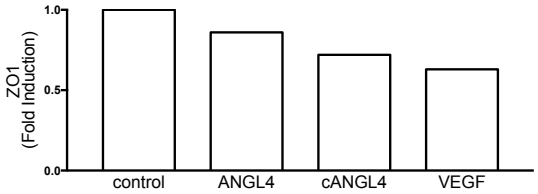
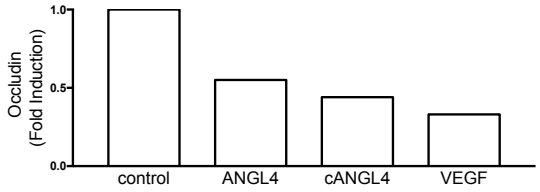
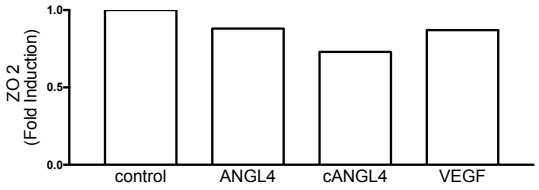
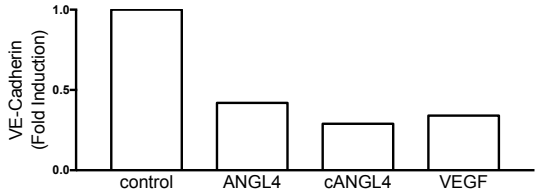
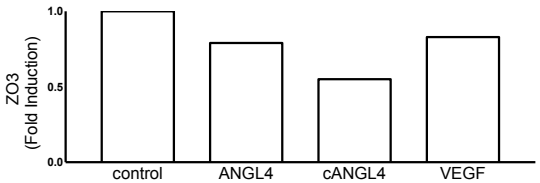
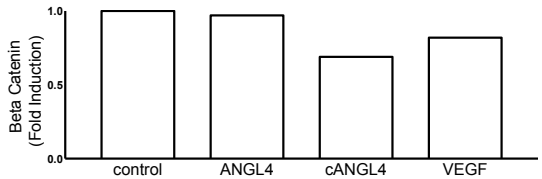

ZO1
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

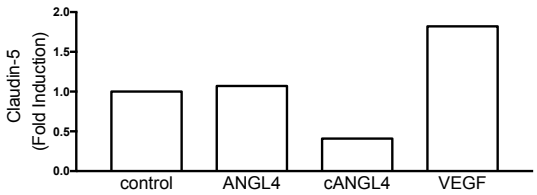

Occludin
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

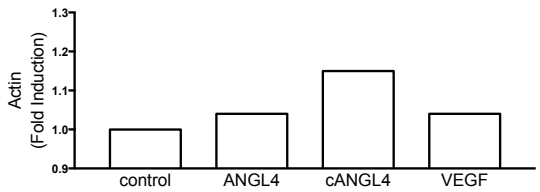

ZO 2
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

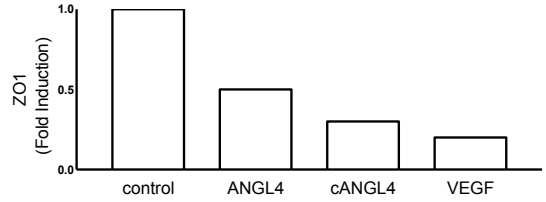

VE-Cadherin
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

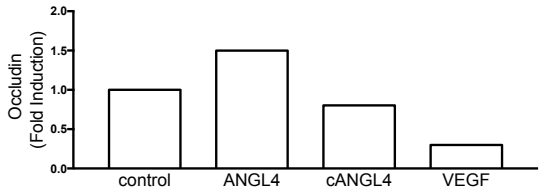

ZO3
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

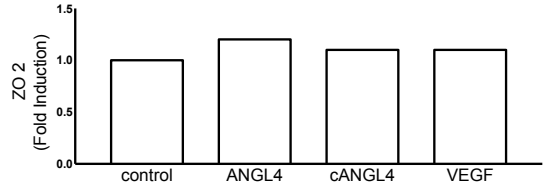

Beta Catenin
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

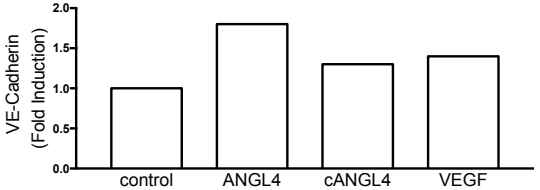

Claudin-5
(Fold Induction)
2.0
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

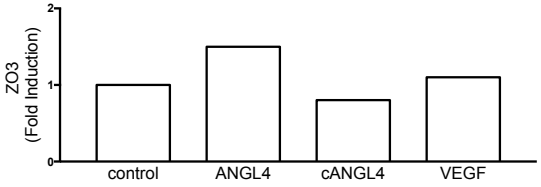

Actin
(Fold Induction)
1.3
1.2
1.1
1.0
0.9
control
ANGL4
cANGL4
VEGF

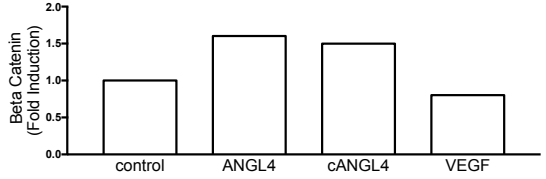

ZO1
(Fold Induction)
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

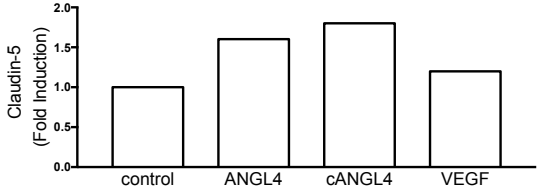

Occludin
(Fold Induction)
2.0
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

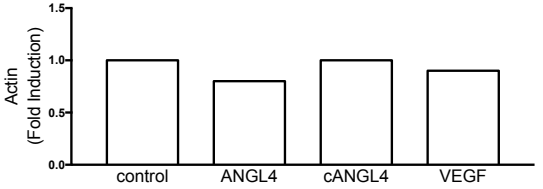

ZO 2
(Fold Induction)
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF


VE-Cadherin
(Fold Induction)
2.0
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF


ZO3
(Fold Induction)
2
1
0
control
ANGL4
cANGL4
VEGF


Beta Catenin
(Fold Induction)
2.0
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF


Claudin-5
(Fold Induction)
2.0
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF


Actin
(Fold Induction)
1.5
1.0
0.5
0.0
control
ANGL4
cANGL4
VEGF

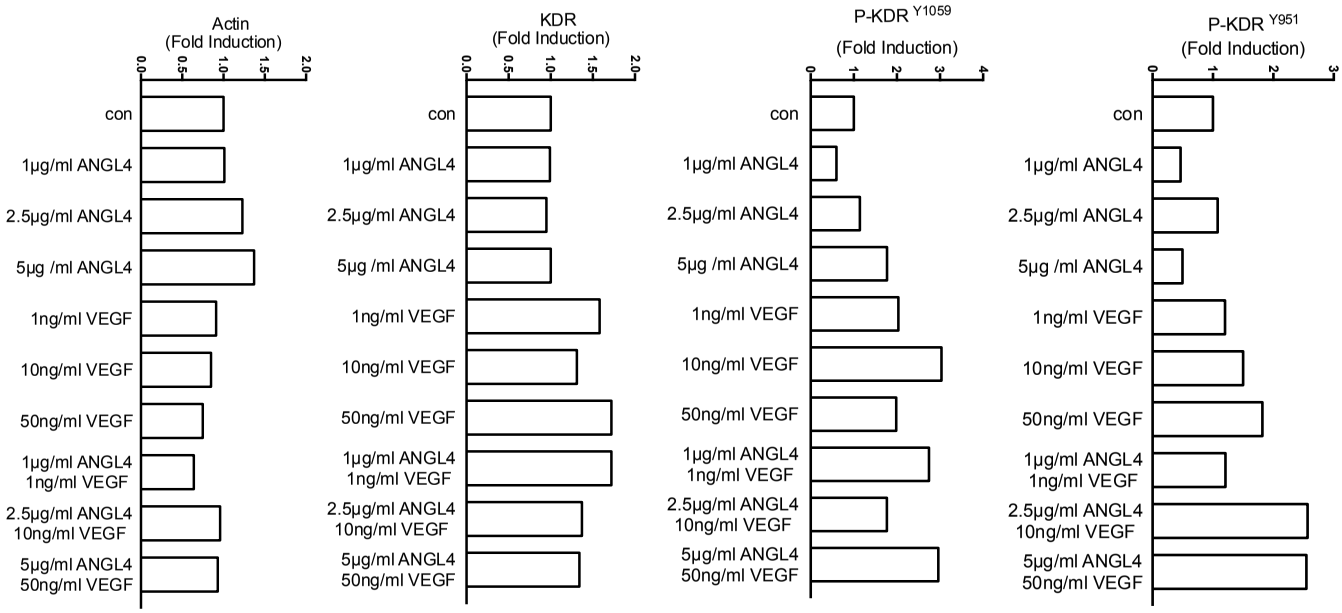
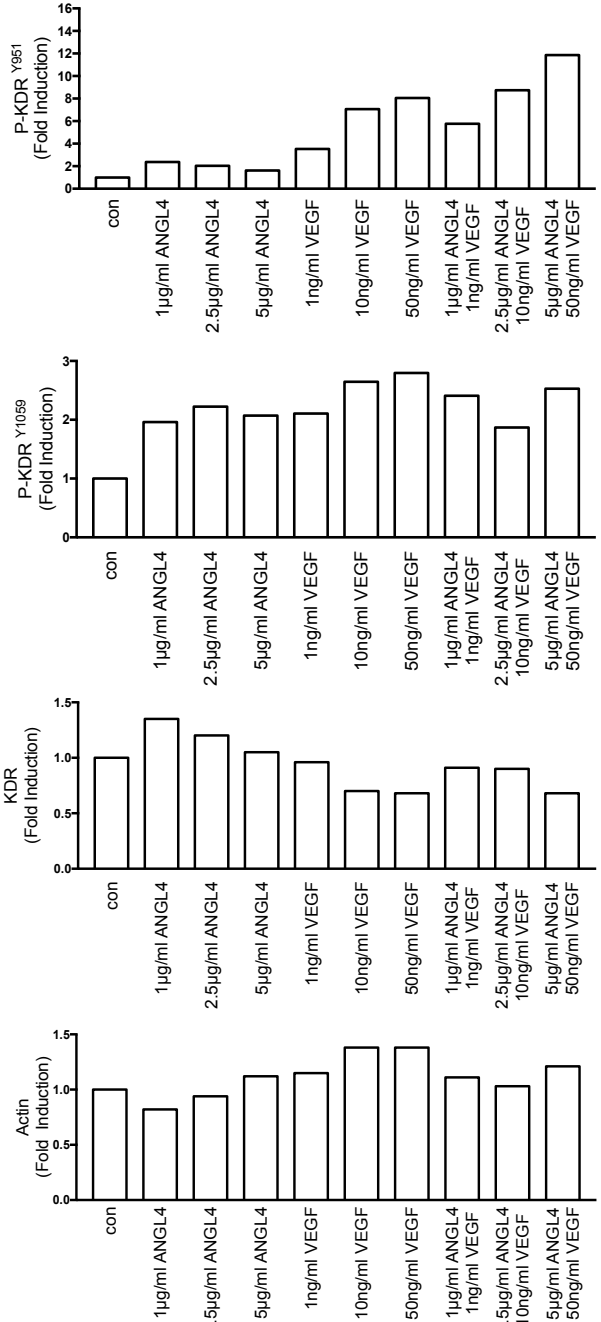
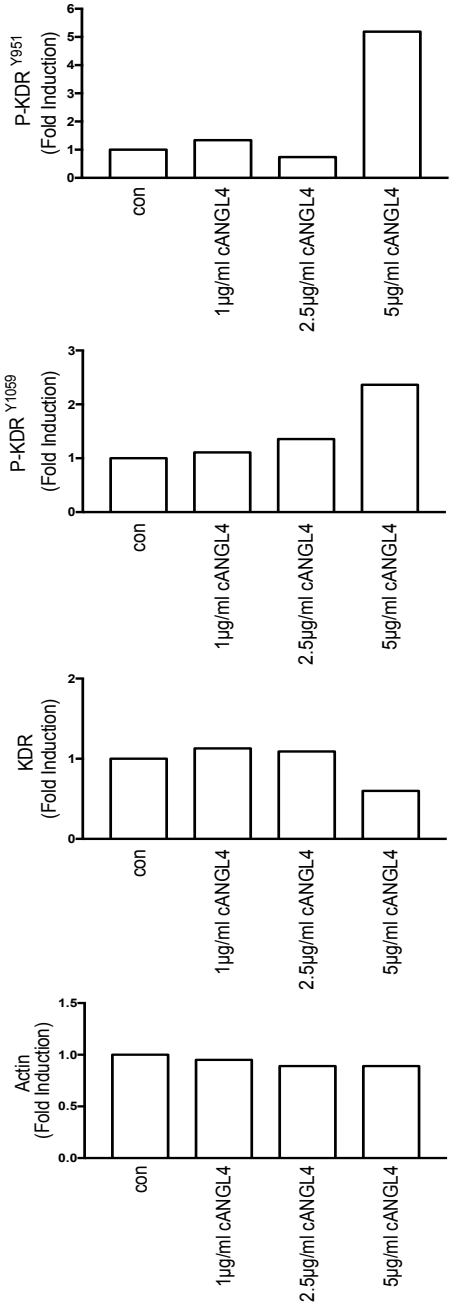

Actin
(Fold Induction)
0.0
0.5
1.0
1.5
2.0
con
1μg/ml ANGL4
2.5μg/ml ANGL4
5μg /ml ANGL4
1ng/ml VEGF
10ng/ml VEGF
50ng/ml VEGF
1μg/ml ANGL4
1ng/ml VEGF
2.5μg/ml ANGL4
10ng/ml VEGF
5μg/ml ANGL4
50ng/ml VEGF
KDR
(Fold Induction)
0.0
0.5
1.0
1.5
2.0
con
1μg/ml ANGL4
2.5μg/ml ANGL4
5μg /ml ANGL4
1ng/ml VEGF
10ng/ml VEGF
50ng/ml VEGF
1μg/ml ANGL4
1ng/ml VEGF
2.5μg/ml ANGL4
10ng/ml VEGF
5μg/ml ANGL4
50ng/ml VEGF
P-KDR Y1059
(Fold Induction)
0
1
2
3
4
con
1μg/ml ANGL4
2.5μg/ml ANGL4
5μg /ml ANGL4
1ng/ml VEGF
10ng/ml VEGF
50ng/ml VEGF
1μg/ml ANGL4
1ng/ml VEGF
2.5μg/ml ANGL4
10ng/ml VEGF
5μg/ml ANGL4
50ng/ml VEGF
P-KDR Y951
(Fold Induction)
0
1
2
3
con
1μg/ml ANGL4
2.5μg/ml ANGL4
5μg /ml ANGL4
1ng/ml VEGF
10ng/ml VEGF
50ng/ml VEGF
1μg/ml ANGL4
1ng/ml VEGF
2.5μg/ml ANGL4
10ng/ml VEGF
5μg/ml ANGL4
50ng/ml VEGF

Fig. 3 Suppl


P-KDR Y951 (Fold Induction)
P-KDR Y1059 (Fold Induction)
KDR (Fold Induction)
Actin (Fold Induction)
con
1µg/ml ANGL4
2.5µg/ml ANGL4
5µg/ml ANGL4
1ng/ml VEGF
10ng/ml VEGF
50ng/ml VEGF
1µg/ml ANGL4 1ng/ml VEGF
2.5µg/ml ANGL4 10ng/ml VEGF
5µg/ml ANGL4 50ng/ml VEGF


P-KDR Y951
(Fold Induction)
0
1
2
3
4
5
6
con
1µg/ml cANGL4
2.5µg/ml cANGL4
5µg/ml cANGL4
P-KDR Y1059
(Fold Induction)
0
1
2
3
con
1µg/ml cANGL4
2.5µg/ml cANGL4
5µg/ml cANGL4
KDR
(Fold Induction)
0
1
2
con
1µg/ml cANGL4
2.5µg/ml cANGL4
5µg/ml cANGL4
Actin
(Fold Induction)
0.0
0.5
1.0
1.5
con
1µg/ml cANGL4
2.5µg/ml cANGL4
5µg/ml cANGL4

Supplemental Fig. 1. Quantification of Western blots of Figure 2D, showing the changes in AJ and TJ protein levels upon treatment of HUVEC with rhANGPTL4 (5 μg/ml), rhcANGPTL4 (5 μg/ml), or rhVEGF (50 ng/ml), for 24 hr. Levels of total actin were used for normalization.

Supplemental Fig. 2. Quantification of Western blots of Figure 2E, showing the changes in AJ and TJ protein levels upon treatment of hREC with rhANGPTL4 (5 μg/ml), rhcANGPTL4 (5 μg/ml), or rhVEGF (50 ng/ml), for 24 hr. Levels of total actin were used for normalization.

Supplemental Fig. 3. Quantification of Western blots of Figure 5D, showing KDR Phosphorylation on Tyr951 and Tyr1059 upon treatment of HUVECs with different doses of rhANGPTL4 (μg/ml), rhVEGFA (ng/ml), or both. Levels of total KDR were used for normalization.

Supplemental Fig. 4. Quantification of Western blots of Figure 5E, showing KDR Phosphorylation on Tyr951 and Tyr1059 upon treatment of hREC with different doses of rhANGPTL4 (μg/ml), rhVEGFA (ng/ml), or both. Levels of total KDR were used for normalization.

Supplemental Fig. 5. Quantification of Western blots of Figure 5H, showing KDR phosphorylation in Tyr951 and Tyr1059 upon treatment of HUVECs with rhcANGPTL4 (μg/ml). Levels of total KDR were used for normalization.

Supplemental Table 1. Characteristics of DME patients from whom aqueous samples were collected for EC permeability assays.

Patient	Age	Sex	*Phakic Status	OCT Central Subfield Thickness (μm)	Best Corrected Visual Acuity (LogMAR)
D1	63	F	P	276	0.301
D2	52	M	PP	240	0.398
D3	56	M	P	290	0.301
D4	55	F	P	338	0.477
D5	67	F	PP	475	0.602
D6	57	M	P	365	0.796
D7	51	M	P	355	0.796
D8	67	M	PP	435	1.398
D9	50	F	P	359	0.097
D10	67	F	P	521	0.602

*Phakic status at the time of sample collection. P, phakic; PP, pseudophakic. DME, diabetic macular edema. OCT, optical coherence tomography. All patients had not been treated with anti-VEGF therapy, steroid, or laser photocoagulation for at least 3 months prior to sample collection.

Supplemental Table 2. Characteristics of DME patients from whom aqueous samples were collected for ELISA analysis for VEGF and ANGPTL4.

Patient Characteristic	Control (N=36)	DME UnTx (N=6)	DME No recent Tx (N=13)	DME Recent Tx (N=6)	*P*
Mean Age in Years ± SD	67.8 ± 11.3	55.3 ± 4.7	61.5 ± 7.5	58.2 ± 5.1	0.0081
Males – no. (%)	17 (47.2)	3 (50.0)	9 (69.2)	5 (83.3)	0.27
Pseudophakic – no. (%)	0 (0)	0 (0)	6 (46.2)	1 (16.7)	0.0001
Prior vitrectomy – no. (%)	0 (0)	0 (0)	2 (15.4)	0 (0)	0.054
Prior laser – no. (%)	0 (0)	0 (0)	8 (61.5)	2 (33.3)	<0.0001
Diabetes mellitus – no. (%)	0 (0)	6 (100)	13 (100)	6 (100)	<0.0001
CVD* – no. (%)	28 (77.8)	6 (100)	13 (100)	6 (100)	0.094

DME, diabetic macular edema. CVD, cardiovascular disease. UnTx, untreated. Tx, treatment
DME UnTx, DME patients without any prior history of anti-VEGF therapy in sample eye
DME No recent Tx, DME patients who have not received anti-VEGF therapy for 12 weeks or longer in sample eye.
DME Recent Tx, DME patients treated with their first anti-VEGF injection in sample eye within 6 weeks of sample collection.
*Includes any patient with a history of hypertension, hypercholesterolemia, coronary artery disease, or cerebral vascular accident.

Supplemental Table 3: Kinetics of the binding of ANGPTL4 and ANGPTL4 to NRP1 and NRP2. The association (ka) and dissociation (kd) rate constants and the equilibrium association (KA) and dissociation (KD) constants were calculated with the BIAeval 3.2 evaluation software, using different concentrations of analytes onto the NRP immobilized sensor chip.

Ligand	Analyte	ka (1/Ms)	kd (1/s)	KA (1/M)	KD (M)	Chi2
NRP1	ANGPTL4	8.94×10^{5}	2.24×10^{-3}	3.99×10^{8}	2.50×10^{-9}	1.81
NRP1	VEGF	4.58×10^{5}	3.05×10^{-3}	1.50×10^{8}	6.65×10^{-9}	0.194
NRP1	cANGPTL4	7.66×10^{3}	2.29×10^{-4}	3.34×10^{7}	2.99×10^{-8}	1.01
NRP2	ANGPTL4	4.53×10^{5}	3.20×10^{-3}	1.42×10^{8}	7.07×10^{-9}	1.41
NRP2	VEGF	3.40×10^{5}	2.10×10^{-3}	1.62×10^{8}	6.18×10^{-9}	0.151
NRP2	cANGPTL4	5.22×10^{5}	4.30×10^{-3}	1.21×10^{8}	8.24×10^{-9}	0.753