SGLT2 inhibition attenuates the dysregulation of Kelch-like 3 and Na-Cl cotransporter in obese diabetic mice

Supplemental Information

Kenichi Ishizawa[1)], Qin Wang[1), 2)], Jinping Li[1)], Ning Xu[1)], Yoshikazu Nemoto[1)], Chikayuki Morimoto[1)], Wataru Fujii[1)], Yoshifuru Tamura[1)], Yoshihide Fujigaki[1)], Kazuhisa Tsukamoto[1)], Toshiro Fujita[3)], Shunya Uchida[1)], Shigeru Shibata[1), 3)]

[1)] Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan. [2)]Department of Nephrology, the Second Affiliated Hospital of Harbin Medical University, Harbin, China. [3)]Division of Clinical Epigenetics, Research Center for Advanced Science and Technology, The University of Tokyo, Japan.

Supplemental Figure Legends

Supplemental Figure 1
Characterization of monoclonal KLHL3^{S433-P} antibody.
(Left panels) Cell lysates from HEK cells expressing no KLHL3, KLHL3^{WT}, or KLHL3^{S433N} were analyzed by Western blotting. Serine mutation at position 433 in KLHL3 completely eliminates the signal in HEK cells expressing KLHL3, confirming the specificity of the antibody. (Right panel) Total kidney lysates from indicated animals were analyzed using KLHL3^{S433-P} antibody. HEK cells expressing KLHL3^{WT} served as a positive control. Arrows indicate KLHL3^{S433-P}.

Supplemental Figure 2
KLHL3 antibody does not recognize KLHL2.
(A) Comparison of hKLHL3 and hKLHL2 protein sequence. Shown above is the immunogen sequence used to produce KLHL3 antibody (corresponding to a.a. 2 to 51 of hKLHL3).
(B) Mouse kidney tissues were separated into cortex and medulla, and the lysates were analyzed by Western blotting using the indicated antibodies. The KLHL3 antibody recognized a strong signal in cortex (where KLHL3 is abundant) but not in medulla (where KLHL2 is predominantly present).

Supplemental Figure 3
Immunofluorescence study using anti-NCC^{T53-P} and KLHL3^{S433-P} antibodies in the kidney of db/db mice.
(A) High power images of kidney sections stained with anti-NCC^{T53-P} antibody in the indicated animals. In db/db mice, signal is increased at the apical membrane in DCT cells. (B) Low power image of kidney sections stained with anti-KLHL3^{S433-P} antibody. The phosphorylation signal is highly detected in the renal cortex in db/db mice, consistent with the phosphorylation of KLHL3^{S433} (arrows). A modest signal was occasionally seen in the renal medulla (arrowheads). (C) High power images of kidney sections stained with anti-KLHL3^{S433-P} in the indicated animals. A part of the image also appeared in Fig.1C. Arrows indicate KLHL3^{S433-P} signal.

Supplemental Figure 4
Effect of insulin on NCC levels in db/db mice.

(A) Blood glucose levels in the indicated group. (B) Effects of insulin (Ins) on NCC levels in the plasma membrane-enriched fraction of the kidneys in db/db mice. Blots show biological replicates. Dot-plot graphs show the results of densitometric quantitation. Data are expressed as means ± SEM.

Supplemental Figure 5

Evaluation of plasma renin activity in db/+ and db/db mice.

Plasma renin activity was measured in the indicated animals. Data are expressed as means ± SEM.

Supplemental Figure 6

Role of angiotensin II signaling in the kidney in the regulation of NCC in db/db mice.

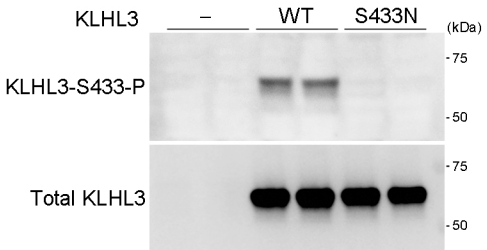
(A) Renal angiotensinogen mRNA expression (*Agt*) was evaluated in the indicate animals. (B) Effects of candesartan (ARB) on NCC levels in the plasma membrane-enriched fraction of the kidneys in db/db mice. Blots show biological replicates. Dot-plot graphs show the results of densitometric quantitation. Data are expressed as means ± SEM.

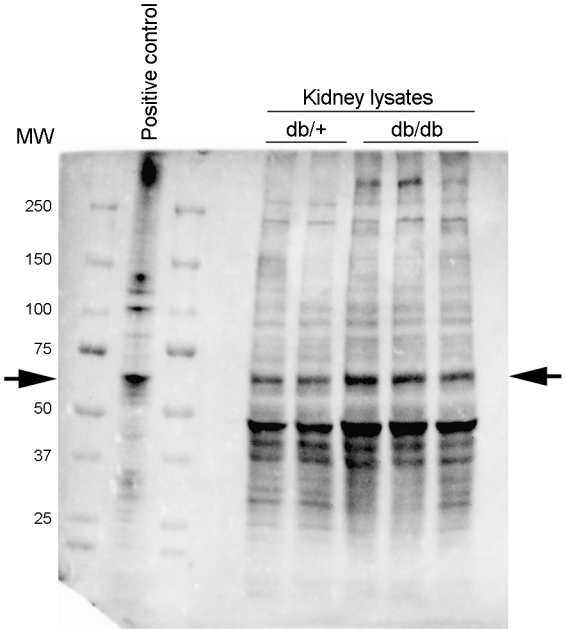
Supplemental Figure 7

Effects of bisindolylmaleimide I (BIM) in db/+ mice.

(A) Effects of BIM on NCC levels in the plasma membrane-enriched fraction of the kidneys in db/+ mice. Blots show biological replicates. Dot-plot graphs show the results of densitometric quantitation. (B) Effects of BIM on urinary Na^+ levels in the db/+ mice. Data are expressed as means ± SEM.

Supplemental Figure 1





Supplemental Figure 2

A

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Immunogen EGESVKLSSQTLIQAGDDEKNQRTITVNPAHMGKAFKVMNELRSKQLLCD
   hKLHL3 EGESVKLSSQTLIQAGDDEKNQRTITVNPAHMGKAFKVMNELRSKQLLCD
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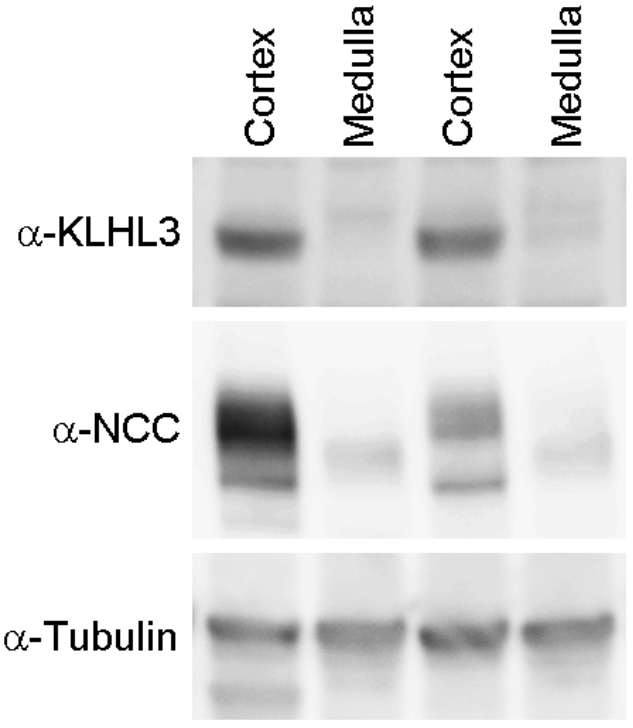
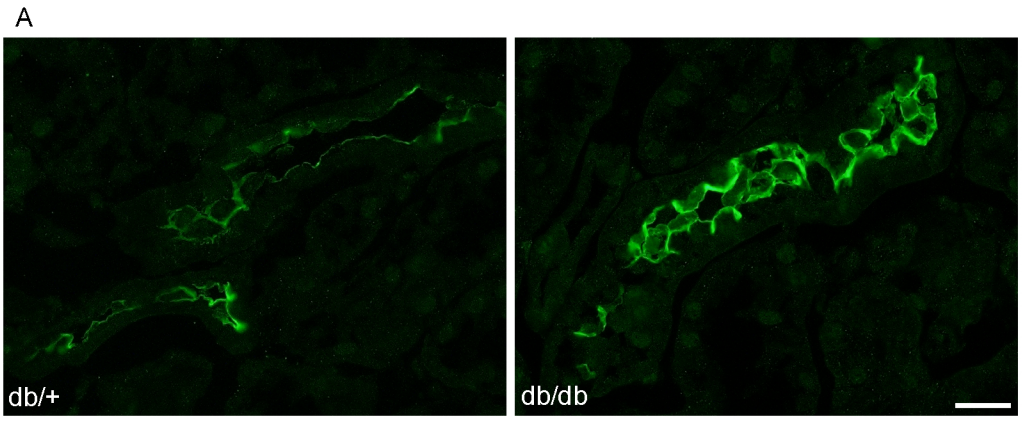
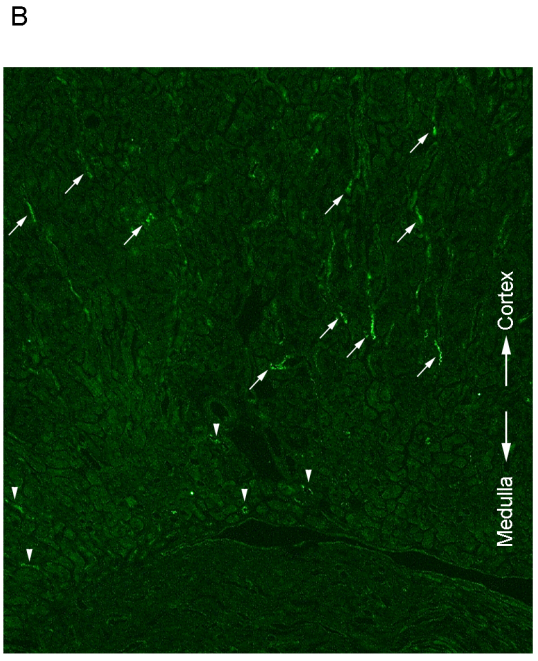
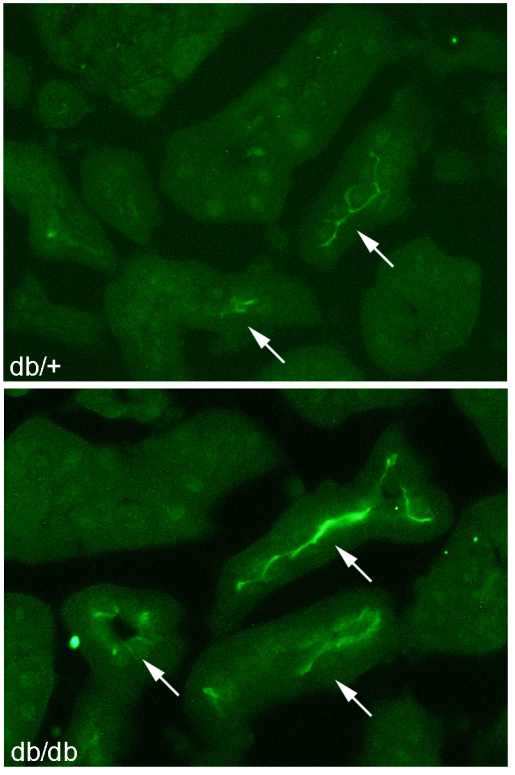
B



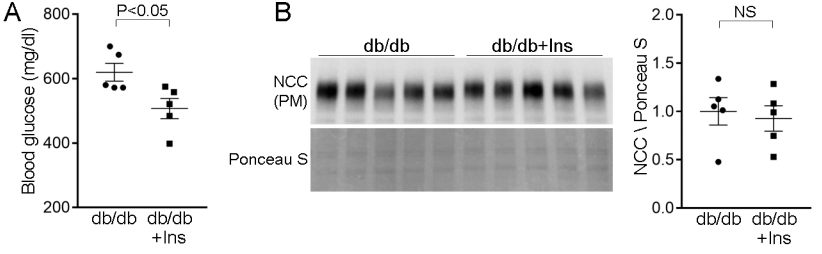
Supplemental Figure 3



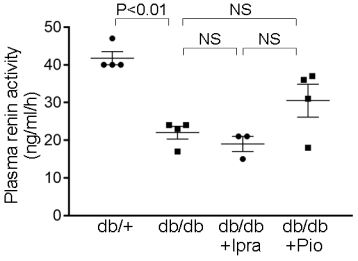




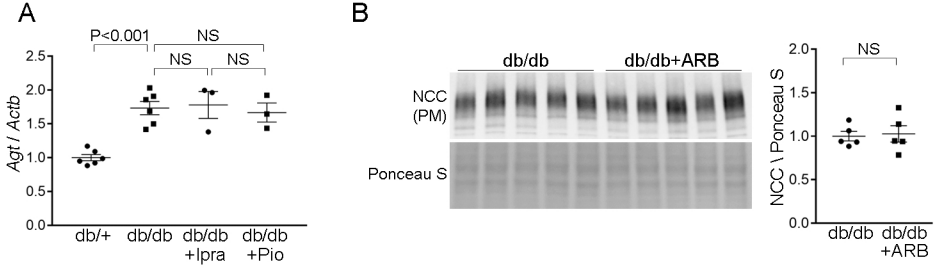
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7