**Supplementary material**

**SIRT6 suppresses NFATc4 expression and activation in cardiomyocyte hypertrophy**

**Running title:** SIRT6 suppresses NFATc4 in cardiomyocyte hypertrophy

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**Table S1 Primer sequences for qRT-PCR**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Sequences** | **Species** |
| **SIRT6** | Forward:5'-GCCGTCTGGTCATTGTCA-3'Reverse:5'-AGCCTTGGGTGCTACTGG-3' | Rattus norvegicus |
| **BNP** | Forward:5'-GGAAGTCAACCCGTCTCA-3'Reverse:5'-AGCCCTCAGTTTGCTTTT-3' | Rattus norvegicus |
| **NFATc4** | Forward:5'-TCTTAACCTGGGTGCTGGCT-3'Reverse:5'-CACACCCTCAGTCCCTTTCAA-3' | Rattus norvegicus |
| **β-actin** | Forward:5'-TCGTGCGTGACATTAAAGAG-3'Reverse:5'-ATTGCCGATAGTGATGACCT-3' | Rattus norvegicus |

**Abbreviations:** SIRT6, Sirtuin 6; BNP, brain natriuretic polypeptide; NFATc4, nuclear factor of activated T cells c4

**Table S2 Small interference RNAs**

|  |  |
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| **Name** | **Primer Sequences** |
| **SIRT6** | Forward:5'-GCCGUCUGGUCAUUGUCAATT-3'Reverse:5'-UUGACAAUGACCAGACGGCTT-3' |
| **NFATc4** | Forward:5'-GGAGUCUGAACUUAAUGAATT-3'Reverse:5'-UUCAUUAAGUUCAGACUCCTT-3' |

**Table S3 Echocardiographic parameters from all groups of rats**

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Sham****(*n* = 6)** | **AAC****(*n* = 6)** |
| **LVAWd (mm)** | 1.48 ± 0.05 | 2.52 ± 0.29\*\* |
| **LVAWs (mm)** | 2.12 ± 0.10 | 3.84 ± 0.13\*\*\* |
| **LVIDd (mm)** | 7.47 ± 0.22 | 5.73 ± 0.56\* |
| **LVIDs (mm)** | 5.02 ± 0.19 | 2.80 ± 0.19\*\*\* |
| **LVPWd (mm)** | 1.73 ± 0.05 | 2.57 ± 0.20\* |
| **LVPWs (mm)** | 2.52 ± 0.09 | 3.49 ± 0.22\*\* |
| **EF (%)** | 59.56 ± 1.75 | 80.46 ± 2.28\*\*\* |
| **FS (%)** | 32.84 ± 1.24 | 50.12 ± 2.63\*\*\* |
| **CO (mL)** | 59.13 ± 4.11 | 48.46 ± 8.22 |
| **SV (mL)** | 176.8 ± 10.72 | 142.1 ± 30.34 |
| **HR (bpm)** | 335.8 ± 17.42 | 379.2 ± 44.8 |

**Abbreviations:** AAC, abdominal aortic constriction; LVAW, left ventricular anterior wall thickness; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall thickness; -d, diastolic; -s, systolic; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; SV, stroke volume; HR, heart rate. All values are presented as means ± SEM. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 *vs.* normal saline (NS) group.



**Fig. S1. Change of p38 expression in SIRT6 overexpression.** NRCMs were transiently transfected with plasmid encoding the wildtype SIRT6 (WT-SIRT6) and mutant of SIRT6 (H133Y) for 48h. Western blot analysis was conducted to determine the protein expression of p38. Data were normalized by α-tubulin for protein expression and presented as mean ± SEM, *n* = 4.

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**Fig. S2. Changes of miR-29a and miR-133a expression in SIRT6 overexpression.** NRCMs were transiently transfected with plasmid encoding the wildtype SIRT6 (WT-SIRT6) for 48h and the expression of miR-29a and miR-133a were determined by qRT-PCR. The data were presented as mean ± SEM. \*\**P* < 0.01 *vs.* Vector. *n* = 4.

A



B



**Fig. S3. The transfection efficiency of cardiomyocytes. (A)** Cells were transfected with GFP-SIRT1 plasmid for 48 h using Lipofectamine 2000. The image was captured by fluorescence microscope. **(B)** Cells were transfected with WT-SIRT6 and WT-NFATc4 plasmids for 48 h using Lipofectamine 2000. The protein expression of NFATc4 and SIRT6 were measured by Western blotting. The data were presented as mean ± SEM. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 *vs.* Vector. *n* = 4.