[Supporting information]

Adhesion of Epithelial Cells to PNIPAm Treated Surfaces for Temperature-Controlled Cell Sheet Harvesting

Hyejeong Kim[1], Hannes Witt[1], Tabea A. Oswald[2], Marco Tarantola[1,3*]

Corresponding Author:

Dr. Marco Tarantola, e-mail: Marco.Tarantola@ds.mpg.de, phone: +49-551-5176-316

[1] Max Planck Institute for Dynamics and Self Organization (MPIDS), Am Fassberg 17, 37077 Göttingen, Germany

[2] Institute of Organic and Biomolecular Chemistry, Georg August Universität Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany

[3] Institute for Dynamics of Complex Systems, University of Göttingen, Friedrich-Hund Platz 1, 37073 Göttingen, Germany

Contents

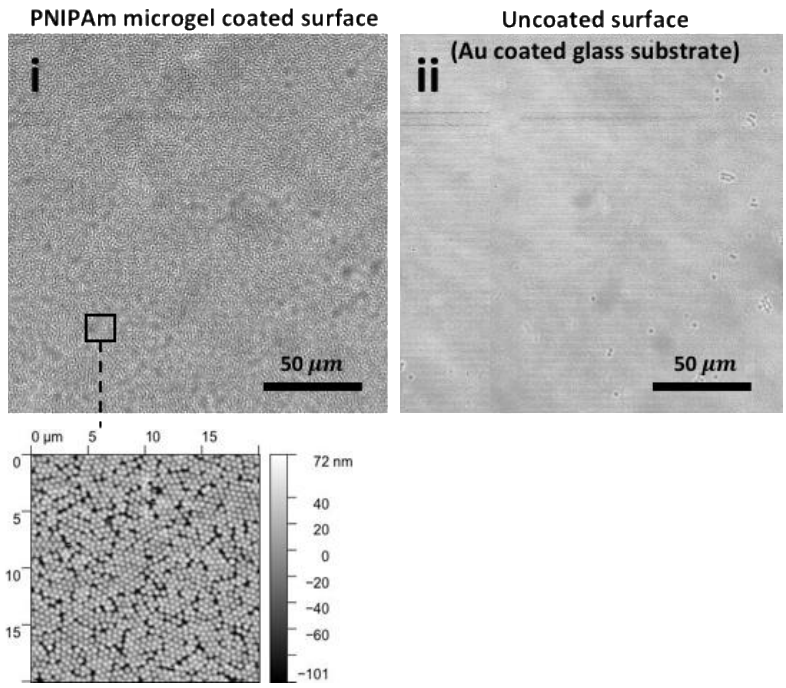


Figure S1. Optical images of i) PNIPAm microgel coated surface and ii) bare gold coated glass substrate, both recorded in bright field mode. Zoom-in (i) highlights the corresponding QI mode imaging area

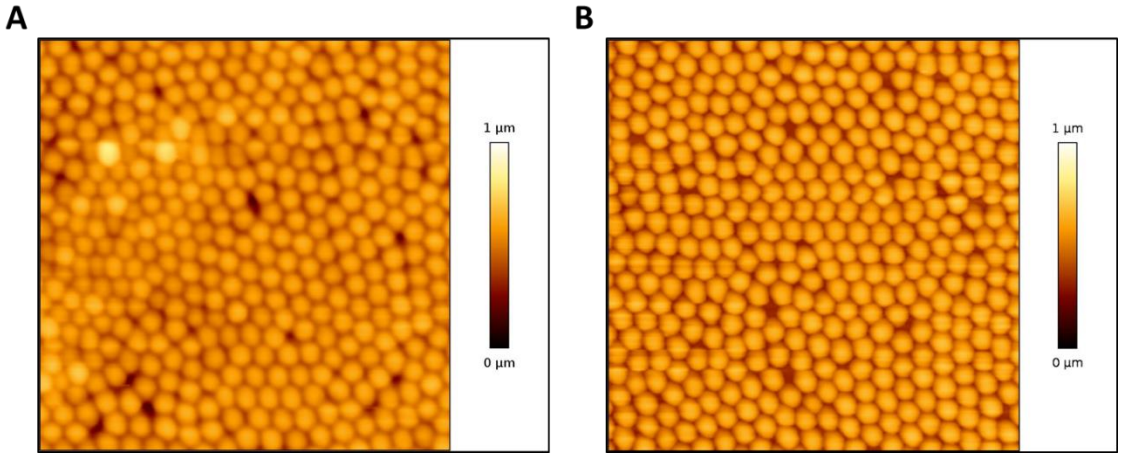


Figure S2. QI-mode-imaging based 2D topology of microgels at **A**) 28 °C, and **B**) 45 °C. 10 x 10 µm scan area.

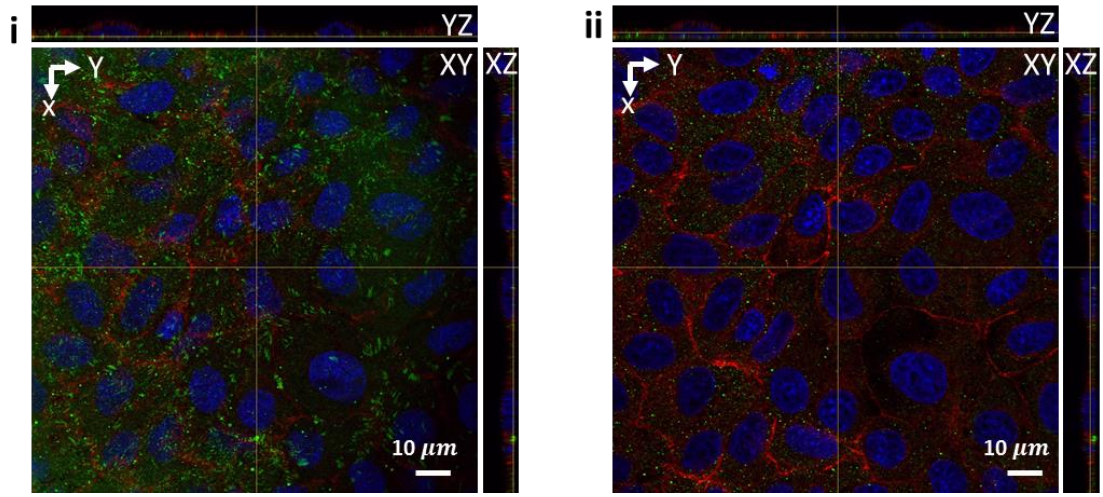
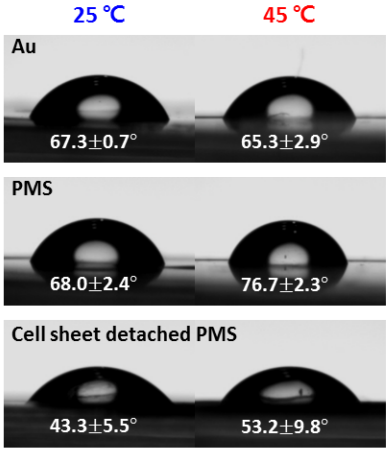
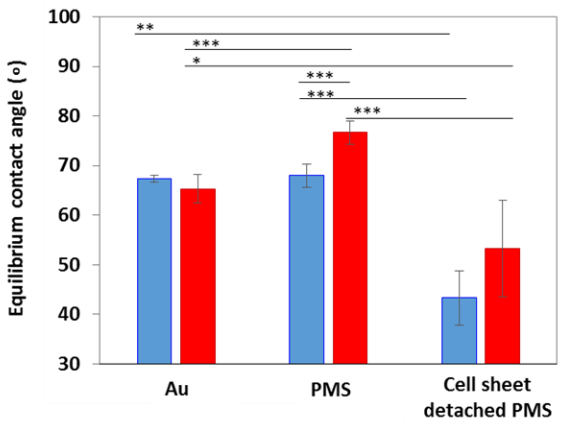


Figure S3. Confocal images of fixated MDCK II cell sheet stained for nuclear DNA (blue), paxillin (green) and E-cadherin (Red) on gold substrate after 48 h. i) Paxillin is well distributed on the ventral surface of the cell sheet at the surface-proximal focal plane. ii) E-cadherin is distributed between individual cells at the focal plane of the apical cell membrane height.

A



B



C

	Au25	Au45	PMS25	PMS45	Cell sheet detached PMS 25	Cell sheet detached PMS 45
Au25	-	**0.42**	**0.57**	-	0.018 (**)	-
Au45	-	-	-	**0.015(**)**	-	**0.07 ***
PMS25	-	-	-	**0.0003 (***)**	**0.0004 (***)**	-
PMS45	-	-	-	-	-	0.0002 (***)
Cell sheet detached PMS 25	-	-	-	-	-	**0.12**
Cell sheet detached PMS 45	-	-	-	-	-	-
# measurement	3	3	7	7	5	5

Figure S4. Contact angle measurement on the gold surface PMS and PMS with remaining ECM after cell sheet lift-off. (A) Side view images of static droplets on the surfaces. (B) Equilibrium water contact angles of gold, PMS, and PMS after cell sheet peeling at different temperatures below (blue) and above (red) LCST. (C) Correlation matrix and number of independent test samples of contact angle measurements. (*: p value <0.1, **: p value < 0.05, ***: p value <0.01; bold: T-test, normal: Wilcoxon Rank Test)

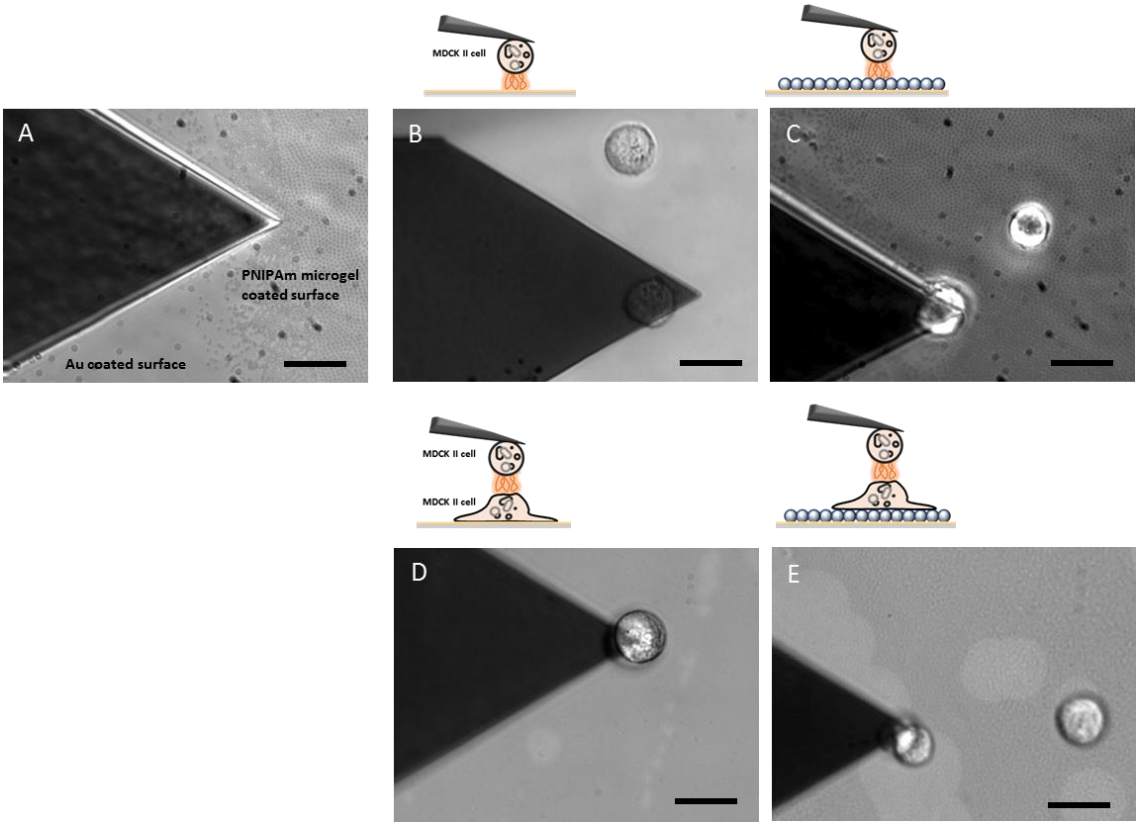


Figure S5. Bright field images while conducting SCFS. **A**. Cantilever tip and a petri dish coated with PNIPAm microgels on one half-side only. Cantilever tip with a single *MDCK II* cell while recording cell-substrate adhesion force curves (**B**) on gold substrate or (**C**) on PMS. Cantilever tip with a *MDCK II* cell while recording cell-cell adhesion force curves (**D**) on gold substrate or (**E**) on PMS. The *MDCK II* cells adhered on the cantilever tip are out of focus while the cell on the substrate or the polymer substrates are in focus. Scale bar: 20 µm.

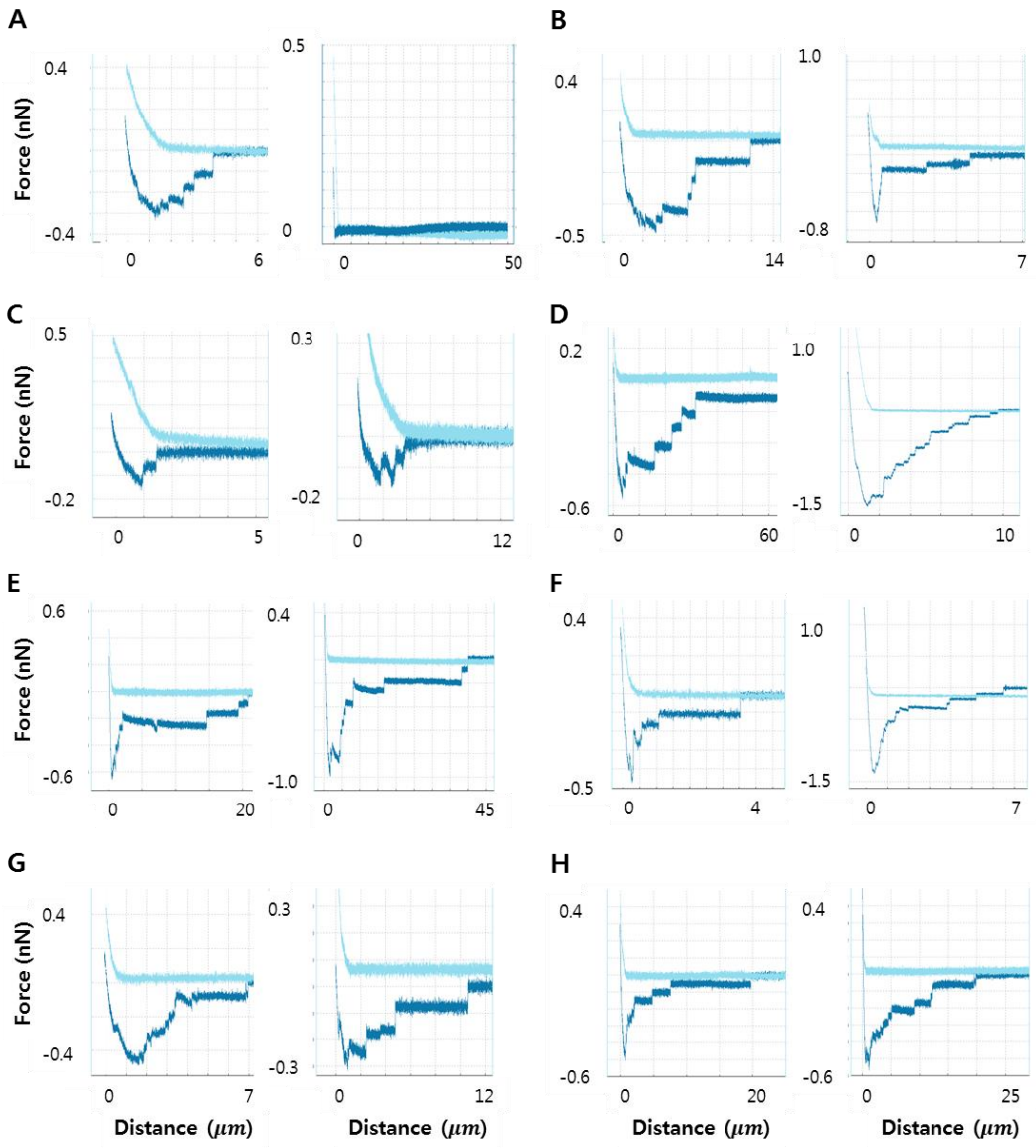


Figure S6. Representative F-D curves of SCFS for the cases of (A)ACC, (B)ACH, thus cell-substrate interaction at low and high temperatures on gold (C)PCC, (D)PCH, thus cell-substrate interaction at low and high temperatures on PMS (E)ACCC, (F)ACCH, thus cell-cell interaction at low and high temperatures on gold (G)PCCC, and (H)PCCH, , cell-cell interaction at low and high temperatures on PMS, the latter 4 categories at 90s dwell time, the first at 30 s. Note that the dwell time in not plotted, thus upon contact, trace and retrace sometimes don't overlap.

Chapter S1. Early cell-cell adhesion on PMS upon temperature changes monitored via SCFS

We expect for cell sheet removal that besides cell-substrate interaction also cell-cell adhesion is of uttermost relevance. We therefore next looked at the strength of initial cell-cell adhesion on PMS compared to gold substrate. While we still pick cells with the AFM cantilever as described above, we now search optically for cells adhering either to the gold substrate or PMS and bring the two cells into contact using SCFS for two time intervals, 30 s (Figure S13, center) as well as 90s as shown in Figure S7A. Here, ACCH/ACCC refers to cell - cell interactions on gold at heated/cooled states; PCCH/PCCC for cell-cell interactions on PMS at heated/ cooled state, respectively. For F_{max}, significant effects are not detectable upon cooling for cell-cell contacts at both contact times, the forces we observe all lay between 100 to 2500 pN and are similar to the cell-substrate interaction. In terms of W_{adh} for cell-cell contact interaction on different substrates, 30 s contact times lead to an adhesion work significantly decreasing on gold upon temperature switching, but not on PMS (see figure S913 center). When looking at the increased contact times of 90 s (see figure S7 C), we now see a significant temperature-based reduction for both gold and PMS, with also significantly increased W_{adh} on PMS over gold at both temperatures. As mentioned above, for the cell-cell contact case, F_{step} between an epithelial cell at the cantilever and the cell attached to the substrate can relate to adherens junction formation typically based on cadherins. F_{step} significantly increases with decreasing temperatures for all substrates at 30 s, and also decreases on PMS for 90s, which was not observed for gold surfaces. (Figures S7D and S13E, center). As for the cell-substrate case, PMS is generally leading to higher F_{step}. For the parameter $l_{pulling}$, l_{step}, and step number on gold and at 30 s, please refer to Figures. S13C, D and G, but the few temperature dependent changes are found on gold. The slopes of step events are summarized in Figure S7E and S13F: based on the temperature reduction, the slope on PMS increases at 90s and decreases at 30 s, which is the opposite of the situation found for F_{step}. Slopes on gold are not affected significantly. Regarding the absolute step number (Figure S7 F), on gold surface, temperature decrease induces fewer overall adhesion points between the cells and equal numbers of jumps and tethers. On PMS cases we also find a decrease of total number of adhesion spots between the cells, but less tether and therefore unspecific adhesion to the lower cell. The cells cultured on PMS thus form more tether junctions with cytoskeletal anchoring to the cantilever-adherent cells at lower temperature. The ratio of jump to tether steps - not including 30 s cell-cell contact cases – is shown in Figure S9B: upon cooling, only on PMS cell show more jumps, while on gold the ratio is reduced to an equal jump to tether probability. We can therefore summarize that a

temperature decrease on PMS substrates - as opposed to gold surfaces - increases F_{step} and decreases the step slope at 30 s, while this situation inverts for 90 s, leading to induction of jumps, thus similar to the cell-substrate case.

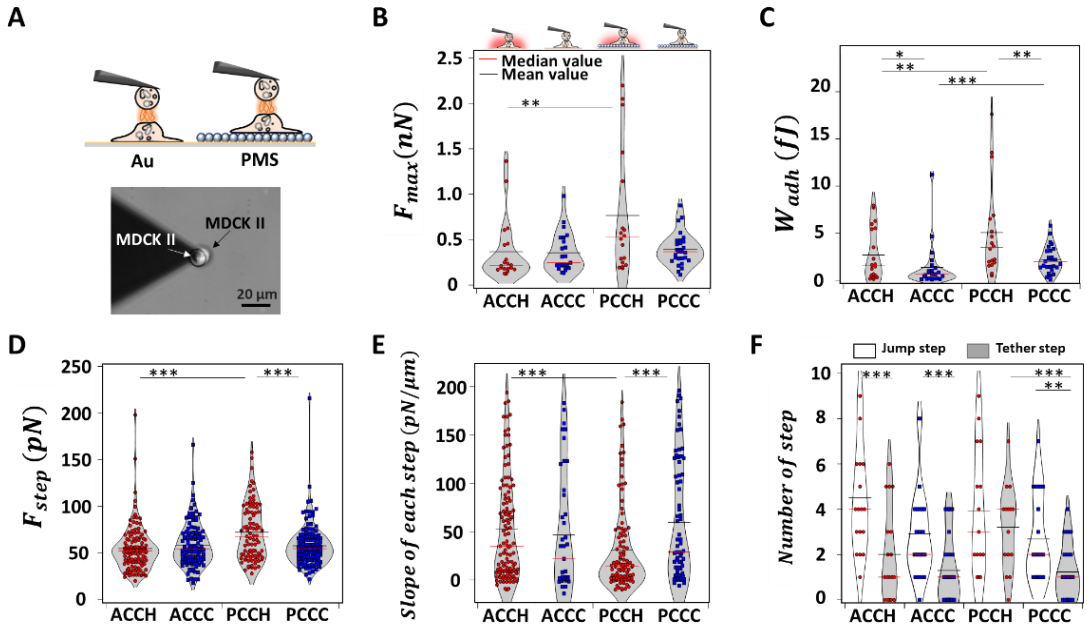


Figure S7. Cell-cell adhesion on bare gold and PMS upon temperature changes monitored via SCFS. A. Schematic of AFM-based cell-cell adhesion experiments. A bright field image of the cantilever tip with an attached single MDCK II cell and a second surface-attached cell while recording cell-cell adhesion force curves. F_{max} (B), W_{adh} (C), step force F_{step} (D), step slope (E) and ratio of jump steps to tether-like steps (F) of MDCK II cells on another MDCK II cell cultured on gold substrates and PMS, all upon temperature switch, corresponding categories schematically given at the top. (red line: median value; black line: mean value). ACCH/ACCC for cell - cell interactions on gold at heated/cooled states; PCCH/PCCC for cell-cell interactions on PMS at heated/ cooled state, respectively. Significance test only shown for temperature changes, for all additional categories refer to Fig. S13 and Table. S6

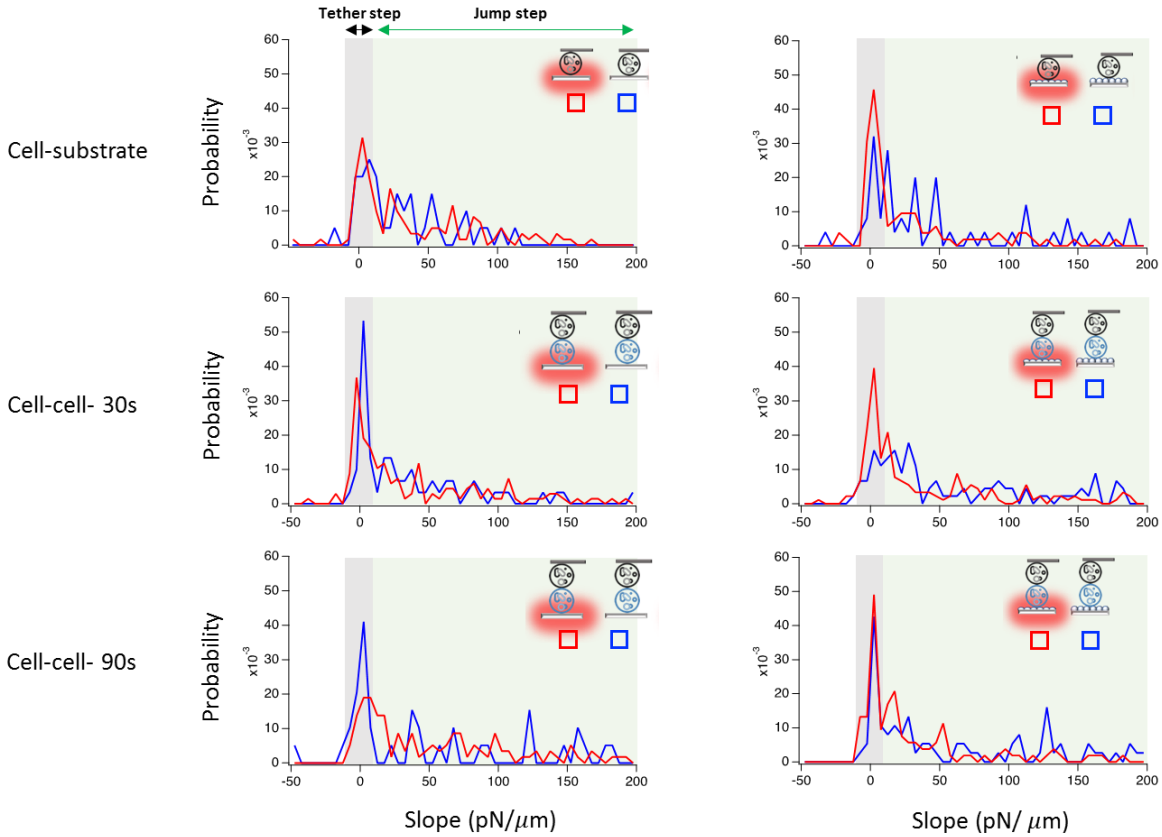


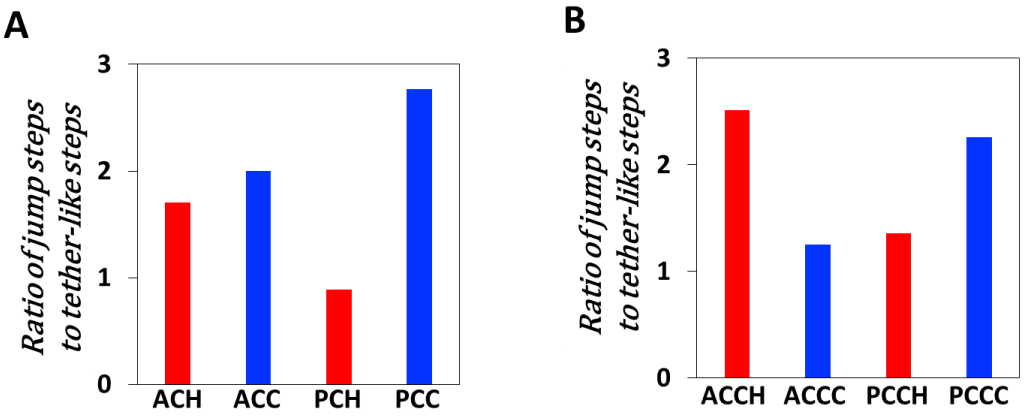
Figure S8. Histogram of the probability distribution of slopes of each step, corresponding to figure 3 E and S7 E.

Table S1. F_{max}, W_{adh}, F_{step}, step slope, l_{step} , N_{step} and $l_{pulling}$ for SCFS base cell liftoff from PMS and gold substrates and the cell liftoff from another cell cultured on PMS and gold substrates above and below the LCST, given as mean (M), median (MD) and degree of change.

		ACCH30	ACCC30	PCCH30	PCCC30	ACCH90	ACCC90	PCCH90	PCCC90
F_{max}	Mean, M, (nN)	0.21	0.21	0.37	0.25	0.37	0.36	0.77	0.39
	M(C)/M(H) (%)		100		69		97		51
	SD (nN)	0.14	0.09	0.39	0.16	0.34	0.22	0.69	0.18
	Median, MD (nN)	0.15	0.20	0.23	0.22	0.22	0.25	0.53	0.37
	MD(C)/MD(H) (%)		130		93		110		70
W_{adh}	M (fJ)	1.77	1.16	1.76	1.82	2.68	1.37	5.05	2.19
	M(C)/M(H) (%)		65.5		103		51.0		43.3
	SD (fJ)	2.15	1.90	2.10	1.97	2.73	2.40	4.89	1.42
	MD (fJ)	0.91	0.40	1.32	1.26	1.54	0.65	3.48	2.00
	MD(C)/MD(H) (%)		44		95.3		42		57.3
F_{step}	M (pN)	32.69	45.17	44.96	54.19	54.74	58.38	72.26	57.69
	M(C)/M(H) (%)		138.2		120.5		106.6		79.8
	SD (pN)	22.62	24.68	29.01	24.49	24.50	23.35	29.89	22.15
	median (pN)	24.12	37.73	38.30	53.80	52.35	54.30	67.70	54.70
	MD(C)/MD(H) (%)		156.4		140.5		103.7		80.80
Slope	M (pN/um)	38.19	36.73	36.17	57.76	52.39	46.61	30.93	59.73
	M(C)/M(H) (%)		96.19		159.7		88.96		193.1
	SD (pN/um)	50.25	44.16	50.95	57.67	53.08	65.92	44.41	62.18
	MD (pN/um)	16.00	20.50	12.50	30.50	35.00	22.00	14.00	29.00
	MD(C)/MD(H) (%)		128.1		244.0		62.86		207.1
l_{step}	M (um)	2.53	3.15	4.11	3.48	2.07	1.69	3.41	3.02
	M(C)/M(H) (%)		124		84.7		82.0		88.6
	SD (um)	4.07	6.68	8.94	6.58	4.23	3.55	7.73	5.21
	MD (um)	0.83	0.63	1.14	1.05	1.04	0.45	0.68	1.25
	MD(C)/MD(H) (%)		76		92.1		43.3		180
N_{step}	M	7.05	3.97	4.76	3.93	6.00	4.83	8.38	5.19
	M(C)/M(H) (%)		56.4		82.5		80.4		61.9
	SD	3.68	1.78	2.91	2.63	3.97	2.25	4.22	3.17
	MD	7	4	4	4	5	4	8	4
	MD(C)/MD(H) (%)		60		100		80		50
$l_{pulling}$	M (um)	17.18	12.41	20.57	14.79	14.06	7.70	25.65	16.16
	M(C)/M(H) (%)		72.26		71.88		54.8		62.98
	SD (um)	14.17	14.79	18.78	12.96	12.02	9.25	17.92	10.35
	MD (um)	14.50	5.08	14.30	9.55	10.20	3.76	33.70	11.90
	MD(C)/MD(H) (%)		35.0		66.80		36.86		35.31

Table S2. Number of F-D curves for step slope analysis

	Number of F-D curve
ACH	24
ACC	10
PCH	40
PCC	13
ACCH 90	18
ACCC 90	23
PCCH 90	15
PCCC 90	22



151

152 **Figure S9**. Ratio of jump steps to tether steps of MDCK II cells (A) on gold surfaces and PMS
153 and (B) on another MDCK II cell cultured on gold substrates and PMS, all upon temperature
154 switch.

155

156

Table S3. Correlation matrix for number of jump steps and tether steps (*: p value <0.1, **: p value < 0.05, ***: p value <0.01; bold: T-test, normal: Wilcoxon Rank Test). The test is only applied for the categories at least two conditions are the same.

	ACH_J	ACH_T	ACC_J	ACC_T	PCH_J	PCH_T	PCC_J	PCC_T
ACH_J	-	0.017 **	**0.74**		**0.027 ****			
ACH_T	-	-		**0.24**		**0.27**		
ACC_J	-	-	-	0.008 ***			**0.38**	
ACC_T	-	-	-	-				**0.65**
PCH_J	-	-	-	-	-	**0.47**	**0.41**	
PCH_T	-	-	-	-	-	-	-	0.026 **
PCC_J	-	-	-	-	-	-		0.04 **
	ACCH_J	ACCH_T	ACCC_J	ACCC_T	PCCH_J	PCCH_T	PCCC_J	PCCC_T
ACCH_J	-	0.002 ***	**0.37**		**0.53**			
ACCH_T	-	-		**0.29**		0.08 *		
ACCC_J	-	-	-	**0.002 *****			0.68	
ACCC_T	-	-	-	-				**0.87**
PCCH_J	-	-	-	-	-	0.6	**0.14**	
PCCH_T	-	-	-	-	-	-		**0.001 *****
PCCC_J	-	-	-	-	-	-	-	0.003 **

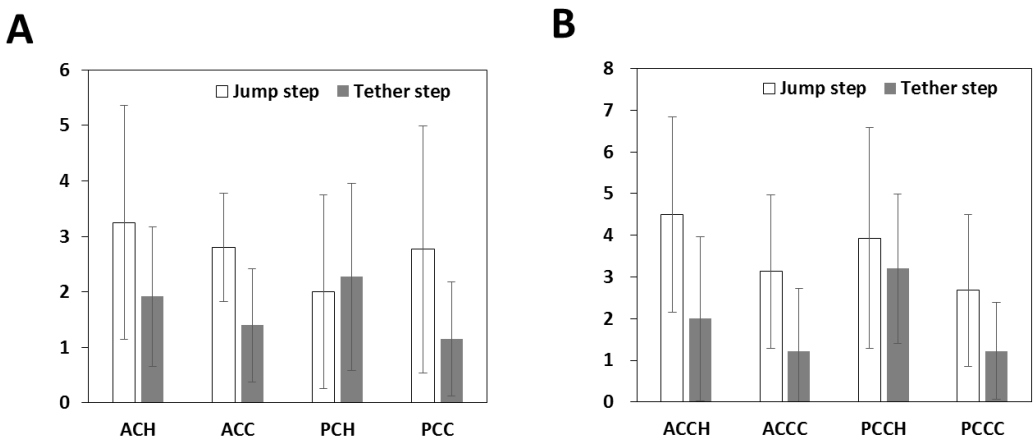


Figure S10. Average number of jump steps and tether steps of MDCK II cells (A) on gold surfaces and PMS and (B) on another MDCK II cell cultured on gold substrates and PMS, all upon temperature switch.

	ACH	ACC	PCH	PCC	ACCH	ACCC	PCCH	PCCC
Jump step	3.25	2.80	2.00	2.77	4.50	2.81	3.93	2.68
Tether step	1.92	1.40	2.28	1.15	2.00	1.27	3.20	1.23

Table S4. Average number of jump and tether step in single F-D curve.

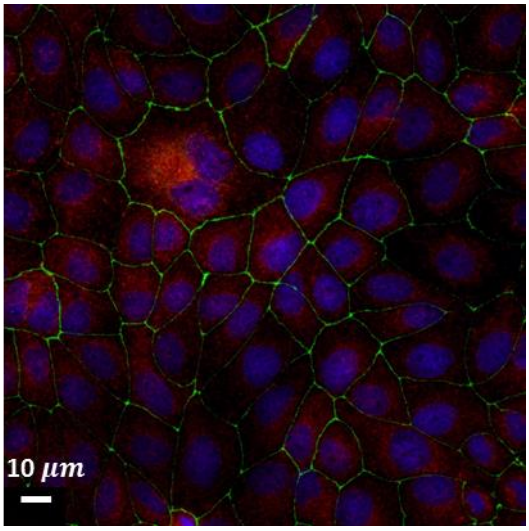


Figure S11. Confocal images of MDCK II cell sheet culture on the gold substrate, thereupon fixated and stained for nuclear DNA (blue), ZO-1 (green) and the ECM component fibronectin (Red).

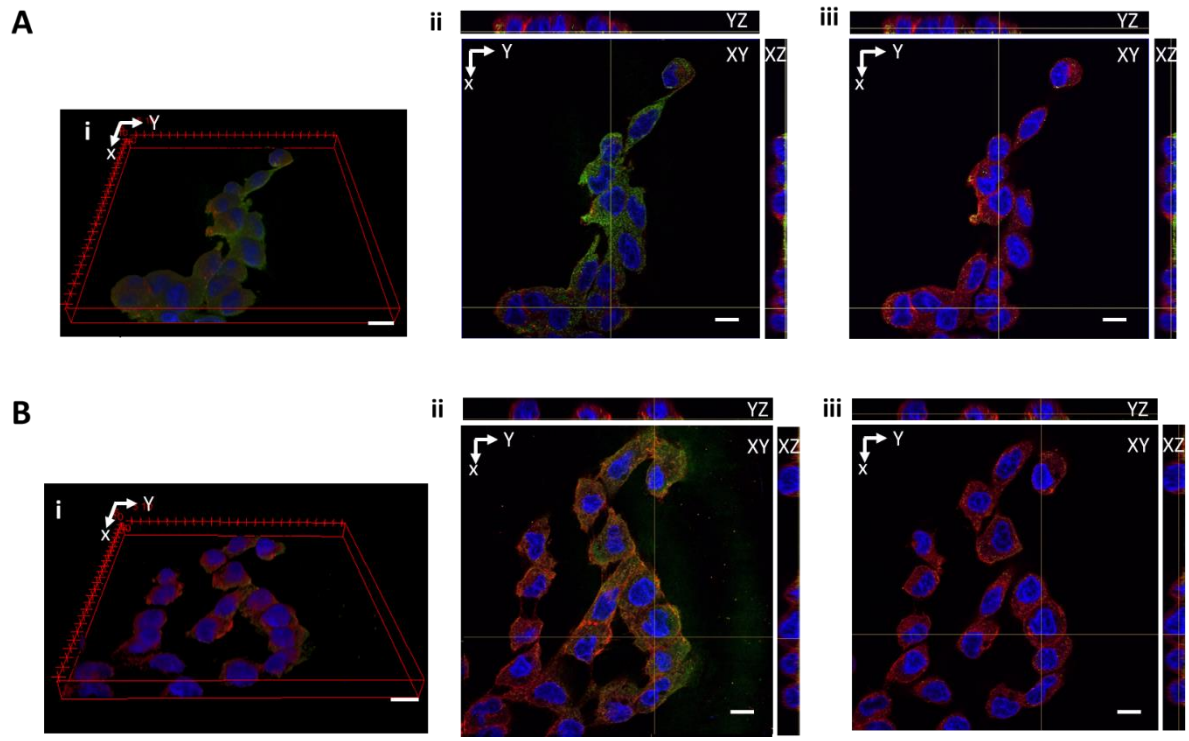


Figure S12. Cell morphology at the early stage (6h) of incubation on **A**. gold substrate and **B**. PMS. Confocal images of fixated MDCK II cells stained for nuclear DNA (blue), paxillin (green) and E-cadherin (Red) on PMS substrate. i) Merged image of the cells on the substrates ii) Paxillin is distributed on the ventral surface of the cells at the surface-proximal focal plane. iii) E-cadherin is distributed between individual cells at the focal plane of the apical cell membrane height. The cells adhere and spread well to both surfaces, indicating that the PMS are adherent at physiological temperature. Scale bar: 10 µm.

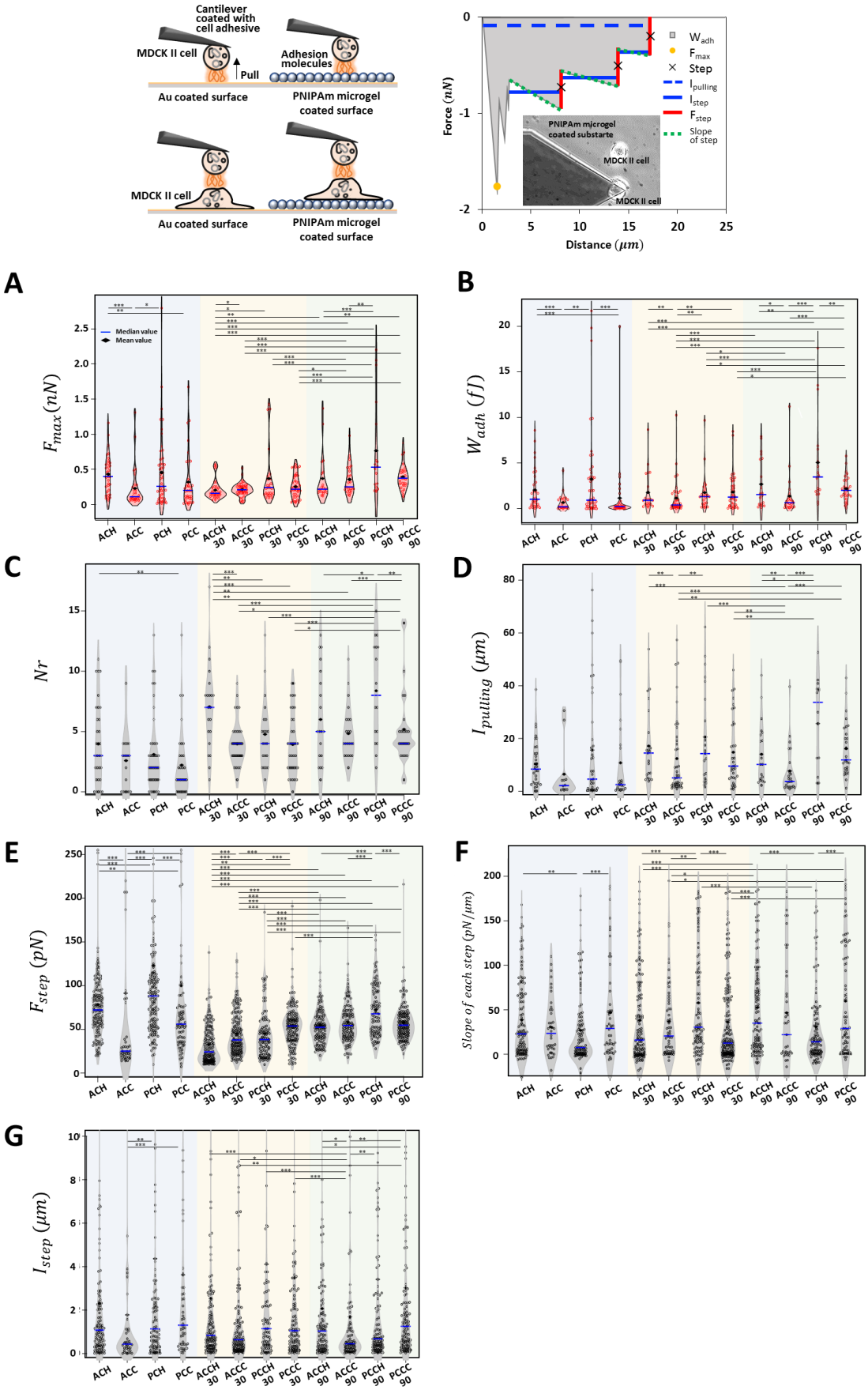


Figure S13. Results of SCFS with all results of significance tests.

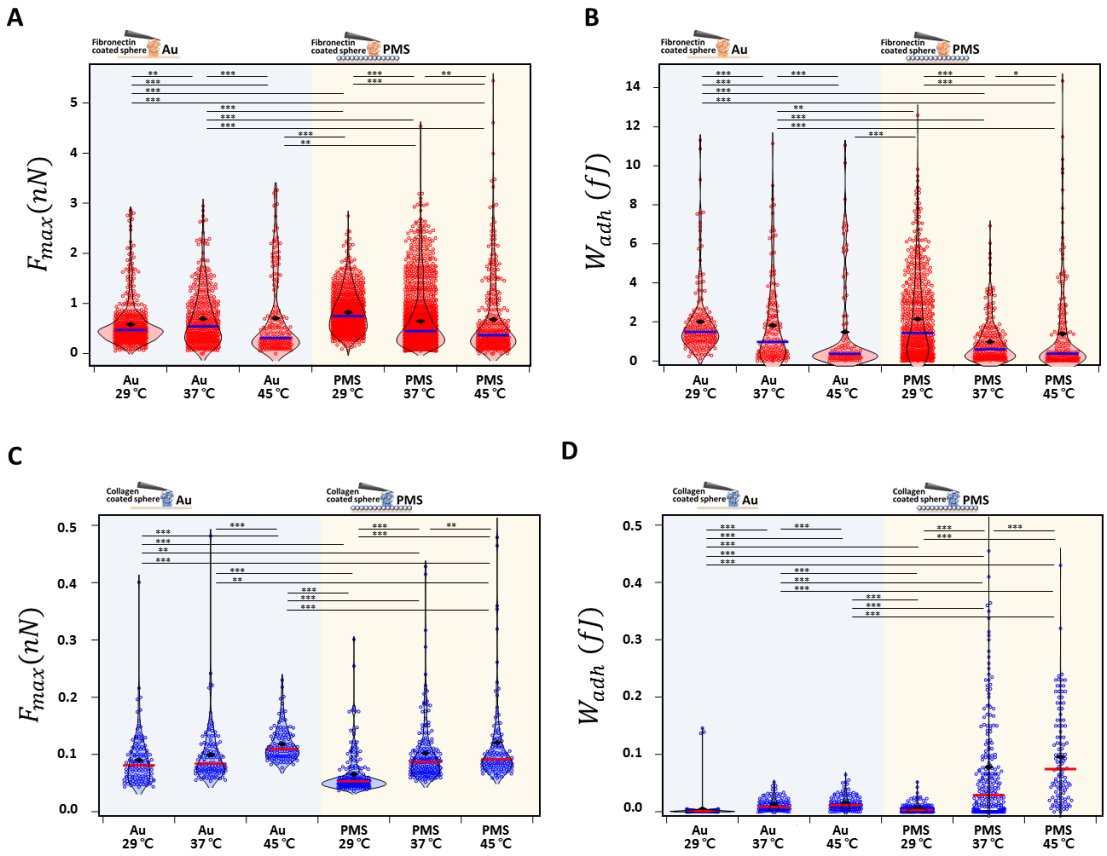


Figure S14. Interaction between ECM components and gold or PMS upon temperature switching. 45 ℃ cases are conducted as an additional control. **A.** Maximal adhesion forces F_{max} and **B**. work of adhesion W_{adh} of colloidal, fibronectin coated AFM cantilever to the gold substrate and PMS. **C.** Maximal adhesion forces F_{max} and **D**. work of adhesions W_{adh} colloidal, collagen coated AFM cantilever to the gold substrate and PMS upon temperature switching. (—: median value; ◆: mean value)

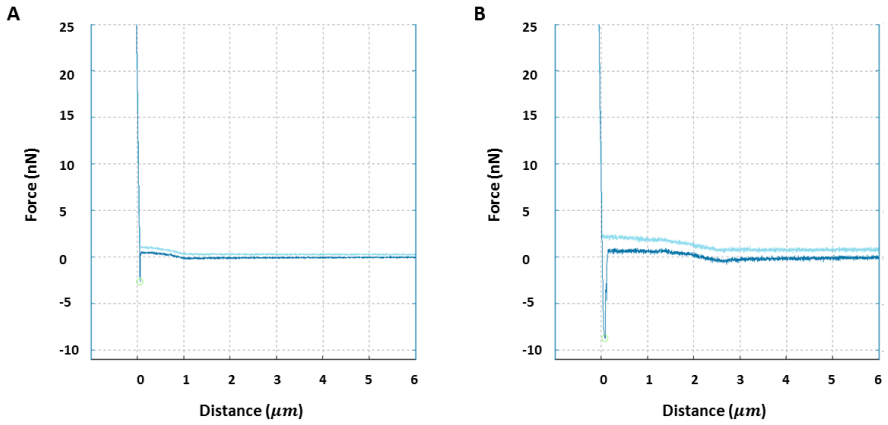


Figure S15. F-D curves for the A. Poly-L-Lysin and B. CellTak functionalized cantilevers.

Table S5. Correlation matrix

(*: p value <0.1, **: p value < 0.05, ***: p value <0.01; bold: T-test, normal: Wilcoxon Rank Test)

		ACH	ACC	PCH	PCC
ACH	F_{max}	-	**0.007 (***)**	0.137	0.011 (**)
	W_{adh}	-	0.008 (***)	0.864	1.69e-04 (***)
	Nr	-	**0.12**	**0.255**	**0.028 (**)**
	Slope of each step	-	0.646	0.014 (**)	0.484
	F_{step}	-	**4.256e-06 (***)**	9.998e-03 (***)	0.03 (**)
	IP	-	**0.28**	0.632	0.168
	I_{step}	-	0.491	0.542	0.23
ACC	F_{max}	-	-	0.083 (*)	0.52
	W_{adh}	-	-	0.039 (**)	0.4622
	Nr	-	-	**0.526**	**0.656**
	Slope of each step	-	-	0.134	0.304
	F_{step}	-	-	1.2e-07 (***)	4.86e-04 (***)
	IP	-	-	0.448	0.692
	I_{step}	-	-	0.011 (**)	0.004 (***)
PCH	F_{max}	-	-	-	0.215
	W_{adh}	-	-	-	0.003 (***)
	Nr	-	-	-	**0.211**
	Slope of each step	-	-	-	0.009 (***)
	F_{step}	-	-	-	3.8e-04 (***)
	IP	-	-	-	0.844
	I_{step}	-	-	-	0.604
PCC	F_{max}	-	-	-	-
	W_{adh}	-	-	-	-
	Nr	-	-	-	-
	Slope of each step	-	-	-	-
	F_{step}	-	-	-	-
	IP	-	-	-	-
	I_{step}	-	-	-	-

		ACCH30	ACCC30	PCCH30	PCCC30	ACCH90	ACCC90	PCCH90	PCCC90
ACCH30	F_{max}	-	0.092 (*)	0.068 (*)	0.154	0.01 (**)	5.0e-04 (***)	1.32e-06 (***)	2.42e-06 (***)
	W_{adh}	-	0.037 (**)	0.633	0.892	0.352	0.171	0.002 (***)	0.049 (***)
	Nr	-	3.22e-04 (***)	0.021 (**)	8.0e-04 (***)	0.363	0.022 (**)	0.344	0.032 (**)
	Slope of each step	-	**0.839**	**0.009 (***)**	**0.725**	0.006 (***)	0.696	0.66	0.005 (***)
	F_{step}	-	9.422e-08 (***)	2.76e-05 (***)	7.76e-16 (***)	3.33e-16 (***)	0 (***)	0 (***)	0 (***)
	IP	-	0.022 (**)	0.95	0.5	0.344	2.0e-04 (***)	0.316	0.694
	I_{step}	-	0.206	0.614	0.638	0.432	0.003 (***)	0.46	0.404
ACCC30	F_{max}	-	-	0.368	0.373	0.184	0.009 (***)	6.21e-06 (***)	1.79e-07 (***)
	W_{adh}	-	-	0.03 (**)	0.041 (**)	0.009 (***)	0.679	7.958e-07 (***)	9.744e-05 (***)
	Nr	-	-	0.405	0.516	0.103	0.149	1.58e-04 (***)	0.067 (*)
	Slope of each step	-	-	0.022 (**)	0.334	0.051 (*)	0.946	0.244	0.067 (*)

	F_{step}	-	-	0.578	1.132e-04 (***)	5.196e-05 (***)	5.226e-07 (***)	3.64e-13 (***)	2.598e-08 (***)
	IP	-	-	0.027 (**)	0.146	0.108	0.248	0.005 (***)	0.012 (**)
	I_{step}	-	-	0.17	0.118	0.774	0.091 (*)	0.584	0.048 (**)
PCCH30	F_{max}	-	-	-	0.715	0.522	0.212	0.002 (***)	0.009 (***)
	W_{adh}	-	-	-	0.958	0.492	0.089 (*)	8.52e-04 (***)	0.055 (*)
	Nr	-	-	-	0.256	0.442	0.706	0.009 (***)	0.653
	Slope of each step	-	-	-	1.22e-04 (***)	0.474	0.114	1.08e-04 (***)	0.792
	F_{step}	-	-	-	5.72e-05 (***)	2.229e-05 (***)	3.66e-07 (***)	1.31e-13 (***)	2.87e-08 (***)
	IP	-	-	-	0.316	0.392	0.003 (***)	0.538	0.974
	I_{step}	-	-	-	0.958	0.262	0.007 (***)	0.366	0.756
PCCC30	F_{max}	-	-	-	-	0.432	0.083 (*)	3.0e-04 (***)	0.002 (***)
	W_{adh}	-	-	-	-	0.343	0.127	7.9e-04 (***)	0.082 (*)
	Nr	-	-	-	-	0.054 (*)	0.101	1.845e-04 (***)	0.067 (*)
	Slope of each step	-	-	-	-	8.2e-04 (***)	0.678	0.862	0.002 (***)
	F_{step}	-	-	-	-	0.76	0.2	1.86e-05 (***)	0.268
	IP	-	-	-	-	0.95	0.012 (**)	0.04 (**)	0.318
	I_{step}	-	-	-	-	0.26	0.002 (***)	0.348	0.684
ACCH90	F_{max}	-	-	-	-	-	0.364	0.007 (***)	0.046 (**)
	W_{adh}	-	-	-	-	-	0.051 (*)	0.0493 (**)	0.749
	Nr	-	-	-	-	-	0.542	0.087 (*)	0.635
	Slope of each step	-	-	-	-	-	0.17	4.0e-04 (***)	0.744
	F_{step}	-	-	-	-	-	0.138	1.208e-05 (***)	0.106
	IP	-	-	-	-	-	0.017 (**)	0.09 (*)	0.226
	I_{step}	-	-	-	-	-	0.0578 (*)	0.794	0.092 (*)
ACCC90	F_{max}	-	-	-	-	-	-	0.036 (**)	0.27
	W_{adh}	-	-	-	-	-	-	3.55e-05 (***)	0.001 (***)
	Nr	-	-	-	-	-	-	0.007 (***)	0.828
	Slope of each step	-	-	-	-	-	-	0.6	0.171
	F_{step}	-	-	-	-	-	-	0.001 (***)	0.893
	IP	-	-	-	-	-	-	0.002 (***)	2.4e-04 (***)
	I_{step}	-	-	-	-	-	-	0.038 (**)	3.16e-04 (***)
PCCH90	F_{max}	-	-	-	-	-	-	-	0.175
	W_{adh}	-	-	-	-	-	-	-	0.025 (**)
	Nr	-	-	-	-	-	-	-	0.018 (**)
	Slope of each step	-	-	-	-	-	-	-	2.0e-04 (***)
	F_{step}	-	-	-	-	-	-	-	4.0e-04 (***)
	IP	-	-	-	-	-	-	-	0.162

	I_{step}	-	-	-	-	-	-	-	0.172
PCCC90	F_{max}	-	-	-	-	-	-	-	-
	W_{adh}	-	-	-	-	-	-	-	-
	Nr	-	-	-	-	-	-	-	-
	Slope of each step	-	-	-	-	-	-	-	-
	F_{step}	-	-	-	-	-	-	-	-
	IP	-	-	-	-	-	-	-	-
	I_{step}	-	-	-	-	-	-	-	-

			Au 29 ℃	**Au 37 ℃**	**Au 45 ℃**	**PMS 29 ℃**	**PMS 37 ℃**	**PMS 45 ℃**
Au 29 ℃	Fibronectin	F_{max}	-	0.016 (**)	0.001 (***)	0 (***)	0.318	0.001 (***)
		W_{adh}	-	4.72E-04 (***)	2.06E-14 (***)	0.24	0 (***)	4.44E-16 (***)
	Collagen	F_{max}	-	**0.133**	1.754E-14 (***)	2.22E-15 (***)	0.015 (**)	7.10E-05 (***)
		W_{adh}	-	0 (***)	0 (***)	0.001 (***)	4E-13 (***)	0 (***)
Au 37 ℃	Fibronectin	F_{max}	-	-	0.001 (***)	2.2E-14 (***)	0.005 (***)	2.20E-04 (***)
		W_{adh}	-	-	3.6E-4 (***)	0.019 (**)	9.38E-05 (***)	1.10E-05 (***)
	Collagen	F_{max}	-	-	7.74E-11 (***)	0 (***)	0.96	0.017 (**)
		W_{adh}	-	-	0.177	1.31E-14 (***)	2.06E-05 (***)	0 (***)
Au 45 ℃	Fibronectin	F_{max}	-	-	-	0 (***)	0.02 (**)	0.68
		W_{adh}	-	-	-	7.54E-09 (***)	0.252	0.456
	Collagen	F_{max}	-	-	-	0 (***)	3.34E-13 (***)	3.62E-07 (***)
		W_{adh}	-	-	-	0 (***)	2E-4 (***)	0 (***)
PMS 29 ℃	Fibronectin	F_{max}	-	-	-	-	0 (***)	0 (***)
		W_{adh}	-	-	-	-	4.44E-16 (***)	2.264E-14 (***)
	Collagen	F_{max}	-	-	-	-	0 (***)	0 (***)
		W_{adh}	-	-	-	-	0 (***)	0 (***)
PMS 37 ℃	Fibronectin	F_{max}	-	-	-	-	-	0.016 (**)
		W_{adh}	-	-	-	-	-	0.084 (*)
	Collagen	F_{max}	-	-	-	-	-	0.014 (**)
		W_{adh}	-	-	-	-	-	3.22E-07 (***)
PMS 45 ℃	Fibronectin	F_{max}	-	-	-	-	-	-
		W_{adh}	-	-	-	-	-	-
	Collagen	F_{max}	-	-	-	-	-	-
		W_{adh}	-	-	-	-	-	-

212 **Table S6** Number of measurement information of SCFS and CP-AFM

	Measurement days	Number of cells	Number of force curves
ACH 30	5	12	32
ACC 30	3	7	24
PCH 30	5	9	49
PCC 30	5	7	32
ACCH 30	3	7	36
ACCC 30	3	13	50
PCCH 30	4	9	26
PCCC 30	3	13	42
ACCH 90	3	8	20
ACCC 90	2	11	23
PCCH 90	3	8	17
PCCC 90	2	11	28
ECM	**Substrate**		**Number of force curve**
Fibronectin	Au	29 ℃	551
		37℃	448
		45℃	188
	PMS	29℃	818
		37℃	1628
		45℃	352
Collagen I	Au	29 ℃	124
		37℃	129
		45℃	126
	PMS	29℃	195
		37℃	190
		45℃	127

213