Supplementary Figure Legends

Fig. S1. The expression level of Brk in HeLa cell derivatives and the effect of Brk on cell spreading. (A) HeLa cells were infected with lentivirus carrying various siRNAs as indicated and then selected with puromycin. Cells were lysed for Western blot with Brk or tubulin antibody as indicated. (B) Cells as in (A) were plated at a density of 1×10^5 cells/well in a 6-well plate, and representative cell images at 1 and 5 hr are shown (left panel). Cell images at 1, 3 and 5 hr after plating were captured by phase contrast microcopy, and cell areas were analyzed by the Olympus Analysis LS Research software and plotted (right panel). Data shown are means ± S.D. of 30 cells (**, $p<0.005$; ***; $p<0.0005$, as compared with parental HeLa cells).

Fig. S2. Association of tyrosine phosphorylated proteins with Brk. 293T cells transfected with Flag-Brk were lysed for immunoprecipitation with M2 agarose beads. The bound proteins were analyzed by Western blot with the anti-phosphotyrosine antibody. The positions of Brk and paxillin are indicated, whereas asterisk marks the protein band larger than 172 kDa.

Fig. S3. Brk interacts with p190. HeLa cells transfected with control vector or Flag-Brk were lysed for immunoprecipitation with anti-p190. The immunoprecipitates and cell lysate were analyzed by Western blot with anti-p190 or anti-Brk as indicated.

Fig. S4. Characterization of the anti-Brk antibody by immunoprecipitation analysis. T47D cells mock transfected or transfected with Flag-Brk were lysed for

immunoprecipitation analysis with anti-Brk antibody, M2 agarose beads (Flag), or a control antibody (IgG). The immunoprecipitates were analyzed by Western blot with anti-Brk antibody. Arrow marks the position of Brk.

Fig. S5. Purity of recombinant Brk protein. Flag-Brk immunoprecipitated from lysate of baculovirus by M2 agarose beads and then eluted by Flag peptide was run on SDS-PAGE and stained with Coomassie blue.

Fig. S6. Quantitative analyses of GTP-bound Rho and GTP-bound Ras. Data from experiments described in Fig. 2C, D were quantified as means ± S.D. (*$p<0.05$; **$p<0.005$, as compared with cells without receiving siRNA (A) or cells carrying control vector (B); n=3).

Fig. S7. Quantitative data for experiments described in Fig. 3. A431 cells (A, B) or T47D cells (C, D) expressing indicated siRNA were analyzed for the level of Brk or Src (A, C), or for tyrosine phosphorylated p190 (B, D). Data shown are means ± S.D. (*$p<0.05$; **$p<0.005$, as compared with cells without receiving siRNA (-); n=3).

Fig. S8. Stably expression of Brk and/or p190 wild type or mutant in p190 null MEFs. p190-/- MEFs were infected with retrovirus expressing p190 or its Y1105F mutant (YF) and then selected with hygromycin. The resulting three cell lines were infected with retrovirus carrying Brk or control vector and then selected with puromycin. Cells were lysed for Western blot with antibodies as indicated.

Fig. S9. Quantitative analyses of GTP-bound Rho and GTP-bound Ras. Data from

experiments described in Fig. 6B were quantified as means ± S.D. (*$p<0.05$; **$p<0.005$, as compared with cells carrying control vectors; n=3).

Fig. S10. Soft agar colony formation capabilities of indicated cell populations. MDA-MB231 cells stably expressing Brk and/or $p120^{2\text{-}3\text{-}3}$ were seeded in soft agar, and colonies were stained with crystal violet after 4 weeks.

Fig. S11. MDA-MB231 cells stably expressing Brk and/or $p120^{2\text{-}3\text{-}3}$ were injected subcutaneously into nude mice. Representative photographs of mice and tumors at 67 days after injection are shown.

Fig. S12. Synergistic activation of Rac1 by Brk and p190. HeLa cells were transfected with Brk and/or p190. Cells were lysed for assaying GTP-bound Rac1 as described in the Materials and Methods, or for Western blot with antibodies as indicated. The amount of GTP-bound Rac was normalized by using that of total Rac in cell lysates, and is expressed as the fold of induction relative to cells transfected with the control vector.