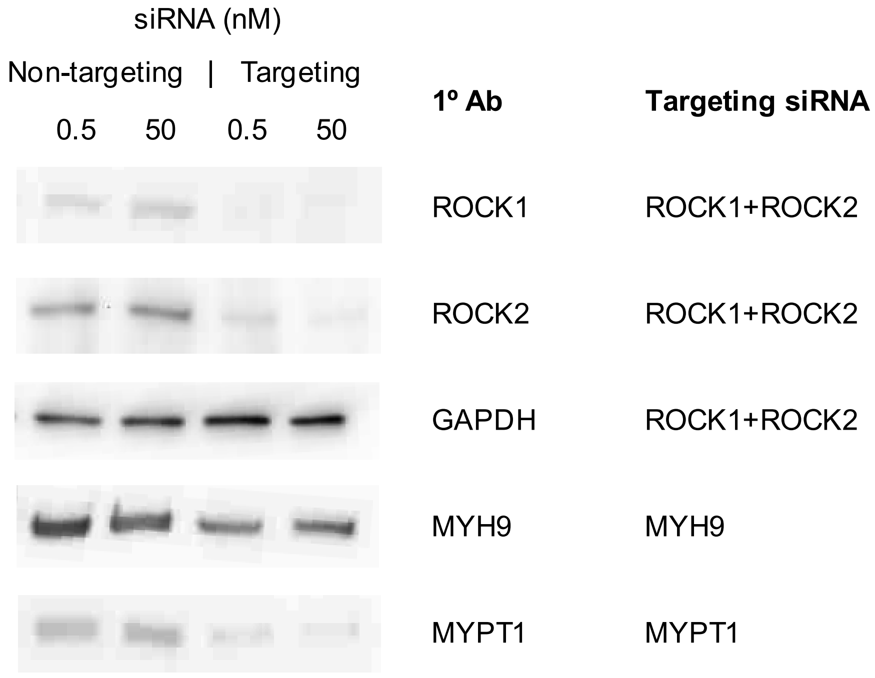

Fig. S1. Western blotting evaluation of siRNA knockdown. Fibroblasts grown on rigid (glass) substrates were transfected with the indicated concentrations of non-targeting or targeting siRNA. Similar siRNA knockdown efficacy is observed on soft hydrogels (data not shown). For ROCK1+ROCK2 co-transfections, 0.25 or 25 nM of each siRNA was used. Protein was isolated 72 hours after transfection. GAPDH serves as a loading control for the non-targeting and ROCK1+ROCK2 conditions.

Movie 1. Phase contrast image sequence of IMR-90 fibroblasts on a 1 kPa substrate before and after addition of 10 µM Y27632 (at frame 31, 2 sec).

Movie 2. Phase contrast image sequence of IMR-90 fibroblasts following washout of Y27632. Images were acquired at 2 min intervals, 200x magnification, and compiled at 15 frames/sec.

Movie 3. Phase contrast image sequence of IMR-90 fibroblasts on a 1 kPa substrate after addition of 0.3 µM cantharidin. Images were acquired at 10 min intervals, 200x magnification, and compiled at 2 frames/sec.

Table S1. Profile of cell cycle gene expression in IMR-90 fibroblasts. For each condition, changes in transcript levels are normalized to a panel of housekeeping genes, and then expressed relative to the 1 kPa, no treatment control.

Symbol	**Description**	**1 kPa**	**1 kPa + Y27632**	**Rigid**	**Rigid +Y27632**
ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	1	0.485	1.0345	0.6522
ANAPC2	Anaphase promoting complex subunit 2	1	0.4412	1.178	1.1432
ATR	Ataxia telangiectasia and Rad3 related	1	0.6424	0.5195	0.5656
AURKA	Aurora kinase A	1	0.5688	0.9732	0.6778
AURKB	Aurora kinase B	1	0.7715	0.5515	0.7635
BCCIP	BRCA2 and CDKN1A interacting protein	1	0.4579	1.4387	1.4579
BCL2	B-cell CLL/lymphoma 2	1	0.9058	0.8427	0.6226
BIRC5	Baculoviral IAP repeat containing 5	1	0.7923	1.447	1.3317
BRCA1	Breast cancer 1, early onset	1	0.8837	1.0382	0.7845
BRCA2	Breast cancer 2, early onset	1	0.709	1.2939	1.078
CASP3	Caspase 3, apoptosis-related cysteine peptidase	1	0.6996	1.6761	0.8295
CCNA2	Cyclin A2	1	0.9015	1.3686	0.8332
CCNB1	Cyclin B1	1	1.0724	1.2683	0.8197
CCNB2	Cyclin B2	1	0.9239	1.2286	0.7428
CCNC	Cyclin C	1	0.6458	1.5687	1.3432
CCND1	Cyclin D1	1	0.9607	1.8183	1.283
CCND2	Cyclin D2	1	0.3938	1.3734	0.7584
CCND3	Cyclin D3	1	0.3481	2.5126	1.8493
CCNE1	Cyclin E1	1	1.0795	1.1718	0.9893
CCNF	Cyclin F	1	0.8067	0.9553	0.9025
CCNG1	Cyclin G1	1	0.8925	1.0701	0.7013
CCNG2	Cyclin G2	1	0.505	1.2193	0.704
CCNH	Cyclin H	1	0.5612	0.7453	0.5837
CCNT1	Cyclin T1	1	0.9036	0.7322	0.5243
CDC16	Cell division cycle 16 homolog (S. cerevisiae)	1	1.1951	1.8278	0.8269
CDC20	Cell division cycle 20 homolog (S. cerevisiae)	1	0.6473	1.2699	0.8903
CDC25A	Cell division cycle 25 homolog A (S. pombe)	1	0.3856	1.3874	1.0553
CDC25C	Cell division cycle 25 homolog C (S. pombe)	1	0.9675	0.9028	0.6351
CDC34	Cell division cycle 34 homolog (S. cerevisiae)	1	0.9291	0.9389	0.6426
CDC6	Cell division cycle 6 homolog (S. cerevisiae)	1	0.3727	1.2094	0.5952
CDK1	Cyclin-dependent kinase 1	1	0.5083	1.0164	0.8214
CDK2	Cyclin-dependent kinase 2	1	0.4739	1.2037	0.6686
CDK4	Cyclin-dependent kinase 4	1	0.7919	0.9038	0.5471
CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	1	0.8243	0.9794	0.5834
CDK5RAP1	CDK5 regulatory subunit associated protein 1	1	0.7477	0.8799	0.5438
CDK6	Cyclin-dependent kinase 6	1	0.6167	1.7037	1.485
CDK7	Cyclin-dependent kinase 7	1	1.1416	1.0338	1.0919
CDK8	Cyclin-dependent kinase 8	1	0.9365	1.0323	0.658
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1	0.8961	1.9026	1.2463
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	1	0.8755	1.0965	0.7509
CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	1	0.7876	0.9557	0.5916
CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1	1.0204	1.2935	1.0065
CDKN3	Cyclin-dependent kinase inhibitor 3	1	0.8378	1.5588	1.1824
CHEK1	CHK1 checkpoint homolog (S. pombe)	1	0.8634	0.8742	0.7349
CKS1B	CDC28 protein kinase regulatory subunit 1B	1	0.8844	0.7246	0.6131
CKS2	CDC28 protein kinase regulatory subunit 2	1	0.3982	1.1877	1.0142

CUL2	Cullin 2	1	1.0267	0.9997	0.9892
E2F1	E2F transcription factor 1	1	0.9856	1.1033	0.7181
E2F4	E2F transcription factor 4, p107/p130-binding	1	0.8477	1.3832	0.9555
GADD45A	Growth arrest and DNA-damage-inducible, alpha	1	1.1697	1.0231	0.8399
GTSE1	G-2 and S-phase expressed 1	1	0.7593	1.44	0.9906
HUS1	HUS1 checkpoint homolog (S. pombe)	1	0.8684	0.7826	0.5811
KNTC1	Kinetochore associated 1	1	0.6607	0.9556	0.606
KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	1	0.8675	1.4081	1.0181
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	1	0.4383	1.5059	1.2541
MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)	1	0.1298	1.4487	1.3456
MCM2	Minichromosome maintenance complex component 2	1	0.5905	1.0974	0.7266
MCM3	Minichromosome maintenance complex component 3	1	0.5879	1.4588	0.9143
MCM4	Minichromosome maintenance complex component 4	1	0.4273	1.089	0.8579
MCM5	Minichromosome maintenance complex component 5	1	0.5808	1.6935	0.8643
MDM2	Mdm2 p53 binding protein homolog (mouse)	1	0.8872	1.0891	0.5905
MKI67	Antigen identified by monoclonal antibody Ki-67	1	0.5137	1.4869	1.497
MNAT1	Menage a trois homolog 1, cyclin H assembly factor (Xenopus laevis)	1	0.7794	1.0918	0.7652
MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	1	1.6962	2.4657	1.5792
NBN	Nibrin	1	0.9891	0.7195	0.5762
RAD1	RAD1 homolog (S. pombe)	1	0.9127	0.757	0.6637
RAD17	RAD17 homolog (S. pombe)	1	0.974	0.9555	0.8911
RAD51	RAD51 homolog (S. cerevisiae)	1	0.4441	0.7951	0.7601
RAD9A	RAD9 homolog A (S. pombe)	1	0.9363	1.6467	0.9611
RB1	Retinoblastoma 1	1	1.1108	1.2121	0.706
RBBP8	Retinoblastoma binding protein 8	1	0.6596	1.2618	0.9633
RBL1	Retinoblastoma-like 1 (p107)	1	0.9453	0.8861	1.0161
RBL2	Retinoblastoma-like 2 (p130)	1	0.8969	1.1147	0.8919
SERTAD1	SERTA domain containing 1	1	0.4999	0.8991	0.6486
SKP2	S-phase kinase-associated protein 2 (p45)	1	0.7572	1.2387	0.7905
STMN1	Stathmin 1	1	0.9171	0.9505	0.7916
TFDP1	Transcription factor Dp-1	1	0.4876	1.5845	1.3417
TFDP2	Transcription factor Dp-2 (E2F dimerization partner 2)	1	0.8007	0.8305	0.6302
TP53	Tumor protein p53	1	0.6244	2.0388	1.5771
WEE1	WEE1 homolog (S. pombe)	1	0.3332	1.1666	1.2536

Table S2. siRNA sequences.

Non-targeting
Duplex #1: 5'-UGGUUUACAUGUCGACUAA-3’
Duplex #2: 5'-UGGUUUACAUGUUGUGUGA-3’
Duplex #3: 5'-UGGUUUACAUGUUUUCUGA-3’
Duplex #4: 5'-UGGUUUACAUGUUUUCCUA-3’

Target: *MYH9*
Duplex #5: 5’-GUAUCAAUGUGACCGAUUU-3’
Duplex #6: 5’-CAAAGGAGCCCUGGCGUUA-3’
Duplex #7: 5’-GGAGGAACGCCGAGCAGUA-3’
Duplex #8: 5’-CGAAGCGGGUGAAAGCAAA-3’

Target: *MYPT1*
Duplex #7: 5’-CAACUAAACAGGCCAAAUA-3’
Duplex #8: 5’-GCUAAAUAGUGGUCAUAUA-3’
Duplex #9: 5’-ACAAAGAGACGUUGAUUAU-3’
Duplex #10: 5’-CGGAUUCCAUUUCUAGAUA-3’

Target: *ROCK1*
Duplex #6: 5’-CUACAAGUGUUGCUAGUUU-3’
Duplex #7: 5’-UAGCAAUCGUAGAUACUUA-3’
Duplex #8: 5’-CCAGGAAGGUAUAUGCUAU-3’
Duplex #9: 5’-GCCAAUGACUUACUUAGGA-3’

Target: *ROCK2*
Duplex #6: 5’-GCAACUGGCUCGUUCAAUU-3’
Duplex #7: 5’-UAGAAUAUGUGGCCUAGAA-3’
Duplex #8: 5’-GAAACUAAUAGGACACUAA-3’
Duplex #9: 5’-CAAACUUGGUAAAGAAUUG-3’