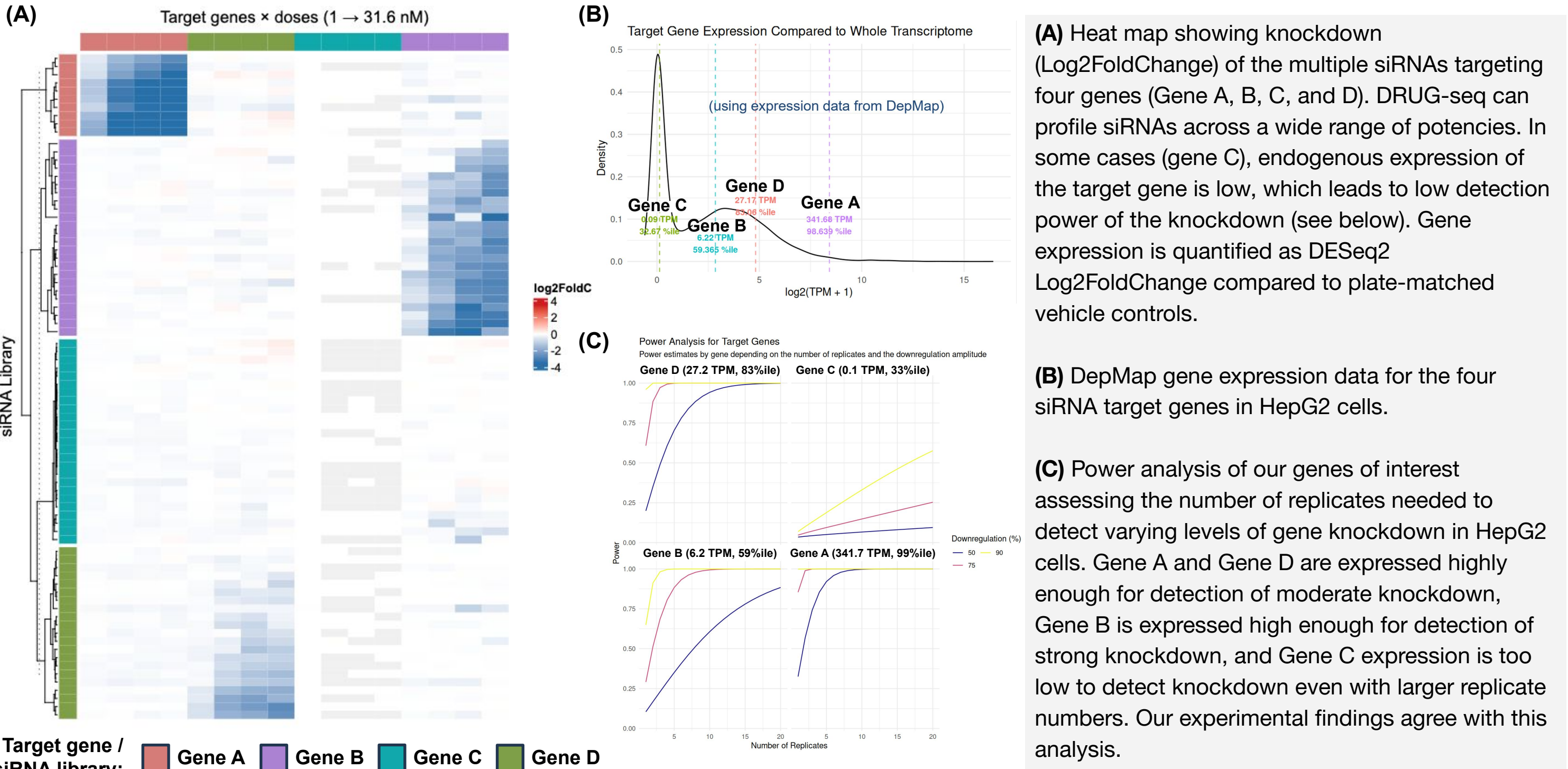


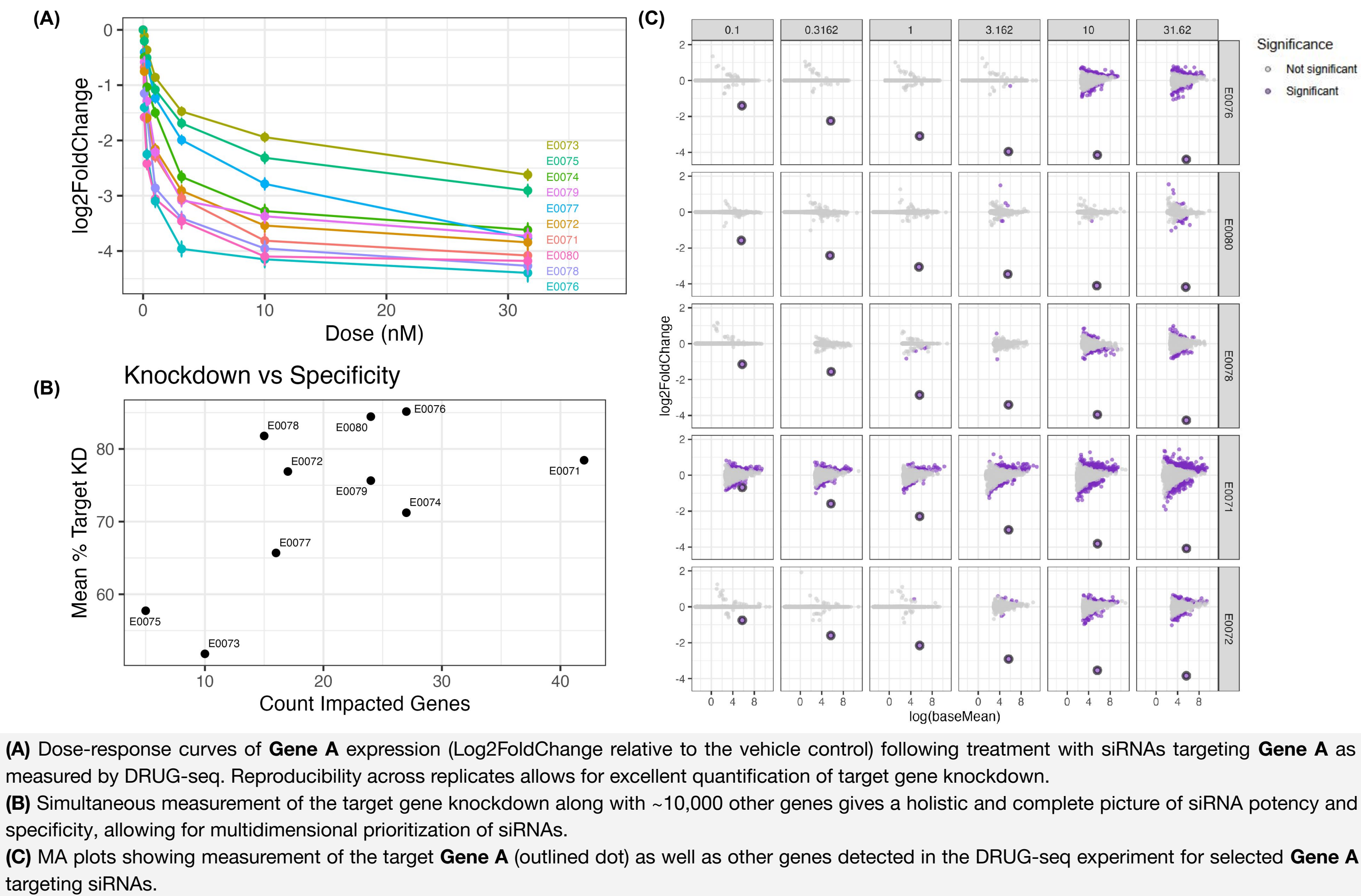
1. Abstract

Oligonucleotide drugs can induce broad transcriptional changes, including downstream and off-target changes beyond the target gene. While *in silico* evaluations can flag some liabilities, most ancillary effects are unpredictable and require empirical measurement. Bulk RNA-seq remains the gold standard for unbiased transcriptome profiling, but its high cost typically limits use to a few late-stage candidates. By incorporating broader transcriptional responses earlier in discovery, drug developers can make faster, more informed decisions and capture key properties contributing to both efficacy and safety. We demonstrate that DRUG-seq, our high-throughput transcriptomics platform, can quantify dose-responsive gene expression changes from an 80-member siRNA library in HepG2 liver cells. This study elucidated siRNA-specific transcriptomic signatures that could inform candidate prioritization, as well as cell-specific transcriptomic responses toward oligo-introduction. DRUG-seq is not only significantly cheaper than bulk RNA-seq, but also more cost-effective per gene than traditional qPCR-based screening while providing richer information across the transcriptome. The high-throughput nature of DRUG-seq enables full-library dose-response profiling in human cells, leading to faster prioritization and thereby expediting the process of lead candidate selection. Such an approach also generates datasets of sufficient size and quality for training advanced machine learning models, enabling more predictive and efficient therapeutic discovery.

3. Overview of siRNA library target gene perturbation



4. DRUG-seq reports on dose-dependent target knockdown and siRNA specificity



7. Impact of DRUG-seq on siRNA screening workflows

Typical siRNA screening process

Learn slowly and expensively

- Each target requires a custom assay
- Hit validation relies on follow-up RNA-seq, delaying or confusing DC nomination
- At best, iteration between potency and specificity consumes time and budgets
- At worst, sunk cost and urgency to get into the clinic lead to hoping for the best, moving forward assets with higher risk

siRNA screening at Ginkgo

Learn everything at once

- Single DRUG-seq readout quantifies both potency and specificity for every library member
- Miniaturized, automated, parallelized cell culture and transfection workflows → ready to go
- Integrated QC & analytics pipelines ensure reproducible results across hundreds of plates
- Faster, de-risked path to clinic

Comparison

Screening of 80 siRNA constructs in dose-response against 4 gene targets (14x 384-well plates)¹

RT-qPCR
3 weeks
~\$40K
Low discovery power

Bulk RNA-seq¹
7 weeks
~\$360K
Slow, expensive. Only suitable for validation

DRUG-seq
3 weeks
~\$50K
High discovery power

¹ Assumptions: a) duplex TaqMan qPCR assays (4 gene targets total)

b) Bulk RNA-seq capacity is ~4 x 384-well plate / week. DRUG-seq capacity at Ginkgo is ~100 x 384-well plate / week.

c) Price and timeline include cell treatment & siRNA perturbation, library prep, sequencing, data processing & analysis.

2. Study Design

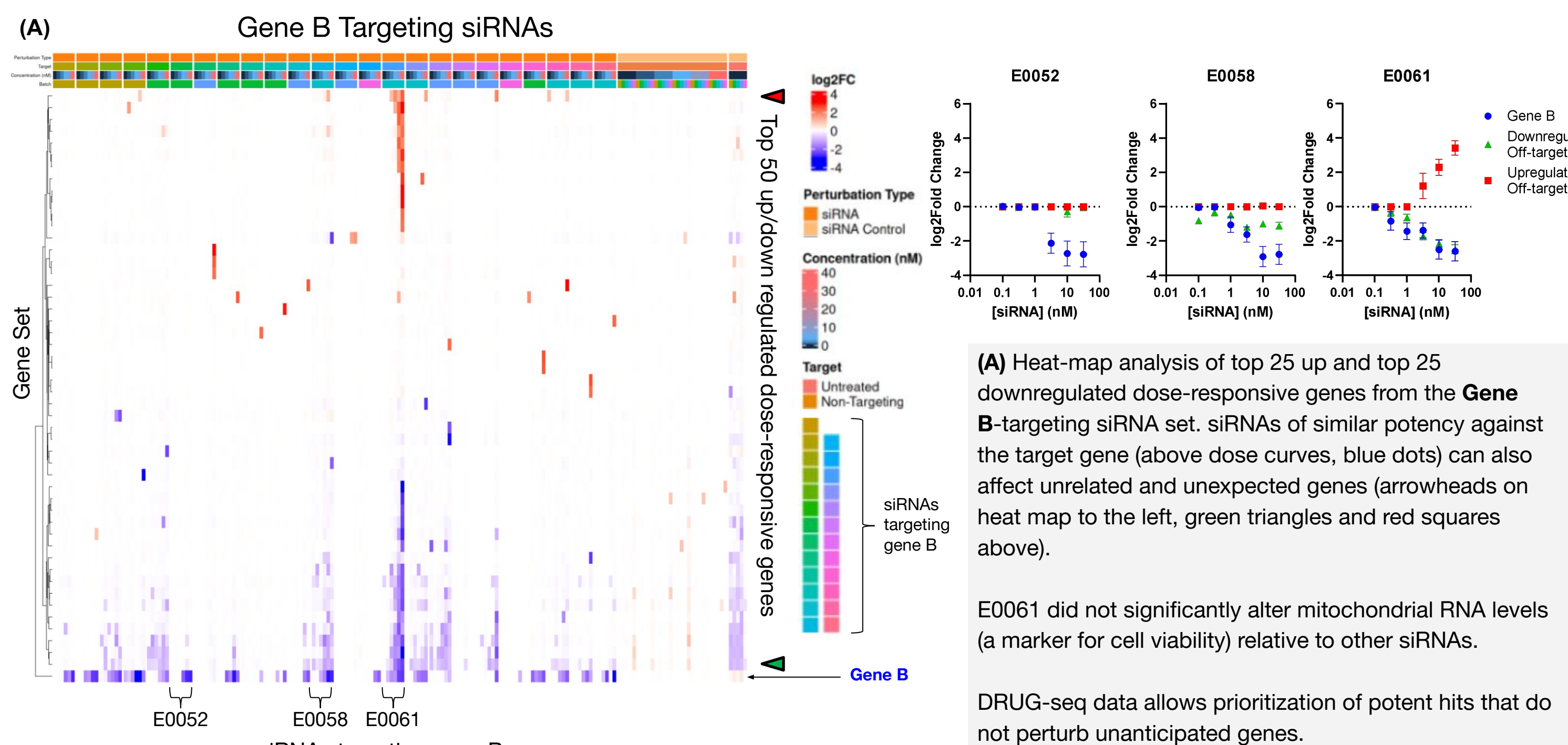
Treatment Name	Replicates	Concentration Range	Function
Untreated	28	N/A	Negative control (present on all plates)
DharmaFECT-4	22	0.15% (v/v)	Vehicle control; reagent used for siRNA transfection (present on all plates)
PPIB siRNA	6	31.6 nM	Positive control for siRNA transfection and gene knockdown (present on all plates)
Non-targeting siRNA	6	0.1, 0.316, 1, 3.16, 10, 31.6 nM	Non-targeting negative control siRNA (present on all plates)
80 member siRNA library	6	0.1, 0.316, 1, 3.16, 10, 31.6 nM	siRNA library spanning 4 gene targets

This study is a collaboration with Tangram Therapeutics, who provided the siRNA libraries.

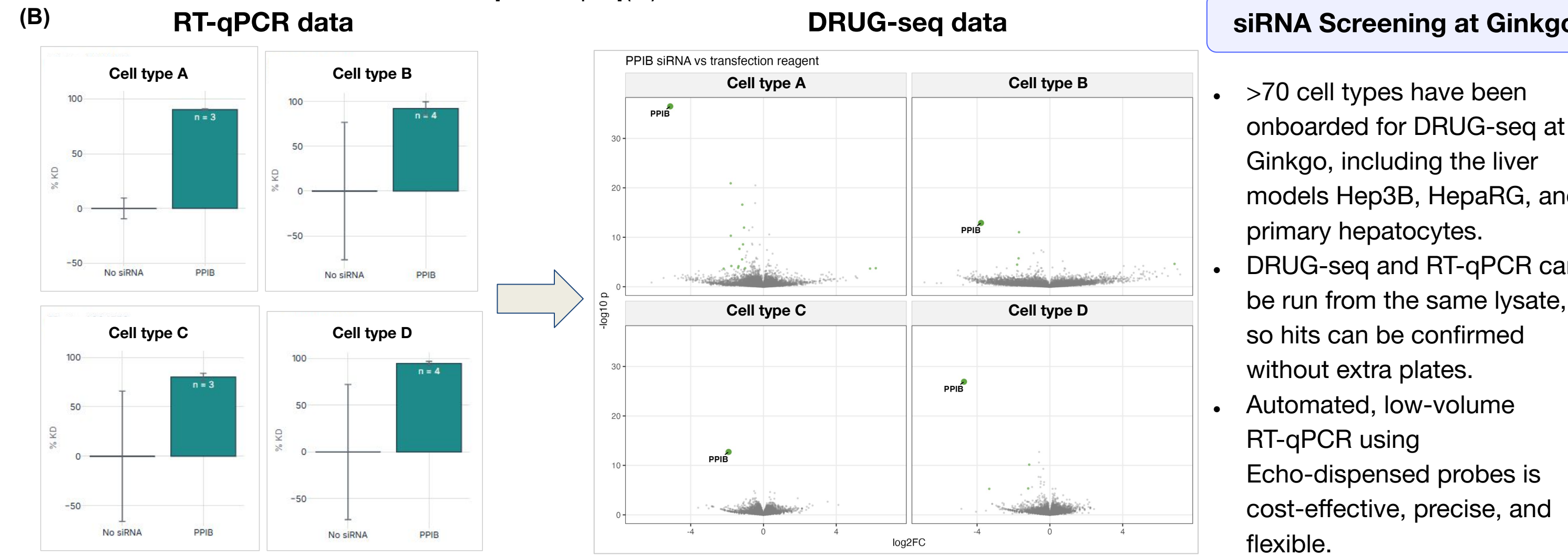
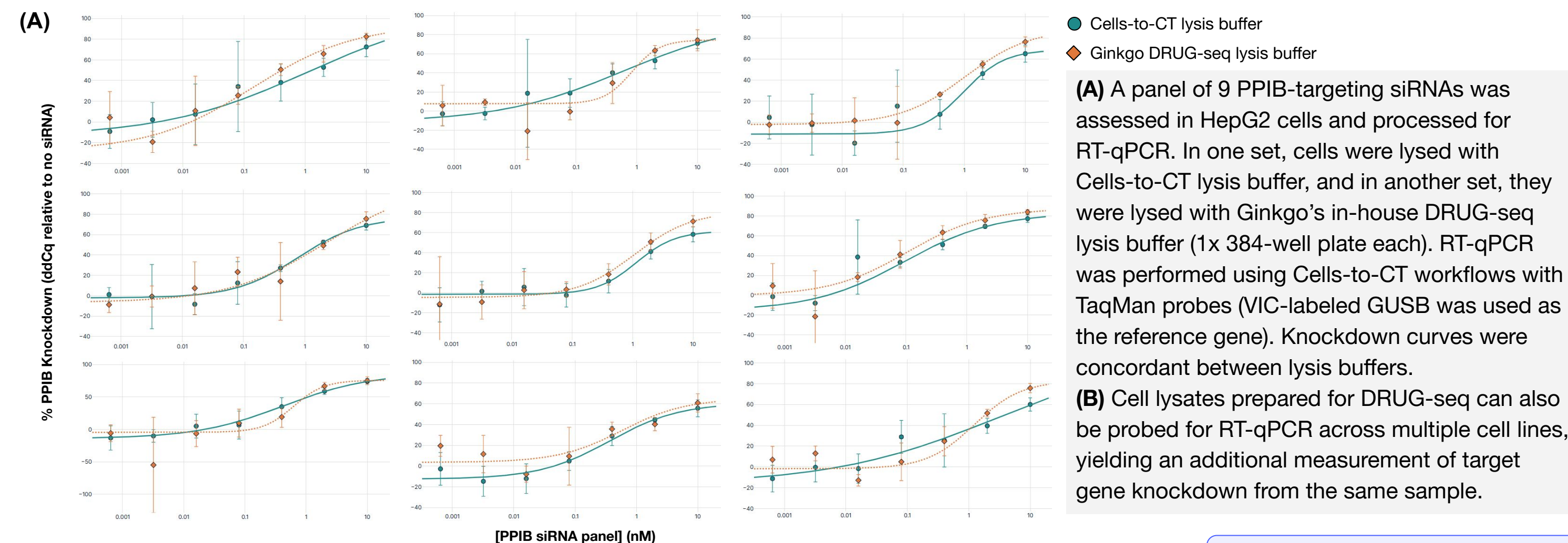
For this high-throughput screen, we used HepG2 liver cells, and optimized transfection conditions for maximal dose-dependent knockdown of a PPIB positive control gene. HepG2 cells were reverse transfected with eighty siRNAs targeting four genes at six concentrations across fourteen 384-well plates (6 siRNAs per plate, with 2 siRNAs on the last plate).

We applied DRUG-seq to generate transcriptomic profiles for every condition and processed the data using our automated analysis pipeline.

5. Consideration of dose-responsive genes for siRNA ranking



6. HTS-ready RT-qPCR/DRUG-seq workflows at Ginkgo



8. Conclusions and Acknowledgements

Our study demonstrates the power of DRUG-seq to generate **scalable, high-resolution transcriptomic profiles** of therapeutically relevant siRNA candidates. By profiling **80 siRNAs in dose-response in HepG2 liver cells**, we quantified siRNA-specific transcriptomic signatures in a single experiment, yielding:

- One-pot measurement of siRNA potency and selectivity**
- Cost-effective derisking of drug candidates**
- Foundational resource for AI and discovery**

This dataset, and others like it, lay the groundwork for training next generation machine learning models that can understand the driving forces behind siRNA selectivity.

We acknowledge and thank Tangram Therapeutics for providing the siRNA libraries tested in this study.

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