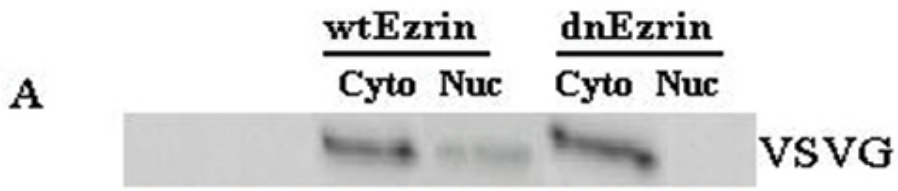
Figure S1

Rat VSMC E19P

Figure S2


A
wtEzrin
dnEzrin
Cyto Nuc
Cyto Nuc
VSVG

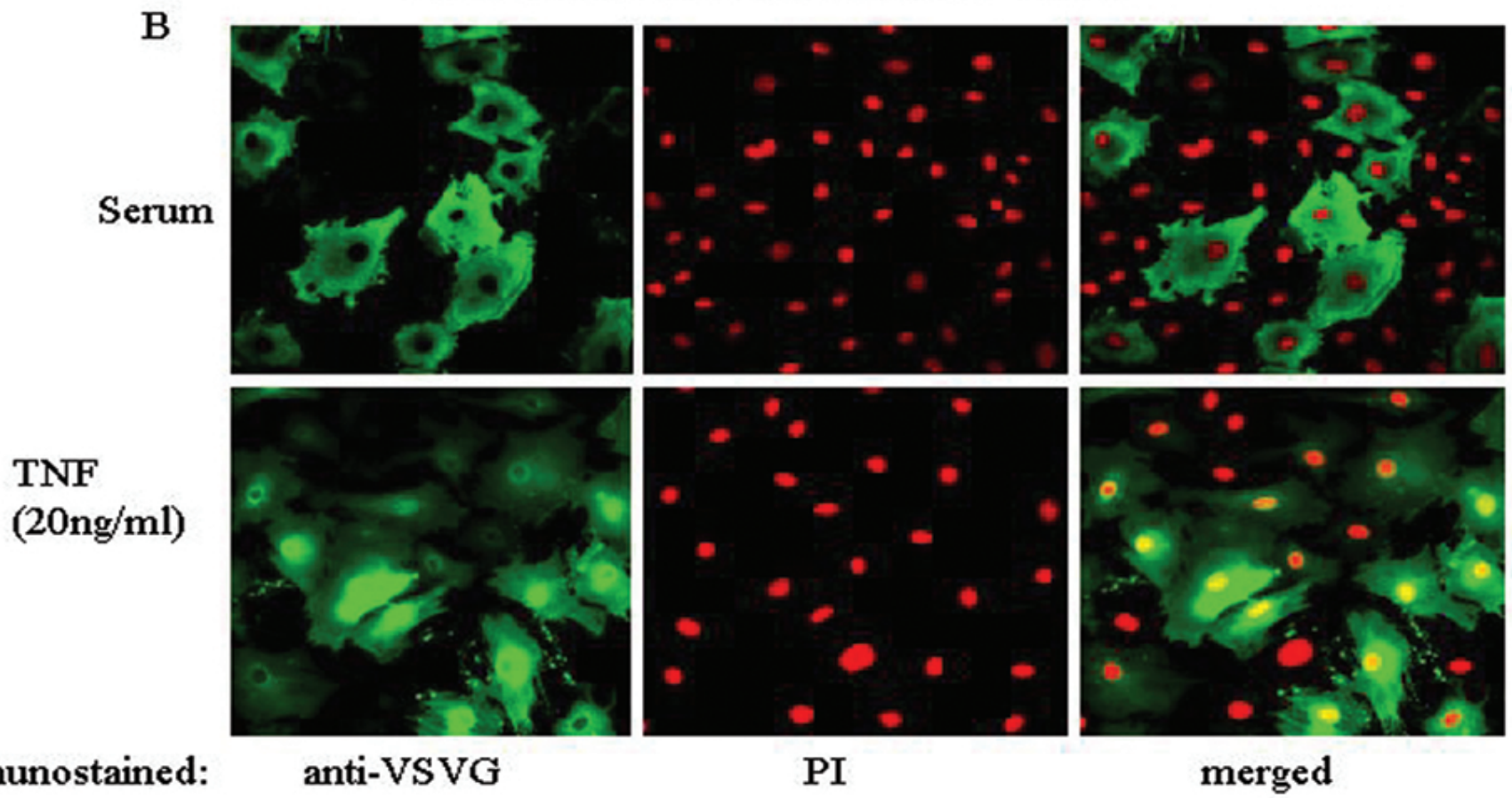
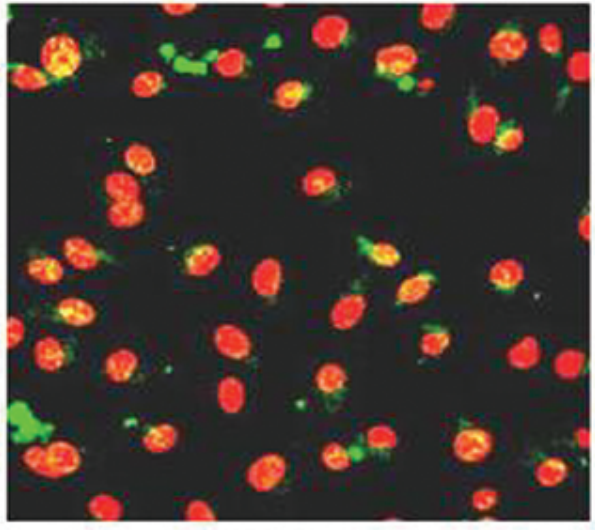
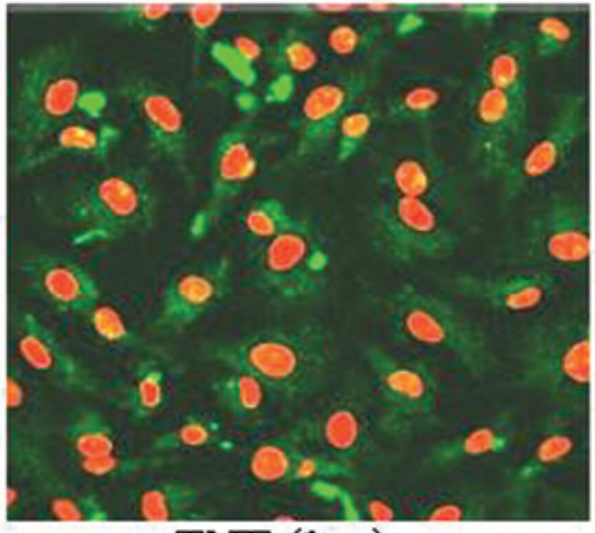

Transfected: Full-length wtEzrin-VSVG
B
Serum
TNF
(20ng/ml)
Immunostained:
anti-VSVG
PI
merged

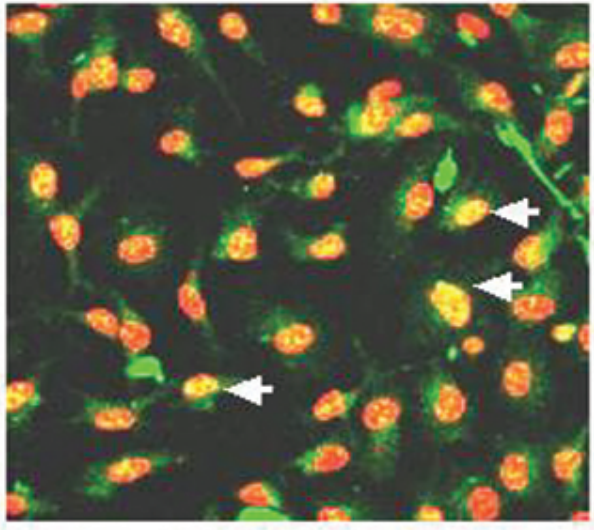
Figure S3



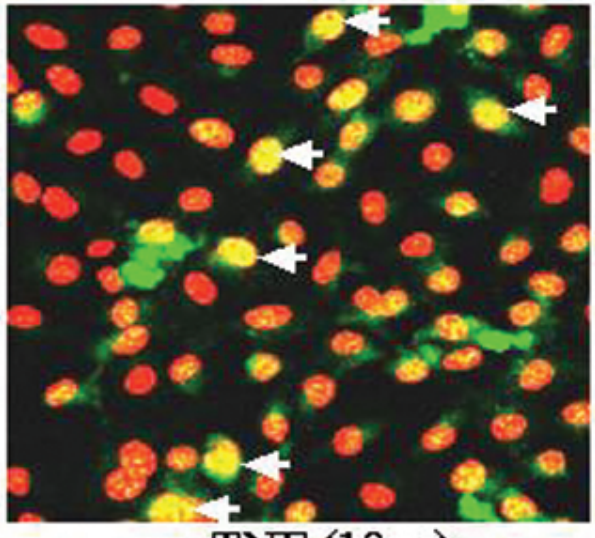
Serum



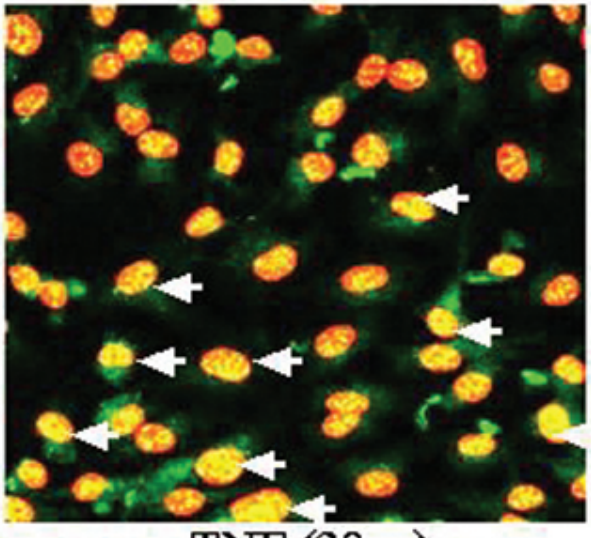
TNF (1ng)



TNF (5ng)

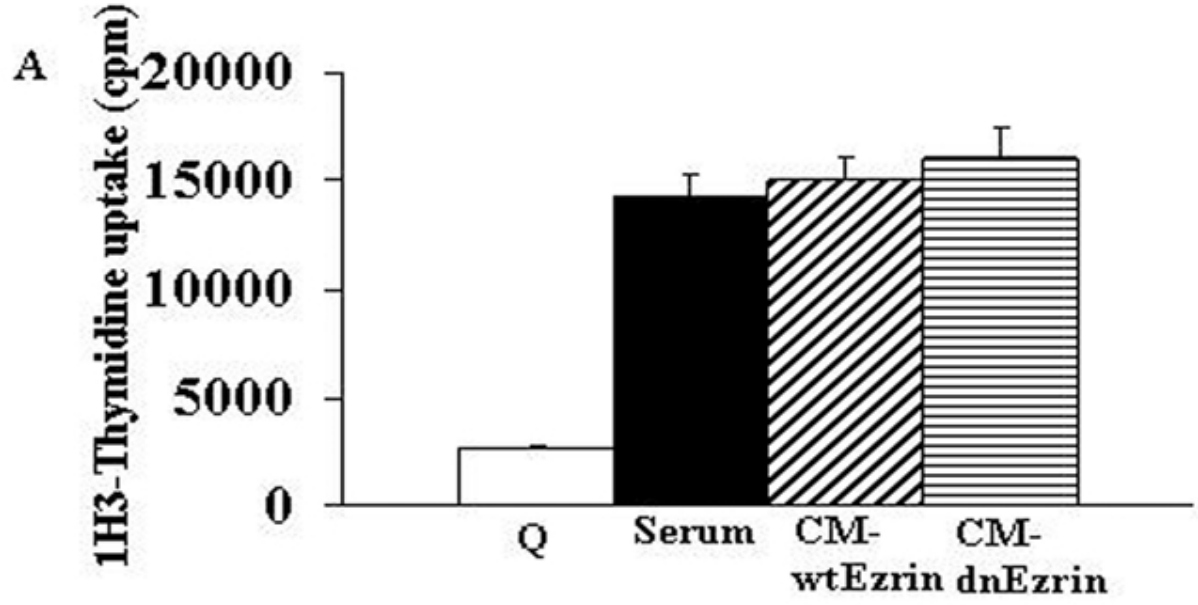


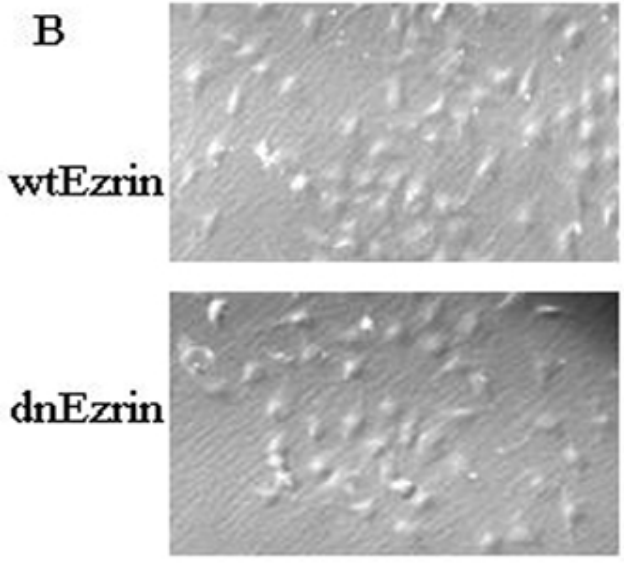
TNF (10ng)



TNF (20ng)

Figure S4


A
1H3-Thymidine uptake (cpm)
20000
15000
10000
5000
0
Q
Serum
CM-wtEzrin
CM-dnEzrin


B
wtEzrin
dnEzrin

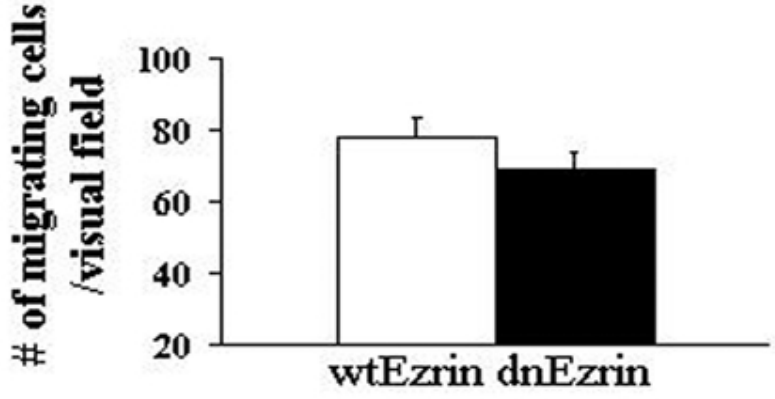
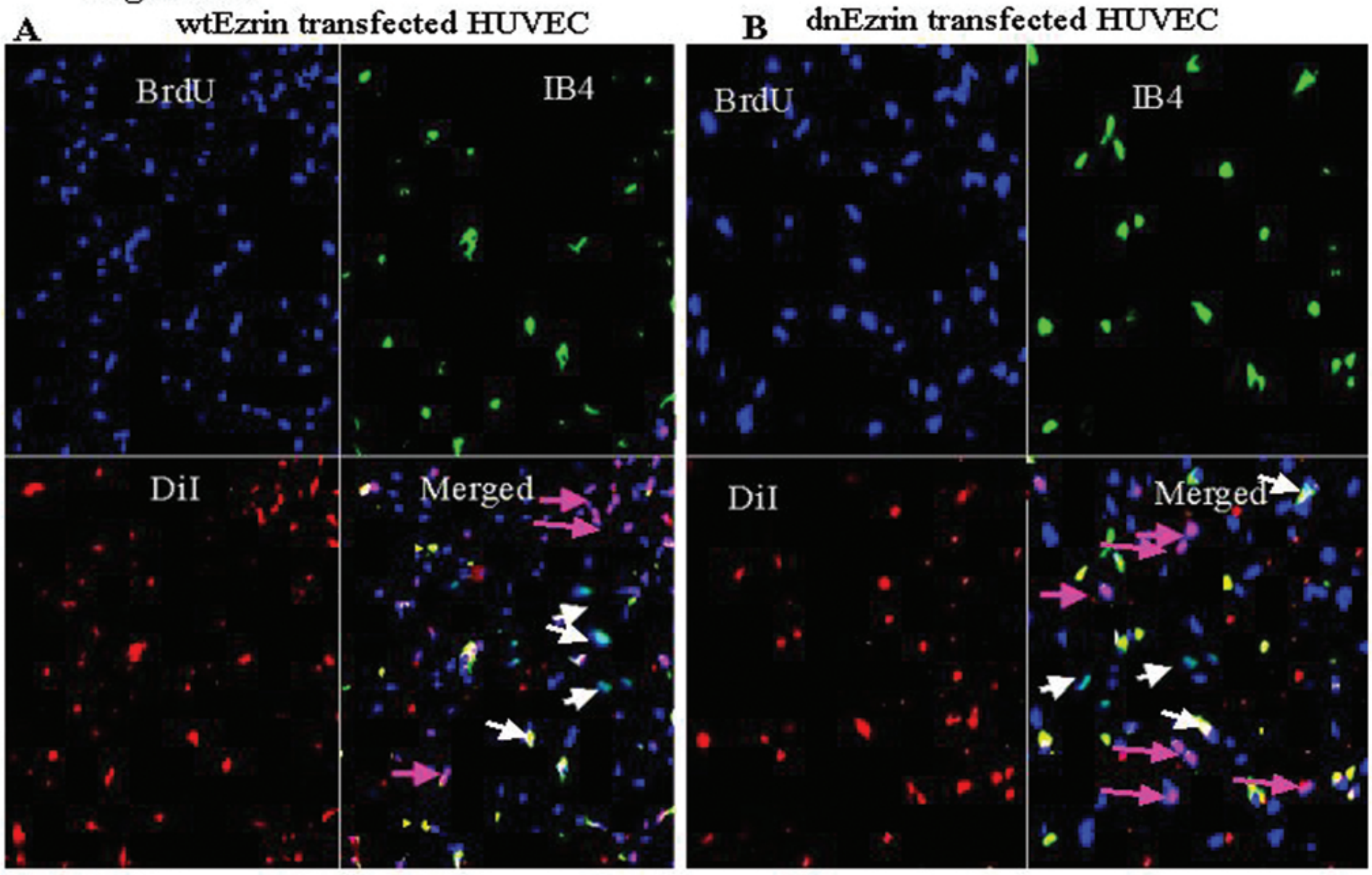

# of migrating cells /visual field
100
80
60
40
20
wtEzrin
dnEzrin

Figure S5


A
wtEzrin transfected HUVEC
BrdU
IB4
DiI
Merged
B
dnEzrin transfected HUVEC
BrdU
IB4
DiI
Merged

LEGENDS TO SUPPLEMENT FIGURES

Figure S1. TNF induced ezrin nuclear translocation is EC specific. Rat vascular smooth muscle cells E19P were cultured as described before (55) and were treated with 20ng/ml TNF. Cytoplasmic and nuclear extracts were analyzed for ezrin protein expression (SC=serum cytoplasmic, TC=TNF cytoplasmic; SN serum nuclear; TN=TNF nuclear).

Figure S2. Ezrin transgene is translocated into the nuclei in response to TNF. HUVEC were transfected with wtEzrin or dnEzrin vectors tagged with VSVG protein (wtEzrin-VSVG; dnEzrin-VSVG). (A) A subset of transfected cells were treated with TNF and cytoplasmic and nuclear extracts were analyzed for tagged VSVG protein expression. (B) In a subset of wtEzrin-VSVG transfected cells immunofluorescent staining was performed to stain for the VSVG protein and for propidium idodide to stain nuclei. Nuclear translocation of exogenously transfected full length ezrin is seen in cells treated with TNF.

Figure S3. TNF-induced ezrin translocation is dose-dependent. Propidium stained nuclei (red) from BAEC treated with increasing doses of TNF were analysed for co-localization of ezrin (green). Yellow fluorescence in the nuclei at TNF doses 5ng/ml indicates ezrin nuclear translocation.

Figure S4. Ezrin modulation does not affect secreted growth factor release (A) Conditioned medium (CM) from wtEzrin or dnEzrin transfected HUVEC was used to

evaluate HUVEC proliferation by thymidine uptake assay. No effect on HUVEC proliferation was observed in the presence of either conditioned medium (Q=quiescent cells). (B). HUVEC were transfected with either wtEzrin or dnEzrin expression vectors. Using sterile razor a 1 cm square scratch was created in the culture disk (EC monolayer was completely scraped in this area ; cells were looked under microscope to assure no cells were present within the scraped area). Cells were continued to be cultured for 6 additional hours and were then subjected to phase contrast microscopy to count the cells that had migrated to the scratched area and were counted. Bar graph represents average number of migrating cells/visual field.

Figure S5: Over-expression of dnEzrin enhances transplanted HUVEC proliferation in vivo. Ischemic hindlimb tissue sections from mice transplanted with wtEzrin transfected HUVEC (A) or dnEzrin transfected HUVEC (B) were immunostained for IB4 (green), BrdU (purple) and DiI (red). Bottom lower panel represents merged images for double stained cells. Representative figures from immunofluorescent staining, showing BrdU+DiI (pink arrow) and IB4+BrdU (white arrow), double positive cells in the ischemic hindlimb tissue from mice receiving either wt-ezrin or dn-ezrin transfected HUVECs are shown.