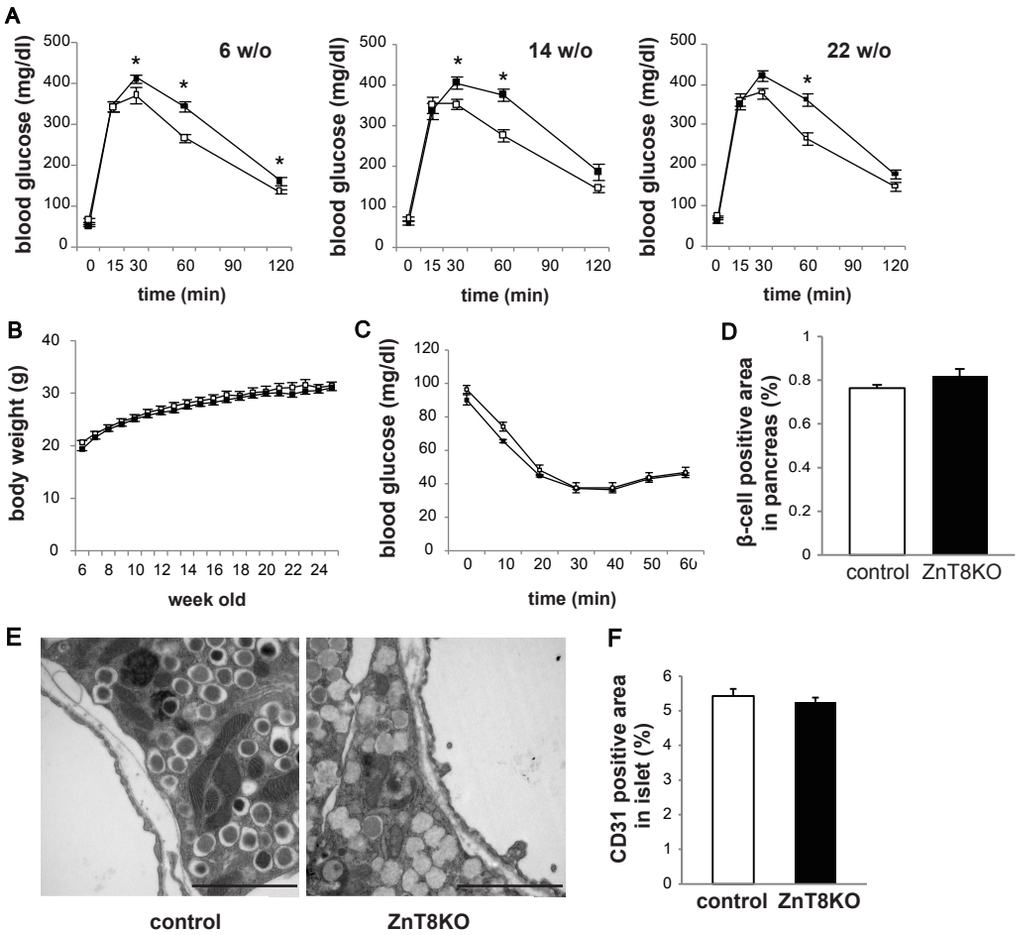
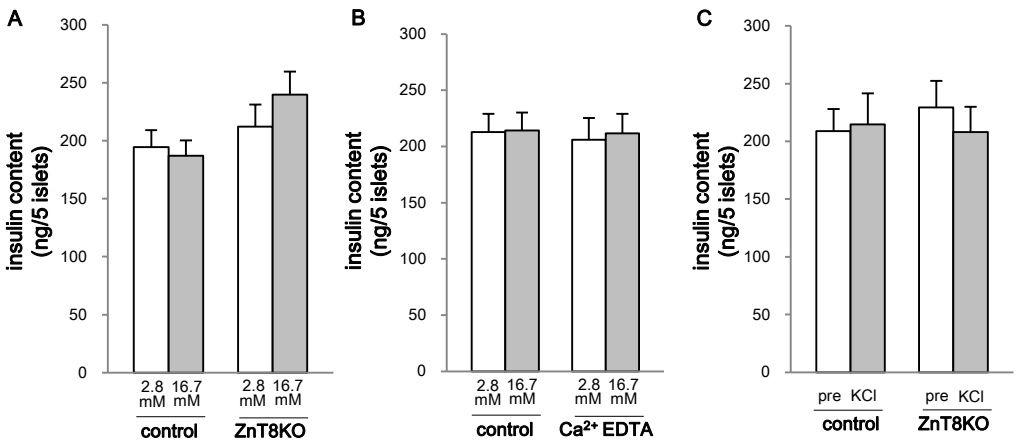
Supplemental Figure 1.



Supplemental Figure 1. Characteristics of β-cell-specific Slc30a8/ZnT8 deficient mice

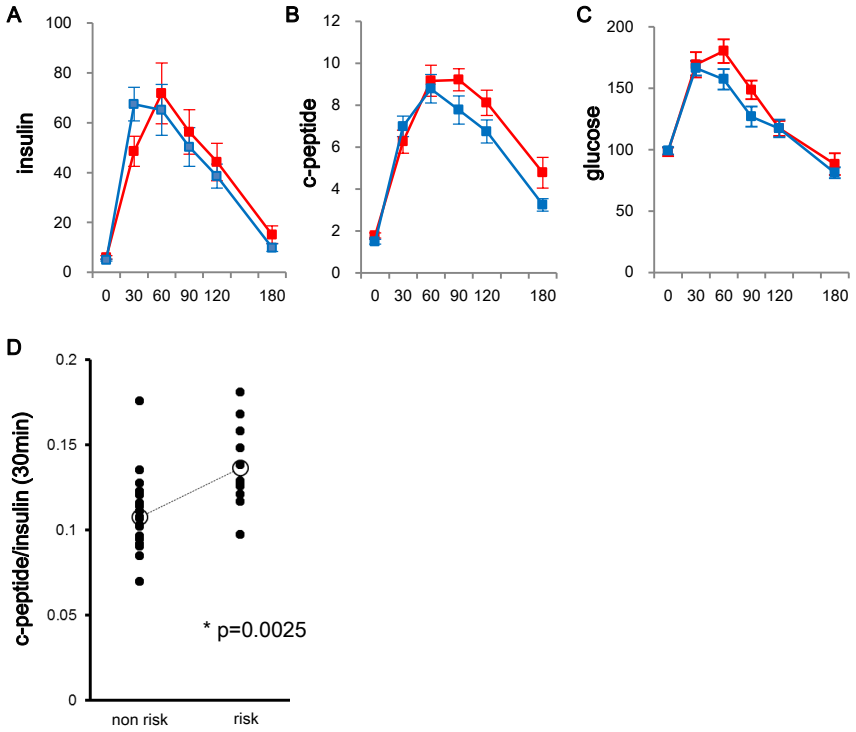
(A) Glucose tolerance after intraperitoneal injection of glucose (2.0 g/kg body weight) in 6-week-old (left), 14-week-old (center) and 22-week-old (right) control mice (open squares, n=12) and ZnT8KO mice (closed squares, n=15). (B) Body weight of control mice (open squares, n=21) and ZnT8KO mice (closed squares, n=16). (C) Insulin tolerance test in overnight fasted control mice (open squares, n=6) and ZnT8KO mice (closed squares, n=6). (D) Relative area of β-cells in pancreas (%) of control mice (open bars, n=11) and ZnT8KO mice (solid bars, n=11). (E) Electron microscopic analysis of vessel endothelial structure in islets. Representative micrographs of vessel endothelium. Bar = 2 μm. There were no differences in vessel endothelial structure or basement membrane. (F) Relative area of CD31-positive cells in islets (%) of control mice (open bars) and ZnT8KO mice (solid bars). Data are mean ± SEM. *p<0.05, versus control mice (by unpaired t test).

Supplemental Figure 2.



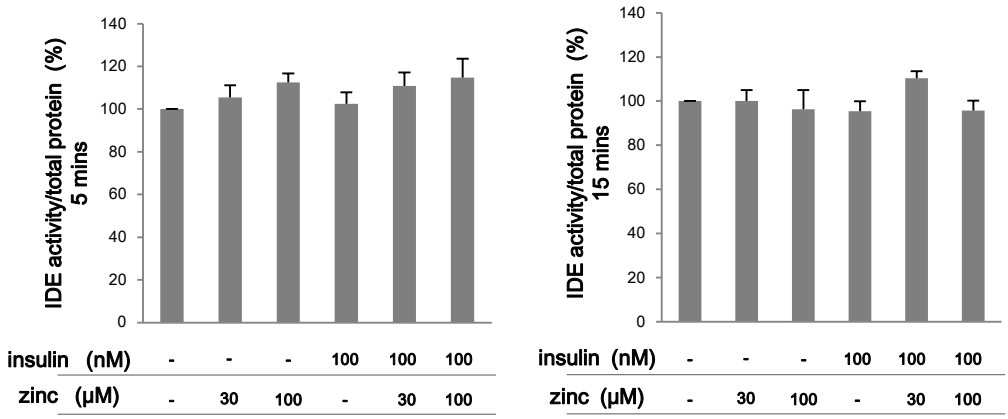
Supplemental Figure 2. Insulin content of isolated islets.
Islets were incubated with (A) 2.8 mM and 16.7 mM glucose, (B) the same concentrations of glucose and 2.5 mM calcium-EDTA, or (C) 2.8 mM glucose (pre) and 50 mM KCl (KCl). Data are mean ± SEM.

Supplemental Figure 3.



Supplemental Figure 3. Results of 75 g OGTT in humans.
(A) Insulin, (B) C-peptide, and (C) glucose in T/T allele (n=19, blue) and C/C allele (n=12, red) individuals. (D) C-peptide/insulin ratio measured at 30 min in T/T allele and C/C allele individuals. Data are the mean ± SEM. *p<0.05, between the T/T and C/C allele groups (by unpaired t-test).

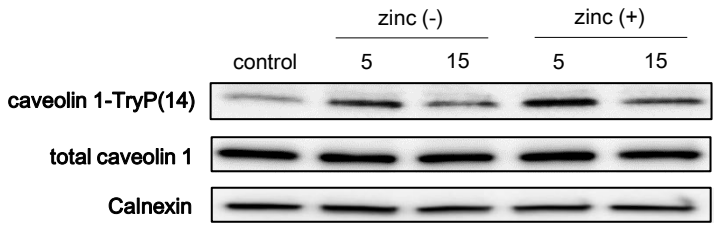
Supplemental Figure 4.



Supplemental Figure 4. IDE activity in HepG2 cells

The cells were incubated with insulin (100 nM) and/or zinc chloride (0, 30, 100 μM) for 5 or 15 mins. IDE activity is expressed relative to the total protein concentration. Data are mean ± SEM.

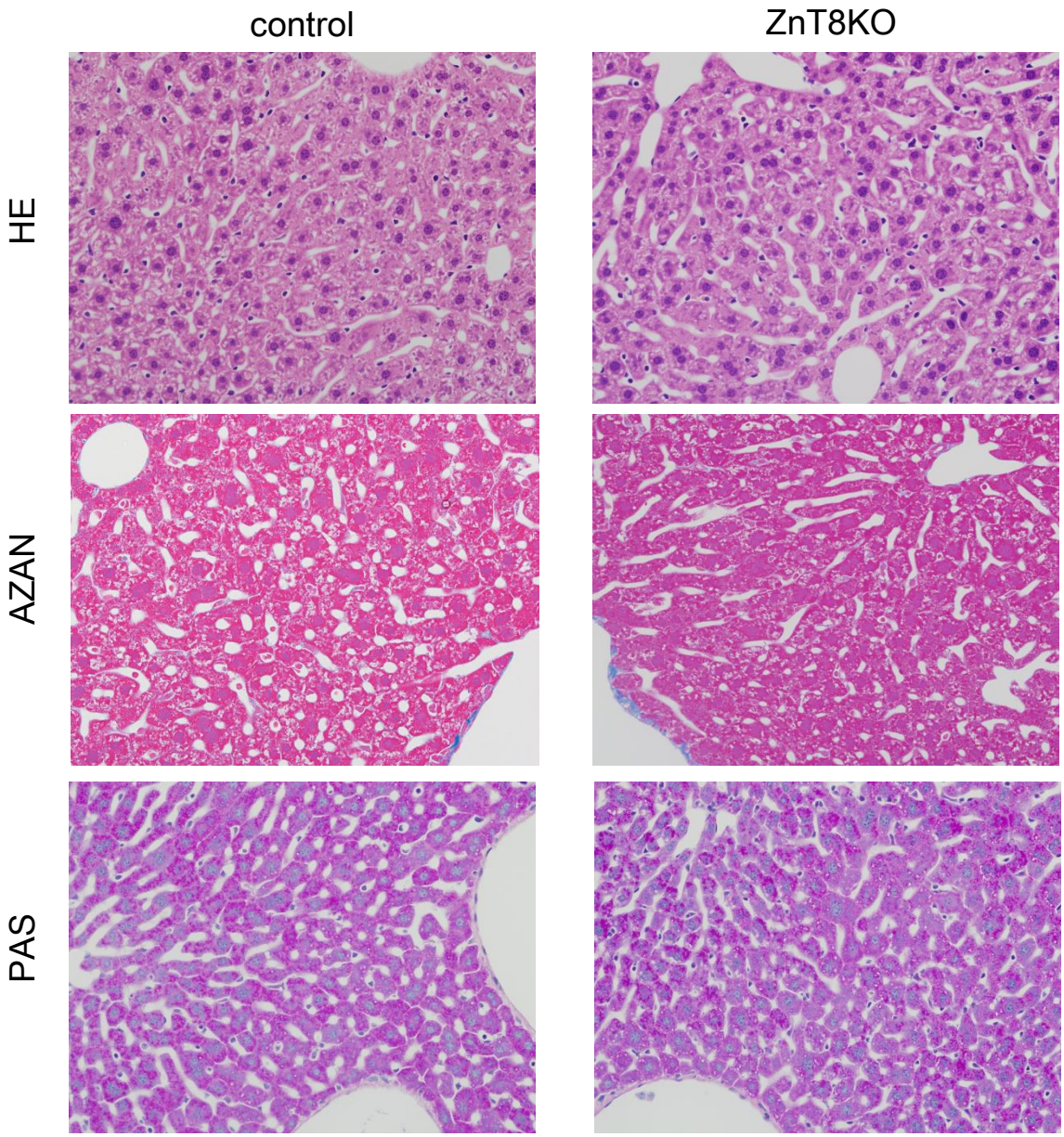
Supplemental Figure 5.



Supplemental Figure 5. Zinc effect on caveolin-dependent insulin receptor internalization

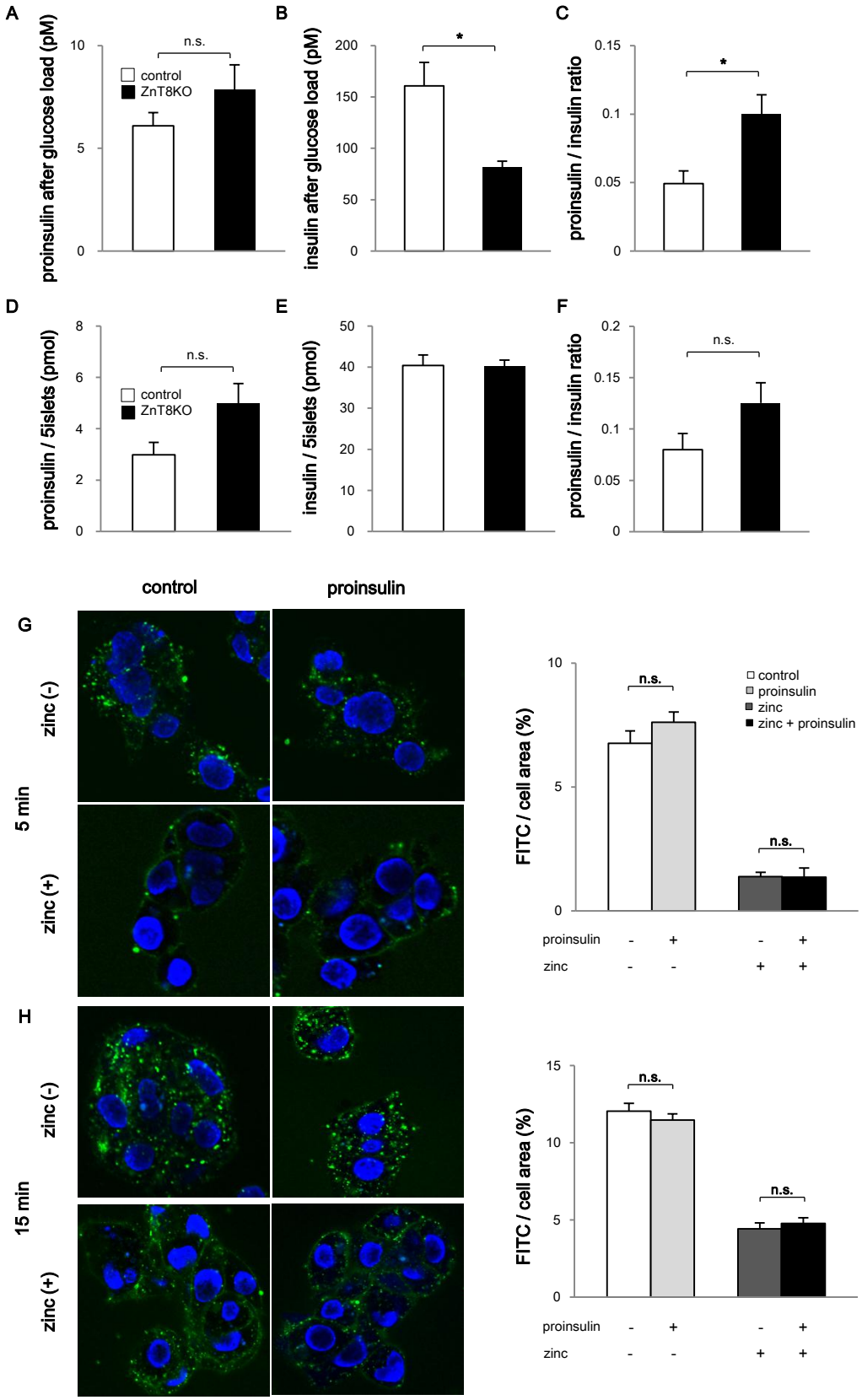
HepG2 cells were treated with 100 nM insulin with or without 100 μM zinc. The cells' plasma membrane fraction was collected and immunoblotted for caveolin 1-TryP(14), total caveolin 1, and calnexin.

Supplemental Figure 6.



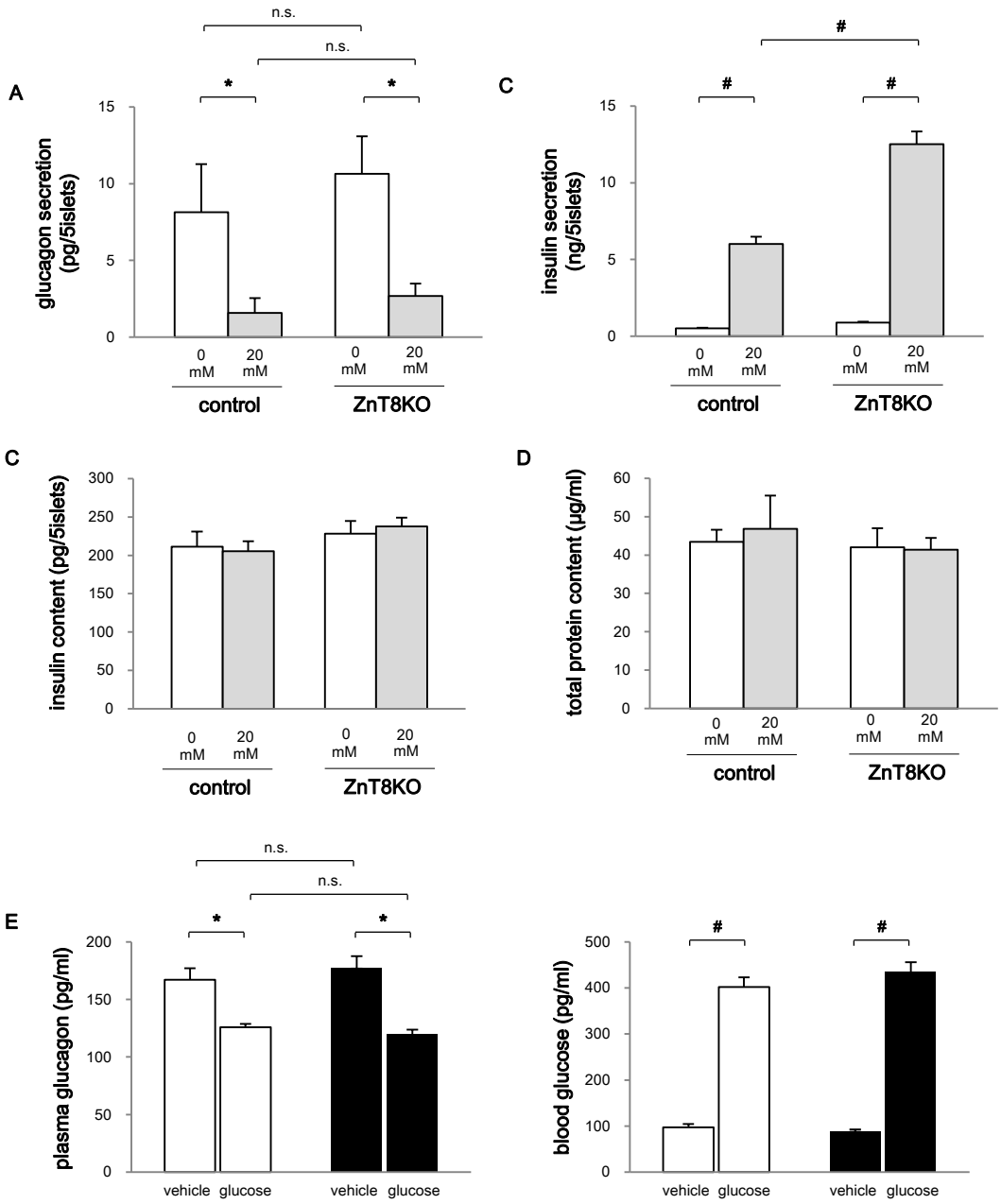
Supplemental Figure 6. Liver staining
HE (upper), AZAN (middle) and PAS (lower) staining for liver of control and ZnT8KO mice.

Supplemental Figure 7.



Supplemental Figure 7. Increased proinsulin level in ZnT8KO mice.
(A, B) Blood proinsulin (A) and insulin (B) levels in 15 min after glucose i.v.. (C) Proinsulin / Insulin ratio after glucose i.v.. (D, E) Proinsulin (D) and Insulin (E) content in islets. (F) Proinsulin / Insulin ratio in islets. (G, H) Effect of zinc and proinsulin on endocytosis of FITC-conjugated insulin in HepG2 cells at 5min (G) and 15 min (H). Bar graph represents FITC-positive area/whole cell area (%). Bar graph represents FITC-positive area/whole cell area (%). Data are mean ± SEM. *P<0.05, by unpaired t-test.

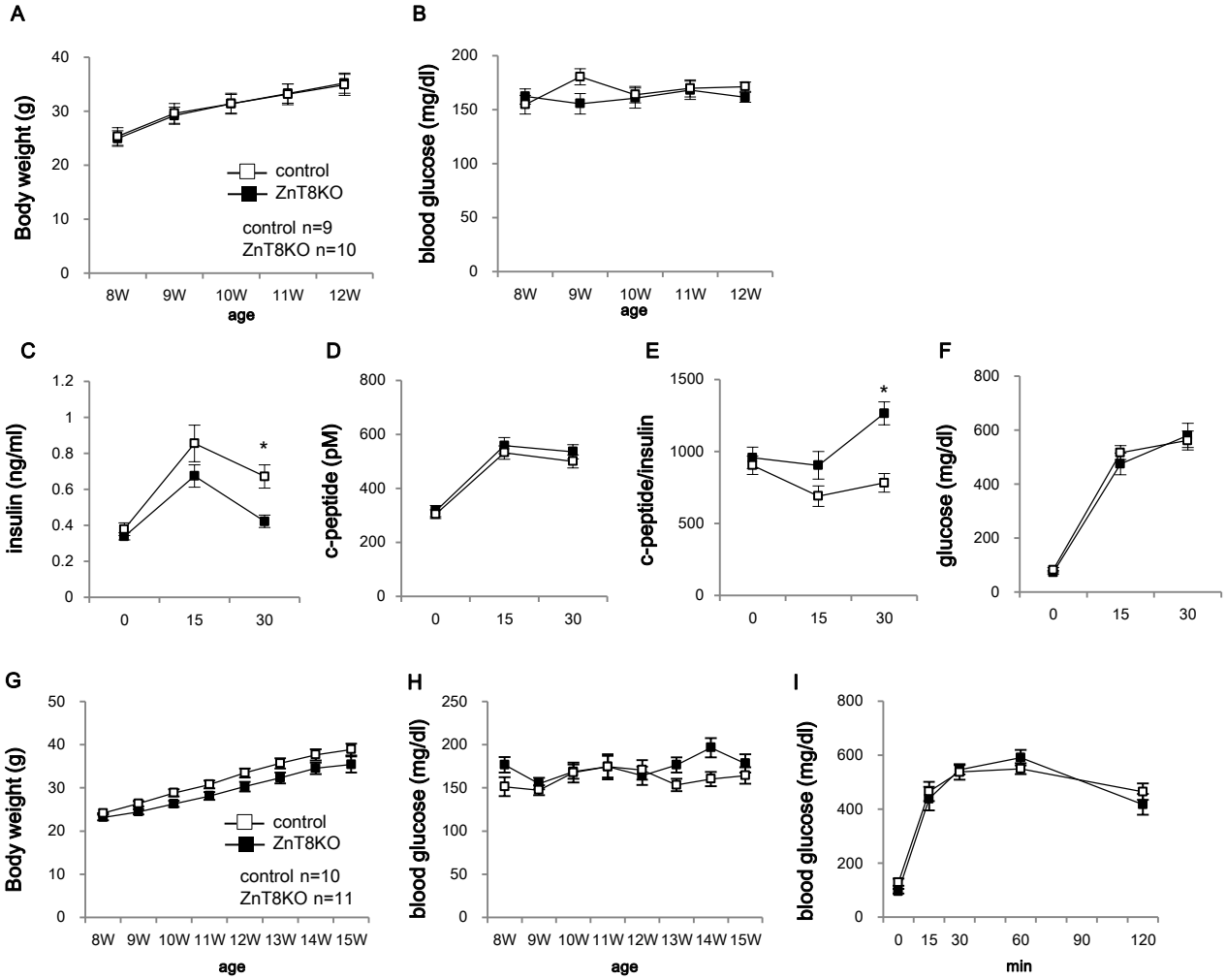
Supplemental Figure 8.



Supplemental Figure 8. Glucagon secretion

(A) Glucagon secretion from isolated islets incubated with 0 mM (white bars, n=8) and 20 mM glucose (gray bars, n=8). (B) Insulin secretion from isolated islets. Same specimen were handled in (A) and (B). (C, D) Insulin content (C) and total protein content (D) in each islets. In all panels, data are mean ± SEM. (E) Glucagon (left) and blood glucose (right) levels were measured at 30 min after vehicle (control mice; open bars, n=7. ZnT8KO mice; solid bars, n=6) or glucose i.p. (2 g/kg body weight (control mice; open bars, n=5. ZnT8KO: solid bars, n=8). *P<0.05, versus *control* mice (by unpaired t-test). #p<0.05 (by non-repeated ANOVA). *n.s.:* no significant difference (by unpaired t-test and non-repeated ANOVA).

Supplemental Figure 9.

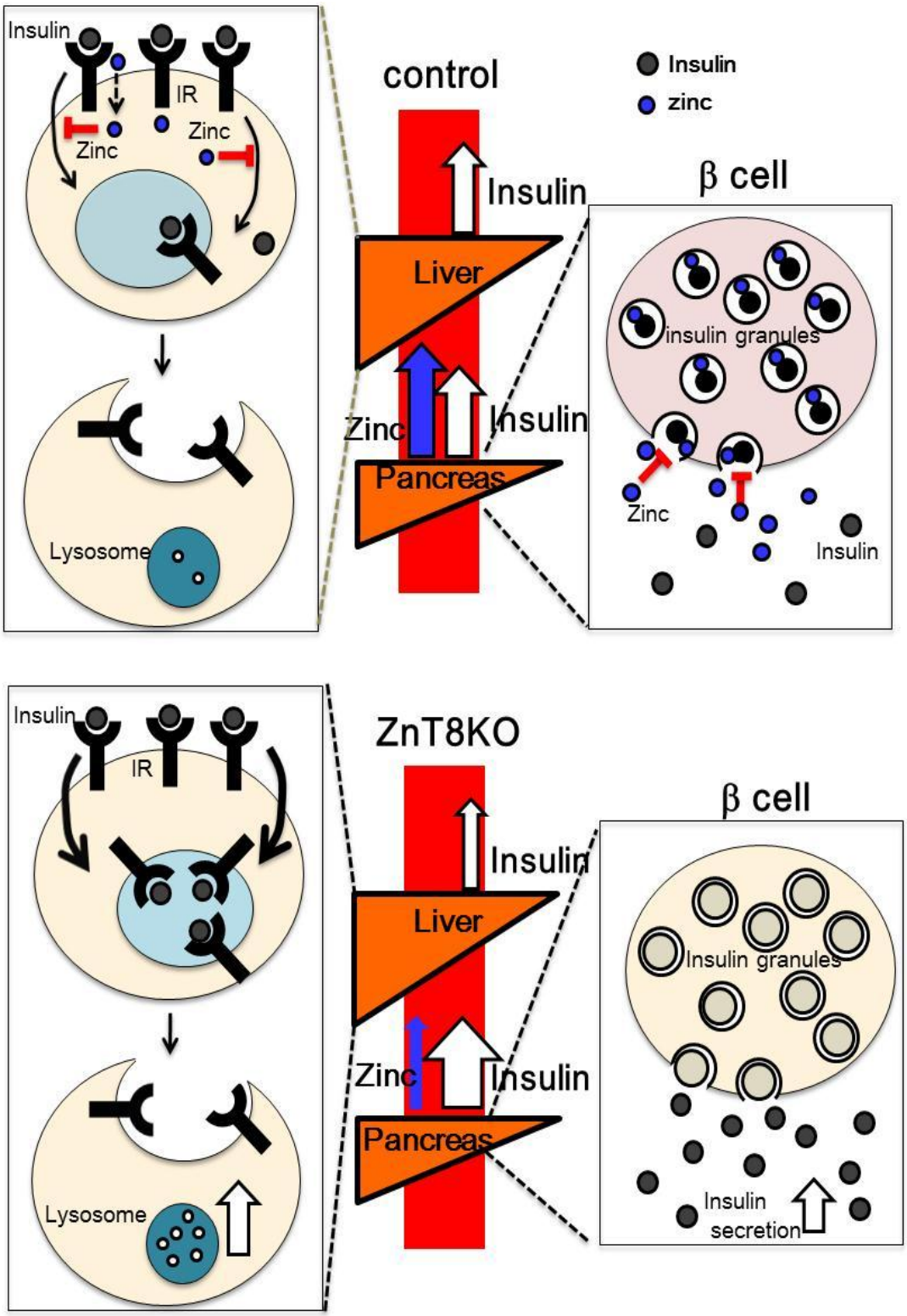


Supplemental Figure 9. High fat diet

(A) Body weight, (B) fed blood glucose levels were repeatedly measured in High Fat diet fed control (n=9, white) and ZnT8KO mice (n=10, black). (C-F) After 4 week HF diet presented in (A, B), Insulin (C), c-peptide (D), c-peptide insulin ratio (E), and glucose levels (F) after 2.0 mg/kg glucose i.p. were measured.

(G-I) Effect of longer High Fat Diet. Body weight (G), fed blood glucose levels (H) and blood glucose levels after glucose i.p. (I) were repeatedly measured in High Fat diet fed control (n=10, white) and ZnT8KO mice (n=11, black). In all panels, data are mean ± SEM. *P<0.05, versus control, by unpaired t-test.

Supplemental Figure 10.



Supplemental Figure 10. Schematic representation of insulin clearance in *control* and *ZnT8KO* mice

Zinc co-secreted with insulin suppresses the insulin secretion from pancreatic β cells (upper-right) and inhibits hepatic insulin clearance (upper-left) in *control* mice. While the reduced zinc secretion allows enhanced insulin secretion from b cells (lower-right), the hepatic insulin clearance is not suppressed (lower-left) in *ZnT8KO* mice. Thus, the peripheral insulin level in *ZnT8KO* mice is maintained at a lower level than that in *control* mice.

Supplemental Table 1. Biochemical markers of ZnT8KO mice

	control (n=4)	ZnT8KO (n=5)	*p* value*
Total protein (g/dL)	4.5 (0.04)	4.6 (0.04)	0.46
Albumin (g/dL)	3.1 (0.2)	3.1 (0.03)	0.20
Albumin/Globlin	2.1 (0.00)	2.2 (0.03)	0.23
serum Fe (μg/dL)	120.0 (5.68)	112.8 (4.89)	0.50
AST（IU/L)	36.8(1.91)	39.0 (2.09)	0.82
ALT（IU/L)	19.0 (0.63)	21.3 (0.91)	0.26
Alkaline phosphatase（IU/L)	166.3 (5.70)	187.8 (6.35)	0.09
Leucine aminopeptidase（IU/L)	49.0 (0.55)	54.8 (1.83)	0.12
γ-GTP（IU/L)	3>	3>	n.s.
Cholinesterase（IU/L)	20.8 (0.97)	24.0 (0.70)	0.08
Total cholesterol (mg/dl)	86.3 (3.10)	91.8 (4.08)	0.50
Triglyceride (mg/dl)	51.0 (2.93)	61.8 (10.40)	0.48
Low-density lipoprotein-cholesterol (mg/dl)	5.0 (0.32)	5.0 (0.48)	0.80
High-density lipoprotein-cholesterol (mg/dl)	44.0 (1.48)	47.5 (2.70)	0.41
Total bilirubin (mg/dL)	0.11 (0.02)	0.08 (0.01)	0.33
Direct bilirubin (mg/dL)	0.03 (0.01)	0.06 (0.00)	0.67
Indirect bilirubin (mg/dL)	0.08 (0.01)	0.06 (0.00)	0.22
Total bile acid (μmol/L)	18.0 (11.67)	7.0 (3.25)	0.45

Data are mean (SD).

*by unpaired t-test.