Nrf2 activation protects against lithium-induced nephrogenic diabetes insipidus

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Supplemental Figures:

Suppl. Fig. 1: Effect of 7 consecutive days of LiCl administration through diet on weight, food intake and kidney gene and protein expression. (A) Animal weight as a % of initial weight and (B) 24hr body weight-normalized food intake over 7 days. (●) control chow and (o) 0.17 % LiCl diet. Results plotted as mean ± standard error of 4 (Control) or 6 (LiCl) animals per group and statistical significance assessed by two-way ANOVA with Dunnet correction for multiple comparisons, * denotes $p<0.05$. C) Full blot image for renal AQP2 expression, re-shown uncropped from Fig. 1C. (D) Nrf2 gene target expression evaluated by RT-PCR; control and LiCl not statistically different by t-test. $P<0.05$ was considered to be statistically significant.

Suppl. Fig. 2: Li induces NDI and not primary polydipsia. Mice receiving LiCl diet on baseline-clamped water intake develop significant volume depletion with hypernatremia, polycythemia. (A) Experimental design. Water and food intake were measured for 4 days for each animal; beginning at day 4, mice were randomized to control chow or LiCl chow. Mice receiving LiCl were provided their pre-determined baseline water amount daily in small sipper tube. (B) Weight (% Day 0), (C) food intake (g/day), and (D) water intake (mL/24hr). Dark area between dashed lines shows *ad libitum* water intake from LiCl treated animals in Fig. 1. Plasma Na^+, K^+, Cl^-, Hematocrit, spot urine osmolality, plasma iCa^{++}, tCO_2, glucose, BUN, anion gap (E-N). Results (B-D) plotted as mean ± standard error of 6 animals per group and statistical significance assessed by two-way ANOVA with Dunnet correction for multiple comparisons. Results (E-N) plotted as mean ± standard error of 6 animals per group, statistical significance assessed by t-test, with $p<0.05$ considered significant.

Suppl. Fig. 3: Li does not induce renal NQO1 expression. WT (C57BL6 albino) mice fed control chow or 0.17% LiCl chow followed by IF for NQO1, MUC1, actin, DAPI. Two panels of WT-control chow are re-shown from Fig. 1F and Fig. 5A-B, along with additional representative images.

Suppl. Fig. 4: Basal phenotype of $Nrf2^{-/-}$ mice. (A) renal NQO1 expression and quantification. (B) 24-hour metabolic cage urine collection normalized to animal body weight. Statistical analysis by t-test with $p<0.05$ considered significant.

Suppl. Fig. 5: Keap1 hypomorphism does not induce NDI. (A) Kidney:body mass ratio of WT and $Keap1^{hm}$ mice. (B) Spot urine analysis reveals mild baseline hyposthenuria in $Keap1^{hm}$. 12-hour water deprivation reveals normal urine concentrating function (C) and appropriate elevation

in plasma renin activity (D). Results are plotted as mean ± SEM. Statistical analysis by t-test (A-B) or one-way ANOVA with Tukey correction for multiple comparisons (C-D), $p<0.05$ was considered significant.

Suppl. Fig. 6: (A) Plasma renin activity and (B) blood urea nitrogen (BUN) in WT and Keap1^{hm} mice after 7d Li or chow. Statistical analysis by one-way ANOVA with Tukey correction for multiple comparisons with $p<0.05$ considered significant.

Suppl. Fig. 7: (A-B) Full blot images including molecular weight markers for Fig. 3J. (C) AQP1 expression and quantitation, including full immunoblot with molecular weight markers. Results plotted as mean ± SEM. Statistical analysis by one-way ANOVA with Tukey correction for multiple comparisons, with $p<0.05$ considered significant.

Suppl. Fig. 8: Western blot images. (A-G) Full blots including molecular weight markers for Fig. 4A**.** (H) Immunoblot and quantitative densitometry of renal ATP6V1B1 protein abundance. Statistical analysis by one-way ANOVA with Tukey correction for multiple comparisons, with $p<0.05$ considered significant.

Suppl. Fig. 9: Basal ion transporter expression of Keap1^{hm} mice. (A) Immunoblotting and densitometric quantitation of tNCC, NKCC2, and CA-II abundance in Li-naïve WT and Keap1^{hm} mice. Statistical analysis by t-test. (B) tNCC and activating phosphorylation (pNCC, T53) in WT and Keap1^{hm} mice after low sodium (LS) or high sodium (HS) diet.

Suppl. Fig. 10: Nrf2 effects on COX expression and PG biosynthesis in Li-naïve mice. (A) Immunoblotting for renal COX-1 and COX-2 expression and corresponding densitometry (B) in Li-naïve WT and Keap1^{hm} mice. (C) COX-1 and COX-2 mRNA transcript abundance is reduced in Keap1^{hm} (D) Immunohistochemistry for COX-2 in kidney sections from WT and Keap1^{hm} mice. (E-F) Immunoblotting and densitometry for COX-1 and COX-2 in kidneys of WT and Nrf2$^{-/-}$ mice. (G) Decreased levels of renal 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin (PGI_2) as assessed by stable isotope-dilution HPLC-MS/MS. Statistical analysis by t-test, $p<0.05$ considered significant.

Suppl. Fig. 11: Expression of renal ion transporters and COX isoforms in CDDO-Me treated mice. Representative immunoblots and quantitative densitometry; statistical analysis by one-way ANOVA with Tukey correction for multiple comparisons, $p<0.05$ considered significant.

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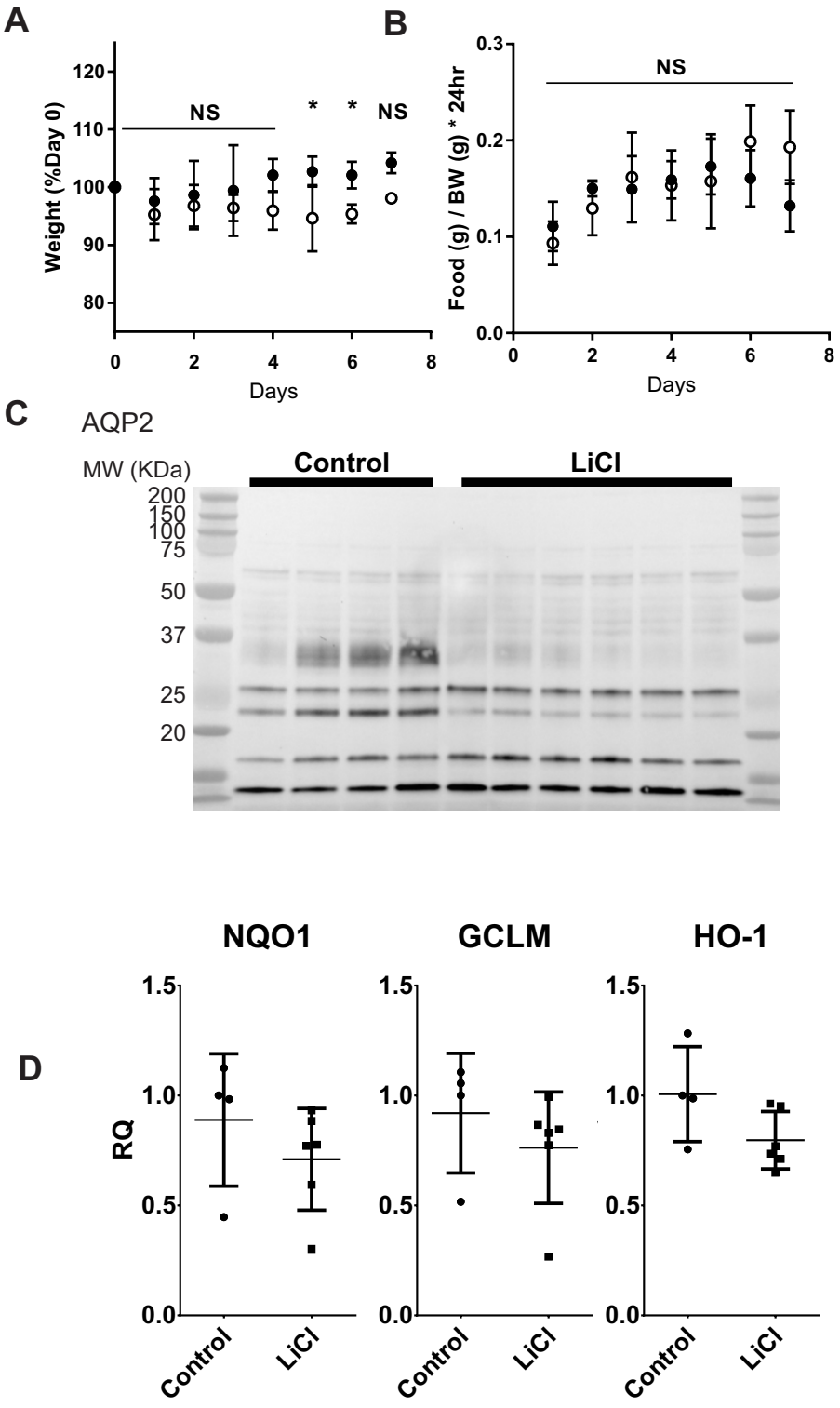
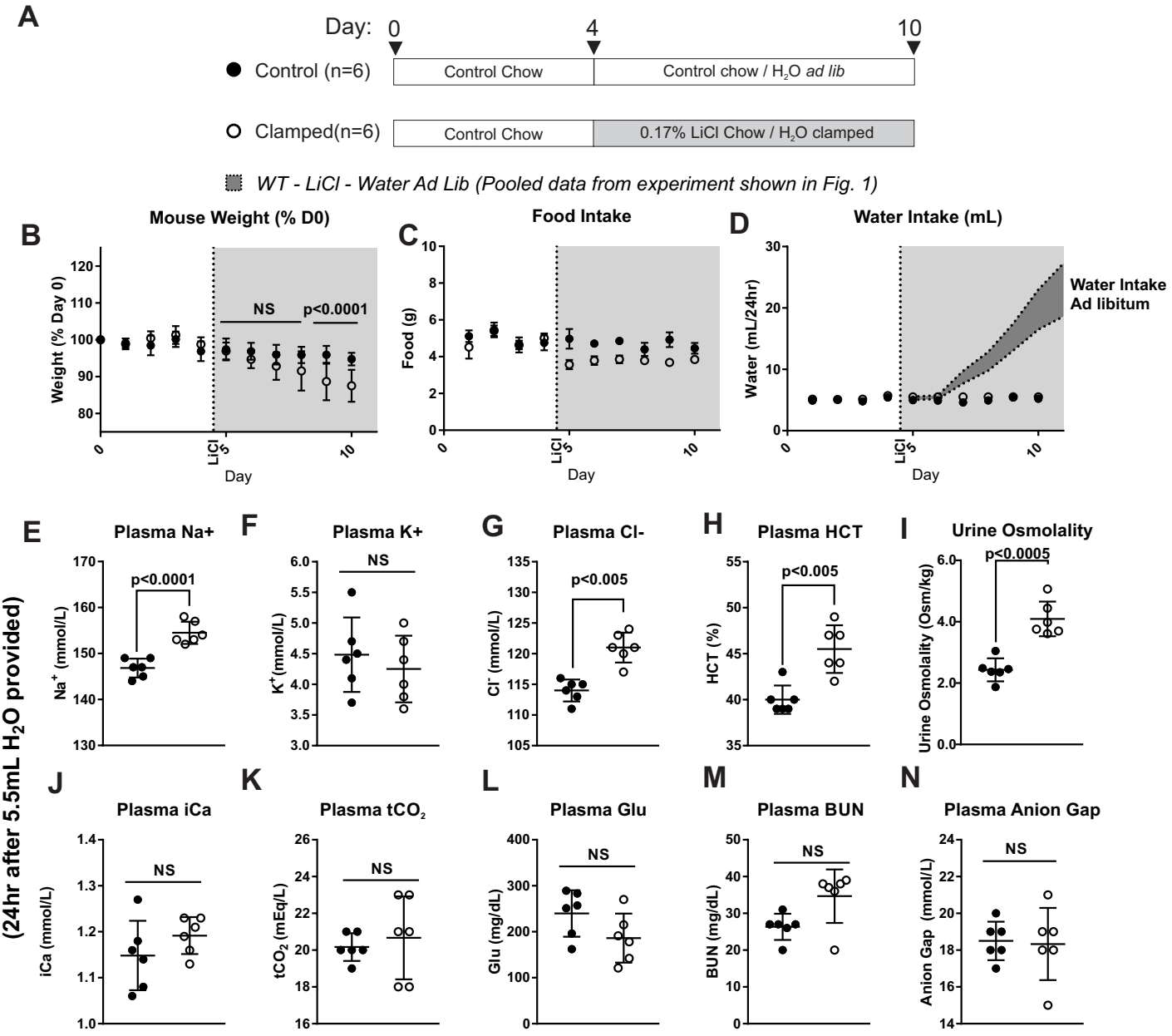
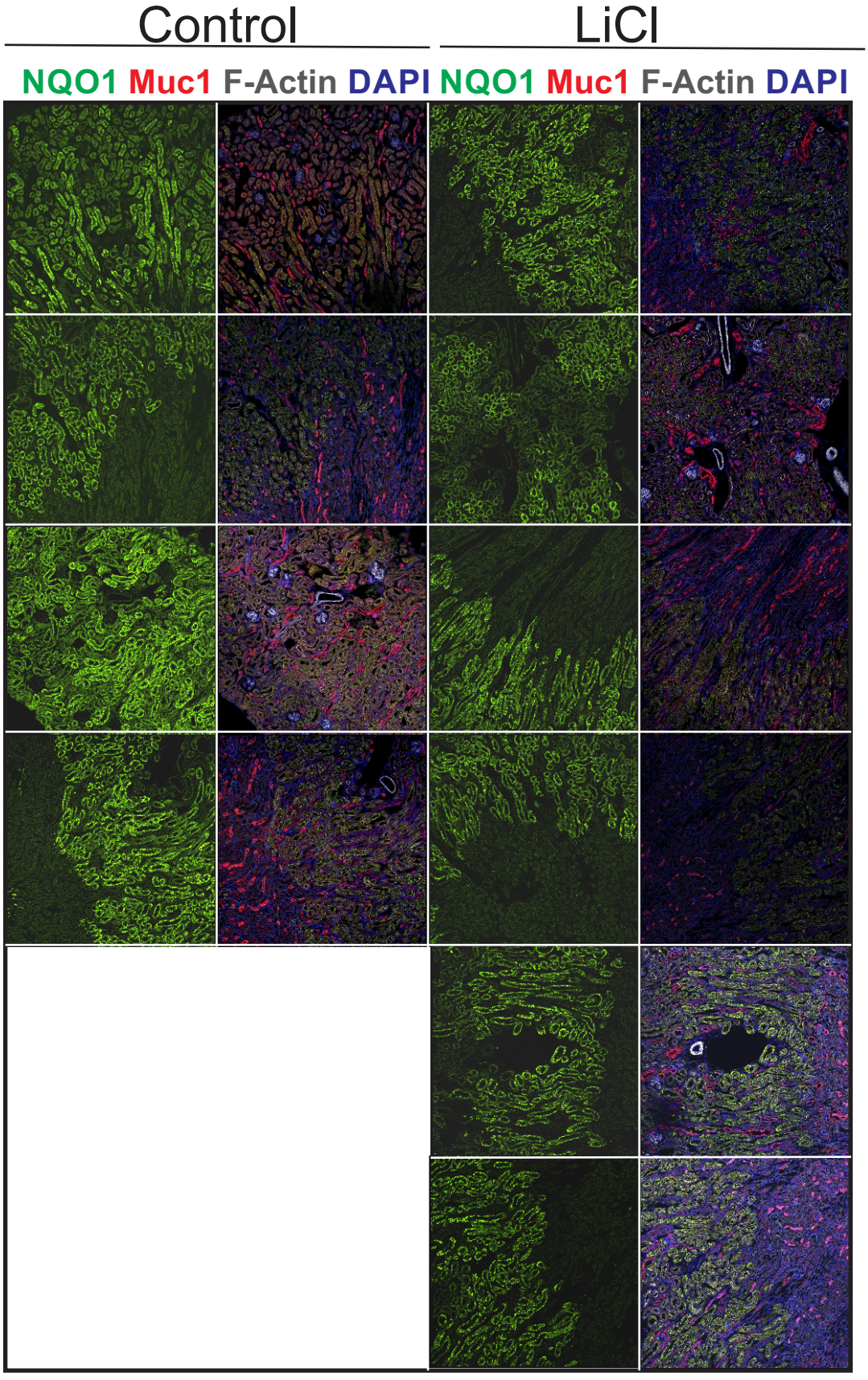

A
B
Weight (%Day 0)
120
110
100
90
80
NS
*
*
NS
0
2
4
6
8
Days
Food (g) / BW (g) * 24hr
0.3
0.2
0.1
0.0
NS
Days
C
AQP2
MW (KDa)
Control
LiCl
200
150
100
75
50
37
25
20
D
NQO1
GCLM
HO-1
RQ
1.5
1.0
0.5
0.0
Control
LiCl

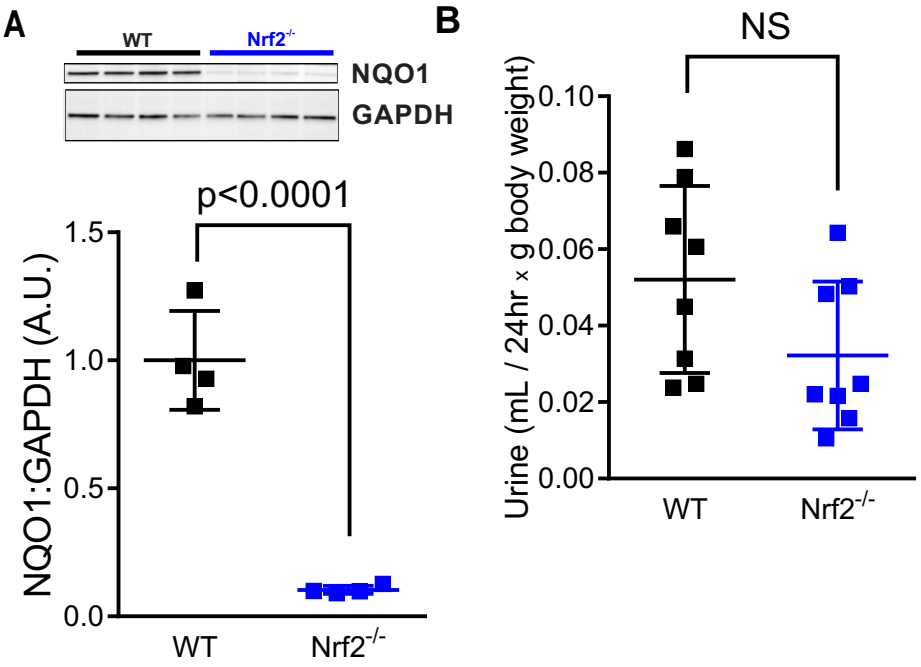
Suppl. Fig. 1


A
Day: 0 4 10
Control (n=6)
Control Chow
Control chow / H2O ad lib
Clamped(n=6)
Control Chow
0.17% LiCl Chow / H2O clamped
WT - LiCl - Water Ad Lib (Pooled data from experiment shown in Fig. 1)
B
Mouse Weight (% D0)
Weight (% Day 0)
NS
p<0.0001
LiCl
Day
C
Food Intake
Food (g)
D
Water Intake (mL)
Water (mL/24hr)
Water Intake
Ad libitum
Plasma Chemistry
(24hr after 5.5mL H2O provided)
E
Plasma Na+
Na+ (mmol/L)
p<0.0001
F
Plasma K+
K+(mmol/L)
NS
G
Plasma Cl-
Cl- (mmol/L)
p<0.005
H
Plasma HCT
HCT (%)
p<0.005
I
Urine Osmolality
Urine Osmolality (Osm/kg)
p<0.0005
J
Plasma iCa
iCa (mmol/L)
NS
K
Plasma tCO2
tCO2 (mEq/L)
NS
L
Plasma Glu
Glu (mg/dL)
NS
M
Plasma BUN
BUN (mg/dL)
NS
N
Plasma Anion Gap
Anion Gap (mmol/L)
NS

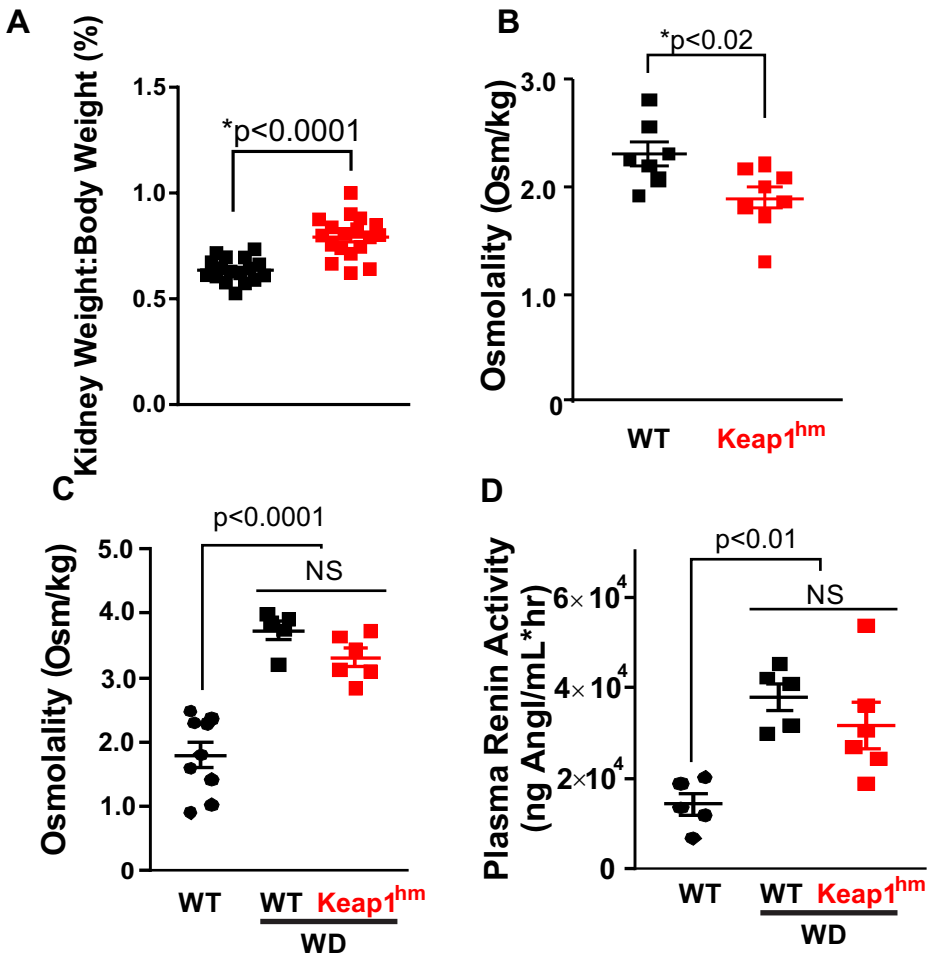
Suppl. Fig. 2



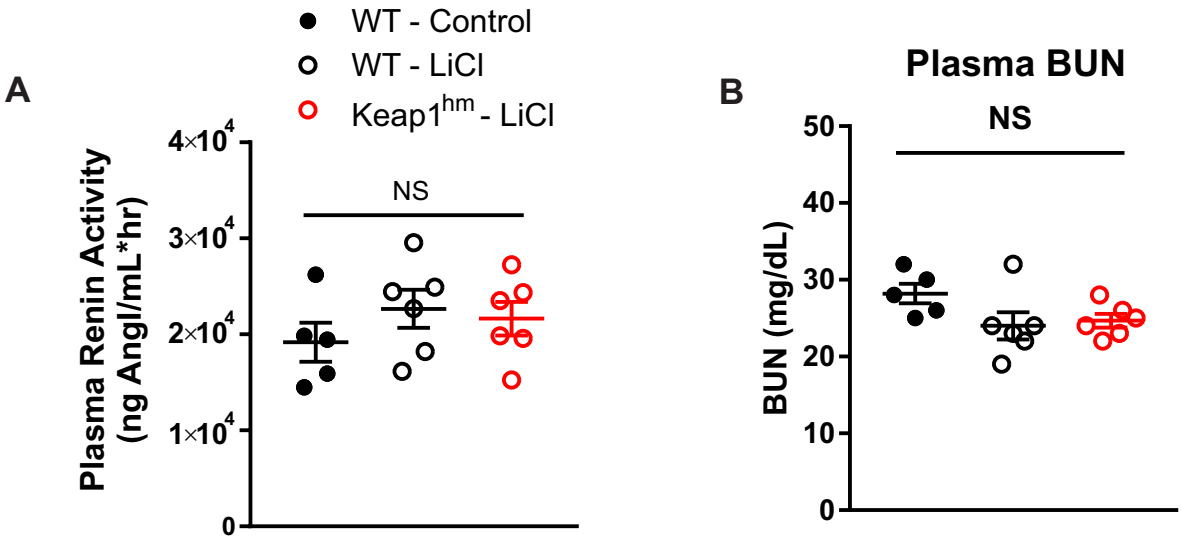
Suppl. Fig. 3


A
WT
Nrf2-/-
NQO1
GAPDH
p<0.0001
NQO1:GAPDH (A.U.)
1.5
1.0
0.5
0.0
WT
Nrf2-/-
B
NS
Urine (mL / 24hr x g body weight)
0.10
0.08
0.06
0.04
0.02
0.00
WT
Nrf2-/-

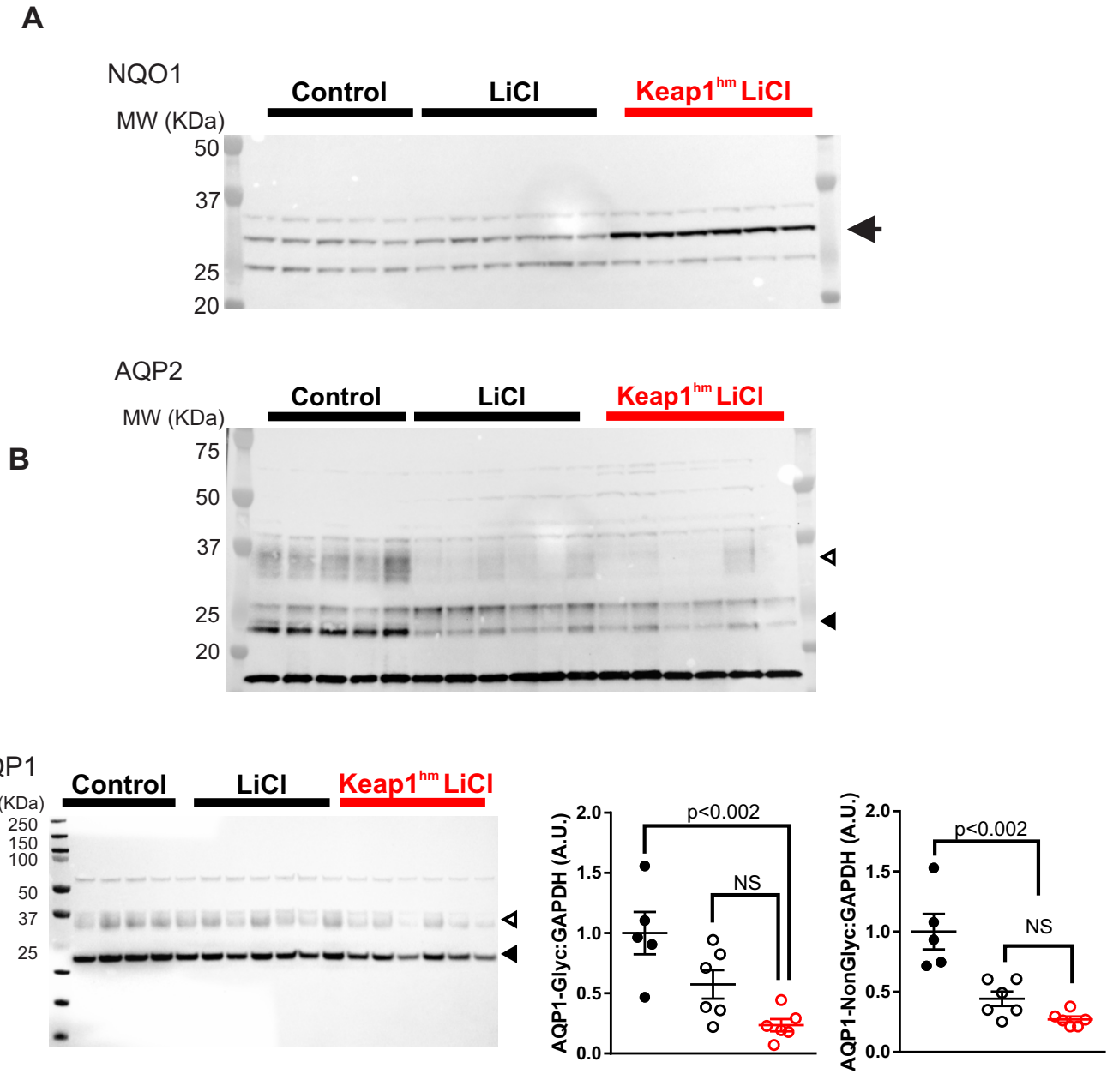
Suppl. Fig. 4


A
Kidney Weight:Body Weight (%)
1.5
1.0
0.5
0.0
*p<0.0001
B
*p<0.02
Osmolality (Osm/kg)
3.0
2.0
1.0
0
WT
Keap1hm
C
Osmolality (Osm/kg)
p<0.0001
NS
5.0
4.0
3.0
2.0
1.0
0
WT
WT
Keap1hm
WD
D
Plasma Renin Activity
(ng Angl/mL*hr)
p<0.01
NS
6× 10^4
4× 10^4
2×10^4
0
WT
WT
Keap1hm
WD

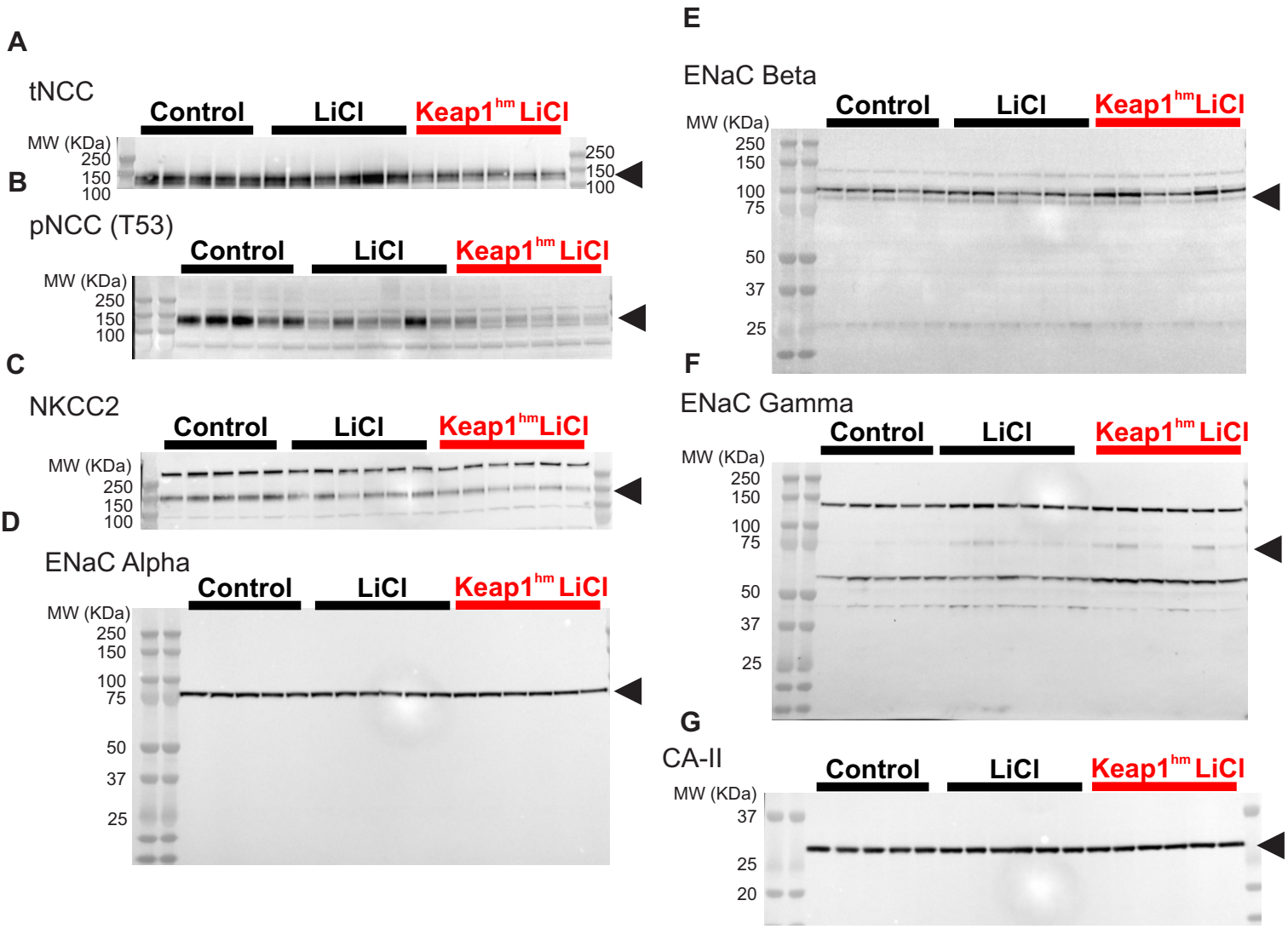
Suppl. Fig. 5

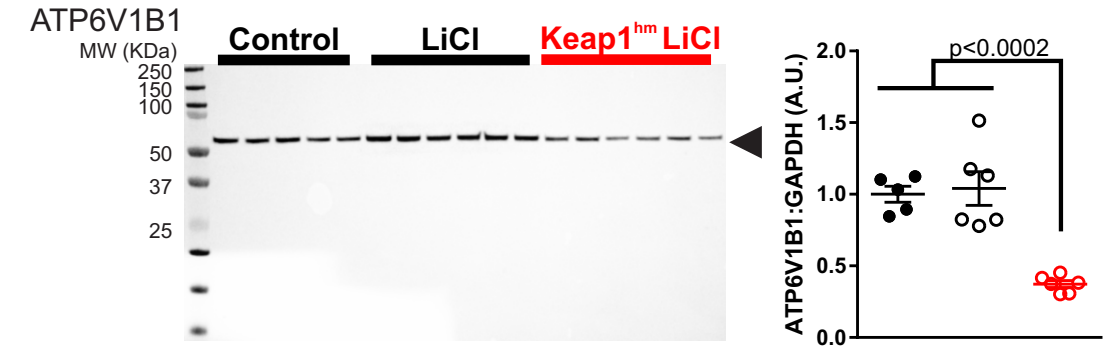

A
WT - Control
WT - LiCl
Keap1hm - LiCl
Plasma Renin Activity
(ng Angl/mL*hr)
4×10^4
3×10^4
2×10^4
1×10^4
0
NS
B
Plasma BUN
NS
BUN (mg/dL)
50
40
30
20
10
0

Suppl. Fig. 6

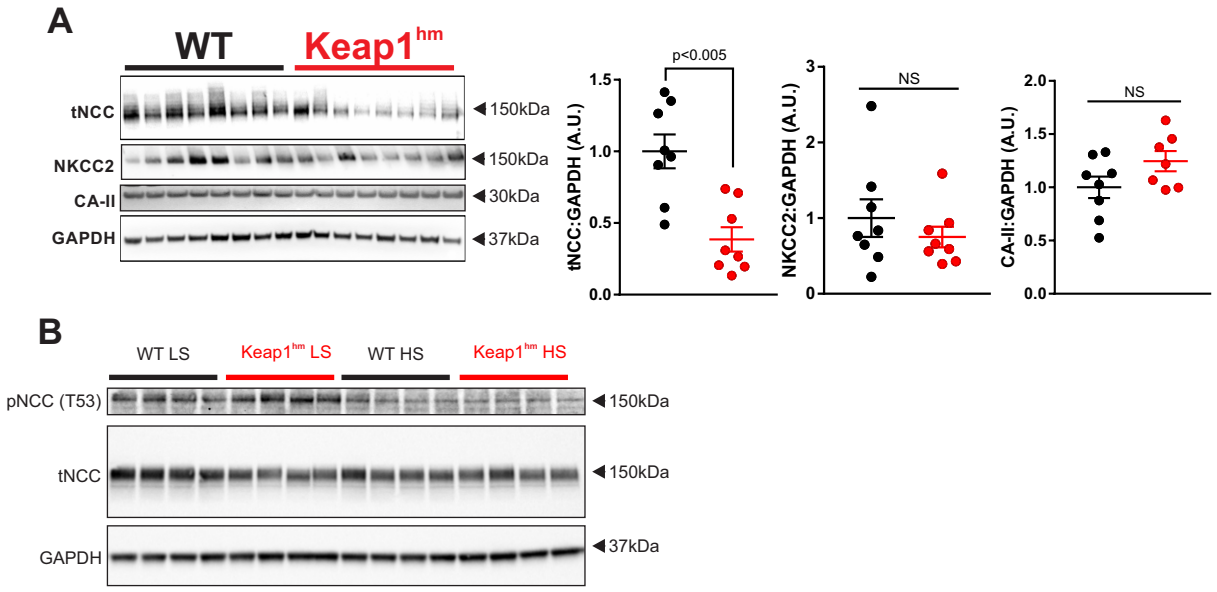

A
NQO1
Control
LiCl
Keap1hm LiCl
MW (KDa)
50
37
25
20
AQP2
Control
LiCl
Keap1hm LiCl
MW (KDa)
B
75
50
37
25
20
C
AQP1
Control
LiCl
Keap1hm LiCl
MW (KDa)
250
150
100
50
37
25
AQP1-Glyc:GAPDH (A.U.)
p<0.002
NS
2.0
1.5
1.0
0.5
0.0
AQP1-NonGlyc:GAPDH (A.U.)
p<0.002
NS
2.0
1.5
1.0
0.5
0.0

Suppl. Fig. 7

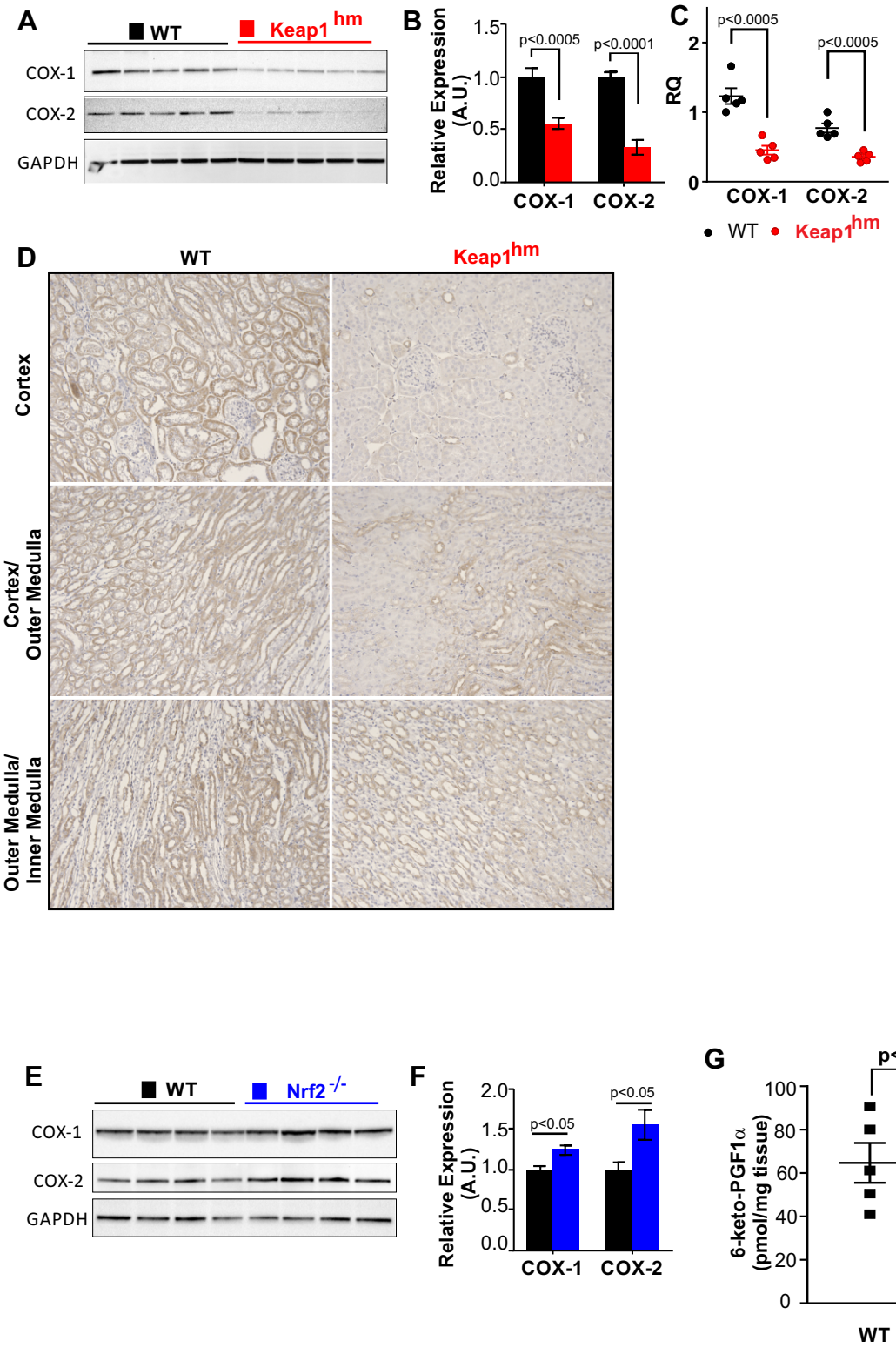

A
tNCC
Control
LiCI
Keap1hm LiCI
MW (KDa)
250
150
100
B
pNCC (T53)
C
NKCC2
D
ENaC Alpha
E
ENaC Beta
F
ENaC Gamma
G
CA-II
75
50
37
25
20


H
ATP6V1B1
Control
LiCI
Keap1hm LiCI
MW (KDa)
250
150
100
50
37
25
p<0.0002
ATP6V1B1:GAPDH (A.U.)
2.0
1.5
1.0
0.5
0.0

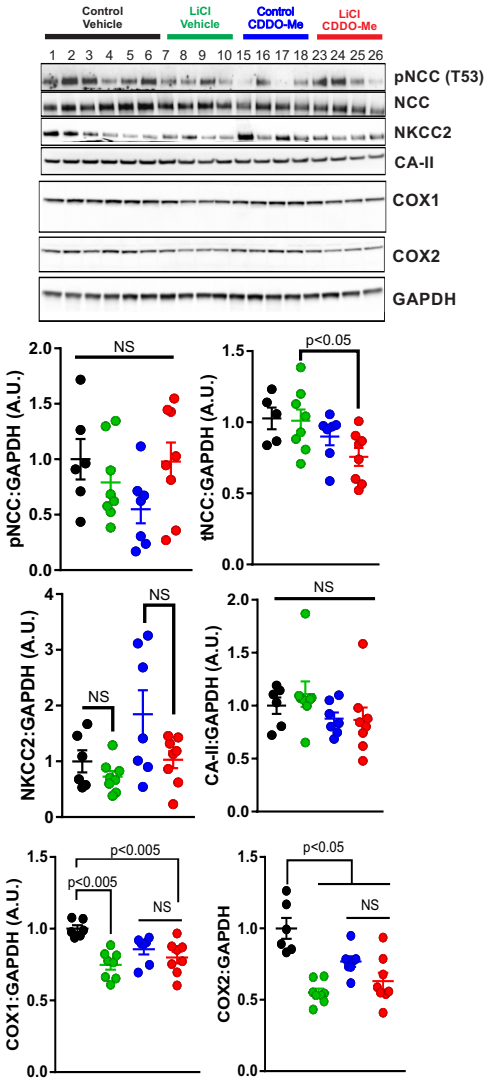
Suppl. Fig. 8


A
WT
Keap1hm
tNCC
NKCC2
CA-II
GAPDH
150kDa
150kDa
30kDa
37kDa
p<0.005
NS
NS
tNCC:GAPDH (A.U.)
NKCC2:GAPDH (A.U.)
CA-II:GAPDH (A.U.)
B
WT LS
Keap1hm LS
WT HS
Keap1hm HS
pNCC (T53)
tNCC
GAPDH
150kDa
150kDa
37kDa

Suppl. Fig. 9


A
WT
Keap1 hm
COX-1
COX-2
GAPDH
B
Relative Expression (A.U.)
1.5
1.0
0.5
0.0
p<0.0005
p<0.0001
COX-1
COX-2
C
RQ
2
1
0
p<0.0005
p<0.0005
COX-1
COX-2
WT
Keap1hm
D
WT
Keap1hm
Cortex
Cortex/ Outer Medulla
Outer Medulla/ Inner Medulla
E
WT
Nrf2 -/-
COX-1
COX-2
GAPDH
F
Relative Expression (A.U.)
2.0
1.5
1.0
0.5
0.0
p<0.05
p<0.05
COX-1
COX-2
G
p<0.0001
6-keto-PGF1α (pmol/mg tissue)
100
80
60
40
20
0
WT
Keap1hm

Suppl. Fig. 10


Control
Vehicle
LiCl
Vehicle
Control
CDDO-Me
LiCl
CDDO-Me
1 2 3 4 5 6 7 8 9 10 15 16 17 18 23 24 25 26
pNCC (T53)
NCC
NKCC2
CA-II
COX1
COX2
GAPDH
pNCC:GAPDH (A.U.)
NS
tNCC:GAPDH (A.U.)
p<0.05
NKCC2:GAPDH (A.U.)
NS
NS
CA-II:GAPDH (A.U.)
NS
COX1:GAPDH (A.U.)
p<0.005
p<0.005
NS
COX2:GAPDH
p<0.05
NS

Suppl. Fig. 11