Molecular Pharmacology

PDE3B, PDE4, and PDE8A Cooperatively Regulate cAMP-dependent Processes in Mouse Brown Adipose Tissue

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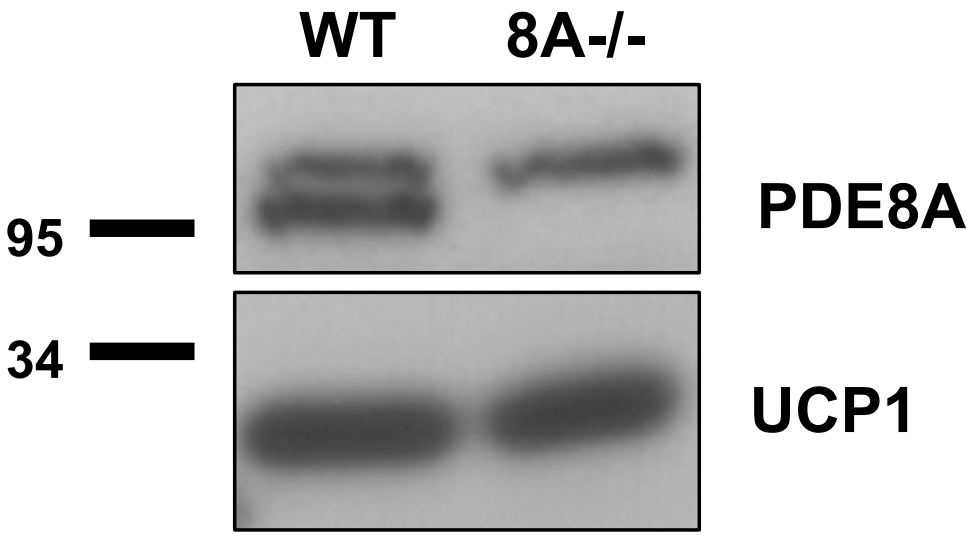

Supplemental Figure S1. PDE8A protein is present in mouse brown adipose tissue. Mouse brown adipose tissue was excised and homogenized as described in Materials and Methods. 30 μg of protein were loaded onto SDS-PAGE, transferred onto PVDF and blotted for PDE8A and UCP1. An immunoreactive band towards a PDE8A antibody at 95 kDa is present in the wildtype but absent in the PDE8A-/- (n=5).

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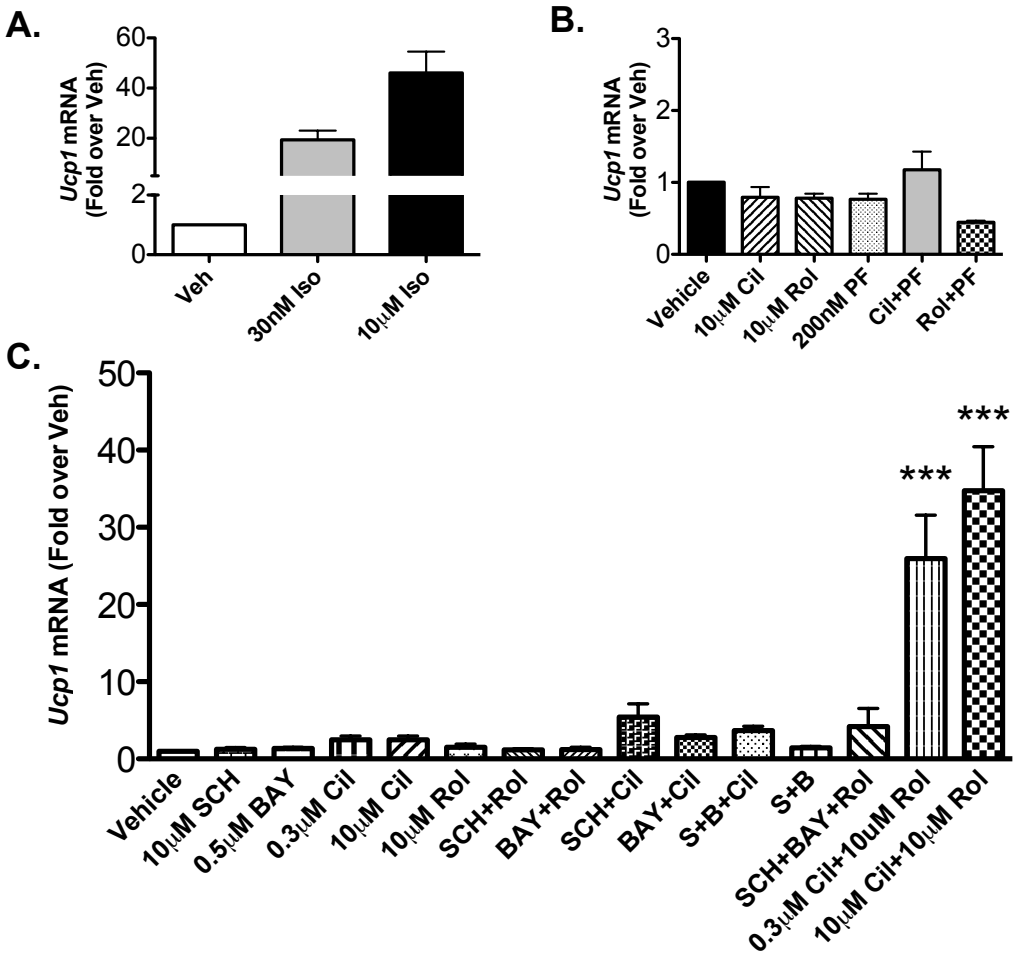

Supplemental Figure S2. The effects of isoproterenol and PDE inhibitor combinations on UCP1 mRNA expression in differentiated brown adipocytes. **[**A] Differentiated brown adipocyte precursors were stimulated with increasing doses of isoproterenol for 4 hours (n=5). [B] Differentiated brown adipocyte precursors were treated with 10 μM cilostamide, 10 μM and/or 200 nM PF-04957325 as described in materials and methods (n=3). [C] Differentiated brown adipocyte precursors were treated with SCH51866, BAY 60-7550, cilostamide, and/or rolipram at the indicated doses as described in materials and methods (n=3-8). RNA was isolated and UCP1 mRNA was quantified relative to 18S mRNA using RT-PCR as described in Materials and Methods. Data are presented as mean fold over vehicle control ± S.E.M., and statistical analyses were performed by One-way ANOVA with Dunnet post hoc test: ***, $p<0.001$ versus vehicle control in each group.

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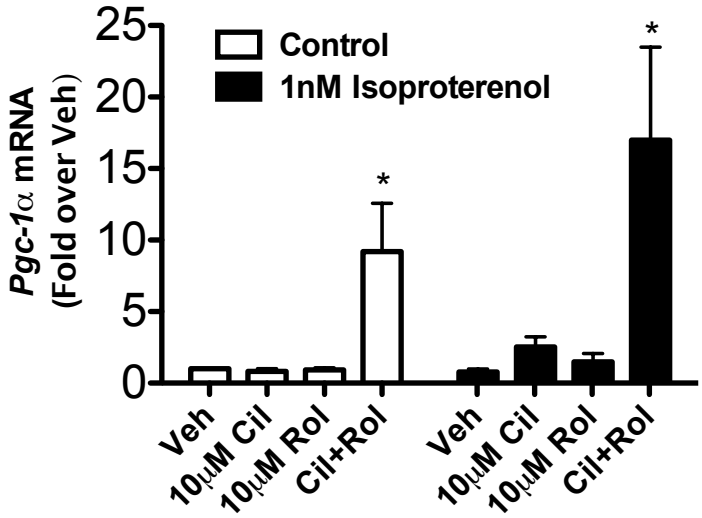

Supplemental Figure S3. PDE3 and PDE4 inhibitors increase PGC-1α mRNA expression in differentiated brown adipocytes. PGC-1α mRNA expression was measured in differentiated brown adipocytes that were pretreated with PDE inhibitors for 30 min, then stimulated by vehicle or 1 nM isoproterenol for 2 h. RNA was isolated and UCP1 mRNA was quantified relative to 18S mRNA using RT-PCR as described in Materials and Methods. Data are presented as mean fold over vehicle control ± S.E.M. (n=3), and statistical analyses were performed by One-way ANOVA with Dunnet post hoc test: *$p<0.05$ versus vehicle in each group.

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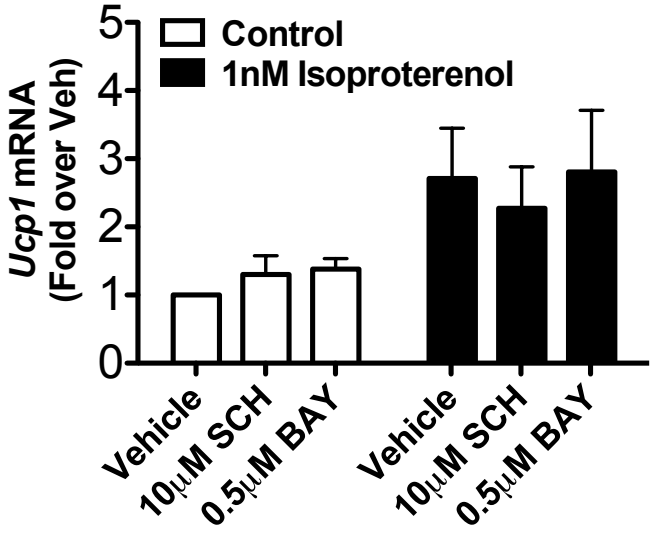

Supplemental Figure S4. PDE1 or PDE2 inhibitors did not potentiate isoproterenol-stimulated UCP1 mRNA induction. Differentiated brown adipocyte precursors were pretreated with 10 μM SCH51866 or 0.5 μM BAY 60-7550 for 30 min, then stimulated with 1 nM Isoproterenol for 4 h. RNA was isolated and UCP1 mRNA was quantified relative to 18S mRNA as a reference using RT-PCR as described in materials and methods. Data are presented as mean fold over vehicle control ± S.E.M (n=3).

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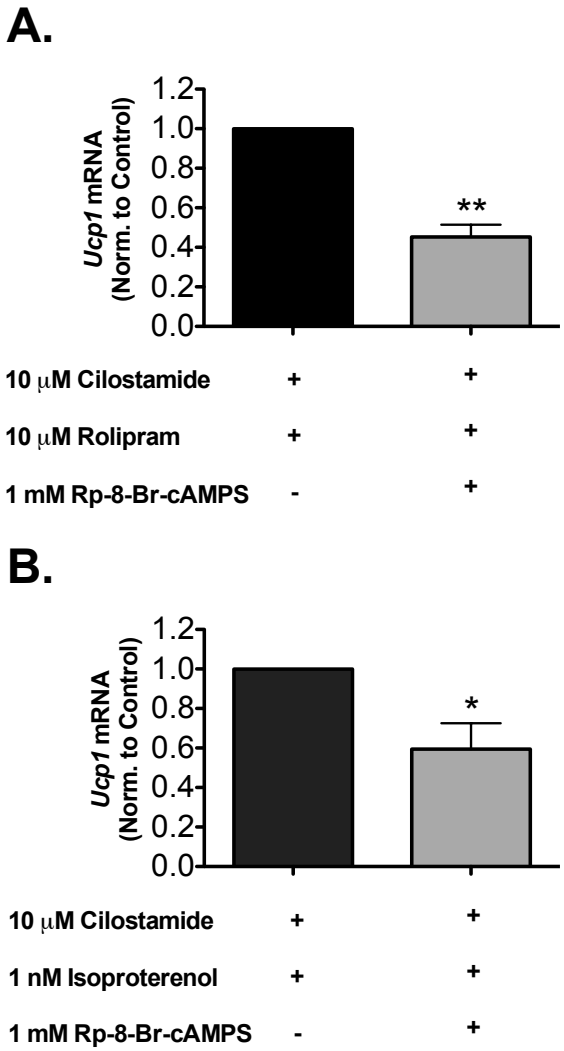

Supplemental Figure S5. The selective PKA antagonist, Rp-8-Br-cAMPS inhibited UCP1 mRNA induction by PDE inhibitors. Differentiated brown adipocyte precursors were pretreated with vehicle or 1 mM Rp-8-Br-cAMPS for 60 min, followed by [A] 10 μM cilostamide and 10 μM rolipram or [B] 10 μM cilostamide alone for 30 minutes, and finally stimulated with vehicle or 1 nM Isoproterenol for 4 h (n=4). RNA was isolated and UCP1 mRNA was quantified relative to 18S mRNA as a reference using RT-PCR as described in materials and methods. Data are presented as mean fold over vehicle control ± S.E.M., and statistical analyses were performed by paired Student's t-test: **p<0.01, *p<0.05 versus control.

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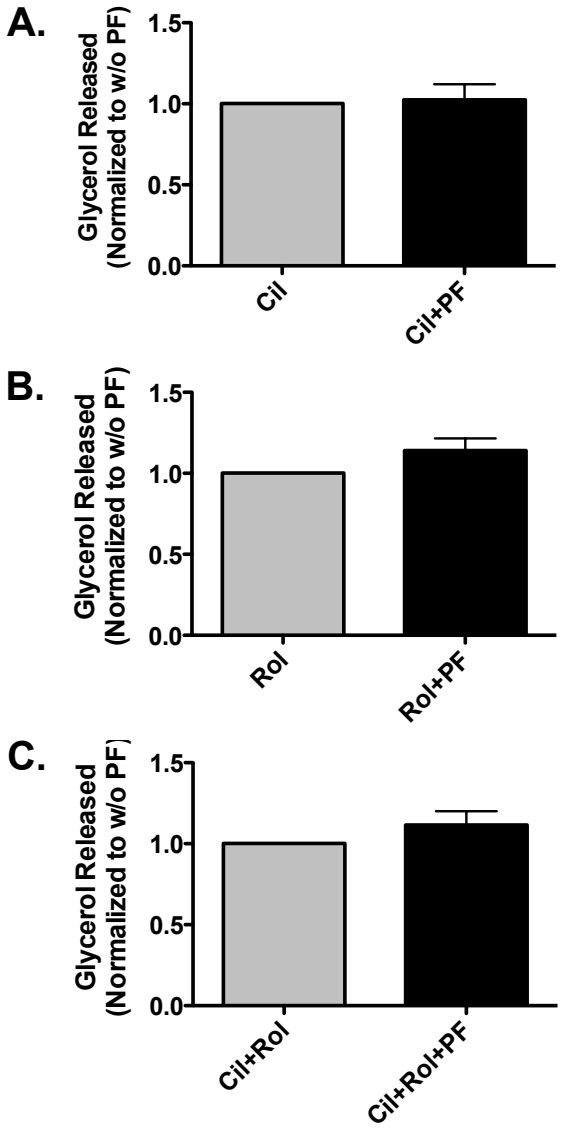

Supplemental Figure S6. PF-04957325 did not potentiate glycerol release when combined with cilostamide, rolipram or both in primary mouse brown adipocytes. Primary mouse brown adipocytes were treated for 80 minutes with: [A] 10 μM cilostamide +/- 200nM PF, [B] 10 μM rolipram +/- 200nM PF, or [C] both 10 μM cilostamide and 10μM rolipram +/- 200nM PF while shaking for 90 cycles/min at 37°C (n=3). Glycerol was measured as described in Materials and Methods. Data are normalized to the amount of glycerol released by PDE inhibitors in the absence of PF, and presented as mean ± S.E.M.

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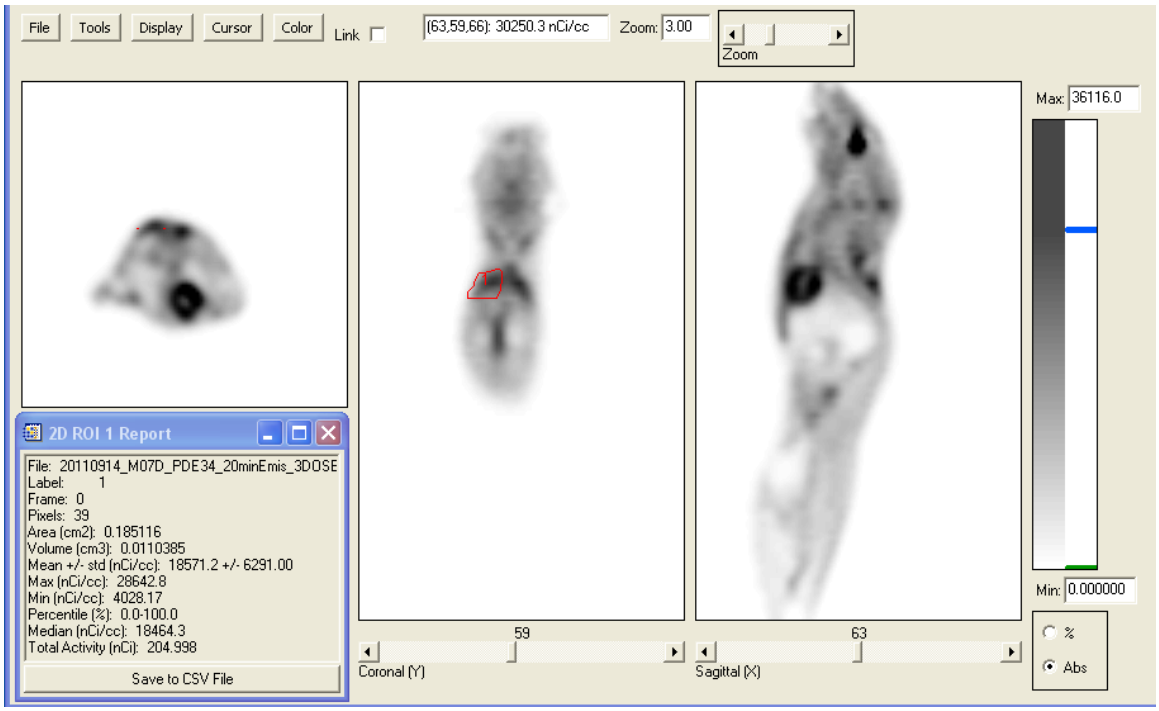

Supplementary Figure S7. FDG uptake was quantified using Siemen's ASPIRO analysis software. Shown is a screen capture image of a typical ASPIRO analysis of a mouse injected with FDG. A region of interest was drawn around the left (red trace) and right halves of the interscapular brown adipose tissue in a coronal plane that represented the center of BAT glucose uptake. The maximum activity concentrations [Max (nCi/cc)] between the two regions were averaged and used to calculate the SUV for each animal as described in Materials and Methods.

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Supplemental Table 1 – Primer Sequences for RT-PCR

Primer Name	Sequence 5' -> 3'
PDE1A forward:	GGGCATTTTCAGCAAATTAAA
PDE1A reverse:	CAGCTGCATGGAGTATCAGG
PDE1B forward:	CCAGCAAGTGAAGACTATGAAGA
PDE1B reverse:	CTGATGTCAGCAGCATGAAG
PDE1C forward:	TTGAGATGGTAATGGCCACA
PDE1C reverse:	ATAAGGCTTTCGGCTTCTCA
PDE2A forward:	GGCTGCAATATCTTTGACCA
PDE2A reverse:	GTGGTGTGCCAGGTCTGTAG
PDE3A forward:	CGACTCCGATTCTGACAGTG
PDE3A reverse:	ATATTCCCAGACAGGCATCC
PDE3B forward:	ACGGAAACCAAAGCAGATTC
PDE3B reverse:	GCAGCCATAACTCATATCTGGA
PDE4A forward:	CAAGCGCCAGAAGCAGAG
PDE4A reverse:	CATAGTCTTCAGGTCAGCCAGA
PDE4B forward:	AATGTGGCTGGGTACTCACA
PDE4B reverse:	AAGGTGTCAGATGAGATTTTAAACG
PDE4C forward:	ATGGGGACTTGATGTGTTCA
PDE4C reverse:	TCTTGAGGAGGTCTCGTTCC
PDE4D forward:	CGTTTTCCGAATAGCAGAGC
PDE4D reverse:	TTTTAAACGTTTTTAACAAATCTCG
PDE5A forward:	AAATCAATTCAGTTTTGAAGATCC
PDE5A reverse:	TGTTGAATAGGCCAGGGTTT
PDE6A forward:	CCAGGAGTGGACCCAGTACA
PDE6A reverse:	GGTTTGGTGATGGCTGAGAG
PDE6B forward:	TGAAGATAAGAAGAGTTGGGTTG
PDE6B reverse:	AGCAGACAGGTCACATGCAG
PDE6C forward:	CGAGCAGATGCAAAGTGAAG
PDE6C reverse:	CAGACAGGTCACAGGCAGTC
PDE7A forward:	AAAGGTGACTTACACCTTGACGA
PDE7A reverse:	CCAGTTCCGACATGGGTTAC
PDE7B forward:	AAAGCTCACCTCCACAATAAAGA
PDE7B reverse:	GGATTGCAAATGTCAGCACA
PDE8A forward:	GACAGAAACACCTGCAGCA
PDE8A reverse:	GTTAGGCAGGTCAACGAAG
PDE8B forward:	GTGACTCCGGGGACAACTCT
PDE8B reverse:	TGCCCCGAGAAGATATTGAT
PDE9A forward:	AATTTTGACTGCAGCAACGAG
PDE9A reverse:	ACCTCCATGGGACGGACT
PDE10A forward:	TACCAGACAGGGTCGCTGA
PDE10A reverse:	TGGCCATAGTTTGGTCACAG
PDE11A forward:	CGAGCTTGTCAGGAAAGGAG
PDE11A reverse:	ACGGCTCCAAGGTCACAG
UCP1 forward:	CGATGTCCATGTACACCAAGGA
UCP1 reverse:	TCGCAGAAAAGAAGCCACAA
PGC1α forward:	GCTTTGAAGTTTTTGGTGAAA
PGC1α reverse:	ACGGTAGGTGATGAAACCATAGC
18S forward:	GTAACCCGTTGAACCCCATT
18S reverse:	CCATCCAATCGGTAGTAGCG