SUPPLEMENTAL MATERIAL

Coordinated Targeting of Cold and Nicotinic Receptors Synergistically Improves Obesity and Type 2 Diabetes

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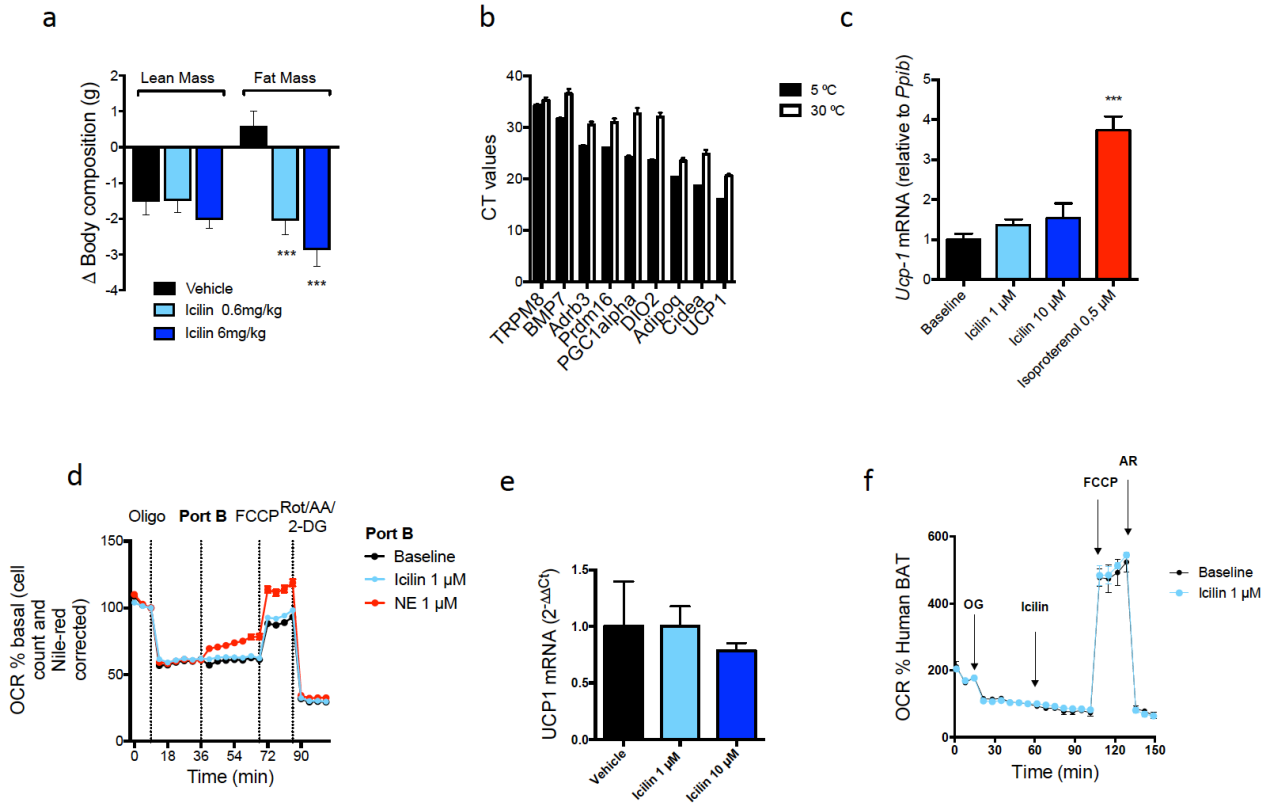
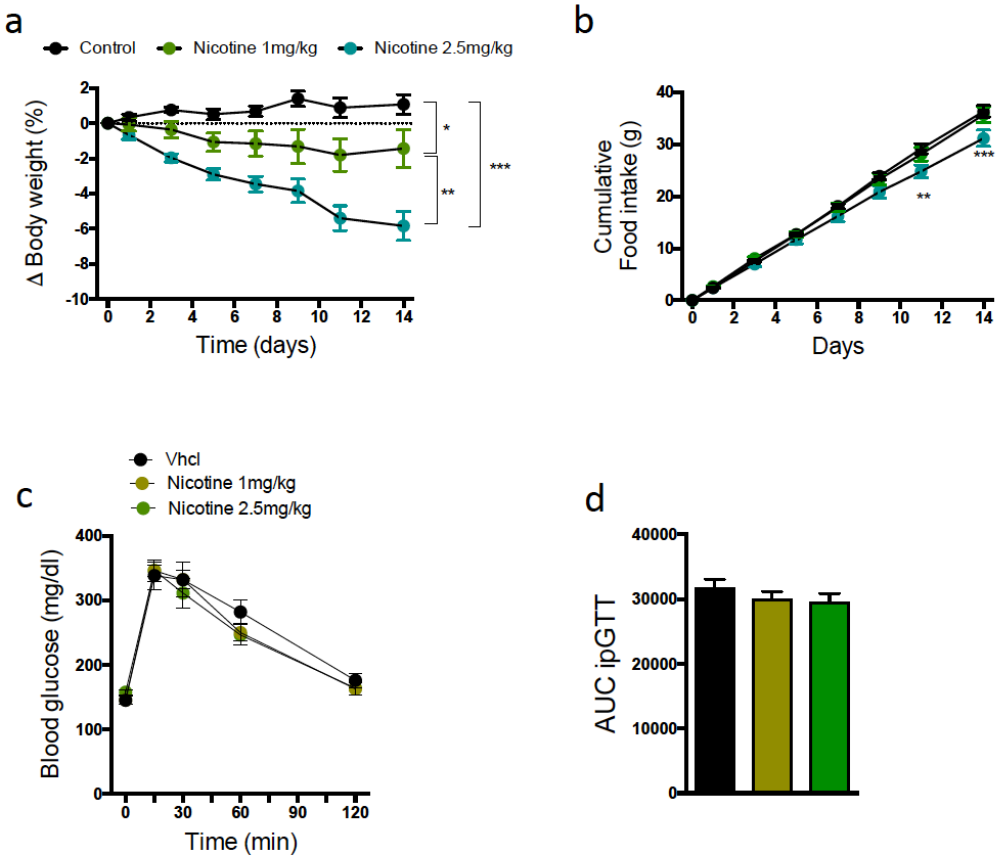


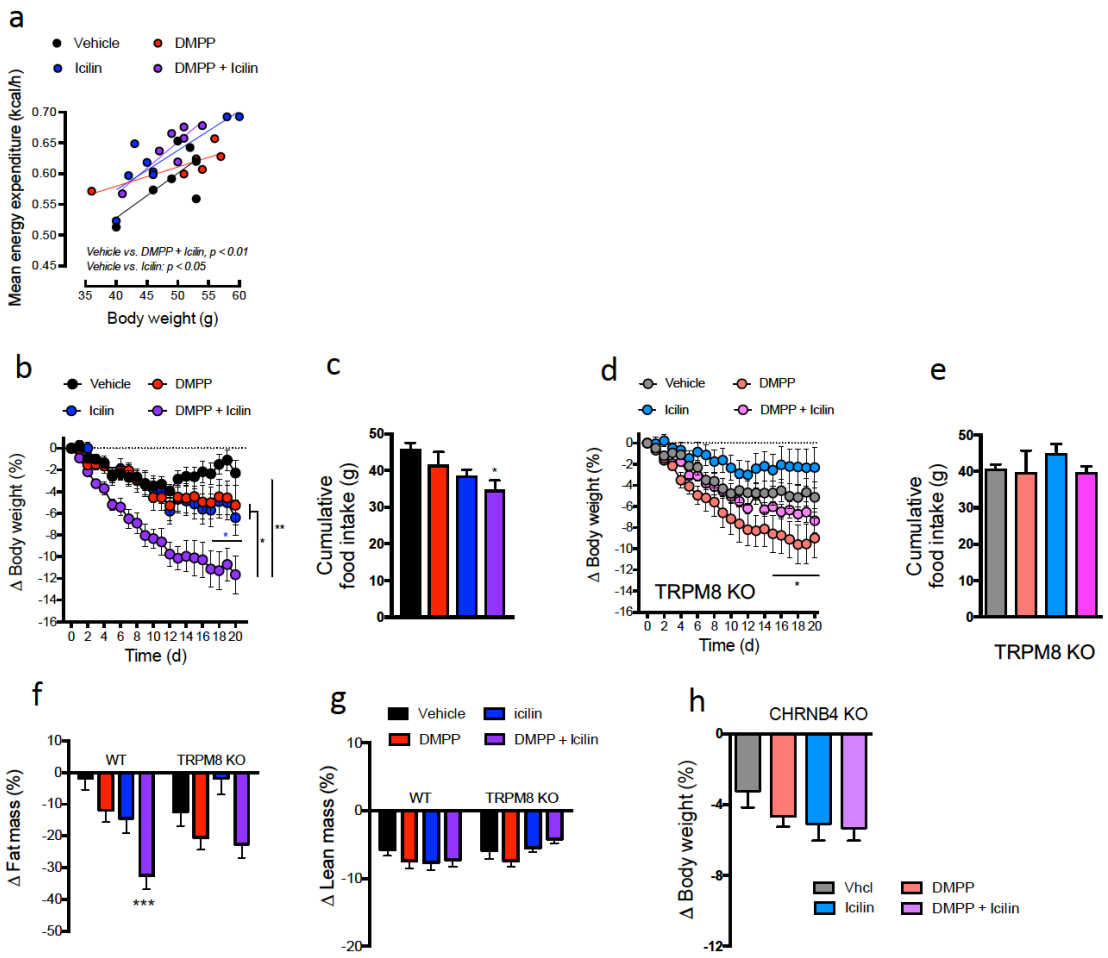
Supplemental Figure 1. TRPM8 is not expressed in murine brown adipocytes and icilin has no direct effect on brown fat thermogenesis in mouse or human primary adipocytes

(a) Effect on body lean and fat mass after daily s.c. administration to DIO male C57Bl6j mice of vehicle (black, n=8), icilin 0.6 mg/kg (light blue, n=8), or icilin 6 mg/kg (dark blue, n=6) for 14 days. (b) CT values of gene transcripts in BAT of female warm (30°C, n=8) and cold (5°C, n=7) acclimatized mice. (c) Effects of vehicle (black, n=3), icilin 1 µM (light blue, n=3), icilin 10 µM (dark blue, n=3), or isoproterenol (red, n=3) on UCP1 mRNA levels in primary brown adipocytes from C57Bl6j mice. (d) Effects of vehicle (black, n=22), icilin 1 µM (light blue, n=22), or norepinephrine 1 µM (red, n=24) on oxygen consumption rate (OCR) of primary brown adipocytes from C57Bl6j mice. (e) Effect of vehicle (black, n=3), icilin 1 µM (light blue, n=3), or icilin 10 µM (dark blue, n=3) on UCP1 mRNA expression in cultured adipocytes derived from human BAT. (f) Effects of vehicle (black, n=3) and icilin 1 µM (light blue, n=3) on OCR of primary adipocytes derived from human BAT. ***$p < 0.001$ by one-way ANOVA (a, c, e) with Tukey post-hoc test. All data are presented as mean ± SEM.



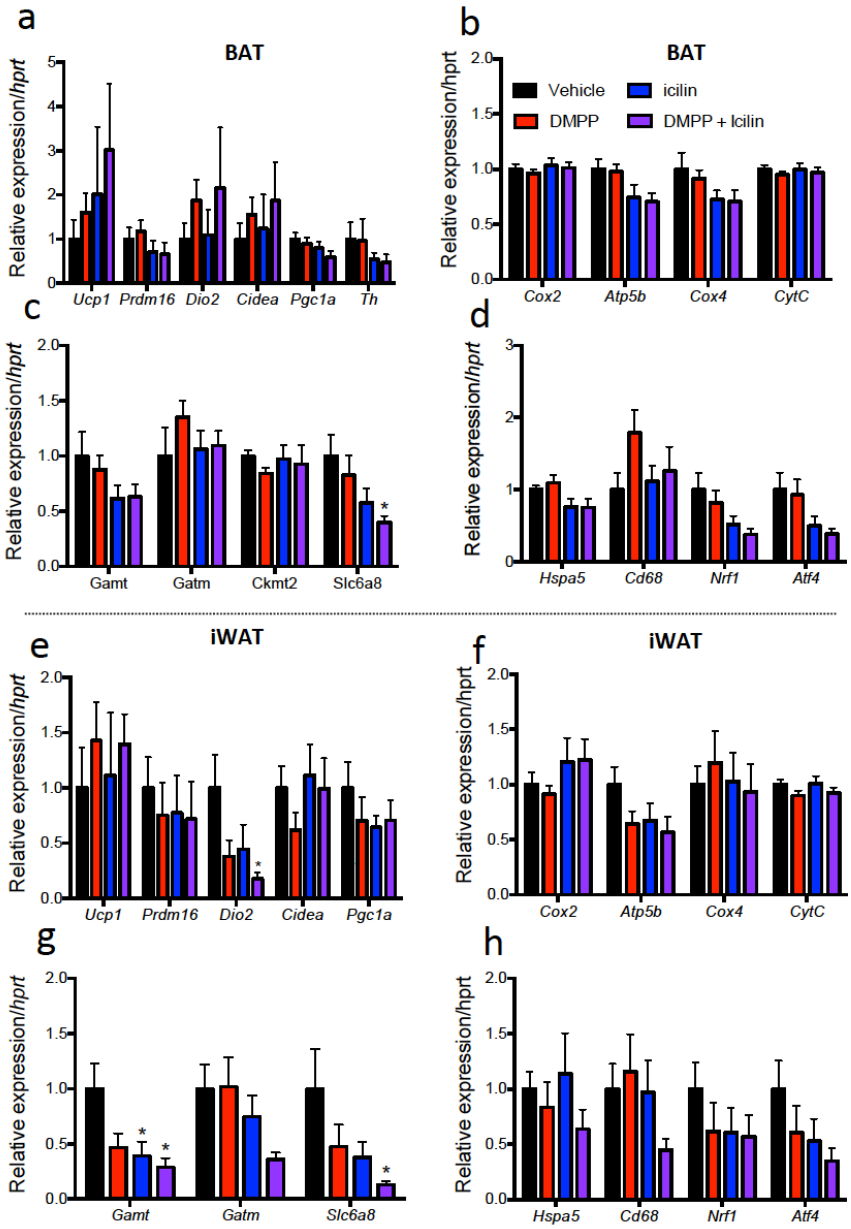
Supplemental Figure 2. Nicotine lowers body weight but has no impact on glucose tolerance in diet-induced obese mice

(a) Effects on body weight, (b) food intake, and (c-d) glucose tolerance following daily s.c. injections to DIO male C57Bl6j mice of vehicle (black, n=8), nicotine 1 mg/kg (green, n=8), and nicotine 2.5 mg/kg (turquoise, n=8) for 14 days. *p < 0.05, **p < 0.01, ***p < 0.001 by two-way ANOVA (a, b, c) and one-way ANOVA (d) with Tukey post-hoc test. All data are presented as mean ± SEM.



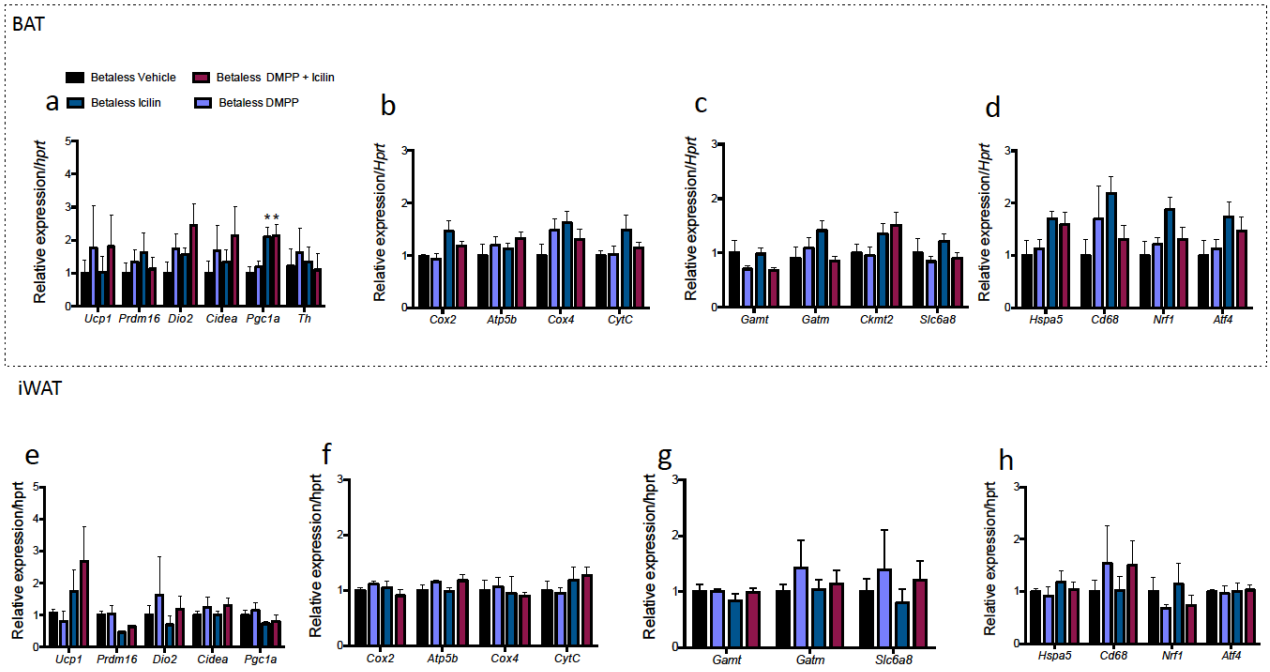
Supplemental Figure 3. Chronic DMPP and icilin co-administration increases energy expenditure by activating TRPM8 and CHRNB4

(a) Effect on mean energy expenditure with body weight as covariate following daily s.c. injections to DIO male C57Bl6j mice of vehicle (black, n=7), DMPP 5 mg/kg (red, n=6), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=7) for 3 days. (b) Effects on body weight and (c) cumulative food intake in DIO male WT mice compared to (d) effects on body weight and (e) cumulative food intake in DIO male TRPM8 KO mice. (f-g) Effects on fat and lean mass following daily s.c. injections to DIO WT and TRPM8 KO mice of vehicle (black, n=8/grey, n=8), DMPP 5 mg/kg (red, n=8/light red, n=8), icilin 5 mg/kg (blue, n=8/light blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=8/pink, n=8) for 21 days. (h) Effects on body weight in DIO male CHRNB4 KO mice treated daily with s.c. injections of vehicle (grey, n=7), DMPP 5 mg/kg (light red, n=6), icilin 5 mg/kg (light blue, n=7), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (light purple, n=7) for 7 days. *p < 0.05, **p < 0.01, ***p < 0.001 by ANCOVA (vehicle vs. DMPP: p=0.52; vehicle vs. icilin: p=0.048, vehicle vs. DMPP/icilin: p=0.009), two-way ANOVA (b, d), and one-way ANOVA (c, e, f, g, h) with Tukey post-hoc test. All data are presented as mean ± SEM.



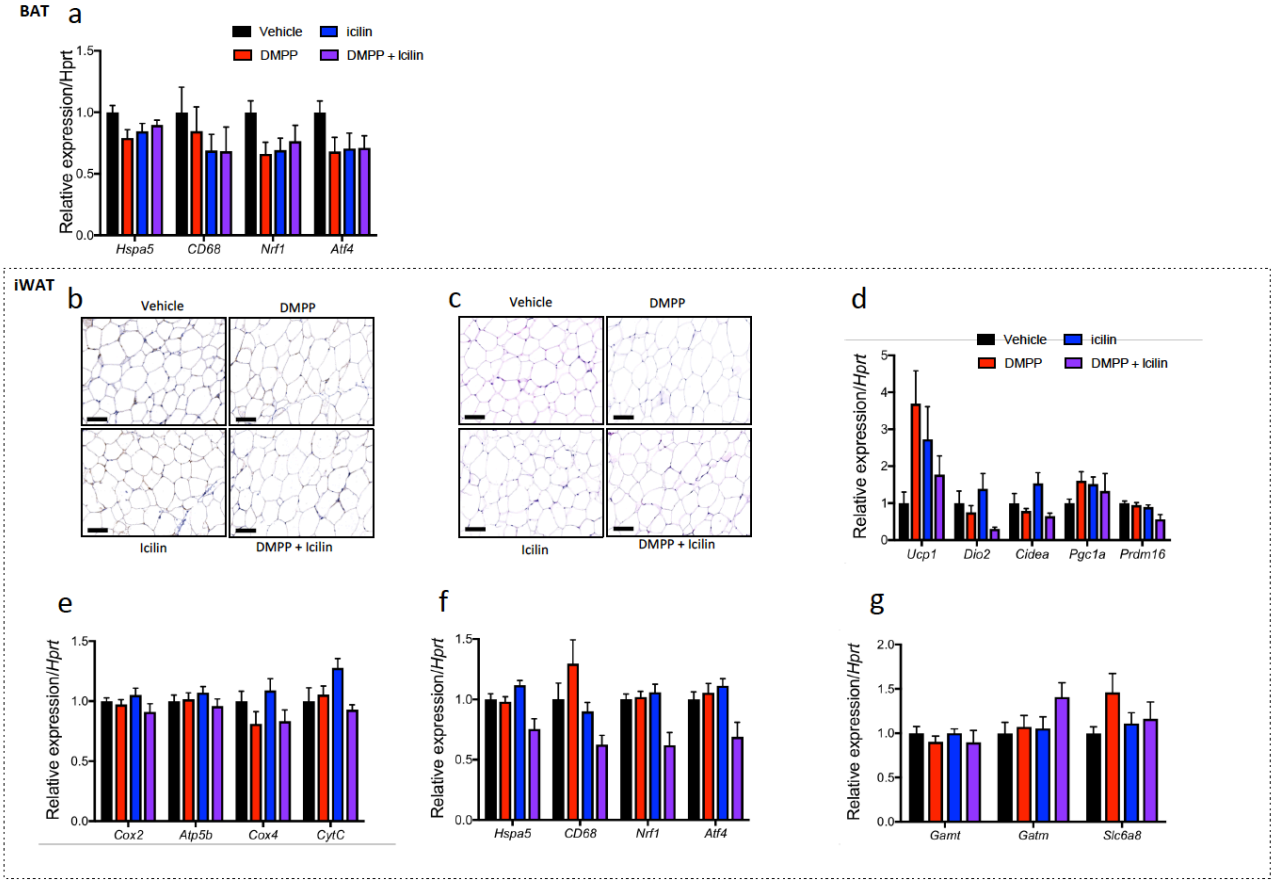
Supplemental Figure 4. Effects of sub-chronic administration of DMPP and icilin on thermogenic gene programs in DIO WT mice

Expression of genes in the BAT involved in (a) thermogenesis, (b) mitochondrial respiration chain, (c) creatine signaling pathway, and (d) proteasomal signaling in DIO male C57Bl6j mice. Expression of genes in the iWAT involved in (e) thermogenesis, (f) mitochondrial respiration chain, (g) creatine signaling pathway, and (h) proteasomal signaling in DIO male C57Bl6j mice. Tissues were analyzed following daily s.c. injections of vehicle (black, n=8), DMPP 5 mg/kg (red, n=8), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=8) for 7 days. *$p < 0.05$ by one-way ANOVA (a-h) with Tukey post-hoc test. All data are presented as mean ± SEM.



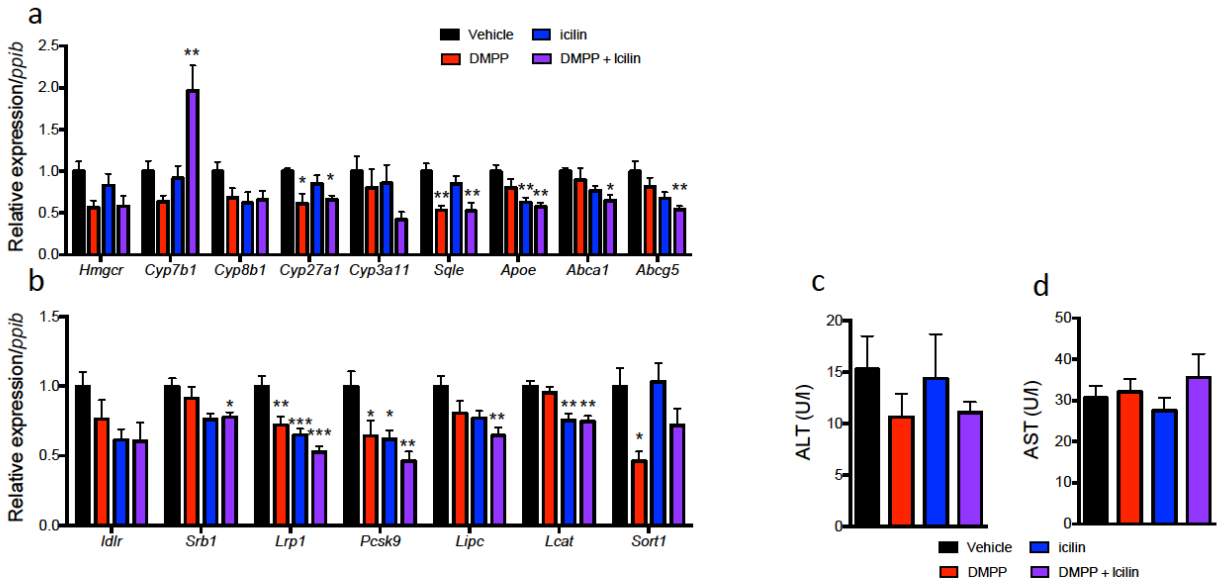
Supplemental Figure 5. Effects of sub-chronic administration of DMPP and icilin on thermogenic gene programs in betaless mice

Expression of genes in the BAT involved in (a) thermogenesis, (b) mitochondrial respiration chain, (c) creatine signaling pathway, and (d) proteasomal signaling in DIO male betaless mice. Expression of genes in the iWAT involved in (e) thermogenesis, (f) mitochondrial respiration chain, (g) creatine signaling pathway, and (h) proteasomal signaling in DIO male betaless mice. Tissues were analyzed following daily s.c. injections of vehicle (black, n=6), DMPP 5 mg/kg (orchid, n=6), icilin 5 mg/kg (ocean-blue, n=6), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (bordeaux, n=6) for 7 days. *p < 0.05 by one-way ANOVA with Tukey post-hoc test. All data are presented as mean ± SEM.



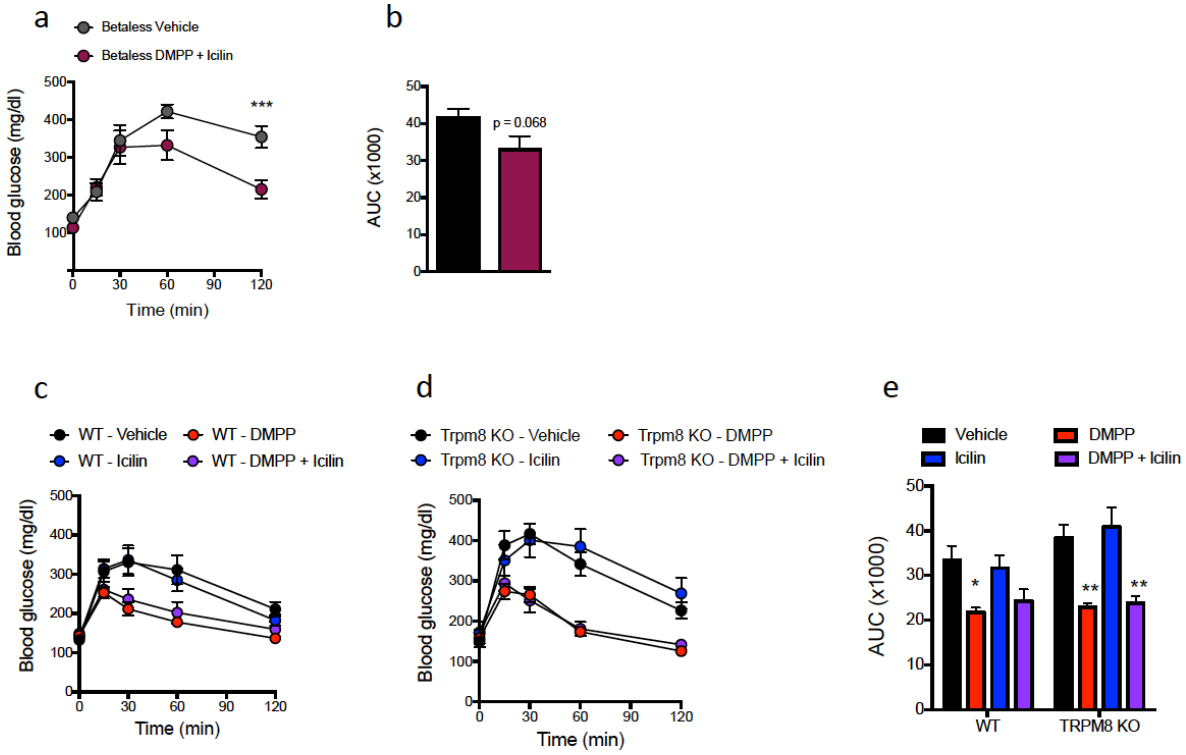
Supplemental Figure 6. Chronic administration of DMPP and icilin has no impact on iWAT thermogenic programs in DIO WT mice housed at thermoneutral conditions

(a) BAT expression of genes involved in proteasomal signaling following daily s.c. injections of vehicle (black, n=8), DMPP 5 mg/kg (red, n=7), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=7) to DIO male C57Bl6j chronically housed at 30ºC for 14 days. (b) UCP1 immunoreactivity staining in iWAT and (c) iWAT H & E staining. iWAT expression of genes involved in (d) thermogenesis, (e) mitochondrial respiration chain, (f) proteasomal signaling, and (g) creatine signaling pathway following daily s.c. injections of vehicle (black, n=8), DMPP 5 mg/kg (red, n=7), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=7) to DIO male C57Bl6j chronically housed at 30ºC for 14 days. All scale bars are 100µm. Data (a, d, e, f, g) were analyzed by one-way ANOVA with Tukey post-hoc test. All data are presented as mean ± SEM



Supplemental Figure 7. Effects of sub-chronic administration of DMPP and icilin on hepatic gene expression, lipid content, and liver function

Hepatic expression of genes involved in (a) bile acid metabolism and (b) lipoprotein uptake following daily s.c. injections to DIO male C57Bl6j mice of vehicle (black, n=8), DMPP 5 mg/kg (red, n=8), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=8) for 7 days. Effects on plasma (c) ALT and (d) AST following daily s.c. injections to DIO male C57Bl6j mice of vehicle (black, n=7), DMPP 5 mg/kg (red, n=7), icilin 5 mg/kg (blue, n=6), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=7) for 21 days *p < 0.05, **p < 0.01, ***p < 0.001 by one-way ANOVA (a-d) with Tukey post-hoc test. All data are presented as mean ± SEM.



Supplemental Figure 8. Beta adrenergic receptors and TRPM8 receptors are dispensable for the glycemic benefits of DMPP and icilin co-administration

(a-b) Effects on glucose tolerance following daily s.c. injections to DIO male betaless mice of vehicle (black, n=6) or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (bordeaux, n=6) for 7 days. (c-e) Effects on glucose tolerance following daily s.c. injections to DIO male TRPM8 WT and KO mice of vehicle (black, n=7 (WT) and n=8 (KO)), DMPP 5 mg/kg (red, n=8), icilin 5 mg/kg (blue, n=8), or the combination of DMPP 5 mg/kg and icilin 5 mg/kg (purple, n=8) for 21 days. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by two-way ANOVA (a, c, d), one-way ANOVA (e) with Tukey post-hoc test, or two-tailed Student's *t*-test (b). All data are presented as mean ± SEM.

Supplemental Table 1: List of primers

Gene name	Forward sequence (5' – 3')	Reverse sequence (3' – 5')
Abca1	AAAACCGCAGACATCCTTCAG	CATACCGAAACTCGTTCACCC
Abcg5	GTACATCGAGAGTGGCCAGA	CTGTGTATCGCAACGTCTCG
Apoe	GATCAGCTCGAGTGGCAAAG	TAGTGTCCTCCATCAGTGCC
Atf4	GATGAGCTTCCTGAACAGCG	GCCAAGCCATCATCCATAGC
Atp5b	CCGGGCAAGAAAGATACAGC	GTCCCACCATGTAGAAGGCT
Cd68	ACAAAACCAAGGTCCAGGGA	ATTCTGCGCCATGAATGTCC
Cidea	AATGGACACCGGGTAGTAAGT	CAGCCTGTATAGGTCGAAGGT
Cox2	GCCCTTCAAGCTCCTAGGTA	CTGGATCCTCTGCTTAGCGA
Cox4	CTAGAGGGACAGGGACACAC	TGGTTCATCTCTGCGAAGGT
Cyp3a11	CTCTCACTGGAAACCTGGGT	TCTGTGACAGCAAGGAGAGG
Cyp7b1	TCTGGGCCTCTCTAGCAAAC	GCACTTCTCGGATGATGCTG
Cyp8b1	CAGCGGACAAGAGTACCAGA	TGGATCTTCTTGCCCGACTT
Cyp27a1	CTTCATCGCACAAGGAGAGC	CCAAGGCAAGGTGGTAGAGA

CytC	GTTCAGAAGTGTGCCCAGTG	GTCTGCCCTTTCTCCCTTCT
Dio2	TGCCACCTTCTTGACTTTGC	GGTTCCGGTGCTTCTTAACC
Hmgcr	AGCTTGCCCGAATTGTATGTG	TGTGTTGTGAACCATGTGACTTC
Hprt	AAGCTTGCTGGTGAAAAGGA	TTGCGCTCATCTTAGGCTTT
Hspa5	GACTGCTGAGGCGTATTTGG	AGCATCTTTGGTTGCTTGTCG
Lcat	GTGCTCCACTTCTTACTGCG	GAACACATGGTCTTCAGGCC
Ldlr	TCAGACGAACAAGGCTGTCC	CCATCTAGGCAATCTCGGTCTC
Lipc	ATGTGGGGTTAGTGGACTGG	TTGTTCTTCCCGTCCATGGA
Lrp1	AACCTTATGAATCCACGCGC	TTCTTGGGGCCATCATCAGT
Nrf1	ACCCAAACTGAACACATGGC	GCAGTTACCTCATCAGCTGC
Pgc1a	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Ppib	GCATCTATGGTGAGCGCTTC	CTCCACCTTCCGTACCACAT
Prdm16	CCGCTGTGATGAGTGTGATG	GGACGATCATGTGTTGCTCC
Sort1	ATCCCAGGAGACAAATGCCA	AACCTTCCGCCACAGACATA
Sqle	TGTTGCGGATGGACTCTTCT	GAGAACTGGACTGGGGTTGA

Supplemental Table 2: List of TaqMan probes

Gene name	Assay ID
Ckmt1	Mm00438221_m1
Ckmt2	Mm01285553_m1
Gamt	Mm00487473_m1
Gatm	Mm00491879_m1
Hprt	Mm01545399_m1
Ppib	Mm00478295_m1
Slc6a8	Mm0050623_m1
Trpm8	Mm01299593_m1
UCP1	Mm01244861_m1