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Supplemental information

Strong humoral immune responses against SARS-CoV-2 Spike after BNT162b2 mRNA vaccination with a 16-week interval between doses

Alexandra Tauzin, Shang Yu Gong, Guillaume Beaudoin-Bussières, Dani Vézina, Romain Gasser, Lauriane Nault, Lorie Marchitto, Mehdi Benlarbi, Debashree Chatterjee, Manon Nayrac, Annemarie Laumaea, Jérémie Prévost, Marianne Boutin, Gérémy Sannier, Alexandre Nicolas, Catherine Bourassa, Gabrielle Gendron-Lepage, Halima Medjahed, Guillaume Goyette, Yuxia Bo, Josée Perreault, Laurie Gokool, Chantal Morrisseau, Pascale Arlotto, Renée Bazin, Mathieu Dubé, Gaston De Serres, Nicholas Brousseau, Jonathan Richard, Roberta Rovito, Marceline Côté, Cécile Tremblay, Giulia C. Marchetti, Ralf Duerr, Valérie Martel-Laferrière, Daniel E. Kaufmann, and Andrés Finzi

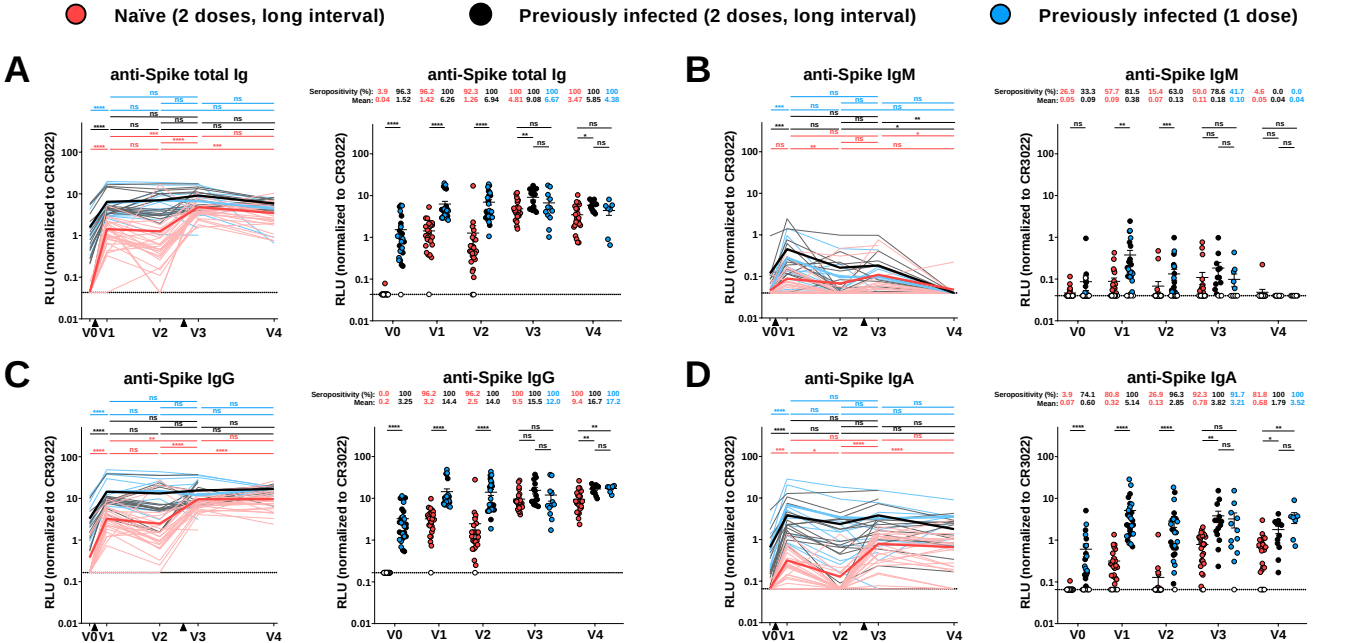

Figure S1 : Elicitation of Spike-specific antibodies in SARS-CoV-2 naïve and previously-infected individuals, Related to Figures 1 and 6.
(**A-D**) Cell-based ELISA was performed by incubating plasma samples from naïve and PI donors collected at V0, V1, V2, V3 and V4 with HOS cells expressing full-length SARS-CoV-2 S. Anti-S Ab binding was detected using HRP-conjugated (**A**) anti-human IgM+IgG+IgA (**B**) anti-human IgM, (**C**) anti-human IgG, or (**D**) anti-human IgA. RLU values obtained with parental HOS (negative control) were subtracted and further normalized to the signal obtained with the CR3022 mAb present in each plate. Naïve and PI donors with a long interval between the two doses are represented by red and black points respectively and PI donors who received just one dose by blue points. (Left panels) Each curve represents the normalized RLUs obtained with the plasma of one donor at every time point. Mean of each group is represented by a bold line. The time of vaccine dose injections is indicated by black triangles. (Right panels) Plasma samples were grouped in different time points (V0, V1, V2, V3 and V4). Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. (* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns, non-significant). For naïve donors, n=26 at V0, V1, V2 and V3 and n=22 at V4. For previously infected donors vaccinated with two doses, n=15 at V0, V1, V2 and V3 and n=12 at V4. For previously infected donors vaccinated with one dose, n=12 at V0, V1, V2 and V3 and n=7 at V4.

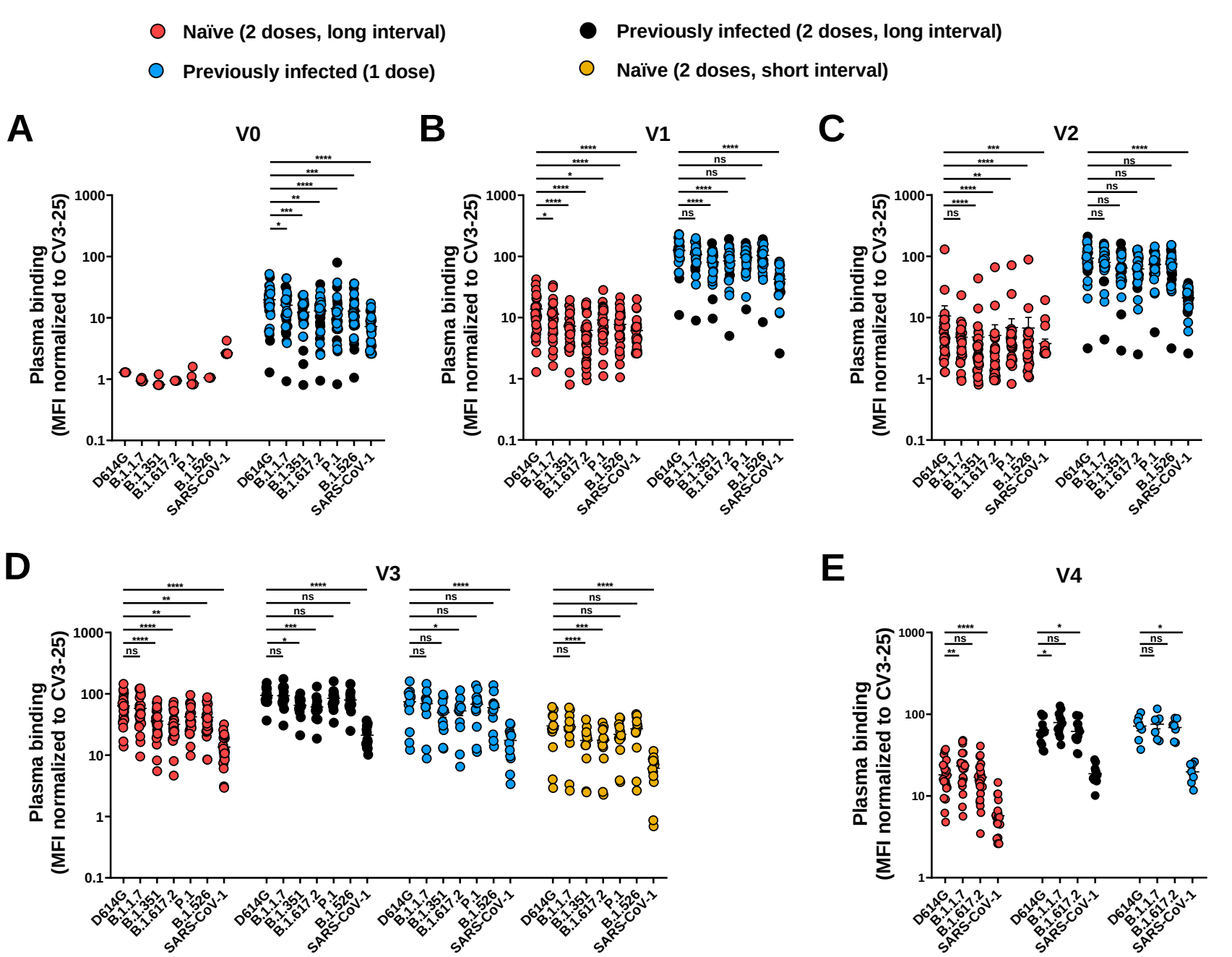

Figure S2 : Recognition of SARS-CoV-2 Spike variants and SARS-CoV-1 Spike by plasma from naïve and PI donors at each time point, Related to Figures 2, 5 and 6.

293T cells were transfected with the indicated *Betacoronavirus* Spike and stained with the CV3-25 Ab or with plasma collected at V0 (**A**), V1 (**B**), V2 (**C**), V3 (**D**) and V4 (**E**) and analyzed by flow cytometry. Plasma recognitions are normalized with CV3-25 binding. Naïve and PI donors with a long interval between the two doses are represented by red and black points respectively, PI donors who received just one dose by blue points and naïve donors with a short interval between the two doses by yellow points. Error bars indicate means ± SEM. (* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns, non-significant). For naïve donors vaccinated withe the long interval, n=26 at V0, V1, V2 and V3 and n=22 at V4. For naïve donors vaccinated withe the short interval, n=12. For previously infected donors vaccinated with two doses, n=15 at V0, V1, V2 and V3 and n=12 at V4. For previously infected donors vaccinated with one dose, n=12 at V0, V1, V2 and V3 and n=7 at V4.

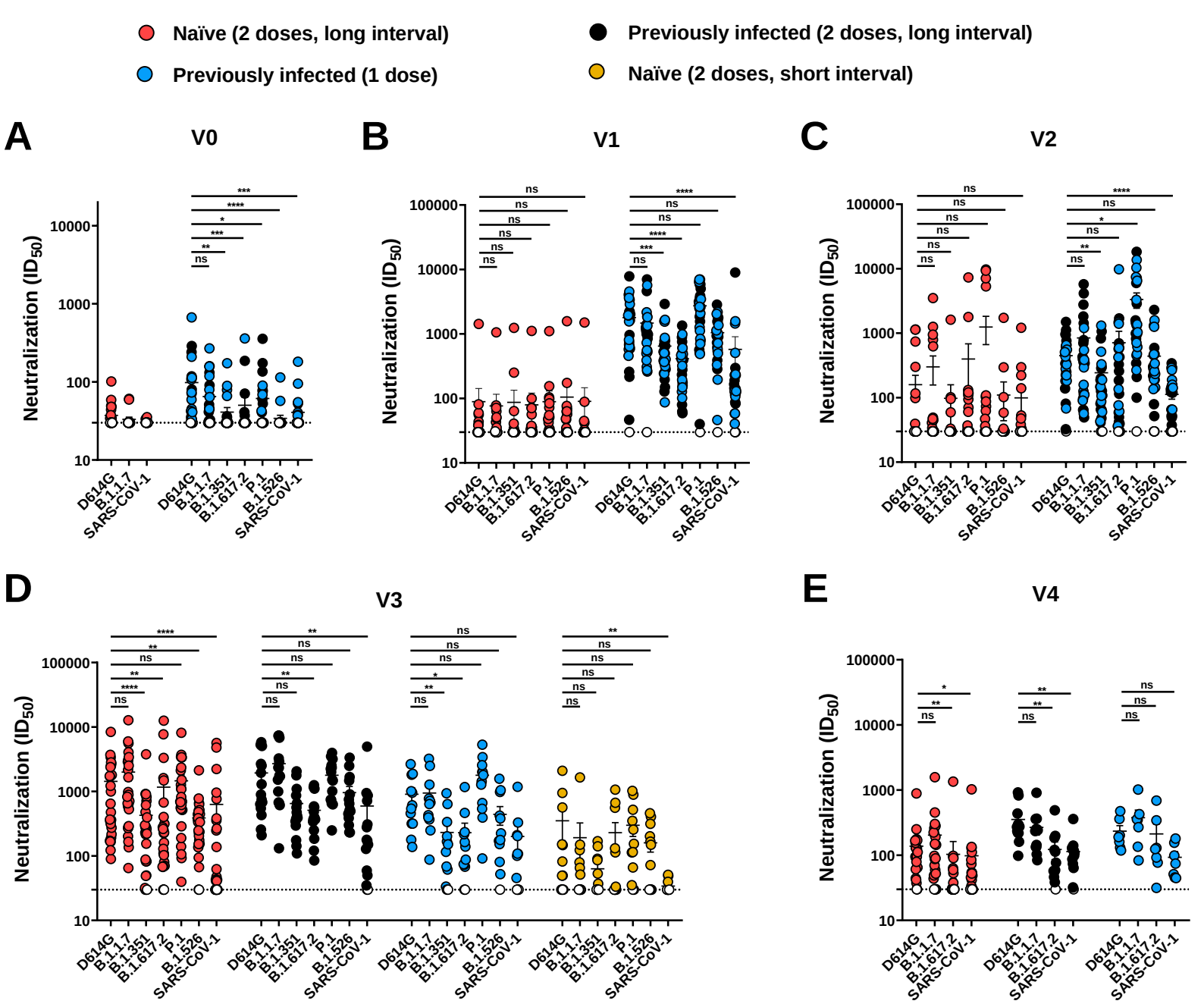

Figure S3 : Neutralization of SARS-CoV-2 Spike variants and SARS-CoV-1 Spike by plasma from naïve and PI donors at each time point, Related to Figures 3, 5 and 6.

Neutralizing activity was measured by incubating pseudoviruses bearing SARS-CoV-2 S variant or SARS-CoV-1 S glycoproteins, with serial dilutions of plasma collected at V0 (**A**), V1 (**B**), V2 (**C**), V3 (**D**) and V4 (**E**) for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID50) values were determined using a normalized non-linear regression using GraphPad Prism software. Naïve and PI donors with a long interval between the two doses are represented by red and black points respectively, PI donors who received just one dose by blue points and naïve donors with a short interval between the two doses by yellow points. Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. (* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns, non-significant). For naïve donors vaccinated withe the long interval, n=26 at V0, V1, V2 and V3 and n=22 at V4. For naïve donors vaccinated withe the short interval, n=12. For previously infected donors vaccinated with two doses, n=15 at V0, V1, V2 and V3 and n=12 at V4. For previously infected donors vaccinated with one dose, n=12 at V0, V1, V2 and V3 and n=7 at V4.

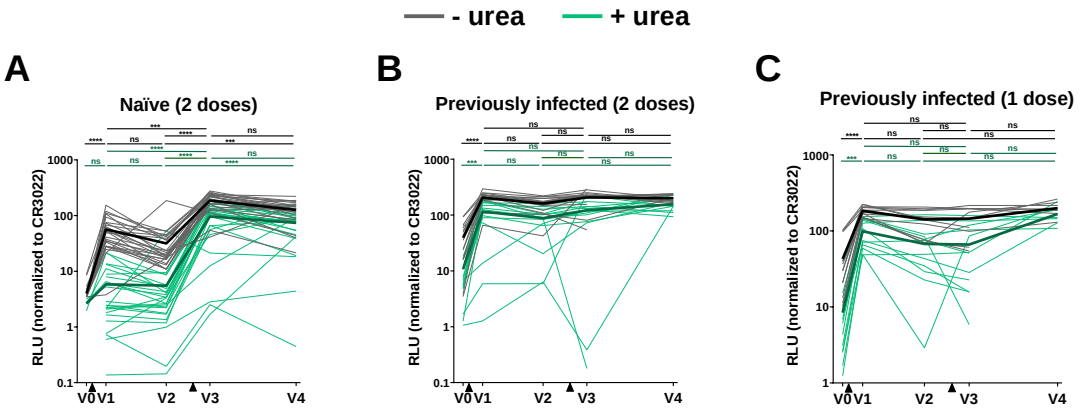

Figure S4 : Comparison of the detection of RBD specific antibodies between ELISA and stringent ELISA in SARS-CoV-2 naïve and previously infected individuals, Related to Figure 4.

(**A-C**) Indirect ELISA was performed by incubating plasma samples from naïve (**A**) PI vaccinated with two (**B**) or one dose (**C**) donors collected at V0, V1, V2, V3 and V4 with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. Relative light unit (RLU) values obtained were normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate. For ELISA (black curves), all the wash steps were made with washing buffer and for stringent ELISA (green curves), the wash steps were made with 8M of urea. Each curve represents the normalized RLUs obtained with the plasma of one donor at every time point. Mean of each group is represented by a bold line. The time of vaccine dose injections is indicated by black triangles. Error bars indicate means ± SEM. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, non-significant). For naïve donors vaccinated withe the long interval, n=26 at V0, V1, V2 and V3 and n=22 at V4. For previously infected donors vaccinated with two doses, n=15 at V0, V1, V2 and V3 and n=12 at V4. For previously infected donors vaccinated with one dose, n=12 at V0, V1, V2 and V3 and n=7 at V4.