Supporting Information

for

Comparative Immunological Studies of Tumor-Associated Lewis X, Lewis Y, and KH-1 Antigens

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1. ELISA data

Table S1. Total antibody titers of pooled day 0, 21, and 43 sera derived from mice immunized with conjugates **1a, 1b** and **1c**, respectively

	Total antibody titer		
	1a	**1b**	**1c**
d0	33.01±16.02	114.12±25.78	29.48±25.21
d21	3005.45±352.22*	2819.41±662.82*	676.09±348.20*
d43	8815.03±320.52*	17591.98±1133.63*	4690.68±420.70*

*Significantly different ($p < 0.05$) from the antibody titer of mouse serum obtained before the initial immunization.

Table S2. The IgG antibody titers of pooled day 0, 21 and 43 sera from mice immunized with conjugates **1a, 1b** and **1c**, respectively

	IgG antibody titer		
	1a	**1b**	**1c**
d0	2.70±2.66	0.23±0.20	0.42±0.41
d21	9.20±7.89	3912.44±539.92*	3.30±1.64
d43	3400.64±586.23*	33827.38±1634.39*	12252.78±1167.76*

*Significantly different ($p < 0.05$) from the antibody titer of mouse serum obtained before the initial immunization.

Table S3. The IgM antibody titers of pooled day 0, 21 and 43 sera from mice immunized with conjugates **1a, 1b** and **1c**, respectively

	IgM antibody titer		
	1a	**1b**	**1c**
d0	36.47±36.46	1.43±0.02	61.49±59.90
d21	5762.77±1407.26*	209.92±83.01	790.76±369.07
d43	10851.59±950.93*	650.93±227.87*	3186.76±1177.96*

*Significantly different ($p < 0.05$) from the antibody titer of mouse serum obtained before the initial immunization.

Table S4. Results of the cross-reactivity of pooled day 43 antisera from mice immunized with conjugates **1a, 1b** and **1c**.

Coating antigen	Total antibody titer			IgG antibody titer		
	1a	**1b**	**1c**	**1a**	**1b**	**1c**
2a	8815.03±320.52	20710.31±2054.89	5257.24±2393.74	8003.22±646.66	39084.32±719.88	21332.28±943.68
2b	5219.07±1902.36	17591.98±1133.60	5473.40±1436.61	6221.09±573.94	32458.46±1079.17	21001.82±2358.34
2c	5906.11±2387.21	22601.55±2236.12	4690.68±420.70	3598.32±823.69	38851.45±970.79	20385.16±1779.32

*Significantly different ($p < 0.05$) between the two indicated groups.

Table S5. ELISA results of the cross-reactivity of each pooled day 43 antiserum with mannose-HSA containing the same linker.

Coating antigen	Total antibody titer		
	1a	**1b**	**1c**
2a	8815.03±320.52		
2b		17591.98±1133.60	
2c			4690.68±420.70
Mannose-HSA	3215.33±594.55*	4393.55±875.52*	2602.28±642.89*

*Significantly different ($p < 0.05$) from the antibody titers obtained with antigen-HSA conjugates **2a**, **2b**, and **2c**, respectively.

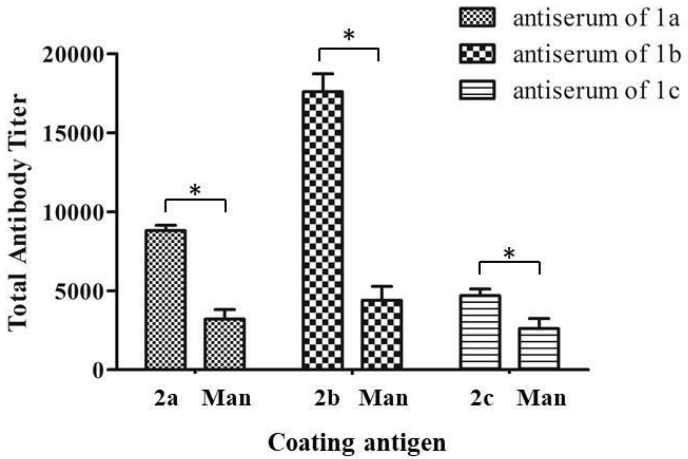

Figure S1. ELISA results of the cross-reactivity of each pooled day 43 antiserum with mannose-HSA conjugate containing the same linker (original data are shown in Table S5). The mannose-HSA conjugate (22 glycans/conjugate) was used as coating antigen and anti-kappa-AP was used as secondary antibody to detect total antibodies. * Statistically different ($p < 0.05$) between the two groups.

Table S6. ELISA results of the cross-reactivity of each pooled day 43 antiserum with lactose-HSA conjugate.

Coating antigen	Total antibody titer		
	1a	**1b**	**1c**
2a	8815.03±320.52		
2b		17591.98±1133.60	
2c			4690.68±420.70
Lactose-HSA	365.27±48.46*	3070.10±125.5*	1263.73±383.43*

*Significantly different ($p < 0.05$) from the antibody titers obtained with antigen-HSA conjugates **2a**, **2b**, and **2c**, respectively.

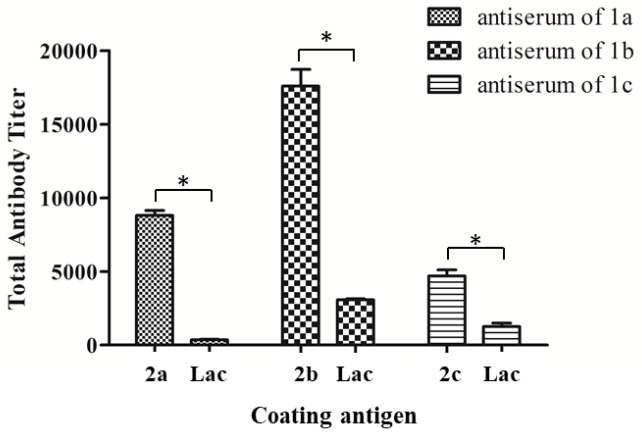

Figure S2. ELISA results of the cross-reactivity of each pooled day 43 antiserum with lactose-HSA conjugate containing the same linker (original data are shown in Table S6). The lactose-HSA conjugate (10 glycans/conjugate) was used as coating antigen and anti-kappa-AP was used as secondary antibody to detect total antibodies. * Statistically different ($p < 0.05$) between the two groups.

2. FACS data

Table S6. Median fluorescence intensity (MFI) results of FACS

Cell line	MFI			
	NS	**1a**	**1b**	**1c**
MCF-7	153.67±53.20	2039.67±382.79*	1851.67±346.87*	959.67±198.48*
SKMEL	148.67±60.70	361.67±125.56	350.00±115.72	307.33±126.33

*Significantly different ($p < 0.05$) from the MFI of NS.

3. Antibody-mediated CDC data

Table S7. Results of antibody-mediated CDC expressed as cell lysis percentage.

Cell line	Cell lysis percentage (%)			
	NS	**1a**	**1b**	**1c**
MCF-7	5.32±1.57	38.17±8.12*	30.17±3.97*	20.58±2.33*
SKMEL	2.39±0.92	3.25±1.27	2.07±0.52	5.03±2.09

* Significantly different ($p < 0.05$) from the cell lysis percentage caused by NS.

4. Mass spectra of glycoconjugates 2a, 2b, and 2c

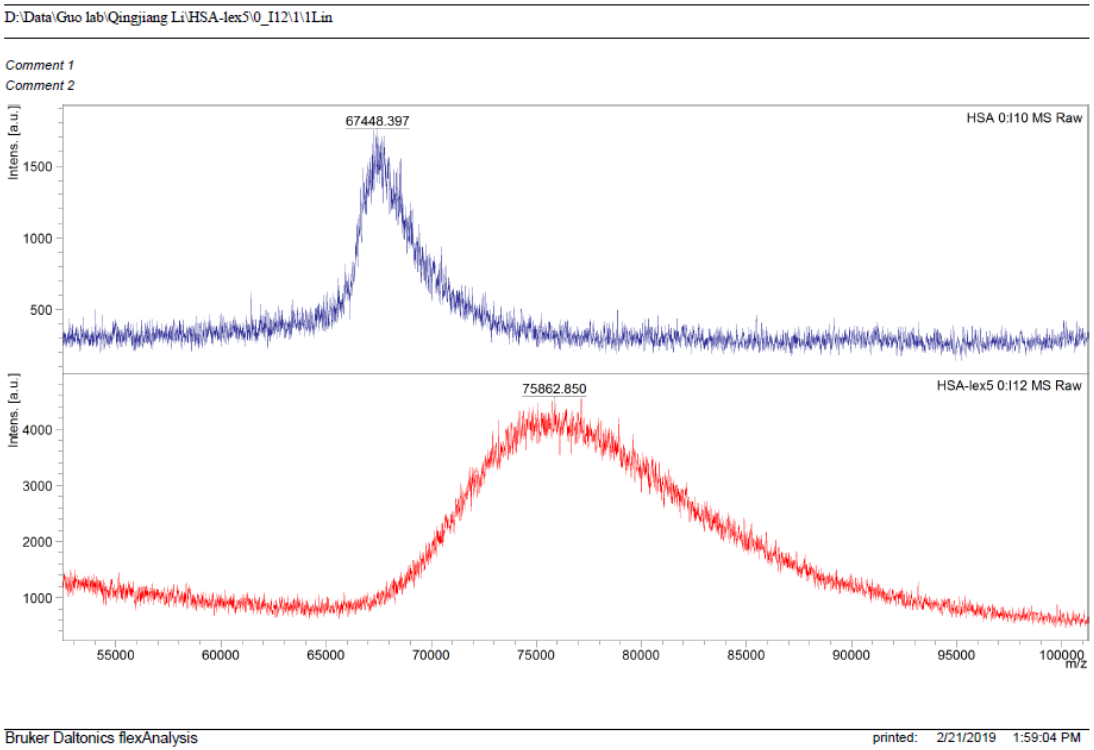

Figure S2. MALDI-TOF mass spectra of HSA (top) and conjugate **2a** (bottom) that were used for the calculation of carbohydrate loading of **2a**.

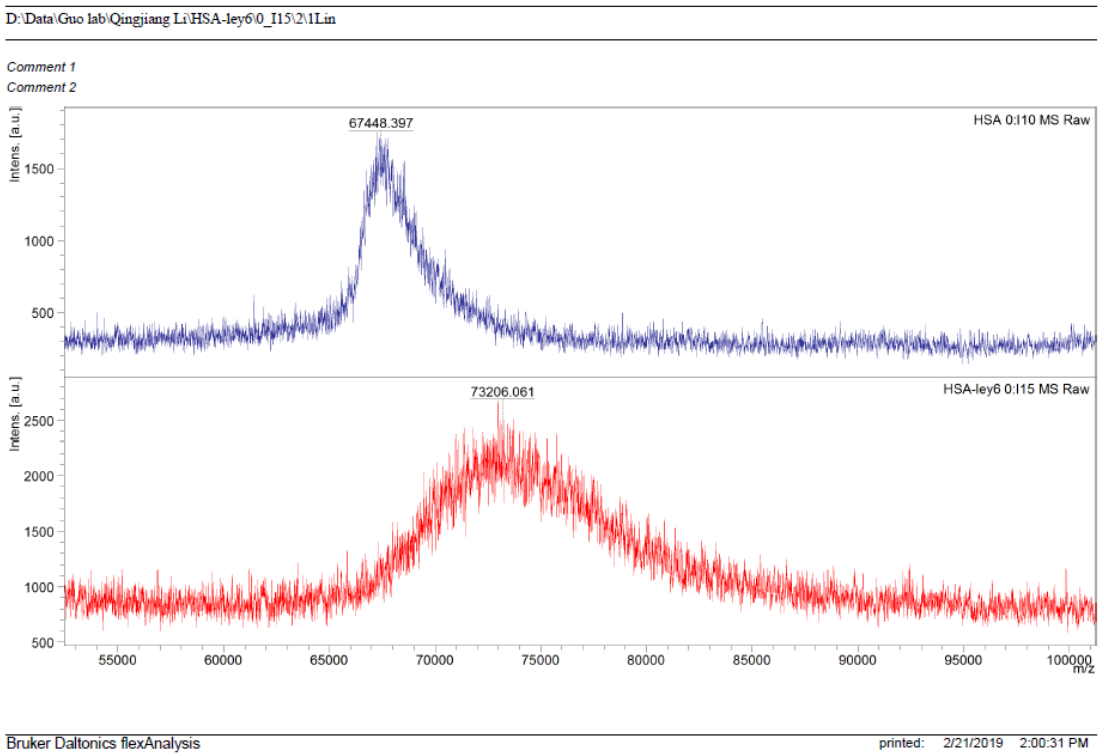

Figure S3. MALDI-TOF mass spectra of HSA (top) and conjugate **2b** (bottom) that were used for the calculation of carbohydrate loading of **2b**.

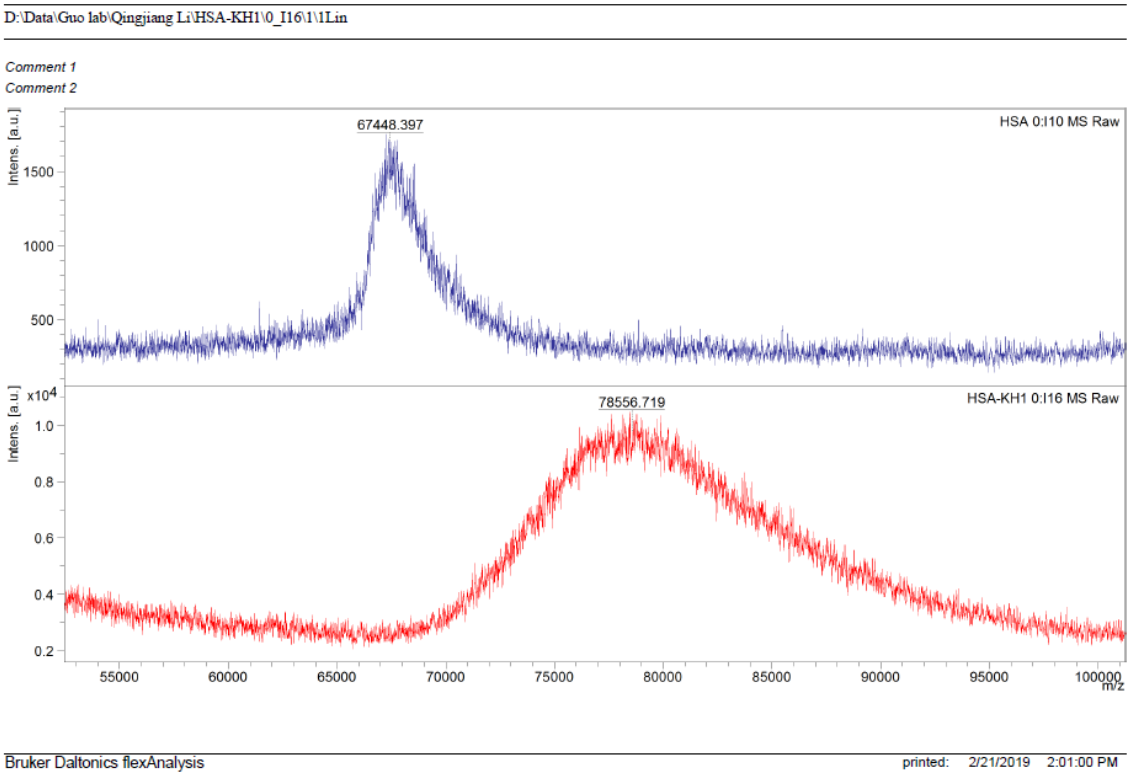

Figure S4. MALDI-TOF mass spectra of HSA (top) and conjugate **2c** (bottom) that were used for the calculation of carbohydrate loading of **2c**.