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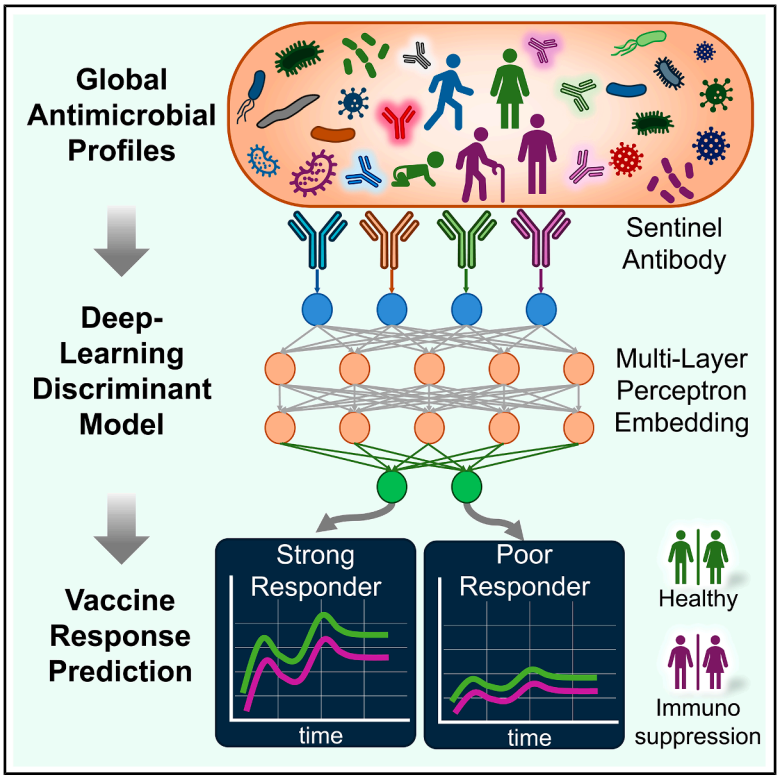
Pre-vaccine sentinel antibodies predict blunted vaccine responses

Graphical abstract



Highlights

- Pre-existing antibodies to common microbes predict new vaccine response robustness
- These sentinel antibodies are stable markers of humoral immune readiness
- AI models use global antibody profiles to stratify high and low vaccine responders
- Blunted vaccine responses occur in both immunosuppressed and healthy individuals

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In brief

LaBaer and colleagues demonstrate that pre-existing antibodies to common microbes predict the strength of new vaccine responses. By analyzing thousands of individuals, they identified "sentinel antibodies" that reflect baseline immune robustness. This framework uses AI to identify poor responders, offering a transformative approach to personalized vaccination strategies.

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Pre-vaccine sentinel antibodies predict blunted vaccine responses

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SUMMARY

Predicting which individuals will mount poor antibody responses before vaccination could improve personalized vaccination strategies. Here, we conducted a national longitudinal study of humoral immune responses to 185 antigens, including SARS-CoV-2 ($n = 3$), common microbial pathogens ($n = 157$), and autoantigens ($n = 25$), in 1,644 immunosuppressed patients and 2,445 healthy individuals before and after COVID-19 vaccination. Although blunted COVID-19 vaccine responses were more frequent in solid organ transplant recipients and individuals with multiple myeloma, autoimmune disease, inflammatory bowel disease, and human immunodeficiency virus, responses were highly heterogeneous within every cohort, and approximately 5%–6% of healthy individuals also mounted weak responses. Pre-existing antibodies to common microbes, including *Staphylococcus aureus*, respiratory syncytial virus, and human respirovirus 3, consistently predicted post-vaccination antibody responses in both healthy and immunosuppressed populations. These broadly prevalent antimicrobial antibodies represent sentinel antibodies that may serve as biomarkers of system-level humoral immune competence. Using global antimicrobial antibody profiles, we developed a deep-learning predictive model that stratified individuals according to their likelihood of mounting blunted vaccine responses. Together, these findings identify pre-existing antimicrobial antibody profiles as scalable biomarkers of humoral immune responsiveness and provide a framework for predicting vaccine responses before immunization.

INTRODUCTION

Individuals' immune responses to vaccination lead to heterogeneous response magnitude, duration of antibody levels, and cellular immunity against multiple pathogens,[1–5] including SARS-CoV-2.[6] Multiple factors have been reported to associate with vaccine-induced response, including age,[7,8] gender,[9,10] genetics,[4] pre-existing immunity,[2,11] and comorbidities.[12,13] Antibody titer is commonly used to evaluate vaccine efficacy and is associated with protection and durability.[1,4] After spike protein

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vaccination, antibody titers to SARS-CoV-2 receptor-binding domain (RBD) strongly associate with the neutralizing antibody levels,[14,15] as well as a reduction in COVID infection,[15,16] risk of severity and/or hospitalization,[15] long COVID,[17] and morbidity,[15] along with other associated complexities involving cellular immunity,[18] variant specificity,[19] and individual demographic factors and immune status.[17,20–22] Elder age associates with reduced antibody response via vaccination and a higher risk.[7,8,10] Women commonly have a higher antibody response than men but with little or no significance.[9,10] Systematic analysis identified specific genetic and immunologic factors that can predict the vaccine-induced antibody response, including the pre-perturbation cell population,[23] positive and negative baseline signatures of lymphocyte and monocyte inflammation,[24] gene signatures,[25] pre-vaccination inflammation and B cell signaling,[26] and pre-vaccination innate immune endotypes characterized by genes associated with pre-inflammatory and time-adjusted signatures identified through a large-scale analysis of transcriptional data of over 3,000 samples via 13 vaccines.[4,5] Prediction models based on these genetic signatures predict the post-vaccination response with area under the receiver operating characteristic (ROC) curves (AUCs) ranging from 0.618 to 0.790.[4,5,25,26] However, evaluating these signatures requires gene expression or genomic analysis, limiting their use in clinical settings. A simple clinical test, such as a serological assay, would make it easier to assess humoral immune health.

In addition to demographic and genetic factors, immunosuppressed individuals are at a significantly higher risk for severe COVID-19 infection, hospitalization, and death compared to healthy individuals.[21,22,27] The National Health Interview Survey (NHIS) estimated that 6.6% of US adults had immunosuppression due to disease, medication, or both in 2021.[28] Nationwide analysis (INFORM)[21] found that immunocompromised individuals accounted for 3.9% of the population of England but 22% of COVID-19 hospitalizations, 28% of COVID-19 ICU admissions, and 24% of COVID-19 deaths, despite >80% having received $\geq$3 doses of COVID-19 vaccines. COVID-19 vaccine response studies in immunosuppressed individuals have not evaluated responses to other pathogens. Such responses, reflecting prior immune history, might inform us about immune health and expected response to SARS-CoV-2. Moreover, earlier studies have been limited to a single clinical type of immunosuppression rather than comparisons across multiple groups. Although immune disorder-specific antibody biomarkers have been reported, their association with immunosuppression status and the subsequent vaccine-induced response remains unclear. Patients with these diseases, including those with disease-specific antibody markers, exhibit heterogeneous antibody responses following vaccination, and while some have blunted vaccine responses, many have normal immune function.

Antibodies to other infectious and microbiome components may act as sentinels by predicting or even contributing to an individual's response to the virus and the vaccine.[29,30] Studies evaluating the impact of pre-existing antibodies on COVID-19 vaccination have focused on SARS-CoV-2 antigens (spike protein and nucleocapsid protein [NC]) and seasonal coronavirus proteins based on the theories of immunological imprinting,[31] antigen-dependent enhancement,[32] and possible cross-reactivity via similar epitopes.[33] Previous infection with SARS-CoV-2 itself led to hybrid immunity that generated higher anti-RBD levels following vaccination.[34] Although pre-existing antibodies correlated with stronger overall responses, they did not show strain-specific responses, suggesting baseline immunity imprinting.[31] Cross-reactive immunity between SARS-CoV-2 and common coronaviruses (HCoV, alpha coronavirus [229E and NL63], and beta coronavirus [OC43 and HKU1]) has been evaluated. Although pre-existing humoral and T cell cross-reactivity was observed in some pre-COVID-19 samples,[11,35] a relationship between baseline HCoV antibody levels and protection against SARS-CoV-2 infection or vaccination remains in question.[11,36]

Antimicrobial antibodies against 77 viruses and 3 bacteria between mild and severe COVID-19 were compared in 350 samples and found elevated in severe COVID patients, along with increased anti-HCoV-229E and NL63 but not HCoV-OC43 and HKU1 levels.[37] Saito et al.[38] evaluated antimicrobial antibodies in 47 samples against 5,000 microbe-derived proteins after the COVID-19 mRNA vaccine and found limited changes after the vaccine, although they observed some trends.[38] Additionally, COVID-19 infection may involve autoimmunity (AIM). High titers of neutralizing autoantibodies against type I IFN-α2 and IFN-ω were found in about 10% of patients with severe COVID-19 pneumonia, which impaired innate and intrinsic antiviral immunity against SARS-CoV-2.[39] Anti-nuclear antibodies (ANAs) and other autoimmune antibody markers associated with systemic AIM were found in hospitalized COVID-19 patients with combined reactivity.[40] The ANA was also persistently positive at 12 months post-COVID, suggesting a role in long COVID.[41] Most of these correlations were found among healthy individuals; their impact on COVID-19 vaccination in immunosuppressed individuals was seldom reported.

The National Cancer Institute (NCI) Serological Sciences Network (SeroNet) established four capacity-building centers (CBCs) across the US to comprehensively evaluate COVID-19 vaccine efficacy in individuals with conditions linked to immunosuppression. Participants with diverse immunosuppressive conditions and healthy controls were recruited and monitored over their initial two-dose mRNA COVID-19 vaccination and/or booster (Table S1). The humoral immune response against antigens from SARS-CoV-2, other pathogens or commensal microbes, and autoantigens was profiled using a high-throughput multiplexed assay that measures antibodies to full-length folded protein antigens, capturing both linear and conformational epitopes.[42] A customized protein library was developed to incorporate antigens from respiratory pathogens, other common pathogens, chronic viruses, antigens related to human immunodeficiency virus (HIV), autoimmune conditions, and additional microorganisms of interest, accommodating the study of various immunosuppressed cohorts and vaccination.

RESULTS

High-throughput, comprehensive antimicrobial and autoantibody profiling

Four CBCs, including Arizona State University (ASU), Icahn School of Medicine at Mount Sinai, Feinstein Institutes for Medical Research-Northwell Foundation, and University of

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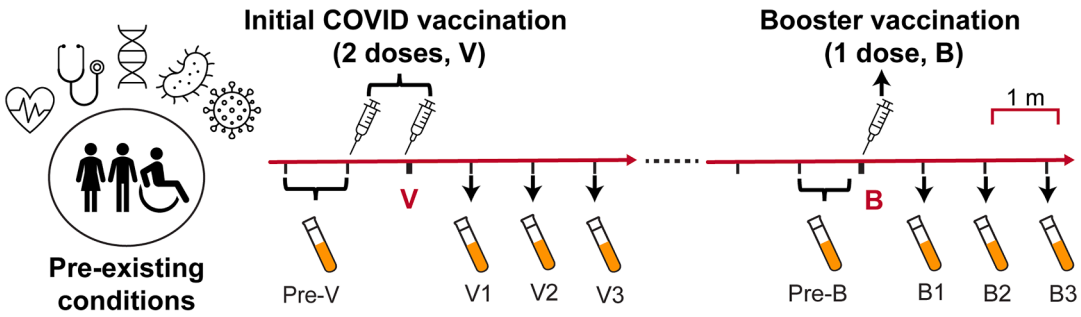

COVID vaccine assessment vs. pre-existing conditions

Primary condition cohorts

Capacity Building Center (CBC)	Healthy	Autoimmune		Cancer		HIV	SOT[4]	Total
		IBD	AIM[1]	MM[2]	SOM[3]			
Mount Sinai	0	351	10	828	33	0	180	1402
ASU	2715	0	69	0	72	612	16	3484
UMN	0	0	1	3	190	43	213	450
Feinstein	1602	0	1654	0	91	4	0	3351
Total sample (participant)	4317 (2445)	351 (125)	1734 (644)	831 (293)	386 (204)	659 (172)	409 (206)	**8687** (4089)

Multiplexed antibody profiling (MISPA), 185 antigens

Coronavirus	Respiratory virus	Herpes virus	Other virus	Bacteria	Autoantigens
• SARS-CoV-2	• Influenza	• HHV1	• HIV	• *Streptococcus*	• p53
• HCoV-229E	• RSV	• HHV2	• Enterovirus	• *Staphylococcus*	• TRIM21
• HCoV-NL63	• HMPV	• EBV	• Coxsackievirus	• *E.coli*	• Interferon
• HCoV-HKU1	• HRV3	• HCMV	• Norovirus	• *Shigella*	• Interleukin
• HCoV-OC43	• ...	• ...	• ...	• ...	• ...

AIM[1]: Other autoimmune; MM[2]: Multiple Myeloma; SOM[3]: Solid organ malignancy; SOT[4]: Solid organ transplant;

Figure 1. High-throughput, comprehensive antimicrobial analysis using MISPA

Schematic workflow for longitudinal sample collection for the COVID-19 vaccine and booster. Samples were allocated across four capacity-building centers (CBCs), and seven cohorts were grouped. The analysis included medical history, demographic information, and antibody levels against viruses, bacteria, and autoantigens.

Minnesota Medical School (UMN), participated in the SeroNet program in response to the high-capacity testing needs during the COVID-19 pandemic.[43] To understand the relationship between individuals' humoral immune status and their response to the COVID-19 vaccine, especially for participants with pre-existing immunosuppressed status, longitudinal samples around COVID-19 vaccination were collected in seven primary cohorts: healthy (n = 4,317), HIV (n = 659), multiple myeloma (MM) (n = 831), solid organ malignancy (SOM) (n = 386), inflammatory bowel disease (IBD) (n = 351), AIM (n = 1,734), and solid organ transplant (SOT) (n = 409). A total of 8,687 individual samples were collected, including pre- and post-vaccination samples from 4,089 participants who received the initial COVID-19 vaccination (2 doses for mRNA or 1 dose for Johnson & Johnson [J&J]) and/or booster (1 dose after the initial vaccination). To evaluate antimicrobial and autoantibodies and their association with the response to the COVID-19 vaccine, a high-throughput, multiplexed assay, multiplexed in solution protein array (MISPA), was used to measure antibodies against a broad range of 185 protein antigens from viruses (coronaviruses, respiratory viruses, herpes viruses, etc.), bacteria (*Streptococcus*, *Staphylococcus*, *Shigella*, etc.), autoantigens of interest (autoimmune antigens, immune modulators, cancer antigens, etc.), and a negative antigen control, green fluorescent protein (GFP) (Figure 1).

The 185 full-length microbial- and autoantigens, as well as GFP, were individually prepared (Figure S1; Table S2) and uniquely barcoded (Figure S2). A total of 12,110 samples, including serum samples from participants (n = 8,687), positive/negative controls (n = 471), and technical replicates (n = 2,952, see STAR Methods section), were analyzed, and the overall antibody response profiles of the serum samples are shown in Figure 2. To ensure reproducibility, a common bridging set of 81 serum samples and 192 blind samples were analyzed in each of the eight assay rounds and compared. They showed an average coefficient of variation (CV) of 18.1% (13.0%–26.0%) for the 81 serum samples (Figure S3) and an average CV of 23.5% (16.0%–29.0%) for the 192 blind samples among the eight replicates (Figure S4).

When compared with other quantitative antibody assays for SARS-CoV-2, antibody levels against SARS-CoV-2 Wuhan

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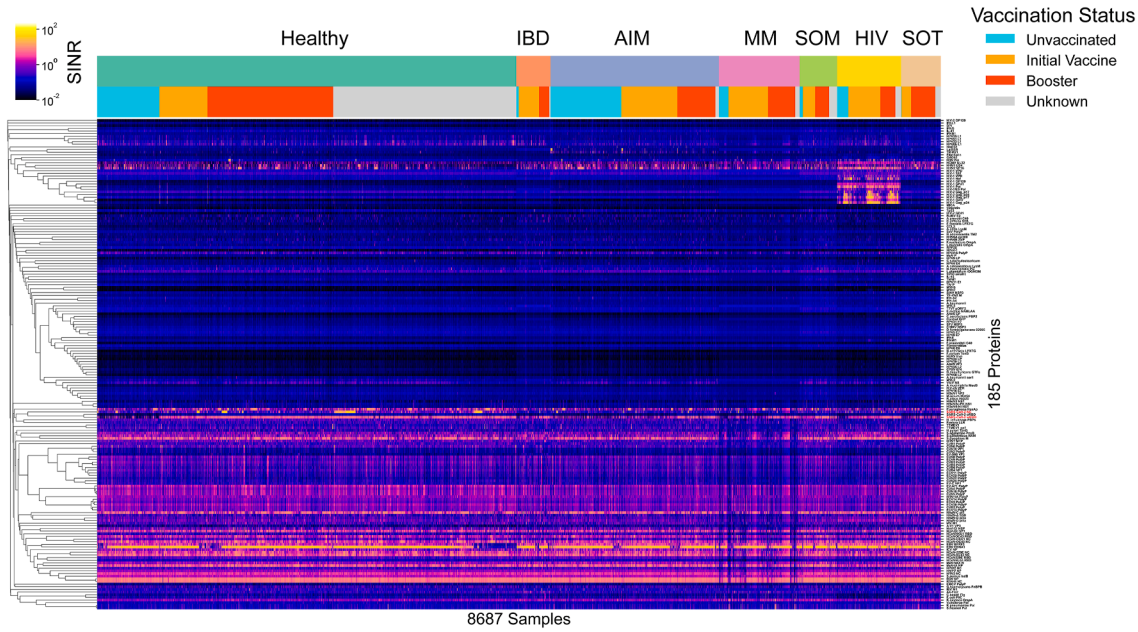

Figure 2. The multiplexed humoral immune profiling of 8,687 individual samples among seven cohorts

The cohorts are labeled on the top, and the antigen names are labeled on the right, with their corresponding vaccination status. Antibodies were hierarchically clustered by Euclidean distance and average linkage. The SARS-CoV-2 antigens were highlighted in red on the right.

RBD (wRBD) from MISPA showed an overall percentage agreement (OPA) of 95.1% with five different commercial or lab-developed assays (Figure S5, ACCESS SARS-CoV-2 Spike IgGII [Beckman, DXI], LIAISON XL SARS-CoV-2 TrimericS IgG [Diasorin], LIAISON XL SARS-CoV-2 S1/S2 IgG [LIAISON XL], Kantaro SeroKlir Semi-Quant Assay [Kantaro], and UMN Spike IgG assay [UMN]).

Blunted anti-RBD response to COVID-19 vaccine in individuals with suppressed immune status

To determine if the pre-existing immune status and/or disease conditions affected the response to SARS-CoV-2 vaccination, anti-wRBD antibody levels were compared before and after both the initial vaccination (V, 2 doses, n = 1,569, pre-V and post-V) and the booster shots (B, single dose, n = 2,412, pre-B and post-B) between the cohorts (Figure 3). We observed significant differences in anti-wRBD antibody response across cohorts during both vaccinations. We defined the seroprevalence thresholds of <0.5 (weak), >3.5 (high), and 0.5–3.5 (moderate) AU/ml, which corresponded to the binding antibody units per milliliter (BAU/ml) thresholds to define robust vaccine/booster responses used previously in a study of 463 patients[44] and another of >2,500 patients,[12] and the samples were classified accordingly as having weak, moderate, or high seroreactivity responses (Figure 3A).

Following vaccination (post-V), compared to healthy patients (6.2%; Figures 3A and 3B), several immunosuppressed clinical cohorts had significantly larger fractions of weak responders (lower than 0.5 AU/ml during the initial vaccination): 71.6%, 48.5%, 43.0%, and 39.0% of the SOT, MM, IBD, and AIM cohorts (chi-squared test, $p = 2.3 \times 10^{-21}$, 1.9×10^{-11}, 1.4×10^{-9}, and 2.9×10^{-8}), respectively. In comparison, relatively smaller fractions of the patients with SOM (17.6%, p = 0.012) and HIV (9.8%, not significant) showed blunted vaccine response, albeit still larger than the healthy population. On the other hand, many in the immunosuppressed cohorts (9.9%–43.1% of participants) had strong responses after the initial vaccination, in some cases approaching levels of the healthy cohort (53.7%), indicating highly heterogeneous vaccine responses among individuals in the same clinical cohort. As previously reported, COVID-19 infection prior to initial vaccination led to a stronger response, except in the SOT cohort (Figure S6).

After the booster (post-B), most healthy individuals (95.0%) had high responses, while 5% fell within the moderate range. Most of those among the immunosuppressed cohorts also responded well to the booster. Despite their immunosuppressed status, only a small portion showed weak or no responses, including the HIV (3.6%), SOM (3.6%), IBD (2.7%), and MM (9.1%). Notably, AIM (17.5%) and SOT (37.9%) showed a significant fraction of weak or no responses to the COVID-19 booster. Taken together, belonging to an immunosuppressed cohort was a strong risk factor for a weak or absent vaccine response, but it was not a sufficient predictor of poor booster response (Figure 3). COVID-19 infection prior to the booster vaccination led to slightly stronger responses in the AIM and SOT cohorts (Figure S6).

Population-level seroprevalence and correlations of antibody responses to microbial antigens

Multiplexed antibody profiling across all samples enabled exploration of global serological patterns, including seroprevalence and correlations among antibody responses. We measured the

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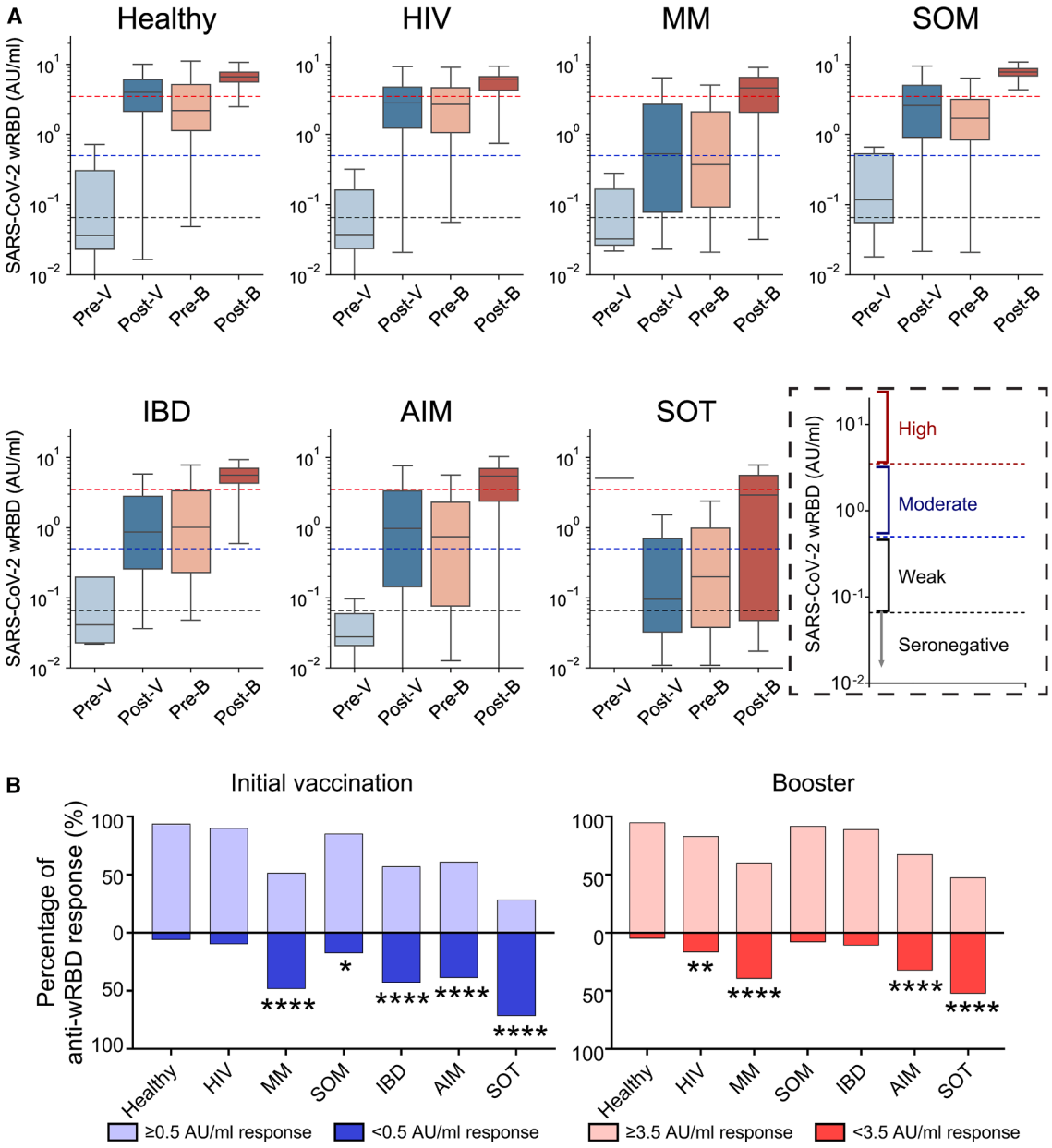

Figure 3. Blunted anti-RBD response to the COVID-19 vaccine among different cohorts during the initial vaccination and the booster vaccination

(A) Anti-wRBD antibody levels (AU/ml) before and after the initial vaccination (2 doses, pre-V and post-V, $n = 1,569$) and the booster vaccination (1 dose, pre-B and post-B, $n = 2,412$) among seven cohorts. The black dotted lines represent the seropositive cutoff for anti-wRBD. The red and blue dotted lines represent high, moderate, and weak anti-wRBD levels at cutoffs of 3.5 and 0.5 AU/ml. Boxes indicate the interquartile range (IQR; 25th–75th percentiles) with median lines; whiskers extend to 1.5 × the IQR.

(B) The percentage of participants who fell into the three categories of anti-wRBD levels at the cutoffs of 3.5 and 0.5 AU/ml. The percentage of participants who had lower anti-RBD responses (percentage less than 0.5 and 3.5 AU/ml during the initial and booster vaccinations, respectively) was assessed using a chi-squared test compared with the healthy cohort. The light and dark blue or red colors represented the percentage of not optimal and optimal anti-wRBD responses during the initial and booster vaccinations. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

seroprevalence for all 185 antigens in all cohorts, and 114 antibodies showed more than 10.0% seroprevalence (seroprevalent antibodies) in at least one cohort using the technical cutoff (Figure S7; Table S3; see STAR Methods). We examined the general stability/variability of antibodies by assessing changes relative to the first sample collected for each participant who had 3 or more samples collected across 18 months ($N = 743$; February 2021 to September 2022). Most antibodies (103/114,

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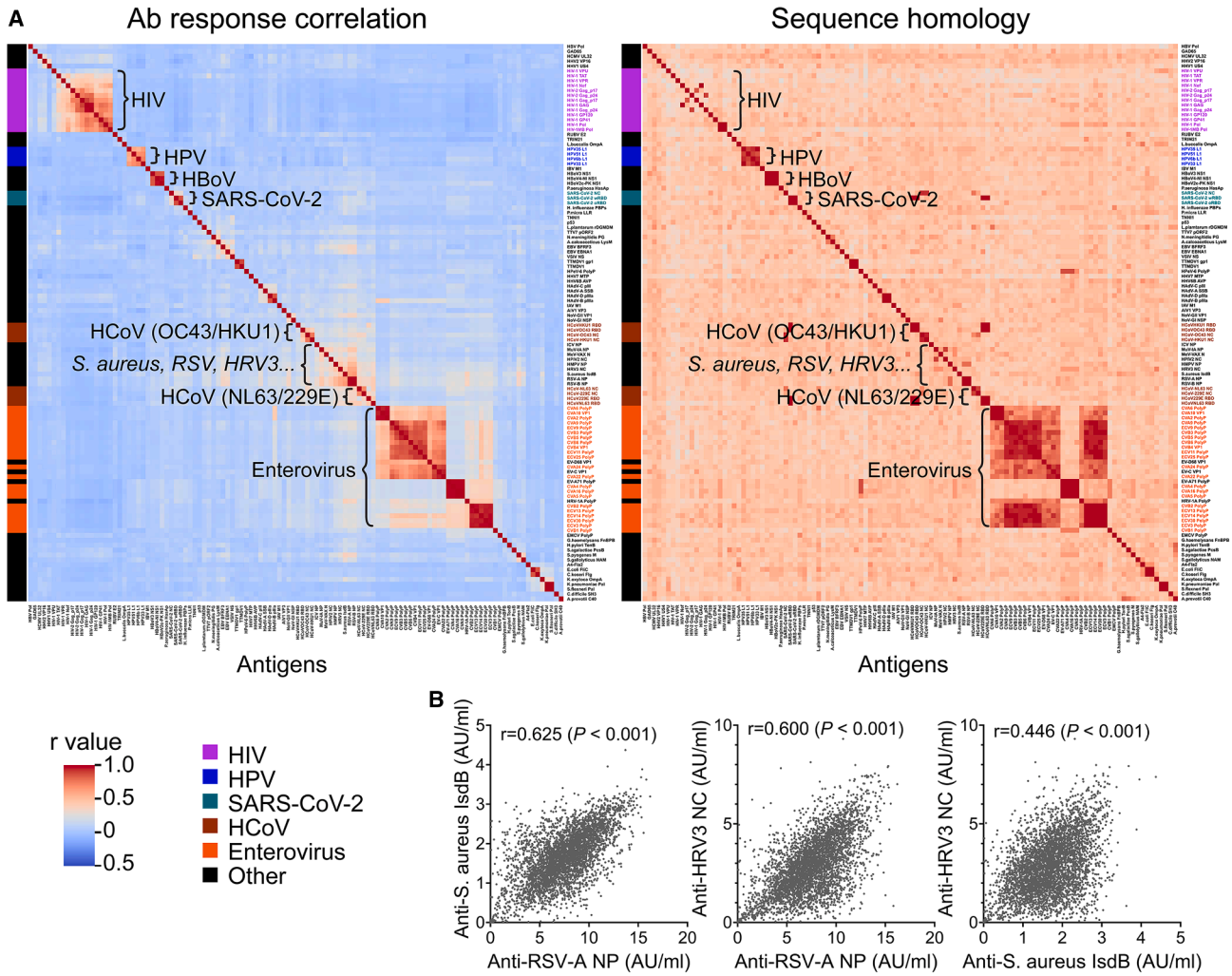

Figure 4. Correlated antibody responses and contribution of antigen sequence homology in the first sample per participant (n = 4,089)

(A) Seroprevalent antibodies (n = 114) were clustered by the response profiles (left, Pearson correlation), and, in the same order of antibodies, pairwise sequence homologies (defined as maximum identity across 20-amino acid sliding windows with 10-amino acid overlap) were overlaid (right).

(B) Correlation between antibodies against RSV-A, *S. aureus*, and HRV3. r, Pearson's correlation coefficient; p value, two tailed p value for Pearson's correlation test.

90.4%) did not change over time, with less than 2% of participants experiencing any change more than 4-fold (Figure S8; Table S4), as noted previously.[42] Given the short half-life of IgG,[45] these results indicate that serum antibody levels are tightly regulated, and antibody profiles reliably retain individuals' infection history.

We therefore used the first collected sample as a representative for each participant (n = 4,089) to assess antibody responses. Nineteen antibodies had more than 10.0% seroprevalence in only a single cohort, either the HIV (anti-HIV antibodies, n = 14) or SOM (n = 5, e.g., anti-p53) cohorts (Figure S7). Among the 95 antibodies that showed seroreactivity across multiple cohorts, more than half (51/95, 53.7%) showed greater than 50.0% median seroprevalence and 14 antibodies had a median seroprevalence exceeding 90.0% across all cohorts, including antigens from *Staphylococcus aureus* (*S. aureus*), respiratory syncytial virus (RSV; RSV-A and RSV-B), human metapneumovirus (HMPV), human respirovirus 3 (HRV3), seasonal coronaviruses (HCoV-NL63, 229E, and OC43), Mumps orthorubulavirus (MuV-IA), norovirus GII (NoV-GII), rhinovirus A1 (HRV-1A), Epstein-Barr virus (EBV), and echovirus E30 (ECV30). Among the 71 antibodies with less than 10.0% seroprevalence in individual cohorts, 58 (82.9%) demonstrated seroreactivity in at least 5 samples across all cohorts, confirming their antigenicity, whereas low or undetectable seroprevalence was observed for 13 antigens, including GFP.

To examine whether antibody responses to different antigens were intercorrelated, we performed a clustering analysis based on Pearson's correlation coefficients among the 114 seroprevalent antibodies, revealing several clusters of correlated antibodies (Figure 4A, left). As correlations between antibodies can arise from high sequence homology among antigens from related organisms, we compared the antibody correlation matrix with a matched heatmap of pairwise protein sequence homology, or

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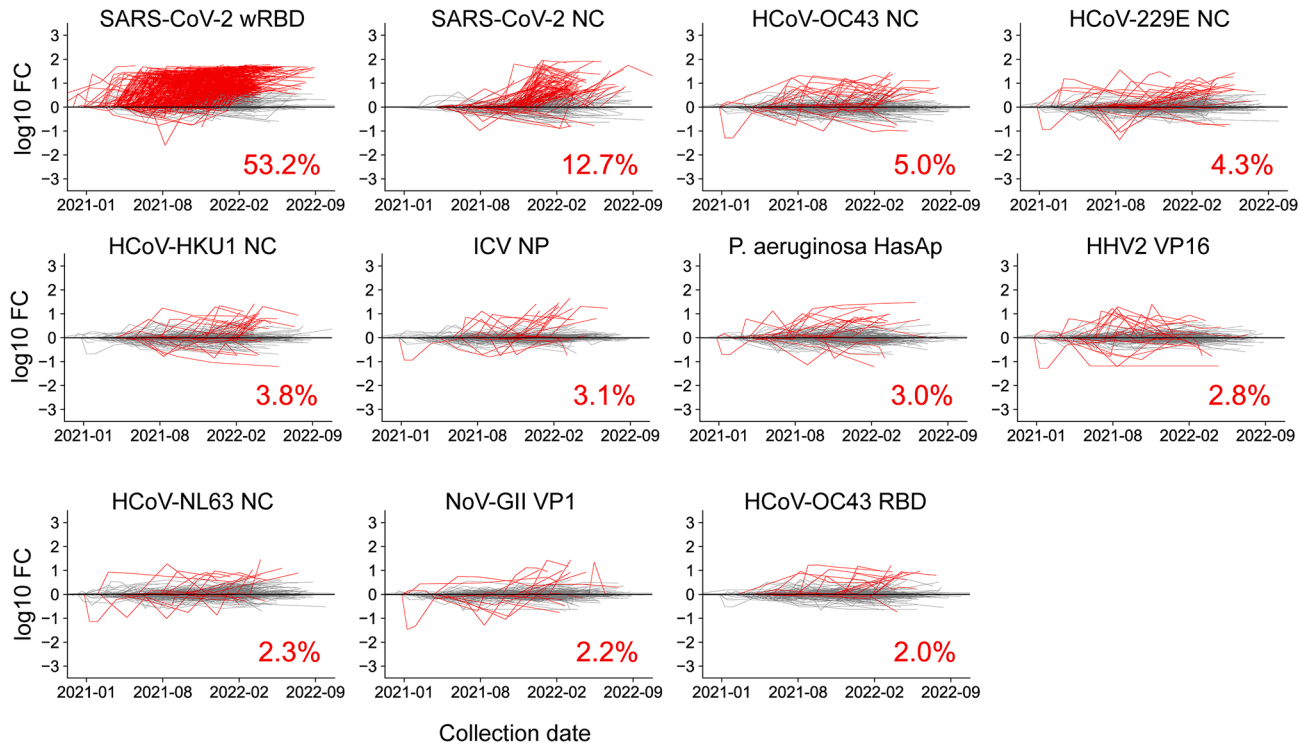

Figure 5. Antibodies with the greatest changes among participants during the study period (February 2021 to September 2022)
Log2 fold changes of individual longitudinal samples over the first collected samples for each participant are shown. Lines for participants with >4-fold change are highlighted in red. The percentage of participants with at least one 4-fold antibody titer change is labeled in the bottom right corner for each antibody.

due to antibodies to antigens derived from the same pathogen that would arise together (Figure 4A, right). Correlation of the enterovirus (EV), human papillomavirus (HPV), and human bocavirus (HBoV) clusters was driven primarily by antigens with high sequence homology (PolyP, L1, and NS1, respectively). There was also a cluster of correlated HIV antigens attributable to multiple antigens from the same virus. The SARS-CoV-2 and HCoV clusters were driven by both homologous proteins (e.g., RBD from Wuhan and Omicron variants of SARS-CoV-2) and multiple proteins from the same or related organisms (e.g., RBD and NC from HCoV-OC43 or HKU1). No correlation was observed between antibody responses for SARS-CoV-2 RBD and seasonal coronaviruses (Figure 4A), despite partial sequence homologies between them. We also observed a group of correlated antibodies against antigens from *S. aureus*, RSV, HRV3, and HMPV with no apparent sequence homology (Figures 4A, near the middle, and 4B). Notably, these antibodies were the most prevalent, with seroprevalence rates over 98% (Figure S7; Table S3).

Some frequently recurring pathogens showed changes over time

Although the majority of antibody levels did not change appreciably during the course of this study (Figure S8), some displayed longitudinal changes. As expected, 51.4% and 12.7% of participants had more than 4-fold changes in anti-wRBD (i.e., vaccination and/or infection) and anti-SARS-CoV-2 NC (i.e., infection) antibody levels over at least one pair of consecutive time points, respectively (Figure 5). Aside from SARS-CoV-2, antibodies with over 4-fold changes in 2%–5% of participants targeted antigens from commonly recurring pathogens (n = 9), including seasonal coronaviruses, human alphaherpesvirus 2 (HHV-2), influenza C virus, norovirus, and *Pseudomonas aeruginosa*, which were among the most seroprevalent antibody targets (Figures 5 and S7).

Pre-existing antimicrobial antibody levels associate with COVID-19 vaccine responses

Despite a greater prevalence of poor responders, responses within the immunosuppressed cohorts were highly heterogeneous, including many strong responders (Figure 3). Conversely, even after a booster, 5% of the healthy cohort failed to develop a strong response. Given that non-SARS-CoV-2 antibody response levels remain stable over time but vary inherently between individuals, we hypothesized that if pre-existing antibody levels against these common antigens reflect general humoral response magnitude, they might also associate with vaccine response, thereby acting as sentinel antibodies. To look for such sentinel antibodies, we performed a binned analysis of different antibody levels in the top 25% and bottom 25% of anti-wRBD responders in each cohort. We excluded participants previously infected with COVID-19 from the analysis, as prior infection influenced the response to initial vaccination or booster (Figure S6).[34]

Overall, among the 111 non-SARS-CoV-2 seroprevalent antibodies, a total of 62 were significantly different between the top

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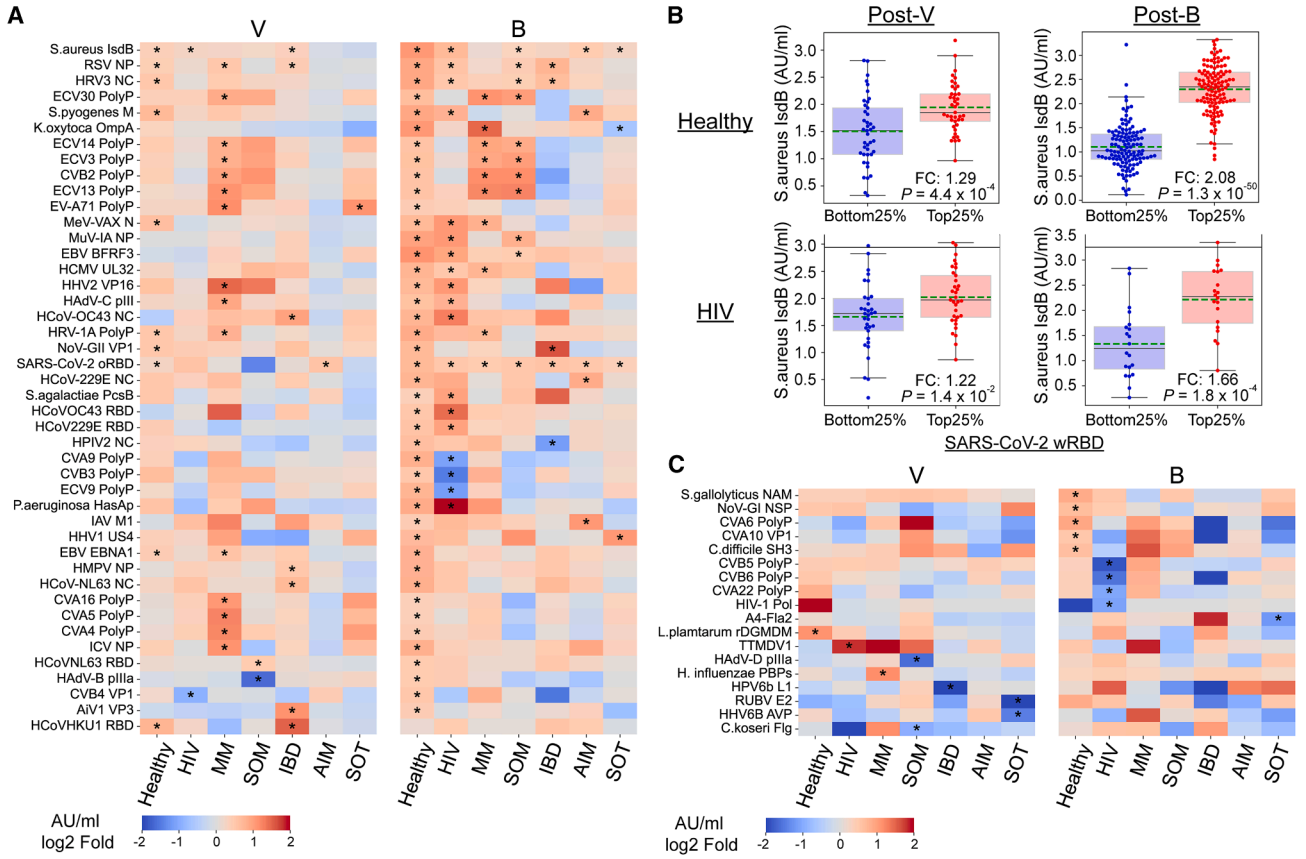

Figure 6. Non-SARS-CoV-2 antibodies that differentiate between top and bottom 25% of anti-wRBD responders across cohorts in both initial vaccinations and booster shots

(A) Heatmap of antibodies with significant differences between anti-wRBD top 25% and bottom 25% responders across two or more cohorts; The red and blue color indicates an AU/ml ratio color scale with positive and negative associations; * indicates an association with significance per *t* test.

(B) Anti-*S. aureus* IsdB was positively associated with top 25% responders during both vaccinations in the healthy and HIV cohorts. Fold change (FC) of the mean response between top 25% and bottom 25% responders was compared using a *t* test, and the *p* value was labeled. Boxes indicate the interquartile range (IQR; 25th–75th percentiles) with median lines; whiskers extend to 1.5× IQR.

(C) Differential antibodies unique to specific cohorts. Color scale indicates an AU/ml ratio with a *t* test *p* value of less than 0.05.

and bottom 25% of vaccine responders (with $p < 0.05$ by *t* test) for either the initial vaccination or the booster response (Table S5), with many antibodies associated with the booster (Figure 6A). The vaccine response-associated antibodies mostly had positive associations, and the majority ($n = 44$) were shared among at least 2 cohorts. Notably, the healthy cohort had the most associations ($n = 44$), especially for the booster ($n = 43$), followed by HIV ($n = 20$), MM ($n = 19$), SOM ($n = 13$), and IBD ($n = 11$). The least shared cohorts were AIM ($n = 4$) and SOT ($n = 4$). The most commonly shared antibodies across four or more cohorts were those against *S. aureus* iron-regulated surface determinant B (IsdB), a known virulence factor, RSV nucleoprotein (NP), and HRV3 NC, which were among the antibodies with the highest seroprevalence in all studied populations (median seroprevalence $\geq$ 98.0%, Table S3). There were 15 antibodies shared among three cohorts, including *Streptococcus pyogenes* (*S. pyogenes*), *Klebsiella oxytoca* (*K. oxytoca*), EVs (ECV14, ECV3, coxsackievirus B2 [CVB2], ECV13, and EV-A71), measles morbillivirus (MeV-Vax), MuV-IA, herpes viruses (EBV, human betaherpesvirus 5 [HCMV], and HHV-2), human mastadenovirus C (HAdV-C), HCoV-OC43, and HRV-1A (Figure 6A).

The antibody most strongly associated with vaccine response was anti-*S. aureus* IsdB (Figures 6A and 6B), which had a significantly higher antibody level in the top responders in the healthy, HIV, SOM, IBD, AIM, and SOT cohorts for either the initial vaccination (V) or booster (B) (mean fold change, *p* values; 1.29, $p = 4.4 \times 10^{-4}$, V healthy; 1.22, $p = 0.014$, V HIV; and 1.26, $p = 0.026$, V IBD) or the booster shots (2.08, $p = 1.3 \times 10^{-50}$, B healthy; 1.66, $p = 1.8 \times 10^{-4}$, B HIV; 1.24, $p = 0.023$, B SOM; 1.36, $p = 0.017$, B AIM; and 1.22, $p = 0.032$, B SOT). Another strongly associated antibody was anti-RSV-A NP (Figure 6A; Table S5), which also had a significantly higher antibody level in the top responders in healthy, HIV, MM, SOM, and IBD cohorts for the initial vaccination (1.32, $p = 2.6 \times 10^{-4}$, V healthy; 1.44, $p = 0.025$, V MM; and 1.34, $p = 4.7 \times 10^{-3}$, V IBD) or the booster shots (1.91, $p = 9.1 \times 10^{-50}$, B healthy; 1.50, $p = 3.7 \times 10^{-3}$, B

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HIV; 1.35, $p = 2.8 \times 10^{-4}$, B SOM; and 1.69, $p = 3.5 \times 10^{-4}$, B IBD) (Table S5). Further, regression analyses showed significant positive linear trends between the pre-V/B antibody levels for RSV-A NP, *S. aureus* IsdB, and HRV3 NC (Figure 4B).

Eighteen antibodies showed a single cohort-specific vaccine response association, and six of these were positively associated with anti-wRBD levels in the healthy cohort (Figure 6C). All but two of the remaining cohort-specific antibodies were negatively associated with the vaccine response. In general, anti-EV antibodies were correlated positively with vaccine response, but the HIV cohort showed an inverse association (Figures 6A, 6C, and S9A–S9E). Although the sample numbers were small, the negative trend held up for both vaccination and booster. The SOM cohort shared most of the antibodies associated with higher anti-wRBD vaccine-induced response (Figure 6A). However, there were three antibodies against HAdV-C penton protein (HAdV-C pIII), HAdV-D pIII, and flagellin protein of *Citrobacter koseri* (*C. koseri*) that were negatively associated with vaccine response following the initial vaccination (Figures 6A and 6C).

Pre-existing antimicrobial antibody levels can stratify healthy individuals with low vaccine responses

The observed associations between pre-existing antibody levels and the vaccine responses implied that the relative strength of generic B cell immunity in individuals was reflected in the global antibody profiles, which could thus inform a serological signature for predicting antibody response levels for future unknown infections and vaccinations.

To test this, we applied machine-learning approaches to construct predictive models to classify individuals with suboptimal vaccine response. When the participants were dichotomized into the low (weak and moderate) and high (high) response groups using the positivity thresholds (Figure 3), the number of samples from low responders in both pre-V and pre-B sample sets was too small to support robust modeling. This was because most patients in both the immunosuppressed and healthy cohorts had received a vaccine prior to the initiation of sample collection. As most non-SARS-CoV-2 antibodies showed no change over 18 months (Figure S8),[42] we therefore used the antibody levels for these non-SARS-CoV-2 antigens from the post-B sample set as proxies for model training and testing, with or without demographic features (age, sex, and race).

To reduce overfitting and minimize feature redundancy, we implemented a deep-learning-based dimensionality reduction framework, where the 2-dimensional embeddings extracted from the output layer of the supervised multi-layer perceptron (MLP) model were used to construct the final classifiers (referred to as the MLP-DA [discriminant analysis] models; Figure 7A). To account for potentially heterogeneous clustering patterns between low- and high-responder groups in the reduced feature space, we evaluated multiple modeling approaches, including logistic regression (MLP-linear), support vector machine (MLP-SVM), and nearest centroid classification (MLP-NC). For robust estimation of model performance, the mean AUC and the sensitivity at 80% specificity derived from repeated 5-fold stratified cross-validation (CrVal; $n = 100$) were used as the main performance metric.

We first built and tested universal cohort-agnostic models on training post-B samples from all cohorts using a feature set comprising the 98 non-SARS-CoV-2 and non-HIV seroprevalent antibodies with demographic information and cohort labels. CrVal of models showed an overall mean AUC of 0.766 and a sensitivity at 80% specificity of 59.4% across held-out post-B samples from all cohorts (Figures 7B and 7C, "universal"). Evaluation of the universal models across individual cohorts revealed higher predictive performance in the healthy, HIV, IBD, MM, and SOM groups, with mean CrVal AUCs of 0.803, 0.717, 0.718, 0.685, and 0.670, respectively (Figures 7B and 7C) and greater sensitivity at 80% specificity (73.4%, 63.3%, 66.8%, 46.3%, and 55.2%) (Table S6). In contrast, antibody responses in the immunosuppressed autoimmune and transplant cohorts were poorly predicted. Furthermore, despite the limited number of samples from low responders, individual models trained on each cohort using the same MLP-DA modeling framework yielded comparable results for these cohorts (Table S7).

We next evaluated whether the universal pan-cohort model trained on the post-B antibody profiles could accurately predict vaccine responses using pre-B samples, given the temporal stability of antibody levels. When the universal post-B-trained model was applied to the pre-B samples, AUC values of 0.660, 0.680, 0.808, 0.638, and 0.644 were observed in healthy, HIV, IBD, MM, and SOM groups (Table S8). The healthy cohort showed a slightly lower CrVal AUC (Table S8) than that for post-B samples (Figure 7B), while HIV, MM, and SOM showed comparable mean AUC values, respectively (Table S8). This result indicates the antibody signatures predictive of vaccine response were stable over time.

To identify the antibodies that contributed most to the predictive performance in the modeling framework, we then conducted an additional dimensionality reduction using partial least squares DA (PLS-DA), a method previously applied in a study for identifying key functional genes from high-dimensional omics data.[46] As shown in Figure 7D, the antibodies most informative for distinguishing high and low vaccine responders included *S. aureus* IsdB and RSV NP in the healthy cohort, consistent with the top vaccine response-associated antibodies identified in the binned differential analysis (Figure 6).

Overall, these modeling results demonstrate that the global profile of pre-existing antibody levels is a robust indicator of generic humoral immune strength, which can predict weak antibody levels upon vaccination or infection.

DISCUSSION

Immunosuppression is a common comorbidity associated with a weaker antibody response to vaccination, which increases the risk of infection, breakthrough infection, disease severity, and mortality.[12,21,22,27,28,47,48] Previous studies have focused on a single cause of immunosuppression, making it challenging to compare their observations across different assays or strategies for multiple types. Meta-analyses have compared antibody responses among different studies by estimating the assay variance, sample size, and analysis method,[13,49] although multiple immunosuppressed groups have not been compared directly using the same platform.

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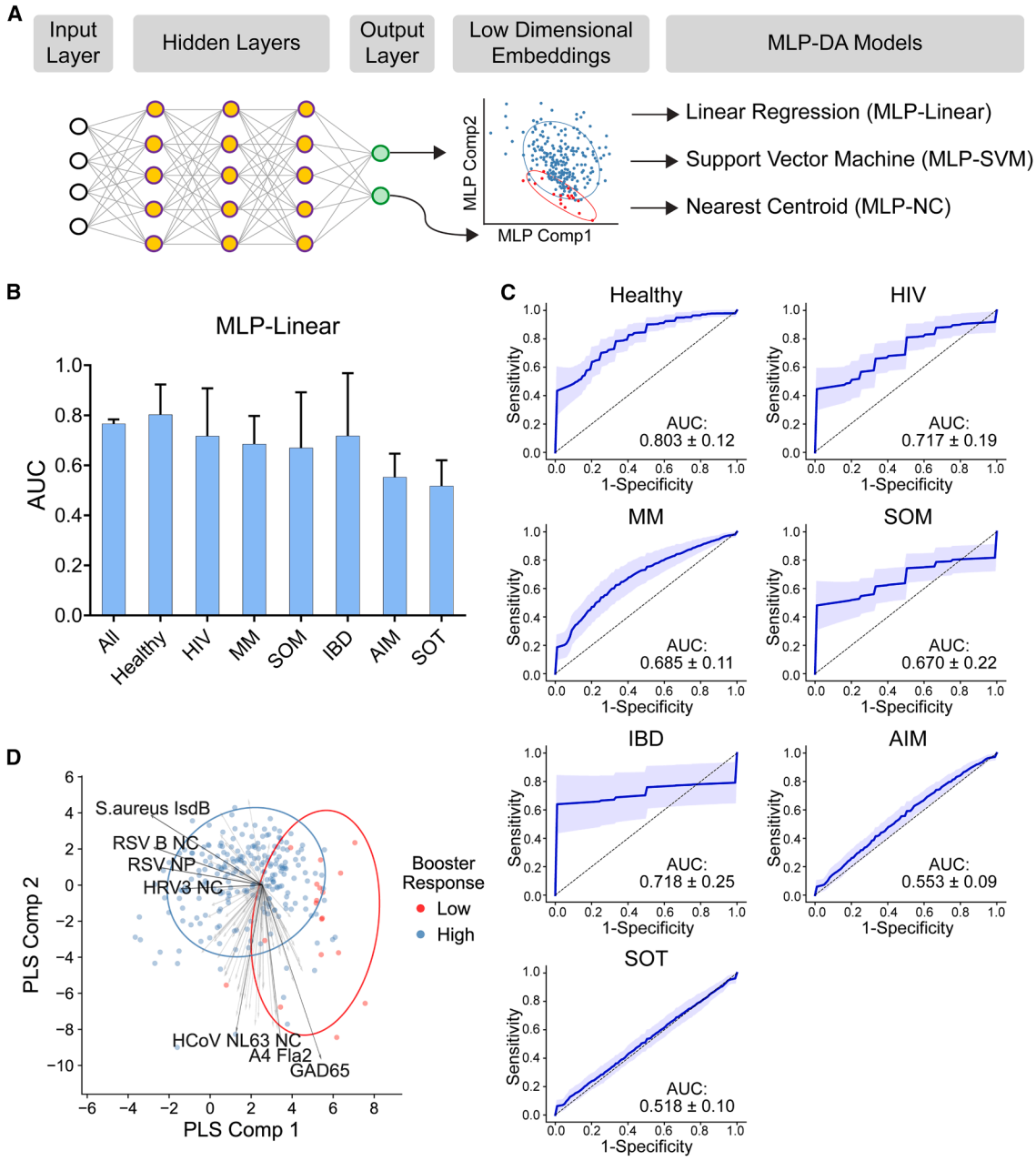

Figure 7. Pre-existing antimicrobial antibody profiles predict healthy individuals with low vaccine response to COVID-19 booster

(A) Schematic overview of the MLP-DA modeling framework. An MLP-based deep-learning model was trained using the response values of 98 seroprevalent antibodies (excluding SARS-CoV-2 and HIV antibodies) from post-B samples to predict the anti-SARS-CoV-2 RBD antibody levels after booster vaccination. The low-dimensional embeddings or components in the last hidden layer were then used to train the final classifiers to predict low/high responders.

(B) Performance of the post-B data-trained universal model tested on held-out post-B samples in individual cohorts. Mean AUC values of repeated (n = 100) 5-fold cross-validation are shown.

(C) The ROC curves for the universal model tested on individual cohorts.

(D) The universal PLS regression model was trained using the identical post-B sample and feature sets used for the MLP models, and the 2D embedding of the samples and the loadings of antibodies were projected onto a 2-component space. Ellipses represent the 95% interval for each group. The loading vectors are shown as arrows, and the top 7 contributing antibodies are labeled.

We observed heterogeneously reduced anti-wRBD response to the COVID-19 vaccine in six immunosuppression types/cohorts in both the initial vaccine series and the booster using a single high-throughput MISPA assay. Razael et al. performed a meta-analysis of 26 studies on 1,726 healthy controls and 3,207 immunocompromised patients and found the lowest

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seroconversion rate was observed in organ transplant recipients, which agrees with our observations. The requirement for immunosuppressive medications likely affects their ability to respond to vaccination.[47,50] In general, B cell depletion, immunosuppressants, and chemotherapy, as may be found in SOT, MM, AIM, and IBD, were among the most frequently reported factors associated with a blunted COVID-19 vaccination response.[13,44,49,51] Individuals with SOM showed a slightly lower response compared to the healthy population.[13] The humoral response to COVID-19 in patients with HIV has been reported with controversy, which may reflect CD4 recovery levels in different cohorts.[49,52] We observed no significant difference between healthy and HIV cohorts during initial vaccination but a lower response during the booster shots. Although a dynamically blunted anti-wRBD response was observed among multiple immunosuppressed cohorts in the current study, there were also many robust (moderate and high) antibody responses within each of these groups, indicating a wide range and heterogeneous humoral immune responses (Figure 2). Most participants received an mRNA vaccine (Moderna or Pfizer), with less than 5% receiving the J&J adenovirus vaccine (Figure S10). There was no significant difference between the mRNA1273 and BNT162b2 vaccines in the initial vaccine and booster responses in the healthy cohort, the only cohort for which there were appreciable numbers for both vaccines (Figure S11).

We have longitudinally documented the seroprevalence and antibody levels for 97 viruses, 40 bacteria, and 25 autoantigens in 4,089 participants including both healthy and immunosuppressed cohorts, resulting in 2 million data points. Most antibodies (n = 172, 172/185 = 93.0%) showed seropositive reactivity in multiple participants. During the study period (February 2021 to September 2022), nearly 90% of antibodies (103/114, 90.4%) showed little or no change across 18 months. The mechanisms of such precise regulation of antibody levels warrant further investigation. Stable responses do not appear to rely on the presence of the target antigen, as most stable responses related to uncommon infections. Notably, vaccination had no effect on non-SARS-CoV-2-related antigens. As expected, antibodies against SARS-CoV-2 antigens showed the greatest change in this study. The other frequently changing antibodies targeted common pathogens in 2.0%–5.0% of participants, although these detection rates were lower than expected, possibly due to the social-distancing practices of the pandemic.[53]

There were 114 antibodies with seroreactivity greater than 10% in at least one cohort in our study, of which 19 were specific to either the HIV or SOM cohorts. The anti-HIV antibodies were mostly restricted to the HIV cohort, as expected. However, careful examination of the heatmap revealed that some individuals who self-reported as healthy had antibodies to multiple HIV proteins (Figure 2). These are presumably occult cases that demonstrate the power of multiplexed testing. We also found elevated anti-glutamic acid decarboxylase 65-kilodalton isoform (GAD65) in patients with HIV, which has been linked to autoimmune complications of diabetes in patients on antivirals.[54] We also observed two autoantibodies, anti-p53 and anti-TNNI1, in the SOM cohort, both of which have previously been associated with AIM in cancer.[55,56] In addition to autoantibodies, we also identified antibodies against Torque teno virus 7, HBoV2, and *Acinetobacter calcoaceticus* in patients with cancer. Most autoantibodies studied here focused on autoimmune diseases and were less than 10.0% in any cohort, except anti-p53, TNN1, and 52 kDa ribonucleoprotein autoantigen Ro/SS-A (TRIM21). Anti-TRIM21 has been used as an autoantibody biomarker for multiple autoimmune diseases, including Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, and idiopathic inflammatory myopathies.[57] While expected in the autoimmune cohorts, we also found it in MM and other SOMs, which has not been reported before, representing its vital role in cancer immunity.[58] Several of the immunosuppressed cohorts showed evidence of reduced antibody titers to many infectious agents, as evidenced by vertical shadows in Figure 2 (MM, AIM, and HIV). We do not know what is responsible for this observation. In part, this may be due to immunosuppressive medication, either as prior or ongoing treatment, and it would be especially interesting to compare these patterns to the subjects' medication histories. This would be less likely to explain reduced titers in the HIV cohort, so the underlying diseases themselves likely contribute as well.

Although membership in an immunosuppressed cohort was linked to blunted antibody responses, it was not sufficient to predict a poor vaccine response; many such participants had robust antibody responses. Moreover, around 5% of individuals from the healthy cohort had blunted anti-wRBD responses during both vaccination and booster, potentially due to sub-clinical immunosuppression, both here and in other vaccine efficacy studies.[59,60] The notion that pre-existing antibody levels might reflect overall humoral health aligns with evidence that immunity against microbes is both positively and negatively associated with the COVID-19 vaccine response.[20,29,30,33,38–40] Most of these studies focused on COVID-19 itself, with evidence of a previous infection showing a higher antibody response to the COVID-19 vaccine, known as hybrid immunity, providing better protection with a longer duration of the antibody.[34,61]

We found that non-SARS-CoV-2 antimicrobial antibody levels, especially those to common organisms, predict response to the COVID-19 vaccine. We identified universal antimicrobial signatures that were positively associated with the top 25% high COVID-19 vaccine responders (Figure 6A). The most predictive antibodies were anti-*S. aureus*, -RSV, and -HRV3, which were also among the most seroprevalent antibodies studied. In a small study using microbe-derived proteins, a correlation between the antibodies against a staphylococcal complement inhibitor and COVID-19 vaccine response was also reported by Saito et al.[38] RSV and HRV3 are common respiratory viruses with very high seroprevalence, consistent with a possible measure of generalized immunity assessment. We evaluated these antimicrobial antibodies' association with the COVID-19 vaccine response for Moderna (N = 155) and Pfizer (N = 366), separately and combined, in the healthy cohort during booster vaccination. As shown in Figure S12, the feature associations between antimicrobial antibody and anti-RBD were consistent. These findings suggest interesting mechanisms behind individuals' differences in antibody responses to new vaccines. Individuals with strong responses may have developed more reactive humoral immune

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responses through more lifetime exposure to general or specific antigens. Or they may have genetically inherited attributes in their immune systems that enable them to respond more vigorously to new exposures. Or most likely, a combination of both. Future studies could compare strong and weak sentinel responders for clinical health history, pre-perturbation cell populations, pre-vaccination inflammation and B cell signaling, signaling response to stimulation, and pre-vaccination innate immune endotypes characterized by genes associated with stronger immunity and HLA associations.

We identified 40 additional antimicrobial antibodies that correlated with vaccine response in at least two cohorts. These antibodies exhibited high seroprevalences, indicating they were common exposures or vaccine-related agents. In addition to the four most tightly correlated antibodies, we also observed antibodies against *S. pyogenes*, *K. oxytoca*, EVs, and herpes viruses, all with stable antibody levels across the 18-month study period. Associations of seasonal coronaviruses with a stronger COVID-related response have been attributed to their homology and cross-reactivity between the RBD proteins.[2,11,29,33,37] While we also observed positive associations between NC and RBD antibodies with post-COVID-19 vaccination response (Figure 6), antibodies against NC and RBD for seasonal coronaviruses and SARS-CoV-2 did not intercorrelate within samples or longitudinally per participant (Figures 4 and S7). The association of antibodies to seasonal coronaviruses, which are highly prevalent, with COVID-19 vaccine response may instead reflect a measure of humoral immune health. We are interested in assessing how generalizable this signature will be for other vaccines. Although we did not have any information about other vaccines received by our study's participants, we did test for responses to the measles vaccine strain (MeV-Vax) NP. Measles is rare in the US, so most responses will be due to vaccination. Interestingly, anti-MeV-Vax levels showed a strong association with the COVID-19 vaccine response (Figure 6A), shared across multiple cohorts, indicating that the two vaccinations shared the sentinel signatures. It will be important to formally test these signatures in the context of other vaccines.

The MLP-DA stratified low responders among healthy individuals with >75% accuracy (Figure 7), using serological measurements and demographic features, comparable to or modestly above prior models built on demographic and/or genetic features.[4,5,25,26] Benchmarked against simpler classifiers on the same feature set (Table S9), the MLP yielded only a marginal in-distribution gain (post-B to post-B AUC 0.766, vs. 0.756 for a Gaussian-kernel SVM and 0.742 for logistic regression). This is expected given the low ratio of minority-class (low-responder) examples to input dimensionality and the near-linear separability of the two classes, a setting in which the inductive bias of lower-capacity models is sufficient and the added expressivity of the MLP adds minor benefit. The MLP, however, significantly outperformed these baselines under the post-B to pre-B train/test split, retaining an AUC of 0.747 vs. 0.698 (Gaussian SVM) and 0.673 (logistic regression) (Table S9), rendering it the smallest cross-time-point degradation among the models evaluated. We therefore characterize the MLP as a nonlinear feature extractor whose value lies in cross-time-point generalization rather than in-distribution discriminative power and not as a prerequisite for the central result that the pre-existing antimicrobial antibody profiles are themselves predictive of vaccine response. We also evaluated the value of including antibody levels, demographics, seropositivity, and cohort label (i.e., immunosuppression status) in model development, and the model based on the antibody levels plus demographics and cohort label performed the best (Table S10). This model is the first to utilize a global antibody profile that predicts the vaccine-induced response without prior knowledge of the immunosuppression status of healthy individuals. These results indicate that the strength of humoral immunity among individuals can be robustly estimated from the relative magnitudes of antibody responses against previously infected common pathogens, such as *S. aureus*, RSV (RSV-A and RSV-B), and HRV (Figure 7B). This type of approach could prove useful in vaccine development, vaccine testing, and clinical management of individuals with immunosuppression. Both the trained universal model and cohort-specific models were tested on specific cohorts (Figure S13). The universal model ("all") performed best compared with models trained and tested on individual cohorts. When validated across orthogonal cohorts, models built for the healthy, HIV, and MM cohorts performed well cross-cohort, whereas models built for the AIM and SOT did not perform well during testing in any cohort, including their own. The SOT cohort had the weakest overall response to vaccine and booster, although it had a markedly wide range of responses to booster. This suggests the possibility of predictors within that cohort. There was a trend toward many of the same sentinel responses seen in other cohorts, including several that were statistically significant, such as *S. aureus* IsdB. However, most were not significant, and neither the universal MLP model nor a cohort-specific model was a strong predictor for this cohort (AUC < 0.6) (Figure S13). An important next step would be to study this cohort along with information about surgical, clinical, and pharmacological history.

We investigated the humoral immune response to 185 different antigens that reflect 97 viruses, 40 bacteria, and 25 autoantigens in response to COVID-19 vaccination in various immunosuppressed and healthy individuals. We found overall blunted anti-wRBD responses in all immunosuppressed cohorts but to different extents and with widely heterogeneous responses among individuals. Some pre-existing antibody levels to common microbes predicted stronger COVID-19 vaccine responses in both healthy and immunosuppressed individuals, suggesting biomarkers for individuals' humoral immune competence. Deep-learning models predict post-vaccination responses based on these pre-existing antibodies to other microbes. This approach reveals that we can learn about exposure history as well as general humoral immune health by broadly surveying antibody responses.

Limitations of the study

The current study has some limitations. Although >8,000 unique samples from >4,000 participants were collected and analyzed together, the sample collection was not evenly distributed, resulting in some specific clinical groups, such as patients with SOT, who received early vaccination before baseline samples could be obtained. We analyzed 185 antigens,

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selected based on our knowledge and previous protein array data. These focused on seroprevalent antigens from microorganisms, which may not include important differentiating antigens. For example, multiple anti-EBV antibodies associated with cancer were not tested due to low seroprevalence, missing an opportunity to understand these anti-EBV antibodies in relation to cancer. Because of potential cross-reactivity, some of the responses (i.e., EV) might provide evidence of infection by more than one microbe. Further evaluation of these responses specific to known infection/exposure information would help distinguish them further. As antibody levels provide useful information regarding prior immune exposures, it would be valuable to evaluate the breadth of the pre-existing antibody responses related to age, gender, and other metadata. Such a study would benefit from a more determined and demographically matched sample collection than was possible under these circumstances. Conducting longitudinal studies is essential for determining the causal relationship between immune conditions and antibody profiles. Our study focused on antibody levels rather than functional immunity, and more comprehensive immune profiling, including cellular responses, would enhance these findings. Antibody levels associate with vaccine protection and durability[1,4]; however, it will be important to demonstrate whether the antibody-level predictions identified here also correlate with infection and/or clinical outcomes. Due to the limited information collected, several demographic factors, such as socioeconomic status and lifestyle, were not analyzed.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Joshua LaBaer (jlabaer@asu.edu).

Materials availability

Plasmids generated in this study have been deposited to DNASU as listed in Table S2.

Data and code availability

- All MISPA data, original codes, and modeling results have been deposited and are publicly available at GitHub (https://github.com/Lee-CBG/Leidos; https://doi.org/10.5281/zenodo.21079244).
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.L., L. Song, J.G.P., J.Q., V.M., and D.M.M.; ASU CBC, J.L. (PI), L. Song, J.G.P., J.Q., V.M., and D.M.M.; Feinstein-Northwell CBC, P.G. (PI), A.D., M. Ryan, and E. Kowalsky; Mt Sinai CBC, C.C.-C. (PI), D.R.M., V.S., and K. Srivastava; UMN CBC, A.K. (PI), B.T., and S.N.T.; sample processing, V.M., K.C., M. Ritchie, B.B., I.G., G.S.T.S., G.C., E. Katergaris, K. Seifert, V.B., P.B., D.F., A.K., L.N., M.D.W., R.S., C.M.V., N.M., C.W., R.W., D.C., N.K., P.A., K.C., A.P., L. Stiene, and I.F.; MISPA analysis, L. Song, J.G.P., D.A., B.G., C.M.V., and T.T.; data analysis, L. Song, S.M., Z.C., Y.C., S.W., B.G., and H.L.; investigation, J.L., L. Song, J.G.P., J.Q., V.M., and D.M.M.; visualization, L. Song and J.G.P.; funding acquisition, J.L., J.G.P., J.Q., and V.M.; project administration, J.G.P., J.Q., V.M., D.M.M., G.R., T.H., N.R., L.P., T.K., and J.L.; supervision, J.L.; writing – original draft, J.L., L. Song, J.G.P., J.Q., V.M., and D.M.M.; writing – review & editing, J.L., L. Song, J.G.P., J.Q., V.M., D.M.M., S.M., C.-W.H., Z.C., Y.C., A.K., and S.N.T.

DECLARATION OF INTERESTS

J.L. and J.Q. are founders of Gila Diagnostics. A disclosure (M26-200L) related to the identified antibodies and the developed model described in this work has been filed by the Biodesign Institute.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cpblue.2026.100088.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-HaloTag® pAb	Promega	Cat# G9281
Biological samples		
Sera collected from SeroNet	This paper, four Capacity Building Center and the autoimmune samples from Feinstein Institutes for Medical Research	N/A
Chemicals, peptides, and recombinant proteins		
Tris-buffered Saline (10*X*)	Cell Signaling	Cat# 12498S
Glycerol	Fisher Scientific	Cat# G334
Dynabeads Protein G	Invitrogen	Cat# 10009D
Glycine	Sigma	Cat# G7126
nuclease-free water	Invitrogen	Cat# AM9938
Tris Base	Sigma	Cat# 252859
2*x* Sapp master mix	Takara	Cat# RR350
QIAquick PCR purification kit	Qiagen	Cat# 28106
Qubit™ 1*X* dsDNA HS Assay Kit	Thermo Fisher	Cat# Q33231
Sodium Hydroxide	Thermo Fisher	Cat# C990H87
NextSeq 500/550 High Output Kit v2.5 (75 Cycles)	Illumina	Cat# 20024906
Oligonucleotides		
Forward and reverse primer	IDT	Customized
Spike-in control-1	IDT	Customized
Spike-in control-2	IDT	Customized
Recombinant DNA		
pJFT7_cHalo-3xFlag_DC	DNASU	Cat# EvNO00595408
pcDNA3.4_DEST_TPA-cHalo-3xFlag	DNASU	Cat# EvNO00967317
Software and algorithms		
GraphPad Prism V10	Graphpad Software Inc.	RRID:SCR_002798
Python Programming Language v3.11	Python Software Foundation	RRID:SCR_008394
Seaborn	https://seaborn.pydata.org/	RRID:SCR_018132
Clustal Omega	https://www.ebi.ac.uk/jdispatcher/msa/clustalo?stype=protein	RRID:SCR_001591
PyTorch	https://pytorch.org/	RRID:SCR_018536
Pandas	https://pandas.pydata.org/	RRID:SCR_018214
Other		
96-well PCR Plate, Semi-Skirted	Fisher Scientific	Cat# AB-1400/150
KingFisher 96 deep-well plate, v-bottom	Thermo Fisher	Cat# 95040450
Biomek i7 Automated Liquid Handler	Beckman-Coulter	Cat# i-7
KingFisher™ Flex Purification System	Thermo Fisher	Cat# 5400610
Allegra X-15R Centrifuge	Beckman-Coulter	Cat# X-15R
Microplate Shaker non-heated	Southwest Science	Cat# SBT1500
Illumina NextSeq550Dx	Illumina	Cat# NextSeq550Dx

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EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human samples

In 2020, the National Cancer Institute (NCI) launched the Serological Sciences Network (SeroNet) to address the need for sensitive serology testing in response to the COVID-19 pandemic. The initiative established strategically located collection centers across the eastern, midwestern, and western regions of the United States, forming a robust infrastructure for epidemiological surveillance. These centers monitored infections, enrolled volunteer donors, and collected whole blood samples along with essential metadata.

When COVID-19 vaccines became available in 2021, SeroNet's Capacity Building Centers (CBCs) were well-positioned to conduct longitudinal studies tracking vaccine efficacy and durability. SeroNet also funded the development of cutting-edge technologies to assess immune responses to both the pandemic virus and emerging variants of concern.

Before the pandemic, MISPA technology was in early development for measuring serum antibody reactivities to a wide range of bacterial-, viral-, and auto-antigens.[42] During the SeroNet program, MISPA was adapted to assess immune responses to SARS-CoV-2, seasonal coronaviruses, and numerous other pathogens and autoantibodies. It was then employed to monitor immunosuppressed populations, including individuals with HIV, cancer, transplants, and autoimmune conditions.

By leveraging both the logistical capabilities of its collection centers and MISPA's both large-scale testing potential and precise quantification, SeroNet conducted extensive studies on vaccine responses and immune histories. These efforts included populations at high risk and followed 4,089 participants up to 18 months, generating 8,687 serum samples tested against a library of 185 antigens. This initiative resulted in a comprehensive immune surveillance study, providing critical insights into COVID-19 vaccine responses and pathogen exposures.

To ensure consistency across sites, the CBCs followed harmonized protocols for sample collection and preservation. Trained professionals, including certified phlebotomists, nurses, and nurse aides, conducted all collections following best practices in biosafety and environmental safety protocols.

Whole blood samples were collected using red-top Vacutainer tubes (for serum) or EDTA tubes (for plasma) under biosafety level 2 (BSL-2) conditions. Blood samples were allowed to clot for 30–60 min at room temperature before centrifugation at 1,300 x g for 20 min at 20°C–25°C.

After centrifugation, the serum or plasma was carefully pipetted into aliquots and stored at −80°C until further analysis. This process ensured the integrity of the samples, minimizing variability across sites and facilitating reliable large-scale testing.

Samples were randomized and their location was predetermined by an external NCI/SeroNet statistician. The test center received the blinded samples, aliquoted and deposited into an automated −80°C Biostore before retrieving as per the randomization schema. All samples went through a single freeze thaw at testing center, before analysis.

Capacity Building Centers

The NCI-supported CBCs were established across the United States, each developing specialized cohorts based on the expertise and clinical populations at their respective institutions. These centers ensured the inclusion of both healthy and immunosuppressed individuals, reflecting the diverse patient populations served.

Eastern Regional Centers

Feinstein Institutes for Medical Research and Northwell Health

Located in Manhasset, New York, Feinstein-Northwell serves as New York's largest healthcare provider. It covers the broader northeastern region, including New Jersey, Pennsylvania, and Connecticut, using LabFly, a mobile blood draw and courier service launched during the pandemic. This center developed cohorts focusing on healthy individuals, autoimmune diseases, cancer, and HIV.

Mount Sinai, Icahn School of Medicine

Situated in Manhattan, New York, Mount Sinai boasts extensive expertise in cancer, transplantation, and autoimmune treatments. It developed specialized cohorts, including those with multiple myeloma, other cancers, transplants, inflammatory bowel disease (IBD), and other chronic conditions.

Midwestern Regional Center

University of Minnesota Medical School (UMN)

Located in Minneapolis, Minnesota, UMN Medical School served as the midwestern collection center, leveraging its clinics and academic community to develop diverse cohorts. These include healthy individuals, and populations with cancer, autoimmunity, HIV, and organ transplants, along with patients receiving treatment for breast cancer and other chronic conditions.

Western Regional Center

Arizona State University

Based in the Phoenix-Tempe metropolitan area, ASU—one of the largest public universities in the U.S. by enrollment—served as the western collection center. Its large and diverse population enabled the creation of a broad Healthy Cohort. ASU collaborated with Valleywise Health (the hospital for Maricopa County) and Midwestern University Clinics to establish additional cohorts for autoimmunity, cancer, HIV, and COVID-19 convalescent patients.

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Ethics approval and informed consent

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and applicable institutional and regulatory requirements. The Arizona sites, including Arizona State University, Dignity Health, Midwestern University, and Valleywise Health, participated under a centralized Institutional Review Board (IRB) administered by WCG IRB (Study ID WCG-1300400; approved January 20, 2021). Additional participating institutions and Capacity Building Centers obtained approval from their respective Institutional Review Boards, including the Feinstein Institutes for Medical Research Institutional Review Board (Study ID 20–1007; approved November 16, 2020), the University of Minnesota Institutional Review Board (Study ID STUDY00012418; approved April 26, 2021), the Feinstein Institutes for Medical Research Institutional Review Board (Study ID 21-0212-FIMR; approved March 4, 2021), the Mount Sinai Hospital IRB (STUDY-16-01215; approved December 02, 2016), the Columbia University IRB (Study ID IRB-AAAS4052; approved August 31, 2020), and the Phoenix Children's IRB (Study ID IRB-21-119; approved May 24, 2021). Written informed consent was obtained from all participants at every participating site prior to study enrollment.

METHOD DETAILS

MISPA assay

Protein library generation

The MISPA protein library was generated using protein cloning, expression, and barcoding as described previously. In brief, all proteins except the receptor binding domain (RBD) of spike protein were cloned into the pJFT7_cHaloTag-3xFLAG destination vector (DNASU). The RBD proteins were cloned into modified pCDNA3.4-cHaloTag-3xFLAG vector (DNASU) with a signal peptide (MDAMKRGLCCVLLLCGAVFVSP) at the N terminal and a linker peptide ((GGGGS)3) between the protein and the cHaloTag. All proteins were expressed[42] and quality-controlled (QC) using SDS-PAGE with in-gel fluorescence.[42] The MISPA library was assembled to act as a serological sensor for "humoral immune readiness", requiring a library that, in addition to SARS-CoV-2 proteins, captured a number of elements including: common human pathogens, especially respiratory viruses, but also some gastrointestinal viruses; chronic viruses of interest (HSVs, HAdVs, HPVs); seasonal coronaviruses; common vaccine proteins; and common bacteria of interest. To identify which antigens would be most useful, we relied on data from our protein microarray studies that examined 6500 microbial antigens from 50 bacterial strains and 100 viral strains across 1200 human sera, in which most of these species were tested with either full proteomic coverage or minimally 10 antigens/organism. The most seroprevalent antigens were then selected to ensure the library could robustly capture individuals' exposure histories. The library also intentionally included markers relevant to specific participants, such as the 14 HIV antigens for HIV, and 25 autoantigens tailored to the solid organ malignancy and autoimmune cohorts. For seasonal coronaviruses and SARS-CoV-2, we included both RBD and NC proteins to differentiate between vaccine-induced response and hybrid immunity from prior infections. Each protein was uniquely barcoded using a DNA oligomer-linked Halo ligand and quality controlled using NGS to verify the unique barcode and stoichiometry.[42] Barcoded proteins that passed QC (the stochiometric of each barcoded protein amount had to exceed the average read number of a blank control (no protein) plus three times the standard deviation (SD), and the relative amount of the expected target barcode had to exceed the 2nd highest barcode value by at least 100-fold) were mixed in equal volume and used as the protein library. To assess the input values for the protein library, it was diluted 100 times and used as a mock sample.

MISPA serology assay

The MISPA serology assay was performed in the same manner as previously described using a Biomek i-7 Automated Liquid Handler system (Beckman-Coulter) and a KingFisher Flex system (Thermo Fisher), except as specifically noted.[42] A 20 μL protein library was aliquoted into a 300 μL 96-well plate (Semi-skirted, Fisher Scientific) for use and mixed with 5 μL of 1:25 diluted serum samples in Tris-buffered Saline (1*X*, TBS, Cell Signaling) and 20% glycerol (v, Fisher Scientific), incubated for 1 h at room temperature (RT), shaken at 850 rpm, and then added to 100 μL of protein G-coated magnetic beads (containing 20% slurry, Invitrogen), pre-aliquoted into KingFisher 96 deep-well plate (v-bottom, Thermo Fisher), to pull down complexes of antibody and DNA-barcoded proteins using a KingFisher Flex system (Thermo Fisher). The interacted barcoded proteins were eluted by incubating with 100 μL glycine (pH2.7, Sigma) in another KingFisher 96 deep-well plate (Thermo Fisher), shaken at 850 rpm for 20 min and 30 μL of 1M Tris (Sigma) in nuclease-free water (Invitrogen) was added to neutralize the solution after removing the protein G beads. A further PCR reaction of 5 μL of neutralized solution, 5 μL of both forward and reverse primer (1 μM) (IDT), 0.5 μL of 10 pM spike-in control oligo (IDT), and 12.5 μL of 2*x* sapphire PCR master mix (Takara) was constructed to amplify the barcoded protein and append the sample index (sample barcode) to the final DNA product. The PCR product was purified through a QIAquick PCR purification kit (Qiagen). The DNA product was quantified by Qubit 1*X* dsDNA HS Assay Kit (Thermo Fisher), denatured using 0.2 N NaOH (Thermo Fisher), and analyzed with NextSeq 500/550 High Output Kit v2.5 (75 Cycles) (Illumina) using Illumina NextSeq550Dx (Illumina).

A total of 12,110 samples were analyzed through 8 MISPA runs, including 8,687 unique samples, 2,184 assay controls (192 blinded serum controls*8 runs and 81 internal serum controls* 8 runs), 471 SARS-CoV-2 RBD antibody assay serum controls (1 Positive, 1 Negative, and 1 Blank control per 96-well plate), and 768 calibrators (3 replicates of 16 SeroNet standard serum calibrators and 3 replicates of 16 Rabbit anti-halo antibody calibrators (Promega)).

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QUANTIFICATION AND STATISTICAL ANALYSIS

MISPA data processing and statistical analysis

All MISPA raw sequencing counts were divided by the number of spike-in control oligo reads in each sample to create a spike-in-normalized ratio, referred to as an arbitrary unit (AU)/ml. To correct for batch effects across eight NGS runs, we applied a 95% global trimmed-mean normalization, in which scaling factors were computed from the run-specific means after trimming the top and bottom 2.5% of values. This procedure reduced the inter-run coefficient of variation (CV) of 81 control serum samples from 21.4% to 17.1%. Plasma samples collected at Mount Sinai exhibited lower background signals (i.e., values below the positivity cutoffs) than serum samples collected at Mount Sinai and other sites, with the differences proportional to the abundance of individual antigens in the MISPA library. To account for this matrix-dependent effect, we added small background correction offset, defined by the protein amount of each antigen in the library divided by a global correction factor of 5, to the MISPA values of plasma samples, reducing the CV between plasma and serum samples from Mount Sinai from 27.8% to 19.3%. We then excluded serum samples (n = 38) with high background (above the mean of AU/ml values plus 3 SDs for GFP) from downstream data analysis. The seropositive cutoffs for anti-SARS-CoV-2 RBD and NC were determined by consolidated pre-2019 serum samples that were tested repeatedly in each MISPA run plate, and merged cutoffs were generated as average plus three times the SDs of all replicates. The average CVs within and between run days were calculated by dividing the SD by the average of replicated samples via the same antigens, and average CVs across all antigens were calculated with a 95% confidence interval (CI). The cutoffs of the individual commercial or lab-developed anti-SARS-CoV-2 spike, S1/S2, or RBD assays were determined by individual CBCs, and the positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) between them and MISPA were performed separately based on available samples analyzed by both assays.

Pre- and post-vaccination anti-SARS-CoV-2 wRBD response through cohorts

To compare the COVID-19 vaccine response in healthy and immunosuppressed individuals, anti-SARS-CoV-2 wRBD antibody levels before and after vaccination were examined for both the initial vaccination (V, 2-doses) and the booster shot (B, single dose). The pre-initial vaccination (Pre-V) group included the samples collected closest to the first dose of V and at least 3 weeks before the first dose of the full vaccination. In contrast, the post-initial vaccination (Post-V) group included the samples between 2 and 18 weeks after the second dose of V and the closest sample to 2 weeks if more than one sample was collected post-vaccination. For the booster vaccination (B), the pre-booster (Pre-B) group included the sample collected closest to the vaccination date if more than one sample was collected before vaccination, while the post-booster (Post-B) group contained samples collected at least 2 weeks after B and the closest sample to 2 weeks if more than one sample collected Post-B. The organ transplant cohort did not have Pre-V samples and was thus excluded from the analysis for the full vaccination analysis.

The percentage of non-optimal post-vaccination anti-wRBD responses for the initial and booster vaccinations was calculated using cutoffs of 0.5 AU/ml and 3.5 AU/ml, respectively. The odds ratio between the percentage of non-optimal response for the Healthy and the specific cohort were compared and the significancy was assessed with the Chi-square test. The difference between pre-vax infection and or no history of infection of post-vax anti-wRBD levels and the antibodies changes between pre- and post-vaccination were evaluated using a two-tailed t test.

Technical cutoffs of antibody levels

The technical seropositivity cutoff for each individual antigen was calculated by multiplying eight times of the median serological response of GFP through all samples ($8*Med_{GFP}$) and the ratio of the target antigen's input ($Input_{antigenX}$) and the input of GFP ($Input_{GFP}$): $Cutoff_{antigenX}=(8*Med_{GFP})*(Input_{antigenX}/Input_{GFP})$. To ensure an adequate sample size when we were analyzing the post-vaccination response in seven cohorts, we focused on 114 antibodies with at least 10% seroprevalence for all samples merged or within each cohort.

Antibody stability analysis

MISPA data of 114 seroprevalent antibodies in longitudinal samples for 743 participants were analyzed to examine the variability of antibody titers. For each antibody, the fraction of participants that had any longitudinal samples with absolute changes greater than 4-fold over the first collected sample were calculated.

Comparison of antibody response correlation with sequence similarities

To determine antibody responses that correlated with other antibodies, the Pearson's correlation coefficient was used as the distance metric for clustering analysis of all the first collected sample for each participant (n = 4,089). To determine antigens that are correlated with other antigens by sequence, the sequence homology was assessed using the similarity of the antigen protein sequence calculated by *Clustal Omega*.[62] Pearson correlation was calculated using the "*corr()*" function from the *pandas* package, and the heatmaps were created by the *seaborn* package in *Python* v3.11 (Python Software Foundation).

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Binned analysis on pre-existing antibodies vs. COVID-19 vaccine response

To examine the association of pre-existing antibody levels vs. post-vaccination/booster response, participants with samples collected at 2 timepoints of Pre-V/Post-V and Pre-B/Post-B were selected. Participants with evidence for previous infection were identified as having Pre-V AU/ml values above the technical seroprevalence cutoff for the relevant antibody.

MLP-DA modeling

We utilized predictive modeling to forecast Post-B anti-wRBD responses. In addition to the SARS-CoV-2 antibodies, all HIV antibodies were excluded from all modeling analyses, since the HIV cohort was highly class-imbalanced with predominantly (96.4%) strong anti-wRBD responders after booster and would thus act as a confounding factor and inflate the model performance for the high-responder class. Additionally, we excluded participants who reported positive COVID-19 infections. Training and testing were conducted on three levels: (1) Training on all cohorts and testing on all cohorts, (2) Training on all cohorts and testing on each cohort, and (3) Training and testing within each cohort.

For each task we trained a Multi-Layer Perceptron (MLP) with 3 hidden layers (128, 64, 2). Categorical input features are also embedded in an 8-dimensional learnable space (one for each feature) and concatenated with antibody features before being fed to the MLP. Given a d-dimensional input vector $x \in \mathbb{R}^d$, this model learns a non-linear mapping to the binary vaccine response label $y \in \{0, 1\}$ using intermediary latent variables $\left\{h^{[l]} \in \mathbb{R}^{d^{[l]}} : h^{[l]} = ReLU\left(W^{[l]}h^{[l-1]} + b^{[l]}\right), h^{[0]} = x, l \in \{1,2,3\}\right\}$, where $W^{[l]} \in \mathbb{R}^{d^{[l]} \times d^{[l-1]}}$ is a learnable weight matrix, and $b^{[l]} \in \mathbb{R}^{d^{[l]}}$ is a learnable bias vector, and $ReLU$ is a non-linear activation function: $ReLU(z) = max(z,0)$, and $d^{[l]}$ corresponds to the dimensionality of layer l. The MLP model outputs $\hat{y} \in (0,1), \hat{y} = \sigma\left(U^T h^{[3]} + c\right)$, where $U \in \mathbb{R}^{d^{[3]}}$ is a learnable weight vector, and $c \in \mathbb{R}$ is a learnable bias scalar, and σ is the sigmoid function: $\sigma(z) = 1/(1 + exp(-z))$.

The parameters of the MLP model were optimized using gradient descent with a Binary Focal Loss cost function. To take into account the imbalance in training data between positive and negative classes, this cost function penalized the MLP more for incorrectly-classified data points of the minority class. We used the validation set (20% of the training set in each fold) for early stopping to avoid overfitting the training set within a 5-fold cross-validation setting. We used the harmonic mean of the accuracies of the low and high responders, with coefficients of 4 and 1, respectively. This process gave us 5 models, each hyperparameter-tuned to 20% of the training set. The analysis was repeated across 100 random seeds to ensure robustness of the models. We averaged the predictions of these 5×100 models and reported their performance on the held-out test set in each fold across all the seeds.

To allow for a higher degree of interpretability, we trained a supervised MLP feature extractor with a latent dimensionality of 2, we chose the last layer's dimensionality of 2 due to its visualization advantage and its higher or similar performance to dimension sizes 16, 32, and 64. Using the training data in each fold, we trained a binary-classifier MLP, then discarded its predictions and used the latent variable $h^{[3]} \in R^2$ as a new set of features. We then used two supervised classical models for binary predictions.

For MLP- support vector machine (MLP-SVM) model, using the 2-D features extracted from MLP, we fitted an SVM model with a Gaussian kernel on the train set. Since data were imbalanced in all tasks, we assigned a higher weight to the low-responder class in training. We also loosened the threshold for classifying a data point as low-responder by drawing the decision boundary away from the margin and toward the high-responders.

For MLP- nearest centroid classification (MLP-NC) model, taking inspiration from clustering algorithms, on the 2-day space of the features extracted from the MLP, we defined the mean point of each of the classes from the training set as two centroids for each class. We then classified test data points based on their distance from each of these centroids. We used Euclidean distance as a metric.

We also conducted the same experiment with other baselines, a simple Logistic Regression model, an SVM with a linear kernel, an SVM with a Gaussian kernel, and a 5-nearest neighbor classifier. The performance results are shown in Table S9.