Supporting Information

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SI Experimental Procedures

***Drosophila* Strains and Cell Culture.** Late third instar larvae (12 h before wandering) grown in normal food at 25 °C with density control in a mixture of both male and female were used in this study. RNAi lines against *w* (JF01545), *ND-75* (JF02791), *ND-49* (HM05059), *ND-42* (HMS05104), *Actβ* (JF03276), *daw* (HMS01110), *dpp* (JF02455), *gbb* (HMS01243), *mav* (HMS01125), *myo* (JF01587), *Rel* (HMS00070), *babo* (JF01953), *put* (HMS01944), *cyt-c1* (HMS01057), *COX5A*(JF02700), *cype* (HMS00815), *ATPsynβ* (JF02892), *blw* (JF02896), *CG12262* (HMS00434), *Thiolase* (HMS01017), and *Mtpα* (HMS00660) were obtained from the TRiP at Harvard Medical School (https://fgr.hms.harvard.edu/fly-in-vivo-rnai), and *ImpL2* (15009R-3) were obtained from National Institute of Genetics at Japan (https://shigen.nig.ac.jp/fly/nigfly/). *UAS-Actβ*, *UAS-PHGPx*, *UAS-foxo*, *UAS-hep-CA*, and *UAS-babo-CA* have been described (1, 2) and *UAS-Rel-68* (BLM 55778) was obtained from Bloomington Stock Center. *CG-Gal4*, *dMef2-Gal4*, *Mhc-Gal4, w^{1118}, Dpt-GFP*, and *Tub-Gal^{80}, UAS-ND-75-RNAi; dMef2-Gal4* have been described (1, 2). To knockdown TGF-β family ligands or transcriptional factors in *ND-75*-deficient muscles, *UAS-RNAi* lines were crossed to *Tub-Gal80, UAS-ND-75-RNAi; dMef2-Gal4* for 24 h at 18 °C to inactivate Gal4. Progenies were grown at 29 °C until the third instar stage (12 h before wandering). Because *Mhc-Gal4* is expressed after the second instar larval stage, it was used in most overexpression experiments to avoid potential developmental problems.

Mouse myoblasts C2C12 were purchased from American Type Culture Collection. C2C12 myoblasts (<15 passages) were cultured in DMEM with 10% FBS and antibiotics and differentiated into myotubes in DMEM containing 2% horse serum and antibiotics (Thermo Fisher Scientific) for 5 d at 37 °C in 5% CO_2.

Fluorescence Staining, Microscopy, and Western Blot. Third instar larval fat bodies or muscles were dissected in PBS and fixed for 15 min in PBS containing 4% formaldehyde. After fixation, the tissues were washed with PBS containing 0.2% Triton X-100 and incubated in primary antibodies overnight at 4 °C. Secondary antibodies together with DAPI (1:1,000, Invitrogen) were used to incubate tissues for 1 h at room temperature. Tissues were washed and mounted in Vectashield (Vector). Rabbit anti-human-p-SMAD3 (1:1,000, Epitomics, 1880-1) antibody for detection of *Drosophila* p-MAD and mouse anti-ATP5A (1:1,000, Mitosciences, MS507) for mitochondria detection were used. Bodipy 493/503 (1 μg/mL, Invitrogen) was used for neutral lipid staining for 30 min at room temperature. C2C12 myoblasts were treated with 100 nM Rotenone (Sigma, R8875) for 1 h and incubated with CellROX Green Reagent (1:1,000, Thermo Fisher, C10444) for 30 min to detect ROS generation. Fluorescent microscopy was performed on a Zeiss Axioskop 2motplus upright, and confocal images were obtained by using a Leica TCS SP2 AOBS system. Ten larval fat bodies and muscles were homogenized with buffer (50 mM Tris·HCl [pH 7.5], 5 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1% Nonidet P-40). Protein extracts were immunoblotted with indicated antibodies: rabbit anti-p-SMAD2 (S465/467, 1:1,000, Cell Signaling, 3108) for p-dSmad2 detection and mouse anti-Tubulin (1:5,000, Sigma).

Mitochondrial O_2 Consumption. Mitochondria from 20 third instar larval fat bodies or muscles were isolated using Mitochondria Isolation Kit for Tissue (Abcam, ab110168) following manufacturer's protocol. To avoid physical injury to isolated mitochondria, larval tissues were gently homogenized with disposable pellet pestles (Thermo Fisher, 12-141-364). Freshly isolated mitochondria were resuspended in isolation buffer (mitochondrial concentration > 1 mg/mL) and immediately used for next measurements. Mitochondrial O_2 consumption rates with or without ADP were measured in 96-well plate using Extracellular Oxygen Consumption Reagent (Abcam, ab197242).

ATP Measurement. Five muscles or fat bodies from third instar larvae were homogenized in 100 μL of extraction buffer (6 M Guanidine Chloride, 100 mM Tris·HCl pH 8.0, 4 mM EDTA), immediately heated at 70 °C for 5 min, then centrifuged at 18,400 × *g* for 10 min at 4 °C to remove cuticle and cell debris. The levels of ATP were measured by using ATP Determination Kit (Thermo Fisher, A22066) following the manufacturer's protocol and were further normalized to protein levels that were measured by using Bradford Reagent (Sigma).

Quantitative Lipidomic Analysis. Five third instar larval fat bodies in triplicate were freshly isolated and homogenized in 200 μL of PBS. Five-microliter homogenates were used to measure protein content of each sample by using Bradford Reagent (Sigma). For lipid profiling, lipids in the remaining homogenates were extracted by following standard protocol from the BIDMC Mass Spectrometry Facility (www.bidmcmassspec.org) and concentrated completely to dryness using a SpeedVac. Spectrophotometric determination was performed at the BIDMC Mass Spectrometry Facility, and lipid concentrations in the samples were normalized to measured protein content.

Triglyceride Measurement. Five third instar larval fat bodies or five third instar larvae were homogenized in 500 μL of PBS containing 0.2% Triton X, heated at 70 °C for 5 min, and centrifuged at 18,400 × *g* for 10 min. Ten microliters of supernatant was used to measure TG by using Serum TG determination kits (Sigma, TR0100-1KT). Protein amounts were measured using Bradford Reagent (Sigma). TG storage was normalized to protein amount.

RNA-seq Transcriptome Analysis. Ten fat bodies from third instar larvae from *dMef2 > ND-75-i* and control were collected on ice. Total RNA was extracted by using TriZol reagent, whereas RNA integrity was assessed using Agilent Bioanalyzer (RIN > 6.6). Sequencing libraries were constructed using Illumina Hi-seq kits following standard protocol, and 100-bp, single-end reads were generated at the Columbia Genome Center. Sequence reads were mapped back to the *Drosophila* genome (flybase genome annotation version r5.51) using Tophat. With the uniquely mapped reads, we quantified gene expression levels by using Cufflinks [fragments per kilobase million (FPKM) values] and HTSeq (read counts per gene). Next, we performed data normalization on the read counts and applied a negative binomial statistical framework using the Bioconductor package "DESeq" to quantify differential expression between experimental and control data. Differentially expressed genes were identified with FDR adjusted *P* values by using Bioconductor package *q* value of 0.1.

Bioinformatics Analysis. Differentially expressed genes were selected if 2 or more-fold changes were consistently observed among the replicates. For genes with less than 2 but greater than 1.5-fold changes, only those with an adjusted *P* value of 0.01 or better were selected. Hits were assigned a confidence value based on both fold changes and *P* values. Genes with at least twofold changes and adjusted *P*

values ≥0.05 were assigned high confidence. GO enrichment analysis was performed with all differentially expressed genes (up- and down-regulated) using DAVID Bioinformatics Resources (https://david.ncifcrf.gov), and then visually displayed for selected metabolic terms by using heat map representation of FPKM. To obtain the target gene list of specific signaling pathways, we collected published or available microarray or Next-Generation Sequencing datasets of ligand treatment, gain- or loss-of-function of components, and ChIP assay for transcriptional factors (ChIP-seq or DroID [www.droidb.org]). We set the overlapping genes between at least two datasets as "moderate-confident" target genes for a signaling pathway. Then we searched the genes that have been validated by qPCR, immunostaining, chemical binding, reporter assays, or genetic interaction and set them as "high-confident" target genes. In this study, we only showed "high-confident" target genes for TGF-β signaling.

RT-qPCR. RNAs from 10 third instar larval fat bodies or muscles or treated C2C12 cells were isolated by using TRIzol (Invitrogen), and cDNA was transcribed by using the iScript cDNA Synthesis Kit (Bio-Rad). qPCR was then performed by using iQ SYBR Green Supermix on a CFX96 Real-Time System/C1000 Thermal Cycler (Bio-Rad). Gene expression was normalized to internal control *RpL32* (*Drosophila*) or *Actin*-β (*Actb*, mouse). qPCR primers used are as follows:

Drosophila:

RpL32-F: GCTAAGCTGTCGCACAAATG

RpL32-R: GTTCGATCCGTAACCGATGT

Actβ-F: ACGGCAAATTTTGACAAAGC

Actβ-R: TTGGTATCATTCGTCCACCA

daw-F: ATCCTTCGTCCGCATCCTAAG

daw-R: CGGTTCCAGGTGTTTCAGC

dpp-F: GACCAGCACAGCATTAGCAAA

dpp-R: AACTGTCGGTTCGCGTCAC

gbb-F: CATCGACGAGAGCGACATCA

gbb-R: TAGTTGTCGTTGGGCACGTT

mav-F: AGCATTACCACAAACGGATTCA

mav-R: CTGTTCGCCACGTAGTAGGT

myo-F: ATTCTTCCAACAACGATAGTCCG

myo-R: CCCCGGTTTACTTTGTACTTTCA

Mouse:

Inhba-F: GTAAAGTGGGGGAGAACGGG

Inhba-R: CCTGACTCGGCAAAGGTGAT

Inhbb-F: GGAAGGTACGGGTCAAGGTG

Inhbb-R: ATGGGAAAGGTATGCCAGCC

Actb-F: CGGTTCCGATGCCCTGAGGCTCTT

Actb-R: CGTCACACTTCATGATGGAATTGA

Statistical Analyses. The data are presented as the mean ± SEM. Student's *t* tests were used for comparisons between two groups. EASE Score was used for evaluating the gene enrichment. $P < 0.05$ was considered statistically significant.

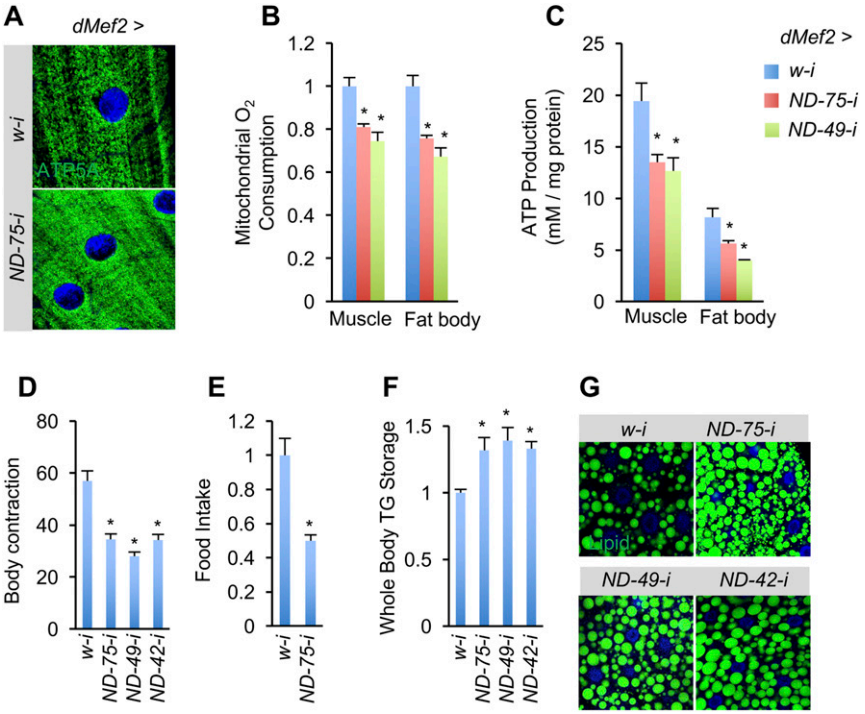

Fig. S1. Muscle complex I perturbation remotely decreases mitochondrial activity in the fat body. (*A*) Representative confocal images of mitochondrial morphology, indicated by ATP5A immunostaining, in larval muscle (green, ATP5A; blue, DAPI). (*B* and *C*) ADP-induced mitochondrial O_2 consumption rates (*B*, $n = 3$, 60 tissues per group) and ATP levels (*C*, $n = 3$, 5 pooled tissues per replicate) in indicated third instar larvae. (*D–G*) Body contraction rates (*D*, $n = 10$), food intake rates (*E*, $n = 3$, 5 pooled larvae per replicate), whole-body TG levels (*F*, $n = 3$, 5 pooled larvae per replicate), and staining of fat body neutral lipids (*G*) in indicated third instar larvae. Genotype: *dMef2 > ND-49-i: dMef2-Gal4/UAS-ND-49-i. dMef2 > ND-42-i: dMef2-Gal4/UAS-ND-42-i.* Data are presented as means ± SEM, $*P < 0.05$.

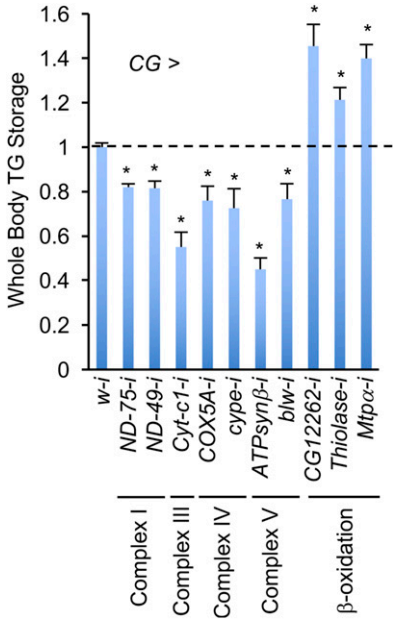

Fig. S2. Differential mitochondrial regulation of lipid metabolism in the fat body. TG levels in indicated third instar larvae. UAS-RNAi lines were crossed to *CG-Gal4* and progenies were grown at 25 °C until third instar stage ($n = 3$, 5 pooled larvae per replicate). Data are presented as means ± SEM, $^*P < 0.05$.

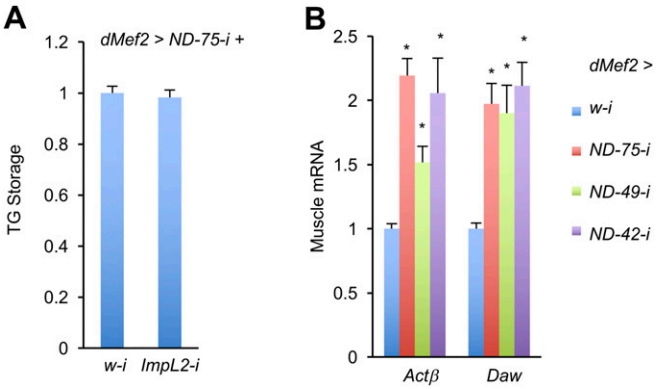

Fig. S3. Muscle expression of TGF-β ligands. (*A*) TG levels in the third instar larvae with indicated genotypes. *UAS-RNAi* lines were crossed to *UAS-ND-75-i, tub-Gal80; dMef2-Gal4*, and the progenies were grown at 29 °C until third instar stage ($n = 3$, 5 pooled larvae per replicate). (*B*) Relative mRNA levels of TGF-β ligands in indicated larval muscles ($n = 3$, 10 pooled larval muscles per replicate). Data are presented as means ± SEM, $^*P < 0.05$.

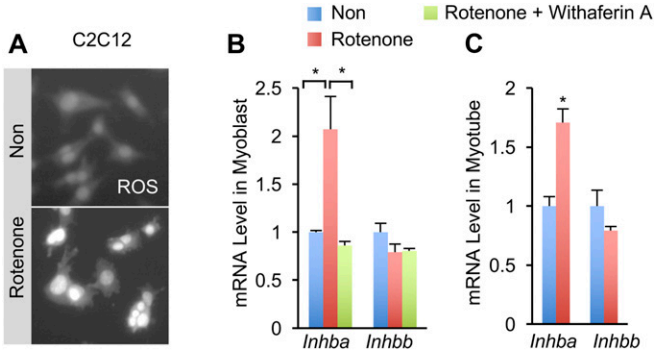

Fig. S4. Mitochondrial complex I perturbation induces *Inhba* expression via NF-κB activity in C2C12 muscle cells. (*A*) Representative images of ROS levels in C2C12 myoblasts with or without 100 nM Rotenone treatment for 1 h. (*B* and *C*) Relative mRNA levels of *Inhba* and *Inhbb* in C2C12 myoblasts (*B*) and myotubes (*C*) stimulated or not by 100 nM Withaferin A for 3 h before 100 nM Rotenone treatment for 1 h ($n = 3$). Data are presented as means ± SEM, $^*P < 0.05$.

Dataset S1. Lipidomics analysis of lipid composition in in the fat body of *dMef2* > *ND-75-i* third instar larvae

Dataset S1

Dataset S2. FPKM Value of differentially expressed genes in the fat body of *dMef2* > *ND-75-i* third instar larvae

Dataset S2

Dataset S3. GO Ontology enrichment for differentially expressed genes in the fat body upon muscle mitochondrial injury

Dataset S3