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Supplemental Material to:

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Multiplexed screening of natural humoral immunity identifies antibodies at fine specificity for complex and dynamic viral targets

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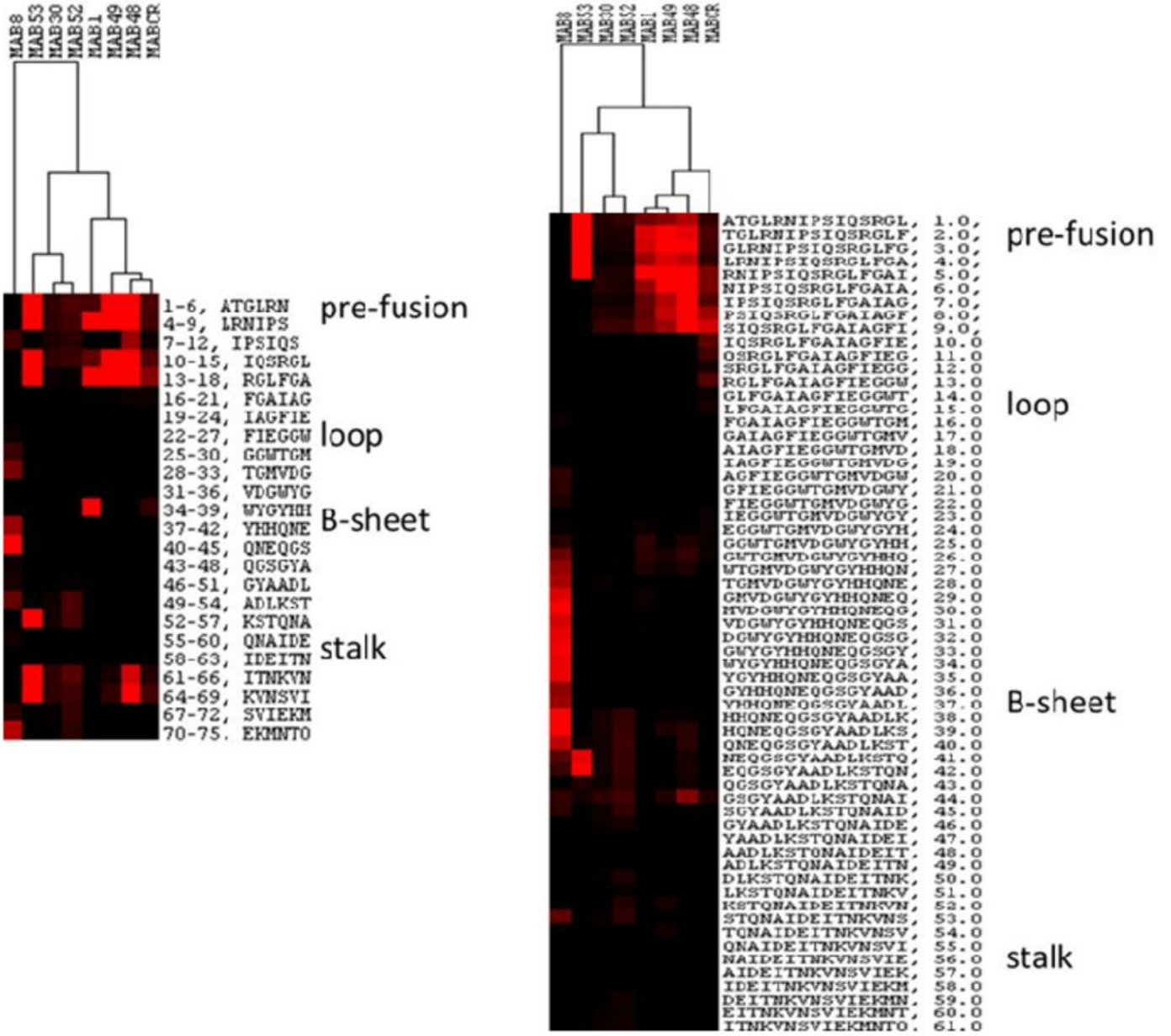
SUPPLEMENTAL MATERIALS

Figure S1. PepScan heat maps for group 1 antibodies directed to the fusion region of HA. For each antibody, 5808 different CLIPS peptides covering linear, conformational and discontinuous epitopes on a 75 amino acid region of HA were designed and synthesized. Representative heat maps are shown for group 1 HA using data aggregated from peptide matrix groups, which gave the highest reliability and signal-to-noise ratios show the different binding strengths of the analyzed peptides.The binding patterns of the six antibodies (mAb 1, 8, 30, 48, 49, 52 and 53) are compared using hierarchical clustering (Pearson correlation), which groups antibodies showing the strongest similarities in binding patterns (by this analysis mAb 8 was an outlier within the cohort of the experiments).

Figure S2. A comparison of group 1 cross-reactive mAb binding to monomeric HA of different clades. IgG was captured on anti-human Fc sensor tips and 200 nM of each HA monomer was allowed to associate and then dissociate. The affinity of mAb 30 was too low for solution phase binding analysis and anti-rabbit Fc sensor tips were not available. In these two cases, ELISA analyses are shown instead, with 0.1 ml of HA coated in PBS overnight at a constant concentration of 0.5 μg/ml (or 10 μg/ml of fusion peptide) followed by BSA blocking and binding of up to 100 nM of antibody for 2 hours. Detection was done with corresponding secondary antibody HRP conjugates and TMB substrate.

Figure S3. A lack of correlation between kinetic binding and neutralization between four mAb, closely related in sequence, from the same blood donor, directed to group 2 HA antigens. (A) Sensorgrams show high-affinity binding of Fc captured mAb 455, 560, 620 and 663 to 200 nM of three H3N2 HA strains (New York /2004, Hong Kong /1968 and Perth /2009) and H7 (Netherlands/2003) trimers (with the exception of mAb 560, which does not bind H3 Perth well). (B) Neutralization activity on the corresponding H3N2 viruses in a plaque formation assay.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MAB30
MAB52
MAB1
MAB49
MAB48
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4-9, LRNIPS
7-12, IPSIQS
10-15, IQSRGL
13-18, RGLFGA
16-21, FGAIAG
19-24, IAGFIE
22-27, FIEGGW
25-30, GGWTGM
28-33, TGMVDG
31-36, VDGWYG
34-39, WYGYHH
37-42, YHHQNE
40-45, QNEQGS
43-48, QGSGYA
46-51, GYAADL
49-54, ADLKST
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58-63, IDEITN
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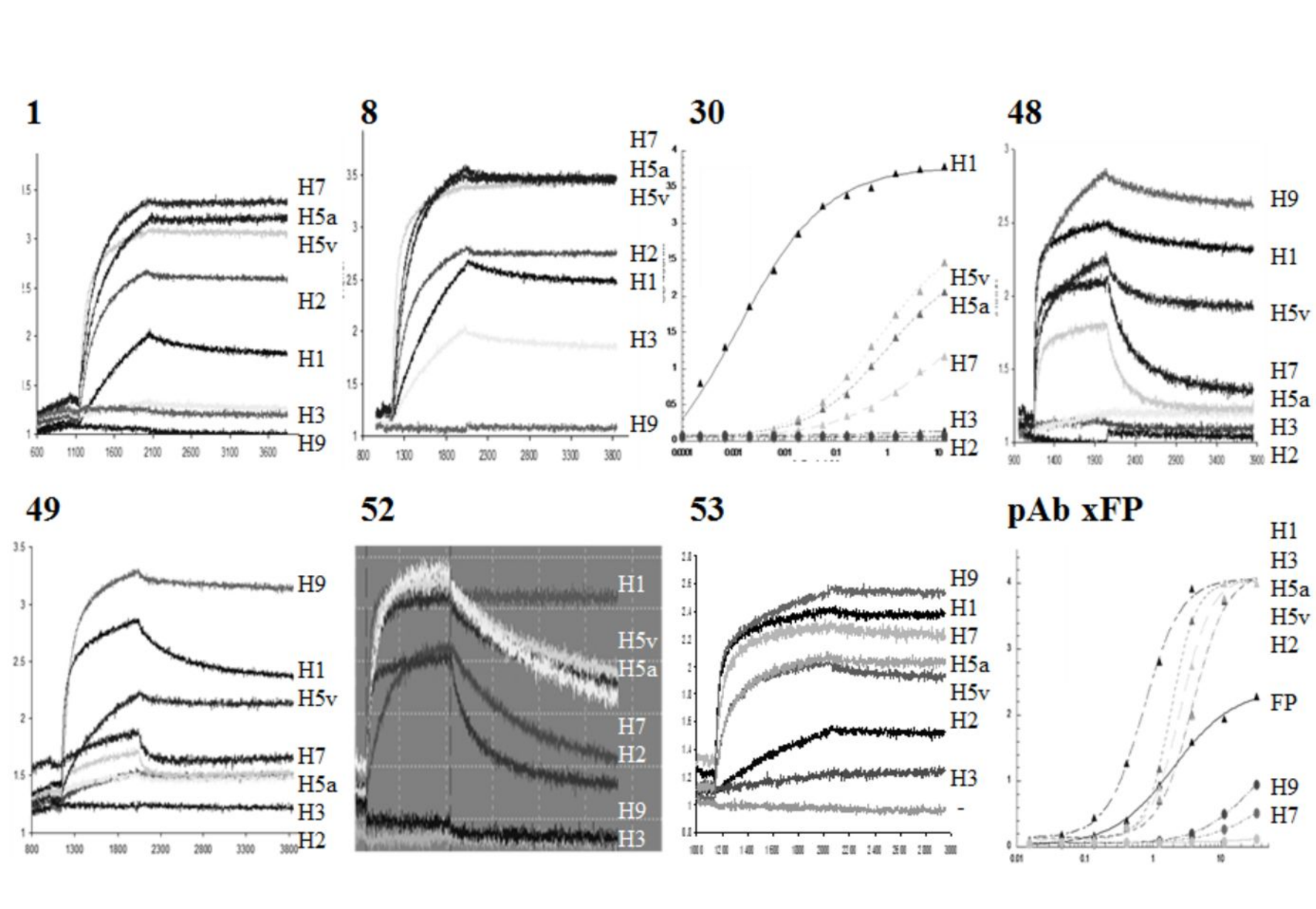
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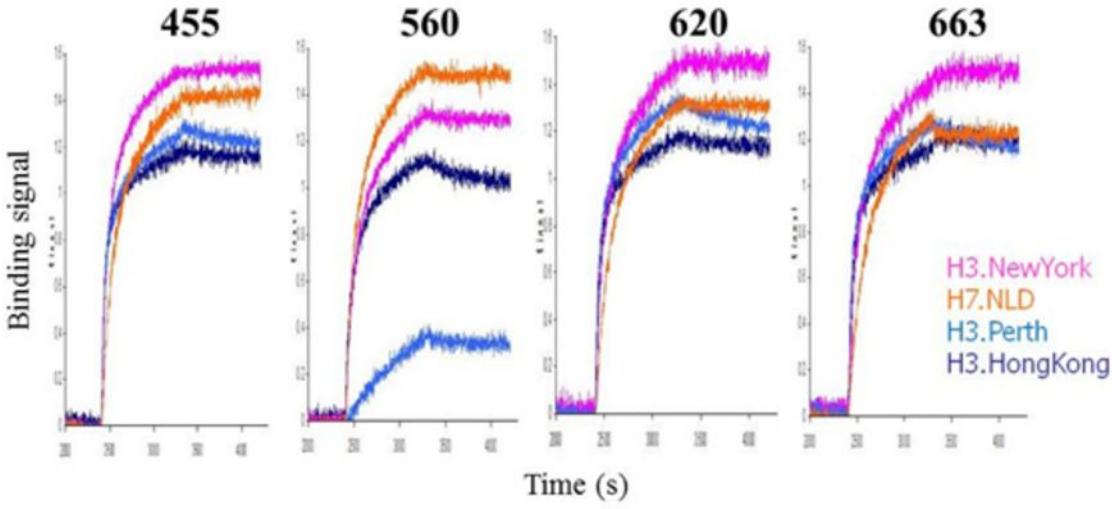
A.



B.

Virus Plaque Bioassay IC_{50}

mAb	H3N2 New York 2004	H3N2 Hong Kong 1968	H3N2 Perth 2009
455	15 µg/mL	10 µg/mL	0.6 µg/mL
560	>20 µg/mL	>20 µg/mL	>20 µg/mL
620	15 µg/mL	2.5 µg/mL	0.2 µg/mL
663	20 µg/mL	15 µg/mL	0.1µg/mL