Supporting information

Precise Immunological Evaluation Rationalized the Design of Self-Adjuvanting Vaccine Composed of Glycan Antigen, TLR1/2 Ligand, and T-helper Cell Epitope

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1. General information

$^1$H and $^{13}$C NMR spectra were recorded in an indicated solvent with a 500 MHz spectrometer equipped with a cryoprobe. For $^1$H NMR analysis, HDO ($\delta = 4.65$ ppm) is used as an internal standard for the measurement in D$_2$O. CHD$_2$OD ($\delta = 3.30$ ppm) is used as references for the measurements in CD$_3$OD. Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra were obtained on an ESI-LTQ-Orbitrap XL (FTMS) mass spectrometer. Unless otherwise noted, reactions in anhydrous solvent were carried out under Ar atmosphere. Distilled CH$_2$Cl$_2$ was distilled from calcium hydride. All other commercially available reagents and solvents were used as purchased.

Materials and reagents:
96 well Microwell$^{\text{TM}}$ MaxiSorp$^{\text{TM}}$ microtiter plates for the ELISA were purchased from Sigma Aldrich. Horseradish peroxidase (HRP)-linked goat anti-mouse IgG and IgM antibodies were purchased from Sigma Aldrich. HRP-linked goat anti-mouse IgG1, IgG2a, IgG2b, and IgG3 antibodies were purchased from Abcam. Alexa Fluro®488-goat anti-mouse IgG antibodies were purchased from Molecular Probes.
2. Synthesis procedures and characterization data for compounds 2 and 3.

**Scheme S1.** Synthesis of compound S2. (a) LiOH, MeOH, H$_2$O, rt, 14 h, 87%.

**Compound S2**

To a solution of compound S1$^1$ (28.0 mg, 9.99 x 10$^{-3}$ mmol) in MeOH/H$_2$O (v/v = 1/1, 2.0 mL), and then was treated with lithium hydroxide monohydrate (25.1 mg, 0.60 x 10$^{-1}$ mmol). After the solution was stirred at room temperature for 14 h, the reaction was neutralized with 90 mM HOAc$_{\text{aq}}$ and concentrated *in vacuo*. The crude product was purified by RP-HPLC on a Nacalai Tesque HILIC column (4.6 x 150 mm) at a flow rate of 1 mL/min using a mobile phase of 25 mM aqueous sodium acetate (Solvent A) and acetonitrile (Solvent B) (90% to 10% B gradient over 30 min, UV detection at 215 nm; retention time: 29.0 min) to afford S2 as a white solid following lyophilisation (17.0 mg, 85%). $^1$H NMR (500MHz, D$_2$O): $\delta$ 4.84 (d, $J = 4.0$ Hz, 1H), 4.77 (d, $J = 4.0$ Hz, 1H), 4.57 (d, $J = 3.0$ Hz, 1H), 4.42 (d, $J = 9.0$ Hz, 1H), 4.31-4.27 (m, 1H), 4.21-4.14 (m, 2H), 4.03-3.95 (m, 6H), 3.89-3.70 (m, 17H), 3.64-3.39 (m, 25H), 3.36-3.32 (m, 1H), 3.15-3.10 (m, 1H), 2.63-2.58 (m, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.92 (s, 9H), 1.90 (s, 3H), 1.62-1.55 (m, 3H), 1.26-1.22 (m, 4H), 1.16 (d, $J = 6.0$ Hz, 3H). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for C$_{77}$H$_{129}$N$_{13}$O$_{48}$ [M+2H]$^{2+}$: 1001.9021. Found: 1001.9069.
Scheme S2. Synthesis of compound 3. (a) CuSO₄, Sodium ascorbate, 80% DMSO in H₂O, rt, 3 h, 53%.

**Compound 3**

CuSO₄ (96 μg, 6.0 x 10⁻⁴ mmol) and sodium L-ascorbate (357 μg, 1.8 x 10⁻³ mmol) were mixed in oxygen-free water (57 μL). And then, the resultant solution was added to a solution of compound S2 (1.0 mg, 5.0 x 10⁻⁴ mmol) and S3¹ (1.05 mg, 6.0 x 10⁻⁴ mmol) in oxygen-free DMSO (255 μL) under Ar atmosphere at room temperature. After the mixture was stirred for 3.0 h at this temperature, DOTA (1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid, 2.42 mg, 6.0 x 10⁻³ mmol) was added, and the resulting solution was stirred for another 20 min. The mixture was lyophilized prior to purification. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C₁₈-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1 to 100% B gradient over 180 min; UV detection at 215 nm; retention time: 46.1 min) to afford 3 as a white solid following lyophilisation (1.0 mg, 53%). The compound 3 was analyzed by analytical RP-HPLC (see Figure S1). ¹H NMR (500MHz, D₂O): 7.79 (s, 1H), 7.30-7.23 (m, 3H), 7.18 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.0 Hz, 2H), 4.89 (d, J = 3.5 Hz, 1H), 4.83-4.81 (m, 1H), 4.77-4.73 (m, 2H), 4.59-4.51 (m, 3H), 4.45 (s, 1H), 4.38-4.31 (m, 4H), 4.26-4.01 (m, 14H), 3.97-3.74 (m, 24H), 3.67-3.51 (m, 17H), 3.45-3.38 (m, 3H), 3.13-3.05 (m, 2H), 3.00-2.70 (m, 7H), 2.67-2.64 (m, 8H), 2.42-2.39 (m, 2H), 2.29-2.12 (m, 4H), 2.10-1.92 (m, 18H), 1.91-1.53 (m, 15H), 1.44-1.00 (m, 25H), 0.87-0.70 (m, 17H). HRMS (ESI-LTQ-Orbitrap XL, positive, see Figure S2) calcd for C₁₅₀H₂₅₇N₂₂O₇₂ [M+3H]³⁺: 1255.9150. Found: 1255.9153.
Figure S1. HPLC data of compound 3 by analytical column (Nacalai Tesque 5C18-AR300, 4.6 x 250 mm; MeCN in H2O containing 0.1% TFA (1-100% gradient over 180 min, 1 mL/min); UV detection at 215 nm).

Figure S2. ESI-LTQ-Orbitrap MS data of compound 3.
Scheme S3. Synthesis of compound 2, (a) Zn, Ac₂O, HOAc, THF, 40 °C, 6 h, 90% (b) 20% piperidine/DMF, rt, 20 min; (c) LiOH, MeOH, H₂O, rt, 14 h, 91% (2 steps); (d) S7, NMM, CH₃CN, H₂O, rt, 13 h, 67%; (e) (I) 50 mM LiOH, H₂O, rt, 30 min, and then acidification by 90 mM HOAc/H₂O; (II) 20 mM TCEP, H₂O, rt, 30 min, 81% (2 steps) (f) S10, Et₃N, DMSO, 40 °C, 13 h, 47%.

Compound S5
To a solution of the compound S4¹ (28.0 mg, 9.99 x 10⁻³ mmol) in AcOH/THF (v/v = 1:1, total 1.0 mL) was added Zu powder (26.0 mg, 0.4 mmol) and acetic anhydride (0.3 ml) at room temperature. After the mixture was stirred for 6 h at room temperature, the
Zn powder was removed by filtration and the filtrate was concentrated in vacuo. The residue was extracted with AcOEt and the organic layer was washed with saturated aqueous NaHCO₃ solution and brine, dried over Na₂SO₄, and then concentrated in vacuo to give crude product. The residue was purified with silica-gel column chromatography (MeOH/CHCl₃ = 1/10) to achieve compound S5 as a white syrup (27.0 mg, 90 %). Rf 0.20 (MeOH/CHCl₃ = 1/10). ¹H NMR (500MHz, CD₃OD): δ 7.82 (d, J = 7.0 Hz, 2H), 7.70 (t, J = 7.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 5.38-5.29 (m, 10H), 5.21-5.14 (m, 2H), 5.10-5.03 (m, 2H), 4.97-4.94 (m 2H), 4.83-4.60 (m, 4H), 4.69 (d, J = 1.5 Hz, 1H), 4.59-4.55 (m, 1H), 4.50-4.39 (m, 5H), 4.32-4.24 (m, 9H), 4.18-4.03 (m, 9H), 3.98-3.90 (m, 3H), 3.88-3.77 (m, 12H), 3.62-3.46 (m, 9H), 3.40-3.38 (m, 2H), 3.30-3.27 (m, 2H), 3.12-3.08 (m, 1H), 2.61-2.51 (m, 3H), 2.16-2.14 (m, 9H), 2.10-2.05 (m, 21H), 1.99-1.96 (m, 27H), 1.93-1.90 (m, 9H), 1.85-1.72 (m, 12H), 1.37-1.27 (m, 9H). ¹³C NMR (125 MHz, CD₃OD): δ 173.6, 173.4, 173.2(x2), 172.7(x2), 172.3(x2), 172.2(x2), 172.1, 172.0(x3), 171.7(x3), 171.5, 171.4(x3), 169.1(x2), 169.0, 145.4, 145.1, 142.7(x2), 128.8(x2), 128.2(x2), 126.2, 126.1, 121.0, 120.9, 101.0, 100.6, 99.9(x2), 79.6, 78.4, 78.3, 73.2(x2), 71.3, 71.2, 70.6(x2), 70.4(x2), 70.3, 69.3(x2), 69.1, 69.0, 68.4(x3), 67.7, 64.7, 64.6(x2), 63.3(x2), 60.2, 58.4, 58.1, 53.4(x2), 50.0, 40.5, 38.9, 38.8, 38.7, 23.5, 23.3(x2), 22.7, 22.6, 21.2, 21.0, 20.9(x2), 20.8(x2), 20.7(x2), 19.7, 19.6, 19.5. HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for C₁₃₁H₁₈₁N₁₁O₆₈Na₃ [M+3Na]³⁺: 1022.0251. Found: 1022.0253.

**Compound S8**

The compound S5 (30.0 mg, 1.0 x 10⁻² mmol) was treated with 20% piperidine in DMF (0.5 mL) at room temperature. The solution was then stirred for 20 min at room temperature. Evaporation of DMF in vacuo was followed by purification by flash chromatography on silica gel to give the amine of triSTn. To a solution of the amine of triSTn in MeOH/H₂O (v/v = 1/1, 8.0 mL) was treated with lithium hydroxide monohydrate (16.8 mg, 0.4 mmol) at room temperature. After the solution was stirred at room temperature for 14 h, the reaction was neutralized with 90 mM HOAcₐq and concentrated in vacuo. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C₁₈-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1% to 100% B gradient over 150 min; UV detection at 215 nm; retention time: 11.2 min) to afford S6 as a white solid following lyophilisation (18.0 mg, 91% for two steps). The compound S6 was analyzed by analytical RP-HPLC (see Figure S3). LRMS (ESI-LTQ-
To a solution of compound S6 (18.0 mg, 9.11 x 10⁻³ mmol) in acetonitrile (3.0 mL) and distilled water (3.0 mL) was added N-methylmorpholine (4 µL, 3.82 x 10⁻² mmol) and the linker S7¹ (19.6 mg, 3.82 x 10⁻² mmol). After the solution was stirred at room temperature for 12 h, the mixture was concentrated in vacuo. The crude product was purified by RP-HPLC on a Nacalai Tesque 5C₁₈-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1% to 100% B gradient over 150 min; UV detection at 215 nm; retention time: 46.1 min) to afford S8 as a white solid following lyophilisation (14.5 mg, 67%). ¹H NMR (500MHz, D₂O): δ 7.82-7.80 (m, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.42-7.38 (m, 2H), 7.35-7.31 (m, 2H), 4.86-4.78 (m, 3H), 4.72 (d, J = 3.5 Hz, 1H), 4.41-4.39 (m, 1H), 4.32-4.20 (m, 3H), 4.13 (d, J = 6.5 Hz, 1H), 4.01-3.92 (m, 5H), 3.88-3.65 (m, 21H), 3.60-3.32 (m, 28H), 3.28 (t, J = 5.0 Hz, 2H), 3.23-3.17 (m, 2H), 3.12-3.06 (m, 1H), 2.68-2.49 (m, 7H), 1.97 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H), 1.60-1.50 (m, 3H), 1.23 (d, J = 6.0 Hz, 3H), 1.17 (d, J = 6.5 Hz, 3H), 1.13 (d, J = 6.5 Hz, 3H). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for C₉₉H₁₅₁N₁₁O₅₅SNa₂ [M+2Na]²⁺: 1210.4499. Found: 1210.4491.

**Figure S3.** HPLC data of compound S6 by analytical column (Nacalai Tesque 5C₁₈-AR300, 4.6 x 250 mm; MeCN in H₂O containing 0.1% TFA (1-100% gradient over 150 min, 1 mL/min); UV detection at 215 nm).

**Compound S9**

To a solution of compound S8 (6.2 mg, 2.60 x 10⁻³ mmol) in MeOH/H₂O (v/v = 1/1,
1.18 mL) was treated with lithium hydroxide monohydrate (2.47 mg, 5.9 x 10^{-2} mmol) at room temperature. After the solution was stirred at room temperature for 30 min, the reaction was neutralized with 90 mM HOAc_{(aq)} and concentrated in vacuo. The residue was dissolved in aqueous 20 mM tris(2-carboxyethyl)phosphine hydrochloride (2.0 mL). After 40 min, the solution was subjected to purification by RP-HPLC on a Nacalai Tesque 5C18-AR300 column (4.6 x 250 mm) at a flow rate of 1.0 mL/min using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) (1 to 1% B isocratic over 50 min; UV detection at 215 nm; retention time: 16.5 min) to afford S9 as a white solid following lyophilisation (4.5 mg, 81%). The compound S9 was analyzed by analytical RP-HPLC (see Figure S4). HRMS (ESI-LTQ-Orbitrap XL, positive) calcd for C_{84}H_{143}N_{11}O_{51}S [M+2H]^2+: 1076.9322. Found: 1076.9331.

**Figure S4.** HPLC data of compound S9 by analytical column (Nacalai Tesque 5C18-AR300, 4.6 x 250 mm; MeCN in H_{2}O containing 0.1% TFA (1-1% isocratic over 50 min, 1 mL/min); UV detection at 215 nm).

**Compound 2**

To a solution of compound S9 (3.50 mg, 1.62 x 10^{-3} mmol) and S10 (2.35 mg, 1.30 x 10^{-3} mmol) in DMSO (1.6 mL) was treated with triethylamine (266 nL, 1.62 x 10^{-3}
mmol) under Ar atmosphere. After the reaction was reacted at 40 °C for 15 h under Ar atmosphere, the mixture was concentrated to dryness in vacuo. The residue was purified by RP-HPLC on a Nacalai Tesque 5C4-AR300 column (4.6 x 250 mm) at a flow rate of 1 mL/min using a mobile phase of 0.1% TFA in water/acetonitrile/isopropanol (v/v/v = 8/1/1, Solvent A) and 0.1% TFA in acetonitrile/isopropanol (v/v = 1/1, Solvent B) (50 to 100% B gradient over 120 min; UV detection at 215 nm; retention time: 51.6 min) to afford 2 as a white solid following lyophilisation (2.91 mg, 47%). The compound 2 was analyzed by analytical RP-HPLC (see Figure S5). HRMS (ESI-LTQ-Orbitrap XL, positive, see Figure S6) calcd for C_{173}H_{312}N_{23}O_{66}S_{2} [M+3H]^{3+}: 1278.0419. Found: 1278.0486

![Figure S5. HPLC data of compound 2 by analytical column (Nacalai Tesque 5C4-AR300, 4.6 x 250 mm; solvent A: 0.1% TFA in water/acetonitrile/isopropanol (v/v/v = 8/1/1) and solvent B: 0.1% TFA in acetonitrile/isopropanol; (50-100% gradient B over 120 min, 1 mL/min); UV detection at 215 nm).](image-url)
Figure S6. ESI-LTQ-Orbitrap MS data of compound 2.
3. Immunization procedure and ELISA method

Mouse immunization
Male BALB/c mice were obtained from Tokai University, School of Medicine. Experiments using BALB/c mice were approved in compliance with the Guidelines for the Care and Use of Laboratory animals, and all animal studies were approved by the committees of the Tokai University School of Medicine. The numbers of approval are #185018 and #191075. Each group of five female wild-type BALB/c mice (8 weeks age) were inoculated with intraperitoneally (i.p.) injection of vaccine 1 (3.5 nmol), 2 (3.5 nmol), or 3 (3.5 nmol) diluted in 0.1 mL PBS on day 1. The immunization schedule included boosting each mouse with three times on days 14, 28, and 42, respectively, by injection of the same vaccine preparation. As for the V1’, the first immunizations were performed with complete Freund’s adjuvant (CFA), the others with incomplete Freund’s adjuvant (IFA). Blood were collected from each mouse before immunization on day 0 (blank controls) and on day 8, 21, 35, and 49, and were clotted to obtain plasmas that were stored at -80 °C before use.

Protocol for ELISA
ELISA plates were coated with a solution of the triSTn-BSA, N-propionyl-triSTn-BSA, STn-BSA, linker-BSA, or BSA (10 μg/mL, 50 μL per well) in the coating buffer (50 mM carbonate, pH 9.5) at 4 °C for 16 h. Nonspecific sites were blocked with 1% BSA in coating buffer at 37 °C for 2 h, then washed three times with phosphate-buffered saline (PBS) at pH 7.4. Subsequently, an individual mouse plasma with serial half-log dilutions from 1:100 to 1:102400 in PBS containing 1% BSA were added to the coated plates (50 μL per well). The plates were incubated at 25 °C for 2 h and then washed three times with PBS containing 0.05% Tween-20 (PBST) and incubated at room temperature for 1 h with a 1:1000 diluted solution of HRP-linked goat anti-mouse IgG or IgM antibody or a 1:3000 diluted solution of HRP-linked goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 antibody (50 μL per well), respectively. After the plates were washed five times with 0.05% PBST, 0.4 mg/mL α-phenylenediamine dihydrochloride (OPD) in 0.05 M phosphate-citrate buffer at pH 5.0 with 0.4 μL/mL 30% H₂O₂ was added to the plates (100 μL per well), and after 25 min at room temperature, 2.5 M aqueous sulfuric acid (50 μL per well) was added to stop the colorimetric reaction. Optical density (OD) was measured at 492 nm on an ELISA plate reader. For titer analysis, the OD values were plotted against the serum dilution numbers to obtain a
best-fit logarithm line. The equation of this line was used to calculate the dilution number at which an OD value of 0.1 was achieved, and this dilution number is defined as the antibody titer.
4. Detailed data of ELISA

**Fig. S7.** IgG antibody titers induced by the immunization of V1, V1’ and the mixture of STn antigen, Pam3CSK4, and Th epitope. \(^1\)**: \(p<0.01\), ***: \(p<0.005\)

**Fig. S8.** IgG antibody titers induced by V1’ on days 0, 7, 35, and 49. \(^1\)
Fig. S9. IgM antibody titers induced by V1 and V2 on days 0, 7, 35, and 49.

Fig. S10. IgG antibody titers of each IgG subclass on day 49 induced by V1*.1
Fig. S11. ELISA of IgG antibody induced by V1 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

Fig. S12. ELISA of IgG antibody induced by V2 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.
Fig. S13. ELISA of IgG antibody induced by V3 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

Fig. S14. ELISA of IgM antibody induced by V1 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.
Fig. S15. ELISA of IgG antibody induced by V2 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.

Fig. S16. ELISA of IgG antibody induced by V3 specific for A) BSA-triSTn, B) BSA-STn, C) BSA-linker, or D) BSA on day 49 of the immunization schedule.
**Fig. S17.** ELISA of IgG antibody induced by V1 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

**Fig. S18.** ELISA of IgG antibody induced by V1 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.
**Fig. S19.** ELISA of IgG antibody induced by V2 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

**Fig. S20.** ELISA of IgG antibody induced by V2 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.
Fig. S21. ELISA of IgM antibody induced by V1 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

Fig. S22. ELISA of IgM antibody induced by V1 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.
Fig. S23. ELISA of IgM antibody induced by V2 specific for BSA-STn on day A) 35, B) 21, or C) 7 of the immunization schedule.

Fig. S24. ELISA of IgM antibody induced by V2 specific for BSA-triSTn on day A) 35, B) 21, or C) 7 of the immunization schedule.
Fig. S25. ELISA of BSA-triSTn-specific A) IgG 1, B) IgG 2a, C) IgG 2b, and D) IgG 3 antibody induced by V1 on day 49 of the immunization schedule.

Fig. S26. ELISA of BSA-triSTn-specific A) IgG 1, B) IgG 2a, C) IgG 2b, and D) IgG 3 antibody induced by V2 on day 49 of the immunization schedule.
Fig. S27. ELISA of IgG antibody specific for A) BSA-STn or B) BSA-TriSTn on day 0 of the immunization schedule. ELISA of IgM antibody specific for C) BSA-STn or D) BSA-TriSTn on day 0 of the immunization schedule.
5. Protocol and data of flow cytometry

Protocol for flow cytometry

The spleen of each mouse was collected on day 49, filtered with mesh (77 μm), and centrifuged (800×g, 5 min). The precipitation was suspended with RBC Lysis Buffer (10 mL) and centrifuged (800×g, 5 min). The spleen cells were obtained as a precipitation, suspended in CELLBANKER, and stored in liq. N₂ before use. After the spleen cells were washed with PBS buffer two times, the cells (3×10⁵) were stained with the respective marker antibodies (1:9) 15 min at 4 °C. After washing with PBS buffer, the cells were analyzed using BD FACS Verse™ (BD Biosciences). Each gate was defined as a polygon in the panel and a line in the histogram. Mean fluorescence intensity (MFI) was shown in the upper right of each histogram.

<The following antibodies were used for cell staining>

| Antibody | Iso type | Company | Clone | Cat.No. |
|----------|----------|---------|-------|---------|
| CD3e FITC | Hamster IgG | TONBO biosciences | 145-2C11 | 35-0031-U100 |
| CD4 PE | Rat IgG2b k | eBioscience | GK1.5 | 12-0041-83 |
| CD8a APC | Rat IgG2a k | eBioscience | 53-6.7 | 17-0081-81 |
| CD11b PE | Rat IgG2b k | BD Biosciences Pharmingen | M1/70 | 557397 |
| CD11c PE-cy7 | Hamster IgG | TONBO biosciences | N418 | 60-0114-100U |
| CD19 APC | Rat IgG2a k | TONBO biosciences | 1D3 | 20-0193-100U |
| CD80 FITC | Hamster IgG2 k | BD Biosciences Pharmingen | 16-10A1 | 553768 |
| CD86 PE | Rat IgG2a k | BD Biosciences Pharmingen | GL1 | 553692 |
| CD138 PE | Rat IgG2a k | BD Biosciences Pharmingen | 281-2 | 09345B |

Fig. S28. Number of spleen cells on day 49 of the immunization schedule. a) Total number of spleen cells. b) Number of spleen cells classified to each cell type (lymphocyte, large lymphocyte, small lymphocyte, and monocyte).
6. Gate of flow cytometry analysis

<Dendritic cell analysis, CD11b+ cell analysis>
<T cell analysis>
<B cell analysis>

CD19 gate

NT

SSC

FSC

Count

CD19

CD80

CD138

V1

V1'

V2

V3
7. Reference

1) T.-C. Chang, Y. Manabe, Y. Fujimoto, S. Ohshima, Y. Kametani, K. Kabayama, Y. Nimura, C.-C. Lin and K. Fukase, Angew. Chem. Int. Ed., 2018, **57**, 8219-8224.
8. \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra

\(^1\text{H}\) NMR spectrum for \(\text{S2 (D}_2\text{O, 500 MHz)}\)

\(^1\text{H}\) NMR spectrum for \(\text{3 (D}_2\text{O, 500 MHz)}\)
$^1$H NMR spectrum for S5 (CD$_3$OD, 500 MHz)

$^{13}$C NMR spectrum for S5 (CD$_3$OD, 500 MHz)
$^1$H NMR spectrum for S8 (D$_2$O, 500 MHz)