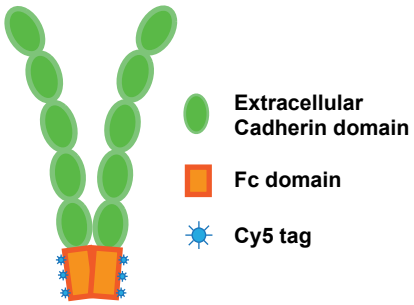
**Fig. S1.** (A) A schematic of the extracellular domain (EC1-5) of Dsg2 and Dsc2a fused to Fc protein, and tagged with Cy5 for visualization. (B) Coomassie Blue-stained SDS-PAGE of Dsg2Fc and Dsc2aFc with expected molecular masses of ~120 kD. A lane between the samples has been deleted for simplification purposes (dashed line) (C) Cy3 labeled collagen IV was printed onto a silanized coverslip using PDMS stamps, and Cy5 labeled Dsg2Fc or Dsc2aFc was then backfilled onto the non-collagen IV coated areas, making a dual-striped surface where a single MDCK cells can spread onto multiple stripes (D) Immunofluorescence staining of desmoplakin (DP I/II) between MDCK cells. (E) Epifluorescence images of Cy5 tagged Dsg2Fc or Dsc2aFc printed on the surface, corresponding DP I/II TIRF images from Fig.1 and a merged image of the two. Scale bar is 10 μm.

**Fig. S2.** Histograms showing the distribution of rupture forces between desmosomal cadherins for each loading rate. As the loading rate is increased a larger rupture force is necessary to break the bonds. This relationship is seen in all conditions above nonspecific binding (A) Dsc2a-Dsc2a, (B) Dsc2a-Dsg2 with $Ca^{2+}$ and Dsc2a-Dsg2 in the presence of the $Ca^{2+}$ chelator EGTA.

**Fig. S3.** Still images from movies 5-7 showing localization of the integrin focal adhesions; visualized with VinGFP. Focal adhesion clusters are localized over collagen IV stripes, and excluded from stripes containing: (A) Dsg2Fc, (B) Dsc2aFc or (C) Dsg2Fc+Dsc2aFc. Scale bar is 10 μm.

# SUPPLEMENTAL FIGURE 1

A.

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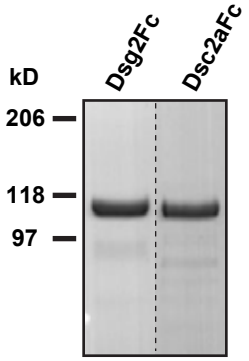
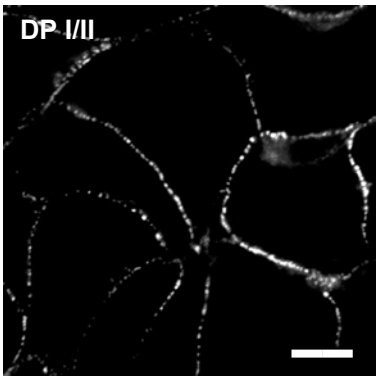



C.


D.

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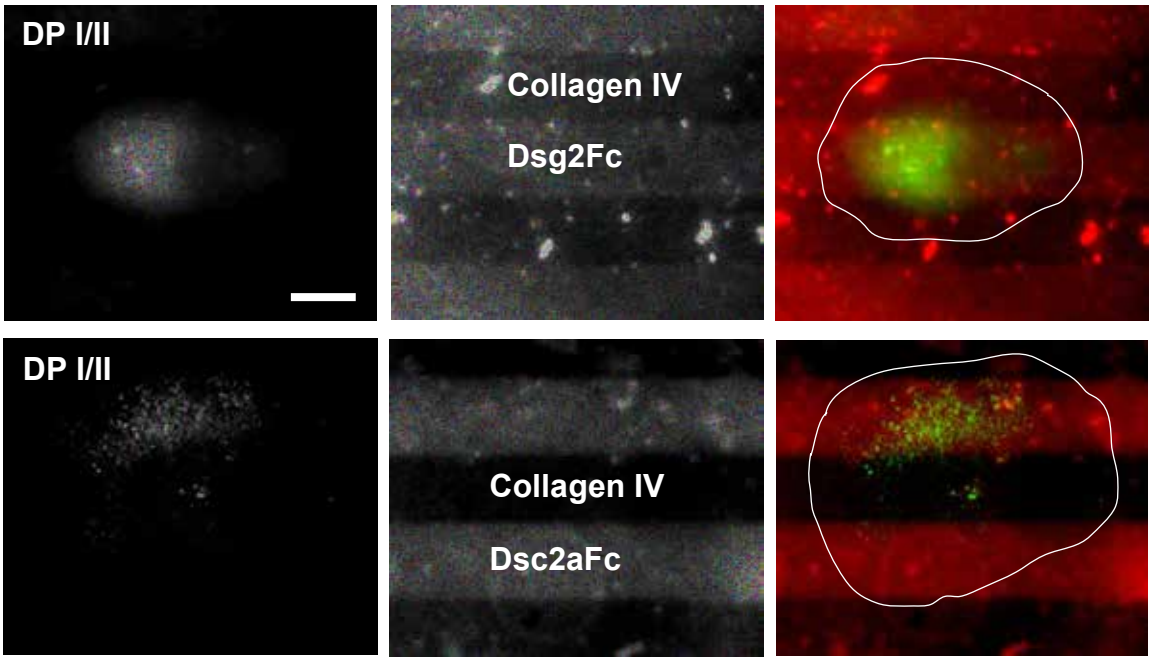
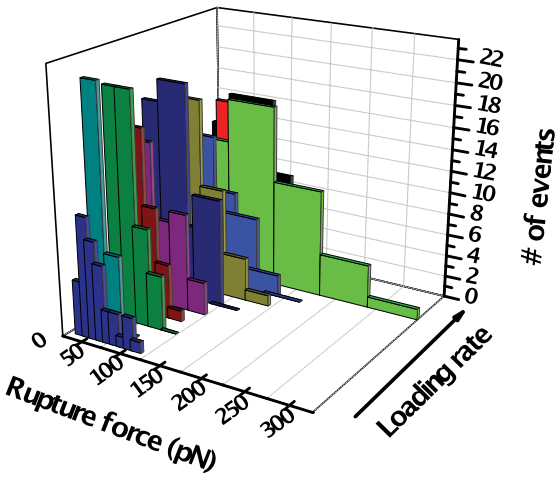


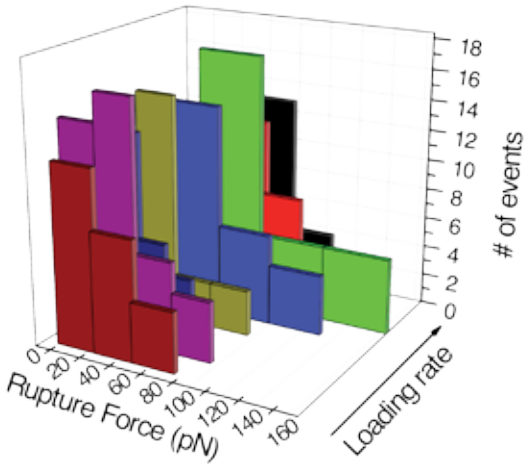
# SUPPLEMENTAL FIGURE 2

A.

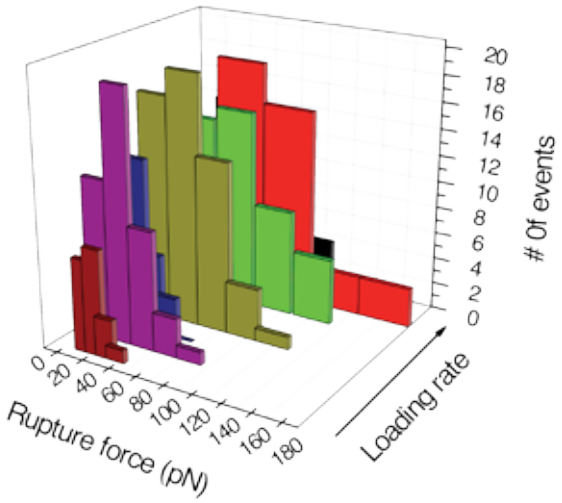



Dsc2a-Dsc2a w/ $Ca^{++}$

B.

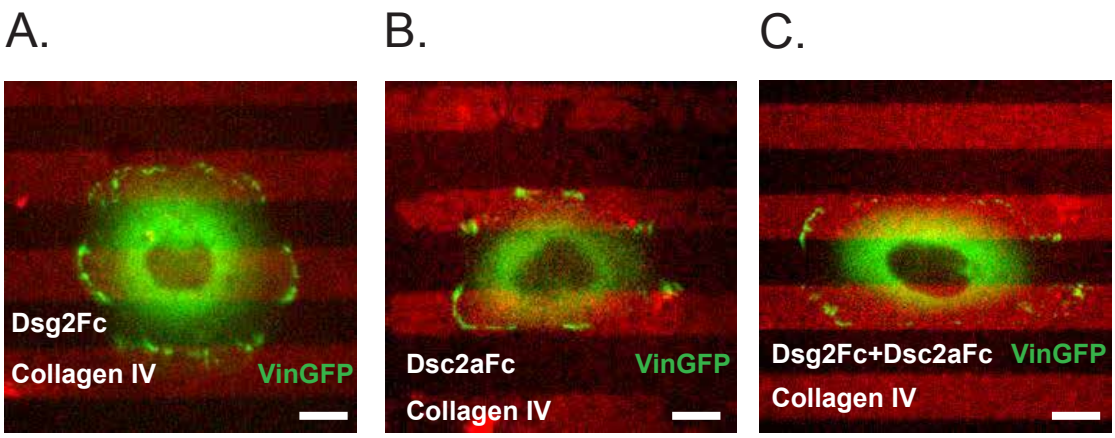



Dsc2a-Dsc2a w/ $Ca^{++}$



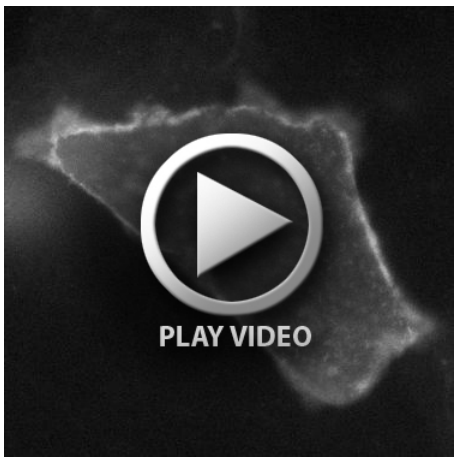

Dsc2a-Dsc2a w/ EGTA

# SUPPLEMENTAL FIGURE 3



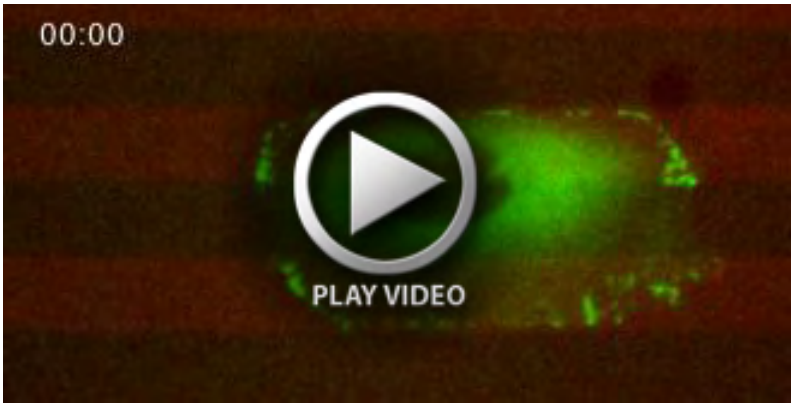

**Movie 1. Membrane mobility of Dsg2W2A_GFP.** FRAP movie of MDCK cells transfected with GFP-tagged proteins Dsg2W2A_GFP. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M). Frames were taken every 10 s for 10 min. An arrow marks the spot where the photobleaching took place.


**Movie 2. Membrane mobility of Dsg2_GFP.** FRAP movie of MDCK cells transfected with GFP-tagged proteins Dsg2_GFP. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M). Frames were taken every 10 s for 10 min. An arrow marks the spot where the photobleaching took place.

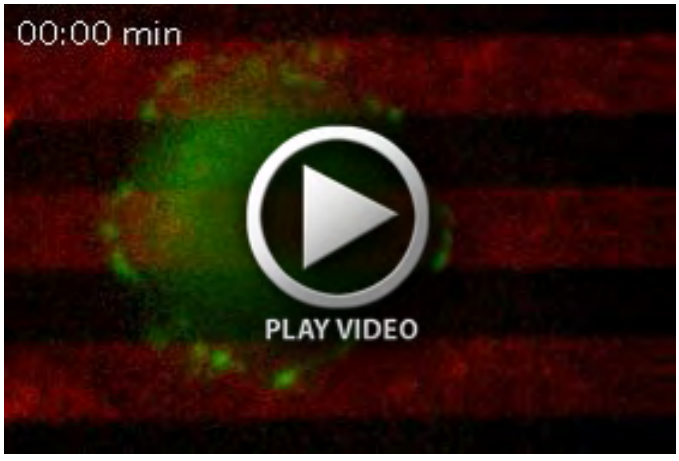



**Movie 3. Membrane mobility of Dsc2aW2A_GFP.** FRAP movie of MDCK cells transfected with GFP-tagged proteins Dsc2aW2A_GFP. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M). Frames were taken every 10 s for 10 min. An arrow marks the spot where the photobleaching took place.


**Movie 4. Membrane mobility of Dsc2a_GFP.** FRAP movie of MDCK cells transfected with GFP-tagged proteins Dsc2a_GFP. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M). Frames were taken every 10 s for 10 min. An arrow marks the spot where the photobleaching took place.




**Movie 5. MDCK migration on dual-patterned substrates of collagenIV and Dsg2Fc+Dsc2aFc.** Single MDCK cells stably expressing VinGFP migrating on surfaces functionalized with collagenIV-Cy3 stripes (red) and backfilled with Dsg2Fc+Dsc2aFc. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M) with a 40X objective. Frames were taken every 10 min for 6-10 hrs. Still of the first frame of each movie are shown in supplemental figure 3.




**Movie 6. MDCK migration on dual-patterned substrates of collagen IV and Dsg2Fc.** Single MDCK cells stably expressing VinGFP migrating on surfaces functionalized with collagenIV-Cy3 stripes (red) and backfilled with Dsg2Fc. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M) with a 40x objective. Frames were taken every 10 min for 6-10 hrs. Still of the first frame of each movie are shown in supplemental figure 3.


**Movie 7. MDCK migration on dual-patterned substrates of collagen IV and Dsc2aFc.** Single MDCK cells stably expressing VinGFP migrating on surfaces functionalized with collagenIV-Cy3 stripes (red) and backfilled with Dsc2aFc. Images were analyzed by time-lapse epifluorescence microscopy on a custom-built inverted microscope (Intelligent Imaging Innovations (3i); Zeiss Axiovert 200M) with a 40X objective. Frames were taken every 10 min for 6-10 hrs. Still of the first frame of each movie are shown in supplemental figure 3.