Supplemental Files:

Article Title: *Unique functions for Notch4 in murine embryonic lymphangiogenesis*

Journal Name: *Angiogenesis*

Authors and Affiliations: Ajit Muley[1]*, Minji Kim Uh[1,2]*, Jennifer M. James[3], Aino Murtomaki[1,4,5], Joseph D. McCarron[1], Chris Kitajewski[1], Maria Gnarra[1], Gloria Riitano[1,6], Yoh-suke Mukouyama[3], Jan Kitajewski[7], Carrie J. Shawber[1,8]

[1]Department of Obstetrics and Gynecology, Columbia University Medical Center, New York, NY 10032, USA.

[2]Department of Pharmacology, Columbia University Medical Center, New York, NY 10032, USA.

[3]Laboratory of Stem Cell and Neuro-Vascular Biology, Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.

[4]Wihuri Research Institute, Biomedicum Helsinki, Haartmaninkatu, Helsinki, 8,00290 Finland

[5]Translational Cancer Medicine Program, Faculty of Medicine and Helsinki Institute of Life Science, University of Helsinki, Helsinki, , FI-00014, Finland

[6]Departments of Molecular Medicine and Experimental Medicine, Sapienza University, Rome, 00185, Italy.

[7]Department of Physiology and Biophysics, University of Illinois Chicago, Chicago, IL 60612, USA.

[8]Department of Surgery, Columbia University Medical Center, New York, NY 10032, USA.

Corresponding Author:

Carrie J. Shawber, PhD

cjs2002@cumc.columbia.edu

Supplemental Tables

Table S1. Quantitative RT-PCR primers

Gene	Upper Primer	Lower Primer
β-actin	5' CGA GGC CCA GAG CAA GAG AG 3'	5' CTC GTA GAT GGG CAC AGT GTG 3'
Dll4	5' CGG GTC ATC TGC AGT GAC AAC 3'	5' AGT TGA GAT CTT GGT CAC AAA ACA G 3'
Hes1	5' CCC AAC GCA GTG TCA CCT TC 3'	5' TAC AAA GGC GCA ATC CAA TAT G 3'
Hey1	5' ACG AGA ATG GAA ACT TGA GTT C 3'	5' AAC TCC GAT AGT CCA TAG CAA G 3'
Hey2	5' ATG AGC ATA GGA TTC CGA GAG TG 3'	5' GGC AGG AGG CAC TTC TGA AG 3'
Notch1	5' CTC ACC TGG TGC AGA CCC AG 3'	5' GCA CCT GTA GCT GGT GGC TG 3'
Notch4	5' GGT GAC ACC CCT GAT GTC AG 3'	5' AGC CTG GCA GCC AGC ATC 3'

Table S2. Antibodies

Antigen	Supplier	Catalogue #
CD31	Pharmingen	553370
DLL4	R&D Systems	AF1389
GFP	Invitrogen	A-11122
JAG1	R&D Systems	AF599
LYVE1	Abcam	ab14917
LYVE1	Ebiosciences	14-0443
PROX1	Angiobio	11-002
NOTCH1	R&D Systems	AF1057
NOTCH4	J. Kitajewski	RB2-2

Supplemental Figures

Fig. S1. Quantification of ectopic Dll4 and Jag1 transcript levels in HeLa cells. *Dll4* and *Jag1* qRT-PCR of HeLa cell lines used in co-culture (Fig. 3c). Data presented as relative transcript levels ± s.e.m.

Fig. S2 Validation of the *Notch4* nullizygous mice. P4 dorsal dermal tissue cross-sections from *Notch4*$^{-/-}$ and wild-type littermates were stained for VEGFR3 and either NOTCH4 or NOTCH1. **a**) NOTCH4 expression was absent in the lymphatic endothelium (white arrowheads) and epithelial cells of the hair follicle (yellow arrowheads) and dermis in *Notch4*$^{-/-}$ tissues. **b**) NOTCH1 expression was unchanged in the VEGFR3+ lymphatics (white arrowheads). Scale bar, 50μm.

Fig. S3 Lymphatic branching was unchanged in E14.5 *Notch4*$^{-/-}$ dermis. Quantification of the average number of branch-points normalized to unit of vessel length. Data presented ± sem. wt (n=7), *N4*$^{+/-}$ (n=13), *N4*$^{-/-}$ (n=7).

Fig. S4 Dermal blood vasculature was unaffected in *Notch4*$^{-/-}$ mice. **a**) CD31 staining of E14.5 dorsal dermal whole-mounts from *N4*$^{-/-}$ and control *N4*$^{+/-}$ littermates. **b**) Quantification of average CD31 intensity normalized by area. Data presented ± s.e.m. *N4*$^{+/-}$ (n=3), *N4*$^{-/-}$ (n=4). **c**) Quantification of the average number of branch-points per field of view. Data presented ± sem. *N4*$^{+/-}$ (n=3), *N4*$^{-/-}$ (n=5).

Fig. S5 Dermal blood vasculature was unaffected in in mice with LEC loss of canonical Notch signaling**.** *Prox1CreER^{T2}* and *DNMAML$^{fl/fl}$* mice were crossed and tamoxifen administered at E12.5 and dorsal dermis analyzed at E14.5. **a**) CD31 staining of *Prox1CreER^{T2};DNMAML$^{fl/+}$* (*DNMAML^{LEC}*) mutant and *DNMAML$^{fl/+}$* (control) dermis. White dashed line denotes the midline. Scale bars, 1000μm. **b**) Quantification of average CD31 intensity normalized by area. Data presented ± s.e.m. control (n=3), *DNMAML^{LEC}* (n=6)

Muley/KimUh et al., Fig. S1

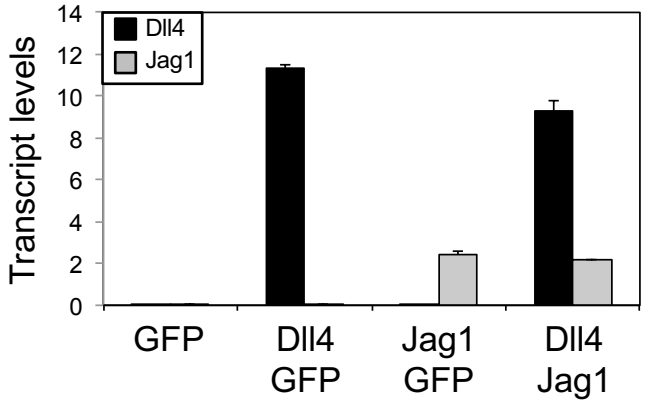

Fig. S1. Quantification of ectopic Dll4 and Jag1 transcript levels in HeLa cells. *Dll4* and *Jag1* qRT-PCR of HeLa cell lines used in co-culture (Fig. 3c). Data presented as relative transcript levels ± s.e.m.

Muley/KimUh et al., Fig. S2

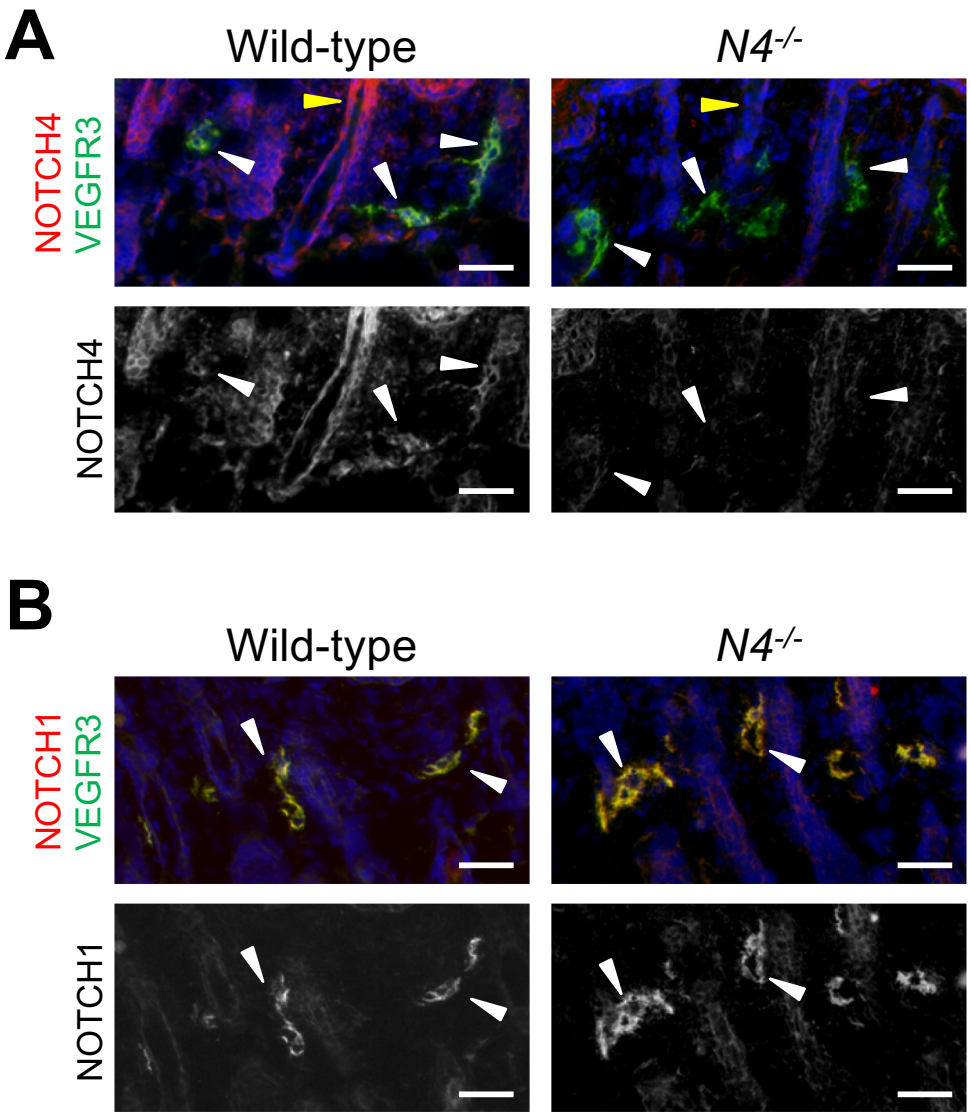

Fig. S2 Validation of the *Notch4* nullizygous mice. P4 dorsal dermal tissue cross-sections from *Notch4*$^{-/-}$ and wild-type littermates were stained for VEGFR3 and either NOTCH4 or NOTCH1. **a**) NOTCH4 expression was absent in the lymphatic endothelium (white arrowheads) and epithelial cells of the hair follicle (yellow arrowheads) and dermis in *Notch4*$^{-/-}$ tissues. **b**) NOTCH1 expression was unchanged in the VEGFR3+ lymphatics (white arrowheads). Scale bar, 50μm.

Muley/KimUh et al., Fig. S3

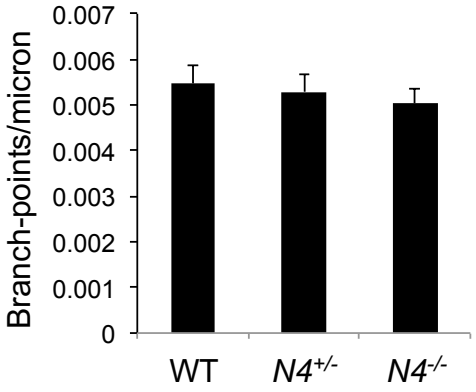

Fig. S3 Lymphatic branching was unchanged in E14.5 *Notch4*$^{-/-}$ dermis. Quantification of the average number of branch-points normalized to unit of vessel length. Data presented ± sem. wt (n=7), *N4*$^{+/-}$ (n=13), *N4*$^{-/-}$ (n=7).

Muley/KimUh et al., Fig. S4

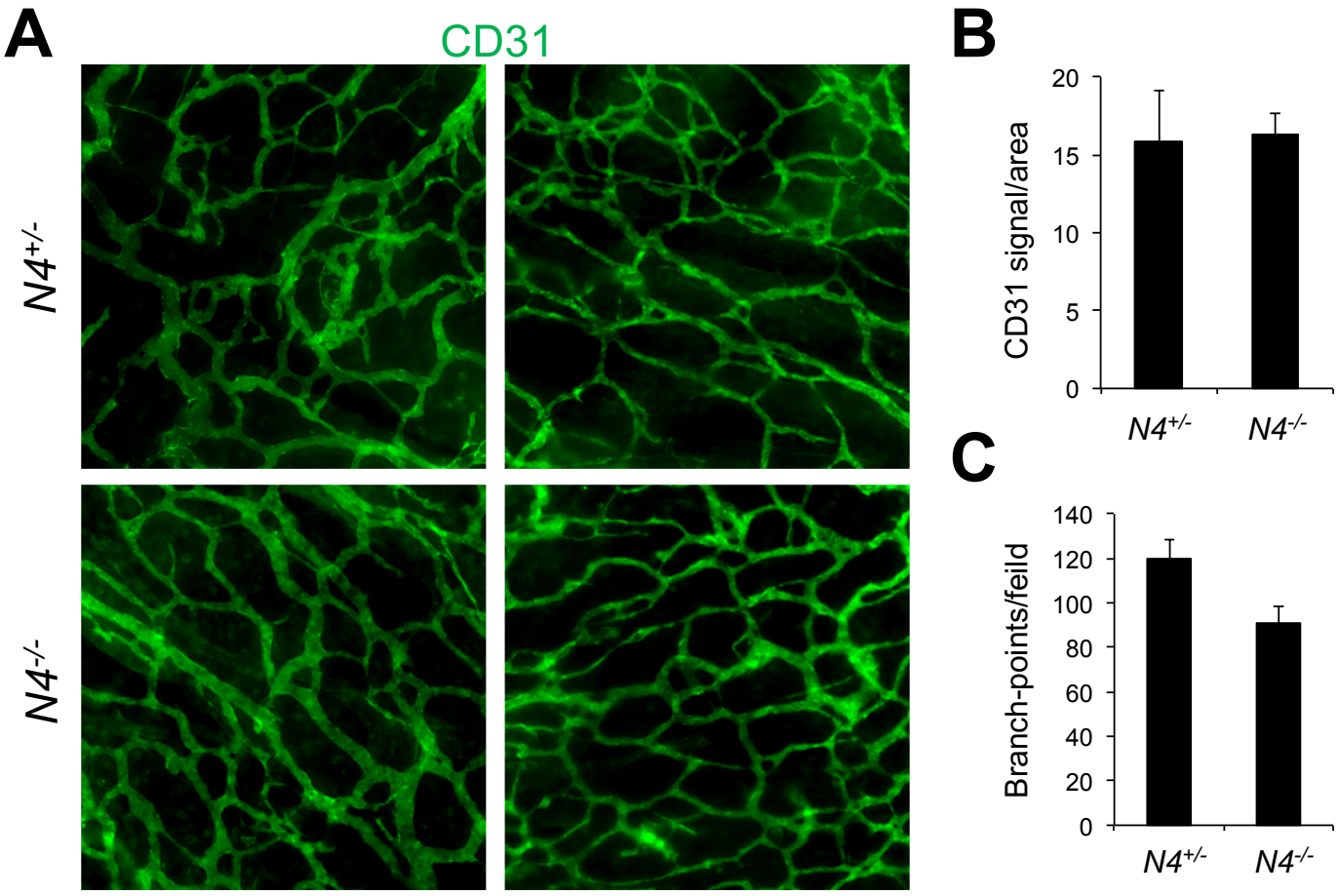

Fig. S4 Dermal blood vasculature was unaffected in *Notch4*$^{-/-}$ mice. **a**) CD31 staining of E14.5 dorsal dermal whole-mounts from *N4*$^{-/-}$ and control *N4*$^{+/-}$ littermates. **b**) Quantification of average CD31 intensity normalized by area. Data presented ± s.e.m. *N4*$^{+/-}$ (n=3), *N4*$^{-/-}$ (n=4). **c**) Quantification of the average number of branch-points per field of view. Data presented ± sem. *N4*$^{+/-}$ (n=3), *N4*$^{-/-}$ (n=5).

Muley/KimUh et al., Fig. S5

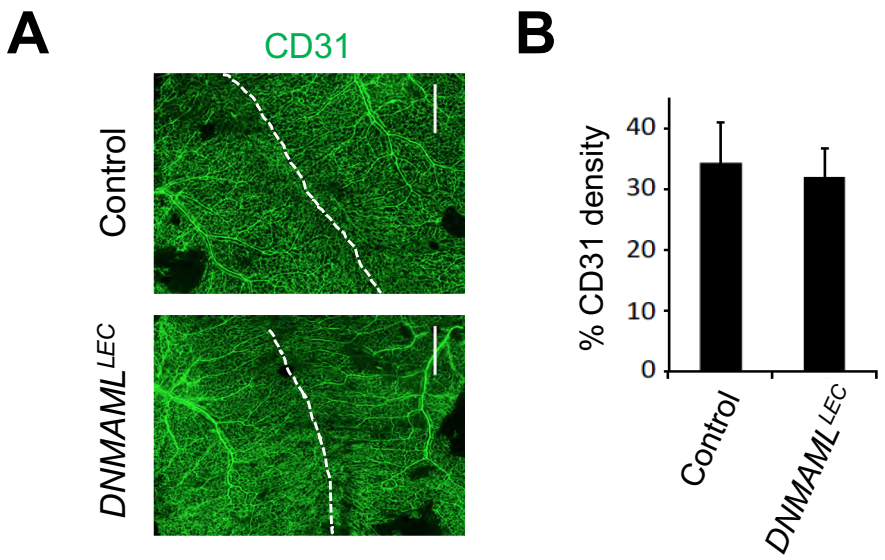

Fig. S5 Dermal blood vasculature was unaffected in in mice with LEC loss of canonical Notch signaling**.** *Prox1CreER^{T2}* and *DNMAML$^{fl/fl}$* mice were crossed and tamoxifen administered at E12.5 and dorsal dermis analyzed at E14.5. **a**) CD31 staining of *Prox1CreER^{T2};DNMAML$^{fl/+}$* (*DNMAML^{LEC}*) mutant and *DNMAML$^{fl/+}$* (control) dermis. White dashed line denotes the midline. Scale bars, 1000µm. **b**) Quantification of average CD31 intensity normalized by area. Data presented ± s.e.m. control (n=3), *DNMAML^{LEC}* (n=6).