

1 **Novel protein-based pneumococcal vaccine elicits**
2 **broad immunity through IgG subclass dominance and**
3 **multifunctional antibody responses**

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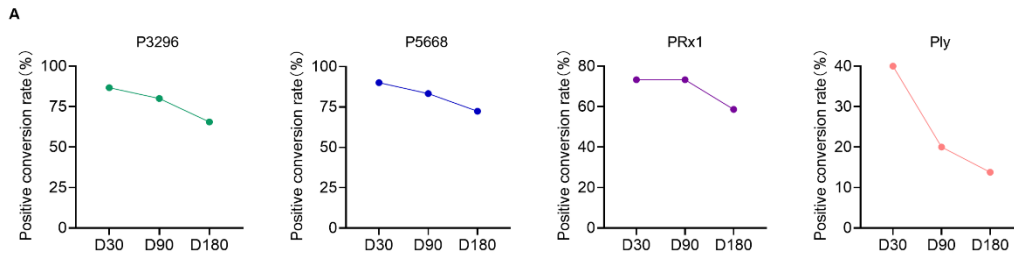
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40 This Supplementary material contains:

41 Supplemental Figure 1 to 9.

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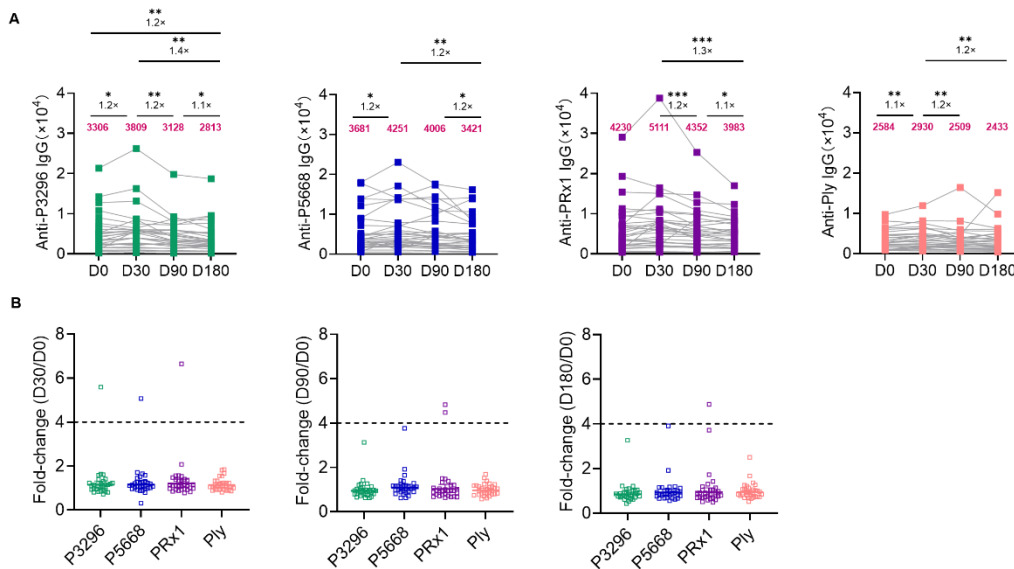


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45 **Supplemental Figure 1. The positive conversion rate in the PBPV group.**

46 **(A)** Positive conversion rates for P3296, P5668, PRx1, and Ply were assessed
 47 at D30, D90, and D180, positive response defined as a ≥ 4 -fold increase in
 48 antibody titers compared to D0 levels.

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51 **Supplemental Figure 2. PPV23 fails to induce the effective P3296-, P5668-,**

52 **PRx1- and Ply-specific antibody production. (A)** Specific antibody titers

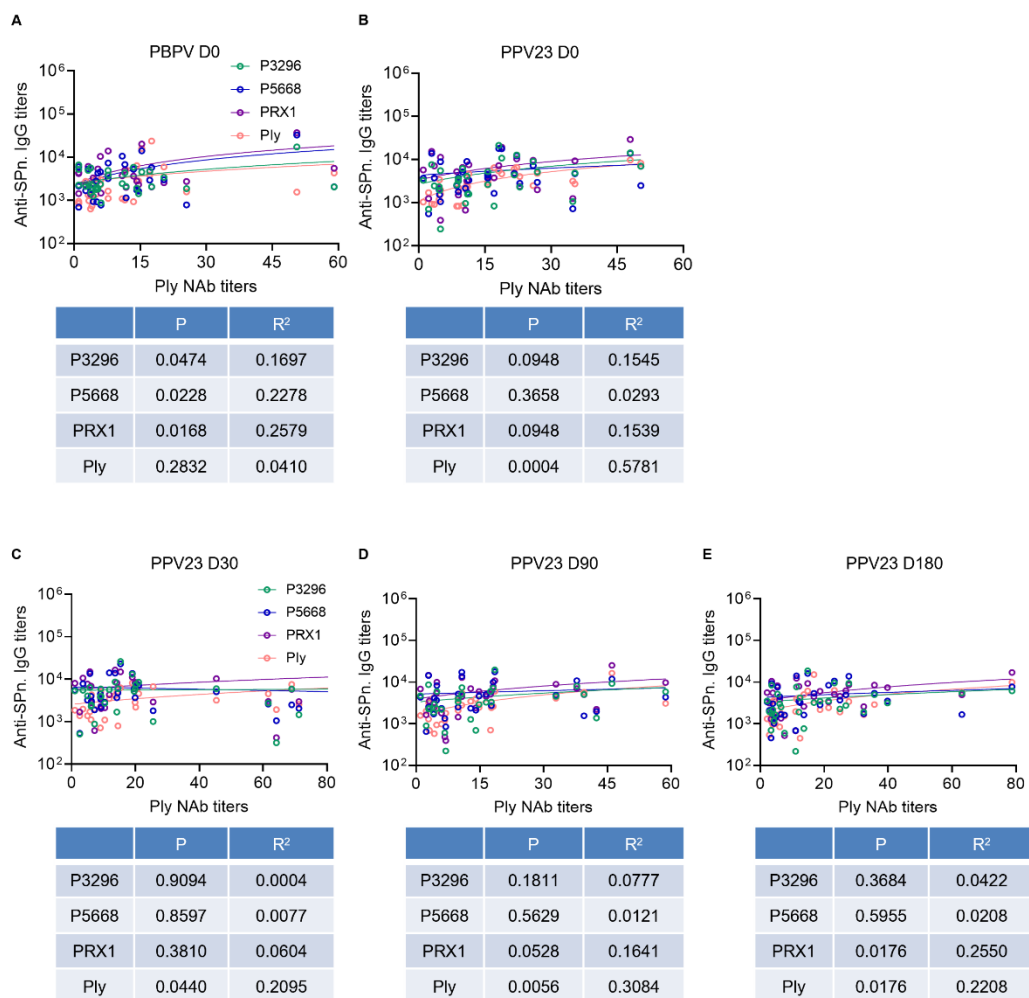
53 against P3296 (green), P5668 (blue), PRx1 (purple), and Ply (pink) were

54 measured in the PPV23 group (n = 30) at D0, D30, D90 and D180. **(B)** Fold

55 changes for P3296, P5668, PRx1, and Ply were assessed at D30, D90 and

56 D180, defined a ≥ 4 -fold increase in antibody titers compared to D0 levels as
 57 positive cutoff. The error bars defined geometric mean with 95% CI. The
 58 numbers in magenta indicated the geometric mean titers (GMT) in **A**.
 59 Significance was measured using the Wilcoxon rank-sum test in **A**. All p-values
 60 were adjusted for multiple testing using the Benjamini–Hochberg (BH) method.
 61 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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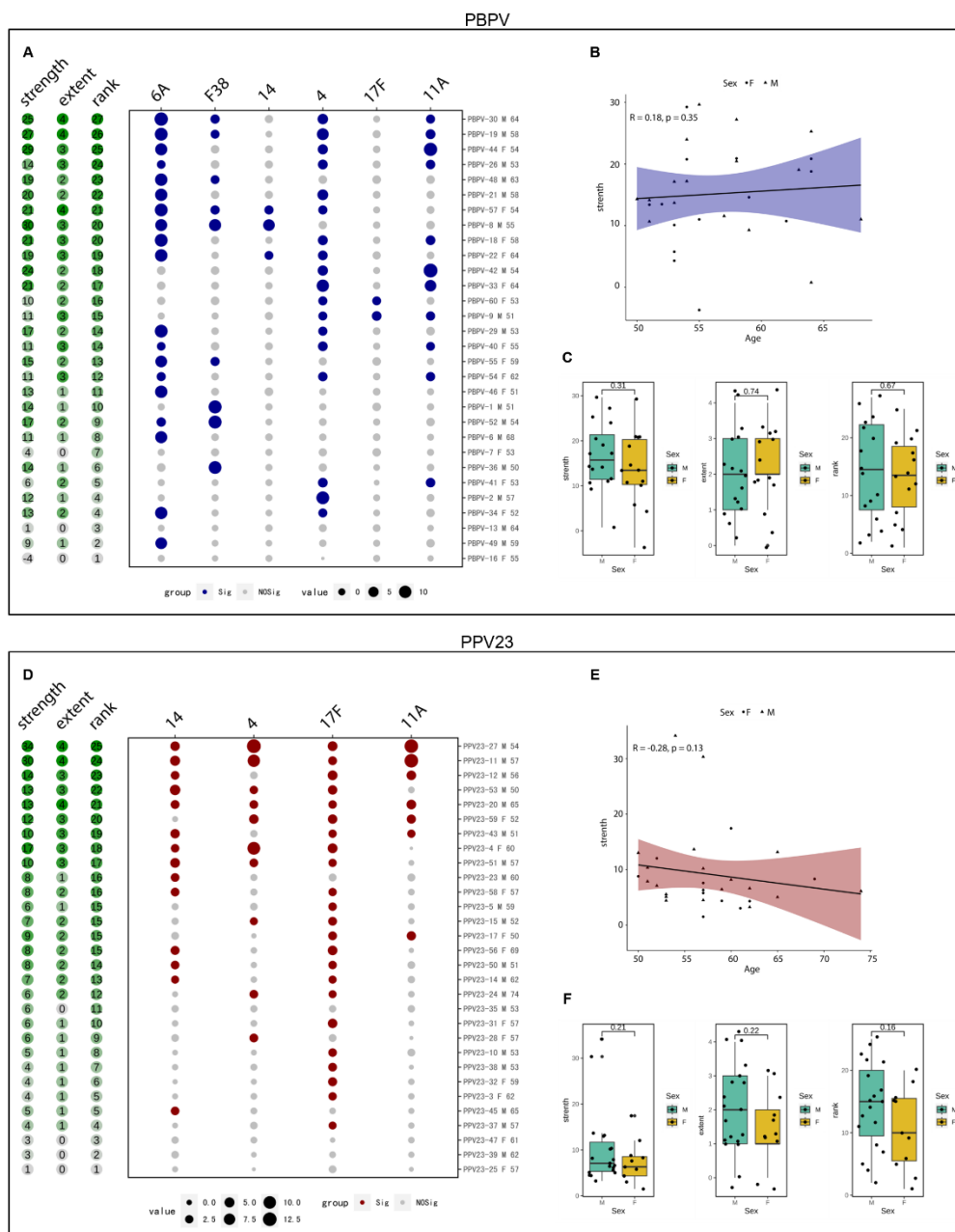


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64 **Supplemental Figure 3. Correlation analysis between titers of four**
 65 **protein-specific antibodies and Ply-neutralizing antibody titers induced**

66 **by PPV23. (A-E)** Correlation analysis between specific antibody titers of the
 67 four PBPV immunogens (P3296, P5668, PRx1 and Ply) and neutralizing
 68 antibody titers against Ply in the PBPV group at D0 and in the PPV23 group at
 69 D0, D30, D90, D180. All p-values were adjusted for multiple testing using the
 70 Benjamini–Hochberg (BH) method.

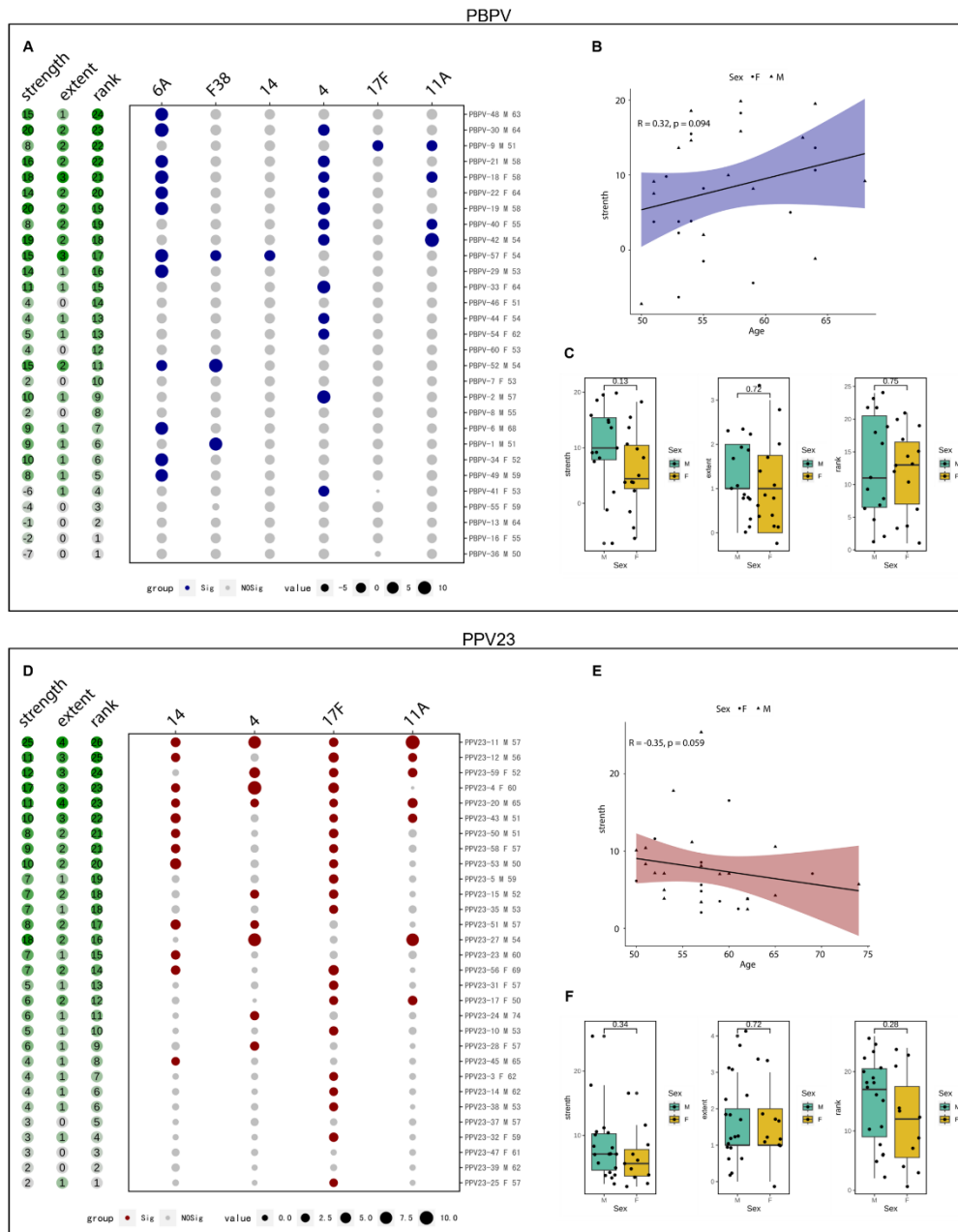
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73 **Supplemental Figure 4 Comparative analysis of OPA induced by PBPV**
74 **and PPV23 at day 90 post-vaccination. (A and D)** The OPA elicited by PBPV
75 and PPV23 at D90 were presented. The left panel illustrates the strength
76 ($\log_2(\text{sum fold change})$), extent, and rank of OPA reactions (strength means the
77 sum of fold change values between baseline and D90 for all serotypes; extent
78 means the number of serotypes (of 4) to which the donor elicits marked (that is,
79 an OPA titer of ≥ 2) responses; rank means an individual's vaccine
80 responsiveness in the cohort based on aggregate responses for all serotypes,
81 where higher ranks represent stronger responses. The middle bubble plot uses
82 color coding to indicate marked responses ($\text{Log}_2 \text{ fold change [FC]} > 1$): blue/red
83 denotes a marked enhancement of OPA functionality ($\text{D90/D0 OPA value} \geq 2$),
84 while gray indicates a value ≤ 2 . Bubble size represents the magnitude of the
85 response. The right panel provides information on participant ID, gender (F for
86 female, M for male), and age. **(B and E)** Correlation between OPA response
87 intensity and age at D90 with PBPV and PPV23. **(C and F)** Gender-based
88 differences in OPA response intensity, magnitude, and grade at D90 with PBPV
89 and PPV23. Boxplots display the median and interquartile range (IQR, 25–75%),
90 with whiskers indicating values within $1.5 \times \text{IQR}$ above and below the
91 quartiles.

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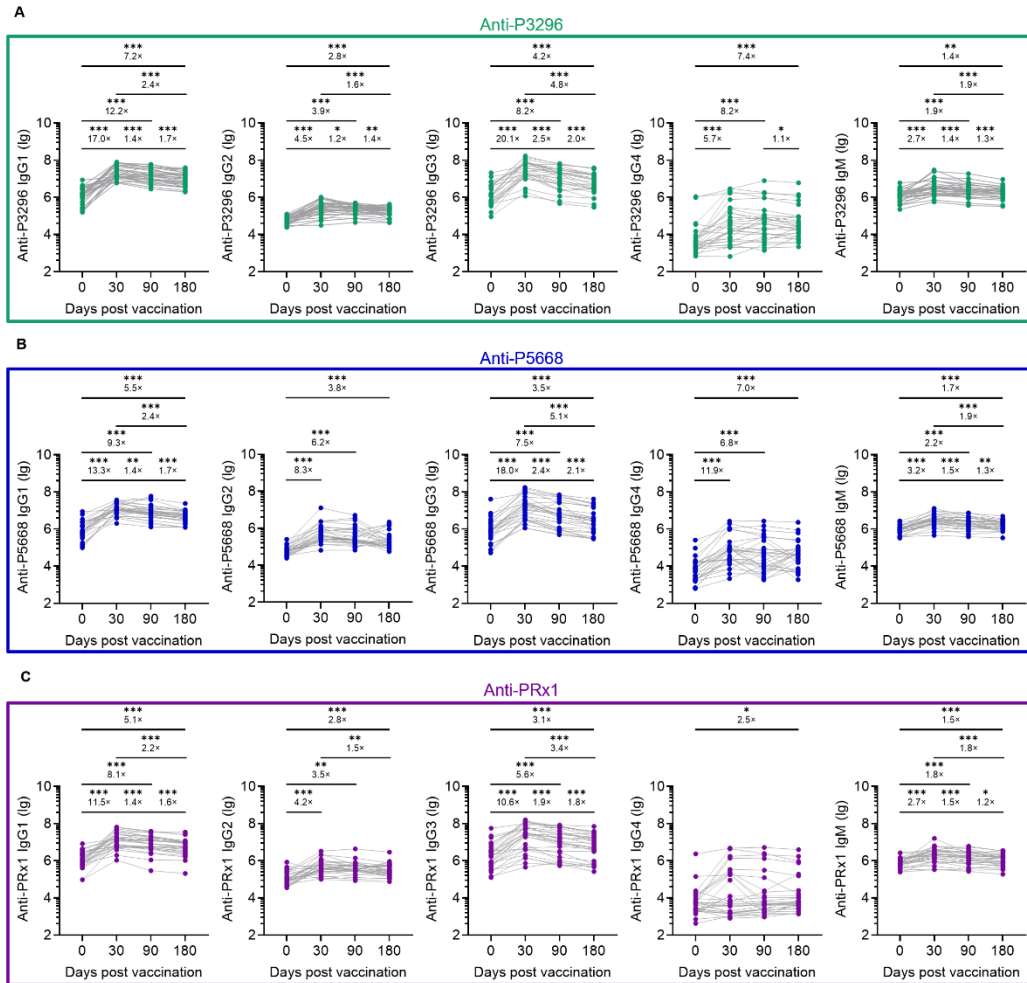


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94 **Supplemental Figure 5 Comparative analysis of OPA induced by PBPV**
 95 **and PPV23 at day 180 post-vaccination. (A and D) The OPA elicited by PBPV**
 96 **and PPV23 at D180 were presented. The left panel illustrates the strength**
 97 **(log₂(sum fold change)), extent, and rank of OPA reactions (strength means the**
 98 **sum of fold change values between baseline and D180 for all serotypes; extent**
 99 **means the number of serotypes (of 4) to which the donor elicits marked (that is,**

100 an OPA titer of ≥ 2) responses; rank means an individual's vaccine
101 responsiveness in the cohort based on aggregate responses for all serotypes,
102 where higher ranks represent stronger responses. The middle bubble plot uses
103 color coding to indicate marked responses (Log2 fold change [FC] > 1): blue/red
104 denotes a marked enhancement of OPA functionality (D180/D0 OPA value ≥ 2),
105 while gray indicates a value ≤ 2 . Bubble size represents the magnitude of the
106 response. The right panel provides information on participant ID, gender (F for
107 female, M for male), and age. **(B and E)** Correlation between OPA response
108 intensity and age at D180 with PBPV and PPV23. **(C and F)** Gender-based
109 differences in OPA response intensity, magnitude, and grade at D180 with
110 PBPV and PPV23. Boxplots display the median and interquartile range (IQR,
111 25–75%), with whiskers indicating values within $1.5 \times$ IQR above and below
112 the quartiles.

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115 **Supplemental Figure 6 Kinetics of P3296-, P5668-, and PRx1-specific**

116 **antibody subclass titers from day 30 to 180 post-vaccination. (A-C) Titers**

117 **of antibody subclasses (IgG1, IgG2, IgG3, IgG4, and IgM) induced against**

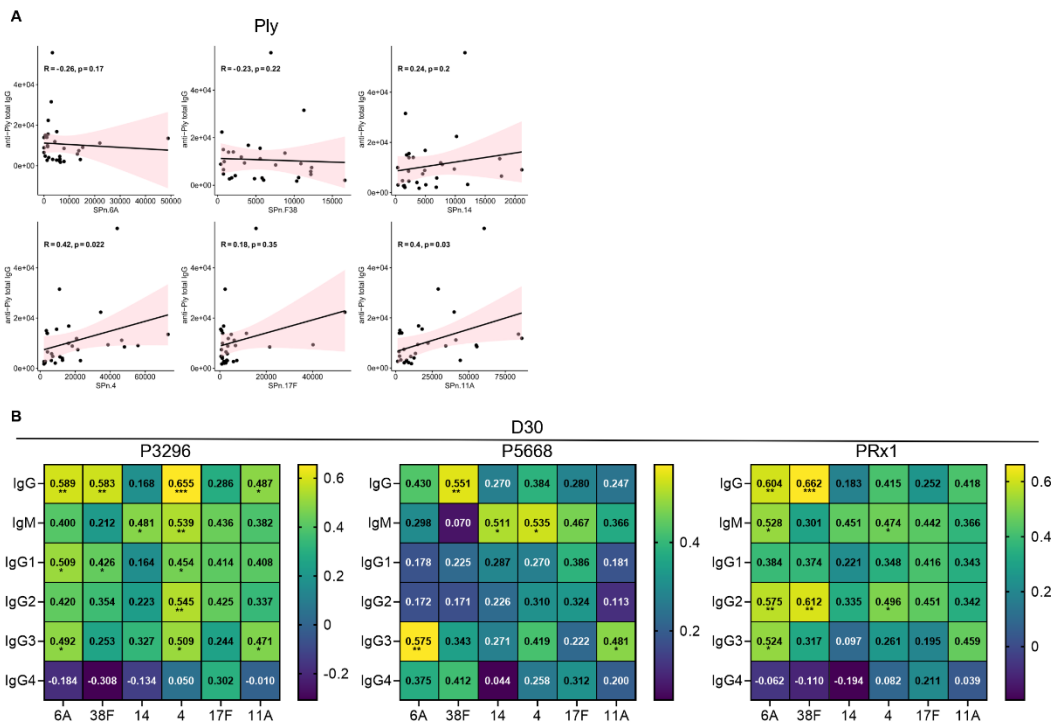
118 **P3296, P5668 and PRx1 in vaccinated subjects. Significance was measured**

119 **using the Wilcoxon rank-sum test in A-C. All p-values were adjusted for multiple**

120 **testing using the Benjamini–Hochberg (BH) method. *p < 0.05, **p < 0.01, ***p**

121 **< 0.001.**

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124 **Supplemental Figure 7 Correlation between antibody titers and OPA**

125 **indices induced by PBPV. (A)** The correlation between Ply-specific antibody

126 titers and the OPA indices of the six serotypes at D30. **(B)** The correlation

127 between the titers of antibody subclasses (IgG, IgM, IgG1, IgG2, IgG3 and IgG4)

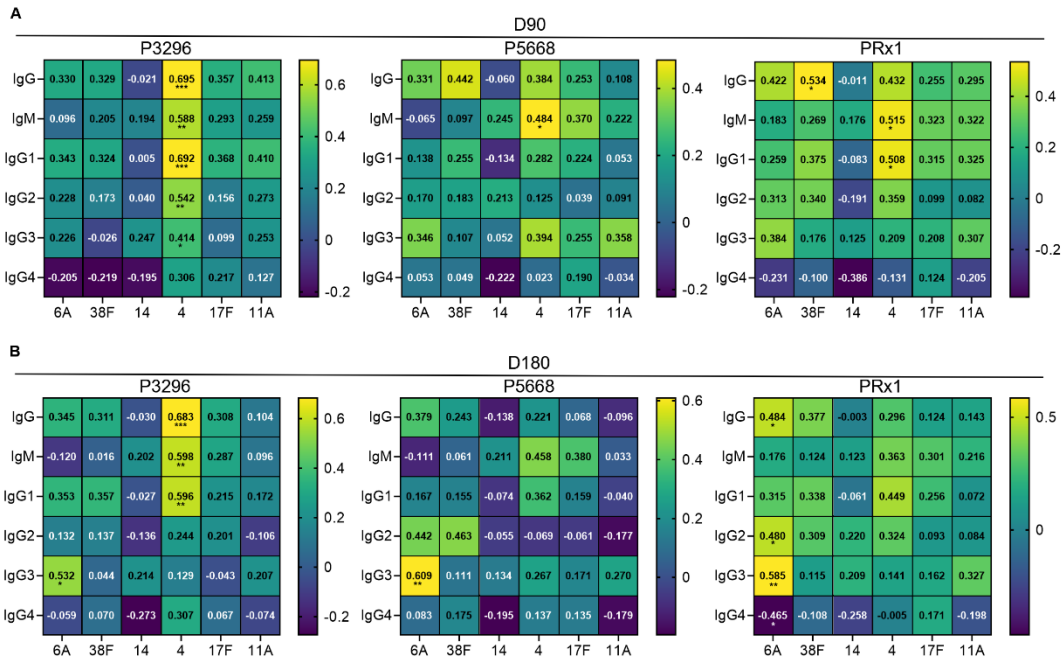
128 of three immunogens and the OPA indices for six serotypes at D30. Significance

129 was measured using the Spearman correlation analysis in **A** and **B**. All p-values

130 were adjusted for multiple testing using the Benjamini–Hochberg (BH) method.

131 *p < 0.05, **p < 0.01, ***p < 0.001.

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134 **Supplemental Figure 8 Correlation between antibody titers and OPA**

135 **indices at day 90 and 180 post-vaccination induced by PBPV. (A and B)**

136 The correlation between the titers of antibody subclasses of three PspA proteins

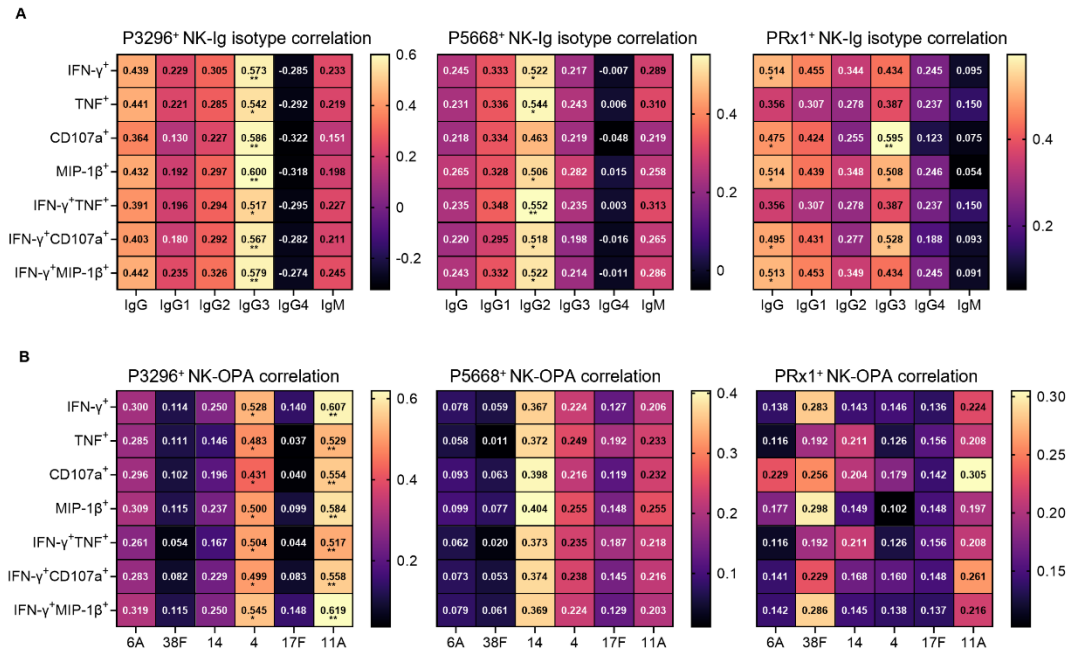
137 and the OPA indices for six serotypes at D90 and D180. Significance was

138 measured using the Spearman correlation analysis in **A** and **B**. All p-values

139 were adjusted for multiple testing using the Benjamini–Hochberg (BH) method.

140 *p < 0.05, **p < 0.01, ***p < 0.001.

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143 **Supplemental Figure 9 Functional NK cell responses induced by PBPV.**

144 **(A)** Correlation analysis between the positive rate of cytokines secreted by NK

145 cells activated by P3296, P5668, and PRx1-specific antibodies and the titers of

146 corresponding different antibody subclasses. **(B)** Correlation analysis between

147 the positive rate of cytokines secreted by NK cells activated by P3296, P5668,

148 and PRx1-specific antibodies and the OPA indices of 6 serotypes. Significance

149 was measured using the Spearman correlation analysis in **A** and **B**. All p-values

150 were adjusted for multiple testing using the Benjamini–Hochberg (BH) method.

151 *p < 0.05, **p < 0.01, ***p < 0.001.