ONLINE METHODS

Cell culture and reagents. HUVECs (Lonza) were cultured in EBM-2 media (Lonza) supplemented with bullet kit containing various growth factors (Lonza) and 10% FCS (Hyclone). bEnd.3 cells were obtained from American Type Culture Collection and cultured in complete DMEM. For starvation experiments, serum-free and growth factor–free medium was used. MDA-MB-231Lm2-4 Luc$^+$ cells, a fast-growing variant of human breast carcinoma cells[35], were a gift from R. Kerbel (University of Toronto). FG human pancreatic adenocarcinoma cells were a gift from S. Kajiji (Scripps Research Institute) and are described elsewhere[40]. R40P mouse pancreatic carcinoma cells were derived from a primary tumor in a pancreas-specific *Ink4a*-deficient, oncogenic K-Ras G12D–expressing mouse[1], and RCP30 cells were derived from a primary tumor in a pancreas-specific *Csk*-deficient, oncogenic K-Ras G12D–expressing mouse (D.J.S. and D.A.C., unpublished observations). WA09 (H9) human embryonic stem cells were cultured in KO-DMEM (Gibco) supplemented with 15% Knockout (KO) Serum Replacement (SR) (Gibco) and 20 ng ml^{-1} bFGF (Chemicon) on gamma-irradiated hs27 human fibroblast cells (American Type Culture Collection). Undifferentiated human embryonic stem cell colonies were manually dissected and allowed to form embryoid bodies in suspension for 5–7 d in KO-DMEM with 15% KO-SR. Embryoid bodies were seeded onto collagen-coated dishes and allowed to differentiate. Cultures were grown in DMEM supplemented with N2 supplement (Invitrogen), 20 ng ml^{-1} bFGF (Chemicon) and 25 ng ml^{-1} VEGF (Peprotech). Fresh medium was added every other day for up to 28 d.

Antibodies to p120RasGAP (SC-63), angiomotin (SC-82491), SMAD5 (SC-26418), TMEFF1 (SC-98956), SIRT1 (SC-15404) were from Santa Cruz Biotechnology. Antibodies to mouse CD31 (550274), human CD31 (550389), VE cadherin (555661) and GCF2 (also known as LRRFIP1) (612161) were from BD Biosciences. β-actin–specific antibody (A5316) was from Sigma. Antibodies to CREB (9104) and p-CREB (9191) were from Cell Signaling. SiRNAs targeting p120RasGAP were from Qiagen. The MEK inhibitor PD0325901 was from Chemietek.

Vectors and plasmids. The mutant *Rasa1* 3′ UTR was generated by replacing both copies of the miR-132 seed sequence GACTGTT with TGTCAAG in a ~70-bp fragment of the wild type 3′ UTR as shown below (the seed sequence is underlined) and by ligation into the pmiR-REPORT vector (Ambion).

Wild-type 3′ UTR: 5′-TGTGTATAACTGGATTGCAGACTGTTCTTACTG TAACTACTTCCTGATTAGGAATATGACCATTTGACTGTTC-3′

Mutant UTR: 5′-TGTGTATAACTGGATTGCATGTCAAGCTTACTGTAA CTACTTCCTGATTAGGAATATGACCATTTTGTCAAGC-3′

The miR-resistant *Rasa1* construct was generated by cloning the *Rasa1* cDNA from the start codon until the stop codon, thereby excluding the 3′ UTR region, into the pCDH vector backbone (CD511-B1 from System Biosciences). An empty backbone vector was used as a control.

MicroRNAs and anti-microRNAs. Control miRNA, control anti-miRNA, miR-132 and anti–miR-132 were from Ambion: miR-132, 5′-UAACAGUCUACAG CCAUGGUCG-3′; Anti–miR-132: 5′-CGACCATGGCTGTAGACTGTTA-3′. For *in vivo* studies that involved large doses of anti-miRNA treatments, oligomers with the same sequence were synthesized on a larger scale from Sigma.

Scrambled anti-miRNA: 5′-[mA][mU][mU][mU][mC][mA][mU][mG] [mA][mC][mU][mG][mU][mU][mA][mC][mU][mG][mA][mC][mC] [mU]-3′

Anti–miR-132: 5′-[mC][mG][mA][mC][mC][mA][mU][mG][mG][mC][mU] [mG][mU][mA][mG][mA][mC][mU][mG][mU][mU][mA]-3′

For some experiments, the miRNAs and anti-miRNAs were obtained with a 5′ Cy3 label. There was no appreciable difference between the control oligomers from Ambion or scrambled oligomers from Sigma in the assays (proliferation, tube formation and Matrigel) in which they were used as controls.

RNA extraction, reverse transcription PCR and miRNA profiling. RNA was extracted with the miRVana microRNA isolation kit (Ambion), and RT-PCR was performed with multiplexed TaqMan primers (Applied Biosystems). The miRNA profiles were generated with a 384-well microfluidic card–based TaqMan human microRNA panel (Applied Biosystems) amplified on a 7900 HT Fast Real Time PCR system (Applied Biosystems). Data were normalized to the internal control small RNA *RNU48*. Individual RT-PCRs were performed with TaqMan Assays (Applied Biosystems) on a SmartCycler (Cepheid) according to the manufacturers' instructions.

Proliferation assay. HUVECs were transfected with miRNAs or anti-miRNAs with siPORT-Neofx reagent (Ambion). BrdU was pulsed 48 h after transfection and cell proliferation was measured using an ELISA kit (Millipore). Ras activity was measured using a Ras Activity ELISA kit (Millipore) according to the manufacturer's instructions. An ELISA assay to measure phospho-MEK abundance was performed with a PathScan ELISA kit (Cell Signaling Technologies) according to the manufacturer's instructions.

Three-dimensional collagen tube formation assay. Tube formation assays were done as previously described[36]. Briefly, 24 h after transfection of miRNAs or anti-miRNAs, HUVECs were seeded on a 3.75 mg ml^{-1} type I collagen matrix (BD Biosciences) in a half-area 96-well plate (Sigma). Complete EBM-2 containing 10 μg ml^{-1} *Ulex europaeus* lectin (Vector Labs) was added 30 min later to enable live cell imaging. Tube lengths were measured and quantified with MetaMorph software (Molecular Devices).

MicroRNA *in situ* hybridization. The human hemangioma tissue array, human breast carcinoma tissue array and normal human tissue array were purchased from US Biomax. *In situ* hybridization was performed as previously described[37] with a digoxigenin-labeled miR-132 Locked Nucleic Acid (LNA) probe (probe sequence 5′-CGACCATGGCTGTAGACTGTTA-3′ Exiqon). Digoxigenin was detected by an digoxigenin-specific, horseradish peroxidase–labeled antibody (Roche) and amplified with a TSA-Plus Cy3 system (Perkin Elmer). miRNA expression was scored with Metamorph software (Molecular Devices).

Nanoparticle preparation. Liposomes incorporating an $\alpha_{v\beta}3$-targeting cyclic RGD peptide were prepared as previously described[1]. Lipid-RNA complexes were formulated with a molar ratio of 4:1 calculated based on the *N*-[1-(2,3-dioleoyloxy)]-*N*,*N*,*N*-trimethylammonium propane (DOTAP) content of the lipsomes. Nucleic acids (miRNAs or anti-miRNAs) and lipids were separately diluted in 100 μl RNase-free water. The RNA solution was added to the liposomes, mixed gently and the mixture was incubated at 25 °C for 5 min before injection into mice.

***In vivo* assays.** Mouse experiments were performed under approval by the University of California–San Diego Institutional Animal Care and Use Committee. Typically, 6- to 8-week-old female mice (Jackson Labs) were used for all experiments. *Ert2*-ubiquitin-Cre mice were a gift from E. Brown (University of Pennsylvania), and *Rasa1*$^{fl/fl}$ mice have been described elsewhere[20]. Growth factor–reduced Matrigel (BD) containing either PBS or 400 ng ml^{-1} recombinant human bFGF (Millipore) was injected subcutaneously into C57BL/6 mice. Mice were injected intravenously with 10 μg control or anti-miRNAs. After 5 d, mice were injected with 10 μg *Griffonia simplificola* lectin–FITC (Vector Labs), the plugs were collected and lysed in RIPA-buffer and the FITC content was measured on a spectrophotometer (Tecan). Retinal neovascularization studies were performed as previously described[38]. Briefly, 5 μg of control anti-miRNA or anti–miR-132 (both from Ambion) was injected into the vitreous cavity of 6-d-old neonatal BALB/c mice under light anesthesia. After 6 d, mice were killed and the retinas were dissected, fixed and stained with CD31-specific antibody. Mouse ID-8 VEGF ovarian carcinoma cells were a gift from G. Coukos (University of Pennsylvania) and have been previously described[39]. Mice were implanted with Matrigel plugs containing 50,000 GFP$^+$ ID8-VEGF cells and treated with intravenous injection of 10 μg of scrambled anti-miRNA or anti–miR-132 (both from Sigma) in vascular targeted RGD-nanoparticles[1] 2, 4, 6 and 8 d after implantation. Plugs were collected and processed as described above.

MDA-MB-231 human breast carcinoma cells (2×10^6) were injected into the mammary fat pad (fat pad no. 4) of nude mice. Mice were treated with 50 μg of scrambled anti-miRNA or anti–miR-132 (both from Sigma) in RGD-nanoparticles intravenously every 2 d starting from day 12 until the

end of the experiment. Injection of a single dose of anti–miR-132 in the RGD nanoparticles decreased tumor size transiently with a half-life of biological efficacy of 2 d (data not shown). Tumor volumes were calculated by the formula $V = (LW^2)/2$, where L and W denote the longer and shorter diameter, respectively.

For the orthotopic pancreatic tumor studies, 1×10^6 human pancreatic carcinoma cells were injected in 50 µl saline into the tails of pancreases of 6- to 8-week-old male nude mice as previously described[40]. The primary tumors were collected 6 weeks after injection and sectioned for immunofluorescent staining.

Immunofluorescence and microscopy. Imaging was performed on a Nikon Spectral C1 confocal microscope (Nikon C1si with EZC1 acquisition software, Nikon Instruments) with Plan Apo 10×/0.45 air, Plan Apo 20×/0.75 air and Plan Apo 60×/1.40 oil objective lenses (Nikon). All images were recorded with a sequential acquisition of the fluorescent channels to prevent fluorescence bleed-through. Images were analyzed with MetaMorph software (Molecular Devices) for determination of tube lengths, vessel density and colocalization. Image contrast and brightness parameters were adjusted across the whole image or equally across all the comparison groups when necessary. Staining intensity on immunohistochemistry slides was quantified on an arbitrary scale (0 (no staining) to 4 (strong staining)) by observers blinded to the sample identity.

Statistical analyses. All statistical analyses were performed with Excel (Microsoft) or Prism (GraphPad). Two-tailed Student's *t* test or Mann-Whitney *U* test was used to calculate statistical significance. A P value < 0.05 was considered to be significant.

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