ThermoMouse: an *in vivo* model to identify modulators of UCP1 expression in brown adipose tissue

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SUPPLEMENTARY INFORMATION

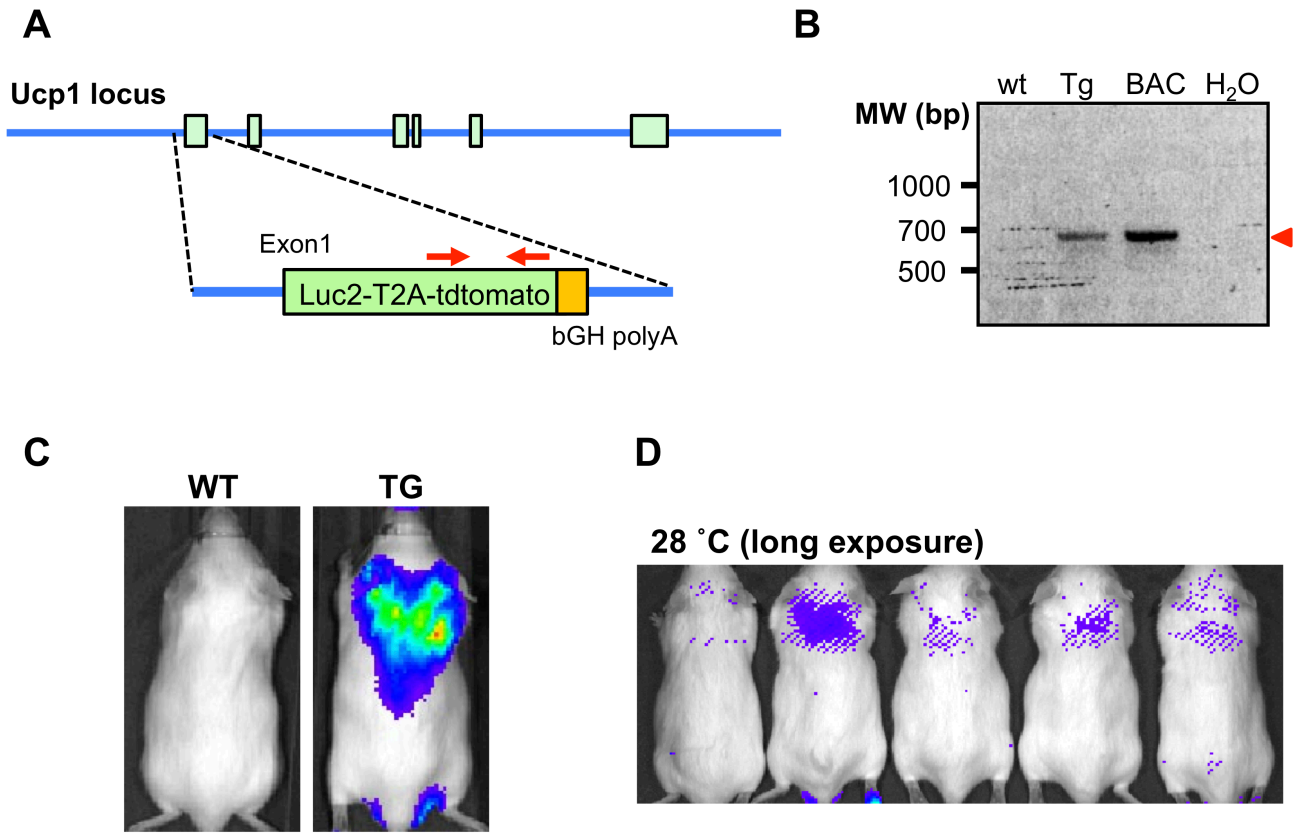

Figure S1 related to Figure 1. Generation of *Ucp1*-luciferase reporter mice
A: Genomic structure of the *Ucp1*-luciferase BAC transgene. The coding sequence of luciferase2-T2A-tdTomato followed by a bovine growth hormone (bGH)-derived polyadenylation signal (polyA) was inserted into the initiation codon of *Ucp1* located in exon 1.
B: BAC transgenic mice (Tg) were identified by PCR analysis using the primers shown as red arrows in (A). The BAC construct (BAC) was used as a positive control. Mouse wild-type (wt) DNA and water (H_20) were used as negative controls.
C: Luciferase signal in wild-type (wt) and *Ucp1*-luciferase transgenic mice (Tg) imaged using the IVIS Spectrum Imaging system at 9˚C.
D: Long-exposure image of luciferase signal in *Ucp1*-luciferase reporter mice maintained at 28°C.

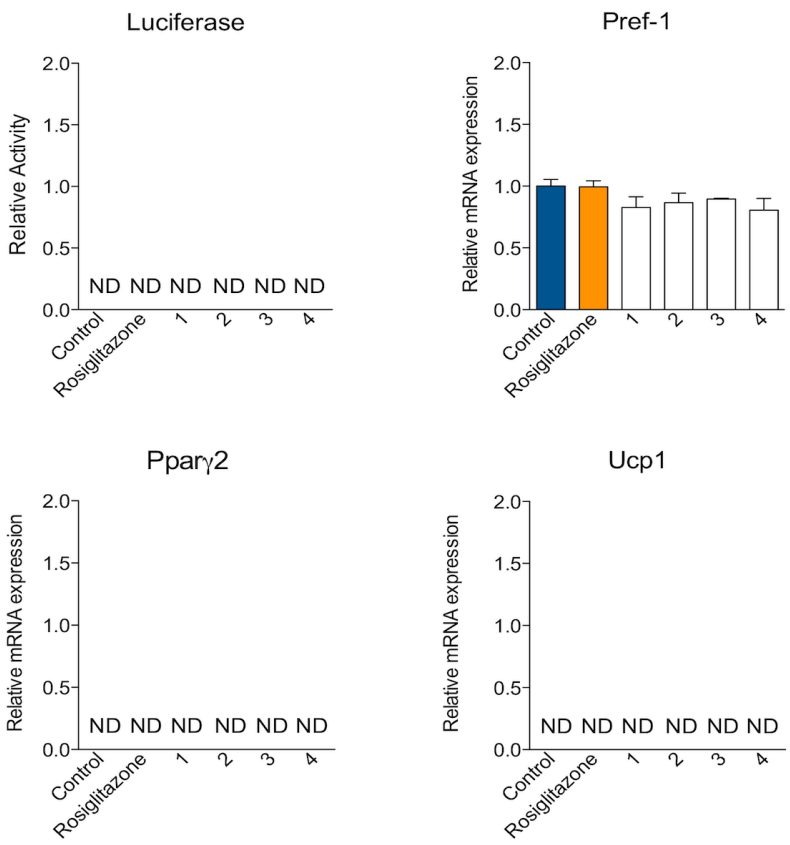

Figure S2 related to Figure 3. Effect of screening hits on *Ucp1*-luciferase preadipocytes
Subconfluent *Ucp1*-luciferase preadipocytes were treated with selected screen hits (10 μM) or rosiglitazone (0.5 μM) for 24 hr prior to luciferase and gene expression analysis (n=4). ND, not detectable.

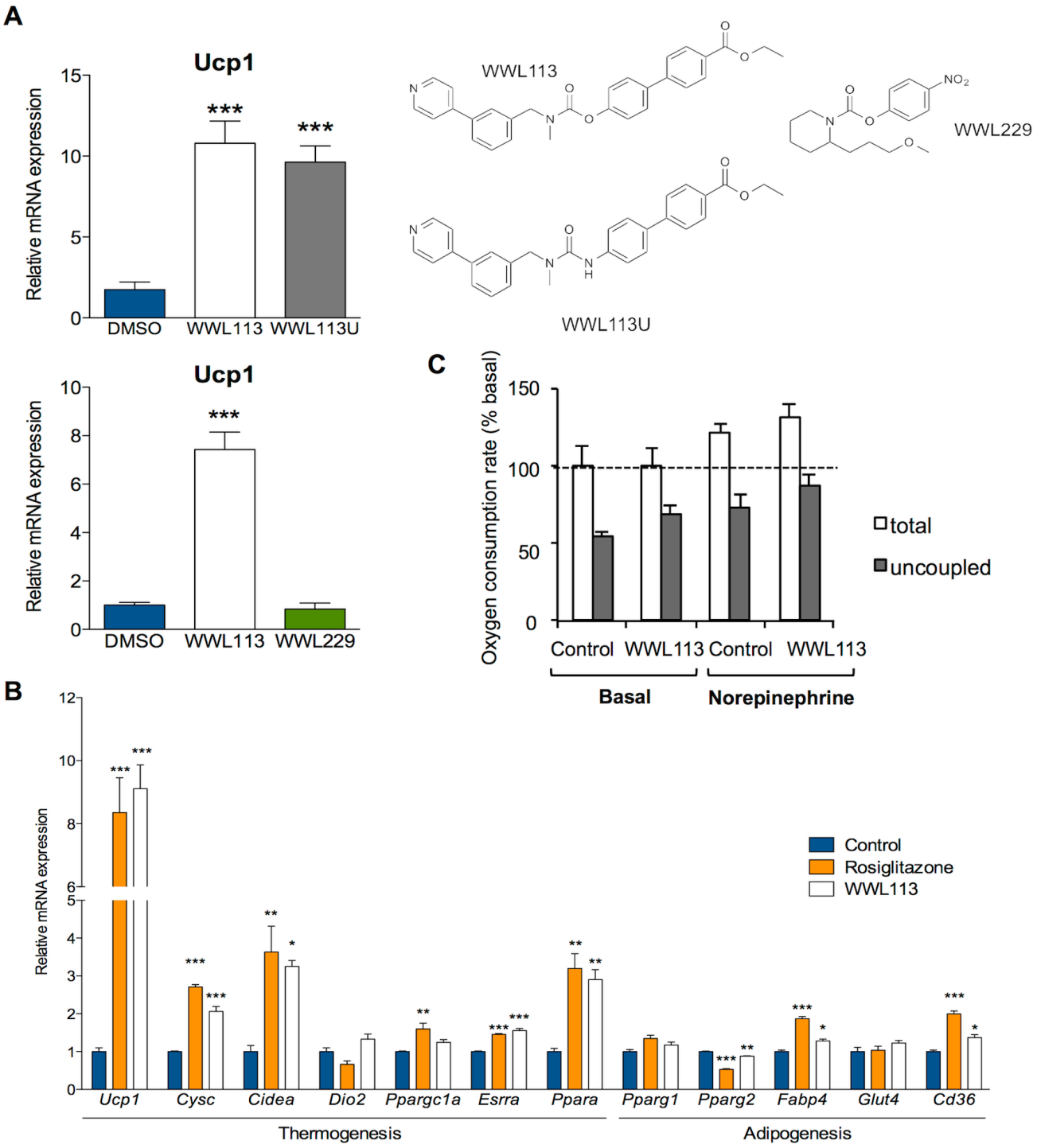

Figure S3 related to Figure 3. Induction of *Ucp1* expression and cellular respiration by WWL113 is not mediated by Ces3 inhibition

A. *Ucp1* mRNA in differentiated primary brown adipocytes treated with WWL113, WWL113U or WWL229 (all at 10 μM) for 24 hr. WWL113 and WWL229 inhibit Ces3, while WWL113U does not (n=4). Structures are shown on the right. *** $P < 0.001$ vs. control.

B: Gene expression in differentiated primary brown adipocytes treated with vehicle, WWL113 (10 μM) or rosiglitazone (0.5 μM) for 48 hr (n=3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

C: Total and uncoupled (oligomycin-insensitive) respiration measured in differentiated brown adipocytes (5 x 10^5 cells/sample) treated with WWL113 (10 μM) in the presence or absence of norepinephrine (0.1 μM) (n=3-4). Data is expressed as relative to basal for each condition. * P <0.05 vs. control.

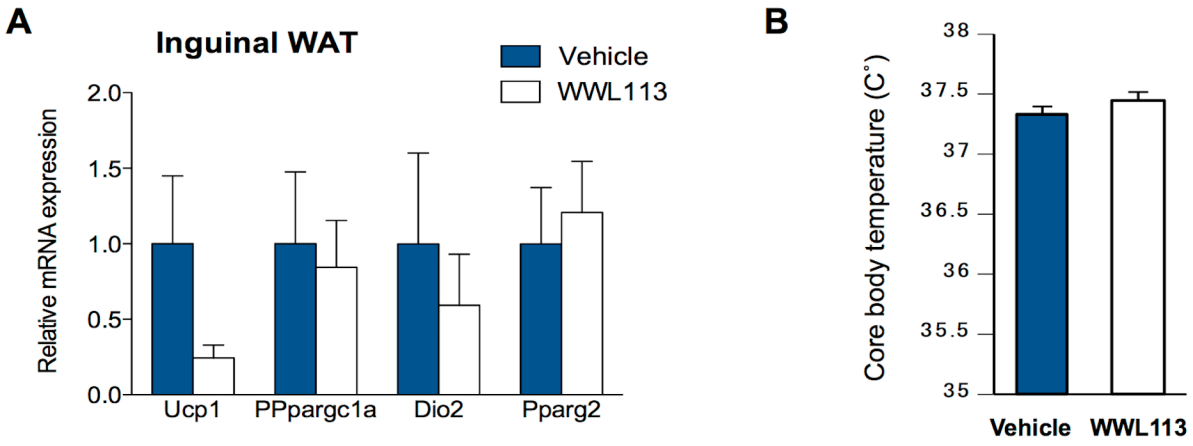

Figure S4 related to Figure 5. WWL113 does not enhance *Ucp1* expression in inguinal WAT
A: Expression of thermogenic genes in inguinal WAT of C57BL/6 mice treated daily with WWL113 (50 mg/kg) or vehicle for 5 days (n=5).
B: Core body temperature in wild-type mice treated with vehicle or WWL113 (50 mg/kg) for 7 days (n=6).

Supplementary Table related to Experimental Procedures: Primer sequences

Gene Id	Forward primer	Reverse Primer
Adipoq	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Pparg	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
Ppargc1a	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Prdm16	GGCGAGGAAGCTAGCCAAA	GGTCTCCTCCTCGGCACTCT
Tbp	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
Ucp1	CACCTTCCCGCTGGACACT	CCCTAGGACACCTTTATACCTAATGG
Ucp1-luciferase genotyping	CATGAACGGCCACGAGTT	TGACGGCCATGTTGTTGT