Supplemental Materials

Molecular Biology of the Cell

Gulvady et al.

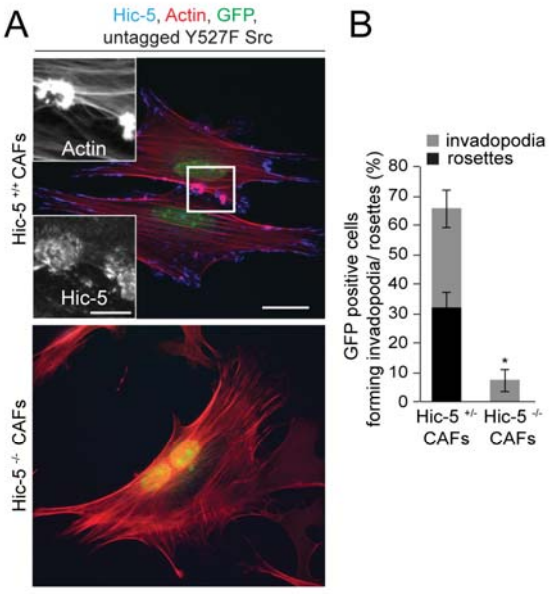

Figure S1. Hic-5 is required for formation of rosettes in active Src-transfected cancer-associated fibroblasts (CAFs). (A) Representative images of the control and Hic-5 knockout cancer-associated fibroblasts (CAFs) transfected with Y527F Src. Scale bar = 30μm. Inset showing F-actin and Hic-5 staining at the rosettes. Scale bar = 5μm (B) Quantitation of GFP-positive CAFs forming either invadopodia or rosettes (n= at least 80

cells). An unpaired Student's t test was performed. Data represent mean ± s.e.m. of at least three independent experiments. p*<0.05.

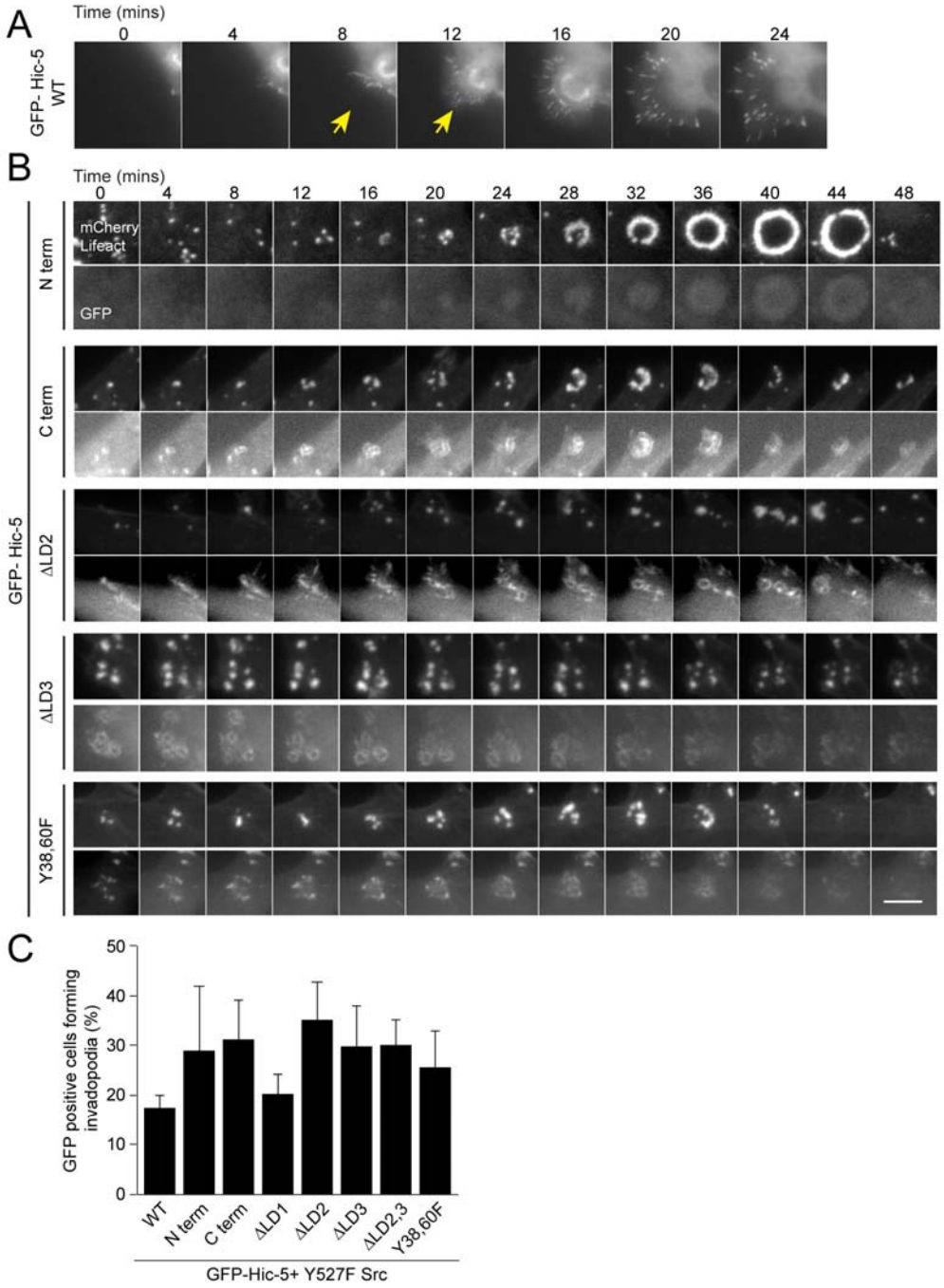

Figure S2. Hic-5 LD2 and LD3 motifs, along with the Y38,60 phosphorylation sites are required for rosette formation. (A) Time-lapse imaging of Y527F Src-transfected cells

expressing GFP-Hic-5 WT. Yellow arrows pointing at rapid, local lamellipodial bursts closely correlated with rosette disassembly. (B) Time-lapse imaging of Y527F Src-transfected cells expressing mCherry-Lifeact and GFP-Hic-5 N-terminus, C-terminus, or GFP-Hic-5 ΔLD2, ΔLD3 or Y38,60F mutants. Scale bar = 5μm. (C) Quantitation of cells expressing GFP-Hic-5 WT, N-terminus, C-terminus, or ΔLD1, ΔLD2, ΔLD3, ΔLD2,3 or Y38,60F mutants of Hic-5 forming invadopodia (n= at least 90 cells). A one-way ANOVA with a Dunnet's multiple comparison test was performed. Data represent mean ± s.e.m. of at least three independent experiments.

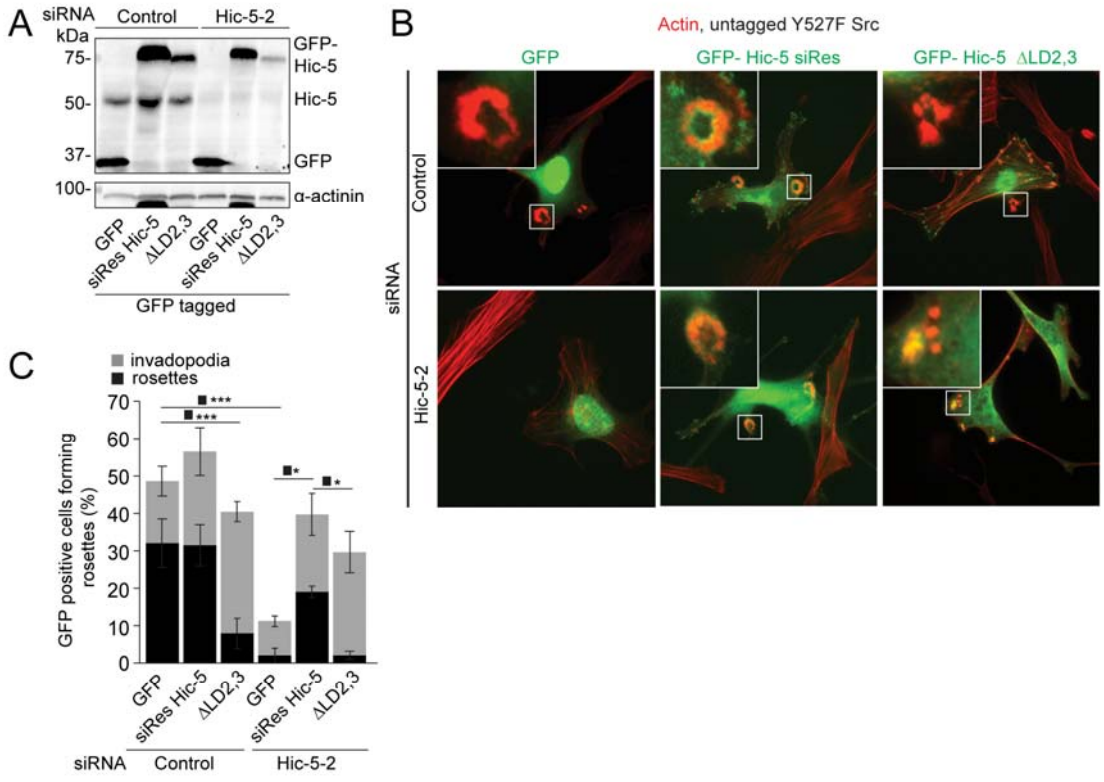

Figure S3. The Hic-5 N-terminal LD2,3 motifs are required for rosette formation in Y527F Src-transfected, Hic-5-deficient NIH3T3 fibroblasts. (A) Western blot of Y527F Src-transfected NIH3T3 fibroblast cell lysates post RNAi-mediated knockdown of Hic-5 and expressing either GFP, siRNA-resistant GFP-Hic-5 WT (siRes Hic-5) or GFP-Hic-5 ΔLD2,3 along with Y527FSrc. (B) Representative images of cells post RNAi-mediated

knockdown of Hic-5 and expressing either GFP, siRNA-resistant GFP-Hic-5 WT (siRes Hic-5) or GFP-Hic-5 ΔLD2,3. (C) Quantitation of GFP-positive cells forming either rosettes or invadopodia. (n= at least 70 cells). A one-way ANOVA with Sidak's multiple comparison test was performed. Data represent mean ± s.e.m. of at least three independent experiments. $p^{*}<0.05$ and *** $p<0.001$. The indicated p values on the graphs represent statistical significance performed between cells forming rosettes.

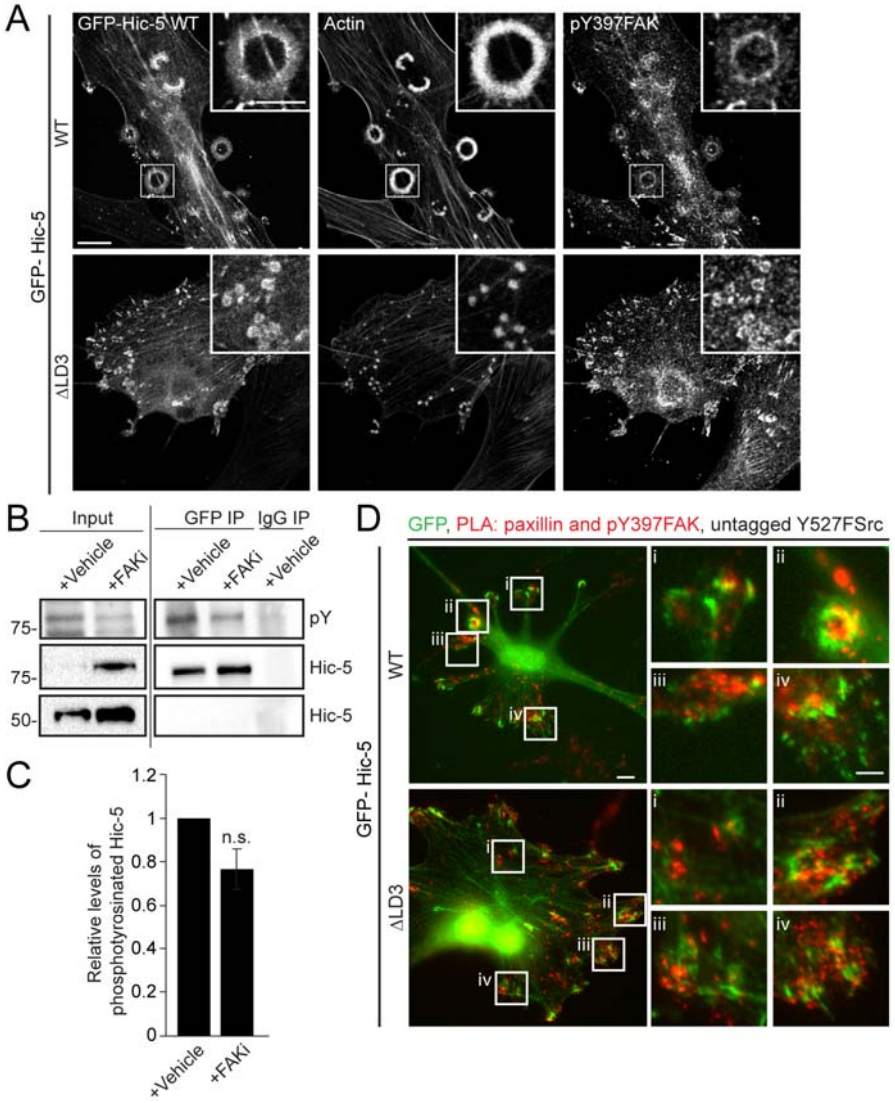

Figure S4. FAK-Src-mediated signaling induces downstream phosphorylation of Hic-5. (A) Representative images of Y527F Src-transfected NIH3T3 fibroblasts expressing GFP-Hic-5 WT or ΔLD3 mutant showing individual channels of Hic-5, actin and pY397FAK. Scale bar = 10μm. Insets showing magnified images of rosettes and invadopodia clusters. Scale bar = 5μm. (B) Western blot of a GFP-immunoprecipitation

assay using cells expressing GFP-Hic-5 WT treated with or without FAK inhibitor (10μM), probed for pan tyrosine phosphorylation levels and Hic-5. (C) Quantitation of the relative levels of tyrosine-phosphorylated Hic-5 post-FAK inhibition. (D) Representative images of PLA-positive spots between endogenous paxillin and pY397FAK in cells expressing GFP-Hic-5 WT or GFP-Hic-5 ΔLD3 mutant. Scale bar = 10μm. Insets showing higher magnification of adhesions, invadopodia and rosettes. Scale bar = 5μm. An unpaired Student's t test was performed. Data represent mean ± s.e.m. of at least three independent experiments.

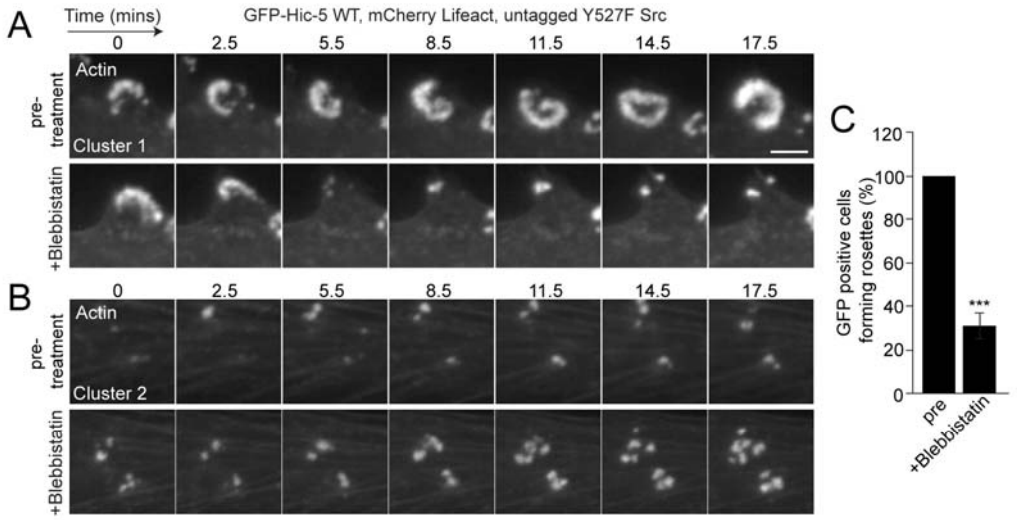

Figure S5. Myosin-II mediated contractility is required for rosette organization. (A) Montage of a rosette formed by a Y527F Src-transfected NIH3T3 fibroblast expressing GFP-Hic-5 WT and mCherry-Lifeact before and after treatment with the myosin-II inhibitor, Blebbistatin (5μM). (B) Montage of invadopodia clusters formed by a Y527F Src-transfected NIH3T3 fibroblast expressing GFP-Hic-5 WT and mCherry-Lifeact before

and after treatment with Blebbistatin (5μM). (C) Quantitation of GFP-Hic-5 WT expressing cells forming rosettes before and after Blebbistatin treatment (5μM). (n= at least 13 cells). An unpaired Student's t test was performed. Data represent mean ± s.e.m. of at least three independent experiments. *** $p<0.001$.