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ELECTRONIC SUPPLEMENTARY INFORMATION

Detection of mSiglec-E, in solution and expressed on the surface of Chinese hamster ovary cells, using sialic acid functionalised gold nanoparticles

Claire L. Schofield,[a] María J. Marín,[a] Martin Rejzek,[b] Paul R. Crocker,[c] Robert A. Field[b*] and David A. Russell[a*]

[a]*School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK. E-mail: d.russell@uea.ac.uk*

[b]*Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK. E-mail: rob.field@jic.ac.uk*

[c]*Division of Cell Signalling and Immunology, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK.*

Introduction

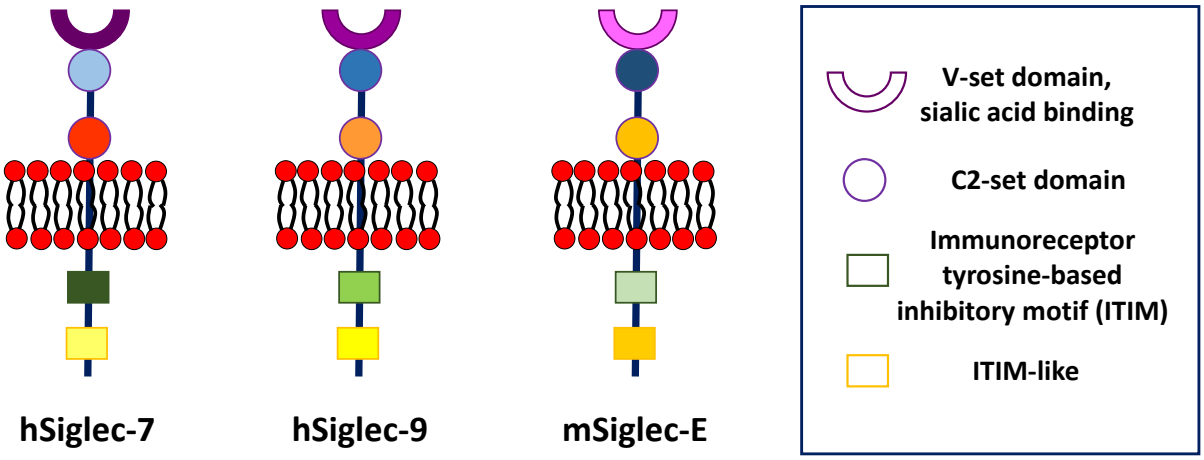

Fig. S1 Schematic representation of three *s*ialic acid-binding *i*mmuno*g*lobulin-like *lec*tins (Siglecs): human Siglec-7 (hSiglec-7), human Siglec-9 (hSiglec-9) and murine Siglec-E (mSiglec-E). The three Siglecs contain one V-set immunoglobulin domain for the binding of sialic acid and two C2-set immunoglobulin domains.[1] Both the V-set and the C2-set domains contain invariant amino acid residues that contribute to the sequence similarity between these Siglecs (indicated by the same colour family) and highly variable peptide sequences (indicated by the difference in colour). Refer to Crocker and Zhang for more information regarding sequence similarities and differences of the Siglecs represented in this figure.[2]

Materials and methods

General methods

All reagents were obtained from Acros (UK), Sigma Aldrich (UK), Alfa Aesar (UK) and Fluka (UK), and used without further purification. Thin layer chromatography was performed on precoated silica plates (Merck 60 F254, 0.25 mm) containing a fluorescence indicator. Compounds were visualised under UV light (254 nm) and by heating after dipping in a solution of 5% sulfuric acid in ethanol, in a solution of ninhydrin (200 mg) in butanol (95 mL) and acetic acid (10%, 5 mL) or in 15 % aqueous sulfuric acid saturated with cerium (IV) sulfate. Flash column chromatography was performed on silica gel (Biotage KP-SIL 60A, 40–63 μm). Standard column chromatography was performed on silica gel (Fluka 60, 63-200 μm). NMR spectra were recorded on a Varian spectrometer at 400 MHz (^{1}H) or 100 MHz (^{13}C). ^{1}H NMR spectra recorded at 400 MHz were referenced to δ_H 7.26 for $CDCl_3$ and δ_H 3.34 for CD_3OD. ^{13}C NMR spectra recorded at 100 MHz were referenced to δ_C 77.0 for $CDCl_3$ and δ_C 49.05 for CD_3OD. Assignments were made with the aid of COSY and HSQC experiments. Multiplicity of signals in ^{13}C NMR spectra was determined from HSQC spectra. Optical rotations were measured at ambient temperature on a Perkin-Elmer model 141 polarimeter using a sodium lamp. Accurate mass electrospray ionization mass spectra (ESI-MS) were obtained from the EPSRC National Mass Spectrometry Service Centre, Swansea (UK) using positive ionization mode on a Finnigan MAT 900 XLT mass spectrometer. The gold nanoparticles were purified by centrifugation using a Beckman Avanti J-25 centrifuge. UV-Visible spectra were recorded using a Hitachi U-3010 UV-Visible spectrometer at room temperature. Quartz cuvettes with a 1 cm path length were used. Transmission electron microscopy images of the glyconanoparticles and of the cells were obtained using a Jeol 2000EX transmission electron microscope.

Synthesis of the S-linked sialic acid derivative ligand 1

S-linked sialic acid derivative ligand **1** was synthesised following a previously reported protocol.[3] Briefly, the mono-*N*-Boc-protected diamine **3** was synthesised starting from *N*-Boc-2,2'-(ethylenedioxy)bis(ethylamine) following a reported protocol.[4] Compound **3** was then reacted with iodoacetic anhydride to give the corresponding iodoacetamide compound **4**. Chemoselective de-S-acetylation of the known α-thioacetate **5**[5, 6] under low temperature

Zemplén conditions followed by low temperature quenching by acidic (H^+) resin generated the corresponding α-configured sialic acid thiol.[5, 7] The thiol was alkylated with compound **4** in dichloromethane in the presence of Hunig's base[8] yielding the thioglycoside **6**. Global deprotection and subsequent reaction of the resulting free amine with γ-thiobutyrolactone in a buffered (pH *ca.* 9.0) ethanolic solution[9] afforded the sodium salt of **1**, which upon acidification gave the desired monovalent sialic acid derivative ligand **1**.

Characterisation of the thiolated polyethylene glycol derivative ligand (PEG ligand 2)

*Characterisation of the t-butyl 2-(2-(2-(2-iodoacetamido)ethoxy)ethoxy)ethylcarbamate (**4**)*

^{1}H NMR (400 MHz; $CDCl_3$): δ = 8.09 and 6.77 (2 bs, 1H, C-N rotamers N*H*C(O)CH_2), 5.55 and 5.01 (2 bs, 1H, C-N rotamers N*H*C(O)O), 3.66 (s, 2H, $H_2$1), 3.57 (bs, 4H, $H_2$5, $H_2$6), 3.51 (t, 4H, $^3J_{3,4}$ = $^3J_{7,8}$ = 4.4 Hz, $H_2$4, $H_2$7), 3.43-3.39 (m, 2H, $H_2$3), 3.29-3.22 (m, 2H, $H_2$8), 1.40 (s, 9H, $(CH_3)_3CO$); ^{13}C NMR (100 MHz; $CDCl_3$): δ = 167.6 (s, 1C, C2), 156.3 (s, 1C, C9), 79.5 (s, 1C, $(CH_3)_3CO$), 70.7, 70.5, 70.4, 69.6 (4xt, 4C, C4, C5, C6, C7), 40.6 (t, 1C, C8), 40.4 (t, 1C, C3), 28.7 (3xq, 3C, $(CH_3)_3CO$), -0.3 (t, 1C, C1); m/z (CI^+) 434 ($[M+NH_4]^+$, 12 %), 417 ($[M+H]^+$, 68%), 217 (100%); HR-MS calcd for $C_{13}H_{26}IN_2O_5^+$ $[M+H]^+$ 417.0881, found 417.0882.

*Characterisation of the N-(2-(2-(2-(2-hydroxyacetamido)ethoxy)ethoxy)ethyl)-4-mercaptobutanamide (**2**)*

R_f = 0.28 (chloroform/methanol 10:1); ^{1}H NMR (400 MHz; CD_3OD): δ = 8.03 and 7.93 (2bs, 2H, 2 x N*H*C(O)CH_2), 3.97 (s, 2H, $H_2$1), 3.63-3.51 (m, 9H, $H_2$4, $H_2$5, $H_2$6, $H_2$7, O*H*), 3.46-3.41, 3.37-3.33 (2m, 4H, $H_2$3, $H_2$8), 2.51-2.48 (m, 2H, $H_2$12), 2.33-2.28 (m, 2H, $H_2$10), 1.90-1.82 (m, 2H, $H_2$11); ^{13}C NMR (100 MHz; CD_3OD): δ = 176.4, 176.1 (2xs, 1C, C-N rotamers C9), 164.1, 163.8 (2xs, 1C, C-N rotamers C2), 72.2, 72.1, 71.5, 71.4 (4xt, 4C, C4, C5, C6, C7), 63.4 (t, 1C, C1), 41.1, 40.5 (2xt, 2C, C3, C8), 36.3 (t, 1C, C10), 32.1 (t, 1C, C11), 25.3 (t, 1C, C12); m/z (ESI^+) 331 ($[M+Na]^+$, 100%), 309 ($[M+H]^+$, 5%), 112 (100%); HR-MS calcd for $C_{12}H_{25}N_2O_5S^+$ $[M+H]^+$ 309.1479, found 309.1480.

Results and discussion

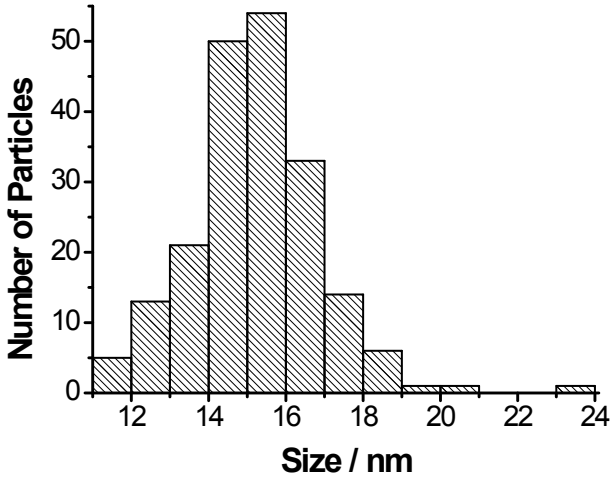

Fig. S2 Size distribution of the glyconanoparticles **1** with an average size 14.9 ± 1.7 nm (n = 200).

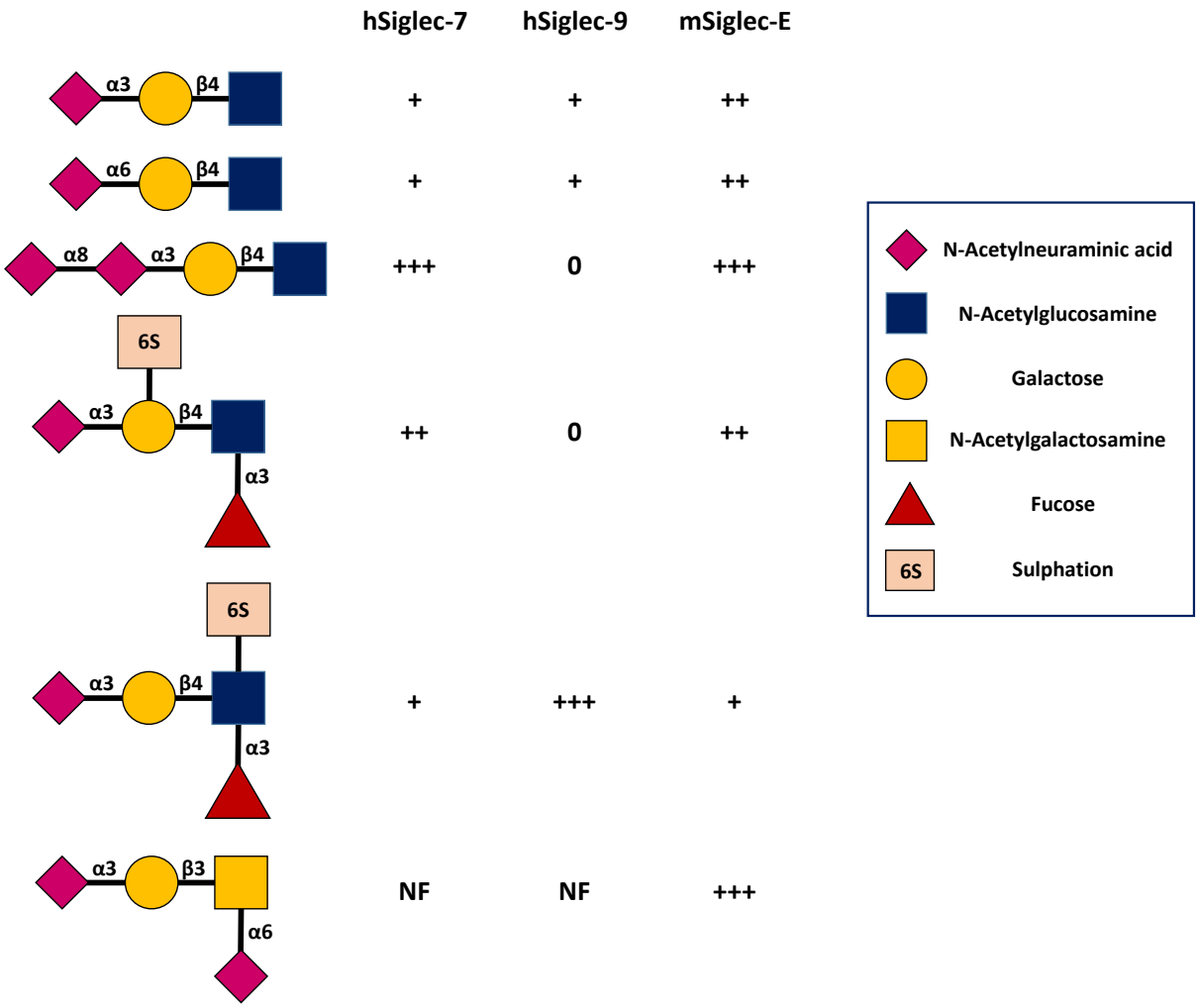

Fig. S3 Sialoside preference of hSiglec-7, hSiglec-9 and mSiglec-E.[1, 10-12] +++: strong binding; ++: moderate binding; +: low binding; 0: no detectable binding; NF: not found in the literature.

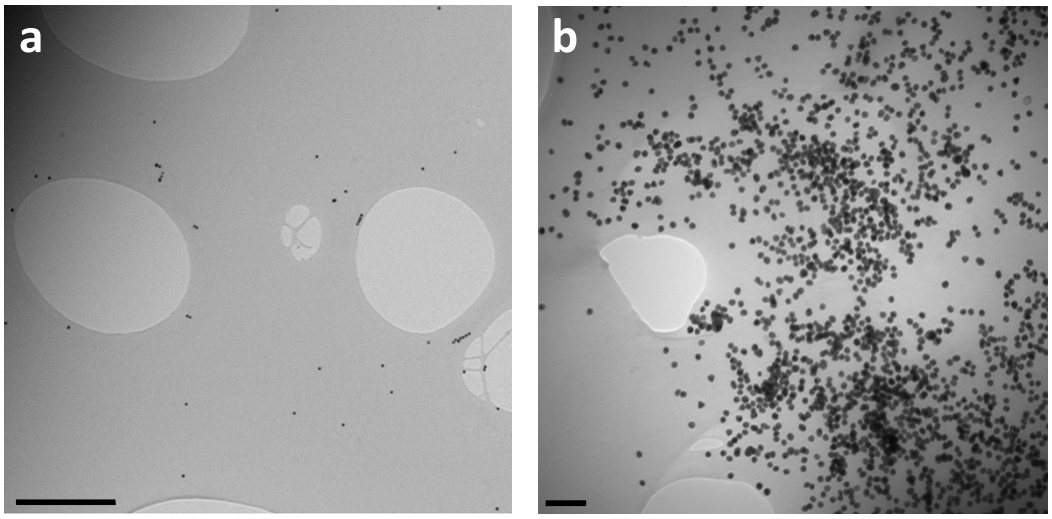

Fig. S4 Transmission electron micrographs of a sample of the glyconanoparticles **1**: **a)** before and **b)** after addition of 2.35 µg of mSiglec-E-Fc/antibody complex. The scale bars represent **a)** 500 nm and **b)** 100 nm.

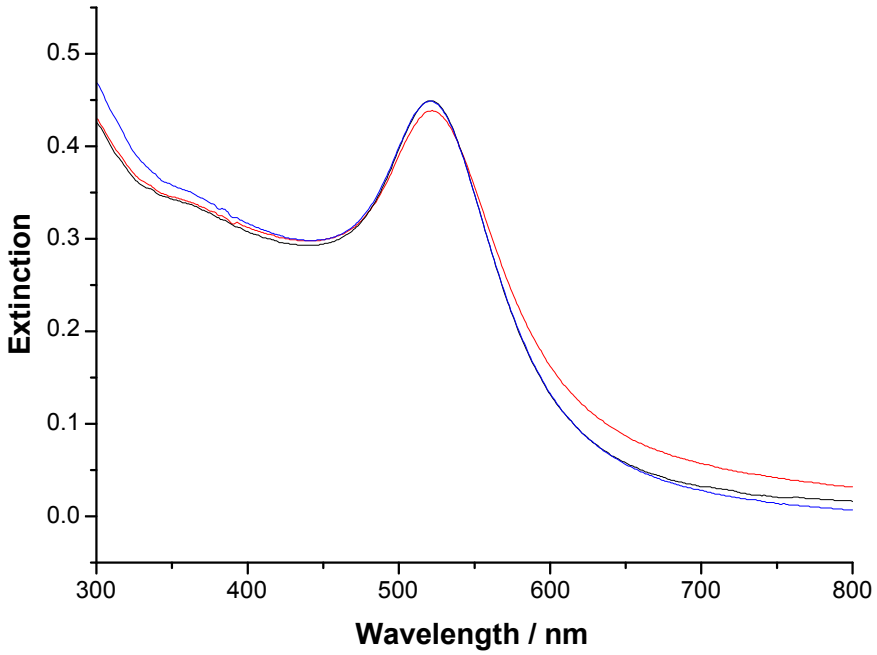

Fig. S5 UV-Vis extinction spectra of glyconanoparticles **1** before (black line) and following addition of 2.35 µg mSiglec-E-Fc/antibody complex (red line) and 2.35 µg hSiglec-7-Fc/antibody complex (blue line).

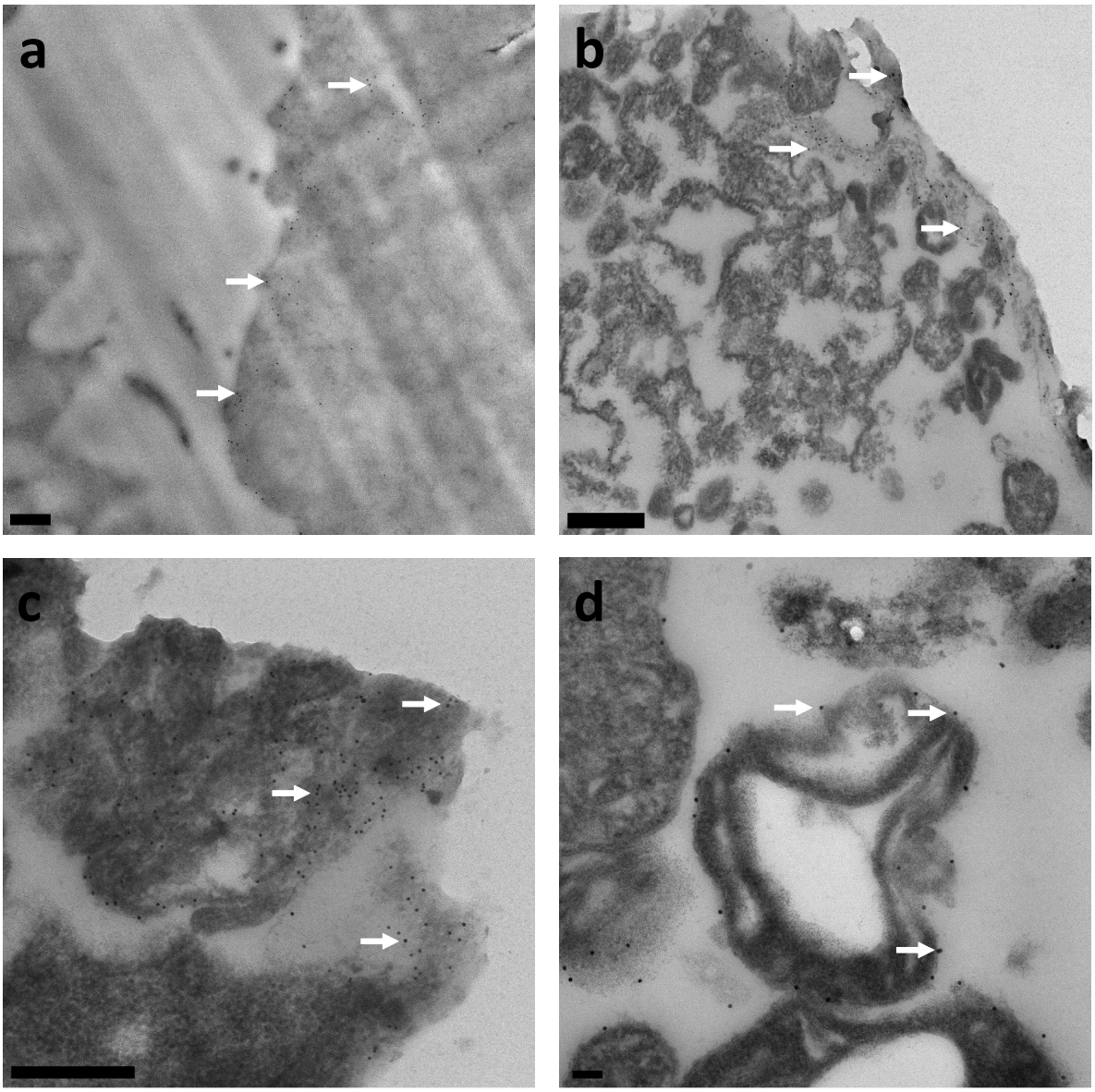

Fig. S6 TEM images of stained sample of CHO cells expressing mSiglec-E in the presence of glyconanoparticles **1**: **a)** scale bar: 500 nm, magnification: 4000x; **b)** scale bar: 500 nm, magnification: 7500x; **c)** scale bar: 500 nm, magnification: 12000x; and **d)** scale bar: 100 nm, magnification: 15000x. White arrows highlight some of the glyconanoparticles bound to the cell surface.

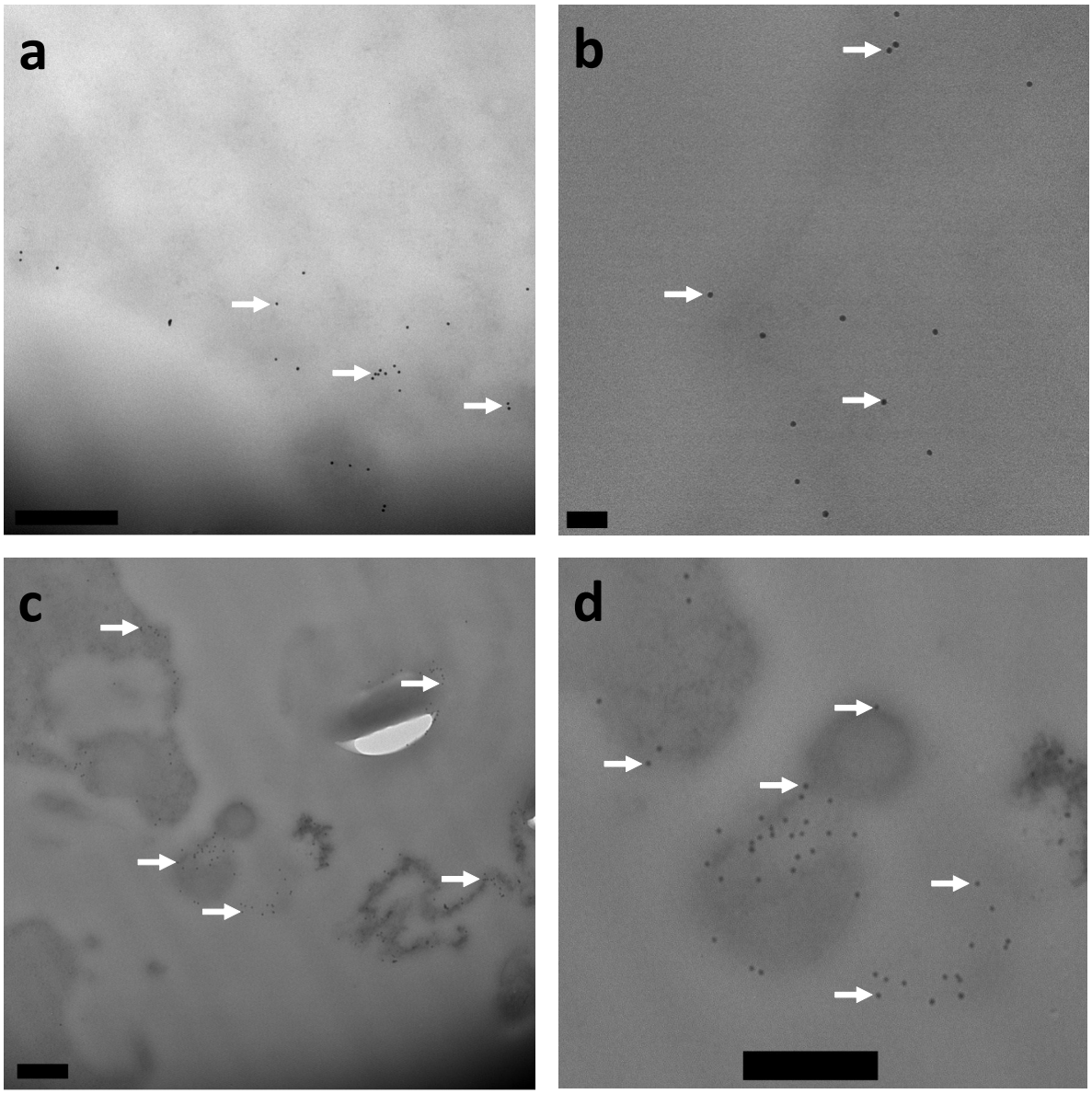

Fig. S7 TEM images of non-stained sample of CHO cells expressing mSiglec-E in the presence of glyconanoparticles **1**: **a)** scale bar: 500 nm, magnification: 10000x; **b)** scale bar: 100 nm, magnification: 20000x; **c)** scale bar: 500 nm, magnification: 5000x; and **d)** focused image of a selected area of **c)**, scale bar: 500 nm. White arrows highlight some of the glyconanoparticles bound to the cell surface.

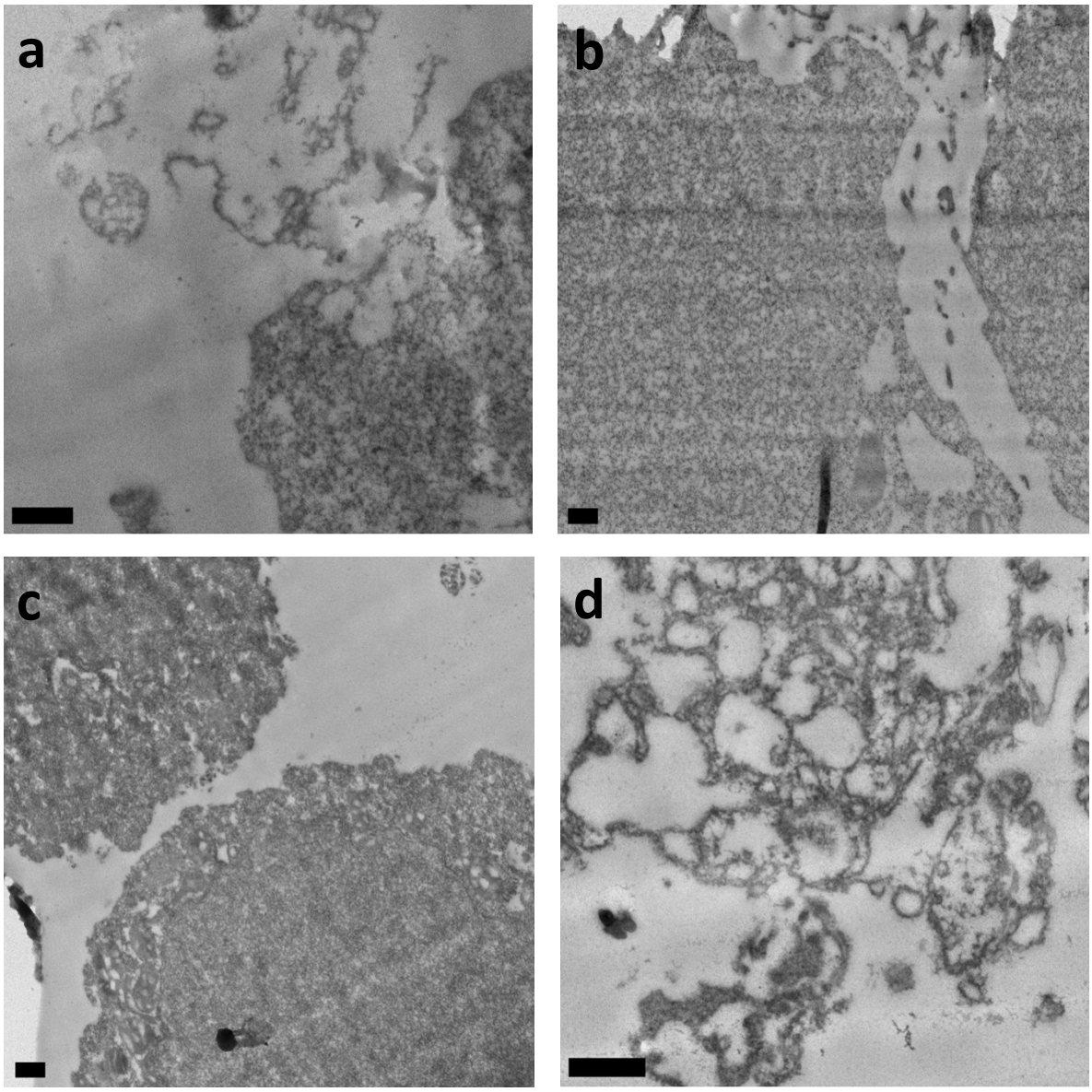

Fig. S8 TEM images of stained sample of wild-type CHO cells in the presence of glyconanoparticles **1**: **a)** scale bar: 500 nm, magnification: 6000x; **b)** scale bar: 500 nm, magnification: 3000x; **c)** scale bar: 500 nm, magnification: 3000x; and **d)** scale bar: 500 nm, magnification: 7500x. No bound glyconanoparticles are observed.

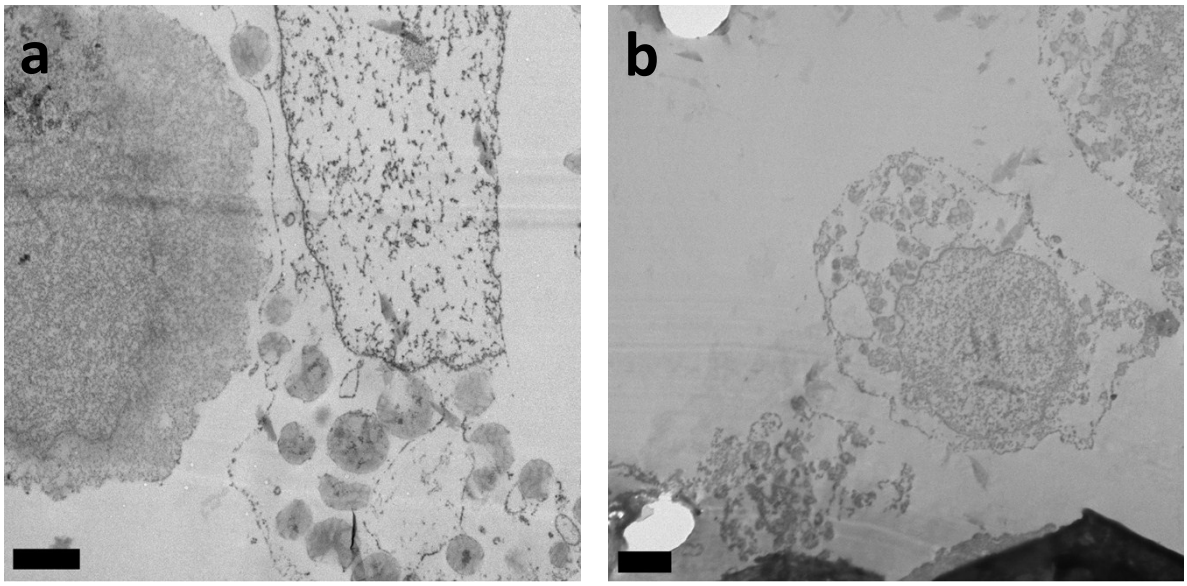

Fig. S9 TEM images of stained sample of CHO cells expressing mSiglec-E without nanoparticles present: **a)** scale bar: 2 µm, magnification: 1500x; and **b)** scale bar: 2 µm, magnification: 1200x.

References

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