Supplementary Information:

Fig. S1. Four inhibitors did not influence the morphology of the OP9 feeder cells

After 1 day of sub-culture of OP9 cells, the indicated chemical inhibitors were added at a concentration of 5 μM. DMSO was added as a control. Cells were further cultured for 3 days, and stained with phalloidin (green) and DAPI (blue). Scale bars indicate 50 μm. Gamma settings were adjusted for clearness of images. The experiment was repeated three times with similar results.
Fig. S2. PI3K/Akt inhibition did not affect the elongation induced by mTORC1 inhibitor

Flk1+ VPCs derived from F10-EGFP/ES cells or Foxo1(-/-) ES cells expressing EGFP driven by F10 enhancer were seeded on an OP9 cell layer to induce the differentiation of ECs in the presence of VEGF (10 ng/ml). One day after seeding, the indicated chemical inhibitors were added at a concentration of 1 μM. DMSO was added as a control. Cells were further cultured for 3 days. (A) Immunostaining of wild-type EC colonies (red: VE-cadherin, green: EGFP) and Foxo1(-/-) EC colonies (red: VE-cadherin, green: EGFP-F). Scale bars indicate 50 μm. Gamma settings were adjusted for clearness of images. The experiment was repeated two times with similar results. (B) Proportion of the two types of EC colonies (thread-like and sheet-like) found in each group of cultures. EC colonies generated in two independent experiments were analyzed. The total number of colonies examined is indicated in the labels of each group. (C) The length of branches of thread-like EC colonies in each group. EC colonies generated in two (WT) or three (Foxo1(-/-)) independent experiments were analyzed. The total number of colonies and branches examined is indicated in the labels of each group (colonies / branches). The length is presented as a ratio relative to the DMSO control (mean ± s.d.). The data were analyzed by ANOVA, followed by Dunnett’s test (* p < 0.05, vs. DMSO control).
Table S1. Screening of chemical inhibitors that induce elongation of endothelial cells

Flk1+ VPCs derived from two F10-EGFP/SM22-DsRed.T4/ES cell clones (Clone No.1 and No.2) were seeded on an OP9 cell layer to induce the differentiation of ECs. One day after seeding, the indicated chemical inhibitors were added at a concentration of 5 µM. DMSO was added as a control. VEGF (10 ng/ml) was added as a positive control for EC elongation. After 3 days, the number of the two types of EC colonies (thread-like and sheet-like) in each group was counted. Blanks in some compounds indicate that the colonies had an unclassifiable shape, or that the number of colonies was less than 10.

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