Supplementary Information

Supplementary Figure 1. Cell adhesion and Rho GTPase regulation by □1 and □3 integrins on FN. (**a**) Representative experiment showing the levels of active and total RhoA (*top*) and Rac (*bottom*) in GE□1, GE□3, and GE□1/□3 cells on FN (5 □g/ml). (**b**) Adhesion assay for the indicated cell lines on FN (5 □g/ml) for 30 mins. Values shown represent the averages +SD from 3 independent experiments, expressed relative to GE□3. (**c**) Representative experiment showing the levels of active and total RhoA (*top*) and Rac (*bottom*) in GE□1, GE□3, and GE□$^{\Delta 759}$ cells. (**d**) Western blot showing depletion of kindlin-2 using two different shRNAs in GE□3 cells (*left*). Adhesion assay on FN (5 □g/ml) for GE□3 cells expressing non-targeting sequences (sh_Ctrl) and GE□3 cells in which kindlin-2 was depleted using two different shRNAs (*right*). Values represent the means +SD from 3 independent experiments. Statistically significant differences are denoted by ** ($p<0.01$), and *** ($p<0.001$). AU; arbitrary units.

Supplementary Figure 2. Regulation of cell spreading and GTPase activity in HUVECs by □1 and □3 integrins on FN. (**a**) Representative images of HUVECs transduced with non-targeting sequences (sh_Ctrl) or shRNAs targeting integrin □1 (sh_□1) or □3 (sh_□3), cultured on FN (5 □g/ml). Bar, 60 □m. (**b**) Representative experiment showing the levels of active and total Rac (*left*) and RhoA (*right*) in sh_Ctrl, sh_□1, and sh_□3 HUVEC monolayers, cultured on FN (5 □g/ml) and treated with thrombin (1 U/ml) for the indicated time-points.

Supplementary Figure 3. Cell spreading and migration □1 depletion in HUVECs. (**a**) Knockdown efficiency of 3 different shRNAs against □1 in HUVECs. (**b**) Single-cell

migration tracks of HUVECs on FN (5 □g/ml), monitored by time-lapse microscopy. (**c**) Cell area of sparsely seeded HUVECs transduced with 3 different hairpins against □1 on FN (5 □g/ml). Results are means +SD of 82-175 cells from 3 independent experiments. (**d**) Velocity and (**e**) directionality of single-cell migration. Values represent the means +SD of 108-145 cells from 3 independent experiments. Statistically significant differences are denoted by * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$). AU; arbitrary units.

Supplementary Movie 1. Scratch assay for GE□1, GE□3, and GE□1/□3 cells. The indicated cell lines were grown to confluency, serum-starved overnight, and scratched with a pipette tip. Scratch closure was stimulated with 10% FCS, and cells were imaged by time-lapse video microscopy. Image intervals; 10 mins, imaging time 16 hours.

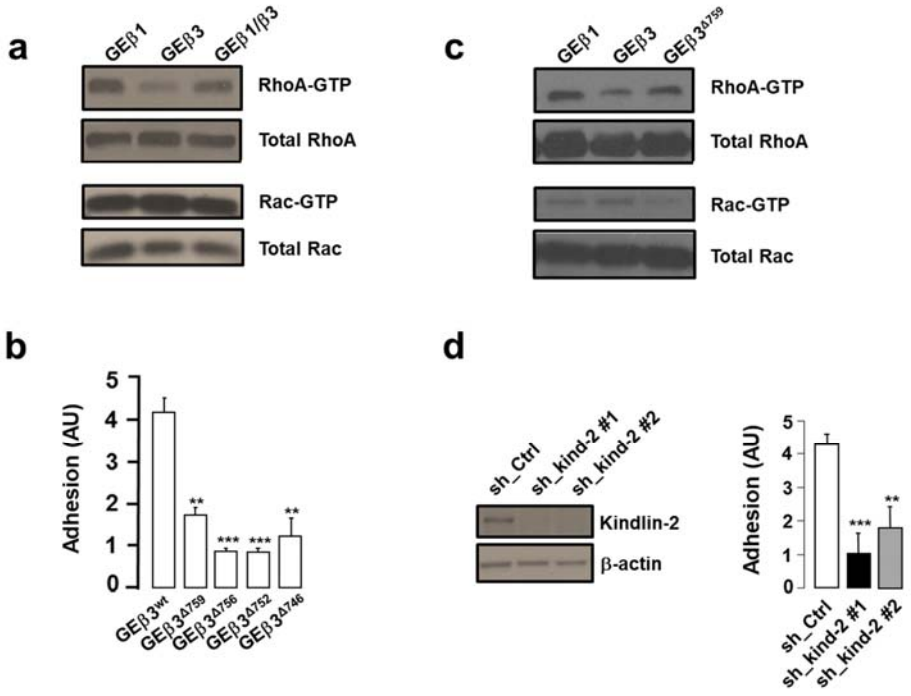
Supplementary Movie 2. Single-cell migration assay for GE□1, GE□3, and GE□1/□3 cells on FN. The indicated cell lines were sparsely seeded on FN and monitored by time-lapse video microscopy. Image intervals; 10 mins, imaging time 15 hours.

Supplementary Movie 3. Scratch assay for cells expressing □1 or □3 mutants. The indicated cell lines were grown to confluency, serum-starved overnight, and scratched with a pipette tip. Scratch closure was stimulated with 10% FCS, cells were imaged by time-lapse video microscopy. Image intervals; 10 mins, imaging time 15 hours.

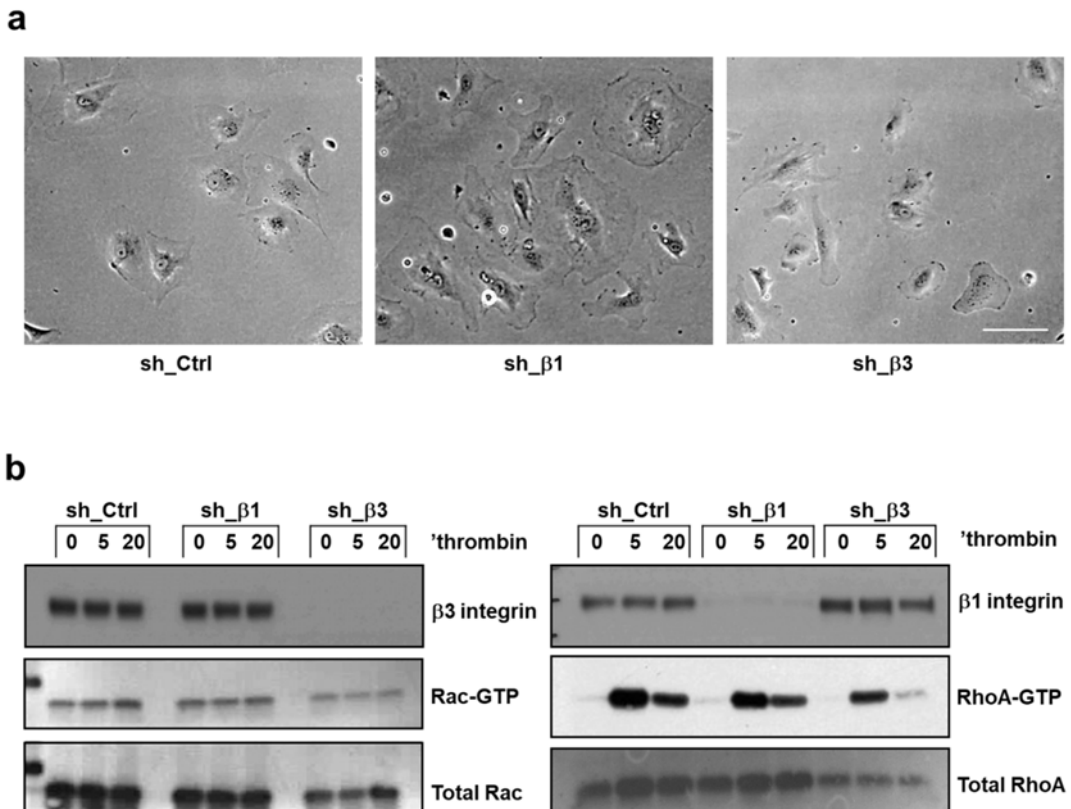
Supplementary Movie 4. Single-cell migration assay for cells expressing □1 or □3 mutants on FN. The indicated cell lines were sparsely seeded on FN (5 □g/ml) and monitored by time-lapse video microscopy. Image intervals; 10 mins, imaging time 15 hours.

Supplementary Movie 5. Single-cell migration assay for cells expressing □1 together with □3 mutants on FN. The indicated cell lines were sparsely seeded on FN (5 □g/ml) and monitored by time-lapse video microscopy. Image intervals; 10 mins, imaging time 15 hours.

Supplementary Figure 1



Supplementary Figure 2

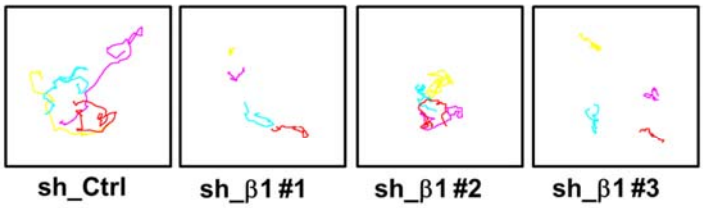


Supplementary Figure 3

a

hairpin	% β1 expression
Control	100
#1	48
#2	14
#3	23

b



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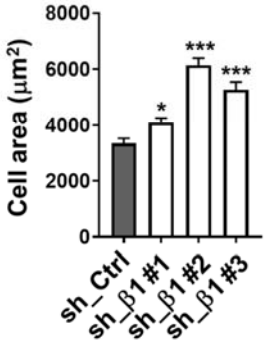

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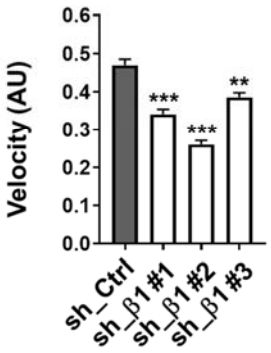

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