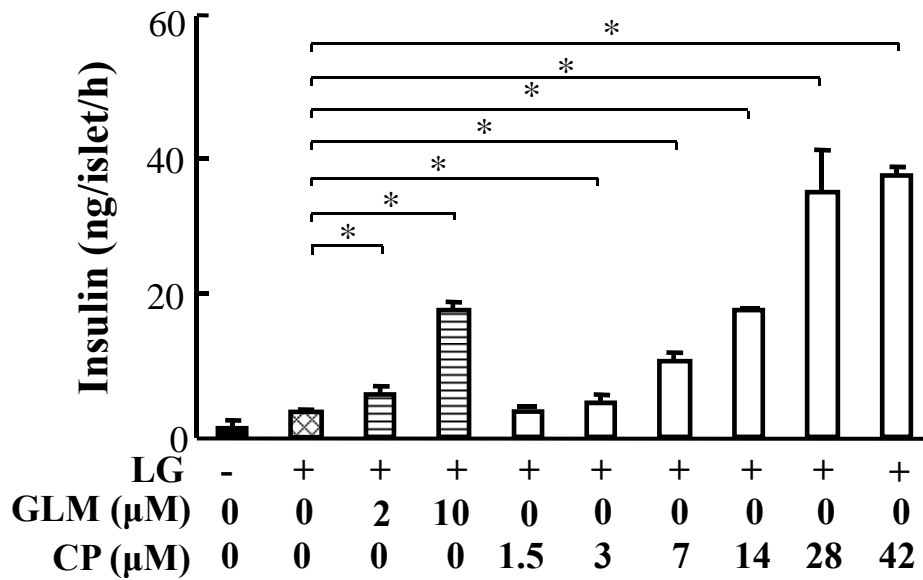
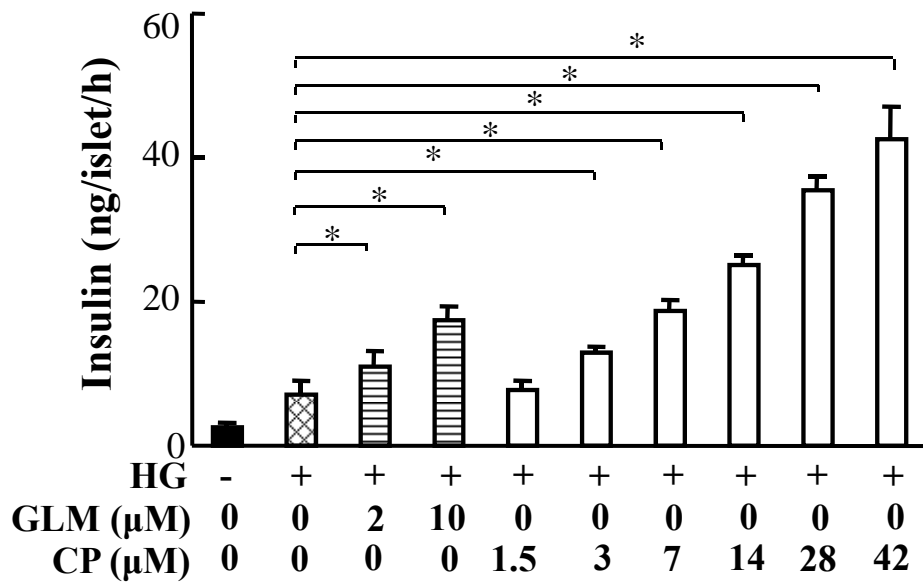
A



B



### Supplemental Figure3



# Supplemental Figure 4

