Supplementary information, Data S1 Materials and Methods

siRNA Transfection and Invasion Screening

Pooled siGENOME siRNAs (Dharmacon, Lafayette, CO) were reverse transfected into 1 x 10^5 1CTP and 1CTR HCECs using RNAiMAX per manufacturer's instructions. Briefly, siRNAs and RNAiMAX were diluted in OptiMEM medium and cells were resuspended and plated in 2% serum conditions. Twenty-four hours later, transfected cells were washed with PBS and replaced with serum-free medium overnight for starvation. For invasion assays, 24-well Matrigel-coated transwell filters (BD Biosciences, San Jose, CA) were thawed and rehydrated according to manufacturer's instructions. Transfected cells were collected, resuspended in 650μL serum-free medium, and 300μL were added to the top chamber in duplicates. The bottom chamber is filled with 600μL 2% serum medium plus growth supplements as a chemoattractant. After overnight incubation, non-invaded cells were scraped off with a cotton swab and wells were washed with PBS. Invaded cells were fixed for 5min in 10% neutral buffered formalin and stained for 10min with 10μg/mL Hoechst (Invitrogen, Grand Island, NY). Images were taken at 10X with five fields imaged per transwell.

Statistical Analysis

Two independent investigators counted the number of cells per field per transwell. These counts were averaged to calculate the fold change relative to control siRNAs. Any siRNA pools that induced a fold change in invasion greater than 3x standard deviations were considered significant.

F-actin Staining

1CTP HCECs were reverse transfected with siRNAs according to methods described above for three days before re-seeding into chamberslides. Cells were washed with PBS, fixed in 4% paraformaldehyde for 10min, and permeabilized with 0.1% Triton X-100. Fixed cells were then stained for F-actin using Alexa Fluor 488 phalloidin (Invitrogen, Grand Island, NY) according to manufacturers instructions.

Lentiviral shRNA Infection

293FT cells were transfected using PolyJet DNA transfection reagent (SignaGen Laboratories, Rockville, MD) with 1ug of the appropriate pGIPZ library vector (and 1ug of helper plasmids (pMD2G and psPAX2). Viral supernatants were collected 24 and 48 hrs post-transfection and cleared through a 0.45uM filter. 1CTP HCECs were infected for 4-6hrs with viral supernatants containing 4ug/mL polybrene (Sigma, St. Louis, MO) and selected with puromycin (Sigma, St. Louis, MO). Successfully infected cells were GFP positive and resistant to three-daypuromycin selection.

Quantitative Real-Time PCR

Total RNA were isolated from semi-confluent HCECs using RNeasy Plus Mini Kit (Qiagen, Valencia, CA) and reverse transcribed using iScriptcDNA Synthesis Kit (BioRad, Hercules, CA) according to manufacturers instructions. Primers were designed using PrimerBank (http://pga.mgh.harvard.edu/primerbank) andobtained from Sigma. Primer sequences are listed in **Supplementary information, Table S4**. Quantitative PCR were conducted in triplicate reactions using LightCycler 480 SYBR Green I Master mix (Roche, Basel, Switzerland) and run on a LightCycler 480 Real-Time PCR System using the following amplification settings: 45 cycles at 95°C for 10sec, 60°C for 15sec, and 72°C for 40sec. The housekeeping gene GAPDH was used as an internal control and data were normalized to a non-silencing shRNA.

Proliferation Assay

HCECs were reverse transfected with pooled siRNAs in clear-bottom 96-well plates using the previously described method. Fresh culture medium were added to each well 48hrs post-transfection and cell viability were measured 96hrs post-transfection using CellTiter-Glo Luminescent Assay (Promega, Madison, WI) according to manufacturer's instructions. Luminescent ATP levels were detected by an Envision plate reader (Perkin-Elmer, Waltham, MA) and normalized to a non-targeting siRNA.