Effects of doxycycline in cell viability and VEGF production by RPE cells.

A) Monkey RPE cells were incubated for 24 hours with increasing concentrations of doxycycline (x-axis). Cell viability was followed by CellTiter-Glo® and is measured by luminescence of intracellular ATP production by luciferase (y-axis). Each bar in the graph represents the average of cell viability measurements of 8 wells.

B) VEGF standard curve in media and in the presence or absence of 50 μM doxycycline measured by ELISA.

C) Effects of doxycycline on VEGF production by RPE cells after 6 hours incubation with media containing increasing concentrations of doxycycline (x-axis). Each bar in the graph represents the average of VEGF levels determined from 8 wells. The percentage of the ratio of VEGF protein levels in media per viable cells in each well was plotted (y-axis).
Effects of doxycycline on PEDF protein production by RPE cells.
A) PEDF standard curves in the present or absence of 50 μM doxycycline measured by ELISA.
B) Effects of doxycycline (x-axis) in PEDF production by mRPE cells after 6 hours incubation. Each bar in the graph represents the average of PEDF concentration values in RPE conditioned media from 12 wells. The percentage of the ratio of PEDF protein levels in media per viable cells measured with CellTiter-Glo® was plotted (y-axis).
C) The ratio of VEGF and PEDF (y-axis) vs. doxycycline concentration (x-axis) was plotted.
Supplementary Methods:

**Monkey RPE cell cultures.** Non-transformed monkey retinal pigment epithelial cells (RPE) from primary cultures (a generous gift from Bruce Pfeffer) (B.A. Pfeffer, Improved methodology for cell culture of human and monkey retinal pigment epithelium. In: N.N. Osborne and G.J. Chader, Editors, *Progress in Retinal Research* vol. 10, Pergamon Press, Oxford, UK (1990), pp. 251–291.) at passages 6-7 were seeded on 24-well plates. Seeding density was $15 \times 10^4$ cells/well as determined by hemocytometer counting. Cells were cultured at $37^\circ$C with 5% CO$_2$ with a humid atmosphere. The nutrient media consisted of DMEM/F12, 50/50 without glutamine supplemented with 200 mM L-glutamine, 100 mM Na-pyruvate, 10 mM non-essential amino acids, 10,000 units/ml Penicillin, 10,000µg/ml Streptomycin and 5% fetal bovine serum. Once confluent, cells were washed with PBS 1X, and serum-free media was replaced at a volume of 450 µl per well. Cells were continued to culture for the indicated period of time.

**Cell viability assay:** The CellTiter-Glo® (Promega, Madison, WI) a Luminescent Cell Viability Assay was used following manufacturer’s instructions to determine the number of viable cells in culture. The method is based on quantification of cellular ATP as an indicator of metabolically active cells. Briefly, cells were washed with HBS buffer (10 mM Hepes, 150 mM NaCl, pH 7.4) and incubated 10 minutes in 200 µl of HBS plus 200 µl of CellTiter-Glo® before determining luminescence with a plate reader. All measurements were performed using the Envision 2104 multilabel reader (Perkin Elmer, Waltham, Mass.). The results analyzed using an Excel spreadsheet (Microsoft).

**Enzyme Linked Immunoabsorbent (ELISA) Assay:** Conditioned media were collected, centrifuged at 13,000 rpm for 15 minutes and used immediately or stored frozen until used. VEGF and PEDF protein concentrations were quantified in the conditioned media by a sandwich ELISA using the Quantikine humanVEGF kit (R&D Systems, Inc., Minneapolis, MN, USA) and the ELISA quant PEDF kit (BioProducts MD), respectively. All of the assays were according to the manufacturer’s instructions. Each assay was performed in more than four replicates per condition. Standard curves of recombinant human VEGF, recombinant human PEDF were prepared.