Supporting Information

Li et al. 10.1073/pnas.1112122108

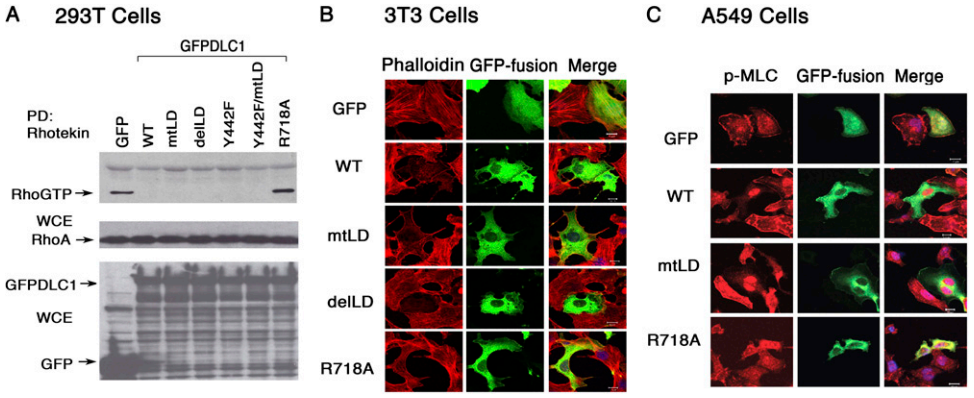

Fig. S1. DLC1 mutants deficient in talin and/or tensin binding retain GAP activity. (*A*) Rho-GAP activity of DLC1 and derived mutants. The in vivo level of GFP-tagged WT or mutant DLC1 in 293T cells was analyzed by Rhotekin pull-down assay as described. Total RhoA and transfected DLC1 are shown as loading controls. (*B* and *C*) Rho-GAP activity-associated immune staining. Cells expressing WT or mutant GFP-DLC1 were stained in red with phalloidin (*B*) or anti-phosphomyosin light chain 2 (MLC2; *C*) in GFP-fusion transfected cells (green) to show stress fiber formation in NIH 3T3 cells (*B*) and activated MLC2 in A549 NSCLC line (*C*).

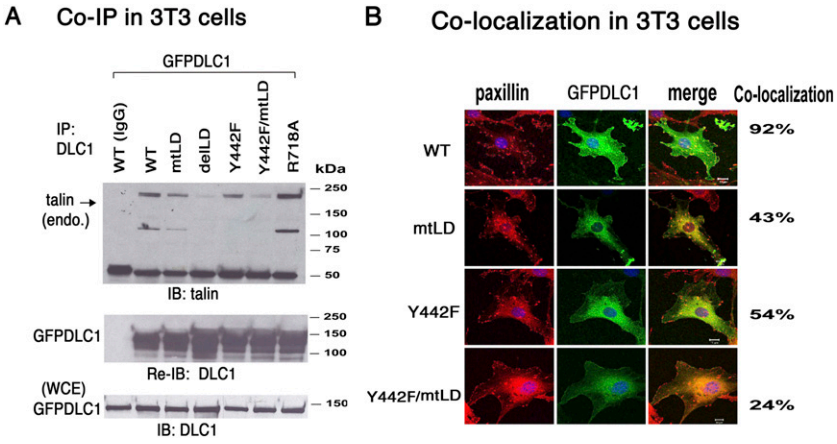

Fig. S2. DLC1 mutants deficient in talin and/or tensin binding display impaired colocalization with the focal adhesion marker paxillin. (*A*) Coimmunoprecipitation of endogenous talin with WT or mutant GFPDLC1 in NIH 3T3 cells. The transfected GFPDLC1 was immunoprecipitated and then followed by anti-talin immunoblotting. (*B*) Colocalization of transfected GFPDLC1 with endogenous paxillin. WT or mutant GFPDLC1 (green) was expressed in NIH 3T3 cells, and the focal adhesion marker paxillin (red) was stained. The confocal images are representative of more than 50 cells observed. The frequency of each colocalization was scored as shown. (Scale bar: 10 μm.)

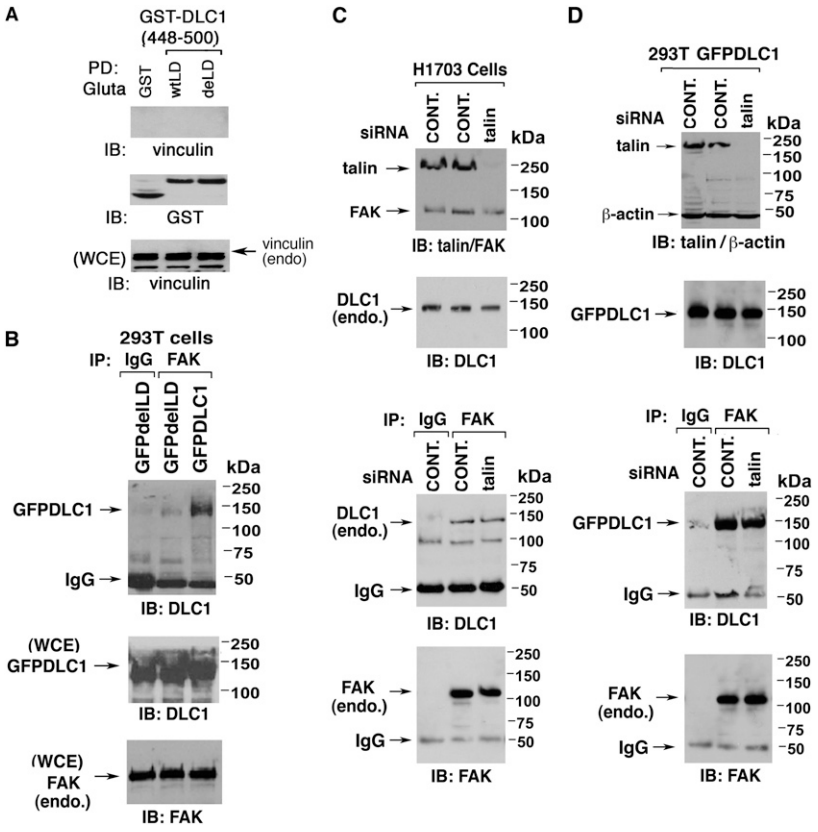

Fig. S3. No detection of LD-like domain-related complex with vinculin; the complex between DLC1 and FAK depends on the LD-like motif and is independent of talin binding. (*A*) Interaction between the LD-like motif of DLC1 vinculin was undetectable. The endogenous vinculin was not detected after pull-down assay with GST-DLC1 (448–500) in 293T cells under the tested condition. (*B*) Deletion of the LD-like motif in DLC1 impairs complex formation with FAK; 293T cells were transfected with WT or delLD GFPDLC1 followed by coimmunoprecipitation as described (*Top*). The input of transfected GFPDLC1 or endogenous FAK is shown. (*C*) The endogenous DLC1/FAK complex is talin-independent; H1703 cells were transfected with control or talin siRNA for 4 d. The efficiency of talin knockdown and the expression of endogenous FAK and DLC1 are shown (*Upper*). The cell extracts were immunoprecipitated by control IgG or anti-FAK antibody followed by anti-DLC1 or anti-FAK immunoblot (*Lower*). (*D*) The complex of transfected GFPDLC1 and endogenous FAK is also talin-independent; 293T cells were first transfected with siRNA as indicated for 2 d and then transfected with GFPDLC1 for another 2 d. The expression of talin, β-actin, and FAK are shown (*Upper*). The cells extracts were then used for coimmunoprecipitation as described in *B* (*Lower*).

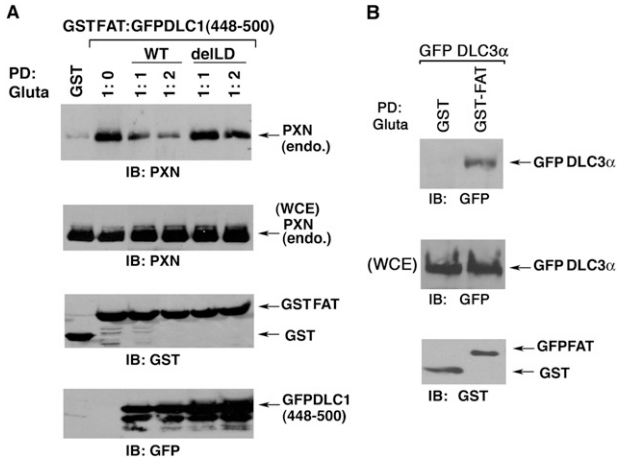

Fig. S4. Competition between paxilin and DLC1 binding to FAT domain of FAK is dependent on the LD-like domain; detection of LD-like motif-related complex formation between FAK and GFPDLC3α. (*A*) GFPDLC1 fragment, but not the delLD fragment, can compete with paxillin for binding to the FAT domain of FAK. GST-FAT with or without increasing amounts of WT or delLD fragment GFPDLC1 (448–500) was cotransfected by GST-FAT at the indicated ratios in 293T cells. The endogenous paxillin binding to GST-FAT was efficiently reduced by increasing levels of GFPDLC1 (448–500), and the effect was greatly attenuated by the fragment carrying the delLD mutation. The expression of transfected proteins and loading controls are shown. (*B*) Pull-down assay of GST-FAT with GFPDLC3α gave a positive signal.

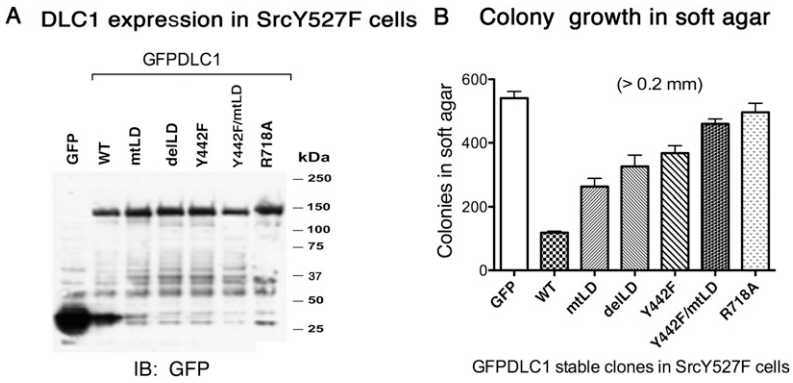

Fig. S5. DLC1 mutants display reduced inhibition of anchorage-independent growth. (*A*) GFP or GFPDLC1 expression in Src (Y527F) transformed cells. NIH 3T3 cells transformed by active Src Y527F were transfected with GFP or WT or mutant GFPDLC1. Cell extracts were analyzed by anti-GFP immunoblot. (*B*) Inhibition of anchorage-independent growth. GFP or GFPDLC1 WT and mutant stable clones generated from Src (Y527F) transformed NIH 3T3 cells shown in *A* were seeded in soft agar plates and grown for 3 wk. Colonies were then stained and counted.

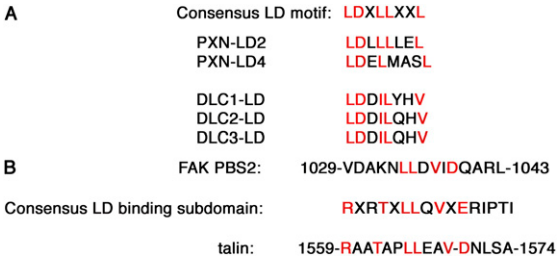

Fig. S6. Sequence comparison of potential binding sites in DLC proteins and its binding partners. (*A*) Comparison with consensus LD motif. The conserved residues in the consensus LD motif are marked in red. The two LD motifs (LD2 and LD4 of paxillin) that bind to the FAT domain FAK are shown. The highly conserved LD-like motif in DLC1–3 is also shown. (*B*) Comparison with consensus LD binding site. The conserved residues in the consensus LD binding sequence are marked in red as reported (1). The consensus LD binding site is compared with the paxillin binding subdomain 2 (PBS2) in the FAT domain of FAK and a sequence in the smallest talin fragment that was positive for binding to DLC1.

1. Tachibana K, Sato T, D'Avirro N, Morimoto C (1995) Direct association of pp125FAK with paxillin, the focal adhesion-targeting mechanism of pp125FAK. *J Exp Med* 182:1089−1099.