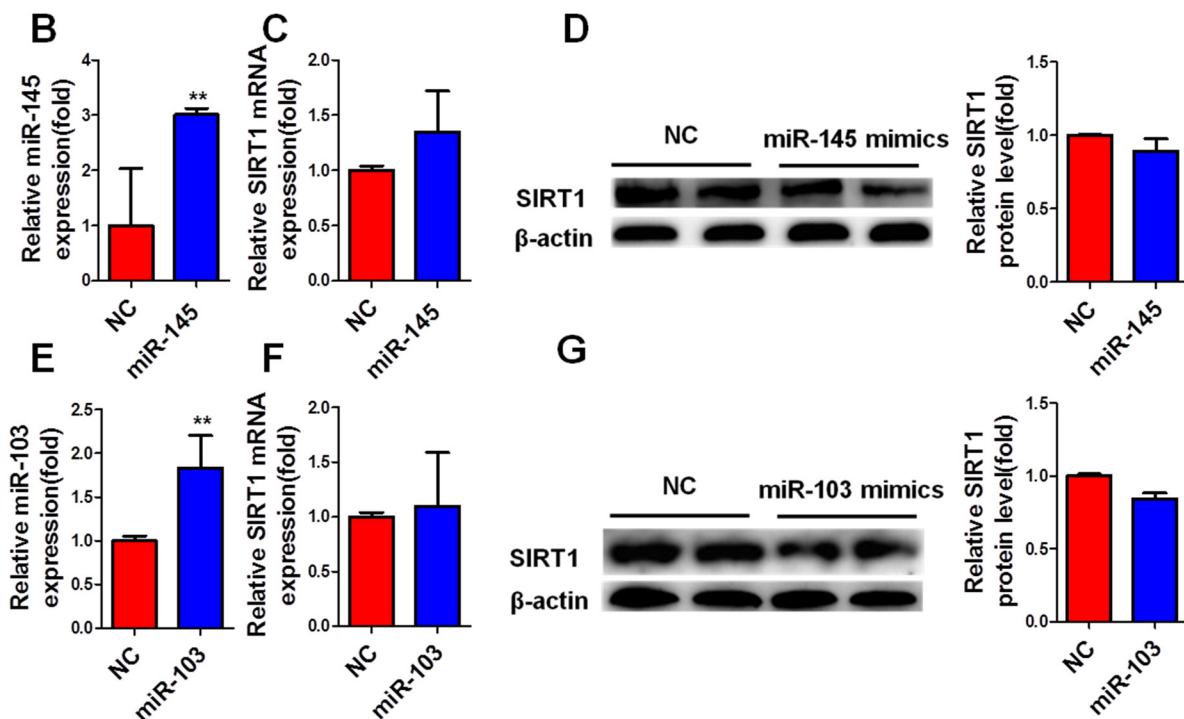
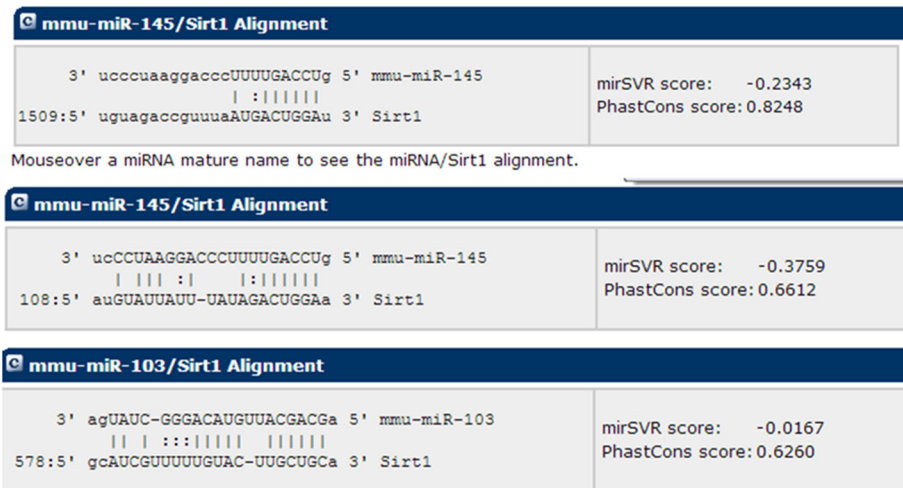


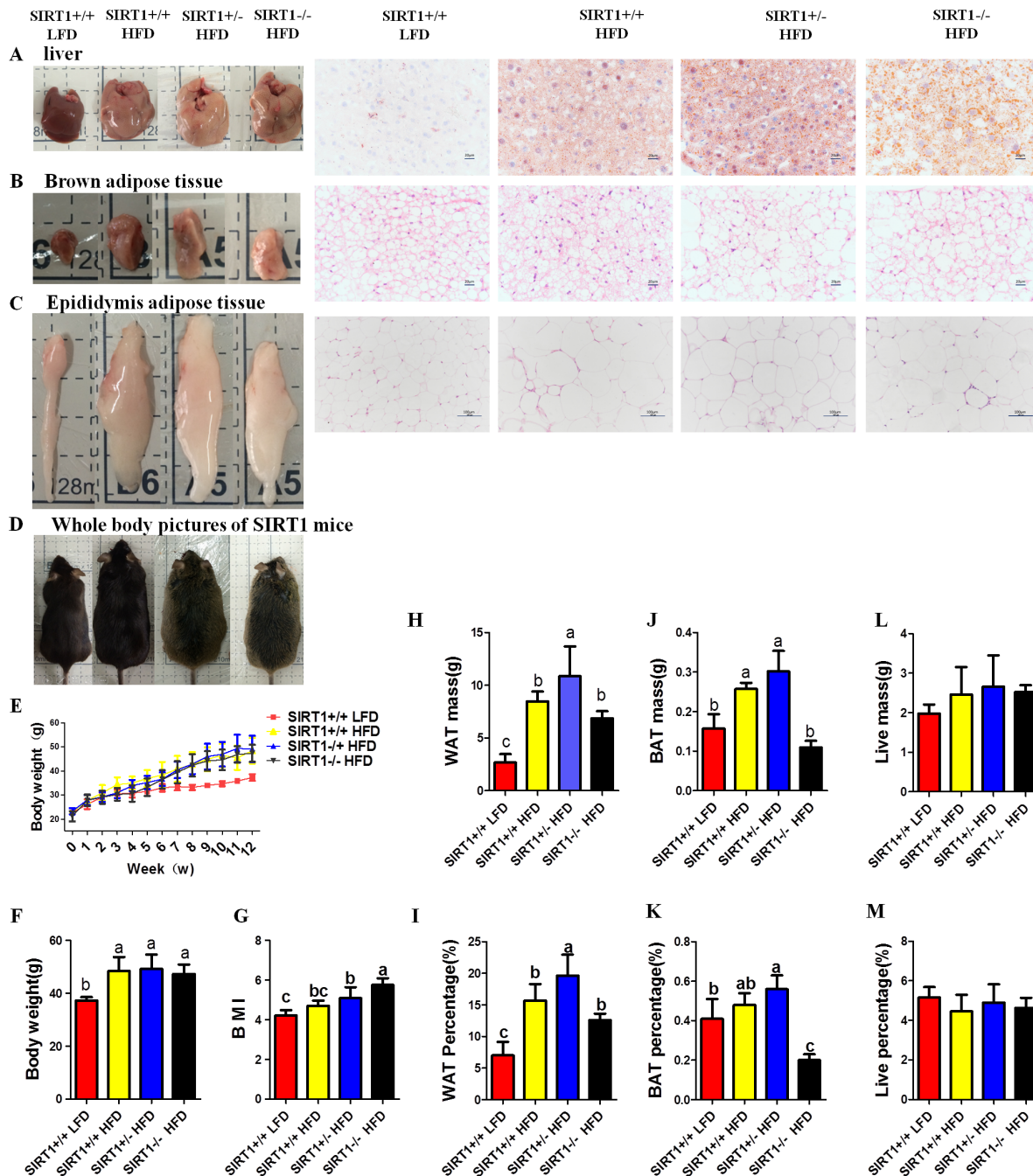
# MiR-377 promotes white adipose tissue inflammation and decreases insulin sensitivity in obesity via suppression of sirtuin-1 (SIRT1)

## SUPPLEMENTARY MATERIALS

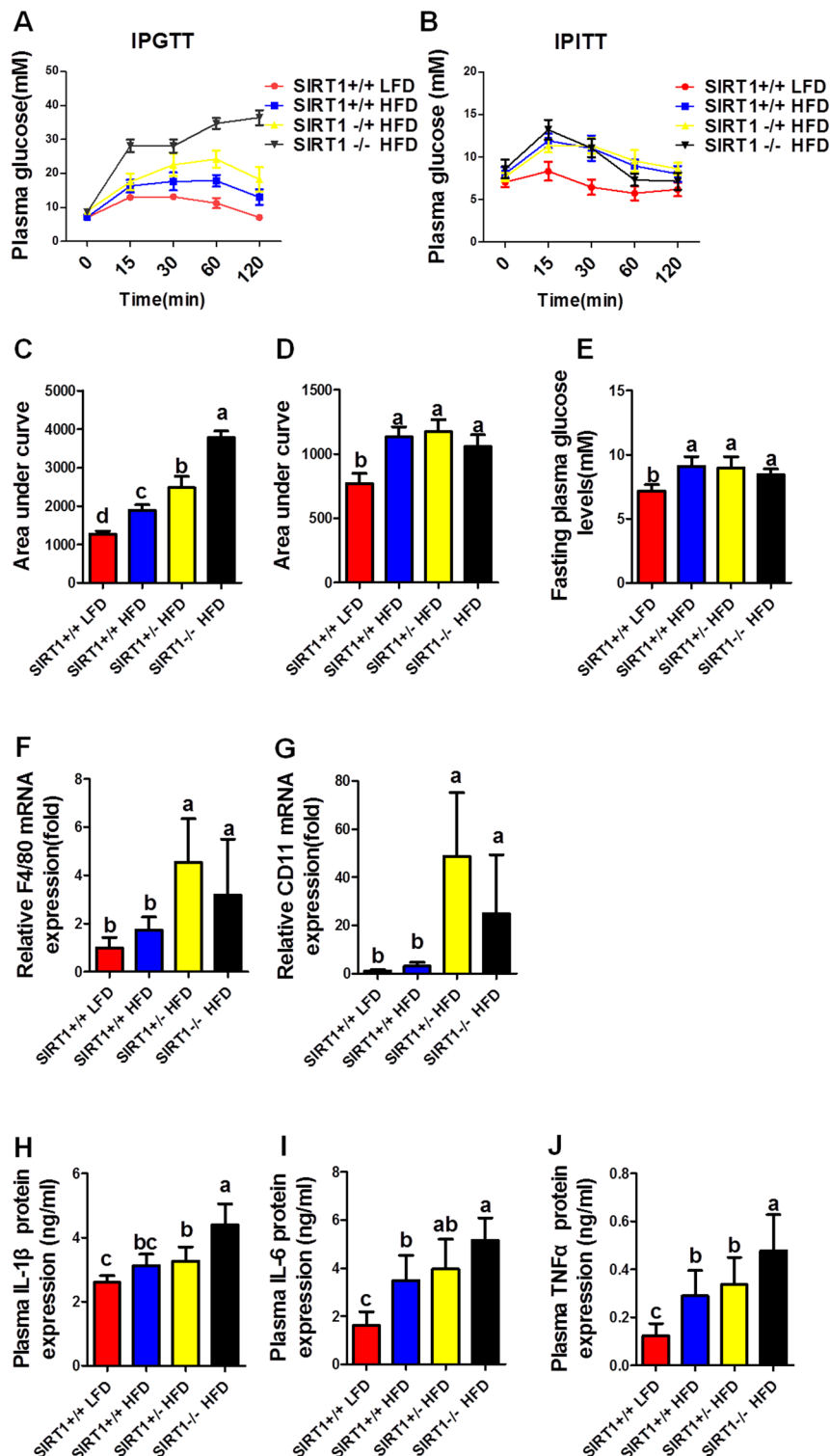
A



**Supplementary Figure 1: Overexpression of miR-145 and miR-103 did not inhibit the translation of SIRT1 in differentiated 3T3-L1 cells.** (A) The potential target sites were predicted by MICRORNA. (B–G) Mature 3T3-L1 cells were transfected with 100 nM miR-145 or miR-103 mimics, respectively, for 24 h and then harvested for real-time PCR and immunoblotting analyses. (B) MiR-145 expression. (C) SIRT1 mRNA levels. (D) SIRT1 protein levels. (E) MiR-103 expression. (F) SIRT1 mRNA levels. (G) SIRT1 protein levels.  $**P < 0.01$  vs. NC (n=3).



**Supplementary Figure 2: SIRT1-deficiency affects adipogenesis and results in hepatic steatosis in HFD-fed mice.** Mice with different genotypes received a LFD or a HFD for 12 weeks. **(A)** Livers and oil red-O staining of liver tissue; scale bar, 20  $\mu$ m (n=3). **(B)** Brown adipose tissue (BAT) and histological (hematoxylin and eosin) staining of sections; scale bar, 20  $\mu$ m (n=3). **(C)** Epididymal adipose tissue and histological (hematoxylin and eosin) staining of sections; scale bar, 100  $\mu$ m (n=3). **(D)** Whole body images of SIRT1 mice. **(E)** Body weight during 12 weeks of HFD (n=6-8). **(F)** Body weight at week 12 (n=6-8). **(G)** Body mass index (BMI) (n=6-8). **(H and I)** White adipose tissue mass and percentage (n=6-8). **(J and K)** BAT mass and percentage. **(L and M)** Liver mass and percentage (n=6-8). Different letters denote significant differences, P < 0.05.



**Supplementary Figure 3: SIRT1-deficiency intensifies adipose tissue inflammation and insulin-resistance in HFD-fed mice.** Mice with different genotypes received a LFD or a HFD for 12 weeks prior to IPGTT or IPITT evaluation (n = 6–8). (A) IPGTT, 2 g/kg glucose. (B) IPITT, 0.6 IU/kg insulin. (C) Area under the curve from IPGTT. (D) Area under the curve from IPITT. (E) Fasting plasma glucose. (F and G) mRNA levels of macrophage specific antigen F4/80 and CD11 from epididymal adipose tissue. (H–J) Serum levels of inflammatory factors IL-1β and IL-6 and TNFα. Different letters denote significant differences, P < 0.05.

Supplementary Table 1: The primer used in this study

Gene	Forward Primer	Reverse Primer
F4/80	5' AGGGCAGGGATCTTGTTATG 3'	5' AGCTGCACTCTGTAAGGACAC 3'
Sirt1	5' CCTGACTTCAGATCAAGAGACGGTA 3'	5' CTGATTAAAAATGTCTCCACGAACAG 3'
CD11	5' TGGATGCAGAGAAGCTGACAC 3'	5' CAACCACCACCCAGGAAC TAT 3'
$\beta$ -actin	5' GGCACCACACCTTCTACAATG 3'	5' GGGGTGTTGAAGGTCTCAAAC 3'
TNF $\alpha$	5' CGGAGTCCGGGCAGGTCTACTTT 3'	5' GTCCAGGTCACTGTCCCAGCATC 3'
IL-1 $\beta$	5' CAAGCAATACCCAAAGAAGAAGA 3'	5' ATTAGAAACAGTCCAGCCCATAC 3'
IL-6	5' CCAGAGATACAAAGAAATGATGG 3'	5' ACTCCAGAAGACCAGAGGAAAT 3'
MCP-1	5' AGCACCAGCCAACCTCTCAC 3'	5' TCTGGACCCATTCCTTCTTG 3'
miR-377-loop	5' CTCAACTGGTGTCTGGAGTCGGCAAT TCAGTTGAGACAAAAGT 3'	
miR-377	5' GTCGTGGAGTCGGCAATT 3'	5' GGCATCACACAAAGGCAAC 3'
miR-145-loop	5' CTCAACTGGTGTCTGGAGTCGGCAATT CAGTTGAGAGGGATTTC 3'	
miR-145	5' CTGGTAGGGTCCAGTTTTTC 3'	5' TCAACTGGTGTCTGGAG 3'
miR-103-loop	5' CTCAACTGGTGTCTGGAGTCGGCA ATTCAGTTGAGTCATAGCCC 3'	
miR-103	5' CTGGTAGGAGCAGCATTGTA 3'	5' TCAACTGGTGTCTGGAG 3'
u6-loop	5' CTCAACTGGTGTCTGGAGTCGGCAATTCA GTTGAGAAAAATATGGAACGCT 3'	
u6	5' CTGGTAGGGTGCTCGCTTCGGCAG 3'	5' CAACTGGTGTCTGGAGTCGGC 3'