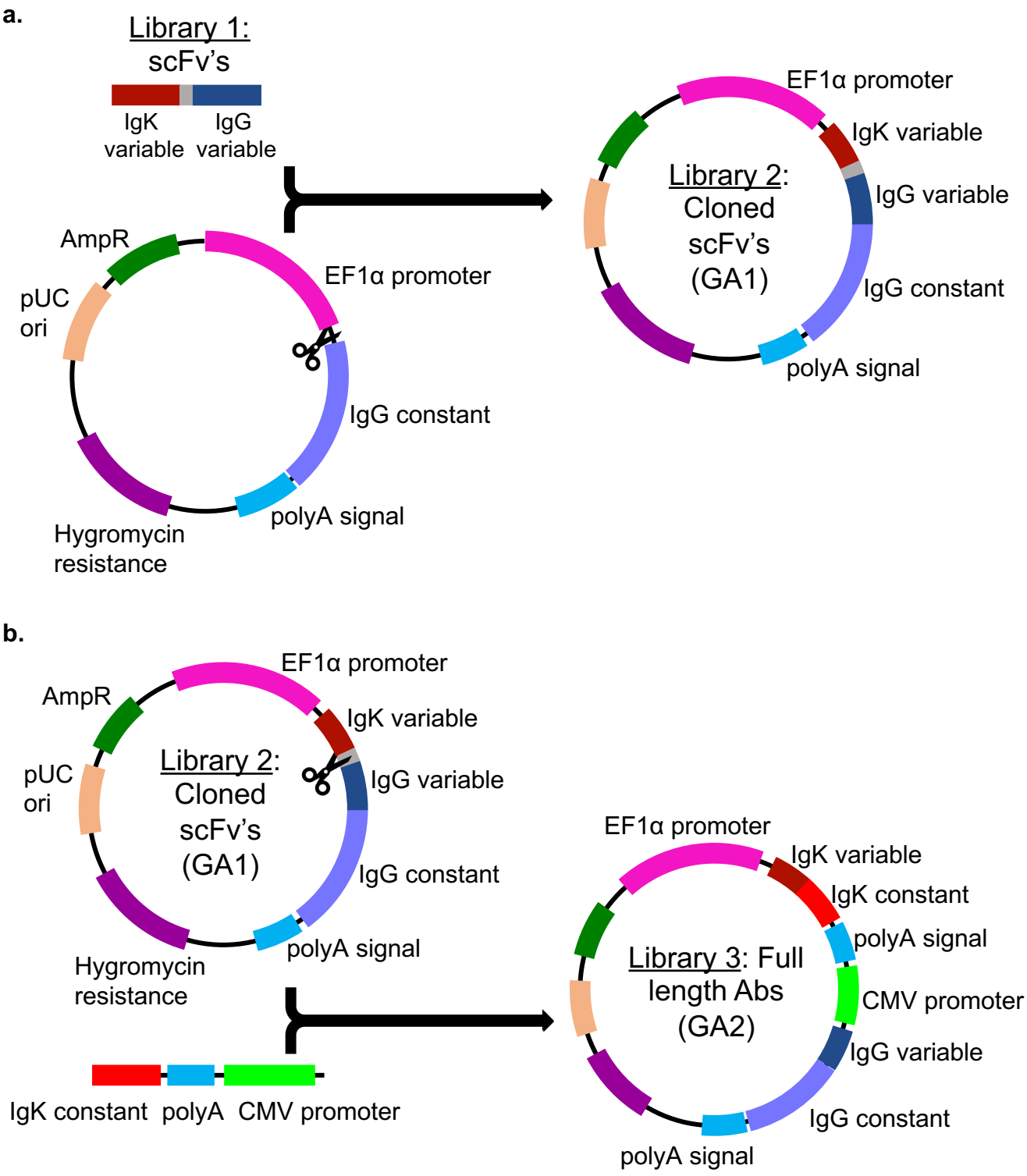
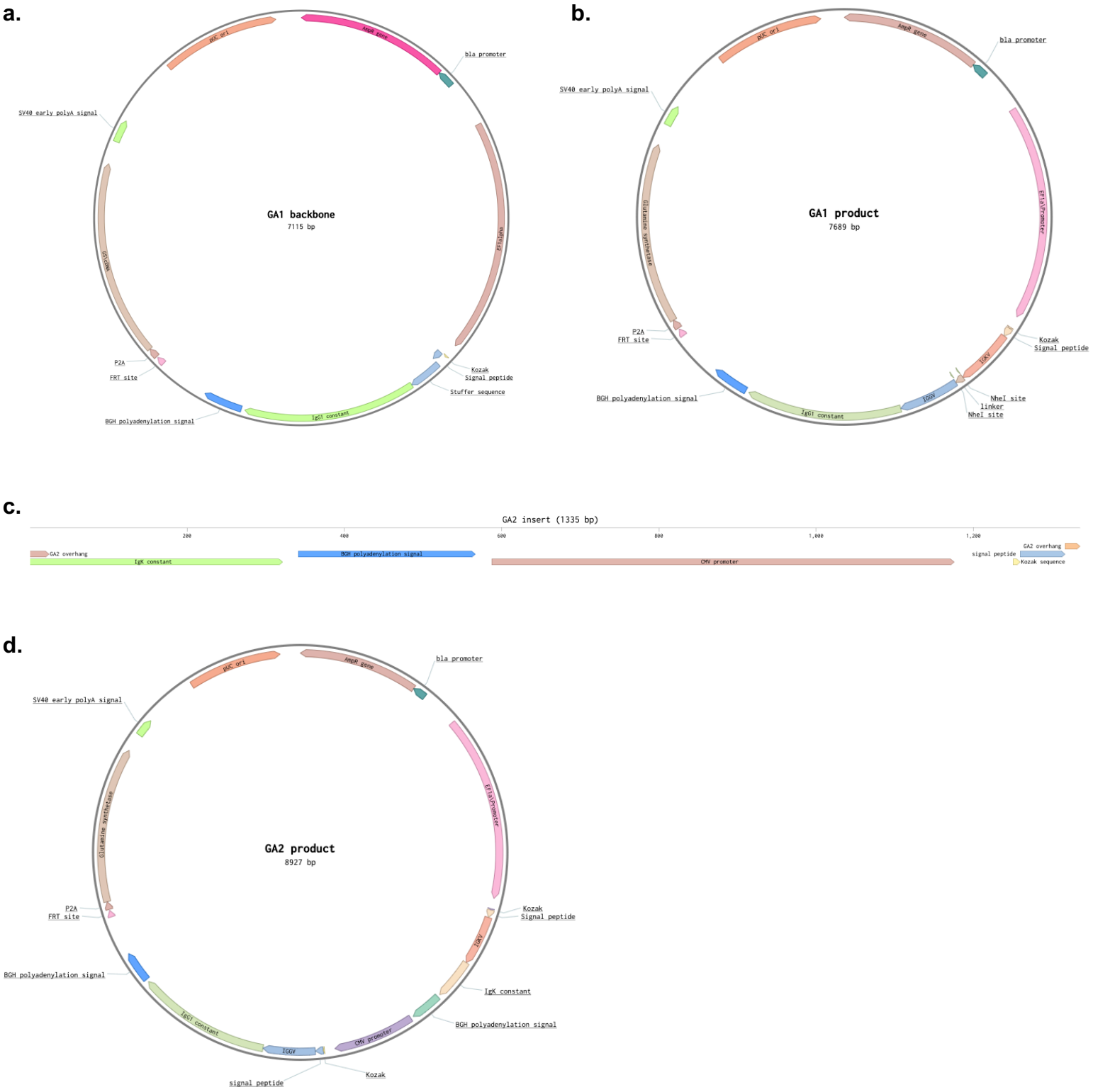
Supplementary Figure S1



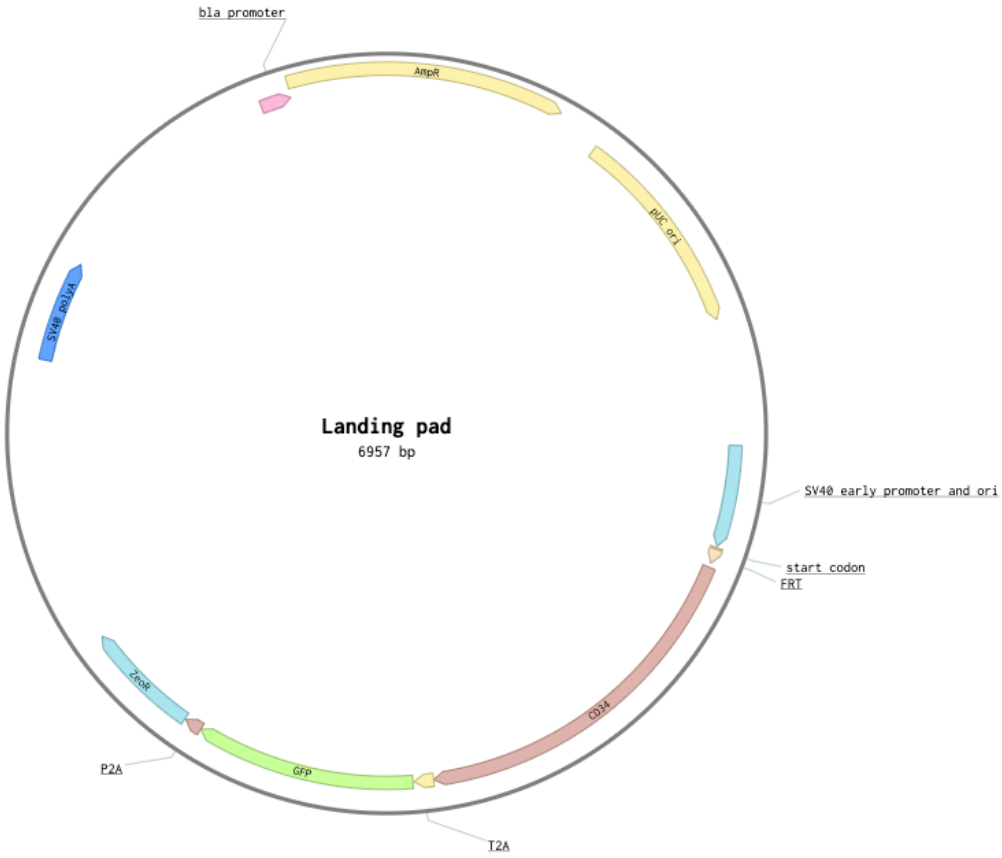
Schematic of Gibson assembly processes. (**a**) Product of Gibson Assembly 1 (GA1), comprising the based plasmid vector with an scFv (from Library 1) cloned between the EF1α promoter and the IgG constant domain sequence. (**b**) Product of Gibson Assembly 2 (GA2), with the scFv linker excised and replaced with a sequence that comprises an IgK constant domain sequence, a poly(A) signal sequence, and a CMV promoter.

Supplementary Figure S2



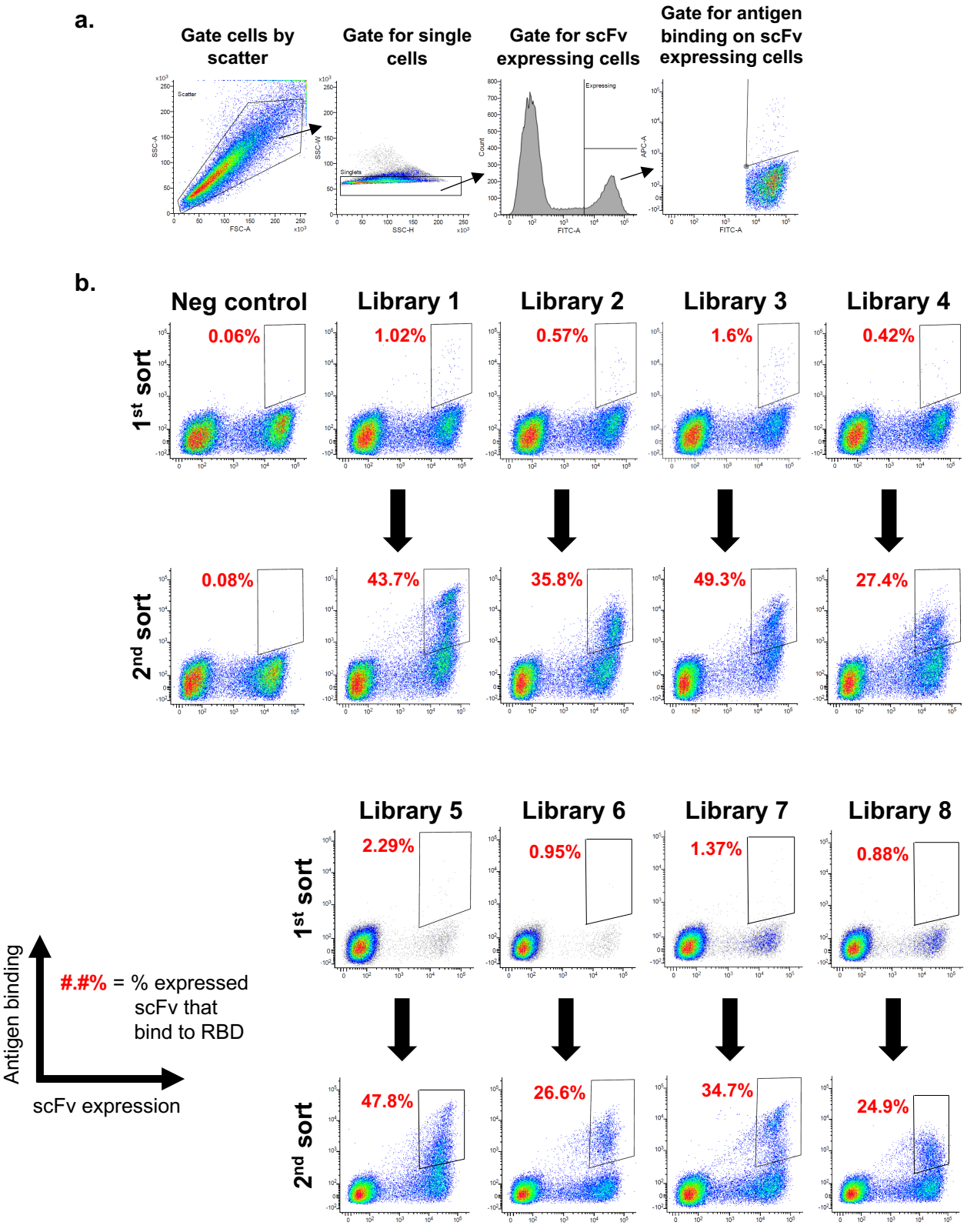
Full-length cloning plasmid and insert maps. (**a**) Gibson Assembly 1 (GA1) backbone (GenBank accession MW079271). (**b**) Product of Gibson Assembly 1 (GA1) after insertion of an scFv sequence (GenBank accession MW079272). (**c**) The linear Gibson Assembly 2 (GA2) insert sequence (GenBank accession MW079275). (**d**) Product of Gibson Assembly 2 (GA2) after insertion of the GA2 insert (GenBank accession MW079273).

Supplementary Figure S3



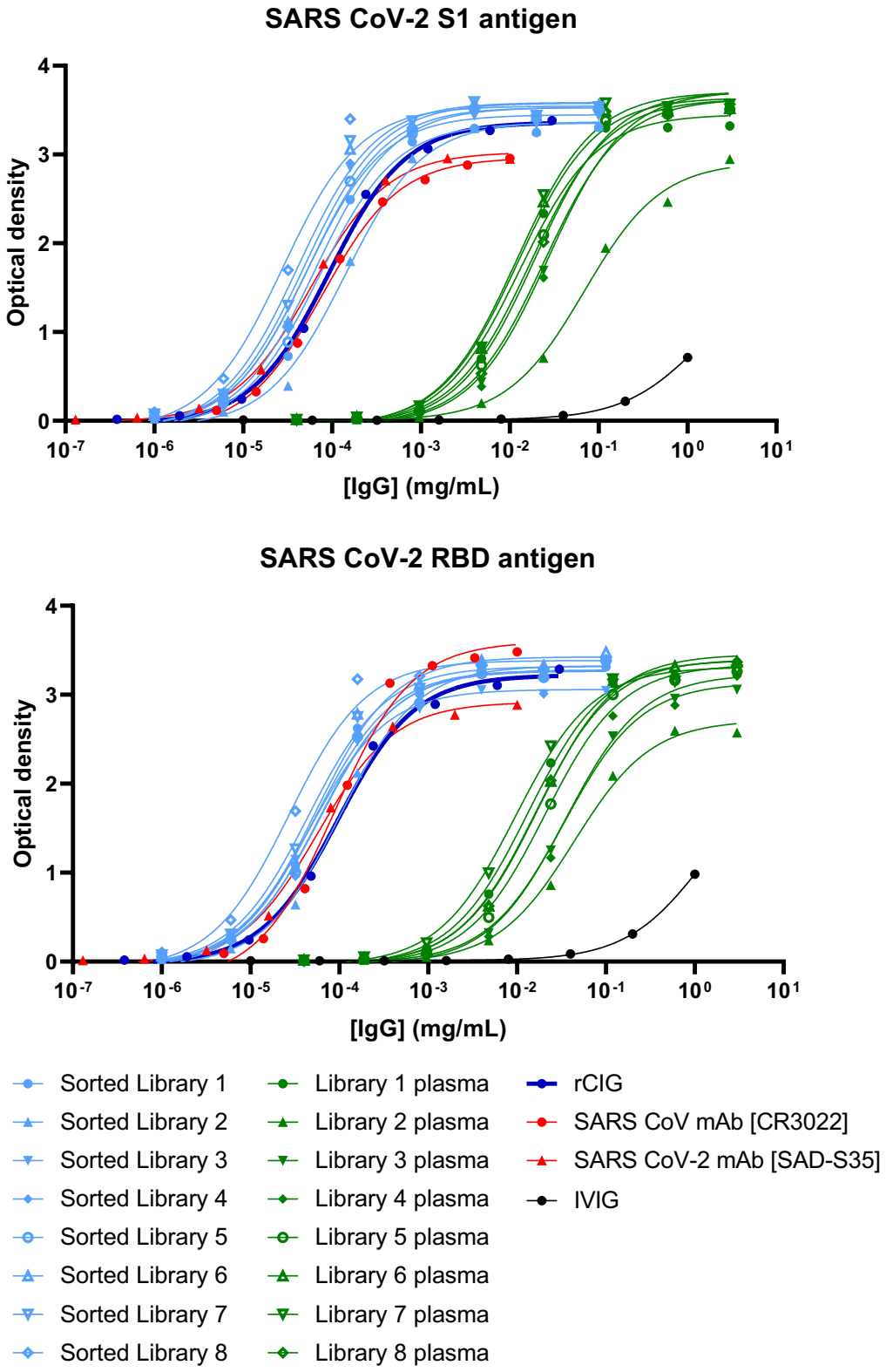
Landing pad plasmid sequence. Landing pad plasmid used to generate a custom CHO landing pad cell line (GenBank accession MW079274).

Supplementary Figure S4



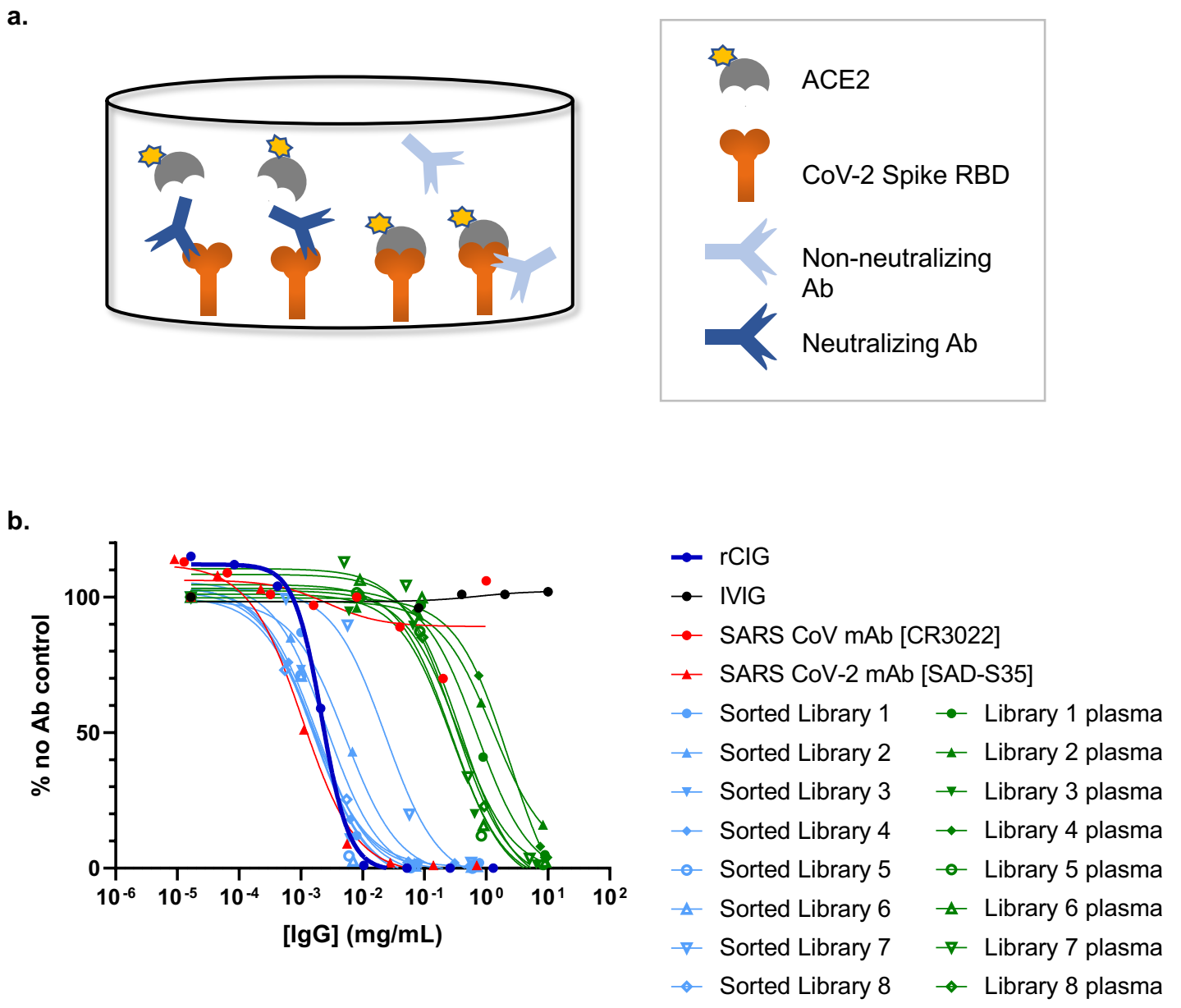
Flow sorting for SARS-CoV-2 specific antibodies using yeast scFv display. (**a**) Gating strategy used for each yeast sort. (**b**) The x-axis measures presence of a C-terminal c-Myc tag, indicating expression of an scFv on the surface of the cell. The y-axis measures binding of antigen to the scFv-expressing cells. The gates used for yeast selection (double positive) are indicated, with the percentage of scFv-expressed RBD binders in red. An irrelevant negative control and the eight rCIG convalescent donor libraries were stained with 1200 nM biotinylated CoV-2 RBD-His. An average of 1.1% of the expressed antibodies were RBD-specific on the first sort. After the second sort, the RBD-specific scFv were amplified and then cloned into full-length antibody expression plasmids. The data for the negative control and Library 1 are the same as that used in Figure 1b.

Supplementary Figure S5



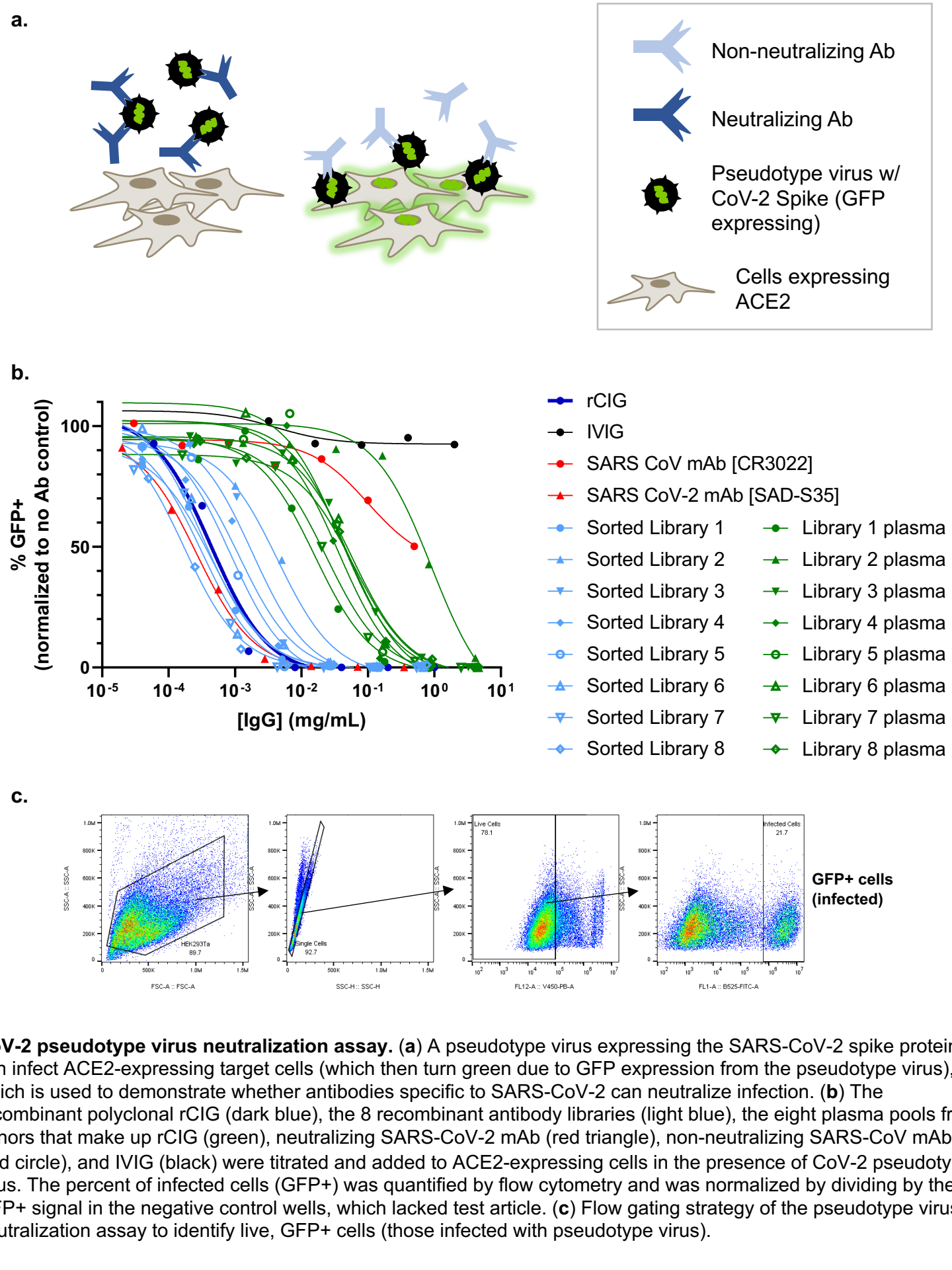
CoV-2 S1- and RBD-specific antibody binding by ELISA. rCIG (dark blue), the 8 recombinant libraries (light blue), the 8 plasma pools from donors that made up rCIG (green), neutralizing SARS-CoV-2 mAb (red triangle), non-neutralizing SARS-CoV mAb (red circle), and IVIG (black) were titrated relative to total IgG concentration.

Supplementary Figure S6



CoV-2 Spike:ACE2 inhibition assay. (**a**) The blocking ability of SARS-CoV-2 specific antibodies were measured using a plate-based ELISA method. Spike RBD was used to coat the plate and after the antibody samples are co-incubated, ACE2 was added and measured for binding to RBD; antibodies that block the interaction demonstrate low or no binding of ACE2 and are considered neutralizing. (**b**) The recombinant polyclonal rCIG (dark blue), the 8 recombinant antibody libraries (light blue), the 8 plasma pools from donors that make up rCIG (green), neutralizing SARS-CoV-2 mAb (red triangle), non-neutralizing SARS-CoV mAb (red circle), and IVIG (black) were titrated and added to the RBD coated plate. The data are reported as "% no Ab control", i.e., dividing the signal of the test article by the signal of a no Ab control.

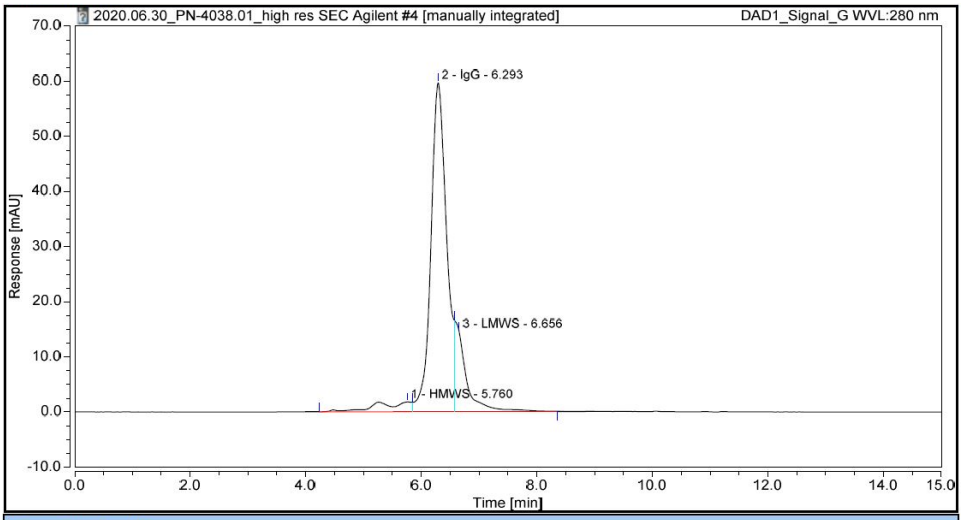
Supplementary Figure S7



CoV-2 pseudotype virus neutralization assay. (**a**) A pseudotype virus expressing the SARS-CoV-2 spike proteins can infect ACE2-expressing target cells (which then turn green due to GFP expression from the pseudotype virus), which is used to demonstrate whether antibodies specific to SARS-CoV-2 can neutralize infection. (**b**) The recombinant polyclonal rCIG (dark blue), the 8 recombinant antibody libraries (light blue), the eight plasma pools from donors that make up rCIG (green), neutralizing SARS-CoV-2 mAb (red triangle), non-neutralizing SARS-CoV mAb (red circle), and IVIG (black) were titrated and added to ACE2-expressing cells in the presence of CoV-2 pseudotype virus. The percent of infected cells (GFP+) was quantified by flow cytometry and was normalized by dividing by the GFP+ signal in the negative control wells, which lacked test article. (**c**) Flow gating strategy of the pseudotype virus neutralization assay to identify live, GFP+ cells (those infected with pseudotype virus).

Supplementary Figure S8

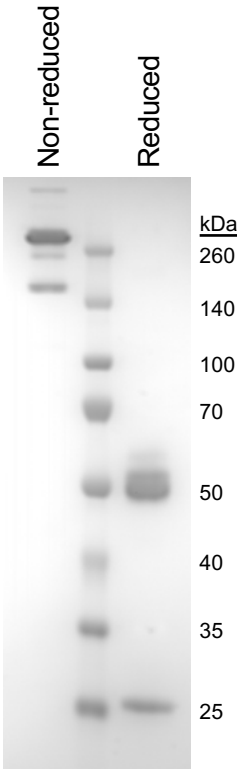
a.



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.760	1.263	1.794	5.34	2.36	n.a.
2	IgG	6.293	18.573	59.684	78.57	78.52	n.a.
3	LMWS	6.656	3.802	14.538	16.09	19.12	n.a.
Total:			**23.639**	**76.016**	**100.00**	**100.00**	

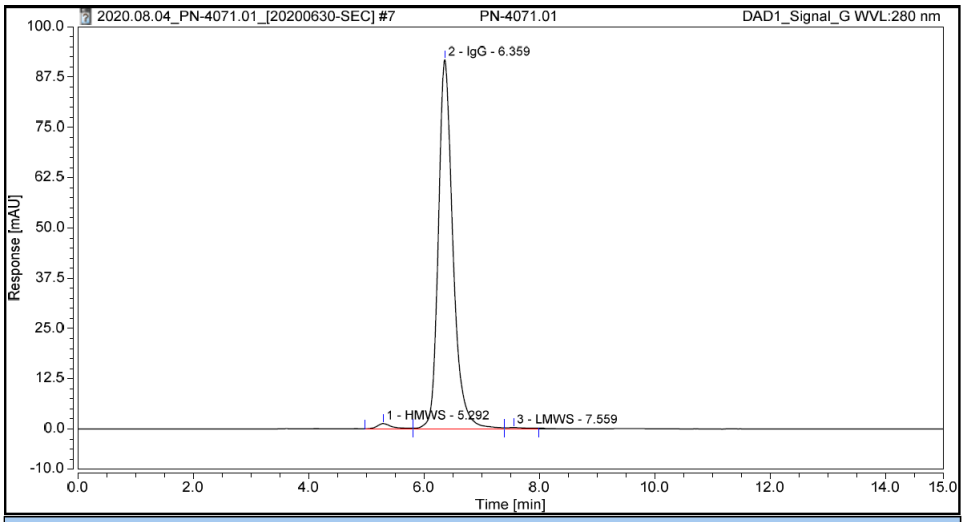
b.



Quality control analysis of purified rCIG protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S9

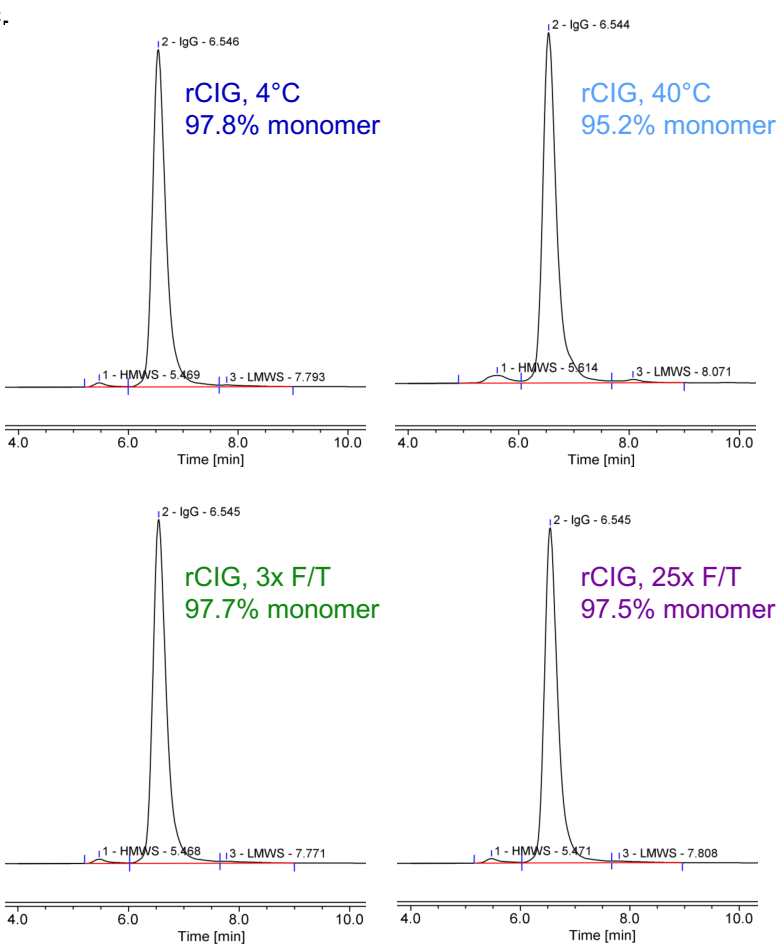
a.



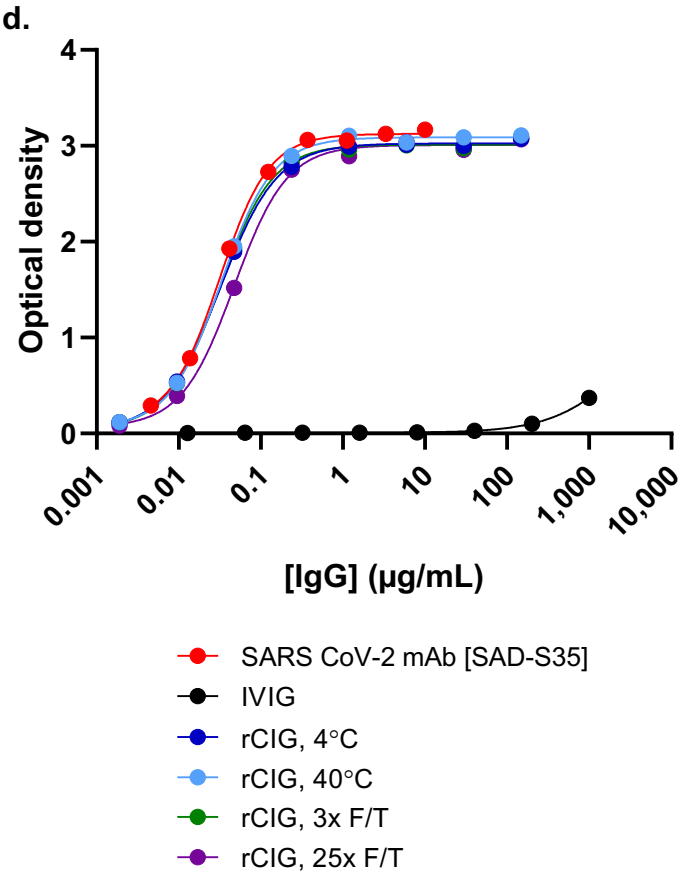
Integration Results							
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.292	0.361	1.277	1.32	1.37	n.a.
2	IgG	6.359	26.934	91.791	98.28	98.33	n.a.
3	LMWS	7.559	0.111	0.278	0.40	0.30	n.a.
Total:			**27.405**	**93.345**	**100.00**	**100.00**	

b.

c.

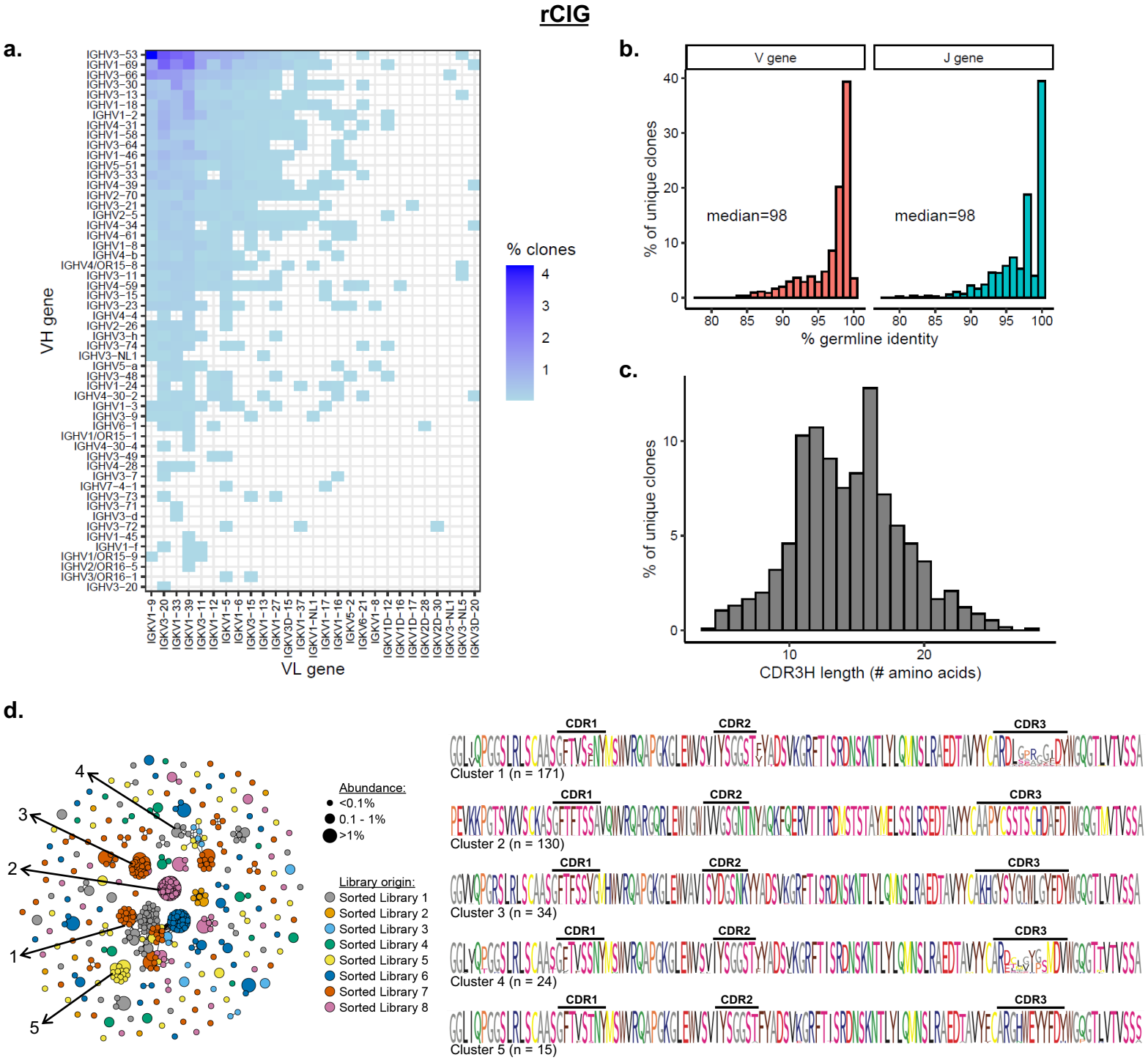


d.



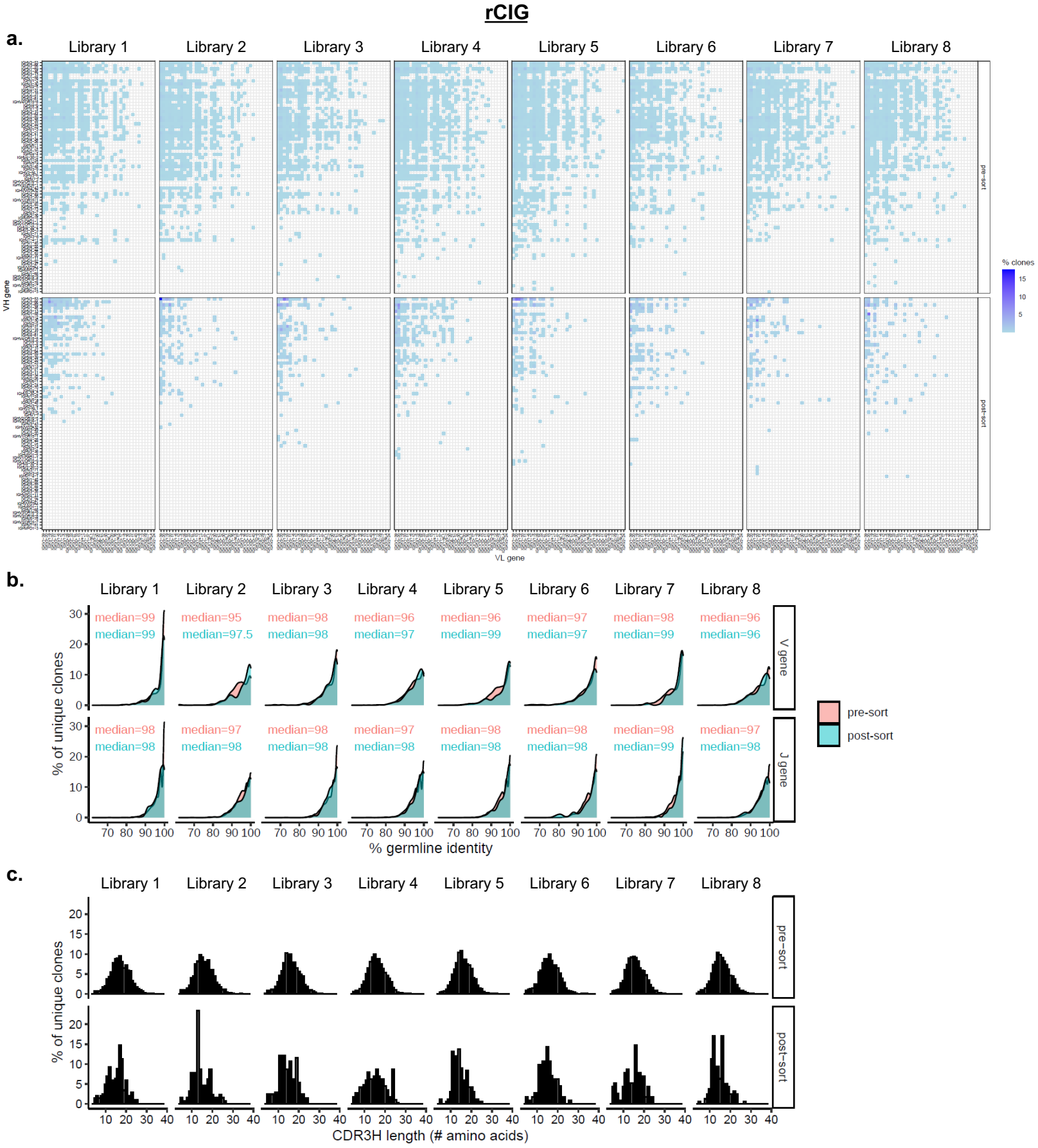
Stability of polished rCIG protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the polished rCIG protein. (**c**) SEC-HPLC and (**d**) SARS-CoV-2 S1 ELISA were used to assess the purity and function of the rCIG protein after incubation at 4°C or at 40°C for 14 days, or after undergoing 3 or 25 freeze-thaw cycles (F/T). 40°C incubation weakly increased the high molecular weight species with no change in SARS CoV-2 ELISA binding, while 25x F/T had no change in purity but had ~50% reduced binding to SARS CoV-2.

Supplementary Figure S10



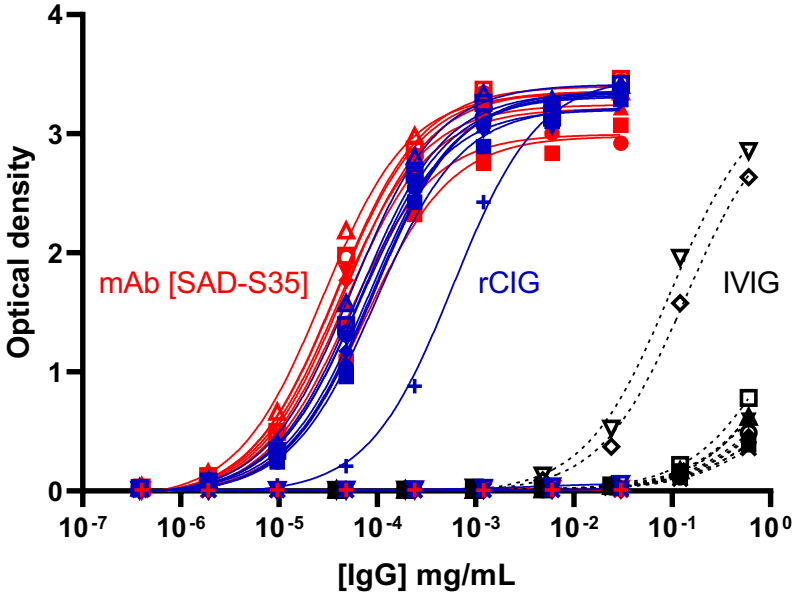
Repertoire sequencing analysis of rCIG. (**a**) Heatmap showing antibody variable (V) gene usage from the linked scFv library (the 8 sorted libraries combined). The x-axis and y-axis show light and heavy chain V genes, respectively. The color represents percent unique clone abundance in the library. (**b**) Histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel), from the final CHO library. (**c**) Histogram showing the distribution of heavy chain CDR3 amino acid length, from the final CHO library. (**d**) Left: The clonal cluster analysis of rCIG antibodies from Figure 2c (from the final CHO library). Right: Sequence logos of all heavy chain sequences from the top five clusters (based on clone count). The first 8 amino acids (variable region primer binding sites) are not shown.

Supplementary Figure S11



Repertoire sequencing analysis of pre- and post-sort rCIG. (**a**) Heatmap showing antibody variable (V) gene usage from each pre-sort and post-sort linked scFv library. (**b**) Histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel) from each pre-sort and post-sort scFv library. (**c**) Histogram showing the distribution of heavy chain CDR3 amino acid length from each pre-sort and post-sort scFv library.

Supplementary Figure S12



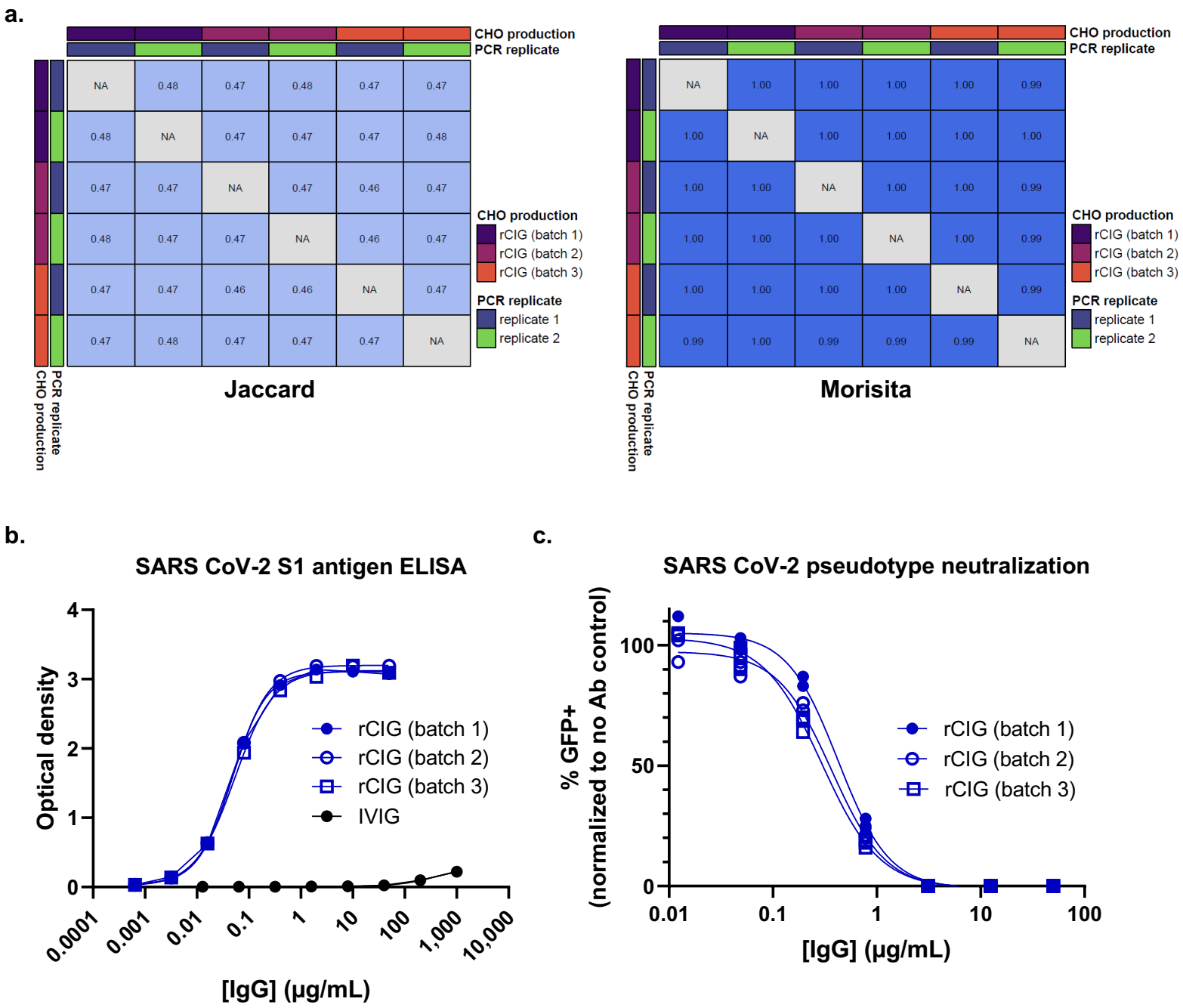
- CoV-2 S1
- CoV-2 RBD
- CoV-2 S (R683A, R685A)
- CoV-2 RBD (N354D)
- CoV-2 RBD (N354D, D364Y)
- CoV-2 RBD (V367F)
- CoV-2 RBD (W436R)
- CoV-2 S (D614G)
- *HCoV-229E S1
- *HCoV-NL63 S1
- *MERS CoV S1
- *SARS CoV RBD

- CoV-2 S1
- CoV-2 RBD
- CoV-2 S (R683A, R685A)
- CoV-2 RBD (N354D)
- CoV-2 RBD (N354D, D364Y)
- CoV-2 RBD (V367F)
- CoV-2 RBD (W436R)
- CoV-2 S (D614G)
- *HCoV-229E S1
- *HCoV-NL63 S1
- *MERS-CoV S1
- SARS-CoV RBD

- CoV-2 S1
- CoV-2 RBD
- CoV-2 S (R683A, R685A)
- CoV-2 RBD (N354D)
- CoV-2 RBD (N354D, D364Y)
- CoV-2 RBD (V367F)
- CoV-2 RBD (W436R)
- CoV-2 S (D614G)
- HCoV-229E S1
- HCoV-NL63 S1
- MERS-CoV S1
- SARS-CoV RBD

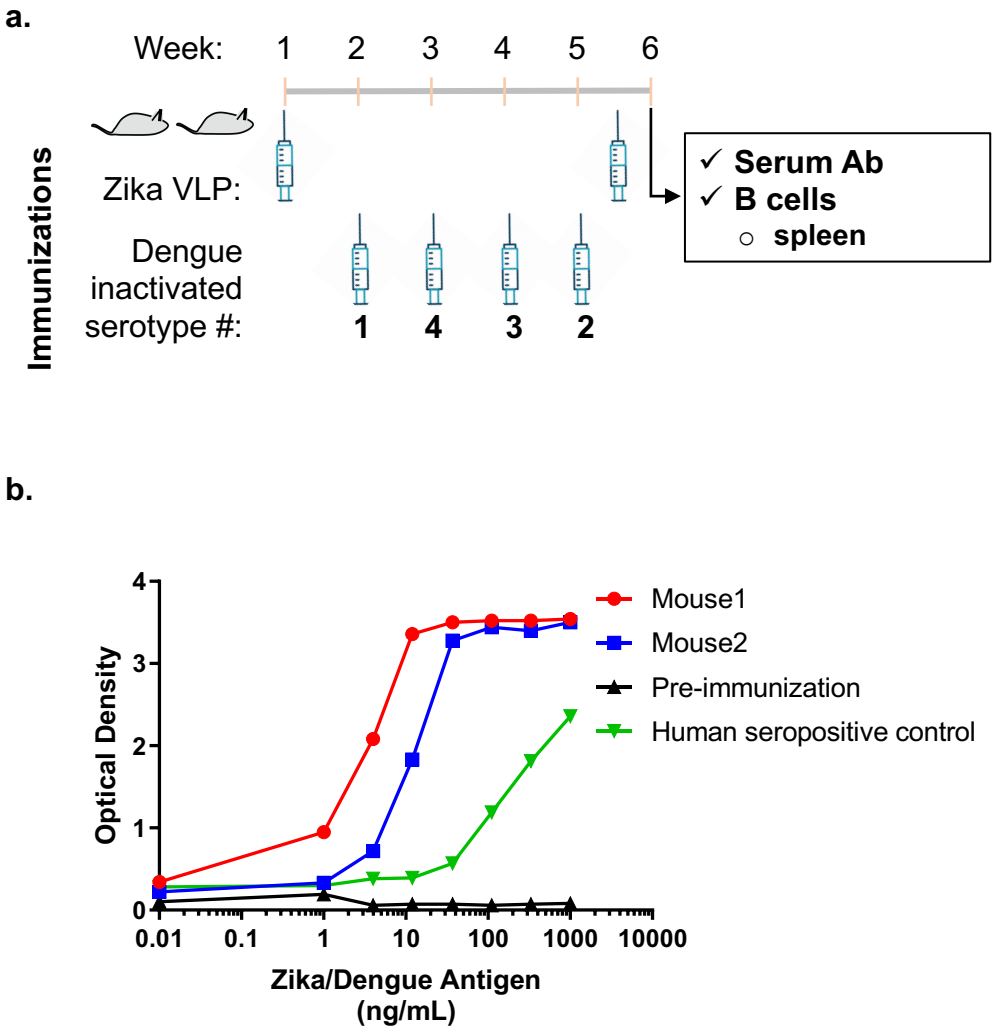
Antibody reactivity to SARS-CoV-2 variants and other coronaviruses. ELISA plates were coated with 2 µg/mL of spike or RBD proteins from known circulating variants of SARS-CoV-2, SARS-CoV, MERS, and other human coronaviruses (HCoV). The binding ability of rCIG (blue), SARS-CoV-2 neutralizing mAb (SAD-S35, red), and IVIG (black) was determined for each antigen. *, No binding was observed against the indicated antigen. While rCIG and the mAb had poly-variant specific responses to all SARS-CoV-2 variants, only rCIG bound SARS-CoV RBD while the mAb did not. IVIG had no specific responses to SARS-CoV-2, SARS-CoV or MERS but did have a weak response to HCoV-229E and HCoV-NL63.

Supplementary Figure S13



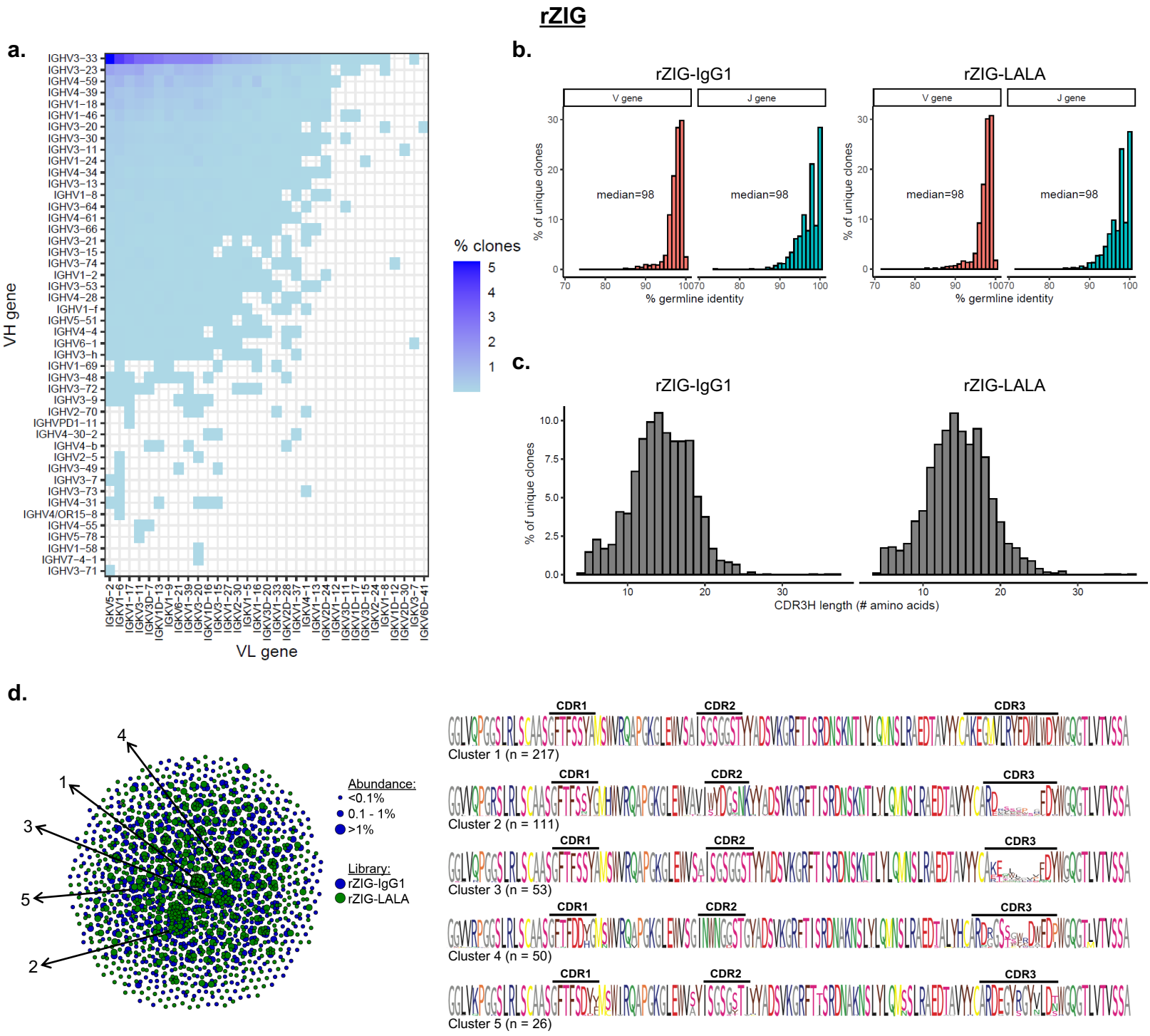
Batch-to-batch variation of rCIG. (**a**) RNA antibody sequencing was performed (in duplicate) on RNA isolated from the end of replicate CHO bioreactor production runs. Jaccard (left) and Morisita (right) analyses showed that the amount of antibody clone variation between production batches and between PCR replicates performed on each batch. Wilcoxon rank sum tests showed that the indices from the PCR replicates came from the same population as the indices from the batch replicates ($p>0.05$), suggesting that the variability inherent to the batches was no worse than the variability between PCR replicates. (**b**) Binding of the indicated batch of serially diluted rCIG (blue) and IVIG (black) to SARS CoV-2 S1 antigen was measured by ELISA. (**c**) The indicated batch of rCIG was titrated and added to ACE2-expressing cells in the presence of CoV-2 pseudotype virus. The percent of infected cells (GFP+) was quantified by flow cytometry and was normalized by dividing by the GFP+ signal in the negative control wells, which lacked test article. Feltz & Miller's asymptotic test was used to test whether the three bioproduction batch IC50 measurements had a different coefficient of variation from eight IC50 measurements on a fourth bioproduction batch (18% vs. 17%, respectively). A p-value > 0.05 suggested that the variation inherent to the batches was no worse than the variation inherent to the pseudotype neutralization assay.

Supplementary Figure S14



Immunization induced antibody responses to Zika and Dengue in humanized mice. (**a**) Two Trianni mice were immunized weekly with Zika virus like particles (VLP), inactivated Dengue 1, Dengue 4, Dengue 3, or Dengue 2 as indicated in the figure. (**b**) After week 5, serum from the mice was tested for antibody response against a mixture of Zika and Dengue antigens and compared to pre-immunization or human seropositive controls to confirm antigen-specific antibody responses.

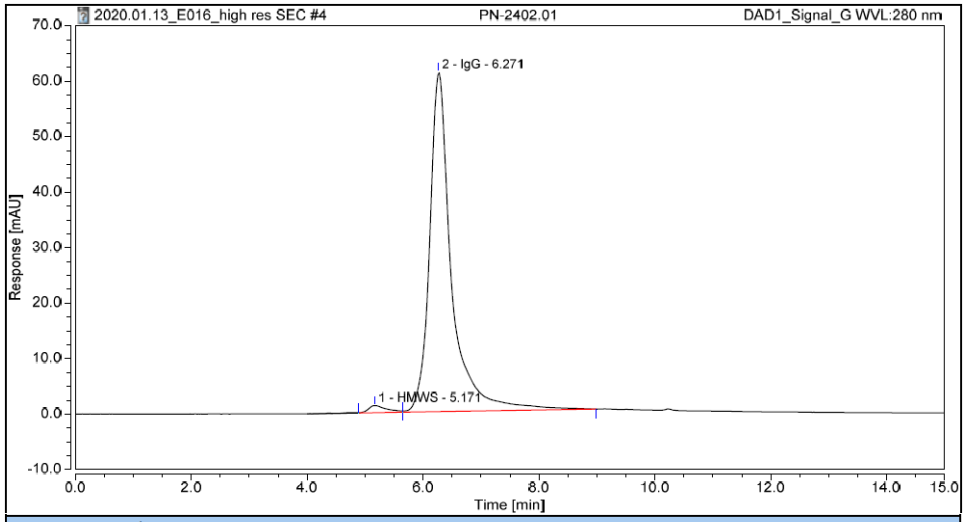
Supplementary Figure S15



Repertoire sequencing analysis of rZIG. (**a**) Heatmap showing antibody variable (V) gene usage from the linked scFv library. The x-axis and y-axis show light and heavy chain V genes, respectively. The color represents percent unique clone abundance in the library. (**b**) For each indicated rZIG library, histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel), from the final CHO library. (**c**) For each indicated rZIG library, histogram showing the distribution of heavy chain CDR3 amino acid length, from the final CHO library. (**d**) Left: The clonal cluster analysis of rZIG antibodies from Figure 3a (from the final CHO library). Right: Sequence logos of all heavy chain sequences from the top five clusters (based on clone count). The first 8 amino acids (variable region primer binding sites) are not shown.

Supplementary Figure S16

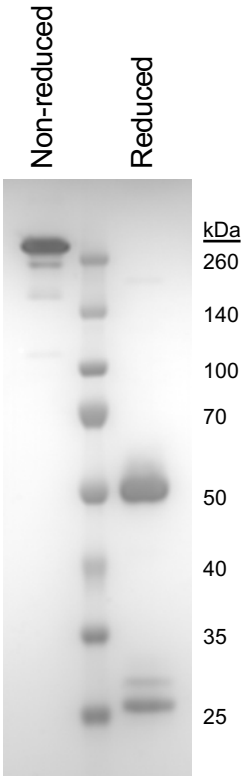
a.



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.171	0.476	1.308	1.74	2.09	n.a.
2	IgG	6.271	26.797	61.146	98.26	97.91	n.a.
Total:			**27.273**	**62.455**	**100.00**	**100.00**	

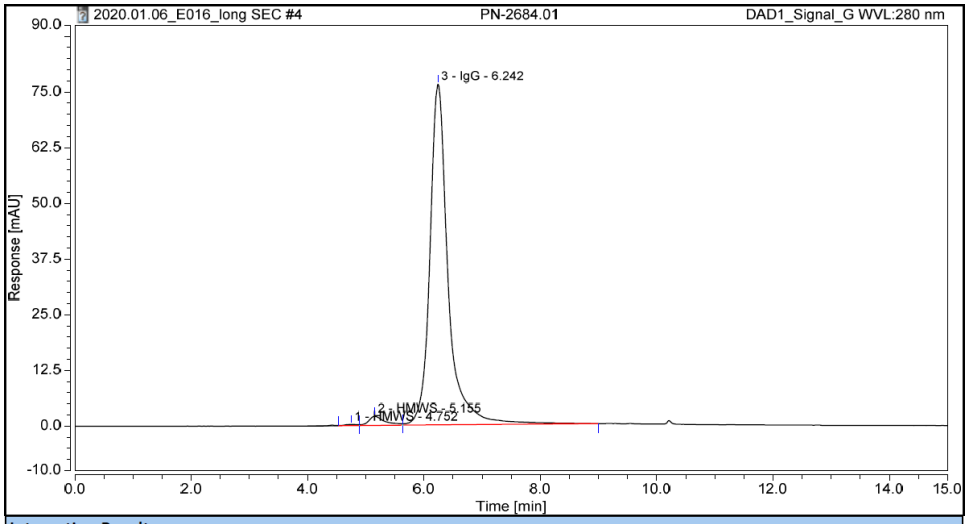
b.



Quality control analysis of purified rZIG-IgG1 protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S17

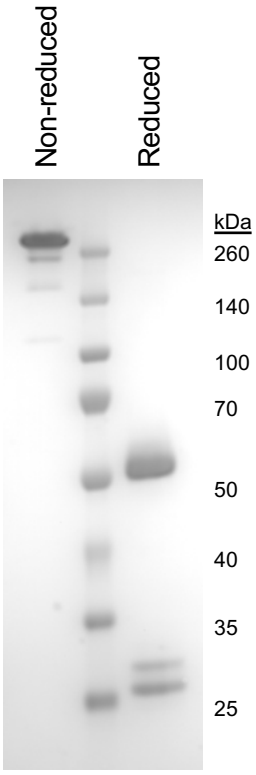
a.



Integration Results

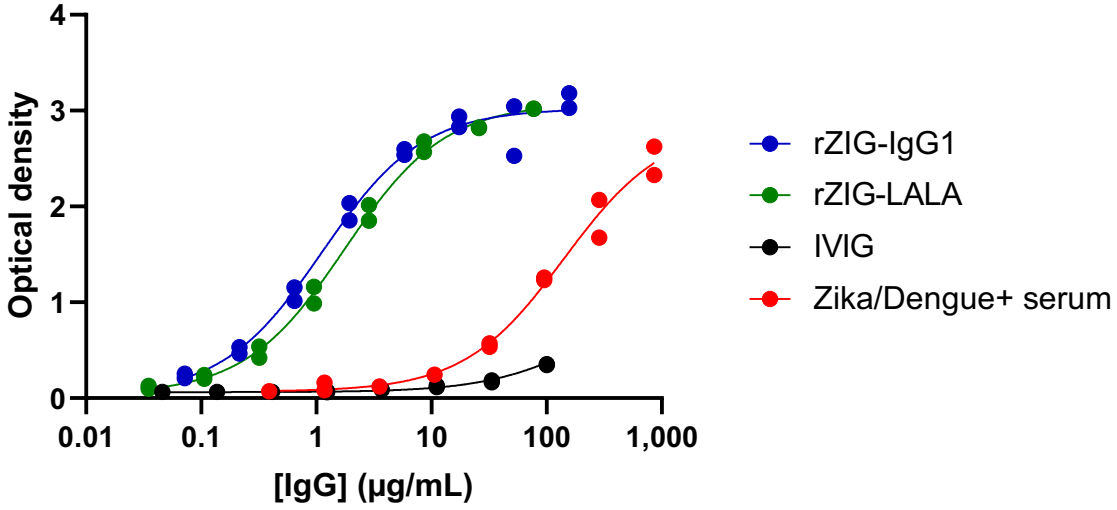
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	4.752	0.071	0.280	0.25	0.36	n.a.
2	HMWS	5.155	0.668	2.054	2.34	2.61	n.a.
3	IgG	6.242	27.850	76.469	97.41	97.04	n.a.
Total:			**28.589**	**78.803**	**100.00**	**100.00**	

b.



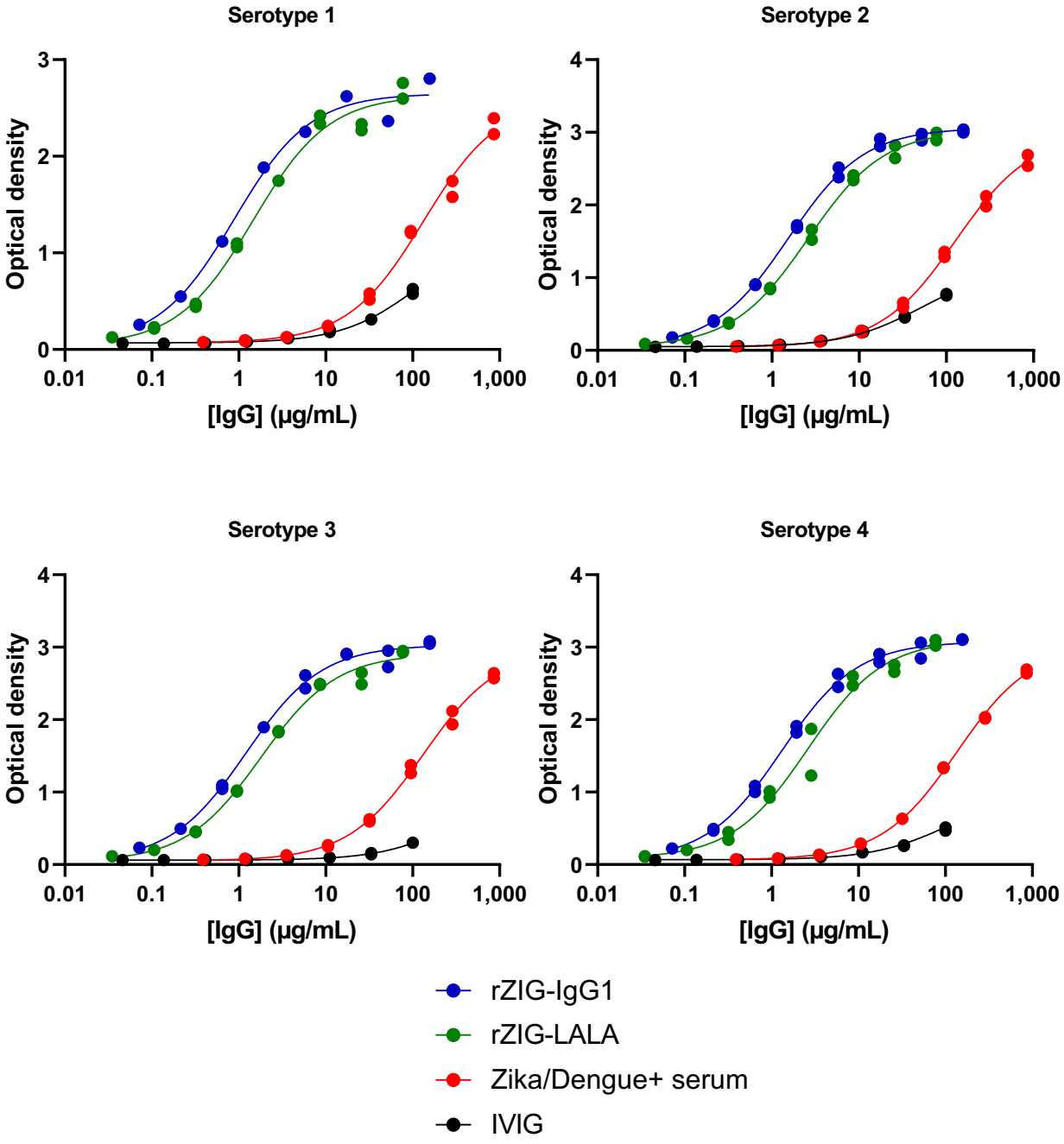
Quality control analysis of purified rZIG-LALA protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S18



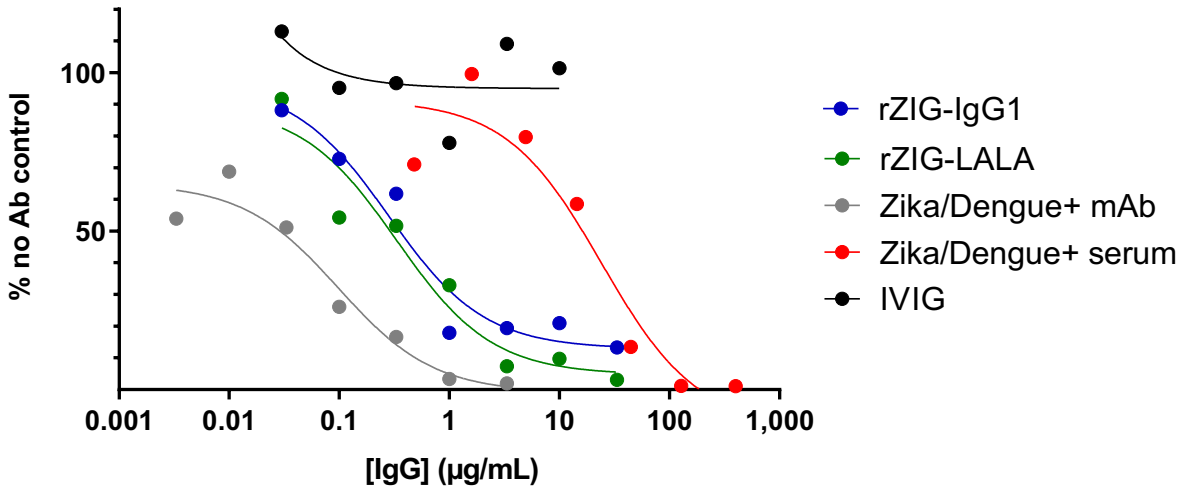
rZIG binding to Zika by ELISA. rZIG-IgG1 (blue), rZIG-LALA (green), negative control IVIG (black), and positive control Zika/Dengue positive serum (red) were serially diluted and added to a Zika envelope-coated plate. Antigen-specific responses were quantified by an anti-human-HRP secondary antibody.

Supplementary Figure S19



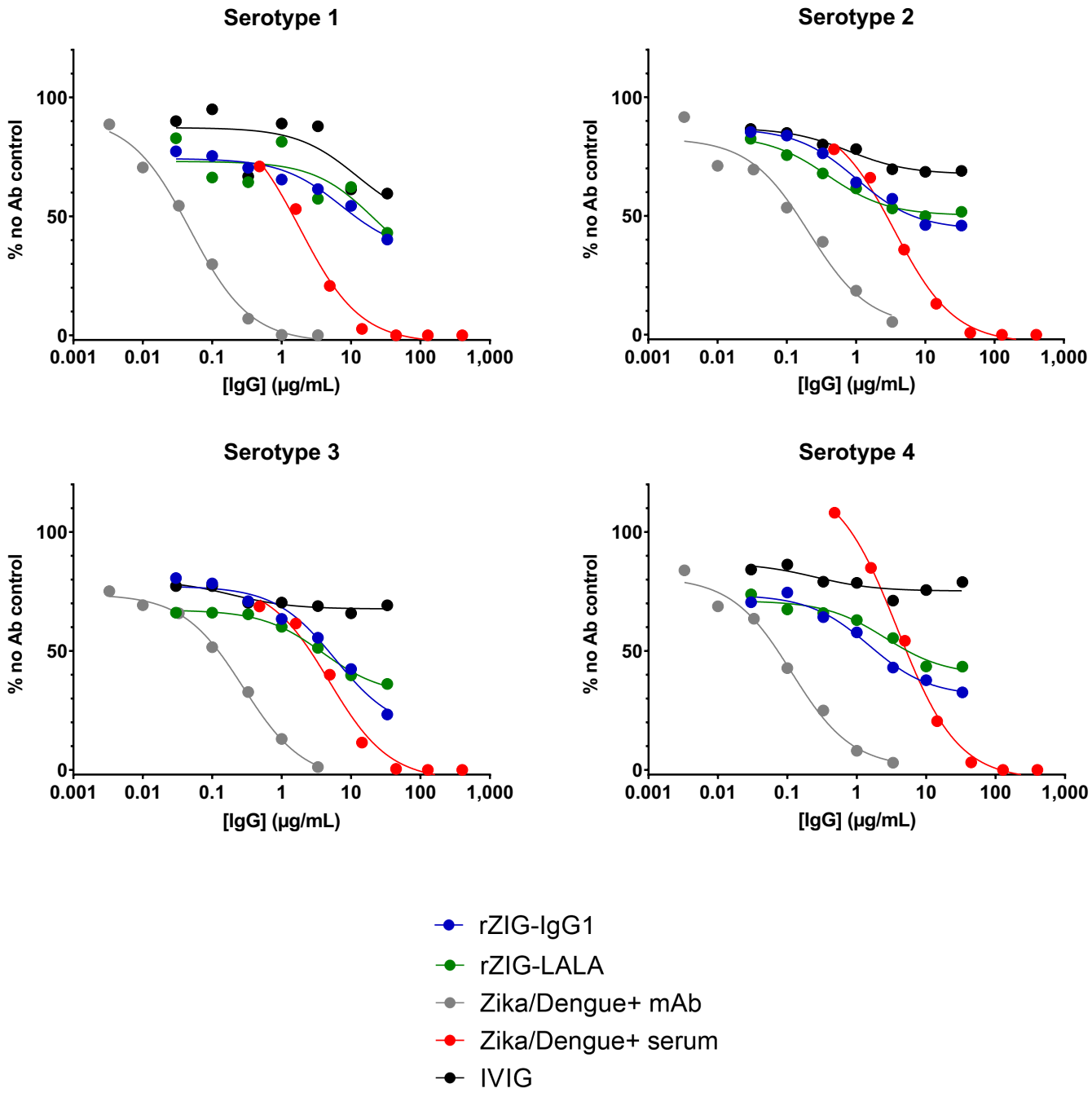
rZIG binding to Dengue serotypes by ELISA. rZIG-IgG1 (blue), rZIG-LALA (green), negative control IVIG (black), and positive control Zika/Dengue positive serum (red) were serially diluted and added to a Dengue serotypes 1, 2, 3, or 4 envelope-coated plates. Antigen-specific responses were quantified by an anti-human-HRP secondary antibody.

Supplementary Figure S20



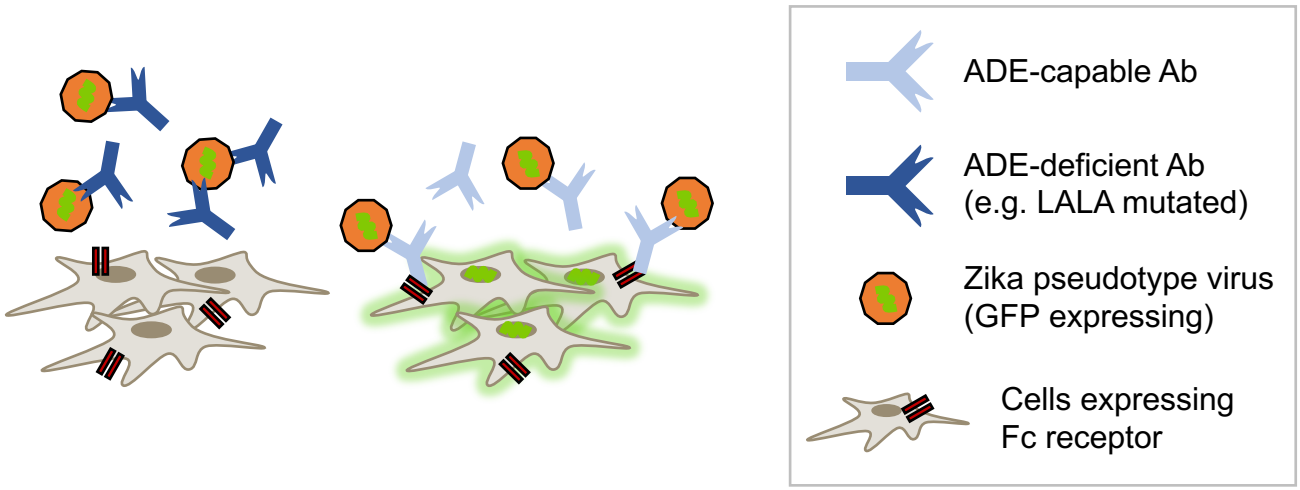
Zika pseudotype virus neutralization assay. rZIG-IgG1 (blue), rZIG-LALA (green), negative control IVIG (black), positive control Zika/Dengue mAb (gray), and positive control Zika/Dengue positive serum (red) were serially diluted, co-incubated with Zika pseudotype virus, and added to BHK/DC-SIGN target cells. Antibody induced neutralization was quantified by the infection-induced luciferase expression divided by luciferase expression in the no Ab control.

Supplementary Figure S21



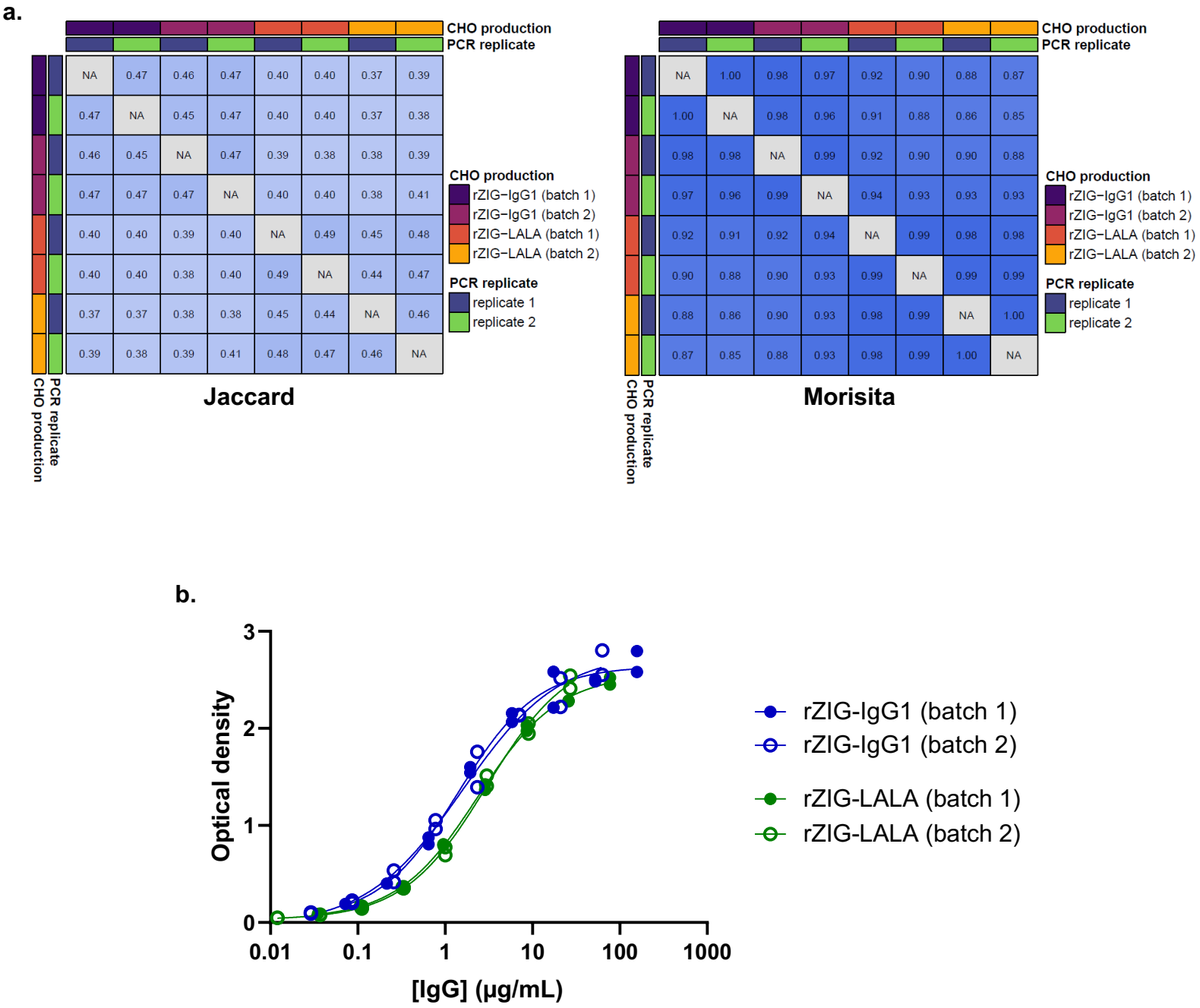
Dengue serotype pseudotype virus neutralization assay. rZIG-IgG1 (blue), rZIG-LALA (green), negative control IVIG (black), positive control Zika/Dengue mAb (gray), and positive control Zika/Dengue positive serum (red) were serially diluted, co-incubated with pseudotype virus expressing the indicated Dengue serotype envelope antigens, and added to BHK/DC-SIGN target cells. Antibody induced neutralization was quantified as "% no Ab control", or infection-induced luciferase expression divided by the luciferase expression in the no Ab control.

Supplementary Figure S22



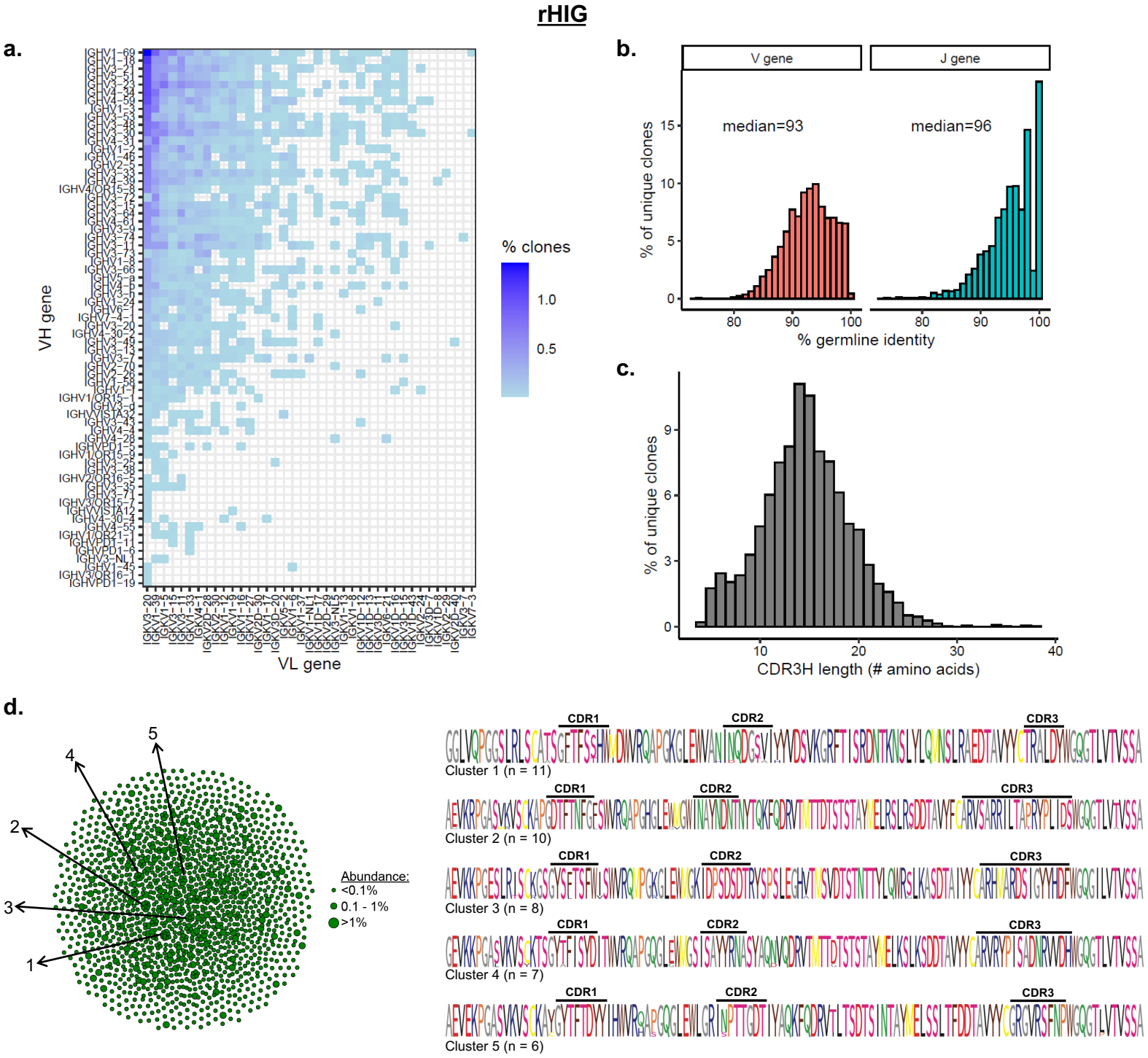
Schematic of antibody dependent enhancement (ADE) assay. Antibody bound to pseudotype virus can infect cells through the Fc receptor expressed on target cells. By introducing an Fc mutation that prevents FcR binding (e.g. LALA), antibody-induced viral infection is abrogated.

Supplementary Figure S23



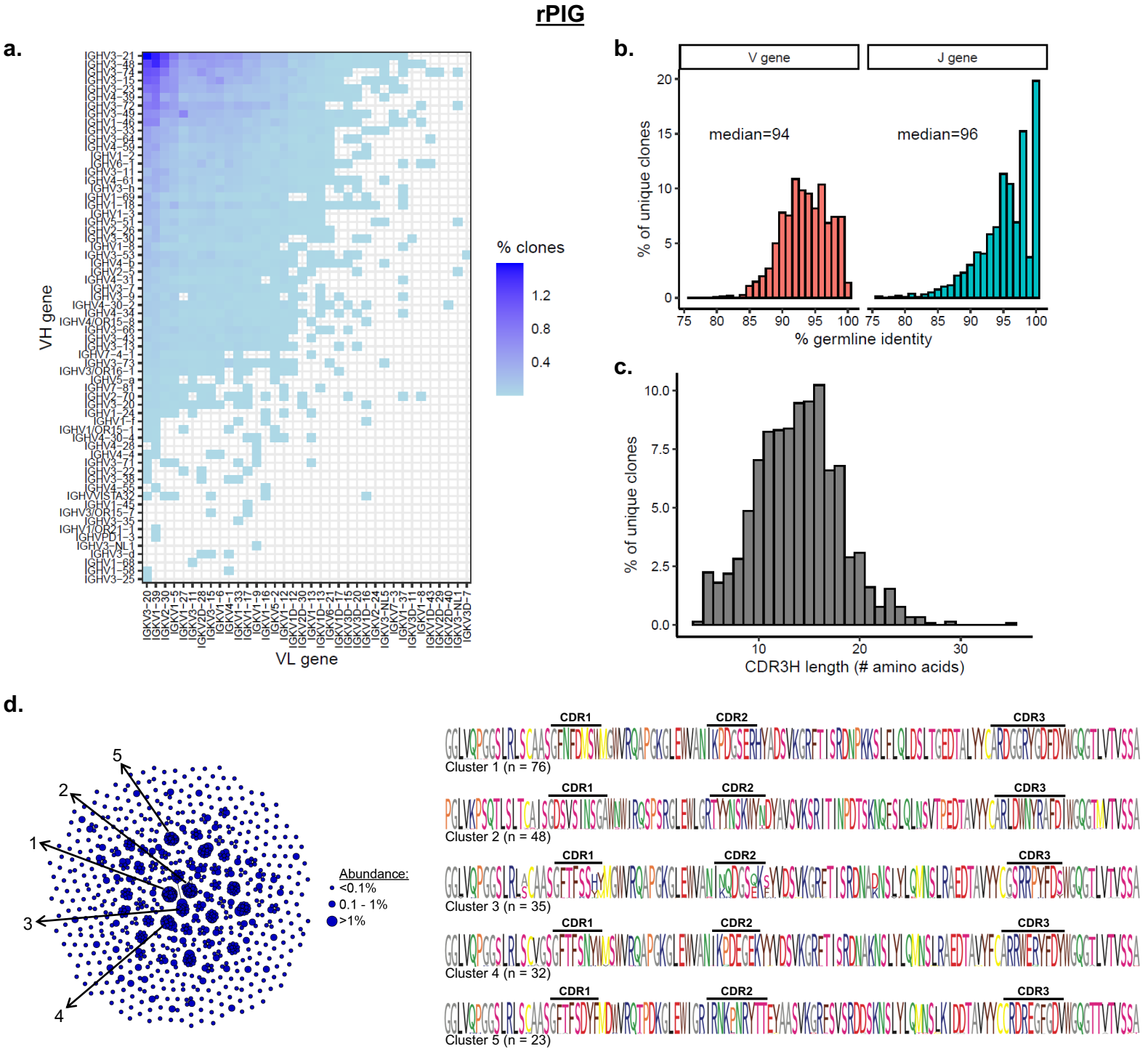
Batch-to-batch variation of rZIG. (**a**) RNA antibody sequencing was performed (in duplicate) on RNA isolated from the end of replicate CHO bioreactor production runs. Jaccard (left) and Morisita (right) analyses showed that the amount of antibody clone variation between production batches and between PCR replicates performed on each batch. Wilcoxon rank sum tests showed that the indices from the PCR replicates came from the same population as the indices from the batch replicates, suggesting that the variability inherent to the batches was no worse than the variability between PCR replicates. (**b**) Binding of the indicated batch of serially diluted rZIG-IgG1 (blue) and rZIG-LALA (green) to a Zika envelope-coated plate was measured by ELISA.

Supplementary Figure S24



Repertoire sequencing analysis of rHIG. (**a**) Heatmap showing antibody variable (V) gene usage from the linked scFv library. The x-axis and y-axis show light and heavy chain V genes, respectively. The color represents percent unique clone abundance in the library. (**b**) Histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel), from the final CHO library. (**c**) Histogram showing the distribution of heavy chain CDR3 amino acid length, from the final CHO library. (**d**) Left: The clonal cluster analysis of rHIG antibodies, modified from Figure 4a (from the final CHO library). Right: Sequence logos of all heavy chain sequences from the top five clusters (based on clone count). The first 8 amino acids (variable region primer binding sites) are not shown.

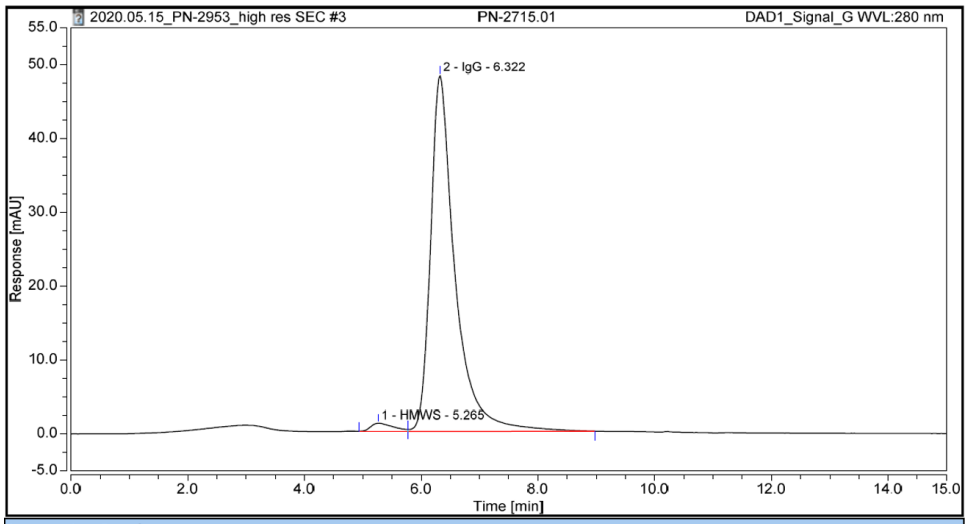
Supplementary Figure S25



Repertoire sequencing analysis of rPIG. (**a**) Heatmap showing antibody variable (V) gene usage from the linked scFv library. The x-axis and y-axis show light and heavy chain V genes, respectively. The color represents percent unique clone abundance in the library. (**b**) Histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel), from the final CHO library. (**c**) Histogram showing the distribution of heavy chain CDR3 amino acid length, from the final CHO library. (**d**) Left: The clonal cluster analysis of rPIG antibodies, modified from Figure 4a (from the final CHO library). Right: Sequence logos of all heavy chain sequences from the top five clusters (based on clone count). The first 8 amino acids (variable region primer binding sites) are not shown.

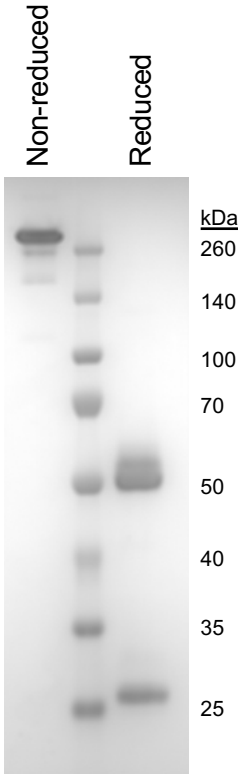
Supplementary Figure S26

a.



Integration Results							
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.265	0.495	1.112	2.07	2.26	n.a.
2	IgG	6.322	23.366	48.178	97.93	97.74	n.a.
Total:			**23.861**	**49.290**	**100.00**	**100.00**	

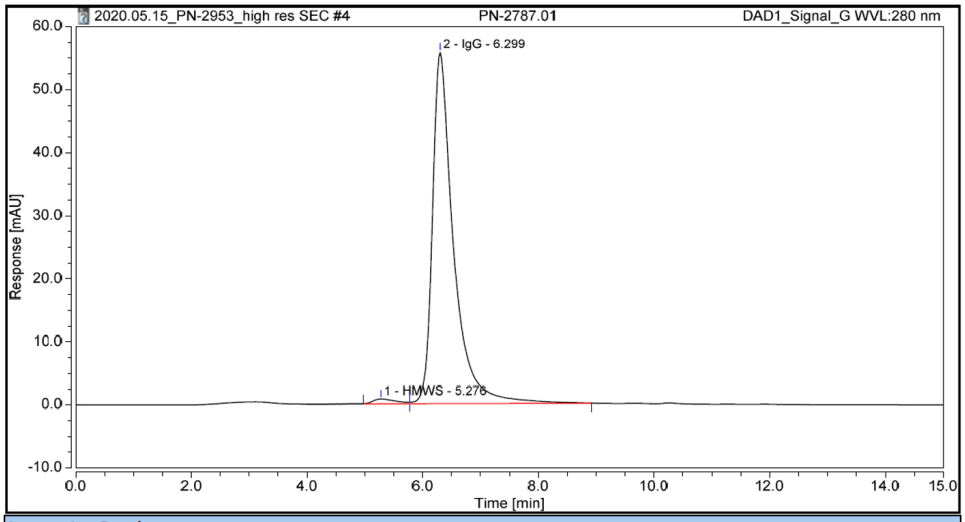
b.



Quality control analysis of purified rHIG protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S27

a.



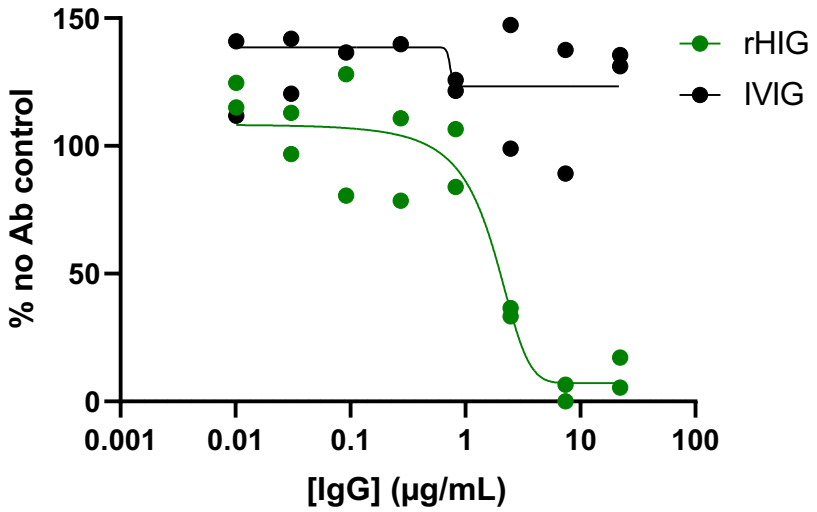
Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.276	0.358	0.780	1.49	1.38	n.a.
2	IgG	6.299	23.617	55.677	98.51	98.62	n.a.
Total:			**23.975**	**56.457**	**100.00**	**100.00**	

b.

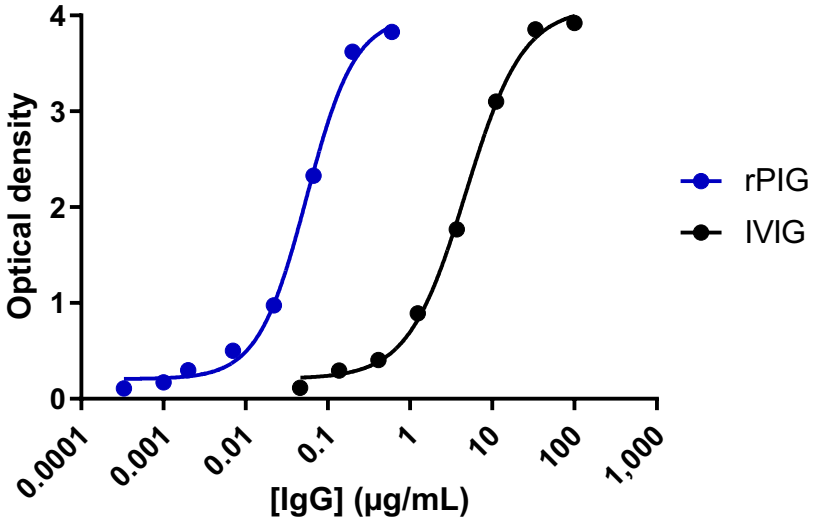
Quality control analysis of purified rPIG protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S28



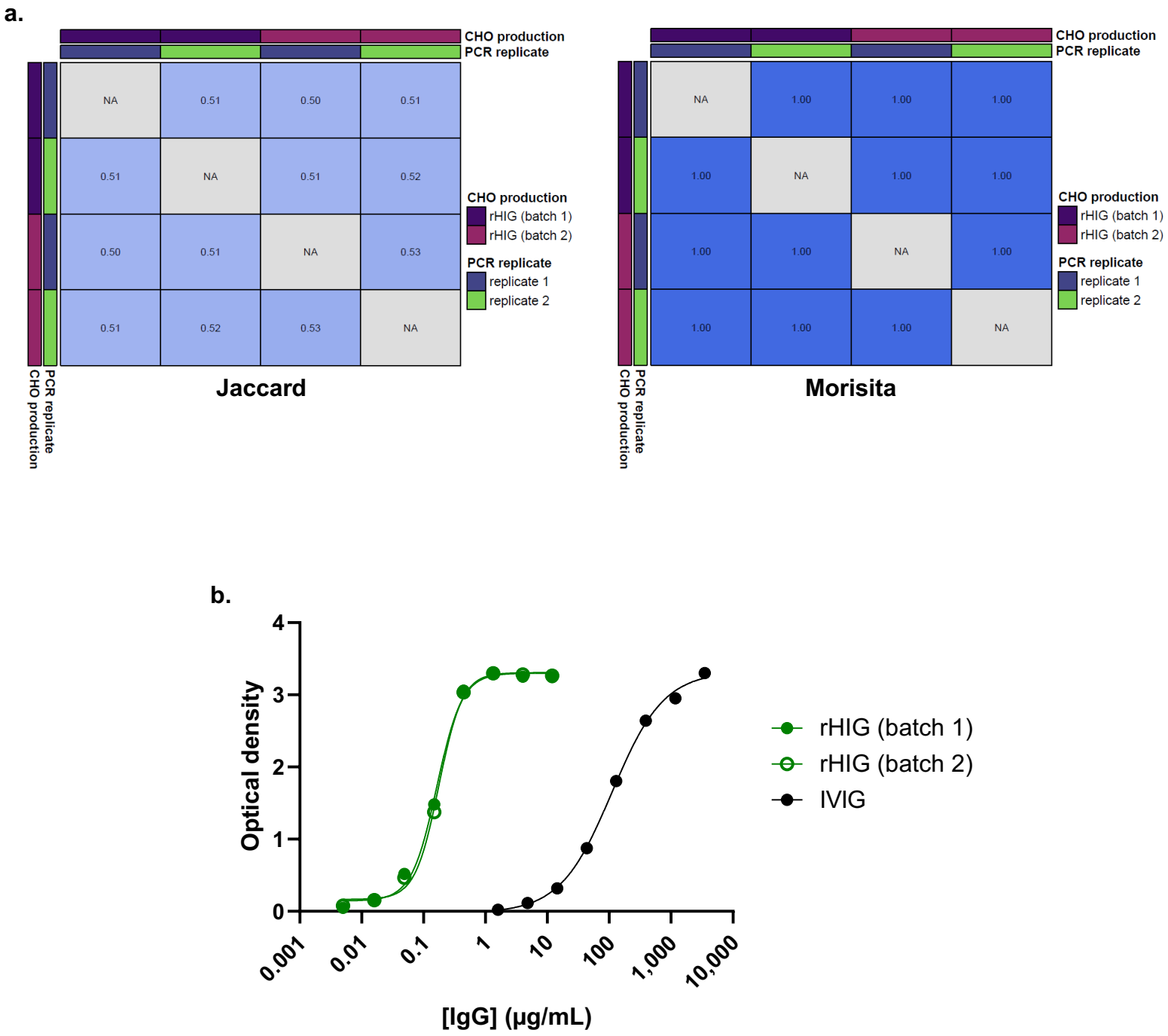
Haemophilus influenzae serum bactericidal assay (SBA). rHIG (green) and IVIG (black) were serially diluted and co-incubated with $5x10^4$ CFU/mL *Haemophilus influenzae* Eagan strain. After incubation, complement was added, incubated, and test samples were plated on chocolate agar. After 16 hours incubation, bacteria colony counts for each serial dilution were quantified and divided by the bacteria colony counts in the no Ab control.

Supplementary Figure S29



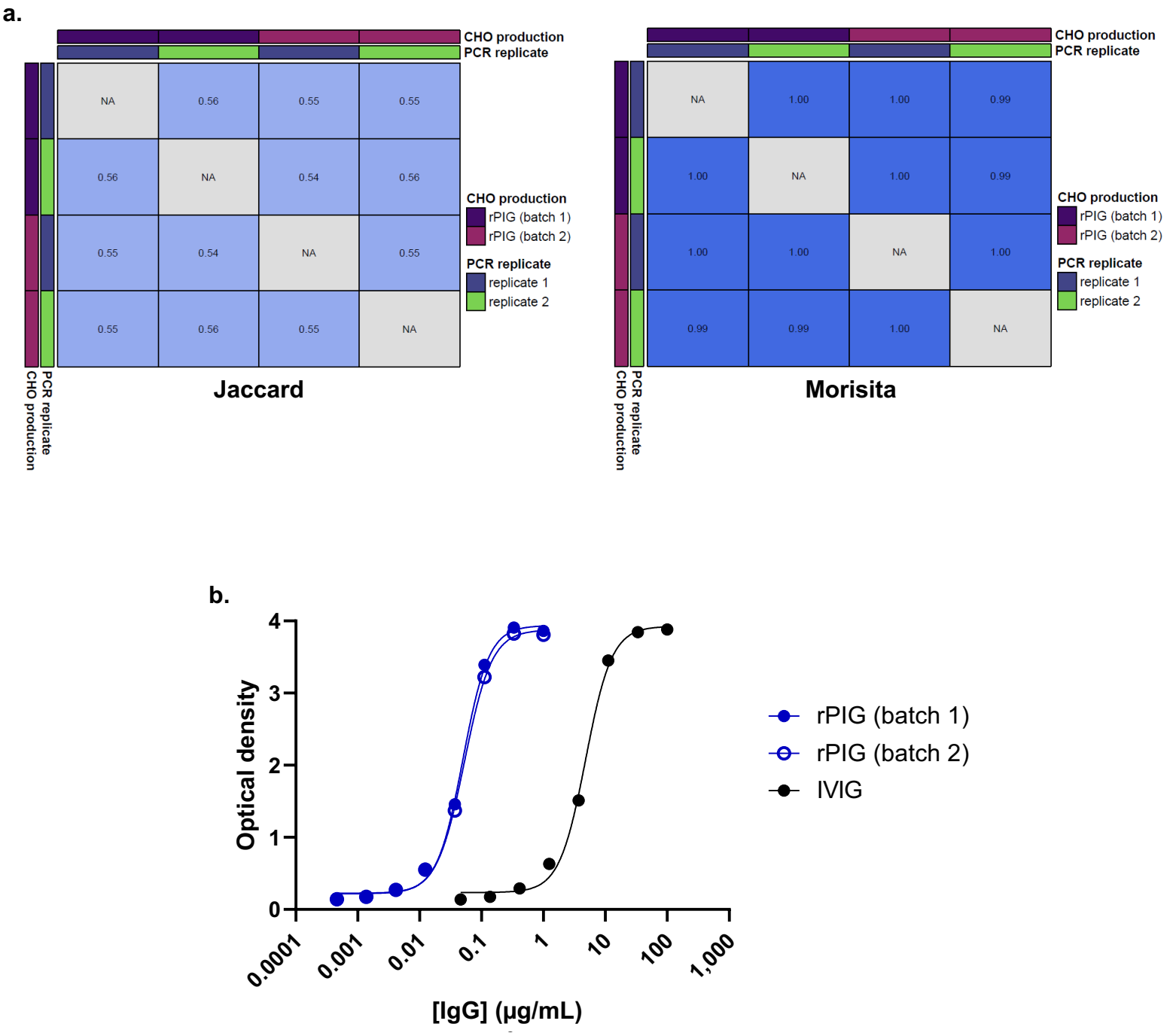
Pneumococcal antibody binding by ELISA. Binding of serially diluted rPIG (blue) and IVIG (black) to a pool of 23 pneumococcal polysaccharides was measured by ELISA.

Supplementary Figure S30



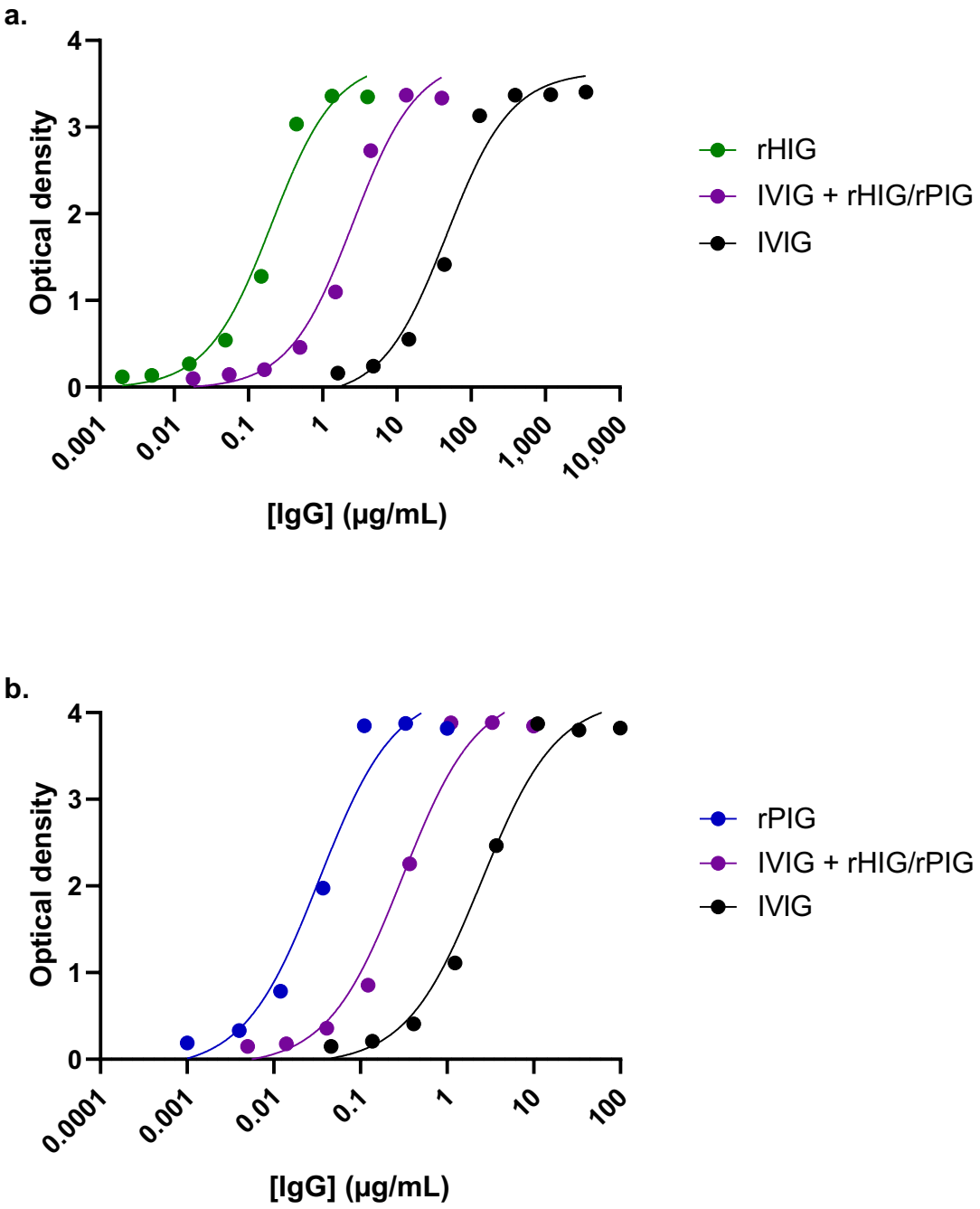
Batch-to-batch variation of rHIG. (**a**) RNA antibody sequencing was performed (in duplicate) on RNA isolated from the end of replicate CHO bioreactor production runs. Jaccard (left) and Morisita (right) analyses showed that the amount of antibody clone variation between production batches and between PCR replicates performed on each batch. Wilcoxon rank sum tests showed that the indices from the PCR replicates came from the same population as the indices from the batch replicates ($p>0.05$), suggesting that the variability inherent to the batches was no worse than the variability between PCR replicates. (**b**) Binding of the indicated batch of serially diluted rHIG (green) and IVIG (black) to Hib was measured by ELISA.

Supplementary Figure S31



Batch-to-batch variation of rPIG. (**a**) RNA antibody sequencing was performed (in duplicate) on RNA isolated from the end of replicate CHO bioreactor production runs. Jaccard (left) and Morisita (right) analyses showed that the amount of antibody clone variation between production batches and between PCR replicates performed on each batch. Wilcoxon rank sum tests showed that the indices from the PCR replicates came from the same population as the indices from the batch replicates ($p>0.05$), suggesting that the variability inherent to the batches was no worse than the variability between PCR replicates. (**b**) Binding of the indicated batch of serially diluted rPIG (blue) and IVIG (black) to a pool of 23 pneumococcal polysaccharides was measured by ELISA.

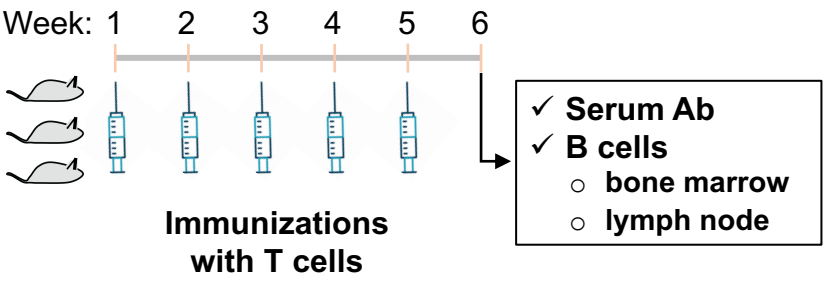
Supplementary Figure S32

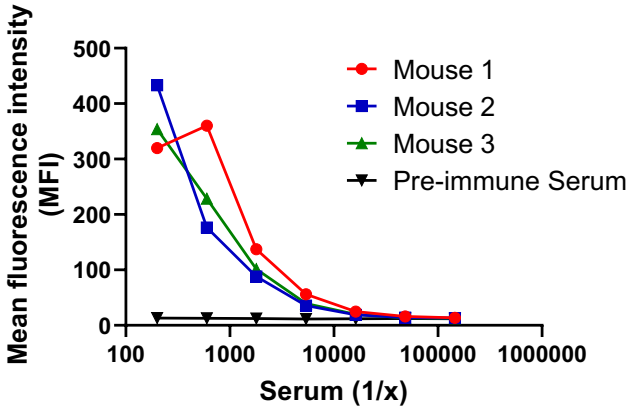


Pneumococcal or Hib antibody binding of IVIG + rHIG/rPIG by ELISA. (**a**) Binding of serially diluted rHIG (green), IVIG + rHIG/rPIG (purple), and IVIG (black) to Hib was measured by ELISA. (**b**) Binding of serially diluted rPIG (blue), IVIG + rHIG/rPIG (purple), and IVIG (black) to a pool of 23 pneumococcal polysaccharides was measured by ELISA.

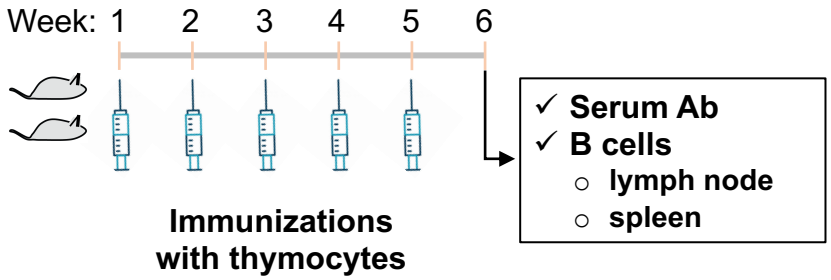
Supplementary Figure S33

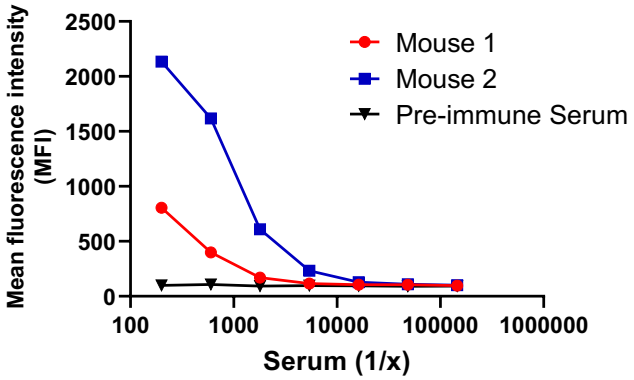
a.





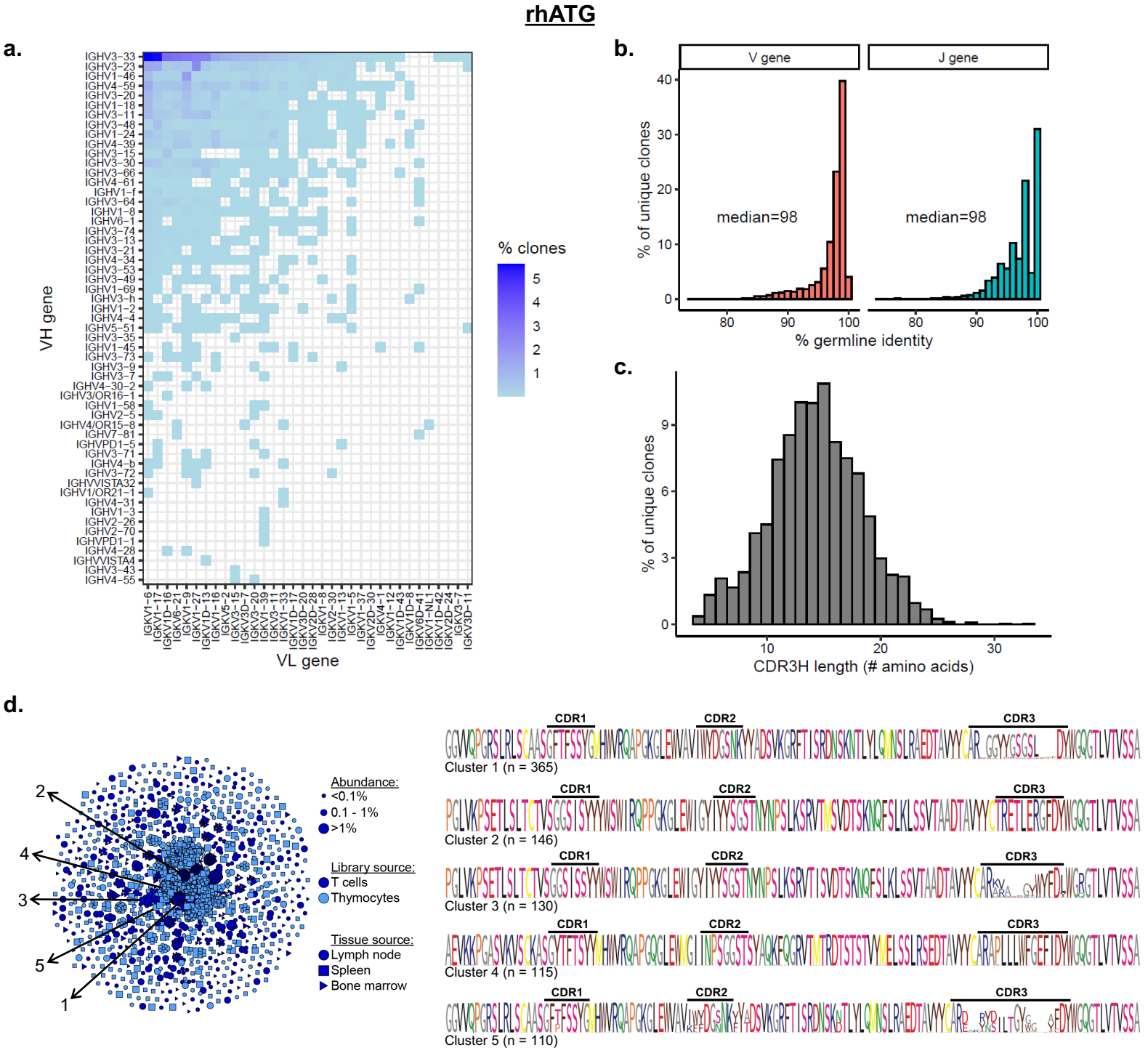
b.





Immunization induced antibody responses to human T cells and thymocytes. (**a**) Three Trianni mice were immunized weekly with T cells isolated from one human donor with ALD/MDP adjuvant. After week 5, serum from the mice was tested to confirm binding to T cells before a final boost without adjuvant 5 days prior to harvesting the organ B cells. (**b**) Two Trianni mice were immunized weekly with thymocytes isolated from five separate human donors with ALD/MDP adjuvant. After week 5, serum from the mice was tested to confirm binding to T cells before a final boost without adjuvant 5 days prior to harvesting the organ B cells.

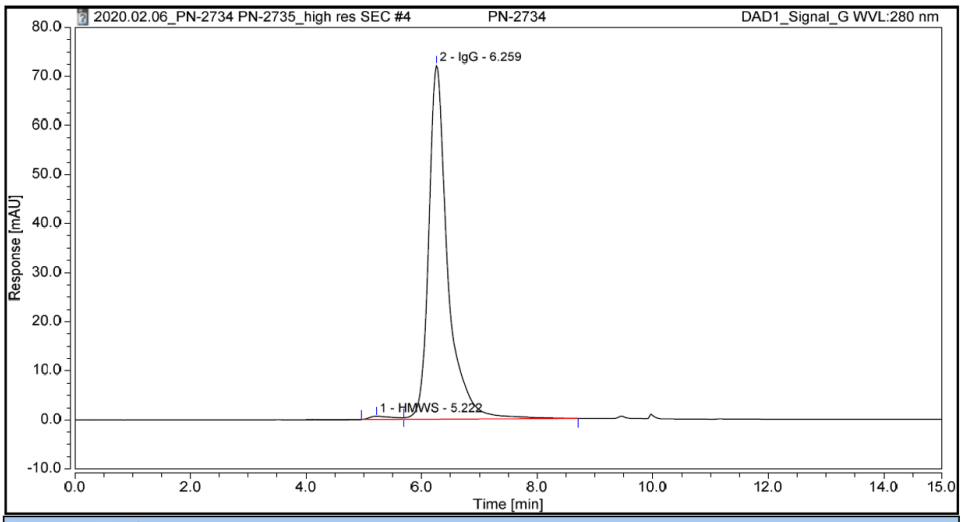
Supplementary Figure S34



Repertoire sequencing analysis of rhATG. (**a**) Heatmap showing antibody variable (V) gene usage from the linked scFv library (the 4 libraries combined). The x-axis and y-axis show light and heavy chain V genes, respectively. The color represents percent unique clone abundance in the library. (**b**) Histogram showing distribution of percent germline identity for variable gene (V; left panel) and joining gene (J; right panel), from the final CHO library. (**c**) Histogram showing the distribution of heavy chain CDR3 amino acid length, from the final CHO library. (**d**) Left: The clonal cluster analysis of rhATG antibodies from Figure 5a (from the CHO libraries). Right: Sequence logos of all heavy chain sequences from the top five clusters (based on clone count). The first 8 amino acids (variable region primer binding sites) are not shown.

Supplementary Figure S35

a.



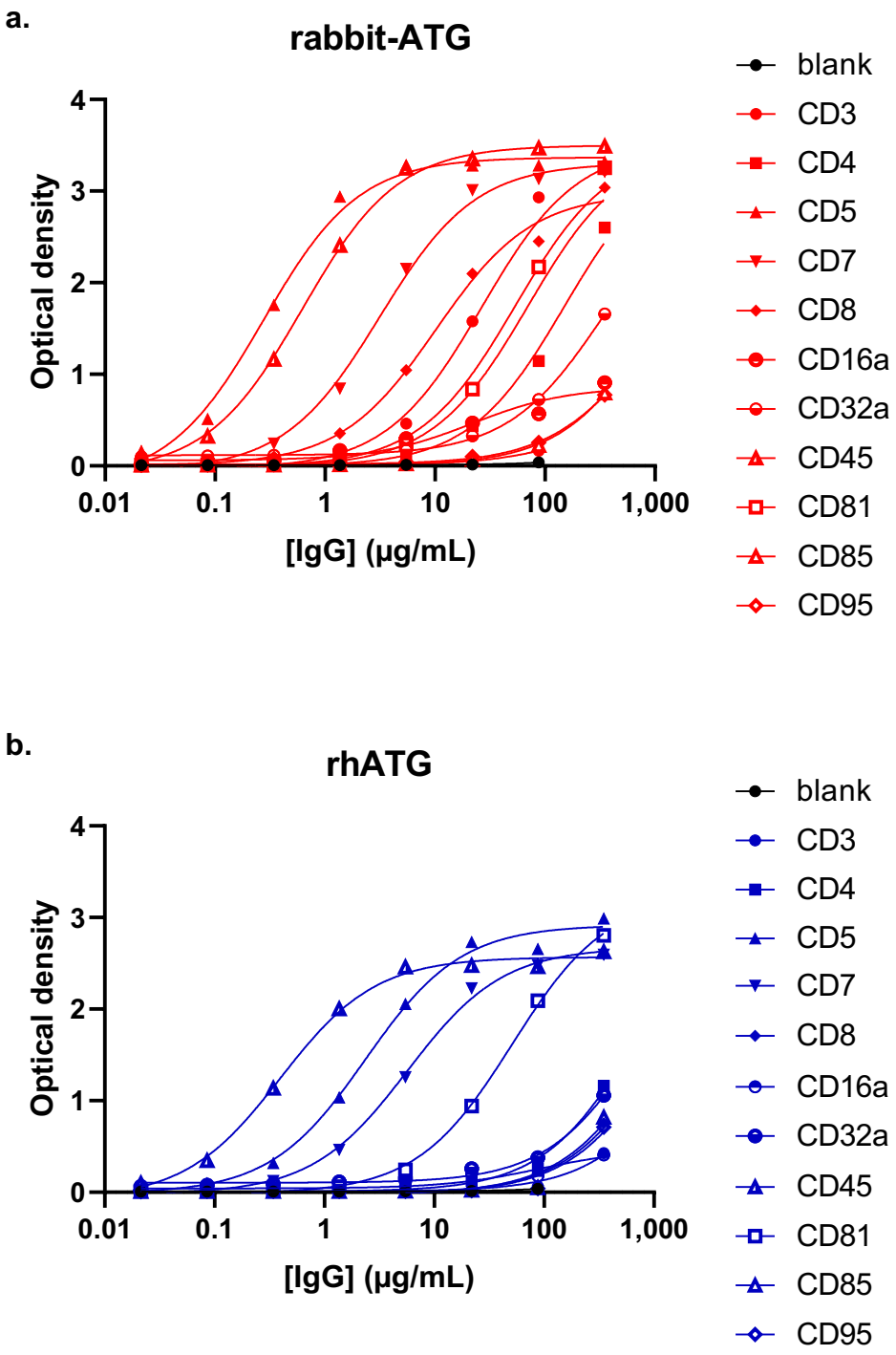
Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	HMWS	5.222	0.303	0.654	1.09	0.90	n.a.
2	IgG	6.259	27.528	72.139	98.91	99.10	n.a.
Total:			**27.831**	**72.792**	**100.00**	**100.00**	

b.

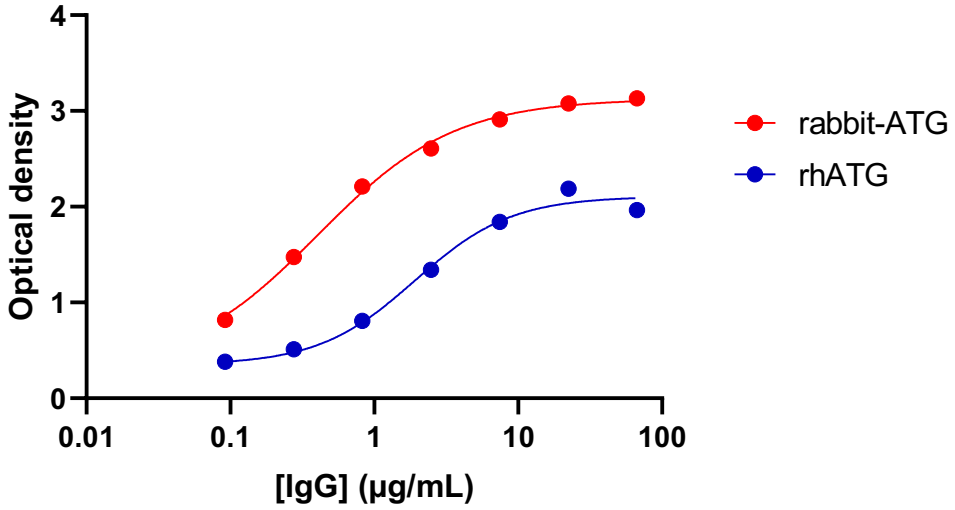
Quality control analysis of purified rhATG protein. (**a**) SEC-HPLC and (**b**) SDS-PAGE analysis were used to assess the purity of the Protein A-purified protein.

Supplementary Figure S36



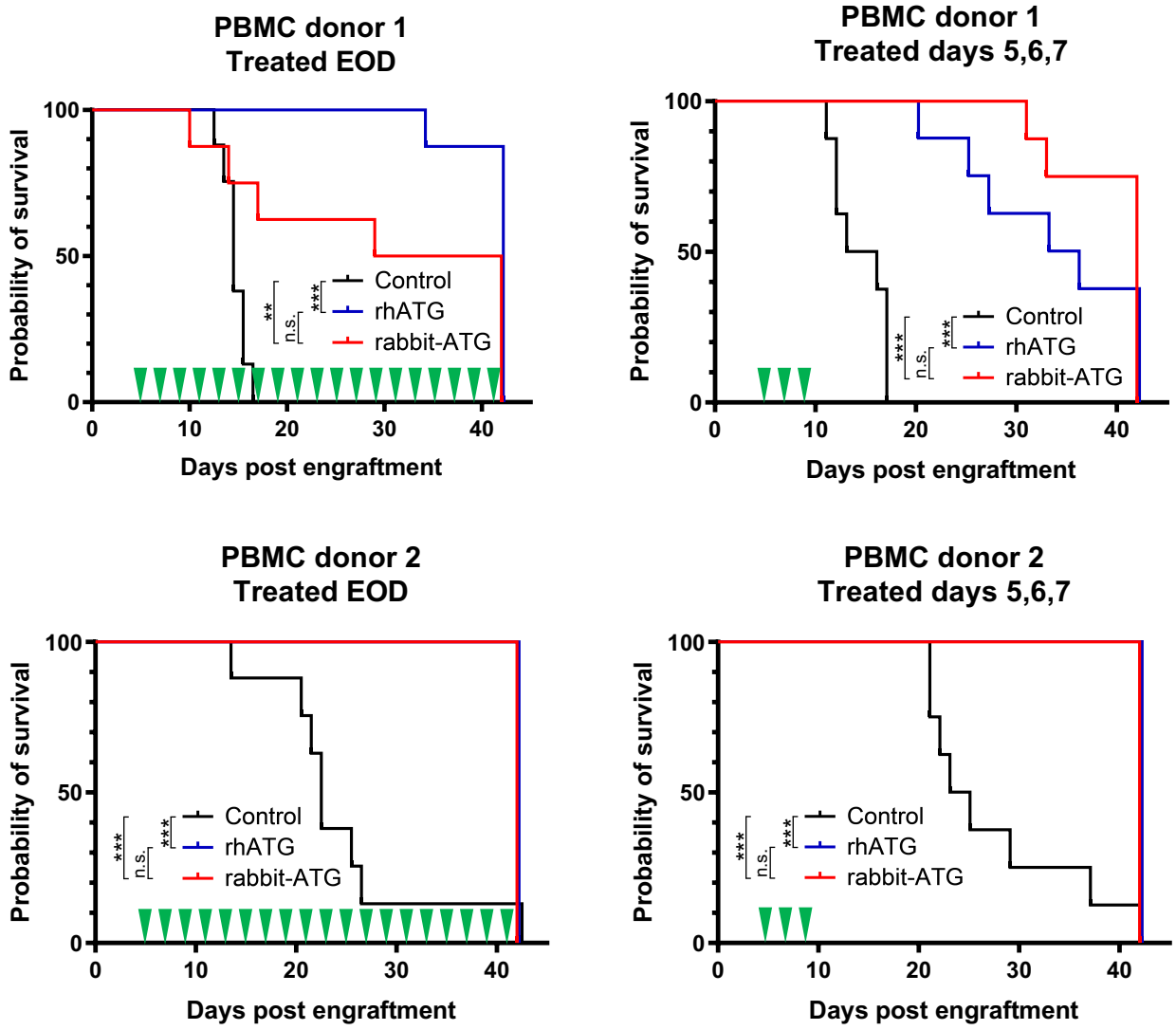
ATG immune cell-specific antibody responses measure by ELISA. The indicated Immune cell antigens were coated onto ELISA plates. **(a)** rabbit-ATG (red) and **(b)** rh-ATG (blue) were serially diluted and added to the plate. Antibody bound to antigens were quantified by anti-rabbit-HRP or anti-human-HRP, respectively.

Supplementary Figure S37



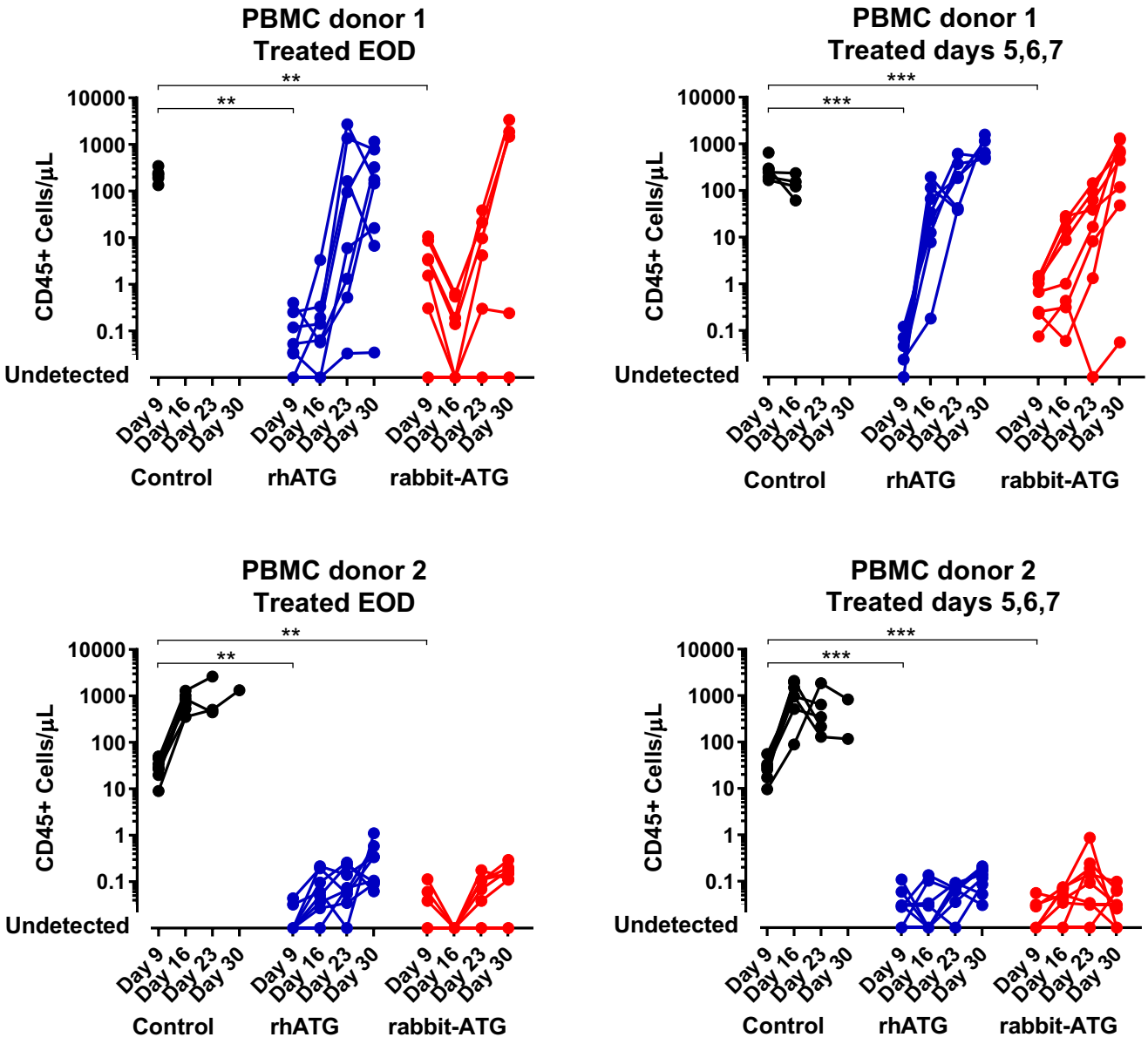
ATG binding to red blood cells by ELISA. RBC-specific antibody response was measured by Immucor Capture-R ELISA. rabbit-ATG (red) and rhATG (blue) were serially diluted and added to the Immucor Capture-R plate. RBC-bound antibodies were quantified by anti-rabbit-HRP or anti-human-HRP, respectively.

Supplementary Figure S38



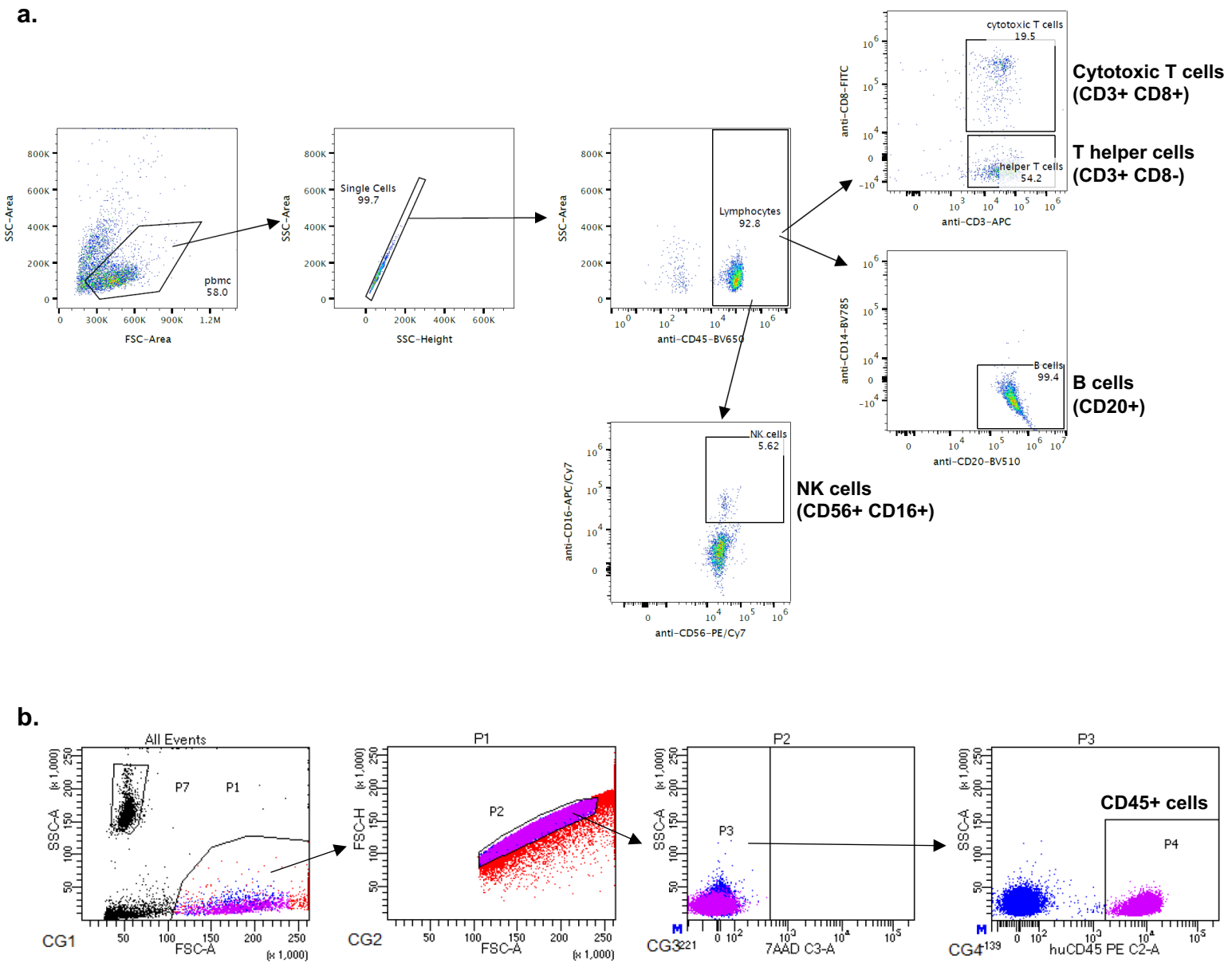
Survival of mice in the GVHD study after ATG treatment. Eight animals per treatment group were engrafted with 10^7 PBMC from one of two donors. Animals were treated with rhATG (blue), rabbit-ATG (red), or vehicle control (black) either every other day beginning at day 5 or on days 5, 6, and 7 (treatment days are indicated by green triangles), then monitored for progression to GVHD and death. ** $p<0.01$, *** $p<0.001$, n.s. not significant.

Supplementary Figure S39



Flow cytometry of CD45+ cells from the ATG GVHD study. Eight animals per treatment group were engrafted with 10^7 PBMC from one of two donors. Animals were treated with rhATG (blue), rabbit-ATG (red), or vehicle control (black) either every other day beginning at day 5 or on days 5, 6, and 7. Flow cytometry was used to determine the concentration of CD45+ cells from each alive mouse on Days 9, 16, 23, and 30. Lines connect measurements from each mouse. No CD45+ cells were observed where circles intercept the x-axis. ** $p<0.01$, *** $p<0.001$.

Supplementary Figure S40



ATG assay flow gating strategies. (**a**) Flow gating strategy for the ATG PBMC killing assay to quantify cytotoxic T cells, T helper cells, B cells, and NK cells. (**b**) Flow gating strategy of the GVH study to quantify CD45+ cells.

SUPPLEMENTARY METHODS

Sourcing Human Materials

Local ethical regulations were followed and informed consent was obtained for all human sample collection.

rCIG: A contract research organization (CRO; Access Biologics, New Orleans, LA, USA) recruited under sample collection protocol #PRO00026464 (Advarra, Columbia, MD, USA) approved by Institutional Review Board (IRB) and included if donors were 12-46 days (average 24 days +/- 14 days) from the onset of two or more COVID-19 symptoms (fever, cough, shortness of breath, sore throat, and pneumonia). Sixty mL of whole blood was collected in ACD tubes, de-identified, and transported overnight to GigaGen for processing. 16 donors with high SARS CoV-2 Spike antigen-specific antibodies by ELISA (as described below) were included in rCIG and were predominantly Caucasian (87.5%), female (75%), aged 49 (+/- 17 years), collected 21 days (+/- 6 days) from onset of symptoms, and were approximately equally distributed, with 6 donors with 2 symptoms, 6 donors with 3 symptoms, and 4 donors with 4 symptoms.

rHIG: A contract research organization (BloodCenter Wisconsin, Milwaukee, WI, USA) vaccinated two donors (Donor 1, a 26-year-old Caucasian female, and Donor 2, a 21-year-old Asian male) with PedvaxHIB vaccine (Merck, Kenilworth, NJ, USA). Leukapheresis was performed eight or nine days later to obtain PBMCs. In parallel, plasma was isolated from separate blood draws on the day of leukapheresis and prior to vaccination. We performed ELISA against Hib (Alpha Diagnostics, San Antonio, TX,

USA; see methods below) on the plasma samples to confirm a response to the vaccine as compared to plasma from the same donors prior to vaccination. Sample collection protocols were approved by IRB protocol #PRO00028063 (Medical College of Wisconsin/Froedtert Hospital IRB) to GigaGen. Informed consent was obtained from all participants and samples were shipped to GigaGen de-identified.

rPIG: A contract research organization (AllCells, Alameda, CA, USA) vaccinated three donors (Donor 1, 57-year-old Caucasian male; Donor 2, 44-year-old Caucasian male; Donor 3, 35-year-old Caucasian/Asian male) with Pneumovax®23 vaccine (Merck, Kenilworth, NJ, USA). We performed a 60 mL blood draw eight days later. Plasma and pan-B cells were isolated from whole blood (see methods below). We performed ELISA against a mixture of all 23 pneumococcal polysaccharides (Alpha Diagnostics, San Antonio, TX, USA; see methods below) on the plasma samples to confirm response to the vaccine. Sample collection protocols were approved by IRB protocol #7000-SOP-045 (Alpha IRB, San Clemente, CA, USA) to AllCells. Informed consent was obtained from all participants and samples were shipped to GigaGen de-identified.

Processing Human Materials

For whole blood, PBMCs and plasma were isolated using density gradient centrifugation SepMate tubes with Lymphoprep medium (StemCell Technologies, Vancouver, BC, Canada). To isolate pan-B cells from PBMCs (from either whole blood or a leukopak), we used the Human EasySep Pan-B Cell Enrichment Kit (StemCell, Vancouver, BC, Canada). After isolation, the cells were cryopreserved using CryoStor® CS10 (StemCell Technologies, Vancouver, BC, Canada). Immediately prior to

generating paired heavy and light chain libraries, cells were thawed, washed in cold DPBS+0.5% BSA, assessed for viability with Trypan blue on a disposable hemocytometer (Bulldog Bio, Portsmouth, NH, USA) or with AOPI on a Cellometer K2 (Nexcelom Bioscience, Lawrence, MA, USA), and then re-suspended in 12% OptiPrep™ Density Gradient Medium (Sigma, St. Louis, MO, USA) at 5,000-10,000 cells per μl. This cell mixture was used for microfluidic encapslation as described below.

Immunization of Trianni Mouse® Mice

Humanized mice were engineered by Trianni (San Francisco, CA, USA). Trianni mice were bred and obtained from Charles River Laboratories (Wilmington, MA, USA). All mice were male and 12-15 weeks old at the start of the immunization process. Antibody Solutions (Santa Clara, CA, USA) performed all Trianni Mouse immunizations. Local ethical regulations were followed for mouse immunizations by the Antibody Solutions IACUC. The mice were ear marked for identification by the breeder and housed in individually ventilated cages (Innovive, San Diego, CA, USA) and racks with HEPA filtered air at a density of up to 5 mice per cage. The animal room was lighted entirely with artificial fluorescent lighting, with a controlled 12 h light/dark cycle (7 am to 7 pm light). The normal temperature and relative humidity ranges in the animal rooms were 20-22.2°C and 30-70%, respectively. The animal rooms were set to have up to 10 air exchanges per hour. Sunnyvale municipal tap water and rodent chow (Teklad Global, Indianapolis, IN, USA) were provided *ad libitum*.

rZIG: Two Trianni humanized mice were immunized consecutively weekly with Zika VLP, inactivated Dengue 1, inactivated Dengue 4, inactivated Dengue 3, then inactivated Dengue 2 with alhydrogel/muramyl dipeptide (ALD/MDP) adjuvant. Animals

were checked for antibody titer and boosted with Zika VLPs without adjuvant 5 days before harvest (Antibody Solutions, Santa Clara, CA, USA).

rhATG: Two Trianni humanized mice were immunized weekly with human thymocytes from 5 de-identified specimens acquired from a CRO (Vitalant Research Institute, San Francisco, CA, USA) for 5 weeks with ALD/MDP adjuvant and boosted on week 6 without adjuvant. Three Trianni mice were immunized weekly for 5 weeks with Pan T cells (StemCell, Vancouver, Canada) in ALD/MDP isolated from PBMCs from 1 de-identified donor (StemCell), checked for an elevated antigen-specific antibody titer, and boosted with the same cells 5 days before harvest without adjuvant (Antibody Solutions, Santa Clara, CA, USA).

After sacrifice, spleen, lymph nodes, and/or bone marrow were harvested and processed into a single cell suspension. Samples from multiple mice were pooled together by tissue and pan-B cells were isolated from spleen and lymph node tissue using the EasySep Mouse Pan-B Cell Enrichment Kit (StemCell Technologies, Vancouver, BC, Canada). CD138+ cells were isolated from bone marrow using Miltenyi CD138+ mouse microbeads (Miltenyi, Bergisch Gladbach, Germany). After isolation, the cells were cryopreserved using CryoStor® CS10 (StemCell Technologies, Vancouver, BC, Canada).

Cell Line Used for rHIG and rhATG

We adapted the adherent Flp-In™-CHO cell line with a genetically integrated FRT site (Thermo Fisher Scientific, Waltham, MA, USA) to suspension culture. For all

steps in the adaptation process, “Ham’s F-12” refers to Ham’s F-12 (with L-glutamine, Thermo Fisher Scientific, Waltham, MA, USA) plus 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA), and “BalanCD” refers to BalanCD CHO Growth A (Irvine Scientific, Santa Ana, CA, USA) with 4 mM Glutamax (Thermo Fisher Scientific, Waltham, MA, USA). To adapt this cell line to suspension, we first passaged the cells into a mixture of 50% Ham’s F-12 plus 50% BalanCD in T-flasks. Cells were next passaged into 25% Ham’s F-12 plus 75% BalanCD and switched to shaking Erlenmeyer flasks. Cells were then passaged into 10% Ham’s F-12, 90% BalanCD + 0.2% anti-clumping agent (Irvine Scientific, Santa Ana, CA, USA) and banked for future use.

Approximately 100 million of the adapted Flp-In CHO cells were transfected per recombinant hyperimmune globulin library using an Amaxa Nucleofector 4D (SG buffer, pulse DU133; Lonza, Basel, Switzerland). These cells were plated into shaking Erlenmeyer flasks and recovered in an incubator at 37ºC, 5% CO_2, 125 rpm. After 48 hours, the cells were counted to determine viability, cells were seeded at 1 million cells/mL, and selection was started using 600 μg/mL Hygromycin-B (Gemini Bio, West Sacramento, CA, USA) in fresh media. Cells were counted and media was changed every 2-3 days during the 7-day selection. The libraries were kept on 600 μg/mL Hygromycin-B (Gemini Bio, West Sacramento, CA, USA) during expansion until viability exceeded 95%. When cells were >95% viable and doubling every 24 hours, the adapted Flp-In™-CHO cell line was banked for liquid nitrogen storage. Before banking, cells were sampled from each library, RNA was purified, and antibody RNA-seq (Illumina, San Diego, CA, USA) was performed to assess the diversity of the libraries (**Supplementary Tables S8, S12**).

Cell Line Used for rPIG, rZIG, and rCIG

A landing pad construct (PMD-4681; **Supplementary Figure S3**; GenBank accession number MW079274) was designed and cloned at GigaGen. PMD-4681 was based on pFRT-lacZeo (Thermo Fisher Scientific, Waltham, MA, USA), with some modifications. In place of the LacZ expression construct a cassette was inserted coding for expression of CD34 and GFP. The CD34, GFP, and downstream Zeocin resistance genes (ZeoR present in pFRT-lacZeo) were separated by 2A motifs (T2A or P2A) to allow for translation of three separate polypeptide chains. The CD34 sequence was sourced as a gBlock from IDT (Coralville, IA, USA). The GFP sequence was sourced from ATUM (DasherGFP; Newark, CA, USA).

The GMP suspension CHO line CHOZN® GS-/- was obtained from MilliporeSigma (St. Louis, MO, USA). PMD-4681 was linearized using ScaI-HF and purified via ethanol precipitation. Cells were transfected with the linearized DNA using Amaxa Nucleofector 4D, SE kit, pulse CM-150 (Lonza, Basel, Switzerland). Cells recovered overnight in an incubator and were plated the next day into minipools at approximately 5,000 cells per well, across ten 96-well plates in selective media. The remaining cells were plated and selected together as a bulk pool control. Wells were topped off with fresh media every seven days until at least 80% confluency was reached.

A total of 236 minipools grew out and were screened in parallel for high GFP expression via flow cytometry and low copy number with a quantitative PCR Copy Number Variation (CNV) assay. Minipools with a copy number less than 2.5 and GFP

expression at least 50% of the bulk pool were expanded into shaking adaptation. Expanded pools were re-tested for GFP expression via flow cytometry.

Cells were then adapted to BalanCD CHO Growth A in preparation for plating into semi-solid media. Minipools were deemed fully adapted when cells showed consistent doubling times and high viability (>90%). Adapted cells were plated into semisolid media for the Molecular Devices (Fremont, CA, USA) ClonePix3 single cell cloning platform. Single cell imaging was obtained on day 0 of cell plating in semisolid media to confirm monoclonality. After 14 days, clonal cell colonies were picked and deposited as one colony per well of a 96-well plate. Each clone was then expanded, re-adapted to selection media, and cryopreserved. Doubling times were calculated and clones with less than a 30-hour doubling time were chosen for further development. Expanded clones were retested for GFP expression and copy number.

Remaining clones were transfected in duplicate using the Gene Pulser Xcell Total System (BioRad, Hercules, CA, USA) per guidelines from MilliporeSigma (St. Louis, MO, USA) for use with CHOZN GS with a mAb-cyan fluorescent protein (FrostyCFP, ATUM, Newark, CA, USA) construct to test expression titer. CFP expression was evaluated via flow cytometry 3 days post transfection to confirm transfection efficiency >35%. After full selection and recovery, cell lines were tested in a 10-day fed batch TPP shaking production run in duplicate. Titers for candidate cell lines ranged from 50–100 mg/L. A single clone (CSS-1286) was selected to use for recombinant hyperimmune globulin expression.

For transfection of recombinant hyperimmune globulins into CSS-1286, approximately 50 million cells were transfected per recombinant hyperimmune globulin

library using the BioRad Gene Pulser Xcell Total System (Hercules, CA, USA), per guidelines from MilliporeSigma (St. Louis, MO, USA) for use with CHOZN GS. The cells were plated into T-75 flasks (approximately 10 million cells per flask) and recovered in an incubator at 37ºC, 5% CO_2 for 72 hours. After 72 hours, the cells were counted to determine viability and then seeded into 100 mL fresh media without glutamine (EX-CELL CD CHO Fusion, MilliporeSigma, St. Louis, MO, USA) in a 500 mL Erlenmeyer flask. Cells were counted and media was changed every 2-3 days during the ~14-day selection. When cells were >95% viable and doubling every 24 hours, the cell line was banked for liquid nitrogen storage. Before banking, cells were sampled from each library, RNA was purified, and antibody RNA-seq (Illumina, San Diego, CA, USA) was performed to assess the diversity of the libraries (**Supplementary Tables S4, S6, S9**).

Medium-scale Production, Polishing, and Stress Testing of rCIG

For bioreactor production at 3 L scale, a seed train protocol was devised to mimic the number of passages required for production at up to 2,000 L scale. For each bioreactor production run, a single vial of rCIG cell bank was thawed at 37°C and the contents of the vial were transferred to 10 mL of EX-CELL CD CHO Fusion media (MilliporeSigma, St. Louis, MO, USA). The cells were then centrifuged for 5 minutes at 500 × *g*. The supernatant was aspirated and discarded, and the cells were re-suspended in 5 mL of EX-CELL CD CHO Fusion. The entire volume of cells was seeded into a 250 mL non-baffled, vented shake flask at a final volume of 50 mL. The shake flask was incubated at 37°C, 5% CO_2, ≥ 80% humidity and 125 RPM (25 mm orbital diameter). Three days post thaw the viable cell density (VCD) was 4.0–6.0 × 10^6

vc/mL with a viability ≥ 90%. At this point the cells were passaged using EX-CELL CD CHO Fusion into a 1000 mL shake flask at a seeding density of 0.4×10^6 vc/mL. In a similar manner, the culture was passaged once more into a 1000 mL shake flask, and then two more times into 1000 mL spinner flasks. The final passage before bioreactor inoculation was done in EX-Cell Advanced CHO Fed Batch media (MilliporeSigma, St. Louis, MO, USA).

Three days after completing the fifth passage, three 3 L Mobius single-use bioreactors (MilliporeSigma, St. Louis, MO, USA) were seeded using the culture. Each bioreactor was prepared with 1300 mL of EX-Cell Advanced CHO Fed Batch media and then seeded at a VCD of $0.4 \pm 0.1 \times 10^6$ vc/mL. Additional media was added if required to have an initial working volume of 1600 mL. Each bioreactor was controlled using the following set points: temperature setpoint Days 0-4 setpoint 37°C; temperature Days 4-14 setpoint 32°C; dissolved oxygen setpoint 30%; pH Days 0-3 7.0 ± 0.2; pH Days 3-14 7.0 ± 0.1. EX-Cell Advanced CHO Feed (MilliporeSigma, St. Louis, MO, USA) and Cellvento 4Feed COMP (MilliporeSigma, St. Louis, MO, USA) were added on Days 3, 5, 7, 9, and 11. Feed volumes were determined as a percentage of the current bioreactor volume, such that EX-Cell Advanced CHO Feed was added at 4% of the volume of the bioreactor and Cellvento 4Feed COMP was added at 2% of the volume of the bioreactor. Glucose levels were monitored daily, and starting on Day 3 were maintained above 4 g/L by adding a 45% Glucose solution until levels reached 6 g/L. The bioreactors were harvested after 14 days of culture. 5-10 million cells were collected from each bioreactor for antibody repertoire sequencing (**Supplementary Figure S13**).

For purification and polishing, an empty column was packed with MabSelect Sure PrismA resin (Cytiva, Marlborough, MA, USA) and equilibrated with 20 mM phosphate, 150 mM NaCl, pH 7.4. Harvested cell-culture fluid was loaded at 20-40 g/L, washed with 20 mM phosphate, 500 mM NaCl, pH 7.4 and 50 mM phosphate, pH 6.0, and eluted with 50 mM sodium acetate, pH 3.5. The pH of the Protein A eluate was adjusted to 3.5 using 1 M acetic acid and the material was subjected to a 1 hour viral hold, after which it was adjusted to pH 5 using 1 M Tris-HCl, pH 9 and filtered to remove particulate. A second column was packed with POROS XS (Thermo Fisher Scientific, Waltham, MA, USA) cation exchange (CEX) resin and equilibrated with 50 mM sodium acetate, pH 5.0. The filtered neutralized low pH hold pool was loaded on this column at 14-21 g/L, washed with 50 mM sodium acetate, 100 mM sodium chloride, pH 5.0, and eluted over a 20 CV gradient to 50 mM sodium acetate, 400 mM sodium chloride, pH 5.0. The product eluted with several distinct peaks, of which only the first was collected. The pooled CEX eluate was diluted with 20 mM tris-acetate, pH 7.4 to <8 mS/cm, then flowed through a Sartobind Q (Sartorious, Göttingen, Germany) anion-exchange membrane. The flowthrough was concentrated using a 30K molecular weight cutoff cellulose acetate tangential flow filtration cartridge (MilliporeSigma, St. Louis, MO, USA), then diafiltered with 200 mM glycine pH 4.5 and sterile-filtered.

For stress testing, polished rCIG at 15 mg/mL in 200 mM glycine, pH 4.5 was incubated at 40°C for 14 days. Separately, aliquots were subjected to 3 or 25 freeze-thaw cycles in a -80°C freezer or dry ice/ethanol bath. The stressed samples were run on SEC-HPLC and SARS CoV-2 S1 ELISA and compared to a control that was stored at 4°C.

Deep Antibody Repertoire Sequencing

Deep antibody sequencing libraries were prepared as described previously,[18] quantified using a KAPA quantitative PCR Illumina Library Quantification Kit (Roche, Mannheim, Germany), and diluted to 17.5 pM. Libraries were sequenced on a MiSeq (Illumina, San Diego, CA, USA) using a 500 cycle MiSeq Reagent Kit v2, according to the manufacturer's instructions. To make sequencing libraries, we used tailed-end PCR to add Illumina sequencing adapters to the 5' and 3' ends of the constructs of interest. For scFv libraries (after droplet emulsion breaking or yeast plasmid isolation), a forward read of 340 cycles was used to capture the light chain CDR3 sequence, and a reverse read of 162 cycles was used to capture the linked heavy chain CDR3 sequence. For CHO libraries, the full-length heavy chain sequence was obtained using overlapping forward and reverse reads of 251 cycles. To determine the number of antibody clones in the final CHO cell libraries and for generating the clonal cluster analysis figures, 5-10 million CHO cells were harvested prior to a production run. For the batch-to-batch variation analysis, 5-10 million CHO cells were harvested at the conclusion of the replicate production runs, and a median of 855,746 sequence reads were obtained for each sequencing library (range: 658,013 to 1,113,968). To determine the Fc subtype of a library, the heavy chain was amplified with a primer that binds further into the constant domain to add the Illumina sequencing adapter to the 3' end; the first 60 bp of the constant domain was sequenced to determine the subtype, which was linked to the corresponding CDR3H that was simultaneously sequenced. Each library was sequenced one time.

Sequence analysis, including error correction, reading frame identification, and FR/CDR junction calls was performed using our previously reported bioinformatics pipeline.[18] Reads with E > 1 (E is the expected number of errors) were discarded, such that we retained sequences for which the most probable number of base call errors is zero. Clones are defined as sequences with unique CDR3 amino acid sequences (CDR3K + CDR3H for scFv clones, CDR3H only for CHO clones). A more conservative clone count is also provided (combined CDR3), where unique clones were combined if they had 1 amino acid difference for 5-6 amino acid long CDR3s, or if they had 1-2 amino acid differences for >6 amino acid long CDR3s; this applies to a concatenation of CDR3K + CDR3H for scFv clones or CDR3H only for CHO clones). For the clonal cluster analysis, we used USEARCH[49] to compute the total amino acid differences between each pairwise alignment of full-length heavy chain sequences with abundance ≥0.01% in each CHO cell bank. We then used the R package igraph[50] (version 1.2.4.1) to generate clustering plots for the pairwise alignments. The sequences were represented as “nodes”, with the color (and sometimes shape) defined in the respective figures. The size of the nodes reflects the frequency of the clone (small, <0.1%; medium, 0.1-1%; large, >1%). “Edges” are the links between nodes, which indicate pairwise alignments with ≤5 amino acid differences. The layout_with_graphopt (niter = 3000, charge = 0.03) option was used to format the output. To visualize the sequences within a library, we selected the top five clusters containing the highest number of connected nodes/sequences. These sequences were aligned and visualized as sequence logos using the R package msa 1.16.0.

To assess antibody repertoire overlap between libraries, we computed Jaccard and Morisita indices using the R package tcR (version 2.3.2).[51] Shannon entropy and Simpson diversity indices were calculated using the R package vegan 2.5.5. Heavy chain V and J gene identities were measured using the USEARCH[49]-local algorithm with the germline database as reference. For the heatmaps showing the pairing of VH and VL genes, the germline divergence histograms, and the histograms showing the distribution of heavy chain CDR3 length, each unique clone was plotted once (i.e., not scaled by sequencing read abundance).

To estimate the error rate due to RNA/cDNA amplification, we sequenced a region of the heavy chain constant domain from the rCIG libraries (the region used to determine the heavy chain subtype), after performing the same amplification process as the final polyclonal libraries. The total error rate for this region was 0.3%, then using Illumina's quality (Q) score for each sequenced base, the estimated sequencing error rate for this region was determined to be 0.052%. Thus, the estimated maximum error rate from our amplification process is 0.3% - 0.052% = 0.248% (which we note may still contain some error due to natural sequence variation in the constant domain).

Sequencing data are available in the Short Read Archive (SRA) under project identifier PRJNA649279.

In vitro *Efficacy Studies*

rCIG: Anti-SARS CoV-2 antibody reactivities were measured using a protocol based on published ELISA methods.[52] In brief, SARS CoV-2 Spike and RBD (wild type and variant proteins; Sino Biological, Wayne, PA, USA) were used to coat ELISA plates at 2 μg/mL. Serial dilutions of antibody preparations including test plasma and

recombinant products, positive control monoclonal antibodies (CR3022; Absolute Antibody, San Diego, CA, USA, and SAD-S35; Acro Biosystems, Newark, DE, USA) and negative control IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) were performed in dilution buffer (1× PBS + 0.05% Tween + 0.3% dry milk) in singlet. Quantitative measurements were performed on a plate reader (Molecular Devices, Fremont, CA, USA) and analyzed using Softmax Pro (Version 7.1; Molecular Devices, Fremont, CA, USA) to calculate the EC50 concentrations of samples. The concentration of total IgG was calculated by Cedex Bioanalyzer Human IgG assay (Roche, Mannheim, Germany).

Blocking of binding between Spike RBD and ACE2 was demonstrated by ELISA (BPS Bioscience, San Diego, CA, USA). In brief, SARS CoV-2 Spike RBD protein was coated onto an ELISA plate, serial dilutions of test plasma and recombinant products, positive control monoclonal antibodies (CR3022; Absolute Antibody, San Diego, CA, USA, and SAD-S35; Acro Biosystems, Newark, DE, USA) and negative control IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) were performed in singlet in dilution buffer (1× PBS + 0.05% Tween + 0.3% dry milk). After incubation, ACE2-His was added at 2.5 ng/mL. After further incubation, anti-His-horseradish peroxidase was added. The plate was developed for a chemiluminescent readout. Quantitative measurements were performed on a plate reader (Molecular Devices, Fremont, CA, USA) and analyzed using Softmax Pro (Version 7.1; Molecular Devices, Fremont, CA, USA) to calculate the EC50 concentrations of samples.

The SARS CoV-2 pseudotype virus neutralization assay was performed in a 96-well plate using ACE2 expressing HEK-293T target cells (CRL-11268; ATCC, Manassas, VA, USA) transiently transfected with TMPRSS-2 expression plasmid. The

GFP reporter pseudotype virus expressing SARS-CoV-2 spike (Integral Molecular, Philadelphia, PA, USA) was mixed with test plasma, test rCIG, positive control monoclonal antibodies (CR3022; Absolute Antibody, San Diego, CA, USA, and SAD-S35; Acro Biosystems, Newark, DE, USA) and negative control IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) at a five-fold dilution series in singlet. After one-hour incubation, 4×10^4 cells target cells were added to each well and incubated at 37°C for 48 hours. After incubation, the media was removed from all wells without disturbing the adherent cells. TrypLE (Thermo Fisher Scientific, Waltham, MA, USA) was added to each well and incubated for 3 minutes at 37°C. Media was added to stop trypsinization and cells were stained with DAPI and passed through a 30-40 μm filter (Pall Corporation, Port Washington, NY, USA) before quantifying GFP+ cells using a Cytoflex LX (Beckman Coulter, Indianapolis, IN, USA). Flow cytometry data were analyzed by FlowJo (BD Biosciences, San Jose, CA, USA).

SARS CoV-2 microneutralization assays were performed at the Regional Biocontainment Laboratory at Duke University Medical Center (Durham, NC, USA) in a 96-well plate format using Vero E6 cells (CRL-1586; ATCC, Manassas, VA, USA) infected with 100 $TCID_{50}$ dose of the 2019-nCoV/USA-WA1/2020 strain. Test and control samples were initially diluted to 1:50, then a 12-step, two-fold serial dilution of test antibodies was performed before infection of the cells; every test or control was run in duplicate. IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) was used as negative control. Cell-only control wells were included alongside virus-only treated wells. Following 4 days of infection, culture media was removed, and cell monolayer was fixed with 10% neutral buffered formalin (NBF) and stained with 0.1% Crystal

Violet. Absorbance at 590 nm or visual inspection was used to measure the monolayer condition/level of infection. Neutralization was reported as the lowest concentration of sample that prevents cytopathic effect in the monolayer of cells.

rZIG: Zika- and Dengue-specific antibodies were measured by ELISA. A 96-well microtiter plate was coated with either 2 µg/ml Zika or Dengue Serotype 1, 2, 3, or 4 recombinant envelope proteins (ProSpec Bio, East Brunswick, NJ, USA) in 1× carbonate coating buffer (BioLegend, San Diego, CA, USA) and incubated overnight at 4°C. After blocking the coated plate with ultrablock buffer (Bio-Rad, Hercules, CA, USA) and washing with PBS + 0.05% Tween-20 (Teknova, Hollister, CA, USA), eight-step three-fold serial dilutions in assay buffer (1× PBS + 0.05% Tween + 0.3% dry milk) were performed on rZIG-IgG1, rZIG-LALA, Zika/Dengue+ serum positive control (Seracare, Milford, MA, USA), and a negative control IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain). Dilutions were added in duplicate and incubated at 37°C for 1 hour. Next, 1:2500 secondary rabbit anti-human IgG horseradish peroxidase conjugate (Southern Biotech 6140-05, Birmingham, AL, USA) was added, and the plate was washed and developed using 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (Thermo Fisher Scientific, Waltham, MA, USA). The reaction was halted after 8 minutes using sulfuric acid stopping solution (Southern Biotech, Birmingham, AL, USA). Quantitative absorbance measurements were performed on a SpectraMax i3x plate reader (Molecular Devices, Fremont, CA, USA) at 450 nm and 620 nm. Standard curves ($OD_{450-620}$) were artificially set to max out at 2.97 absorbance value. EC50 values were calculated by non-linear regression analysis using GraphPad Prism v8 (San Diego, CA, USA).

Zika and Dengue *in vitro* pseudotype neutralization assays were performed at Vitalant Research Institute (VRI, San Francisco, CA, USA). rZIG, a Zika/Dengue-specific immune sera (UWIS; de-identified sample screened positive for Zika and Dengue 1-4 by University of the West Indies), monoclonal antibody positive control (UWI-mAb1; IgG1 isotype cloned from de-identified donor by University of the West Indies, found to be cross-reactive to Zika and Dengue 1-4), and IVIG negative control (Gamunex; Grifols, S.A., Sant Cugat, Spain) were co-incubated with reporter virus particles (RVPs; Integral Molecular, Philadelphia, PA, USA) expressing both luciferase and flavivirus-specific glycoproteins as previously described.[53] Briefly, BHK/DC-SIGN cells (CRL-325; ATCC, Manassas, VA, USA) were seeded in black 96-well plates and then incubated with a 7-step, 3-fold serial dilution of antibodies pre-incubated for one hour at 37°C with RVPs and tested in duplicate. After 72 hours cells were lysed and luciferase activity measured using lysis buffer and firefly luciferase substrate following manufacturer's guidelines (Promega, Madison, WI, USA). Infection-induced relative light units (RLU) in the presence of test articles were calculated as the RLU of the test article divided by the RLU of a no-serum control infection. The amount of protein required to inhibit 50% of the maximum untreated Zika or Dengue RLUs (IC50) was calculated by non-linear regression analysis using GraphPad Prism v8 (San Diego, CA, USA).

Zika pseudotype assays for *in vitro* antibody-dependent enhancement (ADE) were performed at VRI. rZIG, the Zika/Dengue-specific immune sera UWIS, the monoclonal antibody positive control UWI-mAb1, and IVIG negative control (Gamunex; Grifols, S.A., Sant Cugat, Spain) were serially diluted and co-incubated with Zika pseudotype RVPs at 37°C for 1 hour before addition to K562 chronic myelogenous

leukemia cells (CCL-243; Manassas, VA, USA) in U-bottom 96-well plates in triplicate. After a 72 hour incubation at 37°C, cells were harvested, lysed, and infection-induced relative light units (RLU) in the presence of test articles were calculated as the RLU of the test article divided by the RLU of a no antibody control infection (to determine the reported fold-increase in infection).

rHIG: The Human Anti-Hib-PRP IgG ELISA kit (#980-100-PHG, Alpha Diagnostics, San Antonio, TX, USA) was used for anti-Hib ELISA titers. Serial dilutions of test articles were performed in Low NSB (non-specific binding) sample diluent in singlet. IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) was used as a reference control. Quantitative measurements were performed on a plate reader (Molecular Devices, Fremont, CA, USA) at 450 nm. EC50 values were calculated using SoftMax Pro v8 (Molecular Devices, Fremont, CA, USA).

In vitro serum bactericidal assay neutralization studies for Hib were performed at ImQuest (Frederick, MD, USA). The *Haemophilus influenzae* strain ATCC 10211 was obtained from ATCC (Manassas, VA, USA) as a lyophilized stock and was propagated as recommended by the supplier. The Eagan strain was obtained from Zeptometrix (Buffalo, NY, USA). Colonies from an overnight incubation on chocolate agar plates were inoculated into growth media (Brain Heart Infusion, or BHI broth; BD Biosciences, San Jose, CA, USA, with 2% Fildes enrichment; Remel, San Diego, CA, USA) and allowed to achieve an optical density of 625 nm (OD_{625}) of approximately 0.4. The culture was adjusted to an OD_{625} of 0.15, which is equivalent to approximately 5×10^8 colony forming units (CFU)/mL. The culture was further diluted to 5×10^4 CFU/mL in dilution buffer (Hanks Balanced Salt Solution; Gibco, Waltham, MA, USA, with 2%

Fildes enrichment; Remel, San Diego, CA, USA). The density of the bacterial culture used in the assay was confirmed by plating 50 μL of the 5×10^3 and 5×10^2 dilutions in duplicate on chocolate agar and enumerating the colonies following incubation at 37°C/5% CO_2 for 24 hours. rHIG and IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) reference control were diluted three-fold in buffer, starting at 200 μg/mL such that a total of ten total dilutions were evaluated in singlet. 10 μL of each dilution of test article was added in duplicate to a 96-well microtiter plate. ATCC 10211 bacteria at a concentration of approximately 5×10^4 CFU/mL were then added to the plate in a volume of 20 μL, such that the total in-well bacterial density would be 1×10^4 CFU/20 μL. Following an incubation of 15 minutes at 37°C/5% CO_2, 25 μL of baby rabbit complement (Pel-Freez; Rogers, AR, USA) and 25 μL of dilution buffer was added to each well. The plate was incubated at 37°C/5% CO_2 for 60 minutes. Following the incubation, 5 μL of each reaction mixture was diluted in 45 μL of dilution buffer and the entire 50 μL was plated on chocolate agar plates. The plates were incubated for approximately 16 hours at 37°C/5% CO_2. Following incubation, bacterial colonies were enumerated. The fold-dilution of the test article that killed >50% of the bacteria is the serum bactericidal index (SBI).

rPIG: The Human Anti-S. Pneumococcal vaccine (Pneumovax/CPS23) IgG ELISA kit (Alpha Diagnostics #560-190-23G, San Antonio, TX, USA) was used in parallel with the human anti-S. pneumoniae CWPS/22F IgG ELISA kit (#560-410-C22, Alpha Diagnostics, San Antonio, TX, USA) for initial assessment of anti-pneumococcal titers against a pool of all 23 polysaccharides included in the vaccine.[18]

Serotype-specific antibodies were measured by ELISA and opsonophagocytosis. The concentrations of serotype-specific IgG antibody were calculated using the standard reference serum, lot 007SP (National Institute for Biological Standards and Control; Hertfordshire, UK), using the standardized pneumococcal reference ELISA as previously described.[54] Briefly, 96-well flat-bottomed microtiter plates were coated with capsular polysaccharide antigens (LGC Standards, Teddington, UK) from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F, 14, 18C, 19A, 19F, 22F, 23F, and 33F. All samples were tested in duplicate and double absorbed with CWPS and with purified serotype 22F polysaccharide to neutralize the anti-cell wall polysaccharide and nonspecific homologous antibodies to serotype 22F, except for the 22F assay which was absorbed with CWPS Multi, as described in the WHO reference ELISA protocol.[55] Plates were washed, and a titration of rPIG and reference control IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain) was performed. Plates were incubated and washed again, and prediluted alkaline phosphatase-conjugated goat anti-human IgG (Thermo Fisher Scientific, Waltham, MA, USA) was added to each well. After another incubation, the plates were washed a final time and *p*-nitrophenyl phosphate substrate (MilliporeSigma, St. Louis, MO, USA) was added. Following a final incubation, the reaction was stopped by adding 3 M NaOH (Thermo Fisher Scientific, Waltham, MA, USA) to each well. Plates were read using a microtiter plate reader (SPECTROstar Omega; BMG Labtech, Buckinghamshire, UK) at 405 and 620 nm.

The opsonophagocytic indices (OI) to the same pneumococcal serotypes were evaluated by multiplexed opsonophagocytic assay, as previously described.[56] In brief, frozen aliquots of target pneumococci were thawed, washed twice with opsonization

buffer B (HBSS with Ca and Mg, 0.1% gelatin, and 10% fetal bovine serum), and diluted to the proper bacterial density (approximately 2×10^5 CFUs/mL each serotype). Equal volumes of four bacterial suspensions chosen for simultaneous analysis were pooled. Duplicate serially diluted test articles (20 μL/well) were mixed with 10 μL of bacterial suspension in each well of a microplate. After 30 minutes of incubation at room temperature with shaking at 700 rpm, 10 μL of 3- to 4-week-old rabbit complement (Pel-Freeze, Rogers, AR, USA) and 40 μL of differentiated HL60 cells (10^7 cells; CCL-240, ATCC, Manassas, VA, USA) were added. Plates were incubated in a 37°C/5% CO_2 incubator with shaking at 700 rpm. After being incubated for 45 minutes, plates were placed on ice for 20 minutes, and an aliquot of the final reaction mixture (10 μL) was spotted onto four different Todd-Hewitt broth with 0.5% yeast extract and 0.75% agar (THY) plate. When the fluid was absorbed into the agar, an equal volume of overlay agar containing one of four antibiotics (optochin, spectinomycin, streptomycin, or trimethoprim) was applied to each THY agar plate. After overnight incubation at 37°C, the number of bacterial colonies in the agar plates was enumerated. IVIG was used as reference control (Gamunex; Grifols, S.A., Sant Cugat, Spain). The OI was defined as the test product dilution that kills 50% of bacteria and was determined by linear interpolation.

rhATG: To assess relative amount and specificity of rhATG, we performed an ELISA on antigens known to be expressed on thymocytes and previously described as having rabbit-ATG reactivity.[41] Rabbit-ATG positive control was from Sanofi Genzyme (Thymogobulin; Cambridge, MA, USA). T cell antigens (CD3, CD4, CD5, CD7, CD8, CD16a, CD32a, CD45, CD81, CD85 CD95) were purchased from Sino Biological

(Wayne, PA, USA) and individually coated onto 96-well ELISA plates (Thermo Fisher Scientific, Waltham, MA, USA) at 1μg/mL in 1× carbonate coating buffer (BioLegend, San Diego, CA, USA). After an overnight incubation at 4°C, coated plates were washed and blocked (Bio-Rad, Hercules, CA, USA) for 1 hour. Polyclonal products were diluted to 200 μg/mL of total IgG and an 8-step 1:3 titration in assay buffer (1× PBS + 0.05% Tween + 0.3% dry milk) was performed. The antibody titrations were added to each antigen and incubated for 1 hour at 37°C. 1:2500 polyclonal HRP goat anti-rabbit IgG (E28002; Novodiax, Hayward, CA, USA) or 1:2500 mouse anti-human IgG HRP (109-035-088; Jackson ImmunoResearch, West Grove, PA, USA) were diluted 1:2500 and incubated on the plate for 1 hour. Plates were washed, developed using 1-step ultra TMB substrate (Thermo Fisher Scientific, Waltham, MA, USA), and stopped with 1 N HCl. Plates were read by a spectrophotometer (Molecular Devices, Fremont, CA, USA) at 450 nm and analyzed with Softmax Pro (v7; Molecular Devices, Fremont, CA, USA).

To assess off-target antibody binding we performed a red blood cell antigen binding assay, using the Capture-R kit (Immucor, Norcross, GA, USA). Using a dilution of test article (Thymoglobulin or rhATG) or positive control from the Immucor kit, samples were added to the plate and incubated for 1hr at 37°C in singlets. ELISA plates were washed and incubated with 1:2500 polyclonal HRP goat anti-rabbit IgG (E28002; Novodiax, Hayward, CA, USA) or 1:2500 mouse anti-human IgG HRP (109-035-088; Jackson ImmunoResearch, West Grove, PA, USA). Subsequently, plates were washed and developed with ultra-TMB substrate (Thermo Fisher Scientific, Waltham, MA) and the reaction was stopped with 3 M NaOH (Thermo Fisher Scientific, Waltham, MA, USA) and read the plate on a spectrophotometer at 450 nm.

To determine *in vitro* function of rhATG, peripheral blood mononuclear cells were isolated from whole blood acquired from a CRO (StemCell Technologies, Vancouver, Canada) and frozen. PBMCs were thawed, washed, and plated at 1.5×10^5 cells/well in singlets. Thymoglobulin or rhATG were diluted five-fold starting at 40 μg/mL total IgG and co-incubated with each donor PBMC. Cells were co-incubated overnight at 37°C. After incubation, cells were washed, FcR blocked, and stained for CD45 (clone H130; BioLegend #304008, San Diego, CA, USA), CD3 (clone UCHT1; BioLegend #300439, San Diego, CA, USA), CD8 (clone BW135/80; Miltenyi #130-113-157, Bergisch Gladbach, Germany), CD20 (clone 2H7; BioLegend, San Diego, CA), CD56 (clone 5.1H11; BioLegend #362509, San Diego, CA, USA), and CD16 (clone 3G8; BioLegend #302017, San Diego, CA, USA). 1 μL of each antibody was used per 1.5×10^5 cells. Flow cytometry was performed using a Cytoflex LX (Beckman Coulter, Indianapolis, IN, USA) with CytExpert (2.3.1.22) and a consistent collection volume of 150 seconds per well was implemented for every sample. The data were analyzed by FlowJo v10 (BD Biosciences, San Jose, CA, USA). Cell counts after antibody co-incubation relative to no-antibody control (% cells) were calculated. Results were graphed in GraphPad Prism v8 (San Diego, CA, USA). The gating strategy is outlined in **Supplementary Figure S40a**.

In Vivo *Mouse Efficacy Studies*

Ethical approval was obtained by Institutional Animal Care and Use Committees (IACUCs) at either SSI (Copenhagen, Denmark) for the Hib challenge model or Jackson Laboratory (Sacramento, CA, USA) for the GVH model.

IVIG + rHIG/rPIG: For IVIG + rHIG/rPIG *in vivo* challenge studies, the *Haemophilus influenza* strain ATCC 10211 was grown on chocolate agar plates overnight at 35°C and 5% CO_2. Single overnight colonies were resuspended in sterile saline to 1.5×10^8 CFU/mL. This suspension was diluted in BHI broth to 1.5×10^7 CFU/mL and further diluted in BHI broth with 5% mucin and 2% hemoglobin to 1.5×10^4 CFU/mL. In an IACUC-approved protocol (SSI, Copenhagen, Denmark), Balb/cJ mice (Taconic, Rensselaer, NY, USA; n=6 per group) were inoculated with single 0.5 mL intraperitoneal doses of 10^5 CFU/mL ATCC 10211, and then randomized into treatment groups by animal identifier. All mice were female, age 6-8 weeks. The temperature and humidity were registered daily in the animal facilities. The temperature was 22°C +/- 2°C and can be regulated by heating and cooling. The humidity was 55 +/- 10%. The air changes per hour were approximately 8-12 times (70-73 times per hours inside cages), and light/dark period was in 12-hours interval of 6 am - 6 pm / 6 pm – 6 am. The mice had free access to domestic quality drinking water and food (Teklad Global diet 2916C, Envigo, Indianapolis, IN, USA) and occasionally peanuts and sunflower seeds (Køge Korn A/S, Køge, Denmark). The mice were housed in IVC cages , 6-8 mice per cage, with bedding from Tapvei. Further, the animals were offered Enviro-Dri nesting material and cardboard houses (Bio-Serv, Flemington, NJ, USA).

Approximately 1 hour before inoculation, mice were treated orally with 45 μL Nurofen (30 mg/kg) as pain relief. Twenty-four hours prior to Hib inoculation, mice were intravenously administered 200 mg/kg IVIG + rHIG/rPIG mixture, 500 mg/kg IVIG + rHIG/rPIG mixture, 500 mg/kg IVIG (Gamunex; Grifols, S.A., Sant Cugat, Spain), or saline (no treatment). For the ciprofloxacin positive control, one hour after Hib

inoculation, mice were dosed with 20 mg/kg ciprofloxacin. Mice were scored for clinical signs of infection, then after 6 hours all animals were sacrificed and blood and peritoneal fluid was collected for CFU determination by serial dilution and plating of 0.02 ml spots on chocolate agar plates.

rhATG: We contracted with Jackson Laboratory (Sacramento, CA, USA) under an IACUC-approved protocol to Jackson Laboratory to test rhATG for ability to delay GVHD in immunodeficient NOD *scid* gamma (NSG) mice (genotype: NOD.*Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), compared against rabbit-ATG (Thymoglobulin; Sanofi Genzyme, Cambridge, MA) and a vehicle control. All mice were female, age 6-8 weeks. The mice were ear notched for identification and housed in individually ventilated polysulfonate cages with HEPA filtered air at a density of up to 5 mice per cage. The animal room was lighted entirely with artificial fluorescent lighting, with a controlled 12 hour light/dark cycle (6 am to 6 pm light). The normal temperature and relative humidity ranges in the animal rooms were 22-26°C and 30-70%, respectively. The animal rooms were set to have up to 15 air exchanges per hour. Filtered tap water, acidified to a pH of 2.5 to 3.0, and standard rodent chow was provided *ad libitum*.

Each animal was grafted with approximately 1×10^7 PBMC of a single human donor. On Day 5 after PBMC engraftment, animals were randomized by weight and dosed intravenously every other day for two weeks with 5.5 mg/kg rhATG (n=8), 6.5 mg/kg Thymoglobulin (n=8), or a vehicle control (n=8), or Days 5, 6, and 7 post-engraftment with 5.5 mg/kg rhATG (n=8), 6.5 mg/kg Thymoglobulin (n=8), or a vehicle control (n=8). Two PBMC donors were tested for each dosing regimen (2 PBMC donors $\times$ 2 dosing regimens $\times$ 3 treatment groups $\times$ 8 animals per group = 96 animals). Animals

were assessed for clinical signs of mortality daily. Mice were euthanized by CO_2 asphyxiation before final study take down if they showed >20% weight loss from their starting weight or a combination of the following clinical signs: >10-20% weight loss from their starting weight, cold to touch, lethargic, pale, hunched posture and scruffy coat. 50 μL of blood was drawn from all alive animals on Days 9,16, 23, and 30 post-engraftment via retro-orbital bleed, and flow cytometry stained for Human (hu)CD45-PE (clone HI30; BioLegend #304008, San Diego, CA, USA) and 7AAD (BioLegend, San Diego, CA, USA); 50 μL of CountBright beads (Thermo Fisher Scientific, Waltham, MA, USA) were added to each sample prior to acquisition. Flow cytometric data acquisition was performed using the BD Biosciences FACSCanto flow cytometer (San Jose, CA, USA), and data were acquired and analyzed using BD Biosciences FACSDiva™ software (version 8; San Jose, CA, USA); lymphocytes, singlet, live cells, and CD45+ cells were gated and cell numbers quantified relative to CountBright beads. The gating strategy is outlined in **Supplementary Figure S40b**.

SUPPLEMENTARY METHODS REFERENCES

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