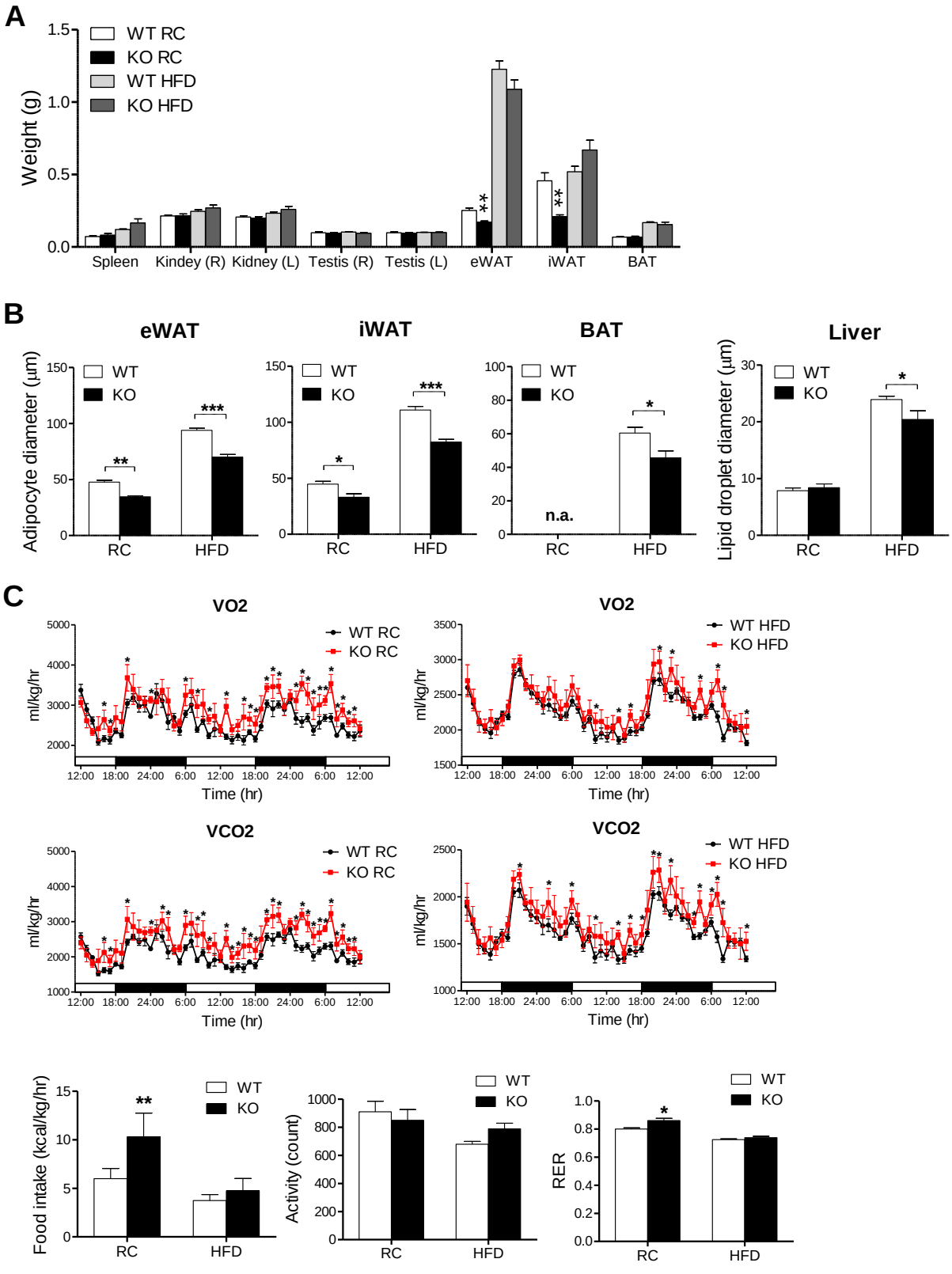
Fig. S1.



www.pnas.org/cgi/doi/10.1073/pnas.1904480116

Fig. S1. Phenotypes of middle-aged WT and viperin KO mice fed RC or an HFD for a long period. (*A*-*C*) WT and viperin KO mice were fed RC or an HFD from age 15 wk to 30 wk (15 wk on diet). Organ weights (n = 4-7) (*A*), sizes of adipocytes and hepatic lipid droplets from hematoxylin and eosin (H&E)-stained sections of adipose tissues (eWAT, iWAT, and BAT) and liver, respectively (n = 4-7; 5 spots each) (*B*), and oxygen consumption rates (VO_2), carbon dioxide production rates (VCO_2), food intake rates, activity, and respiratory exchange ratios (RER) (n = 4-7; indirect calorimetry) (*C*) of WT and viperin KO mice fed RC or an HFD. Data are represented as mean ± SEM of biologically independent samples. *P < 0.05, ** P < 0.01, and *** P < 0.001 vs. WT on the same diet. eWAT, epididymal white adipose tissue; iWAT, inguinal WAT; BAT, brown adipose tissue. RC, regular chow; HFD, high-fat diet.

Fig. S2.

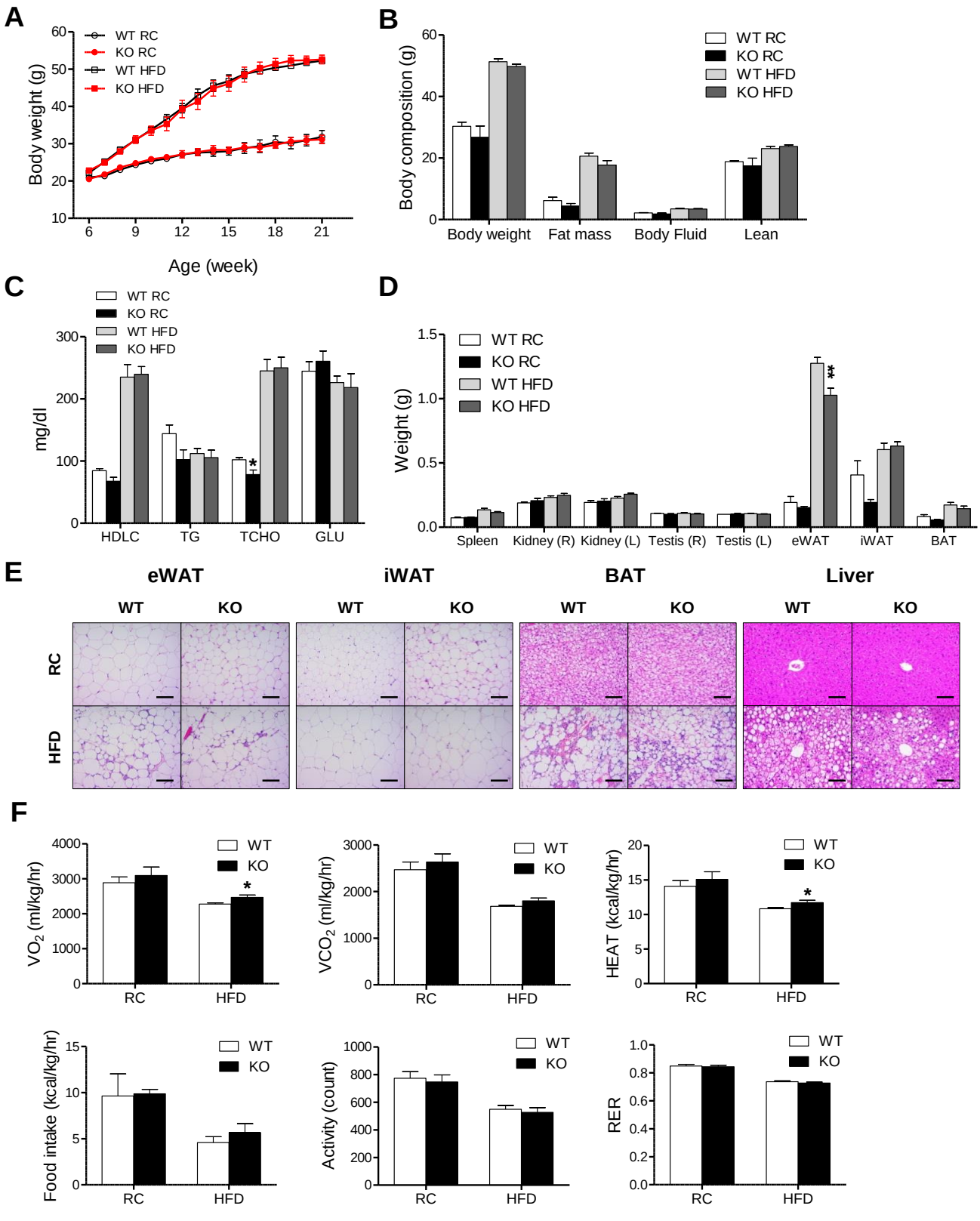

A
Body weight (g)
Age (week)
WT RC
KO RC
WT HFD
KO HFD
B
Body composition (g)
Body weight
Fat mass
Body Fluid
Lean
C
mg/dl
HDLC
TG
TCHO
GLU
D
Weight (g)
Spleen
Kidney (R)
Kidney (L)
Testis (R)
Testis (L)
eWAT
iWAT
BAT
E
eWAT
iWAT
BAT
Liver
WT
KO
RC
HFD
F
VO2 (ml/kg/hr)
VCO2 (ml/kg/hr)
HEAT (kcal/kg/hr)
Food intake (kcal/kg/hr)
Activity (count)
RER

Fig. S2. Phenotypes of young WT and viperin KO mice fed RC or an HFD for a long period. (*A*-*F*) WT and viperin KO mice were fed RC or an HFD from age 6 wk to 21 wk (15 wk on diet). Body weights (n = 3-6) (*A*), body compositions (n = 3-6) (*B*), serum metabolites (HDLC, TG, TCHO, and GLU) (n = 3-6) (*C*), organ weights (n = 3-6) (*D*), representative H&E-stained sections from adipose tissues and liver (Scale bar: 200 μm) (*E*), and VO_2, VCO_2, heat production rates, food intake rates, activity, and RER (n = 3-6) (*F*) of WT and viperin KO mice fed RC or an HFD. Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. WT on the same diet. HDLC, high-density lipoprotein cholesterol; TG, triglyceride; TCHO, total cholesterol; GLU, glucose (*C*).

Fig. S3.

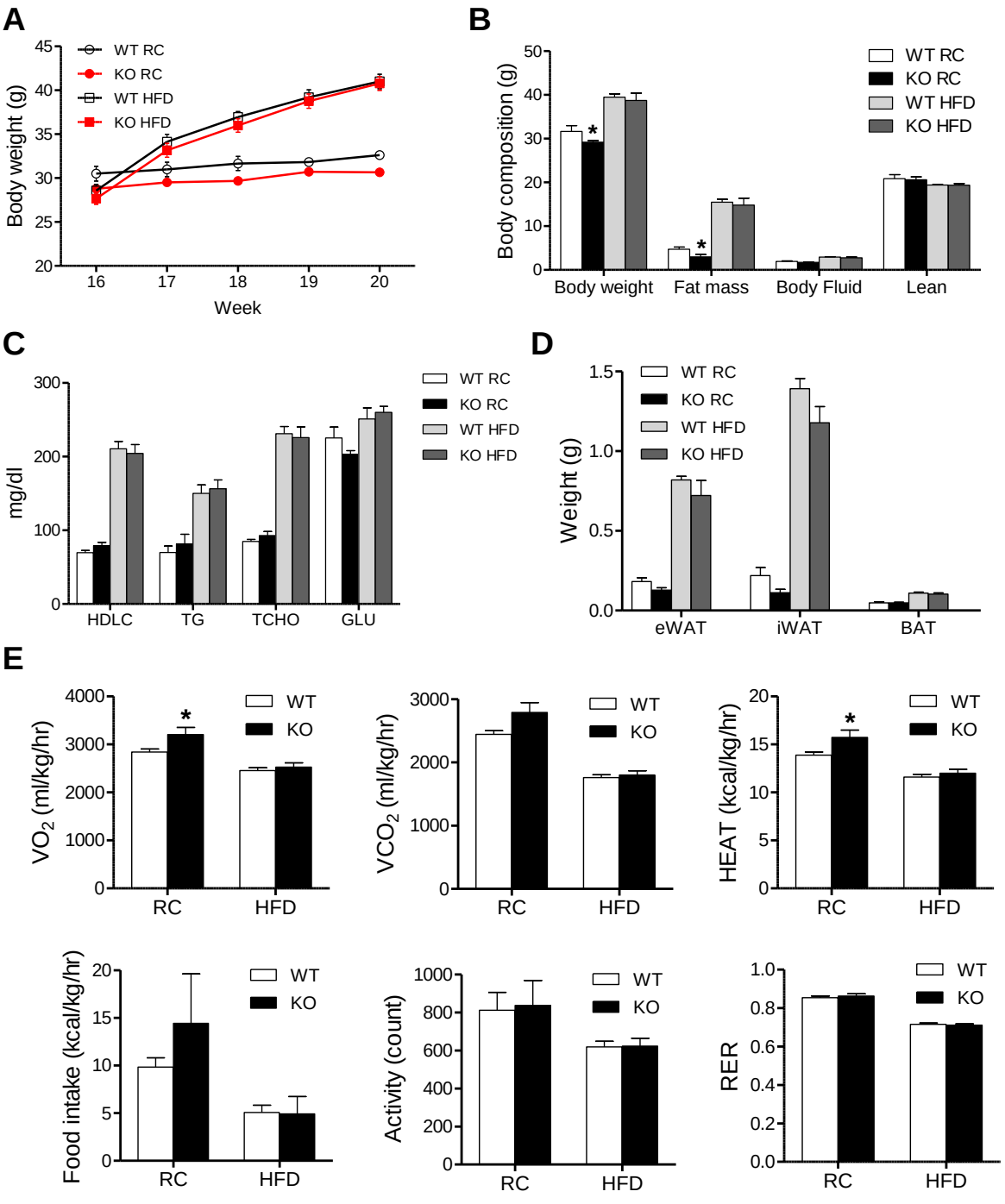

A
Body weight (g)
WT RC
KO RC
WT HFD
KO HFD
Week
B
Body composition (g)
Body weight
Fat mass
Body Fluid
Lean
C
mg/dl
HDLC
TG
TCHO
GLU
D
Weight (g)
eWAT
iWAT
BAT
E
VO2 (ml/kg/hr)
VCO2 (ml/kg/hr)
HEAT (kcal/kg/hr)
Food intake (kcal/kg/hr)
Activity (count)
RER
WT
KO
RC
HFD

Fig. S3. Phenotypes of middle-aged WT and viperin KO mice fed RC or an HFD for a short period. (*A*-*E*) WT and viperin KO mice were fed RC or an HFD from age 16 wk to 20 wk (4 wk on diet). Body weights (n = 5-7) (*A*), body compositions (n = 5-7) (*B*), serum metabolites (HDLC, TG, TCHO, and GLU) (n = 5-7) (*C*), organ weights (n = 5-7) (*D*), and VO_2, VCO_2, heat production rates, food intake rates, activity, and RER (n = 5-7) (*E*) of WT and viperin KO mice fed RC or an HFD. Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$ vs. WT on the same diet.

Fig. S4.

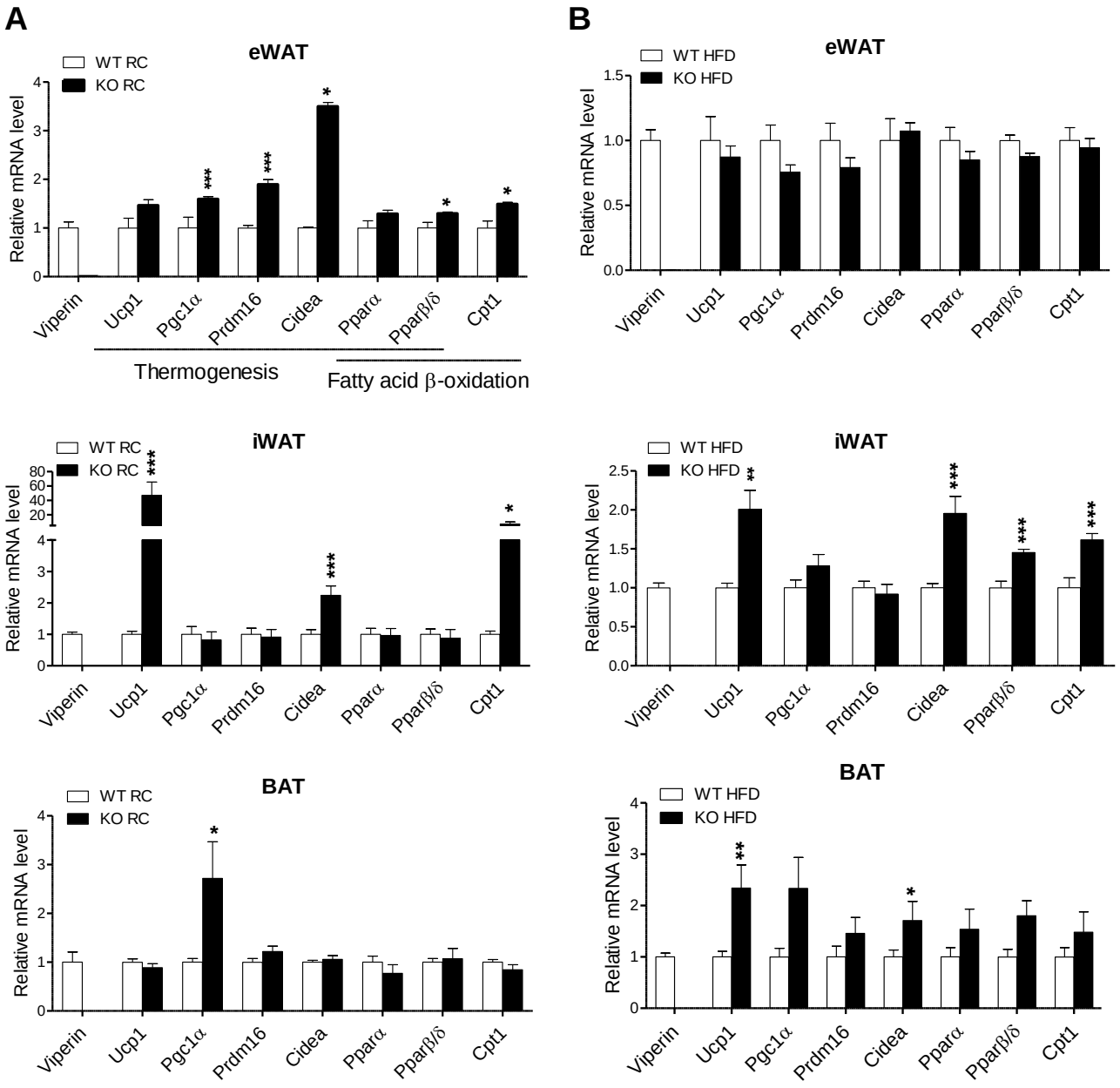


Fig. S4. Expression of thermogenesis-related genes in adipose tissues of young mice fed RC or an HFD for a long period. WT and viperin KO mice were fed RC or an HFD from age 6 wk to 21 wk (15 wk on diet). (*A* and *B*) Relative mRNA levels of thermogenesis- and fatty acid β-oxidation-related genes in adipose tissues of mice fed RC (*A*) or an HFD (*B*) (n = 3-4). Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. WT on the same diet.

Fig. S5.

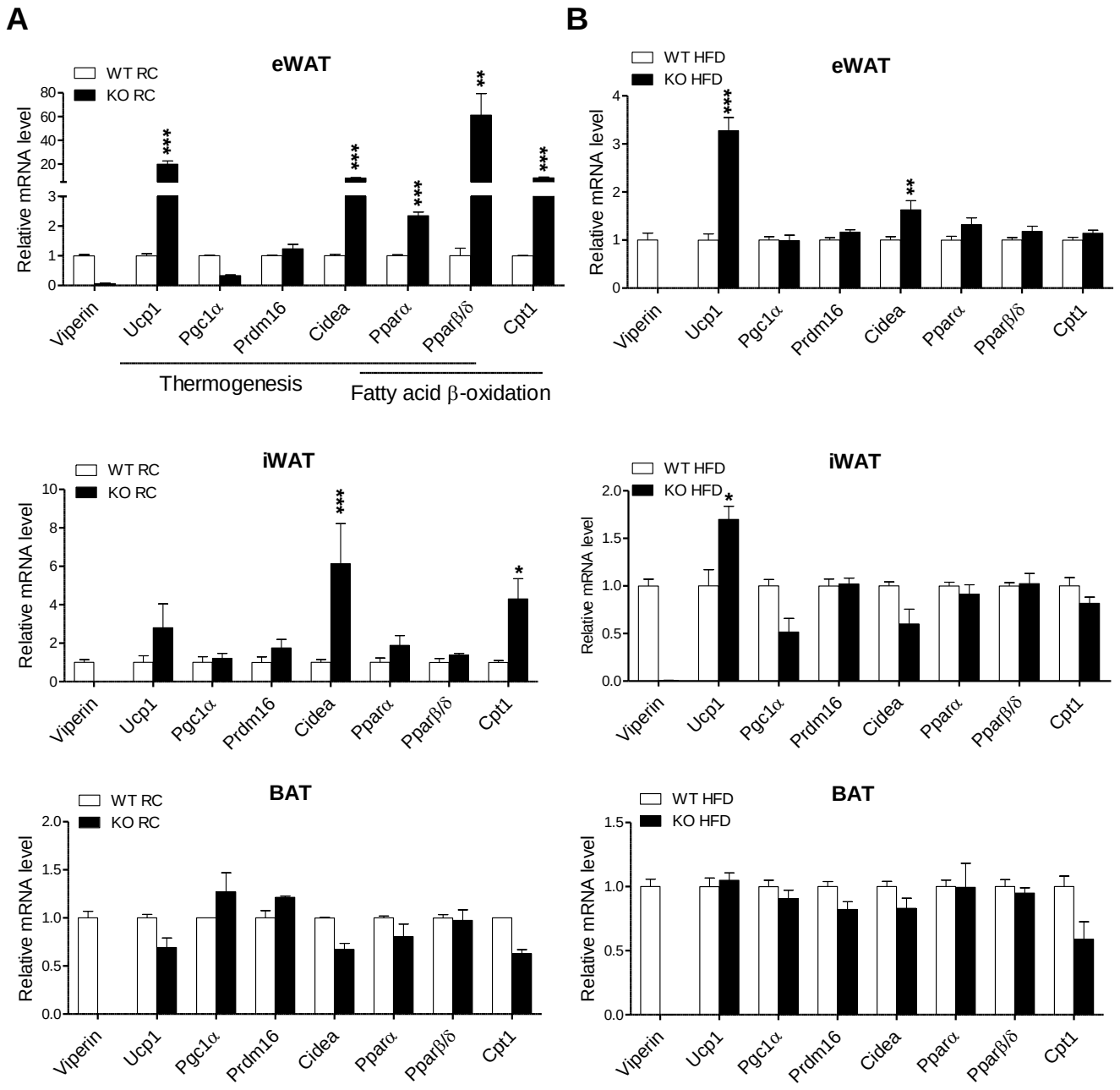


Fig. S5. Expression of thermogenesis-related genes in adipose tissues of middle-aged mice fed RC or an HFD for a short period. WT and viperin KO mice were fed RC or an HFD from age 16 wk to 20 wk (4 wk on diet). (*A* and *B*) Relative mRNA levels of thermogenesis- and fatty acid β-oxidation-related genes in adipose tissues of mice fed RC (*A*) or an HFD (*B*) (n = 3-4). Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. WT on the same diet.

Fig. S6.

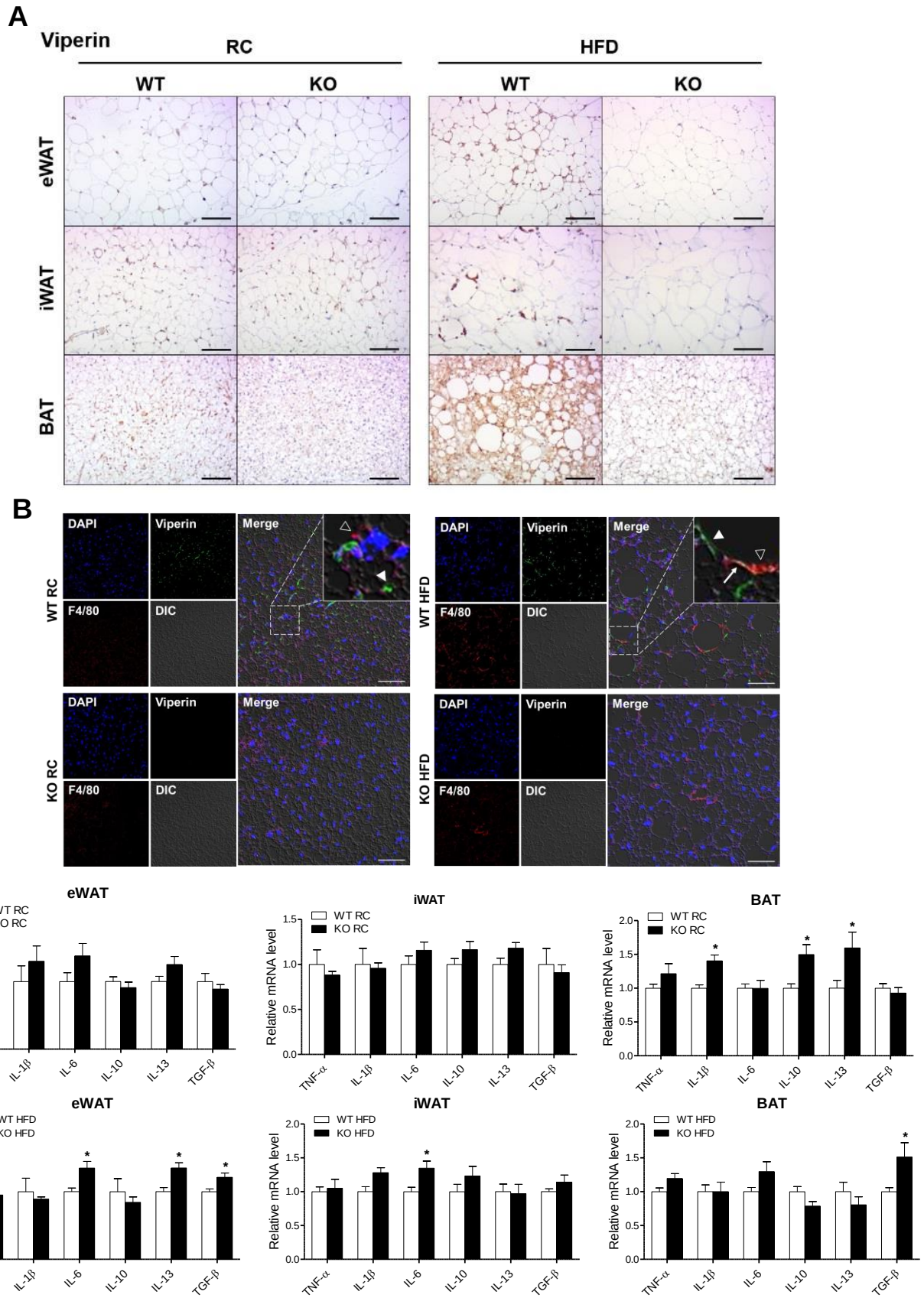

A
Viperin
RC
HFD
WT
KO
WT
KO
eWAT
iWAT
BAT
B
DAPI
Viperin
Merge
F4/80
DIC
WT RC
WT HFD
KO RC
KO HFD
C
eWAT
iWAT
BAT
WT RC
KO RC
Relative mRNA level
TNF-α
IL-1β
IL-6
IL-10
IL-13
TGF-β
D
eWAT
iWAT
BAT
WT HFD
KO HFD
Relative mRNA level
TNF-α
IL-1β
IL-6
IL-10
IL-13
TGF-β

Fig. S6. Expression of cytokines in adipose tissues of middle-aged mice fed RC or an HFD for a long period. WT and viperin KO mice were fed RC or an HFD from age 15 wk to 30 wk (15 wk on diet). (*A*) Immunohistochemical staining for viperin in adipose tissues (Scale bar: 200 μm). (*B*) Immunofluorescence staining of adipose tissues. DAPI, nucleus (blue); viperin (green); F4/80, a marker for macrophages (red). Arrow indicates viperin localized to the macrophage. Closed arrowheads indicate viperin. Open arrowheads indicate macrophage. (*C* and *D*) Relative mRNA levels of cytokines in adipose tissues of mice fed RC (*C*) or an HFD (*D*) (n = 3-4). Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$ vs. WT on the same diet.

Fig. S7.

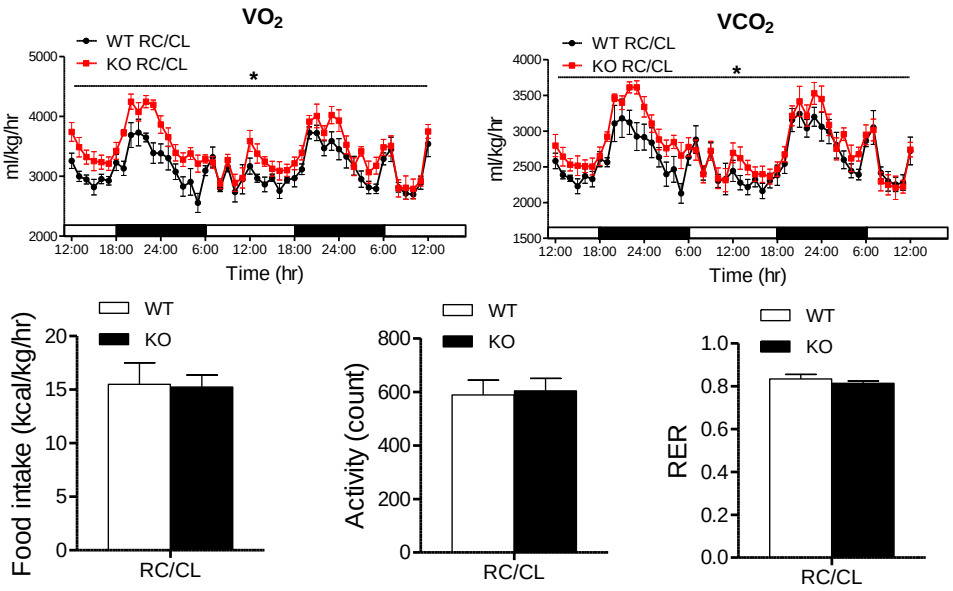


Fig. S7. Metabolic phenotypes of WT and viperin KO mice after CL treatment. WT and viperin KO mice were administrated intraperitoneally with CL-316243, a β3-adrenergic receptor (ADRB3)-agonist (1 mg/kg body weight/d) for 3 d. VO_2, VCO_2, food intake rates, activity, and RER (n = 6, indirect calorimetry) of CL-treated 21-wk-old mice. Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$ vs. WT for the same treatment.

Fig. S8.

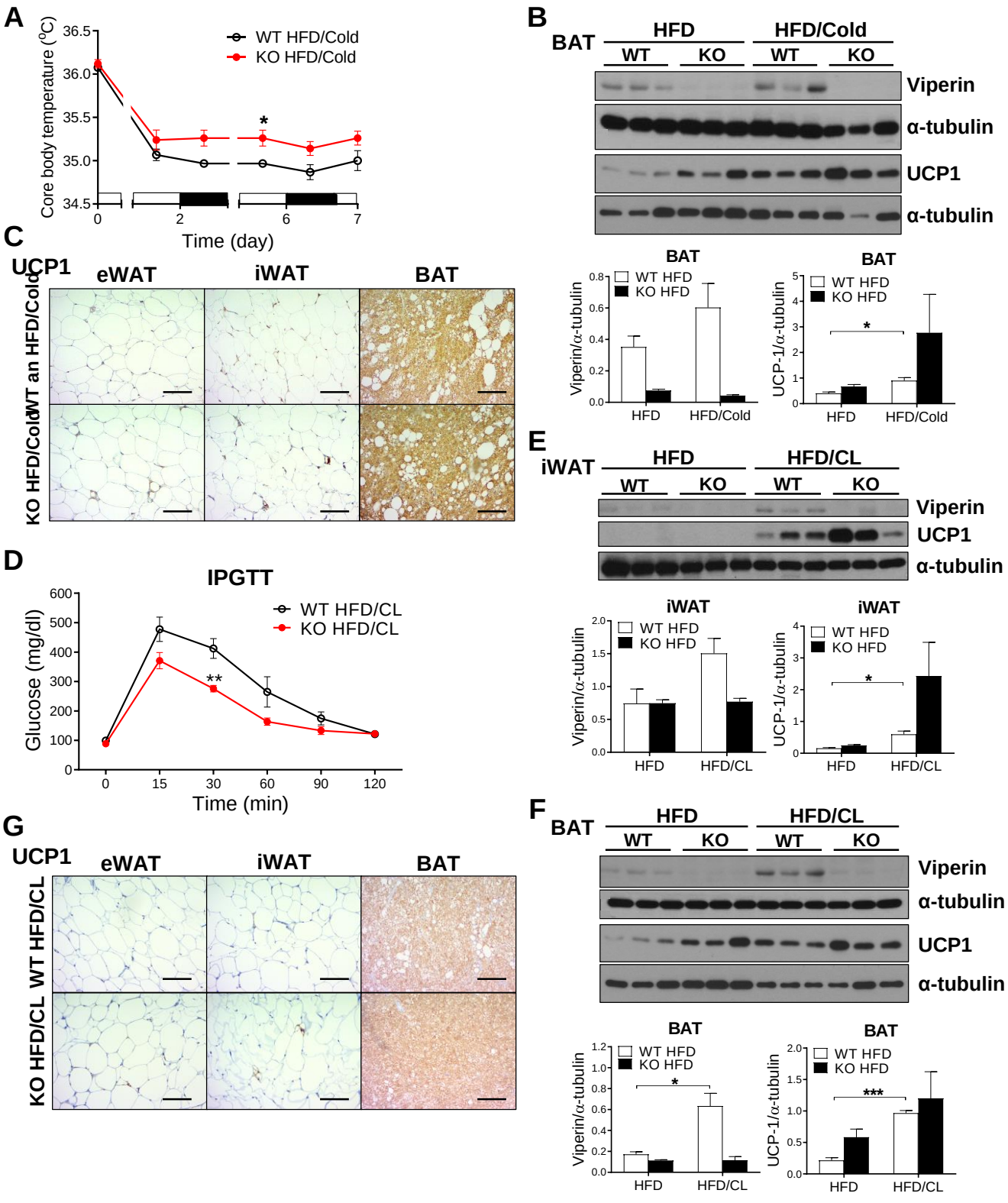

A
Core body temperature (°C)
36.5
36.0
35.5
35.0
34.5
0
2
6
7
Time (day)
WT HFD/Cold
KO HFD/Cold
*
B
BAT
HFD
HFD/Cold
WT
KO
WT
KO
Viperin
α-tubulin
UCP1
α-tubulin
BAT
Viperin/α-tubulin
WT HFD
KO HFD
0.8
0.6
0.4
0.2
0.0
HFD
HFD/Cold
BAT
UCP-1/α-tubulin
WT HFD
KO HFD
5
4
3
2
1
0
*
HFD
HFD/Cold
C
UCP1
eWAT
iWAT
BAT
WT an HFD/Cold
KO HFD/Cold
D
IPGTT
Glucose (mg/dl)
600
500
400
300
200
100
0
0
15
30
60
90
120
Time (min)
WT HFD/CL
KO HFD/CL
**
E
iWAT
HFD
HFD/CL
WT
KO
WT
KO
Viperin
UCP1
α-tubulin
iWAT
Viperin/α-tubulin
WT HFD
KO HFD
2.0
1.5
1.0
0.5
0.0
HFD
HFD/CL
iWAT
UCP-1/α-tubulin
WT HFD
KO HFD
4
3
2
1
0
*
HFD
HFD/CL
F
BAT
HFD
HFD/CL
WT
KO
WT
KO
Viperin
α-tubulin
UCP1
α-tubulin
BAT
Viperin/α-tubulin
WT HFD
KO HFD
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
HFD
HFD/CL
BAT
UCP-1/α-tubulin
WT HFD
KO HFD
2.0
1.5
1.0
0.5
0.0
***
HFD
HFD/CL
G
UCP1
eWAT
iWAT
BAT
WT HFD/CL
KO HFD/CL

Fig. S8. Thermogenic phenotypes of WT and viperin KO mice fed an HFD after cold exposure or CL treatment. WT and viperin KO mice were fed an HFD from age 6 wk to 21 wk (15 wk on diet). (*A*) Rectal temperature of 21-wk-old HFD-fed WT and viperin KO mice during chronic cold exposure for 7 d (n = 4-5). (*B* and *C*) Protein expression of viperin and UCP1 in BAT (n = 3) (*B*) and immunohistochemical staining for UCP1 in adipose tissues (Scale bar: 200 μm) (*C*) of cold-exposed 21-wk-old mice fed an HFD. (*D*-*G*) WT and viperin KO mice were injected i.p. with CL-316243 (1 mg/kg body weight/d) for 3 d. I.p. glucose tolerance test (n = 4-5) (*D*), protein expression of viperin and UCP1 in iWAT and BAT (n = 3) (*E* and *F*), and immunohistochemical staining for UCP1 in adipose tissues (Scale bar: 200 μm) (*G*) of CL-treated 21-wk-old mice fed an HFD. Data are represented as mean ± SEM of biologically independent samples. * $P < 0.05$ and ** $P < 0.01$ vs. WT for the same treatment.

Fig. S9.

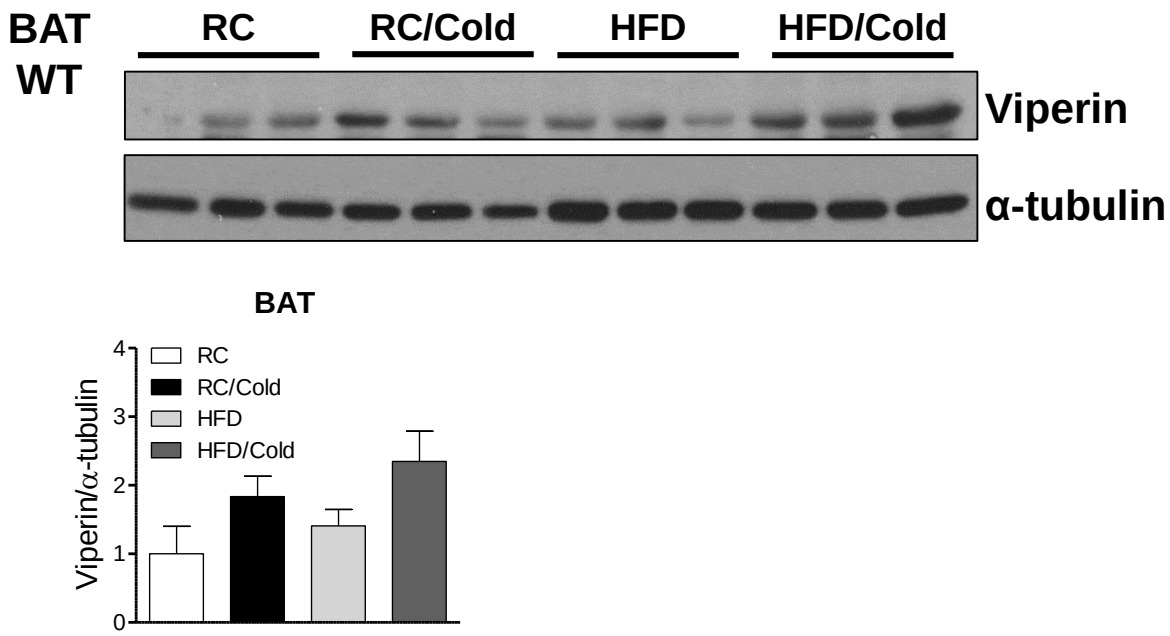


Fig. S9. Comparison of viperin expression levels in the brown adipose tissue of WT mice after feeding an HFD and/or cold exposure. WT mice fed RC or an HFD for 15 wk and/or exposed to cold for 7 d. Protein expression of viperin in BAT (n = 3). Data are represented as mean ± SEM of biologically independent samples.

Fig. S10.

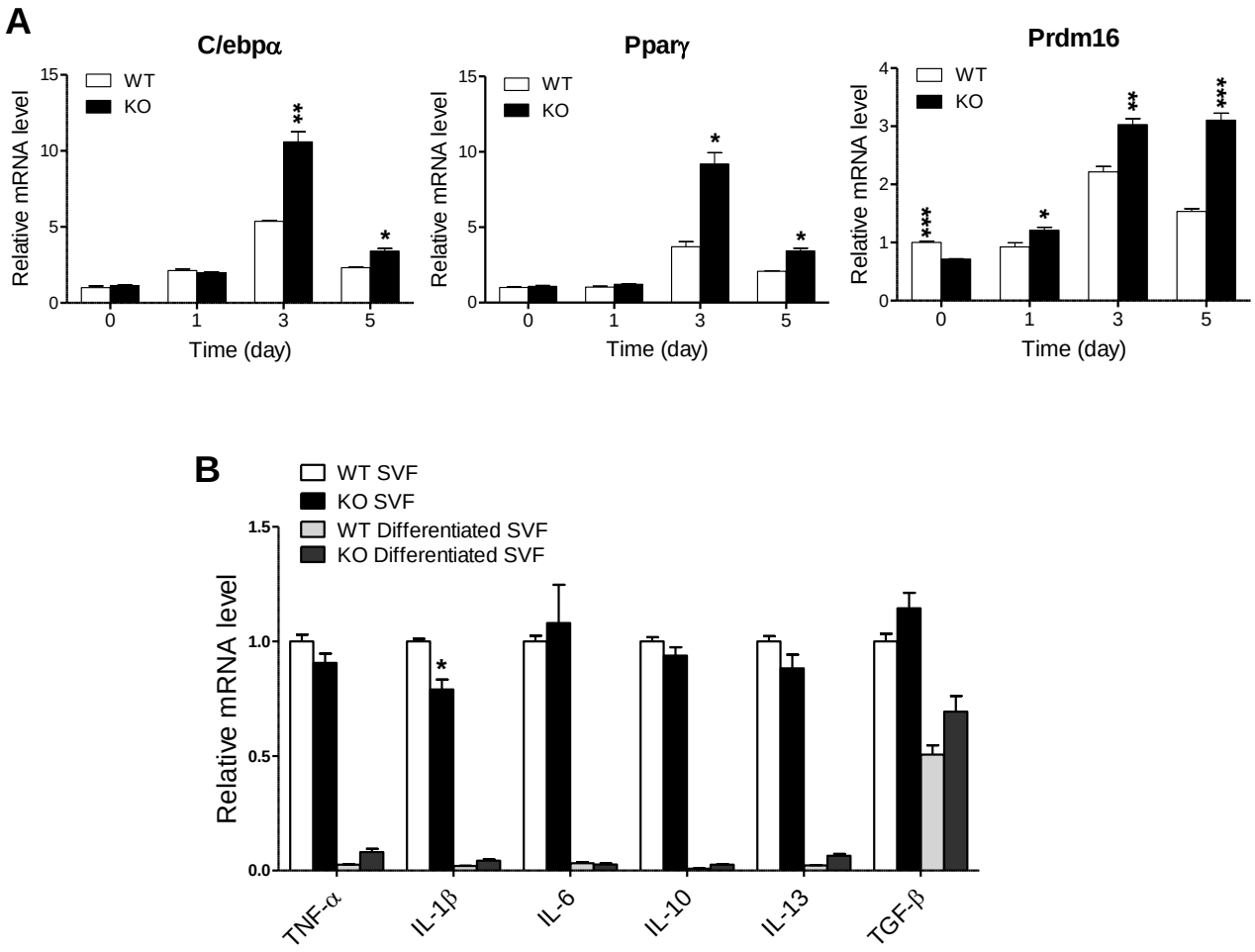


Fig. S10. Cell-autonomous function of viperin in brown adipocytes. SVF is isolated from BAT and differentiated into mature brown adipocytes. (*A*) Relative mRNA levels of adipogenesis- and thermogenesis-related genes in brown adipocytes during differentiation. (*B*) Relative mRNA levels of cytokines in the isolated SVF and the SVF-differentiated brown adipocytes. Data are represented as mean ± SEM of 2 independent experiments. *$P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. WT for the same treatment.

Fig. S11.

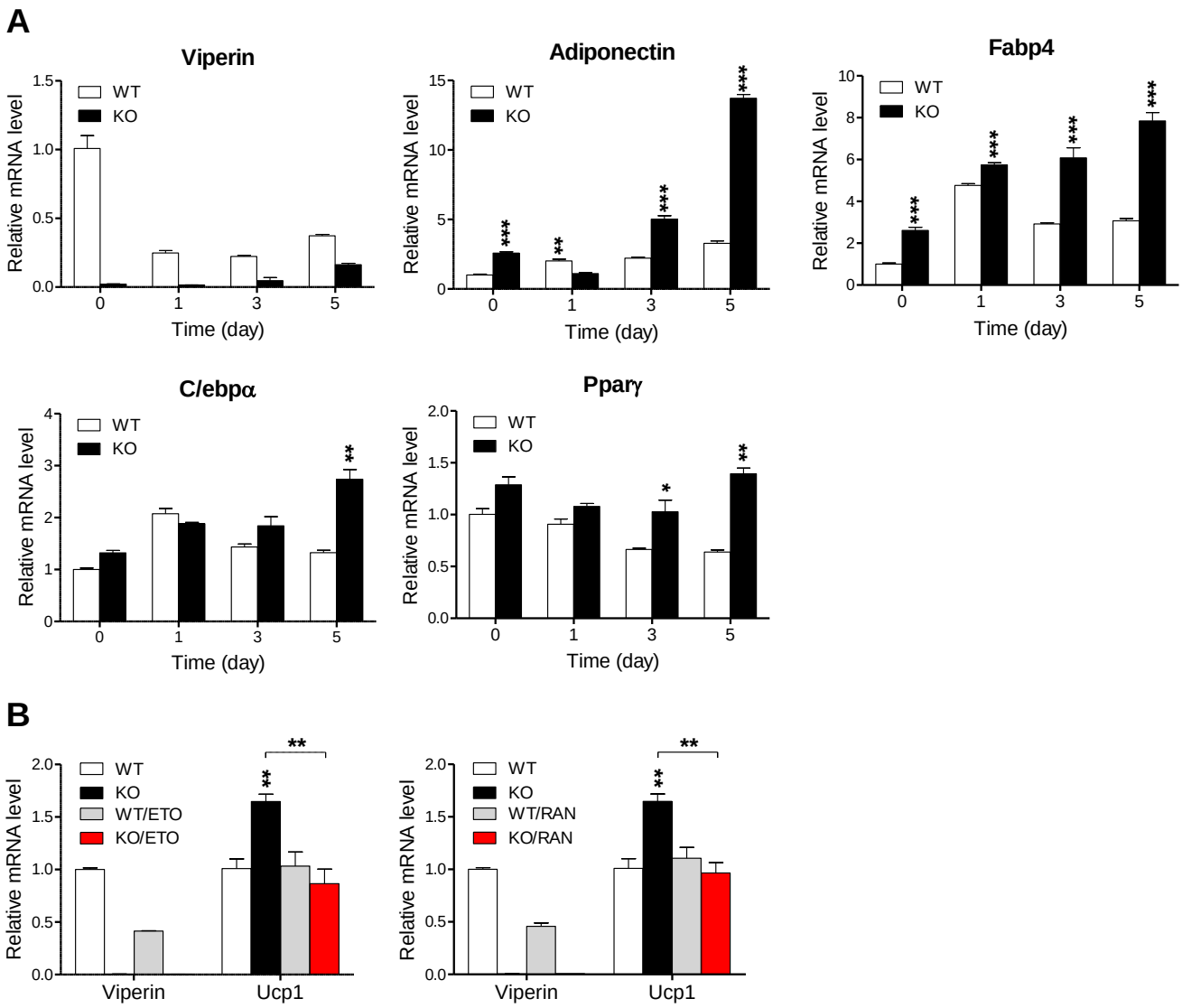


Fig. S11. Cell-autonomous function of viperin in white adipocytes. SVF is isolated from eWAT and differentiated into mature white adipocytes. (*A*) Relative mRNA levels of adipogenesis- and thermogenesis-related genes in white adipocytes during differentiation. (*B*) Relative mRNA levels of viperin and UCP1 in etomoxir (ETO)- or ranolazine (RAN)-treated white adipocytes. Data are represented as mean ± SEM of 2 independent experiments. *$P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. WT for the same treatment.

Table S1

Table S1. Mouse-specific primer sequences used for quantitative real-time PCR.

Primer name	Forward	Reverse
Viperin	GTGAATACTTGGGCAAGCT	CAAATACTCCCCATAGTCC
Ucp1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT
Pgc1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Pparγ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
Pparα	TCGGCGAACTATTCGGCTG	GCACTTGTGAAAACGGCAGT
Pparβ/δ	TTGAGCCCAAGTTCGAGTTTG	CGGTCTCCACACAGAATGATG
Cpt1b	TCTATGAGGGCTCGCG	CGTCAGGGTTGTAGCA
IL-6	CCTCTGGTCTTCTGGAGTACC	ACTCCTTCTGTGACTCCAGC
IL-10	ATAACTGCACCCACTTCCCA	GGGCATCACTTCTACCAGGT
IL-13	GCAGCATGGTATGGAGTGTG	TGGCGAAACAGTTGCTTTGT
IL-1β	GTGGCTGTGGAGAAGCTGTG	GAAGGTCCACGGGAAAGACAC
TNF-α	ATGAGCACAGAAAGCATG	AGTAGACAGAAGAGCGTGGT
TGF-β	CCTGCAAGACCATCGACATG	TGTTGTACAAAGCGAGCACC
C/ebpα	CAAAGCCAAGAAGTCGGTGGACAA	TCATTGTGACTGGTCAACTCCAGC
Adiponectin	CCGGGACTCTACTACTTCTCTT	TTCCTGATACTGGTCGTAGGT
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTC
β-actin	GCTCCGGCATGTGCAA	AGGATCTTCATGAGGTAGT