

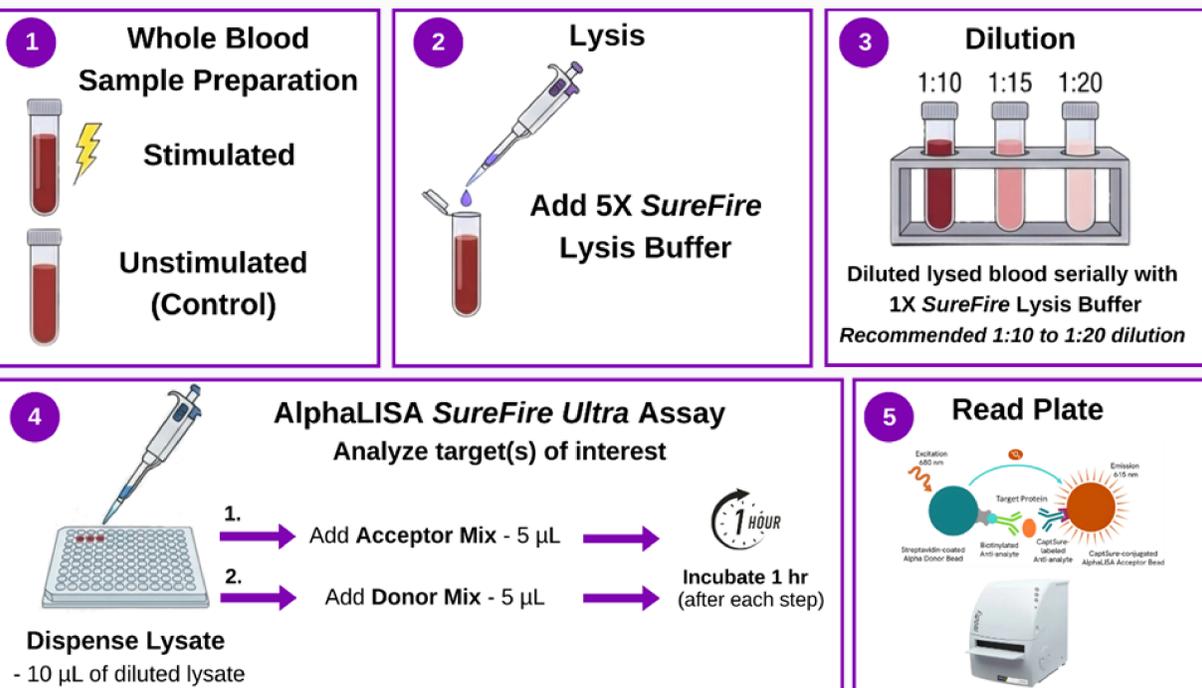
# Measurement of cellular proteins in human whole blood using the AlphaLISA™ SureFire® platform: Next-generation assay technology for translational research

## 1 Overview

- Alpha *SureFire Ultra* technology is well known for measuring cellular targets in 'endogenous' cell systems, often for high throughput screening.
- Translational research is increasingly seeking highly sensitive technologies able to detect target proteins in clinically relevant matrices with minimal sample preparation.
- From immortalised cell lines to pluripotent stem cells to microglia to PBMCs to primary macrophages to tissues – *SureFire* is helping bridge basic science with clinical applications.
- We have now expanded this to detection of cellular proteins in human whole blood - arguably the most complex and challenging matrix of all.
- Here-in is described a best practice workflow that showcases the sensitivity, specificity and simplicity of *SureFire* assay technology for measuring various intracellular target proteins in human whole blood.

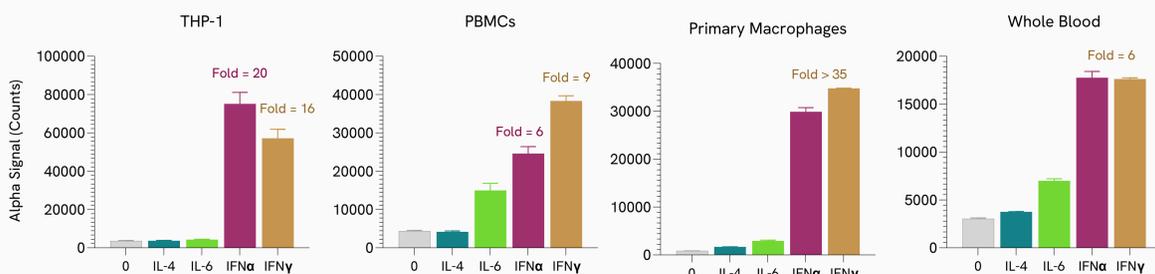
## 2 Alpha SureFire Blood Workflow

- Concentrated human whole blood was collected from healthy donors.
- The anticoagulant citrate-phosphate-dextrose (CPD) was added (i.e. 14 mL CPD to 100 mL blood).
- Blood samples (approximately 2-fold concentrated) were then treated as described below.

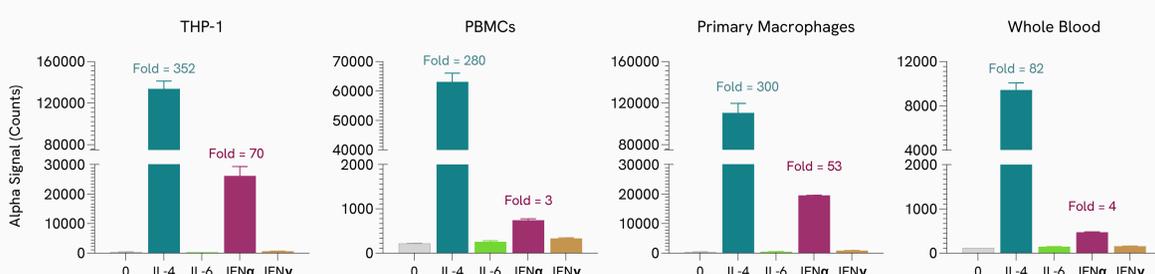


## 3 Specific detection of p-STATs from immortalized cell lines to whole blood

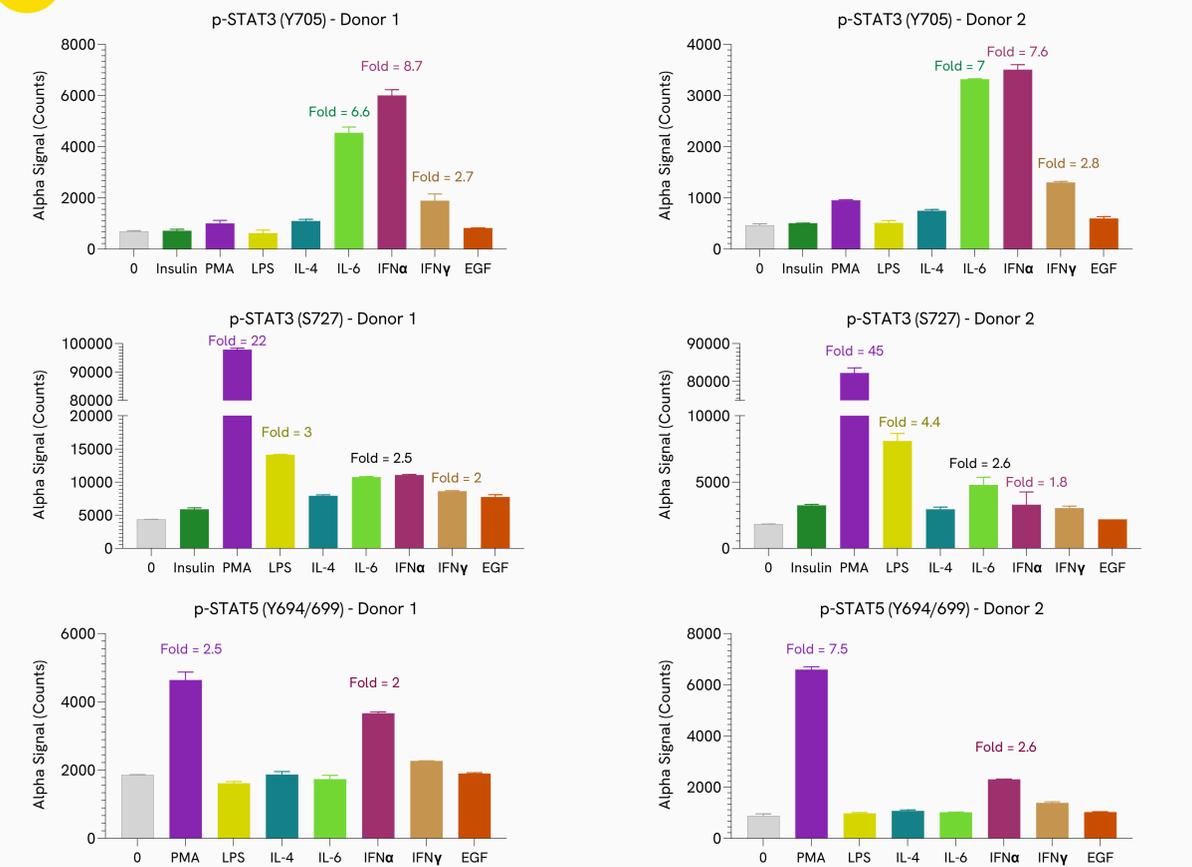
### Specific induction of p-STAT1 (Y701) upon treatment with Type I & 2 IFNs



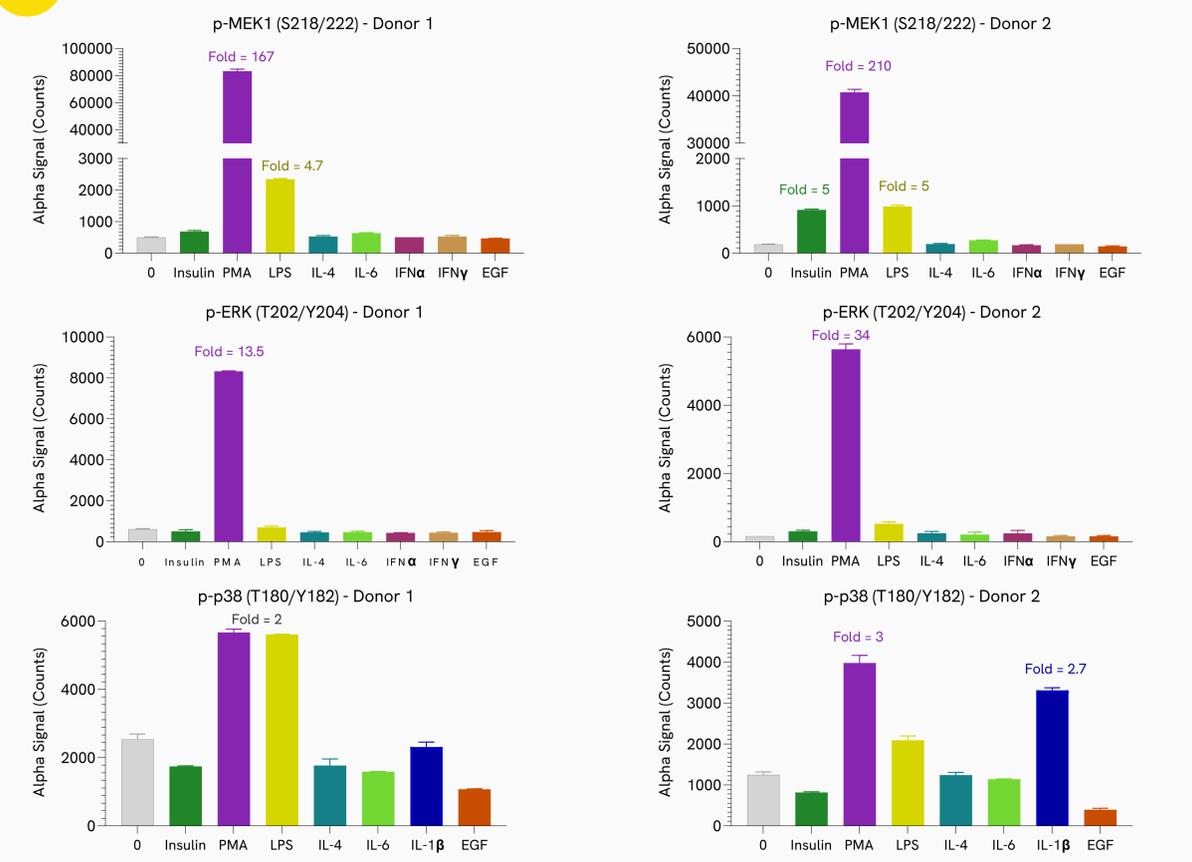
### Specific induction of p-STAT6 (Y641) upon treatment with IL-4 and IFNα



## 3 STAT pathway activation in human whole blood: data from two different donors



## 4 MAPK pathway activation in human whole blood: data from two different donors



## 5 Conclusions

- This study demonstrates the utility of Alpha *SureFire* cell-based assays to measure cellular proteins in human whole blood without the need for cell isolation or washing steps, presenting a powerful alternative to traditional detection methods.
- The impressive sensitivity demonstrated here using whole blood samples highlights its value as a highly differentiated and valuable technology for translational research, well beyond the basic research and screening applications it was originally designed for.