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Longitudinal proteomic and autoantibody signatures after mRNA vaccination in healthy individuals

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ABSTRACT

Background: Longitudinal analysis of the circulating proteome after SARS-CoV-2 mRNA vaccination provides insights into immune adaptation, optimizing vaccination strategies, and understanding long-term molecular effects.

Methods: We analyzed 120 paired plasma samples from 114 healthy participants at six time points: pre-vaccination, 3 days and 7–21 days post-dose 1, 7–21 days post-dose 2, and at 8-, 16-, and 24-weeks post-dose 2. IgG antibody responses to SARS-CoV-2 structural proteins and autoantibody profiles were assessed using a multiplex platform, and plasma proteome changes were quantified by mass spectrometry.

Results: IgG antibodies against spike protein regions (RBD, S1, S2) produced sustained responses for up to 24 weeks, with no detection of nucleocapsid or membrane antibodies, confirming that there was no prior infection. Younger participants (<45 years) presented stronger responses, with no significant sex differences. Proteomics identified 342 proteins, of which 214 proteins were significantly altered, predominantly at 16–24 weeks. Key changes included upregulation of C1 complex proteins (C1R, C1QC, C1S) and downregulations of the C1 inhibitor SERPING1, indicating complement activation. Platelet-associated proteins showed minimal changes, suggesting a limited thrombosis risk. Altered proteins were enriched in carbohydrate metabolism, cofactors/vitamin metabolism, and thyroid hormone pathways. Autoantibody profiling showed stable responses for most self-antigens, with modest increased in several interleukin specific autoantibodies, including IL-1B, at later time points.

Conclusions: Integrated plasma proteomics and autoantibody profiling demonstrated sustained immunogenicity and safety of SARS-CoV-2 mRNA vaccines, characterized by durable spike-specific antibody responses, late-onset complement activation, and limited autoantibody induction. These findings enhance our understanding of vaccine-induced immunity and inform long-term monitoring and optimization of mRNA vaccine strategies.

1. Introduction

The outbreak of COVID-19, which began in December 2019, rapidly spread worldwide, creating an urgent need for effective mitigation strategies. The development of a safe and protective vaccine was soon recognized as the most promising approach to curbing the pandemic. Unprecedentedly, the vaccine development process was conducted on a global scale and accelerated at an unparalleled pace. By late December

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2020, the World Health Organization (WHO) authorized the emergency use of the Pfizer/BioNTech COVID-19 vaccine BNT162b2, making it the first mRNA vaccine to be widely deployed [1–3]. Phase 3 clinical trials have demonstrated that the BNT162b2 vaccine is highly beneficial for preventing severe COVID-19 and can elicit robust humoral and cellular immune responses against SARS-CoV-2 [4]. Despite these successes, the molecular and cellular mechanisms by which mRNA vaccines stimulate the immune system remain poorly understood [5,6]. Understanding these mechanisms is critical for optimizing vaccine design and tailoring immunization strategies. Previous studies have focused on immune cell and cytokine responses, but protective immunity also relies on proteome reprogramming [7,8].

Viruses are well-recognized environmental factors that can trigger autoantibody production and contribute to the development of autoimmune diseases. For example, Epstein–Barr virus (EBV) has been strongly implicated in multiple autoimmune conditions, including Hashimoto's thyroiditis; parvovirus B19 has been linked to autoimmune thyroiditis and lupus-like syndromes; and herpes simplex virus (HSV) infection can precede postinfectious autoimmune encephalitis, such as anti-NMDA receptor encephalitis [9] [10];. Similarly, SARS-CoV-2 infection has been associated with autoantibody generation and autoimmune-like manifestations, and its clinical features, immune responses, and pathogenic mechanisms show considerable overlap with those of established autoimmune diseases [11–13]. Indeed, autoantibodies, markers of an increased risk for autoimmune disorders, have been detected in patients with COVID-19, and several studies have reported associations between SARS-CoV-2 infection and heightened susceptibility to autoimmune conditions [14–16] [17,18],. While uncommon, case reports and observational studies have also described temporal associations between COVID-19 vaccination and autoimmune manifestations, primarily presenting as severe immune-mediated adverse events [19] [20],. Importantly, large-scale epidemiological studies consistently demonstrate that mRNA vaccines are safe and effective, with immune-mediated complications remaining rare [21,22,23]. Nevertheless, these observations highlight the importance of investigating whether mRNA vaccination may influence autoantibody production or immune tolerance in specific contexts [24,25].

In parallel, new clinical studies have reported that modified SARS-CoV-2 mRNA from vaccines may persist in blood and tissues for several weeks, while recombinant spike protein may circulate for months at low levels [26,27]. These findings raise important questions regarding the kinetics of vaccine components and their potential immunological consequences.

Comprehensive plasma proteomic analysis offers valuable insights into the body's response to vaccination and paves the way toward personalized immunization strategies [24,25]. Previous large-scale studies have identified proteomic and metabolomic signatures associated with immune responses in healthy individuals vaccinated against SARS-CoV-2 [19] [20],; however, many of these investigations relied on cross-sectional designs, limiting the ability to capture temporal dynamics. In contrast, our approach follows the same group of individuals over several months, enabling a detailed assessment of dynamic changes through repeated sampling at multiple time points. The purpose of this study was not to question the overall benefit of vaccination but rather to advance mechanistic understanding of the immune response. To this end, we employed a longitudinal multi-omics strategy that integrates plasma proteomics, autoantibody profiling, and antigen-specific IgG detection [28]. By collecting plasma samples before vaccination and up to 24 weeks after the second dose, we sought to capture dynamic proteome changes and evaluate whether vaccination influences autoantibody production. This work may provide insight into the mechanisms of protective immunity and identify potential biomarkers of vaccine-related immune alterations, thereby informing safer and more personalized immunization strategies.

2. Experimental procedures

The primary objective of this study was to characterize longitudinal host responses to SARS-CoV-2 mRNA vaccination, focusing on plasma proteome dynamics, antigen-specific IgG antibody responses, and the prevalence of autoantibodies against a broad panel of antigens.

2.1. Participants and Samples

Initially, 120 healthcare workers were recruited (>18 years old) at Cedars Sinai Medical Center (California, USA) to provide plasma samples before and after Pfizer-BioNTech vaccination. Participants completed a survey before vaccination to collect demographic and medical history, including autoimmune, cardiovascular conditions, and in prior COVID-19 exposure and infection, in addition to data on symptoms experienced after each dose of vaccine [29]. Previous COVID-19 infection and timing with the date of the first vaccine dose were determined on the basis of the COVID-19 diagnosis documented in the electronic health records, the presence of anti-N IgG antibodies at baseline (i.e. before the first vaccination), and the use of self-report survey information. Six participants were excluded from the study because of the evidence of a previous COVID-19 infection, resulting in a final cohort composed of 114 healthy individuals. All participants received two doses of BNT162b2 COVID-19 mRNA (Pfizer/BioNTech) and were invited for follow-up visits with serial blood collection at six timepoints: (1) before or up to 3 days after the 1st dose of vaccine ("Dose 1" or "baseline"), (2) 7 to 21 days after the 1st dose of vaccine ("Dose 2"), (3) 14 days ("2-week"), (4) 8 weeks, (5) 16 weeks, and (6) 24 weeks after the 2nd dose of vaccine. Subject information for vaccinated recipients is summarized in Table 1. All participants provided written informed consent, and all protocols were approved by the Cedars-Sinai institutional review board.

2.2. Blood sample collection and plasma preparation

The blood was collected into standard EDTA-K2 (EDTA dipotassium salt) Vacuette Blood collection tubes and stored on ice until processing within 90 min. The plasma was obtained from the centrifugation of the blood at 2500 rpm for 20 min and aliquoted and stored in a − 80 °C freezer until further use in the study.

2.3. Protein Extraction and Digestion

The detailed proteomics methods can be found in McArdle et al. [30]. Briefly, the samples were subjected to reduction, alkylation, trypsin digestion (4 h at +42 °C) and peptide desalting via an automated workstation (i7 BioMek, Beckman Coulter), as previously described [31]. Briefly, the samples were subjected to reduction, alkylation, trypsin digestion and desalting via an automated workstation (i7 BioMek, Beckman Coulter), as previously described [31].

2.4. LC–MS/MS analysis

Data-independent acquisition-mass spectrometry (DIA-MS) was performed as outlined previously [18]. Briefly, tryptic peptides were analyzed on an Orbitrap Exploris 480 instrument (Thermo Fisher Scientific) interfaced with a flex source coupled to an Ultimate 3000 ultrahigh-pressure chromatography system with mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1 % formic acid in acetonitrile). The peptides were separated on a linear gradient on a C18 column (15 cm, 3 μm) over the course of 60 min at a flow rate of 9.5 μL/min. Fragmented ions were detected across 50 DIA nonoverlapping precursor windows of 12 Da in size.

Table 1
Demographic data for the cohorts included in the study at each timepoint and over the whole study. The table present information on the age (median value and intervals), on the sex (count of individuals and percentage compared to the cohort of a given timepoint) and on the ethnicity (count of individuals and percentage compared to the cohort of a given timepoint).

	Visit 1 (Dose 1) (n = 114)	Visit 2 (Dose 2) (n = 114)	Visit 3 (2 Weeks) (n = 114)	Visit 4 (8 weeks) (n = 114)	Visit 5 (16 weeks) (n = 97)	Visit 6 (24 Weeks) (n = 91)	Whole cohort (n = 644)
Age							
Mean (SD)	44.9 (12.7)	44.9 (12.7)	44.9 (12.7)	44.9 (12.7)	46.1 (12.8)	45.6 (12.6)	45.2 (12.7)
Median [Min, Max]	42.0 [24.0, 79.0]	42.0 [24.0, 79.0]	42.0 [24.0, 79.0]	42.0 [24.0, 79.0]	44.0 [24.0, 79.0]	44.0 [24.0, 79.0]	43.0 [24.0, 79.0]
Sex							
Female (%)	89 (78.1 %)	89 (78.1 %)	89 (78.1 %)	89 (78.1 %)	75 (77.3 %)	72 (79.1 %)	503 (78.1 %)
Male (%)	25 (21.9 %)	25 (21.9 %)	25 (21.9 %)	25 (21.9 %)	22 (22.7 %)	19 (20.9 %)	141 (21.9 %)

2.5. Bioinformatic data analysis

DIA-MS raw files were converted into mzML, and the raw intensity data for peptide fragments were extracted from DIA files via the open-source OpenSWATH workflow [32,33], where experimental MS spectra were matched against a human twin population plasma peptide assay library [34]. The target and decoy peptides were then extracted, scored, and analyzed via the mProphet algorithm [35] to determine scoring cutoffs consistent with a 1 % false discovery rate (FDR). The peak group extraction data from each DIA file were combined via the "feature alignment" script, which performs data alignment and modeling analysis across an experimental dataset [32], and the transition-level data were normalized via MS2TIC. For batch correction BIRCH, an automated workflow based on the R framework (R 3.6.3) was used as described previously [36]. Significant changes between the individual timepoints and the baseline ("Dose 1") were identified via pairwise comparisons for a differential expression analysis via a Bayesian latent variable model with a Markov random field model as previously described [37]. Proteins with a computed Bayesian FDR of less than 0.05 were considered significantly different. The data have been uploaded to Pride. Visualization of the data regulation over time was performed via the MetaboAnalyst webtool, version 6.0, via Pearson correlation [38]. The enriched pathways were determined via DAVID enrichment via the Functional Annotation tool [39]. Significantly enriched pathways were determined by an FDR < 5 %.

2.6. Luminex autoantibody array design

Bead-based antigen arrays were used for multiplex analysis of IgG antibody reactivity against (1) proinflammatory cytokines, chemokines, and growth factors associated with autoimmune disorders such as myositis, systemic sclerosis, type I diabetes, rheumatoid arthritis and overlap syndrome (387 antigens) and (2) five viral structural proteins (spike: S1, S2, S1S2, RBD, N, and M) on the Luminex platform, as previously described [16]. Briefly, MagPlex Microsphere and xMAP® Antibody Coupling (AbC) kits were purchased from Luminex Corporation (Austin, TX, Supplement Table 1). The recombinant antigens were covalently coupled to magnetic carboxylated color-coded beads following the manufacturer's recommended protocol. The antigen-coupled beads were combined, incubated with probands' plasma at a ratio of 1:100 in assay buffer (PBS, 0.5 % BSA, 50 % Low-Cross buffer (Candor Biosciences, Wangen, Germany)) and, after washing procedures, incubated with a secondary PE-labeled anti-human IgG antibody. The beads were washed again and then analyzed with a FlexMap3D instrument (Luminex Corporation). The median fluorescence intensity (MFI) values, reflecting semiquantitative autoantibody levels, were obtained for each color-coded antigen-coupled bead and each sample. This procedure was carried out once for each sample.

2.7. Statistical analysis

The MFI values for each sample/time point were normalized and log2-transformed. Autoantibody positivity was defined as the value of the mean of controls (i.e. baseline samples named "Dose 1") plus 3 standard deviations for each antibody. The evolution of antibody levels over time was assessed via paired t-tests. For a single comparison of two groups, Student's t-test was used after evaluation of normality. If the data distribution was not normal (Kolmogorov–Smirnov normality test), a Wilcoxon signed rank test was performed. To compare multiple groups, ANOVA was used. When the comparison of all groups revealed a significant difference, we performed pairwise comparisons via a parametric two-tailed t-test. Correlations between the tested parameters were performed via Spearman correlation. Hypothesis testing was two-sided, and we considered FDRs <5 % to be significant.

For all the data generated and presented, ProEpiC, R and Prism (version 9, Graph Pad, San Diego, CA) were used. The data are presented as the means±the standard errors for the mean (SEM) or median with a 95 % confidence interval (CL), as stated in the corresponding, as stated in the corresponding Figure legends or in the text.

3. Results

The study included 120 participants aged 24–79 years whose blood samples were collected over a time span of up to six months following the second dose of the vaccination against SARS-CoV-2. Among them, 6 participants were previously infected with SARS-CoV-2 and were excluded from the final analysis. The final cohort included 114 participants who donated blood at the first four timepoints, then 97 participants and 91 participants who donated blood at 16 and 24 weeks after the 2nd dose of vaccine (Table 1, Supplemental Table 7).

Human IgG antibodies to the SARS-CoV-2 structural protein remain stable at 24 weeks post-vaccination.

This study assessed the longitudinal production of IgG antibodies against key SARS-CoV-2 structural proteins, including full-length spike (S), nucleocapsid (N), and membrane (M) proteins. Antibody response patterns before and after vaccination in 114 healthy individuals are illustrated in Fig. 1. Consistent with expectations, the level of antibodies targeting the N or M antigens were low or under the limit of detection both before and after vaccination, suggesting the absence of SARS-CoV-2 infection. In contrast, robust and universal production of antibodies against the receptor-binding domain (RBD), S1, and S2 regions of the spike protein was observed post-vaccination (Fig. 1). Furthermore, statistical analysis via the Kruskal–Wallis one-way ANOVA with Dunn's post hoc test for pairwise comparisons revealed significant increases in IgG levels against the RBD, S1, and S2 (adjusted p-value<0.0001), with the RBD eliciting the strongest antigenic response, generating significantly higher antibody levels than S1 or S2 (Supplemental Tables 1–6).

Adjusting for sex and age, revealed that antibody production

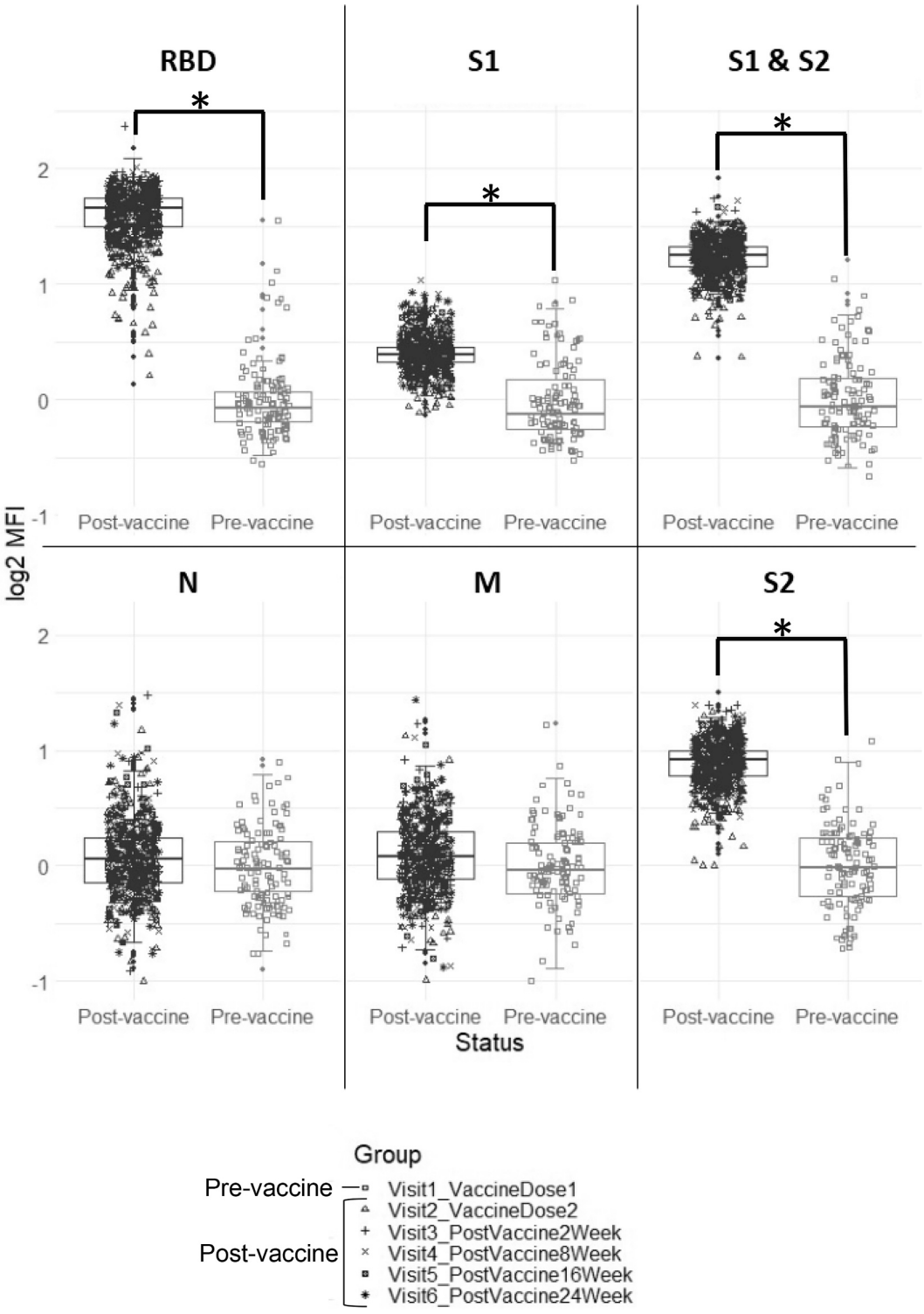

Fig. 1. Comparative analysis after Pfizer mRNA vaccine demonstrates significant increase of IgG against SARS-CoV-2 proteins. Antibodies quantification was performed by the Luminex SARS-CoV-2 IgG multiplex panel, in plasma of 114 healthy individuals across 6 timepoints, up to 24 weeks after the second dose of vaccine. Results represent individual values per collection timepoint (icons) and means ± the SEM (bars) for IgG antibodies recognizing RBD, S1, S2, S1,S2, N, and M SARS-CoV-2 antigens. Significant changes were statistically determined using an unpaired and paired t-test (details in Supplementary Tables 1–6). *p-value<0.05.

increased in all groups following the second vaccine dose, with younger participants (<45 years) showing higher and more sustained responses compared to older participants (>45 years) (Fig. 2). In contrast, no significant differences were observed between male and female participants, which is consistent with previous reports [40–42](Fig. 2).

Plasma proteome profiling reveals major adaptations in the plasma proteome starting at week 16.

Mass spectrometry–based analysis was performed on 643 plasma samples collected across six timepoints from healthy individuals. In total, 342 unique proteins were quantified on the basis of 22,044 unique peptides (Supplemental Table 8). A global heatmap revealed district clustering of the earliest (baseline through 8 weeks) versus later (16 and

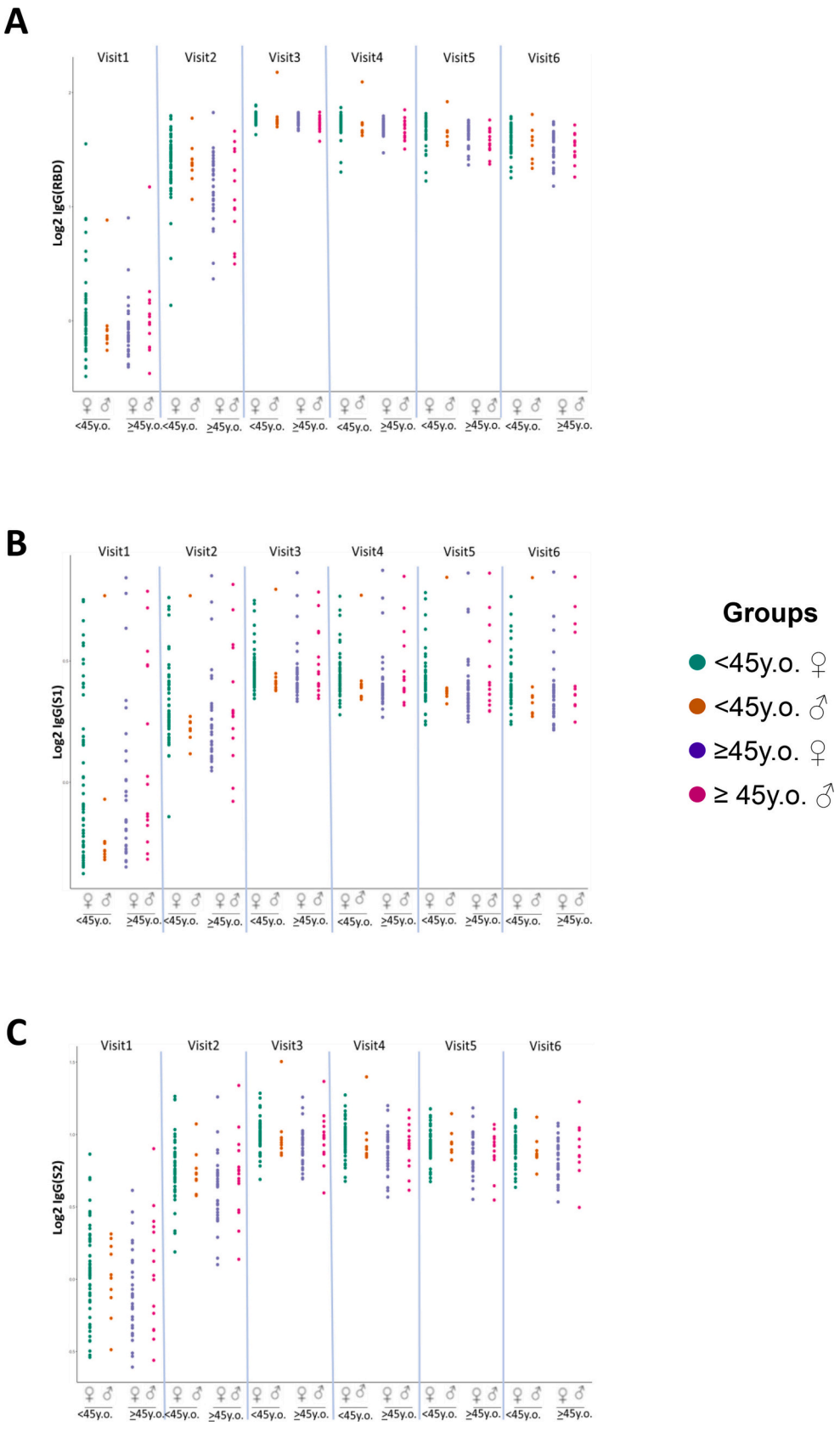

Fig. 2. Quantifications of IgG antibodies against virus-specific antigens in plasma samples of participants monitored over 24 weeks. Each dot represent individual quantification of IgG antibodies targeting RBD (panel A), S1 (panel B) and S2 (panel C) by the Luminex SARS-CoV-2 IgG multiplex panel. Participants were categorized in four groups based on gender and age (<45y.o. & >45y.o.). No major differences between the groups for any of the three monitored IgG antibodies were highlighted.

24 weeks) timepoints (Fig. 3, panel A).

Statistical analysis identified significant changes in protein expression patterns over time, including 214 proteins that were significantly differently expressed (FDR < 5 %) in at least one timepoint compared with the baseline ("Dose 1"). While only 10–11 proteins were altered at early timepoints, 192 and 199 proteins showed significant changes at 16 and 24 weeks, respectively. ANOVA statistical test across all timepoints confirmed 240 proteins with significantly alterations (FDR < 5 %), with the most pronounced changes occurring after week 16 and persisting through week 24 (data not shown). These findings highlight sustained remodeling of the plasma proteome long after the second vaccine dose.

Additionally, a time series analysis of 82 individuals with complete longitudinal sampling identified 25 proteins that were significantly correlated with time (Fig. 3, panel B). Of these 20 proteins increased, and 5 decreased progressively. The KEGG pathway annotation revealed enrichment in carbohydrate metabolism (pentose and gluconate interconversions; pentose phosphate pathway: UTP glucose-1-phosphate uridylyltransferase (UGP2), transaldolase (TALDO1), lambda-crystallin homolog (CRYL1)), metabolism of cofactors and vitamins, Nicotinate Phosphoribosyltransferase (NAPRT), and the thyroid hormone synthesis pathway (TTR).

Proteins involved in immune adaptation/response and thyroid hormone transport are highly regulated in plasma proteomes.

DAVID enrichment and KEGG mapping identified three pathways of particular relevance: coronavirus disease (hsa: 05171), ii. complement and coagulation cascades (hsa: 04610), and iii. Thyroid hormone synthesis (hsa: 04918).

Within the coronavirus disease pathway (hsa: 05171), 31 proteins were mapped, with 1, 24, and 24 significantly altered at weeks 2, 16, and 24, respectively, compared with the baseline ("Dose 1")(Fig. 4). Most proteins were upregulated after week 16, with the exception of complement proteins (C9, complement component C9 and CFB, complement factor B) whose expression decreased compared with baseline.

Analysis of complement and coagulation cascades, with 50 proteins associated with our proteome dataset, revealed dynamic changes in the classical pathway (hsa: 04610). The expression of the structural C1

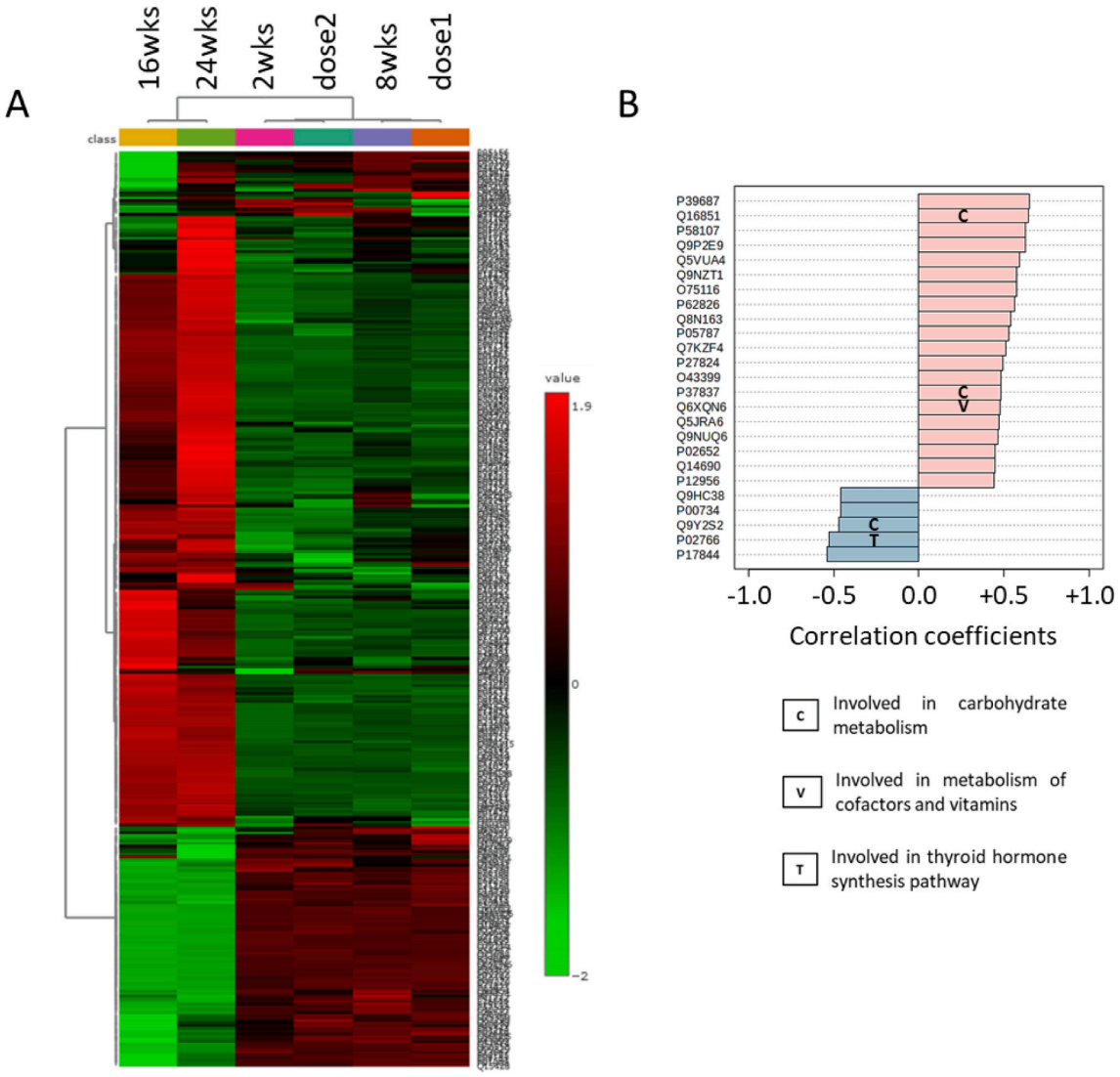

Fig. 3. Plasma proteome regulations observed up to 6 months after SARS-CoV-2 mRNA vaccination. The heatmap allows the visualization of the relative protein intensities obtained for all individuals across 6 timepoints (average values) including at baseline (dose 1), after the first vaccination (dose 2) or several days after vaccination (panel A). In total, 342 uniquely quantified proteins were included. Clustering analysis highlight close plasma proteome signature at 16 weeks and 24 weeks, differentiated from the proteome signatures which are similar between the four others timepoints. A correlative analysis (Pearson correlation) was performed to determine a list of 25 proteins for which MS-based intensities correlated with the time course (panel B). Three proteins were associated to carbohydrate metabolism (Q16851, P37837, Q9Y2S2), one protein was associated to the metabolism of cofactors and vitamins (Q6XQN6), and one protein was associated to the thyroid hormone synthesis pathway (P02766).

Gene name	Median value of protein quantification per timepoint (log2)					
	dose1	dose2	2week	8week	16week	24week
C1R	15.71	15.65	15.58	15.62	**15.84**	**15.80**
C3	22.09	22.04	22.03	22.09	**22.21**	**22.30**
C5	16.41	16.41	16.39	16.45	**16.77**	**16.75**
C1QA	10.08	9.82	9.99	10.14	10.72	10.57
C1QB	14.85	14.68	14.70	14.77	14.71	14.80
C1QC	15.95	15.93	15.92	16.00	**16.17**	**16.19**
C9	16.29	16.25	16.29	16.26	**16.23**	**16.33**
C8A	14.97	14.92	14.96	14.91	**15.36**	**15.43**
C8B	15.12	15.00	15.13	15.14	15.50	**15.48**
C8G	14.19	14.11	14.13	14.16	**14.49**	**14.66**
C1S	15.75	15.70	15.63	15.69	**16.14**	**16.20**
C7	14.48	14.44	14.46	14.46	**15.09**	**15.24**
C6	16.02	15.95	15.97	15.99	**16.26**	**16.31**
MASP2	11.24	11.27	11.20	11.24	**11.43**	**11.48**
F2	19.20	19.22	19.20	19.21	**19.53**	**19.62**
FGA	22.83	22.88	22.84	22.84	**23.40**	**23.24**
FGB	22.98	23.01	23.03	23.01	**23.76**	**23.66**
FGG	21.34	21.28	21.37	21.35	**22.13**	**22.07**
F13B	15.81	15.92	15.83	15.88	**16.11**	**16.11**
CFD	12.13	12.08	12.18	12.22	**12.68**	**12.62**
CFB	18.91	18.87	18.84	18.85	**18.71**	**18.82**
VWF	11.91	12.30	**11.43**	11.29	**14.33**	**14.17**
RPLP0	13.24	13.35	13.09	13.18	13.29	13.37
C2	14.19	14.21	14.18	14.19	**14.39**	**14.41**
C4B	11.71	11.61	11.58	11.66	**12.69**	**12.68**
RPS3	15.61	15.77	15.72	15.69	16.07	16.14
RPS9	13.26	13.30	13.07	13.25	**13.73**	13.50
RPS13	18.73	18.73	18.79	18.72	19.19	19.09
RPS25	13.70	13.75	13.66	13.64	**14.68**	**14.27**
RPL31	12.33	12.37	12.24	12.31	11.02	11.36
RPS21	12.71	12.75	12.78	12.81	**13.03**	**13.02**

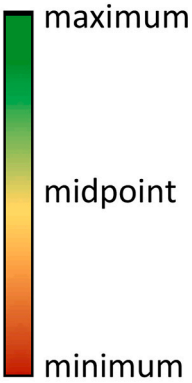

Fig. 4. Table summarizing the relative MS-based intensity of 31 proteins involved in the "coronavirus disease" pathway (KEGG pathway database, map: hsa05171). Dark green: highest protein intensity of a given protein over the 6 timepoints; dark red: lowest protein intensity (horizontal color scale). Bold numbers represent regulations considered significant at a given timepoint (FDR < 5 %) compared to the baseline ("dose 1"). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

complex proteins (C1R, C1QC, C1S, C1QA) increased significantly at weeks 16–24, whereas that of SERPING1 (a C1 inhibitor) decreased, suggesting a shift toward complement activation (Fig. 5). Platelet-related proteins (n = 50) were also evaluated, revealing significant changes in four proteins (variation over ±15 % of the timepoint median value), including the upregulation of von Willebrand factor (VWF) and the downregulation of epiplakin(EPPK1), ras-related nuclear protein (RAN), and ATP Synthase Peripheral Stalk Subunit OSCP (ATP5PO) (Fig. 6)

Finally, although thyroid hormone synthesis (hsa: 04918) was not significantly enriched at the global level, several proteins showed notable changes (FDR > 5 %). TTR (Transthyretin), ALB (Albumin) and GPX3 (Glutathione peroxidase 3) were significantly increased at weeks 16 and 24, whereas SERPINA7 (Thyroxine-binding globulin) and GSR (Glutathione reductase) demonstrated reduced expression levels, without reaching significance threshold (FDR < 5 %), compare with the baseline values ("Dose 1") (Fig. 7).

Changes in the autoantibody profile before and after SARS-CoV-2 vaccination.

Plasma samples were analyzed with a multiplexed bead-based assay using Luminex FlexMAP 3D technology [16] allowing the measurement of 387 targets, including cytokines and interferons (Supplemental Table 9). This strategy was designed to evaluate potential changes in auto-antibodies reactivity that could be triggered following mRNA COVID-19 vaccination. Compared within the six longitudinal timepoints, the majority of tested the self-antigens showed no evidence of altered reactivity, suggesting the absence of broad vaccine-induced autoantibody responses (data not shown).

Among the scrutinized targets, a panel of autoantibodies against 25 interleukins were quantified (Fig. 8). Most remained stable over time, however the level of three interleukins (IL27, IL6 and IL36A) modestly increased beginning 8 weeks after vaccination and peaked at 16–24 weeks after vaccination. A broader pattern of upregulation was observed for 21 interleukin specific autoantibodies, which increased from 16 weeks onward post-vaccination and remained elevated at 24 weeks after vaccination. In contrast, the level of IL-1B autoantibodies, increased earlier, two weeks after the second dose of the vaccine, followed by a modest decrease at last three timepoints.

Finally, a subset of 11 interferons was also evaluated, including type I interferons (IFN-α subtypes INF2, 4, 6, 13, 16, 17 and 21), IFN-β (IFNB1), IFN-λ (IFNL2, 3), IFN-κ (IFNK), and IFN-ω (IFNW1) (Fig. 9). No significant or consistent changes were observed in autoantibody reactivity against any interferons across the six timepoints. (Fig. 10.)

4. Discussion

This study provides important insights into the dynamic immunological and proteomics adaptations elicited by mRNA vaccination against SARS-CoV-2 in a cohort of 114 healthy individuals, which were sampled longitudinally over six time points over a six-month period.

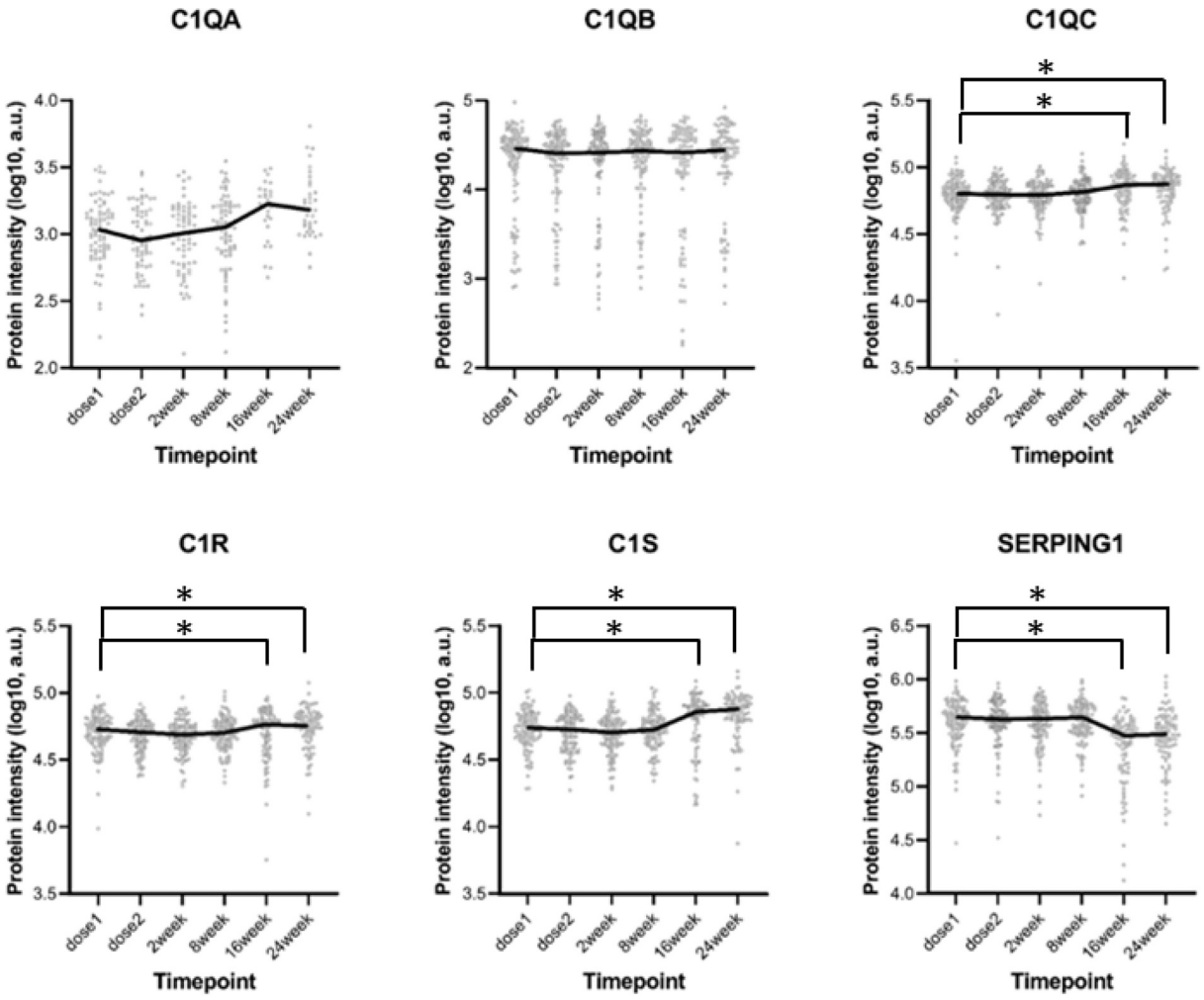

Fig. 5. Among the 50 proteins involved in the complement and coagulation cascade (KEGG Pathway database, map hsa04610), 6 proteins are part of the "classical pathway" (also named antigen-antibody complex, C1) and relative protein intensities are represented across the timepoints. Structural proteins (C1QA, C1QB, C1QC, C1R and C1S) demonstrated MS-based intensity increases with time, while SERPING1 (a known inhibitor of C1 complex) demonstrated a significant reduction in protein intensity. Grey dots represent individual values, and black lines represent the median protein intensity obtained per timepoint including all healthy individuals. *FDR < 5 %.

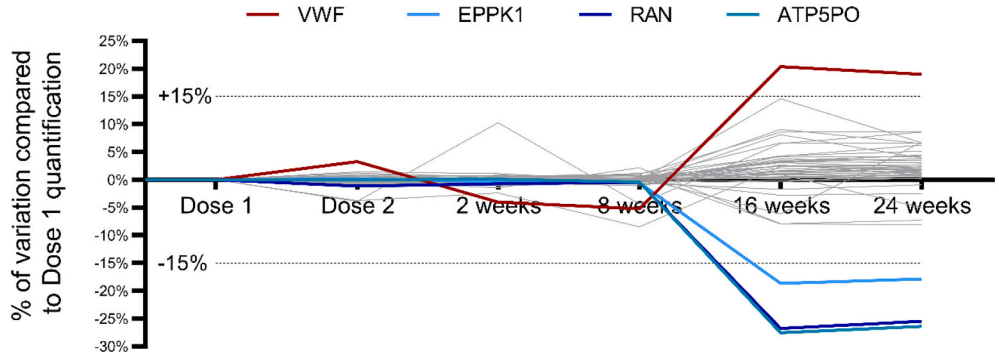

Fig. 6. Proportional changes of protein intensity based on baseline MS quantification ("Dose 1") of 50 proteins associated with the term "platelet". The grey lines represent the median value of a given protein across the 6 timepoints which do not present major regulations. The red line is associated to VWF protein which demonstrated significant protein intensity increase (+15 %) starting at 16 weeks, compared to the baseline ("Dose 1"). Conversely, blues lines correspond to EPPK1, ATP5PO and RAN proteins which demonstrated a significant protein intensity decrease (−15 %)starting at 16 weeks, compared to the baseline ("Dose 1"). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This longitudinal sampling design allowed us to track temporal antibody responses, proteomic changes, and autoantibody reactivities with high resolution.

As expected, IgG levels against the RBD, S1, and S2 antigens were significantly increased after vaccination (Fig. 1), with the RBD elucidating the strongest reactivity, peaking shortly thereafter, and remained

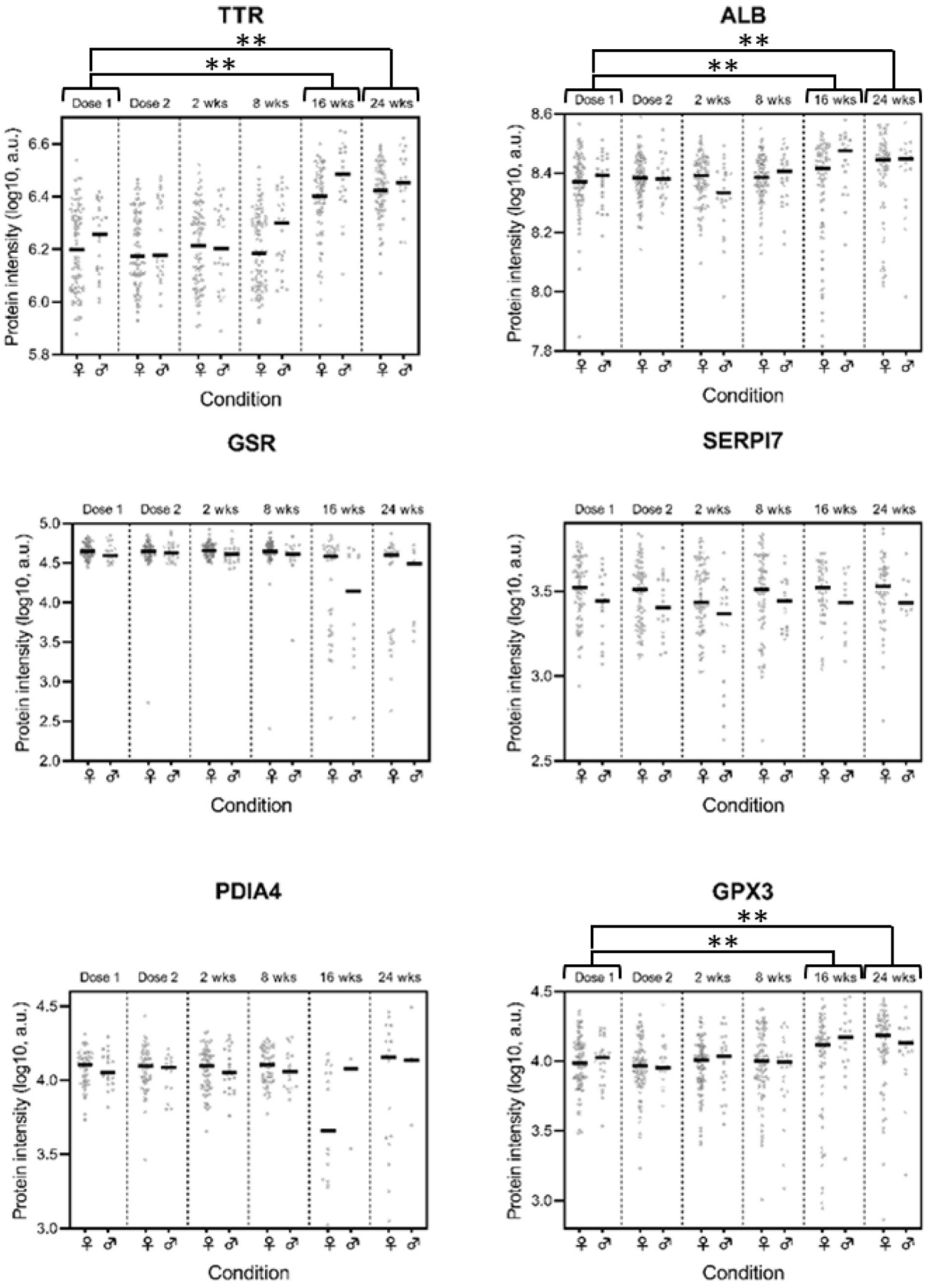

Fig. 7. Visualization of the MS-based quantifications of six proteins involved in the "thyroid hormone synthesis" pathway (KEGG database, map: hsa04918). The grey dots represent individual values, and the black bars represent the median value per gender group and per timepoint. ** FDR < 1 %, compared to the baseline.

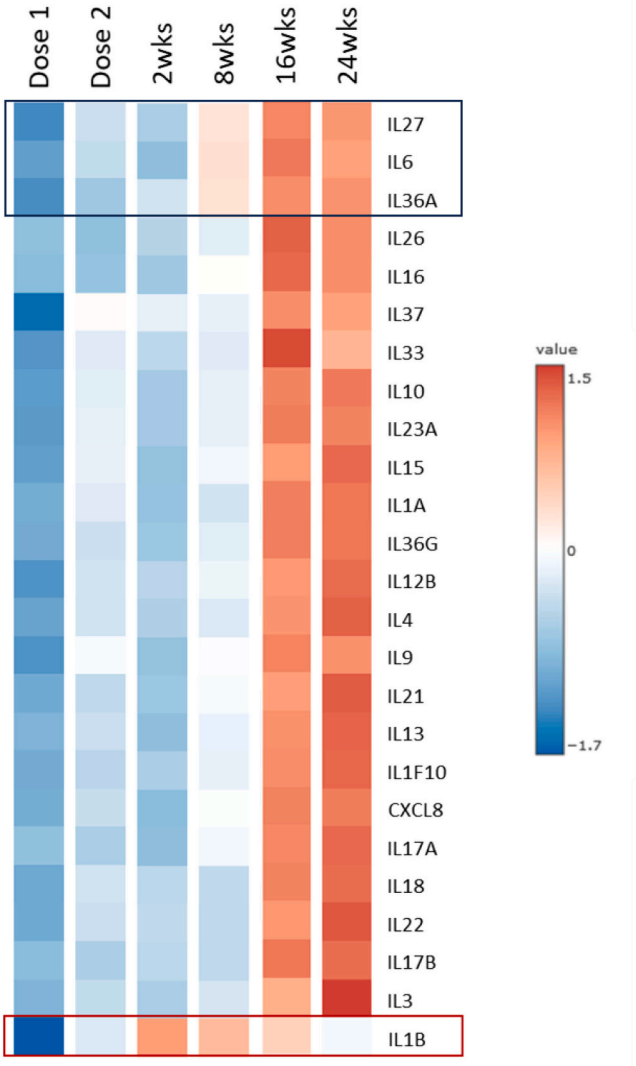

Fig. 8. Relative quantifications of autoantibodies against 25 interleukins obtained by Luminex FlexMAP 3D technology across 6 timepoints (average values). Most of the autoantibodies presented an important increase starting at 16 weeks, maintained at 24 weeks, compared to the baseline ("Dose 1"). Autoantibodies targeting IL27, IL6 and IL36A demonstrated a slight increase starting 8 weeks after vaccination before increasing noticeably at 16 weeks (black box). Conversely, autoantibody targeting IL1B demonstrated a higher intensity 2 weeks after vaccination, before a slight decrease over the last three timepoints.

within the positive range for at least 6 months. Younger participants (<45 years) mounted higher and more sustained responses than older individuals did, which is consistent with published observations linking age to reduced vaccine immunogenicity [43]. These findings reinforce the importance of anti-RBD-IgG as a sensitive serological marker of vaccine-induced immunity [44].

Analysis of the plasma proteome revealed significant alterations emerging around 16 weeks post-vaccination (Fig. 3A), particularly in carbohydrate metabolism pathways (Fig. 3B), consistent with immune cell activation reported previously [45,46]. Williams et al., reported that metabolic pathways, including carbohydrate metabolism, were among the most robustly altered proteomic signatures, especially when comparing vaccine-induced responses to breakthrough infection. These metabolic shifts were linked to immune cell activation and systemic adaptation after exposure. Together, our studies support the idea that metabolic rewiring, particularly in carbohydrate utilization, is a conserved hallmark of mRNA vaccine–induced immune readiness across independent cohorts. Furthermore, our DAVID enrichment analysis of proteins showing significant regulation between baseline ("Dose 1") and later timepoints identified several enriched pathways (FDR < 5 %), including coronavirus disease and complement/coagulation cascades. Notably, 31 proteins mapped to the coronavirus disease pathway, with most showing increased abundance beginning at 16 weeks and persisting through 24 weeks, suggested that the plasma proteome can mimic features of viral infection and highlight the adaptive efficacy of vaccination.

Within these pathways, multiple proteins of the C1 complex were detected, along with SERPING1, its primary inhibitor. Interestingly, five C1 subunits were elevated while SERPING1 was reduced after week 16 compared to baseline, pointing to an increased representation of the C1 complex and reduced inhibition. Such regulation matches responses observed during SARS-CoV-2 infection but at a lower amplitude, consistent with the controlled antigen exposure elicited by vaccination. This pattern underscores the ability of vaccination to "train" the immune system by inducing durable, systemic adaptations. Importantly, these findings are consistent with longitudinal proteomic and transcriptomic studies showing persistent immune gene and protein modulation months after mRNA immunization [43,44,47–49].

Even if limited, few studies have focused on the risk of developing thrombosis-related pathologies such as thrombocytopenia [50], characterized by a low blood platelet count. To visualize the proportional changes in the plasma of healthy individuals after vaccination, a subset of 50 proteins associated with the term "Platelet" was constructed from the quantified plasma proteome dataset. Our platelet-associated protein analysis indicated stability, supporting the absence of thrombocytopenia in line with prior reports [51–53]. Aid et al., 2023 showed that while COVID-19 vaccination can activate coagulation and inflammatory cascades, the pathological thrombosis with thrombocytopenia syndrome (TTS) involves a distinct, dysregulated profile, was not observed in healthy people vaccinated with BNT162b2. Together, these findings suggest that complement and coagulation remodeling after mRNA vaccination represents a controlled, adaptive response, contrasting sharply with the aberrant signatures linked to TTS.

Finally, we explored plasma proteome changes related to thyroid function as a few case reports have described thyroid abnormalities following COVID-19 vaccination [54,55]. In our dataset, seven proteins involved in thyroid hormone synthesis and transport were quantified. Notably, TTR and ALB, two major thyroid hormone carriers, substantially increased beginning at week 16 post-vaccination and remained elevated at week 24. In addition, retinol-binding protein 4 (RBP4), detected by mass spectrometry, also increased significantly at week 16 and remained elevated through week 24 (data not shown). TTR is responsible for transporting thyroxine (T4) and retinol-binding protein, whereas ALB carries triiodothyronine (T3), T4, drugs, and other metabolites. Thus, elevated TTR and ALB levels may indicate systemic proteome adaptations to enhance hormone transport, possibly in response to transient increases in thyroid hormone production or altered acute-phase/metabolic regulation after vaccination. These findings are consistent with reports showing increased thyroid hormone levels in vaccinated patients up to 37 days (i.e., 5 weeks) after the second Pfizer-BioNTech dose [54,56]. The later onset of protein-level changes observed in our study (>16 weeks) compared to hormone elevations (~5 weeks) could reflect the time required for proteome turnover following hormonal regulation. This interpretation is supported by the relative magnitude of changes, as TTR levels increased more markedly than those of ALB did, which is consistent with its shorter half-life (2–2.5 days) compared to ALB (20–22 days) [57–59]. Our study design, which included measurements at 2, 8, and 16 weeks after the second dose, may also have delayed the detection of earlier proteomic shifts. Because our follow-up extended only to 24 weeks (~5.5 months), longer-term trends remain unknown. A recent large cohort study (>2 million individuals) [56] reported increased risk of both hyper- and hypothyroidism up to 12 months after mRNA vaccination. Overall, we

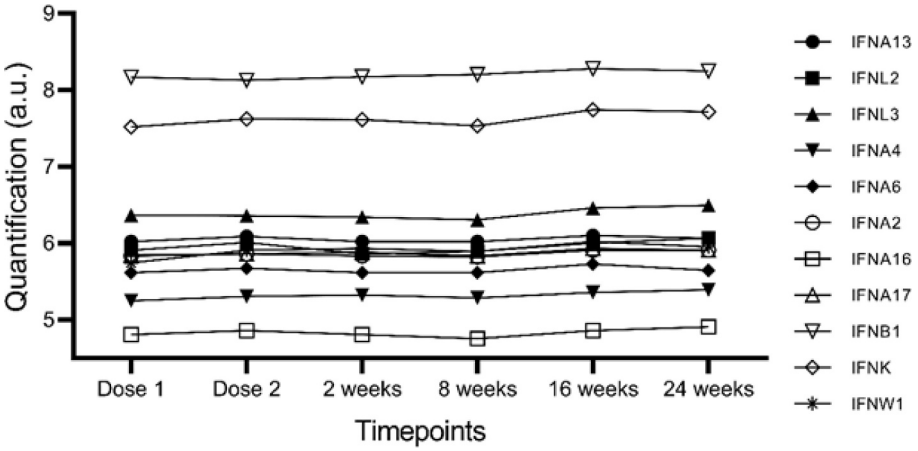

Fig. 9. Quantification of autoantibodies targeting 11 interferons across six timepoints by Luminex FlexMAP 3D technology. The represented values are the median quantifications obtained for a given interferon at a given timepoint.

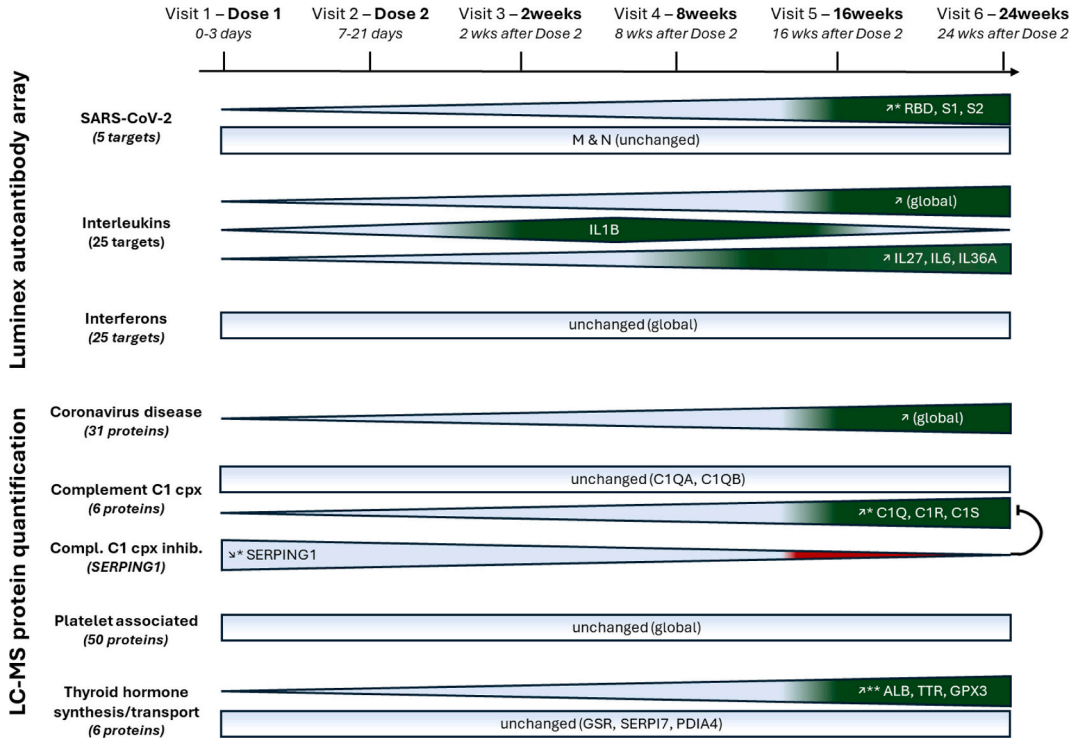

Fig. 10. Graphical summary of the plasma regulations in response to mRNA vaccination in healthy individuals, using complementary strategies: autoantibodies detection (Luminex) and protein quantification (LC-MS). Only significant changes are highlighted in green (upregulation) or in red (downregulation) compared to the baseline. * FDR < 5 %, **FDR < 1 %. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

can hypothesize that thyroid hormone related protein alterations may be transient and could normalize beyond the 6-month period, but extended longitudinal studies are needed to confirm this trajectory.

Using Luminex technology, we also explored the presence of autoantibodies that target cytokines and interferons, motivated by prior evidence that such autoantibodies can arise after infections and may modulate immune responses [60,61]. Autoantibodies are produced by the immune system to react with self-molecules that participate in defense against infection, immune system homeostasis and serve in housekeeping functions [62]. COVID-19 infection, in particular, has been associated with autoantibodies against type I interferons and select cytokines, which correlate with severe outcomes [16,63],. Importantly, our longitudinal analysis revealed no induction of interferon autoantibodies over 24 weeks, after vaccination, providing reassurance that mRNA vaccination does not elicit this potentially harmful response, in contrast to both severe COVID-19 infection and certain live-attenuated vaccines [64].

Notably, we observed dynamic but non-pathogenic regulation of select cytokine-targeting autoantibodies. IL-1β autoantibodies increased immediately after the second vaccine dose but returned to baseline by week 24, which is consistent with a model of transient immune activation [65,66]. This regulation pattern may support the hypothesis of transient immune activation following vaccination [67]. By comparison, autoantibodies against IL-6, IL-27, and IL-36α increased later (beginning at week 8 or 16) and persisted through 24 weeks, suggesting a role in longer-term immune adaptation [68]. Importantly, IL-27 is known to enhance T-cell responses during vaccination, and its delayed autoantibody induction could reflect a physiological feedback mechanism that

tempers T-cell activation over time. This aligns with reports showing that CD8+ T cell frequencies peak within days after mRNA vaccination [69] and then gradually decline over the subsequent 80–120 days [70], raising the possibility that IL-27 autoantibodies contribute to calibrating this trajectory. Similarly, IL-36 family cytokines regulate TNF-α and IL-17 release, and the observed increased in IL-36α autoantibodies in our study beginning at week 16 may represent a neutralization strategy to restore immune homeostasis after the peak vaccine response [71,72]. Together, these findings indicate that while mRNA vaccination can transiently modulate cytokine autoantibodies, these responses appear to be regulated, self-limiting, and distinct from the pathogenic autoantibody profiles observed in COVID-19 infection. By integrating longitudinal profiling with contextual immunological data, our study supports the view that vaccine-induced autoantibody fluctuations are part of a broader, coordinated immune-metabolic adaptation rather than evidence of harmful autoimmunity.

5. Limitations of the study

While the study is strengthened by paired sampling across multiple post-vaccination intervals, certain constraints should be noted. First, the cohort size remains modest and was restricted to healthcare professionals with relatively homogeneous ages, ethnic backgrounds, and occupational exposure to SARS-CoV-2. Importantly, our participants did not include individuals with chronic conditions or those receiving immunomodulatory therapies, factors that could influence immune responses to vaccination and potentially alter autoantibody profiles. Furthermore, only the BNT162b2 mRNA vaccine was studied; therefore, our findings may not be directly generalizable to other vaccine platforms.

Next, while we provide statistical evidence of significant changes in cytokine-targeting autoantibodies, we did not perform functional neutralization assays to determine whether these autoantibodies exert biological effects. These assays, however, were beyond the scope of this study, and similar investigations have been conducted by other groups and have shown that neutralizing antibodies interact with antigenic proteins and that their titers correlate with cytokine levels and vaccine type [73,74].

While our proteomic profiling revealed alterations in metabolic pathways based on protein-level changes, the absence of complementary metabolomics data, such as organic acids, alcohols and sugars, which are involved in core biochemical processes would provide new level of information for determining better the metabolic alterations induced after mRNA vaccination.

Furthermore, our study did not assess the persistence of spike protein in the blood of vaccinated individuals. Recent reports indicate that modified SARS-CoV-2 mRNA may persist for up to a month post-injection and be detected in cardiac and skeletal muscle at sites of inflammation and fibrosis, while recombinant spike protein can remain in circulation for over half a year [26,27]. Incorporating such targeted analyses alongside global proteomic profiling would provide valuable complementary insights into vaccine-induced molecular changes.

Finally, although the strength of this study lies in its true longitudinal design, with six time points sampled from the same individuals and quantitative measurement of autoantibodies against more than 300 antigens, the results remain predominantly explanatory. Future studies integrating larger and more diverse cohorts, additional vaccine platforms, and multi-omics approaches will be required to fully delineate the mechanisms linking mRNA vaccination to immune and metabolic adaptation.

CRediT authorship contribution statement

Blandine Chazarin: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Archana Subramanya Bhat:** Visualization, Methodology, Investigation, Data curation. **Niveda Sundararaman:** Visualization, Software, Methodology, Investigation, Data curation, Writing – review & editing. **Yunxian Liu:** Writing – review & editing, Visualization, Formal analysis. **Jana Gajewski:** Methodology, Data curation. **Ann-Sophie Lindemann:** Formal analysis, Writing – review & editing. **Petra Budde:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Hans-Dieter Zucht:** Formal analysis, Writing – review & editing. **Sandy Joung:** collect and coordinate sample logistic, Writing – review & editing, Resources, Project administration. **Joseph E. Ebinger:** Writing – review & editing, Conceptualization. **Rowann Mostafa:** collect and coordinate sample logistic, writing - review & editing. **Brian Walker:** collect and coordinate sample logistic, writing - review & editing. **Aleksandra Binek:** Formal analysis. **Lindsey Becker:** collect and coordinate sample logistic, writing - review & editing. **Koen Raedschelders:** Project administration. **Kimia Sobhani:** Writing – review & editing, Conceptualization. **Susan Cheng:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Jennifer E. Van Eyk:** Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Justyna Fert-Bober:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kimia Sobhani, Susan Cheng, Jennifer E. Van Eyk, Justyna Fert-Bober reports financial support was provided by NIH. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127888.

Data availability

The mass spectrometry based data will be available on a free-access repository.

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