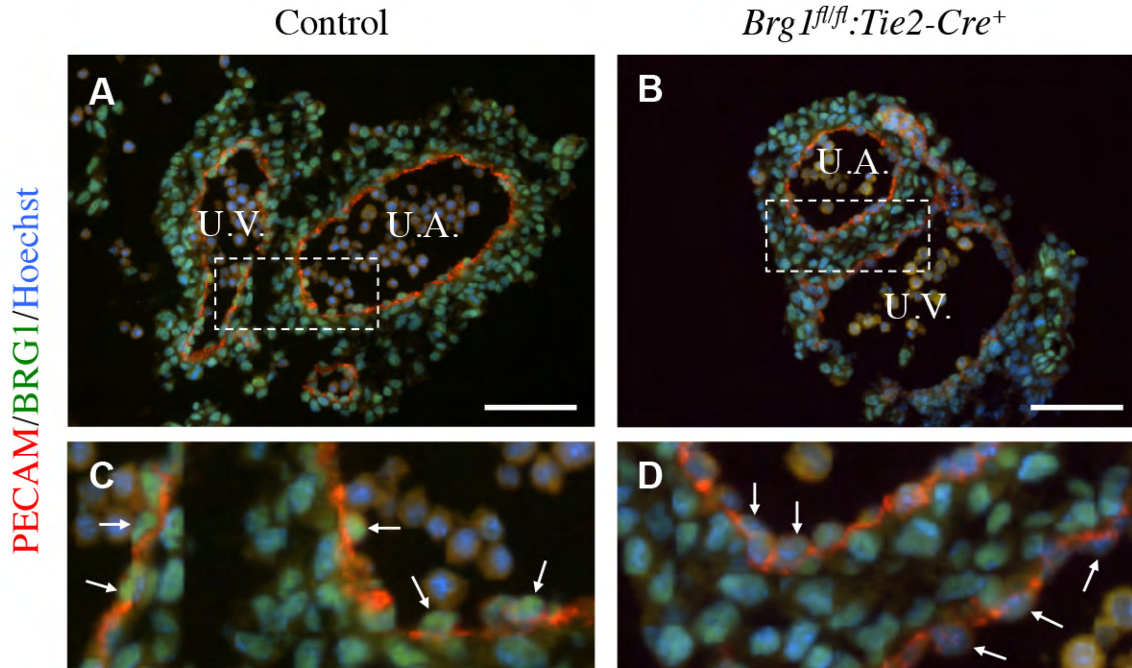


Fig. S1. BRG1 is expressed in arterial and venous endothelial cells, and is excised in *Brg1$^{fl/fl}$:Tie2-Cre^{+}* umbilical vessels. (A-D) E10.5 control and *Brg1$^{fl/fl}$:Tie2-Cre^{+}* umbilical vessels were cross-sectioned and immunostained for expression analysis. To determine BRG1 vascular expression and excision efficiency, sections were co-immunostained for the endothelial cell marker PECAM1 (red), BRG1 (green) and the nuclear marker Hoechst (blue). Endothelial cells in the control artery and vein expressed BRG1 (C), but BRG1 was significantly reduced in *Brg1$^{fl/fl}$:Tie2-Cre^{+}* endothelial cells (D). Boxed regions in A and B are shown magnified in C and D, respectively. Arrows indicate individual endothelial cells. Scale bar: 100 µm.

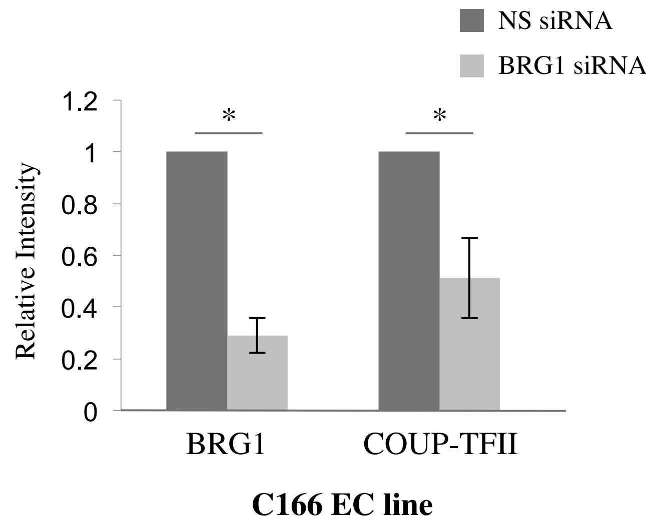
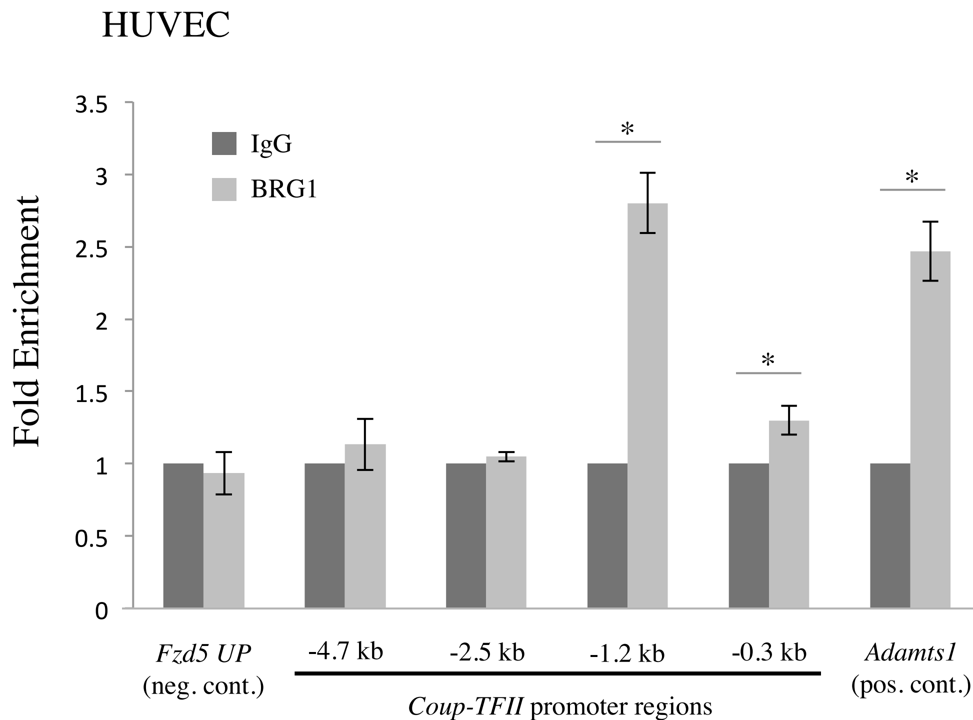


Fig. S2. Quantification of western blots showing diminished COUP-TFII expression in BRG1 knockdown endothelial cells. Four independent western blots analyzing BRG1 and COUP-TFII expression, as shown in Fig. 4H, were analyzed for band intensity. BRG1 and COUP-TFII intensities were normalized to the intensity of GAPDH. Data were combined and are presented as the relative intensities compared with NS siRNA-treated cells. Error bars represent s.e.m. Significant differences were calculated using a two-tailed Student's *t*-test (*P<0.05).



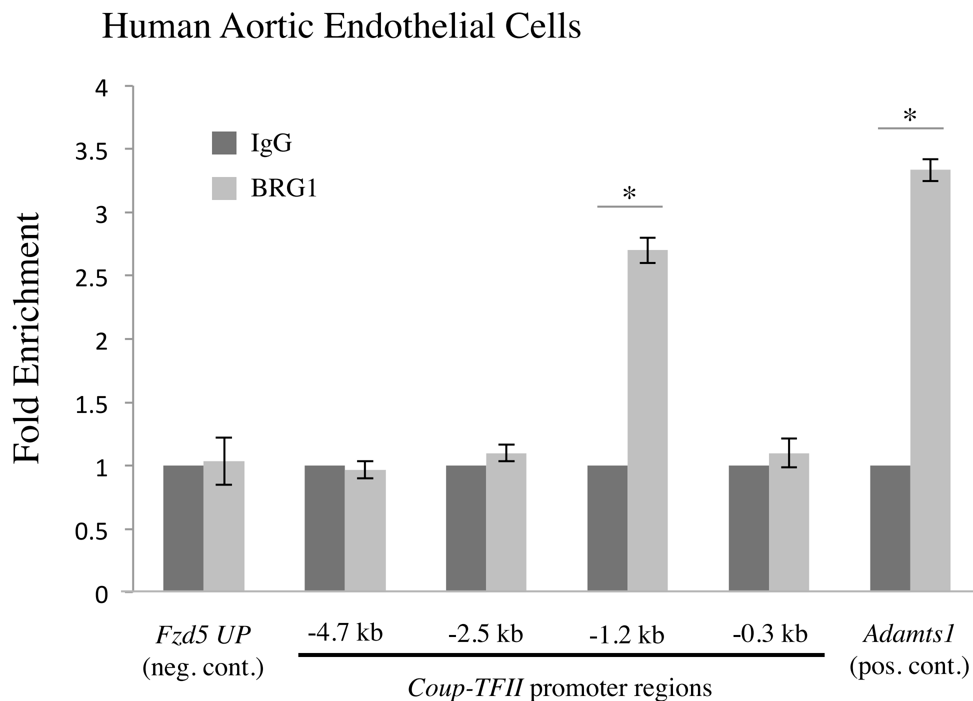


Fig. S3. BRG1 binding profile at the *COUP-TFII* promoter in HUVEC versus human aortic endothelial cells. ChIP assays were performed on HUVEC and primary human aortic endothelial cells using antibodies against BRG1 or isotype-matched non-specific IgG as a negative control. DNA was isolated and amplified by qPCR to determine whether BRG1 bound to various *COUP-TFII* promoter regions corresponding to those assessed in murine C166 cells (See Fig. 5B). Significant BRG1 binding was seen at the –1.2 kb and –0.3 kb regions in HUVECs and at the –1.2 kb region in aortic endothelial cells. A region upstream of the *Fzd5* promoter (*Fzd5 UP*) was used as a negative control BRG1-binding region, and the *Adamts1* promoter served as a positive control BRG1-binding region. Data from three independent experiments were combined and are presented as fold enrichment over the level of ChIP with negative control IgG antibodies. Significant differences were calculated using a two-tailed Student's *t*-test (*$P<0.05$).

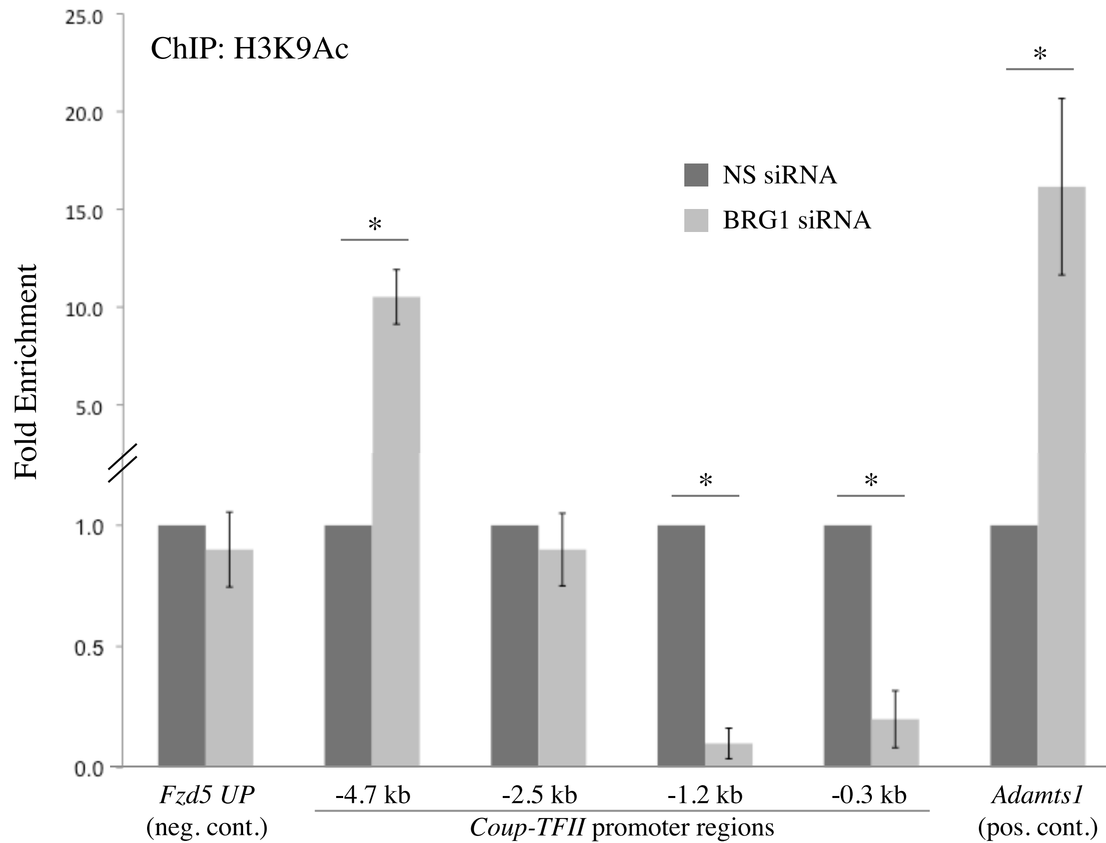


Fig. S4. Regions of the *COUP-TFII* promoter at which BRG1 binds undergo differential H3K9 acetylation upon depletion of BRG1. C166 endothelial cells were transfected with nonspecific (NS) or BRG1-specific siRNA oligos for 24 hours prior to processing for ChIP assays. ChIP with an antibody against acetylated H3K9 (H3K9Ac) was used to assess enrichment of this covalent epigenetic mark at various regions of the *Coup-TFII* promoter. H3K9Ac was enriched at the –4.7 kb region of the *COUP-TFII* promoter but was significantly decreased at the –1.2 kb and –0.3 kb promoter regions following BRG1 knockdown. BRG1 binds to all three of these regions in C166 cells (see Fig. 5B). A region upstream of the *Fzd5* promoter (*Fzd5 UP*) was used as a negative control region, and the *Adamts1* promoter served as a positive control region for H3K9 acetylation, respectively. Data from four independent experiments were combined and are presented as fold enrichment over the levels of ChIP with the H3K9Ac antibody in NS siRNA transfected cells. Significant differences were calculated using a two-tailed Student's *t*-test (*P<0.05).

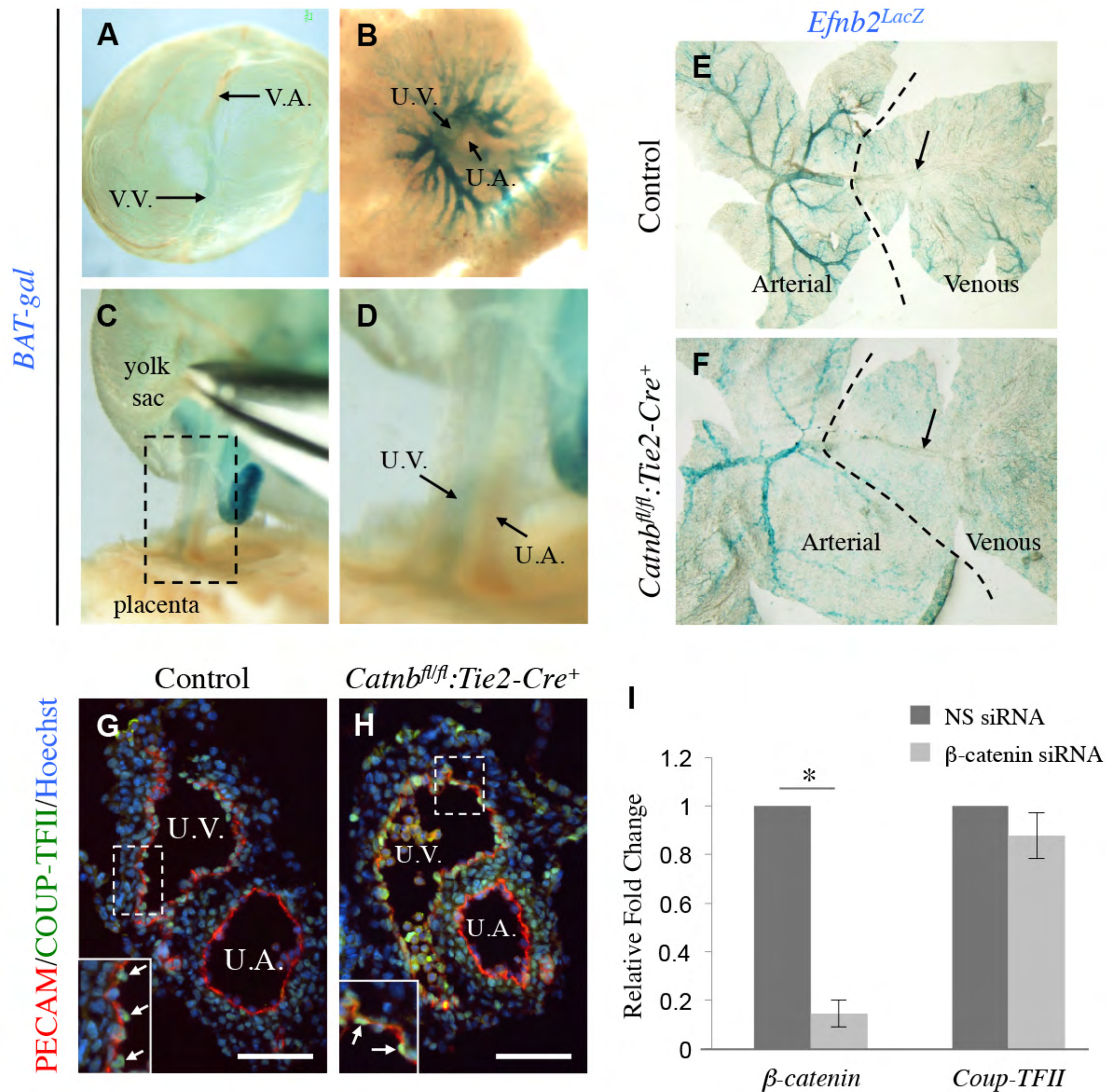


Fig. S5. Wnt signaling does not impact COUP-TFII expression in extra-embryonic veins. (**A-D**) The *BAT-gal* Wnt signaling reporter transgenic mouse line was analyzed for Wnt signaling in extra-embryonic tissues. E10.5 yolk sac (A), placenta (B) and umbilical vessels (C,D) were X-gal stained to identify sites of β-galactosidase/Wnt signaling activity (detected by blue color). (A) Yolk sac vitelline veins (V.V.) had more Wnt signaling reporter activity than vitelline arteries (V.A.). (B-D) Likewise, umbilical veins (U.V.) had more Wnt signaling reporter activity than umbilical arteries (U.A.). The boxed region in C corresponds to the magnified picture of umbilical vessels in D. (**E**,**F**) Control and β-catenin vascular mutant embryos (*Catnb$^{fl/fl}$:Tie2-Cre^{+}*) were crossed onto an *Efnb2^{LacZ}* arterial reporter line and stained with X-gal solution to reveal sites of *Efnb2* (*LacZ*) expression (detected by blue color). Flat-mounted E10.5 yolk sacs displayed comparable *Efnb2* expression in control (E) and *Catnb$^{fl/fl}$:Tie2-Cre^{+}* (F) vitelline arteries and no aberrant *Efnb2* expression in vitelline veins (arrows). (**G**,**H**) Cross-sections of umbilical vessels from littermate control and *Catnb$^{fl/fl}$:Tie2-Cre^{+}* embryos were immunostained for expression analysis. Sections were co-stained for the endothelial cell marker PECAM1 (red), COUP-TFII (green) and the nuclear marker Hoechst (blue). COUP-TFII expression was detected in endothelial cells from both control (G) and *Catnb$^{fl/fl}$:Tie2-Cre^{+}* (H) umbilical veins. Boxed regions are magnified in the insets. Arrows indicate individual endothelial cells. Scale bar: 100 µm. (**I**) C166 endothelial cells were transfected with nonspecific (NS) or β-catenin-specific siRNA for 24 hours. RNA was isolated, cDNA was synthesized and qPCR for β-*catenin* or *COUP-TFII* was performed. No significant difference was detected in *COUP-TFII* expression between NS and β-catenin knockdown cells. Data from three independent experiments were combined and are presented as relative fold change over the expression level in NS siRNA-treated cells. Significant differences were calculated using a two-tailed Student's *t*-test (*$P<0.05$).

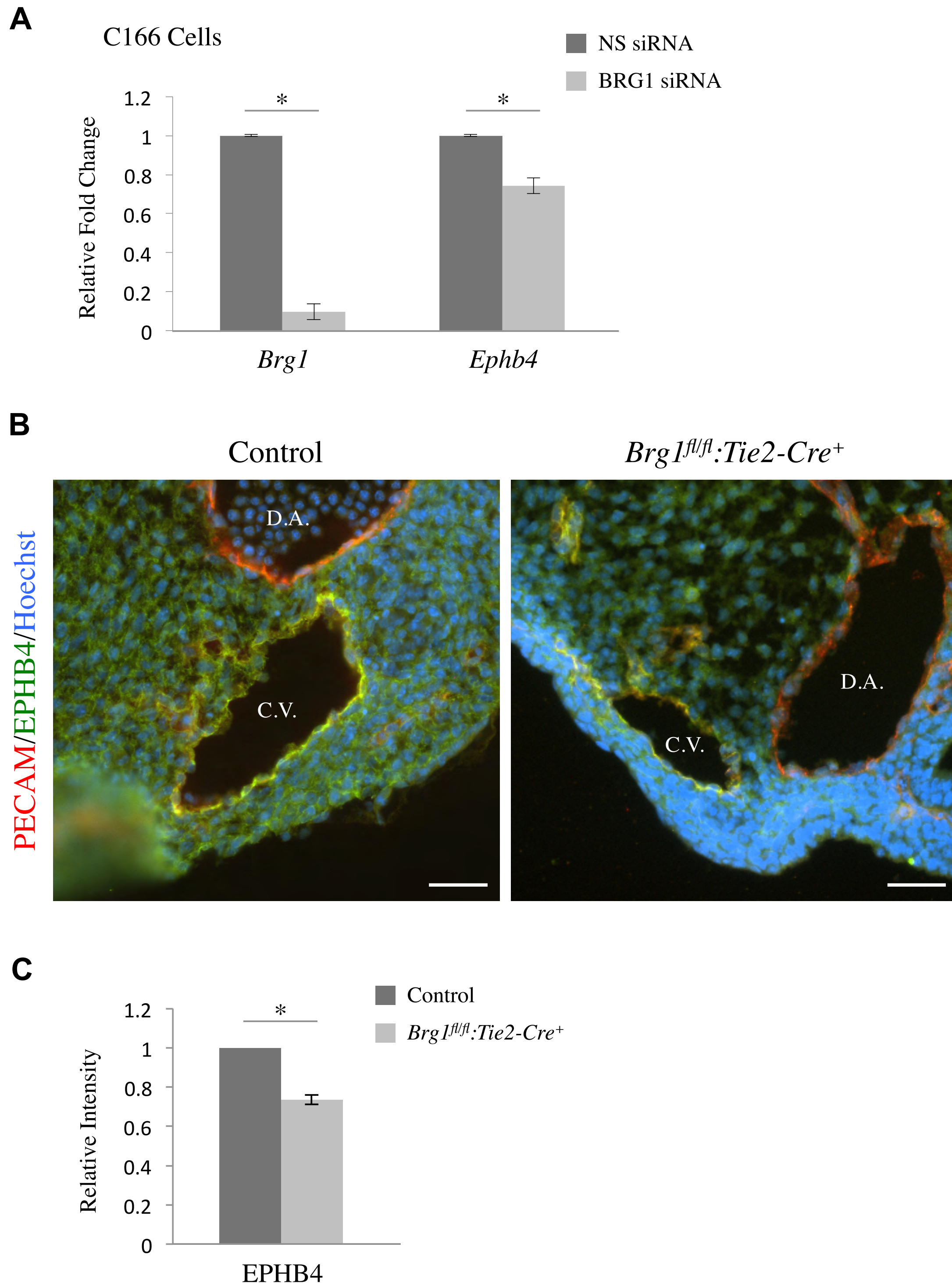


Fig. S6. The venous marker *Ephb4* is downregulated in BRG1 knockdown endothelial cells and in *Brg1$^{fl/fl}$:Tie2-Cre^{+}* cardinal veins. (**A**) C166 endothelial cells were transfected with nonspecific (NS) or BRG1-specific siRNA for 24 hours. RNA was isolated, cDNA was synthesized and qPCR for *Brg1* or *Ephb4* was performed. Data from three independent experiments were combined and are presented as relative fold change over the expression levels in NS siRNA-treated cells. Significant differences were calculated using a two-tailed Student's *t*-test (**P*<0.05). (**B**) E9.75 littermate control and *Brg1$^{fl/fl}$:Tie2-Cre^{+}* embryos were cross-sectioned, and sections containing a dorsal aorta (D.A.) and cardinal vein (C.V.) were immunostained for the venous marker EPHB4 (green), the endothelial cell marker PECAM1 (red) and the nuclear marker Hoechst (blue). EPHB4 staining levels were slightly diminished in *Brg1$^{fl/fl}$:Tie2-Cre^{+}* cardinal vein endothelial cells, which is similar to the slight downregulation of EPHB4 reported in *COUP-TFII$^{fl/fl}$:Tie2-Cre^{+}* embryonic veins (You et al., 2005). Scale bar: 100 µm. (**C**) Fluorescent intensity was calculated for EPHB4-immunostained sections such as those shown in B. Data from three independent sets of littermate control and *Brg1$^{fl/fl}$:Tie2-Cre^{+}* embryos were combined and are presented as relative intensity compared with the normalized fluorescence intensity of EPHB4 in control cardinal vein endothelial cells. Significance was calculated using a two-tailed Student's *t*-test (**P*<0.05).

Table S1. qPCR primers

β-actin	5′-TGTTACCAACTGGGACGACA-3′ and 5′-GGGGTGTTGAAGGTCTCAAA-3′
Gapdh	5′-TCAACGGCACAGTCAAGG-3′ and 5′-ACTCCACGACATACTCAGC-3′
Brg1	5′-CAGTGGCTCAAGGCTATCG-3′ and 5′-TGTCTCGCTTACGCTTACG-3′
Hey2	5′-GGTAGTTGTCGGTGAATTGGAC-3′ and 5′-AAGCGCCCTTGTGAGGAAAC-3′
COUP-TFII	5′-AGTACTGCCGCCTCAAAAAG-3′ and 5′-CGTTGGTCAGGGCAAACT-3′
Nrp1	5′- GACAAATGTGGCGGGACCATA-3′ and 5′-TGGATTAGCCATTCACACTTCTC-3′
Nrp2	5′-CAGTTCTGGTGGGAGGGATA-3′ and 5′-GAGAAGCCAGCAAGATCCAC-3′
Ephb4	5′-CATCAAGGTGGACACAGTGG-3′ and 5′-AAAGAGATGCAGGGAGAGCA-3′
Hey1	5′-GGTACCCAGTGCCTTTGAGA-3′ and 5′-GTGCGCGTCAAAATAACCTT-3′
Dll4	5′-AGCTGGGACTCAGCAAGTGT-3′ and 5′-TAGTAGCCTGGGGGACACAG-3′
Foxc1	5′-AGCAGCAGAACTTCCACTCGGT-3′ and 5′-TCGGCTTTGAGGGTGTGTCA-3′
β-catenin	5′-TGGCAGCAGCAGTCTTAC-3′ and 5′-GAGGTGTCAACATCTTCTTCC-3′.

Table S2. ChIP primers used

Mouse primers

mFzd5UP	5′-GGTGACTTAGGGCAAAACCA-3′ and 5′-AGGCCACCATACCAGGTTCT-3′
mCOUPP-TFII −0.3 kb site	5′-TCCTCCTCCTCTTCCTCCTC-3′ and 5′-AAGATCTCGCTCGCTCTCAC-3′;
mCOUP-TFII −1.2 kb site	5′-GCTAAGTTGCAGCAGTCGTG-3′ and 5′-GAGGGAATGCGATTTATAGG-3′;
mCOUP-TFII −2.5 kb site	5′-CACACACACGAAGCTTCTCC-3′ and 5′-CAGCCATTTCCATGTCAAGA-3′;
mCOUP-TFII −4.7 kb site	5′-AAGCCAGCGAGAGAGACGACA-3′ and 5′-TGGACTGTGCTGGGATCTCG-3′
mAdamts1 promoter	5′-CACTCTTTTCCCCAGAGCAG-3′ and 5′-CGCCCCTTTATAGCCACATA-3′

Human primers

hFzd5UP	5′-CACCATGCCTGGCTACTTTT-3′ and 5′-ATTTTTGCCATCAGGGAACA-3′
hCOUP-TFII −0.3 kb site	5′- GATTTCGATGGCTTTCCTGA-3′ and 5′-GAAGAGGAGGTGGTGGTGAA-3′
hCOUP-TFII −1.2 kb site	5′-GCTAAGTTGCAGCAGTCGTG-3′ and 5′-GAGGGAATGCGATTTATAGG-3′
hCOUP-TFII −2.5 kb site	5′- CCAAGGATGTTTTCTGTGGTC-3′ and 5′-AAAAACCTCATGCCGGTAAA-3′
hCOUP-TFII −4.7 kb site	5′-GGCAGCGAGGACTCTTACAC-3′ and 5′-CAGGGGCTGTCAGAGTTAGG-3′
hAdamts1 promoter	5′-TTCGGTTGGAGAACGCAGTCC-3′ and 5′-GAAGGTGGAGAAGTGGGGTGAG-3′