CHCHD10 Modulates Thermogenesis of Adipocyte by Regulating Lipolysis

Meng Ding[1], Yin-jun Ma[1], Ruo-qi Du[1], Wei-yu Zhou[1], Xin Dou[1], Qi-qi Yang[1], Yan Tang[1], Shu-wen Qian[1], Yun Liu[1], Dong-ning Pan[1], Qi-Qun Tang[1]*, Yang Liu[1]*

Research design and materials

Luciferase reporter assays

HEK293T cells were plated in 24-well plates and transfected with vectors of pGL3-basic-*Chchd10* (250 ng), pcDNA3.1-*Pparγ* (200 ng), and pcDNA3.1-*Pgc1a* (200 ng). At 36th h after transfection, luciferase activity was measured using the dual-luciferase reporter assay (E1910, Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

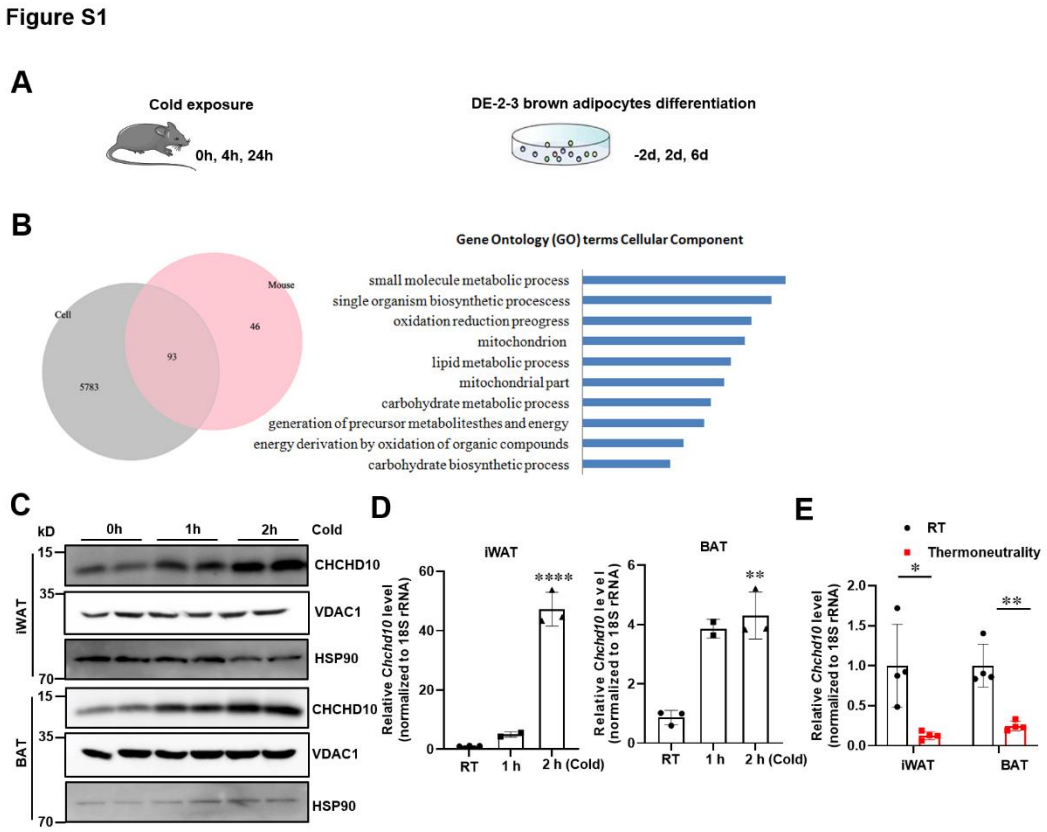
Glucose tolerance test and insulin tolerance test

Male *Chchd10*-WT and *Chchd10*-AKO mice were given an HFD for 12 weeks. The glucose tolerance test was performed after 16 h of fasting via intraperitoneal injection of glucose at a dose of 2 g/kg body weight. The insulin tolerance test was performed after 4 h of fasting via intraperitoneal injection of insulin at a dose of 1.5 IU/kg body weight. The glucose levels in tail blood were measured at 0, 15, 30, 60, 90, and 120 min after glucose or insulin administration by a glucometer (Accu-Chek, Roche).

Generation of recombination adenovirus

Recombinant adenovirus containing sh*Pparg* or sh*Pgc1a* was produced through the BLOCK-iT Adenoviral RNAi Expression System (Invitrogen). An adenoviral expression vector pAd/BLOCK-iT encoding shRNA against sh*Pparg* or sh*Pgc1a* was constructed, while an shRNA against LacZ served as the control. The sh*Pparg* sequence

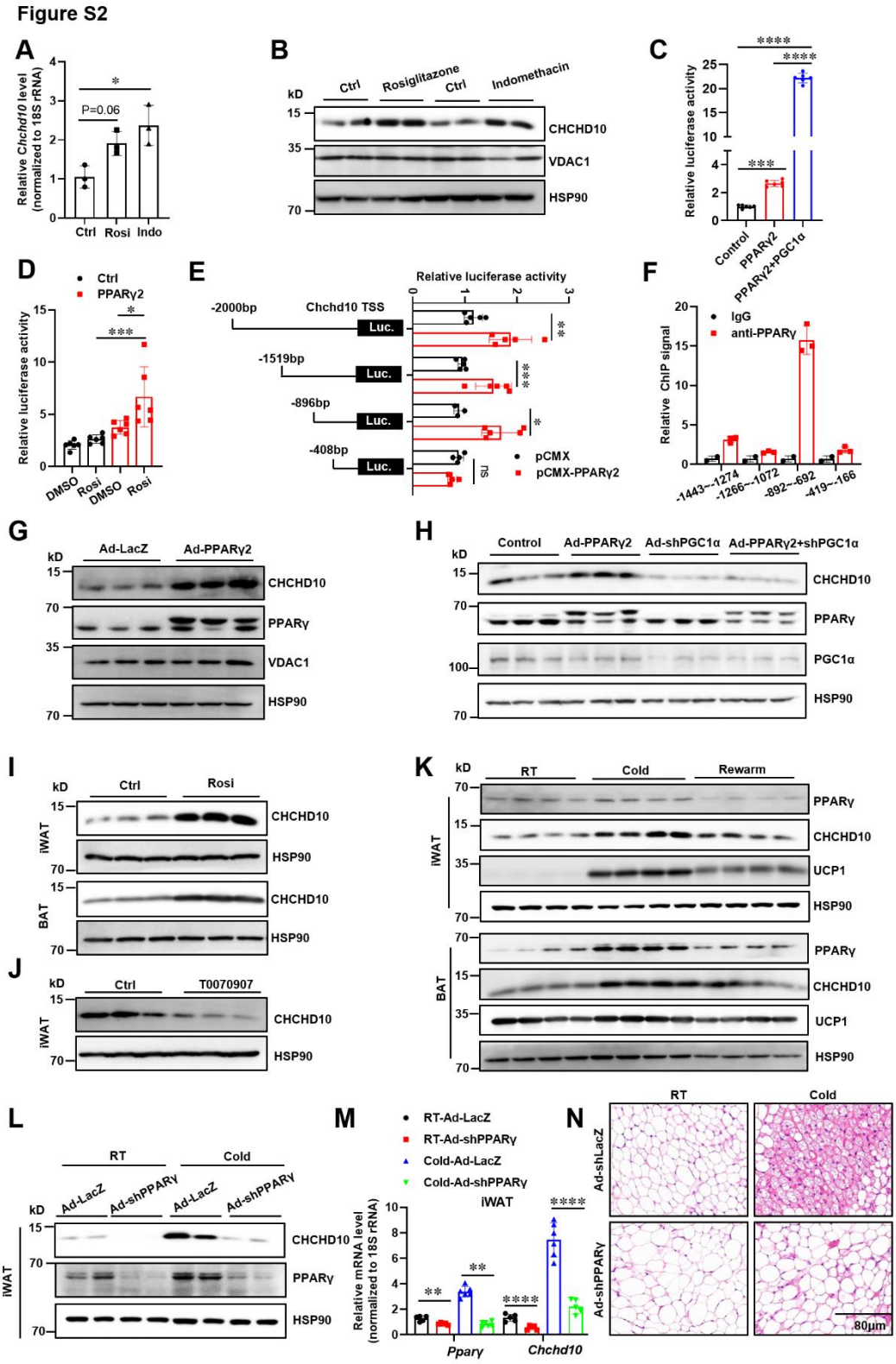
was as follows: 5′-CACCGGGTGAAACTCTGGGAGATTCCGAAGAATCTCCCAGAGTTTCACCC-3′. The sh*Pgc1a* sequence was as follows: 5′-CACCGGTGGATTGAAGTGGTGTAGACGAATCTACACCACTTCAATCCACC -3′. Recombinant adenovirus was produced and amplified in HEK293A cells and purified using adenovirus purification kits (VS-AVPQ022, Sartorius, Germany).



Supplemental Figure 1: CHCHD10 was upregulated during thermogenic adipocytes activation.

(*A-B*) Eight-week-old C57BL/6J male mice were exposed at 4℃ for 0 h, 4 h and 24 h, and the iWAT was dissected. Immortalized brown adipocytes were harvested at day -2, 2, and 6 post-differentiation. RNA-seq was used to analyze the up-regulated genes in these two models (n=3/group). (*C-D*) Protein and mRNA levels of CHCHD10 were

detected in iWAT and BAT of mice housed at 25°C or 4°C for 1 h and 2 h. *Chchd10* level in iWAT was analyzed by one-way ANOVA with Bonferroni's multiple comparisons test; *Chchd10* in BAT was analyzed by Kruskal-Wallis test. (*E*) *Chchd10* mRNA level in iWAT and BAT of mice housed at 30°C or room temperature. (n=4/group). Data in (*E*) was analyzed by two-tailed unpaired Student's *t*-test with Welch's correction. Bar graph data were presented as mean ± SD. ✱, $p < 0.05$; ✱✱, $p < 0.01$; ✱✱✱✱, $p < 0.0001$.

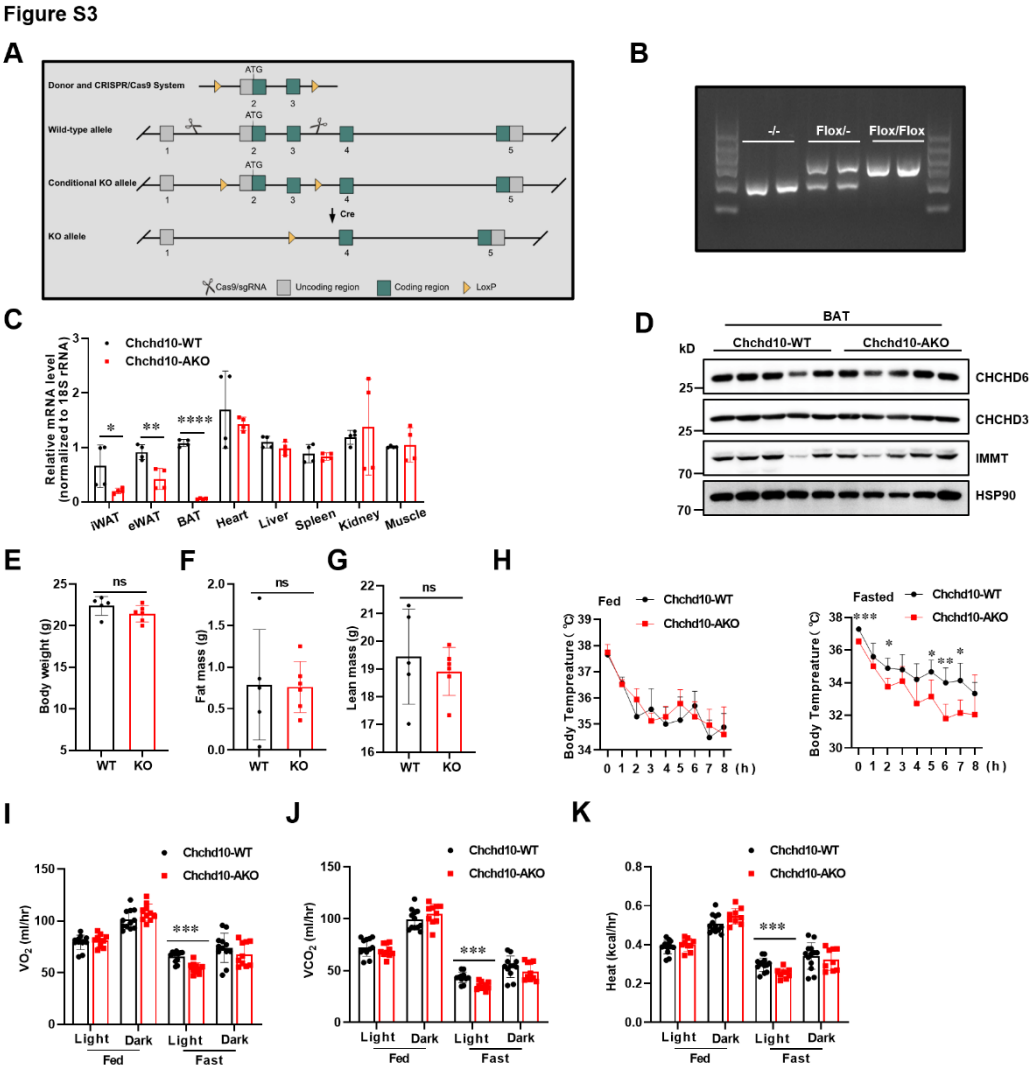


Supplemental Figure 2: PPARγ regulated the expression of CHCHD10.

(*A-B*) The mRNA and protein levels of CHCHD10 in immortalized brown adipocytes with or without rosiglitazone and indomethacin for 24 h. Data was analyzed by one-

way ANOVA with Bonferroni's multiple comparisons test. (*C*) HEK293T cells were transiently transfected with *Chchd10* promoter reporter construct, along with PPARγ and PGC1α, and luciferase activities were measured and normalized to Renilla activity. Data was analyzed by one-way ANOVA with Bonferroni's multiple comparisons test. (*D*) HEK293T cells were transiently transfected with *Chchd10* promoter reporter construct, along with PPARγ expression vector, together treated with rosiglitazone, with DMSO as control, and luciferase activities were measured and normalized to Renilla activity. Data was analyzed by one-way ANOVA with Bonferroni's multiple comparisons test. (*E*) HEK293T cells were transiently transfected with reporter construct harboring different truncated *Chchd10* promoter, together with PPARγ expression vector. pCMX plasmid was used as control. Luciferase activity was then measured and normalized to Renilla activity. Data in (*E*) was analyzed by two-tailed unpaired Student's *t*-test. (*F*) ChIP was conducted with PPARγ antibody at fourth day after immortalized brown preadipocytes differentiation. qPCR analysis of bind of PPARγ on *Chchd10* promoter. Data were normalized to IgG in each group. (*G*) Protein level of CHCHD10 was detected in mature immortalized brown adipocytes treated with Ad-PPARγ or Ad-LacZ. (*H)* Immortalized brown adipocytes treated with Ad-PPARγ, Ad-shPGC1α or Ad-shLacZ. Protein levels of CHCHD10, PPARγ, PGC1α were detected. (*I*) The protein level of CHCHD10 in iWAT and BAT from mice treated with rosiglitazone. (*J*) CHCHD10 protein expression was detected in iWAT from mice treated with or without T0070907 (PPARγ inhibitor). (*K*) Protein levels of PPARγ, CHCHD10, UCP1 in iWAT and BAT under RT, cold and rewarm condition. (*L-N*) Mice were injected with Ad-PPARγ or Ad-LacZ into iWAT, and then housed at room temperature or 4°C. Protein and mRNA levels of CHCHD10 in iWAT were then detected, and H&E staining of iWAT was shown. Bar graph data were presented as

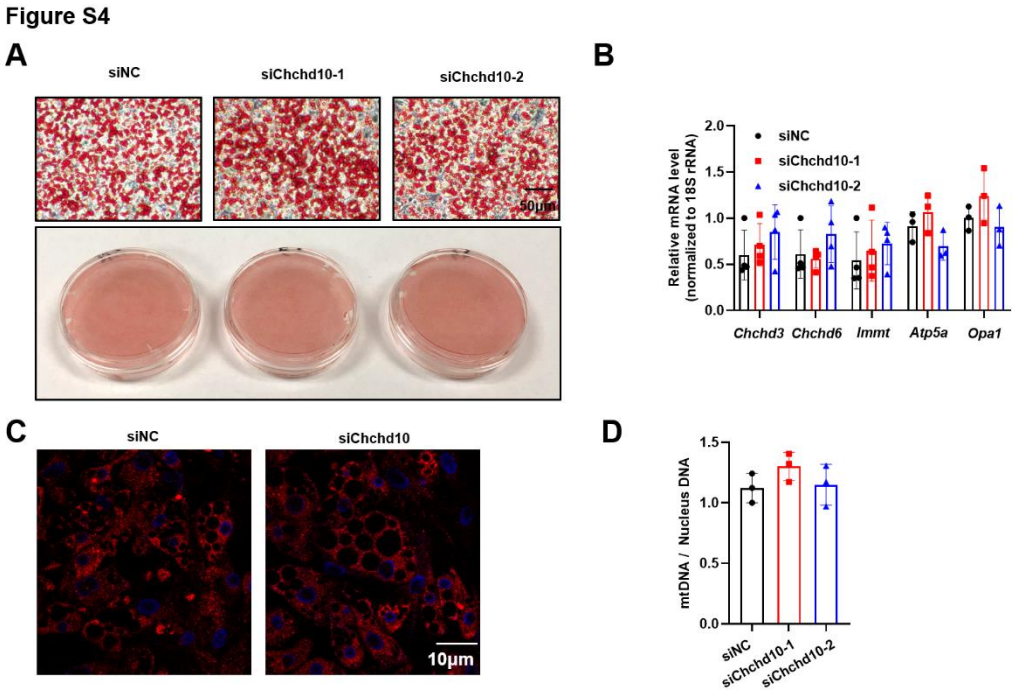
mean ± SD. ✱, p < 0.05; ✱✱, p < 0.01; ✱✱✱, p <0.001; ✱✱✱✱, p <0.0001.



Supplemental Figure 3: Construction of *Chchd10*-AKO mice.

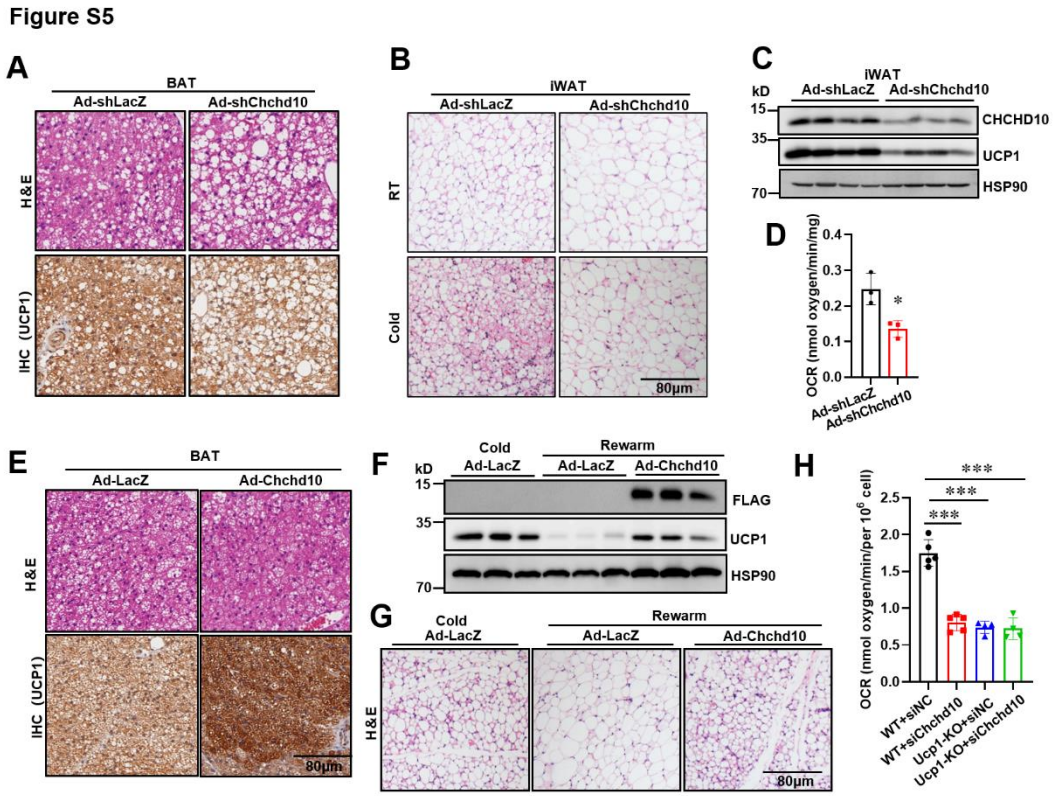
(*A*) Diagram of the construction of *Chchd10* flox/flox mice. (*B*) Genotyping of *Chchd10* flox/flox mice. (*C*) Detection of *Chchd10* mRNA level in different tissues of male *Chchd10*-WT and *Chchd10*-AKO mice (n=4/group). *Chchd10* level in iWAT was analyzed by Mann-Whitney test; other data in (*C*) was analyzed by two-tailed unpaired Student's *t*-test or with Welch's correction. (*D*) Western blot analysis of CHCHD3, CHCHD6 and IMMT protein expression in BAT of *Chchd10*-WT and *Chchd10*-AKO mice. (*E*) Body weight of *Chchd10*-WT and *Chchd10*-AKO mice fed with NCD (n=5-

6/group). Data was analyzed by two-tailed unpaired Student's *t*-test. (*F*-*G*) Body composition analysis by MRI for *Chchd10*-WT and *Chchd10*-AKO mice fed with NCD (n=5-6/group). Data was analyzed by two-tailed unpaired Student's *t*-test. (*H*) *Chchd10*-WT and *Chchd10*-AKO mice under fed or overnight fasted (16 h) conditions were exposed at 4°C, with rectal temperature detected every hour (WT, n = 6 mice; *Chchd10*-AKO, n = 5 mice). Data in (*H*) were analyzed using two-way ANOVA with Bonferroni post hoc multiple comparison test. (*I*-*K*) Oxygen consumption (VO_2), CO_2 generation and energy expenditures of mice under fasted or fed condition at room temperature. (WT, n = 12 mice; *Chchd10*-AKO, n = 10 mice). Data in (*I*-*K*) was analyzed by two-tailed unpaired Student's *t*-test. Bar graph data were presented as mean ± SD. ✱, $p < 0.05$; ✱✱, $p < 0.01$; ✱✱✱✱, $p < 0.0001$.



Supplemental Figure 4: Detection of mitochondria content by Chchd10 disruption. (*A*-*D*) *Chchd10* was disrupted in immortalized brown preadipocytes by siRNAs. After adipogenic induction for 6 days, mature adipocytes were subjected to indicated

detection. (*A*) Oil red o staining showing the adipogenic differentiation. (*B*) qPCR analysis of gene expression in MICOS. *Chchd3*, *Chchd6*, *Immt* level were analyzed by Kruskal-Wallis test; other data was analyzed by one-way ANOVA with Bonferroni's multiple comparisons test. (*C*) Mitotraker staining showing mitochondria content. (*D*) mtDNA measurement (n=3/group). Data was analyzed by one-way ANOVA with Bonferroni's multiple comparisons test. Bar graph data were presented as mean ± SD.



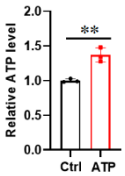
Supplemental Figure 5: Knockdown of *Chchd10* in fat pads inhibited thermogenic adipocyte activation.

(*A*) H&E staining of BAT after injection with Ad-shLacZ or Ad-sh*Chchd10* in BAT. (*B*-*C*) Mice injected with Ad-shLacZ or Ad-sh*Chchd10* in iWAT were exposed to 4°C for 24 h and then subjected to further analysis. (*B*) H&E staining of iWAT. (*C*) Protein levels of CHCHD10 and UCP1. (*D*) Detection of the oxygen consumption rates in

iWAT (n=3/group). Data in (*D*) was analyzed by two-tailed unpaired Student's *t*-test. (*E*) Mice were injected with Ad-LacZ as control or Ad-*Chchd10* in BAT. H&E and IHC staining of BAT were shown. (*F*-*G*) Mice were injected with Ad-LacZ as control or Ad-*Chchd10* in iWAT. All the mice were exposed at 4°C for 72 h and then rewarmed at 25°C for 72 h. (*F*) Protein expression of UCP1 was detected by western blot. (*G*) Representative H&E staining analysis of iWAT in mice. (*H*) Oxygen consumption rates of *Ucp1*-WT and *Ucp1*-KO primary adipocytes transfected with siNC or si*Chchd10*. Data were normalized to cell number and analyzed by one-way ANOVA with Bonferroni's multiple comparisons test. Bar graph data were presented as mean ± SD. ✱, $p < 0.05$, ✱✱✱, $p < 0.001$.

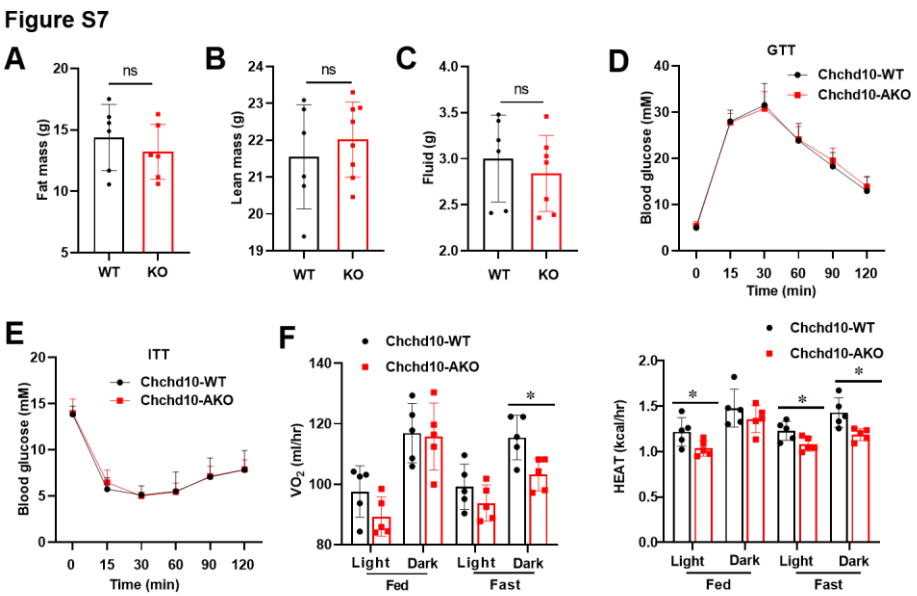
Figure S6

A



Supplemental Figure 6: ATP-liposome was used to deliver ATP to adipocytes.

(*A*) The ATP level of immortalized brown adipocytes treated with ATP-liposome or not. Data in (*A*) was analyzed by two-tailed unpaired Student's *t*-test. ✱✱, $p < 0.01$.



Supplemental Figure 7: Phenotype of *Chchd10*-AKO mice by feeding with HFD. (*A*-*C*) Analysis of body composition of *Chchd10*-WT and *Chchd10*-AKO mice fed with HFD using MRI (n=5-8/group). (*D*) Mice were starved for 16 h, intraperitoneally injected with 50% glucose. Blood glucose was measured for 0, 15, 30, 60, 90 and 120 min (n=6-9/group). (*E*) Mice were starved for 4 h, injected with 1.5 IU insulin intraperitoneally, blood was collected from tail vein. Blood glucose were measured for 0, 15, 30, 60, 90 and 120 min (n=6-9/group). (*F*) The oxygen consumption and energy expenditure of mice for a circadian cycle (n=5/group). Data in (*A*), (*B*), (*C*) and (*F*) were analyzed by two-tailed unpaired Student's *t*-test. Data in (*D*) and (*E*) were analyzed using two-way ANOVA with Bonferroni post hoc multiple comparison test. Bar graph data were presented as mean ± SD. ✱, $p < 0.05$.

Supplementary table 1

The primers used in the study:

Mouse *18s rRNA*	GTAACCCGTTGAACCCCATT
	CCATCCAATCGGTAGTAGCG

Mouse *Chchd10*	CTCAGAGCGACCTAACCCTGT
	AGCTCAGACCGTGATTGTATTTG
Mouse *Chchd3*	GCGGACGAGAACGAGAACAT
	TCGCTGAGACTTAGAGCCAGA
Mouse *Chchd6*	TGTCTGAAAGTGTTGTGAACCG
	GATGGCTGGTAGAGGGACAGT
Mouse *Immt*	CTGCGGGCCTGTCAGTTATC
	GGAGGACGAACTTCCCACA
Mouse *Ucp1*	AGGCTTCCAGTACCATTAGGT
	CTGAGTGAGGCAAAGCTGATTT
Mouse *Hsl*	TGTGGCACAGACCTCTAAAT
	GGCATATCCGCTCTC
Mouse *Atgl*	GGAGACCAAGTGGAACATCTCA
	AATAATGTTGGCACCTGCTTCA
Mouse *Pparg*	GTGCCAGTTTCGATCCGTAGA
	GGCCAGCATCGTGTAGATGA
Mouse *Pgc1a*	ACTGAGCTACCCTTGGGATG
	TAAGAATTTCGGTGGTGACA
Mouse *Fabp4*	CCTTTGTGGGAACCTGGAA
	CTGTCGTCTGCGGTGATT
Mouse *Adiponectin*	TGTTCCTCTTAATCCTGCCCA
	CCAACCTGCACAAGTTCCCTT
Mouse *Cidea*	TGACATTCATGGGATTGCAGAC
	GGCCAGTTGTGATGACTAAGAC

Mouse *Prdm16*	ACAGGCAGGCTAAGAACCAG
	CGTGGAGAGGAGTGTCTTCAG
Mouse *Dio2*	AATTATGCCTCGGAGAAGACCG
	GGCAGTTGCCTAGTGAAAGGT
Human *18s rRNA*	GTAACCCGTTGAACCCCATT
	CCATCCAATCGGTAGTAGCG
Human *Chchd10*	GGGCTCATGGCTCAGATGG
	CAGGAACTGCCTGATCTCGTA
Human *Ucp1*	AGGTCCAAGGTGAATGCCC
	TTACCACAGCGGTGATTGTTC