nature portfolio

https://doi.org/10.1038/s41551-021-00777-y

Supplementary information

Prevention of vascular-allograft rejection by protecting the endothelial glycocalyx with immunosuppressive polymers

In the format provided by the authors and unedited

Contents

List of abbreviations

CIS	cold ischemia storage
CSE	cell-surface engineering
CAR-T cell	chimeric antigen receptor T cell
CuAAc	Cu(I) catalyzed azide alkyne cycloaddition
DAMPs	danger associated molecular patterns
DGF	delayed graft function
gtTGase	guinea pig liver tissue transglutaminase
H&E	hematoxylin and eosin
HLA	human leukocyte antigen
IRI	ischemia-reperfusion injury
LDH	lactate dehydrogenase
LPG	linear polyglycerol
NK cells	natural killer cells
PBMCs	human peripheral blood mononuclear cells
RNA-seq	RNA sequencing
ROS	reactive oxygen species
SCS	static cold storage
Siglec	sialic acid–binding, immunoglobulin (Ig)-like lectin
TLR	toll-like receptor
UT	Untreated
UW solution	organ preservation solution
WGA	wheat germ agglutinin

1.1 Materials

All chemicals were purchased from Sigma-Aldrich (ON, Canada) unless otherwise mentioned. University of Wisconsin (UW) solution was purchased from Organ Recovery Systems (Itasca, IL, USA). Blood was collected from healthy and consenting donors at the Centre for Blood Research with protocol approval from the University of British Columbia clinical ethics committee in vials containing EDTA or sodium citrate. All peptides were purchased fully characterized (RP-HPLC, ESI-MS) by Canpeptide Inc. (Montreal, QC, Canada) and used without further purification. Guinea pig tissue transglutaminase and Histopaque®-1077 were purchased from Sigma-Aldrich Canada. Recombinant human TNF alpha protein was purchased from Abcam and recombinant human IL-2 was purchased from Cedarlane. Anti–SHP-1 mAb, Human IL-10 DuoSet ELISA Kit, Human IL-6 Quantikine ELISA Kit and Proteome Profiler, Panel A were obtained from R&D Systems. EasySep™ isolation kit for CD56+ cells was obtained from STEMCELL Technologies. Antibodies were obtained as follows; PE-Cy™5 mouse anti-human CD54, FITC-conjugated anti-human CD45, APC-conjugated anti–CD8a mAb and FITC-conjugated anti–CD69 mAb are from BD Biosciences; FITC-conjugated anti-human CD8a is from STEMCELL Technologies; CellTracker™ Green (CTG) CMFDA Dye is from Invitrogen; APC-conjugated anti-human siglec-7 is from Biolegend; and CellMask™ deep red plasma membrane stain, Anti-Human CD8+ Dynabeads and rabbit anti-goat Dylight®800 secondary antibody are from Thermo Scientific.

1.2 Cell culture materials

All cell culture-related media and supplements (Trypsin-EDTA, Dulbecco's phosphate-buffered saline (DPBS), HI fetal bovine serum (FBS), penicillin/streptomycin (P/S), and Dulbecco's modified eagle medium (DMEM)) were received from Life Technologies Inc. unless otherwise specified. Ea.hy926 cells were purchased from American Type Culture Collection (ATCC CRL-2922 Manassas, VA) and used up to a passage number of 50. The cells were cultured using DMEM cell media containing 10% FBS and 1% P/S in tissue culture treated T-75 flasks at 37 °C and 5% CO_2. Upon reaching 70% confluence, cells were dissociated with 0.25% trypsin and 0.05% EDTA (Gibco, 25300062), pelleted by centrifugation at 300 *g* and resuspended with complete DMEM medium. HMEC-1 cells were obtained from ATCC and used up to a passage number of 20. The cells were cultured using MCDB 131cell media containing 10 ng/mL epidermal growth factor, 1 µg/mL hydrocortisone, 10 mM glutamine, 10% FBS and 1% P/S in tissue culture treated T-75 flasks at 37 ºC and 5% CO_2. HUVEC cells were obtained from ATCC and used up to a passage number of 10. The cells were cultured using EBM-2 basal media (CC-3156, Lonza) completed with EGM-2 SingleQuots Supplements (CC-4176, Lonza) in tissue culture treated T-75 flasks at 37 ºC and 5% CO_2. Purified human $CD8^+$ T cells (from two donors) were activated and transduced with lentivirus encoding an HLA-A2-specific CAR and a truncated nerve growth factor receptor (DNGFR) as described.[1] DNGFR-positive cells were isolated using magnetic bead sorting, and cryopreserved until use.

1.3 Techniques

All reactions with air and/or water sensitive reagents were performed in a Schlenk flask under dry argon atmosphere. Absolute molecular weights of the polymers were determined by Gel Permeation Chromatography (GPC) on a Waters e2695 separation module fitted with a DAWN HELEOS-II multiangle laser light scattering (MALLS) detector coupled with Optilab T-rEX refractive index detector, both from Wyatt Technology. GPC analysis was performed using Waters ultrahydrogel 7.8 x 300 columns (guard 250 and 120) and 0.1 N $NaNO_3$ at pH 8.5 (10 mM phosphate buffer) as the mobile phase. The dn/dc (0.12) for polyglycerols was used from previously measured literature values.[2] ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer using deuterated solvents (Cambridge Isotope Laboratories, 99.8% D). NMR spectra were obtained by scanning 128 times at atmospheric conditions. Chemical shifts were referenced to the residual solvent peak. The ACD/NMR processor spectroscopic software was used for data handling. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Bruker TENSOR II FTIR spectrometer with a resolution of 4 cm^{-1}. Polymer samples were incorporated into a KBr pellet prior to analysis.

FTIR spectra were obtained by scanning 64 times at atmospheric conditions. The OPUS spectroscopic software was used for data handling. Absorbance and fluorescence readings were acquired using a SpectraMAX multi-mode M3 plate reader from Molecular Devices LLC. Flow cytometry profiles were acquired using a 3-laser CytoFLEX flow cytometer from Beckman Coulter Life Sciences (10,000 events).

Chemical synthesis of linear polyglycerol and its modification:

1.4 Preparation of ethoxy ethyl glycidyl ether monomer (EEGE).

In a round bottom flask, dry glycidol (40 mL, 0.63 mol) was dissolved in ethyl vinyl ether (250 mL, 2.19 mol) and the solution was cooled in an ice bath to 0 °C. Under constant stirring, *p*-toluenesulfonic acid (1.15 g, 5.8 mmol) was added portion wise, maintaining the temperature below 20 °C. The reaction was allowed to reach room temperature after the addition of *p*-toluenesulfonic acid was done and stirred for 4 h. The reaction was then quenched by addition of 100 mL saturated $NaHCO_3$ solution. The organic phase was then separated and dried over Na_2SO_4. Fractionated vacuum distillation yielded the product as a colorless liquid (percent yield = 90%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.9(m, 1 H), 3.76-3.32 (m, 4 H), 3.07 (m, 1 H), 2.73 (m, 1 H,), 2.53 (m, 1 H *J* = 5.03, 2.74 Hz), 1.25 (t, 3 H, *J* = 6.0 Hz), 1.1 ppm (t, 3 H, *J* = 6.0 Hz)

1.5 Preparation of α-azido linear polyglycerol (N_3-LPG)

The preparation of linear polyglycerol was carried out according to the previously published method by Carlotti, S. et al.[3] Tetrabutylammonium azide (0.11 g, 0.39 mmol) was added to a flame dried Schlenk flask and dried under reduced pressure for three hours at 90 °C. Toluene (5 mL) and EEGE monomer (16.5 mL, 0.11 mol) were added via syringe under argon. The reaction mixture was cooled down in an ice/salt bath to -10 °C and activated by the fast addition of triisobutylaluminum (TIBA, 1 M in hexane, 1.60 mL, 1.59 mmol). After addition of TIBA, the mixture was stirred overnight, returning to room temperature. Upon reaction completion, 1 mL of methanol was used to quench the reaction and the solution was stirred for another 30 minutes. Removal of aluminum as the insoluble $Al(OH)_3$ salt was carried out by cooling the solution to 0 °C followed by the addition sodium hydroxide solution (15 wt. %). The resulting solution was dried using $MgSO_4$, followed by filtration and removal of the solvent *in vacuo.* The viscous, light yellow residue was characterized by ^{1}H-NMR and carried forward without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.70 (m, 1 H), 3.70 - 3.41 (m, 7 H), 1.29-1.14 (m, 6 H)

In order to remove the protecting group, the polymer was dissolved in a solution of 3.7% HCl in ethanol (100 mg/mL) and left overnight to deprotect. The light yellow solution was neutralized with saturated $NaHCO_3$, dialyzed immediately against 1K MWCO tubing and lyophilized to generate α-azido linear polyglycerol (LPG-N_3).

^{1}H-NMR (300 MHz, D_2O): δ=3.72-3.59 ppm (m, LPG backbone, 5H).

M_n = 14, 900 Da, M_w/M_n = 1.23.

1.6 Preparation of propargylated polyglycerols (N_3-LPG-Alkyne)

LPG-N_3 (14,900 Da, 830 mg, 0.06 mmol) was added to a 50 mL flame dried Schlenk flask, dried overnight at 50 ºC and purged in argon. The polymer was subsequently dissolved in 33 mL anhydrous dimethylformamide (DMF). After complete dissolution of the polymer, NaH (96 mg, 3.9 mmol) was added in three separate batches under vigorous stirring to afford a cloudy solution. The temperature was elevated to 65ºC and allowed to react for 3 hours under argon. Propargyl bromide (330 uL, 3.0 mmol) was added dropwise to the solution and the mixture was allowed to react for 24 hours under argon. Methanol was added to quench the reaction and the mixture was precipitated 2 times in cold ether. The precipitate was collected via centrifugation. The pellet was then re-dissolved and dialyzed against 1K MWCO tubing in MeOH. Following dialysis, the residual MeOH was removed *in vacuo* and subjected to structural analysis.

^{1}H-NMR (300 MHz, MeOD): δ= 4.17 pp (m, C$\underline{H_2}$C≡CH, 2H) δ=3.66-3.51 ppm (m, LPG backbone, 5H). ~35 alkynes per LPG (71% conversion)

1.7 Preparation of α-amino propargylated linear polyglycerol (NH_2-LPG-Alkyne)

N_3-LPG-Alkyne (650 mg, 0.045 mmol) was dissolved in 15 mL pyridine and PPh_3 (140 mg, 0.52 mmol) was added to the solution. The reaction was stirred vigorously under ambient conditions for 48 hours. Upon reaction completion, one volume equivalent of methanol was added to the flask and the solution was dialyzed against 1 K MWCO tubing in methanol and dried *in vacuo* to afford the product. Conversion of azide to amine was confirmed using fourier transform infrared spectroscopy (FTIR).

1.8 Attachment of Q-tag to NH_2-LPG-Alkyne (LPG-Alkyne-Q)

NH_2-LPG-Alkyne (865 mg, 0.06 mmol NH_2 groups) was added to a 50 mL flame dried Schlenk flask (Flask A) dried overnight and purged in argon. The polymer was subsequently dissolved in 9 mL anhydrous DMF. Another 4 mL of DMF was added to another flame dried flask (Flask B). Trityl (trt) protected peptide, Ac-GQ(trt)Q(trt)Q(trt)LGGGGG-OH (0.072 mmol), 1-hydroxybenzotriazole (HOBt, 11 mg, 0.072 mmol), benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP 32 mg, 0.072 mmol), and N-ethyldiisopropylamine (DIPEA 60 μL, 0.72 mmol) was added to flask B and allowed to stir for 30 min at room temperature, after which the contents were transferred to Flask A by cannula over 5 minutes with 1 mL DMF wash. The new mixture in Flask A was left at room temperature under argon for 48 hours. Acetic anhydride (17 μL, 0.18mmol) was added to quench the reaction and the mixture was stirred for 60 min. The final mixture was precipitated 3 times in cold ether:acetone (50:50 v/v) and the precipitate was collected via centrifugation. The trt protected polymer-peptide conjugate was dissolved in a deprotection mixture containing trifluoroacetic acid (TFA) and triisopropylsilane (TIS) in water (92:6:2 TFA:TIS:H_2O v/v) to a final concentration of 50 mg/mL and the solution was left to react for 3 hours at room temperature. Upon reaction completion, the solution was dried *in vacuo* to remove TFA dissolved in 5 mL methanol and centrifuged to remove any insoluble ppt. The supernatant was dialyzed (2 MWCO) in methanol overnight, followed by water for a further 24 hours filtered and freeze dried. Conjugates were characterized by ^{1}H NMR.

^{1}H NMR (300 MHz, MeOD): δ=3.92-3.59 ppm (m, LPG backbone, 5H), δ=0.91-0.89 ppm (m, leucine -CH_3, 6H)

1.9 Fluorescent tag modification of LPG-Alkyne-Q

Aldehyde groups were generated on the LPG-Alkyne-Q terminus through the oxidation of 1,2 diol groups on one end of linear polyglycerol polymer scaffold using $NaIO_4$. LPG-Alkyne-Q (190 mg, 0.010 mmol) was dissolved in MES buffer (0.1 M, pH 6.5) followed by the addition of $NaIO_4$ (3.7 mg, 0.017 mmol). The solution was stirred overnight at room temperature protected from light. Following periodate oxidation, the reaction was quenched through the addition of glycerol to a final concentration of 20 mM. BODIPY hydrazide (0.012 mmol) dissolved in DMSO was added to the solution followed by the addition of an aniline stock solution (1 M in DMSO) to a final aniline concentration of 10 mM. The mixture was left to react overnight at ambient temperature in the dark. Following reaction completion, the reducing agent $NaCNBH_3$ (7.2 mg, 0.12 mmol) was added to the solution and left to stir overnight at room temperature. Following reduction, an excess of glycine was added to a final concentration of 10 mM to quench any remaining aldehyde groups and stirred for another hour. The conjugate solution was then purified through either dialysis (3.5 K MWCO) or Zeba™ spin desalting columns (7 K MWCO) until a negative silver nitrate test was obtained. This process afforded approximately 1 BODIPY-FL group per polymer (100% BODIPY-FL) as assessed by measuring the intensity of a known concentration of polymer solution against as BODIPY-FL standard curve.

1.10 Sialylation of Q-tagged polyglycerols (LPG-Q-Sia3Lac, LPG-Q)

The synthesis of clickable 2,3 and 2,6 sialyl oligosaccharides (2,3-Sia-LacC6N_3, 2,6-Sia- LacC6N_3) was carried out through enzymatic elongation with sialyltransferases according to previously published methods using 6-azidohexanyl-β-lactoside (LacC6N_3) as an acceptor.[4–6] LPG-Alkyne-Q

(115 mg, .00705 mmol) was dissolved in 4.6 mL anhydrous, degassed DMF. To the polymer solution, α2,3-Sia-LacC6N_3, α2,6-Sia-LacC6N_3 or LacC6N_3 (1.25 eq per alkyne) was added followed by a 1:1 CuBr: N,N,N′,N′′,N′′-pentamethyldiethylenetriamine (PMDETA) solution (20 mol% relative to alkyne content). The vial was purged with argon, capped and allowed to react at room temperature for 24 hours. Excess 2-azidoethanol was added after 24 hours to react with any remaining alkynes. To generate LPG-Q from LPG-Q Alkyne, 3 eq of 2-azidoethanol per alkyne was used to convert all alkyne groups to hydroxyl groups. Excess EDTA was then added to quench the reaction and the solution was dialyzed against 2 MWCO tubing for 24 hours in 1 M NaCl followed by 24 hours in water. The resulting solution was then lyophilized and subjected to structural analysis.

^{1}H NMR LPG-Q-Sia3Lac (300 MHz, D_2O): δ=1.99 ppm (s, -C$\underline{H}_3$, sialic acetyl group, 3H) δ=3.73-3.59 ppm (m, LPG backbone, 5H), δ=0.88-0.84 ppm (m, leucine -CH_3, 6H)

^{1}H NMR LPG-Q-Sia6Lac (300 MHz, D_2O): δ=1.99 ppm (s, -C$\underline{H}_3$, sialic acetyl group, 3H) δ=3.74-3.60 ppm (m, LPG backbone, 5H), δ=0.89-0.84 ppm (m, leucine -CH_3, 6H)

^{1}H NMR LPG-Q-Lac (300 MHz, D_2O): δ=1.54-1.26 ppm (m, -C$\underline{H}_2$ $\underline{CH}_2$ $\underline{CH}_2$ $\underline{CH}_2$ $\underline{CH}_2$ C$\underline{H}_2$, C6 linker, 8H) δ=3.73-3.62 ppm (m, LPG backbone, 5H), δ=0.89-0.84 ppm (m, leucine -CH_3, 6H)

1.11 Carboxy functionalization of LPG (LPG-COOH)

LPG-NH2 was generated by subjecting LPG-N3 to the protocol in section 1.6. LPG-NH_2 (672 mg, 0.047 mmol,) was dried *in vacuo* at 90 °C overnight and subsequently dissolved in anhydrous pyridine (19 mL). Ten molar equivalents of succinic anhydride (470 mg, 0.470 mmol) was added to the reaction which was then allowed to react under argon at room temperature overnight. Upon reaction completion, the polymer was precipitated from the reaction medium with cold acetone and isolated via centrifugation (14,000 *g,* 4 °C). The polymer was then re-dissolved in distilled water and dialyzed against 1K MWCO tubing, lyophilized and subjected to structural analysis.

1.12 Preparation of linear polyglycerol succinimidyl succinate (LPG-SS)

LPG-COOH (161 mg, 0.0113 mmol) and N-hydroxysuccinimide (7.97 mg, 0.0678 mmol) were re-dissolved in anhydrous DMF (9 mL) followed by the addition of N,N′-diisopropylcarbodiimide (8.75 mg, 0.0678 mmol). The solution was left to stir overnight at room temperature under argon atmosphere. Upon reaction completion, the solution was precipitated in acetone (30 mL), isolated by centrifugation (14,000 *g,* 4 °C), and dried for 10 minutes under reduced pressure. Once isolated, LPG-SS was immediately dissolved in a PBS stock solution and used for cell derivatization.

^{1}H-NMR (300 MHz, D_2O): δ=3.71-3.63 ppm (m, LPG backbone, 5H), 2.48 (s, COCH2$\underline{CH2}$CO)

1.13 General procedure for cell-surface engineering (enzymatic and chemical) of endothelial surface

Ea.hy 926 cells at 60-80% confluence were trypsinized and plated in a 96 well plate at 15,000 cells/well. Cells reached 100% confluence at day 3 and allowed to grow for a further 4 days at 37 °C and 5% CO_2. Cells were washed twice with cold DPBS and incubated with UW solution freshly supplemented with 3 mM GSH, 5 mM $CaCl_2$, 0.2 U/mL gtTGase and 0.5 mM LPG-Q or 0.56 mM glycopolymers (LPG-Q-Sia3LacLPG-Q-Sia6Lac and LPG-Q-Lac) unless otherwise noted. The solutions were mixed thoroughly and incubated at 4 °C for 30 minutes under static conditions. The cell supernatant was collected and the cells were washed three times with PBS and subjected to further analysis. The assessment of polymer attachment to endothelial cells are assessed by ammonia assay, flow cytometry and polymer mediated cell-surface camouflage of surface proteins. Polymer attachment using succinimidyl-succinate functionalized LPG (SS-LPG) was carried out under identical conditions without gtTGase (UW solution freshly supplemented with 3 mM GSH, 5 mM $CaCl_2$, and 0.5 mM SS-LPG).

1.14 Ammonia generation assay for enzyme reactivity

The extent of gtTGase ligation was assessed using an ammonia assay kit (Sigma, MAK310) and used according to the manufacturer's protocols. This kit is used for the quantitative determination of ammonia (NH_3), a side product in the gtTGase reaction.[7] This assay is based on the *o*-phthalaldehyde method in which the reagent reacts with NH_3 producing a fluorescent product (λ_{ex} = 360 nm /λ_{em} = 450nm), proportional to the NH_3 concentration in the sample.[8,9] Briefly, PBS was supplemented with 5 mM $CaCl_2$ and 3 mM dithiothreitol (DTT). In a 96 well plate, containing either endothelial cells or glycine ethyl ether (GEE, amine donor), Q-tagged polymer (acyl donor) and guinea pig liver transglutaminase (gtTGase, Sigma T5398) were added to the PBS solution to a final concentration of 5 mM, 0.5 mM and 0.2 U/mL respectively (Vf = 100 μL). The reaction was thoroughly mixed and rocked gently at 4 ºC for 30 minutes. The sample was diluted four-fold and a 10 μL aliquot of the reaction mixture was then transferred to a tube containing 90 μL complete ammonia assay reagent and mixed thoroughly. The solution was left to react for 15 minutes at ambient temperature in the dark. The fluorescence intensity was measured at λ_{ex} = 360 nm /λ_{em} = 450nm using a spectrophotometer and the quantity of ammonia in solution was calculated according to Eq. 1:

$$[NH_3]\ generated = \frac{F_{sample} - F_{blank}}{slope} \qquad \text{(Eq. 1)}$$

Where the slope represents the results of a NH_4Cl standard curve generated under identical conditions to the samples and F_{blank} is the fluorescent intensity of supernatants from endothelial cells or GEE alone in the presence of enzyme.

1.15 Flow cytometry on polymer modified endothelial cells (lifetime measurements, ICAM-1 labeling)

To quantify the extent of cell surface engineering, polymers were attached to endothelial surfaces according to procedures outlined in section 1.13 using polymer in which 10% were labelled with BODIPY FL (ratio of non-labeled polymer: BODIPY labeled polymer = 9:1). The cell monolayers were trypsinized and subjected to flow cytometry (CytoFLEX, Beckman Coulter) in which 10,000 events were acquired and the median fluorescent intensity (MFI) in the 488 channel was recorded.

For lifetime measurements, polymers were attached to endothelial surfaces according to procedures outlined in section 1.13 using 0.5 mM LPG-Q (10% BODIPY FL) and exchanged into phenol-free DMEM (5% FBS, pen/strep) and incubated at 37 °C and 5% CO_2. For each time point the supernatant was removed and saved for further analysis and the cell monolayers were trypsinized and subjected to flow cytometry (CytoFLEX, Beckman Coulter) in which 10,000 events were acquired and the median fluorescent intensity (MFI) in the 488 channel was recorded. The fluorescent intensity of the BODIPY-FL Dye in the supernatant was measured (λ_{ex}/λ_{em}= 503/520 nm using) a plate reader (Spectramax i3, Molecular Devices). Intensity values were normalized to the total cell number in each well as assessed by a trypan blue staining assay.

For ICAM-1 labelling, Ea.hy926 cells were activated with TNF-α (2000 U/mL, 3 hours at 37 °C and 5% CO_2)[10] in serum deficient DMEM to stimulate the expression of ICAM-1 on the cell surface before polymer attachment. To account for differences in reactivity between Q-tagged polymers, concentrations of polymer added to the solution were adjusted using NH_3 generation data to ensure that a similar number of polymers attached to the cell surface. Following polymer attachment, the cells were fixed, and labelled with PE-Cy™5 Mouse Anti-Human CD54 (1:20, BD Biosciences) for 20 minutes at room temperature in the dark. The cell monolayers were trypsinized and subjected to flow cytometry (CytoFLEX, Beckman Coulter) in which 10,000 events were acquired and the median fluorescent intensity in the 667 channel was recorded

1.15.1 Confocal microscopy experiments

Ea.hy926 endothelial cells were plated at a concentration of 10,000 cells/well and cultured on IBIDI μ-Slide 8 well chamber slides in DMEM (10% FBS, 1% P/S) for 7 days. Ea.hy926 cells were washed three times with DPBS and stained with CellMask™ deep red plasma membrane stain

(1:1000 x dilution) for 15 minutes at 37 °C, 5% CO_2. Cells were washed three times with cold DPBS and treated with 1 mM LPG-Q (containing 10% BODIPY FL) for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM $CaCl_2$, 0.2 U/mL gtTGase. Following polymer attachment, cells were washed twice in DPBS, exchanged into phenol-free DMEM (5% FBS, 1% P/S) and immediately imaged. All confocal images were acquired using an inverted Zeiss Axiovert 200M spinning disk confocal microscope equipped with a QuantEM 512SC Photometrics camera (512x512 pixels size) and an incubator platform for live-cell imaging. Images were captured in series using a 60x Oil Plan-Fluor objective lens coupled to a spherical aberration correction unit. Z-stacks were acquired in 0.2 μm increments using the 640 and 488 laser channels and Cy5 and FITC bandpass emission filters. All experiments were repeated three times (3 independent experiments) and the results were pooled. Images of live cells were acquired within one hour of staining.

1.16 Enzyme-mediated glycocalyx removal from endothelial surface

Ea.hy926 endothelial cells were treated with 0.5 mM LPG-SS or LPG-Q (containing 10% BODIPY FL) for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM $CaCl_2$, 0.2 U/mL gtTGase. Following incubation, monolayers were washed twice with room temperature DPBS then incubated with serum starved cell media containing 0.1 mM H_2O_2 and 1 nM epinephrine for 1 hour at 37 °C according to previous procedures.[11] The stimulation of the cells with reactive oxygen species (H_2O_2) and catecholamines (epinephrine) has been demonstrated to upregulate the expression of glycocalyx degrading extracellular proteases including human matrix metalloprotease's (MMP). Cells were washed, trypsinized and immediately subjected to flow cytometry. Controls with no enzyme treatment were used to set gates for 100% labeled cell populations.

1.17 Quantification of glycocalyx on cell surfaces

Ea.hy926 cells at 60-80% confluence were trypsinized and plated in a 48 well plate at 30,000 cells/well. Cells reached 100% confluence at day 3 and allowed to grow for a further 12 days at 37 °C and 5% CO_2. Cells were washed twice with cold PBS and subjected to various conditions. CSE and TNF-α treatment were performed as described previously. After subjecting the cells to the various conditions, the cells were then washed extensively, labelled with WGA-FITC for 15 minutes at room temperature in the dark. The cell monolayers were trypsinized and subjected to flow cytometry in which 10,000 events were acquired and the median fluorescent intensity was recorded.

1.18 Transendothelial protein passage across cell monolayers

HMEC-1 cells at 60-80% confluence were trypsinized and seeded in a tissue culture inserts (3415, Corning) with polycarbonate supports (3 µm pore size, 0.33 cm^2 surface area) at 100,00 cells/cm^2. The tissue culture inserts were placed in 24 well plates. HMEC-1 cells were allowed to grow for 1 week in tissue culture inserts for 1 week with media changed every 2 days prior to performing transendothelial protein passage experiments as reported previously.[12] The passage of FITC-labeled BSA (A92771, Sigma) across the monolayer was tracked over 3 hours. To begin, the media in both compartments was replaced with serum-free media for 1 hour. After, 0.5 mg/mL FITC-BSA in media was added to the upper compartment and 0.5 mg/mL unlabelled BSA (A-7888, Sigma) in media was added to the lower compartment. Every hour for 3 hours, 100 µL aliquots were collected from the lower compartment and replaced with media containing 0.5 mg/mL unlabeled BSA. The fluorescence of the collected media was recorded and the concentration was calculated against a standard curve of FITC-labeled BSA.

1.19 PBMC adhesion to endothelial surfaces

Confluent Ea.hy926 monolayers seeded in a 48 well plate (30,000 cells/well) were activated with TNF-α as described in section 1.14. To obtain PBMCs, EDTA anticoagulated blood from healthy donors was subjected to ficoll density gradient centrifugation Histopaque®-1077 (Sigma Aldrich). PBMCs were cultured for 24 h at 37 °C and 5% CO_2 in RPMI-1640 containing 10% FBS (v/v), 1000 U/mL recombinant human IL-2 (Cedarlane)[13] and 1% P/S. Prior to fluorescent labelling, cultured PBMCs were tested for absence of platelets by labelling an aliquot of cells

with FITC-labelled anti-human CD45 (1:200 Beckman Coulter) and analyzed by flow cytometry (CytoFLEX, Beckman Coulter). PBMCs containing <1% platelets were implemented for further study. The PBMC composition was also measured using a hematological analyzer (XN-550™ automated hematology analyzer, Sysmex Canada Inc.). Following IL-2 treatment the typical composition of the PBMCs were ~80% lymphocytes, 5% immature granulocytes, 2% basophils and 13% monocytes. PBMCs were then suspended in Ca^{2+} and Mg^{2+} free PBS (1 x 10^6 cells/mL), labelled with 5 μM CellTracker™ Green (CTG) CMFDA Dye (Invitrogen) for 30 minutes at 37 °C, washed twice with PBS, and resuspended in binding buffer (HBSS containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1% BSA) to a final concentration of 2.5 x 10^6 cells/mL. PBMCs were then slowly added to activated monolayers (with or without treatment with 0.5 mM LPG-Q or 0.56 mM LPG-Q-Sia3Lac) to a final concentration of 0.75 x 10^6 cells/mL and left to incubate for 1 hour at 37 ºC. Following PBMC attachment, Ea.hy 926 monolayers were washed with PBS and fixed with 4% PFA for 15 minutes at room temperature. Images were acquired using confocal microscopy at confluent regions focused on the PBMC containing plane in confluent areas. PMBC adhesion was quantified by lysing the cells with RIPA buffer, spinning at 15,000 *g* to remove cell debris and measuring the fluorescent intensity of the CMFDA Dye in the lysate ($\lambda_{ex}/\lambda_{em}$= 492/517 nm using a plate reader (Spectramax i3, Molecular Devices).

1.20 PBMC-mediated cytotoxicity

Ea.hy 926 cells were engineered with polymers in UW solution as outlined in section 1.13. IL-2 activated PBMCs (see section 1.17) were pelleted and resuspended in phenol-free DMEM (5% FBS, pen/strep) at an effector (PBMC) to target (EA.hy 926) ratio (E:T) of 10:1 (V_f = 100 μL). After 18 hours at 37 °C and 5% CO_2, supernatants were removed from the well and PBMCs were removed through centrifugation (300 *g* x 5 minutes) and measured for lactate dehydrogenase (LDH) content to probe cell lysis using and LDH cytotoxicity assay (Biovision Inc.). PBMC-mediated cytotoxicity was calculated according to Eq. 2 which was adapted from a similar experiment conducted by Hudak et al.[14] In some circumstances, values were expressed as fold decrease compared to non-polymer engineered cells to account for PBMC donor variability.

$$\text{cytotoxicity} = \frac{\text{(total LDH rel.)} - \text{(LDH rel. after polymer att)} - \text{(PBMC LDH rel.)} - \text{(LDH rel. 100\% alive)}}{\text{(LDH rel. 100\% dead EA.hy 926)}} \quad \text{(Eq. 2)}$$

Total LDH rel. = Total LDH content released into the cell culture media following 18 hour co-culture

LDH rel. after polymer att. = LDH released after polymer attachment under SCS;

PBMC LDH rel. = LDH released by PBMCs following 18 hour culture period in the absence of Ea.hy 926 cells;

LDH rel. 100% alive = LDH released by Ea.hy 926 following 18 hour culture period that have not undergone polymer attachment and cultured in the absence of PBMCs;

LDH rel. 100% dead = LDH released by Ea.hy 926 following treatment with RIPA buffer for 10 minutes at ambient temperature.

1.21 NK cell/macrophage/CD8+ T-cell depletion in PBMC isolates

Following PBMC isolation from whole blood (see section 1.17), IL-2 activated PBMCs were selectively depleted for NK cells by immunomagnetic separation using an EasySep™ isolation kit (STEMCELL Technologies Inc) through positive selection for CD56+ cells. NK content in depleted and non-depleted populations was assessed through flow cytometry (Cytoflex, Beckman Coulter) by labeling lymphocyte populations with both a general Anti-Human CD45 (FITC conjugated, BD Biosciences, 1:40 dilution) and Anti Human Siglec-7 (APC conjugated, Biolegend, 1:20 dilution). CD8+ T-cells were depleted from IL-2 activated PBMCs through positive selection using Anti-Human CD8+ Dynabeads (Thermo Scientific). CD8+ T-cell content in depleted and non-depleted populations was assessed through flow cytometry (Cytoflex, Beckman Coulter) by labeling lymphocyte populations with Anti-Human CD8a (FITC conjugated, STEMCELL Technologies, 1:40 dilution). To generate macrophage/monocyte depleted PBMC populations, isolated PBMCs were

incubated in serum-starved RPMI media for 3 hours and non-adherent cells were removed and subsequently, subjected to IL-2 activation. After the extent of depletion was quantified, Ea.hy926 cells were subject to cytotoxicity assays (see section 1.18). The amount of PBMCs added to the target cells was calculated to account for NK, T-cell or macrophage/monocyte depletion. For example, PBMC populations containing 20% NK cells (population A) were added to target cells in a 10:1 E:T ratio (6.1 x 10^5 cells/well). PBMCs isolated from the same donor and depleted for NK cells (population B) were added to target cells at a quantity of 4.8 x 10^5 cells/well which is the number of non-NK cells present in cytotoxicity studies using population A PBMCs. As such, the impact of non-NK cytotoxicity is normalized for both populations A and B.

1.22 Cytokine profiling and assessment of CD8+ T-cell activation

PBMC-containing supernatants from Ea.hy 926/PBMC co-cultures were removed and pelleted (300 *g*, 5 minutes). The resulting supernatant was diluted 4-fold with PBS containing 1% BSA. Then, IL-10 or IL-6 content was quantified using a Human IL-10 DuoSet ELISA Kit (R&D Systems) and a Human IL-6 Quantikine ELISA Kit, respectively. All studies were carried out according to the manufacturer's protocols. For T-cell activation, PBMC pellets were resuspended in PBS containing 1% BSA to a final concentration of 2 x 10^6 cells/mL and treated concurrently with APC-conjugated anti–CD8a mAb (BD Biosciences, 1:40 dilution) and FITC-conjugated anti–CD69 mAb (BD Biosciences, 1:40 dilution) for 20 minutes at ambient temperature. Flow cytometry profiles were acquired using a 3-laser Cytoflex flow cytometer from Beckman Coulter Life Sciences (10,000 events).

1.23 Assessment of endothelial cytotoxicity imparted by A2-CAR CD8+ T cells

The experiment was performed similar to previously described PBMC/Ea.hy926 co-culture experiments by replacing the effector with CAR-T cells. Ea.hy926 cells were engineered with polymers in UW solution as outlined. Cryopreserved CAR T cells were thawed using standard protocol[1] and were pelleted and resuspended in phenol-free DMEM (5% FBS, pen/strep) at an effector (CAR-T cell) to target (Ea.hy926) ratios of 2:1, 1:2 and 1:10 (V_f = 100 μL). After 18 hours, supernatants were removed from the wells and PBMCs were removed through centrifugation (300 *g* x 5 minutes) and measured for LDH content to probe cell lysis using an LDH cytotoxicity assay (Biovision Inc.). CAR-T cell-mediated cytotoxicity was calculated according to Eq. 2 replacing PBMC LDH release with CAR-T cell LDH release. LDH released by Ea.hy926 following 18 hour culture period that have not undergone polymer attachment and cultured in the absence of CAR-T cell is the 100% alive control and LDH released by Ea.hy926 following treatment with RIPA buffer for 10 minutes at ambient temperature is 100% dead control.

1.24 Animals

C57BL/6J and Balb/c mice were obtained from Jackson Laboratories (Bar Harbor, Maine), bred and used for experimentation at 8 to 12 weeks of age. The mice were maintained at the animal facility of the Simon Fraser University (Vancouver, BC, CA) or the Center for Comparative Medicine of Northwestern University (Chicago, IL, USA). All protocols used in this study were reviewed and approved by the Simon Fraser University Animal Care Committee following the guidelines set out by the Canadian Council on Animal Care or the Institutional Animal Care and Use Committee of Northwestern University. The mice used in the skin transplant studies were between 8 to 12 weeks of age, to reflect the same age group used for the artery transplantation protocols. The skin grafts were also gender matched to the artery transplanted animal, to avoid minor histocompatibility mismatch.

1.25 Murine aortic interposition grafting

Murine aortic interposition grafting was performed as described previously.[15,16] Segments of abdominal aorta from Balb/c (H2d) donor mice were excised, flushed with UW solution and then incubated in UW solution alone (containing 5 Mm $CaCl_2$, 0.2 U/Ml gTGase and 3 mM GSH) or UW solution fortified with 0.2 U/mL gtTGase and 0.5 Mm polymer (LPG-Q, LPG-QSia3Lac) for 1 hour at 4˚C. Aortic segments were then flushed with saline and interposed into the resected infra-renal aorta of C57BL/6 (H2b) recipient mice.

1.26 Morphological analysis of allograft arteries

At day 2, 15 and 42 post-transplantation, grafted artery segments were perfusion fixed with 4% (v/v) paraformaldehyde, excised, and frozen in optimal cutting temperature medium. Eight-micron sections were prepared and stained with hematoxylin and eosin (H&E). Tissue sections were stained with H&E. Histological features of arterial injury and inflammation at day 2 post-transplantation were graded in a blinded manner on a 0 – 4 scale that considered the amount of medial necrosis and leukocyte infiltration. Medial thickness was quantified using ImageJ software (NIH) as described.[17] Histological features of acute rejection at day 15 post-transplantation were graded in a blinded manner on a 0 – 12 scale because acute rejection involves more extensive features of immune reaction and injury compared to day 2 post-transplantation. Specifically, there is extensive infiltration of the arterial media by host leukocytes that causes medial damage characterized by swelling, death of smooth muscle cells, damage to the elastic laminae, fibrin deposition in severe cases, and early development of intimal thickening. Leukocyte infiltration, medial injury and intimal thickening were each graded on a 0 – 4 scale and an aggregate score calculated. For evaluation of chronic rejection, intimal thickening at day 42 was quantified using ImageJ software [15,16,18]

1.27 Cytokine array analysis

To assess the immune modulatory effect of enzymatic polymer grafting, an antibody array kit based on a sandwich ELISA principle (Proteome Profiler, Panel A; R&D Systems) was used to screen the cytokine and chemokine expression level in sera of animals according to the manufacturer's protocol. Relative expression of 40 murine cytokines and chemokines captured by corresponding antibodies spotted on a nitro-cellulose membrane was determined.

The antibodies for the following cytokines were spotted on the membrane: BLC, C5a, G-CSF, GM-CSF, I-309, Eotaxin, sICAM-1, IFN-g, IL- 1a, IL-1b, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-12 p70, IL-16, IL-17 IL-23, IL-27, CXCL10 (IP-10), I- TAC, KC, M-CSF, CCL2 (MCP-1), CCL12 (MCP-5), MIG, CCL3 (MIP-1a), CCL4 (MIP-1b), CXCL2 (MIP-2), CCL5 (RANTES), SDF-1, TARC, TIMP-1, TNF-a, TREM-1. In brief, serum samples were collected at day 2 and 42 post-transplantation from mice after murine aortic interposition grafting (see section 1.23) and spun for 15 min at 15,000 *g* at room temperature (RT) to remove debris. Following the blocking step for 1 h at 4 ^{o}C, a mixture of detection antibody cocktail and 100 uL of serum sample was incubated overnight at 4 ^{o}C with the membrane. Captured proteins were labeled with a streptavidin-functionalized IR-Dye (IRDye 680RD Streptavidin; LI-COR) (1:20,000 dilution in 1 % bovine serum albumin, 30 min at RT) and scanned using a LI-COR Odyssey Infrared Imaging System. The software ImageJ (Version 1.52s) was used to determine the integrated density of the spots on the array images after background subtraction.

1.28 Donor specific antibody quantification

To quantify donor specific antibodies, sera were collected from transplant mice at 42 days post-transplant, serially diluted, and incubated with splenocytes from BALB/c mice. This was followed by staining with a polyclonal Goat-anti-mouse secondary-FITC antibody (BioLegend) and staining for CD3-PE (17A2, BD Biosciences). DSA reactivity on CD3-positive cells was analyzed via flow cytometry using a BD LSRFortessa X-20 (BD Biosciences). Data is presented as the MFI of DSA reactivity on CD3-positive cells.[17]

1.29 Murine skin grafting of aortic interposition grafted mice

Murine skin grafting was performed as described previously.[19,20] Donor mice were anesthesized and the back hair shaved, depilated and cleaned using iodopovidone and alcohol wipes. The mice were then euthanized and the skin harvested. The panniculus carnosus, subcutaneous fat and connective tissue were removed, leaving the dermal layer for grafting. 8 mm diameter skin grafts were prepared using a biopsy punch. Mice that received aortic transplants were used as skin transplant recipients 28 days after artery transplantation. Skin graft recipients were fully anesthetized and the hair from the neck to the hip at the back shaved, depilated and cleaned. The area was subsequently prepared using iodopovidone followed by alcohol wipe. A 6 mm biopsy

punch was used to carefully resect the skin at three different areas (minimum 0.5 mm apart) while preserving the underlying panniculus carnosus layer of the hypodermis. The donor grafts were positioned and then secured onto the resected areas using 7-0 Prolene sutures. The skin grafts were evaluated daily and rejection was determined by the physical appearance of the graft (colour, shape and texture).

1.30 Syngeneic murine kidney transplantation

Kidneys from C57BL/c mice were transplanted into syngeneic recipients by using the surgical techniques previously described by Zhang et al.[21–23] Briefly, the donor kidney was procured along with renal vessels attached to a segment of the abdominal aorta and inferior vena cava (IVC), perfused with either UW or polymer, and cold static storage in UW or UW + LPG-Q-Sia3Lac for 4 hours prior to being transplanted into the recipients (N=5). In the recipient surgery, the kidney was flushed with saline, followed by anastomoses between donor aorta and inferior vena cava (IVC) and the recipient abdominal aorta and IVC in an end-to-side manner, respectively. To complete the ureteral reconstruction, the donor bladder patch was anastomosed the bladder dome of the recipient. Both native kidneys of the recipients were removed during the surgery. Therefore, the recipients' survival depended upon the transplanted kidney. For sham control, the age matched B6 underwent unilateral nephrectomy.

1.30.1 Blood analysis

The whole blood sample was collected from recipient mice at both day 2 and day 7 post-surgery and were used for the measurement of blood urea (BUN) and creatinine using i-STAT bioanalyzer (i-STAT CHEM8+ cartridges).

1.30.2 Histological analysis of graft damage

Grafts were harvested from recipients at humane (graft failed) or experimental endpoint (at day 7 post-surgery), followed by formalin fixation and paraffin embedding. Tissue sections were stained with H&E. The tubular damage of each graft was semi-quantitatively scored as follows:[24] 0, no difference from a sham kidney; 1, up to one third of tubules showing cell swelling, brush border or nuclear loss; 2, as for score 1, but greater than one third but less than two third of tubular damage; and 3, greater than two third of tubular damage. Two sections from each graft were examined, and the tubular damage was scored in total 20 microscopic fields (200x magnification) in a blinded fashion. The data represented the average score of these 20 fields.

1.31 Allogeneic murine kidney transplantation

Kidney transplantation was performed similarly to the process described above; kidneys from Balb/c mice were transplanted into allogeneic B6 recipients (N=8) using the same method as described above, including organ procurement and modification.

1.31.1 Blood analysis

The whole blood sample was collected from recipient mice at both day 7, day 14 and day 30 post-surgery and were used for the measurement of blood urea (BUN) and creatinine using i-STAT bioanalyzer (i-STAT CHEM8+ cartridges).

1.31.2 Histological analysis of graft damage

Kidney tissues at day 30 post-transplantation were preserved by formalin-fixed and paraffin-embedded. For histological scoring of severity of cellular infiltration, tissue sections (~4 μm thickness) were routinely stained with hematoxylin and eosin (HE). The stained tissue slides were scanned with Leica SCN400 Slide scanner (Leica Microsystens Inc., Concord, ON, Canada). The cellular infiltration in each high-powered field (hpf) (200× magnification) was scored as follows in a blinded fashion: 1 (0%–24% of the view affected with infiltrates), 2 (25%–49%), 3 (50%–74%), and 4 (>75%). An average number of at least 20 randomly selected fields in the cortex of kidney sections represented the score of the cellular infiltration of a transplant.

The mesangial expansion (ME) of the glomeruli as a marker of the transplant rejection was examined by using a semi-quantitative scoring system in Masson's trichrome (MT)-stained tissue

sections. The scoring system consisted of 1 to 4 scale to determine the severity of the extracellular matrix (ECM) deposition into both the capillary walls (the incrassation of basement membrane) and the mesangium based on the percentage of the area stained strongly with MT, indicating the ME in an affected glomerulus: 1 (0%–24% of the area affected with densely stain), 2 (25%–49%), 3 (50%–74%), and 4 (>75%) (see supplementary Fig. S27). A range of 100 to 120 glomeruli were counted for each transplant, and were averaged for the severity of ME.

1.32 Statistical and regression analysis

Data are expressed as the mean ± 95% confidence interval using the sample standard deviation. Statistical analyses were performed using GraphPad Prism version 7.0 software, using unpaired t-tests with Welch's correction. Figure 3f used one way ANOVA test. Samples were denoted as statistically significant. $p \leq 0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***). Experiments were performed in triplicate and results were pooled into a single dataset unless otherwise stated. Regression analysis using least-squares was also performed using GraphPad Prism version 7.0 software.

1.33 Ethics approval

Procedures involving human subjects in the Centre for Blood Research at the University of British Columbia have been approved by the Institutional Review Board (IRB) within the University of British Columbia (UBC Ethics approval no: H10-01896 H20-00084 and H18-02553). Animal experiments were performed in accordance with The Canadian Council on Animal Care (CCAC) guidelines and were approved by the University of British Columbia Animal Care Committee (certificate number A16-0271) or the Simon Fraser University Animal Care Committee (certificate number 1235MB-08 and 1319MB-08) or the Institutional Animal Care and Use Committee of Northwestern University (certificate number IS00002313).

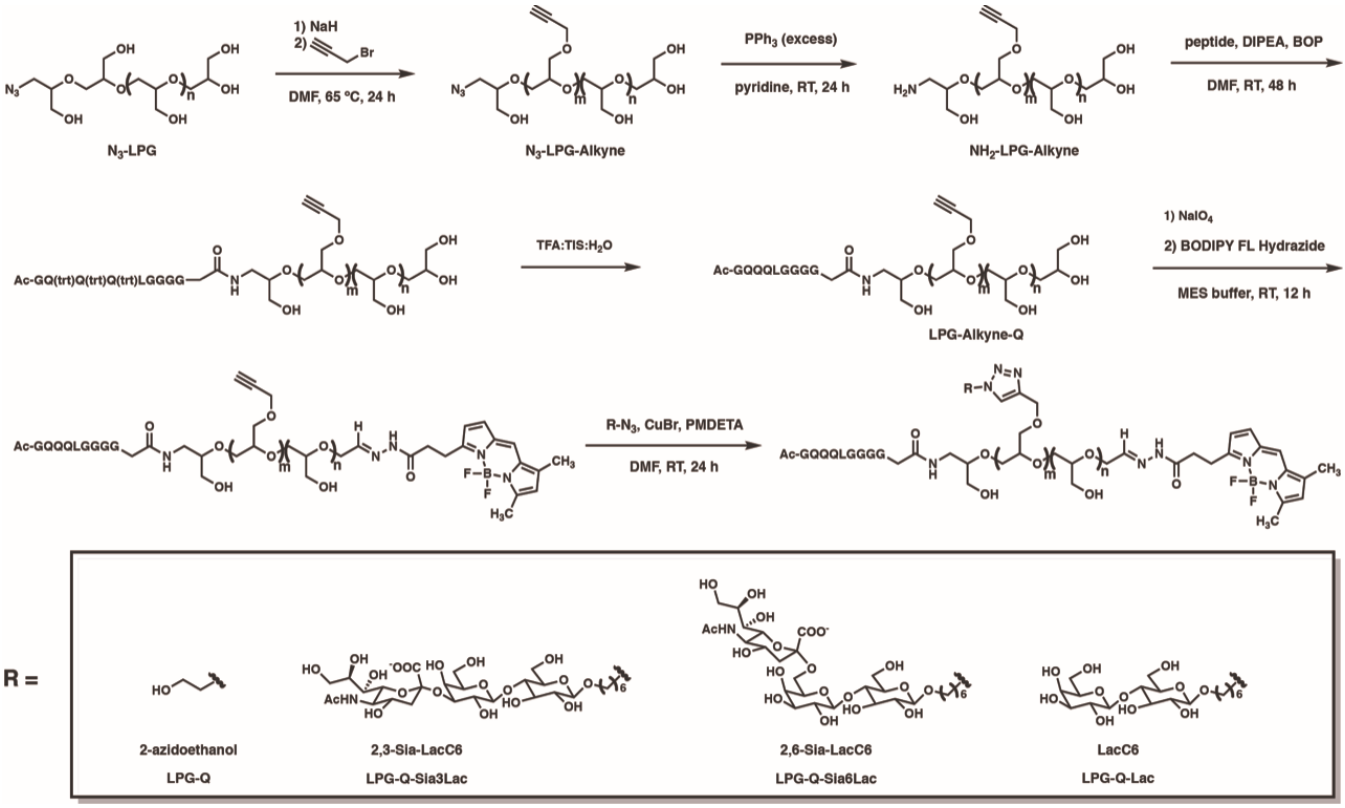

Figure S1: Synthetic overview of LPG-Q glycopolymers. Figure was created using ChemDraw 17.0.

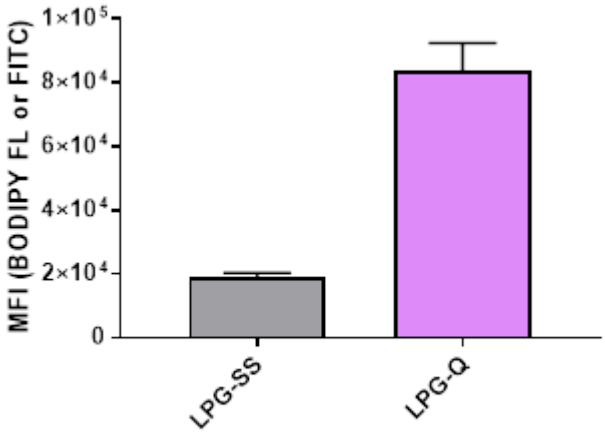

Figure S2: LPG-Q has a higher reactivity towards the endothelial cell surface compared to LPG-SS. Ea.hy 926 cells were incubated with either LPG-Q (10% BODIPY FL-labelled LPG-Q) or LPG-SS (10% FITC-labelled LPG-SS) for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM $CaCl_2$, 0.2 U/mL gtTGase, cells were washed and the incorporation was measured by flow cytometry. Error bars represents 95% confidence intervals.

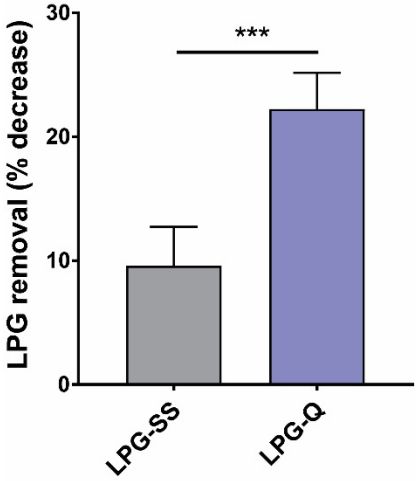

Figure S3: Loss of polymer attached under chemical (LPQ-SS) and enzyme (LPG-Q) ligation strategies on the endothelial cell surface following partial glycocalyx removal. Ea.hy 926 cells were incubated with either LPG-Q (10% BODIPY FL-labelled LPG-Q) or LPG-SS (10% FITC-labelled LPG-SS) for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2, 0.2 U/mL gtTGase. Following polymer attachment, washed cells were incubated in serum starved cell media containing 0.1mM H_2O_2 and 1 nM epinephrine for 1 hour at 37 °C. Following treatment, cells were washed and analyzed by flow cytometry. The stimulation of the cells with reactive oxygen species (H_2O_2) and catecholamines (epinephrine) has been demonstrated to upregulate the expression of glycocalyx degrading extracellular proteases including human matrix metalloprotease's (MMP). Higher amount of LPG was retained on the endothelial surface when the conjugation (ligation) was performed using chemical ligation by succinimidyl succinate LPG (LPG-SS) in comparison to the enzymatic ligation using gtTGase as assessed by the amount of polymer removed from the cell surface following enzyme-mediated glycocalyx removal. The experiment provide evidence for higher glycocalyx specificity for enzymatic ligation. Error bars represents 95% confidence intervals. Unpaired comparisons using a non-parametric t-test are significant with $p > 0.05$ (ns), $p < 0.05$ (*), $p < 0.01$(**), $p < 0.001$ (***).

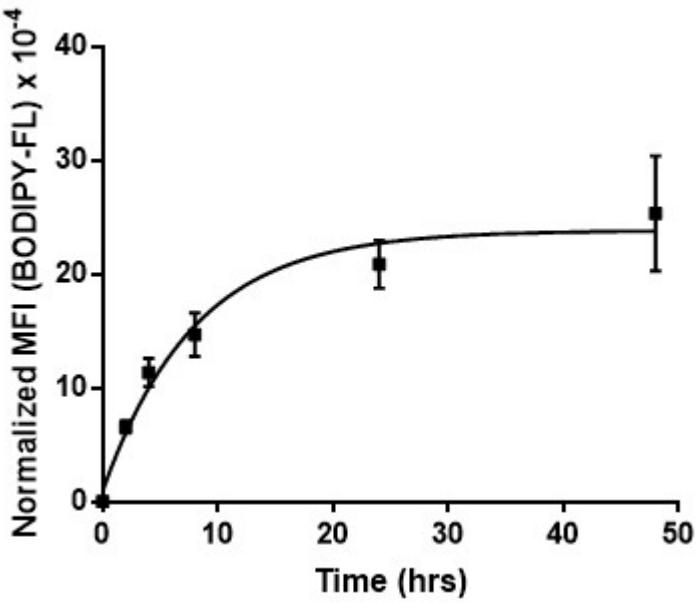

Figure S4: Shedding of LPG-Q into the cell culture media over time following polymer attachment. EaHy.926 cells were incubated with 0.5 mM LPG-Q (10% BODIPY FL-labelled LPG-Q) for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2, 0.2 U/mL gtTGase and media was replaced with phenol-free DMEM cell culture media supplemented with 10% FBS at 37 °C and 5% CO_2. Normalized fluorescent intensity ($\lambda_{ex}/\lambda_{em}$= 503/520 nm) of the cell media was collected at various time points and normalized to cell media collected from non-polymer labelled cells collected at the same time point.

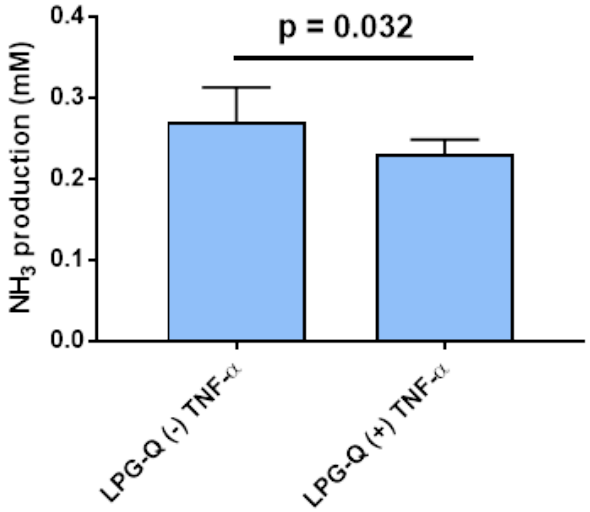

Figure S5: Assessment of enzyme-mediated polymer attachment in unactivated and TNF-α activated Ea.hy 926 endothelial cells as quantified by ammonia (NH_3) generation. Ea.hy 926 cells were activated with TNF-α (2000 U/mL, 3 hours) in serum deficient DMEM and then incubated with 0.5 mM LPG-Q for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. The supernatant was collected and assays for NH_3 content. Error bars represents 95% confidence intervals.

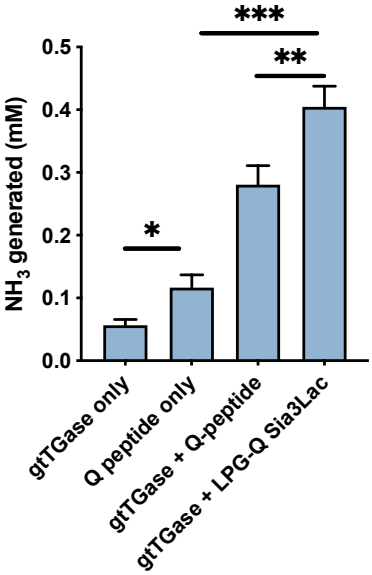

Figure S6: Assessment of enzyme-mediated polymer attachment in HMEC-1 endothelial cells as quantified by ammonia (NH_3) generation. HMEC-1 cells were incubated with 0.5 mM LPG-Q for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. The supernatant was collected and assays for NH_3 content. Unpaired comparisons using a non-parametric t-test are significant with $p > 0.05$ (ns), $p < 0.05$ (*), $p < 0.01$(**), p .001 (***).

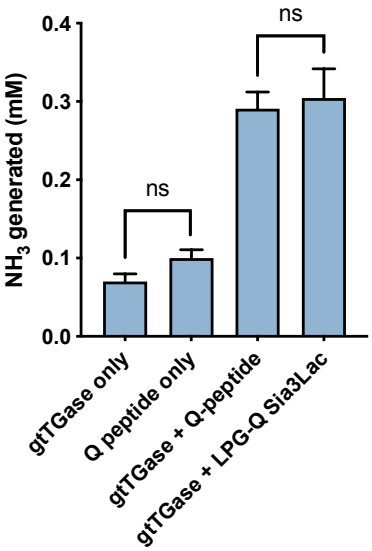

Figure S7: Assessment of enzyme-mediated polymer attachment in HUVEC endothelial cells as quantified by ammonia (NH_3) generation. HUVEC cells were incubated with 0.5 mM LPG-Q for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. The supernatant was collected and assays for NH_3 content. Unpaired comparisons using a non-parametric t-test are significant with $p > 0.05$ (ns).

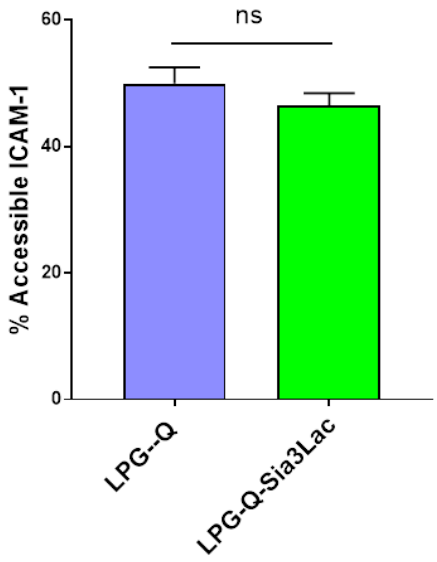

Figure S8: Comparative immunocamouflage of a similar number of LPG-Q or LPG-Q-Sia3Lac polymers grafted to Ea.hy 926 endothelial surfaces. Ea.hy 926 cells were activated with TNF-α (2000 U/mL, 3 hours) in serum deficient DMEM and then incubated with 0.5 mM LPQ-Q or 0.56 mM LPG-Q-Sia3Lac for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. The cells were washed and then labelled with PE-Cy™5 Mouse Anti-Human CD54 and assayed using flow cytometry. Error bars represents 95% confidence intervals.

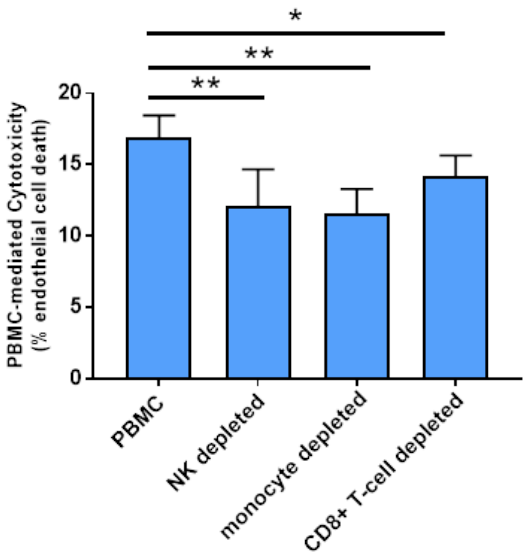

Figure S9: Comparative endothelial toxicity of TNF-α activated Ea.hy 926 endothelial cells cultured with IL-2 activated PBMCs that have been depleted of various immune cell populations. Ea.hy 926 cells were activated with TNF-α (2000 U/mL, 3 hours) in serum deficient DMEM and then incubated with 0.5 mM LPG-Q for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. PBMCs were cultured for 24 h in RPMI-1640 containing IL-2 (1000U/mL). After cell depletion, immune cells were coincubated with endothelial cells at an effector to target ratio of 10:1 for 18 hours and cytotoxicity was evaluated by an LDH assay. Error bars represents 95% confidence intervals. Unpaired comparisons using a non-parametric t-test are significant with $p > 0.05$ (ns), $p < 0.05$ (*), $p < 0.01$(**), $p < 0.001$ (***).

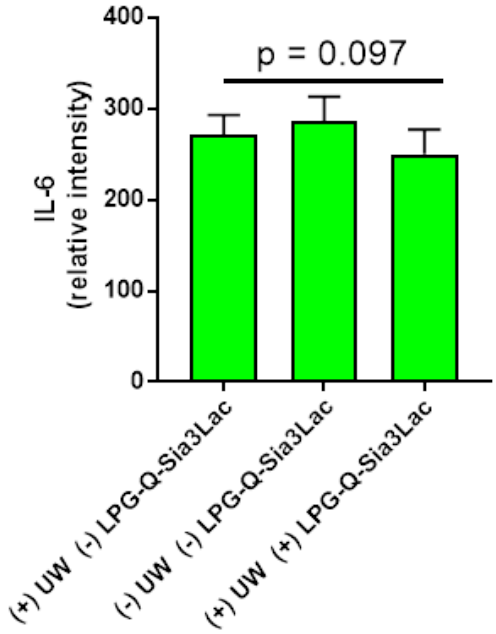

Figure S10: Assessment of IL-6 released into the cell media following PBMC/endothelial co-culture. All polymer attachment was done using 0.56 mM LPG-Q-Sia3Lac for 30 min at 4 °C in UW solution fortified with 3 mM GSH, 5 mM CaCl2 and 0.2 U/mL gtTGase. Following attachment, endothelial cells were co-cultured with IL-2 activated PBMCs for 18 hours and IL-6 secretion was measured by ELISA. Error bars represents 95% confidence intervals.

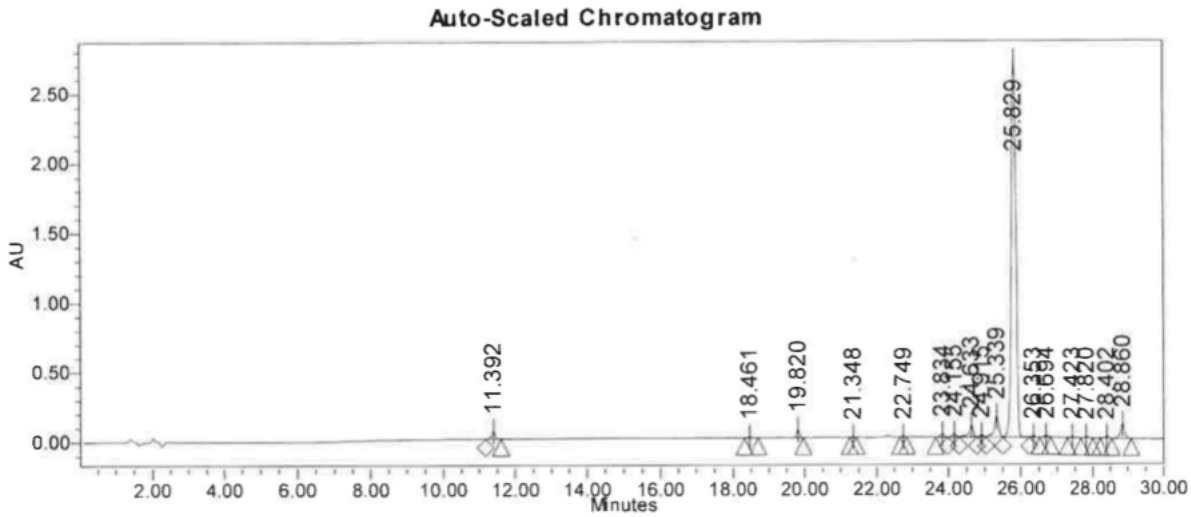

Figure S11: RP-HPLC chromatogram of Q tag as analyzed by CanPeptide Inc. Trityl (trt) protected peptide, Ac-GQ(trt)Q(trt)Q(trt)LGGGGG-OH, purity was assessed to be 83.28% pure as shown in peak eluted at 25.829 minutes. A Waters 600 Multi-Solvent System using a Phenomenex Jupiter Proteo (C18, 4u, 4.6 x 250mm) column. The mobile phase is composed of 0.1% trifluoro acetic acid in acetonitrile.

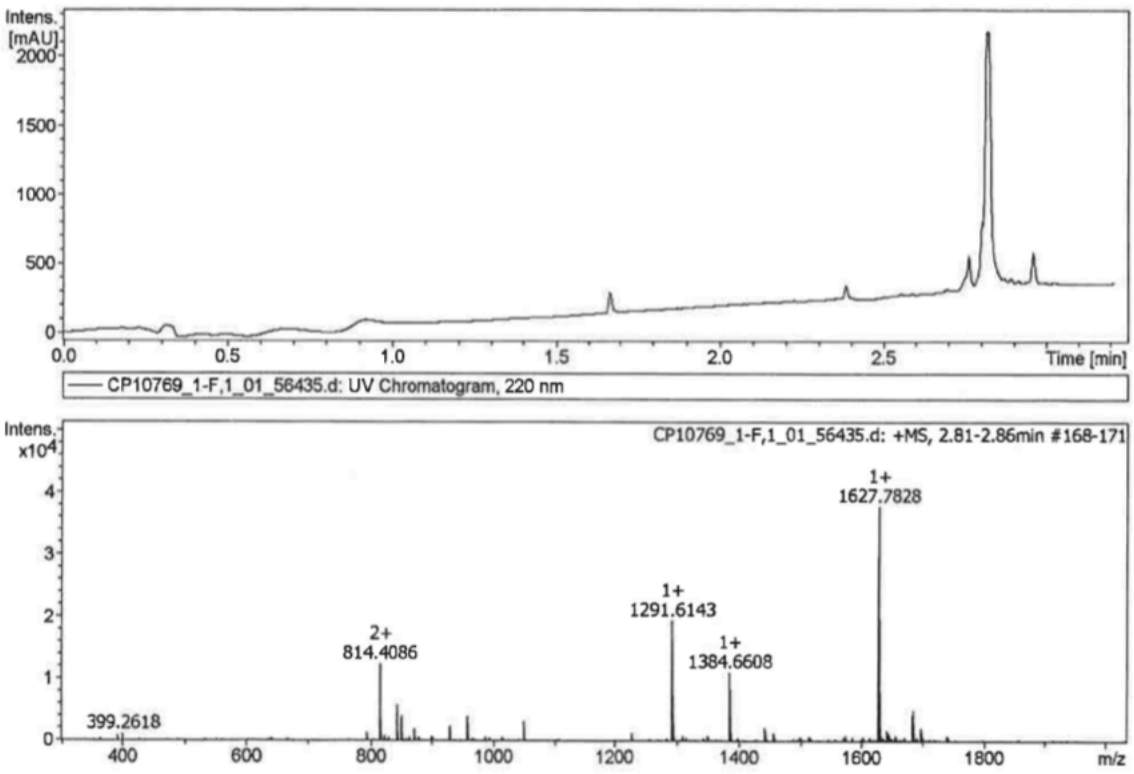

Figure S12: Electrospray ionization mass spectrometry (ESI-MS) chromatogram of Q-tag as analyzed by CanPeptide Inc. Trityl (trt) protected peptide, Ac-GQ(trt)Q(trt)Q(trt)LGGGGG-OH, identity was confirmed as shown in peak 1627.7828 ([M+1]).

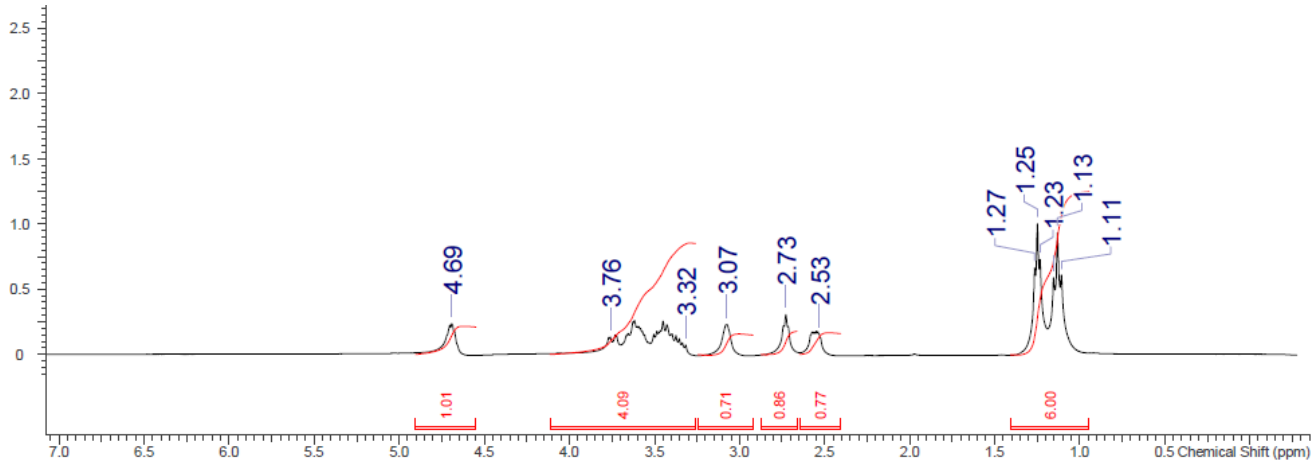

Figure S13: ^{1}H NMR of ethoxy ethyl glycidyl ether (EEGE) monomer in $CDCl_3$.

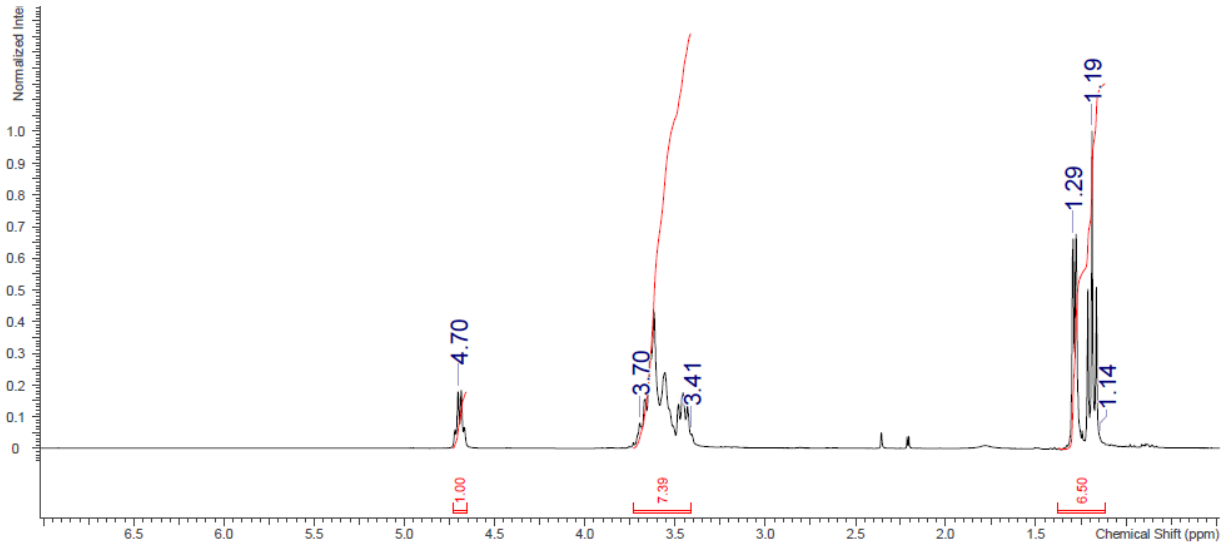

Figure S14: ^{1}H NMR analysis of pEEGE-N_3 in $CDCl_3$.

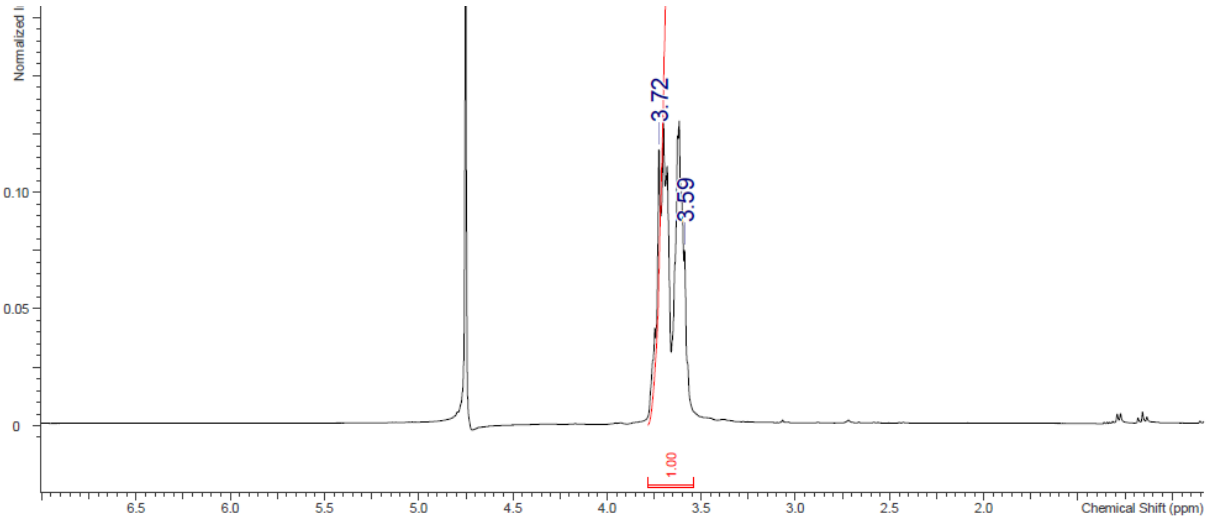

Figure S15: ^{1}H NMR analysis of LPG-N_3 in D_2O (M_n = 14 900 Da).

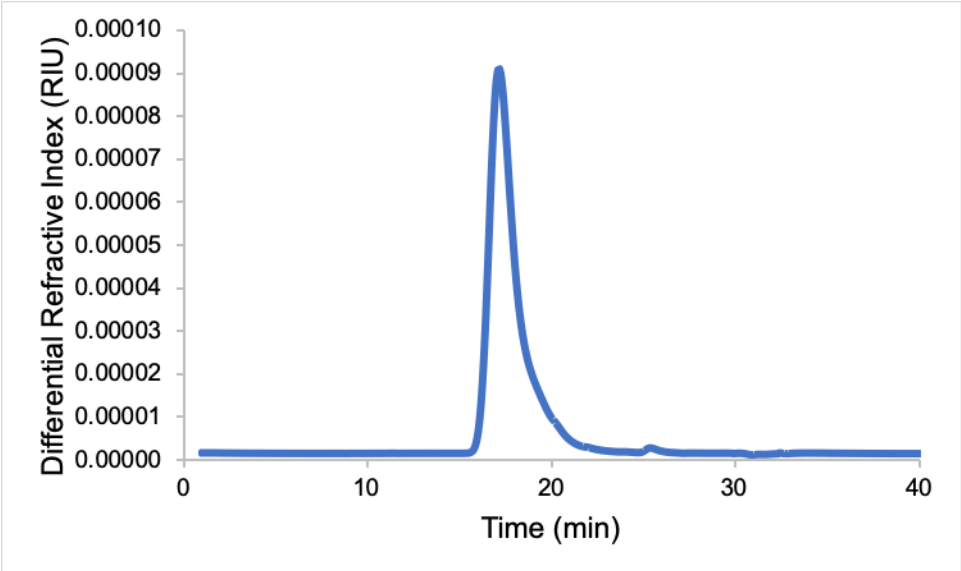

Figure S16: GPC chromatogram of the unfunctionalized LPG-N_3 scaffold.

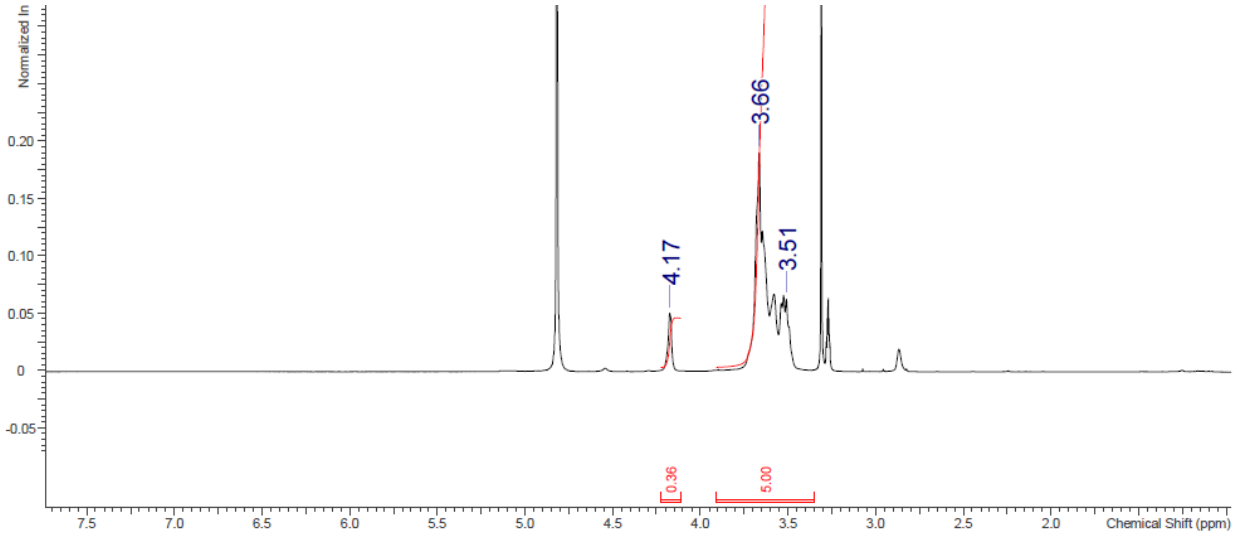

Figure S17: ^{1}H NMR analysis of propargylated LPG-N_3 in MeOD. The singlet at 4.17 represent the methylene of the propargyl group.

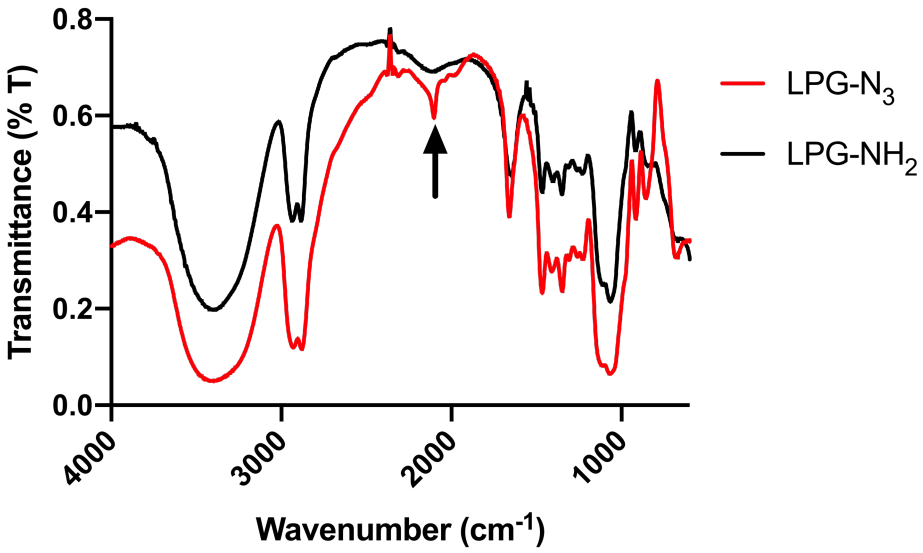

Figure S18: Representative FTIR characterization of LPG-N_3 before (red) and after (black) treatment with PPh_3. FTIR spectra were recorded on a Bruker TENSOR II FTIR spectrometer with a resolution of 4 cm^{-1}. The sharp absorption band at 2100 cm^{-1} in LPG-N_3 is characteristic of asymmetric stretching of the azido (-N_3) group (see arrow). The polymer samples were analyzed using the KBr pellet method. FTIR spectra were obtained by scanning 64 times at atmospheric conditions. The OPUS spectroscopic software was used for data handling.

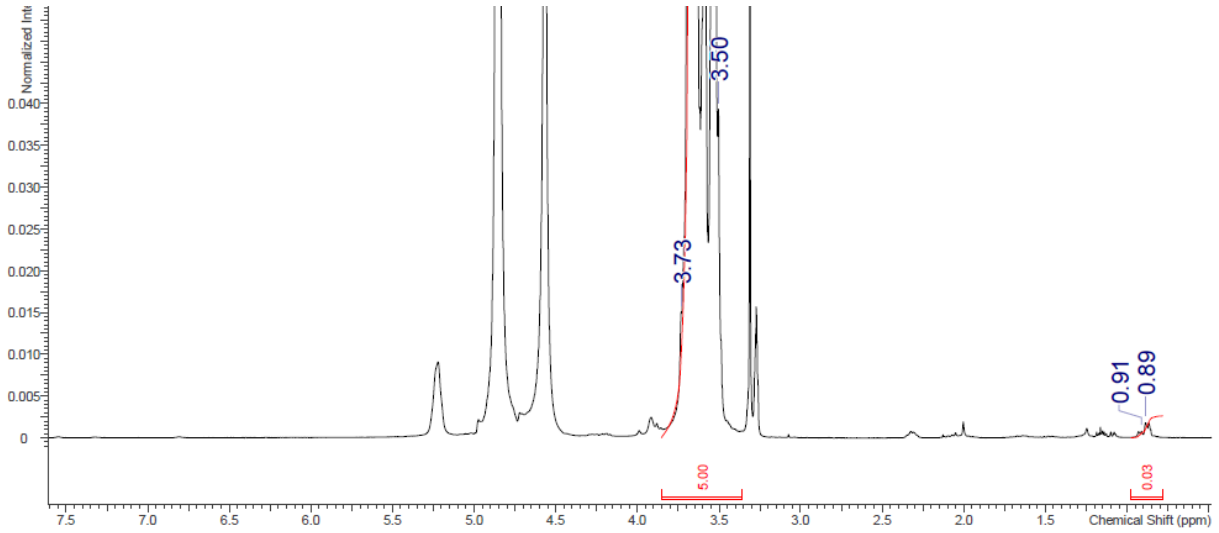

Figure S19: ^{1}H NMR analysis of propargylated LPG-Q in MeOD. The multiplet between 0.91-0.89 ppm represent the leucine methyl groups of the Q-tag (Ac-GQQQLQQQQQ). The amount of peptide per LPG molecule is ~1.0.

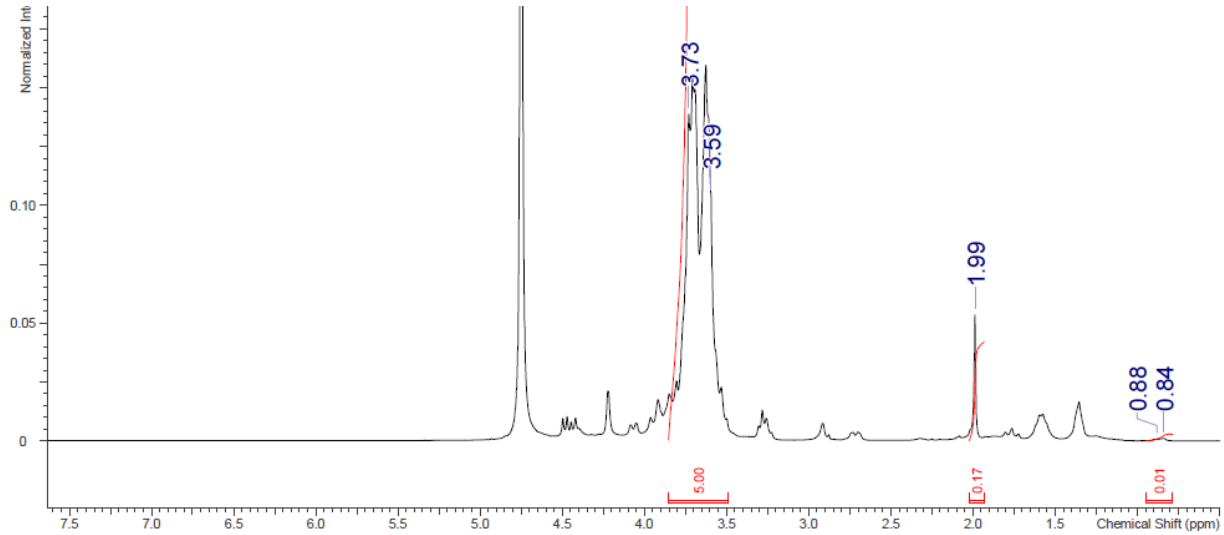

Figure S20: Representative ^{1}H NMR analysis of LPG-Q-Sia3Lac in D_2O. The separated peaks at 1.99 ppm refer to the N-acetyl group of the α2,3-Sialylactose substrate. The calculated degree of functionalization from NMR is ~9.0 %(17 groups).

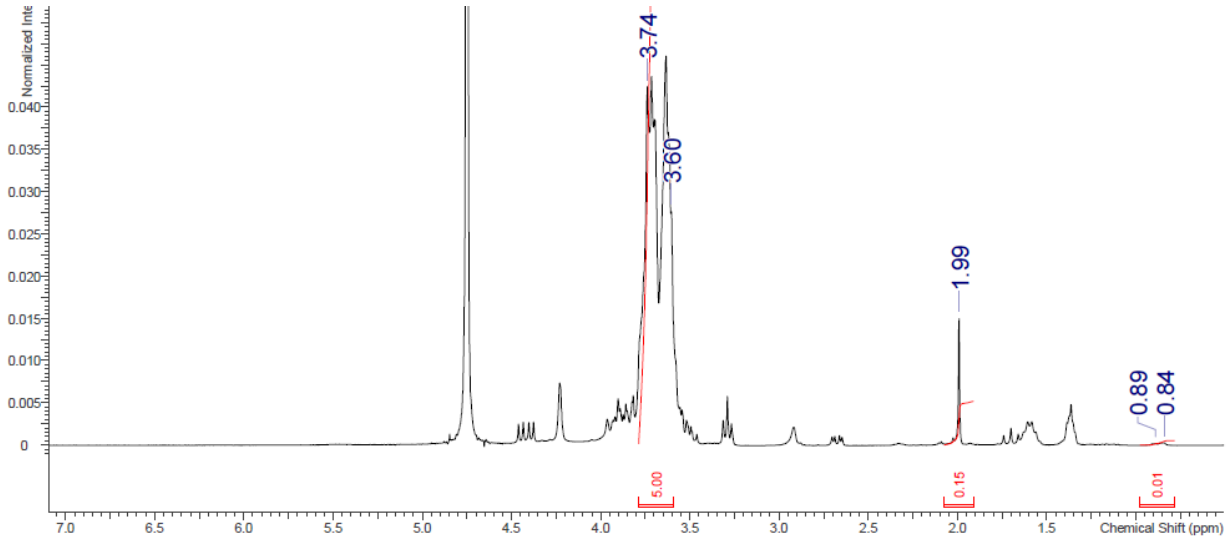

Figure S21: Representative ^{1}H NMR analysis of LPG-Q-Sia6Lac in D_2O. The separated peaks at 1.99 ppm refer to the N-acetyl group of the α2,6-Sialylactose substrate. The calculated degree of functionalization from NMR is ~9.0 % (16 groups).

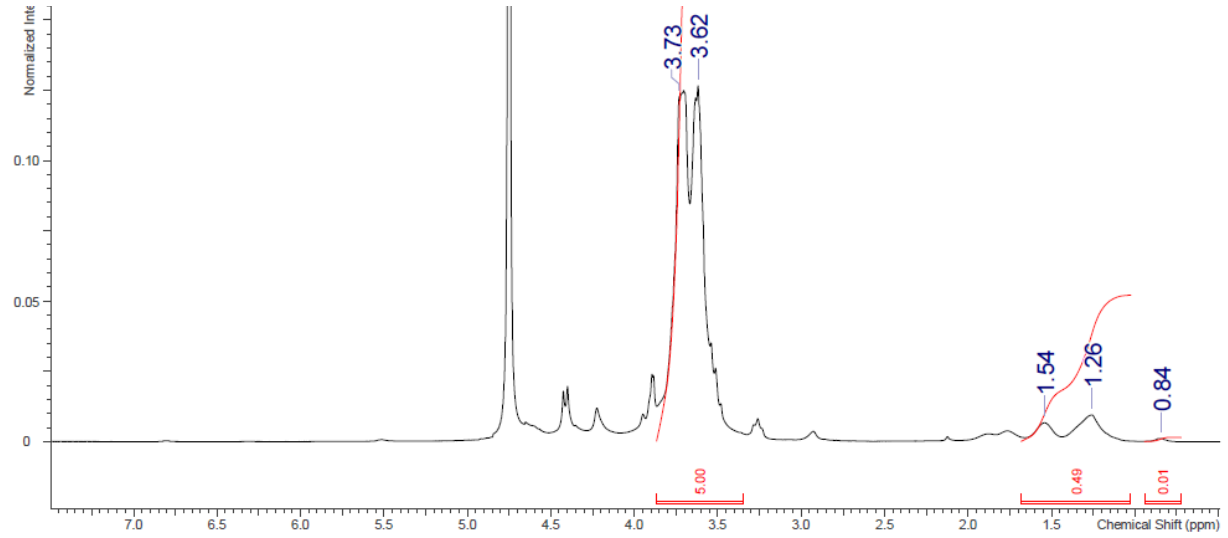

Figure S22: Representative ^{1}H NMR analysis of LPG-Q-Lac in D_2O. The multiplet from 1.54-1.26 ppm refer to the 8 internal protons of the C6 linker on the lactose substrate. The calculated degree of functionalization from NMR is ~8.0 %(15 groups).

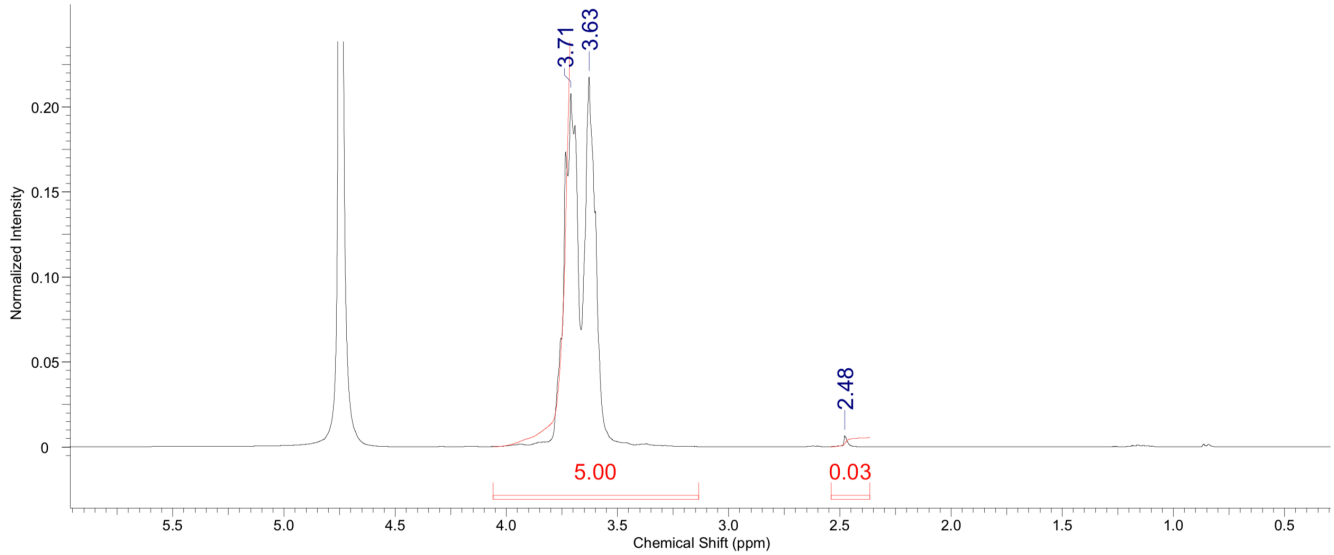

Figure S23: ^{1}H NMR analysis of LPG-SS in D_2O.

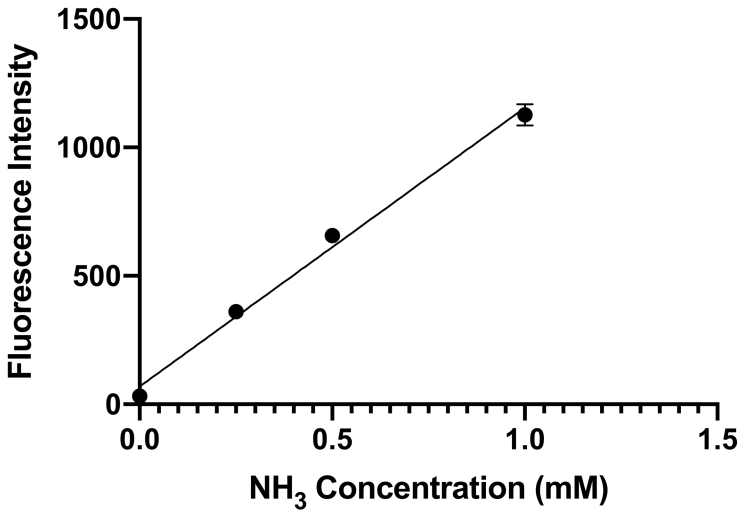

Figure S24: NH_4Cl standard curve generated using ammonia assay kit (Sigma, MAK310). The fluorescence intensity was measured at λ_{ex} = 360 nm /λ_{em} = 450nm using a spectrophotometer. The slope of the standard curve is used to calculate the concentration of NH_3 generated using Eq. 1.

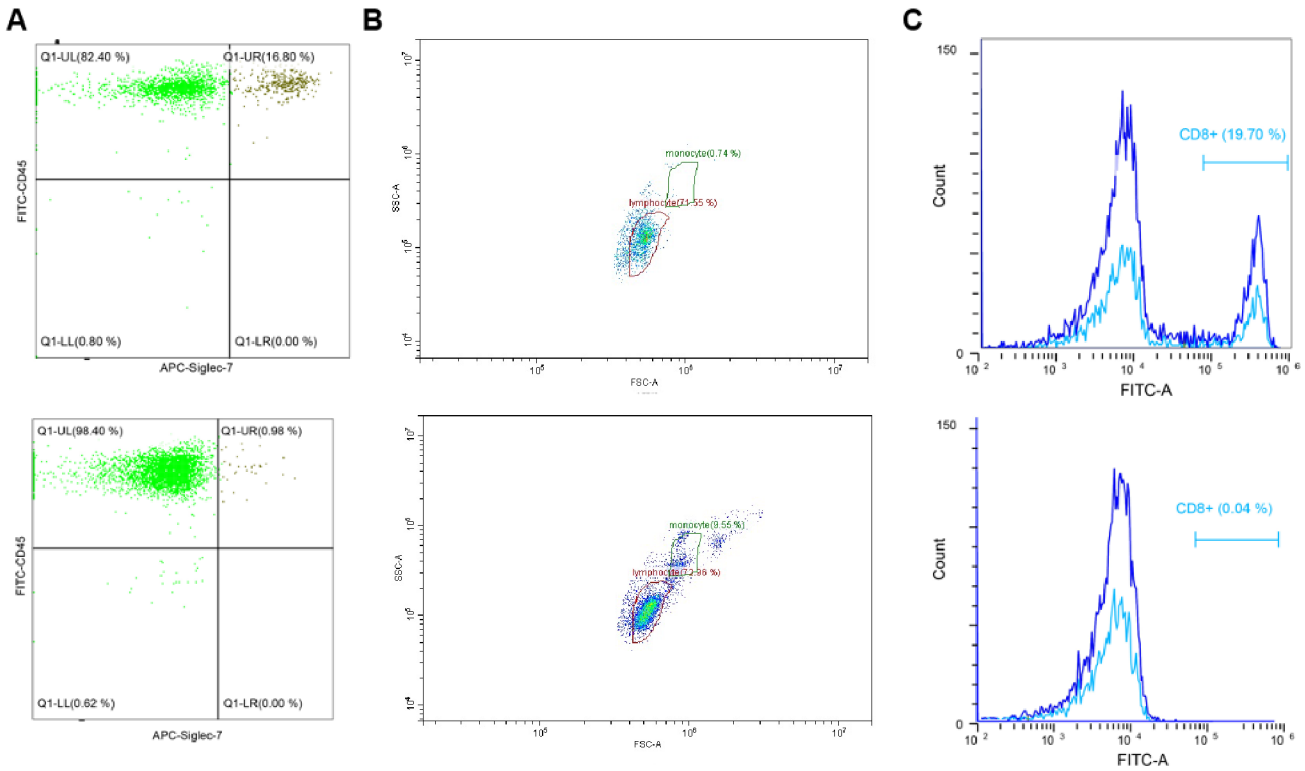

Figure S25: Representative flow cytometry profiles to validate the removal of various immune cells from the PBMC milieu. A) CD45+ CD328+ (Siglec-7) NK cells (Q1-UR) before (top) and after depletion. B) Dot plots of PBMC milieu before (top) and after (bottom) monocyte depletion. Monocytes have forward scattering (FSC, x-axis) and side-scattering (SSC, y-axis) that is unique from other PBMCs. With this, no identifying fluorescent marker was used). C) Population distribution of cells within the 'lymphocyte' gate of the dot plot (Figure 63b, light green) that have been stained with CD8+ mAb before (top) and after (bottom) CD8+ T-cell depletion.

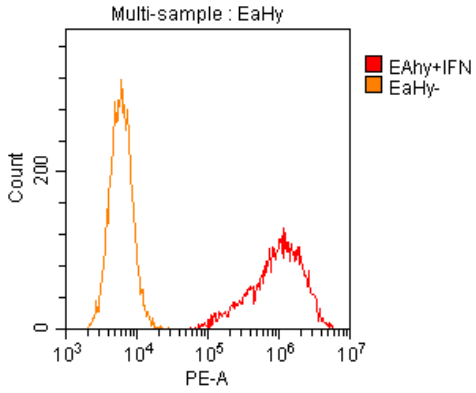

Figure S26: Flow Cytometry profile to validate the expression of HLA-A2 antigen on native Ea.hy926 cells (orange) and TNF-α-stimulated Ea.hy926 cells (red) using anti-HLA-A2. Ea.hy926 cells were activated using TNF-α (2000U/mL for 3 hours).

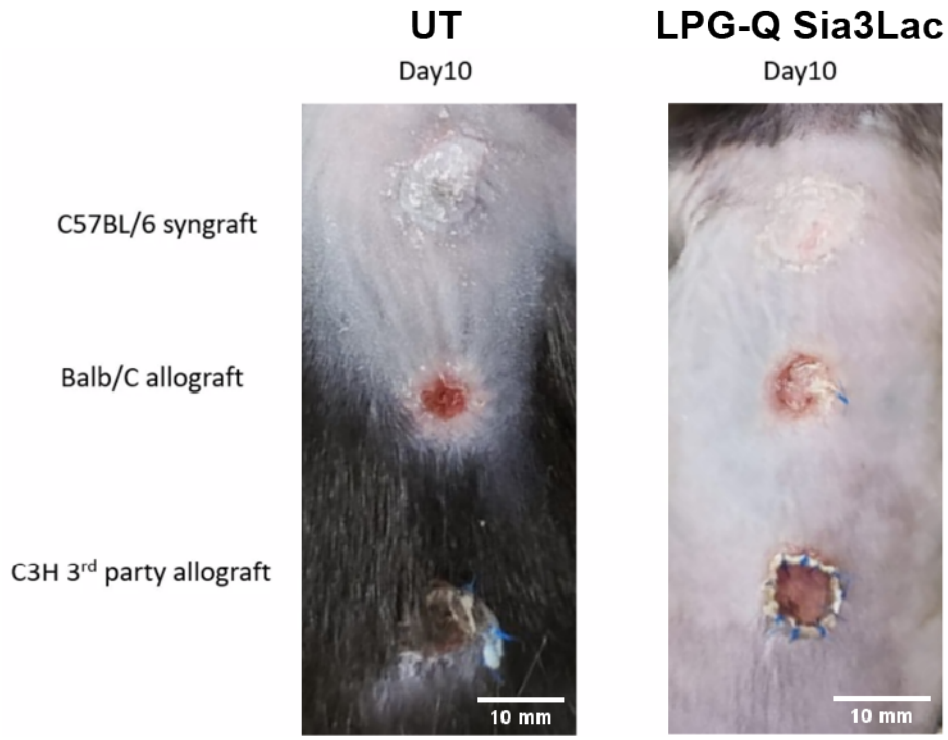

Figure S27: Representative images of skin grafts transplantation. Skin from syngeneic (C57BL/6), allogeneic donor (Balb/c) and allogeneic third party donor (C3H) were grafted onto recipients of either untreated or LPG-Q-Sia3Lac treated artery allografts 28 days after artery transplantation.

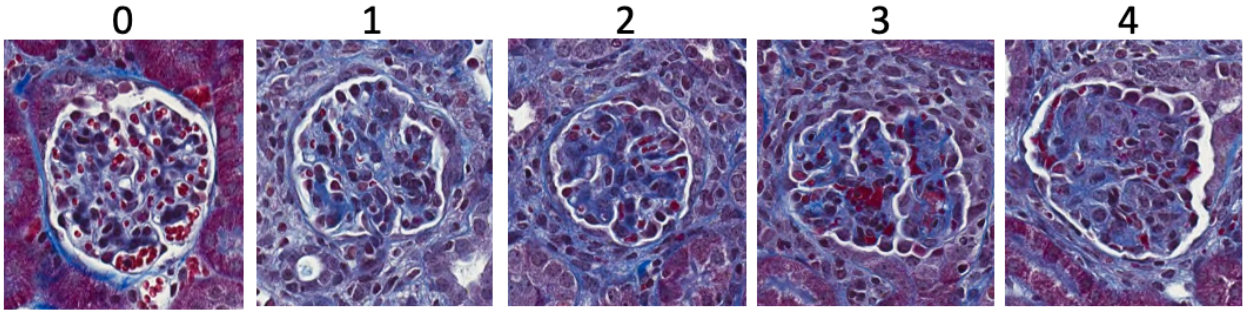

Figure S28: Representative figures showing scoring mesangial expansion of the glomeruli. 1 (0%–24% of the area affected with densely stain), 2 (25%–49%) , 3 (50%–74%), and 4 (>75%).

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Supplementary Table 1. RNASeq analysis of surface modified endothelial cells. *Attached separately as a .xlsx file.*