

Supplementary Materials For

Patulin Ameliorates Hypertrophied Lipid Accumulation and Lipopolysaccharide-Induced Inflammatory Response by Modulating Mitochondrial Respiration

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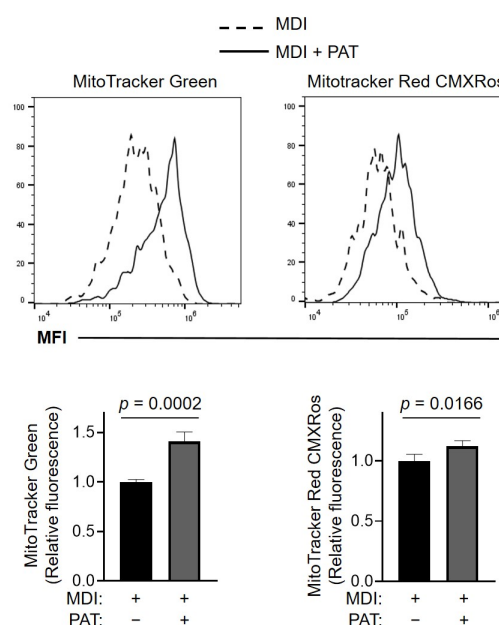


Figure S1. Analysis of mitochondrial function in 3T3-L1 adipocytes. 3T3-L1 cells were seeded and induced to differentiate in the presence of patulin (PAT, 1 or 5 μ M) for 8 days. Mitotracker Green and Mitotracker red-stained cells were detected using flow cytometer, and the relative fluorescence intensity was calculated by normalizing to the MFI values of the PAT-untreated control. Statistical significance (p value) was determined using unpaired two-tailed Student's t -test.

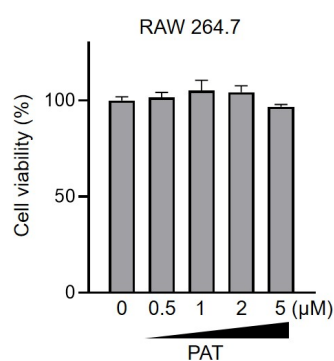


Figure S2. Effects of patulin (PAT) on RAW 264.7 cell viability. RAW 264.7 cells were incubated in a medium containing different concentrations of PAT for 6 h. Cell viability was measured using WST-1 kit.

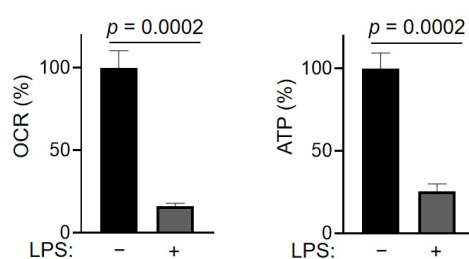


Figure S3. Effects of lipopolysaccharide (LPS) treatment on mitochondrial oxidative function in RAW 264.7 macrophages. Oxygen consumption rate (OCR) and ATP production levels were determined as described in the Materials and Methods. The data were normalized to the OCR values of the unstimulated control. Statistical significance (p value) was determined using unpaired two-tailed Student's t -test.

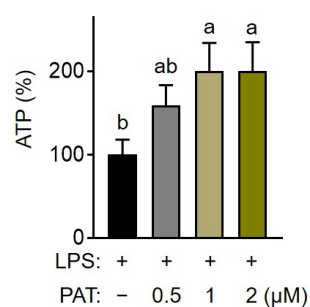


Figure S4. Effects of patulin (PAT) on the mitochondrial ATP production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. ATP generation was calculated as described in the Materials and Methods. The data were normalized to calculated OCR values for the PAT-untreated control. Different letters indicate significantly different values at $p < 0.05$, as determined by one-way ANOVA followed by Tukey's post-hoc test.

Table S1. Primer sequences of mouse mRNA used in RT-PCR analysis

Gene	Direction	Sequence (5'-3')
<i>Pparγ</i>	forward	GCGGGCTGAGAAGTCACGTT
	reverse	CCATCACGGAGAGGTCCACA
<i>C/ebpa</i>	forward	TACCGAGTAGGGGGAGCA
	reverse	TCATTTTTCTCACGGGGCC
<i>Fasn</i>	forward	AGAAGCCATGTGGGGAAGATT
	reverse	AGCAGGGACAGGACAAGACAA
<i>Acly</i>	forward	CTTGGGCCGGAACAAAA
	reverse	GCCGAGGTGGTGCAGAT
<i>Fabp4</i>	forward	GGGAACCTGGAAGCTTGTCT
	reverse	ACTCTCTGACCGGATGGTGA
<i>Cd36</i>	forward	GTGCAAAACCCAGATGACGT
	reverse	TCCAACAGACAGTGAAGGCT
<i>Dgat1</i>	forward	GGCCCAAGGTAGAAGAGGAC
	reverse	GATCAGCATCACACACACC
<i>Gpat</i>	forward	GTAGTTGAACTCCTCCGACA
	reverse	ATCCACTACCACTGAGAGGA
<i>Pgc1α</i>	forward	AAGTGGTGTAGCGACCAATCG
	reverse	AATGAGGGCAATCCGTCTTCA
<i>Cpt1α</i>	forward	CTCCGCCTGAGCCATGAAG
	reverse	CACCAGTGATGCCATTCT
<i>Il-6</i>	forward	CAAAGCCAGAGTCCTTCAGA
	reverse	TTGGTCCTTAGCCACTCCTT
<i>Tnf-α</i>	forward	AGCCACGTCGTAGCAAACCAC
	reverse	AGGTACAACCCATCGGCTGGCA
<i>inos</i>	forward	CCTGTGTTCCACCAGGAGAT
	reverse	CCCTGGCTAGTGCTTCAGAC
<i>Cox-2</i>	forward	TGACCCCCAAGGCTCAAAT
	reverse	GAACCCAGGTCCTCGCTTATG
<i>Rplp0</i>	forward	AGGTCCTCCTTGGTGAAC
	reverse	GTGCTGATGGGCAAGAAC
<i>Gapdh</i>	forward	AGTATGACTCCACTCACGGCAAAT
	reverse	GTCTCGCTCCTGGAAGATGGT
<i>β-actin</i>	forward	GGCTGTATTCCCCTCCATCG
	reverse	CCAGTTGGTAACAATGCCATGT
<i>Eef2</i>	forward	CGGGACACGGCTCTTAACAT
	reverse	CTTCCTGGAGGCACTTACCC

Note: peroxisome proliferator-activated receptor- γ , Ppar- γ ; CCAAT/enhancer-binding protein α , C/ebpa; fatty acid synthase, Fasn; ATP-citrate lyase, Acly; fatty acid-binding protein 4, Fabp4; cluster of differentiation 36, Cd36; diacylglycerol O-acyltransferase 1, Dgat1; glycerol-3-phosphate O-acyltransferase, Gpat; Peroxisome proliferator-activated receptor γ coactivator 1 α , Pgc1 α ; carnitine palmitoyltransferase 1 α , Cpt1 α ; interleukin-6, Il-6; tumor necrosis factor- α , Tnf- α ; inducible nitric oxide synthase, inos; cyclooxygenase-2, Cox-2.