

Figure S1

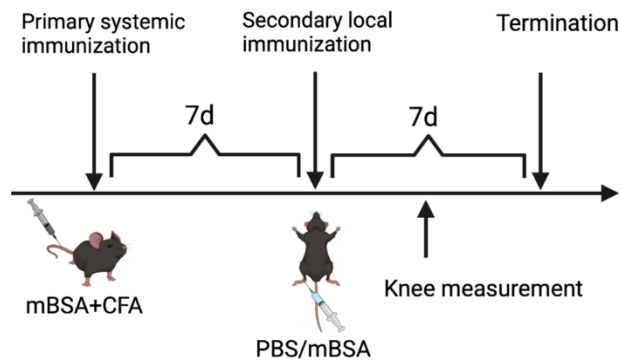


Figure S1: Experimental scheme of antigen-induced arthritis (AIA) mouse model. The mice were immunized by intradermal injection of methylated bovine serum albumin (mBSA) emulsified in complete Freund's adjuvant (CFA), followed seven days later by an intra-articular injection of mBSA in knee joints. Control mice received intra-articular PBS injection in the knee joint. Seven days after the IA injection experiment was terminated.

Supplementary Figure 2

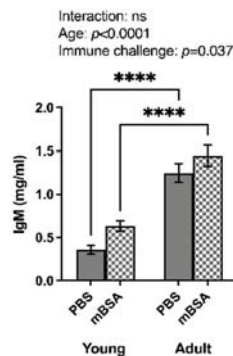







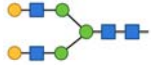







Figure S2: The level of IgM is age-dependent rather than immune induction by mBSA. Serum was collected at termination on day 14, and the IgM (mg/ml) level was measured. Statistical evaluations were performed using a two-way analysis of variance (2way ANOVA) to evaluate the effect of age, the effect of immune challenge and the interaction between age and immune challenge. This was followed by Fisher's LSD multiple comparisons to calculate statistical differences between individual groups.

Table S1: Detected glycoforms in murine IgG2b (Uniprot entry P01867, peptide sequence EDYNSTIR) by LC-MS mass spectrometry analysis.

Glycoform composition ^a	Depiction ^b	Relative % intensity			
<i>A-galactosylated (G0)</i>		Young		Adult	
		PBS	m-BSA	PBS	m-BSA
N1		0.15 ±0.01	0.06 ±0.03	0.07± 0.01 [#]	0.04±0.02
H2N3F		0.56 ±0.10	0.35 ±0.03 [*]	0.32±0.02 [#]	0.23±0.06
H3N3F		1.86±0.12	1.90 ±0.08	1.82±0.03	1.74 ±0.06
H3N4F		11.89±0.79	8.94 ± 0.52 ^{**}	9.76 ± 0.64 [#]	8.56±0.79
H4N3F		1.25 ±0.12	1.73 ±0.04	2.02±0.19 [#]	2.63 ±0.33 ^{***}
<i>Galactosylated (G1/G2)</i>					
H4N4		0.00±0.00	0.09±0.02	0.00±0.00	0.41±0.07 ^{****###}
H4N4F		47.1±2.67	51.06 ±0.51	49.76±0.83	48.03±1.71
H5N4		0.00±0.00	0.02±0.00	0.01 ±0.00	0.06 ± 0.03 [*]
H5N4F		13.67±0.91	18.38±0.29 ^{***}	17.03±0.71 ^{##}	19.33± 0.68 [*]
<i>Sialylated (G1S/G2S)</i>					
H4N3FG1		0.07±0.01	0.12±0.01	0.29±0.01 ^{###}	0.36 ±0.05 ^{###}
H4N4FG1		4.90±0.47	3.50±0.22 [*]	4.57±0.31	3.57±0.32
H5N4FG1		11.09±1.56	8.77±0.53	10.02 ±0.72	9.21 ±0.84
H5N4FG2		3.92±0.28	2.43±0.14 ^{***}	2.59±0.06 ^{###}	1.63±0.25 ^{***}

^aH: hexose, N: N-acetyl hexosamine, F: fucose, G: N-glycolylneuraminic acid. ^bSymbols used: green circle: mannose, yellow circle: galactose, blue square: N-acetylglucosamine, red triangle: fucose, blue diamond: N-glycolylneuraminic acid (Neu5Gc). *Represents the significant difference between PBS to mBSA. #Represents the significant difference between young to adults. The proposed glycan structures are based on fragmentation analysis and literature. Statistical analysis was performed with the Fisher LSD test. Error bar denotes \pm SEM.

Table S2: Detected glycoforms in murine IgG3 (Uniprot entry P03897, peptide sequence EAQYNSTFR) by LC-MS mass spectrometry analysis.

Glycoform composition ^a	Depiction ^b	Relative % intensity			
<i>A-galactosylated (G0)</i>		Young		Adult	
		PBS	m-BSA	PBS	m-BSA
H2N3F		0.85±0.12	0.44±0.13	0.98±0.26	0.44±0.22
H3N3F		3.21±0.11	3.15±0.37	3.12±0.67	2.25±0.95
H3N4F		40.09±1.54	36.43±1.20	42.72±3.58	46.93±1.66 ^{##}
<i>Galactosylated (G1/G2)</i>					
H4N3F		0.31± 0.17	0.00±0.00	0.33±0.19	0.26±0.17
H4N4F		33.46±1.39	39.80±1.17*	36.33±1.96	39.70±1.69
H5N4F		10.07±1.03	13.95±0.87	12.16±2.10	4.54±1.58 ^{**###}
<i>Sialylated (G1S/G2S)</i>					
H4N4FG1		6.98±0.78	2.78±0.44 ^{***}	1.44±0.37 ^{###}	1.86±0.72
H4N5FG1		4.13±0.39	3.46±0.48	2.92±0.69	3.99±2.19
H5N4FG2		0.89± 0.35	0.00± 0.00 ^{**}	0.00± 0.00 ^{##}	0.00±0.00

^aH: hexose, N: N-acetyl hexosamine, F: fucose, G: N-glycolylneuraminic acid. ^bSymbols used: green circle: mannose, yellow circle: galactose, blue square: N-acetylglucosamine, red triangle: fucose, blue diamond: N-glycolylneuraminic acid (Neu5Gc). *Represents the significant difference between PBS to mBSA. [#]Represents the significant difference between young to adults. The proposed glycan structures are based on fragmentation analysis and literature. Statistical analysis was performed with the Fisher LSD test. Error bar denotes ± SEM.