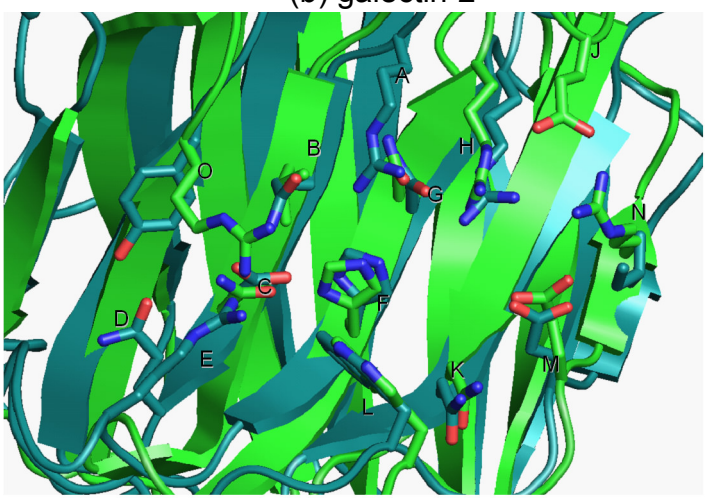
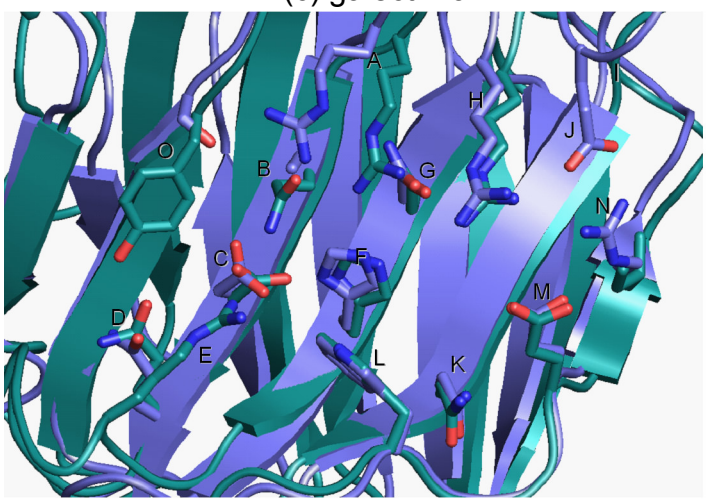
(a) galectin-1

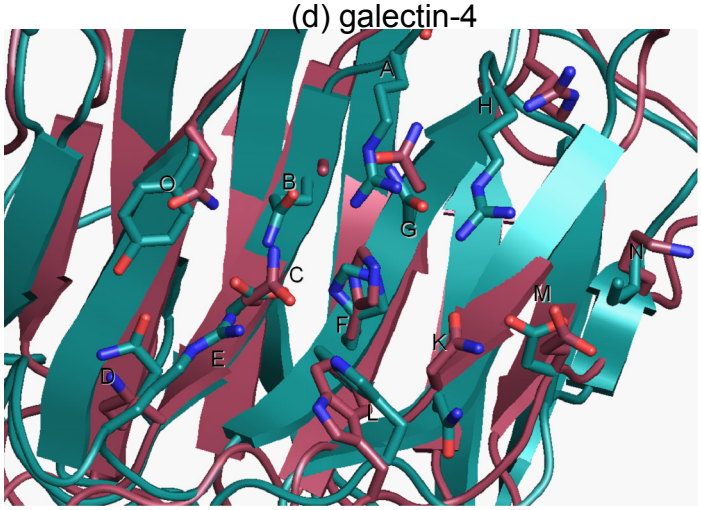
(b) galectin-2



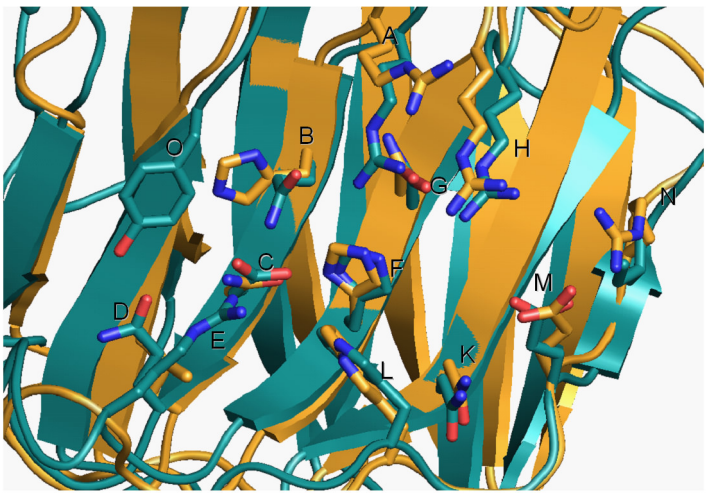
(c) galectin-3



(d) galectin-4



(e) galectin-7



	Galectin-8	Galectin-1	Galectin-2	Galectin-3	Galectin-4	Galectin-7
A	Arg45	Ser29	Gly30	Arg144	Ser220	Arg31
B	Gln47	Val31	Val32	Ala146	Ala222	His33
C	Asp49	Asn33	Asn34	Asp148	Asn224	Asn35
D	Gln51	Gly35	Gly36	Gln150	Lys226	Leu37
E	Arg59					
F	His65	His44	His45	His158	His236	His49
G	Asn67	Asn46	Asn47	Asn160	Asn238	Asn51
H	Arg69	Arg48	Arg49	Arg162	Arg240	Arg53
I		His52				
J		Asp54	Gln52	Glu165		
K	Asn79	Asn61	Asn58	Asn174	Asn249	Asn62
L	Trp86	Trp68	Trp65	Trp181	Trp256	Trp69
M	Glu89	Glu71	Glu68	Glu184	Glu259	Glu72
N	Ile91	Arg73	Arg70	Arg186	Lys261	Arg74
O	Tyr141		Arg120	Ser237	Gln313	Gly124

Fig. S1.

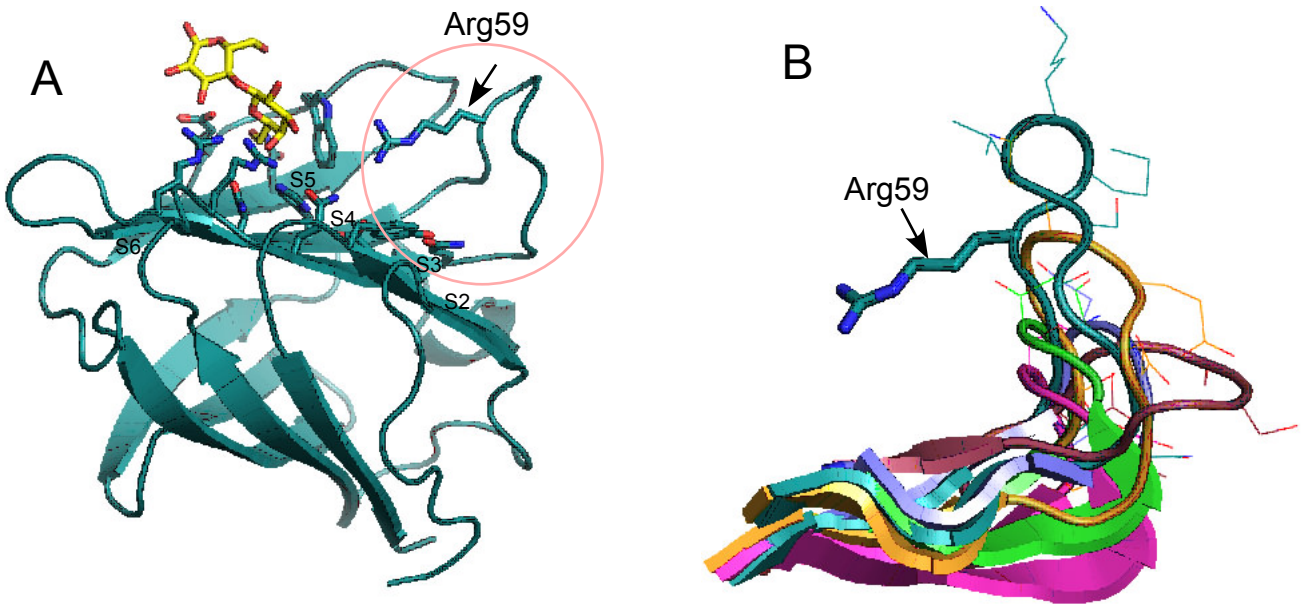


Fig. S2.

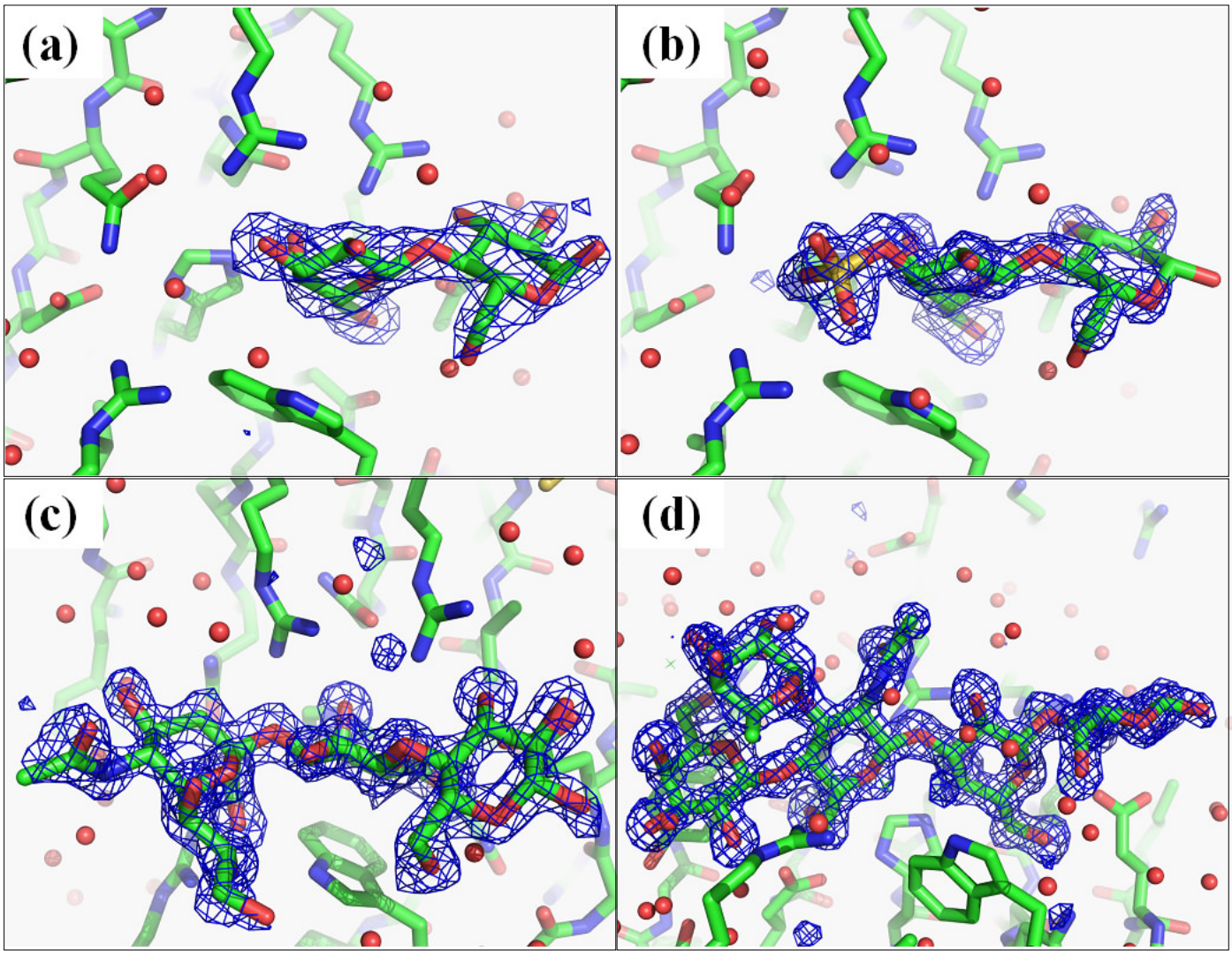
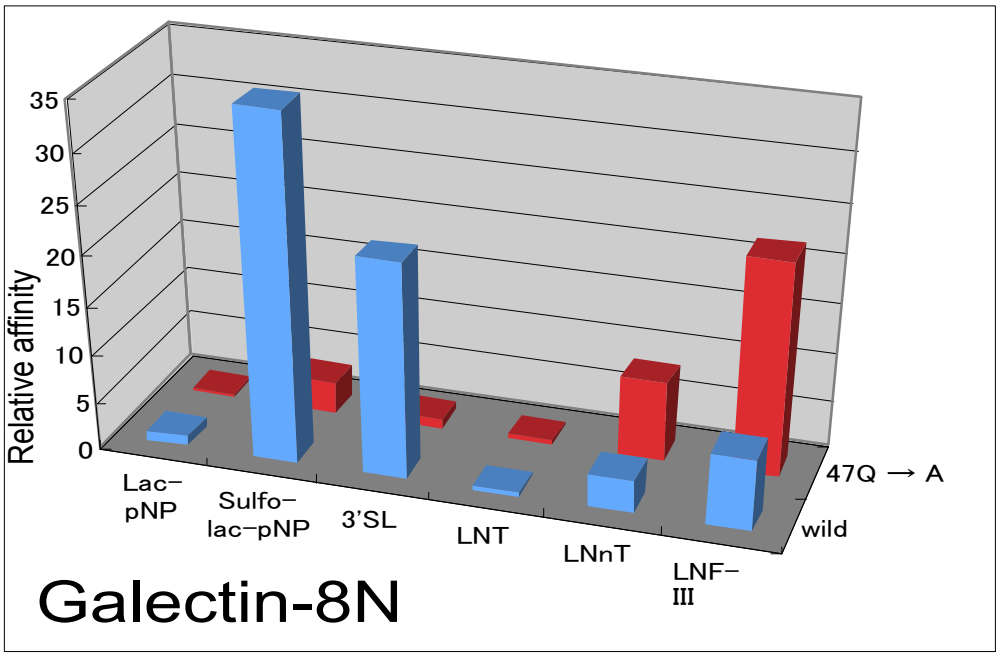
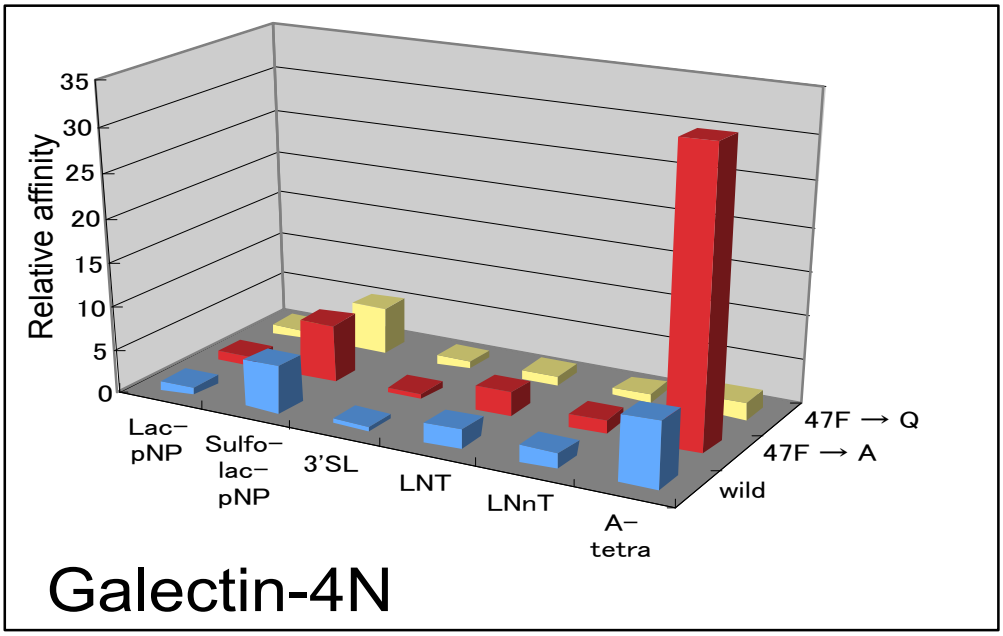


Fig. S3.


Relative affinity
35
30
25
20
15
10
5
0
Lac–pNP
Sulfo–lac–pNP
3'SL
LNT
LNnT
LNF–III
47Q → A
wild
Galectin-8N


Relative affinity
35
30
25
20
15
10
5
0
Lac–pNP
Sulfo–lac–pNP
3'SL
LNT
LNnT
A–tetra
47F → Q
47F → A
wild
Galectin-4N

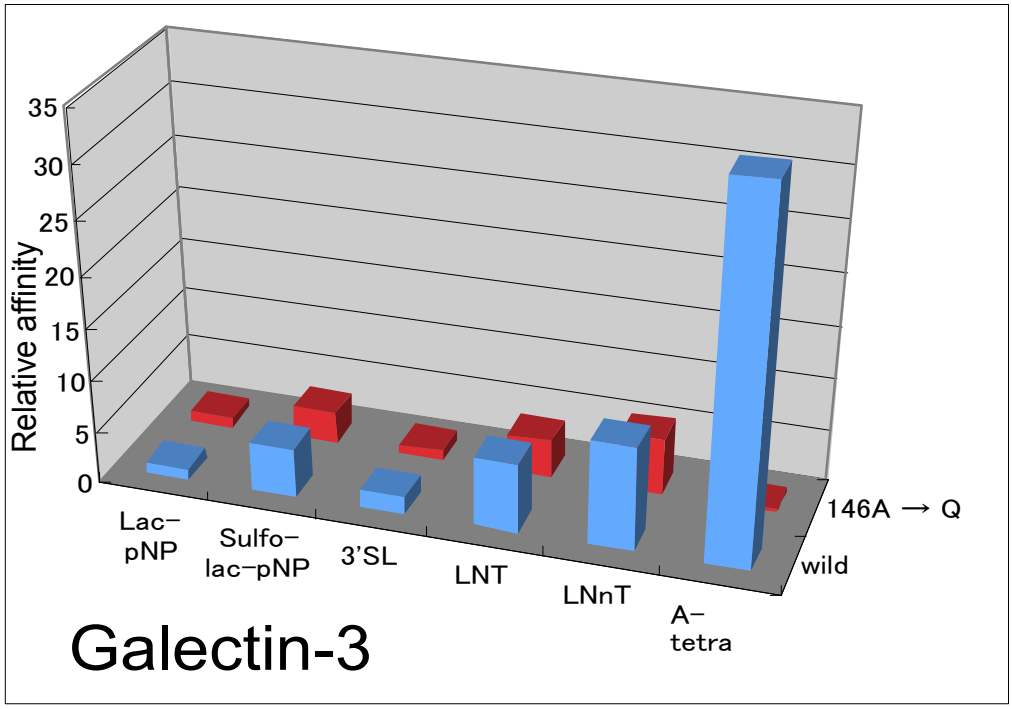

Relative affinity
35
30
25
20
15
10
5
0
Lac–pNP
Sulfo–lac–pNP
3'SL
LNT
LNnT
A–tetra
146A → Q
wild
Galectin-3

Fig.S4

Supplemental Figure Legends

Fig. S1. The backbone structures are shown in a ribbon model and amino acids involved in carbohydrate recognition are shown in a stick model. Galectin-8N is shown in blue-green and other galectins are shown in different colors (galectin-1: pink, galectin-2: yellow-green, galectin-3: blue, galectin-4: red, galectin-7: ocher). Amino acids that directly interact with lactose are shown in red, and amino acids that interact with lactose via water-mediated hydrogen bonds are shown in blue. (a) Comparison with galectin-1 [PDB-ID: 1GZW, (1), RMSD: 0.32 Å] (b) Comparison with galectin-2 [PDB-ID: 1HLC, (2), RMSD: 0.50 Å]. (c) Comparison with galectin-3 [PDB-ID: 1KJL, (3), RMSD: 0.29 Å] (d) Comparison with galectin-4 [PDB-ID: 1X50 (4), RMSD: 1.50 Å.] (e) Comparison with galectin-7 [PDB-ID: 4GAL, (5), RMSD: 0.42 Å].

Fig. S2. Comparison of the crystal structures of galectin-1, -2, -3, -4, -7, and -8N *A*, overall structure of galectin-8N. The loop region between S3 and S4 is indicated by the pink circle. The bound lactose is shown in yellow in the stick model. *B*, Comparison of the loop-region crystal structures. Superimposition of the backbone structures of galectin-8N (blue green), galectin-1 (pink), galectin-2 (yellow green), galectin-3 (light blue), galectin-4N (red), and galectin-7 (orange). Arg59 of galectin-8N is shown in the stick model. Amino acids in the loop region are shown in the line model.

Fig. S3. The omit electron-density maps with the bound (a) lactose, (b) 3′sulfoL, (c) 3′SL and (d) LNF-III. Contours are shown at 3.0σ level. Carbon, oxygen, and nitrogen atoms are shown in *green*, *red*, and *blue* respectively.

Fig. S4. Relative binding abilities of the wild and mutant galectin-8N, galectin-4N, and galectin-3 for various oligosaccharides. The relative binding abilities of the wild and mutant galectin-8N were calculated by dividing the K_D value between Lac-*O*-pNP and galectin-8N by the K_D values for the oligosaccharides. The relative binding abilities of galectin-4N for oligosaccharides were calculated in the same manner. The relative affinities of galectin-3 were calculated by dividing the concentration (M) of lac-pNP yielding 50% inhibition by the concentration (M) of the oligosaccharides yielding 50% inhibition.

Supplemental references

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