# Insights into the heterogeneity of pneumococcal polysaccharide vaccine immunogenicity in older adults

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## SUPPLEMENTARY

**Supplementary table 1: Participant characteristics.**

| | All participants | Responders | Non-responders | P-value | FDR-adjusted p-value |
|---|---|---|---|---|---|
| Total n | 188 | 81 [/170] | 89 [/170] | | |
| **Demograpic variables** | | | | | |
| Age at PPV23 vaccination (years) (median [IQR]) | 75.4 [73.9 - 77.2] | 75.3 [73.8 - 76.9] | 75.5 [74.0 - 76.9] | 0.655 | 1.000 |
| Sex (female) | 38.3% | 37.0% | 41.6% | 0.837 | 1.000 |
| **Lifestyle factors** | | | | | |
| Alcohol consumption (≥1 glass/week) | 68.6% | 65.4% | 71.9% | 0.456 | 1.000 |
| Adherence to Dutch healthy exercise norm (NNGB) | 68.6% | 70.4% | 66.3% | 0.685 | 1.000 |
| CPAI (Cambridge Physical Activity Index): | | | | | |
| Inactive | 7.5% | 9.9% | 4.5% | 0.233 | 1.000 |
| Moderately inactive | 29.3% | 28.4% | 30.3% | 0.913 | 1.000 |
| Moderately active | 25.5% | 25.9% | 27.0% | 1.000 | 1.000 |
| Active | 37.2% | 35.8% | 38.2% | 0.869 | 1.000 |
| Frailty index (median [IQR]); range: 0-1 | 0.06 [0.03 - 0.13] | 0.06 [0.03 - 0.13] | 0.07 [0.03 - 0.13] | 0.602 | 1.000 |
| **(Cardio)metabolic factors** (median [IQR]) | | | | | |
| Waist circumference (cm) | 99.0 [91.4 - 106.1] | 99.3 [93.0 - 107.5] | 99.0 [90.0 - 105.8] | 0.365 | 1.000 |
| BMI (kg/m$^2$) | 26.4 [24.0 - 28.4] | 26.4 [25.1 - 28.7] | 26.4 [23.5 - 28.4] | 0.253 | 1.000 |
| Systolic blood pressure (mmHg) | 136.0 [121.0- 145.5] | 139.0 [124.0 – 150.0] | 136.0 [120.0 – 143.0] | 0.115 | 0.733 |
| Total cholesterol (mmol/L) | 5.1 [4.3 - 5.8] | 5.1 [4.3 - 5.9] | 5.2 [4.3 - 5.9] | 0.849 | 1.000 |
| HDL cholesterol (mmol/L) | 1.39 [1.10 - 1.70] | 1.40 [1.10 - 1.68] | 1.37 [1.12 - 1.72] | 0.620 | 1.000 |
| Creatinine (mmol/L) | 84 [74 - 98] | 82 [74 – 93] | 85 [74 - 99] | 0.290 | 1.000 |
| Glucose (mmol/L) | 5.6 [5.2 - 6.2] | 5.6 [5.2 - 6.2] | 5.5 [5 - 6.2] | 0.376 | 1.000 |
| eGFR | 0.85 [0.72 - 0.95] | 0.88 [0.75 - 0.99] | 0.83 [0.69 - 0.91] | **0.020** | 0.256 |
| CRP (ng/ul)[#] | 1191.1 [569.5 - 2674.5] | 1016.1 [539.8 – 2075.3] | 1463.3 [737.8 - 2939.0] | 0.126 | 0.733 |
| **Comorbidities and clinical history** | | | | | |
| Any comorbidity | 89.9% | 87.7% | 92.1% | 0.474 | 1.000 |
| Osteoporosis[$] | 10.7% | 11.1% | 11.2% | 1.000 | 1.000 |
| Joint inflammation | 13.3% | 17.3% | 11.2% | 0.363 | 1.000 |
| Osteoarthritis | 33.0% | 38.3% | 31.5% | 0.441 | 1.000 |
| Severe back problems or herniated disc | 16.0% | 16.1% | 15.7% | 1.000 | 1.000 |
| Migraine or severe headache[$] | 7.0% | 7.4% | 6.7% | 1.000 | 1.000 |
| Diabetes | 8.5% | 12.4% | 4.49% | 0.092 | 0.654 |
| Hypertension | 63.8% | 67.9% | 61.8% | 0.502 | 1.000 |
| Ever high blood pressure[@] | 53.0% | 55.6% | 49.4% | 0.520 | 0.994 |
| Cardiovascular disease | 14.4% | 12.4% | 15.7% | 0.680 | 1.000 |
| Inflammatory disease | 43.6% | 49.4% | 42.7% | 0.472 | 1.000 |
| Cardiometabolic disease | 76.6% | 77.8% | 74.2% | 0.710 | 1.000 |
| Number of comorbidities (median [IQR]) | 2 [1 - 4] | 3 [1 - 4] | 2 [1 - 3] | **0.000** | **0.000** |
| **Immune cell subsets (absolute count; number/ul whole blood, median [IQR])** [@] | | | | | |
| Lymphocytes | 1814.5 [1429.7 - 2215.6] | 1818.6 [1554.9 - 2274.3] | 1784.3 [1400.9 - 2148.8] | 0.299 | 1.000 |
| B cells | 149.1 [111.5 - 223.5] | 154.2 [105.7 - 235.9] | 142.0 [117.3 - 217.8] | 0.726 | 1.000 |
| CD38$^{dim}$ B cells | 139.7 [105.8 - 213.1] | 143.6 [99.3 - 220.7] | 135.6 [109.3 - 207.7] | 0.657 | 1.000 |
| Naïve mature B cells (CD27-IgD+) | 69.6 [46.8 - 109.7] | 76.4 [48.4 - 118.3] | 69.9 [48.6 - 99.4] | 0.334 | 1.000 |
| Double negative B cells (CD27-IgD-) | 11.9 [7.4 - 17.1] | 12.6 [7.9 - 17.4] | 10.9 [7.4 - 16.7] | 0.563 | 1.000 |
| Non-switched memory B cells (CD27+IgD+) | 18.1 [11.5 - 28.8] | 17.2 [12.0 - 28.4] | 21.2 [12.6 - 31.1] | 0.234 | 1.000 |
| Switched memory B cells (CD27+IgD-) | 28.8 [17.9 – 46.0] | 29.5 [18.0 – 44.0] | 28.0 [16.1 - 45.3] | 0.633 | 1.000 |
| Transitional B cells (CD27-CD38+) | 5.6 [2.9 - 8.6] | 5.6 [2.9 - 9.2] | 5.7 [3.1 - 8.5] | 0.872 | 1.000 |
| Plasmablasts (CD27+CD38+ ) | 1.1 [0.7 - 1.8] | 1.3 [0.8 – 2.0] | 0.9 [0.6 - 1.6] | **0.002** | **0.032** |
| T cells | 1167.4 [932.8 - 1450.1] | 1187.0 [1005.4 - 1466.4] | 1099.2 [924.9 - 1446.8] | 0.611 | 1.000 |
| CD4+ T cells | 749.2 [594.7 - 928.1] | 767.5 [605.8 - 931.5] | 745.5 [610.4 - 922.1] | 0.786 | 1.000 |
| CD8+ T cells | 368.5 [246.2 - 555.7] | 366.0 [274.0 - 538.6] | 366.2 [229.4 - 561.6] | 0.767 | 1.000 |
| NKT cells | 52.6 [27.6 - 113.5] | 53.2 [30.8 - 110.6] | 50.6 [27.3 - 124.3] | 0.817 | 1.000 |
| CD4+ NKT cells | 15.1 [10.0 - 23.6] | 16.5 [11.2 - 22.8] | 14.6 [9.3 - 24.9] | 0.586 | 1.000 |
| CD8+ NKT cells | 47.0 [18.4 - 85.3] | 46.8 [22.0 - 79.6] | 46.9 [17.8 – 106.0] | 1.000 | 1.000 |
| Granulocytes | 3455.4 [2687.3 - 4314.2] | 3649.6 [2986.6 - 4119.5] | 3340.6 [2570.2 - 4490.9] | 0.377 | 1.000 |
| Monocytes | 428.3 [335.3 - 517.8] | 456.9 [352.1 - 545.4] | 401.5 [323.8 – 496.0] | **0.028** | 0.265 |
| Classical monocytes | 395.3 [316.7 - 483.7] | 428.2 [332.7 – 520.0] | 382.2 [296.8 - 474.5] | **0.029** | 0.265 |
| CD16$^{+}$ monocytes | 15.5 [9.7 - 24.1] | 15.4 [10.8 – 25.0] | 15.3 [9.6 – 24.0] | 0.418 | 1.000 |
| Intermediate monocytes | 11.8 [7.8 - 17.7] | 11.8 [8.3 - 19.3] | 11.7 [7.0 – 16.0] | 0.254 | 1.000 |
| Non-classical monocytes | 3.2 [1.5 - 6.9] | 3.4 [1.9 - 6.5] | 3.1 [1.1 - 7.4] | 0.688 | 1.000 |
| NK CD56$^{dim}$ | 292.1 [182.4 - 382.5] | 326.6 [233.0 - 427.7] | 253.3 [149.2 - 341.8] | **0.002** | **0.032** |
| NK CD56$^{bright}$ | 10.2 [6.1 – 15.0] | 11.4 [6.7 – 16.0] | 9.1 [5.6 - 12.7] | 0.046 | 0.368 |
| **Serology chronic infections** | | | | | |
| CMV serostatus postive (≥7.5 AU/ml) | 53.2% | 54.3% | 50.6% | 0.737 | 1.000 |
| CMV IgG concentration (AU/ml) (median [IQR]) | 27.7 [1.3 - 135.5] | 32.6 [1.5 - 105.8] | 9.7 [1.0 - 196.7] | 0.943 | 1.000 |
| EBV serostatus postive (≥22 AU/ml) | 79.3% | 80.3% | 77.5% | 0.806 | 1.000 |
| EBV IgG concentration (AU/ml) (median [IQR]) | 152.5 [35.9 - 340.7] | 136.1 [52.8 - 339.1] | 158.7 [32.2 - 334.3] | 0.568 | 1.000 |
| VZV IgG concentration (AU/ml) (median [IQR]) | 2.8 [1.3 - 4.3] | 2.8 [1.3 - 3.8] | 2.9 [1.3 - 4.9] | 0.299 | 1.000 |

Characteristics are shown for all participants enrolled at baseline and included in the serology analyses (n=188), as well as stratified by PPV23 responder status for participants with complete data who were included in the association analyses (total n=170; responder (n = 81) versus non-responder (n = 89)). CPAI: Cambridge Physical Activity Index, assessing participants' habitual physical activity. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or use of antihypertensive medication. Cardiometabolic disease included diabetes (type I or II), hypertension, stroke, heart failure, myocardial infarction, bypass surgery, balloon dilation, cardiac catheterization, pacemaker implantation, or vascular surgery. Inflammatory disease included gastrointestinal disorders, psoriasis, osteoarthritis, joint inflammation, osteoporosis, or nervous system disease. All health- and immune-related variables were compared between PPV23 responders and non-responders. For categorical variables, p-values were obtained using the Chi-square test. For numeric variables, p-values were determined using the Wilcoxon Rank-Sum test. Both raw and FDR-adjusted (Benjamini-Hochberg) p-values are shown. Significant (raw and FDR-adjusted) p-values are shown in bold. AU = arbitrary units. [$]n=187; [#]n=186; [@]n=183

**Supplementary table 2. Overview of number of participants for whom IgG concentrations, IgM concentrations and opsonophagocytic activity (OPA) titers were measured per timepoint.**

| | | *Timepoint* | | | | |
|---|---|---|---|---|---|---|
| | | ***Baseline*** | ***4-6 weeks*** | ***6-9 months$*** | ***1 year*** | ***2 years*** |
| | **Serotype(s)** | **n** | **n** | **n** | **n** | **n** |
| **IgG** | All | 188 | 186 | 141 | 176 | 151 |
| **IgM** | All | 187 | 183 | *n.d.* | 174 | 145 |
| **OPA** | 1 | 39 | 39 | *n.d.* | 37 | *n.d.* |
| | 3 | 39 | 38 | *n.d.* | 37 | *n.d.* |
| | 8 | 39 | 40 | *n.d.* | 38 | *n.d.* |
| | 19A | 39 | 40 | *n.d.* | 38 | *n.d.* |

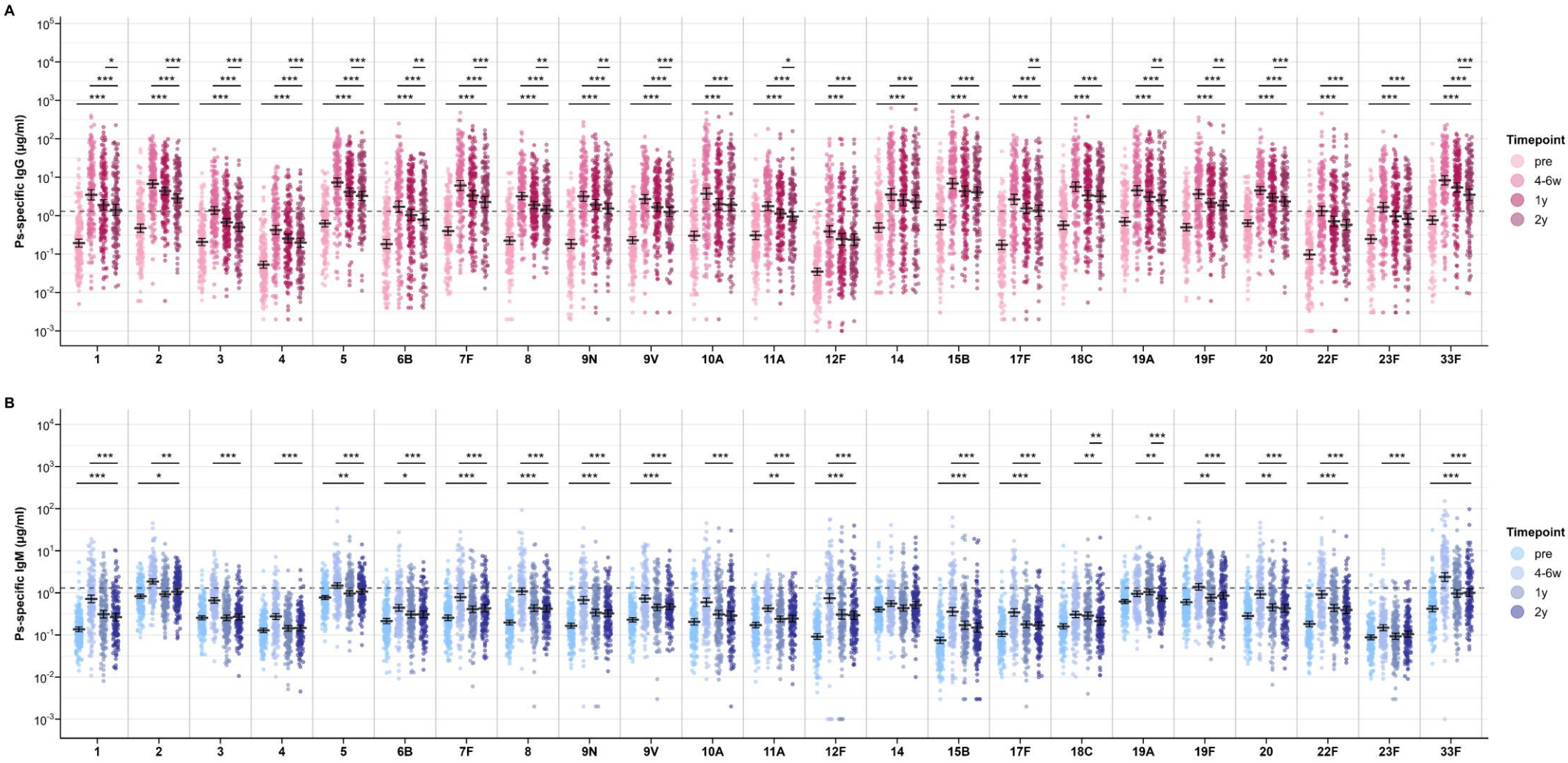
In case of IgG and IgM, antibody measurements were performed for all PPV23 serotypes, whereas for OPA, titers were determined for serotypes 1, 3, 8 and 19A. $^{\$}$At six to nine months post-PPV, solely ps-specific IgG responses have been assessed for the purpose of antibody decay modelling. n.d = not determined.

**Supplementary table 3. PPV23 serotype-specific IgG and IgM geometric mean concentrations (GMCs) before and after vaccination**

| | IgG GMC [95% CI] (µg/ml) Timepoint | | | IgM GMC [95% CI] (µg/ml) | | |
|---|---|---|---|---|---|---|
| | **baseline** | **4-6 weeks** | **1 year** | **baseline** | **4-6 weeks** | **1 year** |
| **N** | 188 | 186 | 176 | 187 | 183 | 174 |
| **Serotype** | | | | | | |
| **1** | 0.19 [0.15-0.24] | 3.47 [2.56-4.71]*** | 1.89 [1.39-2.57]$$$,### | 0.14 [0.12-0.16] | 0.72 [0.58-0.88]]*** | 0.31 [0.25-0.38]$$$,### |
| **2** | 0.47 [0.37-0.60] | 6.65 [5.28-8.37]*** | 4.37 [3.47-5.51]$$$,### | 0.83 [0.74-0.93] | 1.86 [1.62-2.14]]*** | 0.93 [0.81-1.06]### |
| **3** | 0.21 [0.17-0.25] | 1.36 [1.09-1.71]*** | 0.67 [0.54-0.84]$$$,### | 0.25 [0.23-0.29] | 0.66 [0.57-0.76]]*** | 0.26 [0.22-0.30]### |
| **4** | 0.05 [0.04-0.06] | 0.42 [0.32-0.55]*** | 0.25 [0.20-0.33]$$$,### | 0.13 [0.11-0.14] | 0.27 [0.23-0.32]]*** | 0.14 [0.12-0.17]### |
| **5** | 0.62 [0.51-0.76] | 7.33 [5.64-9.54]*** | 4.11 [3.16-5.34]$$$,### | 0.77 [0.69-0.86] | 1.49 [1.28-1.74]]*** | 0.97 [0.84-1.12]$,### |
| **6B** | 0.18 [0.14-0.24] | 1.70 [1.21-2.40]*** | 1.04 [0.74-1.44]$$$,### | 0.21 [0.19-0.25] | 0.44 [0.37-0.53]]*** | 0.30 [0.25-0.36]$$$,### |
| **7F** | 0.40 [0.31-0.51] | 6.06 [4.49-8.16]*** | 3.33 [2.48-4.47]$$$,### | 0.26 [0.22-0.30] | 0.79 [0.65-0.96]]*** | 0.41 [0.34-0.49]$$$,### |
| **8** | 0.22 [0.18-0.28] | 3.21 [2.57-4.00]*** | 1.89 [1.53-2.34]$$$,### | 0.20 [0.17-0.23] | 1.09 [0.91-1.30]]*** | 0.43 [0.36-0.52]$$$,### |
| **9N** | 0.18 [0.14-0.24] | 3.17 [2.37-4.23]*** | 1.95 [1.45-2.62]$$$,### | 0.17 [0.14-0.19] | 0.67 [0.55-0.82]]*** | 0.34 [0.28-0.41]$$$,### |
| **9V** | 0.23 [0.18-0.28] | 2.70 [2.07-3.52]*** | 1.67 [1.29-2.15]$$$,### | 0.23 [0.20-0.26] | 0.73 [0.60-0.88]]*** | 0.45 [0.38-0.54]$$$,### |
| **10A** | 0.30 [0.23-0.39] | 3.72 [2.67-5.18]*** | 1.97 [1.42-2.75]$$$,### | 0.21 [0.17-0.24] | 0.60 [0.47-0.75]]*** | 0.31 [0.25-0.39]$$,### |
| **11A** | 0.30 [0.24-0.39] | 1.76 [1.36-2.27]*** | 1.13 [0.87-1.48]$$$,### | 0.17 [0.15-0.20] | 0.43 [0.36-0.50]]*** | 0.24 [0.20-0.28]$$$,### |
| **12F** | 0.03 [0.03-0.04] | 0.39 [0.28-0.55]*** | 0.24 [0.17-0.35]$$$,### | 0.09 [0.08-0.11] | 0.74 [0.57-0.96]]*** | 0.31 [0.24-0.40]$$$,### |
| **14** | 0.49 [0.36-0.65] | 3.55 [2.48-5.06]*** | 2.53 [1.78-3.60]$$$,### | 0.40 [0.35-0.46] | 0.56 [0.48-0.65]]*** | 0.43 [0.37-0.51]# |
| **15B** | 0.58 [0.43-0.77] | 6.83 [5.04-9.25]*** | 4.33 [3.19-5.87]$$$,### | 0.07 [0.06-0.09] | 0.36 [0.29-0.45]]*** | 0.17 [0.14-0.21]$$$,### |
| **17F** | 0.17 [0.13-0.23] | 2.65 [1.94-3.61]*** | 1.54 [1.13-2.10]$$$,### | 0.11 [0.09-0.12] | 0.34 [0.28-0.42]]*** | 0.18 [0.15-0.21]$$$,### |
| **18C** | 0.56 [0.44-0.72] | 5.65 [4.23-7.53]*** | 3.36 [2.51-4.51]$$$,### | 0.16 [0.14-0.18] | 0.31 [0.26-0.36]]*** | 0.29 [0.24-0.35]$$$ |
| **19A** | 0.70 [0.55-0.89] | 4.51 [3.36-6.05]*** | 3.08 [2.33-4.05]$$$,### | 0.62 [0.56-0.70] | 0.95 [0.82-1.11]]*** | 1.05 [0.90-1.22]$$$ |
| **19F** | 0.50 [0.40-0.62] | 3.61 [2.75-4.75]*** | 2.16 [1.66-2.80]$$$,### | 0.60 [0.52-0.70] | 1.39 [1.16-1.66]]*** | 0.77 [0.64-0.92]### |
| **20** | 0.63 [0.52-0.77] | 4.50 [3.57-5.69]*** | 2.98 [2.37-3.75]$$$,### | 0.28 [0.24-0.33] | 0.92 [0.76-1.12]]*** | 0.45 [0.37-0.55]$$$,### |
| **22F** | 0.10 [0.07-0.13] | 1.31 [0.99-1.74]*** | 0.71 [0.53-0.96]$$$,### | 0.18 [0.16-0.21] | 0.92 [0.75-1.12]]*** | 0.44 [0.36-0.52]$$$,### |
| **23F** | 0.24 [0.19-0.31] | 1.65 [1.23-2.20]*** | 0.96 [0.71-1.29]$$$,### | 0.09 [0.08-0.10] | 0.15 [0.13-0.17]]*** | 0.09 [0.08-0.11]### |
| **33F** | 0.76 [0.59-0.97] | 8.31 [6.30-10.94]*** | 5.41 [4.10-7.14]$$$,### | 0.42 [0.36-0.49] | 2.38 [1.88-3.01]]*** | 0.96 [0.79-1.18]$$$,### |

PPV23 ps-specific IgG and IgM geometric mean concentrations (GMCs [95%CI]; µg/ml) were determined at baseline, four to six weeks, one year and two years following PPV23 vaccination. Following log10-transformation, antibody responses were compared between timepoints using linear mixed-effects models with a random intercept for each participant, followed by post-hoc pairwise comparisons of estimated marginal means with Benjamini-Hochberg correction for multiple testing. The symbols indicate the comparisons of the different timepoints: * = baseline vs. 4-6w; $ = baseline vs. 1y; # = 4-6w vs. 1y; and the number of symbols indicate the level of significance: p-value <0.05*, <0.01**, <0.001***.

**Supplementary figure 1. Pneumococcal serotype-specific IgG and IgM responses before and up to two years after PPV23 vaccination.** PPV23 polysaccharide (ps)-specific IgG (A) and IgM (B) concentrations were determined at baseline (pre), and four to six weeks (4-6w), one year (1y) and two years (2y) after PPV23 vaccination. For IgG, the horizontal dashed line indicates a serum concentration of 1.3 µg/ml. Geometric mean antibody concentrations (GMCs) are indicated in black, with error bars representing the 95% confidence intervals. Serotype-specific antibody concentrations at two years post-PPV23 were compared to preceding timepoints (pre vs. 2y, 4-6w vs. 2y and 1y vs. 2y) using linear mixed-effects models with a random intercept for each participant, followed by post-hoc pairwise comparisons of estimated marginal means with Benjamini-Hochberg correction for multiple testing. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



**Supplementary Table 4: Effect of age, sex and frailty on post-vaccination PPV23 ps-specific IgG responses.**

| | | *4-6w post-vaccination (n = 186)* | | *1y post-vaccination (n = 176)* | |
|---|---|---|---|---|---|
| **Serotype** | **Variable** | **Estimate** | **Adj. p-value** | **Estimate** | **Adj p-value** |
| **1** | baseline IgG conc. | **0,918** | **<0,0001** | **0,956** | **<0,0001** |
| | Sex (female) | 0,217 | 0,160 | 0,155 | 0,486 |
| | Age at vaccination | 0,022 | 1,000 | 0,030 | 1,000 |
| | Frailty index | -1,457 | 1,000 | -2,190 | 1,000 |
| | Age at vaccination*Frailty index | 0,018 | 1,000 | 0,028 | 1,000 |
| **2** | baseline IgG conc. | **0,638** | **<0,0001** | **0,595** | **<0,0001** |
| | Sex (female) | 0,168 | 0,179 | 0,053 | 1,000 |
| | Age at vaccination | -0,006 | 1,000 | -0,004 | 1,000 |
| | Frailty index | -0,257 | 1,000 | -0,288 | 1,000 |
| | Age at vaccination*Frailty index | 0,003 | 1,000 | 0,004 | 1,000 |
| **3** | baseline IgG conc. | **0,575** | **<0,0001** | **0,717** | **<0,0001** |
| | Sex (female) | 0,223 | 0,068 | 0,133 | 0,453 |
| | Age at vaccination | 0,025 | 1,000 | -0,002 | 1,000 |
| | Frailty index | 0,056 | 1,000 | 0,511 | 1,000 |
| | Age at vaccination*Frailty index | -0,002 | 1,000 | -0,007 | 1,000 |
| **4** | baseline IgG conc. | **0,955** | **<0,0001** | **0,909** | **<0,0001** |
| | Sex (female) | 0,124 | 0,795 | 0,093 | 1,000 |
| | Age at vaccination | -0,025 | 1,000 | -0,031 | 1,000 |
| | Frailty index | 1,498 | 1,000 | 1,726 | 1,000 |
| | Age at vaccination*Frailty index | -0,020 | 1,000 | -0,023 | 1,000 |
| **5** | baseline IgG conc. | **0,989** | **<0,0001** | **1,001** | **<0,0001** |
| | Sex (female) | 0,062 | 1,000 | -0,046 | 1,000 |
| | Age at vaccination | -0,008 | 1,000 | -0,001 | 1,000 |
| | Frailty index | 0,587 | 1,000 | 0,214 | 1,000 |
| | Age at vaccination*Frailty index | -0,008 | 1,000 | -0,002 | 1,000 |
| **6B** | baseline IgG conc. | **0,968** | **<0,0001** | **0,928** | **<0,0001** |
| | Sex (female) | -0,030 | 1,000 | -0,144 | 0,904 |
| | Age at vaccination | 0,031 | 1,000 | 0,037 | 1,000 |
| | Frailty index | -2,117 | 1,000 | -2,839 | 1,000 |
| | Age at vaccination*Frailty index | 0,028 | 1,000 | 0,038 | 1,000 |
| **7F** | baseline IgG conc. | **0,924** | **<0,0001** | **0,933** | **<0,0001** |
| | Sex (female) | 0,033 | 1,000 | -0,030 | 1,000 |
| | Age at vaccination | -0,017 | 1,000 | -0,029 | 1,000 |
| | Frailty index | 0,001 | 1,000 | 0,449 | 1,000 |
| | Age at vaccination*Frailty index | <0,0001 | 1,000 | -0,006 | 1,000 |
| **8** | baseline IgG conc. | **0,583** | **<0,0001** | **0,557** | **<0,0001** |
| | Sex (female) | -0,024 | 1,000 | -0,035 | 1,000 |
| | Age at vaccination | **0,127** | **0,049** | **0,121** | **0,046** |
| | Frailty index | **-8,023** | **0,011** | **-7,915** | **0,007** |
| | Age at vaccination*Frailty index | **0,107** | **0,011** | **0,105** | **0,007** |
| **9N** | baseline IgG conc. | **0,853** | **<0,0001** | **0,827** | **<0,0001** |
| | Sex (female) | -0,020 | 1,000 | -0,097 | 1,000 |
| | Age at vaccination | 0,011 | 1,000 | 0,014 | 1,000 |
| | Frailty index | -0,725 | 1,000 | -0,841 | 1,000 |
| | Age at vaccination*Frailty index | 0,009 | 1,000 | 0,010 | 1,000 |
| **9V** | baseline IgG conc. | **0,896** | **<0,0001** | **0,890** | **<0,0001** |
| | Sex (female) | -0,004 | 1,000 | -0,027 | 1,000 |
| | Age at vaccination | 0,018 | 1,000 | 0,025 | 1,000 |
| | Frailty index | -1,762 | 1,000 | -2,273 | 1,000 |
| | Age at vaccination*Frailty index | 0,024 | 1,000 | 0,031 | 1,000 |
| **10A** | baseline IgG conc. | **0,981** | **<0,0001** | **1,022** | **<0,0001** |
| | Sex (female) | 0,048 | 1,000 | 0,061 | 1,000 |
| | Age at vaccination | -0,029 | 1,000 | -0,024 | 1,000 |
| | Frailty index | 1,817 | 1,000 | 1,256 | 1,000 |
| | Age at vaccination*Frailty index | -0,025 | 1,000 | -0,018 | 1,000 |
| **11A** | baseline IgG conc. | **0,842** | **<0,0001** | **0,852** | **<0,0001** |
| | Sex (female) | 0,008 | 1,000 | -0,100 | 1,000 |
| | Age at vaccination | 0,037 | 1,000 | 0,031 | 1,000 |
| | Frailty index | -2,236 | 1,000 | -2,001 | 1,000 |
| | Age at vaccination*Frailty index | 0,030 | 1,000 | 0,027 | 1,000 |

Table continues on the next page.

| | | *4-6w post-vaccination (n = 186)* | | *1y post-vaccination (n = 176)* | |
|---|---|---|---|---|---|
| **Serotype** | **Variable** | **Estimate** | **Adj. p-value** | **Estimate** | **Adj p-value** |
| **12F** | baseline IgG conc. | **0,986** | **<0,0001** | **1,192** | **<0,0001** |
| | Sex (female) | 0,058 | 1,000 | 0,020 | 1,000 |
| | Age at vaccination | -0,088 | 0,729 | 0,048 | 1,000 |
| | Frailty index | 2,217 | 1,000 | -2,620 | 1,000 |
| | Age at vaccination*Frailty index | -0,030 | 1,000 | 0,034 | 1,000 |
| **14** | baseline IgG conc. | **0,798** | **<0,0001** | **0,962** | **<0,0001** |
| | Sex (female) | 0,020 | 1,000 | 0,036 | 1,000 |
| | Age at vaccination | 0,066 | 1,000 | -0,107 | 0,326 |
| | Frailty index | -3,364 | 1,000 | 2,931 | 1,000 |
| | Age at vaccination*Frailty index | 0,046 | 1,000 | -0,040 | 1,000 |
| **15B** | baseline IgG conc. | **0,874** | **<0,0001** | **0,804** | **<0,0001** |
| | Sex (female) | -0,070 | 1,000 | -0,045 | 1,000 |
| | Age at vaccination | -0,027 | 1,000 | 0,064 | 1,000 |
| | Frailty index | 1,023 | 1,000 | -3,138 | 1,000 |
| | Age at vaccination*Frailty index | -0,014 | 1,000 | 0,043 | 1,000 |
| **17F** | baseline IgG conc. | **0,887** | **<0,0001** | **0,897** | **<0,0001** |
| | Sex (female) | 0,059 | 1,000 | -0,071 | 1,000 |
| | Age at vaccination | -0,006 | 1,000 | 0,010 | 1,000 |
| | Frailty index | -1,781 | 1,000 | -0,673 | 1,000 |
| | Age at vaccination*Frailty index | 0,023 | 1,000 | 0,009 | 1,000 |
| **18C** | baseline IgG conc. | **0,940** | **<0,0001** | **0,896** | **<0,0001** |
| | Sex (female) | 0,030 | 1,000 | -0,071 | 1,000 |
| | Age at vaccination | 0,007 | 1,000 | 0,001 | 1,000 |
| | Frailty index | -0,931 | 1,000 | -2,399 | 1,000 |
| | Age at vaccination*Frailty index | 0,013 | 1,000 | 0,032 | 1,000 |
| **19A** | baseline IgG conc. | **0,914** | **<0,0001** | **0,894** | **<0,0001** |
| | Sex (female) | -0,085 | 1,000 | -0,065 | 1,000 |
| | Age at vaccination | 0,004 | 1,000 | 0,008 | 1,000 |
| | Frailty index | -0,471 | 1,000 | -0,709 | 1,000 |
| | Age at vaccination*Frailty index | 0,007 | 1,000 | 0,011 | 1,000 |
| **19F** | baseline IgG conc. | **0,859** | **<0,0001** | **0,859** | **<0,0001** |
| | Sex (female) | -0,026 | 1,000 | -0,111 | 1,000 |
| | Age at vaccination | 0,019 | 1,000 | -0,014 | 1,000 |
| | Frailty index | -0,438 | 1,000 | 0,174 | 1,000 |
| | Age at vaccination*Frailty index | 0,006 | 1,000 | -0,001 | 1,000 |
| **20** | baseline IgG conc. | **0,625** | **<0,0001** | **0,878** | **<0,0001** |
| | Sex (female) | 0,228 | 0,156 | 0,015 | 1,000 |
| | Age at vaccination | 0,029 | 1,000 | 0,036 | 1,000 |
| | Frailty index | -0,528 | 1,000 | -1,260 | 1,000 |
| | Age at vaccination*Frailty index | 0,007 | 1,000 | 0,016 | 1,000 |
| **22F** | baseline IgG conc. | **0,862** | **<0,0001** | **0,698** | **<0,0001** |
| | Sex (female) | 0,047 | 1,000 | 0,103 | 1,000 |
| | Age at vaccination | -0,103 | 0,280 | -0,002 | 1,000 |
| | Frailty index | 5,163 | 0,378 | 0,202 | 1,000 |
| | Age at vaccination*Frailty index | -0,068 | 0,400 | -0,002 | 1,000 |
| **23F** | baseline IgG conc. | **0,855** | **<0,0001** | **0,912** | **<0,0001** |
| | Sex (female) | -0,023 | 1,000 | -0,072 | 1,000 |
| | Age at vaccination | 0,008 | 1,000 | -0,104 | 0,239 |
| | Frailty index | 1,774 | 1,000 | 4,490 | 0,554 |
| | Age at vaccination*Frailty index | -0,024 | 1,000 | -0,059 | 0,594 |
| **33F** | baseline IgG conc. | **0,986** | **<0,0001** | **0,844** | **<0,0001** |
| | Sex (female) | 0,058 | 1,000 | -0,092 | 1,000 |
| | Age at vaccination | -0,088 | 0,729 | -0,012 | 1,000 |
| | Frailty index | 2,217 | 1,000 | 2,536 | 1,000 |
| | Age at vaccination*Frailty index | -0,030 | 1,000 | -0,034 | 1,000 |

Multivariable generalized linear models (GLMs) were used to evaluate the effects of sex, age at vaccination and frailty on IgG responses at four to six weeks (4-6w) and 1 year (1y) following PPV23 vaccination. Models were adjusted for baseline ps-specific IgG concentrations. An interaction term between age at vaccination and frailty was included to examine their combined effect on post-vaccination IgG responses. Bonferroni corrections were applied to account for multiple testing. Significant effects (adjusted (adj.) $p < 0.05$) are indicated in bold.

**Supplementary Table 5: Effect of age, sex and frailty on post-vaccination PPV23 ps-specific IgM responses.**

| | | *4-6w post-vaccination (n = 183)* | | *1y post-vaccination (n = 174)* | |
|---|---|---|---|---|---|
| **Serotype** | **Variable** | **Estimate** | **Adj. p-value** | **Estimate** | **Adj p-value** |
| **1** | baseline IgM conc. | 0,216 | 0,424 | **0,949** | **<0,0001** |
| | Sex (female) | -0,060 | 1,000 | -0,028 | 1,000 |
| | Age at vaccination | -0,027 | 1,000 | 0,011 | 1,000 |
| | Frailty index | -0,655 | 1,000 | -1,259 | 1,000 |
| | Age at vaccination*Frailty index | 0,009 | 1,000 | 0,017 | 1,000 |
| **2** | baseline IgM conc. | 0,082 | 1,000 | **0,967** | **<0,0001** |
| | Sex (female) | -0,006 | 1,000 | -0,030 | 1,000 |
| | Age at vaccination | -0,051 | 1,000 | 0,008 | 1,000 |
| | Frailty index | 1,547 | 1,000 | 0,240 | 1,000 |
| | Age at vaccination*Frailty index | -0,020 | 1,000 | -0,003 | 1,000 |
| **3** | baseline IgM conc. | 0,092 | 1,000 | **1,054** | **<0,0001** |
| | Sex (female) | -0,016 | 1,000 | -0,014 | 1,000 |
| | Age at vaccination | -0,037 | 1,000 | -0,001 | 1,000 |
| | Frailty index | 1,851 | 1,000 | 0,246 | 1,000 |
| | Age at vaccination*Frailty index | -0,026 | 1,000 | -0,003 | 1,000 |
| **4** | baseline IgM conc. | **0,313** | **0,021** | **1,030** | **<0,0001** |
| | Sex (female) | 0,019 | 1,000 | 0,041 | 1,000 |
| | Age at vaccination | -0,038 | 1,000 | 0,029 | 1,000 |
| | Frailty index | 1,954 | 1,000 | -1,659 | 1,000 |
| | Age at vaccination*Frailty index | -0,026 | 1,000 | 0,022 | 1,000 |
| **5** | baseline IgM conc. | 0,226 | 0,206 | **1,074** | **<0,0001** |
| | Sex (female) | 0,068 | 1,000 | -0,030 | 1,000 |
| | Age at vaccination | 0,008 | 1,000 | 0,012 | 1,000 |
| | Frailty index | -0,639 | 1,000 | -1,168 | 1,000 |
| | Age at vaccination*Frailty index | 0,009 | 1,000 | 0,015 | 1,000 |
| **6B** | baseline IgM conc. | 0,235 | 0,073 | **0,985** | **<0,0001** |
| | Sex (female) | 0,198 | 0,108 | -0,055 | 1,000 |
| | Age at vaccination | 0,012 | 1,000 | 0,002 | 1,000 |
| | Frailty index | -0,330 | 1,000 | 0,194 | 1,000 |
| | Age at vaccination*Frailty index | 0,004 | 1,000 | -0,002 | 1,000 |
| **7F** | baseline IgM conc. | 0,204 | 0,188 | **0,900** | **<0,0001** |
| | Sex (female) | 0,019 | 1,000 | 0,003 | 1,000 |
| | Age at vaccination | -0,019 | 1,000 | -0,007 | 1,000 |
| | Frailty index | 0,540 | 1,000 | 1,131 | 1,000 |
| | Age at vaccination*Frailty index | -0,007 | 1,000 | -0,015 | 1,000 |
| **8** | baseline IgM conc. | 0,208 | 0,225 | **0,822** | **<0,0001** |
| | Sex (female) | -0,007 | 1,000 | -0,093 | 1,000 |
| | Age at vaccination | 0,039 | 1,000 | 0,067 | 0,474 |
| | Frailty index | -1,538 | 1,000 | -3,194 | 0,679 |
| | Age at vaccination*Frailty index | 0,019 | 1,000 | 0,043 | 0,646 |
| **9N** | baseline IgM conc. | 0,090 | 1,000 | **0,963** | **<0,0001** |
| | Sex (female) | -0,018 | 1,000 | -0,017 | 1,000 |
| | Age at vaccination | -0,035 | 1,000 | 0,043 | 1,000 |
| | Frailty index | 1,213 | 1,000 | -3,067 | 0,510 |
| | Age at vaccination*Frailty index | -0,015 | 1,000 | 0,040 | 0,548 |
| **9V** | baseline IgM conc. | 0,200 | 0,520 | **1,052** | **<0,0001** |
| | Sex (female) | 0,036 | 1,000 | -0,068 | 1,000 |
| | Age at vaccination | 0,034 | 1,000 | 0,051 | 0,563 |
| | Frailty index | -2,789 | 1,000 | -2,487 | 0,742 |
| | Age at vaccination*Frailty index | 0,037 | 1,000 | 0,033 | 0,770 |
| **10A** | baseline IgM conc. | 0,190 | 0,311 | **1,012** | **<0,0001** |
| | Sex (female) | 0,060 | 1,000 | -0,046 | 1,000 |
| | Age at vaccination | -0,093 | 0,765 | -0,038 | 1,000 |
| | Frailty index | 5,237 | 0,656 | 1,533 | 1,000 |
| | Age at vaccination*Frailty index | -0,068 | 0,691 | -0,020 | 1,000 |
| **11A** | baseline IgM conc. | **0,229** | **0,031** | **0,847** | **<0,0001** |
| | Sex (female) | 0,112 | 0,828 | 0,042 | 1,000 |
| | Age at vaccination | -0,011 | 1,000 | 0,028 | 1,000 |
| | Frailty index | 1,310 | 1,000 | -1,718 | 1,000 |
| | Age at vaccination*Frailty index | -0,017 | 1,000 | 0,023 | 0,988 |

Table continues on the next page.

| | | *4-6w post-vaccination (n = 183)* | | *1y post-vaccination (n = 174)* | |
|---|---|---|---|---|---|
| **Serotype** | **Variable** | **Estimate** | **Adj. p-value** | **Estimate** | **Adj p-value** |
| **12F** | baseline IgM conc. | 0,260 | 0,229 | **1,040** | **<0,0001** |
| | Sex (female) | 0,213 | 0,432 | -0,105 | 1,000 |
| | Age at vaccination | 0,022 | 1,000 | 0,004 | 1,000 |
| | Frailty index | -2,872 | 1,000 | -0,952 | 1,000 |
| | Age at vaccination*Frailty index | 0,037 | 1,000 | 0,013 | 1,000 |
| **14** | baseline IgM conc. | 0,171 | 0,308 | **1,056** | **<0,0001** |
| | Sex (female) | -0,017 | 1,000 | -0,017 | 1,000 |
| | Age at vaccination | 0,004 | 1,000 | 0,001 | 1,000 |
| | Frailty index | 0,569 | 1,000 | -0,384 | 1,000 |
| | Age at vaccination*Frailty index | -0,007 | 1,000 | 0,005 | 1,000 |
| **15B** | baseline IgM conc. | **0,337** | **0,003** | **0,834** | **<0,0001** |
| | Sex (female) | 0,076 | 1,000 | 0,085 | 1,000 |
| | Age at vaccination | -0,046 | 1,000 | 0,011 | 1,000 |
| | Frailty index | 3,475 | 1,000 | 0,959 | 1,000 |
| | Age at vaccination*Frailty index | -0,046 | 1,000 | -0,012 | 1,000 |
| **17F** | baseline IgM conc. | 0,169 | 0,688 | **0,837** | **<0,0001** |
| | Sex (female) | 0,053 | 1,000 | 0,054 | 1,000 |
| | Age at vaccination | -0,042 | 1,000 | -0,011 | 1,000 |
| | Frailty index | 2,655 | 1,000 | -0,174 | 1,000 |
| | Age at vaccination*Frailty index | -0,036 | 1,000 | 0,003 | 1,000 |
| **18C** | baseline IgM conc. | 0,158 | 0,488 | **1,028** | **<0,0001** |
| | Sex (female) | 0,132 | 0,592 | -0,028 | 1,000 |
| | Age at vaccination | 0,032 | 1,000 | 0,006 | 1,000 |
| | Frailty index | -1,872 | 1,000 | -1,369 | 1,000 |
| | Age at vaccination*Frailty index | 0,025 | 1,000 | 0,018 | 1,000 |
| **19A** | baseline IgM conc. | 0,223 | 0,225 | **0,898** | **<0,0001** |
| | Sex (female) | 0,041 | 1,000 | -0,020 | 1,000 |
| | Age at vaccination | -0,036 | 1,000 | -0,018 | 1,000 |
| | Frailty index | 1,468 | 1,000 | 1,976 | 1,000 |
| | Age at vaccination*Frailty index | -0,019 | 1,000 | -0,026 | 1,000 |
| **19F** | baseline IgM conc. | 0,202 | 0,183 | **0,944** | **<0,0001** |
| | Sex (female) | 0,076 | 1,000 | 0,012 | 1,000 |
| | Age at vaccination | 0,039 | 1,000 | 0,024 | 1,000 |
| | Frailty index | -1,707 | 1,000 | -1,797 | 1,000 |
| | Age at vaccination*Frailty index | 0,022 | 1,000 | 0,024 | 1,000 |
| **20** | baseline IgM conc. | 0,096 | 1,000 | **0,921** | **<0,0001** |
| | Sex (female) | 0,047 | 1,000 | -0,035 | 1,000 |
| | Age at vaccination | -0,053 | 1,000 | 0,014 | 1,000 |
| | Frailty index | 3,487 | 1,000 | -1,553 | 1,000 |
| | Age at vaccination*Frailty index | -0,047 | 1,000 | 0,021 | 1,000 |
| **22F** | baseline IgM conc. | 0,172 | 0,454 | **0,842** | **<0,0001** |
| | Sex (female) | 0,115 | 1,000 | 0,062 | 1,000 |
| | Age at vaccination | -0,033 | 1,000 | 0,031 | 1,000 |
| | Frailty index | 1,443 | 1,000 | -1,925 | 1,000 |
| | Age at vaccination*Frailty index | -0,019 | 1,000 | 0,025 | 1,000 |
| **23F** | baseline IgM conc. | 0,102 | 1,000 | **0,893** | **<0,0001** |
| | Sex (female) | 0,031 | 1,000 | 0,037 | 1,000 |
| | Age at vaccination | -0,030 | 1,000 | -0,005 | 1,000 |
| | Frailty index | 1,897 | 1,000 | -0,608 | 1,000 |
| | Age at vaccination*Frailty index | -0,026 | 1,000 | 0,008 | 1,000 |
| **33F** | baseline IgM conc. | 0,208 | 0,403 | **0,879** | **<0,0001** |
| | Sex (female) | 0,059 | 1,000 | 0,015 | 1,000 |
| | Age at vaccination | -0,089 | 0,968 | 0,026 | 1,000 |
| | Frailty index | 5,065 | 0,811 | -0,231 | 1,000 |
| | Age at vaccination*Frailty index | -0,068 | 0,794 | 0,003 | 1,000 |

Multivariable generalized linear models (GLMs) were used to evaluate the effects of sex, age at vaccination and frailty on IgG responses at four to six weeks (4-6w) and 1 year (1y) following PPV23 vaccination. Models were adjusted for baseline ps-specific IgM concentrations. An interaction term between age at vaccination and frailty was included to examine their combined effect on post-vaccination IgM responses. Bonferroni corrections were applied to account for multiple testing. Significant effects (adjusted (adj.) $p < 0.05$) are indicated in bold.

**Supplementary table 6. Serotype 1-, 3-, 8- and 19A-specific opsonophagocytic activity (OPA) geometric mean titers (GMTs) before and after PPV23 vaccination**

| | OPA GMT [95% CI] | | |
|---|---|---|---|
| | **baseline** | **4-6 weeks** | **1 year** |
| **Serotype** | | | |
| **1** | 6.91 [4.76-10.02] | 93.28 [43.56-199.75]**** | 47.59 [23.29-97.23]$$$$,# |
| **3** | 6.78 [4.77-9.63] | 80.01 [48.70-131.46]**** | 17.39 [11.35-26.64]$$$$,#### |
| **8** | 13.54 [7.49-24.50] | 820.80 [533.62-1262.53]**** | 155.55 [86.57-279.47]$$$$,#### |
| **19A** | 68.43 [34.94-134.02] | 820.36 [434.52-1548.84]**** | 518.10 [269.44-996.23]$$$$ |

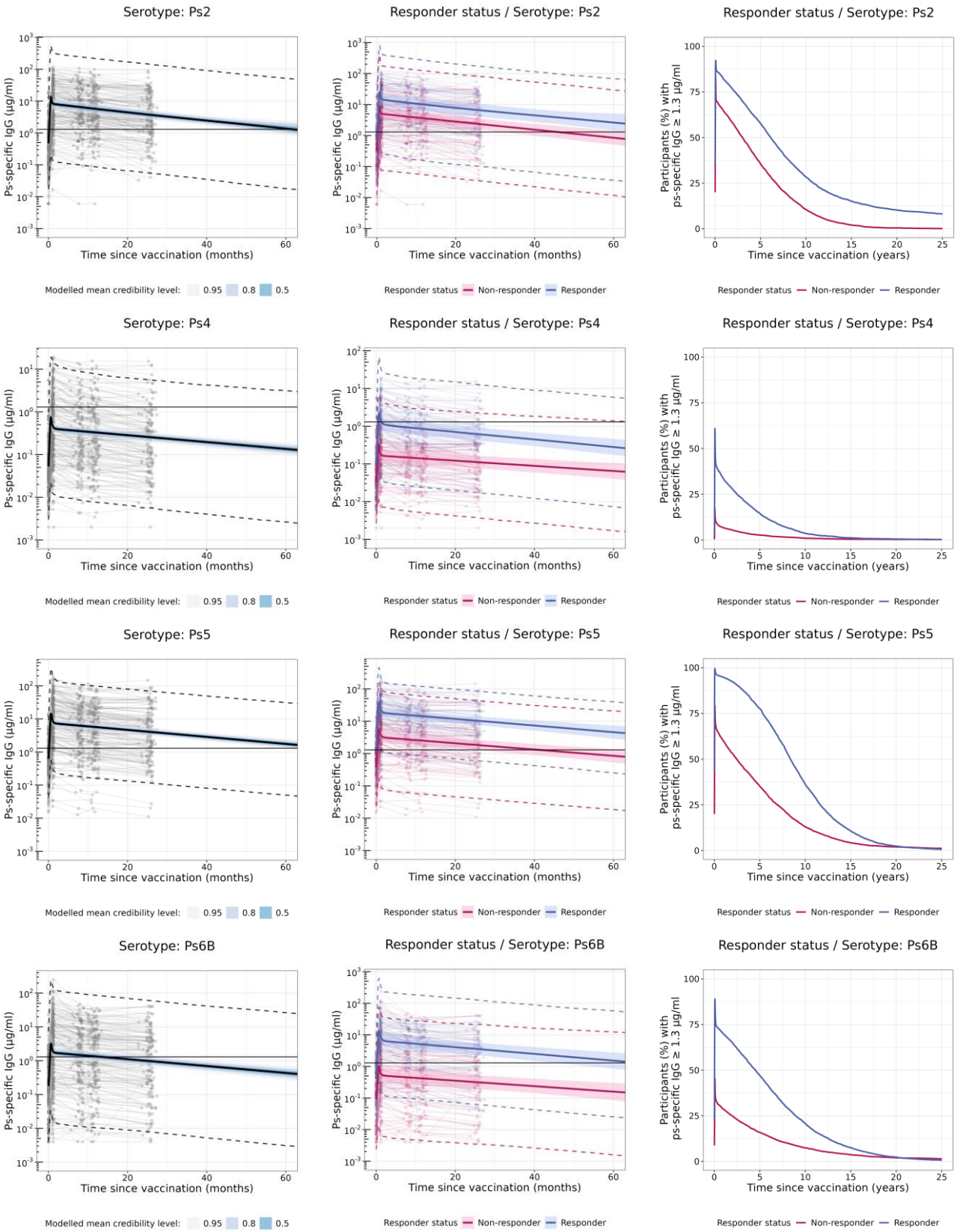
Serotype 1-, 3-, 8- and 19A-specific OPA geometric mean titers (GMTs [95%CI]l) were determined at baseline, four to six weeks and one year following PPV23 vaccination in a subset of participants (n=40). Following log10-transformation, OPA responses were compared between timepoints using linear mixed-effects models with a random intercept for each participant, followed by post-hoc pairwise comparisons of estimated marginal means with Bonferroni correction for multiple testing. The symbols indicate the comparisons of the different timepoints: * = baseline vs. 4-6w; $ = baseline vs. 1y; # = 4-6w vs. 1y; and the number of symbols indicate the level of significance: *p*-value <0.05*, <0.01**, <0.001***, <0.0001****.

**Supplementary Table 7: Effect of age, sex and frailty on post-vaccination PPV23 ps-specific OPA responses.**

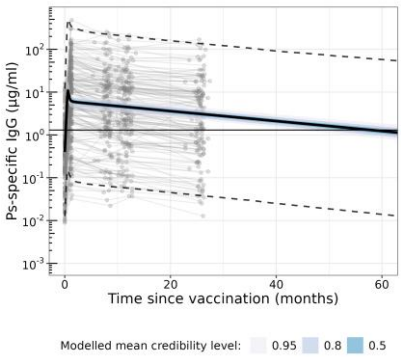
| | | *4-6w post-vaccination (n = 38-40)* | | *1y post-vaccination (n = 37-38)* | |
|---|---|---|---|---|---|
| **Serotype** | **Variable** | **Estimate** | **Adj. p-value** | **Estimate** | **Adj p-value** |
| **1** | Baseline OPA titer | 0,883 | 0,057 | **1,048** | **0,003** |
| | Sex (female) | 0,327 | 1,000 | 0,198 | 1,000 |
| | Age at vaccination | 0,288 | 0,431 | 0,313 | 0,148 |
| | Frailty index | -9,280 | 1,000 | -12,262 | 0,494 |
| | Age at vaccination*Frailty index | 0,124 | 1,000 | 0,163 | 0,496 |
| **3** | Baseline OPA titer | 0,280 | 1,000 | **0,796** | **0,001** |
| | Sex (female) | 0,156 | 1,000 | 0,250 | 0,726 |
| | Age at vaccination | 0,069 | 1,000 | -0,018 | 1,000 |
| | Frailty index | -3,573 | 1,000 | -1,195 | 1,000 |
| | Age at vaccination*Frailty index | 0,048 | 1,000 | 0,014 | 1,000 |
| **8** | Baseline OPA titer | 0,019 | 1,000 | **0,628** | **0,018** |
| | Sex (female) | 0,042 | 1,000 | -0,325 | 1,000 |
| | Age at vaccination | 0,132 | 1,000 | 0,370 | 0,146 |
| | Frailty index | -7,887 | 1,000 | -18,810 | 0,152 |
| | Age at vaccination*Frailty index | 0,106 | 1,000 | 0,251 | 0,151 |
| **19A** | Baseline OPA titer | 0,721 | **<0,0001** | 0,782 | **<0,0001** |
| | Sex (female) | -0,576 | 0,315 | -0,661 | 0,146 |
| | Age at vaccination | 0,055 | 1,000 | 0,046 | 1,000 |
| | Frailty index | -4,045 | 1,000 | -1,595 | 1,000 |
| | Age at vaccination*Frailty index | 0,058 | 1,000 | 0,025 | 1,000 |

Multivariable generalized linear models (GLMs) were used to evaluate the effects of sex, age at vaccination and frailty on OPA responses at four to six weeks (4-6w) and 1 year (1y) following PPV23 vaccination. Models were adjusted for baseline serotype-specific OPA titers. An interaction term between age at vaccination and frailty was included to examine their combined effect on post-vaccination OPA titers. Bonferroni corrections were applied to account for multiple testing. Significant effects (adjusted (adj.) $p < 0.05$) are indicated in bold.

**Supplementary Figure 2. Predicted persistence of ps-specific IgG concentrations following PPV23 vaccination over time for the remaining 18 serotypes.** Long-term persistence of ps-specific IgG concentrations following PPV23 vaccination were estimated for the whole cohort (n = 188) (**left**) and stratified by responder status (responder (n = 86) in blue; non-responder (n = 100) in pink) (**middle**) using a bi-exponential decay model under a Bayesian statistical framework**.** Individual IgG measurements are shown as dots and connected by grey (or light-colored) lines; if applicable, colors reflect responder status. The bold lines represents the modelled median IgG concentrations over time (in months), with its corresponding 95% credibility interval (light-colored ribbons). In panel A (the whole cohort), 50% and 80% credible intervals are also shown (blue ribbons). The dashed lines represent the 2.5th- and 97.5th-percentile of predicted IgG concentrations. The black horizontal line marks an IgG concentration of 1.3 µg/ml. Panels on the **right** shows the model-derived proportion of participants with IgG concentrations ≥1.3 µg/mL over time (in years), stratified by responder status, based on the predicted individual IgG decay trajectories.

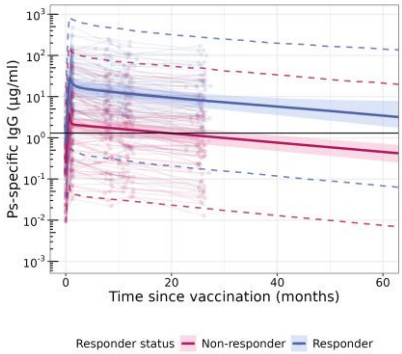


Serotype: Ps7F



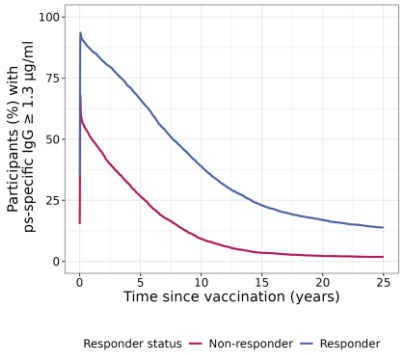

Modelled mean credibility level: 0.95 0.8 0.5

Responder status / Serotype: Ps7F




Responder status Non-responder Responder

Responder status / Serotype: Ps7F




Responder status Non-responder Responder

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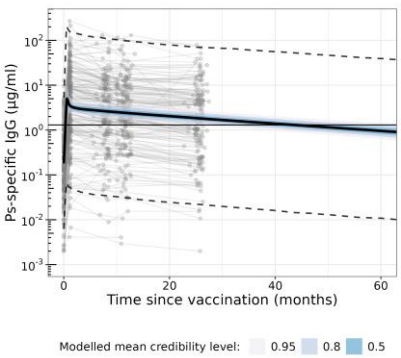


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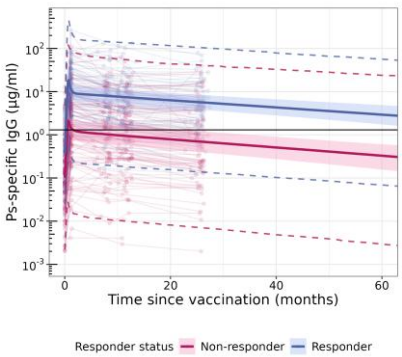


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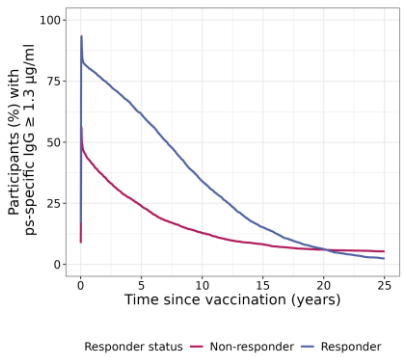


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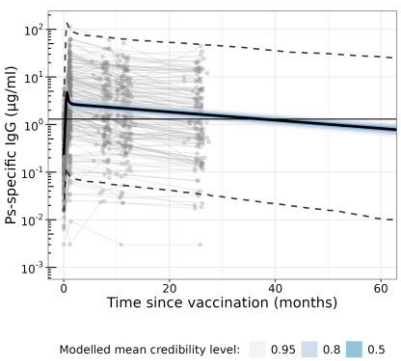


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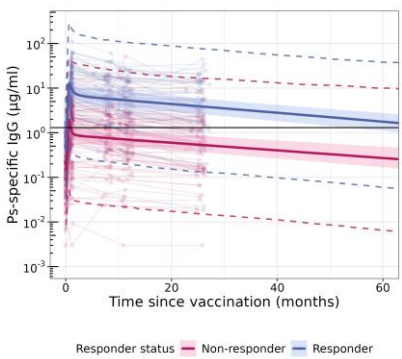


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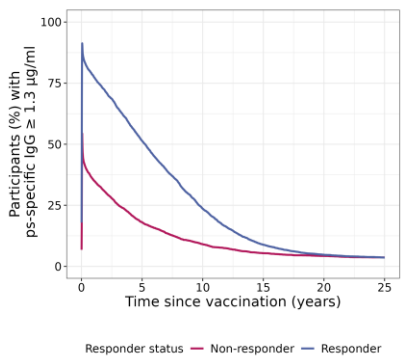


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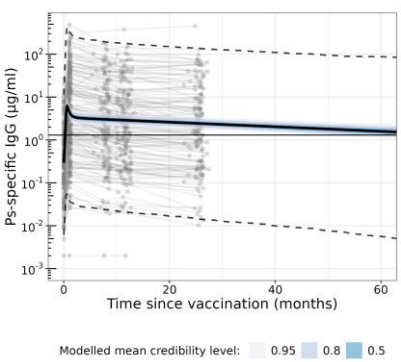


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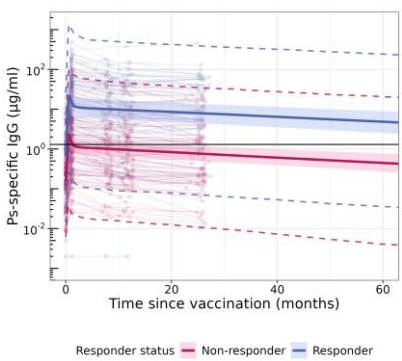


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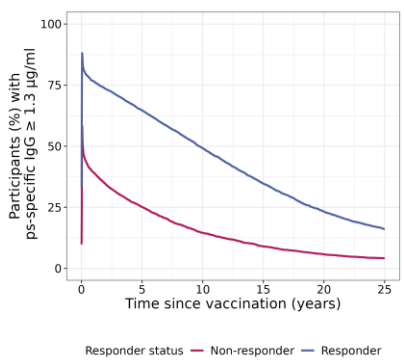


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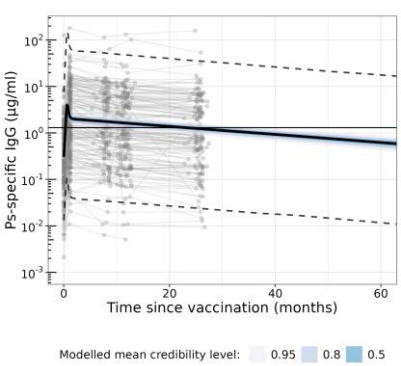


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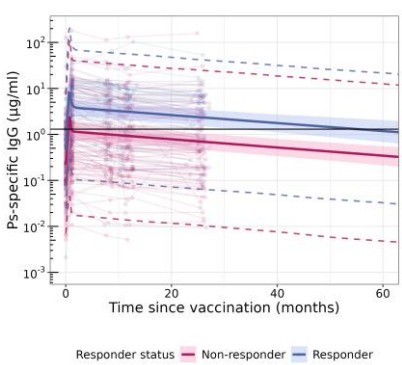


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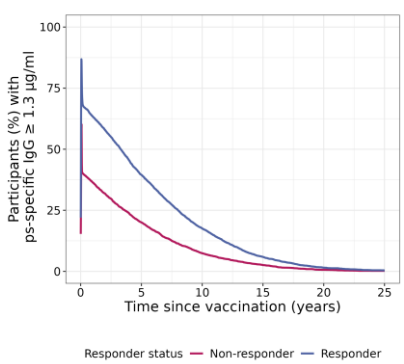


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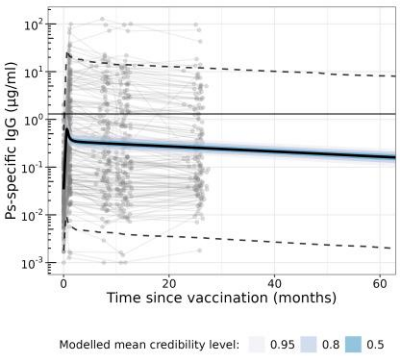


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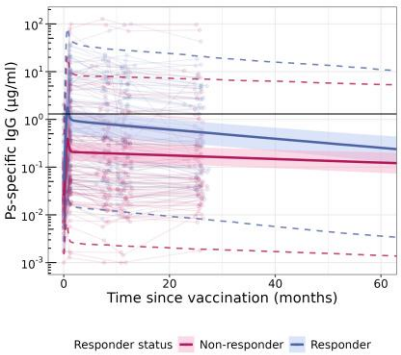


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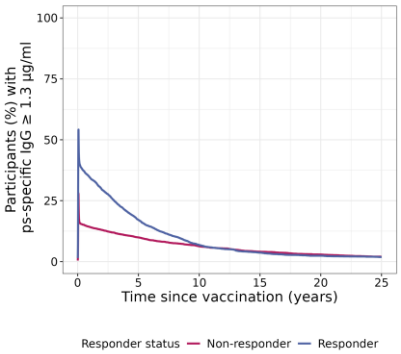


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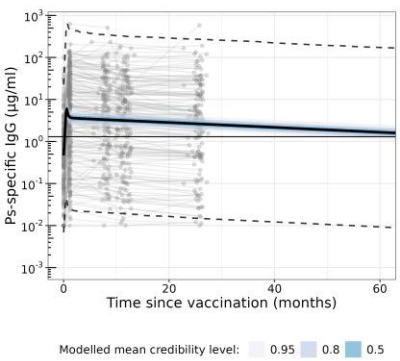


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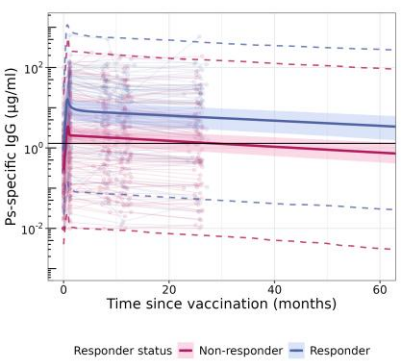


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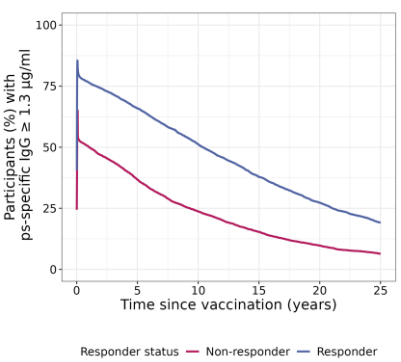


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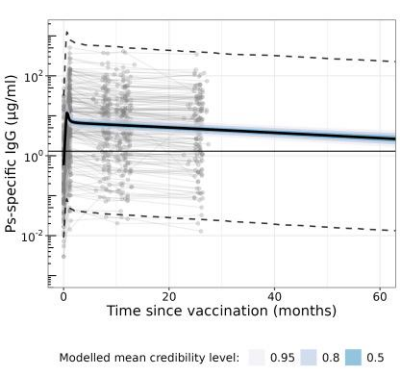


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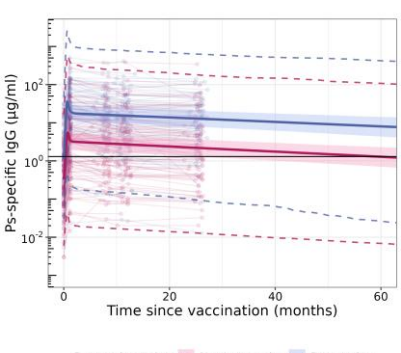


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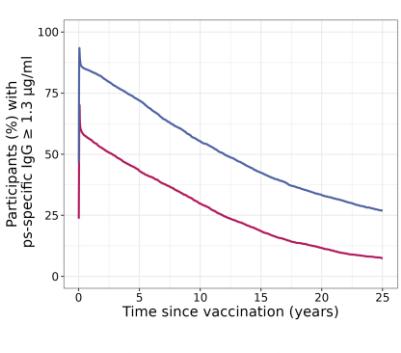


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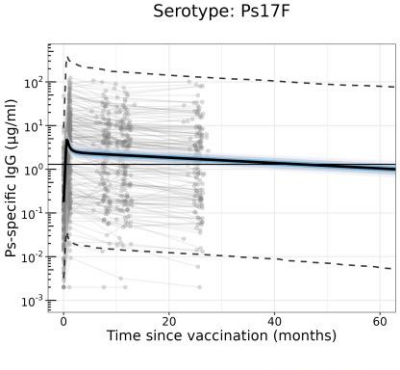


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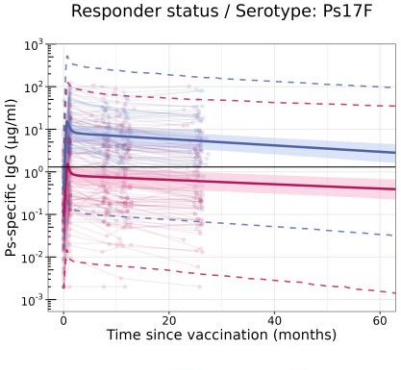


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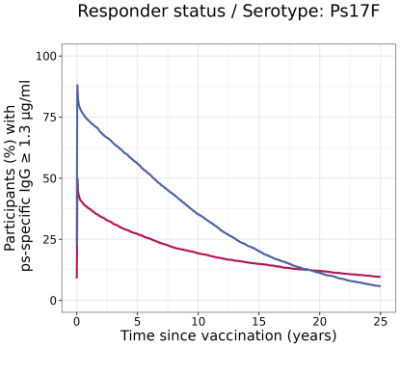


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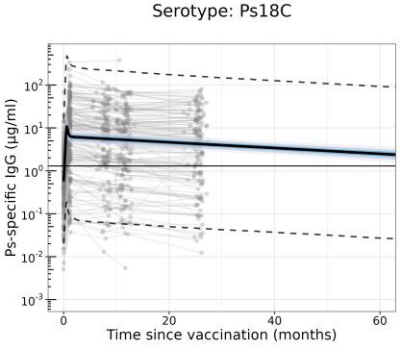


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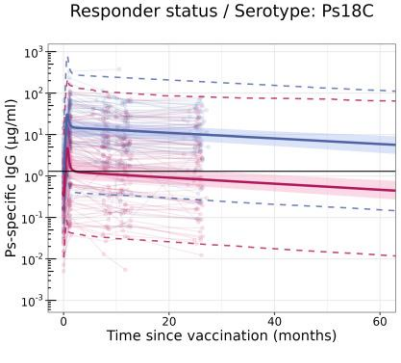


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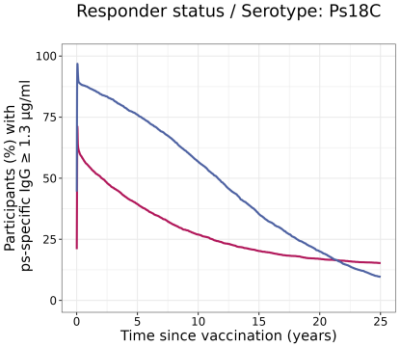


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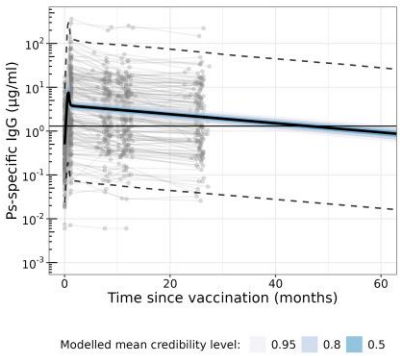


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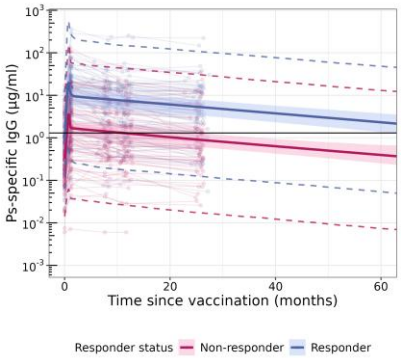


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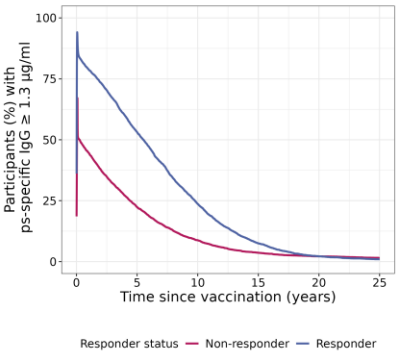


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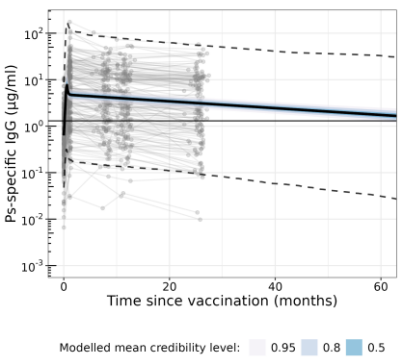


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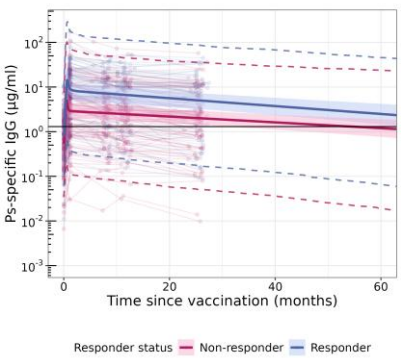


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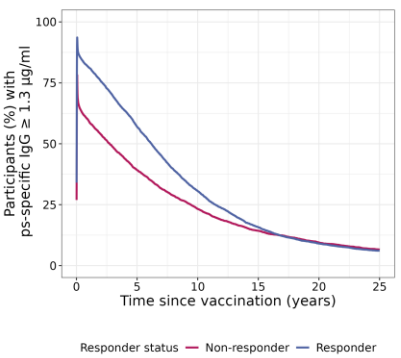


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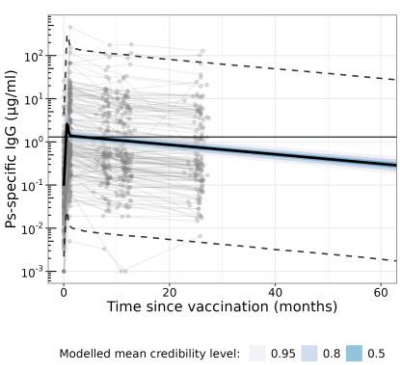


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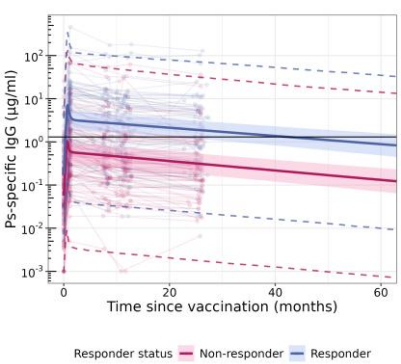


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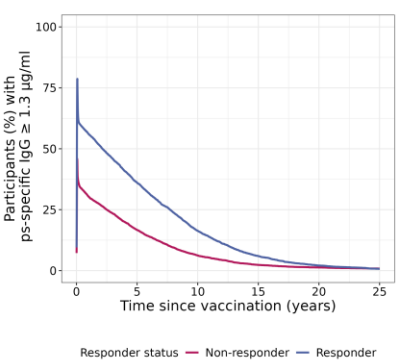


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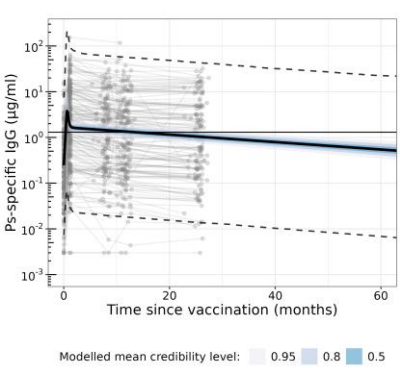


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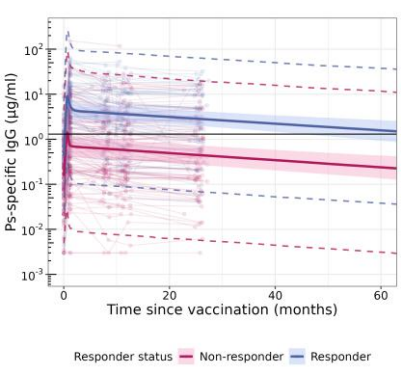


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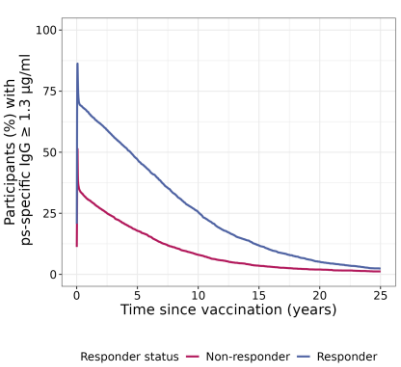


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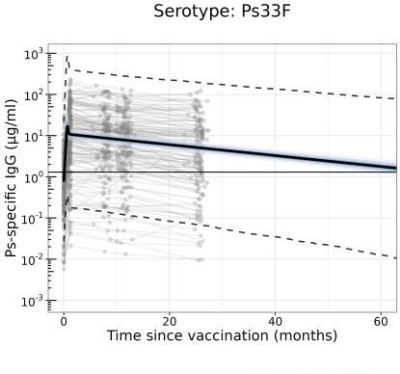


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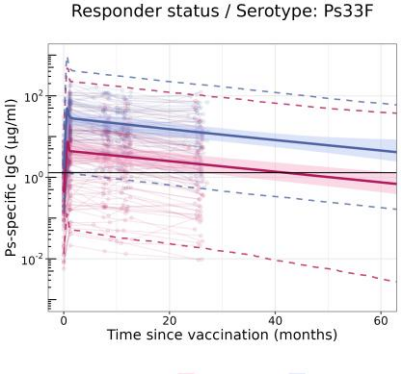


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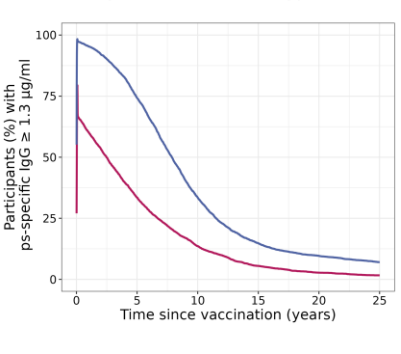
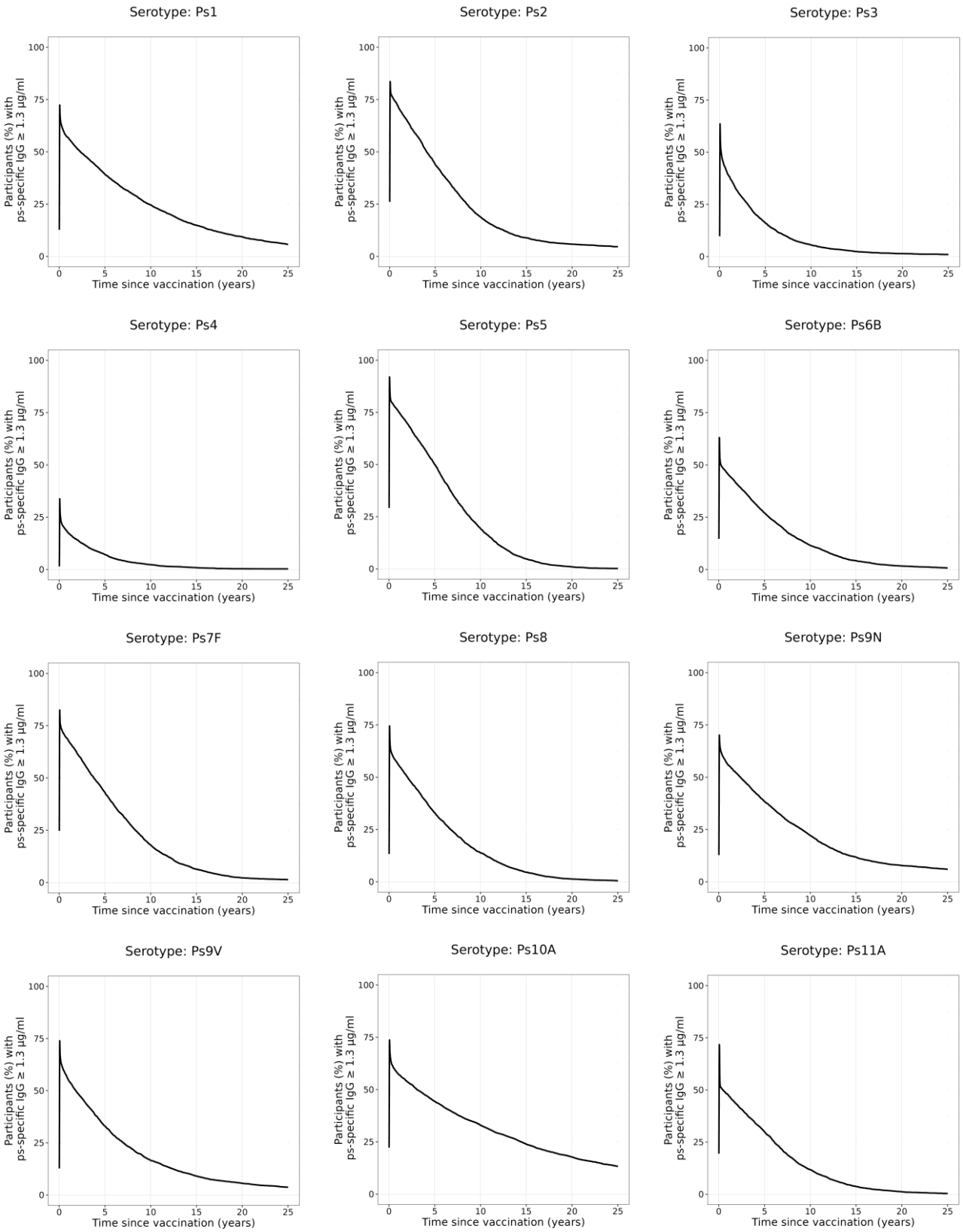



Responder status Non-responder Responder

**Supplementary Figure 3. Estimated proportion of participants in the total cohort with IgG concentrations ≥1.3 µg/mL over time (years) following PPV23 vaccination.** The long-term persistence of serotype-specific IgG concentrations above the threshold of ≥1.3 µg/mL was assessed for the entire cohort (n = 188) using a bi-exponential decay model within a Bayesian statistical framework.



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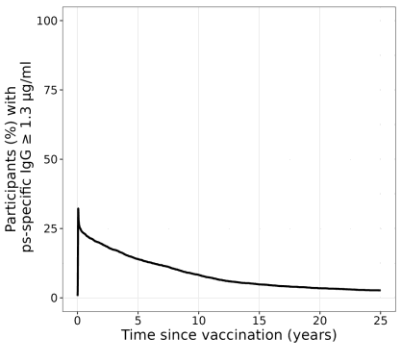


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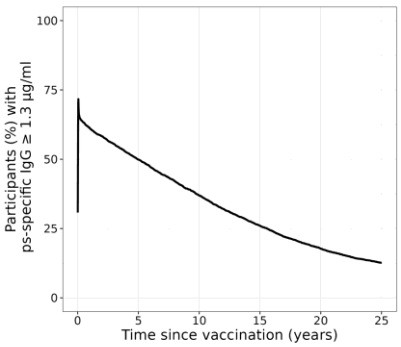


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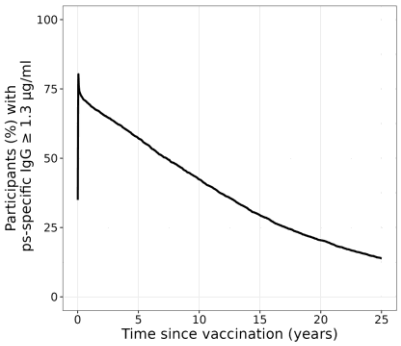


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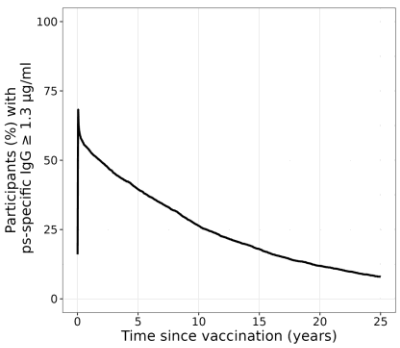


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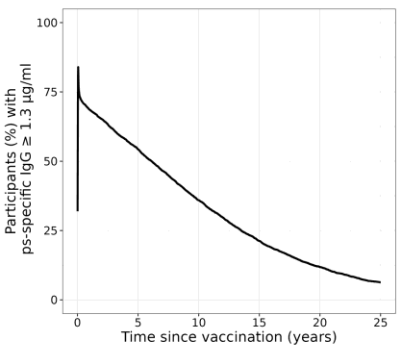


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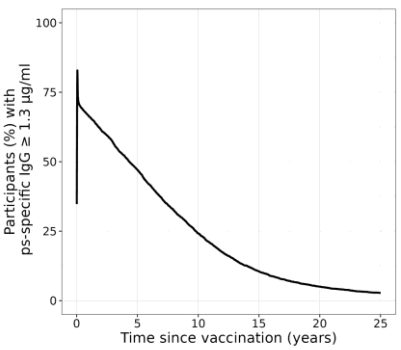


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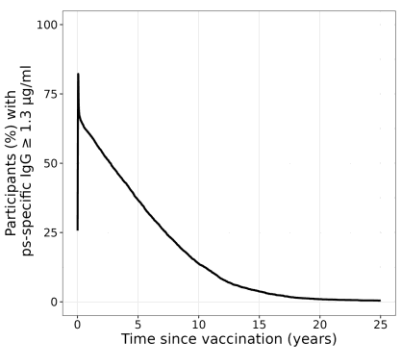


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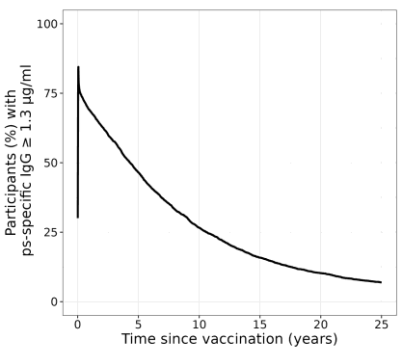


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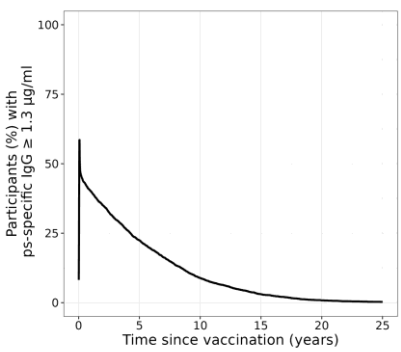


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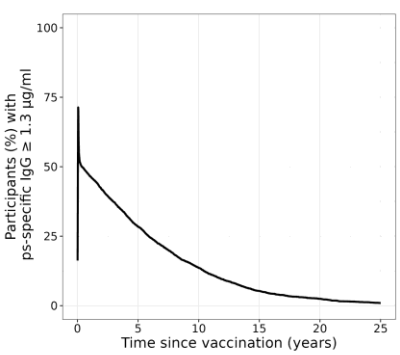
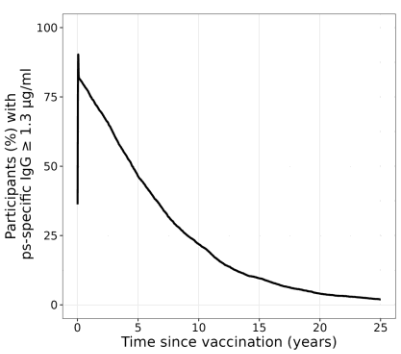
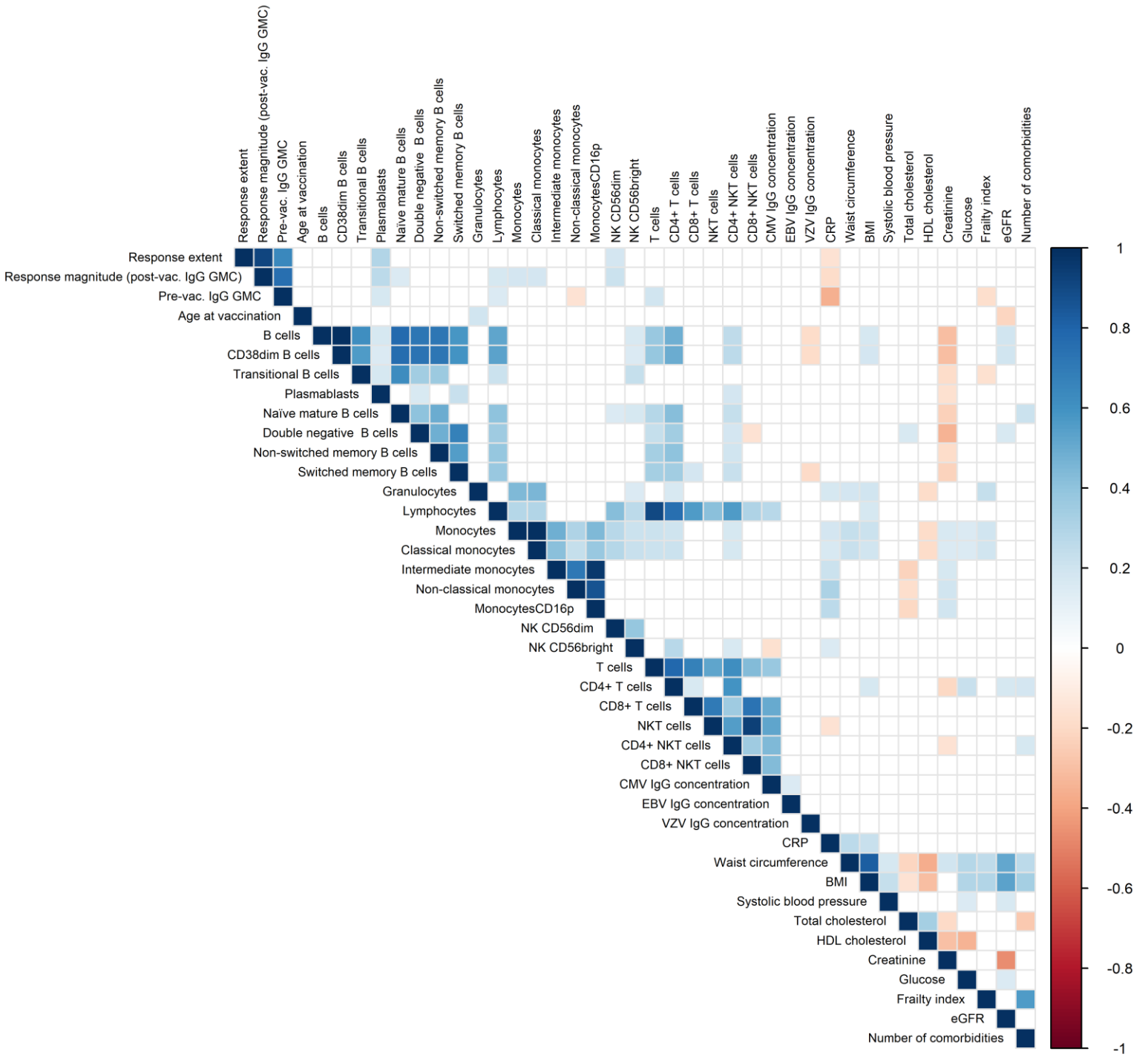



Serotype: Ps33F



**Supplementary Figure 4: Pairwise Spearman correlations between numeric baseline participant characteristics prior to PPV23 vaccination,** shown for individuals included in the elastic net regression analyses (n = 170). The color gradient reflects the strength and direction of the correlation, from negative (red) to positive (blue). Only statistically significant correlations ($p < 0.05$) are displayed; non-significant correlations are left blank. Included variables are demographic and anthropometric measures, clinical and chronic infection serology parameters, and absolute counts of immune cell subsets.



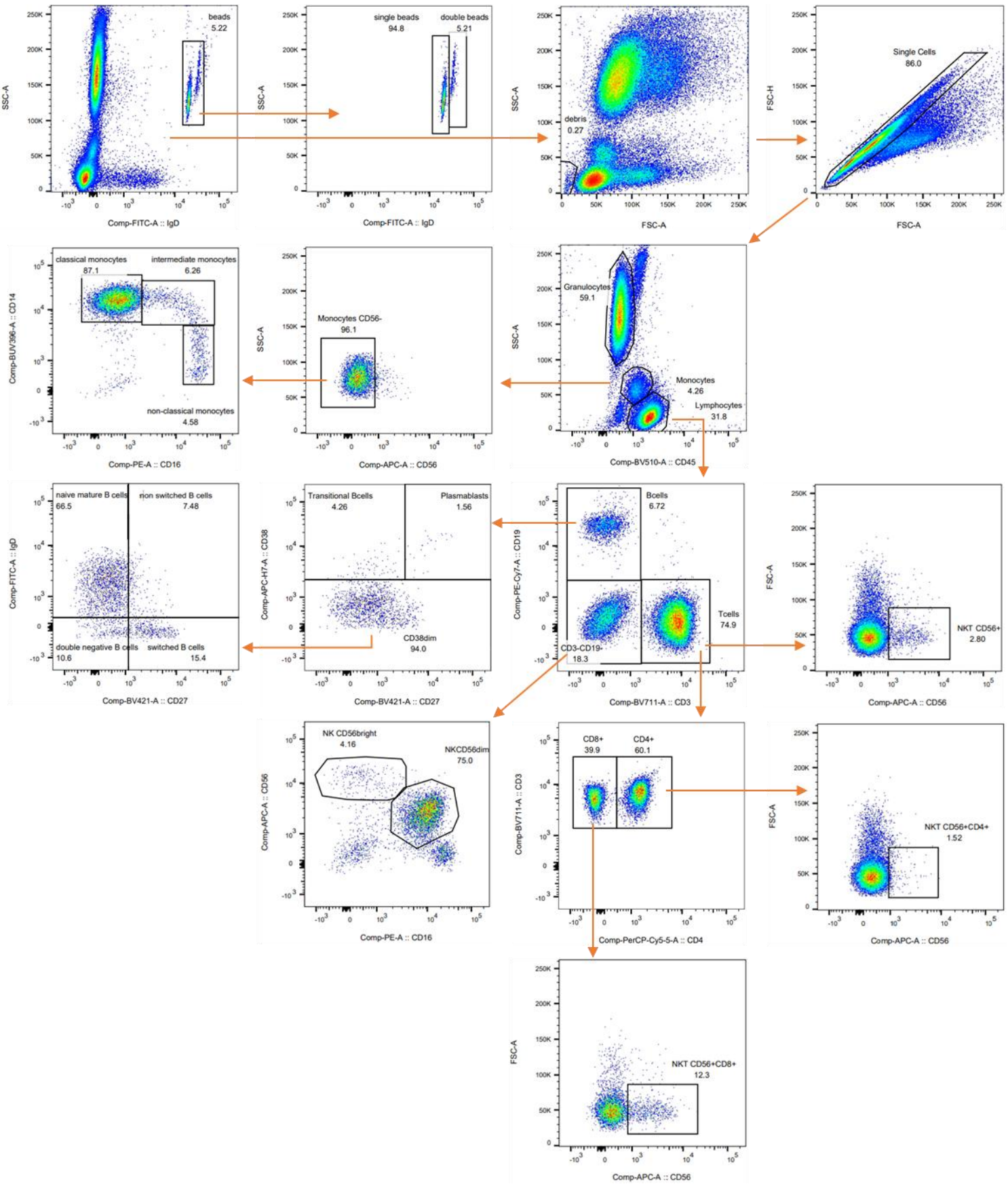
**Supplementary Table 8: Exclusion-, temporary exclusion- and withdrawal during study criteria.**

| **Exclusion criteria** |
| --- |
| • Previous pneumococcal vaccination;<br>• Known or suspected allergy to any of the vaccine components or having experienced a previous severe adverse reaction to any vaccine;<br>• Receipt of any high-dose (≥ 20 mg of prednisone daily or equivalent) daily corticosteroids (locally applied including inhaled steroids are acceptable) within 2 weeks of study entry, or, as medically prescribed, within two weeks after the vaccination;<br>• Repeated use of any high dose of corticosteroids (a dose of > 30 mg of prednisone or equivalent per day for multiple days) in the last month;<br>• Receipt of an organ- or bone marrow transplant during the last 5 years;<br>• Having an anatomical or functional asplenia;<br>• Receipt of blood products or immunoglobulin, within one month of study entry;<br>• Known or suspected coagulation disorder that in the judgement of the investigator would contraindicate against receiving an intramuscular injection or undergo frequent blood sampling;<br>• Known to be positive for human immunodeficiency virus (HIV), and/or hepatitis C virus (HCV) and/or hepatitis B virus (HBV) (presence of HBsAg or anti HBc antibodies).<br>• Start of a treatment with chemotherapy during the last three months; |
| **Temporary exclusion criteria** |
| • If a subject has an elevated body temperature fewer than 48 hours before a visit with blood sampling the visit should be postponed until this criterion is no longer met;<br>• Receipt of any other vaccine(s) 2 weeks before a blood collection. |
| **Withdrawal criteria during study** |
| • Receipt of any high-dose (≥ 20 mg of prednisone daily or equivalent) daily corticosteroids (local incl. inhaled steroids are acceptable) within 2 weeks of study entry, or, as medically prescribed, within two weeks after the vaccination;<br>• Receipt of blood products or immunoglobulins. |

**Supplementary Information 1: Sample size calculation**

The sample size for this study was determined based on the original primary objective: to compare pneumococcal vaccine-specific IgG responses between frail and non-frail older adults, and to assess the relationship between antibody responses and specific characteristics of frailty. Specifically, the calculation focused on differences in pneumococcal polysaccharide-specific serum IgG antibody concentrations measured four to six weeks after administration of the 23-valent pneumococcal polysaccharide vaccine (PPV23). We hypothesized a two-fold difference in geometric mean IgG concentrations (GMC ratio = 2) between frail and non-frail participants. Based on data from the CAPiTA study involving individuals aged 65 years and older (Van Deursen et al., Clinical Infectious Diseases, 2017), we assumed a standard deviation of 1.5 on the natural logarithmic scale. Under these assumptions, a total sample size of 150 participants (75 per group) was required to detect the specified difference with 80% power and a two-sided significance level ($\alpha = 0.05$). This sample size ensures adequate power to detect clinically meaningful differences in immune response to PPV23 between frail and non-frail groups in the older adult population.

**Supplementary Figure 5. TruCount gating strategy.**



**Supplementary Table 9. Pneumococcal serotype polysaccharide-specific IgM assignments in the international pneumococcal standard reference serum 007sp.**

| Serotype | Reference | |
|---|---|---|
| | lot 89S (µg/ml) | 007sp (µg/ml) |
| 2 | 5.1 | 7.5 |
| 8 | 2.0 | 6.2 |
| 9N | 2.4 | 4.9 |
| 10A | 3.9 | 6.4 |
| 11A | 1.0 | 2.7 |
| 12F | 1.4 | 8.1 |
| 15B | 1.6 | 2.8 |
| 17 | 1.7 | 3.4 |
| 20 | 1.7 | 6.9 |
| 22F | 3.1 | 5.4 |
| 33F | 2.6 | 12.3 |

Polysaccharide-specific IgM concentrations for pneumococcal serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17, 20, 22F, and 33F were assigned in the international pneumococcal standard reference serum 007sp. These assignments were established by bridging from previously assigned IgM concentrations in standard reference serum lot 89S.

IgM concentrations for the remaining serotypes were published by us previously :

*Visser, M., van Beek, J., Tcherniaeva, I., van Rooijen, D. M., Beckers, L., Bijvank, E., de Jonge, M. I., Lockhart, S. P., Pride, M. W., Rots, N., van Baarle, D., den Hartog, G., & Buisman, A. M. (2025). Age-related decline in IgM responses associate with reduced opsonophagocytic activity following PCV13 vaccination. NPJ Vaccines, 10(1), 95. https://doi.org/10.1038/s41541-025-01152-7*