Supporting Information

Mesenchymal stem cells resist mechanical confinement through the activation of cortex during cell division

*Chuanchuan Lin[1], Ye He[1,2], Kun Xu[1], Qian Feng[1], Xuan Li[1], Songyue Zhang[1], Ke Li[1], Ruqing Bai[4], Hong Jiang[5], Kaiyong Cai[1,3]**

[1] Key Laboratory of Biorheological Science and Technology, Ministry of Education College of Bioengineering, Chongqing University
Chongqing 400044, China

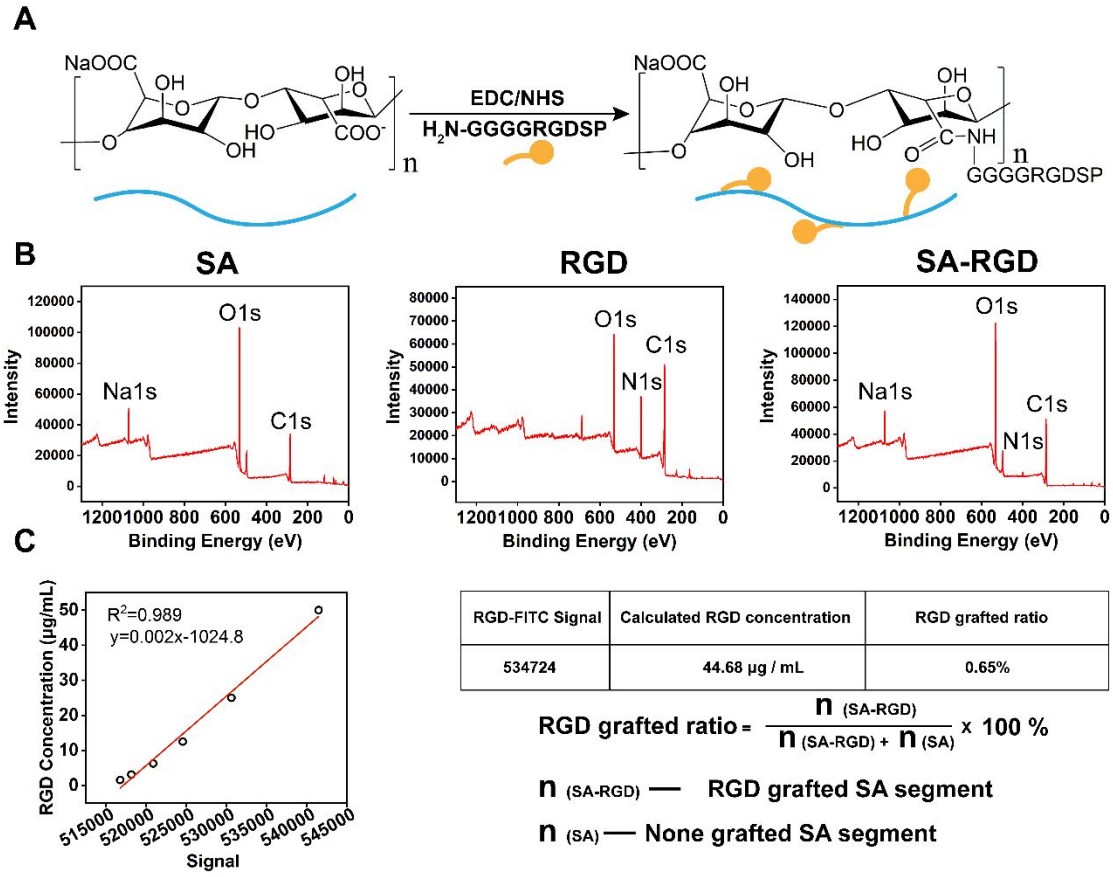
[2] Thomas Lord Department of Mechanical Engineering and Material Science, Duke University
Durham, NC 27708, USA

[3] Chongqing Key Laboratory of Soft-Matter Material Chemistry and Function Manufacturing, Chongqing 400715, China

[4] College of Mechanical and Vehicle Engineering, Chongqing University
Chongqing 400044, China

[5] Department of Biomedical Materials Science, School of Biomedical Engineering, Third Military Medical University (Army Medical University), Chongqing 400038, China.

E-mail: kaiyong_cai@cqu.edu.cn



RGD-FITC Signal	Calculated RGD concentration	RGD grafted ratio
534724	44.68 μg / mL	0.65%

Figure S1 (A) Reaction scheme of RGD peptide with SA. (B) XPS survey spectra of SA, RGD, and SA-RGD. (C) The grafted ratio of SA-RGD.

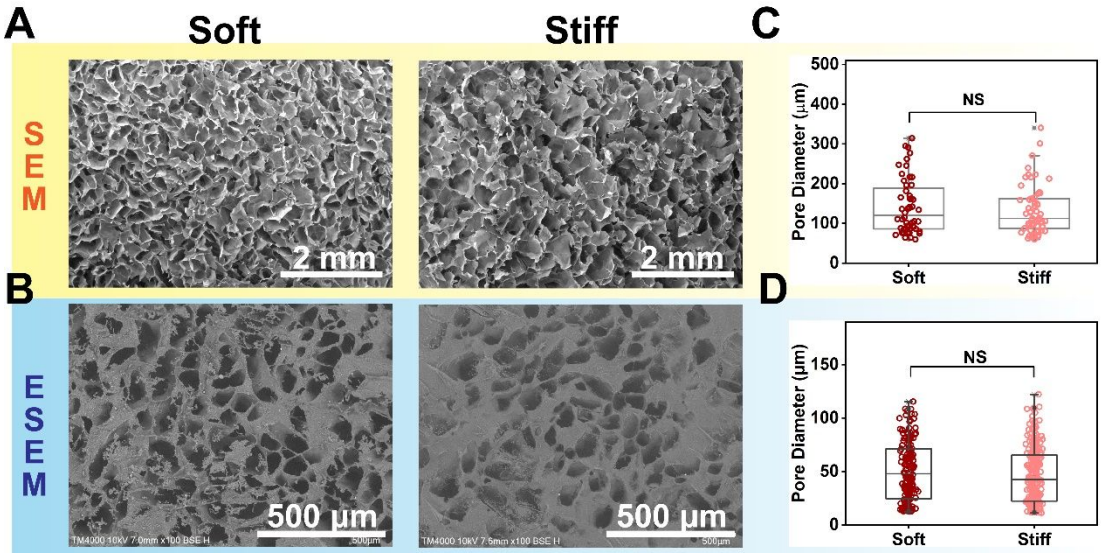


Figure S2: The inner structure of the hydrogel was observed by imaging the cross-section of the lyophilized sample *via* SEM (A) and wet sample *via* ESEM (B); the diameter of pore in sample was calculated and presented in (C) and (D).

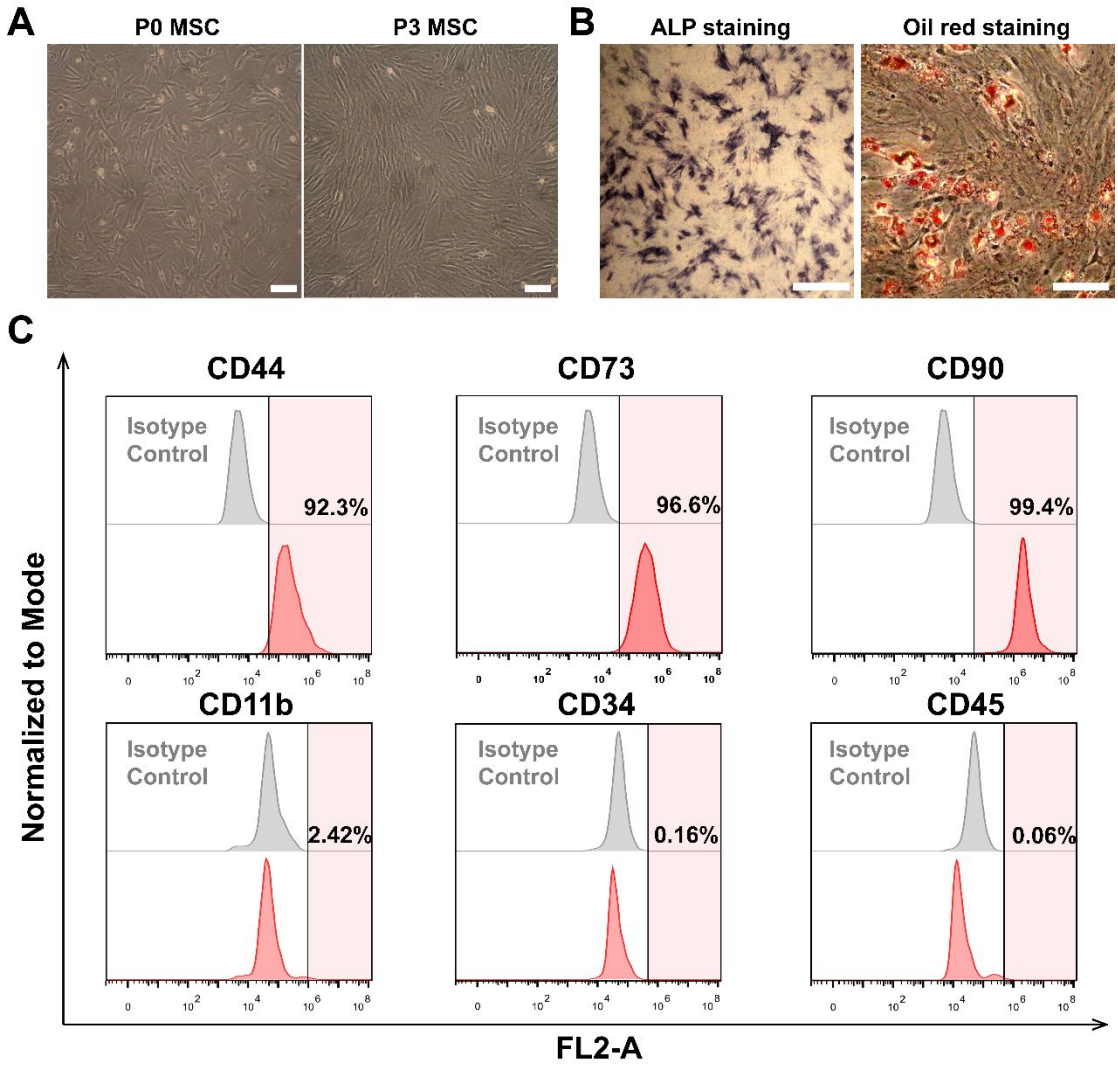


Figure S3 Bone Marrow Mesenchymal stem cells (MSCs) characterization: (A) P_0 and P_3 MSCs; (B) Multi- differentiation capacity identification and (C) Flow characterization of MSCs. Scale bar = 100 μm.

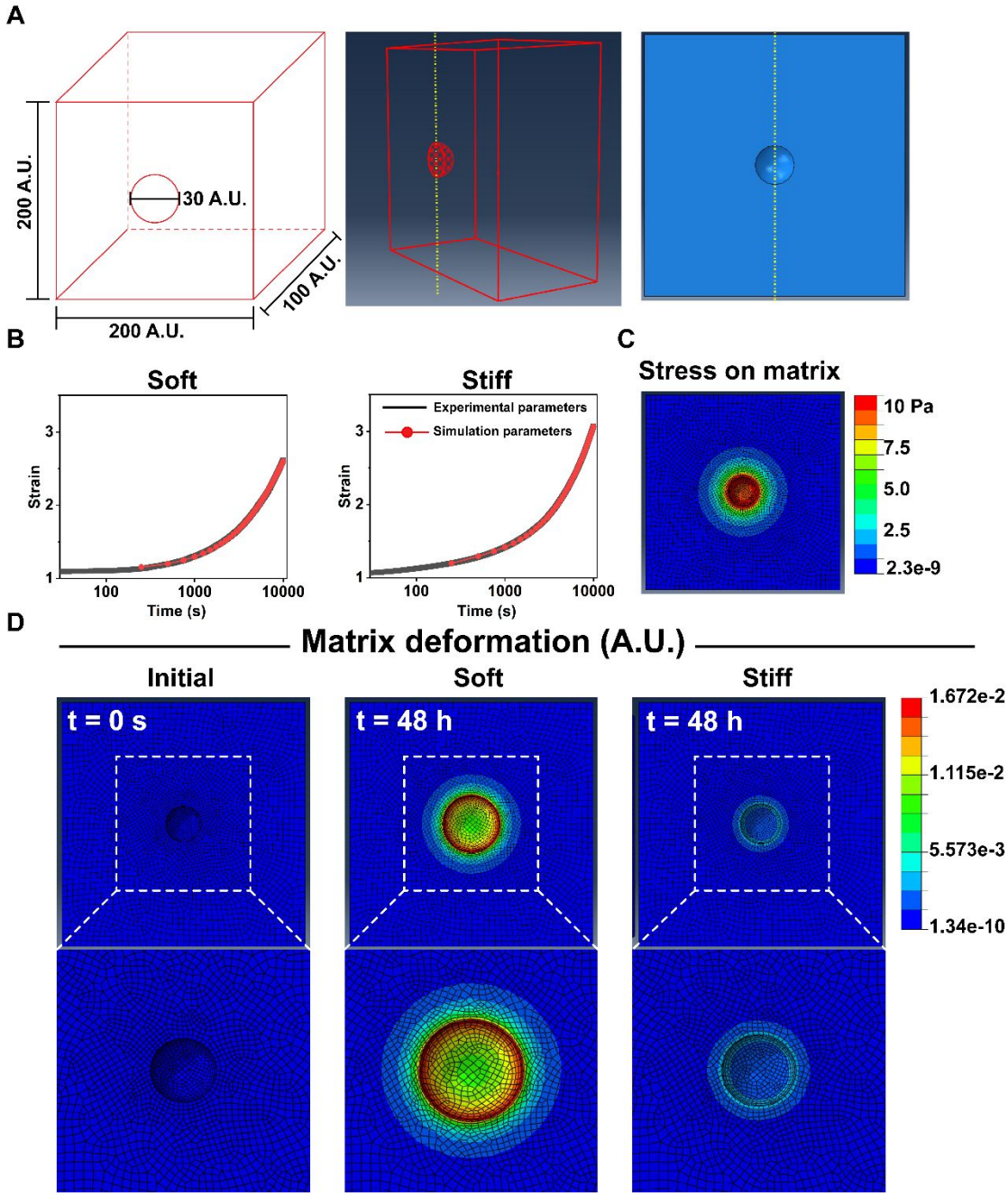


Figure S4 (A) Geometric structure of a simulation model of single cell spreading in a matrix. (B) The prony series which used in simulation model were come from the creep experimental data. (C) Stress field exerted on the simulation model which possessed the mechanical properties of SA hydrogels. (D) Under constant stress, compared with the stiffer matrix, cells in the soft matrix can obtain a larger spreading area.

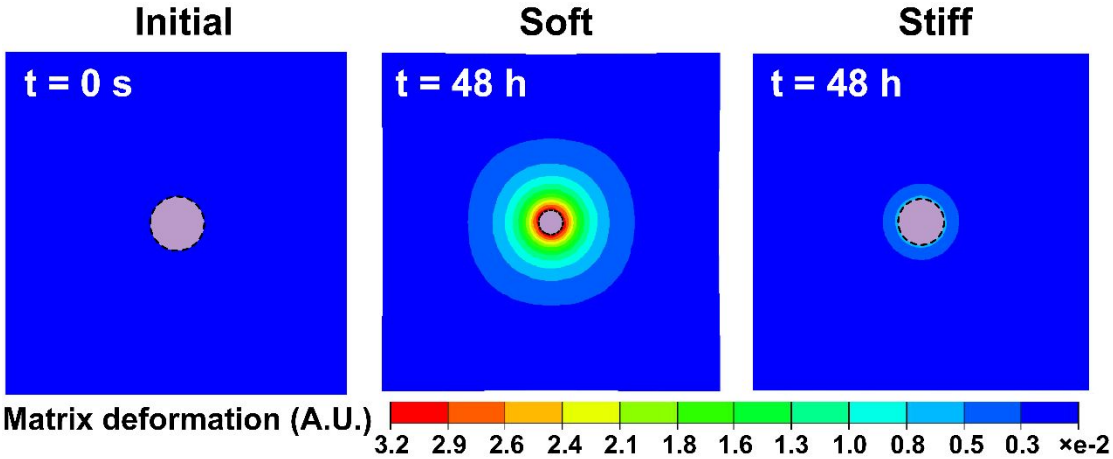


Figure S5. Under constant stress, compared with the stiffer matrix, cells in the soft matrix can obtain a larger deformation.

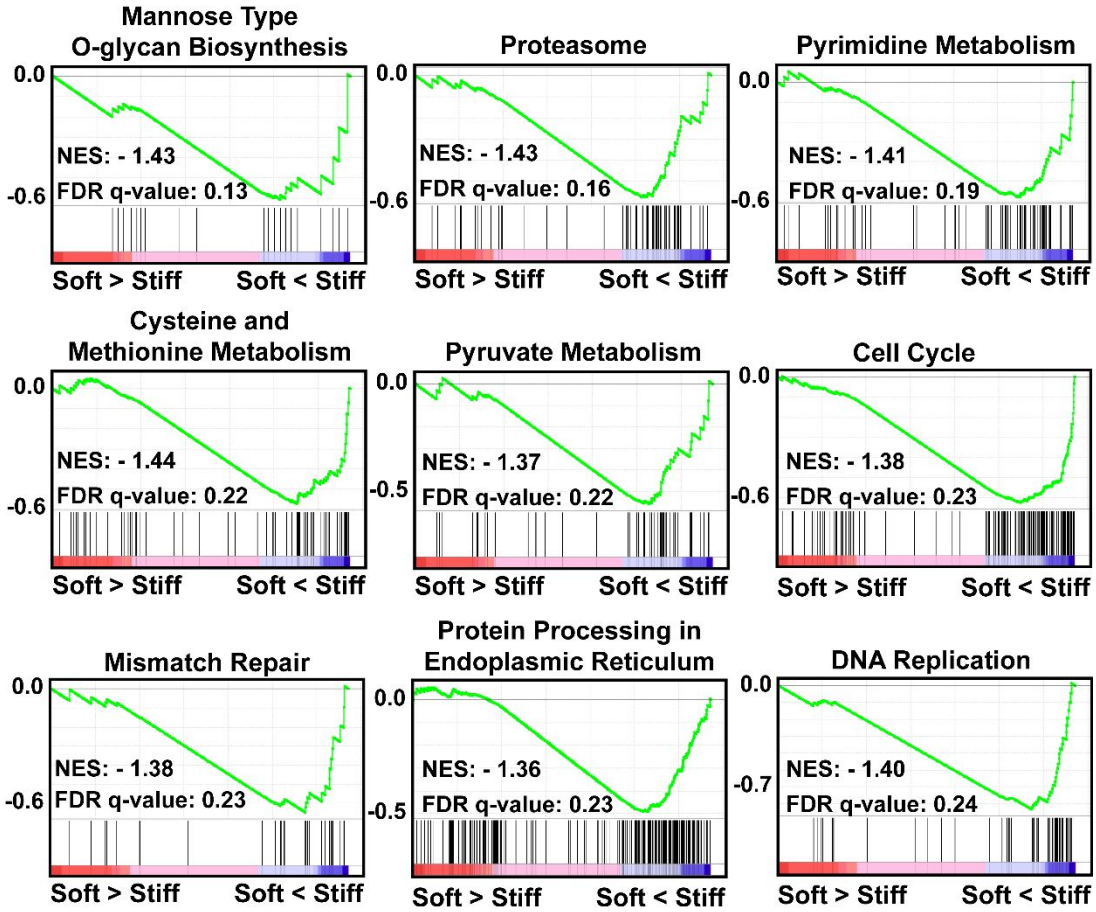


Figure S6 The GSEA enrichment analysis of RNA--seq of MSCs cultured in soft and stiff matrix.

Table S1 Differentiation Expression Genes

Gene Name	|Log2fold change|	Adjust *p-value*
LOC103692169	4.83421975	0.017372123
LOC103694867	4.302629735	0.027858793
novel.679	2.405697778	0.005819081
Pak2	1.774065102	0.004142048
Cnn1	1.454198045	2.79E-21
AABR07025328.1	1.412539237	0.013463477
Acta2	1.393254149	6.82E-58
Actg2	1.32528506	9.72E-09
LOC100911727	1.270228974	0.030241587
Depdc1	1.205245137	0.026170125
Cd55	1.203847478	9.62E-28
Mustn1	1.178704778	0.027933707
Slco2a1	1.144435899	3.19E-20
Cryab	1.136865939	0.019383775
Susd5	1.1217248	0.000423277
Knstrn	1.101498396	0.000147006
Lgr5	1.097590525	7.17E-07
Trib3	1.076543492	2.53E-09
Tagln	1.076039996	2.04E-42
Selp	1.052143397	0.012970629
Pimreg	1.049732392	0.00101949
Troap	1.017792179	0.00326372
Kif18b	1.001711446	0.001710282
Bmp6	0.98997287	0.001786054
Chac1	0.967391808	0.000148592
Cdkn3	0.967080814	0.000285302
Cdca2	0.954940171	0.026686813
Kif4a	0.950030841	0.002793302
Dmrt2	0.945227381	0.002914637
Ccn2	0.944610562	4.46E-61
Dync1i1	0.939219121	0.015152861
AABR07000658.1	0.931881926	0.022123589
Scx	0.925743923	0.001963864
Rnd1	0.925686537	0.01438513
Gask1b	0.913586855	6.93E-19
Plk1	0.898314331	6.02E-07
Cdca3	0.885009882	0.000592371
AABR07028352.1	0.87651617	0.00454503

Gene Name	|Log2fold change|	Adjust *p-value*
Klhl41	0.876304283	0.001484823
LOC100359539	0.873977093	6.51E-05
Kif20b	0.864641856	0.004137708
Kifc1	0.864575783	0.000232757
Mfap5	0.856802415	0.002203254
AC118957.2	0.855024051	0.013567778
Tpx2	0.855017271	2.57E-07
C1qtnf3	0.849889044	9.65E-06
Top2a	0.837114719	1.54E-09
Myl9	0.833379268	3.29E-18
Ccnb1	0.830015247	7.55E-05
AC118957.1	0.821437534	0.008777276
Esm1	0.816385643	6.68E-15
Rexo5	0.811513512	0.046314395
Kif11	0.8112706	0.002195283
Kif23	0.798426687	0.000130311
Nusap1	0.795118022	0.010838096
Rgs16	0.79351072	1.95E-05
Rtn4rl1	0.781009083	0.02124612
Tnfrsf11b	0.775617223	0.034090739
Phf19	0.772424989	0.0496622
Tk1	0.767244525	4.35E-06
Diaph3	0.763378373	0.000426222
Slc38a4	0.752733597	3.69E-10
Dner	0.747965242	3.13E-08
Ttk	0.745551809	0.029884958
Spag5	0.74552836	0.011618878
Ccna2	0.737931616	0.000393316
Serpine1	0.734072322	1.20E-35
Klf2	0.733209067	0.001138239
Hmmr	0.731810188	0.02000841
Cdca8	0.730872122	0.001548633
Spon2	0.730822946	3.69E-10
Cenpf	0.730555309	0.000109773
Bub1	0.730450558	0.00080855
Arsi	0.729542543	0.006942584
Stmn1	0.729161383	2.51E-08
Cdc20	0.728705948	1.50E-05
Prc1	0.728606356	1.17E-06
Chrdl1	0.725466463	0.000104941

Gene Name	|Log2fold change|	Adjust *p-value*
Abi3bp	0.722259126	1.76E-06
Fndc1	0.716630354	7.81E-05
Bmp3	0.712451304	0.008753296
Cdk1	0.709178364	3.95E-05
Kntc1	0.708383544	0.01221886
Nuf2	0.707055375	0.042899066
Acan	0.702416505	1.57E-07
Lvrn	0.69336831	4.79E-05
Cemip	0.691409934	1.75E-21
Thbs1	0.690955166	1.76E-26
Npr3	0.685033973	0.013463477
Sema3d	0.676975766	2.44E-05
Ect2	0.672033404	0.02799381
Psat1	0.670023952	8.72E-09
Lmod1	0.665771268	0.013055927
Anln	0.660134514	1.23E-05
Fgf1	0.656462094	0.048719088
St3gal6	0.656186542	0.012627388
Cenpe	0.65062508	0.003768974
LOC100910996	0.649137636	0.033490714
Ccl12	0.635620849	0.000945438
Ckap2	0.627925576	0.000996217
Kif20a	0.626055444	6.76E-05
Ccdc80	0.625670176	6.36E-26
Iqgap3	0.621895417	0.004580989
Avpr1a	0.618453489	3.18E-10
Kcnk2	0.61742206	6.24E-06
Pik3cg	0.615450632	0.011992126
Rrm2	0.609869943	0.000231527
Scube3	0.603547423	4.75E-06
Pmaip1	0.600221909	0.001816811
Grb14	0.595392257	5.77E-05
Ube2c	0.59073196	0.048506806
Hvcn1	0.586822624	0.012627388
RT1-CE5	0.604508187	0.02309572
Lama1	0.61425676	2.28E-08
Ston2	0.615963981	0.013994334
Cd53	0.616332212	0.000906591
AABR07006310.1	0.621319936	0.000216119
Igfbp5	0.622435827	7.11E-16

Gene Name	|Log2fold change|	Adjust *p-value*
novel.648	0.625242106	0.000203985
AC134224.2	0.635644458	0.00769729
Gpnmb	0.639415998	8.94E-26
novel.69	0.651901499	0.003920972
Tgm1	0.697803208	0.028739415
Rasa4	0.738597677	0.005643592
Rgs9	0.745577618	0.014557779
Fcgr2b	0.752235402	3.50E-07
AABR07035722.1	0.758439092	0.008356125
Tiam1	0.772120591	0.027821429
Ccn3	0.787118935	2.47E-20
Wdfy4	0.787624629	0.049827943
Slamf6	0.822239366	0.046471018
Rbm47	0.83230655	0.04929027
Col15a1	0.834043251	1.76E-10
Siglec10	0.843623261	0.002913854
Bhlhe41	0.846856235	0.002514346
Cxcr4	0.899448219	0.005360486
Fat3	0.946517351	7.70E-08
Hspa1b	0.977245317	0.004627832
novel.647	1.007585403	0.000592371
Map3k7	1.032500803	0.043194912
Ccl6	1.062450134	2.21E-15
Best1	1.122859573	2.95E-19
AC114233.2	1.155491158	0.000835101
Clec7a	1.32473551	0.008066028
Galnt6	1.378685647	0.048527004
Rgs1	1.53458128	0.000188097
Ccl9	1.538458813	0.001425581
Cd300lf	1.63285205	0.006715566
AABR07035776.1	2.429965954	0.009822404
NEWGENE_1559832	2.757288091	0.017320599
LOC103690033	2.96662939	1.65E-13
LOC103694902	3.895838546	0.018012428
LOC100912008	4.427878115	1.26E-12
LOC100911130	4.437340547	3.73E-14
novel.634	5.322566754	0.001098164

Red for Stiff vs Soft up-regulated DEGs;

Blue for Stiff vs Soft down-regulated DEGs.

Table S2 DNA replication

Gene Name	Rank Metric Score	Gene Name	Rank Metric Score
Rnaseh1	0.28946048	*Mcm2*	-0.33164
AABR07039146.2	0.257102817	*Ssbp1*	-0.33955
LOC100362333	0.235597253	*Pola1*	-0.34929
Rnaseh2b	0.222285256	*Rpa1*	-0.37392
AABR07070270.1	0.170595244	*Pole2*	-0.38236
Pold2	0.162055925	*Prim1*	-0.41009
Rfc5	0.008920078	*Fen1*	-0.42113
AABR07014311.1	0	*Rpa3*	-0.42563
AABR07052692.1	0	*Mcm7*	-0.43429
AABR07006258.1	0	*Prim2*	-0.44846
AABR07027368.1	0	*Rfc4*	-0.46405
Pcna	-0.013343581	*Mcm4*	-0.47241
Rnaseh2c	-0.061464112	*Rfc3*	-0.47874
Rfc1	-0.085286587	*Mcm6*	-0.53207
Pold4	-0.092934944	*Pole3*	-0.54966
Pole	-0.09530098	*AC129365.1*	-0.63789
Pold1	-0.10595005	*Pola2*	-0.64164
AC095390.1	-0.216156185	*Rfc2*	-0.67453
Lig1	-0.219104752	*Mcm5*	-0.7305
Rpa2	-0.226019651	*Mcm3*	-0.78455
Pold3	-0.239844456	*LOC100911727*	-0.84869
Pole4	-0.251704216	*AABR07069490.1*	-0.88945
Rnaseh2a	-0.255467921		

White for Stiff vs Soft no change genes;

Blue for Stiff vs Soft down-regulated genes;

Red for Stiff vs Soft up-regulated genes.

Table S3 Primer sequence

Gene	Forward 5'-3'	Revise 5'-3'
Cdk1	*CAGGACTCCAGGCTGTATCTCAT*	*CTTATCGGTATTCCAAACGCTCT*
Cdc20	*GGCCACGGATGATATGTAGTCCC*	*CAGAAGGCTACCAGAACAGATTGAA*
Bub1	*TCTGATAGCGAAAGTAATGACGA*	*AGAATGGCAGCATATTAGTAGGG*
Kif11	*GGTTCGTTCGTGGTTACAAAGTC*	*AAATGGGTCACATCAGGTTCAGT*
Cdca8	*AGGACGAGTCGGCTGAGACGCTA*	*TCCCTGTTCTGTTCTGTTGTTATTTC*
Mhy9	*AGGAGTTCCGGCAGAGGTAT*	*CCTCCAGGTGAGCTAGGACT*
Ras	*TGGCACTCAAGGCTGTATGG*	*AGGCTTGAAAGTGGCTCGTT*