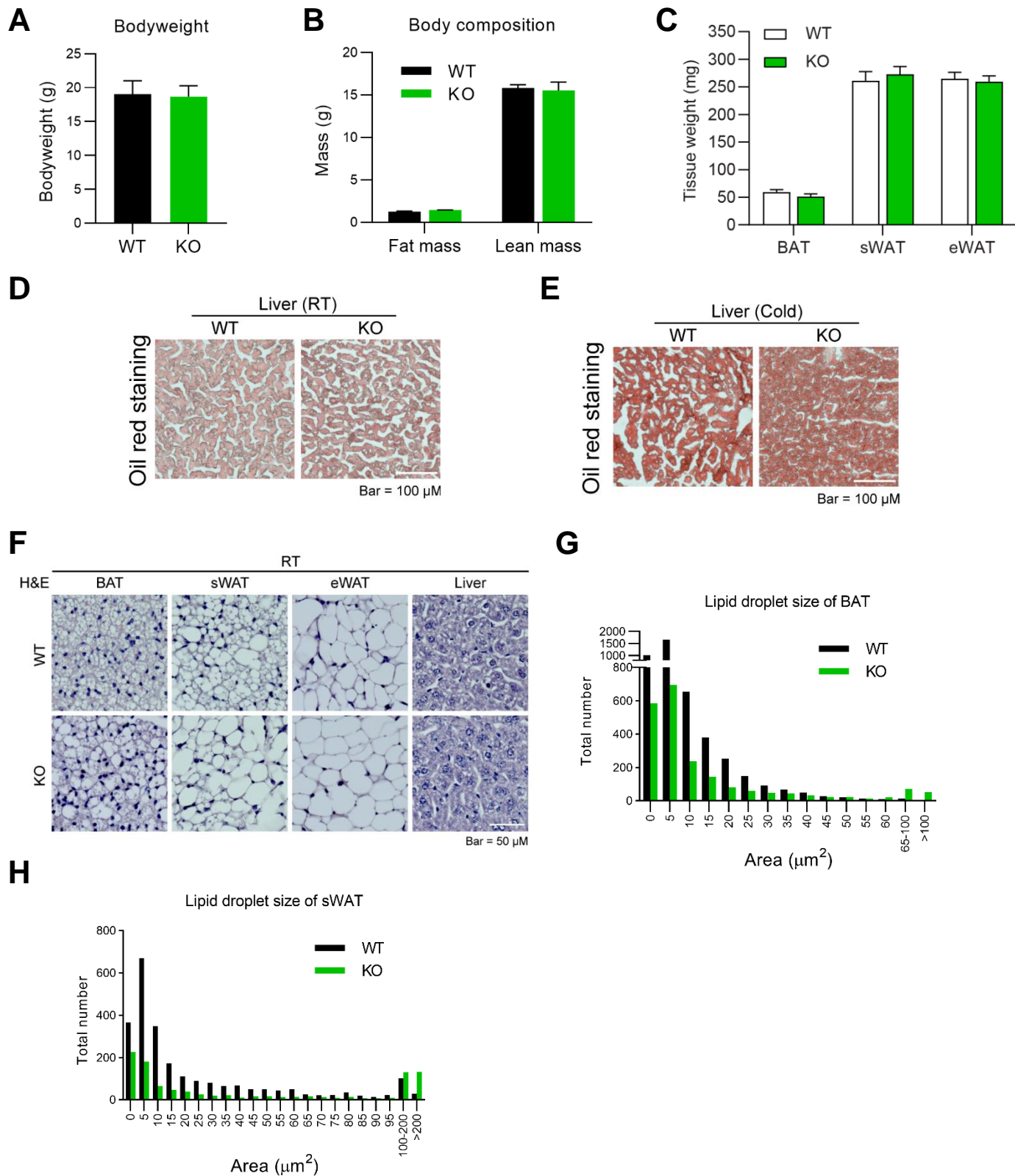


# Figure S1

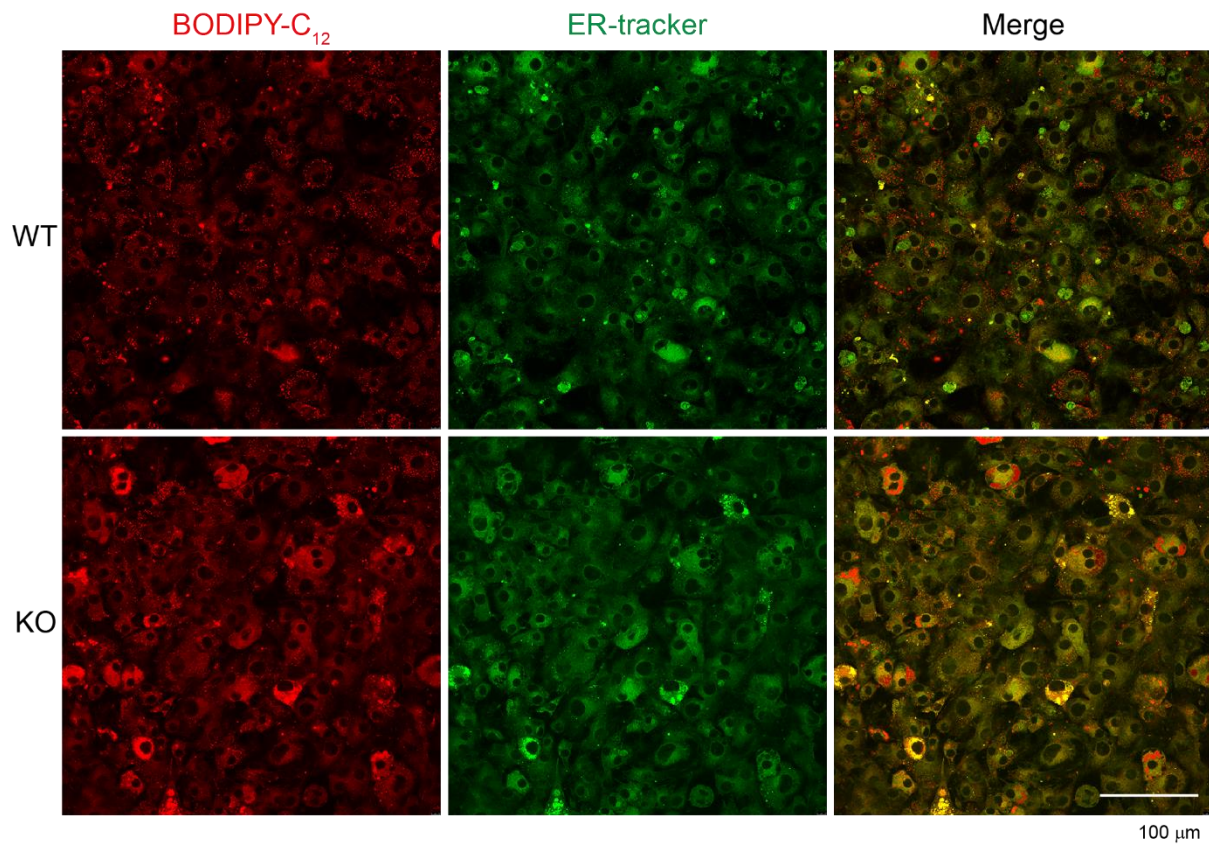


## Figure S1

- (A)** Body weights of Adipo-*Drp*<sup>flx/flx</sup> mice and their littermate controls at room temperature (RT) (n = 6 per group, data are represented as mean ± SEM, *Student's t-test*).
- (B)** Body composition for fat mass and lean mass of Adipo-*Drp*<sup>flx/flx</sup> mice and their littermate controls housed RT (n = 6 per group, data are represented as mean ± SEM, *Student's t-test*).
- (C)** Tissue weights of the BAT, sWAT and eWAT from Adipo-*Drp*<sup>flx/flx</sup> mice and their littermate controls (WT) after cold exposure (n = 6 per group, data are represented as mean ± SEM, *Student's t-test*).
- (D)** Oil red staining on the liver of Adipo-*Drp*<sup>flx/flx</sup> mice and their littermate controls at RT (Representative of 6 mice).
- (E)** Oil red staining on the liver of Adipo-*Drp*<sup>flx/flx</sup> mice and their littermate controls after cold exposure at 6°C (Representative of 6 mice).
- (F)** H&E staining of the BAT, sWAT, eWAT and liver of Adipo-*Drp*<sup>flx/flx</sup> and their littermate controls at RT (Representative of 6 mice).
- (G)** Lipid size distribution of BAT in (F).
- (H)** Lipid size distribution of sWAT in (F).

# Figure S2

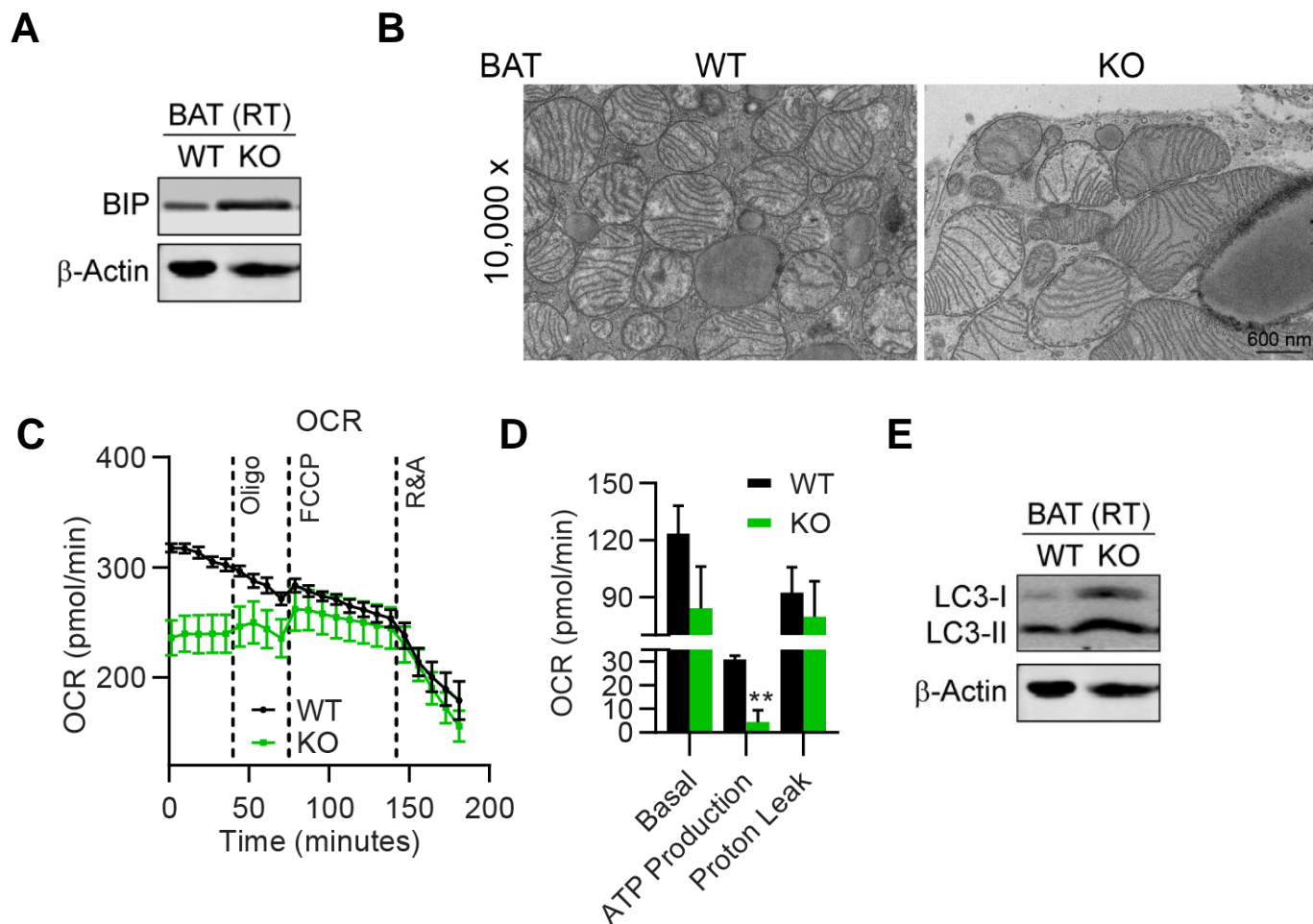
A



## Figure S2

**(A)** Co-staining of BODIPY-C<sub>12</sub> (red) and ER-tracker (green) in differentiated SVF cells from Adipo-*Drp1<sup>flx/flx</sup>* mice and their littermate controls. The cells were staining with BODIPY-C<sub>12</sub> for 10 min and chased in BODIPY-C<sub>12</sub> free media for 1 hour. The cells were stained with ER-tracker during the 1 hour chase phase (Representative of three trials are shown).

## Figure S3



### Figure S3

**(A)** Western blotting analysis of BIP and  $\beta$ -Actin (as loading control) protein levels in the BAT of Adipo-Drp1<sup>flx/flx</sup> mice and their littermate controls at RT (n = 3 per group, the sample loaded in each lane contains pooled samples from 3 mice, representative for three trials).

**(B)** The transmission electron microscopy images for BAT of Adipo-Drp1<sup>flx/flx</sup> mice and their littermate controls after cold exposure at 6°C (Representative of 3 mice).

**(C)** Oxygen consumption rate (OCR) in the BAT measured by a Seahorse XFe24 instrument. The BAT explants were collected from Adipo-Drp1<sup>flx/flx</sup> mice and their littermate controls after cold exposure (n=5 per group, data are represented as means  $\pm$  SEM).

**(D)** Mitochondrial respiration parameters including basal respiration, ATP production, and proton leak were calculated based on the OCR readings in (C) (n=5 per group, data are represented as means  $\pm$  SEM, Student's *t*-test, \*\*p<0.01).

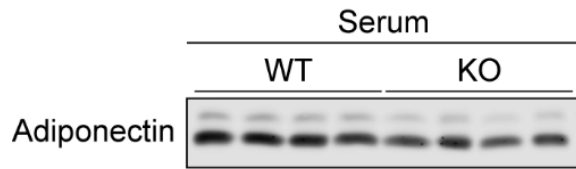
# Figure S3

## Figure S3

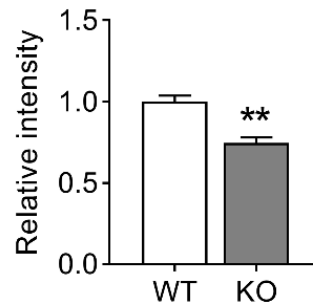
(E) Western blotting analysis of LC3-I/II and b-Actin (as loading control) protein levels in the BAT Adipo-*Drp1*<sup>flx/flx</sup> mice and their littermate controls at RT (n = 3 per group, the sample loaded in each lane contains pooled samples from 3 mice, representative for three trials).

# Figure S4

**A**



**B**

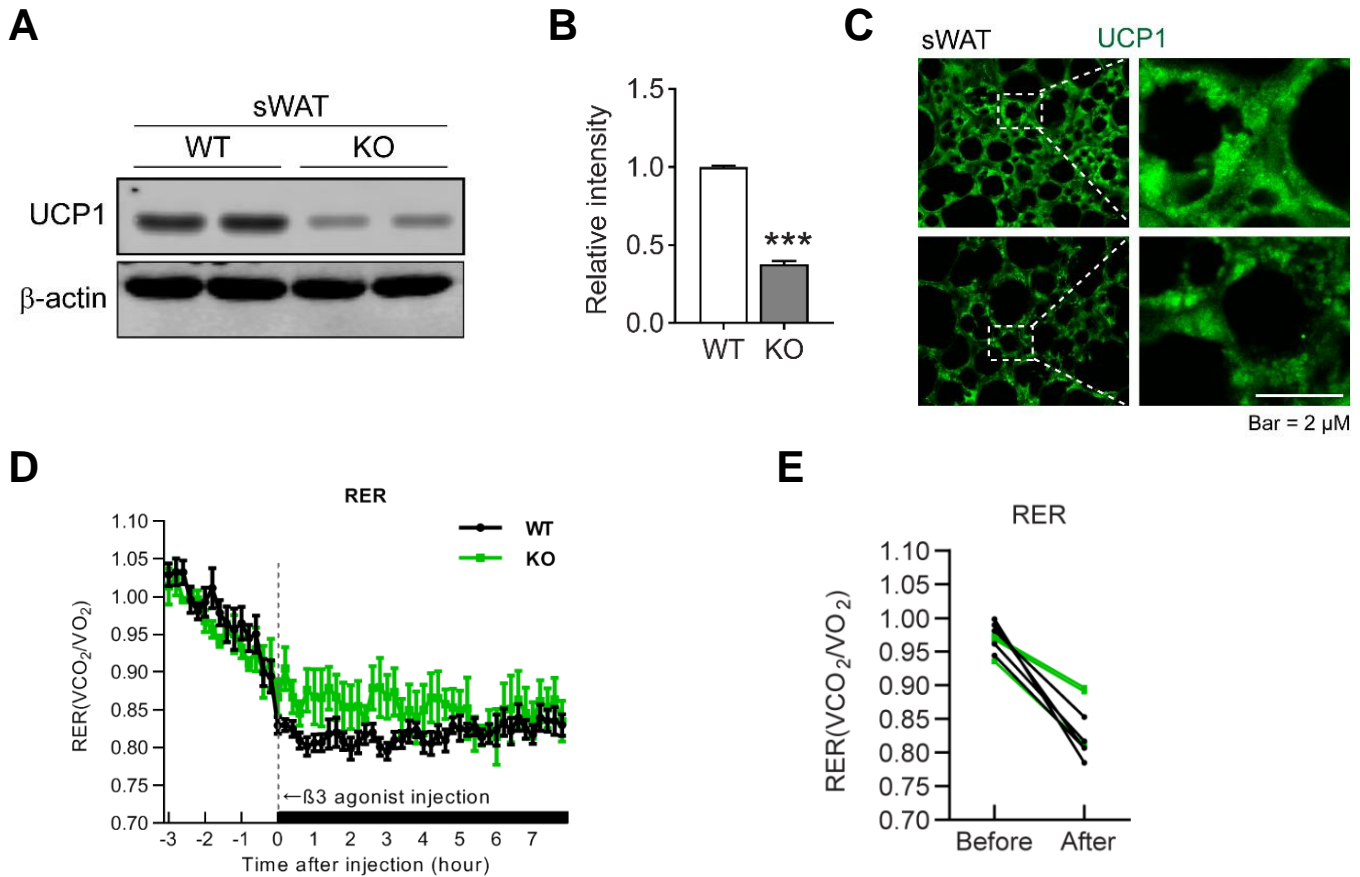


## Figure S4

**(A)** Western blotting analysis of Adiponectin protein levels in the serum of *Adipo-Drp1<sup>flx/flx</sup>* mice and their littermate controls after cold exposure (n = 4 per group, representative of three trials)

**(B)** The quantification of band intensity in (A) (n = 4 per group, representative of three trials, data are represented as mean  $\pm$  SEM, *Student's t-test*, \*\*p<0.01 ).

# Figure S5



## Figure S5

**(A)** Western blotting analysis of UCP1 protein levels in the sWAT of Adipo-*Drp1<sup>flx/flx</sup>* mice and their littermate controls after cold exposure. (n = 6 per group, the sample loaded in each lane contains pooled samples from 3 mice. Representative of three trials )

**(B)** The quantification of relative band intensity in (A) (n = 6 per group, the sample loaded in each lane contains pooled samples from 3 mice. Representative of three trials. The data are represented as mean  $\pm$  SEM, *Student's t-test*, \*\*\*p<0.001).

**(C)** IF staining with anti-UCP1 antibody in the sWAT from Adipo-*Drp1<sup>flx/flx</sup>* mice and their littermate controls after cold exposure (Representative of three trials are shown).

**(D)** RER for indirect calorimetry analysis of Adipo-*Drp1<sup>flx/flx</sup>* mice and their littermate controls upon treatment of  $\beta$ 3 agonist CL-316,243 (n = 3-5 per group, data are represented as mean  $\pm$  SEM)

**(E)** The average of 3-hour RER before and after CL-316,243 treatment for each mouse is shown (n = 3-5, *Student's t-test*, no differences).