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Supplementary appendix

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Supplementary Appendix

Antibody Durability at One Year after Sputnik V Vaccination: antibody levels, neutralizing capacity and Omicron escape

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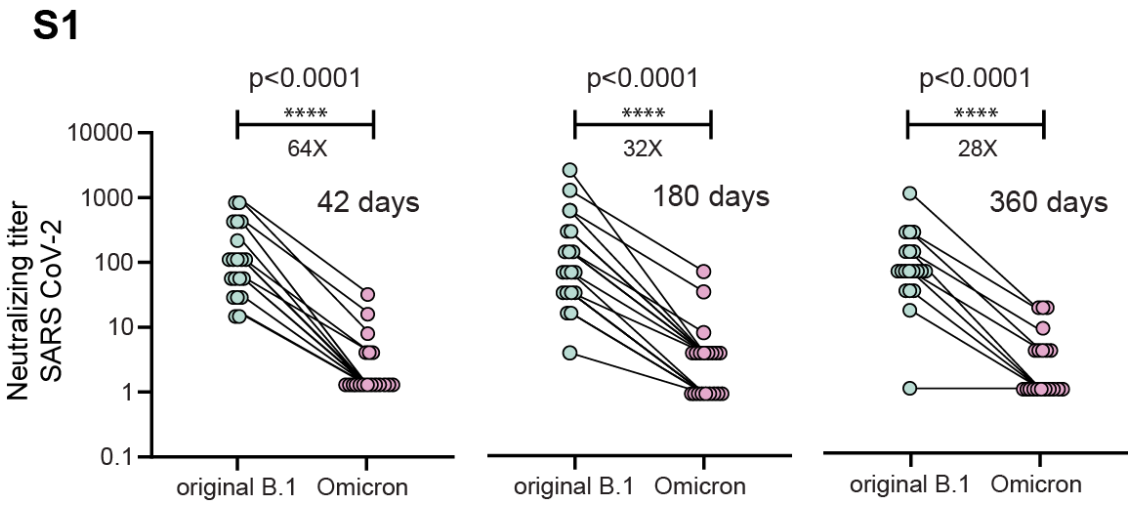

Figure S1. Neutralizing titers of serum samples against the original B.1 and local omicron isolates over time after Sputnik V vaccination. Wilcoxon matched-pair test was used. Statistical significance (****; $P < 0.0001$).

S2. METHODS

General description of the study and cohort: we monitored the humoral immune response over time post immunization with Sputnik V vaccine in 100 health care workers from Buenos Aires province, Argentina. Vaccination included the administration of the heterlogous two-dose regime. The second dose was applied 21 days after the first dose. The cohort included 39% male and 61% female participants, with an average age of 53 years (range, 22 to 85 years). Information about ethnicity was not collected.

Blood was collected by venipuncture into SST tubes (BD Sciences) for serum and stored at -20º C. Study enrollment started in January 2021 and sequential serum samples were collected at

four time points: before vaccination (baseline) and at 42, 180 and 360 days after the initial vaccination. Volunteers showed no evidence of SARS-CoV-2 infection at baseline. Presence of anti-nucleocapsid IgG antibodies was tested to identify possible infections during the study. Thus, we excluded 1 volunteer that tested positive for nucleocapsid.

Ethical approval was obtained from the central committee of the Ministry of Health of Buenos Aires, and all participants provided written informed consent prior to collection of data and specimens (Cod#2021-00983502). All specimens were de-identified prior to processing and antibody testing for all serum specimens.

Cell lines: Vero-CCL81 cells (ATCC) and 293T ACE2/TMPRSS2 cells (kindly provided by Benhur Lee) were used. Cells were cultured at 37°C in 5% CO 2 in Dulbecco's modified Eagle's high-glucose medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (GIBCO).

SARS-CoV-2 pseudotyped VSV: CoV2pp-GFP virus stocks carrying vesicular stomatitis virus as the viral backbone where the glycoprotein gene (G) was replaced with the full-length wild-type spike protein of original Wuhan SARS-CoV-2 (VSV-eGFP-SARS-CoV-2) were provided by Dr. Sean Whelan. CoV2pp-GFP stocks were amplified in our laboratory using 293T ACE2/TMPRSS2 cells at an MOI of 0.01 in Dulbecco's modified Eagle's medium containing 2% FBS at 37°C. Viral supernatants were harvested upon extensive CPE and GFP-positive cells. The medium was clarified by centrifugation at 1,000 g for 5 min. Viral stocks were titrated by fluorescence-forming units per milliliter (FFU/ml) in the Vero cell line. Aliquots were maintained at -80°C.

SARS-CoV-2 original B.1 lineage and Omicron variant: SARS-CoV-2 ancestral reference strain 2019 (GISAID accession ID EPI_ISL_499083) B.1 was obtained from Sandra Gallegos (InViV working group). Omicron variant was isolated from an Argentinean patient sample and the genome was completely sequenced. It belongs to the BA.1 PANGO lineage (GISAID accession ID EPI_ISL_10633761). Viruses were amplified in Vero E6 cells, and stock identity was confirmed by whole-genome sequencing in an Illumina sequencer. Nucleic acid sequence for each viral stock was uploaded to GISAID and completely matched reference sequences for each variant, discarding acquisition of mutations during isolation and amplification processes. Work with SARS-CoV-2 was approved by the INBIRS Institutional Biosafety Committee at biosafety level 3 with negative pressure.

SARS-CoV-2 antibody ELISA: Antibodies to SARS-CoV-2 spike protein were detected using an established, commercially available, two-step ELISA (COVIDAR). Briefly, the assay uses plates coated with a mixture of spike and the receptor binding domain (RBD). The conjugated

monoclonal antibody used for human IgG detection in the COVIDAR ELISA is G18-145, which specifically binds to the heavy chain of all four human immunoglobulin G subclasses: IgG1, IgG2, IgG3, and IgG4. The IgG concentration of each sample, expressed in international units per milliliter was calculated by extrapolation of the optical density at 450 nm (OD450) on a calibration curve built using serial dilutions of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Antibodies to SARS-CoV-2 nucleocapsid protein were detected using a two-step ELISA test.

SARS-CoV-2 spike pseudotyped VSV neutralization assay: neutralization assays were carried out using SARS-CoV-2 pseudotyped particles, provided by Dr. Sean Whelan. First, Vero cells maintained with Dulbecco's modified Eagle's medium (DMEM) high glucose with 10% FBS were seeded in a 96-well plate the day before infection. Patient sera were heat inactivated at 56°C for 30 min and serially diluted in DMEM high-glucose medium. Serum neutralizations were performed by first diluting the inactivated sample 2-fold and continuing with a 2-fold serial dilution. A pre-titrated amount of pseudotyped particles was incubated with a 2-fold serial dilution of patient sera for 1 h at 37°C prior to infection. Subsequently, cells were fixed in 4% formaldehyde containing 2 mg/ml 49,6-diamidino-2-phenylindole (DAPI) nuclear stain (Invitrogen) for 1 h at room temperature, and fixative was replaced with PBS. Images were acquired with the InCell 2000 Analyzer (GE Healthcare) automated microscope in both the DAPI and fluorescein isothiocyanate (FITC) channels to visualize nuclei and infected cells (i.e., eGFP-positive cells), respectively (4 objective, 4 fields per well, covering the entire well). Images were analyzed using the multitarget analysis module of the InCell Analyzer 2000 workstation software (GE Healthcare). GFP-positive cells were identified in the FITC channel using the top-hat segmentation method and subsequently counted within the InCell Workstation software. Absolute inhibitory concentration (absIC) values were calculated for all patient serum samples by modeling a 4-parameter logistic (4PL) regression with GraphPad Prism 8. The 4PL model describes the sigmoid-shaped response pattern. For clarity, it is assumed that the response can be expressed so that the slope increases as the concentration increase. absIC was calculated as the corresponding point between the 0% and 100% assay controls. Fifty percent inhibition was defined by the controls for all samples on the same plate. For example, the absIC50 would be the point at which the curve matches inhibition equal to exactly 50% of the 100% assay control relative to the assay minimum. A 4PL regression with GraphPad Prism 8 was used.

SARS-CoV-2 neutralization assay: neutralization assays were performed using authentic SARS CoV-2 virus isolates. Serum samples were heat inactivated at 56°C for 30 min and serial dilutions from 1/4 to 1/8192 were incubated for 1 h at 37°C in the presence of ancestral or omicron variants in DMEM, 2% FBS. Fifty microliters of the mixture was then deposited over Vero cell monolayers for an hour at 37°C (MOI, 0.01). Infectious medium was removed and

replaced for DMEM, 2% FBS. After 72 h, cells were fixed with 4% paraformaldehyde (4°C, 20 min) and stained with crystal violet solution in methanol. The cytopathic effect (CPE) on the cell monolayer was assessed visually. If damage to the monolayer was observed in the well, it was considered as manifestation of CPE. Neutralization titer was defined as the highest serum dilution without any CPE in two of three replicable wells.

Quantification and statistical analysis: Antibody concentration and neutralizing titer from volunteers were analyzed collectively. Neutralization assays were performed in biological duplicates. All statistical tests and plots were performed using GraphPad Prism 8.0 software. Comparisons of antibody concentration and neutralizing titer were made using two-tailed Wilcoxon matched-pair test. Statistical significance is shown in the figure legends with the following notations: ****, P , 0.0001; ***, P , 0.001; ns, not significant. Geometric means with 95% confidence intervals were calculated.

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S5. Declaration of interests: All authors declare no competing interests.

S6. **Author group that participated in this study**

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