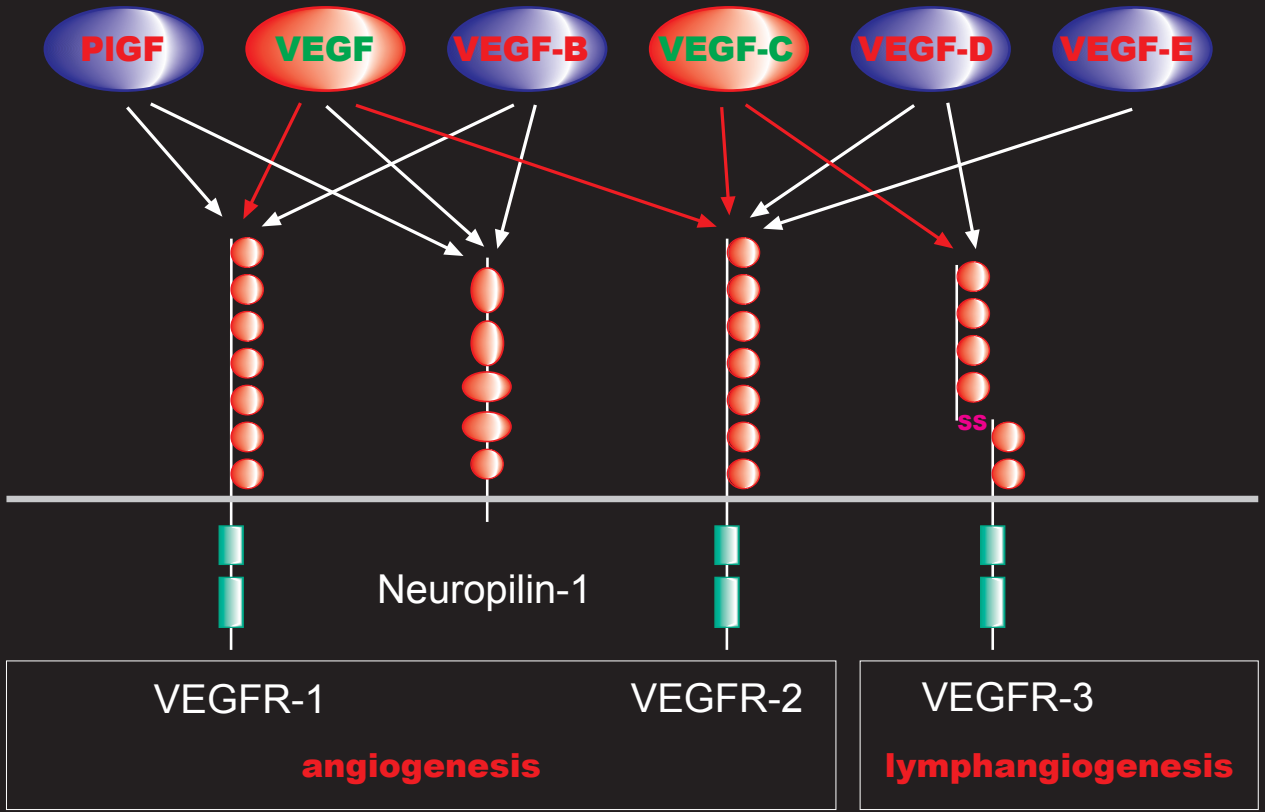
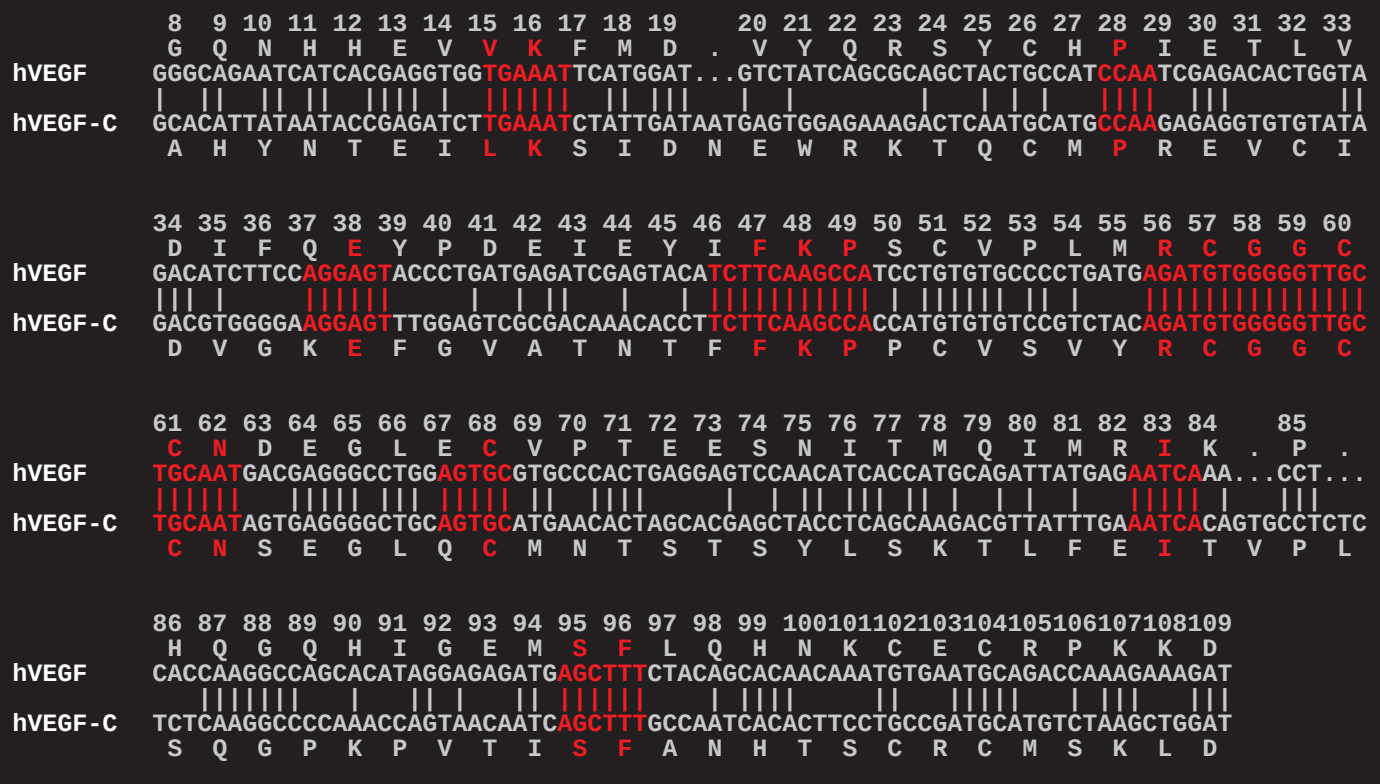
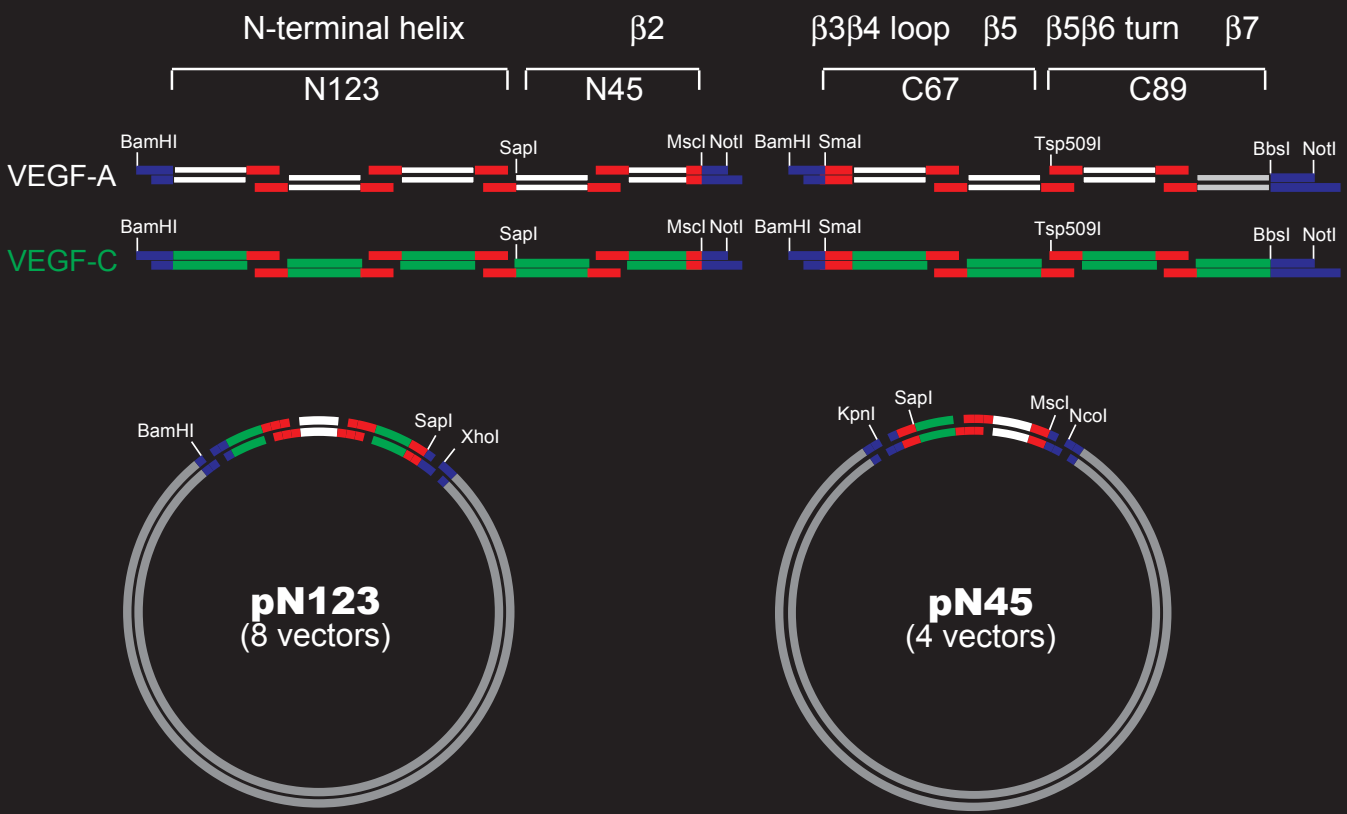
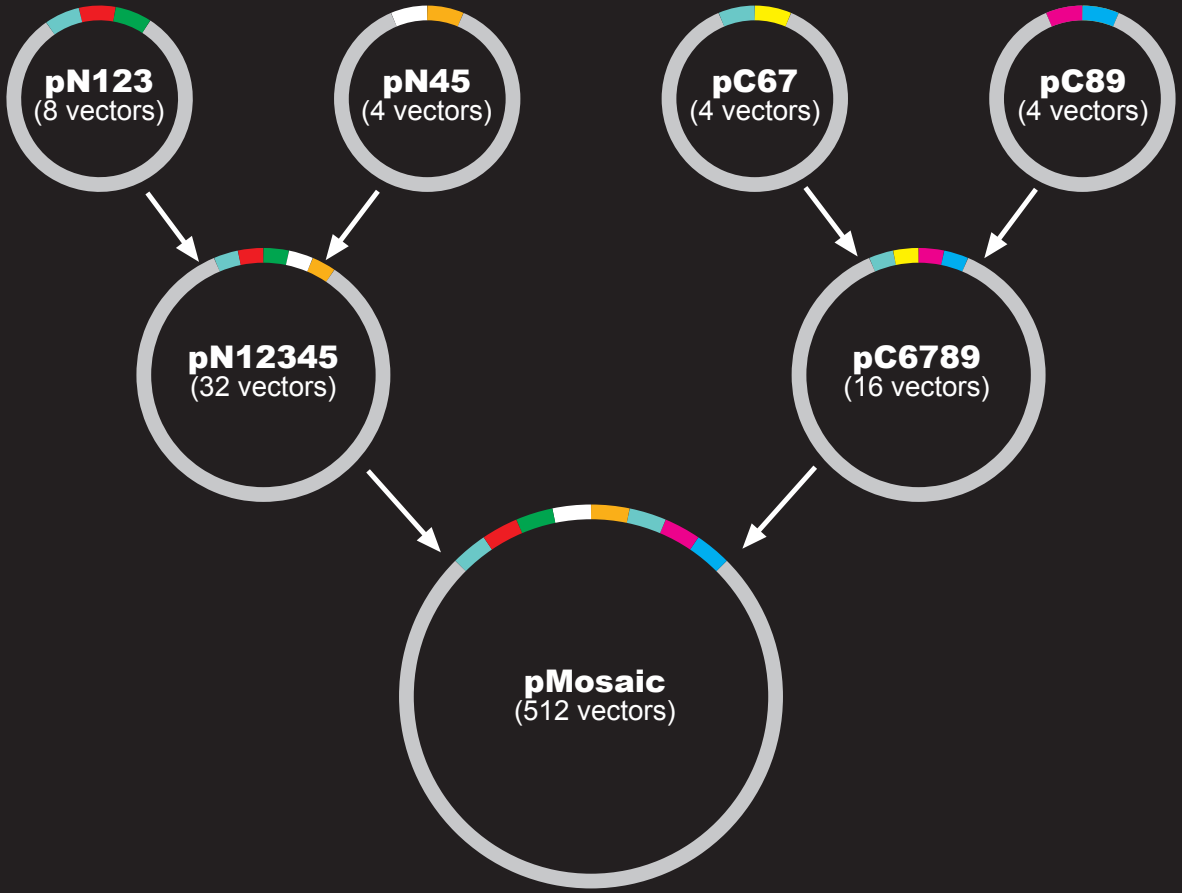

PlGF
VEGF
VEGF-B
VEGF-C
VEGF-D
VEGF-E
ss
Neuropilin-1
VEGFR-1
VEGFR-2
angiogenesis
VEGFR-3
lymphangiogenesis

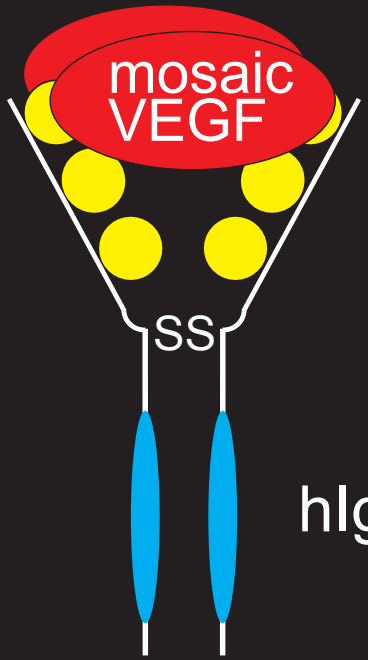
DNA family shuffling (= molecular evolution by in vitro recombination)


10 20 30 40 50
hPDGF-A RPL PIRRKRSIE.E AVPAVCKTRT VIYEIPRSQVDP TSANFLIWPP CVEVKRCT
hPDGF-B RGR RSLGSLTIAEP AMIAECKTRT EVFEISRRLIDR TNANFLVWPP CVEVQRCS
hVEGF-C AHY NTEILKSIDNE WRKTQCMPRE VCIDVGKEF..G VATNTFFKPP CVSVYRCG
mVEGF-C AHY NTEILKSIDNE WRKTQCMPRE VCIDVGKEF..G AATNTFFKPP CVSVYRCG
hVEGF-D TFY DIETLKVIDEE WQRTQCSPRE TCVEVASEL..G KSTNTFFKPP CVNVFRCG
mVEGF-D TFY DTETLKVIDEE WQRTQCSPRE TCVEVASEL..G KTTNTFFKPP CVNVFRCG
hVEGF GQN HHEVVKFMD.V YQRSYCHPIE TLVDIFQEY..P DEIEYIFKPS CVPLMRCG
mVEGF EQK SHEVIKFMD.V YQRSYCRPIE TLVDIFQEY..P DEIEYIFKPS CVPLMRCA
hVEGF-B PGH QRKVVSWID.V YTRATCQPRE VVVPLTVEL..M GTVAKQLVPS CVTVQRCG
hPlGF GSS EVEVVPFQE.V WGRSYCRALE RLVDVVSEY..P SEVEHMFSPS CVSLLRCT
60 70 80 90 100 109
hPDGF-A GC CNTSSVKCQP SRVHHRSVKV AKVEYVRKKP.K LKEVQVRLEEHL ECACATTSL
hPDGF-B GC CNNRNVQCRP TQVQLRPVQV RKIEIVRKKPIF KKATVT.LEDHL ACKCETVAA
hVEGF-C GC CNSEGLQCMN TSTSYLSKTL FEITVPLSQGPK PVTISF..ANHT SCRCMSKLD
mVEGF-C GC CNSEGLQCMN TSTGYLSKTL FEITVPLSQGPK PVTISF..ANHT SCRCMSKLD
hVEGF-D GC CNEESLICMN TSTSYISKQL FEISVPLTSVPE LVPVKV..ANHT GCKCLPTAP
mVEGF-D GC CNEEGVMCMN TSTSYISKQL FEISVPLTSVPE LVPVKI..ANHT GCKCLPTGP
hVEGF GC CNDEGLECVP TEESNITMQI MRIK.P.HQGQH IGEMSF..LQHN KCECRPKKD
mVEGF GC CNDEALECVP TSESNITMQI MRIK.P.HQSQH IERMSF..LQHS RCECRPKKD
hVEGF-B GC CPDDGLECVP TGQHQVRMQI LMIRYPSSQ... LGEMSL..EEHS QCECRPKKK
hPlGF GC CGDENLHCVP VETANVTMQL LKIRSG..DRPS YVELTF..SQHV RCECRPLRE


8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
G Q N H H E V V K F M D . V Y Q R S Y C H P I E T L V
hVEGF GGGCAGAATCATCACGAGGTGGTGAAATTCATGGAT...GTCTATCAGCGCAGCTACTGCCATCCAATCGAGACACTGGTA
hVEGF-C GCACATTATAATACCGAGATCTTGAAATCTATTGATAATGAGTGGAGAAAGACTCAATGCATGCCAAGAGAGGTGTGTATA
A H Y N T E I L K S I D N E W R K T Q C M P R E V C I
34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
D I F Q E Y P D E I E Y I F K P S C V P L M R C G G C
hVEGF GACATCTTCCAGGAGTACCCTGATGAGATCGAGTACATCTTCAAGCCATCCTGTGTGCCCCTGATGAGATGTGGGGGTTGC
hVEGF-C GACGTGGGGAAGGAGTTTGGAGTCGCGACAAACACCTTCTTCAAGCCACCATGTGTGTCCGTCTACAGATGTGGGGGTTGC
D V G K E F G V A T N T F F K P P C V S V Y R C G G C
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
C N D E G L E C V P T E E S N I T M Q I M R I K . P .
hVEGF TGCAATGACGAGGGCCTGGAGTGCGTGCCCACTGAGGAGTCCAACATCACCATGCAGATTATGAGAATCAAA...CCT...
hVEGF-C TGCAATAGTGAGGGGCTGCAGTGCATGAACACTAGCACGAGCTACCTCAGCAAGACGTTATTTGAAATCACAGTGCCTCTC
C N S E G L Q C M N T S T S Y L S K T L F E I T V P L
86 87 88 89 90 91 92 93 94 95 96 97 98 99 100101102103104105106107108109
H Q G Q H I G E M S F L Q H N K C E C R P K K D
hVEGF CACCAAGGCCAGCACATAGGAGAGATGAGCTTTCTACAGCACAACAAATGTGAATGCAGACCAAAGAAAGAT
hVEGF-C TCTCAAGGCCCCAAACCAGTAACAATCAGCTTTGCCAATCACACTTCCTGCCGATGCATGTCTAAGCTGGAT
S Q G P K P V T I S F A N H T S C R C M S K L D


N-terminal helix
β2
β3β4 loop
β5
β5β6 turn
β7
N123
N45
C67
C89
VEGF-A
VEGF-C
BamHI
SapI
MscI
NotI
BamHI
SmaI
Tsp509I
BbsI
NotI
BamHI
SapI
MscI
NotI
BamHI
SmaI
Tsp509I
BbsI
NotI
BamHI
SapI
XhoI
pN123
(8 vectors)
KpnI
SapI
MscI
NcoI
pN45
(4 vectors)


pN123
(8 vectors)
pN45
(4 vectors)
pC67
(4 vectors)
pC89
(4 vectors)
pN12345
(32 vectors)
pC6789
(16 vectors)
pMosaic
(512 vectors)

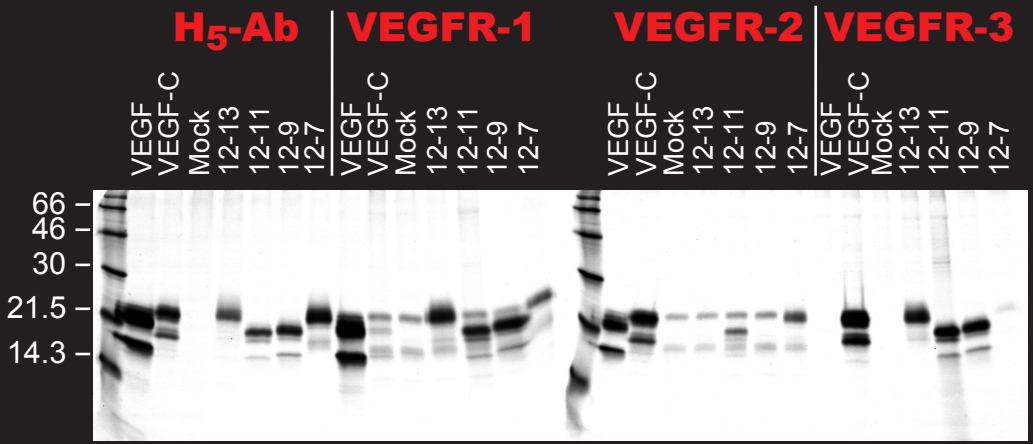


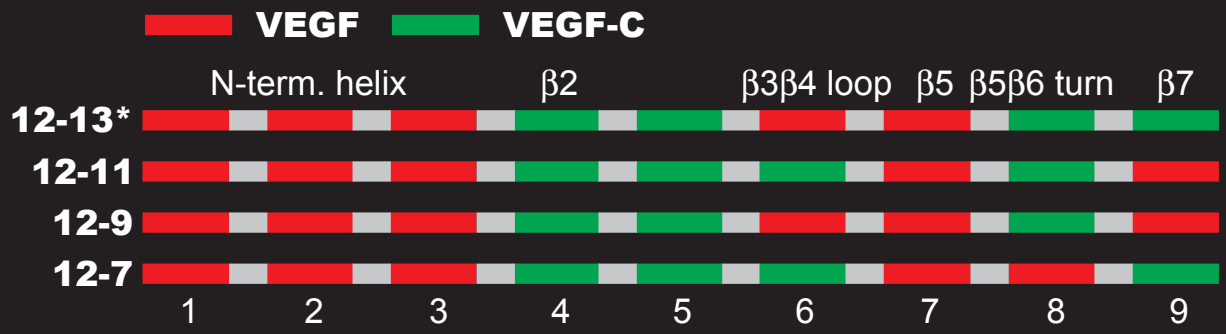
conditioned medium from transfected and S^{35}-labelled 293T cells

hVEGFR loops 1-3 (R-2 and R-3 produced in 293T, R-1 produced in S2 cells)

$hIgG_1$ Fc

Binding of VEGF-VEGF-C mosaic molecules to soluble VEGF receptor bodies




VEGF
VEGF-C
N-term. helix
β2
β3β4 loop
β5
β5β6 turn
β7
12-13*
12-11
12-9
12-7
1
2
3
4
5
6
7
8
9

512 possible mosaic molecules

400 expressed (286 don't bind any VEGFR)

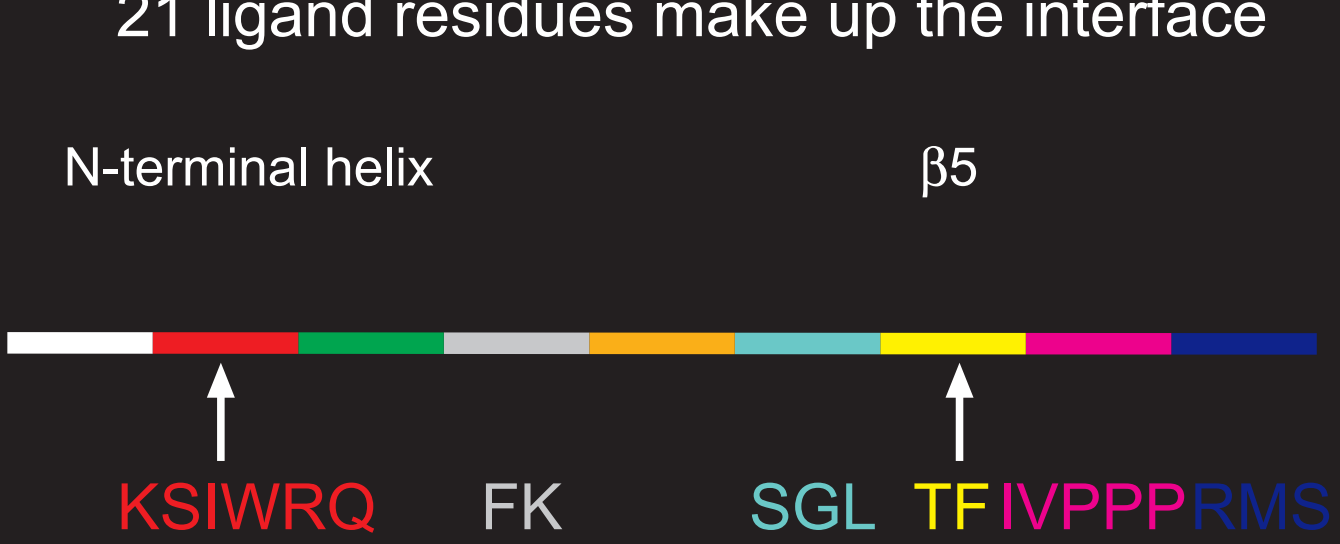
25 VEGFR-1 and -2 (VEGF analogues)
20 VEGFR-2 and -3 (VEGF-C analogues)

23 VEGFR-1 only
21 VEGFR-2 only
15 VEGFR-3 only

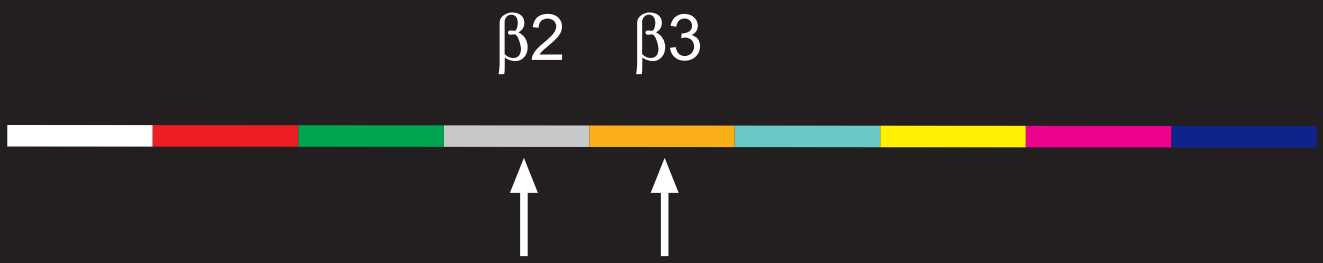
6 VEGFR-1 and -3, but not VEGR-2
4 all three VEGF receptors

VEGFR-1 binding

21 ligand residues make up the interface

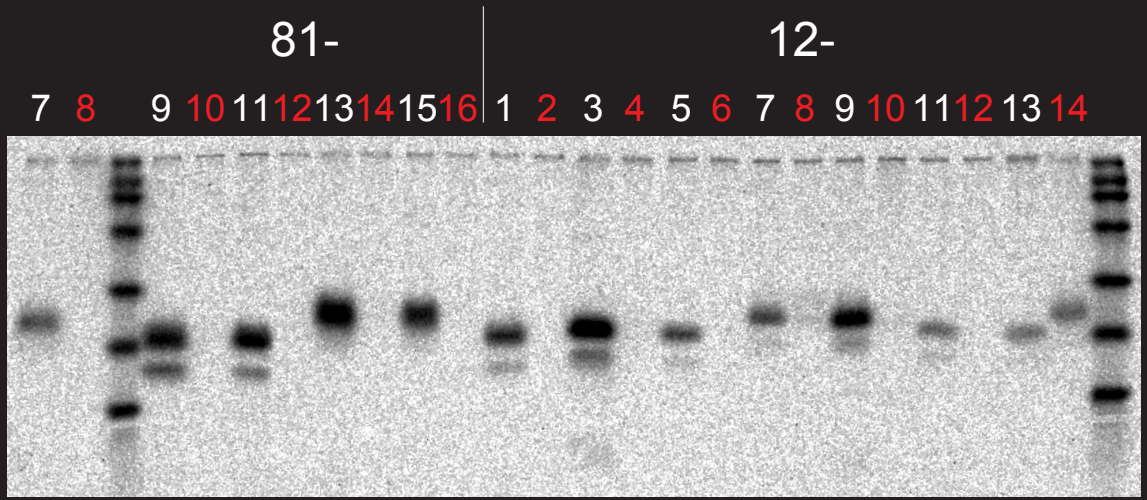


VEGFR-3 binding



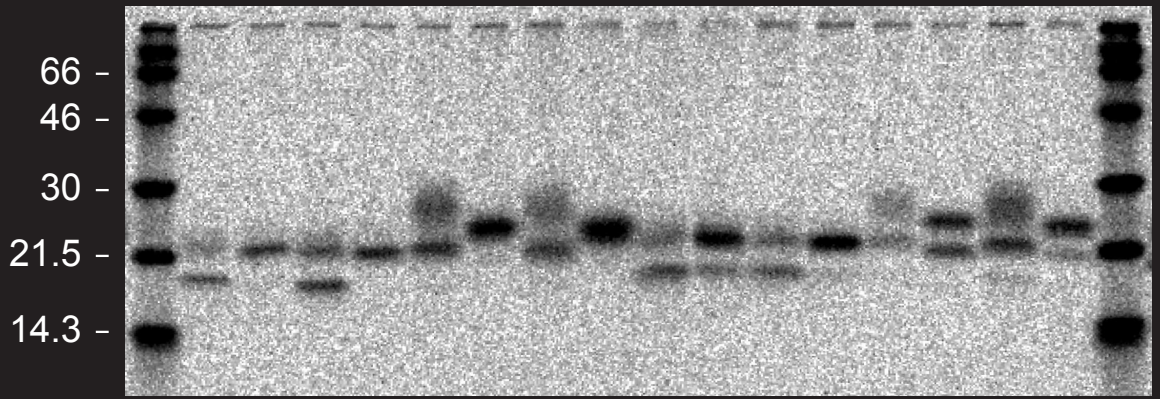
hVEGF-C	FGVATNTFF	KPPCVSVYR
hVEGF	YPDEIEYIF	KPSCVPLMR
hVEGF-D	LGKSTNTFF	KPPCVNVFR
qVEGF-C	FGATTNTFF	KPPCVSIYR
bVEGF-C	FGAATNTFF	KPPCVSVYR
zVEGF-C	FG-ATNTFY	KPPCVSVYR

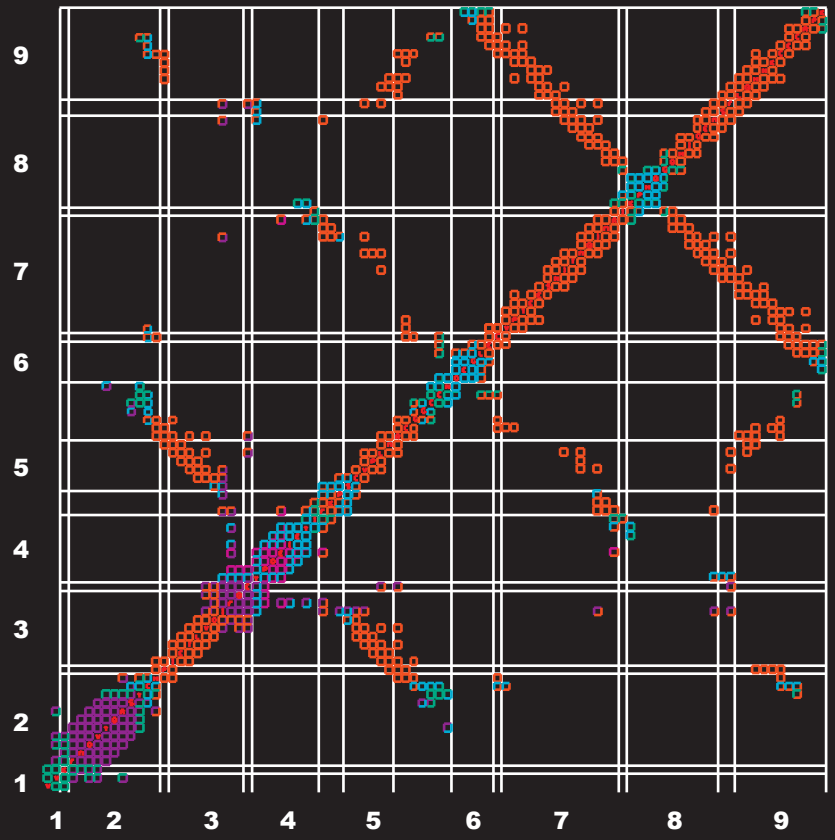
Incompatibility between VEGF-A-derived fragment 3 and VEGF-C-derived fragment 7



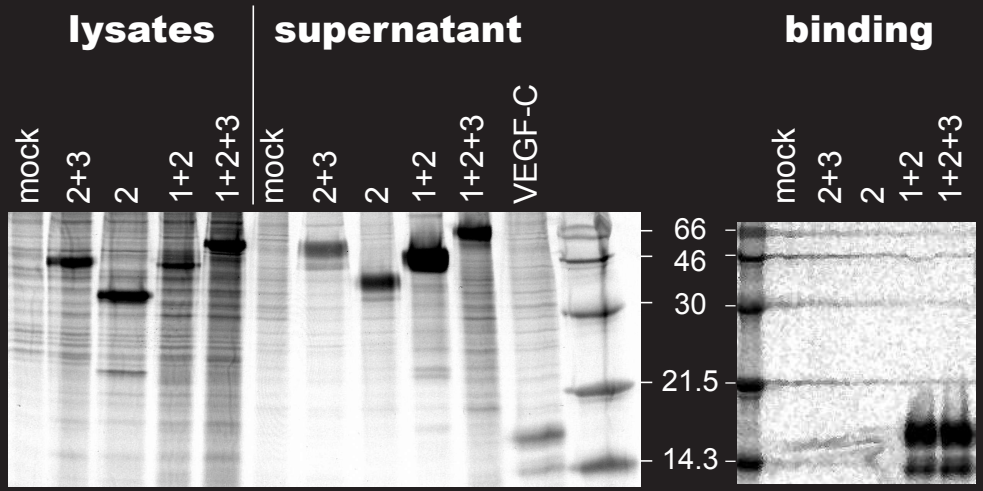
Differential glycosylation

fragment 7 A C A C A C A C A C A C A C A C
fragment 9 A A A A C C C C A A A A C C C C



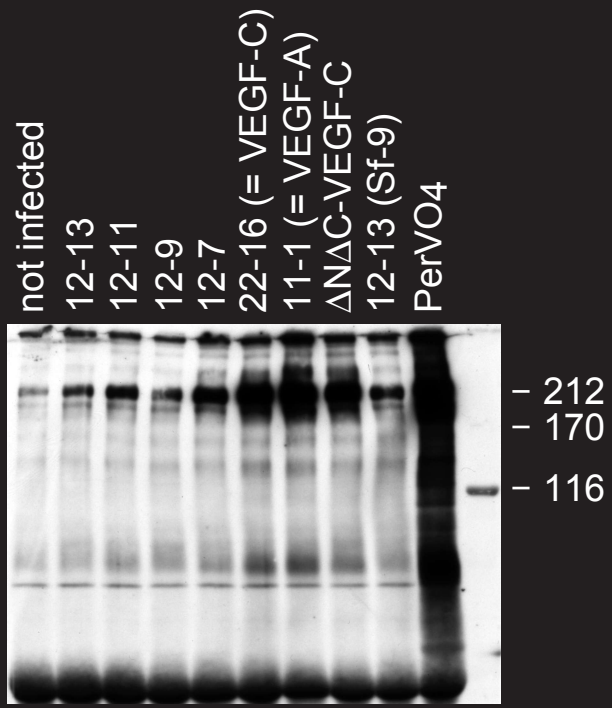

9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9

Expression of different Flt4-Ig fusion proteins and binding of ΔNΔC-hVEGF-C

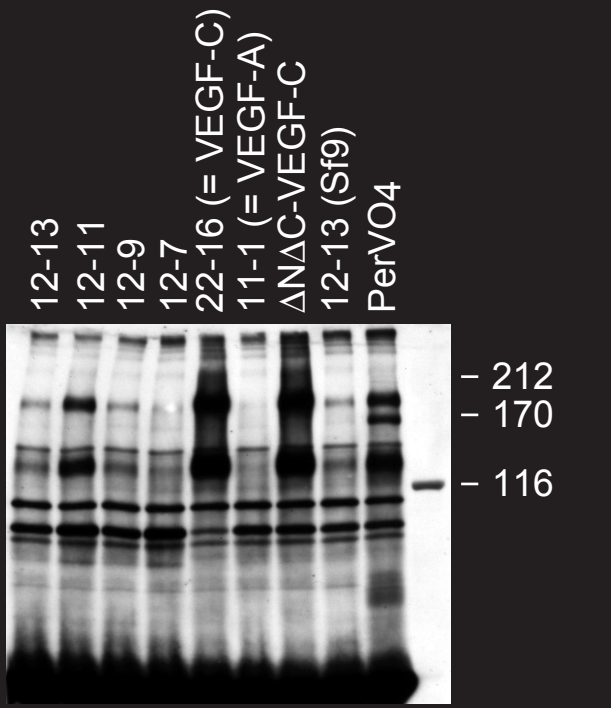


Stimulation of tyrosine phosphorylation

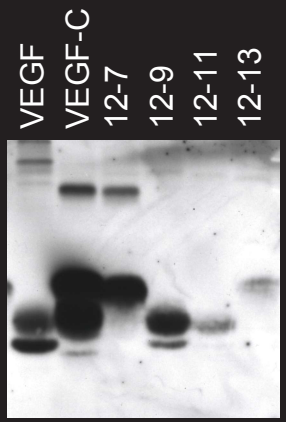
VEGFR-2



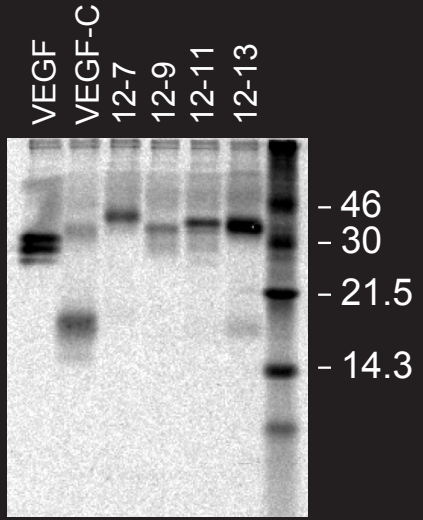
VEGFR-3



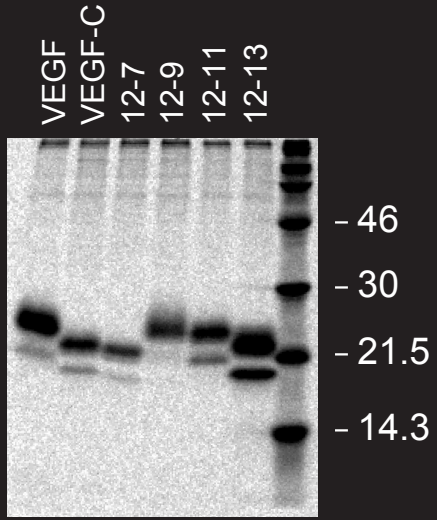
Expression in HF cells



non-reducing



reducing

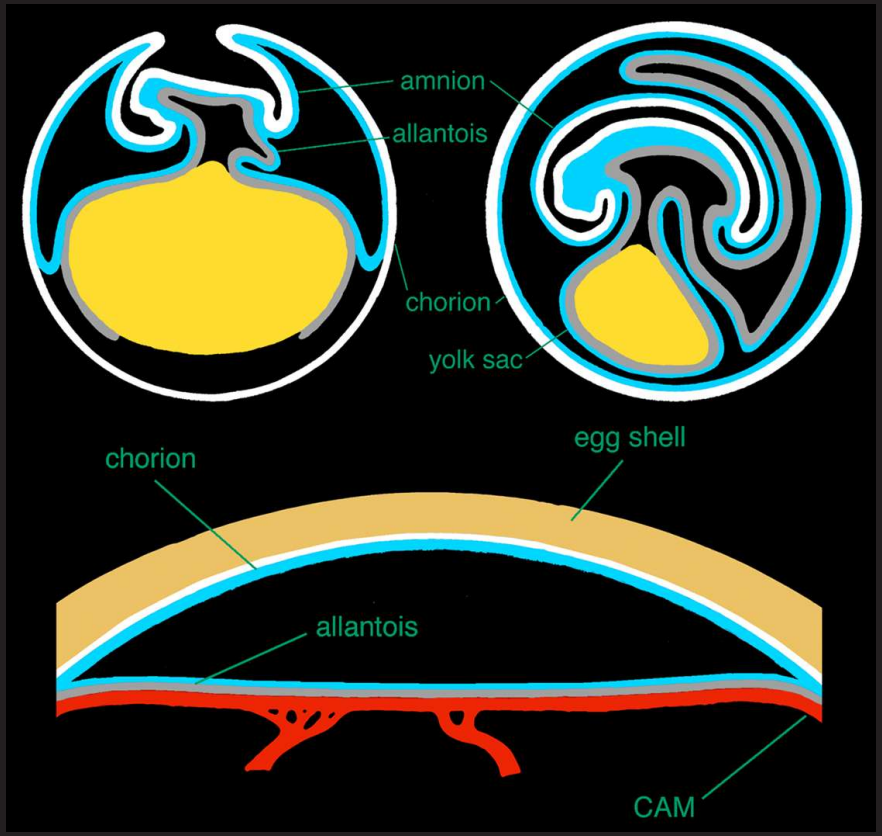


Did we miss something?

1. 36 clones were fully sequenced to estimate the amount of non-productive ligations: one point mutation & one 2-bp-loss
2. Some weak binding towards VEGFR-1 & VEGFR-2 is masked by endogenous VEGF
3. Internal control via redundancy of fragment 1

What next?

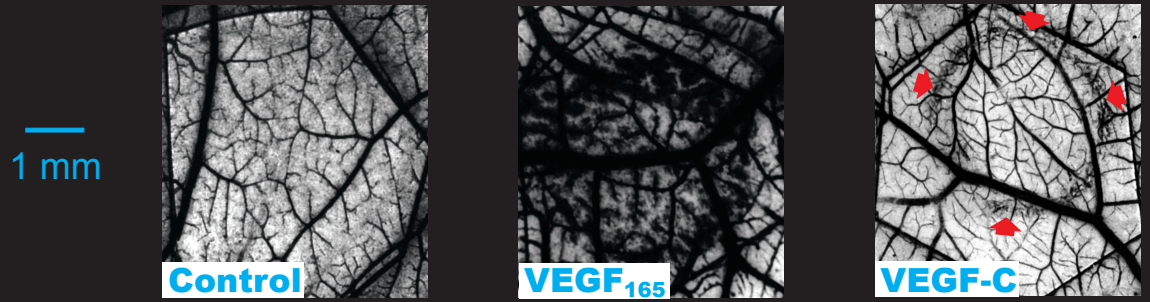
- → Determination of affinities
- → Quantitative biological assay (CAM assay)
- → Improvement (rational vs. random: backcrossing & point mutations)


amnion
allantois
chorion
yolk sac
chorion
egg shell
allantois
CAM

m1 ΔNΔC-melSP-hVEGF-C-H6 (...MSKL*)

6.3 29/31 kDa form of hVEGF-C

Effect of VEGF and VEGF-C on CAM



A artery, **L** lymphatics, **V** vein

Summary

- → In-vitro evolution of the VEGF family yields polypeptides with novel receptor binding profiles and possibly modified biological activities
- → Different structural elements of VEGFs mediate receptor binding, but only some of them confer receptor specificity

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