Supporting Information

Yang *et al*. 10.1073/pnas.0802896105

Experimental Procedures

Reagents and Antibodies. L-histidinol and anti-flag antibodies were purchased from Sigma. Antibodies against MAPK, phospho-MAPK, Akt, phospho-Akt, and phospholipase Cγ were purchased from Cell Signaling Technology. Antiphosphotyrosine (4G10) antibodies were from Upstate Technology. Antiubiquitin antibodies (P4D1) were from Santa Cruz. Antibodies against PDGFRβ were produced by immunization of rabbit with synthetic peptides from the cytoplasmic domain of PDGFRβ. PDGF BB cDNA was obtained from Stuart Aaronson (Mount Sinai School of Medicine, New York). PDGF BB was purchased from Invitrogen and produced in bacteria as described (1). ^{125}I radionuclide was purchased from Perkin–Elmer. Bolton–Hunter reagent and IODO-GEN precoated iodination tubes were from Pierce. FITC-phalloidin was purchased from Invitrogen.

Sequence Alignment and Homology Modeling. Amino acid sequence alignment was performed using the CONSEQ server (2) and according to the IgSF fold characteristics (3) as well as according to the core residues of the Ig-fold of D4 of human KIT structure (4). The Protein Data Bank (PDB) ID codes of each sequence are: PDGFRα human (P16234), mouse (P26618), chicken (Q9PUF6), frog (P26619) and fugu (Q8AXC7); PDGFRβ human (P09619), dog (Q6QNF3), mouse (P05622), fugu (P79749), and KIT human (P10721). A homology model of D4 of PDGFRβ was generated on the basis of D4 KIT structure (PDB ID code: 2E9W) using the WHAT IF server (5). Figures were generated using PyMOL (Delano Scientific, www.pymol.org).

Immunoprecipitation and Immunoblotting. Unstimulated or PDGF-stimulated cells were lysed in a buffer solution containing 50 mM Hepes, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 25 mM sodium fluoride, 1 mM orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 5 μg of aprotinin and leupeptin (pH 7.5). Equal amounts of cell lysates were immunoprecipitated with indicated antibodies, immunopellets were resolved by SDS/PAGE and transferred to nitrocellulose membrane. Membranes were immunoblotted with different antibodies.

1. Hoppe J, Weich HA, Eichner W, Tatje D (1990) Preparation of biologically active platelet-derived growth factor isoforms AA and AB. Preferential formation of AB heterodimers. *Eur J Biochem* 187:207–214.
2. Berezin C, *et al*. (2004) ConSeq: the identification of functionally and structurally important residues in protein sequences. *Bioinformatics* 20:1322–1324.
3. Harpaz Y, Chothia C (1994) Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. *J Mol Biol* 238:528–539.
4. Yuzawa S, *et al*. (2007) Structural basis for activation of the receptor tyrosine kinase KIT by stem cell factor. *Cell* 130:323–334.
5. Rodriguez R, Chinea G, Lopez N, Pons T, Vriend G (1998) Homology modeling, model and software evaluation: three related resources. *Bioinformatics* 14:523–528.

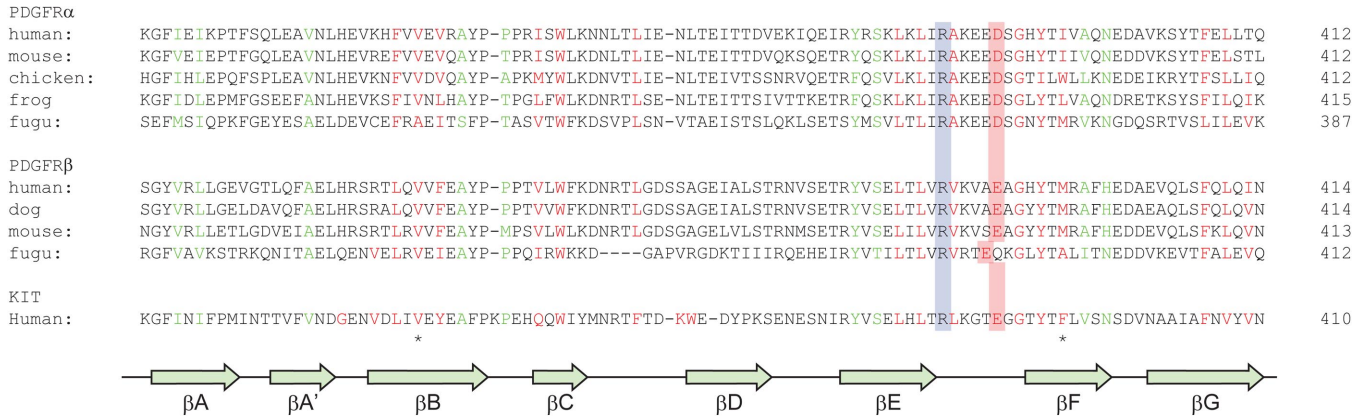

Fig. S1. Structure-based sequence analysis of membrane proximal region of PDGF receptors. Alignment of amino acid sequences of D4 of PDGFRα, PDGFRβ and KIT. The amino acids of key residues of the IgSF fold and the core residues of the Ig-fold of D4 of human KIT structure are colored in red and green, correspondingly. The two key basic and acidic residues responsible for D4 homotypic interaction are boxed in blue and red, respectively. Positions corresponding to the conserved disulfide bond-forming cysteine residues on the Ig-like domain (B5 and F5) are marked by asterisks. β-strands are labeled by arrows below KIT sequence. Secondary structure elements are marked according to the IgSF nomenclature.

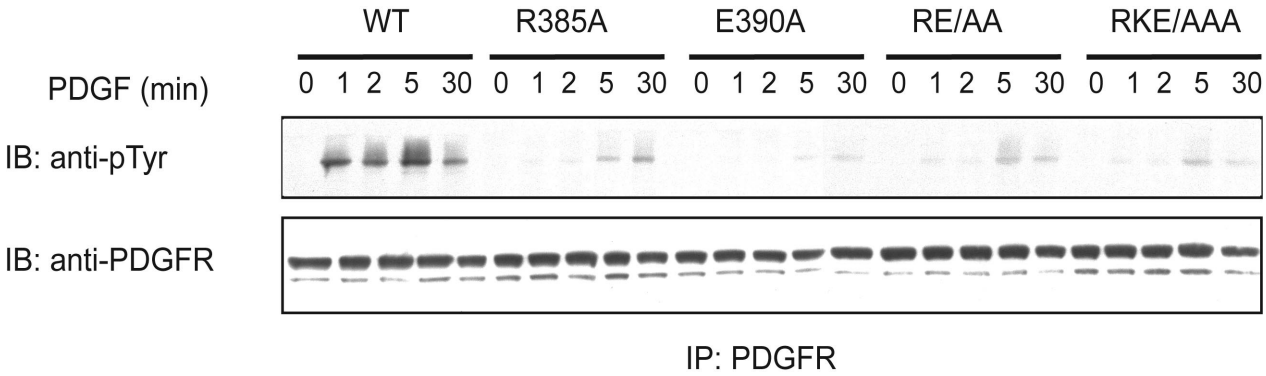

Fig. S2. PDGFRα/β-/- MEFs expressing WT PDGFRβ, and various PDGFRβ D4 mutants were serum starved overnight and stimulated with 25 ng/ml PDGF for indicated time at 37°C. Cell lysates were immunoprecipitated with anti-PDGFR antibodies, followed by immunoblotting with anti-phosphotyrosine antibodies 4G10. Membranes were stripped off, and re-blotted with anti-flag antibodies.

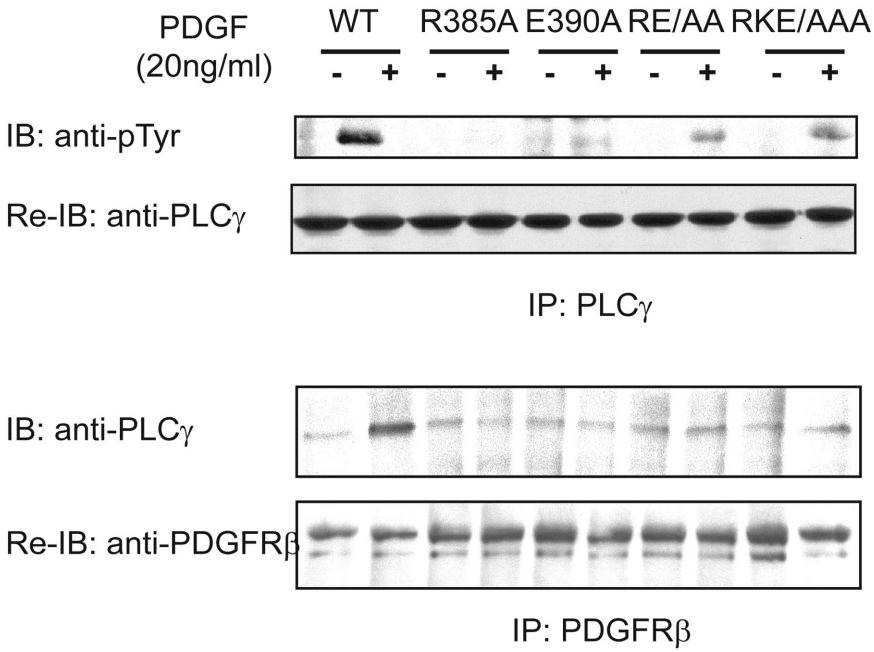

Fig. S3. Cells were stimulated with 10 ng/ml PDGF for 5 min as described above. Equal amount of lysates were immunoprecipitated with anti-PDGFR antibodies, followed by immunoblotting with anti-PLCγ. PLCγ phosphorylation was detected by immunoprecipitation with anti- PLCγ antibodies followed by immunoblotting with anti-phosphotyrosine antibodies.

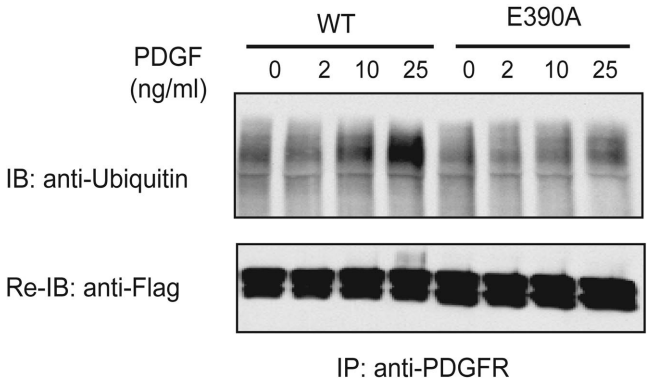

Fig. S4. Cells were serum starved overnight and treated with different concentration of PDGF (0–25 ng/ml). Cell lysates were immunoprecipitated with anti-PDGFR antibodies, and immunoblotted with anti-ubiquitin antibodies.