Novel cell-based Myddosome protein-protein interaction assays: High sensitivity AlphaLISA™ *SureFire® Ultra™* assays for measuring endogenous MyD88, IRAK1 & IRAK4 interactions and associated signalling events

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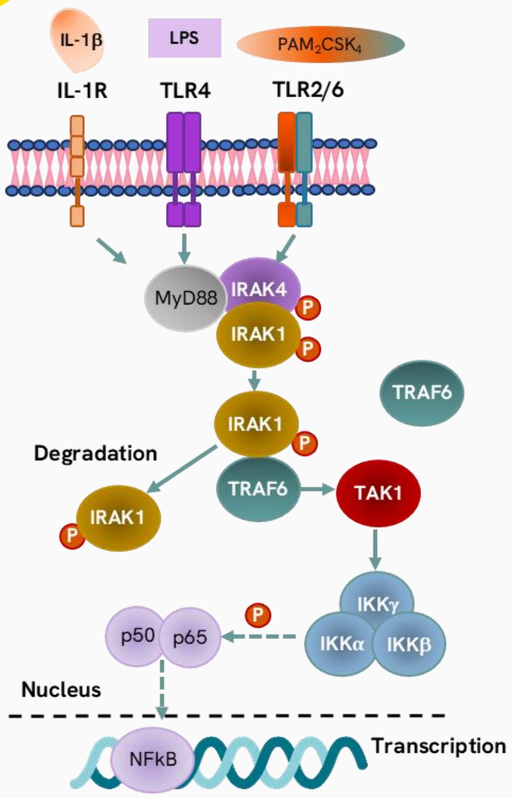
1 Overview

- MyD88, IRAK1 and IRAK4 are central players of the immune system and their dysregulation is implicated in autoimmune, inflammatory and neoplastic diseases. Upon activation of TLRs and IL-1Rs, MyD88 recruits IRAK proteins to form the Myddosome complex. IRAK4 binds first to MyD88 followed by the recruitment, dimerization and phosphorylation of IRAK1 and IRAK4. The formation of a stable Myddosome leads to activation of NF-κB and MAPK pathways and induction of pro-inflammatory cytokines. Hyperactive signalling related to the Myddosome has been linked to autoimmune and inflammatory diseases which has driven vast amounts of research into the development of IRAK1/IRAK4 inhibitors, degraders and other therapies aimed at perturbing or inhibiting signalling from this key junction.
- AlphaLISA™ *SureFire® Ultra*™ assays are well known for their high sensitivity and specificity to detect cellular proteins either associated to cell membranes or localized in cellular compartments. Here we showcase the first-in-class assays for measuring the cellular events related to the Myddosome. High levels of complex formation were detected in cells stimulated with IL-1β. In addition, specific disruption of the Myddosome assembly was measured in cells treated with IRAK1 PROTAC.

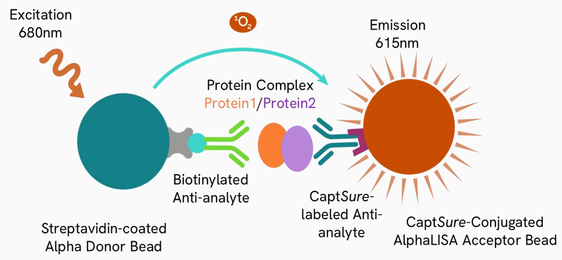
2 Signalling Pathway



3 General Experimental Procedure

- Cells seeded in 96-well plate and treated as indicated.
- Cells lysed & single lysate used in various assays.
- Performed standard Alpha *SureFire* protocol as per below.

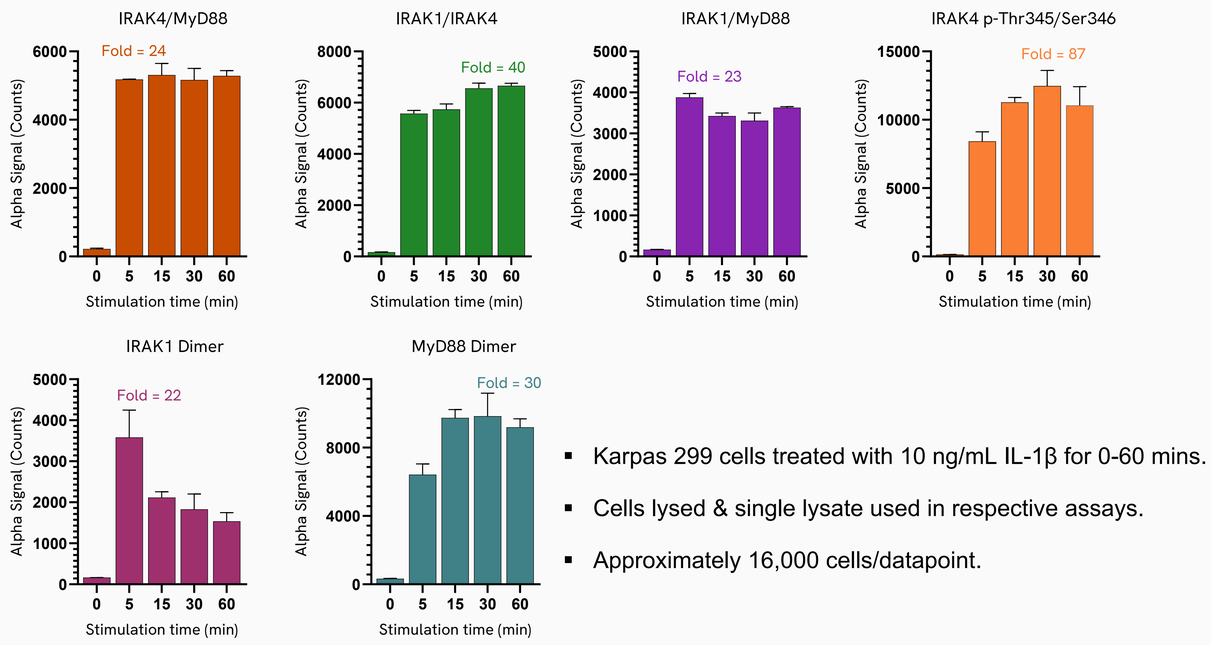
*Alpha SureFire Protein-Protein Interaction Schematic**



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

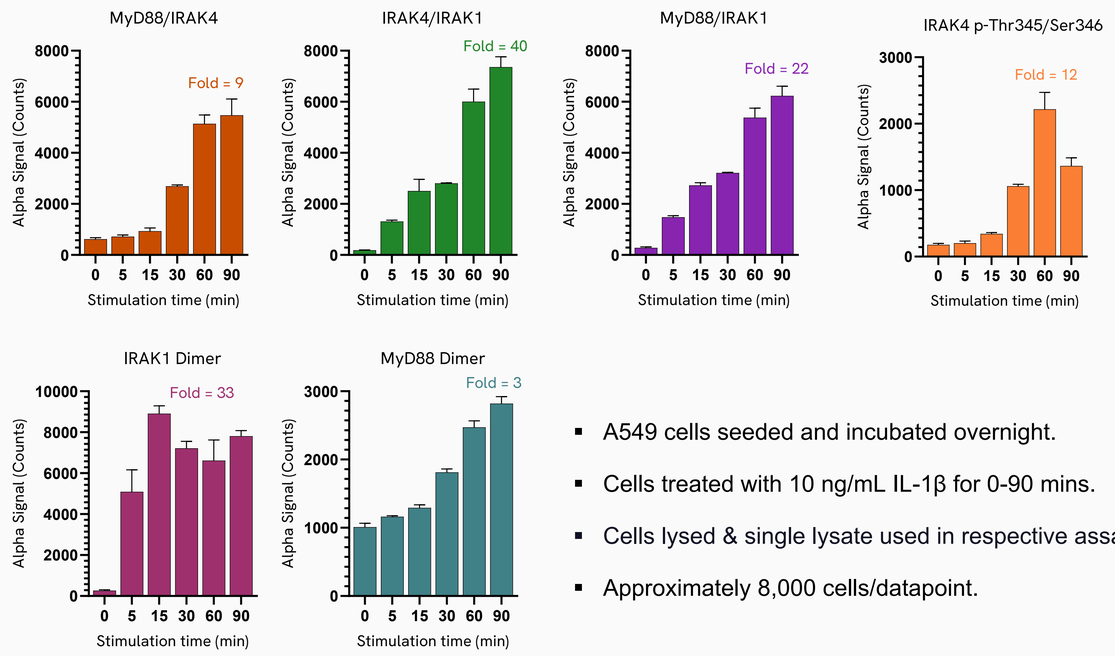
4 Results

IL-1β rapidly induces Myddosome formation in Karpas 299 cells



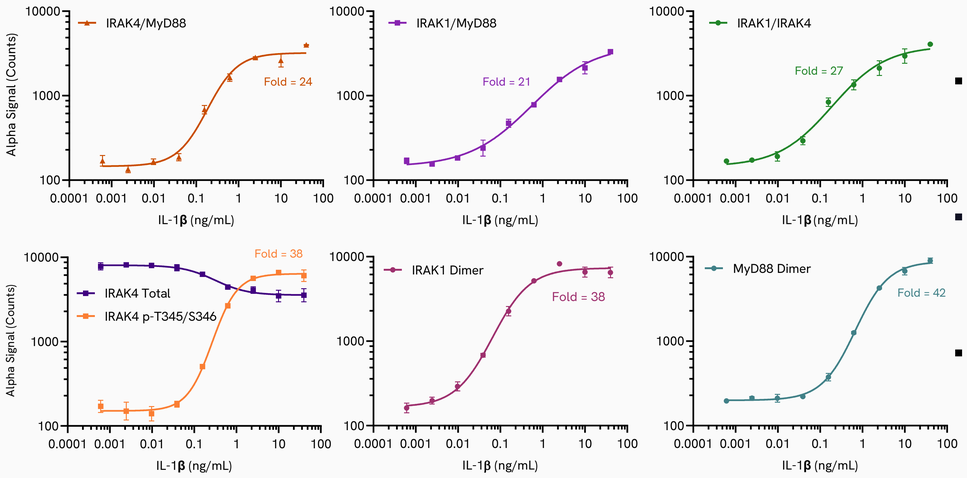
- Karpas 299 cells treated with 10 ng/mL IL-1β for 0-60 mins.
- Cells lysed & single lysate used in respective assays.
- Approximately 16,000 cells/datapoint.

Kinetics of Myddosome induction in IL-1β treated A549 cells



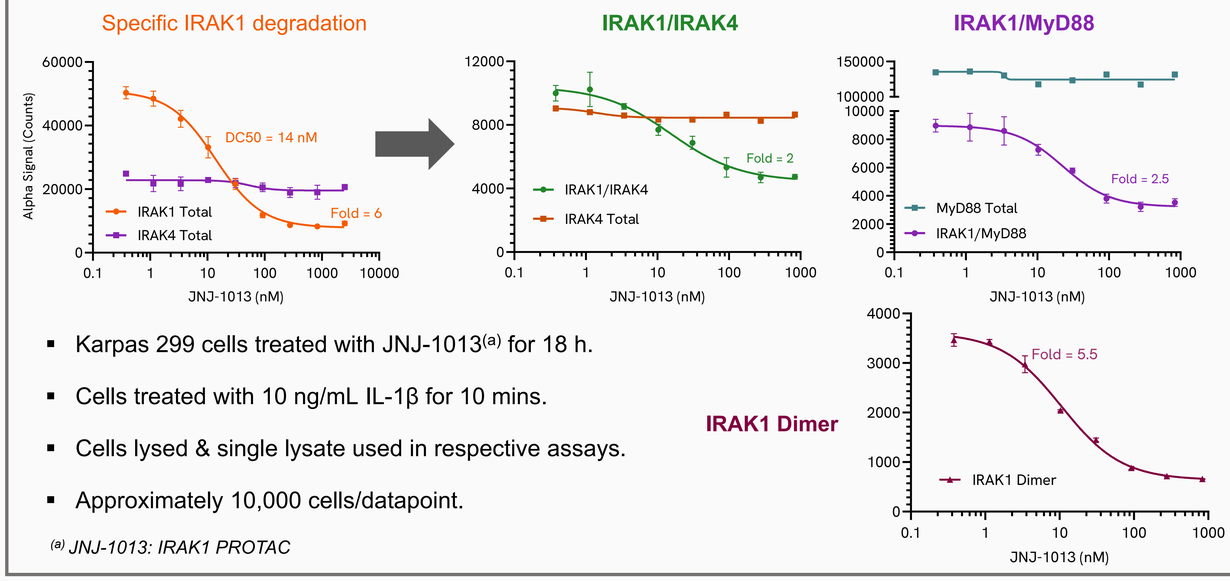
- A549 cells seeded and incubated overnight.
- Cells treated with 10 ng/mL IL-1β for 0-90 mins.
- Cells lysed & single lysate used in respective assays.
- Approximately 8,000 cells/datapoint.

Induction of the Myddosome occurs in a dose-dependent manner



- Karpas cells treated with a dose range of IL-1β for 10 mins.
- Cells lysed & single lysate used in respective assays.
- Approximately 16,000 cells/datapoint.

Perturbance of the Myddosome via IRAK1 degradation



- Karpas 299 cells treated with JNJ-1013[(a)] for 18 h.
- Cells treated with 10 ng/mL IL-1β for 10 mins.
- Cells lysed & single lysate used in respective assays.
- Approximately 10,000 cells/datapoint.

[(a)] *JNJ-1013: IRAK1 PROTAC*

5 Conclusions

- The data presented highlight the general utility of Alpha *SureFire* technology for interrogating key signalling pathways in basic/drug discovery research, high throughput screening, pre-clinical and clinical applications.
- These first-in-class protein-protein interaction Alpha *SureFire* assays specifically demonstrate their potential for identifying new therapies aimed at controlling Myddosome activation which is central to the pathogenesis of various autoimmune and inflammatory diseases.