

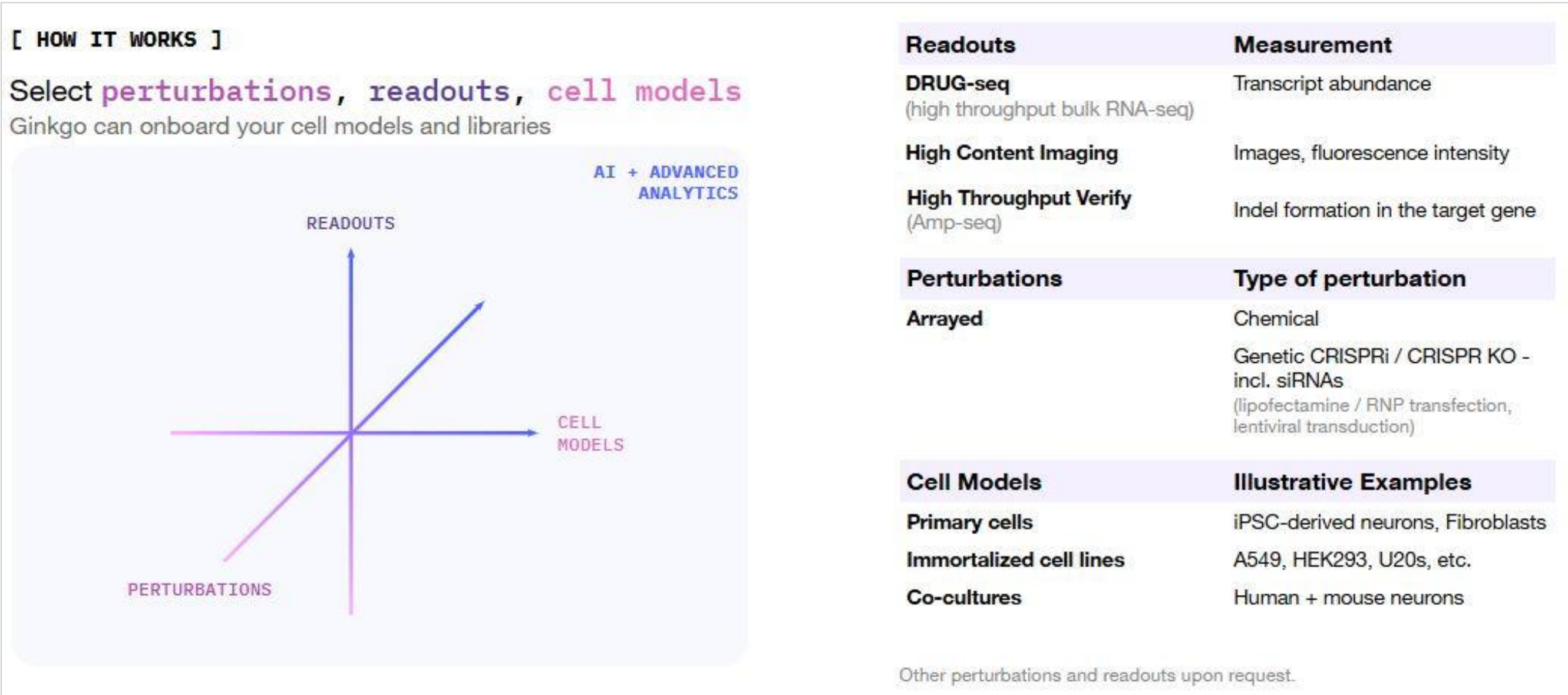
Mapping the Transcriptional Landscape of Drug Responses in Primary Human Cells Using High-Throughput DRUG-seq

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1. Our high throughput data generation platform for AI-driven Drug Discovery

RAPID-Profiling Platform. Cost-Efficient. Insight-Rich.

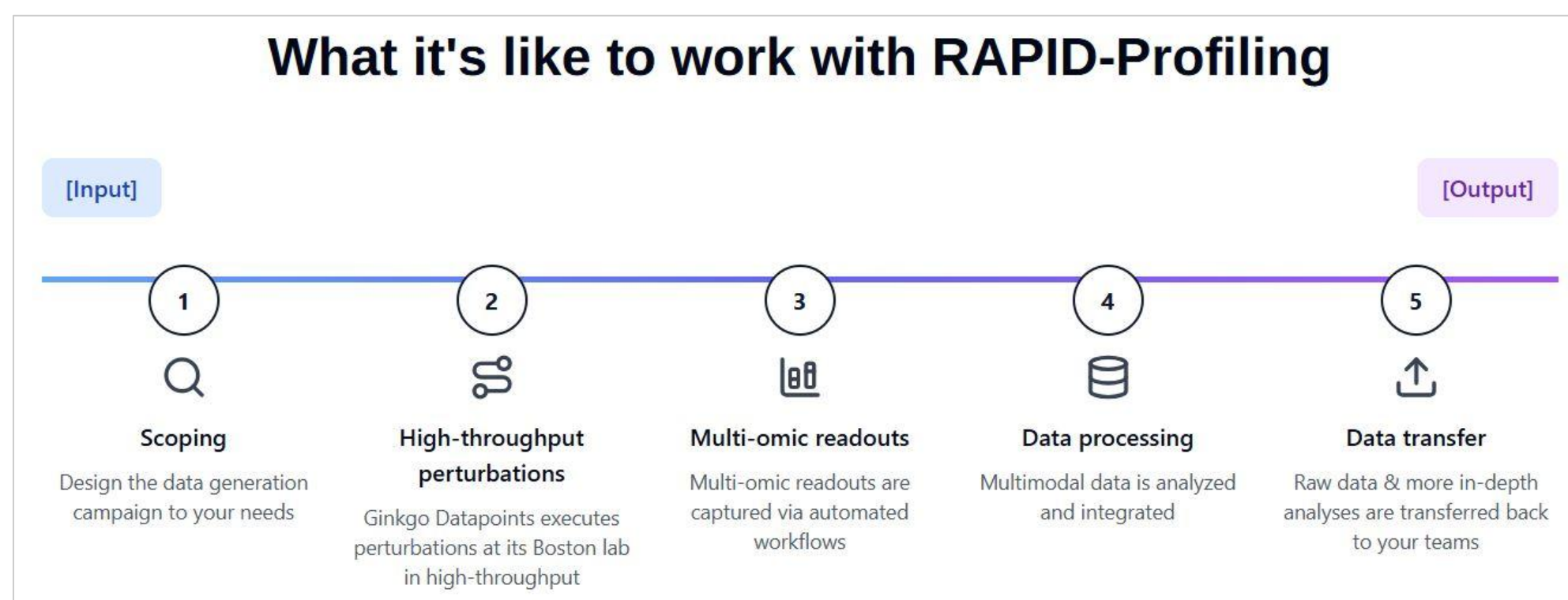
Our high-throughput platform enables profiling of cellular responses to chemical, biological, and genetic perturbations. By leveraging advanced automation and analytics, we deliver comprehensive insights into compound effects and pathway modulation—providing the data you need to make informed decisions quickly and confidently. We provide an end-to-end data generation approach from cell culture to perturbation to data. Our customers select perturbations, readouts and cell models of choice - we generate high-quality data.



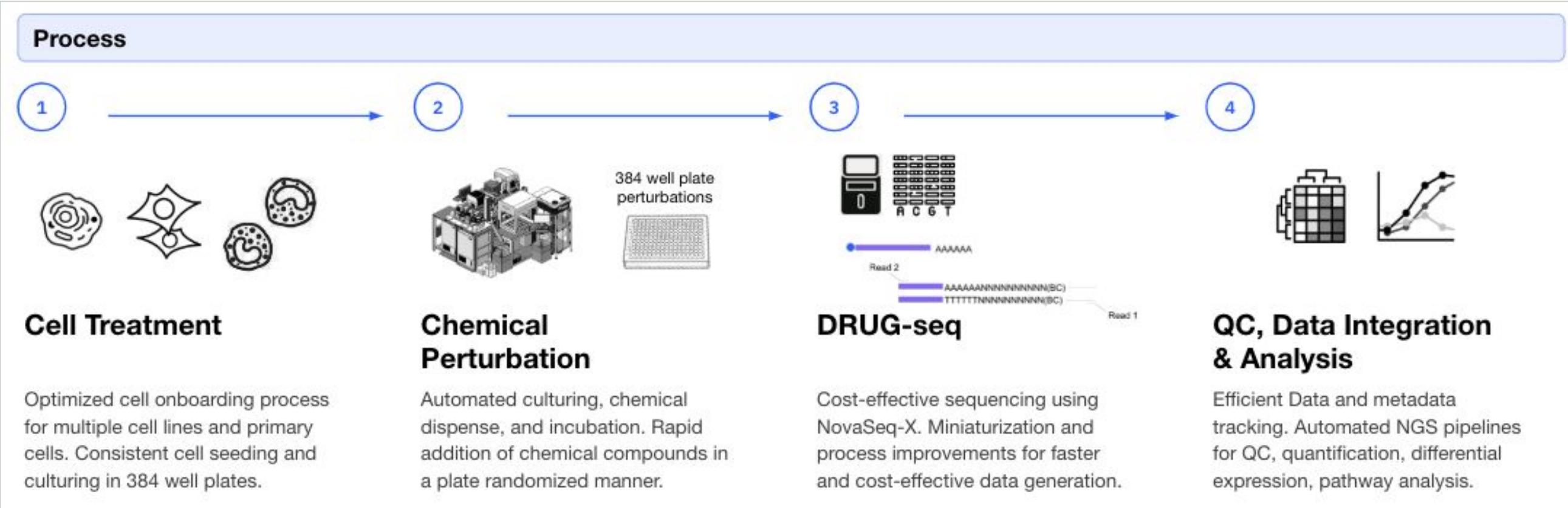
DRUG-seq for Perturbation Response Profiling

DRUG-seq is a miniaturized high-throughput transcriptome profiling assay; it tags polyadenylated mRNAs with no extraction required. DRUG-seq provides unparalleled molecular insights for drug discovery. It's cheaper and higher throughput than bulk RNA-seq, and more versatile than single cell RNA-seq.

Fast turnaround time from cell culturing to perturbation to data (2-3 weeks), unbeatable scale (screen 10,000s of perturbations at a time), and outstanding unit economics (less than 30x the price of traditional bulk RNA-seq) make DRUG-seq the premier transcriptomic screening assay for various data generation needs, from target ID use cases to tox profiling. DRUG-seq readouts can be applied across chemical, genetic (CRISPR KO), and biological perturbation projects.



2. Experimental design

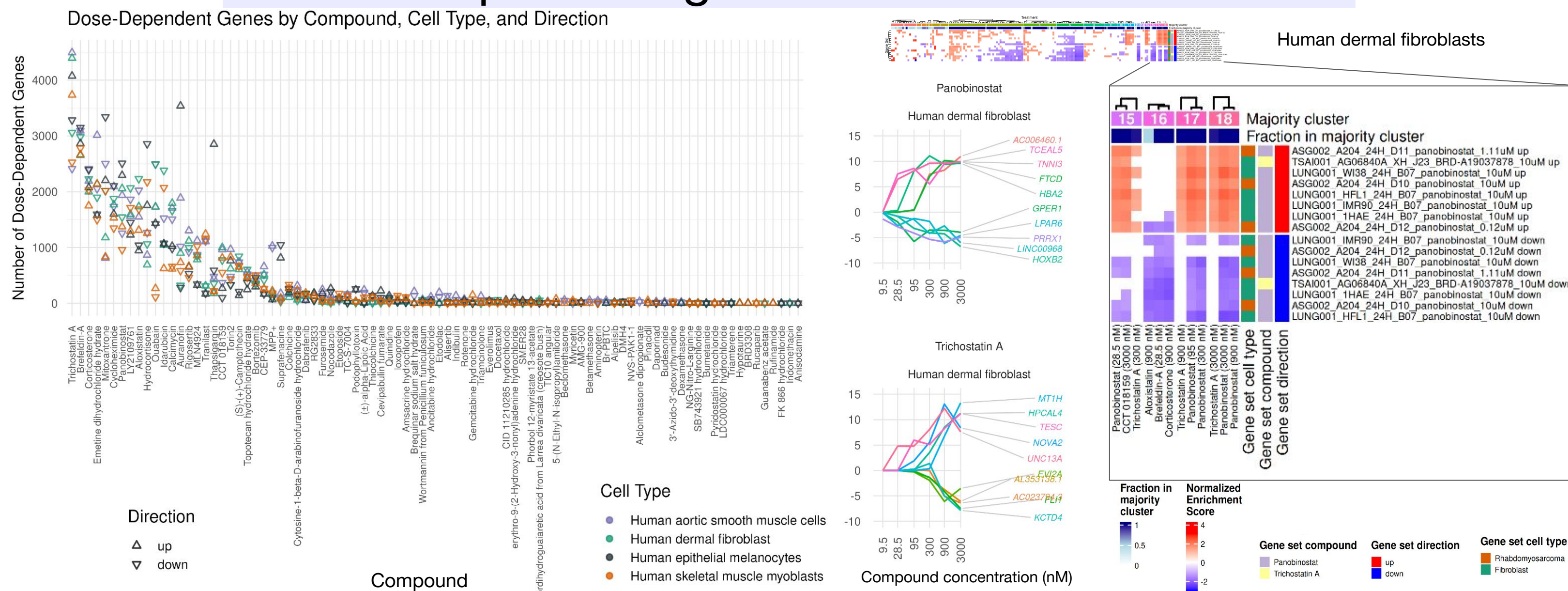


Treatment Name	Replicates per Dose	Concentration Range	Function
DMSO	6	0.0625%, 0.1875%, 0.625%	Vehicle control, added at different volumes to match drug concentrations.
brefeldin A	4	9.5, 28.5, 95, 300, 900,3000 nM	Positive control of transcriptional response. ER-Golgi trafficking inhibitor
dexamethasone	4	9.5, 28.5, 95, 300, 900,3000 nM	Positive control of transcriptional response. Anti-inflammatory
trichostatin A	4	9.5, 28.5, 95, 300, 900,3000 nM	Positive control of transcriptional response. HDAC inhibitor
86 Test compounds	4	9.5, 28.5, 95, 300, 900,3000 nM	11 compounds per plate (8 compounds on last plate)

For this high-throughput screen, we used four primary human cell types—**melanocytes, aortic smooth muscle cells, dermal fibroblasts, and skeletal muscle myoblasts**—cultured across thirty-two 384-well plates. Each cell type was treated with 89 compounds across six concentrations. We applied DRUG-seq to generate transcriptomic profiles for every condition and processed the data using our automated analysis pipeline.

Each plate included **three transcriptionally active positive controls** and DMSO as a vehicle control, tested consistently across all cell types.

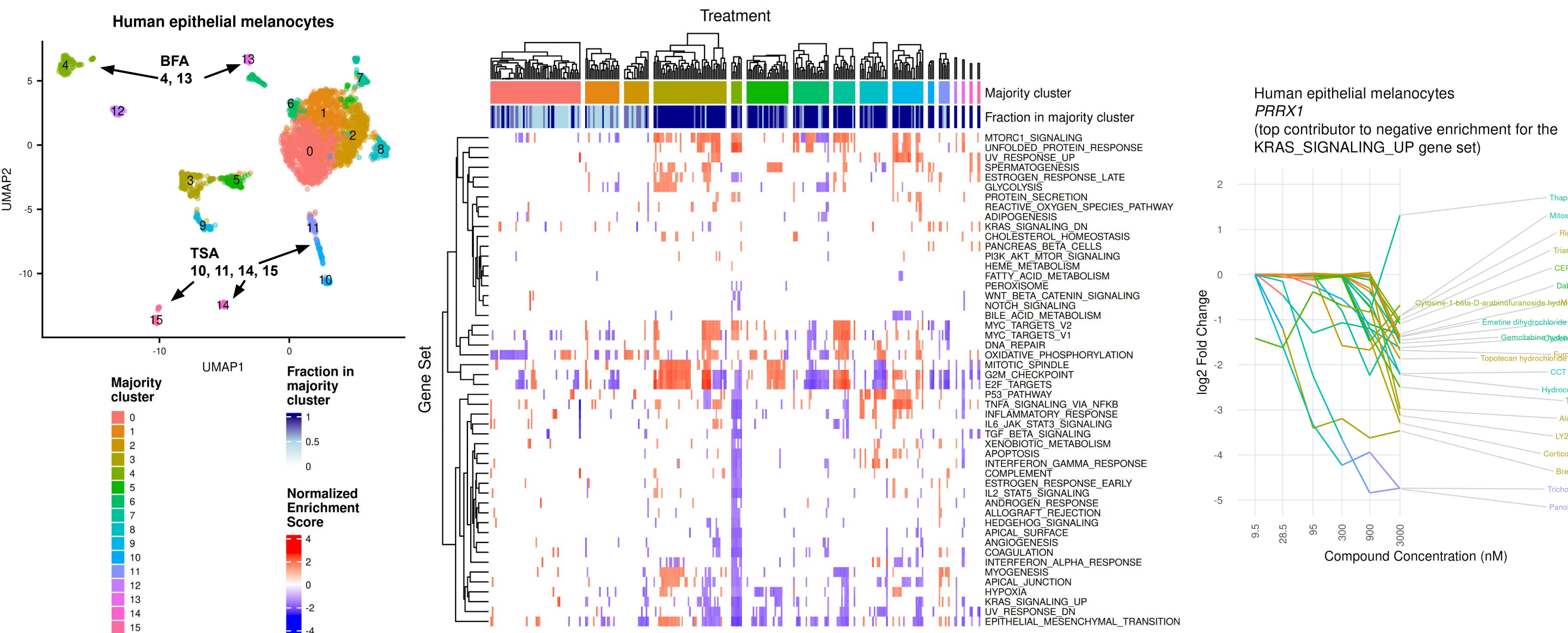
5. Dose-dependent genes and external validation



For all tested compounds, we **quantified the number of dose-dependent genes** (top left). Our analysis revealed that HDAC inhibitors (**TSA** and **panobinostat**) induced strong, dose-dependent transcriptional changes in all four primary human cell types, each affecting over **2,000 genes**; the top and bottom five of which are shown in the dose-dependent gene-expression plots (top middle). **Brefeldin A**, another broadly active control compound, induced over **2,500 dose-responsive genes** across all cell types. Beyond the controls, we **observed a long tail of compounds eliciting substantial dose-dependent transcriptional responses** across cell types, highlighting the sensitivity of our approach.

To validate these responses, we **compared our HDAC inhibitor data to gene expression signatures from the L1000 dataset**. Enrichment analysis showed **strong concordance across cell types**, with TSA and panobinostat clustering together in a concentration-dependent manner. In dermal fibroblasts (top right), we observed distinct clusters corresponding to low (cluster 11), medium (clusters 15 and 17), and high concentrations (cluster 18), confirming the reproducibility and dose-responsiveness of our DRUG-seq results.

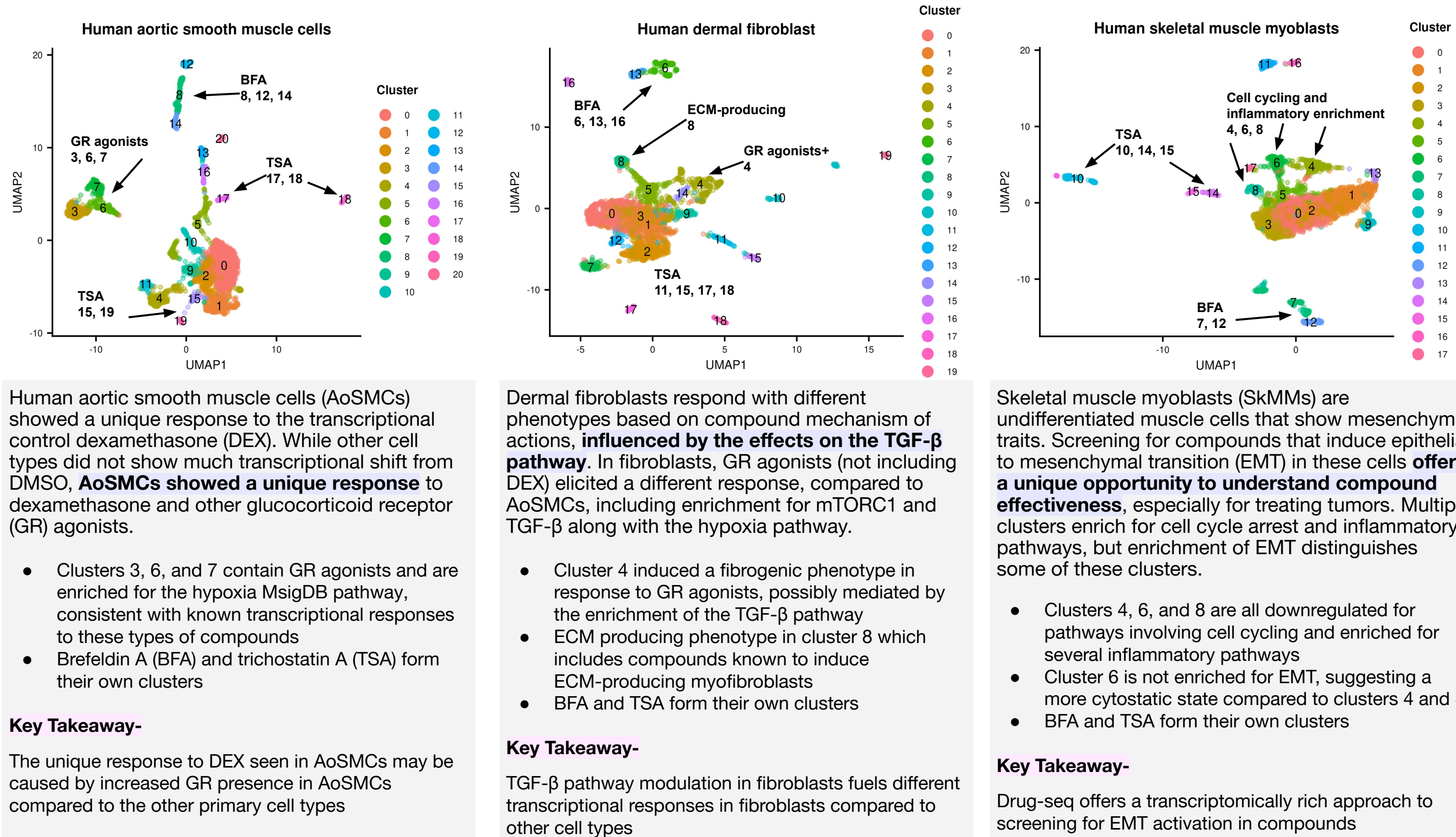
3. Benchmarking with transcriptional controls



We included three well-characterized transcriptional controls—brefeldin A (BFA), dexamethasone (DEX), and trichostatin A (TSA)—on every plate across all four primary human cell types. **BFA and TSA elicited broad and consistent transcriptional responses**, as illustrated here in melanocytes (also shown in panel 6). BFA, which disrupts Golgi function, broadly downregulated MSigDB pathways (top middle; clusters 4 and 13), reflecting its global impact on cellular transport and stress responses. TSA, a histone deacetylase inhibitor, led to increased histone acetylation and transcriptional activation, resulting in distinct clustering patterns in UMAP space (top left; clusters 10, 11, 14, and 15). Further, **many compounds showed clear dose-dependent gene responses** (also shown in panel 5), including *PRRX1*, a gene in the MSigDB KRAS signaling pathway, which is plotted here in melanocytes (top right).

In contrast, **DEX**, a synthetic glucocorticoid, **did not induce a broad transcriptional response across all cell types**. Instead, transcriptional effects were observed **exclusively in aortic smooth muscle cells**. This selective sensitivity likely reflects cell type-specific differences in glucocorticoid receptor signaling, chromatin landscape, and pathway interactions—underscoring the importance of evaluating compounds across diverse primary cell types to fully capture biologically relevant responses.

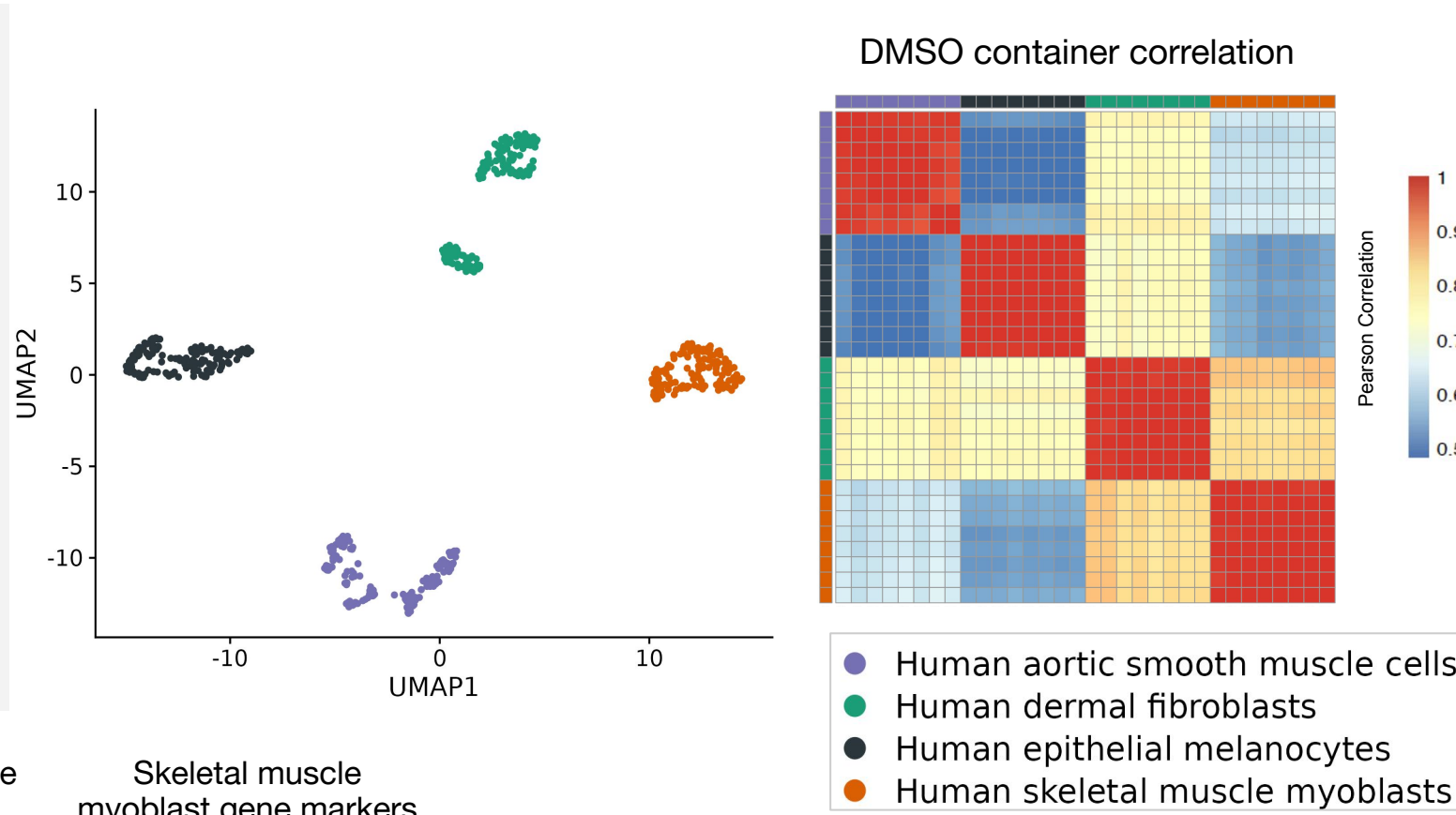
6. Unique and biologically relevant cellular responses



4. DRUG-seq data capture known cell type differences

Using wells treated with the lowest concentration of DMSO, UMAP visualization (right) revealed **clear transcriptional separation of all four primary human cell types**, confirming DRUG-seq's ability to resolve cell type-specific profiles. Additionally, genome-wide Pearson correlation values across DMSO wells (and control-treated replicates, not shown) within each cell type demonstrated strong internal consistency, with a median correlation coefficient exceeding 0.99 (far right).

To further validate cell type identity, we compiled a panel of known marker genes from external sources. Marker gene expression (shown in the heatmap below) was most **distinct in melanocytes and dermal fibroblasts**, while, as expected, **the two muscle-derived cell types, skeletal muscle myoblasts and aortic smooth muscle cells, showed overlapping markers, but retained detectable transcriptional differences** reflective of their unique lineages.



7. Conclusions and future directions

Our study demonstrates the power of DRUG-seq to generate **scalable, high-resolution transcriptomic profiles** in physiologically relevant human primary cells. By profiling **89 compounds across four diverse cell types and six concentrations**, we captured both conserved and cell type-specific transcriptional responses with strong reproducibility and biological fidelity.

- Physiologically relevant insights:** Primary melanocytes, dermal fibroblasts, aortic smooth muscle cells, and skeletal muscle myoblasts revealed lineage-specific drug responses.
- Mechanism-aware profiling:** DRUG-seq reliably identified dose-dependent responses to canonical compounds and uncovered new insights into glucocorticoid, HDAC, and other pathway modulation.
- Benchmark-validated platform:** Our transcriptional signatures showed strong concordance with L1000 data, validating both compound effects and platform sensitivity.
- Foundational resource for AI and discovery:** This dataset lays critical groundwork for building next-generation machine learning models that predict drug responses across cell types and disease states.

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Download our preprint and the full DRUG-seq dataset here!

