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Supplemental Information

Loss of Adipose Growth Hormone Receptor in Mice Enhances Local Fatty Acid Trapping and Impairs Brown Adipose Tissue Thermogenesis

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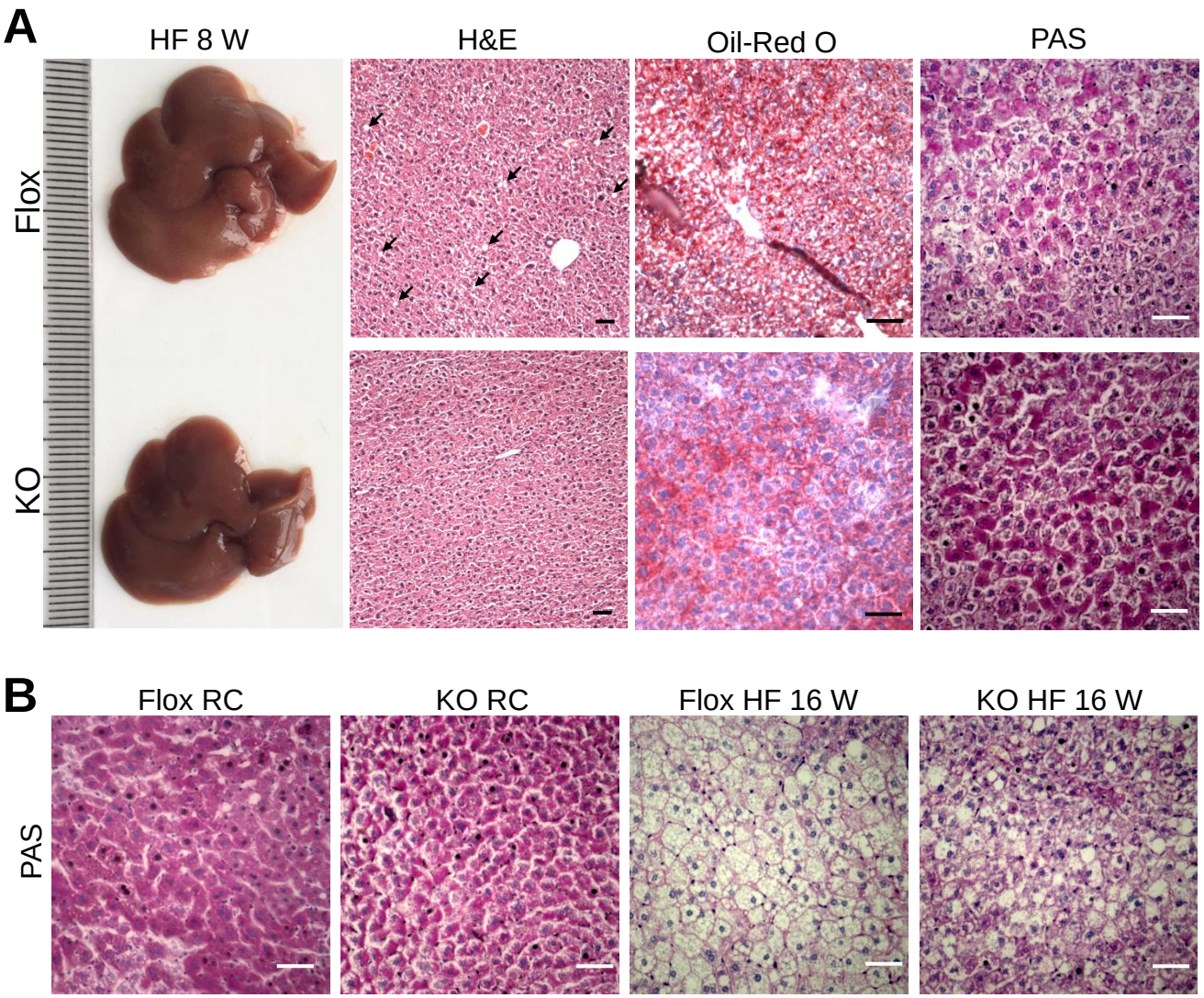

Figure S1: Ad-GHRKO Alleviates Hepatic Steatosis and Promotes Glycogen Synthesis during HF Feeding. Related to Figure 2 and 5.

(A) Representative images of liver from 16-week-old male Ad-GHRKO and Flox mice (HF fed for 8weeks), and H&E, Oil-Red O and PAS staining of liver sections. Scale bar, 50 μm.

(B) PAS staining of liver sections of 24-week-old male Ad-GHRKO and Flox mice (HF fed for 16 weeks). Scale bar, 50 μm.

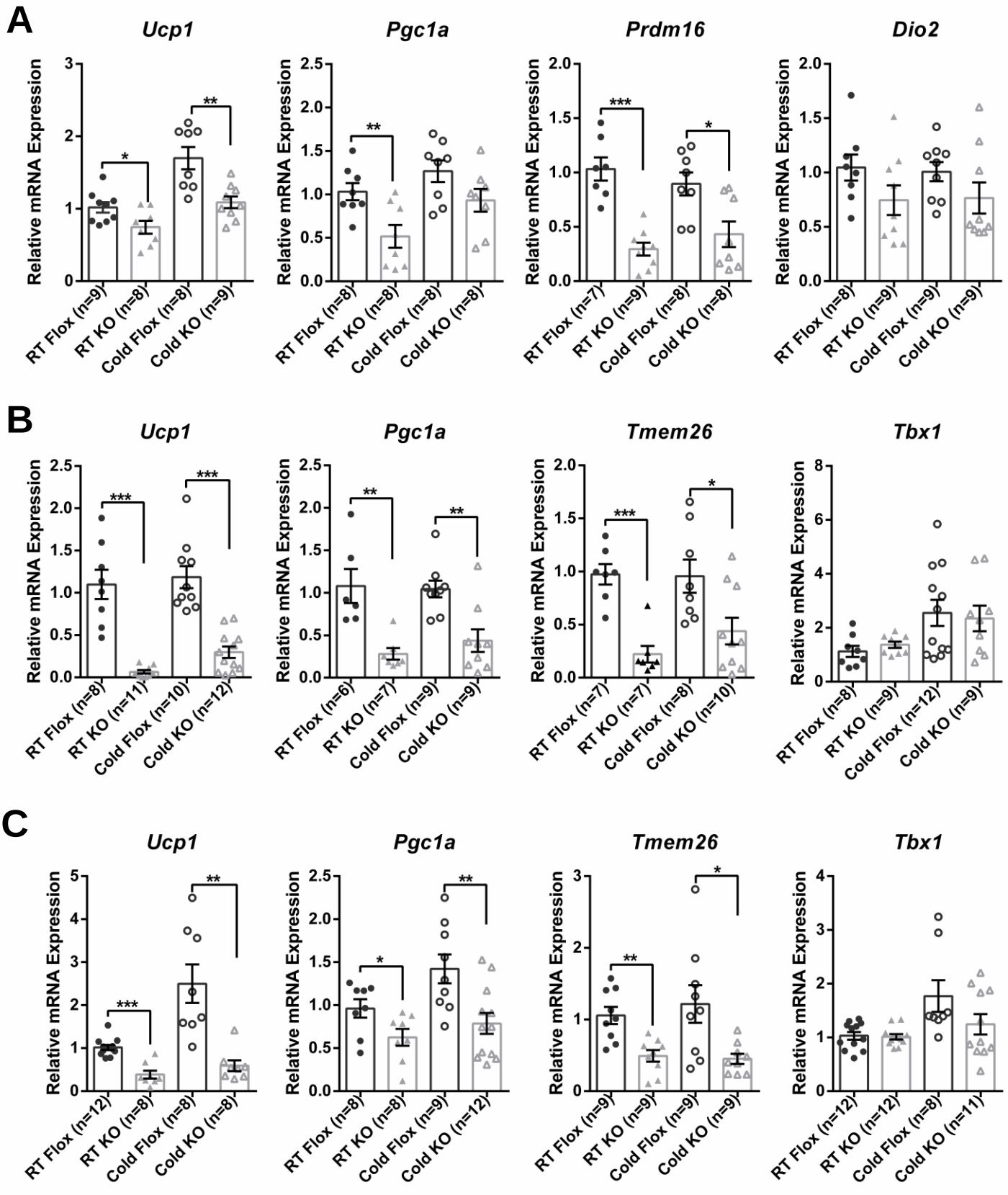

Figure S2: Relative mRNA Expression Levels of Genes in BAT (A), SubQ WAT (B) and Epi WAT (C) of 16-week-old male Ad-GHRKO and Flox mice (HF fed for 8 weeks). Data are represented as mean ± SEM. Related to Figure 6.

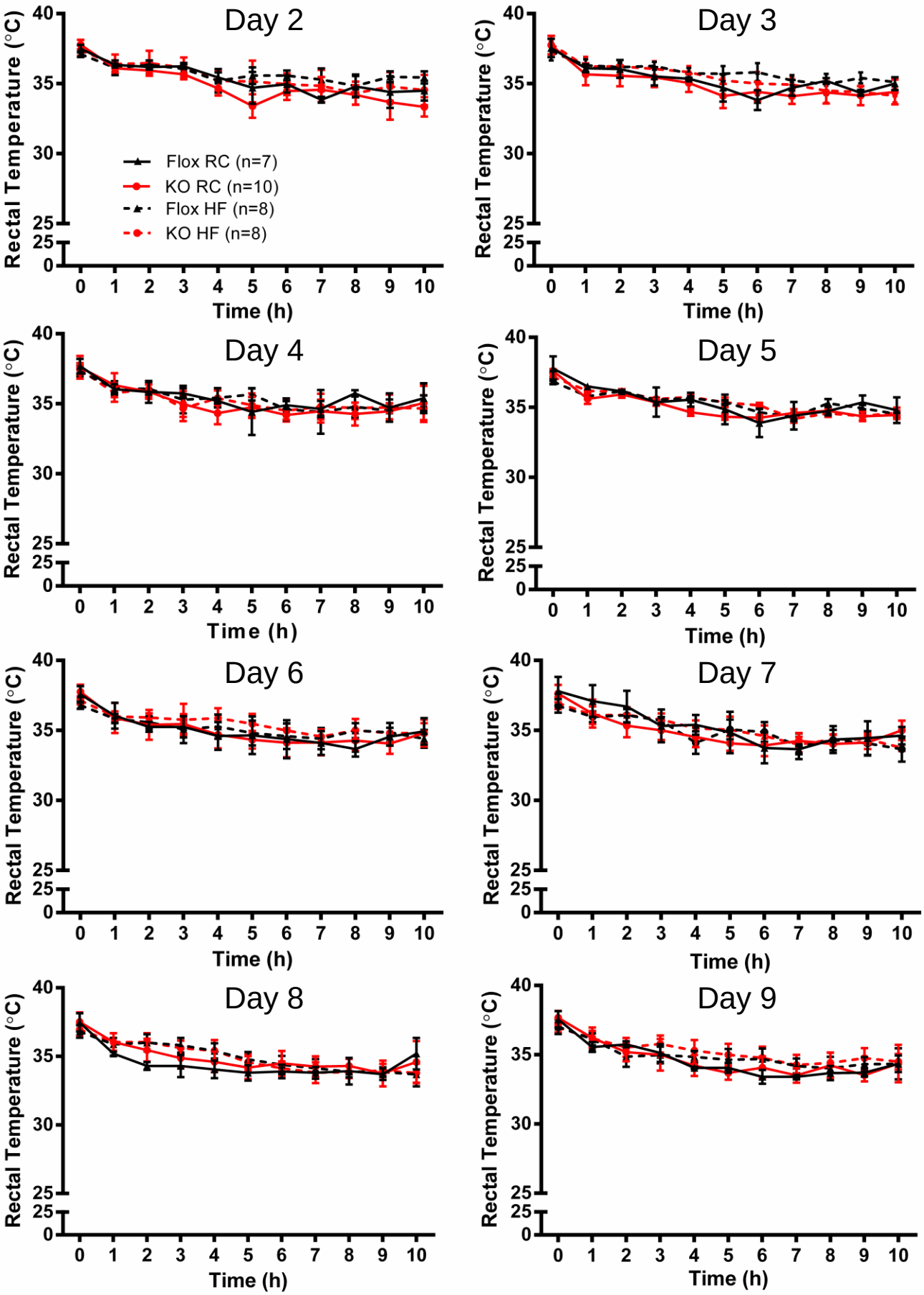

Figure S3: Body Temperature from Day 2 to Day 9 of Cold Stimulation. Data are represented as mean ± SEM. Related to Figure 6.

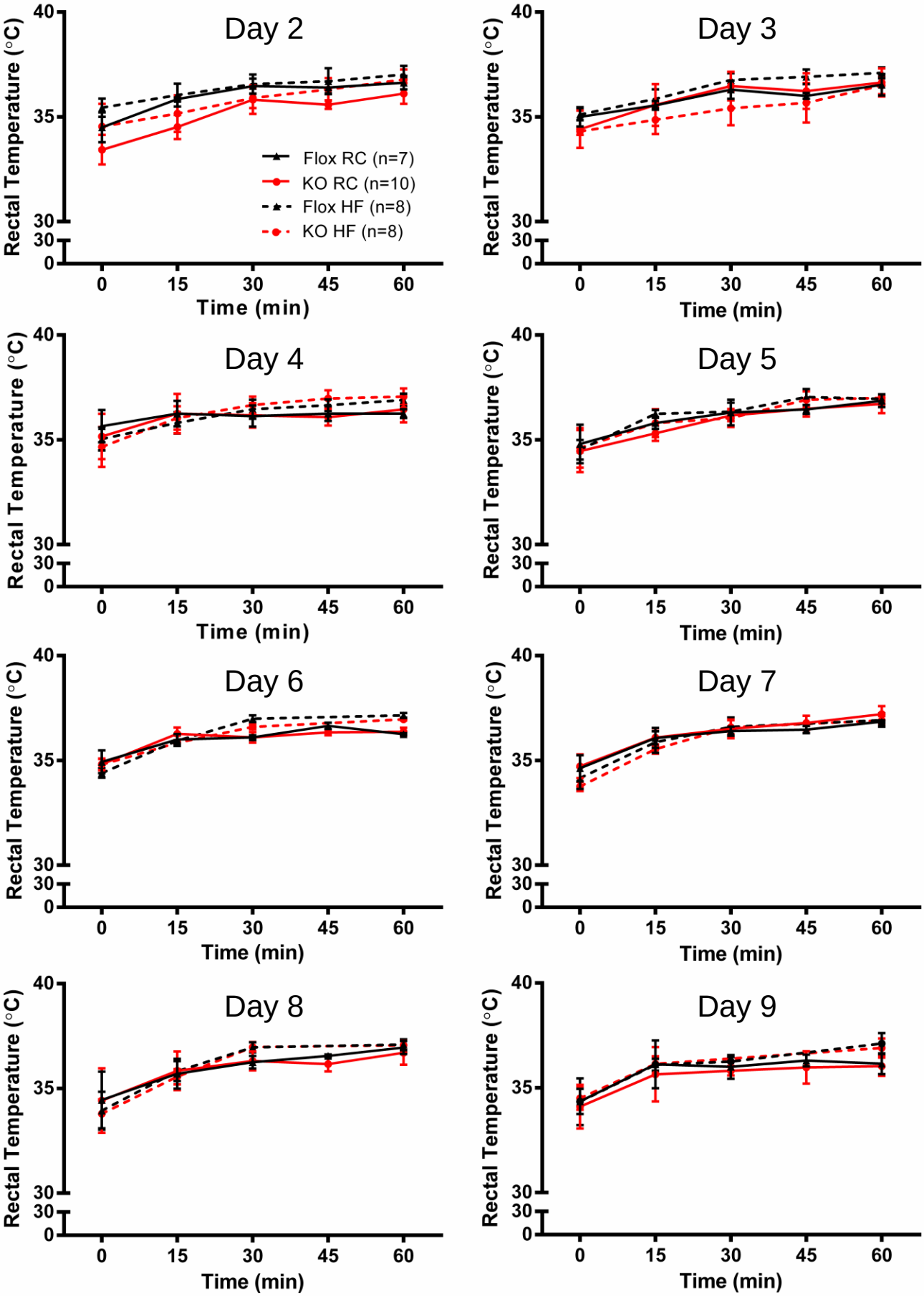

Figure S4: Body Temperature Recovery of the Mice at 25°C from Day 2 to Day 9 of Cold Stimulation. Data are represented as mean ± SEM. Related to Figure 6.

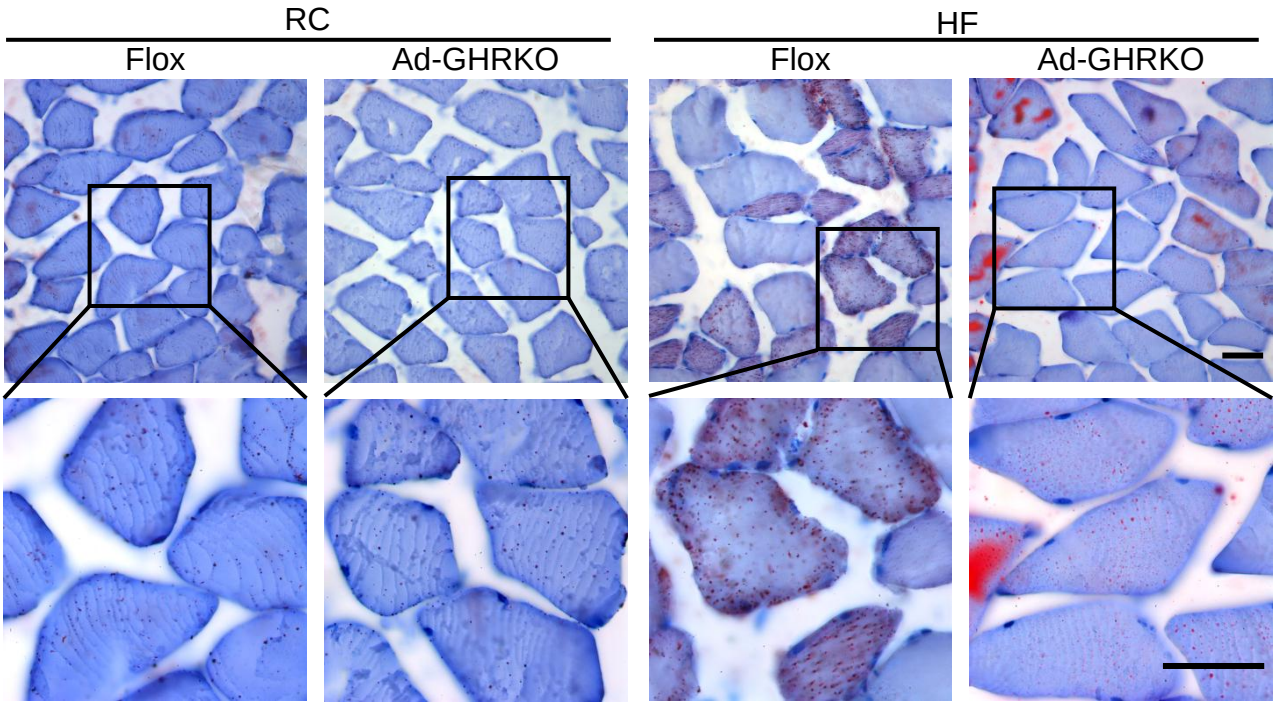

Figure S5: Lipid Accumulation Visualized by Oil-Red O Staining on Muscle Sections of 24-week-old Male Ad-GHRKO and Flox Mice. Related to Figure 2 and 5.
No obvious difference in muscles was found between RC groups. HF diet induced evidently more lipid accumulation in muscles of Flox mice than that of the KO mice. Scale bar, 50 μm.

Table S1. Primer sequences. Related to Figure 1, 3, 4, 5 and Figure S2.

Gene		primer
Ghr	Forward	5'-CATTCTTTTCTGGGATGCTAT-3'
	Reverse	5'-CGGACATTGCATCTGTGATT-3'
Adipo-Cre	Forward	5'-GGATGTGCCATGTGAGTCTG-3'
	Reverse	5'-ACGGACAGAAGCATTTTCCA-3'
Acc1	Forward	5'-TGCCACCACCTTATCACTATGTA-3'
	Reverse	5'-CCTGCCTGTCTCCATCCA-3'
Fas	Forward	5'-TCGTCTATACCACTGCTTACTAC-3'
	Reverse	5'-ACACCACCTGAACCTGAG-3'
Atgl	Forward	5'-GCTGTGGAATGAGGACATAGGA-3'
	Reverse	5'-GCATAGTGAGTGGCTGGTGAA-3'
Hsl	Forward	5'-TGTGTCAGTGCCTATTCAG-3'
	Reverse	5'-GAACAGCGAAGTGTCTCT-3'
Mgl	Forward	5'-GCTGTGGAATGAGGACATAGGA-3'
	Reverse	5'-GCATAGTGAGTGGCTGGTGAA-3'
Pparγ	Forward	5'-TGTGGACCTCTCCGTGATGG-3'
	Reverse	5'-GGTTCTACTTTGATCGCACTTTGG-3'
Fsp27	Forward	5'-TTGATGTGGCCCGTGTAACGTTTG-3'
	Reverse	5'-AAGCTTCCTTCATGATGCGCTTGG-3'
Prdm16	Forward	5'-TGAGGAAGCATTTGAAGTTAAAG-3'
	Reverse	5'-GTTCTTAGCCTGCCTGTAC-3'
Pgc1α	Forward	5'-CCGAAGACACTACAGGTTCCATAG-3'
	Reverse	5'-GGGAGGGAGAGAGGAGAGAGG-3'
Ucp1	Forward	5'-AAGACAGAAGAGCATAGCATTCAC-3'
	Reverse	5'-CCAGTCATACACTCCCACCTC-3'
Tbx1	Forward	5'-CACCGGATCACGCAGCTTAAG-3'
	Reverse	5'-GCAGCGTCTTTGTCTGAGCC-3'
Tmem26	Forward	5'-GTGGTGTGGACGTGGAGTATGC-3'
	Reverse	5'-GTAGATCATCAGGACGAGGCGC-3'
Cide a	Forward	5'-TGACATTCATGGGATTGCAGAC-3'
	Reverse	5'-GGCCAGTTGTGATGACTAAGAC-3'
Dio2	Forward	5'-AATTATGCCTCGGAGAAGACCG-3'
	Reverse	5'-GGCAGTTGCCTAGTGAAAGGT-3'
Srebp1	Forward	5'-CGGAGCCATGGATTGCACTTTC-3'
	Reverse	5'-GATGCTCAGTGGCACTGACTCTTC-3'
Ppara	Forward	5'-AGAGCCCCATCTGTCCTCTC-3'
	Reverse	5'-ACTGGTAGTCTGCAAAACCAAA-3'
Cpt1b	Forward	5'-AAGAGACCCCGTAGCCATCAT-3'
	Reverse	5'-GACCCAAAACAGTATCCCAATCA-3'
Cpt2	Forward	5'-CAAAAGACTCATCCGCTTTGTTC-3'
	Reverse	5'-CATCACGACTGGGTTTGGGTA-3'
Atp5a	Forward	5'-TCTCCATGCCTCTAACACTCG-3'

Fabp3	Reverse	5'-CCAGGTCAACAGACGTGTCAG-3'
	Forward	5'-ACCAAGCCTACTACCATCATCG-3'
	Reverse	5'-CCTCGTCGAACTCTATTCCCAG-3'
Cd36	Forward	5'-TTGAAAAGTCTCGGACATTGAG-3'
	Reverse	5'-TCAGATCCGAACACAGCGTA-3'
Fabp1	Forward	5'-GTCAGAAATCGTGCATGAAGGG-3'
	Reverse	5'-GAACTCATTGCGGACCACTTT-3'
Fatp2	Forward	5'-GATGCCGTGTCCGTCTTTTAC-3'
	Reverse	5'-GACTTCAGACCTCCACGACTC-3'
Fatp5	Forward	5'-GTTCTCCCGTCCAAGACCATT-3'
	Reverse	5'-GCTCCGTACAGAGTGTAGCAAG-3'
Gapdh	Forward	5'-GGGCTGGCATTGCTCTCAATG-3'
	Reverse	5'-CATGTAGGCCATGAGGTCCAC-3'

Table S2. Phenotype summary of *Ghr* knockout mouse lines. Related to Figure 1, 2, 3 and 5.

	GHRKO[a]	**FaGHRKO[b]**	**AdGHRKO[c]**	**Ad-GHRKO[d]**
Body Weight	↓	↑	≈	≈, HF↑
Body Length	↓	↑	≈	≈
GH	↑	≈	≈	≈
IGF-1	↓	↑	≈	≈
Insulin	↓	≈	↓	≈
Blood Glucose	≈↓	≈	≈	≈, HF↓
Glucose Tolerance	Impaired	≈	≈	≈, HF Enhanced
Insulin Sensitivity	Enhanced	≈	Enhanced	≈, HF Enhanced
Fat/BW %	↑	↑	↑	↑
SubQ WAT Mass	↑	↑	↑	↑
Adipocyte Size	↑	↑	↑	↑
Liver/BW %	↓	≈	↓	↓
Liver TG	↑	≈	↓	↓

Note: [a]GHRKO: *Ghr* gene total knockout mouse model (Berryman et al., 2010, Zhou et al., 1997, Berryman et al., 2006, Liu et al., 2004, Coschigano et al., 2003); [b]FaGHRKO: Fat-specific *Ghr* gene knockout mouse model driven by ap2 promoter (List et al., 2013); [c]AdGHRKO: Adipose-specific *Ghr* gene knockout mouse model driven by adiponectin promoter (List EO et al., 2018); [d]Ad-GHRKO: Adipose-specific *Ghr* gene knockout mouse modelin this study. BW: body weight; TG, triglyceride; ↑, increased; ↓, decreased; ≈, no significant changes; HF, high-fat diet.

Transparent Methods

Generation of Adipose Tissue-specific *Ghr* Knockout Mice

All animal experiments were performed under the guidelines for the treatment of laboratory animals and were approved by the Committee on the Ethics of Animal Experiments of Dalian Medical University. Heterozygous mice were generated by crossing *Ghr*-Floxed mice (Wu et al., 2011, Wu et al., 2009b) with mice expressing Cre recombinase under the control of the adiponectin promoter (B6; FVB-Tg (Adipoq-Cre)1Evdr/J, Jackson Laboratory, Stock No 010803). Adipose tissue-specific *Ghr* knockout mice (Ad-GHRKO) were created by crossing the heterozygous mice. The floxed littermates were used as control. Only the male Ad-GHRKO and control mice were examined in the subsequent experiments. Mouse genotyping was performed by PCR using primers shown in Table S1. Mice were fed with RC (D12450J, 10% kcal from fat, Research Diets, Inc., New Brunswick, NJ) or HF diet (D12492, 60% kcal from fat, Research Diets, Inc., New Brunswick, NJ) and maintained in a temperature- and humidity-controlled room on a 12-h light/dark cycle at Specific Pathogen Free Experimental Animal Center of Dalian Medical University.

Body Weight, Organ Weight and Serum Analysis

Body weights were measured every two weeks during the entire experimental period. Blood glucose concentrations were measured with glucometers (ACCU-CHEK). Glucose Tolerance Tests (GTT, 2.0 g glucose per kg of body weight) and Insulin Tolerance Tests (ITT, 0.75 unit of recombinant human insulin (Becton Dickinson and Company) per kg of body weight) were performed as described previously (Wu et al.,

2009a). Organ dissections were weighed on ananalytical balance and snap-frozen in liquid nitrogen before being stored at -80^{o}C. Serum was separated from the whole blood by centrifugation at 4000 rpm for 15 min at 4^{o}C and stored at -80^{o}C for further use. Serum triglyceride (TG) (A110-1), total cholesterol (T-CHO) (A111-1), high-density lipoprotein cholesterol (HDL-C) (A112-2), low-density lipoprotein cholesterol (LDL-C) (A113-2), non-esterified fatty acid (NEFA) (A042-2), aspartate aminotransferase (AST) (C010-1) and alanine aminotransferase (ALT) (C009-1) levels were measured using ELISA kits (Jian Cheng Biological Engineering Institute, Nanjing, China) according to the manufacturer's instructions. Serum GH (BPE20916), IGF-1 (BPE20004), insulin (BPE20353) and β-Hydroxybutyric acid (β-HBA) (BPE20760) were measured using ELISA kits (Lengton Bioscience Co.LTD, Shanghai, China), according to the manufacturer's instructions.

Gene Expression Assessment by Real Time PCR

Tissue samples were saved in RNAhold (EH101, TransGen). Total RNA was extracted from frozen tissues using RNAiso Plus kit (9109, Takara) following the manufacturer's protocol and then reverse transcribed using a primeScript™ RT reagent kit (RR047A, Takara). The levels of mRNA expression were quantified by real-time PCR using a TransStart Tip Green qPCR SuperMix kit (AQ142, TransGen). *Gapdh* was used as the endogenous control. The primers used for qPCR were listed in Supplementary Table S1.

Protein Extraction and Western Blot

Proteins were extracted from frozen tissues using RIPA lysis buffer (R0020, Solarbio) containing protease inhibitor cocktail (P1006, Beyotime). Protein concentrations were quantified by BCA Assay Kit (P0012, Beyotime). Equal amount of proteins was separated with SDS-PAGE, and then transferred to nitrocellular membrane for probing with the indicated antibodies. The following primary antibodies were used: GAPDH (10494-1-AP, Proteintech), Tubulin (Abp52656, Abbkine), ACC1 (4190S, CST), FAS (60196-1-IG, Proteintech), PPARα (15540-1-AP, Proteintech), Lipin1 (GTX115676, GeneTex), PPARγ (16643-1-AP, Proteintech), ATGL (bs-3831R, Bioss), CD36 (18836-1-AP, Proteintech) and SREBP1 (14088-1-AP, Proteintech). Anti-rabbit secondary antibody and ECL detection reagent were purchased from Proteintech.

H&E, IHC, Oil Red-O and PAS Staining

Fat and liver tissues were fixed with 10% formalin for at least 24 hrs. For H&E staining, tissues were embedded in paraffin and sectioned into 8 μm intervals (Leica), and then stained with H&E. For immunohistochemistry (IHC), tissue sections were deparaffinized, rehydrated and permeated using Triton X 100 (0694, Amresco), and followed by antigen retrieval using heat-induced citrate solution (C1032, Solarbio). IHC staining was performed using SP link Detection kit (SP-9001, Zsgb-Bio) and DAB kit (ZLI-9017, Zsgb-Bio) according to the instructions. Antibodies used: anti-UCP1 (GTX112784, GeneTex) and anti-PGC1α (bs-7535R, Bioss). For lipid staining, liver tissues were cut into 10 μm sections at -20^{o}C and sucrose dehydrated, followed by staining with Oil Red-O (D027, Njjcbio). The glycogen accumulation in

the liver was detected by staining the dewaxed liver tissue sections with periodic acid-schiff (PAS, G1281, Solarbio) according to the manufacturer's instructions. The slides were observed using Nikon Ni-E microscope to collect images. The average area of adipocyte cells was analyzed using Image J software on H&E slides from at least three 200× fields of three mice per genotype.

Liver TG Assay

Equal weights of frozen liver tissues were homogenized in ethanol. Samples were centrifuged at 12000 rpm for 10 min and supernatants were collected for TG determination with TG Assay kit (A110-1, Jian Cheng Biological Engineering Institute, Nanjing, China).

SVF Cell Differentiation

Primary stromal vascular fraction (SVF) cells from WAT and BAT of 20-week-old Ad-GHRKO or Flox mice were isolated with the method described previously (Ghorbani and Abedinzade, 2013). The collected SVF cells were seeded at a density of 5,000 cells per cm^2 in DMEM (SH30081.01, Hyclone) with 10% fetal bovine serum (Catalog 100-500, GemCell™), 1× Penicillin-Streptomycin solution (P1400, Solarbio) and 2.5 μg/ml Amphotericin B (CA2021, Coolaber) and cultured for two passages. The cells were then reseeded at a density of 40,000 cells per well in 12-well plates. Differentiation of SVF cells was achieved by culturing the cells in the adipogenic induction medium (MUBMD-90031, Cyagen) for 9 days in a humidified incubator at 37^oC with 5% CO_2. Palmitic acid (PA, H8780, Solarbio) was used to

simulate the HF environment. Once mature adipocytes formation was determined, the cells were fixed with 10 % formalin and stained with Oil Red-O.

Cold Exposure Experiment

The 16-week-old male Ad-GHRKO and Flox mouse groups were each randomly divided into two parallel subgroups. While one sub-group remained at room temperature, the matched subgroup was exposed to 4^{o}C on a 10-h/14-h cycle for 9 days. Mice in all groups were individually housed in a clean cage, with free access to water but no food during the cold episode. The rectal temperature of the mice was measured at various times from the start of cold exposure until back to room temperature for 2 hrs.

Statistical Analyses

All data are shown as mean ± SEMs. Statistical analysis was performed by a Student's t test using GraphPad Prism software. Statistical significance was indicated by *P≤0.05, **P≤0.01 and ***P≤0.001.

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