Geiger et al., http://www.jcb.org/cgi/content/full/jcb.200901105/DC1

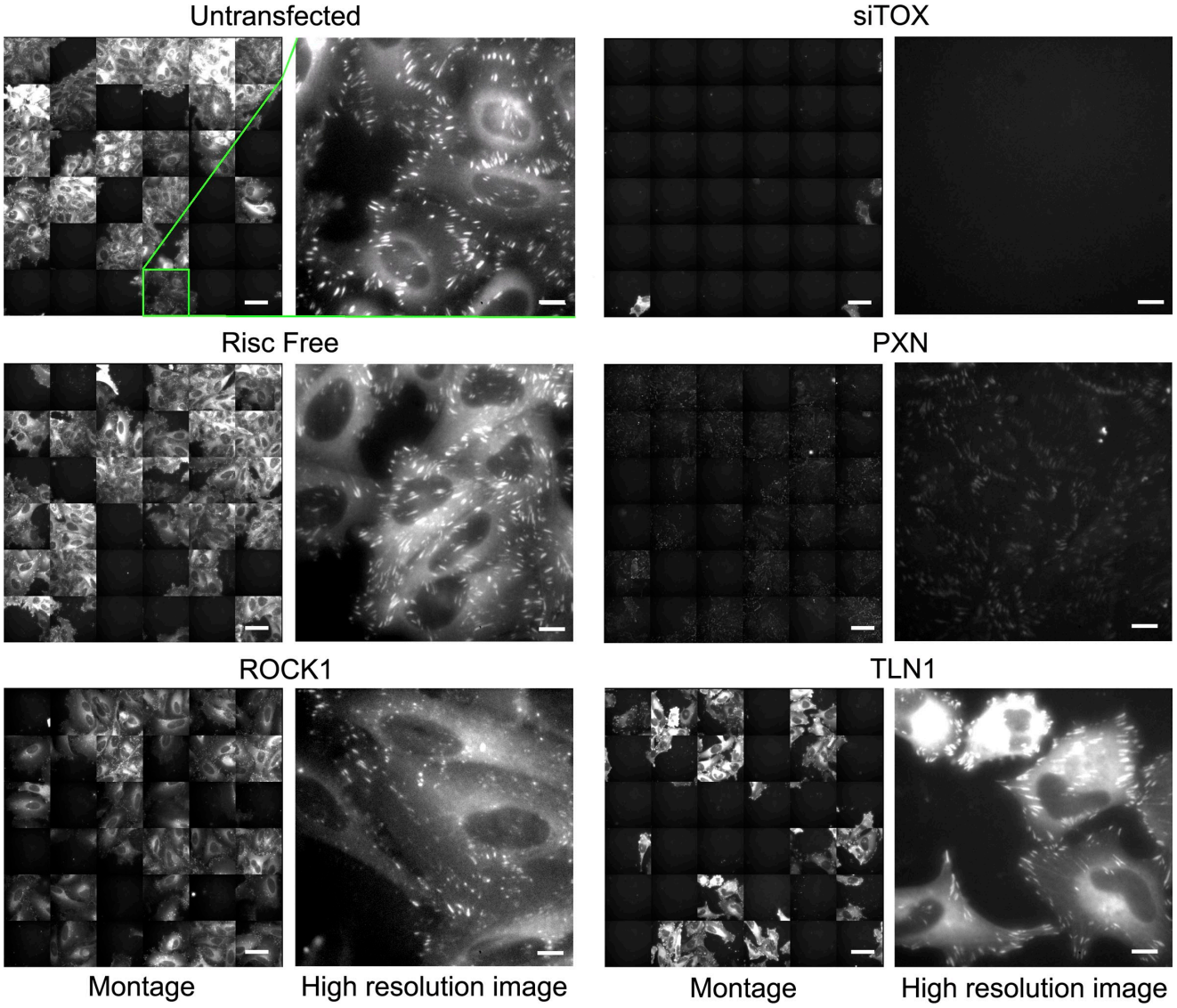


Figure S1. **Images from control wells and selected adhesion-related siRNA wells.** Montages of 6 × 6 images from untransfected HeLa-YFP-paxillin–expressing cells, or cells transfected with the indicated siRNA: Risc-free (control), ROCK1, siTOX (transfection control leading to cell death), PXN, and TLN1. For each montage, one single high-resolution image is also shown. Bars: (montages) 50 µm; (high-resolution images) 10 µm.

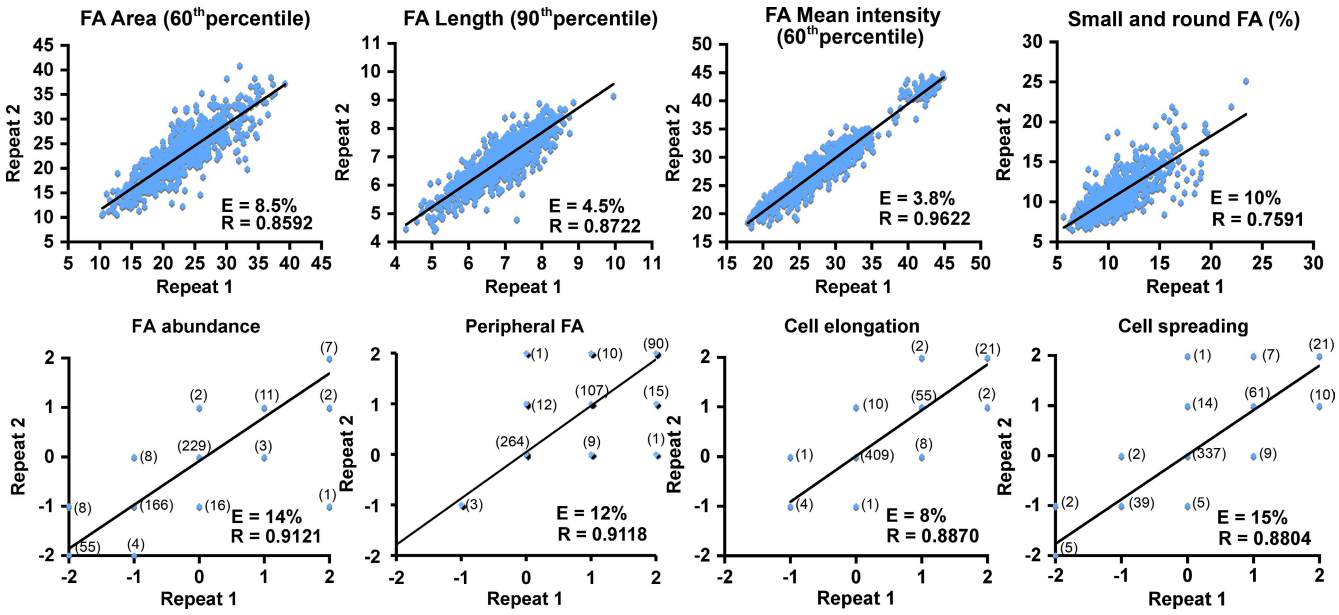


Figure S2. **Reproducibility of measured features.** Each point in the top panel represents a single siRNA, and the number of data points represented by each dot in the bottom panel is indicated. The correlation (R), as well as mean relative error (E) of each feature (difference between measurements divided by the mean), is shown.

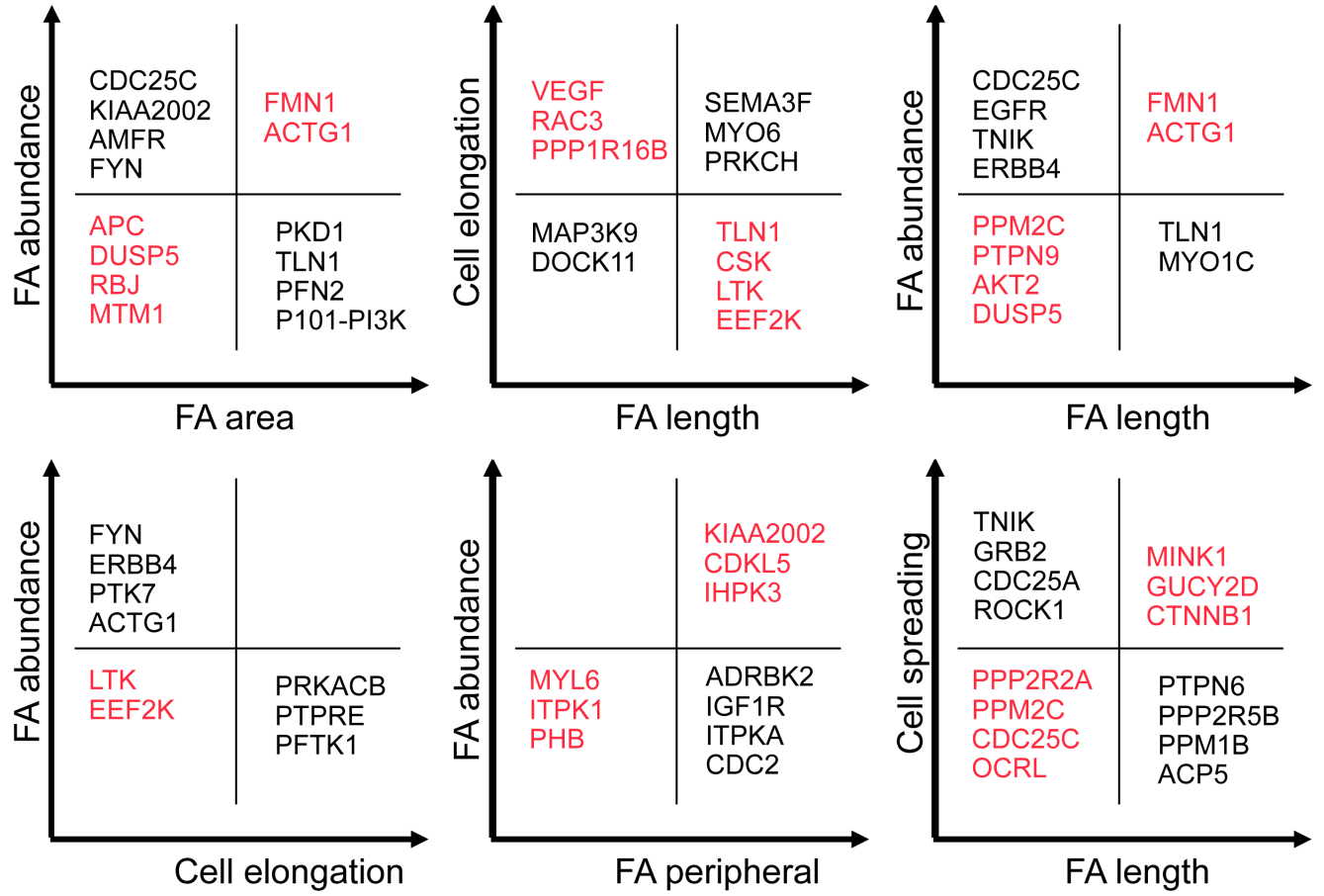


Figure S3. **Examples of siRNAs that produce changes in correlated features, and siRNAs that break the correlations.** Schematic graphs of each of the significant correlations are shown. Four quadrants are designated in each graph, indicating low or high scores for each feature. The siRNAs that produce phenotypes changing the correlated features in a concerted manner are shown in black. The siRNAs that break the correlations are shown in red. Images of all the siRNA phenotypes are available at http://www.cellmigration.org/resource/discovery/geiger/geiger2009_rnai.cgi.

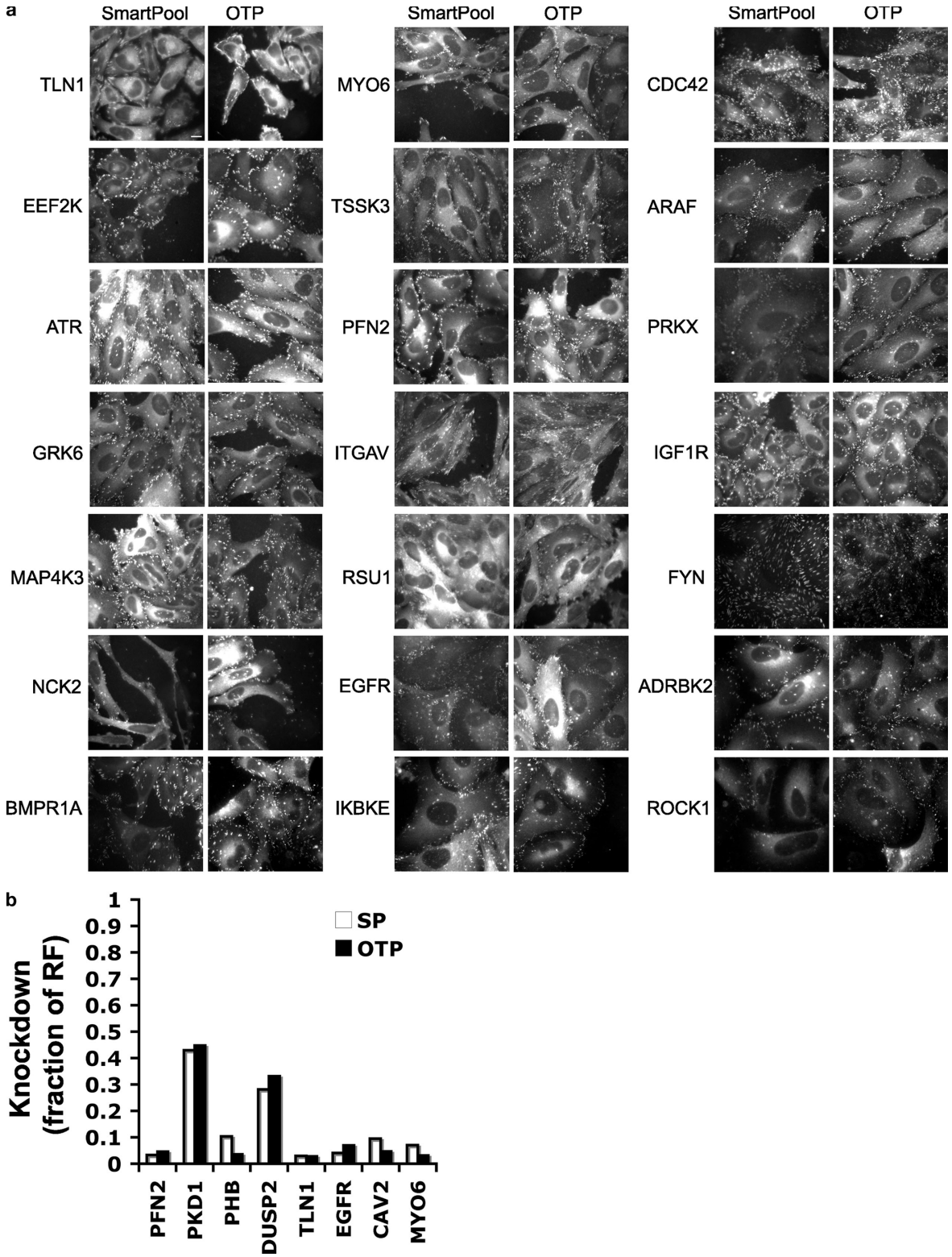
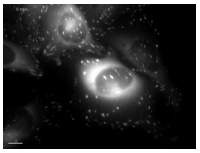
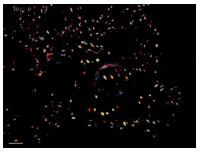


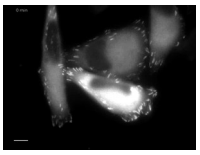
Figure S4. **Representative images of the validated siRNA hits and knockdown measurements.** (a) Sample images of 21 of 44 validated hits, showing the SMARTpool siRNA phenotype compared with the OTP siRNA phenotype. (b) Graph showing the knockdown measurements (the fraction of expression compared with RF) of eight siRNAs (SMARTpools [SP] and OTP). The RNA amount was normalized to hypoxanthine-guanine phosphoribosyltransferase and to the amount in the RF. Quantitative PCR was performed with the following primers: TLN1 forward, 5′-TCTCCCAAAATGCCAAGAAC-3′; TLN1 reverse, 5′-CTCCACTAGCCCTTGCTGTC-3′; EGFR forward, 5′-AGGCACGAGTAACAAGCTCAC-3′; EGFR reverse, 5′-CAATGAGGACATAACCAGCCAC-3′; MYO6 forward, 5′-AGAAGGAGGAGGAATCCCAA-3′; MYO6 reverse, 5′-ATCACTGATGAGCTCGGCTT-3′; PFN2 forward, 5′-GGCAGAGCTGGTAGAGCATT-3′; PFN2 reverse, 5′-AGGTAGATGGGGAGAGGCTG-3′; CAV2 forward, 5′-CTCAACTCGCATCTCAAGGA-3′; CAV2 reverse, 5′-CGTCCTACGCTCGTACACAA-3′; PKD1 forward, 5′-CGTGGTTGTCTATCCCGTCT-3′; PKD1 reverse, 5′-CTGTCCAACAAAGGCCTCA-3′; PHB forward, 5′-CTCCCTACCAAAAATTGCCA-3′; PHB reverse, 5′-CACGTGGATCTAGGCAGACA-3′; DUSP2 forward, 5′-AGCTGCAGTCACTCGTCAGA-3′; and DUSP2 reverse, 5′-ATCTGGTTGTCCTCCACAGG-3′. Bar, 10 μm (applies to all images).



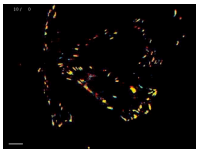
Video 1. **HeLa cells expressing YFP-paxillin and transfected with RF siRNA.** Images were collected every 2 min for 3 h, and the display rate is 10 frames/s. Cropped images from this video are shown in Fig. 7. Bar, 10 μm.



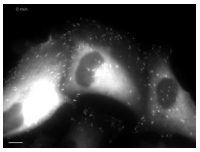
Video 2. **Temporal ratio video, derived from Video 1.** For each siRNA, the ratio between time points, 10 min apart (as displayed), is presented on a color scale, such that "new pixels" are blue, pixels that disappeared are red, and unchanged pixels are represented by variable colors, depending on the specific local intensity ratio. Yellow indicates identical intensities at the two time points. A cropped image from this video is shown in Fig. 7. The display rate is 10 frames/s. Bar, 10 μm.



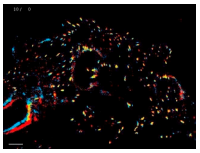
Video 3. **HeLa cells expressing YFP-paxillin and transfected with TLN1 siRNA.** Images were collected every 2 min for 3 h, and the display rate is 10 frames/s. Cropped images from this video are shown in Fig. 7. Bar, 10 μm.



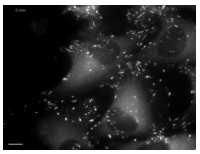
Video 4. **Temporal ratio video derived from Video 3.** For each siRNA, the ratio between time points, 10 min apart (as displayed), is presented on a color scale, such that "new pixels" are blue, pixels that disappeared are red, and unchanged pixels are represented by variable colors, depending on the specific local intensity ratio. Yellow indicates identical intensities at the two time points. A cropped image from this video is shown in Fig. 7. The display rate is 10 frames/s. Bar, 10 μm.



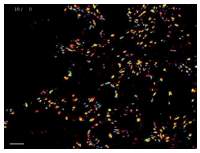
Video 5. **HeLa cells expressing YFP-paxillin and transfected with EGFR siRNA.** Images were collected every 2 min for 3 h, and the display rate is 10 frames/s. Cropped images from this video are shown in Fig. 7. Bar, 10 μm.



Video 6. **Temporal ratio video derived from Video 5.** For each siRNA, the ratio between time points, 10 min apart (as displayed), is presented on a color scale, such that "new pixels" are blue, pixels that disappeared are red, and unchanged pixels are represented by variable colors, depending on the specific local intensity ratio. Yellow indicates identical intensities at the two time points. A cropped image from this video is shown in Fig. 7. The display rate is 10 frames/s. Bar, 10 μm.



Video 7. **HeLa cells expressing YFP-paxillin and transfected with CAV2 siRNA.** Images were collected every 2 min for 3 h, and the display rate is 10 frames/s. Cropped images from this video are shown in Fig. 7. Bar, 10 μm.



Video 8. **Temporal ratio video derived from Video 7.** For each siRNA, the ratio between time points, 10 min apart (as displayed), is presented on a color scale, such that "new pixels" are blue, pixels that disappeared are red, and unchanged pixels are represented by variable colors, depending on the specific local intensity ratio. Yellow indicates identical intensities at the two time points. A cropped image from this video is shown in Fig. 7. The display rate is 10 frames/s. Bar, 10 μm.

Table S1. **Biological features enriched in the low-coverage cluster**

Set	Enriched set	Enriched value	P-value	Set hits	Set size	Set hits	Total hits	Total size	Total hits
						%			%
Low coverage	Protein kinase activity	1	0.000348	25	39	64.1	395	1080	36.58
Low coverage	Phosphorylation	1	0.000381	25	39	64.1	397	1080	36.76
Low coverage	NFκB activation by nontypeable *Hemophilus influenzae*	1	0.0004	4	39	10.3	11	1080	1.02
Low coverage	ATP binding	1	0.000572	25	39	64.1	406	1080	37.6
Low coverage	Kinase activity	1	0.00163	25	39	64.1	431	1080	39.91
Low coverage	Nucleotide binding	1	0.0024	25	39	64.1	441	1080	40.84
Low coverage	Protein serine/threonine kinase activity	1	0.00301	19	39	48.7	295	1080	27.32
Low coverage	Cell cycle: G2/M checkpoint	1	0.00315	3	39	7.7	9	1080	0.84
Low coverage	Protein modification	1	0.01	25	39	64.1	484	1080	44.82
Low coverage	Biopolymer metabolism	1	0.02	25	39	64.1	500	1080	46.3
Low coverage	Cellular protein metabolism	1	0.04	25	39	64.1	526	1080	48.71
Low coverage	M phase	1	0.04	4	39	10.3	37	1080	3.43

Output table from Genomica Software. Each row denotes a significant overlap between the members of the "Low Coverage" cluster, and the gene set that harbors the biological feature. Columns indicate the enriched set names (the biological feature), the p-value of the enrichment, the number of genes in the overlap, the percentage of genes in the overlap, the total number of genes in the gene set, the total number of genes in the dataset, and the total percentage of genes that belong to the gene set. In general, the greater the difference between the percentage of genes in the overlap (Set hits), and the total percentage of genes in the dataset (Total hits), the lower and more significant the p-value. NFκB, nuclear factor κB.