

## Supplementary information

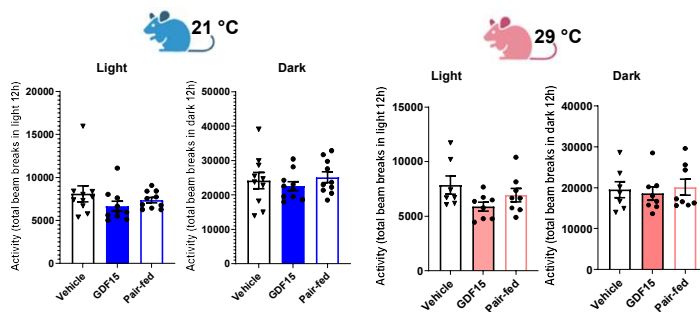
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# GDF15 promotes weight loss by enhancing energy expenditure in muscle

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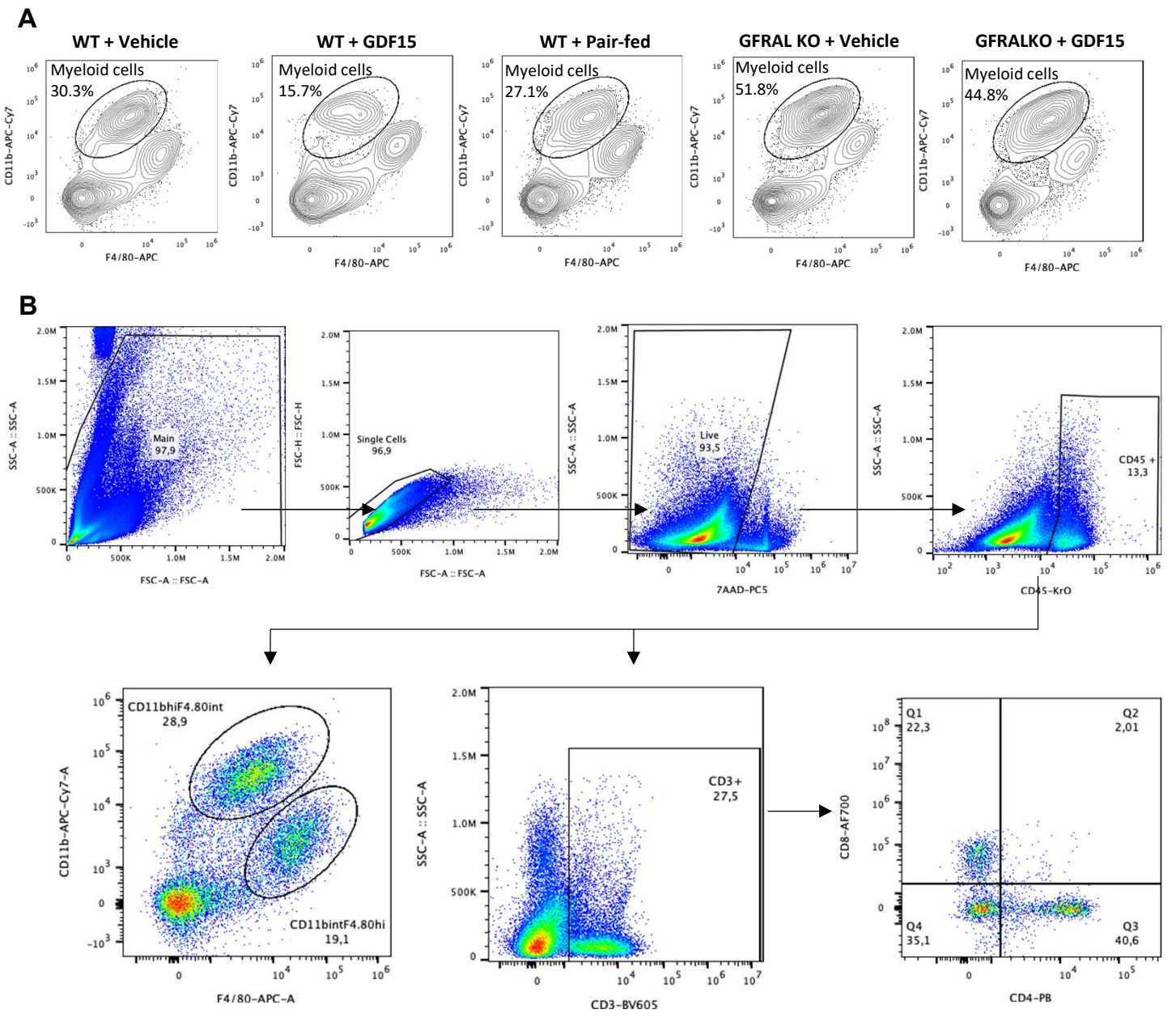
In the format provided by the authors and unedited

### Supplementary Information Figure 1. Activity in CLAMS (related to Fig. 2A-E)

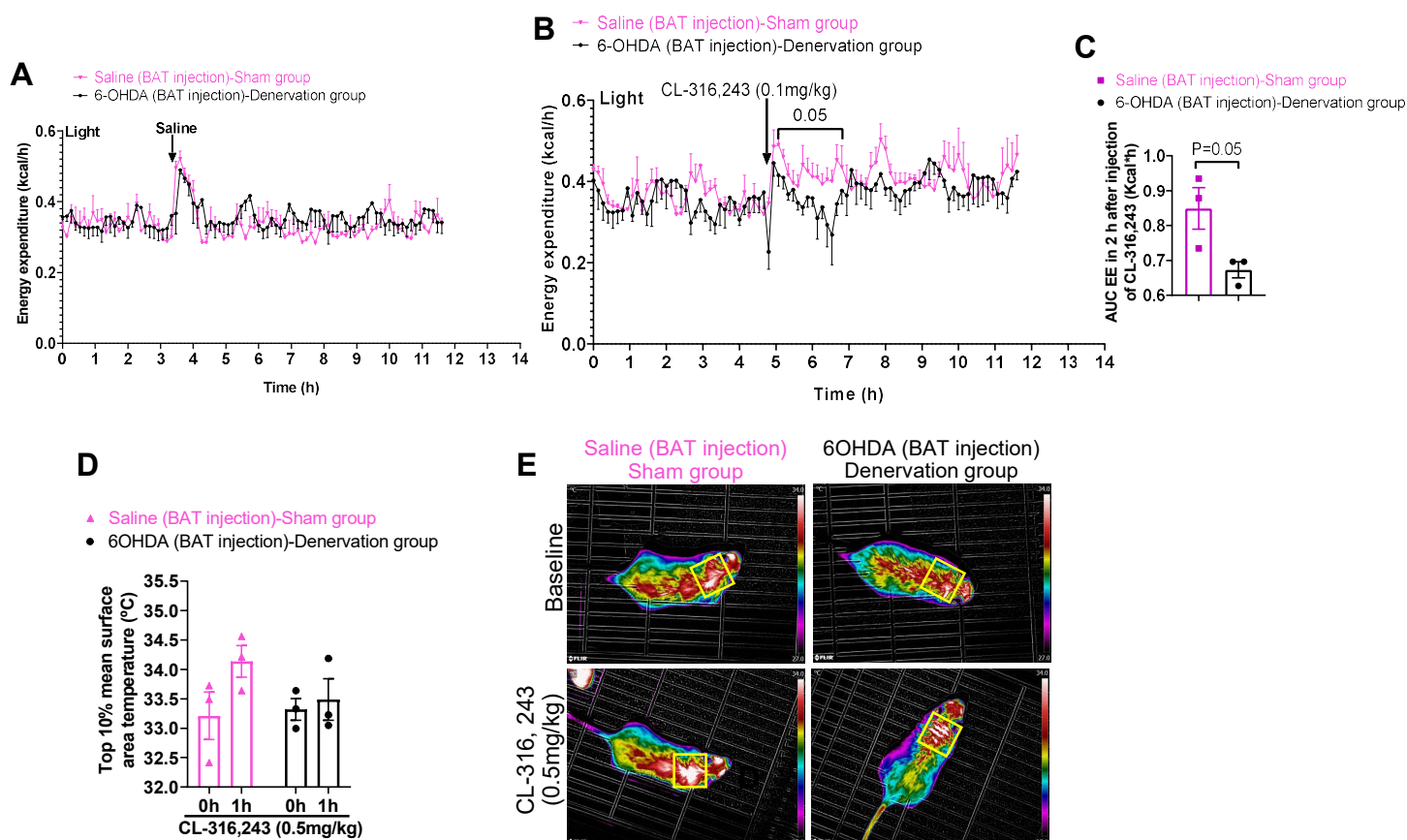


SI Figure 1. Animal activity during 12h-light/dark cycle. Data are mean ± SEM, n = 10 mice/group. Mice illustrations are obtained from BioRender.com (publication license number T2259QRW6B).

**Supplementary Information Figure 2. A and B Gating strategy for flow cytometry analysis of immune cell populations stained with CD45, CD11b, F4/80, CD3 and CD4 antibodies. (related to Extended Data Fig. 5)**

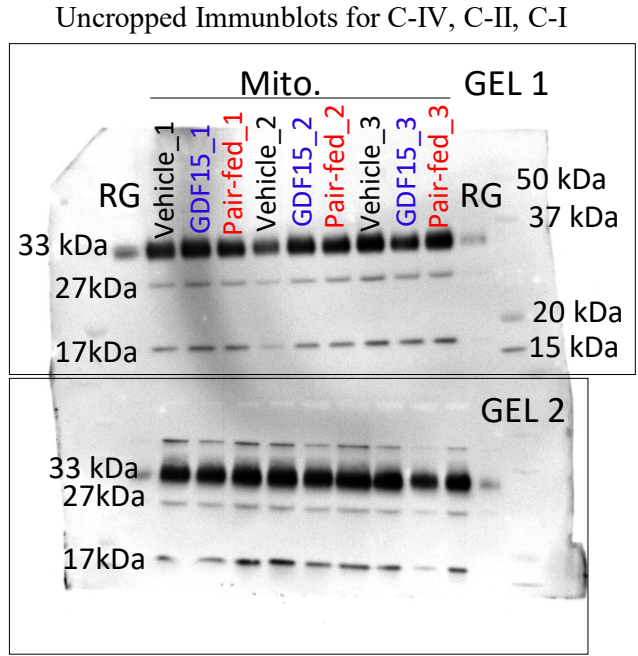
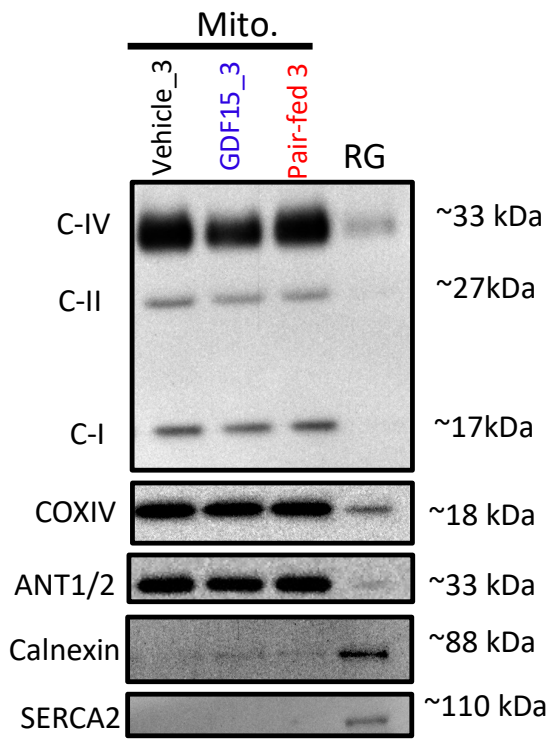


**Supplementary Information Figure 3. Confirmation of denervation of BAT by injection with 6-hydroxydopamine hydrobromide (6OHDA) directly to BAT (related to Extended Data Fig. 9)**



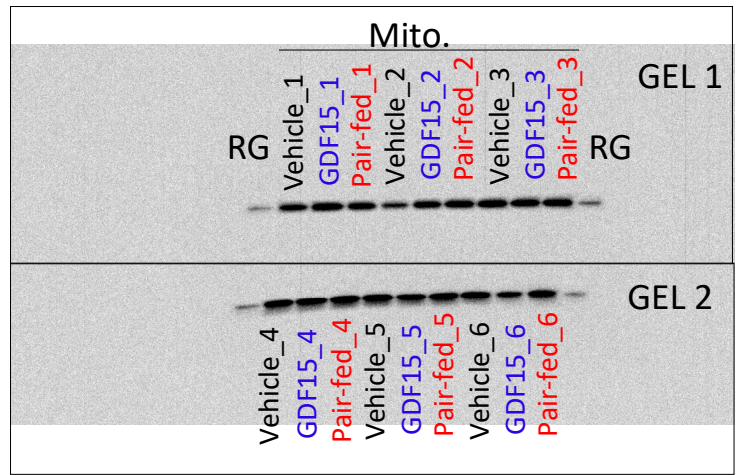
**SI Figure 3. Confirmation of denervation of BAT by injection with 6-hydroxydopamine hydrobromide (6OHDA) directly to BAT.** **A and B**, Experimental scheme for the effects of GDF15 and matched caloric restriction in pair-fed control on body weight, energy expenditure in C57BL/6J mice fed western diet and housed at thermoneutrality (TN, 29 °C). The BAT of mice were denervated by 6-hydroxydopamine hydrobromide (6OHDA). The BAT of mice was directly injected with 6-hydroxydopamine hydrobromide (6OHDA, BAT denervation group) or saline (Sham group). After 48 h of denervation, the mice were put in Comprehensive Laboratory Animal Monitoring System (CLAMS) and treated with saline on 1<sup>st</sup> day in CLAMS (**A**) or beta-3 adrenergic agonist (CL-316,243, 0.1mg/kg, ip) on 2<sup>nd</sup> day in CLAMS (**B**). Compared to Sham group, BAT denervation abolished the effect of CL-316,243 on energy expenditure. Data are mean  $\pm$  SEM, n = 3 mice/group. P values by multiple unpaired t test (two-sided). **C**, AUC of average energy expenditure in 2 h after injection of CL-316,243 (0.1mg/kg, ip). Data are mean  $\pm$  SEM, n = 3 mice/group. P values by unpaired t test (two-sided). **D**, Top 10% mean surface area temperature in the interscapular BAT area of the mice. Data are mean  $\pm$  SEM, n = 3 mice/group. **E**, Representative infrared images of mice. Images are displayed using the rainbow high contrast color palette in the FLiR Research IR program.

**Supplementary Information Figure 4. Mitochondrial cleanliness checking and their related uncropped Immunblots (related to Extended Data Fig. 10H-K).**

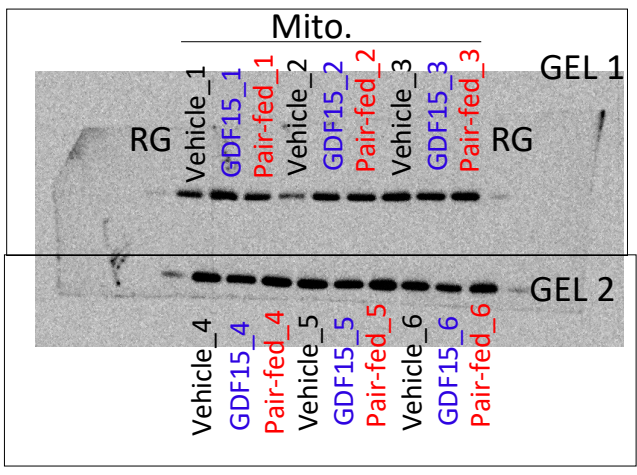


Vehicle\_1: vehicle, mouse #1  
 GDF15\_1: GDF15 treatment, mouse #1  
 Pair-fed\_1: Pair-feeding group, mouse #1

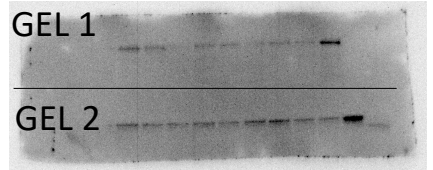
**Uncropped Immunoblots for COXIV – Cytochrome C oxidase, subunit 4**



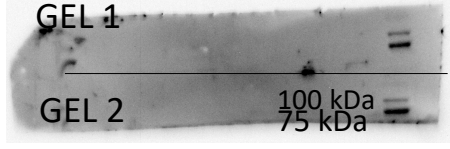
**Uncropped Immunoblots for ANT 1/2**



**Uncropped Immunoblots for Calnexin**



**Uncropped Immunoblots for SERCA2**



**SI Figure 4. Mitochondrial cleanliness checking.** mitochondrial proteins: COXI, COXIV; non-mitochondrial proteins: GLUT4, calnexin, SERCA2. (RG: red gastrocnemius muscle). Results were reproduced in 6 mice.

**Supplementary Information Figure 5. Absolute JO<sub>2</sub> in the permeabilized fibers during ADP titration.** Data are means  $\pm$  SEM, n = 6 mice/group except GDF15 group, n = 5 mice. We utilized the one-phase association curve from this ADP titration to estimate the ADP generated during the Ca<sup>2+</sup> titration as an index of SERCA efficiency. (related to Figure 4G and Extended Data Fig. 100).

