Expanded View Figures

Figure EV1. FUNDC1 is regulated by PGC-1α.

A–C Oil red O staining (A) analysis of differentiated brown adipocytes (5 days) and Western blotting analysis (B) of brown preadipocytes (0 days) and differentiated cells (5 days), scale bar, 10 μm. The expression levels of the indicated proteins are quantified in (C). Data information: experiments were repeated three times, data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. ns: no significant difference; $*P < 0.05$.

D–F Brown fat preadipocytes were infected with control adenovirus and adenovirus expressing PGC-1α. GFP expression was used as an indicator for cell infection, scale bar, 10 μm (D). Levels of the indicated proteins were analyzed by Western blotting and quantified (E, F). Data information: experiments were repeated three times, data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. ns: no significant difference; $*P < 0.05$.

G, H Western blotting analysis of the indicated proteins in HeLa (G) and 293 (H) cells transfected with control vector and a Flag-PGC-1α expression construct. Quantification of the expression levels of the indicated proteins is shown in the panel below. Data information: Experiments were repeated three times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. $*P < 0.05$.

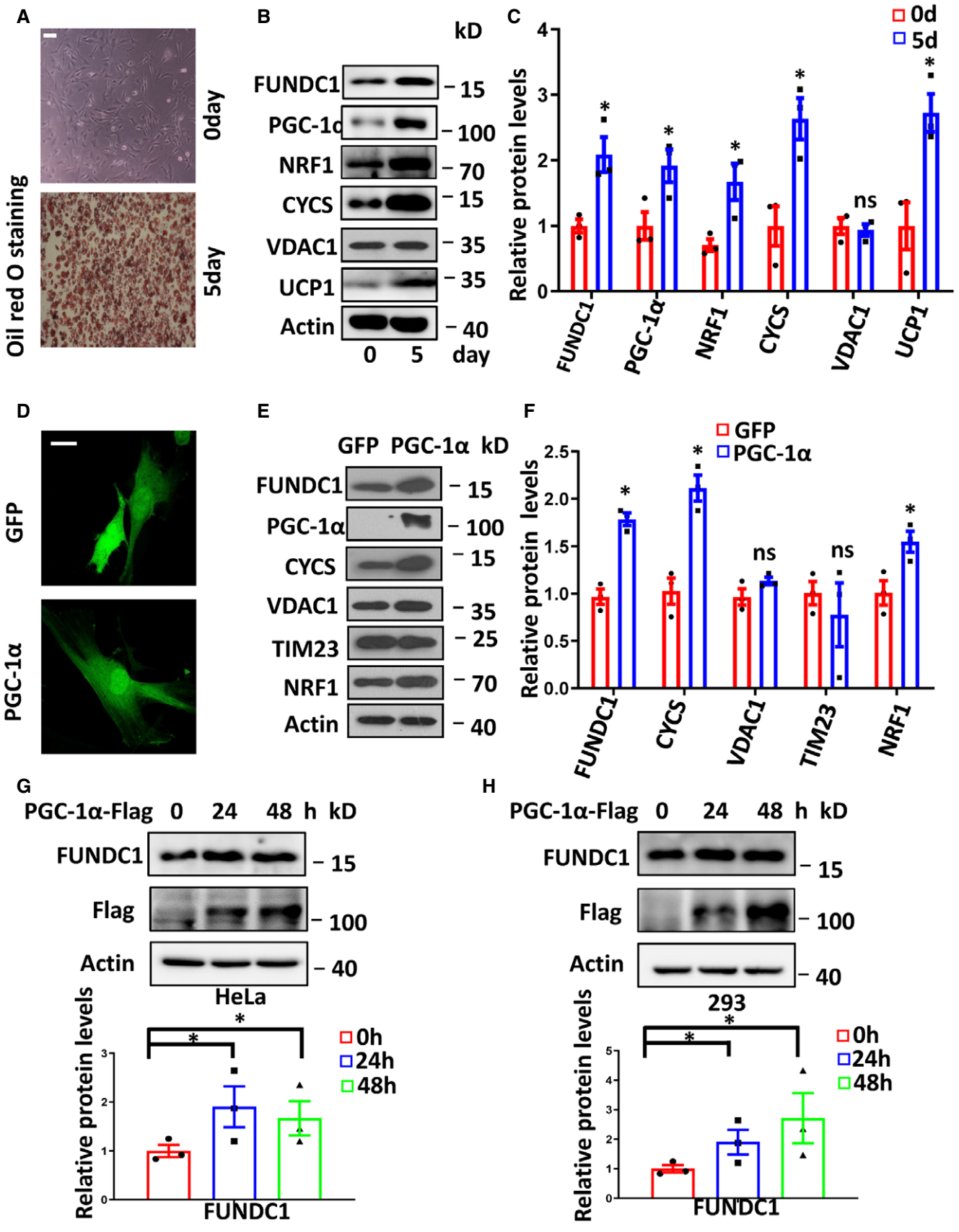

Figure EV1.

Figure EV2. PGC-1α and NRF1 are essential for expression of *Fundc1*, and the autophagic flux is enhanced by cold exposure in BAT.

A–C Western blotting analysis of the indicated proteins in scramble, PGC-1α knockdown, and PGC-1β knockdown brown fat preadipocytes (A). Expression levels of the indicated proteins were quantified (B). Real-time PCR analysis of the expression of the indicated genes in scramble, *PGC-1α* knockdown, and *PGC-1β* knockdown brown fat preadipocytes (C). Data information: Experiments were repeated three times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. ns: no significant difference; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

D, E Scramble and NRF1 knockdown preadipocytes were induced to differentiate into mature adipocytes, and samples were collected for Western blotting analyses and quantification of the indicated proteins. Data information: Experiments were repeated three times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. ns: no significant difference; **$P < 0.01$.

F Expression of the indicated genes was analyzed by real-time PCR in BAT before and after cold exposure (4°C) for 72 h. Data information: $n = 3$ biological replicates, data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. *$P < 0.05$.

G, H The WT mice were treated with chloroquine (CQ; 30 mg/kg/day) for 72h in normal or cold conditions, and the expression of LC3B was detected by Western blotting. The expression of LC3BII was quantified and plotted (H). Data information: $n = 6$ biological replicates, data are represented as the mean ± SEM. Statistical analysis was performed using two-way ANOVA test. **$P < 0.01$.

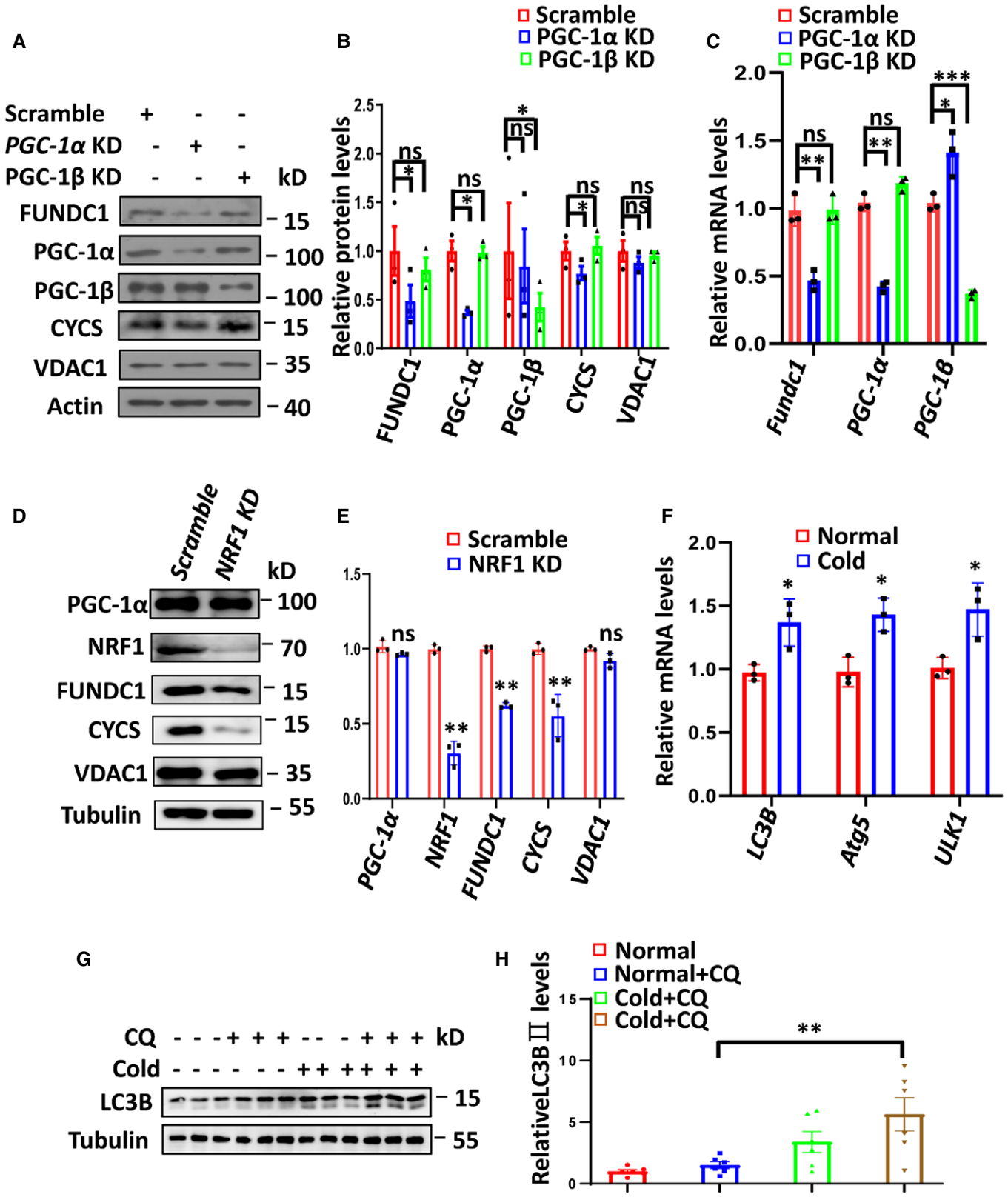

Figure EV2.

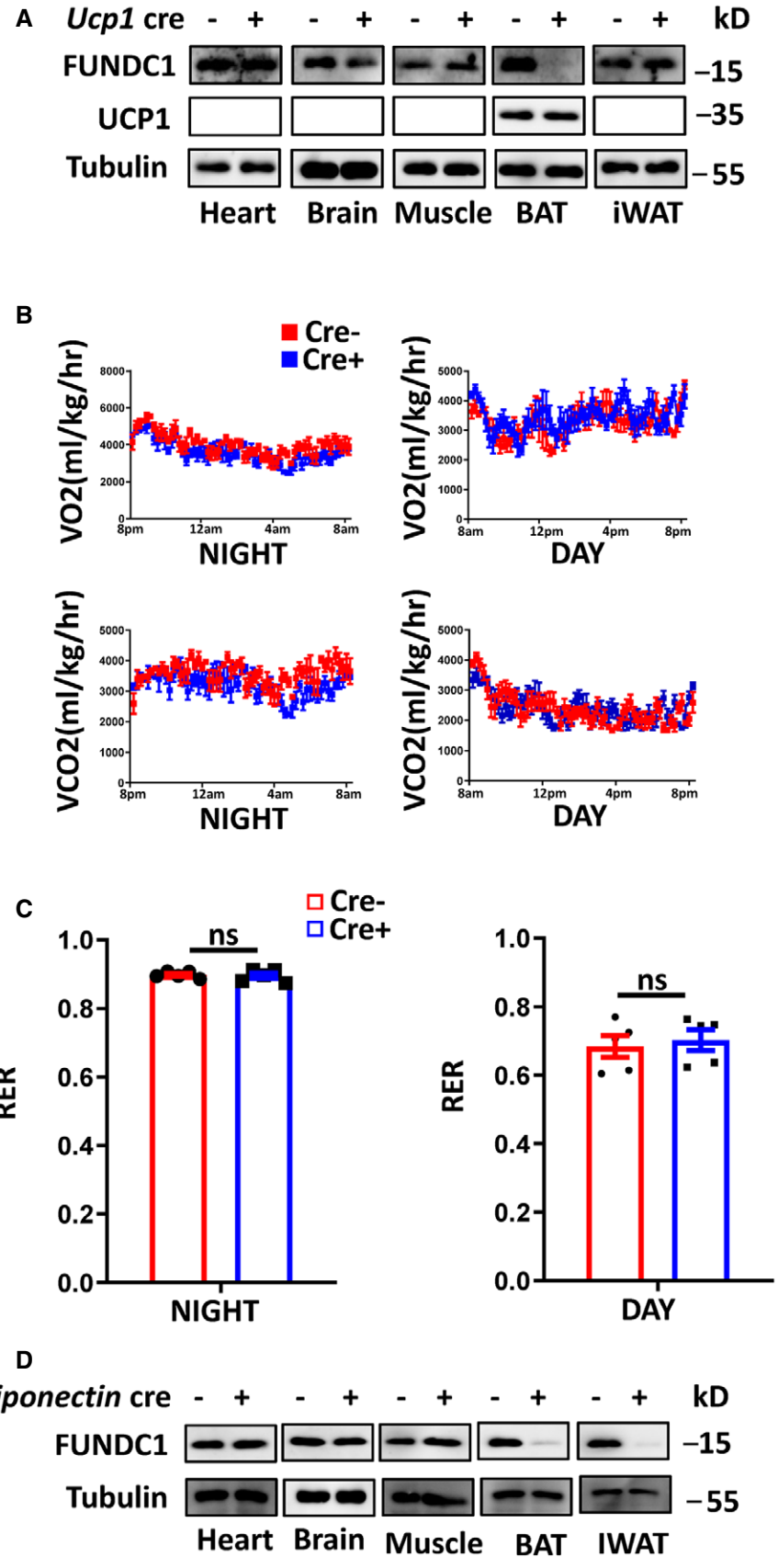

Figure EV3. Generation of mice with brown adipocyte-specific or adipocyte-specific knockout of *Fundc1*.

A Western blot analysis of FUNDC1 in different tissues in *Fundc1*$^{fl/fl}$/*Ucp1*$^{cre-}$ (*Ucp1* Cre-) and *Fundc1*$^{fl/fl}$/*Ucp1*$^{cre+}$ (*Ucp1* Cre+) mice. BAT, brown adipose tissue; iWAT, inguinal white adipose tissue.

B Rates of O_2 consumption (VO_2) and CO_2 production (VCO_2) were measured in *Fundc1*$^{fl/fl}$/*Ucp1*$^{cre-}$ (Cre–) and *Fundc1*$^{fl/fl}$/*Ucp1*$^{cre+}$ (Cre+) mice. Data information: $n = 5$ biological replicates, data are represented as the mean ± SEM.

C Respiratory exchange rate (RER) of *Fundc1*$^{fl/fl}$/*Ucp1*$^{cre-}$ (Cre–) and *Fundc1*$^{fl/fl}$/*Ucp*$^{cre+}$ (Cre+) mice. Data information: $n = 5$ biological replicates, data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. ns: no significant difference.

D Western blot analysis of FUNDC1 in different tissues in *Fundc1*$^{fl/fl}$/*Adiponectin*$^{cre-}$ (*Adiponectin* Cre–) and *Fundc1*$^{fl/fl}$/*Adiponectin*$^{cre+}$ (*Adiponectin* Cre+) mice.

Figure EV4. Ablation of *Fundc1* suppresses mitochondrial biogenesis without affecting autophagy proteins.

A–D *Ucp1* Cre- and *Ucp1* Cre+ mice were exposed to 4°C for 72 h and control mice were kept at 30°C. Autophagy-related protein (A) and mitochondrial protein (C) levels were analyzed by Western blotting. Protein expression levels were quantified and normalized to tubulin and plotted (B, D). Data information: $n = 6$ biological replicates, data are represented as the mean ± SEM. Statistical analysis was performed using two-way ANOVA test. ns: no significant difference; $^{**}P < 0.01$; $^{***}P < 0.001$.

E Wild-type (WT) and *Ucp1* Cre+ WT mice were exposed to 4°C for 72 h (Cold), and control mice were kept at 30°C (Normal). Western blotting analysis of UPC1 protein levels.

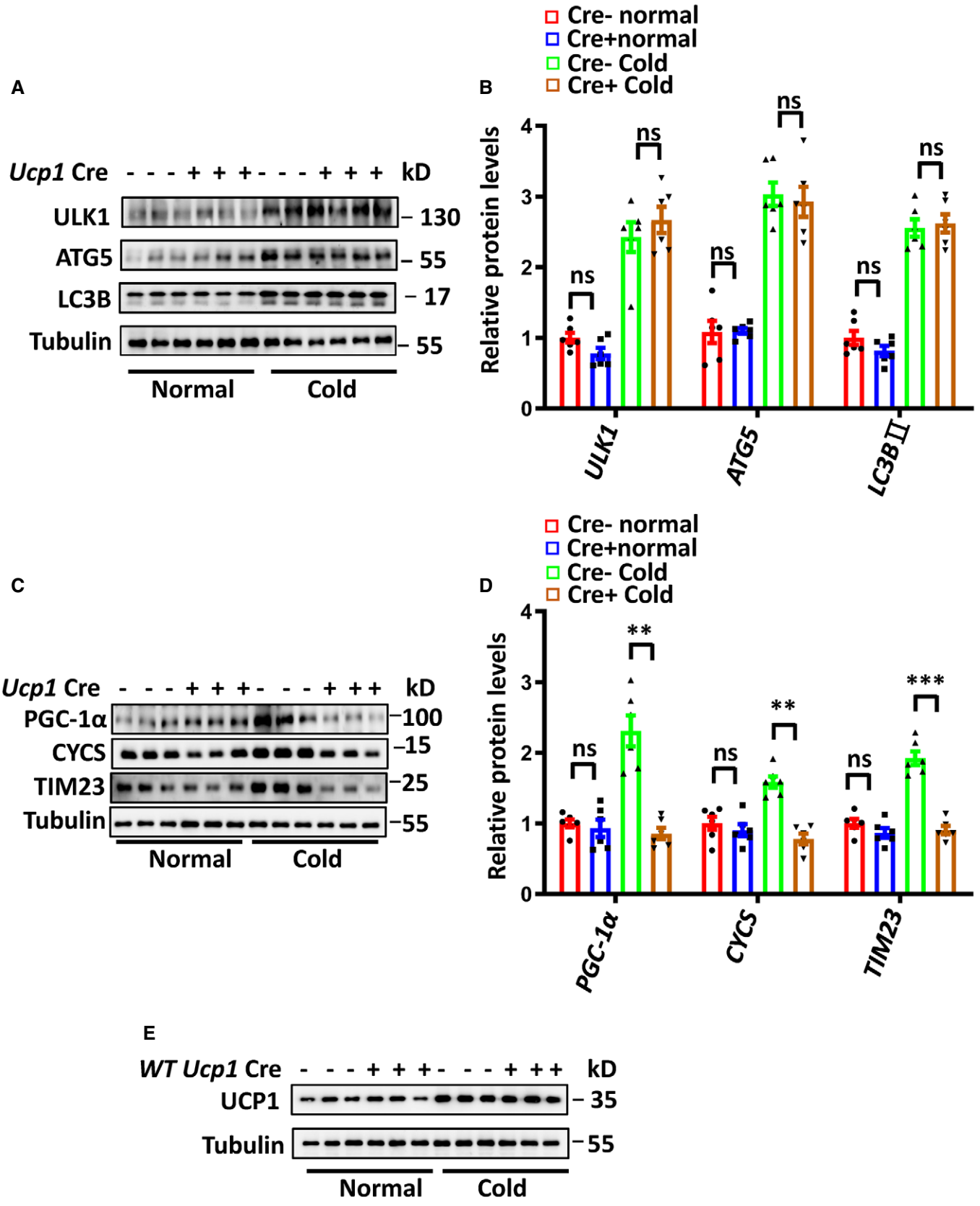
A
Ucp1 Cre - - - + + + - - - + + + kD
ULK1 – 130
ATG5 – 55
LC3B – 17
Tubulin – 55
Normal
Cold
B
Cre- normal
Cre+normal
Cre- Cold
Cre+ Cold
Relative protein levels
ns
ULK1
ATG5
LC3BII
C
Ucp1 Cre - - - + + + - - - + + + kD
PGC-1α –100
CYCS –15
TIM23 –25
Tubulin –55
Normal
Cold
D
Cre- normal
Cre+normal
Cre- Cold
Cre+ Cold
Relative protein levels
ns
**
***
PGC-1α
CYCS
TIM23
E
WT Ucp1 Cre - - - + + + - - - + + + kD
UCP1 – 35
Tubulin – 55
Normal
Cold

Figure EV4.

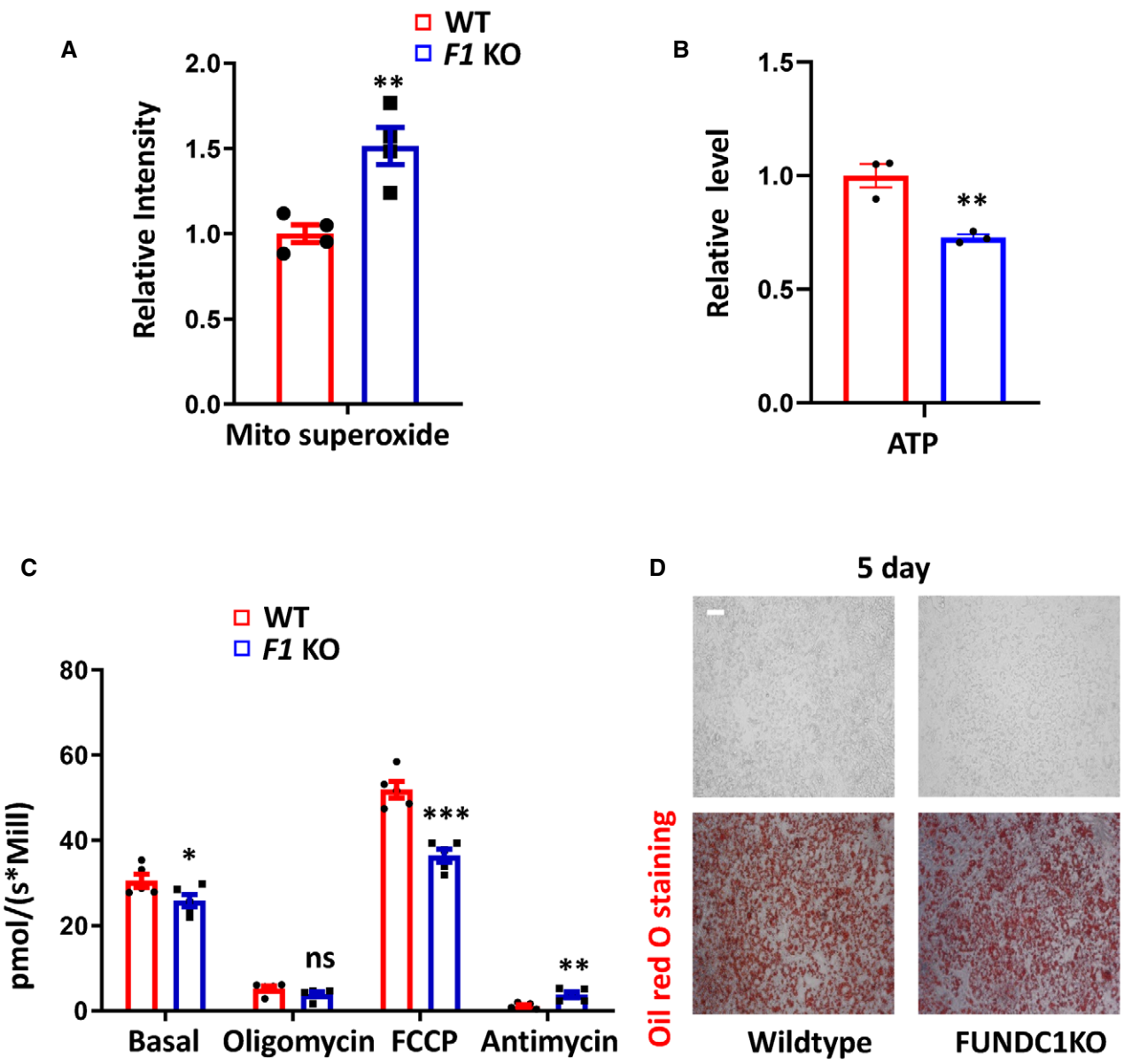

Figure EV5. FUNDC1-dependent mitophagy controls mitochondrial quality.

A Mitochondrial ROS levels were measured by Mito-Sox staining in WT and *Fundc1* knockout (*F1* KO) brown fat preadipocytes. Data information: Experiments were repeated four times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. $^{**}P < 0.01$.

B ATP levels were measured with a luciferase assay kit in WT and *Fundc1* KO preadipocytes. Data information: Experiments were repeated three times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. $^{**}P < 0.01$.

C OCR was measured in WT and *Fundc1* KO preadipocytes. Data information: Experiments were repeated four times, and data are represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

D Oil red O staining analysis of wild-type and *Fundc1* KO brown fat cells after differentiation for 5 days, scale bar, 10 μm.

