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Supplementary Data

Supplemental Table 1. List of antibodies

Supplemental Fig. 1. DNMAML1-GFP is expressed in cerebral vSMCs from SM22-Cre$^+$/DNMAML1$^+$ embryos. Middle cerebral arteries of E13.5 control (A) and SM22-Cre$^+$/DNMAML1$^+$ (B) embryonic brains visualized with Dylight 594-lectin (red) (arrows). (C-H) In transverse sections above the level of the optic chiasm, anti-GFP (green) and lectin co-staining reveals expression and nuclear localization of DNMAML1 in vSMCs from mutant (D, arrowheads) but not control vessels (C). DNMAML1-GFP (green) expression co-localizes with SMC markers SMA (F) and NG-2 (H) in mutant arteries while GFP signal is undetectable in the control vessels (E and G). Nuclei identified by DAPI counterstain (blue). Scale bar: 10 µm.

Supplemental Fig. 2. Preserved EC proliferation in perineural vessels o**f SM22-Cre$^+$/DNMAML1$^+$ embryos**. Ki67 antibody reactivity (green) identifies proliferating lectin-positive ECs (red) in the perineural regions (arrowheads) of E9.5 (A-D), E11.5 (E-H), E13.5 (I and J) and E15.5 (K and L) embryos. Cartoon inserts in A, E, I, and K illustrate the orientation of the region of interest: (A-D) sagittal plane, midline; (E-H) transverse plane; (I-L) coronal plane. (M) Quantification of Ki67$^+$Lectin$^+$ cell counts normalized to controls for each stage. Data represent mean±SEM percentage of cell numbers per high power field normalized to controls (N=6 sections per embryo, N=3 embryos per stage, *p*>0.05 at all stages). Nuclei identified by DAPI counterstain (blue). Scale bars: 50 µm in A, B, E and F and 10 µm in C, D, G, H and I-L).

Supplemental Fig. 3. Absence of vSMC apoptosis in SM22-Cre$^+$/DNMAML1$^+$ embryos. (A-H) Cleaved Caspase 3 (C-Cas-3, green) signal does not co-localize with SMA-positive (red) vascular structures in E11.5, E13.5 and E15.5 embryos. Cartoon inserts in A, E and G illustrate plane of section for respective stages (A-D, transverse; E-H, coronal). Clusters of C-Cas-3 stained apoptotic cells appear only in non-vascular neuronal tissue in the developing cortical plate as well as in the ventral telencephalon of both control and mutant embryos (A, B and E, F, arrowheads). N=3 per group/stage. Scale bar: 10 µm.

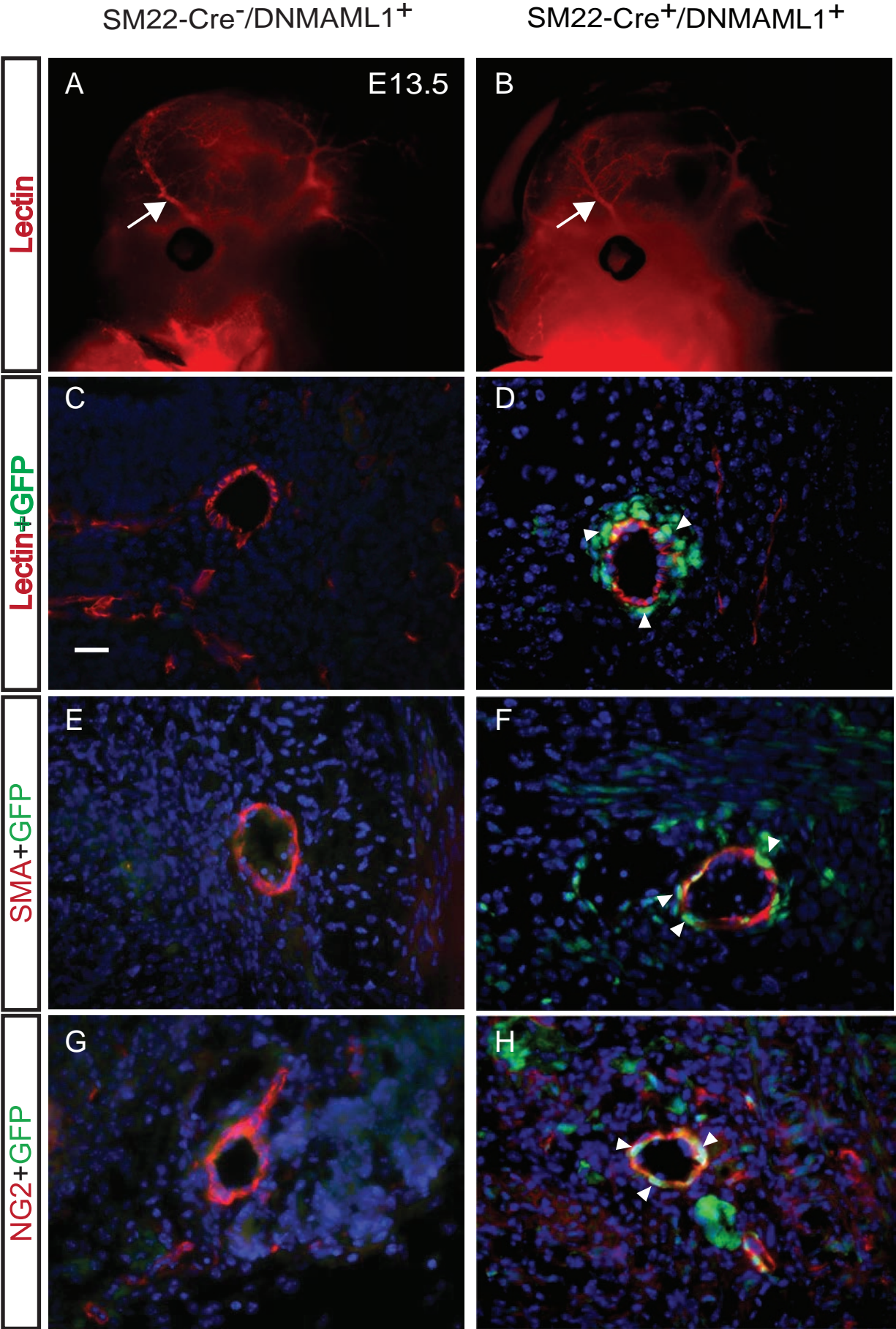**Supplemental Fig. 4. Notch ligand and receptor expression within arterial segments of the anterior cW.**

Panels are representative sections containing ACA/AComA conduit assembly in E13.5 control, SM22-Cre⁻/DNMAML1⁺ embryos. Immunoreactivity against Notch1 (A-C), Notch3 (D-F), and Jagged1 (G-I) is detected in SMCs as shown by co-localized expression with the smooth muscle specific marker, αSMA. Notch ligand Dll4 expression co-localizes with the endothelial cell marker, CD31 (J-L). Panels C, F, I and L are merged images of panels A-B, D-E, G-H and J-K, respectively. Nuclei identified by DAPI counterstain (blue in merged panels). A representative cartoon insert (A) illustrates coronal plane at level of AComA. Scale bar: 10 μm.
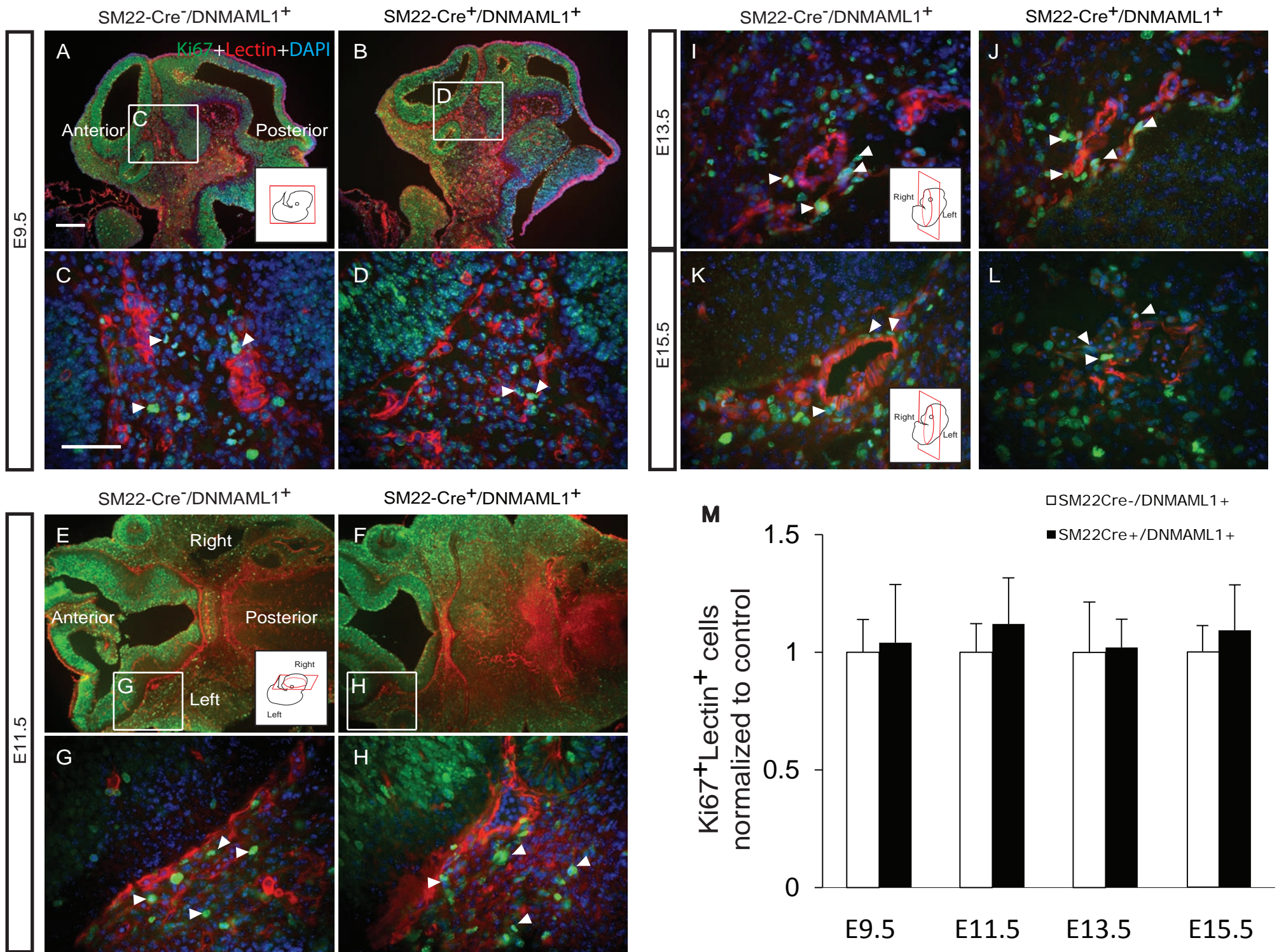
Supplemental Table 1. List of antibodies

Primary Antibody	Source	Cat#	Dilution
Anti-alpha smooth muscle actin	Sigma (Mouse)	C6198	1:200
Anti-alpha smooth muscle actin	Sigma (Mouse)	F3777	1:200
Anti-CD31	BD Pharmingen (Rat)	553370	1:50
Anti-Cleaved Caspase-3	Cell Signaling (Rabbit)	9661	1:200
Anti-Dll4	R&D (Mouse)	1389	1:200
Anti-GFP	Molecular Probes (Rabbit)	A11122	1:400
Anti-GFP	Abcam(Goat)	ab6673	1:400
Anti-Jagged1	Santa Cruz (Rabbit)	SC8303	1:100
Anti-Jagged1	Santa Cruz (Goat)	SC6011	1:100
Anti-Ki67	Abcam (Rabbit)	ab66155	1:100
Anti-NG2	Millipore(Rabbit)	AB5320	1:200
Anti-Notch1	Santa Cruz (Goat)	sc6014	1:400
Anti-Notch3	Santa Cruz (Rabbit)	sc32347	1:100
Anti-Pdgfrβ	Cell signaling (Rabbit)	3169	1:200
Anti-Vegfa	Santa Cruz (Rabbit)	sc152	1:100

Secondary Antibody and Lectin	Source	Cat#	Dilution
Anti-Goat IgG DyLight 594	Jackson Immunoresearch (Donkey)	705-585-003	1:200
Anti-rabbit IgG DyLight 488	Jackson Immunoresearch (Donkey)	705-545-003	1:200
Biotinylated anti-rabbit IgG	Vector Lab (Donkey)	BA-1100	1:200
Streptavidin, Alexa Fluor® 350 conjugate	Molecular Probes	S-11249	1:200
DyLight 594 labeled Lycopersicon Esculentum (Tomato) Lectin	Vector Lab	DL-1177	1:100

Supplemental Fig. 1

SM22-Cre⁻/DNMAML1⁺ | SM22-Cre⁺/DNMAML1⁺

Supplemental Fig. 2

Supplemental Fig. 3

Columns (left to right): SM22-Cre$^-$/DNMAML1$^+$ and SM22-Cre$^+$/DNMAML1$^+$

Rows (top to bottom): E11.5, E13.5, E15.5

Panel A label: SMA+C-Cas-3+DAPI

Supplemental Fig. 4

SM22-Cre⁻/DNMAML1⁺