SUPPLEMENTARY DATA

Supplementary Figure S1. Ang1 induces Dll4 upregulation in HAECs and HMVECs under confluent culture condition. Confluent HAECs (A) and HMVECs (B) were starved for 12 h, and stimulated with vehicle (-) or COMP-Ang1 (+) for 1 h. Dll4 mRNA levels were analyzed by real-time RT-PCR as described in the legend of Fig. 1A. Values are expressed relative to that observed in the unstimulated cells, and shown as means ± s.d. of three (A) and four (B) independent experiments. Significant differences between two groups are indicated as **, $P<0.01$, ***, $P<0.001$.

Supplementary Figure S2. Inhibitors for PI3K and AKT block Ang1-induced AKT activation. (A) Confluent HUVECs starved for 12 h were pretreated with vehicle (control) or 60 nM wortmannin for 30 min, and subsequently stimulated with vehicle (-) or COMP-Ang1 (+) for 20 min. Cell lysates were subjected to Westerin blot analysis with anti-phospho-AKT (P-AKT) and anti-AKT (AKT) antibodies. (B) Effect of the pretreatment with 8 μM AKT inhibitor for 10 min on Ang1-induced AKT activation was examined as described in A.

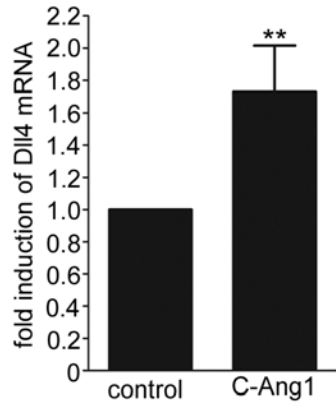
Supplementary Figure S3. GSK3β inhibitor, SB216763, induces upregulation of Dll4 mRNA in confluent but not sparse cultures of HUVECs. Sparse and confluent HUVECs starved for 12 h were stimulated with 10 μM SB216763 for the periods indicated at the bottom (h). Dll4 mRNA levels were analyzed by real-time RT-PCR as described in the legend of Fig. 1A. Values are expressed as fold induction relative to that in the confluent unstimulated cells, and shown as means ± s.d. of three independent experiments.

Supplementary Figure S4. Ang1 does not stimulate the -3.7 kb Dll4 promoter. (A) Confluent HUVECs transfected with the Dll4-3.7k-Luc reporter plasmid together with the pRL-SV40 vector were starved in medium 199 containing 1% BSA for 12 h, and subsequently stimulated with vehicle (control) or COMP-Ang1 (C-Ang1) for 3 h. After the stimulation, the cells were collected, and the lysates were assayed for firefly and renilla luciferase activities as described in the legend of Fig. 4A. Values are expressed relative to that observed in the cells treated with vehicle, and shown as mean ± s.d of three independent experiments. (B) Confluent HUVECs were transfected with Dll4-3.7k-Luc plasmid and pRL-SV40 vector together with either empty vector (control) or the plasmid encoding CA-βCat or Foxc2 as indicated at the bottom. Cell lysates were assayed for firefly and renilla luciferase activities as described in the legend of Fig. 4A. Values are expressed relative to that observed in the control cells, and shown as mean ± s.d of four independent experiments. (C) Confluent HUVECs were transfected with

TOPflash reporter construct and pRL-SV40 vector together with either empty vector (control) or the plasmid encoding CA-βCat. Firefly and renilla luciferase activities in each cell lysate were determined as described in B. Values are expressed relative to that observed in the control cells, and shown as mean ± s.d of four independent experiments. Significant differences between two groups (B) or from control (A, C) are indicated as *, $p<0.05$, ***, $p<0.001$. n.s. indicates no significance between two groups or from control.

Supplementary Figure S5. Depletion of Dll4 by siRNA prevents Ang1-induced deposition of collagen type IV. HUVECs were transfected with either control siRNA (control) or two independent siRNAs targeting Dll4 (Dll4#1 and Dll4#2) as indicated at the top, and cultured to form tube structures in 3D collagen matrices for 48 h. During this period, the cells were stimulated with vehicle (-) or COMP-Ang1 (+) as indicated at the top. To detect the extracellular deposition of collagen type IV, the cultures were fixed, immunostained with anti-collagen type IV antibody, and visualized with Alexa 488-conjugated secondary antibody. After permeabilization, the cells were further stained with rhodamine-phalloidin to visualize filamentous actin. Alexa 488 and rhodamine images were obtained through a confocal microscope. Alexa 488 (collagen IV) and rhodamine (F-actin) images and the merged images (merge) are shown as indicated at the left. Scale bar. 100 μm.

A



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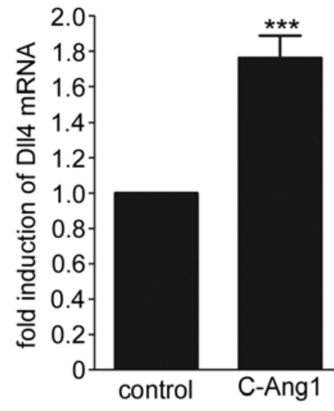

Suplementary Figure S1
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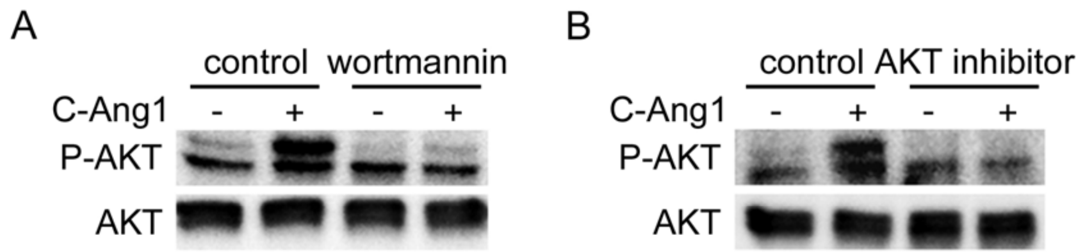

Suplementary Figure S2
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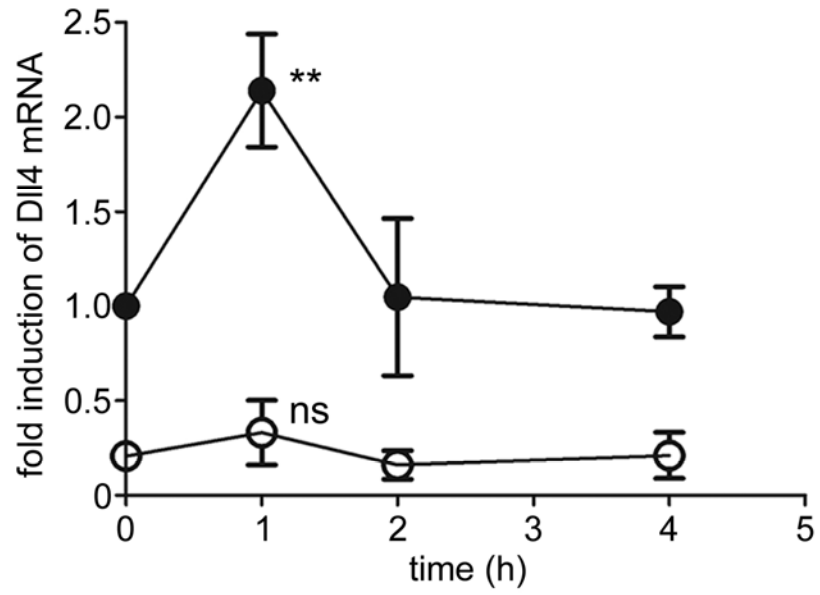

Suplementary Figure S3
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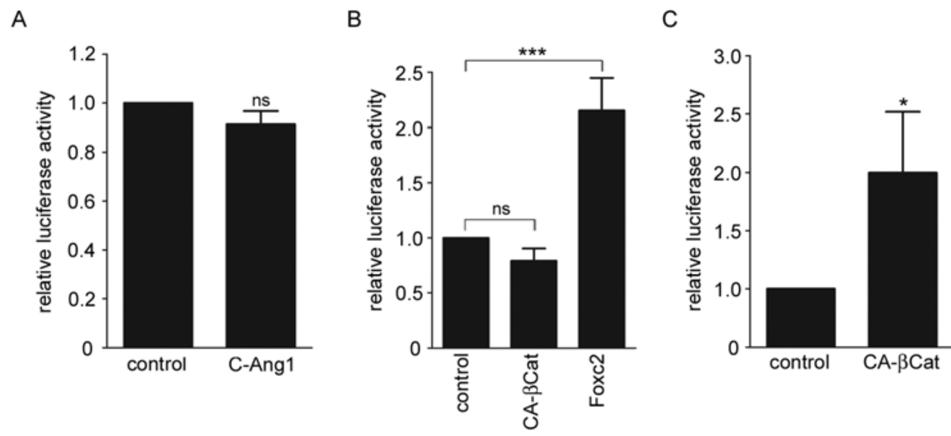

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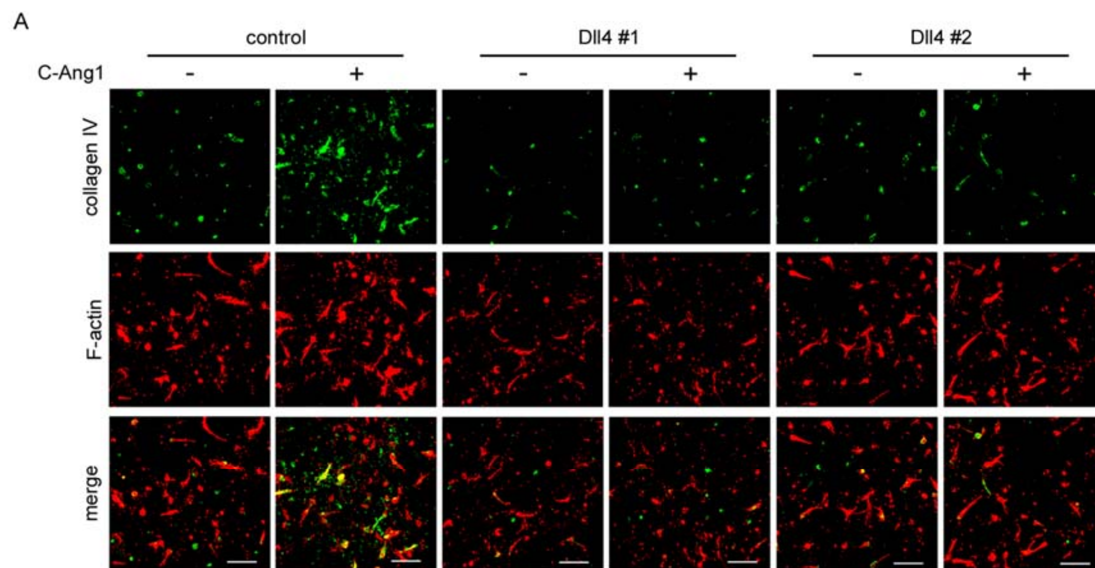

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