Table S1. Data collection and refinement statistics

	VEGF-D
Data collection	
Space group	$P6_122$
Cell dimensions	
a, *b*, *c* (Å)	95.72, 95.72, 70.94
abg (°)	90, 90, 120
Resolution (Å)	40.0 – 2.90 (3.05-2.90) *
R_{sym}	9.0 (76.8)
I / sI	18.4 (3.8)
Completeness (%)	99.7 (99.7)
Redundancy	8.8 (9.2)
Refinement	
Resolution (Å)	40 – 2.90
No. reflections	4543
R_{work} / R_{free}	25.5 / 33.3
No. atoms	
Protein	774
Glycan	100
Water	13
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.540

*Values in parentheses are for highest-resolution shell.

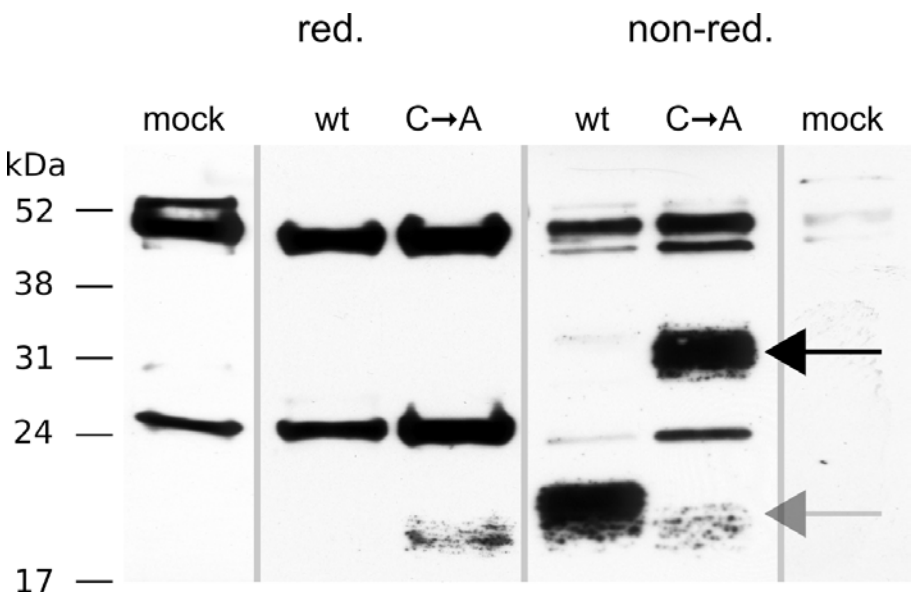

Figure S1. Cys117Ala mutant of human VEGF-D is a more stable covalent dimer
Western blotting of wild-type and Cys117Ala mutant of human VEGF-D under reducing *vs.* non-reducing conditions. The Cys117Ala mutation shifts the ratio of non-covalent (grey arrow) to covalent dimeric (black arrow) form of VEGF-D towards the covalent dimeric form (See also Rissanen *et al.*,[1] Anisimov *et al.*[2] and Toivanen *et al.*[3]) The VD1 antibody[4] apparently recognizes a conformational epitope of the native protein that mostly disappears upon reduction as well as to a varying degree nonspecific bands of approximately 24 and 50 kDa.

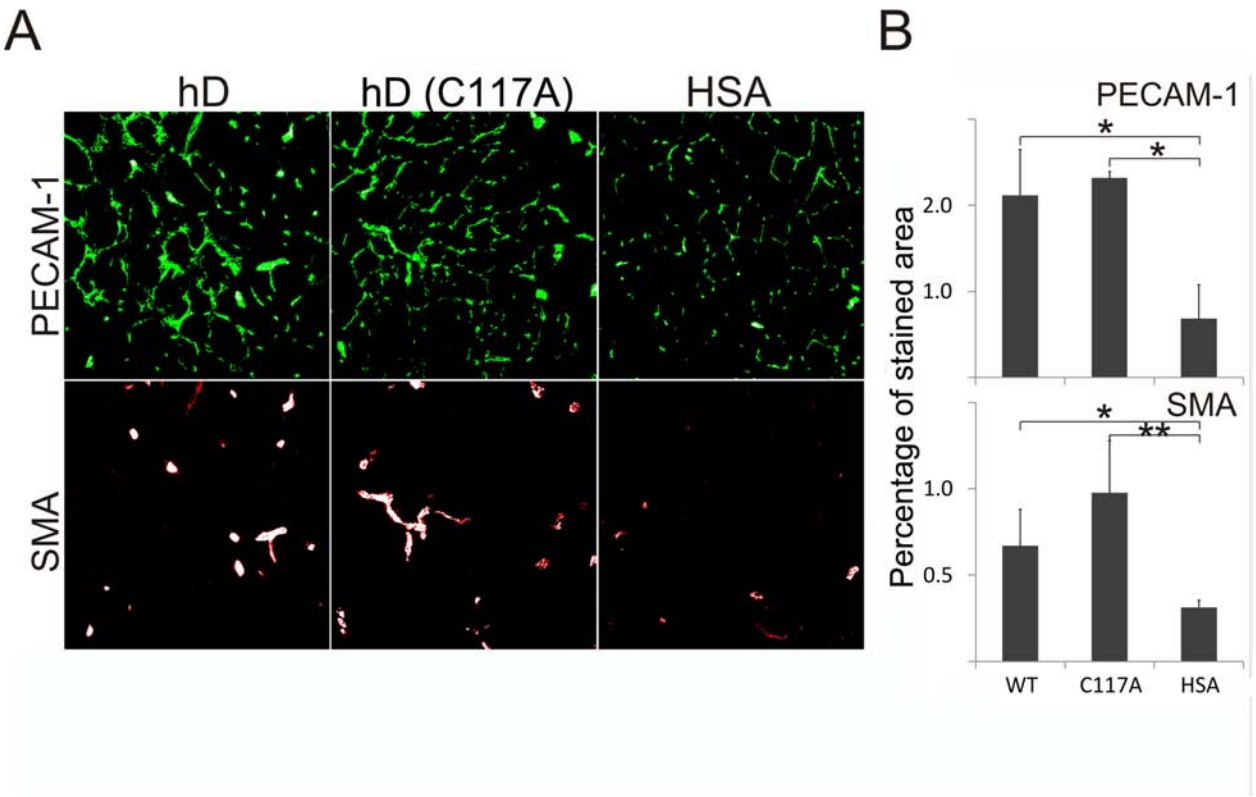

Figure S2. *In vivo* activity of wild-type and Cys117Ala mutant of VEGF-D proteins
Tibialis anterior muscles of NMRI female mice were injected with rAAVs encoding the indicated cDNAs (hD, major form of the mature human VEGF-D, residues 89-205; hD (C117A), major form of the mature human VEGF-D with Cys117Ala mutation and HSA, human serum albumin as a control) and analyzed two weeks later by immunohistochemistry of frozen sections using antibodies against PECAM-1 (platelet endothelial adhesion molecule-1) and SMA (smooth muscle actin). A). Representative images of the staining. B). Quantification of stained area from five or more randomly chosen view fields. Statistical significance is indicated by * where $p<0.05$ and ** where $p<0.01$ compared to rAAV-encoded HSA. Error bars represent +/− SD.

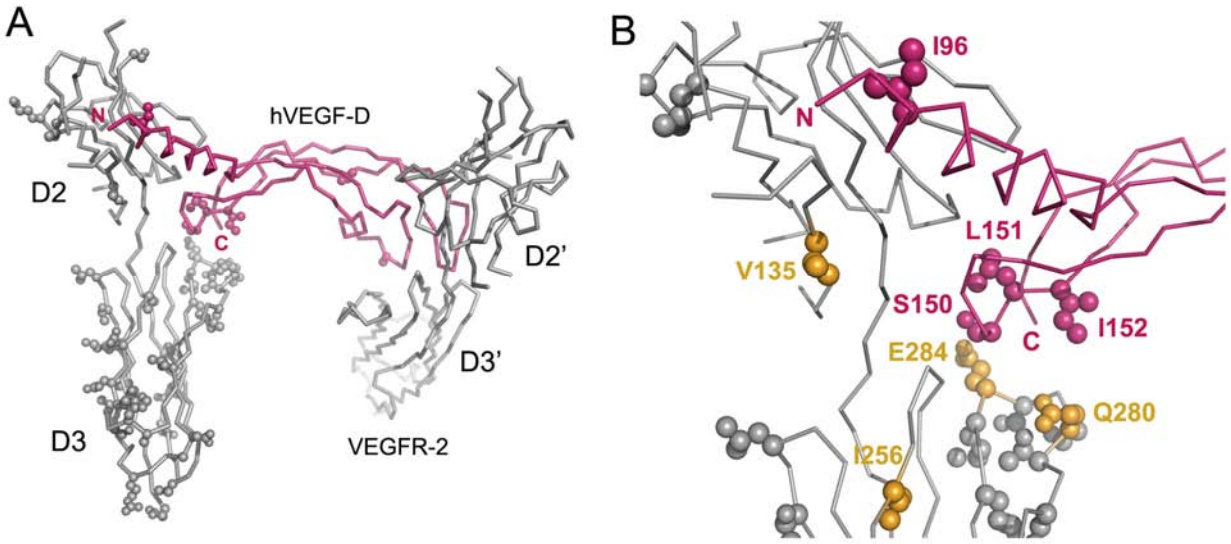

Figure S3. Differences between mouse and human VEGF-D and VEGFR-2D23
(A). A ribbon diagram of the VEGF-D structure (magenta) superimposed with VEGF-C in the VEGFR-2D23 (grey) complex structure. For clarity, only one VEGF-D chain is shown. The differences in the human and the mouse VEGF-D (Figure 3A) and VEGFR-2 (data not shown) sequences are indicated by highlighting the corresponding human residues as spheres. (B). A close-up of (A) with the key VEGF-D differences labeled. Human VEGF-D Ala195 is not shown because it was not visible in the crystal structure. The VEGFR-2 sequence differences at ligand-binding surface are highlighted in orange.

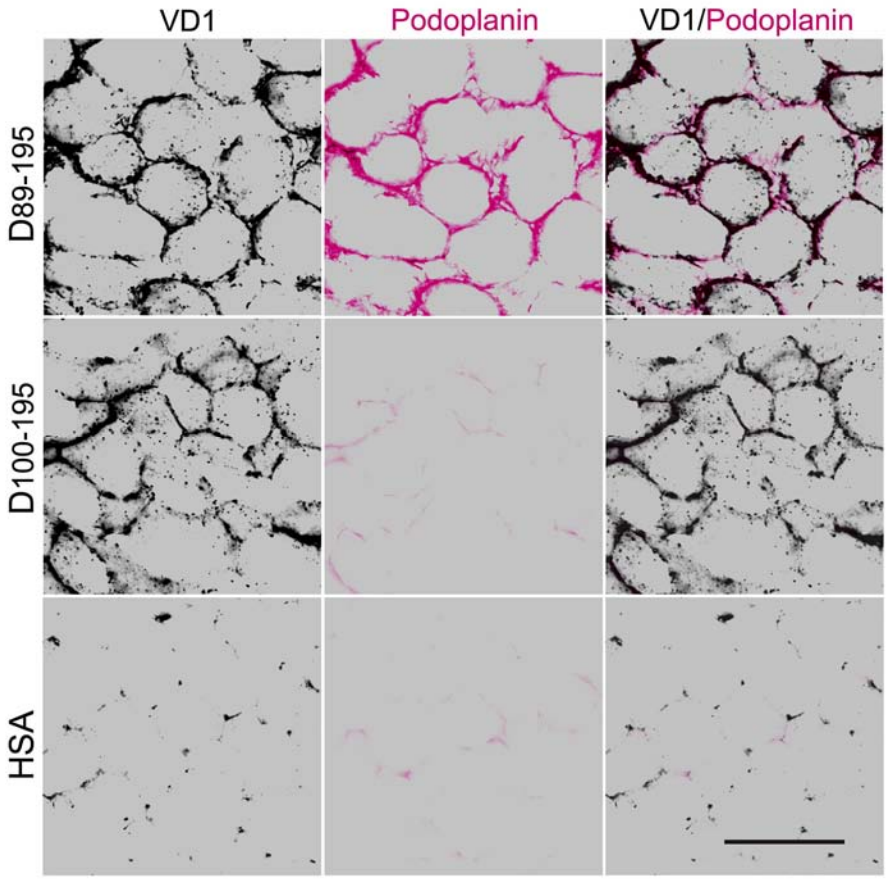

Figure S4. *In vivo* expression and activity of the major and minor forms of human VEGF-D proteins

Tibialis anterior muscles of Balb/c male mice were injected with rAAVs encoding the indicated cDNAs (D_{89-195}, residues 89-195, the N-terminal major form of the human VEGF-D; $D_{100-195}$, residues 100-195, the N-terminal minor form of the human VEGF-D and HSA, human serum albumin as a control) and analyzed two weeks later by immunohistochemistry of frozen sections. Representative images of the staining are shown. Antibodies against human VEGF-D (VD1[4]; first panel from the left) and mouse Podoplanin antibodies (the panel in the middle; lymphangiogenesis) were used for immunostaining. The 3rd panel from the left represents the VD1/Podoplanin overlay. Scale bar represents 100 μm.

REFERENCES

1. Rissanen TT, Markkanen JE, Gruchala M, et al. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. *Circ Res*. 2003;92:1098-1106.
2. Anisimov A, Alitalo A, Korpisalo P, et al. Activated forms of VEGF-C and VEGF-D provide improved vascular function in skeletal muscle. *Circ Res*. 2009;104:1302-1312.
3. Toivanen PI, Nieminen T, Viitanen L, et al. Novel vascular endothelial growth factor D variants with increased biological activity. *J Biol Chem*. 2009;284:16037-16048.
4. Achen MG, Roufail S, Domagala T, et al. Monoclonal antibodies to vascular endothelial growth factor-D block its interactions with both VEGF receptor-2 and VEGF receptor-3. *Eur J Biochem*. 2000;267:2505-2515.