Supplementary information 1

Plasmids encoding full-length cDNAs of mouse EphB2 and human Ephrin-B1 were donated by T. Pawson and R. Klein, respectively. The plasmid encoding full-length EphA2 was described previously (Wang *et al*., 2002). Expression constructs of EphB2-Fc, EphA2-Fc and EphA4-Fc were generated by fusing the EphB2, EphA2 or EphA4 coding sequence (EphB2$^{1\text{-}560}$, EphA2$^{1\text{-}540}$ and EphA4$^{1\text{-}547}$) respectively to a mouse IgG2b Fc domain. The Fc-fusion protein expression constructs of ephrin-B1 and ephrin-A1 were described previously (Wang *et al*., 2002). Mutants of Ephrin-B1 and EphA2, which lack the cytoplasmic domain (Ephrin-B1$^{1\text{-}265}$ and EphA2$^{1\text{-}563}$, respectively), were generated using a PCR-based technique and tagged with EGFP at the carboxy terminus by cloning into pEGFP-N3 (Clontech). The plasmid encoding human Tiam1 was donated by N. Kawazoe and K. Nakaya. Mutants of Tiam1 (C1199, C682, N392; nomenclature refers to the number of COOH-terminal or NH2-terminal amino acids of the encoded Tiam1 proteins, PHnTSS$^{431\text{-}669}$ and Tiam1$^{1\text{-}669}$, Fig. 1b) were also generated by the PCR-based technique as described before. The plasmid encoding PHnTSS fragment of STEF (STEF$^{464\text{-}780}$) was provided by M. Hoshino. All constructs were cloned into pAlterMax (Promega) or pGEX-2T (Amersham Pharmacia) to prepare glutathione-S transferase (GST) fusion proteins in bacteria. The PHnTSS$^{431\text{-}669}$ fragment of Tiam1 and Tiam1$^{1\text{--}669}$ were also cloned into pEGFP-N3. The plasmid pGEX2T-PBD was generated by cloning a PCR-amplified fragment of putative p21 binding domain of human PAK1 (amino acids 70-133) into pGEX-2T for affinity precipitation as described previously (Otsuki *et al*., 2001). The monoclonal antibodies for the myc epitope tag (9E10) and flag tag (M2) were from Santa Cruz Biotechnology, Inc. and Sigma, respectively. The polyclonal antibodies for Tiam1 (C16), ephrin-B1 (C18), EphA4 (S20), EphB2 (C20), Rac1, FAK (C20), p130^{cas} (C20), GFP and GST were purchased from Santa Cruz Biotechnology, Inc. The goat polyclonal antibody for ephrin-B1 was also purchased from R&D systems. Monoclonal antibodies for EphA2 and phosphotyrosine (4G10) were from Upstate Biotechnology. Phosphothreonine and phosphoserine-specific antibodies were obtained from Zymed Laboratories Inc. FITC-conjugated second antibodies of anti-goat IgG, anti-human IgG and anti-rabbit IgG, and rhodamine-conjugated anti-rabbit IgG and anti-mouse

IgG were from Santa Cruz. Fusion proteins of Eph and ephrin with Fc-region of immunoglobulin were purified from culture medium of 293T cells transfected with plasmids encoding those Fc-fusion proteins by passing them through a Protein A sepharose column as described before. (Wang *et al*., 2002) For some experiments, EphB2-Fc, ephrin-A1-Fc and EphA4-Fc were also purchased from a company (R&D systems).