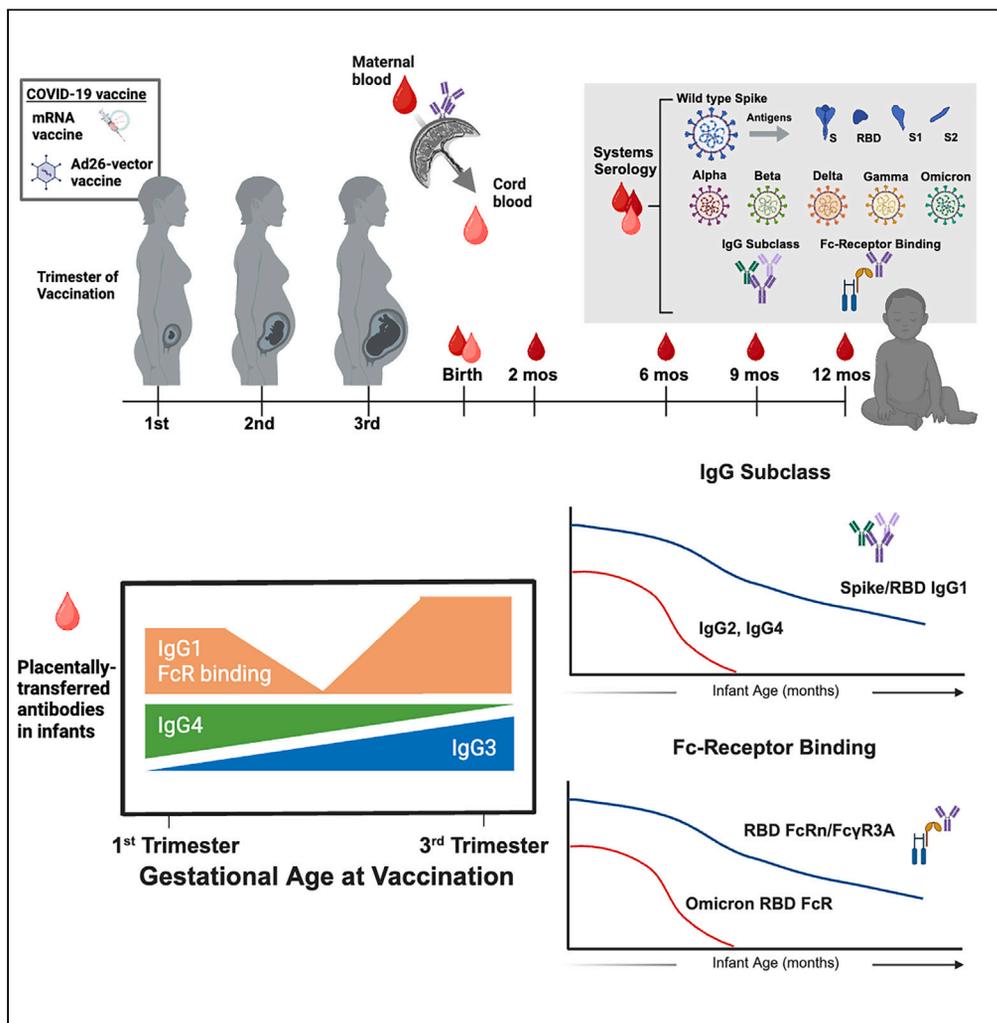


Article

# Placental transfer dynamics and durability of maternal COVID-19 vaccine-induced antibodies in infants



Paola A. Lopez,  
Nadège Nziza,  
Tina Chen, ...,  
Douglas A.  
Lauffenburger,  
Boris D. Julg,  
Andrea G. Edlow

aedlow@mgh.harvard.edu

**Highlights**  
Maternal COVID-19  
vaccine-induced  
antibodies persist in most  
infants through 6 months

IgG1 and IgG3 antibodies  
had the highest  
persistence in 2- and  
6-month infants

Antibodies binding  
placental neonatal Fc-  
receptor and FcγR3A had  
high persistence

Timing of maternal  
vaccination and fetal sex  
influenced antibody  
persistence

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## Article

## Placental transfer dynamics and durability of maternal COVID-19 vaccine-induced antibodies in infants

Paola A. Lopez,<sup>1,9</sup> Nadège Nziza,<sup>2,9</sup> Tina Chen,<sup>2</sup> Lydia L. Shook,<sup>3,4,5</sup> Madeleine D. Burns,<sup>6</sup> Stepan Demidkin,<sup>4</sup> Olyvia Jasset,<sup>4</sup> Babatunde Akinwunmi,<sup>7</sup> Lael M. Yonker,<sup>5,6</sup> Kathryn J. Gray,<sup>5,7</sup> Michal A. Elovitz,<sup>8</sup> Douglas A. Lauffenburger,<sup>1,10</sup> Boris D. Julg,<sup>2,5,10</sup> and Andrea G. Edlow<sup>3,4,5,10,11,\*</sup>

## SUMMARY

**Completion of a COVID-19 vaccination series during pregnancy effectively reduces COVID-19 hospitalization among infants less than 6 months of age. The dynamics of transplacental transfer of maternal vaccine-induced antibodies, and their persistence in infants at 2, 6, 9, and 12 months, have implications for new vaccine development and optimal timing of vaccine administration in pregnancy. We evaluated anti-COVID antibody IgG subclass, Fc-receptor binding profile, and activity against wild-type Spike and RBD plus five variants of concern (VOCs) in 153 serum samples from 100 infants. Maternal IgG1 and IgG3 responses persisted in 2- and 6-month infants to a greater extent than the other IgG subclasses, with high persistence of antibodies binding placental neonatal Fc-receptor and Fc $\gamma$ R3A. Lowest persistence was observed against the Omicron RBD-specific region. Maternal vaccine timing, placental Fc-receptor binding capabilities, antibody subclass, fetal sex, and VOC all impact the persistence of antibodies in infants through 12 months of age.**

## INTRODUCTION

The U.S. Centers for Disease Control and Prevention (CDC) recommends that everyone over the age of 6 months receive both doses of the mRNA COVID-19 vaccine (BNT162b2 and mRNA-1273).<sup>1,2</sup> Large population-based studies have shown that a two-dose series of the mRNA COVID vaccine provides moderate protection against symptomatic infection in children,<sup>3</sup> as well as in the development of severe COVID-19-associated outcomes.<sup>4</sup> However, there is a lack of knowledge regarding how maternal vaccine timing, placental Fc-receptor binding, and fetal characteristics drive transplacental antibody transfer and persistence of antibodies in infants over the first year of life.

While infants under the age of 6 months remain ineligible to receive the COVID-19 vaccine, they are one of the populations at highest risk for severe COVID-19 in the setting of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Infants younger than 6 months not only have higher COVID-19 hospitalization rates compared to other pediatric groups, but also are at higher risk for acquiring life-threatening complications from COVID-19, including acute respiratory failure.<sup>5,6</sup> Maternal immunization is a key strategy to protect infants younger than 6 months from infectious diseases through passive transplacental antibody transfer.<sup>7</sup> Additionally, maternal immunization shapes infant immune development by priming the cellular immune response.<sup>8</sup> Although the level of antibody necessary to achieve infant protection against severe COVID-19 remains unknown, one study showed that the completion of the COVID-19 vaccination during pregnancy is correlated with reduced COVID-19 hospitalization rates in infants younger than 6 months of age, with greater protection for infants conferred when the mother received the COVID-19 mRNA vaccine after 20 weeks gestational age.<sup>9–11</sup> Knowledge is lacking, however, regarding how the timing of not only the first, but also the second dose of the mRNA vaccine in pregnancy impacts the persistence of antibody in infants over the first year of life.

<sup>1</sup>Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

<sup>2</sup>Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA

<sup>3</sup>Massachusetts General Hospital, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Boston, MA 02114, USA

<sup>4</sup>Massachusetts General Hospital, Vincent Center for Reproductive Biology, Boston, MA 02114, USA

<sup>5</sup>Harvard Medical School, Boston, MA 02115, USA

<sup>6</sup>Massachusetts General Hospital for Children, Department of Pediatric, Boston, MA 02114, USA

<sup>7</sup>Brigham and Women's Hospital, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Boston, MA 02115, USA

<sup>8</sup>Women's Biomedical Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

<sup>9</sup>These authors contributed equally

<sup>10</sup>Senior authors

<sup>11</sup>Lead contact

\*Correspondence: aedlow@mgh.harvard.edu

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**Table 1. Clinical characteristics of included infants**

	Overall unique infant (N = 100)	2-month-old infants (N = 55)	6-month-old infants (N = 80)	9-month-old infants (N = 14)	12-month-old infants (N = 4)
<b>Vaccine platform, N (%)</b>					
mRNA-1274	31 (31)	17 (31)	28 (35)	8 (57)	1 (25)
BNT162b.2	59 (59)	30 (55)	48 (60)	5 (36)	3 (75)
Ad.26.COVS.S	10 (10)	8 (15)	4 (5)	1 (7)	0 (0)
<b>Infant sex, N (%)</b>					
Female	49 (49)	24 (44)	41 (51)	6 (43)	3 (75)
<b>Gestational age at 1<sup>st</sup> mRNA vaccine in maternal series, N (%)</b>					
<20 weeks	26 (29)	23 (49)	20 (26)	1 (7)	0 (0)
20–28 weeks	29 (32)	16 (34)	26 (34)	7 (54)	0 (0)
>28 weeks	35 (39)	8 (17)	30 (40)	5 (36)	4 (100)
<b>Gestational age at 2<sup>nd</sup> mRNA vaccine in maternal series, N (%)</b>					
<20 weeks	15 (17)	12 (25)	13 (17)	0 (0)	0 (0)
20–28 weeks	27 (30)	21 (45)	22 (29)	3 (23)	0 (0)
>28 weeks	48 (53)	14 (30)	41 (54)	10 (77)	4 (100)
<b>Gestational age at Ad26.COVS.S vaccine, N (%)</b>					
<20 weeks	3 (30)	3 (37.5)	0 (0)	0 (0)	0 (0)
20–28 weeks	2 (20)	2 (25)	1 (25)	0 (0)	0 (0)
>28 weeks	5 (50)	3 (37.5)	3 (75)	1 (100)	0 (0)
<b>Gestational age at delivery in weeks median (IQR)</b>	39.3 (38.4–40.1)	39.4 (38.6–40.1)	39.2 (38.4–40.0)	39.0 (38.4–39.6)	39.1 (39.0–39.5)

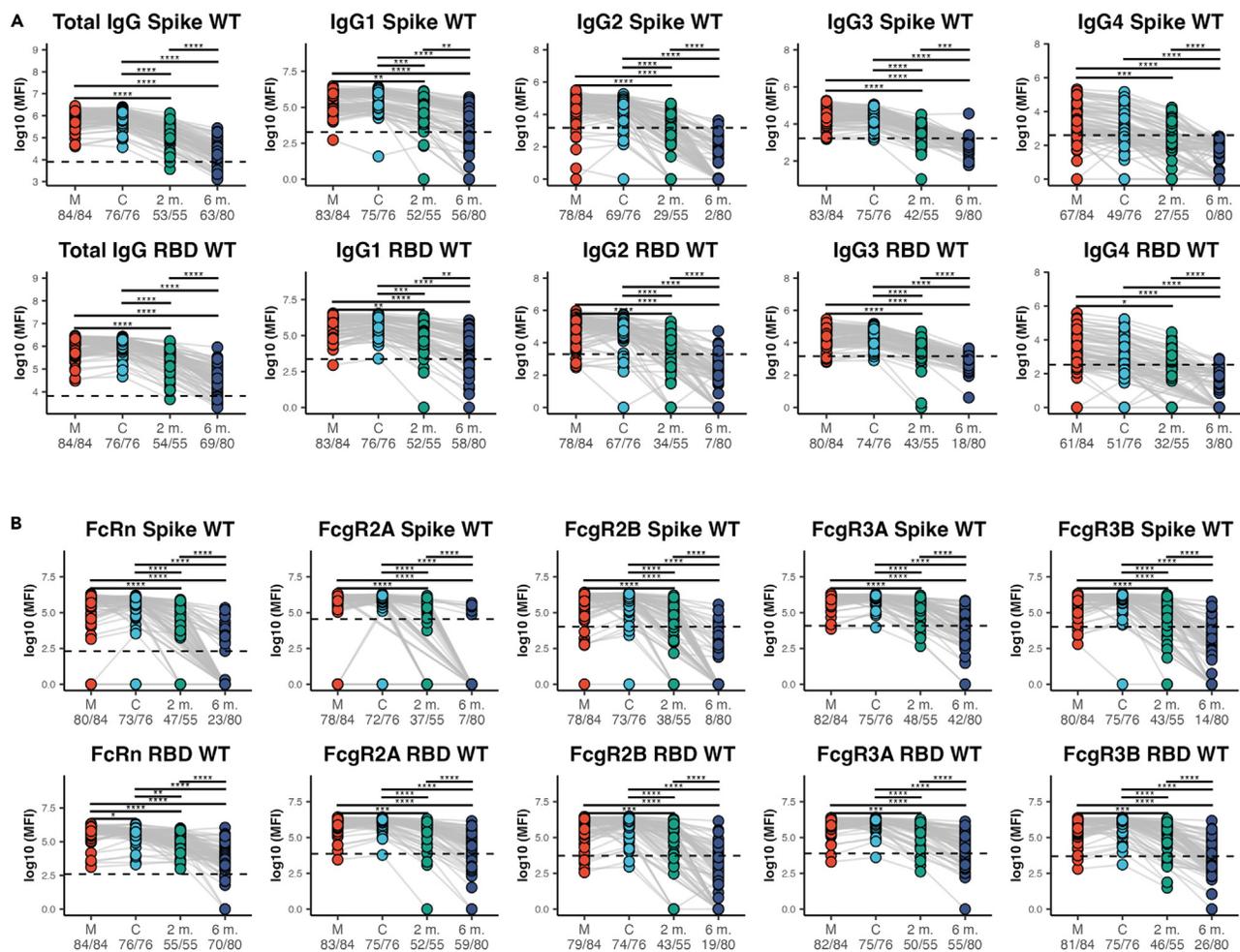
All included infants ( $N_p = 100$ ) reflect the first maternal COVID-19 vaccination series. Some infants' samples were collected at multiple time points, thus there are a total of 153 infant samples from 100 unique infants. All infants were born to mothers who were fully vaccinated in pregnancy as defined at the time (received both doses of the original COVID-19 mRNA vaccine series, or a single dose of the Ad26.COVS.S vaccine). None of the included infants had symptoms of COVID-19 nor did they ever test positive for SARS-CoV-2.

Recent studies examining COVID-19 vaccination during pregnancy have demonstrated the transplacental antibody transfer of anti-spike immunoglobulin (Ig) G antibodies.<sup>12–16</sup> While there are limited data on the persistence of maternal anti-spike IgG antibodies in infants,<sup>16,17</sup> no study has performed comprehensive profiling of maternal COVID-19 vaccine-induced antibodies that persist in infants through 12 months of age, including all IgG subclasses, Fc-receptor (FcR) binding profiles, and against both SARS-CoV-2 wild-type and variants of concern (VOCs) including B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), P.1 (Gamma), and B.1.1.529 (Omicron) variants. FcR binding is a critical consideration, as the Fc region of the antibodies plays an important role in protection against infection and the resolution of SARS-CoV-2 infection<sup>18,19</sup> by inducing the antiviral activity of the innate immune system.<sup>20,21</sup> The Fc region of antibodies also plays a critical role in the transfer of antibodies across the placenta,<sup>22,23</sup> and a more granular understanding of antibodies that persist in infants after maternal COVID vaccination may have broader implications for understanding optimal vaccine timing and features of vaccine-induced antibodies that maximize neonatal and infant protection. Such an understanding may be relevant to new vaccines with specific indications for pregnant individuals, including the respiratory syncytial virus (RSV) vaccine and others still in development. In this study, we performed unbiased systems serology to evaluate the levels and FcR binding profile of SARS-CoV-2-specific antibodies transferred from mother to infants, as well as their persistence in infants up to 12 months of age. Our analyses reveal differences in antibody profiles of infants whose mothers were vaccinated at different stages of gestation. This work further highlights the importance of understanding the timing of vaccination in pregnancy to provide optimal protection to susceptible infants during their first critical months of life.

## RESULTS

### Maternal COVID-19 vaccine-induced antibodies are detected in infants up to 12 months of age

To comprehensively profile COVID-19 vaccine-induced antibodies in infants up to 6 months of age, we evaluated 153 plasma/serum samples from a prospectively collected cohort of 100 unique infants, including  $N = 55$  infants at two months,  $N = 80$  infants at 6 months,  $N = 14$  infants at 9 months, and  $N = 4$  infants at 12 months. Maternal ( $N=84$ ) and cord ( $N = 76$ ) blood was available for a subset of the infants. During the course of the study, none of the included infants tested positive for COVID-19 (see STAR methods and Figure S1). The mothers in this study all received both doses of the first-generation (2020–2021) mRNA vaccinations (mRNA-1273 or BTN162b) or one dose of the Ad26.CoV2.S vaccine during pregnancy, and were not infected with SARS-CoV-2 prior to vaccination or during pregnancy. Table 1 details relevant clinical characteristics of mothers and infants in the study.



**Figure 1. Maternal vaccine-induced SARS-CoV-2 antibodies persist in 2-month and 6-month infants**

Dot plots showing (A) Spike-specific WT (top) and RBD-specific WT antibody levels and (B) FcR profiles of maternal (M), cord (C), 2-month-old infants (2 m), and 6-month-old infants (6 m). Significance was determined by a Kruskal-Wallis test followed by Benjamini-Hochberg procedure for multiple hypotheses correction. If statistically significant, a two-sided Mann-Whitney U test was performed. (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ). The dashed line represents the level of detection for each feature, defined as one standard deviation above the mean value for assay negative biological controls (SARS-CoV-2 negative, unvaccinated samples).

We first plotted the Spike- and receptor-binding domain (RBD)-specific wild-type (WT) IgG antibody titer of mother-cord dyads, and 2-month-old and 6-month-old infants (Figure 1A). Expanding upon previous reports examining primarily total IgG against Spike,<sup>14,24–26</sup> our results show cord samples are similar to maternal with respect to anti-spike and anti-RBD IgG subclasses and IgG FcR binding responses. We observed that anti-spike WT IgG1 was the most persistent antibody at 6 months of age, with 70% (56/80) of 6-month-old infants having detectable anti-spike WT IgG1, while only 11% (9/80) had detectable IgG3 antibody levels, 2/80 with detectable anti-spike IgG2, and no infants showing detectable levels of IgG4.

We also observed similar persistence trends between Spike- and RBD-specific antibodies. 73% (58/80) of 6-month-old infants had detectable anti-RBD WT IgG1, 23% (18/80) had detectable IgG3, 9% (7/80) had detectable IgG2, and 3/80 infants had detectable anti-RBD WT IgG4. These findings are overall consistent with previous reports of a transfer hierarchy of subclass-specific IgG transfer through the placenta from the mother to cord, with IgG1 > IgG3 > IgG4.<sup>27–29</sup>

Furthermore, we observed detectable Spike-specific and RBD-specific FcR-binding IgG levels in infants through 6 months of age (Figure 1B). Interestingly, 47/55 2-month infants demonstrated detectable antibody binding profiles against Spike-specific and 55/55 against RBD-specific neonatal Fc-receptor (FcRn), with similarly high detection of antibodies binding canonical Fc-receptors FcγR2A and FcγR3B at 2 months of age (Figure 1B). However, greater differences in the persistence of the FcR-binding against Spike versus RBD in infants begin to emerge at 6 months of age. We observed only 29% (23/80) of infants had detectable antibodies binding Spike-specific FcRn, 9% (7/80) binding FcγR2A, 10% (8/80) binding FcγR2B, 53% (42/80) binding FcγR3A, and 18% (14/80) binding to FcγR3B. RBD-specific FcR binding was more persistent than that against Spike, with 88% (70/80) of infants having detectable RBD-specific antibodies binding FcRn, 74%

(59/80) binding FcγR2A, 24% (19/80) binding FcγR2B, 69% (55/80) binding to FcγR3A, and 33% (26/80) binding to FcγR3B. Combined, these data demonstrate higher persistence of WT RBD-specific FcR binding IgGs compared to WT Spike at 6 months, with highest persistence of antibodies binding FcRn.

We also analyzed the levels of the same Spike- and RBD-specific WT antibodies in infants 9 months ( $N = 12$ ) and 12 months ( $N = 4$ ) of age (Figure S2). We observed 10/12 infants at 9 months, and 2/4 infants at 12 months had detectable Spike-specific IgG1 levels. Similar persistence was observed for IgG1 against RBD (8/14 at 9 months, 1/4 at 12 months of age). There was negligible persistence of other IgG subclasses at 9 and 12 months of age. We also observed detectable FcR binding levels of RBD-specific antibodies only, with 13/14 9-month infants demonstrating detectable binding to FcRn, and 2/14 and 3/14 demonstrating detectable RBD-specific binding to FcγR3AV and FcγR2AR, respectively.

Of note, 12 infants whose mothers received the Ad26.CoV2.S vaccine were enrolled in the study and systems serology was performed on their samples, including 8 at 2 months, 3 at 6 months, and 1 at 9 months. Of these 12, 75% (9/12) mothers were vaccinated at >20 weeks' gestation. These infants were included in all analyses except those pertaining to maternal timing of vaccine dose 1 and dose 2, which is only relevant to the mRNA vaccines (Figures S3–S5, 3 and 4). Understanding antibody persistence in infants whose mothers received Ad26.CoV2.S may be relevant for future adenovirus-based vaccine engineering or viral-based therapeutics; we therefore report here that 6/9 infants had detectable IgG Spike-WT antibodies at 2 months, 1/3 infants at 6 months, and none at 9 months of age.

### Persistence of maternal COVID-19 vaccine-induced antibodies at 6 months of age differs by VOCs

Next, we examined the cross-reactivity of the maternal COVID-19 vaccine-induced antibodies and antibody FcR binding profile against WT SARS-CoV-2 Spike and RBD, as well as Spike and RBD VOCs in cord and infant samples (Figure 2A). Of note, the vaccines were administered to mothers between 12/18/20 and 6/22/21, and thus represent the first-generation BNT162b2 and mRNA-1273 vaccines. To evaluate the persistence of the maternal vaccine-induced antibodies in infants against the VOCs, we compared the percent of infants in each group with detectable IgG subclasses and FcR binding profiles against Spike-specific and RBD-specific regions of the VOCs (Figure 2A).

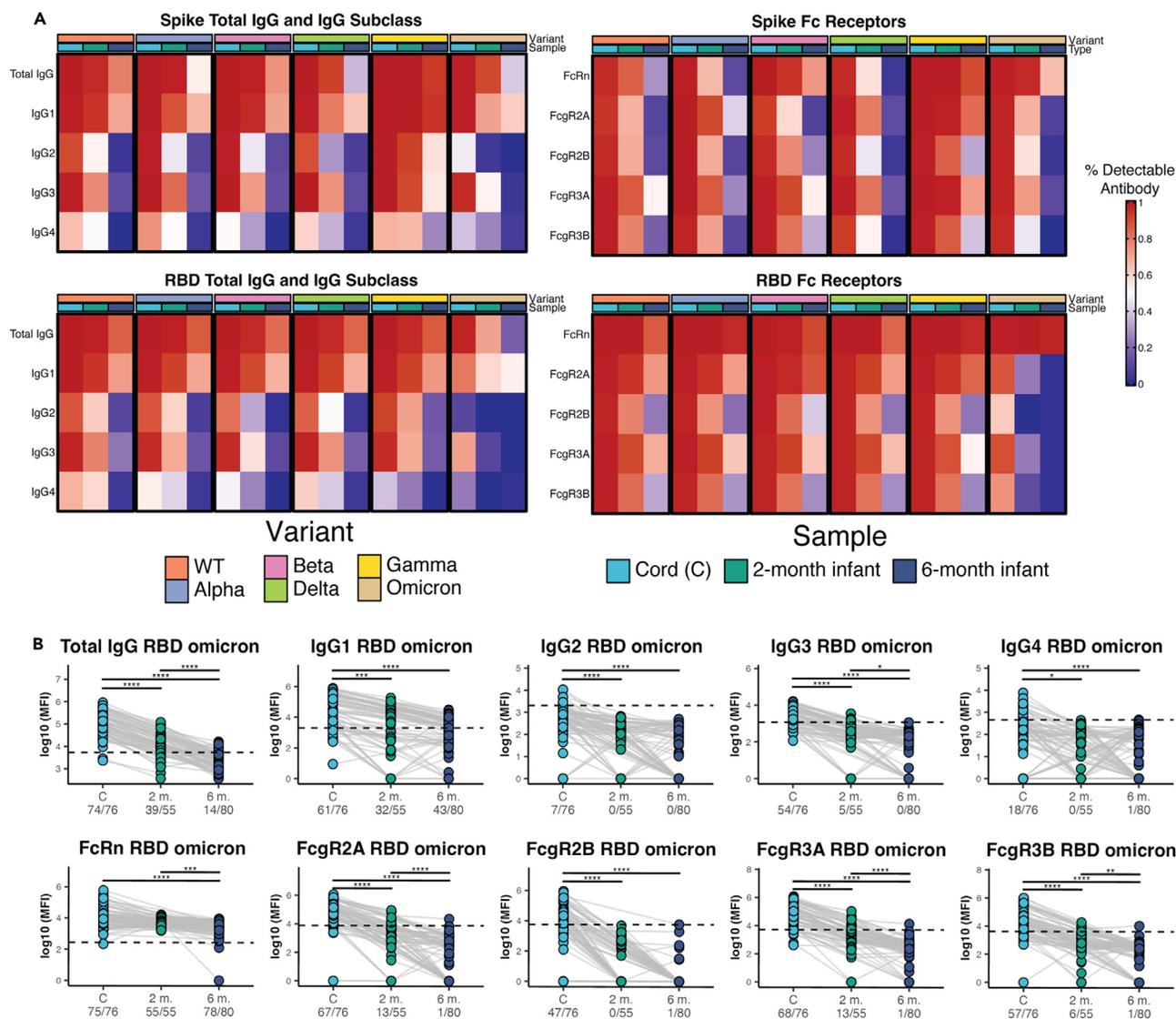
The first-generation COVID-19 mRNA vaccines generated antibodies cross-specific against most VOCs that persisted to 6 months, with highest persistence of antibodies cross-binding to Delta and to Gamma and lowest to Omicron. For all IgG subclasses and FcR profiles apart from IgG2 and IgG4, we observed detectable levels of antibodies and FcR binding against either the RBD- or Spike-specific antigen of all VOCs except Omicron in infants up to 6 months of age (Figure 2A). These findings are consistent with previous work from our group, demonstrating that the Spike-specific vaccine-induced antibodies were able to robustly bind to other VOCs, with lower affinity observed for the RBD-specific region of the VOCs.<sup>30</sup> As seen for WT Spike and RBD (Figure 1A), IgG2 and IgG4 subclasses overall had low persistence, including against the different VOCs. While there was relatively high persistence of IgG1 at 6 months against Spike-specific Omicron (70% of infants showing detectable IgG1 antibodies), very few ( $N = 9/80$ ) 6-month infants had detectable IgG3 against Spike-specific Omicron, and only 14/80 6-month infants had detectable Spike-specific Omicron antibodies with FcR binding capabilities, with highest persistence of antibodies binding FcγR3A (8/80). While IgG1 against Omicron RBD persisted at 6 months in 54% (43/80) of infants, a sharp decrease in the persistence of IgG3 against RBD-specific Omicron was observed, with only 5/55 infants demonstrating detectable levels of this antibody even at 2 months (Figure 2B). We also saw strikingly low persistence of RBD-specific Omicron FcR binding in 2- and 6-month infants, with only FcRn-binding antibodies persisting to any significant extent. The reduced ability of vaccine-generated antibodies to bind the highly mutated<sup>31</sup> RBD region of the Omicron Spike protein relative to other VOCs has been demonstrated previously.<sup>32</sup>

### The timing of maternal vaccination influences antibody levels in 2-month-old infants

We next wanted to examine specifically how the timing of both first and second mRNA vaccine dose impacted the profile of passively transferred maternal antibodies in infants. The 2-month cohort was selected for this subanalysis, given the higher and more uniformly persistent levels of all antibody features in this group, relative to the 6-month-old infant cohort.

First, we grouped the 2-month infant cohort into five groups, based on when the pregnant individual received dose 1 and dose 2 of the primary mRNA COVID-19 vaccination series (Figure 3A). For this analysis, we excluded infants whose mother received the Ad26.CoV.2 vaccine, as only a single dose of this vaccine was administered ( $N = 8$  2-month infants). Time points of <20 weeks, 20–28 weeks, and >28 weeks were chosen as cut points for analysis, given previous work from the CDC demonstrating that maternal vaccination after 20 weeks' gestation was more effective in preventing COVID-19-associated infant hospitalizations in the first 6 months of life, relative to when maternal vaccination was completed prior to 20 weeks.<sup>9,10</sup> The 20–28 weeks and >28 weeks window were selected to gain increased trimester-specific granularity on optimal timing of maternal vaccine administration.

Levels of Spike-specific IgG subclasses and FcR binding antibodies in 2-month-old infants by maternal vaccine timing are plotted in Figure 3B. These analyses demonstrated that for most subclasses and FcR binding features, infants whose mother received at least one dose of mRNA vaccine after 28 weeks (Groups 4 and 5, maroon and rose groups) had the highest levels of maternal COVID-19 vaccine-induced antibodies. Interestingly, the lowest levels of IgG1 and IgG2 subclasses and FcR binding features were seen in infants whose mothers received both doses of the mRNA vaccine in the 20–28 weeks window. Given the prior data demonstrating enhanced protection against COVID-19-associated hospitalization when the vaccine series was completed after 20 weeks' gestation,<sup>9,10</sup> we hypothesized we would observe the lowest antibody titers in infants whose mothers started and/or completed the entire vaccine series prior to 20 weeks. Instead, we observed that receiving both doses of the mRNA vaccine in the mid-to-late second trimester (Group 3, yellow) was associated with the lowest levels of anti-spike IgG1 and IgG2 in infants, which was consistent with prior work in a separate cohort, suggesting less robust maternal antibody



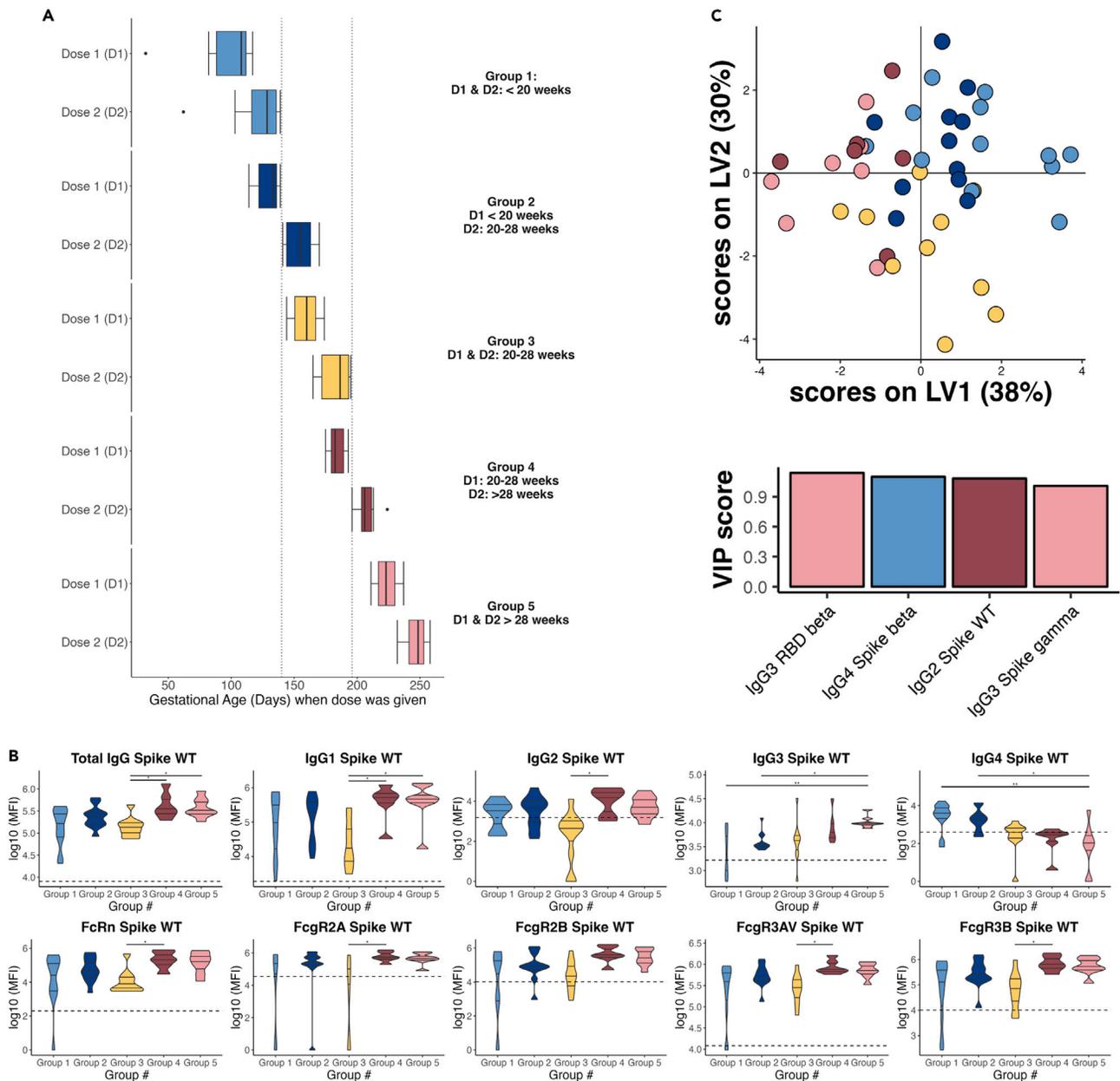
**Figure 2. IgG subclasses and Fc-receptor binding capacity of antibodies against SARS-CoV-2 variants of concern in umbilical cord blood, 2-month, and 6-month infants**

(A) The heatmap shows the ratio of infants with detectable antibody titer or FcR binding levels  $N = 76$  for cord (C),  $N = 55$  for 2-month-old infants (2M), and  $N = 80$  for 6-month-old infants (6M) against the Spike or RBD region of wild-type (WT) strain and variants of concern (VOCs) including Alpha, Beta, Delta, Gamma, and Omicron.

(B) Univariate plots showing RBD-specific Omicron antibody IgG levels (top) and FcR binding profiles in cord (C), 2-month-old infants (2m), and 6-month-old infants (6m). Significance was determined by a Kruskal-Wallis test followed by Benjamini-Hochberg procedure for multiple hypotheses correction. If statistically significant, a two-sided Mann-Whitney U test was performed. ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ ). The dashed line represents the level of detection for each feature, defined as one standard deviation above the mean value for assay negative biological controls (SARS-CoV-2 negative, unvaccinated samples).

responses in second trimester vaccination relative to first and third.<sup>14</sup> These less robust 20–28 weeks responses were hypothesized to be secondary to the known relative maternal immune quiescence of the second trimester, which occurs at least in part to facilitate rapid fetal growth.<sup>33</sup>

However, the finding that the lowest antibody levels were seen in infants whose mothers started and finished the vaccine in the 20–28 weeks window should be interpreted with an important caveat: Group 3 included 9 males and only 1 female infant, whereas other groups in this analysis were comprised by nearly equal numbers of males and females. Univariate analyses revealed that male infant sex was associated with significantly reduced levels of Spike-specific IgG2 antibodies at 2 months of age relative to female infants (Figure S3A). We also observed reduced levels of Spike-specific IgG1 antibodies in male infants compared to female infants, although this finding did not achieve statistical



**Figure 3. Maternal vaccine timing drives Ig levels and Fc-receptor binding profiles in 2-month infants**

Infants whose mothers were vaccinated in the late second and early third trimester (received at least one dose of mRNA vaccine after 28 weeks) had the highest levels of antibodies at 2 months.

(A) Diagram showing the grouping strategy for 2-month-old infants based on the dose 1 (D1) and dose 2 (D2) COVID-19 vaccination. Group 1:  $N = 12$ , Group 2:  $N = 11$ , Group 3:  $N = 10$ , Group 4:  $N = 6$ , Group 5:  $N = 8$ .

(B) Univariate plots showing Spike-specific WT antibody and Fc-receptor (FcR) levels of each 2-month-old infant group (as shown in A) Significance was determined by a Kruskal-Wallis test followed by Benjamini-Hochberg procedure for multiple hypotheses correction. If statistically significant, a two-sided Mann-Whitney U test was performed. ( $*p < 0.05$ ,  $****p < 0.0001$ ). The middle line represents the median, whereas the other lines represent the first and third quartiles. The dashed line represents the level of detection for each feature, defined as one standard deviation above the mean value for assay negative biological controls (SARS-CoV-2 negative, unvaccinated samples).

(C) (Top) A partial-least squares discriminant model (PLSDA) was built using LASSO feature regularization and variable selection of the infant cohort group, resulting a reduced set of features that were consistently in at least 80 of the 100 LASSO models. (Bottom) Variable importance in projection (VIP) score of the selected features. The magnitude indicates the importance of the features in driving the separation in the model. The color of the feature corresponds to the group to which the feature was enriched.

significance (Figure S3A). Uniform manifold approximation and projection analysis also revealed antibody and FcR variation by timing of maternal vaccination and infant sex (Group 3, Figure S3B). It is therefore possible that the lower titers noted in infants after late second trimester maternal vaccination are a function of either maternal vaccine timing, male infant sex, or both.

Finally, we observed that infants whose mother received at least one dose of mRNA vaccine prior to 20 weeks' gestation had the highest levels of IgG4, while all other groups had relatively low levels. IgG4 is one of the IgG subclasses that is least efficiently transferred across the placenta.<sup>27–29</sup> Previous work demonstrated that IgG4 antibodies after COVID-19 vaccination were less inflammatory and had more muted effector functions (antibody-dependent phagocytosis and complement deposition) than other subclasses, and IgG4 levels rose with longer interval from 2<sup>nd</sup> mRNA vaccination.<sup>34</sup> Thus, the elevated IgG4 levels in infants whose mothers were vaccinated at <20 weeks may be present due to increased passage of time and increased opportunity for antibody class switching in the mother, and these antibodies may have more muted effector functions and therefore may be less desirable to pass to infants than other subclasses of IgG. Consistent with this concept, we observed the highest levels of IgG3, the subclass most associated with an effective early antiviral response<sup>35</sup> in infants whose mothers received the first vaccine dose after 20 weeks' gestation.

Given the observed differences in infant antibody levels by timing of maternal vaccination, we next sought out to determine the combination of features that best separated infants based on timing of vaccination using partial least squares discriminant analysis (PLSDA), followed by least absolute shrinkage and selection operator (LASSO) feature regularization to prevent overfitting (Figure 3C). The LASSO-selected features that are important (based on variable importance in projection analyses) in building a model to distinguish groups based on maternal vaccine timing are highlighted in Figure 3C, and include not only the Spike-specific WT antibody levels and FcR binding profiles, but also some RBD-specific and VOC-specific features. Other features used in building the PLSDA model, as well as the model validation results, are depicted in Figures S4A and S4B. Among the top features that distinguish the groups, three (anti-RBD Beta IgG3, anti-spike Gamma IgG3, and anti-spike WT IgG2) were enriched in either Group 4 or Group 5 (at least one vaccine dose administered >28 weeks), and 2 features (anti-Spike beta IgG4 and anti-RBD delta IgG4) were enriched in Group 1 or 2 (at least one vaccine dose administered <20 weeks, Figures 3C and S4A). Similar to levels of IgG subclasses, numerous FcR binding features were enriched in Group 4 or Groups 4 and 5, primarily relative to Group 3 (fully vaccinated 20–28 weeks window).

Given that LASSO selects a minimal set of features to prevent overfitting of the data, we generated a correlation heatmap to determine additional features that correlate with the top features used in the PLSDA model (Figure S4C). IgG3 and IgG4 features, regardless of antigen target, were correlated with other IgG3 and IgG4 antibodies, respectively (Spearman correlation  $\rho > 0.75$ ). Spike- and RBD-specific IgG3 and Spike-specific IgG2 both were positively correlated with FcR binding levels. Overall, these analyses demonstrated that the timing of not only the 1<sup>st</sup> but also the 2<sup>nd</sup> dose of maternal COVID-19 vaccination influences antibody subclass levels in 2-month-old infants.

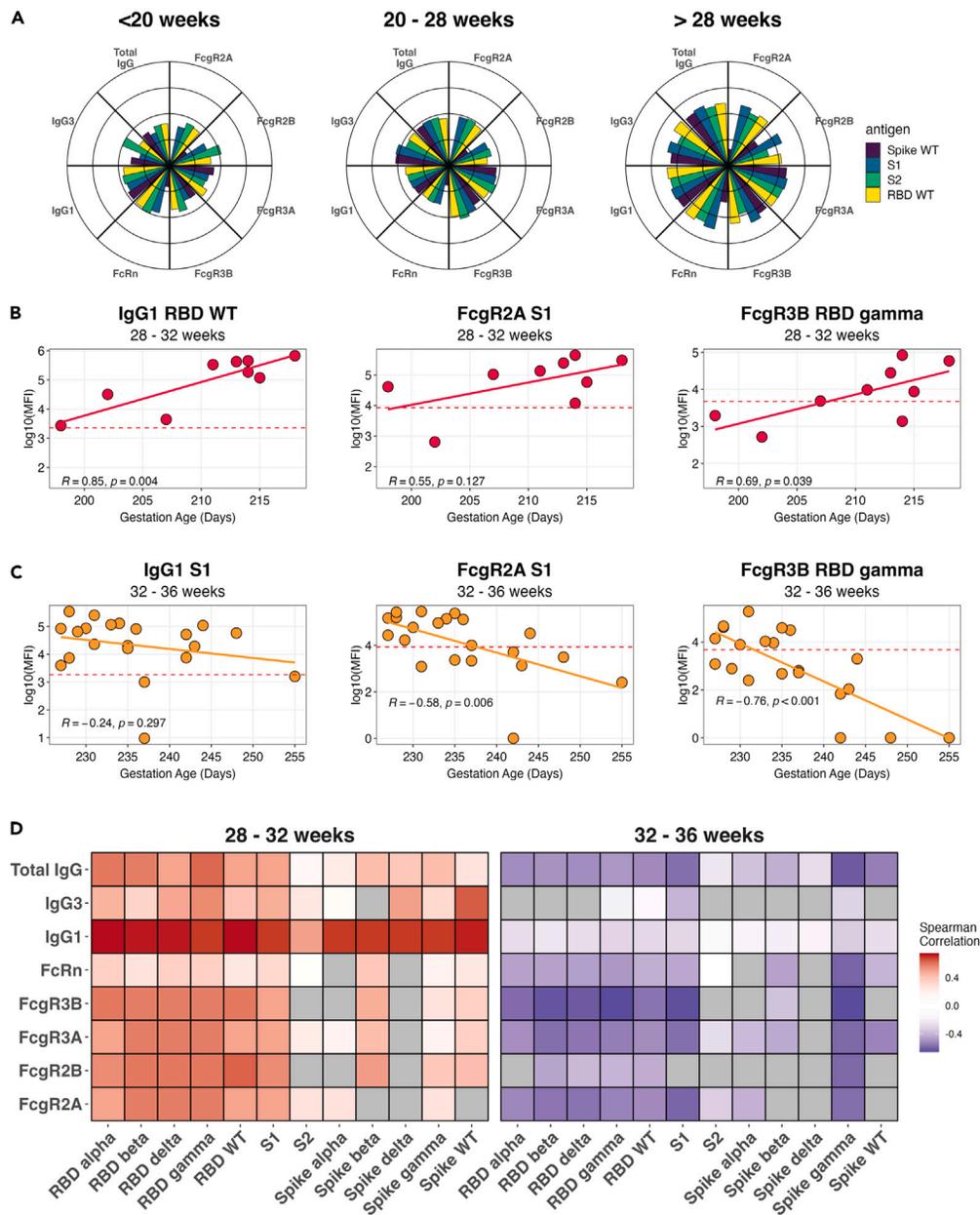
To evaluate the impact of maternal vaccine timing on antibody features in 6-month infants, we sought to determine a set of features that best separate 6-month infants based on maternal vaccination timing, as was done with the 2-month infant cohort, using LASSO feature regularization in conjunction with PLSDA classification (Figures S5A–S5C). Similar to the results in 2-month infants, the LASSO-selected features show an enrichment of antibody levels in 6-month infants whose mother was vaccinated >28 weeks (Figures S5B and S5C). Although the univariate plots of these features did not demonstrate statistically significant differences, these plots demonstrate that infants whose mothers received the vaccine >28 weeks had elevated antibody and FcR binding levels compared to those vaccinated <20 or 20–28 weeks (Figure S5D).

### Evaluating the persistence of maternal vaccine-induced antibodies in 6-month infants by timing of the maternal COVID-19 vaccine series in early versus later 3<sup>rd</sup> trimester: Implications for RSV vaccination in pregnancy

Building on the finding of enriched antibody levels in 2-month infants whose mother was vaccinated in the third trimester (>28 weeks), we next sought to examine antibody persistence in 6-month infants after maternal administration of the COVID-19 vaccines at 28–32 weeks' versus 32–36 weeks' gestation in our sample. The time points of 28–32 weeks and 32–36 weeks were chosen as cut points for this analysis due to the recent recommendation by the CDC's Advisory Committee that pregnant individuals receive bivalent recombinant RSV prefusion F protein (RSV preF) vaccine at 32–36 weeks' gestation, to prevent RSV lower respiratory tract disease in infants.<sup>36</sup> While the COVID-19 mRNA vaccines represent a different vaccine platform with a two-dose rather than one-dose regimen, given the lack of data on antibody persistence in infants when maternal vaccines are administered at 32+ weeks of gestation, this analysis of our data can add significantly to existing knowledge.

First, we evaluated antibody features in 6-month-old infants as a function of timing of maternal vaccination in pregnancy using polar plots (Figure 4A). Because of their low persistence in the 6-month infant cohort (Figure 1A), we excluded IgG2 and IgG4 from these analyses (overall more than 70% of the samples for IgG2 and IgG4 fell below the limit of detection threshold). Comparing the mean percentile ranks of antibody subclasses and FcR binding features persistent in 6-month-old infants by maternal vaccine timing in pregnancy, it was clear that maternal vaccination at >28 weeks was associated with the most robust persistence of antibodies in 6-month-old infants (relative to <20 weeks or 20–28 weeks). These results were thus consistent with the prior results for 2-month-old infants (Figure 3).

To parse more specifically how timing of maternal vaccination within the 3<sup>rd</sup> trimester influenced persistence of infant antibody titers at 6 months, we then evaluated levels of IgG subclasses and FcR binding antibody levels in 6-month infants as a function of maternal vaccination from 28 to 32 weeks (Figure 4B), versus maternal vaccination from 32 to 36 weeks (Figure 4C). We found that the majority of antibody features, including IgG subclasses and FcR binding, were significantly positively correlated with gestational age at maternal vaccine administration



**Figure 4. Maternal vaccine administration at >32 weeks is associated with reduced antibody persistence in 6-month infants**

(A) The polar plots depict the mean percentile ranks of the antibody features within 6 months infants whose mothers were vaccinated with the first dose of the mRNA COVID-19 vaccine <20 weeks ( $N = 20$ ), 20–28 weeks ( $N = 26$ ), and >28 weeks ( $N = 30$ ). Each wedge represents an antibody feature, and the size of the wedge depicts the mean percentile ranging from 0 to 1.

(B) Linear regression models fit the relationship between gestation age of dose 1 (days) (those vaccinated between 28 and 32 weeks) and anti-RBD WT IgG1, anti-S1 FcγR2AR, and anti-RBD Gamma FcγR3B. The rho and p values are reported in the plot. The dashed line represents the level of detection for each feature, defined as one standard deviation above the mean value for assay negative biological controls (SARS-CoV-2 negative, unvaccinated samples).

(C) Linear regression models fit the relationship between gestation age of dose 1 (days) (those vaccinated between 32 and 36 weeks) and anti-RBD WT IgG1, anti-S1 FcγR2AR, and anti-RBD Gamma FcγR3B. The rho and p values are reported in the plot. The dashed line represents the level of detection for each feature, defined as one standard deviation above the mean value for assay negative biological controls (SARS-CoV-2 negative, unvaccinated samples).

(D) The heatmap shows the Spearman correlation score for each feature against gestational age (days) within infant samples whose mothers received the first dose of the mRNA COVID-19 vaccine between 28 and 32 weeks or 32–36 weeks. The red boxes indicate samples with a positive Spearman correlation and blue boxes indicate samples with a negative Spearman correlation. Within each group, gray boxes are features which were excluded from the analysis (if >70% of values fell below the threshold of detection, defined as less than one standard deviation above the mean of SARS-CoV-2 negative, unvaccinated control samples).

across the 28–32 weeks' gestation period, particularly IgG1 against RBD and Spike WT and VOCs, as well as FcγR2AR-, FcγR3AV-, and FcγR3B-binding antibodies (Figures 4B and 4D). In contrast, many of the same features decreased steadily as a function of time across the vaccine window when maternal vaccination was initiated from 32 to 36 weeks (Figures 4C and 4D). In summary, these analyses demonstrate a significant positive correlation between infant antibody levels at 6 months and gestational age at vaccination across the 28–32 weeks gestational age window. However, this relationship reverses across the 32–36 weeks window, with a significant negative correlation between 6-month infant antibody levels and gestational age at maternal vaccination. These limited data suggest that the efficiency of transplacental transfer of maternal antibodies and durability in infants through 6 months of age may be reduced across the 32–36 weeks gestational age window.

## DISCUSSION

Maternal vaccination against COVID-19 has been shown to effectively reduce COVID-19-related hospitalization rates in infants younger than 6 months,<sup>9,10</sup> through the transplacental transfer of maternal COVID-19 vaccine-induced antibodies.<sup>16,17</sup> Here, using systems serology, we profiled maternal antibodies in 100 infants up to 12 months of age in unprecedented detail. These experiments quantified not only total IgG level, but also IgG subclass and FcR binding profile of antibodies against WT Spike and RBD epitopes of SARS-CoV-2, as well as a wide range of VOCs including Omicron. Although the COVID-19 pandemic is ongoing and protecting both mothers and vulnerable infants under 6 months of age who cannot yet be vaccinated against COVID-19 remains critical, our findings have relevance far beyond COVID-19. Our findings that antigen specificity (e.g., Spike versus RBD), IgG subclass (e.g., IgG1 and IgG3), and canonical (FcRn) versus non-canonical (Fcγ) placental receptor binding work in concert to drive persistence of antibodies in infants through 6 months of age may have implications for both future vaccine development and timing of administration in pregnancy. Our findings that both timing of maternal vaccination and fetal sex were significantly associated with persistence of maternally transferred antibody in infants may be used to guide both pregnancy vaccination strategies, and our understanding of infant protection. Finally, our granular examination of the impact of maternal vaccine timing within the mid-to-late 3<sup>rd</sup> trimester demonstrates enhanced antibody persistence in 6-month infants when vaccine occurs across the 28–32 weeks window, yet reduced persistence across the 32–36 weeks window, with potential immediate relevance for maternal RSV vaccination strategies. Thus, the detailed antibody map presented here provides the basis for a more mechanistic understanding of maternal antibody generation, transplacental transfer, and persistence of antibodies across the mother-infant dyad.

While limited data are available regarding the persistence of maternal COVID-19 vaccine-induced antibodies in infants,<sup>16,17</sup> this represents the largest study examining infant antibody persistence, with comprehensive infant follow-up at 2, 6, 9, and even 12 months of age, albeit with more limited numbers profiled after 6 months. In addition, these represent the only available data regarding the persistence of IgG subclasses and FcR binding profiles in infants through 12 months of age. Our observation of IgG1 and IgG3 having the highest persistence in 6 months infants is consistent with the known transfer hierarchy of subclass-specific IgG transfer through the placenta from the mother to cord, with IgG1 > IgG3 > IgG4 > IgG2.<sup>27–29</sup> Subclass-specific IgG transfer hierarchy is mediated not only by the relative abundance of each subclass, but also FcR expression levels and Fc-FcR interaction affinity.<sup>28</sup> As an example, IgG3, one of the most persistent antibodies in 6-month infants, has high binding affinity to placental FcγR3A.<sup>37</sup> In addition to facilitating IgG3 transfer across the placenta, the finding that the most persistent FcR binding profiles at 6 months were for antibodies binding FcγR3A suggests preferential transfer of natural killer (NK) cell-activating antibodies<sup>23,38</sup> to arm neonates with the most effective immunity from birth, as NK cells play a crucial role in neonatal first-line innate immune defenses.<sup>39</sup>

With respect to timing of maternal COVID-19 vaccination in pregnancy, previous studies have examined maternal antibody levels at delivery and transplacental transfer to the umbilical cord by gestational age at maternal administration,<sup>14,24,40,42</sup> but these studies did not comprehensively examine IgG subclasses, FcR binding, or activity against VOCs, nor did they quantify antibody persistence in infants. Prior epidemiologic studies demonstrated that maternal COVID-19 vaccination after 20 weeks' gestation conferred the greatest infant protection against the clinical outcome of COVID-19-associated hospitalizations in the first 6 months of life.<sup>9,10</sup> These studies did not examine antibody levels in infants, nor did they examine the impact of vaccination timing within the second half of pregnancy on infant protection. While there are limited data available on antibody levels in infants after maternal COVID-19 vaccination,<sup>16,17</sup> these studies have not examined how timing of vaccination across a range of trimesters or within trimester impacts infant antibody levels. Knowledge is also lacking regarding how the timing of not only the first, but also the second dose of the mRNA vaccine in pregnancy impacts maternal antibody levels and persistence of antibody in infants. Our finding of the highest levels of IgG1-3 and FcR binding in infants whose mother was vaccinated with at least one dose of the mRNA vaccine after 28 weeks of gestation, and significantly lower IgG levels and FcR binding in infants born to mothers fully vaccinated in the 20–28 weeks window, builds upon our prior work demonstrating enhanced IgG levels, FcR binding, and functions when mothers are vaccinated in 3<sup>rd</sup> relative to 2<sup>nd</sup> trimester.<sup>14,41</sup> The finding that IgG4 was highest in infants born to mothers vaccinated prior to 20 weeks likely can be at least partially explained by antibody class switching and a time effect, with <20 weeks vaccinees having more time for class-switching to IgG4 and transfer of Ig4 across the placenta. IgG4 may be a less advantageous antibody subclass for newborns and early infants with immature immune systems, given that IgG4 responses are T cell dependent<sup>14,41</sup> and have reduced effector functions (e.g., antibody-dependent cellular phagocytosis and complement deposition).<sup>43,44</sup>

Also relevant to considerations of timing of maternal vaccination is our focused subanalysis examining durability of placentally transferred antibodies in 6-month-old infants after maternal vaccination in mid-to-late third trimester. Recently, the CDC's Advisory Committee on Immunization Practices recommended the RSV preF vaccine for seasonal use in pregnant individuals at 32–36 weeks' gestation, to prevent RSV lower respiratory tract disease in infants.<sup>36,45–47</sup> While maternal RSV vaccination from 24 to 36 weeks was demonstrated to have 69.4% efficacy

in protecting against severe lower respiratory tract disease in infants in a large randomized controlled trial,<sup>48</sup> durable infant protection was not well studied across the narrower approved window of 32–36 weeks' gestation. Our data suggest that most effective transfer occurs in the context of maternal vaccination in the early third trimester (28–32 weeks), with progressively less efficacious placental transfer (reflected in lower levels of IgG and FcR binding) as vaccination occurs from 32 to 36 weeks. These limited data point to maternal RSV vaccination in the earlier part of the 32–36 weeks window potentially being more optimal to maximize infant protection through 6 months of age. These data should be interpreted with caution as the RSV vaccine is a different platform (recombinant protein) and a one- rather than two-shot regimen, with likely different transfer kinetics. However, given the current dearth of evidence to guide vaccine timing within the recommended window, we offer these analyses while we await further prospective data from pregnant RSV vaccine recipients and their infants.

With respect to the impact of infant sex on infant antibody persistence at 2 and 6 months of age after maternal vaccination, our finding that male infants had reduced persistence of antibodies at 2 and 6 months relative to females resonates with prior published data. Prior work by our group found that pregnant individuals carrying male infants had lower maternal levels of COVID-specific antibodies and reduced FcR binding antibody levels, compared to individuals carrying female infants after natural COVID infection.<sup>49</sup> Sex-specific anti-COVID antibody glycan profiles and sex-specific patterns of placental FcR expression that may be less favorable for transplacental antibody transfer to male infants were also previously reported.<sup>49</sup> Maternal immune response, specific antibody properties such as glycan profiles, and placental FcR expression all are influenced by fetal sex, making this a critical area for further research.

Lastly, we examined whether maternal vaccination with the first-generation COVID-19 vaccines induced antibodies against other VOCs, persistent in 2- and 6-month-old infants, finding the most consistent cross-immunity was induced for the Gamma and Delta variants, but with persistent levels of antibodies and FcR engagement against all VOCs in infants up to 6 months of age with the exception of Omicron. Persistence of Omicron RBD-specific IgG3 was negligible, with nearly no detectable levels of this antibody in even 2-month infants. Similarly, there was strikingly low Omicron RBD-specific Fcγ-receptor binding in 2- and 6-month infants in comparison to other VOCs or even to Omicron Spike-specific Fcγ-receptor binding. This could be due to the disproportionately high mutation burden in the RBD region of the Omicron Spike protein.<sup>31</sup> The extent to which these potential deficits in activity against Omicron could be overcome by infant or childhood vaccination with updated Omicron-specific COVID-19 vaccines is an important question. Additionally, it is likely that maternal vaccination with an updated COVID-19 booster against Omicron could augment transfer of Omicron Spike- and RBD-specific antibodies to infants, and this is a critical area of ongoing investigation.<sup>50</sup> Finally, these data highlight the importance of comprehensive antibody profiling to better understand the complexity of immunity transferred to the infant by maternal vaccination; such data clearly have implications for vaccine design and/or implementation strategies, particularly against pathogens with high mutation rates.

These results demonstrate that COVID-19 vaccination during pregnancy provides not only a robust antibody response in the mother, but also antibodies detectable in infants through >6 months of age. A fine-grained understanding of antibody subclasses and FcR binding profiles that persist in infants through 1 year of life is critical not only for understanding protection-afforded infants by maternal COVID-19 vaccination, but also for the development of rational vaccines and data-driven maternal vaccination strategies. Our findings related to the critical impact of vaccine timing across gestation and of infant sex on antibody durability at 2 and 6 months of age demonstrate the importance of ongoing investigations of these unique aspects of maternal-fetal immunity that should inform vaccine development, and can drive strategies to optimize maternal and neonatal immunity against a wide array of pathogens.

### Limitations of the study

Limitations of the study include the relatively small number of infants with available data beyond 6 months of age, and the lack of data regarding antibody functions, glycosylation profiles, and whether there are cell-mediated responses to maternal vaccination in the infant. Future studies will focus on the potentially synergistic role of breastmilk antibodies in conferring infant protection, and probing the contribution of infant sex in larger cohorts.

### 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.isci.2024.109273>.

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## AUTHOR CONTRIBUTIONS

A.G.E., P.A.L., B.D.J., N.N., and D.A.L. developed the concept, designed the experiments, and analyzed and interpreted the data. A.G.E., D.A.L., M.A.E., and B.D.J. obtained funding. N.N. and T.C. performed the experiments. P.A.L. and D.A.L. performed the statistical modeling. P.A.L. and A.G.E. wrote the main paper. L.L.S., M.D.B., S.D., O.J., B.A., K.J.G., and A.G.E. collected and prepared samples. L.L.S., M.D.B., O.J., B.A., L.M.Y., K.J.G., and A.G.E. recruited and enrolled participants. O.J., M.D.B., B.A., K.J.G., L.M.Y., and A.G.E. assisted with clinical data collection. M.A.E. gave conceptual advice and edited the manuscript. All authors approved of the final manuscript.

## DECLARATION OF INTERESTS

The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp. K.J.G. has consulted for Illumina, BillionToOne, and Aetion outside the scope of the submitted work. A.G.E. and M.A.E. serve as medical advisors for Mirvie. M.A.E. has equity in Mirvie outside the submitted work. B.D.J. is a part time employee of Leyden Laboratories B.V., Netherlands. B.D.J.'s immediate family member, Galit Alter, is a co-founder of SeromYx Systems, Inc., and has a patent on Systems Serology Platform pending.

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**STAR★METHODS**

**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Mouse Anti-Human IgG-Fc PE	Southern Biotech	CAT # 9040-09
Mouse Anti-Human IgG1-Fc PE	Southern Biotech	CAT # 9052-09
Mouse Anti-Human IgG2-Fc PE	Southern Biotech	CAT # 9060-09
Mouse Anti-Human IgG3-Fc PE	Southern Biotech	CAT # 9210-09
Mouse Anti-Human IgG4-Fc PE	Southern Biotech	CAT # 9200-09
Mouse Anti-Human IgGA1-Fc PE	Southern Biotech	CAT # 9130-09
Mouse Anti-Human IgGA2-Fc PE	Southern Biotech	CAT # 9140-09
Mouse Anti-Human IgM-Fc PE	Southern Biotech	CAT # 9020-09
<b>Chemicals, peptides, and recombinant proteins</b>		
SARS-CoV-2 Spike WT	Sino Biological	CAT # 40589-V08H4
SARS-CoV-2 Spike alpha	Sino Biological	CAT # 40589-V08B6
SARS-CoV-2 Spike beta	Sino Biological	CAT # 40589-V08B7
SARS-CoV-2 Spike gamma	Sino Biological	CAT # 40589-V08B10
SARS-CoV-2 Spike delta	Sino Biological	CAT # 40589-V08B16
SARS-CoV-2 Spike omicron	Sino Biological	CAT # 40589-V08H26
SARS-CoV-2 RBD WT	Sino Biological	CAT # 40592-V08H
SARS-CoV-2 RBD alpha	Sino Biological	CAT # 40592-V08H82
SARS-CoV-2 RBD beta	Sino Biological	CAT # 40592-V08H59
SARS-CoV-2 RBD gamma	Sino Biological	CAT # 40592-V08H86
SARS-CoV-2 RBD delta	Sino Biological	CAT # 40592-V08H115
SARS-CoV-2 RBD omicron	Sino Biological	CAT # 40592-V08H121
SARS-CoV-2 NTD	Sino Biological	CAT # 40591-V49H
SARS-CoV-2 S1 WT	Sino Biological	CAT # 40591-V08H
SARS-CoV-2 S2 WT	Sino Biological	CAT # 40590-V08B
HKU1	Sino Biological	CAT # 40606-V08B
Human FC receptors	Produced at the Duke Human Vaccine Institute (Boesch, 2014 #15)	NA
Streptavidin-R Phycoerythrin	ProZyme	CAT # PJ31S
EDC	ThermoFisher	CAT # A35391
Pierce Sulfo-NHS	ThermoFisher	CAT # A39269
<b>Deposited data</b>		
Generated Code	This paper	Mendeley Data: <a href="https://doi.org/10.17632/35zy6p92f6.1">https://doi.org/10.17632/35zy6p92f6.1</a>
<b>Software and algorithms</b>		
Intellicyt ForeCyt Software	Sartorius	<a href="https://www.sartorius.com/en/products/flow-cytometry/flow-cytometry-software">https://www.sartorius.com/en/products/flow-cytometry/flow-cytometry-software</a>
R programming language	Version 4.1.0	<a href="http://www.r-project.org">http://www.r-project.org</a>
<b>Other</b>		
MagPlex microspheres	Luminex corporation	

## RESOURCE AVAILABILITY

### Lead contact

Requests for information should be directed to the lead contact, Andrea G. Edlow ([aedlow@mgh.harvard.edu](mailto:aedlow@mgh.harvard.edu))

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- All dataset generated during this study is available upon reasonable request.
- All code generated during this study has been deposited at Mendeley Data and is publicly available as of the date of publication. The DOI is listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

### Study population

The study included  $N = 108$  unique infants (49 females and 51 males) born to mothers who received their initial COVID-19 vaccine series in pregnancy (dates of maternal vaccination Dec 18, 2020–June 22, 2021). Mothers enrolled their infants in this study conducted from July 23, 2021 to July 1, 2022. Ultimately samples collected from pregnant individuals infected with SARS-CoV-2 before vaccination or babies known to be infected with SARS-CoV-2 prior to sample collection were excluded ([Figure S1](#)). Matched maternal and umbilical cord serum samples were collected at birth for a subset of mothers co-enrolled in a complementary pregnancy cohort (MGB IRB # 2020P003538). Infant capillary serum samples were collected via microneedle device (TAP II blood collection device, YourBio Health, Medford, MA) at 2 months, 6 months, 9 months and 12 months after birth for infants of vaccinated mothers. All infants were singletons except for 2 pairs of twins. All included pregnant participants signed informed consent for sample collection, and birthing parents signed consent for their infants (MGB IRB #s 2020P003538 and 2020P000955).

## METHOD DETAILS

### Antigens

Spike and RBD proteins of SARS-CoV-2 D614G or variants of concern, along with S1 and S2 were obtained from Sino Biological. These antigens were expressed in mammalian HEK293 cells. To biotinylate these antigens, NHS-Sulfo-LC-LC kits were used following the manufacturer's instructions (ThermoFisher Scientific).

### Multiplexed Luminex assay

Antigen-specific antibody levels and Fc $\gamma$  receptor binding were measured using a custom multiplexed Luminex assay, as previously described.<sup>51</sup> To couple the antigens to magnetic carboxylated fluorescent Luminex beads (Luminex Corporation), carbodiimide-NHS ester coupling was performed, with one bead region assigned to each antigen. The beads were first activated in an activation buffer, which consisted of 100 mM monobasic sodium phosphate (pH 6.2), 50 mg/mL N-hydroxysulfosuccinimide (Sulfo-NHS; Pierce) resuspended in H<sub>2</sub>O, and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide-HCl (EDC; Pierce) resuspended in the activation buffer. Following a 30-min incubation at room temperature (RT), the activated beads were washed in coupling buffer (50 mM morpholineethanesulfonic acid, MES; pH 5.0) and then incubated with antigens for 2 h at RT. Subsequently, the beads were blocked during a 30-min incubation at RT in phosphate-buffered saline (PBS)-TBN (containing 0.1% bovine serum albumin [BSA], 0.02% Tween 20, and 0.05% azide [pH 7.4]). Lastly, PBS-Tween (0.05% Tween 20) was used to wash the beads coupled with the proteins, then the beads were stored in PBS with 0.05% sodium azide at 4°C.

### Antigen-specific immunoglobulin quantification

Antigen-coupled beads were incubated with different sera dilutions (between 1:50 and 1:100, depending on the secondary reagent) at 4°C for 18 h of shaking. After the incubation, the beads were washed three times with PBS-Tween (0.05% Tween 20) and mixed to phycoerythrin (PE)-conjugated mouse anti-human antibodies (IgG, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgM) from Southern Biotech at a concentration of 1.3  $\mu$ g/mL. Following an additional 1-h incubation at RT with shaking, the beads underwent another three washes with PBS-Tween (0.05% Tween 20) and were then resuspended in sheath fluid (Luminex Corporation). To ensure accuracy, each serum sample was tested in duplicate. The levels of PE median fluorescence intensity (MFI) were measured using the iQue screener plus (Intellicyt) analyzer.

### Antigen-specific Fc $\gamma$ -receptor binding

To investigate the relative binding levels of antibodies to individual Fc $\gamma$ -receptors (Fc $\gamma$ Rs), Avi-tagged Fc $\gamma$ R2AR (used to measure Fc $\gamma$ R2A), Fc $\gamma$ R2B (used to measure Fc $\gamma$ R2B), Fc $\gamma$ R3AV (used to measure Fc $\gamma$ R3B), and Fc $\gamma$ R3B were obtained from Duke Human Vaccine Institute.<sup>51</sup> Biotinylation of these receptors was carried out using a BirA biotin-protein ligase (BirA500; Avidity), and any excess biotin was removed using

Zeba spin desalting columns (7K MWCO; Thermo Fisher Scientific). Serum samples were diluted at a ratio of 1:500 and mixed with antigen-coupled beads to form immune complexes. Following an 18-h incubation at 4°C, the beads were washed three times with PBS-Tween (0.05% Tween 20). Streptavidin-R-phycoerythrin (ProZyme) was then added to each biotinylated FcyR at a 4:1 M ratio and allowed to incubate for 20 min at RT. Fluorescent labeled FcyRs (at a concentration of 1 µg/mL in 0.1% BSA-PBS) were subsequently added to the immune complexes, followed by an incubation of 1 h at RT. Lastly, three washes with PBS-Tween (0.05% Tween 20) were done, then beads were resuspended in sheath fluid (Luminex Corporation) for analysis. The median PE intensity was measured using the iQue screener plus (Intellicyt) system, and all samples were tested in duplicate.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Analyses were divided between mothers vaccinated at < 20 weeks' gestation, versus 20–28 weeks (window fully in the 2<sup>nd</sup> trimester) versus 28 weeks to delivery (3<sup>rd</sup> trimester) to reflect the known increased protection against severe infant COVID-19 disease conferred by maternal vaccination after 20 weeks' gestation (e.g., < 20 weeks, >20 weeks),<sup>9,10</sup> and to further subset vaccination after 20 weeks' gestation into biological windows that coincide with the trimesters of pregnancy. All statistical analyses and figure generation were performed using R (version 4.1.0). For Luminex samples, all features were subtracted by phosphate-buffered saline (PBS) control values and were log<sub>10</sub>-transformed. As in previous studies,<sup>17</sup> detectable Luminex-derived features were defined as any value greater than the sum of the mean of the SARS-CoV-2 unvaccinated negative control adult samples (collected before the pandemic) and one standard deviation of those samples.

For univariate analyses of differences between maternal, cord, and infant samples, and as well as 2-month-old infant groups that vary between vaccination timing, significance was determined by Kruskal-Wallis followed by posthoc Benjamini-Hochberg adjustment. For analysis of the transfer between maternal and cord dyads, significance was calculated by a Wilcoxon signed-rank test followed by Benjamini-Hochberg.

Multivariate analyses of the systems serology data were performed in R (version 4.2.1). All log<sub>10</sub> data were then centered and scaled. Luminex-derived features for which 70% of values fell below one standard deviation above the mean of SARS-CoV-2 unvaccinated negative control samples were pruned. Uniform Manifold Approximation and Projection (UMAP), unsupervised dimensionality reduction technique,<sup>52</sup> was performed to evaluate the effect of maternal vaccination timing and fetal sex. Prior to UMAP analysis, the first 20 principal components (PCs) that explain 95% of the data were determined using principal component analysis (PCA).<sup>53</sup> These PCs were then mapped used to reduce the high-dimensional serological data into a two-dimensional space with fine-tuned parameters (n.neighbor = 30, min.dist = 0.2). UMAP was performed using the R package 'Seurat'.

For Partial Least Squares Discriminant Analysis (PLSDA) classification models, Least absolute shrinkage and selection operator (LASSO) feature selection<sup>54</sup> was performed to extract the most significant features in distinguishing our groups. LASSO feature selection was implemented in the function `select_lasso` in the `systemseRology` package (version 1.0) and was performed 100 times, and only features that were chosen repeatedly were further analyzed in downstream analyses. PLSDA was performed using the LASSO-selected features. To evaluate model robustness, the permuted control models were first built by randomly shuffling the labels 100 times. These control models were then cross-validated 10 times to determine the accuracy of our model. Robustness was then calculated as the effect size of the actual and control distributions and defined as the exact p values of the tail probabilities of the actual distributions within the control distributions.