Supplementary Information

Identification of a rhodanine derivative BML-260 as a potent stimulator of UCP1 expression

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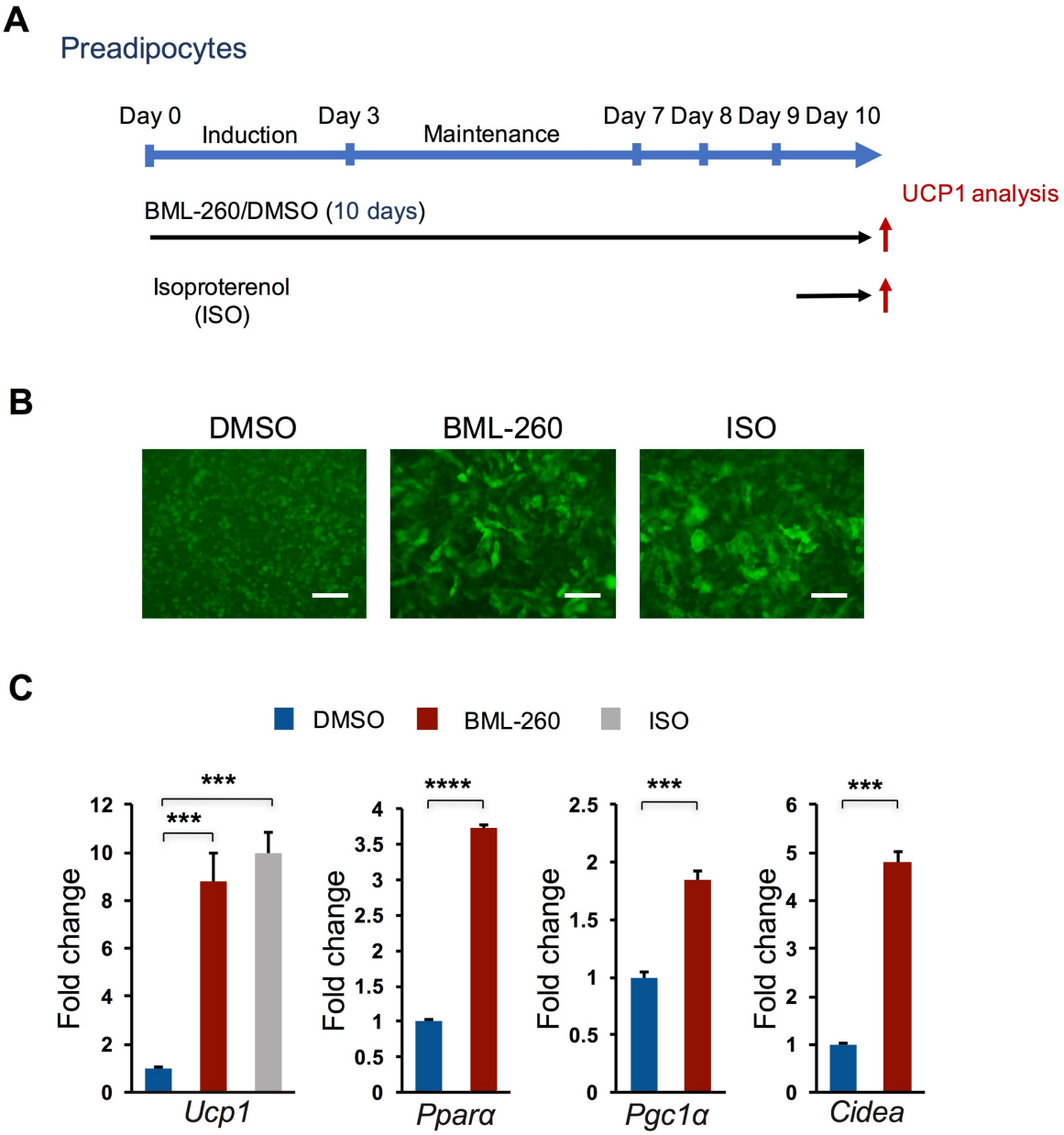
Supplementary table. Information of the phosphatase inhibitor library.

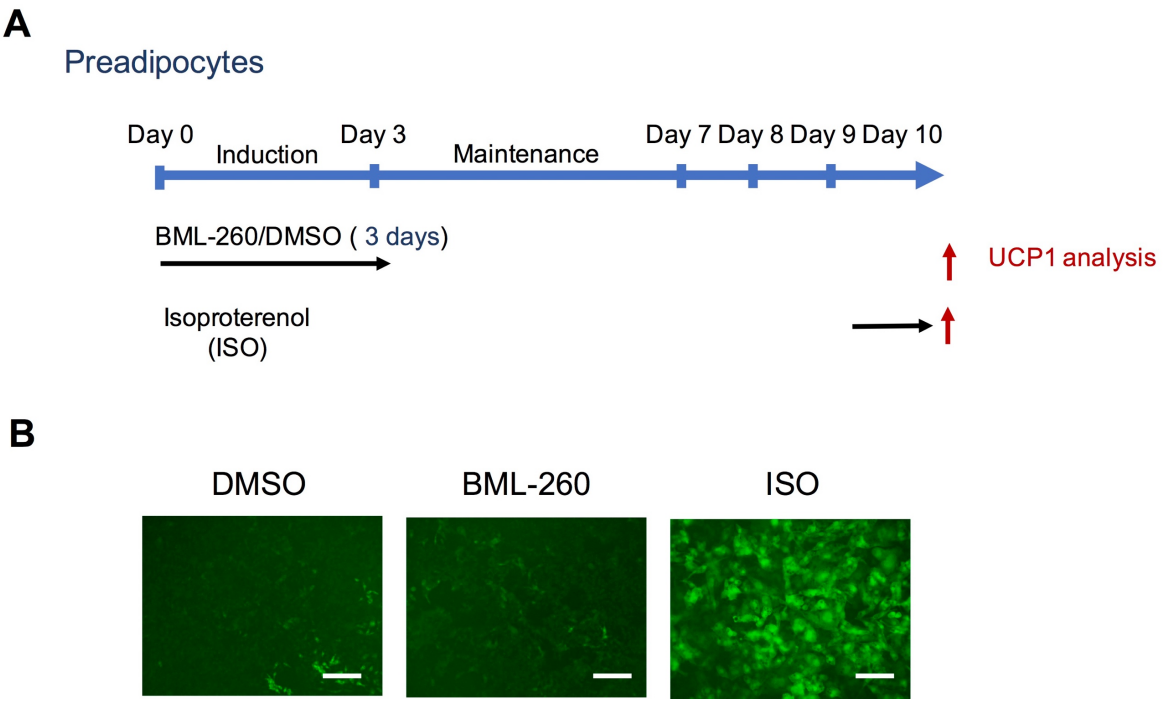
CATALOG #	CAS #	COMPOUND	M.W.	Molecular Formula
PR-106	28874-45-5	Cantharidic acid	214.2	$C_{10}H_{14}O_5$
PR-105	56-25-7	Cantharidin	196.2	$C_{10}H_{12}O_4$
PR-107	145-73-3	Endothall	186.2	$C_8H_{10}O_5$
EI-244	6881-57-8	Benzylphosphonic acid	172.1	$C_7H_9O_3P$
T-100	62284-79-1	L-p-Bromotetramisole oxalate	373.2	$C_{13}H_{13}BrN_2O_4S$
PR-112	150627-37-5	RK-682	368.5	$C_{21}H_{36}O_5$
PR-109	n.a.	RWJ-60475	404.1	$C_{13}H_{11}BrNO_7P$
PR-110	n.a.	RWJ-60475 (AM)3	620.3	$C_{22}H_{23}BrNO_{13}P$
AC-1102	16595-80-5	Levamisole HCl	240.8	$C_{11}H_{13}ClN_2S$
AC-1104	5086-74-8	Tetramisole HCl	240.8	$C_{11}H_{13}ClN_2S$
PR-100	52315-07-8	Cypermethrin	416.3	$C_{22}H_{19}Cl_2NO_3$
PR-101	52918-63-5	Deltamethrin	505.2	$C_{22}H_{19}Br_2NO_3$
PR-102	51630-58-1	Fenvalerate	419.9	$C_{25}H_{22}ClNO_3$
PR-113	3785-90-8	Tyrphostin 8	160.2	$C_{10}H_6N_2O$
PR-114	n.a.	CinnGEL	490.5	$C_{23}H_{30}N_4O_8$
PR-115	n.a.	CinnGEL 2 Me	518.6	$C_{25}H_{34}N_4O_8$
PR124	396073-89-5	BN-82002	359.4	$C_{19}H_{25}N_3O_4$
CT115	517-89-5	Shikonin	288.3	$C_{16}H_{16}O_5$
PR-116	383907-43-5	NSC-663284	321.8	$C_{15}H_{16}ClN_3O_3$
A-105	59865-13-3	Cyclosporin A	1203	$C_{62}H_{111}N_{11}O_{12}$
PR-118	100-33-4	Pentamidine	340.4	$C_{19}H_{24}N_4O_2$
PR-125	39674-97-0	BVT-948	241.2	$C_{14}H_{11}NO_3$
PR-126	n.a.	B4-Rhodanine	485.2	n.a.
PR-127	n.a.	BML-268	741.5	n.a.
PR-128	n.a.	Dioxophenanthrene	307.3	$C_{19}H_{17}NO_3$
PR-119	101439-76-3	BML-260	341.4	$C_{17}H_{11}NO_3S_2$
PR-120	148550-96-3	PD-144795	281.3	$C_{13}H_{15}NO_4S$
PR-121	n.a.	BML-267	284.4	$C_{11}H_{12}N_2O_5S$
PR-129	n.a.	BML-267 Ester	340.4	n.a.
PR-122	n.a.	OBA	209.2	$C_9H_7NO_5$
PR-130	n.a.	OBA Ester	251.2	n.a.
EI-130	303-45-7	Gossypol	518.6	$C_{30}H_{30}O_8$
PR-123	121268-17-5	Alendronate	325.1	$C_4H_{18}NNaO_{10}P_2$

n.a., not available

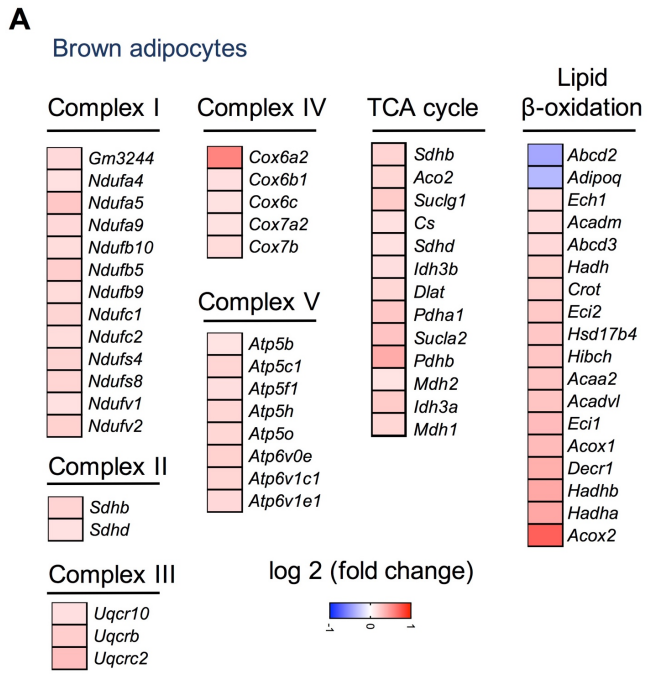
Supplementary figure and figure legends



Supplementary figure 1. BML-260 treatment activates UCP1 expression in brown adipocytes. (**A**) Schematic view of the adipocyte differentiation procedure and compound treatment protocol. Red arrows indicate time points of cell collection for analysis. (**B**) Representative images of GFP intensity of cells upon treatment with BML-260, ISO or DMSO. Scale bar = 200 μm. (**C**) mRNA expression analysis in above cells with ISO or BML-260 treatment. Data are represented as means with SEM. *** P<0.001, **** P<0.0001.



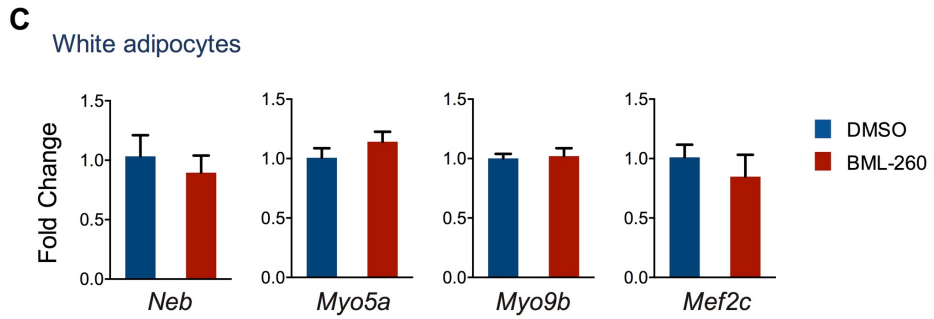
Supplementary figure 2. BML-260 treatment at the differentiation induction stage only does not induce UCP1 expression in brown adipocytes. (**A**) Schematic view of the adipocyte differentiation procedure and compound treatment protocol. Red arrows indicate time points of cell collection for analysis. (**B**) Representative images of GFP intensity of cells upon treatment with BML-260 or ISO. Scale bar = 200 μm.



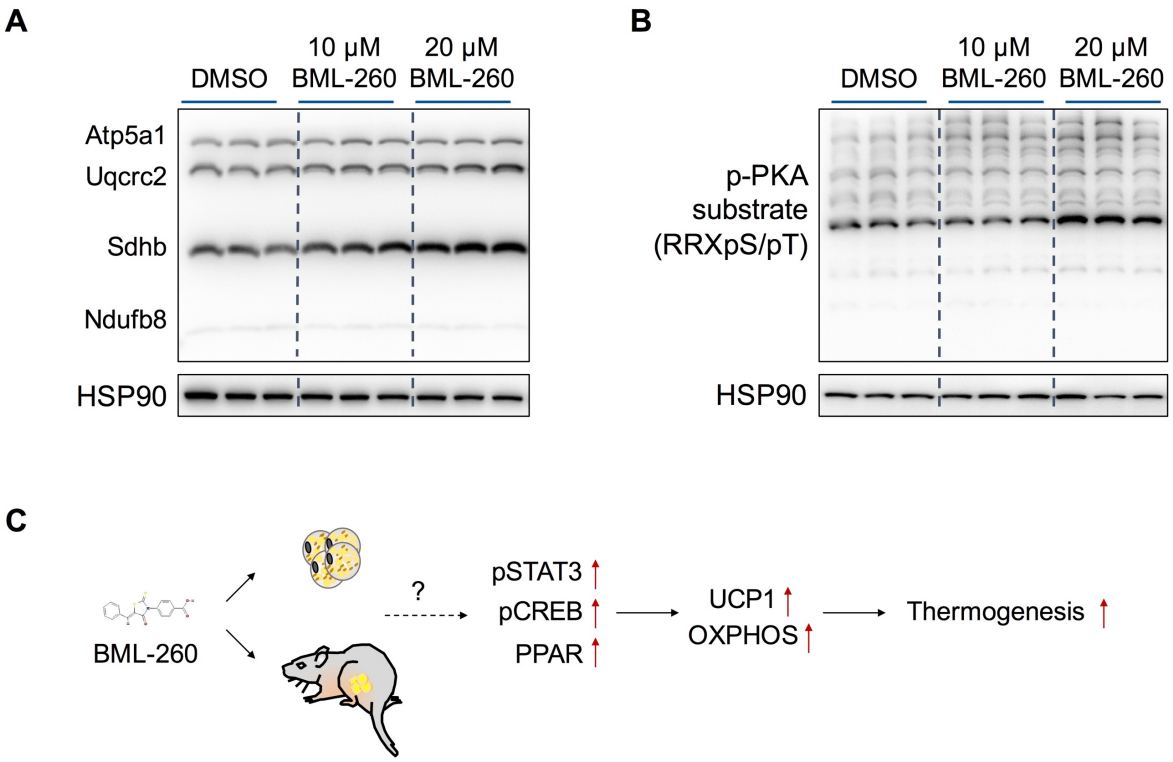
B

Common genes upregulated in BML-260 and ISO treated brown adipocytes

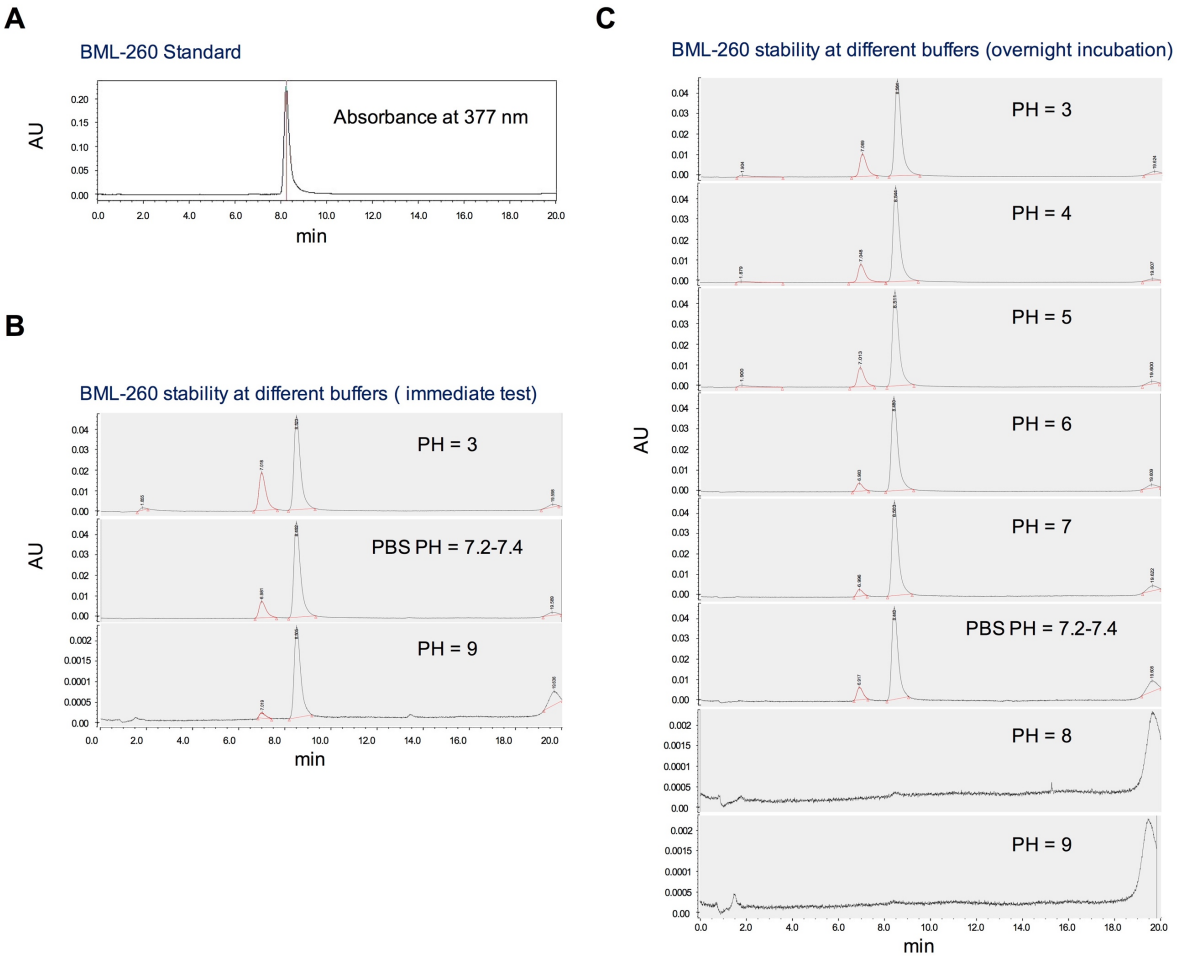
Biological Processes	p-value
Primary metabolic process	1.14 E-13
Catabolic process	2.79 E-07
Protein localization	9.30 E-13
G protein-coupled receptor signaling pathway	2.18 E-10
Negative regulation of autophagy	3.86 E-02



Supplementary figure 3. BML-260 upregulates thermogenic genes. (**A**) Heatmap depicting of upregulated genes after BML-260 treatment in brown adipocytes identified in Gene Ontology analysis. (**B**) Gene Ontology analysis of commonly upregulated genes in brown adipocytes treated by BML-260 or ISO. (**C**) Expression levels of muscle program genes in *in vitro* cultured white adipocytes treated by BML-260 or DMSO.



Supplementary figure 4. BML-260 enhances PKA signaling pathway and OXPHOS proteins in white adipocytes. (**A**) Western analysis of OXPHOS proteins in *in vitro* cultured white adipocytes treated by BML-260 or DMSO. (**B**) Western analysis of PKA signaling pathway in *in vitro* cultured white adipocytes treated by BML-260 or DMSO. (**C**) The schematic diagram of the discoveries in this study. OXPHOS, oxidative phosphorylation.



Supplementary figure 5. HPLC analysis of BML-260 stability in buffers with different pH values. (**A**) HPLC profile of BML-260 standard at 377 nm absorbance. (**B**) Immediate tests of BML-260 dissolved in different buffers as indicated. (**C**) Tests of BML-260 dissolved in different buffers as indicated after overnight incubation.