

Supplemental Material

Supplemental Figure Legends

Supplemental Fig. 1. Brush Cells Are Eliminated in the GI Tract of *Skn-1* KO Mice

(a) Immunostaining revealed that the Trpm5 protein was present in the duodenum of WT mice (upper panel) but absent in *Skn-1* KO mice (lower panel). By contrast, of ChgA and GLP-1 signals were observed in WT (upper panels) and *Skn-1* KO mice (lower panels). Arrowheads indicate signals.

(b) Double immunostaining indicated that nearly all Trpm5-positive cells were also positive for Dclk1. Arrows indicate cells expressing Trpm5 and Dclk1. Scale bars: 100 μm .

Supplemental Fig. 2. Metabolic Phenotypes of *Skn-1* KO Mice Fed a Normal Chow Diet

(a) Although body weight at birth did not differ significantly between *Skn-1* KO and WT littermates (n=60–62), *Skn-1* KO mice exhibited lower body weight than WT littermates at 3 weeks of age under normal chow diet conditions (n=25–33).

(b) There were no significant differences in RER between *Skn-1* KO and WT littermates (n=8–9).

(c) There were no significant differences in spontaneous motor activity between *Skn-1* KO and WT littermates (n=8–9). The data are presented as the mean \pm SEM; *p<0.05.

Supplemental Fig. 3. Metabolic Phenotypes of *Skn-1* KO Mice Fed a High-Fat Diet

No differences in food intake (n=8-10) (a), fecal triacylglycerol (n=8-10) (b), or fecal energy (n=5-9) (c) were observed between *Skn-1* KO and WT littermates at 15 weeks of

age. The data are presented as the mean \pm SEM.

Supplemental Fig. 4. Expression of Catecholamine Biosynthetic Enzymes in the Adrenal Gland

qRT-PCR analysis revealed that gene expression of catecholamine biosynthetic enzymes, including tyrosine hydroxylase (Th), dopa decarboxylase (Ddc), dopamine- β -hydroxylase (Dbh), and phenylethanolamine N-methyltransferase (Pnmt), in the adrenal gland was comparable between *Skn-1* KO and WT littermates (n=5). The data are presented as the mean \pm SEM.

Supplemental Fig. 5. Insulin Secretion and Insulin Resistance in *Skn-1* KO Mice Fed a Normal Chow Diet

(a) Plasma total GIP was comparable between *Skn-1* KO and WT littermates for 60 min after glucose gavage (3.0 mg/g of body weight) (n=6–7).

(b) Plasma active GLP-1 was comparable between *Skn-1* KO and WT littermates for 40 min after glucose gavage (5.0 mg/g of body weight) (n=6–7).

(c) Insulin secretion from isolated islets in response to 25 mM glucose was comparable between *Skn-1* KO and WT littermates (n=10).

(d and e) Blood glucose (d) and plasma insulin (e) after intraperitoneal administration of glucose (1.5 mg/g of body weight). Blood glucose was comparable between *Skn-1* KO and WT littermates for 120 min after administration (n=10–13) (d). Plasma insulin was also comparable between *Skn-1* KO and WT littermates before and 15 min after administration (n=17–20) (e).

(f) ITT revealed that insulin resistance was comparable in *Skn-1* KO and WT littermates

(n=12). The data are presented as the mean \pm SEM.

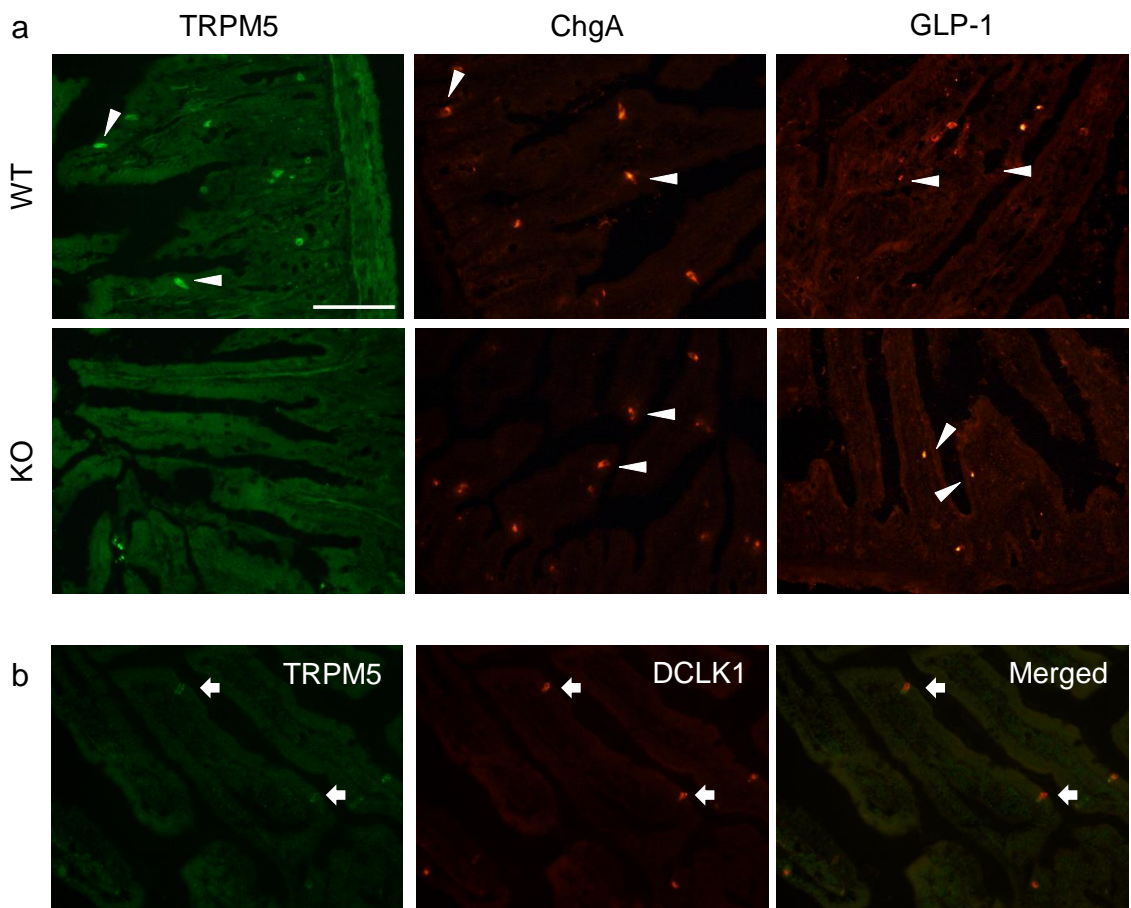


Figure S1 Ushiyama et al.

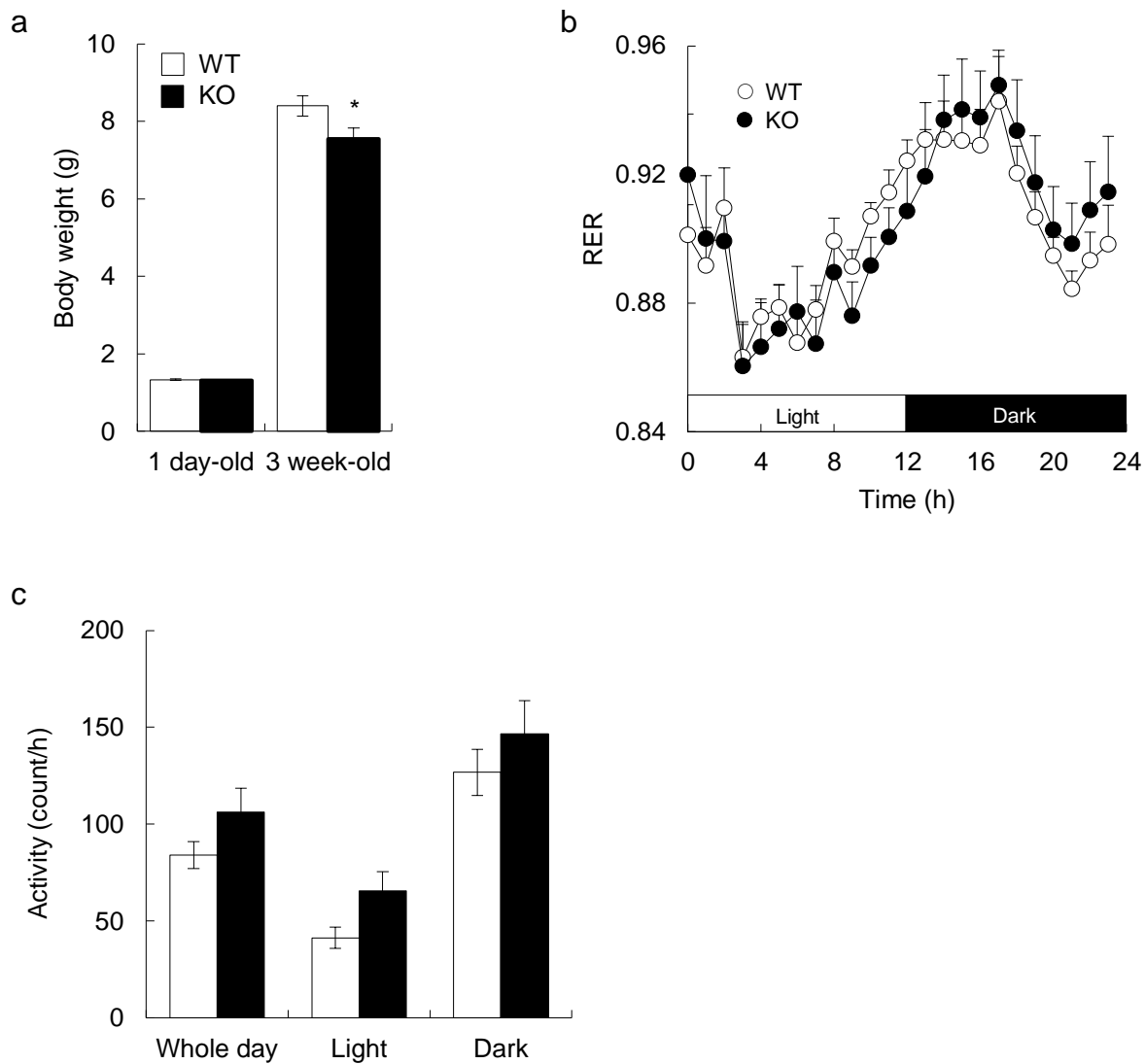


Figure S2 Ushiyama et al.

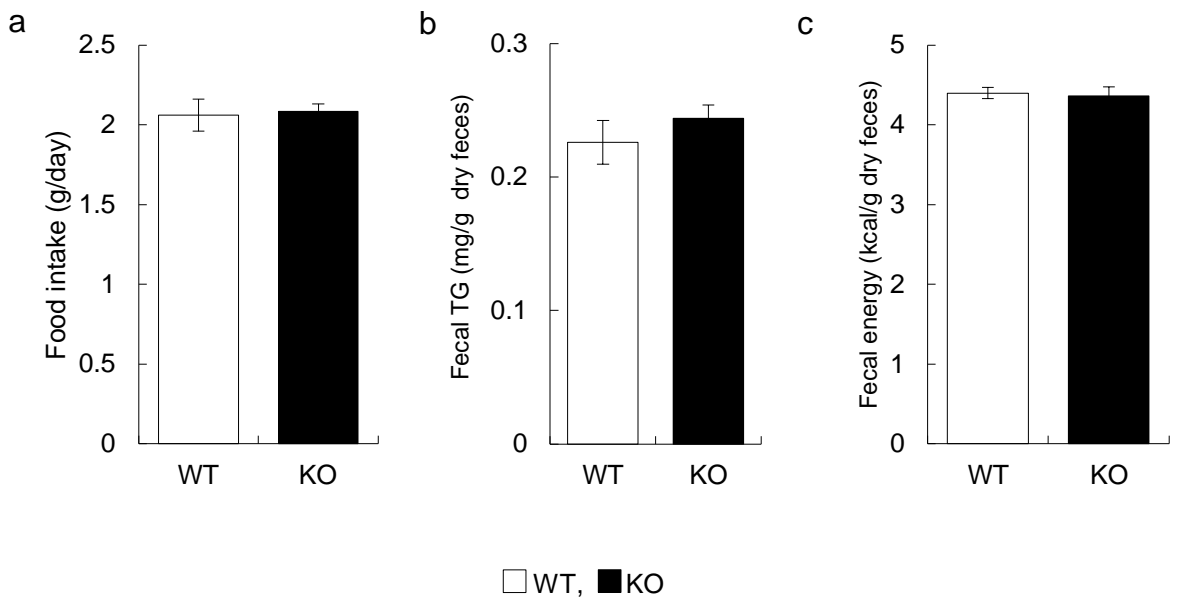


Figure S3 Ushiyama et al.

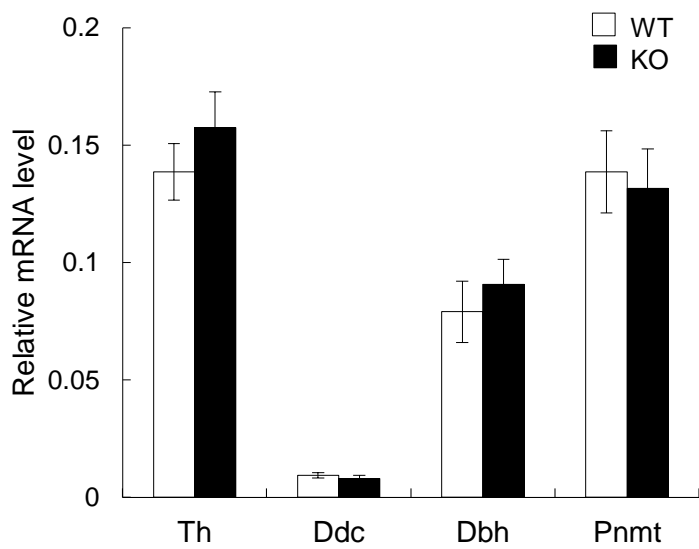
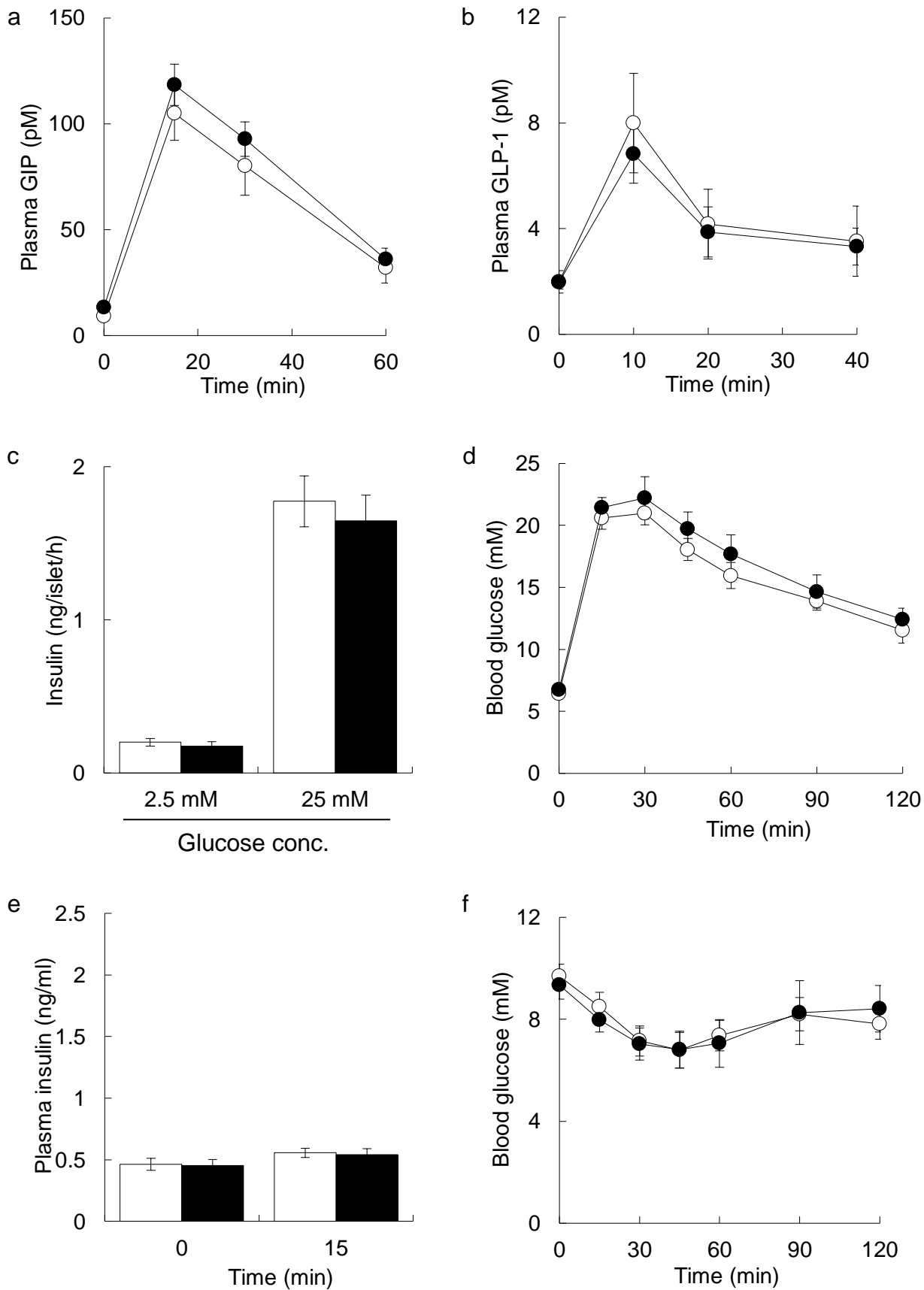


Figure S4 Ushiana et al.



○/□ WT, ●/■ KO

Figure S5 Ushima et al.

Table S1 Blood serum components of CD-fed animals under ad lib feeding schedule

	WT (n=28)	KO (n=18)
Total protein (g/dL)	5.1 ± 0.1	5.3 ± 0.1
AST (IU/L)	184 ± 21	181 ± 22
ALT (IU/L)	40 ± 3	40 ± 3
ALP (IU/L)	221 ± 10	241 ± 15
LDH (IU/L)	528 ± 38	599 ± 72
T-CHO (mg/dL)	103 ± 6	118 ± 7
F-CHO (mg/dL)	28 ± 1	32 ± 2*
E-CHO (mg/dL)	75 ± 5	86 ± 6
Total lipid (mg/dL)	354 ± 25	412 ± 33
Triglyceride (mg/dL)	105 ± 13	124 ± 17
NEFA (μEq/L)	454 ± 35	515 ± 41
Glucose (mg/dL)	203 ± 8	182 ± 10

* indicates p < 0.05.

Table S2 Blood serum components of CD-fed animals under fasting schedule

	WT (n=13)	KO (n=13)
Total protein (g/dL)	5.3 ± 0.0	5.1 ± 0.1
AST (IU/L)	195 ± 23	214 ± 18
ALT (IU/L)	40 ± 3	46 ± 2
ALP (IU/L)	350 ± 21	447 ± 18**
LDH (IU/L)	544 ± 46	529 ± 41
T-CHO (mg/dL)	98 ± 3	98 ± 2
F-CHO (mg/dL)	29 ± 1	29 ± 1
E-CHO (mg/dL)	70 ± 2	70 ± 2
Total lipid (mg/dL)	326 ± 7	349 ± 12
Triglyceride (mg/dL)	94 ± 8	107 ± 10
NEFA (μEq/L)	772 ± 43	871 ± 35
Glucose (mg/dL)	83 ± 4	77 ± 5

** indicates $p < 0.01$.

Table S3 Weight of various organs in HFD fed animals

	Organ weight (mg/g BW)	
	WT (n=6)	KO (n=12)
Liver	45.5 ± 4.6	52.1 ± 1.7
Spleen	2.7 ± 0.2	2.7 ± 0.1
Pancreas	8.7 ± 1.0	8.1 ± 0.6
Kidney	9.1 ± 3.5	7.4 ± 0.6
BAT	4.9 ± 0.3	4.3 ± 0.2
Thigh bone	2.5 ± 0.1	2.4 ± 0.1

Table S4 Plasma hormones of HFD-fed animals

	WT (n=8)	KO (n=7)
Adiponectin ($\mu\text{g/mL}$)	13.4 ± 1.7	11.5 ± 0.9
FGF21 (ng/mL)	0.93 ± 0.18	0.57 ± 0.33
Insulin (ng/mL)	1.99 ± 0.60	1.27 ± 0.39
Leptin (ng/mL)	2.59 ± 0.99	0.55 ± 0.17