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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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The PDF file includes:

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 PlGF from various species. (B) Alignment of L1 sequences from mouse VEGF-B and PlGF against VEGF-A shows substantial variation, whereas the L2 sequence is highly conserved.

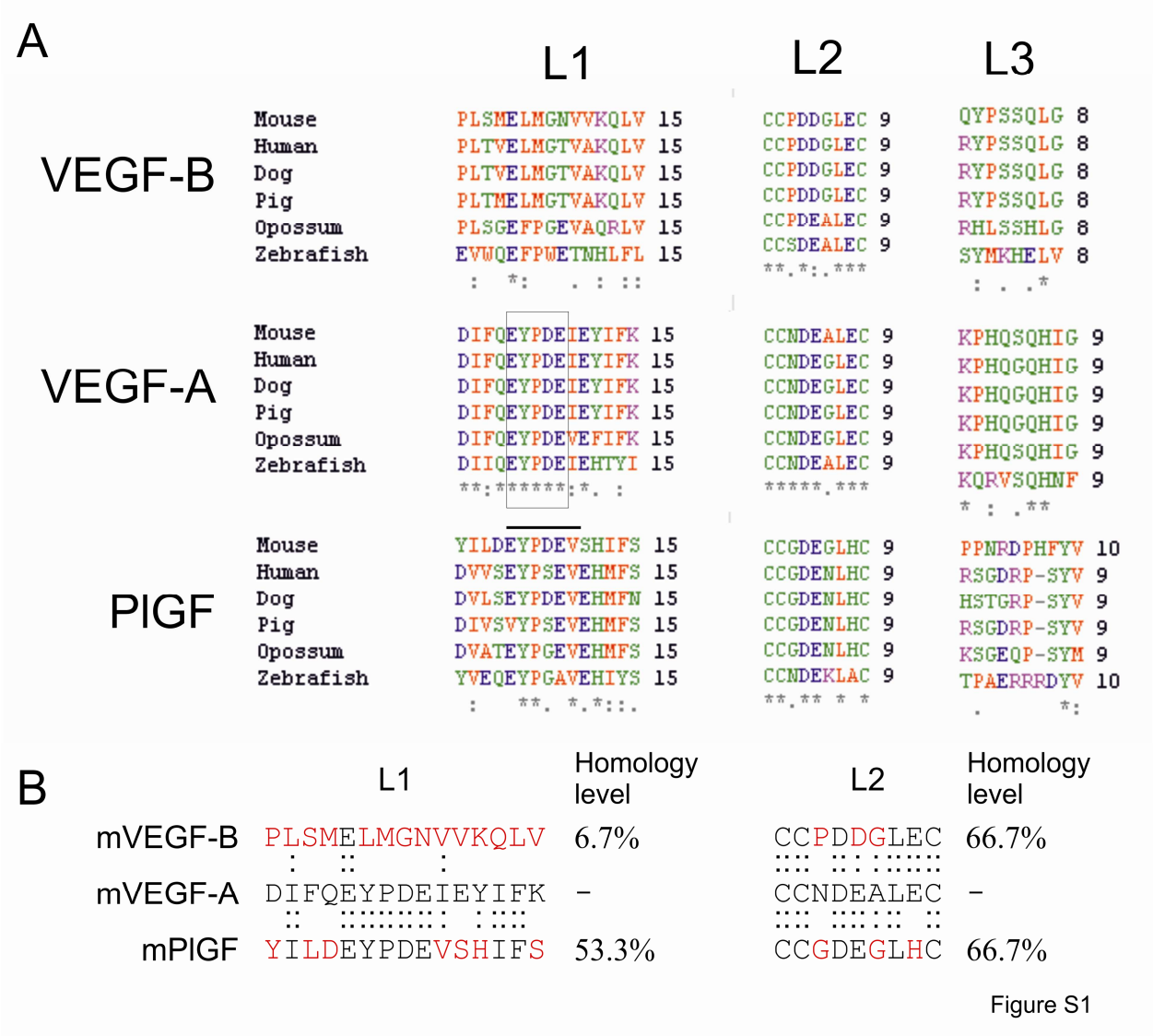

Figure S1

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 ± 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.

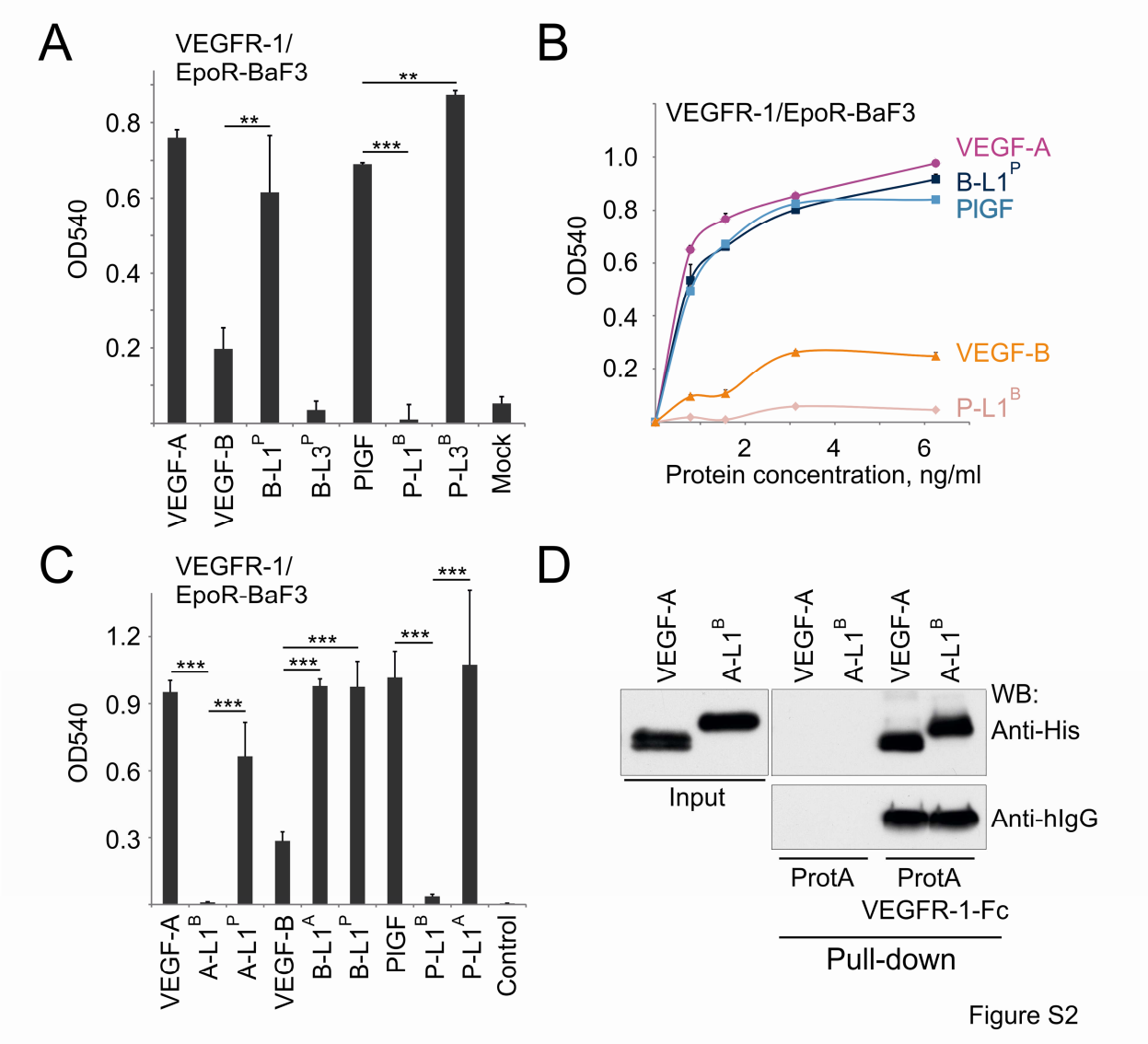

Figure S3. Pretreatment of PAE–VEGFR-2 cells with high molar excess of VEGF-B does not reduce the VEGFR-2 phosphorylation induced by B-L1^{P} or VEGF-A. PAE-VEGFR-2 cells were pretreated with 10 µg/ml VEGF-B for 1 min. Thereafter, VEGF-A (100 ng/ml) or B-L1^{P} (either 100 ng/ml or 500 ng/ml) was added 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.

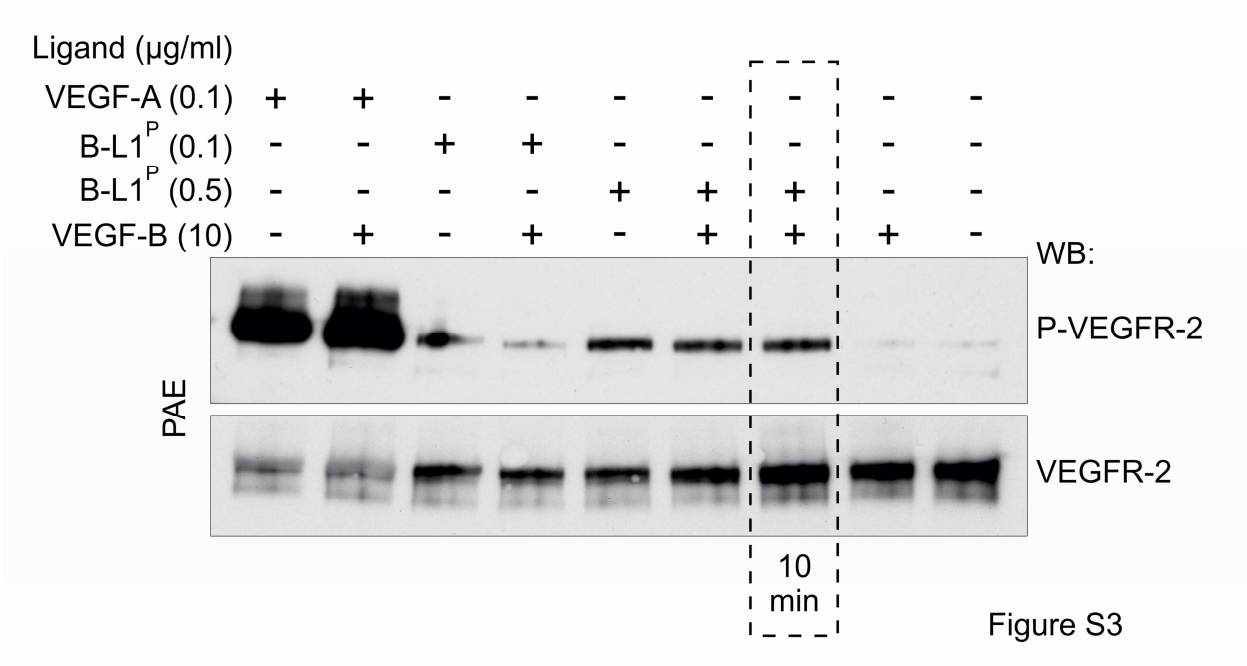

Figure S4. Analysis of L1 swap chimeras between VEGF-A and PlGF. (A) VEGFR-2 tyrosine phosphorylation in BEC cells stimulated by the chimeras B-L1^{A}, B-L1^{P}, A-L1^{P} and P-L1^{A}, and their parental proteins VEGF-A, VEGF-B and PlGF. VEGF-A was used at 50 ng/ml; VEGF-B, A-L1^{P}, and P-L1^{A} were 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} resembles B-L1^{P}. The data in (A) and (B) are representative of two independent experiments.

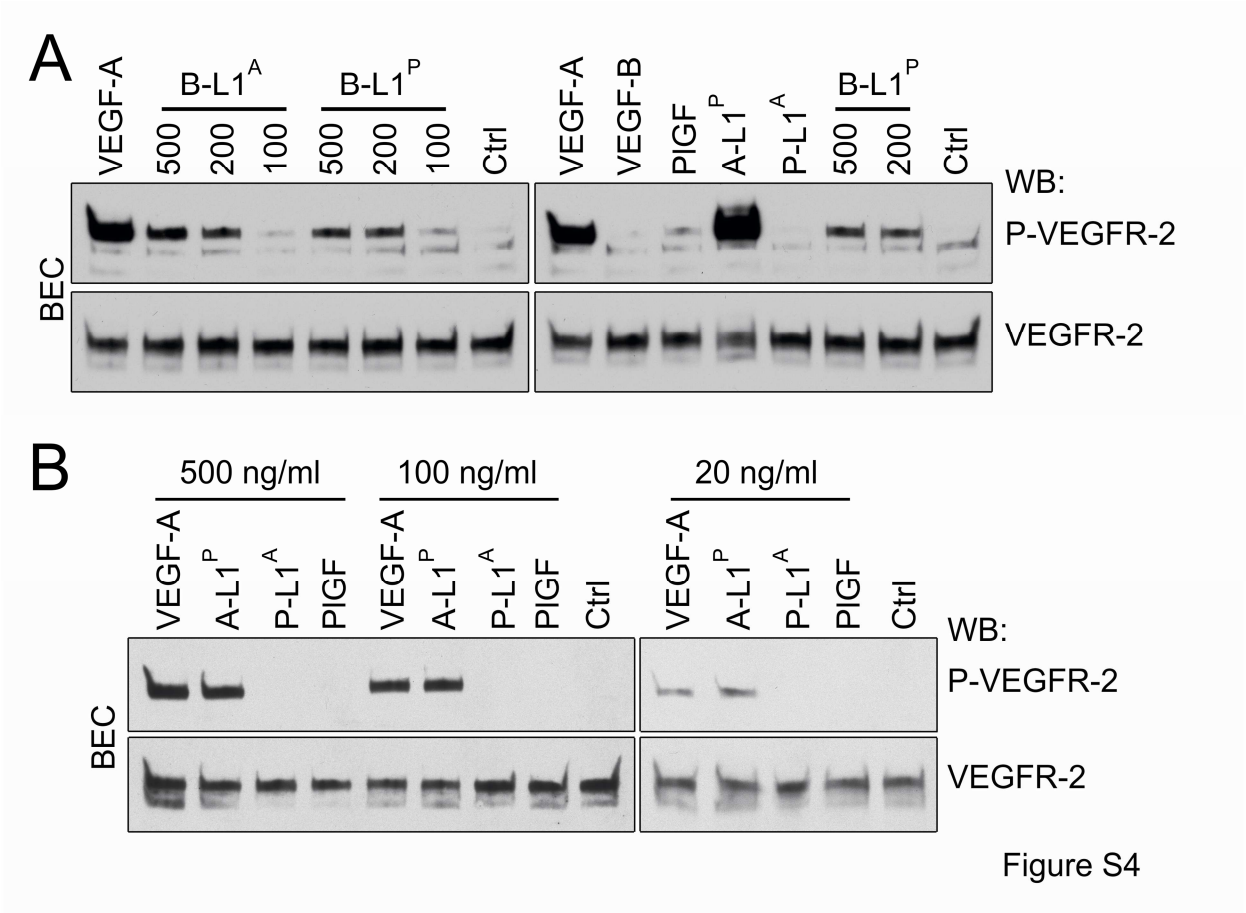
A
VEGF-A
B-L1A
B-L1P
500
200
100
Ctrl
VEGF-B
PlGF
A-L1P
P-L1A
WB:
P-VEGFR-2
VEGFR-2
BEC
B
500 ng/ml
100 ng/ml
20 ng/ml
A-L1P
P-L1A
PlGF

Figure S4