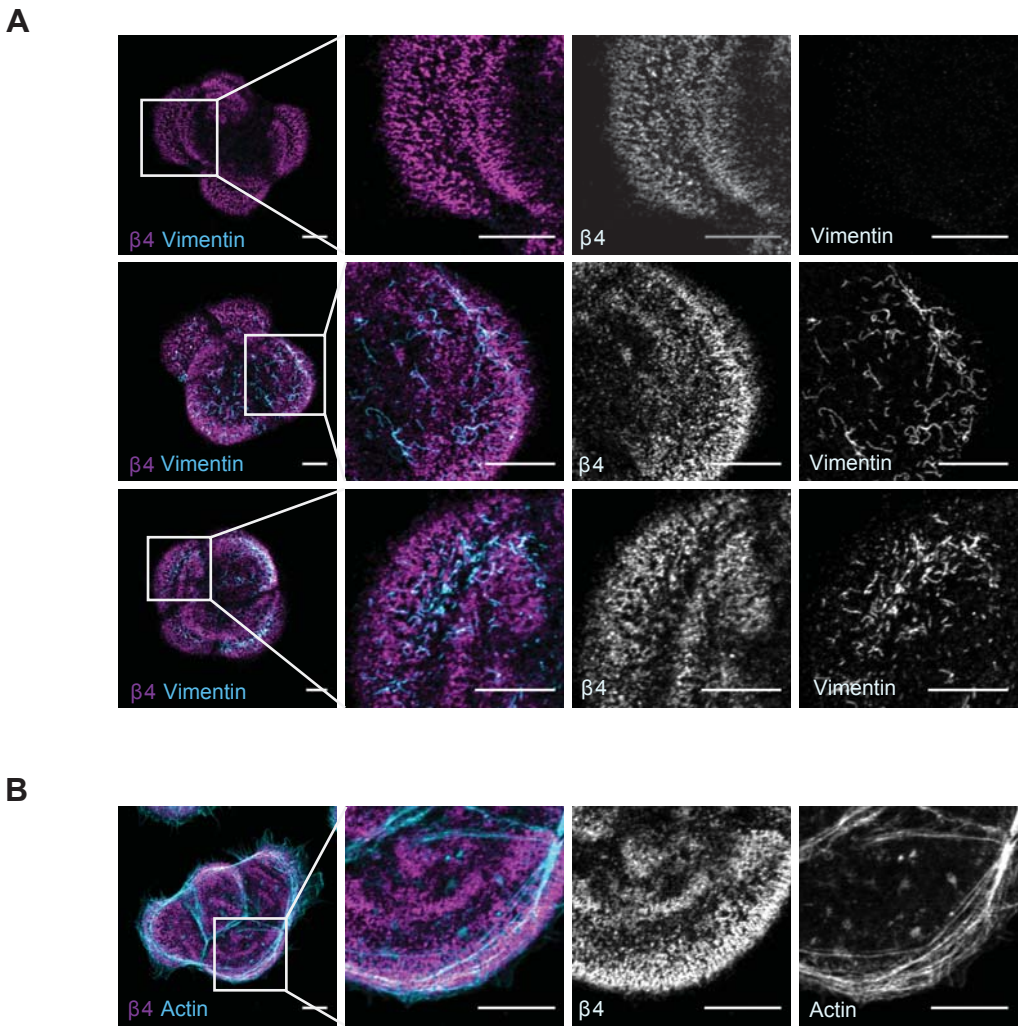

Fig. S1 α6β4 in central HD-like adhesions is not associated with actin or vimentin.
Example overview and zoom-in confocal images of PA-JEB/β4 keratinocytes stained for β4 (magenta) and vimentin (A) or actin (B) (cyan). Top panel of A represents the ~ 50% of PA-JEB/β4 keratinocyt cell islands in which vimentin is absent. Scale bars are 10 µm.

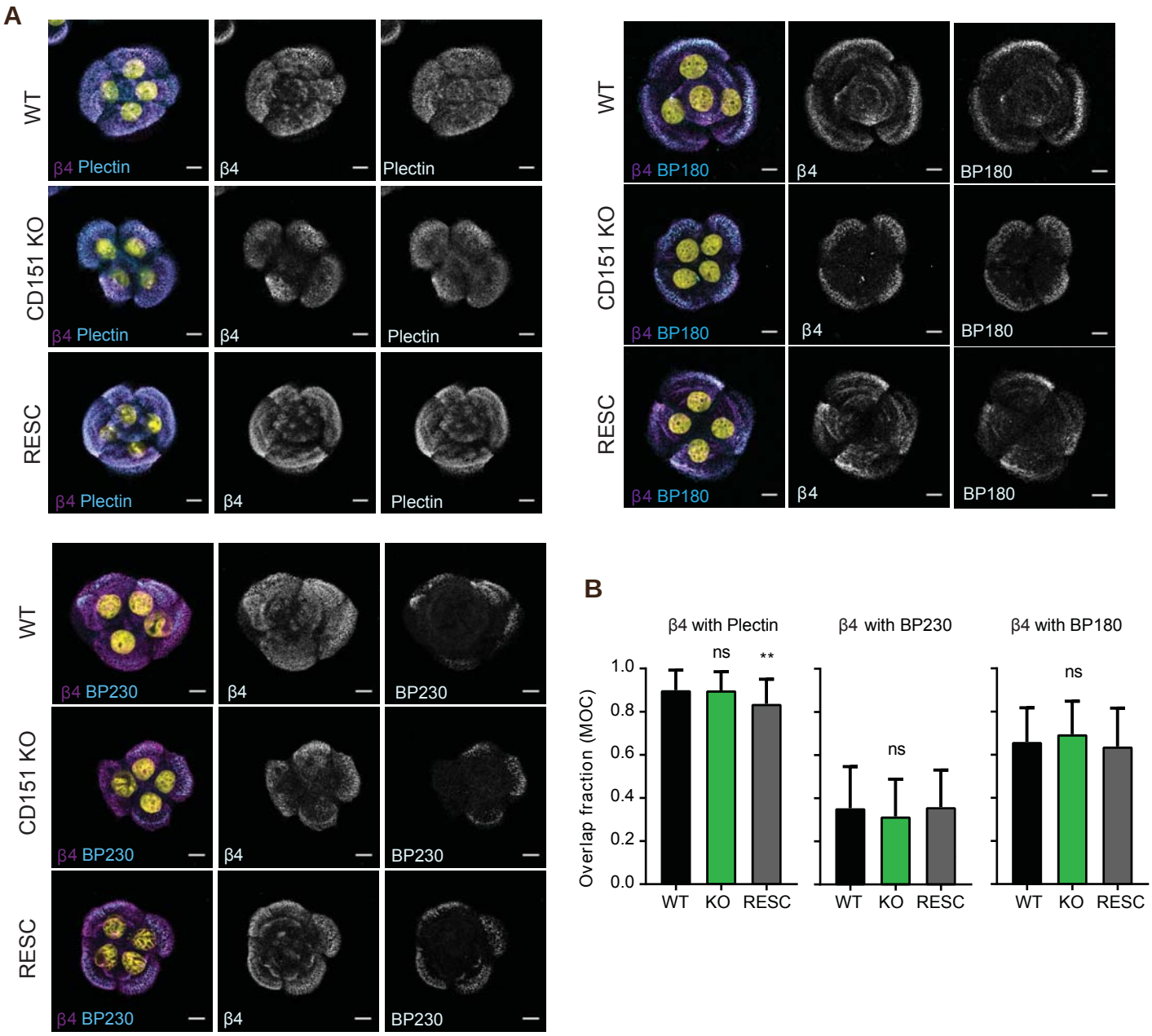

Fig. S2 Type I HDs are formed in the absence of CD151.
(A) Confocal images of WT, CD151-KO and CD151-RESC PA-JEB/β4 keratinocytes stained for plectin, BP180 or BP230 (cyan), β4 (magenta) and DAPI (yellow). Scale bars are 10 µm. B. Quantification of the colocalization (MOC) of β4 with BP180 (WT: 52 images, KO: 47 images, RESC: 48 images; 2 exp. each), BP230 (WT: 97 images, KO: 90 images, RESC: 89 images; 5 exp. each) and plectin (WT: 46 images, KO: 42 images, RESC: 41 images; 3 exp. each) in WT, CD151-KO and CD151-RESC PA-JEB/β4 keratinocytes. Graphs show the mean+s.d.; P value: **< 0.01 (Mann-Whitney U test), ns>0.05.

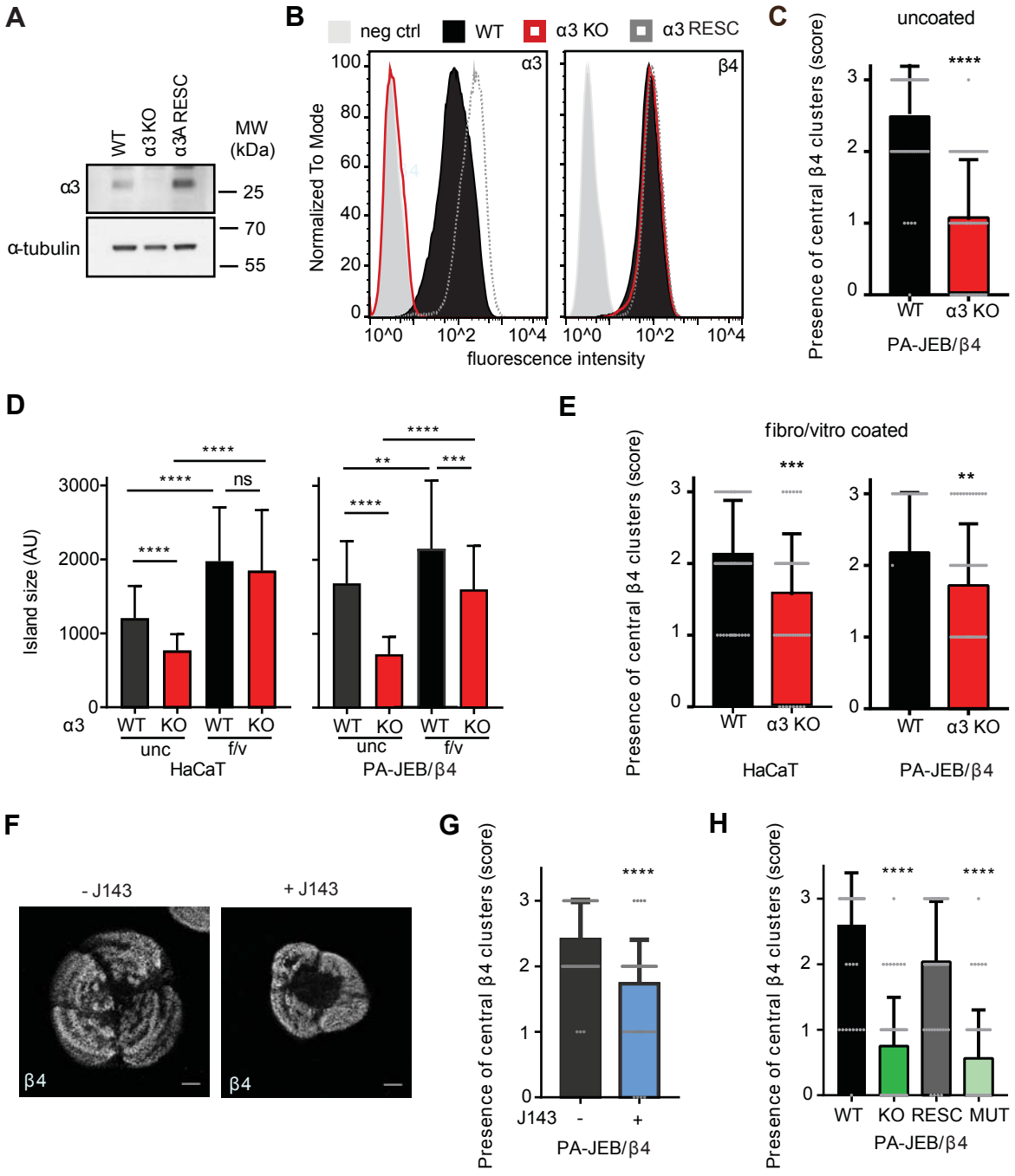

Fig. S3 Confirmation of α3β1 involvement in the maintenance of central HD-like adhesions.
(A) Whole cell lysate of WT, α3-KO and α3-RESC PA-JEB/β4 keratinocytes analyzed by WB for protein levels of α3 and GAPDH (loading control). (B) FACS analyses of α3 and β4 surface expression on WT, α3-KO and α3-RESC PA-JEB/β4 keratinocytes. (C) Quantification of the presence of central β4 clusters (score 0-3) in WT (61 images; 3 exp.) and α3-KO (62 images; 3 exp.) PA-JEB/β4 keratinocytes. (D) Island size of WT and α3-KO HaCaT and PA-JEB/β4 keratinocytes seeded on uncoated (unc) or fibronectin and vitronectin (f/v) coated coverslips (~60 images each; 3 exp.). (E) Quantification of the presence of central β4 clusters (score 0-3) in WT (60 images; 3 exp.) and α3-KO (59 images; 3 exp.) HaCaT keratinocytes, and WT (60 images; 3 exp.) and α3-KO (61 images; 3 exp.) PA-JEB/β4 keratinocytes seeded on fibronectin and vitronectin coated coverslips. (F) Confocal images of β4 in PA-JEB/β4 cells grown on coverslips for two days in the absence (-) or presence (+) of α3 blocking antibody J143. Scale bars are 10 µm. (G) Quantification of the presence of central β4 clusters (score 0-3) in WT PA-JEB/β4 cells grown in the absence (65 images; 3 exp.) or presence (66 images; 3 exp.) of J143. (H) Quantification of the presence of central β4 clusters (score 0-3) in WT (57 images; 4 exp), CD151-KO (56 images; 4 exp.), RESC (55 images; 4 exp.) and MUT (55 images; 4 exp.) PA-JEB/β4 keratinocytes. WT, KO and RESC data is identical to the data depicted in Fig. 2G. Graphs in C, D, E, G and H show the mean+s.d.; P values: **<0.01, ***< 0.001, ****< 0.0001 (Mann-Whitney U test).

Target	Antibody	Application	Source/kind gift from
α-tubulin	Mouse mAb	WB (1:10000)	Sigma-Aldrich #t5168
BP230	5E, human mAb	IF (1:400)	Takashi Hashimoto, Keio University School of Medicine, Shinjuku, Tokyo, Japan
Coll. XVII	VK14, mouse mAb	IF (1:5)	Hendri Pas University Medical Center Groningen, Groningen, The Netherlands
CD151	5C11, mouse mAb	IF (1:1), IP (1:5), WB (1:50)	Fedor Berditchevski University of Birmingham, Birmingham, United Kingdom
CD151	11G5 (IgG1), mouse mAb	IP (1 µg ml^{-1}), FACS (1:400), WB (1:2000)	Jonathan Humphries University of Manchester, Manchester, United Kingdom
CD151	11B1.G4 (p48), mouse mAb	WB (1:2500)	Leonie Ashman University of Newcastle, Newcastle, NSW, Australia.
GAPDH	Rabbit pAb	WB (1:2000)	Cell Signaling #5174
GFP	B34, mouse mAb	WB (1:5000)	Covance
Itg. α3	J143, mouse mAb	IF (1:250), IP (1 µg ml^{-1}), FACS (1:400), blocking (30 µg ml^{-1})	American Type Culture Collection
Itg. α3	A-3, mouse (IgG2a) mAb	IF (1:100)	Santa Cruz #sc-374242
Itg. α3A	Rabbit pAb	WB (1:1000)	In-house
Itg. α6	AA6NT, rabbit pAb	WB (1:500)	Anne Cress University of Arizona, Tucson, Arizona, USA
Itg. α6	GoH3, rat mAb	IP (1 µg ml^{-1})	In-house
Itg. β1	Rabbit pAb	WB (1:2000)	Reinhard Fässler Max Planck Institute of Biochemistry, Martinsried, Germany
Itg. β4	439-9B, rat mAb	IF (1:200)	BD Bioscience #555719
Itg. β4	PE-rat anti-human-CD104	FACS (1:400)	BD Pharmingen #555720
Itg. β4	ASC8, mouse, mAb	Blocking (1:5)	Amy Skubitz University of Minnesota, Minneapolis, MN, USA
Itg. β4	Rabbit pAb	WB (1:2000)	In-house
Keratin 14	Rabbit pAb	IF (1:1000)	Covance #PRB-155P
Laminin-332	R14, rabbit pAb	IF (1:500)	Monique Aumailley University of Cologne, Cologne, Germany
pPaxillin Y31	Rabbit pAb	IF (1:200)	Biosource #44-720G
Plectin	P2, guinea pig pAb	IF (1:400)	Harald Herrmann German Cancer Research Center, Heidelberg, Germany

Table S1. Primary antibodies used in various techniques
Abbreviations used: Immune fluorescence (IF), Immune precipitation (IP), fluorescence-activated cell sorting (FACS) and Western blot (WB).

Movie 1
Movies of a time-lapse experiment using WT (movie S1) and CD151-KO (movie S2) PA-JEB/β4-GFP keratinocytes. Images were taken every hour for 20h, starting from the addition of DMEM+FCS (0h timepoint, 20h after seeding).

Movie 2
Movies of a time-lapse experiment using WT (movie S1) and CD151-KO (movie S2) PA-JEB/β4-GFP keratinocytes. Images were taken every hour for 20h, starting from the addition of DMEM+FCS (0h timepoint, 20h after seeding).

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