

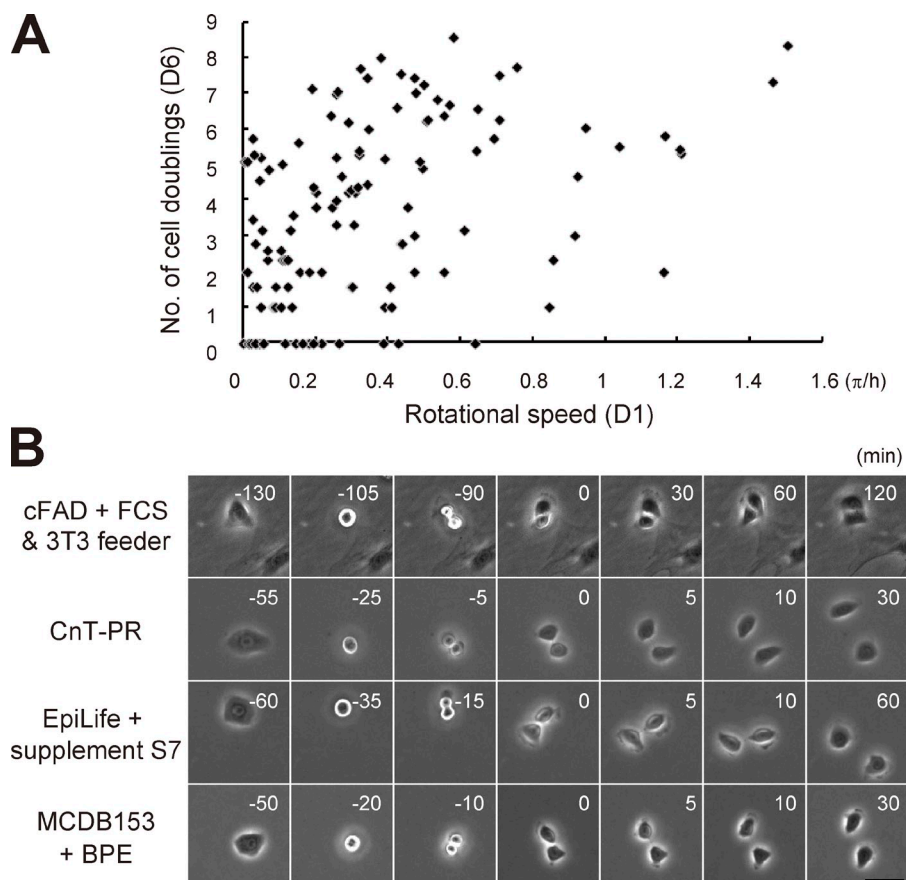
Nanba et al., <http://www.jcb.org/cgi/content/full/jcb.201409024/DC1>

Figure S1. **Motion dynamics of keratinocytes seeded at clonal density in various cell culture media.** (A) Full dataset of Fig. 1 B. Correlation between rotational speed in the two-cell colony stage (D1) and proliferative capacity (number of cell doublings) after 6 d of cultivation with a 3T3 feeder layer and serum-containing medium (Spearman's $\rho = 0.489$, $P = 6.04 \times 10^{-9}$, and $n = 126$ colonies). P-value was calculated by Student's t test. (B) The generation of two-cell colonies from single keratinocytes in various conditions. The time when two-cell colonies were generated after cell division was considered 0 min. Bar, 50 μm .

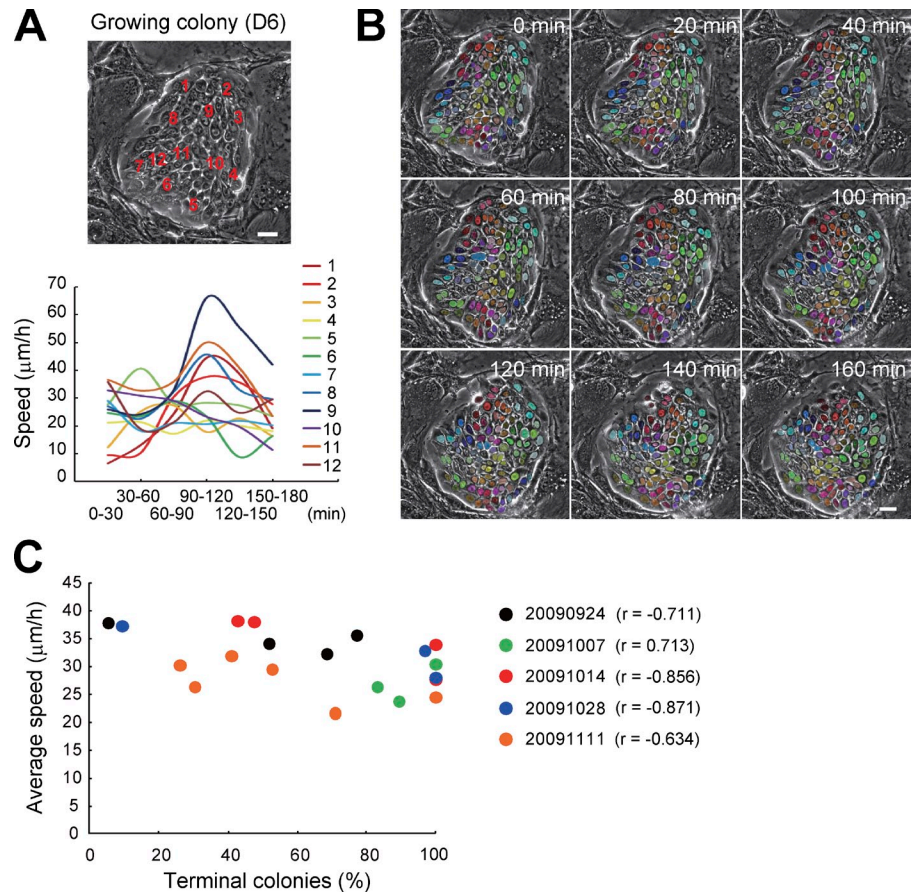


Figure S2. **Collective motion dynamics of keratinocytes in a progressively growing colony.** (A) Motion analysis of individual keratinocytes in a progressive growing colony. Top panel shows location of traced cells in the colony. Bottom panel shows changes in locomotion speed of traced cells. The data shown were obtained from a single representative experiment out of multiple observations. (B) Tracing of cell locomotion in the growing colony presented in A and in Fig. 2 A. Bar, 50 μm . (C) Combination experiments of time-lapse imaging and clonal analysis were performed independently five times. Each experiment, except for an experiment on 20091007, revealed a negative correlation between mean speed of cell locomotion in the colony and the ratio of terminal colonies by clonal analysis.

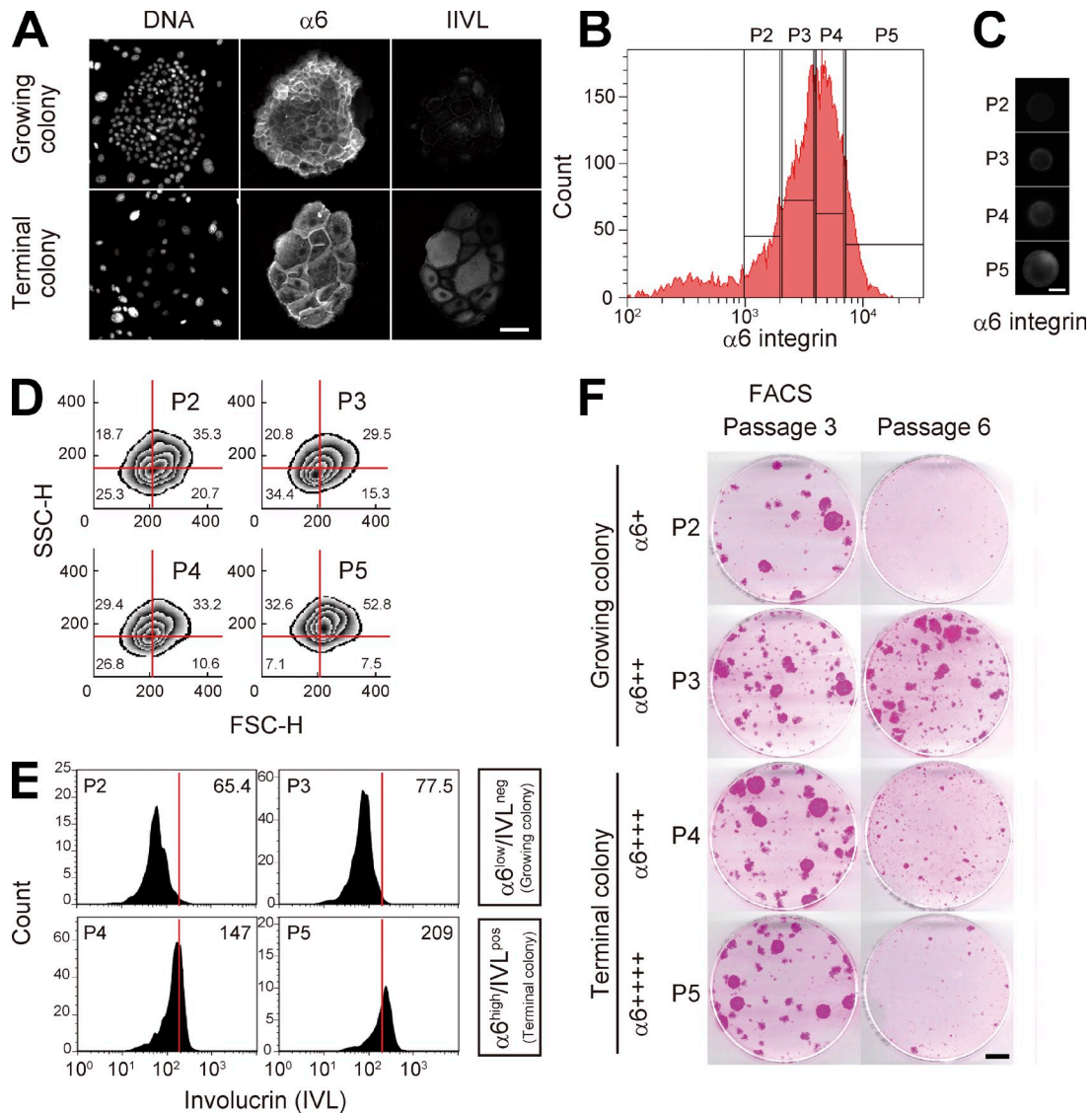


Figure S3. **Fractionation of cultured keratinocytes based on $\alpha 6$ integrin and IVL expression.** (A) Expression of IVL in progressively growing and growth-arrested terminal colonies. Growing colonies were constituted by IVL-negative keratinocytes. Bar, 100 μm . (B) Distribution of $\alpha 6$ integrin expression in cultured human keratinocytes. The cells were fractionated into four subpopulations (P2–P5) by flow cytometry. The data shown were obtained from a single representative experiment out of triplicate repeats. (C) Immunofluorescent images of sorted keratinocytes in each subpopulation. P5 contained a lot of large cells. Bar, 10 μm . (D) Flow cytometric signatures of each subpopulation. The data shown were obtained from a single representative experiment out of triplicate repeats. (E) Flow cytometric analysis of IVL expression in each fraction. Geometrical means of IVL expression are indicated. IVL-negative keratinocytes were predominantly comprised in P2 and P3 subpopulations. The data shown were obtained from a single representative experiment out of triplicate repeats. (F) Detailed results of Fig. 4 C showing colony-forming efficiency of each fraction during serial cultivation. Bar, 10 mm.

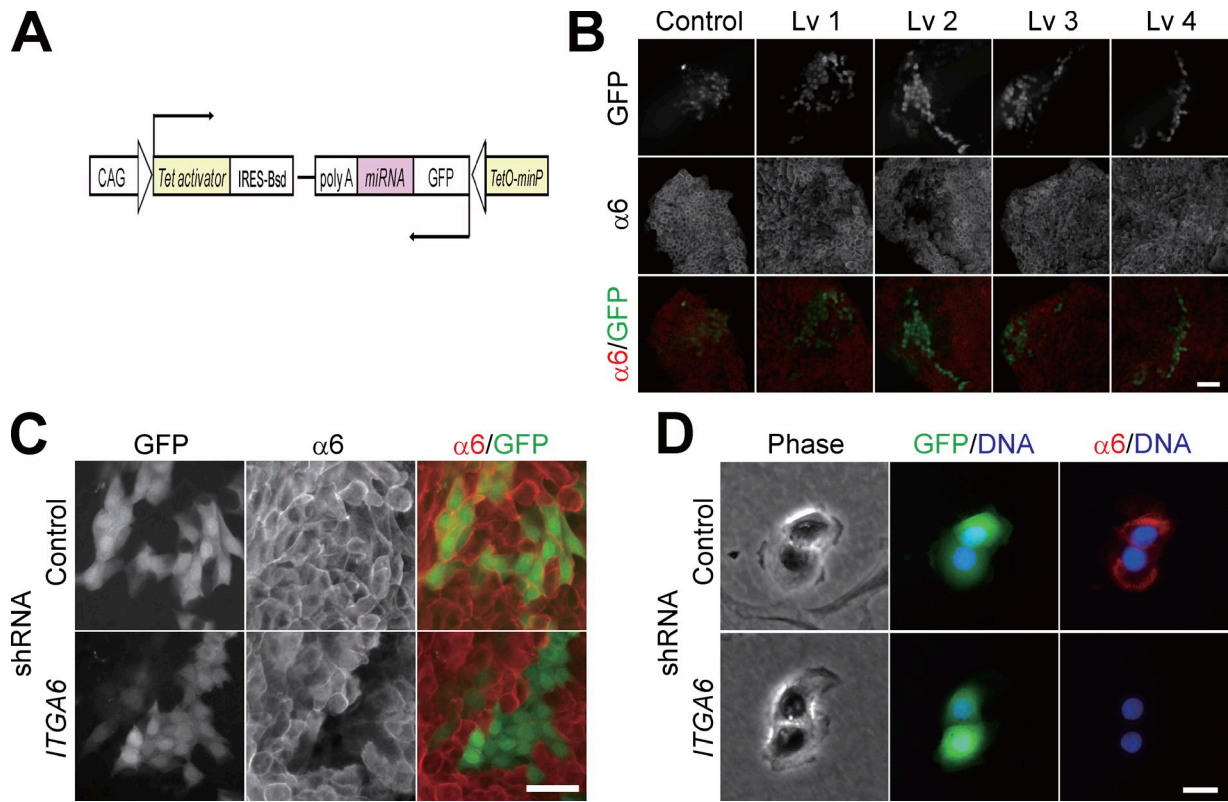


Figure S4. **Characterization and validation of functional shRNA targeted to *ITGA6*.** (A) Structure of doxycycline-inducible shRNA lentiviral vector (See Materials and methods). (B) Lentiviruses (Lv 1–4) carrying shRNA targeted to *ITGA6* under the control of doxycyclin-inducible promoter were infected into HaCaT keratinocytes. 3 d later, shRNA was induced by doxycyclin treatment, and HaCaT cells were maintained for an additional 3 d to then be fixed and immunostained with an $\alpha 6$ integrin antibody (GoH3). Bar, 100 μm . (C) A shRNA targeted to *ITGA6* (Lv 2) efficiently decreased $\alpha 6$ integrin expression in normal human keratinocytes. Bar, 50 μm . (D) Transduction of shRNA targeted to *ITGA6* (Lv 2) into the two-cell colony efficiently decreased expression of $\alpha 6$ integrin. Bar, 20 μm .

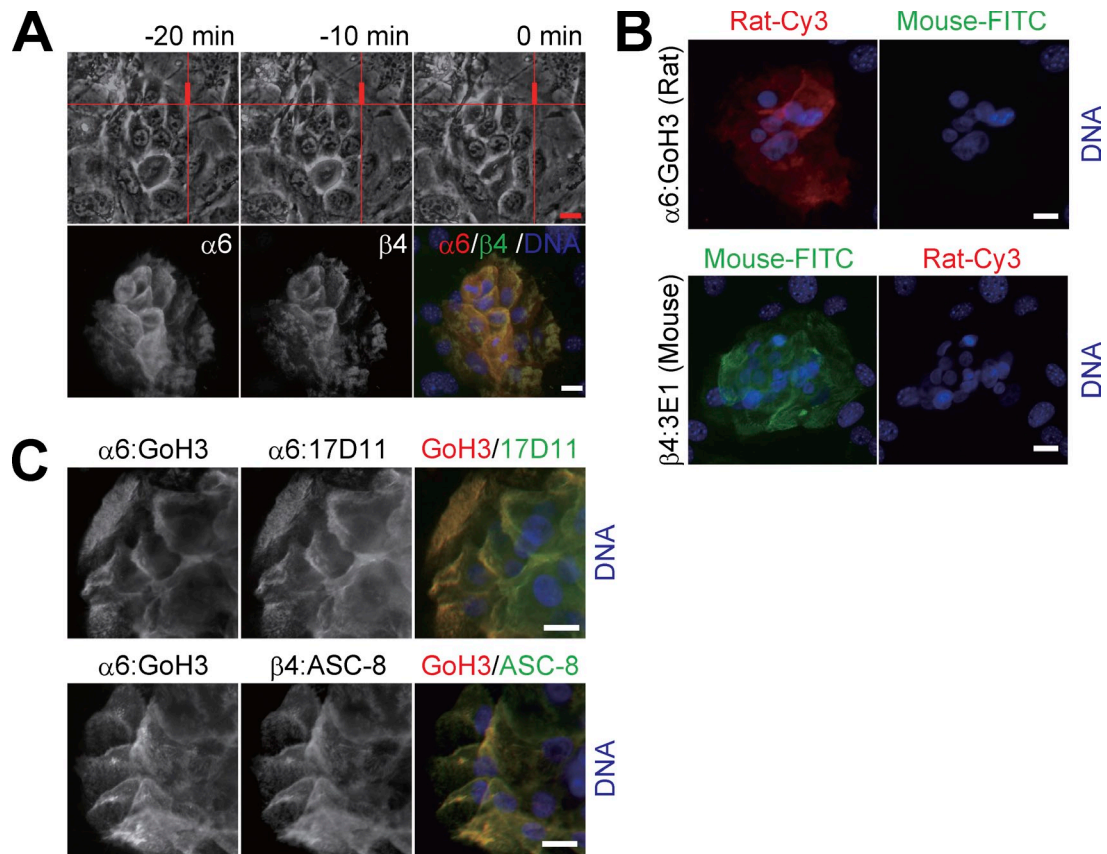
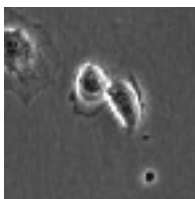
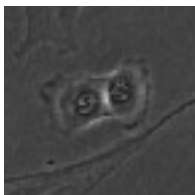


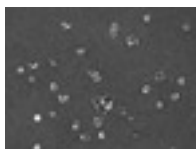
Figure S5. **$\alpha6\beta4$ integrin is involved in collective motion of keratinocytes.** (A) Movement of human epidermal keratinocytes (top) and distribution of $\alpha6\beta4$ integrin (bottom) in two-dimensional cell aggregates. Bars, 20 μm . (B) Secondary antibodies control. Goat antibodies against rat (conjugated with Cy3) and mouse (conjugated with FITC) antibodies did not recognize mouse and rat antibodies, respectively. Bars, 20 μm . (C) Applied antibodies recognized $\alpha6\beta4$ integrin and remained on cell surface after overnight cultivation. Bars, 20 μm .



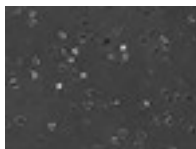
Video 1. **A time-lapse image of a fast-rotating two-cell colony of normal human keratinocytes.** Human epidermal keratinocytes were seeded at clonal density in a 35-mm cell culture dish with mitomycin C-treated 3T3-J2 cells and maintained at 37°C and 10% CO_2 , in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss). Images were collected at 5-min intervals for 30 min without any stimulation.



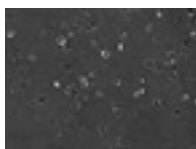
Video 2. **A time-lapse image of a nonrotating two-cell colony of normal human keratinocytes.** Human epidermal keratinocytes were seeded at clonal density in a 35-mm cell culture dish with mitomycin C-treated 3T3-J2 cells and maintained at 37°C and 10% CO_2 , in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss). Images were collected at 5-min intervals for 30 min without any stimulation.



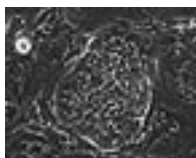
Video 3. **A time-lapse image of motion dynamics of normal human keratinocytes at high density culture in CnT-PR medium.** Human epidermal keratinocytes were seeded into a 35-mm cell culture dish and maintained at 37°C and 10% CO₂, in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss). Images were collected at 5-min intervals for 60 min without any stimulation.



Video 4. **A time-lapse image of motion dynamics of normal human keratinocytes at high density culture in EpiLife medium with supplement S7.** Human epidermal keratinocytes were seeded into a 35-mm cell culture dish and maintained at 37°C and 10% CO₂, in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss). Images were collected at 5-min intervals for 60 min without any stimulation.



Video 5. **A time-lapse image of motion dynamics of normal human keratinocytes at high density culture in MCDB153 medium containing bovine pituitary extract.** Human epidermal keratinocytes were seeded into a 35-mm cell culture dish and maintained at 37°C and 10% CO₂, in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss). Images were collected at 5-min intervals for 60 min without any stimulation.



Video 6. **A time-lapse image of a growing colony of normal human keratinocytes was collected at 5-min intervals for 180 min without any stimulation.** Human epidermal keratinocytes were seeded at clonal density in a 35-mm cell culture dish with mitomycin C-treated 3T3-J2 cells and maintained at 37°C and 10% CO₂, in a chamber mounted on a microscope (Axiovert 200M; Carl Zeiss).

The Fortran program used for the simulation experiments is provided online as an RTF file.