Supporting Information

Mechanistic Investigation and Multiplexing of Liposome-Assisted Metabolic Glycan Labeling

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Table S1. Characterization of liposome size and sugar/lipid molar ratio

Liposome samples	**Effective diameter (nm)***	**Sugar/lipids (molar ratio)**
f-LP-9AzSia	149.8 ± 1.3	1.21
LP-9AzSia	137.5 ± 4.8	1.24
f/DiI-LP-9AzSia	159.6 ± 0.9	1.03
DiI-LP-9AzSia	144.4 ± 11.1	0.90
f/DiI-LP-calcein	160.1 ± 2.1	-
DiI-LP-calcein	144.3 ± 10.3	-
LP-calcein	173.5 ± 4.5	-
Sgc8-LP-9AzSia	207.3 ± 0.8	1.04
tras-LP-9AzSia	242.4 ± 3.6	0.98
RGD-LP-9AzSia	211.3 ± 1.3	1.21
^{BPC}NeuAc-LP-9AzSia	139.6 ± 1.4	1.20
^{MPB}NeuAc-LP-9AzSia	135.2 ± 2.3	0.96
LP-SiaNAl	207.8 ± 0.6	1.17
^{BPC}NeuAc-LP-SiaNAl	209.8 ± 0.7	1.25

*The effective diameter data are mean ± standard deviation of three independent measurements.

Supporting Figures

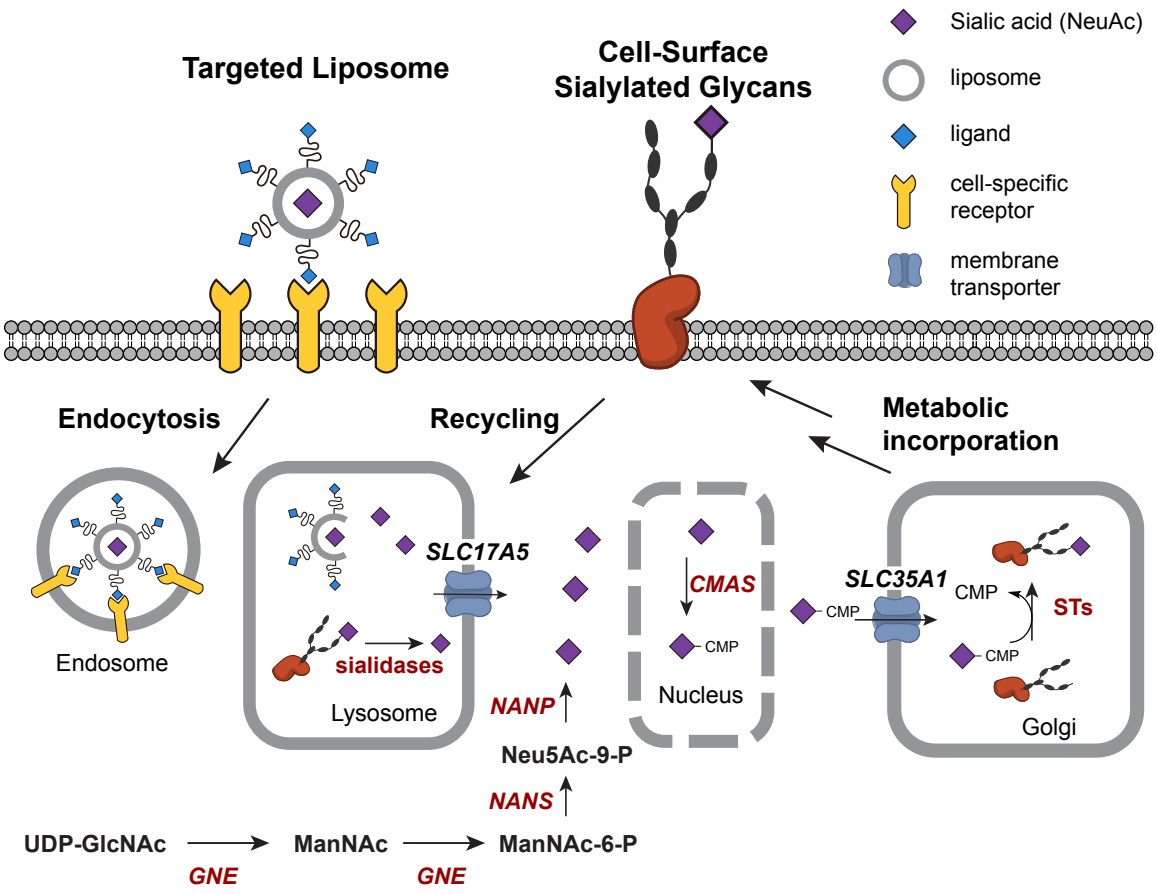

Figure S1. Overview of the *de novo* biosynthetic pathway and salvage pathway of sialic acid. UDP-GlcNAc 2-Epimerase/ManNAc Kinase (*GNE*); NeuAc 9-phosphate synthase (*NANS*); NeuAc 9-phosphate phosphatase (*NANP*); CMP-sialic acid synthase (*CMAS*); STs, sialyltransferases. Uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc); *N*-acetylmannosamine (ManNAc); ManNAc-6-phosphate (ManNAc-6-P); Neu5Ac-9-phosphate (Neu5Ac-9-P).

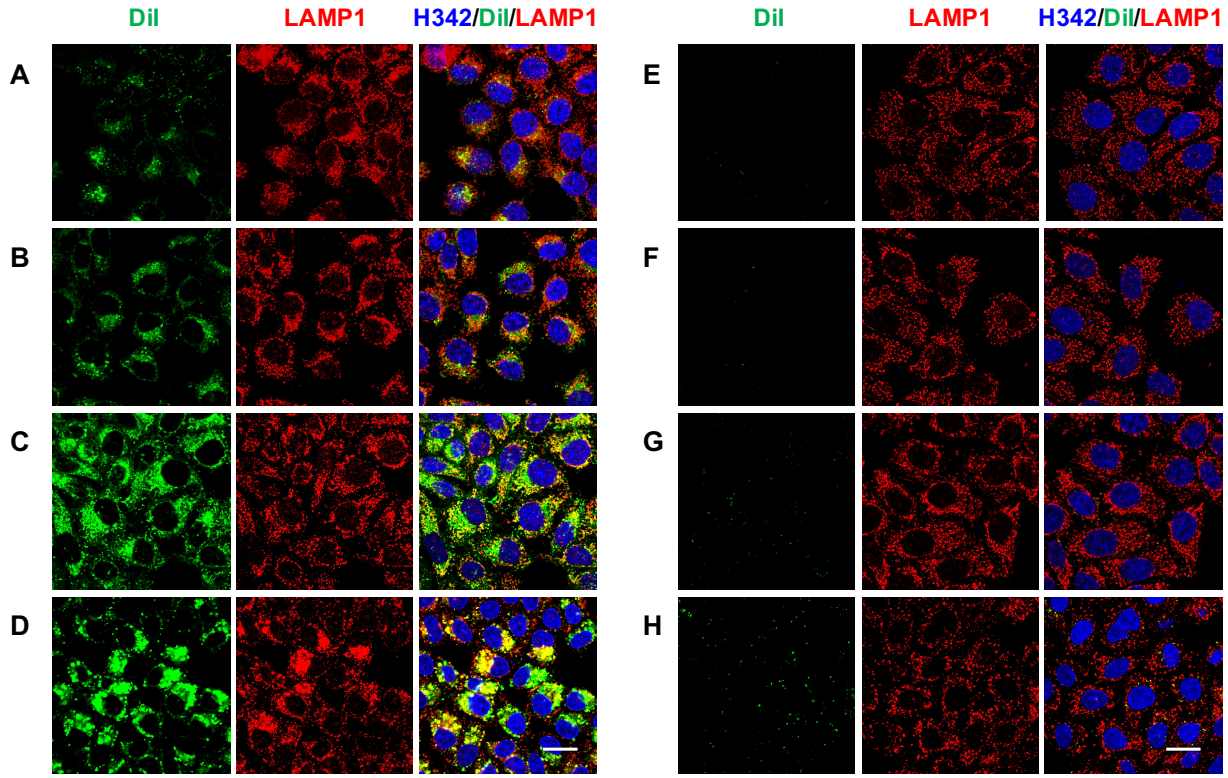

Figure S2. Co-localization of f/DiI-LP-9AzSia with lysosome. FR^+ HeLa cells were incubated with 100 μM f/DiI-LP-9AzSia (A–D) or DiI-LP-9AzSia (E–H) for 0.5 h. The cells were changed into fresh medium and incubated to the time point of 1.5 h (A, E), 3 h (B, F), 6 h (C, G) or 24 h (D, H). The cells were then fixed with 4% PFA and permeabilized with 0.1% Triton X-100, followed by immunostaining with anti-LAMP1 (red) and imaging by confocal fluorescence microscopy. The liposomes were tracked with DiI (green), and the nuclei were stained with Hoechst 33342 (blue). Notably, the DiI fluorescence was not observed on the cell surfaces, presumably due to loss of cell surface-bound liposomes during fixation and permeabilization. Scale bar, 20 μm.

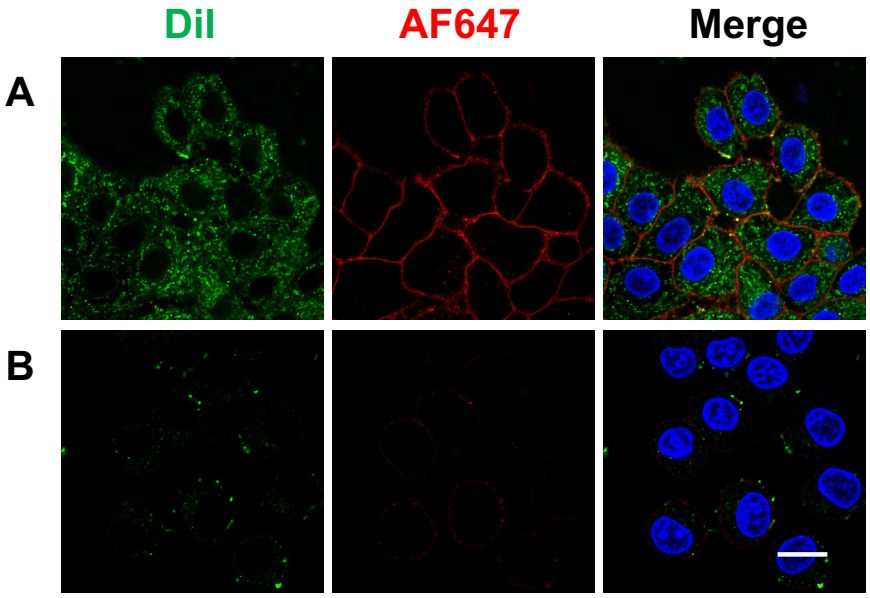

Figure S3. Fluorescence imaging of cell-surface sialoglycans labeled with 9AzSia. FR^+ HeLa cells were incubated with 100 μM f/DiI-LP-9AzSia (A) or DiI-LP-9AzSia (B) for 24 h. After reaction with alkyne-biotin and staining with streptavidin-AF647, the cells were imaged by confocal fluorescence microscopy. The liposomes were tracked with DiI (green), and the nuclei were stained with Hoechst 33342 (blue). Scale bar, 20 μm.

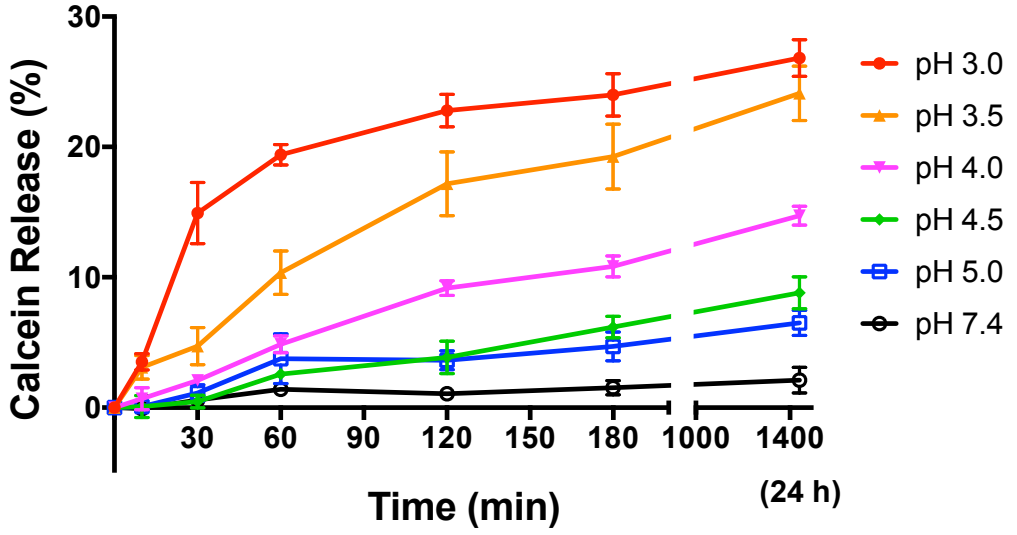

Figure S4. Release of calcein from liposomes at various pH values. The 40 μM LP-calcein (300 mM calcein inside liposomes) solutions were incubated at 37 ºC at varied pH values for a spectrum of time points with a final lipid concentration of 100 μM. Calcein fluorescence was measured at λ_{ex} = 490 and λ_{em} = 520 nm on a plate reader. The percentage of calcein release was calculated according to the following formula: $(I_{pH} - I_0)/(I_{100} - I_0) \times 100\%$, where I_0 is the fluorescence before incubation at 37 ºC, I_{pH} is the intensity measured at various pH, and I_{100} is the totally dequenched calcein fluorescence. Error bars represent ± SD.

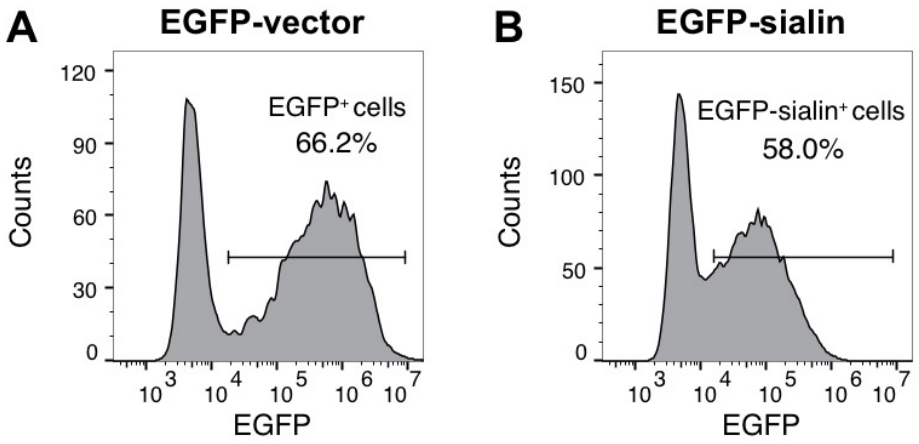

Figure S5. Transfection efficiency analysis of FR^+ HeLa cells transfected with EGFP-vector (A) and EGFP-sialin (B). The cells were transfected by electroporation and cultured for two days, followed by analysis by flow cytometry. The EGFP fluorescence was used to gate the EGFP- or EGFP-sialin-overexpressed cell populations.

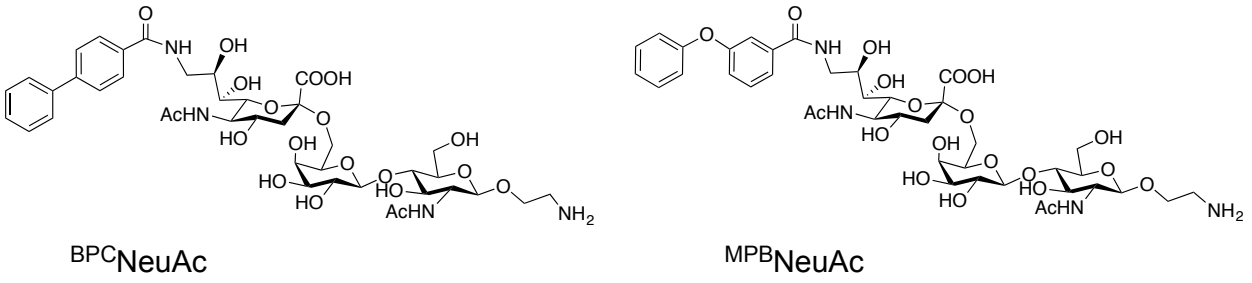

Figure S6. Chemical structures of human CD22 glycan ligands ^{BPC}NeuAc and ^{MPB}NeuAc with a β-O-linked ethylamine linker.

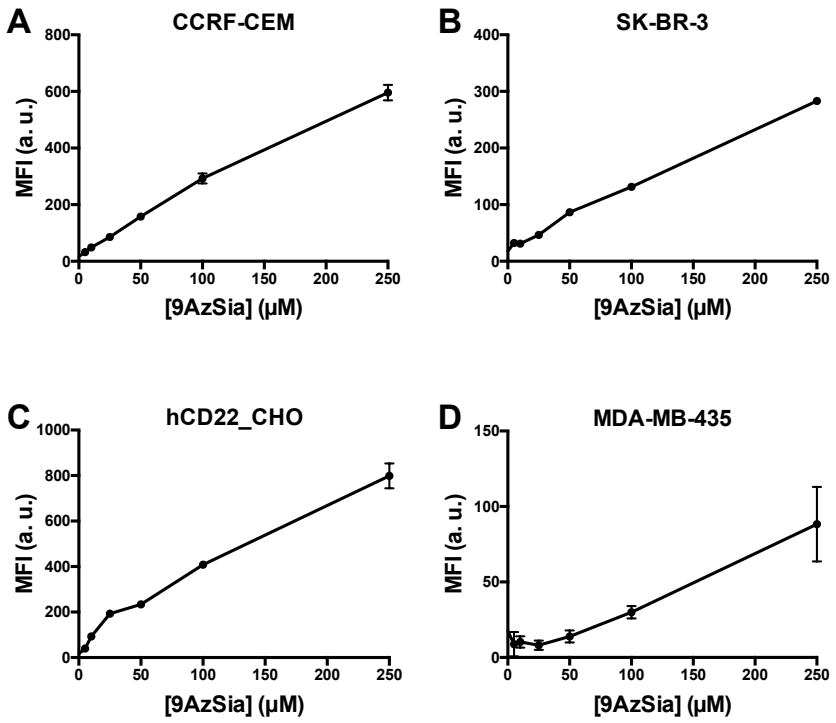

Figure S7. Metabolic incorporation of 9AzSia in CCRF-CEM (A), SK-BR-3 (B), hCD22_CHO (C), and MDA-MB-435 cells (D). The cells were incubated with 9AzSia at varied concentrations for 24 h, followed by reaction with DBCO-biotin and staining with streptavidin-AF647. The cells were then analyzed by flow cytometry. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.

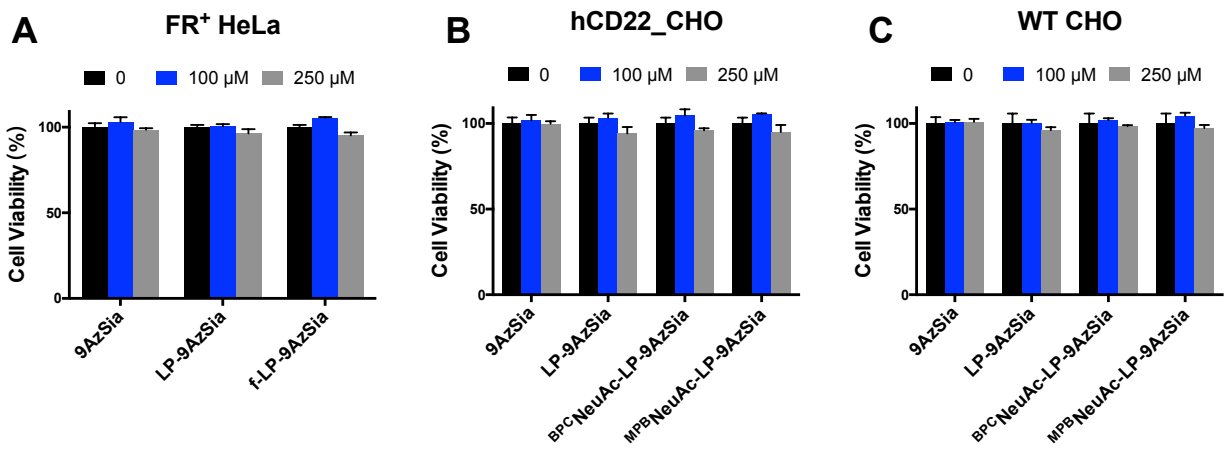

Figure S8. Cell viability analysis. FR^+ HeLa cells (A), hCD22_CHO cells (B), and WT CHO cells (C) were incubated with 9AzSia or various liposomes at varied concentrations for 24 h, followed by analysis of cell viability by the MTS assay. Error bars represent ± SD.

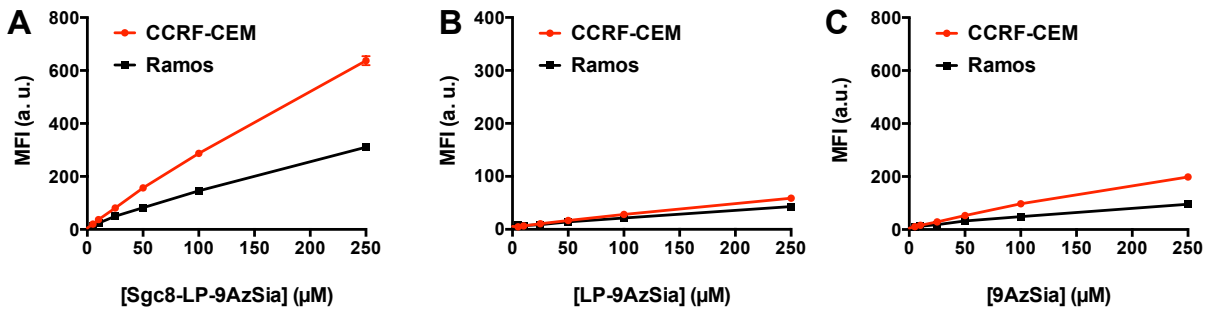

Figure S9. Cell-selective labeling of sialoglycans by Sgc8-LP-9AzSia. CCRF-CEM and Ramos cells were treated with Sgc8-LP-9AzSia (A), LP-9AzSia (B), or 9AzSia (C) at varied concentrations for 24 h. The cells were then reacted with DBCO-biotin, stained with streptavidin-AF647, and analyzed by flow cytometry. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.

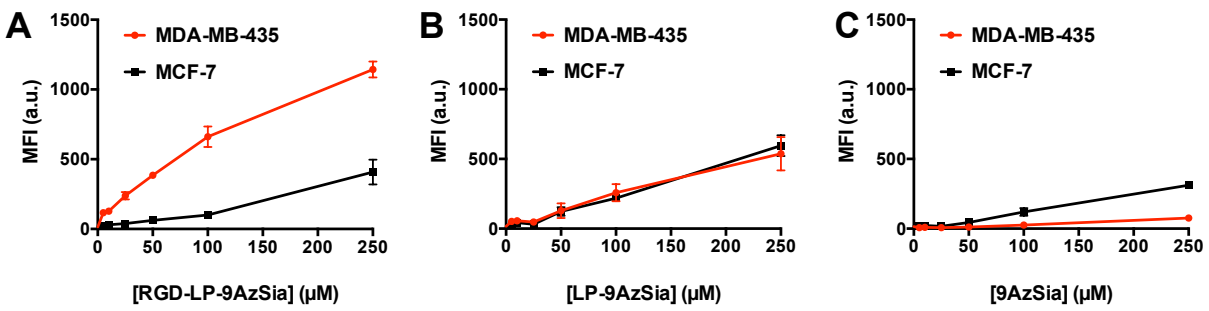

Figure S10. Cell-selective labeling of sialoglycans by RGD-LP-9AzSia. MDA-MB-435 and MCF-7 cells were treated with RGD-LP-9AzSia (A), LP-9AzSia (B), or 9AzSia (C) at varied concentrations for 24 h. The cells were then reacted with DBCO-biotin, stained with streptavidin-AF647, and analyzed by flow cytometry. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.

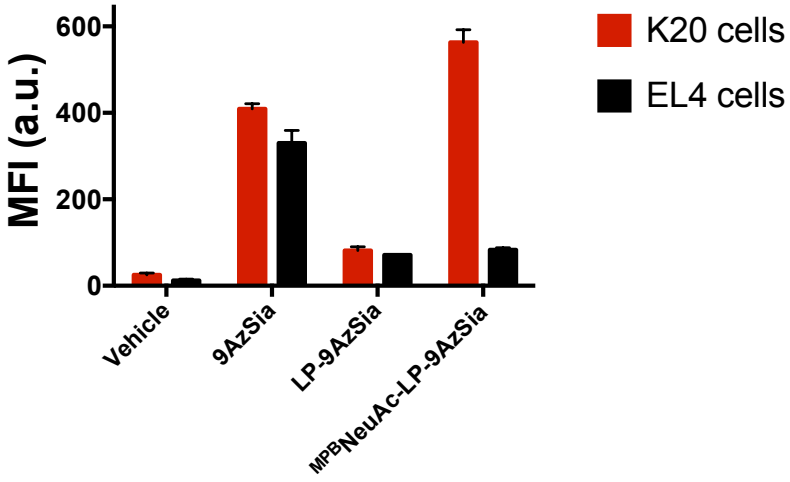

Figure S11. Flow cytometry analysis of 9AzSia metabolic labeling in K20 and EL4 cells. The cells were treated with 1 mM free 9AzSia (positive control), 100 μM LP-9AzSia or 100 μM ^{MPB}NeuAc-LP-9AzSia for 24 h. The cells were then reacted with DBCO-biotin, stained with streptavidin-AF647, and analyzed by flow cytometry. Blank sample referred to untreated cells subject to reaction with DBCO-biotin and streptavidin-AF647. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.

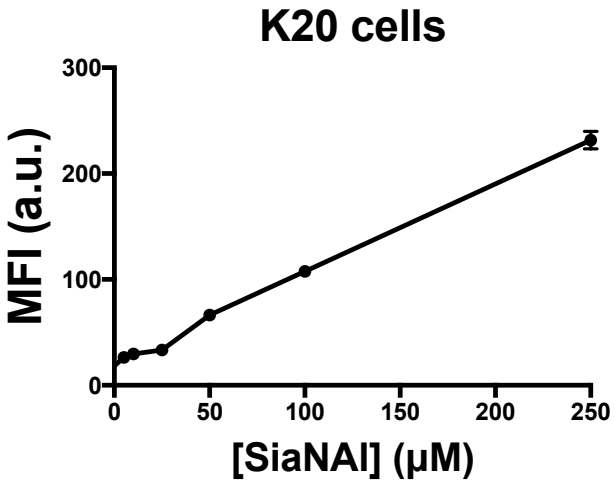

Figure S12. Metabolic incorporation of SiaNAl in K20 cells. The cells were incubated with SiaNAl at varied concentrations for 24 h, followed by reaction with 50 μM azide-AF647 via CuAAC and analyzed by flow cytometry. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.

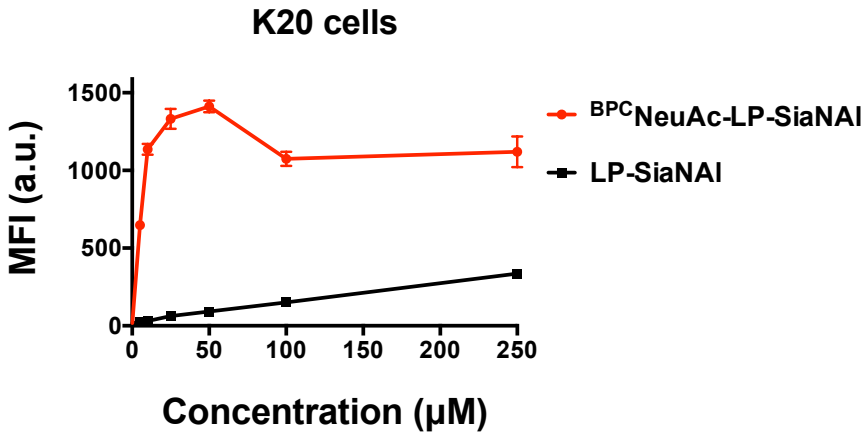

Figure S13. Metabolic incorporation of liposome-encapsulated SiaNAl in K20 cells. The cells were incubated with ^{BPC}NeuAc-LP-SiaNAl or LP-SiaNAl at varied concentrations for 24 h, followed by reaction with 50 μM azide-AF647 via CuAAC and analyzed by flow cytometry. MFI, mean fluorescence intensity. a. u., arbitrary unit. Error bars represent ± SD.