SCIENTIFIC POSTER

Decoding cellular phenotypes with PhenoVue live and endpoint cell painting kits.

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Revvity

Phenotypic screening

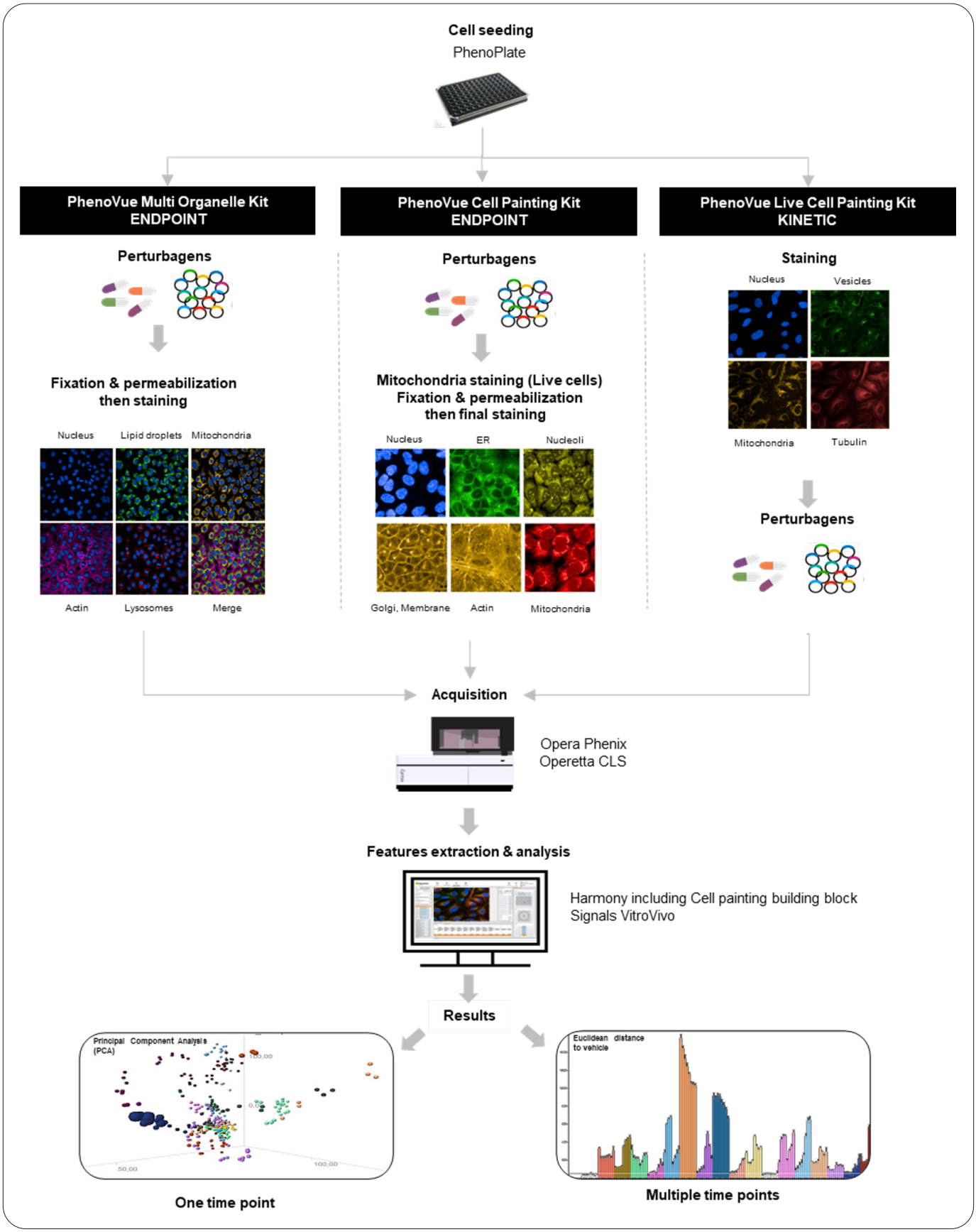
High-content imaging (HCI) enables the multiplexed analysis of cellular features at high resolution, generating rich datasets for identifying phenotypic changes, drug responses, and mechanisms of action. Using platforms such as the Opera Phenix™ Plus and Operetta™ CLS, researchers can acquire high-quality images suitable for deep phenotypic profiling.

The cell painting approach combines fluorescent dyes targeting key organelles, such as the nucleus, mitochondria, ER, Golgi, and cytoskeleton, to reveal subtle and complex phenotypic changes in a single assay.

PhenoVue™ cell painting kits, available in both endpoint and live-cell formats, as well as the multi-organelle staining kit, streamline these assays with validated, ready-to-use reagents optimized for reproducibility, biological relevance, and seamless integration into high-content screening workflows.

Data analysis is powered by Harmony™ software, which offers a dedicated cell painting building block , and Signals™ VitroVivo enabling phenotypic classification through Principal Component Analysis.

Experimental workflow

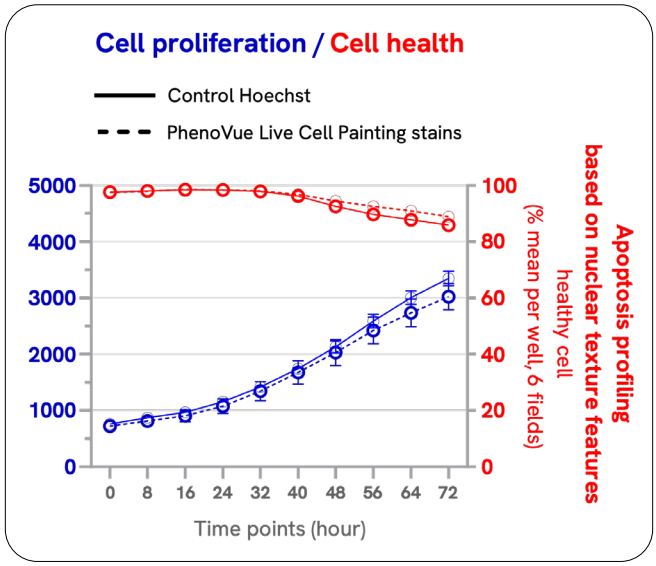


Comparative workflow and key features of the cell painting kits. The table summarizes key differences in protocol complexity, assay type, spectral handling, and suitability for phenotypic screening or kinetic MoA studies.

Features	Multi organelle endpoint	Cell painting endpoint	Live cell painting kinetic-time lapse
Cell type	Fixed	Live then Fixed	Live cells
Number of stains	5	6	4
Organelles covered	Nucleus, actin, mitochondria, lipid droplets, lysosomes	Nucleus, ER, Actin, Mitochondria, Golgi/PM, Nucleoli	Nucleus, Mitochondria, Vesicles, Microtubules
Assay type	Single time point	Single time point	Multi time point
Staining steps	1	2	1 (No-wash)
Spectral overlap	No	Minimal	No
Cytotoxicity	Not applicable (fixed)	Not applicable (fixed)	No
Reversibility / recovery studies	Not possible	Not possible	Enabled
Applications	Hepatotoxicity, disease-profiling (e.g., DILI, lysosomal storage disorders...)	Broad phenotypic screening	MoA profiling, compound kinetics, recovery studies

Live cell painting preserves cell health and proliferation

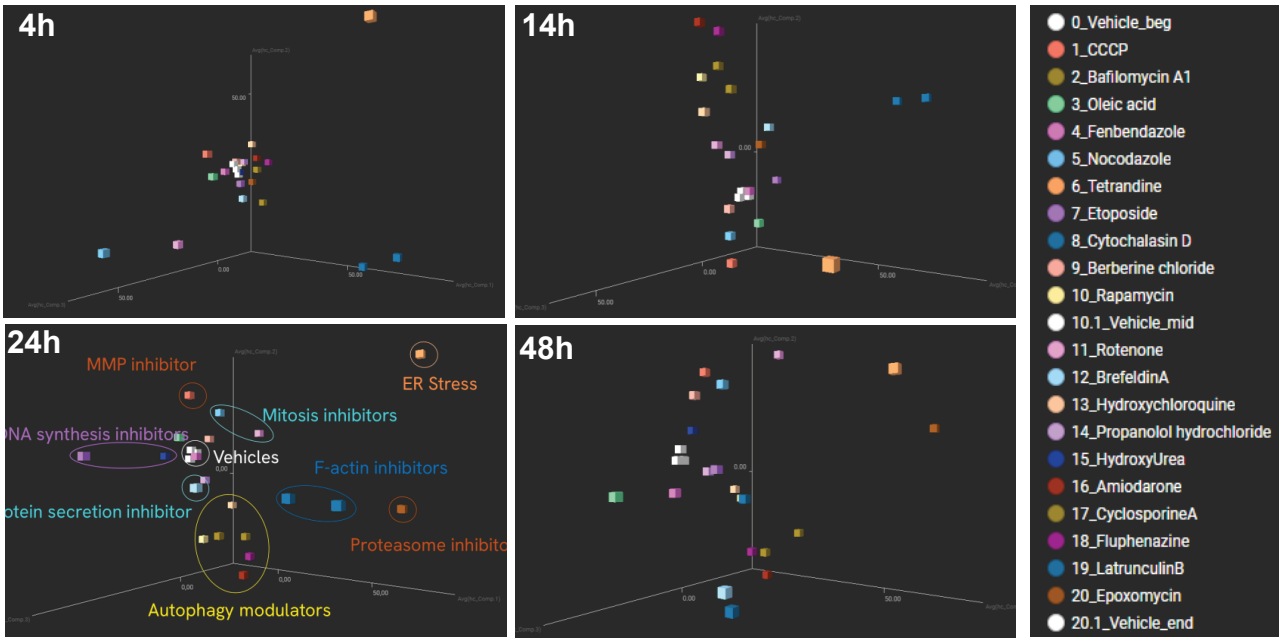
Assessing the impact of staining reagents on cell health is critical for live-cell imaging applications. The PhenoVue live cell painting kit was evaluated for its effects on proliferation and viability up to 72h.



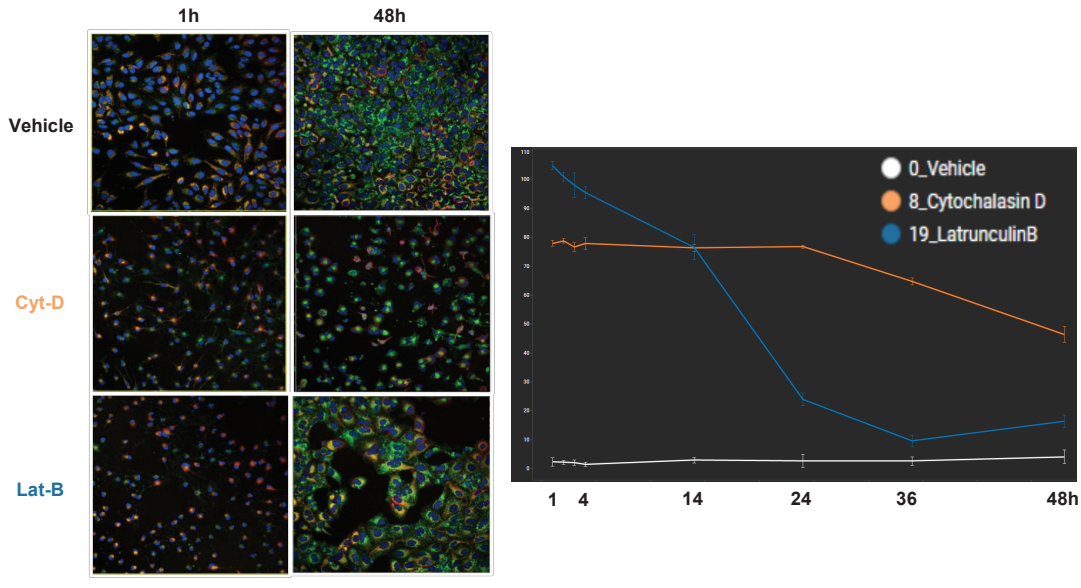
Impact of live cell painting stains on cell health. Cell proliferation (blue, left axis) and cell viability (red, right axis) were measured over 72 hours. U2OS cells stained with the PhenoVue live cell painting kit showed no adverse effects compared to control, confirming compatibility with long-term live-cell assays.

Live cell painting

To explore the kinetics of compound-induced phenotypes, U2OS cells were stained with the PhenoVue live cell painting kit and imaged at multiple time points (1h, 4h, 14h, 24h, 36h and 48h). PCA visualization of high-content imaging data reveals a progressive separation of phenotypic profiles over time.



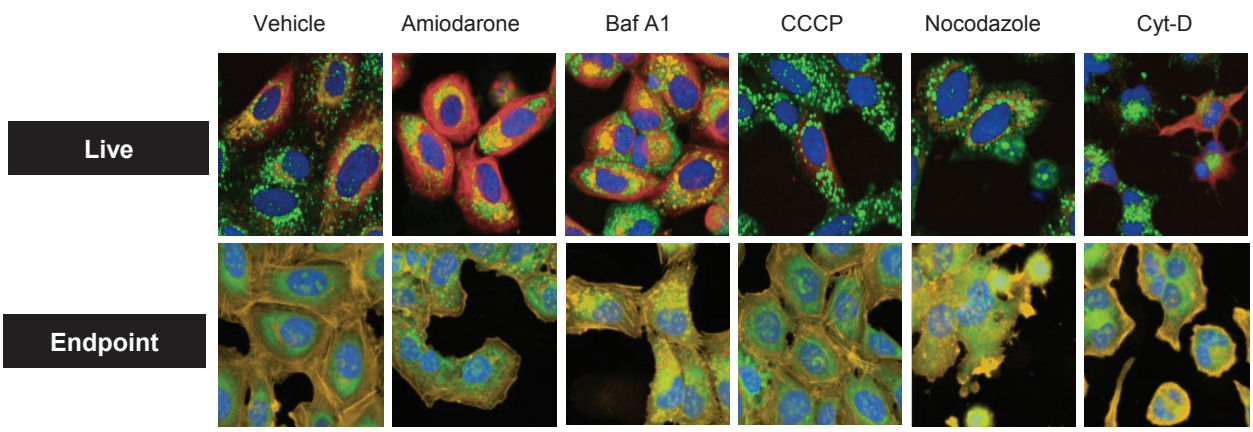
Time-resolved phenotypic clustering reveals MoA-specific segregation in live-cell assays. While early time points show minimal clustering, by 24h and 48h, compounds begin to segregate based on their known mechanisms of action (including autophagy modulators, ER stress inducers, mitosis inhibitors, and F-actin disruptors) highlighting the utility of live-cell multiplexing for dynamic phenotypic profiling.

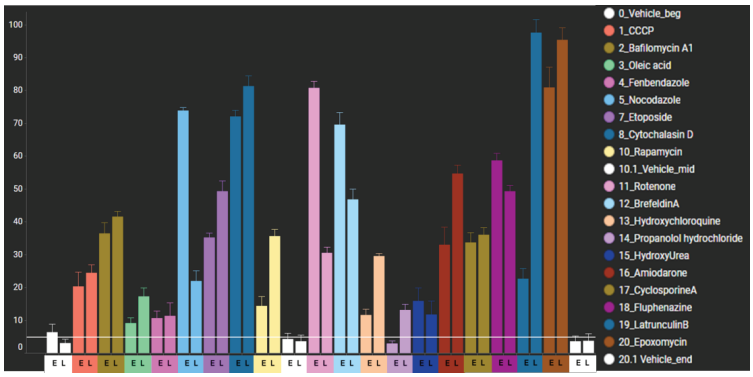


Time-resolved imaging reveals distinct actin remodeling dynamics of Cytochalasin D and Latrunculin B. Representative images, and Euclidean distance analysis show differential actin remodeling dynamics in U2OS cells treated with Cytochalasin D or Latrunculin B. While both compounds disrupt actin structures, Latrunculin B induces a rapid and reversible phenotype, in contrast to the more sustained effect of Cytochalasin D. These results highlight the power of live-cell cell painting to distinguish compound MoA over time.

Endpoint vs. live cell painting

To assess phenotypic resolution, U2OS cells were treated with various compounds (10µM) and analyzed using PhenoVue live or endpoint cell painting kits.



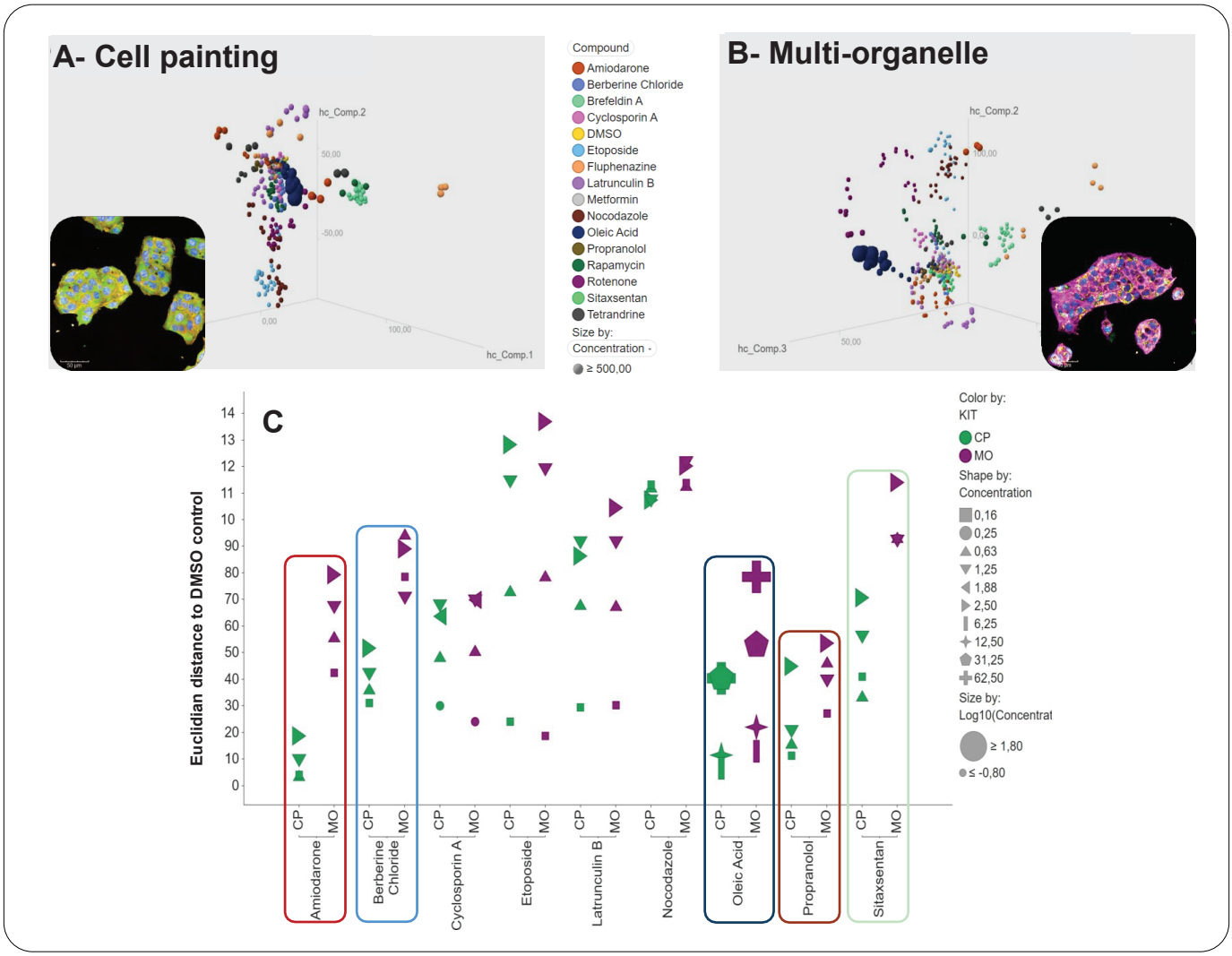


p-value @ 24h (Two-way Anova)	Live	Endpoint
CCCP	****	****
BafilomycinA1	****	**
Oleic acid	****	ns
Fenbendazole	****	**
Nocodazole	****	****
Etoposide	****	****
Cytochalasin D	****	****
Rapamycin	****	****
Rotenone	****	****
BrefeldinA	****	****
Hydroxychloroquine	****	***
Propranolol	****	ns
HydroxyUrea	***	****
Amiodarone	****	****
CyclosporineA	****	****
Fluphenazine	****	****
LatrunculinB	****	****
Epoxomicin	****	****

Comparative phenotypic resolution of live vs endpoint cell painting assays at 24h. Representative images (top) and quantitative (bottom) and statistical (right table) analysis show compound-induced phenotypic changes using the live and endpoint cell painting kits. While both formats discriminate compound-induced phenotypes, the live assay provides enhanced sensitivity and resolution for specific compounds, such as Oleic Acid, Fenbendazole and Propranolol.

Cell painting vs. multi-organelle

To compare phenotypic resolution across platforms, HepG2 cells were profiled using both the PhenoVue cell painting and multi-organelle kits. Dimensionality reduction and distance analysis reveal that the multi-organelle kit enhances separation for specific compounds, supporting its utility in detecting subtle and biologically relevant phenotypes.



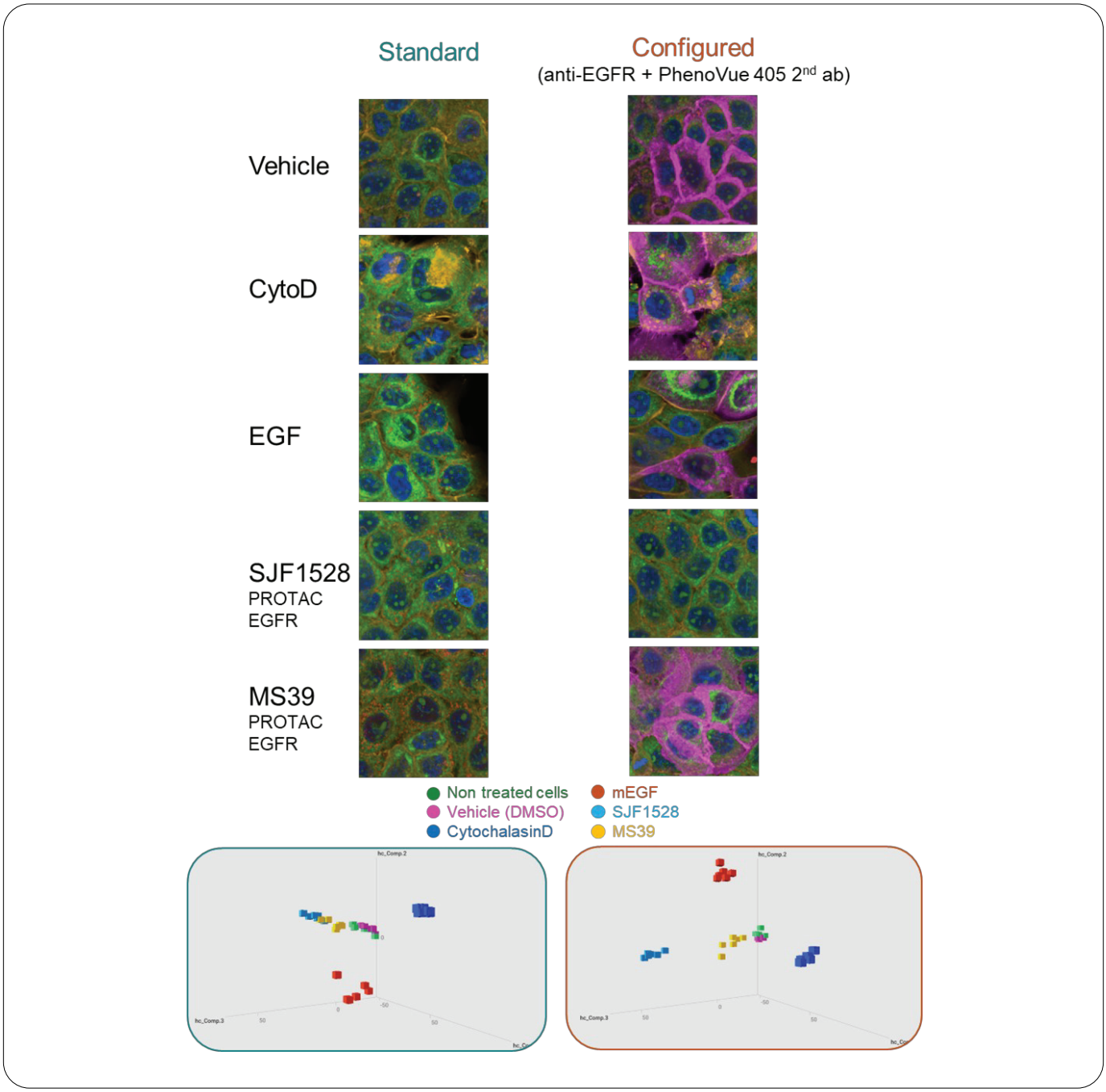
PCA-based clustering and compound discrimination in cell painting (CP) vs multi-organelle (MO) assays. PCA reveals dose-dependent clustering of compounds using both the cell painting kit (A) and the multi-organelle kit (B). Notably, the MO assay shows higher Euclidean distances for five specific compounds (highlighted), indicating enhanced phenotypic separation compared to the CP assay, while reference compounds display similar distances across both methods (C).

Configurable cell painting

The standard PhenoVue cell painting kit enables robust phenotypic profiling using a six-color panel.

To extend assay flexibility and biological relevance, the kit can be configured by incorporating a target-specific primary antibody followed by detection with a PhenoVue Fluor 405 or 400LS-labeled secondary antibody. This format enables the integration of an additional biological marker into the assay.

This advanced multiplexing approach expands imaging channels and enables tailored assays for deeper mechanistic insights.



Improved compound discrimination using a configured cell painting kit with EGFR detection. Images were acquired on the Opera Phenix Plus 5 laser system. PCA profiles from the standard (5-channel) and configured (6-channel) cell painting kits show similar clustering for Cytochalasin D and mEGF. The configured kit, with anti-EGFR and PhenoVue Fluor 405, clearly separates two EGFR-targeting PROTACs (SJF1528 vs MS39), highlighting improved resolution and biological specificity.

Conclusion

- **Endpoint cell painting kit:**
  Enables robust, multiplexed profiling of fixed cells with six validated dyes.
- **Live cell painting kit:**
  Captures dynamic and reversible phenotypes in live cells with a no-wash protocol.
- **Multi-organelle kit:**
  Targets five key organelles, including lysosomes and lipid droplets.
- **Flexible and configurable:**
  Endpoint CP kit supports antibody integration for added specificity.
- **Optimized for high-content imaging:**
  All kits are validated for reproducibility and seamless integration into imaging workflows.

Bibliography

1. Cimini et al, Nature protocols, 2023
2. Njeim et al, ASC Chemical Biology, 2025
3. Spector et al. Cell Motil Cytoskeleton, 1989