Supplementary Information for:

Genome-Wide CRISPR Screens Reveal a Specific Ligand for the Glycan-Binding Immune Checkpoint Receptor Siglec-7

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Supplementary Materials & Methods

PBMC Isolation PBMCs were isolated from Leukoreduction System Chambers obtained from healthy donors at the Stanford Blood Bank. Samples were deidentified prior to isolation. Approximately 7 mL of patient blood was diluted in 60 mL of PBS and layered onto 15 mL Ficol-Paque density gradients (GE Healthcare). Cells were spun 600 *x g* for 30 minutes and PBMCs were carefully isolated from the Ficol/PBS interface. Cells were washed twice with 30 mL of PBS and frozen in heat inactivated FBS containing 10% DMSO.

Immune Cell Isolation PBMC aliquots were quickly thawed and diluted in 10 mL of RPMI containing DNase to break up cell aggregates. Cells were incubated at 5% CO_2, 37 °C for 30 minutes and subsequently counted in duplicate. Cells were then spun down at 600 *x g* and resuspended in RPMI to a final cell concentration of $50x10^6$ cells/mL. Isolation of NK cells was then performed according to manufacturer's instructions using an NK cell magnetic isolation kit (StemCell Technologies). NK cells were then cultured for at least 24 hours before conducting experiments. For killing experiments, NK cells were cultured for 24 hours in RPMI containing 10% Heat Inactivated FBS with 2 μg/mL IL-2 (BioLegend). T cells and monocytes were isolated with the EasySep T cell or Monocyte isolation kits (StemCell Technologies).

Packaging of Genome-Wide CRISPRi Library The CRISPRi-v2 library (top 5 sgRNAs/gene) containing 104,535 sgRNAs was purchased from Addgene (Pooled Library 83969). For lentiviral packaging, HEK293T cells were plated onto six 150 mm dishes at a cell concentration of $7.5x10^6$ cells/dish in 30 mL of medium and allowed to adhere overnight. For every plate, 8 μg of sgRNA library plasmid was mixed with 8 μg total lentiviral packaging plasmids (2 μg pGag/Pol, 2 μg pREV, 2 μg pTAT and pVSVG) in 650 μL of Opti-MEM. 48 μL of LT1 (Mirus Bioscience) transfection reagent was separately diluted in 650 μL of Opti-MEM and allowed to sit at room

temperature for 5 minutes. The two solutions were then combined for a total of 1300 μL transfection complex and the mixture was allowed to incubate at room temperature for 30 minutes. The total volume of each transfection complex was then added to each 150 mm plate dropwise as the media on the plate was swirled constantly. Cells were then placed back in the incubator and virus production was allowed to proceed for 72 hours. Lentiviral media was then removed from each plate, centrifuged at 3000xg for 5 minutes to clear any cellular debris, then filtered through a 0.45 μm syringe filter. Lentiviral media was then snap frozen and stored at -80 °C until the day of library infection.

Lentiviral Transduction of Genome-Wide CRISPRi Library $250x10^6$ K562-dCas9-KRAB cells growing in log phase were spun down and resuspended in 500 mL of complete media containing 8 μg/mL polybrene and a volume of lentiviral media previously determined to give a multiplicity of infection (MOI) of 0.35. MOI was quantitated in a smaller scale experiment by determining the titer of lentiviral media that produced 35% cell viability following infection and treatment with 1 μg/mL of puromycin for 48 hours. Cells were then aliquoted in 10 150 mm plates (50 mL per plate). After 24 hours, cells were pelleted at 600 *x g* and resuspended in media containing 1 μg/mL of puromycin at $0.5x10^6$ cells/mL. Following 48 hours of puromycin selection, cells were spun down and resuspended again in fresh media containing 1 μg/mL of puromycin, maintaining a cell density of $0.5x10^6$ cells/mL. After 96 hours of selection, viability of the infected cell population was greater than 95%, while an uninfected control plate similarly treated with puromycin had cellular viability less than 1%. After this point, cells were spun down and resuspended in fresh media without puromycin and expanded. Cell staining and sorting in all cases were performed within 5 days of removing cells from puromycin selection to avoid dropout of essential genes from extended maintenance of the library in culture.

Siglec Sorting of Genome-Wide CRISPRi Libraries Both Siglec-7 and Siglec-9 screens were performed in duplicate. At all points, all replicates were maintained in culture at a library coverage of 1000x (~$1x10^8$ cells/replicate). On the day of sorting, $1x10^8$ unsorted cells from each replicate were spun down, flash frozen and placed at -80 °C. For sorted samples, 1 μg/mL of Siglec-7 Fc or Siglec-9-Fc (R&D Biosystems,1138-SL &1139-SL) and 1 μg/mL of AlexaFluor488 goat anti-human IgG (Jackson ImmunoResearch, 109-545-003) were diluted in 10 mL of FACS buffer (PBS with 0.5% BSA) and incubated on ice for 1 hour to precomplex. From each CRISPRi library replicate, $1x10^8$ cells were then pelleted at 600 *x g,* washed once with FACS buffer and resuspended in precomplex solutions at 10^7 cells/mL. Cells were incubated on ice for 30 minutes and gently resuspended by pipette every 10 minutes. Following staining, cells were spun down at 600 *x g*, washed twice with FACS buffer and resuspended in FACS buffer containing 1 mM EDTA and 5 nM Sytox Red (Thermo Scientific). Cells were then passed through a cell strainer to remove any aggregates and then placed on the sorter arm, where they were kept at 4 °C and rotated frequently for the duration of the sort. Cell sorting was performed on a BD FACS Aria II. Intact, single cells were selected by gating on FSC/SSC and FSC-A/FSC-H parameters, and live cells were further selected by gating on negative Sytox Red staining. Finally, gates on Siglec staining were constructed such that cells were only sorted if they exhibited a fluorescence intensity value less than 10-fold lower than the average fluorescence value of the stained population. In practice, this usually corresponded to between 10 and 20% of the total stained population depending on the specific Siglec fluorescence distribution. Sorted cells were periodically pelleted and frozen at -80 °C to maximize cell yield and recovery. Sorting was performed until the whole input cell sample had been totally depleted. We were usually able to achieve a sort rate of 4000 cells/second on a 100-micron nozzle, meaning that the full population of $1x10^8$ cells could be sorted in approximately 8 hours. K562

cells were observed to be highly robust, maintaining consistent viability over this extended sort time. Replicates were performed in parallel on two adjacent sorters.

Library Amplification, Sequencing & Data Processing Genomic DNA from unsorted control samples was isolated using the QIAamp DNA Blood Maxi Kit (Qiagen) according to manufacturer's instructions. DNA from sorted samples was isolated using the Sigma GeneElute Genomic DNA Miniprep Kit according to manufacturer's instructions. Libraries were amplified via nested PCR and sequenced on an Illumina NextSeq. Following demultiplexing, FASTQ sequence alignment to library file was performed using MAGeCK (1). sgRNA counts from the two low-staining replicates were compared to sgRNA counts from two control replicates frozen immediately prior to the day of sorting. GO term analysis was performed using GOrilla (2).

CRISPR/Cas9 targeted knockout of CD43 in K562 cells The sgRNA sequence 5'GGCTCGCTAGTAGAGACCAA3' was cloned into the LentiCRISPR-v2 vector (Addgene 52961) using a previously described protocol (3). Briefly, two primers with the sequences 5'-CACCGGGCTCGCTAGTAGAGACCAA-3' and 5'AAACTTGGTCTCTACTAGCGAGCCC-3' were annealed and ligated into a LentiCRISPR-v2 vector that had been linearized by digestion with a BsmBI restriction enzyme. The recA deficient Stbl3 strain was used for all plasmid propagation steps. Lentivirus was prepared and used to infect K562s using the protocol described above. Following puromycin selection, cells were prorogated in culture for one week and assayed for cell-surface expression of CD43 using the MEM-59 antibody (Abcam, ab9088), where a bi-modal population was observed (corresponding to a mix of CD43 knockout and CD43 WT clones). The knockout population was isolated using a BD FACS Aria II using the gating strategy described above.

Generation of CD43-myc-FLAG cell lines The CD43-myc-FLAG construct was purchased in a lentiviral vector from OriGene (Cat # RC214120L3). Deletion constructs were designed and generated using the NEB Q5 Site-Directed Mutagenesis kit according to manufacturer's instructions. Lentivirus was prepared and used to infect K562s using the protocol already described. Puromycin selection was performed as already described. For complementation experiments, a CD43WT-myc-FLAG construct was designed so as to include multiple silent mutations at the site of annealing for the sgRNA used for CD43 knockout. Lentivirus was prepared and used to infect K562s using the protocol described above. Experiments were performed immediately following 48 h of infection.

Western Blotting Following Siglec Pulldowns Bound protein was eluted by boiling beads in 1x SDS-PAGE sample buffer with 25 mM DTT for 5 minutes prior to loading onto a 4-12% Tris-Glycine Gel. Western blotting was performed with an antibody that binds the intracellular domain of CD43 (Bethyl, A304-393A), an anti-Myc antibody (Cell Signaling, 9B11) or an anti-CD45 antibody (Abcam, ab10558). PNA staining was performed using 10 μg/mL PNA-biotin (Vector BioLabs, B-1075) and an IR800-Streptavidin (LiCor 926-32230). All incubations were performed at a 1:500 antibody dilution in PBS-T overnight at 4 °C. Imaging was performed on a LiCor Odyssey CLX.

NK Cell Killing Flow Cytometry Analysis Cells were directly analyzed by flow cytometry following 4 hours of incubation. Staining in the FSC and Far Red channels was used to gate on the target cell population. Sytox Green staining was used to quantitate target cell death under various conditions.

Mass Spectrometry Methods for Siglec 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 target-decoy approach with a reversed decoy database (6). Label free intensity values were log2 transformed and plotted using OriginPro 2018 software.

Mass Spectrometry Methods for CD43 Glyco-Site Mapping The instrument method used an MS1 resolution of 60,000 at FWHM 400 m/z, an AGC target of 3e5, and a mass range from 300 to 1,500 m/z. Dynamic exclusion was enabled with a repeat count of 3, repeat duration of 10 s, exclusion duration of 10 s. Only charge states 2-6 were selected for fragmentation. MS2s were generated at top speed for 3 s. HCD was performed on all selected precursor masses with the following parameters: isolation window of 2 m/z, 30% collision energy, orbitrap (resolution of 30,000) detection, and an AGC target of 1e4 ions. ETD was performed if (a) the precursor mass was between 300-1000 m/z and (b) 3 of 9 HexNAc or NeuAc fingerprint ions (126.055, 138.055, 144.07, 168.065, 186.076, 204.086, 274.092, and 292.103) were present at +/- 0.1 m/z and greater than 5% relative intensity. ETD parameters were as follows: calibrated charge-dependent ETD times, 2e5 reagent target, precursor AGC target 1e4. Data evaluation was performed with Byonic™ (Protein Metrics, Cupertino, USA), where raw files were searched against the CD43 sequence only (Uniprot ID P16150). Search parameters included semi-specific cleavage specificity at the C-terminal site of R, E, and K, with two missed cleavages allowed. Mass tolerance was set at 10 ppm for MS1s, 0.1 Da for HCD MS2s, and 0.35 Da for ETD MS2s. Methionine oxidation (common 2), asparagine deamidation (common 2), and N-term acetylation (rare 1) were set as variable modifications with a total common max of 3, rare max of 1. Peptide hits were filtered using a 1% FDR. O-glycans were also set as variable modifications (common 7), using a custom glycan database, where only HexNAc and HexNAc-Hex were searched. HCD was used to confirm that the peptides were glycosylated, and ETD

spectra were used for site-localization of glycosylation sites. All spectra with glycan modifications were manually annotated.

Microscopy Methods for Super-Resolution Cell-Surface Imaging. For single-molecule localization microscopy, the PBS was replaced by a reducing, oxygen scavenging buffer (7) consisting of 20 mM cysteamine, 2 mL/mL catalase, 560 mg/mL glucose oxidase (all Sigma-Aldrich), 10% (w/v) glucose (BD Difco, Franklin Lakes, NJ), and 100 mM Tris-HCl (Life Technologies). Diffraction-limited imaging was performed at low laser intensity of a few W/cm^2. Then, the laser intensity was increased to 5-8 kW/cm^2 and image acquisition was started after a short delay needed to convert the majority of the fluorophores into a dark state. Exposure time was 50 ms and the calibrated EM gain was 186. First, AF647 was imaged, followed by CF568 (otherwise, the 561 nm excitation would bleach the AF647 molecule). SR reconstructions were reconstructed from approximately 40000 frames for both colors using the ImageJ plugin Thunderstorm (8). Images were filtered with a B-spline filter of order 3 and scale 2.0. Single-molecule signals were detected as local maxima with 8-neighborhood connectivity. The threshold was three times the standard deviation of the first wavelet level. Local maxima were fitted with a 2D-Gaussian using least squares. Post-processing involved drift correction by cross-correlation, followed by filtering (sigma of the fitted Gaussian < 200 nm; uncertainty of localization < 20 nm) and merging of localizations that originated from emitters active in multiple frames (merging radius: 25 nm, max. five off-frames). Images were reconstructed as 2D histograms with a bin size of 32 nm, corresponding to a five-time magnification (camera pixel size 160 nm). The two-color channels were registered by imaging a dense layer of fluorescent beads visible in both channels, followed by affine transformation using Matlab's built-in algorithm imregtform. The determined transformation matrix was then applied to the respective reconstructed image. For cross-correlation analysis, we used the two-color super-resolution reconstructions of K562 cells co-expressing CD43 (labeled with CF568, "green channel") and

Siglec-7/-9 (labeled with AF647, “red channel”). The reconstructions (2D histograms of localizations with 32 nm bin size) of Siglec-7/-9 and CD43 were first blurred with a Gaussian filter (1 pixel radius). Then, identical line profiles with a width of 1.6 µm, covering the whole membrane region, were drawn in both color channels. The thus obtained intensity traces for Siglec-7/-9 and CD43 were stored. The cross-correlation between the red channel trace and the green channel trace were determined. The characteristic cross-correlation peak for low lags was indicative of cross-correlation of the two signals, the absence of this peak indicative of no cross-correlation.

Data Analysis Methods For Immune Synapse Imaging For image analysis, a changepoint finding algorithm was used to determine characteristic steps in the intensity profile, which were caused by different distributions of Siglec-7 across the NK cell membrane. Three scenarios were possible: (i) no step, indicating homogenous Siglec-7 distribution around the membrane; (ii) two steps, one at the beginning of the synapse and one at the end, with a higher intensity between the two steps, indicating accumulation of Siglec-7 signal within the synapse; and (iii) two steps, one at the beginning of the synapse and one at the end, with a lower intensity between the two steps, indicating depletion of Siglec-7 within the synapse. The "step size" was defined for the three scenarios as follows: (i) step size 0; (ii) and (iii) the difference between the mean intensity of the signal within the synapse minus the mean intensity of the signal outside the synapse, leading to positive step sizes for accumulation of Siglec-7 (case (ii)) and negative step sizes for depletion of Siglec-7 (case (iii)).

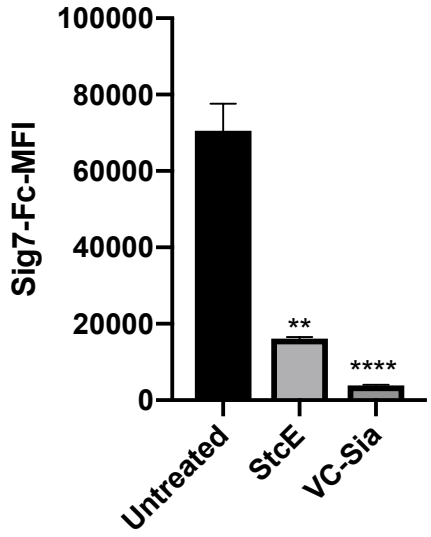
Glycomic Analysis of CD43 CD43-myc-FLAG was isolated as described above and diluted with SDS-PAGE sample buffer with 25 mM DTT and boiled for 5 minutes prior to loading onto a 4-12% Tris-Glycine Gel. Using a razor, a gel slice corresponding to the MW of CD43 (~125 kDa) was removed from the gel. The gel slice was suspended in 1 mL of 5% acetic acid and flash

frozen on dry ice for storage. The CD43 band was subsequently cut into small pieces of about 1 mm^2 and resuspended in 1:1 digestion buffer (50 mM Ammonium Bicarbonate) and 100% acetonitrile. Digestion buffer (50 µl) was added to the gel pieces and the protein was digested with 0.5 µg/µl sequencing-grade trypsin (Promega) at 37 ºC for 12 hours. As the CD43 sequence does not possess cysteines, reduction and alkylation was not necessary. The peptides were extracted with 5% formic acid in 1:2 water-acetonitrile, dried, and subsequently re-dissolved in 0.1% formic acid and stored at -30 ºC. For O-glycan analysis, O-glycans were β-eliminated by treatment of extracted tryptic peptides with NaOH/NaBH4 at 45 ºC for 16 hours. The samples were further neutralized by 10% acetic acid, passed through a Dowex H+ ion exchange column, and lyophilized. Borates were subsequently removed by the addition of 500 µl of methanol:acetic acid (9:1). The samples were dried under a nitrogen stream and the glycans were permethylated for structural characterization by mass spectrometry. Briefly, the sample was dissolved in dimethyl sulfoxide (DMSO) and incubated with methyl iodide in a DMSO/NaOH mixture. The reaction was quenched with nanopore H_2O and permethylated O-glycans were extracted with methylene chloride. The sample was dissolved in methanol and crystallized with α-dihydroxybenzoic acid (DHBA) matrix for MALDI analysis. ESI-MSn analysis of permethylated glycans was performed on an Orbitrap Fusion Tribrid mass spectrometer through a Nanospray Flex™ ion source. The MSn spectra (CID) of the glycans were acquired in the presence of sodium ions for the structure identification and sialic acid linkage determination. Assignment of glycan structures and linkages was done manually with the aid of GlycoWorkbench 1.1, and ChemDraw 18.0 software. All reagents were purchased from Sigma Aldrich unless otherwise mentioned.

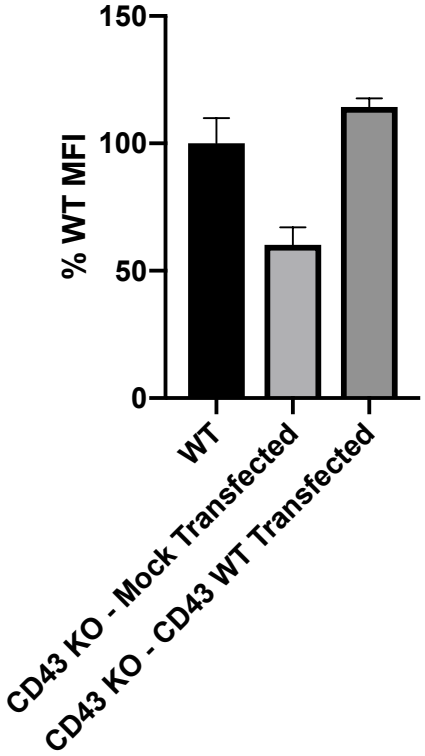
Generation of Fab Fragments 80 µg Mouse anti-MUC1 IgG1 (VU4H5 clone) and mouse anti-CD43 IgG1 (MEM-59 clone) were subjected to Fab digestion using the Pierce Mouse IgG1 Fab and F(ab')2 Preparation Kit (Thermo Scientific) according to manufacturer's instructions.

Following ficin digestion and sample cleanup on an Fc-binding Protein A column, successful Fab generation was confirmed by SDS-PAGE and Coomassie staining that showed a single band running at 50 kDa.

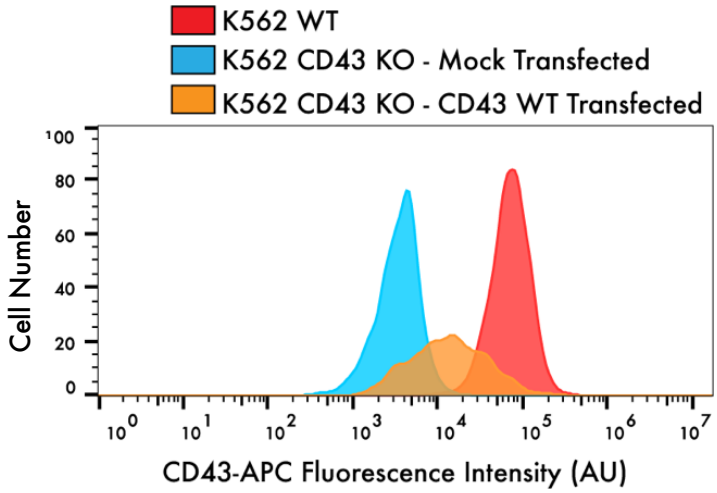
Supplementary Figures



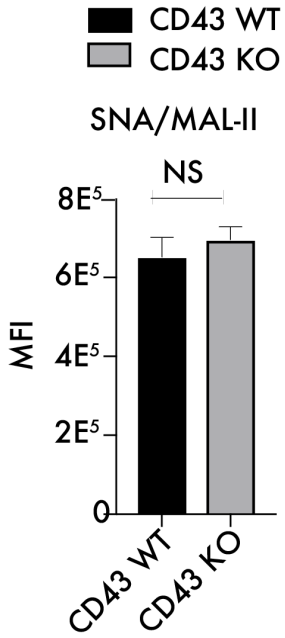
Supplementary Figure 1. K562 cells were treated with either 1 μg/mL of the O-glycoprotein-specific protease StcE or 100 nM of VC-Sialidase for 1 hour, incubated with Siglec-7-Fc precomplexed to an AlexaFluor488 anti-huIgG secondary antibody (100 ng/mL) and subjected to live cell flow cytometry. n=3, ** indicates $p < 0.01$, **** indicates $p < 0.0001$. MFI indicates mean fluorescence intensity.



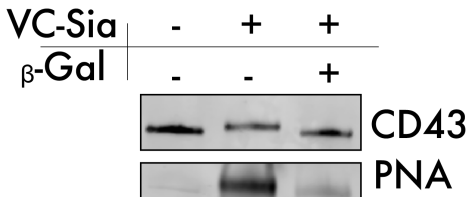
Supplementary Figure 2. K562 CD43 KO cells were either mock transfected or transfected with lentivirus encoding WT CD43. Cells were incubated with Siglec-7-Fc precomplexed to an AlexaFluor488 anti-huIgG secondary antibody (100 ng/mL) and subjected to live cell flow cytometry. Figure panel shows average of 3 replicates for Sig7-Fc staining, error bars indicate SEM. Values are normalized as a percentage of the mean fluorescence intensity (MFI) for Sig7-Fc staining of WT cells.



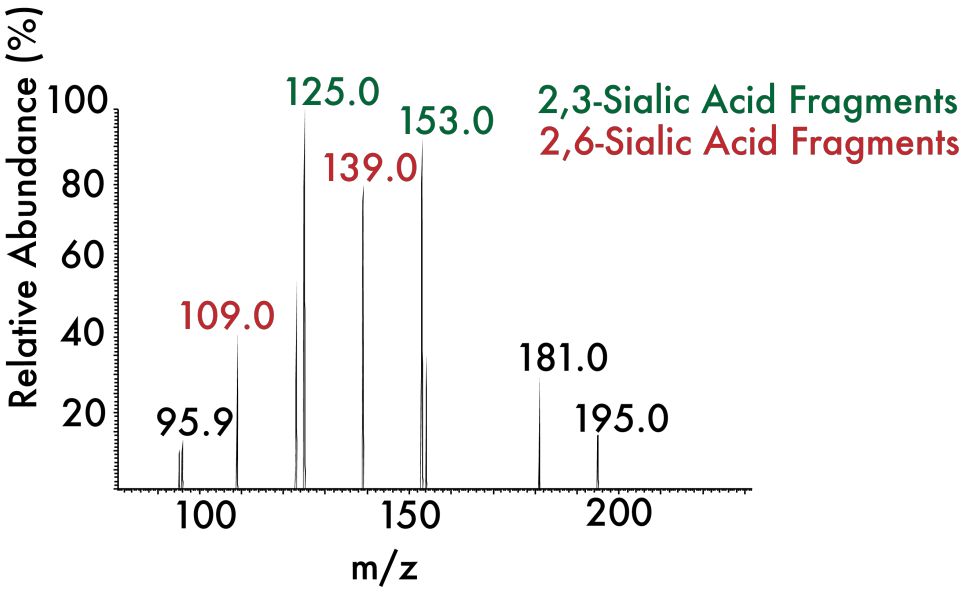
Supplementary Figure 3. K562 CD43 KO cells were either mock infected or infected with lentivirus encoding WT CD43. Cells were incubated with an APC-conjugated anti-CD43 antibody (L10 clone) and subjected to live cell flow cytometry. Representative histogram is shown.



Supplementary Figure 4. K562 CD43 KO cells were generated by CRISPR/Cas9 editing, stained with a mix of the plant lectins SNA-biotin and MAL-II-biotin precomplexed to AlexaFluor488 Streptavidin (1 μg/mL), and subjected to live cell flow cytometry. n=3, NS=not significant. MFI indicates mean fluorescence intensity (arbitrary units).



Supplementary Figure 5. K562 lysates were treated with VC-Sialidase and subjected to western blot with an antibody against the intracellular domain of CD43 and with the Core 1-binding lectin PNA. Lysates were treated with VC-Sialidase to expose the Core 1 epitope. B-Galactosidase treatment further degrades the Core 1 epitope to the GalNAc monosaccharide, eliminating PNA binding.



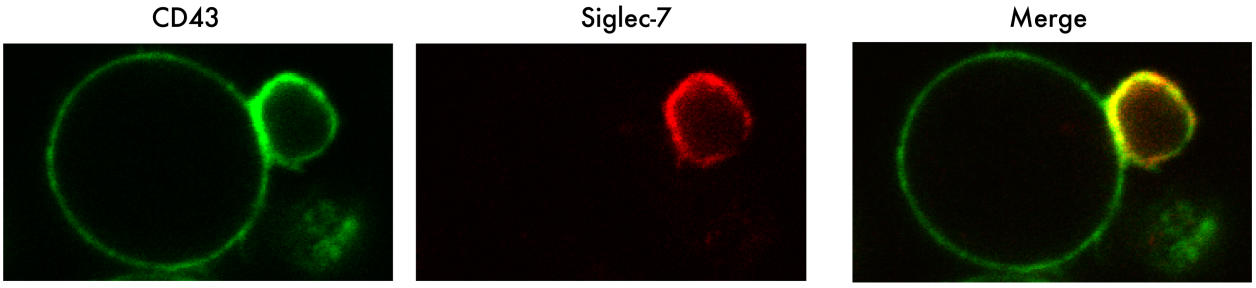
Supplementary Figure 6. MS/MS glycomic analysis was performed to identify linkage patterns of monosialylated glycan fragments attached to CD43.

Possible O-Glycosylation Sites
Some evidence of glycosylation
Definitive evidence of glycosylation

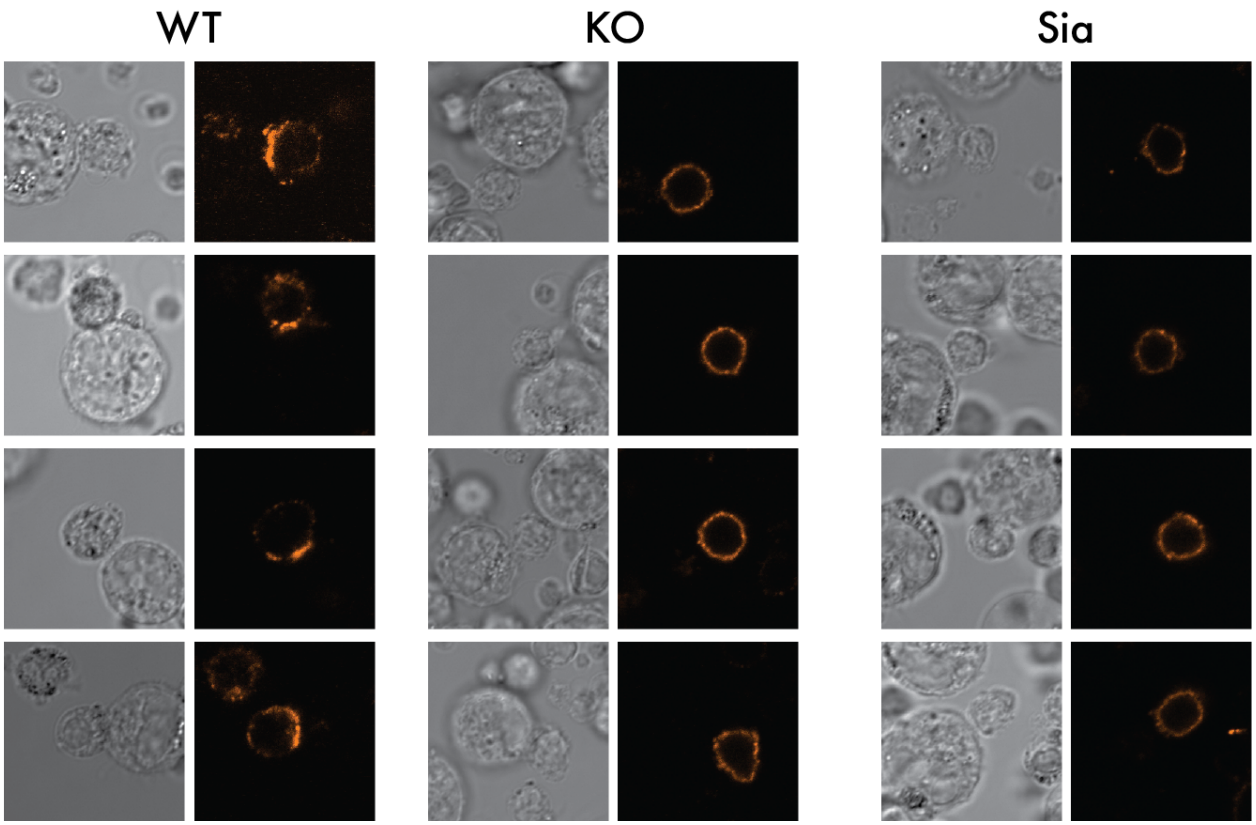
CD43 Extracellular Domain Sequence

STTAVQTPTSGEPLVSTSEPLSSKMYTTSITSDPKADSTGDQTSALPPSTSINEGSPLWTSIGASTGSPLPEPTTYQEV
SIKMSSVPQETPHATSHPAVPTANSLGSHTVTGGTITTNSPETSSRTSGAPVTTAASSLETSRGTSGPPLTMATVSLE
TSKGTSGPPVTMATDSLETSTGTTGPPVTMTTGSLEPSSGASGPQVSSVKLSTMMSPTTSTNASTVPFRNPDENSR

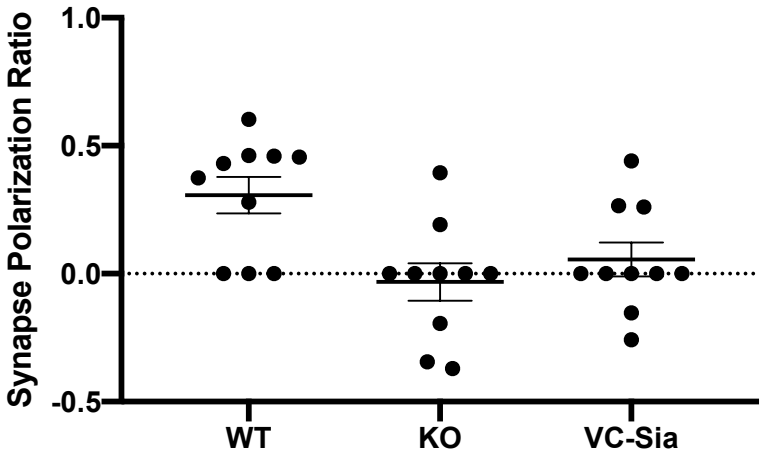
Supplementary Figure 7. Glycosylation sites identified in the N-terminal domain of CD43. Evidence for glycosylation was counted as “definitive” either when there was an ETD spectrum allowing specific site-localization of the glycan or there was an HCD spectrum corresponding to a fully glycosylated CD43 peptide (all possible sites occupied). “Some evidence” indicates that an HCD spectrum was obtained for a peptide from a given region of CD43 that contained GalNAc mass fingerprints, but the specific sites could not be fully localized (partial site occupancy).



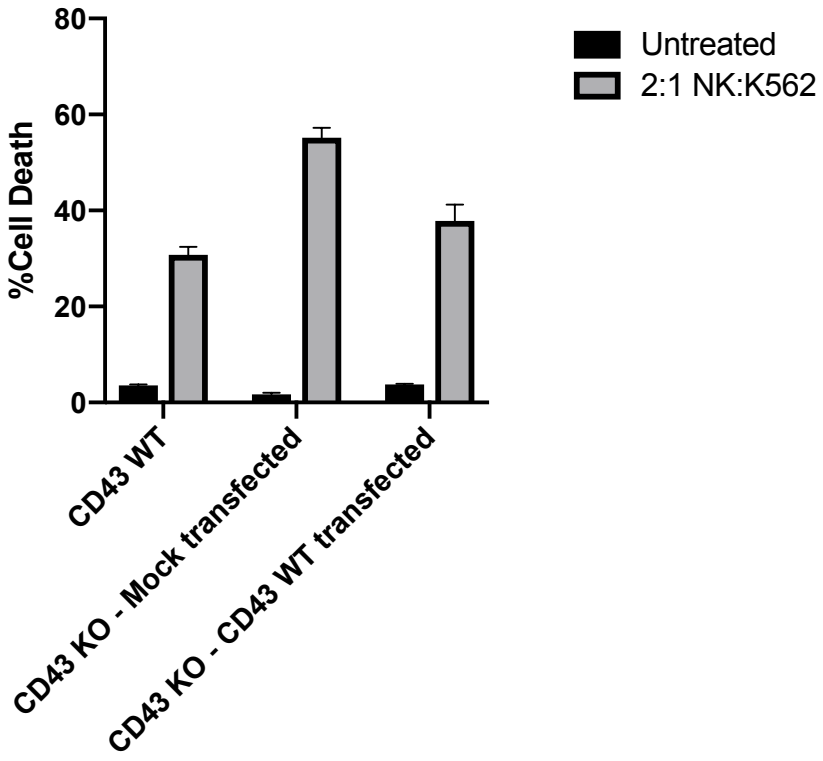
Supplementary Figure 8. A representative immune synapse formed between an NK cell and a K562 cell. Siglec-7 and CD43 both accumulate at the synapse and co-localize in this region (indicated by yellow regions in merged image). The large cell in this image is the K562 target cell, while the smaller cell is the NK cell. Scale of all images is 31.75 microns width and 19.35 microns height.



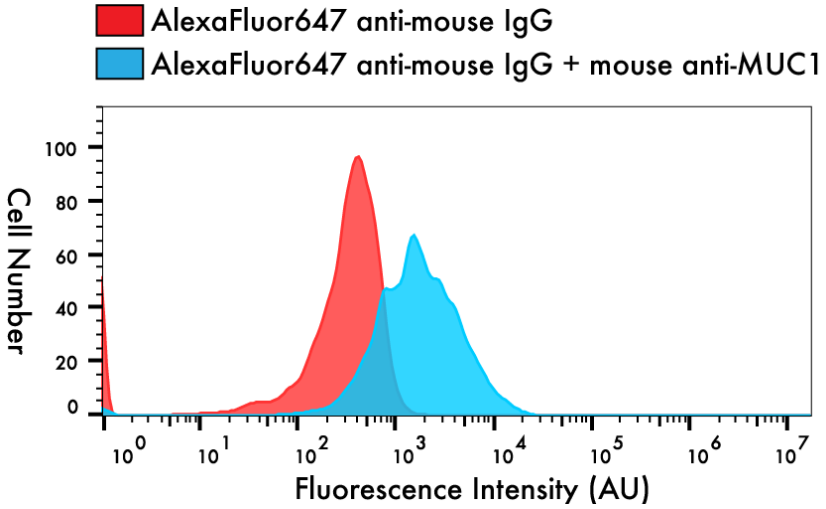
Supplementary Figure 9. Representative images of immune synapses formed between NK cells and WT, CD43 KO and VC-Sia-treated K562 cells. Cell-cell contacts shown in brightfield, Siglec-7 distribution around the NK cell membrane shown in orange. Scaling is same as shown in Fig. 5A.



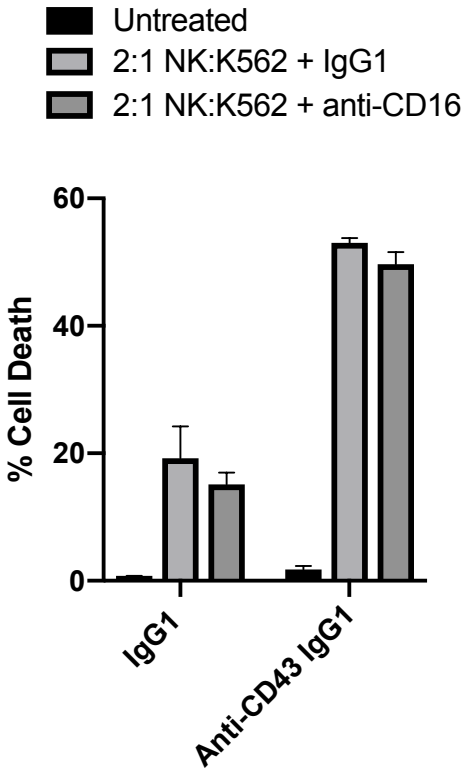
Supplementary Figure 10. Quantitation of Siglec-7 polarization to immune synapses formed between NK cells and WT, CD43 KO and VC-Sia-treated K562 cells in a single biological replicate. Each point represents a single cell. A positive ratio indicates a higher density of Siglec-7 at the synapse vs. outside the synapse. A negative ratio indicates a higher density of Siglec-7 outside the synapse vs. inside the synaptic area. Lines indicate mean, error bars indicate SEM.



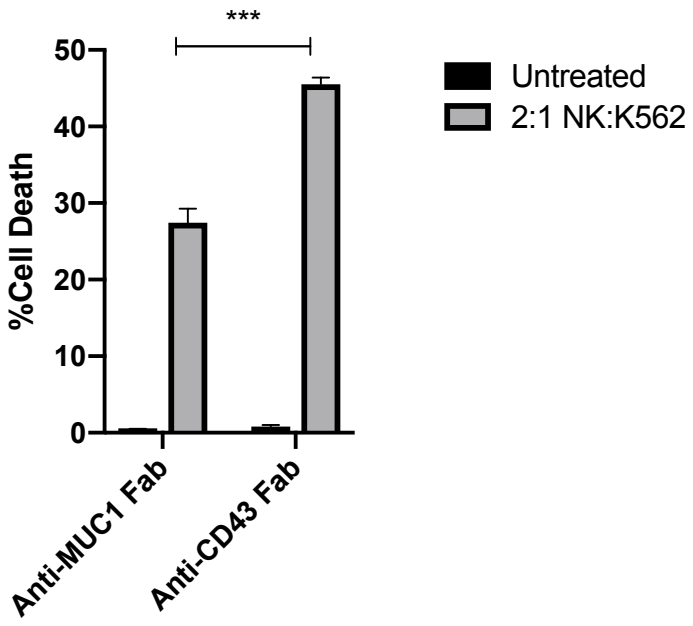
Supplementary Figure 11. Transfection of WT CD43 into CD43 KO cells reduces sensitivity to NK cell lysis. CD43 KO cells were lentivirally transduced for 48 hours with a construct encoding WT CD43, then co-cultured with primary NK cells, incubated at the indicated ratio for 4 h and subjected to flow cytometry using live cell (CellTracker Far Red) and dead cell (Sytox Green) stains to quantitate specific lysis of the target cells. The “Untreated” condition refers to target cells that have not been incubated with NK cells.



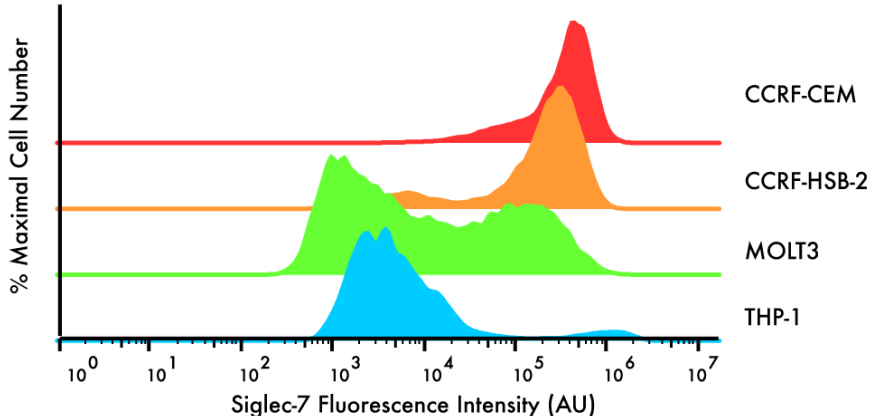
Supplementary Figure 12. K562 WT cells were incubated with 1 μg/mL anti-MUC1 IgG and a secondary Alexa647-conjugated anti-mouse antibody and subjected to live cell flow cytometry. Representative histogram is shown.



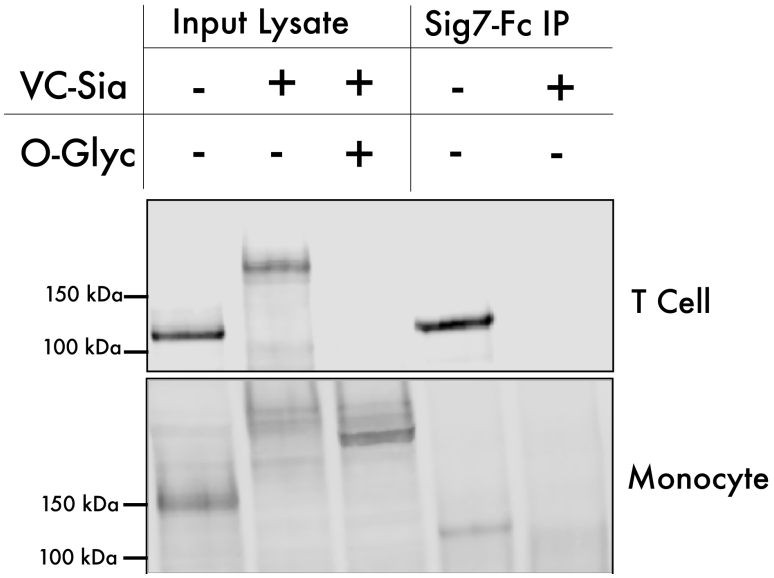
Supplementary Figure 13. K562 cells were pre-treated with intact mouse IgG1 isotype control or anti-CD43 antibodies (10 μg/mL). Cells were then co-cultured with primary NK cells that had been treated either with an isotype control IgG or with a CD16 blocking antibody at 10 μg/mL for 30 min, incubated at the indicated ratio for 4 h and subjected to flow cytometry using live cell (CellTracker Far Red) and dead cell (Sytox Green) stains to quantitate specific lysis of the target cells. The “Untreated” condition refers to target cells that have not been incubated with NK cells.



Supplementary Figure 14. K562 cells were pre-treated with 25 μg/mL Fab fragments targeting either MUC1 or CD43. Cells were then co-cultured with primary NK cells at the indicated ratio for 4 h and subjected to flow cytometry using live cell (CellTracker Far Red) and dead cell (Sytox Green) stains to quantitate specific lysis of the target cells. The “Untreated” condition refers to target cells that have not been incubated with NK cells. Error bars indicate SEM, *** indicates $p < 0.001$.



Supplementary Figure 15: Siglec-7 ligand profiling of leukemia cell lines used for study in Fig. 6. Cells were incubated with Siglec-7-Fc precomplexed to an AlexaFluor488 anti-huIgG secondary antibody (1 μg/mL) and subjected to live cell flow cytometry. Representative histograms shown.



Supplementary Figure 16. Primary T cells and primary monocytes were isolated from donor PBMCs by magnetic isolation and subjected to digestion with 100 nM VC-Sia and a 1:30 dilution of O-Glycosidase from *S. Pneumoniae*. Pulldown of Siglec-7-interacting proteins was then performed and western blotting with an antibody against CD43 was used to assess the interaction of different immune cell glycoforms with Siglec-7.

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