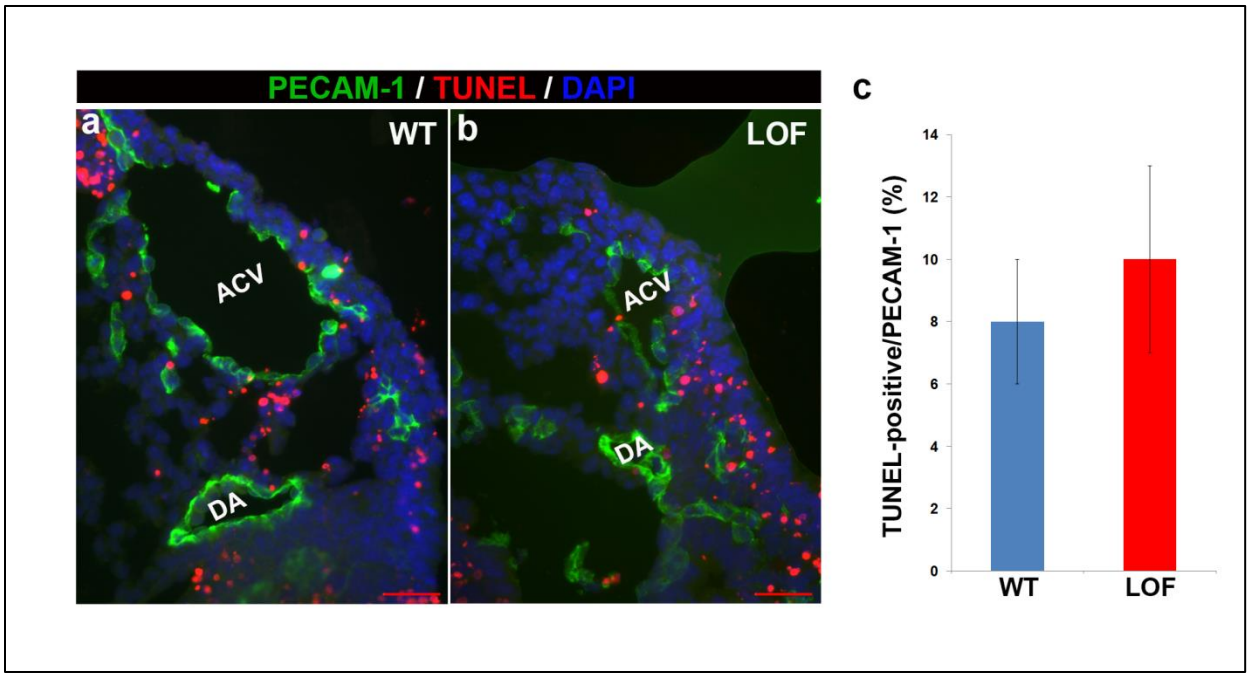
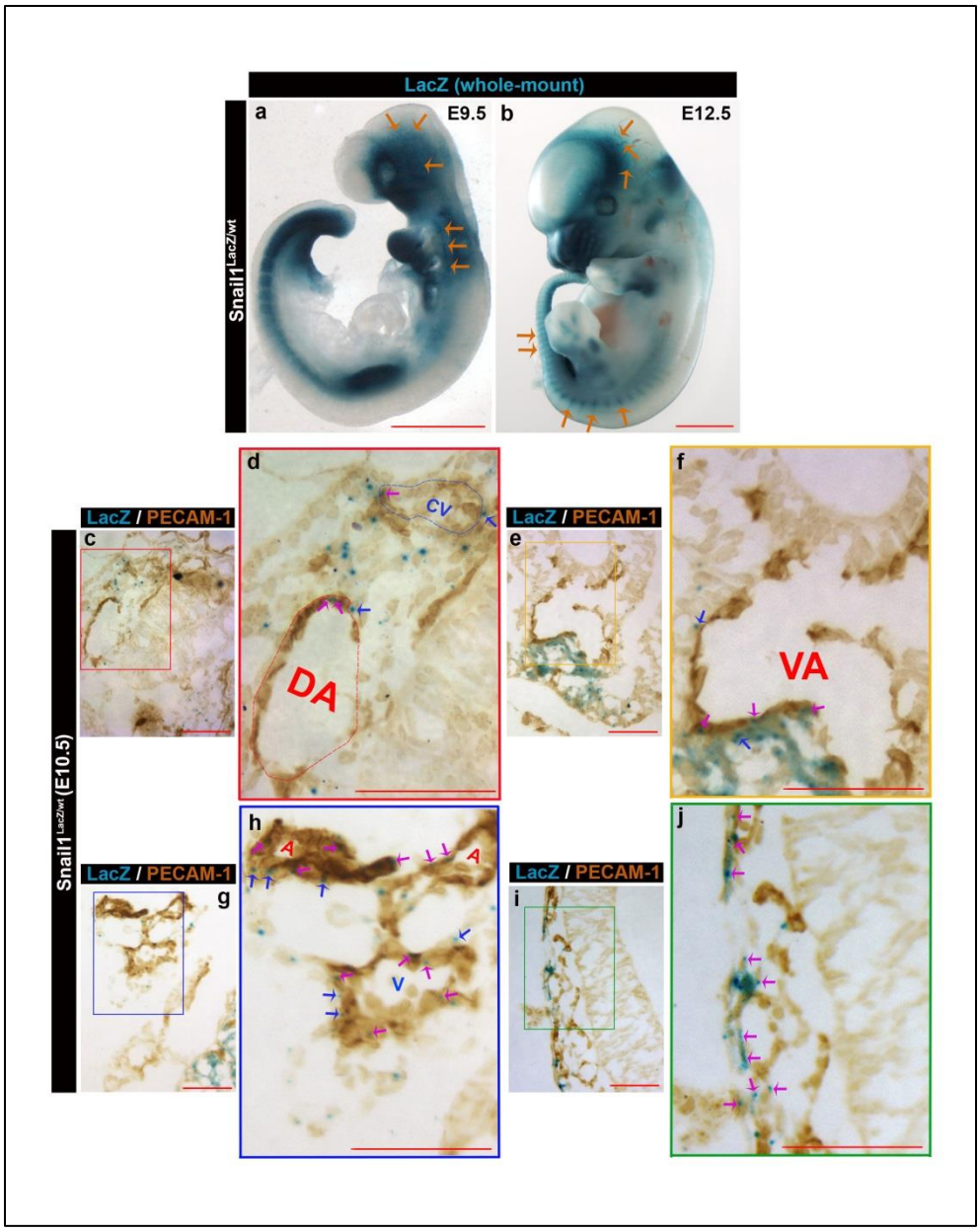
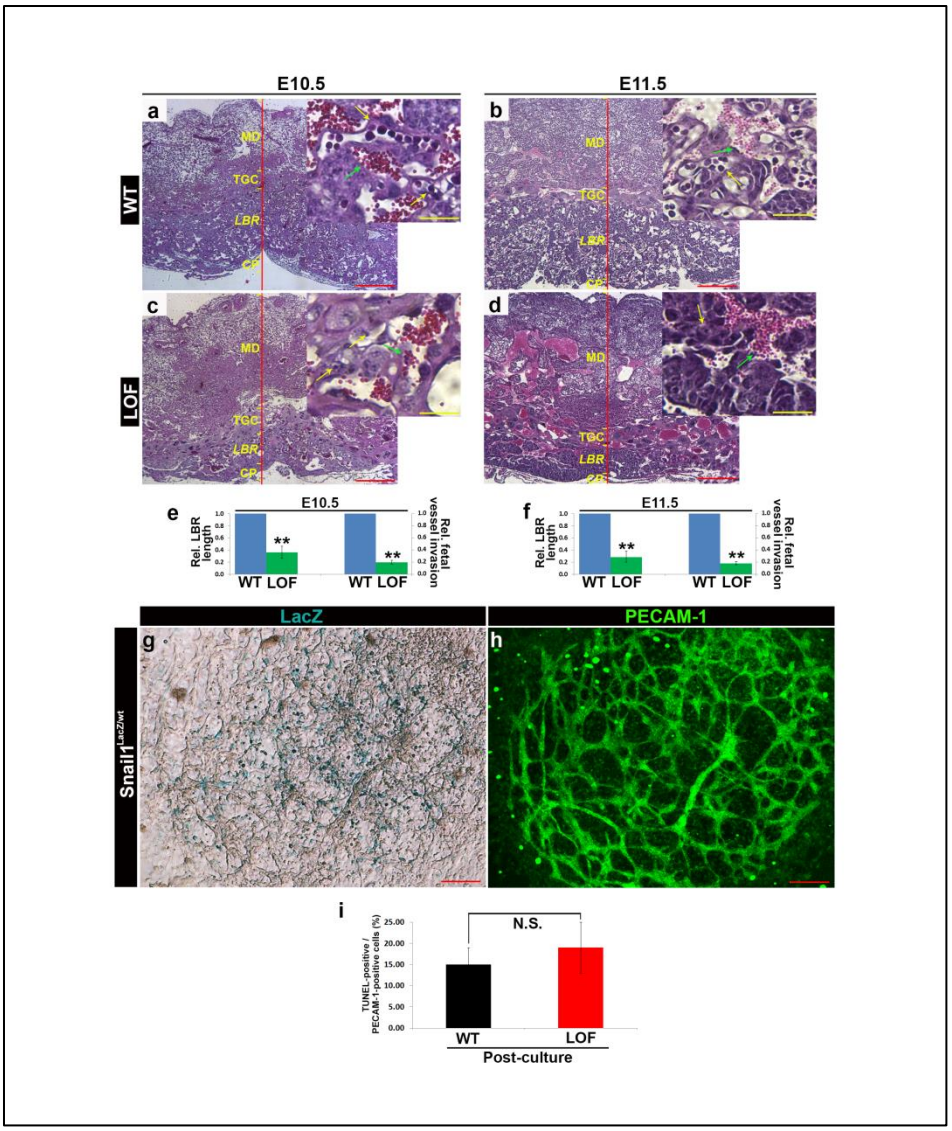


Supplementary Figure 1. EC-specific Deletion of Snail1 Does Not Affect EC Apoptosis. (a,b) Cryo-sections of WT (a) and Snail1 LOF (b) embryos at E10.5 were double-stained for TUNEL (red) and PECAM-1 (green). Nuclei were stained with DAPI (blue). Scale bars: 50 μm. (c) Quantification of apoptotic ECs ($TUNEL^+/PECAM^+$ cells) in the embryos (n = 3 each). Data are presented as mean ± SEM. Not significant, Student's t test.



Supplementary Figure 2. Snail1 Is Expressed in Arterial and Venous Beds During Embryonic Development

(a,b) Whole-mount X-gal/lacZ staining of embryo at E9.5 (a) and E12.5 (b), respectively. Arrows (red) highlights X-gal/lacZ-positive cranial vessels and the DA at E9.5, as well as cranial vessels and ISVs at E12.5. Scale bars: 1 mm. (c-j) E10.5 Snail1$_{LacZ/wt}$ embryos were sectioned and stained with X-gal/lacZ followed by PECAM-1 immunohistochemical staining. X-gal/lacZ-positive ECs and perivascular cells (denoted by green arrows and red arrows, respectively) are localized within the CV and DA [(c,d); the boxed area in panel c is enlarged in panel d] as well as the vitelline artery (VA) [(e,f); the boxed area in panel e is enlarged in panel f]. Additional staining is observed large arteries and veins [(g,h); the boxed area in panel g is enlarged in panel h] as well as small vessels [(i,j); the boxed area in panel i is enlarged in panel j]. DA, dorsal aorta; CV, cardinal vein; VA, vitelline artery; A, artery; V, vein. Scale bars: 50 μm.



Supplementary Figure 3. EC-specific Deletion of Snail1 Alters Vascular Remodeling in Placental Tissues and Allantois Explant Cultures

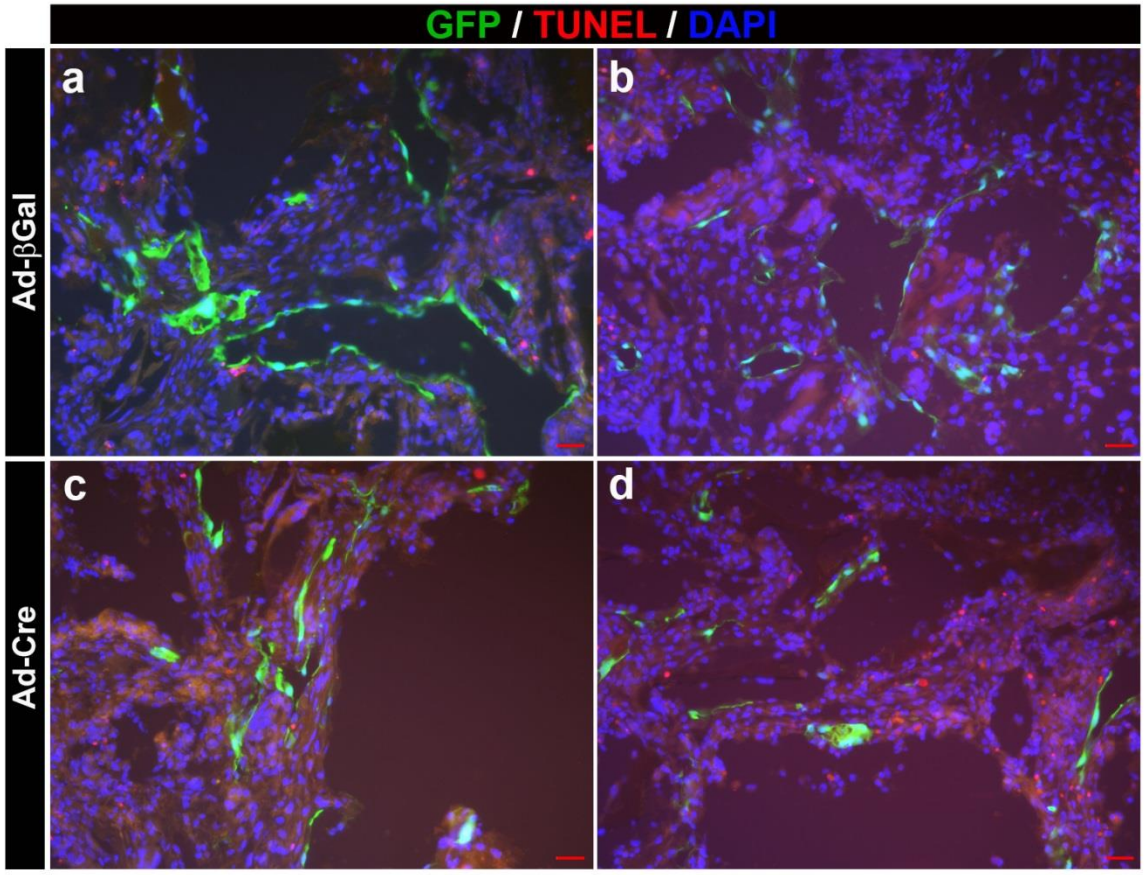
(a-d) H&E staining of placental sections from WT (a,c) and Snail1 LOF (b,d) embryos at E10.5 (*left panel*) and E11.5 (*right panel*). Although the maternal decidua (MD), the chorionic plate (CP), and the trophoblast giant cells (TGC) are comparable, the labyrinthine layer (LBR) is reduced in Snail1 LOF placentas. Insets display higher magnifications of representative fields. Green arrows and yellow arrows denote maternal blood vessels and fetal vessels, respectively. Scale bars: (a-d) 400 μm; (a-d insets) 50 μm.

(e) Quantification of length of LBR layer and invaded fetal blood vessels from E10.5 WT and Snail1 LOF placentas (n = 4). Data are presented as mean ± SEM. **p < 0.01, Student t test.

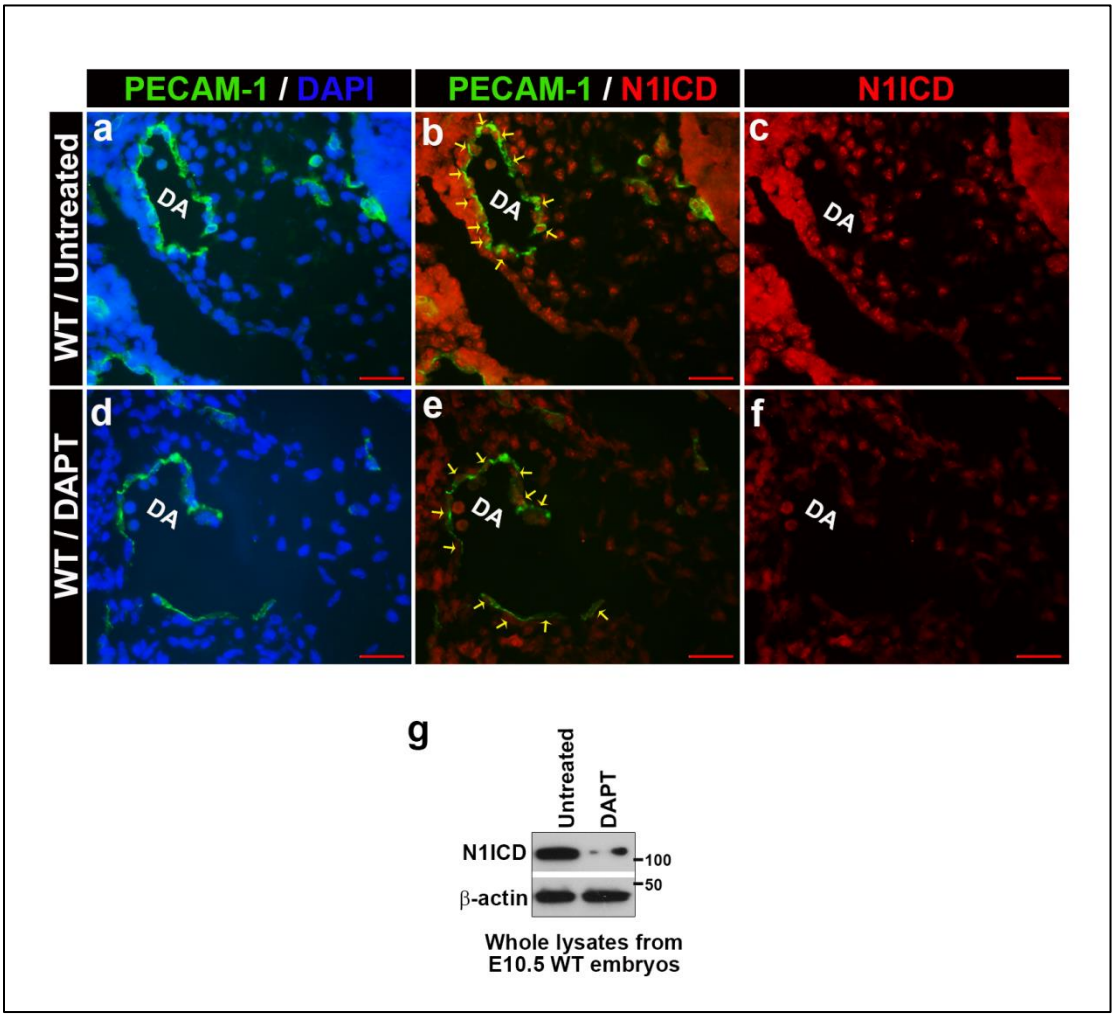
(f) Quantification of length of LBR layer and invaded fetal blood vessels from E11.5 WT and Snail1 LOF placentas (n = 4). Data are presented as mean ± SEM. **p < 0.01, Student t test.

(g,h) Allantoises dissected from Snail1$_{LacZ/wt}$ embryos were subjected to whole-mount X-gal/lacZ staining (g) followed by PECAM-1 immunofluorescent staining (h). Scale bars: 100 μm.

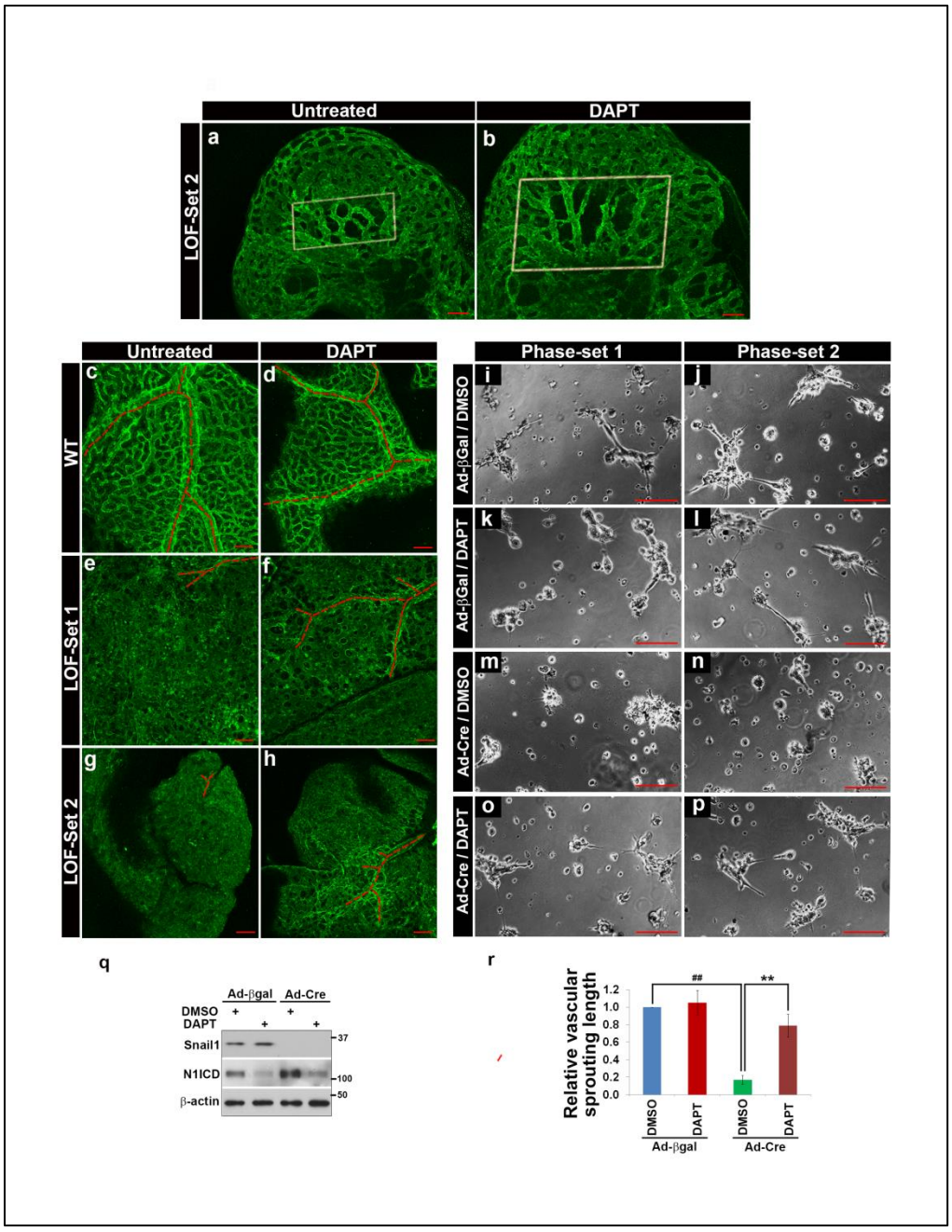
(i) The allantois explant cultures were double-stained for PECAM-1 and TUNEL, and the apoptotic ECs (TUNEL$_+$/PECAM$_+$ cells) were counted (n = 3 each). Data are presented as mean ± SEM. Not significant, Student's t test.



Supplementary Figure 4. Deletion of Snail1 Does Not Affect EC Apoptosis.
(a-d) Cryo-sections of implants originated from Adeno-βGal (a,b) or Adeno-Cre (c,d) treated ECs were stained for TUNEL (red). Nuclei were stained with DAPI (blue). Scale bars: 50μm.



Supplementary Figure 5. DAPT-Dependent Inhibition of Embryonic N1ICD Expression
(a-d) Cross-sections obtained from untreated or DAPT-treated embryos were co-stained with antibodies against PECAM-1 and N1ICD. Cell nuclei were stained with DAPI (blue). Scale bars: 50 μm. Results are representative of 2 experiments performed.
(e) Whole cell lysates obtained from untreated or DAPT-treated embryos were subjected to Western blot analysis. Results are representative of 2 experiments performed.



Supplementary Figure 6. DAPT-Dependent Rescue of Vascular Remodeling Defects in Snail1 LOF Embryos, Yolk Sacs, Allantois Explants and ECs

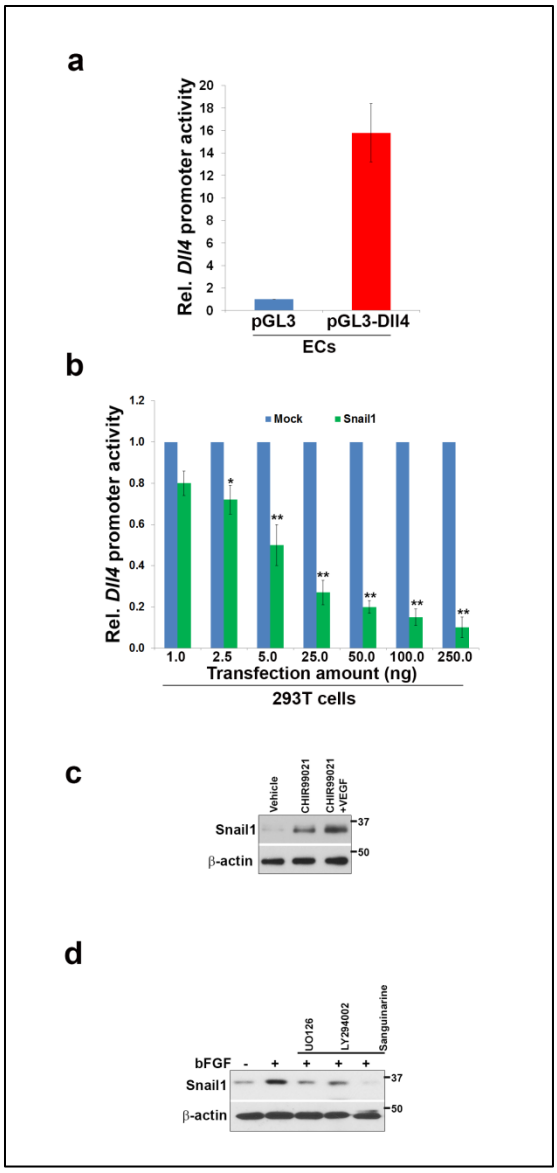
(a-b) Confocal analysis of PECAM-1-stained whole-mounts of untreated (a) or DAPT-treated (b) embryos. Rectangled areas highlight improved vascular remodeling in DAPT-treated Snail1 LOF mutant embryos. Scale bar: 100 μm.

(c-h) Timed-pregnant mice were treated with vehicle or DAPT, and the yolk sacs dissected from the vehicle- or DAPT-treated embryos were subjected to whole-mount PECAM-1 immunofluorescent staining. Scale bars: 100 μm.

(i-p) Adeno-βGal and Adeno-Cre infected ECs were seeded atop Matrigel-coated dishes and cultured for 12 h in the presence of vehicle or DAPT (8 μM). Scale bars: 50 μm.

(q) Adeno-βGal and Adeno-Cre infected ECs were treated with vehicle or DAPT (8 μM) for 12 h and subjected to Western blot analysis.

(r) Quantification of relative vascular sprouting in cultured ECs (n=3). Data are presented as mean ± SEM. ## $p < 0.01$; **$p < 0.01$, ANOVA.



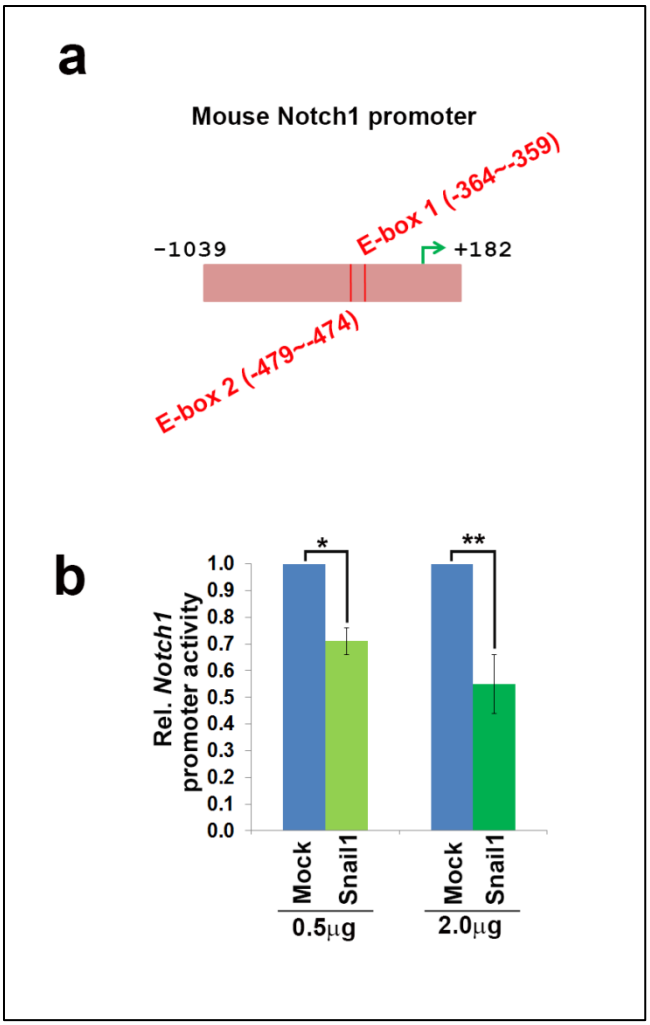
Supplementary Figure 7. Snail1-Dependent Transcriptional Repression of Dll4 Promoter Activity

(a) ECs derived from E10.5 Snail1$_{fl/fl}$ embryos were electroporated with 0.5 μg of pGL3 control vector or a mouse *Dll4* promoter reporter construct and luciferase activity determined. (mean ± SEM; n=3). **p < 0.01, Student's t test.

(b) 293T cells were co-transfected with increasing amounts of a mock or human Snail1 expression vector (1.0 ng-250.0 ng) in combination with a full-length mouse *Dll4* promoter reporter construct (25 ng) and analyzed by luciferase assay. Ectopic expression of Snail1 represses Dll4 promoter activity in a dose-dependent manner. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, Student's t test.

(c) Cultured WT ECs were treated with GSK3 inhibitor, CHIR99021 (3 μM) in the presence or absence of VEGF (100 ng/ml) for 12 h. Cell lysates were prepared and subjected to Western blot analysis.

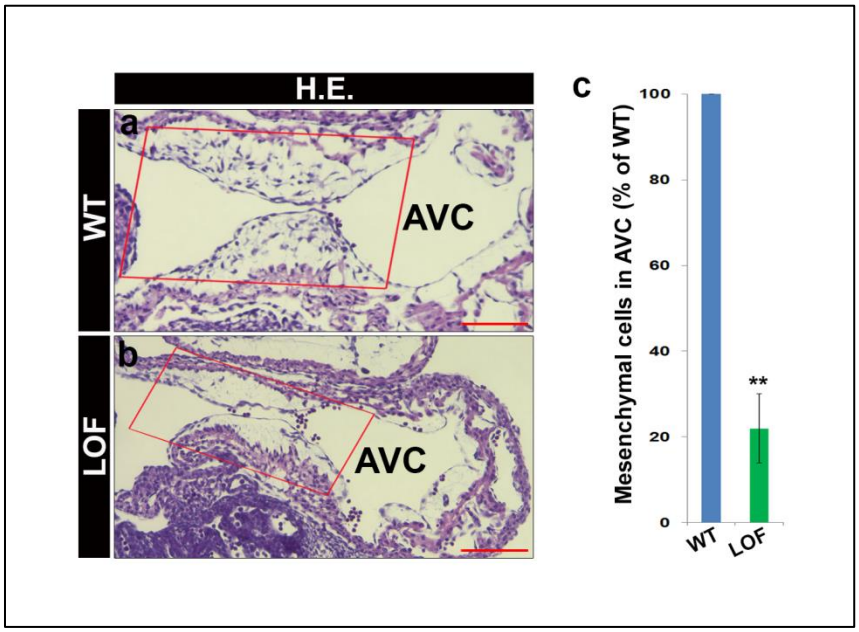
(d) Cultured WT ECs were pretreated with U0126 (20 μM), LY294002 (10 μM) or sanguinarine (2.5 μM) for 1 h followed by stimulation with bFGF (20 ng/ml) for 12 h. Cell lysates were prepared and subjected to Western blot analysis.



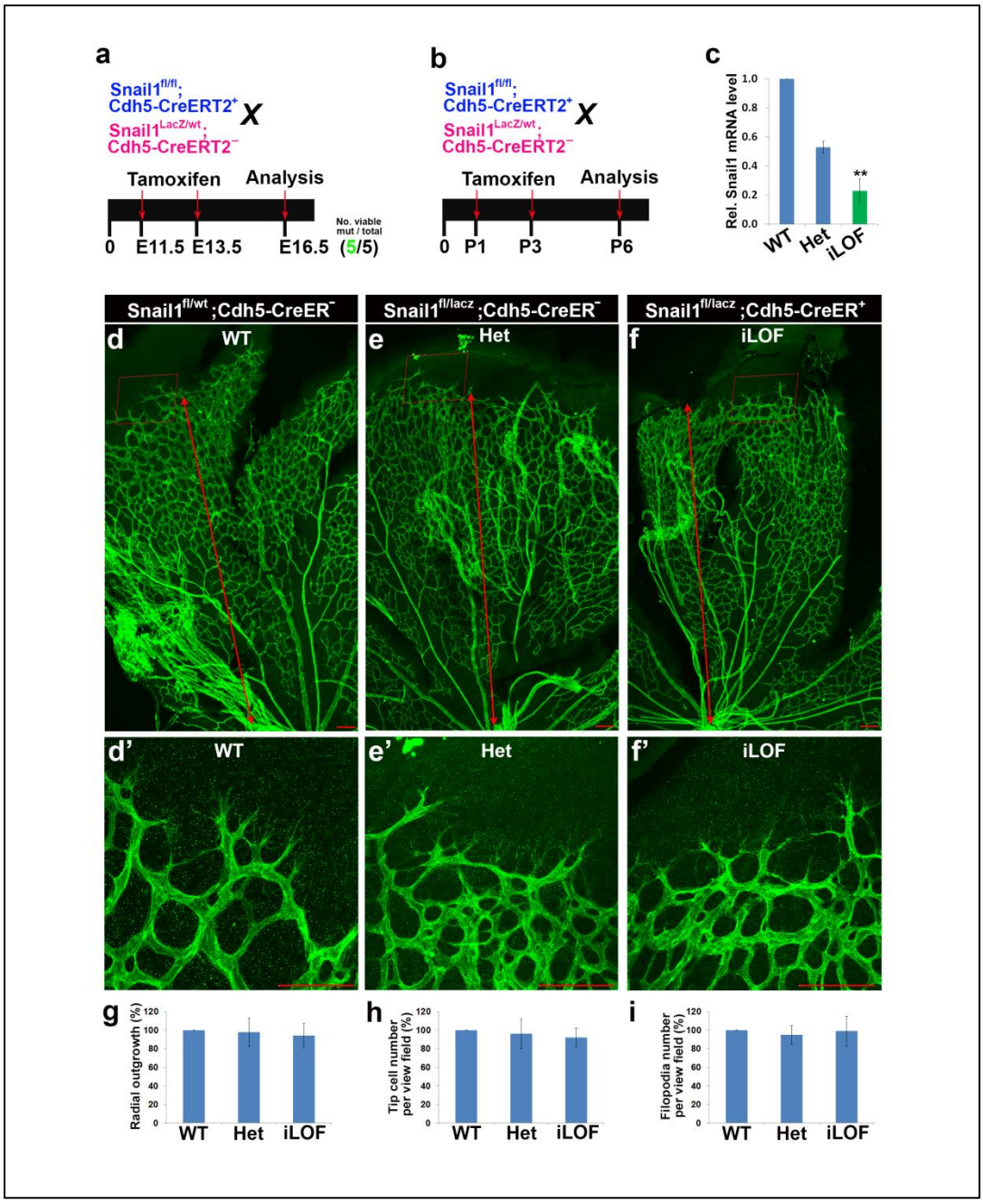
Supplementary Figure 8. Snail1-Dependent Transcriptional Repression of Notch1 Promoter Activity

(a) Diagram depicts the two E-boxes located within the proximal region of the mouse *Notch1* promoter.

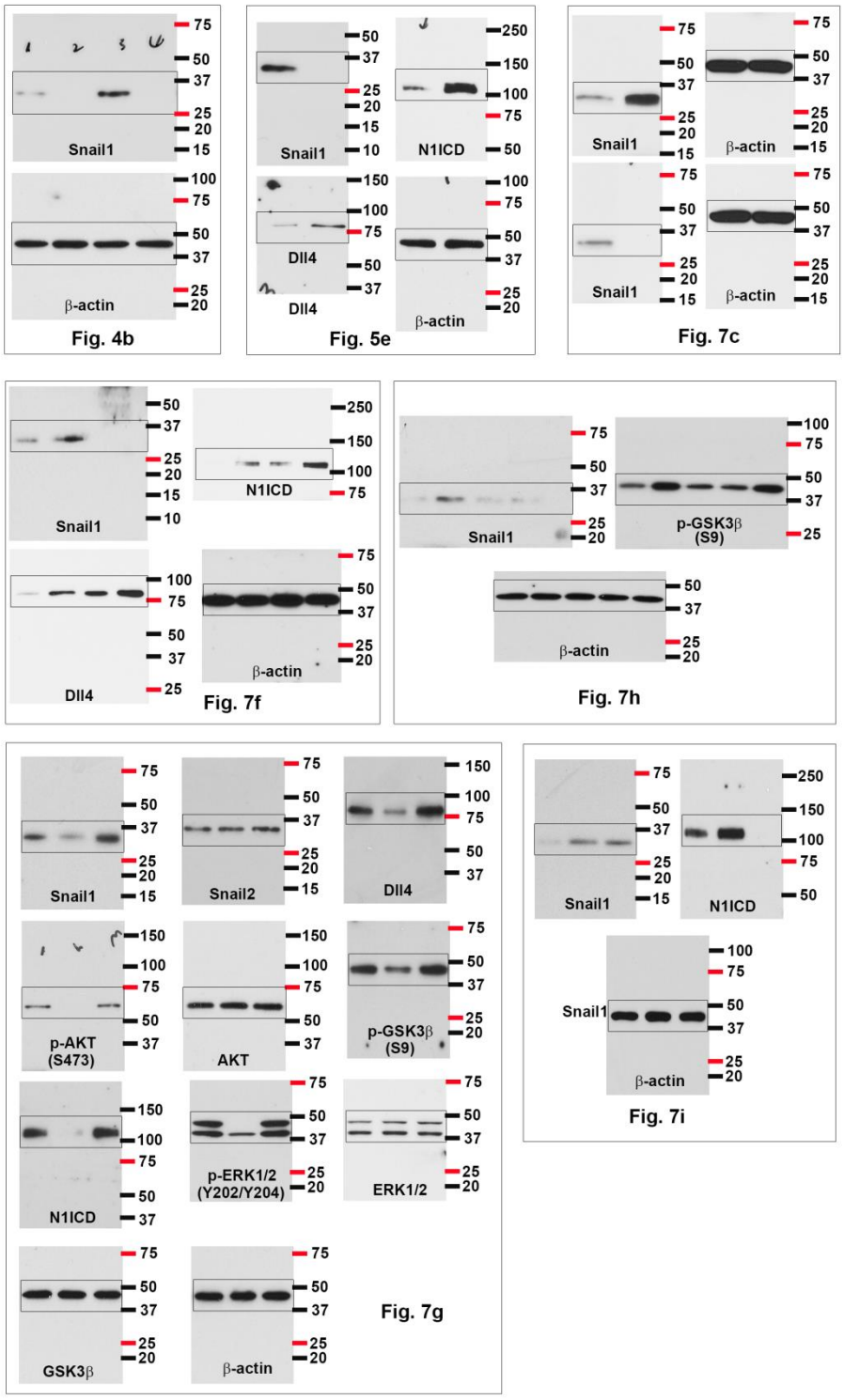
(b) ECs derived from E10.5 Snail1$_{fl/fl}$ embryos were electroporated with a mock or Snail1 expression vector (0.5 μg or 2.0 μg) in combination with 0.5 μg of a mouse *Notch1* promoter reporter construct and luciferase activity determined. (mean ± SEM; n=3). *p < 0.05, **p < 0.01, Student's t test.



Supplementary Figure 9. Cardiac Cushion Development in Snail1 LOF Mutant Embryos
(a,b) Sagittal sections from paraffin-embedded E10.5 WT (a) and Snail1 LOF (b) embryos were evaluated by H&E staining. Representative images show mesenchymal cell invasion into the atrioventricular canal (AVC) (boxed in red). Scale bar: 50 μm.
(c) Quantification of relative mesenchymal cell invasion into AVC regions (boxed in red) (n=3 each). Data are presented as mean ± SEM. **p < 0.01, Student's t-test.



Supplementary Figure 10. Snail1 and Postnatal Retinal Angiogenesis. (a,b) Schematic of Tamoxifen administration strategy in embryos (a) and pups (b). **(c)** Pulmonary ECs isolated from tamoxifen-treated pups were subjected to RT-qPCR analysis. Data are presented as mean ± SEM (n=3). **p < 0.01, ANOVA test. **(d-f)** Whole-mount PECAM-1 immunofluorescent staining of P6 WT (d and d'), Het (e and e') and iLOF (f and f') retina. Scale bar: 100 µm. **(g)** Quantification of radial growth of WT (double-arrow shown in d), Het (double-arrow shown in e) and iLOF (double-arrow shown in f) retinal vasculature is shown [68]. Data are presented as mean ± SEM (n = 4). Not significant, ANOVA. **(h and i)** Quantification of tip cell number **(h)** and filopodia number **(i)** per microscopic field at the angiogenic fronts in WT (d and d' with d´ displaying an enlarged image of the boxed area outlined in d), Het (e and e', e' represents the indicated area in e) and iLOF (f and f', f' represents the indicated area in f) retinas (n = 4). Data are presented as mean ± SEM. Not significant, ANOVA.



Supplementary Figure 11. Full-Length Images of Western Blots
(a) Whole membrane blots show the specific single bands using Snail1, β-actin, Dll4, N1ICD, pGSK3β, Snail2, p-AKT, AKT, p-ERK1/2 and ERK1/2 antibodies for the indicated figures. Blot images of the selected portions shown in each panel were used for the indicated figures in the text.

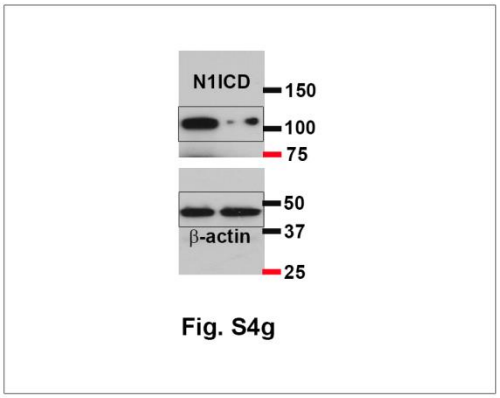


Fig. S4g

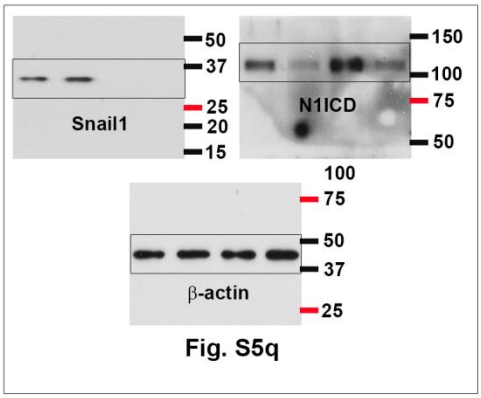


Fig. S5q

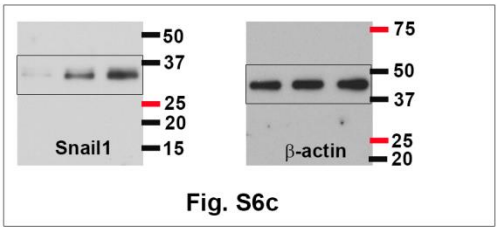


Fig. S6c

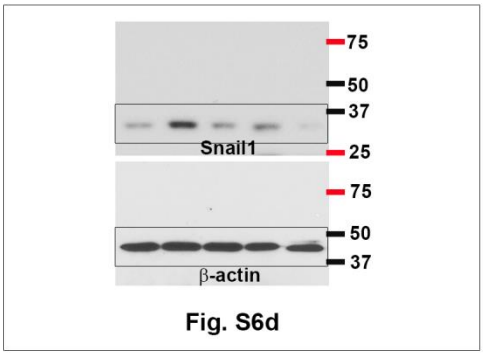


Fig. S6d

Supplementary Figure 11 continued. Full-Length Images of Western Blots
(a) Whole membrane blots show the specific single bands using Snail1, β-actin, Dll4, N1ICD, pGSK3β, Snail2, p-AKT, AKT, p-ERK1/2 and ERK1/2 antibodies for the indicated figures. Blot images of the selected portions shown in each panel were used for the indicated figures in the text.

Supplementary Table 1_Wu

qPCR primer sequences

Gene	Sequence
Mouse Snail1-Forward	AAGATGCACATCCGAAGC
Mouse Snail1-Reverse	ATCTCTTCACATCCGAGTGG
Mouse Dll4-Forward	GACTGAGCTACTCTTACCGGGTCA
Mouse Dll41-Reverse	CTTACAGCTGCCACCATTTCGACA
Mouse Jag1-Forward	CGTGGCAACGACCGTAATCGC
Mouse Jag1-Reverse	ACTGGAATCCCAGGCCTCCAC
Mouse Notch1-Forward	TGCCTGAATGGAGGTAGGTGCGAA
Mouse Notch1-Reverse	GCACAGCGATAGGAGCCGATCTCA
Mouse Hey1-Forward	GGCTGGTACCCAGTGCCTTTG
Mouse Hey1-Reverse	CCTTTCCCTCCTGCAGTGTGC
Mouse Hey2 -Forward	CCAGGCTACAGGGGGTAAAGG
Mouse Hey2-Reverse	CGGGTCAAGGCCTTCCACTGA
Mouse Hes1-Forward	GGAGAAGAGGCGAAGGGCAAG
Mouse Hes1-Reverse	GGTTCCGGAGGTGCTTCACAG
Mouse Ephrin B2-Forward	TGGGTCTTTGGAGGGCCTGGAT
Mouse Eprin B2-Reverse	GGACCGTGATTCCTGGCTGATC
Mouse Eph B4-Forward	CAGGGTACGAGGCTGGGGAA
Mouse Eph B4-Reverse	GCATGGCAGGCAGGACTCGT

β