

Fig. S1. Effects of RPTP α KD on junctional E-cadherin distribution in MCF7 mammary carcinoma cells. MCF7 cells were transiently transfected with siRNA against RPTP α (RPTP α siRNA), or with scrambled control RNA (control siRNA), and analyzed 72 hours after transfection. (A) Immunofluorescence for E-cadherin in control and RNAi-treated cells. Scale bar: 10 μ m. (B) Quantification of intensity and heterogeneity of E-cadherin along junctions, and width of E-cadherin signal across junctions; mean \pm standard deviation (s.d.) relative to one representative experiment, $n=50$ junctions.

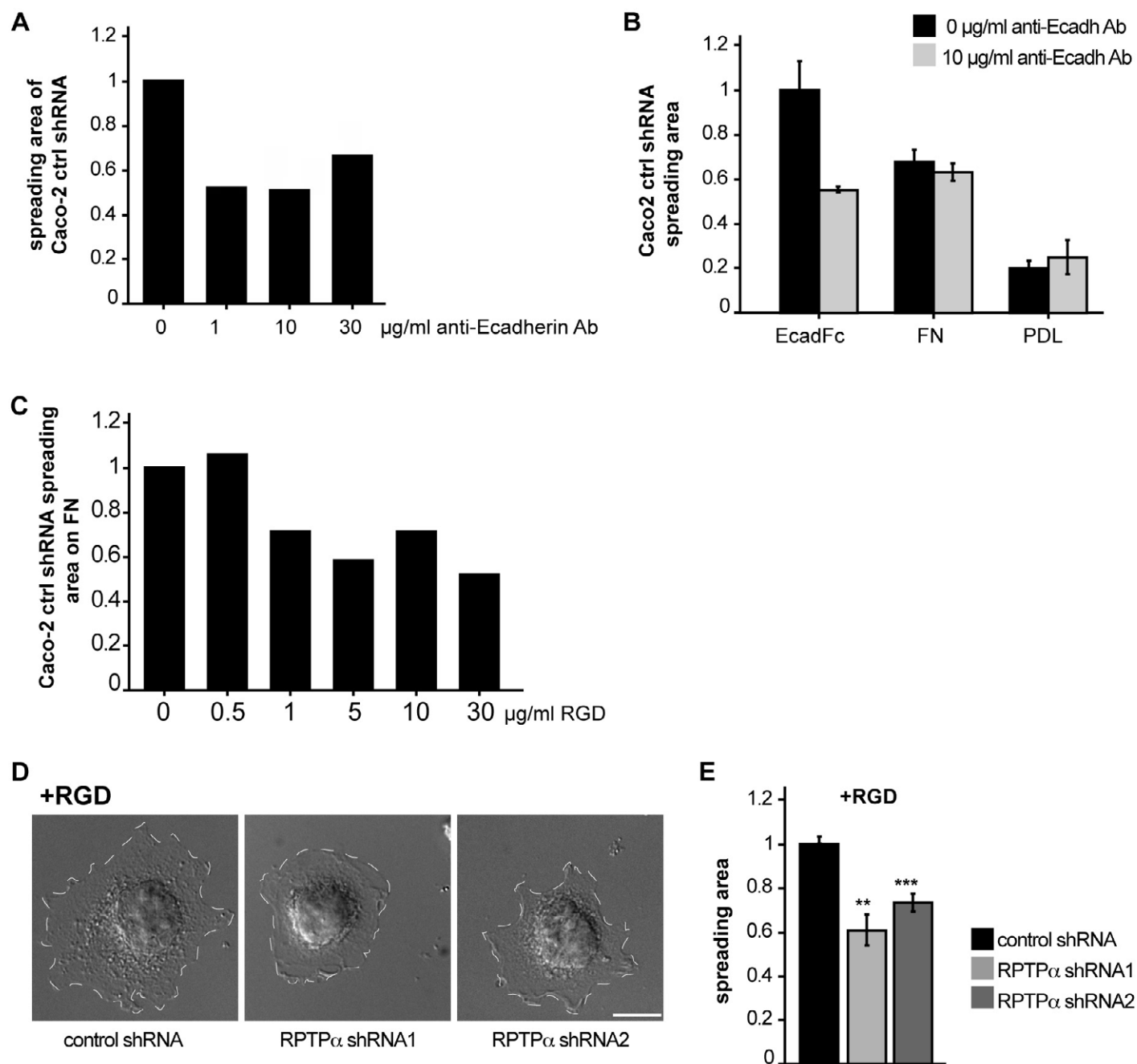


Fig. S2. Validation of E-cadherin-dependence, and analysis of integrin blocking in the spreading assay on immobilized E-cadherin (Ecad-Fc). (A,B) Influence of E-cadherin-function-blocking antibody SHE78-7 on spreading of control shRNA-transduced Caco-2 cells. (A) Quantification of the cell surface area in contact with Ecad-Fc-coated substrate, assayed in presence of various concentrations of the SHE78-7 antibody; at least 100 cells were analyzed per condition. (B) Quantification of cell surface area in contact with Ecad-Fc-, fibronectin (FN)- or poly-D-lysine (PDL)-coated substrates assayed in presence of 0 or 10 µg/ml of SHE78-7; mean \pm s.e.m. of 3 independent experiments; for each at least 60 cells were analyzed. (C–E) Effect of cyclic peptide (cRGD) on Caco-2 cells spreading. (C) Quantification of cell surface area of control shRNA-transduced cells in contact with fibronectin-coated substrate assayed in presence of various concentrations of cRGD; at least 50 cells were analyzed. (D) Representative phase-contrast images of control (control shRNA) and RPTPα KD (RPTPα shRNA1 and RPTPα shRNA2) cells spreading on Ecad-Fc-coated substrate; dashed line delimits surface area in contact with substrate. Scale bar: 10 µm. (E) Quantification of cell surface area in contact with Ecad-Fc-coated substrate, assayed in presence of 10 µg/ml cRGD; expressed as mean \pm s.e.m. of 3 independent experiments; ≥ 60 cells analyzed for each. For panels A and C, note that the baseline for spreading (i.e. cellular surface of unspread cells on PDL) corresponds to ~ 0.2 .

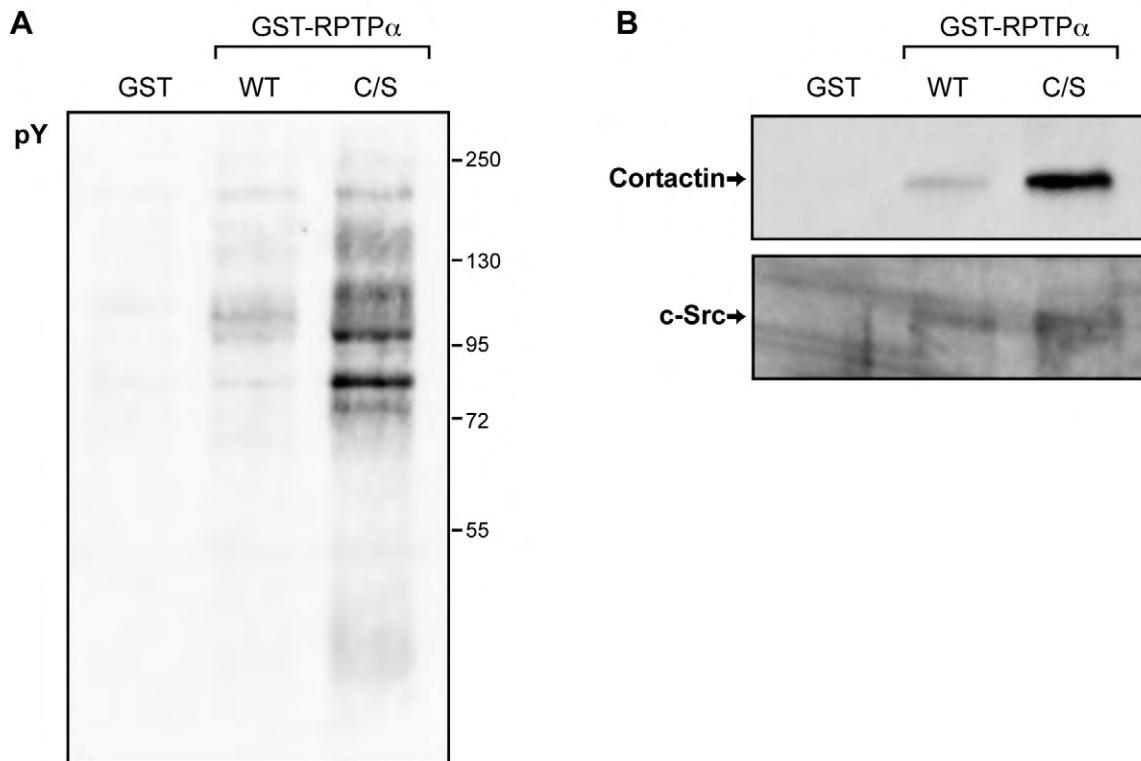


Fig. S3. Interaction of catalytically inactive mutant (C/S) RPTP α with cortactin by substrate-trapping. Caco-2 extract was incubated with equal amounts of GST alone or GST fused with *wild type* (WT) or mutant (C/S) RPTP α intracellular domain. After pull-down, bound proteins together with an aliquot of lysate of pervanadate-treated or untreated cells were analyzed by SDS-PAGE and immunoblotted with anti-phosphotyrosine (pY) (A) or anti-cortactin (B) antibodies. As positive control, immunoblotting against c-Src, a known substrate of RPTP α , was performed.



Movie 1. Time-lapse analysis of RPTP α during cell-cell contact establishment. Intercellular contact formation was followed by time-lapse video-microscopy of A431 cells expressing Venus-tagged RPTP α : phase-contrast and RPTP α -venus epifluorescence images were acquired at 10 minute intervals. White arrows, arrowheads and asterisks show the presence of RPTP α in nascent, early and stable cell-cell junctions respectively. Single Z optical sections are shown for each time point.