Morpholinos: Based on the intron-exon and ATG boundary sequences, *snrk-1* morpholino (MO) phosphorodiamidate oligonucleotides were designed by Gene Tools, Inc. *Dusp-5* splice MO1 sequence: TCGCTTTCTTCTCTCTTACCTGATC. *Dusp-5* ATG (underlined) blocking MO2 sequence: GCAATCTATGCTGGAGACCTTCATG.

PCR: Reverse transcription was performed on 0.7–1.0 µg of total RNA isolated from specific and control siRNA transfected cells or tissue samples using oligo dT primers (0.1 µg). Cycling parameters used for PCR were: 94°C 2 min, 94°C 30 sec, 58°C 30 sec, 72°C 1 min, (34 cycles), 72°C 5 min. Primers used for human *dusp-5*: F, TATCCTGAGTGTTGCGTGGA; R, CGGAACTGCTTGGTCTTCAT; for human *dusp-5* exon 2: F, GGGGATATGAGACTTTCTACTCG; R, CTGGTCATAAGCTGGCCTGT; for human *snrk-1*: F1, ACAGACTTTGGGTTCAGCAACA, R1: TGGTTTCCAGGGCTTCTACAAT; and for human *b-actin*: F1, GGCATCCTCACCCTGAAGTA; R1, CTGCTGTCACCTTCACCGTTCC. Total RNAs from human hemangioma and placenta samples were kind gifts of Dr. Paula North (Medical College of Wisconsin). Genomic DNA from patient samples was isolated by DNeasy® Tissue kit (Qiagen) according to manufacturer's instruction. Primers used for diagnostic PCR for *dusp-5*: F, GCGTGGATGTAAAACCCATTC; R, GCTGAGTGGGGAGAGAACAG. The forward primer for control PCR was F: GCGTGGATGTAAAACCCATT. In the case of *snrk*-1 diagnostic PCR, F: TTGATGGTATAGGAAATCATTTTTG, and R: AGGTGTCCAAAGGCTGTGTT.

***In vitro* apoptosis assay:** The *in vitro* apoptosis assay was performed according to the manufacturer's (BD Biosciences) instructions. Briefly, HUVECs (P3) was contact inhibited for 5 days in endothelial cell growth medium (Lonza). Upon release, cells were transiently transfected with 100 ng of *lacZ* and dusp-5 siRNA as described before. Eight hour post transfection, cells were washed with PBS, collected by trypsin-EDTA, and re-suspended in 1× Annexin V binding buffer [10mM Hepes/ NaOH (pH 7.4) 140mM NaCl, 2.5mM $CaCl_2$]. Each sample was double stained with Annexin V-FITC and Propidium Iodide (PI) and incubated for 30 min in dark at room temperature. Unstained cells, cells stained with Annexin V alone, and cells stained with PI alone served as controls to set up compensation and quadrants; and 5% ethanol served as a positive control. Apoptotic cells were estimated as the number of Annexin V positive and PI negative cells as determined by flow cytometric analysis.

Adhesion assay: Briefly, HUVECs (p3) were collected 24 hr post transfection with 100 ng of *lacZ* and dusp-5 siRNA, washed with PBS, and re-suspended in serum free endothelial cell medium. The adhesion assay was carried out with 7500 cells per well of a 96-well plate pre-coated with laminin and fibronectin (25 µg/mL, Sigma-Aldrich) for 1 h at 30 min at 37°C. After 1 h, non-adherent cells were removed by aspiration and the adherent cells were fixed using 4% paraformaldehyde. Cells were stained with crystal violet and manually counted from 5 random fields at 40× magnification. For negative control, cells were added to uncoated wells.

Statistical analysis: Non-parametric Student's *t*-test and Fisher's exact test were performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. For adhesion and apoptosis assays, an unpaired two-tailed *t* test was used with statistical significance determined at $p<0.05$.

Human patient sample nomenclature: Samples 1, 8, and 23 are tissue from hemangioma patients. Samples 2, 3, 9, 10, 13, 18, 19, and 24 are tissue from lymphatic malformation patients. Samples 4, 14, 15, and 16 are tissue from arteriovenous malformation patients. Samples 5, 6, 12, 17, 20, and 21 are tissue from venous malformation. Sample 7 is from a patient with verrucous hemangioma, sample 11 is from a patient with complex mixed lymphatic-vein malformation and sample 22 is from a patient with a benign tumor of fatty tissue – lipoma.

Western immunoblotting for Phospho-Erk levels

Ten 24 hpf embryos from each group (Uninjected – UI and 6 ng Dusp5 MO1 injected – MO1) were washed once with ice-cold phosphate-buffered saline (PBS) and lysed in 100 µL of RIPA lysis buffer (150 mM NaCl, 1% IPEGAL© CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) for 2 h at 4°C. Lysates were clarified by centrifugation and 30 µL of lysate was separated on a 12% SDS-PAGE gel and transferred on a PVDF membrane. The lysates were probed for phospho and total ERK 1/2 using phospho p44/ 42 (1:1000) and total p44/ 42 antibodies (1:1000) (Cell Signal) respectively. The probed proteins were visualized by coupling with HRP-labeled anti-rabbit secondary antibody (1:2000) (Cell Signal) and developed using West-Pico Chemiluminescent Kit (Pierce Biotechnology).