

Scalable CRISPR Functional Genomics in Human iPSC-Derived Motor Neurons Using Multi-Omics Phenotypic Profiling

Paola Bonetti, Dario Presutti, Roberto Ravasio, Donatella Bardelli, Ilaria Cotellessa, Sara Martin, Silvia Cainarca, Jean-Francois Rolland and Fernanda Ricci
Axxam S.p.A. OpenZone, Via Meucci 3, 20091 Bresso (Milan), Italy

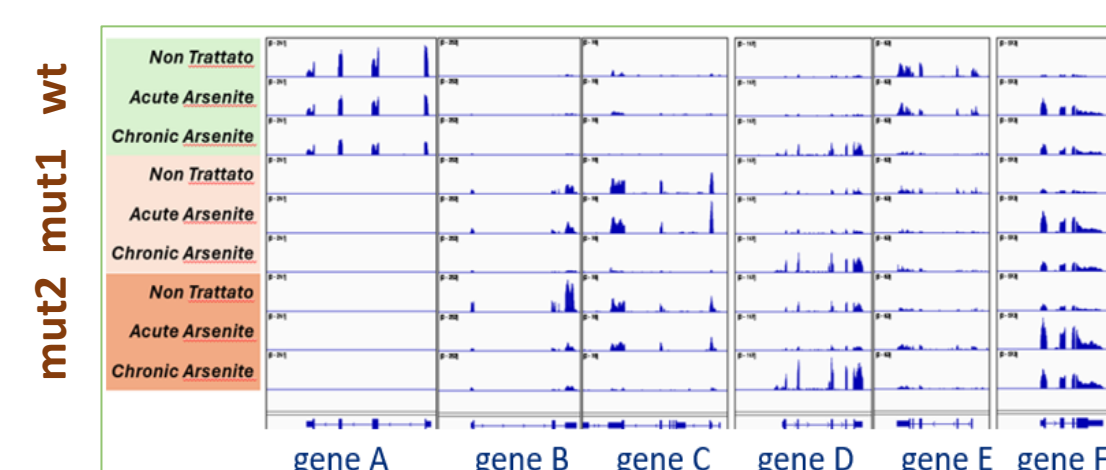
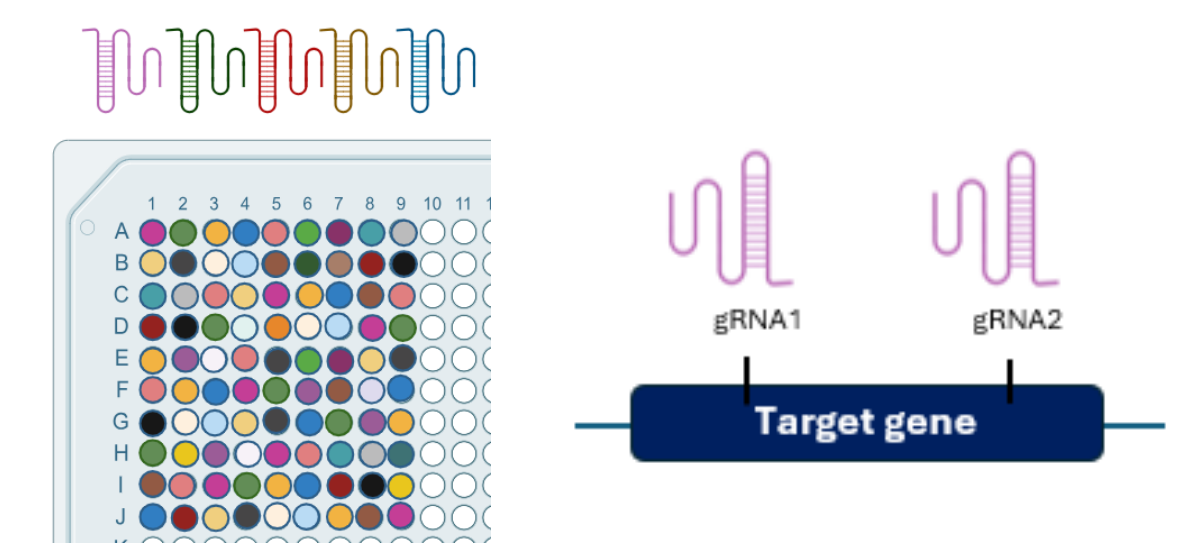
We performed an **arrayed CRISPR knockout screen** to systematically investigate gene function and identify modulators of cellular phenotypes in **CRISPRKO-ready induced pluripotent stem cell (iPSC)-derived motor neurons (bit.bio)** as neuronal disease-relevant model.

The screening workflow was coupled with two complementary multi-omics readouts, high-content morphological profiling (**Cell Painting**) and transcriptomic analysis (**DRUG-seq**) providing a multidimensional framework for target identification and validation.

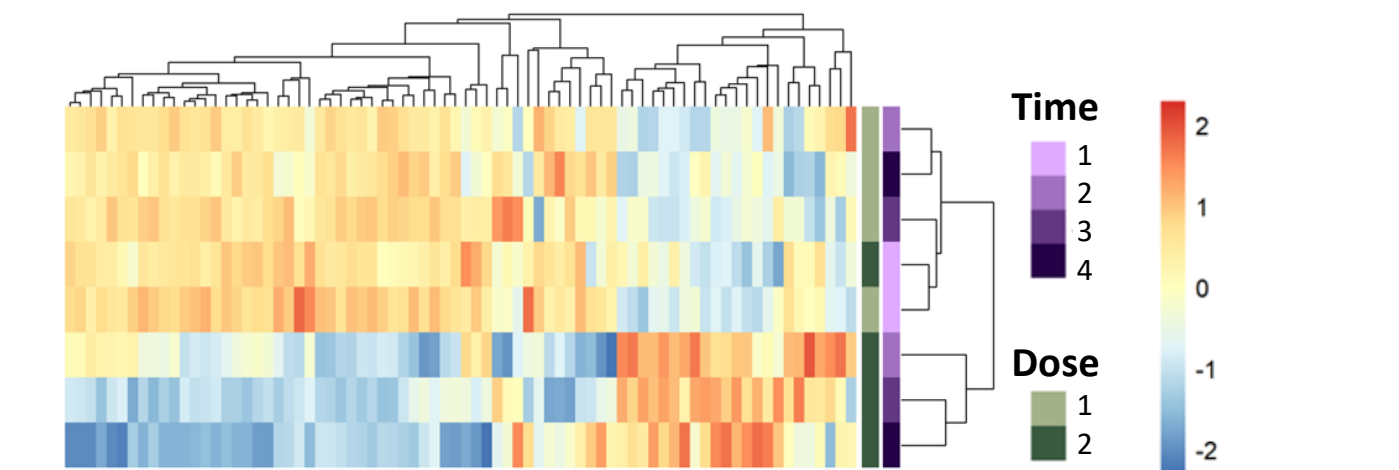
The entire screening campaign was performed in a **miniaturized 384-well plate** format using **fully automated screening station** and **automated liquid handlers**, demonstrating the scalability and robustness of the platform.

Arrayed CRISPR guide RNA (gRNA) library

- A custom Arrayed CRISPR gRNA library (n=92) from a supplier and containing positive and negative controls was used.
- For each target gene, a pool of 2–3 gRNA sequences inducing small deletions in the first coding exons was used.
- Target genes list was defined based on integration of data from public available datasets and dataset internally generated using **Amyotrophic Lateral Sclerosis (ALS)** relevant disease models in presence of stressor (Arsenite)



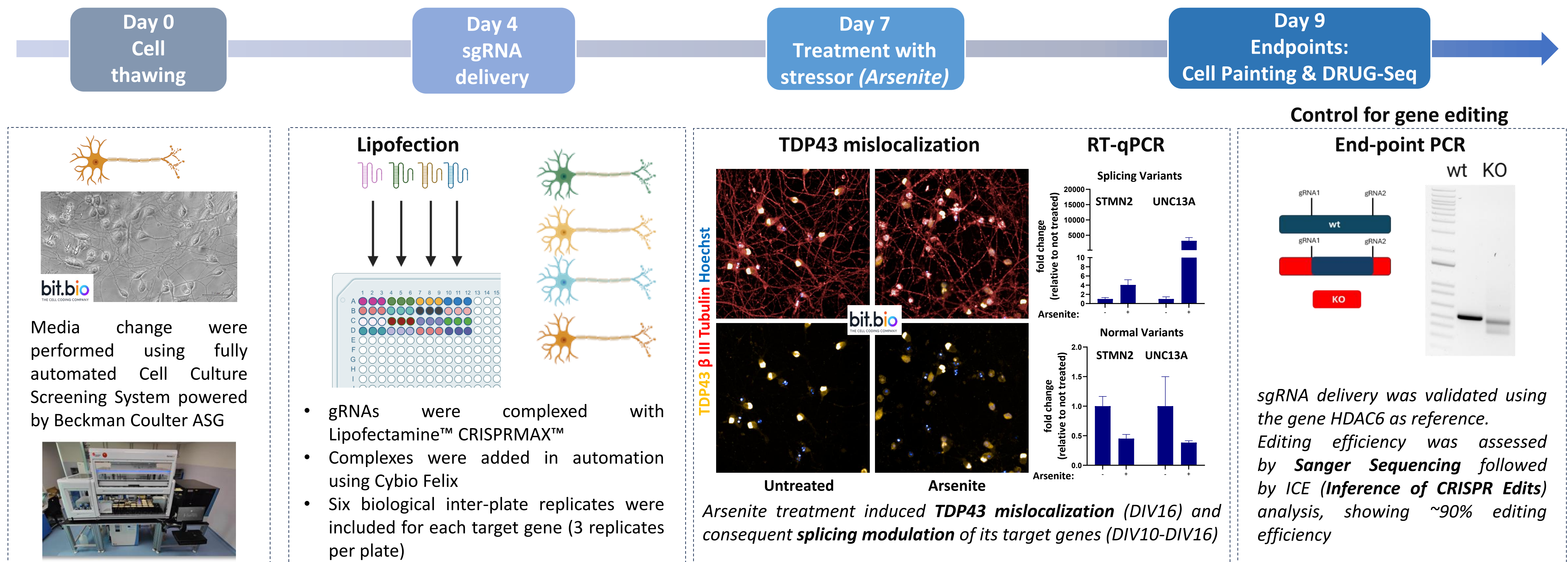
RNA-seq tracks showing differential expressed genes in wt and mutants iPSCs-derived motoneurons



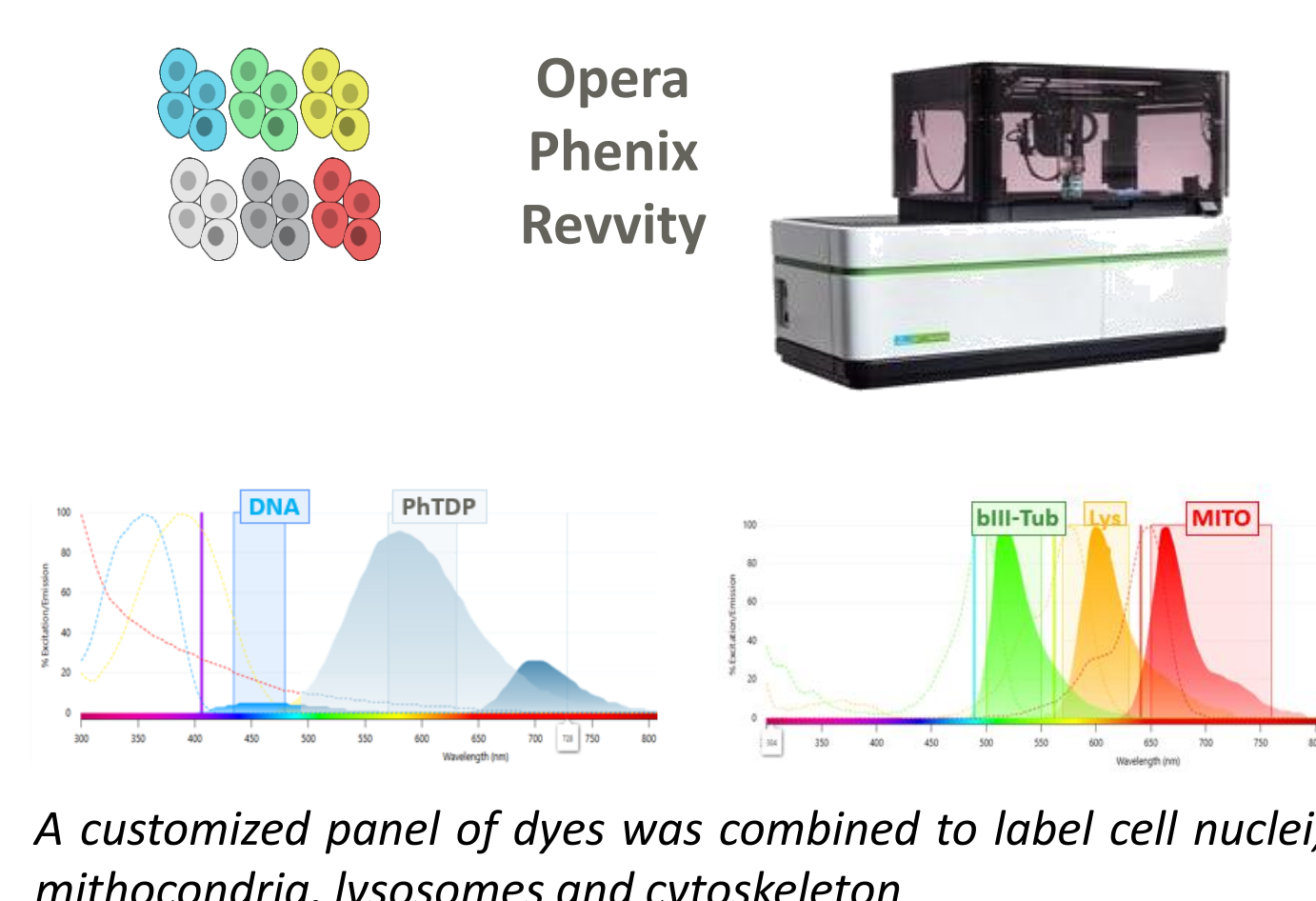
Heatmap showing the regulation of genes selected for CRISPR KO screening after Arsenite treatment

Experimental workflow

Cell line model: CRISPRKO-ready induced pluripotent stem cell (iPSC)-derived motor neurons (bit.bio) constitutively expressing Cas9 nuclease

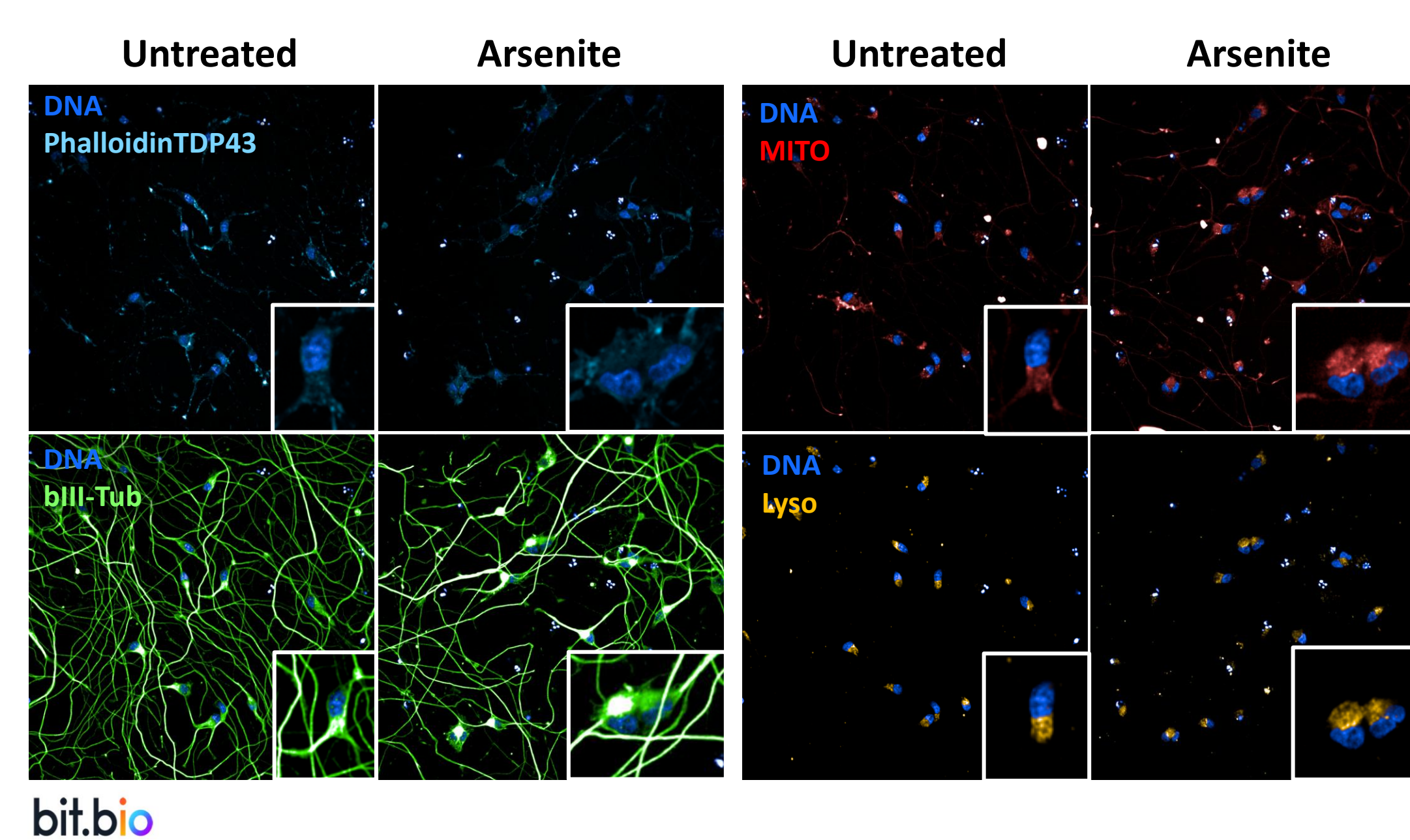


Cell Painting

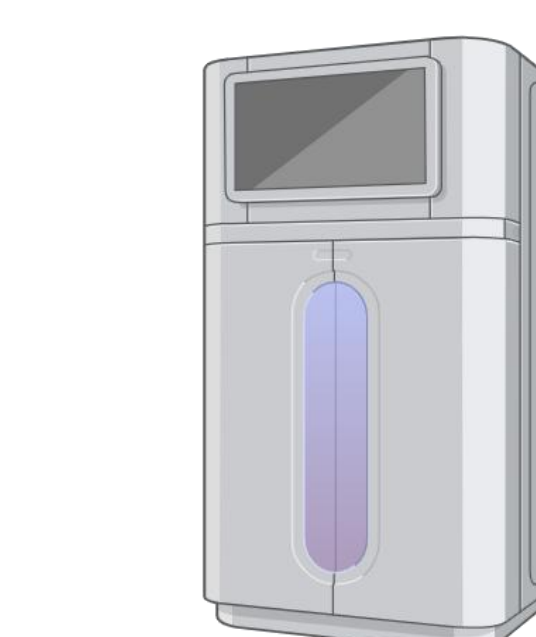


A customized panel of dyes was combined to label cell nuclei, mitochondria, lysosomes and cytoskeleton

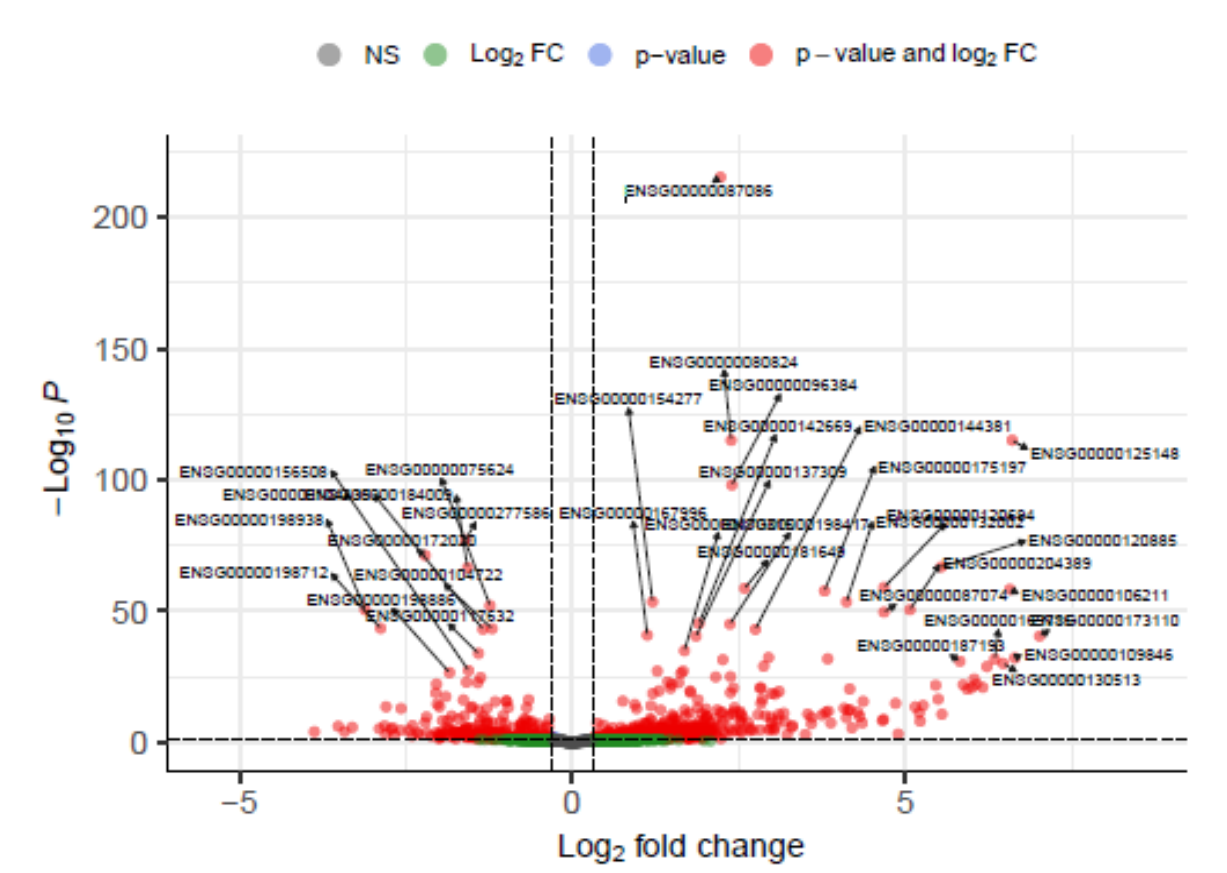
Multi-omics readouts



DRUG-seq

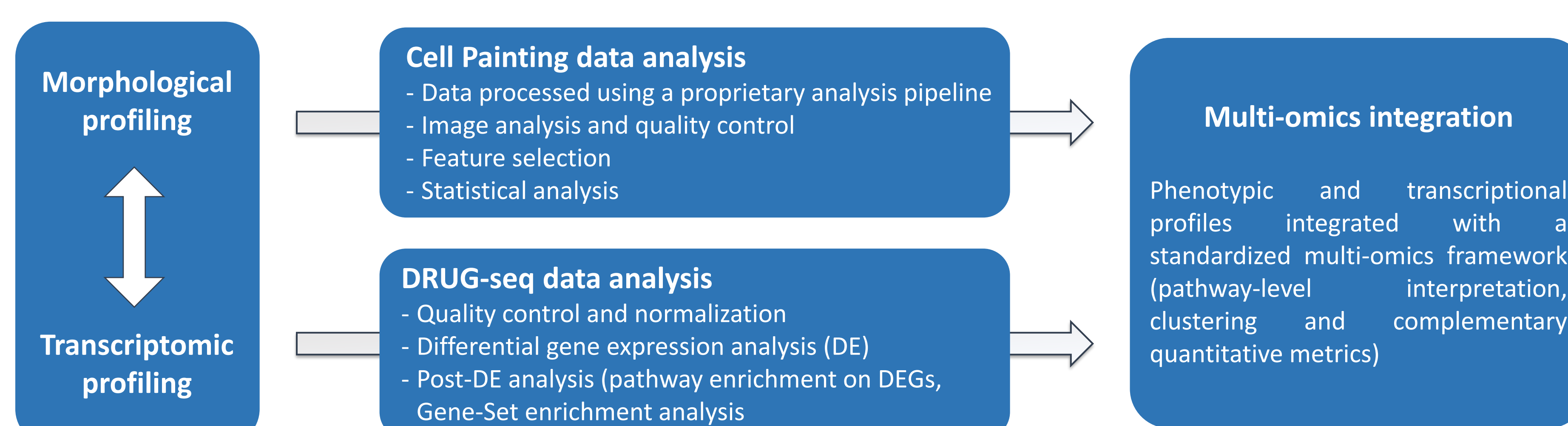


DRUG-seq library preparation and NGS sequencing was performed by Alitheia Genomics SA



Vulcano plot showing differential expressed genes after Arsenite treatment

Cell Painting & DRUG-seq analyses workflow



Our work highlights the power of combining **CRISPR-based functional genomics** with **advanced human cell models** and **multi-omics profiling** to accelerate target discovery. This **scalable and translatable platform** can provide a valuable framework for uncovering novel therapeutic targets and advancing drug discovery in neurologic disease contexts.

