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Supplementary Information

pH-Sensitive branched β-glucan derivative-modified liposomes for activation of antigen presenting cells and induction of antitumor immunity

Shin Yanagihara, Nozomi Kasho, Koichi Sasaki, Naoto Shironaka, Yukiya Kitayama, Eiji Yuba*, Atsushi Harada*

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, 1–1 Gakuen–cho, Naka–ku, Sakai, Osaka 5998531, Japan

*Correspondence: yuba@chem.osakafu-u.ac.jp and harada@chem.osakafu-u.ac.jp

Tel.: +81-72-254-9330; Fax: +81-72-254-9330

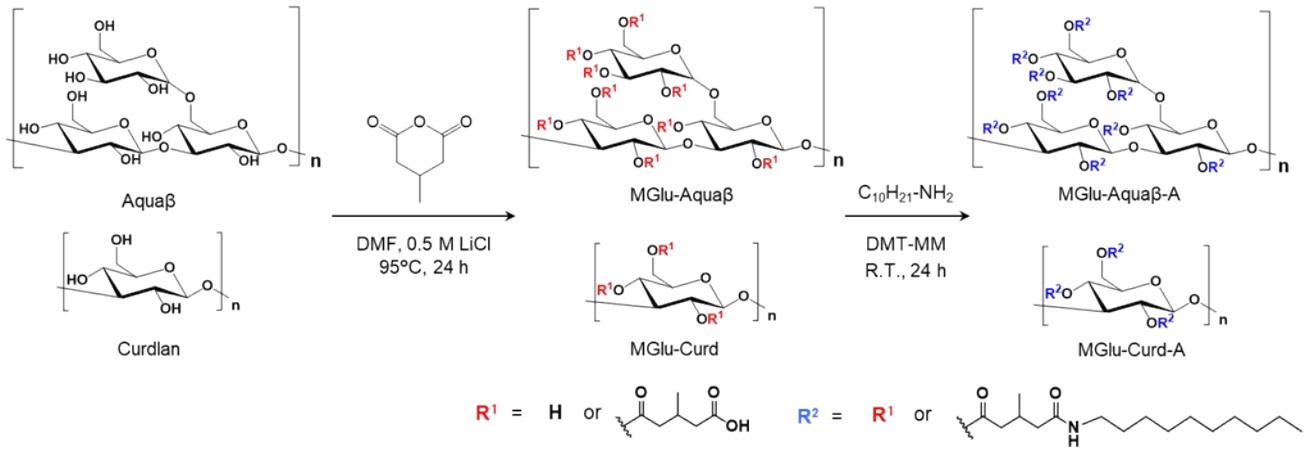


Figure S1. Synthetic route for β-glucan derivatives having carboxy groups and alkyl chains as anchor units to liposomal membrane.

Table S1. Synthesis of β-Glucan Derivatives

Polymer	Aquaβ/g	DMF/mL	LiCl/g	3-Methylglutaric anhydride		Yield		MGlu/%*	MGlu density**
				/g	eq.	/g	/%		
MGlu75-Aquaβ	1.00	20	0.725	3.96	5.01	2.47	89	75	384.8
MGlu63-Aquaβ	1.00	20	0.726	3.12	3.95	1.51	61	63	323.2
MGlu43-Aquaβ	0.500	20	0.724	1.19	3.01	0.825	82	43	220.6
MGlu23-Aquaβ	0.500	20	0.725	0.788	2.02	0.507	66	23	118
MGlu76-Curd	0.298	15	0.316	1.87	7.94	0.675	77	76	228
MGlu61-Curd	0.508	15	0.500	2.58	6.43	0.851	69	61	183
MGlu46-Curd	0.301	15	0.787	2.27	9.54	0.495	78	46	138
MGlu19-Curd	0.302	15	0.786	0.725	3.04	0.34	76	19	57

*Determined by ^{1}H NMR.

**MGlu density is defined as the number of MGlu units per 100 β1,3-linked sugar units. In the case of Aquaβ, total sugar units per 100 β1,3-linked sugar main chin units are calculated as 171 from its degree of branching (0.71).

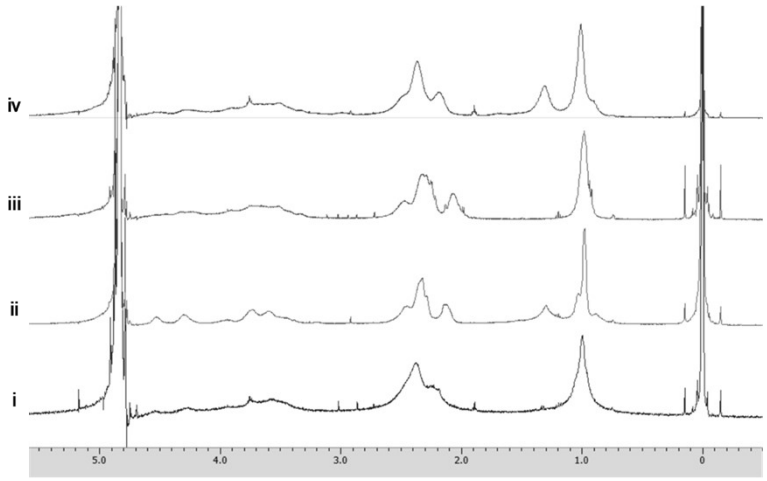
Table S2. Synthesis of β-Glucan Derivatives Having Anchor Moieties

Polymer	MGlu-Aquaβ or MGlu-Curd /g	Decylamine/ mg	DMT-MM /mg	Yield		MGlu /%*	Anchor /%*	MGlu density**	Anchor density**
				/g	/%				
MGlu70-Aquaβ-A6	1.00	94.2	167	0.922	87	70	6	359.1	30.8
MGlu56-Aquaβ-A4	1.01	98.1	174	0.979	95	56	4	287.3	20.5
MGlu38-Aquaβ-A3	0.408	33.0	59.5	0.165	40	38	3	194.9	15.4
MGlu21-Aquaβ-A2	0.210	16.4	21.3	0.108	50	21	2	107.7	10.3
MGlu71-Curd-A6	0.144	28.3	50.3	0.139	90	71	6	213	18
MGlu62-Curd-A4	0.403	41.4	78.4	0.379	85	62	4	186	12
MGlu37-Curd-A4	0.250	32.3	50.4	0.265	108	37	4	111	12
MGlu13-Curd-A4	0.150	28.5	47.7	0.162	106	13	4	39	12

*Determined by ^{1}H NMR.

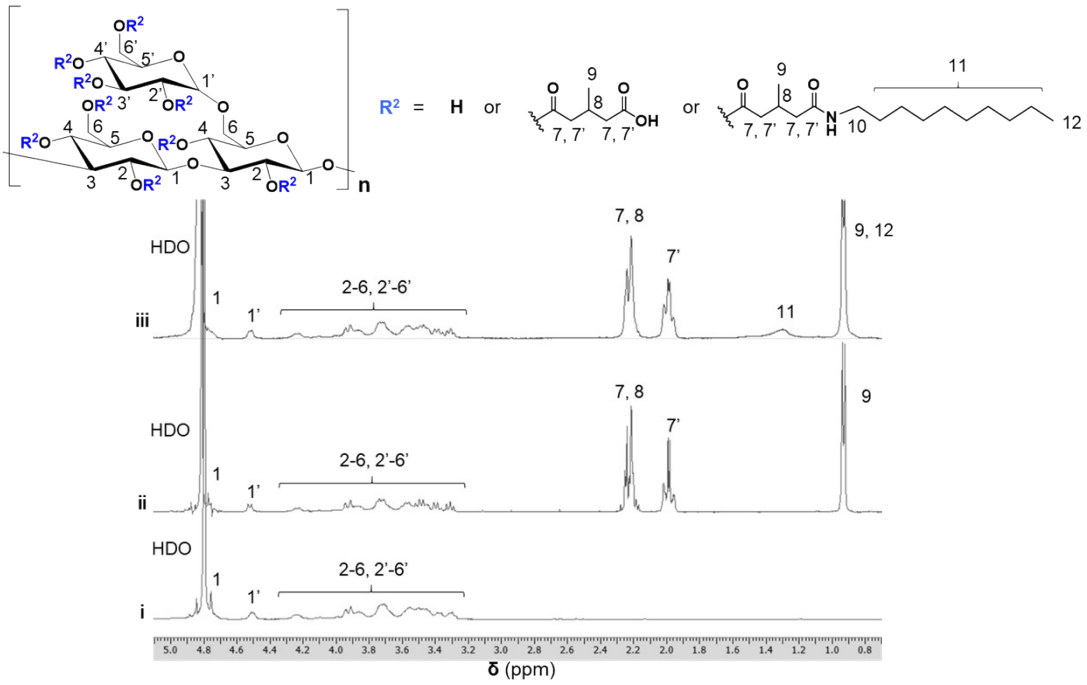
**MGlu and anchor densities are defined as the number of MGlu and anchor units per 100 β1,3-linked sugar units, respectively. In the case of Aquaβ, total sugar units per 100 β1,3-linked sugar main chin units are calculated as 171 from its degree of branching (0.71).

a


iv
iii
ii
i
5.0
4.0
3.0
2.0
1.0
0

δ (ppm)

b


R² = H or 7, 7' 7, 7' OH or 7, 7' 7, 7' N H 10 11 12
n
HDO
iii
1
1'
2-6, 2'-6'
7, 8
7'
11
9, 12
HDO
ii
1
1'
2-6, 2'-6'
7, 8
7'
9
HDO
i
1
1'
2-6, 2'-6'
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8

δ (ppm)

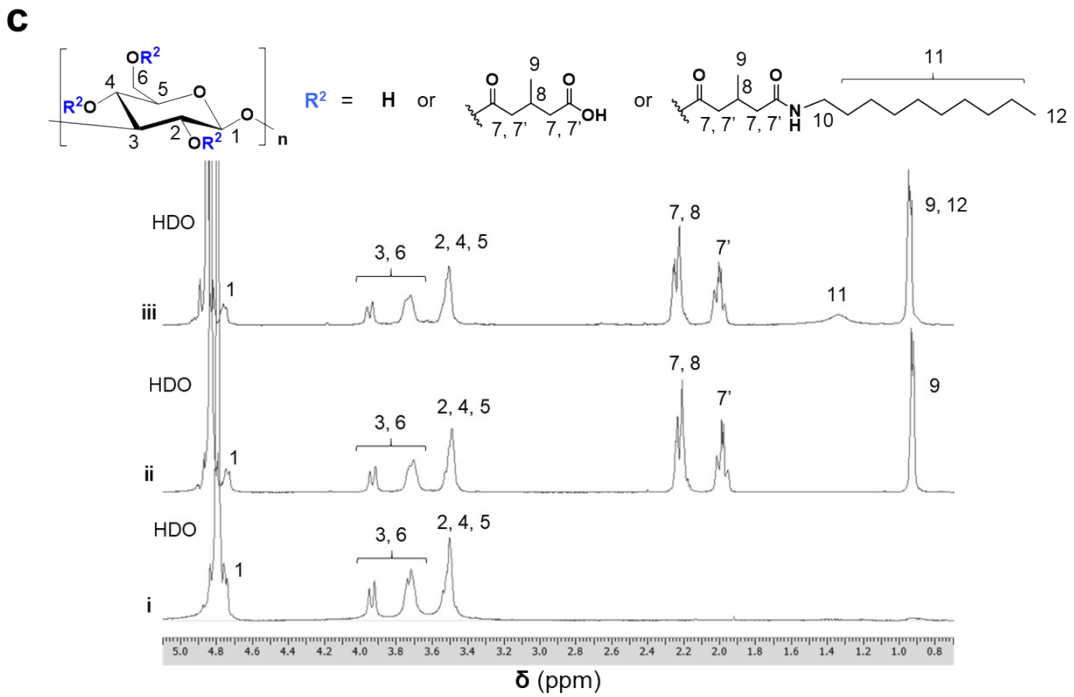


Figure S2. (a) ^{1}H NMR spectra of (i) MGlu46-Curd, (ii) MGlu37-Curd-A4, (iii) MGlu43-Aquaβ, and (iv) MGlu38-Aquaβ-A3 in D_2O. (b) ^{1}H NMR spectra of (i) Aquaβ, (ii) hydrolyzed MGlu43-Aquaβ and (iii) hydrolyzed MGlu38-Aquaβ-A3, and (c) ^{1}H NMR spectra of (i) Curdlan, (ii) hydrolyzed MGlu46-Curd and (iii) hydrolyzed MGlu37-Curd-A4 in D_2O/NaOD, with assigned signals.

Table S3. The peak integration values of Aquaβ, MGlu43-Aquaβ and MGlu38-Aquaβ-A3 (Fig. S2b).

Peak signals	1'	2-6, 2'-6'	7, 8	7'	11	9, 12
i (Aquaβ)	0.37	6.00	-	-	-	-
ii (MGlu43-Aquaβ)	0.37	6.00	3.85	2.55	-	3.87
iii (MGlu38-Aquaβ-A3)	0.37	6.00	3.66	2.44	1.51	3.96

Table S4. The peak integration values of Curdlan, MGlu46-Curd and MGlu37-Curd-A4 (Fig. S2c).

Peak signals	3, 6	2, 4, 5	7, 8	7'	11	9, 12
i (Curdlan)	3.00	2.59	-	-	-	-
ii (MGlu46-Curd)	3.00	2.90	4.02	2.67	-	3.97
iii (MGlu37-Curd-A4)	3.00	2.98	3.75	2.55	1.85	4.14

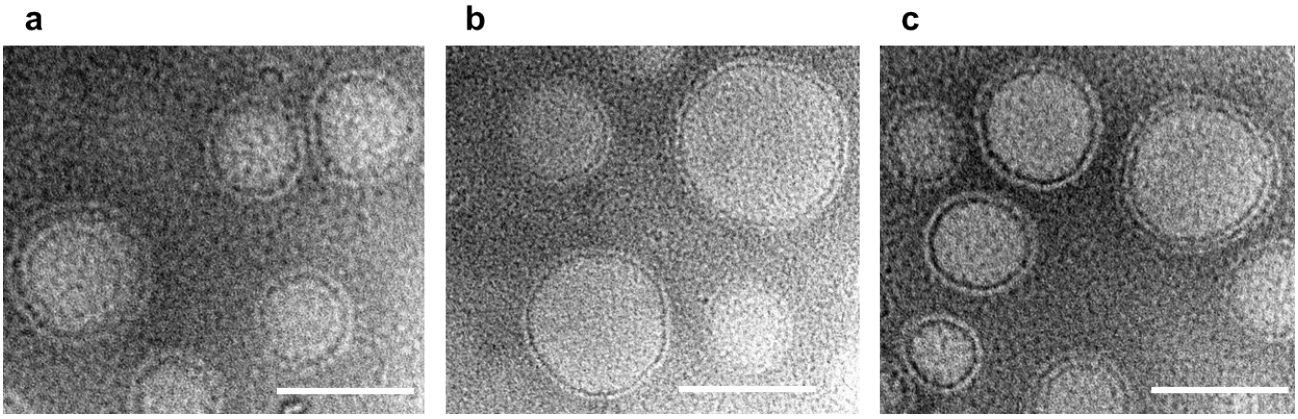


Figure S3. Transmission electron microscopic (TEM) analysis of liposomes modified with (a) MGlu70-Aquaβ-A6, (b) MGlu71-Curd-A6 and (c) unmodified liposomes. Scale bars represent 100 nm.

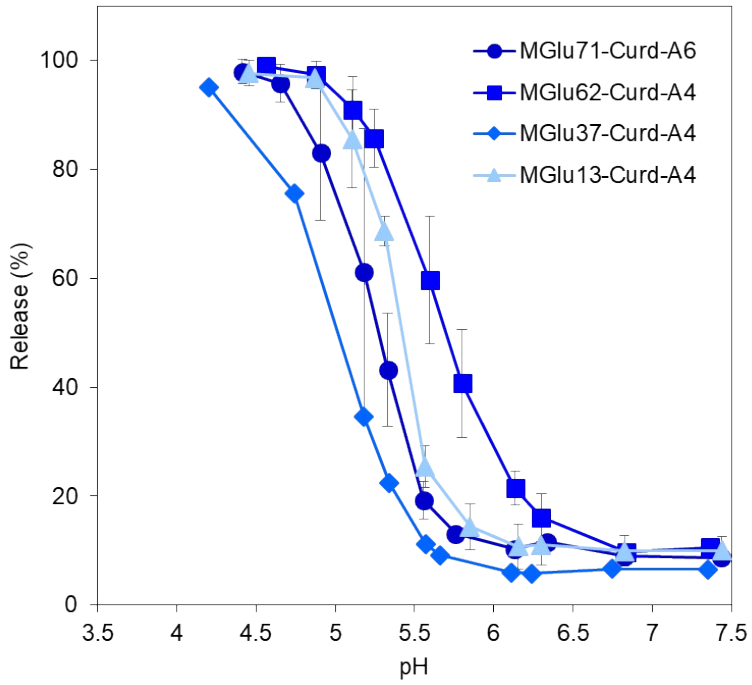


Figure S4. pH-Dependence of pyranine release from liposomes modified with curdlan derivatives after 30 min incubation are shown. Lipid concentration was 2.0×10^{-5} M. Each point is the mean ± SD ($n = 3$).

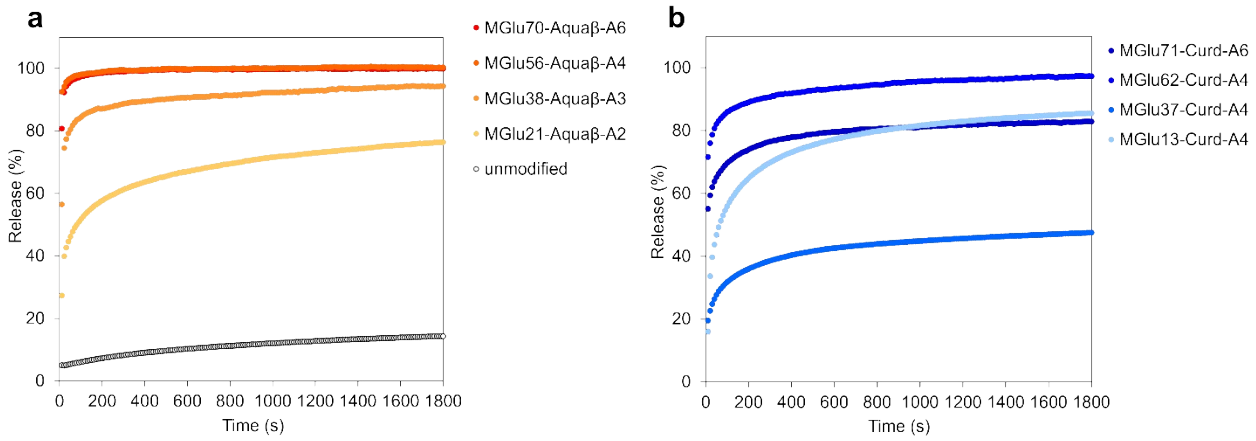


Figure S5. Time-courses of pyranine release from (a) Aquaβ derivative-modified liposomes and unmodified liposomes, and (b) curdlan derivative-modified liposomes at pH 5.

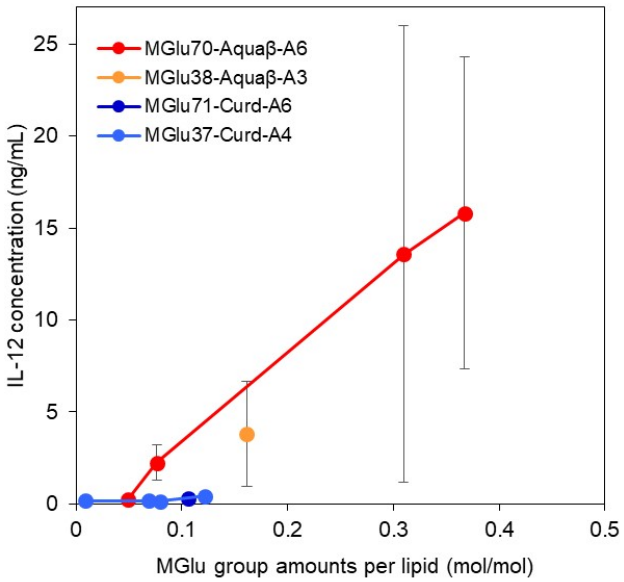


Figure S6. Correlation of IL-12 production with MGlu group amounts of β-glucan derivatives on liposomes. MGlu group amounts of β-glucan derivatives on the liposomes were calculated from MGlu% of each β-glucan derivative and its modification amount on the liposomes.

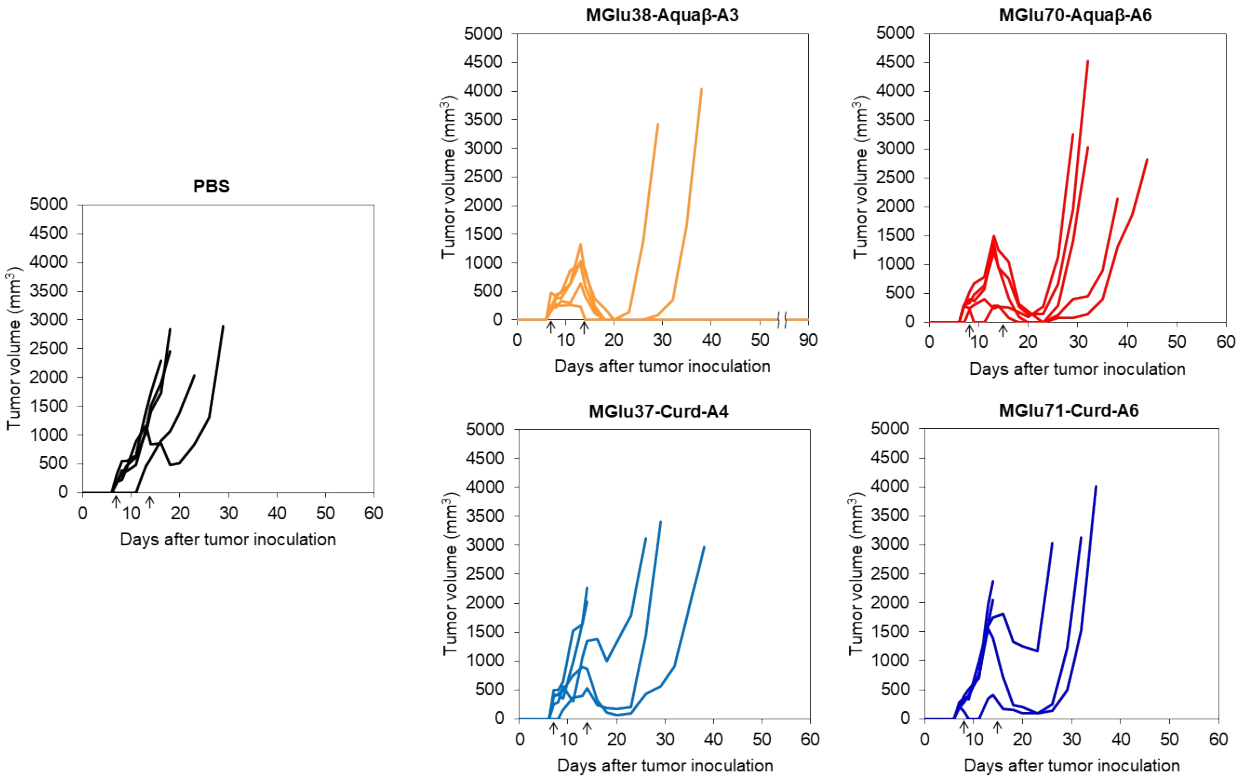


Figure S7. Individual tumor volume change of C57BL/6 mice treated with PBS or liposomes modified with various β-glucan derivatives in Fig. 6. Arrows indicate the sample injection.

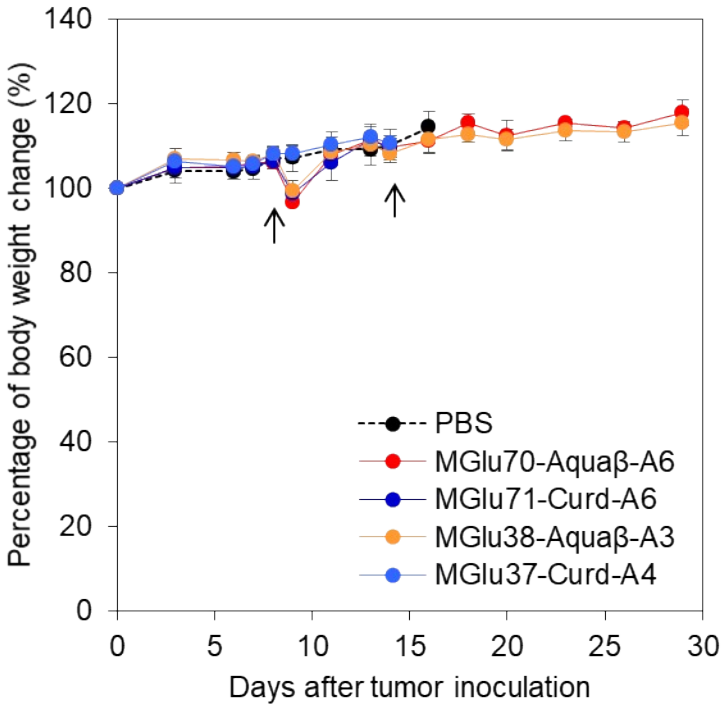


Figure S8. Relative body weight change of mice in Fig. 6. Arrows indicate the sample injection.

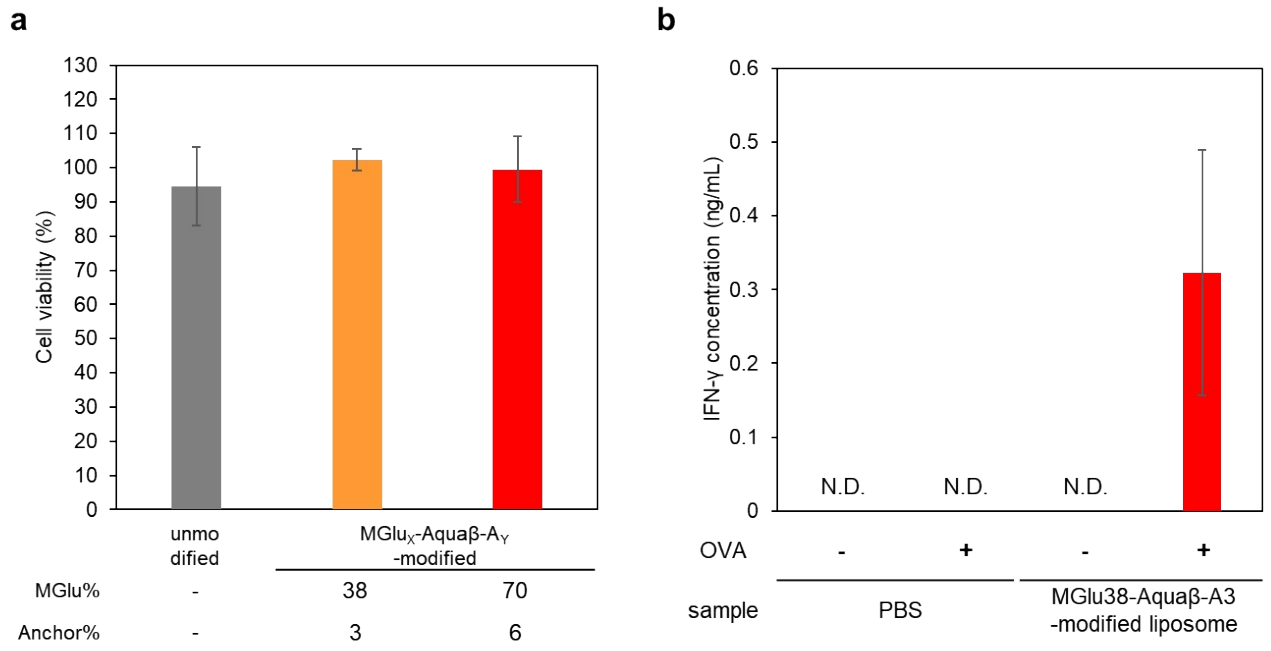


Figure S9. (a) Cell viability of NIH3T3 cells treated with various liposomes (0.2 mM) for 24 h in the presence of 10% FBS. (b) *In vitro* stimulation of splenocytes from mice immunized with PBS or OVA-loaded liposomes. Splenocytes (2×10^6/2 mL) isolated from immunized C57BL/6 mice were incubated with or without 50 μg/mL of OVA for 4 days. IFN-γ secretion in the supernatant was detected by ELISA. Splenocytes were collected at 6 days after the immunizations.

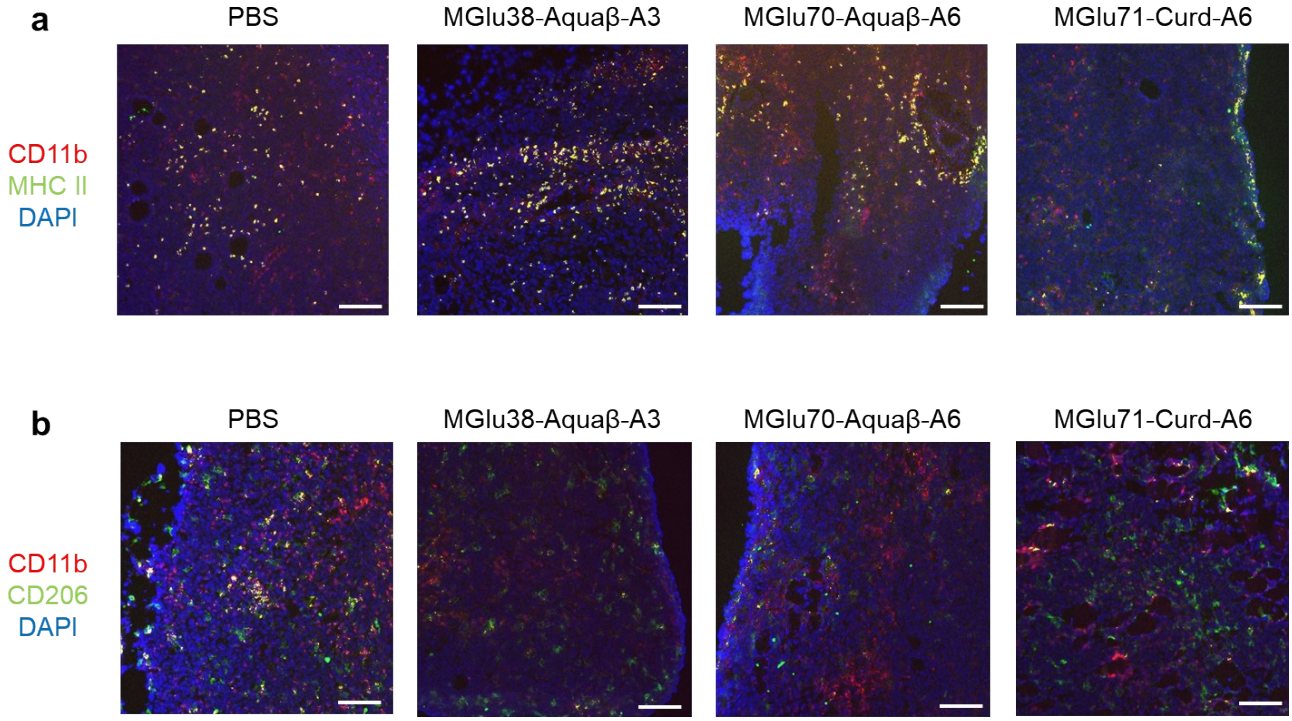


Figure S10. Fluorescence images of tumor sections from the mice immunized with PBS, MGlu38-

Aquaβ-A3-modified liposomes, MGlu70-Aquaβ-A6-modified liposomes and MGlu71-Curd-A6-modified liposomes. Tumor tissues were excised on Day 6 after immunization. Tumor sections were immunohistochemically stained with (a) PE-labeled anti-CD11b (M1/70) and FITC-labeled anti-MHC II (AF6-120.1) and (b) PE-labeled anti-CD11b (M1/70) and Alexa488-labeled CD206 (C068C2). Cell nucleus was also labeled with DAPI. Yellow colors indicate colocalization of green and red fluorescence: M1 macrophages (a) or M2 macrophages (b) in the tumor section. Scale bars represent 100 μm.