JAK/STAT signalling in PBMCs: High sensitivity AlphaLISA™ *SureFire® Ultra™* assays for measuring JAK phosphorylation and downstream activation/inhibition of STAT family proteins

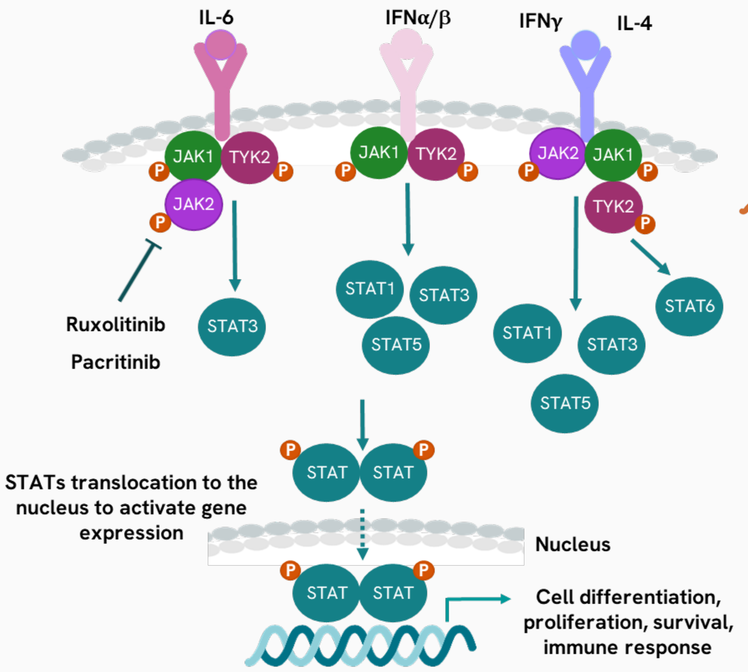
Antony Sheehan, Sheila Falcon (TGR Biosciences, Adelaide, Australia); Michelle Seng, Juliette Seremak (Revvity, Waltham, MA)
antony.sheehan@abcam.com

1 Overview

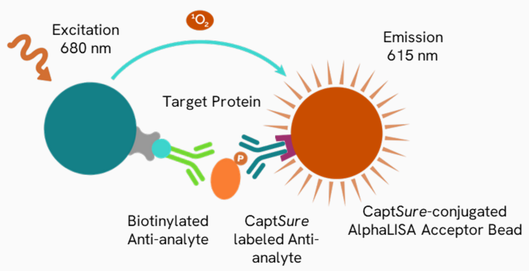
The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway is a key node in the regulation of many cellular processes including inflammation, apoptosis, hematopoiesis and tissue repair. Dozens of cytokines and growth factors including interferons and interleukins have been found to impact JAK/STAT signalling. JAKs mediate the tyrosine phosphorylation of cytokine receptors via the recruitment and activation of STAT proteins. Phosphorylated and dimerized STATs are then translocated to the nucleus where they impart their effects on the regulation of specific genes. Loss of function or mutation of JAK/STAT components have been closely linked to various human cancers and autoimmune diseases and this has driven vast amounts of research into the development of JAK inhibitors and other therapies aimed at inhibiting the JAK/STAT pathway.

AlphaLISA™ *SureFire® Ultra™* assays are traditionally known for measuring target phosphorylation and downstream cascade activation in endogenous cell systems. Here we showcase new *SureFire* assay data for 3 JAK family members: Phospho/Total JAK1, JAK2 and TYK2. High signal windows were generated using heathy human peripheral blood mononuclear cells (PBMCs) showing IFNα, IFNγ and IL-4-mediated activation of JAK proteins combined with the downstream phosphorylation of STAT1, STAT3, STAT5 and STAT6 family members. In addition, specific JAK inhibitor (Ruxolitinib & Pacritinib) data was generated in the immortalised HEL 92.1.7 and THP-1 cell lines using this broad suite of *SureFire* JAK/STAT assays.

2 Signalling Pathway



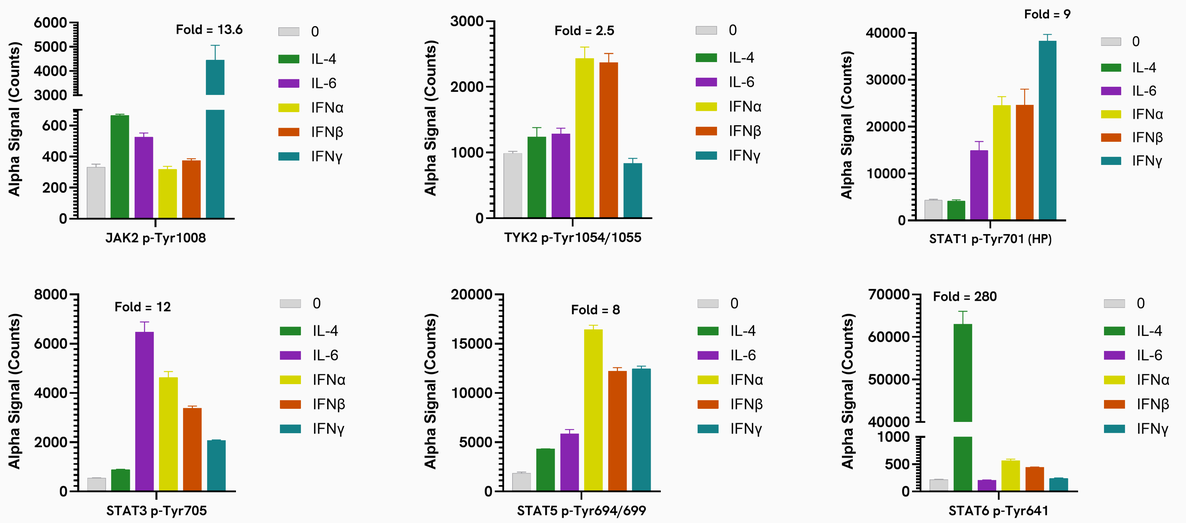
*JAK/STAT family protein levels evaluated by SureFire Ultra**



*For detailed protocols please visit:
https://tgrbiosciences.com
https://www.revvity.com

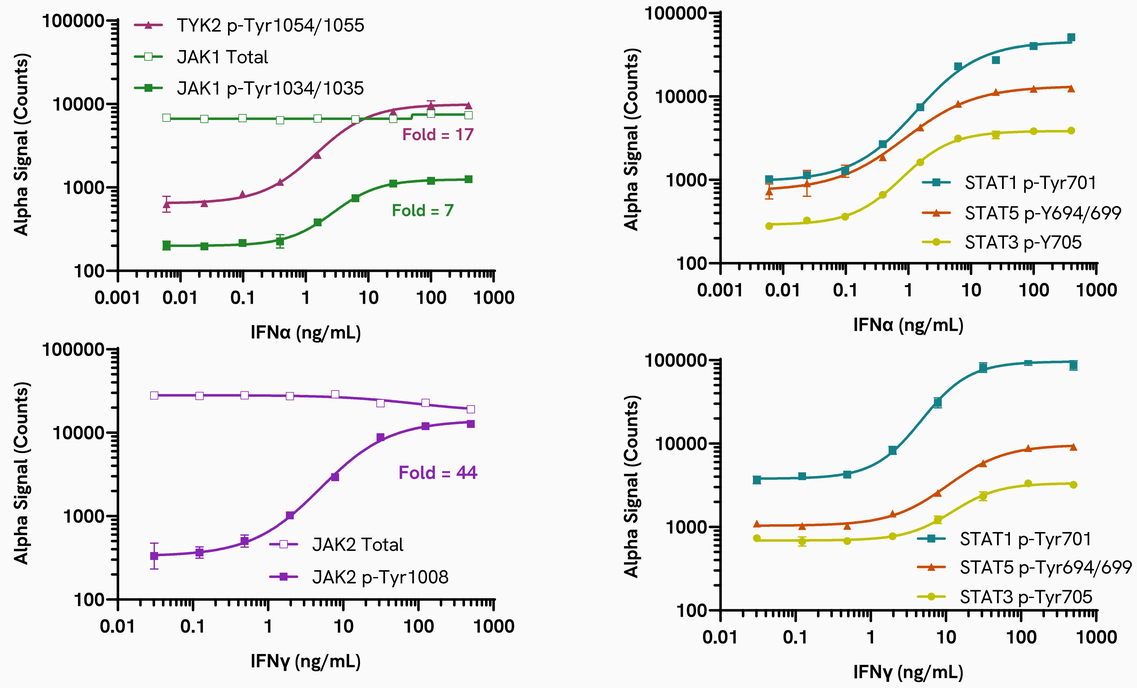
3 Results

Cytokine induction of JAK/STAT signalling pathway in human PBMCs



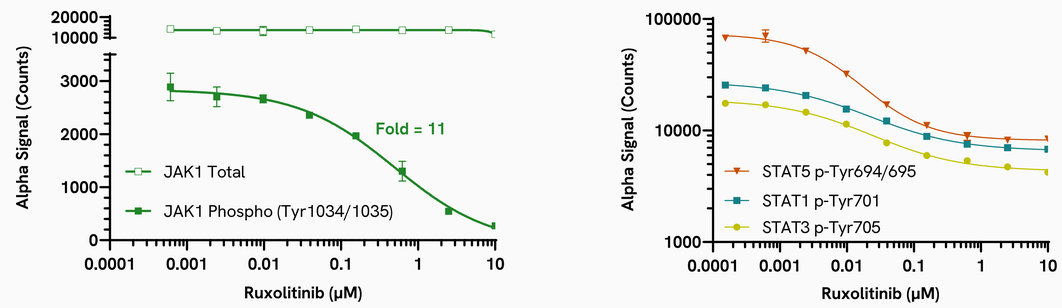
- PBMCs isolated from healthy donors using Ficoll® Plaque Plus and seeded in 96-well culture plates.
- Cells starved for 2 h and treated with the indicated cytokines for 15 mins.
- Cells lysed & single lysate used to assay JAK/STAT family proteins by *SureFire* (40,000 cells/datapoint).

JAK/STAT signalling in macrophages isolated from PBMCs



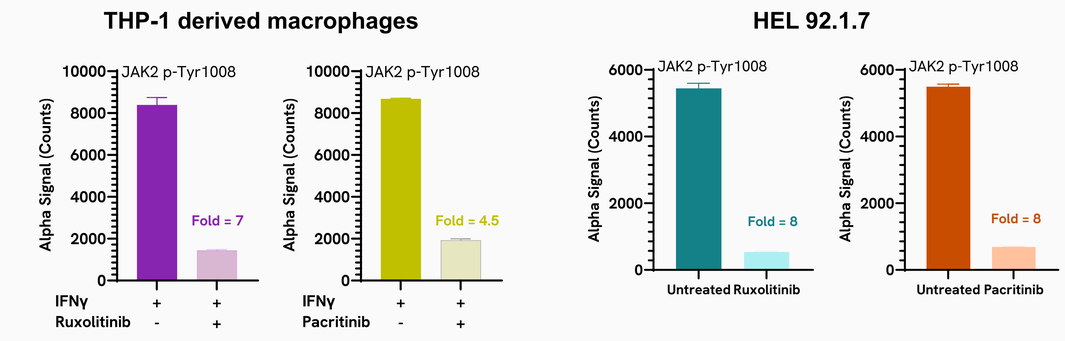
- Monocytes isolated from PBMCs from healthy donors and cultured for 6 days in DMEM.
- Macrophages starved for 2 h and treated with IFNα or IFNγ at the indicated concentrations for 15 mins.
- Cells lysed & single lysate used to assay JAK/STAT family proteins by *SureFire* (approx. 6,000 cells/datapoint).

Ruxolitinib mediated JAK1 inhibition downregulates STAT phosphorylation



- THP-1 derived macrophages stimulated with IFNα for 10 min followed by Ruxolitinib treatment for 15 min.
- Cells lysed & single lysate used to assay JAK/STAT family proteins by *SureFire* (approx. 20,000 cells/datapoint).

Ruxolitinib & Pacritinib mediated JAK2 inhibition



- **IFNγ + 15 mins inhibitor treatment.**
- **15 min inhibitor treatment**

4 Conclusions

- The data presented highlight the utility of Alpha *SureFire* technology with JAK/STAT signalling pathway analysis from basic research to drug discovery, screening, pre-clinical and clinical development.
- The impressive sensitivity demonstrated here using PBMCs highlights how powerful the Alpha *SureFire* technology can be for enabling the identification of new therapies aimed at inhibiting the JAK/STAT pathway in various human cancers and autoimmune diseases.