Supplementary Information for

Glycocalyx Engineering Reveals a Siglec-Based Mechanism for NK Cell Immunoevasion

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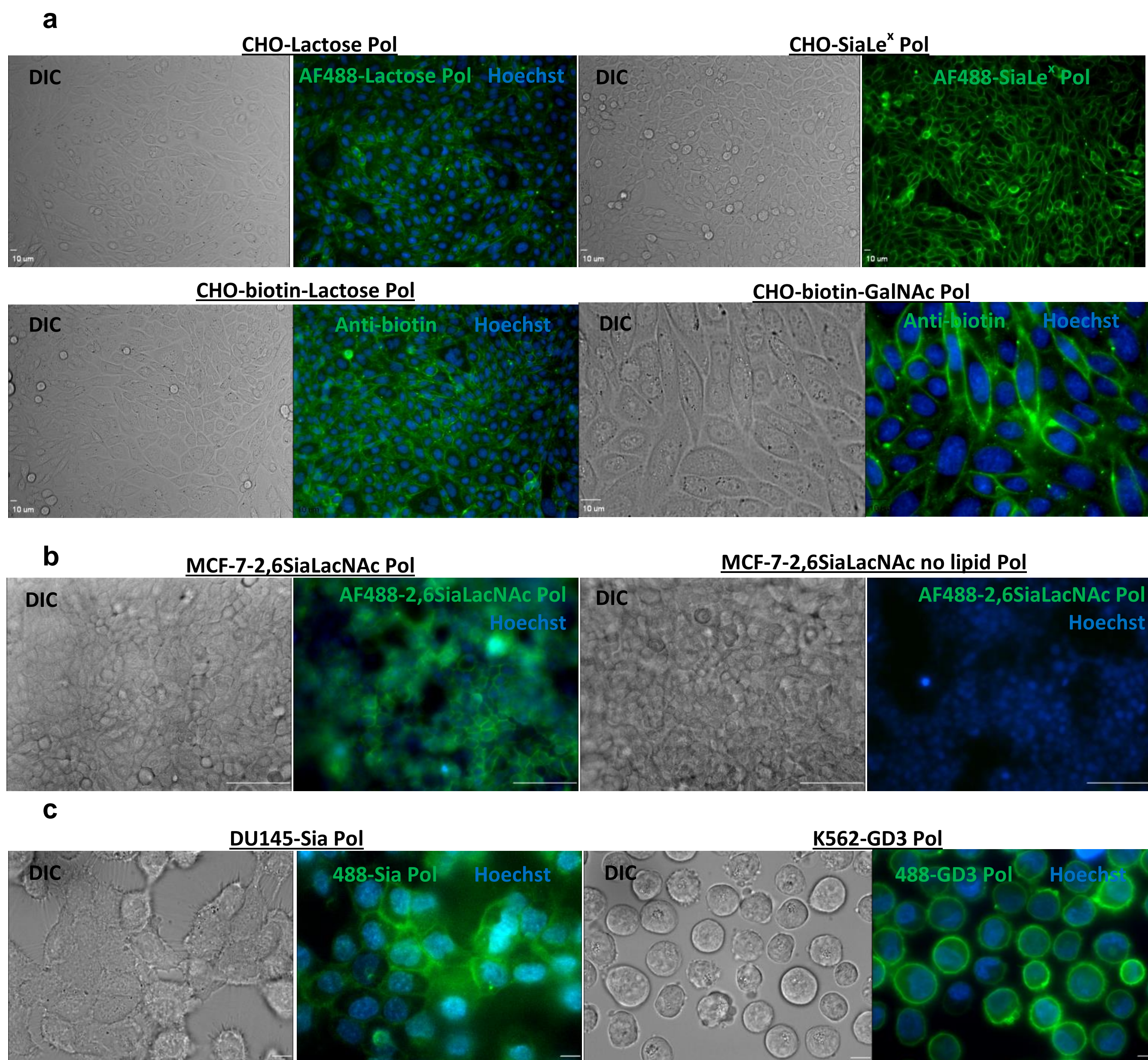
Supplementary Results

Supplementary Figure 1. AF488 labeled glycopolymers can incorporate a wide range of glycan structures into a variety of cell types



(a) Fluorescence microscopy of Chinese Hamster Ovary (CHO) cells treated with Alexa Fluor 488 (AF488) or biotin conjugated polymers. Biotin was further labeled by treatment with anti-bioin-488. Scale bars, 10 μm. (b) MCF-7 breast cancer cells show labeling with 2,6-SiaLacNAc polymer (left panel) while 2,6-SiaLacNAc polymers without the phospholipid tail do not incorporate well (right panel). Scale bars, 100 μm. (c) Fluorescence microscopy of sialic acid and GD3 AF488 conjugated polymers on prostate cancer DU145 cells and myelogenous leukemia K562 cells. Scale bars, 10 μm.

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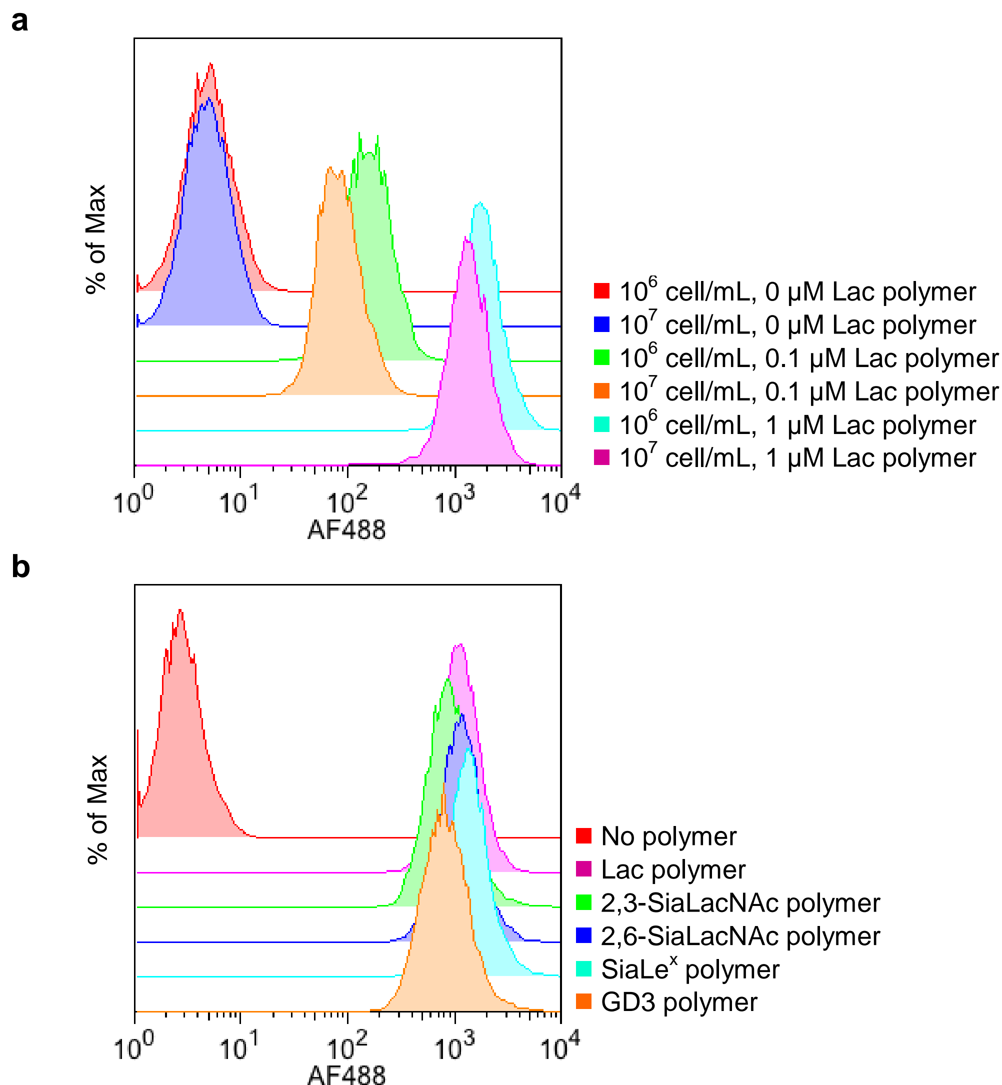
Supplementary Figure 2. Cell density and glycan structure have minor influence on glycopolymer incorporation



(a) Jurkat cells at 10^6 or 10^7 cells/mL were incubated with 0, 0.1, or 1 μM AF488-Lac polymer at room temperature in MEM for 1 h. Cells were washed with 1% FBS in PBS and analyzed by flow cytometry. (b) Jurkat cells at 10^7 cells/mL were incubated with 0.5 μM of the indicated AF488 labeled polymer at room temperature in HBSS for 45 min.

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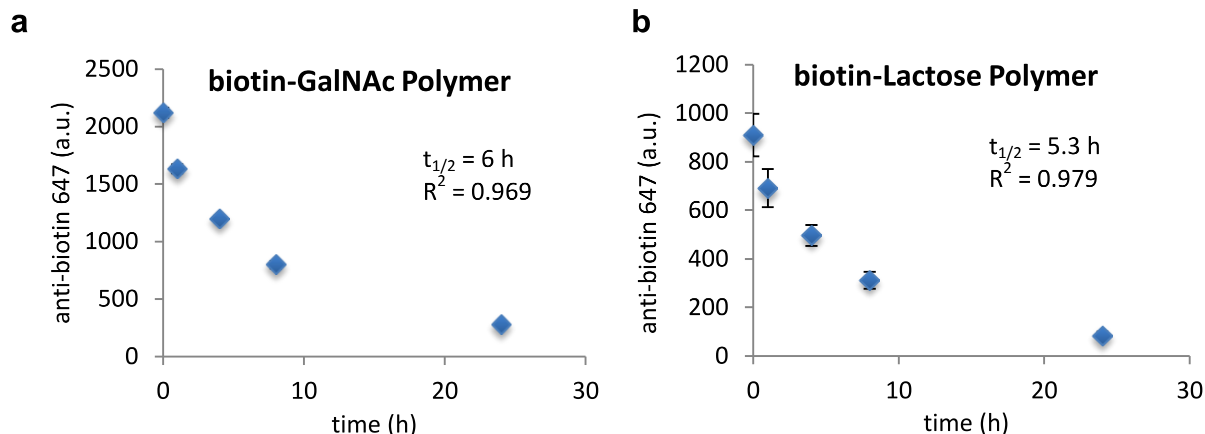
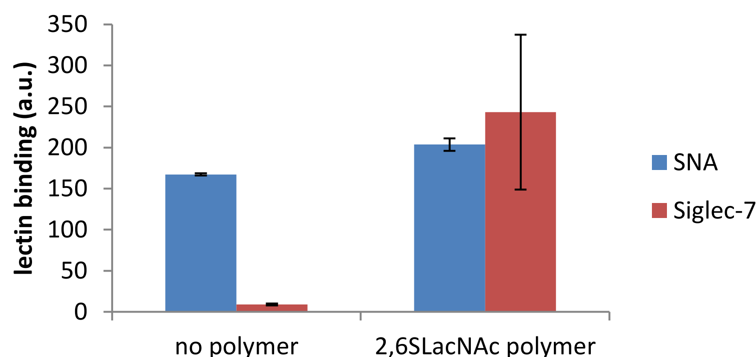
Supplementary Figure 3. Half-life of glycopolymers on Jurkat cell surfaces



Jurkat cells were incubated with 1 μM (a) biotin-GalNAc polymer or (b) biotin-Lac polymer at room temperature in HBSS for 45 min. Cells were washed and cultured in RPMI-1640 supplemented with 10% FBS. An aliquot of 7 × 10^5 cells were removed at the indicated time and washed with cold 1% FBS in PBS. Cells were labeled with 1/100 anti-biotin 647 in 1% FBS in PBS for 20 min on ice. Cells were washed and analyzed by flow cytometry. Experiments were performed in triplicate and half lifes were calculated by best fit to the equation $x = x_o e^{-kt}$ in MATLAB.

Supplementary Figure 4. Glycopolymers are accessible to lectin binding



Binding of SNA-FITC or Siglec-7-hF_c to Jurkat cells after incubation with 1 μm 2,6-SiaLacNAc polymer was assessed by flow cytometry. Data are presented as mean ± s.d. from three independent experiments.

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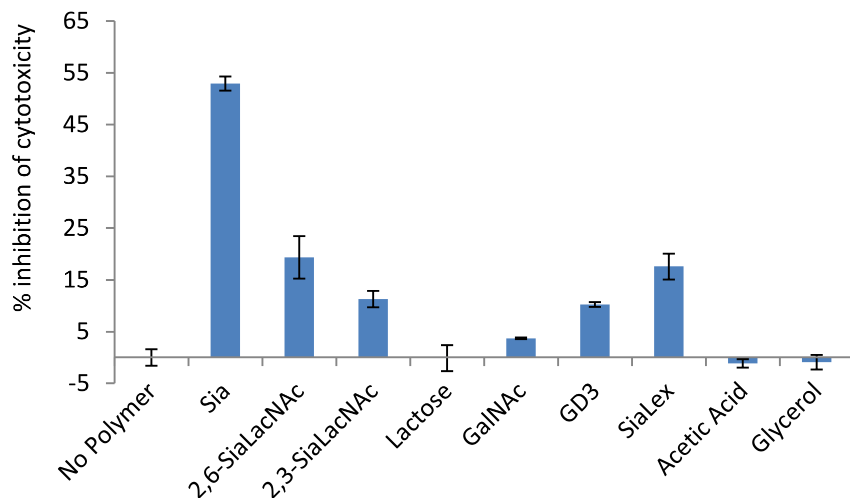
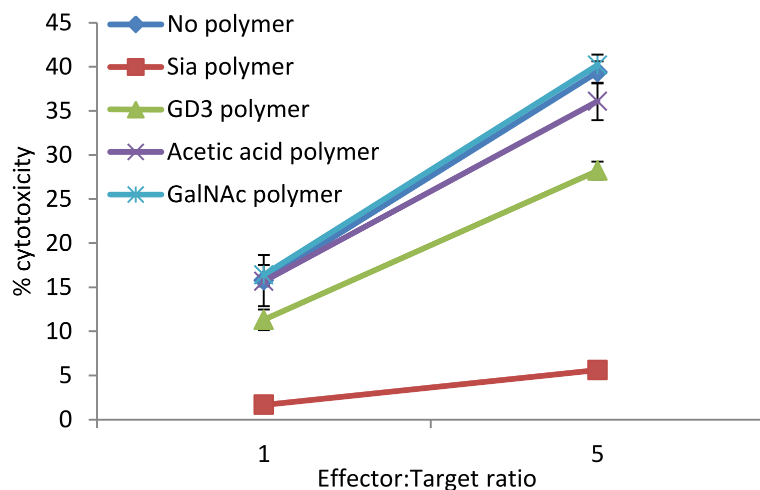
Supplementary Figure 5. Glycopolymers protect target cells from PBMC derived NK-mediated cytotoxicity in a structure dependent manner



Jurkat cells were labeled with 1 μM indicated polymer and incubated with PBMC at an effector to target ratio of 20:1 in a 4 h cytotoxicity assay. The best protection was seen with the Sia polymer while other sialylated ligands provided reduced though still significant inhibition.

Supplementary Figure 6. Glycopolymer protection from purified NK cell cytotoxicity



Jurkat cells were incubated with 1 μM polymer for 45 min at room temperature and mixed with purified NK cells at indicated effector to target ratios. The coculture was incubated for 4 h at 37 ºC in 5% CO_2 and cell death was quantified by propidium iodide staining in flow cytometry.

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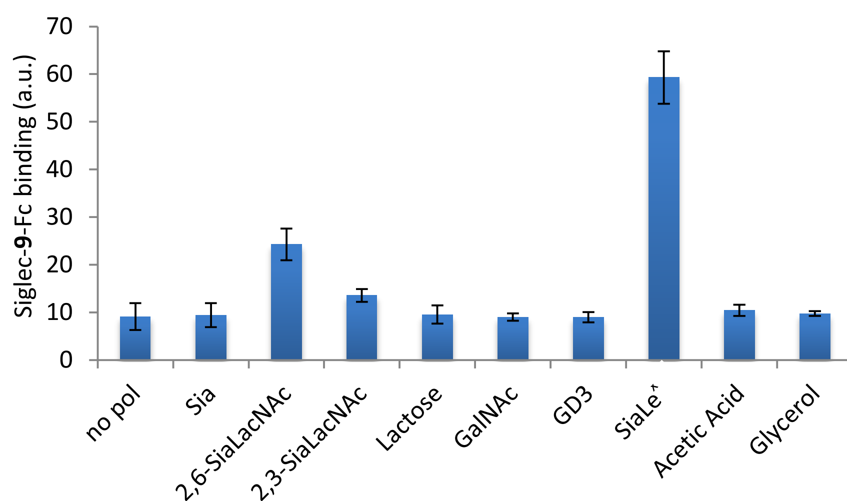
Supplementary Figure 7. Binding of soluble Siglec-9-Fc to cell surface glycopolymers



Jurkat cells were treated with indicated polymer and Siglec-9-F_c binding was assessed by labeling with secondary anti-humanFc-647 followed by flow cytometry.

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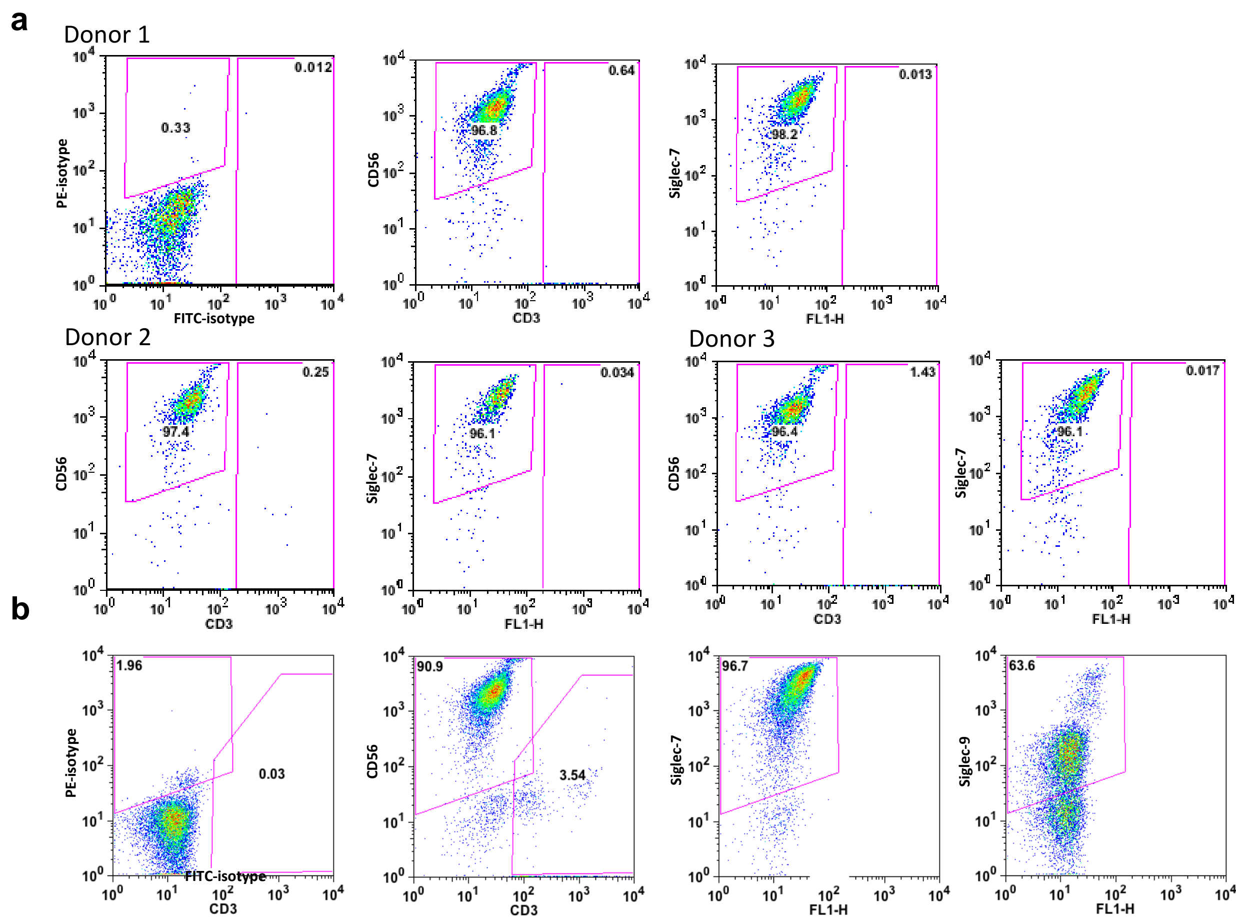
Supplementary Figure 8. Siglec expression on purified human NK cells



(a) Purified human NK cells show ubiquitous Siglec-7 expression at high levels across several donors. (b) Siglec-9 is expressed at lower levels and only in a subset of human NK cells. Cells were phenotyped after isolation from fresh human PBMC by labeling with the indicated antibody and analysis by flow cytometry. Axes are labeled with the indicated marker or by channel (e.g. FL1-H) is no marker was present.

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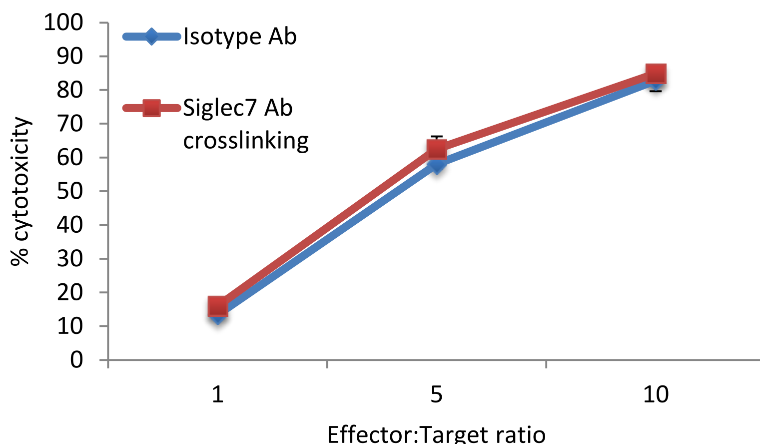
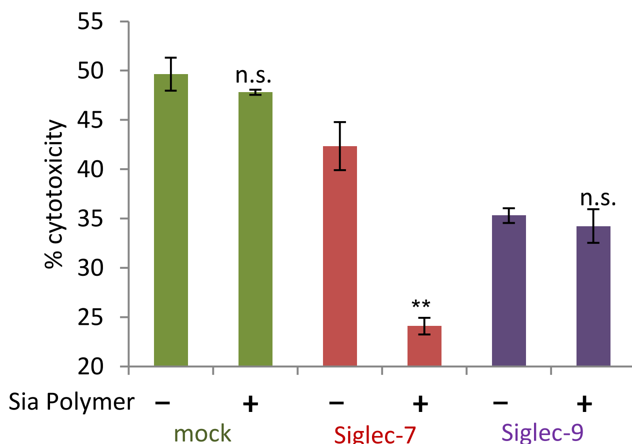
Supplementary Figure 9. Artificially crosslinking Siglec-7 does not affect cytotoxicity



NK cells were incubated with 10 µg/mL of anti-Siglec-7 (6-434, non-function blocking) or isotype antibody at rt for 20 min. followed by addition of 20 µg/mL of goat anti-Ms F(ab')$_2$.[1,2] NK cells with crosslinked Siglec-7 were then cocultured with K562 cells at the indicated effector:target ratios for 4 h and analyzed for cytotoxicity by flow cytometry. As shown, Siglec-7 crosslinking alone did not alter the NK cytotoxic profile.

Supplementary Figure 10. Siglec-7 overexpression promotes Sia polymer inhibition



NK-92 cells overexpressing Siglec-7 showed inhibited cytotoxicity against Sia polymer coated Jurkat cells. Mock treated or Siglec-9 overexpressing cells show insiginficant difference in killing, though killing was overall depressed with Siglec-9 overexpression. Data are presented as mean ± s.d. from three independent experiments (*$P < 0.05$, **$P < 0.01$ for polymer coated versus no polymer control, two-tailed, paired analysis; n.s. not significant).

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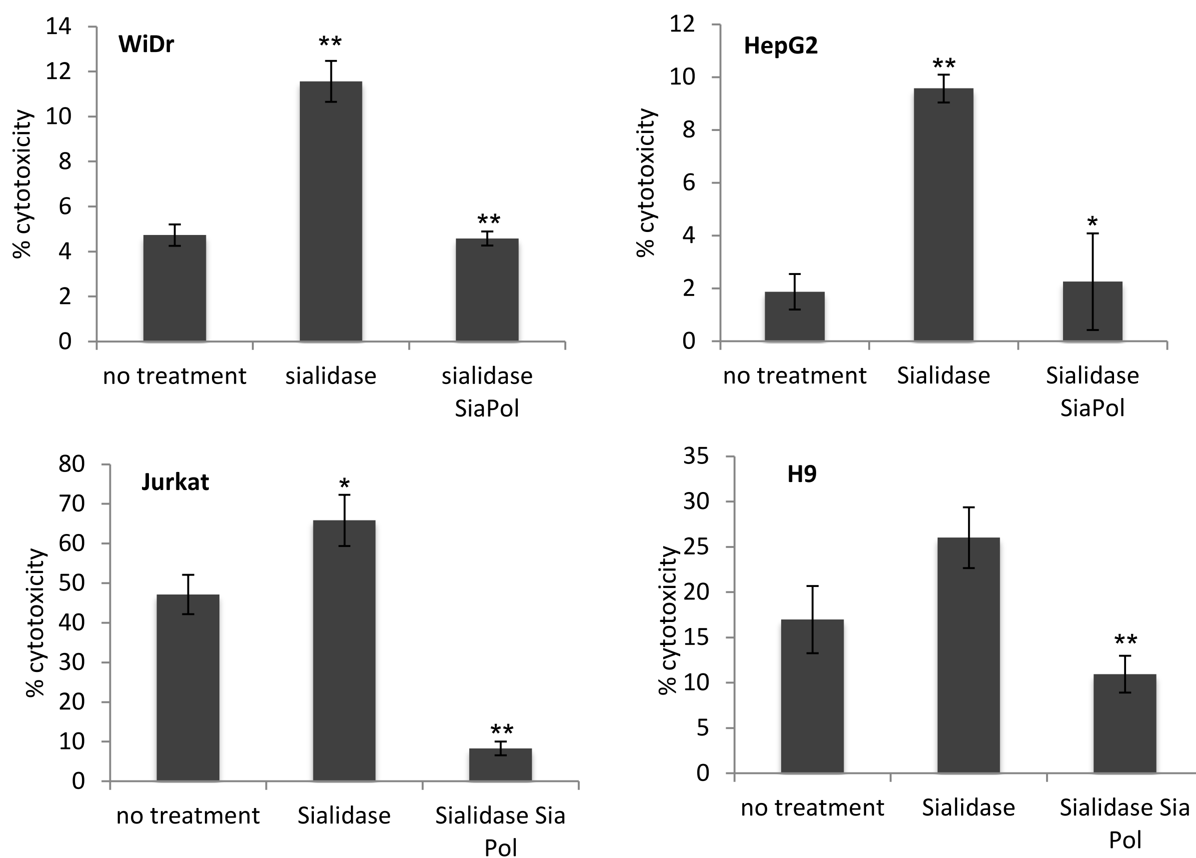
Supplementary Figure 11. Glycopolymers recover sialic acid-based cancer immunoprotection



Removal of cell surface sialic acid from WiDr colon, HepG2 hepatocarcinoma, Jurkat T lymphoma and H9 cutaneous T lymphoma cell lines increases susceptibility to NK cell cytotoxicity and is recovered by treatment with the sialic acid polymer (SiaPol). Cancer target cells were treated with VC sialidase for 1 h at 37 ºC before polymer incorporation at room temperature and coculture with purified NK (5:1, effector:target) in a 4 h cytotoxicity assay. Data are presented as mean ± s.e.m. from three independent experiments (*$P < 0.05$, **$P < 0.01$, for polymer coated versus no polymer control, two-tailed, paired analysis).

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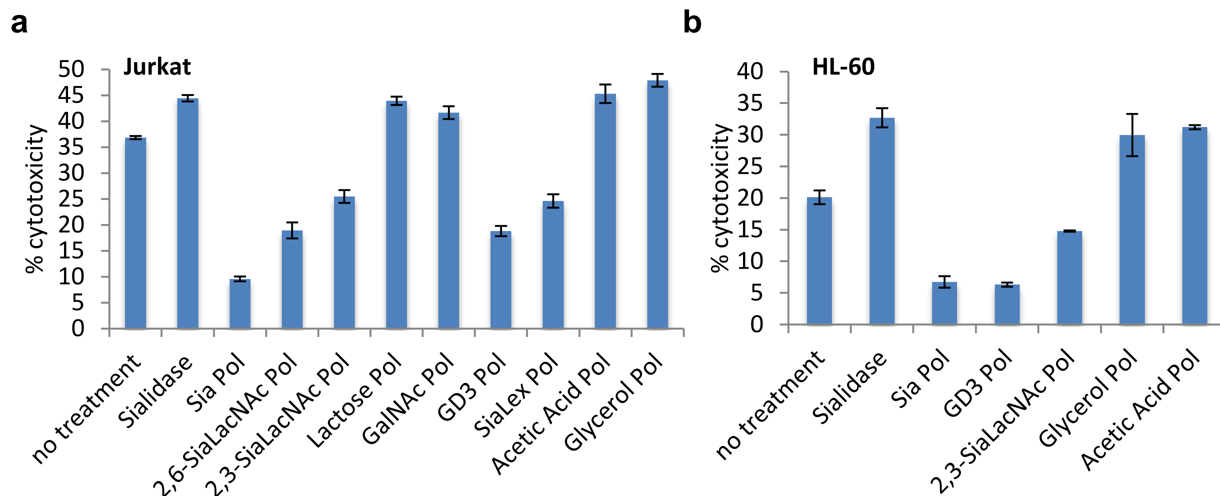
Supplementary Figure 12. Glycopolymers recover sialylation-based protection in a structure dependent manner



(a) Jurkat or (b) HL-60 cells were treated with VC sialidase for 1.5 h at 37 °C and labeled with 1 μM indicated polymer before coculture with primary NK cells at a (a) 3:1 or (b) 5:1 effector to target ratio. Interestingly, the GD3 polymer protected well on HL-60 cells that were sialidase treated but only moderately on Jurkat cells possibly due to a cell type dependent difference in polymer loading after sialidase treatment.

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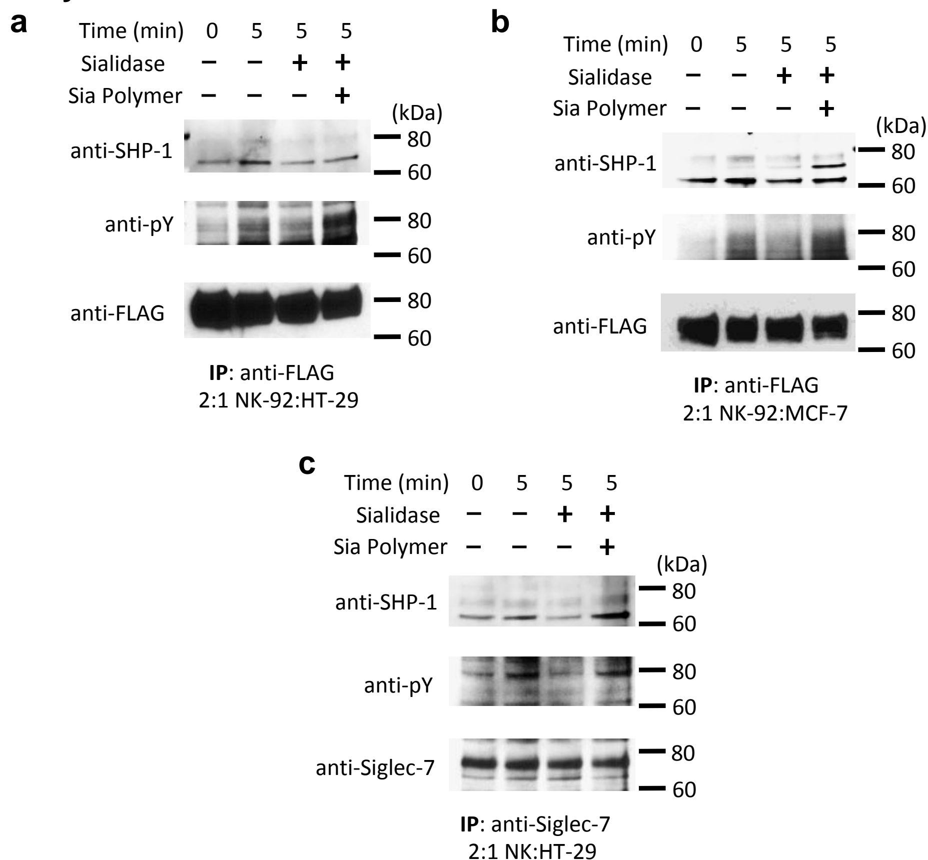
Supplementary Figure 13. Sialylation status of target cell correlates with Siglec-7 phosphorylation and SHP-1 recruitment



Western blot analysis of NK-92 or primary NK cells stimulated with target cells treated with sialidase and coated with Sia polymer. (**a**) Colon HT-29, or (**b**) breast MCF-7 cell lines were cocultured with NK-92 cells overexpressing Siglec-7 at a 2:1 effector to target ratio and lysed immediately or at 5 min. Immunoprecipitation with anti-FLAG M2 agarose beads was used to isolate Siglec-7 before SDS-PAGE and immunoblot analysis. (**c**) HT-29 cells were treated as in (a) but cocultured with primary NK cells and immunoprecipitated with anti-Siglec-7 antibody. Sialidase treatment of the target cancer cell decreased Siglec-7 phosphorylation in all cases which was recovered by coating with the Sia polymer.

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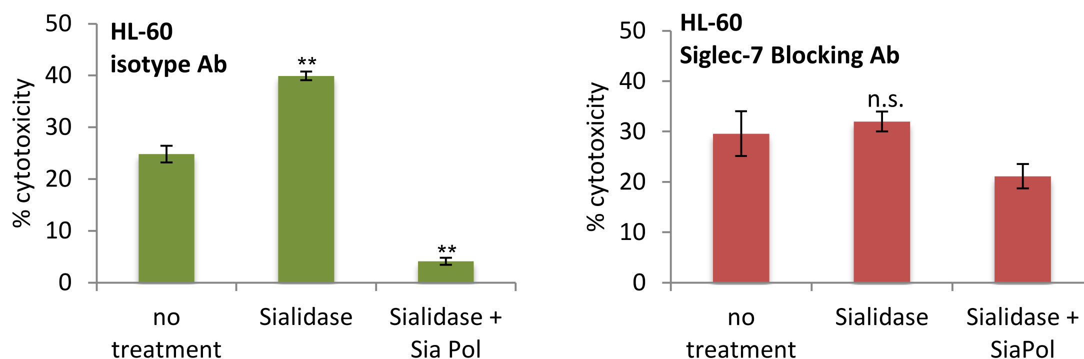
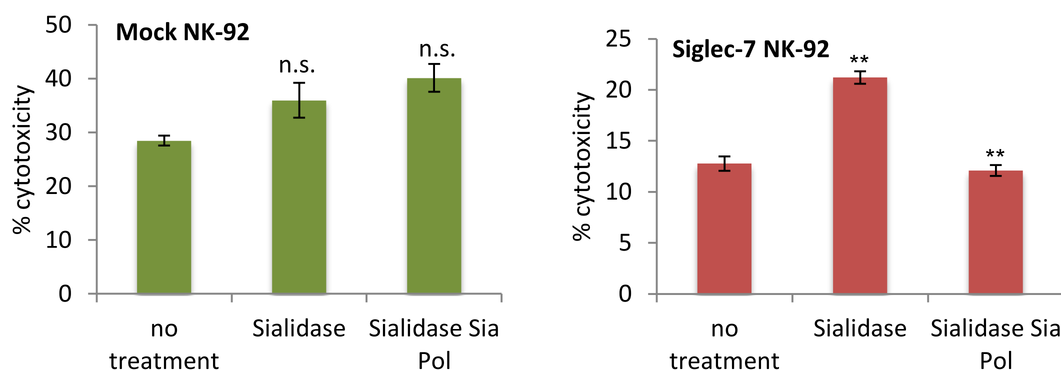
Supplementary Figure 14. Blocking Siglec-7 abrogates sialic acid dependent protection from NK cell cytotoxicity



Purified NK cells were preincubated with 10 µg/mL of Siglec-7-blocking or isotype antibody and mixed with HL-60 cells at a 5:1 NK cell to target ratio in a 4 h cytotoxicity assay. The Siglec-7 blocking antibody significantly decreased the effects of sialidase anf polymer treatment (**$P < 0.01$, for sialidase treated versus no treatment control or Sia polymer coated versus sialidase treated, two-tailed, paired analysis; n.s. not significant).

Supplementary Figure 15. Siglec-7 expression is necessary for sialic acid dependent protection from NK-92 cell killing



NK-92 cells overexpressing FLAG-tagged Siglec-7 were cocultured with HL-60 cells at an 8:1 NK cell to target ratio. Only NK-92 cells expressing Siglec-7 were susceptible to sialylation dependent inhibition while mock treated cells were not affected (**$P < 0.01$, for sialidase treated versus no treatment control or Sia polymer coated versus sialidase treated, two-tailed, paired analysis; n.s. not significant).

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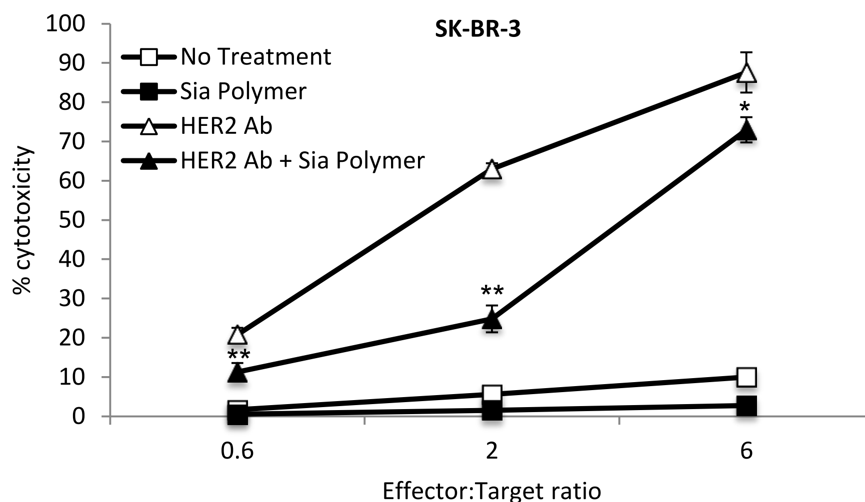
Supplementary Figure 16. Sialic acid polymer inhibits HER2 ADCC of SK-BR-3



Cell surface sialic acid could also dampen NK-mediated ADCC against SK-BR-3 breast adenocarcinoma cells. Cytotoxicity assays were performed in the presence of 2 μg/mL anti-HER2 antibody in increasing NK:target ratios. Data are presented as mean ± s.d. from three independent experiments (* $P < 0.05$, ** $P < 0.01$ for polymer coated versus no polymer control, two-tailed, paired analysis).

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Supplementary Note 1

Additional Synthetic Methods

General materials

Synthetic reagents were purchased from Sigma-Aldrich, Acros, and TCI and used without purification unless noted otherwise. Anhydrous DMF and MeOH, 99.9% purity were purchased from Acros in sealed bottles; all other anhydrous solvents (Fisher brand; HPLC grade) were obtained from an alumina column solvent purification system. EZ-Link Alkoxyamine-PEG4-Biotin (AO-biotin) was purchased from Pierce/Thermo Scientific. All reactions were carried out in flame-dried glassware under N_2 unless otherwise noted. In all cases, solvent was removed by reduced pressure with a Buchi Rotovapor R-114 equipped with a Welch self-cleaning dry vacuum. Products were further dried by reduced pressure with an Edwards RV3 high vacuum. Lyophilization was performed on a LABCONCO FreeZone instrument equipped with an Edwards RV2 pump. Thin layer chromatography was performed with Silicycle 60 Å silica gel plates and detected by UV lamp or charring with *p*-anisaldehyde in acidic EtOH. Flash chromatography was performed using Silicycle 60 Å 230-400 mesh silica. All ^{1}H and ^{13}C NMR spectra are reported in ppm and referenced to solvent peaks. NMR spectra were obtained on Bruker AVQ-400, DRX-500, AV-500, or AV-600 instruments. Size exclusion chromatography (SEC) was performed using a Viscotek TDA 302 SEC fitted with a Shodex SB-803 HQ column with differential refractive index detection. High resolution electrospray ionization (ESI) mass spectra were obtained from the UC Berkeley Mass Spectrometry Facility using an LTQ Orbitrap (Thermo Fisher Scientific).

Synthetic procedures and analytical data for new aminooxy compounds.

Aminooxy 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate (11). To the known protected precursor[3] O-[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate] N-hydroxysuccinimide (210 mg, 0.36 mmol) in 4 mL anhydrous MeOH was added hydrazine hydrate (26 µL, 0.54 mmol) dropwise at 4 ºC and warmed to room temperature over 4 h. The reaction mixture was diluted with CH_2Cl_2 and concentrated under reduced pressure. The crude residue was purified by silica chromatography (1%-20% MeOH/CH_2Cl_2) to give the protected neuraminic acid with the free amniooxy (117 mg, 64%). The intermediate was dissolved in 3 mL anhydrous MeOH and NaOMe (25% in MeOH, 103 µL) was added dropwise while stirring at rt for 1 h followed by the addition of NaOH (1 M, 300 µL). After an additional 1 h, the reaction was neutralized with AcOH and stirred overnight at room temperature to complete hydrolysis. The resulting product was concentrated under vacuum and purified by silica column chromatography (4:2:1 EtOAc/MeOH/H_2O). The desired fractions were pooled and concentrated under vacuum. The purified product was then dissolved in ddH_2O and lyophilized to give the free aminooxy sialic acid **11** (49 mg, 65%) as an off-white powder. ^{1}H NMR (600 MHz, D_2O) δ 3.92 – 3.75 (m, 4H), 3.70 – 3.65 (m, 1H), 3.59 (dt, J = 17.7, 8.8 Hz, 1H), 3.53 (d, J = 9.0 Hz, 1H), 2.57 (dd, J = 12.6, 4.8 Hz, 1H), 2.00 (s, 3H), 1.68 (t, J = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.99, 173.47, 103.16, 72.80, 71.38, 68.23, 68.09, 62.73, 51.78, 37.93, 22.00; HRMS (ESI): calcd for $C_{11}H_{20}N_2O_9$ $[M+H]^+$ m/z = 325.1242, found: 325.1253.

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***N*-hydroxypent-4-eneamide (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→8)-α-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (12).** *N*-Pentenoyl aminooxy 2,3-sialyllactose[4] (19 mg, 0.025 mmol), *N*-acetyl mannosamine (8.4 mg, 0.038 mmol), sodium pyruvate (14 mg, 0.127 mmol), and CTP•Na (21 mg, 0.038 mmol) were combined and dissolved in H_2O (3 mL). A concentrated stock of Tris-HCl buffer pH 8.5 with $MgCl_2$ was added to a final concentration of 100 mM Tris, 20 mM $MgCl_2$. Recombinant *E. coli* K12 sialic acid aldolase (2.5 U), *N. meningitidis* CMP-sialic acid synthetase (2.5 U), and *C. jejuni* α-2,8-sialyltransferase CstII (3 U) were added followed by H_2O to bring the volume to 4 mL. The reaction mixture was incubated at 37 ºC for 4 h followed by shaking at rt for 16 h. The reaction was monitored by TLC (4:2:1 EtOAc/MeOH/H_2O) and upon consumption of the starting material calf alkaline phosphatase was added to remove remaining nucleotide phosphate. After further incubation at 37 ºC for 1 h, the reaction mixture was quenched with cold MeOH (4 mL) and incubated on ice 15 min. The mixture was centrifuged, the precipitates were removed, and solution was concentrated under vacuum. The resulting residue was passed through a BioGel P-2 size exclusion column and eluted with water to obtain **12** (22 mg, 85%) as a white, fluffy powder after lyophilization. ^1H NMR (500 MHz, D_2O) δ 5.79 (dq, *J* = 10.4, 6.4 Hz, 1H), 5.03 (dd, *J* = 21.9, 13.7 Hz, 2H), 4.66 (d, *J* = 8.1 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H), 4.12 (dd, *J* = 12.2, 3.2 Hz, 1H), 4.08 – 3.99 (m, 2H), 3.99 – 3.88 (m, 2H), 3.86 – 3.75 (m, 7H), 3.73 – 3.66 (m, 5H), 3.64 – 3.50 (m, 9H), 3.43 (t, *J* = 8.7 Hz, 1H), 2.74 (dd, *J* = 12.4, 4.5 Hz, 1H), 2.62 (dd, *J* = 12.3, 4.3 Hz, 1H), 2.36 – 2.22 (m, 4H), 2.02 (s, 3H), 1.98 (s, 3H), 1.71 (q, *J* = 11.7 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 174.91, 173.41, 173.27, 172.63, 136.34, 115.95, 105.10, 102.58, 100.45, 100.09, 78.14, 77.27, 75.38, 75.15, 74.99, 73.95, 72.58, 71.67, 70.94, 69.19, 68.41, 68.04, 67.83, 67.37, 62.47, 61.47, 61.36, 61.03, 59.67, 59.24, 52.19, 51.66, 40.42, 39.62, 31.63, 28.86, 22.23, 21.96; HRMS (ESI): calcd for $C_{39}H_{62}N_3O_{28}$ $[M-H]^-$ *m/z* = 1020.3525, found: 1020.3520.

Aminooxy (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranos-ylonic acid)-(2→8)-α-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranos-ylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (GD3) (13). A solution of *N*-pentenoyl aminooxy sialoside **12** (13 mg, 0.013 mmol) in H_2O/MeCN/MeOH/FA (1:1:1:0.001, v:v; 1.2 mL) was stirred at rt with dropwise addition of I_2 (0.064 mmol, 1 M solution in THF). After 1 h, $MeONH_2$ (53 mg, 0.64 mmol) was added to ensure complete deprotection and heated to 38 ºC for 2 h after which the reaction was quenched by the addition of 50 mM $Na_2S_2O_3$ in H_2O until the solution became clear. The solvent was removed and the remaining residue was passed through a short silica chromatography column (EtOAc/MeOH/H_2O). The desired fractions were pooled and concentrated. The material was further purified on a BioGel P-2 size exclusion column and lyophilized to give the free aminooxy GD3 **13** (7.5 mg, 63%) as a white powder. ^1H NMR (500 MHz, D_2O) δ 4.56 (d, *J* = 8.2 Hz, 1H), 4.48 (d, *J* = 6.4 Hz, 1H), 4.21 – 4.01 (m, 3H), 4.01 – 3.89 (m, 2H), 3.89 – 3.73 (m, 7H), 3.69 – 3.48 (m, 13H), 3.31 (t, *J* = 8.5 Hz, 1H), 2.74 (d, *J* = 8.1 Hz, 1H), 2.64 (d, *J* = 12.2 Hz, 1H), 2.02 (s, 3H), 1.99 (s, 3H), 1.70 (t, *J* = 12.0 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.94, 173.10, 108.80, 104.55, 102.60, 99.91, 98.38, 77.87, 75.81, 75.40, 75.31, 75.14, 74.34, 74.21, 73.96, 73.89, 73.62, 72.66, 72.50, 71.71, 71.50, 71.28, 69.87, 69.24, 68.95, 68.35, 68.09, 68.02, 67.97, 67.81, 62.80, 62.50, 62.31, 61.05, 60.26, 59.84, 58.73, 52.10, 52.00, 51.66, 46.79, 43.25, 40.52, 32.41, 22.21, 22.02, 21.97; HRMS (ESI): calcd for $C_{34}H_{57}N_3O_{27}$ $[M-2H]^{-2}$ *m/z* = 468.6517, found: 468.6526.

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3-aminooxy-1,2-propanediol (14). *N*-hydroxyphthalimide (160 mg, 0.97 mmol) and KCO_3 (402 mg, 12.9 mmol) were suspended in 10 mL anhydrous DMF. 3-bromo-1,2-propanediol (170 µL, 1.94 mmol) was added to the suspension and heated at 60 °C for 18 h. The reaction was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by silica column chromatography (Hex/EtOAc) to afford the phthalimide protected aminooxy glycerol (72 mg, 16%). The product was dissolved in 2 mL anhydrous MeOH and hydrazine hydrate (16 µL, 0.33 mmol) was added dropwise with stirring at room temperature. After 2 h, the mixture was dissolved in MeOH, filtered and concentrated under vacuum. The reaction mixture was purified by silica chromatography (10%–20% MeOH in EtOAc) and the fractions were pooled and concentrated. The product was dissolved in ddH_2O and lyophilized to yield the aminooxy glycerol product (24 mg, 83%). ^{1}H NMR (500 MHz, D_2O) δ 3.94 – 3.87 (m, 1H), 3.76 (dd, J = 10.9, 3.7 Hz, 1H), 3.68 (dd, J = 10.9, 7.2 Hz, 1H), 3.60 (dd, J = 11.8, 4.4 Hz, 1H), 3.52 (dd, J = 11.8, 6.2 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 76.35, 69.53, 62.53; HRMS (ESI): calcd for $C_3H_9N_1O_3$ $[M+H]^+$ m/z = 108.0655, found: 108.0654.

Synthetic procedures and analytical data for glycopolymers.

Dipalmitoylphosphatidylethanolamine-poly(methyl vinyl ketone) (DPPE-PolyMVK) (1). DPPE-Poly(MVK) was synthesized as previously described.[51] *N,N*-diisopropylethylamine (15 µL, 0.070 mmol) and the pentafluorophenyl activated ester of 4,4'-azobis(4-cyanovaleric acid) (ACVA-PFP)[51] (15 mg, 0.016 mmol) were added to a solution of DPPE (40 mg, 0.036 mmol) in MeOH (1.5 mL) and $CHCl_3$ (3.5 mL). After stirring at room temperature for 2 h, the resulting phospholipid initiator was concentrated by rotary evaporation. The resulting oil was suspended in anhydrous toluene (750 µL) and methyl vinyl ketone (210 µL, 2.5 mmol) was added followed by degassing with four freeze-pump-thaw cycles. The solution was heated to 80 ºC for 20 h and upon cooling to room temperature the polymer product was dissolved in minimal *p*-dioxane (3 mL) and triturated by dropwise addition to excess diethyl ether (300 mL). The precipitate was isolated by centrifugation (3,700×g, 20 min), washed with diethyl ether, and dried under vacuum to afford DPPE-PolyMVK as an off-white solid (110 mg). Spectral properties matched those previously reported.[51] ^{1}H NMR (500 MHz, $CHCl_3$) δ 4.20 (app t, 2H), 3.78 (app t, 2H), 3.68 – 3.65 (m, 11H), 3.44 (app t, 2H); SEC (DMF, polystyrene standard): $M_w = 26 \times 10^3$ Da, PDI = 2.89.

Aminooxy Glycan-Conjugated Polymers.

All aminooxy compounds were conjugated to the DPPE-PolyMVK backbone by the following method: A mixture of **1** (1 equiv.), aminooxy compound (1.4 equiv. to ketone), and formic acid (1% v/v) in MeCN/MeOH (2:1, v/v) was stirred at 50 ºC for 20 h. To the reaction mixture was added H_2O (to 25% v/v) and stirring was continued at 32 ºC for 18 h. For biotin and AF488 labeling, AO-biotin and AO-AF488 were added to the initial reaction mixture at 0.01 equivalents. After completion, the reaction was diluted in H_2O and MeOH and concentrated under vacuum. The resulting residue was dissolved in H_2O and dialyzed against aqueous 5mM NH_4HCO_3 thrice and H_2O once over 72 h to afford the corresponding oxime phospholipid polymer after lyophilization. Conversion was estimated by comparing the integration

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corresponding to the anomeric proton to that of the methyl vinyl ketone backbone protons. NMR proton integrations are reported as relative amounts. Yields and characterization are as follows:

N-acetylneuraminic acid (Sia) MVK polymer (2). Polymer **2** was given from reaction of polymer **1** with aminooxy sialic acid **11** as an off-white solid (1.7 mg, 55%). Conversion (NMR) = 0.69; M_n (NMR) = 114 kDa; ^{1}H NMR (500 MHz, D_2O) δ 3.99 – 3.36 (m, 7H), 3.01 – 2.81 (m, 1H), 2.77 – 2.40 (m, 3H), 2.00 (s, 4H), 2.37 – 0.55 (m, 9H), 1.81 – 1.74 (m, 1H).

N-acetylneuraminic acid-α2,6-galactose-β1,4-N-acetylglucosamine (2,6-SiaLacNAc) MVK polymer (3). Polymer **1** was reacted with aminooxy 2,6SiaLacNAc[4] to give polymer **3** as a white solid (2 mg, 61%). Conversion (NMR) = 0.84; M_n (NMR) = 256 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.11 – 4.85 (m, 1H), 4.78 – 4.72 (m, 1H), 4.55 – 4.31 (m, 1H), 4.20 – 2.95 (m, 16H), 2.73 – 2.52 (m, 1H), 2.00 (s, 6H), 1.75 – 1.70 (m, 1H), 2.31 – 0.56 (m, 7H).

N-acetylneuraminic acid-α2,3-galactose-β1,4-N-acetylglucosamine (2,3-SiaLacNAc) MVK polymer (4). Polymer **1** was reacted with aminooxy 2,3SiaLacNAc[4] to give polymer **4** as a white solid (2 mg, 49%). Conversion (NMR) = 0.73; M_n (NMR) = 222 kDa; ^{1}H NMR (600 MHz, D_2O) δ 5.04 – 4.86 (m, 1H), 4.77 – 4.33 (m, 2H), 4.23 – 3.09 (m, 14H), 2.73 (d, J = 5.2 Hz, 1H), 2.22 – 1.84 (m, 5H), 2.30 – 0.66 (m, 8H), 1.72 – 1.65 (m, 1H).

Galactose-β1,4-glucose (Lactose) MVK polymer (5). Aminooxy lactose[4] was conjugated to polymer **1** to afford polymer **5** as a white solid (3.2 mg, 60%). Conversion (NMR) = 0.67; M_n (NMR) = 121 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.07 – 4.82 (m, 1H), 4.56 – 4.31 (m, 1H), 4.15 – 3.02 (m, 12H), 2.49 – 0.72 (m, 9H).

N-acetylgalactosamine (GalNAc) MVK polymer (6). Aminooxy GalNAc[5] was conjugated to polymer **1** to afford polymer **6** as a white solid (1.7 mg, 46%). Conversion (NMR) = 0.65; M_n (NMR) = 80 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.32 (d, J = 26.8 Hz, 1H), 4.36 – 4.13 (m, 1H), 4.13 – 3.43 (m, 5H), 2.03 (s, 3H), 2.39 – 0.96 (m, 9H).

N-acetylneuraminic acid-α2,8-N-acetylneuraminic acid-α2,3-galactose-β1,4-glucose (GD3) MVK polymer (7). Polymer **7** was given from reaction of polymer **1** with aminooxy GD3 **13** as a white solid (4.3 mg, 74%). Conversion (NMR) = 0.85; M_n (NMR) = 323 kDa; ^{1}H NMR (500 MHz, D_2O) δ 4.55 – 4.41 (m, 1H), 4.18 – 3.38 (m, 28H), 2.74 (d, J = 9.1 Hz, 1H), 2.62 (d, J = 11.6 Hz, 1H), 2.02 (s, 6H), 2.48 – 0.66 (m, 7H), 1.75 – 1.67 (m, 2H).

N-acetylneuraminic acid-α2,3-galactose-β1,4-(α1,3-fucose)-N-acetylglucosamine ($SiaLe^x$) MVK polymer (8). Polymer **1** was reacted with aminooxy $SiaLe^x$[4] to give polymer **8** as a white solid (2.6 mg, 59%). Conversion (NMR) = 0.75; M_n (NMR) = 275 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.21 – 4.97 (m, 1H), 4.61 – 4.30 (m, 2H), 4.18 – 3.32 (m, 23H), 2.71 (d, J = 7.9 Hz, 1H), 2.00 (s, 6H), 2.37 – 0.69 (m, 8H), 1.79 – 1.71 (m, 1H), 1.14 (s, 3H).

Acetic acid MVK polymer (9). O-(Carboxymethyl)hydroxylamine hemihydrochloride was conjugated to polymer **1** to afford polymer **9** as a white solid (0.5 mg, 25%). Conversion (NMR) = 0.83; M_n (NMR) = 58 kDa; ^{1}H NMR (500 MHz, D_2O) δ 3.85 – 3.70 (s, 2H), 2.24 – 0.93 (m, 6H).

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Glycerol MVK polymer (10). 3-aminooxy-1,2-propanediol **14** was conjugated to polymer **1** to afford polymer **9** as a white solid (1 mg, 44%). Conversion (NMR) = 0.8; M_n (NMR) = 56 kDa; ^{1}H NMR (500 MHz, D_2O) δ 4.21 – 3.84 (m, 3H), 3.74 – 3.49 (m, 2H), 2.24 – 0.80 (m, 7H).

Alexa Fluor 488 N-acetylneuraminic acid-α2,6-galactose-β1,4-N-acetylglucosamine (AF488-2,6-SiaLacNAc) MVK copolymer (15). Polymer **1** was reacted with aminooxy 2,6SiaLacNAc[4] and AO-AF488 to give polymer **15** as a green solid (3.1 mg, 81%). Conversion (NMR) = 0.83; M_n (NMR) = 219 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.03 – 4.87 (m, 1H), 4.47 – 4.34 (m, 1H), 4.03 – 3.37 (m, 19H), 2.77 – 2.63 (m, 1H), 2.00 (s, 3H), 1.74 – 1.69 (m, 1H), 2.29 – 1.10 (m, 7H).

Alexa Fluor 488 N-acetylneuraminic acid-α2,6-galactose-β1,4-N-acetylglucosamine (AF488-2,6-SiaLacNAc) MVK no lipid copolymer (16). Polymer **1** with an N-methyl tail instead of DPPE[6] was reacted with aminooxy 2,6SiaLacNAc[4] and AO-AF488 to give polymer **16** as a green solid (1.2 mg, 55%). Conversion (NMR) = 0.83; M_n (NMR) = 219 kDa; ^{1}H NMR (500 MHz, D_2O) δ 4.98 – 4.82 (m, 1H), 4.40 (d, J = 7.3 Hz, 1H), 4.13 – 3.38 (m, 18H), 2.68 (d, J = 10.1 Hz, 1H), 2.00 (s, 3H), 2.38 – 0.60 (m, 6H), 1.77 – 1.67 (m, 1H).

Alexa Fluor 488 N-acetylneuraminic acid (AF488-Sia) MVK copolymer (17). Polymer **17** was given from reaction of polymer **1** with aminooxy sialic acid **11** and AO-AF488 as a green solid (0.9 mg, 58%). Conversion (NMR) = 0.6; M_n (NMR) = 102 kDa; ^{1}H NMR (500 MHz, D_2O) δ 3.99 – 3.38 (m, 7H), 2.69 – 2.57 (m, 1H), 1.99 (s, 3H), 2.32 – 0.43 (m, 11H), 1.26 – 1.16 (m, 1H).

Alexa Fluor 488 N-acetylneuraminic acid-α2,8-N-acetylneuraminic acid-α2,3-galactose-β1,4-glucose (AF488-GD3) MVK copolymer (18). Polymer **18** was given from reaction of polymer **1** with aminooxy GD3 **13** and AO-AF488 as a green solid (2.4 mg, 59%). Conversion (NMR) = 0.8; M_n (NMR) = 305 kDa; ^{1}H NMR (500 MHz, D_2O) δ 4.46 (d, J = 22.2 Hz, 1H), 4.18 – 3.47 (m, 29H), 2.74 (d, J = 9.3 Hz, 1H), 2.63 (d, J = 12.1 Hz, 1H), 2.01 (s, 3H), 1.98 (s, 3H), 2.37 – 0.75 (m, 7H), 1.72 – 1.66 (m, 1H).

Alexa Fluor 488 N-acetylgalactosamine (AF488-Lac) MVK copolymer (19). Aminooxy lactose[4] and AO-AF488 were conjugated to polymer **1** to afford polymer **19** as a green solid (3.1 mg, 76%). Conversion (NMR) = 0.65; M_n (NMR) = 118 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.04 – 4.83 (m, 1H), 4.51 – 4.31 (m, 1H), 4.14 – 3.13 (m, 12H), 2.49 – 1.05 (m, 9H).

Biotin Galactose-β1,4-glucose (Lactose) MVK copolymer (20). Aminooxy lactose[4] and AO-biotin were conjugated to polymer **1** to afford polymer **20** as a white solid (3.8 mg, 55%). Conversion (NMR) = 0.6; M_n (NMR) = 112 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.07 – 4.85 (m, 1H), 4.50 – 4.32 (m, 1H), 4.03 – 3.36 (m, 12H), 2.41 – 1.07 (m, 10H).

Biotin N-acetylgalactosamine (GalNAc) MVK copolymer (21). Aminooxy GalNAc[5] and AO-biotin were conjugated to polymer **1** to afford polymer **21** as a white solid (0.8 mg, 16%). Conversion (NMR) = 0.75; M_n (NMR) = 98 kDa; ^{1}H NMR (500 MHz, D_2O) δ 5.54 – 5.14 (m, 1H), 4.36 – 4.13 (m, 1H), 4.13 – 3.42 (m, 5H), 2.01 (s, 3H), 2.39 – 0.74 (m, 8H).

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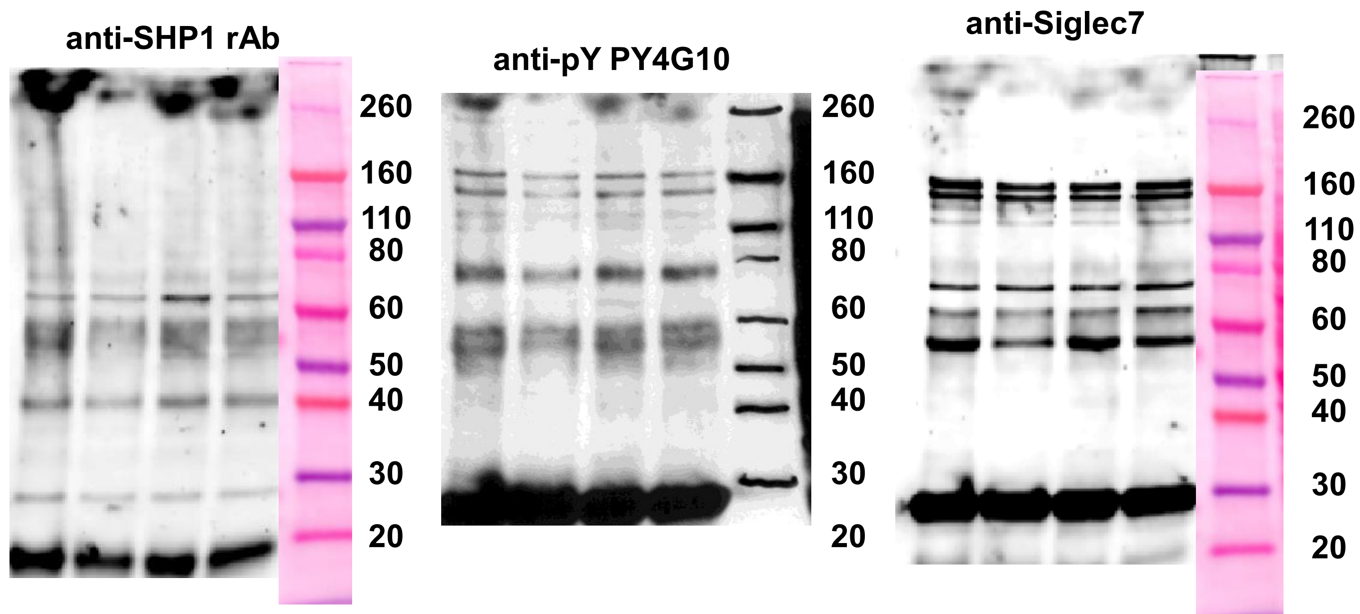
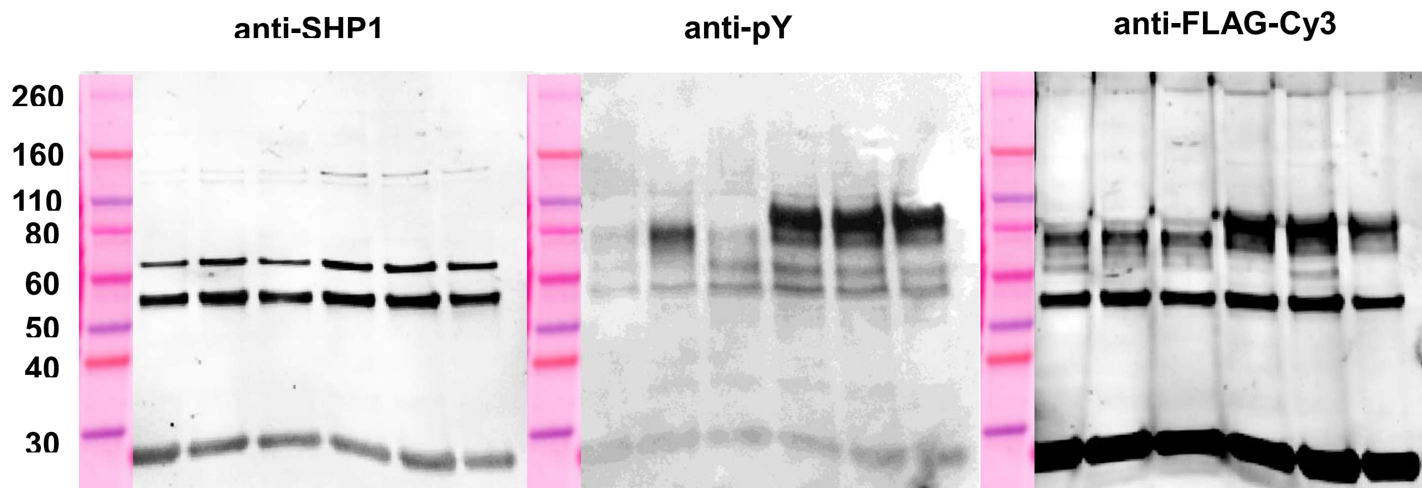
Supplementary Note 2

Full immunoblots used to create indicated Figures

Full blots for Fig. 3c



Full blots for Fig. 3e



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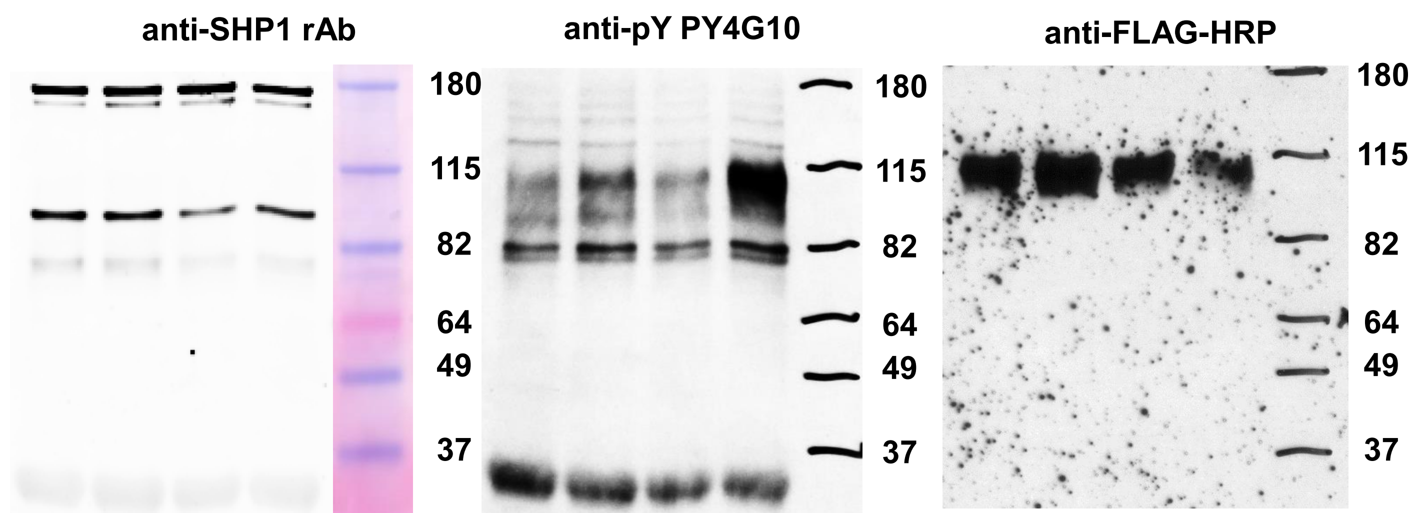
Full blots for Fig. 5b



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