SUPPLEMENT

Cell culture
Human RPE cells obtained from several donor eyes were prepared and cultured as following. After removing the vitreous and the retina, the RPE cells were mechanically harvested, separated by a digestion with 0.05% trypsin and 0.02% EDTA and washed two times with phosphate-buffered saline. The cells were suspended in complete Ham F-10 medium containing 10% fetal bovine serum, glutamax II, and gentamycin, and were cultured in tissue culture flasks (Greiner, Nürtingen, Germany) in 95 % air/5 % CO₂ at 37°C. Cells of passages 3 to 5 were used. The epithelial nature of the RPE cells was routinely identified by immunocytochemistry using the monoclonal antibodies AE1 (recognizing most of the acidic type I keratins) and AE3 (recognizing most of the basic type II keratins), both from Chemicon. All tissue culture components and solutions were purchased from Gibco BRL (Paisley, UK).

RT-PCR
Total RNA was extracted from freshly isolated human RPE cells or from cultured cells by using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The quality of the RNA was analyzed by agarose gel electrophoresis. The $A_{260}/A_{280}$ ratio of optical density was measured using the GeneQuantpro device (Pharmacia, Uppsala, Sweden), and was between 1.9 and 2.1 for all RNA samples, indicating sufficient quality. After treatment with DNase I (Roche), cDNA was synthesized from 1 µg total RNA using the RevertAid H Minus First Strand cDNA Synthesis kit (Fermentas, St. Leon-Roth, Germany). PCR was performed using the Taq PCR Master Mix kit (Qiagen) and the primer pairs described in Table 1. One µl of the first-strand mixture and 0.5 µM of each gene-specific sense and anti-sense primer were used for amplification in a final volume of 20 µl. Amplification was performed for 40 cycles with the PTC-200 Thermal Cycler (MJ Research, Watertown, MA). Each cycle consisted of 30 s at 94°C, 60 s at 60°C, and 2 min at 72°C.

Real-time PCR
RPE cell cultures were treated with thrombin for 2, 4, 6 or 24 hours, and the relative mRNA levels in comparison to unstimulated control was determined. Semi-quantitative real-time RT-PCR was performed with the Single-Color Real-Time PCR Detection System (BioRad, Munich, Germany) using the primer pairs described in Table 1. The PCR solution contained 1
µl cDNA, specific primer set (0.3 nM each) and 10 µl of a 2x mastermix (QuantiTect SYBR Green PCR Kit; Qiagen) in a final volume of 20 µl. The following conditions were used: initial denaturation and enzyme activation (one cycle at 95°C for 15 min); denaturation, amplification and quantification, 45 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for one minute; melting curve, 55°C with the temperature gradually increased (0.5°C) up to 95°C. The amplified samples were analyzed by standard agarose gel electrophoresis. The mRNA expression was normalized to the levels of GAPDH mRNA. The changes in mRNA expression were calculated according to the 2^ΔΔCT method (CT, cycle threshold), with ΔCT = CTTarget gene - CTGAPDH and ΔΔCT = ΔCTtreatment - ΔCTcontrol.

**Chemotaxis**

Suspensions of RPE cells (100 µl; 5 x 10⁵ cells/ml serum-free medium) were seeded onto polyethylene terephthalate filters (diameter 6.4 mm, pore size 8 µm; Becton Dickinson, Heidelberg, Germany) coated with fibronectin (50 µg/ml) and gelatin (0.5 mg/ml). Within 16 hours after seeding, the cells attached to the filter and formed a semiconfluent monolayer. The cells were pre-treated with blocking substances for 30 minutes and thereafter the medium was changed into medium without additives in the upper well and medium containing thrombin and the appropriate blockers in the lower well. After incubation for 6 hours, the inserts were washed with buffered saline, fixed with Karnofsky’s reagent, and stained with hematoxylin. Nonmigrated cells were removed from the filters by gentle scrubbing with a cotton swab. The migrated cells were counted, and the results were expressed relative to the cell migration rate in the absence of test substances.
Table S1. Primer pairs used for PCR experiments.

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<th>Gene and Accession</th>
<th>Primer sequence (5’→3’)</th>
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s, sense. as, anti-sense.