Supplementary figure 1

a, REF52 cells were incubated with RGD-coated beads and stimulated with tensional forces for different amounts of time. Active RhoA (RhoA-GTP) was isolated with GST-RBD and analyzed by western blot. b, MRC5 cells were incubated with beads coated with activating anti-β1 integrin antibody (TS2/16) and stimulated with tensional forces for different amounts of time. Active RhoA (RhoA-GTP) was isolated with GST-RBD and analyzed by western blot. c, REF52 cells were incubated with beads coated with non-activating anti-β1 integrin antibody (P4C10) and stimulated with tensional forces for different amounts of time. Active RhoA (RhoA-GTP) was isolated with GST-RBD and analyzed by western blot. d, REF52 cells were incubated 30 min with FN-coated beads and stimulated with tensional force by using a permanent magnet for different amounts of time. After magnetic separation of the adhesion complex fraction, the lysate and the adhesion complex fraction were analyzed by western blot. e, REF52 cells were incubated with beads coated with anti-TfR antibody and stimulated with tensional forces for different amounts of time. After magnetic separation of the adhesion complex fraction, the lysate and the adhesion complex fraction were analyzed by western blot. All results are representative of at least three independent experiments.

Supplementary figure 2. Magnetic tweezer set up and spring constant calculation.

a, Experimental setup showing a 2.8 micron fibronectin coated magnetic bead on a cell being pulled by the pole tip. The tracker generated by video spot tracker is used to track bead displacement (magnification 60x). b, Tracked radial displacement shown for the bead pulled by an applied 3 seconds force. c, The tracked displacement in (b) is converted to compliance as described in the text and then fitted using a least squares method to a Kelvin-Voigt model shown by the dotted line. d, A modified Kelvin-Voigt or Jeffrey's model is shown. All stiffness values reported are the value of the spring constant of the spring k.

Supplementary figure 3

a, Relative change in stiffness of REF52 cells during application of 5 force pulses on FN-coated bead. Spring constant was calculated for each force pulse and expressed as relative to the spring constant observed during the first pulse (error bars represent s.e.m., *n*=18; * p<0.01).b, Spring constant calculated for the first (white) and second (black) pulse of force applied on FN-coated bead bound to REF52 cells transfected 48 h with control siRNA or RhoA siRNA (error bars represent s.e.m., *n*=20). c, REF52 cells transfected 48 h with control siRNA or RhoA siRNA or RhoA siRNA and a siRNA-resistant mutant of RhoA (myc-RhoA). Expressions of RhoA, myc and tubulin were analyzed by western blot. d, change in stiffness during 2 force pulses applied on FN-coated beads bound to untreated REF52 cells (left panel) or REF52 cells treated for 90 min with cell-permeable C3 toxin (2 µg/ml) (right panel) (error bars represent s.e.m., *n*=15 ,* p<0.01). e, Spring constant calculated for the first (white) and second (black) pulse of force applied on FN-coated bead bound to REF52 cells transfected 48 h with control siRNA or siRNA targeting p115, Gef-H1, LARG, Ect2 or both LARG and GEF-H1 (error bars represent s.e.m., *n*=20). f, REF52 cells were transfected 48 h with control siRNA or p115 siRNA (duplex 1) or p115 siRNA (duplex 2). Expressions of p115 and tubulin were analyzed by western blot.

Supplementary figure 4

a, $SYF^{-/-}$ cells and SYF cells re-expressing Src, Yes and Fyn ($SYF^{+/+}$) or re-expressing Src or Fyn were incubated with FN-coated beads and stimulated with force for 3 min. Src and Fyn expressions and activities were analyzed by western blot. b, REF52 cells untreated or treated with SU6656 (2.5 µM for 30 min) were incubated with FN-coated beads and stimulated with force for 3 min. Hot sample buffer was used for lysis. Samples were then diluted to allow

immunoprecipitation with anti-phosphotyrosine Ab (PY20). Phosphorylated LARG was analyzed by western blot using anti-LARG antibodies.c, Spring constant calculated for the first (white) and second (black) pulse of force applied on FN-coated bead bound to $SYF^{-/-}$ cells and SYF cells re-expressing Src, Yes and Fyn ($SYF^{+/+}$) or re-expressing Src or Fyn (error bars represent s.e.m., *n*=20).d, REF52 cells untreated or treated with taxol (10 µM for 30 min) were incubated with FN-coated beads and stimulated with force for different amounts of time. Active GEF-H1 was sedimented with GST-RhoAG17A and analyzed by western blot. e, REF52 untreated or treated with U1026 (5 µM for 30 min) were incubated with FN-coated beads and stimulated with forces for 3 min. Immunoprecipitation of GEF-H1 was performed and phosphorylation on Threonine was analyzed by western blot using anti-phospho-Threonine-proline antibodies. f, REF52 cells untreated or treated with SU6656 (2.5 µM for 30 min) were incubated with FN-coated beads and stimulated with force for 3 min. Active Ras (Ras-GTP) was sedimented with GST-Raf1.Phosphorylated FAK (Tyr397), phosphorylated Src (Tyr416) and total FAK were analyzed by western blot. g, REF52 cells untreated or treated with U0126 (5 µM for 30 min) were incubated with FN-coated beads. After stimulation with forces for different amounts of time, cells were lysed and active RhoA (RhoA-GTP) was isolated with GST-RBD and analyzed by western blot.

Supplementary figure 5

Full scans of gels/blots that have been cropped in Figures within the primary manuscript.