SENSING SWEET SPOTS: A comprehensive study of SARS-CoV-2-binding lectins

Neetu[1,2,#], Shimona Ahlawat[1,2,#], Rathina Delipan[1], Rajesh P. Ringe[1], Alka Rao[1,2,3*] and T.N.C. Ramya[1,2,*]

[1]CSIR- Institute of Microbial Technology, Sector 39-A, Chandigarh 160036, INDIA

[2]Academy of Scientific & Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh 201002, INDIA

[3] Present address: Food Safety and Standards Authority of India (FSSAI), New Delhi 110002, India

[#]These authors contributed equally to the work and should be considered joint first authors

**Corresponding authors' e-mail: ramya@imtech.res.in, raoalka@imtech.res.in*

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This supplementary data .pdf file contains four supplementary Figures, S1-S4.

A Supplementary Data 1 zipped folder of all the lectin models described in the paper, and a .xlsx Supplementary Data 2 file containing all the source data behind the graphs in the manuscript also accompany this manuscript as supplementary data.

Figure S1

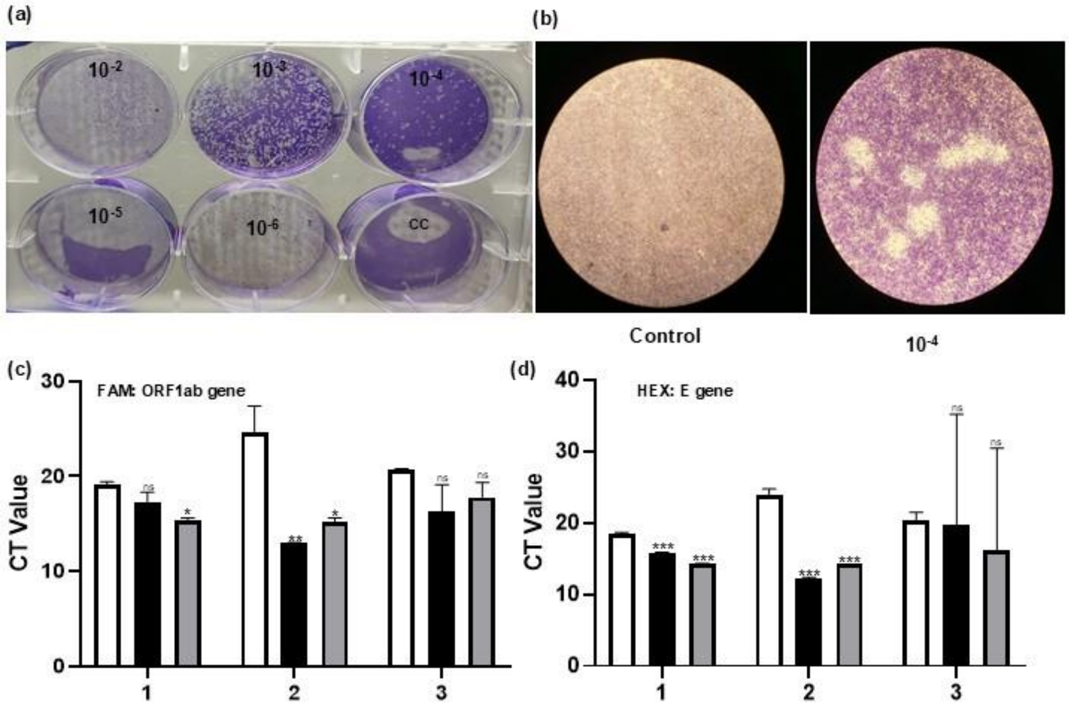


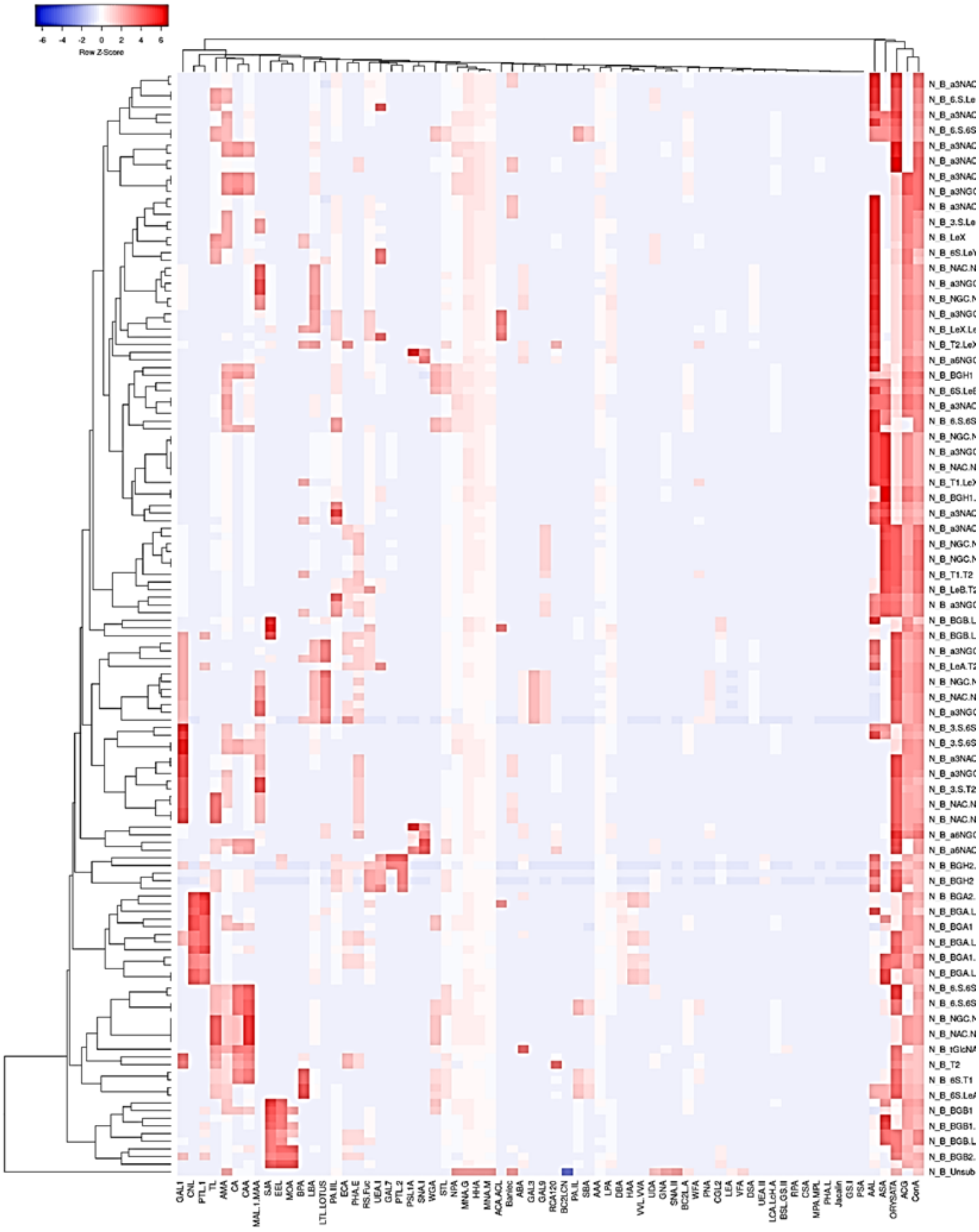
Figure S1: **Propagation and quantitation of enriched SARS-CoV-2. (a)** Quantification of SARS-CoV-2 virus titer propagated in Vero E6 cells. The plaque assay was performed with different dilutions of virus (ranging from 10^{-2} to 10^{-6}) along with uninfected Vero E6 control (CC) in a 6-well plate (72 plaques were observed in dilution 10^{-4}, hence the titer was calculated to be 1.8×10^{6} PFU/ml) **(b)** Plaques observed at 10X magnification (uninfected Vero E6 control and virus plated at 10^{-4} dilution) **(c,d)** RT-PCR to verify enrichment of virus in three biological replicates (referred to as 1, 2, and 3 on the x-axis). Un-enriched virus is indicated by white bars, PEG-enriched virus is indicated by black bars and Dynabead kit-enriched virus is indicated by grey bars. The RT-PCR Ct values were calculated for FAM representing the ORF1ab gene of SARS-CoV-2 **(c)** as well as for HEX representing the E gene of SARS Virus **(d)**, and the RT-PCR was performed with three technical replicates for each biological replicate. Error bars show standard deviation. “ns” indicates non-significant ($P > 0.05$), * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, and *** indicates $P \leq 0.003$.

Figure S2

(a)

Lectins	N-core	O-Core	N-Ext	O-Ext
AAA	No	Yes	No	Yes
AAL	Yes	No	Yes	Yes
ABA	Yes	No	Yes	Yes
ACA/ACL	No	Yes	Yes	Yes
ACG	No	Yes	Yes	Yes
AMA	Yes	No	Yes	No
ASA	Yes	No	Yes	No
Banlec	Yes	No	Yes	No
BC2L-A	Yes	No	Yes	No
BC2LCN	Yes	No	No	No
BPA	No	Yes	Yes	Yes
BSL/GS-II	Yes	No	No	No
CA	Yes	No	Yes	No
CAA	Yes	No	Yes	No
CGL2	No	No	Yes	No
CNL	No	Yes	Yes	Yes
ConA	Yes	No	Yes	No
CSA	No	Yes	No	No
DBA	No	Yes	Yes	Yes
DSA	Yes	No	No	No
ECA	No	No	Yes	No
EEL	No	No	Yes	No
GAL1	No	No	Yes	No
GAL3	No	No	Yes	No
GAL7	No	No	Yes	No
GAL9	No	No	Yes	Yes
GNA	Yes	No	No	No
GS-I	No	No	No	Yes
HAA	No	Yes	Yes	Yes
HHA	Yes	No	Yes	No
Jacalin	No	Yes	No	Yes
LBA	Yes	No	Yes	Yes
LCA	Yes	No	No	No
LEA	Yes	No	No	Yes
LPA	No	No	Yes	No
LTL	Yes	No	Yes	Yes
MAL-1/MAA	No	No	Yes	Yes
MNA-G	Yes	No	Yes	No
MNA-M	Yes	No	Yes	No
MOA	No	Yes	Yes	Yes
MPA/MPL	No	No	No	No
NPA	Yes	No	Yes	No
ORYSATA	Yes	No	Yes	No
PA-IIL	Yes	No	Yes	Yes
PA-IL	No	Yes	Yes	Yes
PHA-E	Yes	No	Yes	No
PHA-L	Yes	No	No	No
PNA	No	Yes	Yes	Yes
PSA	Yes	No	No	No
PSL1A	No	No	Yes	Yes
PTL-1	No	No	Yes	Yes
PTL-2	No	No	Yes	Yes
RCA120	No	No	Yes	Yes
RPA	Yes	No	No	No
RS-Fuc	No	No	Yes	Yes
SBA	No	Yes	Yes	Yes
SJA	No	No	Yes	Yes
SNA-I	No	No	Yes	Yes
SNA-II	Yes	No	No	Yes
STL	No	No	Yes	Yes
TL	Yes	No	Yes	No
UDA	Yes	No	Yes	No
UEA I	No	No	Yes	Yes
UEA-II	No	Yes	Yes	Yes
VFA	Yes	No	No	No
VVL/VVA	No	Yes	Yes	No
WFA	No	Yes	Yes	Yes
WGA	Yes	Yes	Yes	Yes

(b)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-4
-2
0
2
4
6
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N_B_6.S.Le
N_B_a3NAC
N_B_6.S.6S
N_B_a3NAC
N_B_a3NAC
N_B_a3NAC
N_B_a3NG
N_B_a3NAC
N_B_3.S.Le
N_B_LeX
N_B_6S.Le
N_B_NAC.N
N_B_a3NG
N_B_NGC.N
N_B_a3NG
N_B_LeX.Le
N_B_T2.LeX
N_B_a6NG
N_B_BGH1
N_B_6S.Le
N_B_a3NAC
N_B_6.S.6S
N_B_NGC.N
N_B_a3NG
N_B_NAC.N
N_B_T1.LeX
N_B_BGH1.
N_B_a3NAC
N_B_a3NAC
N_B_NGC.N
N_B_NGC.N
N_B_T1.T2
N_B_LeB.T2
N_B_a3NG
N_B_BGB.L
N_B_BGB.L
N_B_a3NG
N_B_LeA.T2
N_B_NGC.N
N_B_NAC.N
N_B_a3NG
N_B_3.S.6S
N_B_3.S.6S
N_B_a3NAC
N_B_a3NG
N_B_3.S.T2
N_B_NAC.N
N_B_NAC.N
N_B_a6NG
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N_B_BGH2.
N_B_BGH2
N_B_BGA2.
N_B_BGA.L
N_B_BGA1
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N_B_BGA1.
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N_B_6.S.6S
N_B_NGC.N
N_B_NAC.N
N_B_tGlcNA
N_B_T2
N_B_6S.T1
N_B_6S.LeA
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N_B_BGB1.
N_B_BGB.L
N_B_BGB2.
N_B_Unsub
GAL1
CNL
PTL1
TL
AMA
CA
CAA
MAL.1.MAA
SJA
EEL
MOA
BPA
LBA
LTL.LOTUS
PA.IIL
ECA
PHA.E
RS.Fuc
UEA.I
GAL7
PTL2
PSL1A
SNA.I
WGA
STL
NPA
MNA.G
HHA
MNA.M
ACA.ACL
Banlec
ABA
GAL3
GAL9
RCA120
BC2LCN
PA.IL
SBA
AAA
LPA
DBA
HAA
VVL.VVA
UDA
GNA
SNA.II
BC2LA
WFA
PNA
CGL2
LEA
VFA
DSA
UEA.II
LCA.LcH.A
BSL.GS.II
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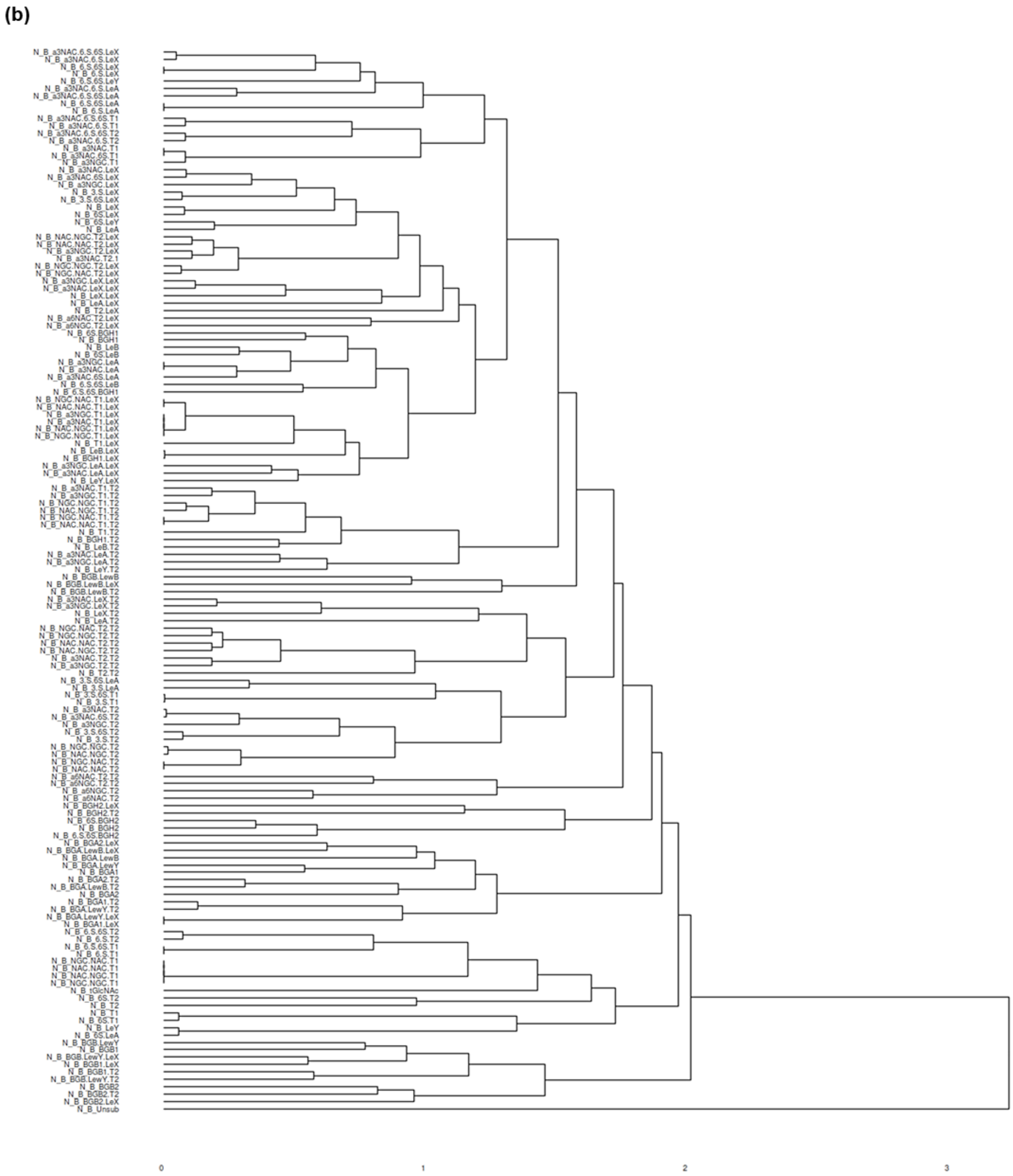
Jacalin
GS.I
PSA
AAL
ASA
ORYSATA
ACG
ConA


(b)
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N_B_6.S.6S.LeY
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N_B_LeX
N_B_6S.LeX
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N_B_LeX.LeX
N_B_LeA.LeX
N_B_T2.LeX
N_B_a6NAC.T2.LeX
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N_B_6S.BGH1
N_B_BGH1
N_B_LeB
N_B_6S.LeB
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N_B_6.S.6S.LeB
N_B_6.S.6S.BGH1
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N_B_T1.LeX
N_B_LeB.LeX
N_B_BGH1.LeX
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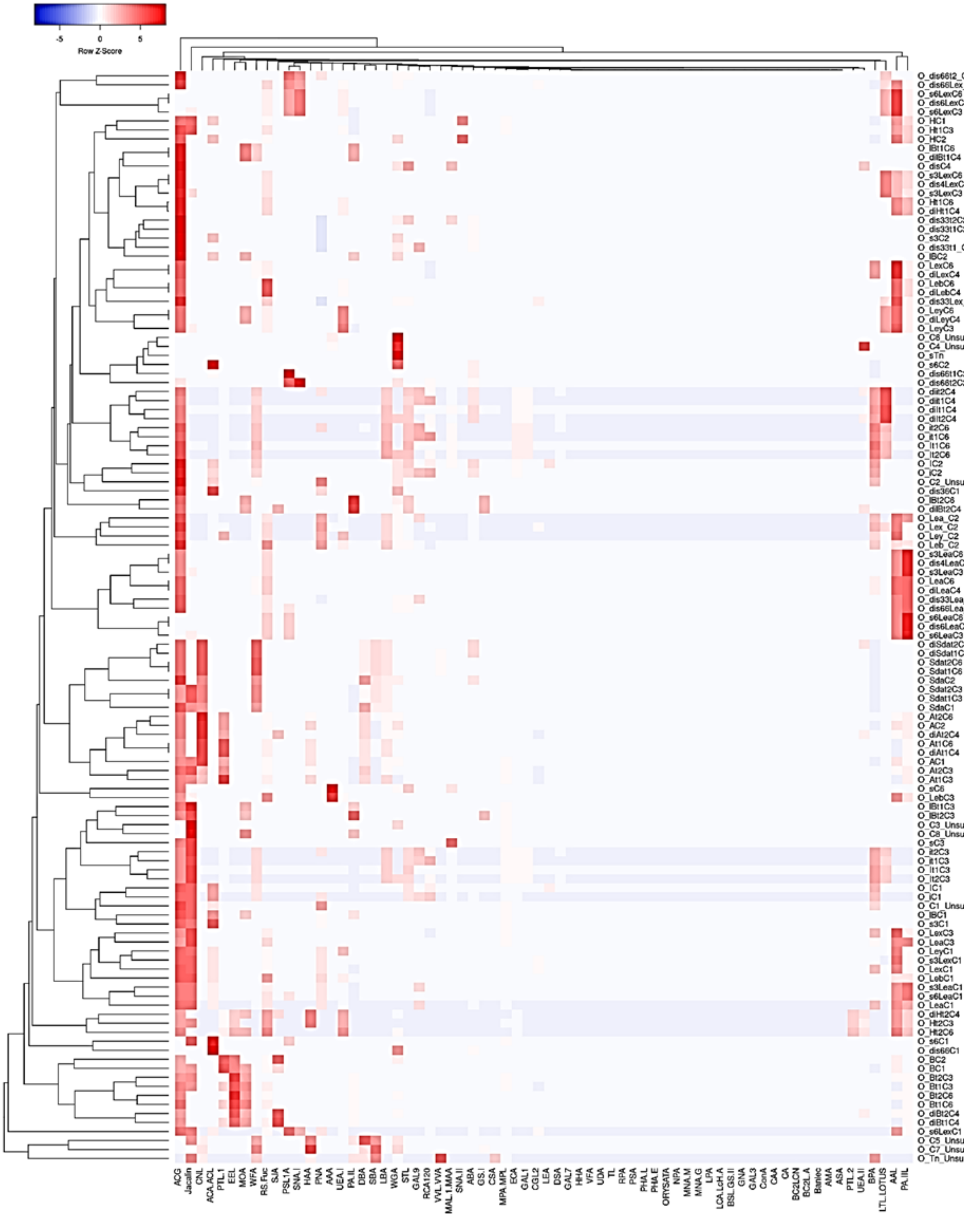
(c)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O_Ht1C3
O_HC2
O_lBt1C6
O_dilBt1C4
O_disC4
O_s3LexC6
O_dis4LexC
O_s3LexC3
O_Ht1C6
O_diHt1C4
O_dis33t2C
O_dis33t1C
O_s3C2
O_dis33t1_
O_lBC2
O_LexC6
O_diLexC4
O_LebC6
O_diLebC4
O_dis33Lex
O_LeyC6
O_diLeyC4
O_LeyC3
O_C6_Unsu
O_C4_Unsu
O_sTn
O_s6C2
O_dis66t1C
O_dis66t2C
O_diit2C4
O_diit1C4
O_dilt1C4
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O_diAt1C4
O_AC1
O_At2C3
O_At1C3
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O_sC3
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O_LebC1
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O_Ht2C6
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O_Tn_Unsu
ACG
Jacalin
CNL
ACA.ACL
PTL.1
EEL
MOA
WFA
RS.Fuc
SJA
PSL1A
SNA.I
HAA
PNA
AAA
UEA.I
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DBA
SBA
LBA
WGA
STL
GAL9
RCA120
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MAL.1.MAA
SNA.II
ABA
GS.I
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MPA.MPL
ECA
GAL1
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VFA
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TL
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ORYSATA
NPA
MNA.M
MNA.G
LPA
LCA.LcH.A
BSL.GS.II
GNA
GAL3
ConA
CAA
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BC2LCN
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AAL
PA.IIL

(c)

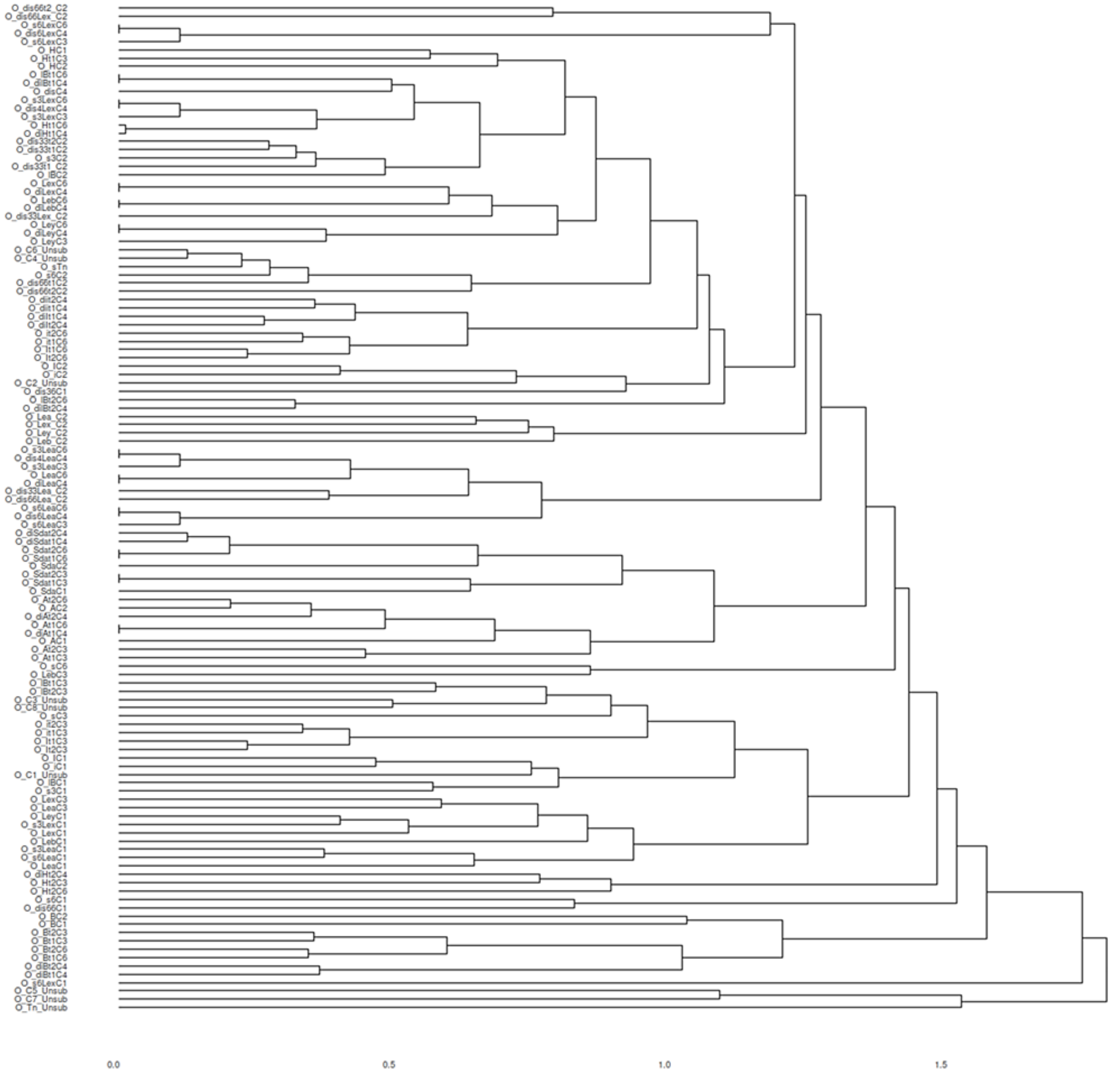
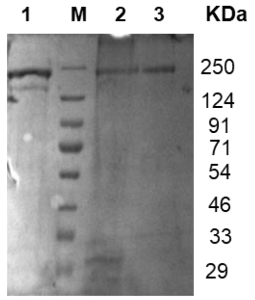


Figure S2: Predicted binding of lectins to generated glycans: (a) Table showing a list of lectins predicted to bind to core N-glycans, N-glycan extension sequences, O-core glycans, and O-glycan extension sequences **(b)** A heat map and a row dendrogram showing the predicted binding propensities of 68 lectins (portrayed on the columns) to different N-extension sequences (portrayed on the rows). **(c)** A heat map and a row dendrogram showing the predicted binding propensities of 68 lectins (portrayed on the columns) to different O-extension sequences (portrayed on the rows).

Figure S3

(a)



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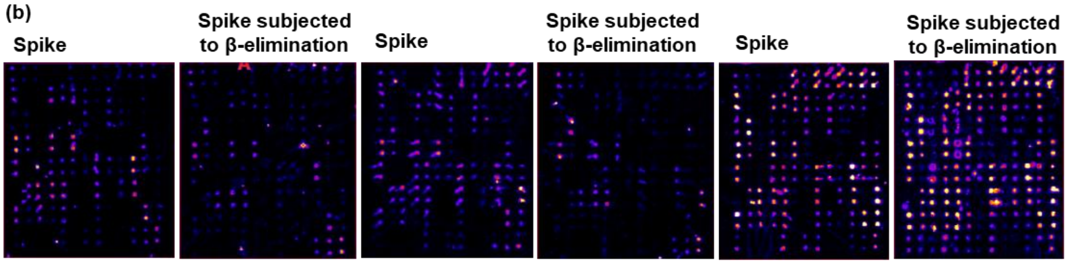


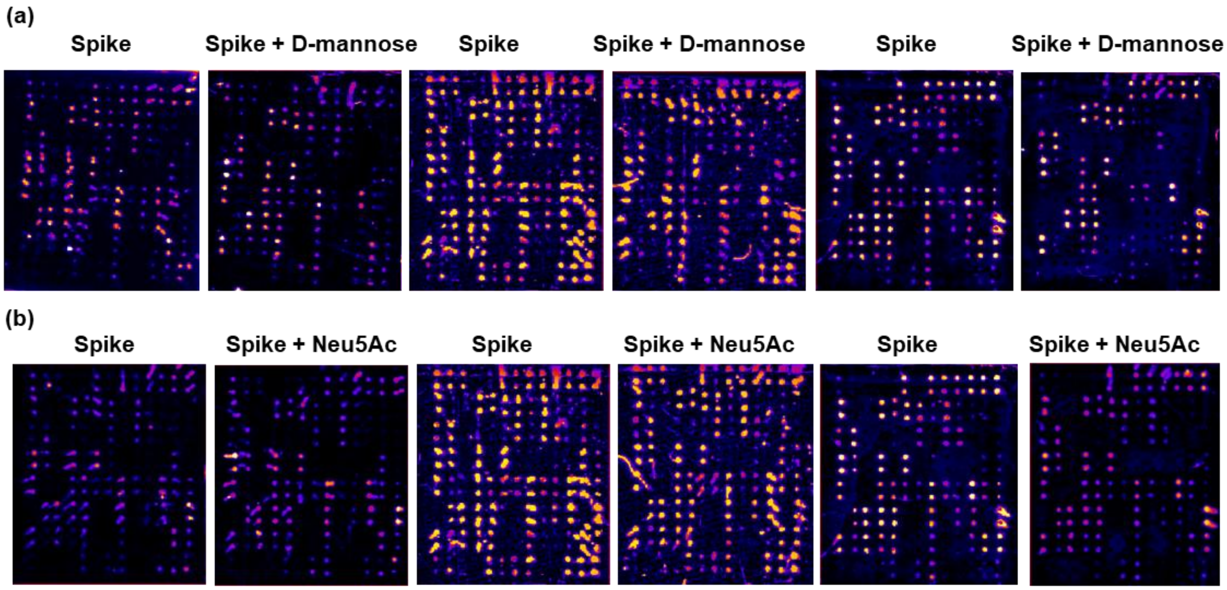
(c) **Differential binding of lectins upon β-elimination of Spike Glycoprotein**

Lectin	Run 1 Fold change (p-value)	Run 2 Fold change (p-value)	Run 3 Fold change (p-value)
ABL	3.024 (0.166)	5.479 (0.164)	1.884 (0.138)
ACL	2.629 (0.513)	6.098 (0.151)	1.148 (0.658)
LSL-N	16.558 (0.203)	6.084 (0.160)	1.538 (0.103)
RCA60	3.985 (0.078)	5.022 (0.102)	1.588 (0.360)
SBA	7.587 (0.005)	4.324 (0.223)	0.533 (0.095)

Lectin	Run 1 Fold change (p-value)	Run 2 Fold change (p-value)	Run 3 Fold change (p-value)
PA-IIL	6.100 (0.080)	5.888 (0.078)	0.922 (0.612)
PTL-2	7.793 (0.055)	2.942 (0.002)	1.701 (0.073)
ORYSATA	7.241 (0.039)	3.923(0.007)	1.115 (0.923)
LENTIL	5.671 (0.020)	3.603 (0.075)	1.643 (0.070)
ConA	5.324 (0.012)	3.624 (0.057)	1.170 (0.216)
MNA-M	6.220 (0.048)	2.059 (0.014)	0.887 (0.502)
CGL2	3.044 (0.076)	4.401 (0.057)	1.065 (0.239)
PSA	3.823 (0.031)	3.987 (0.092)	0.683 (0.320)
MNA-G	3.378 (0.067)	2.606 (0.019)	0.524 (0.061)

Figure S3: Binding of lectins to Spike glycoprotein subjected to β-elimination. (a) SDS-PAGE of Spike glycoprotein (~140 KDa) expressed and purified from HEK293F cell (lane 1) and purified Spike glycoprotein after β-elimination to remove O-glycans (lanes 2 & 3). **b)** Pseudo-coloured images showing the binding of Spike glycoprotein with and without β-elimination (10 µg). The images were generated using the Protein Array Analyzer macro plugin in Image J. **(c)** Fold change and p-values observed for O-glycan binding lectins (left table) and other lectins (right table) in a paired t-test (n=3).

Figure S4



(c) Differential binding upon D-mannose competition

Lectins	Run 1 Fold Change (p-value)	Run 2 Fold Change (p-value)	Run 3 Fold Change (p-value)
GS-II	1.589 (0.018)	3.559 (0.008)	1.930 (0.106)
PSA	2.402 (0.031)	2.351 (0.050)	642.881 (0.042)

(d) Differential binding upon Neu5Ac competition

Lectins	Run 1 Fold Change (p-value)	Run 2 Fold Change (p-value)	Run 3 Fold Change (p-value)
CSA	2.024 (0.006)	5.539 (0.002)	3.388 (0.071)

Figure S4: Binding of lectins to Spike glycoprotein with D-mannose and Neu5Ac competition. (a) Pseudo-coloured images showing the binding of Spike glycoprotein (10 µg) in the presence or absence of 500 mM mannose. **(b)** Pseudo-coloured images showing the binding of Spike glycoprotein (10 µg) in the presence or absence of 100 mM sialic acid. The images were generated using the Protein Array Analyzer macro plugin in Image J. **(c)** Fold change and p-values observed upon D-mannose competition in a paired t-test (n=3). **(d)** Fold change and p-values observed upon Neu5Ac competition in a paired t-test (n=3).