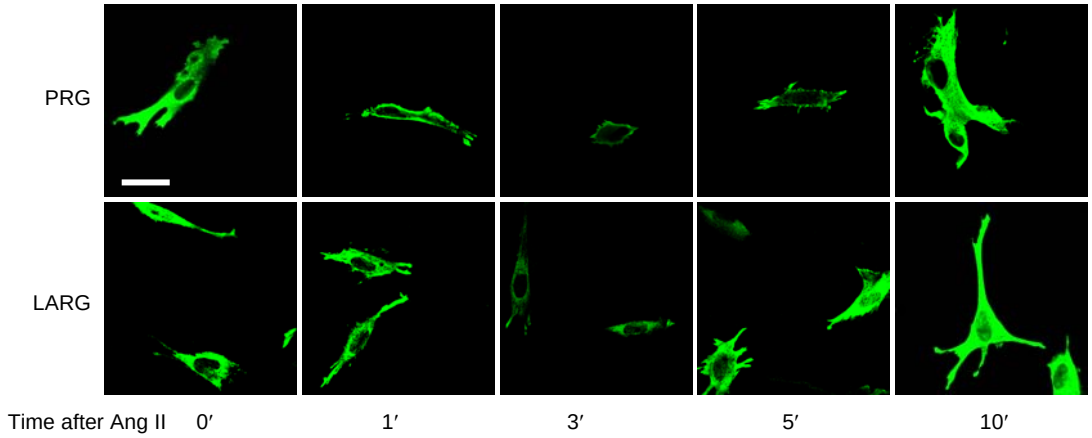
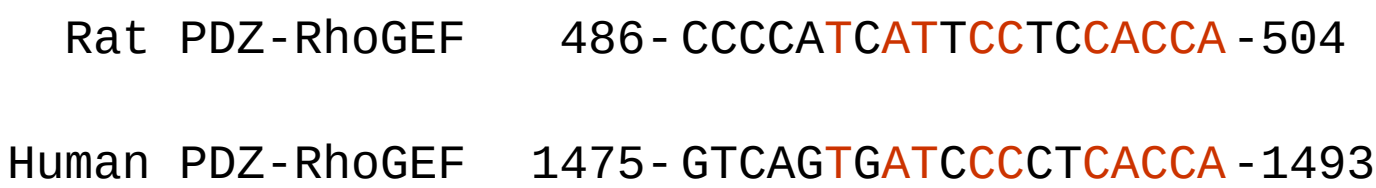
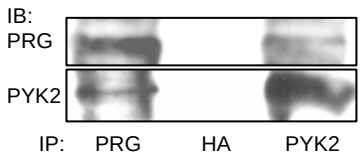
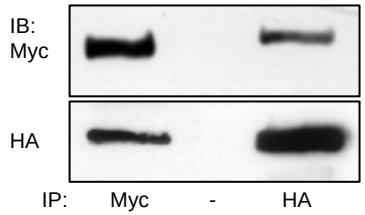


Supplementary figure I. Angiotensin II (AngII) induces translocation of PDZ-RhoGEF (PRG). Rat VSMCs were infected with LARG or PDZ-RhoGEF adenoviral vector. After 40 hours, these cells were stimulated with angiotensin II (100 nM) for the indicated time, and then the subcellular localization of over-expressed LARG or PDZ-RhoGEF is analyzed by immunofluorescent.

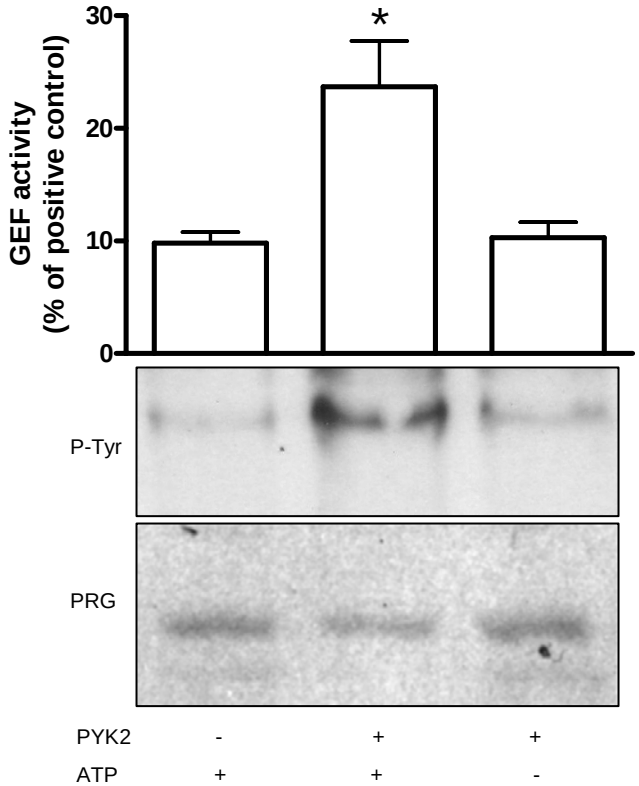


Supplementary figure II. The sequence comparison of human and rat PDZ-RhoGEF at the siRNA target site. Genbank accession number: NM_014784 for human PDZ-RhoGEF and NM_023982 for rat PDZ-RhoGEF. Red indicates the identical sequence.

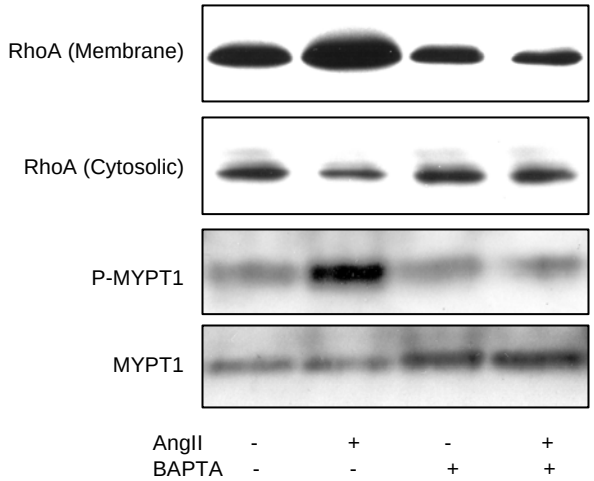




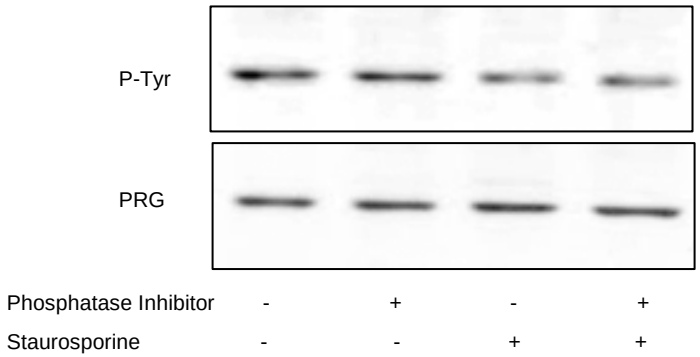
Supplementary figure III. PDZ-RhoGEF interacts with PYK2. A, Cell lysates were prepared from Cultured rat vascular smooth muscle cells, and subjected to immuno-precipitation analysis. B, Myc tagged PDZ-RhoGEF and HA tagged PYK2 were co-expressed in HEK293T cells, and analyzed by immuno-precipitation with anti-Myc and anti-HA. IB, immunoblot; IP, immunoprecipitation.



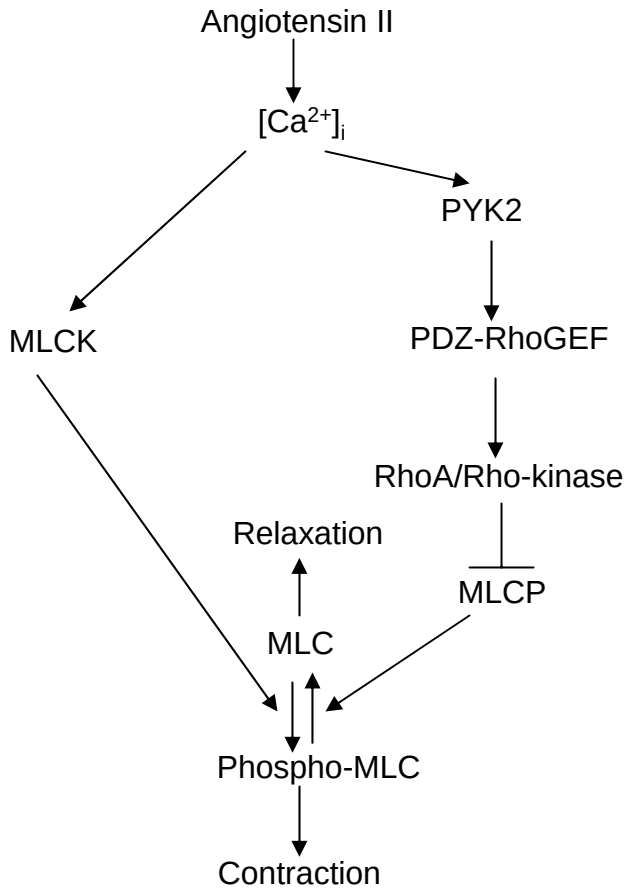
Supplementary figure IV. PDZ-RhoGEF activity was regulated by tyrosine phosphorylation. Immunoprecipitated PDZ-RhoGEF(PRG) was phosphorylated by commercially available constitutively active PYK2 *in vitro*. The GEF activity of PDZ-RhoGEF was measured in triple using RhoGEF exchange assay Biochem Kit (Cytoskeleton), and tyrosine phosphorylation level was analyzed by western blotting.



Supplementary figure V. Angiotensin II activates RhoA in a Ca^{2+}-dependent manner. Cultured rat vascular smooth muscle cells were pre-treated with AM-BAPTA for 15 minutes, and then stimulated with 100 nM angiotensin II (AngII) for 3 minutes. Cell lysates were prepared with RIPA buffer or homogenizing buffer (RhoA translocation), and were analyzed by western blotting.



Supplementary figure VI. Staurosporine efficiently inhibited PDZ-RhoGEF phosphorylation by PYK2. Myc-tagged PDZ-RhoGEF was over-expressed in HEK293T cells, and then immuno-precipitated with anti-myc antibody. After phosphorylated by PYK2, PDZ-RhoGEF was incubated at 37°C for 2 hours in the presence of staurosporine and/or phosphatase inhibitor (Halt Phosphatase Inhibitor Cocktail , Pierce). The resultant products were subjected to western blot analysis with anti-phospho-tyrosine or anti-myc.



Supplementary Figure VII. Proposed mechanism whereby angiotensin II activates RhoA through PYK2/PDZ-RhoGEF signaling.