Chung et al., https://doi.org/10.1083/jcb.201607110

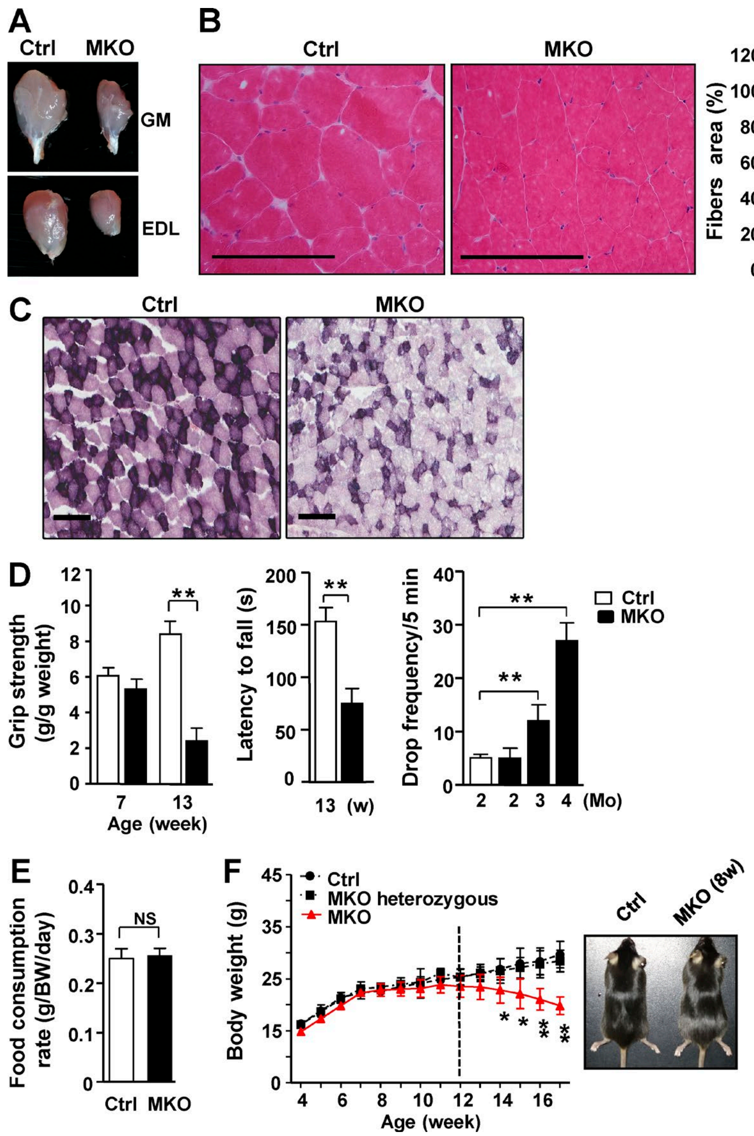

Figure S1. **Muscle phenotypes and metabolic parameters of MKO mice fed a chow diet.** (A) Gross image of gastrocnemius muscle (GM; top) and extensor digitorum longus (EDL) muscle (bottom) from chow-fed 15-wk-old male Ctrl and MKO mice. (B) Hematoxylin and eosin staining of GM (left) and quantitation of GM fiber cross-sectional area in 15-wk-old Ctrl and MKO mice fed a chow diet. Bars, 100 µm. (C) Succinate dehydrogenase staining in EDL muscle in 15-wk-old male Ctrl and MKO mice. Bars, 100 µm. (D) Grip strength (13 wk, *n* = 10), latency to fall (13 wk, *n* = 10), and drop frequency of 2- to 4-mo-old male Ctrl and MKO mice (*n* = 10 per group). (E) Daily food intake on chow diet was measured in 8-wk-old male Ctrl and MKO mice. Mice were housed individually, and food weight was measured three times per week (*n* = 10 per group). (F) Body weight of male Ctrl, MKO heterozygous, and MKO mice on standard chow diet (left, *n* = 10 per group). Representative images of 8-wk-old mice (right). All data represent mean ± SEM; *, $P < 0.05$; **, $P < 0.01$. N.S., not significant.

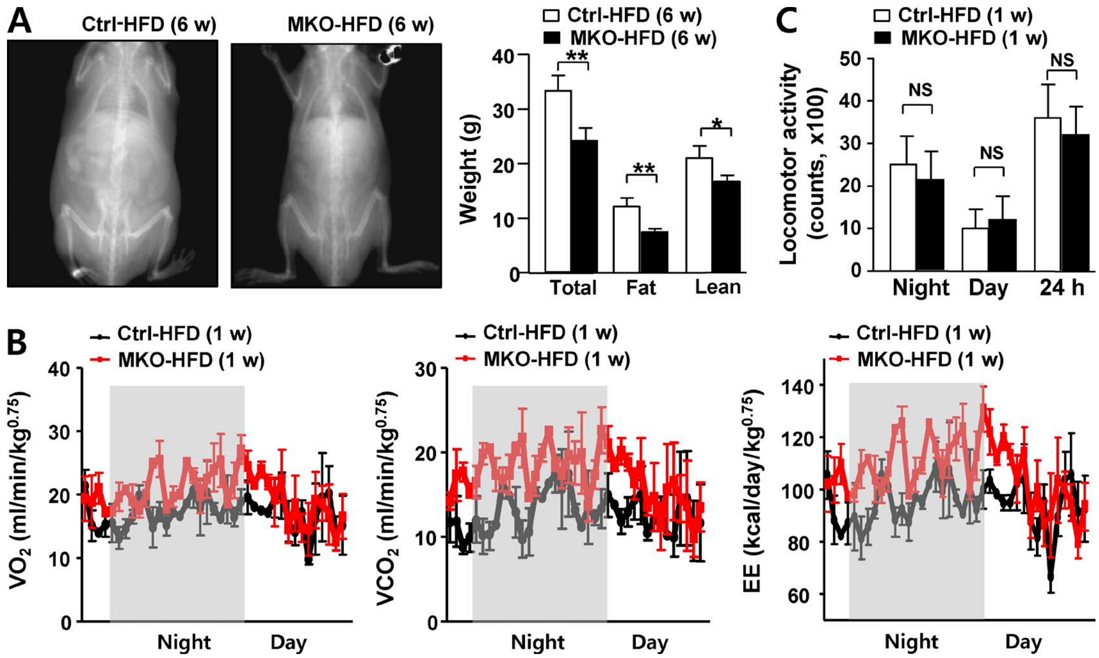

Figure S2. **Reduced muscle and fat mass and elevated EE in HFD-fed MKO mice.** (A) Representative dual-energy x-ray absorptiometry images (left) and body composition of HFD-fed 11-wk-old male Ctrl and MKO mice (*n* = 10 per group). 5-wk-old male mice were fed an HFD for 6 wk. (B) Oxygen consumption (VO_2), carbon dioxide generation (VCO_2), energy expenditure (EE), and locomotor activity (C) of 8-wk-old male Ctrl and MKO mice on 1 wk (short-term) HFD. Mice were analyzed by indirect calorimetry over a period of 12 h light/12 h dark cycles (*n* = 5 per group). Data represent mean ± SEM. *, P < 0.05; **, P < 0.01. NS, not significant.

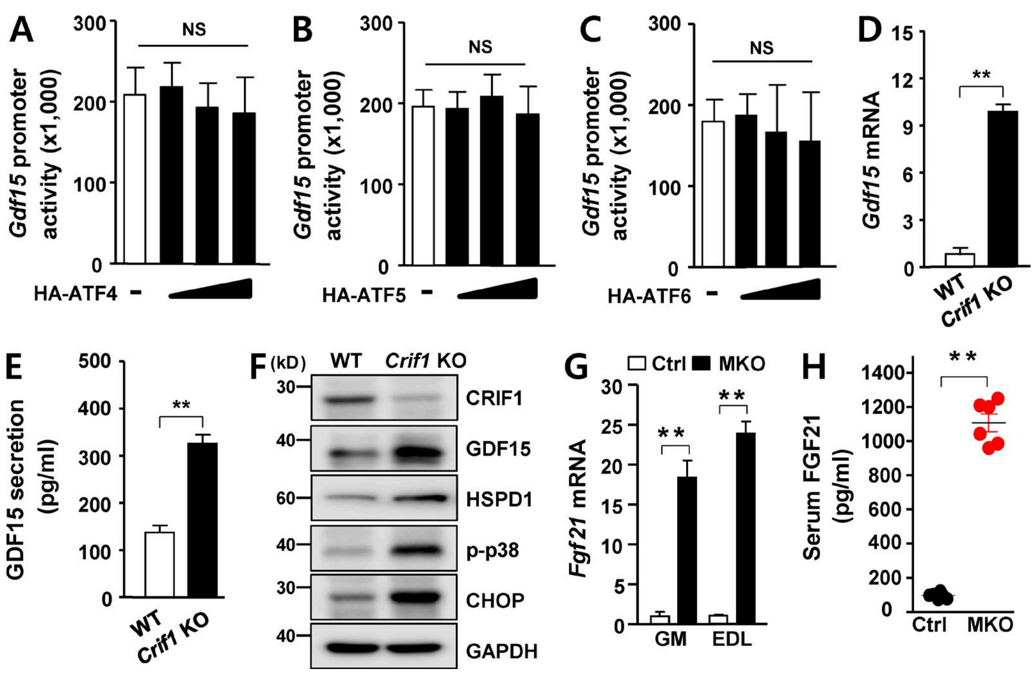

Figure S3. **ATF4/5/6-independent *GDF15* promoter activity and CHOP-dependent induction of GDF15 in *Crif1*-deficient MEF cells.** (A–C) Promoter activity of *GDF15* in C2C12 myoblasts transiently cotransfected with a luciferase reporter driven by the human *GDF15* promoter (−1.7 kb) and mock control or expression vector for HA-tagged human ATF4 (A), human ATF5 (B), or human ATF6 (C). *Gdf15* mRNA expression (D) and GDF15 secretion (E) in *Crif1*-knockout (KO) MEF cells. (F) Immunoblotting to detect CRIF1, GDF15, HSPD1, CHOP, and phospho-p38 T180/Y182 expression in *Crif1*-deficient MEF cells. All data represent mean ± SEM. **, P < 0.01. NS, not significant. (G) *Fgf21* mRNA expression in GM and EDL muscle in 8-wk-old male Ctrl and MKO mice fed a chow diet (*n* = 10 per group). (H) Serum FGF21 concentration in chow-fed 8-wk-old male Ctrl and MKO mice in the fasting state (*n* = 5–6 per group). All data represent mean ± SEM. *, P < 0.05; **, P < 0.01.

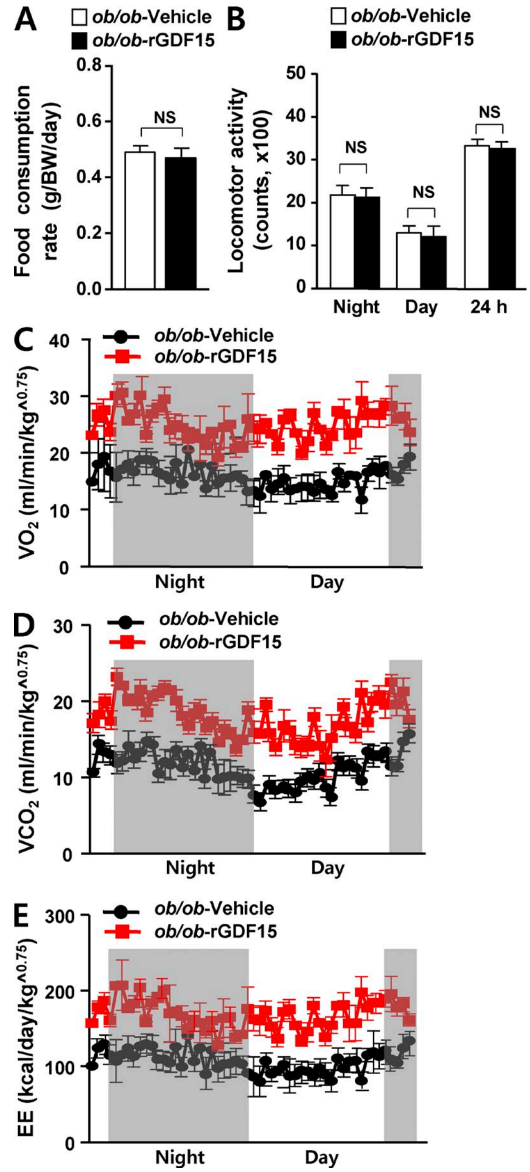

Figure S4. **rGDF15-treated *ob/ob* mice exhibit elevated EE.** Food intake (A), locomotor activity (B), oxygen consumption (VO_2; C), carbon dioxide generation (VCO_2; D), and energy expenditure (EE; E) of *ob/ob*-vehicle and *ob/ob*-rGDF15 mice. Male *ob/ob* mice were injected intraperitoneally three times per week for 3 wk with 0.5 mg/kg rGDF15 or vehicle. Mice were analyzed by indirect calorimetry over a period of 12 h light/12 h dark cycles (*n* = 5 per group). All data represent means ± SEM. NS, not significant.

Table S1. **List of primer sets used for quantitative RT-PCR analyses**

Gene	Forward (5′–3′)	Reverse (5′–3′)
Clpp	GCCATTCACTGCCCAATTCC	TGCTGACTCGATCACCTGTAG
Hspd1	GAGCTGGGTCCCTCACTCG	AGTCGAAGCATTTCTGCGGG
Lonp1	AGCCCTATGTTGGCGTCTTC	CCGGCTGATGTGAATCCTTCT
Tid1	GGAAGCAAGGATAGGCGAGA	GTTGACCGCTTTCCTCAGCAG
Htra2	TCCCCGGAGCCAGTACAAT	GAAAGGGTGCCGGTCTAGG
Chop	AACAGAGGTCACCAGCACAT	ACTTTCCGCTCGTTCTCCTG
Atf4	GGGTTCTGTCTTCCACTCCA	AAGCAGCAGAGTCAGGCTTTC
Grp78	GTGTGTGAGACCAGAACCGT	AGTCAGGCAGGAGTCTTAGG
Ppara	CTCCCTCCTTACCCTTGGAG	GCCTCTGATCACCACCATTT
Acadvl	TTACATGCTGAGTGCCAACAT	CGCCTCCGAGCAAAAGATT
Acadvm	TGACGGAGCAGCCAATGA	TCGTCACCCTTCTTCTCTGCTT
Hsl	AGACACCAGCCAACGGATAC	GCGGTTAGAAGCCACATAGC
Fasn	TACGTACTGGCCTACACCCAA	TGAACTGCTGCACGAAGAAGCAT
Pparg	ATCTTAACTGCCGGATCCAC	TGGTGATTTGTCCGTTGTCT
Pgc1a	TCACACCAAACCCACAGAAA	CTTGGGGTCATTTGGTGACT
Cpt1a	TATAACAGGTGGTTTGAC	CAGAGGTGCCCAATGATG
Cpt1b	TCGCAGGAGAAAACACCATG	AACAGTGCTTGGCGGATGTG
Cox1	ATTCGAGCAGAATTAGGTCA	CTCCGATTATTAGTGGGACA
Ndufa9	ACTGTGTTTGGGGCTACAGG	GATTGATGACCACGTTGCTG
Dio2	AGAGTGGAGGCGCATGCT	GGCATCTAGGAGGAAGCTGTTC
Ucp1	AGGGCCCCCTTCATGAGGTC	GTGAAGGTCAGAATGCAAGC
Xbp1	CAGCACTCAGACTATGTGCA	GTCCATGGGAAGATGTTCTGG
Xbp1 spliced	CTGAGTCCGAATCAGGTGCAG	GTCCATGGGAAGATGTTCTGG
Gdf15	GAGCTACGGGGTCGCTTC	GGGACCCCAATCTCACCT
Fgf21	AGATCAGGGAGGATGGAACA	TCAAAGTGAGGCGATCCATA
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Atgl	CCACCAACATCCACGAGCTT	TTCGAGAGGCGGTAGAGATTG
18S	CTGGTTGATCCTGCCAGTAG	CGACCAAAGGAACCATAACT
Gapdh	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT