Supplementary Information

A Siglec-like sialic acid binding motif found in an adenovirus capsid protein

Christoph Rademacher[1], Thierry Bru[2], Ryan McBride[1], Elizabeth Robison[1], Corwin M. Nycholat[1], Eric J. Kremer[2], James C. Paulson[1]*

Supporting Tables S1 and S2
Supporting Figures S1 and S2

Table S1: List of all complexes analyzed:

1CT1; 1DBN; 1DFQ; 1DQF; 1E8U; 1F31; 1FV2; 1FV3; 1G1T; 1H7T; 1HGE; 1HGG; 1HGH; 1HGI; 1JSH; 1JSI; 1JSN; 1JSO; 1KQR; 1MQM; 1MQN; 1MWE; 1MX1; 1MX5; 1N1Y; 1NSC; 1QDD; 1QFO; 1QWJ; 1RVT; 1RVX; 1RVZ; 1S0I; 1S0J; 1SE3; 1URL; 1USR; 1USX; 1UXA; 1UXB; 1V0F; 1V3C; 1VPS; 1W0O; 1W0P; 1W1X; 1W20; 1W21; 1WGC; 1WW4; 1YA4; 1YA8; 1YAH; 1YAJ; 1YYN; 1Z4X; 2BAT; 2BER; 2BF6; 2C25; 2C4A; 2C4L; 2CHB; 2DF3; 2DQY; 2DQZ; 2DR0; 2DS0; 2EHI; 2H7C; 2HRL; 2HRQ; 2HRR; 2I2S; 2JH7; 2JHD; 2JHL; 2KMB; 2OSX; 2P3I; 2P3J; 2P3K; 2QWB; 2R61; 2RDG; 2RFT; 2RFU; 2V73; 2VU9; 2W68; 2W9L; 2WBV; 2WBW; 2WGC; 2WR1; 2WR2; 2WR3; 2WR4; 2WR7; 2WRB; 2WRE; 2WRF; 2WRG; 2WRH; 2Z8L; 2ZG1; 2ZG3; 2ZOE; 3B50; 3BWR; 3CHB; 3E81; 3F53; 3F5A; 3F5E; 3GVJ; 3GVK; 3GVL; 3H72; 3HMY; 3HN1; 3HTP; 3HTQ; 3HTT; 3JU4; 4HMG; 5HMG

Table S2: Oligosaccharide probes included in the glycan microarray

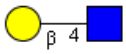
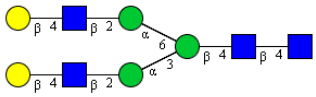
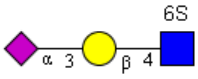
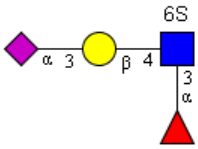
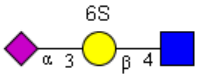
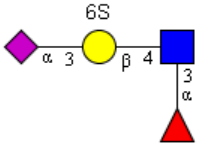
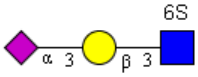
Chart #	Structure	IUPAC Nomenclature
1		Galβ(1-4)-GlcNAcβ-ethyl-NH_2
2		Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
3		NeuAcα(2-3)-Galβ(1-4)-6-sulfo-GlcNAcβ-propyl-NH_2
4		NeuAcα(2-3)-Galβ(1-4)-[Fucα(1-3)]-6-*O*-sulfo-GlcNAcβ-propyl-NH_2
5		NeuAcα(2-3)-6-*O*-sulfo-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
6		NeuAcα(2-3)-6-*O*-sulfo-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ-propyl-NH_2
7		NeuAcα(2-3)-Galβ(1-3)-6-*O*-sulfo-GlcNAcβ-propyl-NH_2
8		NeuAcα(2-3)-Galβ(1-4)-Glcβ-ethyl-NH_2

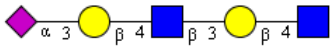
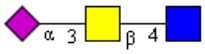
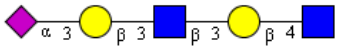
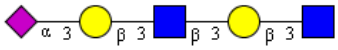
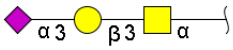
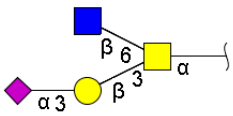
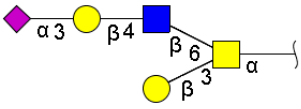
Chart #	Structure	IUPAC Nomenclature
9		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
10		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
11		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
12		NeuAcα(2-3)-GalNAcβ(1-4)-GlcNAcβ-ethyl-NH_2
13		NeuAcα(2-3)-Galβ(1-3)-GlcNAcβ-ethyl-NH_2
14		NeuAcα(2-3)-Galβ(1-3)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
15		NeuAcα(2-3)-Galβ(1-3)-GlcNAcβ(1-3)-Galβ(1-3)-GlcNAcβ-ethyl-NH_2
16		NeuAcα(2-3)-Galβ(1-3)-GalNAcβ(1-3)-Galα(1-4)-Galβ(1-4)-Glcβ-ethyl-NH_2
17		NeuAcα(2-3)-Galβ(1-3)-GalNAcα-Thr-NH_2
18		NeuAcα(2-3)-Galβ(1-3)-[GlcNAcβ(1-6)]-GalNAcα-Thr-NH_2
19		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-6)-[Galβ(1-3)]-GalNAcα-Thr-NH_2

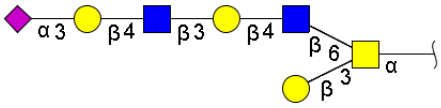
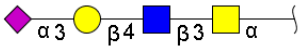
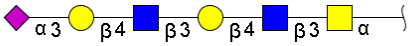
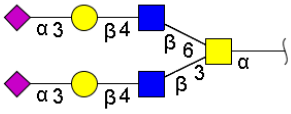
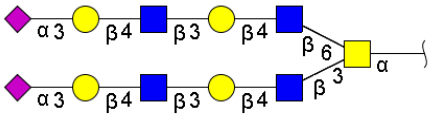
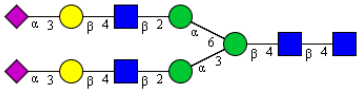
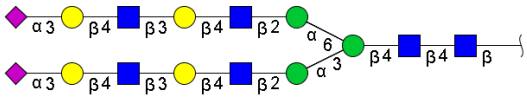
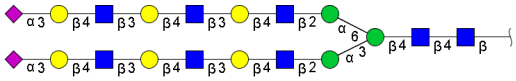
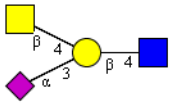
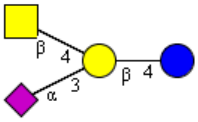
Chart #	Structure	IUPAC Nomenclature
20		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-6)-[Galβ(1-3)]-GalNAcα-Thr-NH_2
21		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAcα-Thr-NH_2
22		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAcα-Thr-NH_2
23		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAcα-Thr-NH_2
24		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAcα-Thr-NH_2
25		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
26		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
27		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
28		NeuAcα(2-3)-[GalNAcβ(1-4)]-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
29		NeuAcα(2-3)-[GalNAcβ(1-4)]-Galβ(1-4)-Glcβ-ethyl-NH_2

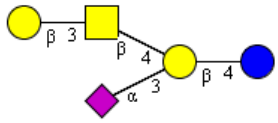
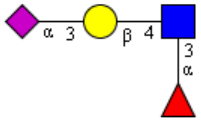
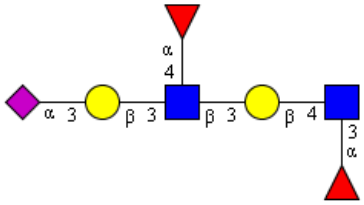
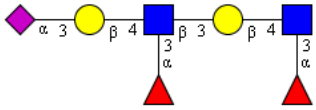
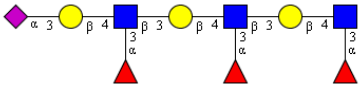
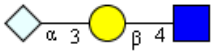
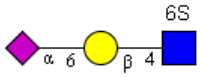
Chart #	Structure	IUPAC Nomenclature
30		Galβ(1-3)-GalNAcβ(1-4)-[NeuAcα(2-3)]-Galβ(1-4)-Glcβ-ethyl-NH_2
31		NeuAcα(2-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ-propyl-NH_2
32		NeuAcα(2-3)-Galβ(1-3)-[Fucα(1-4)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ-ethyl-NH_2
33		NeuAcα(2-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ-ethyl-NH_2
34		NeuAcα(2-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fucα(1-3)]-GlcNAcβ-ethyl-NH_2
35		NeuGcα(2-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
36		NeuAcα(2-6)-Galβ(1-4)-6-sulfo-GlcNAcβ-propyl-NH_2
37		NeuAcα(2-6)-Galβ(1-4)-Glcβ-ethyl-NH_2

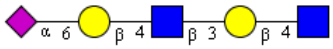
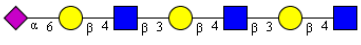
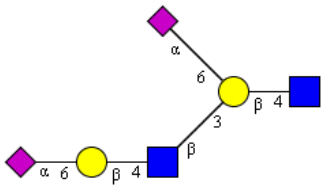
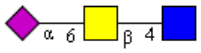
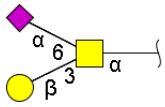
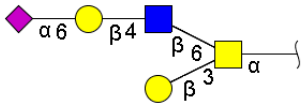
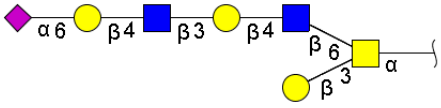
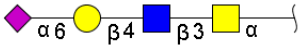
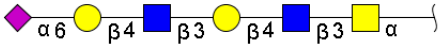
Chart #	Structure	IUPAC Nomenclature
38		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
39		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
40		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
41		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAcα(2-6)]-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
42		NeuAcα(2-6)-GalNAcβ(1-4)-GlcNAcβ-ethyl-NH_2
43		NeuAcα(2-6)-[Galβ(1-3)]-GalNAcα-Thr-NH_2
44		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-6)-[Galβ(1-3)]-GalNAcα-Thr-NH_2
45		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-6)-[Galβ(1-3)]-GalNAcα-Thr-NH_2
46		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAcα-Thr-NH_2
47		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAcα-Thr-NH_2

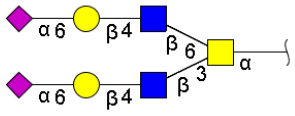
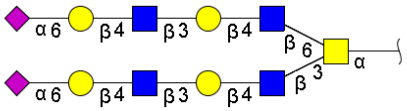
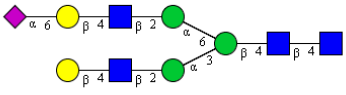
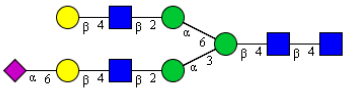
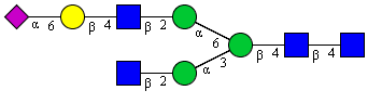
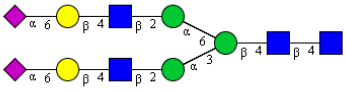
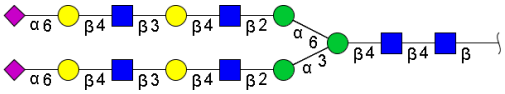
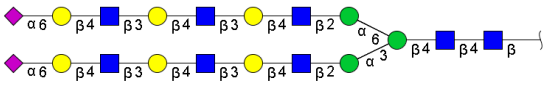
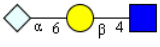
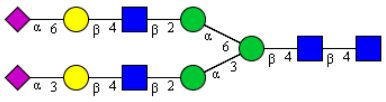
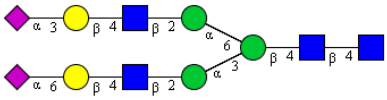
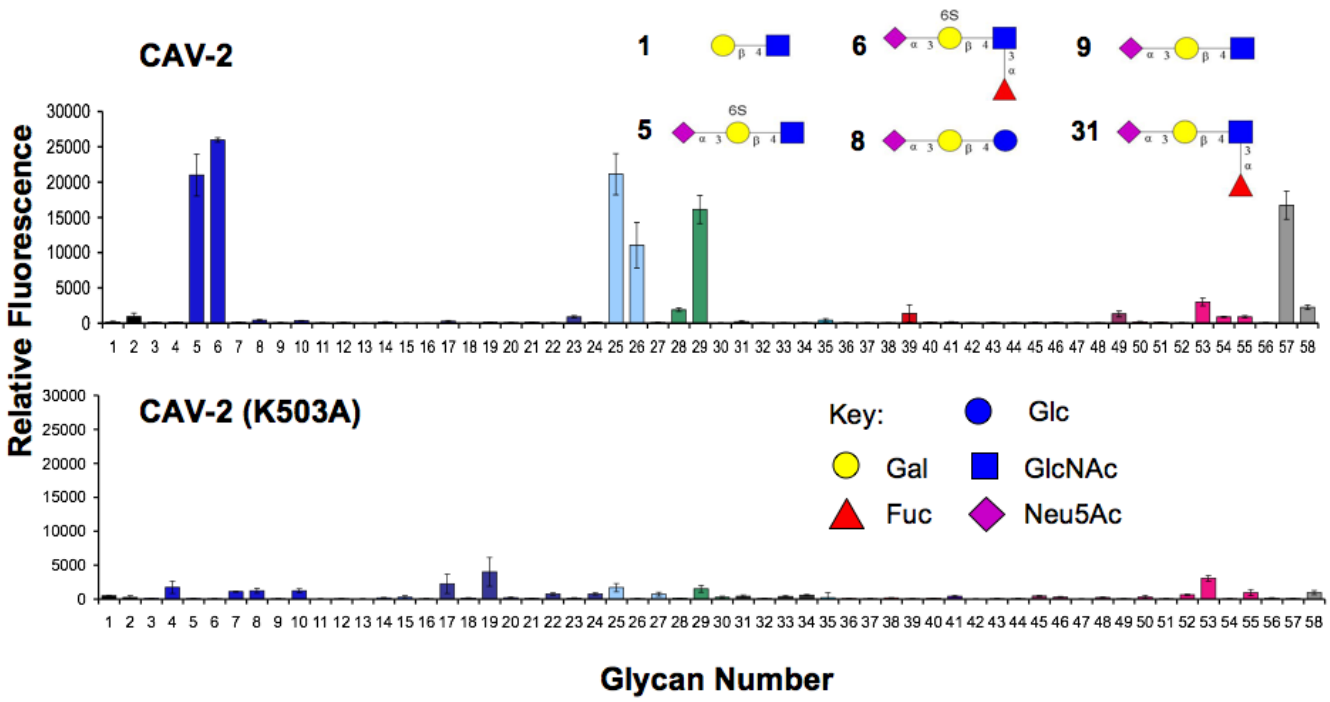
Chart #	Structure	IUPAC Nomenclature
48		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAcα-Thr-NH_2
49		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAcα-Thr-NH_2
50		Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
51		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
52		GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
53		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
54		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
55		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2
56		NeuGcα(2-6)-Galβ(1-4)-GlcNAcβ-ethyl-NH_2
57		NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2

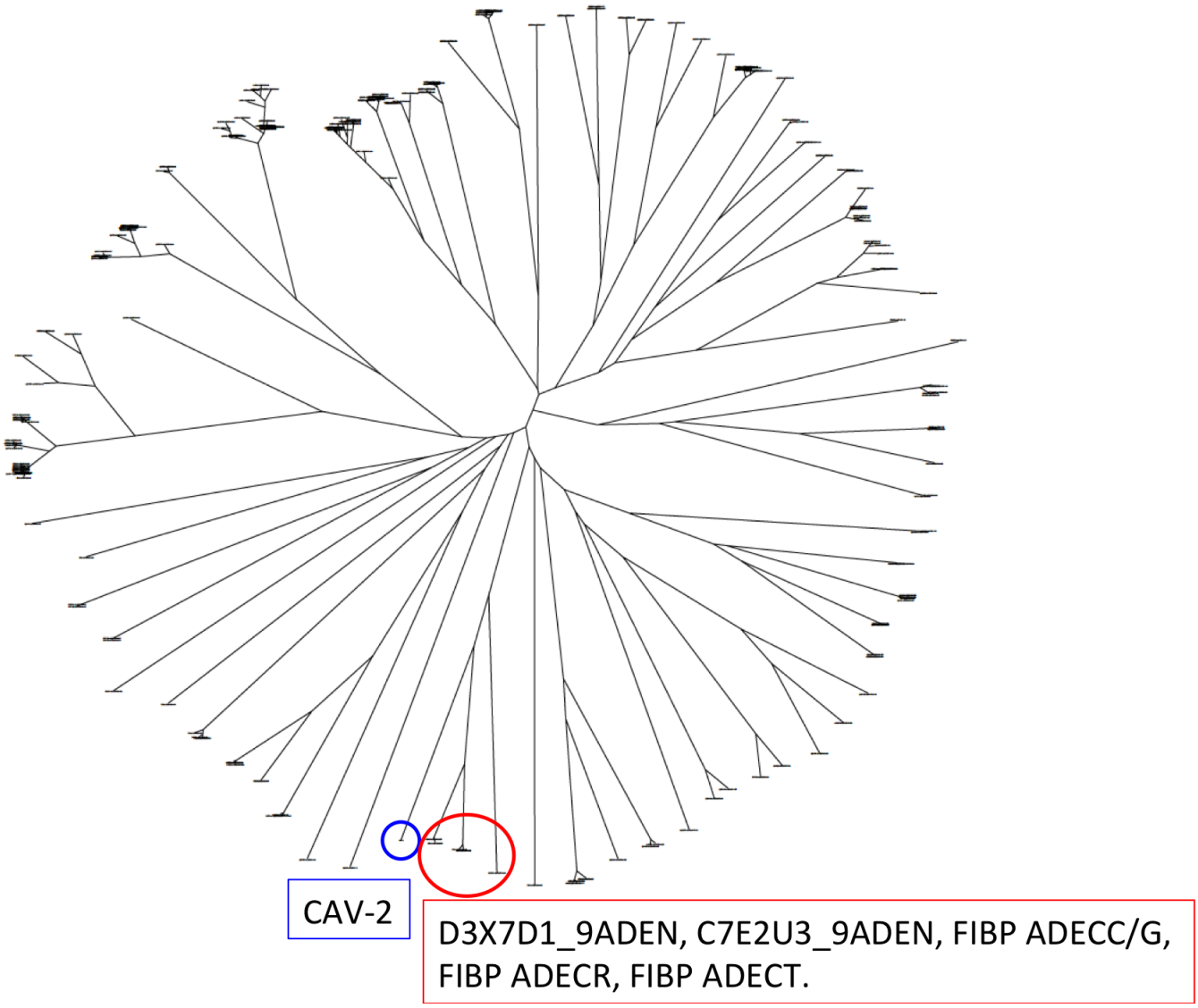
Chart #	Structure	IUPAC Nomenclature
58		NeuAcα(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH_2

Supporting Figure S1:



Glycan array analysis of the WT and K503A CAV-2 fiber knob protein at 500 ug/ml final concentration. (Top) In addition to sialosides **5** and **6** found at lower protein concentrations (50 ug/ml), structures bearing terminal α2,3)-linked Neu5Ac **25**, **26**, **29** and **57** are detected by the fiber knob protein. (Bottom) The mutant CAV-2 (K503A) does not bind significantly to the printed sialoside array at high concentrations.

Supporting Figure S2:



Sequence alignment and family tree of adeno fiber knobs:

A multiple sequence alignment of the adenoviral fibre protein family (PF00541) provided by the PFAM database was extended by the CAV-2 sequence (Uniprot: Q65914) in ClustalX (version 2.0.9). The following sequences contain the essential arginine stretch FRFLN: D3X7D1_9ADEN, C7E2U3_9ADEN, FIBP ADECC, FIBP ADECG, FIBP ADECR, FIBP ADECT. The knobs also contain a lysine residue in a similar position. A family dendrogram resulting from the alignment file was visualized using drawtree as implemented on the mobyle server (Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.).