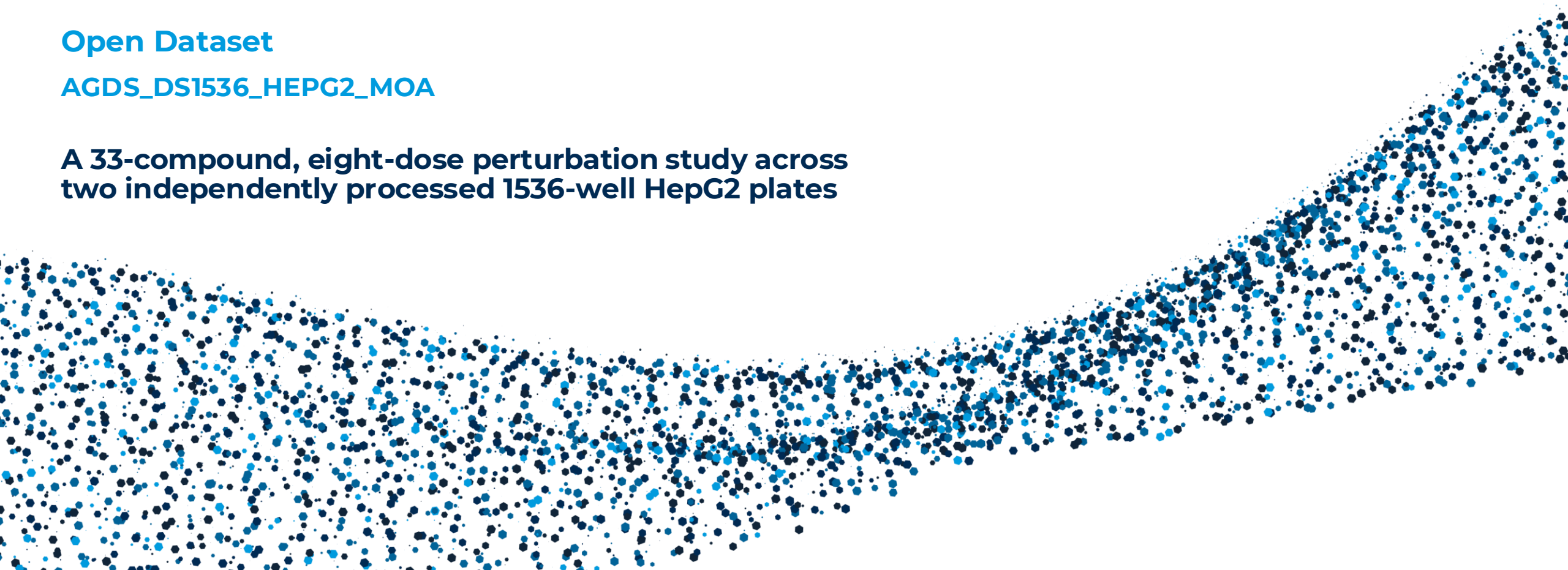


MERCURIUS™ 1536 DRUG-seq™

Open Dataset

AGDS_DS1536_HEPG2_MOA

A 33-compound, eight-dose perturbation study across
two independently processed 1536-well HepG2 plates



Legal Disclaimer

Dataset License Terms & Conditions

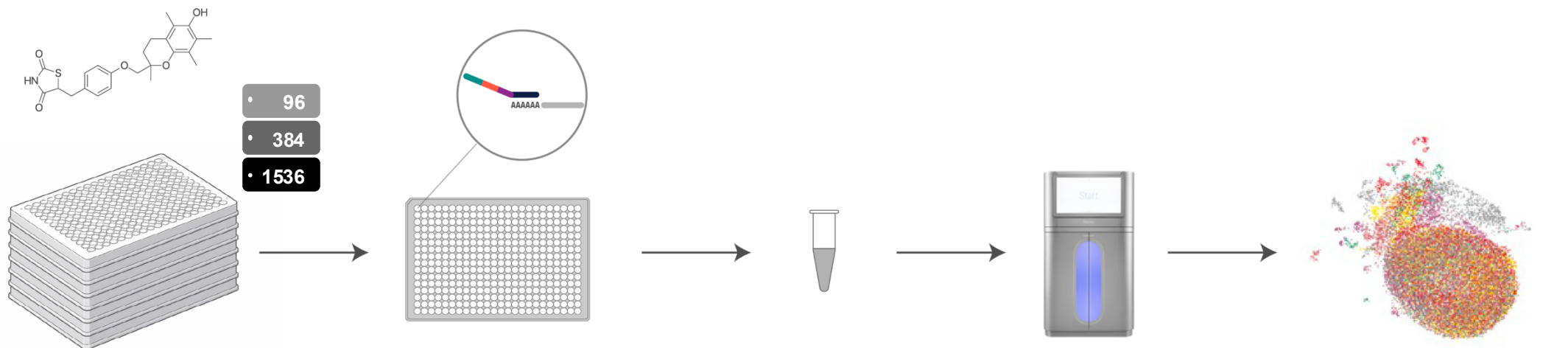
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DRUG-seq is a massively multiplexed, extraction-free 3' mRNA-seq technology



PERTURBATION & FREEZING

Samples in cell culture plates

LYSIS & BARCODING

Each sample is barcoded during the reverse transcription (RT) reaction

POOLING & LIBRARY PREP

Samples are pooled in a single tube, followed by library prep

SEQUENCING

DATA ANALYSIS

Sample demultiplexing and result generation



No PCR machines



Uses incubator
+ oil instead

This dataset presents a 1536-well DRUG-seq experiment designed for automated high-throughput screening

- **800 HepG2 cells** were plated per well across **two 1536-well plates**. A panel of **33 compounds** with known mechanisms of action was tested across eight compound-specific concentrations, with five replicates per condition. Cells were exposed to compounds for 24 hours.
- Following treatment, CellTiter-Blue® was used to measure cell viability directly on the same plates. This provided an orthogonal phenotypic readout that could be compared with the transcriptomic response.
- After the viability assay, cells were prepared for extraction-free DRUG-seq processing. Each of the 1,536 samples was **uniquely barcoded** during reverse transcription, and all samples were subsequently **pooled into a single tube** for library preparation. The full workflow is available [here](#).
- Sequencing data were processed using STAR and DESeq2, with UMAP used to resolve treatment-driven transcriptional structure. MAYA-derived pathway scoring was then applied to characterize compound-specific MoA and toxicity responses.

Two replicate plates of HepG2 cells treated with a curated set of reference compounds with known MoA

33

KNOWN-MOA
COMPOUNDS

8

DOSES /
COMPOUND

5

REPLICATES /
CONDITION

60

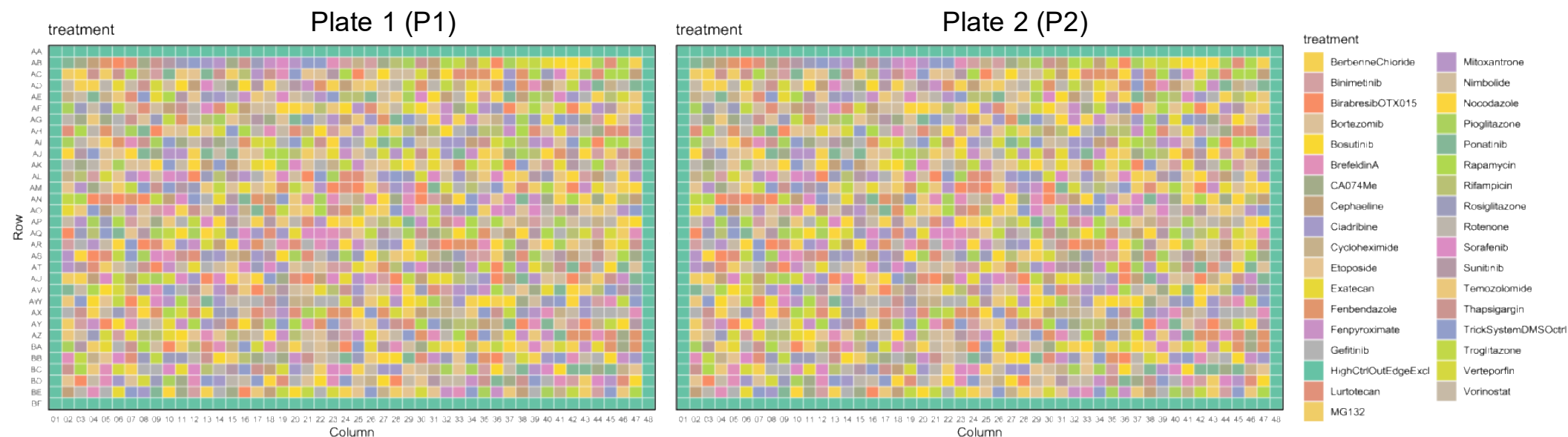
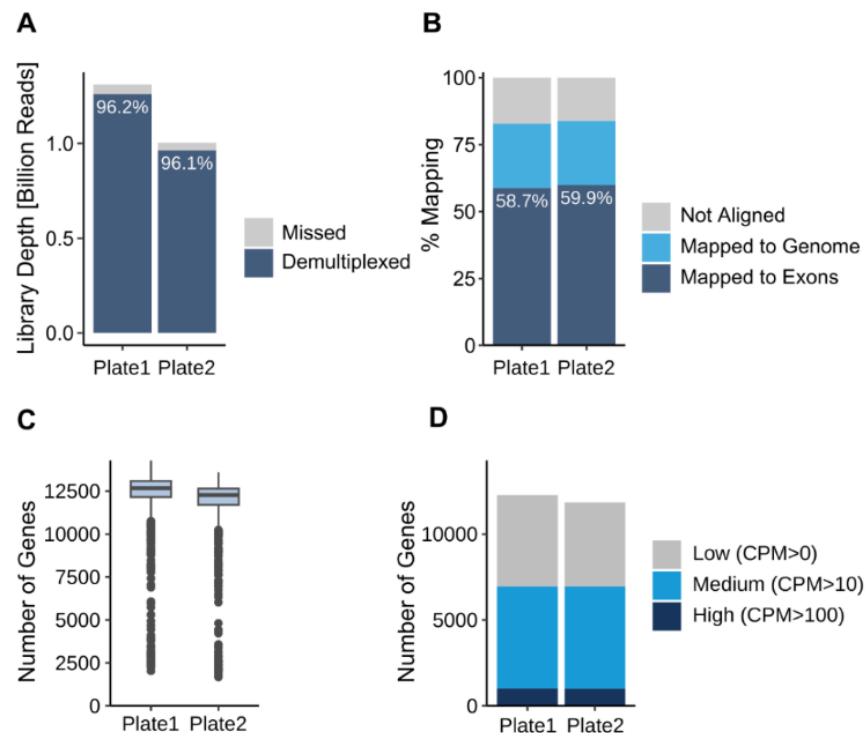
DMSO
CONTROLS /
PLATE

Figure 1. Positioning of samples on each plate. Thirty-three compounds were tested across eight compound-specific dose ranks, selected to capture sub-threshold, threshold, and saturating transcriptional responses across compounds with diverse potencies and mechanisms of action. The samples were positioned randomly on each plate leaving the outer edges empty so to exclude edge effects.

1536-well miniaturization delivers >12,000 detected genes per sample

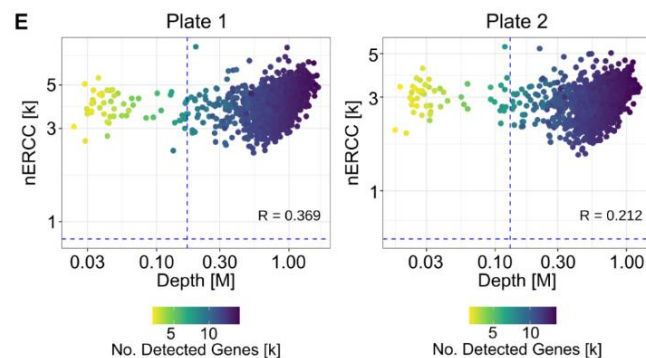
>96% sample assignment

58–60% exonic mapping



>12,000 genes detected at ~0.7M reads/sample

Sensitive for lowly expressed genes

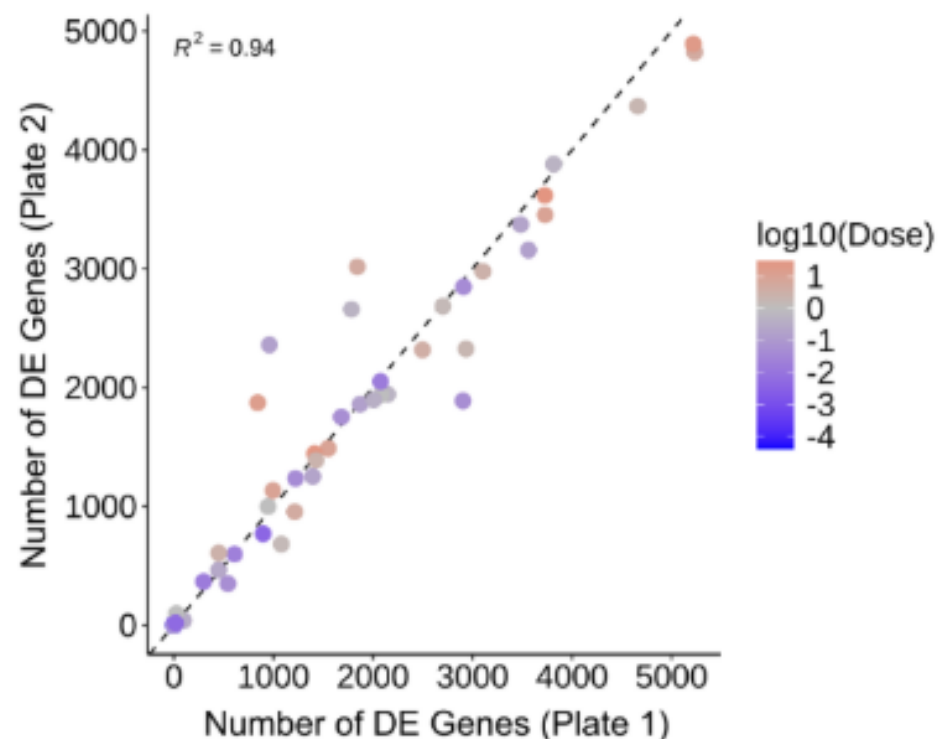


>12,000

genes detected per sample

Figure 2 Quality control and gene detection. **MERCURIUS™ 1536 DRUG-seq** quality control metrics and gene detection across two independently processed replicate plates. Sample assignment exceeded 96%, with median detection of over 12,000 genes per sample at approximately 0.7 million reads per sample. ERCC spike-in analysis showed that variation in sequencing depth was predominantly associated with biological rather than technical differences between samples. More details on ERCC [here](#).

Independent 1536-well plates deliver highly concordant differential expression responses



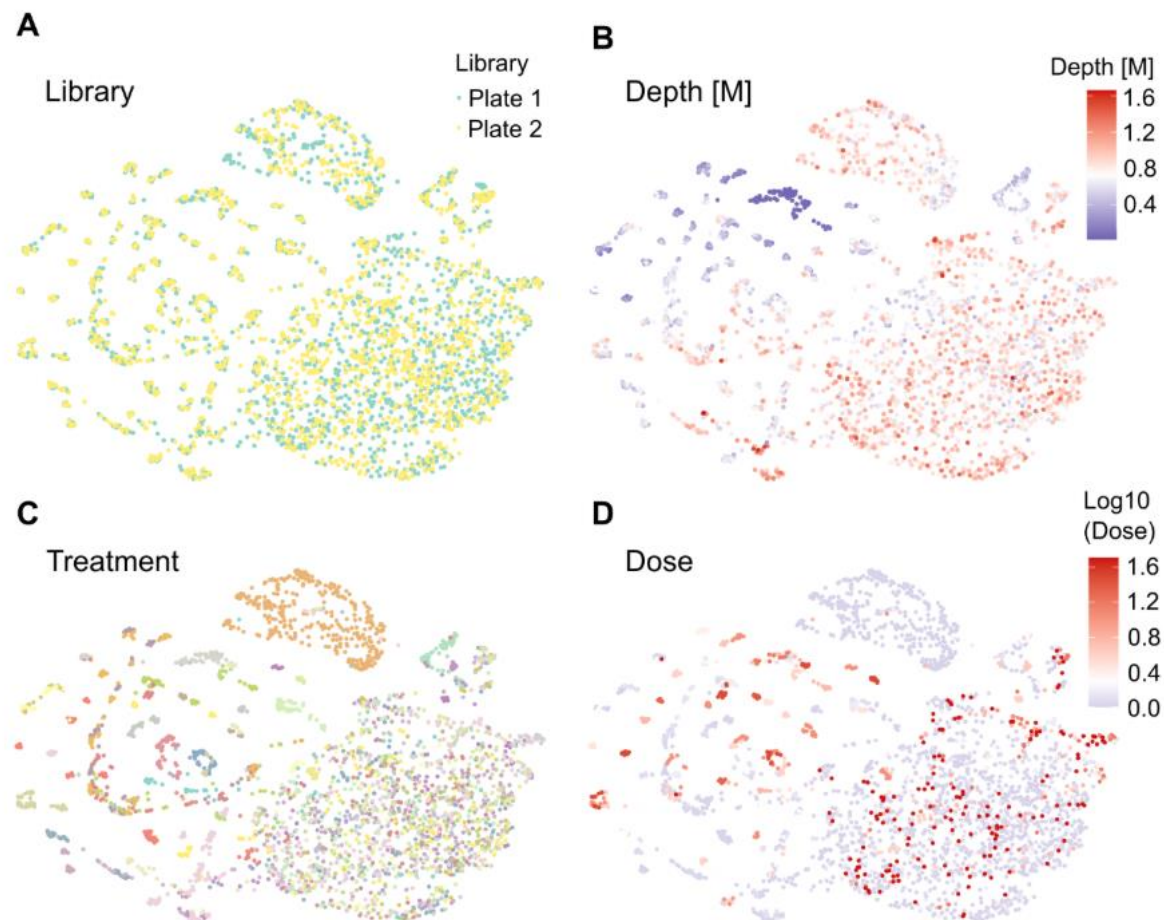
Reproducibility is assessed after independent processing, not by splitting a single library.

$R^2 = 0.94$

inter-plate DE gene count correlation

Figure 3: Inter-plate reproducibility of differential expression across independently processed 1536-well plates. Each point represents a compound-dose combination, comparing the number of differentially expressed genes detected in Plate 1 and Plate 2. The strong correlation between plates ($R^2 = 0.94$) demonstrates highly reproducible transcriptomic responses across independent plate processing.

1536 DRUG-seq profiles retain compound- and dose-dependent biological structure



2,760

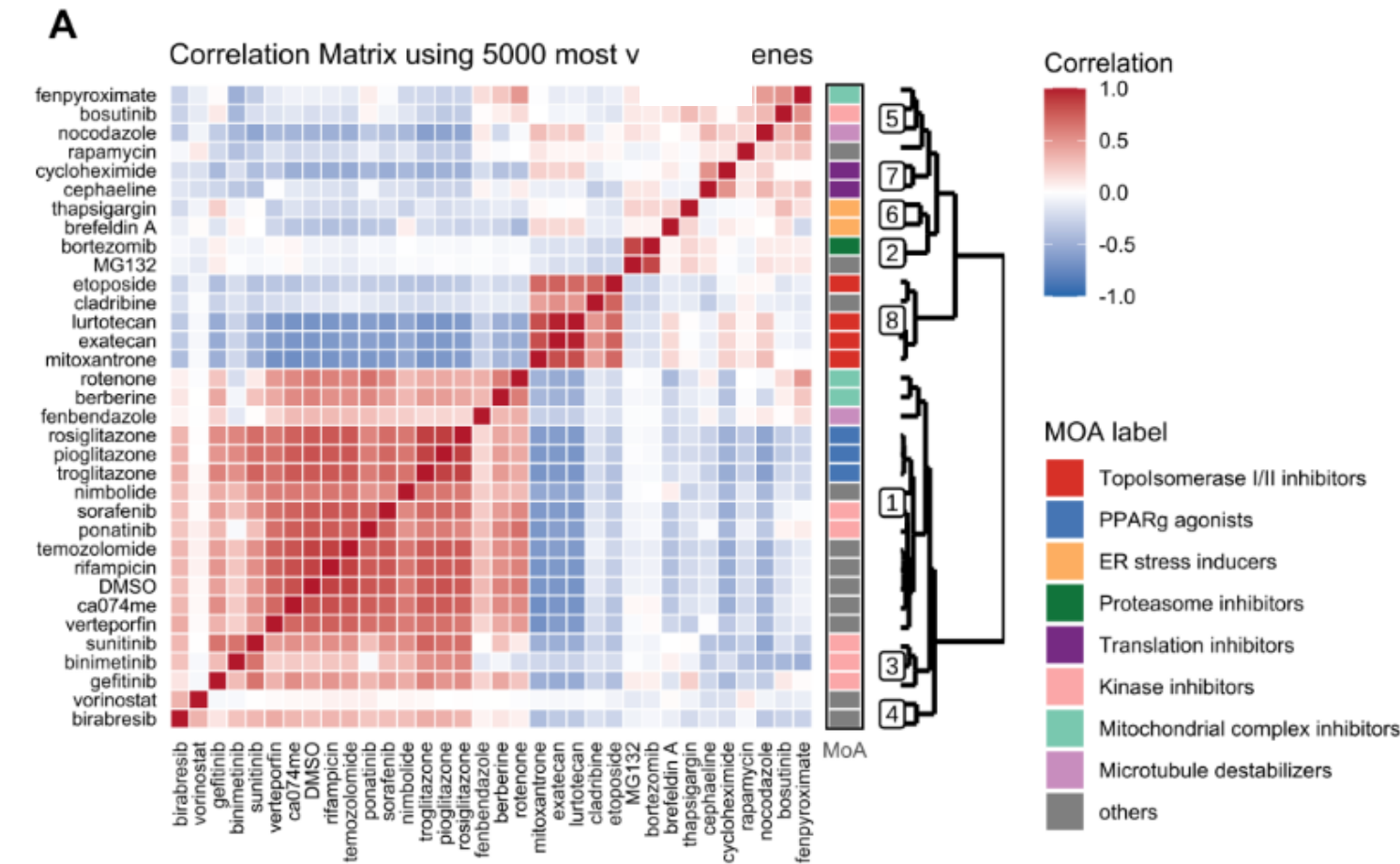
batch-corrected profiles in the UMAP

Figure 4. Global transcriptional structure.

UMAP visualization of 2,760 batch-corrected transcriptional profiles from 33 compounds across eight dose ranks and two replicate plates.

Samples are colored by plate, read depth, treatment identity, and dose. Treatments producing strong transcriptomic responses separate from lower-activity conditions, revealing biologically meaningful compound- and dose-associated structure across the perturbation library.

1536 DRUG-seq clusters compounds by known MoA



5,000

most variable genes used for clustering

Figure 5. Compound similarity and MoA clustering.
Correlation matrix of average gene expression profiles across the 5,000 most variable genes for all 33 compounds. Mechanistically related compounds form discrete regions of high transcriptional correlation, including topoisomerase inhibitors, PPAR γ agonists, ER stress inducers, proteasome inhibitors, and translation inhibitors.

A single 1536-well screen resolves both MoA and toxicity-associated pathway activity

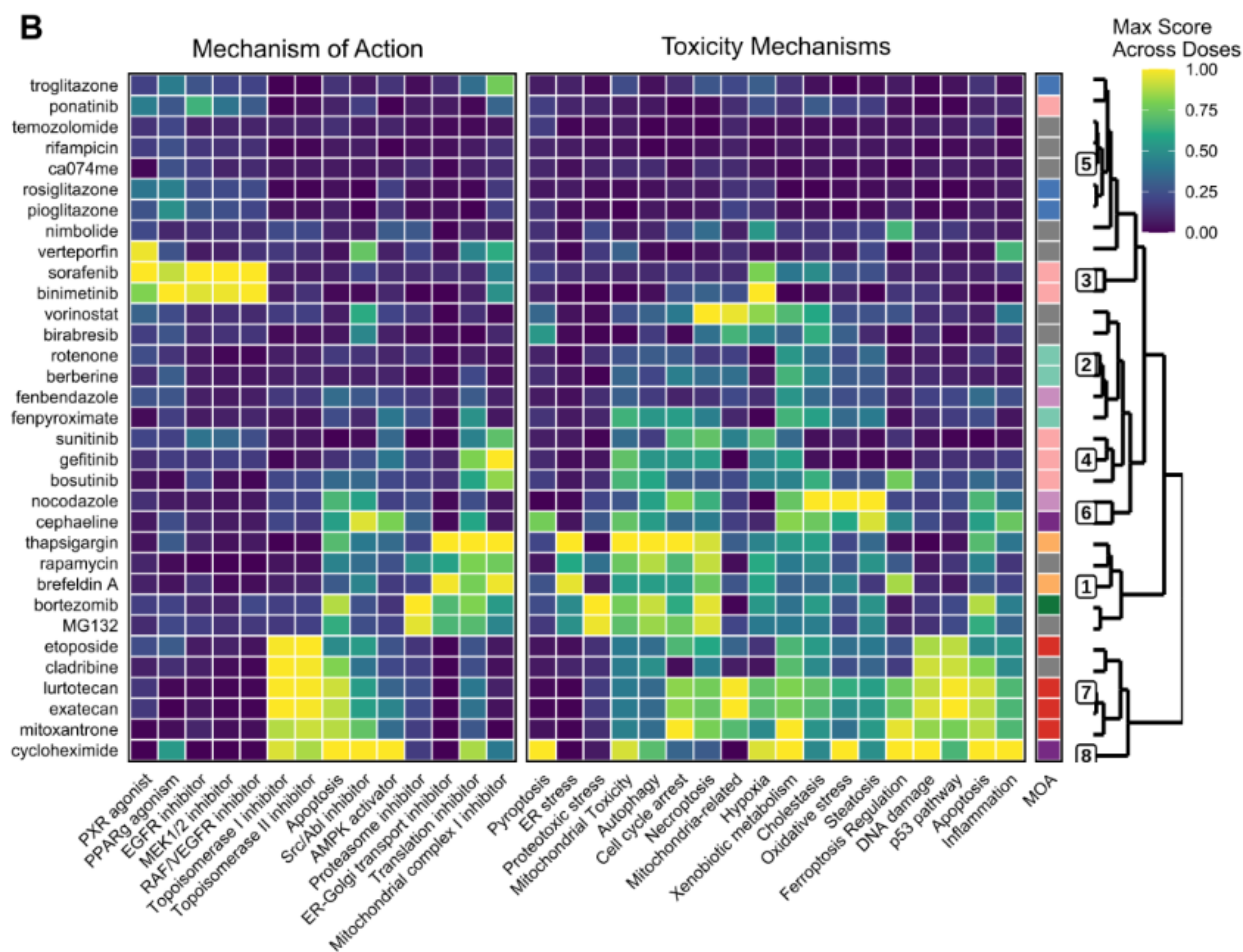


Figure 5. MoA and toxicity pathway scoring.
Maximum normalized mechanism-of-action and toxicity pathway scores across all tested doses for each compound. Activation patterns are compound-specific and complementary, capturing both shared responses among mechanistically related compounds and distinct pathway signatures that differentiate individual treatments.

Gene-level resolution separates compounds that share the same nominal MoA

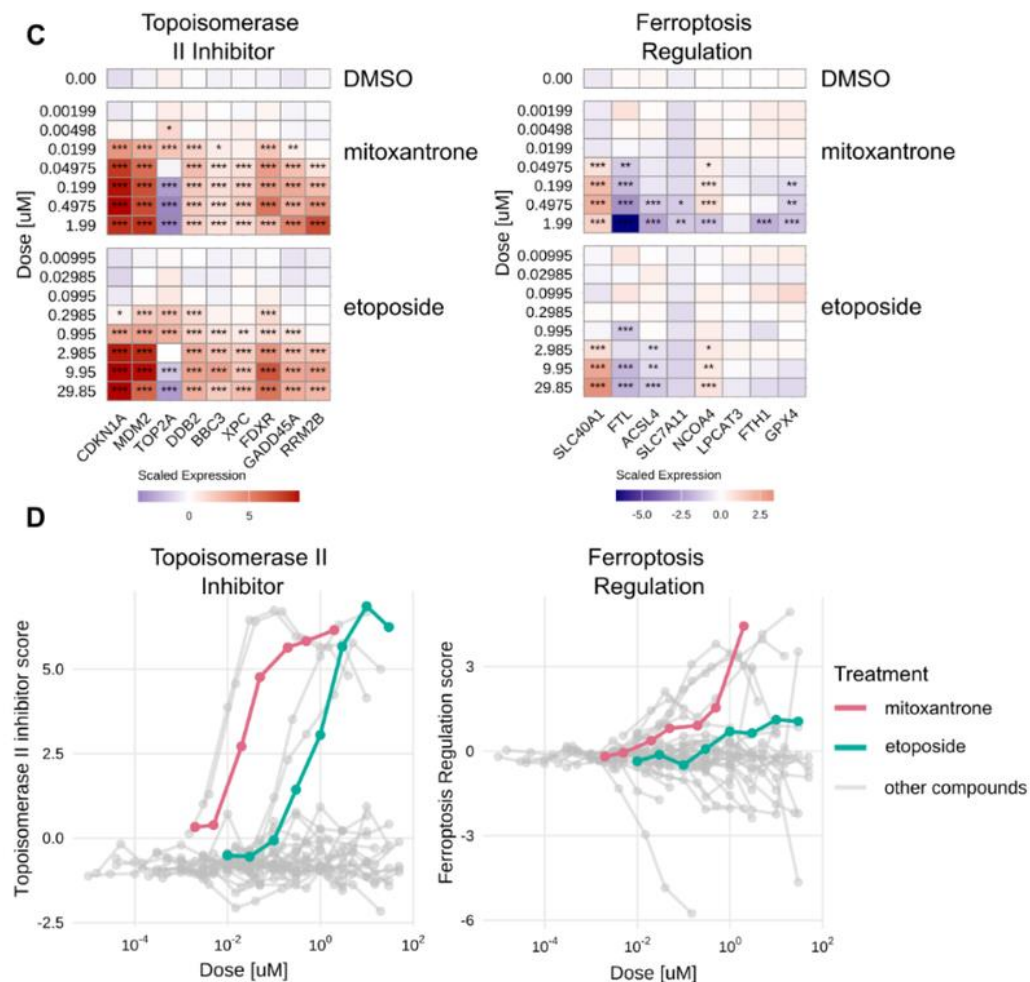


Figure 6. Gene-level resolution of mechanistically related topoisomerase II inhibitors. Mitoxantrone and etoposide share the expected topoisomerase II-associated transcriptional response but show distinct compound-specific profiles. Mitoxantrone activates the MoA signature at lower doses and exhibits a ferroptosis-associated response linked to repression of GPX4 and SLC7A11, demonstrating that 1536 DRUG-seq can resolve mechanistic differences between compounds with the same nominal MoA.

Transcriptomics reveals compound stress before or inline with viability loss on the same 1536-well assay

For safety and dose-selection decisions, transcriptomics can provide an earlier mechanistic window than a single apical endpoint.

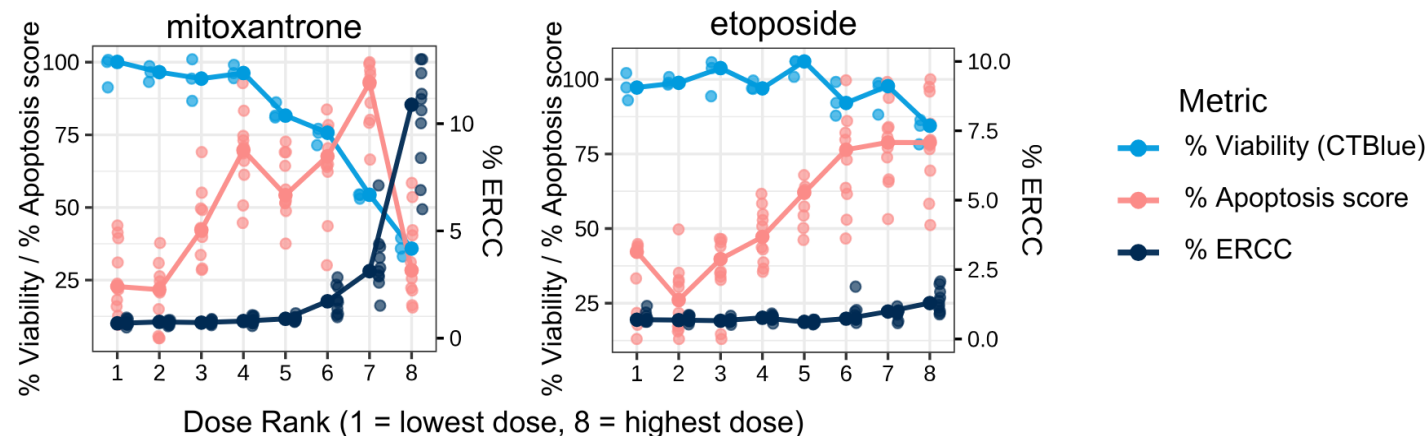


Figure 7. Transcriptomic response versus cell viability. Transcriptomic apoptosis signatures align with, and in some cases precede, measurable cytotoxicity. For mitoxantrone, apoptosis scores increase as CTBlue viability declines. For etoposide, apoptosis activation appears at lower dose ranks before a corresponding reduction in viability, with stable ERCC recovery supporting a genuine biological response rather than a technical artifact.

1536 DRUG-seq generates reproducible dose-response curves and tPODs across independent plates

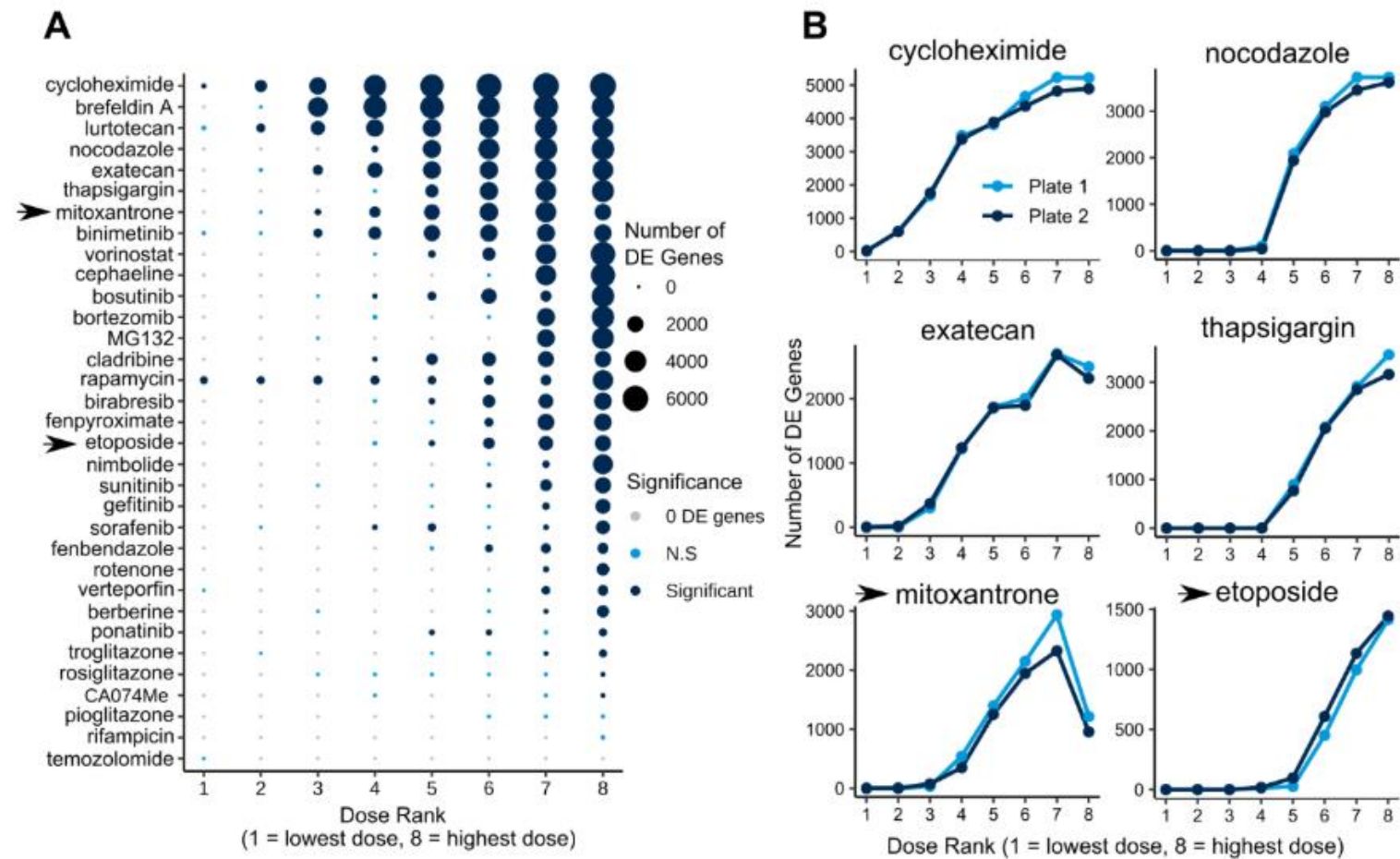


Figure 8. Dose response and tPOD reproducibility. *MERCURIUS™ 1536 DRUG-seq detects reproducible, dose-dependent transcriptional responses across diverse compounds. Compounds exhibit distinct transcriptomic points of departure within their respective dose ranges, while representative dose-response profiles show close agreement between independently processed plates.*

Bubble size = number of DE genes; curves = DE genes across eight dose ranks.

What 1536 DRUG-seq changes in a screening workflow

01

Screening-compatible scale

1536-well input and early multiplexing enable transcriptomics at the throughput of modern perturbation screening.

02

Reproducible data generation

Independent plates produce concordant gene detection, dose-response trajectories, and DE-gene counts.

03

Mechanism-level decision making

Datasets support MoA clustering, pathway-level toxicity, gene-level differentiation, and tPOD estimation.

04

Orthogonal integration

Same-plate viability adds an apical endpoint while transcriptomics exposes earlier and more specific stress biology.

A practical role in the discovery pipeline

Perturbation
screening



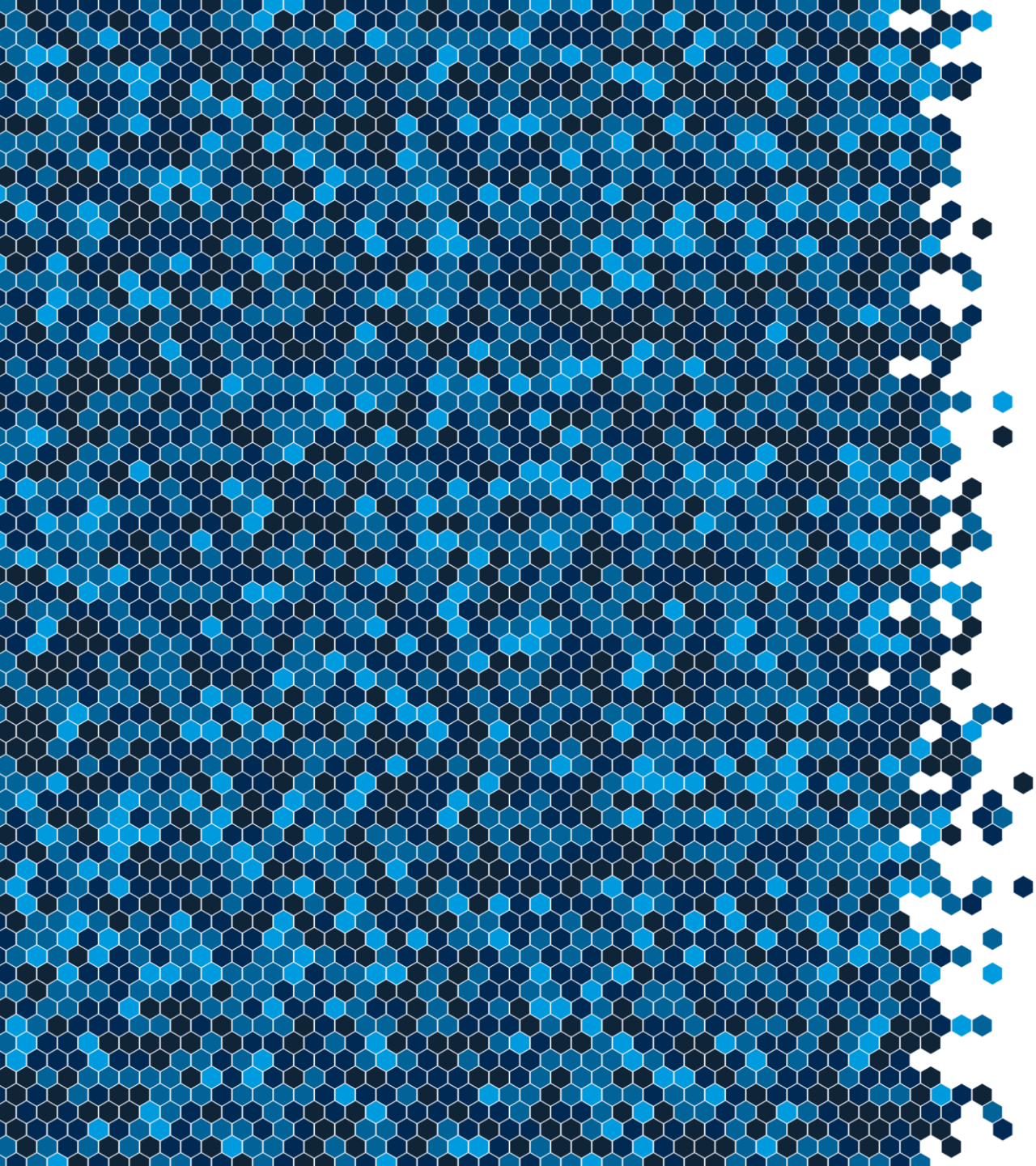
Hit / MoA
triage



Dose & toxicity
de-risking



Predictive model
data generation



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