1 **Supporting Information**

2

3 **Tuning epithelial cell-cell adhesion and collective dynamics with functional**
4 **DNA-E-cadherin hybrid linkers**

5

6 Andreas Schoenit[1,2,3], Cristina Lo Giudice[2], Nina Hahnen[1,2], Dirk Ollech[2,4], Kevin
7 Jahnke[1,5], Kerstin Göpfrich[1,5,*], Elisabetta Ada Cavalcanti-Adam[2,*]

8

9 1 Max Planck Institute for Medical Research, Biophysical Engineering Group,
10 Jahnstraße 29, D-69120 Heidelberg, Germany
11 2 Department of Cellular Biophysics, Growth Factor Mechanobiology Group, Max
12 Planck Institute for Medical Research, Jahnstraße 29, D-69120 Heidelberg, Germany
13 3 Present address: Institut Jacques Monod, CNRS and Université de Paris, 75013
14 Paris, France
15 4 Present address: Applied Physics Department, Science for Life Laboratory and KTH
16 Royal Technical University, Tomtebodavägen 23A, S-17165, Stockholm, Sweden
17 5 Department of Physics and Astronomy, Heidelberg University, D-69120 Heidelberg,
18 Germany

19

20 * correspondence: kerstin.goepfrich@mr.mpg.de, eacavalcanti@mr.mpg.de

21

22 **Materials and Methods**

23

24 Design of the DNA linkers

25

26 All DNA linkers were designed and their hybridization strength was calculated using
27 the Nucleic Acid Package (NUPACK) [1]. It provides a thermodynamic analysis of
28 interacting nucleic acid strands and calculates the free energy in kcal/mol of the
29 secondary structure as well as equilibrium concentrations at a given temperature.
30 We designed our linker systems to consist of a 15-bp long anchor strand functionalized
31 with either cholesterol (chol) or benzylguanine (bg). To the anchor strand,
32 complementary, 26- to 30-bp-long linker strands (Strand 1 and Strand 2) with different
33 hybridization strengths were bound. The complementary part of the linker strands is
34 called binding region and the strength of the linker is calculated only for this sequence.

For the initial design of the 17.7 kcal/mol linker (called DNA linker in the manuscript until **Figure 4**), the *Design* function in NUPACK was used to generate ideal DNA sequences without unwanted self-complementarity. The sequence was designed to achieve the highest hybridization strength at 37 °C. A one base pair mismatch at the end of the sequence increased the binding strength from 17.2 kcal/mol without the mismatch to 17.7 kcal/mol. The hybridization sequence for the 3.2 kcal/mol linker was manually generated by using 11 adenine/thymine bases. The 10.4 kcal/mol linker consists of 13 adenine/thymine bases. The nominal hybridization strength of these two linkers is higher than the indicated values, which were corrected by the bound fraction at 37 °C (see **Figure S3**) resulting in the final values of 10.4 kcal/mol or 3.2 kcal/mol. Example calculation for the 3.2 kcal/mol linker: Nominal hybridization strength at 37 °C is 10.41 kcal/mol, the bound fraction is 0.31, resulting in the final value of 3.23 kcal/mol. The sequence for toehold mediated strand displacement was described elsewhere [2]. This toehold sequence was added to the sequence of the DNA linker 1. The invader strand is the complementary strand to the DNA linker 1 with the toehold overhang (called Reversible DNA linker 1).

<u>DNA sequences</u>

All custom DNA strands were purchased from Biomers (Ulm, Germany, purification: HPLC). Some of the linker strands were modified with a fluorophore. All DNA strands were diluted in Milli-Q water (Merck, Germany) to a stock concentration of 100 µM and stored at -20C until use.

Anchor strand; melting temperature 63.3 °C:

5'/GTTCACAAGAAAGCG/3'/Chol

5'/GTTCACAAGAAAGCG/3'/BG

17.7 kcal/mol linker (called DNA linker) strand 1; melting temperature binding region 57.3 °C:

5'/CGCTTTCTTGTGAACACTCTTTCACTATCT/3'

17.7 kcal/mol linker (called DNA linker) strand 2:

Cy5/5'/CGCTTTCTTGTGAACAGATAGTGAAAGAGA/3'

10.4 kcal/mol linker strand 1; melting temperature binding region 42.9 °C:

5‘/CGCTTTCTTGTGAACTTTTTTTTTTTTT/3'

10.4 kcal/mol linker strand 2:

5'/CGCTTTCTTGTGAACAAAAAAAAAAAAA/3'

3.2 kcal/mol linker strand 1; melting temperature binding region 35.2 °C:

5'/CGCTTTCTTGTGAACTTTTTTTTTTT/3'

3.2 kcal/mol linker strand 2:

5'/CGCTTTCTTGTGAACAAAAAAAAAAA/3'

Reversible DNA linker strand 1:

Atto647N/5’/CGCTTTCTTGTGAACACTCTTTCACTATCTTCTCCATGTCACTTC/3'

Invader strand; melting temperature 85.7 °C:

5'/GAAGTGACATGGAGAAGATAGTGAAAGAGTGTTCACAAGAAAGCG/3'

DNA linker assembly

Duplexes of anchor and linker strand were pre-annealed by mixing 5 µl anchor strand, 5 µl linker strand, 20 µl $MgCl_2$ (100 mM, catalog no. M8226, Sigma-Aldrich) and 20 µl phosphate-buffered saline (PBS). This resulted in a final DNA concentration of 10 µM per strand. For duplex formation, the mix was put in a thermocycler (Bio-Rad), heated to 65 °C for 5 min and cooled down stepwise (10 °C every 30 sec) to 5 °C.

Plasmids and cloning

The plasmid coding for full-length E-cadherin-GFP was kindly provided by Jennifer Stow (Institute of Molecular Biosciences, University of Queensland, Addgene plasmid #28009). The plasmid coding for SNAP-E-cadherin345-mCherry, a truncated E-cadherin with an intracellular mCherry tag, was created via Gibson Assembly (GA). A SNAP-tag was introduced in the E-cadherin sequence between R154 and N376,

103 replacing the extracellular domains EC1 and EC2 while maintaining the ER-import
104 sequence and furin cleavage site of the E-cadherin prodomain.
105 In brief, the plasmid backbone including the E-cadherin prodomain was amplified from
106 the template plasmid E-cadherin-EGFP-Halo (pDO33, unpublished data) via PCR
107 using primers Fwd: *GAA TTC TAG AGG GCC CTA TTC TAT AGT GTC ACC TAA*
108 *ATG CTA GAG CTC GC* and Rev: *TCT CTT CTG TCT TCT GAG GCC AGG AGA*
109 *GGA GTT GGG AAA TGT GAG C*. From the same template the truncated E-cadherin
110 sequence was amplified using primers Fwd: *ATC CGC GTT TAA ACT CGA GGT TAA*
111 *TAA TCC CAC CAC GTA CAA GGG TCA GG* and Rev: *CCT TGC TCA CCA TAC*
112 *TTC CTC CTC CTC CGT CGT CCT CGC CGC CTC CG.* The SNAP and mCherry
113 fragments were amplified from template plasmid SNAPf-mCherry (pDO13,
114 unpublished data) using primers Fwd: *TGG CCT CAG AAG ACA GAA GAG AGA CAA*
115 *AGA CTG CGA AAT GAA GC* and Rev: *CCT TGT ACG TGG TGG GAT TAT TAA*
116 *CCT CGA GTT TAA ACG C* for SNAP and Fwd: *CGGA GGC GGC GAG GAC GAC*
117 *GGA GGA GGA GGA AGT ATG GTG AGC AAG G* and Rev: *TGA CAC TAT AGA ATA*
118 *GGG CCC TCT AGA ATT CTT ACT TGT ACA GCT CGT CCA TGC C*. All four
119 fragments were assembled using the 2 x GA Master Mix (catalog no. E2611, NEB)
120 following the manufacturer's instructions.
121
122 Length of the established DNA-E-cadherin hybrid linker
123
124 We calculate an increased length of ~ 2.8 nm of the fully established *trans*-dimer of the
125 DNA-E-cadherin hybrid compared to the natural E-cadherin based on the following
126 assumptions:
127
128 Length of DNA-SNAP:
129 • 1 bp = ~ 3 Å [3], 35 bp = ~ 135 Å = ~ 13.5 nm
130 • SNAP-tag C-terminal = ~ 2 nm [4]
131 ➔ Total ~ 17.5 nm
132
133 E-cadherin modification:
134 • Full-length E-cadherin *trans*-dimer = 38.5 nm [5]
135 • Overlap EC1-EC2 in *trans*-dimer = 2.9 nm (measured)
136 • EC1 + EC2 = 8.8 nm (measured)

137 ➔ Size reduction by removing EC12 on both sides of *trans*-dimer = 2 x 8.8 nm – 2.9 nm = 14.7 nm
138
139
140 This leads to a total increase in length through DNA-SNAP addition (17.5 nm) and
141 EC12 removal (14.7 nm) of 2.8 nm.
142
143 <u>Cell culture</u>
144
145 Jurkat T-cells (catalog no. TIB-152, ATCC) were grown in culture medium (RPMI,
146 catalog no. 11875093, Gibco) supplemented with 10 % Fetal Bovine Serum (FBS,
147 catalog no. 11140035, Gibco) and 1% Penicillin-Streptomycin (catalog no. P4333
148 Sigma Aldrich) at 37°C and 5% CO_2. They were split every 2-3 days. Before further
149 processing, cells were centrifuged (5 min, 750 rpm), resuspended in phosphate-
150 buffered saline (PBS, catalog no. 10010023, Gibco), centrifuged and finally
151 resuspended in culture medium.
152 A431D cells initially described by [6] were kindly provided by the group of René-Marc
153 Mège (Institut Jacques Monod, Université de Paris). They were grown in culture
154 medium (DMEM, catalog no. 1188002, Gibco) supplemented with 10% Fetal Bovine
155 Serum (FBS, catalog no. 10270106, Gibco) and 1% Penicillin-Streptomycin (catalog
156 no. P4333, Sigma Aldrich) at 37 °C and 5% CO_2. They were passaged every 2-3 days
157 using 0.05% Trypsin (catalog no. 9002077, Merck). Before further processing, the
158 culture medium was aspirated and cells were rinsed with PBS to remove dead cells
159 and debris.
160
161 <u>Generation of SNAP-E-cadherin-mCherry and E-cadherin-GFP cell lines</u>
162
163 A431D cells have been transfected with either E-cadherin-GFP or SNAP-E-cadherin-
164 mCherry via electroporation using an Amaxa Nucleofector I Device (Lonza, Cologne)
165 with the Amaxa Cell line Nucleofector Kit T (program X-01; Lonza) according to the
166 manufacturers protocol. Starting one day after transfection, cells were incubated in
167 antibiotic selection medium (DMEM supplemented with 10 % FBS and 750 µg/ml
168 geneticin (catalog no. 10131035, Gibco) for two weeks. Finally, antibiotic selected cells
169 were sorted via fluorescence activated cell sorting (FACS) using a BD FACS Melody
170 with 3 lasers (488/561/640) and 8 colors (2-2-4) configuration to ensure comparable

171 expression of E-cadherin-GFP or SNAP-E-cadherin-mCherry for the experiments
172 performed in this work.
173
174 Linking of cells
175
176 Cells were incubated with linker medium (cultivation medium containing 10 mM $MgCl_2$
177 and 1 µM pre-annealed linker dsDNA) for 1 h at standard conditions (37 °C, 5% CO2)
178 for all experiments except the force spectroscopy and the subcellular localization of
179 YAP. The pre-annealed linker dsDNA consists of an anchor strand functionalized with
180 either cholesterol or benzylguanine bound to Linker strand 1 or Linker strand 2. Fixed
181 samples were mounted on a laser scanning confocal microscope (Zeiss LSM 900,
182 Oberkochen, Germany) equipped with a 63x oil objective (Plan-Apochromat 63×/1.4
183 Oil DIC M27) and an Airyscan 2 module. Z-stacks of images were taken using the
184 Airyscan mode with as step size of 0.13 µm. Within the ZEN software, an automated
185 deconvolution was performed on the Airyscan data. All images were visualized using
186 Fiji [7]. For the visualization, maximum projections of the z-stacks were generated and
187 brightness and contrast were adjusted. Live-cell timelapse videos were acquired using
188 a laser scanning confocal microscope (Zeiss LSM 880, Oberkochen, Germany)
189 equipped with a 20x air objective (LD A-Plan) and temperature and CO_2 control.
190
191 Sample preparation for single-cell force spectroscopy
192
193 2-well culture inlets (catalog no. 81176, Ibidi) were placed in a glass bottom atomic
194 force microscopy dish (catalog no. GWST-3512, WillCo Wells). 2 000 cells (SNAP-E-
195 cadherin or E-cadherin-GFP) were seeded into each inlet and incubated overnight at
196 standard culture conditions. To prevent cell adhesion, the area outside the inlet was
197 coated with 0.1 µg/ml Poly-L-lysine-Poly-ethylene-glycol (PLL(20)-g[3.5]-PEG(2),
198 SuSoS, Switzerland). On the next day, the area outside the inlet was washed with PBS
199 and dried at 37 °C. If no DNA linker was used for the experiment, adherent cells within
200 the inlet were washed with warm PBS. The inlet was removed and the whole dish was
201 filled with 3 ml medium. Freshly trypsinized cells were added to the dish excluding the
202 area occupied by adherent cells.
203 When using the DNA linker, the adherent cells within the inlet were washed with warm
204 PBS, the cultivation medium was replaced with linker medium containing 1 µM DNA

205 Linker strand 1 and incubated for 1 h at standard conditions. In parallel, freshly
206 trypsinized cells were incubated with linker medium containing 1 µM DNA Linker strand
207 2. The adherent cells were washed with warm PBS. The inlet was removed and the
208 whole dish was filled with 3 ml culture medium. The trypsinized cells were added to the
209 dish excluding the area occupied by adherent cells.
210
211 Single-cell force spectroscopy
212
213 For single-cell force spectroscopy, we used a NanoWizard 3 (JPK instruments, Bruker,
214 Berlin), equipped with a CellHesion module (JPK instruments, Bruker, Berlin) and
215 temperature and CO_2 control. Tipless cantilevers (catalog no. MLCT-O10, Bruker,
216 Berlin) were functionalized with concanavalin-A (ConA-biotin; catalog no. C2272,
217 Sigma) to facilitate cell capturing, adapting the protocol from [8]. Briefly, the cantilevers
218 were cleaned for 15 min in an ultraviolet radiation and ozone (UV-O) cleaner (Jetlight)
219 and placed in a Petri dish covered with parafilm. The cantilevers were then incubated
220 overnight in 50 µL droplets of biotin-BSA (1 mg ml^{-1} in $NaHCO_3$ buffer, 100 mM, pH
221 8.6, catalog no. A6043, Sigma), after which they were washed three times by
222 immersion in fresh PBS. 50 µL droplets of streptavidin (1 mg ml^{-1} in PBS, catalog no.
223 S4762, Sigma) were added to the cantilevers. After 30 min incubation, the cantilevers
224 were washed three times in PBS and placed in 50 µL droplets of ConA-biotin for
225 additional 30 min. Then, the cantilevers were washed three times with PBS and placed
226 in fresh PBS until use.
227 Immediately before use, cantilevers were calibrated with the thermal tune method [9] (k
228 = 0.01 - 0.03 N m^{-1}). The experiment was performed in standard culture medium. For
229 catching cells, the cantilever was brought into contact with a non-adherent cell at 1-3
230 nN constant force for 60-120 sec, after which the cell-functionalized cantilever was
231 retracted and allowed to recover for 10 min before measuring cell-cell interactions.
232 Then, the cell-functionalized cantilever was brought in contact with an adherent cell at
233 1 nN constant force for 2, 5 or 10 sec, before retraction, leading to the acquisition of a
234 force-distance cycle. For each cell-functionalized cantilever, the process was repeated
235 with 5 - 10 adherent cells. Between every measurement, cells were left to recover for
236 1 min. Detachment forces were calculated using the JPKSPM Data Processing
237 software. The values were plotted using GraphPad Prism 9. Error bars show the
238 standard deviation. Plots generated from 3 independent experiments (*N* = 3). Number

239 of measured cells: *n*(E-cadherin-GFP) = 28. *n*(SNAP-E-cadherin) = 37. *n*(SNAP-E-
240 cadherin + DNA linker) = 29.
241
242 Toehold-mediated strand displacement
243
244 A431D-SNAP-E-cadherin cells were seeded on a glass bottom imaging dish (catalog
245 no. 81218, Ibidi) to reach a confluency of about 90%. Before the experiment, they were
246 rinsed with warm PBS. They were incubated with the Reversible linker strand 1 tagged
247 with Atto647N and the complementary DNA linker strand 2 anchored with
248 Benzylguanine for 1 h at 37 °C and 5% CO_2 in the presence of 10 mM $MgCl_2$. After
249 washing the sample with warm PBS and replacing the linking medium with cultivation
250 medium supplemented with 10 mM $MgCl_2$, it was transferred to a laser scanning
251 confocal microscope with temperature monitoring (Zeiss LSM 900, Oberkochen,
252 Germany) equipped with a 63x oil objective (Plan-Apochromat 63×/1.4 Oil DIC M27).
253 The experiment was performed at room temperature (24 °C).
254 Time-lapse videos (every 30 sec) of the linker tagged with Atto657N and SNAP-E-
255 cadherin-mCherry were acquired at for 2 min. Then, the invader strand was added to
256 achieve a concentration of 10 µM (10x excess). The sample was imaged for additional
257 8 min. 8-bit images with a color depth of 255 intensity values were acquired.
258 Using Fiji [7], we defined the background as an intensity value of five and all pixels below
259 this threshold in all images of the time-lapse were set to zero. Then, the mean grey
260 value was calculated for every image in the time-lapse.
261 The intensity values were normalized to the fraction of the maximum intensity. The
262 intensity mean of multiple positions was generated and plotted over time using
263 GraphPad Prism 9. The error bars show the standard deviation of *N* = 3 experiments
264 and *n* = 31 measurements.
265
266 Live imaging of cell-cell adhesion formation
267
268 A431D-SNAP-E-cadherin cells were seeded on a glass bottom imaging dish (catalog
269 no. 81218, Ibidi) to reach a confluency of about 40%. Before the experiment, they were
270 rinsed with warm PBS. To label the actin cytoskeleton, SiR actin and verapamil
271 (catalog no. SC001, Spirochome, diluted 1:1000) were added to the cultivation medium
272 and cells were incubated for 1 h at 37 °C and 5% CO_2.

273 The sample was mounted on an epifluorescence microscope (DeltaVision Imaging
274 System on Olympus IX71 inverted microscope) equipped with temperature and CO_2
275 control and a 60x oil objective (Olympus, Plan Apo, NA = 1.4). E-cadherin-SNAP-
276 mCherry and SiR actin were visualized by using the appropriate filter sets.
277 Furthermore, the bottom of the glass coverslip was imaged by Interference Reflection
278 Microscopy (IRM) by using TRITC excitation and FITC emission. Time-lapse videos of
279 cells without and with the linker (every 10 minutes) were taken at different positions of
280 the dish for multiple hours with constant 37 °C and 5% CO_2. Image acquisition started
281 20 min after linker addition.
282
283 Staining of E-cadherin, actin and β-catenin
284
285 Cells were seeded on clean glass coverslips to reach a confluency of about 50 – 70%
286 on the day of the experiment. They were rinsed with warm PBS and (if applicable)
287 incubated for 1 h at 37 °C and 5% CO_2 with linker medium. Before fixation, the cells
288 were rinsed twice with warm PBS. Fixation was carried out in 4% paraformaldehyde
289 (PFA) for 10 min at room temperature. Cells were permeabilized in 0.4% Triton X-100
290 in PBS for 5 min followed by 3 x 5 min washing in PBS. To stain the actin cytoskeleton,
291 samples were incubated with Phalloidin-coumarin (catalog no. P2495, Sigma) diluted
292 1:200 in 5% BSA in PBS for 1h at room temperature.
293 For indirect immunostaining, samples were blocked with 5% BSA in PBS for 1 h at
294 room temperature. Binding of primary antibodies was achieved by incubating the
295 samples as it follows:
296 Anti-β-catenin mouse antibody (catalog no. 610153, BD) was diluted 1:100 in 5% BSA
297 in PBS and incubated for 1 h at room temperature. Anti-E-cadherin mouse antibody
298 (catalog no. sc-8426, Santa Cruz for E-cadherin-GFP, catalog no. 610181, BD for
299 SNAP-E-cadherin-mCherry) was diluted 1:100 in 5% BSA in PBS and incubated for 1
300 h at room temperature. The samples were washed 3 times 5 min with PBS and
301 incubated with anti-mouse donkey antibody conjugated to AF647 (catalog no. A-
302 31571, Thermo Fisher), diluted 1:200 in 5% BSA in PBS and incubate for 1 h at room
303 temperature. Subsequently, samples were washed 3 times 5 min with PBS. To
304 preserve fluorescence, samples were mounted with Mowiol 488 (catalog no. 81381,
305 Sigma).
306

307 STED microscopy

308

309 Fixed cells stained for E-cadherin-GFP or SNAP-E-cadherin with an antibody
310 conjugated to AF647 were imaged on an Abberior expert line microscope (Abberior
311 Instruments GmbH, Germany) with a pulsed STED line at 775 nm using an excitation
312 laser at 640 nm and spectral detection. The detection window was set to 650-725 nm
313 to detect AF647-conjugated antibodies. Images were acquired with a 100×/1.4 NA
314 magnification oil immersion lens (Olympus). The pixel size was set to 20 nm and the
315 pinhole was set to 1 AU. The confocal as well as the STED laser power were set to
316 10%. For visualization, the contrast was adjusted using Fiji.

317

318 Colocalization of E-cadherin and β-catenin

319

320 Fixed samples were mounted on a laser scanning confocal microscope (Zeiss LSM
321 900, Oberkochen, Germany) equipped with a 63x oil objective (Plan-Apochromat
322 63×/1.4 Oil DIC M27) and an Airyscan 2 module). For the visualization of F-actin and
323 E-cadherin, a background subtraction using a 100-pixel sliding paraboloid was used.
324 The colocalization of β-catenin and E-cadherin was quantified by acquiring line plot
325 profiles (length = 5 µm, width = 20 pixel) at the same positions in both channels in a
326 single z-slice. The lines were manually placed in a way that the maximum intensity was
327 at the middle. The intensity distribution was normalized to the fraction of the maximum
328 value. Multiple line plots were averaged and plotted as intensity over distance using
329 GraphPad Prism 9. The error bars show the standard deviation of N = 3 preparations
330 and n(E-cadherin-GFP) = 20, n(SNAP-E-cadherin-mCherry) = 22 and n(SNAP-E-
331 cadherin-mCherry + DNA linker) = 23 measurements.

332

333 Subcellular localization of YAP

334

335 To assess the localization of Yes associated protein (YAP), cells were seeded on clean
336 glass coverslips to reach a confluency of about 95 - 100% on the day of the experiment.
337 The cells were cultured, incubated with DNA linkers for 3 h, fixed, permeabilized and
338 blocked as described in the section above (Staining of actin and β-catenin). Samples
339 were incubated with Anti-YAP mouse antibody (1:100, catalog no. 101199, Santa Cruz
340 Biotechnology) for 1 h in 5% BSA in PBS and washed 3 times 5 min with PBS.

341 Subsequently, 1 h incubation with anti-mouse donkey antibody conjugated to AF647
342 (catalog no. A-31571, Thermo Fisher) diluted 1:200 in 5% BSA in PBS was performed.
343 After washing (3 times 5 min) with PBS, cells were counter-stained with DAPI (catalog
344 no. D1306, Thermo Fisher) diluted 1:2000 in PBS for 15 min. Afterwards, samples
345 were washed 3 times 5 min with PBS. To preserve fluorescence, samples were
346 mounted with Mowiol 488 (catalog no. 81381, Sigma).
347 Images of 25 – 30 cells per field of view were acquired at a laser scanning confocal
348 microscope using a 63x oil objective (Zeiss LSM 900, Oberkochen, Germany) and
349 visualized using Fiji. Subcellular YAP intensities were quantified using the ImageJ
350 Macro *Intensity Ratio Nuclei Cytoplasm Tool* (RRID:SCR_018573). First, a
351 background correction was performed with all parameters set to zero. The nuclei were
352 segmented based on the DAPI staining. All areas not segmented as nuclei were
353 defined as cytosolic. The cytosolic and the nuclear YAP fractions within the image were
354 quantified and the ratios were calculated. Further data processing was performed in
355 RStudio running R Version 4.0.3. Measurements with a nuclear/cytosolic ratio below
356 0.85 were classified as cytosolic, while a ratio above 1.15 indicated nuclear
357 localization. All measurements in between were classified as uniform. Percentages of
358 the three localization classes were calculated for all conditions (E-cadherin-GFP,
359 SNAP-E-cadherin, 3.2 kcal/mol linker, 10.4 kcal/mol linker, 17.7 kcal/mol linker) and
360 plotted using GraphPad Prism 9.
361
362 <u>Collective migration experiments</u>
363
364 2-well culture inlets (catalog no. 81176, Ibidi) were placed in glass bottom culture
365 dishes. $5x10^4$ cells (A431D cells expressing SNAP-E-cadherin-mCherry or E-cadherin-
366 GFP) were seeded in 80 µl culture medium into the inserts and incubated overnight at
367 standard conditions. The cells were washed with warm PBS and the medium was
368 replaced with medium containing the following DNA linkers: 3.2 kcal/mol, 10.4 kcal/mol
369 and 17.7 kcal/mol linkers anchored with benzylguanine to SNAP-E-cadherin or the
370 17.7 kcal/mol linker anchored with cholesterol into the cell membrane. In the case
371 where no linker was used, fresh culture medium was added. The cells were incubated
372 for 1 h at standard conditions. Then the inlet was removed, the cells were carefully
373 rinsed with PBS and the linker medium was replaced with standard cultivation medium
374 containing 10 mM $MgCl_2$.

375 The cells were transferred to a live cell epifluorescence microscope (Leica DMi8
376 (Wetzlar, Germany) equipped with a 10x phase-contrast air objective and an
377 incubation chamber) and incubated at 37 °C and 5% CO_2. Time-lapse videos (every
378 10 minutes) of the free edge were taken at different positions of the sample for multiple
379 hours. Trajectories of single cells were manually tracked using the Fiji plugin *Manual*
380 *Tracking*.
381
382 <u>Particle image velocimetry analysis and velocity correlation length calculation</u>
383
384 Time-lapse videos were cropped to a ROI of 385 x 1332 µm and background
385 subtraction was performed. Therefore, the minimal intensity of the stack was projected
386 and then subtracted using the *Calculator Plus* plugin. The videos were rotated in a way
387 that the cells migrate from the left to the right.
388 The individual, cropped and background-subtracted images of the stack were loaded
389 into *JPIV* (https://eguvep.github.io/jpiv/index.html) run in a Python environment. Since
390 A431D cells do not migrate fast, we performed particle image velocimetry analysis on
391 images taken 1 h apart (compare image 1 with image 6, image 2 with image 7, etc.)
392 using first a 64x64 and then a 32x32 pixel interrogation window. The vector was placed
393 in the middle of the 32x32 window. This generated a vector field with 32x32 pixel-
394 spaced vectors. The vectors fields were batch-filtered by performing a normalized
395 median test and a median filter, where all invalid vectors were excluded. These invalid
396 vectors were replaced by the median.
397 A custom-written Python script was used to format the JPIV data for further processing.
398 Finally, the velocity of the cell sheet and the velocity correlation were calculated in
399 MATLAB using a script described elsewhere [10], which was modified for the analysis of
400 time-lapse video microscopy [11].
401 In brief, the displacement vectors were divided by the time difference between the two
402 images from which they were generated, resulting in the velocity vector $\boldsymbol{r}_{i,j}$, which was
403 assigned to the central coordinate (*i,j*) of each 32x32 window. Since the axial migration
404 is the dominant migration direction in the described experimental setup, only the lateral
405 component $\boldsymbol{U}_{i,j}$, perpendicular to the migration direction was used to calculate the
406 velocity fluctuations $\boldsymbol{u}_{i,j}$ as:
407

408 $$\boldsymbol{u}_{i,j} = \boldsymbol{U}_{i,j} - \sum_{i=1,m}\sum_{j=1,n} \frac{\boldsymbol{U}_{i,j}}{m \times n} = \boldsymbol{U}_{i,j} - \boldsymbol{U}_{mean}$$

409

410 U_{mean} is the mean velocity along the migration front. The lateral correlation function $\boldsymbol{C}_r$
411 was calculated as:

412

413 $$\boldsymbol{C}_r = \frac{\langle \boldsymbol{u}(r') * \boldsymbol{u}(r'+r)\rangle_{r'}}{\sqrt{\langle \boldsymbol{u}(r')^2\rangle * \langle \boldsymbol{u}(r'+r)^2\rangle}}$$

414

415 $\langle \ldots \rangle$ is the average and $r = \| \boldsymbol{r}_{i,j} \|$ is the norm of $\boldsymbol{r}_{i,j}$. The first crossing of the threshold
416 0.01 with the lateral correlation function $\boldsymbol{C}_r$ was defined as the velocity correlation
417 length. The mean correlation length was calculated for each ROI from 20 consecutive
418 individual measurements corresponding to images acquired between 3.5 and 7.3 h
419 after removing the confinement.

420

421 Statistical analysis

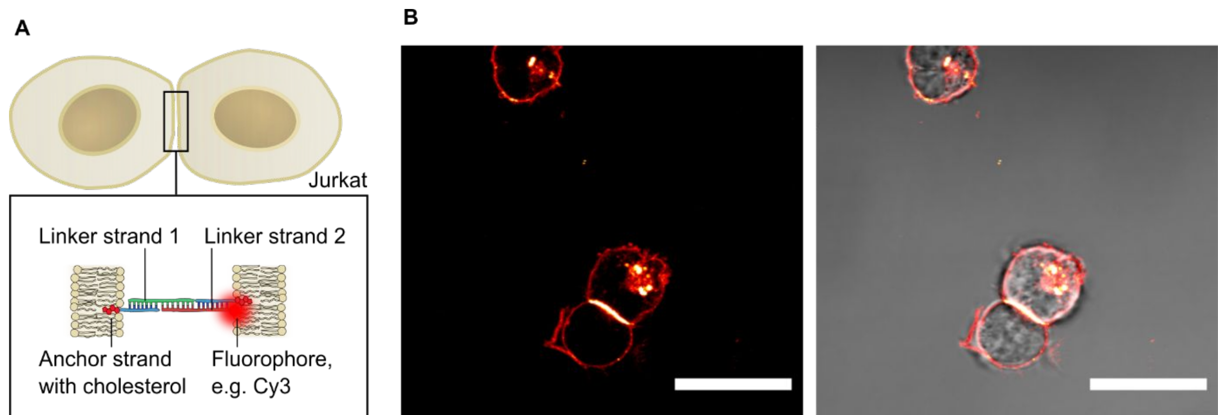
422

423 In GraphPad Prism 9, a one-way ANOVA significance test with Welch's correction was
424 performed on the means of the different experiments using Dunnett's multiple
425 comparison test. Thereby, the threshold for significance was defined as $\alpha = 0.05$. p-
426 values between 0.1 and 0.01 correspond to (*), p-values between 0.01 and 0.001
427 correspond to (**), p-values between 0.001 and 0.0001 correspond to (***) and p-
428 values < 0.0001 correspond to (****). No statistical significance is denoted as (n.s.).
429 The individual datapoints were plotted together with the results of the statistical
430 analysis using GraphPad Prism 9.

431

432 *Supporting Figure 1:* **Cells linked by cholesterol-anchored DNA on their surface.**

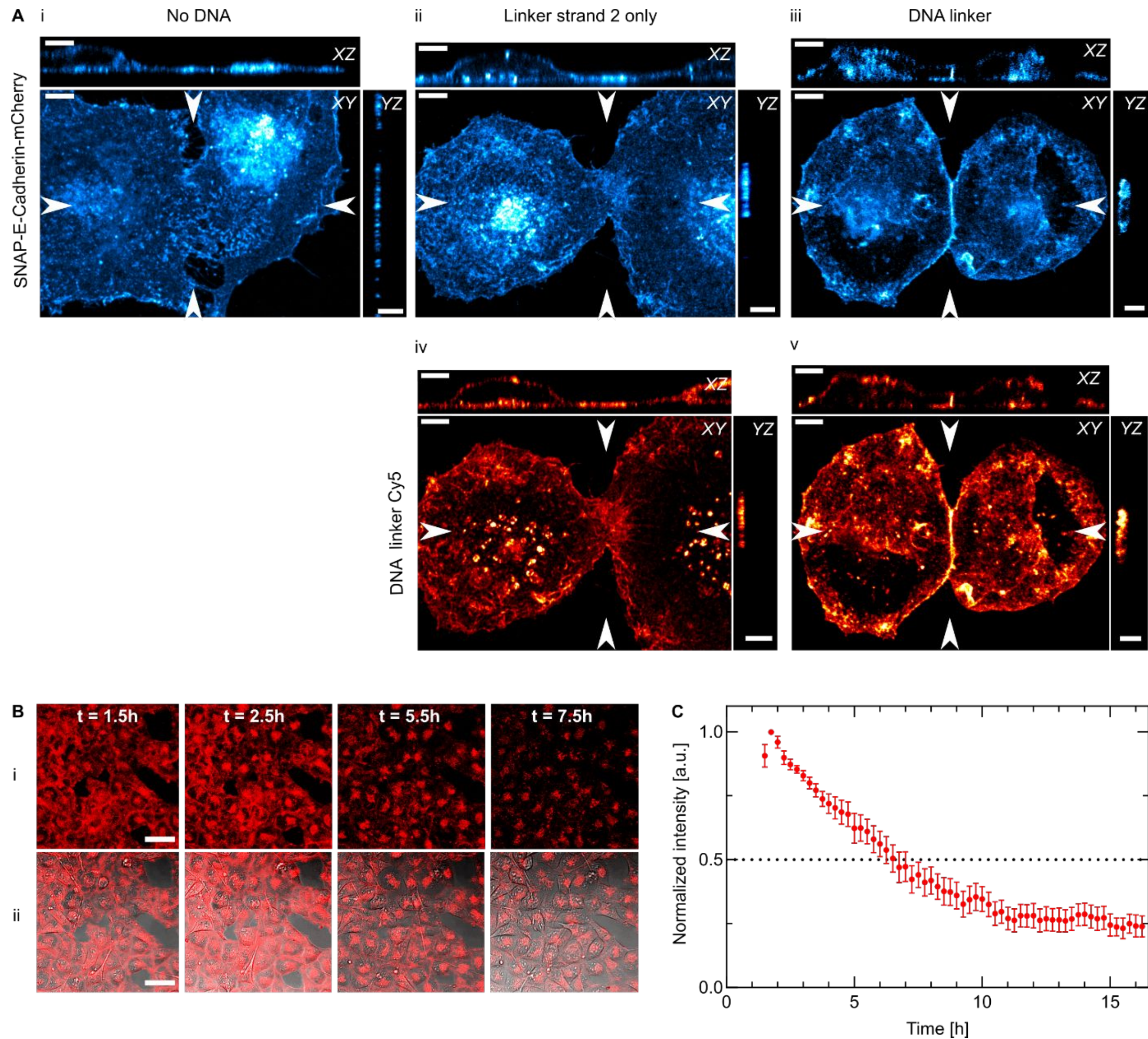
433



Supporting Figure S1: **Cells linked by cholesterol-anchored DNA on their surface. A** Sketch of linked Jurkat cells. Complementary linker strands are bound to an anchor strand which is functionalized with cholesterol. The linker strands can be tagged with a fluorophore, e.g. Cy3 for visualization. **B** Representative live-cell confocal and composite confocal and brightfield images of Jurkat cells incubated with the cholesterol-anchored DNA linker carrying Cy3 (*red*). Images representative of $N = 2$ independent experiments. Scale bars, 20 µm.

434

435 *Supporting Figure 2:* **Expression of SNAP-E-cadherin-mCherry, localization of**
436 **the DNA linker and fluorescence decay over time.**



Supporting Figure S2: **A Expression of SNAP-E-cadherin-mCherry and localization of the DNA linker.** Whole-cell 3D reconstruction of the mCherry signal of A431D cells expressing SNAP-E-cadherin-mCherry (*cyan*) and of the Cy5-tagged DNA linker (*red*). Cells were incubated in absence of DNA (**i**) with linker strand 2 only (**ii, iv**) or the complete DNA linker (**iii, v**). Maximum projection and orthogonal slices through the positions indicated by the dashed line are shown. Scale bars, 5 µm. **B Fluorescence decay over time**. Selected timepoints of live-cell confocal (**i**) and composite brightfield and confocal (**ii**) images of SNAP-E-cadherin expressing cells pre-incubated with the Cy5 tagged DNA linker (*red*). Scale bars, 50 µm. **C** Quantification of the fluorescence decay over time starting at the beginning of image acquisition, 1.5 h after start of the incubation. Data obtained from one preparation (*N = 1*) and *n = 6* measurements. Error bars show the standard deviation.

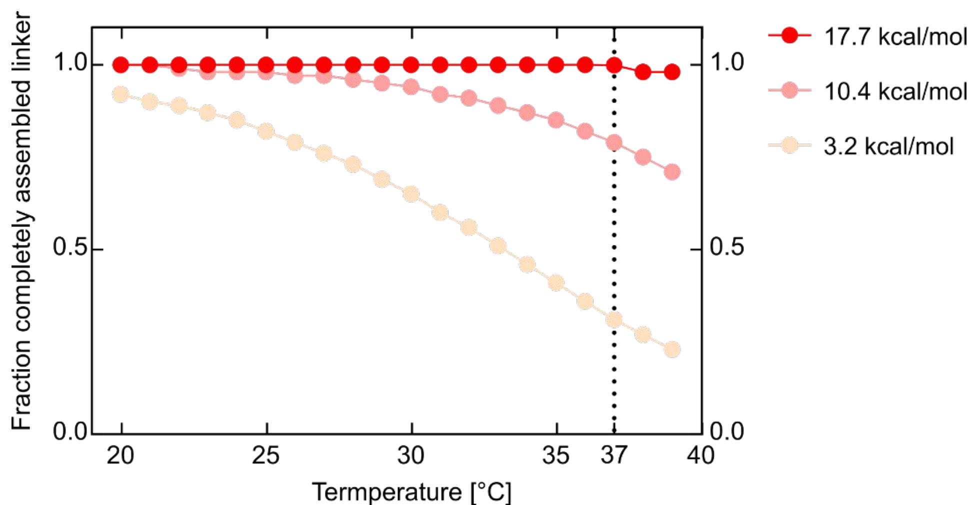
437

438

439

440 *Supporting Figure 3:* **Melting curves of the DNA linkers.**

441



Supporting Figure S3: **Melting curves of the DNA linkers**. The fraction of the completely assembled DNA linkers (17.7 kcal/mol (called DNA linker until mechanotransduction and collective migration experiments (Fig 4)), 10.4 kcal/mol, 3.2 kcal/mol) depending on the temperature were modelled using NUPACK [1]. All experiments except the strand displacement experiment (Fig 2) were performed at a stable temperature of 37 °C, indicated by the dashed line. The final binding strengths of the linkers were corrected by the bound fraction at 37 °C by multiplying the nominal hybridization strength of a linker with its bound fraction.

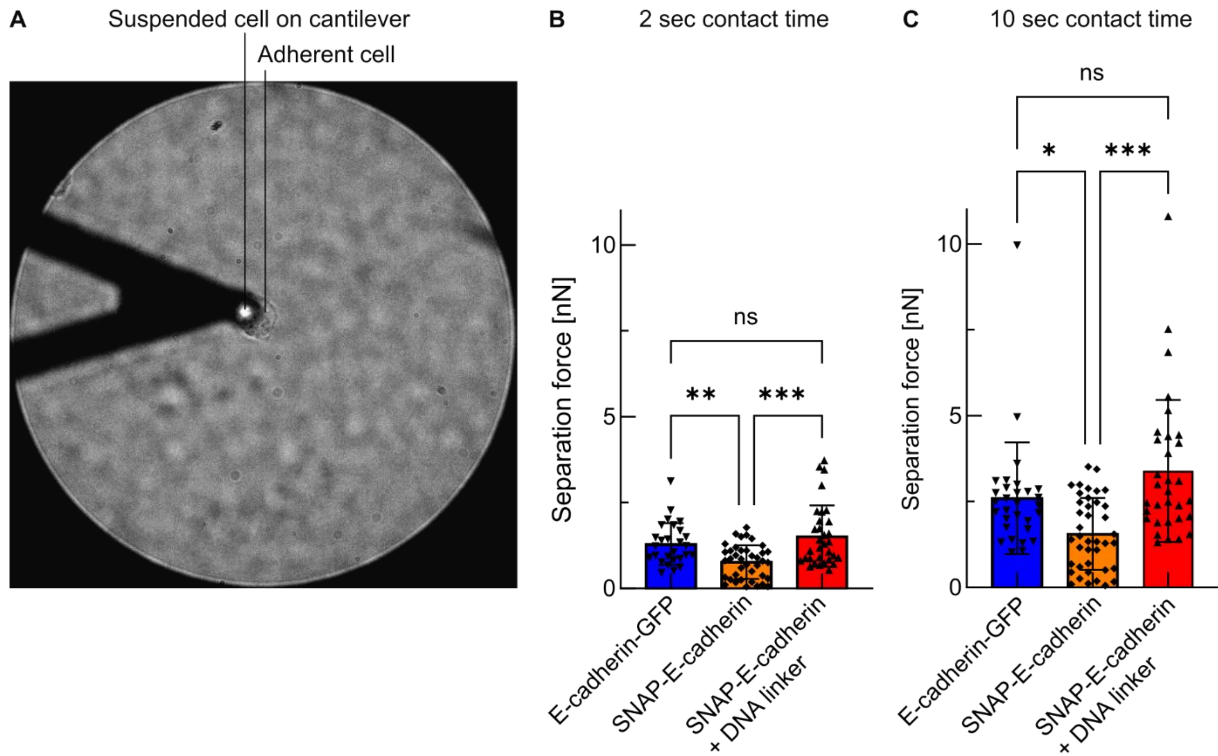
442

443

444

445 *Supporting Figure 4:* **Single cell force spectroscopy**

446

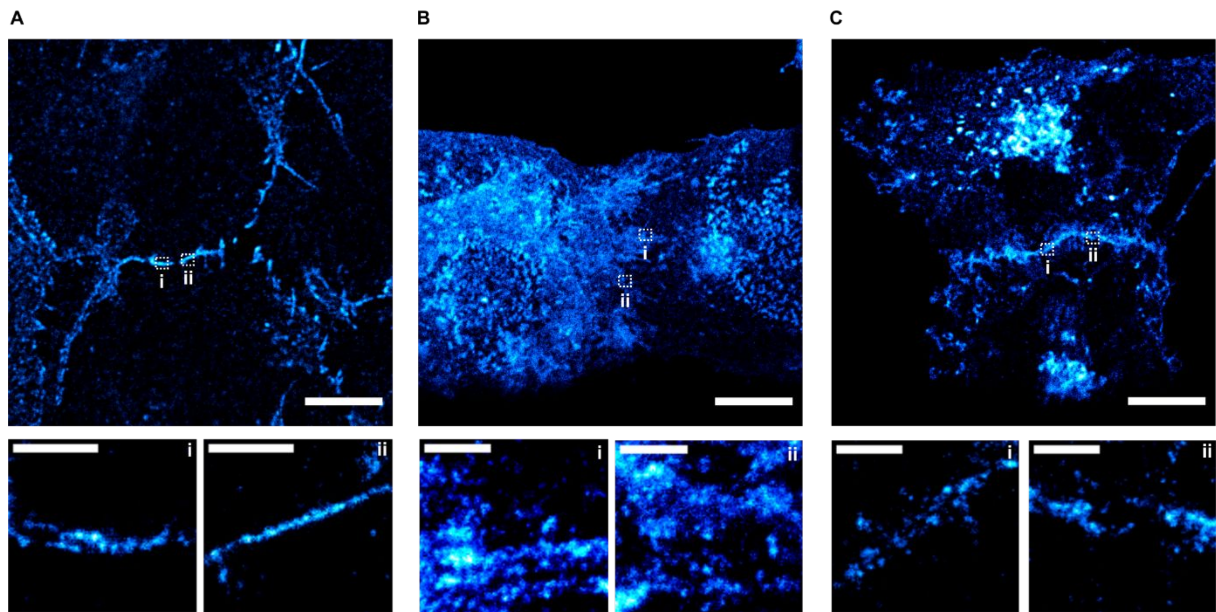


Supporting Figure S4: **Single cell force spectroscopy. A** Brightfield image showing a suspended cell bound to the AFM cantilever functionalized with Concanavalin A on top of an adherent cell. **B** Comparison of the separation forces for 2 seconds contact time. Bars show the mean (E-cadherin-GFP = 1.292 ± 0.607 nN; SNAP-E-cadherin = 0.772 ± 0.49 nN; SNAP-E-cadherin + DNA linker = 1.521 ± 0.894 nN). Error bars show the standard deviation. Plots generated from 3 independent experiments (*N* = 3). Number of measured cells: *n*(E-cadherin-GFP) = 27. *n*(SNAP-E-cadherin) = 39. *n*(SNAP-E-cadherin + DNA linker) = 32. **C** Comparison of the separation forces for *10 seconds contact*. Bars show the mean (E-cadherin-GFP = 2.599 ± 1.619 nN; SNAP-E-cadherin = 1.562 ± 1.045 nN; SNAP-E-cadherin + DNA linker = 3.369 ± 2.065 nN). Error bars show the standard deviation. Plots generated from 3 independent experiments (*N* = 3). Number of measured cells: *n*(E-cadherin-GFP) = 30. *n*(SNAP-E-cadherin) = 40. *n*(SNAP-E-cadherin + DNA linker) = 32. ns no significance. (*) p-value between 0.1 and 0.01. (**) p-value between 0.01 and 0.001. (***) p-value between 0.001 and 0.0001. Multiple ANOVA tests with Welch's correction. Alpha was set to 0.05.

447

448 *Supporting Figure 5:* **Super resolution images of E-cadherin at cell-cell contact.**

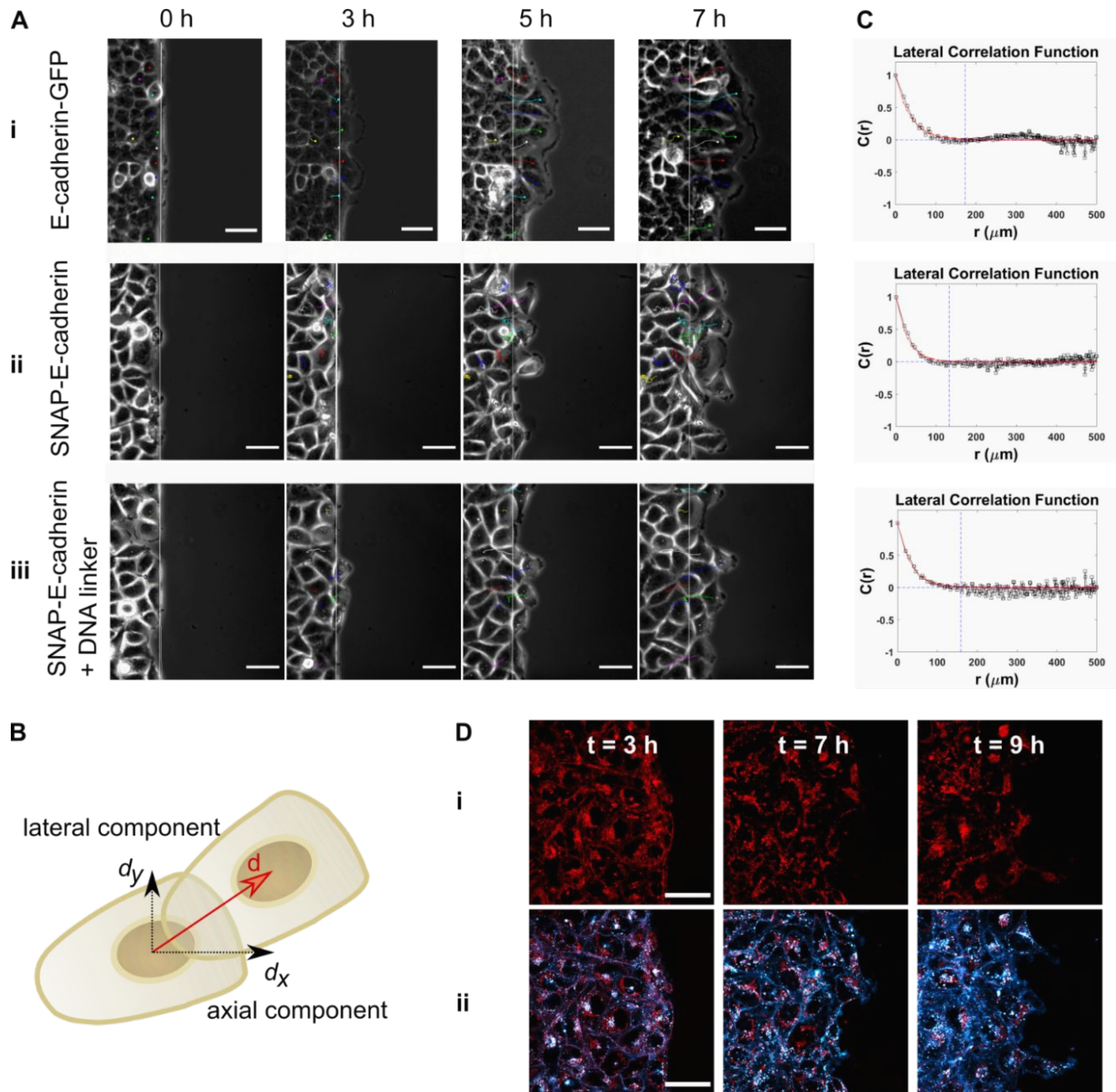
449



Supporting Figure 5: **Super resolution images of E-cadherin at cell-cell contact.** The intracellular domain of E-cadherin is visualized by indirect immunostaining with AF647. Top row: Overview confocal images, scale bar 10 µm. Bottom row: (**i**) and (**ii**) STED images of zoom-ins. The positions are indicated by the dashed lines in the overview image. Scale bar, 1 µm. **A** E-cadherin-GFP. **B** SNAP-E-cadherin. **C** SNAP-E-cadherin + DNA linker. Images representative of N = 2 independent experiments.

450

451 *Supporting Figure 6:* **Effect of the DNA-E-cadherin hybrid system on collective**
452 **migration.**



Supporting Figure S6: **Effect of the DNA-E-cadherin hybrid system on collective migration. A** A431D cells were cultured in confinement for collective migration assays. Live-cell time-lapse phase contrast images of the migration front taken at the indicated timepoints after removing the confinement. The dashed line shows the initial position of the collective front. Trajectories of single cells are shown by colored lines. Scale bars, 50 µm. (**i**) E-cadherin-GFP, (**ii**) SNAP-E-cadherin, (**iii**) SNAP-E-cadherin + DNA linker. **B** Principle of the particle image velocimetry (PIV) analysis: The velocity vector *d* and its axial (d_x) and lateral (d_y) component are calculated. Only the lateral component is used for the calculation of the correlation length. Adapted from *Ollech et. al., 2020*. **C** Representative plots of the lateral correlation function *C(r)* for (**i**) E-cadherin-GFP, (**ii**) SNAP-E-cadherin, (**iii**) SNAP-E-cadherin + DNA linker. The correlation length is defined as the distance *r* at the first zero-crossing of the function, as described previously by *Das et. al., 2015*. **D** Confocal images of the DNA linker (*red*, **i**) and SNAP-E-cadherin-mCherry (*blue;* **ii** overlay) at the migration front at the indicated timepoints. Scale bar, 50 µm.

453

455 **Video descriptions**

456

457 *Supporting Video 1:* **3D projections of single cells.**

458 A431D cells expressing SNAP-E-cadherin-mCherry (*cyan*) were incubated with only
459 linker strand 2 Cy5 (*red*) or the complete, Cy5-tagged DNA linker (*red*). Images
460 correspond to Figure 1 B and Figure S2 A. The scale bar shows 5 µm.

461

462 *Supporting Video 2:* **Toehold-mediated strand displacement.**

463 A431D cells expressing SNAP-E-cadherin-mCherry (*cyan*) were incubated with
464 a toehold-carrying DNA linker tagged Atto647N (*red*). The DNA linker diffuses out of
465 focus after addition of the invader strand (added at t = 1.75 min). Video corresponding
466 to Figure 2 B. The scale bar shows 50 µm.

467

468 *Supporting Video 3:* **Adherens junction formation.**

469 A431D cells expressing SNAP-E-cadherin (*cyan*) were incubated with the DNA linker.
470 The video starts directly after the linker addition. Bottom of the cell (Interference
471 Reflection Microscopy, IRM, *grey*) and actin stained with SiR actin (*red*) are shown.
472 Video corresponding to Figure 2. The scale bar shows 10 µm.

473

474 *Supporting Video 4:* **Migration front of cell collectives.**

475 The collective migration of cells expressing SNAP-E-cadherin, without and with the
476 DNA linker, was imaged in brightfield and individual cells were tracked manually. Video
477 corresponding to Figure S5 A. The scale bar shows 50 µm.

478

479

480 **Methods References:**

481

482 1. Zadeh, J. N.; Steenberg, C. D.; Bois, J. S.; Wolfe, B. R.; Pierce, M. B.; Khan, A.
483 R.; Dirks, R. M.; Pierce, N. A. *Journal of Computational Chemistry* **2011,** 32, (1), 170-
484 173.
485 2. Zhang, D. Y.; Winfree, E. *Journal of the American Chemical Society* **2009,** 131,
486 (47), 17303-17314.
487 3. Griffith, J. D. *Science* **1978,** 201, (4355), 525-7.
488 4. Schmitt S., P. F., Schiltz M., Johnsson K. *Protein Data Bank* **2010**.
489 5. Boggon, T. J.; Murray, J.; Chappuis-Flament, S.; Wong, E.; Gumbiner, B. M.;
490 Shapiro, L. *Science* **2002,** 296, (5571), 1308-1313.
491 6. Lewis, J. E.; Wahl, J. K., III; Sass, K. M.; Jensen, P. J.; Johnson, K. R.;
492 Wheelock, M. J. *Journal of Cell Biology* **1997,** 136, (4), 919-934.
493 7. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch,
494 T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.-Y.; White, D. J.;
495 Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A. *Nature Methods* **2012,** 9, (7),
496 676-682.
497 8. Friedrichs, J.; Helenius, J.; Muller, D. J. *Nature Protocols* **2010,** 5, (7), 1353-
498 1361.
499 9. Schäffer, T. E. *Nanotechnology* **2005,** 16, (6), 664-670.
500 10. Das, T.; Safferling, K.; Rausch, S.; Grabe, N.; Boehm, H.; Spatz, J. P. *Nature*
501 *Cell Biology* **2015,** 17, (3), 276-287.
502 11. Ollech, D.; Pflästerer, T.; Shellard, A.; Zambarda, C.; Spatz, J. P.; Marcq, P.;
503 Mayor, R.; Wombacher, R.; Cavalcanti-Adam, E. A. *Nature Communications* **2020,** 11,
504 (1), 472.
505