Supplemental information

Supplementary Figure 1, Primary cilia in PVN, DMH, LH, and ARC are not affected in IFT88 KO^{SF-1} mice

(A) Comparable expression of primary cilia in PVN, DMH, LH, and ARC in WT and IFT88 KO^{SF-1} mice. Green fluorescence indicates neuronal primary cilia (ACIII) and DAPI (blue) fluorescence indicates nuclei. Scale bar = 10μm.

(B) Schematic images of bregma for mediobasal hypothalamus.

(C) Lengths of the neuronal cilia in each nucleus from (A).

(D) Percentages of the ciliated neurons in each nucleus from (A).

PVN, paraventricular nucleus of the hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; ARC, arcuate nucleus. Number of animals examined is expressed parentheses in each graph. Data are expressed as mean ± SD.

Supplementary Figure 2, Structure and developmental analysis of the IFT88 KO^{SF-1} brain

(A) Representative picture of the brain for both WT and IFT88 KO^{SF-1} mice. Scale bar = 1cm.

(B) Width and length of the brains from (A).

(C) Mediobasal hypothalamic structure analyses of the WT and IFT88 KO^{SF-1} mice by Nissl staining. Scale bar = 200μm.

(D) SF-1 expression in the VMH of WT and IFT88 KO^{SF-1} mice. Scale bar = 150μm.

(E) Number of SF-1 positive cells in the VMH from (D).

(F) The VMH expression of SF1-eGFP. SF1-eGFP transgenic mice were used. Scale bar = 100μm.

(G) Comparable SF1-eGFP positive cells numbers from (F).

(H) Soma size of the SF-1 neurons in WT and IFT88 KO$^{SF\text{-}1}$ littermates.

3V, 3rd ventricle; ARC, arcuate nucleus; AHP, anterior hypothalamic nucleus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; ME, median eminence; TC, tuber cinereum; VMH, ventromedial hypothalamus; ZI, zona incerta.

Number of animals examined is expressed parentheses in each graph. Data are expressed as mean ± SD.

Supplementary Figure 3, No detectable primary cilia in peripheral SF1-expressing cells

(A) Expression of SF-1 (green) and SSTR3 (red), a primary cilia marker, in the anterior pituitary. Note that primary cilia are not detected in pituitary gonadotrophs, where SF-1 is expressed. Scale bar = 10μm.

(B) Expression of SF-1 (green) and ACIII (red), a primary cilia marker, in the adrenal gland. Note that the adrenal cortex, where SF-1 is expressed, does not express primary cilia. Primary cilia exists only in the adrenal medulla. Scale bar = 10μm.

(C) Expression of SF-1 in the testis Leydig and Sertoli (white arrowhead, weak green positive) cells. No noticeable Ac-Tub signals (red) for primary cilia were found in the testis in WT and IFT88 KO$^{SF\text{-}1}$ mice. Scale bar = 20μm.

(D) SF-1 cells in the ovary. The SF-1 (green) was expressed in ovarian theca and interstitial gland. No noticeable ACIII signals (red) for primary cilia were found in ovaries of WT and IFT88 KO$^{SF\text{-}1}$ mice. Scale bar = 20μm.

The SSTR3, ACIII, and Ac-Tub antibodies were used for ciliary markers.

Cor, adrenal cortex; ME, adrenal medulla; St, Sertoli cells; L, Leydig cells; O, oocytes; gr, granulosa cells; t, ovarian theca; ig, interstitial gland.

Supplementary Figure 4, Intact HPA and HPG axes in IFT88 KO^{SF-1} mice

(A)-(D) H&E stainings of the pituitary (A), adrenal gland (B), ovary (C), and testis (D) in WT and IFT88 KO^{SF-1} mice. Scale bar = 100μm.

(E)-(H) Levels of steroid hormones. Corticosterone (E, housed in groups. F, housed individually), aldosterone (G), and estradiol (E2) (H).

Number of animals examined is expressed in parentheses in each graph. The results are expressed as mean ± SD.

Supplementary Figure 5, Metabolic phenotypes of WT and IFT88 KO^{SF-1} female mice

(A) Weekly body weight of female mice. *P<0.05 for two-way ANOVA.

(B) Body compositions of female mice. **P<0.01 for Student's t-test.

(C)-(D), Blood glucose (C) and insulin (D) level of 12-week old female mice. *P<0.05 and **P<0.01 for Student's t-test.

(E) Hypothalamic *Pomc*, *Agrp*, and *Npy* expression in WT and IFT88 KO^{SF-1} female littermates. *P<0.05 for Student's t-test.

Number of animals examined is expressed in parentheses in each graph. All results are expressed as mean ± SD.

Supplementary Figure 6, Metabolic phenotypes of WT and IFT88 KO^{SF-1} mice fed on HFD

(A) Weekly body weight of male mice before and after high-fat diet (HFD). **P<0.01 for two-way ANOVA.

(B) Body compositions of WT and IFT88 KO^{SF-1} littermates at 16-week old.

(C) Cumulative daily food intake from indirect calorimetry.

(D) Food intake during the 12-hours light and dark cycle.

(E)-(J) Changes of VO_2 (E and F), VCO_2 (G and H) and EE (I and J) of WT and IFT88 $KO^{SF\text{-}1}$ littermates were shown. *P<0.05 with blue color for two-way ANOVA and black color for Student's t-test.

(K) Hypothalamic *Pomc, Agrp*, and *Npy* expression. *P<0.05 and **P<0.01 for Student's t-test.

(L) and **(M)**, Total X and Y movement were measured.

Number of animals examined is expressed in parentheses in each graph. The results are expressed as mean ± SD.

Supplementary Figure 7, Metabolic phenotypes of WT and IFT88 $KO^{SF\text{-}1}$ mice after ISO administration

(A) Body weight of male mice before and after ISO administration.

(B)-(E), Temporal changes of VO_2 (B), VCO_2 (C), EE (D), and movement (E) of WT and IFT88 $KO^{SF\text{-}1}$ after ISO injection.

Number of animals examined is expressed in parentheses in each graph. The results are expressed as mean ± SD (ns is used to indicate not significant).

Supplementary Figure 8, Bone phenotypes of female WT and IFT88 $KO^{SF\text{-}1}$ mice

(A)-(D), DEXA analyses were performed and bone parameters were measured. BMD (A), BMC (B), BV (C), and BA (D) of WT and IFT88 $KO^{SF\text{-}1}$ mice are shown.

Number of animals examined is expressed in parentheses in the first graph. Data are expressed as mean ± SD (*P<0.05 for Student's t-test).

Supplementary Figure 9, Hypothalamic expressions of *SF-1*, *ObRb*, and *5-HT2c* genes

(A)-**(B)**, Relative mRNA expressions of *SF-1*, *ObRb*, and *5-HT2c* in male WT and IFT88 KO^{SF-1} mice fed on either normal chow diet (A) or high-fat diet (B).

(C) Relative mRNA expression of *SF-1*, *ObRb*, and *5-HT2c* in female WT and IFT88 KO^{SF-1} mice fed on normal chow diet.

Number of animals examined is expressed in parentheses in each graph. Data are expressed as mean ± SD.

Figure S1

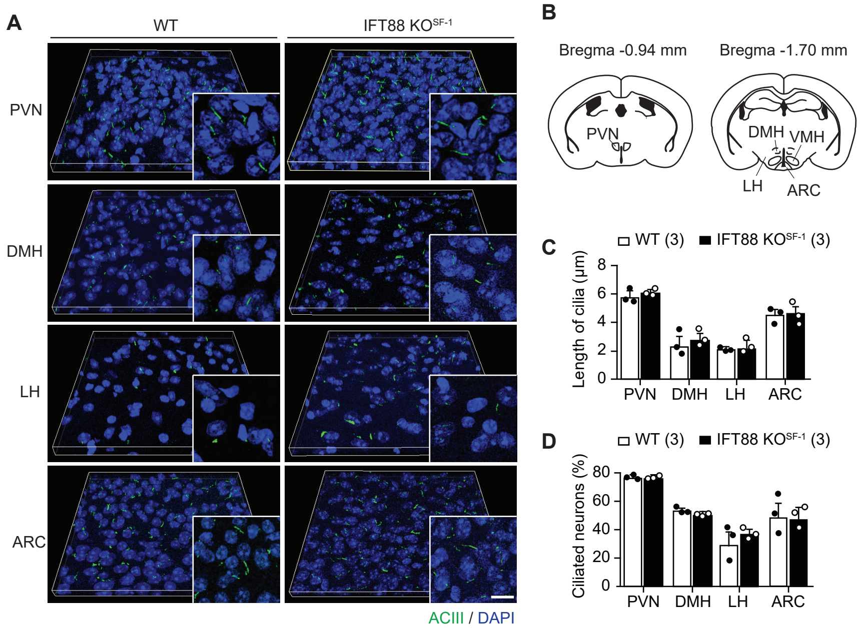

A
WT
IFT88 KO^SF-1
PVN
DMH
LH
ARC
ACIII / DAPI
B
Bregma -0.94 mm
Bregma -1.70 mm
PVN
DMH
VMH
LH
ARC
C
WT (3)
IFT88 KO^SF-1 (3)
Length of cilia (μm)
8
6
4
2
0
PVN
DMH
LH
ARC
D
WT (3)
IFT88 KO^SF-1 (3)
Ciliated neurons (%)
80
60
40
20
0
PVN
DMH
LH
ARC

Figure S2

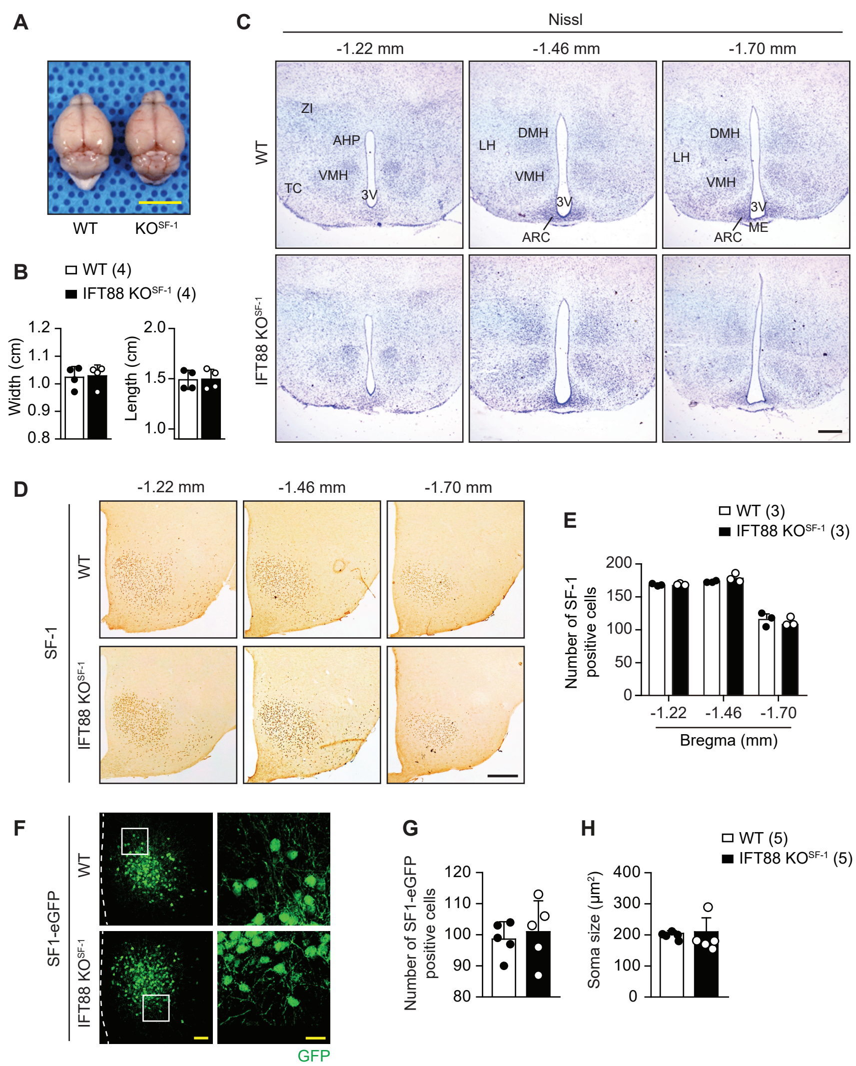

A
WT
KO^SF-1
B
WT (4)
IFT88 KO^SF-1 (4)
Width (cm)
Length (cm)
C
Nissl
-1.22 mm
-1.46 mm
-1.70 mm
WT
IFT88 KO^SF-1
ZI
AHP
VMH
TC
3V
LH
DMH
ARC
ME
D
SF-1
-1.22 mm
-1.46 mm
-1.70 mm
E
WT (3)
IFT88 KO^SF-1 (3)
Number of SF-1 positive cells
Bregma (mm)
F
SF1-eGFP
GFP
G
Number of SF1-eGFP positive cells
H
WT (5)
IFT88 KO^SF-1 (5)
Soma size (μm²)

Figure S3

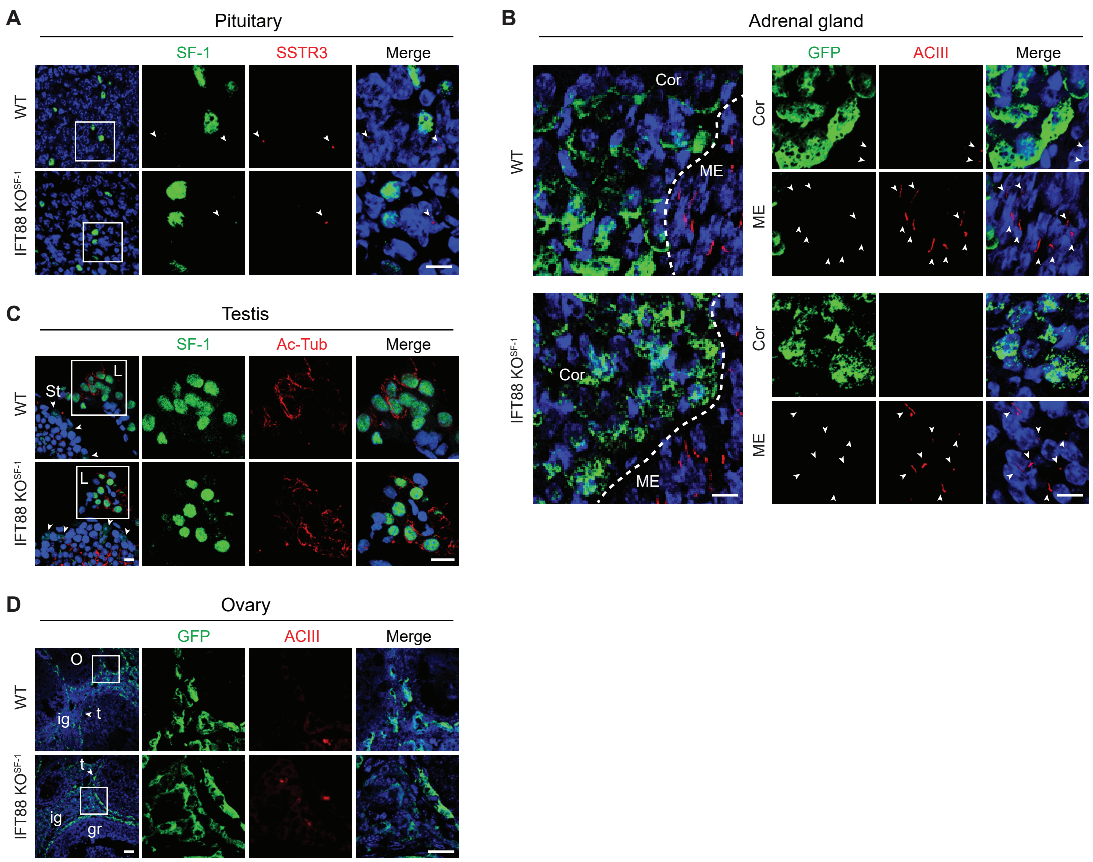
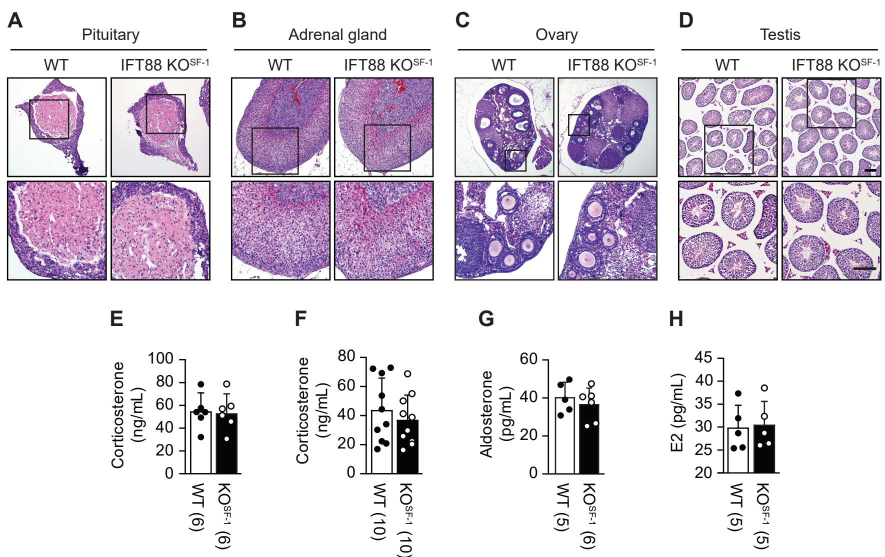

A
Pituitary
SF-1
SSTR3
Merge
WT
IFT88 KO^{SF-1}
B
Adrenal gland
GFP
ACIII
Merge
Cor
ME
C
Testis
SF-1
Ac-Tub
Merge
St
L
D
Ovary
GFP
ACIII
Merge
O
ig
t
gr

Figure S4



A
Pituitary
WT
IFT88 KO^SF-1
B
Adrenal gland
WT
IFT88 KO^SF-1
C
Ovary
WT
IFT88 KO^SF-1
D
Testis
WT
IFT88 KO^SF-1
E
Corticosterone (ng/mL)
100
80
60
40
20
0
WT (6)
KO^SF-1 (6)
F
Corticosterone (ng/mL)
80
60
40
20
0
WT (10)
KO^SF-1 (10)
G
Aldosterone (pg/mL)
60
40
20
0
WT (5)
KO^SF-1 (6)
H
E2 (pg/mL)
45
40
35
30
25
20
WT (5)
KO^SF-1 (5)

Figure S5

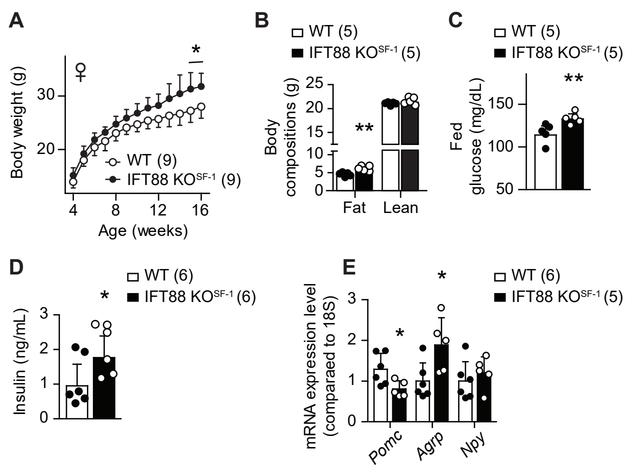

A
♀
*
Body weight (g)
30
20
WT (9)
IFT88 KO^SF-1 (9)
4
8
12
16
Age (weeks)
B
WT (5)
IFT88 KO^SF-1 (5)
Body compositions (g)
25
20
15
10
5
0
**
Fat
Lean
C
WT (5)
IFT88 KO^SF-1 (5)
**
Fed glucose (mg/dL)
150
100
50
D
WT (6)
IFT88 KO^SF-1 (6)
*
Insulin (ng/mL)
3
2
1
0
E
WT (6)
IFT88 KO^SF-1 (5)
mRNA expression level (comparaed to 18S)
*
*
3
2
1
0
Pomc
Agrp
Npy

Figure S6

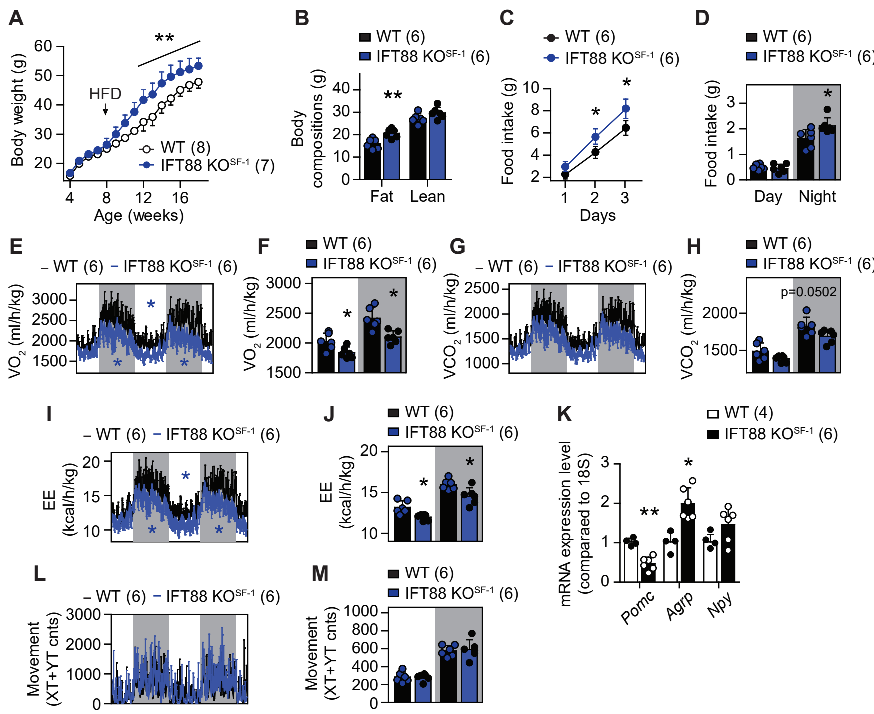
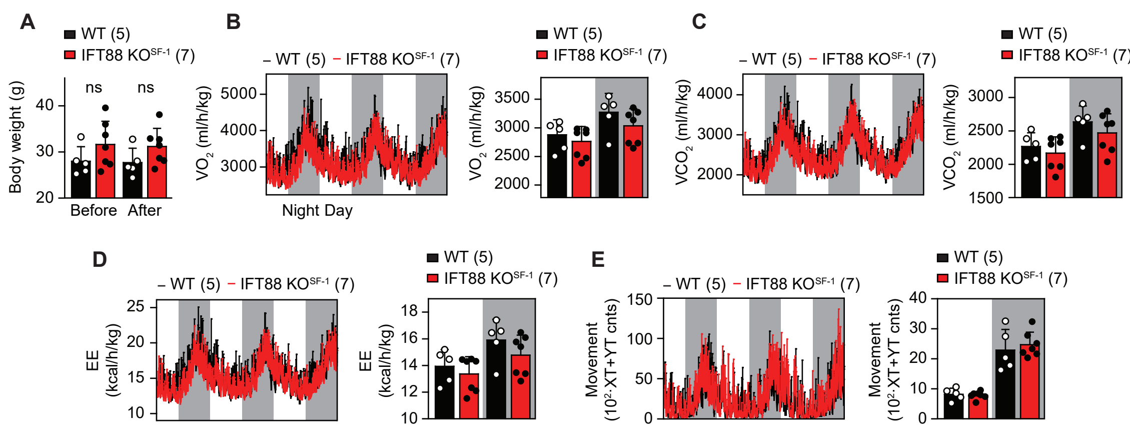
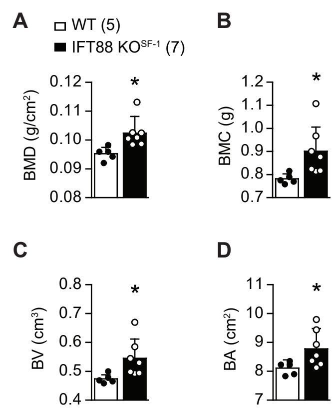

A
B
C
D
E
F
G
H
I
J
K
L
M
HFD
WT (8)
IFT88 KO^SF-1 (7)
Body weight (g)
Age (weeks)
WT (6)
IFT88 KO^SF-1 (6)
Body compositions (g)
Fat
Lean
Food intake (g)
Days
Day
Night
VO2 (ml/h/kg)
VCO2 (ml/h/kg)
p=0.0502
EE (kcal/h/kg)
WT (4)
mRNA expression level (compared to 18S)
Pomc
Agrp
Npy
Movement (XT+YT cnts)

Figure S7



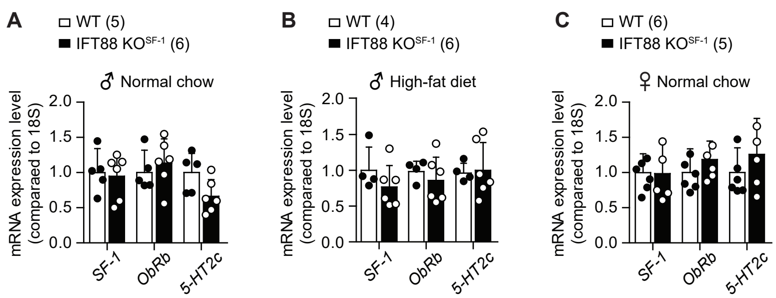
A
WT (5)
IFT88 KO^SF-1 (7)
Body weight (g)
ns
ns
Before
After
B
– WT (5) – IFT88 KO^SF-1 (7)
VO2 (ml/h/kg)
Night Day
WT (5)
IFT88 KO^SF-1 (7)
C
– WT (5) – IFT88 KO^SF-1 (7)
VCO2 (ml/h/kg)
D
– WT (5) – IFT88 KO^SF-1 (7)
EE (kcal/h/kg)
E
– WT (5) – IFT88 KO^SF-1 (7)
Movement (10²·XT+YT cnts)

Figure S8



A
B
WT (5)
IFT88 KO^{SF-1} (7)
BMD (g/cm^2)
0.12
0.11
0.10
0.09
0.08
*
BMC (g)
1.2
1.1
1.0
0.9
0.8
0.7
*
C
D
BV (cm^3)
0.8
0.7
0.6
0.5
0.4
*
BA (cm^2)
11
10
9
8
7
*

Figure S9


A
WT (5)
IFT88 KO^{SF-1} (6)
♂ Normal chow
mRNA expression level (comparaed to 18S)
2.0
1.5
1.0
0.5
0.0
SF-1
ObRb
5-HT2c
B
WT (4)
IFT88 KO^{SF-1} (6)
♂ High-fat diet
C
WT (6)
IFT88 KO^{SF-1} (5)
♀ Normal chow

Supplementary Table 1. Fertility of WT and IFT88 KO^{SF-1} mice

Breeding pair			
Female	Male	Litter size	Litter/month
WT	WT	7.200 ± 1.924	0.940 ± 0.089
WT	IFT88 KO^{SF-1}	7.167 ± 2.137	1.333 ± 0.577
IFT88 KO^{SF-1}	WT	7.250 ± 2.217	1.200 ± 0.447

Both IFT88 KO^{SF-1} male and female have shown comparable fertility during three months breeding period. Data are presented as mean ± SD.