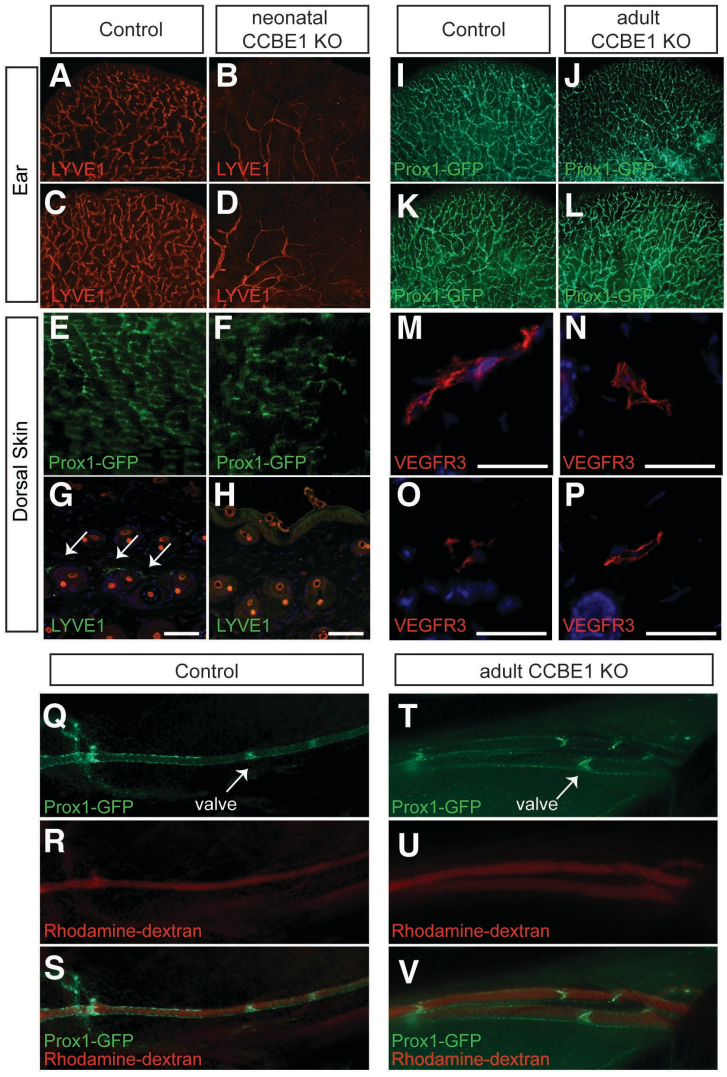
Supplemental Dataset

Supplemental Table 1. Mutant *ADAMTS3* alleles detected in HEK293T clone 4C2.

	DNA sequence	Amino acid sequence
WT	CCTGTCACTTTGGTTGATAGC	MVLLSLWLIAAALVEVR
Allele 1	CCTGTC----------GATAGC	MVLLS-------IAAALVEVR
Allele 2	CCTGTC----------ATAGC	MVLLS*
Allele 3	CCTGTC–CTTTGGTTGATAGC	MVLLS–TLVDSSRSGRD*

Supplemental Figures

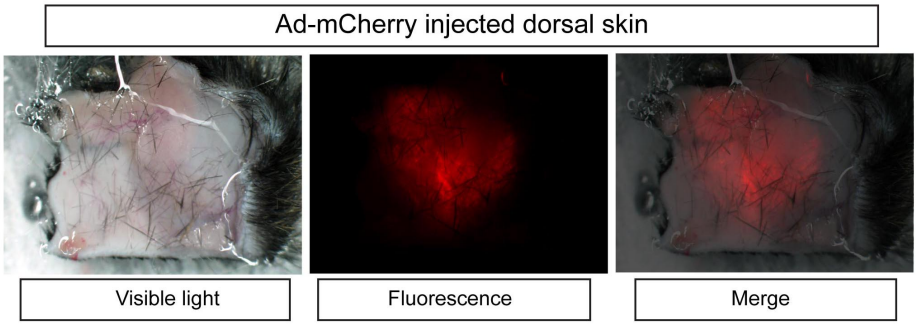
Supplemental Figure 1



Supplemental Figure 1. CCBE1 is required for lymphatic vessel growth but not maintenance. (A-D) Tamoxifen was administered to Ub-CreERT2;*Ccbe1*$^{fl/-}$ ("neonatal CCBE1 KO") and *Ccbe1*$^{fl/-}$ littermate ("control") animals immediately after birth and the lymphatic network of the ear assessed by anti-LYVE1 wholemount staining at age 4 weeks. Images shown are representative of 4 separate experiments. (E-H) Neonatal

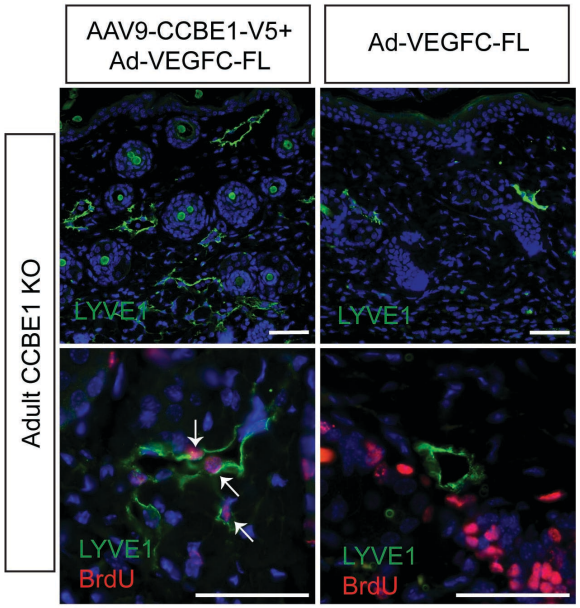
CCBE1 KO animals exhibit reduced lymphatic density in the skin. Imaging of dorsal skin lymphatics in Prox^{GFP} animals is shown in E & F, and LYVE 1 immunostaining of skin sectionsis shown in G & H. Arrows point to cutaneous lymphatic vessels. Images shown are representative of 3 separate experiments. (I-L). Tamoxifen was administered to Ub-CreERT2;*Ccbe1*$^{fl/-}$ ("adult CCBE1 KO") and Ub- *Ccbe1*$^{fl/-}$ control littermates that also carried the Prox^{GFP} allele starting at age 12 weeks, and cutaneous lymphatics assessed by fluorescence microscopy of the ear. Images shown are representative of 4 separate experiments. (M-P) LECs in adult CCBE1 KO animals express normal levels of the VEGFC receptor VEGFR3. Images shown are representative of 4 separate experiments. (Q-S) Lymphatic function in control animals. (T-V) Lymphatic function in adult CCBE1 KO animals. Rhodamine dextran was injected into the hindpaw of control and adult CCBE1 KO animals that carry the *Prox1*^{GFP} allele, and lymphatic uptake in the collecting system imaged 45 minutes later. Images shown are representative of 3 separate experiments. Scale bars indicate 50 microns. Magnification for images shown in Q-V is 10X.

Supplemental Figure 2



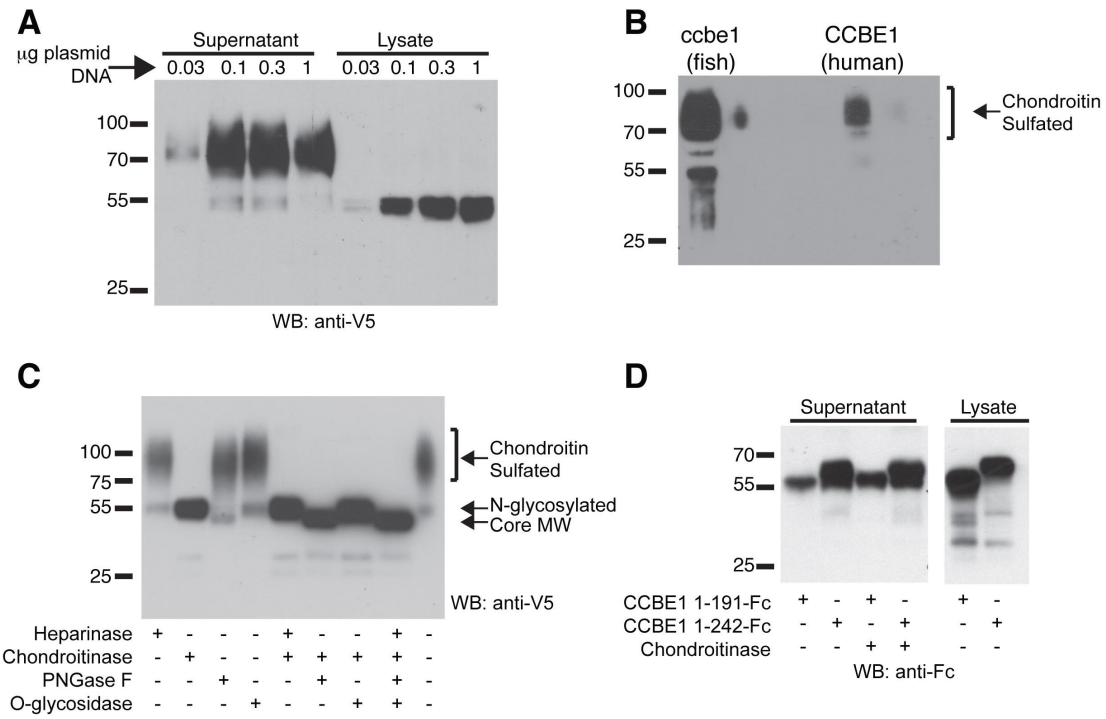
Supplemental Figure 2. Detection of cutaneous adenoviral expression using adeno-mCherry. Adeno-mCherry was added to adeno-VEGFC-FL at the time of injection and the site of expression identified using fluorescent microscopy prior to tissue harvest for assessment of lymphatic proliferation and cell number. Magnification of images shown is 10X.

Supplemental Figure 3



Supplemental Figure 3. CCBE1-V5 rescues lymphangiogenesis in adult CCBE1 KO animals. Pre-treatment of adult CCBE1 KO skin with adeno-associated virus expressing CCBE1-V5 (AAV9-CCBE1-V5) restores the proliferative response of LECs to Ad-VEGFC-FL in adult CCBE1 KO animals. The BrdU+ cells in the adult CCBE1 KO skin that is exposed to only Ad-VEGFC-FL (lower right panel) are in the hair follicle. The data shown are representative of 5 separate experiments. Scale bars indicate 50 microns.

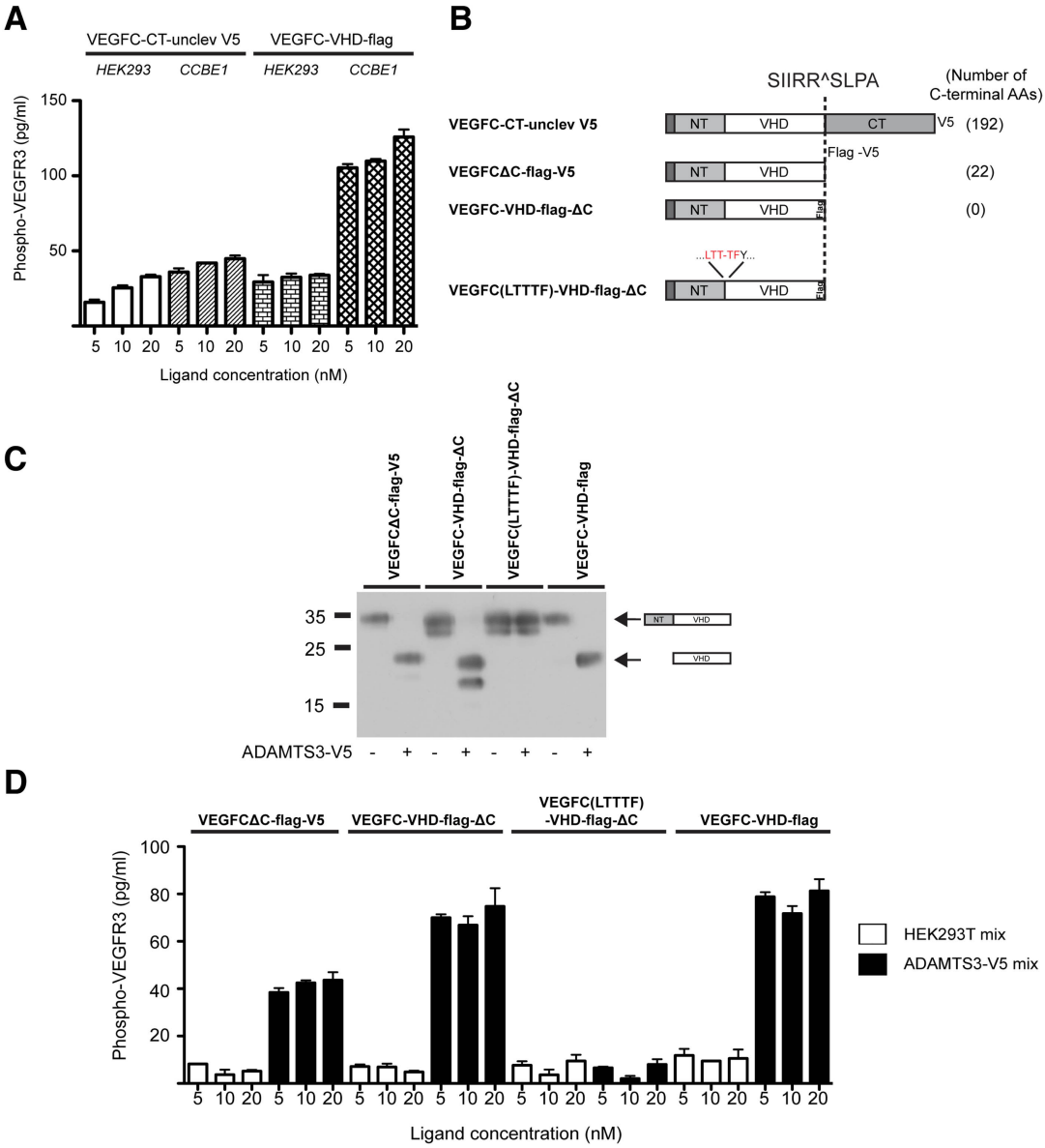
Supplemental Figure 4



Supplemental Figure 4. Secreted CCBE1 is a chondroitin sulfate containing proteoglycan. (A) HEK293T cells were transfected with the indicated amounts of human CCBE1-V5-encoding plasmid and anti-V5 western blots performed on supernatant and cell lysate under denaturing conditions. Note that secreted CCBE1 is of a higher molecular weight than that detected in cell lysate. (B) HEK293T cells were transfected with zebrafish ccbe1-V5-encoding plasmid and anti-V5 western blots performed as described above. (C) CCBE1 is chondroitin sulfated and N-glycosylated. HEK293T conditioned supernatant generated by transfection with CCBE1-V5 plasmid was treated with the indicated enzymes prior to western blot analysis. (D) Chondroitin sulfate is added to the C-terminal, collagen-like region of CCBE1. CCBE1 1-191-Fc and CCBE1 1-252-Fc proteins containing the N-terminal but not the C-terminal region of

CCBE1 were expressed in HEK293T cells and conditioned supernatant analyzed by western blotting using anti-Fc antibodies. The data shown are representative of 3 separate experiments.

Supplemental Figure 5



Supplemental Figure 5. VEGFC activity at VEGFR3 is reduced by increased length of the C terminus. **(A)** C terminal cleavage of VEGFC is required for activation of VEGFR3 independent of N terminal cleavage. LEC phospho-VEGFR3 was measured after exposure to wild-type VEGFC that is tagged in its VHD ("VEGFC-VHD-FLAG") or VEGFC with an uncleavable C terminus (the RR226/227SS mutant) that is tagged at its carboxy terminus ("VEGFC-CT-unclev V5"). **(B)** Schematic of VEGFC mutant

proteins with varying uncleavable C terminal amino acids are shown, with the number of amino acids C terminal of the VHD indicated. VEGFC-CT-unclev V5, the RR226/227SS mutant; VEGFCΔC-FLAG-V5, VEGFC without a C terminus in which serial FLAG and V5 epitopes have been fused in frame to the VHD; VEGFC-VHD -FLAG-ΔC, VEGFC without a C terminus to which a FLAG epitope has been fused in frame to the VHD; VEGFCΔC(LTTTF)-VHD -FLAG-ΔC, VEGFC with a mutation blocking ADAMTS3 cleavage of the N terminus that also lacks a C terminus and to which a FLAG epitope has been fused in frame to the VHD. **(C)** Expression of the VEGFC mutant proteins described in (C) by HEK293T cells and their cleavage following incubation with ADAMTS3-containing conditioned medium. **(D)** The ability of the VEGFC mutants described in (B) to activate VEGFR3 was measured using a phospho-VEGFR3 ELISA. N=3 for each concentration. The biochemical data shown are representative of 2 separate experiments.

Supplemental Methods

Primer Sequences

CCBE1-V5

Forward - TCT AGA TCT GCT TCC CTG ATG GTG CCG

Reverse - AAG CGG CCG CTT AGG TGC TAT CCA GGC CCA GCA GCG GGT TCG GGA TCG GCT TGC CTG GGT AGA AGT CTC TGG GGG CTC

CCBE1$^{1\text{-}175}$-V5

Forward - TCT AGA TCT GCT TCC CTG ATG GTG CCG

Reverse - AAG CGG CCG CTT AAT GAT GAT GAT GGT GGT GGG TGC TAT CCA GGC CCA GCA GCG GGT TCG GGA TCG GCT TGC CGG ATTG GAA GTA CAG GTT CTC GGT ACA TGT CTT

CCBE1^{D170E}

Forward - CAT CCG GGA AGA TGA AGG GAA GAC ATG TAC CCG GGG AGA CAA ATA TCC

Reverse - GTA GGC CCT TCT ACT TCC CTT CTG TAC ATG GGC CCC TCT GTT TAT AGG

VEGFC-V5

Forward - TCT GGA TCC GGT ACC CGG TCC TTC CAC CAT GCA CTT GCT GGG

Reverse - AAG CGG CCG CTT AGG TGC TAT CCA GGC CCA GCA GCG GGT TCG GGA TCG GCT TGC CGG CGC TCA TTT GTG G

VEGFC$^{\Delta C}$-V5

Forward - TCT GGA TCC GGT ACC CGG TCC TTC CAC CAT GCA CTT GCT GGG

Reverse - AAG CGG CCG CTT AGG TGC TAT CCA GGC CCA GCA GCG GGT TCG GGA TCG GCT TGC CCT TGT CAT CGT CGT CCT TGT AGT CAC GTC TAA TAA TGG AAT GAA CTT GTC

VEGFC$^{\Delta N\Delta C}$ (adenovirus)

Forward - TAG GAT CCG CCA CCA TGG AGA CAG ACA CAC TCC TGC TAT GGG TAC TGC TGC TCT GGG TTC CAG GTT CCA CTG GTA CAG AAG AGA CTA TAA AAT TTG C

Reverse - AAG CGG CCG CTT AAC GTC TAA TAA TGG AAT GAA CTT GTC

VEGFC-VHD-flag

The internal primers to insert Flag epitope tag

Reverse (of the N-terminal portion) – GGA TCC CTT GTC ATC GTC GTC CTT GTA GTC AAC TTG TCT GTA AAC ATC C

Forward (of the C-terminal portion) - GGA TCC CAT TCC ATT ATT AGA CGT TCC

VEGFC-RR^{226,227}SS

Forward - CAA GTT CAT TCC ATT ATT TCC TCC TCC CTG CCA GCA ACG TTA

Reverse - TAA CGT TGC TGG CAG GGA GGA GGA AAT AAT GGA ATG AAC TTG

VEGFC-LTTTF

Mutagenesis step 1:

Forward - ACT ATA AAA TTT GCT GCA ACG TTC TAT AAT ACA GAG ATC TTG

Reverse - CAA GAT CTC TGT ATT ATA GAA CGT TGC AGC AAA TTT TAT AGT

Mutagenesis step 2

Forward - ACA GAA GAG ACT ATA AAA TTG ACT ACA ACG TTC TAT AAT ACA GAG

Reverse - CTC TGT ATT ATA GAA CGT TGT AGT CAA TTT TAT AGT CTC TTC TGT

ADAMTS3-V5

Forward - TAG GAT CCG CCA CCA TGG TTC TCC TGT CAC TTT G

Reverse - AAG CGG CCG CTC AGG TGC TAT CCA GGC CCA GCA GCG GGT TCG GGA TCG GCT TGC CTC TTT CTA AGG TGG A

ADAMTS3-HA

Forward - TAG GAT CCG CCA CCA TGG TTC TCC TGT CAC TTT G

Reverse - AAG CGG CCG CTC AAG CGT AAT CTG GAA CAT CGT ATG GGT ATC TTT CTA AGG TGG A

ADAMTS3 gRNA (CRISPR)

5'- CACCGTTGGTTGATAGCAGCCGCTC -3'

Whole-mount microscopy

Imaging of ear lymphatics was performed after manual splitting of the ear leaflets performed after a one hour incubation in PBS. For whole-mount immunostaining ear leaflets fixed overnight in 4% paraformaldehyde at 4 degrees celsius. Tissue was

permeabilized in TBS plus Triton X-100, and stained overnight with anti-LYVE1 antibodies, and detected using secondary antibody conjugated to Alexa-568.

Fluorescent lymphangiography

20 microliters of 10mg/mL rhodamine-dextran (MW 70 kDa, Molecular Probes) was injected into the hindpaw of *Prox1*^{GFP} transgenic mice, and para-aortic lymphatic vessels imaged 45 min later using fluorescence microscropy.