Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2024

Supporting Information for

Bacterial pseudaminic acid binding to Siglec-10 induces macrophage interleukin-10 response and suppresses phagocytosis

I-Ming Lee[1], Hsing-Yu Wu[2], Takashi Angata[2]*, Shih-Hsiung Wu[2]*
[1]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, No.70 Lien-hai Road, Kaohsiung 804201,Taiwan.
[2]Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan

Contents

Experimental material and methods

Supplementary Figures

Figure S1. Raw data of Siglec-10 knock-down THP-1 cells evaluated by western blot…………………………………………………………………………………...S5
Figure S2. Different concentrations of *Ab*-54149 EPS stimulate THP-1 macrophage and Siglec-10 knock-down macrophage to release IL-10……………………………S6
Figure S3. The ΦAB6TSP digested oligosaccharide can induce macrophage THP-1 to release IL-10………………………………………………………….............S7
Figure S4. IL-10 release from WT and and Siglec-10 knockdown THP-1 macrophages incubated with the Pse-coated bacteria and non-Pse coated bacteria…S8
Figure S5. The Pse/Sia glycans unit used for macrophage THP-1 stimulation and modeled Siglec-10 ligand docking…………………………………………………..S9
Figure S6. IL-10 release from WT and Siglec-10 knockdown THP-1 macrophages incubated with the ΦAB6TSP digested product and Sia containing glycans L1~L4...S10

Figure S7. Sequence alignments and structural comparison of Siglec-5,7,8 and 10…………………………………………………………………………………S11
Figure S8. Ligand L1~L4 docking with modeled Siglec-10……………………….S12
Figure S9. Phagocytosis of THP-1 toward *E.coli* BL21 and *Pseudomonas aeruginosa* PA14…………………………………………………………………………...S13
Figure S10. Phagocytosis of *Ab*-54149 EPS treated WT and Siglec-10 knockdown (#666) THP-1 toward *E. coli* BL-21 and *P. aeruginosa* PA14……………………..S14

References………………………………………………………………………S15

Experimental material and methods

Knockdown Siglec-10 on macrophage THP-1 by shRNA

The shRNAs against siglec10 and the pLAS.Void control shRNA were purchased from the National RNAi Core Facility (Academia Sinica, Taipei, Taiwan). The shRNA lentivirus particles were prepared by co-transfection of packing plasmid SPAX2, enveloping plasmid MD2G and shSiglec-10 plasmid into HEK293T cells. The DMEM medium was refreshed after 8 hours post-transfection. Next, we collected the virus particle in the medium after 72 hours and infected the THP-1 cells with adding the polybrene to facilitate the virus infection. Stable clones were selected by using puromycin and the knockdown efficiency was confirmed by western blot.

Bacteria exopolysaccharide extraction

The crude extracts of bacterial surface polysaccharides were obtained based on the protocol reported by Zamze et al.[1] with several modifications. In short, *A. baumannii* strain 54149 cells were cultured with LB medium at 37 °C for 15 h and the cultured cells were collected. The cells were suspended in d.d. water and heated to 100 °C for 20 min to lyse the cells. The cell lysate was clarified by centrifugation at 10,000 rpm for 20 min, and the supernatant containing the bacterial surface polysaccharides was incubated with 80% acetone overnight to precipitate the polysaccharides. The precipitate dissolved in 10 mM Tris-HCl and 1 mM $CaCl_2$, pH 7.5, was treated with ribonuclease (Sigma) and deoxyribonuclease I (Roche) at 37 °C for 6 h and then treated with proteinase K (Bioshop) for 12 hr. Subsequently, the sample was dialyzed against d.d. water by using a 1 kDa-cutoff membrane and then lyophilized. Finally, the crude polysaccharide extracts were further purified by a HW-65F gel-permeation column (TSK-GEL) to remove the contamination of bacterial organisms. The presence and concentration of the extracted polysaccharides were determined by the phenol-sulfuric acid method.[2]

Exopolysaccharide digested by ΦAB6TSP

Twenty mg crude extract of *Ab*-54149 surface polysaccharide dissolved in 25 mM Tris-HCl and 100 mM NaCl, pH 7.5 were incubated with 500 μg of purified ΦAB6 TSP at 37 °C for 6 h; then, the digestion reaction was terminated by heating to 100 °C for 15 min. The denatured proteins were removed by centrifugation. Subsequently, the digested products were loaded onto a P-6 column (Bio-Rad) and the oligosaccharides were eluted with d.d. water. The eluted fractions were pooled and lyophilized.

Mass spectrometry

All mass spectrometry in this study were executed by ESI-MS and ESI-MS-MS. ESI-MS and ESI-MS-MS analyses were done on a LTQ Orbitrap XL ETD mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with standard ESI ion source. 5 μL of sample was injected at a flow rate of 50 μL/min in 80% ACN/H_2O with

0.1% FA by Ultimate 3000 RSLC system from Dionex (Dionex Corporation, Sunnyvale, CA). The conditions for full-scan MS are as follows: mass range m/z 0-6000 and resolution 60,000 at m/z 400. The target ions were sequentially isolated for MS2 by LTQ. Electrospray voltage was maintained at 4 kV and capillary temperature was set at 275 °C. [3]

IL-10 assay

The human macrophage cell line THP-1 was obtained from American Type Culture Collection (Rockville, MD). THP-1cells were cultured in RPMI-1640 medium with 10% FBS. All cells were maintained at 37 °C under a humidified atmosphere of 5% CO_2 in an incubator. Cells (1×10^5/well) were seeded in 24-well plates for 24 hours and then added the 200 nM PMA dissolved in DMSO in order to differentiation for 24 hours. After refresh the medium, THP-1 was treated with various concentration of Pse/Sia derived glycans generated by chemical synthesis[4] or the pathogenic bacteria like *A. baumanni* and *E. cloacae*. The medium was then collected and used to measure the IL-10 concentration by commercial ELISA kit according to the manufacturer instruction (Thermo Fisher Scientific, Rockford, IL)

Fluorescence titration for binding affinity

Steady-state fluorescence spectra have been acquired on Fluorolog-3 (Jobin Yvon) Emission were recorded in the emission range of 290–500 nm upon excitation at 280 nm. The slit widths were fixed at 4 nm for the excitation and 10 nm for the emission wavelength. A quartz cuvette with a path length of 1 cm and a chamber volume of 1 mL was used for detection. A 0.7 mL volume of protein solution at fixed concentration of 0.3 μM was titrated by adding small aliquots (2–70 μL of a ligand stock solution of 500 μM) of ΦAB6TSP digested product and Sia-(1,8)-α-Sia. The PBS buffer at pH 7.4 was used for all solutions. The binding curve was obtained by plotting F/F0 values versus ligand concentration. Data analysis were performed by Graphpad Prism 6.

Siglec-10 expression and purification

A recombinant protein consisting of the two N-terminal immunoglobulin-like domains of Siglec-10 and the Fc region of human immunoglobulin Fc region was prepared, as described in a previous publication (DOI: 10.1007/978-1-0716-0430-4_9)

Ligand docking

The whole structure of Siglec-10 was depicted by computational 3D structure calculation by homology modeling. Siglec-10 is belonged to CD-33 family and therefore we used the structure of CD-33 family members, including Siglec-5, 7 and 8, as the modeling template. The modeling structure of Siglec-10 was obtained by SWISS-MODEL and AlphaFold. The room-mean-square deviation (RMSD) of the structures from these two software is about 0.3Å. The RMSD of carbohydrate binding site of modeled Siglec-10 and the structure of Sigle-5, 7 and 8 is less than 0.8 Å, so we believed that the modeled structure of Siglec-10 is reliable for ligand docking. Ligands (*Ab*-OU

and L1~L5) were generated by ChemOffice chem 3D and minimized their energy with the MM2 parameter. The carbohydrate binding site was selected and gridded by *Autogrid 4.0*. Next, the Ligands were docked against the modeled Siglec-10 grid using the Lamarckian Genetic search algorithm (LGA) with the default parameters by *Autodock* in *PyRx*. The pose with the lowest energy was used for later molecular dynamic (MD) analysis by using NAMD. Briefly, the docked complex structure was solvated with the flexible TIP3P water system in the periodic box. CHARMM force field and psfgen in VMD were used for protein structure file (PSF) and topology parameter. Energy minimization was executed through NVT simulations with NAMD minimization algorithm. The structural representation was created by PyMOL.

Macrophage phagocytosis

Bacteria used for phagocytosis assay were incubated in Luria-Bertani (LB) broth medium at 37°C. Next, the bacteria were centrifuged and collected the pellet. Besides, we dissolved the FITC in DMSO at 5 mg/mL to prepare the FITC stock and prepared fresh for each experiment. The bacterial pellet was suspended with PBS and added the FITC/DMSO to the suspension with the final concentration 50 μg/mL. The sample was incubated at room temperature for 2 hours in the dark with gentle agitation. The FITC labeled bacteria (near 10^8 cfu) was treated with macrophage PMA-differentiated THP-1 for 2 hours. THP-1 was then washed by the PBS for three times and quenched the extracellular fluorescence by trypan blue. Eventually, THP-1 was lysed by lysed buffer and the fluorescence of phagocytosed FITC labeled bacteria was measured by Infinite M1000 Pro (Tecan Group Ltd).

Figures

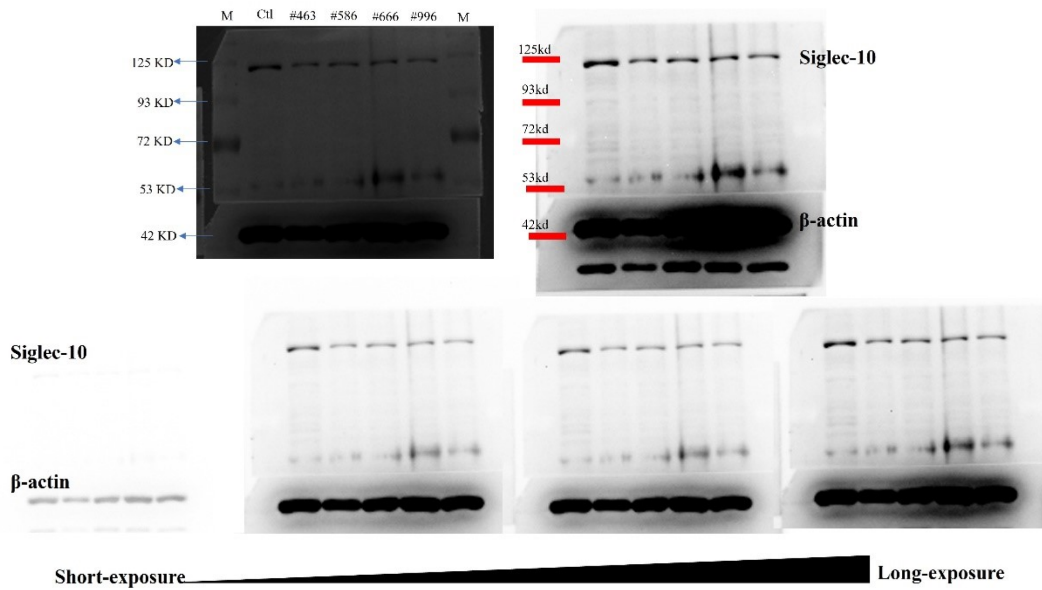

Figure S1. Raw data of Siglec-10 knock-down THP-1 cells evaluated by western blot. Siglec-10 on THP-1 cells was knocked-down by shRNA and observed by western blot with iBright FL 1000 Imaging System. Top left panel : a bright-field image showing the positions of protein markers; top right panel : a composite of the two membranes, stained with anti-Siglec-10 (top) and anti-actin (bottom) antibodies, respectively; bottom panels : the chemiluminescent images of the membranes captured by short to long exposure (left to right). (M: protein marker, Ctl: wild type macrophage)

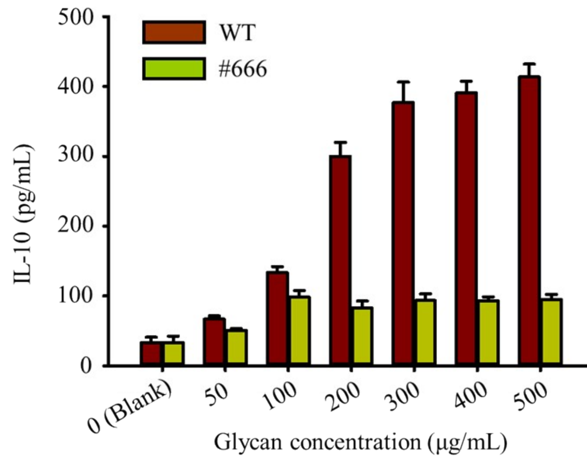

Figure S2. Different concentrations of *Ab*-54149 EPS stimulate THP-1 macrophage and Siglec-10 knock-down macrophage to release IL-10

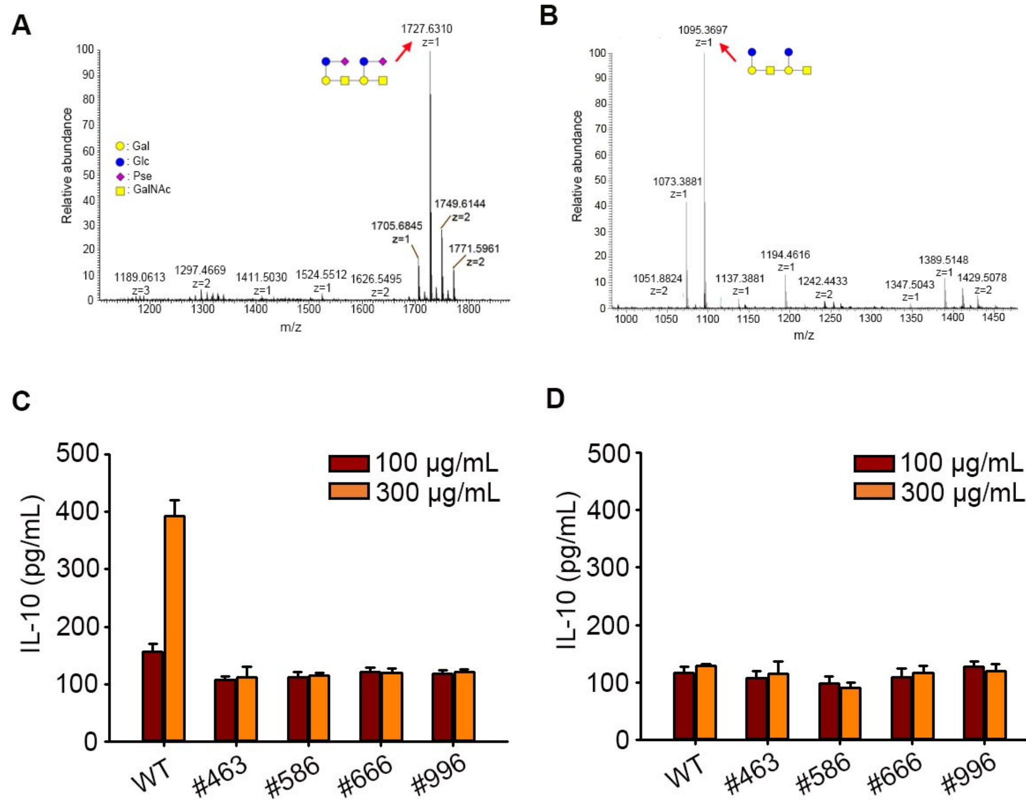

Figure S3. The ΦAB6TSP digested oligosaccharide can induce macrophage THP-1 to release IL-10 (A) Mass analysis of ΦAB6TSP digested product which is comprised of two repeat units of Ab-54149 exopolysaccharide. (B) Mass analysis of ΦAB6TSP digested product with acetic acid treatment. Two Pse on the ΦAB6TSP digested product were removed. (C) THP-1 in WT and Siglec-10 knockdown form (#463, #586, #666, #996) were treated with ΦAB6TSP digested product. (D) THP-1 in WT and Siglec-10 knockdown form (#463, #586, #666, #996) were treated with Pse-removed ΦAB6TSP digested product

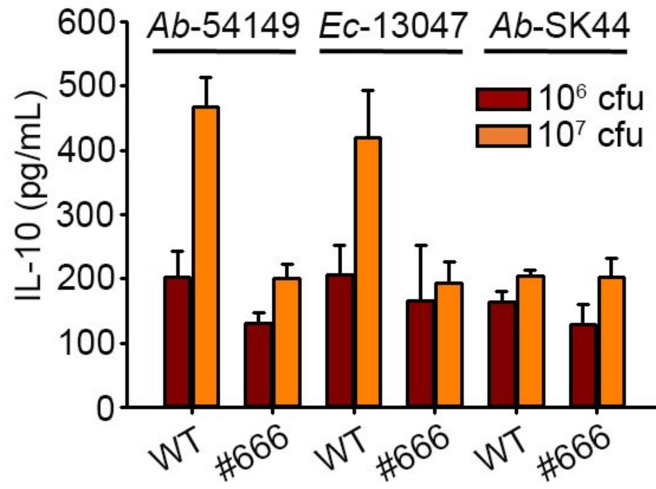

Figure S4. IL-10 release from WT and and Siglec-10 knockdown THP-1 macrophages incubated with the Pse-coated bacteria (*Ab*-54149 and *Ec*-13047) and non-Pse coated bacteria (*Ab*-SK44).

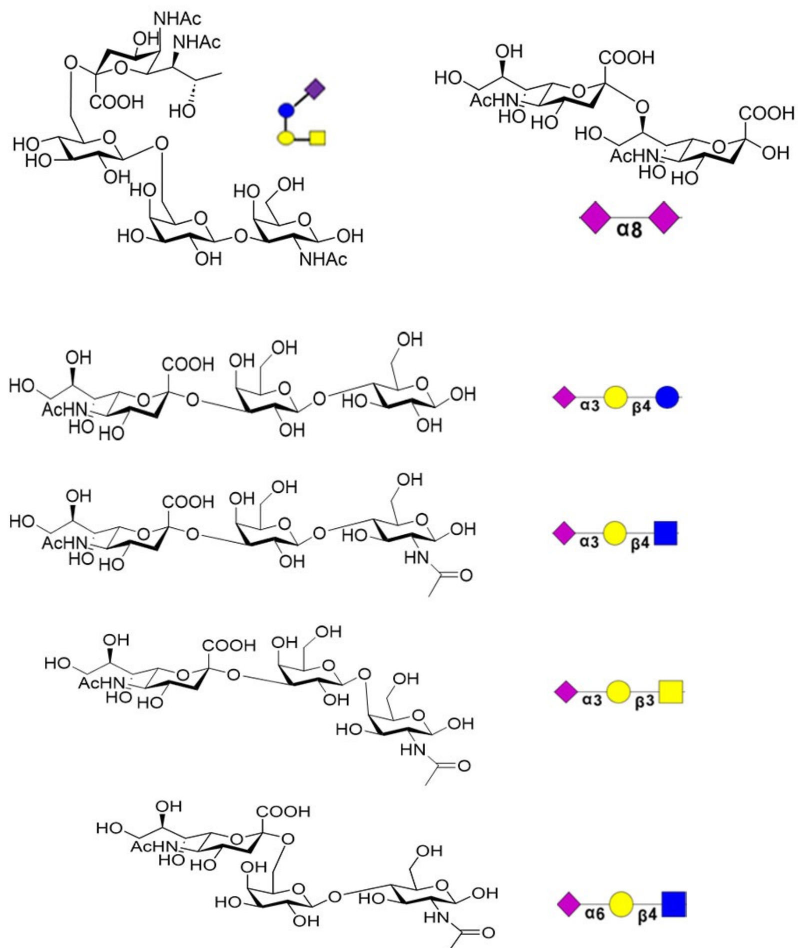

Figure S5. The Pse/Sia-containing glycans used for THP-1 macrophages stimulation and in silico Siglec-10 ligand docking.

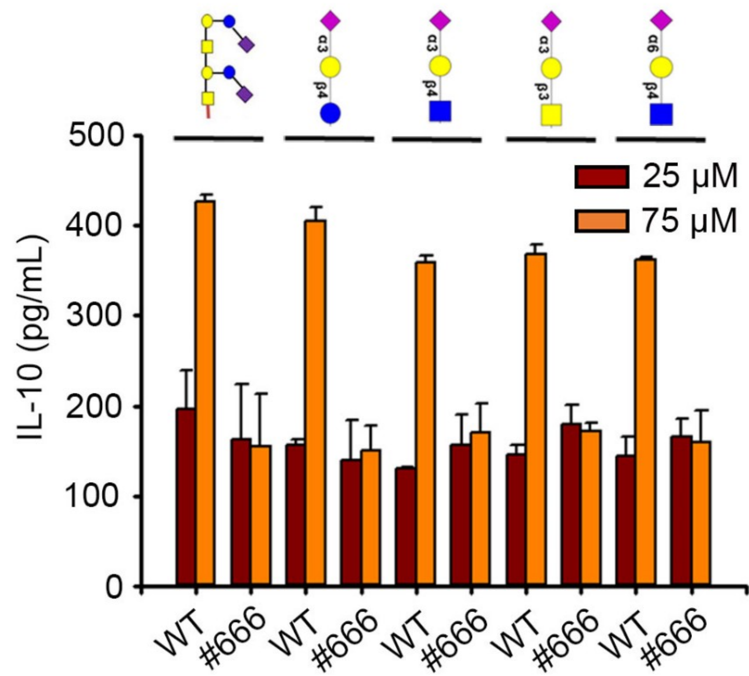

Figure S6. IL-10 release from WT and Siglec-10 knockdown THP-1 macrophages incubated with the ΦAB6TSP digested product and Sia containing glycans L1~L4.

Figure S7. Sequence alignments and structural comparison of Siglec-5,7,8 and 10. (A) Sequence alignment of Siglecs. (B) Structure alignment of Siglec-5,7,8 and modeled Siglec-10 (C) The β–sheets in the N-terminal of the modeled Siglec-10, which can be divided into A, A’, B, C, C’, D, E, F, G, G’ fragment. (D) Modeled Siglec-10 also contains the CC’ and GG’ loops associated with ligand binding. (E) The conserved residues on Siglec-5,7,8 and modeled Siglec-10 for the ligand binding.

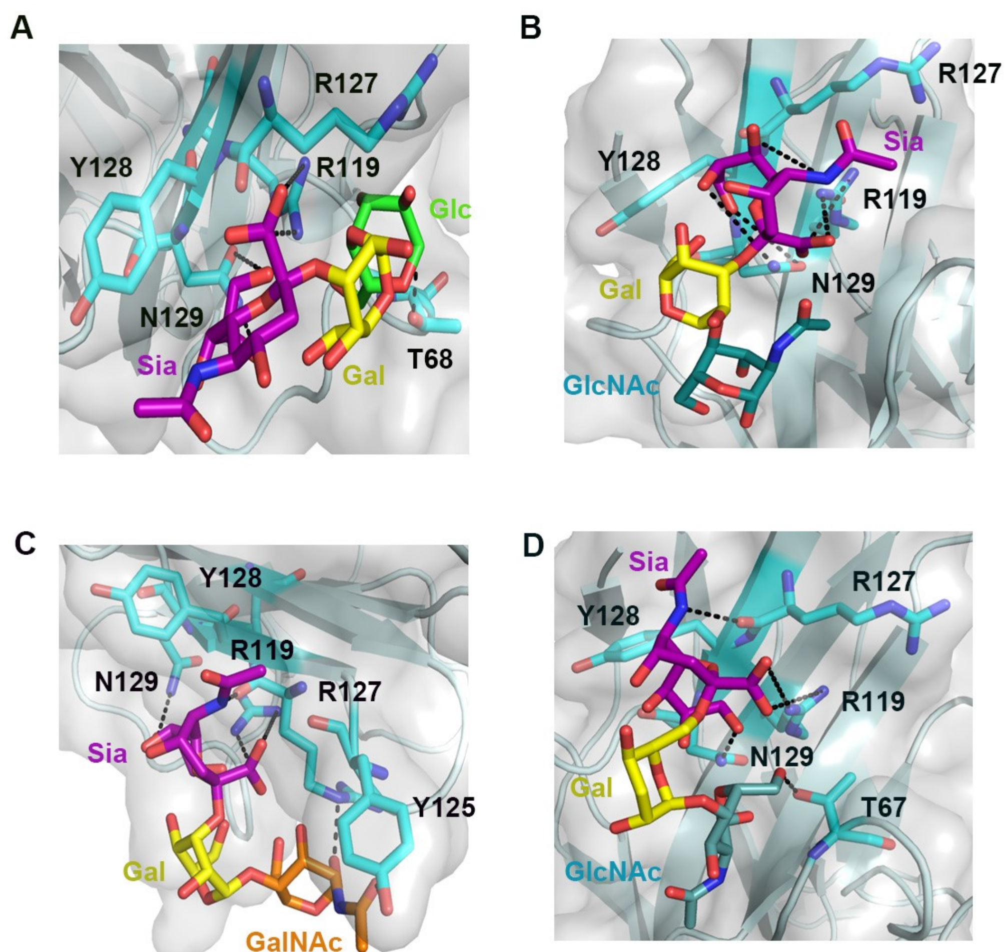

Figure S8. Ligand L1~L4 docking with modeled Siglec-10. Modeled Siglec-10 was docked with L1 (A), L2 (B), L3(C) and L4 (D). The residues on Siglec-10 were colored in cyan and the interactions between ligand and Siglec-10 were represented in black dash lines.

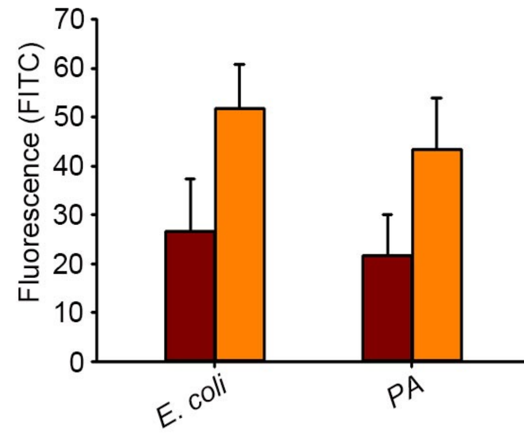

Figure S9. Phagocytosis of *E. coli* BL21 and *Pseudomonas aeruginosa* PA14 by THP-1 macrophages. The bars colored in brown and orange represent the bacteria concentration at 10^7 and 10^8 cfu, respectively.

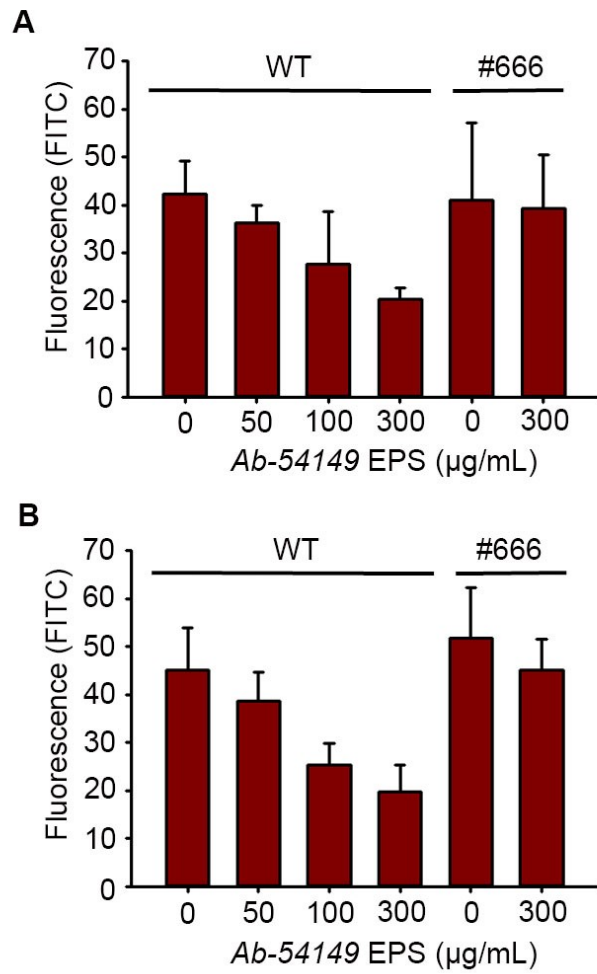

Figure S10. Phagocytosis of *Ab*-54149 EPS treated WT and Siglec-10 knockdown (#666) THP-1 toward (A) *E. coli* BL-21 and (B) *Pseudomonas aeruginosa* PA14 with concentration at 10^8 cfu.

References

1. Zamze, S.; Martinez-Pomares, L.; Jones, H.; Taylor, P. R.; Stillison, R. J.; Gordon, S.; Wong, S. Y. C. *J. Biol. Chem.* **2002,** 277, 41613.
2. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956,** 28, 350.
3. Medzihradszky K. F. *Methods in Enzymology* **2005,** 405, 50.
4. Liang C.H.; Hsu C. H.; Wu C. Y. *Top. Curr. Chem.* **2015,** 367, 125.