Annexin-1-mediated endothelial cell migration and angiogenesis are regulated by VEGF-induced inhibition of miR-196a expression

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Running title: miR-196a regulates endothelial cell migration

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LEGEND TO SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE 1. miR-196a inhibits the time-dependent VEGF-mediated endothelial cell migration in a wound closure assay A) HUVECs were transfected with miR-196a (C and D) or control (A and B) mimics (100 nM, for a total of 48 hours). Cells were processed for wound healing assay in response or not (A and C) to 10 ng/ml of VEGF (B and D). The black lines represents the edge of the wound and pictures were taken after 0, 8, 12 and 18 hours of treatment. Images were captured using an inverted microscope (10X).

SUPPLEMENTAL FIGURE 2A. VEGF-mediated formation of capillary-like structures. HUVECs were firstly transduced with CII-Venus as a control (MOI of 33) and after 48 hours with pmiR-empty (MOI of 65) using lentiviral-mediated infection. 96 hours later, 50 000 transduced cells were plated on a 12 days Normal Human Dermal Fibroblasts (NHDF) monolayer. Co-cultures were maintained for 4 days and media were replaced every 48 hours including VEGF treatment (10 ng/ml) for a total of 2 treatments (n=4). Fluorescent capillary-like structures were observed using an inverted microscope (4X) and pictures were captured every four hours to make movies. Pictures from eight different fields were captured and a representative movie is shown.

SUPPLEMENTAL FIGURE 2B. miR-196a inhibits VEGF-mediated formation of capillary-like structures. HUVECs were firstly transduced with CII-Venus as a control (MOI of 33) and after 48 hours with miR-196a precursor (pmiR-196a) (MOI of 65) using lentiviral-mediated infection. 96 hours later, 50 000 transduced cells were plated on a 12 days Normal Human Dermal Fibroblasts (NHDF) monolayer. Co-cultures were maintained for 4 days and media were replaced every 48 hours including VEGF treatment (10 ng/ml) for a total of 2 treatments (n=4). Fluorescent capillary-like structures were observed using an inverted microscope (4X) and pictures were captured every four hours to make movies. Pictures from eight different fields were captured and a representative movie is shown.

SUPPLEMENTAL FIGURE 2C. Annexin-1 rescues miR-196a inhibition of VEGF-mediated formation of capillary-like structures. HUVECs were firstly transduced with CSII-Venus-ANXA1 (MOI of 33) and after 48 hours with miR-196a precursor (pmiR-196a) (MOI of 65) using lentiviral-mediated infection. 96 hours later, 50 000 transduced cells were plated on a 12 days Normal Human

Dermal Fibroblasts (NHDF) monolayer. Co-cultures were maintained for 4 days and media were replaced every 48 hours including VEGF treatment (10 ng/ml) for a total of 2 treatments (n=4). Fluorescent capillary-like structures were observed using an inverted microscope (4X) and pictures were captured every four hours to make movies. Pictures from eight different fields were captured and a representative movie is shown.