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Supplementary Materials for

The Basis for the Distinct Biological Activities of Vascular Endothelial Growth Factor Receptor-1 Ligands

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Fig. S1. Comparison of the amino acid sequences of VEGFR-1 ligand loops. Fig. S2. Replacement of VEGF-A L1 with the VEGF-B–derived L1 does not affect VEGFR-1 binding, but inhibits VEGFR-1 activation. Fig. S3. Pretreatment of PAE–VEGFR-2 cells with high molar excess of VEGF-B does not reduce the VEGFR-2 phosphorylation induced by B-L1^P or VEGF-A. Fig. S4. Analysis of L1 swap chimeras between VEGF-A and PIGF. **Figure S1. Comparison of the amino acid sequences of VEGFR-1 ligand loops.** (A) Alignments of L1, L2 and L3 amino acid sequences of VEGF-B, VEGF-A and PIGF from various species. (B) Alignment of L1 sequences from mouse VEGF-B and PIGF against VEGF-A shows substantial variation, whereas the L2 sequence is highly conserved.

А		L	1		L2		L3	
VEGF-B	Mouse Human Dog Pig Opossum Zebrafish	PLSMELMGN PLTVELMGT PLTVELMGT PLTMELMGT PLSGEFPGE EVWQEFPWE : *:	VVKQLV 15 VAKQLV 15 VAKQLV 15 VAKQLV 15 VAQRLV 15 TNHLFL 15 . : ::	C C C C *	CPDDGLEC CPDDGLEC CPDDGLEC CPDDGLEC CPDCLEC CPDEALEC CSDEALEC *.*:.***	9 9 9 9 9	QYPSSQLG 8 RYPSSQLG 8 RYPSSQLG 8 RYPSSQLG 8 RHLSSHLG 8 SYMKHELV 8 :*	
VEGF-A	Mouse Human Dog Pig Opossum Zebrafish	DIFQEYPDE DIFQEYPDE DIFQEYPDE DIFQEYPDE DIFQEYPDE DIIQEYPDE **:*****	IEYIFK 15 IEYIFK 15 IEYIFK 15 IEYIFK 15 VEFIFK 15 IEHTYI 15 :*.:	C C C C C *	CNDEALEC CNDEGLEC CNDEGLEC CNDEGLEC CNDEGLEC CNDEGLEC CNDEALEC	9 9 9 9 9	KPHQSQHIG 9 KPHQGQHIG 9 KPHQGQHIG 9 KPHQGQHIG 9 KPHQSQHIG 9 KQRVSQHNF 9 * : .**	
PIGF	Mouse Human Dog Pig Opossum Zebrafish	YILDEYPDE DVVSEYPSE DVLSEYPDE DIVSVYPSE DVATEYPGE YVEQEYPGA : **.	VSHIFS 15 VEHMFS 15 VEHMFN 15 VEHMFS 15 VEHMFS 15 VEHIYS 15 *.*::.	C C C C C *	CGDEGLHC CGDENLHC CGDENLHC CGDENLHC CGDENLHC CNDEKLAC *.** * *	9 9 9 9 9	PPNRDPHFYV J RSGDRP-SYV S HSTGRP-SYV S RSGDRP-SYV S KSGEQP-SYM S TPAERRRDYV J . *:	10)))
В	L1		Homology level	у	L2	2	Homology level	
mVEGF-B mVEGF-A mPIGF	PLSMELMGNV DIFQEYPDEI YILDEYPDEV	VKQLV EYIFK :: SHIFS	6.7% - 53.3%		CCPDDO CCNDEZ CCGDEO	GLEC	66.7% - 66.7% Figure S1	

Figure S2. Replacement of VEGF-A L1 with the VEGF-B-derived L1 does not affect VEGFR-1 binding, but inhibits VEGFR-1 activation. VEGFR-1/EpoR-BaF3 MTT assay using the full-length native and chimeric ligands, produced in 293T cells (A) or with the ligands purified from Sf21 cells (B). Assay conditions are identical to those described in the legends to Figures 2B and D, respectively. The SD bars in (A) and (B) are based on technical replicates. (C) VEGFR-1/Epo-BaF3 MTT assay using the purified ligands. The cells were incubated with the indicated ligands (6 ng/ml) for 3 days. The data represent mean values \pm SE from n=3 independent experiments. (D) A-L1^B and VEGF-A both bind to VEGFR-1. A-L1^B (500 ng/ml) or VEGF-A (500 ng/ml) were incubated (30 min) with BSA-blocked and VEGFR-1-Fc-coated protein A sepharose beads (negative control beads were not VEGFR-1-Fc coated). The beads were extensively washed and analyzed by gel electrophoresis, followed by Western blotting with the anti-5xHis antibody. Input indicates protein loading before addition of protein A sepharose beads. The data are representative of two independent experiments.



Figure S3. Pretreatment of PAE–VEGFR-2 cells with high molar excess of VEGF-B does not reduce the VEGFR-2 phosphorylation induced by B-L1^P or VEGF-A. PAE-VEGFR-2 cells were pretreated with 10 μ g/ml VEGF-B for 1 min. Thereafter, VEGF-A (100 ng/ml) or B-L1^P (either 100 ng/ml or 500 ng/ml) was added and the incubation was continued for 5 min (or 10 min, where indicated by the dotted box). The cells were lysed and analyzed by gel

electrophoresis and western blotting for P-VEGFR-2 (Y1175) or total VEGFR-2. The data are representative of two independent experiments.



Figure S4. Analysis of L1 swap chimeras between VEGF-A and PIGF. (A) VEGFR-2 tyrosine phosphorylation in BEC cells stimulated by the chimeras $B-L1^A$, $B-L1^P$, $A-L1^P$ and P-L1^A, and their parental proteins VEGF-A, VEGF-B and PIGF. VEGF-A was used at 50 ng/ml; VEGF-B, $A-L1^P$, and P-L1^A were used at 500 ng/ml; $B-L1^A$ and $B-L1^P$ – as indicated on the figure (ng/ml). (B) Dose-response effects of increasing concentrations of the native and chimeric ligands in the stimulation of endothelial VEGFR-2 tyrosine phosphorylation. Note that $A-L1^P$, P-L1^A retain receptor-binding and activating properties of the parental molecules, whereas $B-L1^A$

