

SUPPLEMENTARY INFORMATION

Table S1. Description of SPACe extracted features

	Feature name	Channel	Description
Morphology	Area	cell/nucleus/cytoplasm/nucleolus/mitochondria	Area of the object (<i>i.e.</i> number of pixels of the object scaled by pixel-area)
	convex area	nucleus/cell	Area of the convex hull image, which is the smallest convex polygon that encloses the object
	perimeter	nucleus/cell	Perimeter of object which approximates the contour as a line through the centers of border pixels using a 4-connectivity
	perimeter Crofton	nucleus/cell	Perimeter of object approximated by the Crofton formula in 4 directions
	circularity	nucleus/cell	Circularity defines how closely an object resembles a perfect circle
	efc ratio	nucleus	The EFC ratio measures the regularity of nuclei. A larger ratio indicates more circular or ellipsoidal shapes, while a smaller ratio suggests irregular and complex nuclear shape
	eccentricity	nucleus/cell	Eccentricity of the ellipse having the same second moments as the object. The eccentricity is the ratio of the focal distance (distance between focal points) over the major axis length. The value is in the interval [0, 1). When it is 0, the ellipse becomes a circle
	equivalent diameter area	nucleus/cell	The diameter of a circle with the same area as the object
	Feret diameter max	nucleus/cell	Maximum Feret's diameter computed as the longest distance between points around a region's convex hull contour
	Solidity	nucleus/cell	Ratio of pixels in the object to pixels of the convex hull image
Extent	nucleus/cell	Ratio of pixels in the object to pixels in the total bounding box	
Intensity	Intensity percentile 10	nucleus/cytoplasm/nucleolus/mitochondria	intensity value below which 10% of the pixel values in the image are found
	intensity percentile 25	nucleus/cytoplasm/nucleolus/mitochondria	intensity value below which 25% of the pixel values in the image are found
	intensity percentile 75	nucleus/cytoplasm/nucleolus/mitochondria	intensity value below which 75% of the pixel values in the image are found
	intensity percentile 90	nucleus/cytoplasm/nucleolus/mitochondria	intensity value below which 90% of the pixel values in the image are found
	intensity median	nucleus/cytoplasm/nucleolus/mitochondria	middle point of the pixel intensity distribution within the image
	intensity mean	nucleus/cytoplasm/nucleolus/mitochondria	average pixel intensity within the image

	intensity median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria	quantifies the spread or dispersion of pixel intensity values around the median intensity in the image
	intensity std	nucleus/cytoplasm/nucleolus/mitochondria	quantifies the amount of variation or dispersion of pixel intensity values within the image
Textural	contrast percentile 25	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measure of the local variations presents in the image. Specifically evaluates the lower 25% of contrast values
	contrast percentile 50	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measure of the local variations presents in the image. Specifically evaluates the lower 50% of contrast values
	contrast percentile 75	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measure of the local variations presents in the image. Specifically evaluates the lower 75% of contrast values
	contrast mean	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the typical magnitude of contrast values
	contrast median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	midpoint of the contrast values in the image
	contrast std	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the variability or spread of contrast values
	dissimilarity percentile 25	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of difference in pixel intensity. Specifically evaluates the lower 25% of dissimilarity values
	dissimilarity percentile 50	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of difference in pixel intensity. Specifically evaluates the lower 50% of dissimilarity values
	dissimilarity percentile 75	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of difference in pixel intensity. Specifically evaluates the lower 75% of dissimilarity values
	dissimilarity mean	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the typical magnitude of dissimilarity values

dissimilarity median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	midpoint of the dissimilarity values in the image
dissimilarity std	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the variability or spread of dissimilarity values
homogeneity percentile 25	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of similarity in pixel intensity. Specifically evaluates the lower 25% of homogeneity values
homogeneity percentile 50	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of similarity in pixel intensity. Specifically evaluates the lower 50% of homogeneity values
homogeneity percentile 75	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of similarity in pixel intensity. Specifically evaluates the lower 75% of homogeneity values
homogeneity mean	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the typical magnitude of homogeneity values
homogeneity median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	midpoint of the homogeneity values in the image
homogeneity std	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the variability or spread of homogeneity values
energy percentile 25	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the homogeneity or uniformity of pixel intensity. Specifically evaluates the lower 25% of energy values
energy percentile 50	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the homogeneity or uniformity of pixel intensity. Specifically evaluates the lower 50% of energy values
energy percentile 75	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the homogeneity or uniformity of pixel intensity. Specifically evaluates the lower 75% of energy values

energy mean	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the typical magnitude of energy values
energy median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	midpoint of the energy values in the image
energy std	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the variability or spread of energy values
correlation percentile 25	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of linear dependence between the pixel intensities in different regions of an image. Specifically evaluates the lower 25% of energy values
correlation percentile 50	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of linear dependence between the pixel intensities in different regions of an image. Specifically evaluates the lower 50% of energy values
correlation percentile 75	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the degree of linear dependence between the pixel intensities in different regions of an image. Specifically evaluates the lower 75% of energy values
correlation mean	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the typical magnitude of correlation values
correlation median absolute deviation	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	midpoint of the correlation values in the image
correlation std	nucleus/cytoplasm/nucleolus/mitochondria cell mask vs nucleus/cytoplasm/nucleolus/mitochondria imgs	measures the variability or spread of correlation values

Table S2. Features included in the berberine chloride consensus fingerprint

Increased Features	EMD	Decreased Features	EMD
Texture_W5-img-Cell-Mask_Homogeneity_25%	0.3170373	Intensity_W5-img-Cell-Mask_90	-0.352408
Texture_W5-img-Cell-Mask_Homogeneity_mean	0.3150326	Intensity_W5-img-Cell-Mask_75	-0.317043

Texture_W5-img-Cell-Mask_Homogeneity_50%	0.3054184	Texture_W5-img-Cell-Mask_Dissimilarity_75%	-0.307451
Texture_W5-img-Cell-Mask_Homogeneity_75%	0.2802248	Texture_W5-img-Cell-Mask_Dissimilarity_mean	-0.300922
Texture_W5-img-Cell-Mask_Energy_25%	0.2651592	Intensity_W5-img-Cell-Mask_mean	-0.294846
Texture_W5-img-Cell-Mask_Energy_mean	0.2588387	Texture_W5-img-Cell-Mask_Dissimilarity_std	-0.294113
Texture_W5-img-Cell-Mask_Energy_75%	0.2497095	Texture_W5-img-Cell-Mask_Dissimilarity_mad	-0.290659
Texture_W5-img-Cell-Mask_Energy_50%	0.2471353	Texture_W5-img-Cell-Mask_Homogeneity_std	-0.282357
Texture_W5-img-Mito-Mask_Homogeneity_25%	0.1851009	Texture_W5-img-Cell-Mask_Dissimilarity_50%	-0.276137
Texture_W5-img-Mito-Mask_Homogeneity_mean	0.1845715	Intensity_W5-img-Cell-Mask_median	-0.273289
Texture_W5-img-Mito-Mask_Homogeneity_50%	0.183436	Intensity_W5-img-Cell-Mask_mad	-0.267722
Texture_W5-img-Mito-Mask_Homogeneity_75%	0.1631843	Texture_W5-img-Cell-Mask_Contrast_mad	-0.265866
		Texture_W5-img-Cell-Mask_Homogeneity_mad	-0.264881
		Texture_W5-img-Cell-Mask_Contrast_std	-0.264421
		Intensity_W5-img-Mito-Mask_25	-0.263909
		Intensity_W5-img-Mito-Mask_median	-0.254738
		Intensity_W5-img-Mito-Mask_10	-0.251353
		Texture_W5-img-Cell-Mask_Dissimilarity_25%	-0.251204
		Texture_W5-img-Cell-Mask_Contrast_75%	-0.249717
		Intensity_W5-img-Cell-Mask_25	-0.237687
		Texture_W5-img-Cell-Mask_Contrast_mean	-0.237471
		Intensity_W5-img-Mito-Mask_mean	-0.215042
		Intensity_W5-img-Mito-Mask_75	-0.212328
		Texture_W5-img-Cell-Mask_Contrast_50%	-0.210001
		Texture_W5-img-Mito-Mask_Dissimilarity_75%	-0.198229
		Intensity_W5-img-Cell-Mask_std	-0.194447
		Misc_Area-Ratio_mito/cyto	-0.191162
		Texture_W5-img-Mito-Mask_Contrast_75%	-0.187751
		Texture_W5-img-Mito-Mask_Dissimilarity_mean	-0.186542
		Texture_W5-img-Mito-Mask_Dissimilarity_std	-0.178639
		Texture_W5-img-Mito-Mask_Dissimilarity_mad	-0.177346
		Texture_W5-img-Cell-Mask_Contrast_25%	-0.177221
		Texture_W5-img-Mito-Mask_Contrast_mad	-0.175783

		Texture_W5-img-Mito-Mask_Contrast_mean	-0.174393
		Texture_W5-img-Mito-Mask_Dissimilarity_50%	-0.171673
		Texture_W5-img-Mito-Mask_Contrast_std	-0.171529
		Misc_Area-Ratio_mito/cell	-0.167266
		Texture_W5-img-Mito-Mask_Correlation_75%	-0.158939
		Texture_W5-img-Mito-Mask_Dissimilarity_25%	-0.158591
		Intensity_W5-img-Cell-Mask_10	-0.157554
		Texture_W5-img-Mito-Mask_Contrast_50%	-0.155514
		Texture_W5-img-Mito-Mask_Homogeneity_std	-0.153602

SUPPLEMENTARY FIGURES

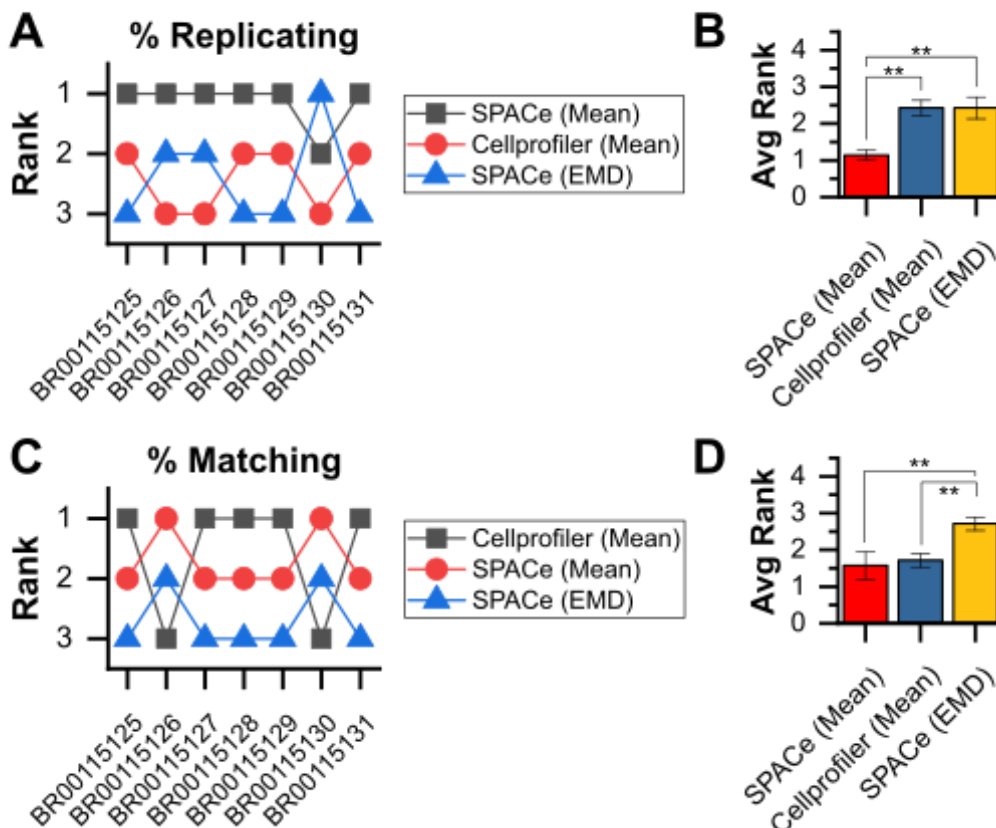


Figure S1. Rank performance of CellProfiler and SPACe analysis of JUMP MoA reference datasets. A) Rank order of percent replicating (“similarity between wells of the same treatment”) performance of CellProfiler and SPACe features across JUMP MoA reference datasets. B) Bar chart and statistical analysis of percent

replicating performance. C) Rank order of percent matching (“similarity between treatments with same MoA”) performance of CellProfiler and SPACe features across JUMP MoA reference datasets. D) Bar chart and statistical analysis of percent matching performance. Brackets and (**) indicates a p-value < 0.01.

Source data are provided in the Source Data file.

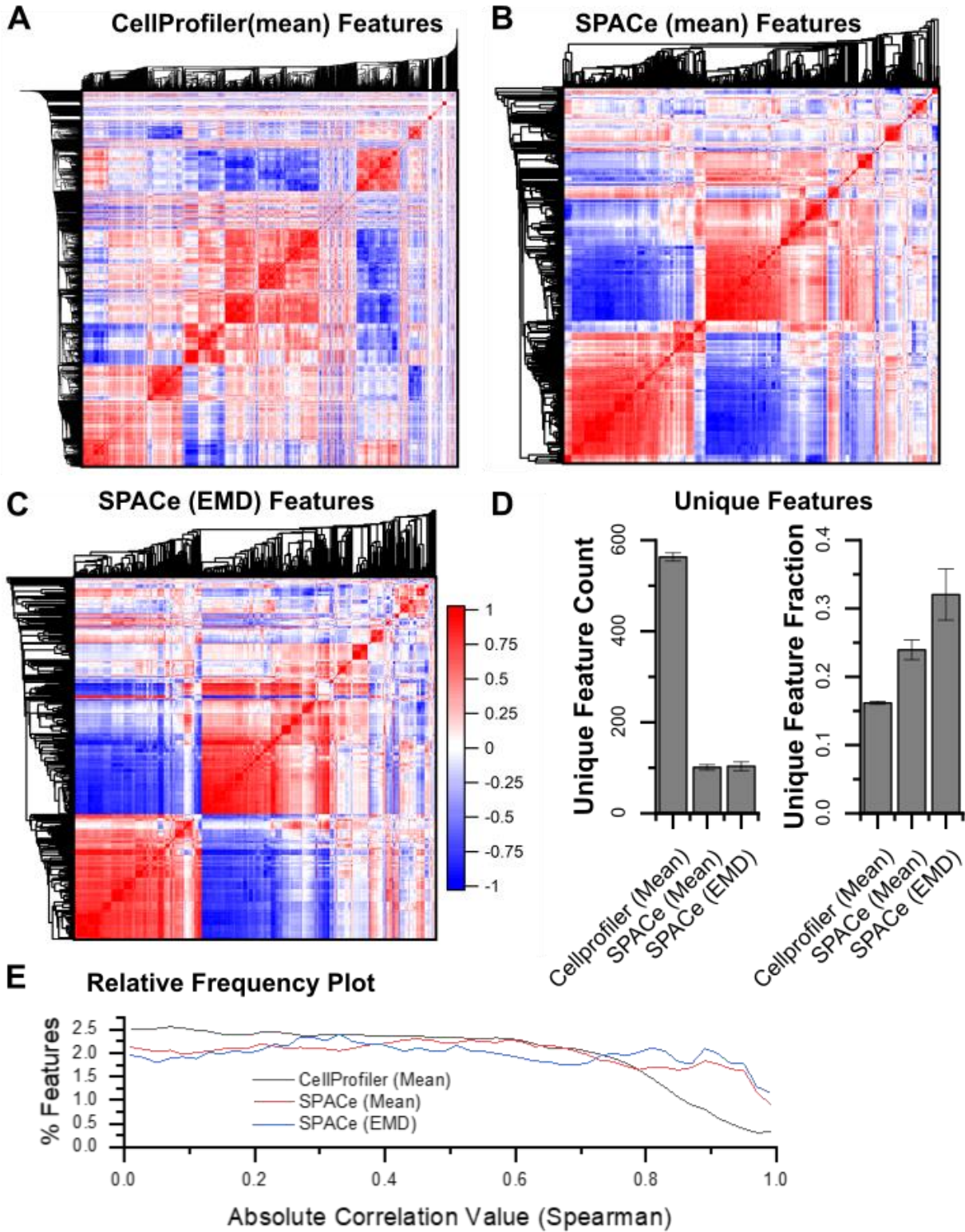


Figure S2. Correlation analysis of extracted features in the JUMP MoA reference datasets. Median correlation (Spearman) matrix across seven JUMP MoA reference datasets for feature extracted using (A) CellProfiler or SPACe (B, mean; C, EMD). Features are clustered using a Euclidian distance nearest neighbor algorithm. D) Absolute count (left) and fraction (right) of unique features for CellProfiler and SPACe analysis. E) Relative frequency plot of absolute Spearman correlation values between features produced by CellProfiler and SPACe. Source data are provided as a Source Data file.

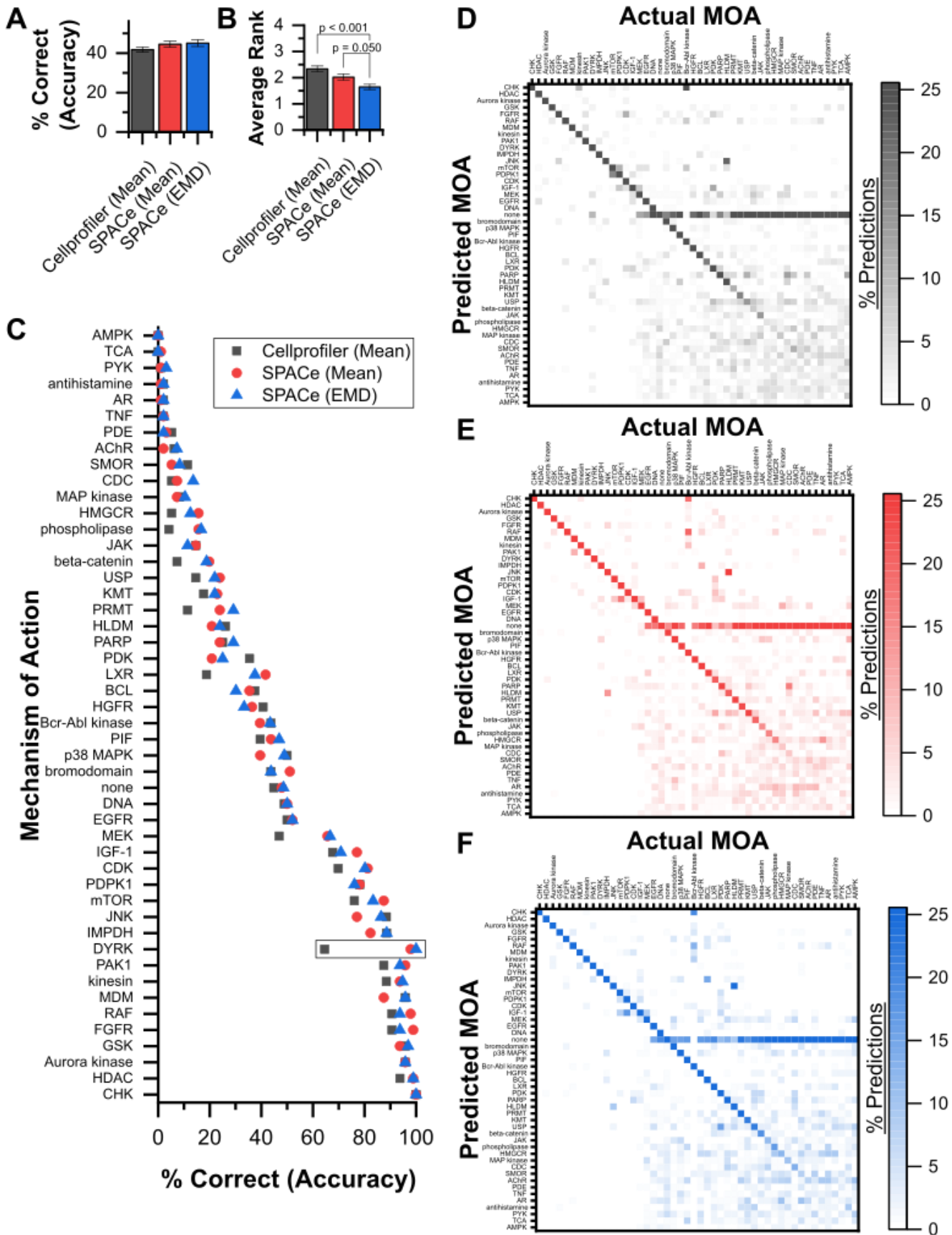


Figure S3. Machine learning model prediction performance using CellProfiler or SPACe extracted features. For six JUMP MoA reference datasets, a set of 5 Random Forest (RF) models were generated to predict MoA

using either CellProfiler (Mean), SPACe (Mean), or SPACe (EMD) values. A) Mean and standard deviation of overall accuracy for RF models. B) Rank analysis showing the mean and standard deviation of model rank (1 – best, 3 – worst) based on the average prediction accuracy for each MOA of RF models trained using either CellProfiler (Mean), SPACe (Mean), or SPACe (EMD) values. Brackets and p-values indicate significant differences observed. C) Average RF model % correct (accuracy) predictions for each MOA contained in the JUMP reference datasets. D-F) Confusion matrices showing the average classification performance of models trained and tested using CellProfiler (Mean), SPACe (Mean), and SPACe (EMD) data, respectively. X and Y axis are ordered based on the order found in (C). Source data are provided as a Source Data file.

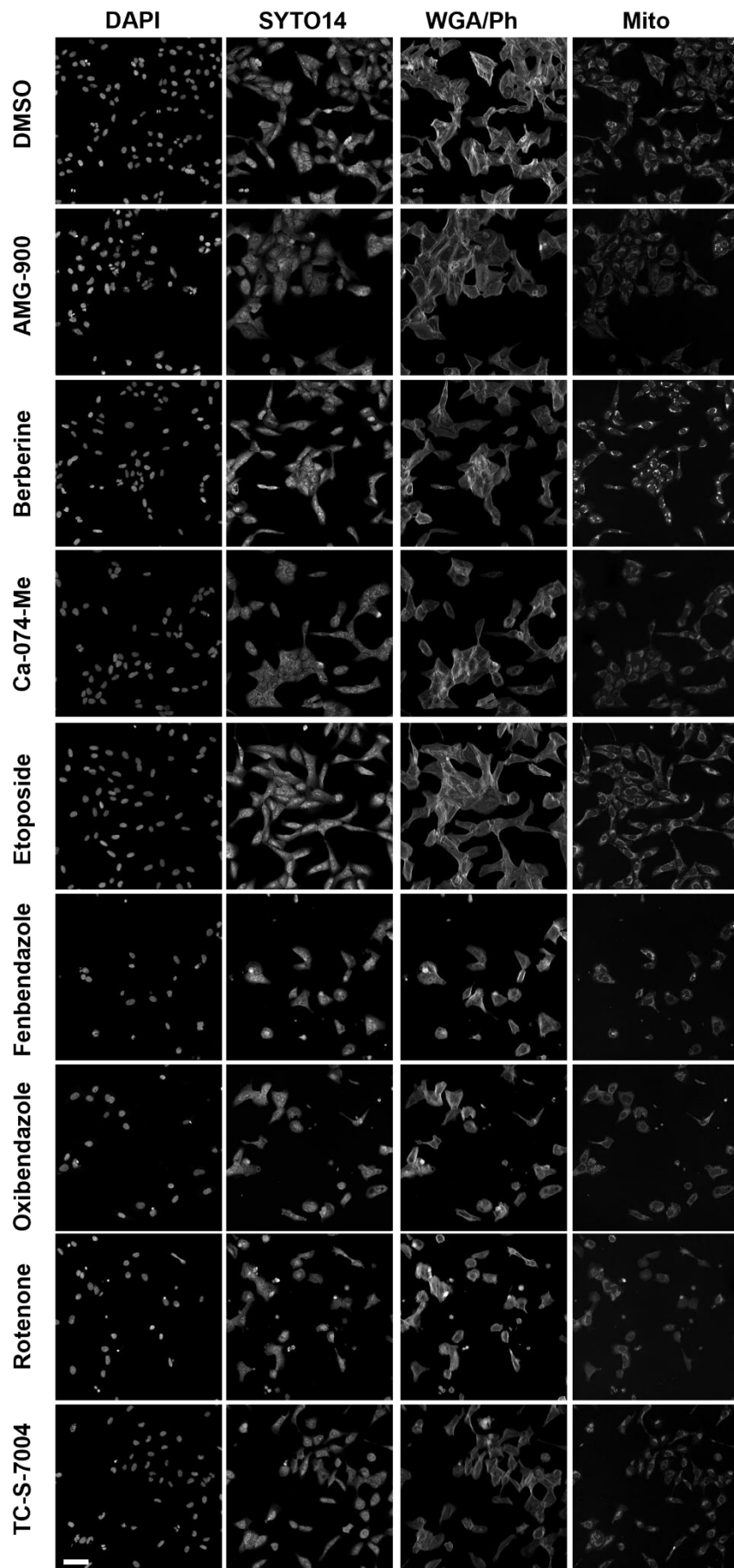


Figure S4. Greyscale images of the treatments described in Figure 2 representing the DAPI, SYTO14, WGA/phalloidin and MitoTracker channels. Scale bar: 50 μm .

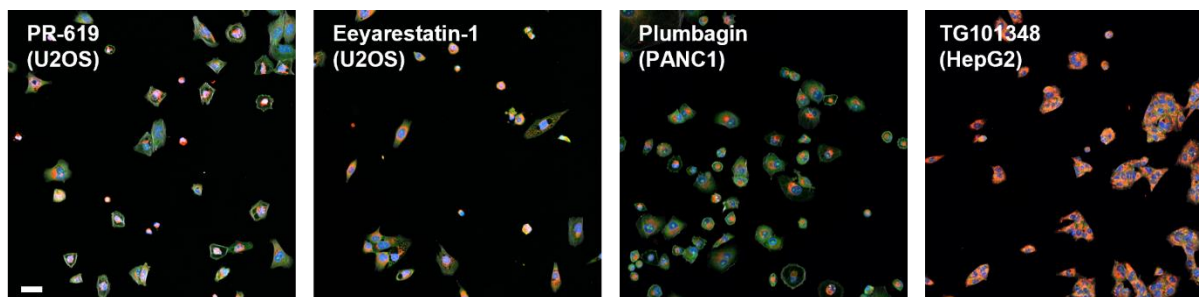


Figure S5. Representative images of the indicated treatments that showed unique phenotypes but also some toxicity in the various cell lines. Scale bar: 20 μm .