Tiliroside Attenuates NLRP3 Inflammasome Activation in Macrophages and Protects against Acute Lung Injury in Mice

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Supplementary Table S1: Sequences of qRT-PCR primers used in this study

Gene	Species	Forward primer (5'-3')	Reverse primer (5'-3')
ACTB	*Homo*	CTCACCATGGATGATGATATCGC	AGGAATCCTTCTGACCCATGC
IL6	*Homo*	TGCAATAACCACCCCTGACC	GTGCCCATGCTACATTTGCC
IL8	*Homo*	AGCCTTCCTGATTTCTGCAG	GTCCACTCTCAATCACTCTCAG
TNFA	*Homo*	ACTTTGGAGTGATCGGCC	GCTTGAGGGTTTGCTACAAC
CCL2	*Homo*	TGTCCCAAAGAAGCTGTGATC	ATTCTTGGGTTGTGGAGTGAG
Actb	*Mus*	CTTTGCCACGGACGAGAC	TCATTGTACTCTGAGGGCTGAC
Il-6	*Mus*	GAACAACGATGATGCACTTGC	CTTCATGTACTCCAGGTAGCTATGGT
Cxcl-2	*Mus*	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
Il-1β	*Mus*	AGTTGACGGACCCCAAAAG	AGCTGGATGCTCTCATCAGG
Inos	*Mus*	CTTTGCCACGGACGAGAC	TCATTGTACTCTGAGGGCTGAC

Supplementary Figure S1

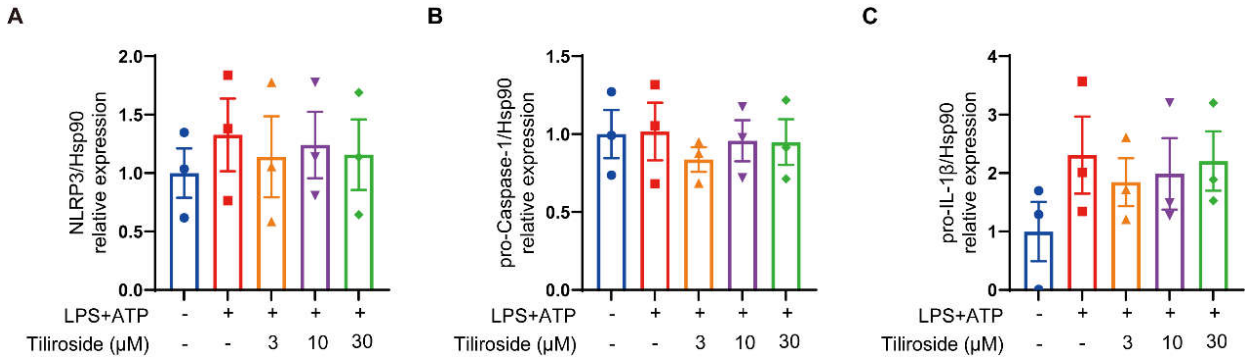

Figure S1. The effect of tiliroside on the expression of NLRP3, pro-Caspase-1, and pro-IL-1β in LPS plus ATP-induced THP-1 macrophages. (A-C) THP-1 macrophages were pretreated with tiliroside or a vehicle for 24 h and then stimulated with LPS (2 μg/mL) for 5 h, followed by ATP (5 mM) treatment for 45 min. NLRP3 **(A)**, pro-Caspase-1 **(B),** and pro-IL-1β **(C)** expression were determined by Western blotting, and the relative expression levels were quantified (n=3). Data are mean ± SEM. These data are related to Figure 1C.

Supplementary Figure S2

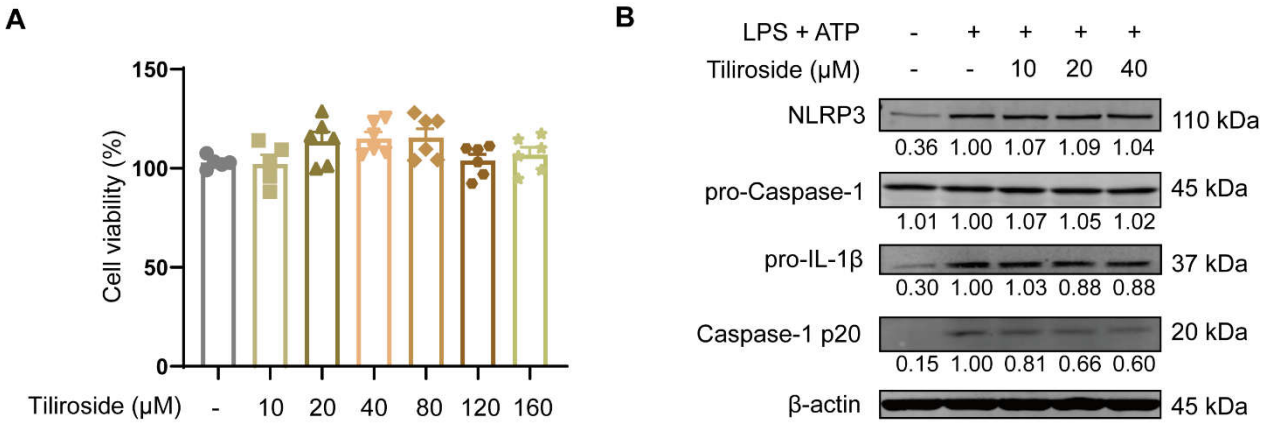

Figure S2. Tiliroside inhibits NLRP3 inflammasome activation in BMDMs. (A) Cell viability was measured by CCK-8 assay using BMDMs treated with different concentrations of tiliroside for 30 h. **(B)** BMDMs were pretreated with tiliroside or a vehicle for 24 h and then stimulated with LPS (100 ng/ml) for 5 h, followed by ATP (5 mM) treatment for 30 min. Protein levels of Caspase-1 p20, NLRP3, pro-Caspase-1, and pro-IL-1β in cell lysates were determined by Western blotting.

Supplementary Figure S3

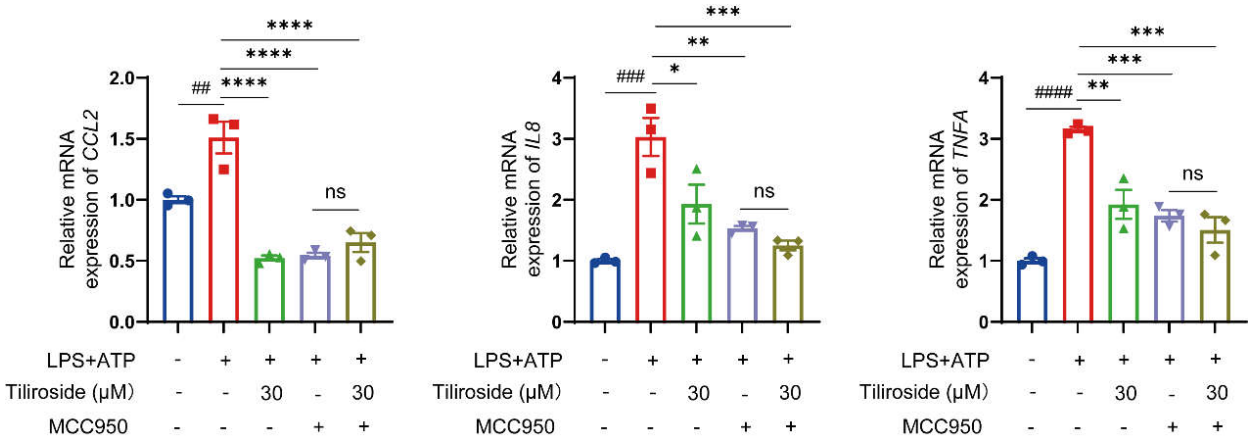

Figure S3. The effect of tiliroside on the expression of pro-inflammatory mediators can be blocked by NLRP3 inhibitor MCC950. THP-1 macrophages were pretreated with tiliroside (30 μM) for 23 h, followed by co-treatment with MCC950 (0.01 μM) for an additional 1 h, and then stimulated with LPS (2 μg/ml) for 5 h, followed by ATP (5 mM) treatment for 45 min. CCL2, IL-8, and TNFA gene expression levels were analyzed by qRT-PCR (n=3). Data are mean ± SEM. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Supplementary Figure S4

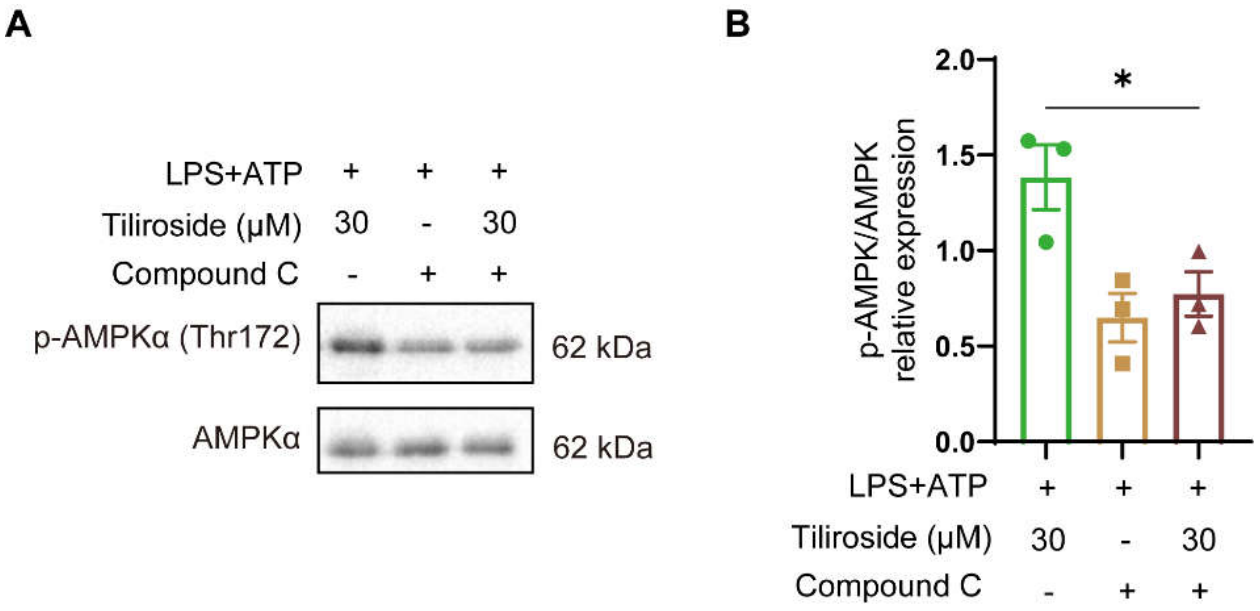

Figure S4. Compound C blocks AMPK activation in THP-1 macrophages. THP-1 macrophages were pretreated with tiliroside (30 μM) for 22 h, followed by co-treatment with Compound C for an additional 2 h, and then stimulated with LPS (2 μg/ml) for 5 h, followed by ATP (5 mM) treatment for 45 min. **(A, B)** Protein levels of p-AMPKα (Thr172) and AMPKα were determined by Western blotting **(A)** and quantification of p-AMPKα (Thr172) normalized to total AMPKα (n=3) **(B)**. Data are mean ± SEM. **P* < 0.05.

Supplementary Figure S5

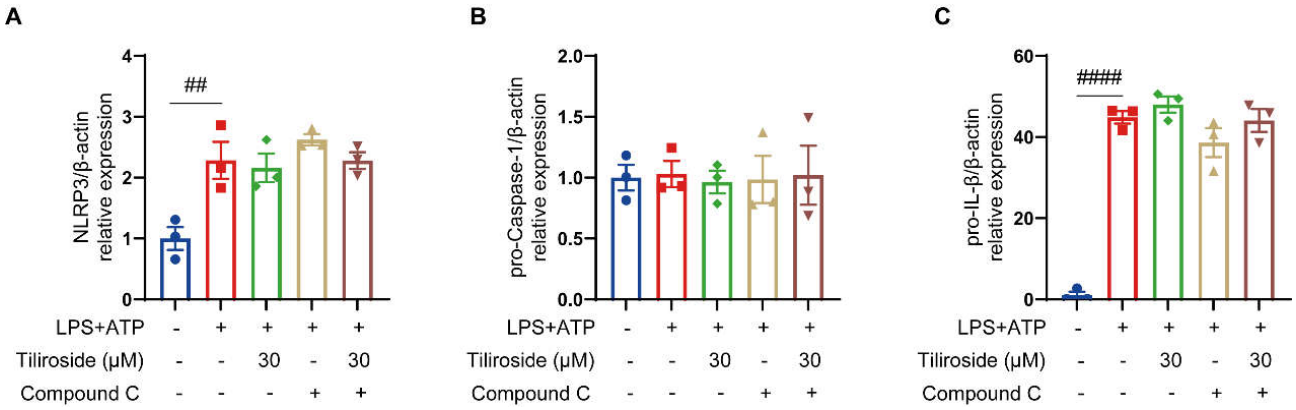

Figure S5. The effect of Compound C on tiliroside-mediated regulation of NLRP3, pro-Caspase-1, and pro-IL-1β in LPS plus ATP-induced THP-1 macrophages. (A-C) THP-1 macrophages were pretreated with tiliroside (30 μM) for 22 h, followed by co-treatment with Compound C for an additional 2 h, and then stimulated with LPS (2 μg/mL) for 5 h, followed by ATP (5 mM) treatment for 45 min. NLRP3 **(A)**, pro-Caspase-1 **(B),** and pro-IL-1β **(C)** were determined by Western blotting, and the relative expression levels were quantified (n=3). Data are mean ± SEM. $^{\#\#}P < 0.01$, $^{\#\#\#\#}P < 0.0001$. These data are related to Figure 3A.