Supplemental Appendix

Generation and characterization of mirikizumab, a humanized monoclonal antibody targeting the p19 subunit of IL-23

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METHODS

In Vitro Analysis of Human Fc Receptor and Complement Binding

CD16a, CD32a, and C1q

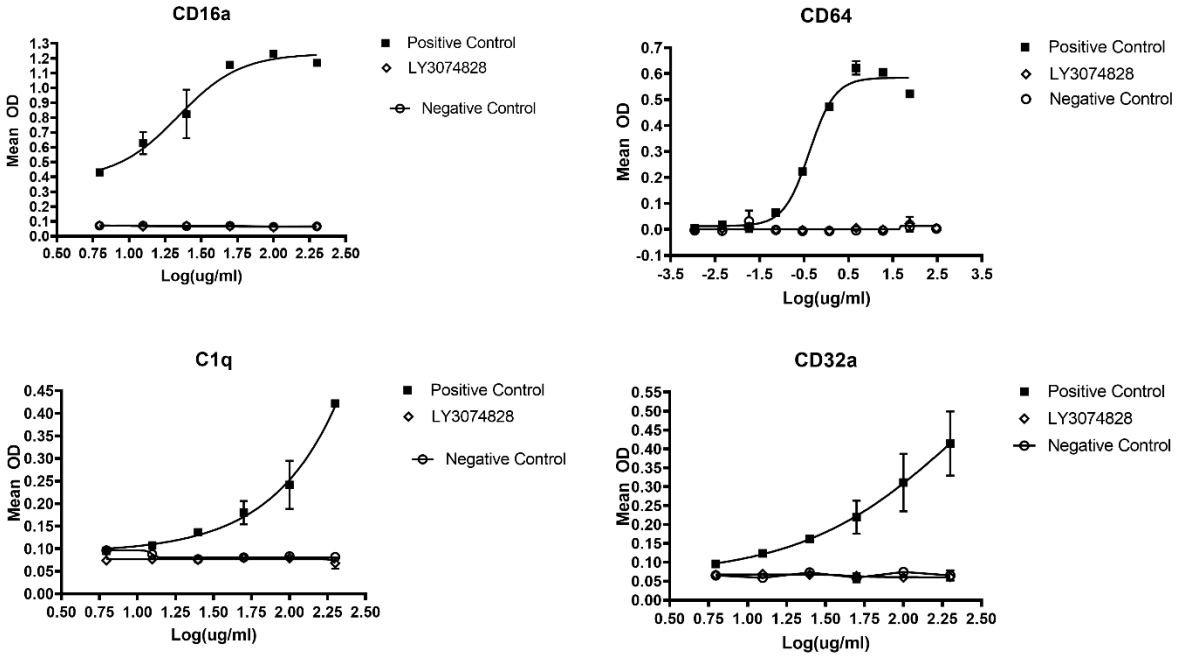
A 96-well microplate was coated with CD-32a with a C-terminal 10-His tag or recombinant human CD-16a with a C-terminal 6-His Tag (R&D Systems for both) at 1 µg/mL in Phosphate Buffered Saline (PBS), or Human C1q (Quidel) at 2 µg/mL in PBS. The plate was incubated overnight at 4°C, then the coating reagent was removed from each well, 200 µL/well of casein blocking reagent (Thermo) was added, and the plate was incubated for 1 hour at room temperature (RT). Each well was washed two times with wash buffer. Serial dilutions of LY3074828, human IgG1 positive control (LSN2436595; Lot No. 17670-16), or human IgG4 negative control (LSN2835015; Lot No. 16396-028) diluted in casein blocking reagent, were added to each well and incubated for 2 hours at RT. Antibodies were tested with a range of 6.25-200 µg/mL in two-fold serial dilutions). Testing was performed in duplicate wells. The plate was then washed three times with wash buffer before a 1:12,500 dilution of HRP-conjugated Goat Anti-Human IgG, F(ab')2 (Jackson ImmunoResearch Catalog No. 109-036-097) in casein blocker was added and incubated for 1 hour at RT. This polyclonal antibody recognizes both human IgG1 and IgG4 (data not shown). The plate was washed four times with wash buffer, and 100 µL/well of TMB Substrate (Pierce) was added, and incubated for 4.5 minutes for CD16a, 9 minutes for CD32a, or 30 minutes for C1q at RT, at which time 100 µL of 1.0 N HCl was added to each well. Optical density was measured using a colorimetric microplate reader set to 450 nm.

CD64

The assay was performed as described above. A 96-well microplate was coated with 100 µL/well of CD64 with a C-terminal 6-His Tag (R&D Systems) at 1 µg/mL in PBS. Antibodies were tested with a concentration range of 0.001 to 300 µg/mL in 4-fold serial dilutions. The plate was incubated with TMB Substrate for 4.5 minutes and developed as described above.

RESULTS

Supplemental Figure 1: Assessment of Fc Receptor Activation and Complement Binding



Abbreviations: IgG1= immunoglobulin G subclass 1; IgG4= immunoglobulin G subclass 4; SD= standard deviation; HRP= horseradish peroxidase

Note: Data are mean±SD of duplicate wells.