Supplementary Materials for

Soft topographical patterns trigger a stiffness-dependent cellular response to contact guidance.

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Tables S1 to S14
Movies S1 to S4

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S4

Fig. S1.

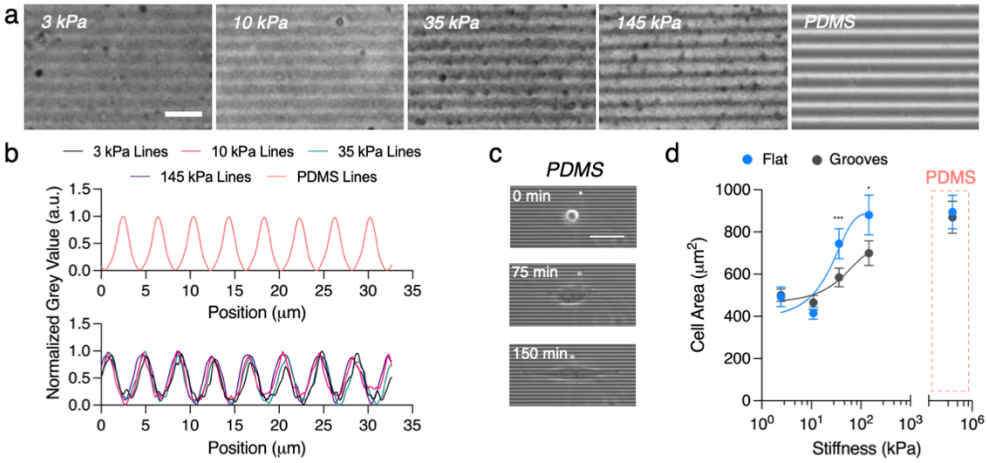

Fig. S1. (**A**) Phase contrast image of 2 µm wide and 1 µm high grooves on PAA gels of different stiffness and PDMS. Scale bar, 10 µm. (**B**) Intensity profiles of the microstructured grooves for PDMS and PAA gels. (**C**) Snapshots of NIH 3T3 fibroblasts adhering, elongating and aligning on PDMS grooves. Scale bar, 35 µm. (**D**) Cell area versus substrate stiffness. See Table S1 for the number of cells and experiments. Data points (Mean ± CI) were fitted as an eye-guide. Statistical significance was assessed by Tukey's tests.

Fig. S2.

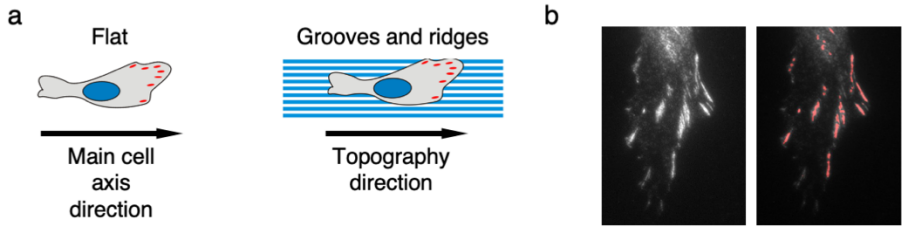

Fig. S2. (**A**) Schematics depicting the reference used to evaluate focal adhesion alignment on flat and grooved substrates. (**B**) Representative image of the detection of focal adhesion used to determine area and orientation.

Fig. S3.

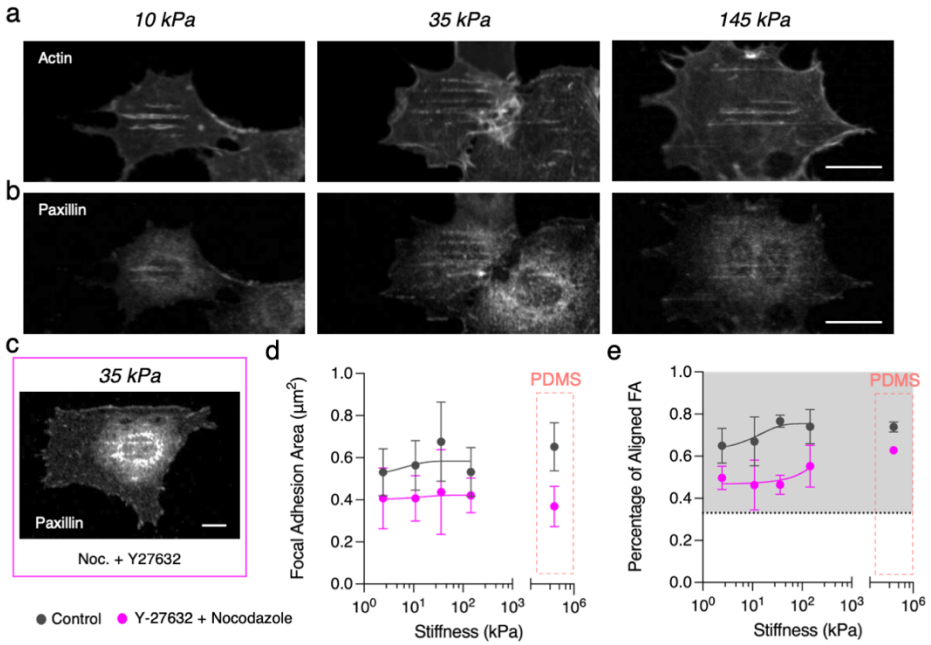

Fig. S3. Representative images of cells treated with Y-27632 and nocodazole on grooves of increasing stiffness. (**A**) F-actin (phalloidin) and (**B**) paxillin. Scale bar, 20 µm. (**C**) Paxillin immunostaining of fibroblast treated with 20 µM Y-27632 and 1 µM nocodazole on 2 µm wide grooves of 35 kPa. Scale bar, 20 µm. (**D**) Focal adhesion area and (**E**) percentage of aligned focal adhesions as a function of increasing grooves stiffness. Grey area in (**E**) corresponds to values above the expected for a random distribution. See Tables S9 for the number of cells and experiments.

Fig. S4.

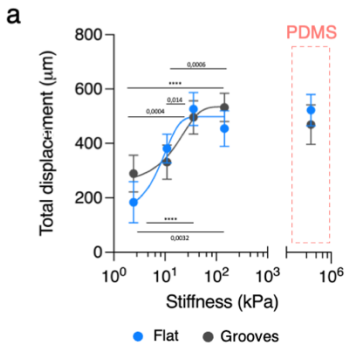

Fig. S4. (**A**) Total displacement of cells migrating for 6h on substrates of different stiffness. See Tables S10 for the number of cells and experiments. Data points (Mean ± CI) were fitted as an eye-guide. Statistical significance was assessed by Tukey's tests (**A**).

Fig. S5.

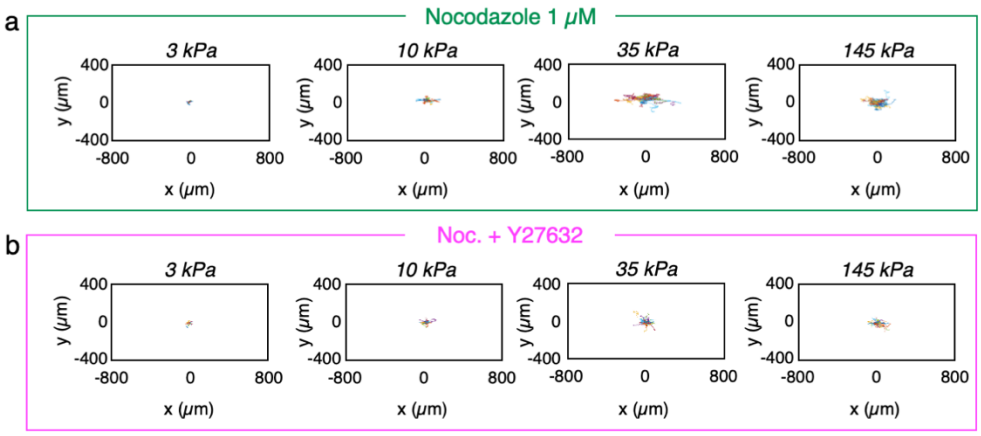

Fig. S5. (**A**) Trajectories of 3T3 fibroblasts treated with 1 μM nocodazole and (**B**) 20 μM Y-27632 and 1 μM nocodazole on grooves of increasing stiffness.

Fig. S6.

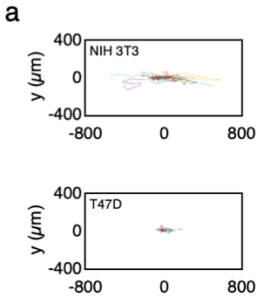

Fig. S6. (**A**) Trajectories of NIH 3T3 fibroblasts and T47D single cells migrating on grooved PDMS substrates.

Fig. S7.

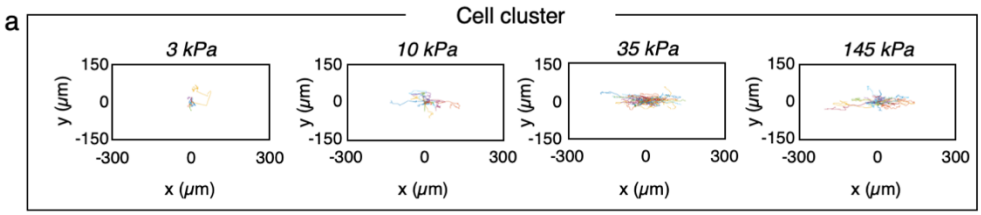

Fig. S7. (**A**) T47D clusters' trajectories on grooves of increasing stiffness.

Table S1.

Table S1 – NIH 3T3 morphology

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	3	52	5	116
10	5	57	10	114
35	6	94	8	134
145	4	67	5	124
PDMS	4	133	6	184

Table S2.

Table S2 – T47D morphology

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	2	10	3	37
10	2	12	4	21
35	3	17	3	42
145	2	9	3	45
PDMS	3	42	3	91

Table S3.

Table S3 – NIH 3T3 focal adhesions

Stiffness (kPa)	Control		Nocodazole+Y27632	
	N experiments	n cells	N experiments	n cells
3	4	21	2	5
10	3	24	2	11
35	4	15	2	4
145	4	25	2	25
PDMS	5	25	2	11

Table S4.

Table S4 – NIH 3T3 – Y27632 morphology

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	3	17	3	57
10	3	14	3	63
35	4	40	4	79
145	4	38	4	83
PDMS	3	75	3	196

Table S5.

Table S5 – NIH 3T3 actin profile

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	--	--	4	31
10	--	--	3	35
35	--	--	3	39
145	--	--	3	36
PDMS	--	--	4	28

Table S6.

Table S6 – NIH 3T3 tubulin profile

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	--	--	3	22
10	--	--	3	36
35	--	--	3	39
145	--	--	3	37
PDMS	--	--	3	27

Table S7.

Table S7 – NIH 3T3 – Nocodazole morphology

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	3	16	3	88
10	4	51	5	102
35	3	30	5	95
145	3	13	4	63
PDMS	3	37	3	137

Table S8.

Table S8 – NIH 3T3 – Nocodazole+Y27632 morphology

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	3	22	3	47
10	3	15	3	63
35	3	28	3	85
145	3	28	3	84
PDMS	3	39	3	54

Table S9.

Table S9 – NIH 3T3 focal adhesions

Stiffness (kPa)	Control		Nocodazole+Y27632	
	N experiments	n cells	N experiments	n cells
3	4	21	2	5
10	3	24	2	11
35	4	15	2	4
145	4	25	2	25
PDMS	5	25	2	11

Table S10.

Table S10 – NIH 3T3 migration

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	4	13	3	24
10	4	23	5	24
35	5	31	5	65
145	4	21	3	69
PDMS	4	30	3	31

Table S11.

Table S11 – NIH 3T3 – Nocodazole migration

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	4	11	3	15
10	4	17	3	28
35	4	28	3	49
145	3	24	3	38
PDMS	4	25	3	25

Table S12.

Table S12 – NIH 3T3 – Nocodazole+Y27632 migration

Stiffness (kPa)	Flat		Grooves	
	N experiments	n cells	N experiments	n cells
3	2	13	2	32
10	2	10	2	52
35	2	26	2	67
145	2	25	2	46
PDMS	2	16	2	29

Table S13.

Table S13 – T47D migration

Stiffness (kPa)	Flat			Grooves		
	N experiments	n cells	n cluster	N experiments	n cells	n cluster
3	2	8	19	3	14	7
10	2	13	16	4	41	19
35	3	4	5	3	47	66
145	2	5	13	3	34	34
PDMS	3	18	22	3	45	24

Table S14.

Table S14 – PAA gels formulation

Stiffness (kPa)	% Acrylamide	% Bis-acrylamide
3	7.5	0.050
10	7.5	0.075
35	12	0.150
145	12	0.600

Movie S1.

NIH 3T3 fibroblasts spreading on topographical grooves and ridges made of PAA gels of different stiffness (3 – 145 kPa). Time hh:mm. Scale bar 50 μm.

Movie S2.

NIH 3T3 fibroblasts migrating on topographical grooves and ridges made of PAA gels of different stiffness (3 – 145 kPa). Time hh:mm. Scale bar 50 μm.

Movie S3.

T47D single cell migrating on 35kPa topographical grooves and ridges. Time hh:mm. Scale bar 50 μm.

Movie S4.

T47D cell cluster migrating on 35kPa topographical grooves and ridges. Time hh:mm. Scale bar 50 μm.