Supplementary Material Figure Legends

Supplementary Figure 1. **Mouse tandem ipsilateral CCA and MCA stroke**. Representative TTC stained mouse brain section 24 h after a tandem common carotid artery and middle cerebral artery occlusive stroke. The white ischemic/stroke core and peri-infarct cortex are designated with arrows, boundary lines and circles, respectively.

Supplementary Figure 2. **Post-stroke perivascular generation of DV**. Domain IV (DIV, red) and DV (green) co-immunohistochemistry of rat stroke brain tissue on PSD3 and PSD7. Note the distinction between perlecan-bound DV (arrowheads) from perlecan-cleaved DV (arrows). Scale bar = 20μm.

Supplementary Figure 3. **ACA-MCA anastomoses comparison between WT and Perlecan deficient mice**. (A) The anastomoses between the middle cerebral arteries (MCA) and the anterior cerebral artery (ACA) in wild-type (WT) littermate control C57Bl6 and perlecan deficient mice (pln -/-) after black latex injection. The anastomoses points are marked with circles and connected by the line of anastomoses. (B) Distance of the line of anastomoses from the midline in WT littermate and pln -/- mice in three coronal planes, located 2 mm, 4 mm and 6 mm from the frontal pole.

Supplementary Figure 4. **DV cloning, purification and purity assessment.** (A) Purified human recombinant DV analyzed by SDS-PAGE silver stain (DV_S), and western blot (DV_W) analysis with anti-DV antibody (R&D systems, shown). 100 micrograms of DV used for each. (B) Fibronectin western blot with varying amounts of loaded fibronectin as indicated demonstrating that the anti-fibronectin antibody (ab80923, Abcam) can readily detect 100 ng or more of fibronectin. (C) Fibronectin western blot (with anti-fibronectin antibody used in (B)) of 500 ng of fibronectin control and 100 μg of three separate preparations of DV demonstrates a fibronectin band at the appropriate size in the control lane but no fibronectin signal in the DV preparations. Based on the determined sensitivity of the anti-fibronectin antibody in (B), this blot demonstrates that any fibronectin contamination of these 3, 100 μg DV samples must be less than 100 ng. (D) Quantification of brain endothelial cell proliferation ± addition of DV (250 nM) or fibronectin (1 mg/ml) ± fibronectin neutralizing antibody at different concentrations shown after 48 hours ± DV in serum free media as measured via MTS assay. Values shown (n=5 separate experiments, each condition performed in triplicate per experiment) means normalized to control proliferation arbitrarily set to 100%) demonstrate that the fibronectin antibodies had no effect on DV enhancement of proliferation but did significantly inhibit FN enhanced proliferation (**$p<0.01$). (E) Representative images of mouse brain microvascular endothelial cells added to Matrigel + DV ± anti-fibronectin antibody (20 μg/ml) for 12 h demonstrating that endothelial cells exposed to DV form interconnected tube-like structures that are unaffected by the fibronectin antibody. Bar is 10 μm. (F) Quantification of tube-like structure formation as in (E) (**$p<0.01$, n=5 separate experiments, each condition performed in triplicate per experiment).

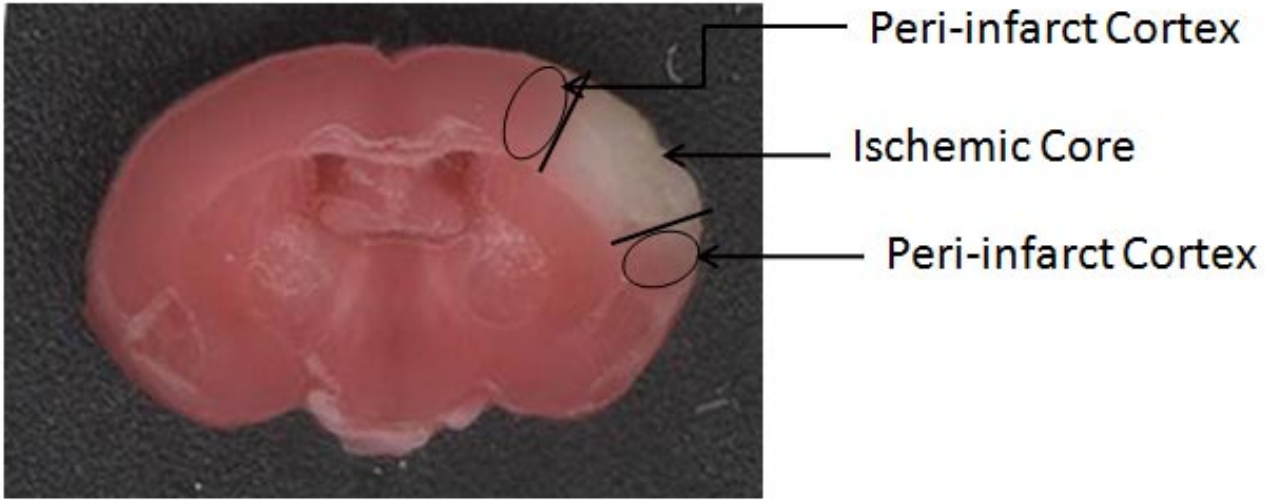
Supplementary Figure 5. **Brain microvascular endothelial cells do not express α2 integrin**. α2 integrin western blot analysis of HUVEC (H) and mouse brain microvascular endothelial cells (B) from C57BL6 mice with GAPDH loading control demonstrating that no α2 integrin could be detected in these brain microvascular endothelial cells.

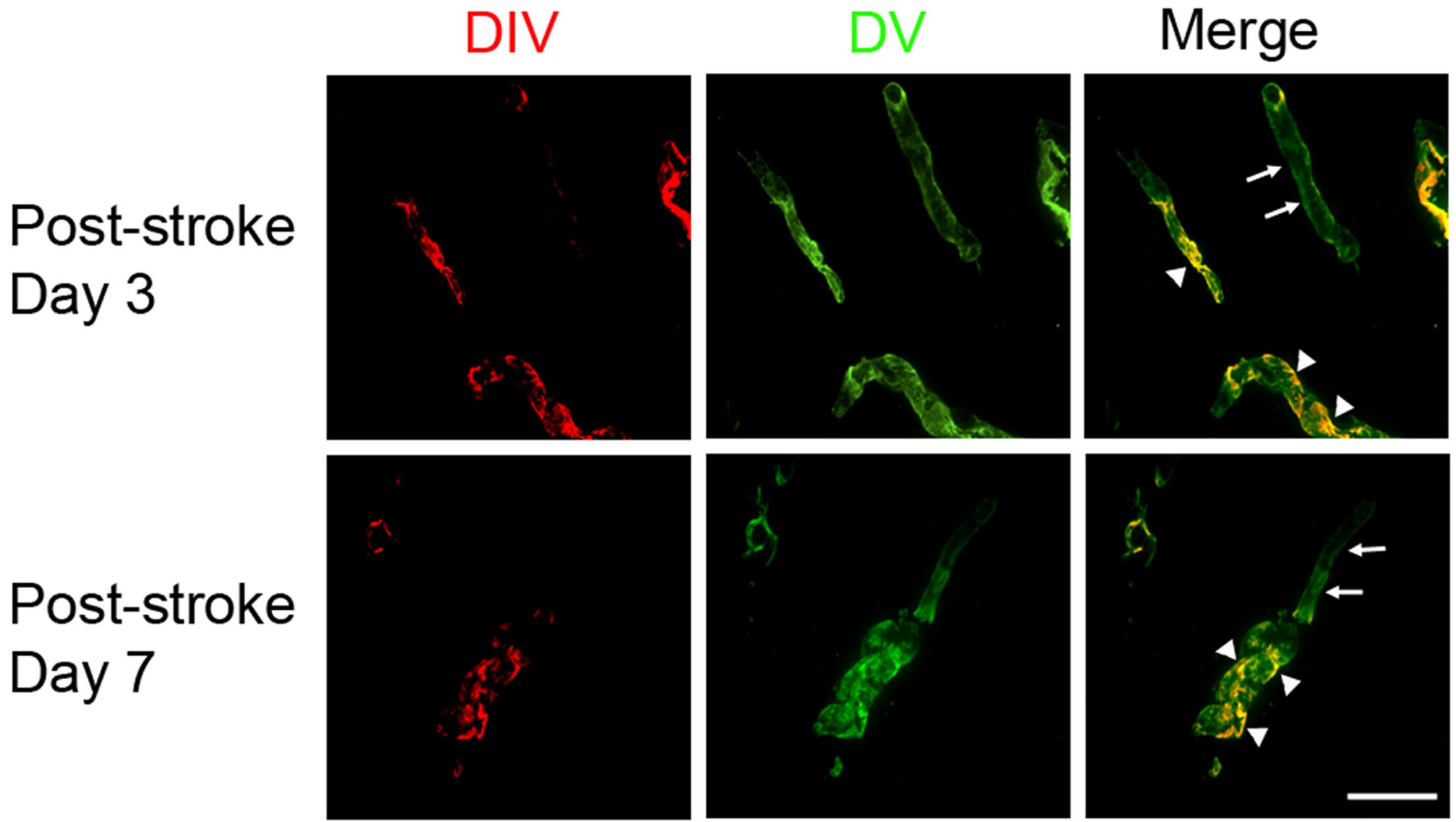
Supplementary Figure 6. DV enhances rat and human brain endothelial cell angiogenesis in vitro. (A) MTS proliferation assay of primary rat brain microvascular endothelial cells after 48 hours in serum free media containing VEGF 20ng/ml ± DV (250 nM). Mean OD at 490nm ± standard deviation shown from n=3 per treatment condition. DV significantly increased rat brain endothelial cells (**p<0.01). (B) Migration assay of human brain microvascular endothelial cells ± DV (250 nM) migrating towards a lower chamber ± the chemo-attractant VEGF (20 ng/ml) over 6h. The mean number of migrated cells ± standard deviation from n=3 wells (10,000 per well) per treatment condition is shown. DV significantly increased human brain microvascular cell migration towards VEGF (**p<0.01).

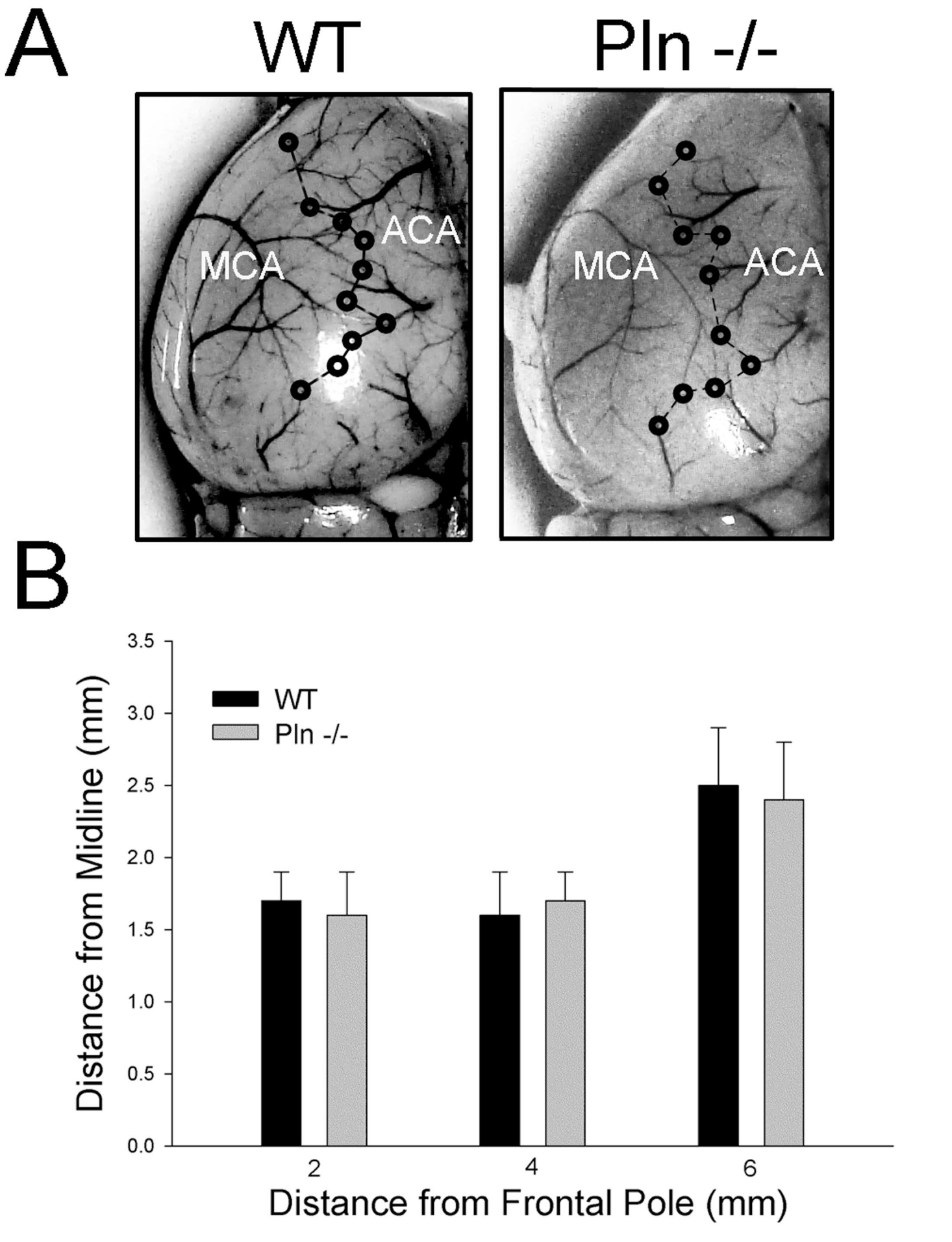
Supplementary Figure 7. **α2 integrin transfection into mouse brain microvascular endothelial cells.** Images (taken with the same exposure times) of α2 integrin immunocytochemistry of mouse brain endothelial cells transfected with a empty mock plasmid (control) demonstrating faint background fluorescence or with an α2 integrin plasmid demonstrating significant α2 integrin expression. Bar is 2 μm.

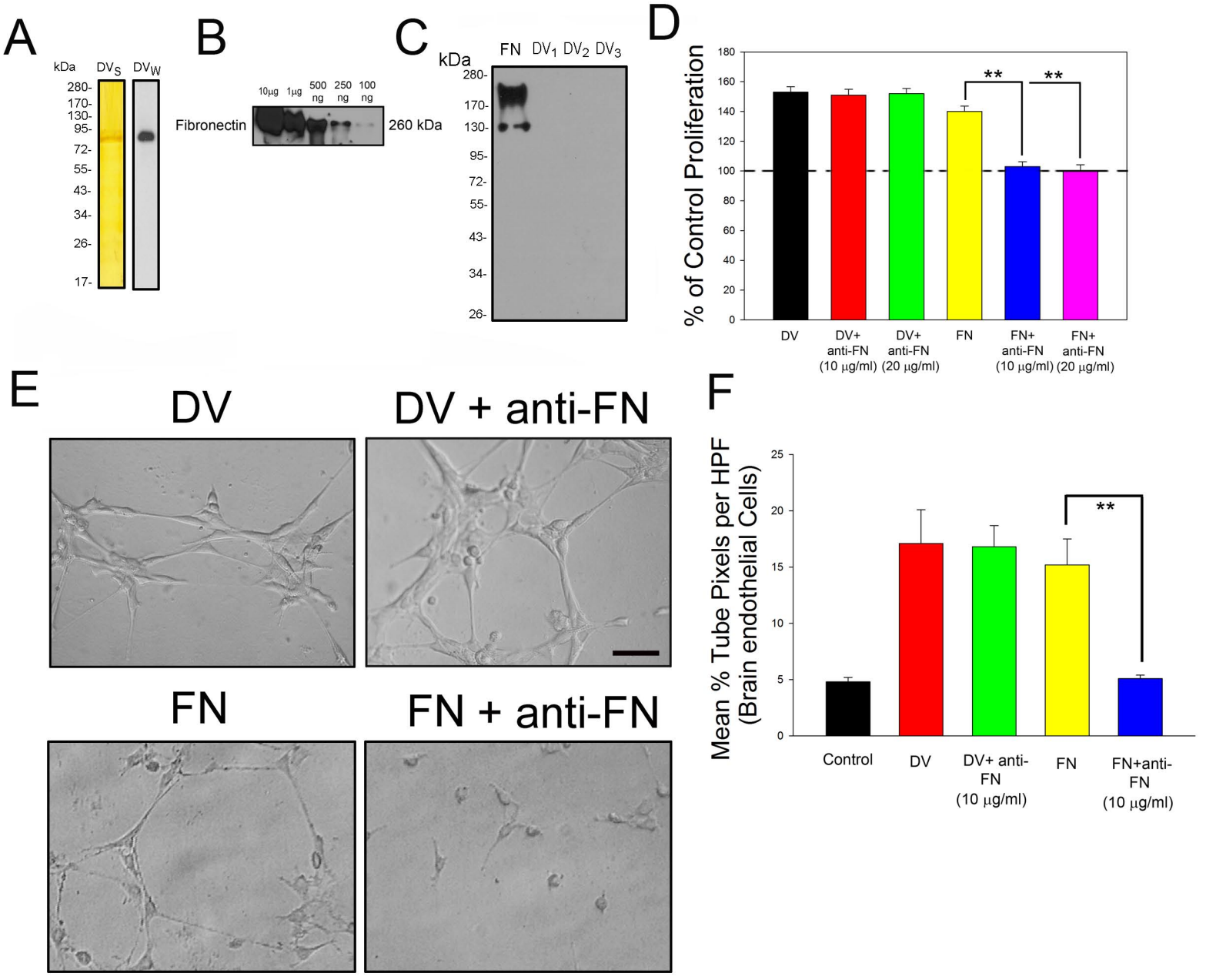
Supplementary Figure 8. **α5 integrin knockdown with siRNA.** α5 integrin (GAPDH loading control also shown) western blot (A) and (B) qPCR of wild type and α5 siRNA treated brain endothelial cells demonstrating a 75% knockdown of α5 protein, and mRNA, respectively. **p<0.01.

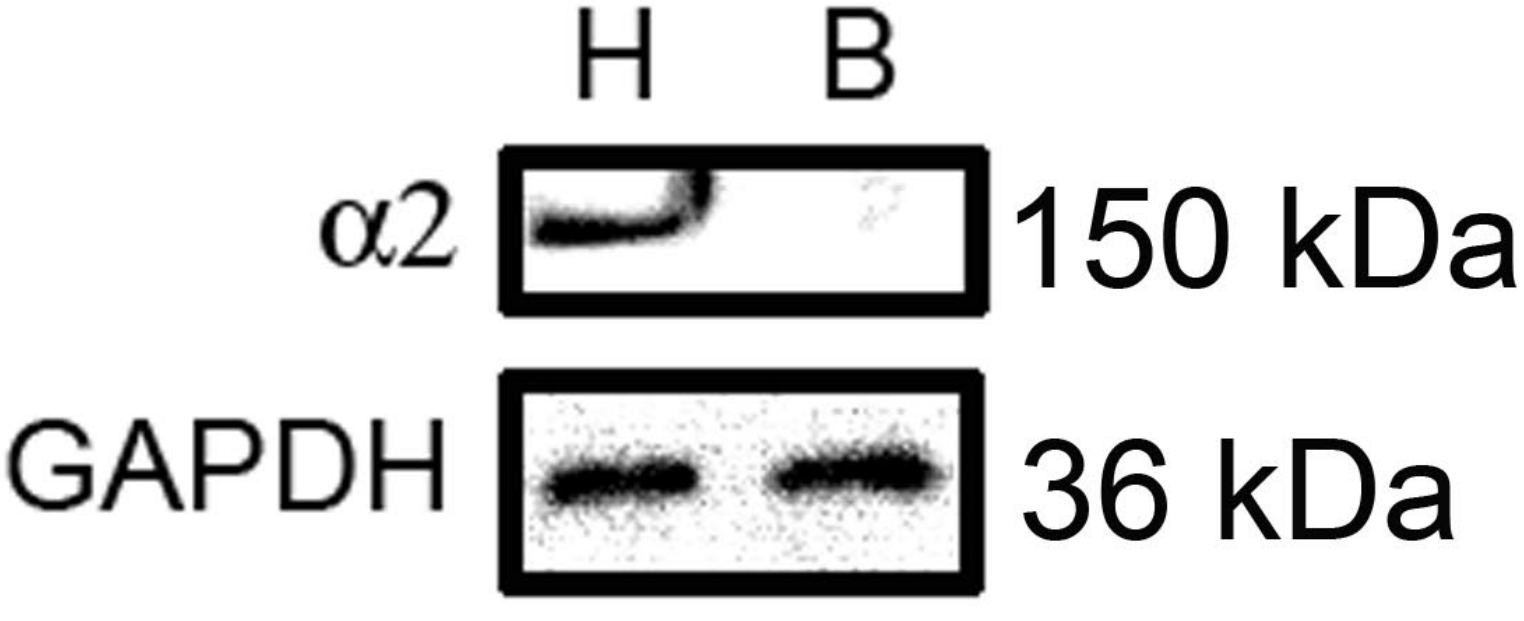
Supplementary Figure 9. **DV activity on brain endothelial cells in the presence of fibronectin**. Mouse brain microvascular brain endothelial cells were grown on plastic or wells coated with DV (20 μg/ml) or fibronectin (FN, 20 μg/ml) for 24 hours ± DV (250 nM) or FN (20 μg/ml) in suspension, followed by MTS proliferation assay. Bars are mean OD_{560nm} ± standard error, n=6 per condition. In suspension, DV significantly increased proliferation of cells grown on plastic (**p<0.01) and grown on fibronectin (*p<0.05). However, DV had no effect on proliferation when cells were directly grown on DV coated wells (as compared to cells on plastic). In contrast, soluble fibronectin significantly enhanced brain endothelial cell proliferation in cells grown on plastic (**p<0.01), DV (*p<0.05) or when cells were grown directly on fibronectin (as compared to cells grown on plastic, p<0.05).

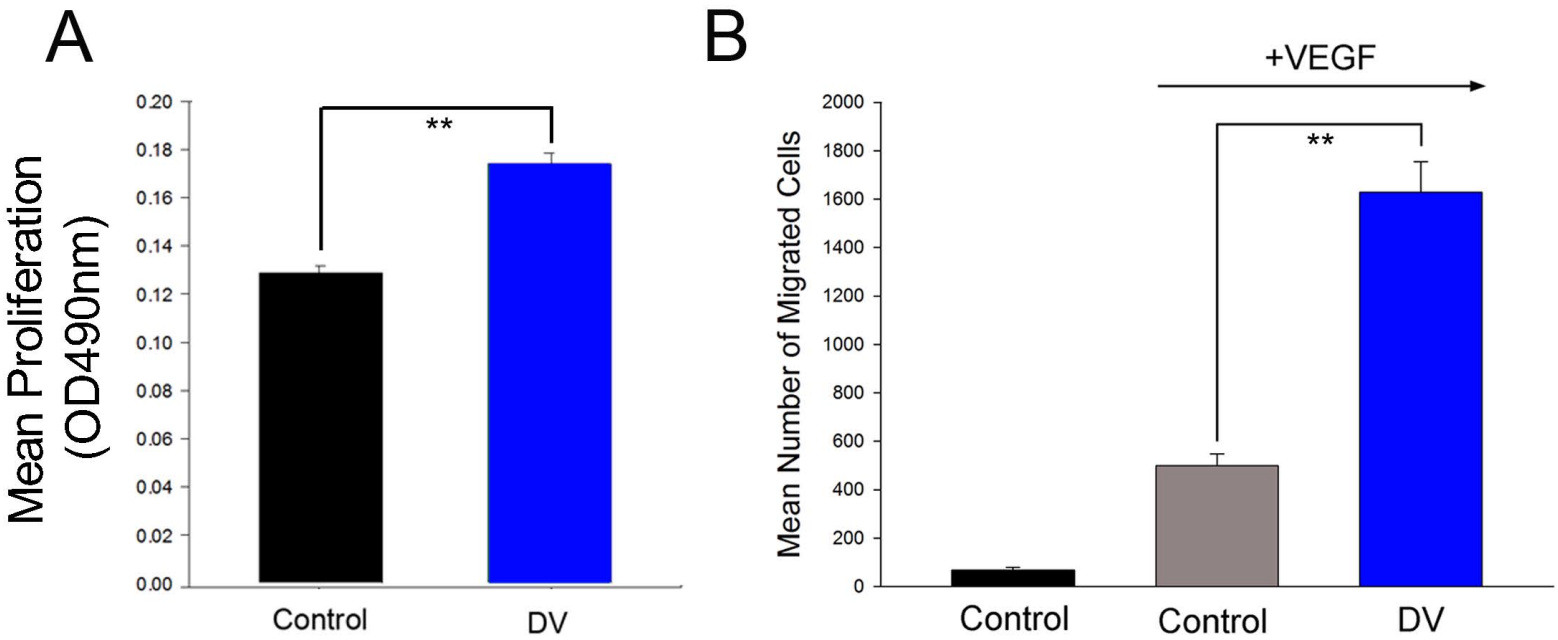

Peri-infarct Cortex
Ischemic Core
Peri-infarct Cortex

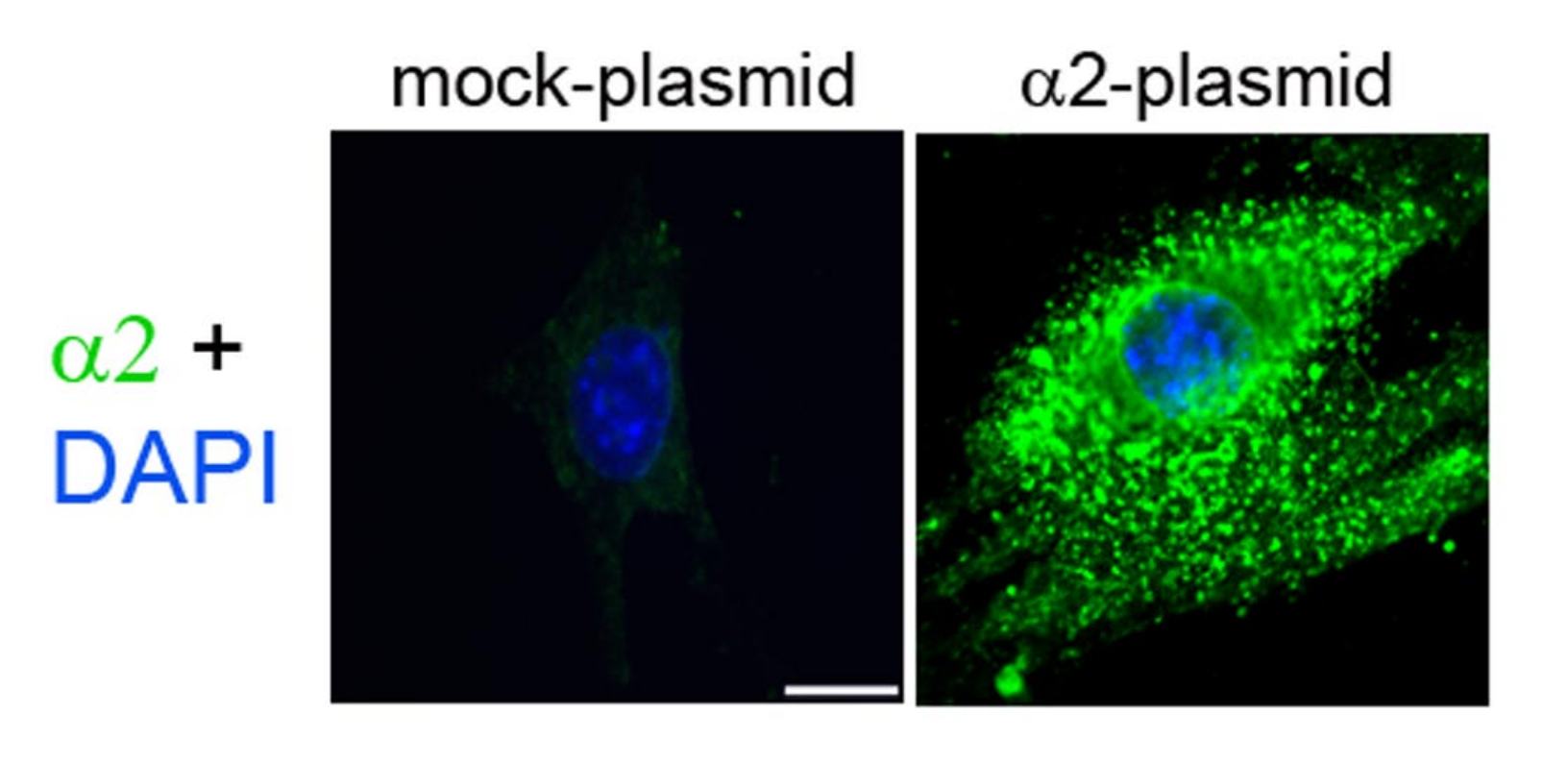

DIV
DV
Merge
Post-stroke
Day 3
Post-stroke
Day 7

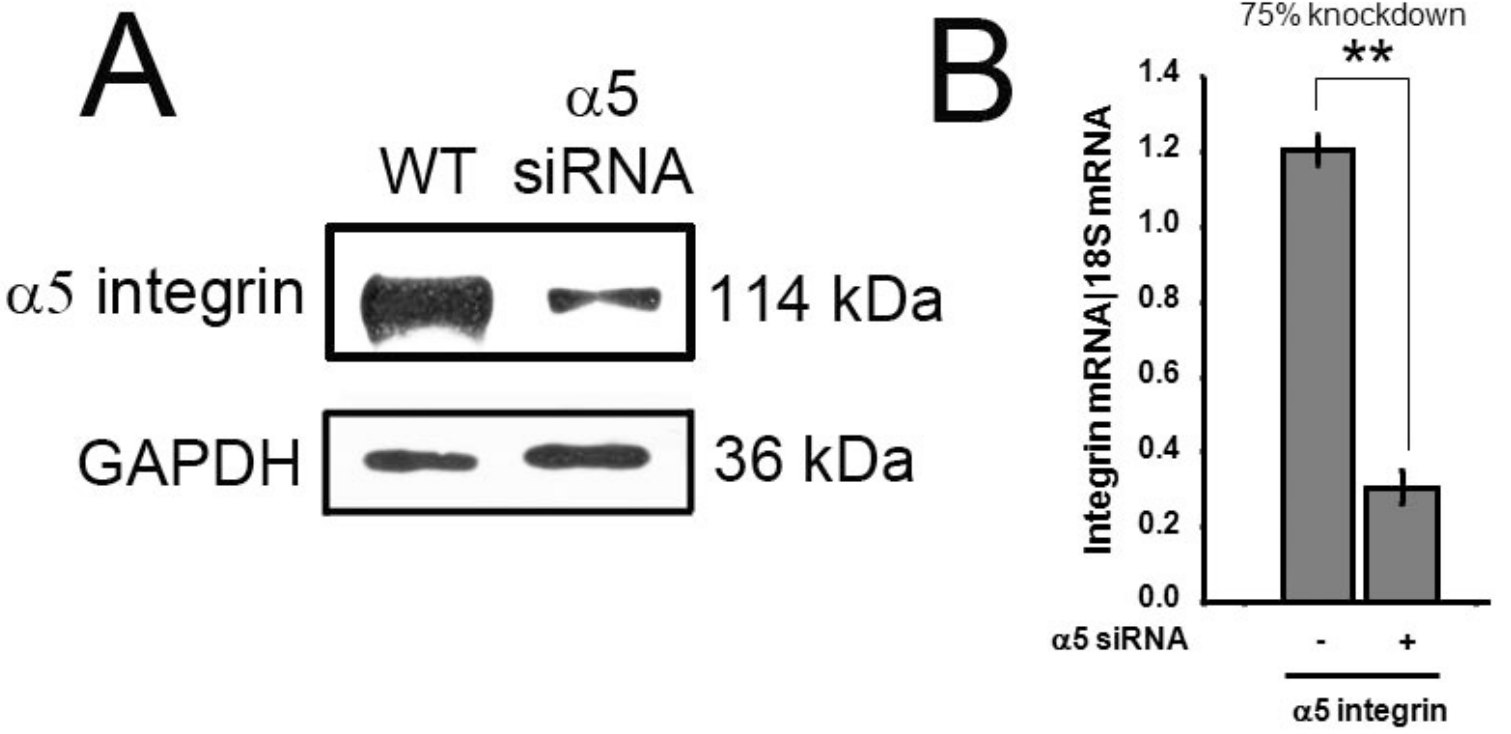

A
WT
Pln -/-
MCA
ACA
MCA
ACA
B
WT
Pln -/-
Distance from Midline (mm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2
4
6
Distance from Frontal Pole (mm)

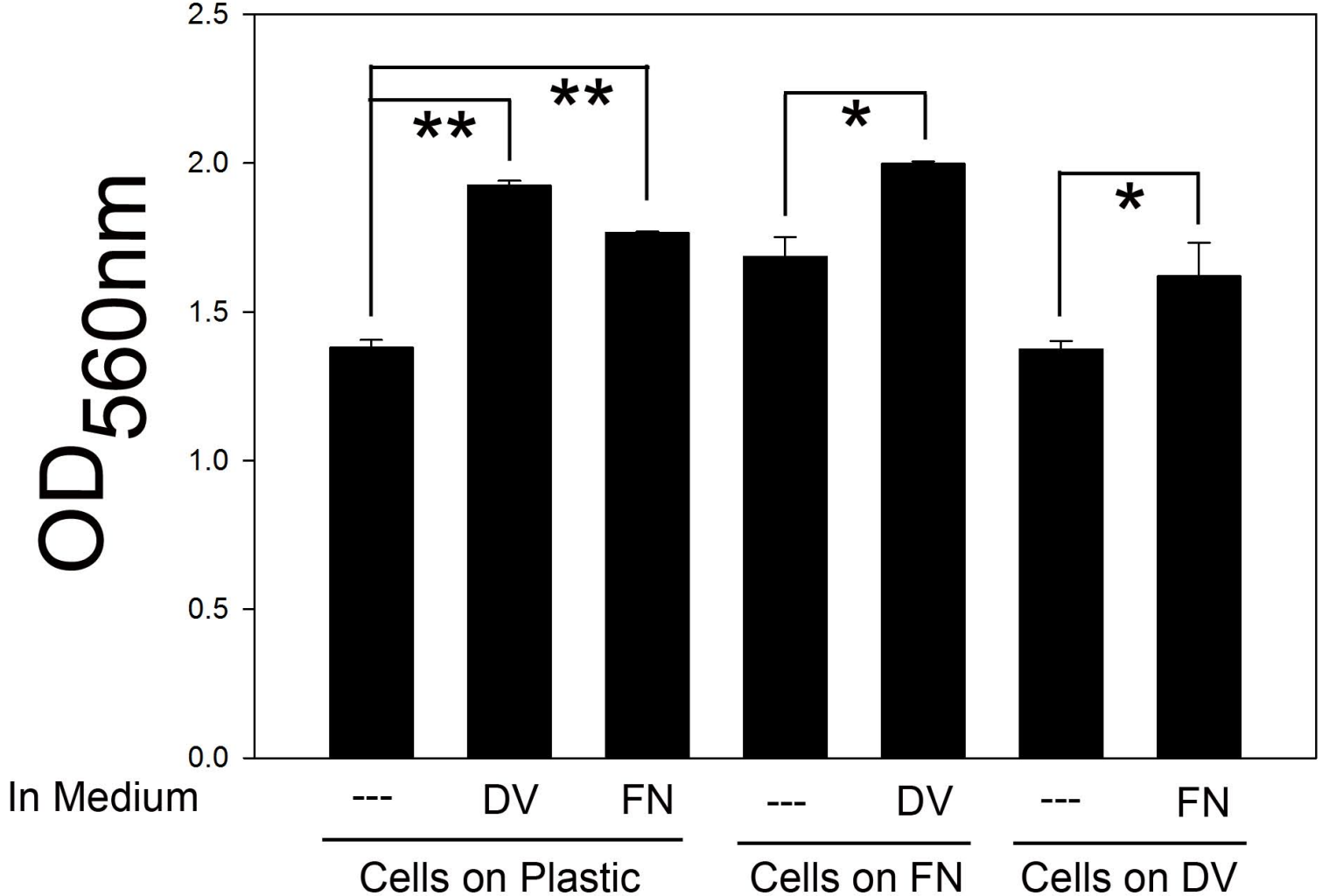

A
kDa
DVS
DVW
280-
170-
130-
95-
72-
55-
43-
34-
26-
17-
B
10μg
1μg
500 ng
250 ng
100 ng
Fibronectin
260 kDa
C
kDa
FN
DV1
DV2
DV3
280-
170-
130-
95-
72-
55-
43-
34-
26-
D
% of Control Proliferation
180
160
140
120
100
80
60
40
20
0
**
**
DV
DV+ anti-FN (10 μg/ml)
DV+ anti-FN (20 μg/ml)
FN
FN+ anti-FN (10 μg/ml)
FN+ anti-FN (20 μg/ml)
E
DV
DV + anti-FN
FN
FN + anti-FN
F
Mean % Tube Pixels per HPF (Brain endothelial Cells)
25
20
15
10
5
0
**
Control
DV
DV+ anti-FN (10 μg/ml)
FN
FN+anti-FN (10 μg/ml)


H
B
α2
150 kDa
GAPDH
36 kDa


A
Mean Proliferation (OD490nm)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
**
Control
DV
B
+VEGF
Mean Number of Migrated Cells
2000
1800
1600
1400
1200
1000
800
600
400
200
0
**
Control
Control
DV


mock-plasmid
α2-plasmid
α2 +
DAPI


A
α5
WT siRNA
α5 integrin
114 kDa
GAPDH
36 kDa
B
75% knockdown
**
Integrin mRNA|18S mRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
α5 siRNA
-
+
α5 integrin


OD560nm
2.5
2.0
1.5
1.0
0.5
0.0
**
**
*
*
In Medium

DV
FN

DV

FN
Cells on Plastic
Cells on FN
Cells on DV