

Integrated DRUG-seq and Cell Painting Profiling of JUMP MoA Compounds in U2OS and HepG2 Cells



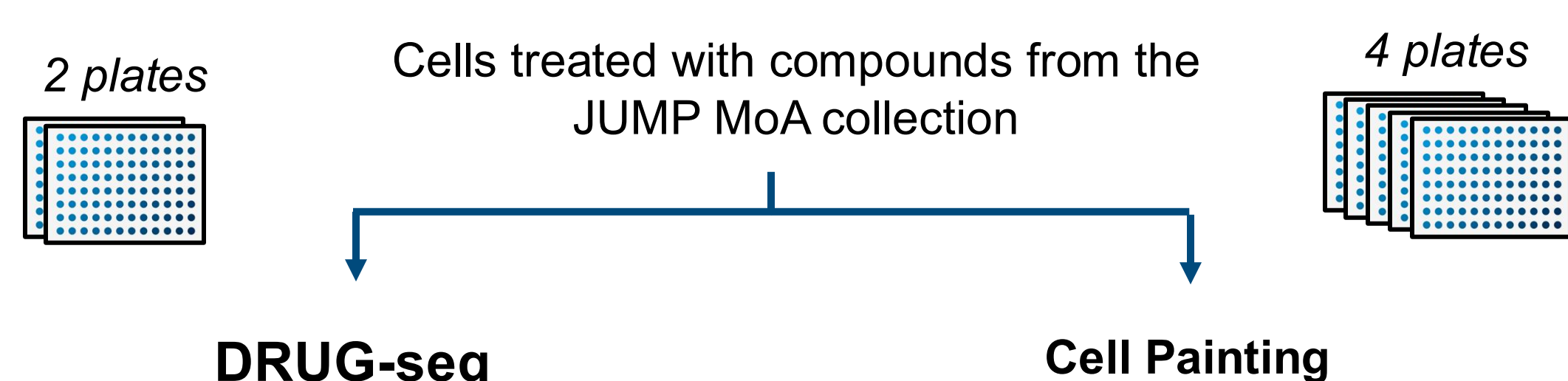
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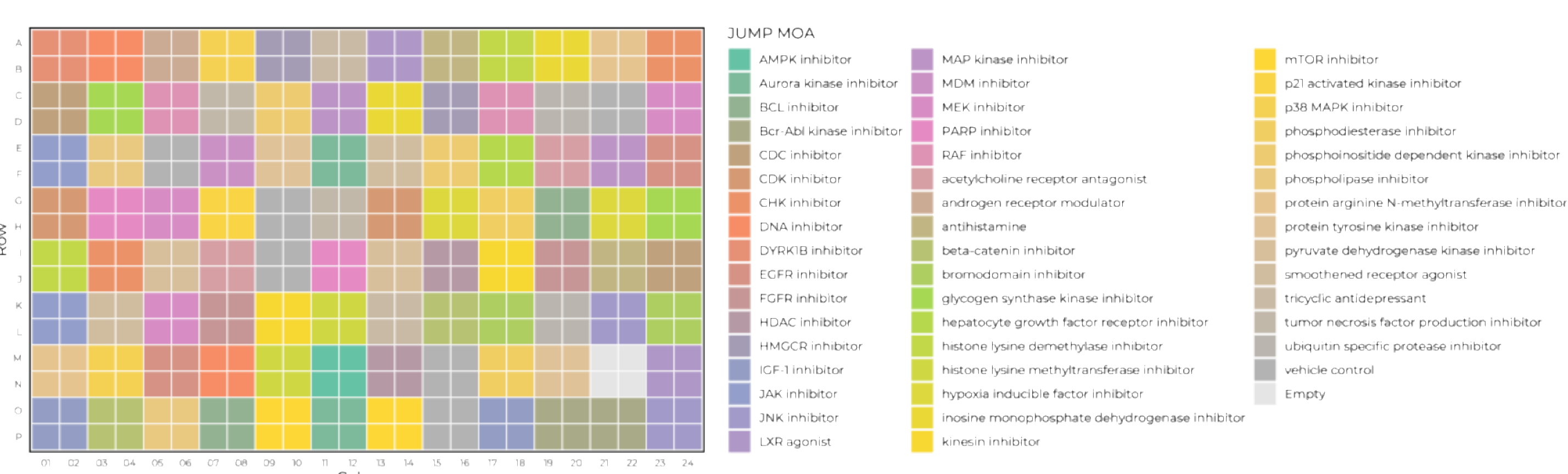
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Experimental Set up of MERCURIUS™ DRUG-seq and Cell Painting

U2OS and HepG2 cells were plated and processed for both DRUG-seq and Cell Painting

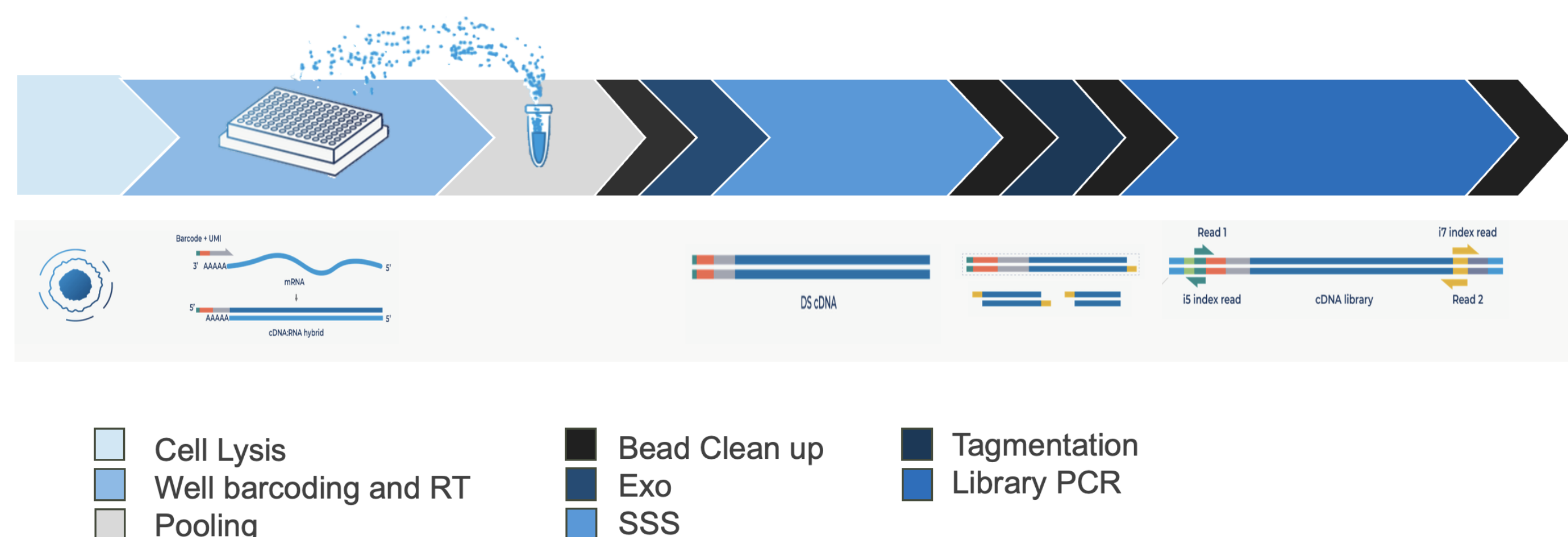


82 JUMP MoA compounds tested in DRUG-seq and Cell Painting



- U2OS and HepG2 cells were treated with 82 compounds in single dose; 4 replicates each.
- Total of 49 mechanisms of action (MoAs) were represented, with one or two treatments assigned to each MoA.

The MERCURIUS™ DRUG-seq protocol enables massively parallel and RNA-extraction free library preparation from 96, 384 and 1536 well plates



Key Takeaways

Aspect	HepG2	U2OS	Overall
QC Metrics	Excellent	Excellent	Acceptable consistency
DEGs per treatment	Up to 2600	Up to 2100	Strong responses in both
Clustering clarity	DRUG-seq > CP	CP ≈ DRUG-seq	DRUG-seq best overall
Cross-cell-type consistency	Moderate	Moderate	CP slightly more consistent
Reproducibility (correlation)	DRUG-seq > CP	–	DRUG-seq superior
Activity scoring (TAS/MAS)	DRUG-seq highest	–	Correlates with feature richness

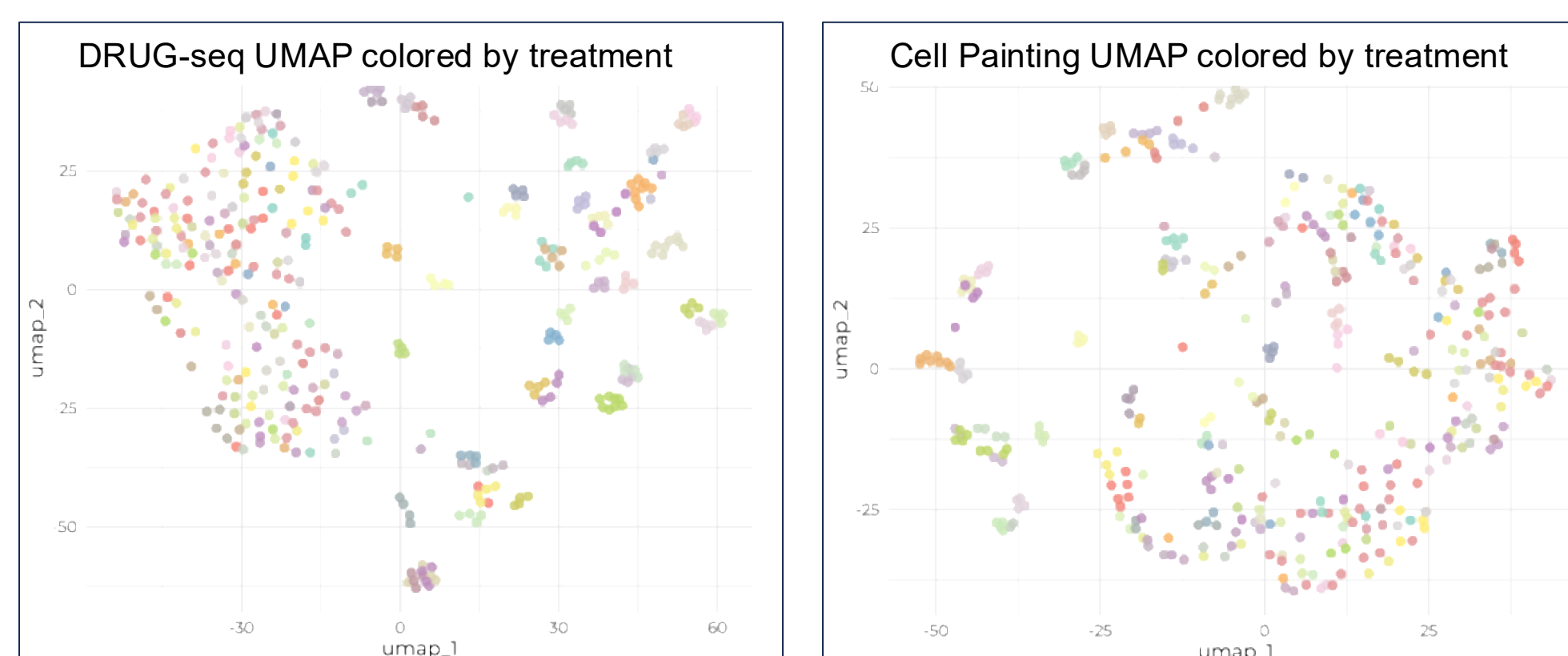
Summary

- DRUG-seq seems to provide stronger and more reproducible clustering than Cell Painting in HepG2, while both perform comparably in U2OS.
- Data quality is robust, with only minor technical anomalies (column 24 ERCC issue).
- Transcriptomic profiling appears to capture broader biological responses, evident in higher DEG counts and stronger inter-replicate correlations.
- Cell Painting remains complementary, offering morphological insights and more consistent cross-cell-type clustering.
- Combined DRUG-seq + Cell Painting integration can enhance mechanism-of-action inference and cross-platform validation.

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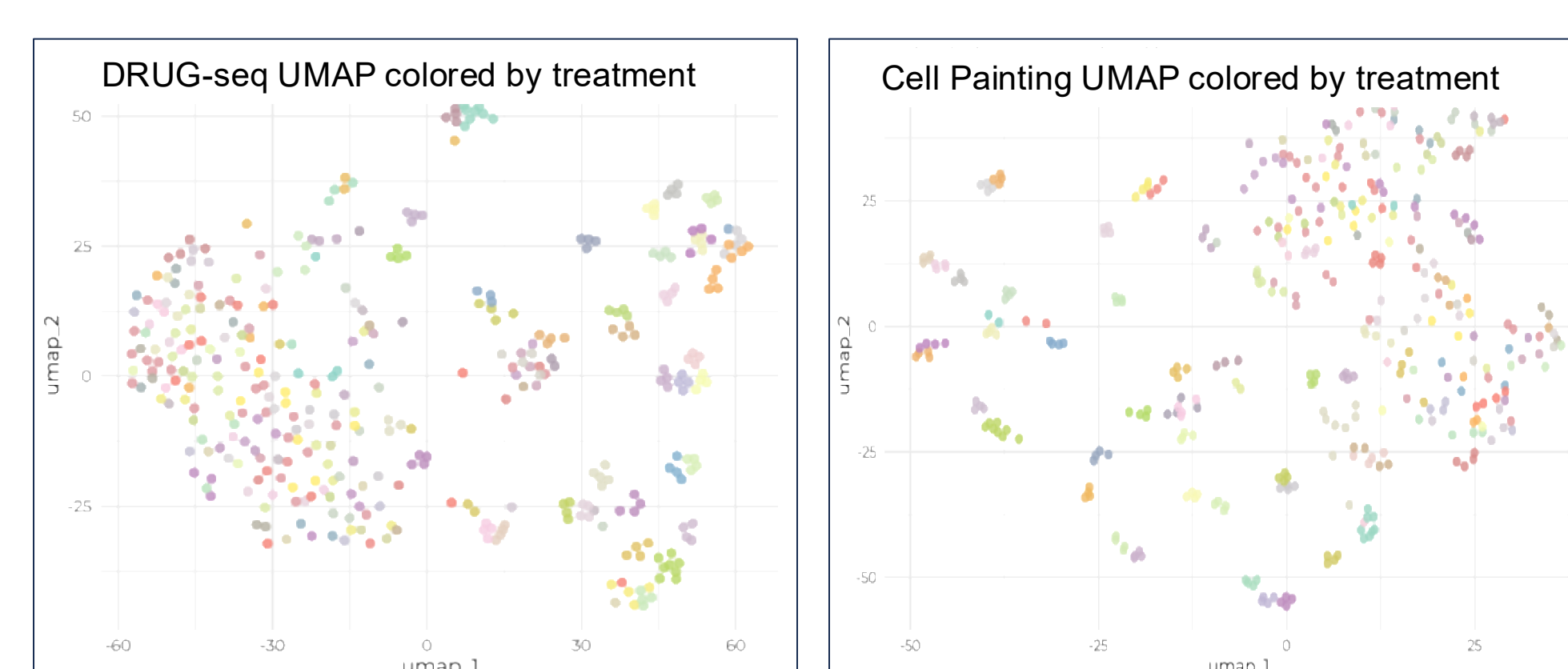
Comparing Performance and Clustering of DRUG-seq and Cell Painting

Treatment Signatures in HepG2: DRUG-seq Outperforms Cell Painting in Clustering Clarity



- Cell Painting processed data included 128 features
- The clustering obtained from DRUG-seq data was visually better than the one obtained from Cell Painting
 - both with UMAP using 30 first PCs and same set of parameters)
- HepG2 cells exhibited significant differential gene expression in 42 out of 82 conditions.
- The number of DE genes per condition reached up to 2600.

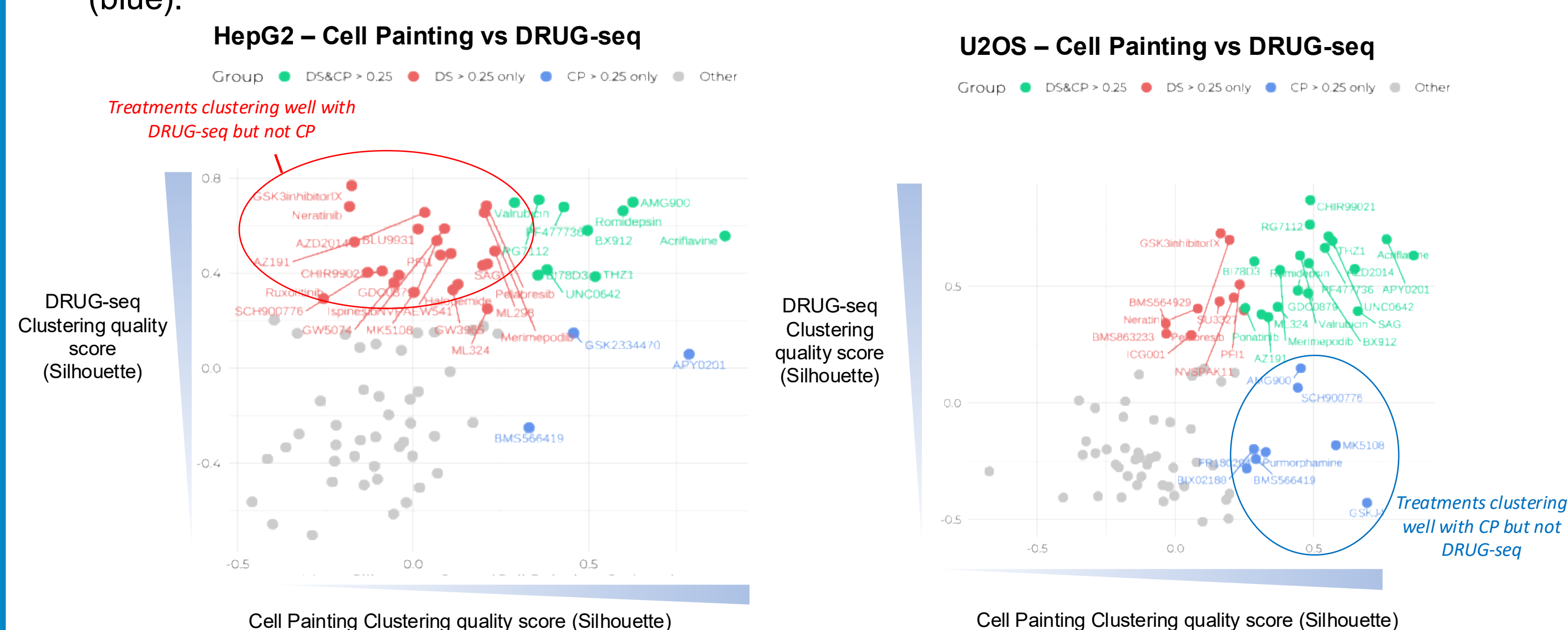
Clustering of Treatment Signatures in U2OS in DRUG-seq and Cell Painting are much more similar



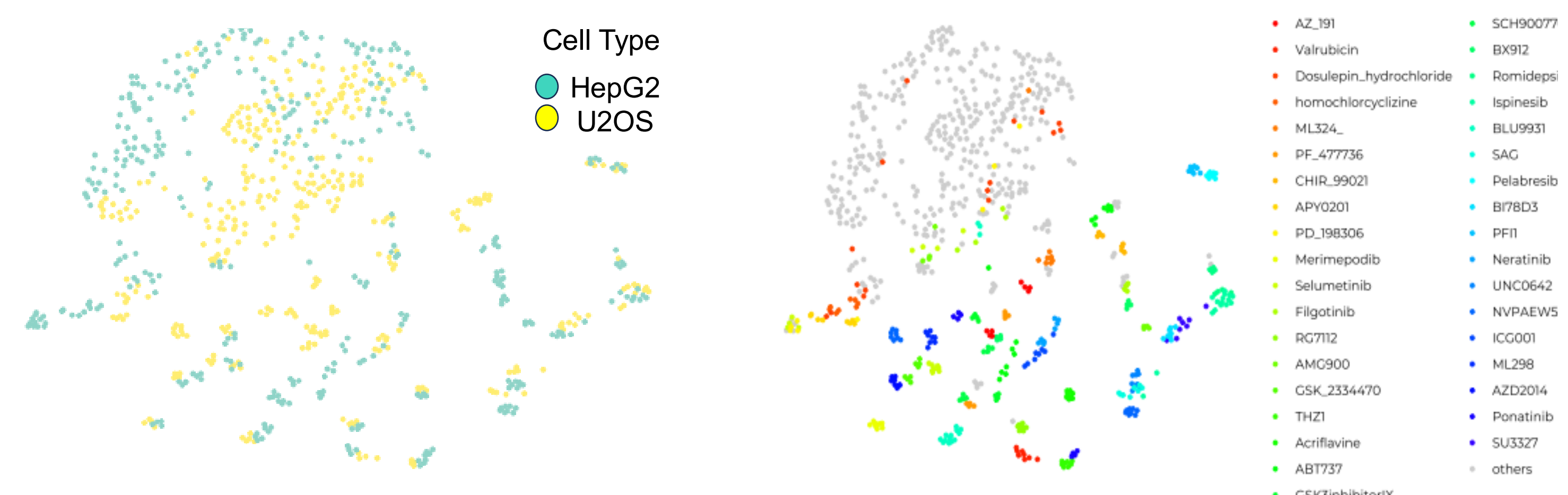
- Cell Painting processed data is composed of 112 features
- The clustering obtained from Cell Painting data was visually similar as the one of DRUG-seq
- U2OS cells showed 40 out of 82 conditions that shows a significant number of DE genes
- The number of DE genes per condition goes up to 2100 DEGs
- Overall, U2OS shows slightly less DEGs than HepG2

Silhouette Scoring Highlights Clustering Differences Between DRUG-seq and Cell Painting

- Silhouette scores use a distance matrix and cluster labels to evaluate how well each point fits within its assigned cluster, and can be aggregated per treatment to quantitatively compare the clustering performance
- In U2OS cells, more treatments are effectively clustered by Cell Painting but not DRUG-seq (blue).

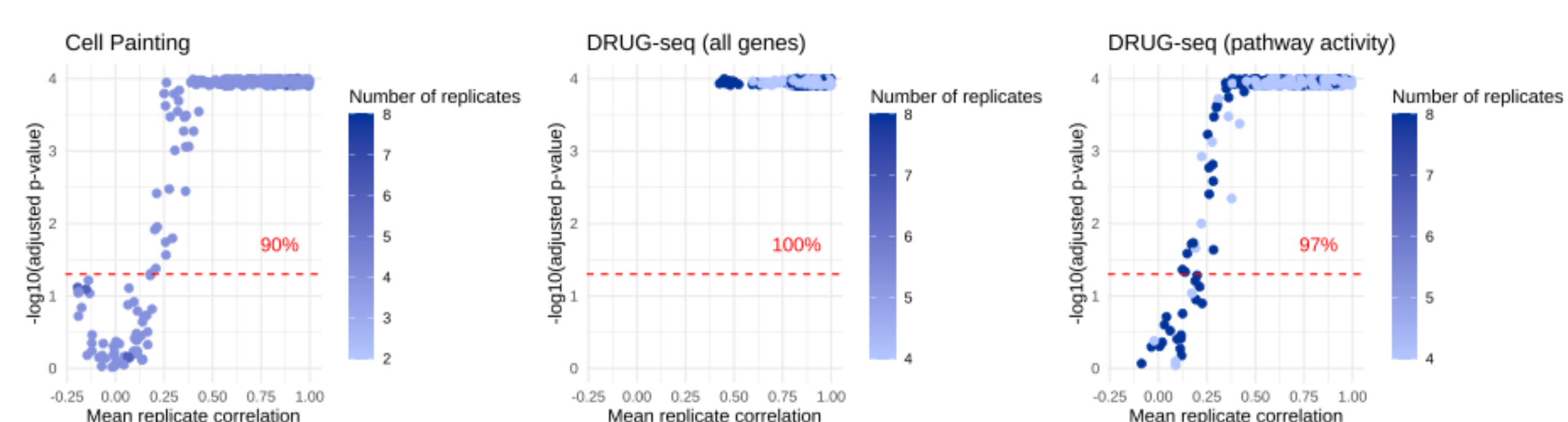


Integrating both U2OS and HepG2 cell types in a single analysis



- Untreated samples tend to cluster by cell type
- Treated samples sometimes form distinct clusters by cell type, while in other cases they aggregate across cell types.
- Differential expression analysis integrating both cell types identified 36 significant conditions out of 82, highlighted in color on the right plot.

Inter-replicate Correlation to Compare Technologies in HepG2



- Reproducing the inter-replicate correlation from Way et al. (2022), we compare technologies based on inter-replicate Spearman correlation, assessed for each treatment.
- DRUG-seq was applied either using all detected genes (>31k genes) or using pathway activity scores computed with MAYA.
- MAYA pathway activity and Cell Painting have a very similar number of features: 107 and 117, respectively.
- DRUG-seq, whether using all genes or pathway activity scores, outperforms Cell Painting in robustness, with 100% and 97% of replicates showing significant correlation, compared to 90% for CP.