Supplemental Figures

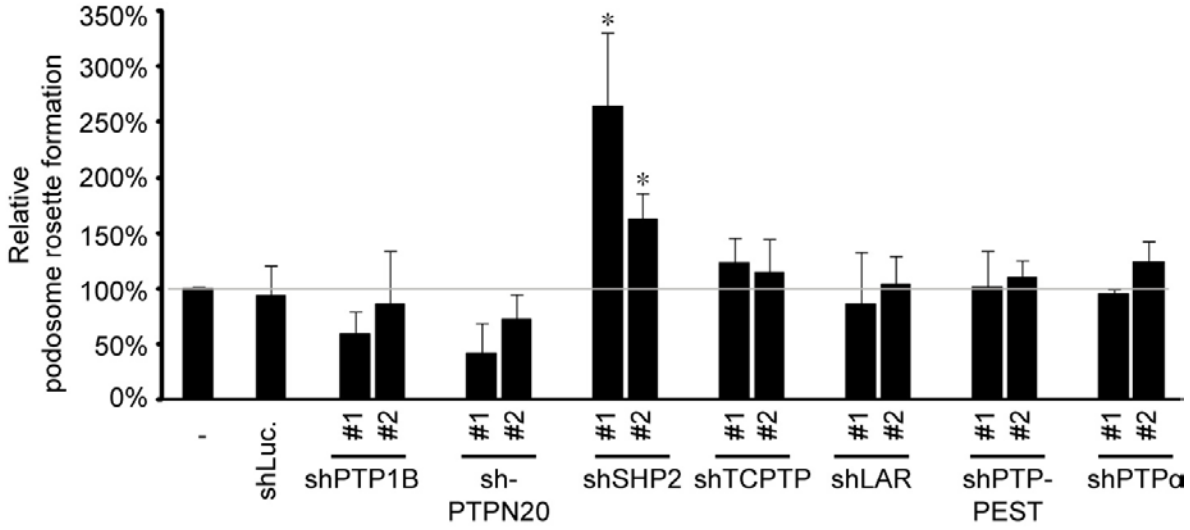

Fig. S1. Examination of the role of PTPs in podosome rosette formation using shRNAs. v-Src-transformed MEFs were infected with recombinant lentiviruses encoding shRNAs specific to luciferase (shLuc.), PTP1B (shPTP1B; clones #1 and #2), SHP2 (shSHP2; clones #1 and #2), TCPTP (shTCPTP; clones #1 and #2), LAR (shLAR; clones #1 and #2), PTP-PEST (shPTP-PEST; clones #1 and #2), or PTPα (shPTPα; clones #1 and #2). Equal numbers (2×10^5) of cells were seeded on fibronectin-coated coverslips for 48 h, fixed, and stained for F-actin. The percentage of cells containing podosome rosettes relative to the total counted cells ($n \geq 400$) was determined and expressed as a percentage relative to the level in control v-Src-transformed MEFs, which was defined as 100%. The values (mean ± s.d.) are from three independent experiments. *, $P < 0.05$ (compared with cells expressing shLuc).

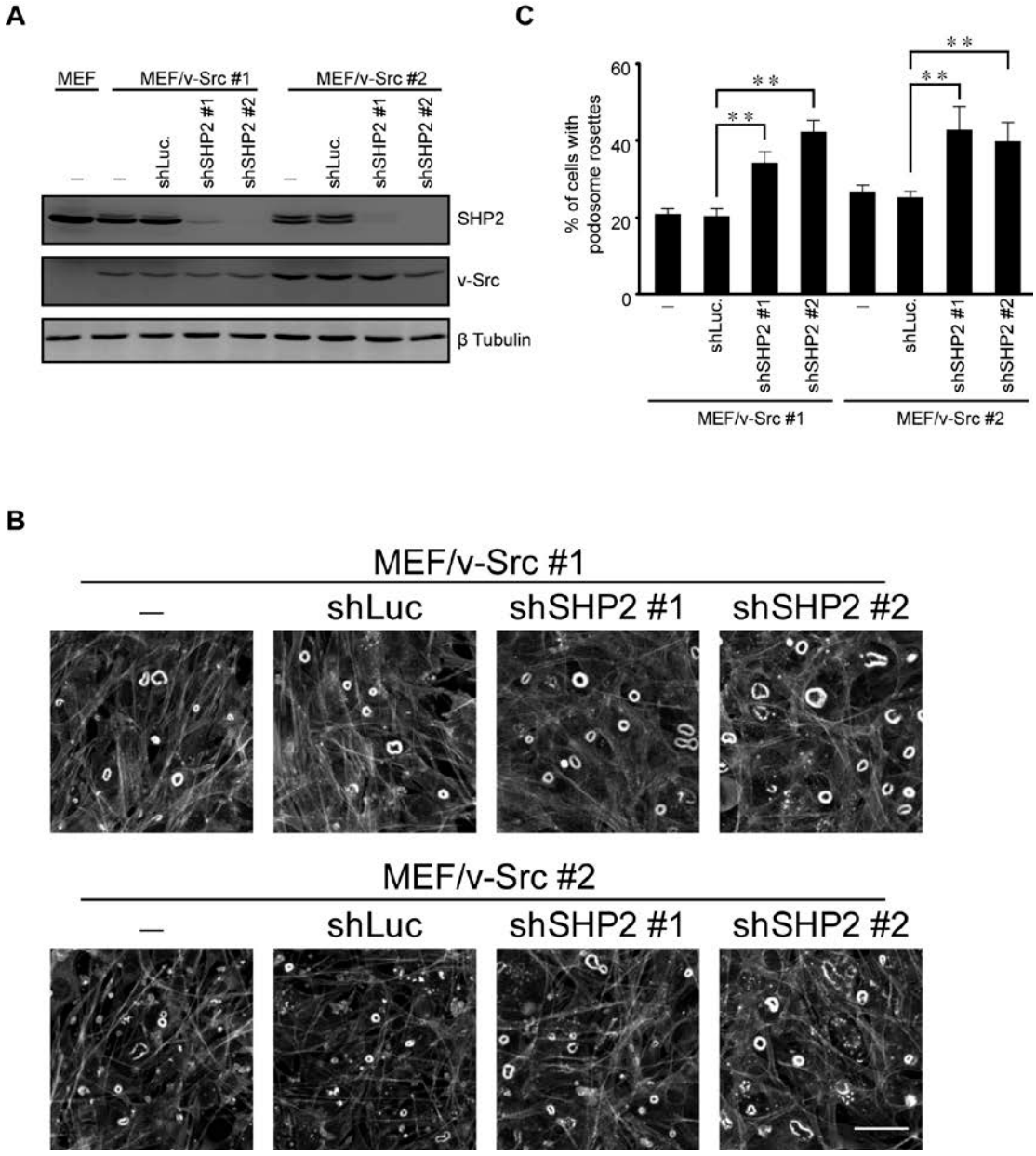

Fig. S2. Knockdown of SHP2 by shRNA increases the formation of podosome rosettes in Src-transformed MEFs. (A) Two independent clones of v-Src-transformed MEFs (MEF/v-Src; clones #1 and #2) were infected with recombinant lentiviruses encoding shRNAs specific to luciferase (shLuc.), or SHP2 (shSHP2; clones #1 and #2). An equal amount of whole cell lysates was analyzed by immunoblotting with the indicated antibodies. (**B**) The cells were grown on fibronectin-coated coverslips for 48 h and then stained for F-actin and cortactin as a marker for podosomes. Scale bar: 50 μm. (**C**) Quantitative results of podosome rosettes. The percentage of the cells containing podosome rosettes in total counted cells ($n \geqq 500$) was determined. Values (means ± s.d.) are from three independent experiments. **, $P < 0.005$.

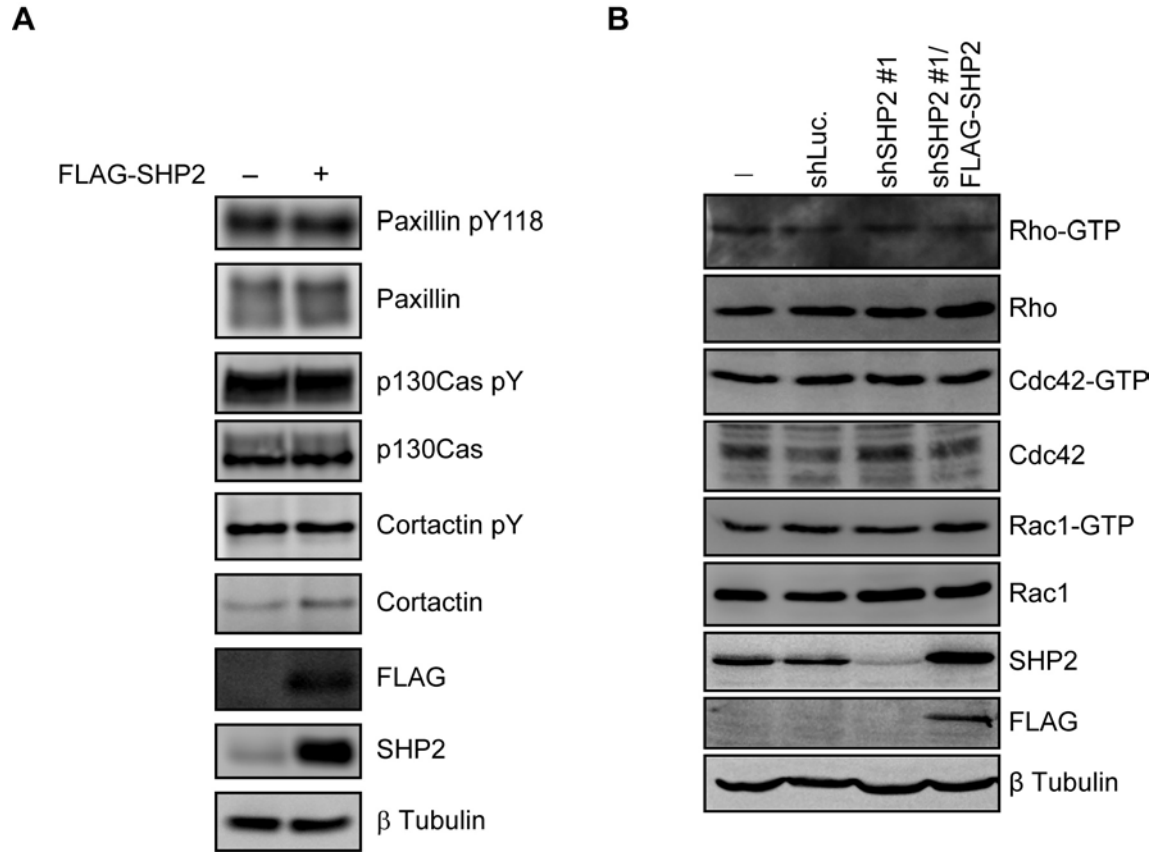

Fig. S3. SHP2 does not affect the tyrosine phosphorylation of paxillin, p130Cas, and cortactin or the activity of Rho, Rac, and Cdc42. (**A**) The tyrosine phosphorylation of paxillin, p130Cas, and cortactin in v-Src-transformed MEFs with or without FLAG-SHP2 overexpression was analyzed. **(B)** shRNAs specific to SHP2 (shSHP2; clone #1) or luciferase (shLuc) were stably expressed in v-Src-transformed MEFs. FLAG-SHP2 was re-expressed in the cells expressing shSHP2#1 (shSHP2#1/FLAG-SHP2). The activities of Rho family proteins (Rho, Rac, and Cdc42) were analyzed.

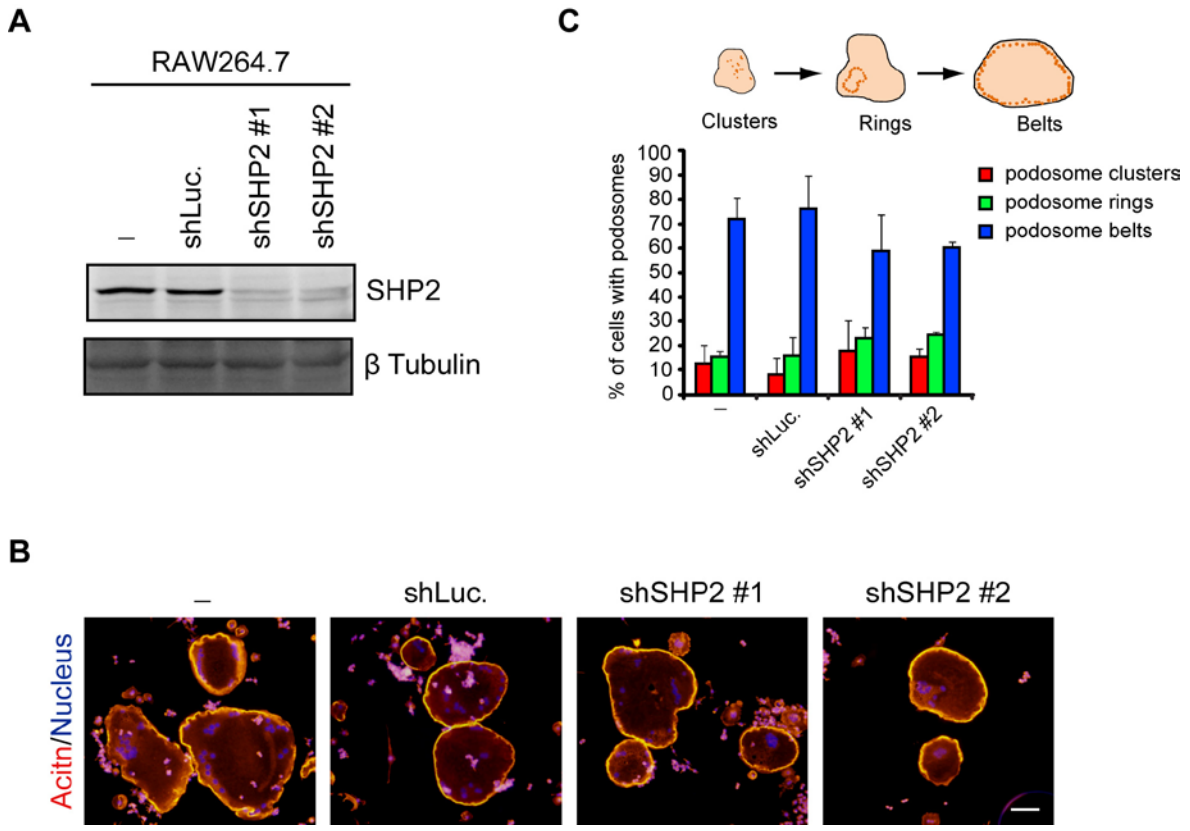

Fig. S4. Knockdown of SHP2 does not affect podosomal organization in RAW264.7 cells. (**A**) RAW264.7 cells were infected with recombinant lentiviruses encoding shRNAs specific to luciferase (shLuc.), or SHP2 (shSHP2; clones #1 and #2). Equal amounts of whole cell lysates were analyzed by immunoblotting with the indicated antibodies. (**B**) Cells were seeded on collagen-coated glass coverslips and treated with GST-RANKL (100 ng/ml) for 7 days. The cells were fixed and stained for F-actin and nuclei. Scale bar: 20 μm. (**C**) Quantitative results of podosomal organization in RANKL-treated RAW264.7 cells. Cells containing more than three nuclei were defined as differentiated osteoclasts. The percentage of cells containing podosomes (clusters, rings, and belts) relative to the total counted osteoclasts (n≥100) was determined. The values (mean ± s.d.) are from three independent experiments.