**Cellular Protein Reagents Preparation**

The soluble membrane protein (SMP) reagent was prepared as previously described (Xu 2013). Briefly, 109 CHO cells (Gibco, A29133) were pelleted and washed sequentially with PBSB (1 mg/mL BSA) and Buffer B (50 mM HEPES, 0.15 M NaCl, 2 mM CaCl2, 5 mM KCl, 5 mM MgCl2, 10% Glycerol, pH 7.2). Buffer B was then supplemented with protease inhibitor (Sigma Aldrich, 4693159001) for all subsequent uses. The cells were pelleted again and resuspended in 5 mL of supplemented Buffer B. The resuspended cells were homogenized for 4 cycles of thirty seconds and then sonicated for three cycles of thirty seconds. The suspension was then centrifuged at 40,000 xg for one hour. The supernatant, containing the soluble cytosolic (SCP) protein fraction and the palette, containing the insoluble membrane protein fraction were collected. The SCP fraction protein concentration was measured via BCA and diluted to a final concentration of 1 mg/mL.

The membrane protein fraction was resuspended in 3 mL of supplemented Buffer B with a Dounce homogenizer kit (thirty strokes). The protein concentration was determined with a detergent compatible BCA (BioRad, 5000116) and diluted to a final concentration of 1 mg/mL with Solubilization Buffer (50 mM HEPES, 0.15 M NaCl, 2 mM CaCl2, 5 mM KCl, 5 mM MgCl2, 1% n-dodecyl-β-D-maltopyranoside). The suspension was mixed overnight, rotating end-over-end, at 4 degrees C. The suspension was centrifuged at 40,000 xg for one hour and the supernatant was collected, containing the solubilized membrane fraction (SMP). The protein concentration was measured with the detergent compatible BCA and diluted to 1mg/mL.

Sulfo-NHS-LC-biotin (Thermo Fisher, PI21335) was dissolved in distilled water at ~11.5 mg/mL. For biotinylation of the cellular protein reagents, the stock solution of Sulfo-NHS-LC-biotin (150 μL) was added to SMP (4.5 mL) or SCP (4.5 mL) and incubated for 45 minutes at room temperature, rotating end-over-end. The reactions were quenched with hydroxylamine (10 μL of 1.5 M at pH 7.2). The biotinylated reagents were aliquoted and stored at -80 degrees for up to six months.

**Ovalbumin and Human Serum Albumin Preparation**

Ovalbumin (Sigma, A5503) and human serum albumin (XXXX) were dissolved at 5 mg/mL in PBS. Sulfo-NHS-LC-biotin (Thermo Fisher, PI21335) was dissolved in distilled water at ~11.5 mg/mL. For biotinylation, the stock solution of Sulfo-NHS-LC-biotin (590 μL) was added ovalbumin (5 mL) and human serum albumin (5 mL) solutions and incubated for 30 minutes at room temperature, rotating end-over-end. The reactions were quenched with hydroxylamine (10 μL of 1.5 M at pH 7.2). The biotinylated reagents were aliquoted and stored at -80 degrees for up to six months.

**Soluble Antibody Production**

-Lina to write

**Specificity Assay**

Protein A magnetic beads (Invitrogen, 10002D) were washed three times and diluted to 54 ug/mL in PBSB (1 mg/mL BSA). Beads (30 μL) were incubated with antibodies (85 μL) overnight. The coated beads were washed twice by centrifugation (3500 xg, 4 minutes) and resuspension with PBSB. Reagents (0.1 mg/mL ovalbumin, 0.1 mg/mL human serum albumin, 0.1 mg/mL SMP, 0.1 mg/mL SCP) were incubated with the washed beads. Ovalbumin and human serum albumin were incubated for three hours at room temperature. SMP and SCP were incubated at 4°C for 20 minutes. The coated beads were washed once and incubated with 1/1000x streptavidin-647 (Invitrogen, S32357) and 1/1000x goat anti-human Fc F(ab’)2 AF-488 (Invitrogen, H10120) on ice for 4 minutes. Coated beads were washed once more, resuspended in PBSB, and analyzed via flow cytometry. Elotuzumab and Ixekizumab expressed on common IgG1 frameworks were analyzed in every experiment as controls.