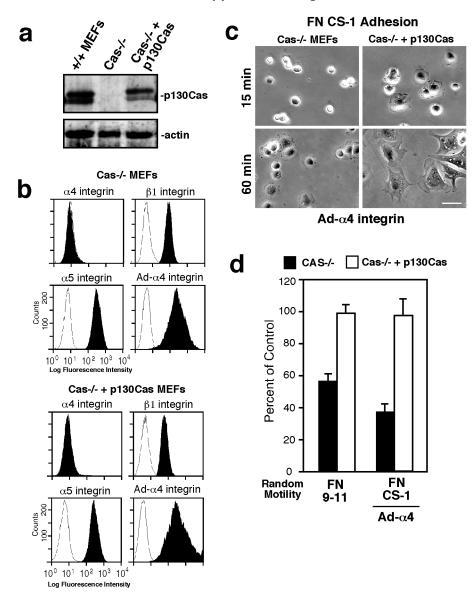


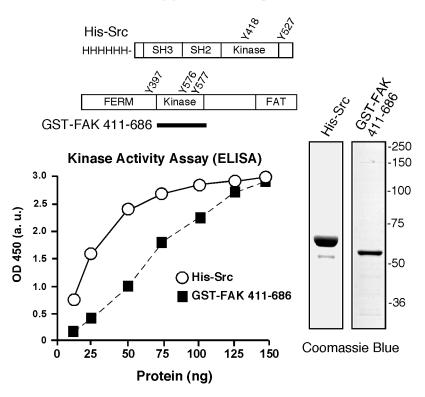
**Supplemental Figure 1.** Recombinant FN (CS-1) or FN (9-11) stimulate  $\alpha 4\beta 1$ - or  $\alpha 5\beta 1$ -mediated NB cell motility, respectively. (a) Quantitative analysis of NB8 and SKNAS cell adhesion after 15 min to cellular FN, FN (CS-1), and FN (9-11). (b) Phase contrast images of NB8 cells adhesion on cellular FN, FN (CS-1), and FN (9-11) after 15 min. Scale bar is 25 μm. (c) NB8 haptotaxis motility performed on cellular FN, FN (CS-1), and FN (9-11) (d) NB cell haptotaxis motility on FN (CS-1) is blocked by anti- $\alpha 4$  mAb (HP2/1, 10 μg/ml) and anti- $\beta 1$  mAb (P4C10, 10 μg/ml) but not by anti- $\alpha 5$  mAb (P1D6, 10 μg/ml). (e) NB haptotaxis cell motility on FN (9-11) is blocked by anti- $\alpha 5$  mAb (P1D6, 10 μg/ml) and anti- $\beta 1$  mAb (P4C10, 10 μg/ml), but not by anti- $\alpha 4$  mAb (HP2/1, 10 μg/ml). (a, c, d, and e) Values are means +/- SD of triplicates from at least two separate experiments.

## Wu et. al., Supplemental Figure 2



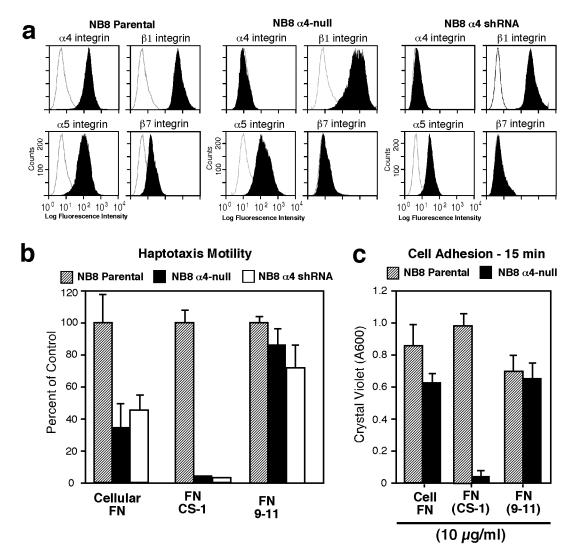
Supplemental Figure 2. Human  $\alpha4$  integrin expression in Cas-/- and Cas-/- +p130Cas fibroblasts. (a) Expression of p130Cas in normal mouse embryonic fibroblasts (MEFs), Cas-/-, and Cas-/- +p130Cas fibroblasts were analyzed anti-p130Cas immunoblotting. Actin blotting verified equal loading. (b) Flow cytometry analyses of endogenous murine  $\alpha4$ ,  $\beta1$ ,  $\alpha5$ , and adenovirus (Ad)-expressed human  $\alpha4$  integrin in Cas-/- and Cas-/- +p130Cas cells (shaded peaks). Staining with control mAb (open peaks). (c) Phase contrast images showing adhesion and spreading on FN (CS-1) of Cas-/- and Cas-/- +p130Cas cells with human  $\alpha4$  integrin expression at indicated time points. Scale bar is 15  $\mu$ m. (d) Random motility assay on FN (9-11) or FN (CS-1) of Cas-/- and Cas-/- +p130Cas cells with or without human  $\alpha4$  integrin expression revealed that p130Cas promotes  $\alpha5\beta1$ - and  $\alpha4\beta1$ -mediated cell motility.

Wu et. al., Supplemental Figure 3



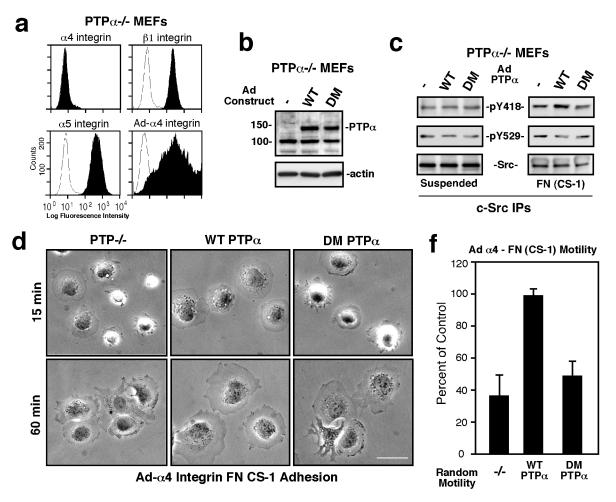
**Supplemental Figure 3**. Activity profile of recombinant His-Src and GST-FAK 411-686. Schematic of His-tagged Src and the FAK kinase domain region (411-686) expressed in baculovirus and initially isolated by Ni-NTA and glutathione agarose, respectively. After size fractionation chromatography, the purity of His-Src and GST-FAK 411-686 was >90% as visualized by SDS-PAGE and Coomassie Blue staining. Shown is an ELISA-based activity assay measuring the phosphorylation of poly Glu:Tyr (4:1).

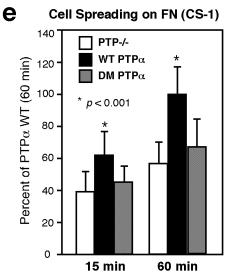
## Wu et. al., Supplemental Figure 4



Supplemental Figure 4. Abolishing  $\alpha 4$  integrin expression inhibits  $\alpha 4\beta 1$ - but not  $\alpha 5\beta 1$ -stimulated NB motility. (a) Flow cytometry analyses of  $\alpha 4$ ,  $\alpha 5$ ,  $\beta 1$ , and  $\beta 7$  integrin expression (shaded peaks) in NB8 parental, NB8  $\alpha 4$ -null sorted, and  $\alpha 4$  shRNA-expressing cells. Open peaks show staining with control mAb. (b) Haptotaxis motility assays show that loss of  $\alpha 4$  integrin expression prevents FN (CS-1) but not FN (9-11)-stimulated NB cell motility and inhibits migration on cellular FN. Values presented are percent of NB8 parental cell motility on cellular FN (Control). (c) Quantitative analysis of NB8 parental and  $\alpha 4$ -null cell adhesion to cellular FN, FN (CS-1), and FN (9-11) after 15 min. (b and c) Values are means +/- SD of triplicates from two separate experiments.

Wu et. al., Supplemental Figure 5





**Supplemental Figure 5.** Human  $\alpha 4$  expression in PTP $\alpha$ -/- fibroblasts reveals that PTP $\alpha$  activity promotes  $\alpha 4\beta 1$ -stimulated cell spreading and motility. (a) Flow cytometric analyses of endogenous murine  $\alpha 4$ ,  $\beta 1$ ,  $\alpha 5$ , and adenovirus (Ad)-expressed human  $\alpha 4$  integrin in PTP $\alpha$ -/- cells (shaded peaks). Open peaks show staining with control mAb. (b) Ad-PTP $\alpha$  reexpression was verified by anti-PTP $\alpha$  blotting of whole cell lysates. Actin blotting verified equal loading. (c) PTP $\alpha$ -/- cells were infected with human Ad- $\alpha 4$  and as indicated, also transduced with either Ad-PTP $\alpha$  (WT) or catalytically-inactive PTP $\alpha$  (DM). After 24 h, cells were serum starved overnight, trypsinized, held in suspension for 45 min or plated onto FN (CS-1) for 45 min prior to cell lysis. Src IPs were sequentially analyzed by Src pY418, Src pY529, and total Src blotting. (d) As in panel C, PTP $\alpha$ -/- cells were transduced with Ad- $\alpha 4$  WT and the indicated Ad-PTP $\alpha$  constructs, and replated onto FN (CS-1). Representative phase contrast images show cell spreading differences at 15 min and 60 min. Scale bar is 15  $\mu$ m. (e) Cell area on FN (CS-1) was measured by ImageJ software and expressed as percentage of WT PTP $\alpha$  cell area at 60 min. Values are means +/- SD from three to four different image fields in which >15 cells were measured per condition. The difference between WT PTP $\alpha$  and PTP-/- or DM PTP $\alpha$  cells is statistically significant (\* p < 0.001). (f) Random motility on FN (CS-1) of PTP $\alpha$ -/- and PTP $\alpha$ -/- re-expression revealed that PTP $\alpha$  activity promotes  $\alpha 4\beta$ 1-mediated cell motility. Values are means +/- SD of triplicates from two separate experiments.