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M8 00617 revision 2

Figure Legends for Supplementary Figures.

Supplementary Fig. 1. GAP activity is critical for the morphological changes induced by DLC-1. Versions of pEGFP-DLC-1 ΔSAM with inactivating mutations in the GAP domain (R677E, K714E, K718E) were transfected into MDA-MB-468 cells and localization and morphology observed by a confocal fluorescence microscope. Scale bar, 10 μM.

Supplementary Fig. 2. Definition of the functional DLC-1 GAP domain. *A*, The DLC-1 amino acid sequence was analyzed by using the PROF structure prediction algorithm. The consensus RhoGAP domain with some flanking sequences at both ends and START domain consist of α-helix and β-sheet structures, respectively. *B,C*, Since transfection of an active DLC-1 RhoGAP caused major changes in cell morphology we used this to delineate the boundaries of the functional RhoGAP domain. The effects of a series of amino-terminal or carboxyl-terminal truncations of DLC-1 (see figure) on cell morphology was examined. MDA-MB-468 cells grown on glass coverslips were transiently transfected with EGFP-tagged (B) amino-terminal or (C) carboxyl-terminal truncation mutants of DLC-1 and imaged by fluorescence microscopy.

Supplementary Fig. 3. Expression of DLC-1 constructs. Several EGFP-DLC-1 truncation and mis-sense mutant constructs were transfected into HEK293 cells. Protein expression was via western blotting with anti-GFP antibody.

Supplementary Fig. 4. DLC-1 induces cellular protrusions. HEK293 cells were transiently transfected with pEGFP-DLC-1 ΔSAM and observed via live cell imaging. Cells that were immobile but exhibited extending protrusions were followed over a 20-30 min time period. The image shown is typical of the majority of cells expressing DLC-1 ΔSAM; this type of behavior was never seen in control transfectants.

Supplementary Figures 5,6,7 show multiple images of vinculin staining in MDA-MB-468 cells transiently expressing DLC-1 ΔSAM, DLC-1 ΔN, and DLC-1 RhoGAP, respectively.

Supplementary Figures 8i and 9 are vinculin staining in MDA-MB-468 cells transiently expressing DLC-1 FL and DLC-1N, respectively, showing focal adhesion localization of these proteins. In Figure 8ii are additional MDA-MB-468 cells expressing DLC-1 FL in an apparent focal adhesion localization pattern.

Supplementary Figure 10 illustrates apparent focal adhesion localization in multiple MDA-MB-468 cells stably expressing DLC-1 FL

Supplementary Table 1 summarizes subcellular localization of DLC-1 and presence or absence of focal adhesions in cells transiently transfected with the indicated DLC-1 constructs.