

Deciphering AAV production dynamics in HEK293 clones through multi-omics profiling

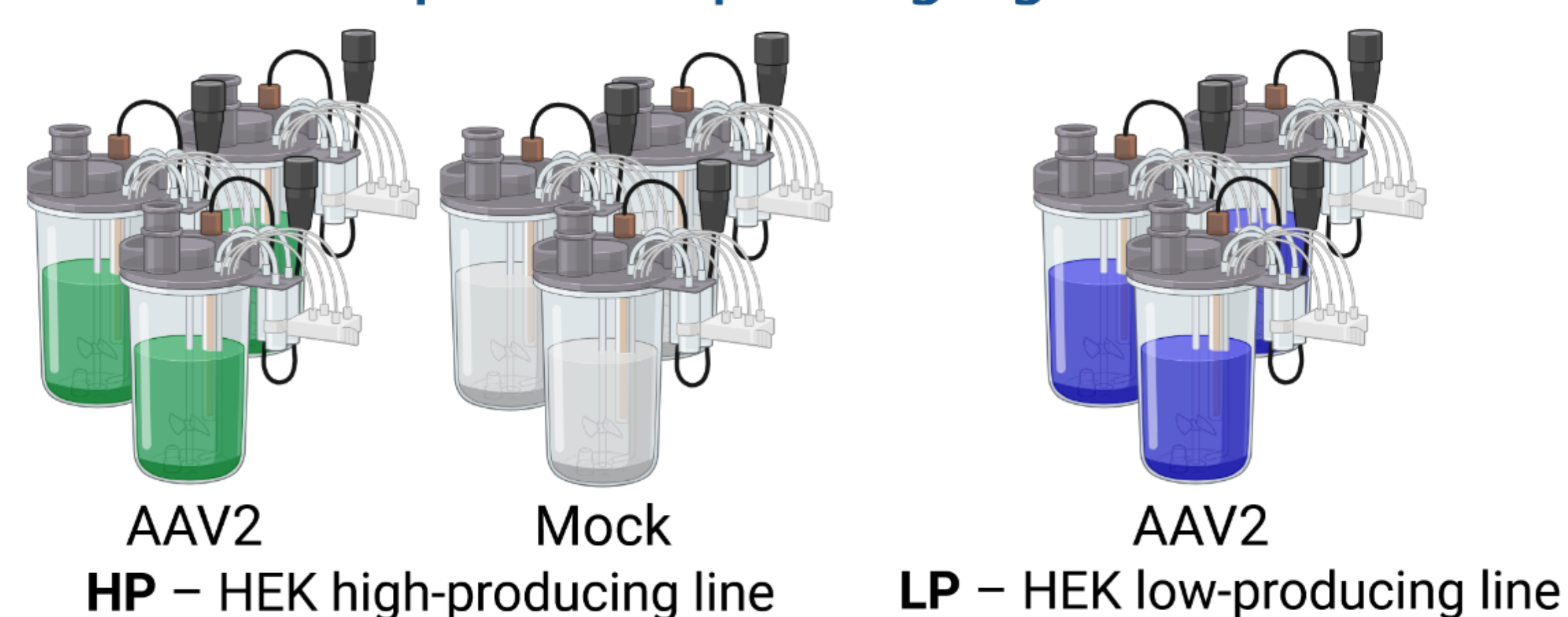
Ruben Esse, Hsin-Yi Lin, Sarah Martin, Isobelle Evie, Ana Sergijenko, Fathema Chowdhury, Ele Zucchelli, Vincenzo Di Cerbo

Cell and Gene Therapy Catapult | Scale Enabling Technologies (SET) | London

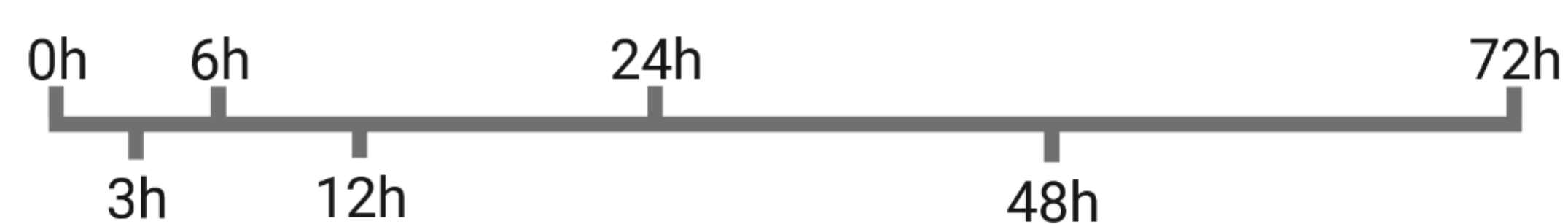
Improvements in manufacturing adeno-associated virus (AAV) vectors have largely centered on refining bioprocesses, with less emphasis placed on understanding and modulating the underlying biological mechanisms orchestrating AAV production. To bridge this knowledge gap, we performed an unprecedented multi-omics study, including transcriptomics, translome analysis, and untargeted metabolomics. These analyses allowed us to examine the cellular response during an industry-scalable AAV2 production. We observed baseline and time-dependent differences in gene expression and translation between high- and low-producing cell lines, as well as between mock-infected and AAV-producing vessels. These differences included the differential activation of the antiviral innate immune response and protein folding mechanisms, alongside previously unreported pathways. Our initial findings pave the way to implementing advanced biology engineering strategies to improve AAV manufacturing.

Our multi-omics approach for deep process characterisation to unlock AAV production potential

① AAV production by transfection of transfer, helper, and packaging constructs



② Time-course sampling



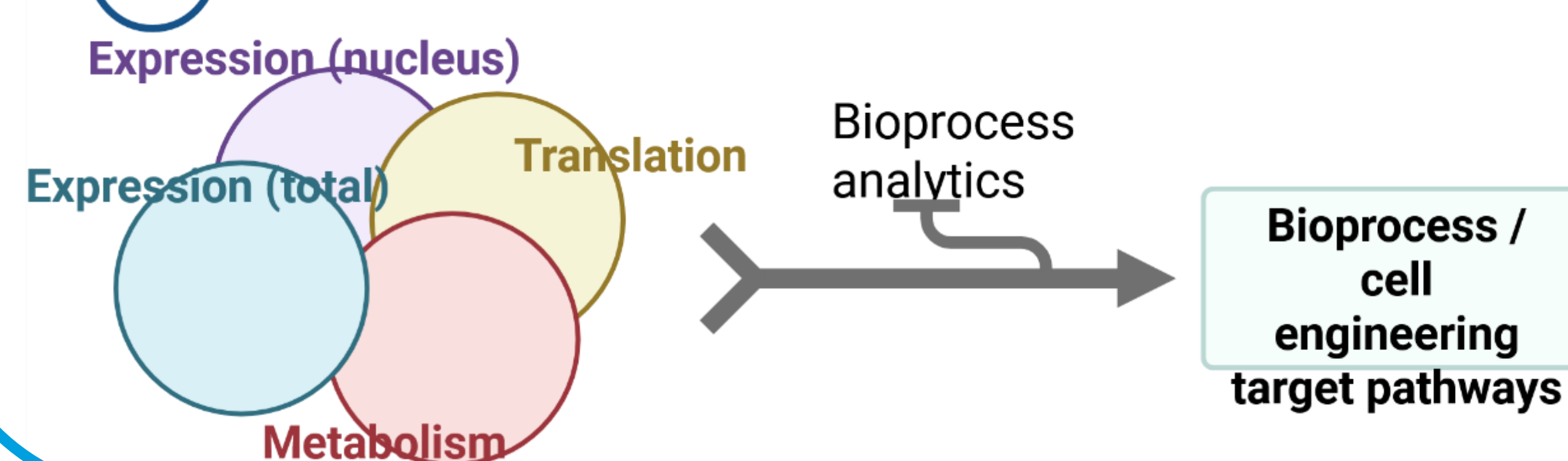
Omics analytics:

- Nuclear RNA sequencing
- Total RNA sequencing
- Ribosome profiling
- Untargeted metabolomics

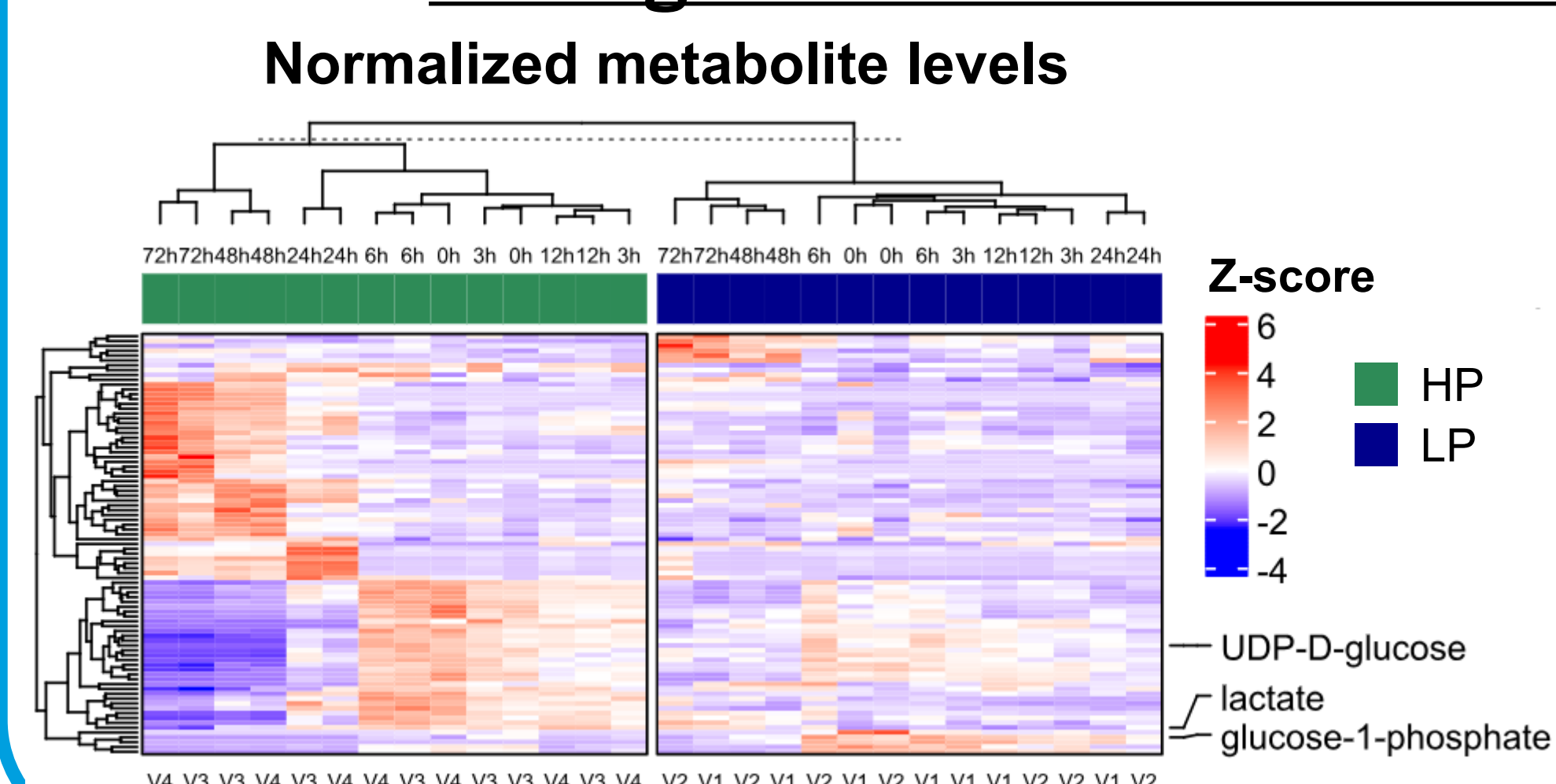
Bioprocess analytics:

- Cell count / viability
- Genome / particle titre
- Transfection efficiency

③ Data mining and target identification



Untargeted metabolomics analysis

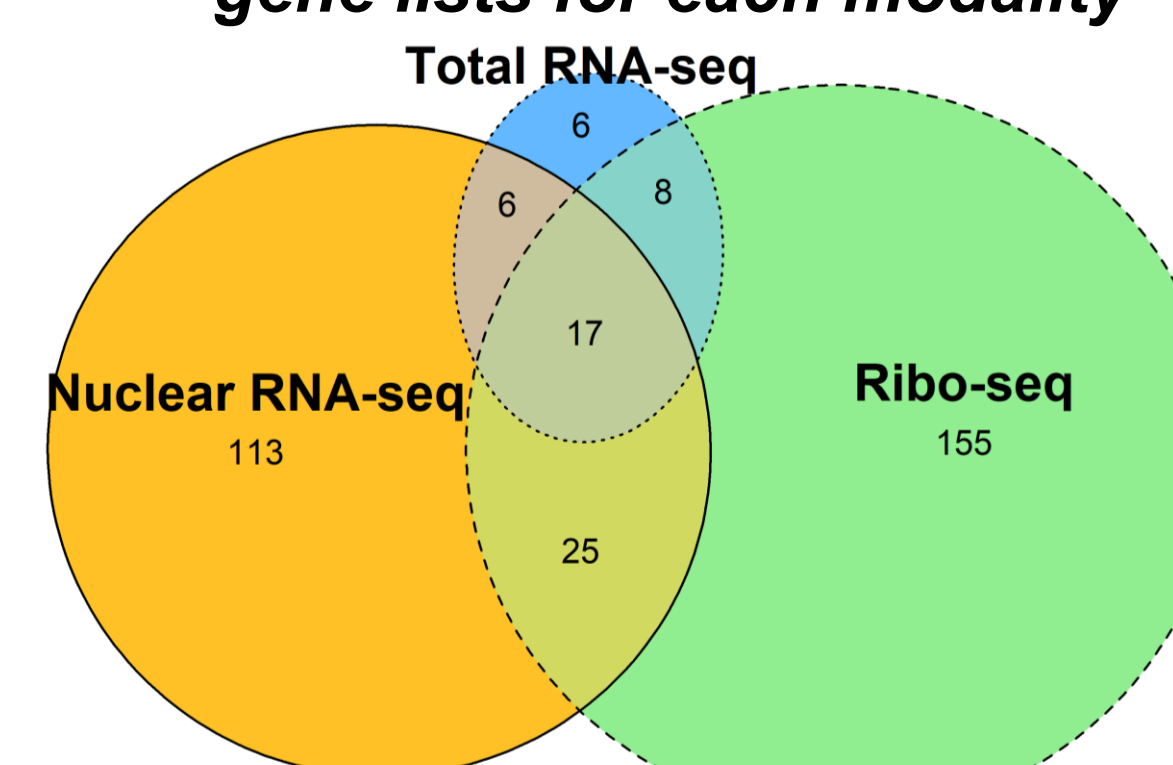


Intracellular metabolite profiling reveals distinct cell line-specific trends throughout the AAV production timeline, highlighting opportunities for media formulation optimisation

Genes and functional themes driving productivity

1) AAV2 producer vessels vs mock controls

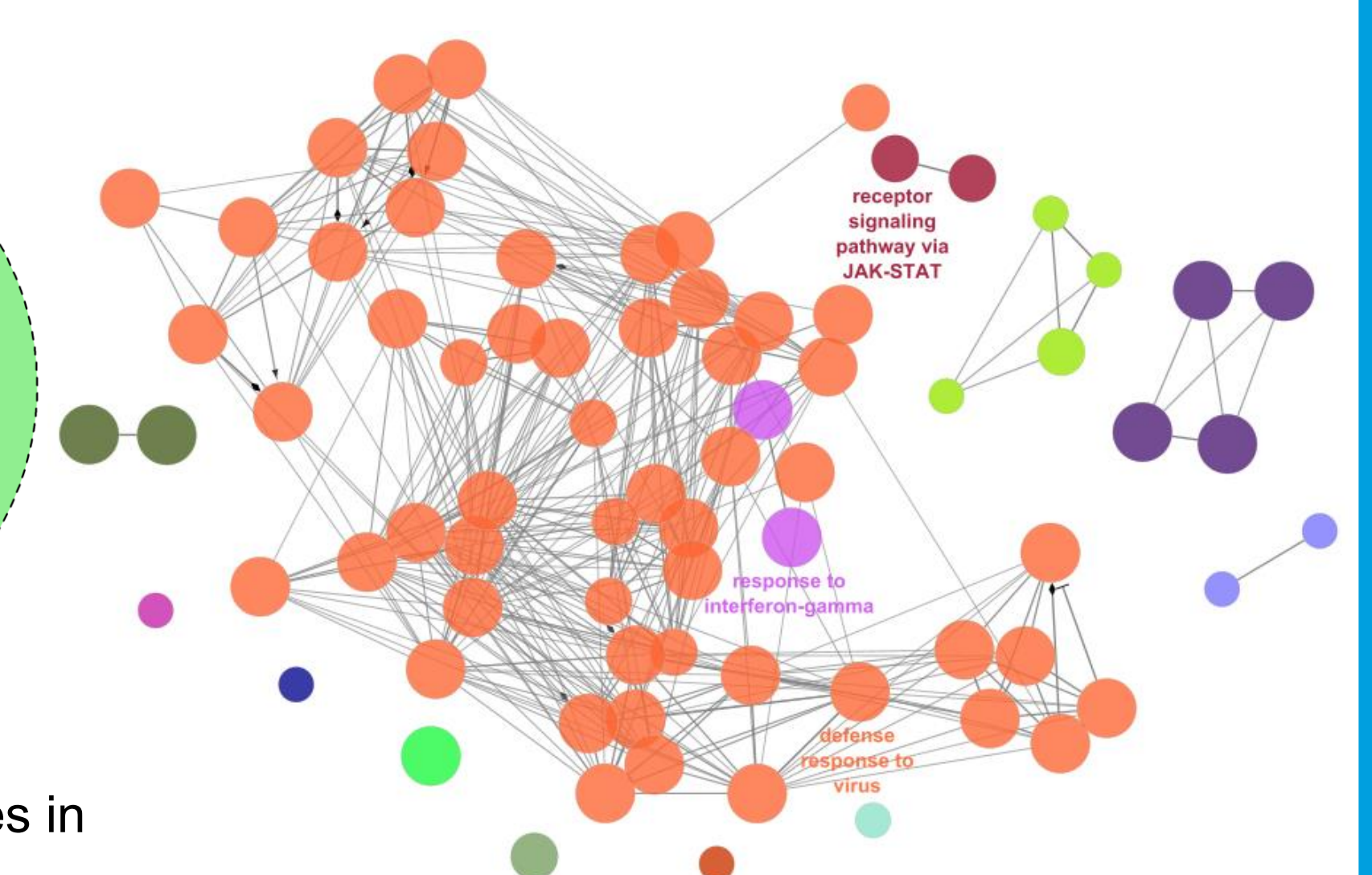
Comparison of significant gene lists for each modality



Genes assayed: 38824

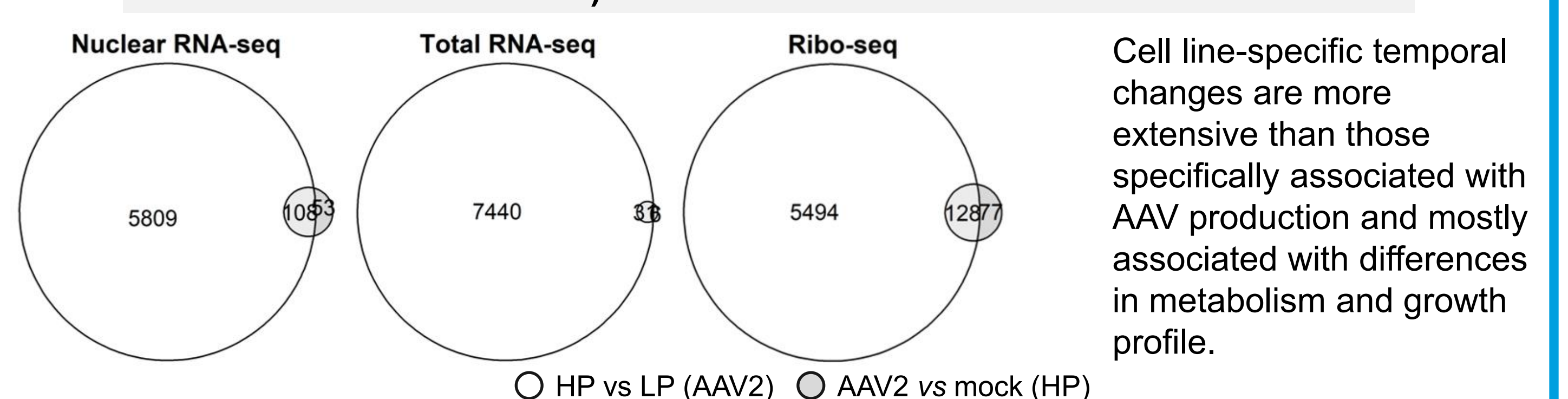
AAV2 production elicits minimal changes in production vessels, with total RNA-seq less sensitive than nuclear RNA-seq and Ribo-seq.

Network of enriched terms



Functional overrepresentation of hits identifies several large functional themes, including innate immunity and protein folding terms.

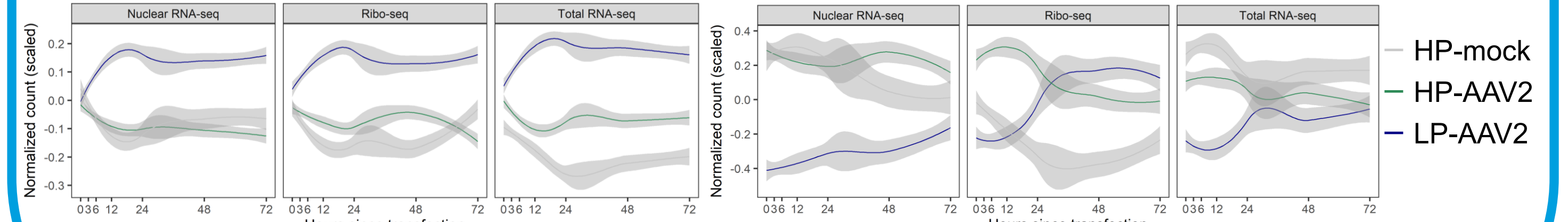
2) Cell line differences



Cell line-specific temporal changes are more extensive than those specifically associated with AAV production and mostly associated with differences in metabolism and growth profile.

'Defence response to virus' theme

Values scaled across sample group for each time point



Innate immunity response and protein folding genes, as well as others in other themes, have a different dynamic response over the production course between the two cell lines.

Conclusions and looking ahead

- Investigation of expression, translation, and metabolic signatures of high- vs low-producing HEK cell lines reveals an AAV production-linked biological fingerprint.
- Antiviral response mechanisms are triggered throughout AAV production. Importantly, many other unreported pathways show changes between high and low producer clones.
- Metabolomics analysis enables better understanding of resource utilization and metabolic engineering strategies.
- Further investigation of the data, including full multi-omics data integration, will generate the full repertoire of candidate targets to apply advanced engineering biology strategies geared to increase yield and quality of AAV production.

References:

- Fu Q et al., Critical challenges and advances in recombinant adeno-associated virus (rAAV) biomanufacturing, *Biotechnol Bioeng.* 2023 Sep;120(9):2601-2621
- Pistek M et al., Comprehensive mRNA-sequencing-based characterization of three HEK-293 cell lines during an rAAV production process for gene therapy applications, *Biotechnol J.* 2023 Aug;18(8):e2200513
- Chung CH et al., Production of rAAV by plasmid transfection induces antiviral and inflammatory responses in suspension HEK293 cells, *Mol Ther Methods Clin Dev.* 2023 Jan 16;28:272-283.
- Patra AT et al., Temporal insights into molecular and cellular responses during rAAV production in HEK293T cells, *Mol Ther Methods Clin Dev.* 2024 Sep;32(3):1-15