# Supplemental Material

## Supplemental Methods

**Cloning.** Genes expressed via the recombinant adeno-associated virus (AAV9) vector were cloned into the psubCAG-WPRE plasmid[1], which is a derivative of psubCMV-WPRE, where the CMV promoter has been replaced with the composite CAG promoter (consisting of the chicken β-actin promoter, cytomegalovirus enhancer and β-actin intron[2]). Cloning of full-length mVEGF-C, ΔNΔC-mVEGF-C and HSA into AAV-vector (psubCAG-WPRE) was described earlier[3]. mCCBE1, fused to a V5 tag (mCCBE1-V5), was cloned as follows: A partial CCBE1 coding DNA sequence (CDS; Genebank #BC152322, Image clone ID 40140631) was inserted as a SacI/XbaI fragment into pVK1 (a pUC19-derived vector[4]). The missing nucleotides were amplified from brown adipose tissue mRNA with primers 5'-GCCGCTAGCGCCACCATGGTGCCGCCGCCT-3' and 5'-GGAGCTTGGGCACAAATGTC-3' and the CDS was completed by inserting the NheI/SacI fragment resulting in vector pVK1-CCBE1. A PCR-amplified V5-tag (obtained with primers 5'-ACCAGGAGCACCAGGAAGAC-3' and 5'-GCCTCTAGAACGCGTCTAGGTGCTGTCCAGGCCCAGCAGAGGGTTAGGGATAGG-CTTGCCTGGATAAAAATTTCTTGGGG-3') was added to the CDS as an Eco81I/XbaI fragment. From the resulting vector, the complete CDS was excised as an MluI fragment and cloned into psubCAG-WPRE.

For the *in vitro* studies, a similar vector was constructed, in which the mouse CCBE1 CDS was replaced by the human CCBE1 CDS (Genebank #NM_133459). For the co-immunoprecipitation study and the protein purification, the sequence coding for a StrepIII-tag[5], an internal ribosomal entry site and the CDS for enhanced green fluorescent protein were inserted immediately behind the CCBE1 CDS and the complete CDS was transferred

into the pMX vector[6] resulting in pMX-hCCBE1-StrepIII-IRES-EGFP. The mammalian expression constructs for VEGF-C and ΔC-VEGF-C have been described[7]. The chimeric VEGF-C/VEGF-D (CDC) expression constructs were assembled by overlapping PCR mutagenesis into the pMosaic vector[8]. The insert comprised sequences coding for amino acid residues Phe32-Ala111 and Ser228-Ser419 from human VEGF-C and intervening residues Thr92-Arg206 from human VEGF-D (version 1), and amino acid residues Phe32-Leu119 and Ser228-Ser419 from human VEGF-C and intervening residues Lys100-Arg206 from human VEGF-D (version 2).

cDNA clones for the ADAMTS constructs were obtained from the Mammalian Gene Collection. ADAMTS1 (GenBank BC040382) and ADAMTS2 (GenBank BC046456) were expression-ready (pCMV-Sport6); the ADAMTS3 cDNA clone (pCR-XL-TOPO, GenBank BC130287) was subcloned as an EcoRI fragment into the expression vector pAc5.1/V5His (Invitrogen) and pCI-neo (Promega), thus adding sequences encoding for a C-terminal V5 and hexahistidine tag. The ADAMTS14 cDNA clone (pENTR223.1, GenBank BC140263) was transferred into the expression vector pEF-DEST51 using the Gateway recombination system.

The constructs for recombinant VEGF-C expression in insect cells cells employed the pMT-Ex vector (a modified version of pMT-BiP-V5His-C[9]) and comprised sequences coding for the signal peptide of the Drosophila BiP, for amino acid residues Phe32-Ala111 for the N-terminal propeptide, Ser228-Ser419 for the C-terminal propeptide and Thr112-Arg227 for ΔNΔC-VEGF-C, followed by sequences coding for a hexahistidine tag. The construct for the expression of full-length VEGF-D comprised sequences coding for amino acid residues 22-354 in the same context. The construct for the expression of histidine-tagged human serum

albumin comprised sequences coding for the signal peptide of the Drosophila BiP and sequences coding for amino acid residues 25-609 from human serum albumin flanked both N- and C-terminally by a hexahistidine tag.

The construct for the expression of VEGF-C from its full-length cDNA was based on pFastBac1 (Invitrogen). In this construct the CDS of VEGF-C was modified by swapping the sequences coding for its endogenous signal peptide against sequences coding for the melittin signal peptide and by adding sequences coding for a C-terminal hexahistidine tag.

For the expression of the N-terminal domain of CCBE1 (CCBE1Δ175), residues 1-175 of human CCBE1 were cloned into the pFastBac (Invitrogen) baculovirus expression vector and equipped with a C-terminal Factor Xa cleavage site (amino acid residues IEGR) and a hexahistidine tag.

**Antibodies.** Anti-VEGF-C antiserum[10], anti-VEGF-C antibodies (R&D Systems, Minneapolis, MN, AF752), anti-V5 antibody (Invitrogen, Carlsbad, CA, #46-0705), anti-phosphotyrosine antibody 4G10 (Merck Millipore, Billerica, MA) and PY20 (BD Transduction Laboratories, Franklin Lakes, NJ), and anti-CCBE1 antibodies (Atlas Antibodies AB, Stockholm, Sweden, #HPA041374) were used for both immunoprecipitation and Western blotting. Anti-VEGF-D antibody VD1[11], anti-VEGFR-3 antibodies (Santa Cruz, Dallas, TX, sc-321), chimeric VEGFR-3/IgGFc[12] or streptactin sepharose (IBA, Göttingen, Germany) were used for immunoprecipitation. Anti-VEGF-D antibody (R&D Systems, AF286), anti-phospho-Erk1/2 antibody (Cell Signaling Technology, Inc., Danvers, MA, #9101), anti-phospho-Akt antibody (Cell Signaling Technology, Inc., #9271), anti-phospho-eNOS (BD Transduction Laboratories, #612392), streptactin-HRP conjugate (IBA and R&D

systems, #890803) were used for Western blots. The hF4-3C5 antibody used to block VEGFR-3 activation was generously provided by ImClone Systems/Eli Lilly[13].

**Cell culture and generation of stable cell lines.** 293T, 293F, 293GPG, 293S GnTI$^-$, PAE-VEGFR-3[14], PAE-VEGFR-3/neuropilin-2[9] and NIH-3T3 cells were grown in D-MEM 10% FCS. PC-3 cells were grown in Ham's F-12 10% FCS, DU-4475 cells in RPMI 1640 20% FCS, CHO cells in α-MEM 10% FCS or EX-CELL ACF CHO Medium and S2 cells in HyClone SFX-Insect (Thermo Scientific, Waltham, MA) or Insect-Xpress (Lonza Group, Basel, Switzerland). 293T cells were transfected with retrovirus vector, that had been produced using standard methods in 293GPG cells using the pMX-hCCBE1-StrepIII-IRES-EGFP construct. The cells were cultured for one week and sorted once with FACS for EGFP. 293F cells were transfected with the expression plasmid pCI-neo-hADAMTS3-V5-H6, selected with G418 and clonal lines were established by the ring cloning technique. Stable expression of CCBE1 and ADAMTS3 was confirmed by Western blotting. LECs, BECs and HUVECs were purchased from Promocell (Heidelberg, Germany) and maintained according to the instructions of the supplier. The Ba/F3-hVEGFR-2/EpoR cell line was generated similarly to the Ba/F3-mVEGFR-2/EpoR[15]; however, pCI-neo was used instead of pEF-BOS. The junctional amino acid sequences of the chimera were ...FFIIEGAQEKTNLEGS (end of VEGFR-2 part) – (start of mEpoR part) LILTLSLILVLISLLLTVLALLSHRRTLQQKIWPGIPSPESEFE... This chimeric construct was electroporated with one 30 ms 1400V pulse (Neon transfection device, Invitrogen) into the Ba/F3 cells. Cells were grown in medium containing 2 ng/ml mIL-3 for 36 hours, after which they were split and selection was started for three weeks with 1.2 mg/ml G418. Cells were maintained with sub-optimal mIL-3 concentration (0.4 ng/ml) and optimal VEGF-A and

VEGF-C concentrations (200 and 300 ng/ml, respectively).

**Protein expression and purification.** S2 cells were transfected using Effectene (Qiagen, Venlo, The Netherlands). Stable cell pools were selected for 3 weeks with 400 µg/ml hygromycin starting 2 days after transfection. For protein production, the cells were adapted to suspension culture and induced for 4-5 days with 1 mM $CuSO_4$. After batch-binding to $Ni^{2+}$NTA sepharose from the pH-adjusted conditioned supernatant, the $Ni^{2+}$NTA sepharose was loaded onto a column, washed with 20 mM imidazole, and eluted with a step-gradient of 250 mM imidazole. The protein was further size-separated on a Superdex 200 column with PBS as a running buffer.

Recombinant baculovirus was produced using the FactBac system (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. Sf9 cells were infected and conditioned medium harvested 4 days post infection. Protein purification was performed as above. CCBE1Δ175 was purified in Hepes-buffered saline supplemented with 2 mM $CaCl_2$.

CCBE1 was enriched from 200 ml conditioned medium of 293T cells expressing StrepIII-tagged CCBE1 using streptactin sepharose (IBA) according to the instructions of the manufacturer, and the eluted peak fraction was dialyzed against TBS. Histidine-tagged ADAMTS3 was purified from 1.5 litres of conditioned medium of stably transfected 293F cells (D-MEM supplemented with 2%FCS, 30µM $ZnCl_2$ and 0.1U heparin/ml). The collected medium was dialyzed against phosphate buffered saline, pH-adjusted to 8.0 and batch-bound protein was eluted with a step-gradient of 250 mM imidazole from the $Ni^{2+}$NTA sepharose (IBA) after washing with 20 mM imidazole and dialyzed against 1xTBS. Biotinylated ΔNΔC-VEGF-C and pro-VEGF-C were generated from purified proteins using sulfosuccinimidyl-6-

[biotin-amido]hexanoate (Thermo Scientific, #21335), according to the instructions of the supplier.

**Cleavage of pro-VEGF-C and pro-VEGF-D.** The indicated amounts of plasmin (Sigma-Aldrich, St. Louis, MO, P1867) were incubated for 18 hours with 2.5 µg of pro-VEGF-C. 1.15 µg of pro-VEGF-C was incubated in TBS, as indicated, with 4.15 µg 293T-cell-derived ADAMTS3 or 15 µg of S2-cell-derived ADAMTS3. 1.7 µg of pro-VEGF-D was incubated with 7.5 µg recombinant 293T-cell-derived ADAMTS3 in TBS for 30 hours with or without 10 µl recombinant CCBE1. Analysis of the cleavage was performed by SDS-PAGE/Western. All incubations were at 37°C and recombinant CCBE1 was included, as indicated, at a concentration of approximately 2.5 µg/ml.

**VEGF-C/CCBE1 co-immunoprecipitation.** 293T cells were transfected with either CCBE1-StrepIII or VEGF-C constructs. Conditioned media were used in a streptactin pull-down analysis either separately or as a mix of CCBE1 and VEGF-C. The mixed media were incubated for 10 min at RT before being applied to the pull-down. The precipitates were analyzed with anti-CCBE1 antibody and anti-VEGF-C antiserum after SDS-PAGE and Western blotting. Input represents 25 µl of VEGF-C conditioned medium, which was loaded as a positive control.

**ADAMTS3/CCEB1 co-immunoprecipitation.** 293T-CCBE1-StrepIII cells were transfected with ADAMTS3 or mock expression constructs. Conditioned media were immunoprecipitated with ADAMTS3 antibodies (Santa Cruz, sc-21486) and protein G sepharose or used in a streptactin pull-down. Precipitates were detected with streptactin-HRP conjugate after SDS-PAGE and Western blotting.

**Mass spectroscopy.** Enriched CCBE1 protein was subjected to liquid chromatography-mass spectrometry as previously described[16]. In short, mass spectrometry analysis was performed on an Orbitrap Elite ETD mass spectrometer (Thermo Scientific) using the Xcalibur version 2.7.1 coupled to a nLCII nanoflow system (Thermo Scientific) via a nanoelectrospray ion source. Peak extraction and subsequent protein identification was achieved using Proteome Discoverer software (Thermo Scientific). Calibrated peak files were searched against human protein databases by the SEQUEST search engine. Database searches were limited to tryptic peptides with a maximum of 1 missed cleavage; carbamidomethyl cysteine and methionine oxidation were set as fixed and variable modifications, respectively.

**Pulse chase.** 293T cells were transfected and grown for 36 hours on 6-cm dishes to near confluency. Cells were starved for 30 min in met-/cys-deficient D-MEM, 5% dialyzed FCS, after which cells were metabolically labeled for 2 hours with [$^{35}$S]-cysteine/[$^{35}$S]-methionine. Thereafter, cells were washed with warm PBS and 5 ml of chase medium was added (D-MEM, 10% FCS + 2 mM cold L-methionine + 2mM cold L-Cysteine). At the indicated time points, the dishes were placed on ice and the medium was removed for analysis.

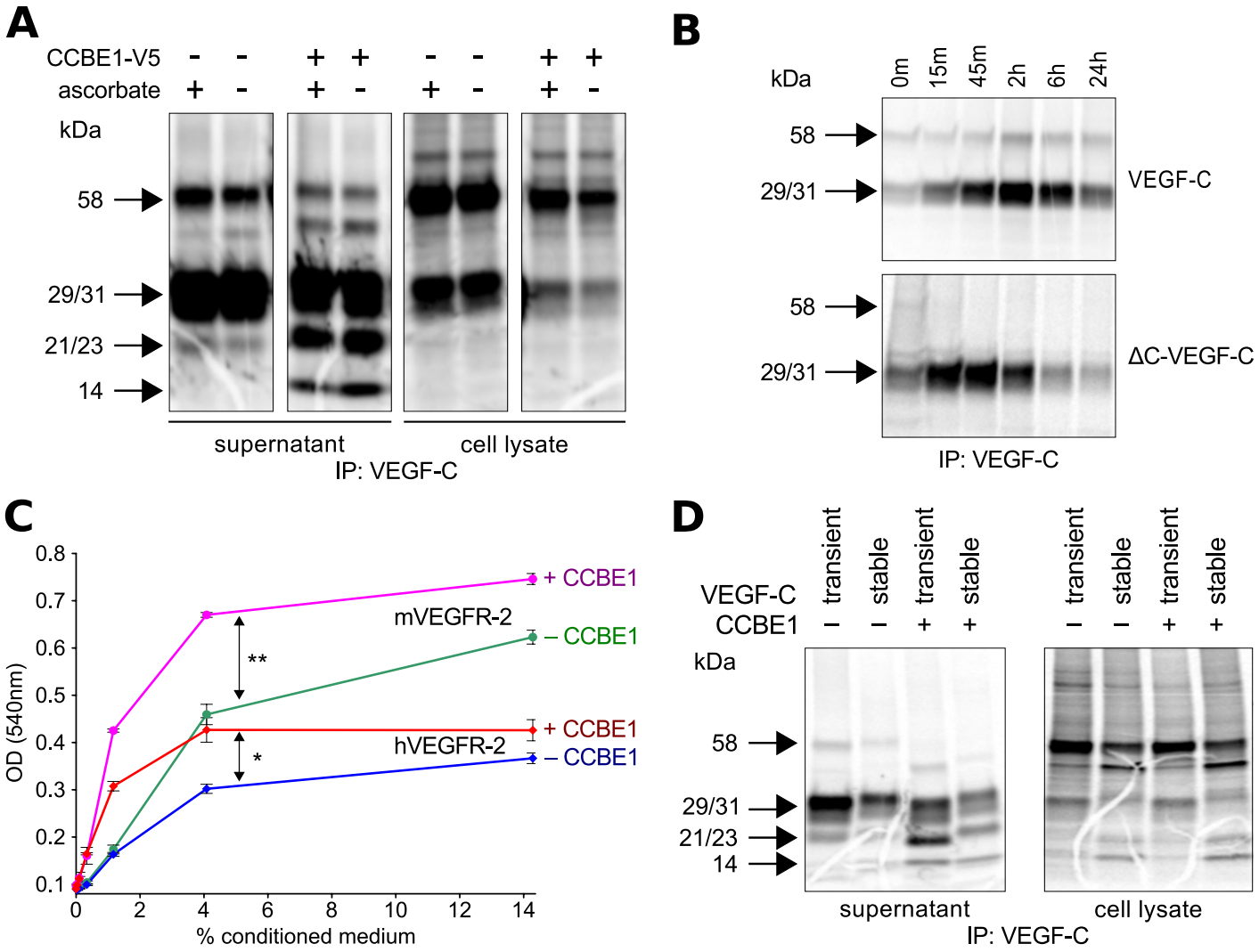
**Competition of VEGF-C cleavage by VEGF-C propeptides.** Purified histidine-tagged proteins were included in the labeling medium of the VEGF-C/CCBE1-cotransfected 293T cells at a concentration of 25 µg/ml. The labeling medium was conditioned for 30-72 hours after transfection, depleted from histidine-tagged proteins with $Ni^{2+}$NTA sepharose, and VEGF-C was immunoprecipitated, separated by PAGE and visualized by exposure to X-ray film. Inhibition of the N-terminal cleavage of VEGF-C was measured by quantitating the 14 kDa N-terminal cleavage product from the laser-scanner read-out.

**Quantitative PCR for ADAMTS2, -3 and -14.** Total RNA was isolated from the cells with NucleoSpin RNA II kit according to the protocol (Macherey-Nagel, Düren, Germany) and cDNA synthesis was performed using iScript cDNA synthase kit (Bio-Rad; Hercules, CA). QPCR was carried out using SYBR green chemistry with Bio-Rad CFX96 Real-Time System. All data were normalized to GAPDH and quantification was performed using the 2-DDCT method. The following primers were used: hADAMTS2 (fwd 5'-AAATCTACCATGACGAGTCC -3', rev 5'- TCATGGACTTTCCATAGCTC -3'), hADAMTS3 (fwd 5'- CCATTCCTATGACTGTCTCC -3', rev 5'-CCAACACCAAAATCAAAACG -3'), hADAMTS14 (fwd 5'-CAACTACTCAATGGATGAGC -3', rev 5'- AAGGTCCTGAATGCCAAG -3') hGAPDH (fwd 5'- CCACTAGGCGCTCACTGTTC -3', rev 5'- CCCCATACGACTGCAAAGAC -3'), mADAMTS2 (fwd 5'- ACTACAACATTGAGGTCCTG -3', rev 5'-TCATGGTAGATTTCGTTGAC -3'), mADAMTS3 (fwd 5'-CGATACATCCATTCCTATGAC -3', rev 5'- GTACACATCTTGTAGCCAAC -3') , mADAMTS14 (fwd 5'- TAGCTTTCAGGACCTTTGAG -3', rev 5'-CTTGGTCTTGCAGAAGTATG -3') m/chGAPDH (fwd 5'-ACAACTTTGGCATTGTGGAA -3', rev 5'- GATGCAGGGATGATGTTCTG -3'), chADAMTS2 (fwd 5'- GGAGCTTGGCCGATACCTAC -3', rev 5'-TGTTTGCAGGGGTCAAAGGT -3'), chADAMTS3 (fwd 5'-GGTCTCCATACCACCCTCCT -3', rev 5'- GGCTGACCTGACTGGATGTC -3') , chADAMTS14(fwd 5'- GGCTGACAGAGGGAAGTGTC -3', rev 5'-GGATTCCTGCAAAGGTTGCG -3').

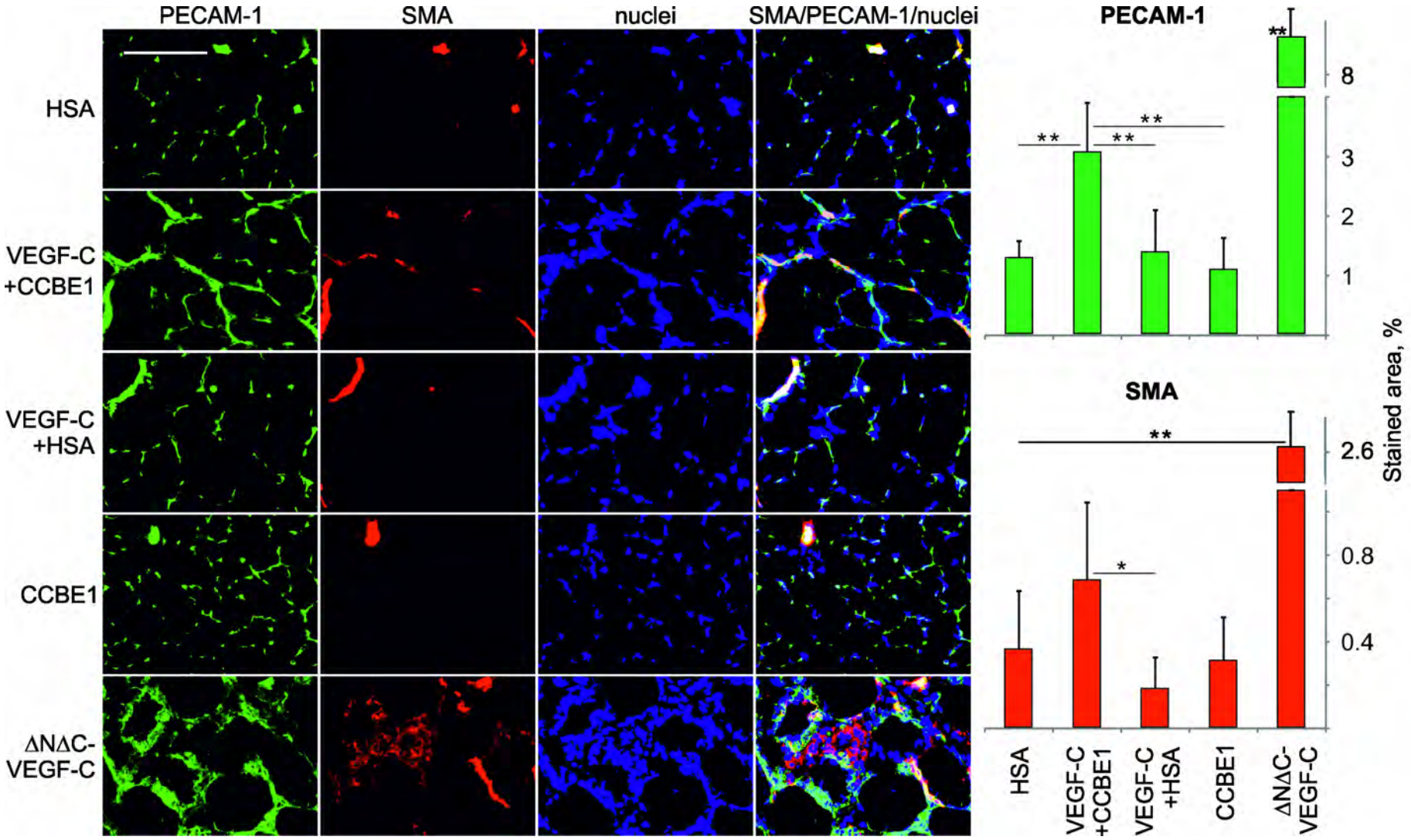
**Downregulation of ADAMTS3 by lentiviral shRNA.** Constructs expressing ADAMTS3

shRNA (TRCN0000050571) and non-target control shRNA were from the RNAi Consortium shRNA library[17]. Lentivirus, generated by using the standard methods according to the RNAi Consortium, was used to transduce 293T cells. After selection of stable pools, the efficiency of VEGF-C cleavage was assayed as described in the *Materials and methods* section under *Transfection, metabolic labeling and protein analysis*.

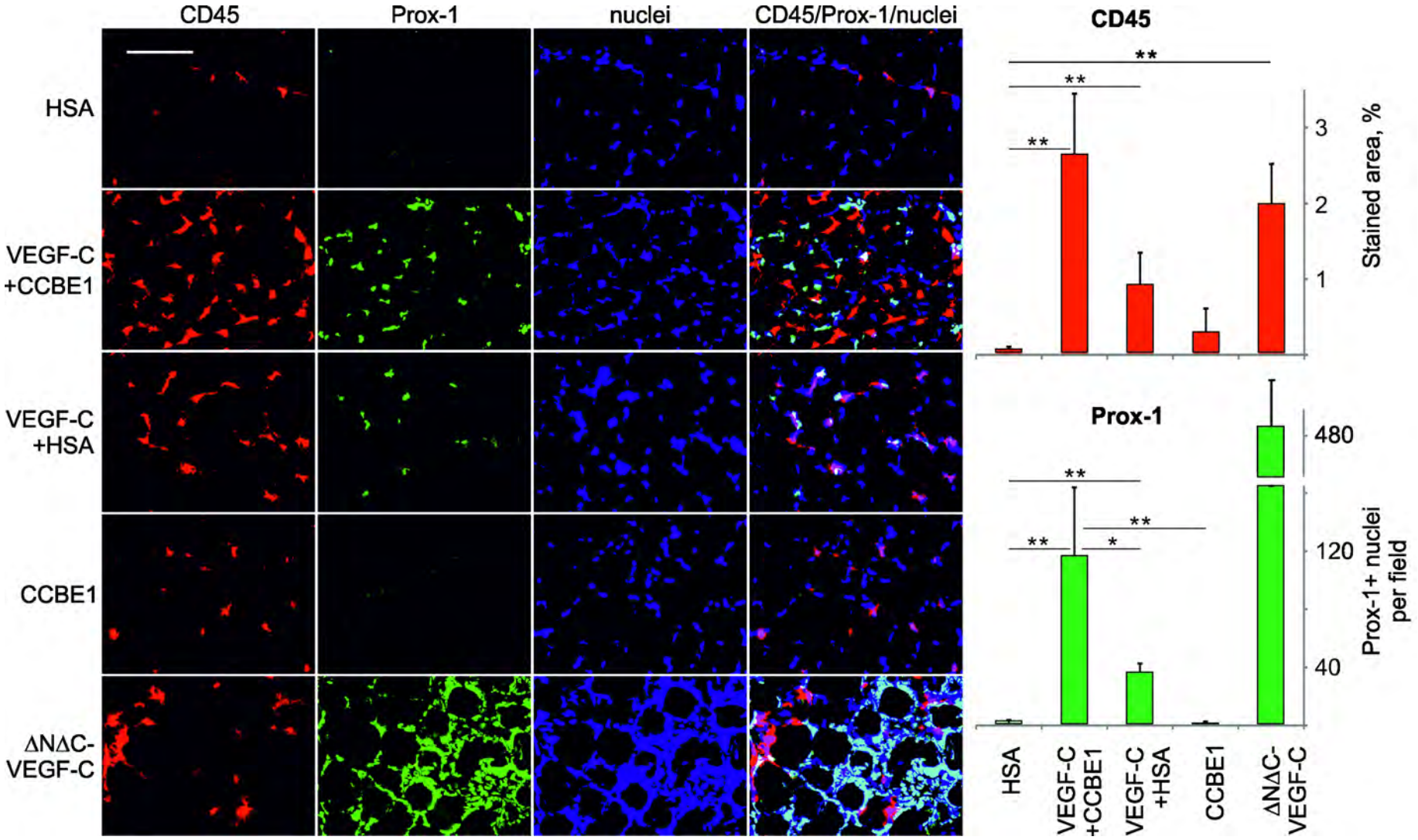
## Supplemental Figures




Jeltsch et al., Fig. S1




Jeltsch et al., Fig. S2

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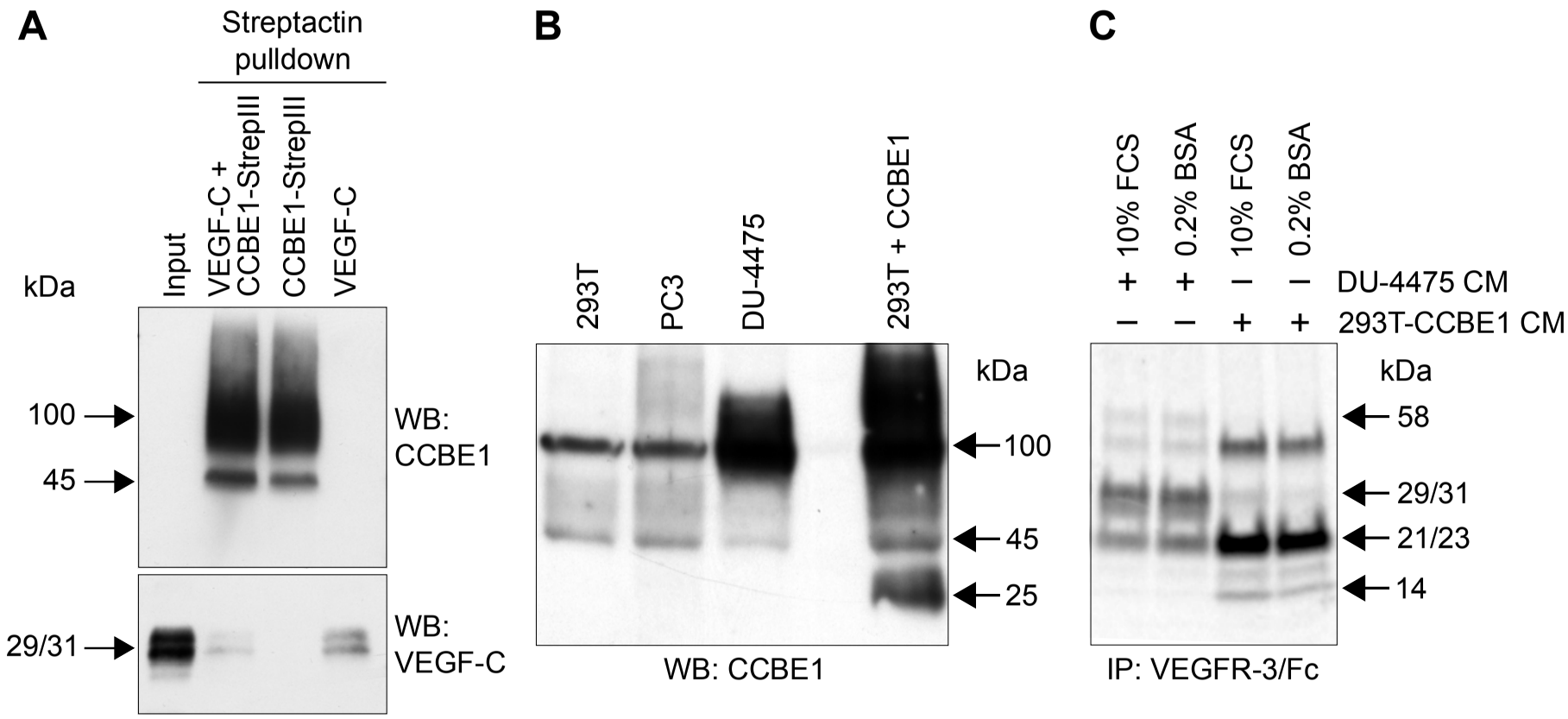
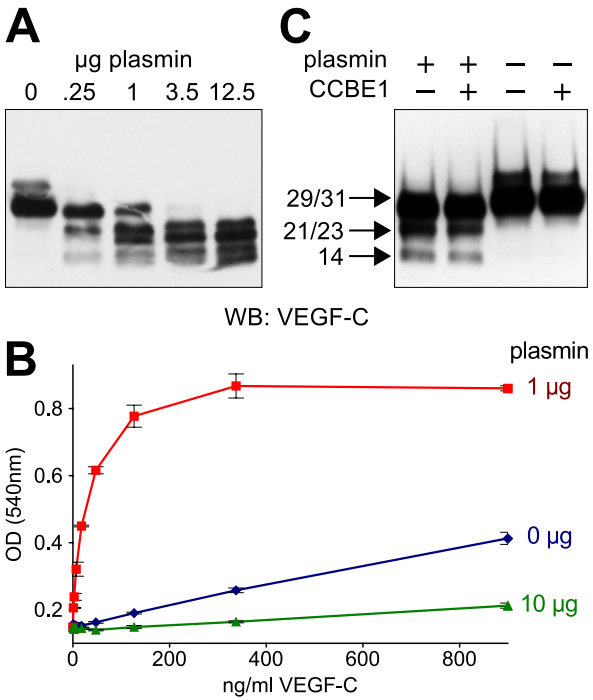
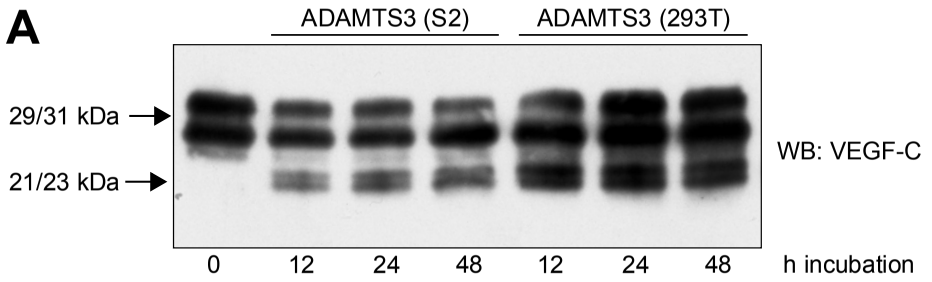
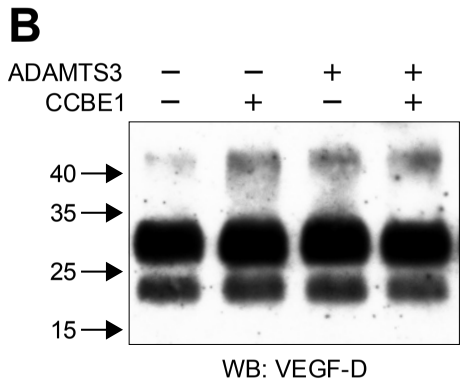


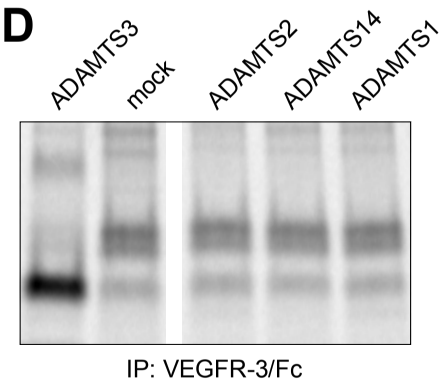

Jeltsch et al., Fig. S4




Jeltsch et al., Fig. S5




C

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ColIa1 Gallus gallus          PPG---LGGNFAP QMSY--GYDEKSAG---VAVP  6.1
ColIa1 Homo sapiens           PPGPPGLGGNFAP QLSY--GYDEKSTGG--ISVP  6.1
ColIIa1 Homo sapiens          PPGPPGLGGNFAA QMAG--GFDEKAGGAQLGVMQ  6.1
ColIa2 Gallus gallus          PPGPPGLGGNFAA QY-----DPSKAADFGPG---  6.1
VEGF-C
  Mus musculus                TRTGDS--VKFAA AHY--NTEILKSIDNEWRKTQ  9.7
  Rattus norvegicus           MRTGDT--VKLAA AHY--NTEILKSIDNEWRKTQ  9.7
  Canis lupus familiaris      ARTEET--IKFAA AHY--NAEILKSIDNEWRKTQ  6.5
  Homo sapiens                SRTEET--IKFAA AHY--NTEILKSIDNEWRKTQ  6.5
  Ornithorhynchus anatinus    ARTEDT--VQFAA AHY--NTEILKSIDNEWRKTQ  3.8
  Anolis carolinensis         AKSEDSNPIKFAA AHY--SPEILKSIDNEWRKTQ  6.5
  Cocturnix japonica          TRSDDS--LKFAA AHY--NAEILKSIDTEWRKTQ  4.3
  Xenopus laevis              TRRDDS--FKFAA AHYNYNADIWKSIENEWRKTQ  6.5
  Danio rerio                 TRSEEAS---FAA AFI--NLELLKSIEIEWRKTL  4.0
VEGF-D
  Gallus gallus               SRSASHRSTR FAAAFY--DIDTLKVIDEEWQKTQ 12.8
  Ornithorhynchus anatinus    SRSAPHRSTR FAAAFY--DIETLKVIDEEWQRTQ 12.8
  Anolis carolinensis         SRSASHRSTR FAAAFY--DIEMLKVIDEEWQRTQ 12.8
  Canis lupus familiaris      SRSASHRATR FAATFY--DIETLKVIDEEWQRTQ 12.8
  Homo sapiens                SRSASHRSTR FAATFY--DIETLKVIDEEWQRTQ 12.8
  Mus musculus                SRSASHRSTR FAATFY--DTETLKVIDEEWQRTQ 12.8
  Rattus norvegicus           SRSTSHRSTR FAATFY--DTETLKVIDEEWQRTQ 12.8
  Xenopus laevis              SRSASHRSTR FAAAFY--DIEILKVIDEEWQKTQ 12.8
  Danio rerio                 TPPENRRSTR YAAASF--SPEMLKDIDDEWQKTQ 12.2
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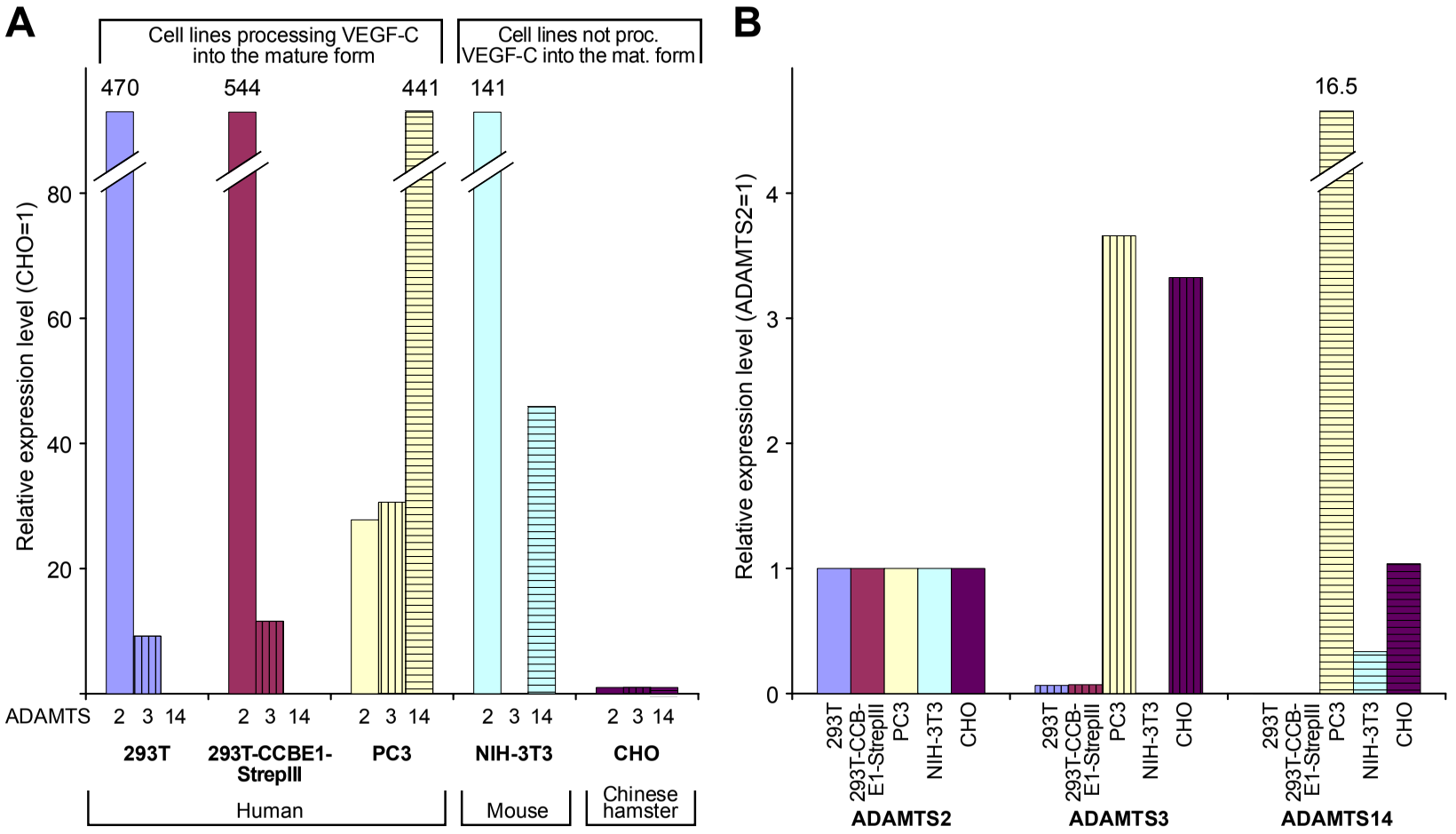
Plasmin (experimental evidence) ADAMTS2 (experimental evidence)
Plasmin (potential) Furin ADAMTS3 (experimental evidence)

Jeltsch et al., Fig. S6




Jeltsch et al., Fig. S7

## Supplemental Figure Legends

**Supplemental Figure 1. CCBE1 enhances VEGF-C release and cleavage**. (A) The figure shows VEGF-C immunoprecipitation from supernatants and lysates of cells transfected with VEGF-C with and without CCBE1, as shown. The molecular weights on the left show the mobility of the major VEGF-C forms. Note that the amount of the mature 21 kDa form of VEGF-C increases (8-fold) in the supernatant and the amounts of the uncleaved VEGF-C and pro-VEGF-C are reduced (by 74% and 51%, correspondingly) upon CCBE1 cotransfection (compare lanes 1, 2 to 3, 4). The 14 kDa fragment resulting from the N-terminal cleavage of VEGF-C appears only in the supernatants of the cotransfected cells. Cotransfection with CCBE1 seems to facilitate the release of VEGF-C as the cell layer associated VEGF-C polypeptides are reduced by 80% in the cell lysates of the CCBE1-cotransfected cells (compare lanes 5, 6 to 7, 8). (B) In a metabolic labeling-pulse-chase analysis, wild-type VEGF-C secretion peaks at 2h, whereas a VEGF-C mutant without the C-terminal propeptide (ΔC-VEGF-C) peaks already between 15 and 45 minutes. (C) Conditioned medium from cultures expressing both CCBE1 and VEGF-C has increased activity in promoting the growth of Ba/F3 cells expressing mouse (m) or human (h) VEGFR-2/EpoR chimeras compared to medium from cultures expressing only VEGF-C. The curves were statistically different from each other at all shown data points except for x=0 ($P<0.05$ marked with * and at $P<0.01$ marked with **; n = 3). (D) Separate cell populations were transfected with VEGF-C or CCBE1 and then mixed for the metabolic labeling period, as indicated. Note also a small difference in the migration of VEGF-C between the stably and transiently transfected cells, resulting from the different glycosylation pattern of VEGF-C produced by the stably transfected 293S GnTI$^-$ cells[18].

**Supplemental Figure 2. CCBE1 co-transduction with VEGF-C stimulates angiogenesis**. Immunohistochemistry for endothelial (PECAM-1) and smooth muscle cell (SMA) markers in *tibialis anterior* muscles. Quantification of the stained areas is shown on the right. Statistically significant differences of $P<0.05$ are marked with *, of $P<0.01$ with ** and of $P<0.001$ with ***; $n \geq 5$.

**Supplemental Figure 3. Recruitment of CD45+ leukocytes and lymphatic vessels by CCBE1/VEGF-C co-transduction.** Prox1 transcription factor was used as the marker for lymphatic endothelial cells. Analysis was done as in the experiments described in Supplemental Figure 2.

**Supplemental Figure 4. CCBE1 and VEGF-C do not interact in a stable manner. CCBE1 is produced by 293T, PC3 and DU-4475 cell lines, but DU-4475-produced CCBE1 does not promote VEGF-C cleavage.** (A) Media conditioned with StrepIII-tagged CCBE1 and VEGF-C were mixed, but no retention of VEGF-C could be detected on the streptactin column (second lane). The faint signal results from non-specific binding of VEGF-C to the column (compare to the fourth lane, for which only media conditioned with VEGF-C was applied to the column). (B) The ConA-bound fraction of conditioned cell culture medium was analyzed by SDS-PAGE/Western blotting. Note that the 293T and PC3 cell lines express only small amounts of endogenous CCBE1, whereas the DU-4475 cells express large amounts. Two species of CCBE1 can be detected in the supernatant, migrating at about 45 and 100 kDa, respectively. In the transfected 293T cells, a short CCBE1 form of 25 kDa is observed. (C) Addition of conditioned medium (CM) from CCBE1-producing 293T cells to VEGF-C-producing cells promotes cleavage of VEGF-C, while CM from DU-4475 cells does not. This effect was observed whether serum-containing (10% FCS) or

serum-free (0.2% BSA) medium was used for the conditioning.

**Supplemental Figure 5. Plasmin cleavage of VEGF-C and the activity of the cleavage products in the Ba/F3-VEGFR-3/EpoR assay.** (A) Pro-VEGF-C is cleaved by plasmin. (B) While low amounts of plasmin resulted in a strong activation of VEGF-C, high amounts led to inactivation of VEGF-C. (C) Cleavage of VEGF-C by plasmin is not enhanced by CCBE1.

**Supplemental Figure 6. Potential cleavage of VEGF-D by ADAMTS3, and VEGF-C by other ADAMTS family members.** (A) VEGF-C is cleaved by recombinant ADAMTS3. (B) VEGF-D was incubated with or without ADAMTS3, but no difference in the ratio between mature VEGF-D and the pro-VEGF-D form was observed. The presence of CCBE1 had no effect. (C) Alignment of the cleavage context for plasmin and ADAMTS2/3 substrates. The cleavage contexts of the four known procollagen targets of ADAMTS2 were aligned with VEGF-C and VEGF-D sequences. The ADAMTS2 cleavage sites are indicated by a yellow triangle, the ADAMTS3 cleavage site by a black triangle, plasmin cleavage sites are indicated by red triangles and the furin cleavage site by a blue triangle. The numbers denote the theoretical isoelectric point of the stretch comprising the 10 amino acid residues N-terminal to the ADAMTS3 cleavage site. Background color-coding for the amino residues: magenta, acidic; red, basic; green, ADAMTS2/3 cleavage motif; yellow, exceptions to the cleavage motif. (D) Transfection of ADAMTS1 and the procollagenase-type ADAMTS2 and ADAMTS14 into VEGF-C expressing 293F cells does not result in increased generation of mature VEGF-C.

**Supplemental Figure 7. Comparison of expression levels of ADAMTS2, ADAMTS3 and ADAMTS14 by quantitative PCR.** (A) Comparison of the expression levels between

different cell types relative to CHO cells =1. (B) Comparison within one cell type relative to ADAMTS2 = 1. Note that the cell lines that do process VEGF-C into its mature form express ADAMTS3, while those cells that are unable or extremely inefficient in processing VEGF-C into its mature form do express very low amounts or no ADAMTS3.

**Supplemental Figure 8. Suppression of VEGF-C processing by ADAMTS3 shRNA and effect of the N- and C-terminal propeptides of VEGF-C on processing.** (A) The processing of pro-VEGF-C into mature VEGF-C was reduced in 293T cells by lentiviral ADAMTS3 shRNA compared to the non-target shRNA. Note that the baseline processing of VEGF-C is increased in the cells transduced with a non-targeting control shRNA compared to the non-transduced cells. (B) The proteolytic processing of VEGF-D and the VEGF-D/VEGF-C chimera version 1 (CDC-V1) in the presence or absence of CCBE1 cotransfection. Processing of CDC-V2 was also not affected by CCBE1 (data not shown). (C) Alignment of the amino acid sequence of the chimeras and their parent proteins. VEGF-C-derived sequences are shown in green, VEGF-D-derived sequences in red. The boxed region marks where the proteolytic cleavages occur in VEGF-C and VEGF-D. (D) Competitive inhibition of CCBE1 action by the VEGF-C propeptides. Note that the amounts of both the mature 21/23 kDa form of VEGF-C and the 14 kDa N-terminal propeptide are reduced by competition with the C- and N-terminal propeptides. C-pp, C-terminal propeptide; N-pp, N-terminal propeptide; HSA, human serum albumin; ΔNΔC, C-and N-terminally truncated form of VEGF-C similar to mature VEGF-C[9].

## Supplemental References

1. Paterna JC, Moccetti T, Mura A, Feldon J, Bueler H. Influence of promoter and WHV post-transcriptional regulatory element on AAV-mediated transgene expression in the rat brain. *Gene Ther*. 2000;7:1304-1311.

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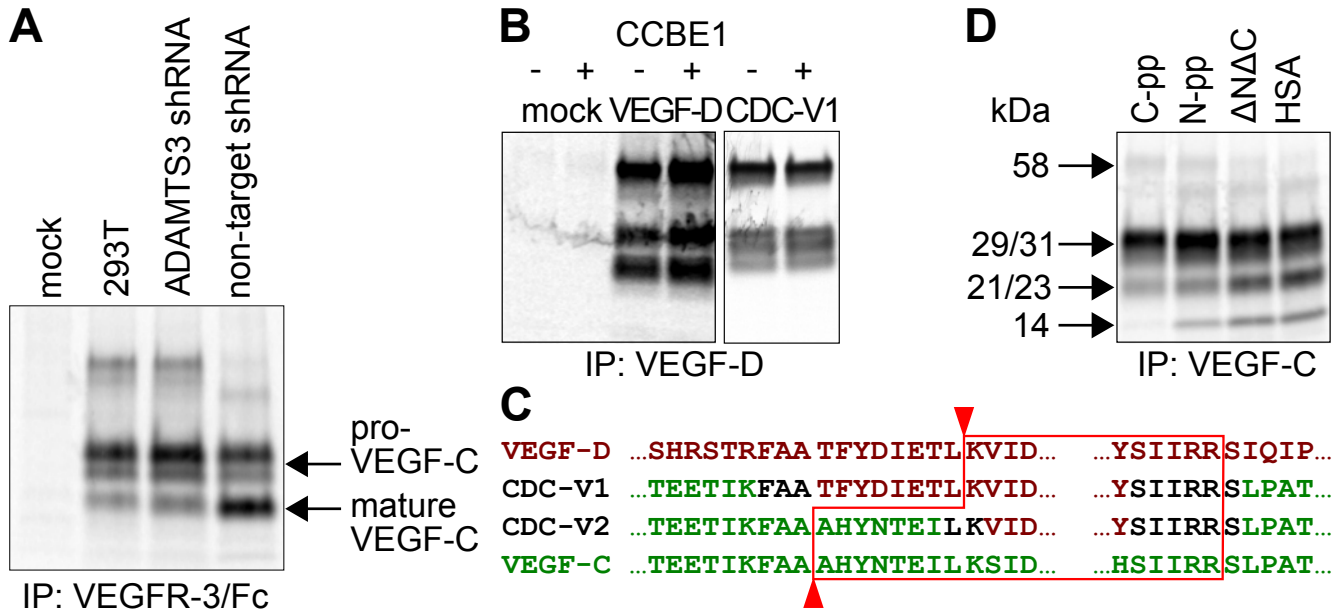


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