Electronic Supplementary Material (ESI) for Lab on a Chip.

Sema4D–plexin-B1 signaling in recruiting dental stem cells for vascular stabilization on a microfluidic platform

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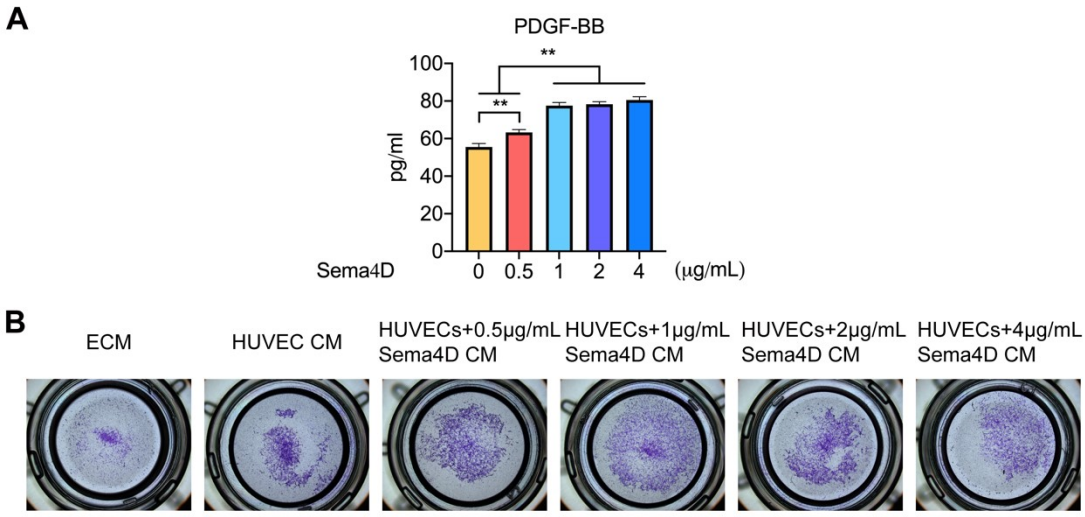

Fig S1. Sema4D concentration. A: ELISA results of PDGF-BB in HUVECs treated with different concentrations of Sema4D. Values are presented as mean ± SD. *P < 0.05. **P < 0.01. **B**: Representative images from the trans-well assay of migration of SHED under different concentrations of Sema4D treated HUVEC CM (2x). Sema4D: Semaphorin 4D; PDGF-BB: Platelet-derived growth factor-BB; HUVECs: human umbilical vein endothelial cells; CM: conditioned medium; SHED: stem cells from human exfoliated deciduous teeth.

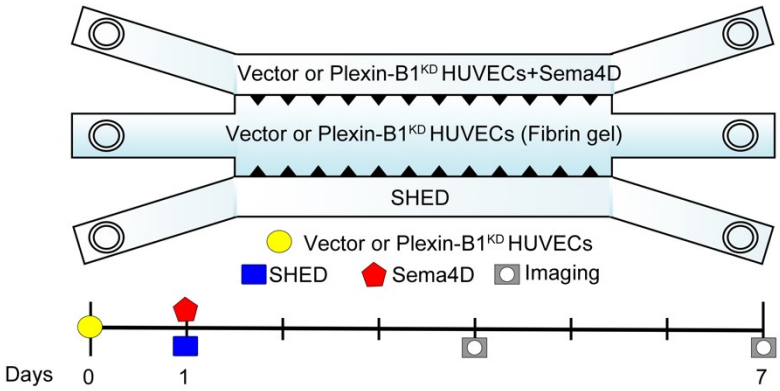

Fig S2. Schematic diagram of the microfluidic assay to investigate the Sema4D–plexin-B1 signaling.

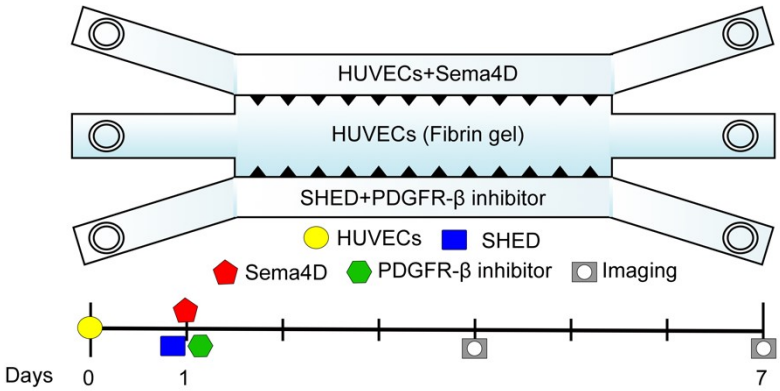

Fig S3. Schematic diagram of the microfluidic assay to investigate the PDGF-BB–PDGFR-β signaling.

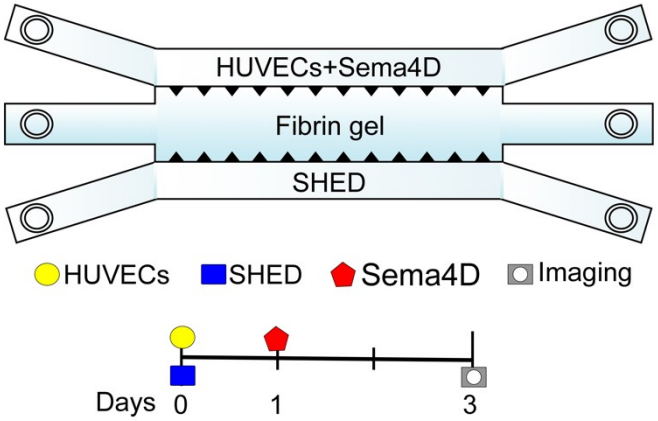

Fig. S4. Schematic diagram of the microfluidic assay to investigate the migration of SHED.

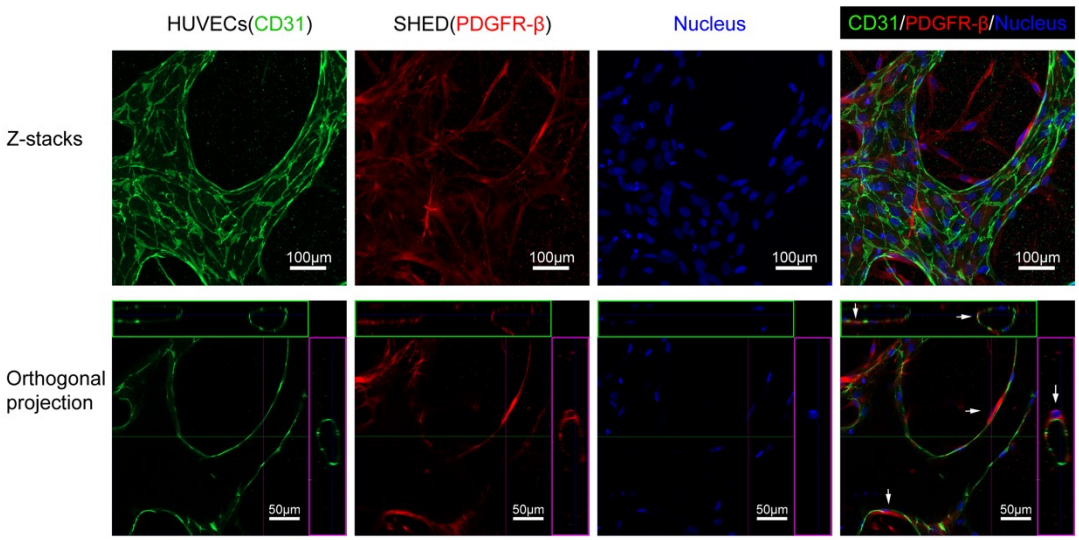

Fig. S5. PDGFR-β positive SHED lining the abluminal surface of the endothelial vascular network formed by $CD31^{+}$HUVECs. White arrows indicate the incorporation of PDGFR-$β^{+}$SHED to the endothelial vascular wall. HUVECs: human umbilical vein endothelial cells; SHED: stem cells from human exfoliated deciduous teeth. PDGFR-β: Platelet-derived growth factor receptor beta.

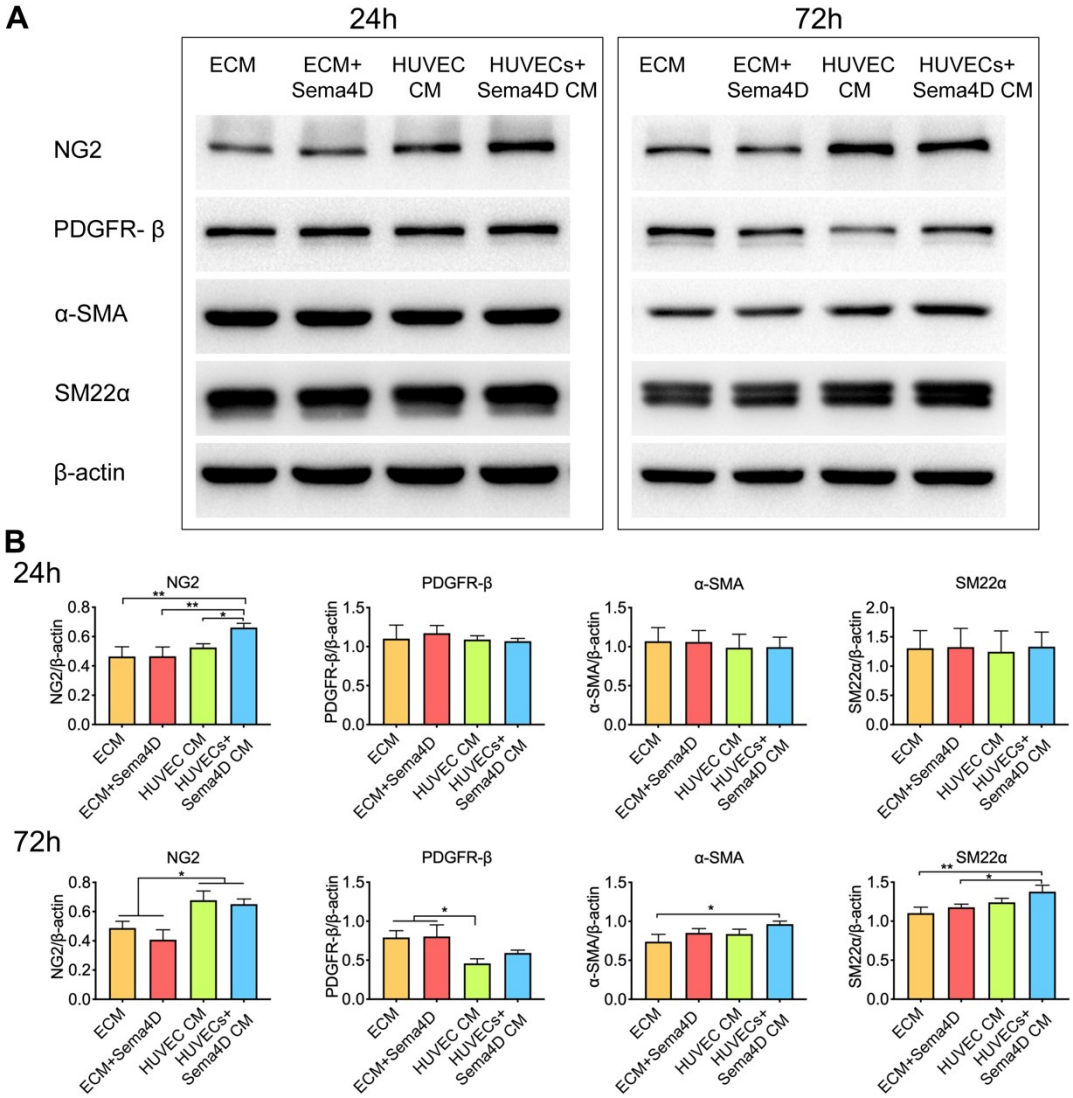

Fig. S6. Differentiation of SHED under Sema4D treated HUVEC CM. A: Western blotting for mural cell markers, NG2, PDGFR-β, α-SMA and SM22α of SHED cultured in Sema4D or Sema4D-treated HUVEC CM for 24h and 72h. **B**: Quantification of the expression of mural cell markers as assessed by western blotting. Values are presented as mean ± SD. *P < 0.05. **P < 0.01. Sema4D: Semaphorin 4D; SHED: stem cells from human exfoliated deciduous teeth; HUVEC CM: human umbilical vein endothelial cell conditioned medium; HUVECs+Sema4D CM: Sema4D treated HUVEC CM; NG2: Neural/glial antigen 2; PDGFR-β; Platelet-derived growth factor receptor beta; α-SMA: α-smooth muscle actin; SM22α: smooth muscle protein 22-alpha.

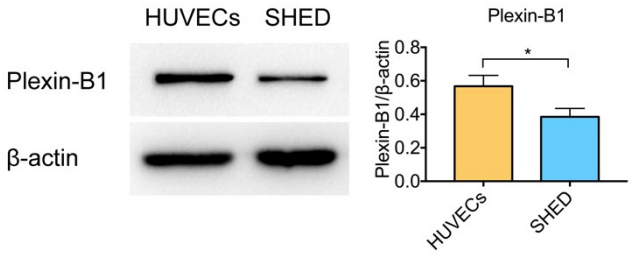

Fig. S7. Expression of plexin-B1 on HUVECs and SHED.

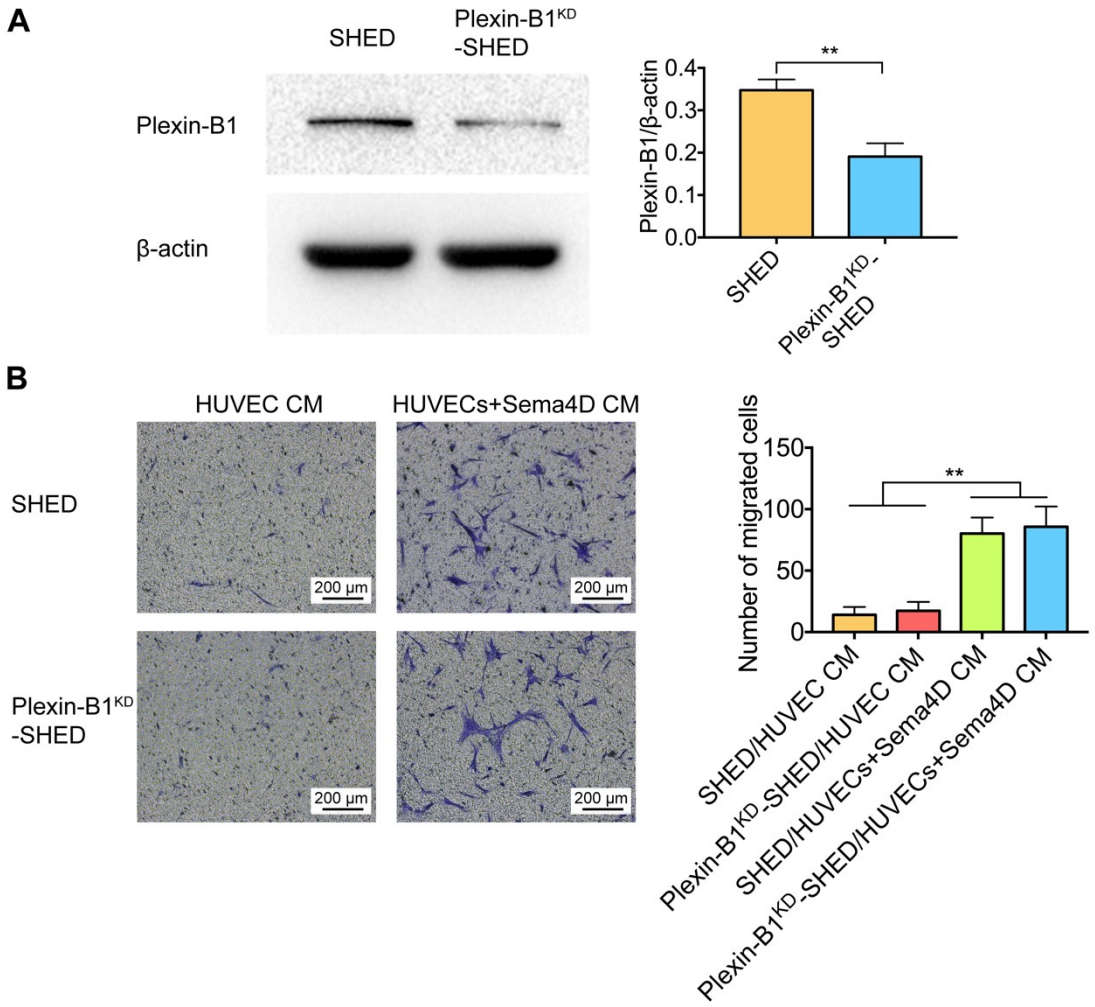

Fig. S8. Knockdown of plexin-B1 on SHED has no effect on migration. A: Western blotting for plexin-B1 expression of SHED treated with plexin-B1 siRNA or negative control siRNA. **B**: Representative images from the trans-well assay of migration and quantification of SHED or Plexin-B1^{KD}-SHED under Sema4D treated HUVEC CM (10x). Sema4D: Semaphorin 4D; siRNA: Small interfering RNA; HUVECs: human umbilical vein endothelial cells; CM: conditioned medium; SHED: stem cells from human exfoliated deciduous teeth.

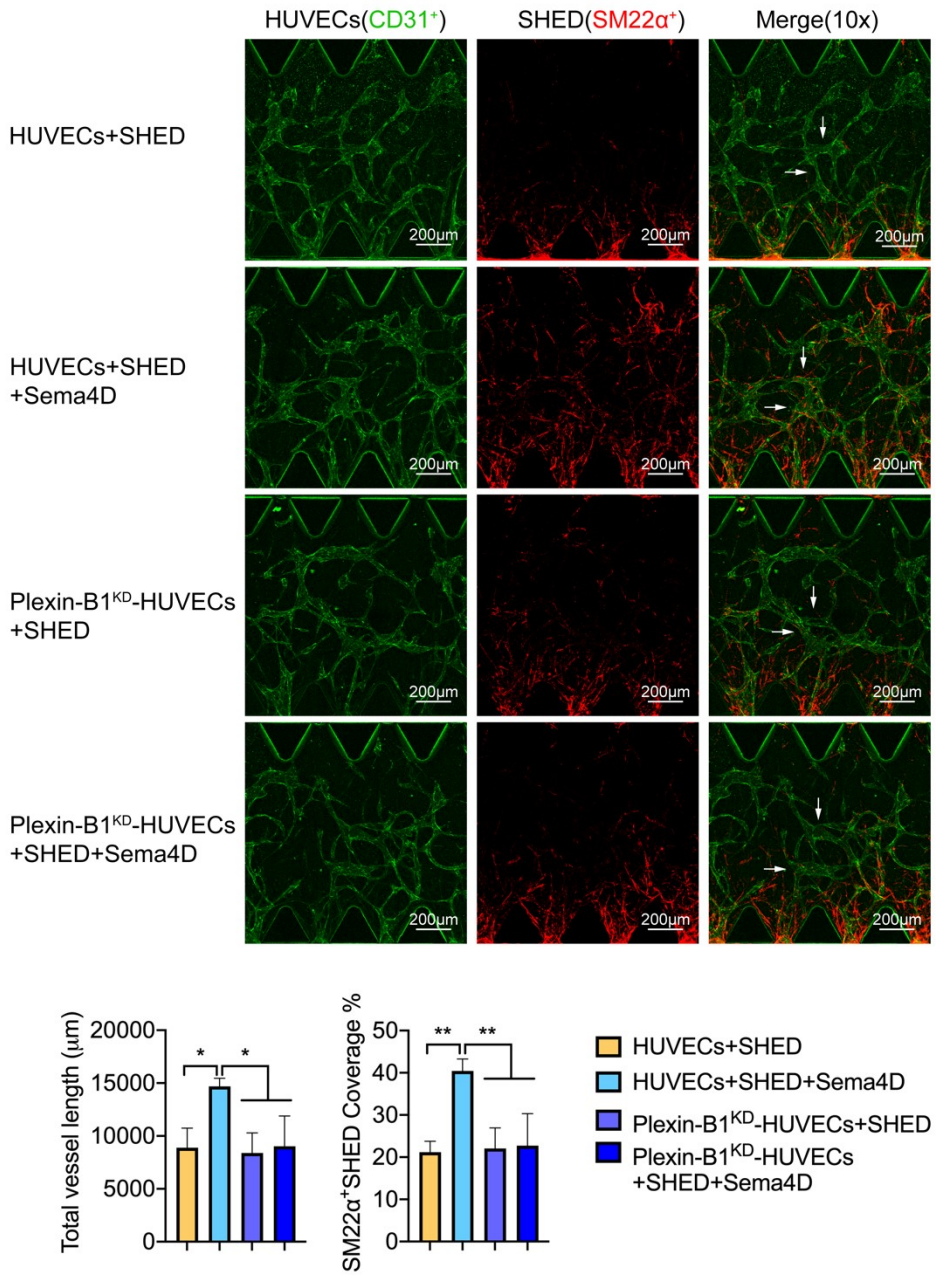

Fig. S9. Knockdown of plexin-B1 on HUVECs impairs the recruitment of SHED. Representative images of the microfluidic assay of HUVECs or Plexin- $B1^{KD}$-HUVECs and SHED in the presence of Sema4D (10x). Quantification of total vessel length and $SM22\alpha^{+}$SHED coverage. Values are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$.

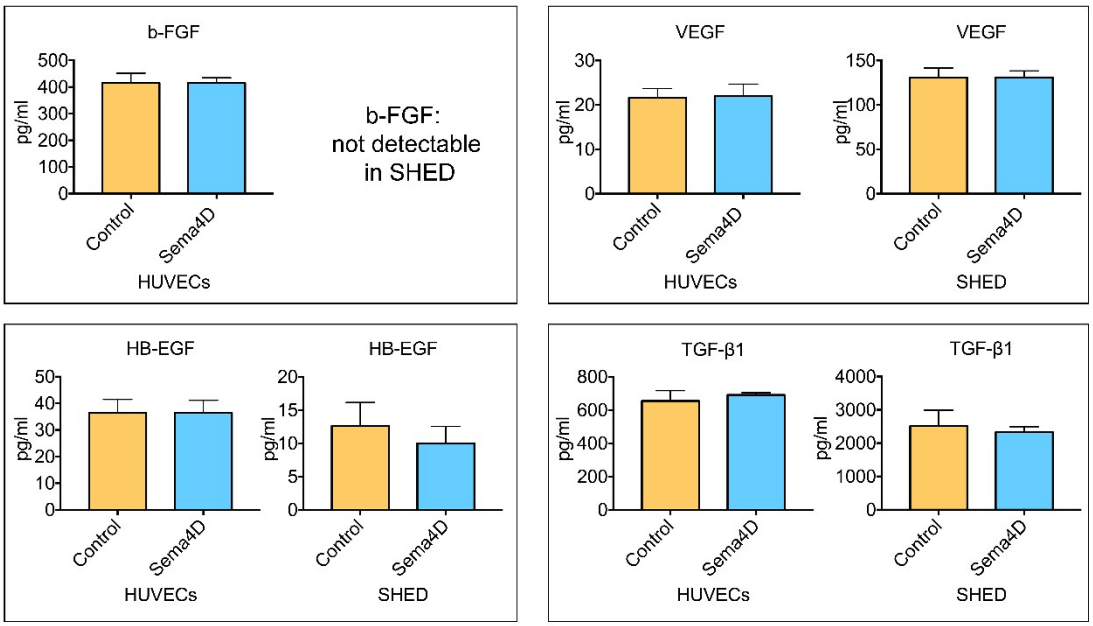

Fig. S10. ELISA results of b-FGF, VEGF, HB-EGF and TGF-β1 in Sema4D-treated HUVECs or SHED. Values are presented as mean ± SD. b-FGF: basic fibroblast growth factor; VEGF: Vascular endothelial growth factor; HB-EGF: heparin-binding epidermal growth factor; TGF-β1: Transforming growth factor β1; HUVECs: human umbilical vein endothelial cells; SHED: stem cells from human exfoliated deciduous teeth.

Video S1. Triple staining for CD31, SM22α, and Collagen IV of HUVECs and SHED cultures with Sema4D treatment in microfluidic device. The video containing different layers of Z-stacks showed that $SM22\alpha^{+}$SHED (red color) line abluminal surface of vessels formed by $CD31^{+}$HUVECs (green color). And both $SM22\alpha^{+}$SHED and $CD31^{+}$HUVECs are localized within the collagen IV positive (purple color) basement membrane. HUVECs: human umbilical vein endothelial cells; SHED: stem cells from human exfoliated deciduous teeth. Scale bar = 50 μm

Video S2. Live imaging of HUVECs and SHED cultures with Sema4D treatment in microfluidic device on day 3. White arrows indicate SHED (CellTracker™ Red CMTPX labeled) migrated from the side channel to central channel where HUVECs (GFP labeled) formed the vascular tubes. The duration of this video is 24 h. Scale bar = 200 μm

Video S3. Live imaging of HUVECs and SHED cultures with Sema4D treatment in microfluidic device on day 6. At later time points, the SHED incorporated onto the abluminal surface of endothelial vessels (GFP-HUVEC) were stable without moving along the vessel walls (indicated by white arrows). The duration of this video is 20 h. Scale bar = 200 μm