ON LINE SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE 1

Primer pairs utilized for gene expression analysis via qPCR

Gene (NCBI mRNA Accession ID)	Forward	Reverse
β-actin, ACTB (NM_001101)	5'-AGTCCCTTGCCATCCTAAAAG-3'	5'- CAATGCTATCACCTCCCCTG-3'
B-actin, ACTB (Porcine)	5'- TGACCTGATGTATGCCAAGC3'	5'- ACAGAATCCACACCAACCTC-3'
FGF2 (NM_ 002006)	5-' ACCCTCACATCAAGCTACAAC-3'	5'- AAAAGAAACACTCATCCGTAACAC -3'
VEGFA, (NM_001025366)	5'-AGTCCAACATCACCATGCAG-3'	5'-TTCCCTTTCCTCGAACTGATTT-3'
Firefly Luciferase (FW_3343311)	5'-GCTATTCTGATTACACCCGAGG-3'	5'- TCCTCTGACACATAATTCGCC-3'

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1. Endorepellin evokes concurrent internalization of α2β1 integrin and VEGFR2. *A*, representative high-magnification confocal images of human endothelial cells before or after endorepellin treatment for 10 and 20 min as indicated. The images represent z-stacks projections (60X oil objective) with xz orthogonal views (bottom panels). The cells were permeabilized with 0.1% Triton X-100 for 10 s, and immunostained with antibody against the α2 integrin subunit (green) and VEGFR2 (red). Notice the progressive co-localization of α2β1 integrin and VEGFR2 within large intracellular vesicles (white arrows). Bar ~ 250 nm. *B*, representative confocal images of endothelial cells incubated with either vehicle (control) or VEGFA (50 ng/ml) for the indicated time intervals. The cells were treated as in panel A. Bar ~ 10 μm.

Figure S2. Endorepellin binding is not due to LG3 and is heparin-independent. *A*, ligand-binding assay using LG3 as a soluble ligand for VEGFR2 as immobilized substrate. LG3 does not bind VEGFR2. (*B*, *C*) Competition binding experiments of endorepellin to either VEGFR1 (*B*) or VEGFR2 (*C*). We used a constant molar amount of endorepellin (100 nM) and increasing heparin concentrations as indicated. Notice that increasing concentrations of heparin has a minimal effect on endorepellin binding to the receptors even at 100 μg/ml heparin (~5 μM heparin based on an average mass of ~20 kDa). ELISAs were performed using a primary anti-endorepellin antibody and HRP-conjugated secondary antibody. The immune complexes were revealed with SIGMA*FAST*™ O-phenylenediamine dihydrochloride. Absorbance at 490 nm was measured in a Perkin Elmer Victor^{3TM}. The values are the mean ±SEM of three independent experiments each run in triplicates.

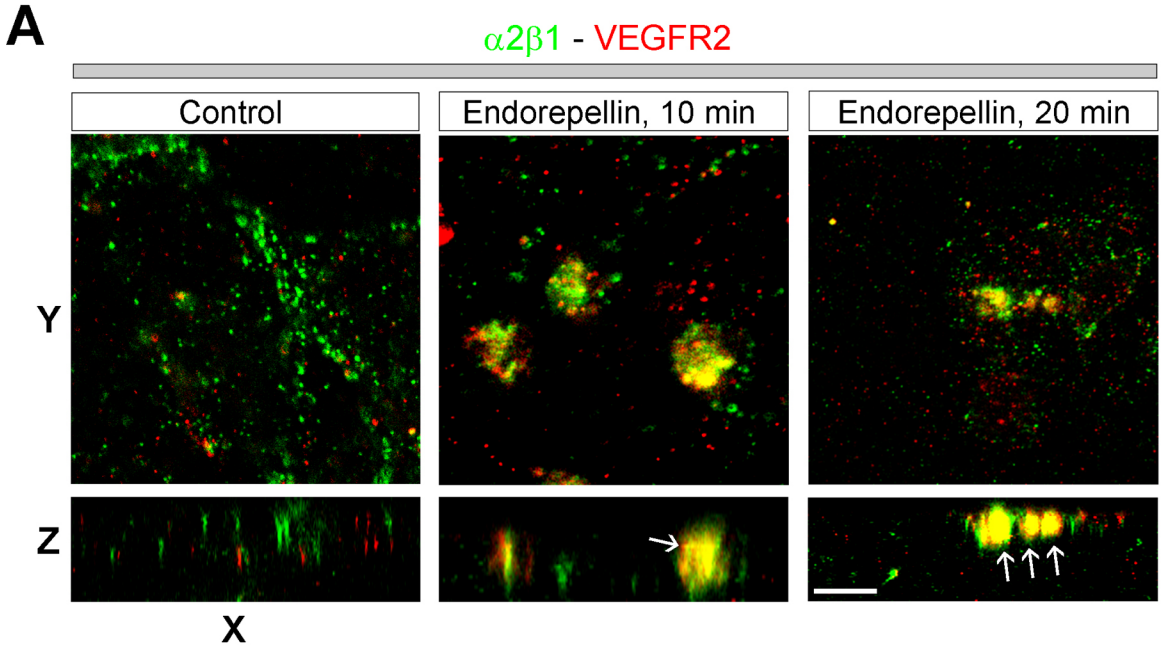
Figure S3. Endorepellin binding to VEGFR1/2 is not affected by excess VEGFE and is independent of cations. *A,B,* competition experiments using a constant molar amount of endorepellin (50 nM) and increasing VEGFE concentration as indicated. Notice that increasing concentrations of VEGFE has no effect on the bound endorepellin. These values represent the mean ±SEM of three independent experiments run in triplicate. *C,D,* ligand-binding assay using endorepellin as a soluble ligand and VEGFR1 or VEGFR2 as immobilized substrate in the absence of cations. Endorepellin binds in the absence of cations. These values represent the mean ±SEM of three independent experiments run in triplicate. Detection was as in Fig. S2.

Figure S4. IR800-endorepellin binding to VEGFR2 is specific and heparin-independent. *A*, ligand-binding assay using endorepellin as a soluble ligand and BSA as immobilized substrate. Notice a non-saturable binding to BSA even at relatively high concentrations of IR800-endorepellin. These values represent the mean ±SEM of three independent experiments run in triplicates. *B*, competition experiment using a constant concentration of

IR800-labeled endorepellin (10 nM) and increasing amounts of heparin as indicated. Notice that increasing concentrations of heparin have no effects on the bound IR800-labeled endorepellin. Even at 4.5 μg/ml (~ 225 nM estimated on an average mass of 20 kDa) there was insignificant displacement by heparin. For both *panels A* and *B*, the fluorescence of IR800-endorepellin was measured in a LI-COR Odyssey. The values are the mean ±SEM of three independent experiments run in triplicates.

Figure S5. **VEGFR2 can be co-immunoprecipitated from HUVECs using anti-α2 integrin subunit antibody only in the presence of endorepellin.** Representative co-immunoprecipitation studies using anti-α2 integrin subunit for the immunoprecipitation (IP) and either anti-VEGFR2 or anti-α2 integrin subunit antibodies for immunoblotting (IB). Notice that the VEGFR2 co-immunoprecipitates with the α2 integrin subunit only in the presence of endorepellin. The experiments were repeated three times with comparable results.

Figure S6. Evidence for *de novo* expression of VEGFR1 and VEGFR2 in PAE cells and endorepellin effects on capillary morphogenesis in PAE-VEGFR2 cells. *A*, Western immunblotting of PAE cells and their transfected counterparts using antibodies specific for either VEGFR1 or VEGFR2 as indicated. The bottom panels represent Coomassie brilliant blue-stained lower parts of the gels for loading. The gels were visualized with the Odyssey Image software (Li-COR) where the blue bands are detected as red bands. ***B,*** **c**apillary morphogenesis assays of PAE-VEGFR2 on Matrigel™. About 5 x 10^5 PAE-VEGFR2 cells were plated on low growth-factor Matrigel™ supplemented with VEGFA (50 ng/ml) and heparin (~2 μg/ml) and increasing concentrations of endorepellin. Notice the dose-dependent inhibition of capillary morphogenesis evoked by endorepellin. The assays were protracted for 4 h and then fixed with ice-cold 10% buffered formaldehyde. The photographs are representative of four independent experiments run in duplicate. Bar = 100 μm.


A
α2β1 - VEGFR2
Control
Endorepellin, 10 min
Endorepellin, 20 min
Y
Z
X

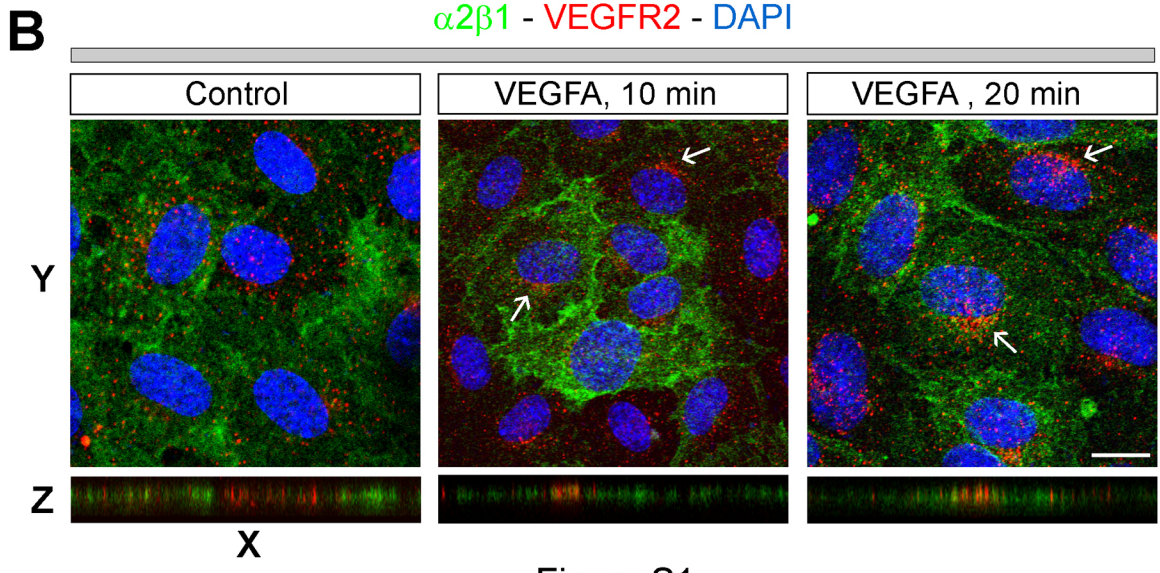
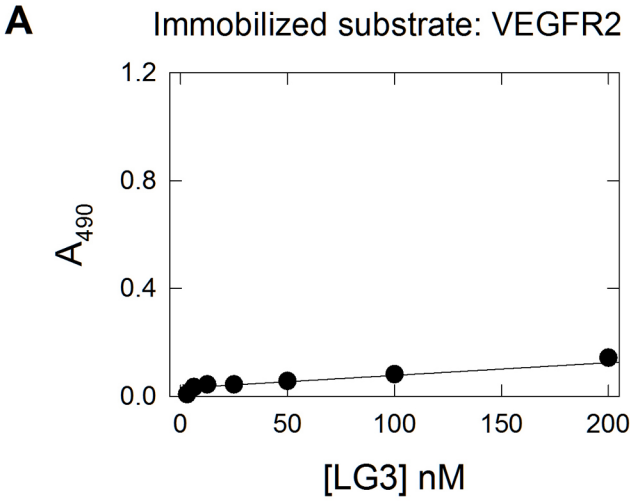
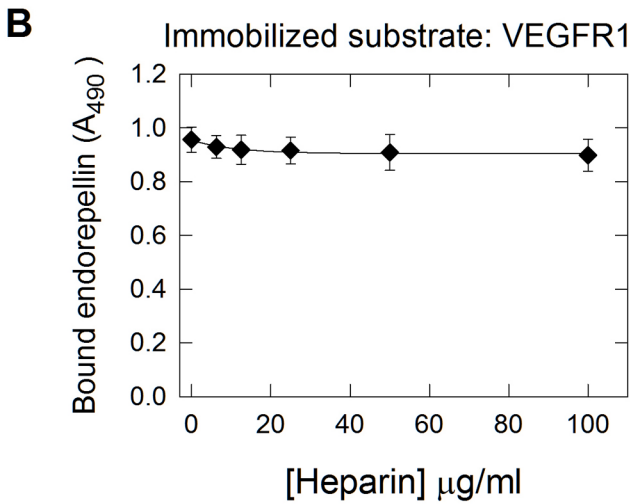

B
α2β1 - VEGFR2 - DAPI
Control
VEGFA, 10 min
VEGFA , 20 min
Y
Z
X

Figure S1


A
Immobilized substrate: VEGFR2
1.2
0.8
0.4
0.0
A490
0
50
100
150
200
[LG3] nM


B
Immobilized substrate: VEGFR1
Bound endorepellin (A490)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
[Heparin] μg/ml

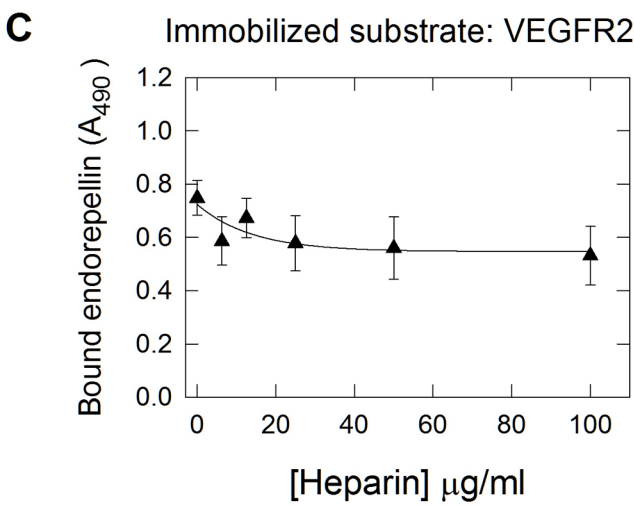

C
Immobilized substrate: VEGFR2
Bound endorepellin (A490)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
[Heparin] μg/ml

Figure S2

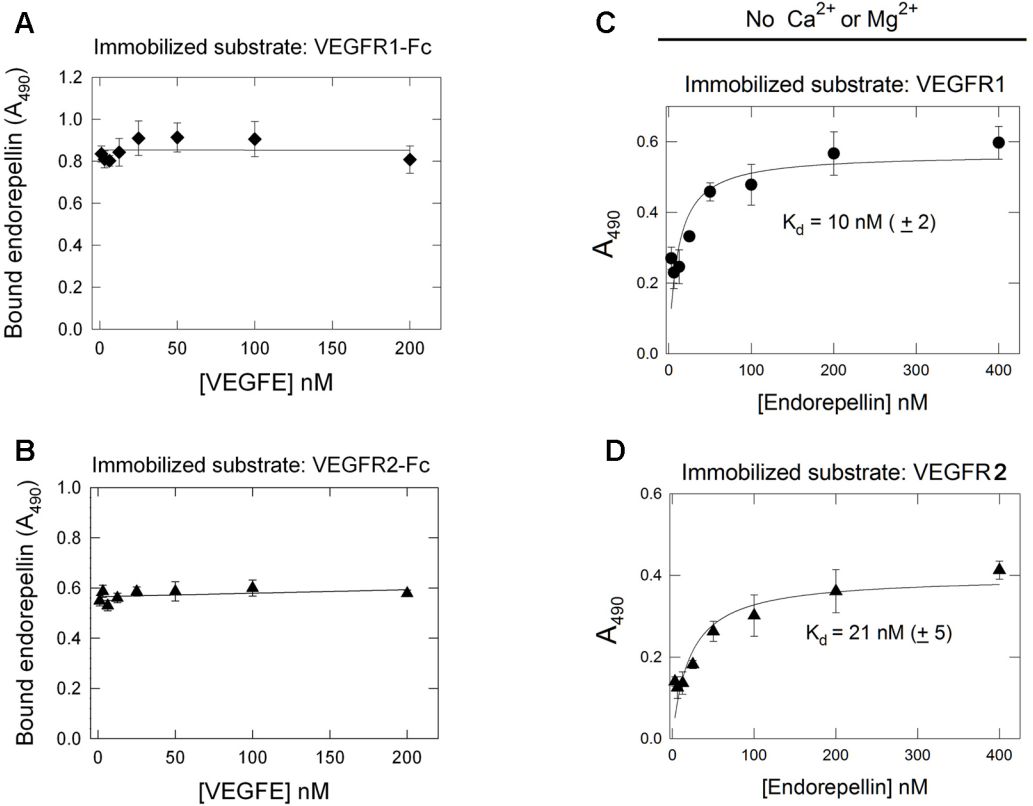
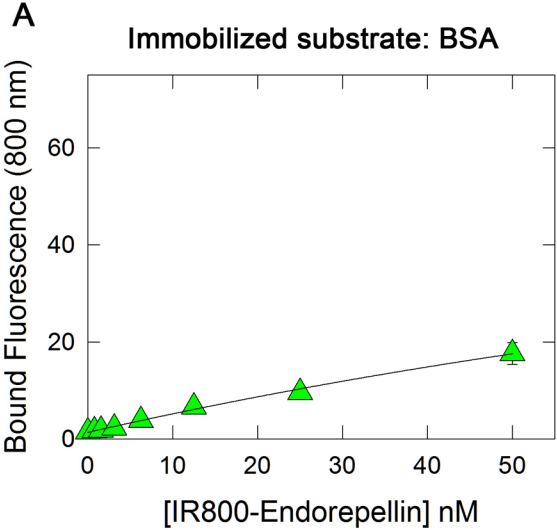

A
Immobilized substrate: VEGFR1-Fc
Bound endorepellin (A490)
[VEGFE] nM
B
Immobilized substrate: VEGFR2-Fc
Bound endorepellin (A490)
[VEGFE] nM
C
No Ca2+ or Mg2+
Immobilized substrate: VEGFR1
A490
Kd = 10 nM (± 2)
[Endorepellin] nM
D
Immobilized substrate: VEGFR2
A490
Kd = 21 nM (± 5)
[Endorepellin] nM

Figure S3


A
Immobilized substrate: BSA
Bound Fluorescence (800 nm)
0
20
40
60
0
10
20
30
40
50
[IR800-Endorepellin] nM

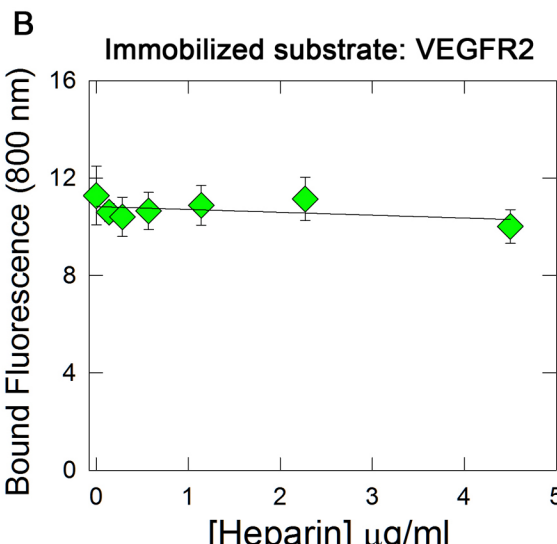

B
Immobilized substrate: VEGFR2
Bound Fluorescence (800 nm)
0
4
8
12
16
0
1
2
3
4
5
[Heparin] μg/ml

Figure S4

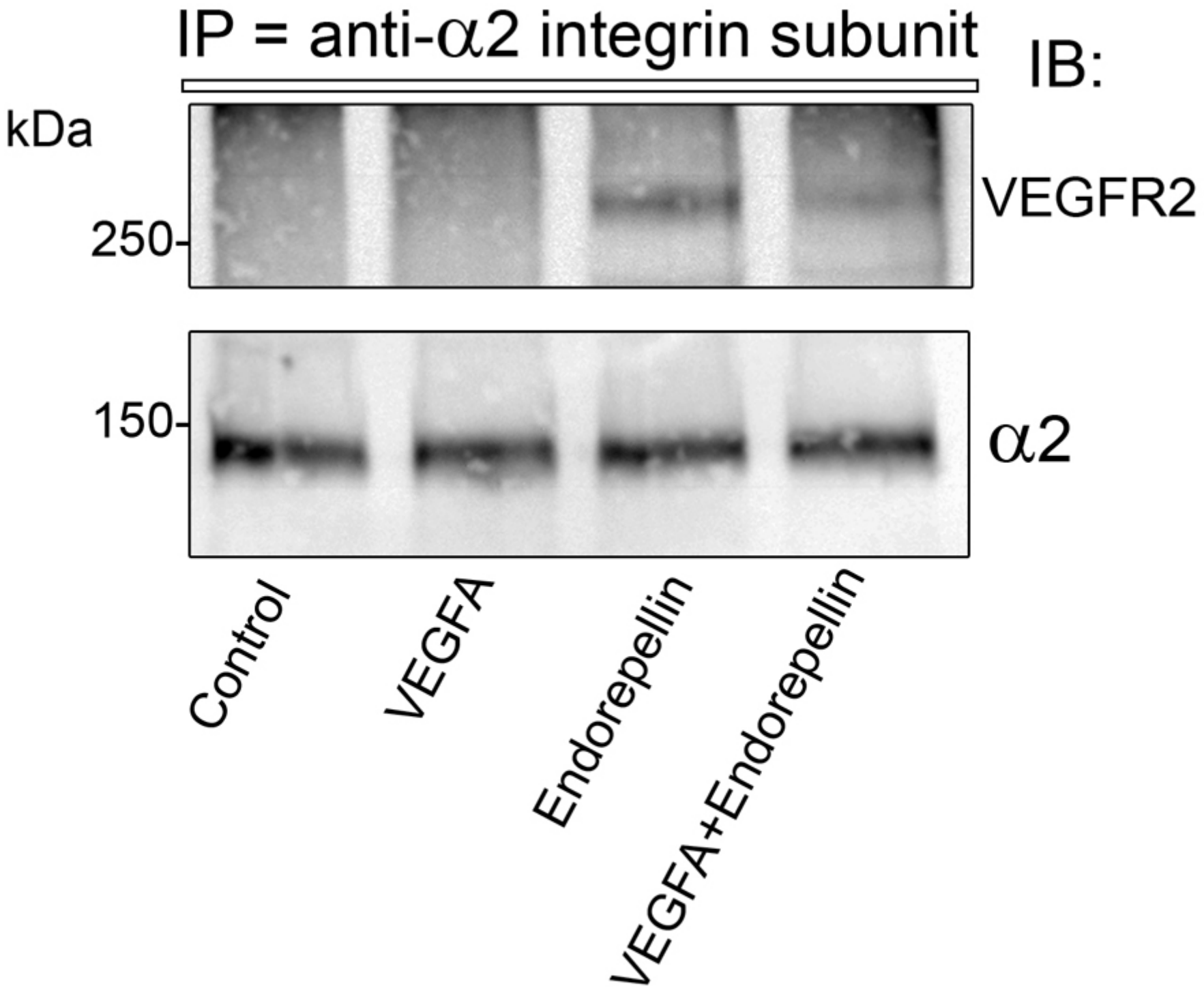

IP = anti-α2 integrin subunit
IB:
kDa
250-
VEGFR2
150-
α2
Control
VEGFA
Endorepellin
VEGFA+Endorepellin

Figure S5

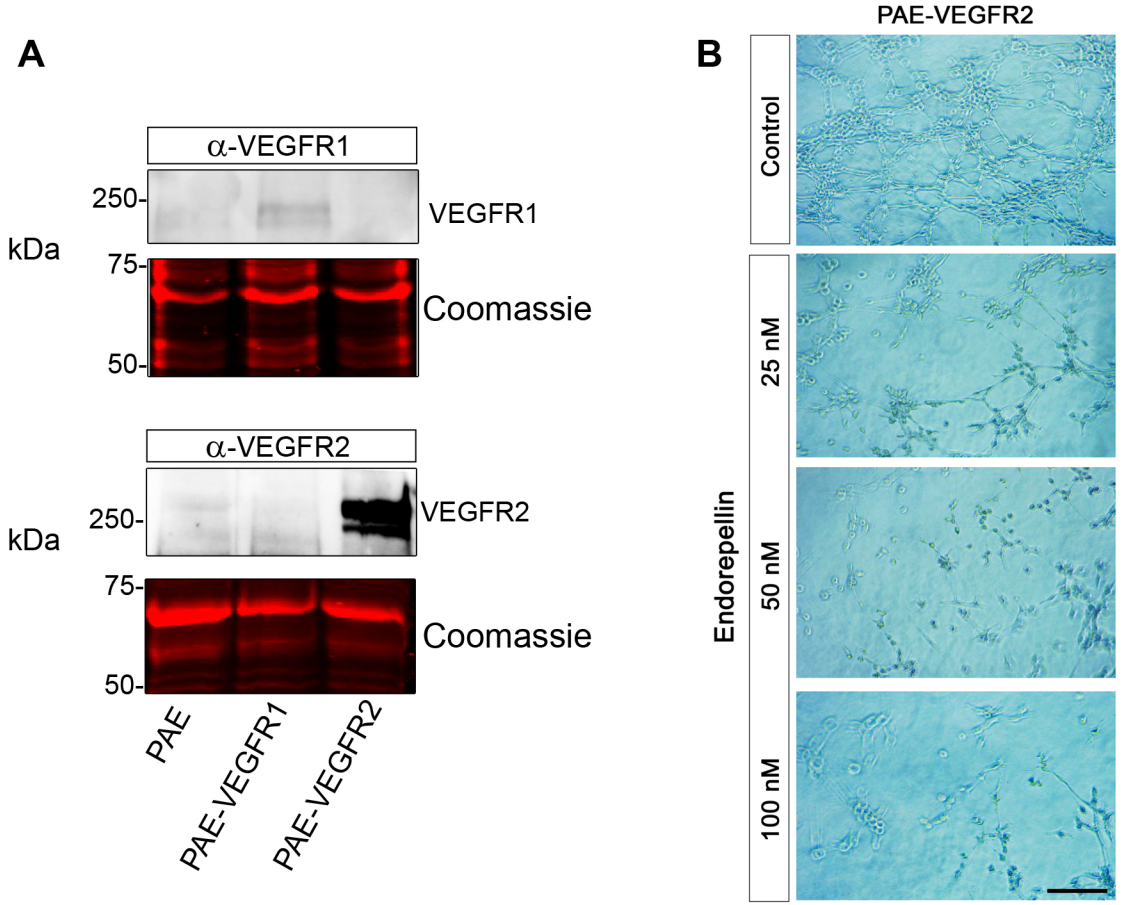

A
α-VEGFR1
250-
kDa
75-
50-
VEGFR1
Coomassie
α-VEGFR2
250-
kDa
75-
50-
VEGFR2
Coomassie
PAE
PAE-VEGFR1
PAE-VEGFR2
B
PAE-VEGFR2
Control
Endorepellin
25 nM
50 nM
100 nM

Figure S6