mouse Tmc4 gene

WT

KOΔ10 (-10 bp)

KOΔ28 (-28 bp)

Figure S1
Figure S2

A

Scale bar 20 μm

B

Tmc4

Entpd2

merged

Tmc4

Plcβ2

merged

Tmc4

Pkd1l3

merged

Sense probe

Plcβ2

merged

Scale bar 20 μm
hTMC4

Figure S3
Figure S4
Figure S5
Table S1.
The compositions of pipette solutions

| Electrolyte (mM) | Solution | NMDG-Cl | KCl |
|------------------|----------|---------|-----|
| NMDG-Cl          | 134      | -       |
| KCI              | -        | 134     |
| BAPTA            | 5        | 5       |
| HEPES            | 10       | 10      |

*N*-Methyl-D-glucamine (NMDG), *O,O*-Bis(2-aminophenyl)ethyleneglycol-\(N,N,N',N'^{-}\)-tetraacetic acid, tetrapotassium salt, hydrate (BAPTA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The pH of all pipette solution was adjusted to pH 7.2 with NMDG-OH. The osmotic pressure of all pipette solution was adjusted to approximately 270 mOsmol/kg.
Table S2.
The compositions of bath solutions

| Electrolyte (mM) | Solution | NaCl (Control) | NMDG | 67 mM NaCl, 67 mM Na-glucuronate | 34 mM NaCl, 34 mM Na-glucuronate | Na-glucuronate | NPPB | Na-glutamate | Asp-Na | Ionomycin |
|------------------|----------|----------------|------|---------------------------------|---------------------------------|----------------|------|--------------|--------|-----------|
| NaCl             | 134      | -              | 67   | 34                              | -                               | 134            | -    | -            | 134    | 134       |
| Na-glucuronate   | -        | -              | 67   | 100                             | 134                             | -              | 2.9  | 2.9          | 2.9    | 2.9       |
| KCl              | 2.9      | 2.9            | 2.9  | 2.9                             | 2.9                             | 2.9            | 2.9  | 2.9          | 2.9    | 2.9       |
| HEPES            | 10       | 10             | 10   | 10                              | 10                              | 10             | 10   | 10           | 10     | 10        |
| D-Glucose        | 15       | 15             | 15   | 15                              | 15                              | 15             | 15   | 15           | 15     | 15        |
| MgCl₂            | 1.2      | 1.2            | 1.2  | 1.2                             | 1.2                             | 1.2            | 1.2  | 1.2          | 1.2    | 1.2       |
| CaCl₂            | 2.1      | 2.1            | 2.1  | 2.1                             | 2.1                             | 2.1            | 2.1  | 2.1          | 2.1    | 2.1       |
| NMDG-Cl          | -        | 134            | -    | -                               | -                               | -              | -    | -            | -      | -         |
| Na-glutamate     | -        | -              | -    | -                               | -                               | -              | 134  | -            | -      | -         |
| Asp-Na           | -        | -              | -    | -                               | -                               | -              | -    | 134          | -      | -         |
| NPPB             | -        | -              | -    | -                               | -                               | -              | 0.1  | -            | -      | -         |
| Ionomycin        | -        | -              | -    | -                               | -                               | -              | -    | 0.005        | -      | -         |

*N*-Methyl-D-glucamine (NMDG), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB). The pH of all bath solution was adjusted to pH 7.4 with NMDG-OH. The osmotic pressure of all bath solution was adjusted to approximately 295 mOsmol/kg.
Table S3.  
Comparison of serum and urine parameters

|                  | WT             | Tmc4 KO        |
|------------------|----------------|----------------|
| **Serum**        |                |                |
| ALB (g/dL)       | 3.20 ± 0.07    | 3.15 ± 0.06    |
| CRE (mg/dL)      | 0.13 ± 0.01    | 0.14 ± 0.01    |
| UA (mg/dL)       | 3.26 ± 0.09    | 3.12 ± 0.45    |
| UA/CRE           | 25.9 ± 1.27    | 23.7 ± 4.14    |
| Na (mEq/L)       | 148 ± 0.79     | 148 ± 0.58     |
| K (mEq/L)        | 9.72 ± 0.46    | 10.1 ± 0.29    |
| Cl (mEq/L)       | 106 ± 0.37     | 105 ± 0.60     |
| Ca (mg/dL)       | 9.66 ± 0.14    | 9.80 ± 0.15    |
| P (mg/dL)        | 9.90 ± 1.30    | 10.8 ± 1.21    |
| Mg (mg/dL)       | 4.08 ± 0.13    | 4.32 ± 0.18    |
| Fe (μg/dL)       | 125 ± 17.0     | 164 ± 14.9     |
| **24h urine**    |                |                |
| weight (g)       | 1.62 ± 0.30    | 1.53 ±0.15     |
| CRE (mg/dL)      | 32.3 ± 2.56    | 35.3 ± 2.32    |
| ALB/CRE          | 0.05 ± 0.00    | 0.04 ± 0.00    |
| TP/CRE           | 10.8 ± 3.45    | 11.4 ± 1.28    |
| BNU/CRE          | 123 ± 7.23     | 119 ± 2.52     |
| UA/CRE           | 0.78 ± 0.35    | 0.24 ± 0.02    |
| Na (mEq/L)       | 99.3 ± 8.79    | 110 ± 10.2     |
| K (mEq/L)        | 380.2 ± 33.1   | 389 ± 21.5     |
| Cl (mEq/L)       | 173 ± 17.0     | 192 ± 10.3     |
| Ca (mg/dL)       | 8.88 ± 1.89    | 6.87 ± 1.31    |
| P (mg/dL)        | 125 ± 22.4     | 114 ± 15.7     |
| Mg (mg/dL)       | 77.5 ± 15.7    | 79.1 ± 8.77    |

Some urine data were shown in ratio to creatine levels. No significant difference in all items using t-test (WT, KO: n = 6 for each). Abbreviations: WT; wild type, KO; knock-out, ALB; albumin, CRE; creatine, UA; uric acid, TP; total protein, BNU; blood urea nitrogen.
### Table S4.
**Property of salt responsive cells**

|                     | Amiloride sensitivity | NaCl  | KCl  | NMDG-Cl | Na-gluconate | Anion dependency |
|---------------------|-----------------------|-------|------|---------|--------------|------------------|
|                     |                       | 100 mM| 500 mM| 500 mM  | 500 mM       | 250 mM           |
| High salt cell      | −                     | −     | +    | +       | +            | weak             |
|                     | −                     | −     | +    | +       | +            | +                |
| Low salt cell       | +                     | +     | +    | −       | −            | +                |

Plus (+) and minus (-) indicate responsive and not responsive respectively. Weak means that the response of 250 mM Na-gluconate is weaker than that of NaCl.