Baeyens et al., http://www.jcb.org/cgi/content/full/jcb.201603106/DC1

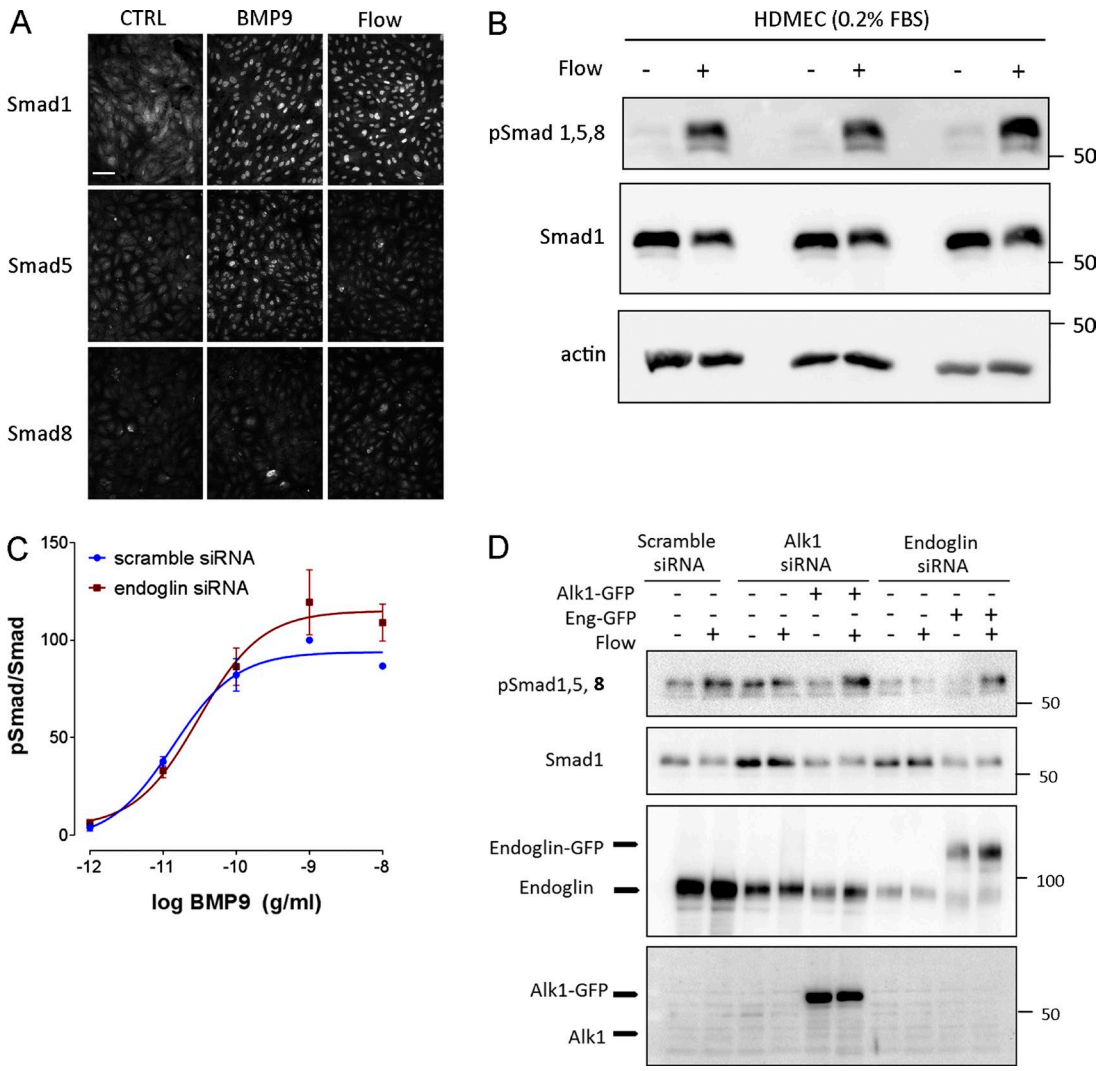

Figure S1. **Fluid shear stress activation of Smad signaling.** (A) Representative staining of Smad1, Smad5, or Smad8 in response to 1 ng/ml BMP9 or 12 dynes/cm^2 for 45 min with a 20× objective. Bar, 25 µm. (B) Western blot of phosphorylated Smad1/5/8, Smad1, and actin with or without flow in human dermal microvascular endothelial cells (HDMEC; 2 h, triplicate). (C) Concentration response curve to BMP9 of HUVECs transfected with a scramble or *Endoglin* siRNA (QIAGEN; $n = 3$). (D) Western blot of phosphorylated Smad1/5/8, Smad1, Alk1, and endoglin after 12 dynes/cm^2 for 15 min, with or without Alk1 or endoglin knockdown with QIAGEN siRNA and rescue with siRNA-resistant GFP-tagged construct.

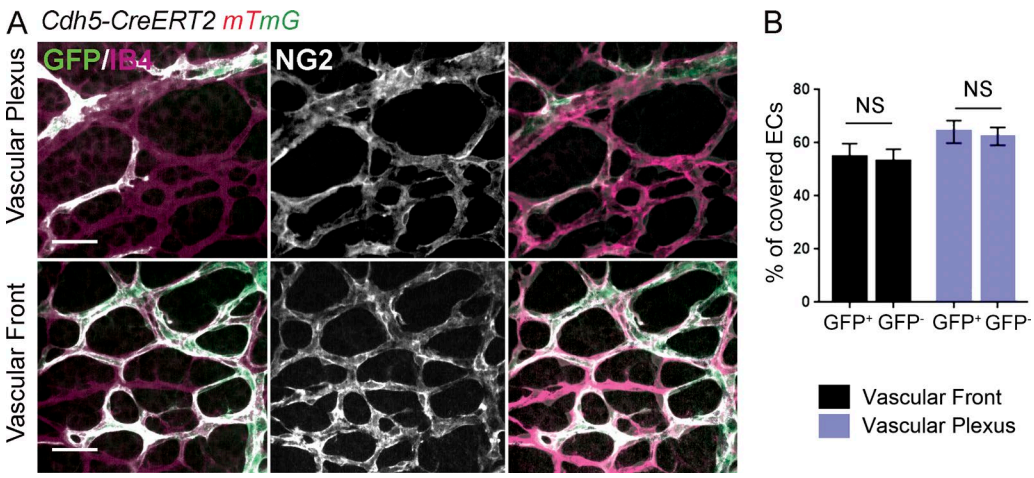

Figure S2. **Analysis of pericyte coverage in control mosaic mice.** (A) Representative images of NG2 staining of P6 retinas after tamoxifen-induced recombination (5 µg tamoxifen at P3) in the vascular plexus and vascular front of control *Cdh5-CerERT2 mTmG* mice. Both GFP+ (recombined) and GFP– (unrecombined) ECs express Alk1. (B) Quantification of pericyte coverage. Graphs show the percentage of EC surface area covered by NG2-positive cells (n = 4 retinas per group, Mann–Whitney; *, $P < 0.05$). Bars, 50 µm. NS, not significant.

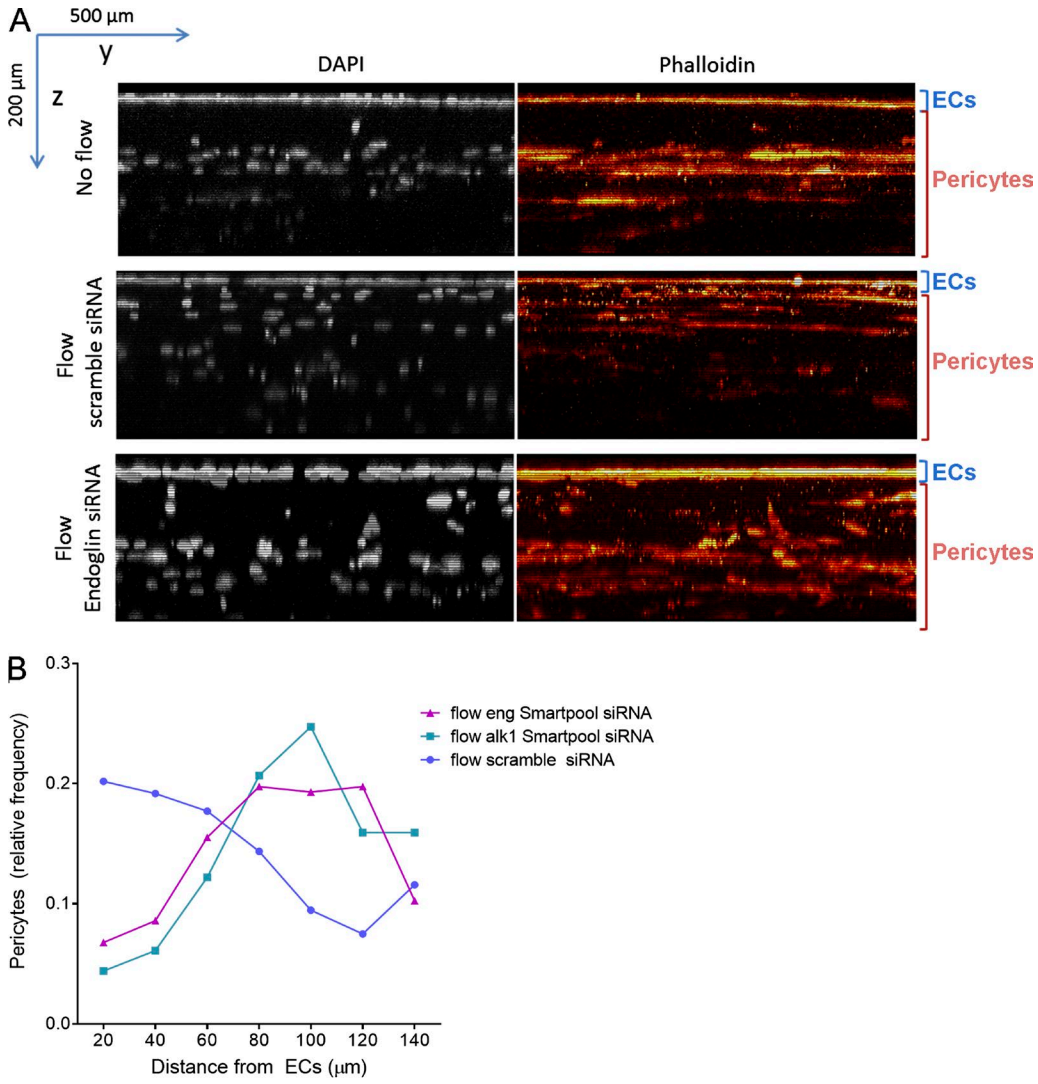

Figure S3. **Pericyte recruitement by flow requires Alk1 and endoglin.** (A) YZ projections of a 200-µm-thick stack from fibrin gels with embedded pericytes and a monolayer of HUVECs on top of the gel. HUVECs were transfected with scrambled or *Endoglin* siRNA. HUVECs and pericytes are stained with phalloidin. (B) Distribution of pericytes localization in a fibrin gel, relative to the endothelial monolayer after no stimulation or 10 dynes/cm^2 flow for 96 h. HUVECs were transfected with scrambled, *Alk1*, or *Endoglin* siRNA (Smartpool; GE Healthcare).