Supplemental Material 3:

Quantitative real-time RT-PCR

Total cellular RNA was isolated from confluent BVEC and LEC cultures after cells were carried through five round of passage using the Trizol reagent (Invitrogen, Carlsbad, CA) and was treated with RQ1 RNase-free-DNase (Promega, Madison, WI). The expression of vascular lineage-specific genes and of HGF-R mRNA was investigated by quantitative real-time RT-PCR, using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) as described (Hong et al., 2004a). The primers and probes for Prox1, LYVE-1, Flt-1 were described previously (Hirakawa et al., 2003). The following primers and probe for HGF-R were used: forward primer 5'-TTAAAGGAGACCTCACCATAGCTAATC-3', reverse primer 5'-CCTGATCGAGAAACCACAACCT-3', and dual labeled TaqMan probe 5'-FAM-TGGGACATCAGAGGGTCGCTTCATG-TAMRA-3'. Each reaction was multiplexed with β-actin primers (forward 5'-TCACCGAGCGCGGCT-3', reverse 5'-TAATGTCACGCACGATTTCCC-3') and probe (5'-JOE-CAGCTTCACCACCACGGCCGAG -TAMRA-3') as an internal control. LEC (passage 5) were also treated with or without recombinant human HGF (30 ng/ml) for 6 hours in endothelial basal medium (EBM; Cambrex, Walkersville, MD) containing 2% fetal bovine serum. Total RNA was extracted, and SYBR-Green based RT-PCR for integrin alpha 9 and for stanniocalcin 1 was performed after addition of Multi-Scribe reverse transcriptase (Applied Biosystems, Foster City, CA). The following primers were used: integrin alpha 9: 5'-CTTTCGCCGAAGGTACAAAG -3' and 5'-TGGTTTTTCTGGACCCAGTC-3'; Stanniocalcin 1: 5'-

CCCAACAACTTAGCGGAAAC -3’ and 5’- TTGCAGAAGCACTGATCACC -3’; β-actin: forward 5’-TCACCGAGCGCGGCT-3’ and reverse 5’-TAATGTCACGCACGATTTCCC-3’. Data were normalized based on the expression levels of β-actin.