Supplemental Materials

Molecular Biology of the Cell

Osmani et al.

Supplementary Material

Figure S1. HD structures in HaCaT cells. (A) HaCaT cell immuno-stained for endogenous ITGA6, Plectin and keratin 14 (CK14). Arrowheads highlight ITGA6 clusters colocalizing with Plectin and growing keratin filaments. Scale bar = 10 µm. (B) Schematic representation of mature (type I) HDs. Note that immature/type II HDs do not contain BPAG1 and Coll XVII.

Figure S2. Arf6 colocalizes with ITGA6 at the plasma membrane. Confocal images of cells expressing EGFP-Arf6(wt) immuno-stained for ITGA6. BM: confocal slice in the XY plane of the basal membrane; +1 µm: confocal slice in the XY plane 1 µm above the BM (+1µm); XZ: confocal slice in the XZ plane along the dashed line; PM: confocal slice in the XY plane of plasma membrane. Scale bar (xy) =10 µm, (xz) = 1 µm.

Figure S3. ITGA6 does not localize at focal adhesions. HaCaT cell immuno-stained for endogenous ITGA6 and (A) βPIX and (B) PAK1. Upper panel: TIRF image, scale bar=10 µm; lower panel: GSD-TIRF image (pixel size = 20 nm), scale bar=1 µm.

Figure S4. Arf6 is required for proper caveolin1 localization at the basal plasma membrane. (A) Quantification of the mean caveolae number per µm of plasma membrane counted on transmission electron microscopy images of 100 µm sections of cells transfected with control, anti-Cav1 or Cavin1 siRNAs (see methods for details) from 1 experiment; number of sections=20, 20 and 20, respectively. Mann-Whitney tests: siCTL vs siCav1 or siCavin1. (B) Representative electron micrograph of the plasma membrane of HaCaT cells transfected with control siRNA, or siRNAs against Cav1 or Cavin1. Scale bar = 100 nm. (C) Confocal images of the basal membrane in cells

transfected with control or indicated siRNAs and immuno-stained for ITGA6 and Cav1. Scale bar=10 µm. (D-E) Quantification of the area covered by Cav1 on the basal membrane of cells (D) transfected by siRNAs against Arf6 or Cavin1 or (E) expressing EGFP-Arf6 constructs from 3 independent experiments; (D) number of cells = 81, 51 and 20, respectively, (E) number of cells = 16, 21 and 21, respectively. T-test (D) siCTL vs siArf6 or siCavin1 and (E) Arf6(wt) vs Arf6(T27N) or Arf6(Q67L).

Figure S5. HD remodeling does not depend on FA signaling. (A) Representative confocal images of the basal membrane of cells treated with vehicle or the FAK inhibitor (PF-228) and immuno-stained for ITGA6 and pY397-FAK. Scale bar=10 µm. (B) Quantification of the amount of HDs at the basal membrane of cells treated with vehicle or the FAK inhibitor (PF-228) from 3 independent experiments; number of cells = 56 and 72. T-test: CTL vs PF-228. (C) Quantification of the amount of HDs on the basal membrane of cells transfected with control siRNA (siCTL) or ITGB1 siRNAs (siITGB1) from 3 independent experiments; number of cells = 54 and 54. T-test: siCTL vs or siITGB1.

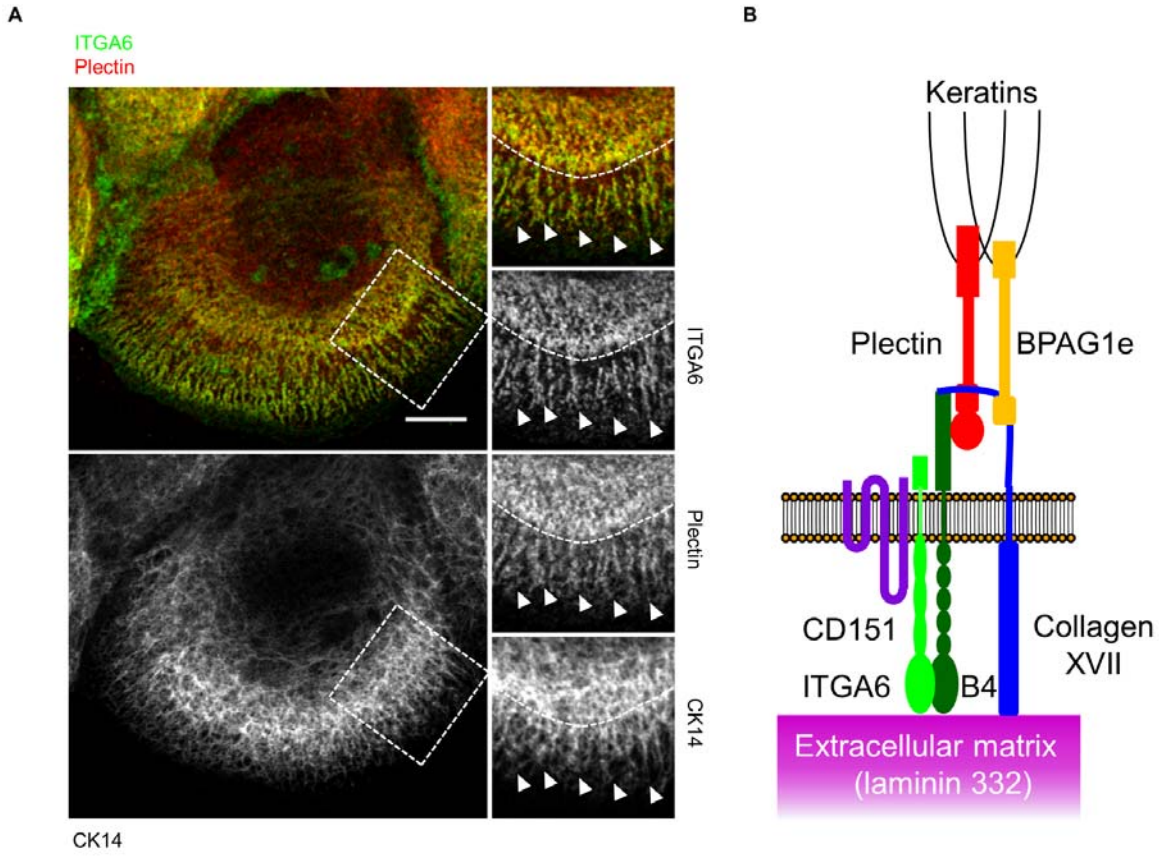
Figure S6. FRAP kinetics of EGFP-ITGB4. Time lapse images extracted from representative FRAP datasets used for the analysis: (A) in the HDs at the basal plasma membrane (related to Fig. 5C-D and 7G) and (B) in ICs (related to Fig. 9G).

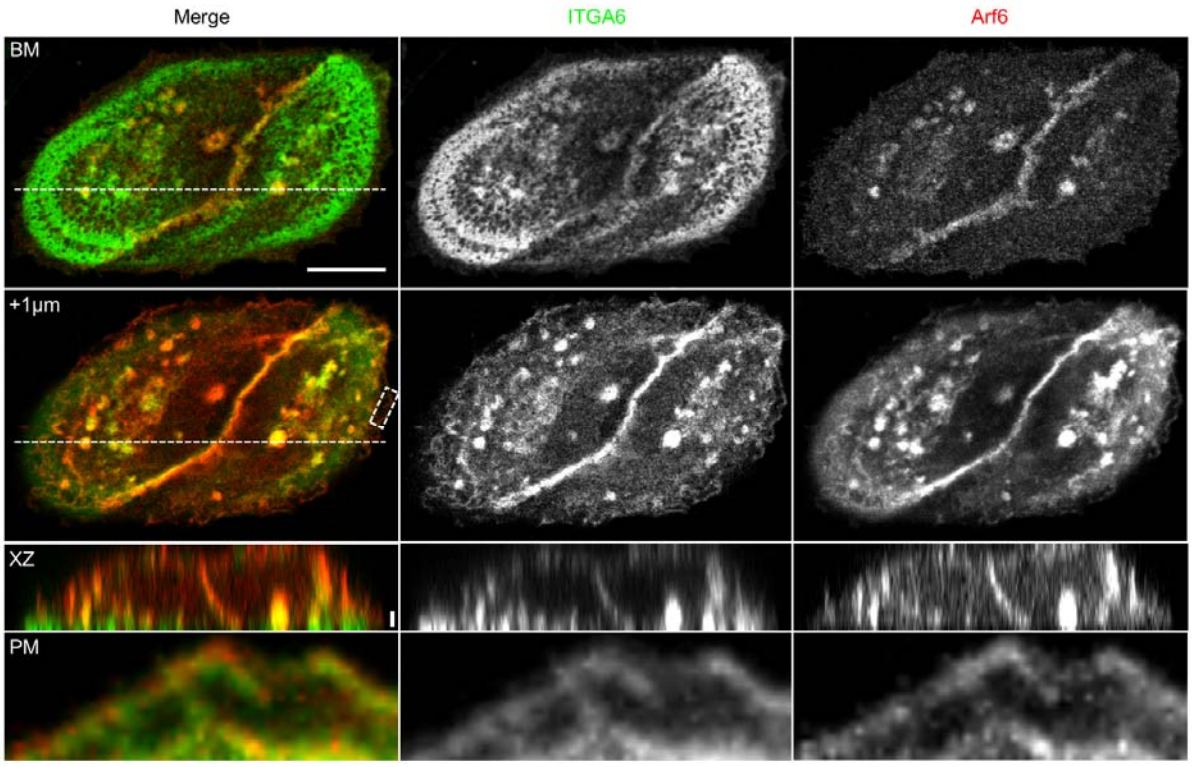
Figure S7. Dynasore inhibits dynamin-dependent endocytosis in HaCaT cells. Confocal slice of HaCaT cells incubated for 1h with EGF-Alexa Fluor 488 on ice in the presence of vehicle (CTL) or Dynasore (Dyna), then washed with cold medium containing vehicle or Dynasore and fixed immediately (t=0) or washed with preheated medium

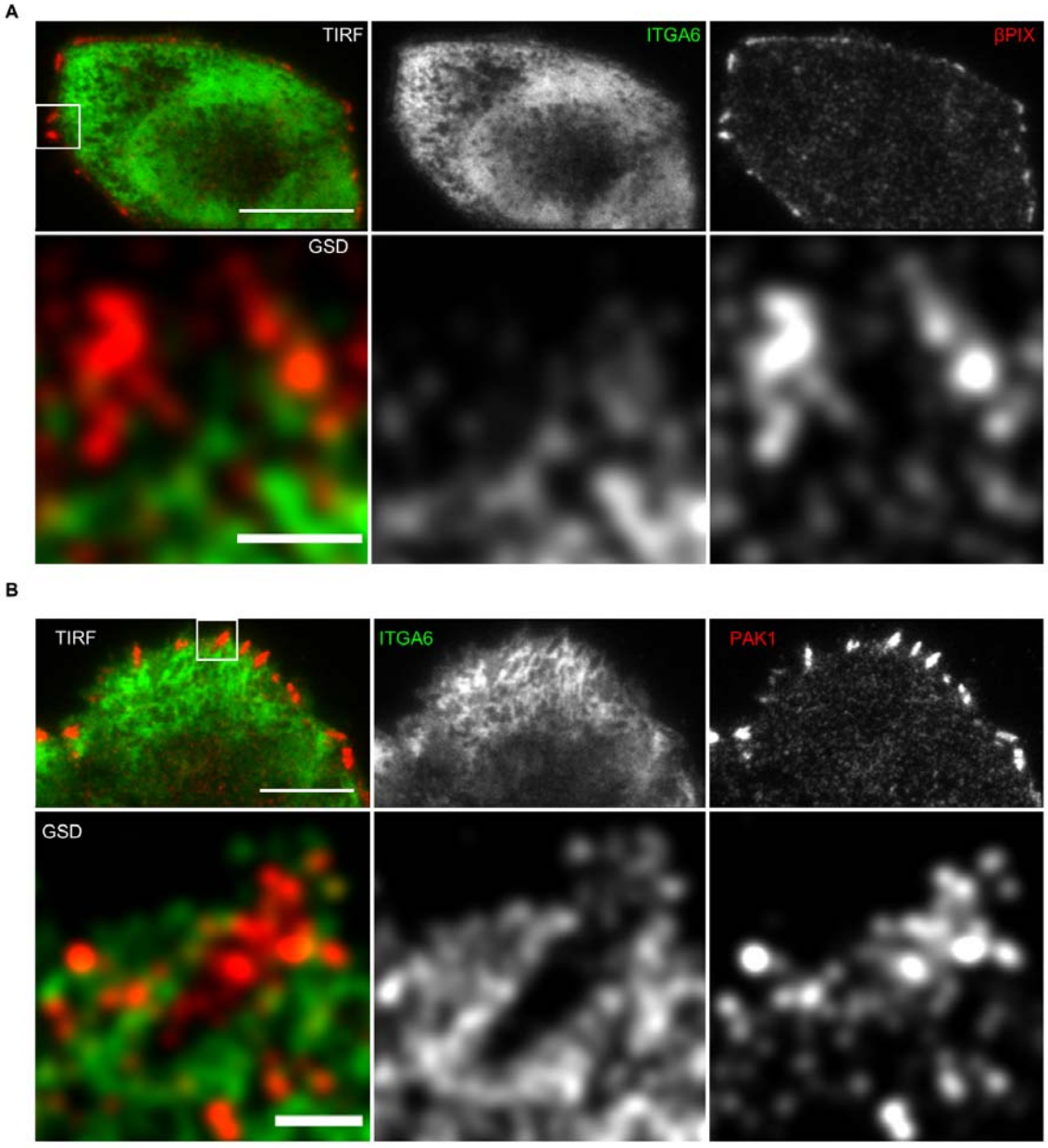
containing vehicle or Dynasore, incubated 10 min at 37°C and fixed (t=10 min). Cells were then immunostained for endogenous EEA1. The coloc panels shows pixels colocalizing in both channels. Scale bar = 10 µm.

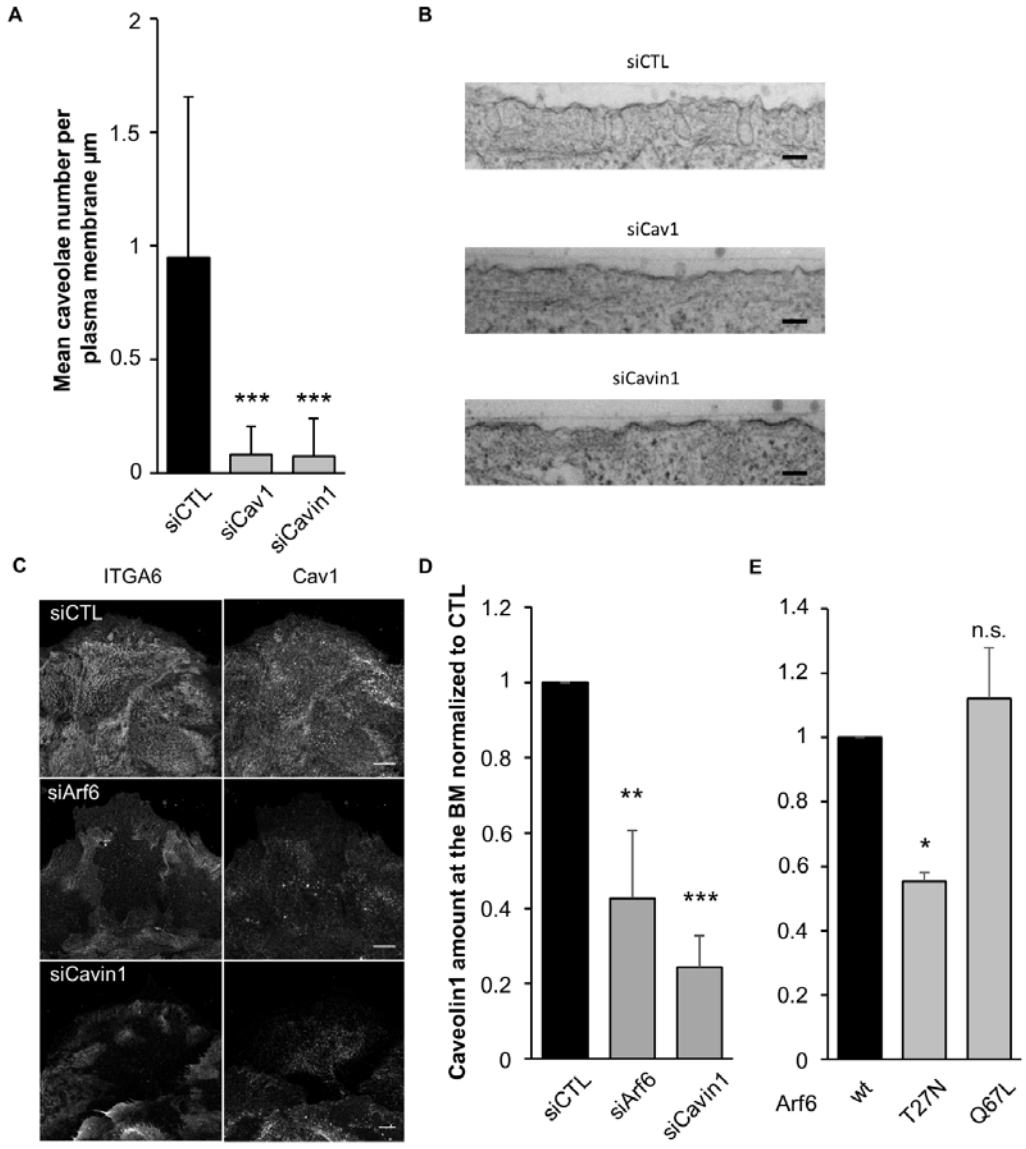
Figure S8. HD grow in response to external mechanical cues. Confocal image of a cell expressing EGFP-ITGB4 under a 10% uniaxial strain for 30 min. The green channel shows the HD signal at the basal membrane immediately after cell stretching is induced and the red channel shows the HD signal after a 30 min stretch. Scale bar = 10 µm.

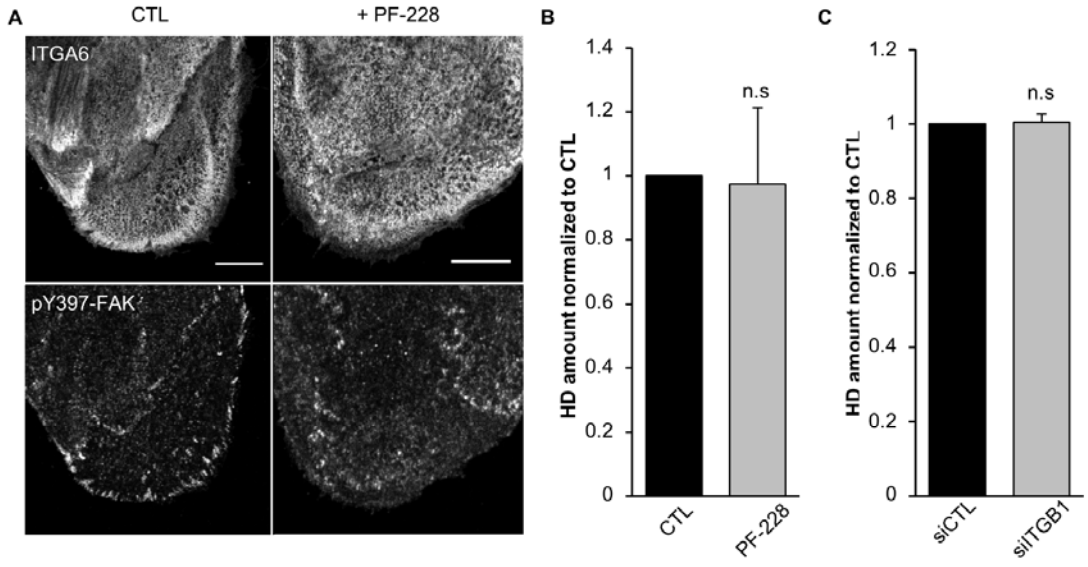
Figure S9. Quantification of siRNA knockdown effect. (A-G) Protein extracts of cells transfected with control siRNAs or siRNAs against (A) ITGA6, (B) ITGB1, (C-D) Arf6, (E-F) Cav1 or (G) Cavin1 were immunoblotted against (A) ITGA6, (B) ITGB1, (C-D) Arf6, (E-F) Cav1 or (G) Cavin1 and (A,E) α-tubulin, (B-D,F) β-actin or (G) GAPDH as a loading control. For rescue experiments (D,F), cells were transfected with (D) Arf6 or (G) Cav1 siRNAs then 48h later nucleofected with the corresponding siRNA-resistant constructs (RNAi^{R}) and protein extracts were prepared 24h later.

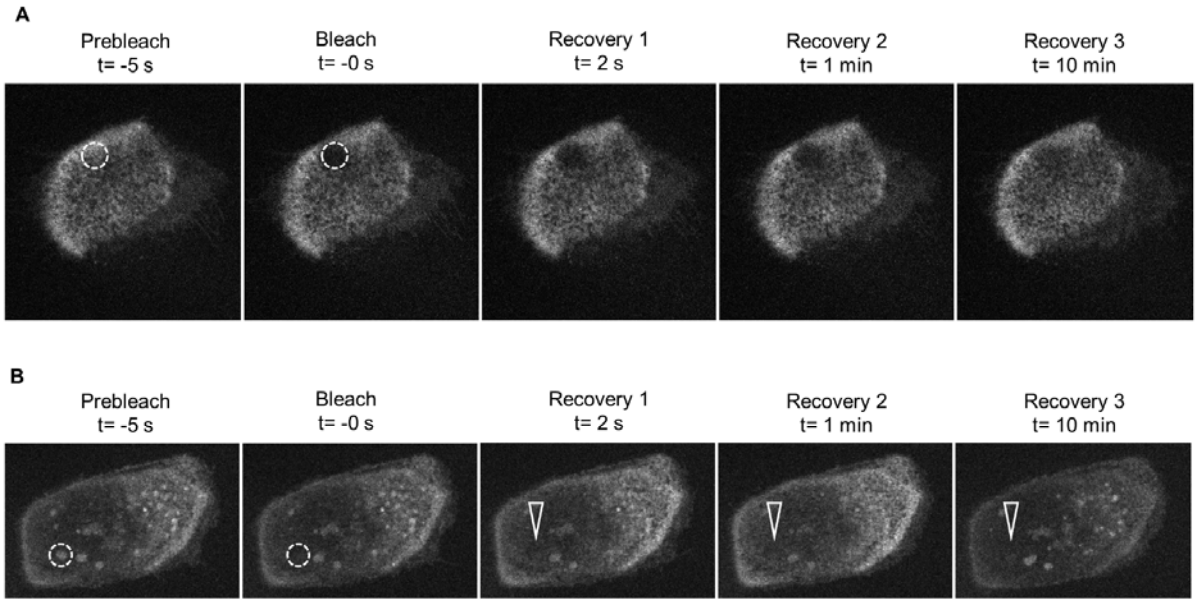

A
ITGA6
Plectin
ITGA6
Plectin
CK14
CK14
B
Keratins
Plectin
BPAG1e
CD151
ITGA6
B4
Collagen
XVII
Extracellular matrix
(laminin 332)


Merge
ITGA6
Arf6
BM
+1μm
XZ
PM


A
TIRF
ITGA6
βPIX
GSD
B
TIRF
ITGA6
PAK1
GSD


A
Mean caveolae number per plasma membrane µm
2
1.5
1
0.5
0
***
***
siCTL
siCav1
siCavin1
B
siCTL
siCav1
siCavin1
C
ITGA6
Cav1
siCTL
siArf6
siCavin1
D
Caveolin1 amount at the BM normalized to CTL
1.2
1
0.8
0.6
0.4
0.2
0
**
***
siCTL
siArf6
siCavin1
E
1.4
1.2
1
0.8
0.6
0.4
0.2
0
n.s.
*
Arf6
wt
T27N
Q67L


A
CTL
+ PF-228
ITGA6
pY397-FAK
B
HD amount normalized to CTL
1.4
1.2
1
0.8
0.6
0.4
0.2
0
n.s
CTL
PF-228
C
HD amount normalized to CTL
1.2
1
0.8
0.6
0.4
0.2
0
n.s
siCTL
siITGB1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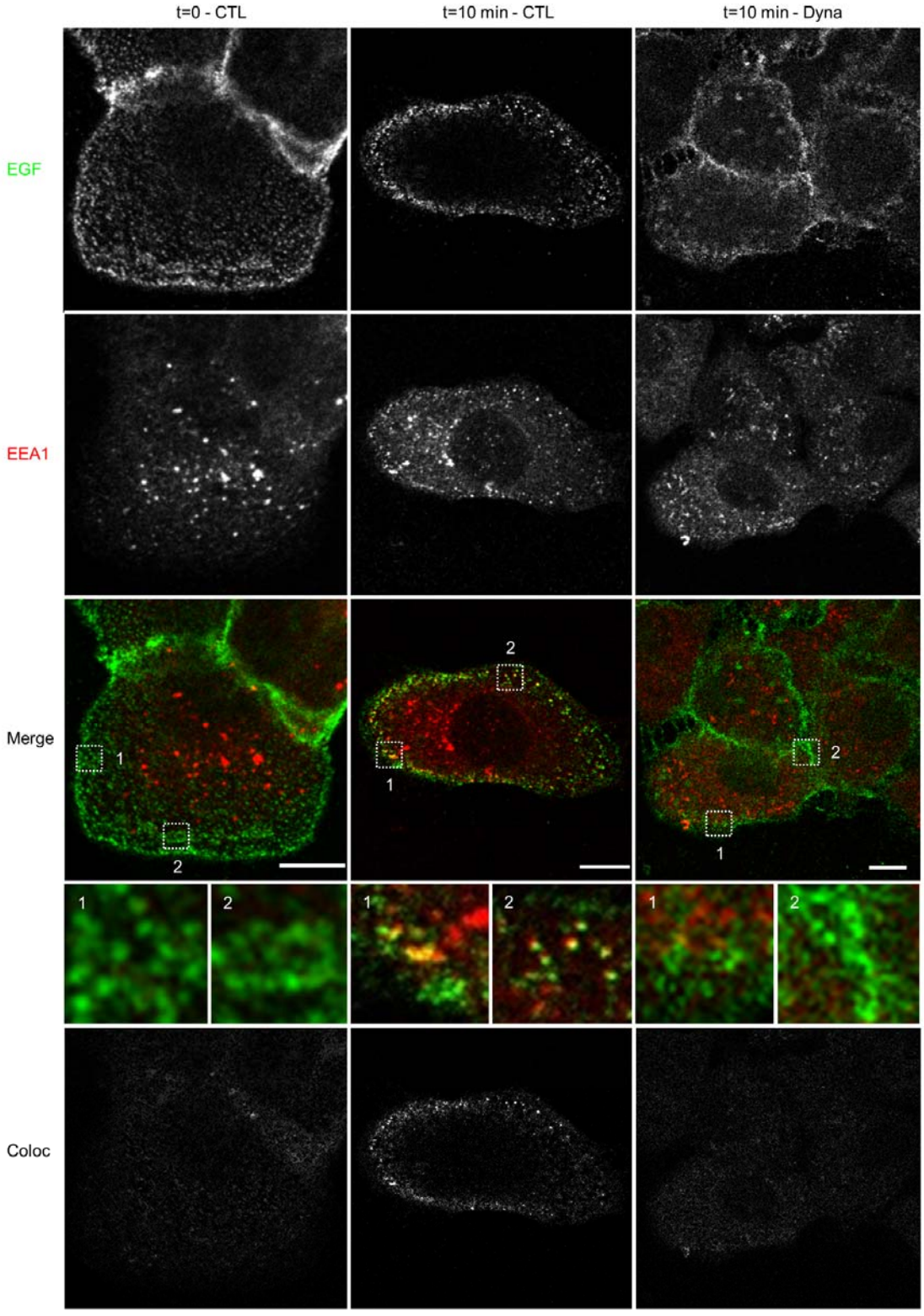
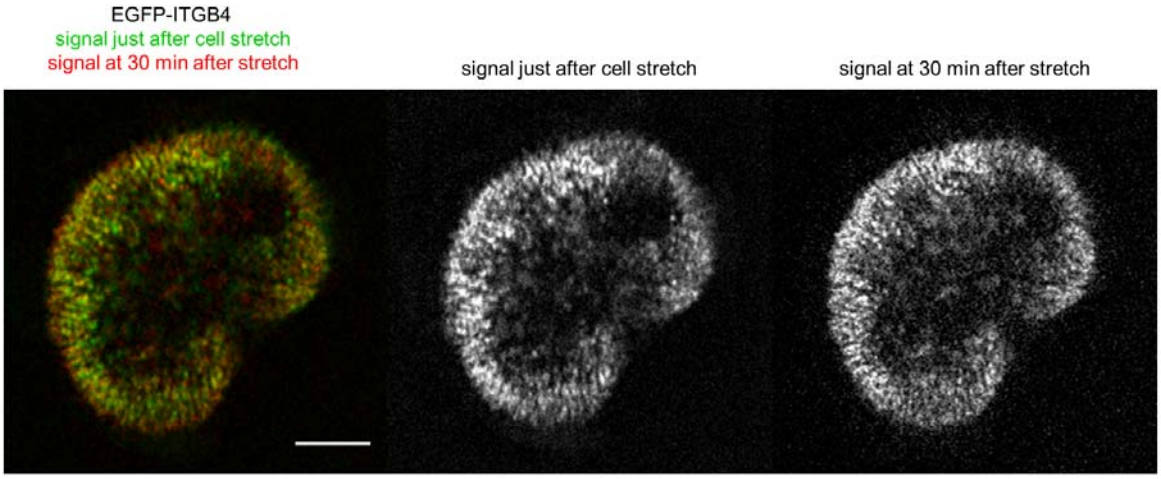
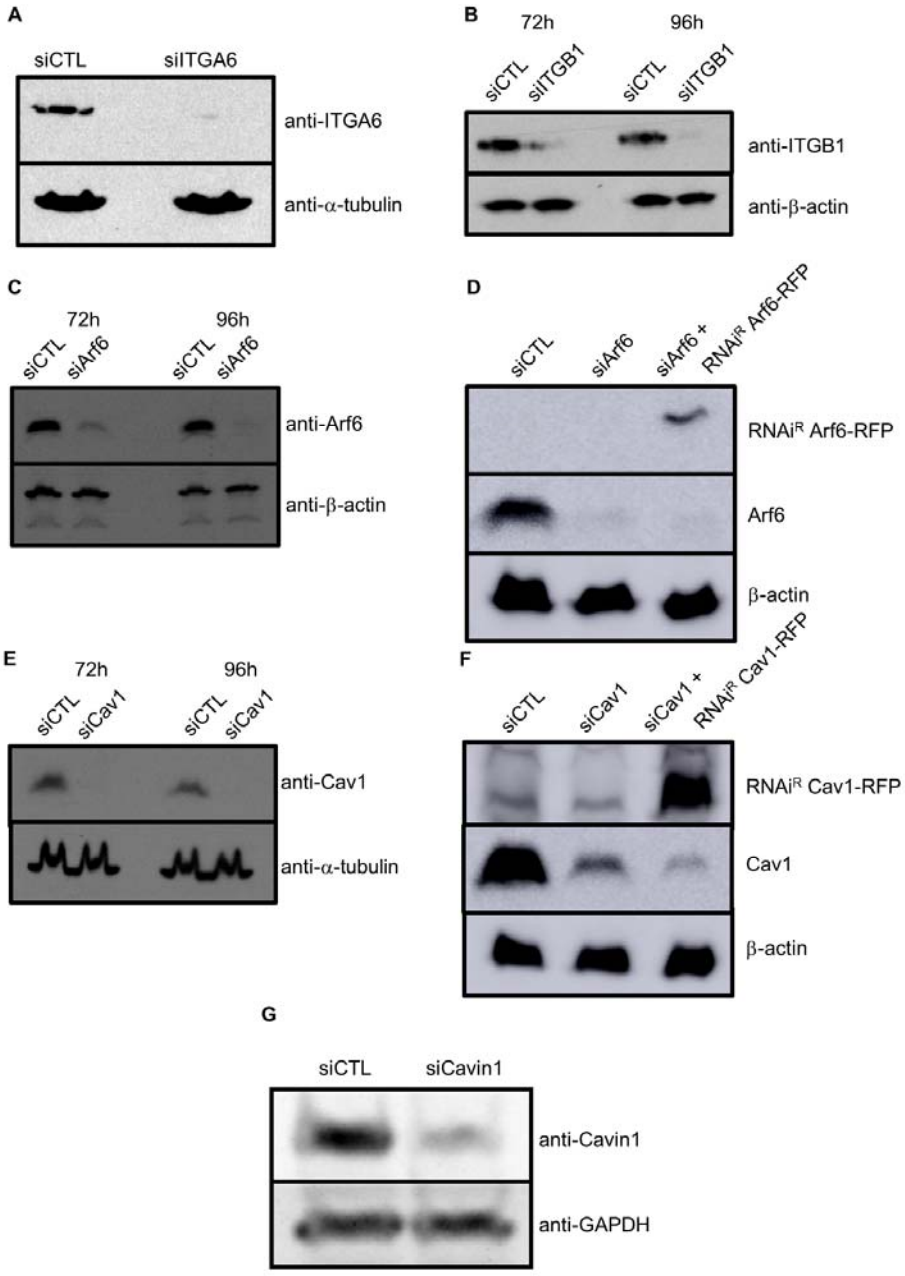
Prebleach
t= -5 s
Bleach
t= -0 s
Recovery 1
t= 2 s
Recovery 2
t= 1 min
Recovery 3
t= 10 min
B
Prebleach
t= -5 s
Bleach
t= -0 s
Recovery 1
t= 2 s
Recovery 2
t= 1 min
Recovery 3
t= 10 min


t=0 - CTL
t=10 min - CTL
t=10 min - Dyna
EGF
EEA1
Merge
Coloc
1
2
1
2
2
1
1
2
1
2
1
2
1
2


EGFP-ITGB4
signal just after cell stretch
signal at 30 min after stretch
signal just after cell stretch
signal at 30 min after stretch


A
siCTL
siITGA6
anti-ITGA6
anti-α-tubulin
B
72h
96h
siCTL
siITGB1
siCTL
siITGB1
anti-ITGB1
anti-β-actin
C
72h
96h
siCTL
siArf6
siCTL
siArf6
anti-Arf6
anti-β-actin
D
siCTL
siArf6
siArf6 +
RNAiR Arf6-RFP
RNAiR Arf6-RFP
Arf6
β-actin
E
72h
96h
siCTL
siCav1
siCTL
siCav1
anti-Cav1
anti-α-tubulin
F
siCTL
siCav1
siCav1 +
RNAiR Cav1-RFP
RNAiR Cav1-RFP
Cav1
β-actin
G
siCTL
siCavin1
anti-Cavin1
anti-GAPDH