Pan et al., http://www.jcb.org/cgi/content/full/jcb.201103016/DC1

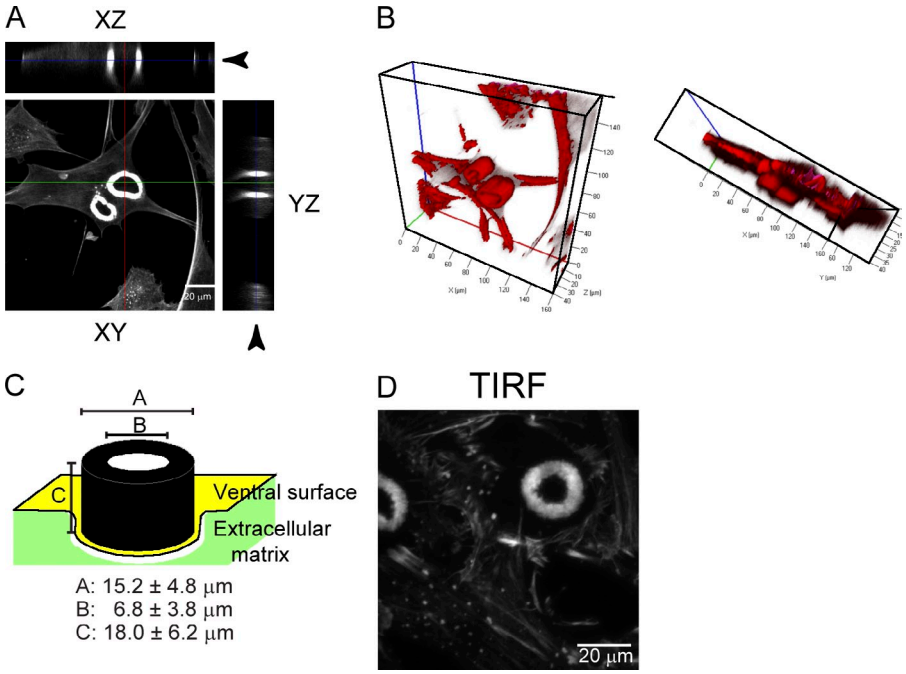

Figure S1. **Podosome rosettes protrude from the ventral surface of cells.** (A) v-Src–transformed MEFs were grown on fibronectin-coated glass coverslips for 24 h and were then stained for F-actin. A representative 3D image is shown, which is reconstituted from 37 confocal slices at 1-µm intervals using laser-scanning microscopy imaging software. The XZ and YZ sections at the indicated positions are shown. (B) The 3D image as shown in A was converted into motion 4D images using VisArt 4D software (LSM 510). Two representative frames are shown. (C) A diagram depicting the structure of podosome rosettes. Values (means ± SD) are from 50 podosome rosettes. Note that approximately one third of the structure is below the ventral surface of cells. (D) v-Src–transformed MEFs were grown on fibronectin-coated glass coverslips for 24 h and were then stained for F-actin. The images were captured by total internal reflection fluorescence (TIRF) microscopy.

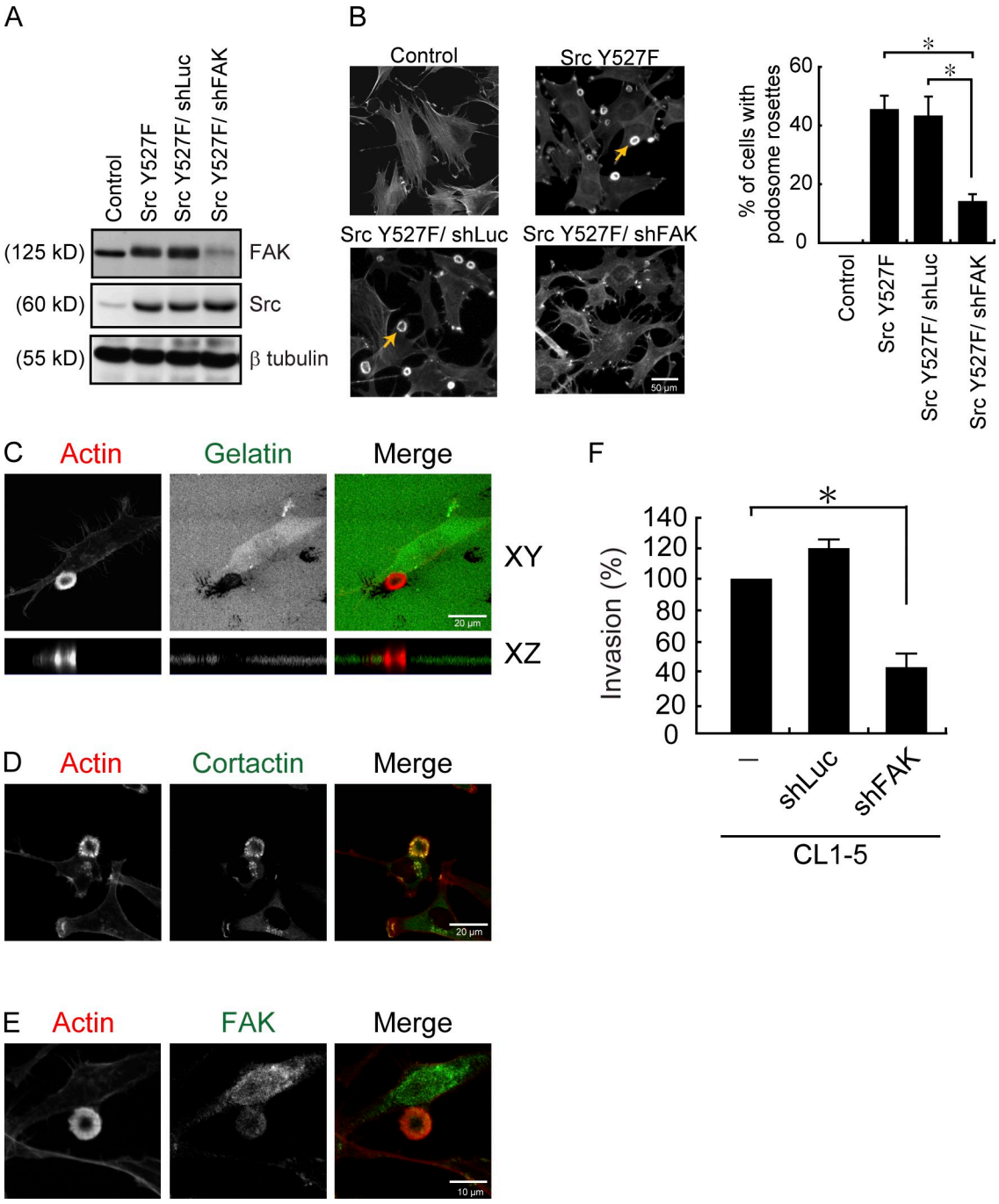

Figure S2. **FAK is crucial for podosome rosettes in SrcY527F-transformed NIH3T3 cells and human lung adenocarcinoma CL1-5 cells.** (A) SrcY527F-transformed NIH3T3 cells were infected with recombinant lentiviruses encoding shRNAs specific to FAK (shFAK) or luciferase (shLuc) as a control. (B) The cells as in A were stained for F-actin. Arrows indicate podosome rosettes. The percentage of the cells with podosome rosettes in the total counted cells was determined. (C) CL1-5 cells were grown on glass coverslips coated with Alexa Fluor 488–conjugated gelatin for 24 h and were then stained for F-actin. Representative images from the XY and XZ sections are shown. (D and E) CL1-5 cells were grown on gelatin-coated glass coverslips and stained for F-actin, cortactin, and FAK. Cortactin served as a marker for podosomes. (F) Control CL1-5 cells and those expressing shLuc, shFAK, or shPYK2 were subjected to an Matrigel invasion assay. Data were quantified and expressed as a percentage relative to the level of the control CL1-5 cells, which was defined as 100%. (B and F) Values (means ± SD) are from three independent experiments. *, $P < 0.005$.

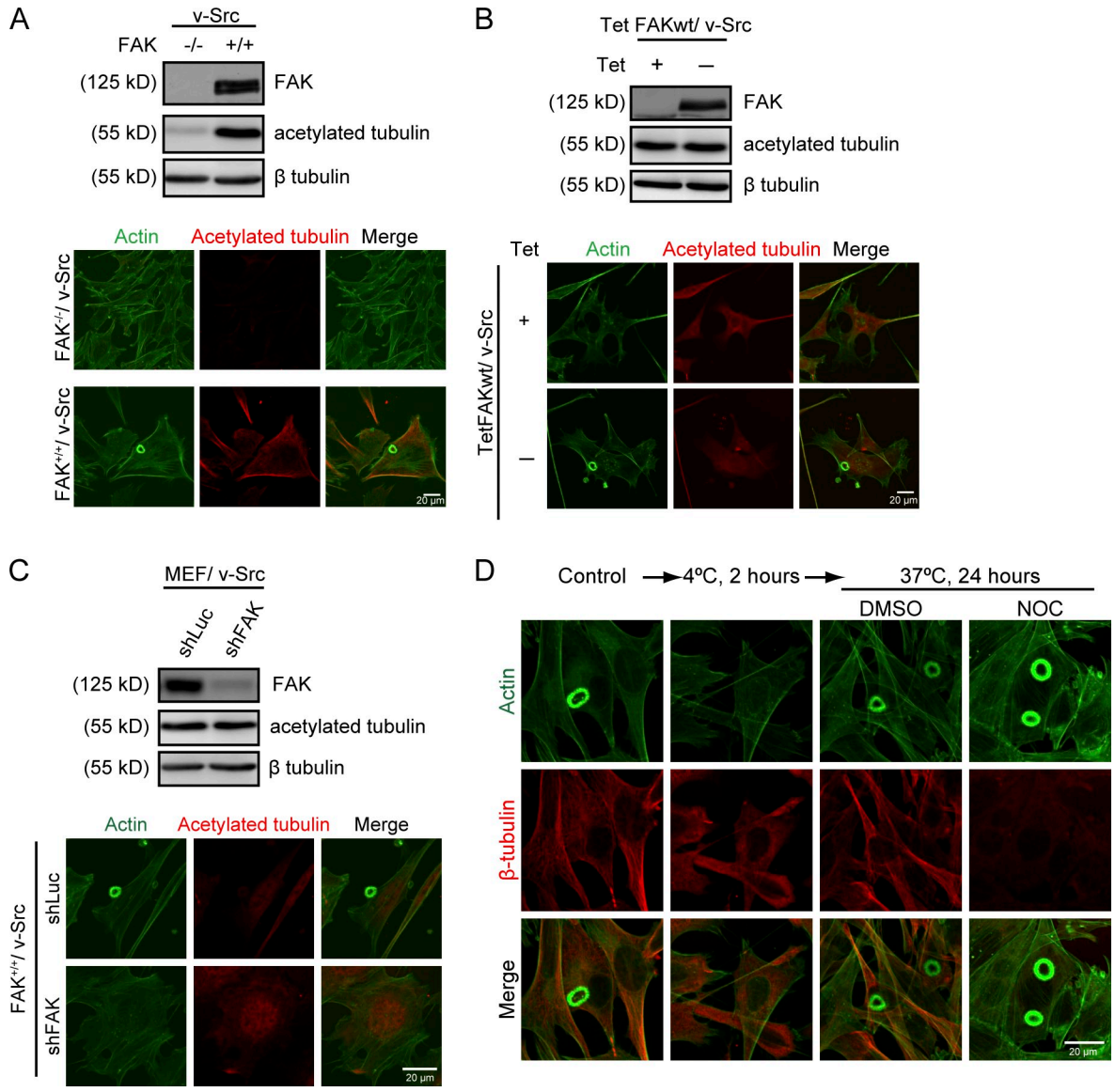

Figure S3. **Assembly of podosome rosettes is independent of the acetylation or integrity of microtubules.** (A) An equal amount of whole-cell lysates from v-Src–transformed FAK$^{+/+}$ MEFs and v-Src–transformed FAK$^{-/-}$ MEFs was analyzed by immunoblotting with antibodies as indicated. The cells were grown on fibronectin for 24 h and stained for F-actin and acetylated tubulin. Representative images from three independent experiments are shown. (B) An inducible (Tet-Off) expression system for the expression of FAK was constructed in v-Src–transformed FAK$^{-/-}$ MEFs (Tet FAK wt/ v-Src). The cells were grown on coverslips in the medium with (+) or without (−) tetracycline for 24 h. The expression of FAK and acetylated tubulin was analyzed by immunoblotting. The cells were fixed and stained for F-actin and acetylated tubulin. (C) v-Src–transformed MEFs were infected with recombinant lentiviruses encoding shRNAs specific to FAK (shFAK) or luciferase (shLuc) as a control. The expression of FAK and acetylated tubulin was analyzed by immunoblotting. Those cells were grown on fibronectin for 24 h and stained for F-actin and acetylated tubulin. (D) v-Src–transformed MEFs were grown on fibronectin-coated glass coverslips for 24 h, and their cytoskeleton including both actin filaments and microtubules was disrupted by cold shock (at 4°C for 2 h) and then recovered at 37°C for 24 h in the presence of 10 μg/ml nocodazole (NOC) or the control solvent DMSO. The cells were fixed and stained for F-actin and β-tubulin.

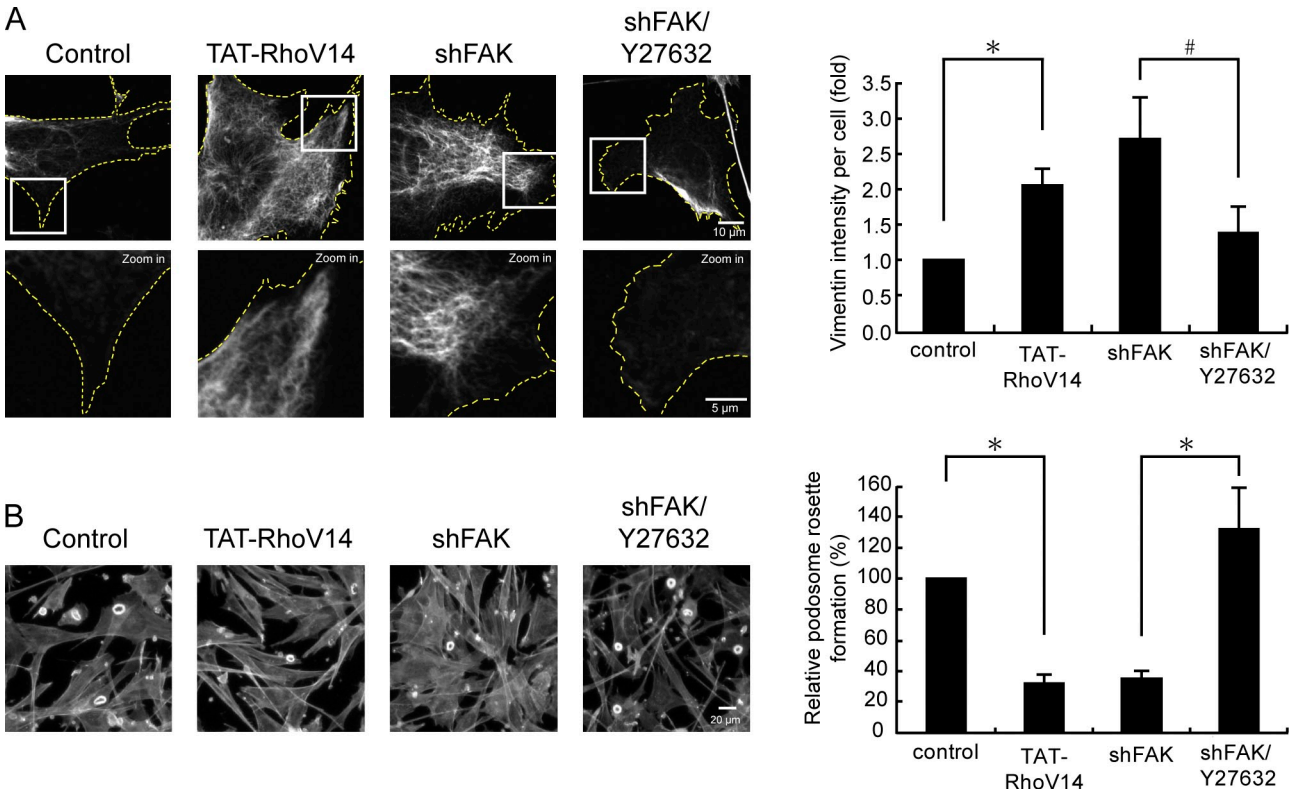

Figure S4. **FAK may promote podosome rosette formation by suppression of Rho signaling and vimentin filaments.** (A) v-Src–transformed MEFs were infected with recombinant lentiviruses encoding shRNAs specific to FAK (shFAK). The cells expressing shFAK were treated with or without 10 μM Y27632 for 12 h. v-Src–transformed MEFs were treated with 5 μg/ml TAT-RhoV14 for 12 h and were then treated with or without 10 μM Y27632 for 12 h. Those cells were fixed and stained for vimentin. The boxed areas in the top images are enlarged. The yellow dashed lines mark the outlines of the cells. The fluorescence intensity of vimentin filaments per cell was measured using the AxioVision Rel. software (version 4.8; $n = 30$) and expressed as fold relative to the level of the control. #, $P < 0.05$. (B) The cells as described in A were grown on fibronectin-coated glass coverslips for 24 h and stained for F-actin. The podosome rosette formation was quantified and expressed as the percentage relative to the level of the control. (A and B) Values (means ± SD) are from three independent experiments. *, $P < 0.005$.