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Supporting Information for:

"Subclass-switched anti-Spike IgG3 oligoclonal cocktails strongly enhance Fc-mediated opsonization"

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Other supporting materials for this manuscript include the following:
Movies S1 to S4
Supplementary information 1 containing plasmid sequences

Materials and Methods

IgG3 plasmid generation, production, and sequencing

The anti–spike monoclonal antibodies used in this work were made from spike-reactive B cells from convalescent patients who were infected 6 weeks earlier with SARS-CoV-2 (verified in the clinic) [5]. Briefly, the V(D)J coding sequences from spike-reactive B cells were generated using 10X Genomics. The variable regions were cloned into pTwist IgG1 vectors (Twist Biosciences) containing the IgG1 constant heavy domain. The plasmid containing the constant heavy domain gene for IgG3 was produced previously. The sequence of the recombinant vectors for the light chain and heavy chain plasmids, including constant domains, are shown in Supplemental material document 1. Primers were designed so the insert (IgG3 heavy chain constant domain) contained overhangs that were complementary to the vector and homologous recombination could occur. This allowed the exchange of IgG1 constant domain into an IgG3 constant domain, leaving the variable region intact. The exchanging of the heavy chain constant domains was done by PCR of the IgG1 vector backbone and the IgG3 constant region to replace the IgG1-specific domain. Subsequent HIFI-DNA assembly (NEB, biolAbs) would mediate the homologous recombination process. PCR products were loaded on agarose gels to assess correct amplification based on size. The bands were excised and purified by QIAGEN PCR clean-up kit according to the manufacturer's instructions. Competent *E. coli* supplied in the kit was transformed with the newly assembled IgG3 plasmids and plated on agar plates containing ampicillin. The purified IgG3 plasmids from transformed colonies were verified by sequencing.

Cell culture and antibody production

Expi293F suspension cells were purchased from Gibco (ThermoFisher) and routinely cultured in 125 ml Erlenmeyer flasks (Nalgene) in 30 ml Expi293 medium (Gibco) in an Eppendorf s41i shaker incubator at 37°C, 8 % CO_2, 120 rpm. Cells were routinely passed and split to a density of 0.5 x 10^6 cells/ml every 3 to 4 days. The day before transfection, the cells were seeded at a density of 2 x 10^6 cells/ml. The next day, cells were seeded at 7.5 x 10^7 cells in 25.5 ml Expi293 medium. Transfection with heavy and light chain plasmids was carried out using the Expifectamine293 kit (Gibco) according to the manufacturer's instructions. Briefly, 20 µg of heavy and light chain plasmid, respectively, were mixed with 2.8 ml OptiMEM (Gibco) and 100 µl Expifectamine and incubated at room temperature for 15 minutes. Afterwards, the transfection mix was added to the Expi293F cells. The following day, 1.5 ml of enhancer 1 and 0.15 ml of enhancer 2 (both from the Expifectamine293 kit) were added and the cells cultured for another 72 hours.

The cells were removed from the cell culture medium by centrifugation (400 x g, 5 min) and the supernatant transferred to a new tube. In order to capture the IgGs from the medium, protein G sepharose 4 Fast Flow (Cytiva) was added to the medium and incubated end-over-end at room temperature for 2 hours. The beads were collected by running the medium bead mix through a gravity flow chromatography column (Biorad) and washed twice with 50 ml PBS. The antibodies were eluted using 5 ml HCl-glycine (0.1 M, pH 2.7). Tris (1 M, pH 8, 1 ml) was used to neutralize the pH. The buffer was exchanged to PBS using Amicon Ultra-15 centrifugal filters (Sigma) with a molecular cut-off of 30,000 Da. The concentration and quality of the purified antibodies was spectrophotometrically measured with the IgG setting of the Nanodrop (Denovix) as well as subjecting the antibodies to an SDS-PAGE and comparing the band intensity to a serial dilution of Xolair (Omalizumab).

Generation of spike-coated beads

Spike protein was generated by transfecting Expi293F cells with 40 µg plasmid containing the gene for the spike protein (CS/PP spike encoding a secretable version of the protein was used to allow purification from cell culture supernatants), donated previously to us by Dr. Florian Krammer's lab [5]. 200 µg of spike protein was biotinylated according to the instructions of EZ-LinkTM Micro Sulfo-NHS-LCBiotinylation Kit (ThermoFisher). Then 500 µl (5x10^7 million beads) of fluorescent (APC) streptavidin microsphere beads (1 µm, Bangs Laboratories) were conjugated with the biotinylated

spike protein (200 µg) according to the manufacturer's instructions.

Flow cytometry-based avidity measurements
The binding assays were performed in a 96-well plate which had been pre-coated with 200 µl of 2% BSA (in PBS) overnight at 4 °C. 100,000 spike-coated beads were used in all wells, with antibody concentrations ranging between 0.02-0.2-2 µg per ml. The beads were opsonized at a volume of 100 µl in 1X PBS at 37 °C for 30 min on a shaking heat block. The wells were washed twice with PBS. To assess antibody binding to spike beads, 50 µl of (1:500 diluted) a Fab-specific fluorescent secondary antibody (#109-546-097, Jackson ImmunoResearch) was used to create a fluorescent signal. The secondary antibody was left to incubate with the spike-bead antibody complex at 37 °Cs for 30 minutes on a shaking heat block. 100 µl of PBS was added to the wells before analysis in the flow cytometer. The beads were analyzed using a Beckman Coulter Cytoflex flow cytometer, which acquired 20000 beads per sample. The data was processed using Flowjo. The gate for spike beads was set based on forward and side scatter. The gate for spike beads positive for antibodies was set based on reactivity to a non-reactive IgG control mAb. The data was later analyzed in Graphpad Prism using a nonlinear regression model: Hill slope was unconstrained, maximum value (Bmax) were set to 100% and K_D was constrained to be any value between 0-10000. The best fit value was presented in the Graph shown with the fitness of the model.

SPR kinetic assay
To study binding kinetics to the spike trimer, RBD or NTD we immobilized a high-capacity amine sensor chip (Bruker) with Anti-human IgG (Fc) antibody (Cytiva BR-1008-39) at 25 µg/ml in 10 mM sodium acetate buffer pH 5 at flow rate 10 µl/min and contacting 300s. This was done in a MASS-16 biosensor instrument (Bruker) with a running buffer consisting of PBS + 0,05%Tween20. The antibodies were diluted in PBS and injected over the surface for 90s at 10 µL/min. The running buffer was PBS with 0.01% Tween 20. The RBD-antigen used was produced in HEK293 acquired from Sinobiological (Beijing, China: RBD WT 40592-V08H). The spike trimer was produced as described above. The NTD-antigens were acquired from Sinobiological (Beijing, China: NTD 40591-V9H and NTD-Omicron 40591-V08H42). The RBD-antigen was diluted from 60 nM to 3.75 through serial halving dilutions. The NTD-antigen was in turn diluted from from 80 to 2.5 nM, by serial halving dilutions. The spike trimer was added at 40 nM. The antigens were injected at these concentrations and were allowed to interact with the sensor for 2 minutes, with a flow rate 30 µl/min, followed by a dissociation for 6 minutes. After each cycle the surface was regenerated with 3M MgCl. All experiments with RBD and NTD were performed three times, and once with the spike trimer. The data was analyzed using Sierra Analyser software version 3.4.3 (Bruker) program to determine apparent dissociation constants (K_D).

ELISA avidity measurements
For ELISA, 1 µg/ml of spike protein in PBS was immobilized onto ELISA High-bind plates (Sarstedt) overnight at 4°C. The wells were washed with PBST (1x PBS with 0.05 % Tween 20) 3 times and then blocked with 2% BSA/PBS for 1 hour at room temperature. After three washes, a serial dilution of primary antibody (anti-spike mAb) was added (100 µl of mAbs with a concentration of 0.08 up to 20 µg/ml) and incubated for 1 hour at room temperature. The wells were washed three times. A rabbit anti-human light chain-HRP secondary antibody (anti-kappa for clone 57, anti-lambda for clone 11 and 66) (Abcam ab202549 and ab200966) at a dilution of 1:5000 in PBS was added and left to incubate for 1 hour at room temperature. The wells were washed 3 more times with PBST. Finally, 100 µl developing reagent (20 ml sodium citrate pH 4.5 + 1 ml ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 0.2 g in 10 ml water, Sigma) + 0.4 ml 0.6 % H_2O_2) was added. OD450 was recorded after 15 – 30 minutes. Data was plotted with GraphPad Prism.

C1q deposition assay
To study complement activation, we opsonized 500,000 spike beads with 0.1-1-10 µg/ml of mAbs with 1% antibody-depleted serum as a source for complement in a 96-well plate previously coated with 2% BSA. After 30 minutes of opsonization at 37 °Cs on a shaking heat block (300 RPM), the

wells were washed 2 times with PBS before a FITC fluorescently labeled anti-C1q Ab was added (ab4223, Abcam) (1:250 dilution from stock, 50 µl). The anti-C1q Ab was left with the beads for 30 min at 37 °C on a shaking heat block (300 RPM). PBS was added to resuspend the beads in a final volume of 150 µl. The gate for spike beads was set based on forward and side scatter, and the gate for C1q deposition was set based on the results of the control-IgG1(Supp Fig. 5A). The data acquired were analyzed with Flowjo and plotted in GraphPad Prism. The EC_{50}-analysis was performed on GraphPad Prism using a non-linear regression model with the bottom constrained to 0 and the top value constrained to 100% for the % beads-C1q deposition analysis. For the MFI EC_{50} analysis, the top was set to be shared and greater than 0.

Flow cytometry-based phagocytosis assays

Phagocytosis experiments were done with the same batch of spike beads used in the binding assay. To assess internalization, the red-fluorescent beads were stained with pHrodo green (P35369, ThermoFisher), a pH-sensitive dye using 5 µl of 10 mM of pHrodo green (ThermoFisher) in 95 µl of Na_2CO_3 (pH 9) for 30 min at 37 °Cs. Excess stain was washed away with PBS. THP-1 cells (Sigma-Aldrich) were cultured as described previously [5]. In all experiments, $1x10^5$ THP-1 cells were used. In one set of experiments, the ratio of cells to beads was 1:30 (Multiplicity of prey, MOP, 30); in the other, it was 1:15 (MOP 15). For the MOP 30 experiments, $3x10^6$ spike beads were opsonized with 10 µg/ml of antibodies in a volume of 100 µl of Sodium media (pH adjusted to 7.3 with NaOH; 5.6 mM glucose, 10.8 mM KCl, 127 mM NaCl, 2.4 mM KH_2PO_4, 1.6 mM $MgSO_4$, 10 mM HEPES, 1.8 mM $CaCl_2$). For the MOP 15 experiments, $1.5x10^6$ spike beads were opsonized with 5 µg/ml of mAbs. Opsonization was performed for 30 minutes at 37 °C on a shaking heat block (300 RPM) in a volume of 100 µl. During this incubation period, THP-1 cells were counted (using a Bürker chamber), and the medium was exchanged from RPMI to Sodium medium. THP-1 cells (50 µl, $2x10^6$/ml) were added to each well, and the cells were allowed to phagocytose beads for 30 minutes at 37 °C on a shaking heat block (300 RPM). The 96-well plate was then incubated on ice for 15 minutes and analyzed directly in a Beckman Coulter Cytoflex flow cytometer. A gate for THP-1 cells was set up based on their forward and side scatter (Supp Fig. 4A). The gate for internalization and association was set with negative control of cells only (Fig. 3A). The analysis stopped after 5000 events were captured in the THP-1-gate. The data was analyzed in the program Flowjo by setting similar gates as described above. The Flowjo-processed data was further analyzed in GraphPad Prism, and internalization and bead signal (APC-A) of the THP-1 gate were plotted to compare the different antibodies.

Monocytes were isolated from the blood of healthy donors first by acquiring a PBMC layer by using Polymorphoprep (Abbot). Monocytes were purified from the PBMC layer through positive selection using CD14 Microbeads (Cat#130-050-201, Miltenyi Biotec) according to the manufacturer's instructions. Following isolation, monocytes were counted using a XN-350 hematology analyser (Sysmex). Neutrophils were, in turn, isolated with Polymorphprep gradient according to the manufacturer's instructions and were counted with a Bürker chamber. Isolated cells were kept on ice for 1 hour after being resuspended in sodium media and adjusted to $2x10^6$ cells/ml. Donors had given written and oral consent to participate in this study. They were provided oral information on the purpose of the donation, which was used only to isolate and use the monocytes and neutrophils in flow and microscopy experiments. This study used a previously approved ethical approval from the Swedish ethical review authority (2020/01747).

For monocyte phagocytosis experiments, spike beads were opsonized with 10 µg/ml of mAbs (IgG control mAb or OctomAb IgG3) but without any serum. Monocytes ($1x10^5$cells) were added in a volume of 50 µl. The cells were left to phagocytose for 30 min at 37 °C on a shaking heat block (300 RPM). Following this, the cells were stained on ice for 20 minutes with a CD14 antibody (Brilliant Violet 421™ anti-human CD14 Antibody, Biolegend) diluted 1:50 to 3 µg/ml and added in a volume of 20 µl. The wells were analyzed in a Beckman Coulter Cytoflex Flow cytometer. Monocytes were gated using size and granularity (Supp. Fig. 6A). Further selection was made by gating for CD14-positive cells. The analysis was stopped after 3000 events were recorded in the CD14+ gate. The percentage of cells associated with spike beads (APC+) and internalized with beads (APC+, FITC+) (Supp. Fig. 6A) were plotted against MOP in Prism Graphpad.

For the neutrophil experiments, the spike beads were opsonized with 10 µg/ml of mAbs with 1% antibody-depleted serum (Pel-Freeze, Cat#34014-10) for 30 min at 37 °C on a shaking heat block (300 RPM) (100 µl final volume). To only study the effects of Fc-mediated phagocytosis, we heat-inactivated the serum at 56 °C for 1 hour. Both the heat-inactivated serum and normal human serum were added to the respective wells. Neutrophils ($1x10^5$ cells) were added to each well with a volume of 50 µl ($2x10^6$/ml). The cells were left to phagocytose for 30 min at 37 °C on a shaking heat block (300 RPM). The process of phagocytosis was stopped by putting the plate on ice. While on ice, the cells were stained with an anti-CD18 antibody (BV421 Mouse anti-Human CD18, BD Biosciences) diluted 1:100 to 2 µg/ml and 20 µl of this diluted antibody was added to the cells. The cells were stained on ice for 20 minutes before being analyzed in a Beckman Coulter Cytoflex flow cytometer. A gate was set for FSC-A and SSC-A to mark the neutrophils (Supp. Fig 6B). This population was further selected for by the CD18 marker (Supp Fig. 6B). The analysis stopped after 3000 events were captured in the CD18+ gate, and the data was processed in the program for Flowjo with similar gates as described above. The percentage of cells with internalized beads and bead signal (APC) in the CD18 gate was used as metrics to compare the mAbs.

IgG mAb aggregation quality control experiments

After production and purification of the mAbs we performed a quality control of the mAbs to test if aggregation could influence the results. We used clone 81 IgG1 and IgG3 as a sample issue. We centrifuged the samples at 16000 x g for 10 minutes at room temperature. After centrifugation, we removed the supernatant and sterile-filtered it through a 0.2-micron syringe filter. We then compared the treated Ab81 IgG1 and IgG3 vs untreated variants of them to see if there was a difference in function. We performed phagocytosis experiments with THP-1 cells at MOP 30, as described above. The results from this quality control can be found in Supplementary figure 4C.

Live imaging phagocytosis assay

Cell preparation and spike bead opsonization

Neutrophils were isolated from 4 healthy donors through Polymorphprep as described above. The cells were then left on ice for 1 h in aliquots of $2x10^6$/ml per donor. Cells were seeded on an 8-well Ibidi-plate (Cat#80827, Ibidi GmbH, Germany) at a density of $1x10^5$ cells/well for 1 hour with 5% CO_2 at 37°C. All Ibidi-plates were pre-coated with 3.3 µg/ml human fibronectin (F0895-1MG, Sigma) diluted in PBS. After the incubation, the cells were stained with Hoechst 1:20000 (ThermoFisher, Germany) and Alexa Fluor 594-conjugated wheat germ agglutinin (WGA) 1:333 diluted in sodium medium to a final volume of 300 µl/well. Excess stain was washed 3 times with 300 µl/well Sodium medium, before 250 µl/well of sodium medium was added.

Spike-biotinylated beads were pre-stained with pHrodo green prior to opsonization with 10 µg/ml antibodies (IgG3 OctomAb, IgG1 OctomAb, Ab94 IgG3, Control-IgG1) and 1% antibody-depleted serum (Pel-Freeze, Cat#34014-10). Beads were opsonized in 100 µl (containing $1x10^6$ beads) for 30 minutes at 37 °C shaking heat block. After opsonization, $5x10^5$ beads/well (50 µl) were added right before the initiation of live imaging to the previously seeded neutrophils.

Live fluorescence imaging and data acquisition

Time-lapse images of phagocytosis were recorded every 10 minutes for 60 minutes using a Nikon N-SIM microscope. Images were acquired through fluorescence microscopy with a 20X Plan Apo λ objective (NA = 0.40) and Perfect Focus System (PFS). All images were taken with an ORCA-Flash 4.0 cCMOS camera (Hamamatsu Photonics K.K) at 8 positions per well. The filter cubes used were DAPI (Ex. 340 – 380 nm, Em. 435 – 485 nm), TxRed (Ex. 540 – 580 nm, Em. 600 – 660 nm), FITC (Ex. 465 – 495 nm, Em. 515 – 555 nm) and Cy5 (Ex. 625 – 650 nm, Em. Peak 670 nm). Samples were imaged live in an environmental chamber (Okolab) at 37°C with 5% CO_2. All image analysis was performed through the Nikon software General Analysis 3. Graphs and statistics were compiled in Microsoft Excel and Prism 9. The ET50 analysis was performed on Prism Graphpad, using a non-linear regression model, hill-slope unconstrained and with bottom value constrained to 0 % and top value to 100%.

SIM Image acquisition

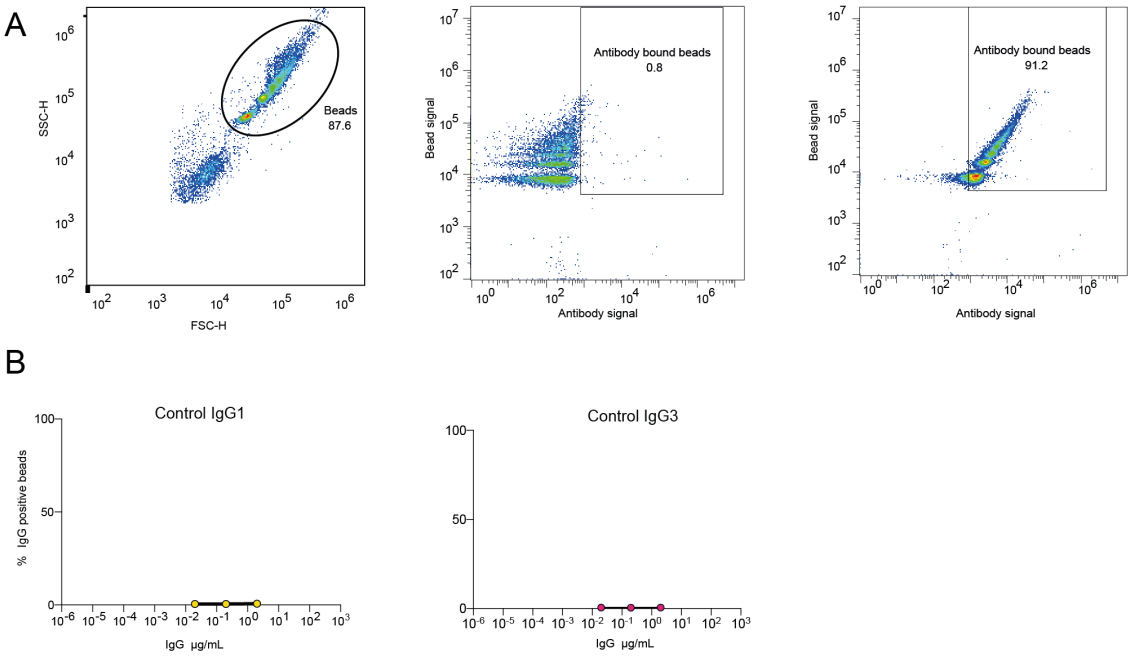
After 60 minutes of phagocytosis, the sample was fixed in 4% paraformaldehyde with PBS at 37°C for 15 minutes. The wells were washed 3 times with sodium medium prior to SIM imaging. All SIM images were acquired using Nikon N-SIM microscope with a LU-NV laser unit (488/561/640 nm), CFI SR HP Apochromat TIRF 100X oil objective (NA = 1.49) and ORCA-Flash 4.0 cCMOS camera (Hamamatsu Photonics K.K). Filter cubes used were N-SIM 488 (Ex. 484 – 496 nm, Em. 500 – 545 nm), N-SIM 561 (Ex. 557 – 567 nm, Em. 579 – 640 nm) and N-SIM 640 (Ex. 629 – 645 nm, Em. 663 – 738 nm). Wide-field fluorescence microscopy was used to acquire images in the DAPI channel in the SIM system. A SPECTRA X light engine (Lumecore inc) was used for wide-field excitation. A Z-stack of 15 steps was collected for each image in all channels. SIM images were reconstructed using the Nikon SIM software through NIS Elements Ar (NIS-A 6D and N-SIM Analysis). Wide-field images were processed with NIS Element Clarify.ai and Denoise.ai to minimize blur from the fluorescence microscope and cropped from a 3x5 grid. All Z-stack images were compiled to a maximum intensity projection image before SIM- and fluorescence images were merged in the software NIS Elements.

Animal experiment

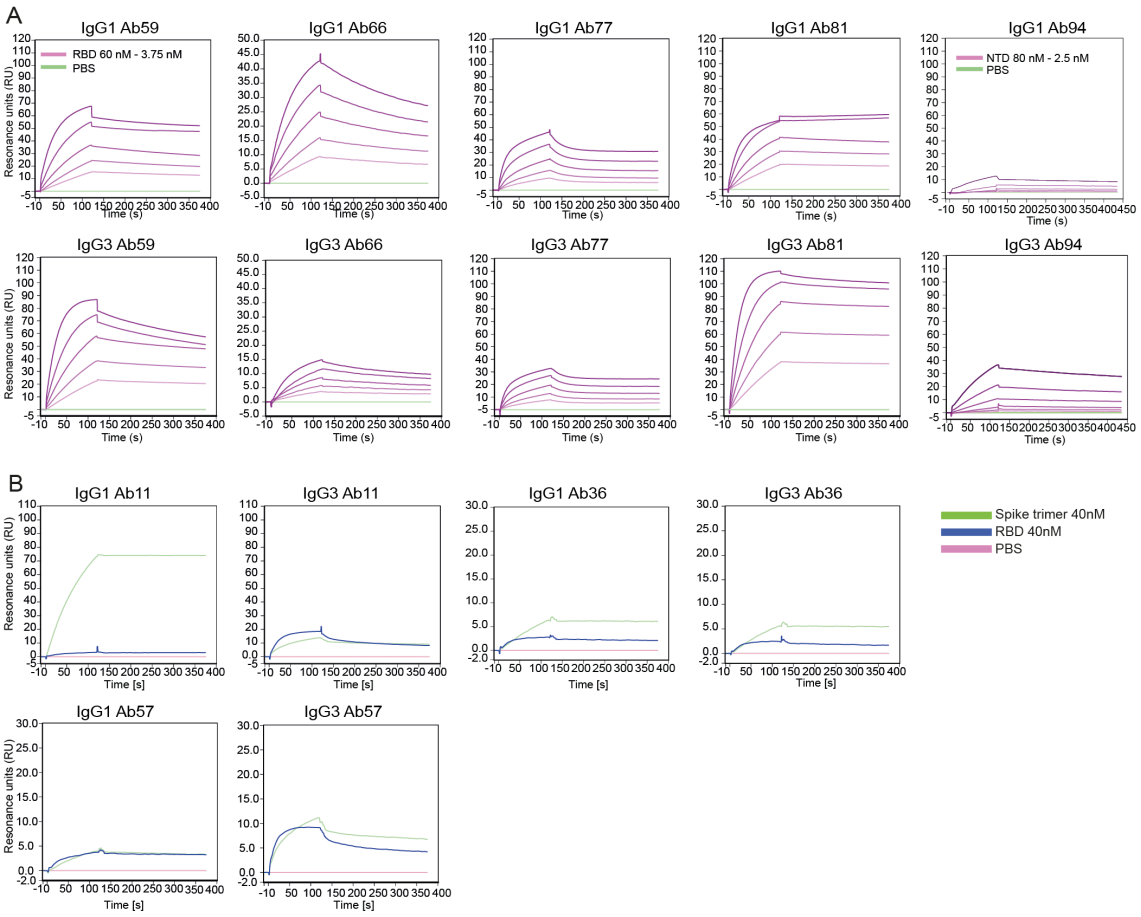
30 seven- to ten-week-old female K18 hACE2 (B6.Cg-Tg(K18-ACE2)2Prlmn/J) mice were split into 5 groups of 6 mice/group and antibodies were administered in one single dose, intraperitoneally. Eight hours after antibody administration, animals were inoculated intranasally with 10^5 PFU of SARS-CoV-2 (Wuhan strain, isolate SARS-CoV-2/01/human/2020/SWE, sourced from the Public Health Agency of Sweden). The mice' body weights and health status were recorded daily, and the animals were euthanized if they lost more than 20% of their body weight or showed severe deterioration in health status. The infection proceeded for 10 days before the animals were euthanized. Blood, tissue, and bronchoalveolar lavage were harvested and stored accordingly. All the animal experiments were performed under the approval of the regional animal experimental ethics committee in Stockholm (2020-2021). BAL-fluid analysis for viral titers was analyzed as described previously using qPCR[5].

Statistical analysis

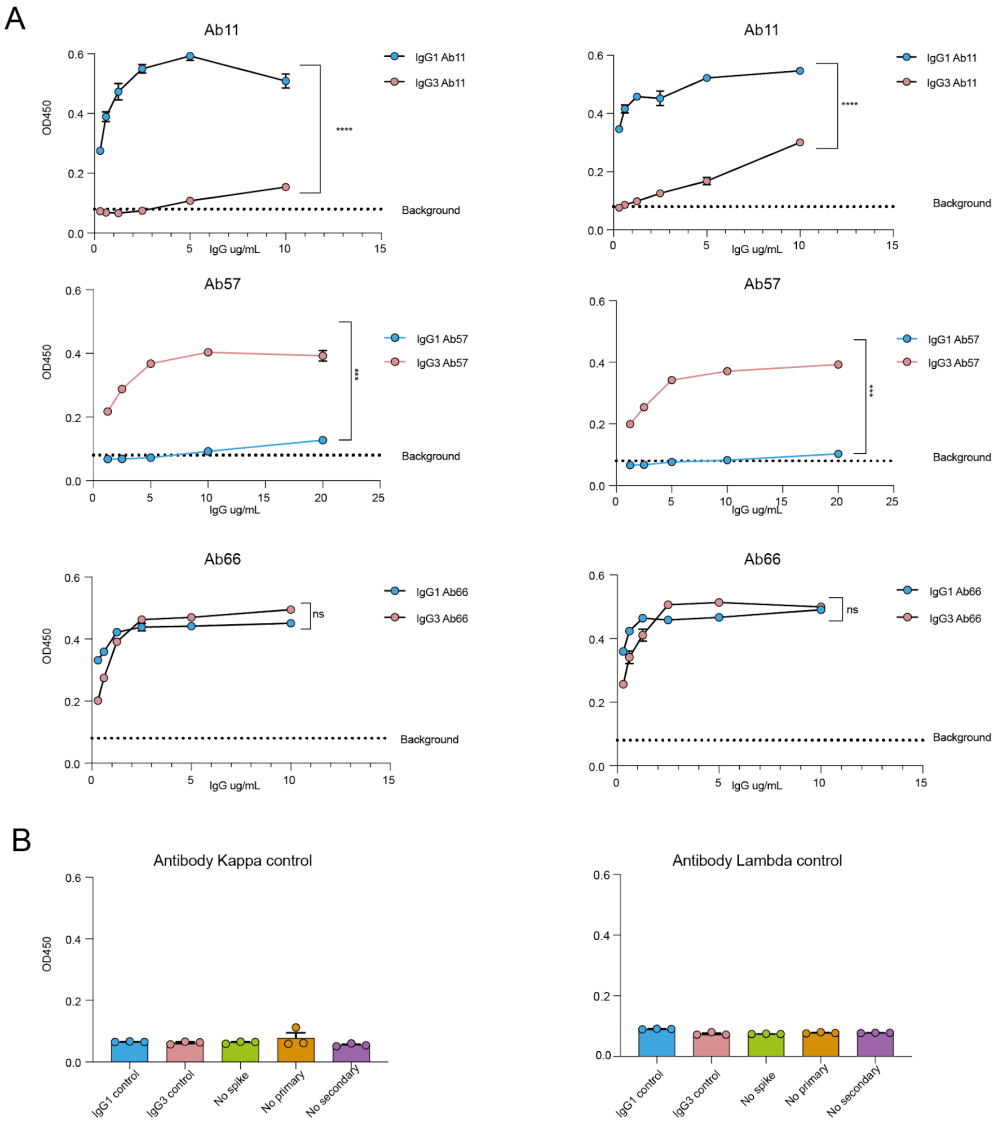
Statistical analysis was performed in GraphPad Prism. For comparisons between subclasses in specific clones or between mixes, a 2-tailed Welch's t-test was used for statistical analysis. When more than 2 treatments were analyzed, a one-way ANOVA with multiple comparisons test was used, with correction for multiple comparisons with Tukey's test.



Supplemental Figure 1. **A**. Gating strategy of spike-coated microspheres. The beads are gated for size and granularity. A negative gate for antibody binding was set by using an IgG control. **B** Isotype control binding curves with concentrations 2-0.2-0.02 ug/mL (X-axis). Y-axis shows percentage IgG+ positve beads.



Supplemental Figure 2. Binding kinetics with RBD and monoclonal binding to spike
A Surface plasmon resonance-based binding kinetics assay with the spike RBD as ligand for clones 59 to 81 with multiple RBD concentrations (60, 30, 15 and 7.5 nM) and constant IgG concentration on the sensor chip. NTD is used for clone 94 (80, 40, 20, 10, 5 and 2.5 nM).
B Binding of 40 nM Spike-protein to immobilized IgG.

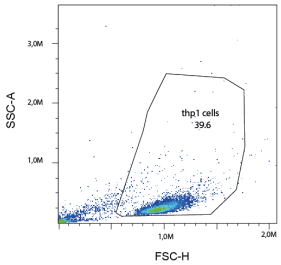


Supplemental figure 3. ELISA experiment repeats 1-2 and secondary control experiments
A First and second repeat of ELISA binding curves of clones 11, 57,66. OD450 plotted on the Y-axis.
B Controls for the anti-kappa and lambda secondaries with either IgG1 isotype control, IgG3 isotype control, no spike protein, no primary antibody and without secondary.

A

Antibody	Internalization	Bead signal
IgG1 Ab11	11	9100
IgG3 Ab11	7	8500
IgG1 Ab36	10	9100
IgG3 Ab36	31	29200
IgG1 Ab57	12	9300
IgG3 Ab57	22	10100
IgG1 Ab59	16	10500
IgG3 Ab59	38	28100
IgG1 Ab66	9	6100
IgG3 Ab66	20	9700
IgG1 Ab77	14	8100
IgG3 Ab77	30	17200
IgG1 Ab81	14	7700
IgG3 Ab81	34	44400
IgG1 Ab94	17	10300
IgG3 Ab94	47	74000

B



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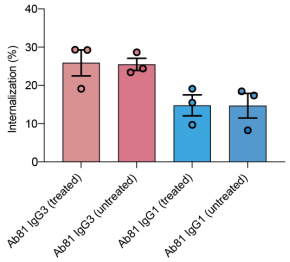
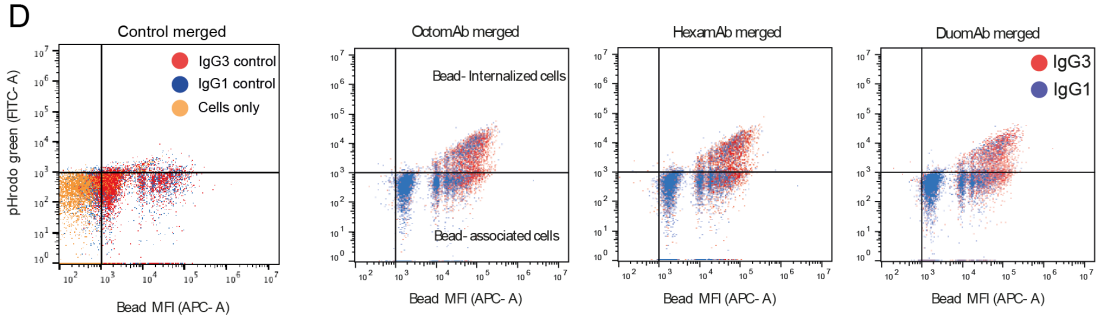
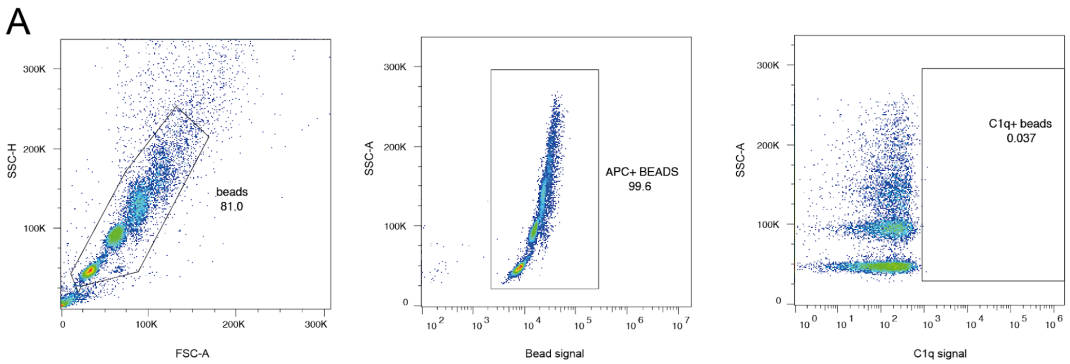


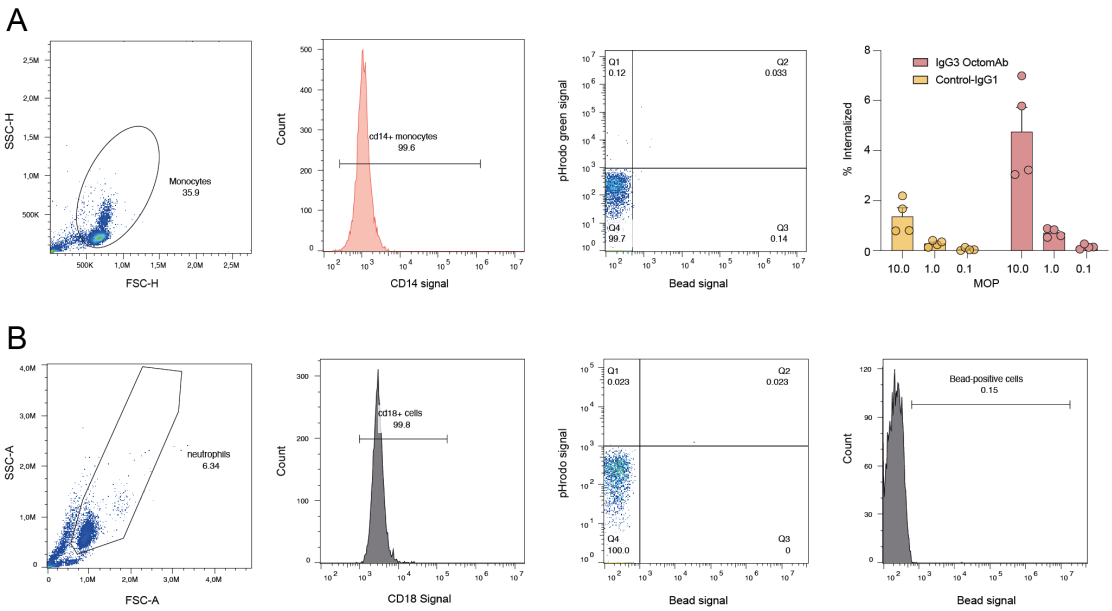
D



Supplemental Figure 4. **Results table, gating, and quality control for mAbs**
A Table showing percentage of THP-1-cells with internalized beads and bead signal for the whole THP-1 population for each respective clone and its subclasses. **B** THP-1 cells were gated for size and granularity. **C** Aggregate quality control of IgG3 and IgG1 mAbs. IgG mAbs were treated by centrifugation, then removal of supernatants, followed by sterile-filtering through a 0.2-micron syringe filter. The treated IgG samples were compared to untreated to observe if there was a functional difference in phagocytosis effect. THP-1 cells were used at an MOP of 30 with 100.000 cells. **D** Flow cytometry data, showing merged data of IgG1 and IgG3 oligoclonal cocktails.The orange cells represent cells only, red cells represent IgG3-treated cells and blue IgG1.



Supplemental Figure 5. Gating strategies for measuring C1q deposition. A Beads were gated for by size and granularity. Beads were further gated for bead-flourescence (APC). A negative control IgG was used to set C1q deposition gate.



Supplemental Figure 6. Gating strategies for monocytes and neutrophils.
A Monocytes were gated for size and granualirity and CD14+. Internalization gate was set with only cells. Graph shows percent monocytes with internalized beads as a function of MOP. 10 µg/ml of mAbs was used to opsonize either 1 million beads (MOP10), 100,000 beads (MOP 1) or 10,000 beads (MOP 0.1).
B Neutrophil gating strategy was started by gating for size and granularity, CD18+. The CD18+ cells were gated for both bead and pHrodo signal to differentiate cells with beads. CD18+ cells were gated for bead signal to determine quantity of beads phagocytoes.

Supp. Movie 1 – Neutrophil phagocytosis of Spike beads opsonized with control IgG1
Live imaging of neutrophil phagocytosis of spike beads over a 60-minute experiment. Images were taken every 10 minutes. The movie depicts neutrophil DNA in blue (Hoechst) and cell membrane in magenta (Alexa Fluor 594 - WGA). Spike beads are shown in red, and upon internalization in the phagolysosome, they turn green (pHrodo green). Beads had been opsonized with IgG1 control.

Supp. Movie 2 – Neutrophil phagocytosis of Spike beads opsonized with Ab94 IgG3
Live imaging of neutrophil phagocytosis of spike beads over a 60-minute experiment. Images were taken every 10 minutes. The movie depicts neutrophil DNA in blue (Hoechst) and cell membrane in magenta (Alexa Fluor 594 - WGA). Spike beads are shown in red, and upon internalization in the phagolysosome, they turn green (pHrodo green). Beads had been opsonized with Ab94 IgG3.

Supp. Movie 3 – Neutrophil phagocytosis of Spike beads opsonized with OctomAb IgG1
Live imaging of neutrophil phagocytosis of spike beads over a 60-minute experiment. Images were taken every 10 minutes. The movie depicts neutrophil DNA in blue (Hoechst) and cell membrane in magenta (Alexa Fluor 594 - WGA). Spike beads are shown in red, and upon internalization in the phagolysosome, they turn green (pHrodo green). Beads had been opsonized with OctomAb IgG1.

Supp. Movie 4 – Neutrophil phagocytosis of Spike beads opsonized with OctomAb IgG3
Live imaging of neutrophil phagocytosis of spike beads over a 60-minute experiment. Images were taken every 10 minutes. The movie depicts neutrophil DNA in blue (Hoechst) and cell membrane in magenta (Alexa Fluor 594 - WGA). Spike beads are shown in red, and upon internalization in the phagolysosome, they turn green (pHrodo green). Beads had been opsonized with OctomAb IgG3.