Supplemental Materials

Molecular Biology of the Cell

Fearnley et al.

SUPPLEMENTARY FIGURE LEGENDS

SUPPLEMENTARY FIGURE S1**:** Statistical analysis of VEGF-A isoform-specific signal transduction events. Ligand-stimulated endothelial cells were subjected to immunoblotting to monitor levels of (A, B) VEGFR2-pY1175, (C, D) ERK1/2-pT202/pY204, (E, F) p38-pT180/pY182 or (G, H) Akt-pS473 levels upon (A, C, E, G) VEGF-A_{165} or (B, D, F, H) VEGF-A_{121} titration. Error bars indicate $\pm$SEM (n≥4). p<0.05 (*), p<0.01 (**), p<0.005 (***), p<0.0001 (****).

SUPPLEMENTARY FIGURE S2. NRP1 is required for ERK1/2-dependent ATF-2 phosphorylation. (A) NRP1 depletion in endothelial cells followed by 1.25 nM VEGF-A_{165} (165) or VEGF-A_{121} (121) stimulation as indicated. (B, C) Effects of NRP1 depletion on 1.25 nM VEGF-A_{165} or VEGF-A_{121}-stimulated intracellular signaling monitored by immunoblotting for (B) ATF-2-pT71 or (C) ERK1/2-pT202/pY204. Error bars indicate $\pm$SEM (n=3). p<0.05 (*), p<0.01 (**), p<0.005 (***).

SUPPLEMENTARY FIGURE S3. VEGF-A isoform-specific regulation of endothelial cell outcomes. (A-D) Endothelial cells were seeded into various cellular assays and subjected to 0, 0.025, 0.25 or 1.25 nM VEGF-A_{165} or VEGF-A_{121} prior to assessment of endothelial cell (A-B) migration or (C-D) tubulogenesis. Error bars indicate $\pm$SEM (n=4). (E) *Ex vivo* angiogenesis using mouse tissues. Aortic ring slices were seeded into a collagen gel (see Materials and methods) prior to stimulation with 0, 0.025, 0.25 or 1.25 nM VEGF-A_{165} or VEGF-A_{121}. (F) Quantification of *ex vivo* aortic endothelial sprouting. Bar, 200 μm, 400 μm and 1000 μm respectively. Error bars indicate $\pm$SEM (n=3). p<0.05 (*), p<0.01 (**), p<0.005 (***), p<0.0001 (****).

SUPPLEMENTARY FIGURE S4. ATF-2 is required for endothelial cell proliferation. Endothelial cells were treated with control, scrambled or ATF-2-specific siRNA duplexes and stimulated with 0.25 nM VEGF-A_{165}, VEGF-A_{121} and grown in full growth media. Endothelial cell proliferation was assessed using a BrdU incorporation ELISA (see Materials and methods). Error bars indicate $\pm$SEM (n=3). p<0.05 (*), p<0.0001 (****).

SUPPLEMENTARY FIGURE S5. VCAM-1 requirement in endothelial tubulogenesis. Endothelial cells treated with control, scrambled or VCAM-1-specific siRNA duplexes were seeded onto a bed of confluent human fibroblasts and stimulated with 0.25 nM VEGF-A_{165} or VEGF-A_{121} over 7 days. (A) Endothelial tubules were stained for the endothelial-specific antigen, PECAM-1 using mouse anti-PECAM-1 antibody followed by anti-mouse AlexaFluor594 conjugate (see Materials and methods). Stained cells were visualized by fluorescence microscopy. (B) Quantification of endothelial tubulogenesis. Bar, 1000 μm. Error bars indicate ±SEM (n=3). NS; non-significant.

Figure S1

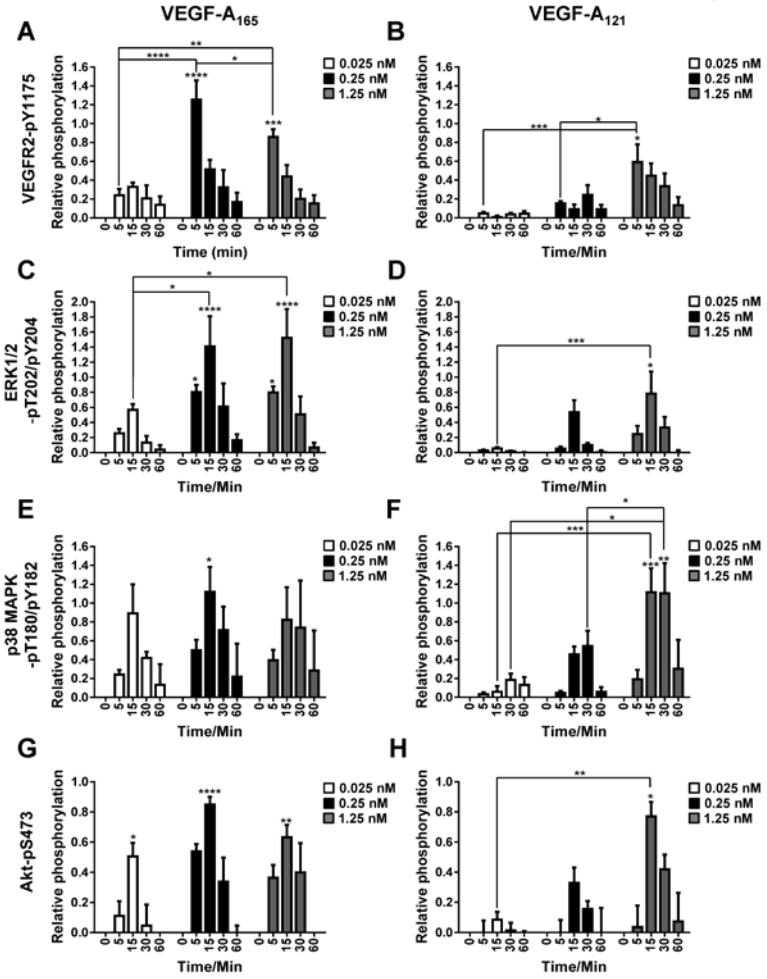

VEGF-A165
VEGF-A121
A
B
C
D
E
F
G
H
VEGFR2-pY1175
ERK1/2
-pT202/pY204
p38 MAPK
-pT180/pY182
Akt-pS473
Relative phosphorylation
Time (min)
Time/Min
0.025 nM
0.25 nM
1.25 nM

Figure S2

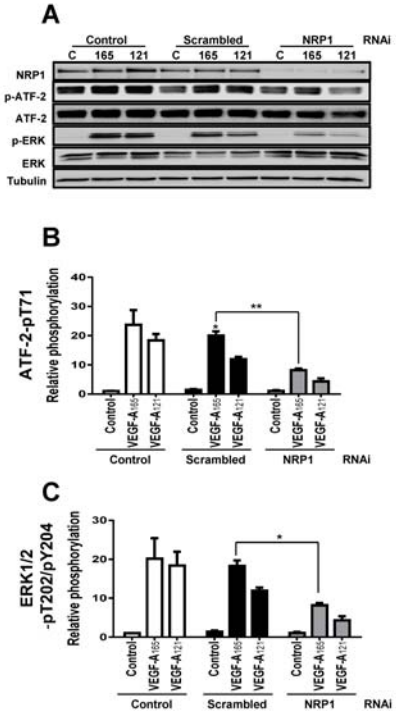

A
Control
Scrambled
NRP1
RNAi
C 165 121 C 165 121 C 165 121
NRP1
p-ATF-2
ATF-2
p-ERK
ERK
Tubulin
B
ATF-2-pT71
Relative phosphorylation
40
30
20
10
0
**
Control
VEGF-A165
VEGF-A121
Control
Scrambled
NRP1
RNAi
C
ERK1/2
-pT202/pY204
Relative phosphorylation
30
20
10
0
*
Control
VEGF-A165
VEGF-A121
Control
Scrambled
NRP1
RNAi

Figure S3

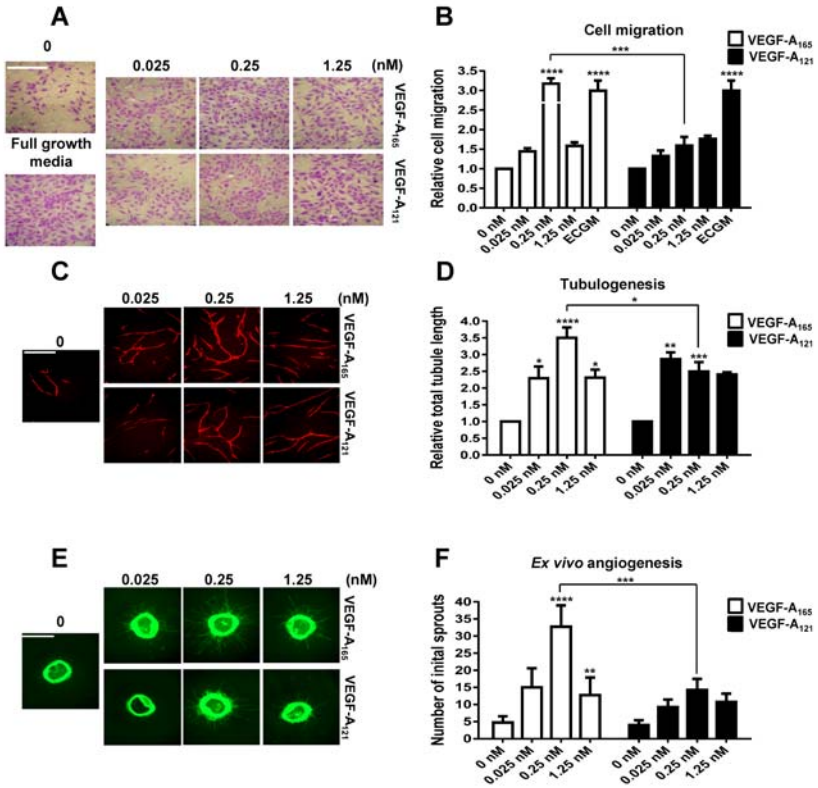

A
0
0.025
0.25
1.25
(nM)
Full growth media
VEGF-A165
VEGF-A121
B
Cell migration
VEGF-A165
VEGF-A121
Relative cell migration
0 nM
0.025 nM
0.25 nM
1.25 nM
ECGM
C
0.025
0.25
1.25
(nM)
0
VEGF-A165
VEGF-A121
D
Tubulogenesis
VEGF-A165
VEGF-A121
Relative total tubule length
0 nM
0.025 nM
0.25 nM
1.25 nM
E
0.025
0.25
1.25
(nM)
0
VEGF-A165
VEGF-A121
F
Ex vivo angiogenesis
VEGF-A165
VEGF-A121
Number of inital sprouts
0 nM
0.025 nM
0.25 nM
1.25 nM

Figure S4

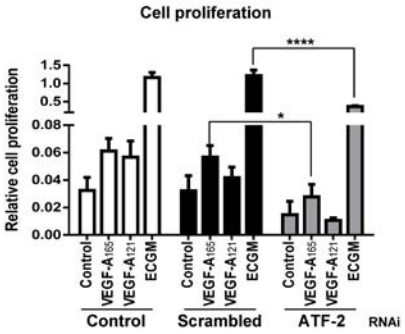
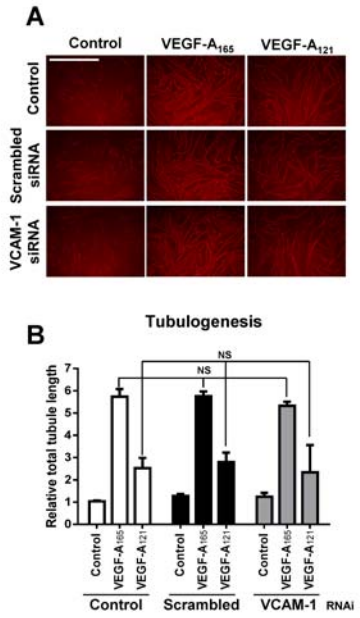

Cell proliferation
Relative cell proliferation
1.5
1.0
0.5
0.08
0.06
0.04
0.02
0.00
****
*
Control
VEGF-A165
VEGF-A121
ECGM
Control
VEGF-A165
VEGF-A121
ECGM
Control
VEGF-A165
VEGF-A121
ECGM
Control
Scrambled
ATF-2
RNAi

Figure S5


A
Control
VEGF-A165
VEGF-A121
Control
Scrambled siRNA
VCAM-1 siRNA
B
Tubulogenesis
Relative total tubule length
NS
NS
Control
VEGF-A165
VEGF-A121
Control
Scrambled
VCAM-1
RNAi