Supplemental Methods

**Cell Culture**

For co-cultures, a suspension of pericytes was added to confluent monolayers of HRECs at various concentrations depending on the experiment. For N-cadherin immunostaining, 50-100 pericytes were added to a confluent layer of HREC (~$1X10^5$ cells) grown on a 10mm diameter glass coverslip. For other co-culture experiments, an equal number of pericytes, endothelial cells or MIO-M1 cells were added to the pre-established endothelial monolayers.

Conditioned media were prepared from pericytes, endothelial cells or MIO-M1 cells passaged one day prior. Fresh complete media was added and the cells were incubated for 16 hours prior to collecting the conditioned media. In some experiments cells were treated with anti-TGF-β antibody (50ng/ml; Abcam ab30838), recombinant human Tie-2 Fc chimera (200ng/ml; R&D Systems 313-TI), purified S1P (1-10μM; Tocris Bioscience), the S1P1 antagonist VPC23019 (0.3μM; Avanti Polar Lipids), or the S1P2 antagonist JTE-013 (1μM; Tocris Bioscience).

**Knockdown of Sphk1, S1P1 and N-cadherin in Pericytes and Endothelial Cells**

The expression of sphingosine kinase 1 (SphK1) in pericytes and S1P1 and N-cadherin in endothelial cells was reduced using morpholino oligos (GeneTools, LLC, Philomath, OR). Cells were incubated for 16 hours with the appropriate Morpholino (10μM) and Endo-Porter as per the manufacturers protocol and collected to confirm the loss of protein expression by Western blotting. Controls included Endo-Porter alone or cells incubated with a 5-mispair oligo (control morpholinos). Sequences of the morpholinos used are as follows;

hSphk1: AAAATCCCAGAACTTGAGCGGACAT
hSphk1 mispair: AAATTGCCACAACTTCACCGGACAT
hS1P1: TGCCAACCCTGTGTCCCCAGGAAGC
hS1P1 mipair: TGCCAACCGTCTGTGCCGACGAAGC
N-cadherin: CTCCCGCTATCCGGCACATGGAGGC
N-cadherin mispair: CTCGCCCTATCCCGCAGATCGAGGC

**Electrical Cell-substrate Impedance Sensing**

HREC ($1X10^5$) were plated into multiwall chambers (8W10E+, Applied Biophysics, Troy, NY) containing 40, 250μm diameter gold microelectrodes per well. The electrodes were coated with fibronectin prior to the addition of cells. The cells were grown for 16 hours until maximum resistance was attained (~1200 Ω). Additional cells (pericytes, endothelial cells or MIO-M1 cells) or conditioned media were added to each well and monitoring was continued for up to 20 hours. The resistance values for multiple wells were normalized to the identical starting resistance value at time 0 and the data from 3 wells were averaged and presented as normalized resistance versus time. In some cases the maximum achieved resistance as a percent over the basal resistance level at time 0 was determined.

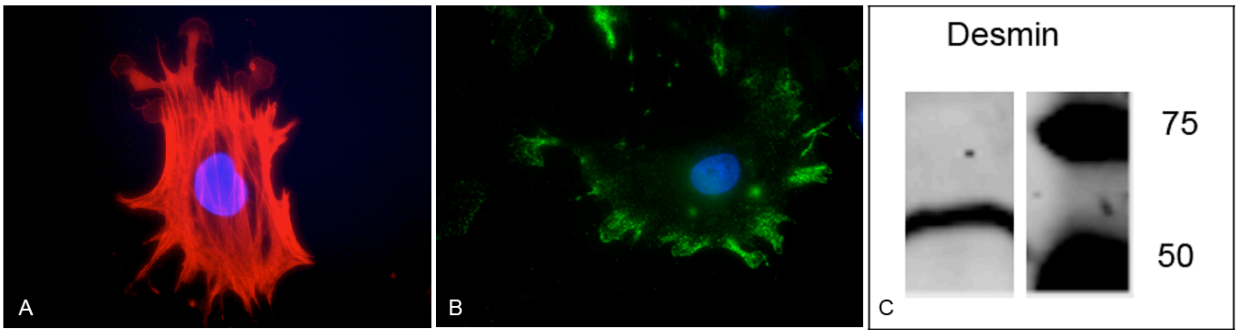
**S1P Quantification**

The S1P concentration in the conditioned media of pericytes was assessed by S1P ELISA (Echelon Biosciences Inc. Salt Lake City, UT). The conditioned media from ~$1X10^6$ cells was concentrated 2.5 fold using a 3kDa filter and applied to the ELISA plate. ELISA was performed according to the manufacturer's instructions.

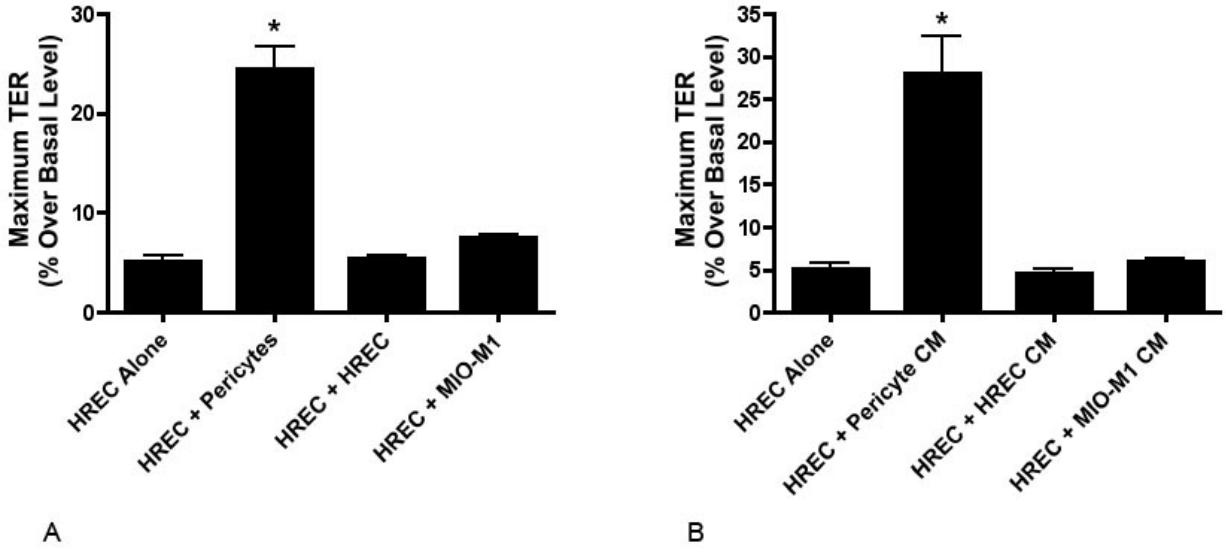
**Delipidation of Pericyte Conditioned Media**

Pericyte conditioned media or fresh complete media was collected and passed over two consecutive solid phase extraction columns containing Octadecyl (C18) sorbant (Burdick and Jackson, VWR) and subsequently used in ECIS experiments.

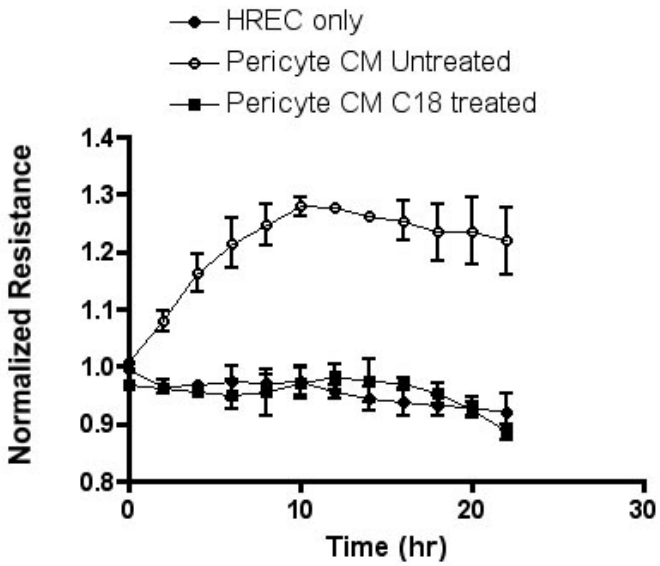
Supplemental Data



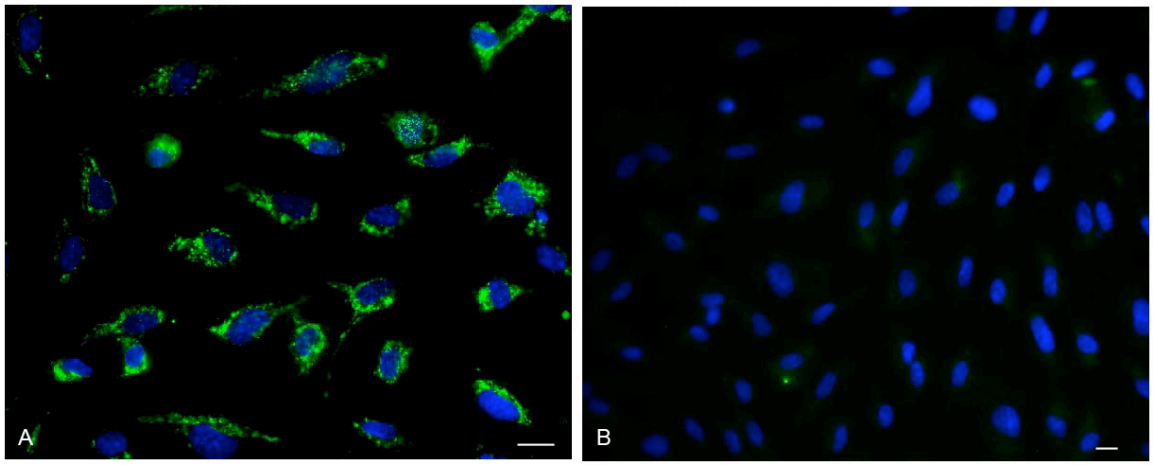

**Supplemental Figure I**. Characterization of pericytes used for these studies. The human retinal pericytes were positive for α-smooth muscle actin (A), the PDGF β-receptor (B) and desmin (C).



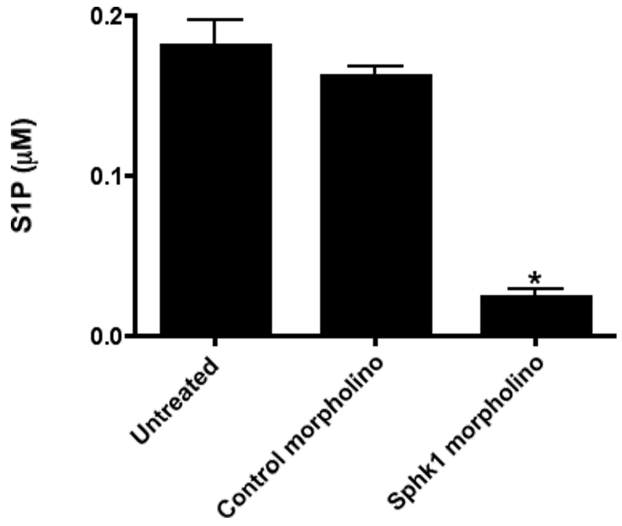

**Supplemental Figure II**. (A) HREC alone reach a maximum resistance of 5.1% above basal levels at time 0, whereas HREC with added pericytes increase resistance 25.8% above basal levels. (B) HREC treated with pericyte conditioned media (CM) also demonstrate a significantly greater change in resistance (28.3%) compared to HREC alone. No significant change in resistance above basal levels was seen in monolayers to which HREC or MIO-M1 cells or conditioned media were added. Bars represent pooled values from three different experiments. * Significanly greater ($P<0.0001$).



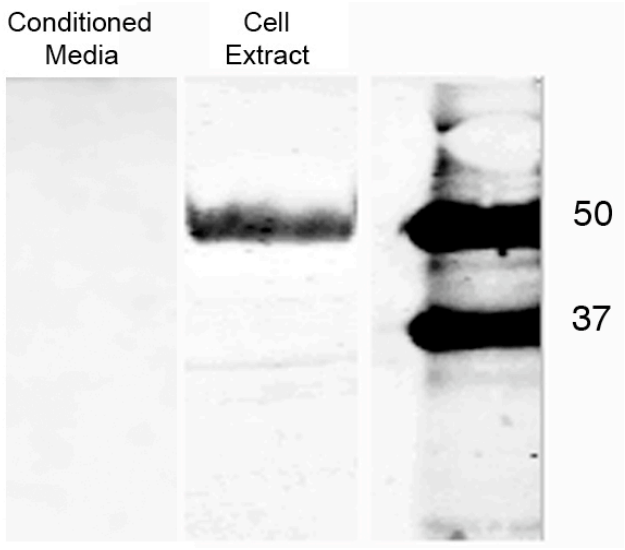

**Supplemental Figure III**. Delipidated pericyte conditioned media looses the ability to enhance the endothelial monolayer resistance. Media were delipidated by passage over a C18 column and used for ECIS analysis. HREC only grown in delipidated fresh complete media and HREC exposed to C18-treated pericyte conditioned media demonstrated significantly lower resistance than untreated pericyte conditioned media (CM).



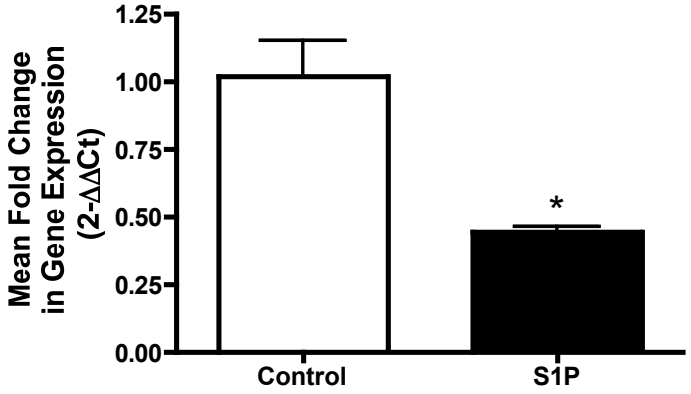

**Supplemental Figure IV**. Confocal image of human retinal endothelial cells 8 hours after the addition of pericyte conditioned media (A) or pericyte conditioned media in the presence of the S1P1 antagonist VPC23019 (0.3μM) (B). Cells are stained for N-cadherin (green) and DNA (blue). Bar = 10μm.




**Supplemental Figure V**. Sphingosine 1-phosphate in the conditioned media of pericytes. Pericyte conditioned media from a culture of approximately $1X10^6$ cells contains approximately 180nM S1P as determined by ELISA. No significant loss of S1P was detected when pericytes were treated with control Sphk1 morpholinos whereas cells treated with the Sphk1 morpholinos demonstrated a significant loss of S1P in the media. *Significantly less than untreated and control morpholino-treated cells ($P<0.001$).




**Supplemental Figure VI**. Western blot analysis of the sphingosine kinase-1 enzyme in cell extracts of human retinal pericytes. The protein was not found in the conditioned media of the cells.




**Supplemental Figure VII**. Sphingosine 1-phosphate decreases the expression of Ang-2 in humanretinal endothelial cells. Human retinal endothelial cells were incubated alone or in the presenceof S1P (10μM) for 12 hours and the level of Ang-2 mRNA determined by real-time RT-PCR. *Significantly less than control (P=0.0125).