



# InTraSeq™ technology: a multimodal single cell method for simultaneous measurement of RNA and Proteins to uncover new single cell biology

## INTRODUCTION

Single cell RNA sequencing (scRNA-seq) is a powerful technique for studying the molecular heterogeneity of cells. However, scRNA-seq does not provide information about protein expression, which is essential for understanding the functional state of cells. Here, we introduce the InTraSeq (Intracellular protein and Transcriptomic Sequencing) method, a novel technology developed by Cell Signaling Technology Inc. (CST), designed to revolutionize single-cell biology with an efficient and simple experimental workflow. The InTraSeq method enables the concurrent measurement of RNA, surface markers, cytoplasmic proteins, and nuclear proteins within individual cells. By leveraging CST's renowned antibodies, the InTraSeq method also enables the study of signaling pathways at the single-cell resolution. This technology was validated by CST in the 10x Genomics Chromium single cell system.

## METHODS

InTraSeq 3' assay kit and protocol is streamlined and requires only one hour of benchwork and uses InTraSeq 3' conjugates with 10x Barcodes. It also allows for multi-day sample storage, making it a practical and efficient tool for high-throughput applications. These samples were then analyzed using the 10x Genomics Chromium Single Cell 3' Kits with Feature Barcoding technology.

## RESULTS

In these studies, InTraSeq technology has been shown to retain RNA in cells and preserve cellular heterogeneity. We show that InTraSeq antibodies conjugated to 10x Genomics barcodes display a robust protein signal that is supported by the RNA expression in a heterogeneous sample. The technology easily identifies cell states within T cells that would otherwise be challenging using only the RNA signal. The drug inhibition experiment in Jurkat cells reveals the expected decrease in signaling pathways, similarly to the stimulation and phosphatase treatment in primary T cells. Furthermore, stimulation of peripheral blood mononuclear cells with LPS reveals the post-translational modification (PTM) changes as well as novel RNA/proteins correlations. This displays the ability for the InTraSeq technology to unravel signaling pathway dynamics in a complex sample.

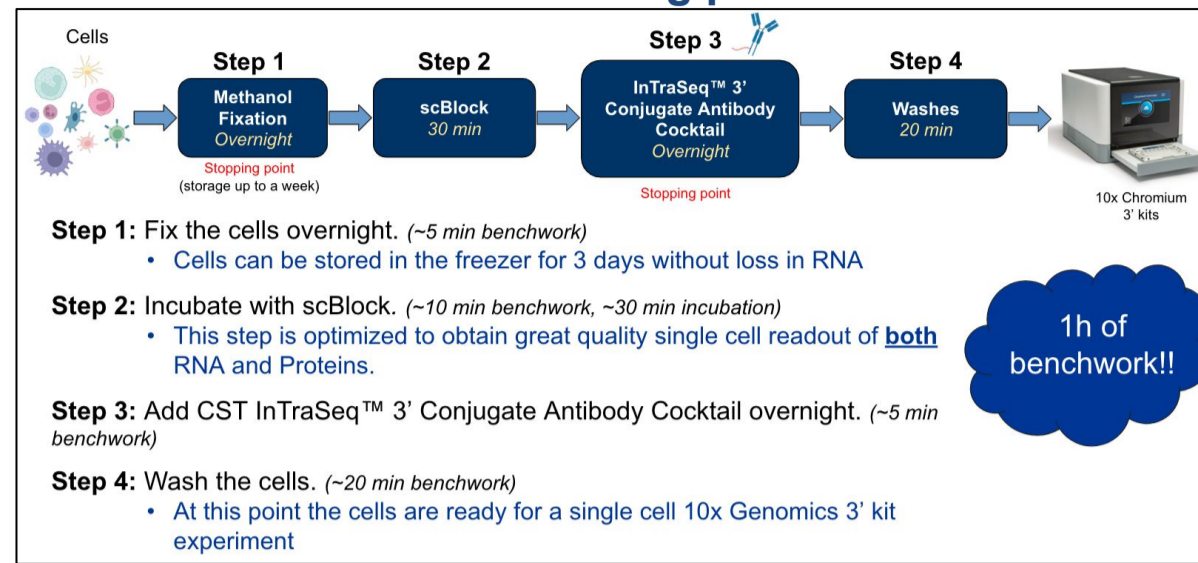
## CONCLUSIONS

InTraSeq technology is a powerful new tool for studying the molecular heterogeneity of cells. It has the potential to identify new druggable targets in cancer, neurodegenerative diseases, and other complex diseases by finding novel correlations between protein/PTMs and other RNA proteins in specific cell types from a heterogeneous tissue. The InTraSeq method can be used to identify new cell subtypes, map signaling pathways in a heterogeneous sample, and identify cell states driven by signaling pathways to offer a comprehensive view of single cell differentiation trajectories. The technique can uncover cellular mechanisms in complex biological tissues that would otherwise be missed in scRNA-seq datasets. Finally, this new technology is a practical and efficient tool for high-throughput applications and has the potential to revolutionize our understanding of cell biology.

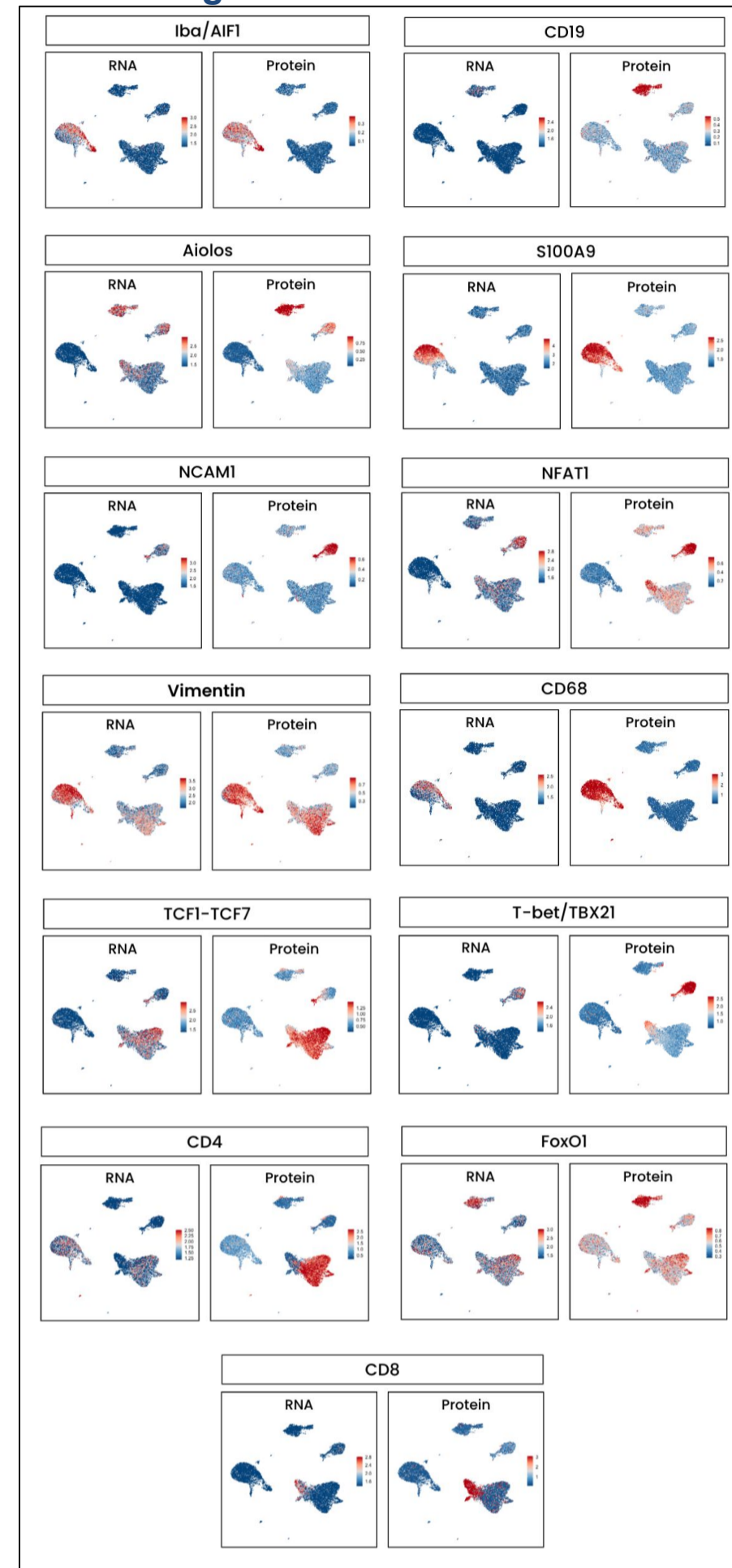
## REFERENCES

- Blair JD, Hartman A, Zenk F, Dalgarno C, Treutlein B, Satija R. Phospho-seq: Integrated, multi-modal profiling of intracellular protein dynamics in single cells. *bioRxiv*. 2023:2023-03.
- Lin Y, Wu TY, Wan S, Yang JY, Wong WH, Wang YR. scJoint integrates atlas-scale single-cell RNA-seq and ATAC-seq data with transfer learning. *Nature biotechnology*. 2022 May;40(5):703-10.

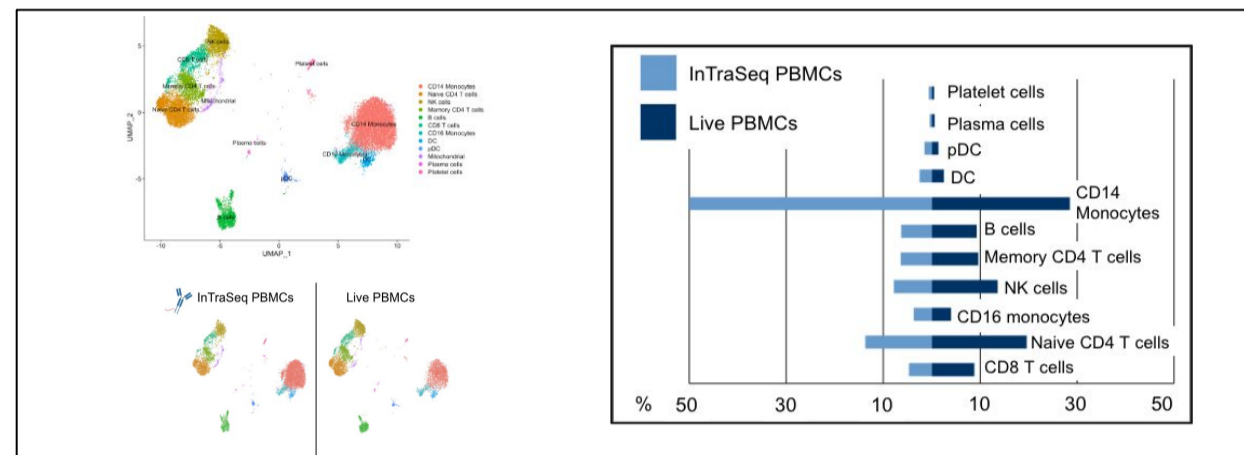
### InTraSeq protocol is a 4-Step straightforward immunostaining protocol



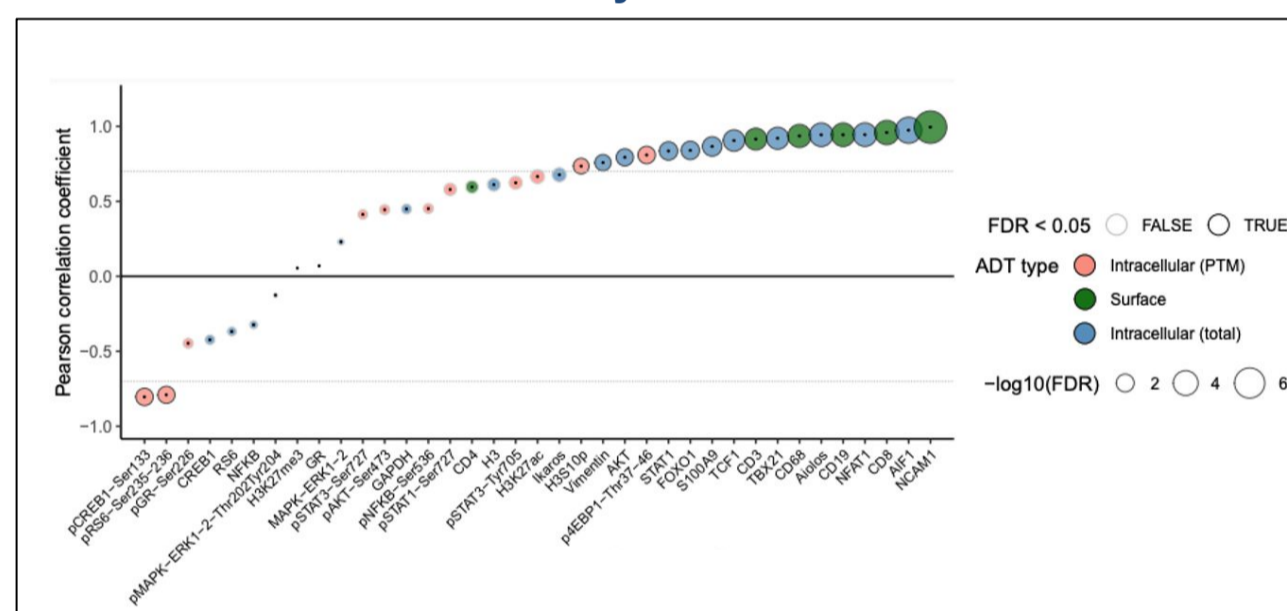
### Robust RNA and Protein signal in individual cells



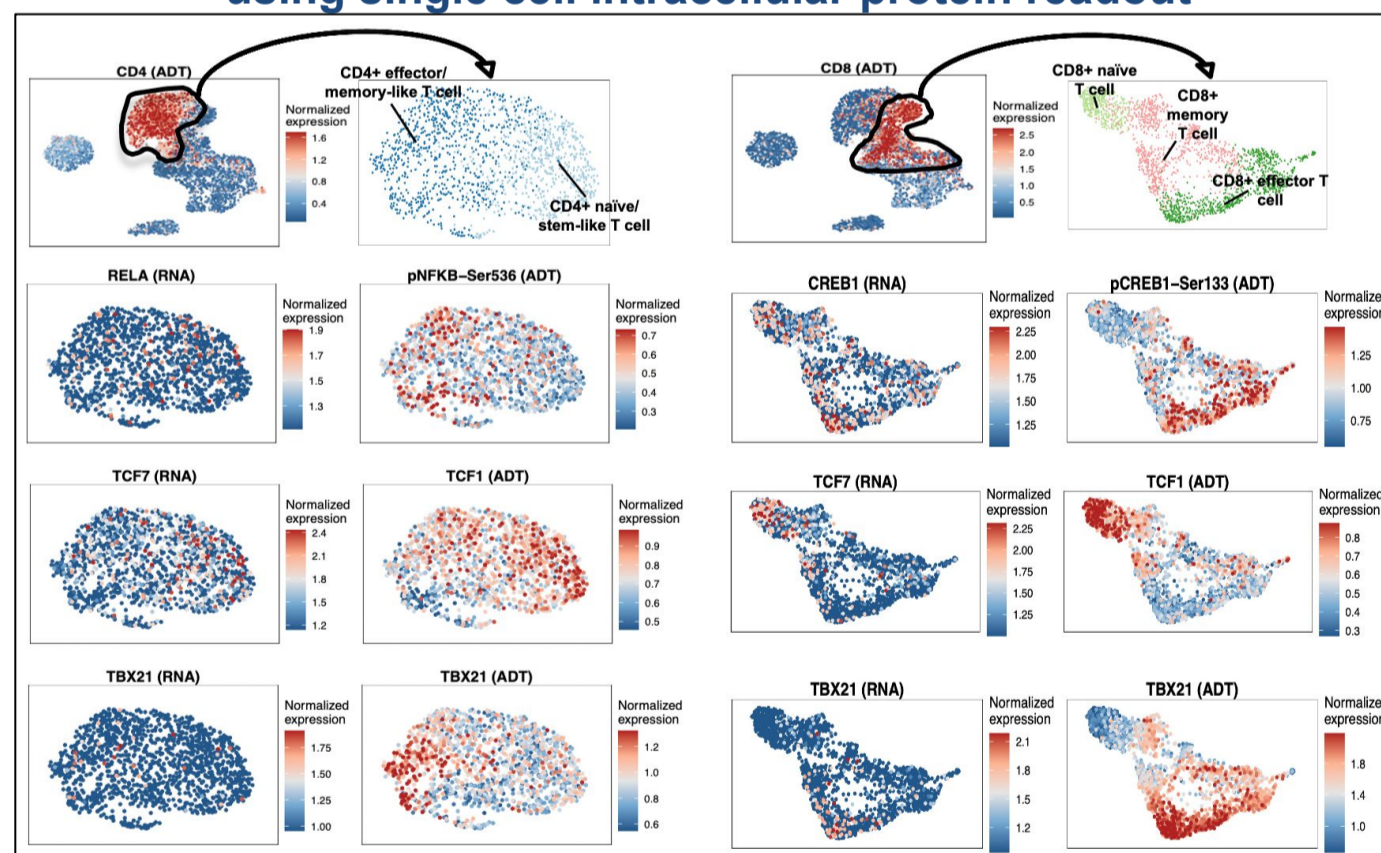
### InTraSeq method preserves cellular heterogeneity



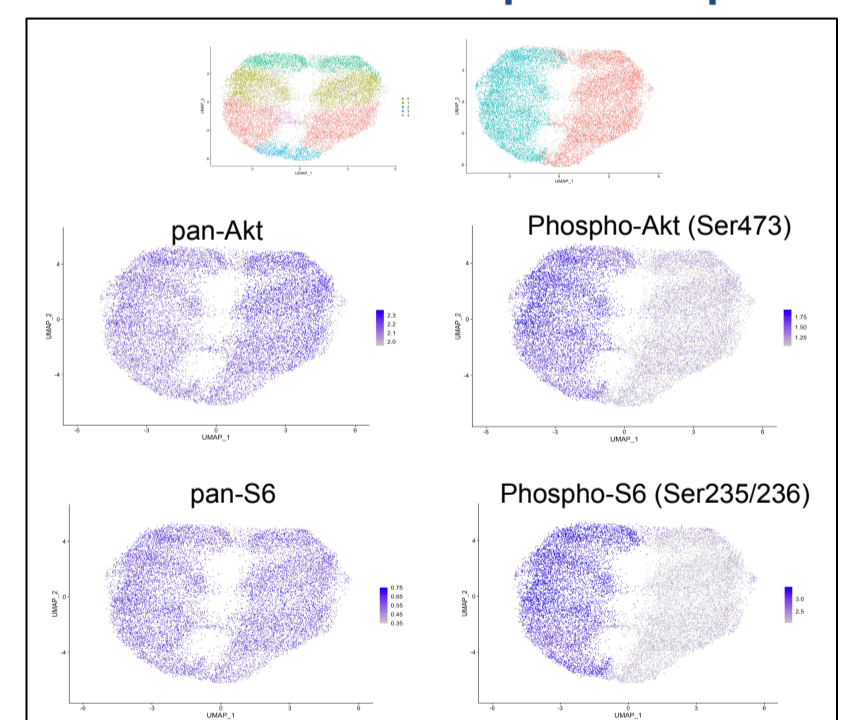
### RNA does not necessarily correlate with Protein levels



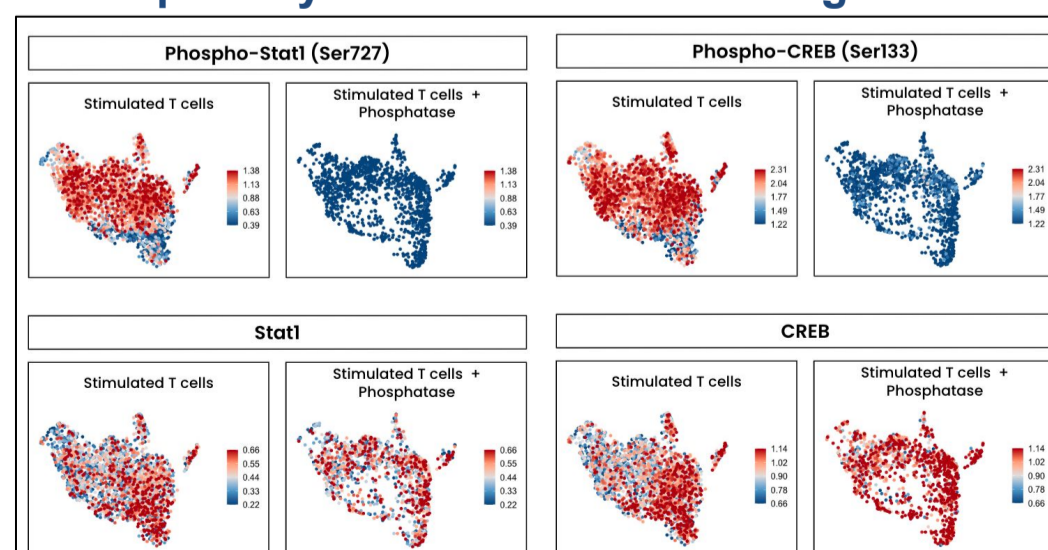
### Identifying cell states within CD4+ and CD8+ cells using single cell intracellular protein readout



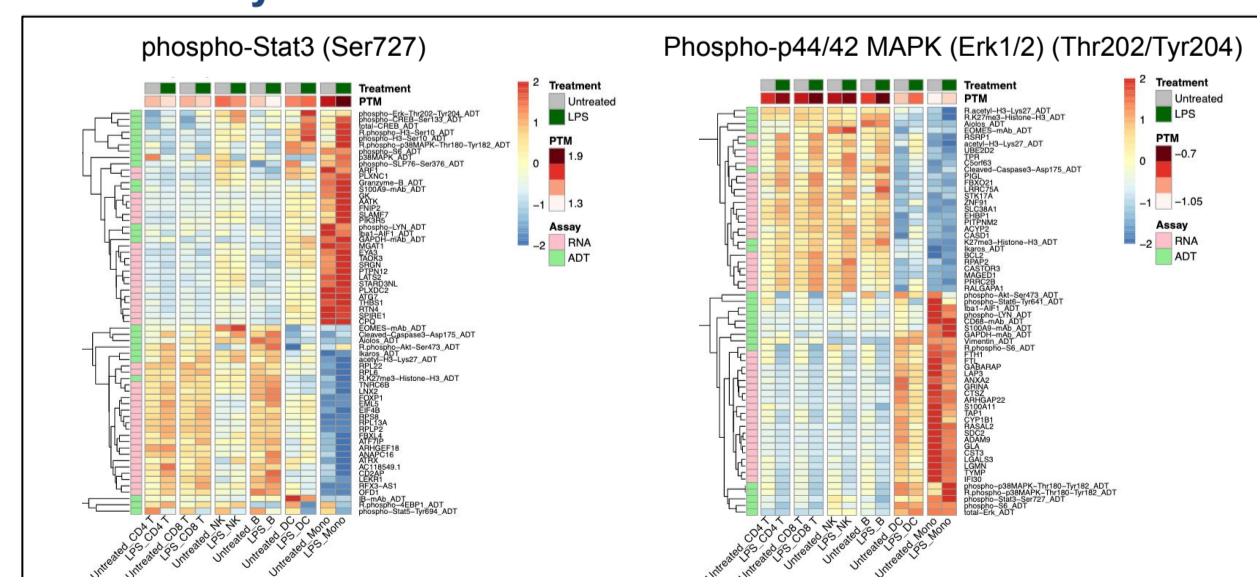
### Acute PI3K inhibition in Jurkat cells shows a decrease in p-S6 and p-Akt



### Phosphatase treatment of stimulated primary T cells reduces PTM signal



### Unbiased PTMs correlation study reveals signaling pathway dynamics in PBMCs after LPS stimulation



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