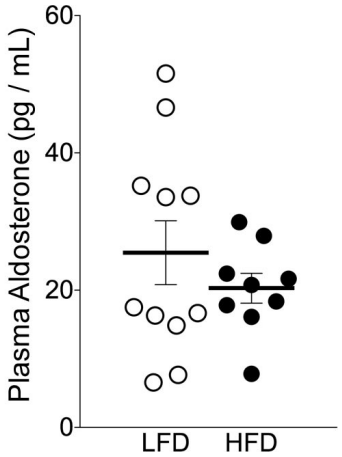
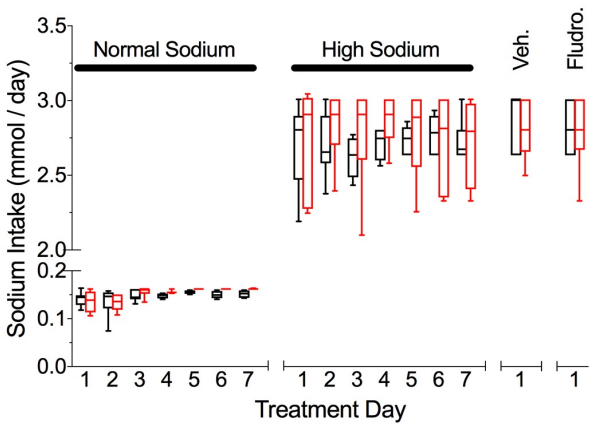


Supplementary Figure S1: Plasma aldosterone concentration in low (open circle, N = 11) and high fat-fed (closed circle, N = 9) mice. After low or high fat feeding for 12 weeks, we supplemented gel food with high sodium and then sacrificed mice. LFD, low fat-fed mice; HFD, high fat-fed mice. Each dot represents data from one mouse with mean and SEM.

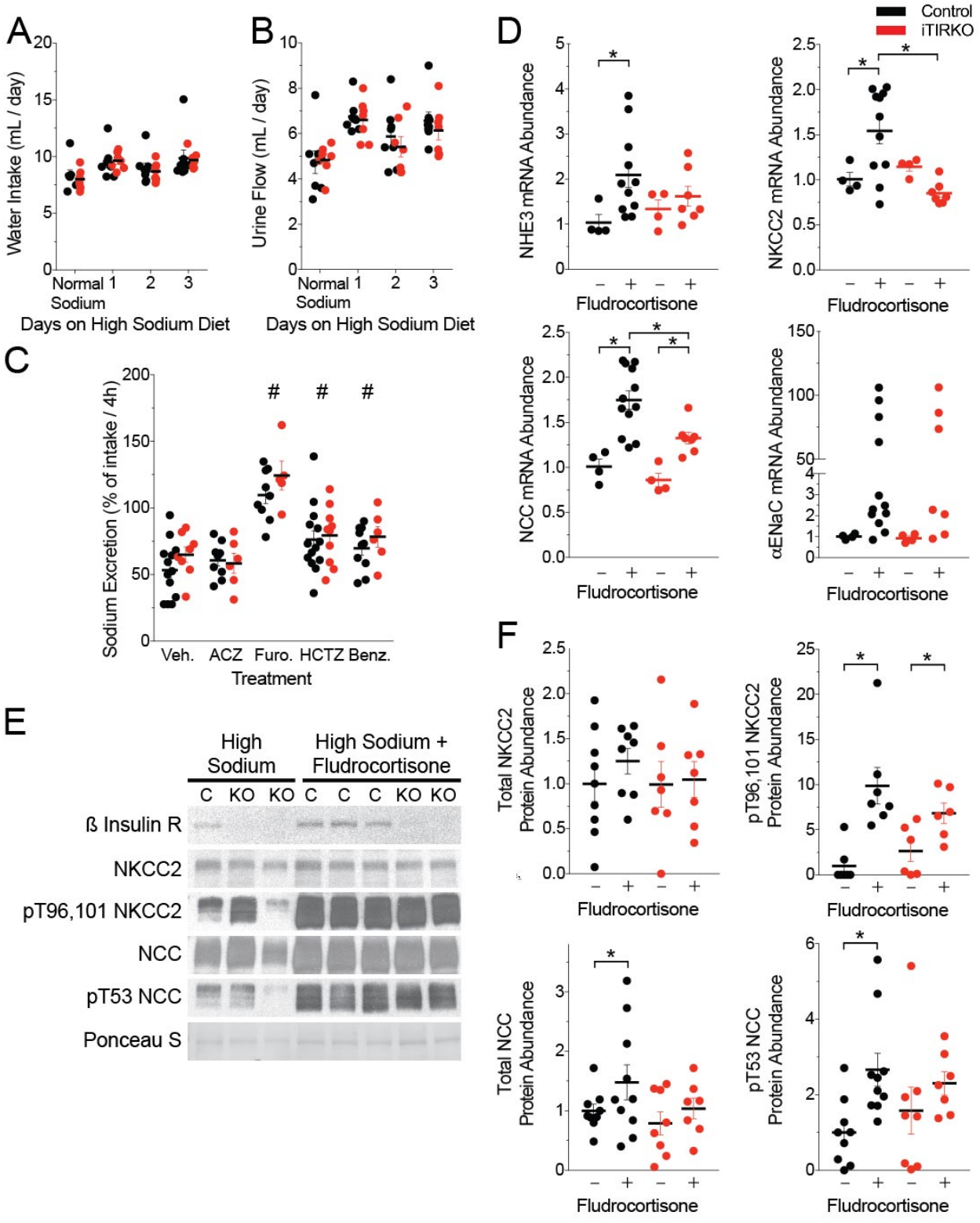


Supplementary Figure S2: Sodium intake during blood pressure recording of control (black, N = 7) and iTIRKO (red, N = 5) mice. After high fat feeding and doxycycline administration to induce Cre expression, we implanted radiotelemetric blood pressure catheters in control (black, N = 7) and iTIRKO (red, N = 5) mice. We measured sodium intake daily. Please see Methods in the main text for further details. Control, age-matched littermates of iTIRKO mice; iTIRKO, inducible renal tubular insulin receptor knockout mice; Veh., Vehicle; Fludro., Fludrocortisone. In box and whisker plots, whiskers represent minimum and maximum values, and box borders represent the 25th percentile, median, and 75th percentile.

To assess the relative activity of sodium transporters and channels after deletion of the tubular insulin receptor, we measured sodium excretion after pharmacological inhibition of channels and transporters that reabsorb the majority of filtered sodium. We placed high fat-fed control and iTIRKO mice on a high sodium and high fat diet for at least 2 weeks and acclimated them to metabolic cages. We provided mice with 2.5 kcals of food and performed intraperitoneal injection of one of several sodium transporter or channel inhibitors, collected urine every 2 hours for 4 hours, and calculated sodium excretion as previously described(22). We used the following diuretics based on prior studies: acetazolamide 60 mg / kg(28), furosemide 75 mg / kg(14), hydrochlorothiazide 30 mg / kg(29), benzamil 1.4 mg / kg(22), or alkalinized saline alone (vehicle for all drugs). Vehicle or drugs were given at a volume of 5 µl / g body weight. After 3 days of washout, we then anesthetized mice with isoflurane, obtained blood by cardiac puncture, perfused them with saline, and collected plasma and tissue.

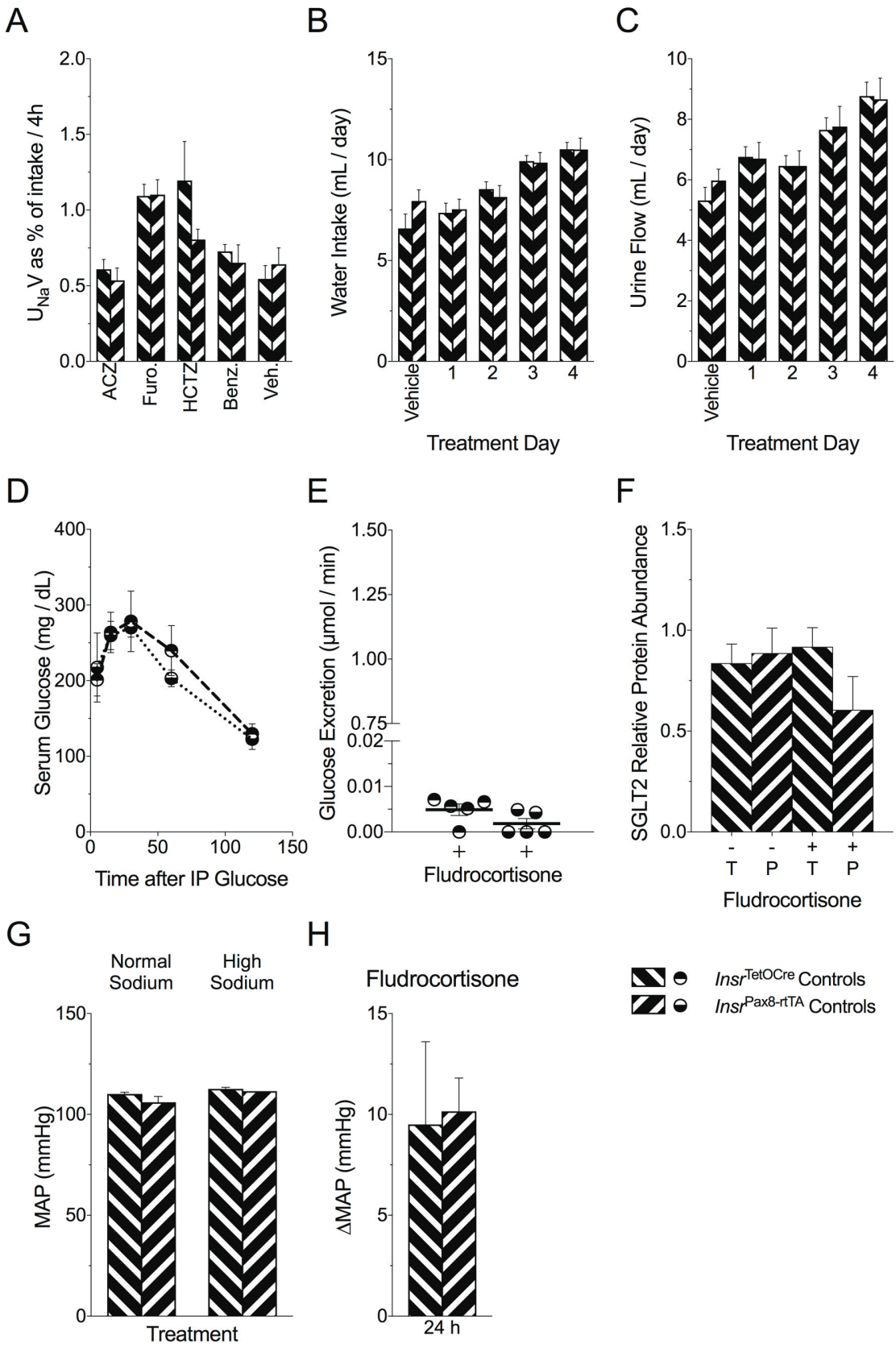
We then compared the mRNA and protein expression of select proximal and distal sodium transporters in fludrocortisone-treated and untreated control and iTIRKO mice.

Expression of NKCC2 mRNA was increased in control but not iTIRKO mice after fludrocortisone treatment (iTIRKO 0.85 ± 0.5 versus control 1.54 ± 0.14 arbitrary units compared to untreated control mice, $P < 0.001$). However, total and phospho$T_{96,101}$ NKCC2 protein abundance was no different between genotypes in fludrocortisone-treated and untreated groups. Fludrocortisone increased NCC gene expression less in iTIRKO compared to control mice (iTIRKO 1.33 ± 0.07 versus control 1.75 ± 0.10 arbitrary units compared to untreated control mice, $P = 0.004$). Plasma potassium, a negative regulator of phospho-T_{53} NCC(30), was also not significantly different between control and iTIRKO mice (**Supplementary Table S2**).

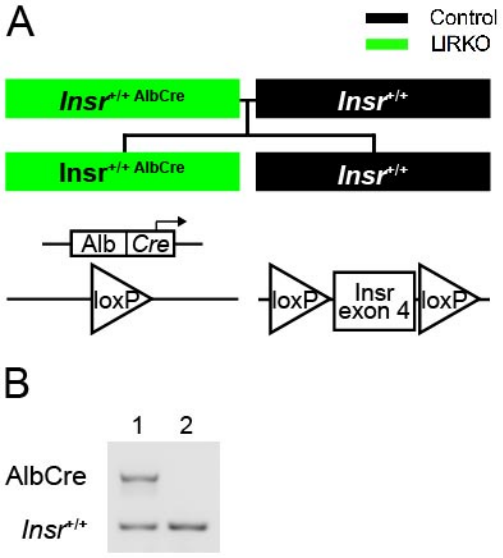


Supplementary Figure S3: No effect of genetic deletion of renal tubular insulin receptor on water and sodium balance on a high sodium diet. After high fat feeding and doxycycline administration to induce Cre expression, we placed mice in metabolic cages and transitioned mice from a normal to high sodium diet. (A)

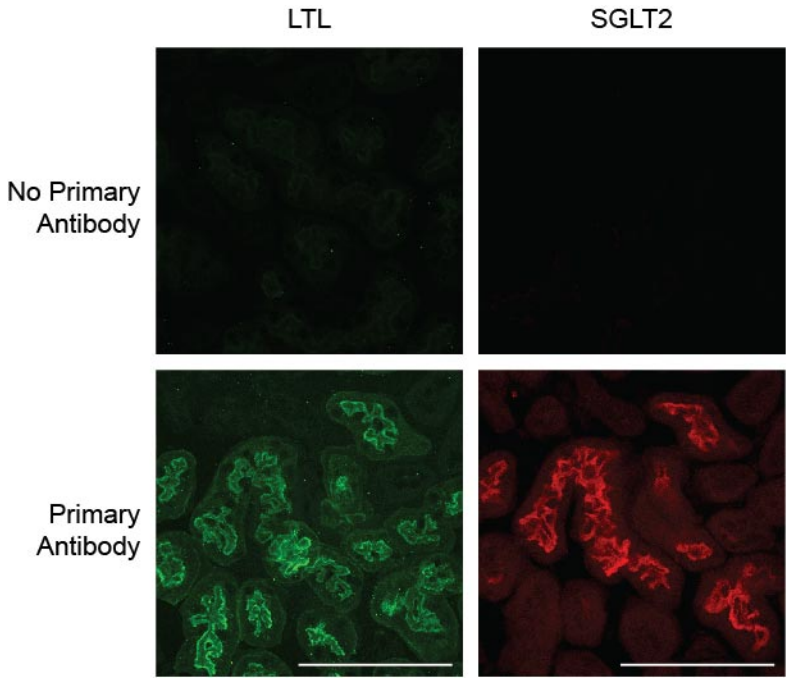
Water intake and (B) urine flow rate in control (black) and iTIRKO (red) mice on a high fat, normal and high sodium diet. (C) Renal channel and transporter inhibition-induced sodium excretion on a high sodium- and high fat-fed control (N = 13) and iTIRKO (N = 8) mice. (D) Associated transporter/channel mRNA abundance relative to GAPDH abundance in high fat-fed control mice with (N = 12) and without (N = 4) mineralocorticoid administration and iTIRKO mice with (N = 7) and without (N = 4) mineralocorticoid administration. (E) Representative immunoblot and (F) densitometry of total and phosphorylated sodium potassium 2 chloride cotransporter (NKCC2) and sodium chloride cotransporter (NCC) on a high sodium diet with (N = 8 - 12 control, 7 iTIRKO) and without (N = 9 - 10 control, 7 iTIRKO) mineralocorticoid administration. Control, age-matched littermates of iTIRKO mice; iTIRKO, inducible renal tubular insulin receptor knockout mice. Veh., Vehicle; ACZ, acetazolamide; Furo., Furosemide; HCTZ, Hydrochlorothiazide; Benz., Benzamil. *, $P < 0.05$ between groups by two-way ANOVA with multiple comparisons. #, $P < 0.05$ compared to vehicle by two-way ANOVA. Each dot represents data from one mouse with mean and SEM.


A
B
C
$U_{Na}V$ as % of intake / 4h
2.0
1.5
1.0
0.5
0.0
ACZ
Furo.
HCTZ
Benz.
Veh.
Water Intake (mL / day)
15
10
5
0
Vehicle
1
2
3
4
Treatment Day
Urine Flow (mL / day)
10
8
6
4
2
0
Vehicle
1
2
3
4
Treatment Day
D
E
F
Serum Glucose (mg / dL)
400
300
200
100
0
0
50
100
150
Time after IP Glucose
Glucose Excretion (µmol / min)
1.50
1.25
1.00
0.75
0.02
0.01
0.00
+
+
Fludrocortisone
SGLT2 Relative Protein Abundance
1.5
1.0
0.5
0.0
-
-
+
+
T
P
T
P
Fludrocortisone
G
H
Normal Sodium
High Sodium
MAP (mmHg)
150
100
50
0
Treatment
Fludrocortisone
ΔMAP (mmHg)
15
10
5
0
24 h
Insr^TetOCre Controls
Insr^Pax8-rtTA Controls

Supplemental Figure S4: Comparison of *Insr*^{TetOCre} (N = 6) and *Insr*$^{Pax8\text{-}rtTA}$ (N = 5) control strains. (A) Renal channel and transporter inhibition-induced sodium excretion on a high sodium diet. (B) 24-hour Water intake and (C) Urine output over 4 days of fludrocortisone administration. (D) Intraperitoneal glucose tolerance testing on a high sodium diet. (E) Urinary glucose excretion on day 5 of fludrocortisone administration. (F) SGLT2 protein abundance in membrane-enriched whole kidney lysate with and without fludrocortisone administration. (G) Mean arterial pressure (MAP) on a normal and high sodium diet. (H) Change in 24-hour MAP between vehicle and fludrocortisone. Veh., Vehicle; ACZ, acetazolamide; Furo., Furosemide; HCTZ, Hydrochlorothiazide; Benz., Benzamil; IP, intraperitoneal; T, TetOCre allele; P, Pax8-rtTA allele. For Panels (D) and (E), each dot represents data from one mouse with mean and SEM.



Supplementary Figure S5: Generation of LIRKO mice. (A) Breeding strategy and schematic of Cre-loxP system to generate liver insulin receptor (Insr) knockout mice. (B) Genotyping DNA gel of $Insr^{+/+;\ AlbCre}$ (green, LIRKO, lane 1) and $Insr^{+/+}$ (black, control, lane 2) mice. Control, age-matched littermates of LIRKO mice. LIRKO, Liver insulin receptor knockout mice; Alb, Albumin promoter



Supplementary Figure S6: Negative controls for LTL and anti-SGLT2 immunofluorescence. Scale bar represents 50 μm.

	Units	Control	iTIRKO	p-value
Food intake	Kcal / day	15.1 ± 0.3	15.2 ± 0.3	0.55
Water intake	mL / day	9.5 ± 1.6	9.3 ± 0.9	0.78
Urine output	mL / day	6.4 ± 1.1	6.1 ± 1.1	0.30
Urine Na^+	mM	367 ± 109	356 ± 95	0.72
Urine K^+	mM	90 ± 26	81 ± 22	0.21
Plasma Na^+	mM	154 ± 4	152 ± 2	0.21
Plasma K^+	mM	4.1 ± 0.7	5.2 ± 0.6	0.72
Plasma Cl^-	mM	111 ± 2	111 ± 3	0.61
Plasma tCO_2	mM	19 ± 2	18 ± 2	0.67
Plasma Glucose	mg / dL	195 ± 31	176 ± 49	0.30
Plasma Aldosterone	pg / mL	516 ± 341	404 ± 32	0.54

Supplementary Table S1: Characteristics of control and iTIRKO mice on a high sodium and high fat diet. Control N = 9, iTIRKO N = 8. Control, age-matched littermates of iTIRKO mice; iTIRKO, inducible renal tubular insulin receptor knockout mice.

	Units	Control	iTIRKO	p-value
Plasma Na^+	mM	145 ± 1	145 ± 1	1.00
Plasma K^+	mM	3.8 ± 0.3	3.5 ± 0.2	0.37
Plasma Cl^-	mM	105 ± 1	104 ± 1	0.74
Plasma tCO_2	mM	26 ± 6	30 ± 7	0.49
Plasma Glucose	mg/dL	203 ± 1	216 ± 4	0.27

Supplementary Table S2: Plasma chemistries of fludrocortisone-treated high sodium and high fat-fed control and iTIRKO mice. Control, N = 7, iTIRKO, N = 6. Control, age-matched littermates of iTIRKO mice; iTIRKO, inducible renal tubular insulin receptor knockout mice.

Genotyping

Gene		Sequence
Insr[29]	Forward	5'-CCCTGGACGCCAAGAGCAGC-3'
	Reverse	5'-GGGCACATCTGCCTGTGTGGG-3'
Pax8-rtTA[28]	Forward	5'-CCATGTCTAGACTGGACAAGA-3'
	Reverse	5'-CTCCAGGCCACATATGATTAG-3'
Cre[28]	Forward	5'-ATGTCCAATTTACTGACCG-3'
	Reverse	5'-CGCCGC ATAACCAGTGAAAC-3'

RT-PCR

Gene		Sequence
NHE3	Forward	5'-CTGTCATTGGCACTATATGGA-3'
	Reverse	5'-TTGTACAAGACCACAGTCAC-3'
NKCC2	Forward	5'-CCGTGGCCTACATAGGTGTT-3'
	Reverse	5'-GGCTCGTGTTGACATCTTGA-3'
NCC	Forward	5'-CAGTGCCTGGTGCTTACAGGGC-3'
	Reverse	5'-CATCATGCAGGACACCAATG-3'
α ENaC	Forward	5'-TGCTCCTGTCACTTCAGCAC-3'
	Reverse	5'-CCCCTTGCTTAGCCTGTTC-3'
SGLT1	Forward	5'-ATGCGGCTGACATCTCAGTC-3'
	Reverse	5'-ACCAAGGCGTTCCATTCAAAG-3'
SGLT2	Forward	5'-TGGCGGTGTCCGTGGCTTGG-3'
	Reverse	5'-CGGACACTGGAGGTGCCAGATAGC-3'
GAPDH	Forward	5'-CATGGCCTTCCGTGTTCCTA-3'
	Reverse	5'-CCTGCTTCACCACCTTCTTGAT-3'

Supplementary Table S3: Primers for genotyping and RT-PCR.