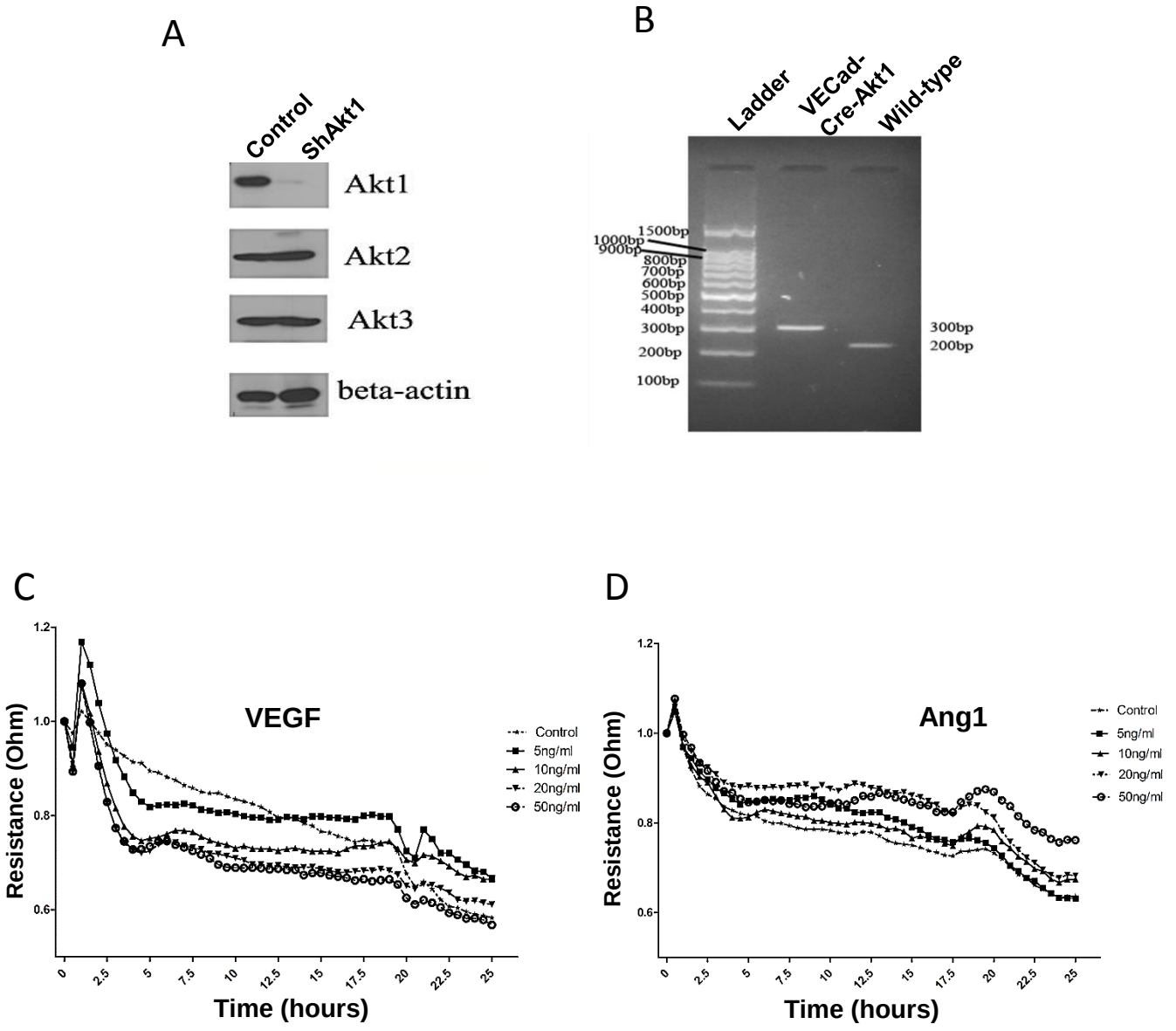
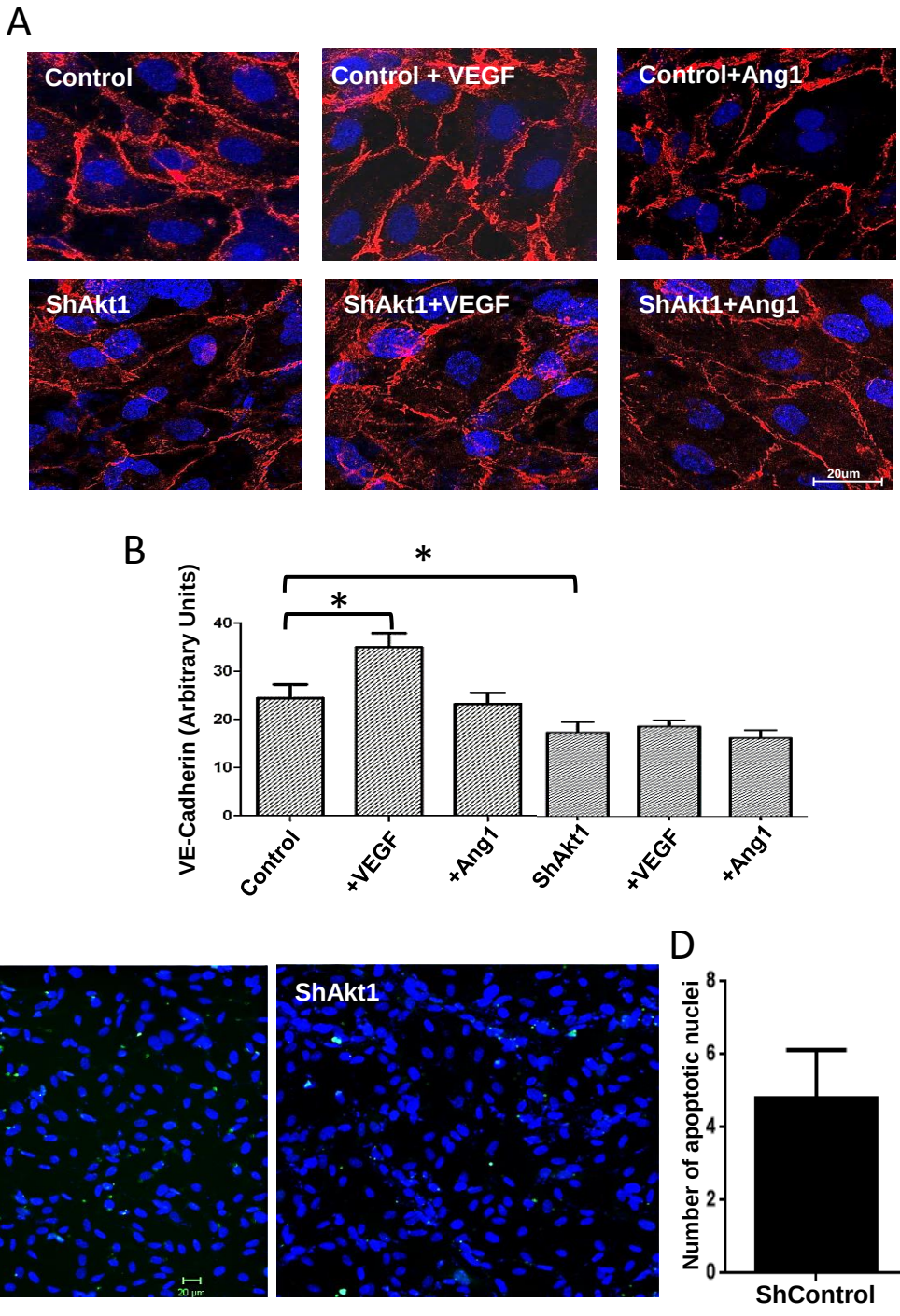
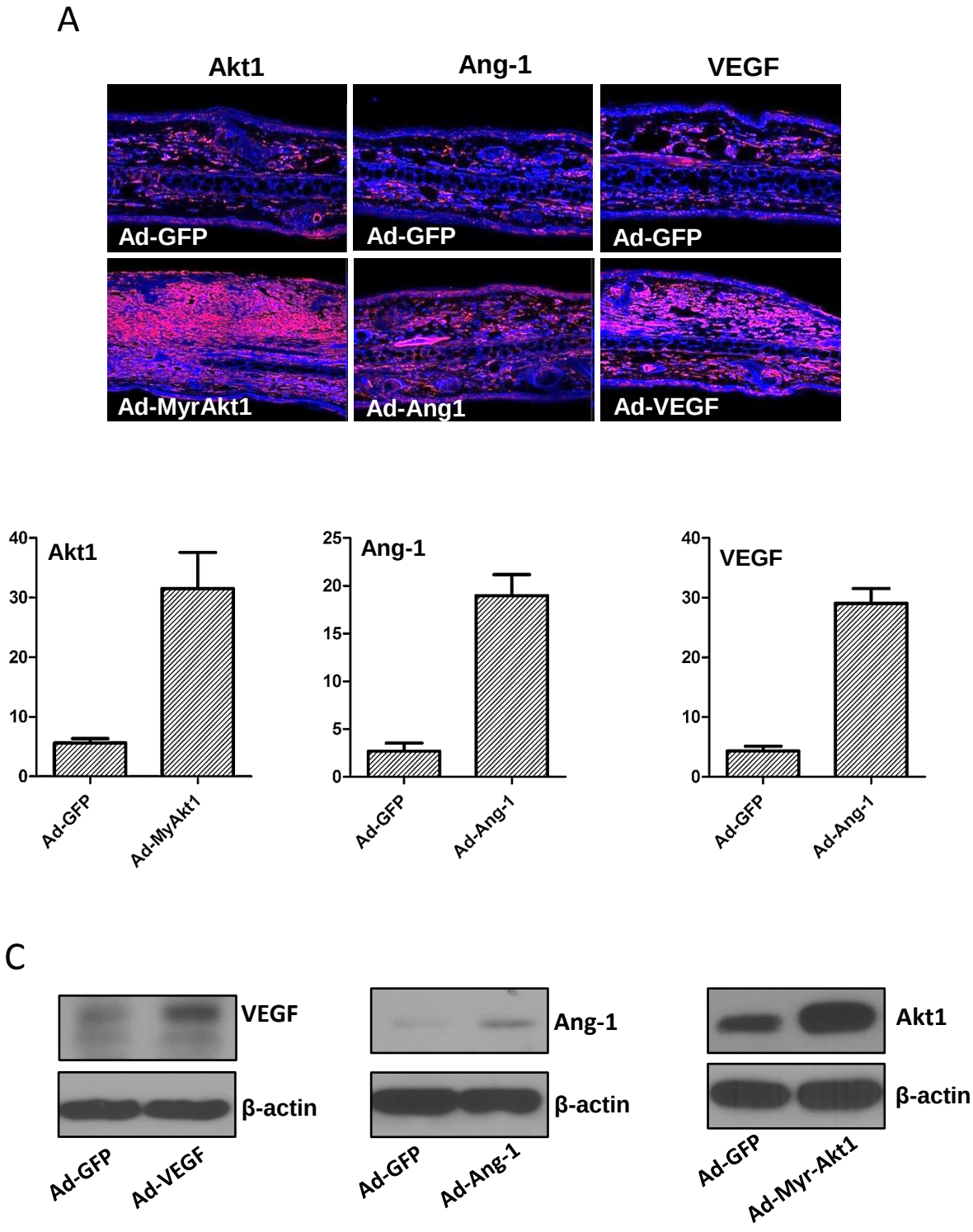


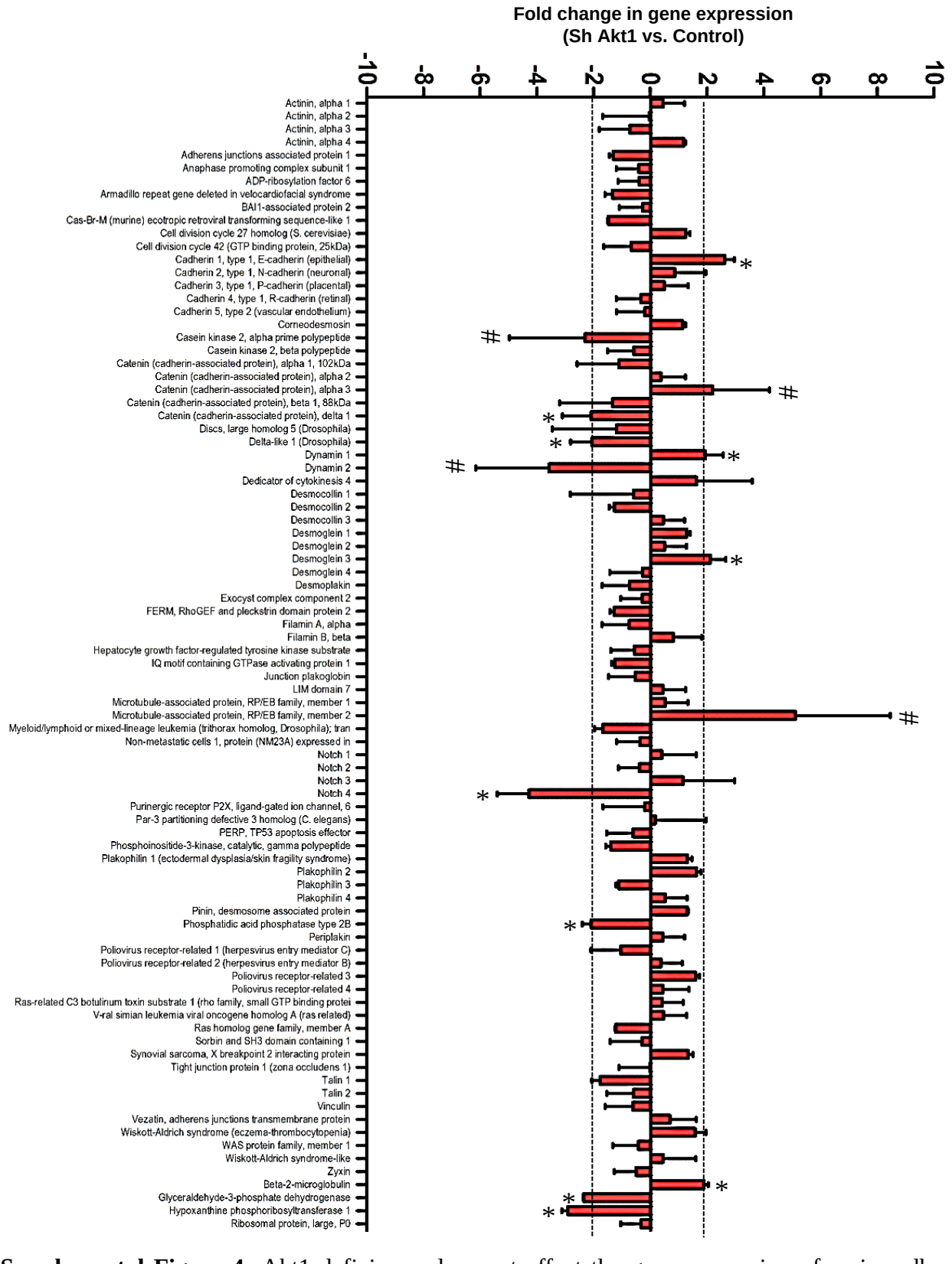
Supplemental Figure 1: (A) Western blot figure showing ShRNA-mediated Akt1 knockdown in HMEC with no changes in the expression levels of Akt2 and Akt3. **(B)** Agarose gel confirms the results of Genotyping; the 200bp band for Wide-type and 300bp for Cre-Akt1 knock down. n=5). **(C and D)** Graphs showing real-time barrier resistance of HMEC monolayers from 0 to 25 h, maintained in 2% FBS and treated with various doses of VEGF and Ang1, respectively, and measured by the electric cell-substrate impedance sensing (ECIS) equipment.



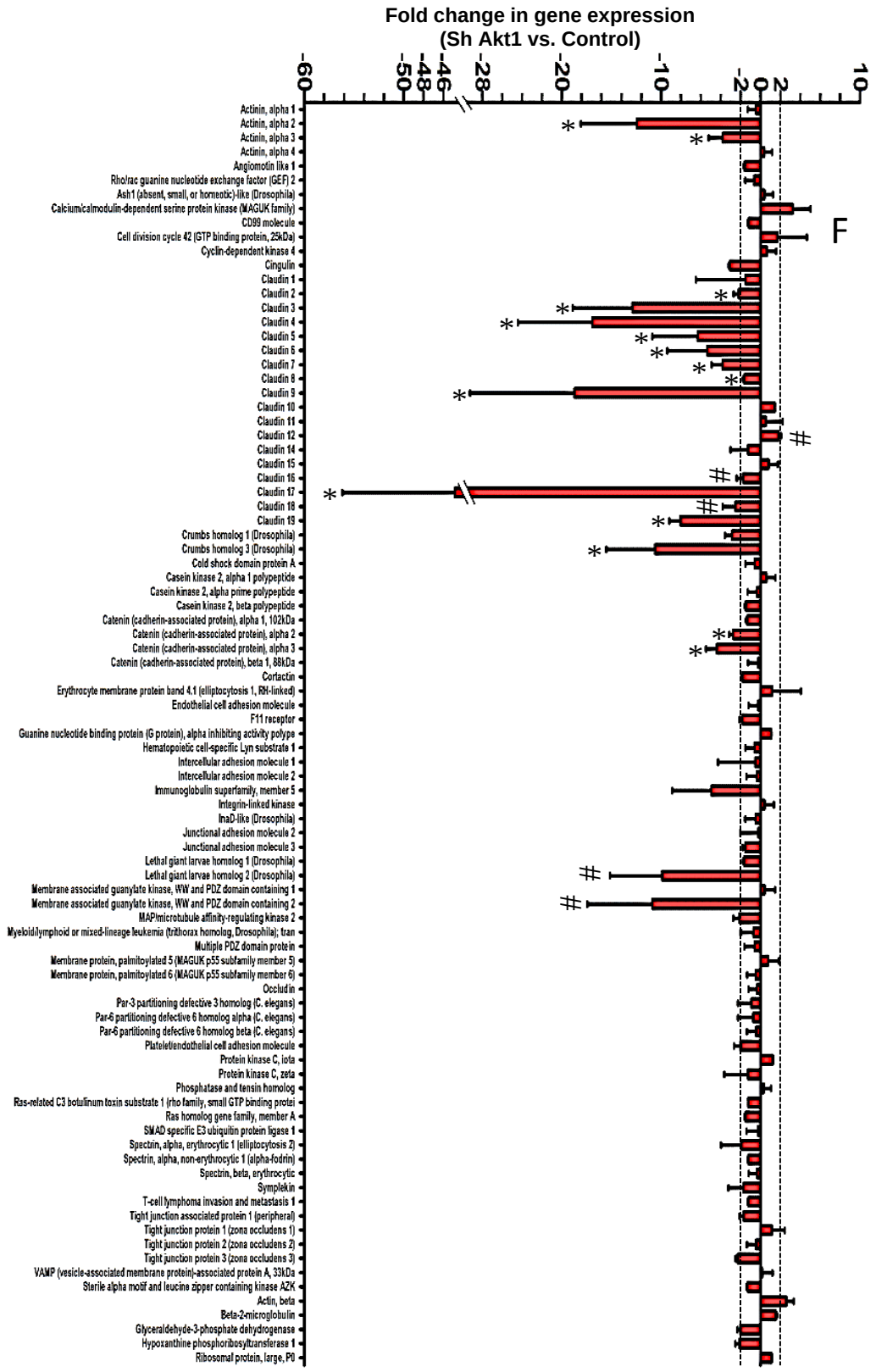
Supplemental Figure 2: Long-time incubation (30 hours) of ShControl and ShAkt1 HMECs in serum free media has no significant effect on apoptosis or VE-cadherin expression between them in the presence or absence of growth factors. **(A)** VE-cadherin staining of ShControl and ShAkt1 cells in the presence and absence of VEGF or Ang1 after 30 hour incubation. **(B)** Bar graphs showing changes in VE-Cadherin expression in ShControl and ShAkt1 HMECs in the presence and absence of VEGF and Ang1 after 30 hour incubation (n=3). **(C and D)** TUNEL staining of ShControl and ShAkt1 HMECs 30 hours after incubation in serum free medium (n=3). *$P < 0.01$. Scale bar: 20 µm.



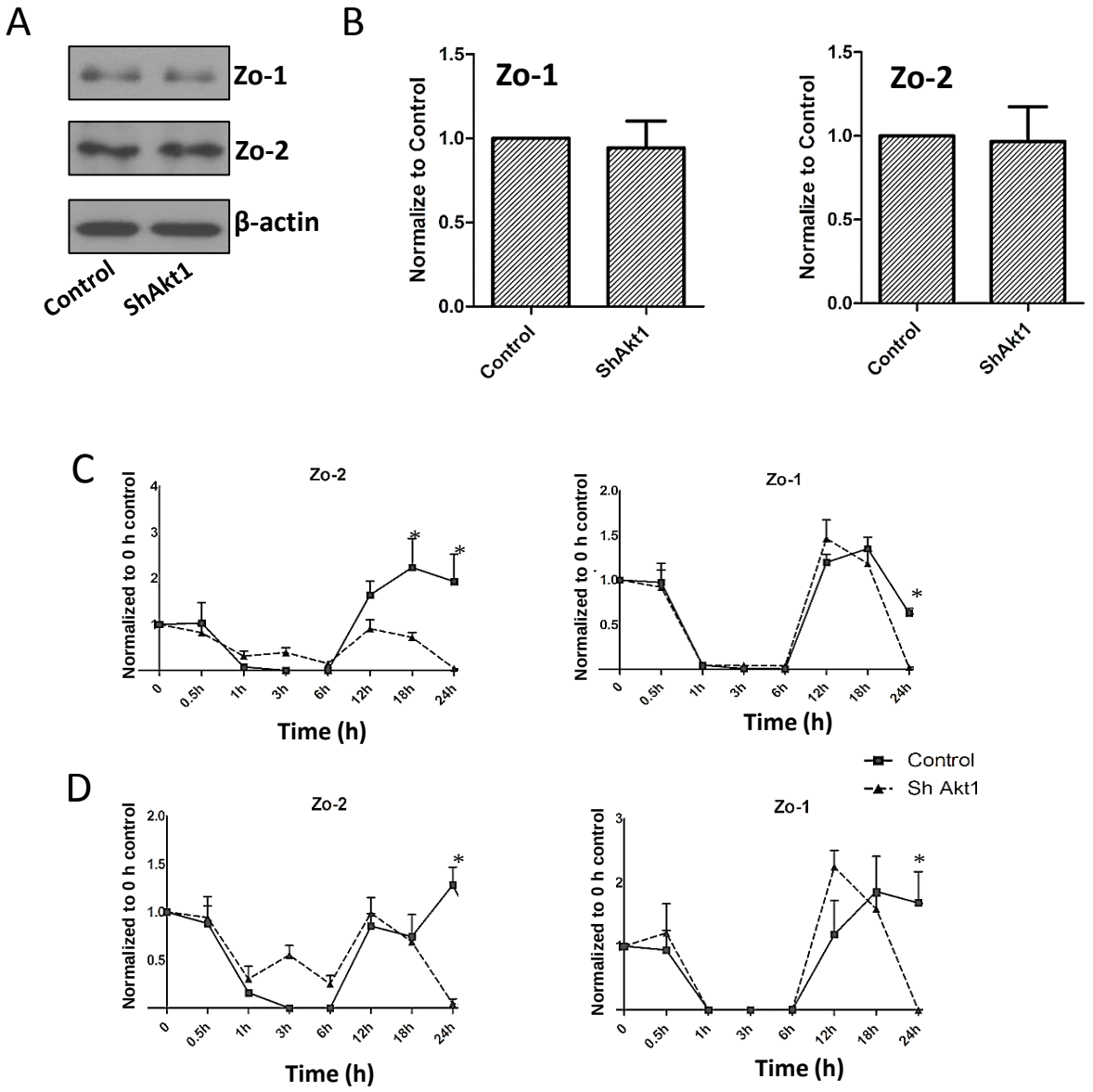
Supplemental Figure 3: (A) Images of mouse ear sections showing increased expression of Akt1, Ang-1 or VEGF upon overexpression with respective Adenoviral particles, compared to Ad-GFP expressing control sections. DAPI is stained in blue. **(B)** Image J analysis of the mouse ear sections indicating increased expression of Akt1, Ang-1 or VEGF upon overexpression with respective Adenoviral particles, compared to Ad-GFP expressing control sections (n=6). **(C)** Western blot images of mouse ear tissues showing increased expression of VEGF, Akt1 or Ang-1 upon overexpression with respective Adenoviral particles, compared to Ad-GFP expressing control sections .



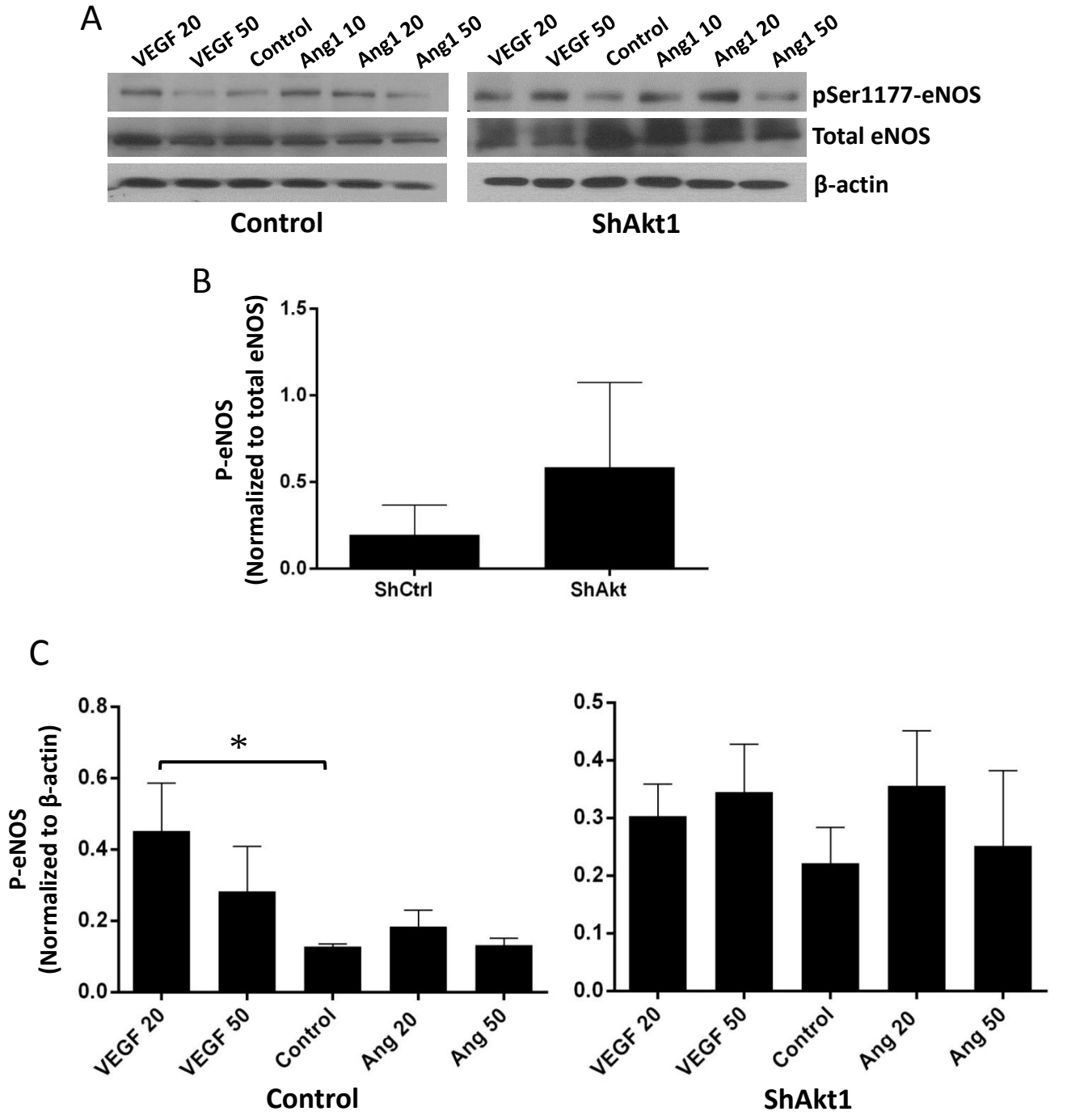
Supplemental Figure 4: Akt1 deficiency does not affect the gene expression of major adherens-junction proteins. Comparative gene array analysis of 85 adherens junction proteins between control and ShAkt1 HMEC (n=3). **P* < 0.01; #*P* < 0.01.



Supplemental Figure 5: Akt1 deficiency affects the gene expression of tight-junction proteins. Comparative gene array analysis of 85 tight junction proteins between control and ShAkt1 HMEC (n=3). *$P < 0.01$; #$P < 0.01$.



Supplemental Figure 6: Akt1 deficiency affects real-time changes in the expression of proteins in endothelial-barrier tight-junctions in response to VEGF and Ang1 treatments. **(A-B)** Western blot images and densitometry analysis of control and ShAkt1 HMEC lysates showing basl levels of Zo-1 and Zo-2 expression levels, respectively (n=3). **(C)** Densitometry analysis of Western blot images of control and ShAkt1 HMEC lysates treated with 20 ng/ml VEGF showing real-time changes in the expression levels of tight junction proteins Zo-1 and Zo-2 determined, and compared to 0 h time point (n=3). **(D)** Densitometry analysis of Western blots of control and ShAkt1 HMEC lysates treated with 50 ng/ml Ang1 showing changes in the expression levels of tight junction proteins Zo-1 and Zo-2, and compared to 0 h time point (n=3). **P* < 0.01, #*P* < 0.05.



Supplemental Figure 7: No significant difference in the levels of phospho-eNOS in ShAkt1 HMEC compared to ShControl HMEC at basal levels and 24 hours after VEGF or Ang1 stimulation. **(A)** Western blot images showing the levels of phosphorylated and total expression of eNOS in ShControl and ShAkt1 HMEC. **(B-C)** Bar graphs showing densitometry analysis of phospho-eNOS in ShControl and ShAkt1 HMEC in the presence and absence of 24 hr treatment with various doses of Ang1 and VEGF (n=3). *$P < 0.05$.