Endomucin inhibits VEGF-induced endothelial cell migration, growth, and morphogenesis by modulating VEGFR2 signaling

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SUPPLEMENTAL FIGURES

Table S1: Sequences for primers for qRT-PCR.

	Forward	Reverse
h*EMCN*:	5'TGCAGGACTTTCTCCTTTTC3'	5'ATTTGTTCTGGTGGGTTTGT3'
h*GAPDH*	5'CAAATTCCATGGCACCGTCA3'	5'GGAGTGGGTGTCGCTGTTGA3'
h*HPRT1*	5'CCTGGCGTCGTGATTAGTGAT3'	5'AGACGTTCAGTCCTGTCCATAA3'
m*Emcn*	5'AATACCAGGCATCGTGTCAGT3'	5'CTGATTCTCAGTCTTGTTCTGGG3'
m*Gapdh*	5'GTGGCAAAGTGGAGATTGTTGCC3'	5'GATGATGACCCGTTTGGCTCC3'
m*Hprt1*	5'TCAGTCAACGGGGGACATAAA3'	5'GGGGCTGTACTGCTTAACCAG3'

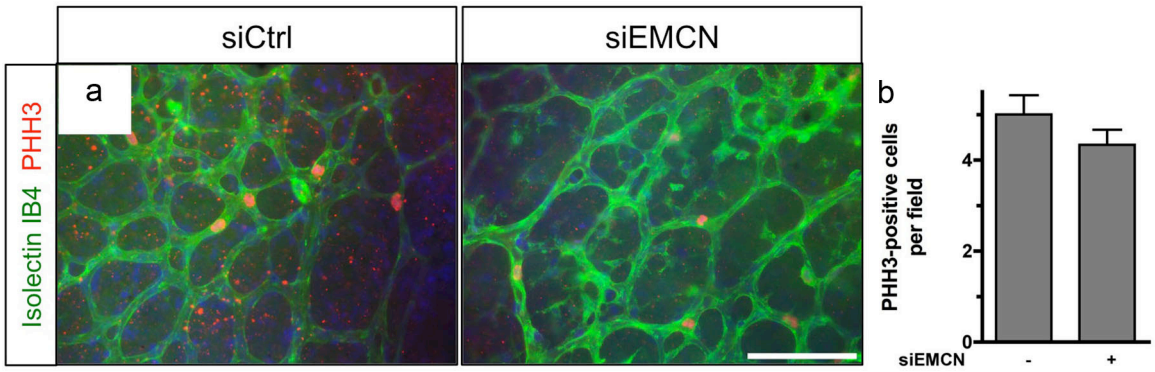

Figure S1: Loss of EMCN shows a reduced apoptotic profile independent of proliferation.

(a) Representative images of a P6 vasculature labeled for IB_4 (green) and mitosis (phospho-histone; PHH3, red) and nuclei (blue) 48 hr after intravitreal injection with siEMCN or siCtrl. **(b)** Quantification shows no change in the number of PHH3-positive cells in siEMCN injected eyes compared to control eyes. Scale bar, 100 μm.

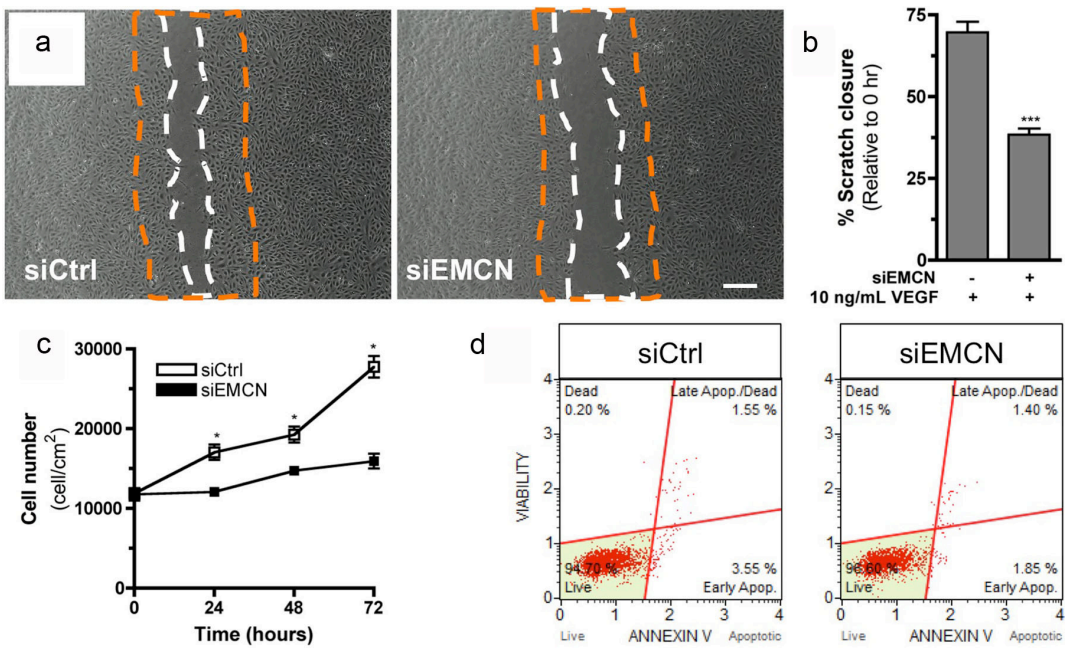

Figure S2: EMCN controls EC migration and proliferation in HUVECs. HUVECs were transfected with siCtrl or siEMCN. **(a, b)** Cell migration was measured by a wound assay and cell growth and viability were measured by **(c)** Trypan blue or assessed for cell death using a **(d)** Muse automated cell analyzer. **(a)** Representative images of the wound margins immediately (orange) and 10 hr (white) after scratch in response to 10 ng/mL VEGF. **(b)** Scratch closure was quantified at 10 hr and data are expressed as a % of the initial scratch width. **(c)** Graph showing cell number of siEMCN (closed black squares) and siCtrl (open squares) transfected cells 0, 24, 48, and 72 hr after treatment with VEGF (10 ng/mL) shows significant reduction in EC proliferation. **(d)** Representative plots of siEMCN and siCtrl experiments performed for annexin V detection shows non-apoptotic live (lower left: 7-AAD negative, apoptosis negative), non-apoptotic dead (upper left: 7-AAD positive, apoptosis negative), apoptotic live (lower right: 7-AAD negative, apoptosis positive), and apoptotic dead (upper right: 7-AAD positive, apoptosis positive) cells. *P<0.05, ***P<0.001 siEMCN vs siCtrl. Error bars represent SEM.

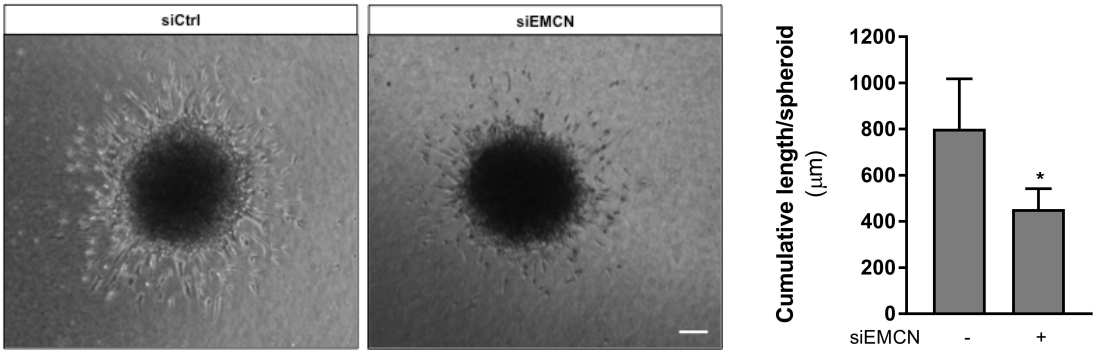

Figure S3: EMCN knockdown attenuates capillary sprouting of endothelial cells in response to VEGF. (Left) Representative images of angiogenic sprouting from spheroids generated from HRECs stimulated (12 hours) by VEGF (5 ng/ml). **(Right)** Quantification of total length of all sprouting processes originating from a single spheroid in the presence of VEGF (2-8 spheroids/well). Quantitative results are from three independent experiments. *P<0.05 siEMCN vs siCtrl. Error bars represent SEM. Scale bar, 100 μm.

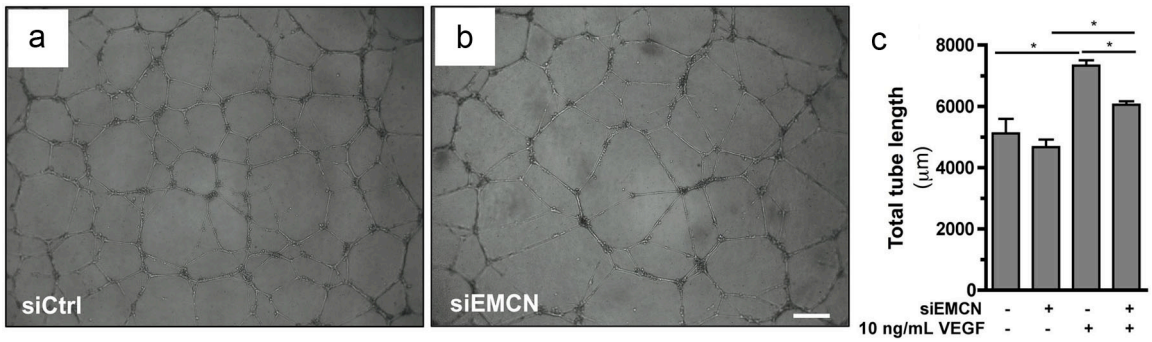

Figure S4: Reduced levels of EMCN render HRECs unable to organize into vessel-like structures at later time points. (a) siCtrl or **(b)** siEMCN cells were plated on polymerized BME in the presence of VEGF (10 ng/mL). Representative phase-contrast images after 24 hr are shown. **(c)** Quantification of total tube length was performed by Image J 24 hr after plating on BME in the presence and absence of VEGF shows knockdown of EMCN expression inhibits ECM-induced tube formation. Quantitative results are from three independent experiments. *P<0.05, ***P<0.001 siEMCN vs siCtrl or AdEMCN vs AdGFP; *P<0.05, **P<0.01 VEGF vs no VEGF. Error bars represent SEM. Scale bar, 100 μm.

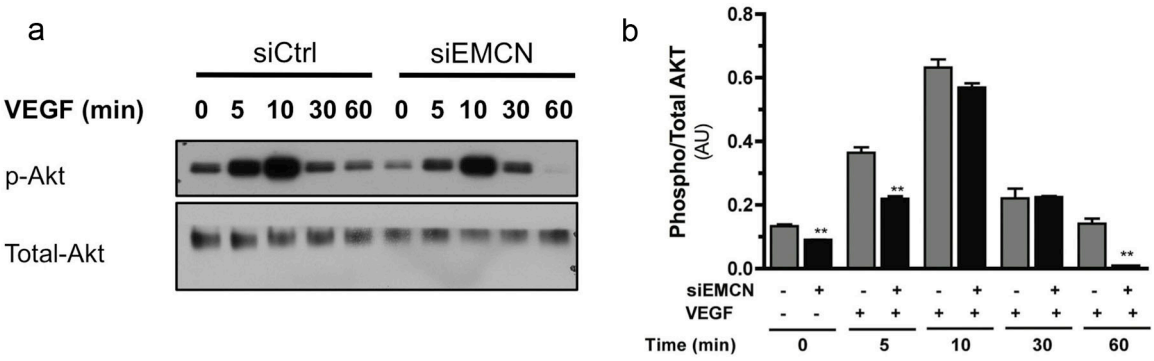

Figure S5: EMCN inhibits VEGF-induced phosphorylation of Akt in HRECs. (a) Immunoblot and **(b)** quantitative analysis of siCtrl and siEMCN cells stimulated with exogenous VEGF (10 ng/mL) at indicated time points and assessed for levels of phosphorylated and total Akt. ***P*<0.01. Error bars represent SEM. Cropped gels are displayed.