Supporting Information for

ORIGINAL ARTICLE

Molecular engineering of antibodies for site-specific conjugation to lipid polydopamine hybrid nanoparticles

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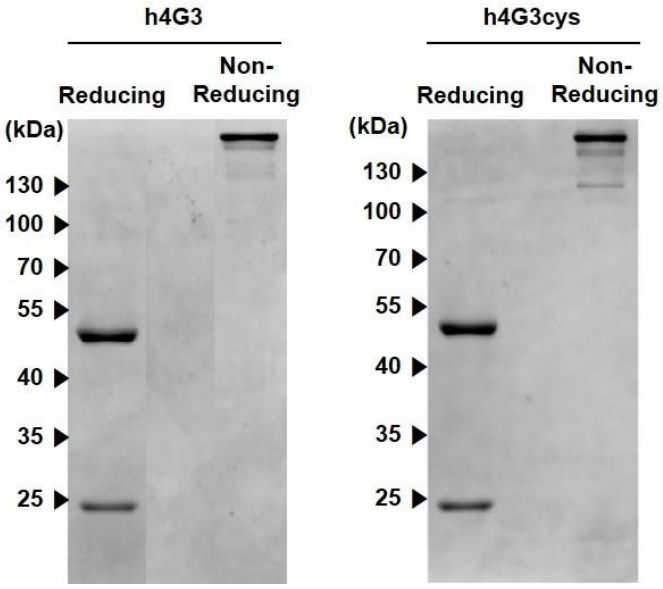

Figure S1 Generation of h4G3 Q124C mutant (h4G3cys) for site-specific conjugation. The h4G3 and h4G3cys were purified from culture fluids using a protein A column and separated by SDS-PAGE under reducing and non-reducing conditions.

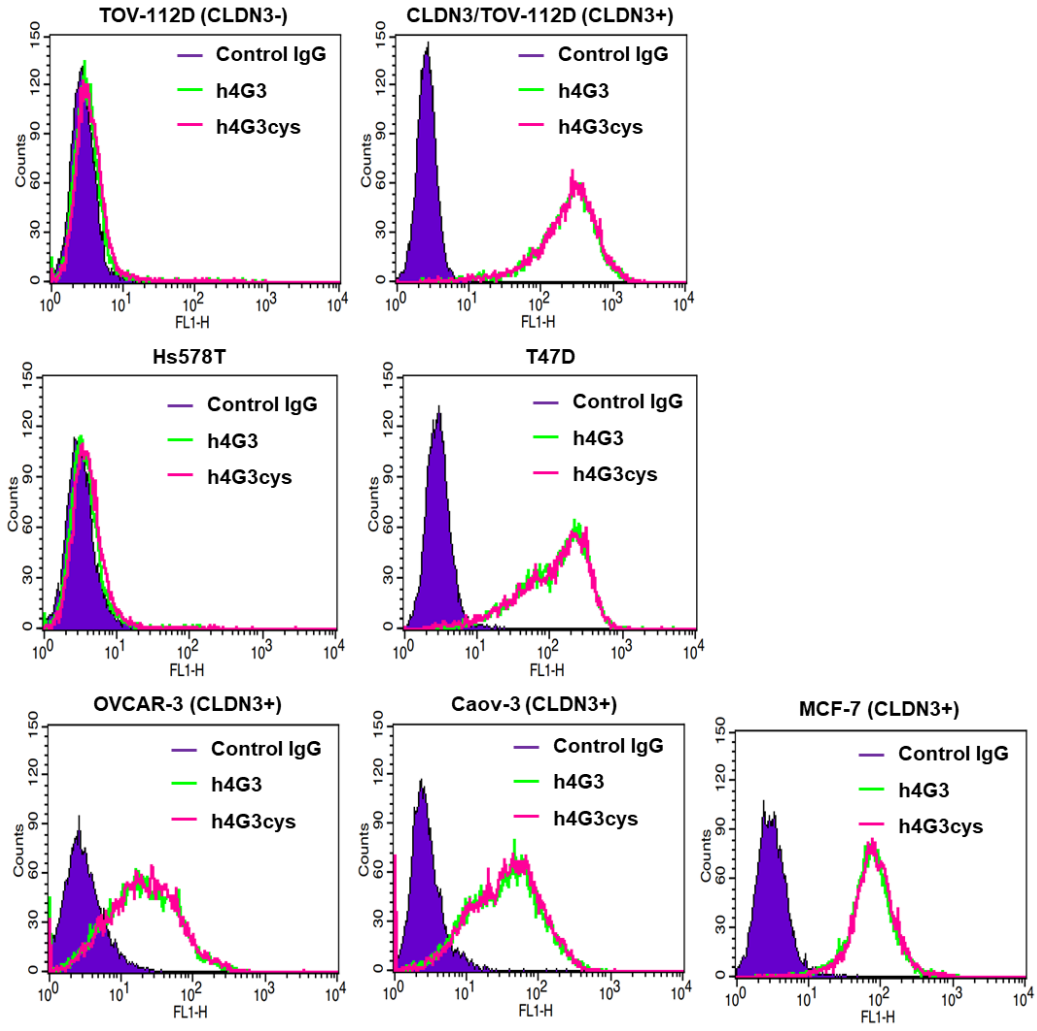

Figure S2 CLDN3-binding affinity of h4G3 and h4G3cys.Various cells with different expression level of CLDN3 were incubated with h4G3 and h4G3cys at 2.5 μg/mL for 1 h. Bound antibodies were detected using FITC-conjugated goat anti-human IgG secondary antibody and analyzed by flow cytometry.

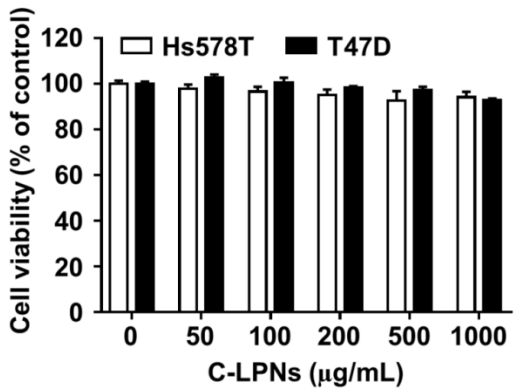

Figure S3 Viability of cells treated with C-LPNs. The cytotoxicity of C-LPNs was determined in Hs578T and T47D cells. The cells were treated with C-LPNs of various concentrations for 1 h, and incubated for 24 h. The cytotoxicity was measured using a WST assay. Data represent means ±SD (*n*=3).

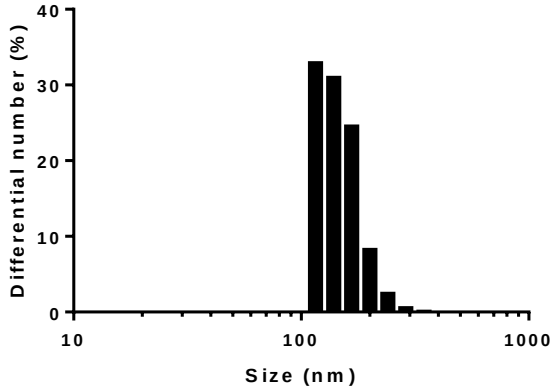

Figure S4 Size distribution of C-LPNs. The size distribution of C-LPNs was measured by light dynamic scattering.

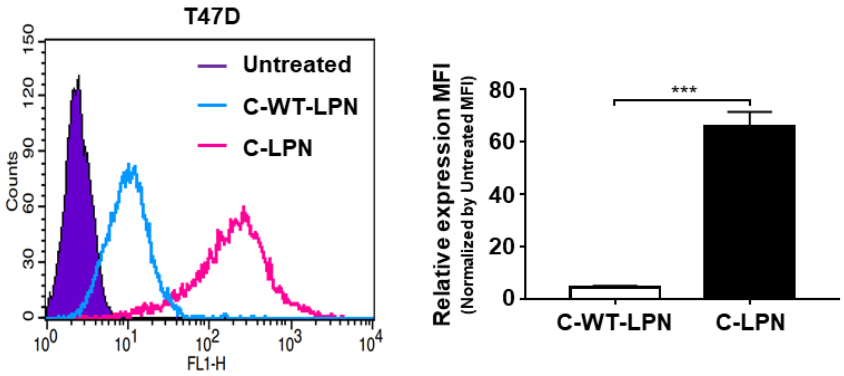

Figure S5 CLDN3-positive cell binding of nanoparticles. T47D cells were treated with LPNs modified with wild type h4G3 (C-WT-LPNs), or with C-LPNs. For flow cytometry, the nanoparticles were labeled with FITC. After treatment of T47D cells with fluorescent nanoparticles for 1 h, flow cytometry analysis was done. The relative mean fluorescence intensity (MFI) was determined by dividing with untreated MFI. Data represent means ±SD (n=3; $^{***}P$<0.001).

Table S1 Binding kinetics of h4G3cys to CLDN3/TOV-112D.

Fitting model	k_{a1} ($M^{-1}\cdot s^{-1}$)	k_{d1} (s^{-1})	k_{a2} (s^{-1})	k_{d2} (s^{-1})	K_D (nmol/L)	
One-to-one two-state	2.05×10^{4}	4.68×10^{-4}	9.01×10^{-5}	2.06×10^{-5}	5.24	
Fitting model	k_{a1} ($M^{-1}\cdot s^{-1}$)	k_{d1} (s^{-1})	K_{D1} (nmol/L)	k_{a2} ($M^{-1}\cdot s^{-1}$)	k_{d2} ($s\cdot^{1}$)	K_{D2} (nmol/L
One-to-two	4.62×10^{4}	5.07×10^{-4}	11	2.03×10^{4}	1.18×10^{-5}	0.578