Supplemental Materials

Molecular Biology of the Cell

Seddiki et al.

Supplementary figure Legends

Figure S1: Anti-vinculin and F-actin staining taken at the cell-substratum interface of α-catenin KD MDCK cells expressing wt or α-catenin mutants

Anti-vinculin immunostaining and phalloidin staining of αE-catenin KD MDCK cells expressing α-cat-WT-GFP, α-cat-L334P-GFP or α-cat-Δmod-GFP. Panels show confocal sections taken at the most ventral position in the cell layers. The expression of mutant proteins does not impact the distribution of vinculin and F-actin at the cell-substratum interface. Scale bar: 5 μm.

Figure S2: Blebbistatin treatment does not displace vinculin from cell-cell contacts of α-cat-Δmod-GFP expressing cells (A) Anti-vinculin immunostaining was performed on αE-catenin KD MDCK cells expressing α-cat-WT-GFP or α-cat-Δmod-GFP grown on glass substrates. Panels show confocal sections taken at the apical cell domain of the monolayers. While vinculin staining was lost from cell–cell contacts of α-cat-WT-GFP expressing cells after blebbistatin treatment (20 μM, 2 hours), it persists in blebbistatin treated α-cat-Δmod-GFP expressing cells. Scale bar: 10 μm. **(**B**)** Quantification of the vinculin/α-catenin intensity ratio measure by line scan over cell-cell contacts (mean values ± SD, **** $p < 0.001$, ns: non-significant, one-way ANOVA test).

Figure S3: The typical half recovery time for the α-cat mutants were not significantly different and did not depend on substrate stiffness. (A) FRAP experiments performed on cell-cell contacts of α-cat-WT-GFP, α-cat-L334P-GFP or α-cat-Δmod-GFP expressing cells grown on glass substrates. Means of recovery characteristic times t½ ± SEM of experiments presented in Figure 2 C,D. ns: non-significant, one-way ANOVA test . **(B)** FRAP experiments performed on MDCK α-catenin-KD cells expressing GFP-tagged wt α-catenin (green), α-cat-

L334P (blue) or α-cat-Δmod (red) cultured for 24 hours on 4.5 (light colors) or 35 kPa (dark colors) PPA gels. Graphs represent means of recovery characteristic times t½ ± SEM of experiments presented in Figure 3 A,B. ns: non-significant, two-way ANOVA test.

Figure S4: The junctional stability of E-cadherin does not depend on α-catenin mutation or on substrate stiffness. (A, B) FRAP experiments were performed on E-cadherin-dsRed at cell-cell contacts of αE-catenin KD MDCK transfecting with α-cat-WT-GFP or α-cat-L334P-GFP expressing and coexpressing E-cadherin-dsRed. **(A)** Fluorescence recovery over time (± SEM, n ≥ 50 out of 3 independent experiments for each condition) fitted with a one-term exponential equation. **(B)** Mobile fraction values (mean values ± SD and 95th percentile) extracted from the fits of individual recovery curves considered in panels **A**. ns: non-significant, unpaired t-test. **(C)** FRAP experiments were performed at cell-cell contacts of E-cadherin-GFP expressing MDCK cells spread on substrates of controlled rigidity (2 and 5 kPa, respectively. Curves represent Mean GFP fluorescence recovery over time (± SEM, n ≥ 50 out of 3 independent experiments for each condition) fitted with a one-term exponential equation.

Movie 1:MDCK cells silenced for α-catenin (α-cat KD) seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 2: α-cat KD MDCK cells expressing α-cat-L334P seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 3: α-cat KD MDCK cells expressing GFP-tagged α-cat-wt seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 4: α-cat KD MDCK cells expressing α-cat-Δmod seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 5: RFP-Ftractin expressing α-cat KD cells seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 6: RFP-Ftractin expressing α-cat-L334P cells seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 7: RFP-Ftractin expressing α-cat-wt cells seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Movie 8: RFP-Ftractin expressing α-cat-Δmod cells seeded on 500 μm Ø FN patterns and phase contrast imaged for 16 hours.

Figure S1: Seddiki

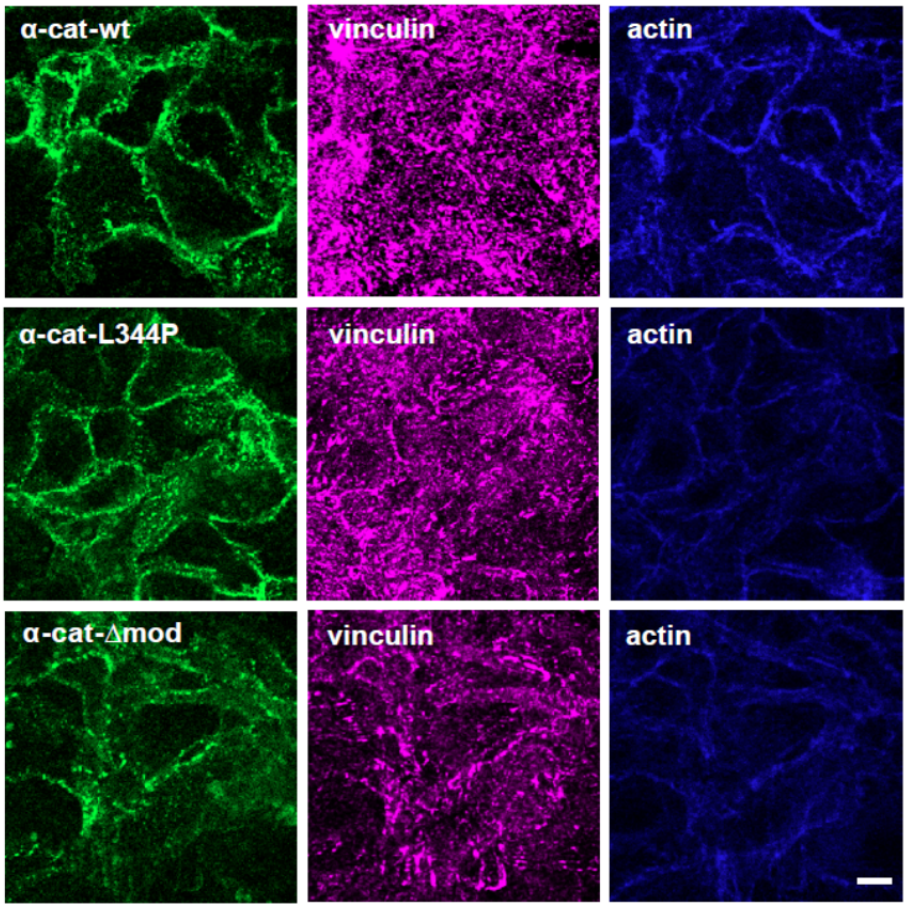

Figure S2: Seddiki

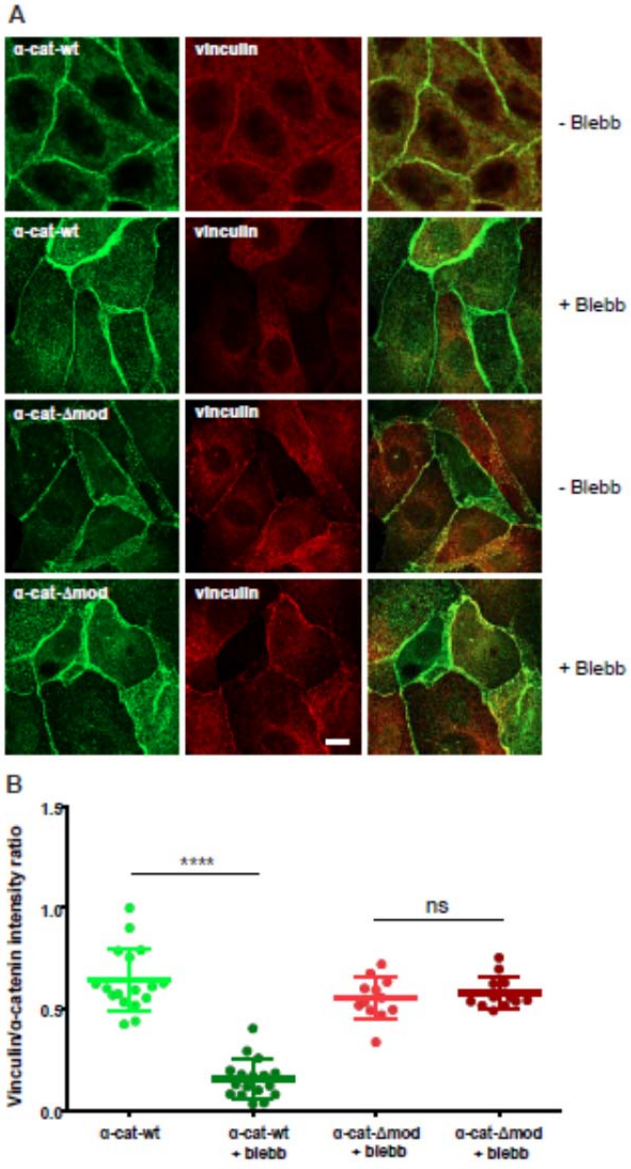

Figure S3: Seddiki

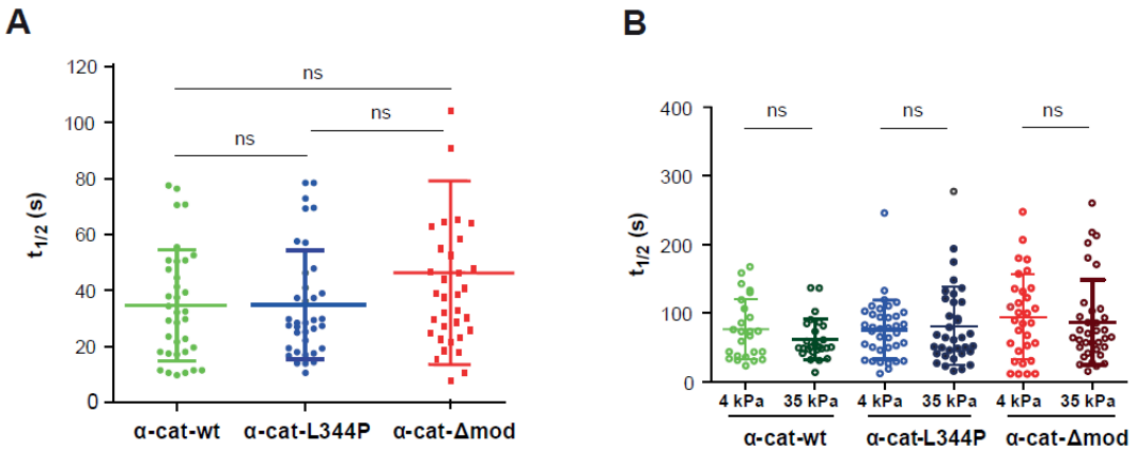

Figure S4: Seddiki