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Supplemental Information

circARF3 Alleviates Mitophagy-Mediated

Inflammation by Targeting miR-103/TRAF3

in Mouse Adipose Tissue

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Supplemental Information:

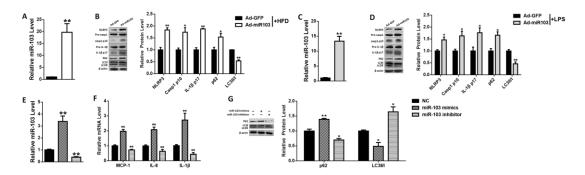


Figure S1. miR-103 promoted adipose inflammation and inhibits mitophagy. (A and B) HFD mice were ip injected with Ad-miR103 or Ad-GFP. (A) The relative miR-103 level in HFD mice adipose tissue. (B) The relative protein levels of NLRP3, Caspase 1, IL-1 β , p62 and LC3 were measured. (C and D) ND mice were ip injected with Ad-miR103/Ad-GFP and LPS. (C) The relative miR-103 level in adipose tissue. (D) Western blot analysis of p62 and LC3 protein levels. (E-F) Primary adipocytes were transfected with miR-103 mimics, miR-103 inhibitor and control. The comparative levels of miR-103 (E), *MCP-1*, *IL-6* and *IL-1\beta* mRNA (F), p62 and LC3 protein (G) were detected. Error bar: SE. Data represented the mean \pm SEM. (*p < 0.05; **p < 0.01. n \geq 3).

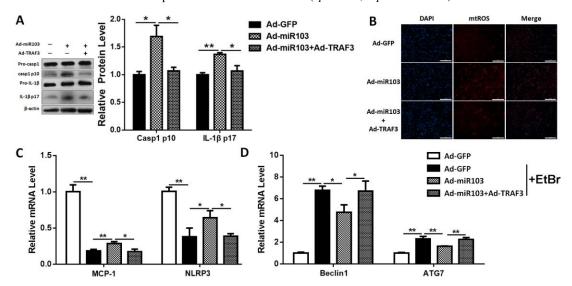


Figure S2. miR-103 promotes inflammation by targeting TRAF3 in adipocytes. (A and B) Adipocytes were treated with Ad-GFP, Ad-miR103 or Ad-miR103+Ad-TRAF3. (A) Western blot analysis of Caspase 1 and IL-1 β protein levels. (B) Relative mtROS amounts were observed after MitoSOX staining. (C and D) Adipocytes were treated with EtBr. QPCR were used to detected the relative mRNA levels of MCP-1, NLRP3, Beclin1 and ATG7. Scale bar: 200µm. Error bar: SE. Data represented the mean \pm SEM. (*P < 0.05; **P < 0.01. n \geq 3).

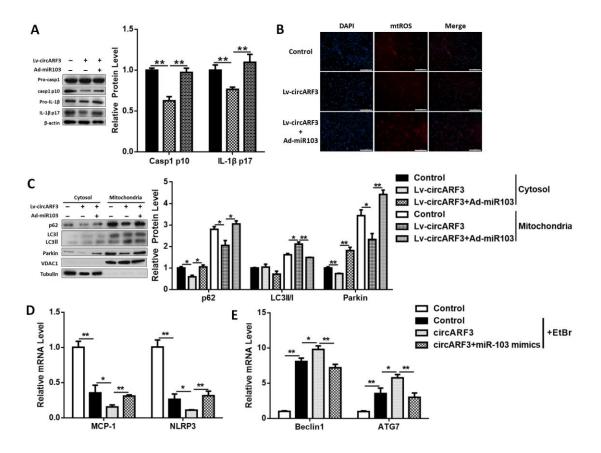


Figure S3. circARF3 alleviates adipose inflammation via activating mitophagy. Adipocytes were treated with circARF3 lentiviruses, circARF3 lentiviruses+miR-103 adenoviruses or control empty viruses. (A) Western blot analysis of Caspase 1 and IL-1 β protein levels. (B) Relative mtROS amounts were observed after MitoSOX staining. (C) Subcellular distribution of p62, LC3, and Parkin in adipocytes. (D and E) Adipocytes were treated with EtBr. QPCR were used to detected the relative mRNA levels of MCP-1, NLRP3, Beclin1 and ATG7. Scale bar: 200µm. Error bar: SE. Data represented the mean \pm SEM. (*P < 0.05; **P < 0.01. n \geq 3).

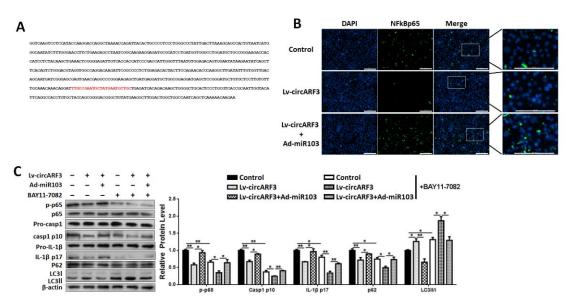


Figure S4. circARF3 alleviates mitophagy mediated inflammation by inhibiting NF- κ B signaling. (A) The sequence of circARF3, and the binding sits with miR-103 were presented in red. (B and C) Adipocytes were treated with circARF3 lentiviruses, circARF3 lentiviruses+miR-103 adenoviruses or control empty viruses. (B) Intracellular distribution of NF- κ Bp65. (C) Immunoblot analysis of p65, Caspase 1, IL-1 β , p62 and LC3 protein levels are shown with or without BAY11-7082 treatment. Scale bar: 200µm. Error bar: SE. Data represented the mean \pm SEM. (*p < 0.05; **p < 0.01. n \geq 3).