Submitted Manuscript: Confidential
Template revised February 2021

Supplementary Materials for

Obesity is associated with an altered baseline and post-vaccination influenza antibody repertoire

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Materials and Methods

Clinical datasets

Participants were recruited as a part of a prospective observational study carried out at the University of North Carolina at Chapel Hill Family Medicine Center, an academic outpatient primary care facility in Chapel Hill, North Carolina. Recruitment criteria for this study included adults 18 years of age and older receiving the seasonal trivalent inactivated influenza vaccine (TIV) for the years 2010-2011 that included the following strains: A/H1N1/California/7/2009, A/H3N2/Perth/16/2009, and B/Brisbane/60/08. Exclusion criteria included immunosuppression, immunomodulatory or immunosuppressive drugs, acute febrile illness, history of hypersensitivity to any influenza vaccine components, history of Guillan-Barre syndrome, use of theophylline preparations, or warfarin. The study cohort included both obese (body mass index bigger than 30 kg/m2, n=104) and healthy-weight (18.5 ≤BMI≤ 24.9, n=101) individuals. Patients recruited to the study provided a blood sample prior to vaccination (baseline - day 0) and one month (28-35 days) post vaccination. Blood was collected via antecubital puncture. Sera were collected using non-heparinized 10 mL vacutainers, which were allowed to clot at room temperature for 2 hours before being separated by centrifugation at 800 x g for 10 minutes. Sera were then frozen at -80oC for subsequent analysis. All procedures were approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill.

We used serum samples from a prospective study of influenza vaccination conducted in 2010-11 [12] which included both obese (body mass index (BMI) bigger than 30 kg/m2, n=105) and healthy-weight (18.5 ≤BMI≤ 24.9, n=100) subjects. Serum samples were obtained before vaccination (baseline - day 0) and 30 days post vaccination with the 2010 trivalent inactivated seasonal influenza vaccine (TIV) that included the following strains: A/H1N1/California/7/2009, A/H3N2/Perth/16/2009, and B/Brisbane/60/08.

Normalization of serum concentration

Since the concentrations of different serum samples of the same individual may be different due to a variety of reasons at different time points, serum concentrations were normalized by measuring the total protein concentration in the serum using Nanodrop, and diluting all the samples to the same total protein concentration by PBS (the average concentration / 10). These diluted samples were considered as diluted 1:10, and additional dilutions were done from them.

Antigens

β-propiolactone (BPL)-inactivated whole influenza viruses were obtained from two resources: (1) Influenza viruses were grown in-house in embryonated chicken eggs, BPL-inactivated and purified on sucrose columns as previously described [13], and their concentrations were determined by the hemagglutinin assay, as previously described [13]. (2) Additional influenza viruses were obtained from the WHO (produced by the National Institute for Biological Standards and Control, NIBSC) as reagents for single radial diffusion (SRD) influenza potency assay with a known concentration. Recombinant HA (rHA) proteins were purchased from Sino biological (China) as purified His-tagged proteins. Most of them were produced in human HEK293 cell cultures, and some in Baculovirus-Insect Cells. Synthetic 20 amino acids (aa) peptides were synthesized at > 90% purity by CPC scientific (CA USA). Each peptide included an N-terminal KK tag as an amine group source for binding to the coated slides.

Antigen microarray design and spotting

To study the anti-influenza antibody repertoire we designed and spotted two types of antigen microarrays: (1) An influenza VP microarray was spotted with a panel of 34 whole inactivated influenza viruses and 23 recombinant HA proteins (Table S1) that were selected to represent the antigenic diversity of vaccine and historical human influenza strains of the H1N1, H3N2 and B subtypes from 1918 to 2016. Whole inactivated viruses were diluted in 0.005% triton and spotted at 2 HAU/µl concentration for viruses that were grown in-house in embryonated eggs, or 4 µgHA/ml for WHO viruses. Recombinant HA proteins were diluted in 0.01% triton and spotted at 16.25 µg/ml concentration. (2) A peptide microarray was spotted with 205 partially overlapping 20 aa peptides (with 15 aa overlap) spanning the full-length HA (H1) and NA (N1) proteins of the pH1N1 2009 california vaccine strain (Cal09) that was included in the TIV vaccine tested in this study. Peptides were dissolved in 20-60% dimethyl sulfoxide (DMSO) to a 2 mg/ml solution, depending on the peptide hydrophobicity, and spotted at a concentration of 1 mg/ml in 0.0025% triton X-100. Microrrays were spotted using a Scienion Sx spotter (Scienion, Germany) on Hydrogel-coated slides that bind amine groups (H slides, Schott, Germany). VP and peptide slides were printed separately, and all slides used for profiling the whole cohort were printed in a single batch to avoid potential batch effects. Each antigen was spotted in triplicate.

Hybridization of antigen microarrays

Human serum samples were diluted 1:3000 for measuring anti-human IgG and 1:300 for measuring anti-human IgA in a hybridization buffer that contained 1% BSA in 0.025% PBST (0.025% tween-20 in PBS). The spotted slides were blocked by 1 hr incubation on a rocker at room temperature (RT) with a chemical blocking solution (50 mM ethanolamine, 50 mM borate, pH 9.0). After blocking the slides were washed twice with 0.05% PBST, twice with PBS and once with DDW (each wash - 3 min on the rocker), and dried by centrifugation at RT for 5 minutes at 800g. Then the microarrays were hybridized with the diluted serum samples in divided trays (PepperPrint) for 2h at RT. Following washings as described above, the microarrays were incubated for 45 min with Alexa Fluor 647 labeled polyclonal anti-human IgG antibody at 1:1000 dilution (Jackson ImmunoResearch cat# 709-605-149) or Alexa Fluor 488- conjugated polyclonal anti-human IgA antibody at 1:6000 dilution (Jackson ImmunoResearch cat# 109-545-011). The secondary antibodies were diluted in 1% BSA in 0.025% PBST. To detect bound antibodies, slides were scanned on a two-laser GenePix 4400A scanner (Molecular Devices). Due to technical problems (high background or limited volume of some of the sera), a small number of the samples were not hybridized or analyzed. The analysis presented here included serum samples from 104 Ob and 99 HW individuals for peptide arrays, 94 Ob and 95 HW samples for IgG VP arrays, 87 Ob and 87 HW samples for IgA VP arrays.

Analysis of microarray results

Scanned slides were annotated using GenePix Pro version 7 (Molecular Devices) to obtain the mean fluorescence intensity ($0 \leq MFI \leq 65{,}000$). The local background fluorescence intensity was subtracted from each spot MFI. The median background-subtracted MFI (median MFI-B) was selected for each triplicate of the same antigen. Data analysis was conducted using an in-house pipeline written in Python. The vaccine-induced change in the antibodies level to a given antigen was calculated as the fold-change rise from the baseline level, or as a baseline-subtracted response.

Magnitude and Breadth summary statistics

Normalized microarray results were analyzed using Python scripts, written by Dr. Tomer Hertz. For a given subject and a set of antigens we defined the 'breadth' and 'magnitude' as followed: (a) Magnitude - denotes the sum of median (MFI-B) results to a given set of antigens. (b) Breadth – denotes the number of antigens in a given set with a median (MFI-B) higher than a selected threshold: (MFI-B) > 2000 for peptides and recombinant HA proteins, and (MFI-B) > 4000 for whole viruses.

The magnitude and breadth were calculated for each subject at each time point and also for the post-vaccination baseline-subtracted responses, for the following sets of antigens: Cal09 H1 peptides, Cal09 N1 peptides, HA proteins of subtype B strains, HA proteins of subtype H1N1 strains, HA proteins of subtype H3N2 strains, whole viruses of subtype B strains, whole viruses of subtype H1N1 strains, and whole viruses of subtype H3N2 strains.

Baseline immune history (BIH) ranking

All subjects (n=205) were ranked according to the baseline magnitude or breadth to H1N1 whole viruses, or rHA proteins or H1N1 strains (rH1), or Cal09 H1 peptides, or Cal09 N1 peptides. For each ranking, the subjects were divided into quartiles, and the two extreme quartiles of highest and lowest scores, termed high-BIH and low-BIH, respectively, were compared. The number of individuals in each group varied by assay due to technical issues such as sample availability and high-background as follows:

Assay	Low-BIH group	High BIH group
Viruses and Proteins IgG	47	47
Viruses and Proteins IgA	44	44
H1 nad N1 peptides IgG	49	50
H1 and N1 peptides IgA	49	47

Vaccine responders ranking

For each individual, we computed the baseline-adjusted response to the vaccine for each peptide in the peptides microarray, by subtracting the baseline binding of antibodies to this peptide from the post-vaccination antibody binding. All subjects (n=205) were ranked according to the magnitude or breadth of baseline-adjusted responses to Cal09 H1 or N1 peptides. The subjects were divided into quartiles, and the two extreme quartiles of highest and lowest baseline-adjusted responses, termed high-responders and low-responders, respectively, were compared.

Statistical analysis

We used 2-sided hypothesis tests (Wilcoxon rank-sum and Fisher's exact test) to test for differences between the distribution of breadth and magnitude scores defined above. Differences between baseline and post-vaccination responses within each group were tested using the Wilcoxon signed rank test. The relative risk (RR)and

95% CI for obese individuals or individuals <65 Y to have the magnitude of their IgG and IgA BIH magnitude of responses to whole viruses, recombinant proteins and H1 or N1 peptides to fall in the lowest quartile compared to healthy weight individuals or individuals >65 Y was calculated using the python statmoldels package. If the RR and 95% CI is smaller than 1, then bring obese or <65 y may be a protective factor, e.g., for not having a BIH magnitude in the bottom quartile. When the 95% CI includes 1, then being obese or <65 y may have no effect on the subject being in the lowest quartile of BIH response.

To predict the group of subjects based on the immune-history profiles, we used a logistic regression model and a generalized linear model (GLM) was used to predict the vaccine-induced immune responses using a logistic model for binary variables and a linear model for continuous. All models were trained using leave-one-out cross validation. Predicted values were collected over all folds for computing the area under the curve (AUC) summary stat. All continuous variables were standardized prior to training. Regularization was implemented using the elastic net package with different alpha and L1 weight parameters for each model. All models were trained using the statsmodels python package.

Human IgG and IgA ELISA Quantization

Commercial enzyme linked immunosorbent assay (ELISA) kits (Bethyl Laboratories, USA, cat# E80-104 for human IgG and cat# E80-1026 for human IgA) were used to quantify the total human IgG and IgA concentrations in the normalised sera, using the manufacturer's instructions with the following modifications. The Normalized serum samples were diluted 1:24375 for human IgA ELISA and 1:243750 for human IgG ELISA. The ELISA assays were performed in 384-well white MaxiSorp Nunc plates (cat# 460372). The wells were coated with 17 µl/well of ELISA coating antibody in coating buffer, blocking and all washes were performed with 100 µl/well, and 30 µl/well diluted sera and standards were added in triplicates. Following washes, 30 µl/well HRP-conjugated detection antibody was also added. Instead of TMB, we used 30 µl/well of the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Scientific, cat# 34579, the two reagents were mixed at 1:1 ration before adding to the plate). Plates were read using a standard luminometer (TECAN infinite M200 PRO) at 600 nm. The average of each triplicate was calculated, and total antibody concentration was concluded from the standard curve.

Scoring HA residues based on the weights of regression model

The logistic regression model was trained based on H1/N1 peptide arrays results and assigned weights to individual features (peptides) from both the HA and NA proteins of the Cal09 vaccine strain. We used the weights from the baseline IgA or the baseline IgG models. We assigned to each residue of the HA protein the maximal weight of peptide containing the given residue. The HA residues were divided into 3 groups based on the distribution of scores of the baseline IgA or IgG.(i) Residues assigned with high positive score were associated with obesity status , (ii) Residues assigned with high negative score negative were associated with HW status and (iii) unweighted residues which had neutral or very low contribution score (below 5% of highest score in the model).

Curating HA domains and sites

Residues comprising the HA domain could be mapped into the following sites based on (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3410141/), The numbering is bellow is according to PDB ID: 3LZG(10.1126/science.1186430) . HA1-chain A, position 11 to 325. HA2-chain B, position 1 to 175. Fusion domain: chain A, position 11 to 64, position 276 to 324. Chain B position 1 to 160. Receptor binding site (RBS)-chain A 115 to 264. Esterase domain - chain A positions 66 to 116 and 166 to 277. Conserved HA glycosylation sites - chain A positions 20-23, 33-35,289-291, Chain B 154-156. Cal09 glycosylation site- chain A positions 278-280. Antigenic sites - all residues comprising Cb, Sb, Sa, Ca1 and Ca2. Cb antigenic site- chain A positions 79-84. Sb antigenic site- Chain A position 187 to 199. Sa antigenic site - chain A positions 128, 129 156-161 and 162-168. Ca1 antigenic site - chain A 168 -174,206-209 and 238 to 241. Ca2 antigenic site -Chain A positions 139-145 and 224-226.

Statistical significance test for enrichment of scored residues in functional domains

A permutation test was used to examine the significance of enrichment of scored residues in each domain. The evaluated value was different between the percentage of positively scored residues and negatively scored residues (indicating OB and HW status, respectively). When N is the number of residue in the HA protein (n =498), S is the number of residue comprising a given domain and D is the difference between positive and negative residues. The permutation test involved 10K iterations, in each iteration we sampled S residues out of N and calculated D. p value was calculated at the number of iterations where (D iteration >= D initial) divided by the number of iterations.

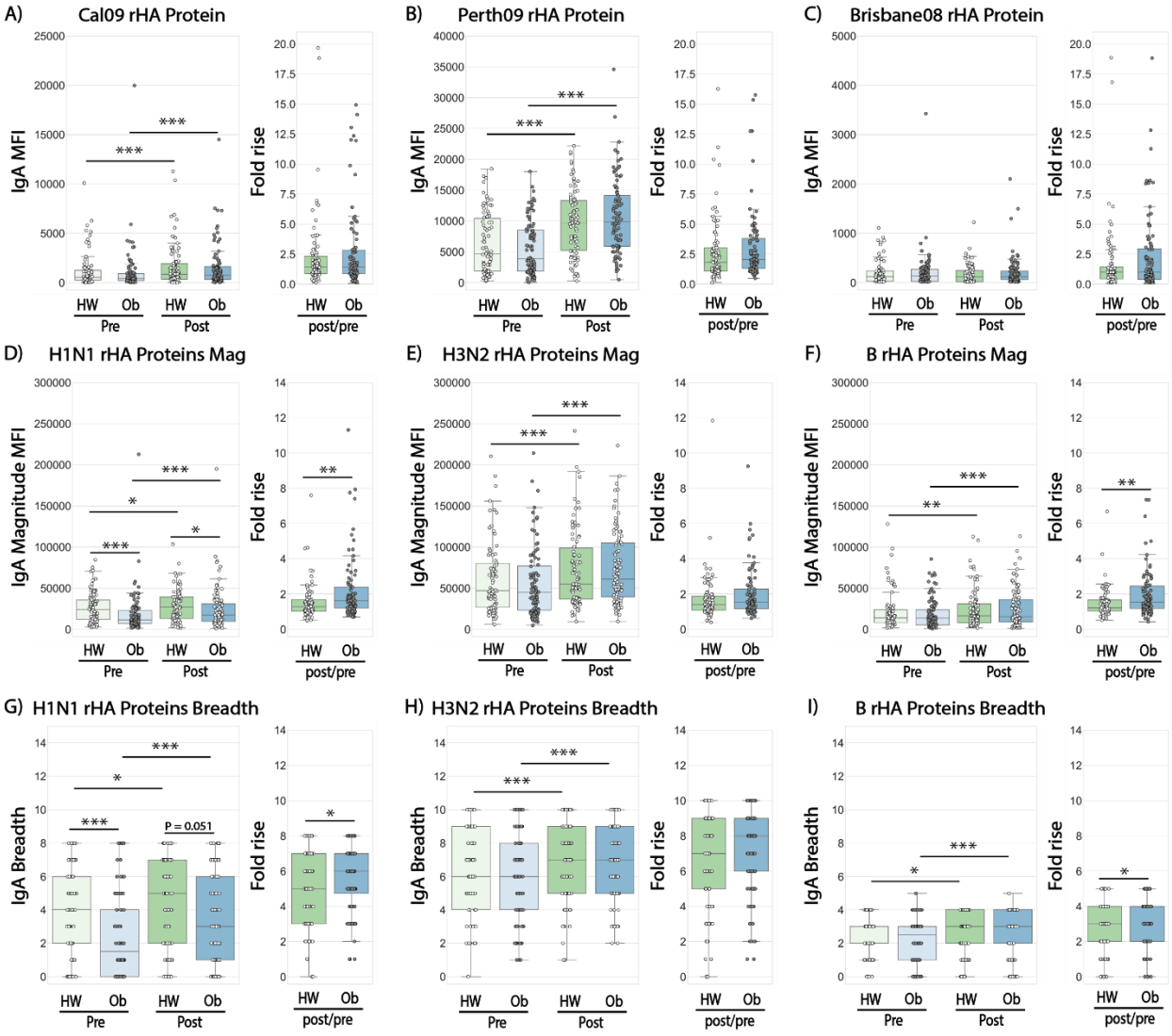

Fig. S1.

Baseline Immune History (BIH) and post vaccination IgA responses in healthy weight (HW) and obese (OB) individuals to a panel of historical influenza recombinant proteins (rHA).

Baseline and post-vaccination serum samples from 82 healthy-weight (HW) and 92 obese (OB) subjects were hybridized with an antigen microarray spotted with 34 BPL-inactivated influenza viruses and 23 recombinant HA (rHA) proteins that included the three vaccine strains used in the study, for profiling of IgA binding. **(A-C)** IgA binding to the recombinant HA of H1N1 A/California/7/2009, H3N2 A/Perth/16/2009 and B/Brisbane/60/2008 vaccine strains. (**D-F**) The magnitude of IgA antibodies bound to a panel of 8 recombinant H1 (rH1) proteins (D); a panel of 10 recombinant H3 (rH3) proteins; (E) and a panel of recombinant HA antigens of 5 B strains (F). **(G-I)** The breadth of IgA antibodies bound to a panel of recombinant HA proteins, including: 8 rH1 proteins (G); 10 rH3 proteins (H); and 5 recombinant HA proteins of 5 B strains (l) proteins. The four box plots in the left portion of each panel summarize the baseline (L->R: HW: light green, Ob: light blue) and the 30-day post-vaccination (L->R: HW: dark green, Ob: dark blue) binding responses. The two boxplots on the right side of each panel represent the fold increase (L->R: HW: green, Ob: blue). Lines represent the median fluorescence intensity (MFI), the boxes denote the 25th and 75th percentiles, and the error bars represent 1.5 times the interquartile range. Statistical significance was assessed using the Wilcoxon signed rank test (pre vs. post) and the Wilcoxon rank-sum test (HW vs. Ob). * $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

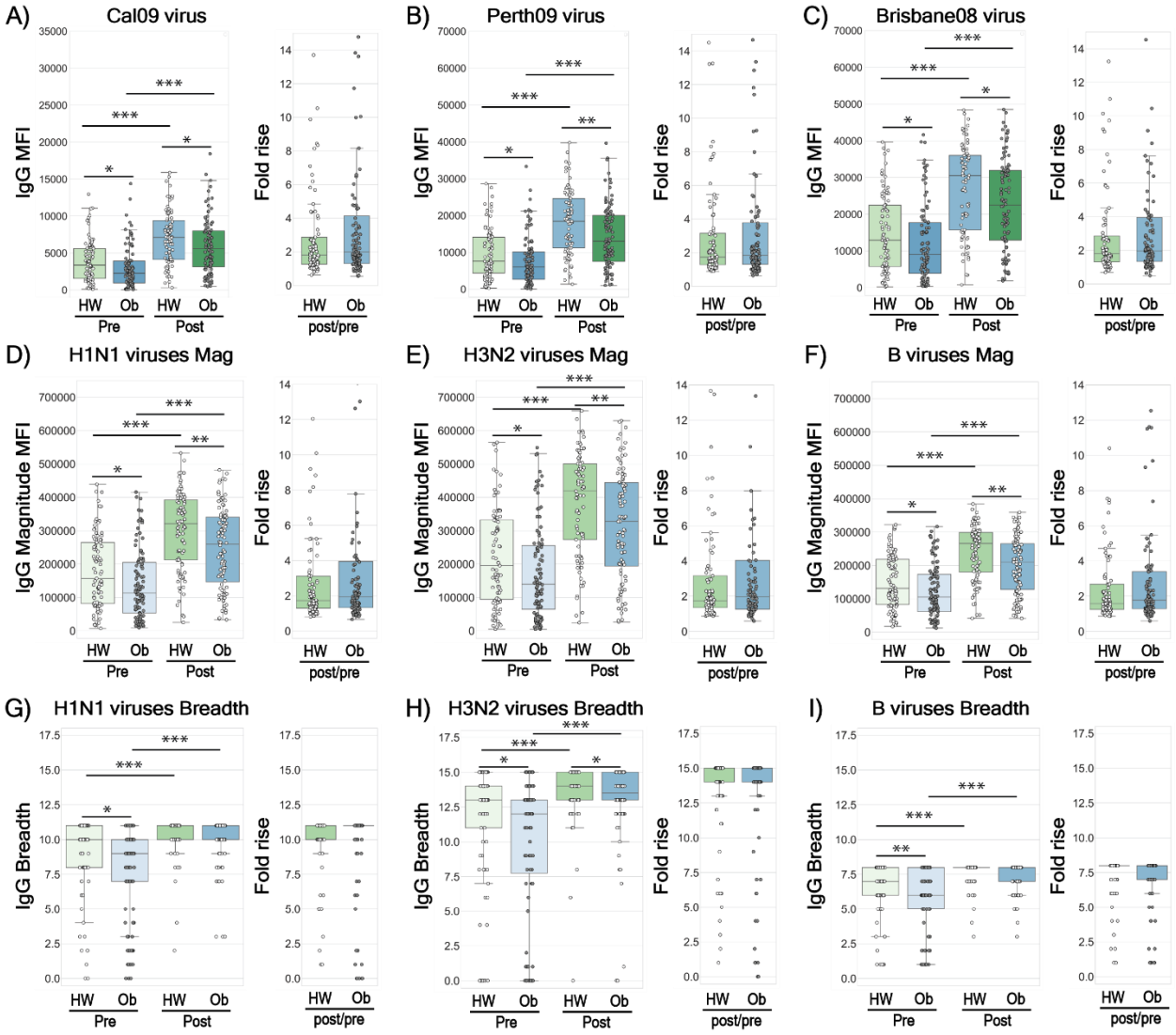

Fig. S2.

Baseline Immune History (BIH) and 30-day post vaccination IgG responses in healthy weight (HW) and obese (Ob) individuals to vaccine strains and a panel of historical influenza viruses.

Baseline and post-vaccination serum samples from 89 healthy-weight (HW) and 100 obese (Ob) subjects were hybridized with an antigen microarray spotted with 34 BPL-inactivated influenza viruses that included the three vaccine strains used in the study, for profiling of IgG binding. **(A-C)** IgG binding to the H1N1 A/California/7/2009, H3N2 A/Perth/16/2009 and B/Brisbane/60/2008 BPL-inactivated viruses. **(D-F)** The magnitude of IgG antibodies bound to a panel of 11 H1N1 virus strains (D); a panel of 15 H3N2 virus strains; (E) and a panel of recombinant HA antigens of 8 B virus strains (F). **(G-I)** The breadth of IgG antibodies bound to a panel of 11 H1N1 virus strains (G); a panel of 15 H3N2 virus strains; (H) and a panel of recombinant HA antigens of 8 B virus strains (I). The four box plots in the left portion of each panel summarize the baseline (L->R: HW: light green, Ob: light blue) and the 30-day post-

vaccination (L->R: HW: dark green, Ob: dark blue) binding responses. The two boxplots on the right side of each panel represent the fold increase (L->R: HW: green, Ob: blue). Lines represent the median fluorescence intensity (MFI), the boxes denote the 25th and 75th percentiles, and the error bars represent 1.5 times the interquartile range. Statistical significance was assessed using the Wilcoxon signed rank test (pre vs. post) and the Wilcoxon rank-sum test (HW vs. Ob). * $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

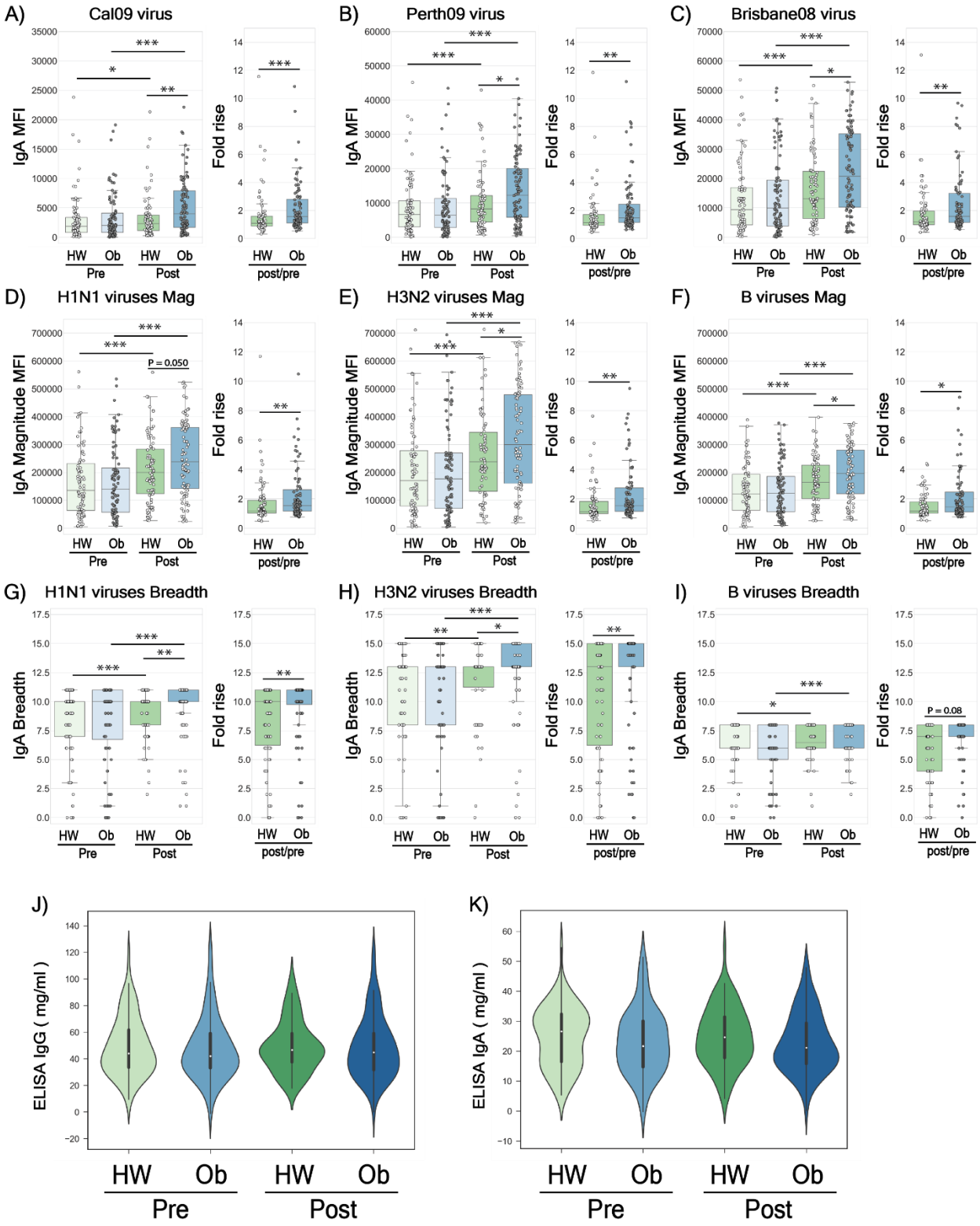

Fig. S3.

Baseline Immune History (BIH) and 30-day and post-vaccination IgA responses to a panel of BPL-inactivated influenza viruses by healthy weight and obese individuals, and Total IgG and IgA titers in the serum of obese and healthy-weight subjects.

Baseline and post-vaccination serum samples from 82 healthy-weight (HW) and 92 obese (Ob) subjects were hybridized with an antigen microarray spotted with 34 BPL-inactivated influenza viruses that included the three vaccine strains used in the study, for profiling of IgA binding. **(A-C)** IgA binding to the H1N1 A/California/7/2009, H3N2 A/Perth/16/2009 and B/Brisbane/60/2008 BPL-inactivated viruses. **(D-F)** The magnitude of IgA antibodies bound to a panel of 11 H1N1 virus strains (D); a panel of 15 H3N2 virus strains; (E) and a panel of recombinant HA antigens of 8 B virus strains (F). **(G-I)** The breadth of IgA antibodies bound to a panel of 11 H1N1 virus strains (G); a panel of 15 H3N2 virus strains; (H) and a panel of recombinant HA antigens of 8 B virus strains (I). The four box plots in the left portion of each panel summarize the baseline (L->R: HW: light green, Ob: light blue) and the 30-day post-vaccination (L->R: HW: dark green, Ob: dark blue) binding responses. The two boxplots on the right side of each panel represent the fold increase (L->R: HW: green, Ob: blue). Lines represent the median fluorescence intensity (MFI), the boxes denote the 25th and 75th percentiles, and the error bars represent 1.5 times the interquartile range. Statistical significance was assessed using the Wilcoxon signed rank test (pre vs. post) and the Wilcoxon rank-sum test (HW vs. Ob). * $p<0.05$, ** $p<0.005$, *** $p<0.0005$. **(J-K)** Cumulative distribution plots comparing the baseline and post-vaccination total level of IgG (J) and IgA (K) in the serum samples of 205 subjects, measured by sandwich ELISA. A white dot represents the median titer.

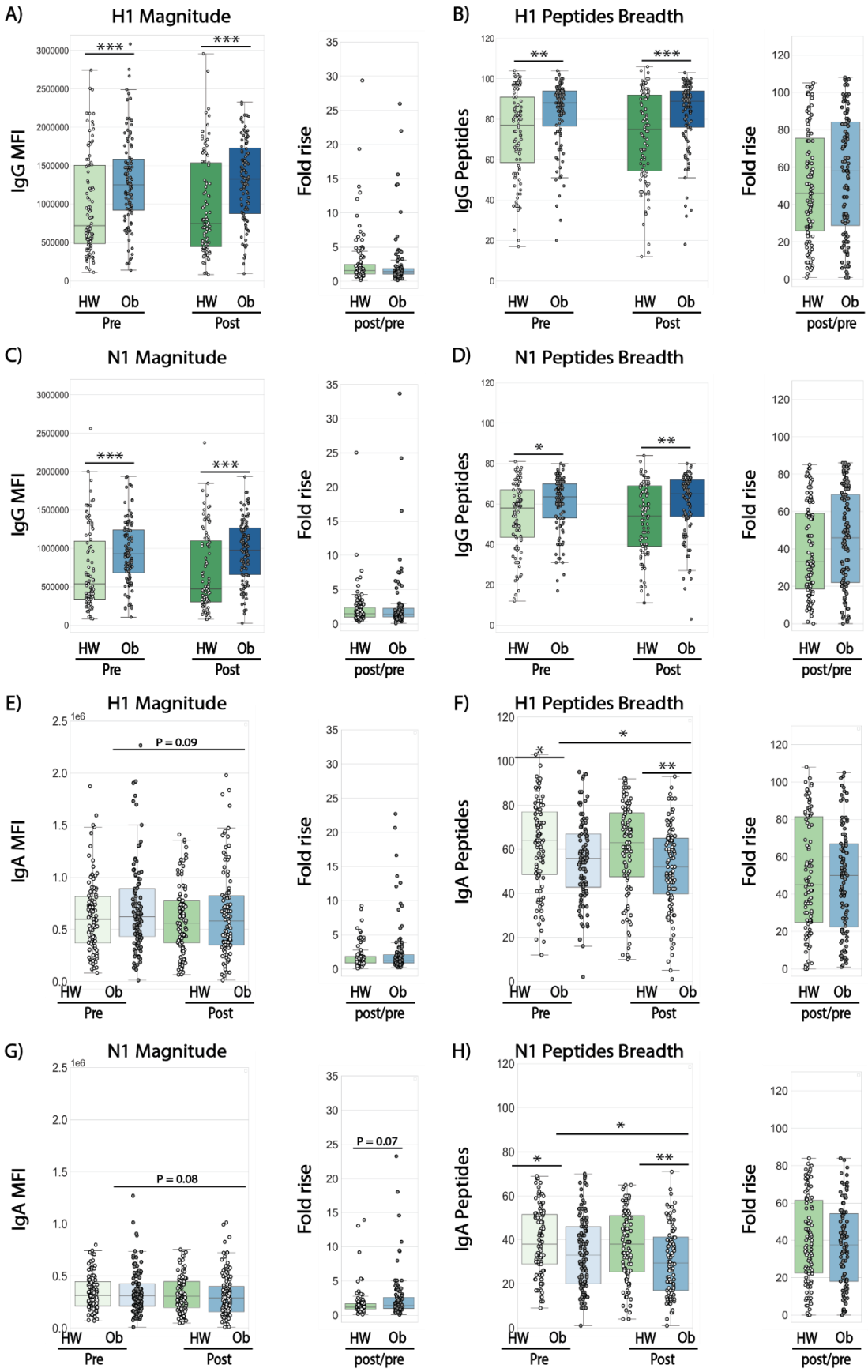

Fig. S4.

Baseline immune history (BIH), obesity- and age-associated magnitude and breadth of response profiles to influenza H1 and N1 peptides of the A/California/7/2009 vaccine strain.

Arrays spotted with 20mer amino acid peptides spanning the HA and NA proteins of the H1N1 vaccine strain (15aa overlap) were used to profile the repertoires of IgG and IgA responses to linear surface epitopes of Cal09. Responses were summarized using magnitude (sum of responses to all peptides of the same protein) and breadth defined as the number of peptides from each protein to which the subject had antibodies. **(A, C)** Magnitude of IgG responses to H1 (A) and N1 (C) peptides at baseline comparing healthy-weight (HW) and obese (Ob) subjects. **(B, D)** Breadth of IgG responses to H1 (B) and N1 (D) peptides at baseline. **(E, G)** Magnitude of IgA responses to H1 (E) and N1 (G) peptides at baseline. **(F, H)** Breadth of IgA responses to H1 (F) and N1 (H) peptides at baseline. The four box plots in the left portion of each panel summarize the baseline (L->R: HW: light green, Ob: light blue) and the 30-day post-vaccination (L->R: HW: dark green, Ob: dark blue) binding responses. The two boxplots on the right side of each panel represent the fold increase (L->R: HW: green, Ob: blue). Lines represent the median fluorescence intensity (MFI), the boxes denote the 25th and 75th percentiles, and the error bars represent 1.5 times the interquartile range. Statistical significance was assessed using the Wilcoxon signed rank test (pre vs. post) and the Wilcoxon rank-sum test (HW vs. Ob). * p<0.05., ** p<0.005, *** p<0.0005.

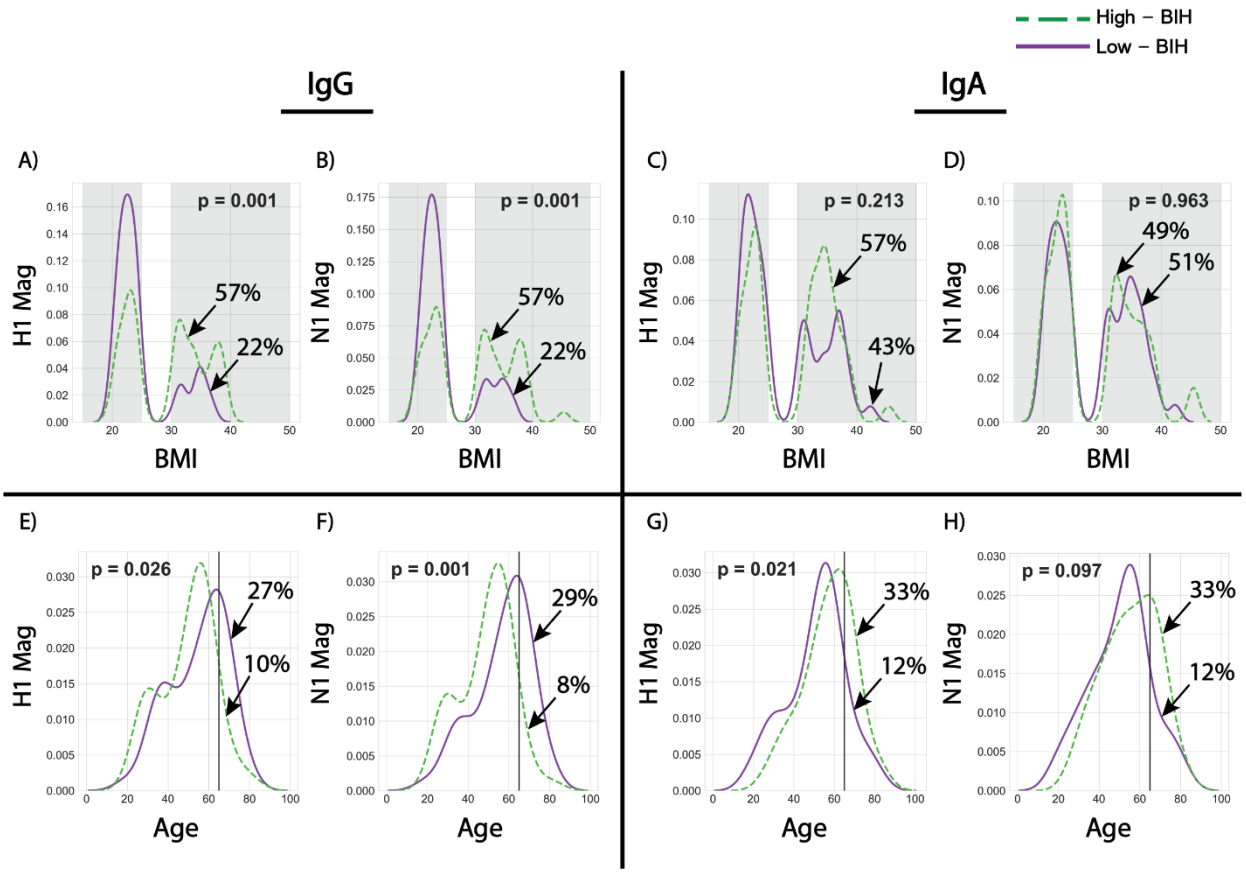

Fig. S5.

Obesity- and age-associated baseline immune history (BIH) profiles to influenza H1 and N1 proteins.

(**A-D**) Distribution of the magnitude response by BMI (Panels A to D). The distributions of BMI within the low-BIH group (n=51, solid purple line) and the high-BIH group (n=51, dashed green line) were plotted for IgG and IgA responses to Cal09 H1 and N1 antigens as follows: (A) IgG magnitude against H1 peptides (B) IgG magnitude to N1 peptides; (C) IgA magnitude to H1 peptides; and (D) IgA magnitude to N1 peptides. The percentages of HW and OB subjects in the high-quartile of BIH responders are listed. Overweight subjects (25 < BMI < 30) were excluded from our analysis. The percentage of obese subjects in the low-BIH responders and high-BIH responders quartiles are listed. (**E-H**) Distribution of the magnitude of response by age. The distributions by subjects age group (<65 y and > 65 y) within the low-BIH group (n=51, solid purple lines) and the high-BIH group (n=51, dashed green lines) were plotted for their IgG and IgA responses to H1N1 antigens as follows: (E) IgG magnitude against H1 peptides; (F) IgG magnitude to N1 peptides; (G) IgA magnitude against H1 peptides; and (H) IgA magnitude to N1 peptides. The percentages of individuals > 65y in the low and high-quartile of BIH responders are listed. p values for differences between the BMI and age distributions of the low-BIH and high-BIH groups were determined using the Wilcoxon ranksum test. Obesity was associated with high IgG-BIH to peptides of the Cal09 H1 and N1 peptides. Age >65 y was associated with low IgG- and IgA-BIH to Cal09 H1 peptides, and low IgG-BIH (but not IgA-BIH) to Cal09 N1.

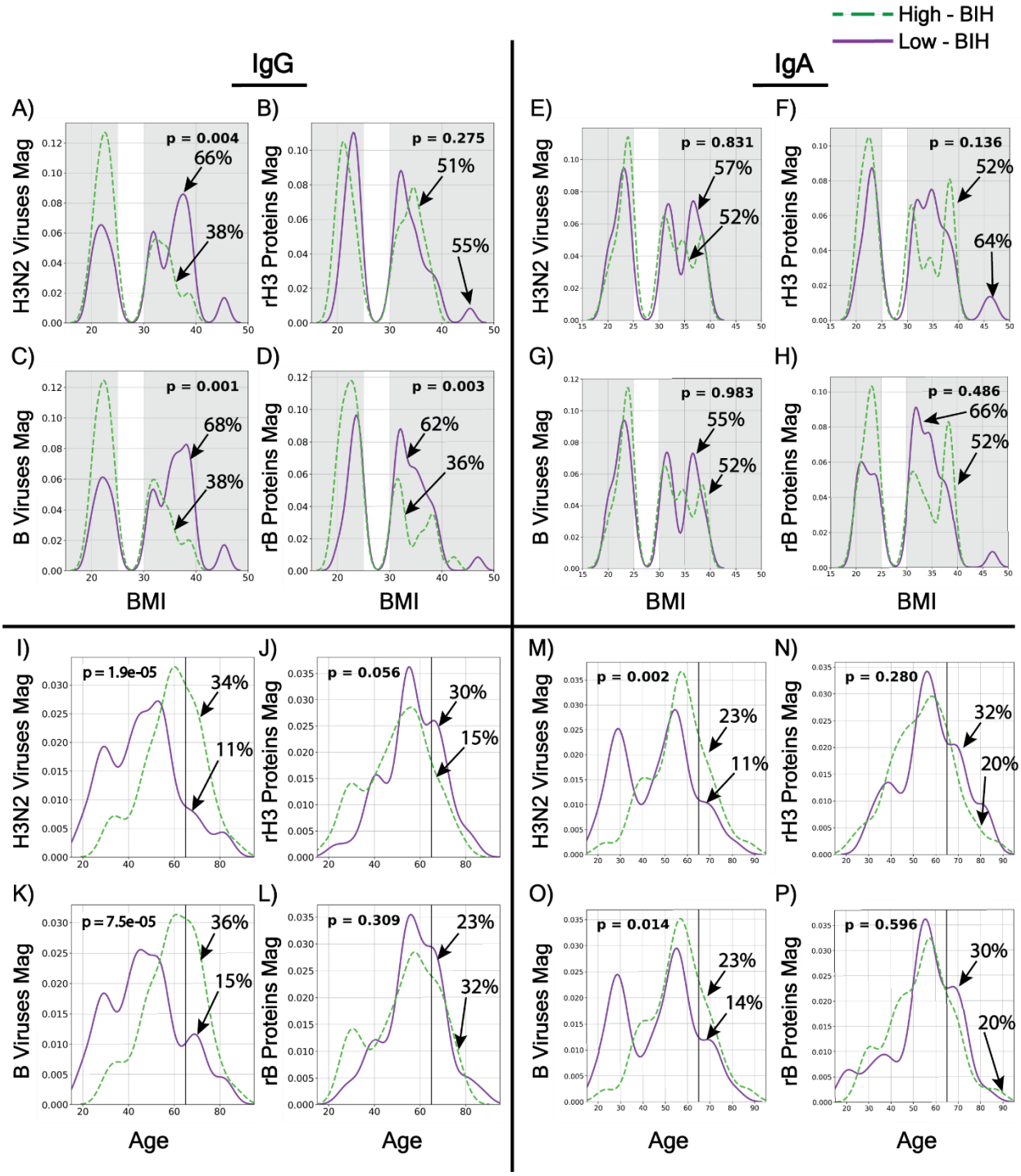

Fig. S6.

Obesity- and age-associated baseline immune history (BIH) profiles to influenza BL-inactivated H3N2 and B viruses and recombinant HA proteins.

(**A-H**) Distribution by BMI. The distributions of BMI within the low-BIH group (n=47 for IgG and n=44 for IgA, solid purple line) and the high-BIH group (n=47 for IgG and n=44 for IgA,

dashed green line) were plotted for IgG and IgA responses to H3N2 and B viruses and recombinant HA proteins as follows: (A) IgG magnitude against H3N2 viruses; (B) IgG Magnitude to rH3 proteins; (C) IgA magnitude to H3N2 viruses; (D) IgA magnitude to rH3 proteins; (E) IgG breadth to H3N2 viruses; (F) IgG breadth to H3 peptides; (G) IgA breadth to B viruses; and (H) IgA breadth to B proteins. (**I-P**) Distribution by age. The distributions by subjects age group (<65 y and > 65 y) within the low-BIH group (n=47 for IgG and n=44 for IgA,, solid purple line) and the high-BIH group (n=47 for IgG and n=44 for IgA,, dashed green line) were plotted for their IgG and IgA responses to H3N2 viruses and recombinant HA proteins as follows: (I) IgG magnitude against H3N2 viruses; (J) IgG Magnitude to rH3 proteins; (K) IgA magnitude to H3N2 viruses; (L) IgA magnitude to rH3 HA proteins; (M) IgG breadth to B viruses; (N) IgG breadth to rB HA proteins; (O) IgA breadth to B viruses; and (P) IgA breadth to rB HA proteins. p values for differences between the age distributions of the low-BIH and high-BIH groups were determined using the Wilcoxon ranksum test.

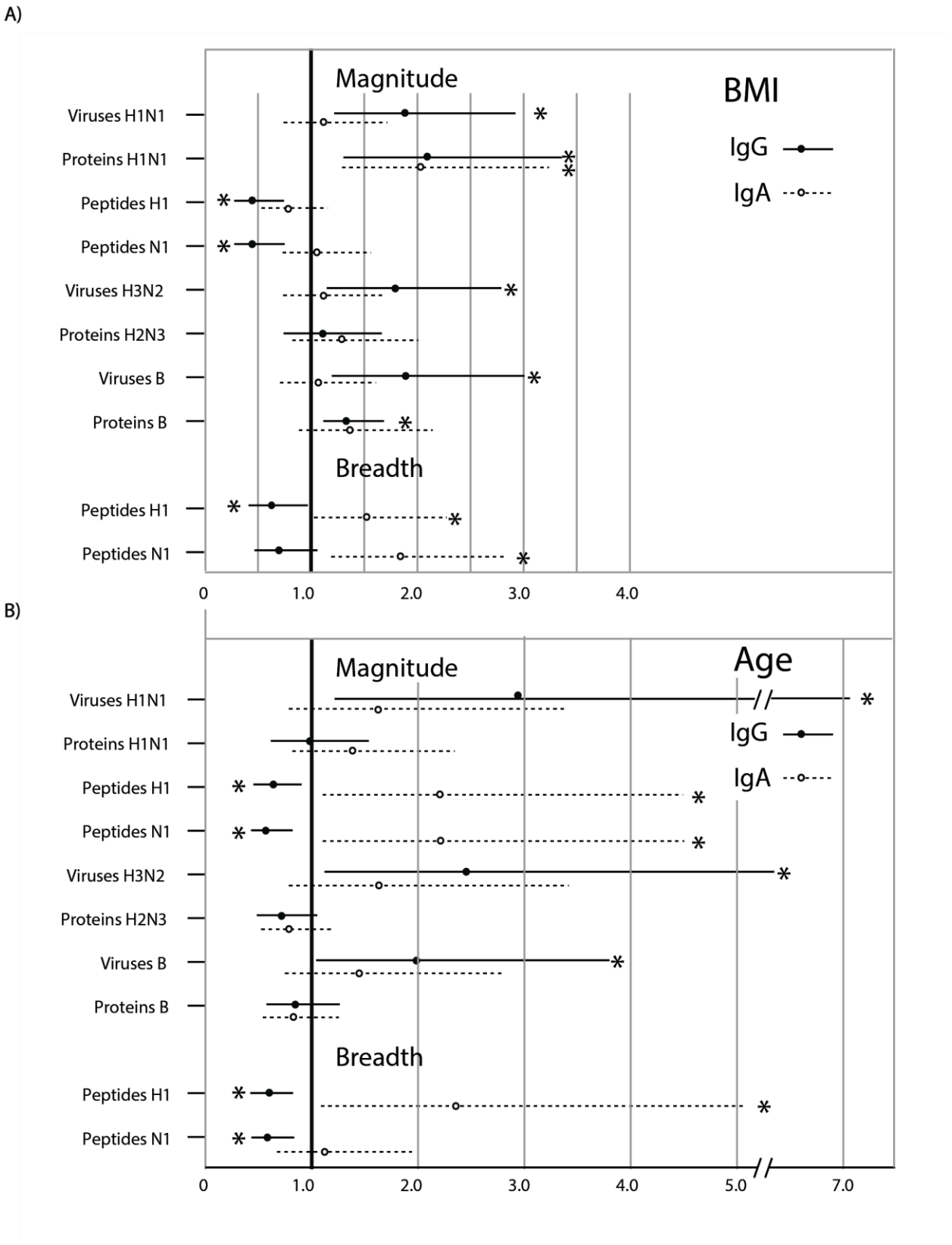

Fig. S7.

Relative risk for being in the lowest quartile of magnitude or breadth of BIH responses.

(A) Upper panel: Relative risk (RR) for obese individuals to have the magnitude of their IgG and IgA BIH responses in the lowest quartile. The relative risk and 95% CI for obese individuals to have the magnitude of their IgG (black filled circles) and IgA (white filed circles) BIH responses in the lowest quartile compared to healthy weight individuals was calculated using an on-line calculator from MedCalk (https://www.medcalc.org/calc/relative_risk.php). The range of the 95% confidence intervals are indicated by the solid and dashed. Lines, respectibvely. **(B)** Lower Panel: Relative risk (RR) for individuals <65 y have the magnitude of their IgG and IgA BIH responses in the lowest quartile compared with individuals >65 y. The relative risk and 95% CI for individuals <65 Y to have the magnitude of their IgG (black filled circles) and IgA (white filled circles) BIH responses in the lowest quartile compared to individuals >65 Y was calculated using the Python statsmodels package. The range of the 95% confidence intervals is indicated by solid and dashed lines, respectively. The dark horizontal line indicates a RR of 1. If the RR and 95% CI is larger than 1, then being obese or <65 y may be a positive risk for having a BIH magnitude in the bottom quartile. If the RR and 95% CI is smaller than 1, then bring obese or <65 y may be a protective factor, e.g., for not having a BIH magnitude in the bottom quartile. When the 95% CI includes 1, then being obese or <65 y may have no effect on the subject being in the lowest quartile of BIH response. The specific targets are listed on the vertical axis. 95% CIs that do not include 1 are indicated by an Asterix.

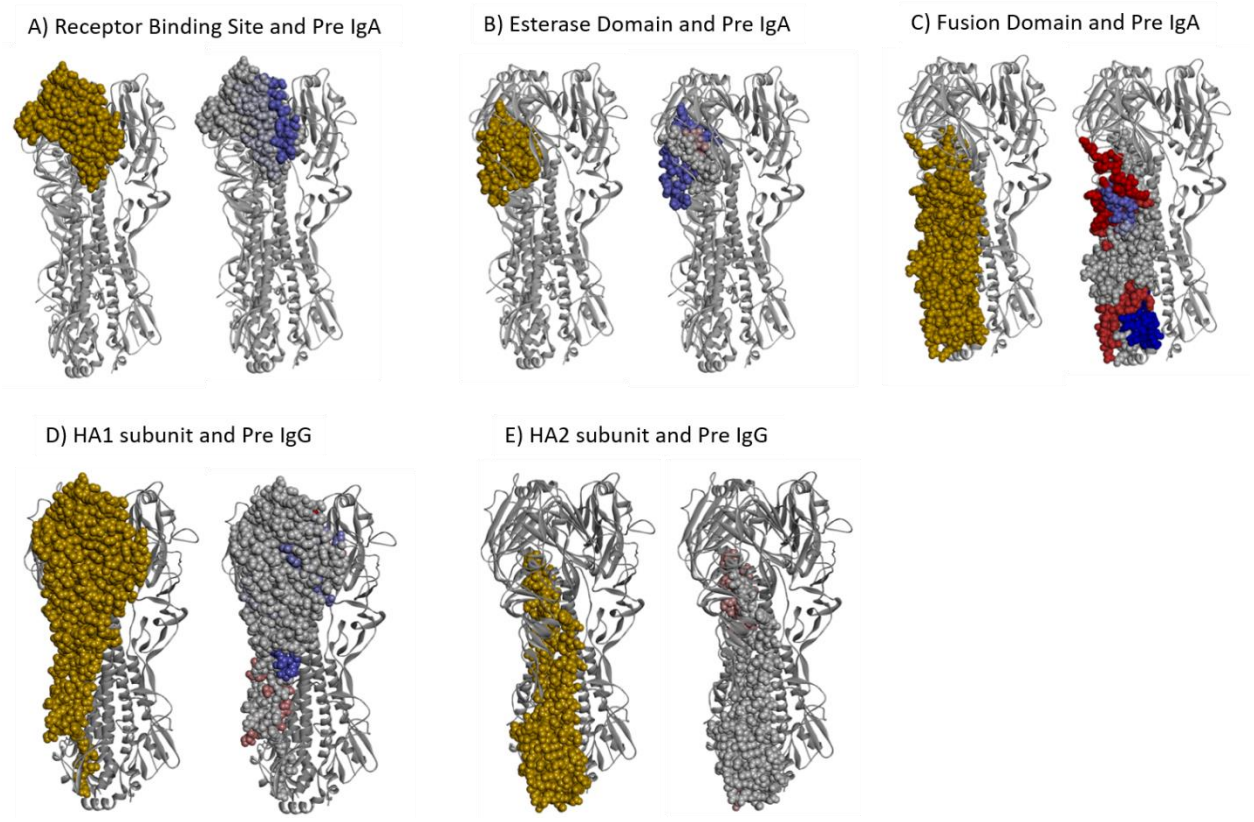

Fig. S8.

Different domains in the Cal09 HA protein are differentially targeted by BIH antibodies of obese and healthy weight subjects.

A logistic regression model was trained to discriminate between HW and OB individuals using the IgG and IgA antibody profiles to the HA peptides of the Cal09 vaccine strain. The weights assigned by the model were used to score individual amino acids on the HA protein based on the maximal weight of a given position across all of the peptides in which it was included (see Methods for details). Figures were created using Discovery Studio Visualizer software (XXref) and the crystal structure of the Cal09 HA trimeric protein PDB ID: 3LZG (10.1126/science.1186430). The HA trimeric protein is presented as a gray ribbon. Residues colored in gold comprise the given site. Residues associated with HW status are colored in blue shades according to their scores. Residues associated with OB status are colored in red shades according to their scores. Sites and scored residues are presented on a single HA subunit. Left side in each panel: Dark gold spheres represent amino acid residues belonging to each of the five regions of interest mapped onto one of the three trimeric proteins: **(A)** Receptor binding site; **(B)** The esterase domain; **(C)** The fusion domain; **(D)** the HA1 subunit; and **(E)** the HA2 subunit. Right side in each Panel: Dark blue spheres represent amino acid residues within the regions of interest preferentially associated with IgG or IgA antibodies in sera from HW individuals (Panels A to D) and dark red spheres represent amino acid residues within the regions of interest

preferentially associated with IgG or IgA antibodies in sera from HW individuals (Panels B, D, and E).

Table S1.

Whole inactivated viruses and recombinant HA proteins antigens spotted on the influenza VP microarrays

Subtype	Strain	Vaccine Year	Whole Virus	Recombinant Protein HA
H1N1	A/WSN/1933	-	X	X
	A/Puerto Rico/8/1934	-		X
	A/USSR/90/1977	-	X	X
	A/Brazil/11/1978	-	X	
	A/Chile/1/1983	1984-1987	X	
	A/Singapore/6/1986	1987-1997	X	
	A/Beijing/262/1995	1998-2000	X	X
	A/New Caledonia/20/1999	2000-2007	X	X
	A/Solomon Islands/3/2006	2007-2008	X	X
	A/Brisbane/59/2007	2008-2010	X	X
	A/California/7/2009 *	2010-2016	X	X
	A/Christchurch/16/2010	2010-2016	X	
H3N2	A/Bangkok/1/1979	-	X	
	A/Leningrad/360/1986	1987-1988	X	
	A/Guizhou/54/1989	-	X	X
	A/Shandong/9/1993	-	X	
	A/Sydney/5/1997	1998-2000	X	X
	A/Panama/2007/1999	2000-2004	X	
	A/New York/55/2004	2005-2006	X	X
	A/California/07/2004	-	X	
	A/Wisconsin/67/2005	2006-2008	X	X
	A/Brisbane/10/2007	2008-2010	X	X

	A/Perth/16/2009 *	2010-2012	X	X
	A/Victoria/210/2009	2011 (S)	X	X
	A/Victoria/361/2011	2012-2013	X	X
	A/Texas/50/2012	2013-2015	X	X
	A/Switzerland/9715293/2013	2015-2016	X	X
B	B/Lee/1940	-	X	
	B/Yamagata/16/1988	-	X	X
	B/Jiangsu/10/2003	2005-2006	X	
	B/Malaysia/2506/2004	2006-2008	X	X
	B/Florida/4/2006	2008-2009	X	X
	B/Brisbane/60/2008 *	2010-2017	X	X
	B/Massachusetts/2/2012	2014-2015	X	
	B/Phuket/3073/2013	2015-2016,	X	X
Number of strains:		34	23	
Number of strains included in seasonal vaccines:		25	18	

(S) - southern hemisphere vaccine

* The strains included in the vaccine given in this trial