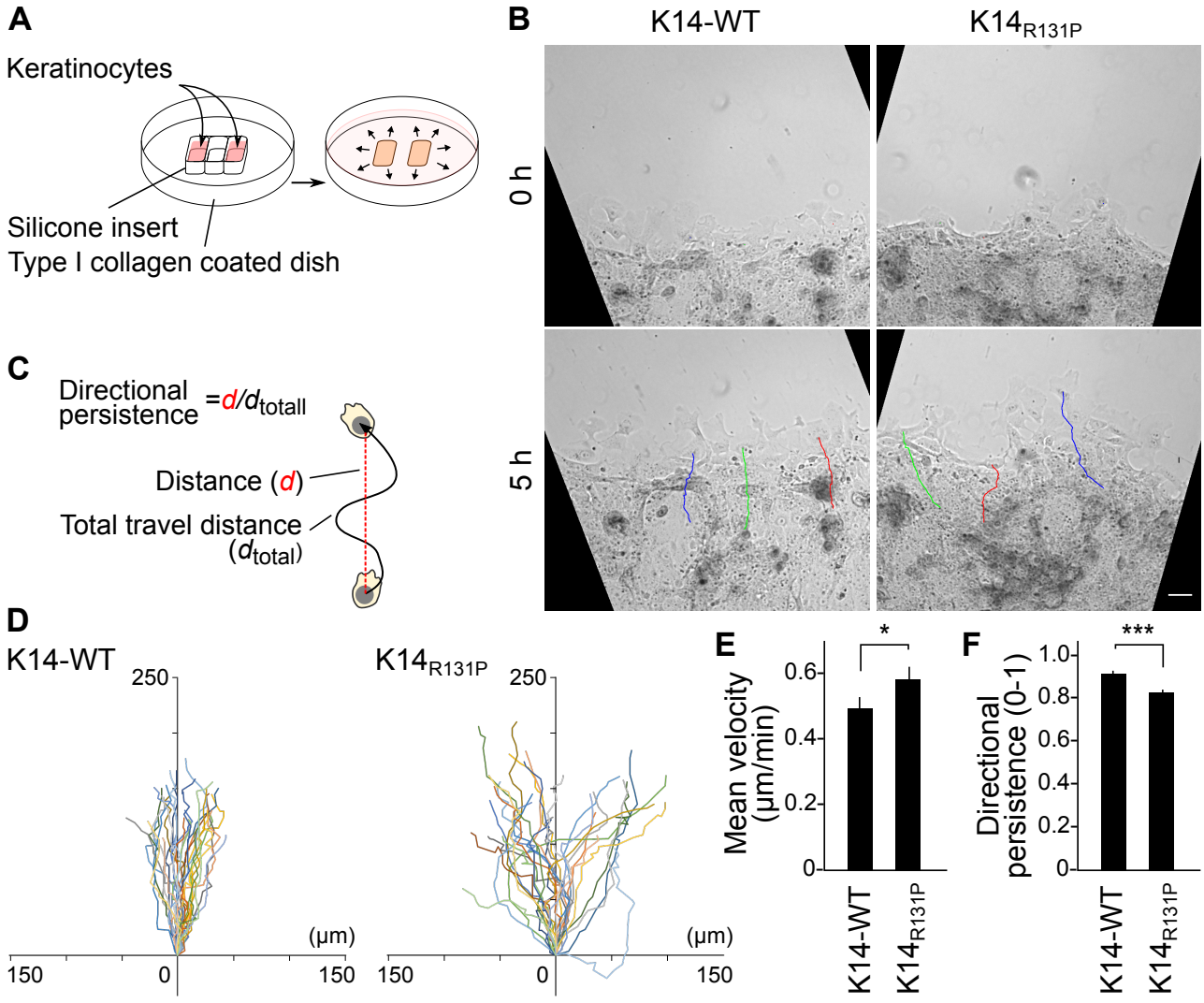


Figure S1. Keratin organization affects directed collective migration

(A) Schematic illustration of methods used in the collective migration assay. Migration of leader cells (black arrows) was analyzed by time-lapse imaging. (B) Phase contrast images of collective cell migration at the time point of 0 and 5 h after the start of observation. The overlaid lines in lower panels show the trajectory of individual cells. Scale bar, 50 μm. (C) For quantitative analysis of cell migration by tracking the nuclei of individual leader cells, see Method section for details. (D) Trajectories of 30 leader cells in a representative experiment. (E) Mean velocity of the leader cells of 5 h migration. (F) Directionality of collective migration. (E, F) Data are shown as the mean ± *SD* of three independent experiments (30 cells per experiment). *, $P<0.05$, ***, $P<0.001$.

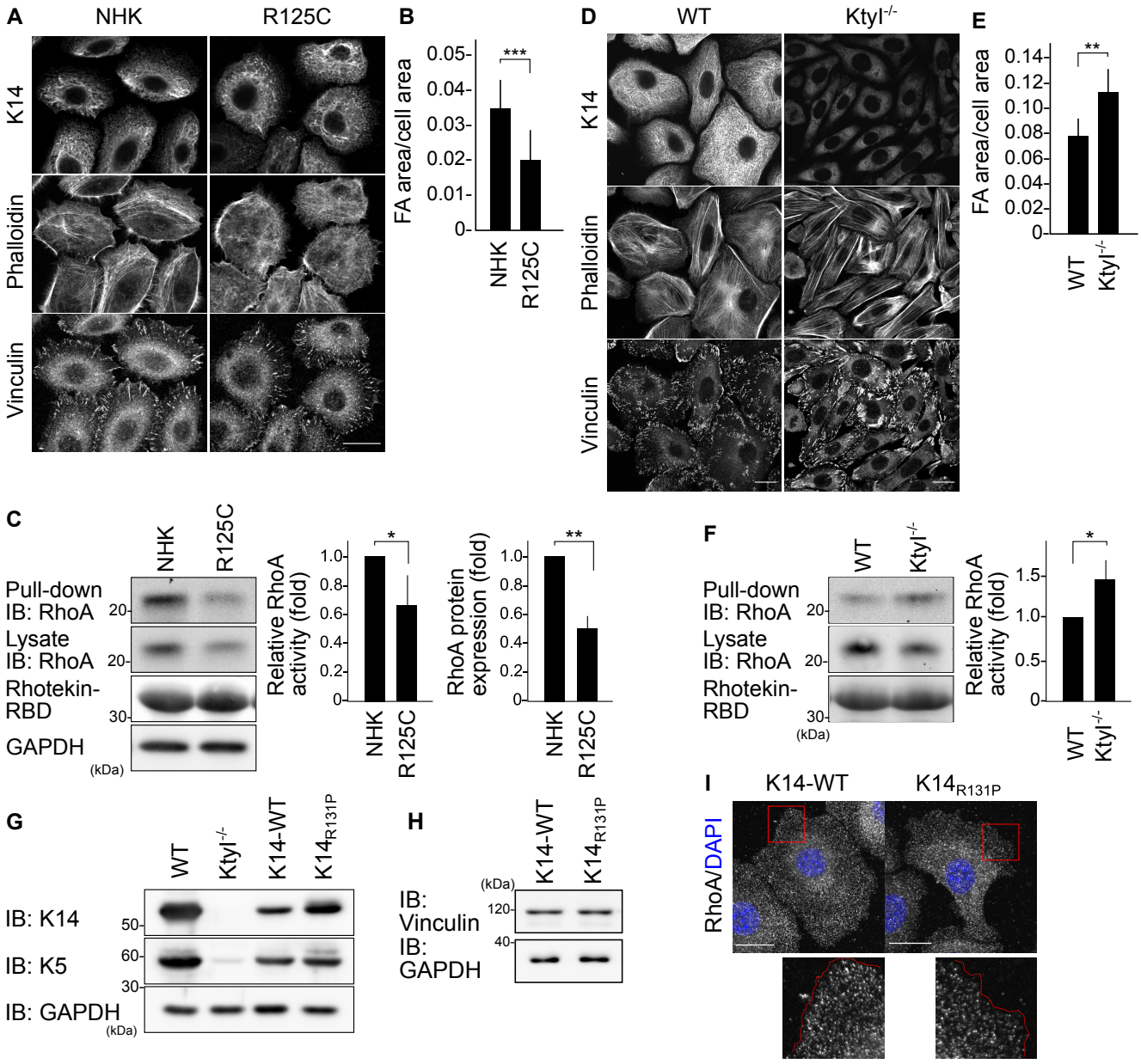


Figure S2. Characterization of human keratinocytes and Ktyl$^{-/-}$ keratinocytes
(A,B,D,E) Immunofluorescence analysis of K14, F-actin, and vinculin in indicated cell lines. The proportion of FA area in the cell area were calculated as Fig. 2. (C and F) RhoA activities in indicated cell lines analyzed by pull-down assays. (B,C,E,F) The data shown represent the mean ± *SD* of at least three independent experiments. *, P<0.05, **, P<0.01, ***, P<0.001. (G,H) Expression levels of K14 and K5 (G) and Vinculin (H) in indicated cell lines. (I) Fluorescence images of RhoA. Bottom panels show the magnified images of the red boxes. Dotted red lines show the cell outlines. Scale bars are shown as 20 μm.

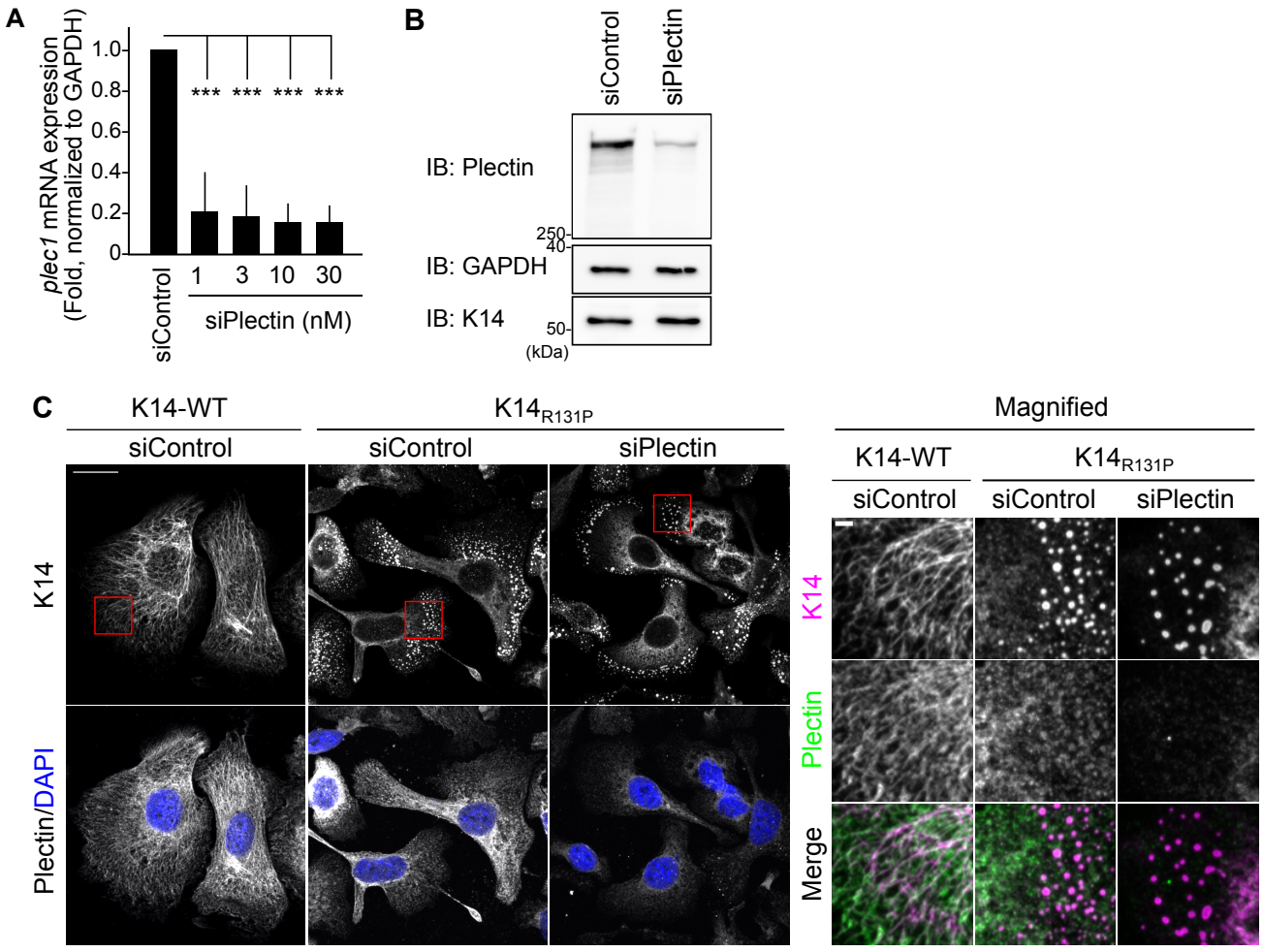


Figure S3. Distribution of RhoA and $K14_{R131P}$-induced keratin network disorganization is independent of plectin

(A) Effects of plectin-targeting siRNA on plectin mRNA expression. N=3, ***, *P*<0.001. (B) Effects of plectin-targeting siRNA on plectin protein expression. (C) Effects of plectin knockdown on localization of K14. Right panels show the magnified images of the red boxes. Scale bars are shown as 20 μm (left) and 2 μm (right).

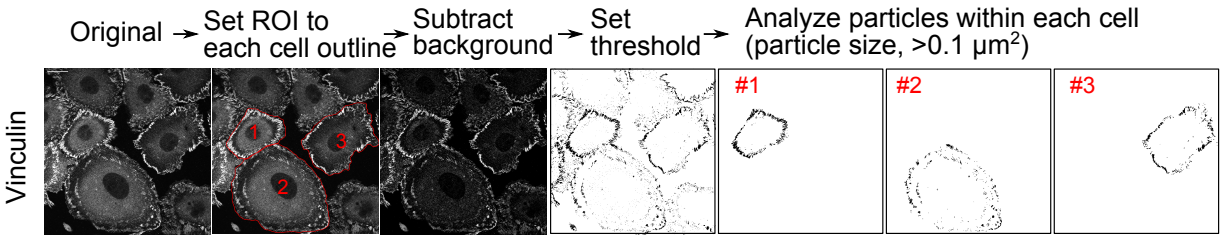
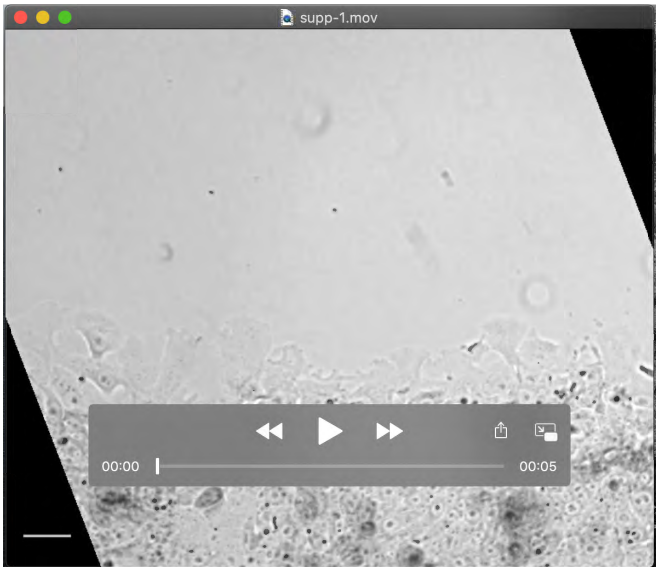


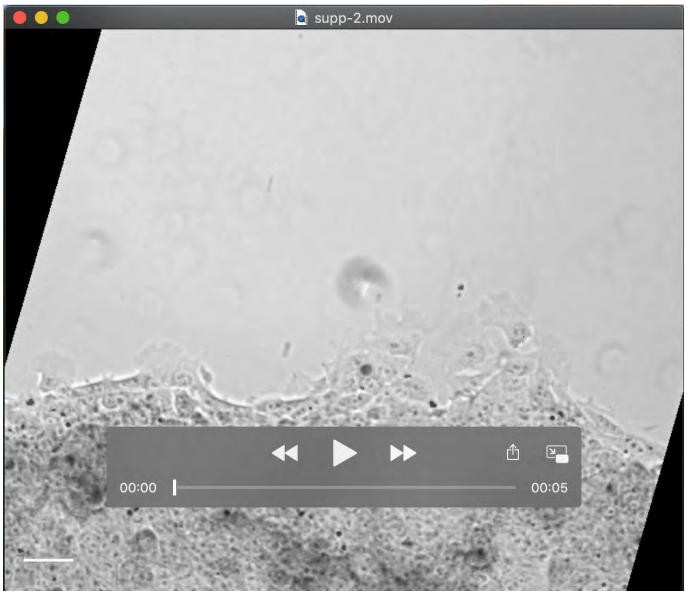
Figure S4. Methods of FA size quantification
The example images of data processing for FA quantification. Images were quantified based on vinculin immunofluorescence signals with ImageJ software. See materials and method section for the details.

Supplementary Movies



Movie 1

Phase contrast images of collective cell migration of K14-WT group. The overlaid lines show the trajectory of individual cells. Scale bar, 50 µm.



Movie 2

Phase contrast images of collective cell migration of $K14_{R131P}$ group. The overlaid lines show the trajectory of individual cells. Scale bar, 50 µm.