

# Rapidly Develop Simple Potency Assays for Interleukins & Their Receptors

# DiscoverX

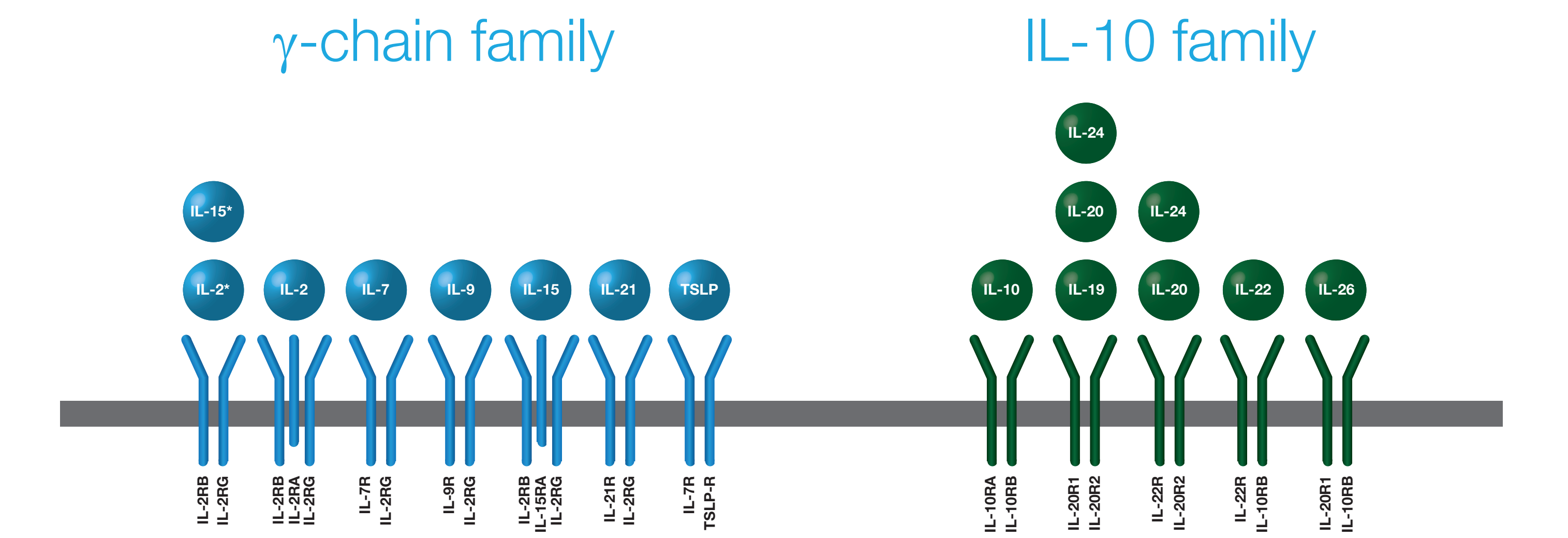
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Abstract

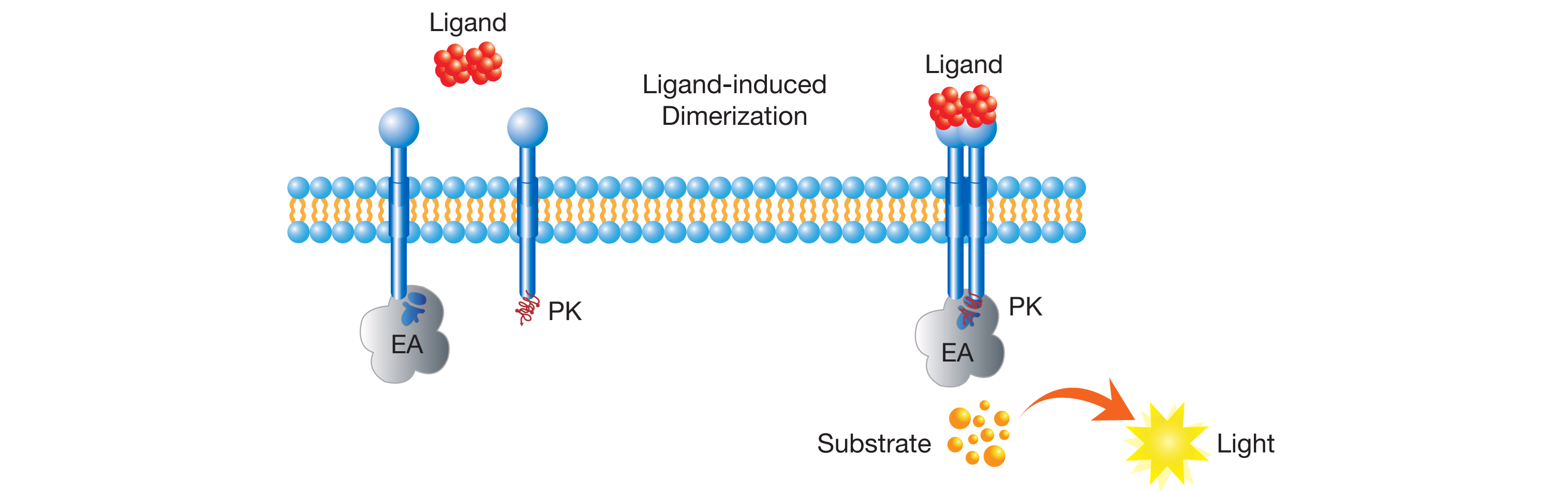
Ligand-induced receptor dimerization is an early functional step in receptor activation, representing the most proximal, functional read-out for Interleukin receptors. It is well understood that the family of Interleukin receptors will dimerize with the other members of its family leading to a complicated signaling cascade that is critically involved in a variety of auto-immune, inflammatory and oncogenic diseases. Here we present a novel application of the PathHunter® technology to monitor receptor-receptor interactions at the surface of intact live cells, applicable to diverse receptor types, with a specific focus on the interleukin family of receptors. The high specificity, simplicity of the assay protocol, large signal to noise ratio, serum tolerance and reproducibility of these assays has enabled their use in cell-based screening, functional characterization and QC lot release assays. Examples discussed here include assays for ~80% of all human interleukins, including assays for Ustekinumab and Tocilizumab. These robust assays have excellent reproducibility, accuracy and precision, demonstrating their suitability for use as QC Lot Release assays.

Structural Diversity in Interleukin Receptor Families

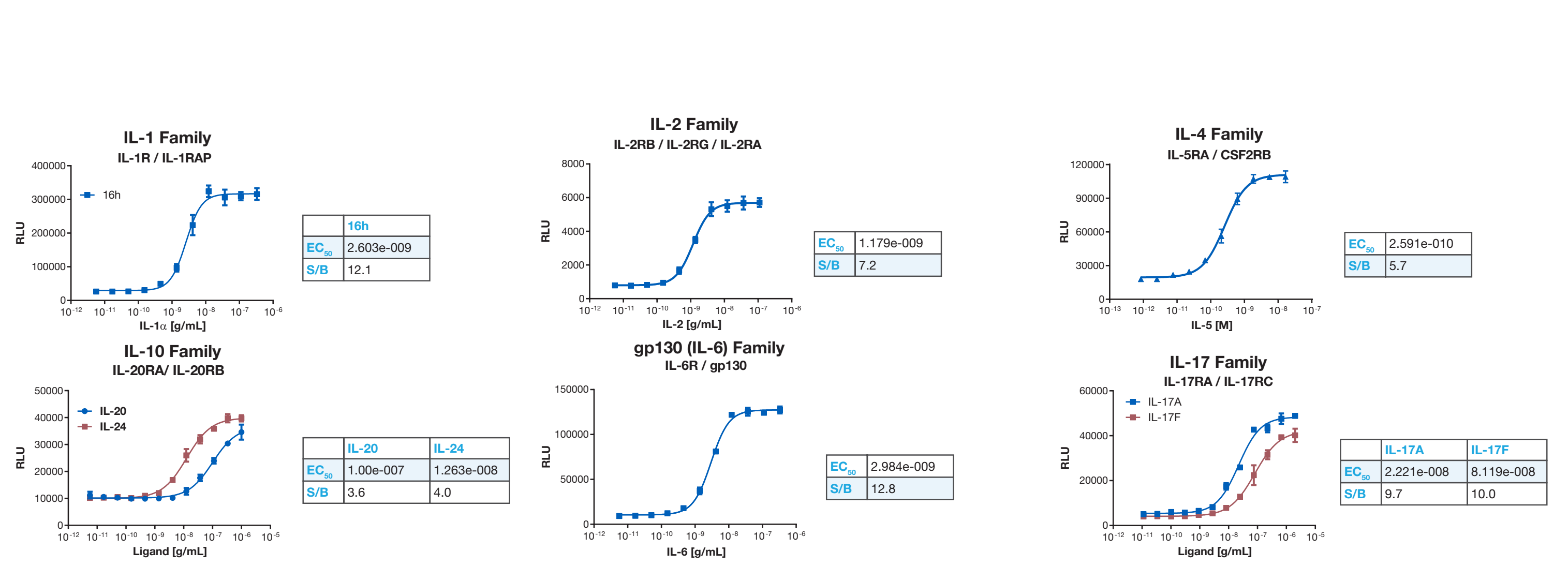


Structural diversity in interleukin receptors. Receptors for interleukins are arranged in families. Each receptor is composed of at least two subunits. All IL-2 family receptors share a subunit, known as the common  $\gamma$  chain subunit, while also encoding a high affinity receptor subunit that recognizes and binds its specific ligand with high affinity. Both receptor subunits are essential for transducing signals to downstream pathways. (Figure adapted from Liao et al., 2011. Curr Opin Immun 23(5): 598-604). IL-10 family receptors involve heterodimerization of multiple receptor subunits to generate responses to different ligands (Figure adapted from Zdanov A., 2010. Cytokine Growth Factor Rev. 21(5): 325-30).

PathHunter Interleukin Assays - Principle

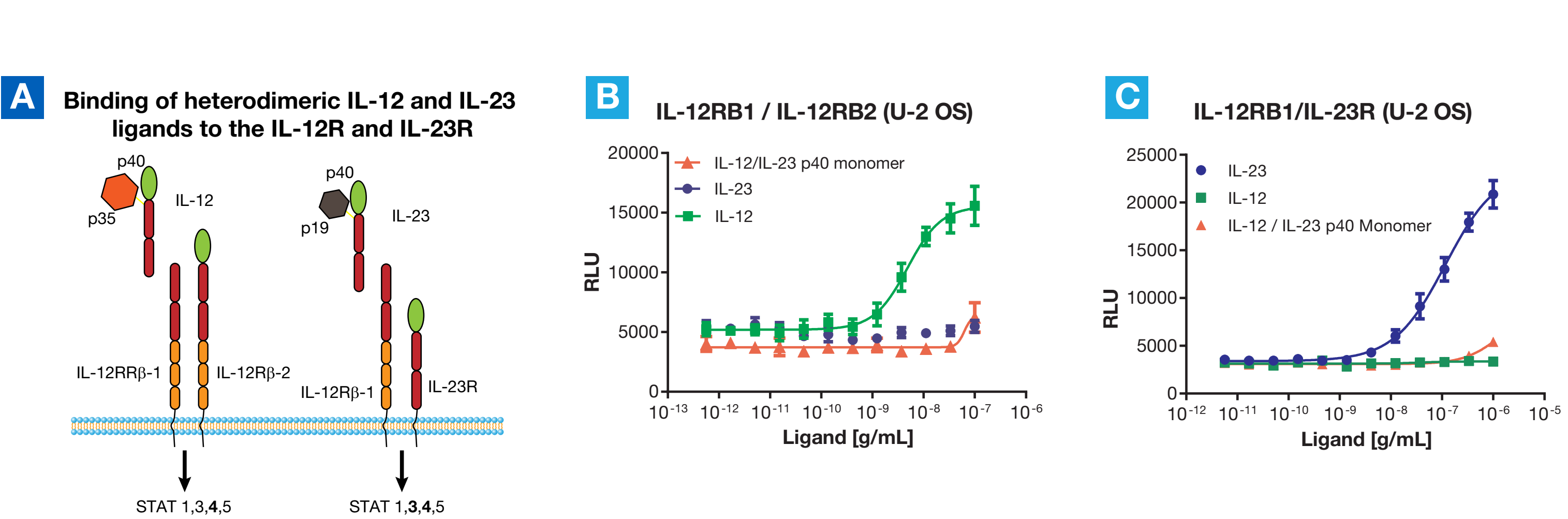


Highly Specific Assays for Interleukin Receptors Across Multiple Families



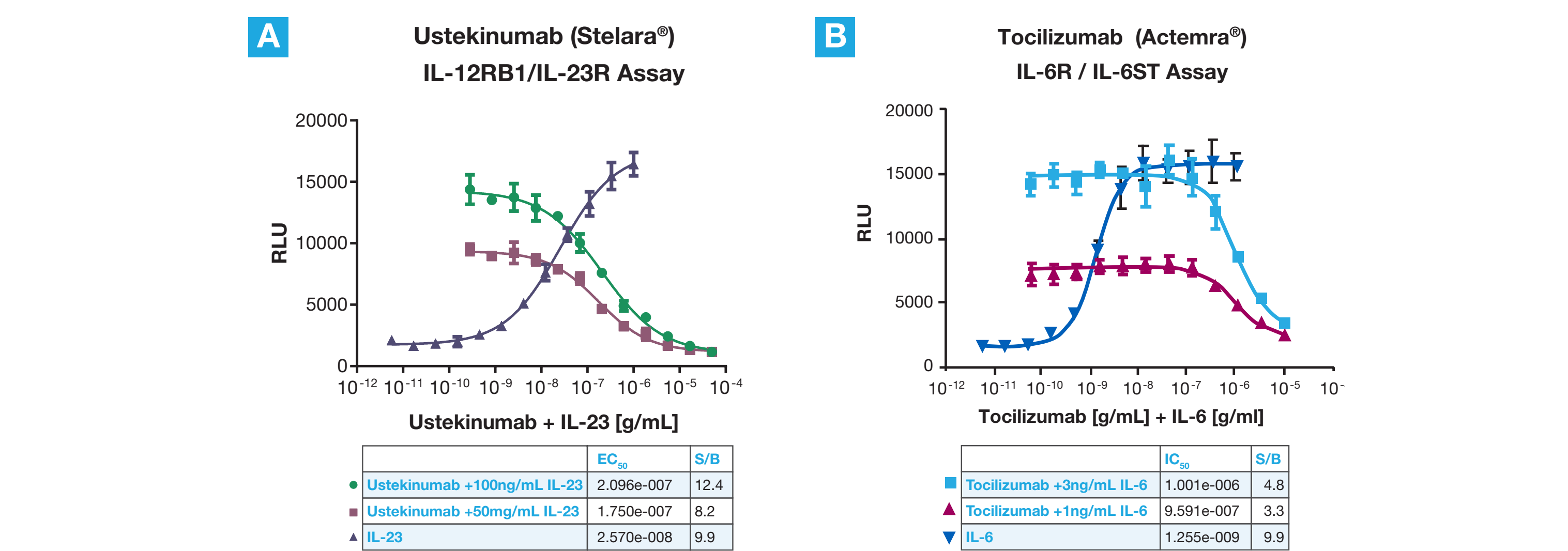
Representative examples of assays for interleukin receptors from 6 different families of interleukins / receptors. Each plot shows a functional dose response for the relevant ligand(s) in a given assay from the indicated family. Data plotted are mean RLU and standard deviation from at least triplicate wells for each dose. These assays are characterized by robust assay windows and high reproducibility.

Dimerization of Inflammation Targets IL-23R and IL-12R is Highly Specific



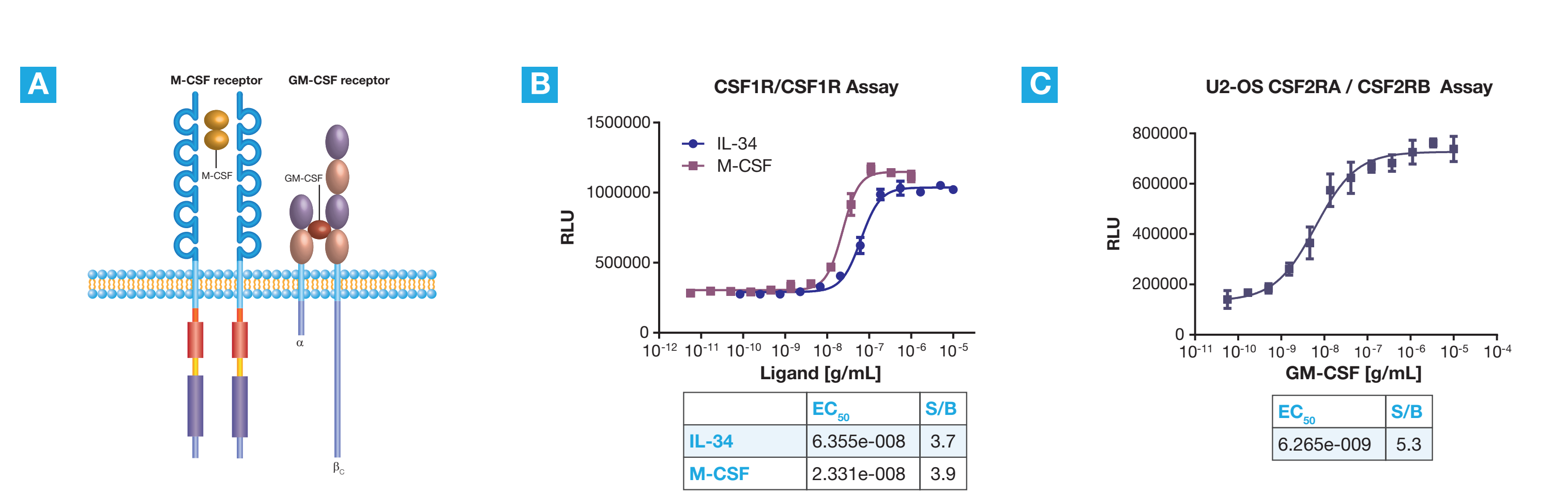
Dimerization of IL-23R and IL-12R is highly specific. **A.** IL23R and IL12R share a common subunit (IL12RB1). Each receptor binds a heterodimeric ligand, which share the p40 subunit. Both receptors have been linked to Crohn's disease. **B.** Dimerization of IL-12R (IL12RB1/IL12RB2) is stimulated by IL-12, but not by IL-23 or p40 monomer. **C.** Dimerization of IL-23R (IL23R/ IL12RB1) is stimulated by IL-23 but not by IL-12 or p40 monomer.

Simple, Functional Cell-based Assays for Ustekinumab & Tocilizumab



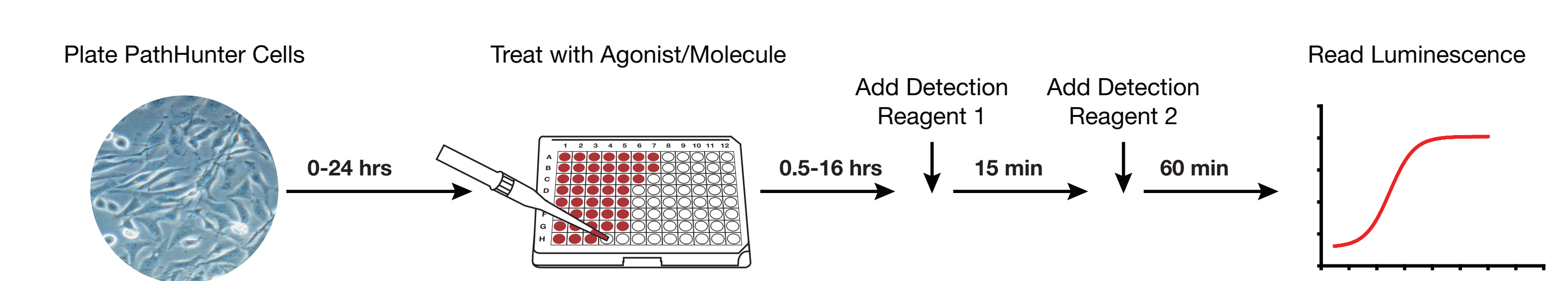
**A.** To treat inflammatory diseases induced by IL-23 or IL-12, antibodies targeting the p40 subunit have been developed as therapeutics, for example Ustekinumab or Stelara® (Stelara is a registered trademark of Janssen/Centacor). Response of IL23 assay to the therapeutic antibody Ustekinumab is shown above. The assay has been tested with two different concentrations of IL-23 agonist, at EC<sub>50</sub> and EC<sub>50</sub> of the agonist response. **B.** Cell-based assay for detecting stimulation and inhibition of IL-6 signaling through the membrane-bound IL-6R. Assay responds to IL-6 with an EC<sub>50</sub> of 1.26ng/ml, while tocilizumab (a therapeutic antibody targeting IL-6R) inhibits IL-6-induced activation of the IL-6R and gp130 (IL-6ST).

Robust cell-based assays for IL-34, M-CSF & GM-CSF



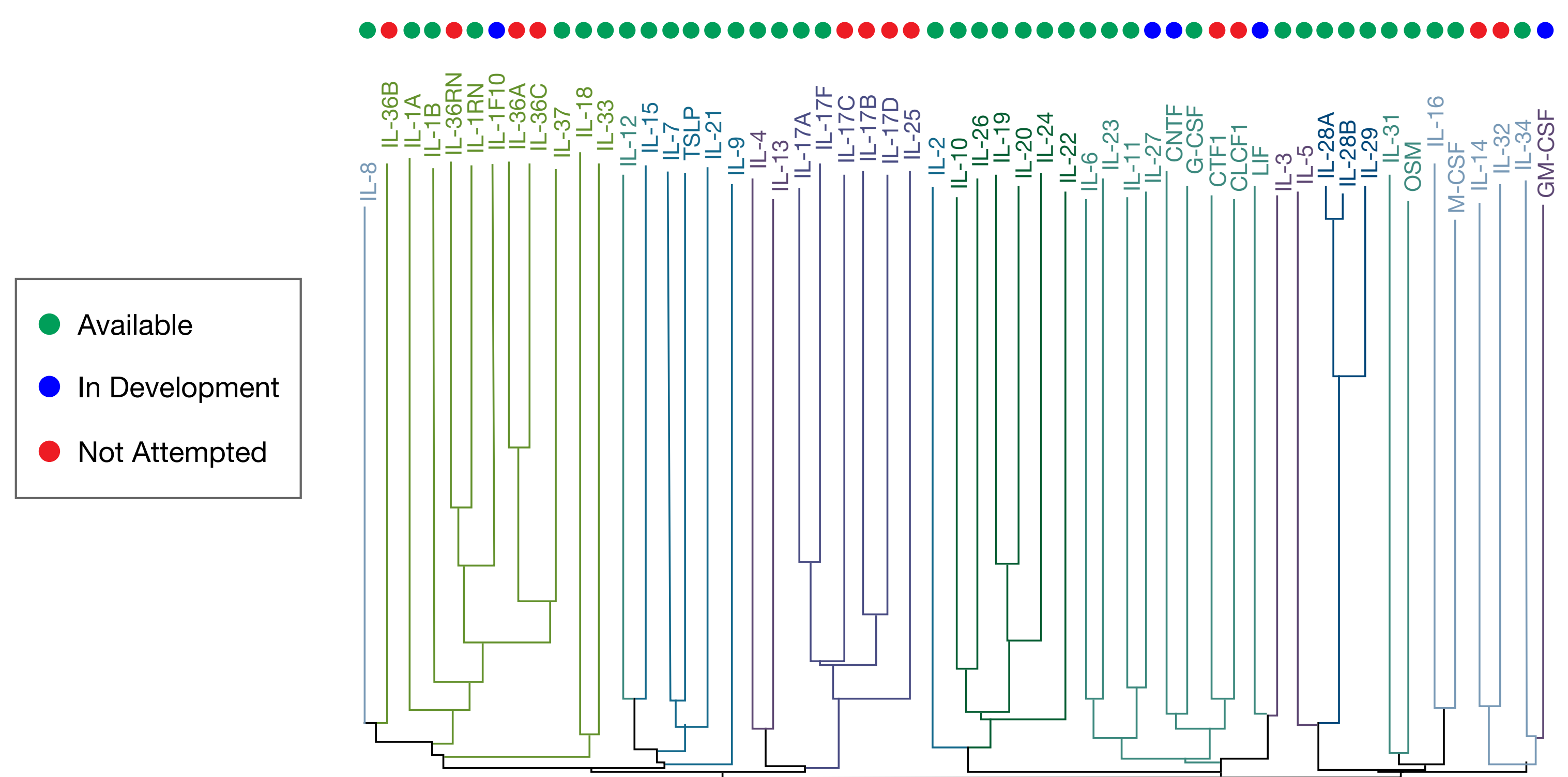
Robust cell-based assays for IL-34, M-CSF & GM-CSF. **A.** Schematic representation of the receptors for macrophage colony-stimulating factor (M-CSF), granulocyte/macrophage CSF (GM-CSF). The M-CSF receptor (CSF1R) is a homodimeric receptor tyrosine kinase; the GM-CSF receptor (CSF2R) is a heterodimeric receptor. **B.** The CSF1R homodimerization assay measures a robust response to IL-34 and M-CSF. **C.** The CSF2R heterodimer assay measures a robust response to GM-CSF.

A Simple Homogenous Protocol With Rapid Results



PathHunter cell-based assays use a simple homogenous protocol with rapid results. Cells are plated on a 96-well plate and incubated for 0-24 hours at 37°C. The agonist/test molecule is added to the plate and incubated for 0.5-16 hours, with specific times optimized for each assay. The detection reagents are added sequentially in two addition steps and the chemiluminescent signal can be detected on any plate-reading luminometer. PathHunter assays for use in QC Lot release testing are available as continuous culture cell lines or can be custom developed into ready-to-assay cells.

Phylogram of Human Interleukins & Available Assays



This phylogram of human interleukins and closely related proteins outlines 8 major groups – IL1-like,  $\gamma$ -chain utilizing, IL4-like, IL6/12-like, IL10-like, IL28-like and IL17-like, and the non-classified cytokines. The blue, green and red dots in front of each name highlights our progress with the development of the assay – green means that we have that assay available, blue means that it is in development and red dots indicate that we have not yet attempted construction of that assay.

[Image adopted from Brocker *et al.*, 2010. Evolutionary divergence and functions of the human interleukin (IL) gene family. *Human Genomics*. 5(1): 30-55]

Summary

- Ready-to-use menu – Save 3-6 months in assay development
- Fast Results – Cut assay time by 3-4 days with results in <24 hrs
- Highly Specific – Results with confidence
- Functional Assay – Identify biologically relevant leads
- Simple Homogenous Protocol – Easy to use & implement with single readout
- Broad Applications – Use for screening & lot release testing