Supplemental materials

Supplementary materials and methods

Rat isolated retinal pigmented epithelial cells

Rat isolated retinal pigmented epithelial (RPE) cells were prepared as described previously with minor modifications. Briefly, the enucleated eyes of Long-Evans rats (Institute for Animal Reproduction, Ibaraki, Japan) were washed with Hank's Balanced Salt Solution (HBSS; 138 mM NaCl, 5.3 mM KCl, 5.6 mM D-glucose, 0.44 mM KH₂PO₄, 4.2 mM NaHCO₃, 0.34 mM Na₂HPO₄, 0.02 mM phenol red) after cleaning with 5% povidone-iodine solution. For the separation of RPE cells from the neural retina, eyes were incubated in HBSS containing 38 U/mL testicular hyaluronidase (Worthington Biochemical, NJ) and 20 U/mL collagenase type II (Worthington Biochemical) for 30 minutes at 37°C, followed by incubation in HBSS containing 0.1% trypsin for 30 minutes at 37°C. The RPE cell suspension was obtained after rinsing with HBSS and then washing twice with phosphate-buffered saline (PBS(-), 137 mM NaCl, 8.1 mM Na₂HPO₄, 2.7 mM KCl, 1.5 mM KH₂PO₄).

Quantitative real-time reverse transcription-polymerase chain reaction
Total RNA from freshly isolated rat RPE cells was prepared by using an RNeasy® Mini kit (QIAGEN, Hilden, Germany). Complementary DNA was prepared from total RNA by ReverTra Ace (TOYOBO, Osaka, Japan). Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed using Mx3000P (Agilent Technologies, Santa Clara, CA) with SYBR® Premix Ex TaqTM II (Takara, Shiga, Japan) and specific primers2 (Table S1) via 40 cycles of 95°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds. A standard curve was generated for each run using a plasmid (pGEM-T Easy Vector; Promega, Madison, WI) containing the target gene. The expression level of each mRNA was normalized with respect to that of β-actin mRNA.

Supplementary table

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>GenBank Accession No.</th>
<th>Primer sequences (Upper, sense primer; Lower, antisense primer)</th>
<th>Product size (bp)</th>
</tr>
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<tbody>
<tr>
<td>mdr1a (abcb1a)</td>
<td>NM_133401</td>
<td>5'-acagaaaaacagagagtccg-3' 5'-cgtcttgatcatgtggcc-3'</td>
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<tr>
<td>mdr1b (abcb1b)</td>
<td>NM_012623</td>
<td>5'-acagaaaaacagagagtccg-3' 5'-agaggcaccaggtgtcact-3'</td>
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<td>β-actin</td>
<td>NM_031144</td>
<td>5'-ctatgaagttggtgccacgtcgtc-3' 5'-cctagaagccattttgcgcggtgcagtg-3'</td>
<td>285</td>
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</table>
Supplementary Figure S1

Supplementary figure legend

Supplementary Figure S1. Quantitative real-time RT-PCR analysis of mdr1a and mdr1b in freshly isolated rat RPE cells. Each column represents the mean ± SE (n=3 measurements).

References
