Differential immune imprinting by influenza virus vaccination and infection in non-human primates

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Table S1 Macaque information

Macaque	Sex	Age
6145	Male	Adult
T651	Female	Adult
T771	Male	Adult
7071	Female	Adult
7072	Female	Adult
7073	Female	Adult

Table S2 Mutations disrupt binding by antibodies with known epitope

	Epitope	Antibody	Fold reduction
X31-mRBS	RBS	C05	367
X31-mRBS	RBS	HC19	12
X31-mInterface	Interface	H2214	65
X31-mHC45	Head	HC45	21
X31-mK1747	Mid HA	K1747	145
X31-Delta Stem	Stem	FI6	>500
X31-mCR8020	Stem	CR8020	63

Loss of affinity, expressed as an n-fold ratio, was determined using KD values from ELISA with mutant and wild type HA with the specified monoclonal antibodies, in order to verify that the mutations introduced into HA for epitope mapping disrupted binding.

Fig. S1 Influenza isolates used in this study

Isolate	Abbrev.	# Diff.	% Div.
A/Aichi/02/1968(H3N2)	H3-HK-1968	0	0
A/Port Chalmers/1/1973(H3N2)	H3-PC-1973	20	4
A/Victoria/3/1975(H3N2)	H3-VI-1975	28	5
A/Texas/1/1977(H3N2)	H3-TX-1977	35	7
A/Bangkok/01/1979(H3N2)	H3-BK-1979	41	8
A/Philippines/2/1982(H3N2)	H3-PH-1982	46	9
A/Shanghai/11/1987(H3N2)	H3-SH-1987	53	10
A/Beijing/353/1989(H3N2)	H3-BJ-1989	55	11
A/Johannesburg/33/1994(H3N2)	H3-JB-1994	63	12
A/Moscow/10/1099(H3N2)	H3-MO-1999	60	12
A/Wisconsin/67/2005(H3N2)	H3-WI-2005	73	14
A/California/04/2009(H1N1)	H1-CA-2009	291	56
A/dfeb/BO/PBV780-781/2011(H18N11)	H18-BO-2011	331	62
B/Phuket/3073/2013	B-PK-2013	400	74
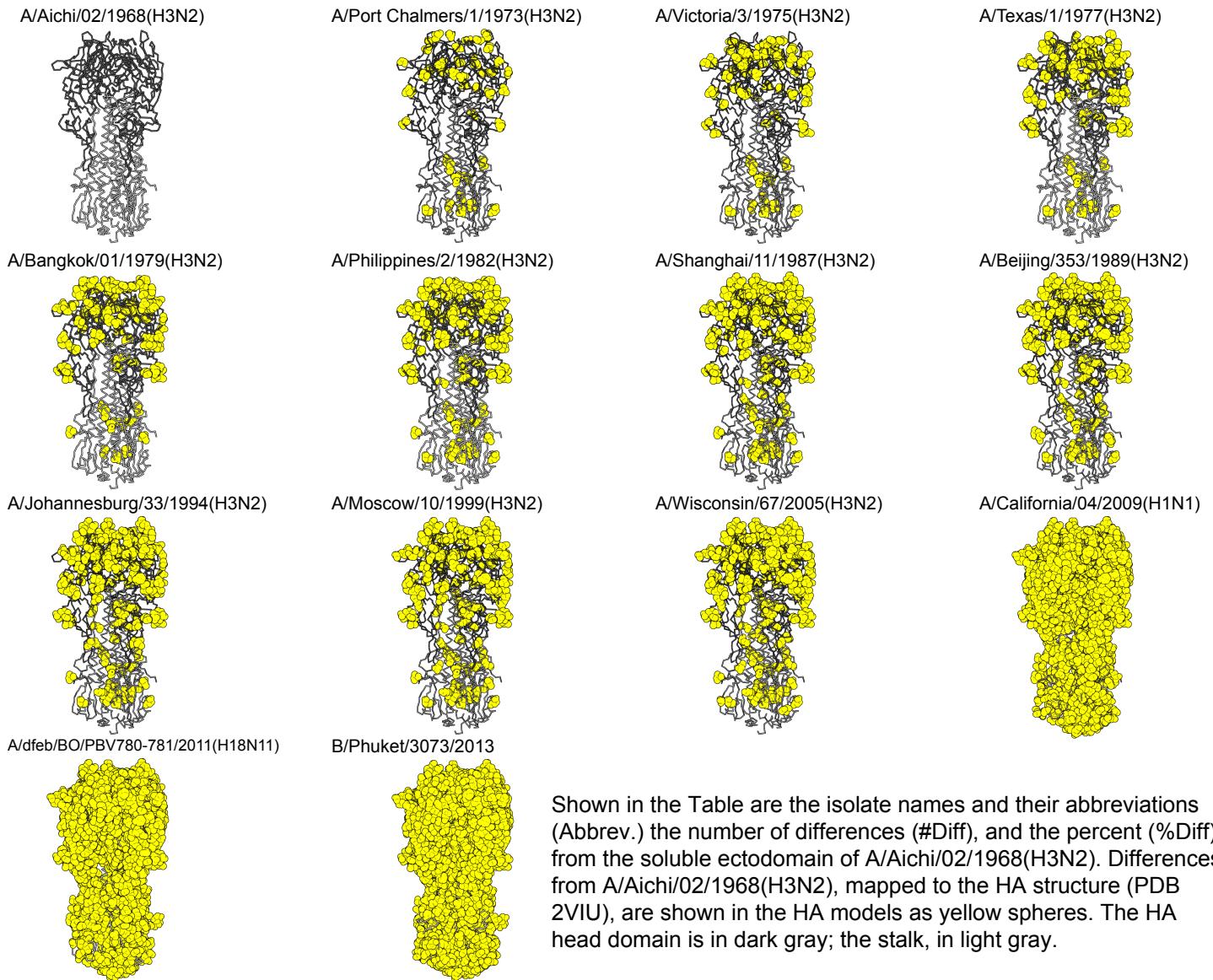
Shown in the Table are the isolate names and their abbreviations (Abbrev.) the number of differences (#Diff), and the percent (%Diff) from the soluble ectodomain of A/Aichi/02/1968(H3N2). Differences from A/Aichi/02/1968(H3N2), mapped to the HA structure (PDB 2VIU), are shown in the HA models as yellow spheres. The HA head domain is in dark gray; the stalk, in light gray.

Fig. S2. Reactivities of macaque sera.
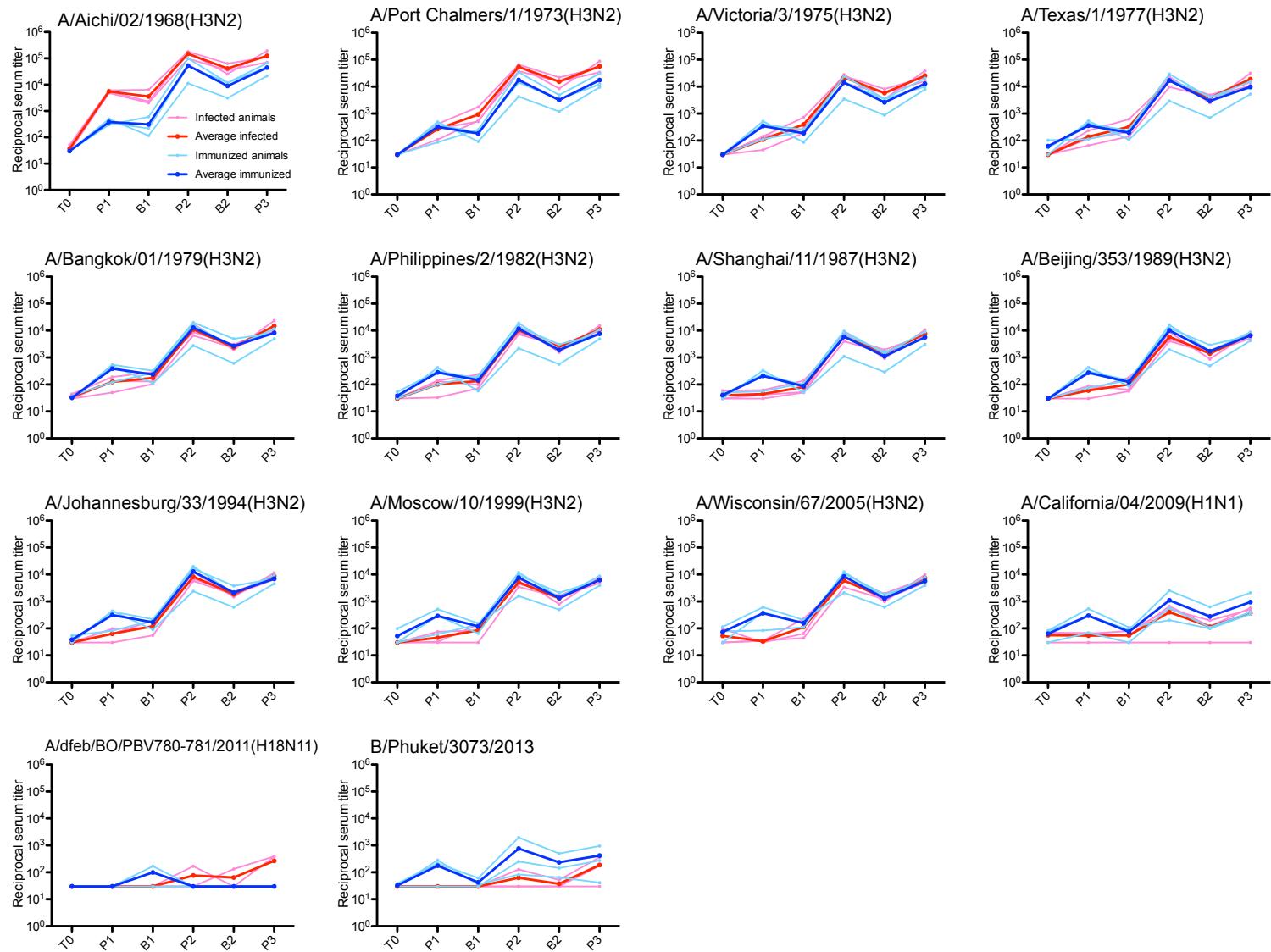
Graphs show reciprocal serum titers for the indicated HA proteins, as measured by ELISA. For each panel the time points on the x-axis correspond to Fig. 1 and are: Time 0 (T0), peak 1 (p1), baseline 1 (b1), peak 2 (p2), baseline 2 (b2), peak 3 (p3).

Fig. S3.Neutralization of influenza viruses
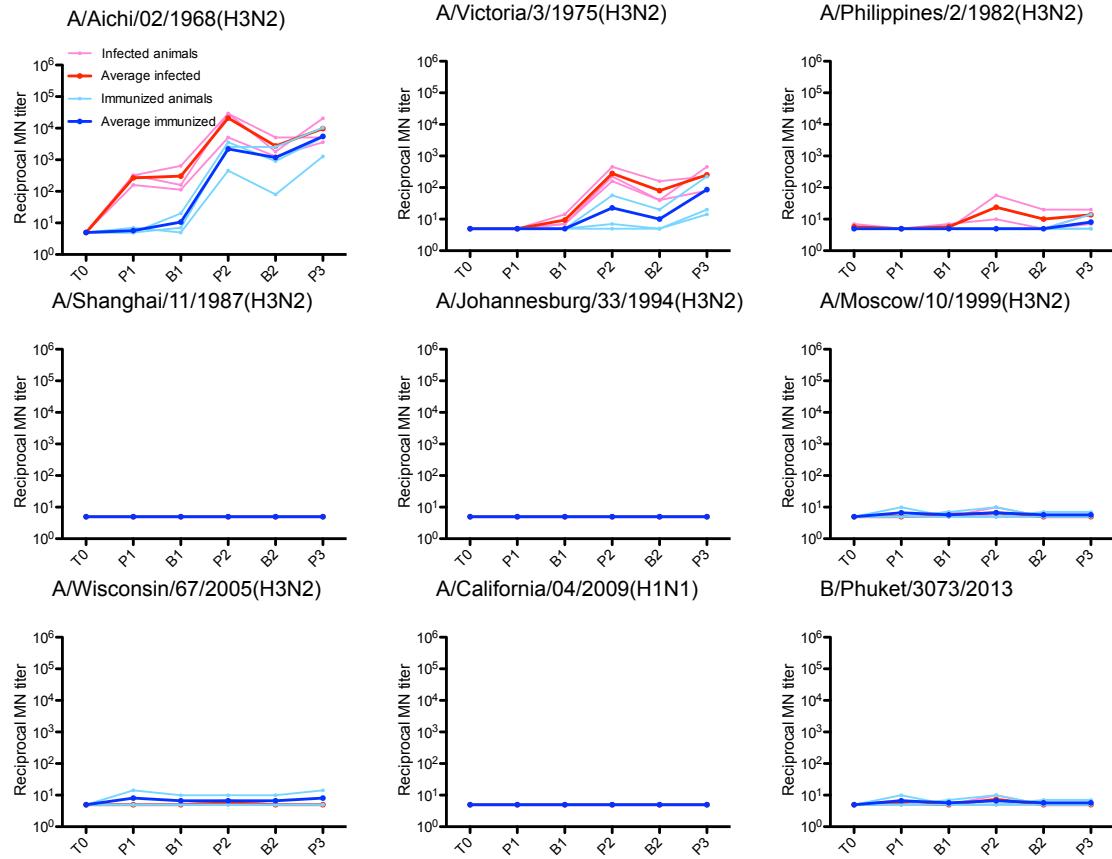
Macaque sera were tested by microneutralization assay; the reciprocal neutralizing titers are graphed. For each panel the time points on the x-axis correspond to Fig. 1 and are: Time 0 (T0), peak 1 (p1), baseline 1 (b1), peak 2 (p2), baseline 2 (b2), peak 3 (p3).

Fig. S4. Cross reactivity of macaque sera
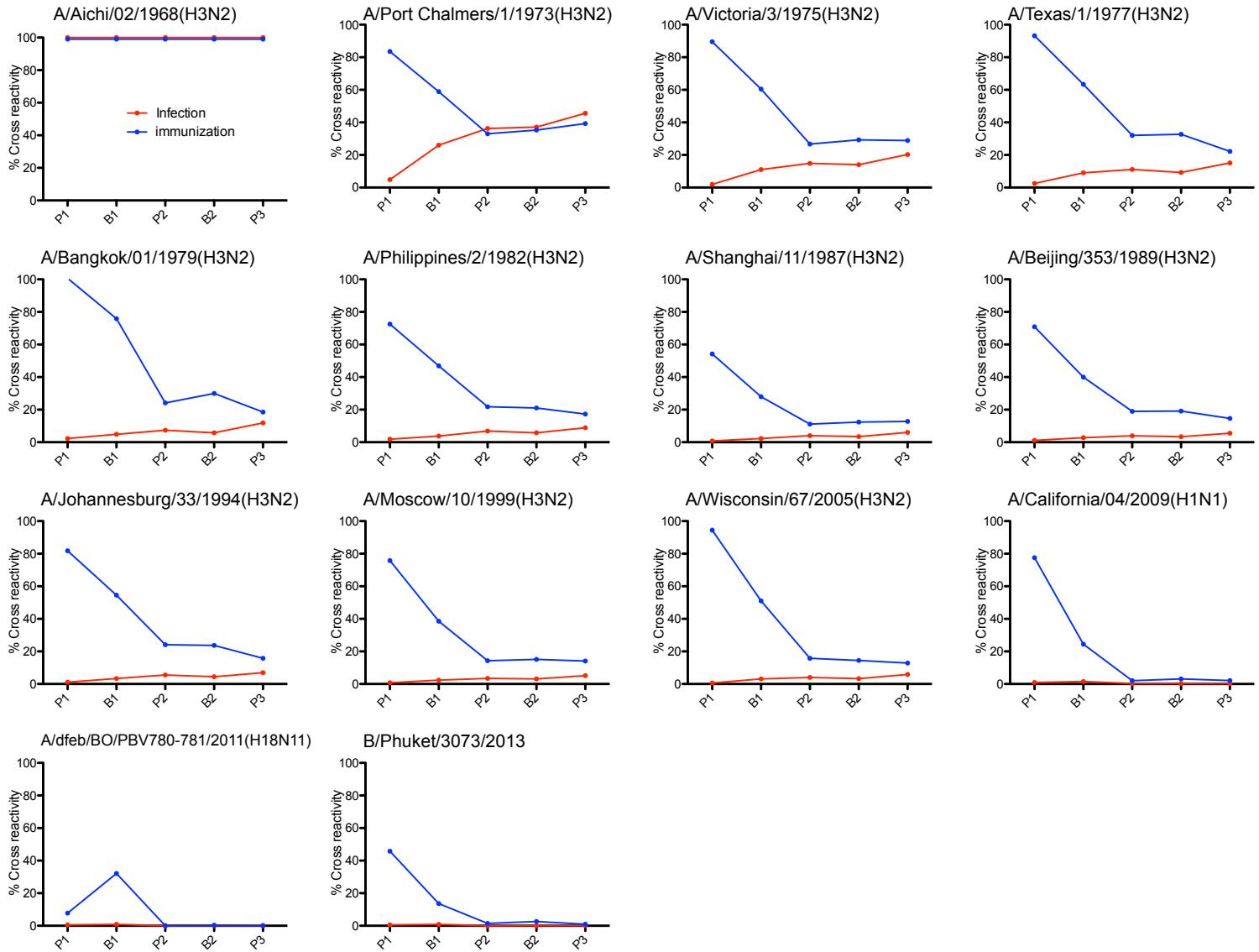
Using the averaged ELISA titers for each cohort, we calculated the percentage of the HA-directed serum response that reacts with heterologous HAs ((Heterologous titer ÷ H3-HK-1968 titer)*100). For each panel the time points on the x-axis correspond to Fig. 1 and are: Time 0 (T0), peak 1 (p1), baseline 1 (b1), peak 2 (p2), baseline 2 (b2), peak 3 (p3).

Fig. S5. Sequence alignment of wild-type and mutant HA variants
Differences from the reference strain, wild type A/Aichi/02/1968(H3N2)(X31), are shown as letters; identities are shown as dots.


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                    1        10        20        30        40        50        60        70        80
X31-WT              QDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGKICNNPHRILDGIDCTLIDALLGDPHCDVFQNETWD
X31-mRBS            ....................................................................................
X31-mInterface     ....................................................................................
X31-mHC45          ...........................................................KN..................R.....
X31-m1747          .........................K..........N.T.............................................
X31-delta stem     ....................................................................................
X31-m8020          ....................................................................................
X31-3xhead         ....................................................................................
X31-3xhead mInterface ....................................................................................

                    90       100       110       120       130       140       150       160       170
X31-WT              LFVERSKAFSNCYPYDVPDYASLRSLVASSGTLEFITEGFTWTGVTQNGGSNACKRGPGSGFFSRLNWLTKSGSTYPVLNVTMPN
X31-mRBS           .............................................T....I.RSNNS..............KN...............
X31-mInterface     ....................................................................................
X31-mHC45          ..........N.........................................................................
X31-m1747          ....................................................................................
X31-delta stem     ....................................................................................
X31-m8020          ....................................................................................
X31-3xhead         ....................................................................................
X31-3xhead mInterface ....................................................................................

                    180       190       200       210       220       230       240       250
X31-WT              NDNFDKLYIWGIHHPSTDQEQTSLYVQASGRVTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSNGNLIAPR
X31-mRBS           .....................R...K..........................R..DQ.........................
X31-mInterface     ....................................................R......G....................
X31-mHC45          ....................................................................................
X31-m1747          ....................................................................................
X31-delta stem     ....................................................................................
X31-m8020          ....................................................................................
X31-3xhead         ....................................................................................
X31-3xhead mInterface .................................................R......G....................

                    260       270       280       290       300       310       320       330       340
X31-WT              GYFKMRTGKSSIMRSDAPIDTCISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGLFGAIAGFIE
X31-mRBS           ....................................................................................
X31-mInterface     ....................................................................................
X31-mHC45          ....................................................................................
X31-m1747          .............................................................R......................
X31-delta stem     ....................................................................................
X31-m8020          ....................................................................................
X31-3xhead         ....................................................................................
X31-3xhead mInterface ....................................................................................

                    350       360       370       380       390       400       410       420
X31-WT              NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNA
X31-mRBS           ....................................................................................
X31-mInterface     ....................................................................................
X31-mHC45          ....................................................................................
X31-m1747          ....................................................................................
X31-delta stem     .............................................R...R..................................
X31-m8020          ......R.................N.T.........................................................
X31-3xhead
X31-3xhead mInterface

                    430       440       450       460       470       480       490       500       510
X31-WT              ELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALNNRFQIKGVELKSG
X31-mRBS           ....................................................................................
X31-mInterface     ....................................................................................
X31-mHC45          ....................................................................................
X31-m1747          ....................................................................................
X31-delta stem     ....................................................................................
X31-m8020          ....................................................................................
X31-3xhead
X31-3xhead mInterface
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Fig. S6. Mode of exposure influences the distribution of epitopes bound by serum antibodies.
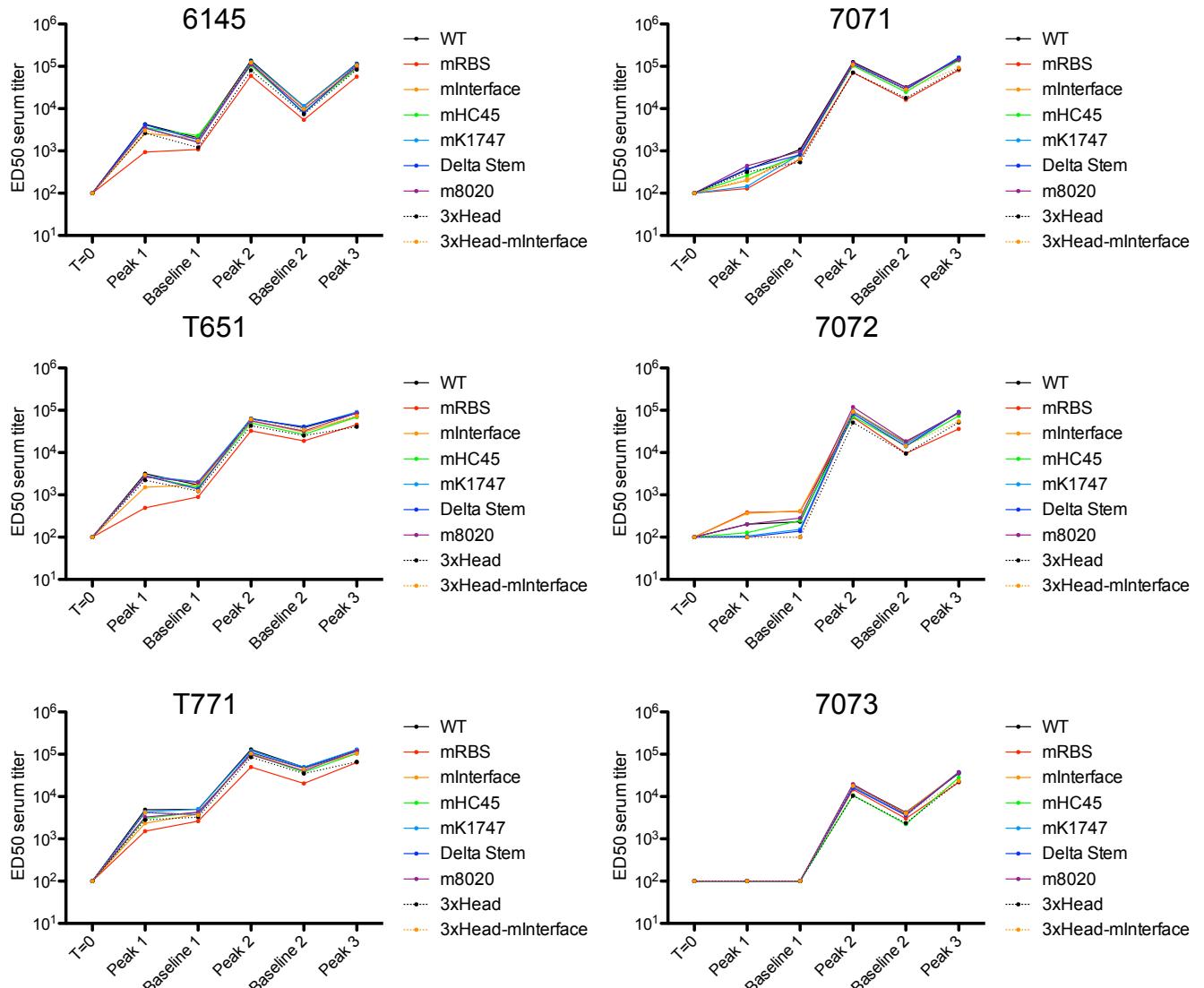
ELISA ED50 data used for Fig 3. For each panel the time points on the x-axis correspond to Fig. 1 and are: Time 0 (T0), peak 1 (p1), baseline 1 (b1), peak 2 (p2), baseline 2 (b2), peak 3 (p3).