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Supplemental information

Adenylate cyclase 10 promotes brown adipose tissue thermogenesis

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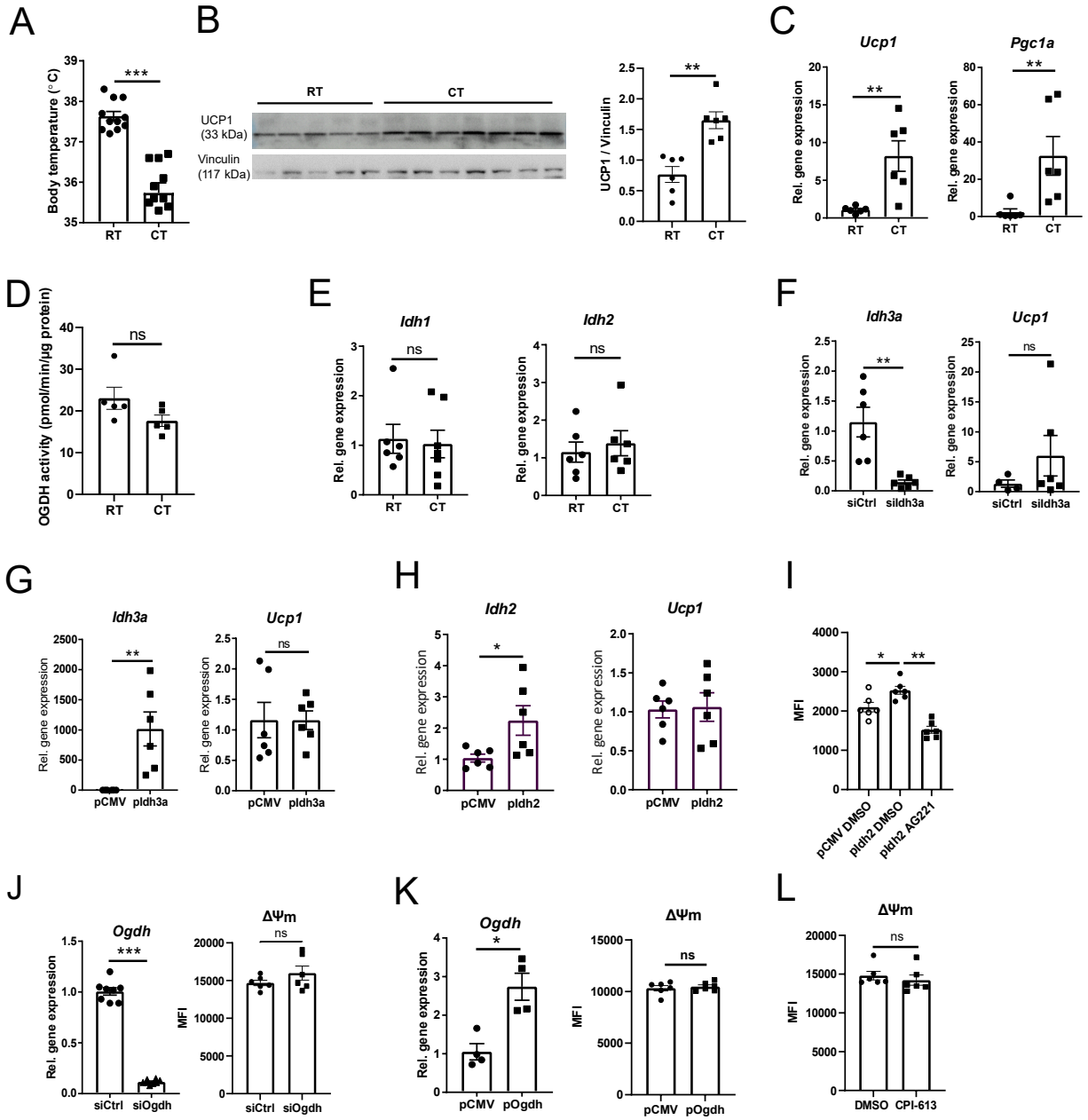
A
Body temperature (°C)
***
RT
CT
B
RT
CT
UCP1
(33 kDa)
Vinculin
(117 kDa)
UCP1 / Vinculin
**
C
Ucp1
Pgc1a
Rel. gene expression
D
OGDH activity (pmol/min/µg protein)
ns
E
Idh1
Idh2
F
Idh3a
Ucp1
siCtrl
sildh3a
G
Idh3a
Ucp1
pCMV
pIdh3a
H
Idh2
Ucp1
pIdh2
I
MFI
pCMV DMSO
pIdh2 DMSO
pIdh2 AG221
J
Ogdh
ΔΨm
siCtrl
siOgdh
K
Ogdh
ΔΨm
pOgdh
L
ΔΨm
DMSO
CPI-613

Figure S1

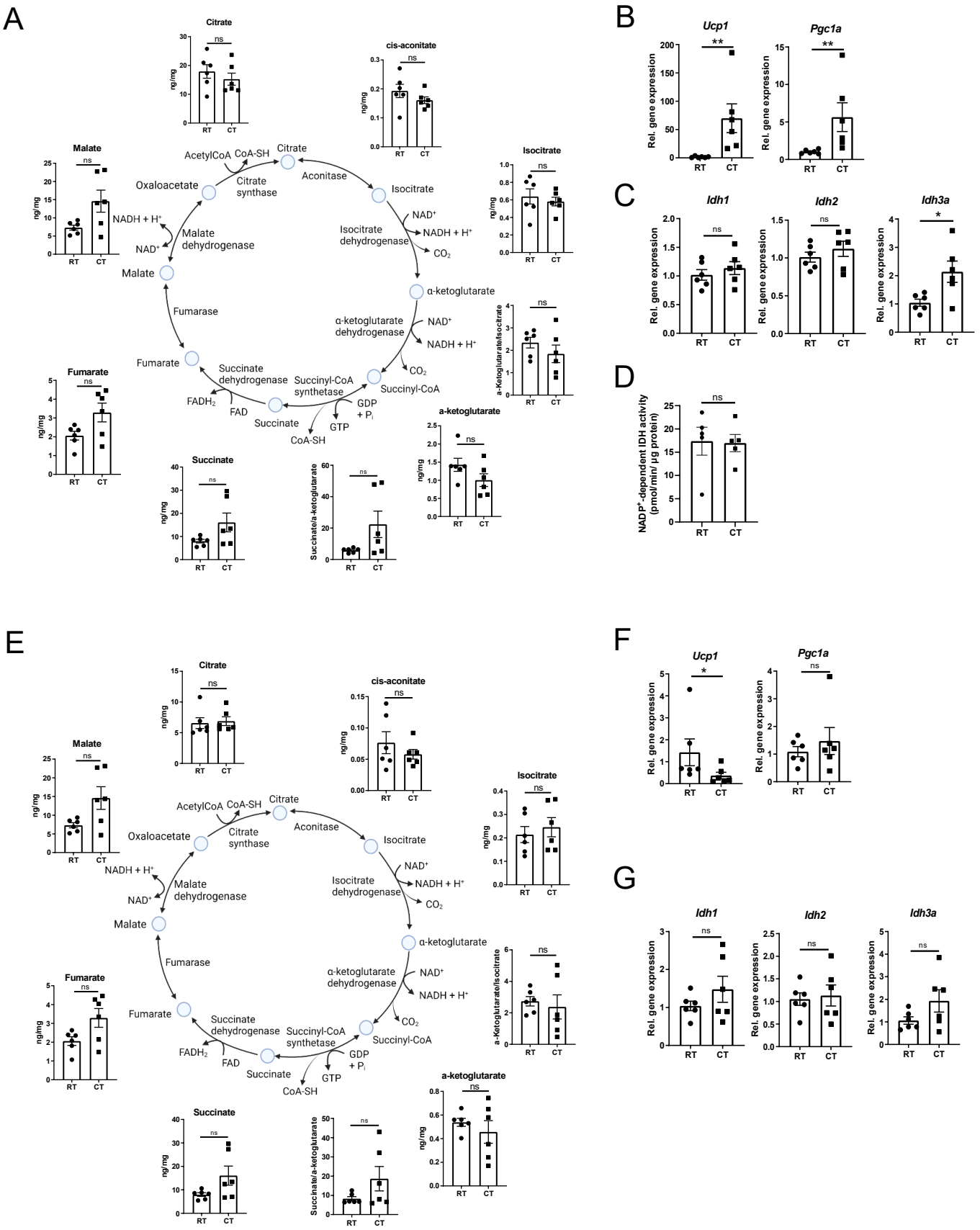
A
Citrate
cis-aconitate
Isocitrate
Malate
Fumarate
Succinate
a-ketoglutarate
Succinate/a-ketoglutarate
a-Ketoglutarate/Isocitrate
ng/mg
ns
RT
CT
AcetylCoA
CoA-SH
Citrate synthase
Aconitase
Oxaloacetate
NADH + H+
NAD+
Malate dehydrogenase
Isocitrate dehydrogenase
CO2
α-ketoglutarate
α-ketoglutarate dehydrogenase
Fumarase
Succinate dehydrogenase
FADH2
FAD
Succinyl-CoA synthetase
GDP + Pi
GTP
Succinyl-CoA
B
Ucp1
Pgc1a
Rel. gene expression
**
C
Idh1
Idh2
Idh3a
*
D
NADP+-dependent IDH activity (pmol/min/ µg protein)
E
F
G

Figure S2

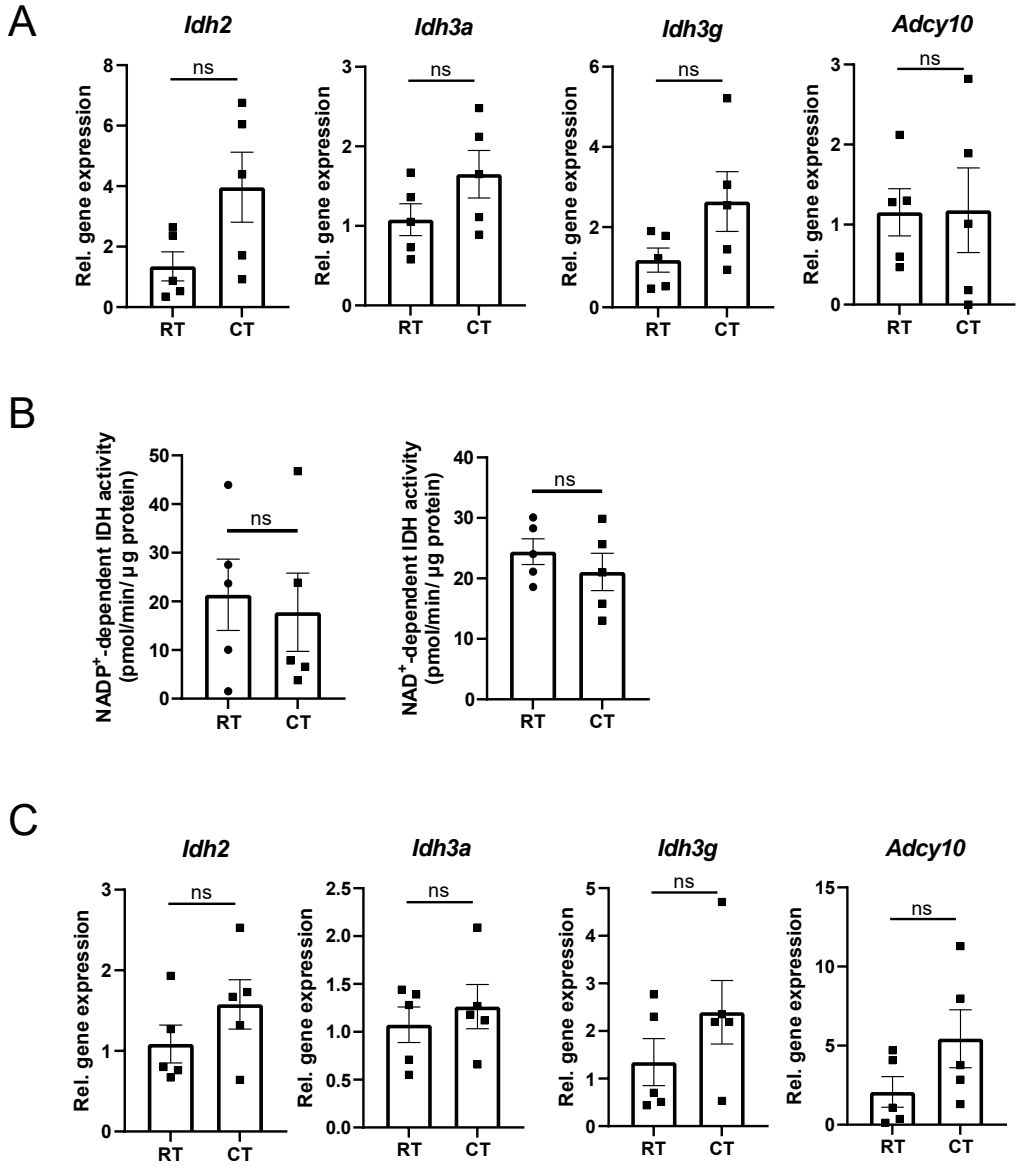
A
Idh2
Idh3a
Idh3g
Adcy10
Rel. gene expression
ns
RT
CT
B
NADP+-dependent IDH activity (pmol/min/ µg protein)
NAD+-dependent IDH activity (pmol/min/ µg protein)
C

Figure S3

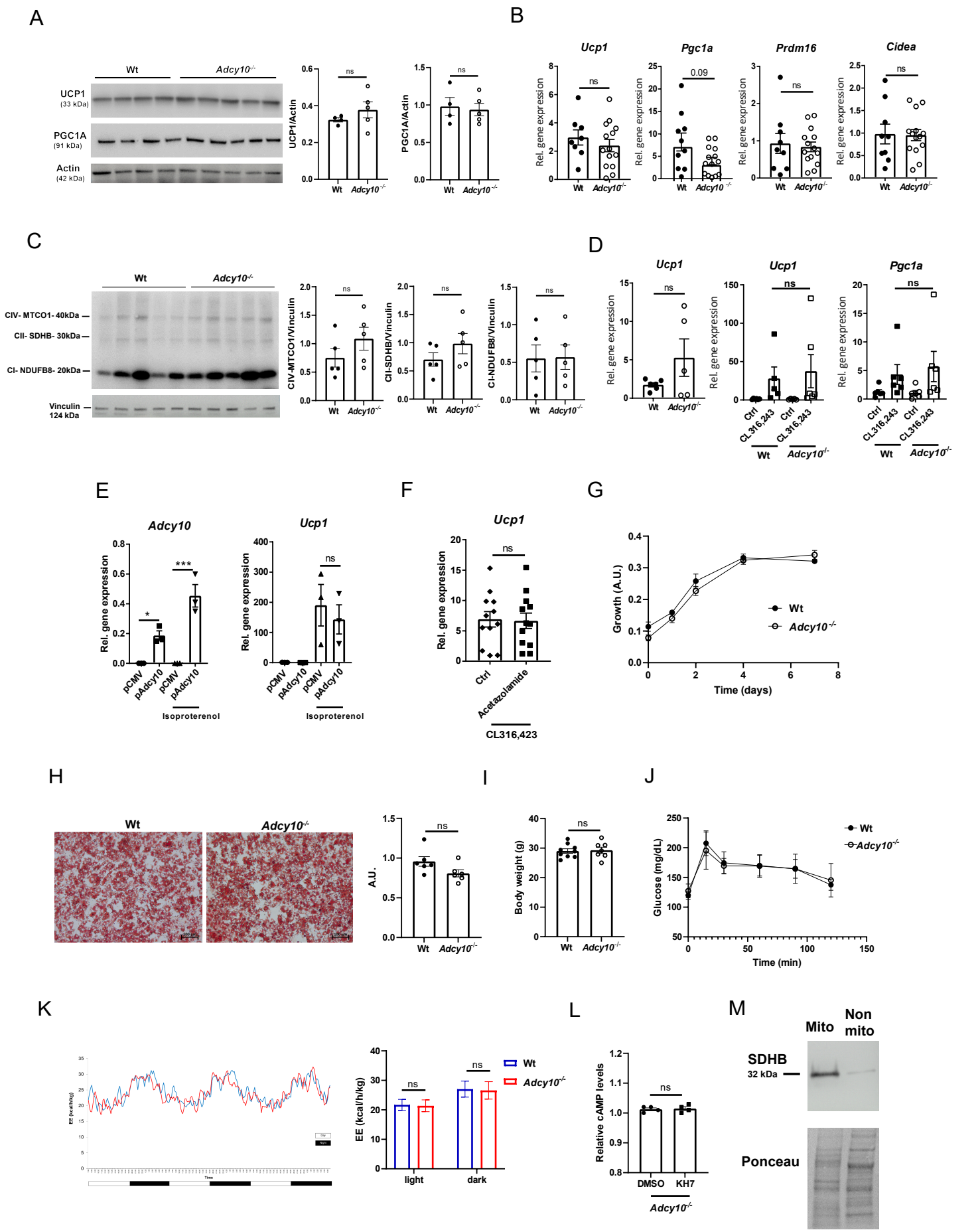
A
Wt
Adcy10-/-
UCP1
(33 kDa)
PGC1A
(91 kDa)
Actin
(42 kDa)
UCP1/Actin
PGC1A/Actin
ns
B
Ucp1
Pgc1a
Prdm16
Cidea
Rel. gene expression
0.09
C
CIV- MTCO1- 40kDa
CII- SDHB- 30kDa
CI- NDUFB8- 20kDa
Vinculin
124 kDa
CIV-MTCO1/Vinculin
CII-SDHB/Vinculin
CI-NDUFB8/Vinculin
D
Ucp1
Pgc1a
Ctrl
CL316,243
E
Adcy10
Ucp1
pCMV
pAdcy10
Isoproterenol
F
Acetazolamide
CL316,423
G
Growth (A.U.)
Time (days)
H
A.U.
I
Body weight (g)
J
Glucose (mg/dL)
Time (min)
K
EE (kcal/h/kg)
light
dark
L
Relative cAMP levels
DMSO
KH7
M
Mito
Non mito
SDHB
32 kDa
Ponceau

Figure S4

Supplemental information

Figure S1. IDH does not affect *Ucp1* expression and OGDH does not affect the mitochondrial membrane potential in brown adipocytes.

A. Body temperature of C57BL/6 wt male mice kept for 8 h at 4 °C (CT) or room temperature (RT) (n=10-11 mice per group). **B**. Western blot for UCP1 protein in BAT of wt mice kept for 8 h at CT or RT. The intensity of the UCP1 and Vinculin bands was quantified and the ratio UCP1/Vinculin was calculated (n=5-7 mice per group). **C,E**. *Ucp1*, *Pgc1a*, *Idh1* and *Idh2* gene expression in BAT of wt mice kept for 8 h at CT or RT. Gene expression is set as 1 in RT samples (n=6 mice per group). **D**. OGDH activity normalized to protein concentration in BAT of wt mice kept for 8 h at CT or RT (n=5 mice per group). **F**. Brown preadipocytes (cell line) were transfected with siRNA against *Idh3a* or control siRNA; *Idh3a* and *Ucp1* gene expression was measured by qPCR. Gene expression is set as 1 in siCtrl samples (n=6). **G**. Brown preadipocytes (cell line) were transfected with plasmid to overexpress *Idh3a* or control pCMV plasmid; *Idh3a* and *Ucp1* gene expression was measured by qPCR. Gene expression is set as 1 in pCMV samples (n=6). **H**. Brown preadipocytes (cell line) were transfected with plasmid to overexpress *Idh2* or control pCMV plasmid; *Idh2* and *Ucp1* gene expression was measured by qPCR. Gene expression is set as 1 in pCMV samples (n=6). **I**. Brown preadipocytes (cell line) were transfected with plasmid to overexpress *Idh2* and the next day they were treated for 18 h with 5 μM AG-221 or DMSO. The ΔΨm was measured by TMRE staining and FACS (n=6). **J**. Brown preadipocytes (cell line) were transfected with siRNA against *Ogdh* or control siRNA; *Ogdh* gene expression was measured by qPCR. Gene expression is set as 1 in siCtrl samples (left, n=8). The ΔΨm was measured by TMRE staining and FACS (right, n=6). **K**. Brown preadipocytes (cell line) were transfected with plasmid to overexpress *Ogdh* or control plasmid; *Ogdh* gene expression was measured by qPCR, gene expression is set as 1 in pCMV samples (left, n=4). The ΔΨm was measured by TMRE staining and FACS (right, n=6). **L**. Brown adipocytes (cell line) were treated for 24 h with 10 μM CPI-613 or carrier (DMSO) and ΔΨm was measured by TMRE staining and FACS (n=6). Data are shown mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001. ns: not significant. MFI: Mean Fluorescence Intensity

Figure S2. No TCA cycle metabolic changes in subcutaneous and gonadal adipose tissue upon cold exposure.

A. TCA cycle metabolite levels or ratios in subcutaneous adipose tissue (SAT) of wt mice kept for 8 h at CT or RT measured by LC-MS/MS (n=6 mice per group). **B**,**C**. *Ucp1*, *Pgc1a*, *Idh1*, *Idh2* and *Idh3a* gene expression in SAT of wt mice kept for 8 h at CT or RT measured by qPCR (n=6

mice per group). **D**. $NADP^+$-dependent IDH activity normalized to protein concentration in SAT of wt mice kept for 8 h at CT or RT (n=5 mice per group). **E**. TCA cycle metabolite levels or ratios in gonadal adipose tissue (GAT) of wt mice kept for 8 h at CT or RT measured by LC-MS/MS (n=6 mice per group). **F**,**G**. *Ucp1*, *Pgc1a*, *Idh1*, *Idh2* and *Idh3a* gene expression in GAT of wt mice kept for 8 h at CT or RT measured by qPCR (n=6 mice per group). Data are shown mean±SEM. *p < 0.05, **p < 0.01. ns: not significant.

Figure S3. No TCA cycle metabolic changes in skeletal muscle upon cold exposure.
A. *Idh2*, *Idh3a*, *Idh3g* and *Adcy10* gene expression in gastrocnemius muscle of wt mice kept for 8 h at CT or RT measured by qPCR (n=5 mice per group). **B**. $NADP^+$- and NAD^+-dependent IDH activity normalized to protein concentration in gastrocnemius muscle of wt mice kept for 8 h at CT or RT (n=5 mice per group). **C**. *Idh2*, *Idh3a*, *Idh3g* and *Adcy10* gene expression in quadriceps muscle of wt mice kept for 8 h at CT or RT measured by qPCR (n=5 mice per group). ns: not significant.

Figure S4. ADCY10 does not regulate UCP1 expression in brown adipocytes.
A. Immunoblot against UCP1, PGC1a and β-actin in BAT of wt and *Adcy10*$^{-/-}$ mice exposed for 8 h to 4°C. The intensity of the UCP1 PGC1a and β-actin bands was quantified and the ratios UCP1/ β-actin and PGC1a/ β-actin was calculated (n=4-5 mice per group). **B***. Ucp1, Pgc1a, Prdm16* and *Cidea* relative gene expression in BAT of wt and *Adcy10*$^{-/-}$ mice exposed for 8 h to 4 °C measured by qPCR (n=8-14 mice per group). **C**. Western blot for oxidative phosphorylation complexes and Vinculin in BAT of wt and *Adcy10*$^{-/-}$ mice exposed for 8 h to 4 °C. The intensity of the CIV-MTCO1, CII-SDHB, CI-NDUFB8 and Vinculin bands was quantified and the ratios CIV-MTCO1/Vinculin, CII-SDHB/Vinculin and CI-NDUFB8/Vinculin was calculated (n=5 mice per group). **D**. *Ucp1* and *Pgc1a* relative gene expression in wt and *Adcy10*$^{-/-}$ primary brown adipocytes treated for 3 h with 1 µM CL316,243 or control solution (PBS) (n=5-6). **E**. *Adcy10* and *Ucp1* relative gene expression in brown preadipocytes (cell line) transfected with plasmid overexpressing *Adcy10* or control plasmid and treated for 3 h with 1 µM isoproterenol or control solution (PBS) (n=3). **F**. *Ucp1* relative gene expression in brown adipocytes treated for 3 h with 1 mM acetazolamide or DMSO and 1 µM CL316,243 (n=12). **G**. Cell growth of wt and *Adcy10*$^{-/-}$ primary brown preadipocytes assessed by PrestoBlue staining (n=3). **H**. Oil Red O staining images and quantification in differentiated wt and *Adcy10*$^{-/-}$ primary brown adipocytes. Left: representative images of brown adipocytes from one out of 3 mice, scale bar: 100 µm. Right: Quantification of Oil Red O staining (n=6). **I**. Body weight of *Adcy10*$^{-/-}$ and wt mice at RT (n=7-9 mice per group). **J**. Glucose tolerance

test in *Adcy10*$^{-/-}$ and wt mice at RT (n=7-9 mice per group). **K**. Energy expenditure of *Adcy10*$^{-/-}$ and wt mice at RT (n= 8 mice per group). **L**. cAMP in mitochondrial fractions of *Adcy10*$^{-/-}$ primary brown adipocytes treated for 2 h with KH7 or DMSO (n=4). **M**. Immunoblot against SDHB and Ponceau staining in the mitochondrial and non-mitochondrial fraction of mouse BAT. Data are shown as mean±SEM. *p < 0.05, ***p < 0.001, ns: not significant. A.U. Arbitary units.

Table S1. Primer sequences

Gene name	**Forward Sequence (5' → 3')**	**Reverse Sequence (5' → 3')**
mouse *18S* rRNA	GTTCCGACCATAAACGATGCC	TGGTGGTGCCCTTCCGTCAAT
mouse *Idh1*	GTGGTGGAGATGCAAGGAGAT	TGGTCATTGGTGGCATCACG
mouse *Idh2*	GATGGACGGTGACGAGATGAC	GGTCTGGTCACGGTTTGGA
mouse *Idh3a*	GAGCGCAATGTCACAGCAAT	CAGCGGCTATTGGGGTCTTT
mouse *Idh3g*	GAGCATTTCCTCACCTCCATCT	CAGCGTTGGAGCTTACATGC
mouse *Adcy10*	GCCATAGTGGAGAAAGTGCTG	CACCACCGTGATGATGTTCT
mouse *Ucp1*	GTGAAGGTCAGAATGCAAGC	AGGGCCCCCTTCATGAGGTC
mouse *Pgc1a*	ATACCGCAAAGAGCACGAGAA	CTCAAGAGCAGCGAAAGCGTCACA
mouse *Prdm16*	TGAGCCCCAAGGAGTCTATG	GACGAGGGTCCTGTGATGTT
mouse *Cidea*	GTGGCTGATAGGGCAGTGAT	TGCAATCCCATGAATGTCAG
mouse *Ogdh*	TGTGCCTGGTTGGAGAATCC	GCTCCAGCATTGGTGTTTCG