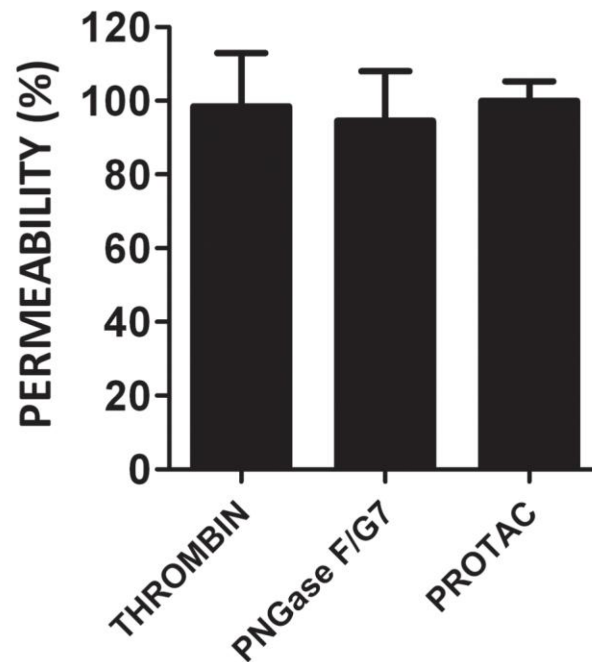


Supplementary Figure 1: PNGase F and Protac do not influence thrombin-induced endothelial cell barrier permeability. Thrombin-induced endothelial cell barrier permeability was determined as described in 'Experimental Procedures'. In this experiment, PNGase F and its buffer G7 or Protac were incubated for 3 hours prior to addition of 5nM thrombin for 10 minutes. Both PNGase/G7 and Protac reagent final concentrations were set to those equivalent to experiments with APC present. Endothelial barrier permeability was detected using Evans Blue-BSA transmigration as described in 'Experimental Procedures'. The data represent experiments performed in triplicate and plotted as the mean ± S.E.M.

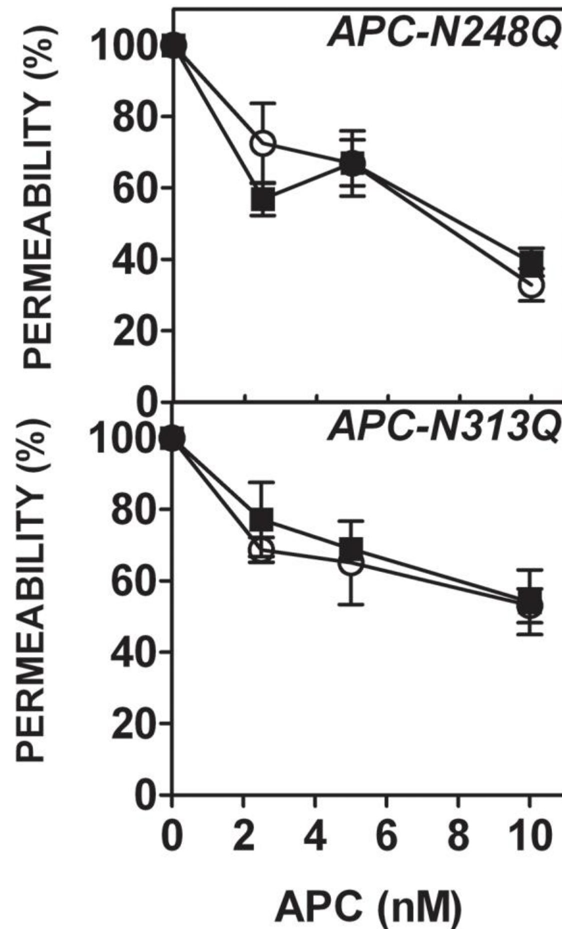
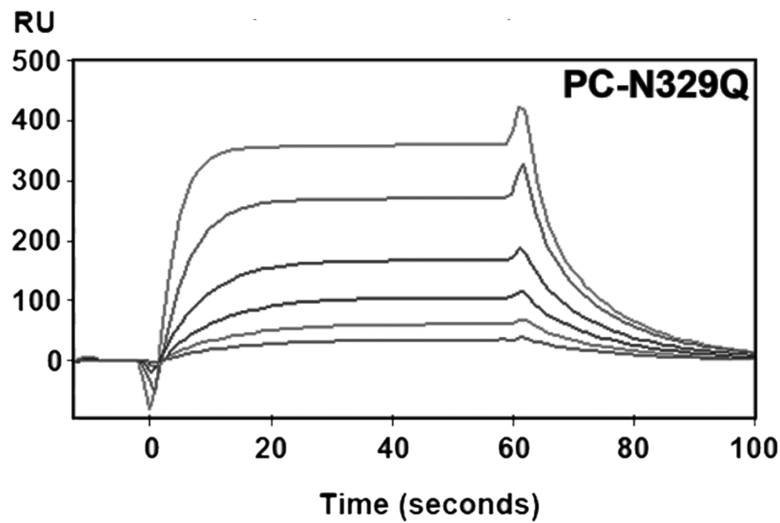


Figure 2: Variants *APC-N248Q* and *APC-N313Q* exhibit comparable endothelial cell barrier protective function to wild type APC: Thrombin-induced endothelial cell barrier permeability was determined as described in 'Experimental Procedures'. EA.hy926 cells were incubated with wild type APC (○), *APC-N248Q* (■; *top panel*) or *APC-N313Q* (■; *bottom panel*) (2-10nM; all final concentrations). After 3 hours, EA.hy926 cells were treated with 5nM thrombin in serum-free media for 10 minutes. The cells were then washed and incubated with 0.67 mg/ml Evans Blue with 4% bovine serum albumin. Changes in endothelial cell barrier permeability were determined by assessment of the increase in absorbance at 650nm in the outer chamber as before.



Supplementary Figure 3: Assessment of *PC-N329Q* Binding to Soluble EPCR. Protein C-sEPCR binding affinity was determined as previously described (19). Briefly, 10 μg/ml monoclonal anti-EPCR antibody, RCR-2 (kind gift of Dr. K. Fukudome, Saga Medical School), was immobilized by amine coupling to both flow cells of a CM5 sensor chip. Soluble EPCR (sEPCR) (19) was dialysed into HBS-P buffer (100mM HEPES, pH 7.4, 150mM NaCl) and bound to the test flow cell via RCR-2. RCR-2 was immobilized on the reference flow cell to detect non-specific binding. *PC-N329Q* (31.25–1000nM) was sequentially injected over both flow cells at a flow rate of 10μl/min for 60 seconds. APC-EPCR binding was dissociated using HBS-EP buffer (HBS-P, but containing 3mM EDTA; BIAcore) The RCR-2 surface was regenerated with 10μl of 10mM glycine-HCl (pH 2).