

Supplemental material

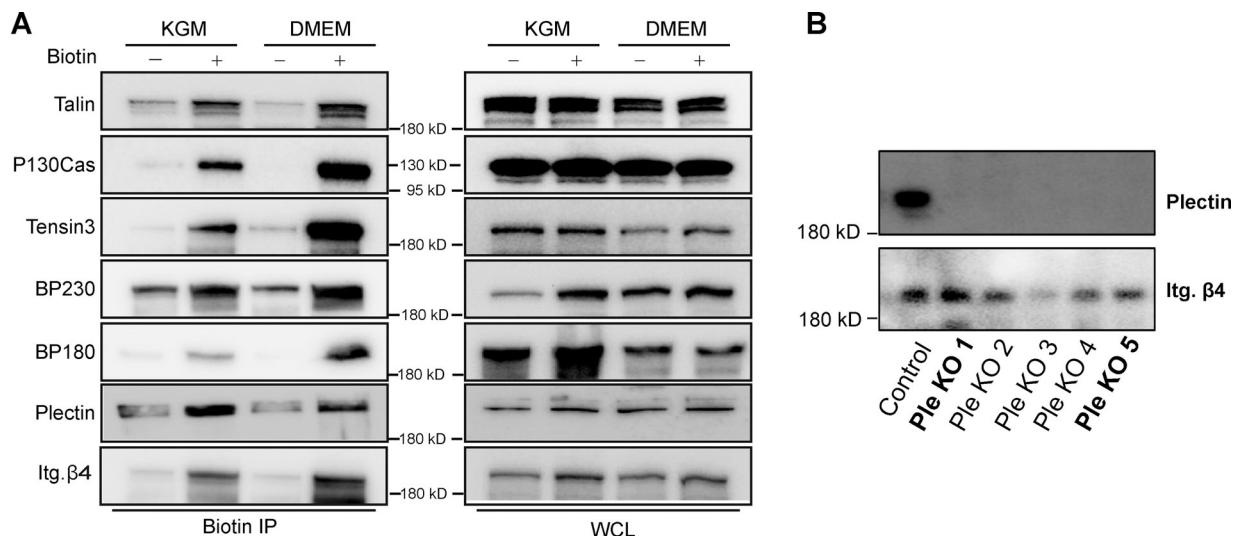


Figure S1. Confirmation of BirA*-β4 interactions by streptavidin precipitation and Western blot. **(A)** PA-JEB/β4 keratinocytes (cultured in KGM or DMEM) expressing integrin β4 fused to the biotin ligase BirA* were used to perform proximity biotinylation assays. The proximity interactors of integrin β4 were pulled down by streptavidin beads and detected by Western blotting with the indicated antibodies. **(B)** Western blot analysis of whole-cell lysates (WCL) of plectin (Ple) knockout (KO) clones, probed with antibodies against plectin and integrin β4. β4 (+) PA-JEB cells were included as a positive control.

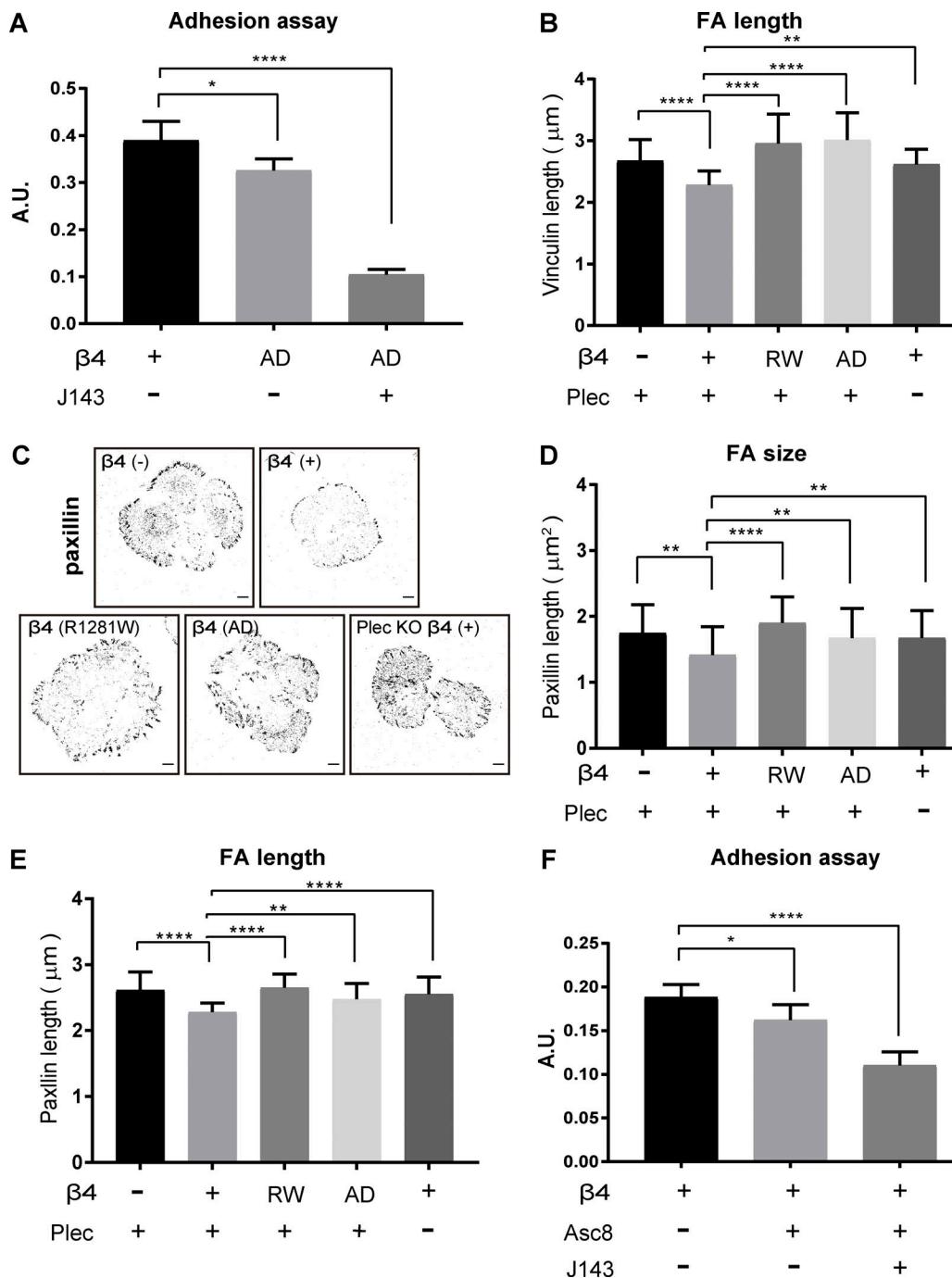


Figure S2. Focal contact area varies depending on the expression and function of integrin $\beta 4$. **(A)** Cells were treated with or without integrin $\alpha 3$ -blocking mAb (J143; 20 $\mu\text{g/ml}$) in suspension before a short-term (45 min) adhesion assay was performed on a laminin-332-rich matrix substrate. Data are presented as the mean ($\pm \text{SD}$) from three independent experiments. *, $P < 0.05$; ****, $P < 0.0001$. **(B)** Quantification of FA length probed by vinculin with ImageJ. Data are presented as the mean ($\pm \text{SD}$) from three independent experiments, with ~20 images per experiment. **, $P < 0.01$; ****, $P < 0.0001$. **(C)** Inverse black-and-white images of confocal micrographs of $\beta 4 (-)$, $\beta 4 (+)$, $\beta 4$ -R1281W, and $\beta 4$ -AD PA-JEB keratinocytes showing cell morphology and paxillin-stained FAs (black). Scale bars: 10 μm . **(D and E)** Quantification of FA size and length probed by paxillin with ImageJ. Data are presented as the mean ($\pm \text{SD}$) from two independent experiments, with ~20 images per experiment. **, $P < 0.01$; ****, $P < 0.0001$. **(F)** Integrin $\beta 4 (+)$ PA-JEB keratinocytes were treated with integrin $\beta 4$ -blocking mAb (ASC-8; supernatant diluted 1:5) alone or together with integrin $\alpha 3$ -blocking mAb (J143; 20 $\mu\text{g/ml}$). Data are presented as the mean ($\pm \text{SD}$) from three independent experiments. *, $P < 0.05$; ****, $P < 0.0001$.

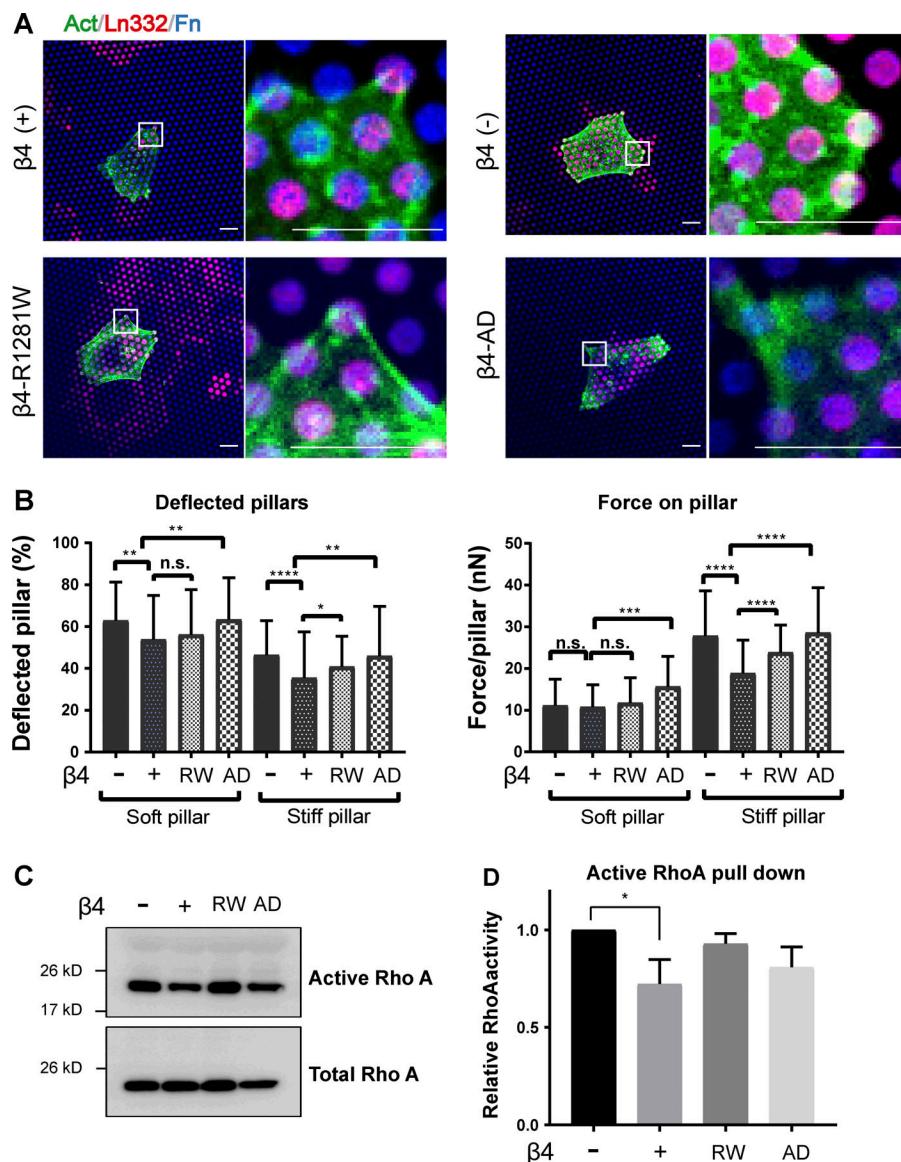


Figure S3. Integrin $\alpha 6\beta 4$ reduces traction force generation through modulation of RhoA activity. **(A)** Confocal images showing the deposition of laminin-332 (red) by $\beta 4$ (-) and $\beta 4$ (+) PA-JEB keratinocytes seeded on micropillars. Cells were visualized by actin staining (green), and pillar tops were coated with fibronectin (blue). Scale bars: 10 μ m. **(B)** Quantifications of percentage of deflected pillars and force per pillar of the indicated cell lines seeded on pillars. **(C)** Representative immunoblot of RhoA activity in integrin $\beta 4$ (-), $\beta 4$ (+), $\beta 4$ -R1281W, and $\beta 4$ -AD PA-JEB keratinocytes. Active RhoA was pulled down from the lysates, using GST-rhotekin-binding domain fusion proteins with the RhoA-binding region of rhotekin. **(D)** RhoA activity is shown as active RhoA/total RhoA, and values were normalized to the $\beta 4$ (-) group. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; n.s., not significant.

Provided online is one supplemental table in Word and one data file in Excel. Table S1 lists primary antibodies. Data S1 shows the mass spectrometry data for the proximity interactors of the integrin $\beta 4$ analysis.