SUPPLEMENTAL MATERIAL

Detailed Methods

Northern Blot
Ten μg of total RNA was loaded onto a denaturing 20% polyacrylamide gel and transferred to a Zetaprobe GT membrane (Bio-Rad), crosslinked by UV irradiation, and baked at 85°C. Blots were hybridized overnight at 39°C with ^{32}P-labeled antisense STARFIRE probes directed against the mature sequence of miR-218 (Integrated DNA Technologies). U6 RNA levels were detected as loading control.

Cell culture and transfection
Retinal endothelial cells (RECs), human umbilical vein endothelial cells (HUVECs) were cultured in EGM2 medium (Lonza) supplemented with penicillin/streptomycin at 31°C and 37°C, respectively. DLD1 cells were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin.

A scratch wound was produced 48 hrs after transfection with a P200 pipet tip. Images of the wound were taken 12 hrs after scratching. Scratch width was quantified using NIH ImageJ software.

COS cells were transfected using Fugene 6.0 (Roche) according to manufacturer's protocol. For 3'UTR target assays, 20ng of pMIR-Report luciferase vector was transfected with increasing amounts of CMV6-miR-218 expression vector. Empty CMV6 expression vector was used as control and to keep total plasmid DNA amounts constant.

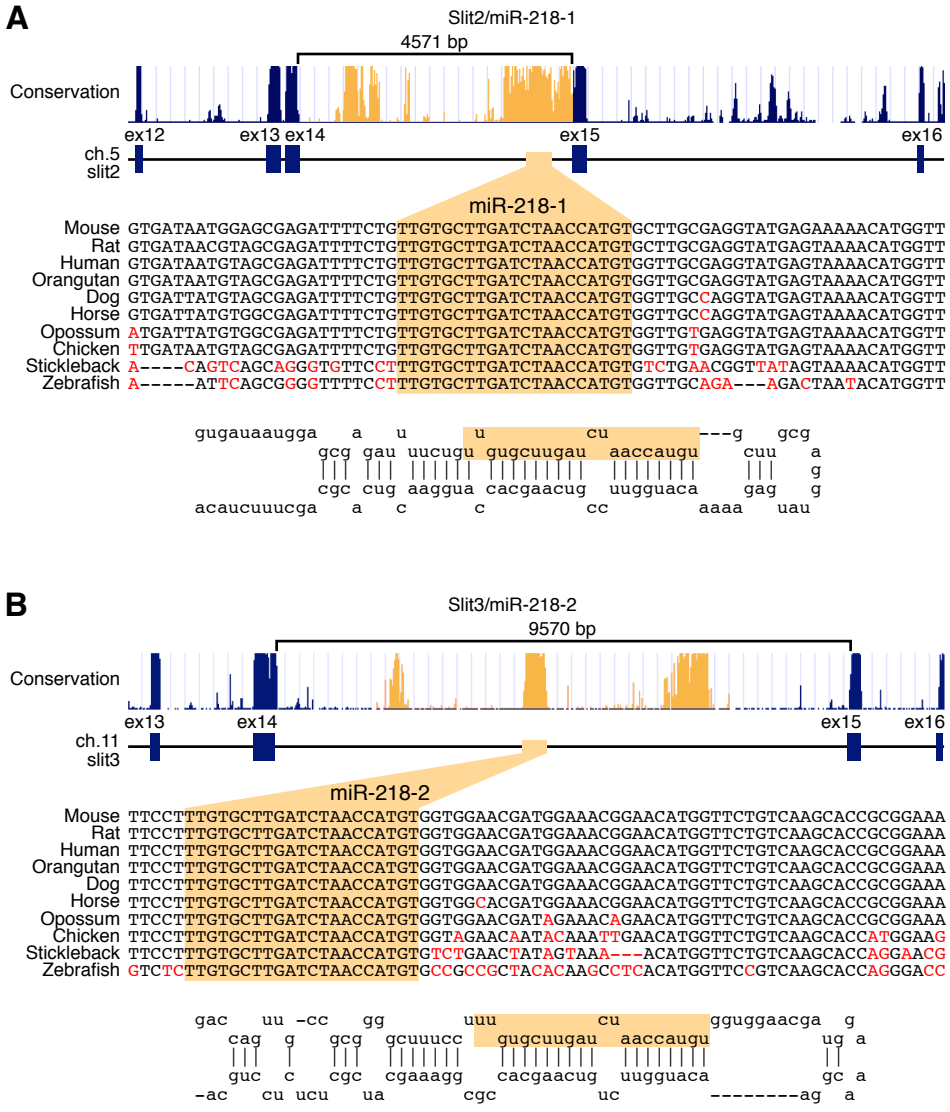
Primer sequences
Robo1 3'UTR: For: 5'-GAGCTCAAGACAACCAGAGAGGCTTAC-3';
Rev: 5'-AAGCTTTGTTCCCTTAGAACTGCACAT- 3';
Robo2 3'UTR: For 5'-GAGCTCTGTATGTTTCTACGAGACTCC-3';
Rev 5'-AAGCTTACTGAACATACTGTGAGCACT- 3';
GLCE1 3'UTR: For 5'-GAGCTCAGCTCGAAACCAAAAGTGCATT- 3';
Rev 5'-AAGCTTCAGAGAAACAGCAAGGACAGAA -3'.

mutRobo1 : 5'-
CTGTTTTTAATCTTTTTTTTTTTGGTTTTAAAATCAGAATCACTAAACTTTATTTG-3';
mutGLCE: 5'-
CGTGTTCAGGTTTCACGGTCACATACATAATCAGAACTGAAATGAAACTT-3';

Analysis of retinal angiogenesis
1 μl of a 5 mg/ml solution of LNA modified anti-miR-218 or universal control oligonucleotides was unilaterally injected in the subretinal space of postnatal day (P) 2 mice in the ICR background. We found that LNA modified oligonucleotides did not require electroporation for efficient uptake into the retina and knockdown of gene expression. Mice were allowed to develop for 3 days prior to RNA and protein isolation at P5, and 5 days prior to isolation of retinas at P7 for histological analysis. Visualization

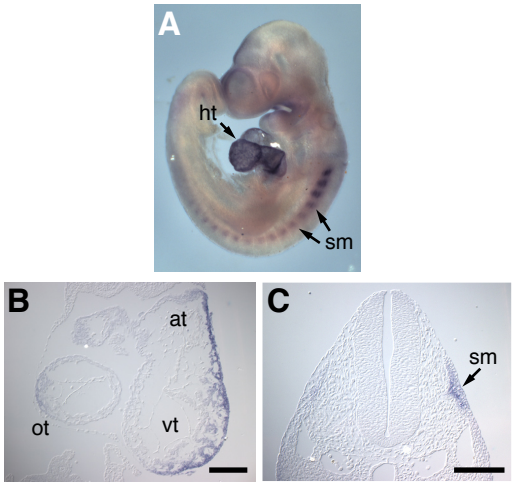
of retinal vasculature was performed as previously described. Briefly, retinas dissected from injected mice were fixed in 4% paraformaldehyde (PFA) for 1hr, permeablilized, and stained with 40 μg/ml FITC conjugated isolectin from Bandeiraea simplicifolia (Sigma-Aldrich) at 4°C overnight. Retinas were then washed five times with PBS and post fixed with 4% PFA prior to flatmounting. Quantification of vessel density was performed on 20-25 random fields at the vascular migration front from 7 control and 5 anti miR-218 injected retinas using NIH ImageJ software. The thickness of the vascular plexus was calculated from z-stacks of images taken at the vascular migration front of 6 control and 6 anti miR-218 injected retinas. The radial length of the vascular network was calculated by measuring the distance form the optic disc to the periphery of the vascular plexus in four quadrants of 12 control and 13 anti miR-218 injected retinas. Student's t-tests were used to determine statistical significance between groups.

Supplemental Figure and Figure Legends



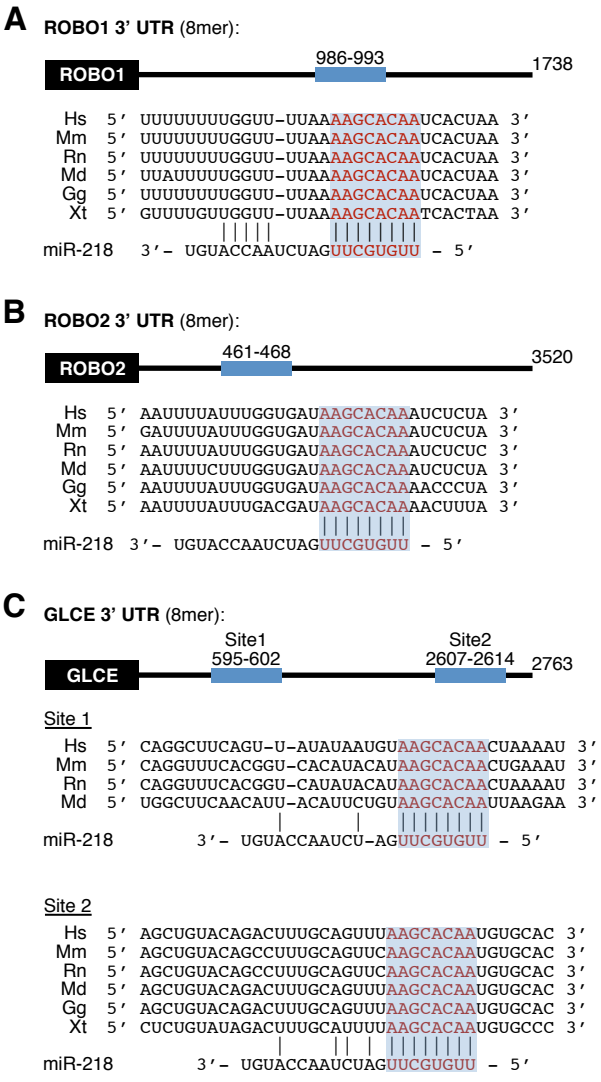
Online Figure I

Evolutionary conservation of miR-218-1 within Intron 14 of the Slit2 (A) and miR-218-2 within intorn 14 of the Slit3 gene (B). Sequence comparison of the pre-miR-218 is shown at bottom of image with mature miR-218 sequence highlighted in blue revealing 100% identity from human to zebrafish.



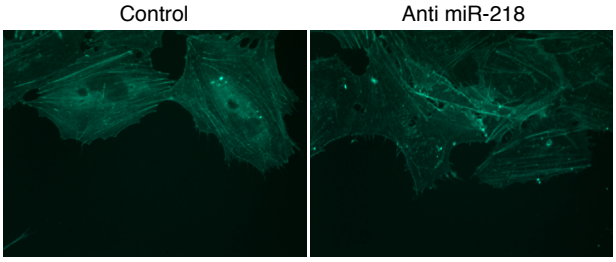
Online Figure II

In situ hybridization of E9.5 mouse embryos reveals specific expression of miR-133 in the heart and somites (**A**). Histological sections of the embryo shown in (**A**) demonstrating heart (**B**) and somite (**C**) expression. Heart (ht), somite (st).



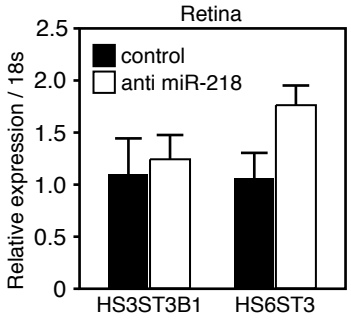
Online Figure III

Evolutionary conservation of miR-218 target sites within the 3'UTRs of the Robo1 (**A**), Robo2 (**B**), and GLCE (**C**) genes.



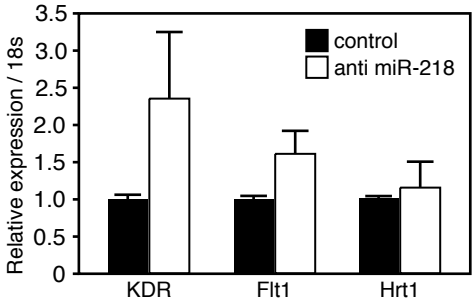
Online Figure IV.

Phalloidin staining of the actin cytoskeleton following transfection of control or anti miR-218 oligonucleotides in HUVECs demonstrates stress fiber formation and lamellapodial extension.



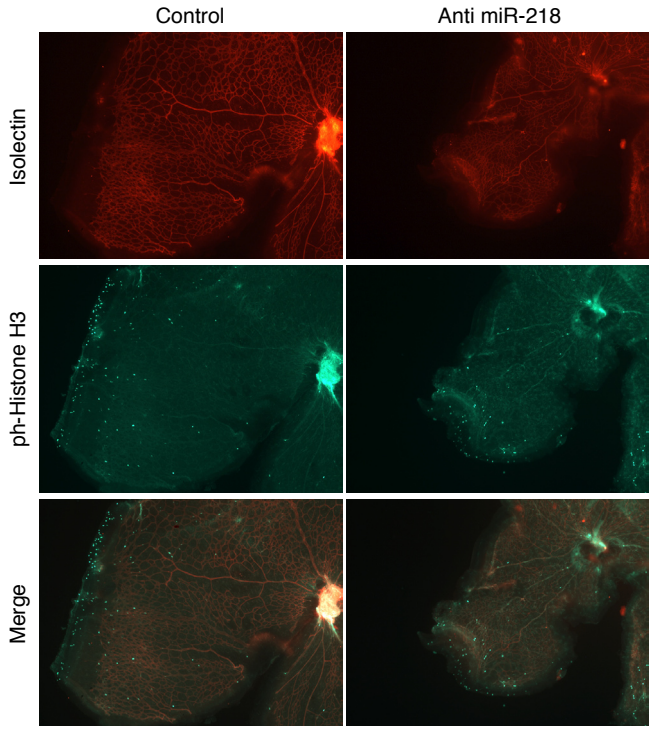
Online Figure V

Retinal expression of heparan sulfotransferases after miR-218 knockdown. Real time RT-PCR on pools of 3 control and 3 anti-miR-218 injected retinas.



Online Figure VI

Real time RT-PCR demonstrates expression of VEGF and Notch pathway genes. The VEGF receptors, KDR and Flt1, and Hrt1 do not display significant alterations upon miR-218 knockdown in the retina.



Online Figure VII.

Normal proliferation in anti miR-218 injected retinas. Phospho-histone H3 / isolectin staining demonstrate proliferating cells and the retinal vasculature 5 days after anti miR-218 injection (P7).