SUPPLEMENTARY TEXTS

Text S1

Data schemas

This section and **Text S3** describe the provided data and computer programs in sufficient detail to reproduce the results of the paper and to make use of the data and programs in the development and evaluation of new methods. The data are stored in four relational tables:

- supplement_Object: one row for each of the 454,793 cells. The primary key (TableNumber, ImageNumber, ObjectNumber) identifies a cell. The pair of columns (TableNumber, ImageNumber) is a foreign key into the supplement_Image table. The remaining 476 columns are the 453 measurements as well as 23 measurements that were not used for profiling (e.g., horizontal and vertical position of the cell in the field of view).
- supplement_Image: one row for each of the 2,528 fields of view. The primary key (TableNumber, ImageNumber) identifies an image. The pair of columns (Image_Metadata_Plate_DAPI, Image_Metadata_Well_DAPI) indicates the well the image came from. The column Image_Metadata_Compound contains the name of the compound the sample was treated with; it is a foreign key into the supplement_Compound table. The column Image_Metadata_Concentration is the concentration of the compound treatment in M. Together, the pair of columns (Image_Metadata_Compound, Image_Metadata_Concentration) is a foreign key into the supplement_GroundTruth table.
- supplement_GroundTruth: one row for each of the 104 compound-concentrations in our ground-truth set. In addition to the primary key (compound, concentration), there is a column moa that contains the mechanism of action of the compound. For the mock treatment DMSO, the moa column contains “DMSO”.
- supplement_Compound: one row for each of the 39 compounds in our ground-truth set. In addition to the primary key compound, there is a column smiles that contains the chemical structure of the compound. For four of the compounds proprietary to AstraZeneca, structures are not available. No structure is provided for the mock treatment DMSO.

We provide the data as tab-delimited text files, together with SQL statements and scripts for importing them into a MySQL database. Although our scripts expect to read the data from a MySQL database, the text files can also be used directly. The ZIP file that contains the text files is 754 MB. Unpacked, the text files are 2.1 GB. Imported into the database, the tables are 835 MB.

The script load_supplement_tables.sh produces a sequence of SQL statements to create the necessary tables and import the data from the text files. It takes as argument the directory that contains the text files. Run the script, then send its output to MySQL. If you prefer, you can create the tables using the .sql files in the zip file, then import the data by issuing LOAD DATA INFILE statements manually to MySQL.

Text S2

Implementations

The profiling scripts are written in Python as part of CellProfiler Analyst (CPA), and depend on the following freely-available python modules:

- numpy 1.5.1
- CellProfiler Analyst (http://github.com/CellProfiler/CellProfiler-Analyst/) rev. e33def16b2
- scipy 0.7.1
- progressbar 2.3
- sklearn (scikits.learn) 0.12
- MySQL-python 1.2.3
- MDP toolkit 3.3

The visualization scripts additionally require matplotlib 1.2. Unless otherwise noted, all dependencies are available through the Python Package Index (PyPi, http://pypi.python.org/pypi/).

Data access is directed by the CellProfiler Analyst properties file supplement.properties. You should modify this file to contain the hostname, etc., for your database server.

All script can be run with the -h option to see a comprehensive list of options and arguments.

Cache: Before you can run any profiling scripts, you must make a binary cache of the per-cell data using cpa.profiling.cache, as follows:

```
python -m cpa.profiling.cache \
  supplement.properties /path/to/cache \
  "Image_Metadata_Compound = 'DMSO'"
```

The arguments are the CPA properties file, the directory in which to store the cache, and a SQL predicate that identifies the images of mock-treated samples, which will be used as reference for normalization (see Online Methods).

Subsampling: The GM profiling method and the preprocessors construct their models using a subsample of the cells. The script subsample creates such a subsample. It takes four positional arguments (CPA properties file, cache directory, subsample output file, and the number of cells to include in the subsample). The -f option can be used to specify a CPA filter to select the cells to sample from.

Preprocessors: The scripts cpa.profiling.factor_analysis, cpa.profiling.pca, and cpa.profiling.fasel construct models that the profiling scripts can use to preprocess the image features. All three scripts take the same positional arguments:

- the filename of a subsample,
- model size (the number of factors or principal components), and
- an output file in which to store the model.

Profiling scripts: The profiling scripts are:

- cpa.profiling.profile_mean
- cpa.profiling.profile_ksstatistic
- cpa.profiling.profile_svmnormalvector
- cpa.profiling.profile_gmm
- cpa.profiling.profile_factoranalysis_mean

All take the following positional arguments:

- CPA properties file
- Cache directory
- CPA group defining sets of images to be profiled together

In addition, all take the following options:

- `--multiprocessing` (run on multiple CPUs or CPU cores)
- `--lsf-directory` *TEMPORARY-DIRECTORY* (run in parallel on a computing cluster using LSF)
- `-o` *OUTPUT-FILENAME* (file to store the profiles in)
- `-f` *FILTER-NAME* (CPA filter indicating which images to profile)

As an example, the following command profiles each well (sample) of the experiment using the "means" method:

```
python -m cpa.profiling.profile_mean \
  -o my_mean_profiles.txt -f noncontrols \
  supplement.properties /path/to/cache Well
```

The profile_ksstatistic and profile_svmnormalvector scripts expect an additional positional argument: the name of a CPA filter that defines the images that are of mock-treated samples, to serve as reference distribution for the KS statistic and negative training samples for the SVM. The profile_svmnormalvector script accepts the additional option `--rfe`, which indicates that recursive feature elimination should be used. The profile_gmm script accepts the additional option `--components` *NCOMPONENTS*, which specifies the number of mixture components. The profile_factoranalysis_mean script accepts the additional options `--factors` *NFACTORS*, which specifies the number of factors, and `--save-model` *FILENAME*, which indicates that the model should be saved to file.

Post-processing scripts: The script cpa.profiling.median_profiles groups a set of profiles according to a CPA filter given as an argument and computes the median profile in each group. The script expects three positional arguments: the properties file, the filename of the profiles to read (generated by one of the profiling scripts), and the name of the CPA filter to group by. The option `-o` *OUTPUT-FILENAME* causes the median profiles to be written to the specified file. As an example, the following command computes profiles for each compound-concentration as the median of the replicate samples:

```
python -m cpa.profiling.median_profiles \
  -o my_mean_profiles_per_treatment.txt \
  supplement.properties /path/to/cache \
  CompoundConcentration
```

The script cpa.profiling.leave_one_out performs nearest-neighbor classification with leave-one-out crossvalidation. It expects three positional arguments: the properties file, the path to the cache, and the name of a CPA filter that specifies the true class of each image. The option `-h` *HOLDOUT-GROUP* specifies a CPA group that defines which profiles to hold out when classifying a profile. As an example, the following command classifies per-treatment profiles, holding out all profiles treated with the same compound as the one being classified, even if those other profiles were treated with a different concentration, and prints the confusion matrix in sparse form to standard output:

```
python -m cpa.profiling.leave_one_out \
  -H Compound supplement.properties \
  my_mean_profiles_per_treatment.txt MOA
```

The scripts plot_profiles and plot_distances provide rudimentary visualization of profiles and the distances between them. To plot per-treatment profiles, group and label them by MOA, and output a PDF file:

```
python -m cpa.profiling.plot_profiles \
  -o profiles.pdf \
  supplement.properties \
  mean_profiles_per_treatment.txt MOA
```

To plot the distance matrix of the profiles, grouped and labeled by MOA, and output a PNG file:

```
python -m cpa.profiling.plot_distances \
  -o distance_matrix.png \
  supplement.properties \
  mean_profiles_per_treatment.txt MOA
```

Text S3

Computer programs

The file `reproduce.zip` contains four directories: `inputs` contains source data for Figure S 9; `src` contains the source code, `properties` contains the CPA properties file, and `outputs` contains the generated data files.

The GNU makefile `src/Makefile` encodes every step to recreate the results, so typing `make` in the `src` directory will recreate all the output files.

The scripts require CellProfiler Analyst (which include the implementations of the profiling method). In addition, most scripts require matplotlib 1.1.0, and calculate_aucs.py requires xalglib 3.4.0 (http://www.alglib.net/).

The scripts in the src directory are as follows:

- mean_confusion_fa.py computes the mean confusion of the 20 factor-analysis models.
- mean_confusion_gmm.py computes the mean confusion of the 20 Gaussian mixture models.
- pretty-confusion-matrix.py renders a confusion matrix as a PDF file.
- gmm_varycomponents_plot.py plots Figure S 5).
- fa_varyfactors_plot.py plots Figure 2.
- loo_confusion.py processes the data from Loo et al.[15] for use in Figure S 9.
- make_features_table.py generates Table S 1.
- make_ground_truth_table.py generates Table S 2.
- make_compounds_table.py generates Table S 3.
- make_aucs_pvalues_table.py generate Table S 10.
- calculate_aucs.py calculates AUCs and p-values for Table S 10.
- misclassified.py lists compound-concentrations that were misclassified.

Text S4

Classification bias from batch and plate effects

The samples were prepared and imaged in 10 batches. If the compounds with one mechanism of action were entirely in a different batch than other mechanisms, there would be a concern that they may be discernible due to differences between batches rather than biological differences[20]. In this experiment, however, all MOAs except two are split between two or more batches (Table S 9). The exceptions are cholesterol-lowering drugs and kinase inhibitors, which are represented only in one batch each, but because other mechanisms are also represented in these batches, there is no reason to believe that the classification is biased by batch effects.

Text S5

Previous comparisons by classification accuracy

Loo et al.[15] evaluated their method, which includes the SVM normal-vector method at its core, applying four label sets to a compound set different from ours. Based on the nearest-neighbor search results in their Supplementary Data 2, we calculated classification accuracies for their experiments, and found them to be in the range of 32–51%, depending on the marker set (Figure S 9). If the best marker set is chosen for each mechanistic class, the accuracy increases to 68%.

Kümmel et al.[26] assessed several dimensionality-reduction methods and well-summary approaches in a screening context: the methods were evaluated by their ability to distinguish cells treated with each of five compounds from mock-treated cells. Although the study did not measure ability to distinguish compounds from each other, it is interesting to note that the KS statistic was found to perform similarly to a simple median method. Factor analysis led to a decrease in accuracy, although they only measured 29 features of each cell, so compared to our experiment it is more likely for useful variation to be restricted to a single measurement, and therefore to be discarded as noise.

Text S6

Comparison by p-value of AUC

The papers that presented the KS, SVM, and GMM methods include comparisons to previous methods. Here, we describe the method of comparison and explain why we compare the methods using a different method. For the sake of completeness, we summarize the previously published comparison results and apply the same method to our dataset, using our implementations of the core parts of the profiling methods.

Method of comparison: In all three papers the performance of a profiling method is quantified in a screening context, using the distances between compounds as follows. If the profiling method worked as desired, the distances between compounds with the same MOA should be smaller than the distances between compounds with different MOA. Therefore, two sets of distances are calculated: pair-wise distance between members of a mechanistic group (the intra-set distance) and pair-wise distances between members of different mechanistic groups (the inter-set distances). To test whether the former are significantly larger than the latter, a Mann–Whitney U test is performed (Ott, R.L., Longecker, L. (2001) *An Introduction to Statistical Methods and Data Analysis*, 5th ed., ISBN 978-0-534-25122-2).

The Mann–Whitney U test assumes that the data are independent and drawn from distributions with identical shape. (The distributions can differ in position.) This assumption is problematic because it is unlikely that the intra-set and inter-set distance will have distributions of similar shape: the intra-set distances most likely form a narrow distribution, whereas the inter-set distances will be more dispersed because some mechanisms are more different than others. Say, for instance, that of the twelve MOAs, ten are very distinct and two are very similar according to the calculated profiles. The p-values for the two similar MOAs calculated using the Mann-Whitney U test are still very small, as the medians of the intra-set and inter-set distances are so different. It is, however, impossible to definitively classify a sample in either one of the two similar MOAs.

We chose instead to compare the methods by classification accuracy because that measure is more relevant for profiling experiments, which aim to distinguish multiple phenotypes. If two MOAs appear identical based on their profiles, their prediction accuracies will be around 50%.

Summary of previous comparison results: Perlman et al.[14] compare the Translation Invariant Similarity Score (TISS) method, which includes the KS method as its core, to the means method. The comparison takes only the total-intensity measurements into account, not the other measurements. The p-values are listed for nine mechanisms of action. The KS method has markedly lower p-values for five of the nine MOAs, and the mean method has markedly lower p-values for three of the nine MOAs.

Loo et al.[15] and Slack et al.[18] compare the SVM and GMM methods, respectively, to TISS. The SVM method using each of four markers sets separately is compared to TISS using all markers sets simultaneously. The SVM method appears to work better overall based on the p-values, but the combining of the marker sets for TISS makes a conclusive comparison impossible. The GMM method is compared to TISS using the same images but different features sets. This makes it difficult to tell if a change in p-value is caused by a different method or a different feature set. The GMM method has significantly lower p-values than TISS for three out of nine MOA; the inverse is true for one MOA. The remaining five MOAs have comparable p-values.

Comparison of core methods by p-value: We computed areas under the ROC for each of the five profiling methods (Table S 10). For factor analysis, the p-values are 0.0001 or below for every MOA. The other four methods yield slightly higher p-values for compounds that cause protein degradation. The means method yields higher p-values for actin disruptors. The GM method yields higher p-values for actin disruptors and cholesterol-lowering compounds.

Text S7

Most influential variables

In order to measure of how influential each variable is in distinguish each MOA from the rest (Table S 5), we first computed per-MOA profiles as element-wise medians of the profiles of the wells treated with compounds labeled as having that MOA. We then rank the variables, for each MOA profile, by the element-wise minimum distance to any other MOA profile.

Text S8

Number of factors

Why is 50 factors optimal in our experiment when Young et al.[19] use 6 factors? First of all, the number 6, which was suggested by the Kaiser criterion, is a somewhat arbitrary cutoff: it is simply the threshold where an additional factor would contain less information than an average feature. It is entirely possible that a few more factors would have led to more discriminative profiles, but in Young et al.'s paper, interpretability of the factors is important, so it makes sense to choose a relatively low number of factors.

Even if Young et al. were to choose their number of factors based on performance in an analytic task (such as classification), they could not have found more than 36 factors because they only measured 36 features. In constrast we measure 453 features. Although there are unquestionably redundancies in our feature set, it seems reasonable to assume that the rich feature set would allow us to detect additional effects on cells—effects that are too subtle/complex to be interpretable, but which nevertheless help in classification. It may also matter that we have an actin stain and a tubulin stain, and that we therefore are able to measure cellular morphology. (Young et al. only measured nuclear features.) A number of factors around 50 is consistent with our experience with training boosting classifiers for particular phenotypes in a screening context, where classifiers with 20 rules work well but adding up to about 50 rules helps[5].

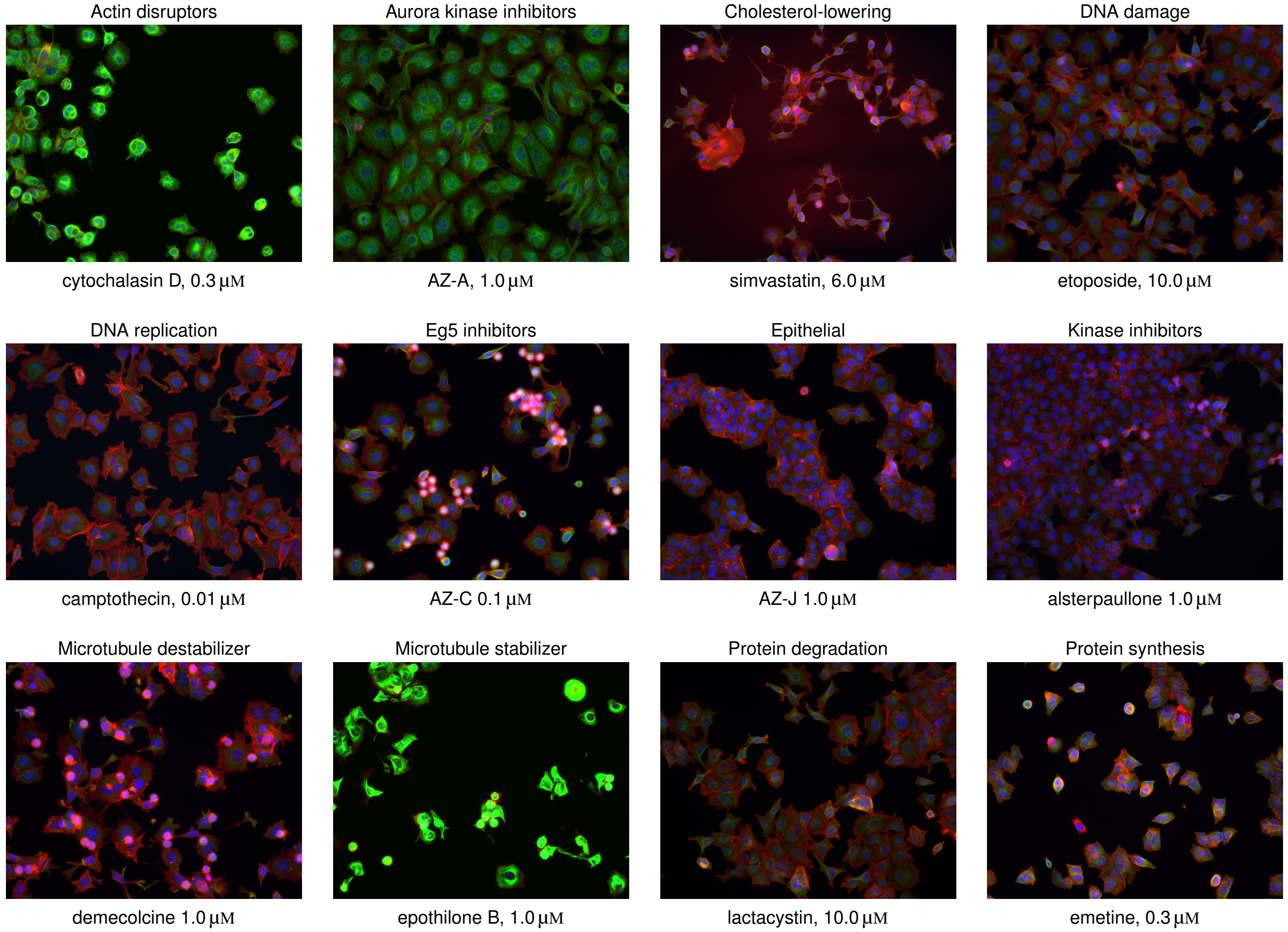
SUPPLEMENTARY DATA

Data S1: `pipelines.zip` – CellProfiler pipelines for processing the images. There are two pipelines, one that computes illumination-correction functions per plate, and one that applies the illumination correction functions to images and analyzes them. The pipelines are provided in both human-readable and machine-readable format.

Data S2: `database.zip` – Measurements and metadata in CSV format, as well as a script for loading them into a MySQL database, as described (**Text S1**).

Data S3: `reproduce.zip` – Input data and source code for regenerating the figures, tables, and results (**Text S3**).

SUPPLEMENTARY FIGURES



Actin disruptors
Aurora kinase inhibitors
Cholesterol-lowering
DNA damage

cytochalasin D, 0.3 μM
AZ-A, 1.0 μM
simvastatin, 6.0 μM
etoposide, 10.0 μM

DNA replication
Eg5 inhibitors
Epithelial
Kinase inhibitors

camptothecin, 0.01 μM
AZ-C 0.1 μM
AZ-J 1.0 μM
alsterpaullone 1.0 μM

Microtubule destabilizer
Microtubule stabilizer
Protein degradation
Protein synthesis

demecolcine 1.0 μM
epothilone B, 1.0 μM
lactacystin, 10.0 μM
emetine, 0.3 μM

Figure S1: Images of one compound-concentration in each of the 12 MOAs spanned by our ground-truth set. Red color shows F-actin, green shows β-tubulin, and blue shows DNA.

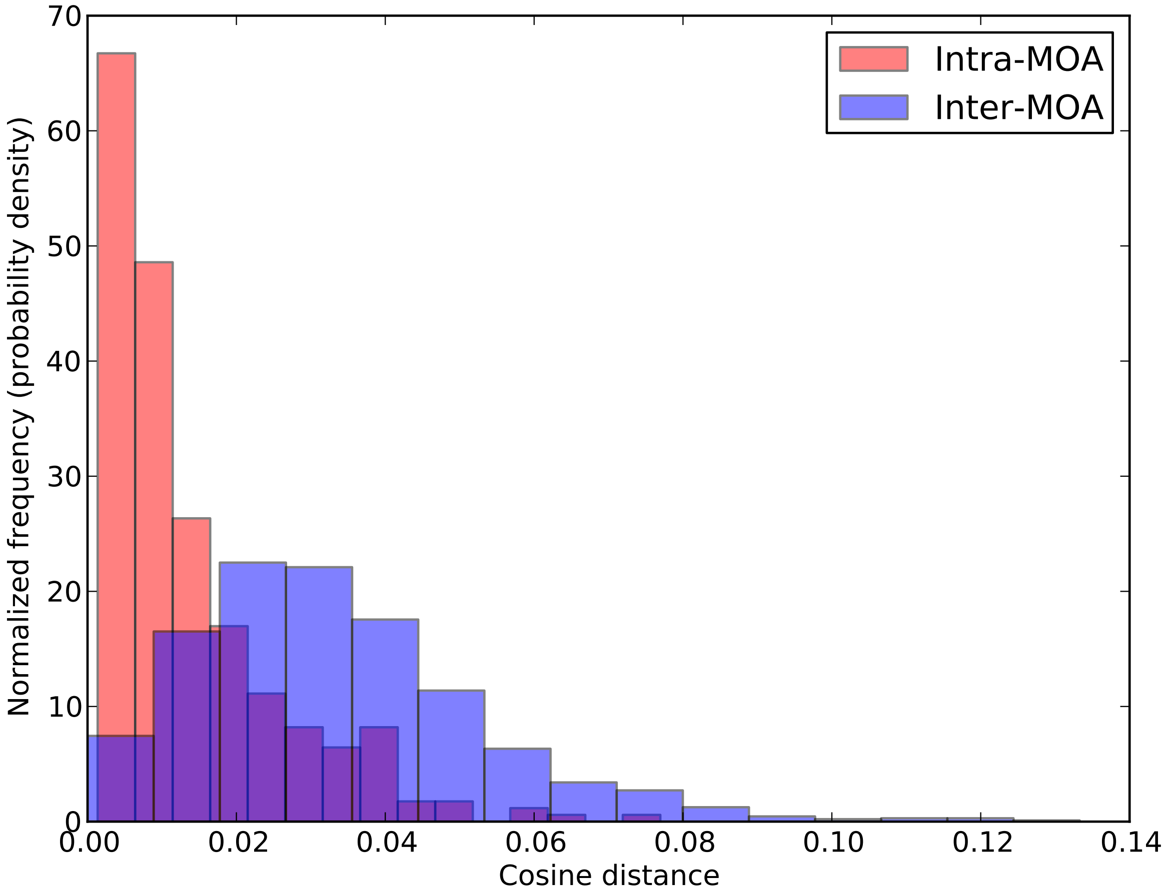
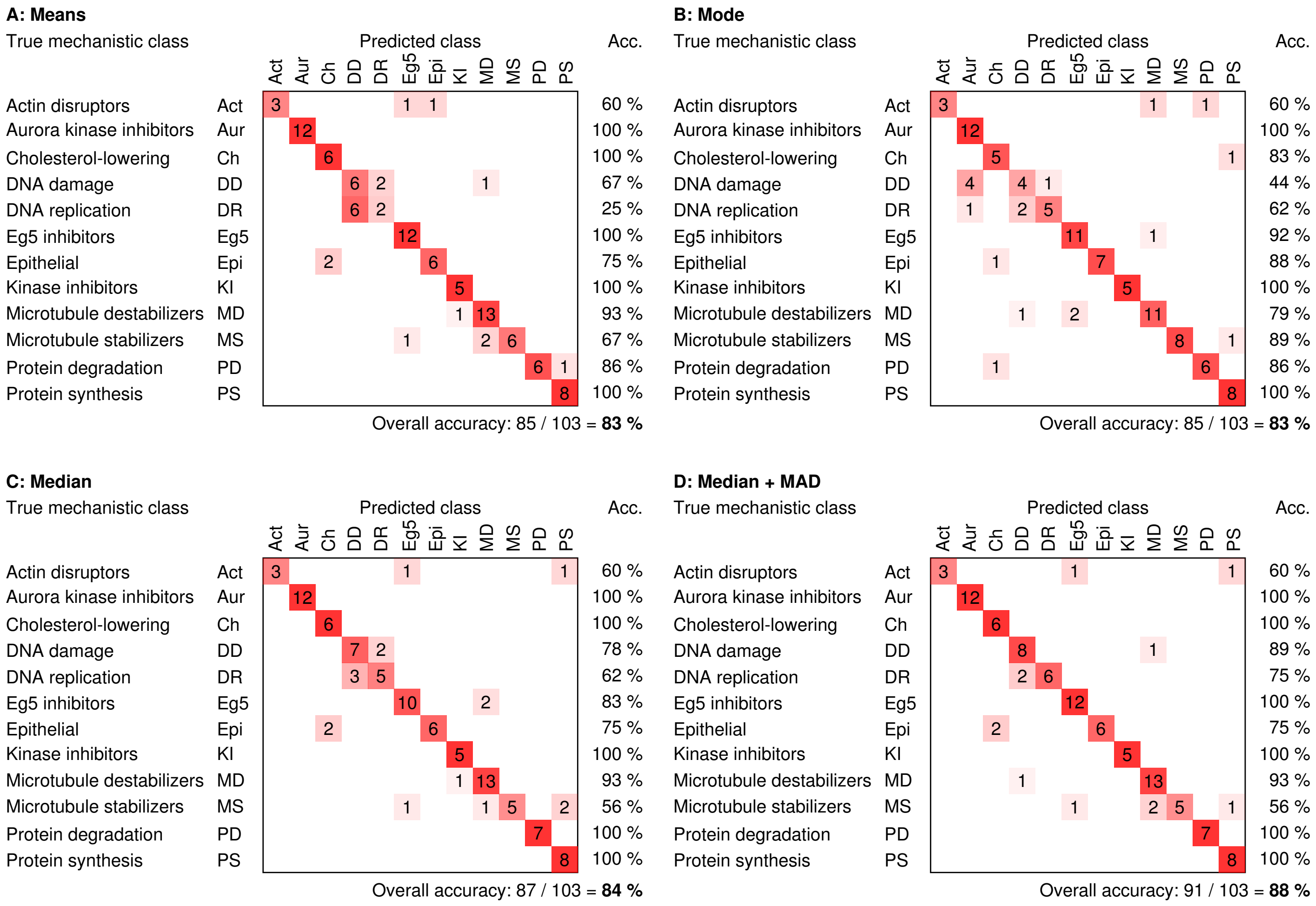


Figure S2: Histograms of distances between compound-concentrations that have the same MOA and between compound-concentrations that have different MOA. These distances are between profiles produced by the "means" method; the other methods produced histograms that were not dramatically different.



A: Means

True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	3					1	1						60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				6	2				1				67 %
DNA replication	DR				6	2								25 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						75 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	13				93 %
Microtubule stabilizers	MS						1			2	6			67 %
Protein degradation	PD											6	1	86 %
Protein synthesis	PS												8	100 %

Overall accuracy: 85 / 103 = **83 %**

B: Mode

True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	3								1		1		60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			5									1	83 %
DNA damage	DD		4		4	1								44 %
DNA replication	DR		1		2	5								62 %
Eg5 inhibitors	Eg5						11			1				92 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1		2			11				79 %
Microtubule stabilizers	MS										8		1	89 %
Protein degradation	PD			1								6		86 %
Protein synthesis	PS												8	100 %

Overall accuracy: 85 / 103 = **83 %**

C: Median

True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	3					1						1	60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				7	2								78 %
DNA replication	DR				3	5								62 %
Eg5 inhibitors	Eg5						10			2				83 %
Epithelial	Epi			2				6						75 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	13				93 %
Microtubule stabilizers	MS						1			1	5		2	56 %
Protein degradation	PD											7		100 %
Protein synthesis	PS												8	100 %

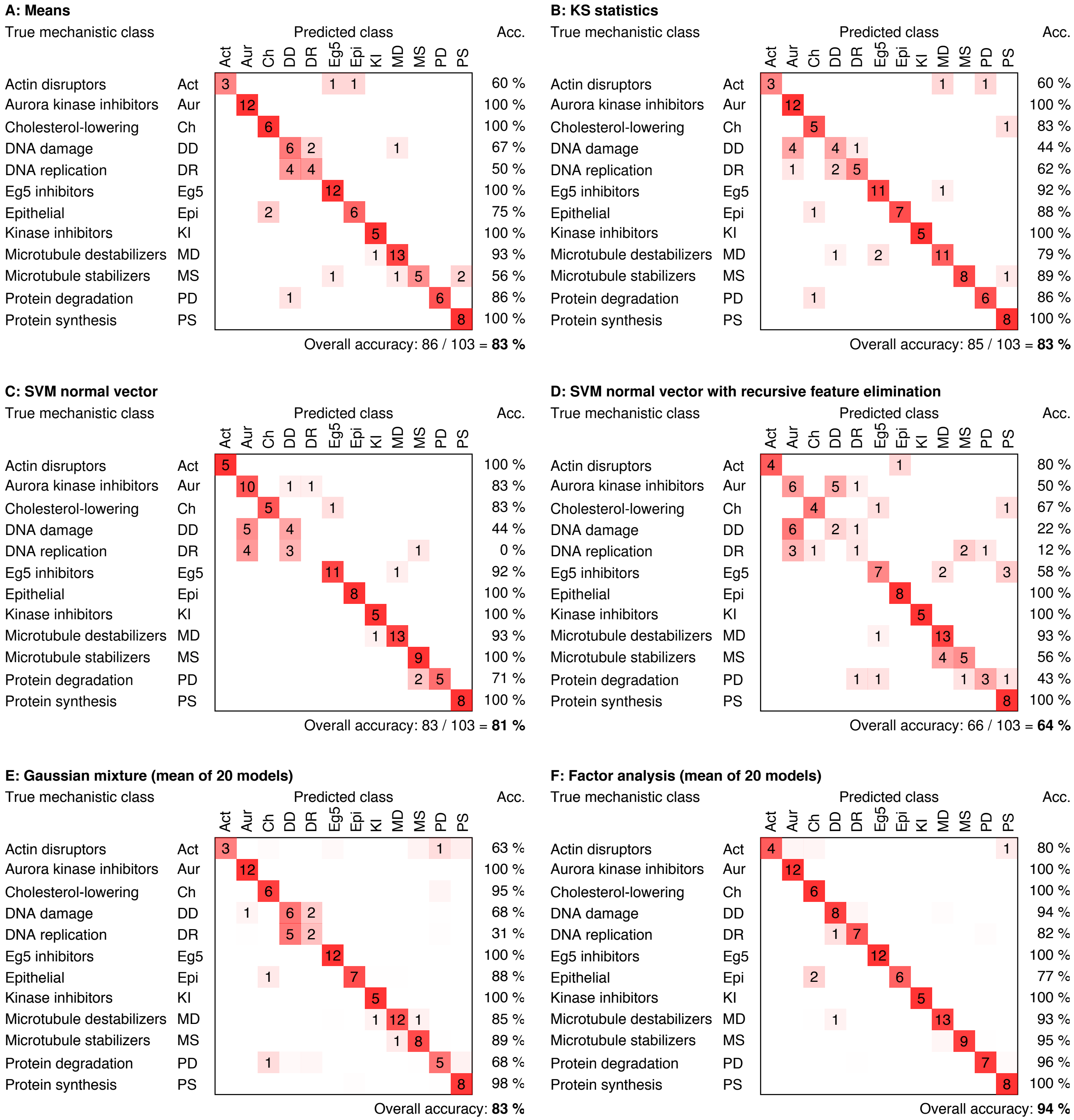
Overall accuracy: 87 / 103 = **84 %**

D: Median + MAD

True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	3					1						1	60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8					1				89 %
DNA replication	DR				2	6								75 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						75 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS						1			2	5		1	56 %
Protein degradation	PD											7		100 %
Protein synthesis	PS												8	100 %

Overall accuracy: 91 / 103 = **88 %**

Figure S3: Confusion matrices for other population statistics.



A: Means

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3					1	1						60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				6	2				1				67 %
DNA replication	DR				4	4								50 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						75 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	13				93 %
Microtubule stabilizers	MS						1			1	5		2	56 %
Protein degradation	PD				1							6		86 %
Protein synthesis	PS												8	100 %

Overall accuracy: 86 / 103 = **83 %**

B: KS statistics

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3								1		1		60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			5									1	83 %
DNA damage	DD		4		4	1								44 %
DNA replication	DR		1		2	5								62 %
Eg5 inhibitors	Eg5						11			1				92 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1		2			11				79 %
Microtubule stabilizers	MS										8		1	89 %
Protein degradation	PD			1								6		86 %
Protein synthesis	PS												8	100 %

Overall accuracy: 85 / 103 = **83 %**

C: SVM normal vector

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	5												100 %
Aurora kinase inhibitors	Aur		10		1	1								83 %
Cholesterol-lowering	Ch			5			1							83 %
DNA damage	DD		5		4									44 %
DNA replication	DR		4		3						1			0 %
Eg5 inhibitors	Eg5						11			1				92 %
Epithelial	Epi							8						100 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	13				93 %
Microtubule stabilizers	MS										9			100 %
Protein degradation	PD										2	5		71 %
Protein synthesis	PS												8	100 %

Overall accuracy: 83 / 103 = **81 %**

D: SVM normal vector with recursive feature elimination

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	4						1						80 %
Aurora kinase inhibitors	Aur		6		5	1								50 %
Cholesterol-lowering	Ch			4			1						1	67 %
DNA damage	DD		6		2	1								22 %
DNA replication	DR		3	1		1					2	1		12 %
Eg5 inhibitors	Eg5						7			2			3	58 %
Epithelial	Epi							8						100 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD						1			13				93 %
Microtubule stabilizers	MS									4	5			56 %
Protein degradation	PD					1	1				1	3	1	43 %
Protein synthesis	PS												8	100 %

Overall accuracy: 66 / 103 = **64 %**

E: Gaussian mixture (mean of 20 models)

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3										1		63 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										95 %
DNA damage	DD		1		6	2								68 %
DNA replication	DR				5	2								31 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	12	1			85 %
Microtubule stabilizers	MS									1	8			89 %
Protein degradation	PD			1								5		68 %
Protein synthesis	PS												8	98 %

Overall accuracy: **83 %**

F: Factor analysis (mean of 20 models)

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	4											1	80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8									94 %
DNA replication	DR				1	7								82 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						77 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS										9			95 %
Protein degradation	PD											7		96 %
Protein synthesis	PS												8	100 %

Overall accuracy: **94 %**

Figure S4: Confusion matrices for the five profiling methods, showing the number of compound-concentrations that were classified correctly (on the diagonals) and incorrectly (off the diagonals), the classification accuracies for each MOA (right columns), and overall classification accuracy (number of correctly classified compound-concentration divided by the total number of compound-concentrations). Panels E and F show average outcomes over 20 models; dimly colored squares without numbers indicate classification outcomes that occurred less than 0.5 times on average.

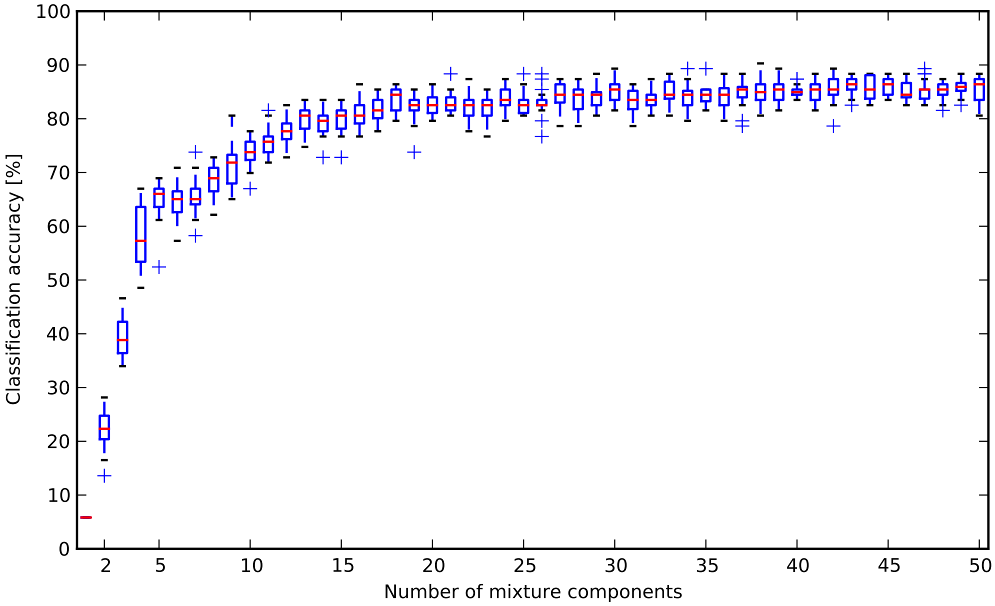
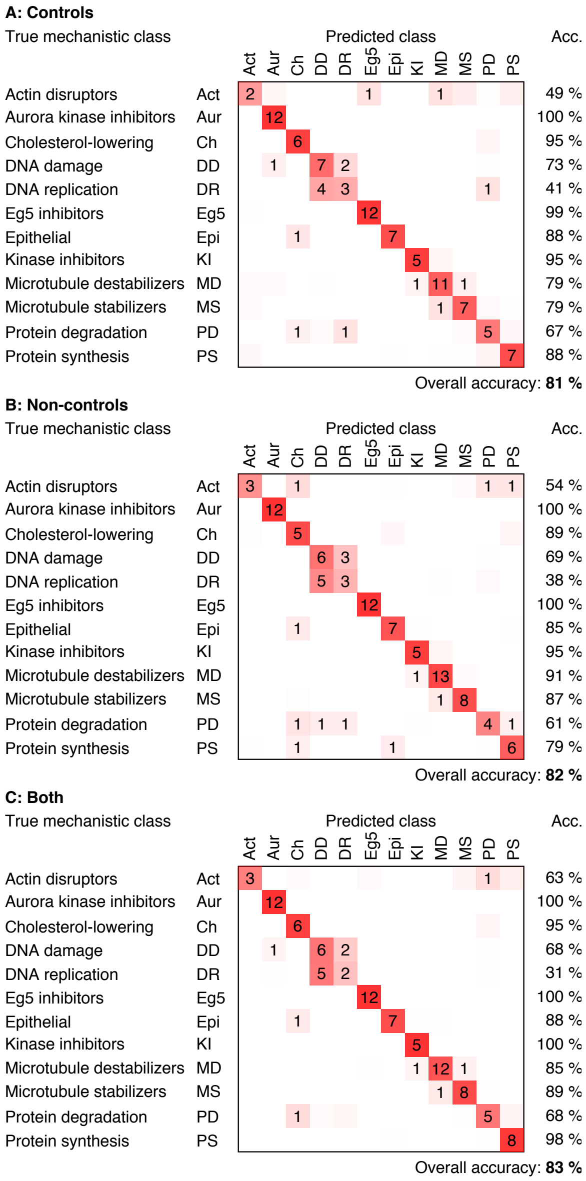


Figure S5: Distributions of classification accuracies for 20 runs of the Gaussian mixture method for each possible choice of the number of mixture components from 2 to 50.



A: Controls

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	2					1			1				49 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										95 %
DNA damage	DD		1		7	2								73 %
DNA replication	DR				4	3						1		41 %
Eg5 inhibitors	Eg5						12							99 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					95 %
Microtubule destabilizers	MD								1	11	1			79 %
Microtubule stabilizers	MS									1	7			79 %
Protein degradation	PD			1		1						5		67 %
Protein synthesis	PS												7	88 %

Overall accuracy: **81 %**

B: Non-controls

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3		1								1	1	54 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			5										89 %
DNA damage	DD				6	3								69 %
DNA replication	DR				5	3								38 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			1				7						85 %
Kinase inhibitors	KI								5					95 %
Microtubule destabilizers	MD								1	13				91 %
Microtubule stabilizers	MS									1	8			87 %
Protein degradation	PD			1	1	1						4	1	61 %
Protein synthesis	PS			1				1					6	79 %

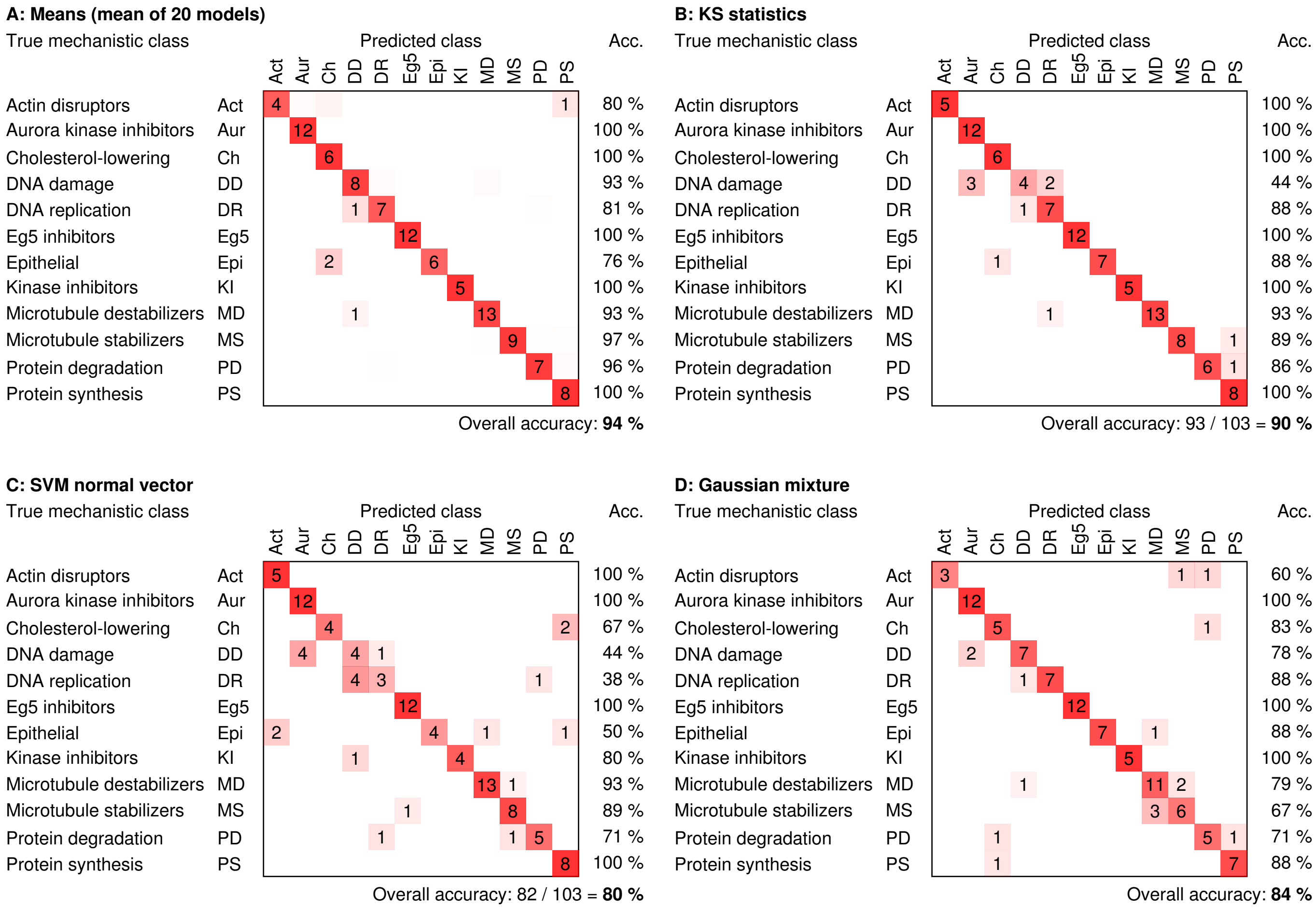
Overall accuracy: **82 %**

C: Both

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3										1		63 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										95 %
DNA damage	DD		1		6	2								68 %
DNA replication	DR				5	2								31 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD								1	12	1			85 %
Microtubule stabilizers	MS									1	8			89 %
Protein degradation	PD			1								5		68 %
Protein synthesis	PS												8	98 %

Overall accuracy: **83 %**

Figure S6: The GM method performs similarly whether the model is built from a subsample of control cells, a subsample of non-control (treated) cells, or a mixture of both.



A: Means (mean of 20 models)

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	4											1	80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8									93 %
DNA replication	DR				1	7								81 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						76 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS										9			97 %
Protein degradation	PD											7		96 %
Protein synthesis	PS												8	100 %

Predicted class

Overall accuracy: **94 %**

B: KS statistics

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	5												100 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD		3		4	2								44 %
DNA replication	DR				1	7								88 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			1				7						88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD					1				13				93 %
Microtubule stabilizers	MS										8		1	89 %
Protein degradation	PD											6	1	86 %
Protein synthesis	PS												8	100 %

Predicted class

Overall accuracy: 93 / 103 = **90 %**

C: SVM normal vector

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	5												100 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			4									2	67 %
DNA damage	DD		4		4	1								44 %
DNA replication	DR				4	3						1		38 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi	2						4		1			1	50 %
Kinase inhibitors	KI				1				4					80 %
Microtubule destabilizers	MD									13	1			93 %
Microtubule stabilizers	MS						1				8			89 %
Protein degradation	PD					1					1	5		71 %
Protein synthesis	PS												8	100 %

Predicted class

Overall accuracy: 82 / 103 = **80 %**

D: Gaussian mixture

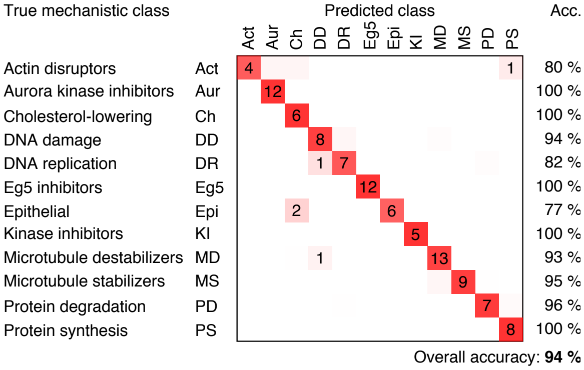
True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	3									1	1		60 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			5								1		83 %
DNA damage	DD		2		7									78 %
DNA replication	DR				1	7								88 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi							7		1				88 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					11	2			79 %
Microtubule stabilizers	MS									3	6			67 %
Protein degradation	PD			1								5	1	71 %
Protein synthesis	PS			1									7	88 %

Predicted class

Overall accuracy: **84 %**

Figure S7: Confusion matrices for each method, using factor analysis as a preprocessing step.

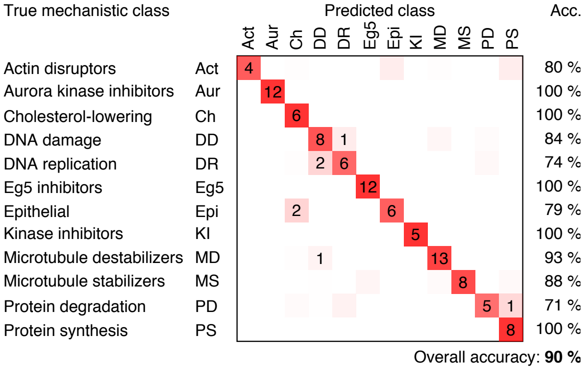
A: Controls



True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	4											1	80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8									94 %
DNA replication	DR				1	7								82 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						77 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS										9			95 %
Protein degradation	PD											7		96 %
Protein synthesis	PS												8	100 %

Overall accuracy: **94 %**

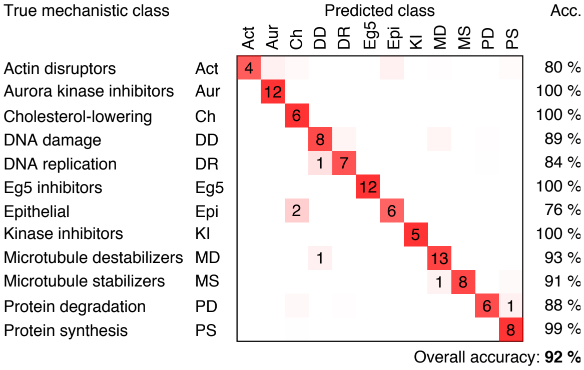
B: Non-controls



True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	4												80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8	1								84 %
DNA replication	DR				2	6								74 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						79 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS										8			88 %
Protein degradation	PD											5	1	71 %
Protein synthesis	PS												8	100 %

Overall accuracy: **90 %**

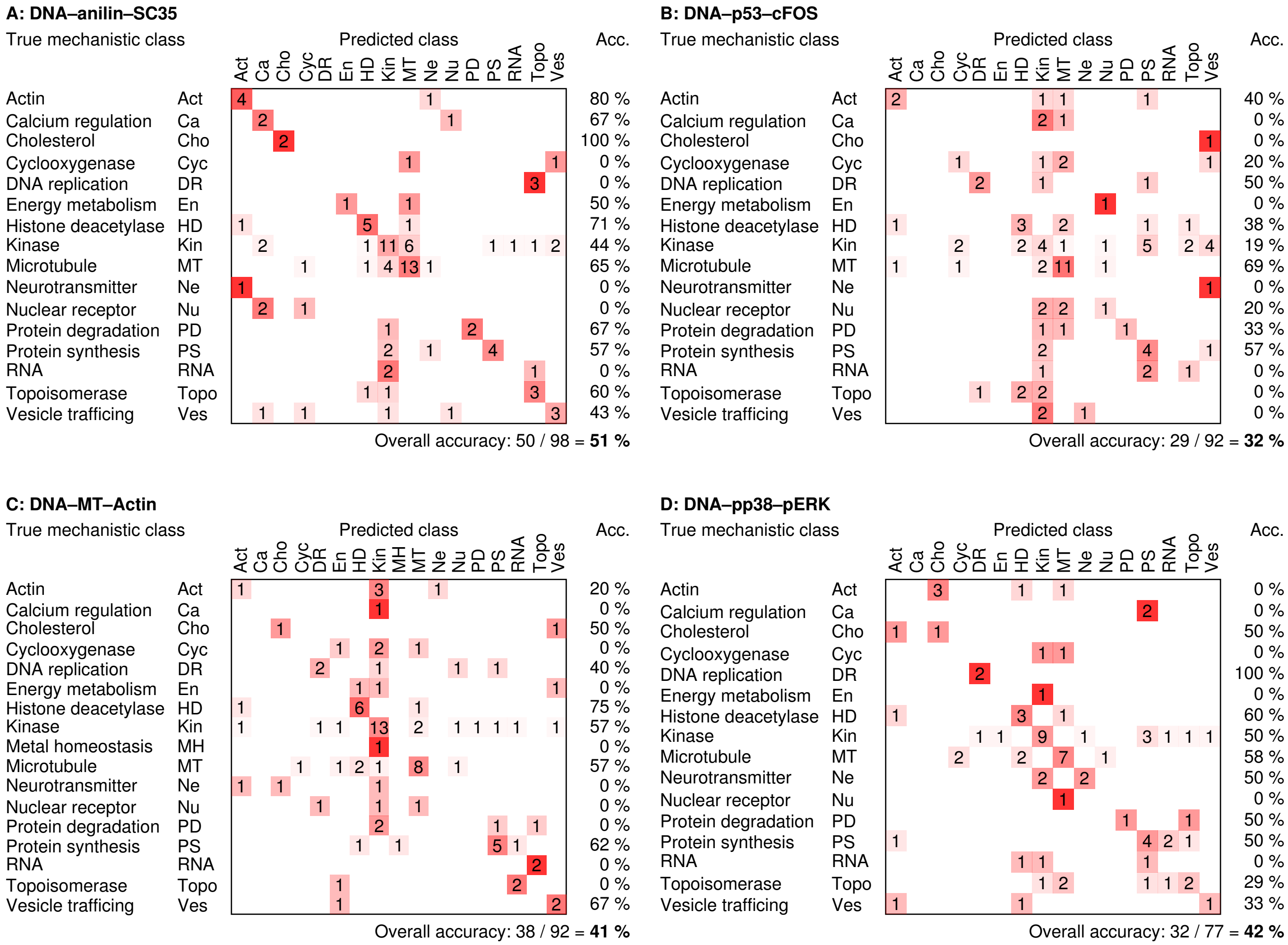
C: Both



True mechanistic class		Predicted class												Acc.
		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	
Actin disruptors	Act	4												80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8									89 %
DNA replication	DR				1	7								84 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi			2				6						76 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD				1					13				93 %
Microtubule stabilizers	MS									1	8			91 %
Protein degradation	PD											6	1	88 %
Protein synthesis	PS												8	99 %

Overall accuracy: **92 %**

Figure S8: The FA method performs similarly whether the model is built from a subsample of control cells, a subsample of non-control (treated) cells, or a mixture of both.



A: DNA–anilin–SC35

True mechanistic class		Act	Ca	Cho	Cyc	DR	En	HD	Kin	MT	Ne	Nu	PD	PS	RNA	Topo	Ves	Acc.
Actin	Act	4									1							80 %
Calcium regulation	Ca		2									1						67 %
Cholesterol	Cho			2														100 %
Cyclooxygenase	Cyc									1							1	0 %
DNA replication	DR															3		0 %
Energy metabolism	En						1			1								50 %
Histone deacetylase	HD	1						5		1								71 %
Kinase	Kin		2					1	11	6				1	1	1	2	44 %
Microtubule	MT				1			1	4	13	1							65 %
Neurotransmitter	Ne	1																0 %
Nuclear receptor	Nu		2		1													0 %
Protein degradation	PD								1				2					67 %
Protein synthesis	PS								2		1			4				57 %
RNA	RNA								2							1		0 %
Topoisomerase	Topo							1	1							3		60 %
Vesicle trafficking	Ves		1		1				1			1					3	43 %

Predicted class

Overall accuracy: 50 / 98 = **51 %**

B: DNA–p53–cFOS

True mechanistic class		Act	Ca	Cho	Cyc	DR	En	HD	Kin	MT	Ne	Nu	PD	PS	RNA	Topo	Ves	Acc.
Actin	Act	2							1	1				1				40 %
Calcium regulation	Ca								2	1								0 %
Cholesterol	Cho																1	0 %
Cyclooxygenase	Cyc				1				1	2							1	20 %
DNA replication	DR					2			1					1				50 %
Energy metabolism	En											1						0 %
Histone deacetylase	HD	1						3		2				1		1		38 %
Kinase	Kin				2			2	4	1		1		5		2	4	19 %
Microtubule	MT	1			1				2	11		1						69 %
Neurotransmitter	Ne																1	0 %
Nuclear receptor	Nu								2	2		1						20 %
Protein degradation	PD								1	1			1					33 %
Protein synthesis	PS								2					4			1	57 %
RNA	RNA								1					2		1		0 %
Topoisomerase	Topo					1		2	2									0 %
Vesicle trafficking	Ves								2		1							0 %

Predicted class

Overall accuracy: 29 / 92 = **32 %**

C: DNA–MT–Actin

True mechanistic class		Act	Ca	Cho	Cyc	DR	En	HD	Kin	MH	MT	Ne	Nu	PD	PS	RNA	Topo	Ves	Acc.
Actin	Act	1							3			1							20 %
Calcium regulation	Ca								1										0 %
Cholesterol	Cho			1														1	50 %
Cyclooxygenase	Cyc						1		2		1								0 %
DNA replication	DR					2			1				1		1				40 %
Energy metabolism	En							1	1									1	0 %
Histone deacetylase	HD	1						6			1								75 %
Kinase	Kin	1				1	1		13		2		1	1	1	1		1	57 %
Metal homeostasis	MH								1										0 %
Microtubule	MT				1		1	2	1		8		1						57 %
Neurotransmitter	Ne	1		1					1										0 %
Nuclear receptor	Nu					1			1		1								0 %
Protein degradation	PD								2						1		1		0 %
Protein synthesis	PS							1		1					5	1			62 %
RNA	RNA																2		0 %
Topoisomerase	Topo						1									2			0 %
Vesicle trafficking	Ves						1											2	67 %

Predicted class

Overall accuracy: 38 / 92 = **41 %**

D: DNA–pp38–pERK

True mechanistic class		Act	Ca	Cho	Cyc	DR	En	HD	Kin	MT	Ne	Nu	PD	PS	RNA	Topo	Ves	Acc.
Actin	Act			3				1		1								0 %
Calcium regulation	Ca													2				0 %
Cholesterol	Cho	1		1														50 %
Cyclooxygenase	Cyc								1	1								0 %
DNA replication	DR					2												100 %
Energy metabolism	En								1									0 %
Histone deacetylase	HD	1						3		1								60 %
Kinase	Kin					1	1		9		1			3	1	1	1	50 %
Microtubule	MT				2			2		7		1						58 %
Neurotransmitter	Ne								2		2							50 %
Nuclear receptor	Nu									1								0 %
Protein degradation	PD												1			1		50 %
Protein synthesis	PS	1												4	2	1		50 %
RNA	RNA							1	1					1				0 %
Topoisomerase	Topo								1	2				1	1	2		29 %
Vesicle trafficking	Ves	1						1									1	33 %

Predicted class

Overall accuracy: 32 / 77 = **42 %**

Figure S9: Confusion matrices from Loo et al.’s experiment, compiled from information in their Supplementary Data 2[15]

SUPPLEMENTARY TABLES

Table S1: The 453 measurements made by CellProfiler for each cell. (See the CellProfiler manual for descriptions of each.)

Image feature name
TableNumber
ImageNumber
ObjectNumber
Nuclei_Children_Cells_Count
Nuclei_Children_Cytoplasm_Count
Nuclei_AreaShape_Area
Nuclei_AreaShape_Eccentricity
Nuclei_AreaShape_Solidity
Nuclei_AreaShape_Extent
Nuclei_AreaShape_EulerNumber
Nuclei_AreaShape_Perimeter
Nuclei_AreaShape_FormFactor
Nuclei_AreaShape_MajorAxisLength
Nuclei_AreaShape_MinorAxisLength
Nuclei_AreaShape_Orientation
Nuclei_Zernike_0_0
Nuclei_Zernike_1_1
Nuclei_Zernike_2_0
Nuclei_Zernike_2_2
Nuclei_Zernike_3_1
Nuclei_Zernike_3_3
Nuclei_Zernike_4_0
Nuclei_Zernike_4_2
Nuclei_Zernike_4_4
Nuclei_Zernike_5_1
Nuclei_Zernike_5_3
Nuclei_Zernike_5_5
Nuclei_Zernike_6_0
Nuclei_Zernike_6_2
Nuclei_Zernike_6_4
Nuclei_Zernike_6_6
Nuclei_Zernike_7_1
Nuclei_Zernike_7_3
Nuclei_Zernike_7_5
Nuclei_Zernike_7_7
Nuclei_Zernike_8_0
Nuclei_Zernike_8_2
Nuclei_Zernike_8_4
Nuclei_Zernike_8_6
Nuclei_Zernike_8_8
Nuclei_Zernike_9_1
Nuclei_Zernike_9_3
Nuclei_Zernike_9_5
Nuclei_Zernike_9_7
Nuclei_Zernike_9_9
Nuclei_Intensity_IntegratedIntensity_CorrDAPI
Nuclei_Intensity_MeanIntensity_CorrDAPI
Nuclei_Intensity_StdIntensity_CorrDAPI
Nuclei_Intensity_MinIntensity_CorrDAPI
Nuclei_Intensity_MaxIntensity_CorrDAPI
Nuclei_Intensity_IntegratedIntensityEdge_CorrDAPI
Nuclei_Intensity_MeanIntensityEdge_CorrDAPI
Nuclei_Intensity_StdIntensityEdge_CorrDAPI
Nuclei_Intensity_MinIntensityEdge_CorrDAPI
Nuclei_Intensity_MaxIntensityEdge_CorrDAPI
Nuclei_Intensity_MassDisplacement_CorrDAPI
Nuclei_Intensity_LowerQuartileIntensity_CorrDAPI
Nuclei_Intensity_MedianIntensity_CorrDAPI
Nuclei_Intensity_UpperQuartileIntensity_CorrDAPI
Nuclei_Intensity_IntegratedIntensity_CorrActin
Nuclei_Intensity_MeanIntensity_CorrActin
Nuclei_Intensity_StdIntensity_CorrActin
Nuclei_Intensity_MinIntensity_CorrActin
Nuclei_Intensity_MaxIntensity_CorrActin
Nuclei_Intensity_IntegratedIntensityEdge_CorrActin
Nuclei_Intensity_MeanIntensityEdge_CorrActin
Nuclei_Intensity_StdIntensityEdge_CorrActin
Nuclei_Intensity_MinIntensityEdge_CorrActin
Nuclei_Intensity_MaxIntensityEdge_CorrActin
Nuclei_Intensity_MassDisplacement_CorrActin
Nuclei_Intensity_LowerQuartileIntensity_CorrActin
Nuclei_Intensity_MedianIntensity_CorrActin
Nuclei_Intensity_UpperQuartileIntensity_CorrActin
Nuclei_Intensity_IntegratedIntensity_CorrTub
Nuclei_Intensity_MeanIntensity_CorrTub
Nuclei_Intensity_StdIntensity_CorrTub
Nuclei_Intensity_MinIntensity_CorrTub
Nuclei_Intensity_MaxIntensity_CorrTub
Nuclei_Intensity_IntegratedIntensityEdge_CorrTub
Nuclei_Intensity_MeanIntensityEdge_CorrTub
Nuclei_Intensity_StdIntensityEdge_CorrTub
Nuclei_Intensity_MinIntensityEdge_CorrTub
Nuclei_Intensity_MaxIntensityEdge_CorrTub
Nuclei_Intensity_MassDisplacement_CorrTub
Nuclei_Intensity_LowerQuartileIntensity_CorrTub
Nuclei_Intensity_MedianIntensity_CorrTub
Nuclei_Intensity_UpperQuartileIntensity_CorrTub
Nuclei_Neighbors_NumberOfNeighbors_10
Nuclei_Neighbors_PercentTouching_10
Nuclei_Neighbors_FirstClosestXVector_10
Nuclei_Neighbors_FirstClosestYVector_10
Nuclei_Neighbors_SecondClosestXVector_10
Nuclei_Neighbors_SecondClosestYVector_10
Nuclei_Neighbors_AngleBetweenNeighbors_10
Nuclei_Neighbors_NumberOfNeighbors_20
Nuclei_Neighbors_PercentTouching_20
Nuclei_Neighbors_FirstClosestXVector_20
Nuclei_Neighbors_FirstClosestYVector_20
Nuclei_Neighbors_SecondClosestXVector_20
Nuclei_Neighbors_SecondClosestYVector_20
Nuclei_Neighbors_AngleBetweenNeighbors_20
Nuclei_Texture_AngularSecondMoment_CorrDAPI_10
Nuclei_Texture_Contrast_CorrDAPI_10
Nuclei_Texture_Correlation_CorrDAPI_10

Continues...

Table S1 continued

Continues...

Table S1 continued

Image feature name
Nuclei_Texture_Variance_CorrDAPI_10
Nuclei_Texture_InverseDifferenceMoment_CorrDAPI_10
Nuclei_Texture_SumAverage_CorrDAPI_10
Nuclei_Texture_SumVariance_CorrDAPI_10
Nuclei_Texture_SumEntropy_CorrDAPI_10
Nuclei_Texture_Entropy_CorrDAPI_10
Nuclei_Texture_DifferenceVariance_CorrDAPI_10
Nuclei_Texture_DifferenceEntropy_CorrDAPI_10
Nuclei_Texture_InfoMeas1_CorrDAPI_10
Nuclei_Texture_InfoMeas2_CorrDAPI_10
Nuclei_Texture_GaborX_CorrDAPI_10
Nuclei_Texture_GaborY_CorrDAPI_10
Nuclei_Texture_AngularSecondMoment_CorrActin_10
Nuclei_Texture_Contrast_CorrActin_10
Nuclei_Texture_Correlation_CorrActin_10
Nuclei_Texture_Variance_CorrActin_10
Nuclei_Texture_InverseDifferenceMoment_CorrActin_10
Nuclei_Texture_SumAverage_CorrActin_10
Nuclei_Texture_SumVariance_CorrActin_10
Nuclei_Texture_SumEntropy_CorrActin_10
Nuclei_Texture_Entropy_CorrActin_10
Nuclei_Texture_DifferenceVariance_CorrActin_10
Nuclei_Texture_DifferenceEntropy_CorrActin_10
Nuclei_Texture_InfoMeas1_CorrActin_10
Nuclei_Texture_InfoMeas2_CorrActin_10
Nuclei_Texture_GaborX_CorrActin_10
Nuclei_Texture_GaborY_CorrActin_10
Nuclei_Texture_AngularSecondMoment_CorrTub_10
Nuclei_Texture_Contrast_CorrTub_10
Nuclei_Texture_Correlation_CorrTub_10
Nuclei_Texture_Variance_CorrTub_10
Nuclei_Texture_InverseDifferenceMoment_CorrTub_10
Nuclei_Texture_SumAverage_CorrTub_10
Nuclei_Texture_SumVariance_CorrTub_10
Nuclei_Texture_SumEntropy_CorrTub_10
Nuclei_Texture_Entropy_CorrTub_10
Nuclei_Texture_DifferenceVariance_CorrTub_10
Nuclei_Texture_DifferenceEntropy_CorrTub_10
Nuclei_Texture_InfoMeas1_CorrTub_10
Nuclei_Texture_InfoMeas2_CorrTub_10
Nuclei_Texture_GaborX_CorrTub_10
Nuclei_Texture_GaborY_CorrTub_10
Nuclei_Texture_AngularSecondMoment_CorrDAPI_3
Nuclei_Texture_Contrast_CorrDAPI_3
Nuclei_Texture_Correlation_CorrDAPI_3
Nuclei_Texture_Variance_CorrDAPI_3
Nuclei_Texture_InverseDifferenceMoment_CorrDAPI_3
Nuclei_Texture_SumAverage_CorrDAPI_3
Nuclei_Texture_SumVariance_CorrDAPI_3
Nuclei_Texture_SumEntropy_CorrDAPI_3
Nuclei_Texture_Entropy_CorrDAPI_3
Nuclei_Texture_DifferenceVariance_CorrDAPI_3
Nuclei_Texture_DifferenceEntropy_CorrDAPI_3
Nuclei_Texture_InfoMeas1_CorrDAPI_3

Continues...

Table S1 continued

Image feature name
Nuclei_Texture_InfoMeas2_CorrDAPI_3
Nuclei_Texture_GaborX_CorrDAPI_3
Nuclei_Texture_GaborY_CorrDAPI_3
Nuclei_Texture_AngularSecondMoment_CorrActin_3
Nuclei_Texture_Contrast_CorrActin_3
Nuclei_Texture_Correlation_CorrActin_3
Nuclei_Texture_Variance_CorrActin_3
Nuclei_Texture_InverseDifferenceMoment_CorrActin_3
Nuclei_Texture_SumAverage_CorrActin_3
Nuclei_Texture_SumVariance_CorrActin_3
Nuclei_Texture_SumEntropy_CorrActin_3
Nuclei_Texture_Entropy_CorrActin_3
Nuclei_Texture_DifferenceVariance_CorrActin_3
Nuclei_Texture_DifferenceEntropy_CorrActin_3
Nuclei_Texture_InfoMeas1_CorrActin_3
Nuclei_Texture_InfoMeas2_CorrActin_3
Nuclei_Texture_GaborX_CorrActin_3
Nuclei_Texture_GaborY_CorrActin_3
Nuclei_Texture_AngularSecondMoment_CorrTub_3
Nuclei_Texture_Contrast_CorrTub_3
Nuclei_Texture_Correlation_CorrTub_3
Nuclei_Texture_Variance_CorrTub_3
Nuclei_Texture_InverseDifferenceMoment_CorrTub_3
Nuclei_Texture_SumAverage_CorrTub_3
Nuclei_Texture_SumVariance_CorrTub_3
Nuclei_Texture_SumEntropy_CorrTub_3
Nuclei_Texture_Entropy_CorrTub_3
Nuclei_Texture_DifferenceVariance_CorrTub_3
Nuclei_Texture_DifferenceEntropy_CorrTub_3
Nuclei_Texture_InfoMeas1_CorrTub_3
Nuclei_Texture_InfoMeas2_CorrTub_3
Nuclei_Texture_GaborX_CorrTub_3
Nuclei_Texture_GaborY_CorrTub_3
Cells_Parent_Nuclei
Cells_Children_Cytoplasm_Count
Cells_AreaShape_Area
Cells_AreaShape_Eccentricity
Cells_AreaShape_Solidity
Cells_AreaShape_Extent
Cells_AreaShape_EulerNumber
Cells_AreaShape_Perimeter
Cells_AreaShape_FormFactor
Cells_AreaShape_MajorAxisLength
Cells_AreaShape_MinorAxisLength
Cells_AreaShape_Orientation
Cells_Zernike_0_0
Cells_Zernike_1_1
Cells_Zernike_2_0
Cells_Zernike_2_2
Cells_Zernike_3_1
Cells_Zernike_3_3
Cells_Zernike_4_0
Cells_Zernike_4_2
Cells_Zernike_4_4

Continues...

Table S1 continued

Image feature name
Cells_Zernike_5_1
Cells_Zernike_5_3
Cells_Zernike_5_5
Cells_Zernike_6_0
Cells_Zernike_6_2
Cells_Zernike_6_4
Cells_Zernike_6_6
Cells_Zernike_7_1
Cells_Zernike_7_3
Cells_Zernike_7_5
Cells_Zernike_7_7
Cells_Zernike_8_0
Cells_Zernike_8_2
Cells_Zernike_8_4
Cells_Zernike_8_6
Cells_Zernike_8_8
Cells_Zernike_9_1
Cells_Zernike_9_3
Cells_Zernike_9_5
Cells_Zernike_9_7
Cells_Zernike_9_9
Cells_Intensity_IntegratedIntensity_CorrActin
Cells_Intensity_MeanIntensity_CorrActin
Cells_Intensity_StdIntensity_CorrActin
Cells_Intensity_MinIntensity_CorrActin
Cells_Intensity_MaxIntensity_CorrActin
Cells_Intensity_IntegratedIntensityEdge_CorrActin
Cells_Intensity_MeanIntensityEdge_CorrActin
Cells_Intensity_StdIntensityEdge_CorrActin
Cells_Intensity_MinIntensityEdge_CorrActin
Cells_Intensity_MaxIntensityEdge_CorrActin
Cells_Intensity_MassDisplacement_CorrActin
Cells_Intensity_LowerQuartileIntensity_CorrActin
Cells_Intensity_MedianIntensity_CorrActin
Cells_Intensity_UpperQuartileIntensity_CorrActin
Cells_Intensity_IntegratedIntensity_CorrTub
Cells_Intensity_MeanIntensity_CorrTub
Cells_Intensity_StdIntensity_CorrTub
Cells_Intensity_MinIntensity_CorrTub
Cells_Intensity_MaxIntensity_CorrTub
Cells_Intensity_IntegratedIntensityEdge_CorrTub
Cells_Intensity_MeanIntensityEdge_CorrTub
Cells_Intensity_StdIntensityEdge_CorrTub
Cells_Intensity_MinIntensityEdge_CorrTub
Cells_Intensity_MaxIntensityEdge_CorrTub
Cells_Intensity_MassDisplacement_CorrTub
Cells_Intensity_LowerQuartileIntensity_CorrTub
Cells_Intensity_MedianIntensity_CorrTub
Cells_Intensity_UpperQuartileIntensity_CorrTub
Cells_Neighbors_NumberOfNeighbors_3
Cells_Neighbors_PercentTouching_3
Cells_Neighbors_FirstClosestXVector_3
Cells_Neighbors_FirstClosestYVector_3
Cells_Neighbors_SecondClosestXVector_3

Continues...

Table S1 continued

Image feature name
Cells_Neighbors_SecondClosestYVector_3
Cells_Neighbors_AngleBetweenNeighbors_3
Cells_Neighbors_NumberOfNeighbors_10
Cells_Neighbors_PercentTouching_10
Cells_Neighbors_FirstClosestXVector_10
Cells_Neighbors_FirstClosestYVector_10
Cells_Neighbors_SecondClosestXVector_10
Cells_Neighbors_SecondClosestYVector_10
Cells_Neighbors_AngleBetweenNeighbors_10
Cells_Texture_AngularSecondMoment_CorrActin_10
Cells_Texture_Contrast_CorrActin_10
Cells_Texture_Correlation_CorrActin_10
Cells_Texture_Variance_CorrActin_10
Cells_Texture_InverseDifferenceMoment_CorrActin_10
Cells_Texture_SumAverage_CorrActin_10
Cells_Texture_SumVariance_CorrActin_10
Cells_Texture_SumEntropy_CorrActin_10
Cells_Texture_Entropy_CorrActin_10
Cells_Texture_DifferenceVariance_CorrActin_10
Cells_Texture_DifferenceEntropy_CorrActin_10
Cells_Texture_InfoMeas1_CorrActin_10
Cells_Texture_InfoMeas2_CorrActin_10
Cells_Texture_GaborX_CorrActin_10
Cells_Texture_GaborY_CorrActin_10
Cells_Texture_AngularSecondMoment_CorrTub_10
Cells_Texture_Contrast_CorrTub_10
Cells_Texture_Correlation_CorrTub_10
Cells_Texture_Variance_CorrTub_10
Cells_Texture_InverseDifferenceMoment_CorrTub_10
Cells_Texture_SumAverage_CorrTub_10
Cells_Texture_SumVariance_CorrTub_10
Cells_Texture_SumEntropy_CorrTub_10
Cells_Texture_Entropy_CorrTub_10
Cells_Texture_DifferenceVariance_CorrTub_10
Cells_Texture_DifferenceEntropy_CorrTub_10
Cells_Texture_InfoMeas1_CorrTub_10
Cells_Texture_InfoMeas2_CorrTub_10
Cells_Texture_GaborX_CorrTub_10
Cells_Texture_GaborY_CorrTub_10
Cells_Texture_AngularSecondMoment_CorrActin_3
Cells_Texture_Contrast_CorrActin_3
Cells_Texture_Correlation_CorrActin_3
Cells_Texture_Variance_CorrActin_3
Cells_Texture_InverseDifferenceMoment_CorrActin_3
Cells_Texture_SumAverage_CorrActin_3
Cells_Texture_SumVariance_CorrActin_3
Cells_Texture_SumEntropy_CorrActin_3
Cells_Texture_Entropy_CorrActin_3
Cells_Texture_DifferenceVariance_CorrActin_3
Cells_Texture_DifferenceEntropy_CorrActin_3
Cells_Texture_InfoMeas1_CorrActin_3
Cells_Texture_InfoMeas2_CorrActin_3
Cells_Texture_GaborX_CorrActin_3
Cells_Texture_GaborY_CorrActin_3

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Table S1 continued

Image feature name
Cells_Texture_AngularSecondMoment_CorrTub_3
Cells_Texture_Contrast_CorrTub_3
Cells_Texture_Correlation_CorrTub_3
Cells_Texture_Variance_CorrTub_3
Cells_Texture_InverseDifferenceMoment_CorrTub_3
Cells_Texture_SumAverage_CorrTub_3
Cells_Texture_SumVariance_CorrTub_3
Cells_Texture_SumEntropy_CorrTub_3
Cells_Texture_Entropy_CorrTub_3
Cells_Texture_DifferenceVariance_CorrTub_3
Cells_Texture_DifferenceEntropy_CorrTub_3
Cells_Texture_InfoMeas1_CorrTub_3
Cells_Texture_InfoMeas2_CorrTub_3
Cells_Texture_GaborX_CorrTub_3
Cells_Texture_GaborY_CorrTub_3
Cytoplasm_Parent_Cells
Cytoplasm_Parent_Nuclei
Cytoplasm_AreaShape_Area
Cytoplasm_AreaShape_Eccentricity
Cytoplasm_AreaShape_Solidity
Cytoplasm_AreaShape_Extent
Cytoplasm_AreaShape_EulerNumber
Cytoplasm_AreaShape_Perimeter
Cytoplasm_AreaShape_FormFactor
Cytoplasm_AreaShape_MajorAxisLength
Cytoplasm_AreaShape_MinorAxisLength
Cytoplasm_AreaShape_Orientation
Cytoplasm_Zernike_0_0
Cytoplasm_Zernike_1_1
Cytoplasm_Zernike_2_0
Cytoplasm_Zernike_2_2
Cytoplasm_Zernike_3_1
Cytoplasm_Zernike_3_3
Cytoplasm_Zernike_4_0
Cytoplasm_Zernike_4_2
Cytoplasm_Zernike_4_4
Cytoplasm_Zernike_5_1
Cytoplasm_Zernike_5_3
Cytoplasm_Zernike_5_5
Cytoplasm_Zernike_6_0
Cytoplasm_Zernike_6_2
Cytoplasm_Zernike_6_4
Cytoplasm_Zernike_6_6
Cytoplasm_Zernike_7_1
Cytoplasm_Zernike_7_3
Cytoplasm_Zernike_7_5
Cytoplasm_Zernike_7_7
Cytoplasm_Zernike_8_0
Cytoplasm_Zernike_8_2
Cytoplasm_Zernike_8_4
Cytoplasm_Zernike_8_6
Cytoplasm_Zernike_8_8
Cytoplasm_Zernike_9_1
Cytoplasm_Zernike_9_3

Continues...

Table S1 continued

Image feature name
Cytoplasm_Zernike_9_5
Cytoplasm_Zernike_9_7
Cytoplasm_Zernike_9_9
Cytoplasm_Intensity_IntegratedIntensity_CorrActin
Cytoplasm_Intensity_MeanIntensity_CorrActin
Cytoplasm_Intensity_StdIntensity_CorrActin
Cytoplasm_Intensity_MinIntensity_CorrActin
Cytoplasm_Intensity_MaxIntensity_CorrActin
Cytoplasm_Intensity_IntegratedIntensityEdge_CorrActin
Cytoplasm_Intensity_MeanIntensityEdge_CorrActin
Cytoplasm_Intensity_StdIntensityEdge_CorrActin
Cytoplasm_Intensity_MinIntensityEdge_CorrActin
Cytoplasm_Intensity_MaxIntensityEdge_CorrActin
Cytoplasm_Intensity_MassDisplacement_CorrActin
Cytoplasm_Intensity_LowerQuartileIntensity_CorrActin
Cytoplasm_Intensity_MedianIntensity_CorrActin
Cytoplasm_Intensity_UpperQuartileIntensity_CorrActin
Cytoplasm_Intensity_IntegratedIntensity_CorrTub
Cytoplasm_Intensity_MeanIntensity_CorrTub
Cytoplasm_Intensity_StdIntensity_CorrTub
Cytoplasm_Intensity_MinIntensity_CorrTub
Cytoplasm_Intensity_MaxIntensity_CorrTub
Cytoplasm_Intensity_IntegratedIntensityEdge_CorrTub
Cytoplasm_Intensity_MeanIntensityEdge_CorrTub
Cytoplasm_Intensity_StdIntensityEdge_CorrTub
Cytoplasm_Intensity_MinIntensityEdge_CorrTub
Cytoplasm_Intensity_MaxIntensityEdge_CorrTub
Cytoplasm_Intensity_MassDisplacement_CorrTub
Cytoplasm_Intensity_LowerQuartileIntensity_CorrTub
Cytoplasm_Intensity_MedianIntensity_CorrTub
Cytoplasm_Intensity_UpperQuartileIntensity_CorrTub
Cytoplasm_Texture_AngularSecondMoment_CorrActin_10
Cytoplasm_Texture_Contrast_CorrActin_10
Cytoplasm_Texture_Correlation_CorrActin_10
Cytoplasm_Texture_Variance_CorrActin_10
Cytoplasm_Texture_InverseDifferenceMoment_CorrActin_10
Cytoplasm_Texture_SumAverage_CorrActin_10
Cytoplasm_Texture_SumVariance_CorrActin_10
Cytoplasm_Texture_SumEntropy_CorrActin_10
Cytoplasm_Texture_Entropy_CorrActin_10
Cytoplasm_Texture_DifferenceVariance_CorrActin_10
Cytoplasm_Texture_DifferenceEntropy_CorrActin_10
Cytoplasm_Texture_InfoMeas1_CorrActin_10
Cytoplasm_Texture_InfoMeas2_CorrActin_10
Cytoplasm_Texture_GaborX_CorrActin_10
Cytoplasm_Texture_GaborY_CorrActin_10
Cytoplasm_Texture_AngularSecondMoment_CorrTub_10
Cytoplasm_Texture_Contrast_CorrTub_10
Cytoplasm_Texture_Correlation_CorrTub_10
Cytoplasm_Texture_Variance_CorrTub_10
Cytoplasm_Texture_InverseDifferenceMoment_CorrTub_10
Cytoplasm_Texture_SumAverage_CorrTub_10
Cytoplasm_Texture_SumVariance_CorrTub_10
Cytoplasm_Texture_SumEntropy_CorrTub_10

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Table S1 continued

Image feature name
Cytoplasm_Texture_Entropy_CorrTub_10
Cytoplasm_Texture_DifferenceVariance_CorrTub_10
Cytoplasm_Texture_DifferenceEntropy_CorrTub_10
Cytoplasm_Texture_InfoMeas1_CorrTub_10
Cytoplasm_Texture_InfoMeas2_CorrTub_10
Cytoplasm_Texture_GaborX_CorrTub_10
Cytoplasm_Texture_GaborY_CorrTub_10
Cytoplasm_Texture_AngularSecondMoment_CorrActin_3
Cytoplasm_Texture_Contrast_CorrActin_3
Cytoplasm_Texture_Correlation_CorrActin_3
Cytoplasm_Texture_Variance_CorrActin_3
Cytoplasm_Texture_InverseDifferenceMoment_CorrActin_3
Cytoplasm_Texture_SumAverage_CorrActin_3
Cytoplasm_Texture_SumVariance_CorrActin_3
Cytoplasm_Texture_SumEntropy_CorrActin_3
Cytoplasm_Texture_Entropy_CorrActin_3
Cytoplasm_Texture_DifferenceVariance_CorrActin_3
Cytoplasm_Texture_DifferenceEntropy_CorrActin_3
Cytoplasm_Texture_InfoMeas1_CorrActin_3
Cytoplasm_Texture_InfoMeas2_CorrActin_3
Cytoplasm_Texture_GaborX_CorrActin_3
Cytoplasm_Texture_GaborY_CorrActin_3
Cytoplasm_Texture_AngularSecondMoment_CorrTub_3
Cytoplasm_Texture_Contrast_CorrTub_3
Cytoplasm_Texture_Correlation_CorrTub_3
Cytoplasm_Texture_Variance_CorrTub_3
Cytoplasm_Texture_InverseDifferenceMoment_CorrTub_3
Cytoplasm_Texture_SumAverage_CorrTub_3
Cytoplasm_Texture_SumVariance_CorrTub_3
Cytoplasm_Texture_SumEntropy_CorrTub_3
Cytoplasm_Texture_Entropy_CorrTub_3
Cytoplasm_Texture_DifferenceVariance_CorrTub_3
Cytoplasm_Texture_DifferenceEntropy_CorrTub_3
Cytoplasm_Texture_InfoMeas1_CorrTub_3
Cytoplasm_Texture_InfoMeas2_CorrTub_3
Cytoplasm_Texture_GaborX_CorrTub_3
Cytoplasm_Texture_GaborY_CorrTub_3

Table S2: The ground-truth set used for comparing profiling algorithms

Mechanism of action	Compound	Concentrations [μM]
Actin disruptors	cytochalasin B	10.0, 30.0
	cytochalasin D	0.3
	latrunculin B	1.0, 3.0
Aurora kinase inhibitors	AZ-A	0.1, 0.3, 1.0, 3.0, 10.0, 30.0
	AZ258	0.1, 0.3, 1.0
	AZ841	0.1, 0.3, 1.0
Cholesterol-lowering	mevinolin/lovastatin	1.5, 5.0, 15.0
	simvastatin	2.0, 6.0, 20.0
DNA damage	chlorambucil	10.0
	cisplatin	10.0
	etoposide	1.0, 3.0, 10.0
	mitomycin C	0.1, 0.3, 1.0, 3.0
DNA replication	camptothecin	0.003, 0.01, 0.03
	floxuridine	10.0, 30.0
	methotrexate	10.0
	mitoxantrone	0.003, 0.01
Eg5 inhibitors	AZ-C	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0
	AZ138	0.03, 0.1, 0.3, 1.0, 3.0
Epithelial	AZ-J	1.0, 3.0, 10.0
	AZ-U	1.0, 3.0, 10.0
	PP-2	3.0, 10.0
Kinase inhibitors	PD-169316	3.0, 10.0
	alsterpaullone	1.0, 3.0
	bryostatin	0.3
Microtubule destabilizers	colchicine	0.03
	demecolcine	0.3, 1.0, 3.0, 10.0
	nocodazole	1.0, 3.0
	vincristine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0
Microtubule stabilizers	docetaxel	0.03, 0.1, 0.3
	epothilone B	0.1, 0.3, 1.0
	taxol	0.3, 1.0, 3.0
Protein degradation	ALLN	3.0, 100.0
	MG-132	0.1, 3.0
	lactacystin	10.0
	proteasome inhibitor I	0.1, 3.0
Protein synthesis	anisomycin	0.3, 1.0
	cyclohexamide	5.0, 15.0, 50.0
	emetine	0.1, 0.3, 1.0

Table S3: The compounds and concentrations with which cells were treated

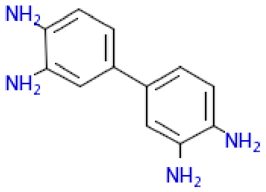
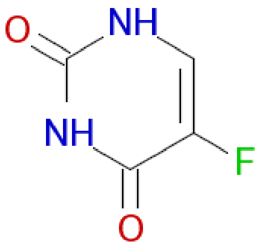
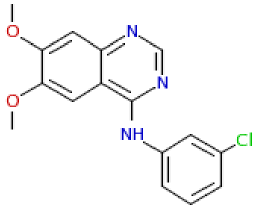
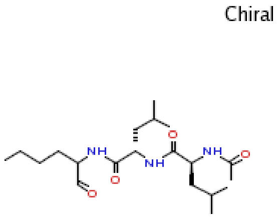
Compound name	Concentrations [µM]	Structure
3,3’-diaminobenzidine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
5-fluorouracil	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
AG-1478	0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1.0, 2.0, 3.0, 6.0, 10.0, 20.0	
ALLN	0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 10000.0	
AZ-A	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-B	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-C	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0	(*not disclosed*)

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Table S3: *continued from the previous page*

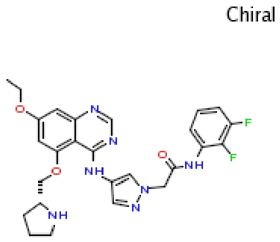
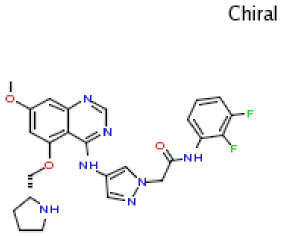
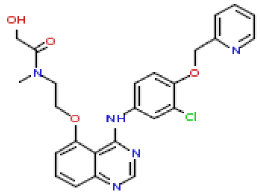
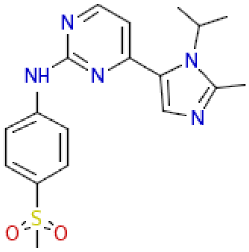
Compound name	Concentrations [μM]	Structure
AZ-D (AZ841)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-E (AZ258)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-F (AZ701)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-G (AZ235)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-H	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-I	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-J	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	(*not disclosed*)
AZ-K	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	(*not disclosed*)
AZ-L	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-M	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)

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Table S3: *continued from the previous page*

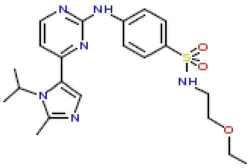
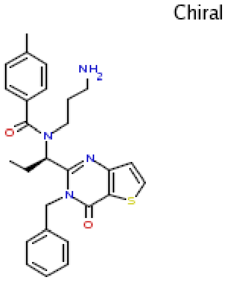
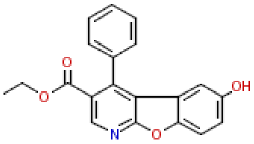
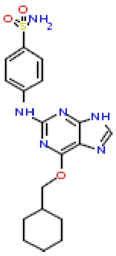
Compound name	Concentrations [μM]	Structure
AZ-N	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	(*not disclosed*)
AZ-O	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
AZ-P (AZ970)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-Q (AZ138)	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
AZ-U	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
Cdk1 inhibitor III	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
Cdk1/2 inhibitor (NU6102)	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	

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Table S3: *continued from the previous page*

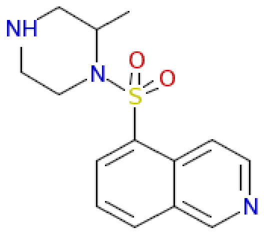
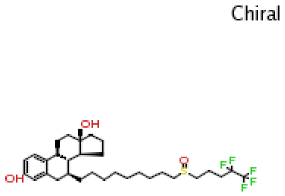
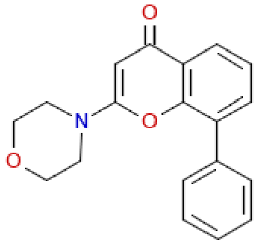
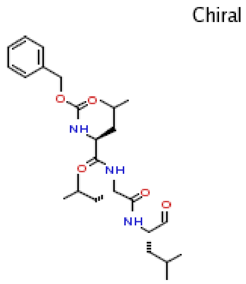
Compound name	Concentrations [μM]	Structure
H-7	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
ICI-182,780	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
LY-294002	0.0006, 0.002, 0.006, 0.02, 0.06, 0.2, 0.6, 2.0	
MG-132	0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0	

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Table S3: *continued from the previous page*

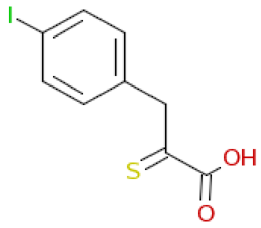
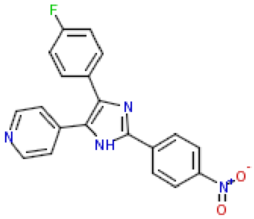
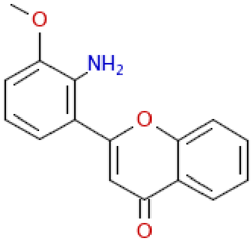
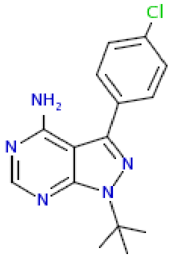
Compound name	Concentrations [μM]	Structure
PD-150606	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
PD-169316	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
PD-98059	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
PP-2	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	

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Table S3: *continued from the previous page*

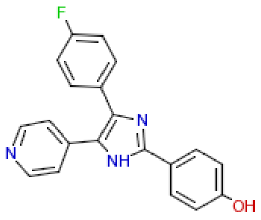
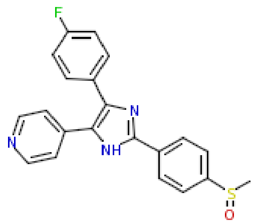
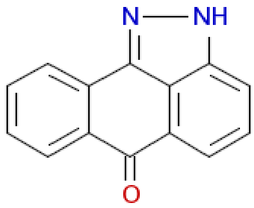
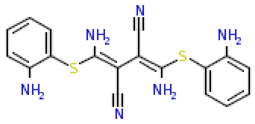
Compound name	Concentrations [μM]	Structure
SB-202190	0.006, 0.02, 0.06, 0.2, 0.6, 2.0, 6.0, 20.0	
SB-203580	0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1.0, 2.0, 3.0, 6.0, 10.0, 20.0	
SP-600125	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0, 10000.0	
TKK	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	(*not disclosed*)
UNKNOWN	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	(*not disclosed*)
UO-126	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	

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Table S3: *continued from the previous page*

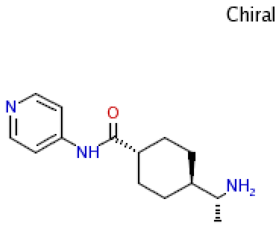
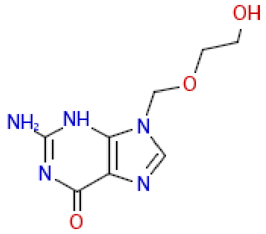
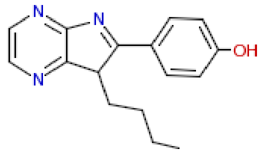
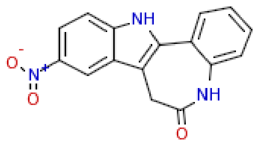
Compound name	Concentrations [μM]	Structure
Y-27632	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
acyclovir	0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5.0	
adenine arabinofuranoside	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	(*not disclosed*)
aloisine A	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
alsterpaullone	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	

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Table S3: *continued from the previous page*

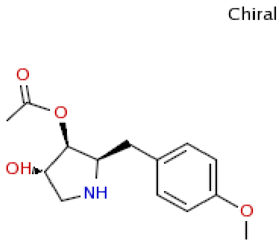
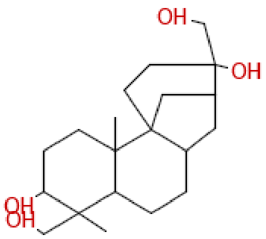
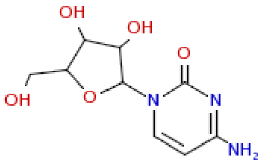
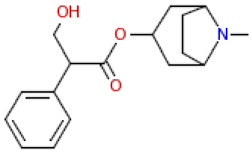
Compound name	Concentrations [μM]	Structure
anisomycin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
aphidicolin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
arabinofuranosylcytosine	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
atropine	0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5.0	

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Table S3: *continued from the previous page*

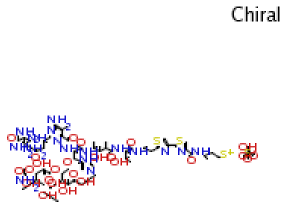
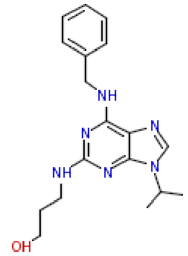
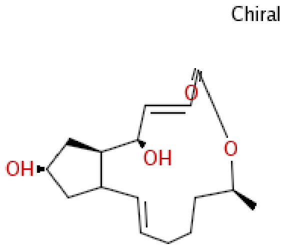
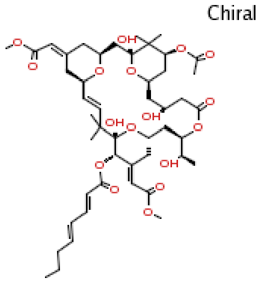
Compound name	Concentrations [μM]	Structure
bleomycin	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0, 10000.0	
bohemine	0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1.0, 2.0, 3.0, 6.0, 10.0, 20.0	
brefeldin A	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
bryostatin	6e-05, 0.0002, 0.0006, 0.002, 0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 1.0, 3.0, 10.0	

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Table S3: *continued from the previous page*

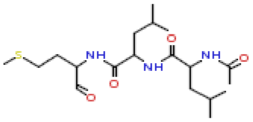
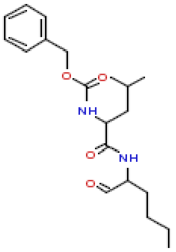
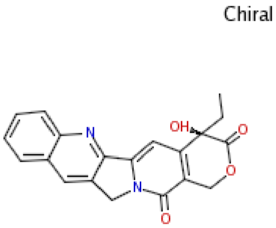
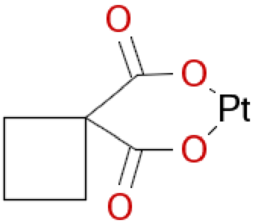
Compound name	Concentrations [μM]	Structure
calpain inhibitor 2 (ALLM)	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
calpeptin	0.006, 0.015, 0.02, 0.05, 0.06, 0.15, 0.2, 0.5, 0.6, 1.5, 2.0, 5.0, 6.0, 15.0, 20.0, 50.0	
camptothecin	0.0015, 0.003, 0.005, 0.01, 0.015, 0.03, 0.05, 0.1, 0.15, 0.3, 0.5, 1.0, 1.5, 3.0, 5.0, 10.0	
carboplatin	0.006, 0.02, 0.06, 0.2, 0.6, 2.0, 6.0, 20.0	

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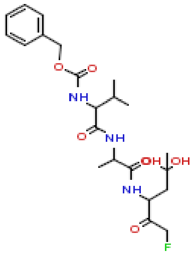
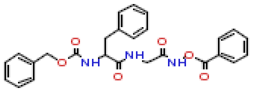
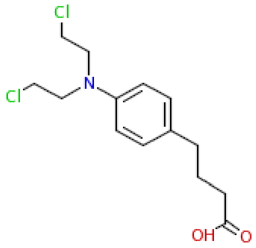
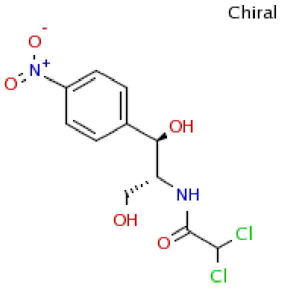
Table S3: *continued from the previous page*

Compound name	Concentrations [μM]	Structure
caspase inhibitor 1 (ZVAD)	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
cathepsin inhibitor I	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
chlorambucil	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
chloramphenicol	0.006, 0.02, 0.06, 0.2, 0.6, 2.0, 6.0, 20.0	

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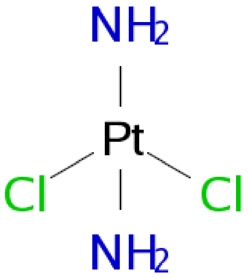
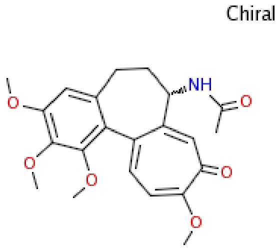
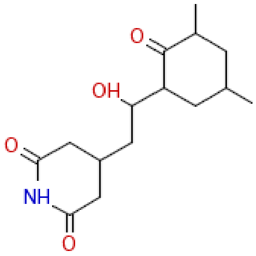
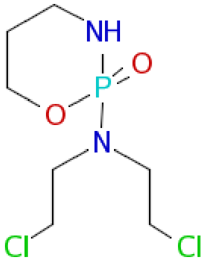
Compound name	Concentrations [μM]	Structure
cisplatin	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
colchicine	1e-05, 3e-05, 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0	
cyclohexamide	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
cyclophosphamide	0.0015, 0.003, 0.005, 0.01, 0.015, 0.03, 0.05, 0.1, 0.15, 0.3, 0.5, 1.0, 1.5, 3.0, 5.0, 10.0	

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Table S3: *continued from the previous page*

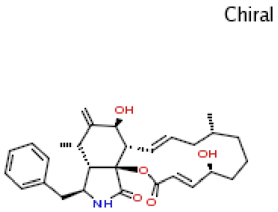
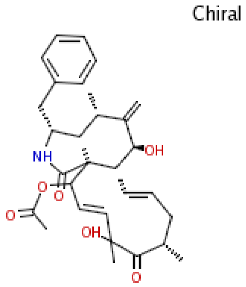
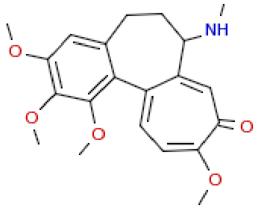
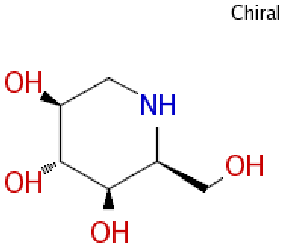
Compound name	Concentrations [μM]	Structure
cytochalasin B	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
cytochalasin D	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 10000.0	
demecolcine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
deoxymannojirimycin	0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0, 1000.0	

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Table S3: *continued from the previous page*

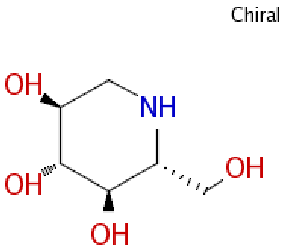
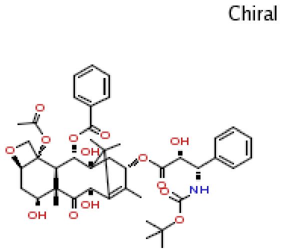
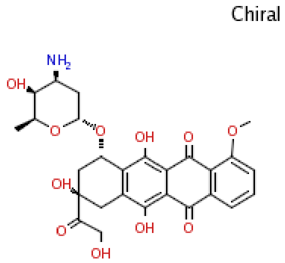
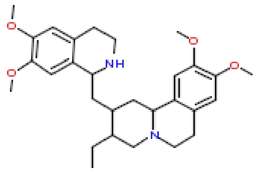
Compound name	Concentrations [μM]	Structure
deoxynojirimycin	0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0, 1000.0	
docetaxel	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0	
doxorubicin	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
emetine	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	

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Table S3: *continued from the previous page*

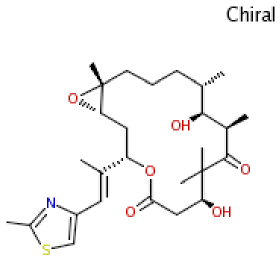
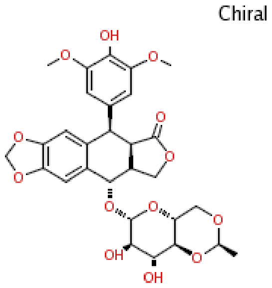
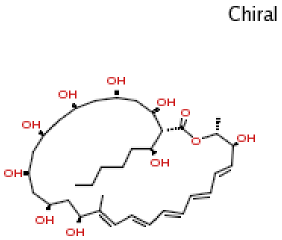
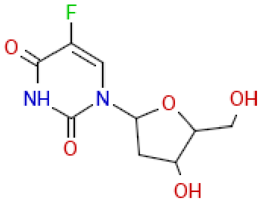
Compound name	Concentrations [µM]	Structure
epothilone B	3e-05, 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0	
etoposide	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
filipin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
floxuridine	0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0	

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Table S3: *continued from the previous page*

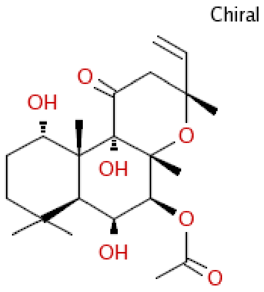
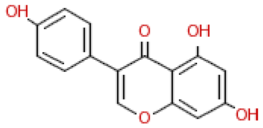
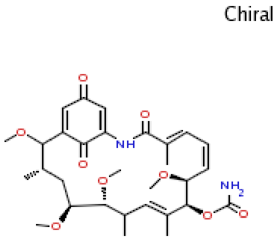
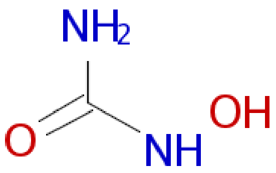
Compound name	Concentrations [μM]	Structure
forskolin	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	
genistein	0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 10000.0	
herbimycin A	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
hydroxyurea	0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0, 1000.0, 10000.0	

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Table S3: *continued from the previous page*

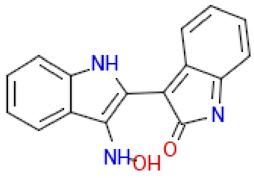
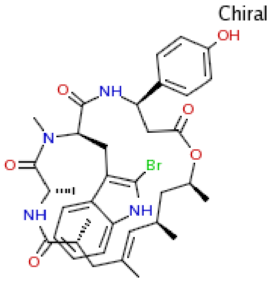
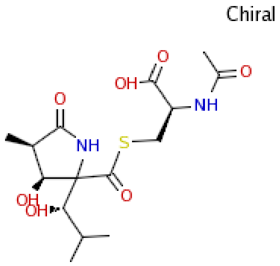
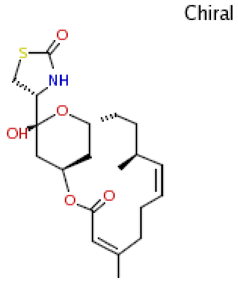
Compound name	Concentrations [μM]	Structure
indirubin monoxime	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
jasplakinolide	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0	
lactacystin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 10000.0	
latrunculin B	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0	

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Table S3: *continued from the previous page*

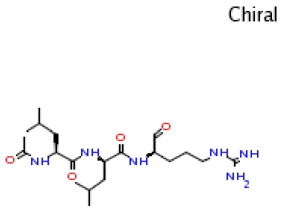
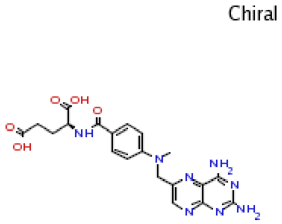
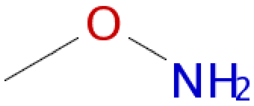
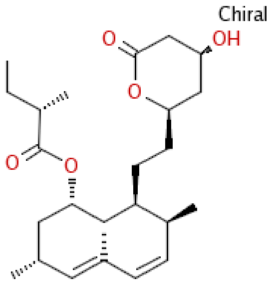
Compound name	Concentrations [μM]	Structure
leupeptin	0.006, 0.015, 0.02, 0.05, 0.06, 0.15, 0.2, 0.5, 0.6, 1.5, 2.0, 5.0, 6.0, 15.0, 20.0, 50.0	
methotrexate	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 10000.0	
methoxylamine	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	
mevinolin/lovastatin	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	

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Table S3: *continued from the previous page*

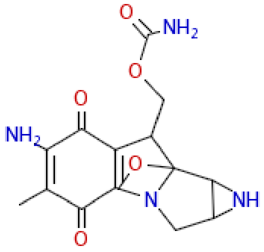
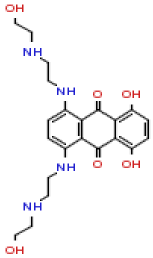
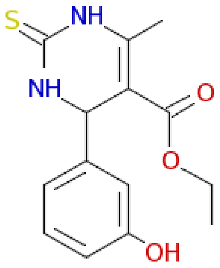
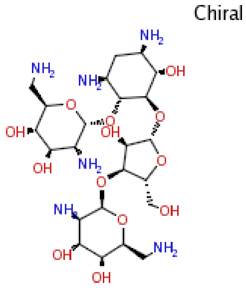
Compound name	Concentrations [μM]	Structure
mitomycin C	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
mitoxantrone	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
monastrol	0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0	
neomycin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	

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Table S3: *continued from the previous page*

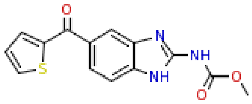
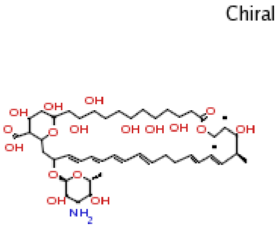
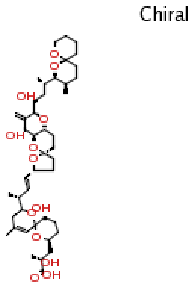
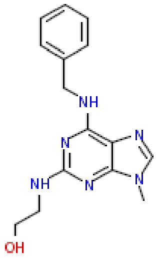
Compound name	Concentrations [μM]	Structure
nocodazole	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
nystatin	0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1.0, 2.0, 3.0, 6.0, 10.0, 20.0	
okadaic acid	6e-05, 0.0002, 0.0006, 0.002, 0.006, 0.02, 0.06, 0.2	
olomoucine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	

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Table S3: *continued from the previous page*

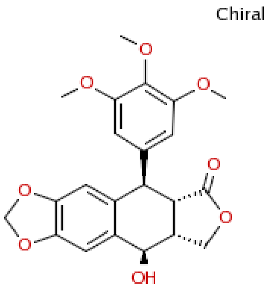
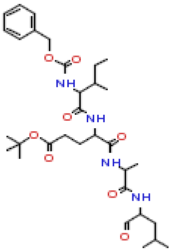
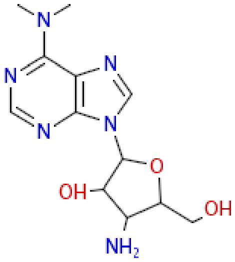
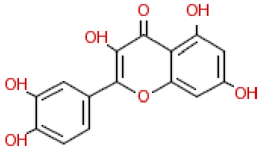
Compound name	Concentrations [μM]	Structure
podophyllotoxin	3e-06, 1e-05, 3e-05, 0.0001, 0.0003, 0.001, 0.003, 0.01	
proteasome inhibitor I	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
puromycin	0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0	
quercetin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	

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Table S3: *continued from the previous page*

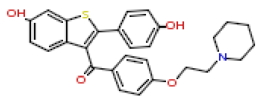
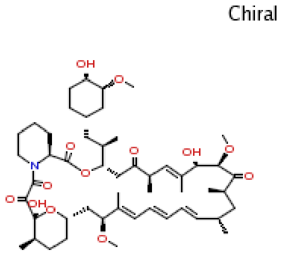
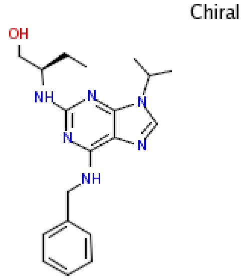
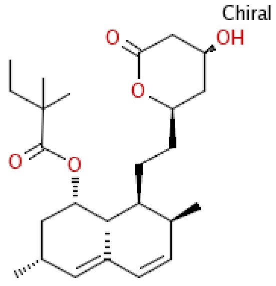
Compound name	Concentrations [μM]	Structure
raloxifene	0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5.0	
rapamycin	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 10000.0	
roscovitine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	
simvastatin	0.006, 0.02, 0.06, 0.2, 0.6, 2.0, 6.0, 20.0	

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Table S3: *continued from the previous page*

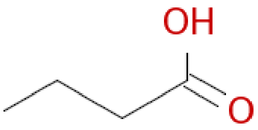
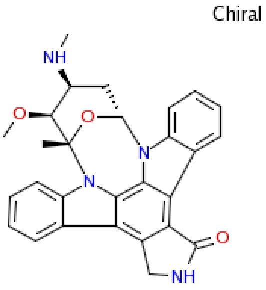
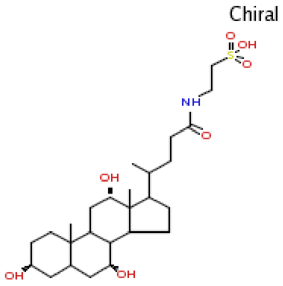
Compound name	Concentrations [μM]	Structure
sodium butyrate	0.083, 0.25, 0.83, 2.5, 8.3, 25.0, 83.0, 250.0	
sodium fluoride	0.15, 0.5, 1.5, 5.0, 15.0, 50.0, 150.0, 500.0, 10000.0	
staurosporine	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0	
taurocholate	0.0083, 0.025, 0.083, 0.25, 0.83, 2.5, 8.3, 25.0	

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Table S3: *continued from the previous page*

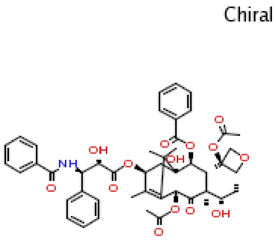
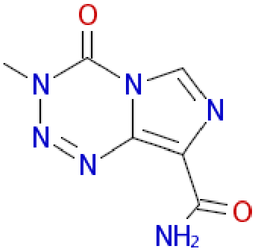
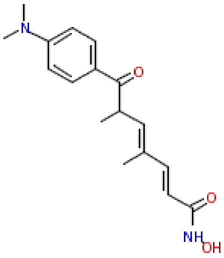
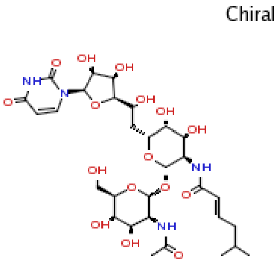
Compound name	Concentrations [μM]	Structure
taxol	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0	
temozolomide	0.006, 0.02, 0.06, 0.2, 0.6, 2.0, 6.0, 20.0	
trichostatin	0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 10000.0	
tunicamycin	0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, 50.0	

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Table S3: *continued from the previous page*

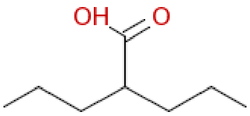
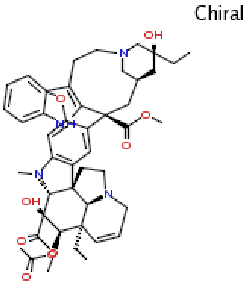
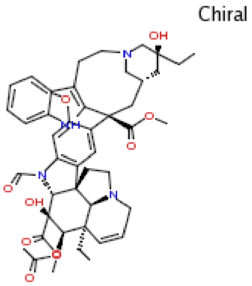
Compound name	Concentrations [μM]	Structure
valproic acid	0.15, 0.5, 1.5, 5.0, 15.0, 50.0, 150.0, 500.0	
vinblastine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 10000.0	
vincristine	0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0	

Table S4: Sizes of the phenotypic subpopulations visible to the eye in four compounds in different mechanistic classes, based on inspection of three fields of view in each of two replicate samples for each compound.

			Prevalence of phenotype [%]							
Mechanism	Compound	Concentration [μM]	Mitotic	Monoaster	Fragmented nucleus	Diffuse tubulin	Control	Large cell, large nucleus	Large cell, multiple nuclei	Large nucleus, multiple micronuclei
Microtubule stabilizer	taxol	1.0	27	3	0	0	0	0	2	24
Microtubule destabilizers	demecolcine	1.0	44	0	27	16	12	0	0	0
Aurora kinase inhibitors	AZ-A	1.0	1	0	3	0	11	35	50	0
Eg5 inhibitors	AZ-C	0.1	13	13	7	9	11	4	2	4

Table S5: The 15 most important image features for distinguishing each mechanism of action using the means method.

Mechanism of action	Rank	Score	Image feature
Actin disruptors	1	0.281215	Cytoplasm_Texture_GaborY_CorrActin_3
	2	0.267669	Cytoplasm_Texture_GaborX_CorrActin_3
	3	0.208613	Cells_Texture_GaborY_CorrActin_3
	4	0.187357	Cells_Texture_GaborX_CorrActin_3
	5	0.167390	Nuclei_Texture_SumVariance_CorrDAPI_3
	6	0.159650	Cytoplasm_Intensity_UpperQuartileIntensity_CorrTub
	7	0.156146	Cells_Intensity_MedianIntensity_CorrTub
	8	0.153290	Nuclei_Texture_Variance_CorrDAPI_3
	9	0.152532	Nuclei_Texture_GaborY_CorrActin_3
	10	0.147827	Nuclei_Texture_SumVariance_CorrDAPI_10
	11	0.138384	Nuclei_Texture_GaborX_CorrActin_3
	12	0.124821	Cytoplasm_Intensity_StdIntensity_CorrTub
	13	0.114793	Nuclei_Texture_SumEntropy_CorrDAPI_10
	14	0.113721	Nuclei_Intensity_LowerQuartileIntensity_CorrTub
	15	0.104418	Cytoplasm_Intensity_MeanIntensity_CorrTub
Aurora kinase inhibitors	1	0.179038	Cells_Intensity_IntegratedIntensityEdge_CorrTub
	2	0.164493	Cells_Intensity_IntegratedIntensity_CorrTub
	3	0.152114	Cytoplasm_Intensity_IntegratedIntensity_CorrTub
	4	0.119348	Nuclei_Texture_DifferenceEntropy_CorrDAPI_10
	5	0.097981	Cytoplasm_Intensity_IntegratedIntensityEdge_CorrTub
	6	0.096588	Cytoplasm_AreaShape_Area
	7	0.088599	Nuclei_Texture_DifferenceVariance_CorrDAPI_10
	8	0.087314	Nuclei_Intensity_MeanIntensityEdge_CorrActin
	9	0.074868	Cells_AreaShape_Area
	10	0.070440	Nuclei_Texture_GaborY_CorrTub_3
	11	0.068690	Cytoplasm_Texture_Correlation_CorrTub_10
	12	0.065225	Cytoplasm_AreaShape_Perimeter
	13	0.065225	Cells_AreaShape_Perimeter
	14	0.063790	Nuclei_Intensity_UpperQuartileIntensity_CorrActin
	15	0.063738	Cells_Intensity_UpperQuartileIntensity_CorrActin
Cholesterol-lowering	1	0.093230	Nuclei_Intensity_IntegratedIntensityEdge_CorrDAPI
	2	0.084346	Cells_AreaShape_Extent
	3	0.081998	Cells_Texture_GaborX_CorrTub_3
	4	0.080526	Nuclei_Intensity_MinIntensityEdge_CorrDAPI
	5	0.080094	Nuclei_Intensity_MinIntensity_CorrDAPI
	6	0.074491	Nuclei_Texture_DifferenceEntropy_CorrDAPI_10
	7	0.072067	Nuclei_Texture_GaborX_CorrTub_10
	8	0.069402	Nuclei_Texture_GaborY_CorrTub_10

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Table S5: *continued from the previous page*

Mechanism of action	Rank	Score	Image feature
	9	0.066591	Cells_Texture_Correlation_CorrTub_10
	10	0.058949	Cells_Zernike_3_1
	11	0.056188	Cytoplasm_Texture_InfoMeas2_CorrTub_3
	12	0.053279	Nuclei_Texture_Contrast_CorrDAPI_10
	13	0.052195	Nuclei_Texture_InverseDifferenceMoment_CorrDAPI_10
	14	0.050724	Cytoplasm_Texture_InfoMeas1_CorrTub_3
	15	0.050655	Cytoplasm_Texture_GaborX_CorrTub_3
DNA damage	1	0.045092	Cytoplasm_Intensity_IntegratedIntensity_CorrTub
	2	0.044668	Cytoplasm_Intensity_MeanIntensity_CorrTub
	3	0.043116	Cells_Intensity_MeanIntensityEdge_CorrTub
	4	0.040310	Nuclei_Intensity_MaxIntensity_CorrActin
	5	0.039393	Cells_Neighbors_PercentTouching_10
	6	0.039011	Cytoplasm_Intensity_MedianIntensity_CorrTub
	7	0.038127	Nuclei_Neighbors_PercentTouching_10
	8	0.035094	Nuclei_Intensity_MeanIntensity_CorrActin
	9	0.034896	Cytoplasm_AreaShape_FormFactor
	10	0.034740	Nuclei_Neighbors_PercentTouching_20
	11	0.033824	Cytoplasm_Intensity_StdIntensityEdge_CorrActin
	12	0.033655	Cells_Intensity_MedianIntensity_CorrTub
	13	0.032402	Cells_Intensity_StdIntensityEdge_CorrTub
	14	0.031690	Cytoplasm_Intensity_StdIntensity_CorrActin
	15	0.031184	Nuclei_Intensity_StdIntensityEdge_CorrDAPI
DNA replication	1	0.079445	Nuclei_Neighbors_PercentTouching_20
	2	0.060039	Nuclei_Neighbors_NumberOfNeighbors_20
	3	0.045092	Cytoplasm_Intensity_IntegratedIntensity_CorrTub
	4	0.044645	Cells_Intensity_UpperQuartileIntensity_CorrTub
	5	0.042304	Nuclei_Texture_SumEntropy_CorrDAPI_3
	6	0.040094	Cytoplasm_Texture_GaborY_CorrActin_3
	7	0.039256	Nuclei_Neighbors_PercentTouching_10
	8	0.037857	Nuclei_Texture_InfoMeas1_CorrDAPI_10
	9	0.037525	Nuclei_Texture_InfoMeas1_CorrDAPI_3
	10	0.035017	Cytoplasm_Intensity_UpperQuartileIntensity_CorrTub
	11	0.033534	Cytoplasm_AreaShape_Solidity
	12	0.032936	Cytoplasm_Intensity_MeanIntensityEdge_CorrTub
	13	0.032238	Cytoplasm_Intensity_IntegratedIntensity_CorrActin
	14	0.032039	Nuclei_Intensity_MedianIntensity_CorrTub
	15	0.031169	Cells_AreaShape_Area
Eg5 inhibitors	1	0.136123	Cytoplasm_Texture_GaborX_CorrTub_10
	2	0.134797	Nuclei_Intensity_StdIntensity_CorrTub
	3	0.133356	Cytoplasm_Texture_GaborY_CorrTub_10
	4	0.101140	Cells_Intensity_StdIntensity_CorrTub
	5	0.095494	Nuclei_Intensity_MedianIntensity_CorrActin
	6	0.092331	Nuclei_Intensity_MeanIntensity_CorrActin
	7	0.088028	Cells_Texture_InfoMeas1_CorrTub_3
	8	0.087820	Nuclei_Intensity_LowerQuartileIntensity_CorrActin
	9	0.082726	Cells_Texture_SumVariance_CorrTub_10
	10	0.080209	Cells_Texture_InfoMeas2_CorrTub_3
	11	0.077260	Nuclei_Intensity_UpperQuartileIntensity_CorrActin
	12	0.075767	Nuclei_Intensity_MeanIntensityEdge_CorrActin
	13	0.075408	Nuclei_Texture_SumEntropy_CorrTub_10
	14	0.072718	Cells_Intensity_StdIntensity_CorrActin

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Table S5: *continued from the previous page*

Mechanism of action	Rank	Score	Image feature
	15	0.071903	Cells_Intensity_UpperQuartileIntensity_CorrActin
Epithelial	1	0.225988	Nuclei_Neighbors_NumberOfNeighbors_20
	2	0.204335	Nuclei_Neighbors_NumberOfNeighbors_10
	3	0.153913	Nuclei_Neighbors_PercentTouching_20
	4	0.135766	Nuclei_Neighbors_PercentTouching_10
	5	0.096608	Cytoplasm_Intensity_MinIntensity_CorrTub
	6	0.091836	Cells_Intensity_MinIntensityEdge_CorrTub
	7	0.087701	Cells_Intensity_MinIntensity_CorrTub
	8	0.085939	Cytoplasm_Intensity_MinIntensityEdge_CorrTub
	9	0.081036	Nuclei_Intensity_IntegratedIntensityEdge_CorrTub
	10	0.066969	Nuclei_Intensity_IntegratedIntensity_CorrTub
	11	0.060913	Cells_AreaShape_FormFactor
	12	0.055912	Cytoplasm_Texture_Contrast_CorrTub_3
	13	0.053228	Nuclei_Texture_Contrast_CorrDAPI_10
	14	0.052187	Cells_Intensity_IntegratedIntensity_CorrTub
	15	0.048274	Cytoplasm_AreaShape_MinorAxisLength
Kinase inhibitors	1	0.134838	Nuclei_Intensity_IntegratedIntensityEdge_CorrTub
	2	0.131200	Cytoplasm_Intensity_IntegratedIntensityEdge_CorrTub
	3	0.130932	Cytoplasm_AreaShape_FormFactor
	4	0.109810	Cells_Intensity_IntegratedIntensityEdge_CorrTub
	5	0.098938	Nuclei_Intensity_MeanIntensity_CorrDAPI
	6	0.098835	Nuclei_Intensity_IntegratedIntensity_CorrDAPI
	7	0.093230	Nuclei_Intensity_IntegratedIntensityEdge_CorrDAPI
	8	0.085660	Nuclei_Intensity_UpperQuartileIntensity_CorrDAPI
	9	0.084234	Nuclei_Texture_InfoMeas1_CorrDAPI_3
	10	0.077387	Nuclei_Intensity_MedianIntensity_CorrDAPI
	11	0.073766	Cytoplasm_AreaShape_Area
	12	0.069723	Nuclei_Intensity_MaxIntensity_CorrDAPI
	13	0.069517	Cytoplasm_Texture_InfoMeas1_CorrActin_10
	14	0.066969	Nuclei_Intensity_IntegratedIntensity_CorrTub
	15	0.066534	Cytoplasm_Texture_GaborX_CorrTub_3
Microtubule destabilizers	1	0.066222	Nuclei_Texture_AngularSecondMoment_CorrTub_10
	2	0.050640	Nuclei_Texture_AngularSecondMoment_CorrTub_3
	3	0.050038	Nuclei_Texture_SumVariance_CorrTub_3
	4	0.047789	Nuclei_Texture_Correlation_CorrDAPI_3
	5	0.047526	Nuclei_Intensity_IntegratedIntensityEdge_CorrActin
	6	0.045564	Nuclei_Texture_InfoMeas1_CorrDAPI_3
	7	0.043635	Cells_Texture_Variance_CorrTub_10
	8	0.039665	Nuclei_Texture_InfoMeas2_CorrDAPI_3
	9	0.036823	Nuclei_Texture_SumVariance_CorrActin_10
	10	0.035998	Nuclei_Intensity_StdIntensityEdge_CorrActin
	11	0.035751	Nuclei_Texture_SumVariance_CorrTub_10
	12	0.035645	Cells_Intensity_MeanIntensityEdge_CorrActin
	13	0.035200	Cells_Intensity_StdIntensityEdge_CorrActin
	14	0.034137	Nuclei_Texture_Variance_CorrTub_3
	15	0.034126	Cells_Texture_InfoMeas1_CorrActin_3
Microtubule stabilizers	1	0.266498	Nuclei_Intensity_StdIntensityEdge_CorrTub
	2	0.230081	Cytoplasm_Texture_AngularSecondMoment_CorrTub_3
	3	0.198321	Cells_Texture_GaborY_CorrTub_10
	4	0.191167	Cytoplasm_Texture_SumEntropy_CorrTub_3

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Table S5: *continued from the previous page*

Mechanism of action	Rank	Score	Image feature
	5	0.190461	Cytoplasm_Texture_AngularSecondMoment_CorrTub_10
	6	0.189933	Cytoplasm_Intensity_StdIntensityEdge_CorrTub
	7	0.185869	Cells_Texture_GaborX_CorrTub_10
	8	0.178724	Cells_Texture_SumAverage_CorrTub_3
	9	0.174168	Cells_Texture_SumAverage_CorrTub_10
	10	0.160962	Cytoplasm_Texture_GaborY_CorrTub_10
	11	0.154275	Nuclei_Intensity_StdIntensity_CorrTub
	12	0.153313	Cytoplasm_Texture_SumAverage_CorrTub_3
	13	0.150168	Cells_Intensity_StdIntensity_CorrTub
	14	0.150092	Cytoplasm_Texture_SumEntropy_CorrTub_10
	15	0.149339	Cytoplasm_Texture_SumAverage_CorrTub_10
Protein degradation	1	0.084139	Cytoplasm_Texture_GaborX_CorrTub_10
	2	0.083899	Cytoplasm_Zernike_3_1
	3	0.081998	Cytoplasm_Intensity_IntegratedIntensity_CorrActin
	4	0.081266	Cytoplasm_Texture_GaborY_CorrTub_10
	5	0.075760	Cytoplasm_AreaShape_Area
	6	0.071945	Cytoplasm_AreaShape_Solidity
	7	0.067088	Cytoplasm_Texture_GaborX_CorrActin_10
	8	0.064361	Cytoplasm_Zernike_0_0
	9	0.056766	Cytoplasm_Texture_GaborY_CorrActin_10
	10	0.056530	Cells_Texture_GaborX_CorrActin_10
	11	0.046923	Cells_Texture_GaborY_CorrActin_10
	12	0.045643	Cells_Texture_Contrast_CorrActin_10
	13	0.045052	Cytoplasm_AreaShape_Extent
	14	0.044919	Cells_Texture_GaborX_CorrTub_10
	15	0.040845	Cells_Texture_DifferenceVariance_CorrActin_10
Protein synthesis	1	0.092005	Cytoplasm_Texture_Correlation_CorrActin_3
	2	0.086052	Cells_Texture_Correlation_CorrActin_3
	3	0.068384	Cytoplasm_Texture_DifferenceVariance_CorrActin_3
	4	0.066499	Cells_Texture_DifferenceVariance_CorrActin_3
	5	0.064255	Cytoplasm_Texture_Contrast_CorrTub_3
	6	0.053710	Cytoplasm_Texture_SumAverage_CorrActin_3
	7	0.053177	Cytoplasm_Texture_Contrast_CorrActin_3
	8	0.052644	Cells_Texture_InfoMeas1_CorrActin_3
	9	0.051165	Cells_Texture_SumVariance_CorrTub_3
	10	0.050724	Cytoplasm_Texture_InfoMeas1_CorrTub_3
	11	0.050487	Cytoplasm_Texture_GaborX_CorrTub_3
	12	0.048193	Cells_Texture_SumVariance_CorrTub_10
	13	0.045031	Cells_Texture_Variance_CorrTub_10
	14	0.041479	Nuclei_Intensity_MinIntensityEdge_CorrActin
	15	0.038174	Cells_Intensity_LowerQuartileIntensity_CorrActin

Table S6: The number of times each feature was selected by the SVMRFE method. Only the 20 most selected features are shown.

Feature	Number of times selected
Cells_Neighbors_NumberOfNeighbors_10	171
Cytoplasm_Texture_GaborY_CorrTub_10	138
Cytoplasm_Texture_GaborX_CorrTub_10	133
Cells_Neighbors_PercentTouching_3	115
Cells_Neighbors_NumberOfNeighbors_3	112
Nuclei_Intensity_LowerQuartileIntensity_CorrDAPI	105
Cells_Intensity_IntegratedIntensityEdge_CorrTub	85
Cytoplasm_Texture_GaborX_CorrActin_10	79
Nuclei_Intensity_MinIntensityEdge_CorrDAPI	78
Nuclei_AreaShape_FormFactor	77
Cells_Neighbors_PercentTouching_10	74
Nuclei_Intensity_MaxIntensity_CorrTub	74
Nuclei_Intensity_MedianIntensity_CorrDAPI	74
Nuclei_Texture_SumAverage_CorrDAPI_3	73
Nuclei_Texture_SumAverage_CorrDAPI_10	72
Nuclei_Intensity_MinIntensity_CorrDAPI	69
Cells_Intensity_MeanIntensityEdge_CorrTub	68
Cytoplasm_Texture_GaborY_CorrActin_10	67
Cells_Intensity_StdIntensityEdge_CorrTub	65
Nuclei_Texture_SumVariance_CorrDAPI_3	61

Table S7: Accuracies for all combinations of dimensionality-reducing preprocessing method and profiling method

	Profiling method			
Dimensionality reduction method	Means	KS	SVM	GM
None	83%	83%	81%	81%
Factor analysis	94%	90%	80%	84%
PCA	81%	90%	73%	73%
Factor-analysis–based feature selection	67%	65%	68%	75%

Table S8: The 15 features most heavily loaded onto each factor in the 50-factor model

Factor number	Measurements
1	Nuclei_Texture_GaborY_CorrDAPI_10 Nuclei_Intensity_StdIntensityEdge_CorrActin Nuclei_Texture_Variance_CorrDAPI_3 Nuclei_Texture_Entropy_CorrActin_10 Nuclei_Texture_GaborX_CorrDAPI_10
2	Nuclei_Texture_DifferenceEntropy_CorrDAPI_10 Nuclei_Texture_Contrast_CorrDAPI_10 Cytoplasm_Texture_InfoMeas2_CorrTub_3 Nuclei_Texture_DifferenceVariance_CorrDAPI_10 Cytoplasm_Texture_SumVariance_CorrTub_3

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Table S8: *continued from the previous page*

Factor number	Measurements
3	Cells_Zernike_1_1
	Cells_Zernike_0_0
	Cells_Neighbors_FirstClosestYVector_10
	Cells_Neighbors_FirstClosestYVector_3
	Cytoplasm_Zernike_1_1
4	Nuclei_Texture_AngularSecondMoment_CorrTub_3
	Nuclei_Texture_Entropy_CorrTub_3
	Nuclei_Intensity_MinIntensity_CorrTub
	Nuclei_Intensity_MinIntensityEdge_CorrTub
	Cells_Texture_AngularSecondMoment_CorrTub_3
5	Nuclei_Texture_SumEntropy_CorrTub_10
	Nuclei_Texture_SumAverage_CorrTub_10
	Cells_Texture_SumAverage_CorrTub_10
	Nuclei_Texture_SumAverage_CorrTub_3
	Nuclei_Texture_Entropy_CorrTub_10
6	Cells_Intensity_IntegratedIntensityEdge_CorrTub
	Cytoplasm_Texture_InfoMeas2_CorrActin_3
	Nuclei_Texture_SumEntropy_CorrActin_3
	Nuclei_Texture_DifferenceEntropy_CorrTub_3
	Cytoplasm_Texture_InfoMeas1_CorrActin_3
7	Nuclei_Zernike_2_0
	Nuclei_Zernike_0_0
	Nuclei_AreaShape_Eccentricity
	Nuclei_Zernike_1_1
	Nuclei_Zernike_2_2
8	Nuclei_Texture_DifferenceEntropy_CorrTub_10
	Nuclei_Texture_Entropy_CorrTub_10
	Nuclei_Texture_SumEntropy_CorrTub_3
	Nuclei_Texture_DifferenceEntropy_CorrDAPI_10
	Nuclei_Texture_Contrast_CorrDAPI_10
9	Cells_AreaShape_FormFactor
	Cytoplasm_Texture_InverseDifferenceMoment_CorrActin_10
	Cells_Neighbors_PercentTouching_3
	Cytoplasm_AreaShape_Perimeter
	Cells_AreaShape_Perimeter
10	Cells_Intensity_MeanIntensityEdge_CorrTub
	Cytoplasm_Intensity_MeanIntensityEdge_CorrTub
	Cytoplasm_Intensity_MeanIntensity_CorrTub
	Cytoplasm_Intensity_LowerQuartileIntensity_CorrTub
	Cytoplasm_Intensity_MedianIntensity_CorrTub

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Table S8: *continued from the previous page*

Factor number	Measurements
11	Nuclei_Texture_Entropy_CorrTub_10
	Nuclei_Texture_Entropy_CorrTub_3
	Cytoplasm_AreaShape_MinorAxisLength
	Nuclei_Texture_AngularSecondMoment_CorrTub_3
	Cells_AreaShape_MinorAxisLength
12	Cells_Neighbors_SecondClosestYVector_3
	Cells_Neighbors_SecondClosestYVector_10
	Cytoplasm_Texture_InfoMeas2_CorrTub_3
	Nuclei_Texture_InfoMeas2_CorrTub_3
	Nuclei_Texture_InfoMeas1_CorrTub_3
13	Nuclei_Texture_DifferenceEntropy_CorrActin_3
	Nuclei_Texture_Contrast_CorrActin_3
	Nuclei_Texture_InverseDifferenceMoment_CorrDAPI_3
	Nuclei_Texture_InverseDifferenceMoment_CorrActin_3
	Nuclei_Texture_DifferenceVariance_CorrActin_3
14	Cells_Texture_Correlation_CorrTub_3
	Cytoplasm_Texture_Correlation_CorrTub_3
	Cells_Texture_InfoMeas2_CorrTub_3
	Cells_Texture_SumEntropy_CorrTub_10
	Cells_Texture_SumEntropy_CorrTub_3
15	Cells_Texture_Variance_CorrActin_3
	Nuclei_Texture_Variance_CorrActin_3
	Cells_Texture_Entropy_CorrActin_3
	Cells_Texture_Variance_CorrActin_10
	Nuclei_Texture_SumVariance_CorrActin_3
16	Cytoplasm_Texture_Correlation_CorrActin_3
	Cytoplasm_Texture_InfoMeas2_CorrActin_3
	Nuclei_Texture_SumVariance_CorrTub_3
	Nuclei_Texture_Variance_CorrTub_3
	Cells_Texture_SumEntropy_CorrActin_3
17	Nuclei_Texture_Entropy_CorrActin_3
	Nuclei_Texture_AngularSecondMoment_CorrActin_3
	Nuclei_Texture_Entropy_CorrActin_10
	Nuclei_Texture_AngularSecondMoment_CorrActin_10
	Nuclei_Texture_SumEntropy_CorrActin_3
18	Cells_Texture_Entropy_CorrTub_3
	Cytoplasm_Texture_Entropy_CorrTub_3
	Cells_Texture_DifferenceEntropy_CorrTub_3
	Cells_Texture_AngularSecondMoment_CorrTub_3
	Cells_Texture_InverseDifferenceMoment_CorrTub_3

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Table S8: *continued from the previous page*

Factor number	Measurements
19	Cells_Intensity_MinIntensityEdge_CorrActin Cytoplasm_Intensity_MinIntensityEdge_CorrActin Cytoplasm_Intensity_MinIntensity_CorrActin Cells_Intensity_MinIntensity_CorrActin Cells_Intensity_MeanIntensityEdge_CorrActin
20	Cells_Texture_Correlation_CorrTub_3 Cells_Texture_InfoMeas1_CorrTub_3 Cytoplasm_Texture_Correlation_CorrActin_3 Cytoplasm_Texture_Correlation_CorrTub_3 Cells_Texture_InfoMeas2_CorrActin_3
21	Cytoplasm_Texture_SumEntropy_CorrActin_10 Cells_Texture_InverseDifferenceMoment_CorrTub_10 Cytoplasm_Texture_Entropy_CorrActin_10 Cytoplasm_Texture_SumEntropy_CorrActin_3 Cytoplasm_Texture_AngularSecondMoment_CorrActin_10
22	Cytoplasm_Intensity_StdIntensityEdge_CorrActin Cells_Intensity_StdIntensityEdge_CorrActin Cells_Intensity_StdIntensity_CorrActin Cytoplasm_Intensity_StdIntensity_CorrActin Nuclei_Intensity_StdIntensityEdge_CorrActin
23	Nuclei_Intensity_UpperQuartileIntensity_CorrActin Nuclei_Intensity_IntegratedIntensity_CorrActin Nuclei_Texture_SumAverage_CorrActin_10 Nuclei_Intensity_MedianIntensity_CorrActin Nuclei_Texture_SumAverage_CorrActin_3
24	Nuclei_Intensity_IntegratedIntensityEdge_CorrTub Nuclei_Intensity_MeanIntensityEdge_CorrTub Nuclei_Intensity_MaxIntensityEdge_CorrTub Nuclei_Texture_Correlation_CorrActin_10 Cytoplasm_Intensity_StdIntensityEdge_CorrTub
25	Cytoplasm_Zernike_0_0 Cytoplasm_Zernike_1_1 Cytoplasm_Intensity_IntegratedIntensity_CorrTub Cells_AreaShape_MinorAxisLength Cytoplasm_Intensity_IntegratedIntensity_CorrActin
26	Nuclei_Intensity_IntegratedIntensityEdge_CorrTub Nuclei_Intensity_MaxIntensityEdge_CorrTub Cytoplasm_Intensity_MaxIntensityEdge_CorrTub Nuclei_Texture_SumEntropy_CorrDAPI_10 Nuclei_Texture_SumVariance_CorrDAPI_10

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Table S8: *continued from the previous page*

Factor number	Measurements
27	Cytoplasm_Intensity_IntegratedIntensityEdge_CorrActin Cells_Intensity_IntegratedIntensityEdge_CorrActin Nuclei_Intensity_MeanIntensityEdge_CorrActin Nuclei_Intensity_MinIntensityEdge_CorrActin Nuclei_Intensity_MinIntensity_CorrActin
28	Nuclei_Texture_AngularSecondMoment_CorrDAPI_3 Nuclei_Texture_Entropy_CorrDAPI_3 Cells_Texture_SumVariance_CorrTub_10 Nuclei_Texture_InverseDifferenceMoment_CorrDAPI_3 Cells_Texture_SumVariance_CorrTub_3
29	Nuclei_Texture_DifferenceEntropy_CorrTub_3 Nuclei_Texture_DifferenceVariance_CorrTub_3 Nuclei_Texture_Contrast_CorrTub_3 Cells_Texture_SumVariance_CorrActin_10 Nuclei_Texture_InverseDifferenceMoment_CorrTub_3
30	Cells_Intensity_MeanIntensityEdge_CorrActin Cells_Intensity_MaxIntensityEdge_CorrActin Cytoplasm_Intensity_MaxIntensity_CorrActin Cytoplasm_Intensity_MeanIntensityEdge_CorrActin Cytoplasm_Intensity_MeanIntensity_CorrActin
31	Cells_Intensity_MaxIntensityEdge_CorrTub Cells_Intensity_MeanIntensityEdge_CorrTub Cytoplasm_Texture_Correlation_CorrActin_3 Cytoplasm_Intensity_MeanIntensityEdge_CorrTub Cytoplasm_Intensity_MaxIntensity_CorrTub
32	Cytoplasm_Texture_Entropy_CorrActin_3 Cytoplasm_Texture_InverseDifferenceMoment_CorrActin_3 Cytoplasm_Texture_AngularSecondMoment_CorrActin_3 Cells_Texture_InverseDifferenceMoment_CorrActin_3 Cytoplasm_Texture_DifferenceEntropy_CorrActin_3
33	Nuclei_Texture_Correlation_CorrActin_3 Nuclei_Texture_InfoMeas2_CorrActin_3 Nuclei_Texture_InfoMeas1_CorrActin_3 Nuclei_Texture_Correlation_CorrActin_10 Cytoplasm_Intensity_MeanIntensity_CorrTub
34	Nuclei_Intensity_IntegratedIntensity_CorrActin Nuclei_Intensity_IntegratedIntensityEdge_CorrActin Cells_Neighbors_FirstClosestYVector_10 Cells_Neighbors_FirstClosestYVector_3 Cells_Neighbors_FirstClosestXVector_10

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Table S8: *continued from the previous page*

Factor number	Measurements
35	Cytoplasm_Intensity_StdIntensity_CorrTub
	Cells_Texture_DifferenceVariance_CorrTub_10
	Cytoplasm_AreaShape_Eccentricity
	Cells_AreaShape_Eccentricity
	Cytoplasm_Zernike_2_2
36	Cells_Intensity_LowerQuartileIntensity_CorrActin
	Cells_Intensity_MedianIntensity_CorrActin
	Cells_Intensity_MeanIntensity_CorrActin
	Cytoplasm_Intensity_MedianIntensity_CorrActin
	Cytoplasm_Intensity_MeanIntensity_CorrActin
37	Nuclei_Intensity_IntegratedIntensityEdge_CorrDAPI
	Nuclei_AreaShape_Solidity
	Nuclei_AreaShape_FormFactor
	Nuclei_AreaShape_Extent
	Nuclei_Intensity_MeanIntensityEdge_CorrDAPI
38	Cytoplasm_AreaShape_Solidity
	Cytoplasm_AreaShape_Extent
	Cytoplasm_Texture_Contrast_CorrActin_3
	Cytoplasm_Intensity_MinIntensity_CorrTub
	Cells_Intensity_MinIntensity_CorrTub
39	Nuclei_Texture_SumAverage_CorrDAPI_10
	Nuclei_Intensity_StdIntensity_CorrTub
	Nuclei_Texture_SumAverage_CorrDAPI_3
	Nuclei_Texture_GaborX_CorrTub_10
	Cells_Intensity_MaxIntensity_CorrTub
40	Nuclei_Texture_Contrast_CorrDAPI_10
	Nuclei_Texture_DifferenceVariance_CorrDAPI_10
	Cells_Texture_DifferenceVariance_CorrActin_10
	Nuclei_Texture_SumVariance_CorrDAPI_3
	Cells_Texture_Contrast_CorrActin_10
41	Cytoplasm_Texture_SumAverage_CorrTub_10
	Cytoplasm_Texture_SumAverage_CorrTub_3
	Cells_Texture_SumAverage_CorrTub_3
	Cells_Texture_SumAverage_CorrTub_10
	Nuclei_Texture_SumAverage_CorrTub_10
42	Cytoplasm_Texture_Contrast_CorrTub_10
	Cytoplasm_Texture_DifferenceVariance_CorrTub_10
	Cytoplasm_Texture_DifferenceEntropy_CorrTub_3
	Cytoplasm_Intensity_UpperQuartileIntensity_CorrTub
	Cells_Texture_Contrast_CorrTub_3

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Table S8: *continued from the previous page*

Factor number	Measurements
43	Cytoplasm_Intensity_IntegratedIntensityEdge_CorrTub
	Cells_Intensity_IntegratedIntensityEdge_CorrTub
	Nuclei_Texture_Entropy_CorrActin_3
	Nuclei_Intensity_IntegratedIntensityEdge_CorrTub
	Nuclei_Texture_SumEntropy_CorrActin_10
44	Nuclei_AreaShape_Perimeter
	Nuclei_AreaShape_Area
	Nuclei_AreaShape_MajorAxisLength
	Nuclei_Intensity_IntegratedIntensity_CorrDAPI
	Nuclei_AreaShape_MinorAxisLength
45	Cells_Zernike_3_1
	Cytoplasm_AreaShape_Eccentricity
	Cells_AreaShape_Eccentricity
	Cells_Zernike_0_0
	Cells_AreaShape_Solidity
46	Nuclei_AreaShape_Eccentricity
	Nuclei_Intensity_IntegratedIntensityEdge_CorrDAPI
	Nuclei_Zernike_0_0
	Nuclei_Intensity_MeanIntensityEdge_CorrDAPI
	Nuclei_Zernike_2_0
47	Cells_Intensity_MinIntensity_CorrActin
	Cytoplasm_Intensity_MinIntensity_CorrActin
	Cytoplasm_Intensity_MinIntensityEdge_CorrActin
	Cells_Intensity_MinIntensityEdge_CorrActin
	Nuclei_Neighbors_PercentTouching_10
48	Cells_Texture_SumAverage_CorrActin_3
	Cells_Texture_SumAverage_CorrActin_10
	Nuclei_Texture_Variance_CorrDAPI_3
	Nuclei_Neighbors_SecondClosestXVector_20
	Nuclei_Neighbors_SecondClosestXVector_10
49	Cells_Texture_DifferenceVariance_CorrTub_3
	Cells_Texture_Contrast_CorrTub_3
	Cytoplasm_Texture_DifferenceVariance_CorrTub_3
	Cytoplasm_Texture_Contrast_CorrTub_3
	Cells_Texture_DifferenceEntropy_CorrTub_3
50	Cells_Texture_Correlation_CorrActin_10
	Cells_Texture_InfoMeas2_CorrActin_3
	Cells_Texture_Correlation_CorrActin_3
	Cells_Texture_InfoMeas2_CorrActin_10
	Nuclei_Texture_Contrast_CorrActin_3

Table S9: Distribution of mechanisms of action across batches

Mechanism of action	Number of compounds	Batches
Actin disruptors	3	02, 01
Aurora kinase inhibitors	3	01, 04, 03
Cholesterol-lowering	2	09
DMSO	1	02, 10, 03, 01, 06, 08, 07, 09, 04, 05
DNA damage	4	04, 03
DNA replication	4	08, 02, 09
Eg5 inhibitors	2	03, 10
Epithelial	3	05, 08, 10
Kinase inhibitors	3	07
Microtubule destabilizers	4	01, 03
Microtubule stabilizers	3	07, 01
Protein degradation	4	07, 02, 06
Protein synthesis	3	04, 03

Table S10: AUCs and p-values for the five profiling methods

	Profiling method									
	Means		KS statistic		SVM normalvector		Gaussian mixture		Factor analysis	
Mechanism	AUC	p-value	AUC	p-value	AUC	p-value	AUC	p-value	AUC	p-value
Actin disruptors	0.674	0.0295	0.914	≤ 0.0001	0.998	≤ 0.0001	0.689	0.0196	0.979	≤ 0.0001
Aurora kinase inhibitors	1.000	≤ 0.0001	1.000	≤ 0.0001	0.988	≤ 0.0001	1.000	≤ 0.0001	1.000	≤ 0.0001
Cholesterol-lowering	0.879	≤ 0.0001	0.966	≤ 0.0001	0.923	≤ 0.0001	0.741	0.0005	0.965	≤ 0.0001
DNA damage	0.834	≤ 0.0001	0.742	≤ 0.0001	0.942	≤ 0.0001	0.868	≤ 0.0001	0.930	≤ 0.0001
DNA replication	0.838	≤ 0.0001	0.708	≤ 0.0001	0.816	≤ 0.0001	0.834	≤ 0.0001	0.903	≤ 0.0001
Eg5 inhibitors	0.987	≤ 0.0001	0.994	≤ 0.0001	0.984	≤ 0.0001	0.995	≤ 0.0001	1.000	≤ 0.0001
Epithelial	0.973	≤ 0.0001	0.966	≤ 0.0001	0.924	≤ 0.0001	0.966	≤ 0.0001	0.978	≤ 0.0001
Kinase inhibitors	0.999	≤ 0.0001	0.995	≤ 0.0001	0.993	≤ 0.0001	0.994	≤ 0.0001	1.000	≤ 0.0001
Microtubule destabilizers	0.852	≤ 0.0001	0.793	≤ 0.0001	0.881	≤ 0.0001	0.854	≤ 0.0001	0.865	≤ 0.0001
Microtubule stabilizers	0.781	≤ 0.0001	0.804	≤ 0.0001	0.981	≤ 0.0001	0.955	≤ 0.0001	0.998	≤ 0.0001
Protein degradation	0.722	0.0002	0.715	0.0003	0.716	0.0003	0.701	0.0007	0.816	≤ 0.0001
Protein synthesis	0.976	≤ 0.0001	0.985	≤ 0.0001	0.997	≤ 0.0001	0.975	≤ 0.0001	0.997	≤ 0.0001