SUPPLEMENTAL FIGURES

Fig. S1. Knockdown of FAK does not affect the formation of peripheral adhesion complexes induced by Palm-PTK6-YF. A) PC3 cells expressing Palm-PTK6-YF were transfected with FAK siRNAs or control siRNAs for 3 days. Cells were stained with anti-phospho-tyrosine antibodies and visualized with FITC (green). Cells were counterstained with DAPI (blue). Size bar denotes 20 μ m. Phase contrast images are also shown. Size bar denotes 50 μ m. B) Immunoblot analysis of total cell lysates of PC3 cells described in A was performed with anti-FAK and β -actin antibodies.

Fig. S2. Tandem mass spectrometry of p130CAS protein phosphorylated by PTK6 in vitro. A) A schematic structure of p130CAS protein shows that it contains four discrete domains: an SH3 domain, a substrate domain, a 4-helix bundle (4HB), and an evolutionarily conserved C-terminal domain. B) Eleven tyrosine residues of p130CAS phosphorylated by PTK6 were identified by microcapillary liquid chromatography tandem mass spectrometry (LC/MS/MS). No phosphorylation was detected in the Y₆₆₄DYVHL motif, probably due to low peptide sequence coverage of this tyrosine residue. * Tyrosine residues 165 and 664 were shown to be directly targeted by PTK6 by using phospho-p130CAS Y165 and Y664 antibodies.

Figure S1



Palm-YF

Figure S2



p130CAS

Β

Tyrosine site	Sequence
Y165*	PATDL Y QVPPG
Y224	GQGYV Y EAAQP
Y234	PEQDE Y DIPRH
Y249	GPQDI Y DVPPV
Y262	LLPSQ Y GQEVY
Y267	YGQEV Y DTPPM
Y287	PLLEV Y DVPPS
Y306	NHHAV Y DVPPS
Y327	LREET Y DVPPA
Y362	PAEDV Y DVPPP
Y387	GPGTL Y DVPRE
Y664*	GWMED Y DYVHL