

Data supplement

Neuraminidase 1 deficiency attenuates cardiac dysfunction, oxidative stress, fibrosis, inflammatory via AMPK-Sirt3 pathway in diabetic cardiomyopathy mice

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Supplementary Table 1: The information of the primary antibodies used in western blot

Antibody description	Company	No. and dilution
NEU1	Abcam	#ab233119; 1:1000
α SMA	Abcam	#ab5694; 1:1000
Collagen I	Abcam	#ab34710; 1:1000
β -actin	Abcam	#ab8227, 1:1000
P-P65	Bio-world	#BS4135, 1:1000
T-P65	Cell Signaling Technology	#8242, 1:1000
P-I κ B α	Abcam	#ab133462, 1:1000
T-I κ B α	Abcam	#ab7217, 1:1000
Bax	Cell Signaling Technology	#2722, 1:1000
Bcl-2	Cell Signaling Technology	# 2870, 1:1000
c-caspase3	Cell Signaling Technology	#9661, 1:1000
P67 phox	Abcam	#ab109366, 1:1000
SOD2	Abcam	#ab13533, 1:1000
SIRT3	Abcam	#ab189860, 1:1000
P-AMPK α	Cell Signaling Technology	#2535, 1:1000
T-AMPK α	Cell Signaling Technology	# 2603P, 1:1000

Supplementary Table 2: The information of the primers used in RT-PCR.

Target	Forward Sequences (5'→3')	Reverse Sequences (5'→3')
Mice-GAPDH	TCATCAACGGGAAGCCCATC	CTCGTGGTTCACACCCATCA
Mice-TGFβ1	ATCCTGTCCAAACTAAGGCTCG	ACCTCTTTAGCATAGTAGTCCGC
Mice-CTGF	CTCGTGGTTCACACCCATCA	AGTTGGCTCGCATCATAGTTG
Mice-Col1	CCCAACCCAGAGATCCCATT	GAAGCACAGGAGCAGGTGTAGA
Mice-Col3	GATCAGGCCAGTGGAATGT	GTGTGTTTCGTGCAACCATC
Mice-fibronectin	CCGGTGGCTGTCAGTCAGA	CCGTTCCCACTGCTGATTTATC
Mice-IL6	GTCAGCTGGATAGCGACA	GAAGCACAGGAGCAGGTGTAGA
Mice-TNFα	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT
Mice-MCP1	TGGCTCAGCCAGATGCAGT	CCAGCCTACTCATTGGGATCA
Mice-p67phox	GCCGGAGACGCCAGAAGAGCTA	GGGGCTGCGACTGAGGGTGAA
Mice-gp91phox	TTCCAGTGCGTGTTGCTCGACA	TGGCGGTGTGCAGTGTATCAT
Mice-SIRT3	GGTCCGGGAGTGTTACAGG	CACCATGACCACCACCCTAC
Mice-Bax	CTGGATCCAAGACCAGGGTG	CCTTTCCCCTTCCCCCATTC
Mice-BCL2	CTGAGTACCTGAACCGGCAT	GGTATGCACCCAGAGTGATG
Rat-GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT
Rat-p67phox	TTCCATCCCCAAATGCAAAG	TCAGATGCCCTAAAACCGGAG
Rat-gp91phox	GACCATTGCAAGTGAACACCC	AAATGAAGTGGACTCCACGCG
Rat-IL6	GTTGCCTTCTTGGGACTGATG	ATACTGGTCTGTTGTGGGTGGT
Rat-TNFα	AGCATGATCCGAGATGTGGAA	TAGACAGAAGAGCGTGGTGCC
Rat-MCP1	AGTCGGCTGGAGAACTACAAGA	CTGAAGTCCTTAGGGTTGATGC

Figure S1. NEU1 expression after administrated with AAV9-shRNA or AAV9-shNEU1

A. mRNA levels of NEU1 after administrated with AAV9-shRNA or AAV9-shNEU1 in NRVMs (n=6). B. NEU1 protein expression and quantitative data (n=6).

Figure S2. NEU1 inhibition did not affect blood glucose level and body weight in diabetic mice

A. Blood glucose of mice at duration of diabetes (n=6). B. Pancreas insulin of mice at duration of diabetes (n=6). C. Glycosylated hemoglobin of mice at duration of diabetes (n=6). D. Body weight at the initial and end of study (n=6). E. Representative image of echocardiography. NS indicates not significant.

Figure S3. ShNEU1 exerted its cardiac protection via activating Sirt3 in vitro

A. mRNA levels of SIRT3 in vehicle and shNEU1 administered mice (n =6). * $P < 0.05$ vs. saline+vehicle group; # $P < 0.05$ vs. stz+vehicle group. B. Western blot for sirt3 and the quantitative data for NRVMs (n= 6). C. The relative mRNA levels of p67*phox* and p22*phox* normalized to GAPDH under HG stimulation in NRVMs (n = 6). D. The relative mRNA levels of BAX and BCL-2 normalized to GAPDH under HG stimulation in NRVMs (n = 6). E. Cell death was detected by an CCK8 assay under H₂O₂ stimulation in NRVMs (n = 6). F. The content of MDA and SOD activity under HG stimulation in NRVMs (n=6). * $P < 0.05$ vs. the matched group; NS indicates not significant.

Figure S4. Knockdown of AMPK α blunted the protective effects of shNEU1 in vitro.

A. Western blot for AMPK α and the quantitative data in NRVMs (n= 6). B-C. The ROS level and SOD activity under HG stimulation in NRVMs (n=6). D-E. The relative mRNA levels of IL-6 and TNF- α normalized to GAPDH under HG stimulation in NRVMs (n = 6). F. Cell death was detected by an CCK8 assay under HG stimulation in NRVMs (n = 6). * $P < 0.05$ vs. the matched group; NS indicates not significant.

Figure S1.

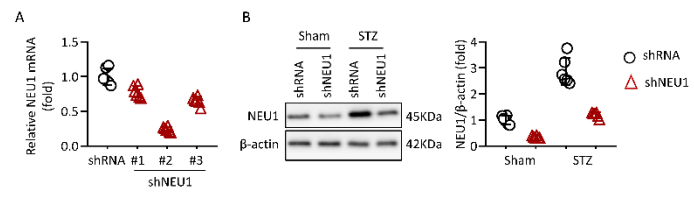


Figure S2.

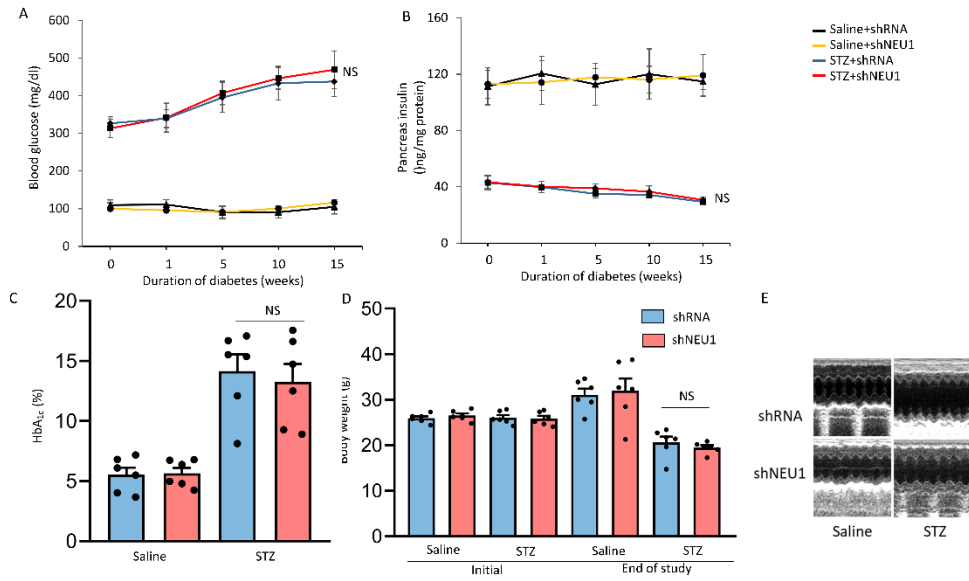


Figure S3.

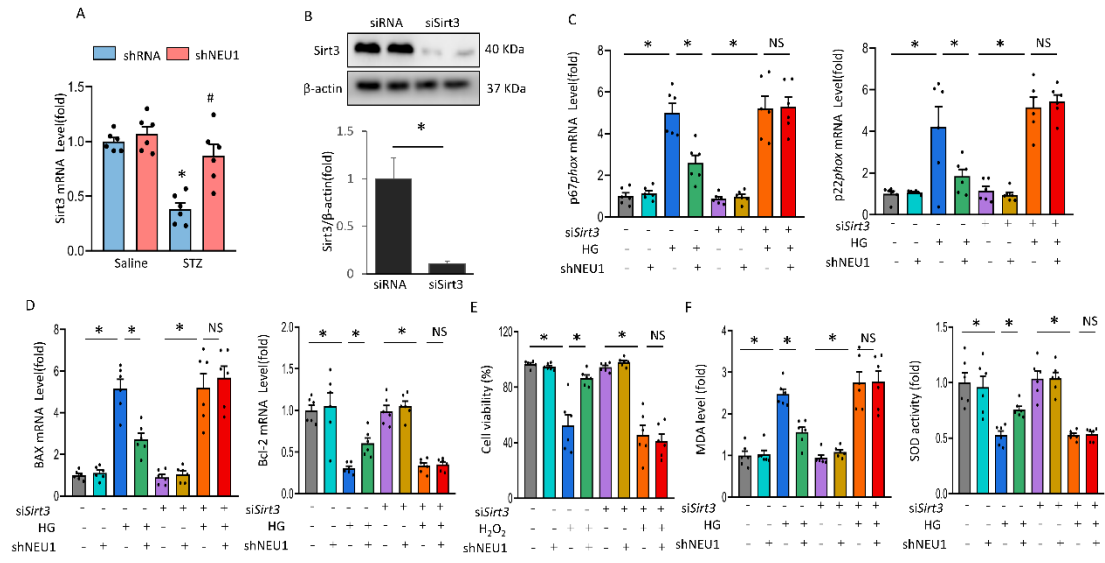


Figure S4.

