VALIDATION OF CRISPR GENE KNOCKOUTS IN U2OS CELL LINES WITH DIA MS-BASED PROTEOMICS

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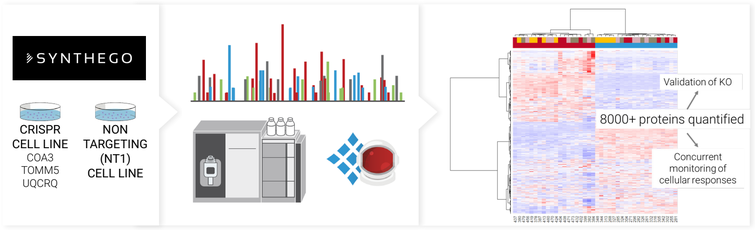
INTRODUCTION

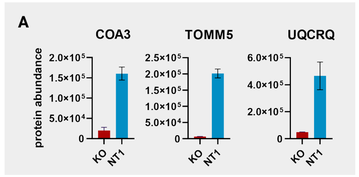
- CRISPR-Cas9 is an essential tool for gene editing in cellular systems with routine applications in functional screening, target validation and identification as well as generation of disease models
- Optimized RNP delivery protocol yields gene knockouts (KOs) which are functional at pool level
- High-throughput confirmation of KOs at protein level is often time-consuming and cost-intensive (e.g. using off-the-shelf antibodies)
- We present a scalable, high-throughput and quantitative proteomics platform based on data independent acquisition (DIA) mass spectrometry (MS) to concurrently validate gene edits and to monitor 8000+ proteins



CONCLUSIONS

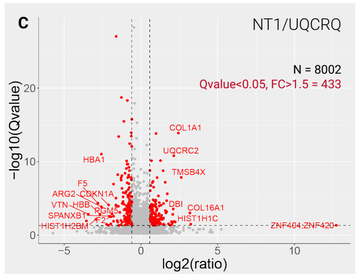
- **DIA-MS: A high-throughput and quantitative approach to concurrently validate CRISPR KO results and to monitor their functional impacts in parallel**
- **Precise quantification of edited genes on the protein level overcome the need for antibodies as well as inconclusive transcript-level information**
- **Profiling of > 8000 proteins in the edited systems delineates downstream cellular responses and can potentially unveil by-effects**
- **Functional insights into the CRISPR-edited systems assist the interpretation of screening results**

RESULTS



B

	UQCRQ	COA3	TOMM5	NT1
UQCRQ		490	599	433
COA3	490		91	28
TOMM5	599	91		116
NT1	433	28	116	



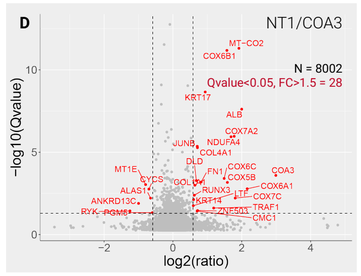


Figure 1: DIA MS Proteomic Analysis Results. **(A)** Protein abundance of COA3, TOMM5 and UQCRQ in target gene KO cell line. Data are represented as mean ± SD (n=2 for KO, n=5 for NT1). **(B)** Number of significantly changing protein (Qvalue < 0.05, FC>1.5) for all comparisons. Volcano-plot representing the comparison between UQCRQ **(C)** and COA3 **(D)** vs. non-targeting (NT1)

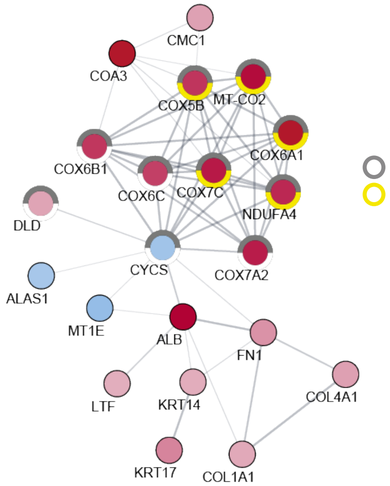
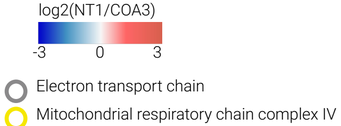


Figure 2: Protein-protein Interaction Network. Protein-protein interactions of significantly changing proteins (Qvalue<0.05, FC>1.5) between NT1 and COA3 were displayed in a network. Nodes were colored based on protein abundance (log2 of the ratio between NT1 and COA3). Proteins were classified by gene ontology enrichment analysis (biological process in gray, cellular component in yellow)



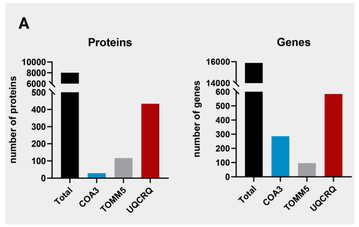


Figure 3: Comparison Between Proteomics and Transcriptomics Results.
(A) Bar plot displaying the number of significantly changing proteins (Qvalue <0.05, FC>1.5) and genes (Qvalue<0.001) for COA3, TOMM5 and UQCRQ KO cell lines.
(B) Transcript abundance for target gene KO in KO vs. NT1 cell line. Data are represented as mean ± SD (for COA3 and TOMM5 n=6 for KO, n=3 for NT1; for UQCRQ n=3 for KO, n=3 for NT1)