Title: Upregulation of α-ENaC induces pancreatic β-cell dysfunction, ER stress, and SIRT2 degradation

Running title: Excessive α-ENaC promotes β-cell dysfunction

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The authors reported no conflict of interests.
Supplementary Data

Supplementary Table 1. Characteristics of study participants

<table>
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<th>Case</th>
<th>Age(years)</th>
<th>Gender</th>
<th>Glucose (mmol/L)</th>
<th>Blood pressure (mmHg)</th>
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<td>F</td>
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<td>F</td>
<td>5.1</td>
<td>123/85</td>
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<td>F</td>
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<td>130/75</td>
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<tr>
<td>4</td>
<td>51</td>
<td>M</td>
<td>5.3</td>
<td>137/65</td>
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<td>68</td>
<td>M</td>
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Supplementary figures and legends

Supplementary Fig. 1 Expression of α-ENaC in islet β-cells. A: Immunofluorescence staining of glucagon and α-ENaC in the human pancreas tissues (n = 5). B: Quantitative analysis of α-ENaC (green) colocalizing with β-cells (insulin) in Fig. 1B and α cells (glucagon) in panel A. C: Immunofluorescence staining of glucagon and α-ENaC in the pancreatic tissues of control and db/db mice. D: Fasting blood glucose levels of db/db mice and control mice (n = 6). E: Fasting blood glucose levels of HFD mice and NCD mice (n = 3). Data are presented as means ± standard error of the mean, *P < 0.05 and ***P < 0.01 (two-tailed Student's t-test). Scale bar: 100 μm. Abbreviations: NCD, normal chow diet; HFD, high-fat diet.

Supplementary Fig. 2 Detection of apoptosis in Min6 cells treated by PA. A: TUNEL staining in Min6 cells treated with PA. B: TUNEL-positive cells in Min6 cells treated with PA. C: Annexin-V/PI double fluorescence staining and flow cytometry analysis in Min6 cells treated with PA. D: Western blotting assay of α-ENaC in Min6 cells treated with glucose for 24 h. E: Quantitation of the Western blotting data in panel D. Data are presented as means ± standard error of the mean (n
Supplementary Fig. 3 Knockout or inhibition of α-ENaC ameliorated PA-induced cell damage. A: The cell viability analysis in WT and α-ENaC knockout (Scnn1a−/−) Min6 cells treated with or without PA. *P < 0.05, **P < 0.01. B: Annexin-V/PI double fluorescence staining and flow cytometry analysis in WT and Scnn1a−/− Min6 cells treated with or without 0.25 mmol/L PA. C: Western blotting assay of cleaved caspase-3 and caspase-3 in Min6 cells treated with amiloride and PA. D: Quantitation of the Western blotting data in panel C. Data are presented as means ± standard error of the mean (n = 3), *P < 0.05, **P < 0.01 (two-tailed Student's t-test). Abbreviations: WT: wild type; Amil: amiloride.

Supplementary Fig. 4 α-ENaC over-expression further injured Min6 cells under the PA condition. A: Western blotting assay of cleaved caspase-3 and caspase-3 in α-ENaC overexpressed Min6 cells treated with PA. B: Quantitation of the Western blotting data in panel A. C: TUNEL staining in α-ENaC overexpressed Min6 cells treated with PA. D: TUNEL positive cells in α-ENaC overexpressed Min6 cells treated with PA. Data are presented as means ± standard error of the mean (n = 3). Scale bar: 50 μm, *P < 0.05, **P < 0.01 (two-tailed Student's t-test).

Supplementary Fig. 5 SIRT2 over-expression attenuated the upregulation of both XBP1s and CHOP induced by PA in Min6 cells. A: Co-immunoprecipitation of BIP and α-ENaC in Min6 cells. B: Quantitation of the α-ENaC expression at time 0 point in Fig. 5D. C: Western blotting assay of XBP1s and CHOP in SIRT2 overexpressed Min6 cells treated with PA. D: Quantitation of the Western blotting data in panel C. Data are presented as mean ± standard error of the mean (n = 3). *P < 0.05, **P < 0.01 (two-tailed Student's t-test).
Figure S3

A

Cell Viability (%)

Time (h) 0 12 18 24 36 48

WT  Scnn1a-/-  WT + PA  Scnn1a-/- + PA

# #

B

DAPI

Annexin-V

WT  Scnn1a-/-  WT + PA  Scnn1a-/- + PA

C

Amil (μM) 0 10 3 1 0 10 3 1
0.5mM PA - - - + + + +
Cleaved Caspase-3

17KD

Caspase-3

35KD

β-actin

43KD

D

Cleaved Caspase-3
(Caspase-3 ratio)

Amil (μM) 0 10 3 1 0 10 3 1
0.5mM PA - - - + + + +
Figure S5

A

<table>
<thead>
<tr>
<th>IP</th>
<th>α-ENaC IgG</th>
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<tr>
<td>Input</td>
<td>α-ENaC</td>
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<tr>
<td></td>
<td>BIP</td>
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α-ENaC 95KD
BIP 78KD

B

Relative α-ENaC Protein Level

pcDNA3.1 pcDNA3.1-α-ENaC

C

pcDNA3.1-SIRT2 pcDNA3.1 PA SIRT2 CHOP XBP1s β-actin

- - + +
+ + - -
- + - +
43KD 27KD 59KD 43KD

D

Relative Protein Level

SIRT2 CHOP XBP1s

pcDNA3.1 pcDNA3.1-SIRT2 pcDNA3.1-PA pcDNA3.1-SIRT2-PA

*** ** *