Supplemental Document 1

Full methodology of Siglec ligand pulldown and subsequent mass spectrometry

Siglec ligand pulldown for proteomics

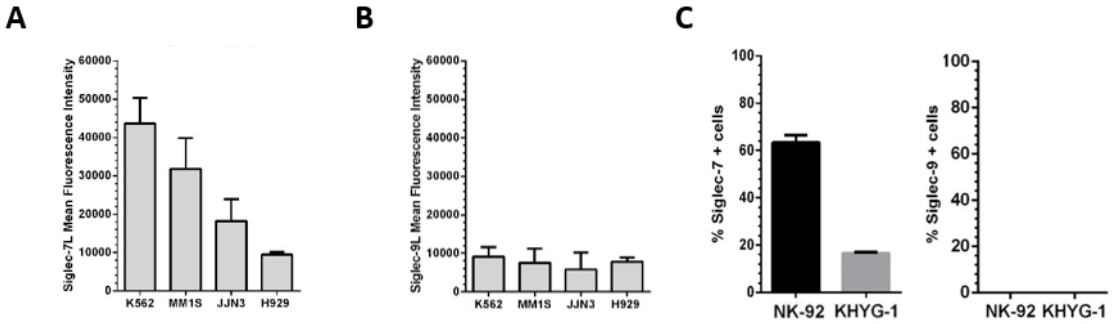
$10x10^6$ MM1S, JJN3 and H929 MM cells were lysed in ice cold 0.1% Nonidet P-40 containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 1× Halt Protease Inhibitor mixture (Thermo Fisher Scientific) and rotated at 4 °C for 1 h. The insoluble fraction was then cleared by centrifugation at 18,000 × g for 15 min. Protein concentration was determined by bicinchoninic acid (BCA) assay, and lysates were diluted further in lysis buffer to 500 µL with a final protein concentration of 1 µg/mL. Lysate was split into two 500-µL aliquots; one aliquot was left untreated, while the other was treated with 100 nM vibrio cholerae (VC)-ialidase for 1 h. In parallel, 5 µg Siglec-Fc was mixed with 50 µL Protein G Dynabeads in 250 µL PBS and rotated at room temperature for 1 h. Beads were isolated with a magnet and washed once with PBS. Treated and untreated cell lysates were then added to the beads and incubated overnight at 4 °C with rotation. Following pulldown, flow-through was removed and beads were washed twice with ice cold 0.1% Nonidet P-40 and twice with ice cold 50 mM ammonium bicarbonate. Trypsin (1 µg, Promega) and GluC (1 µg, Progema) were then added to beads in 100 µL 50 mM ammonium bicarbonate, and beads were incubated overnight with shaking at 37 °C. The following day, digestion was acidified by adding formic acid (FA) to beads to a final concentration of 2%, followed by shaking at 37 °C for 30 min. Supernatant was then removed; beads were washed once with 100 µL 2% FA, and this wash was pooled with the original supernatant. The digested sample was dried down in a speedvac and resuspended in 10 µL 0.1% FA.

Mass spectrometry of Siglec-7L pulldowns.

Peptides were separated over a 25 cm EasySpray reverse-phase LC column (75 μm inner diameter packed with 2 μm, 100 Å, PepMap C18 particles, Thermo Fisher Scientific). The mobile phases (A: water with 0.2% formic acid and B: acetonitrile with 0.2% formic acid) were driven and controlled by a Dionex Ultimate 3000 RPLC nano system (Thermo Fisher Scientific). An integrated loading pump was used to load peptides onto a trap column (Acclaim PepMap 100 C18, 5 um particles, 20 mm length, Thermo Fisher Scientific) at 5 μL/minute, which was put in line with the analytical column 5 minutes into the gradient. The gradient flowed isocratically from at 3% B for the first 6 minutes of the analysis, followed by an increase from 3% to 35% B from 6 to 93 minutes, an increase from 35% to 42% from 93 to 103 minutes, an increase from 35% to 95% B from 103 to 104 minutes, isocratic flow at 90% B from 104 to 109 minutes, a return to 3% B from 109 to 110 minutes, and a re-equilibration at 3% for 30 minutes for a total analysis time of 140 minutes. The eluate was ionized using an EASY-Spray ionization source (Thermo Fisher Scientific) held at +1.95 kV , the column was held at 40 °C, and the inlet capillary temperature was held at 275 °C. Survey scans of peptide precursors were collected in the Orbitrap from 300-1500 Th with an AGC target of 400,000, a maximum injection time of 50 ms, RF lens at 60%, and a resolution of 60,000 at 200 m/z. Monoisotopic precursor selection was enabled for peptide isotopic distributions, and precursors of z = 2-6 were selected for data-dependent MS/MS scans for 3 seconds of cycle time. Dynamic exclusion was set to exclude precursors after being selected three times for an exclusion time of 10 seconds with a ±10 ppm window set around the precursor monoisotope. An isolation window of 2 Th was used to select precursor ions with the quadrupole, and precursors were fragmented using a normalized HCD collision energy of 28. MS/MS scans were collected with an AGC target of 50,000 ions, with a maximum accumulation time of 54 ms and an Orbitrap resolution of 30,000 at 200 m/z. The same method was used for both untreated and sialidase treated

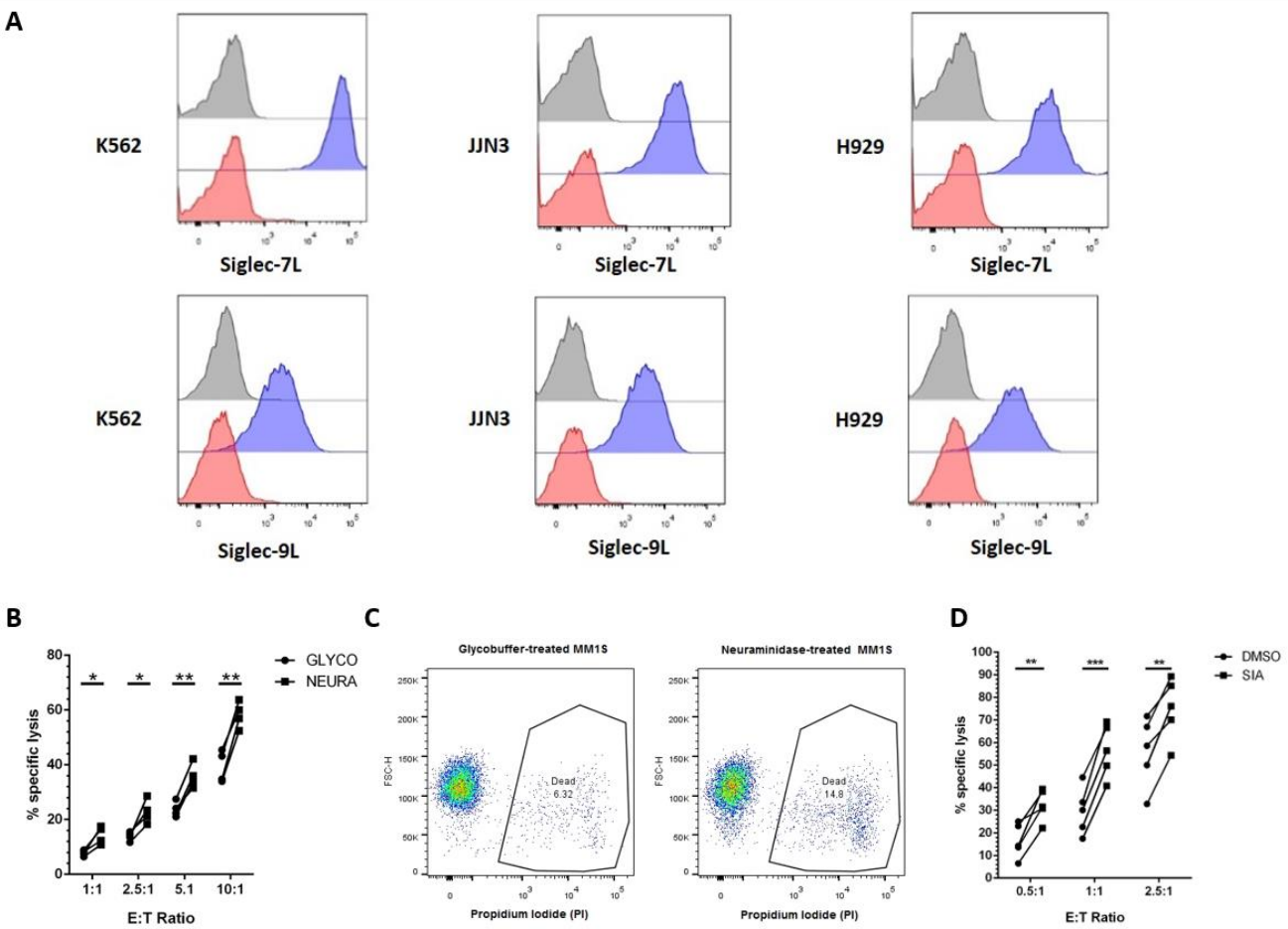
samples. Data was analyzed using MetaMorpheus, and label free quantification was performed using FlashLFQ algorithm built into MetaMorpheus (4, 5). Default settings were used for the search, including precursor and product ion mass tolerances of 5 and 20 ppm, respectively, and variable modifications of oxidized methionine and deamidated asparagine. Match between runs and normalization of quantified results were both enabled. Spectra were searched against a human protein database (reviewed entries only, 20,416 entries total) downloaded from Uniprot, and false discovery rate was controlled ($q < 0.01$) using a targetdecoy approach with a reversed decoy database (6). Label free intensity values were log2 transformed and plotted using OriginPro 2018 software.

Supplemental Figure 1



Supplemental Figure 1. Expression of Siglec-7L, Siglec-9L and their cognate receptors varies across MM and NK cell lines, respectively. (A, B) Determined by Siglec-7 and Siglec-9 Fc chimera staining, MFI of Siglec-7L^{+} and Siglec-9L^{+} tumor cells was determined (**A**, **B**, respectively). **(C)** NK cell lines NK-92 and KHYG-1 were stained with anti-Siglec-7 and anti-Siglec-9 antibodies to determine Siglec-7 and Siglec-9 expression. Data in **A**, **B**, **C** represents mean + standard deviation, n=3 independent biological repeats for all.

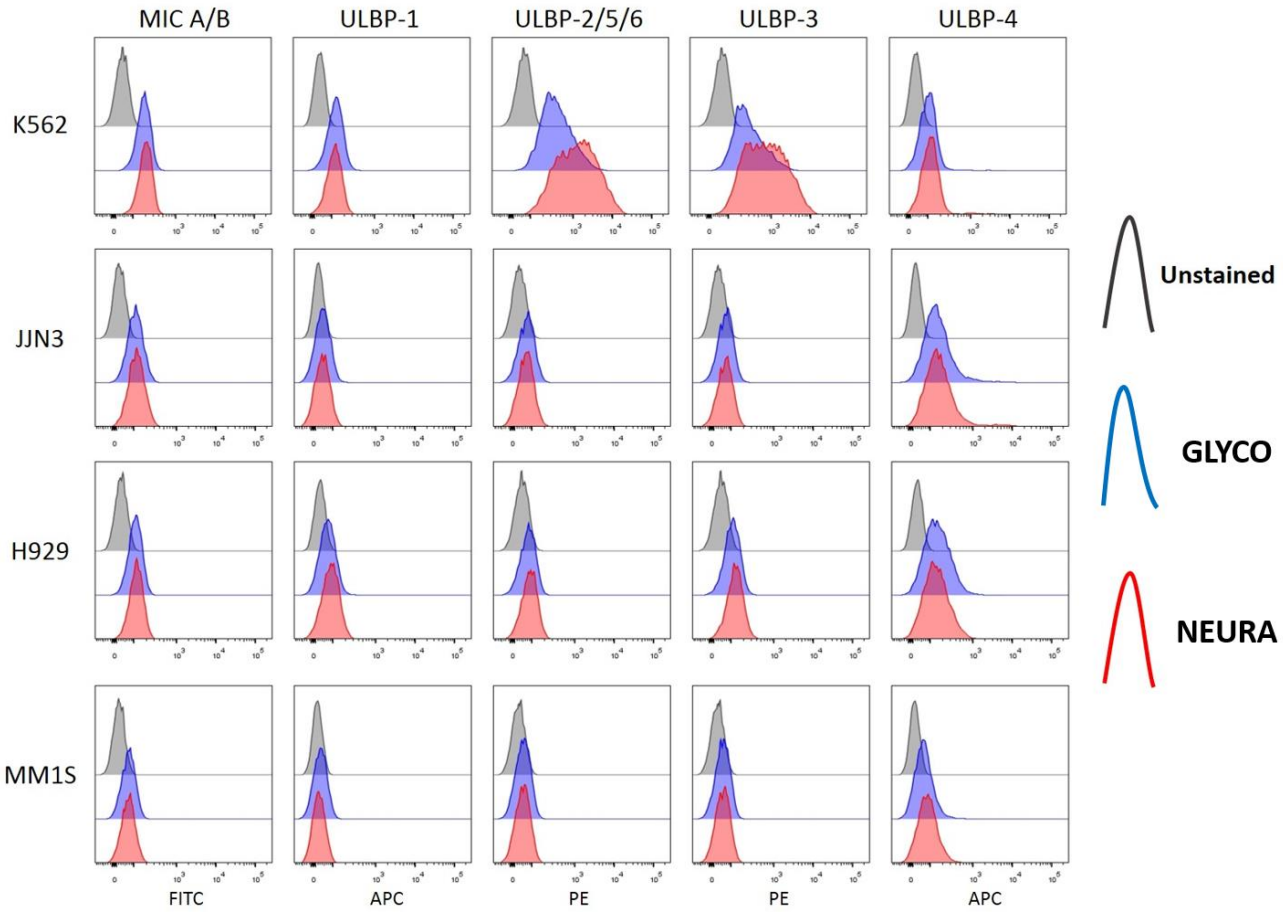
Supplemental Figure 2.



Supplemental Figure 2. Neuraminidase is an effective, but sometimes toxic, method of cell surface desialylation while 3Fax-Peractyl Neu5Ac represents a more tolerable means of MM cell desialylation. (A) Siglec-7L and Siglec-9L expression were measured on K562, H929 and JJN3 cell lines after treatment with GLYCO (blue) or NEURA (red). **(B)** JJN3 MM cells were desialylated using NEURA and co-cultured with KHYG-1 NK cells at various indicated E:T ratios. **(C)** MM1S cells were stained with Propidium iodide (PI) after being treated with GLYCO or NEURA to determine toxicity of NEURA treatment. **(D)** MM1S were treated with 300µM SIA for 72 hours before being co-cultured with expanded primary NK cells at indicated E:T ratios. Data in **B** and **D** were analysed using Student's unpaired t-test (**B**)

or paired t-test (**D**), respectively. **A** is a single representative repeat of a total of n=3 independent biological repeats. **B** and **D** represent exact increases in cytotoxicity for each repeat (**B**) or individual donor (**D**), (**C**) represents one individual repeat in dot blot form of n=5. n=4 for **B** and n=5 for **D**. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Supplemental Figure 3



Supplemental Figure 3. Expression of NKG2D ligands MIC A/B and ULBP-1-6 is not increased after neuraminidase-mediated desialylation, but ULBP-2/5/6 and ULBP-3 expression is increased on the erythroleukemia cell line K562. MM cell lines JJN3, H929 and MM1S and the erythroleukemia cell line K562 were treated with NEURA or a GLYCO control, prior to being screened for MIC A/B and ULBP-1-6 expression using flow cytometry. Figure represents n=1 biological repeat. Assay carried out n=3 biological independent repeats.