

Supplementary Materials: Directed Evolution of Therapeutic Antibodies Targeting Glycosylation in Cancer

Ron Amon, Ronit Rosenfeld, Shahar Perlmutter, Oliver C. Grant, Sharon Yehuda, Aliza Borenstein-Katz, Ron Alcalay, Tal Marshanski, Hai Yu, Ron Diskin, Robert J. Woods, Xi Chen and Vered Padler-Karavani

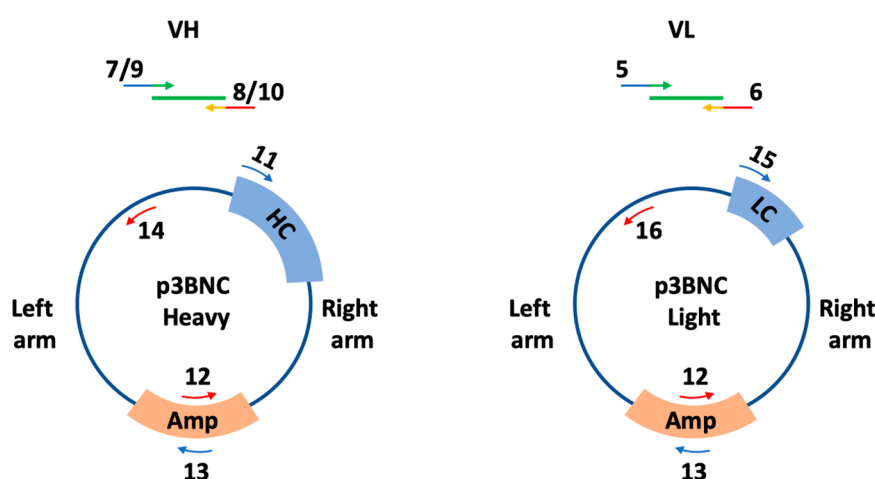


Figure S1. Gibson assembly of variable regions into p3BNC IgG expression vectors. Variable regions were cloned into p3BNC expression vectors by Gibson assembly. Primers number refer to the numbers in Supplementary Table 1b. HC-heavy constant, LC-light constant.

Table S1. List of glycans nanoprined on glycan microarrays.

Glycan ID	Structure
1	Neu5,9Ac α 3Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
2	Neu5Gc9Aca α 3Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
3	Neu5,9Ac α 6Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
4	Neu5Gc9Aca α 6Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
5	Neu5Aca6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
6	Neu5Gca6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
7	Neu5,9Ac α 3Gal β 3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
8	Neu5Gc9Aca α 3Gal β 3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
9	Neu5,9Ac α 3Gal β 3GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
10	Neu5Gc9Aca α 3Gal β 3GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
11	Neu5Aca3Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
12	Neu5Gca3Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
13	Neu5Aca3Gal β 3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
14	Neu5Gca3Gal β 3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
15	Neu5Aca3Gal β 3GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
16	Neu5Gca3Gal β 3GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
17	Neu5Aca6Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
18	Neu5Gca6Gal β 4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
19	Neu5Aca6Gal β 4Glc β O(CH ₂) ₂ CH ₂ NH ₂
20	Neu5Gca6Gal β 4Glc β O(CH ₂) ₂ CH ₂ NH ₂
21	Neu5Aca3Gal β 4Glc β O(CH ₂) ₂ CH ₂ NH ₂
22	Neu5Gca3Gal β 4Glc β O(CH ₂) ₂ CH ₂ NH ₂
23	Neu5,9Ac α 6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
24	Neu5Gc9Aca α 6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
25	Neu5Aca3Gal β O(CH ₂) ₂ CH ₂ NH ₂

26	Neu5Gca3GalβO(CH ₂) ₂ CH ₂ NH ₂
27	Neu5Aca6GalβO(CH ₂) ₂ CH ₂ NH ₂
28	Neu5Gca6GalβO(CH ₂) ₂ CH ₂ NH ₂
29	Neu5,9Acα3GalβO(CH ₂) ₂ CH ₂ NH ₂
30	Neu5Gc9Aca3GalβO(CH ₂) ₂ CH ₂ NH ₂
31	Neu5,9Acα6GalβO(CH ₂) ₂ CH ₂ NH ₂
32	Neu5Gc9Aca6GalβO(CH ₂) ₂ CH ₂ NH ₂
33	Neu5Aca3Galβ3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
34	Neu5Gca3Galβ3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
35	Neu5,9Acα3Galβ3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
36	Neu5Gc9Aca3Galβ3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
37	Neu5,9Acα6Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
38	Neu5Gc9Aca6Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
39	Neu5,9Acα3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
40	Neu5Gc9Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
41	Neu5Aca8Neu5Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
42	Neu5Aca8Neu5Aca8Neu5Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
43	Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
44	Galβ4GlcβNH ₂
45	Galβ4GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
46	GalβNH ₂
47	GalNAcαO(CH ₂) ₂ CH ₂ NH ₂
48	GalβO(CH ₂ CH ₂) ₆ NH ₂
49	GalβO(CH ₂) ₂ CH ₂ NH ₂
50	Galβ4GlcβO(CH ₂ CH ₂) ₆ NH ₂
51	Galβ3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
52	Galβ3GalNAcαO(CH ₂) ₂ CH ₂ NH ₂
53	Galβ3GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
54	Galβ4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
55	Neu5Aca3Galβ4(Fuca3)GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
56	Neu5Gca3Galβ4(Fuca3)GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
57	Neu5Aca3Galβ4(Fuca3)GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
58	Neu5Gca3Galβ4(Fuca3)GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
59	Galβ3GlcNAcβ3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
60	Neu5Aca3Galβ3GlcNAcβ3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
61	Neu5Gca3Galβ3GlcNAcβ3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
62	Neu5Aca3Galβ4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
63	Neu5Gca3Galβ4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
64	Neu5Aca8Neu5Aca3Galβ4GlcβO(CH ₂) ₃ NHCOCH ₂ (OCH ₂ CH ₂) ₆ NH ₂
65	Neu5Aca8Neu5Aca8Neu5Aca3Galβ4GlcβO(CH ₂) ₃ NHCOCH ₂ (OCH ₂ CH ₂) ₆ NH ₂
66	Neu5Aca6(Neu5Aca3)Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
67	Neu5Aca6(Neu5Gca3)Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
68	Neu5Aca6(Kdna3)Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
69	Neu5Gca8Neu5Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
70	Kdna8Neu5Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
71	Neu5Aca8Kdna6Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
72	Neu5Aca8Neu5Gca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
73	Neu5Aca8Neu5Gca6Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
74	KDNα8Neu5Gca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
75	Neu5Gca8Neu5Gc-α3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
76	Neu5Aca8Neu5Aca6Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
77	Neu5GcMeα8Neu5Aca3Galβ4GlcβO(CH ₂) ₂ CH ₂ NH ₂
78	Galα3Galβ4GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
79	Galβ3GalNAcαO(CH ₂) ₂ CH ₂ NH ₂
80	Galβ4(Fuca3)GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
81	Neu5Aca8Neu5Aca3Galβ4GlcO(CH ₂) ₂ CH ₂ NH ₂

82	Neu5Ac α 8Neu5Ac α 3(GalNAc β 4)Gal β 4GlcO(CH ₂) ₂ CH ₂ NH ₂
83	Neu5Ac α 3Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
84	Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
85	Fuca2Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
86	Neu5Gca3Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
87	Neu5,9Ac2 α 3Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
88	Neu9Ac5Gca3Gal β 3(Fuca4)GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂

Table S2. (a) Primers used for cloning and sequencing in YSD system. (b) Primers used for cloning and sequencing of variable regions from scFv into p3BNC vectors.

a. Primers used in YSD system		
Primer #	Primer name	Primer sequence 5→3
1	pETCON2 universal For	TCTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCGGC
2	pETCON2 universal Rev	TTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGATCCGCCCC
3	pCON2-seq-For_primer	GTCCAGACTACGCTCTGCAGG
4	pCON2-seq-Rev_primer	GATTTTGTACATCTACACTGTTG

b. Primers for whole IgG exprssion		
Primer #	Primer name	Primer sequence 5→3
1	p3BNC heavy for seq	GGATCCCCGTGCCAAG
2	p3BNC heavy rev seq	CGGAGATCATGAGGGTG
3	p3BNC light for seq	CTTCGTTAGAACGCGGC
4	p3BNC light rev seq	TCCTTGCTGTCTGCTC
5	Native VL p3BNC for	GTAGCAACTGCAACCGGTGTACATTGACACATTAATAATGACTCAGAGTCCATC
6	Native VL p3BNC rev	GGTGCAGCCACCGTACGTTTGAATTTCTAATTTGTTCACCGC
7	Native VH p3BNC for	GTAGCAACTGCAACCGGTGTACATTGCGAAGTCAAGCTAGAAGA AAGCG
8	Native VH p3BNC rev	GATGGGCCCTTGGTCGACGCGGCAGAAACCGTCACCAG
9	RA9-23 VH p3BNC for	GTAGCAACTGCAACCGGTGTACATTGCAAAGTCAAGCTAGAAGA AAGCG
10	RA9-23 VH p3BNC rev	GATGGGCCCTTGGTCGACGCGGCAGGAACCGTCACCAG
11	p3BNC_H vector right arm for primer	GCGTCGACCAAGGGCCCCATC
12	p3BNC_H/L vector right arm rev primer	CAACCAAGTCATTCTGAGAATAG
13	p3BNC_H/L vector left arm for primer	CTATTCTCAGAATGACTTGGTTG
14	p3BNC_H vector left arm rev primer	GCAATGTACACCGGTTGCAG
15	p3BNC_L vector right arm for primer	CGTACGGTGGCTGCACCATC
16	p3BNC_L vector left arm rev primer	TGAATGTACACCGGTTGCAGTTG

