Supporting Information

Small molecule-driven SIRT3-autophagy mediated NLRP3 inflammasome inhibition ameliorates inflammatory crosstalk between macrophages and adipocytes

Running title: A cycloartane triterpenoid alleviates adipose tissue inflammation

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Table S1. Antibodies for Western blotting analysis.

Antibody	Source	Vendor	Catalog No.	RRID No.
Beclin 1	Rabbit	Cell Signaling Technology	#3495	AB_1903911
LC3	Rabbit	Cell Signaling Technology	#12741	AB_2617131
p-AMPKα （Thr172）	Rabbit	Cell Signaling Technology	#50081	AB_2799368
AMPKα	Rabbit	Cell Signaling Technology	# 5831	AB_10622186
p62	Rabbit	Sigma-Aldrich	P0067	AB_1841064
Atg5	Rabbit	Cell Signaling Technology	#12994	AB_2630393
Atg7	Rabbit	Cell Signaling Technology	#8558	AB_10831194
NLRP3	Rabbit	Cell Signaling Technology	#13158	AB_2798134
Caspase-1	Rabbit	Cell Signaling Technology	#3866	AB_2069051
ASC/TMS1	Rabbit	Cell Signaling Technology	# 13833	AB_2798325
IL-1β	Rabbit	Cell Signaling Technology	# 31202	AB_2799001
Cleaved Caspase-1	Rabbit	Cell Signaling Technology	# 67314	AB_2714037
Cleaved-IL-1β	Rabbit	Cell Signaling Technology	# 83186	AB_2800010
ULK1	Rabbit	Cell Signaling Technology	#8054	AB_11178668
p-ULK1 (Ser555)	Rabbit	Cell Signaling Technology	#5869	AB_10707365
SIRT3	Rabbit	Proteintech	10099-1-AP	AB_2239240
SOD2	Rabbit	Cell Signaling Technology	# 13141	AB_2636921
Acetylated-Lysine	Mouse	Cell Signaling Technology	# 9681	AB_331799
Cytochrome C	Rabbit	Cell Signaling Technology	# 11940	AB_2637071
α-Tubulin	Mouse	Cell Signaling Technology	# 12351	AB_2797891
GAPDH	Rabbit	Santa Cruz Biotechnology	sc-25778	AB_10167668
Perilipin-1	Rabbit	Cell Signaling Technology	#9349S	AB_10829911
Integrin αX	Mouse	Santa Cruz Biotechnolog	sc-46676	AB_626859
F4/80	Mouse	Santa Cruz Biotechnolog	sc-71085	AB_1122717
CD206/MRC1	Rabbit	Cell Signaling Technology	#91992	AB_2800175
CD68	Mouse	Santa Cruz Biotechnolog	Sc-20060	AB_627158
Anti-rabbit IgG		Cell Signaling Technology	#7074	AB_2099233
Anti-Mouse IgG		Cell Signaling Technology	#7076	AB_330924

Table S2. List of oligonucleotide primer pairs used in qRT-PCR.

Gene	Forward	Reverse
18S	AGCCTGCGGCTTAATTTGAC	CAACTAAGAACGGCCATGCA
Arginase-1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Ccl5	TGCAGAGGACTCTGAGACAGC	GAGTGGTGTCCGAGCCATA
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
CD206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
CD68	CCTTATGGACAGCTTACCTTTGG	CTGAGCAGCCTGTAGCCTTAGAG
Cxcl10	GCTGCCGTCATTTTCTGC	TCTCACTGGCCCGTCATC
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Ccl11	AGAGCTCCACAGCGCTTCT	GCAGGAAGTTGGGATGGA
Cxcl10	GCTGCCGTCATTTTCTGC	TCTCACTGGCCCGTCATC
MCP-1	CAACTCTCACTGAAGCCAGCTC	TAGCTCTCCAGCCTACTCATTGG
MIP-1α	CTTCTCTGTACCATGACACTCTGC	ATTCAGTTCCAGGTCAGTGATGTAT
IL-1β	TGTTCTTTGAAGTTGACGGACCC	TCATCTCGGAGCCTGTAGTGC

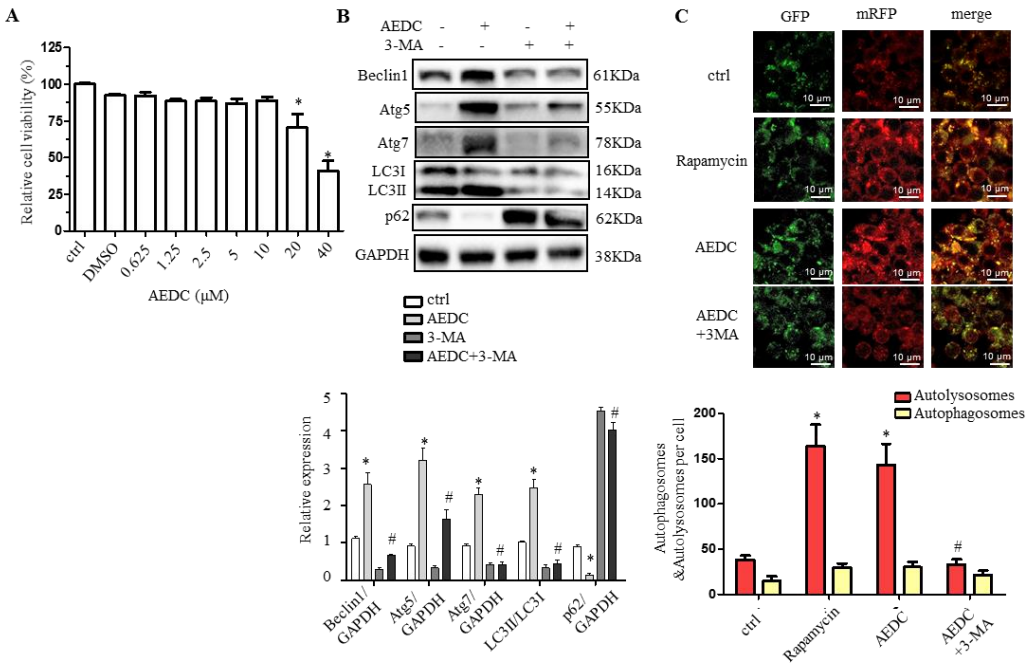

Figure S1. Cytotoxicity and autophagy inducing activity of AEDC on THP-1 cells. (A) THP-1 cells were treated with different concentrations of AEDC for 24 h. Cell viability was detected by MTT assay ($n = 9$). (B) THP-1 cells were treated with or without AEDC (10 μM) for 12 h and 3-MA (5 mM) for 6 h. Expression of autophagy related proteins was detected by Western blotting ($n = 6$). GAPDH was used as an internal loading control. Data were normalized to the mean value of the control group. (E) THP-1 cells were transiently infected with the mRFP-GFP-LC3 lentivirus. mRFP-GFP-LC3 puncta were measured using a confocal microscope ($n = 5$). Scale bar = 10 μm. Data are expressed as means ± SEM. *$P < 0.05$ AEDC vs. ctrl; #$P < 0.05$ AEDC vs. AECD + 3-MA.

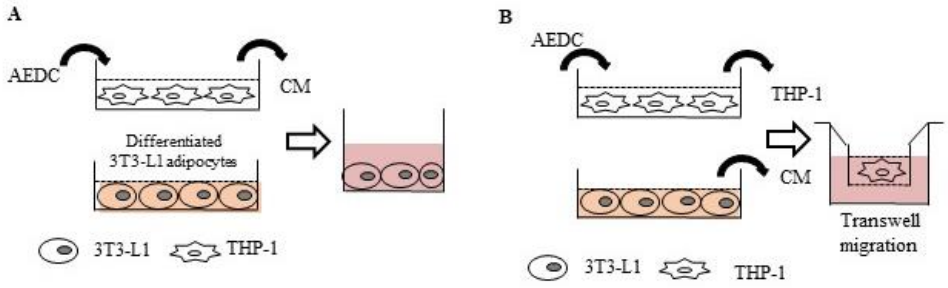

Figure S2. The experimental procedures of the macrophage-adipocyte co-culture experiments (A), and the procedures of the macrophage migration experiments (B).

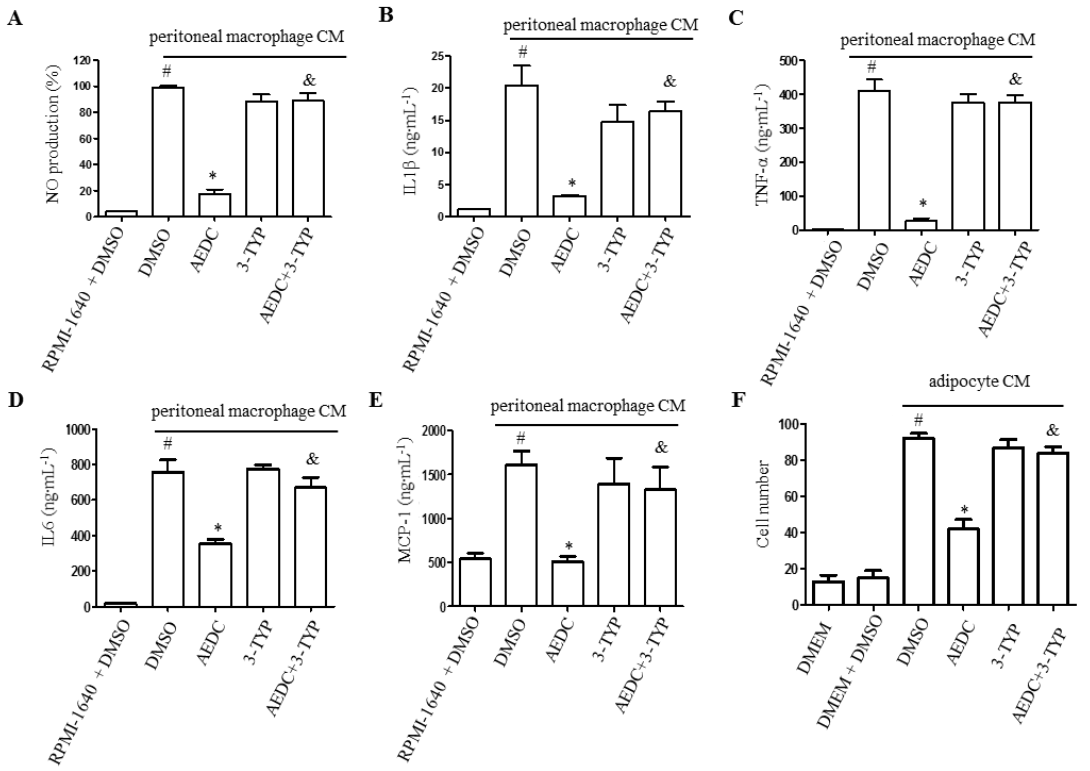

Figure S3. AEDC attenuates the primary peritoneal macrophages-CM induced inflammatory responses in adipocytes. THP-1 cells were treated with or without 10 μM AEDC for 12 h and 50 μM 3-TYP for 6 h. Subsequently, the cells were stimulated with LPS (1 $\mu g \cdot mL^{-1}$) for 4 h and then 1 mM ATP for 1 h. Then, the cells were changed to fresh medium. After 24 h, the medium supernatants were collected as peritoneal macrophage CM. The fully differentiated 3T3-L1 adipocytes were incubated in peritoneal macrophage CM for 24 h. NO production (A) and the levels of IL-β (B), TNF-α (C), IL-6 (D) and MCP-1 (E) in 3T3-L1 adipocytes were determined by ELISA kits. Data are expressed as means ± SEM (n = 6). #P < 0.05, RPMI-1640 + DMSO vs. peritoneal macrophage CM + DMSO; *P < 0.05, peritoneal macrophage CM + AEDC vs. peritoneal macrophage CM + DMSO; &P < 0.05, peritoneal macrophage CM + AEDC vs. peritoneal macrophage CM + AEDC + 3-TYP. (F) The primary peritoneal macrophages were treated with or without 10 μM AEDC for 12 h and 50 μM 3-TYP for 6 h, followed by co-culture with adipocyte CM for 4 h. Migrated primary peritoneal macrophages were visualized by DAPI staining and quantified. Data are expressed as means ± SEM (n = 6). #P < 0.05, DMEM + DMSO vs. adipocyte CM + DMSO; *P < 0.05 adipocyte CM + AEDC vs. adipocyte CM + DMSO; &P < 0.05 adipocyte CM + AEDC vs. adipocyte CM + AEDC + 3-TYP.

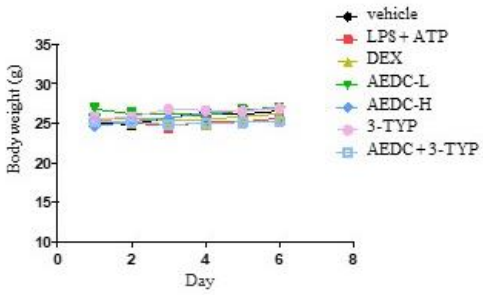

Figure S4. The body weight of different group of LPS plus ATP-induced mice model. DEX: 4 mg·Kg^{-1} dexamethasone; AEDC-L: 5 mg·Kg^{-1} AEDC; AEDC-H: 20 mg·Kg^{-1} AEDC; 3-TYP: 4 mg·Kg^{-1} 3-TYP; AEDC + 3-TYP: 20 mg·Kg^{-1} AEDC and 4 mg·Kg^{-1} 3-TYP.