Figure S5. (a) The level of the introduced PDGFR. Monolayers of BRECs stably expressing the indicated PDGFR construct were lysed, and subjected to Western blot analysis. A “+” and “∗” indicate the immature and mature forms of the receptor, respectively. The blot was then stripped and re-probed with an anti-RasGAP antibody. The normalized level of PDGFR expression was within 3 fold. (b) The recruitment of p85 to PDGFR. Monolayers of BRECs stably expressing the indicated PDGFR construct were starved in EBM with 0.2 % HS for 16-18 h. PDGF (10 ng/ml) or buffer was added for 5 min. Total cell lysates were immunoprecipitated with a PDGFR antibody, and subjected to Western blot analysis followed by anti-p85. The blot was then stripped and re-probed with an anti-PDGFR antibody. A arrow indicates p85. (c) The activation of PLCγ after recruitment to PDGFR. Monolayers of BRECs stably expressing the indicated PDGFR construct were starved in EBM with 0.2 % HS for 16-18 h. PDGF (10 ng/ml) or buffer was added for 5 min. Total cell lysates were immunoprecipitated with a PLCγ antibody. The phosphotyrosine content of PLCγ was assessed by Western blot analysis using an anti-phosphotyrosine antibody. The blot was then stripped and re-probed with an anti- PLCγ antibody.

Material and Methods

Antibody. Rabbit polyclonal anti-p85 antibody was purchased from Upstate Biotechnology Inc (Lake Placid, NY).