Learning From the JUMP CP Pilot Data: Insights for Platform Development

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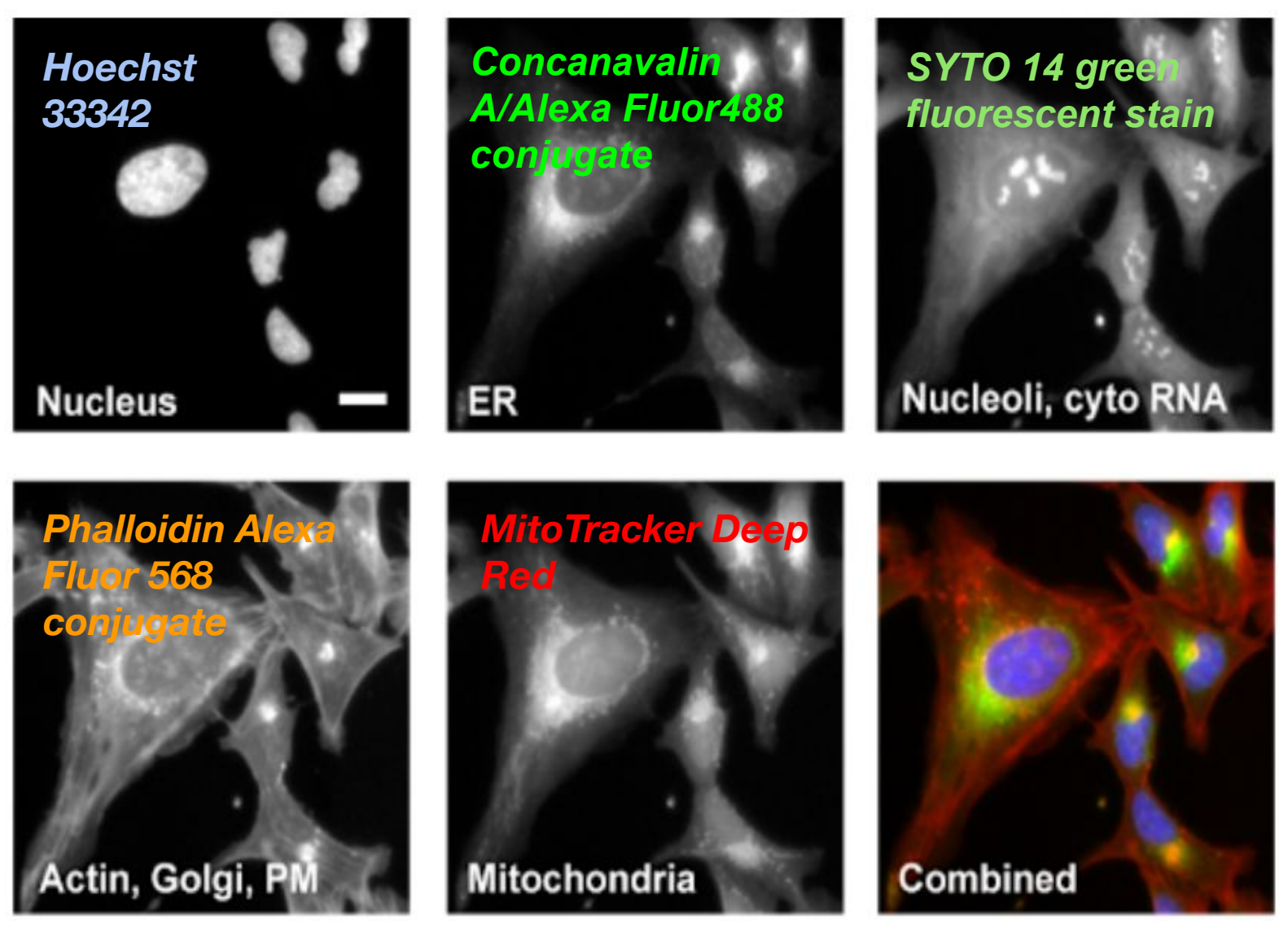
Introduction

There is a growing interest in adopting image-based phenotypic profiling for target and drug discovery processes. Much of the growth has been driven by the use of Cell Painting, a standardized high content profiling method originally developed at the Broad Institute. The JUMP (Joint Undertaking in Morphological Profiling) Cell Painting (CP) consortium has been established to generate a large public reference Cell Painting dataset with the aim to create a new phenotypic approach to drug discovery. Here, we have focused on the preliminary JUMP CP dataset[1], which includes A549 and U2OS cell lines treated with chemical and genetic (CRISPR and ORF) perturbations to explore the CellProfiler output features capturing the variability in this data. We show how our web-based data analytics platform, StratoMineR, can be used to evaluate phenotypic data holistically.


Hoechst 33342
Nucleus
Concanavalin A/Alexa Fluor488 conjugate
ER
SYTO 14 green fluorescent stain
Nucleoli, cyto RNA
Phalloidin Alexa Fluor 568 conjugate
Actin, Golgi, PM
MitoTracker Deep Red
Mitochondria
Combined

Cell Lines: A549, U2OS
Plates: 51, **Replicates:** 2-5
Gene Targets: 175+
Time Points: 1, 2, 4, 14, 28 days
Treatments: Compounds (306), **CRISPR sgRNAs** (335), **ORFs** (175)

Figure 1: The Cell Painting Assay and the JUMP-CP Pilot experimental parameters. The cells were fixed and the standard Cell Painting assay protocol with six fluorescent dyes[2] were used to label various components of the cell. Segmentation and feature extraction was performed using CellProfiler.

Methods

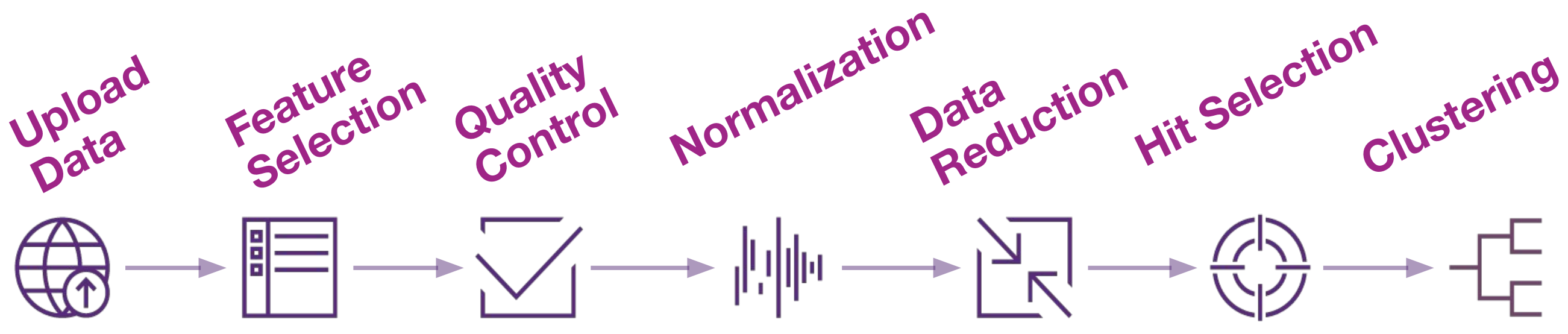


Figure 2: The StratoMineR™ workflow. StratoMineR™ is a web-based platform which guides users through a typical workflow in analysis of high content multi-parametric data[3].

Results

Data visualization and quality control

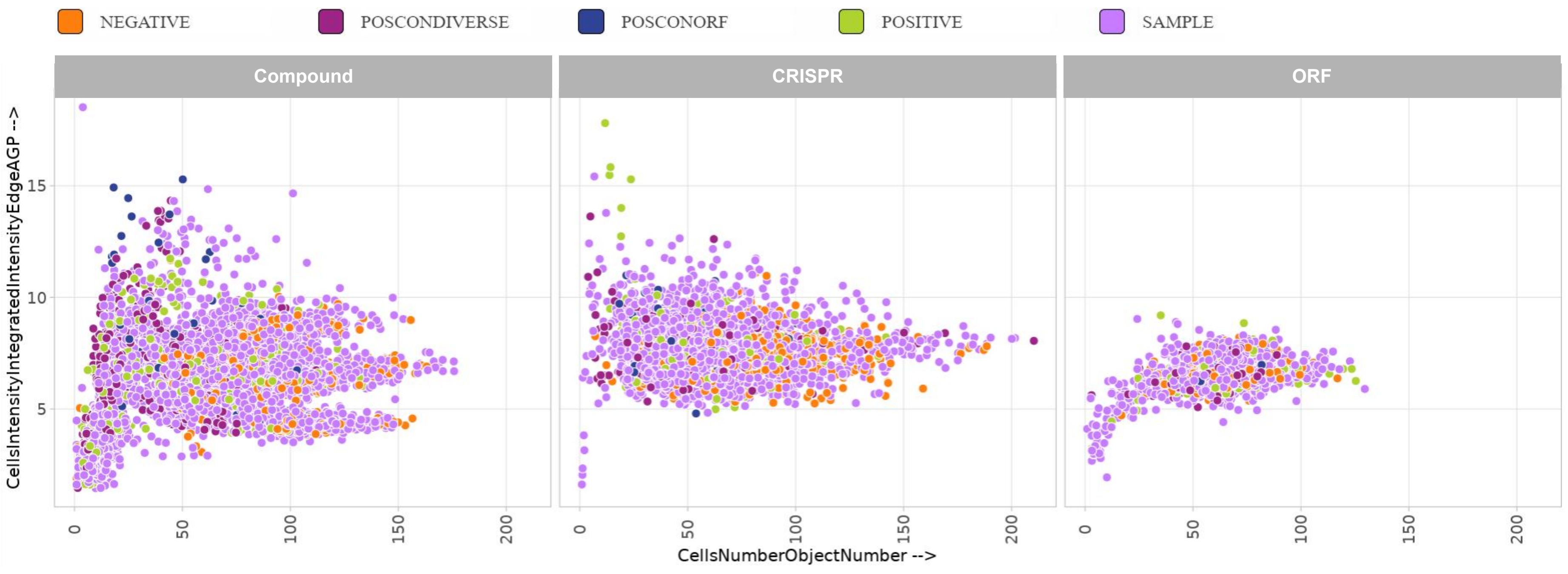
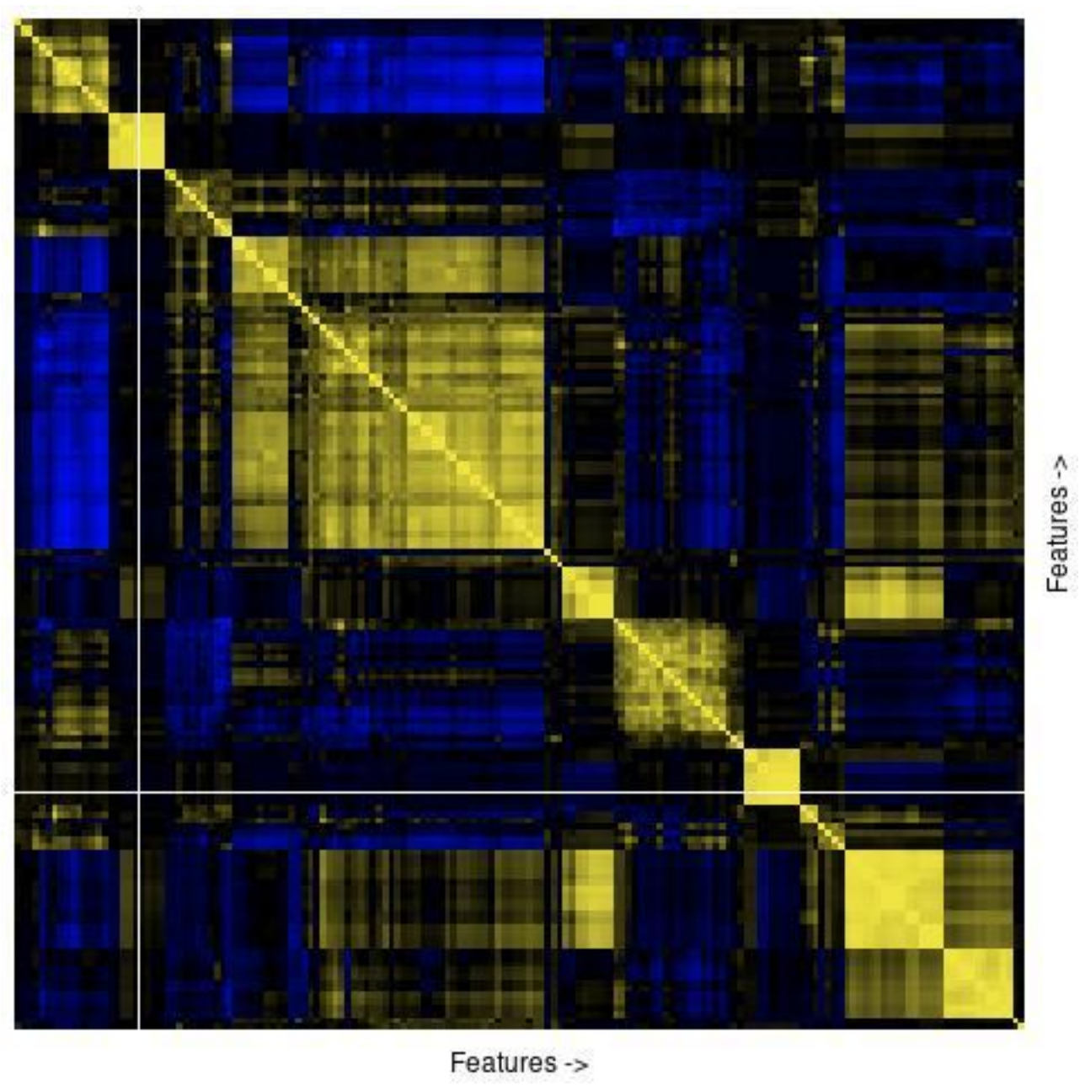


Figure 3: Using the StratoMineR™ Quality Control interactive data visualization module, we can quickly get an overview of the entire preliminary JUMP-CP dataset. We used the merged metadata module to combine an annotation file with the raw data, this supports inclusion of details about the experiment (compound names, time points, reagent classes, etc) which results in more plotting options. For example, here the data points are labeled by reagent class and the data is tiled by perturbation type: Compound, CRISPR or ORF.

Feature Selection

One of the biggest barriers to analyzing the JUMP CP data is the vast amount of features; (5792 in the pilot experiments). Due to our collaboration with KML Vision (*poster number:* ***1095-B***), we were interested in understanding which features contributed the most variance within this data set. Therefore, we grouped the features into the CellProfiler measurement categories: Morphology (AreaShape), Intensity, Texture, Granularity, Correlation, Radial Distribution, Location, Neighbor, Parent and Children. We then performed Feature Selection on these different categories independently. We used Spearman's correlation to understand which features were highly correlated with each other. We performed further downstream analysis which included: plate normalization to the median of the negative control, data transformation to handle skewed features, and feature scaling to normalize the numerical range of independent measurements



Feature	Closest Feature	Correlation Value
CellsAreaShapeBoundingBoxArea	CytoplasmAreaShapeBoundingBoxArea	1
CellsAreaShapeBoundingBoxMaximumX	CytoplasmAreaShapeBoundingBoxMaximumX	1
CellsAreaShapeBoundingBoxMaximumY	CytoplasmAreaShapeBoundingBoxMaximumY	1
CellsAreaShapeBoundingBoxMinimumY	CytoplasmAreaShapeBoundingBoxMinimumY	1
CellsAreaShapeBoundingBoxMinimumX	CytoplasmAreaShapeBoundingBoxMinimumX	1
CellsAreaShapeMinFeretDiameter	CytoplasmAreaShapeMinFeretDiameter	1
CellsAreaShapeMaxFeretDiameter	CytoplasmAreaShapeMaxFeretDiameter	1
CellsAreaShapeCenterY	CytoplasmAreaShapeCenterY	1
CellsAreaShapeCenterX	CytoplasmAreaShapeCenterX	1
CellsAreaShapeMajorAxisLength	CytoplasmAreaShapeMajorAxisLength	0.999

Figure 4: Feature selection with Morphology features in the preliminary JUMP-CP dataset. The correlation matrix shows only 250 features, and the table lists the top 10 features with the highest correlation values with their closest feature, indicating redundancy. The same method was applied for every feature category.

References

1. *Chandrasekaran SN et al. bioRxiv https://doi.org/10.1101/2022.01.05.475090.*
2. *Bray MA et al. Nat Protoc. 2016 Sep; 11(9): 1757-1774.*
3. *Omta W et al. Assay Drug Dev Technol. 2016; 14(8): 439-452.*
4. *KML Vision GmbH, IKOSA (software), 2023, Graz, Austria, software available at https://app.ikosa.ai/*

Dimensionality reduction

We applied Principal Component Analysis (PCA) on each feature category group based on the samples. We determined the number of components to calculate based on a Scree plot (between 8 and 10 components for most of the feature groups). Data reduction is useful for three critical reasons: 1) reduces computational load, 2) reduces redundancy, and 3) reveals the biology behind the data by highlighting important features. Using PCA allowed us to extract feature loading scores which can be subsequently used for making informed decisions on prioritization to create a smaller feature set. This was important for our collaboration with KML Vision, for supporting exploration of different features within their IKOSA AI platform[4] for morphological cell profiling.

Morphology Features

Component 1		Component 2		Component 3	
Feature	Loading	Feature	Loading	Feature	Loading
CellsAreaShapeExtent	-0.902	NucleiAreaShapeZernike55	0.886	NucleiAreaShapeMinFeretDiameter	0.982
CellsAreaShapeZernike00	-0.882	NucleiAreaShapeZernike77	0.858	NucleiAreaShapeEquivalentDiameter	0.960
CellsAreaShapeSolidity	-0.876	NucleiAreaShapeZernike75	0.840	NucleiAreaShapeMaximumRadius	0.958
CellsAreaShapeZernike95	0.872	NucleiAreaShapeZernike33	0.807	NucleiAreaShapePerimeter	0.948
CellsAreaShapeZernike31	0.845	NucleiAreaShapeZernike71	0.803	NucleiAreaShapeArea	0.945

Intensity Features

Component 1		Component 2		Component 3	
Feature	Loading	Feature	Loading	Feature	Loading
NucleiIntensityMaxIntensityEdgeER	0.950	CellsIntensityMeanIntensityEdgeBrightfield	1.000	NucleiIntensityStdIntensityEdgeBrightfield	0.776
NucleiIntensityStdIntensityEdgeER	0.925	CellsIntensityMeanIntensityEdgeHighZBF	0.999	CellsIntensityMaxIntensityHighZBF	0.753
CellsIntensityMaxIntensityER	0.910	CellsIntensityMeanIntensityLowZBF	0.997	CellsIntensityStdIntensityBrightfield	0.753
NucleiIntensityMaxIntensityER	0.900	CellsIntensityMeanIntensityBrightfield	0.997	NucleiIntensityMassDisplacementBrightfield	0.740
NucleiIntensityStdIntensityEdgeAGP	0.859	CellsIntensityUpperQuartileIntensityBrightfield	0.997	CellsIntensityMaxIntensityBrightfield	0.738

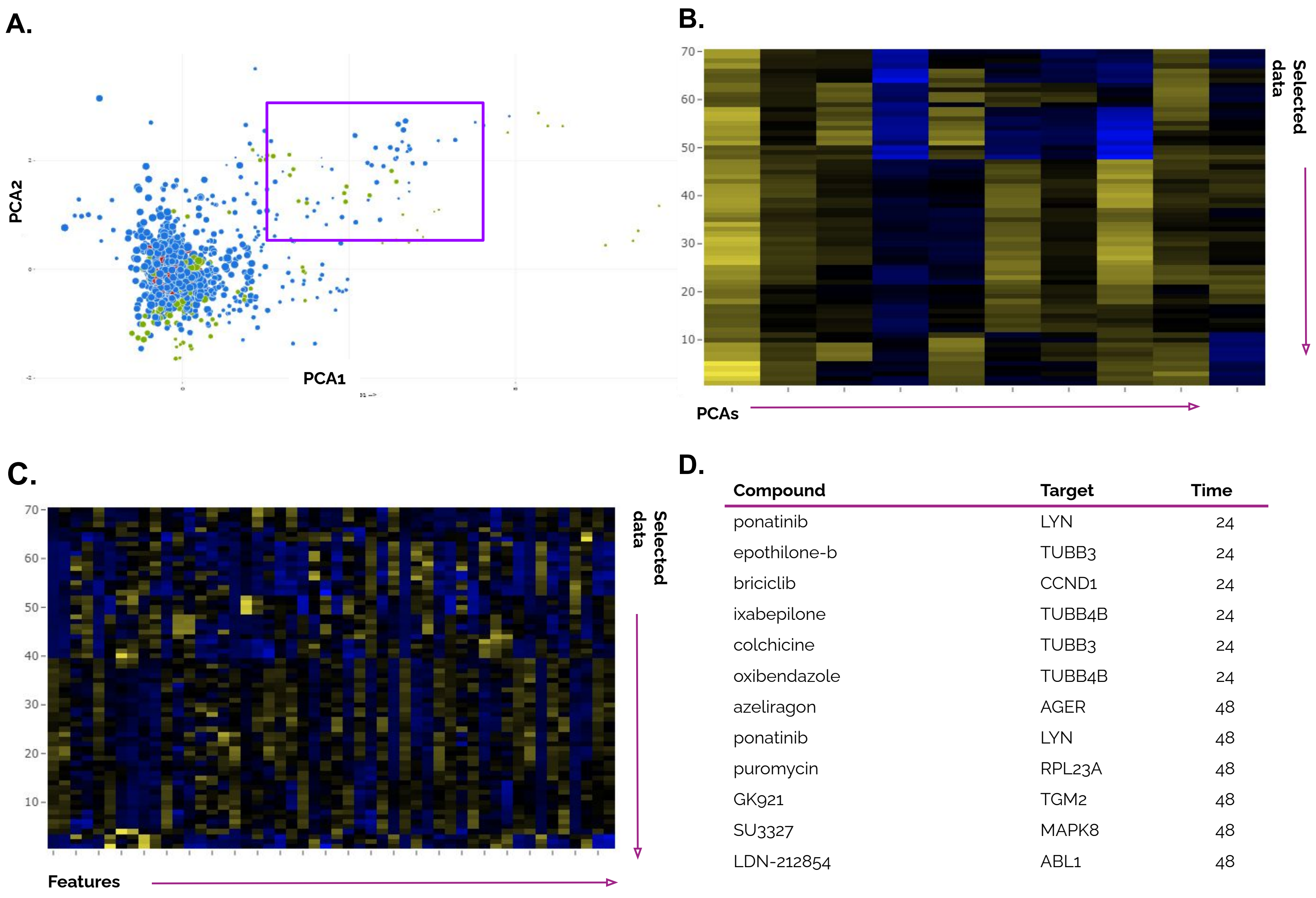
Texture Features

Component 1		Component 2		Component 3	
Feature	Loading	Feature	Loading	Feature	Loading
CellsTextureSumVarianceBrightfield300256	1.028	NucleiTextureCorrelationDNA1003256	-0.801	CellsTextureAngularSecondMomentER1000256	1.022
CellsTextureSumVarianceBrightfield301256	1.021	NucleiTextureInfoMeas1DNA1001256	-0.799	CellsTextureAngularSecondMomentER1001256	1.019
CellsTextureSumVarianceHighZBF301256	1.012	NucleiTextureCorrelationDNA1001256	-0.793	CellsTextureAngularSecondMomentER1002256	1.018
CellsTextureSumVarianceBrightfield302256	1.010	NucleiTextureCorrelationDNA1002256	-0.792	CellsTextureAngularSecondMomentER1003256	1.016
CellsTextureSumVarianceBrightfield500256	1.010	NucleiTextureInfoMeas1DNA1003256	-0.788	CellsTextureDifferenceVarianceER1000256	1.009

Table 1: Principal Component Analysis results.The five features with higher loadings within the first three components are shown. More features were significantly loading within the components (data not shown here). Morphology, Intensity and Texture features are shown here, the same analysis was performed for the other feature groups. Please contact us for more information.

Hit selection & Clustering Analyses

Besides exploring the features and their importance within each category, we used the pilot data to make several phenotypic comparisons between two cell lines, and tracked phenotypic drift over various time points and conditions.



Compound	Target	Time
ponatinib	LYN	24
epothilone-b	TUBB3	24
briciclib	CCND1	24
ixabepilone	TUBB4B	24
colchicine	TUBB3	24
oxibendazole	TUBB4B	24
azeliragon	AGER	48
ponatinib	LYN	48
puromycin	RPL23A	48
GK921	TGM2	48
SU3327	MAPK8	48
LDN-212854	ABL1	48

Figure 6: Hit selection and clustering identified compounds with related and unrelated targets. Unsupervised hit selection using Euclidean distance scoring was used for a subset of data from compound-treated A549 experiment, and distance scores were calculated from the median of the negative controls with $p < 0.05$. This approach identified 57 compound hits that were phenotypically distinct from the negative controls. Shown here is a hit selection scatter plot for A549 cell line (**A**) and selected hit compounds (inset) can be clustered based on 10 PCAs (**B**) or across 50 features (**C**). List of selected hits from compound-treated A549 cells reveals groups of related and unrelated targets (**D**).

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