

# Supporting Information

## *Pre-Vaccination Glycan Markers of Response to Influenza Vaccine Implicate the Complement Pathway*

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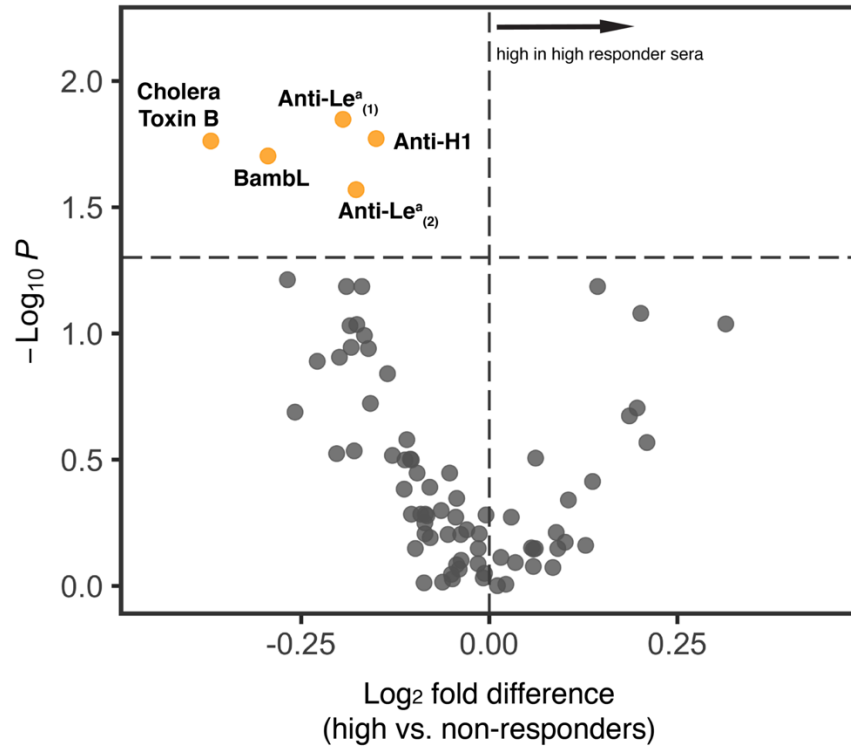
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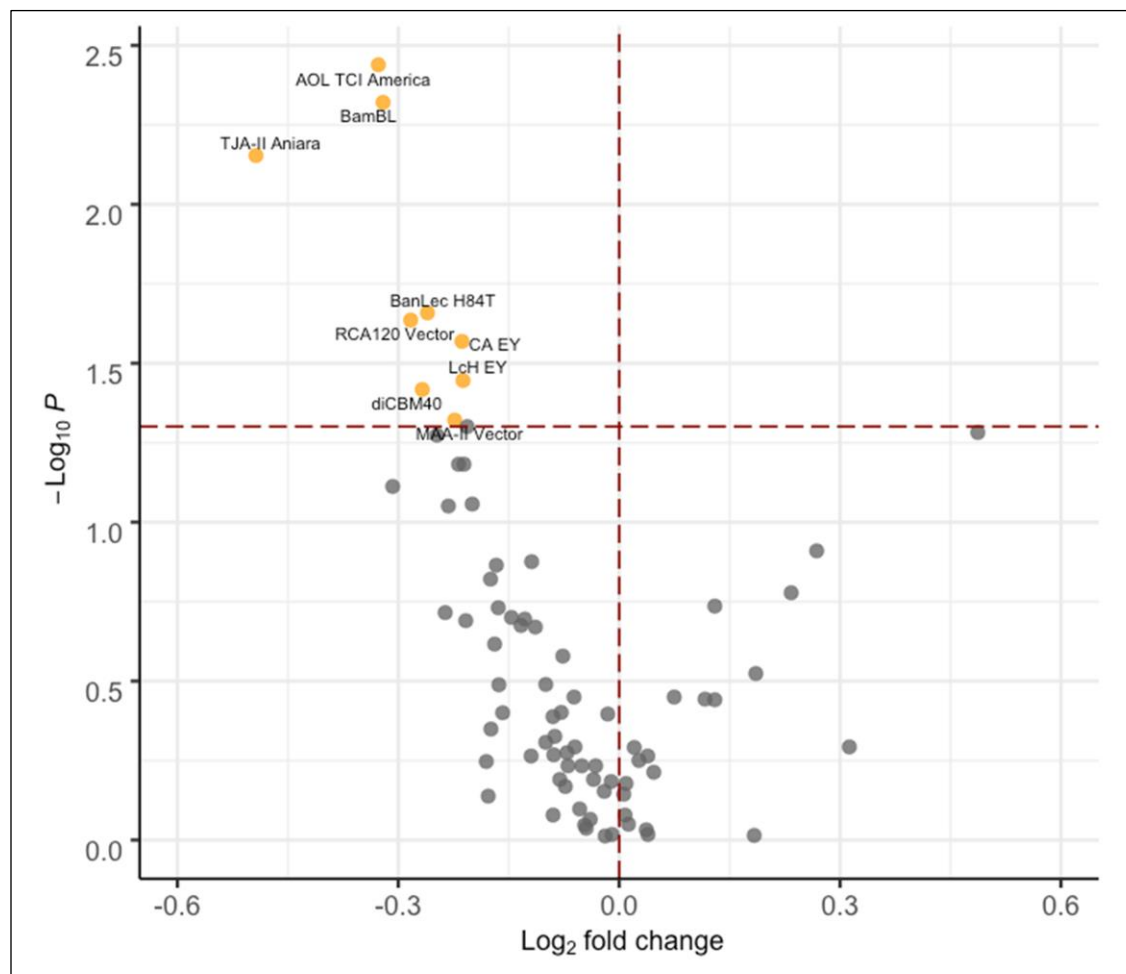
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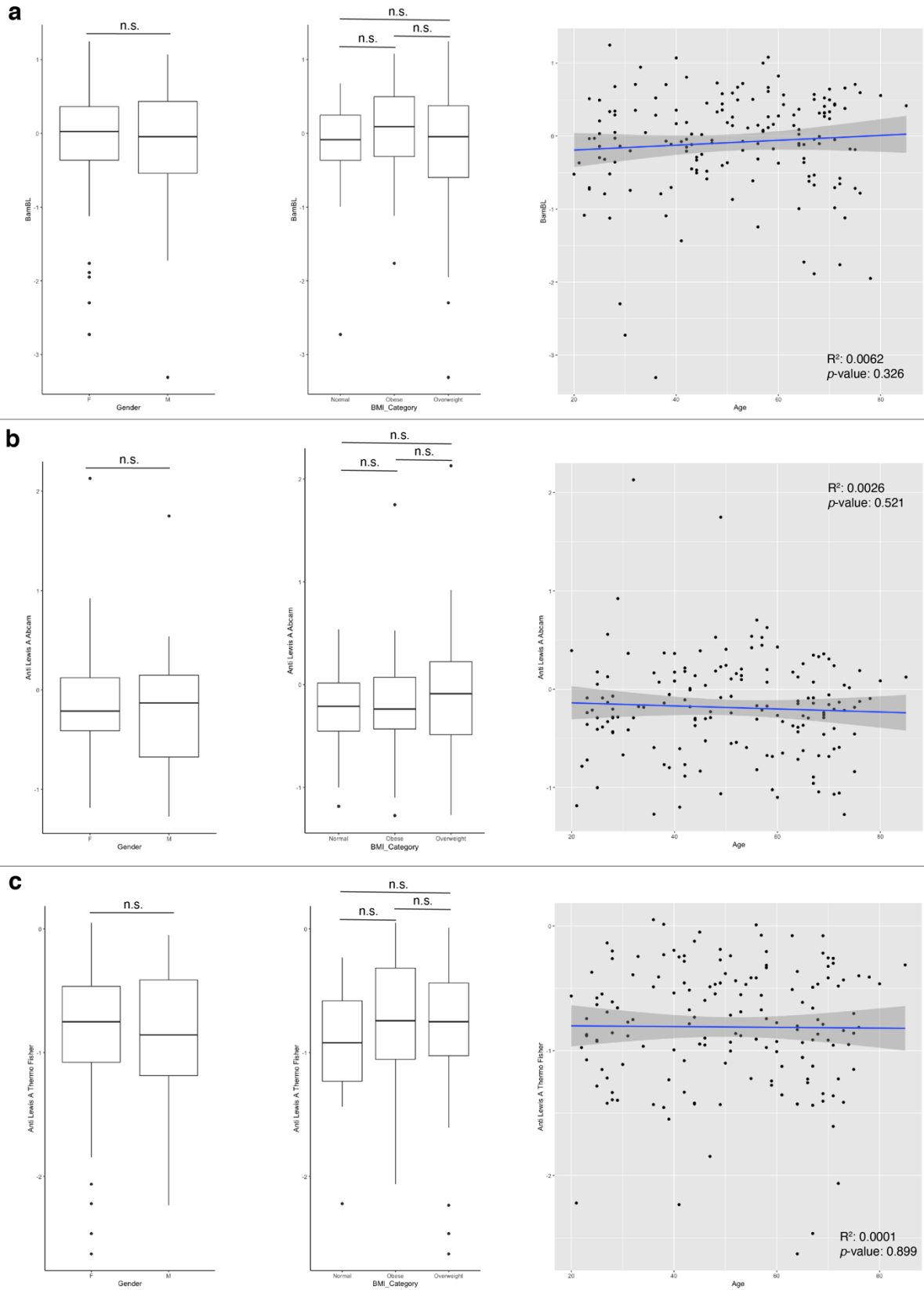
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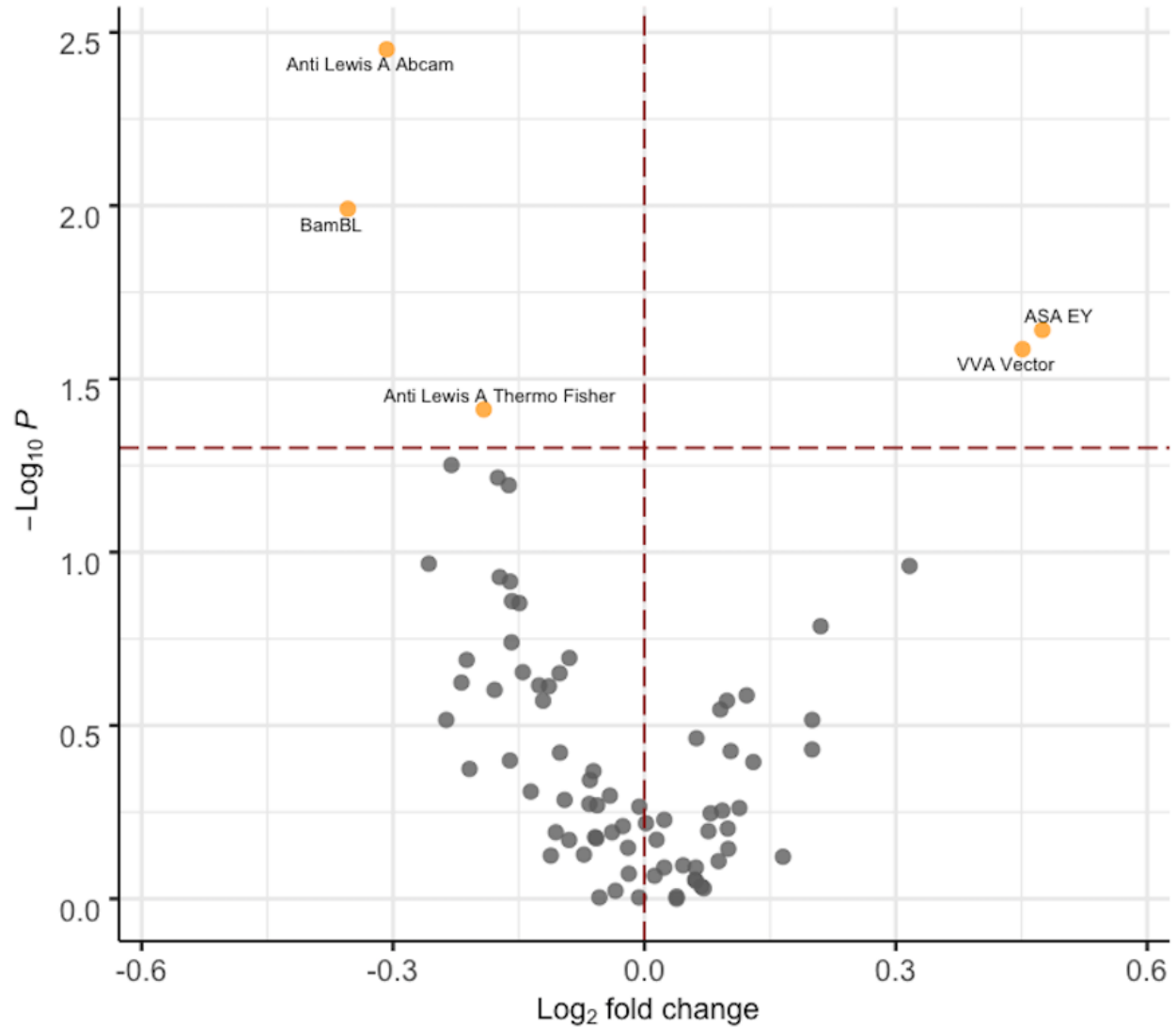


**Figure S2.** Volcano plot comparing lectin microarray data for high responders (N = 66) and low/moderate-responders (N = 39) pre-vaccination. A positive fold change denotes higher binding of the probe in high responders. Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in yellow.



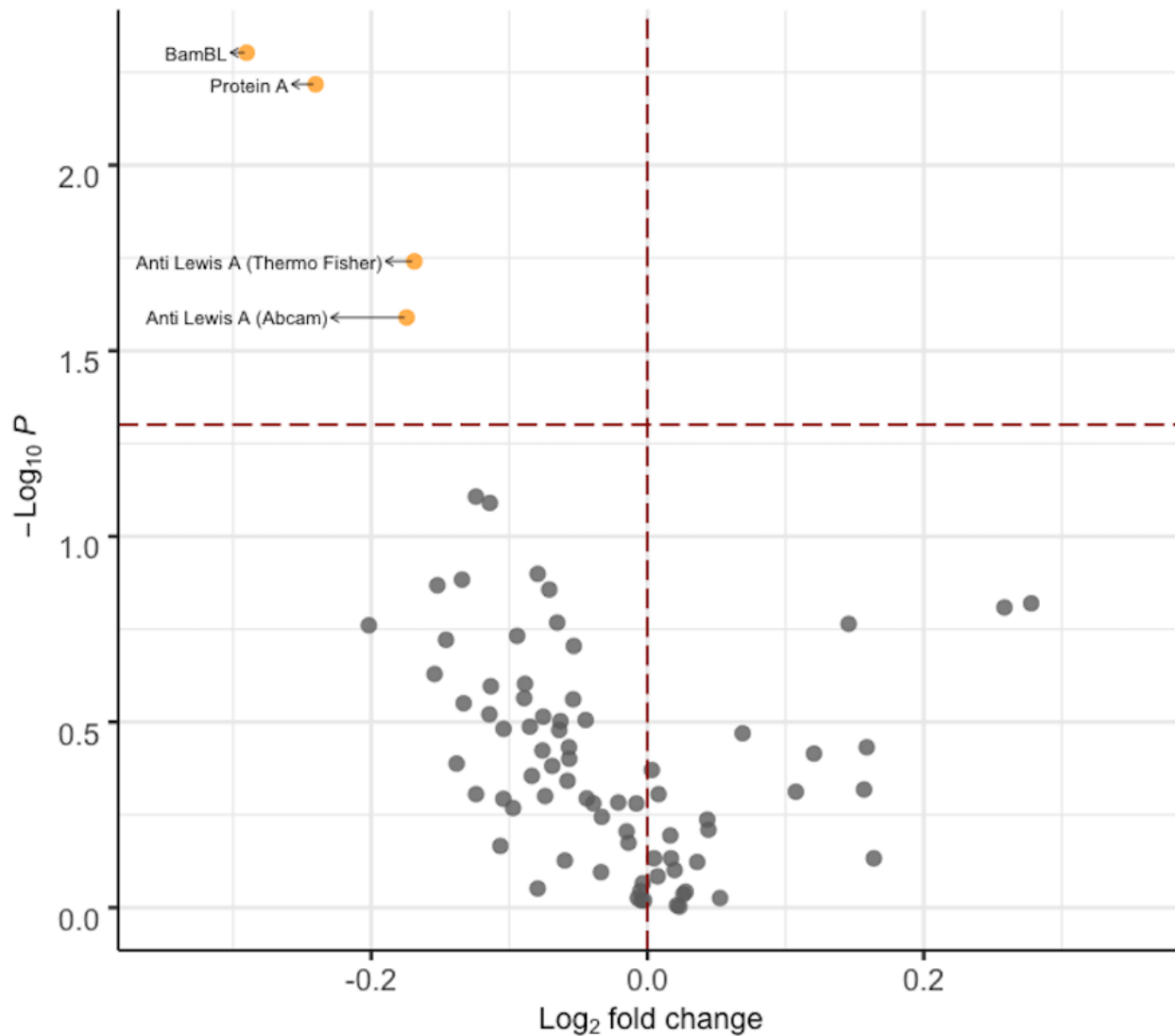
(see next page for Figure caption)

**Figure S3.** Association of multiple factors with microarray data of (a) BambL, (b) anti-Le<sup>a</sup> (Abcam), and (c) anti-Le<sup>a</sup> (Thermo Fisher). For association analysis of gender (left) and body-mass index (BMI, middle), boxplots comparing data between different groups are shown. “n.s.” indicates a p-value > 0.05 derived from Mann-Whitney U test. “Normal”, “Overweight” and “Obese” correspond to  $18 \leq \text{BMI} < 25$ ,  $25 \leq \text{BMI} < 30$  and  $30 \leq \text{BMI}$ , respectively. For association analysis of age (right), linear fitting of data with age is shown, including the fitting line (blue), 95% confidence interval (dark grey), R<sup>2</sup> value and p-value of the linear fit.

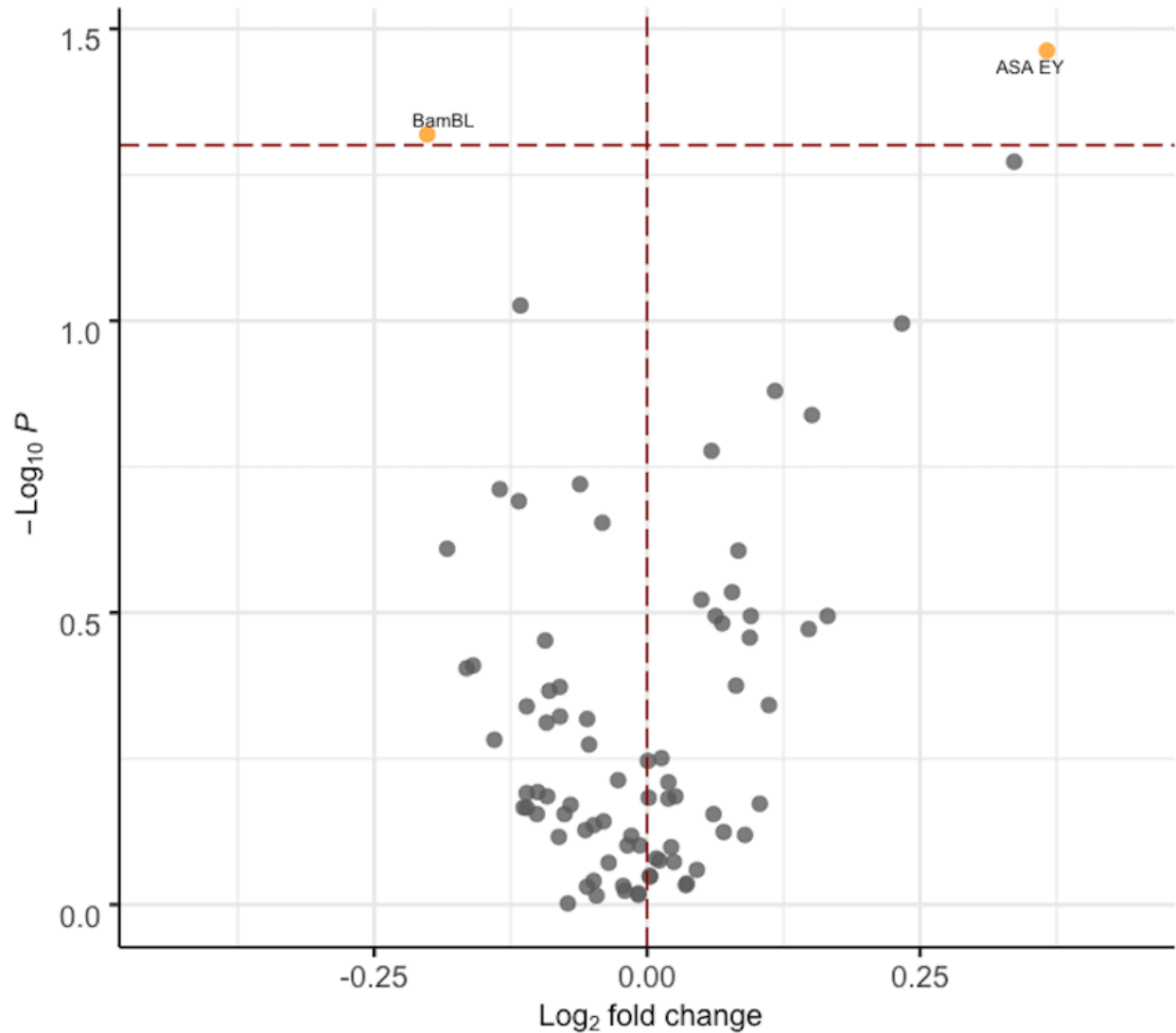


**Figure S4.** Volcano plot comparing lectin microarray data for re-defined high responders (N = 48) and non-responders (N = 48) pre-vaccination. Re-definition of responder groups was based on the response score of Wu et al. (see main paper reference 24) which takes into account BMI, age and gender. Participants who scored among the top tertile were considered high-responders, and participants who scored among the bottom tertile were considered non-responders. A positive fold change denotes higher binding of the probe in high responders. Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in yellow.

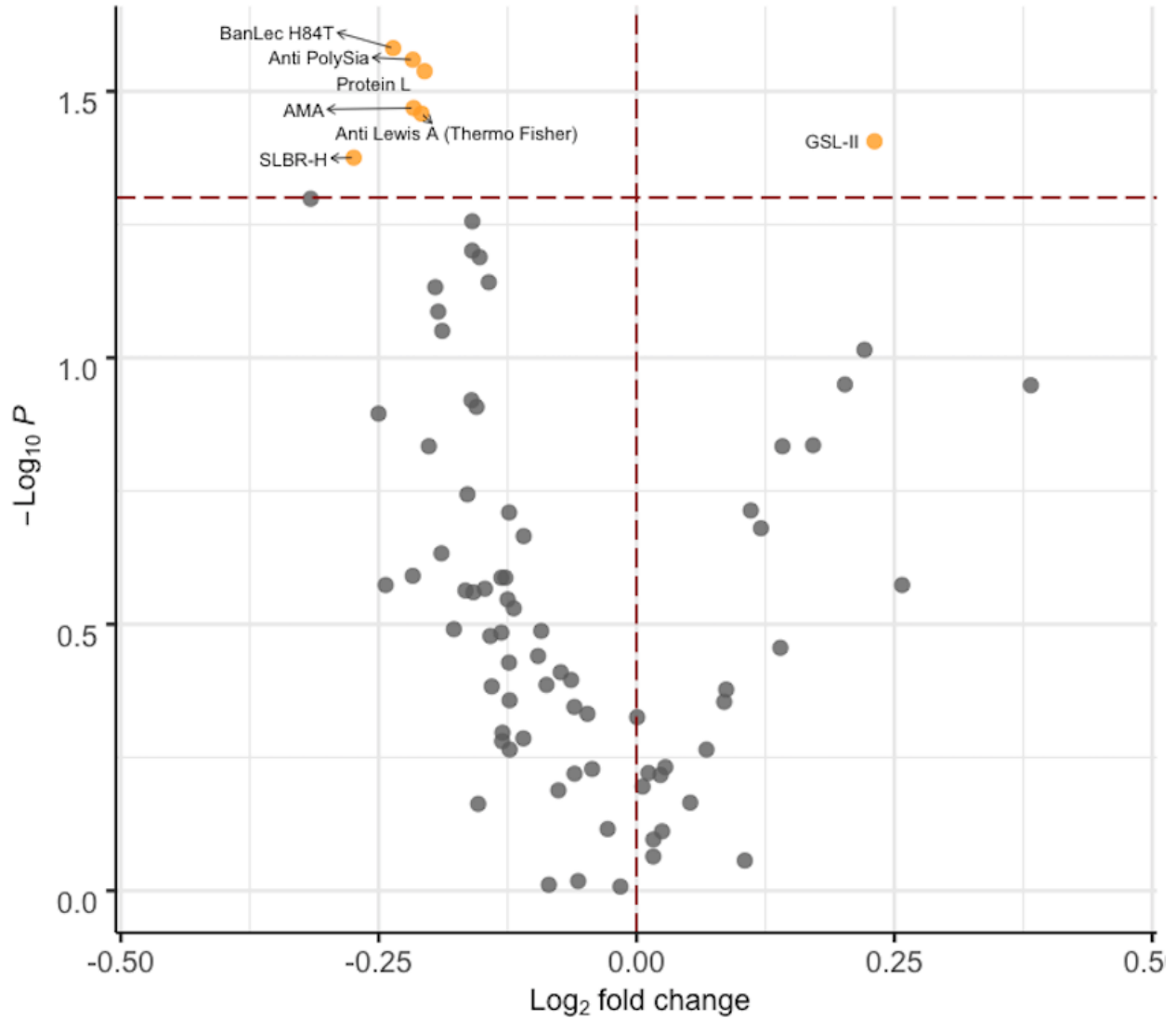




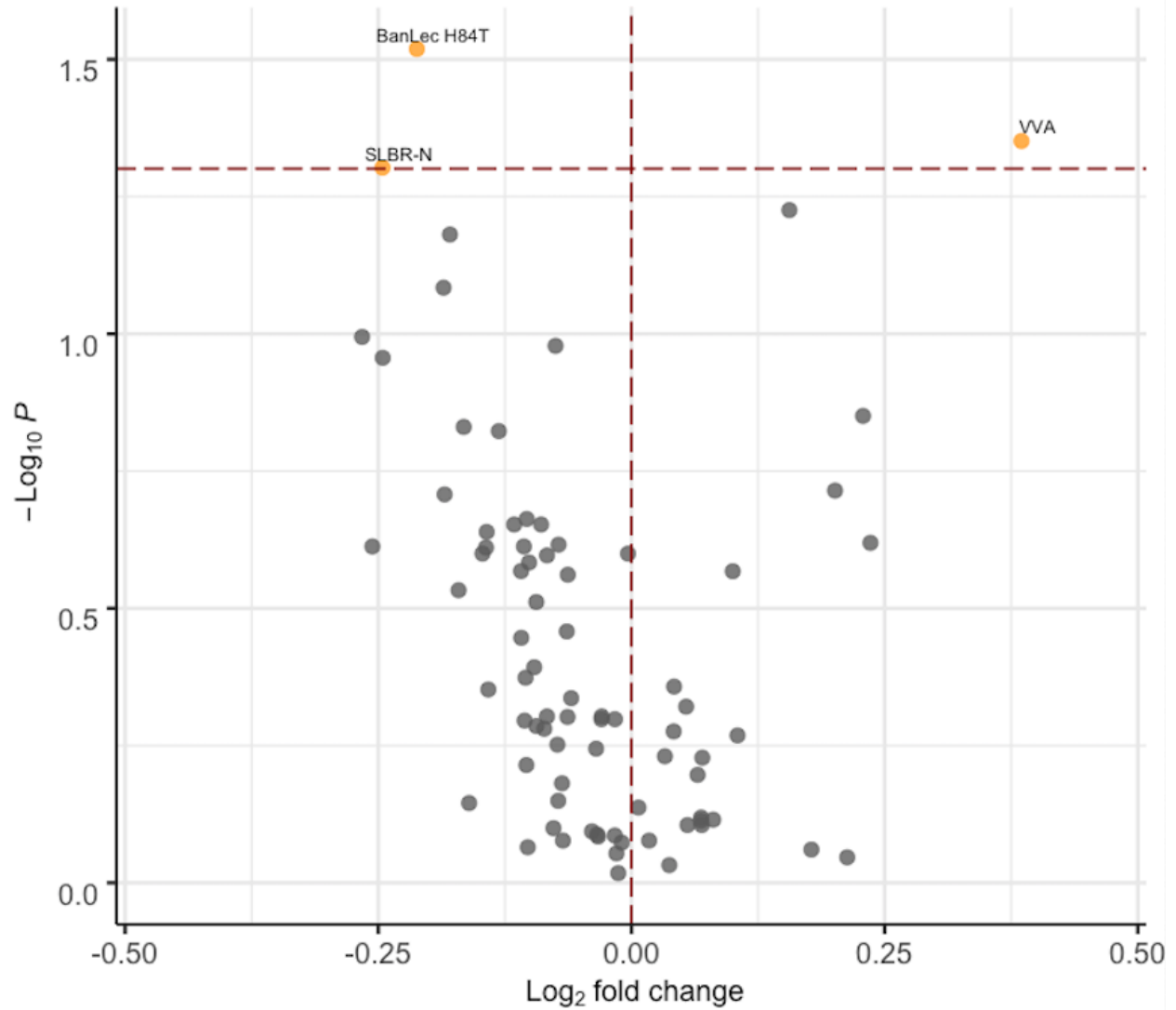
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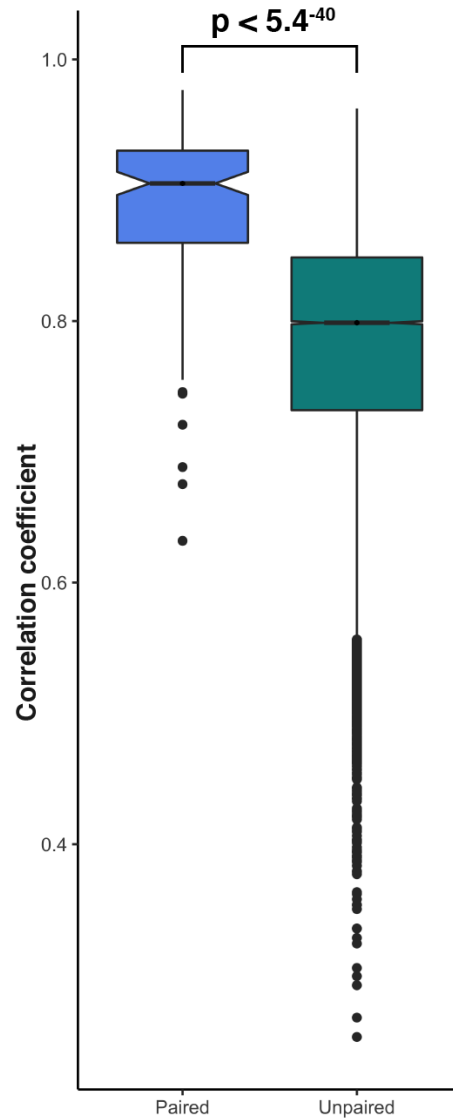
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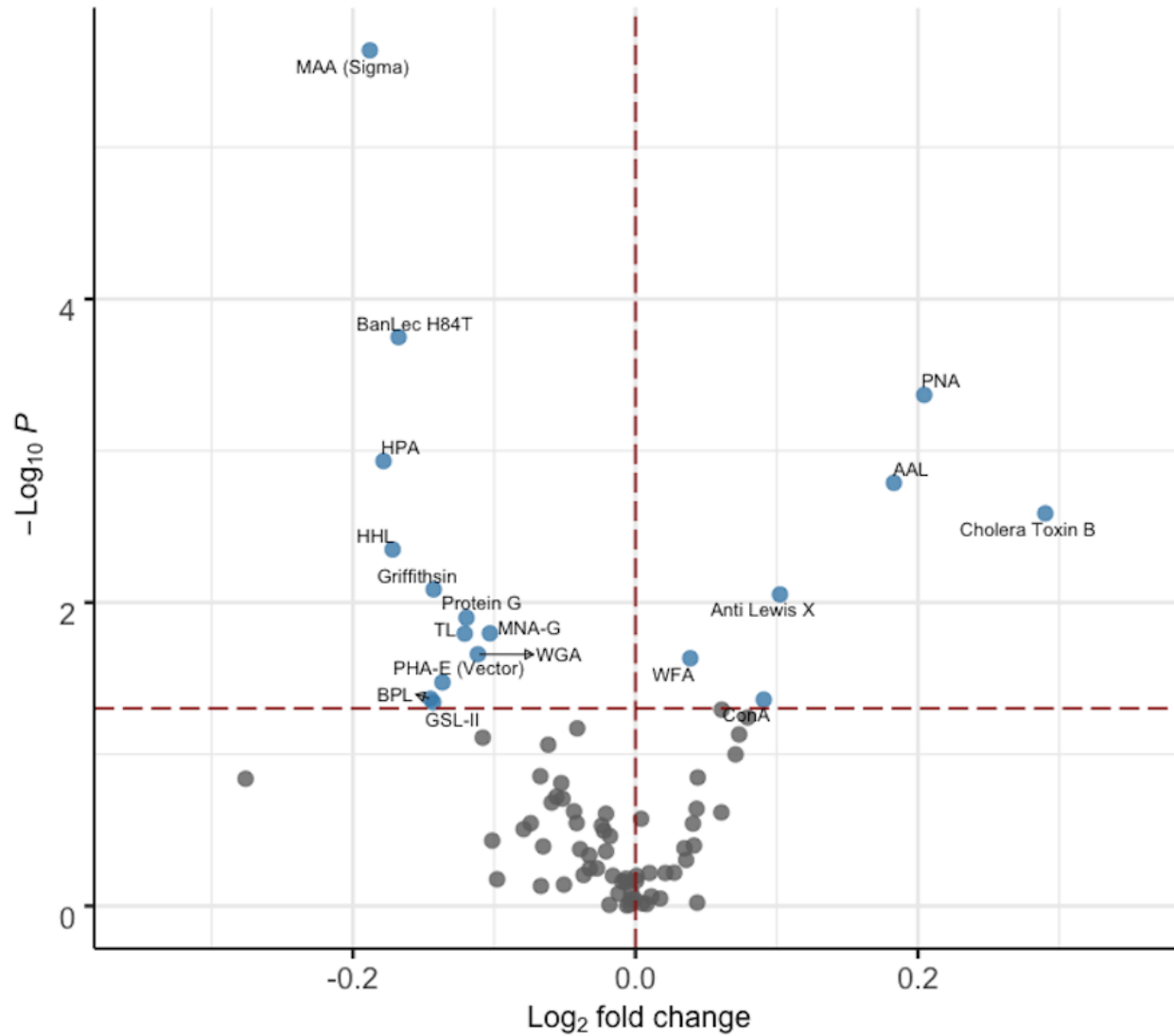
**Figure S7.** Volcano plot comparing lectin microarray data for high responders (N = 65) and non-responders (N = 62) pre-vaccination to the B/Yamagata (B/Phuket/3073/2013) strain. A positive fold change denotes higher binding of the probe in high responders. Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in yellow.



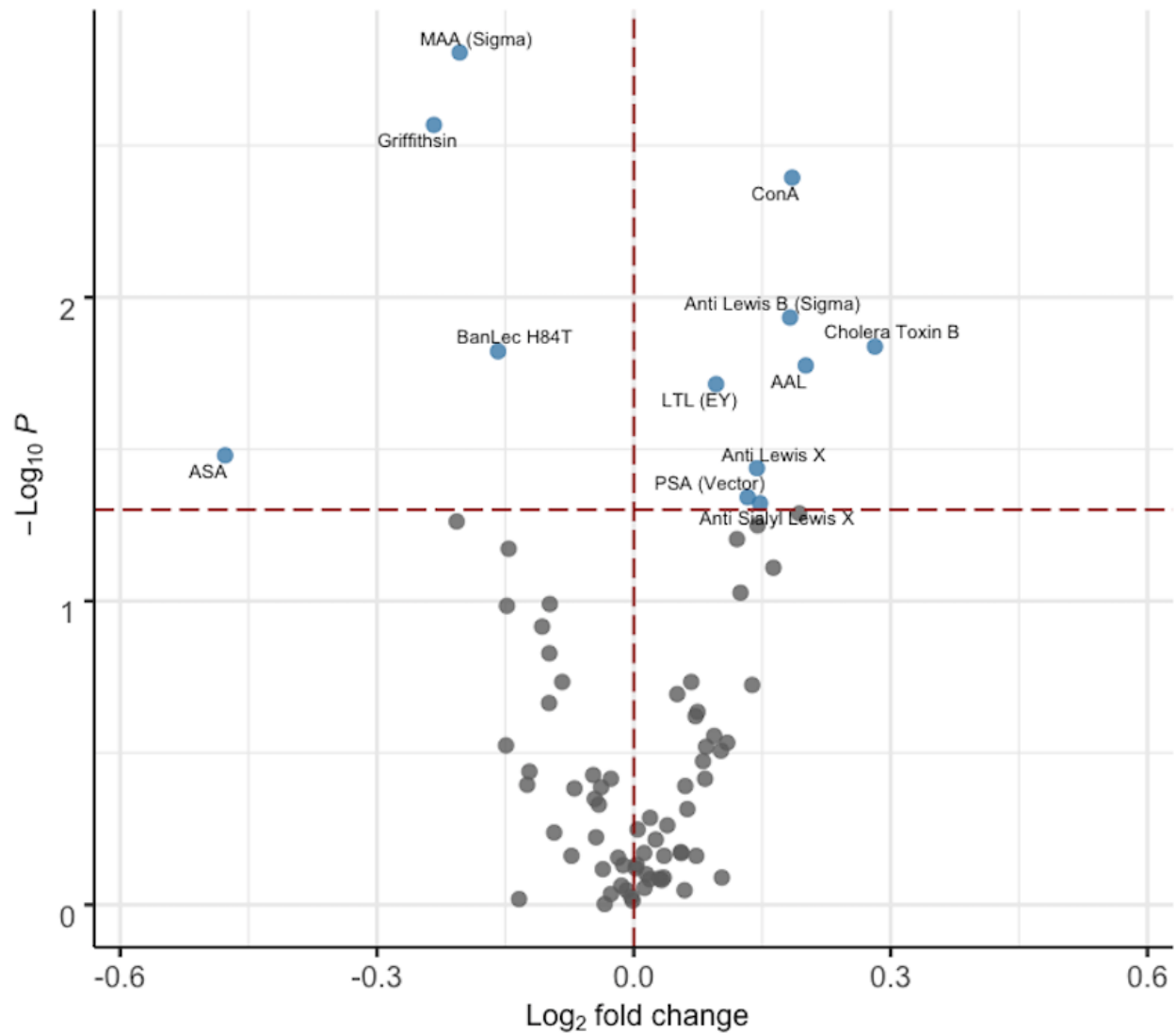
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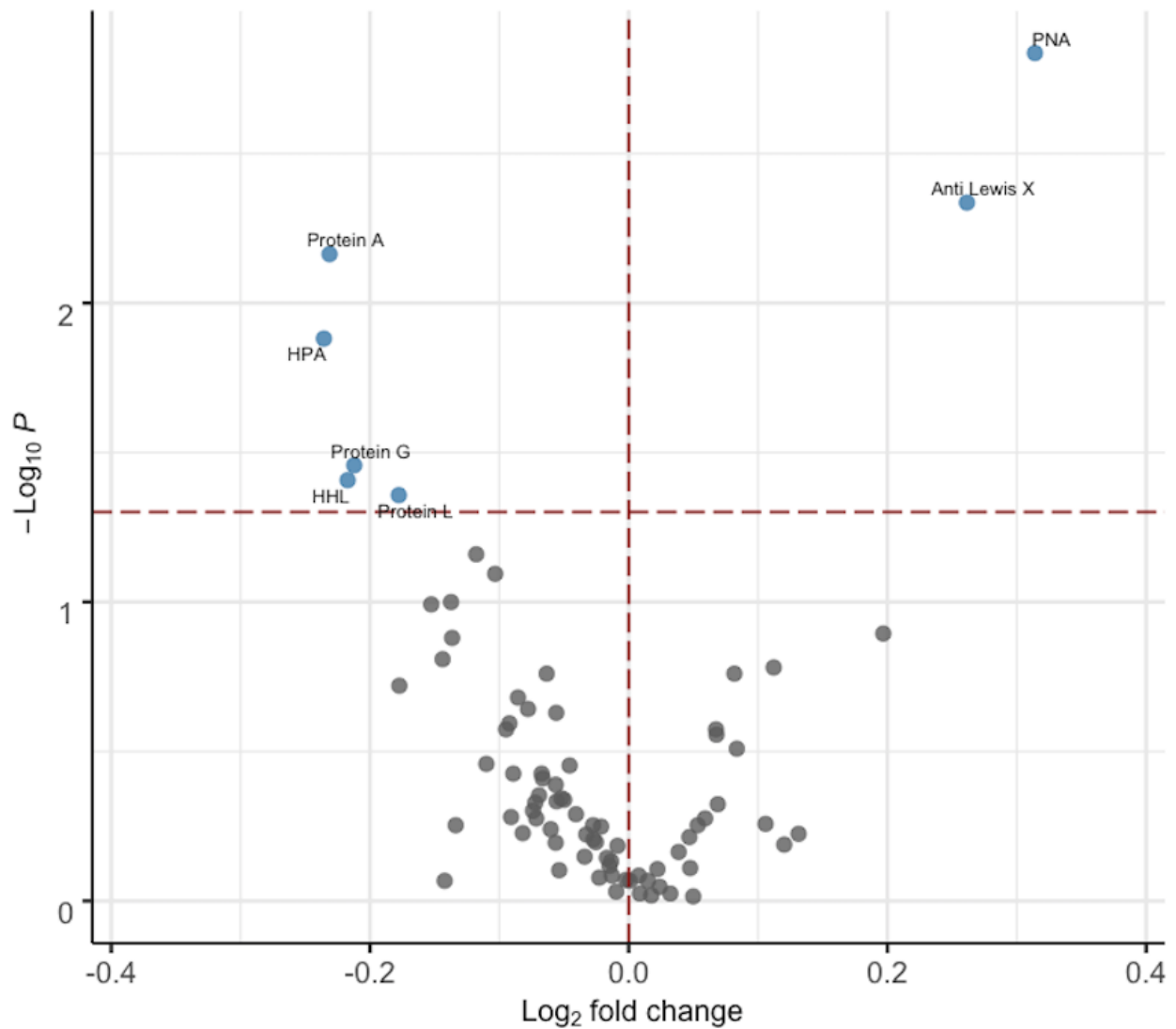
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**Figure S10.** Volcano plot comparing the paired pre- and post-vaccination lectin microarray data of all participants. A positive fold change denotes higher binding of the probe to post-vaccination sera (upregulation). Paired Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in blue.

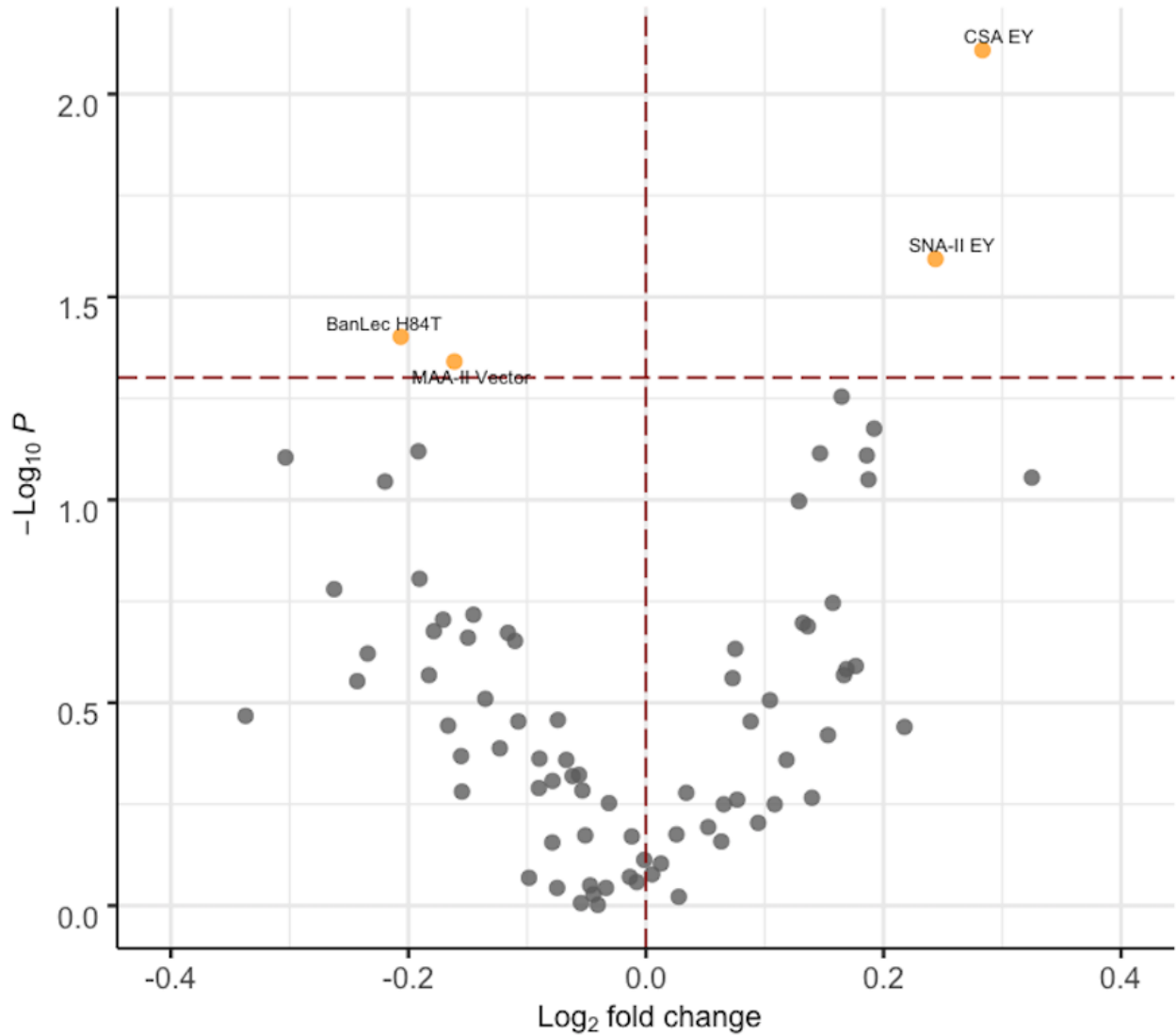


**Figure S11.** Volcano plot comparing the paired pre- and post-vaccination lectin microarray data of high responders. A positive fold change denotes higher binding of the probe to post-vaccination sera (upregulation). Paired Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in blue.



**Figure S12.** Volcano plot comparing the paired pre- and post-vaccination lectin microarray data of non-responders. A positive fold change denotes higher binding of the probe to post-vaccination sera (upregulation). Paired Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in blue.





**Figure S13.** Volcano plot comparing lectin microarray data for high responders (N = 66) and low/moderate-responders (N = 39) post-vaccination. A positive fold change denotes higher binding of the probe in high responders. Mann–Whitney U test was used to determine p-values. Probes with  $p < 0.05$  are colored in yellow.

**Table S1.** List of Probes Printed on Lectin Microarrays.

Probe	Species of origin	Source	Printing concentration (mg/ml)	Inhibiting Sugar*
ConA	<i>Concanavalin A</i>	Millipore Sigma	2	Man
GNA	<i>Galanthus nivalis</i>	Millipore Sigma	2	Man
AIA	<i>Artocarpus integrifolia</i>	GlycoMatrix/Bio-world	2	Gal
PHA-E	<i>Phaseolus vulgaris</i>	Vector Labs	2	GlcNAc
AIA	<i>Artocarpus integrifolia</i>	Vector Labs	2	Gal
LcH	<i>Lens culinaris</i>	Vector Labs	2	Man
SNA	<i>Sambucus nigra</i>	Vector Labs	2	Lac
WGA	<i>Triticum vulgare</i>	Vector Labs	2	GlcNAc
PNA	<i>Arachis hypogaea</i>	Vector Labs	2	Gal
AAL	<i>Aleuria aurantia</i>	Vector Labs	1.5	Fuc
PSA	<i>Pisum sativum</i>	Vector Labs	2	Man
PSA	<i>Pisum sativum</i>	GlycoMatrix/Bio-world	2	Man
UEA-I	<i>Ulex europaeus</i>	Vector Labs	2	Fuc
LEL	<i>Lycopersicon esculentum</i>	Vector Labs	2	GlcNAc
MPL	<i>Machura pomifera</i>	Vector Labs	2	Gal
BPL	<i>Bauhinia purpurea</i>	Vector Labs	2	Gal
MAA-I	<i>Maachia amurensis</i>	Vector Labs	2	Lac
STL	<i>Solanum tuberosum</i>	Vector Labs	2	GlcNAc
WFA	<i>Wisteria floribunda</i>	Vector Labs	2	Gal
DBA	<i>Dolichos Biflorus</i>	Vector Labs	2	Gal
SBA	<i>Glycine max</i>	Vector Labs	2	Gal
HHL	<i>Hippeastrum hybrid</i>	Vector Labs	2	Man
DSA	<i>Datura stramonium</i>	Vector Labs	2	Lac

ECA	<i>Erythrina cristagalli</i>	Vector Labs	2	GlcNAc
GSL-II	<i>Griffonia simplicifolia</i>	Vector Labs	2	GlcNAc
LTL	<i>Lotus tetragonolobus</i>	Vector Labs	2	Fuc
GNA	<i>Galanthus nivalis</i>	Vector Labs	2	Man
NPL	<i>Narcissus pseudonarcissus</i>	Vector Labs	2	Man
MAA-II	<i>Maachia amurensis</i>	Vector Labs	1	Lac
VVA	<i>Vicia villosa</i>	Vector Labs	2	Gal
UEA	<i>Ulex europaeus</i>	Millipore Sigma	2	Fuc
PHA-L	<i>Phaseolus vulgaris</i>	Millipore Sigma	2	GlcNAc
MAA	<i>Maachia amurensis</i>	Millipore Sigma	2	Lac
SNA	<i>Sambucus nigra</i>	Millipore Sigma	2	Lac
MNA-M	<i>Morniga M</i>	EY Labs	2	Man
SNA-I	<i>Sambucus nigra</i>	EY Labs	2	Lac
SNA-II	<i>Sambucus nigra</i>	EY Labs	2	Gal
PHA-E	<i>Phaseolus vulgaris</i>	EY Labs	2	GlcNAc
CA	<i>Colchicum autumnale</i>	EY Labs	2	GlcNAc
MNA-G	<i>Morniga G</i>	EY Labs	2	Gal
UEA-II	<i>Ulex europaeus</i>	EY Labs	2	GlcNAc
CSA	<i>Pure cytisus</i>	EY Labs	2	Gal
UDA	<i>Urtica dioica</i>	EY Labs	2	GlcNAc
LcH	<i>Lens culinaris</i>	EY Labs	2	Man
TL	<i>Tulipa sp.</i>	EY Labs	2	GlcNAc
ACA	<i>Amaranthus caudatus</i>	EY Labs	2	GlcNAc
Blackbean	<i>Phaseolus vulgaris</i>	EY Labs	2	Lac
PHA-L	<i>Phaseolus vulgaris</i>	EY Labs	2	GlcNAc
UEA-I	<i>Ulex europaeus</i>	EY Labs	2	Fuc
LTL	<i>Lotus tetragonolobus</i>	EY Labs	2	Fuc

MAA	<i>Maachia amurensis</i>	EY Labs	2	Lac
PTA GalNAc	<i>Psophocarpus tetragonolobus</i>	EY Labs	2	Gal
AMA	<i>Arum maculatum</i>	EY Labs	2	Man
PTA Gal	<i>Psophocarpus tetragonolobus</i>	EY Labs	2	Gal
ASA	<i>Allium sativum</i>	EY Labs	2	Man
HPA	<i>Helix pomatia</i>	Millipore Sigma	2	Gal
TJA-II	<i>Trichosanthes japonica</i>	Aniara	2	Lac
diCBM40	<i>Clostridium perfringens</i>	expressed in-house	1.5	Lac
AOL	<i>Aspergillus oryzae</i>	TCI America	2	Fuc
Griffithsin	<i>Griffithsia</i>	expressed in-house	1.7	Man
SLBR-N	<i>Streptococcus gordonii</i>	expressed in-house	2	Lac
SLBR-H	<i>Streptococcus gordonii</i>	expressed in-house	2	Lac
SLBR-B	<i>Streptococcus gordonii</i>	expressed in-house	2.3	Lac
BamBL	<i>Burkholderia cepacia</i>	expressed in-house	1.5	Fuc
BanLec H84T	<i>Musa paradisiaca</i>	expressed in-house	1	Man
Protein A		Thermo Fisher	0.5	/
Protein G		Thermo Fisher	1	/
Protein L		Thermo Fisher	0.5	/
Anti Lewis A		Abcam	as received	/
Anti Lewis X		Millipore Sigma	as received	/
Anti Lewis Y		Abcam	as received	/
Anti Lewis B		Millipore Sigma	as received	/
Anti Sialyl Lewis X		GeneTex	as received	/
Anti H1		Thermo Fisher / Invitrogen	as received	/
Anti H2		Santa Cruz Biotechnology	as received	/
Anti PolySia		Absolute Antibody	as received	/

Anti Lewis A		Thermo Fisher / Invitrogen	as received	/
Anti Lewis B		Abcam	as received	/
Anti B.G.A		Abcam	as received	/
Anti B.G.A		Thermo Fisher / Invitrogen	as received	/
Anti B.G.B		Abcam	as received	/
Anti B.G.B		Thermo Fisher / Invitrogen	as received	/
Cholera Toxin B	<i>Vibrio cholerae</i>	Millipore Sigma	1	Lac
RCA120	<i>Ricinus communis</i>	Vector Labs	2	Gal
Ricin B	<i>Ricinus communis</i>	Vector Labs	1	Lac

\*Inhibiting sugars: Man, mannose; Gal, galactose; Fuc, fucose; GlcNAc, N-acetylglucosamine; Lac, lactose.

**Table S2.** Excel file of LFQ Data from Pulldowns.

Available as a separate supplementary excel file.

**Table S3.** Serum Glycoproteins Enriched by BamBL.

Swiss-Prot accession number	Swiss-Prot entry name	Protein name
P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2
P09871	C1S_HUMAN	Complement C1s subcomponent
P04003	C4BPA_HUMAN	C4b-binding protein alpha chain
P01023	A2MG_HUMAN	Alpha-2-macroglobulin
O95445	APOM_HUMAN	Apolipoprotein M
Q06033	ITIH3_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H3
P35542	SAA4_HUMAN	Serum amyloid A-4 protein
Q96PD5	PGLYRP2_HUMAN	N-acetylmuramoyl-L-alanine amidase
P01876	IGHA1_HUMAN	Immunoglobulin heavy constant alpha 1
P01857	IGHG1_HUMAN	Immunoglobulin heavy constant gamma 1
P23142	FBLN1_HUMAN	Fibulin-1
P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein
P01859	IGHG2_HUMAN	Immunoglobulin heavy constant gamma 2
P05156	CFAI_HUMAN	Complement factor I
P10909	CLUS_HUMAN	Clusterin
P14151	LYAM1_HUMAN	L-selectin
P01024	CO3_HUMAN	Complement C3
P20742	PZP_HUMAN	Pregnancy zone protein
P01877	IGHA2_HUMAN	Immunoglobulin heavy constant alpha 2
Q7Z5P9	MUC19_HUMAN	Mucin-19
P08603	CFAH_HUMAN	Complement factor H
P02749	APOH_HUMAN	Beta-2-glycoprotein 1
P02743	SAMP_HUMAN	Serum amyloid P-component
P02671	FIBA_HUMAN	Fibrinogen alpha chain
P13598	ICAM2_HUMAN	Intercellular adhesion molecule 2

P51884	LUM_HUMAN	Lumican
P00747	PLMN_HUMAN	Plasminogen
P05546	HEP2_HUMAN	Heparin cofactor 2
Q96IY4	CBPB2_HUMAN	Carboxypeptidase B2
O75882	ATRN_HUMAN	Attractin
P08519	APOA_HUMAN	Apolipoprotein(a)
P05090	APOD_HUMAN	Apolipoprotein D
P05452	TETN_HUMAN	Tetranectin
Q6UXB8	PII6_HUMAN	Peptidase inhibitor 16
Q9UK55	ZPI_HUMAN	Protein Z-dependent protease inhibitor
Q14520	HABP2_HUMAN	Hyaluronan-binding protein 2
P43121	MUC18_HUMAN	Cell surface glycoprotein MUC18
Q12913	PTPRJ_HUMAN	Receptor-type tyrosine-protein phosphatase eta
P08637	FCG3A_HUMAN	Low affinity immunoglobulin gamma Fc region receptor III-A
P22105	TENX_HUMAN	Tenascin-X
P33151	CADH5_HUMAN	Cadherin-5
P07333	CSF1R_HUMAN	Macrophage colony-stimulating factor 1 receptor
P26927	HGFL_HUMAN	Hepatocyte growth factor-like protein
P15151	PVR_HUMAN	Poliovirus receptor
P13473	LAMP2_HUMAN	Lysosome-associated membrane glycoprotein 2
P15814	IGLL1_HUMAN	Immunoglobulin lambda-like polypeptide 1
P01833	PIGR_HUMAN	Polymeric immunoglobulin receptor
Q9HDC9	APMAP_HUMAN	Adipocyte plasma membrane-associated protein
P55290	CAD13_HUMAN	Cadherin-13
P01033	TIMP1_HUMAN	Metalloproteinase inhibitor 1
P48740	MASP1_HUMAN	Mannan-binding lectin serine protease 1
Q6YHK3	CD109_HUMAN	CD109 antigen
P41222	PTGDS_HUMAN	Prostaglandin-H2 D-isomerase



Q9UNN8	EPCR_HUMAN	Endothelial protein C receptor
O00533	NCHL1_HUMAN	Neural cell adhesion molecule L1-like protein
P12821	ACE_HUMAN	Angiotensin-converting enzyme
P10153	RNAS2_HUMAN	Non-secretory ribonuclease
P13591	NCAM1_HUMAN	Neural cell adhesion molecule 1
P35916	VGFR3_HUMAN	Vascular endothelial growth factor receptor 3
O00187	MASP2_HUMAN	Mannan-binding lectin serine protease 2
P12830	CDH1_HUMAN	Cadherin-1
P10721	KIT_HUMAN	Mast/stem cell growth factor receptor Kit
P62937	PPIA_HUMAN	Peptidyl-prolyl cis-trans isomerase A
P16070	CD44_HUMAN	CD44 antigen
P16109	LYAM3_HUMAN	P-selectin
P12259	FAS_HUMAN	Coagulation factor V
P05164	PERM_HUMAN	Myeloperoxidase
P17813	EGLN_HUMAN	Endoglin
Q6UX71	PLXDC2_HUMAN	Plexin domain-containing protein 2
P05154	IPSP_HUMAN	Plasma serine protease inhibitor
P02786	TFR1_HUMAN	Transferrin receptor protein 1
P07359	GP1BA_HUMAN	Platelet glycoprotein Ib alpha chain
Q15762	CD226_HUMAN	CD226 antigen
Q9BY67	CADM1_HUMAN	Cell adhesion molecule 1
Q8N6C8	LILRA3_HUMAN	Leukocyte immunoglobulin-like receptor subfamily A member 3
P02675	FIBB_HUMAN	Fibrinogen beta chain
Q15166	PON3_HUMAN	Serum paraoxonase/lactonase 3
P17936	IBP3_HUMAN	Insulin-like growth factor-binding protein 3
Q8NBP7	PCSK9_HUMAN	Proprotein convertase subtilisin/kexin type 9

**Table S4.** Serum Glycoproteins Enriched by Anti-Le<sup>a</sup>

Swiss-Prot accession number	Swiss-Prot entry name	Protein name
P08697	A2AP_HUMAN	Alpha-2-antiplasmin
P10586	PTPRF_HUMAN	Receptor-type tyrosine-protein phosphatase F
Q99784	NOE1_HUMAN	Noelin
P51884	LUM_HUMAN	Lumican
P08519	APOA_HUMAN	Apolipoprotein(a)
P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2
P40197	GPV_HUMAN	Platelet glycoprotein V
Q92820	GGH_HUMAN	Gamma-glutamyl hydrolase
P02774	VTDB_HUMAN	Vitamin D-binding protein
P02751	FINC_HUMAN	Fibronectin
Q15485	FCN2_HUMAN	Ficolin-2
P12259	FA5_HUMAN	Coagulation factor V
Q02413	DSG1_HUMAN	Desmoglein-1
P07858	CATB_HUMAN	Cathepsin B
P35222	CTNB1_HUMAN	Catenin beta-1
Q9NQ79	CRAC1_HUMAN	Cartilage acidic protein 1
P10909	CLUS_HUMAN	Clusterin
P00751	CFAB_HUMAN	Complement factor B
P11597	CETP_HUMAN	Cholesteryl ester transfer protein
P02748	CO9_HUMAN	Complement component C9
P07357	CO8A_HUMAN	Complement component C8 alpha chain
P04003	C4BPA_HUMAN	C4b-binding protein alpha chain
Q9NZP8	C1RL_HUMAN	Complement C1r subcomponent-like protein
P06276	CHLE_HUMAN	Cholinesterase

O95445	APOM_HUMAN	Apolipoprotein M
P02749	APOH_HUMAN	Beta-2-glycoprotein 1
P02656	APOC3_HUMAN	Apolipoprotein C-III
P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein
P43652	AFAM_HUMAN	Afamin
P0DP01	HV108_HUMAN	Immunoglobulin heavy variable 1-8

**Table S5.** Characteristics of Study Participants Grouped by Strain-specific Response**Subtable 1: Strain: Influenza A/H1N1 (A/Brisbane/02/2018)**

	High responders (n = 69)	Low responders (n = 25)	Non responders (n = 66)
Male/Female	26/43	10/15	25/41
Median Age in Years (IQR)	51.0 (36.0-64.0)	49.0 (28.5-66.0)	56.0 (40.0-68.0)
Median Body-Mass Index (IQR)	28.8 (25.3-32.8)	27.3 (23.1-36.0)	28.6 (24.4-32.6)

**Subtable 2: Strain: Influenza A/H3N2 (A/Kansas/14/2017)**

	High responders (n = 86)	Low responders (n = 9)	Non responders (n = 65)
Male/Female	27/59	5/4	29/36
Median Age in Years (IQR)	47.0 (30.5-65.0)	44.0 (35.0-55.5)	56.5 (44-69.5)
Median Body-Mass Index (IQR)	29.1 (25.3-33.4)	24.4 (23.1-33.9)	28.0 (25.4-31.5)

**Subtable 3: Strain: Influenza B/Yamagata (B/Phuket/3073/2013)**

	High responders (n = 66)	Low responders (n = 28)	Non responders (n = 66)
Male/Female	21/45	12/16	28/38
Median Age in Years (IQR)	47.5 (31.0-58.0)	43.0 (28.5-62.5)	62 (44.8-70.0)
Median Body-Mass Index (IQR)	28.1 (25.3-32.7)	28.1 (24.2-33.7)	29.0 (25.3-32.8)

**Subtable 4: Strain: Influenza B/Victoria (B/Colorado/6/2017-like)**

	High responders (n = 68)	Low responders (n = 21)	Non responders (n = 71)
Male/Female	23/45	11/10	27/44
Median Age in Years (IQR)	51.0 (34.0-64.0)	49.0 (32.5-67.0)	54.5 (41.8-68.3)
Median Body-Mass Index (IQR)	28.6 (25.2-32.8)	29.4 (24.8-34.9)	27.9 (25.2-31.7)