

## ***Supplemental Information***

### **Culture medium**

- **TR-iBRB2 cells**

DMEM with 10% fetal bovine serum, 20 mM NaHCO<sub>3</sub>, 70 mg/L benzylpenicillin, and 100 mg/L streptomycin

- **RPE-J cells**

DMEM with 4% fetal bovine serum, 20 mM NaHCO<sub>3</sub>, 25 mM D-glucose, 0.1 mM nonessential amino acids, 60 mg/L benzylpenicillin, and 125 mg/L streptomycin

### **Buffer contents**

- **Extracellular fluid (ECF) buffer**

122 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose, and 10 mM HEPES, pH 7.4

- **Na<sup>+</sup>-free ECF buffer**

122 mM LiCl, 25 mM KHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose, and 10 mM HEPES, pH 7.4

- **Cl<sup>-</sup>-free ECF buffer**

122 mM Sodium gluconate, 25 mM NaHCO<sub>3</sub>, 3 mM potassium gluconate, 1.4 mM calcium gluconate, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose, and 10 mM HEPES, pH 7.4

- **K<sup>+</sup>-replacement ECF buffer**

125 mM KCl, 25 mM KHCO<sub>3</sub>, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose, and 10 mM HEPES, pH 7.4

- **Standard oocyte saline (SOS) solution**

100 mM NaCl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM HEPES, 25 µg/mL gentamycin, 2.5 mM pyruvate, and 1% bovine serum albumin, pH 7.5

- **ND96 solution**

96 mM NaCl, 2mM KCl, 1mM MgCl, 1.8 mM CaCl<sub>2</sub>, and 5 mM HEPES, pH 7.4

## **Statistical analysis**

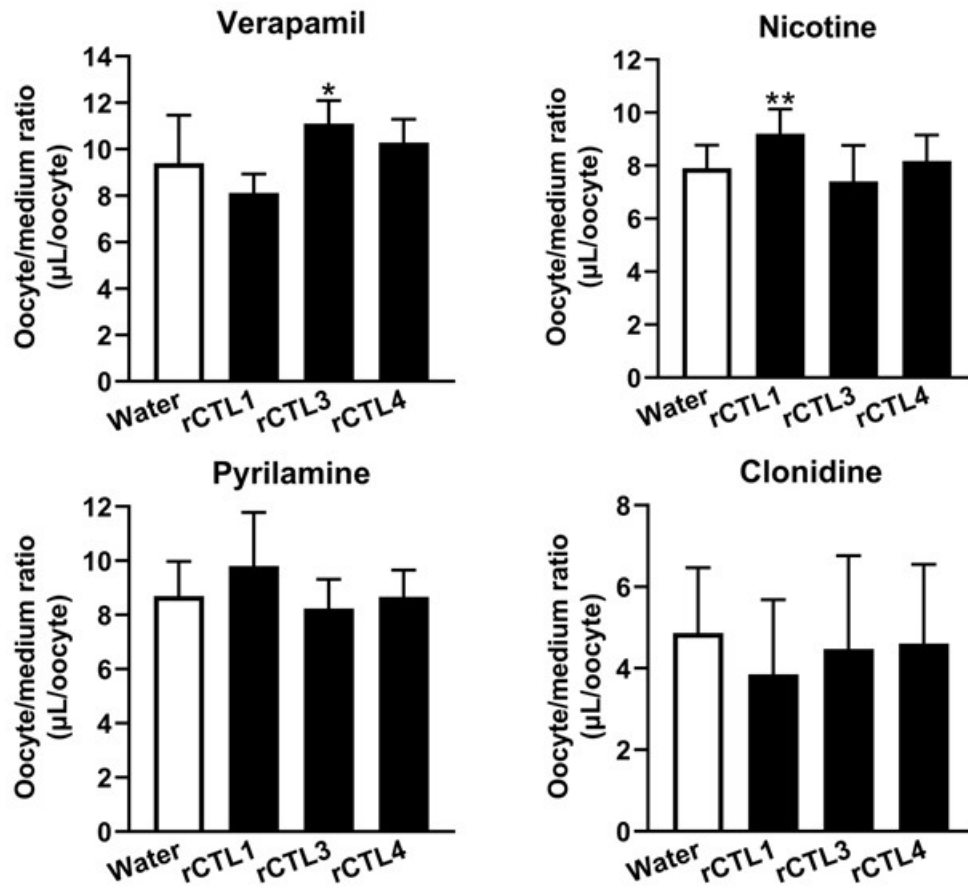
Statistical analyses were carried out using a one way analysis of variance (ANOVA) followed by Dunnett's test and an unpaired two-tailed Student's t-test for several and two groups, respectively. Unless otherwise indicated, all data represent means ± S.D.

**Table S1. Primers for full-length cDNA cloning**

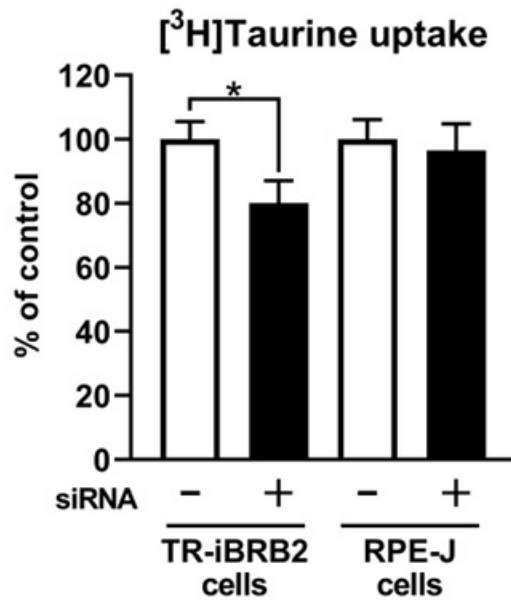
| Genes | Gene Bank Accession # | Orientation | Primer sequence (5' to 3')    | Restriction Enzyme |
|-------|-----------------------|-------------|-------------------------------|--------------------|
| rCTL1 | NM_053492.3           | Forward     | TCTAGAATGGGCTGCTGCAGCTCCGCC   | <i>Xba</i> I       |
|       |                       | Reverse     | AAGCTTTTACCTTTTCTGAGCATCGGCTT | <i>Hind</i> III    |
| rCTL3 | NM_001013914          | Forward     | TCTAGATTCATCATGGGTACTCGGTGGT  | <i>Xba</i> I       |
|       |                       | Reverse     | AAGCTTCTATCTCACAATGGGCCGGAGTT | <i>Hind</i> III    |
| rCTL4 | NM_212541             | Forward     | TCTAGATGAGCCATGGGGAAAAAGCA    | <i>Xba</i> I       |
|       |                       | Reverse     | GCAGTCGTGGGTACGATGA           | <i>Not</i> I       |

**Table S2. Primers for RT-PCR analysis**

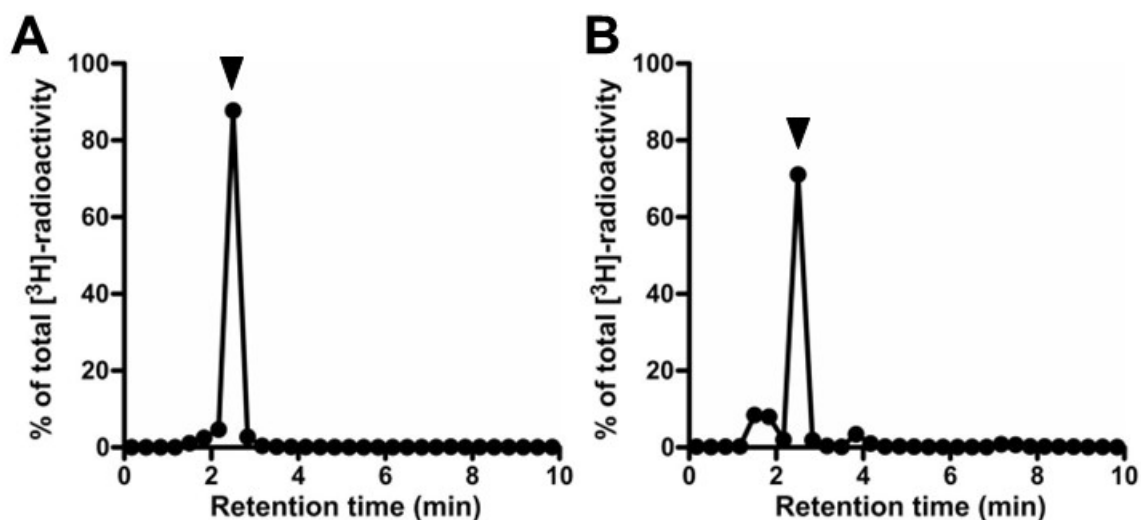
| Target mRNA | Gene Bank Accession # | Orientation | Primer sequence (5' to 3') | Product size (bp) |
|-------------|-----------------------|-------------|----------------------------|-------------------|
| rCTL1       | NM_053492.3           | Forward     | CATGTGGTGGTACCACGTGGTC     | 162               |
|             |                       | Reverse     | CGAATAAGGCGGTTCCTGATGC     |                   |
| rCTL2       | NM_001134715.1        | Forward     | ATGTGCTCCATGCTCTACC        | 176               |
|             |                       | Reverse     | TTCAATGGGAAGGTCTCTGG       |                   |
| rCTL3       | NM_001013914          | Forward     | TCCAAGAATTCAAGTCACCTCACG   | 243               |
|             |                       | Reverse     | GACAGCAAAGCACAGGAAAAGTGT   |                   |
| rCTL4       | NM_212541             | Forward     | ATCTACCACTGCTGGCAACAGTAC   | 278               |
|             |                       | Reverse     | AGGAGGACGAAGGTGACCAGAG-    |                   |



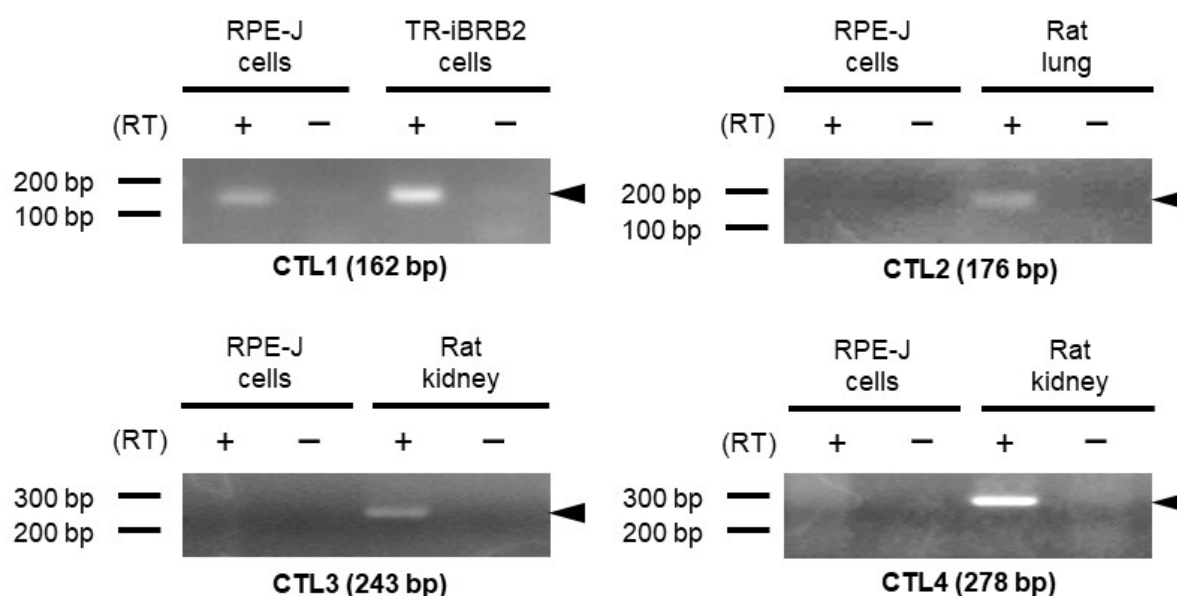
**Figure S1. Uptake of cationic compounds by *Xenopus laevis* oocytes.** Uptake of [<sup>3</sup>H]verapamil (0.45 μCi), [<sup>3</sup>H]nicotine (0.45 μCi), [<sup>3</sup>H]pyrilamine (0.45 μCi), and [<sup>3</sup>H]clonidine (0.45 μCi) by water-, rCTL1, rCTL3 or rCTL4 cRNA-injected oocytes was examined at 20°C for 60 min. Each column represents the mean ± S.D. (n = 9–30). \**p*<0.05, \*\**p*<0.01, significantly different from water-injected oocytes.



**Figure S2. Effect of rCTL1 knockdown on the uptake of [<sup>3</sup>H]taurine.** TR-iBRB2 cells and RPE-J cells were transfected with negative control siRNA (-) or rCTL1 siRNA (+). The uptake of [<sup>3</sup>H]taurine (0.1  $\mu$ Ci) by siRNA-transfected TR-iBRB2 cells and RPE-J cells was measured at 37°C for 10 min. Each column represents the mean  $\pm$  S.D. (n = 3). \* $p$ <0.05, significantly different from negative control siRNA-transfected cells.



**Figure S3. HPLC chromatograms of the  $[^3\text{H}]$ putrescine solution (A) and tissue (retina and vitreous humor) samples (B) after the microdialysis study.** Retina and vitreous humor were obtained after microdialysis study and HPLC analysis was performed at a flow rate of 1.0 mL/min with Inertsil ODS-3<sup>®</sup> (3  $\mu\text{m}$ ) (GL Sciences Inc., Tokyo, Japan) and EP-300 (Eicom, Kyoto, Japan). Mobile phase contains 121 mM sodium acetate, 7.8 mM sodium octanesulfonate, and 22% acetonitrile. The  $[^3\text{H}]$ -derived radioactivity in each eluent was determined using AccuFLEX LSC7400 (Nippon RayTech Co., Ltd., Tokyo, Japan). Arrowheads indicate the retention time of  $[^3\text{H}]$ putrescine.



**Figure S4. mRNA expression of rat CTLs in RPE-J cells.** RT-PCR analysis was performed with total RNA prepared from RPE-J cells in the presence (+) or absence (-) of reverse transcriptase (RT). Total RNA prepared from TR-iBRB2 cells, rat lung, and rat kidney was used as positive control sample. Arrowhead indicates predicted product size. Primers are shown in Table S2.