Supplementary Information

Carboxylated polyamidoamine dendron-bearing lipid-based assemblies for precise control of intracellular fate of cargo and induction of antigen-specific immune responses

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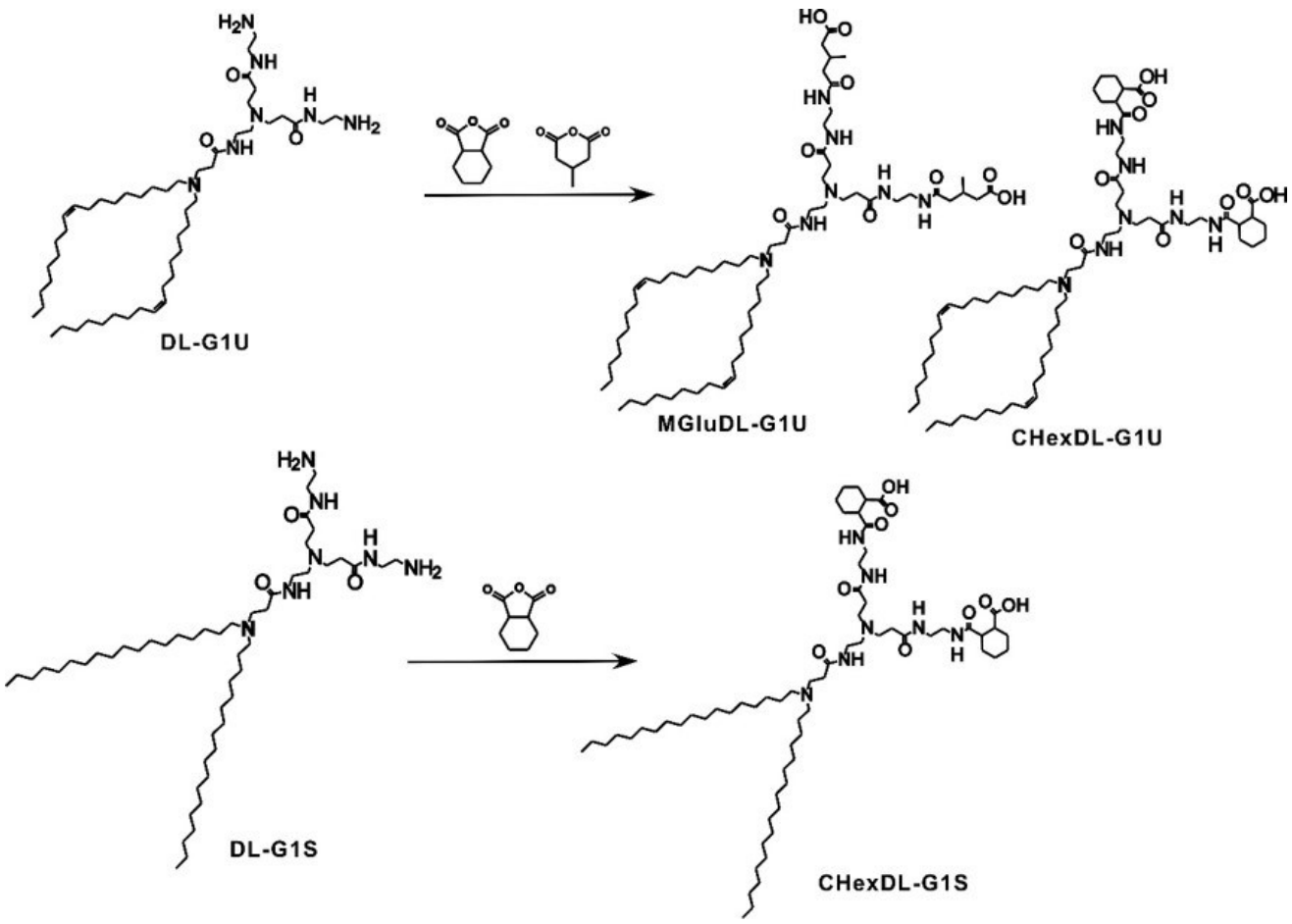
IUPAC name for dendron-bearing lipid.

DL-G1U: 3,3'-((2-(3-(di((Z)-octadec-9-en-1-yl)amino)propanamido)ethyl)azanediyl)bis(N-(2-aminoethyl)propanamide)

DL-G1S: 3,3'-((2-(3-(dioctadecylamino)propanamido)ethyl)azanediyl)bis(N-(2-aminoethyl)propanamide)

DL-G2S: 3,3',3",3'"-((((3,3'-((2-(3-(di((Z)-octadec-9-en-1-yl)aminopropanamido)ethyl)azanediyl)bis(propanoyl))bis(azanediyl))bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(N-(2-aminoethyl)propanamide)



Scheme S1. Synthetic routes for carboxylated dendron-based lipids.

Table S1. Fluorescence intensity of pryranine or calcein encapsulated in various liposomes and OVA encapsulation efficiency for liposomes for immunization

Liposome	Pyranine fluorescence (a.u.)	Calcein fluorescence (a.u.)	OVA encapsulation efficiency (%)
EYPC	203 ± 14.3	331 ± 48.9	21.4 ± 0.9
MGluDL-G1U	204 ± 2.3	388 ± 54.7	21.7 ± 1.0
CHexDL-G1U	186 ± 3.3	322 ± 11.6	23.4 ± 0.9
CHexDL-G1S	170 ± 1.5	338 ± 5.6	-
CHexDL-G2S (10 mol%)	204 ± 14.7	355 ± 23.4	-

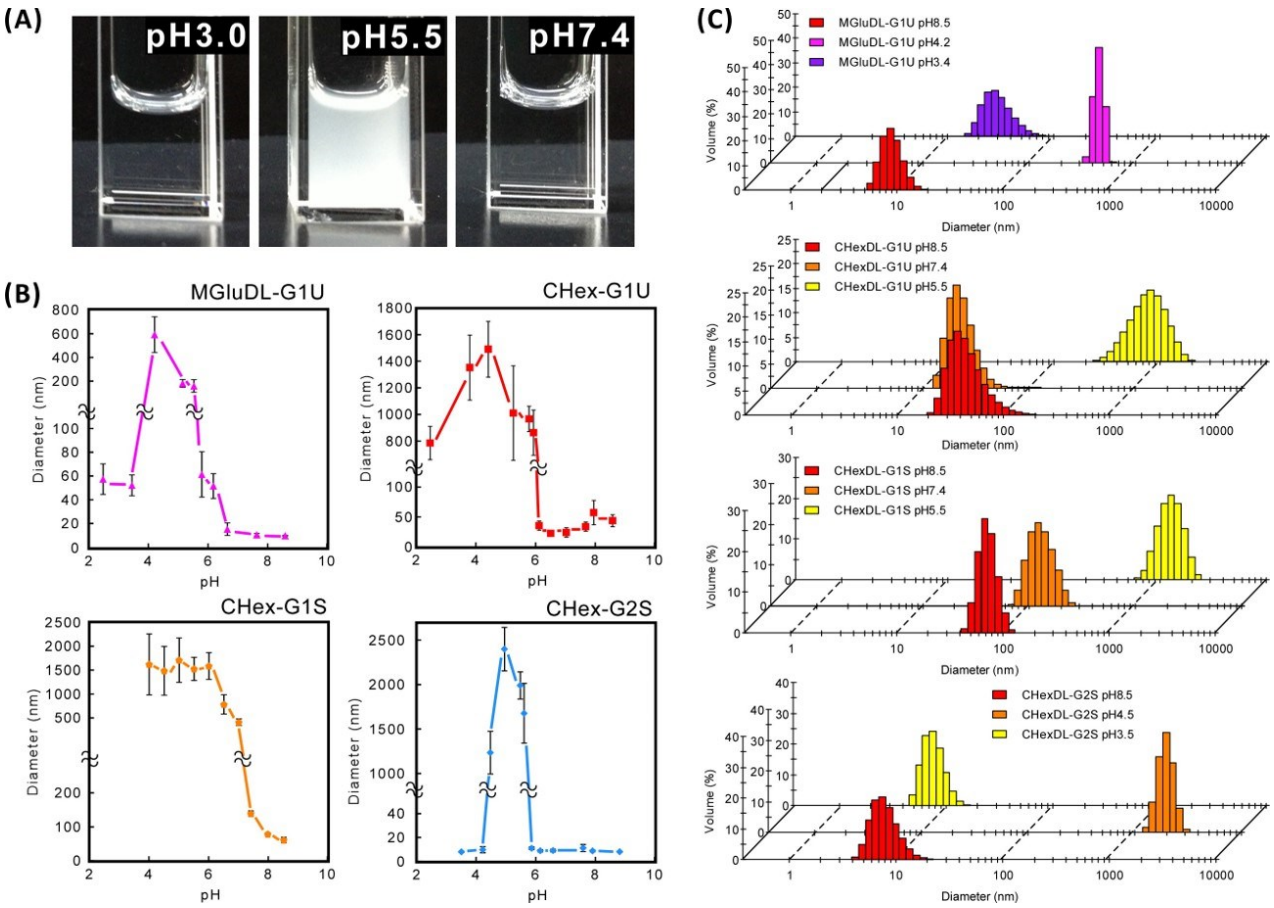


Figure S1. (A) Representative images of CHexDL-G2S solution at varying pH. (B) Particle sizes of various dendron lipid assemblies as a function of pH. Volume average diameters were shown. (C) Size distribution of dendron lipid solution at varying pH at 25℃. Concentration of dendron lipids was 0.1 mM.

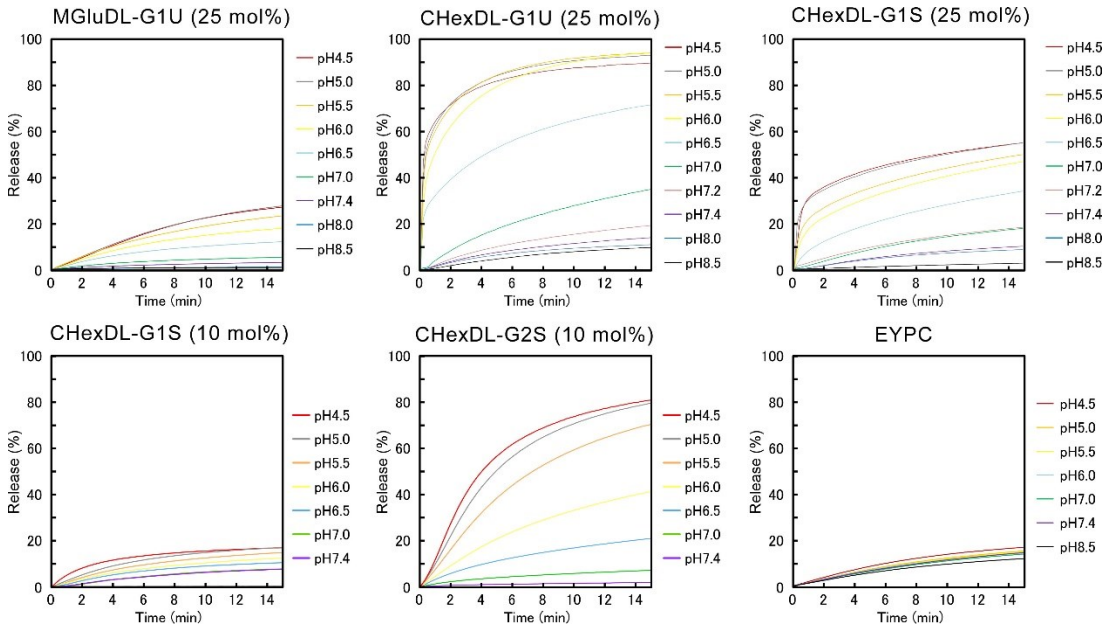


Figure S2. (A) Time courses of pyranine release from various kinds of dendron lipid/EYPC liposomes in PBS of varying pH at 37℃. Lipid concentration was 2.0×10^{-5} M.

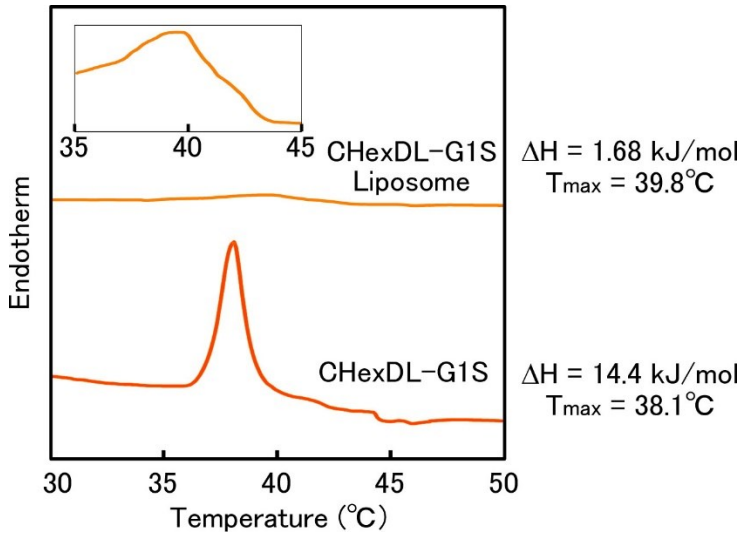


Figure S3. DSC charts of CHexDL-G1S and CHexDL-G1S-containing liposome in PBS at pH 7.4. Rate of temperature increase was 1℃/min.

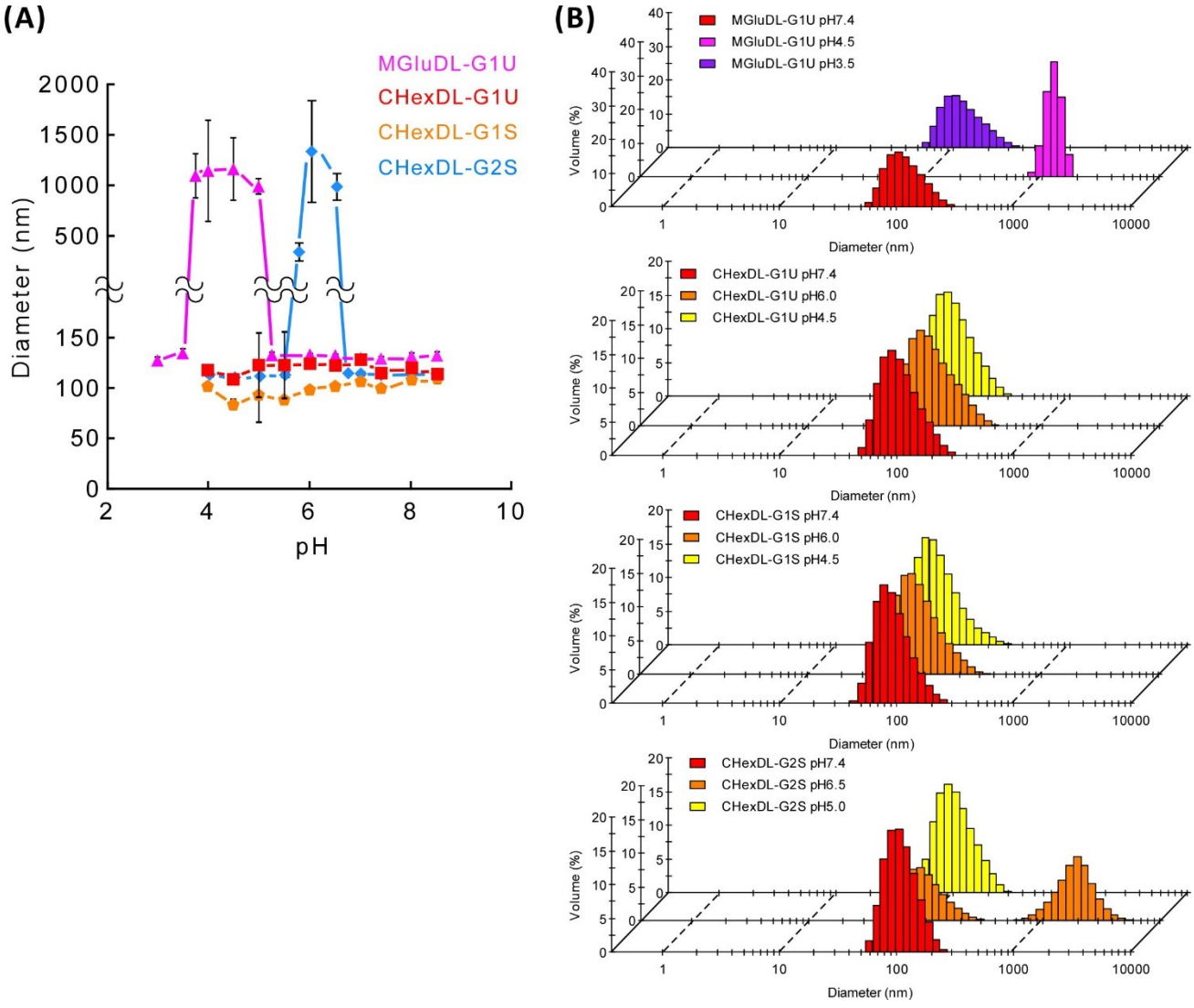


Figure S4. Particle sizes (A) and Size distribution (B) for various dendron lipid/EYPC liposomes in

PBS of varying pH at 25℃.

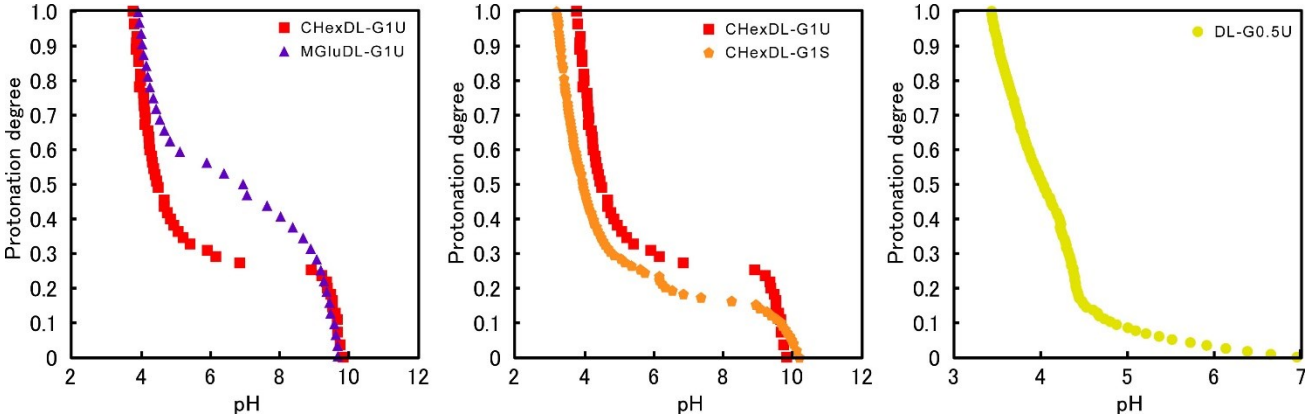


Figure S5. Degree of protonation of various dendron lipids as a function of pH.

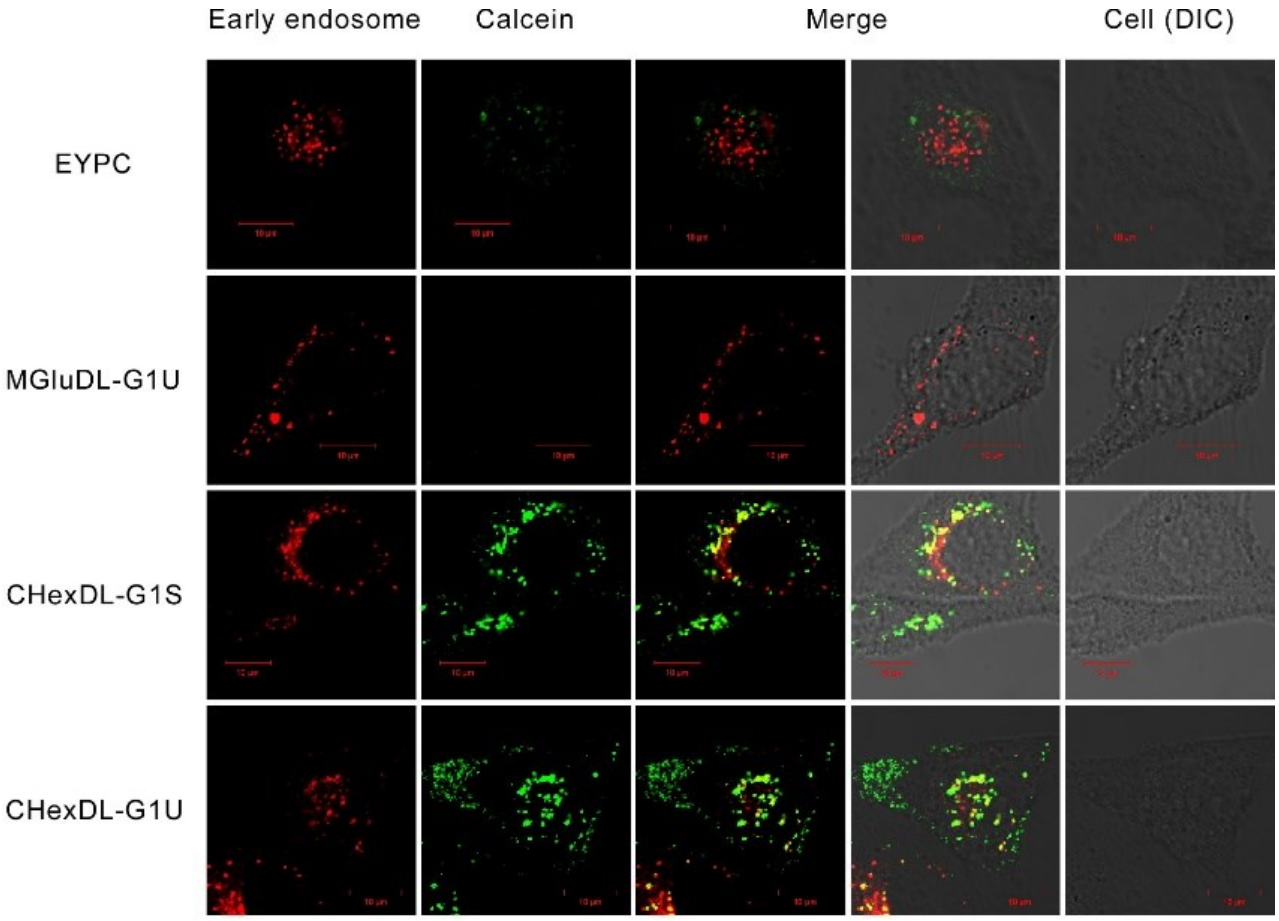


Figure S6. CLSM images of HeLa cells treated with calcein-loaded liposomes of various kinds for 4 h at 37℃ in the presence of serum. Dendron lipid contents in the liposomes were 25 mol%. Intracellular organelles were stained with organelle-specific baculovirus-based staining kits. Scale bar represents 10 μm.

Figure S6. (Continued)

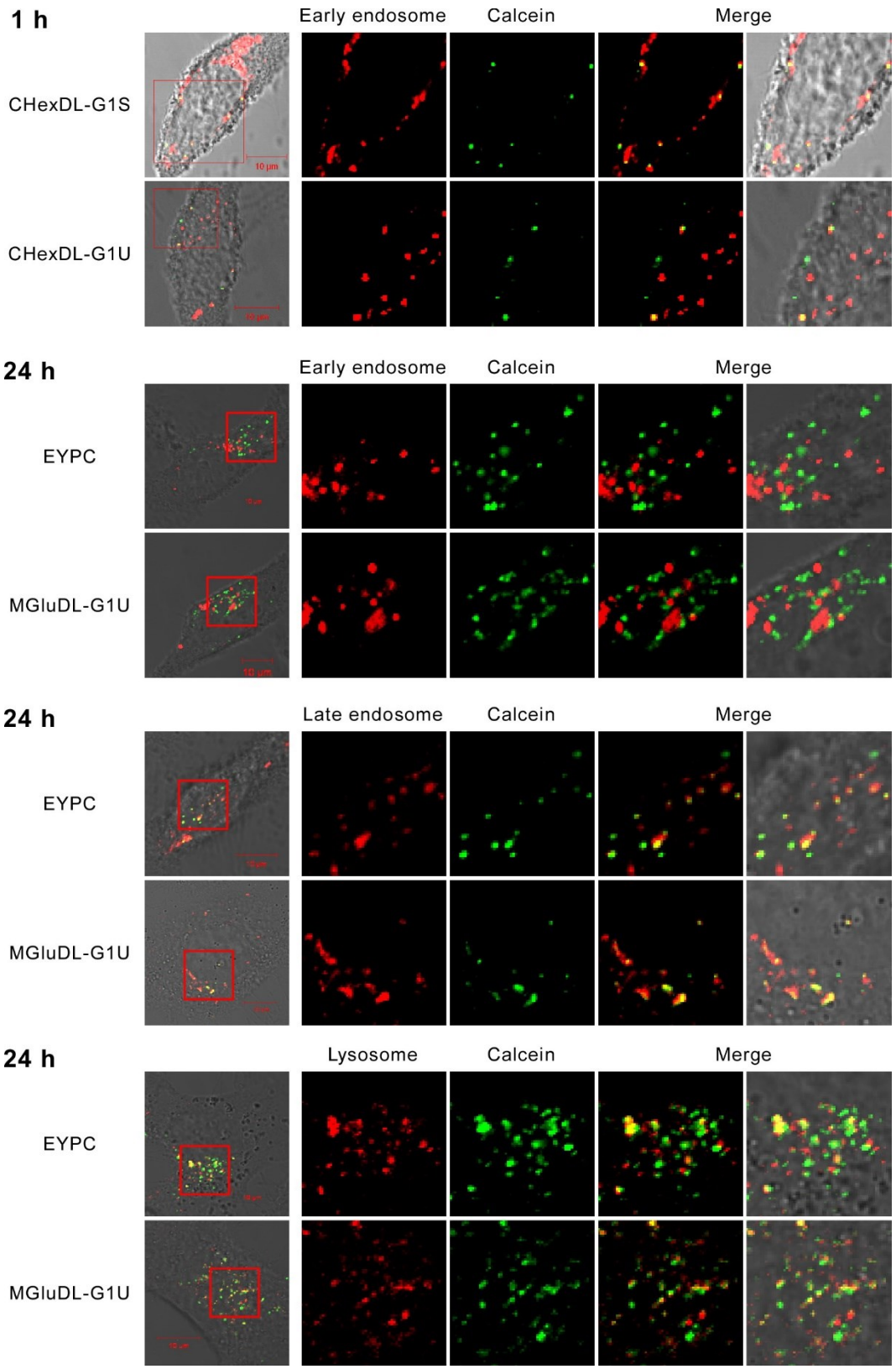


Figure S7. CLSM images of HeLa cells treated with calcein-loaded liposomes of various kinds for 1 h or 24 h at 37℃ in the presence of serum. Intracellular organelles were stained with organelle-specific baculovirus-based staining kits. Scale bar represents 10 μm. Dendron lipid contents in the liposomes were 25 mol%.

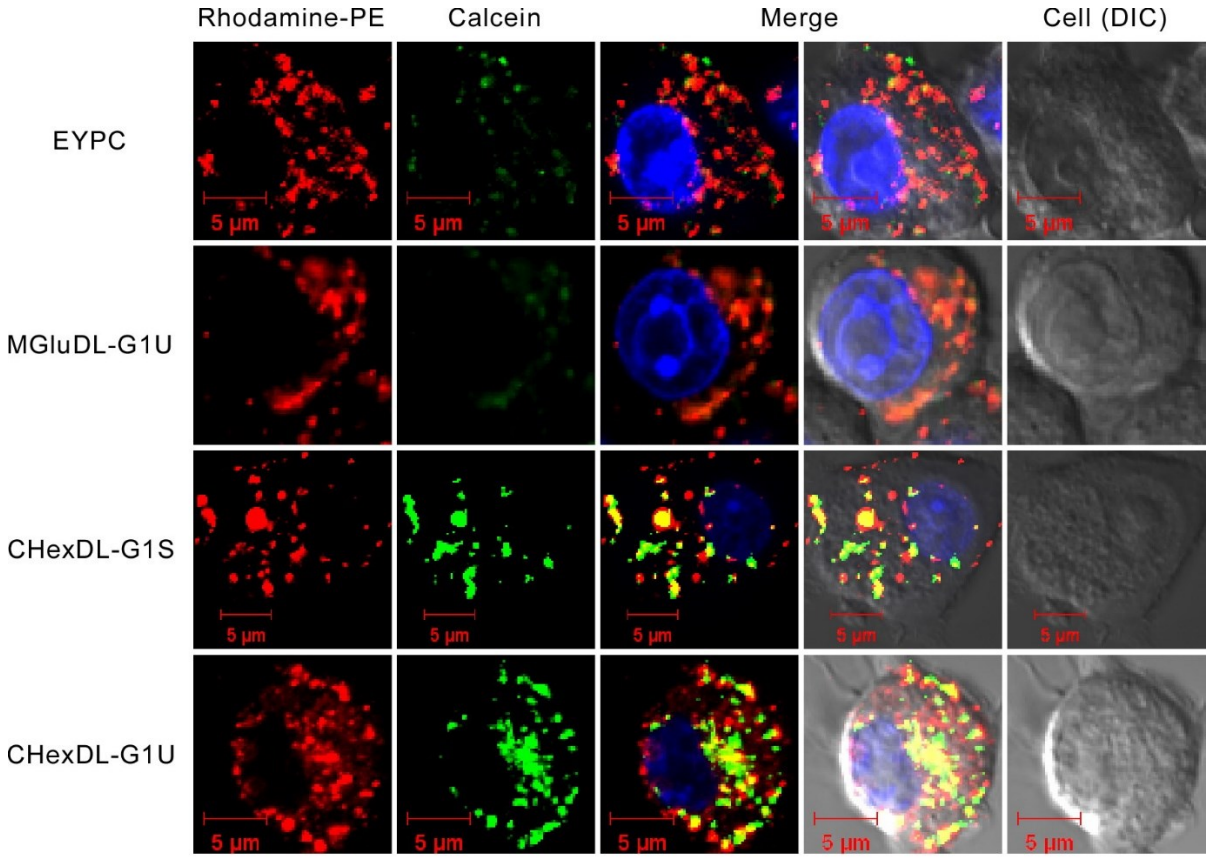


Figure S8. CLSM images of DC2.4 cells treated with Rh-PE-labeled and calcein-loaded liposomes of various kinds for 4 h at 37℃ in the presence of serum. Cell nucleus was stained with Hoechst. Scale bar represents 10 μm. Dendron lipid content was 25 mol%. Lipid concentration was 0.5 mM.

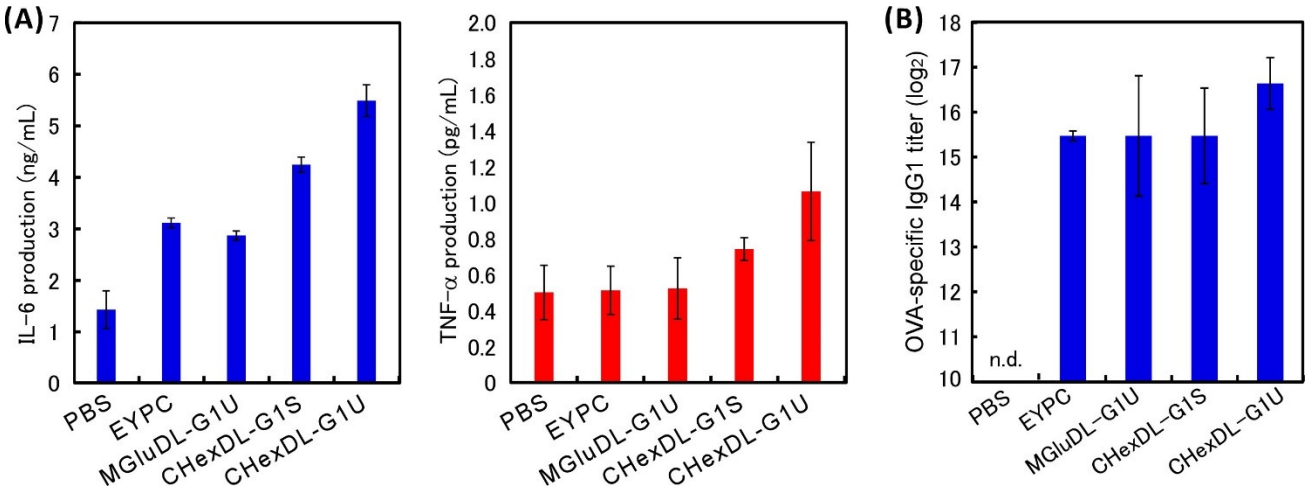


Figure S9. (A) Cytokine production from DC2.4 cells treated with various dendron lipid-containing liposomes. (B) Production of OVA-specific IgG1 in serum of C57BL/6 mice immunized with OVA-loaded liposomes with or without various dendron lipids (25 mol%). Antibody titer was measured by ELISA at 7th day after second administration.

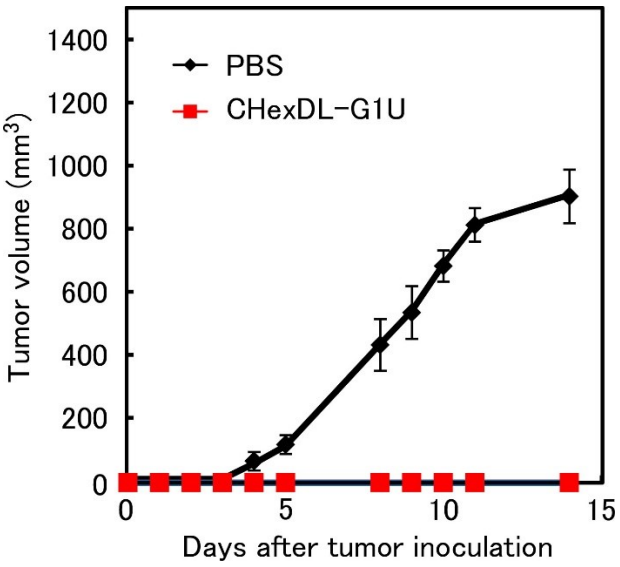


Figure S10. Tumor prophylactic effect. C57BL/6 mice were immunized with OVA-loaded dendron lipid CHexDL-G1U (25 mol%) liposomes or PBS at 7th day and 12th day before tumor cell inoculation. E.G7-OVA cells were subcutaneously inoculated into the back of C57BL/6 mice and tumor volume was monitored.

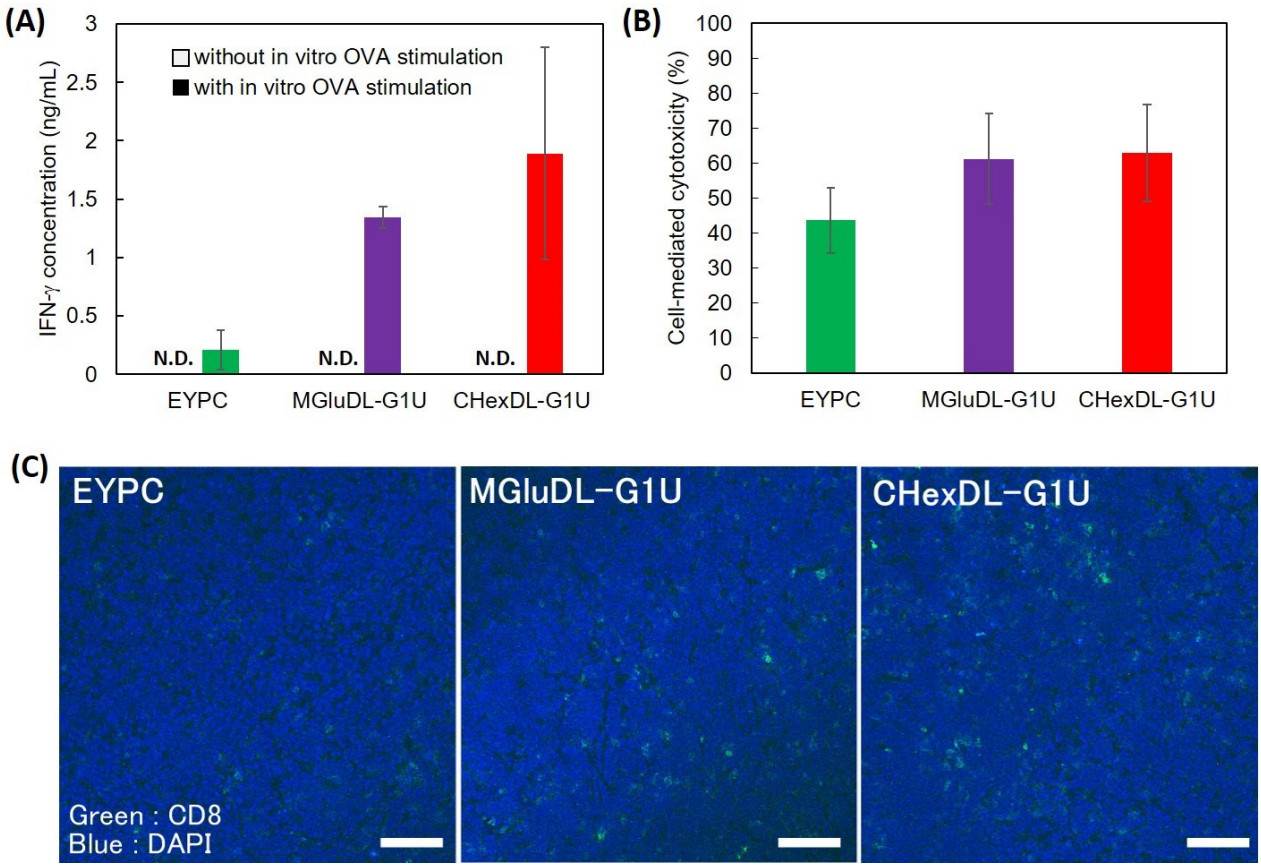


Figure S11. Induction of antigen-specific cellular immune responses. E.G7-OVA cells were inoculated to C57BL/6 mice. At 5th day and 12th day after tumor inoculation, mice were subcutaneously

immunized with OVA-loaded liposomes. At 15th day after tumor inoculation, tumor tissues were excised from a part of mice for detection of CD8-positive cells in tumor cryosection as reported in previous study (*Biomaterials*, **67**, 214-224 (2015)). At 19th day after tumor inoculation, splenocytes was collected from remaining mice for detection of cellular immune responses in spleen as reported in previous study (*Biomaterials*, **120**, 32-45 (2017)). (A) IFN-γ production from splenocytes with or without *in vitro* OVA stimulation (25 μg/mL) for 5 days. (B) Cell-mediated cytotoxicity against E.G7-OVA cells by splenocytes (Effector/target ratio = 50) detected by a lactate dehydrogenase assay kit. (C) Immunofluorescent images of tumor sections stained with CD8-Alexa Fluor488 (green) and DAPI (blue). Scale bars represent 100 μm.