Supplementary Information for

Glycan-modified virus-like particles evoke T helper type 1-like immune responses

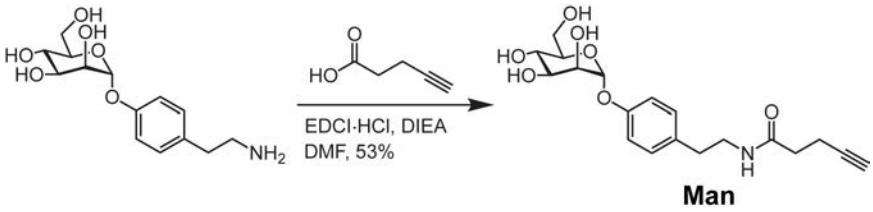
Mohammad Murshid Alam[†], Cassie M. Jarvis[†#], Robert Hincapie[‡#], Craig S. McKay[‡], Jiri Schimer[‡], Carlos A Sanhueza-Chavez[‡¥], Ke Xu[§], Roger C. Diehl[†], M. G. Finn[‡], Laura L. Kiessling[†*]

[†]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, 02139 USA
[‡]School of Chemistry and Biochemistry and School of Biological Sciences, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, GA, 30332, USA.
[¥]Current address: Department of Pharmaceutical Sciences, St. John's University, 8000 Utopia Pkwy. Queens, NY 11439, USA.
[§]Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA.

*__Corresponding Author__: Laura L. Kiessling, e-mail: kiesslin@mit.edu
[#]These authors contributed equally.

General.
Reagents and buffers were used as received from the manufacturer, unless otherwise noted. Particle integrity and homogeneity were assessed through fast protein liquid chromatography (FPLC), performed on a GE ÄKTA Explorer (Amersham Pharmacia) using a Superose-6 column (GE), and through dynamic light scattering (DLS), performed on a Dynapro plate reader (Wyatt Technologies). The extent of incorporation of the extended capsid monomer in the Qβ-Ova particles was determined through microfluidic gel electrophoresis, performed using a Bioanalyzer 2100 Protein 80 chip (Agilent). The extent of modification of virus-like nanoparticle (VLP) conjugates was determined through high resolution mass spectrometry, performed on a 6230B time-of-flight LC/MS, using a Poroshell 300SB-C3 LC column (Agilent).

Synthesis of the arylmannoside antigen

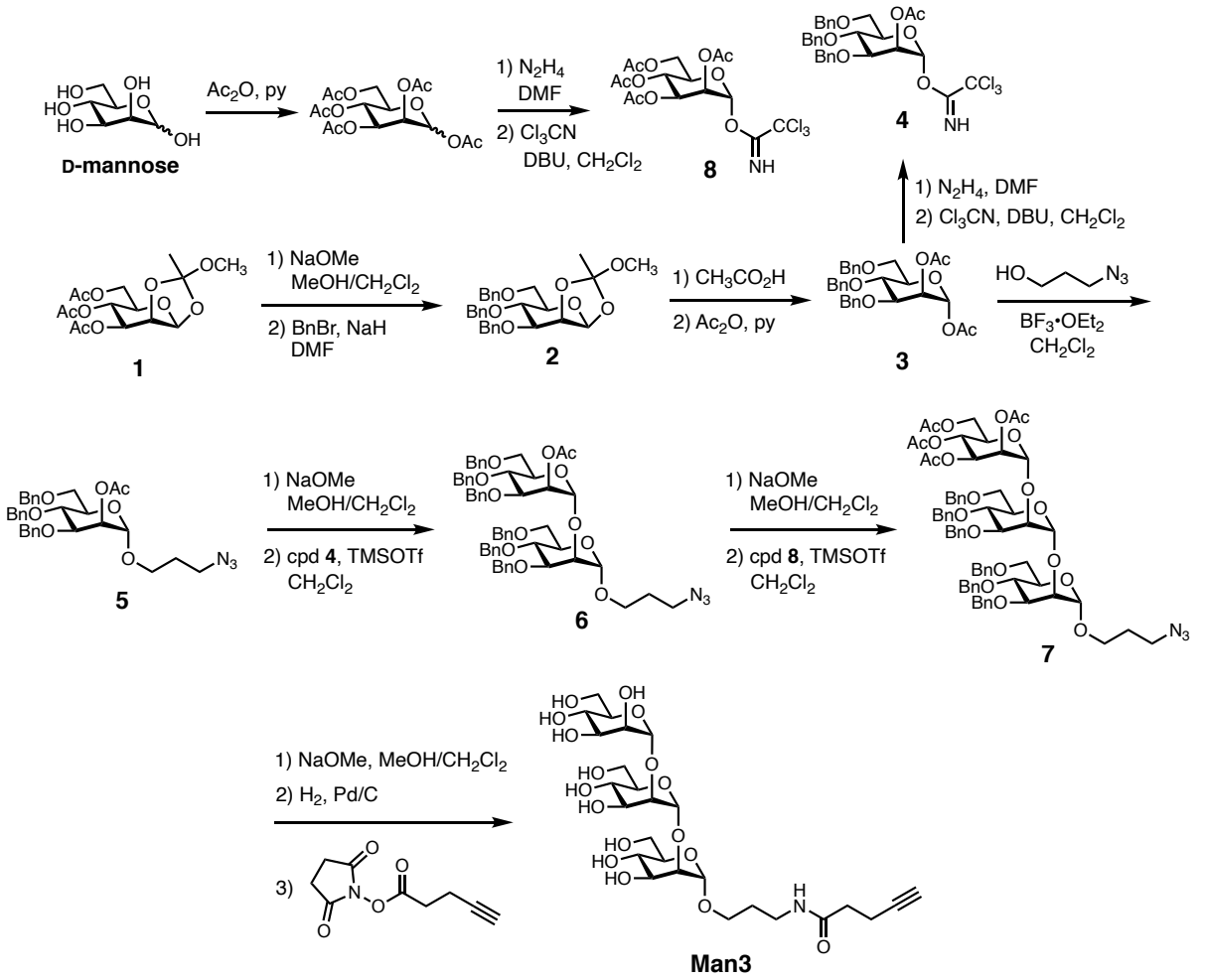


Scheme 1. Synthesis of arylmannoside alkyne

Synthetic procedures and characterization data.

(Scheme 1) *Arylmannoside alkyne, N-(4-(((2R,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetra-hydro-2H-pyran-2-yl)oxy)phenethyl)pent-4-ynamide*, **Man (mannose)**. To a flame-dried flask containing α-1-O-(4-hydroxyphenylethylamino)-D-mannoside (0.100 g,

0.334 mmol) was added 4-pentynoic acid (0.039 g, 0.401 mmol) and N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.154 g, 0.802 mmol). DMF (4.2 mL) and diisopropylethylamine (0.28 mL, 1.604 mmol) were added, and the solution was stirred overnight at rt. The reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (10-30% MeOH/CH_2Cl_2) to afford the desired arylmannoside alkyne (**Man**, 0.528 g, 0.176 mmol, 53%) as a clear residue of >95% purity as judged by NMR. ^{1}H NMR (500 MHz, CD_3OD) δ 7.15 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 2H), 5.43 (d, *J* = 1.9 Hz, 1H), 3.98 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.89 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.78 – 3.67 (m, 3H), 3.60 (ddd, *J* = 9.8, 5.1, 2.6 Hz, 1H), 3.37 (dd, *J* = 7.9, 6.7 Hz, 2H), 2.74 (t, *J* = 7.3 Hz, 2H), 2.44 (tdd, *J* = 7.1, 2.6, 1.1 Hz, 2H), 2.34 (td, *J* = 7.2, 1.1 Hz, 2H), 2.26 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.95, 156.57, 134.38, 130.86, 117.86, 100.33, 83.51, 75.28, 72.42, 72.06, 70.29, 68.35, 62.66, 42.18, 36.04, 35.74, 15.73. HRMS (EMM) calculated for $C_{19}H_{25}NO_7$ $[M+H]^+$: 380.1704, found 380.1697.



Scheme 2. Synthesis of trimannose alkyne.

(Scheme 2) *Acetoxymannose orthoester, (3aS,5R,6R,7S,7aS)-5-(acetoxymethyl)-2-methoxy-2-methyltetrahydro-5H-[1,3]dioxolo[4,5-b]pyran-6,7-diyl diacetate,* **1**. Mannose (50.3 g, 0.28 mol) was dissolved in pyridine (300 mL) and the solution was cooled to 0 °C with an ice bath. After

20 minutes of stirring, acetic anhydride (145 mL, 1.5 mol) was added to the cooled solution and stirring was continued for 12 h as the mixture warmed to rt. The reaction was then concentrated by rotary evaporation and the residue was dissolved in ethyl acetate, transferred to a separation funnel, and washed with 1M hydrochloric acid followed by a saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4, filtered, and the volatiles removed under vacuum. The residue was then dissolved in methylene chloride (560 mL) and added dropwise to cooled (0 °C) solution of hydrogen bromide (204 mL, 0.84 mol, 34% solution in acetic acid) in methylene chloride (200 mL). The reaction mixture was stirred until total consumption of the starting acetate was observed by thin layer chromatography (TLC), requiring approximately 4 h. The mixture was then diluted with ethyl acetate, transferred to a separation funnel and washed sequentially with water and aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4, filtered, and the volatiles removed by rotary evaporation. The resulting material was dissolved in a mixture of acetonitrile (300 mL) together and methanol (28 mL) with 2,6-lutidine (65.0 mL, 0.56 mol) and n-Bu_4NBr (18.1 g, 0.056 mol). The mixture was stirred for 16 h at rt. The volatiles were then removed by rotary evaporation and the resulting material was purified directly by normal phase flash chromatography, eluting with EtOAc/hexanes (3:7) containing 3% triethylamine. Compound **1** (61.0 g, 0.16 mol, 60%) was isolated as a white solid. The recorded ^{1}H NMR spectrum of **1** was consistent with previously reported data. [1] ^{1}H NMR ($CDCl_3$, 500 MHz): 5.51 (d, *J* = 2.6 Hz, H-1), 5.31 (dd, *J* = 9.7 and 9.7 Hz, 4H), 5.16 (dd, *J* = 4.0 and 9.7 Hz, H-3), 4.62 (d, *J* = 2.6 and 4.0 Hz, H-2), 4.25 (dd, *J* = 4.9 and 12.1 Hz, H-6), 4.15 (dd, *J* = 2.6 and 12.1 Hz, H-6'), 3.69 (ddd, *J* = 2.6, 4.9 and 9.5 Hz, H-5), 3.29 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.75 (s, 3H).

(Scheme 2) *Benzylmannose orthoester, (3aS,5R,6R,7S,7aS)-6,7-bis(benzyloxy)-5-((benzyloxy)-methyl)-2-methoxy-2-methyltetrahydro-5H-[1,3]dioxolo[4,5-b]pyran,* **2**. Mannose orthoacetate **1** (28.2 g, 78.0 mmol) was dissolved in methanol (200 mL) and sodium methoxide was added (approx. 500 mg). After 12 hours of stirring at room temperature, TLC showed complete consumption of the starting triacetate. The mixture was then neutralized with Amberlite IR120+, filtered, concentrated by rotary evaporation, and dried under high vacuum for 8 hours. The resulting foam was dissolved in dimethylformamide (300 mL) and cooled to 0 °C. Sodium hydride (20.0 g, 475 mmol, 57% mineral oil dispersion) was added and the suspension was vigorously stirred at 0 °C for 15 minutes. Benzyl bromide (33.4 mL, 280 mmol) was then added dropwise with stirring, and vigorous stirring was continued for 12 h, allowing the system to warm to room temperature. The reaction mixture was cooled in an ice bath and treated with methanol, after which stirring was continued for 20 minutes. The mixture was transferred to a separation funnel, diluted with ethyl acetate and washed with water (3 x 300 mL). The organic layer was dried over Na_2SO_4, filtered, and the volatiles removed by rotary evaoration. The resulting material was purified by normal phase flash chromatography eluting with EtOAc/hexanes (1:4) containing 3% Et_3N. Compound **2** (35.7 g, 70.0 mmol, 90%) was isolated as a colorless wax. The recorded ^{1}H NMR spectrum of **2** was consistent with previously reported data. [1] ^{1}H NMR ($CDCl_3$, 500 MHz): 7.43-7.24 (m, 15H), 5.37 (d, *J* = 2.5 Hz, H-1), 4.91 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.78 (d, *J* = 11.9 Hz, 1H), 4.63 (d, *J* = 11.9 Hz, 1H), 4.63 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.41 (d, *J* = 2.6 and 3.9 Hz, 1H), 3.94 (dd, *J* = 9.3 and 9.3 Hz, 1H), 3.77 (d, *J* = 4.5 and 11.1 Hz, H-6), 3.72 (d, *J* = 2.3 and 11.1 Hz, H-6'), 3.44 (ddd, *J* = 2.3, 4.5 and 9.5 Hz, H-5), 3.30 (s, 3H), 1.57 (s, 3H).

(Scheme 2) *1,2-diacetoxymannose benzyl ether, (2R,3S,4S,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2,3-diyl diacetate,* **3**. Orthoester **2** (13.2 g, 26.0 mmol) was dissolved in glacial acetic acid (100 mL) and the solution was stirred overnight at room temperature. The solvent was removed by rotary evaporation and the residue was dissolved in ethyl acetate, transferred to separation funnel, and washed with saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4, filtered, and the volatiles removed under vacuum. The residue was dissolved in pyridine (20.0 mL) and acetic anhydride (15.0 mL) was added. After 6 hours of stirring at rt, the mixture was cooled in an ice bath and treated with ethanol (20.0 mL). The mixture was stirred for an additional 30 min, the volatiles were then removed by rotary evaporation. The residue was dissolved in ethyl acetate, transferred to separation funnel and washed sequentially with water, 1M HCl, and saturated aqueous sodium bicarbonate. The organic fraction was dried over Na_2SO_4, filtered, and the volatiles removed *in vacuo*. The resulting material was purified by flash chromatography eluting with a gradient EtOAc/hexanes (5:95 to 3:7). Compound **3** (11.7 g, 22.0 mmol, 85%) was isolated as a colorless syrup. The recorded ^{1}H NMR spectrum of **3** was consistent with previously reported data. [2] ^{1}H NMR ($CDCl_3$): 7.31-7.27 (m, 13H), 7.18 (m, 2H), 6.13 (d, J = 1.6 Hz, H-1), 5.37 (s, H-2), 4.87 (d, J = 10.6 Hz, 1H), 4.74 (d, J = 11.2 Hz, 1H), 4.69 (d, J = 12.1 Hz, 1H), 4.56 (d, J = 11.2 Hz, 1H), 4.52 (d, J = 10.6 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 3.99 (m, H-3 and H-4), 3.86 (m, H-5), 3.82 (dd, J = 3.8 and 11.2 Hz, H-6), 3.71 (dd, J = 1.1 and 11.2 Hz,H-6'), 2.18 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$): 169.9, 168.2, 137.9, 137.4, 128.3-127.5 (aromatic Cs), 91.1 (C-1), 77.5, 75.2, 73.7, 73.5, 73.4, 71.8, 68.3 (C-6), 67.4 (C-2), 20.8, 20.7.

(Scheme 2) *Benzyl acetoxy mannosyl imidate, (2R,3S,4S,5R,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3-yl acetate*, **4**. A solution of hydrazine monohydrate (436 mL, 14.1 mmol) in DMF (5.0 mL) was cooled to 0 °C in an ice bath and glacial acetic acid (812 mL, 14.1 mmol) was added dropwise. The resulting solution was then added to a solution of acetate **3** (7.56 g, 14.1 mmol) in DMF (28.0 mL) and the reaction was stirred overnight, allowing it to warm to room temperature. The mixture was then diluted with ethyl acetate, transferred to a separation funnel, and washed with water, 1M HCl, and saturated aqueous sodium bicarbonate. The resulting anomeric alcohol was purified by normal phase flash chromatography eluting with a gradient of ethyl acetate/hexanes (1:9 to 3:7). The respective anomeric alcohol (6.91 g, 14.0 mmol, 99%) was isolated as a colorless syrup. This alcohol (5.93 g, 12.0 mmol) and trichloroacetonitrile (3.6 mL, 36.0 mmol) were dissolved in methylene chloride (50.0 mL) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (270 mg, 1.8 mmol) was added. The resulting amber solution was stirred for 14 h at rt, at which time TLC showed complete consumption of the starting alcohol. The solvents were removed by rotary evaporation and the resulting material purified by normal phase flash chromatography eluting with an EtOAc/hexanes gradient (5:95 to 1:4), giving imidate **4** (6.55 g, 10.3 mmol, 86%). The recorded ^{1}H NMR spectrum of **4** was consistent with previously reported data.[2]

(Scheme 2) *Acetoxy tribenzyl mannose azidopropyl glycoside, (2S,3S,4S,5R,6R)-2-(3-azidopropoxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl acetate,* **5**. Diacetate **3** (6.7 g, 12.5 mmol) and 3-azidopropanol (2.5 g, 25.0 mmol) were dissolved in dry CH_2Cl_2 (40.0 mL) and cooled to 0 °C using an ice bath. Boron trifluoride etherate (1.5 mL, 10.0 mmol) was added dropwise with stirring, and the mixture was then allowed to warm to room temperature. After 12 h, the mixture was cooled in an ice bath and quenched by the addition of

triethylamine (2.0 mL). The volatiles were removed by rotary evaporation. The resulting crude product was purified by normal phase flash chromatography eluting with EtOAc/hexanes. Glycoside **5** (6.9 g, 12.0 mmol, 96%) was isolated as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): 7.40-7.28 (m, 13H), 7.20 (m, 2H), 5.39 (s, H-2), 4.89 (d, *J* = 11.2 Hz, 1H), 4.89 (s, H-1), 4.75 (d, *J* = 10.7 Hz, 1H), 4.72 (d, *J* = 10.7 Hz, -1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 11.8 Hz, 1H), 4.51 (d, *J* = 11.0 Hz, 1H), 4.00 (dd, *J* = 3.1 and 9.2 Hz, H-3), 3.92 (dd, *J* = 9.3 and 9.3 Hz, H-4), 3.82 (m, 3H, H-5, H-6 and H-6'), 3.76 (dd, *J* = 10.0 and 10.0 Hz, 1H), 3.52 (ddd, *J* = 6.2, 6.2 and 12.0 Hz, 1H), 3.38 (dd, *J* = 6.2 and 6.2 Hz, 2H), 2.19 (s, 3H), 1.87 (m, 2H). ^{13}C NMR ($CDCl_3$, 70 MHz): 170.5, 138.3, 138.2, 137.9, 128.4-127.7, 97.9 (C-1), 78.1 (C-3), 75.3, 74.3 (C-4), 73.5, 71.9, 71.6, 68.8, 68.7 (C-2), 64.5, 48.3, 28.8, 21.2.

(Scheme 2) *Protected mannosyl dimer, (2R,3S,4S,5R,6R)-2-(((2S,3S,4S,5R,6R)-2-(3-azido-propoxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl acetate,* **6**. Glycoside **5** (2.4 g, 4.2 mmol) was dissolved in a CH_2Cl_2/MeOH mixture (2:1, 20 mL) and sodium methoxide (20.0 mg) was added. The reaction mixture was stirred at rt overnight and then was diluted with methanol and neutralized with Amberlite IR-120 (H^+). The mixture was filtered, and the volatiles removed by rotary evaporation, followed by three cycles of methylene chloride addition and rotary evaporation. The resulting oil was dried under high vacuum overnight, dissolved in dry methylene chloride and glycosyl imidate **4** (2.45 g, 3.8 mmol) was added. The volatile solvent was removed by rotary evaporation, and trace water was removed by three cycles of toluene addition and rotary evaporation. Then resulting mixture was dissolved in dry methylene chloride and 4 Å molecular sieves (5.0 g) were added. The suspension was stirred at rt for 30 min, cooled to 0 °C with an ice bath, and the glycosylation was started by addition of trimethylsilyl trifluoromethanesulfonate (206 µL, 1.1 mmol). The reaction was monitored by TLC; when complete consumption of the glycosyl donor was observed, the reaction was treated with triethylamine (1.0 mL) and then filtered through a Celite® pad. The volatiles were then removed *in vacuo* and the residue purified by normal phase flash chromatography eluting with EtOAc/hexanes. Disaccharide **6** (3.54 g, 3.5 mmol, 92%) was isolated a colorless oil. ^{1}H NMR ($CDCl_3$): 7.36-7.24 (m, 25H), 7.20 (m, 3H), 7.15 (m, 2H), 5.53 (dd, *J* = 1.8 and 3.2 Hz, H-2'), 5.07 (d, *J* = 1.8 Hz, H-1'), 4.85 (m, 3H), 4.66 (m, 4H), 4.55 (d, *J* = 10.8 Hz, 1H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.46 (d, *J* = 10.9 Hz, 1H), 4.41 (d, *J* = 10.9 Hz, 1H), 3.96 (m, 3H), 3.88 (dd, *J* = 2.9 and 9.3 Hz, 1H), 3.83 (m, 2H), 3.71 (m, 7H), 3.30 (ddd, 6.2, 6.2, and 10.1 Hz, 1H), 3.25 (m, 2H), 2.13 (s, 3H), 1.74 (m, 2H); ^{13}C NMR ($CDCl_3$): 169.9, 138.3, 138.2, 138.1, 137.9, 137.7, 128.2-127.3 (aromatic Cs), 99.3 (anomeric C), 98.5 (anomeric C), 79.3, 77.9, 75.0, 74.9, 74.7, 74.3, 74.2, 73.2, 73.1, 71.9, 71.8, 71.7, 71.6, 69.0, 69.0, 68.5, 64.1, 48.2, 28.6, 20.96.

(Scheme 2) *Protected trimannose azide, (2R,3R,4S,5S,6R)-2-(acetoxymethyl)-6-(((2R,3S,4S,5R,6R)-2-(((2S,3S,4S,5R,6R)-2-(3-azidopropoxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate,* **7**. Disaccharide **6** (1.3 g, 1.3 mmol) was dissolved in a methylene chloride/methanol mixture (2:1, 20 mL) and sodium methoxide (~20.0 mg) was added. The reaction was stirred at rt for 12 h and then diluted with methanol and neutralized with Amberlite IR-120 (H^+). The resin was removed by filtration and the volatiles removed by rotary evaporation. Residual methanol was removed by

three cycles of methylene chloride addition and rotary evaporation, followed by drying under high vacuum overnight. The resulting crude material was dissolved in dry methylene chloride and glycosyl donor **8** was added. The volatile solvent was removed by rotary evaporation, and trace water was removed by three cycles of toluene addition and rotary evaporation. Then resulting mixture was dissolved in dry methylene chloride (8.0 mL) and 4 Å molecular sieves (1.5 g) were added. The suspension was stirred for 30 min, cooled in an ice bath, and the glycosylation reaction initiated with trimethylsilyl triflate (73 µL, 0.4 mmol). The mixture was stirred until complete consumption of the imidate donor was observed by TLC, followed by quenching with triethylamine (0.5 mL). The molecular sieves were removed by filtration through a Celite® pad, and the volatiles were removed by rotary evaporation. The resulting crude prodeuct was purified by normal phase flash chromatography eluting with EtOAc/hexanes. Trisaccharide **7** (1.4 g, 1.08 mmol, 81%) was isolated as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.40-7.20 (m, 30H), 5.44 (m, 2H), 5.29 (dd, *J* = 9.9 and 9.9 Hz, 1H), 5.18 (d, *J* = 1.5 Hz, 1H), 4.94 (d, *J* = 1.5 Hz, 1H), 4.93 (d, *J* = 1.3 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.84 (d, *J* = 10.7 Hz, 1H), 4.71 (d, *J* = 12.1 Hz, 1H), 4.57-4.51 (m, 7H), 4.15 (m, 2H), 4.03 (dd, *J* = 2.1 and 2.1 Hz, 1H), 3.98 (m, 3H), 3.92 (dd, *J* = 2.7 and 9.2 Hz, 1H), 3.87 (m, 2H), 3.77 (m, 8H), 3.63 (ddd, *J* = 5.9, 5.9 and 11.9 Hz, 1H), 3.27 (m, 1H), 3.23 (dd, *J* = 6.9 and 6.9 Hz, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.72 (m, 2H); ^{13}C NMR ($CDCl_3$): 170.4, 169.6, 169.6, 169.6, 138.3, 138.2, 138.1, 138.0, 128.3-127.3 (aromatic Cs), 100.3, 99.0, 98.6, 79.2, 75.4, 75.0, 74.7, 73.1, 73.0, 72.3, 72.2, 72.0, 71.8, 69.4, 69.3, 69.0, 68.9, 68.7, 65.9, 64.1, 62.0, 48.2, 28.6, 20.7, 20.5, 20.5, 20.5.

(Scheme 2) *Trimannose alkyne, N-(3-(((2S,3S,4S,5S,6R)-3-(((2R,3S,4S,5S,6R)-4,5-dihydroxy-6-(hydroxy-methyl)-3-(((2R,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)pent-4-ynamide,* **Man3**. Trisaccharide **7** (531.5 mg, 0.41 mmol) was dissolved in a dichloromethane/methanol mixture (1:1, 10 mL) and sodium methoxide (~20.0 mg) was added. The resulting suspension was vigorously stirred at rt for 24 h, diluted with methanol, and neutralized with Amberlite IR-120 (H^+). The resin was removed by filtration, and the volatiles were removed by rotary evaporation. The resulting material was dissolved in methanol (4.0 mL) followed by addition of trifluoroacetic acid (50 µL, 0.41 mmol) and 10% palladium on activated charcoal (~50 mg). The reaction flask was sealed with a septum and evacuated briefly and backfilled with hydrogen three times to replace the air with a hydrogen atmosphere. The suspension, kept under 1 atm H_2, was vigorously stirred at rt for 48 h, and then filtered through a plug of Celite® to remove the catalyst. The volatiles were removed by rotary evaporation and the crude product was dissolved in dimethylformamide (1.5 mL). *N*-Hydroxysuccinimido pentynoate (200.1 mg, 1.03 mmol) was added and the reaction mixture stirred at rt. After 72 h, ethanolamine (1.0 mL) was added and stirring was continued for 30 min. The volatiles were removed by rotary evaporation under high vacuum and the resulting crude material was purified by automated reversed-phase chromatography using a Biotage Isolera system (12 g SNAP Ultra C18 column, eluting with a MeOH/H_2O gradient from 0:1 to 1:1, ELSD detection). Fractions containing the target trisaccharide (identified by mass spectrometry of aliquots) were pooled, concentrated by rotary evaporation, then dissolved in water and lyophilized. The **Man$_3$** trisaccharide (163.1 mg, 0.25 mmol, 61%) was obtained as a white solid. ^{1}H NMR (CD_3OD, 300 MHz): 5.29 (s, 1H, anomeric), 5.07 (s, 1H, anomeric), 5.01 (s, 1H, anomeric), 4.05 (s, 1H), 4.00 (s, 1H), 3.89-3.86 (m, 5H), 3.78-3.67 (m, 6H), 3.65-3.55 (m, 2H), 3.53-3.48 (m, 3H), 3.36 (m, 1H), 3.29 (dd, *J* =

2.3 and 6.7 Hz, 1H), 2.47 (m, 2H), 2.40 (m, 2H), 2.30 (dd, *J* = 2.5 and 2.5 Hz, 1H), 1.79 (dd, *J* = 6.3 and 6.3 Hz, 2H), 1.35 (m, 2H); 13C NMR (CD_3OD, 70 MHz): 172.7, 101.6 (anomeric C), 101.1 (anomeric C), 98.6 (anomeric C), 79.2, 78.8, 73.7, 73.5, 73.3, 70.9, 70.8, 70.5, 70.4, 68.9, 67.8, 67.8, 67.3, 64.6, 61.9, 61.6, 61.5, 36.1, 34.7, 29.2, 28.9, 14.4.

Virus-like nanoparticle preparation and characterization.

Qβ-(AF488)$_{20}$(N$_3$)$_{700}$. A solution of Qβ (200 μL from 10 mg/mL stock solution in 0.1 M K-Phos, pH 7.4, 0.14 μmol in subunit, 0.56 μmol in reactive amines per subunit) was cooled in an ice bath and wrapped in aluminum foil to protect from light. AlexaFluor 488 succinimidyl ester (10 μL from 10 mM stock, 0.1 μmol) was added, and the tube was gently inverted to mix the reagents and then was placed on a slowly rotating shaker at rt for 3 h. The extent of acylation was determined by electrospray ionization mass spectrometry (ESI-MS) analysis of the reaction mixture (20 AF488 per particle, 0.11 per subunit). The reaction was then cooled in an ice bath and NHS-azide [3] (40 μL from 100 mM stock, 4.0 μmol) was added. The reaction vessel was inverted to mix the reagents and incubated on a rotating shaker at rt for 3 h. The reaction mixture was purified using PD-10 prepacked Sephadex columns (GE Healthcare) according to the manufacturer's instructions and the resulting particles were concentrated using Amicon Ultra-4 100 kDa centrifugal filters (EMD Millipore). The sample was sterile filtered through a 0.2 μm PTFE syringe filter, and the total protein content was quantified by Bradford assay against BSA standards. Recovery of protein was approximately 70%. The extent of modification was determined by ESI-TOF HRMS (700 ± 50 azides per particle, 3.89 per subunit).

Qβ-(AF488)$_{20}$(Man)$_{540}$. To a solution of Qβ(AF488)$_{20}$(N$_3$)$_{700}$ (250 μL from 5.3 mg/mL stock solution in 0.1 M K-Phos, pH 7.4, 87 nmol subunit, 0.35 μmol reactive azides) was added a mixture of arylmannoside alkyne (21 μL from 50 mM stock, 1.05 μmol), a premixed solution of copper sulfate (10 μL from 50 mM stock, 0.5 μmol) and tris(3-hydroxypropyltriazolylmethyl) amine. (THPTA)[4, 5] (50 μL from 50 mM stock, 2.5 μmol), and aminoguanidine (35 μL from 100 mM stock, 3.5 μmol). The tube was slowly inverted to mix all components and the reaction was initiated by the addition of sodium ascorbate (35 μL from 100 mM stock), followed by immediate mixing and incubation at 37 °C for 4 h. The reaction mixture was purified using prepacked PD-10 columns according to the manufacturer's instructions. Particles were concentrated using Amicon Ultra-4 100 kDa centrifugal filters. Samples were sterile filtered through 0.2 μm PTFE syringe filters, and protein content was quantified by Bradford assay against BSA standards. Recovery of protein was approximately 71%. The extent of modification was determined by ESI-TOF HRMS (540 phenyl mannosides per particle, 3 per subunit).

Qβ-(AF488)$_{20}$(pentaerythritol-derived triol (PE)**)$_{540}$.** To a solution of Qβ (AF488)$_{20}$(N$_3$)$_{700}$ (250 μL from 5.3 mg/mL stock solution in 0.1 M K-Phos, pH 7.0, 87 nmol subunit, 0.35 μmol reactive azides) was added a mixture of 2,2-bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol[6] (21 μL from 50 mM stock, 1.05 μmol), a premixed solution of $CuSO_4$ (10 μL from 50 mM stock, 0.5 μmol) and THPTA (50 μL from 50 mM stock, 2.5 μmol), and aminoguanidine (35 μL from 100 mM stock, 3.5 μmol). The tube was slowly inverted to mix all components and the reaction was initiated by the addition of sodium ascorbate (35 μL from 100 mM stock), followed by immediate mixing and incubation at 37 °C for 4 h. The reaction mixture was purified using a PD-10 column according to the manufacturer's instructions, and particles were concentrated

using Amicon Ultra-4 100 kDa centrifugal filters. The sample was sterile-filtered through a 0.2 μm PTFE syringe filter, and the protein content was quantified by Bradford assay against BSA standards; recovery was approximately 77%. The extent of modification was determined by ESI-time of flight (TOF)-high resolution mass spectrometry (HRMS) (540 PE per particle).

Qβ-$(AF488)_{20}$$(PE)_{450}$$(Man)_{90}$. To a solution of Qβ $(AF488)_{20}(N_3)_{700}$ (250 μL from 5.3 mg/mL stock solution in 0.1 M K-Phos, pH 7.0, 87 nmol subunit, 0.35 μmol reactive azides) was added added a mixture of phenyl mannoside alkyne (4.2 μL from a 50 mM stock in dimethylsulfoxide (DMSO), 0.21 μmol), a premixed solution of $CuSO_4$ (10 μL of 50 mM stock, 0.5 μmol) and THPTA (50 μL of 50 mM stock, 2.5 μmol), and aminoguanidine (35 μL of 100 mM stock, 3.5 μmol). The tube was slowly inverted to mix all components and the reaction was initiated by the addition of sodium ascorbate (35 μL of 100 mM stock), followed by immediate mixing and incubation at 37 °C for 4 h. The extent of modification was determined by ESI-TOF HRMS (90 Man per particle, 0.5 per subunit). To cap the remaining azides, a mixture of 2,2-bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol[3] (21 μL from 50 mM stock, 1.05 μmol), a premixed solution of $CuSO_4$ (10 μL of 50 mM stock, 0.5 μmol) and THPTA (50 μL of 50 mM stock, 2.5 μmol), aminoguanidine (35 μL of 100 mM stock, 3.5 μmol), and sodium ascorbate (35 μL of 100 mM stock, 3.5 μmol) were added. The tube was gently inverted to mix the reagents and incubated at 50 °C for 1 h. The reaction mixture was purified using PD-10 prepacked Sephadex columns according to the manufacturer's instructions and particles were concentrated using Amicon Ultra-4 100 kDa centrifugal filters. The sample was sterile-filtered through a 0.2 μm PTFE syringe filter, and the total protein content was quantified by Bradford assay against BSA standards. Recovery of protein was approximately 62%.

Qβ-$(AF488)_{20}$ $(Man_3)_{475}$. To a solution of Qβ $(AF488)_{20}(N_3)_{700}$ (150 μL from 12.5 mg/mL stock solution in 0.1 M K-Phos, pH 7.0, 126 nmol subunit, 0.470 μmol reactive azides) was added added a mixture of trimannose-alkyne (112 μL from 50 mM stock; 5.6 μmol), a premixed solution of $CuSO_4$ (23.5 μL from 200 mM stock, 4.7 μmol) and THPTA (23.5 μL from 1 M stock, 23.5 μmol), and aminoguanidine (93 μL from 100 mM stock, 9.3 μmol). The tube was slowly inverted to mix all components and the reaction was initiated by the addition of sodium ascorbate (93 μL from 500 mM stock; 9.3 μmol), followed by immediate mixing and incubation at 50 °C for 2 h. The tube was gently inverted to mix the reagents and incubated at 50 °C for 2 h. The reaction mixture was purified using PD-10 prepacked Sephadex columns according to the manufacturer's instructions and particles were concentrated using Amicon Ultra-4 100 kDa centrifugal filters. The sample was sterile filtered through a 0.2 μm PTFE syringe filter, and the total protein content was quantified by Bradford assay against BSA standards. Recovery of protein was approximately 67%. The extent of modification was determined by ESI-TOF HRMS (475 trimannose per particle, 2.6 per subunit). Particles following CuAAC conjugation were assessed by dynamic light scattering and found to be stable.

Qβ-$(AF488)_{20}$ $(Man_3)_{200}$$(PE)_{340}$. To a solution of Qβ $(AF488)_{20}(N_3)_{700}$ (100 μL from 12.5 mg/mL stock solution in 0.1 M K-Phos, pH 7.0, 84 nmol subunit, 0.311 μmol reactive azides) was added a mixture of trimannose-alkyne (14.3 μL from 50 mM stock, 0.715 μmol), a premixed solution of copper sulfate (15.5 μL from 200 mM stock, 3.1 μmol) and THPTA (15.5 μL from 1 M stock, 15.5 μmol), and aminoguanidine (62 μL from 500 mM stock, 31 μmol). The tube was slowly inverted to mix all components and the reaction was initiated by the addition of sodium

ascorbate (62 µL from 500 mM stock, 31 µmol), followed by immediate mixing and incubation at 50 °C for 2 h. The extent of modification was determined by ESI-TOF HRMS (200 Man per particle, 1.1 per subunit). To cap the remaining azides, a mixture of 2,2-bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol[3] (31.1 µL from 50 mM stock, 3.11 µmol), a premixed solution of $CuSO_4$ (12.5 µL from 200 mM stock, 2.5 µmol) and THPTA (12.5 µL from 1 M stock, 12.5 µmol), aminoguanidine (62 µL of 500 mM stock, 31 µmol), and sodium ascorbate (62 µL of 500 mM stock, 31 µmol) were added. The tube was gently inverted to mix the reagents and then was incubated at 50 °C for 2 h. The reaction mixture was purified using PD-10 prepacked Sephadex columns according to the manufacturer's instructions and particles were concentrated using Amicon Ultra-4 100 kDa centrifugal filters. The sample was sterile filtered through a 0.2 µm PTFE syringe filter, and the total protein content was quantified by Bradford assay against BSA standards. Recovery of protein was approximately 77%. Particle stability following conjugation is shown by dynamic light scattering (DLS).

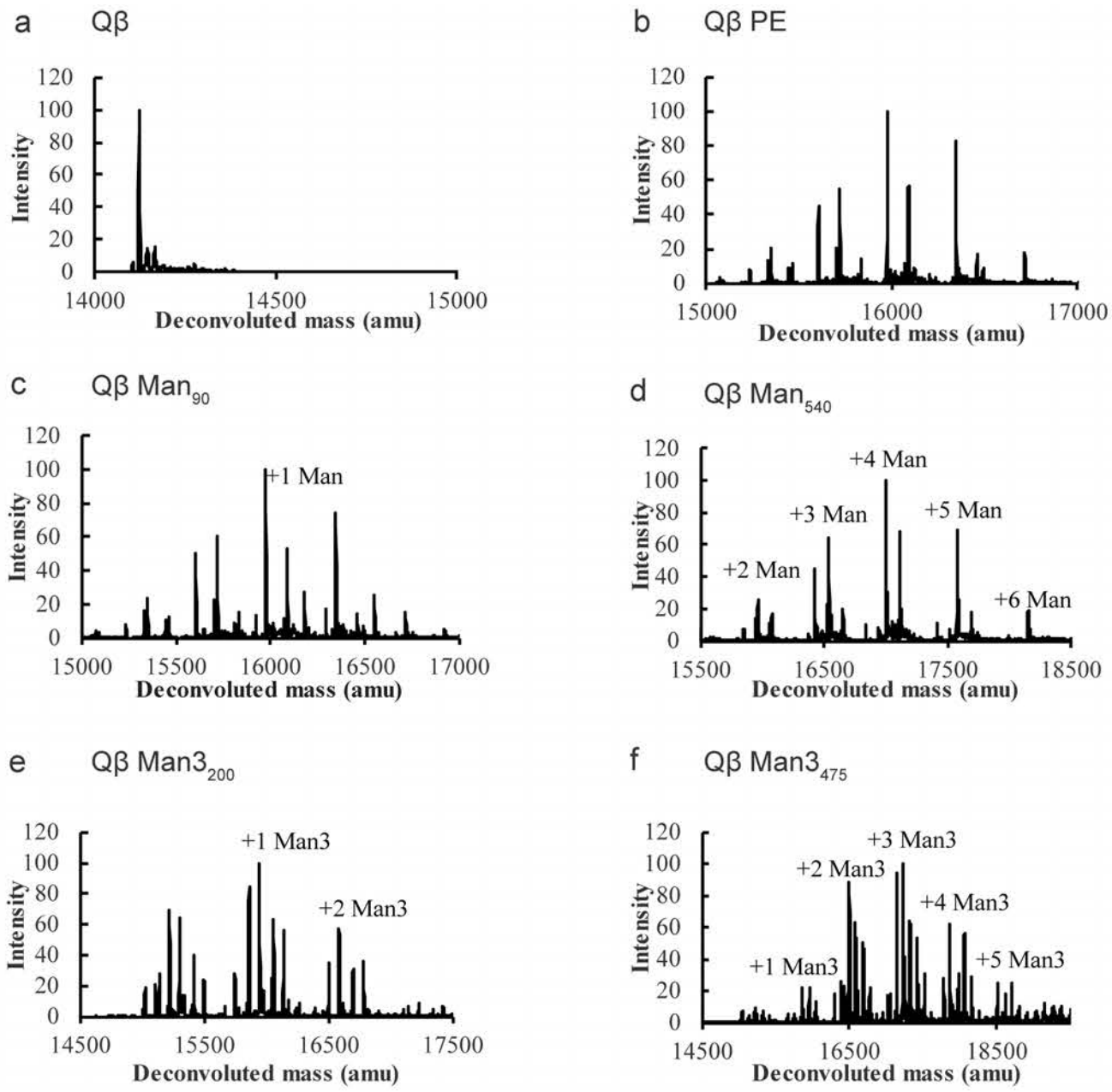
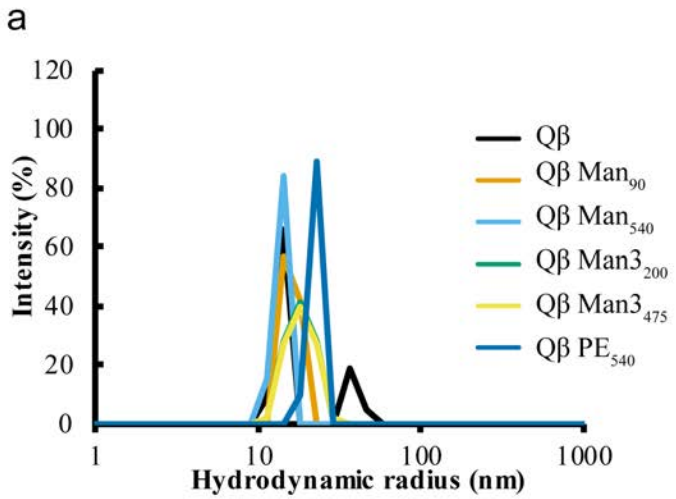


Figure S1. Deconvoluted ESI-TOF high resolution mass spectrometry (HRMS) spectra for Qβ, Qβ-PE, Qβ-Man_{90}, Qβ-Man_{540}, Qβ-$Man3_{200}$ and Qβ-$Man3_{475}$. Each cluster of peaks indicates the presence of protein subunits with differing numbers of attachments to lysine residues. Detection efficiencies are assumed to be similar; thus, peak intensities are assumed to correspond to relative concentrations of the component subunits.



b

VLP	R (nm)	Pd (%)
Qβ	16.2	14.1
Qβ Man_{90}	15.9	16.9
Qβ Man_{540}	18.0	13.9
Qβ $Man3_{200}$	17.9	18.1
Qβ $Man3_{475}$	17.7	15.4
Qβ PE_{540}	18.4	12.8

Figure S2. Dynamic light scattering analysis of Qβ-mannoside and Qβ-PE conjugates. (a) Histogram showing hydrodynamic radius of Qβ conjugates. (b) Hydrodynamic radii and polydispersities of indicated particles provided by the instrument software.

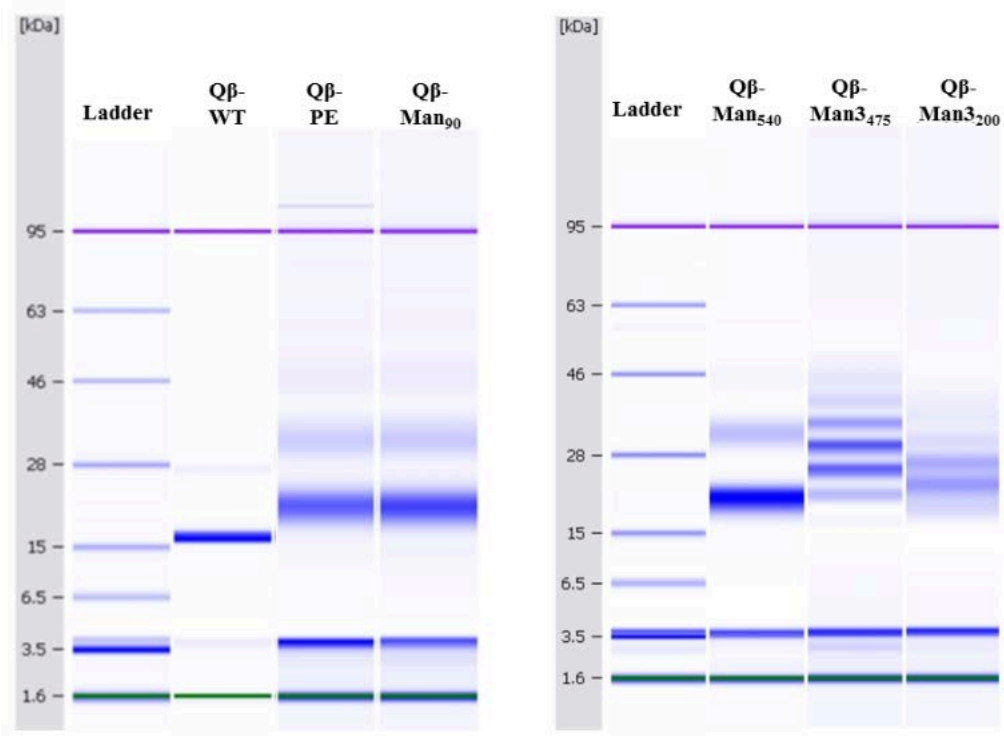


Figure S3. Microchip electrophoretic analysis of Qβ-mannoside and Qβ-PE conjugates.

Qβ-Ovalbumin (Ova) particles: Hybrid Qβ VLPs with C-terminal 'Ova' peptide extensions were recombinantly expressed and prepared as described previously.[7, 8] The incorporation number of the extended capsid protein was determined by protein chip analysis and LCMS analysis (Figure S4 and S6; ~30 copies per particle). The azide, mannose, trimannose, and pentaerythritol modified versions of the Qβ-CatD-Ova hybrid particles were prepared as described above for the wild-type Qβ VLPs.

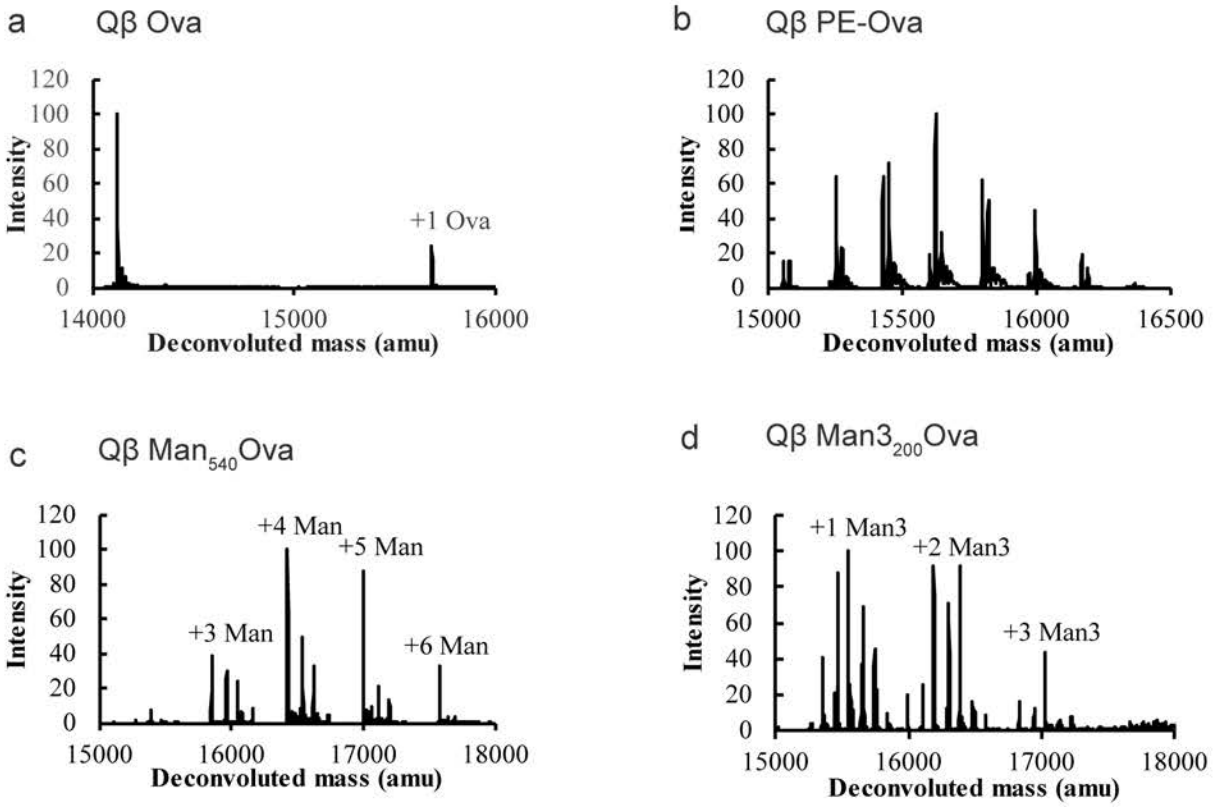
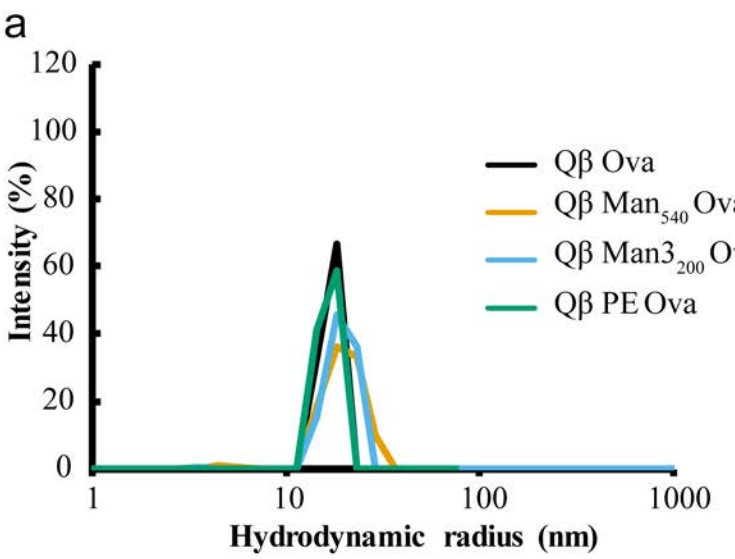


Figure S4. Deconvoluted ESI-TOF HRMS spectra for Qβ-Ova conjugates. Each cluster of peaks indicates the presence of protein subunits with differing numbers of attachments to lysine residues. Detection efficiencies are assumed to be similar; thus, peak intensities are assumed to correspond to relative concentrations of the component subunits.



b

VLP	R (nm)	Pd (%)
Qβ Ova	17.1	14.1
Qβ Man_{540} Ova	18.9	12.6
Qβ $Man3_{200}$ Ova	19.7	17.4
Qβ PE Ova	17.1	7.0

Figure S5. DLS analysis of Qβ-Ova conjugates. (a) Histogram showing hydrodynamic radius of Qβ-Ova conjugates. (b) Hydrodynamic radius and polydispersity of indicated particles provided by the instrument software.

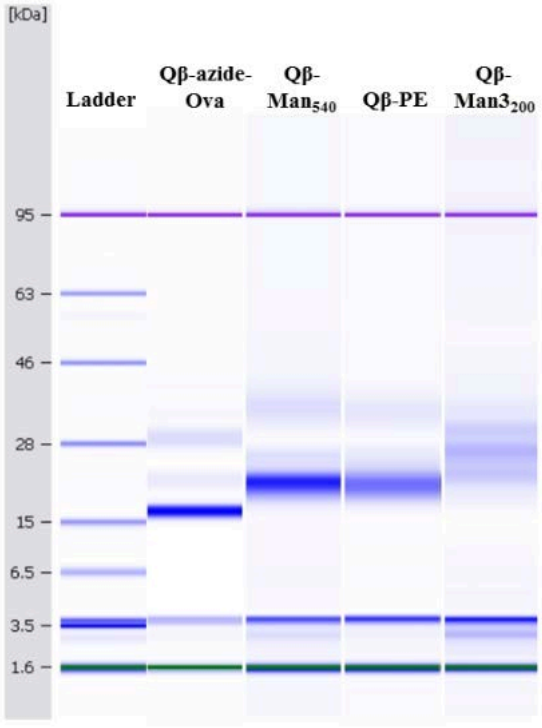


Figure S6. Microchip electrophoretic analysis of Qβ-Ova conjugates.

Table S1. Properties of the mannosylated and control particles used in this study.

Virus-like particle	Mannoside conc. [μM]	μg RNA/mg VLP
Qβ-$(AF488)_{20}$-$(Man)_{540}$	674	448 ± 12
Qβ-$(AF488)_{20}$-$(Man)_{90}$	102	680 ± 10
Qβ-$(AF488)_{20}$-$(Man3)_{475}$	901	592 ± 8
Qβ-$(AF488)_{20}$-$(Man3)_{200}$	403	659 ± 12
Qβ-$(AF488)_{20}$-$(PE)_{540}$	0	507 ± 13

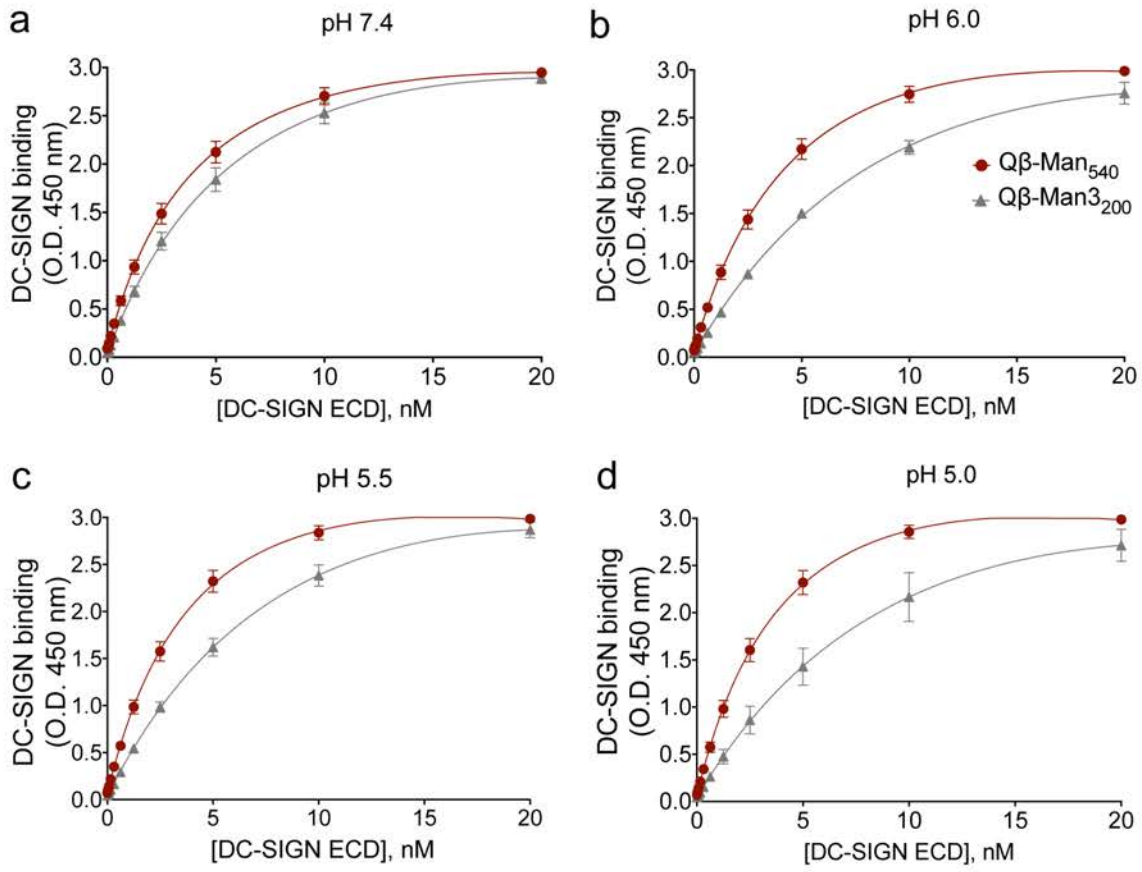


Figure S7. Enzyme-linked immunosorbent assay (ELISA) measurement of binding of Qβ-Man_{540} and Qβ-$Man3_{200}$ to DC-SIGN at different pH values. Mannosylated VLPs (Qβ-Man_{540} or Qβ-$Man3_{200}$) were immobilized on ELISA plates and the binding to an increasing concentration of DC-SIGN extracellular domain (ECD) was measured at pH ranging from 7.4 to 5.0. The error bars represent standard error of the mean (SEM) from at least three independent experiments.

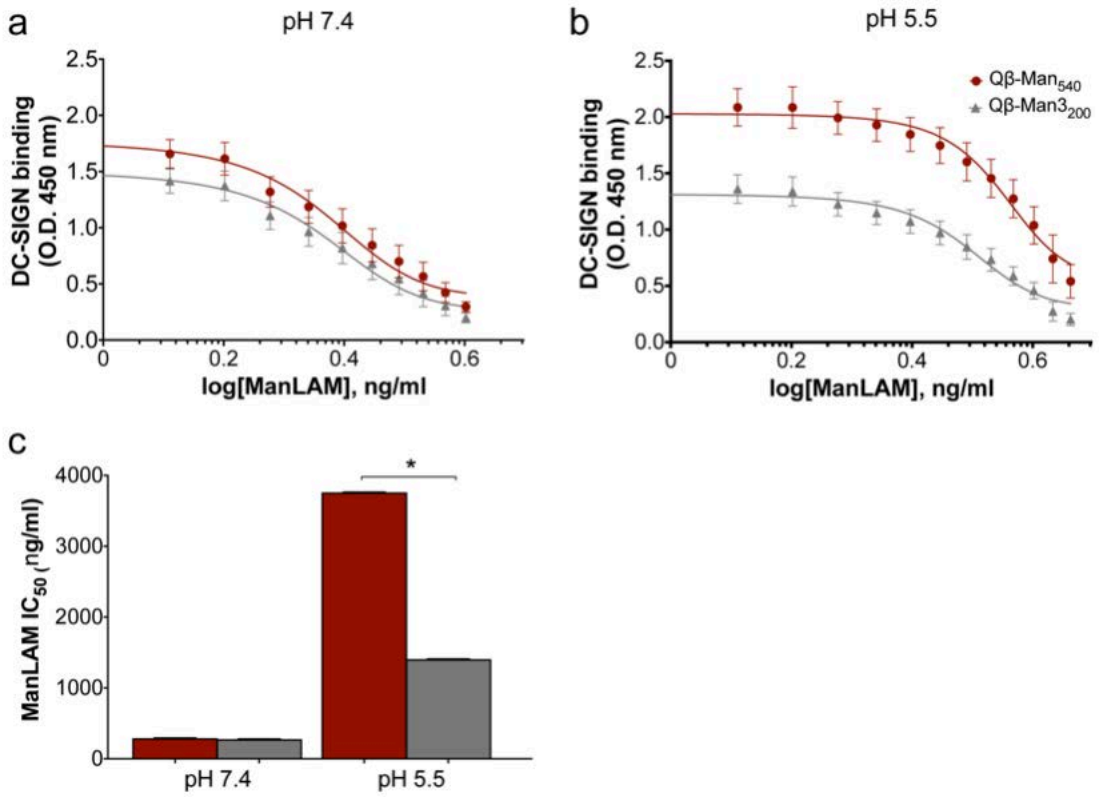


Figure S8. Binding inhibition of DC-SIGN and Qβ VLPs with mannose-capped lipoarabinomannan (ManLAM) at different pH values. Mannosylated VLPs were immobilized on ELISA plates and the binding of DC-SIGN ECD to the VLPs were inhibited by an increasing concentration of ManLAM at (a) pH 7.4 or (b) 5.5. (c) The half maximal inhibitory concentration (IC50) of ManLAM was calculated using Graphpad Prism. The error bars represent standard error of the mean (SEM) from at least three independent experiments. Wilcoxon matched-paired signed rank test was used for data analysis. *P<0.05.

Intracellular trafficking

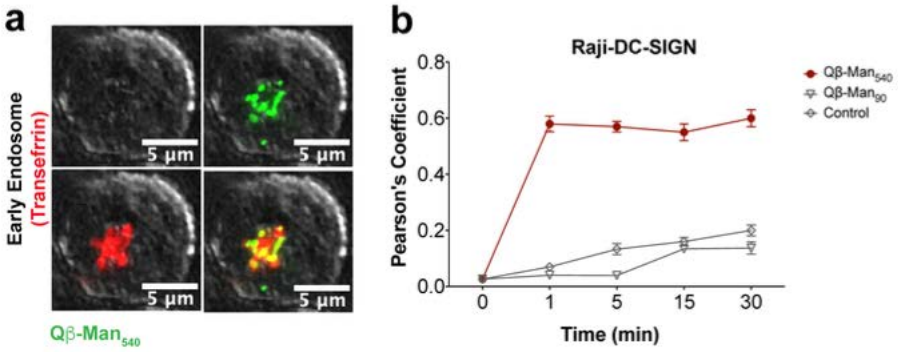


Figure S9. Early endosomal trafficking of VLPs in Raji DC-SIGN cells [9]. Early endosomes were labeled with Cy3- transferrin. Pearson's coefficient was calculated for colocalization of the VLPs and transferrin using colocalization threshold plugin in Fiji.

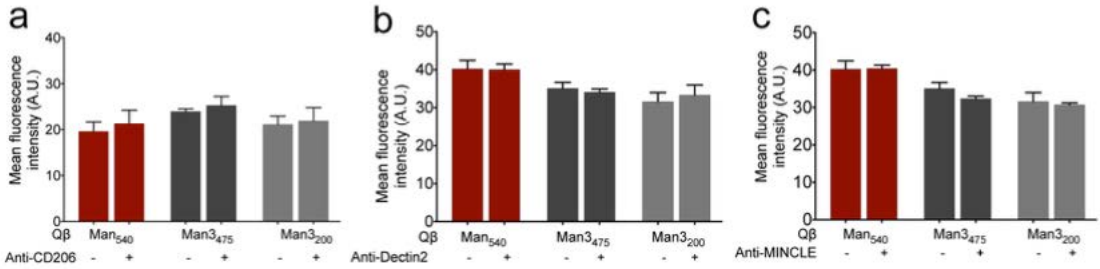
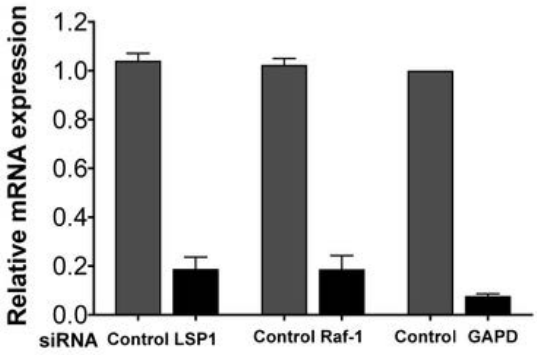


Figure S10. Uptake of the Qβ-Man_{540} is not mannose receptor (CD206), Dectin2 or MINCLE mediated. Human monocyte-derived DCs ($0.5x10^6$ cells/mL) were pretreated with anti-human CD206, anti-human Dectin2 or anti-human MINCLE antibody (20 µg/ml) for 20 min on ice followed by VLP (2 nM) stimulation at 37 °C for 15 min in PBS (pH 7.4) supplemented with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$. Internalization of the Qβ-Man_{540} was measured by flow cytometry.

Gene expression knockdown

Figure S11. Gene silencing using siRNA. Quantitative real time PCR analysis of the expression of LSP, Raf-1 and GAPD mRNA in moDCs treated with control (non-targeting), LSP, Raf-1 or GAPD specific small interfering RNA (siRNA). The error bars represent standard error of the mean (SEM) from at least three independent experiments.



Characterization of gene expression

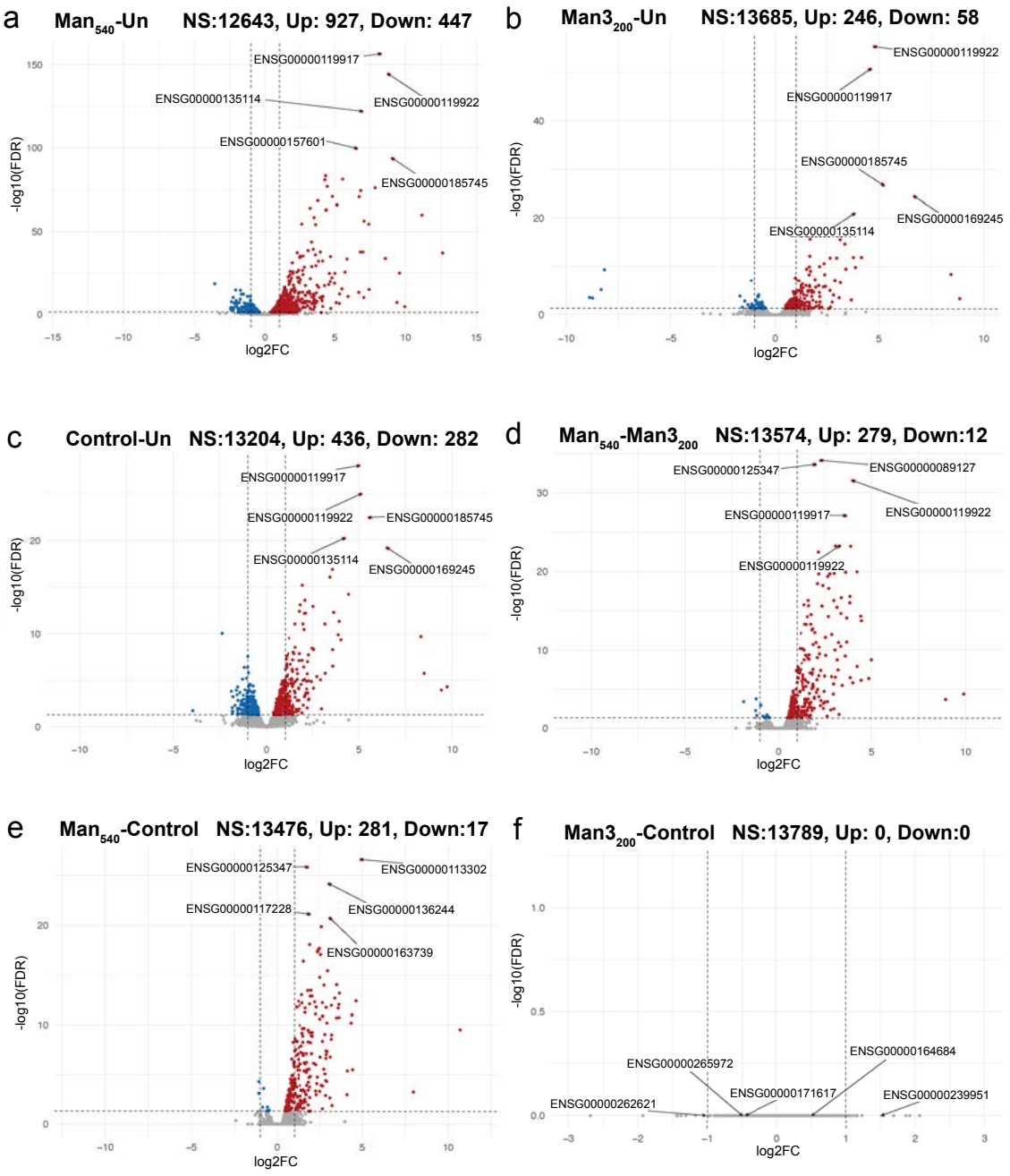


Fig S12. Volcano plot of RNA-Seq data. NS=Not significant, Up and Down = numbers of genes significantly upregulated or downregulated.

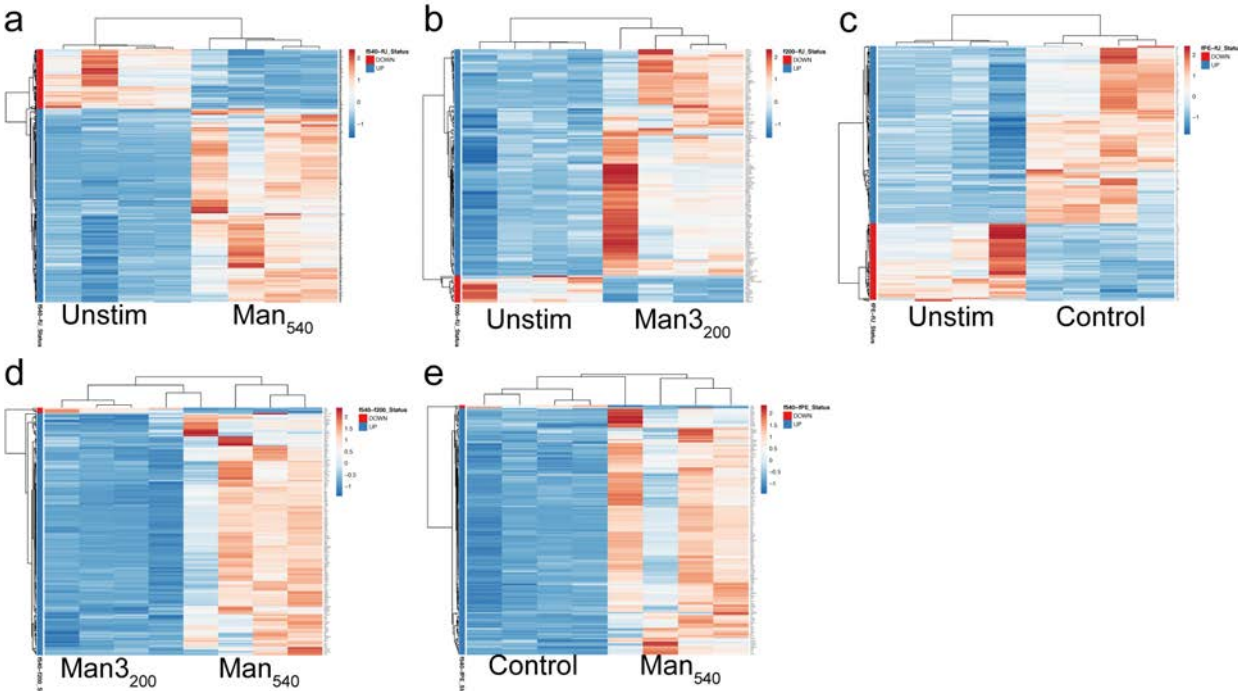


Fig S13. Heat maps of RNA-Seq data. moDCs were stimulated with mannosylated or the control particle (4 nM) for 6 h (n=4). RNAs were extracted using standard methods and sequenced. Comparative heat map data showing relative RNA expression in each individual sample.

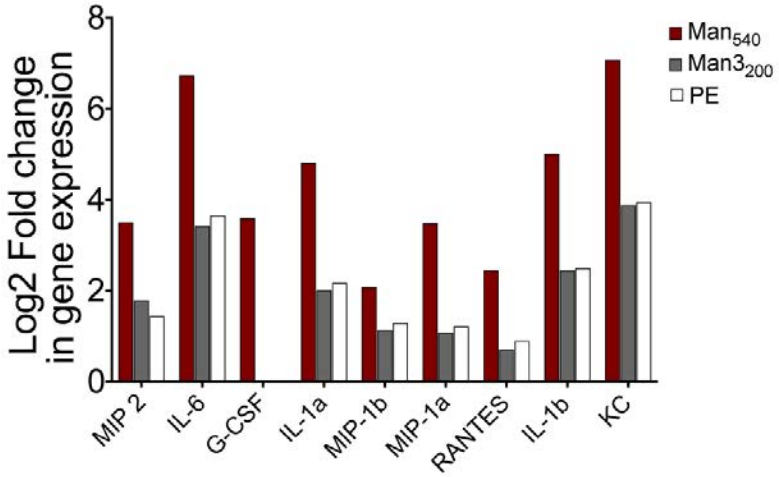


Fig S14. Cytokines and chemokines relevant to anti-tumor immunity are induced in moDC following Qβ-Ova-Man_{540} treatment. Data analyzed from RNA-Seq analysis.

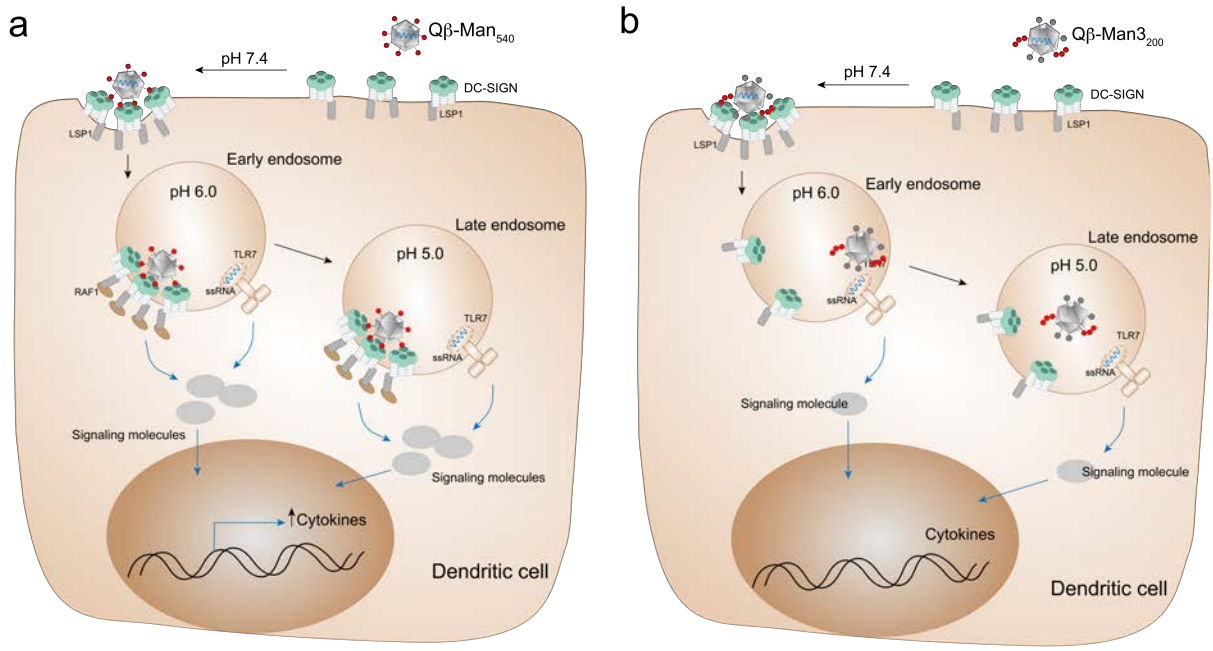


Fig S15. Model depicting the effect of pH on (a) Qβ-Man_{540} and (b) Qβ-$Man3_{200}$ binding and activation of DC-SIGN signaling pathway. Following internalization, the VLPs traffic to the endosomal compartments where they encounter acidic microenvironment (pH 6.0-5.0). In the acidic environment the binding affinity of Qβ-Man_{540} remained unaltered that accounts for a stable activation of signaling events leading to cytokine expression in DCs, whereas the binding affinity of Qβ-$Man3_{200}$ decreased drastically that inhibit the formation of an active signaling complex leading to perturbed cytokine expression in DCs.

Table S2: The top 100 upregulated genes in moDC stimulated with VLPs.

	VLP-Man$_{540}$		VLP-Man3$_{200}$		VLP-PE	
Gene Symbol	**Log_2 Fold change**	**Corrected P values (FDR)**	**Log_2 Fold change**	**Corrected P values (FDR)**	**Log_2 Fold change**	**Corrected P values (FDR)**
CXCL11	12.63	6.08E-38	8.44	3.66E-09	8.32	1.94E-10
CXCL10	11.16	1.15E-60	6.70	3.80E-25	6.51	6.71E-20
AC008695.1	9.96	1.10E-05	NA	NA	9.41	1.08E-04
IFNB1	9.58	8.18E-26	NA	NA	NA	NA
IFIT1	9.07	1.85E-94	5.21	1.73E-27	5.55	3.61E-23
IFIT2	8.79	3.52E-145	4.79	4.50E-56	5.10	1.05E-25
AL445490.1	8.58	1.28E-34	NA	NA	NA	NA
IFIT3	8.21	2.68E-157	4.60	1.87E-51	4.99	9.54E-29
IFI44L	7.86	7.06E-77	4.17	1.17E-12	4.44	6.27E-15
USP41	7.42	5.66E-16	NA	NA	NA	NA
APOBEC3A	7.41	3.09E-55	3.79	1.17E-12	3.89	1.25E-10
CXCL1	7.07	4.55E-57	3.88	1.30E-10	3.94	4.51E-12
GBP1P1	7.05	3.93E-11	NA	NA	NA	NA
IL12B	6.99	1.47E-38	NA	NA	NA	NA
OASL	6.91	9.02E-123	3.82	1.10E-21	4.22	6.05E-21
RSAD2	6.84	4.00E-75	2.99	1.57E-12	3.18	8.53E-12
CCL8	6.81	1.47E-38	2.91	6.64E-08	3.19	1.69E-06
IL6	6.73	1.67E-71	3.43	3.02E-10	3.65	5.61E-13
CXCL9	6.70	3.07E-14	2.69	1.88E-02	2.24	9.47E-02
MX1	6.54	2.03E-100	3.16	2.39E-16	3.58	1.33E-17
IL27	5.94	9.19E-12	NA	NA	NA	NA
TNFSF10	5.87	2.67E-34	2.18	1.24E-04	2.20	8.25E-05
ETV7	5.73	2.12E-26	NA	NA	NA	NA
USP18	5.56	4.65E-82	2.16	1.79E-05	2.57	4.70E-09
TNIP3	5.51	2.31E-16	3.69	6.16E-04	4.03	4.25E-10
CCL1	5.34	2.45E-13	NA	NA	NA	NA
IFNL1	5.33	1.38E-07	NA	NA	NA	NA
CMPK2	5.16	1.16E-66	1.99	1.37E-06	2.25	9.52E-08
CCL15	5.10	4.51E-08	NA	NA	NA	NA
IL1B	5.01	1.20E-25	2.45	9.71E-07	2.49	3.38E-09
PDGFRL	4.98	8.58E-20	NA	NA	NA	NA
ISG15	4.84	1.20E-71	1.81	6.43E-06	2.05	3.56E-07
IL1A	4.80	5.94E-23	2.01	1.79E-03	2.17	2.14E-04
IFNG	4.72	1.51E-04	NA	NA	NA	NA

HERC5	4.70	9.62E-36	1.81	1.79E-05	2.00	1.02E-05
ISG20	4.68	8.98E-21	1.62	2.88E-02	1.72	1.76E-02
HESX1	4.62	1.22E-25	1.37	2.04E-01	1.41	1.20E-01
CCL2	4.57	5.57E-19	2.01	1.36E-02	2.13	3.56E-04
OAS2	4.47	1.19E-77	1.74	7.90E-10	2.14	6.86E-13
MX2	4.41	8.17E-64	1.41	5.25E-05	1.70	7.76E-06
OAS3	4.38	1.35E-63	1.70	6.27E-13	2.08	7.08E-13
IFI44	4.35	4.08E-84	1.68	1.07E-10	2.11	2.78E-14
IFI6	4.30	1.23E-81	1.45	3.14E-04	1.71	1.82E-06
TNFSF18	4.28	2.37E-18	2.67	6.72E-06	2.57	6.98E-05
IL23A	4.18	7.91E-12	NA	NA	NA	NA
TRIL	4.15	9.24E-06	NA	NA	NA	NA
SGPP2	4.14	4.26E-33	3.38	1.90E-15	3.45	8.89E-17
CCL20	4.11	4.60E-09	1.43	5.30E-01	1.54	7.00E-02
IFI27	4.08	1.26E-11	NA	NA	NA	NA
AC083837.1	4.07	2.76E-10	NA	NA	NA	NA
RPL36A-HNRNPH2	4.05	4.16E-02	NA	NA	NA	NA
DDX58	3.98	1.56E-38	1.28	6.62E-07	1.44	1.08E-06
AC093583.1	3.95	3.93E-11	3.11	2.21E-04	3.60	2.90E-07
PLAT	3.86	2.57E-17	1.65	3.31E-02	2.08	5.55E-04
TNFAIP6	3.85	5.62E-33	2.39	2.23E-11	2.54	1.27E-13
TNF	3.81	3.49E-69	2.17	8.93E-11	1.97	6.65E-16
GBP4	3.75	1.57E-18	0.87	6.02E-01	0.91	1.64E-01
DNAAF1	3.75	6.56E-09	NA	NA	NA	NA
NEURL3	3.70	5.88E-14	1.62	7.42E-02	1.75	1.96E-02
HES4	3.68	6.20E-08	NA	NA	NA	NA
PTGS2	3.67	3.04E-30	1.30	1.14E-02	1.26	6.15E-03
BATF2	3.64	5.95E-55	0.99	2.02E-02	1.10	1.03E-02
IFIT5	3.63	9.90E-37	1.39	6.83E-03	1.58	2.20E-04
CXCL8	3.59	2.57E-09	2.16	8.88E-03	2.01	4.60E-03
ZBP1	3.59	1.54E-09	NA	NA	NA	NA
CSF2	3.58	1.90E-07	NA	NA	NA	NA
TRIM31	3.58	2.05E-05	NA	NA	NA	NA
NEXN	3.57	8.22E-10	NA	NA	NA	NA
IDO1	3.55	2.83E-12	NA	NA	NA	NA
KCTD14	3.55	6.35E-06	NA	NA	NA	NA
EPSTI1	3.54	3.54E-59	1.12	1.16E-03	1.51	1.29E-08
G0S2	3.51	4.21E-09	2.83	5.96E-05	3.59	4.92E-09
CCL4L2	3.50	3.75E-09	1.91	2.78E-02	2.33	9.74E-04

CXCL2	3.50	3.55E-11	1.79	3.12E-02	1.43	4.61E-02
GP1BA	3.49	8.20E-04	2.59	4.07E-02	3.00	1.15E-02
LRRC32	3.49	3.19E-13	3.17	1.57E-07	2.75	3.84E-06
HELZ2	3.48	3.71E-40	1.28	1.41E-06	1.40	6.41E-06
CCL3L1	3.48	7.05E-08	1.27	4.60E-01	1.49	7.35E-02
CCL15-CCL14	3.37	1.56E-07	2.28	2.17E-02	2.94	8.64E-06
VCAM1	3.37	1.60E-11	2.19	1.62E-03	2.26	2.24E-04
PMAIP1	3.37	9.54E-30	1.69	6.37E-07	1.81	1.31E-08
IRF7	3.36	1.62E-44	1.22	2.21E-04	1.49	2.28E-05
APOBEC3B	3.31	3.31E-04	0.66	9.61E-01	0.61	6.68E-01
PLSCR1	3.27	1.84E-22	1.06	6.64E-02	1.33	1.53E-03
NFKBIZ	3.26	8.12E-65	2.67	1.57E-12	2.41	9.93E-19
PRAL	3.26	2.04E-13	1.11	3.10E-01	1.47	8.48E-03
DSP	3.25	9.93E-05	NA	NA	-0.69	2.14E-02
CXCL3	3.20	8.42E-09	1.40	2.45E-01	1.45	6.90E-02
IFITM1	3.19	6.33E-15	0.68	6.47E-01	0.88	1.49E-01
GBP1	3.11	2.26E-39	0.93	3.14E-04	1.18	1.31E-08
EXOC3L1	3.09	2.51E-06	NA	NA	NA	NA
GCNT4	3.07	1.49E-08	NA	NA	NA	NA
AC009950.1	3.07	4.26E-14	0.76	6.48E-01	0.88	2.34E-01
TNC	3.00	5.83E-09	1.73	2.14E-02	2.21	1.24E-05
OSM	2.94	2.27E-12	1.42	7.74E-03	1.58	6.42E-04
ADM	2.93	1.95E-18	1.09	1.03E-01	1.67	6.44E-07
GPR84	2.92	3.25E-05	NA	NA	2.23	9.09E-03
C15orf48	2.90	1.16E-07	1.46	4.85E-02	2.46	1.30E-05
TNFRSF4	2.90	1.64E-12	2.41	4.46E-09	2.30	1.77E-08
XAF1	2.85	2.28E-29	1.01	1.24E-02	1.23	4.35E-04

Table S3: 281 upregulated genes in moDC stimulated with VLP-Man_{540}.

Gene Symbol	Log_2 fold change	Corrected P values (FDR)	Description
IFNB1	9.58	8.18E-26	interferon beta 1
AL445490.1	8.58	1.28E-34	
USP41	7.42	5.66E-16	ubiquitin specific peptidase 41
GBP1P1	7.05	3.93E-11	guanylate binding protein 1 pseudogene 1
IL12B	6.99	1.47E-38	interleukin 12B
IL27	5.94	9.19E-12	interleukin 27
ETV7	5.73	2.12E-26	ETS variant 7
CCL1	5.34	2.45E-13	C-C motif chemokine ligand 1
IFNL1	5.33	1.38E-07	interferon lambda 1
CCL15	5.10	4.51E-08	C-C motif chemokine ligand 15
PDGFRL	4.98	8.58E-20	platelet derived growth factor receptor like
IFNG	4.72	1.51E-04	interferon gamma
HESX1	4.62	1.22E-25	HESX homeobox 1
IL23A	4.18	7.91E-12	interleukin 23 subunit alpha
TRIL	4.15	9.24E-06	TLR4 interactor with leucine rich repeats
CCL20	4.11	4.60E-09	C-C motif chemokine ligand 20
IFI27	4.08	1.26E-11	interferon alpha inducible protein 27
AC083837.1	4.07	2.76E-10	
RPL36A-HNRNPH2	4.05	4.16E-02	RPL36A-HNRNPH2 readthrough
GBP4	3.75	1.57E-18	guanylate binding protein 4
DNAAF1	3.75	6.56E-09	dynein axonemal assembly factor 1
HES4	3.68	6.20E-08	hes family bHLH transcription factor 4
ZBP1	3.59	1.54E-09	Z-DNA binding protein 1
CSF2	3.58	1.90E-07	colony stimulating factor 2
TRIM31	3.58	2.05E-05	tripartite motif containing 31
NEXN	3.57	8.22E-10	nexilin F-actin binding protein
IDO1	3.55	2.83E-12	indoleamine 2 3-dioxygenase 1
KCTD14	3.55	6.35E-06	potassium channel tetramerization domain containing 14
CCL3L1	3.48	7.05E-08	C-C motif chemokine ligand 3 like 1
APOBEC3B	3.31	3.31E-04	apolipoprotein B mRNA editing enzyme catalytic subunit 3B
DSP	3.25	9.93E-05	desmoplakin
CXCL3	3.20	8.42E-09	C-X-C motif chemokine ligand 3
IFITM1	3.19	6.33E-15	interferon induced transmembrane protein 1
EXOC3L1	3.09	2.51E-06	exocyst complex component 3 like 1
GCNT4	3.07	1.49E-08	glucosaminyl (N-acetyl) transferase 4 core 2

AC009950.1	3.07	4.26E-14	
IL15RA	2.74	2.46E-14	interleukin 15 receptor subunit alpha
OAS1	2.73	1.96E-27	2'-5'-oligoadenylate synthetase 1
IRF1	2.67	3.09E-55	interferon regulatory factor 1
MIR3945HG	2.55	1.37E-03	MIR3945 host gene
PARP9	2.54	3.45E-35	poly(ADP-ribose) polymerase family member 9
EIF2AK2	2.50	5.26E-20	eukaryotic translation initiation factor 2 alpha kinase 2
EREG	2.46	1.11E-04	epiregulin
RTP4	2.46	3.26E-23	receptor transporter protein 4
SP110	2.44	1.73E-27	SP110 nuclear body protein
CCL5	2.44	1.43E-09	C-C motif chemokine ligand 5
NT5C3A	2.43	4.78E-14	5'-nucleotidase cytosolic IIIA
TNK2-AS1	2.41	1.92E-04	TNK2 antisense RNA 1
SIGLEC1	2.39	2.29E-11	sialic acid binding Ig like lectin 1
AC116407.2	2.37	8.41E-07	
DDX60	2.36	4.13E-23	DExD/H-box helicase 60
HCG4B	2.34	1.58E-02	HLA complex group 4B (non-protein coding)
STAT1	2.32	2.27E-26	signal transducer and activator of transcription 1
IL19	2.32	8.51E-03	interleukin 19
STAP1	2.31	1.66E-04	signal transducing adaptor family member 1
RASGEF1B	2.25	2.19E-09	RasGEF domain family member 1B
AC245128.3	2.23	3.24E-02	
IMPDH1P10	2.22	2.08E-03	inosine monophosphate dehydrogenase 1 pseudogene 10
HAPLN3	2.22	6.60E-08	hyaluronan and proteoglycan link protein 3
C1orf106	2.20	9.51E-04	chromosome 1 open reading frame 106
LTA	2.16	1.20E-05	lymphotoxin alpha
CAMK1G	2.13	5.44E-03	calcium/calmodulin dependent protein kinase IG
TMEM88	2.10	1.38E-03	transmembrane protein 88
HDX	2.10	1.01E-05	highly divergent homeobox
DUSP8	2.10	1.45E-03	dual specificity phosphatase 8
HLA-K	2.09	1.13E-03	major histocompatibility complex class I K (pseudogene)
CCL4	2.07	3.15E-03	C-C motif chemokine ligand 4
CCL3	2.07	8.71E-04	C-C motif chemokine ligand 3
SOCS3	2.06	1.38E-15	suppressor of cytokine signaling 3
SAMD9	2.06	4.42E-08	sterile alpha motif domain containing 9
TNFSF9	2.06	3.84E-07	TNF superfamily member 9
IFITM3	2.05	4.85E-26	interferon induced transmembrane protein 3

PNPT1	2.04	1.68E-25	polyribonucleotide nucleotidyltransferase 1
TRIM25	2.04	1.29E-26	tripartite motif containing 25
AC124319.2	2.03	6.95E-06	
PML	2.00	5.58E-32	promyelocytic leukemia
BCL2A1	2.00	4.14E-03	BCL2 related protein A1
AC004988.1	1.99	8.09E-03	
GAREM1	1.99	3.36E-02	GRB2 associated regulator of MAPK1 subtype 1
AIM2	1.98	8.45E-06	absent in melanoma 2
U62317.5	1.98	4.56E-02	
DDX60L	1.97	3.25E-10	DEAD-box helicase 60 like
GCH1	1.94	1.91E-11	GTP cyclohydrolase 1
TRIM15	1.94	3.07E-02	tripartite motif containing 15
NCF1B	1.90	7.01E-04	neutrophil cytosolic factor 1B pseudogene
KCNA3	1.89	2.38E-04	potassium voltage-gated channel subfamily A member 3
C1orf147	1.89	4.03E-03	chromosome 1 open reading frame 147
SERPING1	1.88	1.51E-06	serpin family G member 1
DTX3L	1.87	3.66E-21	deltex E3 ubiquitin ligase 3L
NT5C3AP1	1.85	6.70E-03	5'-nucleotidase cytosolic IIIA pseudogene 1
TRIM21	1.85	8.57E-27	tripartite motif containing 21
AC116366.1	1.84	4.77E-04	
MASTL	1.84	1.54E-13	microtubule associated serine/threonine kinase like
AC020931.1	1.84	1.57E-03	
ATF3	1.83	5.40E-05	activating transcription factor 3
IFI35	1.83	1.19E-24	interferon induced protein 35
NKD1	1.83	7.57E-03	naked cuticle homolog 1
TAP1	1.83	1.62E-20	transporter 1 ATP binding cassette subfamily B member
MIR155HG	1.82	1.17E-05	MIR155 host gene
AL021707.6	1.82	3.10E-03	
GMPR	1.79	4.27E-05	guanosine monophosphate reductase
U62317.3	1.78	1.96E-11	
FAM46A	1.78	2.57E-12	family with sequence similarity 46 member A
OTOF	1.78	2.68E-02	otoferlin
DHX58	1.76	5.32E-19	DExH-box helicase 58
DUSP5	1.75	3.13E-21	dual specificity phosphatase 5
GRHL1	1.75	6.84E-05	grainyhead like transcription factor 1
SLC12A5-AS1	1.73	3.50E-02	SLC12A5 and MMP9 antisense RNA 1
C5orf56	1.73	7.21E-06	chromosome 5 open reading frame 56

JAK3	1.72	1.32E-08	Janus kinase 3
HSH2D	1.68	9.13E-06	hematopoietic SH2 domain containing
PARP14	1.67	6.49E-12	poly(ADP-ribose) polymerase family member 14
MT1E	1.66	1.59E-02	metallothionein 1E
SAMD9L	1.66	3.80E-07	sterile alpha motif domain containing 9 like
RNF213	1.65	6.94E-11	ring finger protein 213
HIVEP2	1.65	2.61E-05	human immunodeficiency virus type I enhancer binding protein 2
ATP10A	1.65	3.22E-05	ATPase phospholipid transporting 10A (putative)
CFLAR-AS1	1.65	2.63E-04	CFLAR antisense RNA 1
MEFV	1.64	6.13E-05	MEFV pyrin innate immunity regulator
APOL6	1.61	5.88E-10	apolipoprotein L6
ARL5B	1.60	1.01E-05	ADP ribosylation factor like GTPase 5B
PTGER4	1.59	2.71E-14	prostaglandin E receptor 4
NLRC5	1.59	6.07E-12	NLR family CARD domain containing 5
BIRC3	1.59	3.75E-09	baculoviral IAP repeat containing 3
STAT2	1.58	3.24E-12	signal transducer and activator of transcription 2
CFB	1.57	9.40E-06	complement factor B
C19orf66	1.56	1.60E-14	chromosome 19 open reading frame 66
HBEGF	1.55	3.26E-02	heparin binding EGF like growth factor
MYD88	1.54	1.02E-13	myeloid differentiation primary response 88
CD274	1.52	3.59E-10	CD274 molecule
PARP10	1.52	4.57E-13	poly(ADP-ribose) polymerase family member 10
ZNFX1	1.51	2.18E-15	zinc finger NFX1-type containing 1
FAM225A	1.51	3.44E-04	family with sequence similarity 225 member A (non-protein coding)
SP140	1.50	1.13E-04	SP140 nuclear body protein
CSF1	1.50	1.87E-02	colony stimulating factor 1
FTH1P7	1.49	2.64E-02	ferritin heavy chain 1 pseudogene 7
APOBEC3G	1.47	1.44E-10	apolipoprotein B mRNA editing enzyme catalytic subunit 3G
TRIM22	1.46	2.31E-08	tripartite motif containing 22
TRIM10	1.46	3.55E-02	tripartite motif containing 10
INHBA	1.44	3.12E-03	inhibin beta A subunit
CLUHP3	1.43	3.14E-03	clustered mitochondria homolog pseudogene 3
HLA-DOB	1.42	3.21E-02	major histocompatibility complex class II DO beta
HCAR2	1.40	2.31E-04	hydroxycarboxylic acid receptor 2

PLAUR	1.39	6.35E-05	plasminogen activator urokinase receptor
AC073548.1	1.39	1.77E-04	
AC083862.2	1.38	8.63E-04	
FXYD6	1.37	6.85E-03	FXYD domain containing ion transport regulator 6
BISPR	1.36	9.46E-07	BST2 interferon stimulated positive regulator (non-protein coding)
TMEM217	1.36	4.96E-05	transmembrane protein 217
RGS1	1.35	1.59E-06	regulator of G protein signaling 1
IRF9	1.35	6.19E-17	interferon regulatory factor 9
AL136295.5	1.35	8.06E-03	
SAT1	1.34	1.07E-08	spermidine/spermine N1-acetyltransferase 1
LRG1	1.34	4.02E-03	leucine rich alpha-2-glycoprotein 1
CHST7	1.34	9.00E-03	carbohydrate sulfotransferase 7
CNP	1.34	3.04E-16	2'3'-cyclic nucleotide 3' phosphodiesterase
RASGRP1	1.34	1.53E-02	RAS guanyl releasing protein 1
TDRD7	1.34	2.76E-14	tudor domain containing 7
PPP1R15A	1.32	5.21E-12	protein phosphatase 1 regulatory subunit 15A
NCOA7	1.32	4.51E-08	nuclear receptor coactivator 7
TOR1B	1.32	1.10E-14	torsin family 1 member B
BLZF1	1.32	4.34E-08	basic leucine zipper nuclear factor 1
REC8	1.32	1.33E-04	REC8 meiotic recombination protein
FJX1	1.31	3.72E-02	four jointed box 1
MUC1	1.29	3.02E-02	mucin 1 cell surface associated
SLAMF1	1.28	1.07E-08	signaling lymphocytic activation molecule family member 1
KCNJ2	1.28	9.10E-03	potassium voltage-gated channel subfamily J member 2
CRLF2	1.28	3.23E-02	cytokine receptor-like factor 2
TMEM132A	1.28	1.81E-03	transmembrane protein 132A
TGIF2-C20orf24	1.27	9.52E-04	TGIF2-C20orf24 readthrough
MOB3C	1.27	1.62E-13	MOB kinase activator 3C
ELOVL7	1.26	9.16E-04	ELOVL fatty acid elongase 7
TNFAIP2	1.25	1.38E-07	TNF alpha induced protein 2
AC060766.1	1.25	3.54E-02	
KMO	1.25	4.40E-09	kynurenine 3-monooxygenase
AP001189.5	1.25	4.90E-02	
RHOF	1.24	1.62E-09	ras homolog family member F filopodia associated
TRIM56	1.24	9.27E-12	tripartite motif containing 56
TRIM69	1.24	6.80E-09	tripartite motif containing 69
PLEK	1.24	2.80E-07	pleckstrin

MB21D1	1.23	1.72E-09	Mab-21 domain containing 1
FUT4	1.23	6.00E-10	fucosyltransferase 4
GLRX	1.23	2.76E-10	glutaredoxin
HIVEP1	1.22	1.58E-04	human immunodeficiency virus type I enhancer binding protein 1
TNIP2	1.22	2.98E-06	TNFAIP3 interacting protein 2
SLFN5	1.22	5.74E-04	schlafen family member 5
C19orf71	1.21	1.87E-02	chromosome 19 open reading frame 71
AL137003.1	1.21	6.48E-03	
SRXN1	1.21	5.61E-04	sulfiredoxin 1
NABP1	1.21	7.05E-10	nucleic acid binding protein 1
TICAM1	1.21	1.26E-07	toll like receptor adaptor molecule 1
RHBDF2	1.20	7.02E-05	rhomboid 5 homolog 2
IL7	1.20	8.83E-03	interleukin 7
NID1	1.19	1.15E-03	nidogen 1
LAMP3	1.19	5.18E-09	lysosomal associated membrane protein 3
STOML1	1.18	3.89E-04	stomatin like 1
LINC00622	1.18	3.33E-03	long intergenic non-protein coding RNA 622
GBP5	1.18	5.03E-04	guanylate binding protein 5
NR4A3	1.18	5.02E-06	nuclear receptor subfamily 4 group A member 3
LGALS9	1.18	2.71E-05	galectin 9
TEX14	1.18	2.33E-02	testis expressed 14 intercellular bridge forming factor
ELL2	1.18	3.13E-06	elongation factor for RNA polymerase II 2
DENND3	1.18	4.01E-07	DENN domain containing 3
ARG2	1.17	2.21E-02	arginase 2
PSME2P2	1.17	8.83E-03	proteasome activator subunit 2 pseudogene 2
FP565260.3	1.17	1.87E-04	
ZNF107	1.17	1.01E-02	zinc finger protein 107
TAGAP	1.17	5.11E-11	T-cell activation RhoGTPase activating protein
GCNT2	1.17	6.59E-07	glucosaminyl (N-acetyl) transferase 2 I-branching enzyme (I blood group)
BTG2	1.17	7.67E-08	BTG anti-proliferation factor 2
THEMIS2	1.16	4.47E-11	thymocyte selection associated family member 2
LY6E	1.16	7.38E-06	lymphocyte antigen 6 family member E
PLXNA1	1.16	2.74E-05	plexin A1
SCARF1	1.16	1.95E-07	scavenger receptor class F member 1
CABYR	1.16	2.12E-02	calcium binding tyrosine phosphorylation regulated
PPM1K	1.15	8.85E-03	protein phosphatase Mg2+/Mn2+ dependent 1K

AKAP2	1.15	1.39E-04	A-kinase anchoring protein 2
MAFF	1.14	1.46E-04	MAF bZIP transcription factor F
TYMP	1.14	1.54E-04	thymidine phosphorylase
AC004687.1	1.14	8.59E-06	
PPIF	1.13	9.23E-05	peptidylprolyl isomerase F
ZC3HAV1	1.13	6.40E-07	zinc finger CCCH-type containing antiviral 1
SLC22A16	1.13	2.50E-03	solute carrier family 22 member 16
NFKBIE	1.13	7.98E-04	NFKB inhibitor epsilon
CRIM1	1.12	8.01E-12	cysteine rich transmembrane BMP regulator 1
SCO2	1.12	1.25E-05	SCO2 cytochrome c oxidase assembly protein
IL32	1.12	2.56E-03	interleukin 32
TEN1-CDK3	1.11	1.20E-02	TEN1-CDK3 readthrough (NMD candidate)
AL662797.3	1.11	3.37E-02	
FOSL1	1.11	1.11E-02	FOS like 1 AP-1 transcription factor subunit
GTPBP2	1.11	1.96E-11	GTP binding protein 2
APOL3	1.11	4.32E-05	apolipoprotein L3
ADAM19	1.11	1.65E-03	ADAM metallopeptidase domain 19
TMEM268	1.11	2.36E-04	transmembrane protein 268
APOL1	1.11	1.03E-06	apolipoprotein L1
TRIM5	1.10	1.96E-05	tripartite motif containing 5
RNF19B	1.10	4.48E-06	ring finger protein 19B
SPRED2	1.10	1.50E-08	sprouty related EVH1 domain containing 2
C10orf10	1.10	2.66E-03	chromosome 10 open reading frame 10
TRIM16	1.09	6.96E-07	tripartite motif containing 16
AL357060.3	1.09	2.67E-02	
LYPD3	1.09	8.32E-03	LY6/PLAUR domain containing 3
SLC25A28	1.09	2.22E-06	solute carrier family 25 member 28
MMP12	1.09	1.59E-06	matrix metallopeptidase 12
DAPP1	1.09	4.86E-07	dual adaptor of phosphotyrosine and 3-phosphoinositides 1
CCNJ	1.09	4.50E-08	cyclin J
LAP3	1.08	2.95E-08	leucine aminopeptidase 3
ADPRHL2	1.08	2.29E-11	ADP-ribosylhydrolase like 2
UBE2L6	1.08	3.89E-10	ubiquitin conjugating enzyme E2 L6
SKIL	1.08	1.07E-04	SKI like proto-oncogene
FAM72B	1.08	2.85E-02	family with sequence similarity 72 member B
PAPD7	1.07	2.34E-09	poly(A) RNA polymerase D7 non-canonical
SERPINB9	1.07	5.91E-04	serpin family B member 9
ABCA1	1.07	4.82E-02	ATP binding cassette subfamily A member 1
LINC01215	1.07	3.37E-02	long intergenic non-protein coding RNA 1215

JUNB	1.07	2.14E-03	JunB proto-oncogene AP-1 transcription factor subunit
C17orf107	1.06	3.23E-02	chromosome 17 open reading frame 107
TXNRD1	1.06	1.26E-04	thioredoxin reductase 1
AF117829.1	1.06	5.53E-03	
C17orf58	1.05	9.71E-05	chromosome 17 open reading frame 58
AL390066.1	1.05	6.06E-03	
DCP1A	1.04	7.19E-09	decapping mRNA 1A
CD69	1.04	4.77E-02	CD69 molecule
TLR2	1.04	3.25E-05	toll like receptor 2
TNIP1	1.04	1.29E-06	TNFAIP3 interacting protein 1
TNFRSF10A	1.04	2.33E-08	TNF receptor superfamily member 10a
STK17A	1.04	4.56E-08	serine/threonine kinase 17a
DNAH3	1.03	6.72E-04	dynein axonemal heavy chain 3
SLC7A11	1.03	7.79E-05	solute carrier family 7 member 11
MAP2K3	1.03	2.59E-04	mitogen-activated protein kinase kinase 3
RIPK2	1.03	4.81E-06	receptor interacting serine/threonine kinase 2
ACSL1	1.03	2.72E-03	acyl-CoA synthetase long-chain family member 1
FHL2	1.02	1.94E-02	four and a half LIM domains 2
HCAR3	1.02	2.08E-02	hydroxycarboxylic acid receptor 3
SMAD3	1.02	1.21E-03	SMAD family member 3
GTPBP1	1.02	1.95E-08	GTP binding protein 1
FAM225B	1.02	2.45E-02	family with sequence similarity 225 member B (non-protein coding)
SLFN12	1.01	1.08E-03	schlafen family member 12
NFKBID	1.01	4.49E-06	NFKB inhibitor delta
POGLUT1	1.00	3.26E-05	protein O-glucosyltransferase 1
ABTB2	1.00	1.52E-02	ankyrin repeat and BTB domain containing 2

Table S4: Real time qPCR primer sequences.

Gene	Gene ID	Forward Primer	Reverse Primer
IL-1b	3553	CACCTGTACGATCACTGAACTG	ACCACTTGTTGCTCCATATCC
IL-6	3569	TGCAATAACCACCCCTGACC	TGCGCAGAATGAGATGAGTTG
IL-8	3576	CATAAGGCACAAACTTTCAGAGAC	TTACACACAGTGAGATGGTTCC
IL-10	3586	GCTCCAAGAGAAAGGCATCTAC	CCCTGATGTCTCAGTTTCGTATC
IL-12B	3593	CACAGGAGGATGACACAGAAA	ACAATTTCATGTCCTTAGCCATAAC
IFN-b	3456	GCCGCATTGACCATCTATGA	GCCAGGAGGTTCTCAACAATAG
TNF	7124	AGAGGGAGAGAAGCAACTACA	GGGTCAGTATGTGAGAGGAAGA
GAPDH	2597	CCATGTTCGTCATGGGTGTG	GGTGCTAAGCAGTTGGTGGTG
b-Actin	60	TCAGAAGGATTCCTATGTGGGCGA	TTTCTCCATGTCGTCCCAGTTGGT
LSP1	4046	AGTCCCTAAACCGCTCCATA	GTGTATTGTTCCAGCCACTGA
Raf-1	5894	CTCGTGGACAGAGAGATTCAAG	CTCCGTGCCATTTACCCTTAT

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