

Time-resolved proteome profiling of glioblastoma cell response to type I interferon stimulation using DirectMS1 approach

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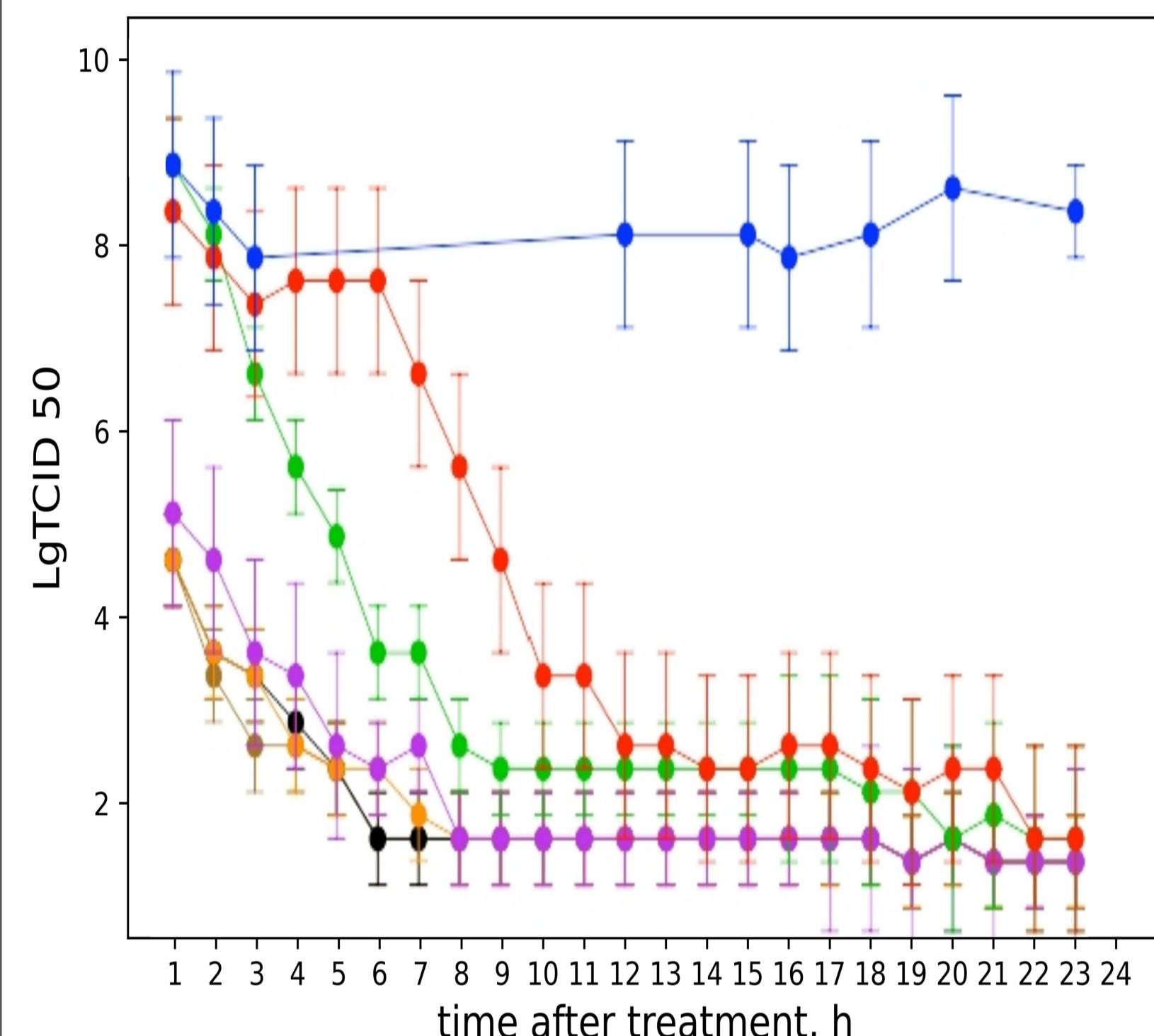
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Introduction

Knowledge of interferon-dependent antiviral mechanisms in tumor cells is important for oncolytic virus therapies. Glioblastoma DBTRG-05MG cell line acquires resistance to the vesicular stomatitis virus (VSV) after interferon treatment. However, existing studies usually show proteins that are differentially expressed at a single time point after the treatment (e.g. 24 hours) and there is a lack of time-resolved proteomics studies.

DBTRG resistance to VSV



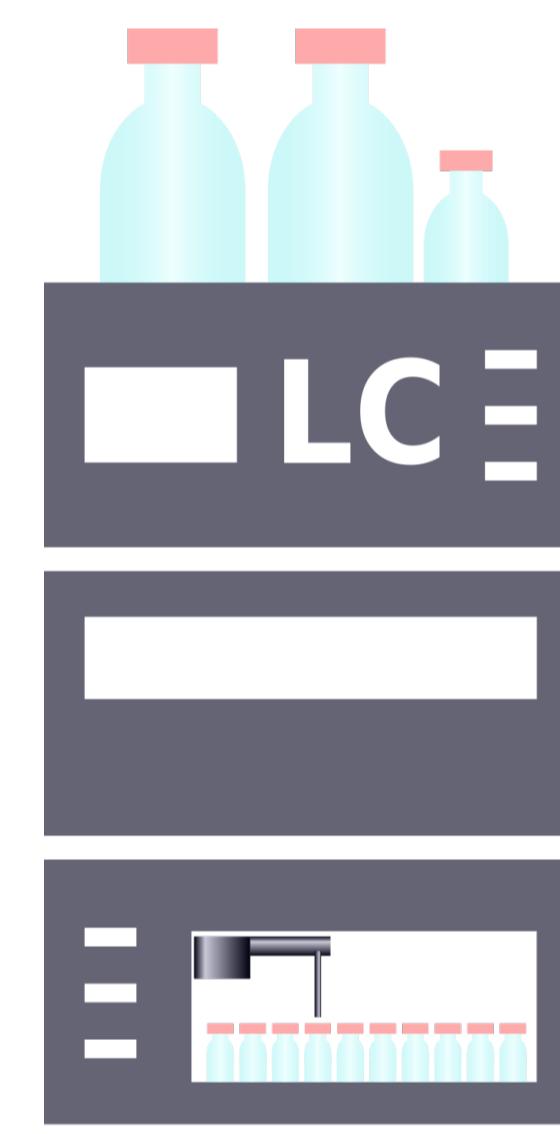
LTCID50 (median tissue culture infectious dose) determines the amount of virus within a sample

We found that virus resistance formation time strongly depends on the concentration of interferon and varies in the range of 7-12 hours

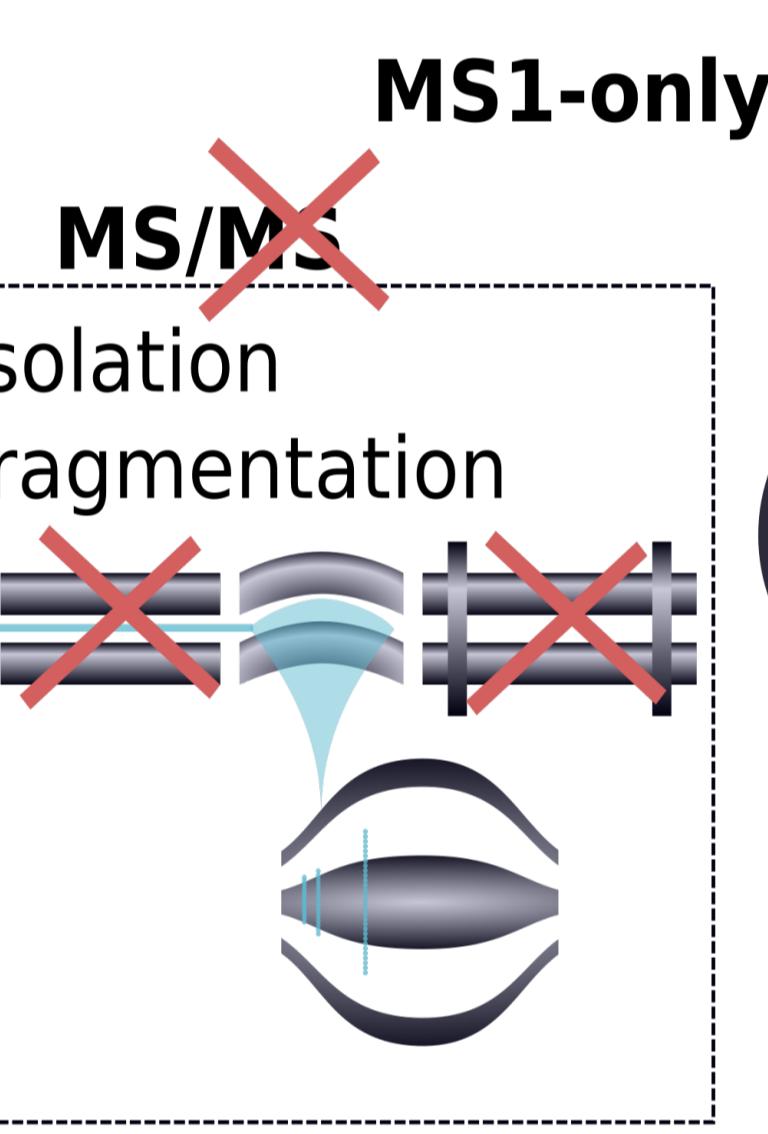
Only two extreme concentrations of interferon (30 and 1000 units/mL) were chosen for proteomics experiments

Key ideas of the project were: 1) to investigate time-resolved interferon-dependent protein regulation and 2) match it with virus resistance profile

Methods



5-min gradient

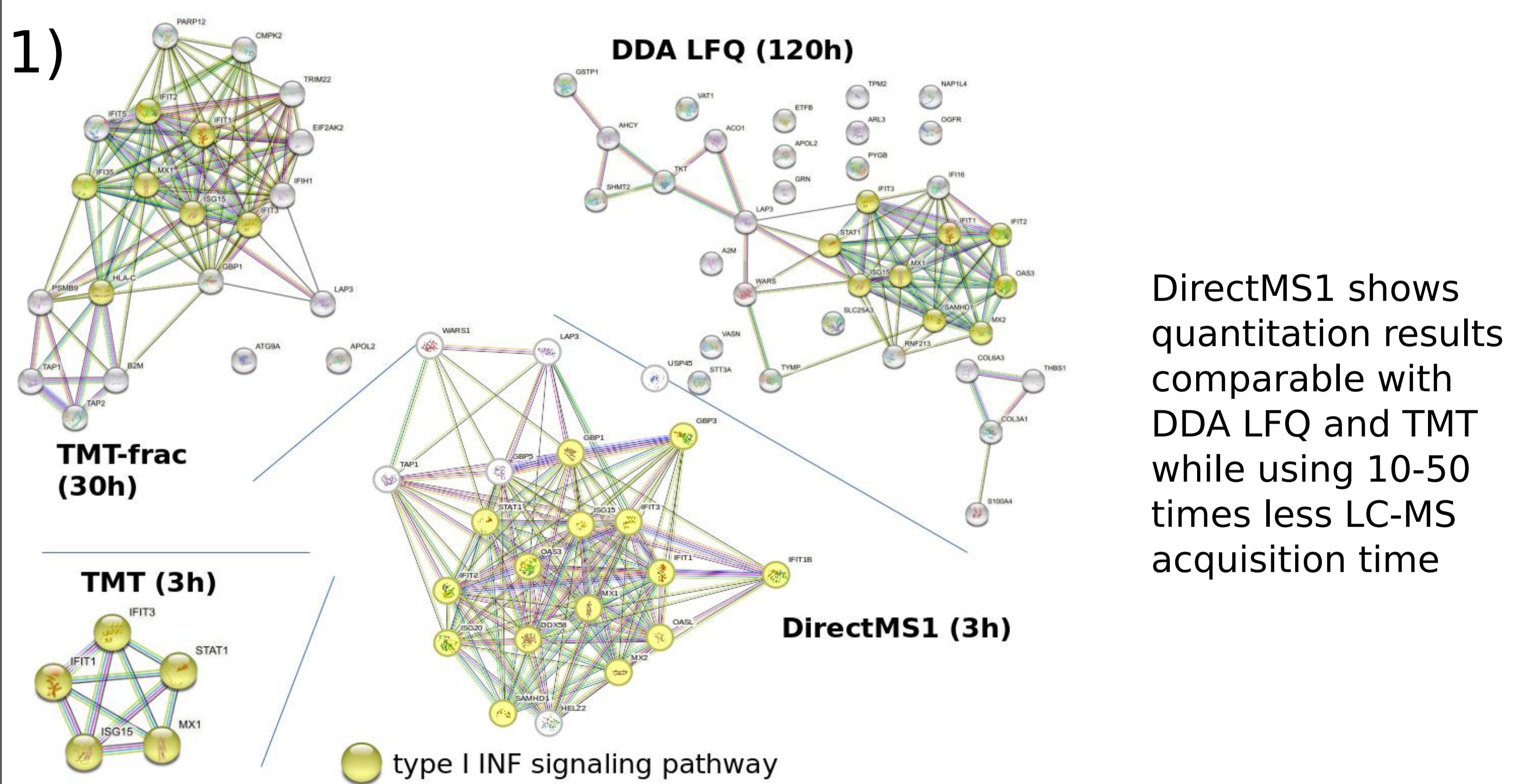


DirectMS1 [1]: protein identification and quantitation using MS1 spectra
LC-E
5-min gradient
ms1
ms/ms
~1h per sample
7min per sample

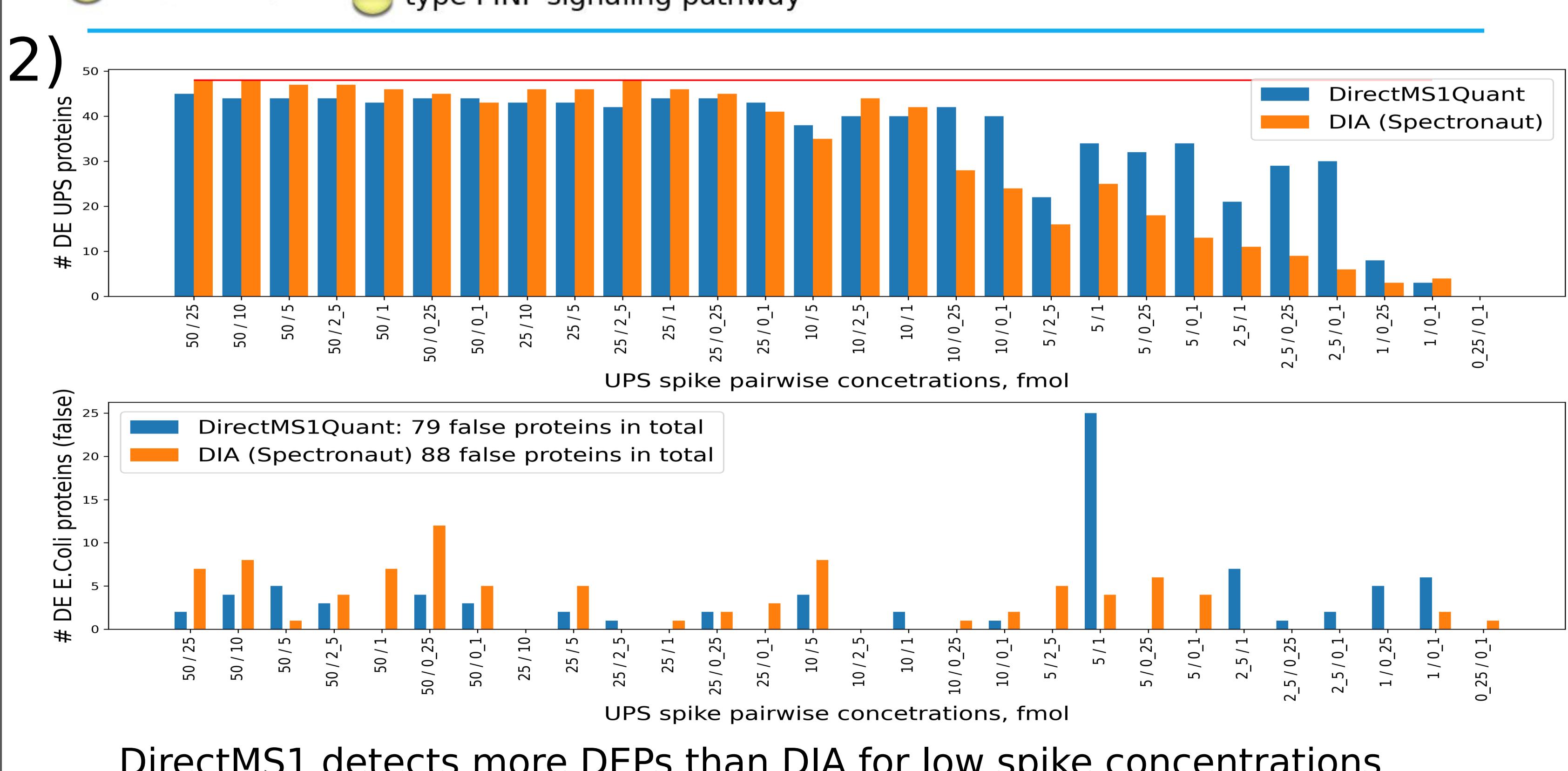
- Orbitrap QE HF and QE HF-X
- 5 min LC gradient using 5 cm C18 column
- glioblastoma DBTRG-05MG and U251 cell lines
- 11 time points in a range of 0-24 hours
- control and treatment using type I interferon

Why DirectMS1 ?

Previously [2], we compared DirectMS1 quantitation with DDA and DIA methods using two samples: 1) glioblastoma cell lines treated using interferon and 2) well-characterized spikes of UPS proteins into E. coli.

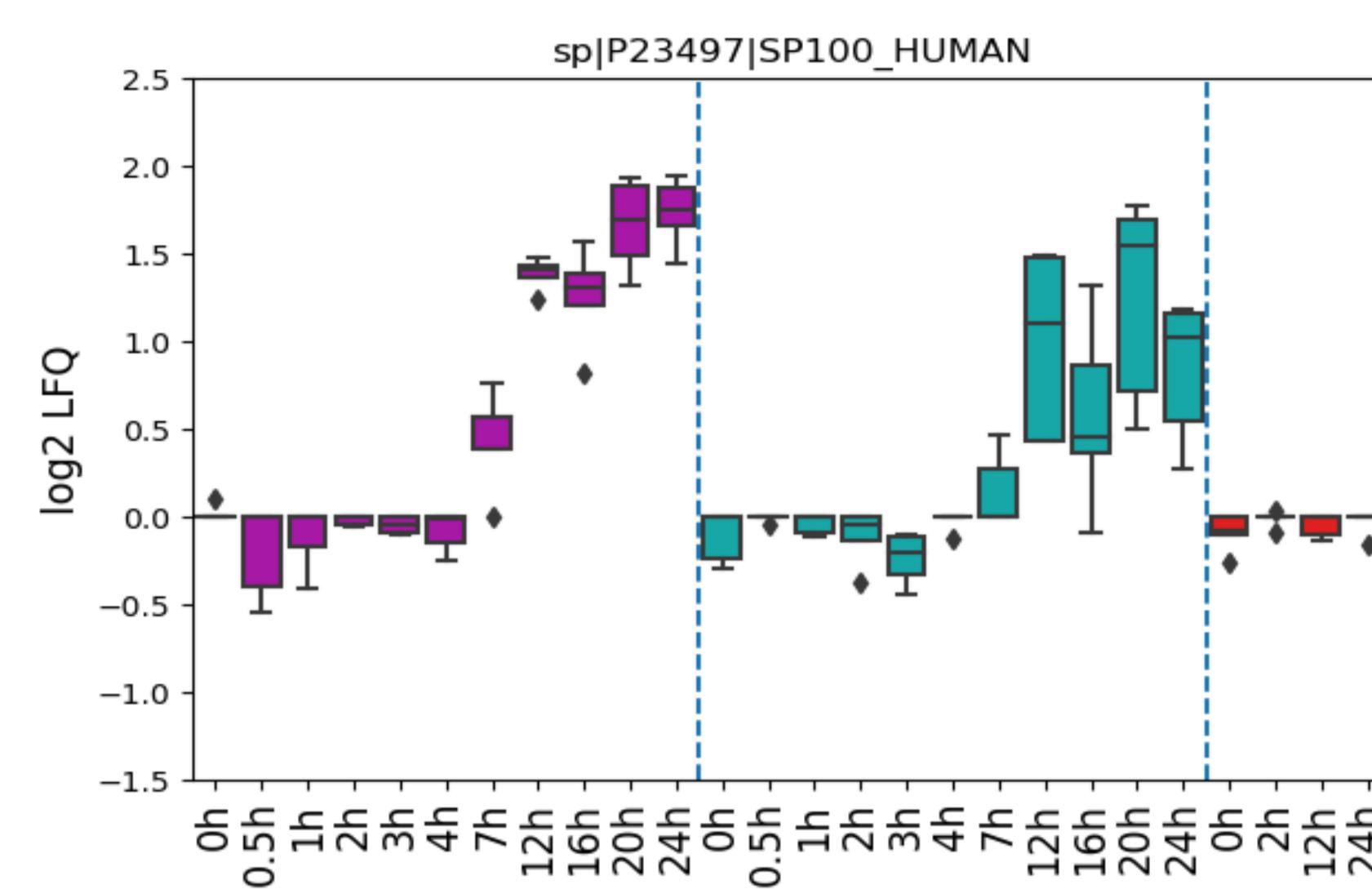


DirectMS1 shows quantitation results comparable with DDA LFQ and TMT while using 10-50 times less LC-MS acquisition time

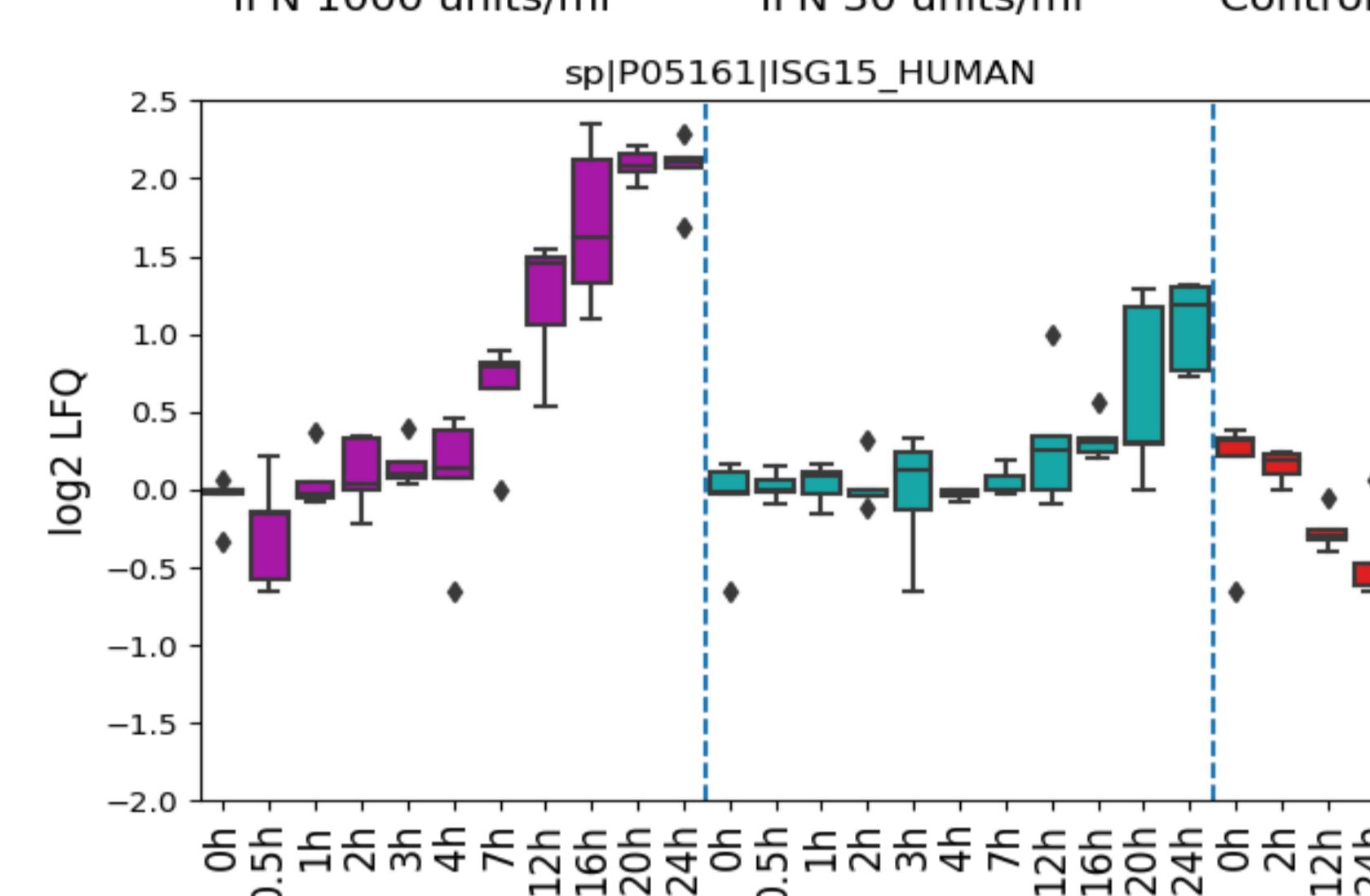


DBTRG Cell line

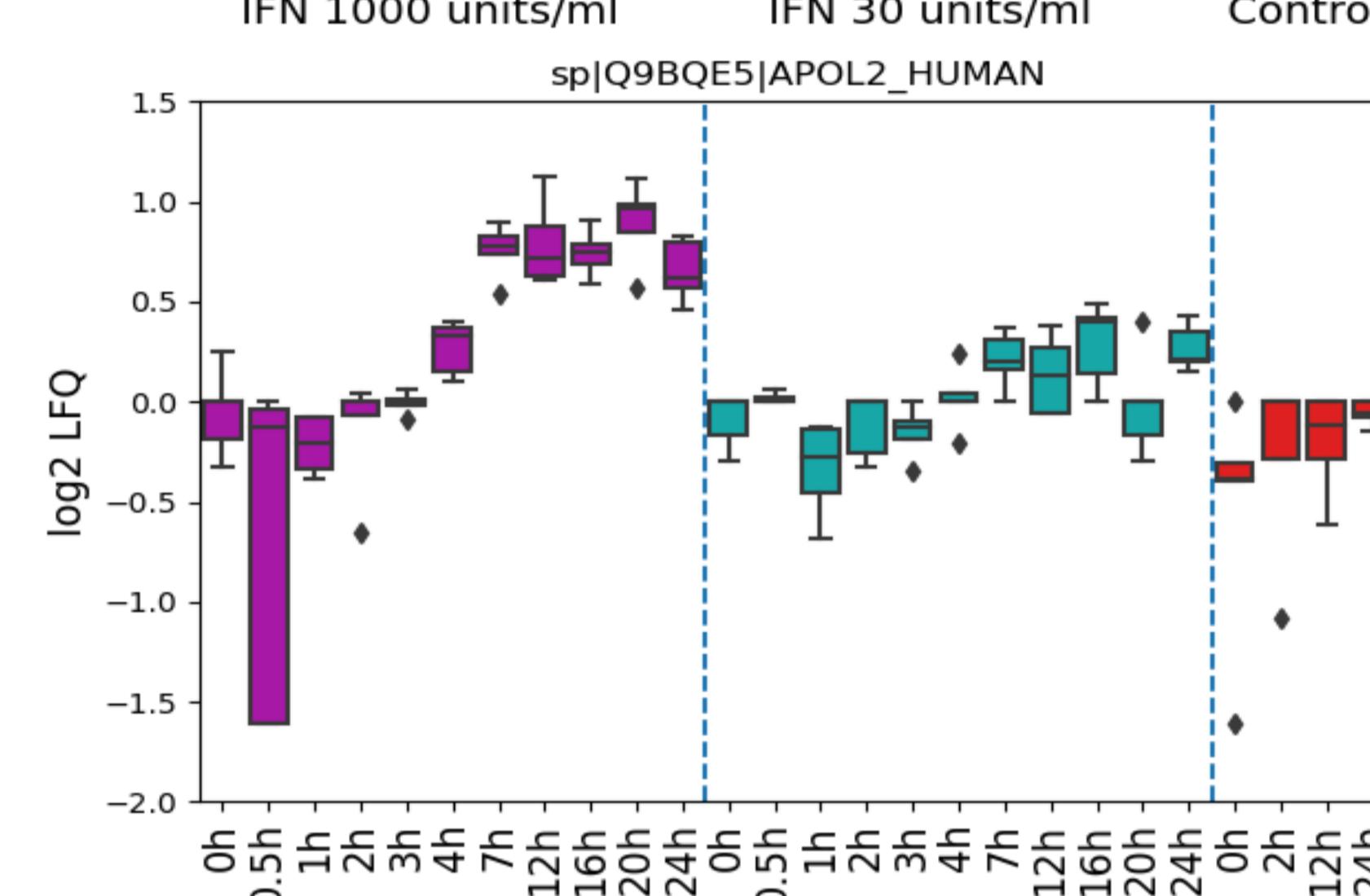
- 1 day of total acquisition time for 150 LC-MS1 runs
- 2500 quantified proteins
- 88 pairwise comparisons against each of 4 control time points
- 235 differentially expressed (DE) proteins were detected under conditions: DE in at least 3 (of 11) time points and DE against at least 2 (of 4) controls



9 proteins with monotonic increasing in concentration after interferon treatment

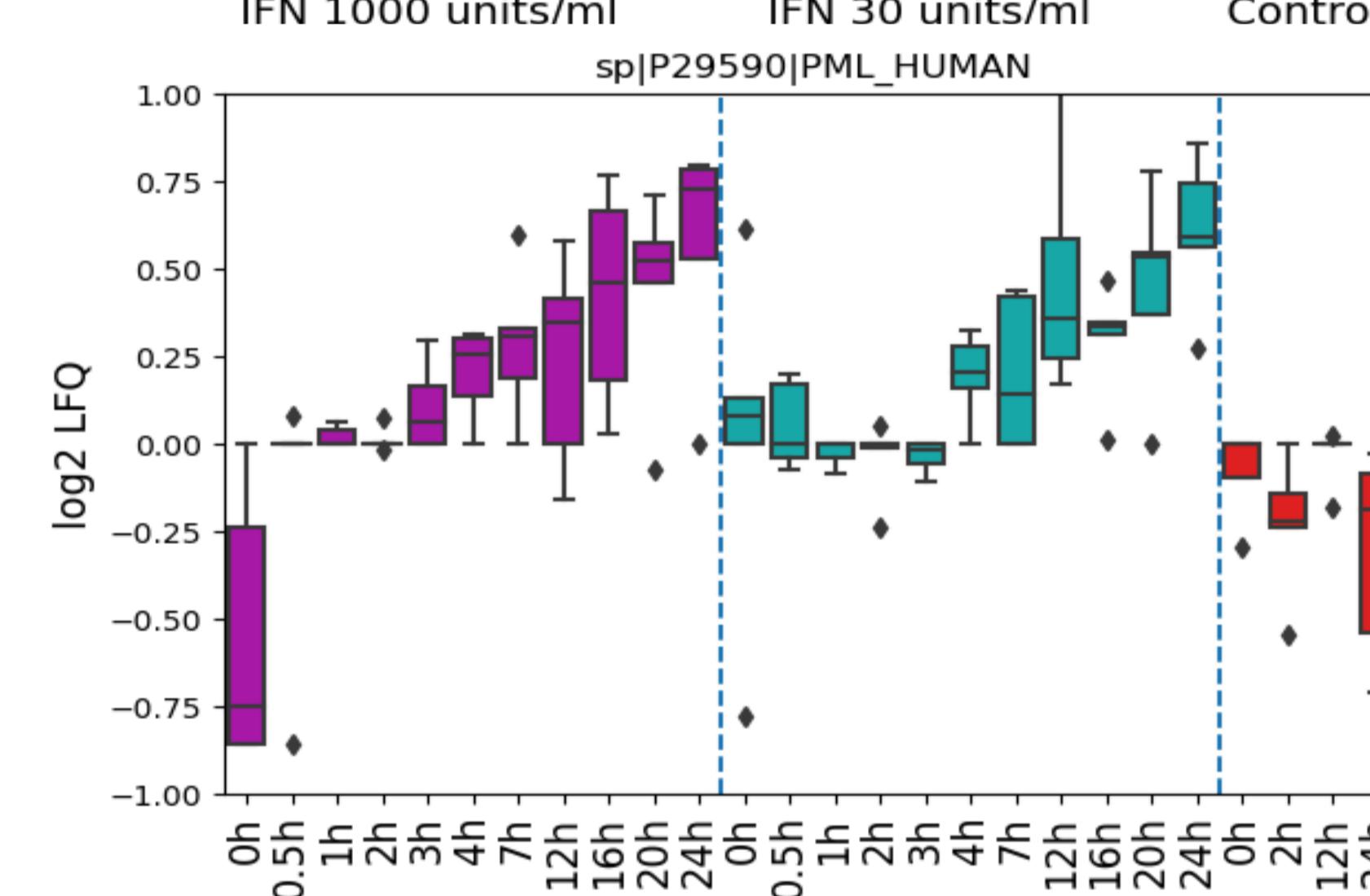


Most of these 9 interferon-regulated proteins start expressing only after 7h when resistance to VSV was already formed for 1000 units/ml of interferon



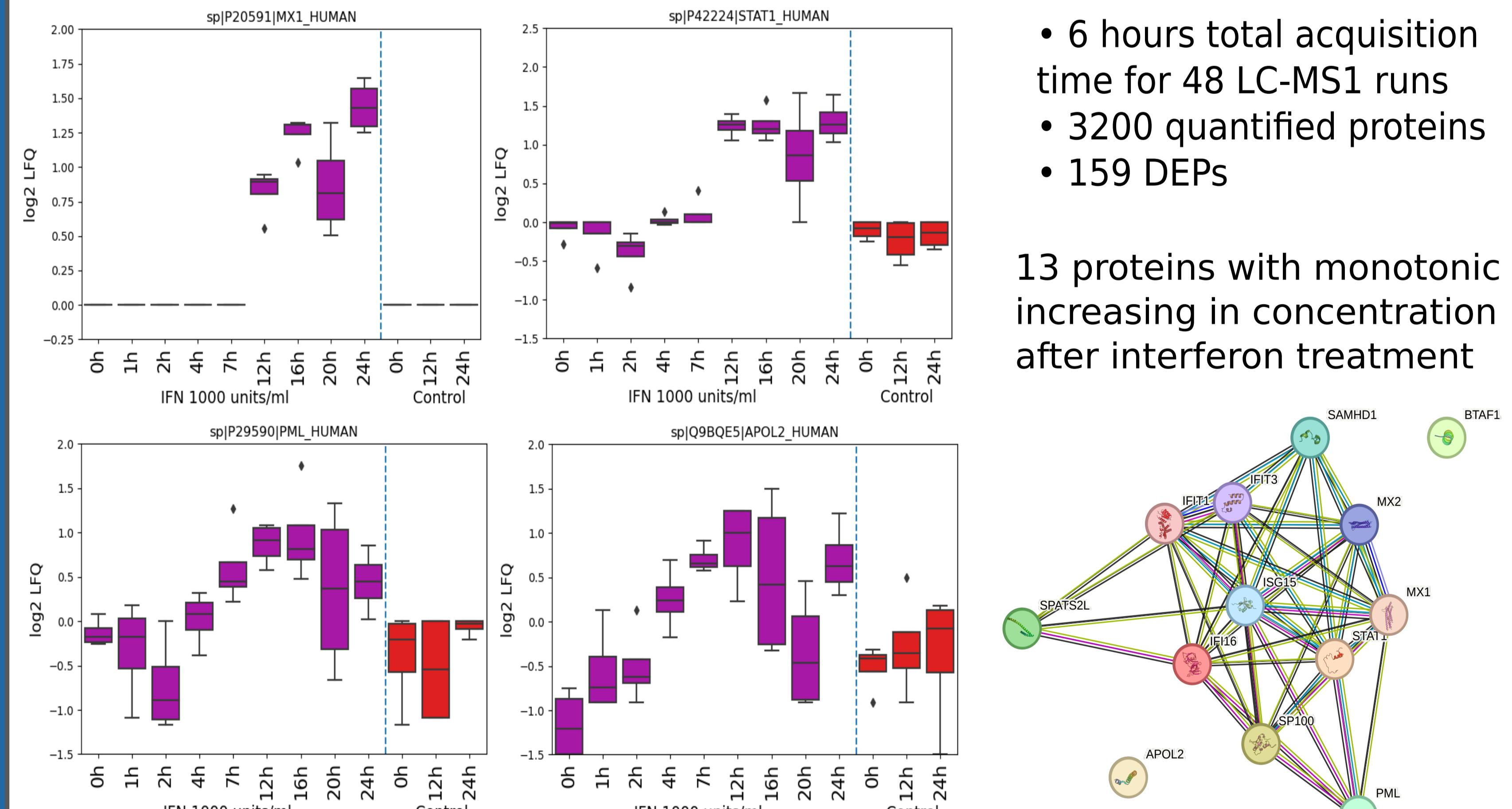
However, PML and APOL2 started expressing earlier for high concentration of interferon and correlate with virus resistance formation

Our previous study [3] showed that DBTRG cell line resistant to VSV even without IFN treatment when IFIT3 and PLSCR1 genes were suppressed. Suppression of those genes caused an increase in the abundances of APOL2, DDX58, ERAP1, HLA-B, HLA-C, GLS, MX1, ISG15, PML, PSME2, and SAMHD1 gene products



Extra confirmation using knockdowns of APOL2 and PML genes is planned

U251 Cell line



Conclusions

Most usually reported interferon-stimulated genes are expressed at proteome level only after virus resistance is fully established

Standard proteomics experiment with single time point after treatment is not enough for complete understanding of biological processes

We found two proteins which correlate with VSV resistance and may play a major role in antiviral mechanisms for particular glioblastoma cells

DirectMS1 method makes it possible to implement large-scale time-resolved proteomic analysis with reasonable total acquisition time and quantification depth

Acknowledgments

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