Mechanosensitive Kinases Regulate Stiffness-Induced Cardiomyocyte Maturation

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Supplemental Information

Supplemental Figures

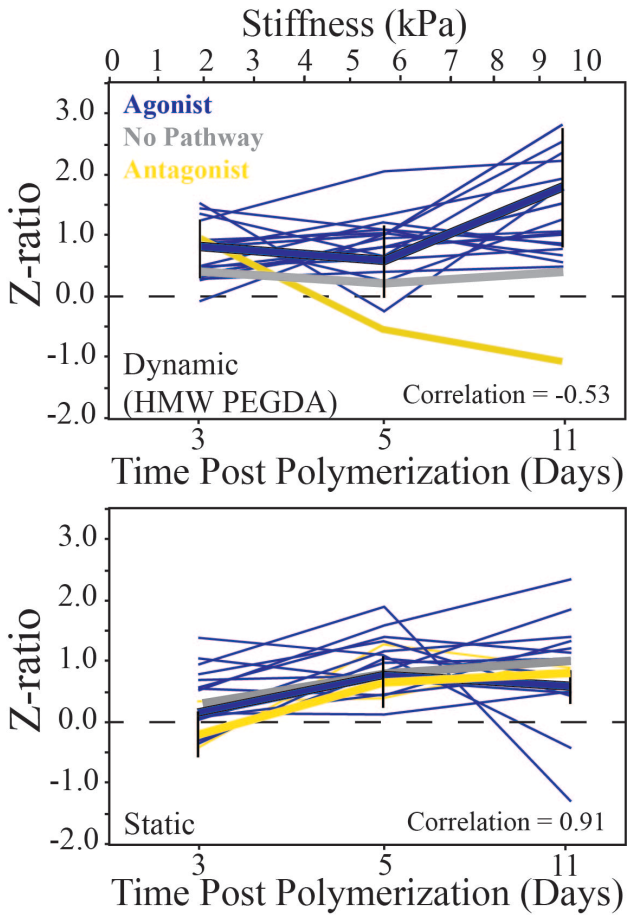

Figure S1: *Agonist and Antagonist Protein Expression Track with Time and Stiffness* Agonist (blue) and antagonist (yellow) expression as a function of time and stiffness on dynamic HA hydrogels (top) and static 11kPa PA hydrogels (bottom). The gray line is an average of all proteins z-ratios not identified as agonists or antagonists. Correlation coefficient is calculated for agonist vs. antagonist, with a negative correlation on dynamic hydrogels (-0.53) and a positive correlation on static hydrogels (0.91).

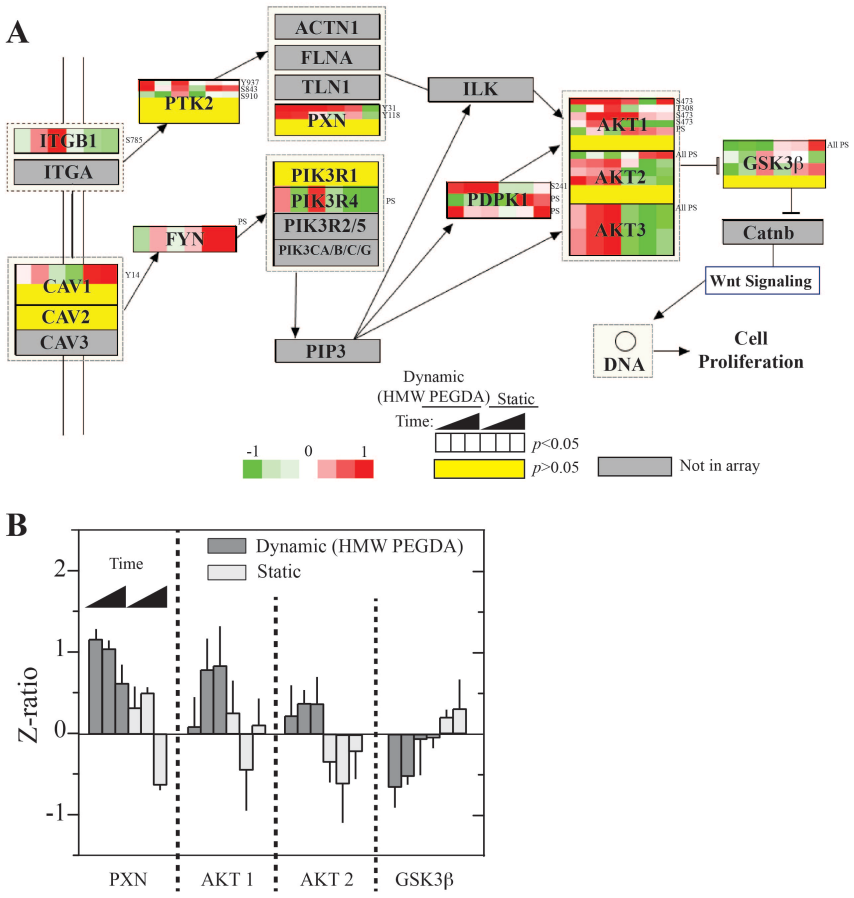

Figure S2: *Focal Adhesion Signaling is Substrate Stiffness-Dependent* (A) A focused view of Figure 4 depicting specific pathways of the focal adhesion signaling WikiPathway, identified by GO-ELITE analysis of microarray data, is shown. For genes with interaction $p < 0.05$, protein boxes were divided into 6 smaller boxes indicating z-ratios from 3, 5 and 11 days in culture compared to cells initially cultured for 1 day (left to right) for dynamic HMW PEGDA/HA (left three boxes) and static 11kPa PA (right three boxes). Rows within each box indicate different antibodies used for each target. For proteins with interaction $p > 0.05$, boxes were shaded yellow. For proteins not included in the array, the protein boxes are shaded grey. The color map indicates down-regulation (-1, green), no change with respect to the global average (0, white) and upregulation (+1, red). Phosphorylated site is denoted for each antibody. (B) Average expression and/or phosphorylation from multiple antibodies displayed (A) for paxillin (PXN), AKT1, AKT2 and GSK3β on dynamic HMW PEGDA/HA (dark grey) and static 11kPa PA (light grey). Note that averages include all antibodies for a protein, irrespective

of significance between materials or time.

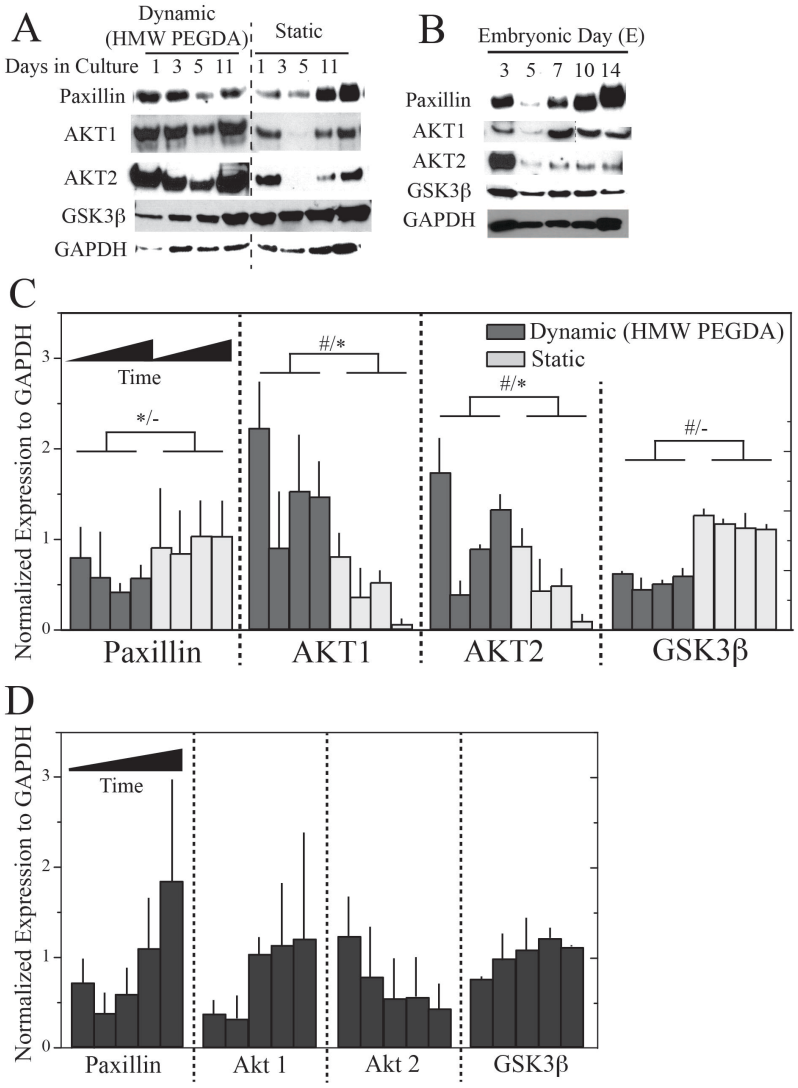

Figure S3: *Microarray Validation for Select Focal Adhesion and Signaling Proteins* (A) Representative western blots for paxillin, AKT 1, AKT 2, GSK3β and GAPDH for dynamic HA and static 11kPA PA hydrogels over 1, 3, 5, 11 days after plating. (B) Representative western blots for paxillin (PXN), AKT 1, AKT 2, GSK3β and GAPDH for chicken lysates at 72, 120, 168, 240, 312 and 336 HPF. (C) Western blot data quantified for dynamic HMW PEGDA/HA (dark grey) and static 11kPa PA (light grey) hydrogels with the same time course as indicated in panel A, which increases to the right as indicated. Significance was determined via 2-way ANOVA as indicated by material *p*-value/time *p*-value. *$p<0.05$, $^{\#}p<10^{-3}$. '–' indicates no significance. (D) Western blot data

quantified for chicken lysates with the same time course as indicated in panel B, which increases to the right as indicated. All western blots were performed in triplicate.

Supplemental Tables

Supplemental Table 1: *Protein Kinases Microarray Indicate Cardiac Agonist and Antagonists*

The data in this table corresponds to the cluster image shown in Figure 3 of the 199 unique proteins that were found to have statistically significant changes in expression or phosphorylation over time and between hydrogels types based on 2-way ANOVA analysis of their z-ratios. Note that only data with a z-ratio greater than 1 or less than -1, i.e. proteins with significant expression or phosphorylation changes, are displayed here; this eliminated 56 unique measures that had statistically significant changes where z-ratios were greater than 1 or less than -1 (these data are used in Figure 4 for GO mapping). All z-ratios have been computed relative to 72 HPF cells cultured for 1 day on a matrix identical to the condition being tested. The boundaries denoted by the thick black lines correspond to the white lines in Figure 3. Agonists are highlighted in blue and antagonists in yellow. Antibodies recognizing phosphorylation sites are specifically noted with the site and type of phosphorylation in the name column.

	Z-ratio vs. day 1					
	HA			11 kPa PA		
Name	D3	D5	D11	D3	D5	D11
RIPK1	3.118561	1.41393	3.460529	1.302378	1.178256	-0.056596
Paxillin 1:Y31	1.41654	1.268163	1.087366	0.842122	0.635712	-0.754733
PKBa (Akt1): S473	0.264489	0.965528	1.06965	1.111093	0.264182	0.1588
PKBa (Akt1): S473	1.229188	2.038357	2.217376	0.661501	-1.260495	0.894316
Acetylated Lysine	1.44282	1.304733	1.770655	1.116746	-0.506789	-0.031211
Hsp70	1.541007	1.037319	1.107019	1.150081	-0.562886	-0.493094
PAK1/2/3	0.776104	1.06329	1.04998	1.172034	-1.184353	-0.623856
CaMK4	0.779123	1.324707	0.95634	0.931652	-1.019233	-0.319275
CASP5	0.598107	1.028487	0.994088	0.390919	-0.728146	-0.041321
CaMK4	0.495511	1.22562	0.834609	0.411212	-0.706381	-0.243526
CaMKK(2)	0.943849	0.248911	1.263717	-0.984113	0.527364	0.127558
CAMK2d	1.523365	-0.229242	1.811499	-0.72495	-0.347124	0.260349
BRD2	1.136803	0.054021	1.203891	-0.452536	-0.653066	-0.326928
Erk4	1.107361	0.055422	1.394372	-0.630889	-0.118246	-0.334087

PAK1/2/3: (S144/141/154)	0.948263	0.369127	1.066551	-0.690516	-0.095569	-0.345316
Plk3	0.672003	-0.134462	1.005197	-0.083173	-0.03673	-0.608942
FKHRL1: T32	1.455873	-0.416982	0.932408	-0.063484	0.080064	-0.251109
Jun: S73	0.842453	0.095988	1.675553	-0.063109	-0.22174	0.298127
DRAK2	0.459267	0.794971	1.376504	-0.274393	0.076223	-0.41308
PDK1: S241	0.803476	1.024977	2.820525	-0.219629	-0.229376	0.072734
CASK/Lin2	1.022953	2.045698	0.802445	-0.505226	-1.15088	0.438138
CASP4	0.295283	1.221252	0.786617	-0.601213	-0.465674	0.522222
TTK	1.321602	0.471881	0.869861	-0.57012	-1.146624	0.306811
Hsp90a/b	1.085824	0.564965	0.41566	-0.255948	-0.798461	0.078978
MAPKAPK2a+b: T334	0.48936	0.587282	1.482418	0.193866	-0.157672	-1.362849
MLC(MLRC2): S19	0.665943	0.120591	0.966125	0.626417	-0.280281	-1.162283
DNAPK	0.104506	-0.319912	1.019119	-0.272631	-0.630259	-0.405866
PI3KR4	0.48408	-0.59191	0.84015	-0.354578	-0.830465	-1.030824
LOK	0.457298	-0.241554	0.909654	-0.0935	-0.809914	-1.021077
PSD-95	0.578636	-0.102043	0.673613	-0.234534	-0.58851	-1.04871
Src	0.725509	-0.368572	0.678135	0.117391	-1.029744	-1.218583
RSK2	0.362122	-0.423058	0.574849	-0.365098	-0.894501	-1.420319
Aurora A (AIK)	0.303025	0.054139	1.08098	-0.294044	-1.32332	-0.609501
Hsp90a/b	1.565056	0.720446	1.484229	-0.579676	-1.711245	-1.567809
CAMK2g	1.364879	0.650535	2.377233	-1.607255	-1.652867	-1.193594
Axl	0.810166	0.65317	1.048305	-0.954094	-0.670458	-0.814457
LATS1	0.537874	0.068121	1.316567	-0.925545	-1.317764	-1.450028
STK33	0.295381	0.277942	0.707861	-0.505754	-1.148913	-0.95651
CDK10	0.516296	0.140963	0.40281	-0.91955	-1.204346	-1.085317
PKBg (Akt3)	0.452398	0.811281	1.052166	-0.596686	-0.954137	-0.432973
PKBg (Akt3)	0.313189	0.815993	1.018104	-0.526271	-1.053984	-0.648539
PKA R2a: S99	0.372615	0.891271	1.262279	-0.284347	-1.469272	-1.207878
TAK1	0.318886	0.964627	1.149757	-0.023813	-0.648555	-0.518719
PKCb2	0.37785	0.805038	1.53164	-0.210172	-0.973237	-0.87325
PKBb (Akt2)	0.485591	0.593907	0.779685	0.137288	-1.237538	-0.425113
PKCa: S657	0.032825	0.7158	0.36242	-0.741567	-1.308193	-1.092303
Striatin	0.399507	0.992305	0.291171	-0.636113	-1.12192	-1.080082
SLK	0.516777	0.238874	0.459154	-0.168609	-1.14005	-1.108244
p38a MAPK: (T180+Y182)	0.575381	0.733064	0.238724	-0.237214	-1.398244	-1.46398
Smac/DIABLO	0.587405	-0.038214	0.010419	-0.157447	-0.944491	-1.053084

PACSIN1	0.514819	0.300631	0.106391	-0.601623	-0.727706	-1.495214
PKBb (Akt2)	0.276853	0.391034	0.491596	-1.018768	-1.401298	-0.554514
CDC2L5 (CHED)	0.281433	0.403158	0.368731	-1.085769	-0.963701	-0.439058
PARP1	0.990711	0.570212	0.938939	-0.978055	-0.268624	-1.21526
p53: S392	1.006419	1.736152	1.023852	-1.248786	-0.200012	-1.100283
DAPK1	0.491915	0.665172	0.506557	-1.140837	-0.202872	-0.791534
Hsp70	1.185353	0.794187	1.056422	-0.111991	-0.442507	-1.282293
Tau: S519	1.297463	0.657086	0.3599	-0.331313	-0.419152	-1.114111
p38a MAPK	0.857238	1.100944	0.877293	-0.221055	-0.027737	-0.553582
p38a MAPK: (T180+Y182)	0.919623	1.0269	0.672235	-0.114266	-0.451576	-0.613434
p38a MAPK	1.446649	1.104376	0.557517	-0.594791	0.011412	-0.718936
Histone H3: S11	1.117656	0.788326	0.682093	0.370793	-0.249005	-0.709561
DNAPK	1.728067	0.597896	0.024918	-0.976747	-0.300776	-1.194556
Cyclin D1	1.512635	0.608261	-0.408179	-0.798171	0.119052	-1.00741
Tau: S717	1.016748	0.511381	0.29606	0.279633	-0.965381	-0.871309
STAT5A: S780	0.744842	1.117934	0.160584	0.836651	-0.731099	-0.953199
p38a MAPK	0.448033	0.689392	0.101709	0.489351	-1.113249	-1.142935
p38a MAPK	-0.080375	1.008698	0.409135	0.387138	-0.123883	-0.847004
NFkappaB p65: S276	0.207677	-0.243458	1.13438	0.706522	-0.840447	-0.414968
Tau: T522	0.320528	0.054567	0.62102	0.843564	-1.034836	-0.819794
S6Kb1: T252	-0.138331	0.297189	0.472441	0.3297	-1.232425	-0.882128
Crystallin aB: S19	-0.677603	-0.186794	0.47217	-0.089992	-1.506035	-1.612321
MEKK4 (MAP3K4)	-0.034323	-0.446795	0.144932	0.971659	-1.564642	-0.732007
MEK5 (MAP2K5)	-0.171479	-0.428684	0.140558	0.258188	-1.524946	-0.785424
MEKK1 (MAP3K1)	-0.196334	-0.557563	-0.080015	0.330578	-1.457612	-0.572964
B23 (NPM): T199	-0.745112	-0.862645	1.004867	1.024493	-0.671971	0.364066
CK1g2	0.93041	0.155272	-0.116395	-0.760403	-1.019973	-0.459042
HspBP1	1.444564	1.41566	-1.327759	-1.361431	-1.661153	-1.431718
p73	0.17493	-0.482951	-1.669515	0.021272	-1.838897	-1.257993
DFF45 + DFF35	-0.632077	0.190245	-0.659683	-1.860874	-0.921992	-0.788212
Tau: S516+S519	1.159635	1.36147	-0.588396	0.567417	-0.486393	-0.324366
p38a MAPK: T180+Y182	0.433488	1.117919	-1.347307	1.760173	-0.982249	0.833823
ROKa (ROCK2)	1.972204	-0.989057	-1.407477	1.413432	-1.307096	1.301044
4E-BP1	1.025276	0.004168	0.471921	1.499449	0.272316	-0.396939
PDK1	1.218788	0.187133	0.238844	-0.081323	0.074075	0.936769
Erk5: T218+Y220	1.367647	-0.781281	0.63823	1.316673	1.402561	1.261759

Ksr1	0.842898	0.840624	0.550829	-0.037351	1.044453	0.390595
Lck	0.757186	0.444438	0.876175	-0.247151	1.716076	0.192929
MEK6 (MAP2K6)	1.614992	-0.151231	0.849289	-0.100568	1.364647	0.21265
PKR1: T451	0.364637	0.033185	-0.979673	-0.369043	1.992409	-0.42783
MKP1	-0.836143	-0.133765	-0.146683	0.035132	-1.06497	1.232768
ZAP70	-0.061521	-0.203611	-0.609243	0.042182	-0.18419	1.393055
APG2	0.150294	-0.472691	-0.299352	0.116513	0.237445	1.321845
Bid	-0.671042	0.532931	0.12821	0.192042	0.510321	1.993602
Cdc25C: S216	0.258885	0.646374	0.22615	0.101978	0.556267	2.556052
Hsc70	-0.057588	0.368266	-0.082892	0.080845	0.336476	1.214685
PITSLRE	-0.146578	1.225894	0.417843	0.378211	1.087375	1.93692
Hsp60	0.210503	0.976806	0.224738	0.485401	0.330767	1.349873
PKCl/i: T564	0.260779	0.502538	-0.88319	-0.608357	0.650599	1.787113
Caveolin 1: Y14	0.061818	0.449019	-0.226282	-0.559367	0.901969	1.739942
CASP7	0.444569	0.5958	0.008601	-0.352635	0.21982	1.172298
IRAK1	0.073512	1.081855	0.885188	-0.622458	0.52171	1.573068
CASP9	-0.009595	0.408844	0.365617	-0.561613	-0.070947	1.21751
CDK1 (CDC2)	0.043758	1.29544	0.234885	-0.034385	0.260234	0.672023
CDK6	-0.434431	-0.863763	-0.718763	0.352504	1.744787	2.415047
Lck	-0.524246	0.116391	-1.183648	0.45455	2.305449	1.799592
CAMK1a	-0.151746	0.037172	-0.043639	0.310736	1.487269	2.336935
AIF	-0.387123	-0.011213	-0.685952	0.164083	1.489236	1.98385
ACK1	-0.125669	0.018257	-0.623166	0.486812	1.519447	1.914611
Mnk2	-0.078268	0.156158	-0.300941	0.392307	0.953456	1.30486
MAPKAPK2	-0.519881	0.341587	-0.530013	0.591909	1.900478	1.880025
Fyn	-0.326917	0.333247	-0.10909	0.227629	1.481577	1.191292
MEK5 (MAP2K5)	-0.207637	0.127849	-0.905763	0.97597	1.220942	1.371272
CASP1	-1.006798	-0.390332	-0.625822	1.006348	1.560494	1.588134
CDK1 (CDC2)	0.059832	0.08199	0.396044	-0.144604	1.225236	1.751633
YSK1	-0.40377	-0.138005	0.337413	-0.255201	1.02004	1.462446
MEK3 (MAP2K3)	0.420992	-0.298495	-0.006241	0.243264	1.201852	1.845549
Nlk	0.375408	0.245514	-0.008121	0.066984	0.741243	1.336288
CDK1 (CDC2)	-1.216324	-0.097755	0.176882	0.146405	1.447098	2.697508
CaMK1d	-0.74525	-0.024432	0.106416	0.501158	0.583972	1.089181
PKCd	-0.876049	-0.311605	0.422427	0.346629	0.219945	1.210451
JNK1/2/3	-1.452539	-0.141892	0.215458	0.35837	1.29291	1.006271

KHS	-1.194769	0.072718	-0.0909	-0.602463	0.602155	1.69426
CDK5	-0.81786	-4.92E-04	-0.148184	-0.877076	1.007051	1.318459
PAK2	-0.552638	0.353208	-0.221834	-0.404794	0.972517	1.082372
Cyclin B1	-1.487178	-0.360937	-0.85401	-0.280898	1.645455	2.230759
Ksr1	-0.98207	0.265598	-0.546646	-0.289714	1.000431	1.750282
CDK2	-1.499435	0.079835	-0.774513	0.44826	2.357549	1.964587
PKC h: T655	-1.186869	0.232441	-0.906284	-0.119242	1.020722	1.278831
Plk2	-1.089996	0.318755	-0.57791	0.113046	1.143469	1.212019
DDIT3(CHOP)	-1.356082	0.25179	-0.52449	0.10793	1.085071	1.116675
BMX (Etk): Y40	-1.245008	0.448983	-0.514794	-0.104603	1.370643	0.908806
MEK7 (MAP2K7)	-1.302749	-0.125739	-1.479917	0.507675	1.199299	1.550629
PAC1	-1.075421	-0.218818	-0.851956	0.076474	1.121872	1.046202
GCK	-1.082652	-0.406333	-1.134822	-0.017798	1.201981	0.911047
ErbB2 (HER2)	-1.391078	-0.106752	-1.732703	0.076242	1.227747	0.648536
Tyk2	-1.153299	0.159261	-1.284704	-0.680616	0.897699	1.250663
CDK1 (CDC2)	-0.520045	0.404827	-0.968281	-0.196117	1.012198	0.857373
Chk1	-0.861002	0.023662	-0.551867	0.0076	0.201694	1.198285
TBK1	-0.747377	-0.050391	-0.765219	-0.622961	0.068058	1.006084
Src	-0.350373	0.896066	-0.017745	-0.284935	1.49196	1.052773
CDK2	-4.233369	1.74415	-1.701654	-0.519198	1.307565	-0.838613
ATF2	-0.364917	-0.18171	0.033055	2.040451	-0.217375	1.721937
Arrestin b1	-0.378173	-2.953097	-2.546699	2.098366	-0.117612	1.608445
MST1	-2.527446	-2.072399	-1.14156	2.662114	0.063739	2.006949
Aurora A (AIK)	-1.854017	-1.7871	-1.055906	0.874698	0.203757	1.981832
MST1	-0.838552	-0.484228	-0.207971	0.491802	0.098773	1.060639
Mnk1: T250+T255	-1.678045	-1.264944	0.054993	2.506058	-0.441717	1.1486
MKP2	-2.485517	-1.802326	-1.20783	2.305037	-2.088473	0.438617
MEK4 (MAP2K4): S257+T261	-0.590291	-0.659351	-0.22697	1.016706	-0.698462	0.226291
MEK6 (MAP2K6):S207	-0.761505	-0.631656	-0.520762	2.03918	-0.966093	0.319338
MEK3b (MAP2K3)	-0.728759	-0.485143	-0.707002	2.757088	2.325353	1.804679
Pyk2: Y579	-1.45977	-1.813892	-0.387773	2.059047	1.397269	1.686875
NMDAR2B:Y1474	-0.821751	-1.189606	-0.55451	1.630476	0.572204	0.648914
Pyk2: Y579	-1.951041	-1.474617	-1.238349	3.531604	2.230718	0.988416
ILK1	-0.543595	-0.989285	0.789326	1.169073	2.010636	0.619987
MST3	-0.810771	-0.856942	0.691949	1.536015	1.186381	0.526412
Smad2/3	-3.503268	-2.395816	-1.954583	-0.71122	0.036338	1.613225

VEGFR2 (KDR):Y1214	-1.679586	-1.751728	-0.87986	0.244951	-0.039532	0.179672
ZIPK	-1.022111	-0.740629	-1.61761	-0.918463	0.822728	1.216546
FasL	-1.867587	-1.541067	-1.609083	-0.951751	1.395385	0.917357
STAT6	-1.575446	-1.300546	-1.597811	-0.547319	0.682964	0.363246
PKCm (PKD)	-1.370068	-0.250952	-1.239103	-0.224799	0.806516	0.900827
CK1d	-1.857824	-0.459427	-0.942899	-0.593865	1.023619	0.658055
PDK1	-1.832064	-0.346846	-1.264894	-0.657584	0.893746	0.611151
PKCb2	-1.154032	-0.115537	-0.622879	-0.197305	0.224934	0.623178
Mcl1	-1.319301	-0.218202	-0.971265	-0.61945	1.424814	0.425025
Abl	-1.399569	-0.321737	-2.307207	0.127413	0.670179	1.309879
NFkappaB p65	-1.057321	-0.885754	-2.051806	0.320045	0.761837	1.006974
STAT5B	-2.272437	-0.722978	-2.952246	0.421225	0.985004	1.278741
STAT3	-2.535036	-0.999121	-2.773464	0.175419	1.415979	1.269999
STAT5A	-1.83704	-0.832961	-1.868501	-0.235185	1.076075	0.634091
STAT2	-2.419463	-0.901293	-3.996701	-0.269609	0.902753	0.895227
STAT4	-1.653014	-0.653023	-2.589441	-0.137029	0.628782	0.794242
Fos	-2.132183	-1.270737	-3.076954	-0.569609	0.602799	0.753295
NFkappaB p50	-1.646434	-0.781109	-1.624122	-0.227187	0.317804	0.732452
GSK3a+b	-1.100833	-0.763927	-1.083164	0.140016	0.193573	0.82517
Nek2	-0.662121	-0.68675	-1.107335	0.051373	0.082896	0.672394
Trail	-2.74235	-1.86363	-3.648386	0.008781	0.172514	0.664456
PAK1	-1.871039	-1.185871	-2.549705	0.032676	-0.065148	0.296416
PKCb1	-1.303128	-0.730947	-1.3375	-0.09781	-0.134037	0.277638
Catenin b1	-1.793364	-1.119445	-2.007365	-0.268435	-0.111805	0.217634
STAT1a+b	-1.729214	-1.294235	-1.765104	-0.119931	0.141772	0.152858
PTEN	-0.987824	-0.644651	-1.215845	-0.285324	-0.232666	0.006768
IkBa	-2.230398	-0.713355	-2.113096	-0.103292	-0.473585	0.115489
PP2A/Ca+Cb	-1.892122	-1.419836	-1.482832	-1.508824	0.598088	-0.322736
PKCm (PKD): S738+S742	-2.131156	-1.222308	-1.830782	0.887954	0.491858	0.251773
PTP1D: S580	-0.975557	-0.971189	-0.988516	1.150545	0.246225	-0.240427
PTP1C	-3.009337	-2.472423	-2.104736	2.531459	1.814942	0.060138
Rac1: S71	-1.37083	-1.245224	-1.129863	1.258873	0.941889	-0.744528
PTP1B	-2.080339	-1.793426	-1.887101	0.031305	1.107104	-0.101787
Tyrosine Hydroxylase: S18	-1.256523	-1.111868	-0.687271	-0.132243	0.391533	-0.169687
Rb	-2.824816	-2.666907	-1.904764	1.054273	1.202195	-0.625483
Rac1	-2.590589	-2.059583	-2.651311	0.780016	1.835245	-1.720305

Raf1: S259	-1.496093	-1.064544	-0.876762	0.717639	0.300768	-1.026208
IkBb	-0.72104	0.312734	-1.713653	0.716927	0.958355	0.410461
PKCq	0.173988	-0.74191	-1.57076	0.611903	1.208336	0.203944
Erk1 + Erk2: T185+Y187	-1.166351	-0.878663	0.636741	-0.39176	0.854204	0.44973
Rb: T356	-0.466729	-0.064906	0.422701	0.514812	1.0059	-0.262417

Supplemental Videos

Supplementary Video 1: *Calcium Imaging for Cells on Dynamic and Static Hydrogels After 1 and 11 Days in Culture.*
Representative cells undergoing calcium imaging shown in four culture conditions of 1) 72 HPF cells plated for 1 day on dynamic HMW HA hydrogels followed by 2) identical cells on static 11 kPa PA hydrogels, 3) 72 HPF cells plated for 11 days on dynamic HMW HA hydrogels, and 4) 72 HPF cells plated for 11 days on static 11 kPa PA hydrogels. Numbering indicated on video.

Supplementary Video 2: *Calcium Imaging for Cells on Dynamic and Static Hydrogels After 11 Days of Chronic Exposure to AKT or GSK3β Inhibitors.*
Representative cells undergoing calcium imaging shown in four culture conditions of 72 HPF cells plated for 11 days on dynamic HMW HA hydrogels that had been treated with 1) MK-2206 or 3) CHIR-99021. The latter two cells were 72 HPF cells plated for 11 days on static 11 kPa PA hydrogels that had been chronically treated with 2) MK-2206 or 4) CHIR-99021. Numbering indicates on video.