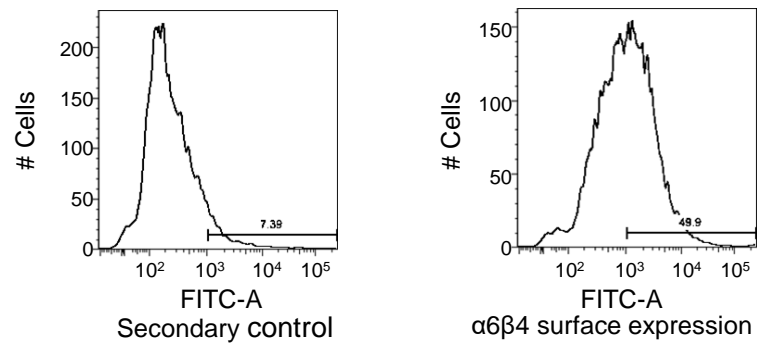


Supplementary Figure S1. Integrin $\alpha 6\beta 4$ is primarily expressed in the arterial vessels of adult mouse retina. (A–B) Integrin $\alpha 6\beta 4$ is expressed in a subset of vessels of adult mouse retina. (A) Immunofluorescence staining of whole mount mouse retina for Collagen IV and $\beta 4$ -integrin subunit. (B) Enlarged image of one petal of retina showing restricted $\beta 4$ expression. (C) Immunofluorescence staining of whole mount mouse retina for α -smooth muscle actin (α -SMA) and $\beta 4$ -integrin subunit. Arrows show examples of α -SMA / $\beta 4$ co-localization in the arterial branch. of Scale = 1 mm.



Supplementary Figure S2. Integrin $\alpha 6\beta 4$ is expressed on the surface of human umbilical endothelial cells (HUVECs). HUVECS were immunostained with antibodies to the integrin $\beta 4$ subunit and analyzed by flow cytometry.

Table S1. Antibodies.

Protein	Antibody/Application*	Manufacturer
CD31 [JC70A, M0823]	Mouse anti-human & mouse/IFM	DAKO
CD31[M-20]	Rabbit anti-mouse /IFM	Santa Cruz
FITC-conjugated -SMA [1A4, A#5228]	Mouse anti-human/mouse/IFM	Sigma
Integrin b4 subunit [346-11A, 553745]	Rat anti-mouse/IFM	BD Biosciences
Integrin b4 subunit [439-9B, 55719]	Rat anti-human/IFM	BD Biosciences
Integrin b4 subunit [# sc-55514]	Mouse anti-human/WB	Santa Cruz
Laminin 4 chain [3H2, ab205568]	Mouse anti-human	Abcam
Laminin 5 chain [4B12, ab77175]	Mouse anti-human	Abcam
Integrin 3 subunit	Mouse anti-human/WB**	Gift from Dr. CM DiPersio
GAPDH [GA1R] [MA5-15738]	Mouse anti-human&mouse/WB	ThermoFisher Scientific
TRITC – UEA Lectin (L4889)	NA/IFM	Sigma
Anti-mouse IgG Alexa Fluor 488 [A21202]	Donkey anti-mouse IgG/IFM	ThermoFisher Scientific
Anti-mouse IgG Alexa Fluor 568 [A10037]	Donkey anti-mouse IgG/IFM	ThermoFisher Scientific
Anti-rabbit IgG Alexa Fluor 488 [A11034]	Goat anti-rabbit IgG/IFM	ThermoFisher Scientific
Anti-Rat IgG Alexa Fluor 647 [A-21247]	Goat anti-rat IgG/IFM	ThermoFisher Scientific
Anti-mouse HRP [ab97046]	Rabbit anti-mouse IgG/WB	Abcam

*IFM = Immunofluorescence microscopy; WB = western blotting.

Table S2. RNAi.

Gene	Manufacturer	Targeting Sequence
Negative Control #1 (siRNA)	Sigma	Sigma SIC001
ITGA3 #1 (siRNA)	Sigma	5'-GUUUGAAGGCUUGGGCAAA-3'
ITGA3 #2 (siRNA)	Sigma	5'-GUGUACAUCUAUCACAGUA-3'
ITGA3 #3 (siRNA)	Sigma	5'-GAGAUCACCGUCCAUGGCA-3
ITGB4 #1 (siRNA)	Sigma	5'-GUCACAUGGUGGGCUUUA-3'
ITGB4 #2 (siRNA)	Sigma	5'-GCCACUACACUAUUGGAUU-3'
ITGB4 #3 (siRNA)	Sigma	5'-GCACGUGUGAGGAAUGCAA-3'
Non-targeting NT (shRNA)	Dharmacon	5'-TGGTTTACATGTTGTGTGA-3'
ITGA3 (shRNA)	Dharmacon	5'-GTGTTTCGTCACGTTGATGC-3'

Table S3. QPCR Primers.

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
LAMA4*	GAAATTGCATTTGAAGTCCG	ACCTGTCCATTTTTCATGTG
LAMA5*	GAAGTGAAAACCTCAAGCGGG	CATCGACATACAGCCAGACTC
ITGA3*	GGGGAATACAAAAAGCCCA	ACCCGTTATTATGTCACGCCA
ITGB4*	AGACCGATCCACTTTGCCAG	AAGTTCAGCCGGCTCTATCG
CXCR4 [#]	AAATCTTCCTGCCCACCATC	GTACTTGTCCGTCATGCTTCT
ANGPT2 [#]	CAAAATAAGCAGCATCAGCCA	AGTACATTCCGTTCAAGTTGGA
DLL4 [#]	CCAACTGCCCTTCAATTTTAC	GGATGGCGATCTTGCTGAT
JAG1 [#]	ACTATGCCTGTGACCAGAATG	CTTAGGACTGCAGCCTTGTC
JAG2 [#]	GTCATCCCCTTCCAGTTCCG	GTGGTATCGTTGTCCCAGTC
ID1 [#]	GCTGTTACTCACGCCTCAAG	CCTGATGTAGTCGATGACGTG
ID2 [#]	ATCAGCATCCTGTCCTTGC	CTTAAAAGATTCCGTGAATTTGTTGT
PDGFB [#]	GAGTTGGACCTGAACATGAC	AGATCTCGAACACCTCGGT
KDR [#]	TGGAATTGACAAGACAGCAAC	GAGGATCTTGAGTTCAGACATGAG
MMP14 [#]	TTCGCCGACTAAGCAGAAG	CTTGAATTCCTAGACCGCTGT
NRP1 [#]	GCAGTATTCCTCCAAACCACT	ATGATCTGGGTCATCTTCTC
ACTB*	GACGACATGGAGAAAATCTG	ATGATCTGGGTCATCTTCTC

*Primers were purchased from Sigma. [#]Primers were purchased from Integrated DNA Technologies.

Table S4. Summary of Gene Expression Analysis.

Gene	$\alpha 6$ siRNA ‡	$\beta 4$ siRNA#1	$\beta 4$ siRNA#2	$\beta 4$ siRNA#3	$\alpha 3$ siRNA #1	$\alpha 3$ siRNA #2	$\alpha 3$ siRNA #3
LAMA5	P< 0.01 ↓	P< 0.05 ↓	P< 0.05 ↓	NS	NS	NS	NS
LAMA4	NS*	NS	NS	NS	NS	NS	NS
CXCR4	P< 0.01 ↓	NS	NS	NS	NS	NS	NS
ANGPT2	P< 0.01 ↓	P< 0.01 ↓	P< 0.01 ↓	P< 0.05 ↓	NS	NS	NS
DLL4	NS*	NS	P< 0.05 ↓	NS	NS	NS	NS
JAG1	NS	NS	NS	NS	P< 0.01 ↓	NS	P< 0.01 ↓
JAG2	NS	NS	NS	NS	P< 0.05 ↓	P< 0.01 ↓	NS
KDR	NS	NS	NS	NS	P< 0.01 ↓	P< 0.05 ↓	NS
MMP14	ND [#]	ND	ND	ND	NS	P< 0.05 ↓	NS
NRP1	NS	P< 0.05 ↓	P< 0.001 ↓	P< 0.05 ↓	P< 0.001 ↓	P< 0.001 ↓	P< 0.001 ↓
ID1	P< 0.01 ↑	NS	NS	NS	P< 0.001 ↓	P< 0.05 ↓	P< 0.001 ↓
ID2	P< 0.05 ↓	NS	NS	NS	P< 0.05 ↓	NS	NS
PDGFB	NS	P< 0.001 ↓	P< 0.001 ↓	NS	P< 0.01 ↓	P< 0.05 ↓	NS

*NS = not significant; [#]ND = not determined; ↓ = RNA expression downregulated; ↑ = RNA expression upregulated; ‡Data is from Xu H,

Pumiglia K, LaFlamme SE. Laminin-511 and $\alpha 6$ integrins regulate the expression of CXCR4 to promote endothelial morphogenesis.

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