

# Incorporating Metabolic Competence into High-Throughput Profiling Assays

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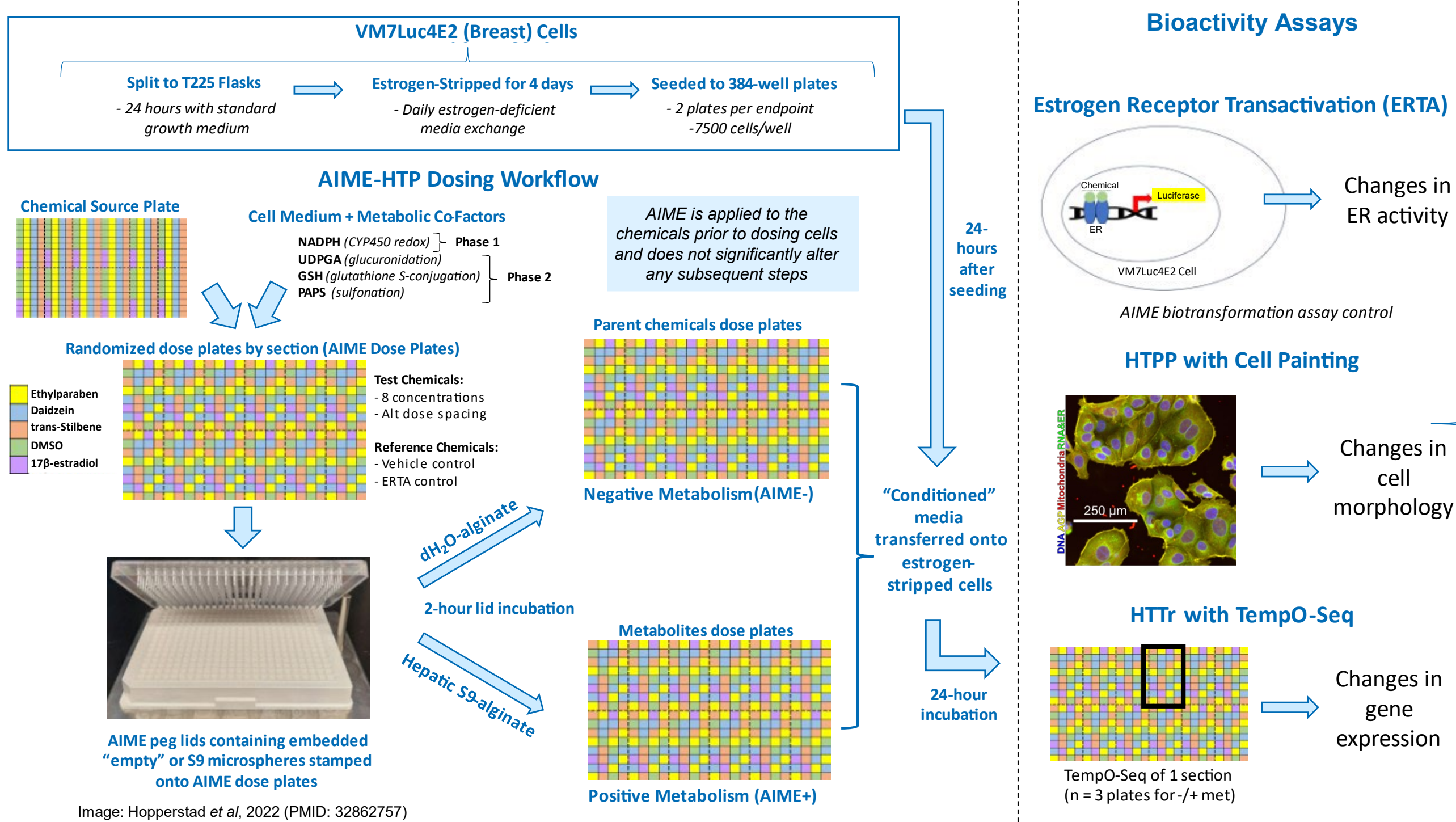
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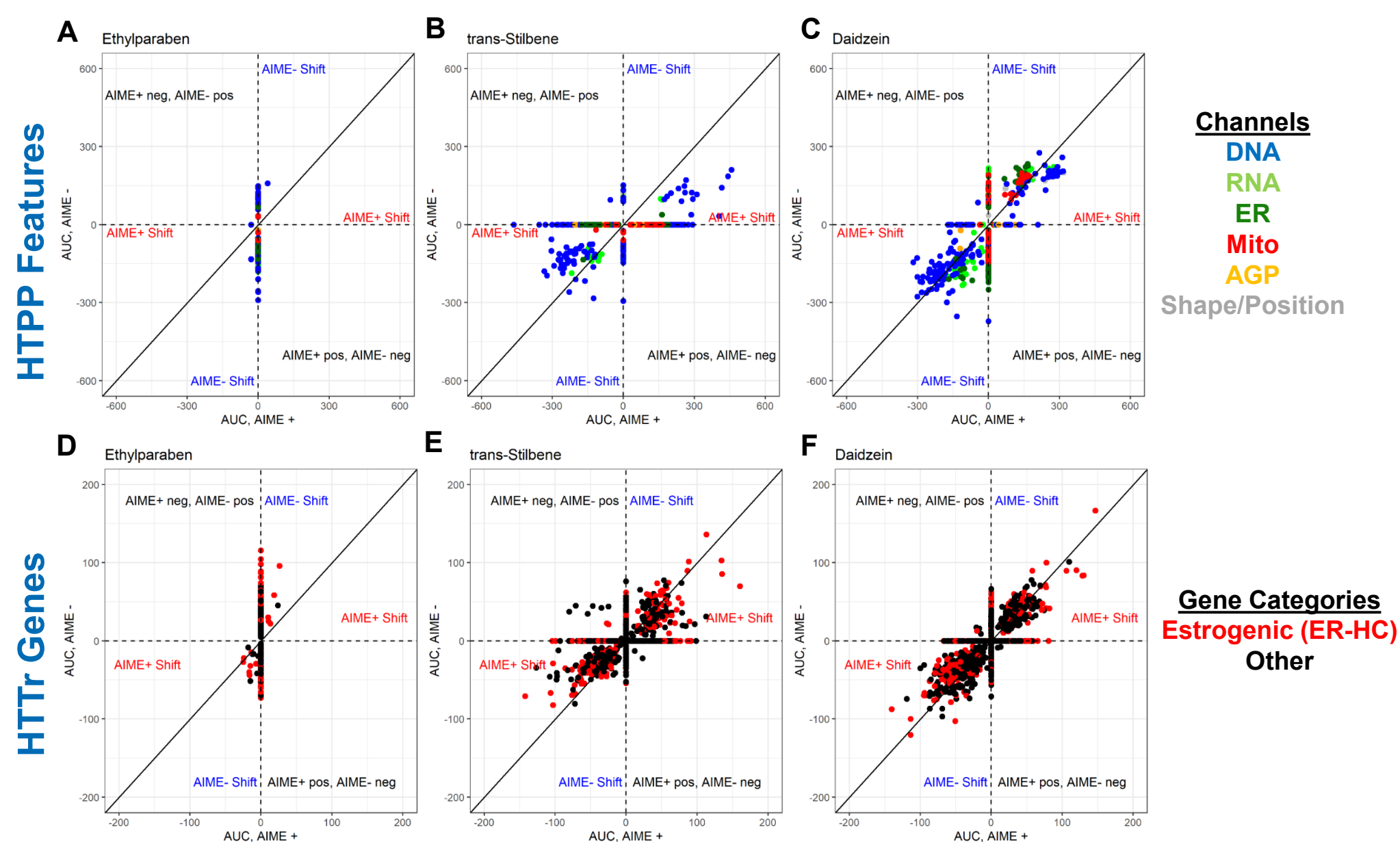
## Proof-of-Concept Experimental Design

**Aim:** To incorporate the **Alginate Immobilization of Metabolic Enzymes (AIME)** *in vitro* metabolism platform **into high-throughput profiling (HTP) assays** used in chemical bioactivity screening and hazard identification to reduce uncertainties in new approach methods (NAMs)-based chemical risk assessments.



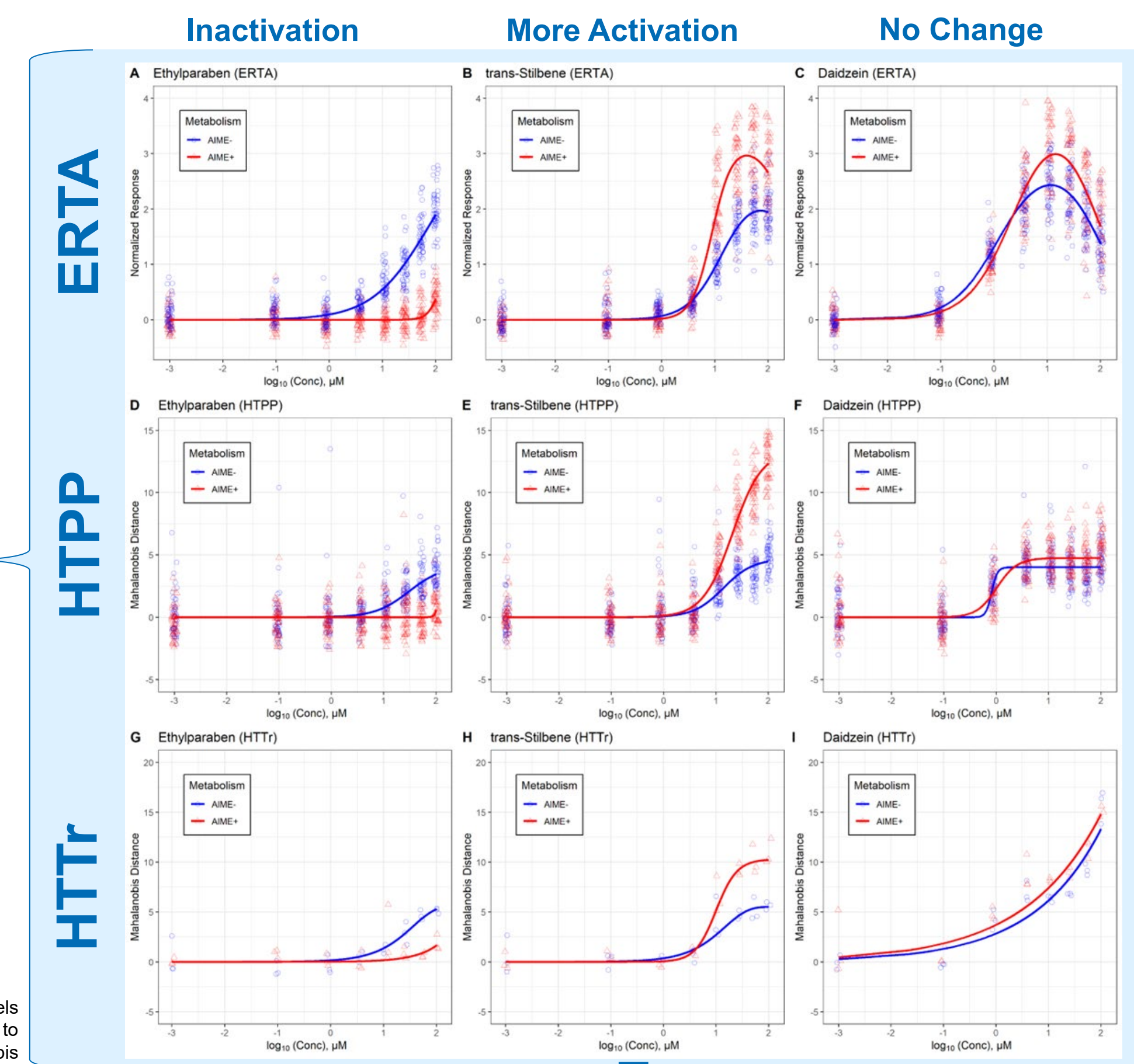
**Fig 1: Workflow schematic and readouts from three bioactivity assays in VM7Luc4E2 (breast) cells.** A luciferase assay was used to measure estrogen receptor activation. Panels A-C show the normalized response to DMSO in the ERTA assay. High-throughput phenotypic profiling (HTPP) plots (D-F) are based on dimensionality reduction of features to Mahalanobis distances calculated relative to DMSO wells. High-throughput transcriptomics (HTTr) plots (G-I) are based on dimensionality reduction of gene counts to Mahalanobis distances calculated relative to DMSO wells. Best fit curves were calculated using *tcp1fit2*, and colors of the lines and associated points indicate that the responses are from the AIME- (blue) or AIME+ (red) condition.

## Area Under the Curve (AUCs) of Shared Active Features and Genes Pivot Toward the More Bioactive Metabolic Condition

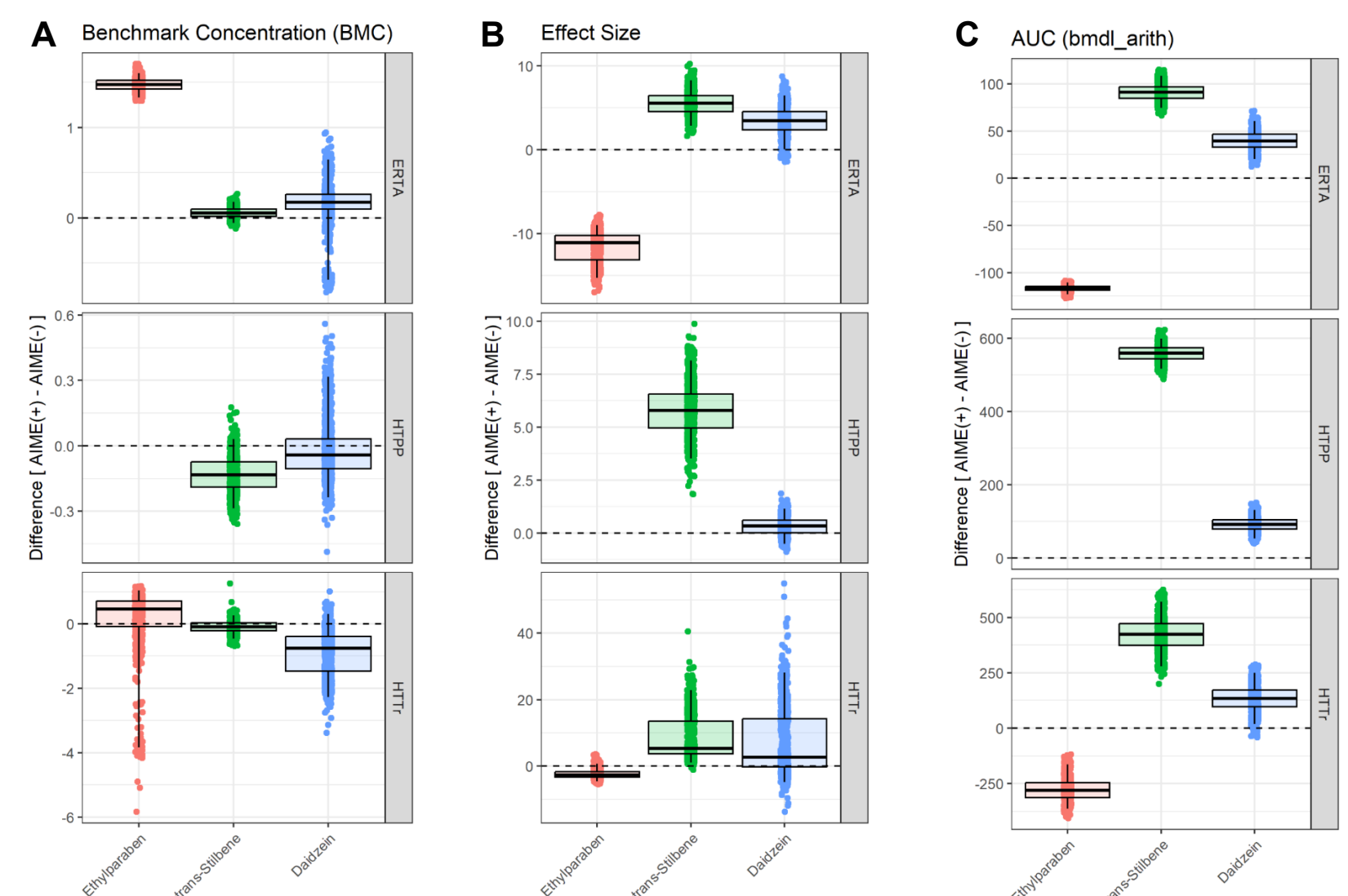


**Fig 3: AUC pivot plots for active HTPP features and HTTr genes.** (A-C) Each dot represents a feature that is active (hitcall > 0.90) in at least 1 condition. Color represents the channel of the active feature. (D-F) Each dot represents a gene that is active (hitcall > 0.90) in at least one condition with a log<sub>2</sub> fold change ≥ 2. Color represents if a gene is a component of an estrogen receptor-high confidence (ER-HC) gene signature (Harrill, et al, 2024; PMID: 39177380) or not. For all plots, AUC was calculated by taking the approximate integral of the best fit curve and setting responses below the *benchmark dose lower bound (bmdl)* to 0. Additionally, if a feature or gene was inactive (hitcall < 0.90) for a condition, then the AUC was assigned as 0.

## Expected Shifts in Overall Bioactivity with Metabolism



## Area Under the Curve (AUC) Detects Shifts with Metabolism Better Than Potency or Effect Size



**Fig 2: Summary of Bootstrapping Results for Benchmark Concentration (BMC), Effect Size and Area Under the Curve (AUC).** Global bioactivity results for all assays were bootstrapped for 1000 iterations and each iteration was analyzed via *tcpflit2* to determine if metabolic shifts were significant for BMC (A), effect size (B) or AUC (C). AUC was calculated by taking the approximate integral of the best fit curve and setting responses below the *bmdl* to 0. Differentials were only calculated if the original endpoint was active (*hitcall* > 0.90) for both conditions and only active bootstraps were utilized for comparison. The differential was calculated by subtracting the AIME- condition from the AIME+ condition. The dashed line represents both conditions being equal, and the stems represent the 5<sup>th</sup> and 95<sup>th</sup> percentile of the differential values. Stems crossing over the dashed line indicate that AIME conditions were not significantly different.