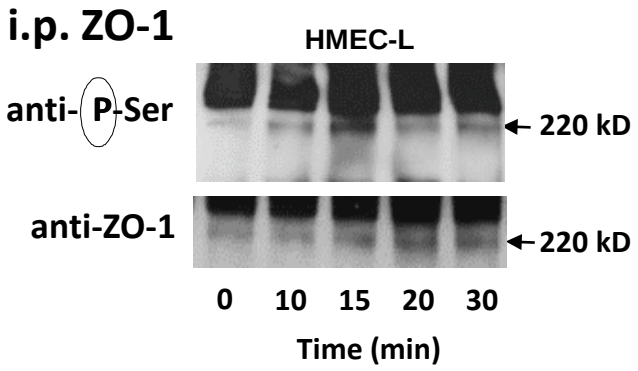
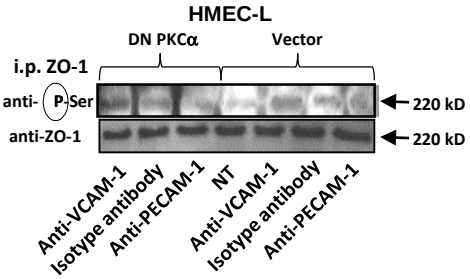
Supplement Figure 1

A (uncropped blot of Figure 2A)



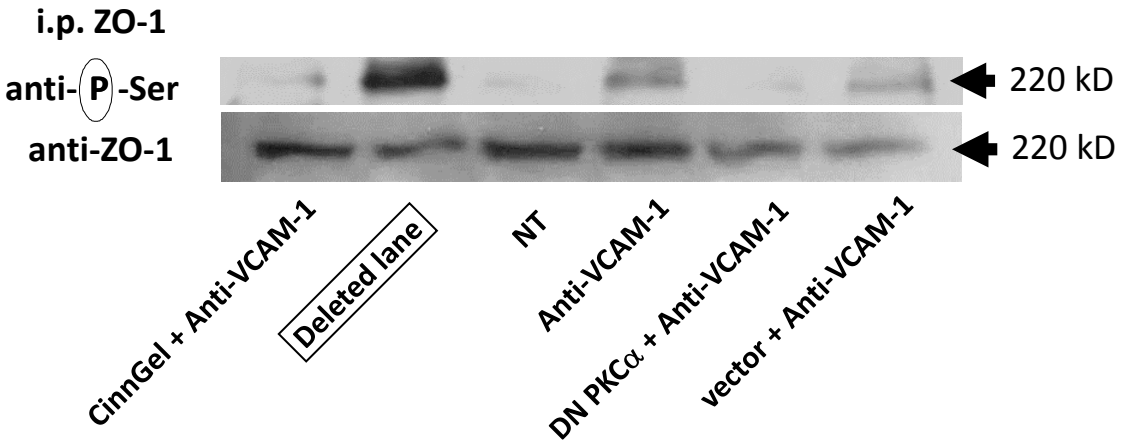
B (blot of Figure 2E)



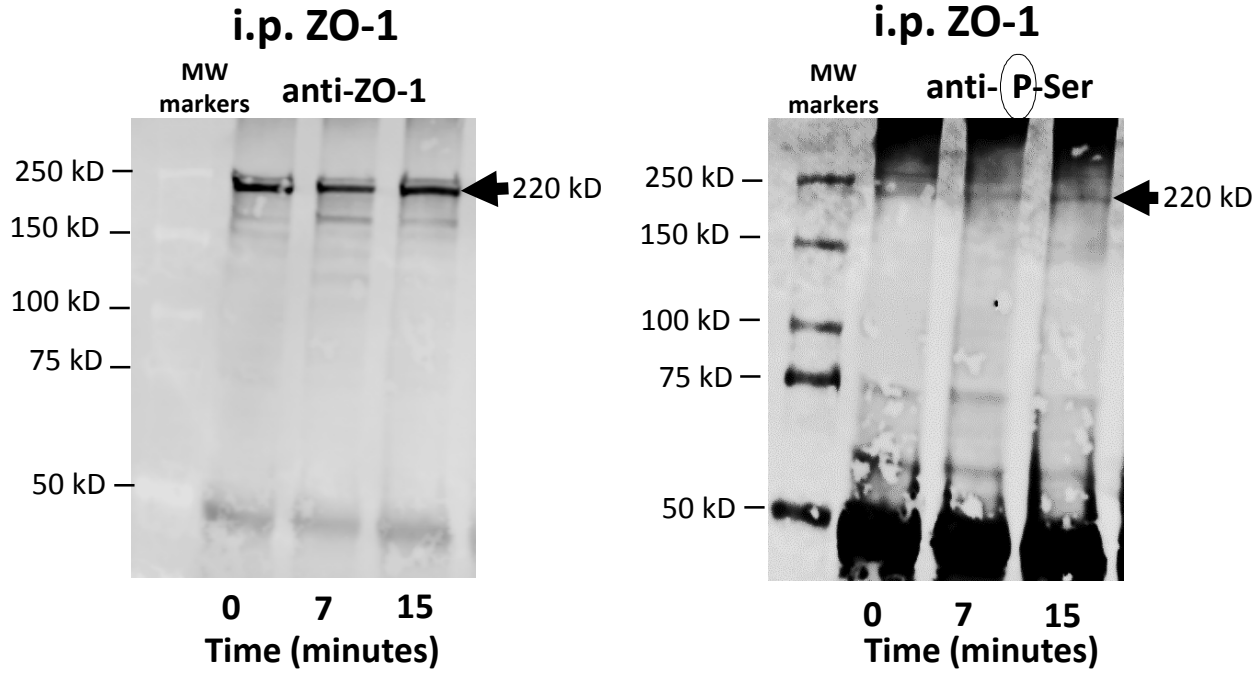
Supplement Figure 1. VCAM-1 signals induce serine phosphorylation of ZO-1 in HMEC-L cells. In panel A, HMEC-L cells were not transfected and in panel B HMEC-L cells were transfected with dominant negative PKCα or control vector. The confluent HMEC-L cells were stimulated with 27 µg/ml anti-VCAM-1 antibody plus 15 µg/ml a secondary antibody. At 0-30 minutes for panel A or 15 minutes for panel B, cells were lysed and ZO-1 was immunoprecipitated (i.p.). ZO-1 and serine phosphorylation were examined by western blot with anti-ZO-1 or anti-phosphoserine. A) Shown are the micrographs from Figure 2A without cropping of the image. The gel was cut at the top just below loading wells and at the bottom of the gel at about 75 kD to remove the lower antibody bands (<50 kD) resulting from the antibodies used in the immunoprecipitations. The antibody bands that are present in all the gels with immunoprecipitation of ZO-1 are evident in an uncut gel in supplement Figure 2B. B) Shown are the micrographs from Figure 2E without removal of the spaces between the lanes 1 and 2 and between lanes 2 and 3 in the upper blot for detection of anti-phosphoserine.

Supplement Figure 2

A (includes lane not removed from blot of Figure 3A)



B (uncropped blots from Figure 3B)



Supplement Figure 2. VCAM-1 signals induce serine phosphorylation of ZO-1 in endothelial cells. mHEV cells were nontreated (NT), pretreated with inhibitors as in Figure 2 and then stimulated with anti-VCAM-1 plus a secondary antibody for 15 minutes (panels A) or with 1μM H_2O_2 (panel B). ZO-1 was immunoprecipitated and examined by western blot. A) Shown is the blot from Figure 3A without removal of the deleted lane. B) Shown is the blot from Figure 3B without cropping. The gel was cut just below antibody chains at 50 kD.