

Supplementary Material

Intramuscular vaccination against SARS-CoV-2 transiently induces neutralizing IgG rather than IgA in the saliva

Stephan Winklmeier^{1,2}, Heike Rübsamen^{1,2}, Ceren Özdemir^{1,2}, Paul R. Wratil^{3,4}, Gaia Lupoli³, Marcel Stern³, Celine Schneider^{1,2}, Katharina Eisenhut^{1,2}, Samantha Ho^{1,2}, Hoi Kiu Wong^{1,2}, Damla Taskin^{1,2}, Marvin Petry^{1,2}, Michael Weigand⁵, Peter Eichhorn⁵, Bärbel U. Foessel⁶, Simone Mader^{1,2}, Oliver T. Keppler^{3,4}, Tania Kümpfel^{1,2}, Edgar Meinl^{1,2*}

¹Institute of Clinical Neuroimmunology, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany.

²Biomedical Center (BMC), Medical Faculty, Ludwig-Maximilians-Universität München, Martinsried, Germany.

³Max von Pettenkofer Institute & GeneCenter, Virology, Ludwig-Maximilians-Universität München, Munich, Germany.

⁴ German Center for Infection Research (DZIF), partner site Munich, Munich, Germany.

⁵Institute of Laboratory Medicine, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany.

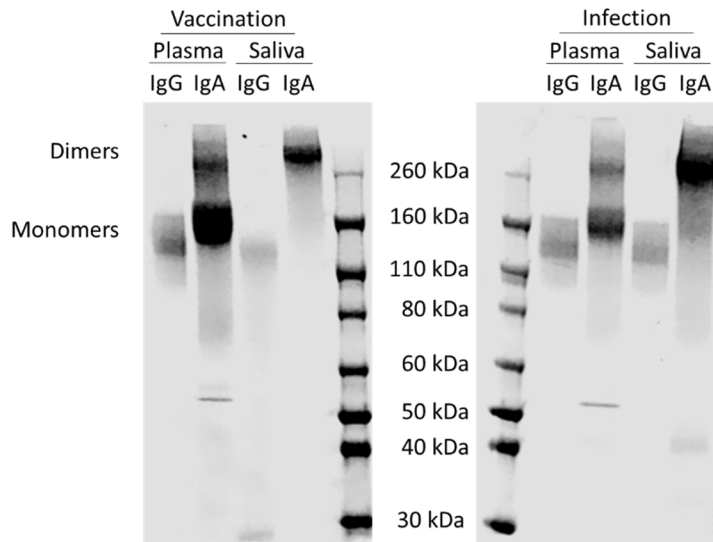
⁶Institute of Epidemiology, Helmholtz Munich, Neuherberg, Germany.

*** Correspondence:**

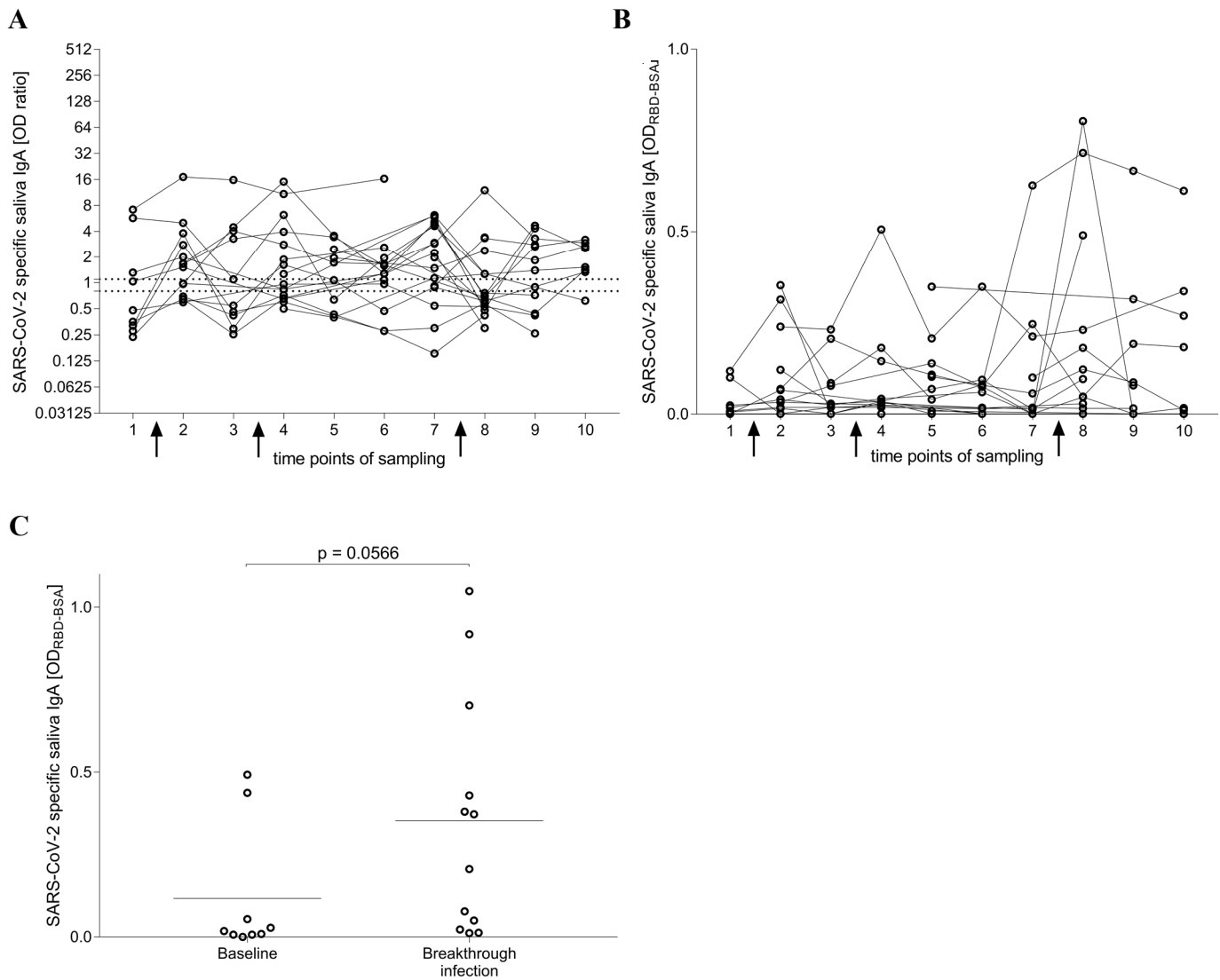
Dr Edgar Meinl

edgar.meinl@med.uni-muenchen.de

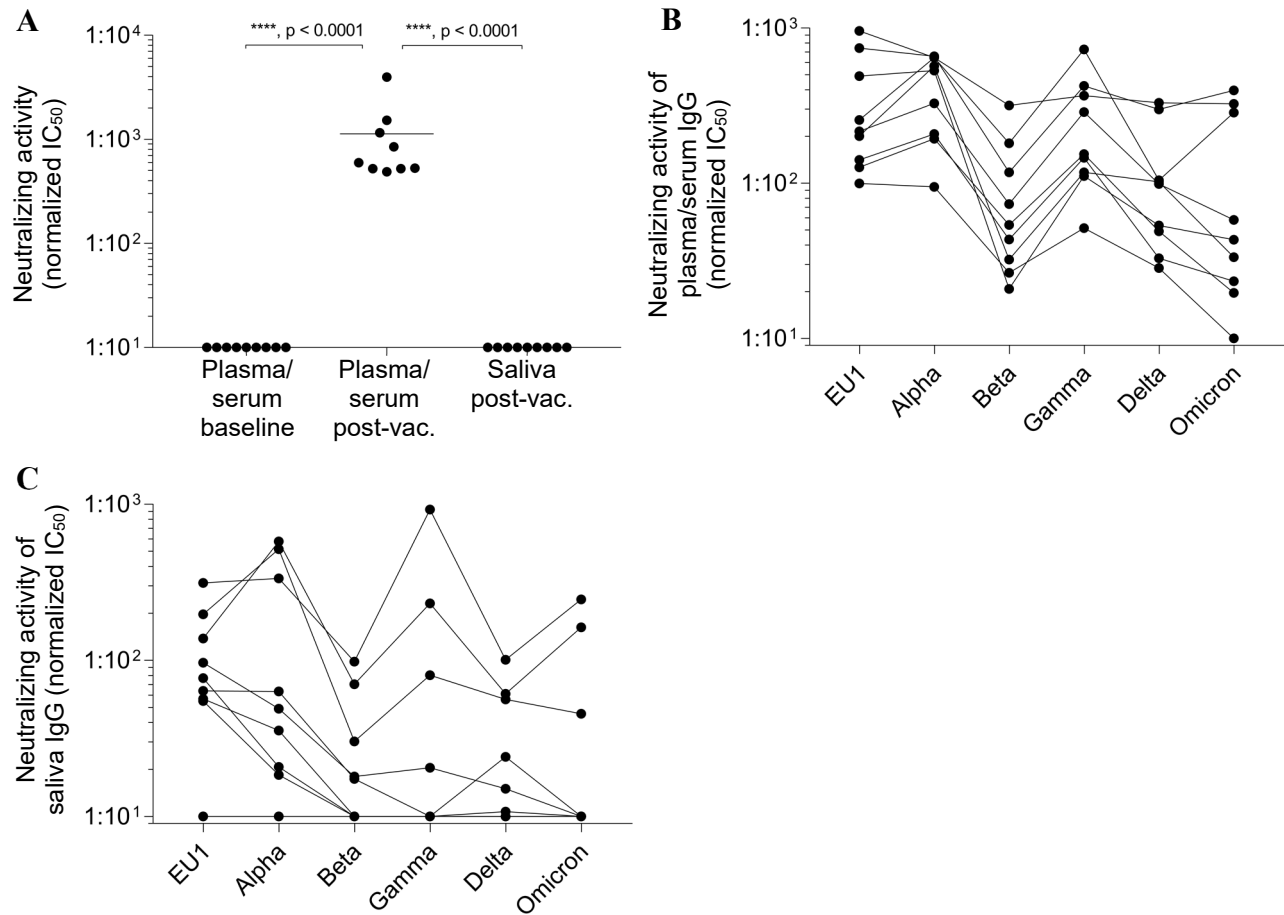
1 Supplementary Figures



Supplementary Figure 1. Analysis of monomeric or dimeric IgA and IgG in blood and saliva. Plasma- and saliva-derived and purified IgG and IgA (obtained after the third vaccination (left) or after an additional breakthrough infection (right)) were separated by non-reducing SDS-PAGE gel and stained by Coomassie Blue.



Supplementary Figure 2. Analysis of SARS-CoV-2 specific IgA in the saliva by ELISA. IgA response to the S1 domain or RBD WT of the SARS-CoV-2 spike protein after BNT vaccination or breakthrough infection obtained by the EUROIMMUN ELISA kit (**A**) or an in-house ELISA following a previous publication (1) (**B** and **C**). (**A** and **B**) Longitudinal reactivity of specific IgA in saliva. Arrows indicate vaccination time points one, two and three. Connection between dots refer to the response within one donor. Time points of sampling: 1 = before first vaccination ($n_A = 9$, $n_B = 9$); 2 = two weeks after first vaccination ($n_A = 14$, $n_B = 14$); 3 = before second vaccination ($n_A = 10$, $n_B = 10$); 4 = two weeks after second vaccination ($n_A = 16$, $n_B = 11$); 5 = six weeks after second vaccination ($n_A = 9$, $n_B = 9$); 6 = three months after second vaccination ($n_A = 13$, $n_B = 13$); 7 = six months after second vaccination ($n_A = 17$, $n_B = 13$); 8 = two weeks after third vaccination ($n_A = 17$, $n_B = 12$); 9 = three months after third vaccination ($n_A = 12$, $n_B = 9$); 10 = six months after third vaccination ($n_A = 8$, $n_B = 8$). The area between the two horizontal dotted lines in (**A**) were considered to represent the borderline zone of reactivity (EUROIMMUN). For comparison, we included these lines for serum (**Figure 1C**) also here in the graph of saliva IgA. (**C**) Detection of SARS-CoV-2 RBD-specific IgA in saliva from baseline ($n = 9$) and after breakthrough infection ($n = 12$) from three times vaccinated participants. Horizontal lines indicate the mean levels of all donors in the respective groups ($p = 0.0566$, Mann-Whitney U test).



Supplementary Figure 3. Analysis of neutralizing activity against variants of concern of SARS-CoV-2. Neutralizing activity before (A) and after the third vaccination (post-vac.; A, B and C). Samples were collected within one month after the third BNT vaccination. S3 neutralization assay was performed as described in materials and methods. (A) The neutralizing activity is displayed as IC₅₀ normalized to 10⁷ viral RNA copies against the EU1/ B.1.177 variant of SARS-CoV-2. The neutralizing activity before Ig purification is shown for baseline plasma/serum, plasma/serum and saliva after the third vaccination (n = 9; $p < 0.0001$, one-way ANOVA, Tukey's multiple comparison test). Each dot represents one donor. Horizontal lines indicate the mean levels of all donors in the respective groups. (B) depicts the neutralizing activity of plasma/serum-derived IgG (n = 9) and (C) of saliva-derived IgG (n = 9) after the third vaccination against different SARS-CoV-2 VoCs (EU1/B.1.177, Alpha/B.1.1.7, Beta/B.1.351, Gamma/P.1/B.1.1.28.1, Delta/B.1.617.2, Omicron/B.1.1.529 sublineage BA.1).

2 References

1. Darwich A, Pozzi C, Fornasa G, Lizier M, Azzolini E, Spadoni I, et al. BNT162b2 vaccine induces antibody release in saliva: a possible role for mucosal viral protection? *EMBO Mol Med.* 2022;14(5):e15326.