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Methods of Mapping Antigen Specificity to Antibody-Secreting Cells

Reference To Sequence Listing

This application includes a Sequence Listing filed electronically as an XML file named
5 381203733SEQ, created on March 10, 2023, with a size of 824 kilobytes. The Sequence Listing is
incorporated herein by reference.

Field

The present disclosure is directed, in part, to antibody capture complexes and methods
10 of mapping antigen specificity to antibody-secreting cells.

Background

B cells express the B cell receptor (BCR) on their surface, thereby allowing the
determination of an antigen-specific B cell repertoire profiling. While multiple platforms are
15 available for antibody discovery from B cells expressing cell-surface BCR, the antibodies isolated
vary from low to high affinity. Following activation by cognate antigen, B cells undergo fine-
tuning of their BCRs and may ultimately differentiate into antibody-secreting cells (ASCs).
Antibody secreting cells are a specialized cell type that represents the end-stage of the B-cell
differentiation program and comprise plasmablasts, short-lived plasma cells, and long-lived
20 plasma cells (or, more commonly, simply “plasma cells” (PCs); Tellier and Nutt, Eur. J. Immunol.,
2019, 49, 30-37). These ASCs can produce high-affinity antibodies with therapeutic or
prophylactic potential. However, ASCs can also be the source of antibody-mediated
pathologies.

While antigen-specific antibodies from B cells expressing BCRs on their cell surface can
25 be readily cloned and sequenced following flow cytometric isolation, PCs, which are ASCs that
do not express cell-surface BCRs, cannot currently be easily profiled in a high-throughput way.
And while PCs may be the source of very high-affinity antibody, the specificity of any given
secreted antibody cannot rapidly be determined. Because there are limitations as to the
application of current platforms to PCs, there is a long-felt but unsolved need to efficiently pair
30 a given antibody with the BCR⁺ PC that secreted it as well as to efficiently determine the antigen
specificity of that antibody.

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Summary

The present disclosure provides an antibody capture complex comprising a first component of a binding pair linked to an antibody-capture molecule; wherein the first component of the binding pair is capable of binding a second component of the binding pair; wherein the antibody-capture molecule is capable of binding to a target antibody, wherein the target antibody is secreted by an antibody secreting cell. In some embodiments, when the second component of the binding pair is biotin and the first component of the binding pair is streptavidin, the antibody-capture molecule does not bind to a kappa light chain of the target antibody.

10 The present disclosure also provides methods of capturing a target antibody secreted by an antibody secreting cell, the methods comprising: contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of the binding pair to bind to the cell surface of the population of antibody secreting cells, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and contacting the population of antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.

The present disclosure also provides methods of capturing a target antibody secreted by an antibody secreting cell, the methods comprising: contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and contacting the population of antibody secreting cells with an

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antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.

The present disclosure also provides methods of capturing an IgE antibody secreted by an antibody secreting cell, wherein the IgE antibody is directed to an allergen, the methods comprising: contacting a population of antibody secreting cells with NHS-biotin to allow biotin to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody; contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises streptavidin linked to an ectodomain of a high affinity IgE receptor (FcεRIa), whereby the antibody-capture molecule binds to the IgE antibody secreted by the antibody secreting cell; and contacting the population of antibody secreting cells with an antigen, wherein the antigen is an antigenic portion or a mixture of a plurality of antigenic portions of the allergen, whereby the IgE antibody captured by the antibody capture complex binds to the antigen.

In some embodiments of the methods disclosed herein, the methods further comprise, after contacting the population of antibody secreting cells with an antigen, sorting the antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody. In some embodiments of the methods disclosed herein, the methods further comprise separating the collected pool of antibody secreting cells into single cells and isolating the antibody secreting cell that secretes the target antibody. In some embodiments, antibody-encoding nucleic acids are isolated from the antibody secreting cell that secretes the target antibody.

In some embodiments of the methods disclosed herein, the step of contacting the population of antibody secreting cells with an antigen comprises: (a) contacting the population of antibody secreting cells with a first labeled form of the antigen to allow the antigen to bind to antibodies captured at the cell surface of the population of antibody secreting cells including the target antibody captured on the cell surface of the antibody secreting cell, wherein the antigen of the first labeled form is conjugated to a first detectable label; (b) washing the population of antibody secreting cells to remove unbound antigen; (c) contacting the population of antibody secreting cells with (i) an unlabeled form of the antigen, (ii) a second labeled form of the antigen, or (iii) the unlabeled form of the antigen and the second labeled

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form of the antigen; (d) washing the population of antibody secreting cells to remove unbound antigen, and (e) collecting a pool of antibody secreting cells remaining bound to the first labeled form of the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, and wherein the target antibody
5 secreted by the antibody secreting cell and captured by the antibody capture complex remains bound to the first labeled form of the antigen.

In some embodiments of the methods disclosed herein, after collecting a pool of antibody secreting cells remaining bound to the first labeled form of the antigen, the methods further comprise separating the collected pool of antibody secreting cells into single cells and
10 isolating the antibody secreting cell that secretes the target antibody. In some embodiments, antibody-encoding nucleic acids are isolated from the antibody secreting cell that secretes the target antibody.

The present disclosure also provides methods of identifying a region of a gene encoding an antigen-binding fragment of a target antibody wherein the target antibody is secreted by a
15 cell comprising a modified cell surface, and wherein the target antibody is subsequently captured on the modified cell surface, the methods comprising: a) providing an antibody secreting cell comprising a modified cell surface, wherein the antibody secreting cell secretes a target antibody, wherein the target antibody is subsequently captured on the modified cell surface, wherein the target antibody captured on the modified cell surface is bound to an
20 antigen, wherein the antigen is linked to a barcode nucleic acid molecule, and wherein the antibody secreting cell further comprises a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface; b) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface; c) hybridizing a portion of the nucleic acid
25 molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface to a portion of a second nucleic acid molecule attached to the solid surface; d) preparing a first library of amplicons of gene expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ) in the antibody secreting cell, and a third library of
30 amplicons of antigen barcode nucleic acid molecules; e) sequencing each of the three libraries of amplicons; and f) identifying the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.

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The present disclosure also provides methods of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the methods comprising: a) contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of the binding pair to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; b) contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; c) contacting the population of antibody secreting cells with a secondary anti-Ig antibody, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the secondary anti-Ig antibody; d) contacting the population of antibody secreting cells with an antigen comprising a barcode nucleic acid molecule, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen; e) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; f) separating the pool of antibody secreting cells into single antibody secreting cells, and for each single antibody secreting cell: 1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell; 2) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface; 3) preparing a first library of amplicons of gene expression, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ), and a third library of amplicons of antigen barcode nucleic acid molecules; and 4) sequencing each of the three libraries of amplicons; and g) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

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The present disclosure also provides methods of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the methods comprising: a) contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting
5 cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the
10 antibody secreting cell; b) contacting the population of antibody secreting cells with a secondary anti-Ig antibody, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the secondary anti-Ig antibody; c) contacting the population of antibody secreting cells with an antigen comprising a barcode nucleic acid molecule, whereby the target antibody secreted by the antibody secreting cell and
15 captured by the antibody capture complex binds the antigen; d) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; e) separating the pool
20 of antibody secreting cells into single antibody secreting cells, and for each single antibody secreting cell: 1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell; 2) hybridizing a portion of the nucleic acid molecule encoding the region of the gene encoding the
25 antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface; 3) preparing a first library of amplicons of gene expression, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ), and a third library of amplicons of antigen barcode nucleic acid molecules; and 4) sequencing each of the three libraries of amplicons; and f) identifying the region of the gene encoding the
30 antigen-binding fragment of the target antibody.

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Brief Description Of The Drawings

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

5 Figure 1 shows a representative workflow of an Ig secretion capture method. Step 1 shows the biotinylation of cells with N-hydroxysuccinimido biotin (NHS-biotin). Step 2 shows the Ig secretion-capture reagent comprising streptavidin coupled with anti-Fc. α IgK was used for antibodies that express kappa light chain or Fc ϵ R1 α ecto-domain for specific IgE capture. Step 3 shows a secreted α IgE or α IgG antibody bound to an oligo-barcoded and fluorescently-
10 tagged antigen bound by an anti-IgG or anti-IgE antibody.

Figure 2 shows a representative workflow of an IgE secretion capture method. Step 1 shows the biotinylation of a PC using NHS-biotin. Step 2 shows a reagent comprising the ectodomain of the high-affinity IgE receptor (ectoFc ϵ R1 α) conjugated to streptavidin (the “secreted IgE capture reagent”) and the streptavidin bound to biotin on the PC. Step 2 also
15 shows the ectoFc ϵ R1 α of the secreted IgE capture reagent capturing an IgE that the PC has secreted. Step 3 shows the captured IgE bound to a barcoded antigen and bound by an anti-IgE antibody.

Figure 3 shows a representative workflow of an IgG secretion capture method. Step 1 shows the biotinylation of cells with NHS-biotin. Step 2 shows the IgG secretion-capture
20 reagent comprising streptavidin-conjugated anti-mouse IgK assembled on the biotinylated cells. Step 3 shows a secreted IgG antibody bound to a barcoded antigen and bound by an anti-IgG antibody.

Figures 4A-4F show, in Figure 4A, the detection of secreted IgG captured on the surface of ARH77 cells. Figure 4B shows the detection of secreted IgE captured on the surface of U266
25 cells. Figure 4C shows that, while secreted IgE captured on the surface of U266 cells was detected, surface IgE as a B-cell receptor was not. Figure 4D shows a titration of streptavidin- α Igk (StAv- α Igk) on ARH77 cells. Figure 4E shows a titration of streptavidin-Fc ϵ R1 α (StAv-Fc ϵ R1 α) on U266 cells. Figure 4F shows high specificity of detection of secreted IgE captured on the surface of U266 cells but not the IgM secreted by Ramos cells.

30 Figures 5A-5G show, in Figure 5A, a representative workflow of a procedure to determine the sequences of V(D)J regions of an antibody specific for a barcoded antigen and that of the barcode followed by confirmation of antigen specificity of the antibody by an

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enzyme-linked immunosorbent assay (ELISA). Figure 5B shows a flow cytometry of antigen-specific IgG captured on the surface of PCs in hIL-4R α immunized mice. Figure 5C shows a visualization of antigen specificity based on a barcode signal of hIL-6R α (non-specific) and hIL-4R α (specific) in bone marrow (BM) PCs and spleen draining lymph node (dLN) B cells and PCs.

5 Figure 5D shows a UMAP of B cells and PCs isolated from spleen/dLN and bone marrow of challenged mice. Figure 5E shows a graph indicating the ELISA antibody levels of total IgG, anti-hIL-4R α and the ratio of anti-hIL-4R α to total IgG in BM-PCs, SP-PCs and SP-B cells. Figure 5F and Figure 5G show graphs from two separate experiments illustrating the binding affinity of antibodies cloned from BM-PCs, SP-PCs and SP-B cells.

10 Figures 6A-6F show, in Figure 6A, a graph illustrating the percent similarity to the germline V region between hIL-4R α -specific and non-specific cells isolated from BM-PCs, spleen/dLN-PCs, and spleen/dLN-B cells from challenged mice. Figure 6B shows a Venn diagram illustrating clonal overlap based on CDR3 nucleotide sequences from heavy and light chains of clones isolated from BM-PCs, spleen/dLN-PCs, and spleen/dLN-B cells. Figure 6C shows a
15 Volcano plot comparison of differential gene expression between hIL-4R α -specific and non-antigen specific BM-PCs. Figure 6D shows a heatmap illustrating the transcriptional profile of differentially expressed genes expressed in hIL-4R α -specific and non-antigen specific BM-PCs. Figure 6E shows a UMAP illustrating the location and binding affinity (Kd) of hIL-4R α -specific antibodies from BM-PCs on total BM-PCs. Figure 6F shows a UMAP illustrating scaled gene
20 expression of Cd24a, Ppob, Fkbp11, Ssr2, Tmem176a, Tmem176b, Ly6d and CD74 in BM-PCs.

Figures 7A-7H show, in Figure 7A, a representative workflow of a procedure to determine the sequences of V(D)J regions of an antibody specific for a barcoded antigen and that of the barcode followed by confirmation of antigen specificity of the antibody by an enzyme-linked immunosorbent assay (ELISA). Figure 7B shows from left to right, the gating of
25 PCs as cells, single cells, live cells, DUMP⁻ cells, and CD138⁺Blimp-1⁺ cells. Figure 7C shows IgE-secreting, IgE^{Venus+} PCs derived from the BM and LDNs of mice that had been challenged with house dust mite (HDM) extract. Figure 7D shows a UMAP of IgE⁺ PCs based on VDJ-BCR expression over non-IgE PCs. Figure 7E shows the fraction of IgE, IgG, IgA and IgM PCs and mean expression of the top 4 genes expressed in different isotype from both dLN and BM.
30 Figure 7F shows a visualization of antigen specificity based on barcode expression of Der p1, Der p2, Der f1 versus the negative control Ole e1. Figure 7G shows representative binding curves of IgE antibodies to Der p 1, Der p 2, Der f 1 and to the negative control antigen Ole e 1.

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Figure 7H shows antigen specificity validated by ELISA for a subset of IgE antibodies generated from IgE-secreting cells.

Figures 8A-8G show, in Figure 8A, flow plots of BM from an allergic bone marrow donor, BM from a non-allergic bone marrow control donor and a non-streptavidin FcεR1α capture control. Figure 8B shows the results of a test performed on serum to detect levels of IgE specific to cat dander e1, dog dander e5, common silver birch t3, dermatoph pteronyssinus d1, timothy g6, and alternaria alternaria m6. Figure 8C shows an expression dot plot illustrating scaled expression of immunoglobulin genes, and PC and B cell markers within total PCs. Figure 8D shows a graph illustrating scaled expression of IGHE transcript in IgE, IgG, IgA and IgM PCs. Figure 8E shows a table of clustered BCR clonotypes indicating 5 IgE clonotypes, their clone size, and the number of IgE versus non-IgE cells within each clonotype. Figure 8F shows an alignment plot providing a comparison between IgE sequence from an IgE PC to IgG sequences in clonotype 21. Figure 8G shows antigen specificity tested by ELISA for one human IgE antibody isolated by the IgE capture complex of the disclosure from an allergic BM. Representative binding curves of human IgE antibody to Fel d1, Can e1, Der p1 and DNP-IgE are shown.

Figure 9 shows single cell capture as well as library preparation for mRNA, V(D)J regions, and antigen barcode. On the lower row of Figure 9: (i) the light-blue rectangles represent template switch oligos; (ii) the beige rectangles represent sequences of three contiguous riboguanosine residues; (iii) the orange rectangles represent unique molecular identifiers (UMIs); (iv) the purple rectangles represent surface barcodes; and (v) the black squares immediately to the right of the purple rectangles represent sequencing primers.

Figure 10 shows a representative flowchart of the analysis of single-cell mRNA, V(D)J region, and antigen barcode libraries.

Figure 11 shows a visualization of antigen specificity. Cells with higher antigen specificity scores, i.e., a strong signal for target antigens and a low signal for control antigens, were prioritized to select antibody candidates. The target antigens are the HDM allergens Der p 1, Der p 2, and Der f 1 and the negative control was olive antigen Ole e 1. The antigen specificity score for each target antigen was determined by subtracting the quantile rank of the control antigen (qC) raised to the x-th power from the quantile rank of the target antigen (qT), i.e., $qT - qC^x$. Cells with the highest antigen specificity score were selected as antigen specific candidates.

Figure 12 shows a table of IgE candidates based on antigen specificity score. For each candidate the table records the sample from which the cell was obtained, the raw antigen

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barcode counts for each of the target antigens, the normalized antigen barcode counts and the antigen specificity score. Data were acquired for 1,069 IgE⁺ cells.

Figures 13A-13B show the binding of IgE antibodies derived from PC-secreted IgE captured with the secreted IgE capture reagent where the PCs were derived from mice challenged with HDM extract. Figure 13A shows representative binding curves of IgE antibodies to Der p 1 (left-hand chart), Der p 2 (middle chart), and Der f 1 (right-hand chart). Figure 13B shows representative binding curves of IgE antibodies to the negative control antigen Ole e 1.

Figures 14A-14C show that antigen-specific, secreted IgG can be captured on the surfaces of PCs derived from challenged Veloclimmune[®] mice. Figure 14A shows a successive flow cytometric gating strategy to detect antigen-specific IgG⁺ splenic B cells. Figure 14B shows a successive flow cytometric gating strategy to identify BM-derived PCs upon which antigen-specific, secreted IgG had been captured. Figure 14C shows, from left to right, four controls used to accurately define antigen-specific PC populations derived from the BM, and a splenic positive control.

Figures 15A-15D show the normalized value of a target antigen barcode counts on the y axis against the normalized barcode counts of a control antigen barcode counts in all PCs derived from immunized mice and colored by isotype. Figure 15A and Figure 15B show data for cells from the bone marrow while Figure 15C and Figure 15D show data for splenic cells.

Figures 16A-16C show, in Figure 16A, a flow cytometry plot of ARH77 cells stained with αIgG. Figure 16B shows a flow cytometry plot of U266 cells stained with αIgE. Figure 16C shows a flow cytometry analysis of Ramos, U266 or a 100:1 mix of these cells in the presence of an embodiment of the StAv-FcεR1α capture complex.

Figures 17A-17D show, in Figure 17A, the immunization regimen mice underwent prior to tissue collection for downstream antibody specificity mapping through antibody secretion TRAP and Sequencing work. Figure 17B shows IgG-secreting PCs gated as live cells, lymphocytes, single cells, DUMP⁻ cells (TCRβ, CD200R3, Ly6G, CD49b, CD11b, and IgM), CD98+TACI+, and IgG+Ag+ cells. Figure 17C shows Ag+IgG+ cells from spleen and dLN gated as live cells, lymphocytes, single cells, CD19+CD3⁻, IgD-IgM⁻, and IgG+Ag+ cells. Figure 17D shows gating controls to identify Ag+IgG+ BMPCs.

Figures 18A-18D show, in Figure 18A, a combined transcriptional analysis and unbiased clustering analysis of spleen plasma cells, BM plasma cells and splenic B cells. Figure 18B and Figure 18C show the three distinct cell populations yielded by scRNA-seq: bone marrow plasma

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cells (BMPCs), spleen/dLN plasmablast/plasma cell-like (Sp/dLN PBs/PCs), and spleen/dLN B cells (Sp/dLN B cells). Figure 18D shows a Biacore analysis of supernatants and purified antibodies of the disclosure showing the affinity of the BMPCs, Sp/dLN PBs/PCs and Sp/dLN B cells as measured by negative log₁₀ of the binding K_ds.

5 Figures 19A-19B show, in Figure 19A, representative binding curves of IgE antibodies to Der p1, Der p2, Der f1 and to the negative control Ole e1. Figure 19B shows an Fel d1 specific ELISA to test binding of a human IgE mAb 12_2 and its cross-reactivity to Fel d1.

Figures 20A-20B show, in Figure 20A, a gating strategy to sort plasma cells from human bone marrow using CD38 staining. Figure 20B shows the results of an exemplary serum

10 ImmunoCAP® test for an allergic donor.

Figures 21A-21D show an antibody secretion TRAP and sequencing methodology disclosed herein allows detection of vaccine-specific IgG-secreting plasma cells from bone marrow donor. Figure 21A, a representative workflow of a procedure to determine the gating of PCs as cells, single cells, live cells, DUMP- cells, and CD20-CD38++ cells. Figure 21B shows
 15 IgG-secreting and antigen-positive cells found within IgG-secreting cells. Figure 21C shows lack of detection of IgG-secreting PCs without NHS-biotin component, or StAv-Igkappa, or anti-IgG antibody. Figure 21D shows lack of detection of antigen-specific IgG-secreting cells without antigen staining.

Figure 22 depicts the workflow of secretion trap combined with antigen chase (STAC).

20 Step 1: antibody secreting cells were biotinylated with amine-reactive NHS-biotin. Step 2: Secreted antibodies were captured on the surface of antibody secreting cells using streptavidin coupled with anti-hIgG. Anti-hIgG was used here since all antibodies have human constants. Step 3: Cells were incubated with monomeric hIL-4Rα conjugated to AlexaFluor647 for 30 min at RT. Cells were washed and then incubated 2x45min at RT with hIL-4Rα antigen pre-
 25 incubated with StAv-PE.

Figures 23A-23D show flow cytometry analysis of cells undergoing the STAC protocol. Figure 23A shows a table for antibodies used in experiments. Antibodies 1-3 are specific for hIL-4Rα with differing affinities (K_D), and antibody 4 is specific to a different target and serves as a negative control for the experiment. Figure 23B shows a model B cell line with no surface
 30 BCR (A25 cell line) was incubated with NHS-biotin and an anti-hIgG trap reagent (Step 1 and 2 of Figure 22). These cells were then incubated with antibodies from the table in Figure 23A followed by the antigen chase incubations (Step 3 of Figure 22). Figure 23C shows flow

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cytometry analysis of cells undergoing STAC protocol shows that antibody secreting cells can be separated based on affinity. Antibodies 1-4 are color coded on the flow cytometry plots according to the colors in Figure 23A. The higher affinity antibodies (ab_1, ab_2) skew towards the monomeric hIL-4R α -AlexaFluor647 (y-axis), the lower affinity antibody (ab_3) skews toward
5 the tetrameric hIL-4R α -biotin-StAv-PE (x-axis), and the negative control antibody (ab_4) is negative for both antigens. Figure 23D shows flow Cytometry analysis of cells undergoing STAC protocol. All cells are hlgK+ indicating all antibodies, including the negative control (ab_4), are bound to the surface by the secretion trap. An anti-hlgK FMO is shown in grey, all other colors coordinate with the colors in Figure 23A.

10

Description Of Embodiments

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Various terms relating to aspects of the present disclosure are used throughout the
15 specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

Unless otherwise expressly stated, it is not intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly,
20 where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is not intended that an order be inferred, in any respect. This holds for any possible non-expressed basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

25 As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the term "about" means that the recited numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical value is used, unless indicated otherwise by the context, the term "about"
30 means the numerical value can vary by $\pm 10\%$ and remain within the scope of the disclosed embodiments.

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As used herein, the term “comprising” may be replaced with “consisting” or “consisting essentially of” in particular embodiments as desired.

As used herein, the terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence”, “polynucleotide”, or “oligonucleotide” can comprise a polymeric form of
5 nucleotides of any length, can comprise DNA and/or RNA, and can be single-stranded, double-stranded, or multiple stranded. One strand of a nucleic acid also refers to its complement.

As used herein, the term “antibody” can comprise an intact immunoglobulin molecule, a fragmented immunoglobulin molecule comprising an antigen-binding fragment, an antigen-binding fragment without any other fragment of an immunoglobulin molecule.

10 The present disclosure provides antibody capture complexes comprising a first component of a binding pair linked to an antibody-capture molecule; wherein the first component of the binding pair is capable of binding a second component of the binding pair; wherein the antibody-capture molecule is capable of binding to a target antibody, wherein the target antibody is secreted by an antibody secreting cell. In some embodiments, when the
15 second component of the binding pair is biotin and the first component of the binding pair is streptavidin, the antibody-capture molecule does not bind to a kappa light chain of the target antibody.

In some embodiments, a first component of a binding pair comprises a first reagent and a second component comprises a second reagent, wherein the first reagent and the second
20 reagent form a bond.

In some embodiments, the first component of the binding pair comprises avidin, streptavidin, or anti-biotin, and the second component of the binding pair comprises biotin. In some embodiments, the first component of the binding pair comprises biotin and the second component of the binding pair comprises avidin and/or streptavidin. In some embodiments, the
25 first component of the binding pair comprises biotin and the second component of the binding pair comprises anti-biotin. In some embodiments, the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos. In some embodiments, the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad
30 and max. In some embodiments, the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max. In some embodiments, the first component of the binding pair comprises one of an azide and an

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alkyne and the second component of the binding pair comprises the other of an azide and an alkyne. In some embodiments, the first component of the binding pair comprises one of an azide and a phosphine and the second component of the binding pair comprises the other of an azide and a phosphine.

- 5 In some embodiments, the second component of the binding pair comprises a surface marker of the antibody secreting cell and the first component of the binding pair comprises an antibody that binds to the surface marker. In some embodiments, the cell surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand
- 10 interactor (TACI). In some embodiments, the cell surface marker comprises CD27. In some embodiments, the cell surface marker comprises CD38. In some embodiments, the cell surface marker comprises CD45. In some embodiments, the cell surface marker comprises CD138. In some embodiments, the cell surface marker comprises CD98. In some embodiments, the cell surface marker comprises CD78. In some embodiments, the cell surface marker comprises
- 15 CD319. In some embodiments, the cell surface marker comprises CXCR4. In some embodiments, the cell surface marker comprises BCMA. In some embodiments, the cell surface marker comprises GPRC5D. In some embodiments, the cell surface marker comprises FCRL5. In some embodiments, the cell surface marker comprises CD19. In some embodiments, the cell surface marker comprises Ly6D. In some embodiments, the cell surface marker comprises
- 20 CD52. In some embodiments, the cell surface marker comprises TACI.

- In some embodiments, the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody, interleukin-6 (IL-6), and/or a fragment of IL-6 capable of binding to IL-6R. In some
- embodiments, the second component of the binding pair comprises CD27, and the first
- 25 component of the binding pair comprises an anti-CD27 antibody, CD70, and/or a fragment of CD70 capable of binding to CD27.

- In some embodiments, the second component of the binding pair comprises fluorescein isothiocyanate (FITC) and the first component of the binding pair comprises an anti-FITC antibody. In some embodiments, the second component of the binding pair comprises
- 30 fluorescein and the first component of the binding pair comprises an anti-fluorescein antibody. In some embodiments, the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody. In some

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embodiments, the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody. In some embodiments, the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody. In some embodiments, the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody. In some embodiments, the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody. In some embodiments, the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody. In some embodiments, the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody. In some embodiments, the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.

In some embodiments, the antibody-capture molecule comprises a capture antibody.

In some embodiments, the capture antibody comprises an anti-Fc antibody. In some embodiments, the capture antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody. In some embodiments, the capture antibody comprises an anti-Fc γ antibody. In some embodiments, the capture antibody comprises an anti-Fc α antibody. In some embodiments, the capture antibody comprises an anti-Fc ϵ antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, and/or an anti-Fc γ RIIIB antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RI antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RIIA antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RIIB antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RIIB1 antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RIIB2 antibody. In some embodiments, the anti-Fc γ antibody comprises an anti-Fc γ RIIIA antibody. In some embodiments, the anti-Fc α antibody comprises an anti-Fc α RI antibody. In some embodiments, the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody. In some embodiments, the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody. In some embodiments, the anti-Fc ϵ antibody comprises an anti-Fc ϵ RII antibody.

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In some embodiments, the capture antibody comprises an anti-light chain kappa antibody and/or an anti-light chain lambda antibody. In some embodiments, the capture antibody comprises an anti-light chain kappa antibody. In some embodiments, the capture antibody comprises an anti-light chain lambda antibody.

5 In some embodiments, the capture antibody comprises an anti-Ig antibody.

In some embodiments, the capture antibody comprises an anti-IgM antibody.

In some embodiments, the capture antibody comprises an anti-IgG antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, and/or an anti-IgG4 antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG1 antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG2 antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG2a antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG2b antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG3 antibody. In some embodiments, the anti-IgG antibody comprises an anti-IgG4 antibody.

15 In some embodiments, the capture antibody comprises an anti-IgA antibody. In some embodiments, the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody. In some embodiments, the anti-IgA antibody comprises an anti-IgA1 antibody. In some embodiments, the anti-IgA antibody comprises an anti-IgA2 antibody. In some embodiments, the anti-IgA antibody comprises an anti-secretory IgA antibody. In some embodiments, the anti-IgA antibody comprises a polymeric anti-IgA antibody.

In some embodiments, the capture antibody comprises an anti-IgE antibody.

In some embodiments, the antibody-capture molecule comprises an Fc receptor or an ectodomain of the Fc receptor. In some embodiments, the Fc receptor comprises an Fcγ receptor, an Fcα receptor, and/or an Fcε receptor. In some embodiments, the Fc receptor comprises an Fcγ receptor. In some embodiments, the Fc receptor comprises an Fcα receptor. In some embodiments, the Fc receptor comprises an Fcε receptor. In some embodiments, the Fcγ receptor comprises an FcγRI receptor, an FcγRIIA receptor, an FcγRIIB receptor, an FcγRIIB1 receptor, an FcγRIIB2 receptor, an FcγRIIIA receptor, an FcγRIIIB receptor, and/or an FcRn receptor. In some embodiments, the Fcγ receptor comprises an FcγRI receptor. In some embodiments, the Fcγ receptor comprises an FcγRIIA receptor. In some embodiments, the Fcγ

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receptor comprises an FcγRIIB receptor. In some embodiments, the Fcγ receptor comprises an FcγRIIB1 receptor. In some embodiments, the Fcγ receptor comprises an FcγRIIB2 receptor. In some embodiments, the Fcγ receptor comprises an FcγRIIA receptor. In some embodiments, the Fcγ receptor comprises an FcγRIIB receptor. In some embodiments, the Fcγ receptor
 5 comprises an FcRn receptor. In some embodiments, the Fcα receptor comprises an FcαRI receptor and/or an Fcα/μR receptor. In some embodiments, the Fcα receptor comprises an FcαRI receptor. In some embodiments, the Fcα receptor comprises an Fcα/μR receptor. In some embodiments, the Fcε receptor comprises an FcεRI receptor and/or an FcεRII receptor. In some embodiments, the Fcε receptor comprises an FcεRI receptor. In some embodiments, the Fcε
 10 receptor comprises an FcεRII receptor. In some embodiments, the Fc receptor comprises a fragment of an Fc receptor capable of binding Fc.

In some embodiments, the antibody-capture molecule comprises protein A, a fragment of protein A capable of binding to a portion of an Fc region, protein G, and/or a fragment of protein G capable of binding to a portion of an Fc region. In some embodiments, the antibody-
 15 capture molecule comprises protein A. In some embodiments, the antibody-capture molecule comprises a fragment of protein A capable of binding to a portion of an Fc region. In some embodiments, the antibody-capture molecule comprises protein G. In some embodiments, the antibody-capture molecule comprises a fragment of protein G capable of binding to a portion of an Fc region. In some embodiments, the antibody-capture molecule comprises protein L and/or
 20 a fragment of protein L capable of binding to a portion of a light chain. In some embodiments, the antibody-capture molecule comprises protein L. In some embodiments, the antibody-capture molecule comprises a fragment of protein L capable of binding to a portion of a light chain.

In some embodiments, the antibody capture complex comprises avidin or streptavidin
 25 linked to an ectodomain of a high affinity IgE receptor (FcεRIa). In some embodiments, the antibody capture complex comprises avidin linked to FcεRIa.

In some embodiments, the antibody capture complex comprises streptavidin linked to FcεRIa.

Target Antibody

In some embodiments, the target antibody comprises an IgM antibody.
 30 In some embodiments, the target antibody comprises an IgG antibody. In some embodiments, the anti-IgG antibody comprises an IgG1 antibody, an IgG2 antibody, an IgG2a

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antibody, an IgG2b antibody, an IgG3 antibody, and/or an IgG4 antibody. In some embodiments, the anti-IgG antibody comprises an IgG1 antibody. In some embodiments, the anti-IgG antibody comprises an IgG2 antibody. In some embodiments, the anti-IgG antibody comprises an IgG2a antibody. In some embodiments, the anti-IgG antibody comprises an IgG2b
5 antibody. In some embodiments, the anti-IgG antibody comprises an IgG3 antibody. In some embodiments, the anti-IgG antibody comprises an IgG4 antibody.

In some embodiments, the target antibody comprises an IgA antibody. In some embodiments, the IgA antibody comprises an IgA1 antibody, an IgA2 antibody, a secretory IgA antibody, or a polymeric IgA antibody. In some embodiments, the IgA antibody comprises an
10 IgA1 antibody. In some embodiments, the IgA antibody comprises an IgA2 antibody. In some embodiments, the IgA antibody comprises a secretory IgA antibody. In some embodiments, the IgA antibody comprises a polymeric IgA antibody.

In some embodiments, the target antibody comprises an IgE antibody.

Target Antigen

15 In some embodiments, the target of the target antibody, i.e., the antigen to which the target antibody binds, is an allergen. In some embodiments, the allergen comprises a component of an animal product, a drug, a foodstuff, a substance present in a venom, a substance present in saliva of a biting insect, a mold spore, a cosmetic, a metal, latex, a wood, and/or a plant pollen. In some embodiments, the allergen comprises a component of an animal
20 product. In some embodiments, the allergen comprises a drug. In some embodiments, the allergen comprises a foodstuff. In some embodiments, the allergen comprises a substance present in a venom. In some embodiments, the allergen comprises a substance present in saliva of a biting insect. In some embodiments, the allergen comprises a mold spore. In some embodiments, the allergen comprises a cosmetic. In some embodiments, the allergen
25 comprises a metal. In some embodiments, the allergen comprises latex. In some embodiments, the allergen comprises a wood. In some embodiments, the allergen comprises a plant pollen.

In some embodiments, the animal product comprises Bla g, Bla g 1, Bla g 2, Bla g 3, Bla g 3, Bla g 4, Bla g 5, Bla g 6, Bla g 7, Bla g 8, Bla g 9, Bla g 11, Per a, Per a 1, Per a 2, Per a 3, Per a 6, Per a 7, Per a 9, Per a 10, Per a 11, Per a 12, Der p 1, Der p 2, Der f 1, Eur m 1, Pso o 1,
30 Equ c 1, Fel d 1, Fel d 4, Mus m 1, Rat n 1, dust mite tropomyosin, shellfish tropomyosin, and/or cockroach tropomyosin. In some embodiments, the animal product comprises Bla g. In some

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embodiments, the animal product comprises Bla g 1. In some embodiments, the animal product comprises Bla g 2. In some embodiments, the animal product comprises Bla g 3. In some embodiments, the animal product comprises Bla g 3. In some embodiments, the animal product comprises Bla g 4. In some embodiments, the animal product comprises Bla g 5. In some
 5 embodiments, the animal product comprises Bla g 6. In some embodiments, the animal product comprises Bla g 7. In some embodiments, the animal product comprises Bla g 8. In some embodiments, the animal product comprises Bla g 9. In some embodiments, the animal product comprises Bla g 11. In some embodiments, the animal product comprises Per a. In some embodiments, the animal product comprises, Per a 1. In some embodiments, the animal
 10 product comprises Per a 2. In some embodiments, the animal product comprises Per a 3. In some embodiments, the animal product comprises Per a 6. In some embodiments, the animal product comprises Per a 7. In some embodiments, the animal product comprises Per a 9. In some embodiments, the animal product comprises Per a 10. In some embodiments, the animal product comprises Per a 11. In some embodiments, the animal product comprises Per a 12. In
 15 some embodiments, the animal product comprises Der p 1. In some embodiments, the animal product comprises Der p 2. In some embodiments, the animal product comprises Der f 1. In some embodiments, the animal product comprises Eur m 1. In some embodiments, the animal product comprises Pso o 1. In some embodiments, the animal product comprises Equ c 1. In some embodiments, the animal product comprises Fel d 1. In some embodiments, the animal
 20 product comprises Fel d 4. In some embodiments, the animal product comprises Mus m 1. In some embodiments, the animal product comprises Rat n 1. In some embodiments, the animal product comprises dust mite tropomyosin. In some embodiments, the animal product comprises shellfish tropomyosin. In some embodiments, the animal product comprises cockroach tropomyosin.

25 In some embodiments, the animal product comprises animal fur, animal dander, cockroach calyx, wool, and/or a dust mite secretion. In some embodiments, the animal product comprises animal fur. In some embodiments, the animal product comprises animal dander. In some embodiments, the animal product comprises cockroach calyx. In some embodiments, the animal product comprises wool. In some embodiments, the animal product comprises a dust
 30 mite secretion.

In some embodiments, the drug comprises penicillin, a sulfonamide, a salicylate, and/or neomycin. In some embodiments, the drug comprises penicillin. In some embodiments, the

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drug comprises a sulfonamide. In some embodiments, the drug comprises a salicylate. In some embodiments, the drug comprises neomycin.

In some embodiments, the foodstuff comprises celery, celeriac, corn, maize, egg, fruit, pumpkin, eggplant, legume, milk, seafood, sesame, soy, tree nut, wheat, and/or balsam of Peru. In some embodiments, the foodstuff comprises celery. In some embodiments, the foodstuff comprises celeriac. In some embodiments, the foodstuff comprises corn. In some embodiments, the foodstuff comprises maize. In some embodiments, the foodstuff comprises egg. In some embodiments, the foodstuff comprises fruit. In some embodiments, the foodstuff comprises pumpkin. In some embodiments, the foodstuff comprises eggplant. In some
10 embodiments, the foodstuff comprises legume. In some embodiments, the foodstuff comprises milk. In some embodiments, the foodstuff comprises seafood. In some embodiments, the foodstuff comprises sesame. In some embodiments, the foodstuff comprises soy. In some embodiments, the foodstuff comprises tree nut. In some embodiments, the foodstuff comprises wheat. In some embodiments, the foodstuff comprises balsam of Peru. In some
15 embodiments, the legume comprises a bean, a pea, a peanut, or a soybean. In some embodiments, the legume comprises a bean. In some embodiments, the legume comprises a pea. In some embodiments, the legume comprises a peanut. In some embodiments, the legume comprises a soybean. In some embodiments, the tree nut comprises a pecan and/or an almond. In some embodiments, the tree nut comprises a pecan. In some embodiments, the
20 tree nut comprises an almond.

In some embodiments, the substance present in a venom comprises a substance present in bee sting venom and/or a substance present in wasp sting venom. In some embodiments, the substance present in a venom comprises a substance present in bee sting venom. In some embodiments, the substance present in a venom comprises a substance
25 present in wasp sting venom.

In some embodiments, the biting insect is a mosquito or tick. In some embodiments, the biting insect is a mosquito. In some embodiments, the biting insect is a tick. In some embodiments, the allergen in the biting insect is present in the saliva. In some embodiments, the allergen comprises an oligosaccharide. In some embodiments, the allergen comprises
30 galactose-alpha-1,3-galactose or galactose-alpha-1,3-galactose-beta-1,4-N-acetyl glucosamine.

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In some embodiments, the cosmetic comprises a fragrance and/or quaternium-15. In some embodiments, the cosmetic comprises a fragrance. In some embodiments, the cosmetic comprises quaternium-15.

In some embodiments, the metal comprises nickel and/or chromium. In some
5 embodiments, the metal comprises nickel. In some embodiments, the metal comprises chromium.

In some embodiments, the plant pollen comprises a grass pollen, a weed pollen, and/or a tree pollen. In some embodiments, the plant pollen comprises a grass pollen. In some
10 embodiments, the plant pollen comprises a weed pollen. In some embodiments, the plant pollen comprises a tree pollen. In some embodiments, the grass pollen comprises ryegrass pollen and/or timothy-grass pollen. In some embodiments, the grass pollen comprises ryegrass pollen. In some embodiments, the grass pollen comprises timothy-grass pollen. In some
15 embodiments, the weed pollen comprises ragweed pollen, plantago pollen, nettle pollen, pollen of the species *Artemisia vulgaris*, pollen of the species *Chenopodium album*, or sorrel pollen. In some embodiments, the weed pollen comprises ragweed pollen. In some
embodiments, the weed pollen comprises plantago pollen. In some embodiments, the weed pollen comprises nettle pollen. In some embodiments, the weed pollen comprises pollen of the species *Artemisia vulgaris*. In some embodiments, the weed pollen comprises pollen of the species *Chenopodium album*. In some embodiments, the weed pollen comprises sorrel pollen.
20 In some embodiments, the tree pollen comprises birch pollen, alder pollen, hazel pollen, hornbeam pollen, pollen of the genus *Aesculus*, willow pollen, poplar pollen, pollen of the genus *Platanus*, pollen of the genus *Tilia*, pollen of the species *Olea*, pollen of Ashe juniper, or pollen of the species *Alstonia scholaris*. In some embodiments, the tree pollen comprises birch pollen. In some embodiments, the tree pollen comprises alder pollen. In some embodiments,
25 the tree pollen comprises hazel pollen. In some embodiments, the tree pollen comprises hornbeam pollen. In some embodiments, the tree pollen comprises pollen of the genus *Aesculus*. In some embodiments, the tree pollen comprises willow pollen. In some
embodiments, the tree pollen comprises poplar pollen. In some embodiments, the tree pollen comprises pollen of the genus *Platanus*. In some embodiments, the tree pollen comprises
30 pollen of the genus *Tilia*. In some embodiments, the tree pollen comprises pollen of the species *Olea*. In some embodiments, the tree pollen comprises pollen of Ashe juniper. In some
embodiments, the tree pollen comprises pollen of the species *Alstonia scholaris*. In some

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embodiments, the plant pollen comprises olive tree pollen. In some embodiments, the allergen comprises Ole e 1.

In some embodiments, the allergen comprises is one from an oak tree (*Quercus albus*), Japanese cedar tree (*Cryptomeria japonica*), or from an Elm tree or Hickory tree.

5 In some embodiments, the allergen comprises a dog allergen, such as Can f proteins. In some embodiments, the allergen comprises a cow's milk allergen or a beef protein, such as Bos d 2-13. In some embodiments, the allergen comprises a chicken allergen (e.g., Gal d proteins), pig allergens (i.e., Sus s proteins), or a serum albumin. In some embodiments, the allergen comprises a fish allergen (e.g., Gad c/Gad m/Pan h/Sals s proteins) In some embodiments, the
10 allergen comprises a parvalbumin.

In any of the embodiments described herein, the allergen can be replaced with a non-allergen compound such as, for example, a foreign antigen. In some embodiments, the foreign antigen comprises a spike protein of a coronavirus. In some embodiments, the foreign antigen comprises a mutated peptide from a host. In some embodiments, the foreign antigen
15 comprises a protein from another species. In some embodiments, the foreign antigen comprises a compound from an infectious disease, such as SARS-CoV-2 or influenza virus.

In some embodiments, the antibody secreting cell is a plasma cell. In some embodiments, the antibody secreting cell is a plasmablast. In some embodiments, the plasma cell is a short-lived plasma cell. In some embodiments, the plasma cell is a long-lived plasma
20 cell.

Methods of Capturing A Target Antibody Secreted By An Antibody Secreting Cell

The present disclosure also provides methods of capturing a target antibody secreted by an antibody secreting cell, the method comprising: contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of
25 the binding pair to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of
30 the binding pair on the cell surface of the antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and

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contacting the population of antibody secreting cells with an antigen to allow the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind the antigen. In some embodiments, the method further comprises, after contacting the population of antibody secreting cells with an antibody capture complex, contacting the
5 population of antibody secreting cells with a secondary anti-Ig antibody. The step of contacting with a secondary anti-Ig antibody can be performed before, with, or after the step of contacting the population of antibody secreting cells with an antigen.

The present disclosure also provides methods of capturing a target antibody secreted by an antibody secreting cell, the method comprising: contacting a population of antibody
10 secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells,
15 and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and contacting the population of the antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen. In some embodiments, the method further comprises, after contacting the population of antibody secreting cells with an antibody capture
20 complex, contacting the population of antibody secreting cells with a secondary anti-Ig antibody. The step of contacting with a secondary anti-Ig antibody can be performed before, with, or after the step of contacting the population of antibody secreting cells with an antigen.

In some embodiments, after the population of antibody secreting cells is contacted with an antigen, the method may further comprise sorting the population of antibody secreting cells
25 to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antigen secreting cell that secretes the target antibody; and in some such embodiments, the method may further comprise separating the collected pool of antibody secreting cells into single cells
30 and isolating the antigen secreting cell that secretes the target antibody.

In some embodiments, the antigen is a barcoded antigen. In some embodiments, the antigen comprises an allergen. In some embodiments, the barcoded antigen comprises a

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barcode nucleic acid molecule. In some embodiments, a portion of the nucleotide sequence of the barcode nucleic acid molecule is unique to the antigen. In some embodiments, a portion of the barcode nucleic acid molecule comprises a sequencing primer. In some embodiments, the sequencing primer is upstream of the portion of the nucleotide sequence of the barcode

5 nucleic acid molecule that is unique to the antigen. In some embodiments, a portion of the barcode nucleic acid molecule is complementary to a template switch oligonucleotide (TSO). In some embodiments, the portion of the nucleotide sequence of the barcode nucleic acid molecule is unique to the antigen, is upstream of the portion of the barcode nucleic acid molecule, and is complementary to the TSO. In some embodiments, the 3' terminus of the
10 barcode nucleic acid molecule comprises three contiguous cytidine or ribocytidine residues. In some embodiments, the barcode nucleic acid molecule comprises DNA. In some embodiments, the barcode nucleic acid molecule comprises RNA.

In some embodiments, the population of antibody secreting cells is contacted with a plurality of barcoded antigens, wherein each barcoded antigen of the plurality of barcoded
15 antigens comprises a unique antigen linked to a unique nucleic acid molecule. In some embodiments, the unique antigen comprises an allergen. In some embodiments, the unique nucleic acid molecule comprises a barcode nucleic acid molecule.

In some embodiments, each barcoded antigen of the plurality of barcoded antigens is detectably labeled. In some embodiments, each barcoded antigen of the plurality of barcoded
20 antigens is labeled with a radioactive compound, a fluorescent compound, or an enzyme. In some embodiments, each barcoded antigen of the plurality of barcoded antigens is labeled with a radioactive compound. In some embodiments, each barcoded antigen of the plurality of barcoded antigens is labeled with a fluorescent compound. In some embodiments, each barcoded antigen of the plurality of barcoded antigens is labeled with an enzyme. . In some
25 embodiments, the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc . In some embodiments, the radioactive compound comprises ^3H . In some embodiments, the radioactive compound comprises ^{14}C . In some embodiments, the radioactive compound comprises ^{19}F . In some embodiments, the radioactive compound comprises ^{35}S . In some embodiments, the radioactive compound comprises ^{125}I . In some embodiments, the radioactive
30 compound comprises ^{131}I . In some embodiments, the radioactive compound comprises ^{111}In . In some embodiments, the radioactive compound comprises ^{99}Tc . In some embodiments, the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-

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dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein. In some embodiments, the fluorescent compound comprises fluorescein. In some embodiments, the fluorescent compound comprises fluorescein isothiocyanate. In some embodiments, the fluorescent compound comprises rhodamine. In some embodiments, the fluorescent compound comprises 5-dimethylamine-1-naphthalenesulfonyl chloride. In some embodiments, the fluorescent compound comprises phycoerythrin. In some embodiments, the fluorescent compound comprises a fluorescent protein. In some embodiments, the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase. In some embodiments, the enzyme comprises alkaline phosphatase. In some embodiments, the enzyme comprises horseradish peroxidase. In some embodiments, the enzyme comprises luciferase. In some embodiments, the enzyme comprises glucose oxidase.

In embodiments where a secondary anti-Ig antibody is employed, in some such embodiments, the secondary anti-Ig antibody comprises an anti-IgM antibody, an anti-IgG antibody, an anti-IgA antibody, and/or an anti-IgE antibody. In some embodiments, the secondary anti-Ig antibody comprises an anti-IgM antibody. In some embodiments, the secondary anti-Ig antibody comprises an anti-IgG antibody. In some embodiments, the secondary anti-Ig antibody comprises an anti-IgA antibody. In some embodiments, the secondary anti-Ig antibody comprises an anti-IgE antibody.

In some embodiments, the secondary anti-Ig antibody is detectably labeled. In some embodiments, the secondary anti-Ig antibody is labeled with a radioactive compound, a fluorescent compound, or an enzyme. In some embodiments, the secondary anti-Ig antibody is labeled with a radioactive compound. In some embodiments, the secondary anti-Ig antibody is labeled with a fluorescent compound. In some embodiments, the secondary anti-Ig antibody is labeled with an enzyme. In some embodiments, the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc . In some embodiments, the radioactive compound comprises ^3H . In some embodiments, the radioactive compound comprises ^{14}C . In some embodiments, the radioactive compound comprises ^{19}F . In some embodiments, the radioactive compound comprises ^{35}S . In some embodiments, the radioactive compound comprises ^{125}I . In some embodiments, the radioactive compound comprises ^{131}I . In some embodiments, the radioactive compound comprises ^{111}In . In some embodiments, the radioactive compound comprises ^{99}Tc . In some embodiments, the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or

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a fluorescent protein. In some embodiments, the fluorescent compound comprises fluorescein. In some embodiments, the fluorescent compound comprises fluorescein isothiocyanate. In some embodiments, the fluorescent compound comprises rhodamine. In some embodiments, the fluorescent compound comprises 5-dimethylamine-1-naphthalenesulfonyl chloride. In some
5 embodiments, the fluorescent compound comprises phycoerythrin. In some embodiments, the fluorescent compound comprises a fluorescent protein. In some embodiments, the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase. In some
embodiments, the enzyme comprises alkaline phosphatase. In some embodiments, the enzyme comprises horseradish peroxidase. In some embodiments, the enzyme comprises luciferase. In
10 some embodiments, the enzyme comprises glucose oxidase.

In some embodiments, the population of antibody secreting cells is obtained by enriching a population of cells obtained from a human for immune cells. In some embodiments, the population of cells obtained from the human is enriched for plasma cells or plasmablasts.

In some embodiments, the population of antibody secreting cells is obtained from a
15 lymph node, lung, bone marrow, and/or blood of a human. In some embodiments, the population of antibody secreting cells is obtained from a lymph node. In some embodiments, the population of antibody secreting cells is obtained from a lung. In some embodiments, the population of antibody secreting cells is obtained from bone marrow. In some embodiments, the population of antibody secreting cells is obtained from blood.

20 In some embodiments, the antigen and/or the secondary anti-Ig antibody are/is detected. In some embodiments, the antibody secreting cells are sorted to collect a pool of antibody secreting cells that are bound by the antigen and/or by the secondary anti-Ig antibody , wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody.

25 In some embodiments, the antibody secreting cells are contacted with an Fc block prior to contacting the population of antibody secreting cells with the second component of the binding pair.

The present disclosure also provides methods of capturing an IgE antibody secreted by an antibody secreting cell, wherein the IgE antibody is directed to an allergen, the method
30 comprising: contacting a population of antibody secreting cells with NHS-biotin to allow biotin to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody; contacting the population of antibody

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secreting cells with an antibody capture complex, wherein the antibody capture complex comprises streptavidin linked to an ectodomain of FcεR1a, and whereby the antibody-capture molecule binds to the IgE antibody secreted by the antibody secreting cell; and contacting the population of antibody secreting cells with an antigen, wherein the antigen is an antigenic
5 portion or a mixture of a plurality of antigenic portions of the allergen, whereby the IgE antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds to the antigen. In some embodiments, the antigen comprises a plurality of barcoded antigens, wherein each of the plurality of barcoded antigens is a different antigenic portion of the allergen. In some embodiments, the method further comprises sorting the population of
10 antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture complex and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antigen secreting cell that secretes the IgE antibody.

In some embodiments, the method further comprises contacting the population of
15 antibody secreting cells with an anti-IgE antibody to allow the IgE antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind to the anti-IgE antibody. In some embodiments, the method may further comprise sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture
20 complex and is bound by the antigen and the anti-IgE antibody, wherein the collected pool of antibody secreting cells comprises the antigen secreting cell that secretes the IgE antibody.

In some embodiments, the method further comprises separating the collected pool of antibody secreting cells into single cells and isolating the antigen secreting cell that secretes the IgE antibody.

25 ***Antigen Chase***

A population of antibody secreting cells may comprise a plurality of antibody secreting cells each secreting an antibody that is captured by the antibody capture complex, wherein the plurality of antibody secreting cells includes the antibody secreting cell that secretes the target antibody. Some of the antibodies secreted by the plurality of antibody secreting cells bind an
30 antigen to which the target antibody binds, although the antibodies may have varying affinities to the antigen. An antigen chase can be employed to obtain a cell population enriched in

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antibody secreting cells that secrete antibodies having high affinity for the antigen, which allows for isolation of an antibody secreting cell that secretes a target antibody having high affinity. Therefore, in some embodiments of the methods disclosed herein, the step of contacting the population of antibody secreting cells with an antigen includes an antigen chase.

- 5 In these embodiments, as an initial binding step, the population of antibody secreting cells is contacted with a first labeled form of the antigen to allow the antigen to bind to antibodies including the target antibody captured at the surface of antibody secreting cells and form an antigen-antibody complex, followed by a "chase" step where the cells are contacted with one of several forms of the antigen: (i) an unlabeled form of the antigen ("cold chase"), (ii) a second
10 labeled form of the antigen ("hot chase"), or a combination of an unlabeled form of the antigen and a second labeled form of the antigen ("combination chase"). The cells that remain bound to the first labeled form of the antigen after the chase represent cells secreting antibodies with high binding affinity. Accordingly, an antigen chase permits selection among cells secreting antibodies with different affinities to enrich for cells secreting antibodies with high affinities
15 and subsequent isolation of an antibody secreting cell that secretes a target antibody of high affinity.

The term "enriching" means increasing the frequency or percentage of desired cells in a cell population, e.g., increasing the percentage of antibody secreting cells secreting high affinity antibodies within an antibody secreting cell population containing cells secreting antibodies
20 having various affinities (e.g., high affinity, medium affinity, and low affinity). Thus, an antibody secreting cell population enriched in cells secreting high affinity antibodies encompasses a cell population having a higher frequency and/or higher percentage of antibody secreting cells secreting high affinity antibodies as a result of an enrichment process. In the present context, the enrichment process is a process that includes an antigen chase, thereby selecting cells that
25 secrete high affinity antibodies to an antigen from a population of cells that secrete antibodies of various affinities to the antigen, and separating cells that secrete high affinity antibodies from cells that secrete antibodies not of high affinity.

The cell population obtained as a result of a chase is enriched in antibody-secreting cells secreting antibodies with high binding affinity to an antigen of interest. In other words, the
30 enriched population of cells contains a greater percentage of cells that secretes an antibody that binds to the antigen with high binding affinity, as compared to a cell population before or without a chase. In some embodiments, at least 40% of the cells collected secrete a high

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affinity antibody, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the enriched cell population may be a population having at least 50% of cells within the population secreting an antibody that binds to the antigen of interest with high binding affinity, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the enriched cell

5 population may be a population having at least 60% of cells within the population secreting an antibody that binds to the antigen of interest with high binding affinity, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the enriched cell population may be a population having at least 70% of cells within the population secreting an antibody that binds to the antigen of interest with high binding affinity, e.g., antibody having a KD from 0.1pM to

10 25nM. In some embodiments, the enriched cell population may be a population having at least 80% of cells within the population secreting an antibody that binds to the antigen of interest with high binding affinity, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the enriched cell population may be a population having at least 90% of cells within the population secreting an antibody that binds to the antigen of interest with high

15 binding affinity, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the enriched cell population may be a population having at least 95% of cells within the population secreting an antibody that binds to the antigen of interest with high binding affinity, e.g., antibody having a KD from 0.1pM to 25nM. In some embodiments, the frequency of cells secreting high affinity antibodies (e.g., antibody having a KD from 0.1pM to 25nM) in the cell

20 population after a chase is increased by at least 30% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies (e.g., antibody having a KD from 0.1pM to 25nM) in the cell population after a chase is increased by at least 40% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high

25 affinity antibodies (e.g., antibody having a KD from 0.1pM to 25nM) in the cell population after a chase is increased by at least 50% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies (e.g., antibody having a KD from 0.1pM to 25nM) in the cell population after a chase is increased by at least 75% as compared to the frequency in the cell population before or

30 without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies (e.g., antibody having a KD from 0.1pM to 25nM) in the cell population after a chase is increased by at least 100% as compared to the frequency in the cell population before or

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without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies (e.g., antibody having a KD of 0.1pM to 25nM) in the cell population after a chase is increased by at least 200% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 1nM (e.g., antibody having a KD from 0.1pM to 1nM) in the cell population after a chase is increased by at least 40% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 1nM (e.g., antibody having a KD from 0.1pM to 1nM) in the cell population after a chase is increased by at least 50% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 1nM (e.g., antibody having a KD from 0.1pM to 1nM) in the cell population after a chase is increased by at least 75% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 1nM (e.g., antibody having a KD from 0.1pM to 1nM) in the cell population after a chase is increased by at least 100% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 1nM (e.g., antibody having a KD from 0.1pM to 1nM) in the cell population after a chase is increased by at least 200% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 0.1nM (e.g., antibody having a KD from 0.1pM to 0.1nM) in the cell population after a chase is increased by at least 40% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 0.1nM (e.g., antibody having a KD from 0.1pM to 0.1nM) in the cell population after a chase is increased by at least 50% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 0.1nM (e.g., antibody having a KD from 0.1pM to 0.1nM) in the cell population after a chase is increased by at least 75% as compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a KD of less than 0.1nM (e.g., antibody having a KD from 0.1pM to 0.1nM) in the cell population after a chase is increased by at least 100% as

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compared to the frequency in the cell population before or without the chase. In some embodiments, the frequency of cells secreting high affinity antibodies having a K_D of less than 0.1nM (e.g., antibody having a K_D from 0.1pM to 0.1nM) in the cell population after a chase is increased by at least 200% as compared to the frequency in the cell population before or
5 without the chase.

"Binding affinity," as that term is known in the art, generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between
10 members of a binding pair (e.g., antibody and antigen). The affinity of a molecule for its binding partner can generally be represented by the dissociation equilibrium constant (K_D or K_D). There is an inverse relationship between K_D (molar) value and binding affinity, therefore the smaller the K_D value (M), the higher the affinity. Thus "higher affinity" refers to antibodies that generally bind antigen stronger and/or faster and/or remain bound longer. Generally, a
15 lower concentration (M) of antigen is needed to achieve the desired effect due to its stronger binding interaction.

The term " k_d " (sec⁻¹ or 1/s) refers to the dissociation rate constant of a particular antibody-antigen interaction, or the dissociation rate constant of an antibody, Ig, antibody-binding fragment, or molecular interaction. This value is also referred to as the k_{off} value.

20 The term " k_a " (M⁻¹ x sec⁻¹ or 1/M) refers to the association rate constant of a particular antibody-antigen interaction, or the association rate constant of an antibody, Ig, antibody-binding fragment, or molecular interaction.

The term " K_D " or " K_D " (M) refers to the equilibrium dissociation constant of a particular antibody-antigen interaction, or the equilibrium dissociation constant of an antibody, Ig,
25 antibody-binding fragment, or molecular interaction. The equilibrium dissociation constant is obtained by dividing the k_a with the k_d .

A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Binding affinities obtained using the method are typically in the range of about 0.1pM to about 25nM as determined by surface
30 plasmon resonance. In some embodiments, binding affinities are less than about 10nM as determined by surface plasmon resonance.

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The term "high affinity" antibody refers to those antibodies having a binding affinity, expressed as K_D , of 25nM or less, e.g., having a numerical value of about 0.1pM to about 25nM. To this end, high affinity antibodies may have a measured K_D about 25×10^{-9} M (25nM) or less, about 10×10^{-9} M (10nM) or less, about 1×10^{-9} M (1nM) or less, about 1×10^{-10} M (0.1nM) or less, about 0.5×10^{-10} M (0.05nM) or less, about 0.05×10^{-10} M (5pM) or less, about 1pM or less, or about 0.5pM or less, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA. Those of skill in the art will recognize that values for K_D of antibodies may be represented numerically either as nE^{-z} , or as $n \times 10^{-z}$, for example, $3.2E^{-12}$ is equivalent to 3.2×10^{-12} and indicates a K_D of 3.2 picomolar (pM). In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 25nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 20nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 15nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 10nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 5nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 1nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 0.5nM. In some embodiments, the high affinity antibodies have a measured K_D in a range of from about 0.1pM to about 0.1nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 20nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about less than about 15nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 10nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 5nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 1nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 0.1nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 0.5nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 0.01nM. In some embodiments, the high affinity antibodies have a measured K_D of less than about 0.001nM (or 1pM). In some embodiments, the high affinity antibodies have a measured K_D of less than about 0.5pM.

In some embodiments, the antigen is a protein that is present in a monomeric form. Examples of proteins that exist in monomers include interleukin molecules, such as IL-13. In

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some embodiments, the antigen is a protein that is present in a multimeric form, including homomers and heteromers. In some embodiments, the antigen is a protein that is present in both monomeric and multimeric forms, in which case a mixture of protein monomers and multimers can be used in the method described herein.

5 Whether the antigen is a protein that is present in a monomeric form, multimeric form, or a mixture thereof, the antigen may be employed in the methods described herein in a monovalent form or a multivalent form. The terms “monovalent” and “multivalent” are used to refer to the number of units of antigen being presented and to differentiate from the antigen itself being a protein in a monomer form, a multimer form or a mixture thereof. Thus, a
10 monovalent form of an antigen refers to a single unit form of the antigen, where the antigen itself may be a protein in a monomeric form, a multimeric form or a mixture thereof. A multivalent form of an antigen refers to multiple units of the antigen being presented, typically by way of a multivalent molecule to which the antigen is bound or linked. A multivalent molecule can be a dimer, trimer, tetramer, pentamer, hexamer, and the like, or a mixture
15 thereof. In some embodiments, the multivalent molecule is a streptavidin multimer (e.g., tetramer), which can be complexed with biotin which is then linked to the antigen, thereby providing a multivalent (e.g., tetravalent form) of the antigen. In some embodiments, a streptavidin multimer includes tetramer and may additionally include trimer and/or dimer. In
20 some embodiments, a streptavidin multimer is conjugated with a fluorophore such as phycoerythrin. In some
20 embodiments, the multivalent molecule is a dimer of an immunoglobulin Fc fragment, to which the antigen can be linked to provide a bivalent form of the antigen. In some embodiments, the multivalent molecule is a trimer of a trimerization molecule, such as foldon, to which the antigen can be linked to provide a trivalent form of the antigen.

 In some embodiments, the step of contacting the population of antibody secreting cells
25 with an antigen comprises: (a) contacting the population of antibody-secreting cells with a first labeled form of the antigen to allow the antigen to bind to antibodies including the target antibody captured on the cell surface, wherein the antigen of the first labeled form is conjugated to a first detectable label; (b) washing the cells to remove unbound antigen; (c)
30 contacting the cells with either (i) an unlabeled form of the antigen, (ii) a second labeled form of the antigen, or (iii) the unlabeled form of the antigen and the second labeled form of the antigen; (d) washing the cells to remove unbound antigen; (e) collecting a pool of antibody secreting cells remaining bound to the first labeled form of the antigen, wherein the collected

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pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex remains bound to the first labeled form of the antigen.

In some embodiments, the first labeled form of the antigen is at a concentration
5 between 0.001nM and 1uM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.01nM and 100nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.05nM to 10nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.05nM to 9nM, 0.05nM to 8nM, 0.05nM to 7nM, 0.05nM to 6nM, 0.05nM to 5nM, 0.05nM to 4nM, 0.05nM to 3nM, 0.05nM to 2nM, or 0.05nM to 1nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.1nM to 7.5nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.1nM to 7nM, 0.1nM to 6nM, 0.1nM to 5nM, 0.1nM to 4nM, 0.1nM to 3nM, 0.1nM to 2nM, or 0.1nM to 1nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.2nM to 7.5nM. In some embodiments, the first labeled form of the antigen is at a concentration between 0.2nM to 7nM, 0.2nM to 6nM, 0.2nM to 5nM, 0.2nM to 4nM, 0.2nM to 3nM, 0.2nM to 2nM, 0.2nM to 1nM, 0.3nM to 7nM, 0.3nM to 6nM, 0.3nM to 5nM, 0.3nM to 4nM, 0.3nM to 3nM, 0.3nM to 2nM, 0.3nM to 1nM, 0.5nM to 7nM, 0.5nM to 6nM, 0.5nM to 5nM, 0.5nM to 4nM, 0.5nM to 3nM, 0.5nM to 2nM, 0.5nM to 1nM. In some embodiments, the first labeled form of the antigen is at a
15 concentration between 1.0nM to 10nM, 1.0nM to 9nM, 1.0nM to 8.0nM, 1.0nM to 7nM, 1.0nM to 6nM, 1.0nM to 5nM, 1.0nM to 4nM, 1.0nM to 3nM, 1.0nM to 2nM, 2.0nM to 10.0nM, or 5.0nM to 10.0nM. In specific embodiments, the antibody-secreting cells can be contacted with a first labeled form of the antigen where the first labeled form of the antigen is at a concentration of 0.2nM. In specific embodiments, the antibody-secreting cells can be
25 contacted with a first labeled form of the antigen where the first labeled form of the antigen is at a concentration of 5.0nM. In specific embodiments, the antibody-secreting cells can be contacted with a first labeled form of the antigen where the first labeled form of the antigen is at a concentration of 7.5nM. In specific embodiments, the antibody-secreting cells can be contacted with a first labeled form of the antigen where the first labeled form of the antigen is
30 at a concentration of 10nM. In specific embodiments, the antibody-secreting cells can be contacted with a first labeled form of the antigen where the first labeled form of the antigen is at a concentration of 0.1nM, 0.2nM, 0.3nM, 0.4nM, 0.5nM, 0.6nM, 0.7nM, 0.8nM, 0.9nM,

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1.0nM, 1.5nM, 2.0nM, 2.5nM, 3.0nM, 3.5nM, 4.0nM, 4.5nM, 5.0nM, 5.5nM, 6.0nM, 6.5nM, 7.0nM, 8.0nM, 8.5nM, 9.0nM, 9.5nM, or greater.

In some embodiments, the contacting of the antibody secreting cells with a first labeled form of the antigen occurs from about 5 to about 60 minutes, e.g., about 20 minutes, about 30
5 minutes, about 40 minutes, or about 50 minutes.

In some embodiments, the first labeled form of antigen is a monovalent form of the antigen. In some embodiments, the first labeled form of antigen is a multivalent form of the antigen. In some embodiments, the first labeled form of antigen is a mixture of monovalent and multivalent forms of the antigen. Whether a monovalent form, a multivalent form or a
10 mixture thereof, the antigen itself can be a protein that is a monomer, multimer, or a mixture of monomer and multimer.

In some embodiments, the first labeled form of the antigen is the antigen conjugated to a first detectable label. The antigen can be labeled with small molecules, radioisotopes, enzymatic proteins and fluorescent dyes. In some embodiments, the detectable label is a small
15 molecule. Detectable small molecule labels allow for easy labeling of proteins and can be used in a number of regularly deployed detection assays known in the art.

In some embodiments, the detectable label is an enzyme reporter. Enzyme labels are larger than biotin, however, they rarely disrupt antibody function. Commonly used enzyme labels are horseradish peroxidase (HRP), alkaline phosphatase (AP), glucose oxidase and β -
20 galactosidase. To use enzyme-labeled antibodies, samples are incubated with an enzyme-specific substrate that is catalyzed by the enzyme to produce a colored product (chromogenic assays) or light (chemiluminescent assays). Each enzyme has a set of substrates and detection methods that can be employed. For example, HRP can be reacted with diaminobenzidine to produce a brown-colored product or with luminol to produce light. In contrast, AP can be
25 reacted with para-Nitrophenylphosphate (pNPP) to produce a yellow-colored product detected by a spectrophotometer or with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT) to produce a purple-colored precipitate.

In some embodiments, the detectable label is a fluorescent label. Fluorescent labels are directly conjugated to the antibody, no enzyme/substrate or binding interactions are required
30 for detection. Therefore, the amount of fluorescent signal detected is directly proportional to the amount of target protein in the sample. Fluorescent tags can be covalently attached to antibodies through primary amines or thiol.

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After incubation of the population of antibody-secreting cells with a first labeled form of the antigen (the initial binding step), any unbound antigen can be removed from the primary antibody-producing cells. In some embodiments, the unbound antigen is removed through washing. As is known in the art, washing is a technique where a wash buffer is used to remove unwanted components including unbound antigen. Wash buffers are known in the art. In some embodiments, the wash buffer is a phosphate buffered saline (PBS) based wash buffer. In some embodiments, the wash buffer is a Tris buffered saline (TBS) wash buffer. In some embodiments, the wash buffer comprises a detergent. In some embodiments, the detergent is Tween-20. The cells are washed with wash buffer for an allotted time in order to remove unbound antigen. This allotted amount of time will be an amount of time sufficient to remove unbound antigen. In some embodiments, this allotted time can be from about 10 minutes to about 60 minutes to remove unbound antigen; multiple washes that total from 10 to 60 minutes may be used, e.g., 3 washes of 10 minutes or one 30-minute wash; 2-4 washes of 5-15 minutes each, etc. After washing and aspirating the supernatant comprising the wash buffer and unbound antigen, the pellet comprising cells bound with antigen can be used in subsequent steps. In some embodiments, the pellet comprising the bound antigen can be resuspended in a buffer and used in subsequent steps. In some embodiments, the buffer used to resuspend the pellet can be the same wash buffer. In some embodiments, the buffer used to resuspend the pellet can be a different buffer than the wash buffer.

After contacting a population of antibody secreting cells with the first labeled form of antigen (initial binding/contacting step) and once the unbound first labeled form of antigen is removed, the cells are subjected to antigen chase, i.e., the cells are contacted again, or "chased," with the antigen to selectively enrich for cells secreting antibodies with high affinities to the antigen. This chase can be performed using any of the several forms of the antigen: (i) an unlabeled form of the antigen ("cold" chase), (ii) a second labeled form of the antigen ("hot" chase); or (iii) an unlabeled form of the antigen and a second labeled form of the antigen (a "combination chase"). The chase allows the chase antigen to bind to the antibody initially bound by the first labeled form of the antigen, thereby chasing the first labeled form of the antigen off the antibody, unless the antibody has high affinity for the antigen and remains bound to the first labeled form of the antigen after the chase.

In some embodiments, the chase is performed using an unlabeled form of the antigen (cold chase). In some embodiments, the unlabeled form of the antigen is a monovalent form of

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the antigen – that is, while the antigen itself can be a protein in a monomeric form, a multimeric form, or a mixture of monomeric and multimeric forms, a monovalent form of the antigen is the antigen itself without further, secondary multimerization. In some embodiments, the unlabeled form of the antigen is a multivalent form of the antigen, where the antigen itself
5 can be a protein in a monomeric form, a multimeric form, or a mixture of monomeric and multimeric forms. In some embodiments, the unlabeled form of the antigen is a mixture of a monovalent form and a multivalent form of the antigen. In embodiments where a multivalent form of the antigen is used, such form can be provided by a multivalent molecule to which the antigen is bound or linked. In some embodiments, the multivalent molecule is a streptavidin
10 multimer (e.g., tetramer), which can be complexed with biotin which is then linked to the antigen, thereby providing a multivalent (e.g., tetravalent) form of the antigen. In some embodiments, a streptavidin multimer may include trimer and/or dimer in addition to tetramer. In some embodiments, the multivalent molecule is a dimer of an immunoglobulin Fc fragment, to which the antigen can be linked to provide a bivalent form of the antigen. In some
15 embodiments, the multivalent molecule is a trimer of a trimerization molecule, such as foldon, to which the antigen can be linked to provide a trivalent form of the antigen.

In some embodiments, the chase is performed using a second labeled form of the antigen (hot chase). In such embodiments, after the initial binding and washing steps, the antibody secreting cells are chased with a second labeled form of the antigen. The second
20 labeled form of the antigen has a label that provides a different detectable signal than the label on the first labeled form of the antigen. Suitable choices of the label have been described herein, as long as the label on the second labeled form of the antigen is different from the label on the first labeled form of antigen. Such labels include small molecules, radioisotopes, enzymatic proteins and fluorescent dyes. In some embodiments, the first label is
25 AlexaFluor647, and the second label is phycoerythrin.

In some embodiments, the second labeled form of the antigen is a monovalent form of the antigen. In some embodiments, the second labeled form of the antigen is a multivalent form of the antigen. In some embodiments, the second labeled form of the antigen comprises a mixture of a monovalent and a multivalent form of the antigen. In embodiments where a
30 multivalent form of the antigen is used, such form can be provided by a multivalent molecule to which the antigen is bound or linked. In some embodiments, the multivalent molecule is a streptavidin multimer (e.g., tetramer), which can be complexed with biotin which is then linked

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to the antigen, thereby providing a multivalent (e.g., tetravalent) form of the antigen. In some embodiments, a streptavidin multimer may include trimer and/or dimer in addition to tetramer. In some embodiments, a streptavidin multimer is conjugated with a fluorophore such as phycoerythrin. In some embodiments, the multivalent molecule is a dimer of an immunoglobulin Fc fragment, to which the antigen can be linked to provide a bivalent form of the antigen. In some embodiments, the multivalent molecule is a trimer of a trimerization molecule, such as foldon, to which the antigen can be linked to provide a trivalent form of the antigen.

The first labeled form of the antigen and the second labeled form of the antigen can be the same or different valent form of the antigen but must have labels that emit different detectable signals from each other. In some embodiments, the first labeled form of the antigen is a monovalent form of the antigen and the second labeled form of antigen is also a monovalent form. In some embodiments, the first labeled form of the antigen is a monovalent form of the antigen whereas the second labeled form of the antigen is a multivalent form.

In some embodiments, the chase is performed with an unlabeled form of an antigen (cold) and a second labeled form of the antigen (hot), also referred herein as a combination chase. In such embodiments, after the initial binding and washing steps, the antibody-secreting cells are chased with an unlabeled form of the antigen and a second labeled form of the antigen. The two forms of chase antigen: the unlabeled form of the antigen ("cold chase antigen") and the second labeled form of the antigen ("hot chase antigen") can be brought into contact with the cells at the same time or sequentially (e.g., with the cold chase antigen being added first, followed by the hot chase antigen, or vice versa). In some embodiments, the unlabeled form of the antigen is a monovalent form. In some embodiments, the unlabeled form of the antigen is a multivalent form. In some embodiments, the unlabeled form of the antigen is a mixture of monovalent form and multivalent form. In some embodiments, the second labeled form of the antigen is a monovalent form. In some embodiments, the second labeled form of the antigen is a multivalent form. In some embodiments, the second labeled form of the antigen comprises a mixture of a monovalent form and a multivalent form. In embodiments where a multivalent form of the antigen is used in a combination chase, such form can be provided by a multivalent molecule to which the antigen is bound or linked. In some embodiments, the multivalent molecule is a streptavidin multimer (e.g., tetramer), which can be complexed with biotin which is then linked to the antigen, thereby providing a multivalent (e.g., tetravalent) form of the antigen. In some embodiments, a streptavidin

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multimer may include trimer and/or dimer in addition to tetramer. In some embodiments, the multivalent molecule is a dimer of an immunoglobulin Fc fragment, to which the antigen can be linked to provide a bivalent form of the antigen. In some embodiments, the multivalent molecule is a trimer of a trimerization molecule, such as foldon, to which the antigen can be
5 linked to provide a trivalent form of the antigen.

For any format of the chase, the chase antigen concentration used is in excess as compared to the concentration of the first labeled form of the antigen, irrespective of the form of antigen used for the chase (i.e., unlabeled form, a second labeled form, or an unlabeled form and a second labeled form). In embodiments where an unlabeled form of the antigen is used
10 (in a cold chase or in a combination chase), the antigen in the unlabeled form is at least 2 fold, i.e., 2 fold to up to, e.g., 2500 fold, in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 2-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 3-fold in molar ratio relative to the first labeled form of the antigen. In some
15 embodiments, the antigen in the unlabeled form is 4-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 5-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 6-fold, 7-fold, 8-fold, or 9-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 10-
20 fold, 15-fold, 20-fold, or 25-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the unlabeled form is 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, or greater in molar ratio relative to the first labeled form of the antigen. In some embodiments, depending on the concentration of the first labeled form of the antigen, the unlabeled form of the antigen may be at a concentration
25 between 0.4nM to 1uM, or 10 to 600nM. In some embodiments, depending on the concentration of the first labeled form of the antigen, the unlabeled form of the antigen is at a concentration between 10nM to 600nM, 10nM to 500nM, 10nM to 400nM, 10nM to 300nM, 10nM to 200nM, 10nM to 150nM, 10nM to 100nM, 10nM to 75nM, 10nM to 65nM, 10nM to 50nM, 10nM to 40nM, 10nM to 30nM, 10nM to 25nM, or 10nM to 20nM. In embodiments
30 where a second labeled form of the antigen is used (in a hot chase or a combination chase), the antigen in the second labeled form is at least 2 fold, i.e., 2 fold to up to, e.g., 2500 fold, in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the

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second labeled form is 2-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the second labeled form is 3-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the second labeled form is 4-fold in molar ratio relative to the first labeled form of the antigen. In some
5 embodiments, the antigen in the second labeled form is 5-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the second labeled form is 6-fold, 7-fold, 8-fold, or 9-fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the second labeled form is 10-fold, 15-fold, 20-fold, or 25-
10 fold in molar ratio relative to the first labeled form of the antigen. In some embodiments, the antigen in the second labeled form is 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, or greater in molar ratio relative to the first labeled form of the antigen. In some embodiments, depending on the concentration of the first labeled form of the antigen, the second labeled form of the antigen may be at a concentration between 0.4nM to 1uM, or 10nM to 600nM. In some embodiments, depending on the concentration of the first labeled
15 form of the antigen, the second labeled form of the antigen may be at a concentration between 10nM to 600nM, 10nM to 500nM, 10nM to 400nM, 10nM to 300nM, 10nM to 200nM, 10nM to 150nM, 10nM to 100nM, 10nM to 75nM, 10nM to 65nM, 10nM to 50nM, 10nM to 40nM, 10nM to 30nM, 10nM to 25nM, or 10nM to 20nM.

In some embodiments where a combination chase is performed, the cold chase antigen
20 and hot chase antigen can be at the same concentration or at different concentrations.

In some embodiments of a combination chase, the cells are contacted with a cold chase antigen and a hot chase antigen sequentially; and in some such embodiments, a washing step can be included between the two chase antigens. In such embodiments, the cells are washed with wash buffer for an allotted time sufficient to remove unbound antigen. In some
25 embodiments, washing the cells for a period of time of about 10 minutes to about 60 minutes, in one or more washes.

The chase is performed for a period of time sufficient to allow the chase antigen to bind to antibodies. In some embodiments, the chase is performed with an unlabeled form or a second labeled form of the antigen for a time of about 5 to about 60 minutes, e.g., about 10
30 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 45 minutes, or about 50 minutes. In some embodiments of a combination chase, the chase is performed with an unlabeled form of the antigen for a time of about 5 to about 60 minutes followed by incubating

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with a second labeled form of the antigen for a time of about 5 to about 60 minutes. The time of incubation with the unlabeled form of antigen and with the second labeled form of antigen can be the same or different length. As a non-limiting example, the unlabeled form of antigen may contact the cells for about 30 minutes while the second labeled form of the antigen may subsequently contact the cells for about 45 minutes, and vice versa. In some embodiments of a combination chase, the chase is performed with a second labeled form of the antigen for a time of about 5 to about 60 minutes. In some embodiments of a combination chase, the chase is performed with an unlabeled form of the antigen and a second labeled form of the antigen at the same time for a time of about 5 to about 60 minutes.

10 In some embodiments, the chase is performed more than once. In some embodiments, the cold chase is performed more than once. In some embodiments, the hot chase is performed more than once. In some embodiments, the cold chase is performed once followed by a hot chase performed more than once. In some embodiments, a cold chase is performed more than once followed by a hot chase performed once. In some embodiments, a cold chase 15 is performed more than once followed by a hot chase performed more than once. In some embodiments, a combination chase is performed more than once. In some embodiments, a wash step is included after each chase step. In some embodiments, more than one wash step is included after each chase step, e.g., 2 washes or 3 washes.

After chase has been completed, unbound antigen is again removed through washing 20 and the cells remaining bound to the first labeled form of the antigen are collected.

In some embodiments where the first detectable label is a first fluorescent label, fluorescence-activated cell sorting (FACS) can be utilized to collect the cells remaining bound to the first labeled form of the antigen. In some embodiments where the first detectable label is a first fluorescent label, and the second detectable label is a second fluorescent label that 25 differentiates from the first fluorescent label, two-dimensional FACS is used to collect cells that remain bound to the first labeled form of the antigen. In specific embodiments, the first detectable label is A647 and the second detectable label is Phycoerythrin.

Isolation of Antibody-Encoding Nucleic Acids

A pool of antibody secreting cells can be sorted or separated into single cells. Protocols 30 for single cell isolation by flow cytometry are well-known (Huang, J. et al, 2013, supra). Single cells may be sorted and collected by alternative methods known in the art, including but not

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limited to manual single cell picking, limited dilution, microfluidics, laser capture microdissection, and Gel Bead Emulsions (GEMs), which are all well-known in the art. See, for example, Rolink et al., *J Exp Med* (1996)183:187-194; Lightwood, D. et al, *J. Immunol. Methods* (2006) 316(1-2):133-43; Gross et al., *Int. J. Mol. Sci.* (2015) 16: 16897-16919; and Zheng et al.,
5 *Nature Communications* (2017) 8: 14049. Gel Bead Emulsions (GEMs) are also commercially available (e.g., 10X Chromium System from 10x Genomics, Pleasanton, CA).

Once obtained, single antibody-secreting cells may be propagated by common cell culture techniques for subsequent DNA preparation. Alternatively, antibody genes may be amplified from single antibody-producing cells directly and subsequently cloned into DNA
10 vectors.

A nucleic acid encoding an antibody or a fragment thereof can be isolated from single antibody-secreting cells obtained herein. For example, genes or nucleic acids encoding immunoglobulin variable heavy and variable light chains (i.e., VH, VL, VL can be V κ or V λ) can be recovered using RT-PCR protocols with nucleic acids isolated from antibody-secreting cells. For
15 example, a nucleic acid encoding an antibody fragment is first reverse-transcribed (RT) to complementary DNA (cDNA). The cDNA is subsequently amplified in PCR reactions using primers specific to antibody gene sequences, e.g., constant regions of an antibody chain. All chains can be amplified using chain-specific primers in multiplex or separately. The amplicons are then sequenced to obtain the nucleic acid sequence encoding the antibody fragment.
20 These RT-PCR protocols are well known and conventional techniques, as described for example, by Wang et al., *J. Immunol. Methods* (2000) 244:217-225 and described herein.

In some embodiments, the nucleic acid encodes a fragment of an antibody, such as a variable domain, constant domain or combination thereof. In certain embodiments, the nucleic acid isolated from an antibody-producing cell encodes a variable domain of an antibody. In
25 some embodiments, the nucleic acid encodes an antibody heavy chain or a fragment thereof. In other embodiments, the nucleic acid encodes an antibody light chain or a fragment thereof.

Once recovered, antibody-encoding genes or nucleic acids can be cloned into IgG heavy- and light-chain expression vectors and expressed via transfection of host cells. For example, antibody-encoding genes or nucleic acids can be inserted into a replicable vector for further
30 cloning (amplification of the DNA) or for expression (stably or transiently) in cells. Many vectors, particularly expression vectors, are available or can be engineered to comprise

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appropriate regulatory elements required to modulate expression of an antibody encoding gene or nucleic acid.

An expression vector in the context of the present disclosure can be any suitable vector, including chromosomal, non-chromosomal, and synthetic nucleic acid vectors (a nucleic acid
5 sequence comprising a suitable set of expression control elements) as described herein. Examples of such vectors include derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral nucleic acid (RNA or DNA) vectors.

In some embodiments, a nucleic acid molecule is included in a naked DNA or RNA
10 vector, including, for example, a linear expression element (as described in, for instance, Sykes and Johnston, Nat Biotech (1997) 12:355-59), a compacted nucleic acid vector (as described in for instance US 6,077,835), or a plasmid vector such as pBR322 or pUC 19/18. Such nucleic acid vectors and the usage thereof are well known in the art. See, for example, US 5,589,466 and US 5,973,972.

15 In certain embodiments, the expression vector can be a vector suitable for expression in a yeast system. Any vector suitable for expression in a yeast system may be employed. Suitable vectors include, for example, vectors comprising constitutive or inducible promoters such as yeast alpha factor, alcohol oxidase and PGH. See, F. Ausubel et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley InterScience New York (1987); and
20 Grant et al., Methods in Enzymol 153, 516-544 (1987).

In some embodiments, an expression vector carrying a nucleic acid isolated from antibody-secreting cell and encoding an antibody or fragment thereof is introduced into a host cell for expression of the antibody or the fragment thereof. Host cells include, e.g., mammalian cells, yeast cell, bacterial cells, or insect cells. In some embodiments, the host cells are cultured
25 under conditions that express the nucleic acid, and the antibody or portion thereof can then be produced and isolated for further use. In some embodiments, host cells comprising one or more of the above nucleic acids are cultured under conditions that express a full-length antibody, and the antibody can then be produced and isolated for further use. In certain embodiments, the host cell comprises a nucleic acid that encodes a variable domain of an
30 antibody, and the cell is cultured under conditions that express the variable domain. In other embodiments, the host cell comprises a nucleic acid that encodes a variable heavy chain (VH) domain of an antibody, and the cell is cultured under conditions that express the VH domain.

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In another embodiment, the host cell comprises a nucleic acid that encodes a variable light chain (VL) domain of an antibody, and the cell is cultured under conditions that express the VL domain. In specific embodiments, the host cell comprises a nucleic acid that encodes a VH domain of an antibody and nucleic acid that encodes a VL domain of an antibody, and the cell is
5 cultured under conditions that express the VH domain and the VL domain.

In some embodiments, the host cell is a bacterial or yeast cell. In some embodiments, the host cell is a mammalian cell. In other embodiments, the host cell can be, for example, a Chinese hamster ovarian cells (CHO) such as, CHO K1, DXB-11 CHO, Veggie-CHO cells; a COS (e.g., COS-7); a stem cell; retinal cells; a Vero cell; a CV1 cell; a kidney cell such as, for example, a
10 HEK293, a 293 EBNA, an MSR 293, an MDCK, aHaK, a BHK21 cell; a HeLa cell; a HepG2 cell; WI38; MRC 5; Colo25; HB 8065; HL-60; a Jurkat or Daudi cell; an A431 (epidermal) cell; a CV-1, U937, 3T3 or L-cell; a C127 cell, SP2/0, NS-0 or MMT cell, a tumor cell, and a cell line derived from any of the aforementioned cells. In a particular embodiment, the host cell is a CHO cell. In a specific embodiment, the host cell is a CHO K1 cell.

15 The present disclosure also provides methods of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell comprising a modified cell surface, and wherein the target antibody is subsequently captured on the modified cell surface, the method comprising: a) providing an antibody secreting cell comprising a modified cell surface, wherein the cell secretes a target
20 antibody wherein the target antibody is subsequently captured on the modified cell surface, wherein the target antibody captured on the modified cell surface is bound to an antigen, wherein the antigen is linked to a barcode nucleic acid molecule, and wherein the antibody secreting cell further comprises a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell
25 surface; b) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface; c) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface to a portion of a second nucleic acid molecule attached to the solid surface; d) preparing a first library of amplicons of gene
30 expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ) in the antibody secreting cell, and a third library of amplicons of antigen barcode nucleic acid molecules; e) sequencing each of the three libraries

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of amplicons; and f) identifying the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.

In the above method, regarding step b) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, in the partition
5 (e.g., droplet), the partition (e.g., bead) dissolves and the cell barcode nucleic acid molecule/TSO oligos are free floating.

In the above method, regarding step c) hybridizing a portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface to a portion of a second nucleic acid molecule
10 attached to the solid surface, the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface (e.g., the BCR mRNA) does not directly hybridize. Rather, it is first made into first strand cDNA, and then the reverse transcriptase adds CCC to the end of the cDNA. The CCC then hybridizes to the rGrGrG part of the TSO. In addition, regarding the second nucleic acid
15 molecule attached to the solid surface, in the partition (e.g., droplet), the partition (e.g., bead) dissolves and the second nucleic acid molecule is free floating.

In some embodiments, the modification of the modified cell surface comprises avidin, streptavidin, anti-biotin, biotin, the jun protein or a portion thereof, the fos protein or a portion thereof, the mad protein or a portion thereof, the max protein or a portion thereof, the myc
20 protein or a portion thereof, an azide, an alkyne, and/or a phosphine. In some embodiments, the modification of the modified cell surface comprises avidin. In some embodiments, the modification of the modified cell surface comprises streptavidin. In some embodiments, the modification of the modified cell surface comprises biotin. In some embodiments, the modification of the modified cell surface comprises the jun protein or a portion thereof. In
25 some embodiments, the modification of the modified cell surface comprises the fos protein or a portion thereof. In some embodiments, the modification of the modified cell surface comprises the mad protein or a portion thereof. In some embodiments, the modification of the modified cell surface comprises the max protein or a portion thereof. In some embodiments, the modification of the modified cell surface comprises the myc protein or a portion thereof. In
30 some embodiments, the modification of the modified cell surface comprises an azide. In some embodiments, the modification of the modified cell surface comprises an alkyne. In some embodiments, the modification of the modified cell surface comprises a phosphine.

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In some embodiments, the portion of the barcode nucleic acid molecule is complementary to a first template switch oligonucleotide (TSO). In some embodiments, the portion of the barcode nucleic acid molecule can be ligated to a first TSO. In some embodiments, a 3' barcoded system can be used. In this case, the antigen barcode will have
5 polyA. VDJ enrichment and sequencing may be performed differently.

In some embodiments, the portion of the first nucleic acid molecule attached to the solid surface comprises a first template switch oligo (TSO). In some embodiments, the 3' terminus of the first nucleic acid molecule attached to the solid surface comprises a first sequence of three contiguous riboguanosine residues. In some embodiments, the first nucleic
10 acid molecule attached to the solid surface further comprises a first unique molecular identifier (UMI). In some embodiments, the first nucleic acid molecule attached to the solid surface further comprises a first surface barcode. In some embodiments, the first nucleic acid molecule attached to the solid surface further comprises a first sequencing primer. In some
15 5', the first sequence of three riboguanosine residues, the first TSO, the first UMI, the first surface barcode, and the first sequencing primer. In some embodiments, the first nucleic acid molecule attached to the solid surface is attached by the 5' terminus of the first nucleic acid molecule attached to the solid surface.

In some embodiments, the first nucleic acid molecule attached to the solid surface
20 comprises a first DNA molecule attached to the solid surface. In some embodiments, the first DNA molecule attached to the solid surface comprises a first single-stranded DNA molecule attached to the solid surface. In some embodiments, the portion of the first single-stranded DNA molecule attached to the solid surface comprises a first TSO and the first single-stranded
25 terminus of the portion of the barcode nucleic acid molecule which is complementary to the first TSO. Reverse transcription also occurs in the other strand as well, wherein the antigen barcode nucleic acid is extended from its 3' and the first nucleic acid molecule attached to the solid surface is also extended from its 3'.

In some embodiments, the barcode nucleic acid molecule is a single-stranded DNA
30 barcode nucleic acid molecule, wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first TSO, and the single-stranded DNA barcode nucleic acid molecule is reverse transcribed beginning from the 3' terminus of the first TSO.

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In some embodiments, the 3' terminus of the portion of the barcode nucleic acid molecule comprises three contiguous cytidine or ribocytidine residues.

In some embodiments, an mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface is reverse transcribed, thereby generating a single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface. The actual chemistry occurs in solution in the partition (e.g., droplet).

In some embodiments, the nucleotide sequence of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface differs from a complement of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface in that the 3' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three additional, contiguous cytidine or ribocytidine residues. In some embodiments, the 3' terminus of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a plurality of contiguous adenine residues. In some embodiments, the 5' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a plurality of contiguous thymine residues. In some embodiments, the 5' terminus of the second nucleic acid molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues.

In some embodiments, the second nucleic acid molecule attached to the solid surface further comprises a second UMI. In some embodiments, the second nucleic acid molecule attached to the solid surface further comprises a second surface barcode. In some embodiments, the second surface barcode is the same surface (cell) barcode as the first nucleic acid molecule, in order to match antigen to BCR. In some embodiments, the second nucleic acid molecule attached to the solid surface further comprises a second sequencing primer. In some embodiments, the second nucleic acid molecule attached to the solid surface comprises, from 3' to 5', the second sequence of three riboguanosine residues, the second TSO, the second UMI, the second surface barcode, and the second sequencing primer. In some embodiments where a 10X genomics system is used, only the UMI is different for each of the nucleic acid

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molecules on the same surface. As long as the cell barcode is the same, the other components (e.g., TSO and sequencing primer) can be different. In some embodiments, the second nucleic acid molecule attached to the solid surface is attached by the 5' terminus of the second nucleic acid molecule attached to the solid surface.

5 In some embodiments, the second nucleic acid molecule attached to the solid surface comprises a second DNA molecule attached to the solid surface. In some embodiments, the second DNA molecule attached to the solid surface comprises a second single-stranded DNA molecule attached to the solid surface. In some embodiments, the 3' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second
10 sequence of three contiguous riboguanosine residues. In some embodiments, the 3' terminus of the portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three contiguous cytidine residues. In some embodiments, the second single-stranded DNA molecule attached to the solid surface is reverse transcribed beginning from the
15 3' terminus of the three contiguous cytidine residues.

 In some embodiments, the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a second single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the antibody captured on the modified cell surface.
20 In some embodiments, the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three contiguous cytidine or ribocytidine residues, and wherein the 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous
25 riboguanosine residues. In some embodiments, the second single-stranded DNA molecule attached to the solid surface is reverse transcribed beginning from the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface. In some embodiments, the single-stranded DNA molecule encoding the region of the gene is reverse
30 transcribed beginning from the 3' terminus of the second single-stranded DNA molecule attached to the solid surface.

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In some embodiments, the antibody secreting cell is disposed within a partition with the solid surface. In some embodiments, the partition contains a single antibody secreting cell. In some embodiments, the partition contains a single plasma cell or plasmablast. In some embodiments, the solid surface comprises a bead. In some embodiments, the antibody
5 secreting cell within the partition is lysed. In some embodiments, the solid surface (e.g., bead) is dissolved upon partitioning and its surface-attached DNA molecules are released into solution within the partition. In some embodiments, the partition is an oil. In some embodiments, the partition is in the form of a droplet.

In some embodiments, the barcoded nucleic acid molecule further comprises a third
10 sequencing primer.

In some embodiments, each amplicon is produced from a first library of amplicons that comprises the gene that encodes the antigen-binding fragment of the target antibody captured on the modified cell surface.

In some embodiments, the first library of amplicons is sample de-multiplexed, aligned,
15 filtered, and UMI counted. In some embodiments, the sequencing of the first library of amplicons comprises next-generation sequencing.

In some embodiments, each amplicon of the first library of amplicons is mapped and aligned to a standard reference genome. In some embodiments, the standard reference genome comprises a human standard reference genome. In some embodiments, the human
20 standard reference genome is GRCh38. In some embodiments, the standard reference genome comprises a murine standard reference genome. In some embodiments, the murine standard reference genome is mm10.

In some embodiments, each amplicon of the first library of amplicons is mapped to the standard reference genome with a single cell alignment software, such as STAR or kallisto (Bray
25 et al., Nat. Biotechnol., 2016, 34, 525-527; Erratum in: Nat. Biotechnol., 2016, 34, 888), wherein each amplicon of the first library of amplicons comprises a unique molecular identifier, wherein all amplicons of the first library of amplicons which map to annotated genes of the standard reference genome are binned, and wherein the binned amplicons are counted for each unique molecular identifier (UMI), thereby generating a single cell count matrix comprising a mapped
30 counts for each annotated gene and a count for each UMI.

In some embodiments, the single cell count matrix is filtered for high quality cells and extensively profiled genes by calculating the ratio of the total number of annotated genes

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divided by the $\log_{10}$ of the total number of UMIs counted. In some embodiments, cells with a gene to UMI ratio below about 0.1 are filtered out. In some embodiments, cells with more than about four times the interquartile range of the total number of UMIs counted are filtered out. In some embodiments, cells with more than about 80% of reads map to a mitochondrial gene
5 are filtered out. In some embodiments, cells with a gene to UMI ratio below about 0.1, cells with more than about four times the interquartile range of the total number of UMIs counted, and cells with more than about 80% of reads map to a mitochondrial gene are filtered out. In some embodiments, any threshold can be set for gene to UMI ratios and mitochondrial threshold. These values are highly dependent on the tissue being used. More stable tissues,
10 such as blood and lymph nodes, require less stringent thresholds whereas tissue such as the gut, lung, or skin would have higher numbers of dead/dying cells with high mitochondrial contamination.

In some embodiments, the single cell count matrix is normalized such that the total number of UMIs counted is about 1,000, 5,000, 10,000, 20,000, 50,000, or 100,000. In some
15 embodiments, the single cell count matrix is normalized such that the total number of UMIs counted is about 10,000. In some embodiments, the single cell count matrix is normalized such that the total number of UMIs counted is about 5,000. In some embodiments, the single cell count matrix is normalized such that the total number of UMIs counted is about 20,000.

In some embodiments, the principle component embeddings of the single cell count
20 matrix are computed by principle component analysis (PCA) and are used as input to compute a uniform manifold approximation and projection (UMAP). Alternatively, tSNE projection could also be calculated from the PCA embeddings (van der Maaten et al., J. Mach. Learning Res., 2008, 9, 2579-2605). Additional iterative clustering of major cell types can be performed to identify similarities across batches. In some embodiments, the UMAP is generated using the
25 methods disclosed in Korsunsky et al., Nat. Methods, 2019, 16, 1289-1296.

In some embodiments, cluster-specific centroids are used to determine a linear adjustment function per cell, applying the linear adjustment function per cell to correct for differences in batches, thereby generating batch corrected embeddings, and using the batch corrected embeddings to generate a uniform manifold approximation and projection (UMAP) in
30 two dimensions. The method is called harmony (Korsunsky et al., Nat. Methods, 2019, 16, 1289-1296), however, several alternatives exist including the calculation of integration anchors from Seurat (Hao et al., Cell, 2021, 184, 3573-3587).

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In some embodiments, a weighted k-neighbor graph is determined and the Leiden algorithm applied to the weighted k-neighbor graph with resolution values of about 0.1, about 0.2, about 0.5, and about 1.0 to determine cell type clusters in an unsupervised manner. In some embodiments, a weighted k-neighbor graph is determined and the Leiden algorithm applied to the weighted k-neighbor graph with resolution values of about 0.1 to determine cell type clusters in an unsupervised manner. In some embodiments, a weighted k-neighbor graph is determined and the Leiden algorithm applied to the weighted k-neighbor graph with resolution values of about 0.2 to determine cell type clusters in an unsupervised manner. In some embodiments, a weighted k-neighbor graph is determined and the Leiden algorithm applied to the weighted k-neighbor graph with resolution values of about 0.5 to determine cell type clusters in an unsupervised manner. In some embodiments, a weighted k-neighbor graph is determined and the Leiden algorithm applied to the weighted k-neighbor graph with resolution values of about 1.0 to determine cell type clusters in an unsupervised manner. Leiden is a community-detection algorithm that performs clustering iteratively by splitting nodes of similar cells to generate well-connected clusters (Traag et al., Scientific Reports, 2019, 9, 5233). Alternative algorithms include Louvain, K-means and NMF clustering.

In some embodiments, a pairwise Wilcoxon test is performed to all of the cells in one cluster and comparing the result of the first pairwise Wilcoxon test, and another pairwise Wilcoxon test is performed to all of the cells in every other cluster to quantify a p-value and fold-change for each gene in each cluster. In some embodiments, when a gene has a low p-value and the high fold-change, the cells are labeled by a common marker of antibody secreting cells and the cells are labeled by clustered subtypes that are named by the top genes from the differential expression result. This can be repeated for the cells with and without antigen specificity in each isotype (primarily IgE). In some embodiments, a test such as DESeq2 can be used for differential expression.

In some embodiments, sample de-multiplexing is performed, de novo assembly of read pairs into contigs accomplished, and the contigs against germline segment V(D)J reference sequences are aligned and annotated for the second family of amplicons.

In some embodiments, the alignment of the contigs against germline segment V(D)J reference sequences comprises aligning against a human germline reference database or a murine germline reference database using the software IgBlast (Ye et al., Nuc. Acids Res., 2013, 41, W34-W40). In some embodiments, the human germline reference database comprises the

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IMGT database of human germline immunoglobulin sequences (world wide web at "imgt.org/IMGTrepertoire/LocusGenes/"). In some embodiments, the murine germline reference database comprises the IMGT germline reference database.

In some embodiments, VDJ sequences are compared against sequences in the human
5 germline reference database or the murine germline reference database, thereby labeling the
VDJ sequences for quality alignments by checking for in-frame alignments, length of the CDR3,
and/or the absence of stop codons in the variable region sequence and the VDJ sequences
filtered based on the labels. In some embodiments, VDJ sequences are compared against
sequences in the human germline reference database or the murine germline reference
10 database, thereby labeling the VDJ sequences for quality alignments by checking for in-frame
alignments. In some embodiments, VDJ sequences are compared against sequences in the
human germline reference database or the murine germline reference database, thereby
labeling the VDJ sequences for length of the CDR3. In some embodiments, VDJ sequences are
compared against sequences in the human germline reference database or the murine
15 germline reference database, thereby labeling the VDJ sequences for the absence of stop
codons in the variable region sequence. Alternately, this step can be omitted to retain all
possible VDJ sequences, which can be aligned to IgBlast to confirm high quality sequences that
are in frame for human or mouse variable regions.

This step may not be possible for less well-studied organisms if the database of germline VDJ
20 sequences is not available.

In some embodiments, the VDJ sequences are mapped to a reference database (e.g.,
IMGT) of immunoglobulin chains.

In some embodiments, the immunoglobulin isotype, the variable region mapping, the
joining region mapping, the diversity region mapping, the accuracy of full-length variable
25 regions for both heavy and light chain sequence per cell are confirmed by the alignment. In
some embodiments, the immunoglobulin isotype is confirmed by the alignment. In some
embodiments, the variable region mapping is confirmed by the alignment. In some
embodiments, the joining region mapping is confirmed by the alignment. In some
embodiments, the diversity region mapping is confirmed by the alignment. In some
30 embodiments, the accuracy of full-length variable regions for both heavy and light chain
sequence per cell is confirmed by the alignment. In some embodiments, the VDJ sequences are
mapped and annotated to the germline through IgBlast.

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In some embodiments, the third library of amplicons is sample de-multiplexed, aligned, filtered, and UMI counted. In some embodiments, a single cell alignment software, such as STAR or kallisto, can be used.

In some embodiments, the third library of amplicons are mapped to a custom short-
5 read reference which comprises the barcode nucleic acid molecule reference associated with each antigen. In some embodiments, the counts for each uniquely mapped barcode nucleic acid molecule are summed for each cell in a barcoded antigen single cell matrix.

In some embodiments, the barcode nucleic acid molecules across all cells are quantified and normalized by taking the centered log-ratio of each barcode nucleic acid molecule of the
10 plurality of barcode nucleic acid molecules across each sample capture. Alternately, either centered log-ratio or denoising and scaling by background (DSB) can be used (Mulè et al., bioRxiv, 2020.02.24.963603).

In some embodiments, the background antigen signal is removed by DSB for each barcode nucleic acid molecule of the plurality of barcoded nucleic acid molecules. Alternately,
15 centered log-ratio can be used.

In some embodiments, an antigen signal distribution is determined, wherein when there is a well separated bimodal distribution in the antigen signal distribution, z-transformed values are used to compute antigen specificity, and wherein when there is not a well separated bimodal distribution in the antigen signal distribution, a quantile value is used. In some
20 embodiments, rank order can be used rather than quantile value.

In some embodiments, the analysis of the first library of amplicons, the second library of amplicons, and the third library of amplicons is simultaneous to obtain at least one candidate sequence of the antigen-positive target antibody captured on the modified cell surface.

In some embodiments, antibody secreting cells with a valid antibody constant region are
25 subset using the analysis of the first library of amplicons and the analysis of the second library of amplicons. In some embodiments, the antibody constant region comprises an IgE constant region. In some embodiments, the subsetting antibody secreting cell comprises detectable levels of IgE heavy chain and CD79a but lacking detectable levels of Ms4a1 and CD19, wherein the subsetting antibody secreting is an IgE⁺ plasma cell or plasmablast.

30 In some embodiments, the differential expression of the IgE⁺ antibody secreting cell is assessed against an IgA-secreting cell isotype, an IgG-secreting cell isotype, and/or an IgM-

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secreting cell isotype. In some embodiments, the assessment of differential expression further characterizes the unique transcriptional signature of the IgE⁺ antibody secreting cell.

In some embodiments, the antigen specificity of the IgE⁺ antibody secreting cell is assessed. In some embodiments, the assessment comprises comparing the antigen specificity of the IgE⁺ antibody secreting cell against a plurality of antigens and a control antigen, and wherein the plurality of antigens comprises the target antigen. In some embodiments, the comparing comprises calculating an empirical score for each antigen of the plurality of antigens and the control antigen by subtracting a quantile value of a signal associated with the antigen (qT) from a quantile value of a signal associated with the control antigen (qC) with a penalty factor (x) to determine an antigen specificity score where the antigen specificity score = $qT - qC^x$. In some embodiments, the penalty factor is the same for all antigens. In some embodiments, the penalty factor is different for different antibody isotypes. In some embodiments, the penalty factor is different when other antigens are used.

In some embodiments, the antigen specificity score for each of the plurality of antigens and the control antigen is determined. In some embodiments, a control antigen specificity score is calculated, wherein when the antigen specificity score is high and the control antigen specificity score is low, selecting the cell. In some embodiments, the VDJ regions identify antigen-specific antibodies.

In some embodiments, antibody candidates are selected from a ranked list of antigen signals using the paired V_H:V_L antibody sequence of the cell.

In some embodiments, differential expression between isotypes, conditions, and/or antigen specificity is determined, wherein determining the differential expression between isotypes comprises performing a pairwise Wilcoxon test for all of the cells in one isotype and performing a pairwise Wilcoxon test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster; and wherein determining the differential expression between antigen specificity comprises performing a pairwise Wilcoxon test for all of the cells in one isotype and performing a pairwise Wilcoxon test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster.

There are many models/statistics for differential expression analysis. Some examples include: 1) "wilcox", which identifies differentially expressed genes between two groups of cells using a Wilcoxon Rank Sum test (as described herein); 2) "bimod", which is a likelihood-ratio test for single cell gene expression, (McDavid et al., Bioinformatics, 2013); 3) "roc", which

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identifies “markers” of gene expression using ROC analysis; for each gene, evaluates (using AUC) a classifier built on that gene alone, to classify between two groups of cells; an AUC value of 1 means that expression values for this gene alone can perfectly classify the two groupings (i.e., each of the cells in cells.1 exhibit a higher level than each of the cells in cells.2); an AUC value of 0 also means there is perfect classification, but in the other direction; a value of 0.5 implies that the gene has no predictive power to classify the two groups; it returns a “predictive power” ($\text{abs}(\text{AUC}-0.5) * 2$) ranked matrix of putative differentially expressed genes; 4) “t”, which identifies differentially expressed genes between two groups of cells using the Student’s t-test; 5) “negbinom”, which identifies differentially expressed genes between two groups of cells using a negative binomial generalized linear model; 6) “poisson”, which identifies differentially expressed genes between two groups of cells using a poisson generalized linear model; 7) “LR”, which uses a logistic regression framework to determine differentially expressed genes; constructs a logistic regression model predicting group membership based on each feature individually and compares this to a null model with a likelihood ratio test; 8) “MAST”, which identifies differentially expressed genes between two groups of cells using a hurdle model tailored to scRNA-seq data; utilizes the MAST package in R to run the DE testing; 9) “DESeq2”, which identifies differentially expressed genes between two groups of cells based on a model using DESeq2 which uses a negative binomial distribution (Love et al., Genome Biol., 2014); and 10) “detection rate”, which identifies differentially expressed genes between two groups of cells based on percent detection rate between cell groups.

In some embodiments, VDJ sequence similarity in an antigen positive target cell are clustered, wherein the clustering VDJ sequence similarity comprises sequence-based alignment, grouping of similar variable regions by amino acid sequence.

In some embodiments, the antigen positive target cell is IgE⁺.

25 In some embodiments, redundant sequences are trimmed.

The present disclosure also provides methods of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the method comprising: a) contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of the binding pair to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; b) contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody

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capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; c) contacting the population of antibody secreting cells with a secondary anti-Ig antibody, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the secondary anti-Ig antibody; d) contacting the population of antibody secreting cells with an antigen comprising a barcode nucleic acid molecule, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen; e) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody; f) separating the pool of antibody secreting cells into single antibody secreting cells, and for each single antibody secreting cell: 1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell; 2) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface; 3) preparing a first library of amplicons of gene expression, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ), and a third library of amplicons of antigen barcode nucleic acid molecules; and 4) sequencing each of the three libraries of amplicons; and g) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

In order that the subject matter disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the claimed subject matter in any manner. Throughout these examples, molecular cloning reactions, and other standard recombinant DNA techniques, were carried out according to methods described in Maniatis et al., *Molecular Cloning - A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press (1989), using commercially available reagents, except where otherwise noted.

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Examples*Materials and Methods**Reagents Used***Mouse flow antibodies (fluorescently conjugated)**

Antibody	AbPID or Clone #	Supplier	REGN# or Catalog #	Lot#
Fc Block (CD16/32)	2.4G2	Tonbo Biosciences	70-0161-M001	P0161032315705
B220	RA3-6B2	BD	563793	121784
CD138	281-2	BD	563193	9309295
IgG1	A85-1	BD	740121	0030366
IgE	R35-72	BD	564207 and 744281	44493 and 1082998
IgM	II/41	Invitrogen	47-5790-82	2297386
IgD	11-26c.2a	BD	565348	9023728
TCR β	H57-597	Biolegend	109220	B249533
Ly6G	1A8-Ly6g	Biolegend	127624	B264760
CD49b	DX5	Biolegend	108920	B241908
CD11b	M1/70	Biolegend	101226	B259668
TACI	8F10	BD	742840	1260588
CD98	4F2	Biolegend	128214	B347216
CD200R3	Ba13	Biolegend	142208	B301161
CD19	1D3	BD	560143	1022418
IgG2a	SB84a	Southern Biotech	1155-02	F1320-WE90
CD3	17A2	Biolegend	100214	B316738

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Human flow antibodies (fluorescently conjugated)

Antibody	AbPID or Clone #	Supplier	REGN# or Catalog #	Lot#
Human FC Block	polyclonal	eBioscience	14-9161-73	2235896
CD20	2H7	BD	560631	6273736
CD38	HB7	BD	562666	8004645
IgE	G7-26	BD	566324	7339778
IgD	IA6-2	BD	555778	357891
IgM	MHM88	BioLegend	314534	B276950
CD3	UHT1	BD	555916	8066963
CD11b	ICRF44	BD	562793	8067863
CD14	M5E2	BioLegend	301804	B309751
CD16	3G8	BioLegend	302006	B256873
CD123	7G3	BD	558663	8032894

Reagents used and lot numbers

Reagent	Brand	Catalog/Lot
House Dust Mite (HDM) extract	Greer	XPB70D3A25/348717
Saline	Sigma	S8776/RNBF2733
Microtainer® tubes	BD Biosciences	365967
OptEIA® mouse IgE ELISA set	BD Biosciences	555248/1138318
EasySep® Human Pan-B cell Isolation Kit	StemCell	19554/1000042494
EasySep® Mouse Pan-B cell Isolation Kit	StemCell	19844A/10000054035

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Reagent	Brand	Catalog/Lot
Streptavidin Conjugation Kit - Lightning-Link®	ABCAM	ab102921/GR3353137-1 and GR3394825
Biotin Conjugation Kit - Lightning-Link®	ABCAM	Ab201795/GR3406889
Bovine serum albumin (BSA)	Sigma	A9576/SLCH0670
Normal rat serum	StemCell	13551/1000039221
Ficoll® density gradient medium	GE Healthcare	17-1440-03/10292123
Dextran Sulfate	NA	NA
NHS-Biotin	Sigma	20311/3526395
ELISA Plate	Thermo Scientific	80040LE/0910
Carbonate-bicarbonate coating buffer	Sigma	C3041-100/SLBW0243
Distilled water	Gibco	15230-170/2337487
Tween® 20	Sigma	P1379/SLBS6187
OptEIA® assay diluent	BD Biosciences,	555213/0301707
Dulbecco's phosphate- buffered saline (DPBS)	Gibco	14190/2276658
OptEIA® TMB substrate	BD Biosciences	555214/9273407
Stop solution	BDH VWR Analytical	BDH7500-1/21A1956065
4',6-diamidino-2- phenylindole (DAPI)	ThermoFisher	62247/VG3030411
Der p 1 antigen	IndoorBiotech	NA-DP1-1/44098
Der p 2 antigen	IndoorBiotech	NA-DP2-1/41384
Der f 1 antigen	IndoorBiotech	NA-DF1-1/44001
Ole e 1 antigen	IndoorBiotech	RP-OE1-1/43138
TotalSeq® C0956	BioLegend	405283/B325046
TotalSeq® C0957	BioLegend	405285/B327470

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Reagent	Brand	Catalog/Lot
TotalSeq® C0958	BioLegend	405293/B320055
TotalSeq® C0959	BioLegend	405159/B323806
TotalSeq® C0951	Biolegend	405261/B313967
TotalSeq® C0952	Biolegend	405263/B322438

Biotinylation of Cell Surfaces

Cells were washed and resuspended in PBS containing 5 %w/v BSA. Surface Fc was blocked with 1:10 diluted Fc Block (eBioscience) along with detection of surface BCR (for cell line assays) for fifteen minutes at 4°C. The cells were washed twice more with PBS, spun down at 500g for 10 minutes, resuspended in 1mL of freshly prepared NHS-biotin (Sigma, 0.5 mg/mL in PBS) and incubated at 37°C for fifteen minutes. The biotinylated cells were washed three times in cold PBS containing 5% w/v BSA with a changed tube for each washing step.

Preparation of Secreted IgE Capture Reagent

10 EctoFcεR1α was conjugated with streptavidin using a Lightning-Link® streptavidin conjugation kit to form the secreted IgE capture reagent. In brief, a solution of 0.5 mg/mL ectoFcεR1α containing 1 mg/mL streptavidin was prepared. One-hundred microliters of Modifier reagent was added to the solution with gentle mixing. The cap from the vial of Streptavidin Conjugation Mix was removed and the mixture, including the Modifier reagent, 15 was added directly onto the lyophilized material. The material was resuspended gently by withdrawing and re-dispensing the added suspension once or twice using a pipette. The cap was replaced on the vial and the vial was stood for three hours in the dark at 20-25°C. Thereafter, 100 μL of Quencher reagent was added with gentle mixing. Thus, the secreted IgE capture reagent was generated.

Incubation of Cells Having Biotinylated Cell Surfaces with Secreted IgE Capture Reagent

20 Washed, biotinylated cells were resuspended at $1-3 \times 10^6$ cells/mL in 1 mL of PBS containing 5% w/v BSA and 30 μg/mL purified secreted IgE capture reagent and placed in six-well plates. In some embodiments, a volume up to 3 mL in six-well plates can be used. The cells were incubated at 37°C for one hour on a rotating platform to allow for capture of secreted IgE. 25 The cells were then washed twice in PBS containing 5 %w/v BSA.

Lung-Cell Preparation

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Tubes and reagents were prepared before lung samples were obtained. Ten milliliters of sterile water was added to 50 mg of Liberase® TH so as to have a final concentration of 26 collagenase Wunsch units/mL or 5.0 mg/mL collagenase. One milliliter aliquots were made into individual tubes and stored at -20 °C. A Liberase® mix (digestion buffer) was prepared as

5 needed:

Reagent	Initial Conc.	Final Conc.	Volume to Add
Liberase TH Research Grade	26 U/mL	0.7 U/mL	1.0 mL
DNase (in 20 mM Tris, 1 mM MgCl ₂ , 50% glycerol)	10 mg/mL	20 µg/mL	74.4 µL
HBSS with Ca and Mg	---	---	36.1 mL

For each mouse, 1.5 mL of the Liberase® mix was pipetted into a separate 5 mL flow cytometry tube. The tubes were kept on ice or in a cool rack until digestion was required.

For each mouse, the largest lobe of the right lung or the whole lung was placed in a
 10 separate 2 mL Eppendorf® tube with 1 mL of the Liberase® mix. In those tubes, the lung material was chopped into small pieces, typically cubes of about 2-3 mm. This material was incubated at 37°C for twenty minutes. The digesting material was added to gentleMACS™ C Tubes and the digestion stopped by the addition of 0.5 M EDTA to a final concentration of 10 mM. Two milliliters of MACS® buffer was also added. The caps were screwed on tightly and the
 15 capped tubes were run on a gentleMACS™ Octo Dissociator using the preloaded program m_lung_02_01. The resulting cells were centrifuged at 400 g for four minutes and the supernatant was decanted. Red-blood cells in the pellet were lysed with 1 mL of Red-Blood Cell Lysing Buffer for three minutes at ambient temperature. Thereafter, 10 mL of DPBS was added to deactivate the lysing buffer. The cells were centrifuged at 400 g for four minutes, the
 20 supernatant decanted, and the pellet resuspended in 1 mL of DPBS. The suspension was filtered through a 100 µm Millipore® plate filter into a 2 mL deep-well plate. The cells were centrifuged at 400 g for four minutes and resuspended in 500 µL DPBS. Fifty microliters from each sample were pooled with 100 µL from other samples and the 150 µL pooled samples were added to round-bottomed 96-well plates.

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Draining Lymph Node-Cell Preparation

To twelve-well, 70 μ m cell strainer, plates 1-2mL of RPMI media was added. The draining lymph nodes were collected and added to the plates. PCR plates were prepared and holes punched into each well with a 20 G needle and were placed on 2 mL deep-well plates.

- 5 The draining lymph nodes were mashed on a 74 μ m cell strainer in 2mL RPMI media using the back end of a 3 mL syringe. The preparation was filtered through a 100 μ m Millipore® plate filter into a 2 mL deep-well plate and the filtered cells centrifuged at 400 x g for four minutes. The pellet was resuspended in 500 μ L DPBS and transferred to a 96-well deep-bottomed plate. This plate was centrifuged at 400 x g for four minutes. The cells were
10 resuspended in 200 μ L of PBS. Into 96-well round-bottomed tubes were plated 200 μ L of the suspension. The plate was centrifuged at 400 x g for four minutes and the supernatant flicked off. The typical cell count from mice that had been administered intranasally 50 μ g of HDM extract diluted in 20 μ L of saline three times a week for fifteen weeks was 230×10^6 cells.

Bone Marrow-Cell Preparation

- 15 To 48-well plates, 200 μ L of RPMI media was added. PCR plates were prepared and holes punched into each well with a 20 G needle and were placed on 2 mL deep-well plates.
The femurs of mice were extracted and the bones cleaned. Both ends of each bone were cut and placed in the PCR plate with holes. The PCR plate was placed on top of a 2 mL deep-well collection plate and centrifuged for four minutes at 500 x g. The centrifugation step
20 was repeated if any BM remained in the bones. The pellets were resuspended in 500 μ L of red blood-cell lysis buffer was added. The suspension was incubated for three minutes at ambient temperature after which 1-2 mL of PBS was added to deactivate the lysis buffer. The resulting cells were centrifuged at 400 x g for four minutes and the pellet resuspended in 1 mL DPBS. The suspension was filtered through a 100 μ m Millipore plate filter into a 2 mL deep-well plate.
25 From each sample, about 100-200 μ L was taken for fluorescence minus one (FMO)s with the pool of the samples used for the FMO. Aliquoted, pooled samples were placed into twelve wells for the FMO. The remaining samples were centrifuged at 400 x g for four minutes.

- The bone-marrow samples were resuspended in 200 μ L of PBS. Half of the bone-marrow sample was plated into 96-well round-bottomed plates. The plates were centrifuged at 400 x g
30 for four minutes and the supernatants flicked off. The typical cell count from mice that had been administered intranasally 50 μ g of HDM extract diluted in 20 μ L of saline three times a week for fifteen weeks was 116×10^6 cells.

Spleen-Cell Preparation

Spleens were extracted from mice and homogenized in 5mL DPBS in gentleMACS™ tubes using the pre-loaded program TBA. Homogenized spleens were centrifuged at 400 x g for four minutes and supernatant was discarded. Spleens were then re-suspended in 4 mL red blood cell lysis buffer and incubated at room temperature for 5 minutes, after which 10 mL DPBS was added to neutralize the lysis buffer. Spleens were then centrifuged at 400 x g for four minutes and the supernatant was discarded. Spleens were then re-suspended in 10 mL DPBS and filtered through 70 µM filters for single cell suspensions. Cell counts and viability were determined and the spleens were then stained for antigen+ B cells.

10 *Pan-B cell isolation*

B cells were isolated using an EasySep® Mouse Pan-B cell Isolation Kit. In brief, the samples were prepared at 1 x 10⁸ cells in 0.5-8 mL of PBS containing 5 %w/v BSA. Fifty microliters of rat serum was added per mL of sample. The samples were added to 14 mL polystyrene round-bottom tubes (catalog no. 35205, lot no. 00421123). The cells were mixed and incubated for five minutes at ambient temperature. Fifty microliters of RapidSpheres® that had been vortexed for thirty seconds were added per milliliter of sample and the mixture was incubated at 2.5 minutes. Phosphate-buffered saline containing 5% w/v BSA was added to a volume of 5 mL and the tubes placed inside The Big Easy EasySep® magnet (StemCell catalog no. 18001) for 2.5 minutes at ambient temperature. The enriched cell suspension was poured into a new tube and counted. An overview of the procedure is set forth below:

			50 µL of Sample			
Cell type	Cell Count	Resuspend to 10 ⁸ cells/mL	Rat serum to add	Cocktail to add	RapidSpheres®	Pan-B cells recovered
BM following HDM challenge	116 x 10 ⁶	1160 µL	58	58	58	68 x 10 ⁶

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Cell type	Cell Count	Resuspend to 10 ⁸ cells/mL	50 μ L of Sample			
			Rat serum to add	Cocktail to add	RapidSpheres [®]	Pan-B cells recovered
DLNs following HDM challenge	230 x 10 ⁶	2300 μ L	115	115	115	30 x 10 ⁶

Example 1: Development of Capture Complexes

To pair secreted antibodies to the ASCs secreting them, immunoglobulin capture complexes were derived that can locally capture secreted antibodies on the surface of ASCs (Figure 1). To achieve this, the cell surface was biotinylated using NHS-biotin, which has a hydrophilic active group that binds to cell surface primary amines (-NH₂). An affinity matrix was then assembled around cells using streptavidin coupled to an Fc binding reagent (α Igk for IgG and Fc ϵ R1 α ectodomain for IgE) to capture secreted immunoglobulins from ASCs (Figure 1). To mark antigen specificity of PCs, this method was coupled with barcoded antigen and/or fluorescent-tagged antigen staining (Figure 1). Single cell transcriptomes (scRNA-seq), barcoded antigen signal and VH:VL antibody sequences were generated using 10X Genomics reagents.

To determine functionality, sensitivity and specificity of IgG and IgE capture complexes, ARH77, an IgG (kappa light chain) secreting cell line, and U266, an IgE (lambda light chain) secreting myeloma line were used for proof of principle experiments (Figures 4A, 4B, 4D and 4E). A lack of BCR surface expression on both cell lines were first confirmed (Figures 16A and 16B). ARH77 and U266 cells were biotinylated using NHS-Biotin and a capture complex was assembled using streptavidin coupled to anti-human Igk for IgG (StAv- α Igk) and the ectodomain of the high affinity IgE receptor, Fc ϵ R1 α (StAv-Fc ϵ R1 α) for IgE. Sensitivity of capture was determined by staining for IgG and IgE (Figures 4A and 4B). While ARH77 and U266 cells missing any part of the affinity matrix lacked IgG and IgE on their surface, successful assembly of all components of the secretion capture complex allowed specific detection of secreted IgG and IgE on the surface of cells that are secreting them (Figures 4A and 4B). To further determine the minimal concentration of StAv- α Igk and StAv-Fc ϵ R1 α required for binding secreted antibodies,

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dose titration of the capture complexes was performed and it was observed that StAv- α Igk saturated at 3.75 μ g/mL while StAv-Fc ϵ RI α saturated between 15-30 μ g/mL (Figures 4D and 4E).

To test specificity of the capture complex, IgM-expressing Ramos cells were mixed with U266 cells 1:1 (Figure 4F) or 100:1 (Figure 16C) and IgE secretion capture complex was
5 assembled on the mixed population. No binding of secreted IgE was observed on the surface of Ramos cells suggesting there is minimal or no cross talk with neighboring cells at the dilution and culture conditions tested (Figure 4F; Figure 16C).

Example 2: Proof of Concept for Secreted IgE Capture

10 *Capture of Secreted IgE: Experimental Procedure*

An approach was made to pair a given secreted IgE antibody with the PC that secreted the antibody. Figure 2 (Step 1) shows the surface of a PC that has been biotinylated with NHS-biotin because the N-hydroxysuccinimido group of NHS-biotin has a hydrophilic active group that nonspecifically reacts with cell-surface primary amines (-NH₂). Step 2 shows a secreted IgE
15 capture reagent bound to the biotinylated PC. Figure 2 (Step 2) also shows the ectoFc ϵ RI α of the secreted IgE capture reagent capturing an IgE that the PC has secreted. Figure 2 (Step 3) shows the captured IgE bound to a barcoded antigen and bound by an anti-IgE antibody.

The Secreted IgE Capture Reagent Successfully Captured Secreted IgE on the Surface of U266 Cells

20 The surfaces of IgE-secreting U266 cells were biotinylated as described herein, washed three times in cold PBS with 5% w/v BSA with the tubes changed for every wash step, and incubated with the secreted IgE capture reagent as described herein. Thereafter, the cells were washed and stained for thirty minutes at 4°C with Brilliant™ Violet 711-labeled anti-IgE (BD) in PBS with 5% w/v BSA, 10 mg/mL dextran sulfate, and 1:50 Fc block. The cells were then washed
25 with 10 x Brilliant™ Violet buffer. Thereafter, the cells were washed twice in PBS and fixed with BD Cytofix™. Subsequently, flow cytometric data was acquired.

The secreted IgE capture reagent was found to specifically capture secreted IgE antibody on the surface of U266 cells (Figure 4B). Importantly, cell-surface IgE antibody was not detected in control cells that were not biotinylated, incubation with the secreted IgE capture reagent, or
30 contacted with Brilliant™ Violet 711-labeled anti-IgE. This confirmed that each step is necessary for detecting captured secreted IgE. Moreover, IgE was not detected on U266 cells as a BCR. This confirmed that captured, secreted IgE antibody was detected on the surface of U266 cells

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rather than IgE as part of a BCR (Figure 4C). To test specificity of the IgE secretion capture reagent, U266 cells were mixed in a 1:1 ratio with Ramos cells, which secrete human IgM. The mixed cells were incubated with the secreted IgE capture reagent as for the U266 cells alone. The cells were then washed in PBS and fixed with Cytofix™ as for the U266 cells alone. Flow
5 cytometric data was again acquired. The secreted IgE capture reagent was found to capture secreted IgE antibody on the surface of U266 cells but not the IgM secreted by Ramos cells. This indicated specificity of the secreted IgE capture reagent (Figure 4C).

Example 3: Capture of Secreted IgE on the Surfaces of Primary Plasma Cells

10 IgE^{Venus} and Blimp-1^{mCherry} reporter mice were used to determine if the secreted IgE capture reagent could capture secreted IgE on the surface of IgE-producing, primary plasma cells. Specifically, the Venus reporter, which had been inserted downstream of membrane IgE locus, identified IgE-secreting cells while the mCherry reporter, which had been inserted downstream of Blimp-1, identified PCs (Asrat et al., Sci. Immunol., 2020, 10, eaav8402). The
15 mice were challenged with 50 µg of HDM extract diluted in 20 µl of saline solution administered intranasally three times a week for fifteen weeks. The mice were anesthetized and sacrificed as described herein. The BM cells were prepared as described herein and the DLNs were prepared as described herein. BM from mice were pooled together and, separately, dLN from mice were pooled together. Pan-B cells were isolated and stained with an antibody mix
20 comprising Brilliant™ Violet 711 labeled anti-IgE (BD) to detect secreted IgE in PBS with 5% w/v BSA for thirty minutes at 4°C. The labeled cells were washed in PBS with 5% w/v BSA, fixed with Cytofix™, and sorted with a BD FACSymphony® S6 flow cytometer (Figures 7A and 7B). Plasma cells were gated in the following order: Cells, single cells, live cells, DUMP⁻ cells, CD138⁺Blimp-1⁺ cells (Figures 7A and 7B). Dump is a collection of markers in the same channel, which includes
25 CD3, CD11b, IgD, IgM, Ly6g, etc. Any cell that expressed these markers was excluded. CD138 was detected using anti-CD138 antibodies, and plasma cells were gated as Blimp1⁺CD138⁺ cells. IgE-secreting PCs were sorted as IgE^{Venus+} cells (Figure 7C).

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Example 4: Single-Cell Library Preparation and Sequencing of Antigen-Specific, IgE-Expressing Plasma Cells

Figure 7A shows a representative overview of the steps of Examples 3 to 5 while Figure 9 shows the steps from the binding of barcode antigen to a secreted IgE captured on the surface of a PC to library selection and sequencing.

Plasma cells were prepared as described in Example 3. Single PCs were suspended in PBS with 0.04% w/v BSA and loaded onto a Chromium® Controller device (10x Genomics) at 15,000 cells per lane. Partitions of the PCs in barcoded beads were formed and the PCs lysed. Reverse transcription was performed followed by breakdown of the partition. RNA-seq, Feature Barcode, and V(D)J libraries were prepared using a Chromium® Next GEM Single Cell 5' Kit, v.2 (10x Genomics). After amplification of the libraries, cDNA was split into separate RNA-seq, Feature Barcode, and V(D)J aliquots. To enrich the V(D)J aliquot for BCR sequences, the Chromium® Automated Single Cell Mouse and Human BCR Amplification & Library Construction Kit (10x Genomics) was used. Feature Barcode libraries for DNA-barcoded Antigens or DNA-barcoded cell surface proteins were prepared using a Chromium® 5' Feature Barcode Library Construction kit (10x Genomics). Paired-end sequencing was performed on an Illumina NovaSeq® 6000 sequencing system for RNA-seq libraries with read 1 being 26 bp for unique molecular identifiers (UMIs) and cell barcodes and read 2 being 80 bp for transcript reads, with 10 bp i7 and 10 bp i5 reads. Pair-end sequencing was also performed feature barcode libraries with read 1 being 26 bp for UMIs and cell barcodes, read 2 being 35 bp for feature barcode read, and with 10 bp i7 and 10 bp i5 reads. Pair-end sequencing was also performed for V(D)J libraries with read 1 being 150 bp, 10 bp for i7, 10 bp for i5, and Read 2 being 150 bp. For RNA-seq and Feature Barcode libraries, the Cell Ranger Single-Cell Software Suite, version 6.1.1 (10x Genomics) was used to perform sample de-multiplexing, alignment, filtering, and UMI counting. The human GRCh38 and mouse mm10 genome assembly and RefSeq gene model for humans and mice, respectively, were used for alignment. For V(D)J libraries, the Cell Ranger software was used to perform sample de-multiplexing, to de novo assemble of read pairs into contigs, to align, and to annotate contigs against all V(D)J germline reference sequences from the germline mouse IMGT reference database.

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Example 5: Single Cell Analysis of Antigen-Specific, IgE-Expressing Plasma Cells

Figure 10 shows a representative flowchart of the steps described in this Example.

Following sequencing, the single cell gene expression library, the secreted antibody V(D)J region library, and the barcoded antigen library were analyzed simultaneously to obtain candidate

5 antigen-positive secreted IgE antibody sequences.

The single cell gene expression library was mapped and aligned to the standard mouse reference genome Mm10. Subsequently, the unique mapped counts for each gene were determined by the CellRanger software. Reads were mapped to the standard reference genome with a single cell alignment software STAR, where each read comprises a unique molecular
10 identifier which maps to annotated genes of the standard Mm10 reference genome. The mapped reads were binned and counted for each unique molecular identifier (UMI), thereby generating a single cell count matrix comprising of mapped counts for each annotated gene and a count for each UMI. The single cell-count matrix was then filtered for by calculating the ratio of the total number of annotated genes divided by the $\log_{10}$ of the total number of UMIs
15 counted. Cells with a gene to UMI ratio below about 0.1 were filtered out. Cells with more than four times the interquartile range of the total number of UMIs were filtered out. Cells with more than about 80% of reads map to a mitochondrial gene were filtered out. High quality cells with a gene to UMI ratio above about 0.1, with less than about four times the interquartile range of the total number of UMIs counted, and with less than 80% of reads mapping to a
20 mitochondrial gene were retained. The count matrices from each individual sample capture were normalized to a total count of 10,000 and batch corrected using the Harmony algorithm to generate a combined uniform manifold approximation and projection (UMAP). Unsupervised clustering by the Leiden algorithm was then used to determine cell type clusters and a Wilcox test was used to identify specific cell type and cluster marker genes.

25 The IgBlast sequence analysis tool (Ye et al., Nucleic Acids Res., 2013, 41,W34-W40) was carried out to align the V(D)J sequences against the germline mouse IMGT reference database. Valid V(D)J sequences were then filtered by productive in-frame alignments, full-length CDR3s, and the absence of stop codons within the sequence. The IgBlast tool also confirmed the immunoglobulin isotype, gene mappings for variable, diverse, and joining regions, and accurate
30 full-length variable regions for both heavy and light chain sequences per cell.

The barcoded antigen library was mapped by the CellRanger software to a custom short-read reference that contained the DNA tag sequences of the four barcoded antigens, namely,

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Der p 1, Der p 2, Der f 1 and Ole e 1. These tag sequences were quantified across all cells and were normalized by taking the centered log-ratio (CLR) per barcoded antigen across each sample capture. In addition, background antigen signal for each barcoded antigen was removed by DSB (Denoised and Scaled by Background). Both the CLR and the DSB normalized values
 5 were used to quantify the target antigen signals, namely, those for Der p 1, Der p 2 and Der f 1, against the control signal, namely, that for Ole e 1.

Antigen specificity score (AgSS) for IgG were calculated by subtracting the quantile of hIL-6R α barcoded UMI counts multiplied by a penalty factor (x) from the quantile of hIL-4R α barcoded UMI counts, $AgSS = Q_{hIL-4R\alpha} - Q_{hIL-6R\alpha}^x$. AgSS for IgE were calculated with a similar
 10 formula for mouse and human single cell data. For IgE scores the target antigens were either Der p1, Der p2 or Der f1 and the control was always Ole e1.

Ig BCR sequences were clustered using scirpy v0.10 using the function `scirpy.pp.ir_dist` based on the amino acid sequence and with a hamming distance of less than 4. Clustered BCRs were collected into clonotypes with the function `scirpy.tl.define_clonotypes` based on the
 15 heavy chain CDR3 amino acid sequence based on the previous criteria of a hamming distance of less than 4. Scirpy documentation can be referenced at the world wide web at “scverse.org/scirpy/latest/api.html”.

Following processing of the three single-cell libraries, the gene expression and V(D)J region sequencing data were used to subset PCs with a valid IgE constant region and detectable
 20 expression of IgE and CD79a but lacking the expression of Ms4a1 and CD19 typically found in non-PC B cells. IgE-expressing PCs were then assessed for differential expression against additional IgA, IgG, and IgM plasma cell isotypes to further characterize the unique transcriptional signature of IgE-secreting PCs. IgE-secreting PCs were then assessed for antigen specificity by comparing the normalized levels of the target antigens, namely, Der p 1, Der p 2,
 25 or Der f 1, against the normalized level of the control antigen Ole e 1. This was performed by calculating an empirical score for each target antigen and subtracting the quantile rank of the target antigen from the quantile rank of the control antigen with a penalty factor. The formula used to do is set forth below:

$$\text{Antigen specificity} = qT - qC^x$$

30 where qT refers to the quantile of the target signal, qC refers to the quantile of the control signal, and x is a penalty factor.

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The antigen specificity score (AgSS) was determined per cell for each of the three target antigens and utilized to prioritize cells with the strongest signal of target antigen while minimizing the signal of the control antigen. Antibody candidates were selected from the ranked lists of target antigen signal using paired VH:VL antibody sequences from the cell in question. Figure 11 shows visualization of antigen specificity.

Example 6: IgE-Binding ELISAs

After selecting allergen-specific antibodies, relative binding to Der p 1, Der p 2, and Der f 1 were quantified by ELISA. Figure 12 shows a table of IgE candidates based on antigen signal. Data were acquired for 1,069 IgE⁺ cells. Specifically, binding microtiter plates were coated with 100 μ L of 2 μ g/mL of natural Der p 1, Der p 2, Der f 1, and Ole e 1 in PBS overnight at 4°C. IgE antibodies were prepared at a concentration of 1 μ g/mL and serially diluted three-fold in PBS with 0.5% w/v BSA. After washing with 0.05% v/v Tween® 20 in PBS and blocking in PBS with 0.5% w/v BSA, 100 μ L of the diluted IgE antibodies were transferred to the plates and incubated for one hour at ambient temperature. Subsequently, 100 μ L of anti-mouse IgE antibody conjugated to horse-radish peroxidase (HRP) diluted at 1:5000 was added. The plates were then incubated for one hour at ambient temperature, and washed in 0.05% v/v Tween®20 in PBS. Subsequently, the HRP substrate 3,3',5,5'-tetramethylbenzidine (TMB) was added. The reaction was stopped (with 100 μ L of sulfuric acid 2.0 N) and optical density values determined using a plate reader at 450 nm. IgEs mAb1, mAb3, and mAb6 were found to bind to Der p 1 and Der f 1 efficiently (Figure 13A). However, these antibodies did not bind to the negative control allergen Ole e 1 (Figure 13B). Compared to IgE mAb1, mAb4, and mAb6, the other IgE mAbs bound to Der p 1 and Der f 1 less efficiently. Overall, these data show that three IgE antibodies, which were captured by the secreted IgE capture reagent and for which the V(D)J regions were determined, bound specifically to Der p 1 and Der f 1.

Example 7: Capture and Profile of IgE Plasma Cells *In Vivo*

For validation of IgE capture *in vivo*, the previously described IgE^{Venus} and Blimp-1^{mCherry} reporter mice (Figure 7A) that were exposed to house dust mite (HDM) extract for 15 weeks and secreted IgE was captured using StAv-Fc ϵ RI α ectodomain as described in Figure 1. Cells were stained with an antibody mix containing PC markers, α IgE for secreted IgE, and oligonucleotide-barcoded HDM-allergens, including Der p1, Der p2, Der f1 and an Olive allergen

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(Ole e1) as a negative control. PCs were gated using Blimp-1 signal (Figures 7B and 7C) and IgE PCs were sorted (Figures 7B and 7C) along with non-IgE PCs (Figures 7B and 7C) for comparison. Consistent with what was observed for U266 and Ramos (Figure 4F), secreted IgE was only detected on IgE PCs, and not on other isotype (non-IgE PCs), confirming specificity of the trap in a mixed cell population (Figures 7B and 7C).

Example 8: IgE Plasma Cells Sequenced from the Bone Marrow and Lymph Nodes of HDM Exposed Mice

To elucidate the biology of tissue resident IgE PCs, the single-cell transcriptomic profiles from lung dLN and BM were merged and cell clustering to compare IgE, and non-IgE PCs was performed. 12,646 IgG+ cells, 17,524 IgA+ cells, 3,503 IgM+ cells and 1,703 IgE+ cells were obtained (Figure 7D). From single cell transcriptomes, immunoglobulin expression was confirmed and specific gene expression programs were identified for each isotype. IgE PCs expressed high levels of *Ighe*, *Slpi*, *Sdf2l1*, and *Pdia4*. IgG PCs expressed *Ighg1* and modestly higher *Cd74* and *B2m* while IgA PCs expressed *Igha* and higher levels of *Cd79a* and *Cd69*. IgM PCs expressed *Ighm* and higher *Slc3a2*, *Ctss* and *Ghg* (Figure 7E).

Using-barcoded HDM allergens, it was possible to directly map IgE PCs to their specificity to Der p1, Der p2, Der f1 (HDM allergens) and Ole e1 (olive allergen, negative control) and compare their AgSS which were calculated as described in Example 4. The antigens were Der p1, Der p2 and Der f1, and the control for all was Ole e1 (olive antigen) (Figure 7F).

A few allergen-specific IgE antibodies were selected based on reactivity profile (see, Table 3) and their relative binding to Der p1, Der p2 and Der f1 as well as to the olive allergen, Ole e1, was tested by ELISA. 2 IgE antibodies (IgE mAb12_2 and mAb 3_1) bound to Der p1 (Figure 6F, Figure 19A), 3 IgEs bound to Der p2 (IgE mAb12_2, mAb8_2 and mAb21_2) and 6 IgEs bound to Der f1 (IgE mAb12_2, mAb1_2, mAb 1_1, mAb3_1, mAb4_1 and mAb 6_1). Ab12_2 bound to Ole e1 in addition to Der p1, Der p2 and Der f1 (Figure 7G) but did not show any binding to Fel d1 (Figure 19B).

To determine if the IgEs that showed HDM-allergen binding had anaphylactic potential, a passive cutaneous anaphylaxis (PCA) assay was performed. Mice were sensitized intradermally with a cocktail of Der p1 or Der p2 specific IgEs (Figure 7G and Figure 19A) in their left ear and an irrelevant DNP-IgE in the right ear as a negative control. After 24 h, mice were intravenously challenged with Evans blue containing Der p1 for group A or Der p2 for group B

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(Figure 7H). Mast cell degranulation was observed only in the ears sensitized with Der p1 or Der p2 specific IgEs (left ear), as measured by Evan's blue dye leakage (Figure 7H), demonstrating that the cocktail of IgE antibodies used induced crosslinking of FcεR1α and mast cell degranulation.

5

Example 9: Human Primary Cells: Identification and Profiling of IgE PCs from the Bone Marrow of Allergic Donors

To determine if IgE secretion capture could be extended to human primary cells, we performed IgE-secretion capture on the BM of self-reported allergic donors. B lineage enriched cells were coated with NHS-biotin and secreted IgE was captured using StAv-FcεR1α ectodomain as previously described in Figure 1. PCs were gated as CD38 high/CD20 low (Figure 20A) and specificity and functionality of StAv-FcεR1α secretion capture was confirmed in non-allergic BM that had full Biotin- StAv-FcεR1α assembly (Figure 8A) as well as allergic BM that lacked StAv-FcεR1α (Figure 8A). To perform full validation of human IgE secretion capture, donors with confirmed cat, timothy grass and HDM reactive IgEs in sera were selected (Figure 8B, Figure 20B), the IgE secretion capture was performed, and all IgE PCs along with non-IgE PCs from the BM were sorted for comparison.

Following sorting, scRNA-seq recovered full transcriptomes and BCR sequences of 5,559 bone marrow PCs from the cat, grass, and dust allergic individual. The specificity of PC capture was confirmed based on the abundant expression of various immunoglobulin genes and PC markers such as *XBP1*, *SLAMF7*, and *CD74*, but B cell markers such as *MS4A1* and *CD19* were not observed (Figure 8C). Ten IgE PCs were identified by VH:VL sequencing and alignment to the human germline database with IgBlast. These IgE PCs correlated very strongly with *IGHE* transcript expression along with a small fraction of cells from IgA and IgG isotypes which also expressed *IGHE*, possibly reflecting sequential class switching between these isotypes or sterile *IGHE* transcription in some IgA and IgG cells (Figures 8C and 8D). This suggests that despite the rarity of IgE PCs in human bone marrow, the capture method of the disclosure enables isolation and profiling of IgE PCs.

To investigate clonal evolution, VH sequences from all 5,559 cells were clustered based on the amino acid sequence of the heavy chain CDR3 region allowing for up to 3 amino acid mismatches, deletions, or insertions. The 10 IgE PCs clustered uniquely into 5 clonotypes, which were defined as a group of PCs with highly similar heavy chain amino acid CDR3s (see, Table 4).

30

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Two clonotypes, denoted 753 (n=4 cells) and 696 (n=3 cells), were made up exclusively of IgE cells and each clonotype displayed identical CDR3 sequences, respectively (Figure 8E). Two other clonotypes denoted 1330 and 576 were single-cell clones from individual IgE cells (Figure 8E). Interestingly, clonotype 21 contained 14 IgG cells and a single IgE (Figure 8E). Closer

5 inspection of the CDR3 nucleotide sequence of clonotype 21 revealed that the lone IgE sequence contains 3-point mutations within the CDR3 region while all IgG members of clonotype 21 carry an identical CDR3 region (Figure 8F). Additionally, the IgE sequence harbored somatic hypermutations in the upstream V gene locus (Figure 8F) compared to IgG clonotype members, suggesting additional rounds of affinity maturation.

10 After selecting the IgE clones, we tested binding of the purified IgEs and a control (DNP-IgE) to relevant allergens to which the donor was allergic to (Fel d1 for cats, Der p1 for dust or Can e1 for dog as a control) by ELISA (Figure 8G). One of the IgE mAbs purified bound to Fel d1, but not Can e1 or Der p1 (Figure 8G), suggesting that human IgE antibodies could successfully be isolated from allergic BM using the IgE capture method of the disclosure.

15

Example 10: Proof of Concept for Secreted IgG Capture

Preparation of Secreted IgG Capture Reagent

An anti-mouse IgK antibody was conjugated with streptavidin using a Lightning-Link® streptavidin conjugation kit to form the secreted IgG capture reagent. In brief, a solution of
20 anti-mouse IgK antibody containing 1 mg/mL streptavidin was prepared. One-hundred microliters of Modifier reagent was added to the solution with gentle mixing. The cap from the vial of Streptavidin Conjugation Mix was removed and the mixture, including the Modifier reagent, was added directly onto the lyophilized material. The material was resuspended gently by withdrawing and re-dispensing the added suspension once or twice using a pipette. The cap
25 was replaced on the vial and the vial stood for three hours in the dark at 20-25°C. Thereafter, 100 µL of Quencher reagent was added with gentle mixing. Thus, the secreted IgK capture reagent was generated.

Capture of Secreted IgG: Experimental Procedure

Figure 3 (Step 1) shows the surface of a PC that has been biotinylated with NHS-biotin.
30 Figure 3 (Step 2) shows a secreted IgG capture reagent bound to the biotinylated PC. Figure 3 (Step 2) also shows the anti-mouse IgK antibody of the secreted IgG capture reagent capturing

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an IgG that the PC has secreted. Figure 3 (Step 3) shows the captured IgG bound to a barcoded antigen and bound by an anti-IgG antibody.

Capture of Secreted IgG on the Surface of Primary Plasma Cells

To detect antigen-specific IgG-secreting PCs, the footpads of VI3/VelocImmune mice
5 were immunized twice a week for four weeks with an immunogen. The mice were rested for a month then boosted with immunogen four days before anesthesia, sacrifice, and procurement of the BM, Spleen, and Draining Lymph Nodes. Cells from the bone marrow, spleen, and draining-lymph nodes were prepared as described herein. Single-cell preparations from the spleen and lymph nodes were pooled and surface Fc receptors blocked with 1:10 diluted Fc
10 Block for fifteen minutes at 4°C. The pooled preparations were then stained using an antibody cocktail to gate for antigen-specific IgG⁺ B cells (Figure 14A). The antibody cocktail for the spleen and lymph nodes includes antibodies against murine IgG1, IgG2a, CD19, IgM, IgD, and CD3. The antibody cocktail for the BM includes antibodies against murine B220, CD138, TACI, CD98, TCRB, CD200R3, Ly6G, CD49b, CD11b, IgM, CD19, IgG1, IgG2a, and IgD. All antibodies are
15 commercially available. The surfaces of the PCs were biotinylated as described herein, washed three times in cold PBS with 5% w/v BSA with the tubes changed for every wash step, and incubated with the secreted IgG capture reagent as described herein with the secreted IgG capture reagent present and the secreted IgE capture reagent absent. The cells were incubated at 37°C for one hour on a rotating platform to allow capture of secreted Ig antibody and stained
20 with an antibody mix containing fluorescein isothiocyanate-labeled anti-IgG (BD Biosciences and Southern Biotech) for detection of captured IgG and barcoded, phycoerythrin-labeled immunogen in PBS with 5% BSA for thirty minutes at 4°C. Labeled PCs were sorted on a BD FACSSymphony® S6 flow cytometer and PCs identified upon which secreted IgG had been captured (Figure 14B). Controls were used to detect an antigen-specific population with
25 secreted IgG captured on the cell surface thereof (Figure 14C).

Example 11: Single-Cell Library Preparation and Sequencing of Plasma Cells with Secreted IgG Captured on the Surface Thereof

For IgG-secreting PCs for which the IgG had been captured, libraries for gene expression,
30 the V(D)J regions of the secreted IgG, and barcoded antigens were prepared and sequenced in the manner described in Example 3. The individual libraries were also mapped, quantified, normalized, and processed in the manner described in Example 4. Owing to the humanized

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nature of the V(D)J region of the VelocImmune® mice, the V(D)J library was mapped to the standard human germline IMGT reference. Following processing of the three single-cell libraries, the gene expression and V(D)J region sequencing data were used to subset PCs with a valid IgG constant region and detectable expression of IgG and CD79a but lacking the expression of Ms4a1 and CD19 typically found in non-PC B cells. In order to select antigen-specific, IgG-expressing PCs, the antigen specificity score was calculated for the target immunogen against a control antigen (hIL-6R α) as described in Example 4. Results are shown in Figure 15, UMAP of the bone marrow in Figures 15A and 15B and the spleen in Figures 15C and 15D. The x axis in Figure 15B and Figure 15D represents the centered log-ratio of the immunogen antigen signal and the y-axis the centered log-ratio of the hIL-6R α antigen signal. Figure 15D only shows the plasma cells as determined by expression of CD79A, but lack of CD19 and MS4A1. Cells are colored by the plasma cell isotype as determined from the VDJ library sequencing.

15 Example 12: IgG and Ag+ ELISAs

To test antigen-specificity and IgG expression of BCR sequences from the IgG secretion trap, hIL-4R α antigen or anti-human IgG (Jackson Labs) was plated at 1 μ g/mL or 2 μ g/mL, respectively, overnight at +4°C. The next day, plates were washed three times in PBS supplemented with 0.05% Tween20 (PBS-T) and blocked with PBS with 0.5% BSA for 1 hour at room temperature. Plates were subsequently washed three times with PBS-T, and diluted supernatants from Expi293F cells transfected with individual BCRs from the BMPC, PB/PC-like, or Spleen B-cell fractions were plated for 1 hour at room temperature. Plates were then washed three times with PBS-T and incubated with an anti-human IgG-HRP antibody (Jackson Labs) at 1:10,000 for 1 hour at room temperature. Plates were washed three times in PBS-T, developed using TMB substrate, stopped using 1N sulfuric acid, and read at 450nm.

Example 13: Identification and Isolation of Antigen-Specific IgG Antibody Secreting Cells from Immunized Mice

As described in Figure 1, an α lgk antibody was conjugated to streptavidin to capture secreted antibodies using kappa light chain. The StAv- α lgk capture complex was then used on NHS-biotin coated ASCs from VelocImmune® mice immunized with human IL-4R α (hIL-4R α).

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Detection of Ag⁺/IgG⁺ secreting plasma cells was achieved by staining cells with anti-mouse IgG and barcoded hIL-4Rα antigen. Barcoded hIL-6Rα was used as a negative control (Figure 5A).

As shown in Figures 5 and 17, to detect Ag⁺/IgG⁺ ASCs, mice were immunized with hIL-4Rα monomeric protein via footpad (Figure 5A and Figure 17A). Single cell suspensions of BM
5 cells were generated, and B lineage cells were enriched by negative selection (Figure 5A). Cells were coated with NHS-biotin and secreted IgG captured with StAv-algk as described herein (Figure 1). Following assembly of the biotin - StAv-algk complex on the surface, enriched B cells/ASCs were stained with an antibody mixture containing PC-specific markers, αIgG for detection of secreted IgG, barcoded hIL-4Rα for detection of Ag⁺ cells and barcoded hIL-6Rα as
10 a negative control. Spleen and draining lymph nodes (dLNs) from the same mice were pooled and stained using a similar antibody cocktail for comparison of VH:VL sequences from Ag⁺ B cells and plasmablasts (PBs) to BMPCs (Figures 17B and 17C). Ag⁺/IgG⁺ PCs from the BM were sorted using staining controls along with B cells and ASCs expressing surface BCR (PBs/PCs) from spleen/dLN (Figure 5B and Figures 17B, 17C, and 17D).

15 scRNA-seq was performed on sorted BMPCs, splenic/dLN B cells, and PBs/PCs to determine transcriptional differences, identify VH:VL repertoire changes and quantify antigen specificity at a single cell level. From barcoded antigen counts of hIL-4Rα and a negative control antigen hIL-6Rα, Ag⁺/IgG⁺ cells were verified (Figure 5C). To prioritize specificity and minimize polyreactivity, an antigen specificity score (AgSS) was determined to select cells with strong
20 specificity for hIL-4Rα and minimal hIL-6Rα binding. Cells with high AgSS were prioritized for hIL-4Rα and had minimal barcoded UMI counts for the control antigen hIL-6Rα (Figure 5C). Combined transcriptional analysis and unbiased clustering indicated that plasma cells from either spleen or BM clustered together and expressed classical PC markers such as *Slamf7*, *Xbp1*, *Sdc1*, *Prdm1* and *Slc3a2* (Figure 5D, Figure 18A). Splenic B cells, however, clustered very
25 distinctly from plasma cells and expressed typical B cell markers such as *Ms4a1*, and *Cd79a* (Figure 18A). Additionally, Ag⁺ cells with high AgSS (>0.5) from both the BM and spleen generally clustered together (Figure 5D). In BM PCs especially, cells with high AgSS clustered together at the bottom of the UMAP (Figure 5D).

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Example 14: Affinity of Antigen-Specific IgG Plasma Cells Isolated from Bone Marrow and Spleen/dLN B Cells

scRNA-seq yielded three distinct cell populations: bone marrow plasma cells (BMPCs), spleen/dLN plasmablast/plasma cell-like (Sp/dLN PBs/PCs), and spleen/dLN B cells (Sp/dLN B cells). From each of these populations, 20 unique VH:VL sequences from cells with the highest hIL-4R α barcode signal were selected for cloning and expression analysis (Figures 18B and 18C). 20 heavy and light chain variable sequences were successfully cloned from BMPCs, 19 from Sp/dLN B cells and 19 from Sp/dLN PBs/PCs into vectors expressing human IgG4 and human Igk constants (see, Table 1).

Consistent IgG expression was observed across all three populations, but anti-hIL-4R α activity was observed in 80% (16/20) of BMPCs compared to 32% (6/19) and 53% (10/19) of Sp/dLN PBs/PCs and Sp/dLN B cells, respectively (Figure 5E; Table 1). When accounting for varying levels of IgG in the supernatants, BMPCs also had a higher median ratio of hIL-4R α :total IgG binding, an indication that these sequences will have higher affinity (Figure 5E). Biacore analysis of the supernatants and purified antibodies confirmed this, with antibodies from the BMPCs having the highest affinity antibodies as measured by negative log₁₀ of the binding K_ds (Figure 5F and Figure 18D).

Example 15: scRNA-seq of Captured IgG cells

Following validation of Ag⁺ cells, we investigated the unique BCR sequences obtained by scRNA-seq of the VDJ region to compare Ag⁺ BMPCs with B and PBs/PCs from the spleen/dLN. BCR sequences from all cells were mapped to specific V, D and J gene segments by IgBlast and the extent of somatic hypermutation was measured by percent similarity to germline V regions. Ag⁺ cells in all three compartments exhibited lower percent similarity to germline V regions and therefore more frequent mutations than non-Ag⁺ cells (Figure 6A). The BCR sequences of Ag⁺ cells were further investigated by identifying BCR clonotypes in cells which shared the same heavy and light chain V gene segment and had identical CDR3 amino acid sequences in both the heavy and light chains (Table 2). 10 unique CDR3 sequence pairs were clonally shared among all 3 compartments, while the majority of each BCR repertoire was specific to each compartment (Table 2).

In addition to BCR sequence analysis, the transcriptomes of Ag⁺ and non-Ag⁺ cells in each of the three compartments were also compared to identify gene signatures related to hIL-

4Rα specificity. In BMPCs, several gene expression programs differed based on hIL-4Rα specificity (Figures 6C and 6D). *Tmsbx4*, *Cd24a*, *Tmsb10* and *Fkbp11* all demonstrated significant upregulation in hIL-4Rα specific BMPCs. Non-Ag+ cells showed increases in *Ly6d*, *Prg2*, *Tmem176a* and *Tmem176b* (Figures 6C and 6D). Sub-clustering of BMPCs indicated *Cd24a*, *Ppib*, *Fkbp11* and *Ssr2* transcripts are all expressed highly toward the bottom of the UMAP projection (Figure 6E), while gene programs marked by *Tmem176a*, *Tmem176b*, *Ly6d* and *Cd74* expression cluster in the middle and top regions of the UMAP projection (Figure 6E). However, despite significant transcriptional differences, BCR sequences cloned from either of the transcriptionally distinct regions resulted in high affinity hIL-4Rα specific antibodies as measured by negative log10 of the binding Kds (Figure 6F).

Example 16: Expanding Trap-N-Seq to human bone marrow to characterize short versus long-lived vaccine-specific IgG plasma cells.

In this Example, TRAPnSeq was used to isolate vaccine-specific IgG+ plasma cells (PCs) from human bone marrow. After enrichment for B cells and antibody secreting cells (ABCs), cells were biotinylated with NHS-biotin, and an affinity matrix was assembled using streptavidin coupled to anti-human Igk (StAv-αlgk) to capture secreted IgG. Following assembly of biotin - StAv-αlgk complex on the surface, enriched B cells/ASCs were stained with an antibody mix containing PC-specific markers, αIgG for detection of secreted IgG, and fluorescent-tagged antigens (listed in the table below) for detection of Ag+ cells.

After gating on PCs as CD20-CD38++ (Figure 21A), the use of secretion trap to isolate vaccine-specific IgG+ PCs from human bone marrow was validated. The necessary components were demonstrated in Figure 21B for the detection of secreted IgG on the surface of PCs, and in Figure 21C for detection of antigen-specific IgG. Complete assembly of all components of the secretion trap allowed detection of secreted IgG antibodies (Figure 21D, left), and detection of those that were vaccine-specific (Figure 21D, right).

Antigen List

		Supplier	Cat	Lot
1	Native VZV	Creative Diagnostics	DAG219	VZ030066A1
2	H1N1 ANTIGEN (A/New Caledonia/20/99)	Creative Diagnostics	DAG3690	C1035

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3	NATIVE IBV (B/Jiangsu/10/03)	Creative Diagnostics	DAGC081	V2971
4	MUV GRADE 2	Creative Diagnostics	DAG- H10369	MU060020A 3
5	MEASLES GRADE 2	Creative Diagnostics	DAG- H10368	ME040037A4
6	RUBELLA VIRUS STRAIN HPV-77	Creative Diagnostics	DAGA-545	C1664
7	Diphtheria Toxin	Abcam	ab188505	GR3400744- 7
8	Tetanus Toxoid	EMD Millipore	582231- 25ug	3845630

Example 17: Secretion Trap In Combination with Antigen Chase

The workflow of a secretion trap combined with antigen chase is illustrated in Figure 22.

5 Biotinylated human IL-4 Receptor α (hIL-4R α) ectodomain was conjugated to PE-labeled streptavidin ("SA-PE") tetramer overnight at 4°C. Separately, a trap antibody (mouse anti-human IgG (BD cat# 555784, Clone# G18-145)) was conjugated to streptavidin overnight using a commercially available kit (abcam cat# ab102921).

10 A25 cells were suspended in NHS-biotin (Sigma cat# 203112) at final concentration of 0.5mg/mL in DPBS/FBS for 15min at 37°C. A25 cells were derived from the commercially available A20 line by knocking out the endogenous surface BCR. Thus, A25 cells are mouse B cells without surface or secreted BCR.

After incubation, the cells were washed 3 times in DPBS/FBS, with transfer to a new conical tube after each wash to remove residual biotin.

15 Biotinylated cells were incubated with or without streptavidin conjugated trap antibody (or "SA-anti-huIgG"), performed at 1:100 dilution) at 10 million cells/mL in DPBS/FBS for about 15 minutes at 4°C. The 1:100 cell versus trap antibody ratio ensured that the trap reagent was bound to all biotinylated cells prior to spiking in the antibodies in the next step. After incubation, the cells were washed twice in DPBS/FBS and then resuspended in DPBS/FBS.

20 The following four antibodies differing in affinity were used. Each individual antibody was spiked into a cell suspension at about 1mg antibody per 2million cells. After incubation, the cells were washed twice in DPBS/FBS.

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	KD	t1/2 pH7.4
ab_1	1.68E-09	>115.5
ab_2	3.26E-09	21.7
ab_3	1.42E-08	10.6
ab_4	neg control	

Next, the cells were incubated with 5nM hIL-4R α labeled with AlexaFluor 647 (“AF647”) for 30min at room temperature (RT) in DPBS/FBS. After incubation, the cells were washed twice in DPBS/FBS.

5 Afterwards, the cells were incubated with pre-conjugated hIL-4R α biotin-StAv-PE twice for 45min (or 2x45min) at RT in DPBS/FBS. The concentration for antigen hIL-4R α was 20 nM. After incubation, the cells were washed twice in DPBS/FBS, and analyzed by flow cytometry. As shown in Figure 23C, the four antibodies show clear separation by affinity.

As a control, after the chase antigen incubations (hIL-4R α -biotin-StAv-PE), cells were
10 washed twice in DPBS/FBS and then stained with an anti-hulgK-PE-Cy7 antibody to detect antibody on the surface of the cells. Ab 4/neg control did not stain for antigen, but it was positive for IgK, indicating that the antibody was still captured on the surface of the cells.

Table 1: Antigen-specific IgG plasma cell sequences isolated from the bone marrow plasma cells and splenic plasma and B cells

Line #	Cell Barcode	Heavy Sequence
1	SEQ ID NO: 1	SEQ ID NO: 2
2	SEQ ID NO: 3	SEQ ID NO: 4
3	SEQ ID NO: 5	SEQ ID NO: 6
4	SEQ ID NO: 7	SEQ ID NO: 8
5	SEQ ID NO: 9	SEQ ID NO: 10
6	SEQ ID NO: 11	SEQ ID NO: 12
7	SEQ ID NO: 13	SEQ ID NO: 14
8	SEQ ID NO: 15	SEQ ID NO: 16
9	SEQ ID NO: 17	SEQ ID NO: 18
10	SEQ ID NO: 19	SEQ ID NO: 20

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11	SEQ ID NO: 21	SEQ ID NO: 22
12	SEQ ID NO: 23	SEQ ID NO: 24
13	SEQ ID NO: 25	SEQ ID NO: 26
14	SEQ ID NO: 27	SEQ ID NO: 28
15	SEQ ID NO: 29	SEQ ID NO: 30
16	SEQ ID NO: 31	SEQ ID NO: 32
17	SEQ ID NO: 33	SEQ ID NO: 34
18	SEQ ID NO: 35	SEQ ID NO: 36
19	SEQ ID NO: 37	SEQ ID NO: 38
20	SEQ ID NO: 39	SEQ ID NO: 40
21	SEQ ID NO: 41	SEQ ID NO: 42
22	SEQ ID NO: 43	SEQ ID NO: 44
23	SEQ ID NO: 45	SEQ ID NO: 46
24	SEQ ID NO: 47	SEQ ID NO: 48
25	SEQ ID NO: 49	SEQ ID NO: 50
26	SEQ ID NO: 51	SEQ ID NO: 52
27	SEQ ID NO: 53	SEQ ID NO: 54
28	SEQ ID NO: 55	SEQ ID NO: 56
29	SEQ ID NO: 57	SEQ ID NO: 58
30	SEQ ID NO: 59	SEQ ID NO: 60
31	SEQ ID NO: 61	SEQ ID NO: 62
32	SEQ ID NO: 63	SEQ ID NO: 64
33	SEQ ID NO: 65	SEQ ID NO: 66
34	SEQ ID NO: 67	SEQ ID NO: 68
35	SEQ ID NO: 69	SEQ ID NO: 70
36	SEQ ID NO: 71	SEQ ID NO: 72
37	SEQ ID NO: 73	SEQ ID NO: 74
38	SEQ ID NO: 75	SEQ ID NO: 76
39	SEQ ID NO: 77	SEQ ID NO: 78
40	SEQ ID NO: 79	SEQ ID NO: 80
41	SEQ ID NO: 81	SEQ ID NO: 82

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42	SEQ ID NO: 83	SEQ ID NO: 84
43	SEQ ID NO: 85	SEQ ID NO: 86
44	SEQ ID NO: 87	SEQ ID NO: 88
45	SEQ ID NO: 89	SEQ ID NO: 90
46	SEQ ID NO: 91	SEQ ID NO: 92
47	SEQ ID NO: 93	SEQ ID NO: 94
48	SEQ ID NO: 95	SEQ ID NO: 96
49	SEQ ID NO: 97	SEQ ID NO: 98
50	SEQ ID NO: 99	SEQ ID NO: 100
51	SEQ ID NO: 101	SEQ ID NO: 102
52	SEQ ID NO: 103	SEQ ID NO: 104
53	SEQ ID NO: 105	SEQ ID NO: 106
54	SEQ ID NO: 107	SEQ ID NO: 108

Table 1 (cont.)

Line #	Light Sequence	v_identity	IR_VJ_1_ locus	IR_VDJ_1_ locus
1	SEQ ID NO: 109		IGK	IGH
2	SEQ ID NO: 110	0.95973	IGK	IGH
3	SEQ ID NO: 111	0.97987	IGK	IGH
4	SEQ ID NO: 112	0.98658	IGK	IGH
5	SEQ ID NO: 113	0.9726	IGK	IGH
6	SEQ ID NO: 114	0.95302	IGK	IGH
7	SEQ ID NO: 115	0.9697	IGK	IGH
8	SEQ ID NO: 116	0.95638	IGK	IGH
9	SEQ ID NO: 117	0.99317	IGK	IGH
10	SEQ ID NO: 118	0.95302	IGK	IGH
11	SEQ ID NO: 119	0.98653	IGK	IGH
12	SEQ ID NO: 120	0.98649	IGK	IGH
13	SEQ ID NO: 121	0.95946	IGK	IGH
14	SEQ ID NO: 122	0.97987	IGK	IGH

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15	SEQ ID NO: 123	0.95623	IGK	IGH
16	SEQ ID NO: 124	0.97651	IGK	IGH
17	SEQ ID NO: 125	0.98311	IGK	IGH
18	SEQ ID NO: 126	0.94898	IGK	IGH
19	SEQ ID NO: 127	0.97306	IGK	IGH
20	SEQ ID NO: 128	0.97973	IGK	IGH
21	SEQ ID NO: 129	0.98986	IGK	IGH
22	SEQ ID NO: 130	0.9698	IGK	IGH
23	SEQ ID NO: 131	0.97635	IGK	IGH
24	SEQ ID NO: 132	0.94631	IGK	IGH
25	SEQ ID NO: 133	0.93919	IGK	IGH
26	SEQ ID NO: 134	0.98355	IGK	IGH
27	SEQ ID NO: 135	0.98316	IGK	IGH
28	SEQ ID NO: 136	0.99324	IGK	IGH
29	SEQ ID NO: 137	0.99658	IGK	IGH
30	SEQ ID NO: 138	0.95638	IGK	IGH
31	SEQ ID NO: 139	0.99327	IGK	IGH
32	SEQ ID NO: 140	0.97651	IGK	IGH
33	SEQ ID NO: 141	0.96949	IGK	IGH
34	SEQ ID NO: 142	0.93581	IGK	IGH
35	SEQ ID NO: 143	1	IGK	IGH
36	SEQ ID NO: 144	0.95973	IGK	IGH
37	SEQ ID NO: 145	0.96207	IGK	IGH
38	SEQ ID NO: 146	0.94595	IGK	IGH
39	SEQ ID NO: 147	0.95973	IGK	IGH
40	SEQ ID NO: 148	0.97611	IGK	IGH
41	SEQ ID NO: 149	0.96309	IGK	IGH
42	SEQ ID NO: 150	0.95946	IGK	IGH
43	SEQ ID NO: 151	0.95302	IGK	IGH
44	SEQ ID NO: 152	0.97987	IGK	IGH
45	SEQ ID NO: 153	0.9798	IGK	IGH

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46	SEQ ID NO: 154	0.94595	IGK	IGH
47	SEQ ID NO: 155	0.93266	IGK	IGH
48	SEQ ID NO: 156	0.97651	IGK	IGH
49	SEQ ID NO: 157	0.92568	IGK	IGH
50	SEQ ID NO: 158	0.96959	IGK	IGH
51	SEQ ID NO: 159	0.98635	IGK	IGH
52	SEQ ID NO: 160	0.95638	IGK	IGH
53	SEQ ID NO: 161	0.9698	IGK	IGH
54	SEQ ID NO: 162	0.98635	IGK	IGH

Table 1 (cont.)

Line #	IR_VJ_1_cdr3	IR_VDJ_1_cdr3	IR_VJ_1_cdr3_nt
1	SEQ ID NO: 163	SEQ ID NO: 164	SEQ ID NO: 165
2	SEQ ID NO: 166	SEQ ID NO: 167	SEQ ID NO: 168
3	SEQ ID NO: 169	SEQ ID NO: 170	SEQ ID NO: 171
4	SEQ ID NO: 172	SEQ ID NO: 173	SEQ ID NO: 174
5	SEQ ID NO: 175	SEQ ID NO: 176	SEQ ID NO: 177
6	SEQ ID NO: 178	SEQ ID NO: 179	SEQ ID NO: 180
7	SEQ ID NO: 181	SEQ ID NO: 182	SEQ ID NO: 183
8	SEQ ID NO: 184	SEQ ID NO: 185	SEQ ID NO: 186
9	SEQ ID NO: 187	SEQ ID NO: 188	SEQ ID NO: 189
10	SEQ ID NO: 190	SEQ ID NO: 191	SEQ ID NO: 192
11	SEQ ID NO: 193	SEQ ID NO: 194	SEQ ID NO: 195
12	SEQ ID NO: 196	SEQ ID NO: 197	SEQ ID NO: 198
13	SEQ ID NO: 199	SEQ ID NO: 200	SEQ ID NO: 201
14	SEQ ID NO: 202	SEQ ID NO: 203	SEQ ID NO: 204
15	SEQ ID NO: 205	SEQ ID NO: 206	SEQ ID NO: 207
16	SEQ ID NO: 208	SEQ ID NO: 209	SEQ ID NO: 210
17	SEQ ID NO: 211	SEQ ID NO: 212	SEQ ID NO: 213
18	SEQ ID NO: 214	SEQ ID NO: 215	SEQ ID NO: 216

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19	SEQ ID NO: 217	SEQ ID NO: 218	SEQ ID NO: 219
20	SEQ ID NO: 220	SEQ ID NO: 221	SEQ ID NO: 222
21	SEQ ID NO: 223	SEQ ID NO: 224	SEQ ID NO: 225
22	SEQ ID NO: 226	SEQ ID NO: 227	SEQ ID NO: 228
23	SEQ ID NO: 229	SEQ ID NO: 230	SEQ ID NO: 231
24	SEQ ID NO: 232	SEQ ID NO: 233	SEQ ID NO: 234
25	SEQ ID NO: 235	SEQ ID NO: 236	SEQ ID NO: 237
26	SEQ ID NO: 238	SEQ ID NO: 239	SEQ ID NO: 240
27	SEQ ID NO: 241	SEQ ID NO: 242	SEQ ID NO: 243
28	SEQ ID NO: 244	SEQ ID NO: 245	SEQ ID NO: 246
29	SEQ ID NO: 247	SEQ ID NO: 248	SEQ ID NO: 249
30	SEQ ID NO: 250	SEQ ID NO: 251	SEQ ID NO: 252
31	SEQ ID NO: 253	SEQ ID NO: 254	SEQ ID NO: 255
32	SEQ ID NO: 256	SEQ ID NO: 257	SEQ ID NO: 258
33	SEQ ID NO: 259	SEQ ID NO: 260	SEQ ID NO: 261
34	SEQ ID NO: 262	SEQ ID NO: 263	SEQ ID NO: 264
35	SEQ ID NO: 265	SEQ ID NO: 266	SEQ ID NO: 267
36	SEQ ID NO: 268	SEQ ID NO: 269	SEQ ID NO: 270
37	SEQ ID NO: 271	SEQ ID NO: 272	SEQ ID NO: 273
38	SEQ ID NO: 274	SEQ ID NO: 275	SEQ ID NO: 276
39	SEQ ID NO: 277	SEQ ID NO: 278	SEQ ID NO: 279
40	SEQ ID NO: 280	SEQ ID NO: 281	SEQ ID NO: 282
41	SEQ ID NO: 283	SEQ ID NO: 284	SEQ ID NO: 285
42	SEQ ID NO: 286	SEQ ID NO: 287	SEQ ID NO: 288
43	SEQ ID NO: 289	SEQ ID NO: 290	SEQ ID NO: 291
44	SEQ ID NO: 292	SEQ ID NO: 293	SEQ ID NO: 294
45	SEQ ID NO: 295	SEQ ID NO: 296	SEQ ID NO: 297
46	SEQ ID NO: 298	SEQ ID NO: 299	SEQ ID NO: 300
47	SEQ ID NO: 301	SEQ ID NO: 302	SEQ ID NO: 303
48	SEQ ID NO: 304	SEQ ID NO: 305	SEQ ID NO: 306
49	SEQ ID NO: 307	SEQ ID NO: 308	SEQ ID NO: 309

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50	SEQ ID NO: 310	SEQ ID NO: 311	SEQ ID NO: 312
51	SEQ ID NO: 313	SEQ ID NO: 314	SEQ ID NO: 315
52	SEQ ID NO: 316	SEQ ID NO: 317	SEQ ID NO: 318
53	SEQ ID NO: 319	SEQ ID NO: 320	SEQ ID NO: 321
54	SEQ ID NO: 322	SEQ ID NO: 323	SEQ ID NO: 324

Table 1 (cont.)

Line #	IR_VDJ_1_cdr3_nt	IR_VJ_1_v_gene
1	SEQ ID NO: 325	IGKV1-12*01
2	SEQ ID NO: 326	IGKV1-17*01
3	SEQ ID NO: 327	IGKV1-5*03
4	SEQ ID NO: 328	IGKV1-5*03
5	SEQ ID NO: 329	IGKV2-28*01,IGKV2D-28*01
6	SEQ ID NO: 330	IGKV1-17*01
7	SEQ ID NO: 331	IGKV1-27*01
8	SEQ ID NO: 332	IGKV1-17*01
9	SEQ ID NO: 333	IGKV2-28*01,IGKV2D-28*01
10	SEQ ID NO: 334	IGKV1-17*01
11	SEQ ID NO: 335	IGKV1-27*01
12	SEQ ID NO: 336	IGKV1-27*01
13	SEQ ID NO: 337	IGKV1-27*01
14	SEQ ID NO: 338	IGKV1-17*01
15	SEQ ID NO: 339	IGKV1-27*01
16	SEQ ID NO: 340	IGKV1-27*01
17	SEQ ID NO: 341	IGKV1-33*01,IGKV1D-33*01
18	SEQ ID NO: 342	IGKV3-20*01
19	SEQ ID NO: 343	IGKV1-5*03
20	SEQ ID NO: 344	IGKV1-39*01,IGKV1D-39*01
21	SEQ ID NO: 345	IGKV1-27*01
22	SEQ ID NO: 346	IGKV3-20*01

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23	SEQ ID NO: 347	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*02
24	SEQ ID NO: 348	IGKV1-39*01,IGKV1D-39*01
25	SEQ ID NO: 349	IGKV1-27*01
26	SEQ ID NO: 350	IGKV3-15*01
27	SEQ ID NO: 351	IGKV1-17*01
28	SEQ ID NO: 352	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*02
29	SEQ ID NO: 353	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*02
30	SEQ ID NO: 354	IGKV1-39*01,IGKV1D-39*01
31	SEQ ID NO: 355	IGKV1-5*03
32	SEQ ID NO: 356	IGKV1-17*01
33	SEQ ID NO: 357	IGKV3-15*01
34	SEQ ID NO: 358	IGKV1-27*01
35	SEQ ID NO: 359	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*02
36	SEQ ID NO: 360	IGKV1-6*01
37	SEQ ID NO: 361	IGKV1-9*01
38	SEQ ID NO: 362	IGKV1-6*01,IGKV1-6*02
39	SEQ ID NO: 363	IGKV3-20*01
40	SEQ ID NO: 364	IGKV1-39*01,IGKV1D-39*01
41	SEQ ID NO: 365	IGKV1-17*01
42	SEQ ID NO: 366	IGKV2-24*01
43	SEQ ID NO: 367	IGKV1-17*01
44	SEQ ID NO: 368	IGKV1-17*01
45	SEQ ID NO: 369	IGKV3-20*01
46	SEQ ID NO: 370	IGKV1-27*01
47	SEQ ID NO: 371	IGKV1-39*01,IGKV1D-39*01
48	SEQ ID NO: 372	IGKV1-17*01
49	SEQ ID NO: 373	IGKV1-39*01,IGKV1D-39*01
50	SEQ ID NO: 374	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*02
51	SEQ ID NO: 375	IGKV3-20*01
52	SEQ ID NO: 376	IGKV1-39*01,IGKV1D-39*01
53	SEQ ID NO: 377	IGKV1-17*01

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54	SEQ ID NO: 378	IGKV1-17*01
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Table 1 (cont.)

Line #	IR_VDJ_1_v_gene	IR_VDJ_1_d_gene
1	IGHV3-21*01	IGHD6-6*01
2	IGHV3-9*01	IGHD6-13*01,IGHD6-25*01,IGHD6-6*01
3	IGHV3-9*01	IGHD6-13*01
4	IGHV3-9*01	IGHD6-13*01
5	IGHV1-2*02	IGHD1-14*01,IGHD6-25*01
6	IGHV3-9*01	IGHD1-26*01
7	IGHV3-9*01	IGHD6-19*01
8	IGHV3-9*01	IGHD6-13*01
9	IGHV1-8*01	IGHD1-1*01,IGHD4-11*01,IGHD4-4*01
10	IGHV3-9*01	IGHD3-16*02
11	IGHV3-9*01	IGHD6-19*01
12	IGHV3-30*18,IGHV3-30-5*01	IGHD7-27*01
13	IGHV3-20*01	IGHD6-13*01
14	IGHV3-9*01	IGHD1-26*01
15	IGHV3-9*01	IGHD6-19*01
16	IGHV3-9*01	IGHD6-13*01
17	IGHV3-48*03	IGHD3-10*01
18	IGHV5-51*01	
19	IGHV3-9*01	IGHD6-19*01
20	IGHV3-21*01	IGHD6-6*01
21	IGHV3-20*01	IGHD6-13*01
22	IGHV3-9*01	IGHD1-26*01
23	IGHV3-23*01,IGHV3-23D*01	IGHD6-6*01
24	IGHV3-9*01	IGHD1-26*01
25	IGHV3-20*04	IGHD6-13*01,IGHD6-19*01
26	IGHV6-1*01	IGHD1-7*01

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27	IGHV3-9*01	IGHD6-13*01
28	IGHV3-23*01,IGHV3-23D*01	IGHD3-10*01,IGHD3-10*02
29	IGHV3-23*01,IGHV3-23D*01	IGHD1-7*01
30	IGHV3-9*01	IGHD3-16*01,IGHD3-16*02
31	IGHV3-9*01	IGHD6-19*01
32	IGHV3-9*01	IGHD1-26*01
33	IGHV3-48*03	IGHD1-7*01
34	IGHV3-9*01	IGHD6-13*01
35	IGHV3-21*01	IGHD6-13*01
36	IGHV3-9*01	IGHD6-19*01
37	IGHV3-33*01,IGHV3-33*06	IGHD1-7*01
38	IGHV3-21*06	IGHD4-17*01
39	IGHV3-9*01	IGHD1-26*01
40	IGHV4-59*08	IGHD3-16*01
41	IGHV3-9*01	IGHD2-15*01,IGHD6-13*01,IGHD6-19*01
42	IGHV3-23*01,IGHV3-23D*01	IGHD1-26*01
43	IGHV3-9*01	IGHD2-15*01,IGHD6-13*01,IGHD6-19*01
44	IGHV3-9*01	IGHD1-26*01
45	IGHV3-9*01	IGHD1-26*01
46	IGHV3-9*01	IGHD6-13*01
47	IGHV3-9*01	IGHD1-26*01
48	IGHV3-9*01	IGHD6-13*01
49	IGHV3-23*01,IGHV3-23D*01	IGHD1-26*01
50	IGHV3-23*01,IGHV3-23D*01	
51	IGHV3-64*07	IGHD6-13*01
52	IGHV3-9*01	IGHD1-26*01
53	IGHV3-9*01	IGHD6-13*01
54	IGHV1-18*01	IGHD1-7*01

Table 1 (cont.)

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Line #	IR_VJ_1_j_gene	IR_VDJ_1_j_gene	IR_VJ_1_c_gene	IR_VDJ_1_c_gene
1	IGKJ4*01	IGHJ3*01,IGHJ3*02	IGKC	IGHG1
2	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
3	IGKJ1*01	IGHJ2*01	IGKC	IGHG2C
4	IGKJ1*01	IGHJ2*01	IGKC	IGHG2C
5	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
6	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
7	IGKJ1*01	IGHJ4*02	IGKC	IGHG1
8	IGKJ3*01	IGHJ5*02	IGKC	IGHG1
9	IGKJ3*01	IGHJ2*01	IGKC	IGHG1
10	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
11	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
12	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
13	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
14	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
15	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
16	IGKJ1*01	IGHJ2*01	IGKC	IGHG1
17	IGKJ3*01	IGHJ3*02	IGKC	IGHG2C
18	IGKJ5*01	IGHJ5*02	IGKC	IGHG2C
19	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
20	IGKJ5*01	IGHJ3*02	IGKC	IGHG2C
21	IGKJ1*01	IGHJ1*01	IGKC	IGHG2C
22	IGKJ2*01	IGHJ4*02	IGKC	IGHG2C
23	IGKJ1*01	IGHJ3*02	IGKC	IGHG2C
24	IGKJ3*01	IGHJ4*02	IGKC	IGHG2C
25	IGKJ1*01	IGHJ4*02	IGKC	IGHG1
26	IGKJ2*01	IGHJ4*02	IGKC	IGHG2C
27	IGKJ5*01	IGHJ3*01	IGKC	IGHG2C
28	IGKJ3*01	IGHJ4*02	IGKC	IGHG2C

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29	IGKJ4*01	IGHJ4*02,IGHJ5*02	IGKC	IGHG1
30	IGKJ3*01	IGHJ6*02	IGKC	IGHG2C
31	IGKJ1*01	IGHJ4*02	IGKC	IGHG1
32	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
33	IGKJ3*01	IGHJ3*02	IGKC	IGHG1
34	IGKJ1*01	IGHJ2*01	IGKC	IGHG2C
35	IGKJ5*01	IGHJ4*02	IGKC	IGHG2C
36	IGKJ1*01	IGHJ5*02	IGKC	IGHG1
37	IGKJ2*01	IGHJ4*02	IGKC	IGHG2C
38	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C
39	IGKJ2*01	IGHJ4*02	IGKC	IGHG2C
40	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
41	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
42	IGKJ4*01	IGHJ4*02	IGKC	IGHG2C
43	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
44	IGKJ4*01	IGHJ6*02	IGKC	IGHG2C
45	IGKJ2*01	IGHJ4*02	IGKC	IGHG2C
46	IGKJ1*01	IGHJ2*01	IGKC	IGHG2C
47	IGKJ3*01	IGHJ6*02	IGKC	IGHG2C
48	IGKJ2*01	IGHJ6*02	IGKC	IGHG2C
49	IGKJ5*01	IGHJ4*02	IGKC	IGHG2C
50	IGKJ3*01	IGHJ3*02	IGKC	IGHG2C
51	IGKJ2*01	IGHJ6*02	IGKC	IGHG2C
52	IGKJ3*01	IGHJ6*02	IGKC	IGHG2C
53	IGKJ2*01	IGHJ6*02	IGKC	IGHG2C
54	IGKJ1*01	IGHJ4*02	IGKC	IGHG2C

Table 1 (cont.)

Line #	REGN560.L15_ab_raw	REGN78.L15_ab_raw	Tissue	TissueCT
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1	11214	271	BM	BM_Plasma_cell
2	6538	26	BM	BM_Plasma_cell
3	6317	236	BM	BM_Plasma_cell
4	5046	95	BM	BM_Plasma_cell
5	4411	214	BM	BM_Plasma_cell
6	4144	18	BM	BM_Plasma_cell
7	4142	224	BM	BM_Plasma_cell
8	3780	524	BM	BM_Plasma_cell
9	3568	890	BM	BM_Plasma_cell
10	3476	16	BM	BM_Plasma_cell
11	3212	115	BM	BM_Plasma_cell
12	3180	348	BM	BM_Plasma_cell
13	2931	33	BM	BM_Plasma_cell
14	2828	50	BM	BM_Plasma_cell
15	2585	158	BM	BM_Plasma_cell
16	2366	69	BM	BM_Plasma_cell
17	2299	881	BM	BM_Plasma_cell
18	2174	55	BM	BM_Plasma_cell
19	2063	50	BM	BM_Plasma_cell
20	2058	681	BM	BM_Plasma_cell
21	17383	236	SP	SP_B_cell
22	13995	5	SP	SP_B_cell
23	10971	152	SP	SP_B_cell
24	10811	11	SP	SP_B_cell
25	10605	16	SP	SP_B_cell
26	10355	167	SP	SP_B_cell
27	9506	149	SP	SP_B_cell
28	9446	129	SP	SP_B_cell
29	9188	93	SP	SP_B_cell
30	9182	179	SP	SP_B_cell
31	8515	57	SP	SP_B_cell

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32	7912	32	SP	SP_B_cell
33	7344	106	SP	SP_B_cell
34	6888	34	SP	SP_B_cell
35	6710	33	SP	SP_B_cell
36	6345	75	SP	SP_B_cell
37	6230	60	SP	SP_B_cell
38	6051	66	SP	SP_B_cell
39	3560	17	SP	SP_B_cell
40	5520	1	SP	SP_Plasma_cell
41	2457	227	SP	SP_Plasma_cell
42	2084	5	SP	SP_Plasma_cell
43	1980	2	SP	SP_Plasma_cell
44	1845	26	SP	SP_Plasma_cell
45	1775	25	SP	SP_Plasma_cell
46	1525	2	SP	SP_Plasma_cell
47	1522	6	SP	SP_Plasma_cell
48	1239	17	SP	SP_Plasma_cell
49	1199	2	SP	SP_Plasma_cell
50	924	0	SP	SP_Plasma_cell
51	897	3	SP	SP_Plasma_cell
52	819	19	SP	SP_Plasma_cell
53	818	5	SP	SP_Plasma_cell
54	815	26	SP	SP_Plasma_cell

Table 1 (cont.)

Line #	Kd	negLog10Kd
1	4.16E-07	6.380906669
2	1.61E-07	6.793174124
3	5.37E-07	6.270025714
4	1.58E-07	6.801342913

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5	2.38E-08	7.623423043
6	1.36E-07	6.866461092
7	1.55E-08	7.809668302
8	0	NA
9	0	NA
10	0	NA
11	2.75E-08	7.560667306
12	3.45E-08	7.462180905
13	5.82E-08	7.235077015
14	0	NA
15	1.99E-08	7.701146924
16	8.04E-09	8.094743951
17	0	NA
18	4.14E-08	7.382999659
19	1.21E-08	7.91721463
20	0	NA
21	1.77E-07	6.752026734
22	0	NA
23	0	NA
24	2.07E-07	6.684029655
25	9.76E-08	7.010550182
26	1.12E-07	6.950781977
27	0	NA
28	1.46E-07	6.835647144
29	5.48E-08	7.261219442
30	0	NA
31	1.33E-07	6.876148359
32	5.81E-08	7.235823868
33	0	NA
34	3.42E-08	7.465973894
35	2.93E-08	7.53313238

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36	0	NA
37	1.97E-07	6.705533774
38	0	NA
39	0	NA
40	1.18E-07	6.928117993
41	1.50E-07	6.823908741
42	1.27E-07	6.896196279
43	0	NA
44	0	NA
45	0	NA
46	4.92E-08	7.308034897
47	0	NA
48	0	NA
49	1.32E-07	6.879426069
50	0	NA
51	0	NA
52	0	NA
53	2.47E-07	6.607303047
54	1.35E-08	7.869666232

Table 2: hLL-Ra IgG-specific BCR sequences shared among bone marrow plasma cells, splenic B and Plasma cells

Line #	Cell_Barcode	heavy_sequence
1	Clonotype #1 (n=10 cells)	
2	SEQ ID NO: 379	SEQ ID NO: 380
3	SEQ ID NO: 381	SEQ ID NO: 382
4	SEQ ID NO: 383	SEQ ID NO: 384
5	SEQ ID NO: 385	SEQ ID NO: 386
6	SEQ ID NO: 387	SEQ ID NO: 388
7	SEQ ID NO: 389	SEQ ID NO: 390

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8	SEQ ID NO: 391	SEQ ID NO: 392
9	SEQ ID NO: 393	SEQ ID NO: 394
10	SEQ ID NO: 395	SEQ ID NO: 396
11	SEQ ID NO: 397	SEQ ID NO: 398
12	Clonotype #2 (n=9 cells)	
13	SEQ ID NO: 399	SEQ ID NO: 400
14	SEQ ID NO: 401	SEQ ID NO: 402
15	SEQ ID NO: 403	SEQ ID NO: 404
16	SEQ ID NO: 405	SEQ ID NO: 406
17	SEQ ID NO: 407	SEQ ID NO: 408
18	SEQ ID NO: 409	SEQ ID NO: 410
19	SEQ ID NO: 411	SEQ ID NO: 412
20	SEQ ID NO: 413	SEQ ID NO: 414
21	SEQ ID NO: 415	SEQ ID NO: 416
22	Clonotype #3 (n=9 cells)	
23	SEQ ID NO: 417	SEQ ID NO: 418
24	SEQ ID NO: 419	SEQ ID NO: 420
25	SEQ ID NO: 421	SEQ ID NO: 422
26	SEQ ID NO: 423	SEQ ID NO: 424
27	SEQ ID NO: 425	SEQ ID NO: 426
28	SEQ ID NO: 427	SEQ ID NO: 428
29	SEQ ID NO: 429	SEQ ID NO: 430
30	SEQ ID NO: 431	SEQ ID NO: 432
31	SEQ ID NO: 433	SEQ ID NO: 434
32	Clonotype #4 (n=9 cells)	
33	SEQ ID NO: 435	SEQ ID NO: 436
34	SEQ ID NO: 437	SEQ ID NO: 438
35	SEQ ID NO: 439	SEQ ID NO: 440
36	SEQ ID NO: 441	SEQ ID NO: 442
37	SEQ ID NO: 443	SEQ ID NO: 444
38	SEQ ID NO: 445	SEQ ID NO: 446

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39	SEQ ID NO: 447	SEQ ID NO: 448
40	SEQ ID NO: 449	SEQ ID NO: 450
41	SEQ ID NO: 451	SEQ ID NO: 452
42	Clonotype #5 (n=6 cells)	
43	SEQ ID NO: 453	SEQ ID NO: 454
44	SEQ ID NO: 455	SEQ ID NO: 456
45	SEQ ID NO: 457	SEQ ID NO: 458
46	SEQ ID NO: 459	SEQ ID NO: 460
47	SEQ ID NO: 461	SEQ ID NO: 462
48	SEQ ID NO: 463	SEQ ID NO: 464
49	Clonotype #6 (n=6 cells)	
50	SEQ ID NO: 465	SEQ ID NO: 466
51	SEQ ID NO: 467	SEQ ID NO: 468
52	SEQ ID NO: 469	SEQ ID NO: 470
53	SEQ ID NO: 471	SEQ ID NO: 472
54	SEQ ID NO: 473	SEQ ID NO: 474
55	SEQ ID NO: 475	SEQ ID NO: 476
56	Clonotype #7 (n=5 cells)	
57	SEQ ID NO: 477	SEQ ID NO: 478
58	SEQ ID NO: 479	SEQ ID NO: 480
59	SEQ ID NO: 481	SEQ ID NO: 482
60	SEQ ID NO: 483	SEQ ID NO: 484
61	SEQ ID NO: 485	SEQ ID NO: 486
62	Clonotype #8 (n=5 cells)	
63	SEQ ID NO: 487	SEQ ID NO: 488
64	SEQ ID NO: 489	SEQ ID NO: 490
65	SEQ ID NO: 491	SEQ ID NO: 492
66	SEQ ID NO: 493	SEQ ID NO: 494
67	SEQ ID NO: 495	SEQ ID NO: 496
68	Clonotype #9 (n=4 cells)	
69	SEQ ID NO: 497	SEQ ID NO: 498

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70	SEQ ID NO: 499	SEQ ID NO: 500
71	SEQ ID NO: 501	SEQ ID NO: 502
72	SEQ ID NO: 503	SEQ ID NO: 504
73	Clonotype #10 (n=3 cells)	
74	SEQ ID NO: 505	SEQ ID NO: 506
75	SEQ ID NO: 507	SEQ ID NO: 508
76	SEQ ID NO: 509	SEQ ID NO: 510

Table 2 (cont.)

Line #	light_sequence	v_identity
2	SEQ ID NO: 511	0.95066
3	SEQ ID NO: 512	0.9375
4	SEQ ID NO: 513	0.96711
5	SEQ ID NO: 514	0.96382
6	SEQ ID NO: 515	0.95066
7	SEQ ID NO: 516	0.95066
8	SEQ ID NO: 517	0.95066
9	SEQ ID NO: 518	0.95724
10	SEQ ID NO: 519	0.95395
11	SEQ ID NO: 520	0.95724
13	SEQ ID NO: 521	0.95987
14	EQ ID NO: 522	0.95987
15	SEQ ID NO: 523	0.96321
16	SEQ ID NO: 524	0.95318
17	SEQ ID NO: 525	0.95652
18	SEQ ID NO: 526	0.95652
19	SEQ ID NO: 527	0.95318
20	SEQ ID NO: 528	0.95652
21	SEQ ID NO: 529	0.95987
23	SEQ ID NO: 530	0.92491

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24	SEQ ID NO: 531	0.95222
25	SEQ ID NO: 532	0.94198
26	SEQ ID NO: 533	0.94198
27	SEQ ID NO: 534	0.92491
28	SEQ ID NO: 535	0.92833
29	SEQ ID NO: 536	0.94881
30	SEQ ID NO: 537	0.94198
31	SEQ ID NO: 538	0.94539
33	SEQ ID NO: 539	0.95563
34	SEQ ID NO: 540	0.95222
35	SEQ ID NO: 541	0.94539
36	SEQ ID NO: 542	0.95904
37	SEQ ID NO: 543	0.94198
38	SEQ ID NO: 544	0.95222
39	SEQ ID NO: 545	0.93515
40	SEQ ID NO: 546	0.94198
41	SEQ ID NO: 547	0.94539
43	SEQ ID NO: 548	0.91156
44	SEQ ID NO: 549	0.93532
45	SEQ ID NO: 550	0.92177
46	SEQ ID NO: 551	0.93197
47	SEQ ID NO: 552	0.93537
48	SEQ ID NO: 553	0.93197
50	SEQ ID NO: 554	0.95638
51	SEQ ID NO: 555	0.95302
52	SEQ ID NO: 556	0.96309
53	SEQ ID NO: 557	0.96309
54	SEQ ID NO: 558	0.94595
55	SEQ ID NO: 559	0.95302
57	SEQ ID NO: 560	0.92282
58	SEQ ID NO: 561	0.93289

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59	SEQ ID NO: 562	0.93289
60	SEQ ID NO: 563	0.93289
61	SEQ ID NO: 564	0.92953
63	SEQ ID NO: 565	0.9215
64	SEQ ID NO: 566	0.94539
65	SEQ ID NO: 567	0.92491
66	SEQ ID NO: 568	0.91126
67	SEQ ID NO: 569	0.91126
69	SEQ ID NO: 570	0.96246
70	SEQ ID NO: 571	0.96587
71	SEQ ID NO: 572	0.96587
72	SEQ ID NO: 573	0.96246
74	SEQ ID NO: 574	0.95066
75	SEQ ID NO: 575	0.96053
76	SEQ ID NO: 576	0.96053

Table 2 (cont.)

Line #	IR_VJ_1_I ocus	IR_VDJ_1_I ocus	IR_VJ_1_cdr3	IR_VDJ_1_cdr3
2	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
3	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
4	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
5	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
6	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
7	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
8	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
9	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
10	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
11	IGK	IGH	SEQ ID NO: 577	SEQ ID NO: 578
13	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
14	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579

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15	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
16	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
17	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
18	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
19	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
20	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
21	IGK	IGH	SEQ ID NO: 250	SEQ ID NO: 579
23	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
24	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
25	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
26	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
27	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
28	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
29	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
30	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
31	IGK	IGH	SEQ ID NO: 580	SEQ ID NO: 581
33	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
34	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
35	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
36	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
37	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
38	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
39	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
40	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
41	IGK	IGH	SEQ ID NO: 582	SEQ ID NO: 583
43	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585
44	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585
45	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585
46	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585
47	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585
48	IGK	IGH	SEQ ID NO: 584	SEQ ID NO: 585

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50	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
51	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
52	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
53	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
54	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
55	IGK	IGH	SEQ ID NO: 586	SEQ ID NO: 587
57	IGK	IGH	SEQ ID NO: 169	SEQ ID NO: 588
58	IGK	IGH	SEQ ID NO: 169	SEQ ID NO: 588
59	IGK	IGH	SEQ ID NO: 169	SEQ ID NO: 588
60	IGK	IGH	SEQ ID NO: 169	SEQ ID NO: 588
61	IGK	IGH	SEQ ID NO: 169	SEQ ID NO: 588
63	IGK	IGH	SEQ ID NO: 589	SEQ ID NO: 590
64	IGK	IGH	SEQ ID NO: 589	SEQ ID NO: 590
65	IGK	IGH	SEQ ID NO: 589	SEQ ID NO: 590
66	IGK	IGH	SEQ ID NO: 589	SEQ ID NO: 590
67	IGK	IGH	SEQ ID NO: 589	SEQ ID NO: 590
69	IGK	IGH	SEQ ID NO: 232	SEQ ID NO: 281
70	IGK	IGH	SEQ ID NO: 232	SEQ ID NO: 281
71	IGK	IGH	SEQ ID NO: 232	SEQ ID NO: 281
72	IGK	IGH	SEQ ID NO: 232	SEQ ID NO: 281
74	IGK	IGH	SEQ ID NO: 591	SEQ ID NO: 592
75	IGK	IGH	SEQ ID NO: 591	SEQ ID NO: 592
76	IGK	IGH	SEQ ID NO: 591	SEQ ID NO: 592

Table 2 (cont.)

Line #	IR_VJ_1_cdr3_nt	IR_VDJ_1_cdr3_nt	IR_VJ_1_v_gene	IR_VDJ_1_v_gene
2	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
3	SEQ ID NO: 601	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
4	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
5	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01

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6	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
7	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
8	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
9	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
10	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
11	SEQ ID NO: 593	SEQ ID NO: 594	IGKV3-15*01	IGHV6-1*01
13	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
14	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
15	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
16	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
17	SEQ ID NO: 597	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
18	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
19	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01
20	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-39*01

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21	SEQ ID NO: 595	SEQ ID NO: 596	IGKV1-39*01,IGKV1D-39*01	IGHV4-39*01
23	SEQ ID NO: 598	SEQ ID NO: 599	IGKV1-16*02	IGHV4-34*01
24	SEQ ID NO: 598	SEQ ID NO: 600	IGKV1-16*01	IGHV4-34*01
25	SEQ ID NO: 598	SEQ ID NO: 602	IGKV1-16*01	IGHV4-34*01
26	SEQ ID NO: 598	SEQ ID NO: 600	IGKV1-16*01	IGHV4-34*01
27	SEQ ID NO: 598	SEQ ID NO: 603	IGKV1-16*02	IGHV4-34*01
28	SEQ ID NO: 598	SEQ ID NO: 603	IGKV1-16*02	IGHV4-34*01
29	SEQ ID NO: 598	SEQ ID NO: 600	IGKV1-16*01	IGHV4-34*01
30	SEQ ID NO: 598	SEQ ID NO: 600	IGKV1-16*01	IGHV4-34*01
31	SEQ ID NO: 598	SEQ ID NO: 600	IGKV1-16*01	IGHV4-34*01
33	SEQ ID NO: 604	SEQ ID NO: 605	IGKV1-16*02	IGHV4-34*01
34	SEQ ID NO: 604	SEQ ID NO: 605	IGKV1-16*02	IGHV4-34*01
35	SEQ ID NO: 604	SEQ ID NO: 606	IGKV1-16*02	IGHV4-34*01
36	SEQ ID NO: 604	SEQ ID NO: 605	IGKV1-16*02	IGHV4-34*01
37	SEQ ID NO: 604	SEQ ID NO: 606	IGKV1-16*02	IGHV4-34*01
38	SEQ ID NO: 604	SEQ ID NO: 605	IGKV1-16*02	IGHV4-34*01
39	SEQ ID NO: 604	SEQ ID NO: 606	IGKV1-16*02	IGHV4-34*01
40	SEQ ID NO: 604	SEQ ID NO: 605	IGKV1-16*02	IGHV4-34*01
41	SEQ ID NO: 607	SEQ ID NO: 606	IGKV1-16*02	IGHV4-34*01
43	SEQ ID NO: 608	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23D*01
44	SEQ ID NO: 610	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23*04,IGHV3-23D*01
45	SEQ ID NO: 611	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23D*01

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46	SEQ ID NO: 611	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23D*01
47	SEQ ID NO: 611	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23D*01
48	SEQ ID NO: 611	SEQ ID NO: 609	IGKV1-17*01	IGHV3-23*01,IGHV3-23D*01
50	SEQ ID NO: 612	SEQ ID NO: 613	IGKV1-27*01	IGHV3-9*01
51	SEQ ID NO: 612	SEQ ID NO: 613	IGKV1-27*01	IGHV3-9*01
52	SEQ ID NO: 612	SEQ ID NO: 613	IGKV1-27*01	IGHV3-9*01
53	SEQ ID NO: 612	SEQ ID NO: 614	IGKV1-27*01	IGHV3-9*01
54	SEQ ID NO: 612	SEQ ID NO: 615	IGKV1-27*01	IGHV3-9*01
55	SEQ ID NO: 612	SEQ ID NO: 613	IGKV1-27*01	IGHV3-9*01
57	SEQ ID NO: 171	SEQ ID NO: 616	IGKV1-5*03	IGHV3-9*01
58	SEQ ID NO: 171	SEQ ID NO: 617	IGKV1-5*03	IGHV3-9*01
59	SEQ ID NO: 171	SEQ ID NO: 617	IGKV1-5*03	IGHV3-9*01
60	SEQ ID NO: 171	SEQ ID NO: 618	IGKV1-5*03	IGHV3-9*01
61	SEQ ID NO: 171	SEQ ID NO: 618	IGKV1-5*03	IGHV3-9*01
63	SEQ ID NO: 619	SEQ ID NO: 620	IGKV6-21*01,IGKV6D-21*01	IGHV3-21*02
64	SEQ ID NO: 619	SEQ ID NO: 620	IGKV6-21*01,IGKV6D-21*01	IGHV3-21*02
65	SEQ ID NO: 619	SEQ ID NO: 620	IGKV6-21*01,IGKV6D-21*01	IGHV3-21*02

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66	SEQ ID NO: 619	SEQ ID NO: 620	IGKV6- 21*01,IGKV6D- 21*01	IGHV3-21*02
67	SEQ ID NO: 619	SEQ ID NO: 620	IGKV6- 21*01,IGKV6D- 21*01	IGHV3-21*02
69	SEQ ID NO: 234	SEQ ID NO: 621	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-59*08
70	SEQ ID NO: 234	SEQ ID NO: 621	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-59*08
71	SEQ ID NO: 622	SEQ ID NO: 621	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-59*08
72	SEQ ID NO: 623	SEQ ID NO: 621	IGKV1- 39*01,IGKV1D- 39*01	IGHV4-59*08
74	SEQ ID NO: 624	SEQ ID NO: 625	IGKV1- 12*01,IGKV1- 12*02,IGKV1D- 12*02	IGHV6-1*01
75	SEQ ID NO: 624	SEQ ID NO: 626	IGKV1- 12*01,IGKV1- 12*02,IGKV1D- 12*02	IGHV6-1*01
76	SEQ ID NO: 624	SEQ ID NO: 626	IGKV1- 12*01,IGKV1- 12*02,IGKV1D- 12*02	IGHV6-1*01

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Table 2 (cont.)

Line #	IR_VDJ_1_d_gene	IR_VJ_1_j_gene	IR_VDJ_1_j_gene	IR_VJ_1_c_gene
2	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
3	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
4	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
5	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
6	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
7	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
8	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
9	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
10	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
11	IGHD7-27*01	IGKJ4*01	IGHJ5*02	IGKC
13	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
14	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
15	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
16	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
17	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
18	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
19	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
20	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
21	IGHD3-10*01	IGKJ3*01	IGHJ4*02	IGKC
23	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
24	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
25	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
26	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
27	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
28	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
29	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
30	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC
31	IGHD2-21*01,IGHD2-21*02	IGKJ1*01	IGHJ4*02	IGKC

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33	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
34	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
35	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
36	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
37	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
38	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
39	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
40	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
41	IGHD2-21*01	IGKJ1*01	IGHJ4*02	IGKC
43	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
44	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
45	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
46	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
47	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
48	IGHD3-16*02	IGKJ3*01	IGHJ4*02	IGKC
50	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
51	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
52	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
53	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
54	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
55	IGHD6-13*01	IGKJ1*01	IGHJ2*01	IGKC
57	IGHD6-13*01	IGKJ1*01	IGHJ4*02	IGKC
58	IGHD6-13*01	IGKJ1*01	IGHJ4*02	IGKC
59	IGHD6-13*01	IGKJ1*01	IGHJ4*02	IGKC
60	IGHD6-13*01	IGKJ1*01	IGHJ4*02	IGKC
61	IGHD6-13*01	IGKJ1*01	IGHJ4*02	IGKC
63	IGHD6-6*01	IGKJ3*01	IGHJ4*02	IGKC
64	IGHD6-6*01	IGKJ3*01	IGHJ4*02	IGKC
65	IGHD6-6*01	IGKJ3*01	IGHJ4*02	IGKC
66	IGHD6-6*01	IGKJ3*01	IGHJ4*02	IGKC
67	IGHD6-6*01	IGKJ3*01	IGHJ4*02	IGKC

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69	IGHD1-26*01	IGKJ3*01	IGHJ6*02	IGKC
70	IGHD1-26*01	IGKJ3*01	IGHJ6*02	IGKC
71	IGHD1-26*01	IGKJ3*01	IGHJ6*02	IGKC
72	IGHD1-26*01	IGKJ3*01	IGHJ6*02	IGKC
74	IGHD1-20*01,IGHD1-7*01,IGHD1/OR15-1a*01	IGKJ4*01	IGHJ5*02	IGKC
75	IGHD1-7*01	IGKJ4*01	IGHJ5*02	IGKC
76	IGHD1-7*01	IGKJ4*01	IGHJ5*02	IGKC

Table 2 (cont.)

Line #	IR_VDJ_1_c_Gene	TissueCT	CDR3L_CDR3H
2	IgG2C	BM_Plasma_cell	SEQ ID NO: 577 - SEQ ID NO: 578
3	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
4	IgG2C	SP_Plasma_cell	SEQ ID NO: 577 - SEQ ID NO: 578
5	IgG2C	SP_Plasma_cell	SEQ ID NO: 577 - SEQ ID NO: 578
6	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
7	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
8	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
9	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
10	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
11	IgG2C	SP_Bcell	SEQ ID NO: 577 - SEQ ID NO: 578
13	IgG2C	BM_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579
14	IgG2C	SP_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579
15	IgG2B	SP_Bcell	SEQ ID NO: 250 - SEQ ID NO: 579
16	IgG2C	SP_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579
17	IgG2C	SP_Bcell	SEQ ID NO: 250 - SEQ ID NO: 579
18	IgG2C	SP_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579
19	IgG2C	SP_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579
20	IgG2B	SP_Bcell	SEQ ID NO: 250 - SEQ ID NO: 579
21	IgG2C	SP_Plasma_cell	SEQ ID NO: 250 - SEQ ID NO: 579

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23	IgG1	BM_Plasma_cell	SEQ ID NO: 580 - SEQ ID NO: 581
24	IgG1	BM_Plasma_cell	SEQ ID NO: 580 - SEQ ID NO: 581
25	IgG2C	SP_Bcell	SEQ ID NO: 580 - SEQ ID NO: 581
26	IgG1	SP_Plasma_cell	SEQ ID NO: 580 - SEQ ID NO: 581
27	IgG1	SP_Plasma_cell	SEQ ID NO: 580 - SEQ ID NO: 581
28	IgG1	SP_Bcell	SEQ ID NO: 580 - SEQ ID NO: 581
29	IgG1	SP_Plasma_cell	SEQ ID NO: 580 - SEQ ID NO: 581
30	IgG1	SP_Bcell	SEQ ID NO: 580 - SEQ ID NO: 581
31			SEQ ID NO: 580 - SEQ ID NO: 581
33	IgG1	BM_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
34	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
35	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
36	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
37	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
38	IgG1	SP_Bcell	SEQ ID NO: 582 - SEQ ID NO: 583
39	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
40	IgG1	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
41	IgG2C	SP_Plasma_cell	SEQ ID NO: 582 - SEQ ID NO: 583
43	IgG2C	BM_Plasma_cell	SEQ ID NO: 584 - SEQ ID NO: 585
44	IgG2C	SP_Plasma_cell	SEQ ID NO: 584 - SEQ ID NO: 585
45	IgG2C	SP_Bcell	SEQ ID NO: 584 - SEQ ID NO: 585
46	IgG2C	SP_Bcell	SEQ ID NO: 584 - SEQ ID NO: 585
47	IgG2C	SP_Plasma_cell	SEQ ID NO: 584 - SEQ ID NO: 585
48	IgG2C	SP_Plasma_cell	SEQ ID NO: 584 - SEQ ID NO: 585
50	IgG2C	BM_Plasma_cell	SEQ ID NO: 586 - SEQ ID NO: 587
51	IgG2C	SP_Bcell	SEQ ID NO: 586 - SEQ ID NO: 587
52	IgG2C	SP_Plasma_cell	SEQ ID NO: 586 - SEQ ID NO: 587
53	IgG2C	SP_Bcell	SEQ ID NO: 586 - SEQ ID NO: 587
54	IgG2C	SP_Bcell	SEQ ID NO: 586 - SEQ ID NO: 587
55	IgA	SP_Bcell	SEQ ID NO: 586 - SEQ ID NO: 587
57	IgG2C	BM_Plasma_cell	SEQ ID NO: 169 - SEQ ID NO: 588

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58	IgG2C	BM_Plasma_cell	SEQ ID NO: 169 - SEQ ID NO: 588
59	IgG2C	BM_Plasma_cell	SEQ ID NO: 169 - SEQ ID NO: 588
60	IgG2C	SP_Plasma_cell	SEQ ID NO: 169 - SEQ ID NO: 588
61	IgG2C	SP_Bcell	SEQ ID NO: 169 - SEQ ID NO: 588
63	IgG1	BM_Plasma_cell	SEQ ID NO: 589 - SEQ ID NO: 590
64	IgG1	BM_Plasma_cell	SEQ ID NO: 589 - SEQ ID NO: 590
65	IgG1	SP_Bcell	SEQ ID NO: 589 - SEQ ID NO: 590
66	IgG2C	SP_Plasma_cell	SEQ ID NO: 589 - SEQ ID NO: 590
67	IgG2C	SP_Plasma_cell	SEQ ID NO: 589 - SEQ ID NO: 590
69	IgG2C	BM_Plasma_cell	SEQ ID NO: 232 - SEQ ID NO: 281
70	IgG2C	SP_Bcell	SEQ ID NO: 232 - SEQ ID NO: 281
71	IgG1	SP_Bcell	SEQ ID NO: 232 - SEQ ID NO: 281
72	IgG1	SP_Plasma_cell	SEQ ID NO: 232 - SEQ ID NO: 281
74	IgG1	BM_Plasma_cell	SEQ ID NO: 591 - SEQ ID NO: 592
75	IgG1	SP_Bcell	SEQ ID NO: 591 - SEQ ID NO: 592
76	IgG2C	SP_Plasma_cell	SEQ ID NO: 591 - SEQ ID NO: 592

Table 3: IgE HDM-specific BCR sequences metadata

Line #	Cell_Barcode	Ab_name	meta_tissue
1	SEQ ID NO: 627	IgE mAb 1_2	BoneMarrow
2	SEQ ID NO: 628	IgE mAb 8_2	BoneMarrow
3	SEQ ID NO: 629	IgE mAb 12_2	BoneMarrow
4	SEQ ID NO: 630	IgE mAb 13_2	LymphNode
5	SEQ ID NO: 631	IgE mAb 17_2	LymphNode
6	SEQ ID NO: 632	IgE mAb 21_2	LymphNode

Table 3 (cont.)

Line #	heavy_sequence	light_sequence
1	SEQ ID NO: 633	SEQ ID NO: 634

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2	SEQ ID NO: 635	SEQ ID NO: 636
3	SEQ ID NO: 637	SEQ ID NO: 638
4	SEQ ID NO: 639	SEQ ID NO: 640
5	SEQ ID NO: 641	SEQ ID NO: 642
6	SEQ ID NO: 643	SEQ ID NO: 644

Table 3 (cont.)

Line #	v_identity	IR_VJ_1_locus	IR_VDJ_1_locus	IR_VJ_1_cdr3
1	0.9966	IGK	IGH	SEQ ID NO: 645
2	0.97578	IGK	IGH	SEQ ID NO: 646
3	0.9932	IGK	IGH	SEQ ID NO: 647
4	1	IGK	IGH	SEQ ID NO: 648
5	0.89796	IGK	IGH	SEQ ID NO: 649
6	0.95918	IGK	IGH	SEQ ID NO: 650

Table 3 (cont.)

Line #	IR_VDJ_1_cdr3	IR_VJ_1_cdr3_nt	IR_VDJ_1_cdr3_nt
1	SEQ ID NO: 651	SEQ ID NO: 652	SEQ ID NO: 653
2	SEQ ID NO: 654	SEQ ID NO: 655	SEQ ID NO: 656
3	SEQ ID NO: 657	SEQ ID NO: 658	SEQ ID NO: 659
4	SEQ ID NO: 660	SEQ ID NO: 661	SEQ ID NO: 662
5	SEQ ID NO: 663	SEQ ID NO: 664	SEQ ID NO: 665
6	SEQ ID NO: 666	SEQ ID NO: 667	SEQ ID NO: 668

5

Table 3 (cont.)

Line #	IR_VJ_1_v_gene	IR_VDJ_1_v_gene	IR_VDJ_1_d_gene	IR_VJ_1_j_gene	IR_VDJ_1_j_gene
1	IGKV3-10*01	IGHV9-3*01	IGHD1-1*01	IGKJ2*01	IGHJ1*03
2	IGKV1-117*01	IGHV2-3*01	IGHD1-1*01	IGKJ4*01	IGHJ3*01

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3	IGKV12-46*01	IGHV1-12*01	IGHD1-1*01,IGHD1-1*02	IGKJ1*01	IGHJ3*01
4	IGKV3-12*01	IGHV1-26*01	IGHD1-1*01	IGKJ1*01	IGHJ3*01
5	IGKV6-32*01	IGHV1-42*01	IGHD3-3*01	IGKJ1*01	IGHJ2*01
6	IGKV8-24*01	IGHV1-82*01	IGHD4-1*01,IGHD4-1*02	IGKJ5*01	IGHJ1*03

Table 3 (cont.)

Line #	IR_VJ_1_c_gene	IR_VDJ_1_c_gene	Derp1_ab_raw	Derp2_ab_raw	Derf1_ab_raw
1	IGKC	IGHE	158	60	37
2	IGKC	IGHE	29	22	32
3	IGKC	IGHE	15	11	57
4	IGKC	IGHE	16	46	15
5	IGKC	IGHE	10	6	184
6	IGKC	IGHE	2	215	1

Table 3 (cont.)

Line #	Olee1_ab_raw	Derp1_ab_clr	Derp2_ab_clr	Derf1_ab_clr
1	0	2.615831	1.6454458	1.3217044
2	0	0.8618727	0.65546227	1.1766725
3	1	0.23326397	0.004874706	1.7406077
4	1	0.98130155	1.6244271	0.8201184
5	3	0.901677	0.5801703	3.7320528
6	4	-0.39760602	4.0095387	-0.7951561

5

Table 3 (cont.)

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Line #	Olee1_ab_clr	Derp1_ab_rank	Derp2_ab_rank	Derf1_ab_rank
1	-1.1156448	1.006892564	1.002825161	0.982488148
2	-1.1272509	0.889548671	0.836672436	0.969734609
3	-0.43410373	0.636937195	0.45274196	0.972788444
4	0.028710008	0.775289528	0.858380754	0.76541155
5	0.6026533	0.434538481	0.33633976	0.544358354
6	0.8257969	-0.245443457	0.411732604	-0.386059378

Table 4: HDM-specific clustered BCR sequences containing
an IgE clone from human bone marrow

Line #	Cell_Barcode	heavy_sequence
1	SEQ ID NO: 669	SEQ ID NO: 670
2	SEQ ID NO: 671	SEQ ID NO: 672
3	SEQ ID NO: 673	SEQ ID NO: 674
4	SEQ ID NO: 675	SEQ ID NO: 676
5	SEQ ID NO: 677	SEQ ID NO: 678
6	SEQ ID NO: 679	SEQ ID NO: 680
7	SEQ ID NO: 681	SEQ ID NO: 682
8	SEQ ID NO: 683	SEQ ID NO: 684
9	SEQ ID NO: 685	SEQ ID NO: 686
10	SEQ ID NO: 687	SEQ ID NO: 688
11	SEQ ID NO: 689	SEQ ID NO: 690
12	SEQ ID NO: 691	SEQ ID NO: 692
13	SEQ ID NO: 693	SEQ ID NO: 694
14	SEQ ID NO: 695	SEQ ID NO: 696
15	SEQ ID NO: 697	SEQ ID NO: 698
16	SEQ ID NO: 699	SEQ ID NO: 700
17	SEQ ID NO: 701	SEQ ID NO: 702
18	SEQ ID NO: 703	SEQ ID NO: 704

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19	SEQ ID NO: 705	SEQ ID NO: 706
20	SEQ ID NO: 707	SEQ ID NO: 708
21	SEQ ID NO: 709	SEQ ID NO: 710
22	SEQ ID NO: 711	SEQ ID NO: 712
23	SEQ ID NO: 713	SEQ ID NO: 714
24	SEQ ID NO: 715	SEQ ID NO: 716

Table 4 (cont.)

Line #	light_sequence	v_identity
1	SEQ ID NO: 717	0.89492
2	Nan	0.92982
3	SEQ ID NO: 718	0.92982
4	SEQ ID NO: 719	0.92857
5	Nan	0.925
6	SEQ ID NO: 720	0.89153
7	Nan	0.89855
8	SEQ ID NO: 721	0.93103
9	SEQ ID NO: 722	0.87854
10	Nan	0.93103
11	Nan	0.92453
12	SEQ ID NO: 723	0.93333
13	nan	0.90625
14	nan	0.93103
15	nan	0.93103
16	nan	0.88889
17	nan	0.90722
18	SEQ ID NO: 724	0.90102
19	SEQ ID NO: 725	0.90722
20	nan	0.924
21	nan	0.94643

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22	nan	0.91818
23	SEQ ID NO: 726	0.925
24	Nan	0.88618

Table 4 (cont.)

Line #	IR_VJ_1_locus	IR_VDJ_1_locus	IR_VJ_1_cdr3	IR_VDJ_1_cdr3
1	IGL	IGH	SEQ ID NO: 727	SEQ ID NO: 728
2		IGH	None	SEQ ID NO: 729
3	IGK	IGH	SEQ ID NO: 730	SEQ ID NO: 729
4	IGL	IGH	SEQ ID NO: 731	SEQ ID NO: 729
5		IGH	None	SEQ ID NO: 729
6	IGL	IGH	SEQ ID NO: 732	SEQ ID NO: 729
7		IGH	None	SEQ ID NO: 729
8	IGL	IGH	SEQ ID NO: 733	SEQ ID NO: 729
9	IGK	IGH	SEQ ID NO: 734	SEQ ID NO: 729
10		IGH	None	SEQ ID NO: 729
11		IGH	None	SEQ ID NO: 729
12	IGL	IGH	CAIWHDSETGWVF (SEQ ID NO: 735)	SEQ ID NO: 729
13		IGH	None	SEQ ID NO: 729
14		IGH	None	SEQ ID NO: 729
15		IGH	None	SEQ ID NO: 729
16		IGH	None	SEQ ID NO: 736
17		IGH	None	SEQ ID NO: 737
18	IGL	IGH	CAAWDDRLNGWVF (SEQ ID NO: 738)	SEQ ID NO: 737
19	IGL	IGH	CAAWDDRLNGWVF (SEQ ID NO: 738)	SEQ ID NO: 737
20		IGH	None	SEQ ID NO: 739
21		IGH	None	SEQ ID NO: 739

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22		IGH	None	SEQ ID NO: 739
23	IGK	IGH	CQQYDKWLITF (SEQ ID NO: 740)	SEQ ID NO: 739
24		IGH	None	SEQ ID NO: 741

Table 4 (cont.)

Line #	IR_VJ_1_cdr3_nt	IR_VDJ_1_cdr3_nt	IR_VJ_1_v_gene	IR_VDJ_1_v_gene
1	SEQ ID NO: 742	SEQ ID NO: 743	IGLV2-14*01	IGHV3-9*01
2	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
3	SEQ ID NO: 745	SEQ ID NO: 744	IGKV3-20*01	IGHV3-9*01,IGHV3-9*02
4	SEQ ID NO: 746	SEQ ID NO: 744	IGLV3-21*02,IGLV3-21*03,IGLV3-21*04	IGHV3-9*01,IGHV3-9*02
5	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
6	SEQ ID NO: 747	SEQ ID NO: 744	IGLV2-14*01	IGHV3-9*01
7	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
8	SEQ ID NO: 748	SEQ ID NO: 744	IGLV3-21*03	IGHV3-9*01,IGHV3-9*02
9	SEQ ID NO: 749	SEQ ID NO: 744	IGKV3-11*01,IGKV3D-11*02	IGHV3-9*01
10	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
11	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02

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12	SEQ ID NO: 750	SEQ ID NO: 744	IGLV1-47*01	IGHV3-9*01,IGHV3-9*02
13	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
14	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
15	None	SEQ ID NO: 744		IGHV3-9*01,IGHV3-9*02
16	None	SEQ ID NO: 751		IGHV4-59*10
17	None	SEQ ID NO: 752		IGHV4-59*01,IGHV4-59*02,IGHV4-59*11
18	SEQ ID NO: 753	SEQ ID NO: 752	IGLV1-44*01	IGHV4-59*01
19	SEQ ID NO: 753	SEQ ID NO: 752	IGLV1-44*01	IGHV4-59*01,IGHV4-59*02,IGHV4-59*11
20	None	SEQ ID NO: 754		IGHV3-7*01
21	None	SEQ ID NO: 754		IGHV3-21*01,IGHV3-21*02,IGHV3-21*05
22	None	SEQ ID NO: 754		IGHV3-7*01
23	SEQ ID NO: 755	SEQ ID NO: 754	IGKV1D-8*02,IGKV1D-8*03	IGHV3-7*01
24	None	SEQ ID NO: 756		IGHV3-23*05

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Table 4 (cont.)

Line #	IR_VDJ_1_d_gene	IR_VJ_1_j_gene	IR_VDJ_1_j_gene	IR_VJ_1_c_gene
1	IGHD3-10*02,IGHD4/OR15-4a*01,IGHD4/OR15-4b*01	IGLJ1*01	IGHJ6*02	IGLC1
2	IGHD4-17*01		IGHJ6*01	
3	IGHD4-17*01	IGKJ3*01	IGHJ6*01	IGKC
4	IGHD4-17*01	IGLJ3*02	IGHJ6*01	IGLC2
5	IGHD4-17*01		IGHJ6*01	
6	IGHD4-17*01	IGLJ1*01	IGHJ6*01	IGLC1
7	IGHD4-17*01		IGHJ6*01	
8	IGHD4-17*01	IGLJ3*02	IGHJ6*01	IGLC2
9	IGHD4-17*01	IGKJ4*01	IGHJ6*01	IGKC
10	IGHD4-17*01		IGHJ6*01	
11	IGHD4-17*01		IGHJ6*01	
12	IGHD4-17*01	IGLJ3*02	IGHJ6*01	IGLC2
13	IGHD4-17*01		IGHJ6*01	
14	IGHD4-17*01		IGHJ6*01	
15	IGHD4-17*01		IGHJ6*01	
16	IGHD5-24*01		IGHJ6*02	
17	IGHD2-15*01,IGHD2-21*01,IGHD2-21*02		IGHJ3*01	
18	IGHD2-15*01,IGHD2-21*01,IGHD2-21*02	IGLJ3*02	IGHJ3*01	IGLC2
19	IGHD2-15*01,IGHD2-21*01,IGHD2-21*02	IGLJ3*02	IGHJ3*01	IGLC2
20	IGHD2-2*02		IGHJ2*01	
21	IGHD2-2*02		IGHJ2*01	
22	IGHD2-2*02		IGHJ2*01	
23	IGHD2-2*02	IGKJ5*01	IGHJ2*01	IGKC
24	IGHD2-21*01,IGHD2-21*02		IGHJ5*02	

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Table 4 (cont.)

Line #	IR_VDJ_1_c_gene	Ig_chain	cc_aa_hamming	cc_aa_hamming_size
1	IGHE	IgE	21	15
2	IGHG4	IgG	21	15
3	IGHG4	IgG	21	15
4	IGHG4	IgG	21	15
5	IGHG2	IgG	21	15
6	IGHG4	IgG	21	15
7	IGHG4	IgG	21	15
8	IGHG4	IgG	21	15
9	IGHG3	IgG	21	15
10	IGHG4	IgG	21	15
11	IGHG3	IgG	21	15
12	IGHG4	IgG	21	15
13	IGHG4	IgG	21	15
14	IGHG4	IgG	21	15
15	IGHG4	IgG	21	15
16	IGHE	IgE	576	1
17	IGHE	IgE	696	3
18	IGHE	IgE	696	3
19	IGHE	IgE	696	3
20	IGHE	IgE	753	4
21	IGHE	IgE	753	4
22	IGHE	IgE	753	4
23	IGHE	IgE	753	4
24	IGHE	IgE	1330	1

The disclosure provides the following illustrative embodiments:

1. An antibody capture complex comprising a first component of a binding pair linked to
5 an antibody-capture molecule;

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wherein the first component of the binding pair is capable of binding a second component of the binding pair;

wherein the antibody-capture molecule is capable of binding to a target antibody, wherein the target antibody is secreted by an antibody secreting cell;

5 wherein when the second component of the binding pair is biotin and the first component of the binding pair is streptavidin, the antibody-capture molecule does not bind to a kappa light chain of the target antibody.

2. The antibody capture complex of embodiment 1, wherein:

the first component of the binding pair comprises avidin, streptavidin, or anti-biotin
10 and the second component of the binding pair comprises biotin;

the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos;

the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad and max;

15 the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max;

the first component of the binding pair comprises one of an azide and an alkyne and the second component of the binding pair comprises the other of an azide and an alkyne; or

the first component of the binding pair comprises one of an azide and a phosphine and
20 the second component of the binding pair comprises the other of an azide and a phosphine.

3. The antibody capture complex of embodiment 1, wherein the first component of the binding pair comprises avidin or streptavidin and the second component of the binding pair comprises biotin.

4. The antibody capture complex of embodiment 1, wherein the second component of
25 the binding pair comprises a surface marker of the antibody secreting cell and the first component of the binding pair comprises an antibody that binds to the surface marker.

5. The antibody capture complex of embodiment 4, wherein the cell surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand
30 interactor (TACI).

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6. The antibody capture complex of embodiment 1, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).
7. The antibody capture complex of embodiment 1, wherein the second component of the binding pair comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody or CD70.
8. The antibody capture complex of embodiment 1, wherein:
the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;
the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody;
the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;
the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;
the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;
the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;
the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody;
the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or
the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.
9. The antibody capture complex of any one of embodiments 1 to 8, wherein the antibody-capture molecule comprises a capture antibody.
10. The antibody capture complex of embodiment 9, wherein the capture antibody comprises an anti-Fc antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.
11. The antibody capture complex of embodiment 10, wherein the anti-Fc antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody.

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12. The antibody capture complex of embodiment 11, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.
- 5 13. The antibody capture complex of embodiment 11, wherein the anti-Fc α antibody comprises an anti-Fc α RI antibody.
14. The antibody capture complex of embodiment 11, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
15. The antibody capture complex of embodiment 9, wherein the capture antibody
10 comprises an anti-IgM antibody.
16. The antibody capture complex of embodiment 9, wherein the capture antibody comprises an anti-IgG antibody.
17. The antibody capture complex of embodiment 16, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b
15 antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
18. The antibody capture complex of embodiment 9, wherein the capture antibody comprises an anti-IgA antibody.
19. The antibody capture complex of embodiment 18, wherein the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a
20 polymeric anti-IgA antibody.
20. The antibody capture complex of embodiment 9, wherein the capture antibody comprises an anti-IgE antibody.
21. The antibody capture complex of any one of embodiments 1 to 8, wherein the antibody-capture molecule comprises an Fc receptor or an ectodomain of the Fc receptor.
- 25 22. The antibody capture complex of embodiment 21, wherein the Fc receptor comprises an Fc γ receptor, an Fc α receptor, or an Fc ϵ receptor.
23. The antibody capture complex of embodiment 22, wherein the Fc γ receptor comprises an Fc γ RI receptor, an Fc γ RIIA receptor, an Fc γ RIIB receptor, an Fc γ RIIB1 receptor, an Fc γ RIIB2 receptor, an Fc γ RIIIA receptor, an Fc γ RIIIB receptor, or an FcRn receptor.
- 30 24. The antibody capture complex of embodiment 22, wherein the Fc α receptor comprises an Fc α RI receptor or an Fc α / μ R receptor.

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25. The antibody capture complex of embodiment 22, wherein the Fcε receptor comprises an FcεRI receptor or an FcεRII receptor.
26. The antibody capture complex of any one of embodiments 1 to 8, wherein the antibody-capture molecule comprises protein A or protein G.
- 5 27. The antibody capture complex of any one of embodiments 1 to 8, wherein the antibody-capture molecule comprises protein L.
28. The antibody capture complex of embodiment 1, wherein the antibody capture complex comprises avidin or streptavidin linked to an ectodomain of a high affinity IgE receptor (FcεRIa).
- 10 29. A method of capturing a target antibody secreted by an antibody secreting cell, the method comprising:
- contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of the binding pair to bind to the cell surface of the population of antibody secreting cells, wherein the population of antibody secreting cells
- 15 comprises the antibody secreting cell that secretes the target antibody;
- contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the antibody secreting
- 20 cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and
- contacting the population of antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.
- 25 30. The method of embodiment 29, wherein:
- the first component of the binding pair comprises avidin, streptavidin, or anti-biotin and the second component of the binding pair comprises biotin;
- the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos;
- 30 the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad and max;

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the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max;

the first component of the binding pair comprises one of an azide and an alkyne and the second component of the binding pair comprises the other of an azide and an alkyne; or

5 the first component of the binding pair comprises one of an azide and a phosphine and the second component of the binding pair comprises the other of an azide and a phosphine.

31. The method of embodiment 29, wherein the first component of the binding pair comprises avidin or streptavidin and the second component of the binding pair comprises biotin.

10 32. The method of embodiment 29, wherein:

the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;

the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody;

15 the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;

the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;

20 the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;

the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;

the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody;

25 the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or

the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.

33. A method of capturing a target antibody secreted by an antibody secreting cell, the
30 method comprising:

contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that

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secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture

5 molecule captures the target antibody secreted by the antibody secreting cell; and
contacting the population of antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.

34. The method of embodiment 33, wherein the second component of the binding pair
10 comprises a surface marker of the population of antibody secreting cells and the first component of the binding pair is an antibody that binds to the surface marker.

35. The method of embodiment 34, wherein the surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI).

15 36. The method of embodiment 33, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).

37. The method of embodiment 33, wherein the second component of the binding pair comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody
20 or CD70.

38. The method of any one of embodiments 29 to 37, wherein the antibody-capture molecule comprises a capture antibody.

39. The method of embodiment 38, wherein the capture antibody comprises an anti-Fc antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.

25 40. The method of embodiment 39, wherein the anti-Fc antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody.

41. The method of embodiment 40, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.

30 42. The method of embodiment 40, wherein the anti-Fc α antibody comprises an anti-Fc α RI antibody.

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43. The method of embodiment 40, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
44. The method of embodiment 38, wherein the capture antibody comprises an anti-IgM antibody.
- 5 45. The method of embodiment 38, wherein the capture antibody comprises an anti-IgG antibody.
46. The method of embodiment 45, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
- 10 47. The method of embodiment 38, wherein the capture antibody comprises an anti-IgA antibody.
48. The method of embodiment 47, wherein the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody.
- 15 49. The method of embodiment 38, wherein the capture antibody comprises an anti-IgE antibody.
50. The method of any one of embodiments 29 to 37, wherein the antibody-capture molecule comprises an Fc receptor or the ectodomain of the Fc receptor.
51. The method of embodiment 50, wherein the Fc receptor comprises an Fc γ receptor, an
20 Fc α receptor, or an Fc ϵ receptor.
52. The method of embodiment 51, wherein the Fc γ receptor comprises an Fc γ RI receptor, an Fc γ RIIA receptor, an Fc γ RIIB receptor, an Fc γ RIIB1 receptor, an Fc γ RIIB2 receptor, an Fc γ RIIIA receptor, an Fc γ RIIIB receptor, or an FcRn receptor.
53. The method of embodiment 51, wherein the Fc α receptor comprises an Fc α RI receptor
25 or an Fc α / μ R receptor.
54. The method of embodiment 51, wherein the Fc ϵ receptor comprises an Fc ϵ RI receptor or an Fc ϵ RII receptor.
55. The method of any one of embodiments 29 to 37, wherein the antibody-capture molecule comprises protein A or protein G.
- 30 56. The method of any one of embodiments 29 to 37, wherein the antibody-capture molecule comprises protein L.

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57. The method of any one of embodiments 29-56, further comprising contacting the population of antibody secreting cells with a secondary anti-Ig antibody, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the secondary anti-Ig antibody.
- 5 58. The method of embodiment 57, wherein the secondary anti-Ig antibody is anti-IgE or anti-IgG.
59. The method of embodiment 57 or 58, wherein the secondary anti-Ig antibody is detectably labeled.
60. The method of embodiment 59, wherein the secondary anti-Ig antibody is labeled with
10 a radioactive compound, a fluorescent compound, or an enzyme.
61. The method of embodiment 60, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
62. The method of embodiment 60, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl
15 chloride, phycoerythrin, or a fluorescent protein.
63. The method of embodiment 60, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
64. The method of any one of embodiments 29-63, wherein in the step of contacting the population of antibody secreting cells with an antigen, the antigen is a barcoded antigen.
- 20 65. The method of any one of embodiments 29 to 64, wherein in the step of contacting the population of antibody secreting cells with an antigen, the population of antibody secreting cells is contacted with a plurality of barcoded antigens, wherein each barcoded antigen of the plurality of barcoded antigens comprises a unique antigen linked to a unique nucleic acid molecule.
- 25 66. The method of embodiment 65, wherein the unique nucleic acid molecule comprises DNA.
67. The method of embodiment 65, wherein the unique nucleic acid molecule comprises RNA.
68. The method of any one of embodiments 65 to 67, wherein each unique nucleic acid
30 molecule comprises from about 10 nucleotides to about 500 nucleotides.
69. The method of any one of embodiments 65 to 68, wherein each barcoded antigen is detectably labeled.

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70. The method of embodiment 69, wherein each barcoded antigen is labeled with a radioactive compound, a fluorescent compound, or an enzyme.
71. The method of embodiment 70, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
- 5 72. The method of embodiment 70, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
73. The method of embodiment 70, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
- 10 74. The method of any one of embodiments 64 to 73, wherein the barcoded antigen comprises an allergen.
75. The method of any one of embodiments 29 to 74, wherein the antibody secreting cell is a plasma cell or plasmablast.
76. The method of any one of embodiments 29 to 75, wherein the population of antibody
15 secreting cells is obtained by enriching a population of cells obtained from a human for immune cells.
77. The method of embodiment 76, wherein the population of cells obtained from the human is enriched for plasma cells or plasmablasts.
78. The method of embodiment 77, wherein the population of enriched immune cells is
20 enriched for plasma cells or plasmablasts using pan-B cell enrichment.
79. The method of any one of embodiments 29 to 78, wherein the population of antibody secreting cells is obtained from the lymph node, lung, bone marrow, or blood of a human.
80. The method of any one of embodiments 29 to 79, further comprising detecting the antigen bound by the target antibody.
- 25 81. The method of any one of embodiments 29 to 80, further comprising sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody.
- 30 82. The method of embodiment 81, wherein separating the collected pool of antibody secreting cells into single cells and isolating the antibody secreting cell that secretes the target antibody.

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83. The method of any one of embodiments 57 to 80, further comprising detecting the secondary anti-Ig antibody.

84. The method of embodiment 83, further comprising sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells
5 in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen and the secondary anti-Ig antibody, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody bound by the antigen and the secondary anti-Ig antibody.

85. The method of embodiment 84, further comprising separating the collected pool of
10 antibody secreting cells into single cells and isolating the antibody secreting cell that secretes the target antibody.

86. The method of any one of embodiments 29 to 85, further comprising contacting the population of antibody secreting cells with an Fc block prior to contacting the population of antibody secreting cells with the second component of the binding pair.

15 87. A method of capturing an IgE antibody secreted by an antibody secreting cell, wherein the IgE antibody is directed to an allergen, the method comprising:

contacting a population of antibody secreting cells with NHS-biotin to allow biotin to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody;

20 contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises streptavidin linked to an ectodomain of a high affinity IgE receptor (FcεR1a), whereby the antibody-capture molecule binds to the IgE antibody secreted by the antibody secreting cell; and

contacting the population of antibody secreting cells with an antigen, wherein the
25 antigen is an antigenic portion or a mixture of a plurality of antigenic portions of the allergen, whereby the IgE antibody captured by the antibody capture complex binds to the antigen.

88. The method of embodiment 87, wherein the antigen comprises a plurality of barcoded antigens, wherein each of the plurality of barcoded antigens is a different antigenic portion of the allergen.

30 89. The method of embodiment 87 or 88, further comprising sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture

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complex and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody.

90. The method of embodiment 87 or 88, further comprising contacting the population of antibody secreting cells with an anti-IgE antibody to allow the IgE antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind to the anti-IgE antibody.

91. The method of embodiment 90, further comprising sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture complex and is bound by the antigen and the anti-IgE antibody, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody.

92. The method of embodiment 89 or 91, further comprising separating the collected pool of antibody secreting cells into single cells and isolating the antibody secreting cell that secretes the IgE antibody.

93. The method of any one of embodiments 29-92, wherein the step of contacting the population of antibody secreting cells with an antigen comprises:

(a) contacting the population of antibody secreting cells with a first labeled form of the antigen to allow the antigen to bind to antibodies including the target antibody captured on the cell surface of the population of antibody secreting cells, wherein the antigen of the first labeled form is conjugated to a first detectable label;

(b) washing the population of antibody secreting cells to remove unbound antigen;

(c) contacting the population of antibody secreting cells with

(i) an unlabeled form of the antigen,

(ii) a second labeled form of the antigen, or

(iii) the unlabeled form of the antigen and the second labeled form of the antigen;

(d) washing the population of antibody secreting cells to remove unbound antigen, and

(e) collecting a pool of antibody secreting cells remaining bound to the first labeled form of the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex remains bound to the first labeled form of the antigen.

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94. The method of embodiment 93, wherein the first labeled form of the antigen is at a concentration between 0.001nM and 1uM.
95. The method of embodiment 93, wherein the first labeled form of the antigen is at a concentration between 0.1 and 7.5nM.
- 5 96. The method according to any one of embodiments 93-95, wherein the first detectable label is a first fluorescent label.
97. The method according to any one of embodiments 93-96, wherein the antigen is a protein in a monomeric form.
98. The method according to any one of embodiments 93-96, wherein the antigen is a
10 protein in a multimeric form.
99. The method according to any one of embodiments 93-96, wherein the antigen is a protein present in both monomeric and multimeric forms.
100. The method according to any one of embodiments 97-99, wherein the first labeled form of the antigen is a monovalent form of the antigen.
- 15 101. The method according to any one of embodiments 97-100, wherein the unlabeled form of the antigen is a monovalent form of the antigen.
102. The method according to any one of embodiments 97-100, wherein the unlabeled form of the antigen is a multivalent form of the antigen.
103. The method of embodiment 102, wherein the multivalent form of the antigen is
20 provided by a multivalent molecule to which the antigen is bound.
104. The method of embodiment 103, wherein the multivalent molecule is selected from a streptavidin multimer such as tetramer, a dimer of an immunoglobulin Fc fragment, or a trimer of a trimerization molecule such as foldon.
105. The method according to any one of embodiments 97-104, wherein the second labeled
25 form of the antigen is a monovalent form of the antigen.
106. The method according to any one of embodiments 99-104, wherein the second labeled form of the antigen is a multivalent form of the antigen.
107. The method of embodiment 106, wherein the multivalent form of the antigen is provided by a multivalent molecule to which the antigen is bound or linked.
- 30 108. The method of embodiment 107, wherein the multivalent molecule is a streptavidin multimer such as tetramer, a dimer of an immunoglobulin Fc fragment, or a trimer of a trimerization molecule such as foldon.

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109. The method according to any of embodiments 106-108, wherein the multivalent form of the antigen is labeled with a second detectable label.
110. The method of embodiment 109, wherein the second detectable label is a second fluorescent label.
- 5 111. The method of embodiment 100, wherein in step (c) the population of antibody secreting cells is contacted with the unlabeled form of the antigen.
112. The method of embodiment 111, wherein the unlabeled form of the antigen is a monovalent form of the antigen.
113. The method of embodiment 111 or 112, wherein the antigen in the unlabeled form is at
10 least 2 to 4 fold in molar ratio relative to the first labeled form of the antigen used in step (a).
114. The method of embodiment 100, wherein in step (c) the population of antibody secreting cells is contacted with the second labeled form of the antigen.
115. The method of embodiment 114, wherein the second labeled form of the antigen is a multivalent form of the antigen.
- 15 116. The method of embodiment 114 or 115, wherein the antigen in the second labeled form is at least 2 to 4-fold in molar ratio relative to the first labeled form of the antigen used in step (a).
117. The method according to embodiments 111-116, wherein the contacting in step (c) and the washing in step (d) are repeated at least once before collecting the cells in step (e).
- 20 118. The method of embodiment 100, wherein in step (c) the population of antibody secreting cells is contacted with an unlabeled form of the antigen and a second labeled form of the antigen.
119. The method of embodiment 118, wherein the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.
- 25 120. The method of embodiment 118, wherein the antigen is a monomeric protein, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.
121. The method of embodiment 118, wherein the antigen is a multimeric protein, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the
30 antigen is a multivalent form of the antigen.

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122. The method of embodiment 118, wherein the antigen is a protein present in both monomeric and multimeric forms, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.

123. The method of any one of embodiments 118-122, wherein the cells are contacted with
5 the unlabeled form of the antigen and the second labeled form of the antigen at the same time.

124. The method according to any one of embodiments 118-123, wherein the unlabeled form of the antigen is at least 2 to 4-fold in molar ratio relative to the first labeled form of the antigen used in step (a).

125. The method according to any embodiments 93-124, wherein the first detectable label is
10 a fluorescent label, and wherein fluorescence-activated cell sorting is used to collect cells that remain bound to the first labeled form of the antigen.

126. The method according to any one of embodiments 111-124, wherein the first detectable label is a first fluorescent label, and the second detectable label is a second fluorescent label that differentiates from the first fluorescent label, wherein two-dimensional
15 fluorescence-activated cell sorting is performed to collect the pool of antibody secreting cells that remain bound to the first labeled form of the antigen, and wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody which remains bound to the first labeled form of the antigen.

127. The method according to embodiments 93-126, wherein the target antibody has an
20 affinity in the range of from about 0.1pM to about 25nM (KD).

128. The method of embodiment 127, wherein the affinity is less than about 10nM (KD).

129. The method according to any one of embodiments 93-128, further comprising separating the pool of antibody secreting cells collected in step (e) into single cells and isolating the antibody secreting cell that secretes the target antibody.

25 130. The method according to embodiment 82, 85, 92 or 129, further comprising isolating antibody-encoding nucleic acids from the isolated antibody secreting cell that secretes the target antibody.

131. A method of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by a cell comprising a modified cell
30 surface, and wherein the target antibody is subsequently captured on the modified cell surface, the method comprising:

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- a) providing an antibody secreting cell comprising a modified cell surface, wherein the antibody secreting cell secretes a target antibody, wherein the target antibody is subsequently captured on the modified cell surface, wherein the target antibody captured on the modified cell surface is bound to an antigen, wherein the antigen is linked to a barcode nucleic acid molecule, and wherein the antibody secreting cell further comprises a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface;
- b) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface;
- 10 c) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface to a portion of a second nucleic acid molecule attached to the solid surface;
- d) preparing a first library of amplicons of gene expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ) in the antibody secreting cell, and a third library of amplicons of antigen barcode nucleic acid molecules;
- 15 e) sequencing each of the three libraries of amplicons; and
- f) identifying the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.
- 20 132. The method of embodiment 131, wherein the portion of the barcode nucleic acid molecule is complementary to a first template switch oligonucleotide (TSO), wherein the portion of the barcode nucleic acid molecule terminates at the 3' terminus of the barcode nucleic acid molecule.
133. The method of embodiment 131 or embodiment 132, wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first template switch oligo (TSO).
- 25 134. The method of any one of embodiments 131-133, wherein the 5' terminus of the first nucleic acid molecule attached to the solid surface comprises a first sequence of three contiguous riboguanosine residues.
- 30 135. The method of any one of embodiments 131 to 134, wherein the first nucleic acid molecule attached to the solid surface further comprises a first unique molecular identifier (UMI).

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136. The method of any one of embodiments 131 to 135, wherein the first nucleic acid molecule attached to the solid surface further comprises a first surface barcode.

137. The method of any one of embodiments 131 to 136, wherein the first nucleic acid molecule attached to the solid surface further comprises a first sequencing primer.

5 138. The method of any of embodiments 131 to 137, wherein the first nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the first sequence of three riboguanosine residues, the first TSO, the first UMI, the first surface barcode, and the first sequencing primer.

139. The method of any one of embodiments 131 to 138, wherein the first nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the first nucleic acid
10 molecule attached to the solid surface.

140. The method of any one of embodiments 131-139, wherein the first nucleic acid molecule attached to the solid surface comprises a first DNA molecule attached to the solid surface.

141. The method of embodiment 140, wherein the first DNA molecule attached to the solid
15 surface comprises a first single-stranded DNA molecule attached to the solid surface.

142. The method of embodiment 141, wherein the portion of the first single-stranded DNA molecule attached to the solid surface comprises a first TSO, wherein the portion of the barcode nucleic acid molecule hybridized to the first single-stranded DNA molecule is complementary to the first TSO, and wherein the method further comprises reverse
20 transcribing the first single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the barcode nucleic acid molecule which is complementary to the first TSO.

143. The method of any one of embodiments 131-142, wherein the barcode nucleic acid molecule is a single-stranded DNA barcode nucleic acid molecule, and wherein the portion of
25 the first nucleic acid molecule attached to the solid surface comprises a first TSO, and the method further comprises reverse transcribing the single-stranded DNA barcode nucleic acid molecule beginning from the 5' terminus of the first TSO.

144. The method of any one of embodiments 131-143, wherein the 3' terminus of the portion of the barcode nucleic acid molecule comprises three contiguous cytidine or
30 ribocytidine residues.

145. The method of embodiment 144, further comprising reverse transcribing an mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target

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antibody captured on the modified cell surface, thereby generating a single-stranded cDNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface, wherein the nucleotide sequence of the single-stranded cDNA molecule encoding the region of the gene encoding the antigen-binding

- 5 fragment of the target antibody captured on the modified cell surface differs from a DNA complement of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface in that the 3' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface
- 10 comprises three additional, contiguous cytidine or ribocytidine residues.

146. The method of embodiment 145, wherein the 3' terminus of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a plurality of contiguous adenine residues.

147. The method of embodiment 145 or embodiment 146, wherein the 5' terminus of the
- 15 single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a plurality of contiguous thymine residues.

148. The method of any one of embodiments 131 to 147, wherein the 5' terminus of the second nucleic acid molecule attached to the solid surface comprises a second sequence of
- 20 three contiguous riboguanosine residues.

149. The method of any one of embodiments 131 to 148, wherein the second nucleic acid molecule attached to the solid surface further comprises a second UMI.

150. The method of any one of embodiments 131 to 149, wherein the second nucleic acid molecule attached to the solid surface further comprises a second surface barcode, wherein
- 25 the nucleotide sequences of the first and second surface barcode nucleic acid molecules are the same.

151. The method of any one of embodiments 131 to 150, wherein the second nucleic acid molecule attached to the solid surface further comprises a second sequencing primer.

152. The method of any of embodiments 131 to 151, wherein the second nucleic acid
- 30 molecule attached to the solid surface comprises, from 5' to 3', the second sequence of three riboguanosine residues, the second TSO, the second UMI, the second surface barcode, and the

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second sequencing primer, wherein the nucleotide sequences of the first and second surface barcode nucleic acid molecules are the same.

153. The method of any one of embodiments 131 to 152, wherein the second nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the second nucleic acid
5 molecule attached to the solid surface.

154. The method of any one of embodiments 131 to 153, wherein the second nucleic acid molecule attached to the solid surface comprises a second DNA molecule attached to the solid surface.

155. The method of embodiment 154, wherein the second DNA molecule attached to the
10 solid surface comprises a second single-stranded DNA molecule attached to the solid surface.

156. The method of embodiment 155, wherein the 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, and wherein the 3' terminus of the portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment
15 of the target antibody captured on the modified cell surface comprises three contiguous cytidine or ribocytidine residues, wherein the method further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the three contiguous cytidine or ribocytidine residues.

157. The method of any one of embodiments 131 to 156, wherein the nucleic acid molecule
20 encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a second single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.

158. The method of embodiment 156, wherein the 3' terminus of the portion of the single-
25 stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three contiguous cytidine or ribocytidine residues, and wherein the 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, wherein the method further comprises
30 reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the single-stranded DNA molecule encoding

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the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.

159. The method of any one of embodiments 131 to 158, wherein the antibody secreting cell is disposed within a partition with the solid surface.

5 160. The method of any one of embodiments 131 to 159, wherein the solid surface comprises a bead.

161. The method of embodiment 159 or 160, further comprising lysing the antibody secreting cell within the partition.

162. The method of any one of embodiments 131 to 161, wherein the barcoded nucleic acid
10 molecule further comprises a third sequencing primer, wherein the third sequencing primer begins at the 5' terminus of the barcoded nucleic acid molecule.

163. The method of any one of embodiments 131 to 162, wherein each amplicon of the first library of amplicons comprises a gene, wherein each amplicon does not comprise a nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target
15 antibody captured on the modified cell surface.

164. The method of embodiment 163, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the first library of amplicons.

165. The method of any one of embodiments 131 to 164, wherein the sequencing of the first library of amplicons comprises next-generation sequencing.

20 166. The method of any one of embodiments 131 to 165, the method further comprising mapping and aligning each amplicon of the first library of amplicons to a standard reference genome.

167. The method of embodiment 166, wherein the standard reference genome comprises a human standard reference genome.

25 168. The method of embodiment 167, wherein the human standard reference genome is GRCh38.

169. The method of embodiment 166, wherein the standard reference genome comprises a murine standard reference genome.

30 170. The method of embodiment 169, wherein the murine standard reference genome is mm10.

171. The method of any one of embodiments 166-170, wherein the mapping and aligning comprises mapping each amplicon of the first library of amplicons to the standard reference

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genome with a splice aware aligner (STAR), wherein each amplicon of the first library of amplicons comprises a unique molecular identifier, wherein all amplicons of the first library of amplicons which map to annotated genes of the standard reference genome are binned, and wherein the binned amplicons are counted for each unique molecular identifier (UMI), thereby
5 generating a single cell count matrix comprising mapped count for each annotated gene and a count for each UMI.

172. The method of embodiment 171, the method further comprising filtering the single cell count matrix for high quality cells and extensively profiled genes by calculating the ratio of the total number of annotated genes divided by the $\log_{10}$ of the total number of UMIs counted,
10 wherein cells with a ratio below about 0.1 are filtered out, wherein cells with more than about four times the interquartile range of the total number of UMIs counted are filtered out, and cells with more than about 80% of reads that map to a mitochondrial gene are filtered out.

173. The method of embodiment 172, the method further comprising normalizing the single cell count matrix such that the total number of UMIs counted is about 10,000.

15 174. The method of embodiment 172 or 173, the method further comprising computing the PCA embeddings of the single cell count matrix, and performing iterative clustering of major cell types to identify similarities across batches, thereby generating a uniform manifold approximation and projection (UMAP).

175. The method of any of embodiments 172 to 174, the method further comprising using
20 cluster-specific centroids to determine a linear adjustment function per cell, and applying the linear adjustment function per cell to correct for differences in batches, thereby generating batch corrected embeddings, and using the batch corrected embeddings to generate a uniform manifold approximation and projection (UMAP) in two dimensions.

176. The method of embodiment 174 or 175, the method further comprising determining a
25 weighted k-neighbor graph and applying the Leiden algorithm to the weighted k-neighbor graph with resolution values of about 0.1, about 0.2, about 0.5, and about 1.0 to determine cell type clusters in an unsupervised manner.

177. The method of embodiment 176, the method further comprising performing a pairwise Wilcoxon test to all of the cells in one cluster and comparing the result of the first
30 pairwise Wilcoxon test, and performing another pairwise Wilcoxon test to all of the cells in every other cluster to quantify a p-value and fold-change for each gene in each cluster, wherein when a gene has the low p-value and the high fold-change, the cells are labeled by a common marker

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of antibody secreting cells and the cells are labeled by clustered subtypes that are named by the top genes from the differential expression result.

178. The method of any of embodiments 131 to 177, the method further comprising performing sample de-multiplexing, de novo assembly of read pairs into contigs, and aligning
5 and annotating the contigs against germline segment V(D)J reference sequences for the second family of amplicons.

179. The method of embodiment 178, wherein alignment of the contigs against germline segment V(D)J reference sequences comprises aligning against a human germline reference database or a murine germline reference database.

10 180. The method of embodiment 179, wherein the human germline reference database comprises the IMGT database of human germline immunoglobulin sequences.

181. The method of embodiment 179, wherein the murine germline reference database comprises the IMGT germline reference database.

182. The method of any one of embodiments 179 to 181, the method further comprising
15 comparing VDJ sequences against sequences in the human germline reference database or the murine germline reference database, thereby labeling the VDJ sequences for quality alignments by checking for in-frame alignments, length of the CDR3, and the absence of stop codons in the variable region sequence, and filtering the VDJ sequences based on the labels.

183. The method of embodiment 182, the method further comprising mapping the VDJ
20 sequences to a reference database of immunoglobulin chains.

184. The method of embodiment 179, wherein the immunoglobulin isotype, the variable region mapping, the joining region mapping, the diversity region mapping, the accuracy of full-length variable regions for both heavy and light chain sequence per cell are confirmed by the alignment.

25 185. The method of any of embodiments 131 to 184, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the third library of amplicons.

186. The method of any one of embodiments 131 to 185, the method further comprising mapping the third library of amplicons to a custom short-read reference which comprises the barcode nucleic acid molecule reference associated with each antigen, wherein the counts for
30 each uniquely mapped barcode nucleic acid molecule are summed for each cell in a barcoded antigen single cell matrix.

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187. The method of embodiment 186, the method further comprising quantifying the barcode nucleic acid molecules across all cells and normalizing the quantification by taking the centered log-ratio of each barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules across each sample capture.
- 5 188. The method of embodiment 187, the method further comprising removing background antigen signal by denoising and scaling by background (DSB) for each barcode nucleic acid molecule of the plurality of barcoded nucleic acid molecules.
189. The method of embodiment 188, the method further comprising determining an antigen signal distribution, wherein when there is a well separated bimodal distribution in the
10 antigen signal distribution, z-transformed values are used to compute antigen specificity, and wherein when there is not a well separated bimodal distribution in the antigen signal distribution, a quantile value is used.
190. The method of embodiment 189, the method further comprising statistically modeling the target antigen and negative control antigen distributions.
- 15 191. The method of any one of embodiments 131 to 190, wherein the analysis of the first library of amplicons, the second library of amplicons, and the third library of amplicons are analyzed simultaneously to obtain at least one candidate sequence of the antigen-positive target antibody captured on the modified cell surface.
192. The method of embodiment 191, the method further comprising subsetting antibody
20 secreting cells with a valid antibody constant region using the analysis of the first library of amplicons and the analysis of the second library of amplicons.
193. The method of embodiment 192, wherein the antibody constant region comprises an IgE constant region.
194. The method of embodiment 192 or 193, wherein the subsetting antibody secreting cell
25 comprises detectable levels of IgE heavy chain and CD79a but lacks detectable levels of Ms4a1 and CD19, wherein the subsetting antibody secreting cell is an IgE⁺ antibody secreting cell.
195. The method of embodiment 194, the method further comprising assessing the differential expression of the IgE⁺ antibody secreting cell against an IgA-secreting cell isotype, an IgG-secreting cell isotype, and/or an IgM-secreting cell isotype.
- 30 196. The method of embodiment 195, wherein the assessment of differential expression further characterizes the unique transcriptional signature of the IgE⁺ antibody secreting cell.

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197. The method of embodiment 196, the method further comprising assessing the antigen specificity of the IgE⁺ antibody secreting cell.
198. The method of embodiment 197, wherein the assessment comprises comparing the antigen specificity of the IgE⁺ antibody secreting cell against a plurality of antigens and a control
5 antigen, and wherein the plurality of antigens comprises the target antigen.
199. The method of embodiment 198, wherein the comparing comprises calculating an empirical score for each antigen of the plurality of antigens and the control antigen by subtracting a quantile value of a signal associated with the antigen (qT) from a quantile value of a signal associated with the control antigen (qC) with a penalty factor (x) to determine an
10 antigen specificity score where the antigen specificity score = $qT - qC^x$.
200. The method of embodiment 199, wherein the penalty factor is the same for all antigens.
201. The method of embodiment 199, wherein the penalty factor is different for different antibody isotypes.
- 15 202. The method of embodiment 199, wherein the penalty factor is different when other antigens are used.
203. The method of embodiment 199, the method further comprising determining the antigen specificity score for each of the plurality of antigens and the control antigen.
204. The method of embodiment 203, the method further comprising calculating a control
20 antigen specificity score, wherein when the antigen specificity score is high and the control antigen specificity score is low, selecting the cell.
205. The method of embodiment 204, wherein the VDJ regions identify antigen-specific antibodies.
206. The method of embodiment 204, the method further comprising selecting antibody
25 candidates from a ranked list of antigen signals using the paired V_H:V_L antibody sequence of the cell.
207. The method of any one of embodiments 191 to 206, the method further comprising determining differential expression between isotypes, conditions, and/or antigen specificity, wherein determining the differential expression between isotypes comprises performing a
30 pairwise Wilcox test for all of the cells in one isotype and performing a pairwise Wilcox test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster; and wherein determining the differential expression between antigen

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specificity comprises performing a pairwise Wilcox test for all of the cells in one isotype and performing a pairwise Wilcox test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster.

208. The method of any one of embodiments 191 to 207, the method further comprising
5 clustering VDJ sequence similarity in an antigen positive target cell, wherein the clustering VDJ sequence similarity comprises sequence-based alignment, grouping of similar variable regions by amino acid sequence.

209. The method of any one of embodiments 191 to 208, wherein the antigen positive target cell is IgE⁺.

10 210. The method of any one of embodiments 191 to 209, the method further comprising trimming redundant sequences.

211. A method of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the method comprising:

15 a) contacting a population of antibody secreting cells with a second component of a binding pair, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, whereby the second component of the binding pair binds to the cell surface of the population of antibody secreting cells;

b) contacting the population of antibody secreting cells with an antibody capture
20 complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell;

25 c) contacting the population of antibody secreting cells with a secondary anti-Ig antibody to allow the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind the secondary anti-Ig antibody;

d) contacting the population of antibody secreting cells with an antigen comprising a
barcode nucleic acid molecule to allow the target antibody secreted by the antibody secreting
30 cell and captured by the antibody capture complex to bind the antigen;

e) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig

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antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody;

f) separating the pool of antibody secreting cells into single antibody secreting cells,

5 and for each single antibody secreting cell:

1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell;

2) hybridizing a portion of the nucleic acid molecule comprising the region of the gene
10 encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface;

3) preparing a first library of amplicons of gene expression, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ), and a third library of amplicons of antigen barcode nucleic acid molecules; and

15 4) sequencing each of the three libraries of amplicons; and

g) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

212. The method of embodiment 211, wherein:

the first component of the binding pair comprises avidin, streptavidin, or anti-biotin

20 and the second component of the binding pair comprises biotin;

the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos;

the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad and max;

25 the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max;

the first component of the binding pair comprises one of an azide and an alkyne and the second component of the binding pair comprises the other of an azide and an alkyne; or

the first component of the binding pair comprises one of an azide and a phosphine and
30 the second component of the binding pair comprises the other of an azide and a phosphine.

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213. The method of embodiment 211, wherein the first component of the binding pair comprises avidin or streptavidin and the second component of the binding pair comprises biotin.

214. The method of embodiment 211, wherein:

5 the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;

the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody;

10 the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;

the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;

the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;

15 the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;

the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody;

20 the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or

the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.

215. A method of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the

25 method comprising:

a) contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first
30 component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell;

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b) contacting the population of antibody secreting cells with a secondary anti-Ig antibody to allow the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind the secondary anti-Ig antibody;

c) contacting the population of antibody secreting cells with an antigen comprising a
5 barcode nucleic acid molecule, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen;

d) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the
10 pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody;

e) separating the pool of antibody secreting cells into single antibody secreting cells, and for each single antibody secreting cell:

1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first
15 nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell;

2) hybridizing a portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface;

20 3) preparing a first library of amplicons of gene expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ) in the antibody secreting cell, and a third library of amplicons of antigen barcode nucleic acid molecules; and

4) sequencing each of the three libraries of amplicons; and

25 f) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

216. The method of embodiment 215, wherein the second component of the binding pair comprises a surface marker of the antibody secreting cell and the first component of the binding pair is an antibody that binds to the surface marker.

30 217. The method of embodiment 216, wherein the surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI).

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218. The method of embodiment 215, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).
219. The method of embodiment 215, wherein the second component of the binding pair
5 comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody or CD70.
220. The method of any one of embodiments 211 to 219, wherein the antibody-capture molecule comprises a capture antibody.
221. The method of embodiment 220, wherein the capture antibody comprises an anti-Fc
10 antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.
222. The method of embodiment 221, wherein the anti-Fc antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody.
223. The method of embodiment 222, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an
15 anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.
224. The method of embodiment 222, wherein the anti-Fc α antibody comprises an anti-Fc α RI antibody.
225. The method of embodiment 222, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
- 20 226. The method of embodiment 220, wherein the capture antibody comprises an anti-IgM antibody.
227. The method of embodiment 220, wherein the capture antibody comprises an anti-IgG antibody.
228. The method of embodiment 227, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
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229. The method of embodiment 220, wherein the capture antibody comprises an anti-IgA antibody.
230. The method of embodiment 229, wherein the anti-IgA antibody comprises an anti-IgA1
30 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody.

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231. The method of embodiment 220, wherein the capture antibody comprises an anti-IgE antibody.
232. The method of any one of embodiments 211 to 219, wherein the antibody-capture molecule comprises an Fc receptor or an ectodomain of the Fc receptor.
- 5 233. The method of embodiment 232, wherein the Fc receptor comprises an Fc γ receptor, an Fc α receptor, or an Fc ϵ receptor.
234. The method of embodiment 233, wherein the Fc γ receptor comprises an Fc γ RI receptor, an Fc γ RIIA receptor, an Fc γ RIIB receptor, an Fc γ RIIB1 receptor, an Fc γ RIIB2 receptor, an Fc γ RIIIA receptor, an Fc γ RIIIB receptor, or an FcRn receptor.
- 10 235. The method of embodiment 233, wherein the Fc α receptor comprises an Fc α RI receptor or an Fc α / μ R receptor.
236. The method of embodiment 233, wherein the Fc ϵ receptor comprises an Fc ϵ RI receptor or an Fc ϵ RII receptor.
237. The method of any one of embodiments 211 to 219, wherein the antibody-capture
15 molecule comprises protein A or protein G.
238. The method of any one of embodiments 211 to 219, wherein the antibody-capture molecule comprises protein L.
239. The method of any one of embodiments 211 to 238, wherein the secondary anti-Ig antibody is anti-IgE or anti-IgG.
- 20 240. The method of any one of embodiments 211 to 239, wherein the secondary anti-Ig antibody is detectably labeled.
241. The method of embodiment 240, wherein the secondary anti-Ig antibody is labeled with a radioactive compound, a fluorescent compound, or an enzyme.
242. The method of embodiment 241, wherein the radioactive compound comprises ^3H ,
25 ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
243. The method of embodiment 241, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
244. The method of embodiment 241, wherein the enzyme comprises alkaline phosphatase,
30 horseradish peroxidase, luciferase, or glucose oxidase.
245. The method of any one of embodiments 211 to 244, wherein the antibody secreting cell is contacted with a plurality of barcoded antigens, wherein each barcoded antigen of the

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plurality of barcoded antigens comprises a unique antigen linked to a unique nucleic acid molecule.

246. The method of embodiment 245, wherein the unique nucleic acid molecule comprises DNA.

5 247. The method of embodiment 245, wherein the unique nucleic acid molecule comprises RNA.

248. The method of any one of embodiments 245 to 247, wherein each unique nucleic acid molecule comprises from about 10 nucleotides to about 500 nucleotides.

249. The method of any one of embodiments 245 to 248, wherein each barcoded antigen is
10 detectably labeled.

250. The method of embodiment 249, wherein each barcoded antigen is labeled with a radioactive compound, a fluorescent compound, or an enzyme.

251. The method of embodiment 250, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .

15 252. The method of embodiment 250, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.

253. The method of embodiment 250, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.

20 254. The method of any one of embodiments 211 to 253, wherein the barcoded antigen comprises an allergen.

255. The method of any one of embodiments 211 to 254, wherein the antibody secreting cell is a plasma cell or plasmablast.

256. The method of any one of embodiments 211 to 255, the method further comprising
25 enriching a population of cells obtained from a human for immune cells prior to contacting the surface of the population of antibody secreting cells with the second component of a binding pair or prior to contacting the surface of the population of antibody secreting cells with the antibody capture complex.

257. The method of embodiment 256, wherein the population of cells obtained from the
30 human is enriched for plasma cells or plasmablasts.

258. The method of embodiment 257, wherein the population of enriched immune cells is enriched for plasma cells or plasmablasts using pan-B cell enrichment.

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259. The method of any one of embodiments 211 to 258, wherein the population of antibody secreting cells is obtained from the lymph node, lung, bone marrow, or blood of a human.
260. The method of any one of embodiments 211 to 259, the method further comprising
5 detecting the secondary anti-Ig antibody and/or one or more of the barcoded antigens.
261. The method of any one of embodiments 211 to 260, the method further comprising sorting the population of antibody secreting cells to obtain the pool of antibody secreting cells that are bound by secondary anti-Ig antibody and/or one or more of the barcoded antigens.
262. The method of any one of embodiments 211 to 261, the method further comprising
10 contacting the population of antibody secreting cells with an Fc block prior to contacting the population of antibody secreting cells with the second component of the binding pair.
263. The method of any one of embodiments 211 to 262, wherein the portion of the barcode nucleic acid molecule is complementary to a first template switch oligonucleotide (TSO).
- 15 264. The method of any one of embodiments 211 to 263, wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first template switch oligo (TSO).
265. The method of any one of embodiments 211 to 264, wherein the 5' terminus of the first nucleic acid molecule attached to the solid surface comprises a first sequence of three
20 contiguous riboguanosine residues.
266. The method of any one of embodiments 211 to 265, wherein the first nucleic acid molecule attached to the solid surface further comprises a first unique molecular identifier (UMI).
267. The method of any one of embodiments 211 to 266, wherein the first nucleic acid
25 molecule attached to the solid surface further comprises a first surface barcode.
268. The method of any one of embodiments 211 to 267, wherein the first nucleic acid molecule attached to the solid surface further comprises a first sequencing primer.
269. The method of any of embodiments 211 to 268, wherein the first nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the first sequence of three riboguanosine
30 residues, the first TSO, the first UMI, the first surface barcode, and the first sequencing primer.

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270. The method of any one of embodiments 211 to 269, wherein the first nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the first nucleic acid molecule attached to the solid surface.

271. The method of any one of embodiments 211 to 270, wherein the first nucleic acid
5 molecule attached to the solid surface comprises a first DNA molecule attached to the solid surface.

272. The method of embodiment 271, wherein the first DNA molecule attached to the solid surface comprises a first single-stranded DNA molecule attached to the solid surface.

273. The method of embodiment 272, wherein the portion of the first single-stranded DNA
10 molecule attached to the solid surface comprises a first TSO and the method further comprises reverse transcribing the first single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the barcode nucleic acid molecule which is complementary to the first TSO.

274. The method of any one of embodiments 211 to 273, wherein the barcode nucleic acid
15 molecule is a single-stranded DNA barcode nucleic acid molecule, and wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first TSO, and the method further comprises reverse transcribing the single-stranded DNA barcode nucleic acid molecule beginning from the 3' terminus of the first TSO.

275. The method of any one of embodiments 211 to 274, wherein the 3' terminus of the
20 portion of the barcode nucleic acid molecule comprises three contiguous cytidine residues.

276. The method of embodiment 275, further comprising reverse transcribing an mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody, thereby generating a single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody, and wherein the nucleotide
25 sequence of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody differs from a complement of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody in that the 3' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three
30 additional, contiguous cytidine residues.

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277. The method of embodiment 276, wherein the 3' terminus of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises a plurality of contiguous adenine residues.
278. The method of embodiment 276 or embodiment 277, wherein the 5' terminus of the
5 single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises a plurality of contiguous thymine residues.
279. The method of any one of embodiments 211 to 278, wherein the 5' terminus of the second nucleic acid molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues.
- 10 280. The method of any one of embodiments 211 to 279, wherein the second nucleic acid molecule attached to the solid surface further comprises a second UMI.
281. The method of any one of embodiments 211 to 280, wherein the second nucleic acid molecule attached to the solid surface further comprises a second surface barcode.
282. The method of any one of embodiments 211 to 281, wherein the second nucleic acid
15 molecule attached to the solid surface further comprises a second sequencing primer.
283. The method of any of embodiments 211 to 282, wherein the second nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the second sequence of three riboguanosine residues, the second TSO, the second UMI, the second surface barcode, and the second sequencing primer.
- 20 284. The method of any one of embodiments 211 to 283, wherein the second nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the second nucleic acid molecule attached to the solid surface.
285. The method of any one of embodiments 211 to 284, wherein the second nucleic acid molecule attached to the solid surface comprises a second DNA molecule attached to the solid
25 surface.
286. The method of embodiment 285, wherein the second DNA molecule attached to the solid surface comprises a second single-stranded DNA molecule attached to the solid surface.
287. The method of embodiment 286, wherein the 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of
30 three contiguous riboguanosine residues, and wherein the 3' terminus of the portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three contiguous cytidine residues, wherein the method

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further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the three contiguous cytidine residues.

288. The method of any one of embodiments 211 to 287, wherein the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody
5 comprises a second single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody.

289. The method of embodiment 287, wherein the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three contiguous cytidine residues, and wherein the
10 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, wherein the method further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding
15 fragment of the target antibody.

290. The method of any one of embodiments 211 to 289, wherein the single antibody secreting cells are each disposed within a partition with a solid surface.

291. The method of any one of embodiments 211 to 290, wherein the solid surface comprises a bead.

20 292. The method of embodiment 290 or embodiment 291, further comprising lysing the single antibody secreting cells within the partitions.

293. The method of any one of embodiments 211 to 292, wherein the barcoded nucleic acid molecule further comprises a third sequencing primer.

294. The method of any one of embodiments 211 to 293, wherein each amplicon of the first
25 library of amplicons comprises a gene, wherein each amplicon does not comprise a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody.

295. The method of embodiment 294, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the first library of amplicons.

30 296. The method of any one of embodiments 211 to 295, wherein the sequencing of the first library of amplicons comprises next-generation sequencing.

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297. The method of any one of embodiments 211 to 296, the method further comprising mapping and aligning each amplicon of the third library of amplicons to a standard reference genome.
298. The method of embodiment 297, wherein the standard reference genome comprises a
5 human standard reference genome.
299. The method of embodiment 298, wherein the human standard reference genome is GRCh38.
300. The method of embodiment 297, wherein the standard reference genome comprises a murine standard reference genome.
- 10 301. The method of embodiment 300, wherein the murine standard reference genome is mm10.
302. The method of any one of embodiments 297 to 301, wherein the mapping and aligning comprises mapping each amplicon of the first library of amplicons to the standard reference genome with a splice aware aligner (STAR), wherein each amplicon of the first library of
15 amplicons comprises a unique molecular identifier, wherein all amplicons of the first library of amplicons which map to annotated genes of the standard reference genome are binned, and wherein the binned amplicons are counted for each unique molecular identifier (UMI), thereby generating a single cell count matrix comprising a mapped count for each annotated gene and a count for each UMI.
- 20 303. The method of embodiment 302, the method further comprising filtering the single cell count matrix for high quality single antibody secreting cells and extensively profiled genes by calculating the ratio of the total number of annotated genes divided by the $\log_{10}$ of the total number of UMIs counted, wherein single antibody secreting cells with a ratio below about 0.1 are filtered out, wherein single antibody secreting cells with more than about four times the
25 interquartile range of the total number of UMIs counted are filtered out, and single antibody secreting cells with more than about 80 % of reads map to a mitochondrial gene are filtered out.
304. The method of embodiment 303, the method further comprising normalizing the single cell count matrix such that the total number of UMIs counted is about 10,000.
- 30 305. The method of embodiment 303 or embodiment 304, the method further comprising computing the PCA embeddings of the single cell count matrix, performing iterative clustering

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of major cell types to identify similarities across batches, thereby generating a uniform manifold approximation and projection (UMAP).

306. The method of any of embodiments 303 to 305, the method further comprising using cluster-specific centroids to determine a linear adjustment function per cell, applying the linear
5 adjustment function per cell to correct for differences in batches, thereby generating batch corrected embeddings, and using the batch corrected embeddings to generate a uniform manifold approximation and projection (UMAP) in two dimensions.

307. The method of embodiment 305 or embodiment 306, the method further comprising determining a weighted k-neighbor graph and applying the Leiden algorithm to the weighted k-
10 neighbor graph with resolution values of 0.1, 0.2, 0.5, and 1.0 to determine cell type clusters in an unsupervised manner.

308. The method of embodiment 305, the method further comprising performing a pairwise Wilcoxon test to all of the single antibody secreting cells in one cluster and comparing the result of the first pairwise Wilcoxon test, and performing another pairwise Wilcoxon test to all of
15 the single antibody secreting cells in every other cluster to quantify a p-value and fold-change for each gene in each cluster, wherein when a gene has the low p-value and the high fold-change, the single antibody secreting cells are labeled by a common marker of plasma cells, plasmablasts, or B cells and the single antibody secreting cells are labeled by clustered subtypes that are named by the top genes from the differential expression result.

20 309. The method of any of embodiments 211 to 308, the method further comprising performing sample de-multiplexing, de novo assembly of read pairs into contigs, and aligning and annotating the contigs against germline segment V(D)J reference sequences for the second family of amplicons.

310. The method of embodiment 309, wherein alignment of the contigs against germline
25 segment V(D)J reference sequences comprises aligning against a human germline reference database or a murine germline reference database.

311. The method of embodiment 310, wherein the human germline reference database comprises the IMGT database of human germline immunoglobulin sequences.

312. The method of embodiment 310, wherein the murine germline reference database
30 comprises the IMGT germline reference database.

313. The method of any one of embodiments 310 to 312, the method further comprising comparing VDJ sequences against sequences in the human germline reference database or the

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murine germline reference database, thereby labeling the VDJ sequences for quality alignments by checking for in-frame alignments, length of the CDR3, and the absence of stop codons in the variable region sequence, and filtering the VDJ sequences based on the labels.

314. The method of embodiment 313, the method further comprising mapping the VDJ
5 sequences to a reference database of immunoglobulin chains.
315. The method of embodiment 310, wherein the immunoglobulin isotype, the variable region mapping, the joining region mapping, the diversity region mapping, the accuracy of full-length variable regions for both heavy and light chain sequence per cell are confirmed by the alignment.
- 10 316. The method of any of embodiments 211 to 315, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the third library of amplicons.
317. The method of any one of embodiments 211 to 316, the method further comprising mapping the third library of amplicons to a custom short-read reference which comprises the barcode nucleic acid molecule reference associated with each antigen, wherein the counts for
15 each uniquely mapped barcode nucleic acid molecule are summed for each cell in a barcoded antigen single cell matrix.
318. The method of embodiment 317, the method further comprising quantifying the barcode nucleic acid molecules across all single antibody secreting cells and normalizing the quantification by taking the centered log-ratio of each barcode nucleic acid molecule of the
20 plurality of barcode nucleic acid molecules across each sample capture.
319. The method of embodiment 318, the method further comprising removing background antigen signal by denoising and scaling by background (DSB) for each barcode nucleic acid molecule of the plurality of barcoded nucleic acid molecules.
320. The method of embodiment 319, the method further comprising determining an
25 antigen signal distribution, wherein when there is a well separated bimodal distribution in the antigen signal distribution, z-transformed values are used to compute antigen specificity, and wherein when there is not a well separated bimodal distribution in the antigen signal distribution, a quantile value is used.
321. The method of embodiment 320, the method further comprising statistically modeling
30 the target antigen and negative control antigen distributions.
322. The method of any one of embodiments 211 to 321, wherein the analysis of the first library of amplicons, the second library of amplicons, and the third library of amplicons are

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analyzed simultaneously to obtain at least one candidate sequence of the antigen-positive target antibody.

323. The method of embodiment 322, the method further comprising subsetting plasma cells or plasmablasts with a valid antibody constant region using the analysis of the first library
5 of amplicons and the analysis of the second library of amplicons.

324. The method of embodiment 323, wherein the antibody constant region comprises an IgE constant region.

325. The method of embodiment 323 or embodiment 324, wherein the subsetting plasma cell or plasmablast comprises detectable levels of IgE heavy chain and CD79a but lacking
10 detectable levels of Ms4a1 and CD19, wherein the subsetting plasma cell or plasmablast is an IgE⁺ plasma cell or plasmablast.

326. The method of embodiment 325, the method further comprising assessing the differential expression of the IgE⁺ plasma cell or plasmablast against an IgA-secreting plasma cell or plasmablast isotype, an IgG-secreting plasma cell or plasmablast isotype, and/or an IgM-
15 secreting plasma cell or plasmablast isotype.

327. The method of embodiment 326, wherein the assessment of differential expression further characterizes the unique transcriptional signature of the IgE⁺ plasma cell or plasmablast.

328. The method of embodiment 327, the method further comprising assessing the antigen specificity of the IgE⁺ plasma cell or plasmablast.

20 329. The method of embodiment 328, wherein the assessment comprises comparing the antigen specificity of the IgE⁺ plasma cell or plasmablast against a plurality of antigens and a control antigen, and wherein the plurality of antigens comprises the target antigen.

330. The method of embodiment 329, wherein the comparing comprises calculating an empirical score for each antigen of the plurality of antigens and the control antigen by
25 subtracting a quantile value of a signal associated with the antigen (qT) from a quantile value of a signal associated with the control antigen (qC) with a penalty factor (x) to determine an antigen specificity score where the antigen specificity score = $qT - qC^x$.

331. The method of embodiment 330, wherein the penalty factor is the same for all antigens.

30 332. The method of embodiment 330, wherein the penalty factor is different for different antibody isotypes.

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333. The method of embodiment 330, wherein the penalty factor is different when other antigens are used.
334. The method of embodiment 331, the method further comprising determining the antigen specificity score for each of the plurality of antigens and the control antigen.
- 5 335. The method of embodiment 334, the method further comprising calculating a control antigen specificity score, wherein when the antigen specificity score is high and the control antigen specificity score is low, selecting the cell.
336. The method of embodiment 335, wherein the VDJ regions identify antigen-specific antibodies.
- 10 337. The method of embodiment 335, the method further comprising selecting antibody candidates from a ranked list of antigen signals using the paired $V_H:V_L$ antibody sequence of the cell.
338. The method of any one of embodiments 322 to 337, the method further comprising determining differential expression between isotypes, conditions, and/or antigen specificity,
- 15 wherein determining the differential expression between isotypes comprises performing a pairwise Wilcoxon test for all of the single antibody secreting cells in one isotype and performing a pairwise Wilcoxon test for all of the single antibody secreting cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster; and wherein determining the differential expression between antigen specificity comprises performing a pairwise Wilcoxon
- 20 test for all of the single antibody secreting cells in one isotype and performing a pairwise Wilcoxon test for all of the single antibody secreting cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster.
339. The method of any one of embodiments 320 to 338, the method further comprising clustering VDJ sequence similarity in an antigen positive target cell, wherein the clustering VDJ
- 25 sequence similarity comprises sequence-based alignment, grouping of similar variable regions by amino acid sequence.
340. The method of any one of embodiments 320 to 339, wherein the antigen positive target cell is IgE^+ .
341. The method of any one of embodiments 320 to 340, the method further comprising
- 30 trimming redundant sequences.

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Various modifications of the described subject matter, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims..

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What Is Claimed Is:

1. An antibody capture complex comprising a first component of a binding pair linked to an antibody-capture molecule;
wherein the first component of the binding pair is capable of binding a second
5 component of the binding pair;
wherein the antibody-capture molecule is capable of binding to a target antibody,
wherein the target antibody is secreted by an antibody secreting cell;
wherein when the second component of the binding pair is biotin and the first
component of the binding pair is streptavidin, the antibody-capture molecule does not bind to
10 a kappa light chain of the target antibody.
2. The antibody capture complex of claim 1, wherein:
the first component of the binding pair comprises avidin, streptavidin, or anti-biotin
and the second component of the binding pair comprises biotin;
the first component of the binding pair comprises one of jun and fos and the second
15 component of the binding pair comprises the other of jun and fos;
the first component of the binding pair comprises one of mad and max and the second
component of the binding pair comprises the other of mad and max;
the first component of the binding pair comprises one of myc and max and the second
component of the binding pair comprises the other of myc and max;
20 the first component of the binding pair comprises one of an azide and an alkyne and
the second component of the binding pair comprises the other of an azide and an alkyne; or
the first component of the binding pair comprises one of an azide and a phosphine and
the second component of the binding pair comprises the other of an azide and a phosphine.
3. The antibody capture complex of claim 1, wherein the first component of the binding
25 pair comprises avidin or streptavidin and the second component of the binding pair comprises
biotin.
4. The antibody capture complex of claim 1, wherein the second component of the
binding pair comprises a surface marker of the antibody secreting cell and the first component
of the binding pair comprises an antibody that binds to the surface marker.
- 30 5. The antibody capture complex of claim 4, wherein the cell surface marker comprises
CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D,
CD52, or transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI).

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6. The antibody capture complex of claim 1, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).
7. The antibody capture complex of claim 1, wherein the second component of the binding pair comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody or CD70.
8. The antibody capture complex of claim 1, wherein:
the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;
the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody;
the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;
the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;
the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;
the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;
the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody;
the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or
the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.
9. The antibody capture complex of any one of claims 1 to 8, wherein the antibody-capture molecule comprises a capture antibody.
10. The antibody capture complex of claim 9, wherein the capture antibody comprises an anti-Fc antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.
11. The antibody capture complex of claim 10, wherein the anti-Fc antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody.

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12. The antibody capture complex of claim 11, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.
13. The antibody capture complex of claim 11, wherein the anti-Fc α antibody comprises an anti-Fc α RI antibody.
14. The antibody capture complex of claim 11, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
15. The antibody capture complex of claim 9, wherein the capture antibody comprises an anti-IgM antibody.
16. The antibody capture complex of claim 9, wherein the capture antibody comprises an anti-IgG antibody.
17. The antibody capture complex of claim 16, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
18. The antibody capture complex of claim 9, wherein the capture antibody comprises an anti-IgA antibody.
19. The antibody capture complex of claim 18, wherein the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody.
20. The antibody capture complex of claim 9, wherein the capture antibody comprises an anti-IgE antibody.
21. The antibody capture complex of any one of claims 1 to 8, wherein the antibody-capture molecule comprises an Fc receptor or an ectodomain of the Fc receptor.
22. The antibody capture complex of claim 21, wherein the Fc receptor comprises an Fc γ receptor, an Fc α receptor, or an Fc ϵ receptor.
23. The antibody capture complex of claim 22, wherein the Fc γ receptor comprises an Fc γ RI receptor, an Fc γ RIIA receptor, an Fc γ RIIB receptor, an Fc γ RIIB1 receptor, an Fc γ RIIB2 receptor, an Fc γ RIIIA receptor, an Fc γ RIIIB receptor, or an FcRn receptor.
24. The antibody capture complex of claim 22, wherein the Fc α receptor comprises an Fc α RI receptor or an Fc α / μ R receptor.
25. The antibody capture complex of claim 22, wherein the Fc ϵ receptor comprises an Fc ϵ RI receptor or an Fc ϵ RII receptor.

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26. The antibody capture complex of any one of claims 1 to 8, wherein the antibody-capture molecule comprises protein A or protein G.

27. The antibody capture complex of any one of claims 1 to 8, wherein the antibody-capture molecule comprises protein L.

5 28. The antibody capture complex of claim 1, wherein the antibody capture complex comprises avidin or streptavidin linked to an ectodomain of a high affinity IgE receptor (FcεRIa).

29. A method of capturing a target antibody secreted by an antibody secreting cell, the method comprising:

10 contacting a population of antibody secreting cells with a second component of a binding pair to allow the second component of the binding pair to bind to the cell surface of the population of antibody secreting cells, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody;

15 contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and

20 contacting the population of antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.

30. The method of claim 29, wherein:

the first component of the binding pair comprises avidin, streptavidin, or anti-biotin and the second component of the binding pair comprises biotin;

25 the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos;

the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad and max;

30 the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max;

the first component of the binding pair comprises one of an azide and an alkyne and the second component of the binding pair comprises the other of an azide and an alkyne; or

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the first component of the binding pair comprises one of an azide and a phosphine and the second component of the binding pair comprises the other of an azide and a phosphine.

31. The method of claim 29, wherein the first component of the binding pair comprises avidin or streptavidin and the second component of the binding pair comprises biotin.

5 32. The method of claim 29, wherein:

the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;

the second component of the binding pair comprises phycoerythrin (PE), and the first component of the binding pair comprises an anti-PE antibody;

10 the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;

the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;

15 the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;

the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;

the second component of the binding pair comprises BV650, and the first component of the binding pair comprises an anti-BV650 antibody;

20 the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or

the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.

25 33. A method of capturing a target antibody secreted by an antibody secreting cell, the method comprising:

contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell; and

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contacting the population of antibody secreting cells with an antigen, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen.

34. The method of claim 33, wherein the second component of the binding pair comprises
5 a surface marker of the population of antibody secreting cells and the first component of the binding pair is an antibody that binds to the surface marker.
35. The method of claim 34, wherein the surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI).
- 10 36. The method of claim 33, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).
37. The method of claim 33, wherein the second component of the binding pair comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody or CD70.
- 15 38. The method of any one of claims 29 to 37, wherein the antibody-capture molecule comprises a capture antibody.
39. The method of claim 38, wherein the capture antibody comprises an anti-Fc antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.
40. The method of claim 39, wherein the anti-Fc antibody comprises an anti-Fc γ antibody,
20 an anti-Fc α antibody, or an anti-Fc ϵ antibody.
41. The method of claim 40, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.
42. The method of claim 40, wherein the anti-Fc α antibody comprises an anti-Fc α RI
25 antibody.
43. The method of claim 40, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
44. The method of claim 38, wherein the capture antibody comprises an anti-IgM antibody.
- 30 45. The method of claim 38, wherein the capture antibody comprises an anti-IgG antibody.

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46. The method of claim 45, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
47. The method of claim 38, wherein the capture antibody comprises an anti-IgA antibody.
- 5 48. The method of claim 47, wherein the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody.
49. The method of claim 38, wherein the capture antibody comprises an anti-IgE antibody.
50. The method of any one of claims 29 to 37, wherein the antibody-capture molecule
- 10 comprises an Fc receptor or the ectodomain of the Fc receptor.
51. The method of claim 50, wherein the Fc receptor comprises an Fcγ receptor, an Fcα receptor, or an Fcε receptor.
52. The method of claim 51, wherein the Fcγ receptor comprises an FcγRI receptor, an FcγRIIA receptor, an FcγRIIB receptor, an FcγRIIB1 receptor, an FcγRIIB2 receptor, an FcγRIIIA
- 15 receptor, an FcγRIIIB receptor, or an FcRn receptor.
53. The method of claim 51, wherein the Fcα receptor comprises an FcαRI receptor or an Fcα/μR receptor.
54. The method of claim 51, wherein the Fcε receptor comprises an FcεRI receptor or an FcεRII receptor.
- 20 55. The method of any one of claims 29 to 37, wherein the antibody-capture molecule comprises protein A or protein G.
56. The method of any one of claims 29 to 37, wherein the antibody-capture molecule comprises protein L.
57. The method of any one of claims 29-56, further comprising contacting the population
- 25 of antibody secreting cells with a secondary anti-Ig antibody, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the secondary anti-Ig antibody.
58. The method of claim 57, wherein the secondary anti-Ig antibody is anti-IgE or anti-IgG.
59. The method of claim 57 or 58, wherein the secondary anti-Ig antibody is detectably
- 30 labeled.
60. The method of claim 59, wherein the secondary anti-Ig antibody is labeled with a radioactive compound, a fluorescent compound, or an enzyme.

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61. The method of claim 60, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
62. The method of claim 60, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
63. The method of claim 60, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
64. The method of any one of claims 29-63, wherein in the step of contacting the population of antibody secreting cells with an antigen, the antigen is a barcoded antigen.
- 10 65. The method of any one of claims 29 to 64, wherein in the step of contacting the population of antibody secreting cells with an antigen, the population of antibody secreting cells is contacted with a plurality of barcoded antigens, wherein each barcoded antigen of the plurality of barcoded antigens comprises a unique antigen linked to a unique nucleic acid molecule.
- 15 66. The method of claim 65, wherein the unique nucleic acid molecule comprises DNA.
67. The method of claim 65, wherein the unique nucleic acid molecule comprises RNA.
68. The method of any one of claims 65 to 67, wherein each unique nucleic acid molecule comprises from about 10 nucleotides to about 500 nucleotides.
69. The method of any one of claims 65 to 68, wherein each barcoded antigen is
- 20 detectably labeled.
70. The method of claim 69, wherein each barcoded antigen is labeled with a radioactive compound, a fluorescent compound, or an enzyme.
71. The method of claim 70, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
- 25 72. The method of claim 70, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
73. The method of claim 70, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
- 30 74. The method of any one of claims 64 to 73, wherein the barcoded antigen comprises an allergen.

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75. The method of any one of claims 29 to 74, wherein the antibody secreting cell is a plasma cell or plasmablast.
76. The method of any one of claims 29 to 75, wherein the population of antibody secreting cells is obtained by enriching a population of cells obtained from a human for immune
5 cells.
77. The method of claim 76, wherein the population of cells obtained from the human is enriched for plasma cells or plasmablasts.
78. The method of claim 77, wherein the population of enriched immune cells is enriched for plasma cells or plasmablasts using pan-B cell enrichment.
- 10 79. The method of any one of claims 29 to 78, wherein the population of antibody secreting cells is obtained from the lymph node, lung, bone marrow, or blood of a human.
80. The method of any one of claims 29 to 79, further comprising detecting the antigen bound by the target antibody.
81. The method of any one of claims 29 to 80, further comprising sorting the population of
15 antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody.
82. The method of claim 81, wherein separating the collected pool of antibody secreting
20 cells into single cells and isolating the antibody secreting cell that secretes the target antibody.
83. The method of any one of claims 57 to 80, further comprising detecting the secondary anti-Ig antibody.
84. The method of claim 83, further comprising sorting the population of antibody secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells
25 in the pool each secrete an antibody that is captured by the antibody capture molecule and is bound by the antigen and the secondary anti-Ig antibody, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody bound by the antigen and the secondary anti-Ig antibody.
85. The method of claim 84, further comprising separating the collected pool of antibody
30 secreting cells into single cells and isolating the antibody secreting cell that secretes the target antibody.

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86. The method of any one of claims 29 to 85, further comprising contacting the population of antibody secreting cells with an Fc block prior to contacting the population of antibody secreting cells with the second component of the binding pair.

87. A method of capturing an IgE antibody secreted by an antibody secreting cell, wherein
5 the IgE antibody is directed to an allergen, the method comprising:

contacting a population of antibody secreting cells with NHS-biotin to allow biotin to bind to the cell surface, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody;

10 contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises streptavidin linked to an ectodomain of a high affinity IgE receptor (FcεR1a), whereby the antibody-capture molecule binds to the IgE antibody secreted by the antibody secreting cell; and

15 contacting the population of antibody secreting cells with an antigen, wherein the antigen is an antigenic portion or a mixture of a plurality of antigenic portions of the allergen, whereby the IgE antibody captured by the antibody capture complex binds to the antigen.

88. The method of claim 87, wherein the antigen comprises a plurality of barcoded antigens, wherein each of the plurality of barcoded antigens is a different antigenic portion of the allergen.

89. The method of claim 87 or 88, further comprising sorting the population of antibody
20 secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture complex and is bound by the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody.

90. The method of claim 87 or 88, further comprising contacting the population of
25 antibody secreting cells with an anti-IgE antibody to allow the IgE antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind to the anti-IgE antibody.

91. The method of claim 90, further comprising sorting the population of antibody
30 secreting cells to collect a pool of antibody secreting cells, wherein the antibody secreting cells in the pool each secrete an IgE antibody that is captured by the antibody capture complex and is bound by the antigen and the anti-IgE antibody, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the IgE antibody.

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92. The method of claim 89 or 91, further comprising separating the collected pool of antibody secreting cells into single cells and isolating the antibody secreting cell that secretes the IgE antibody.

93. The method of any one of claims 29-92, wherein the step of contacting the population
5 of antibody secreting cells with an antigen comprises:

(a) contacting the population of antibody secreting cells with a first labeled form of the antigen to allow the antigen to bind to antibodies including the target antibody captured on the cell surface of the population of antibody secreting cells, wherein the antigen of the first labeled form is conjugated to a first detectable label;

10 (b) washing the population of antibody secreting cells to remove unbound antigen;

(c) contacting the population of antibody secreting cells with

(i) an unlabeled form of the antigen,

(ii) a second labeled form of the antigen, or

15 (iii) the unlabeled form of the antigen and the second labeled form of the antigen;

(d) washing the population of antibody secreting cells to remove unbound antigen, and

(e) collecting a pool of antibody secreting cells remaining bound to the first labeled form of the antigen, wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the target antibody secreted
20 by the antibody secreting cell and captured by the antibody capture complex remains bound to the first labeled form of the antigen.

94. The method of claim 93, wherein the first labeled form of the antigen is at a concentration between 0.001nM and 1uM.

95. The method of claim 93, wherein the first labeled form of the antigen is at a
25 concentration between 0.1 and 7.5nM.

96. The method according to any one of claims 93-95, wherein the first detectable label is a first fluorescent label.

97. The method according to any one of claims 93-96, wherein the antigen is a protein in a monomeric form.

30 98. The method according to any one of claims 93-96, wherein the antigen is a protein in a multimeric form.

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99. The method according to any one of claims 93-96, wherein the antigen is a protein present in both monomeric and multimeric forms.
100. The method according to any one of claims 97-99, wherein the first labeled form of the antigen is a monovalent form of the antigen.
- 5 101. The method according to any one of claims 97-100, wherein the unlabeled form of the antigen is a monovalent form of the antigen.
102. The method according to any one of claims 97-100, wherein the unlabeled form of the antigen is a multivalent form of the antigen.
103. The method of claim 102, wherein the multivalent form of the antigen is provided by a
10 multivalent molecule to which the antigen is bound.
104. The method of claim 103, wherein the multivalent molecule is selected from a streptavidin multimer such as tetramer, a dimer of an immunoglobulin Fc fragment, or a trimer of a trimerization molecule such as foldon.
105. The method according to any one of claims 97-104, wherein the second labeled form of
15 the antigen is a monovalent form of the antigen.
106. The method according to any one of claims 99-104, wherein the second labeled form of the antigen is a multivalent form of the antigen.
107. The method of claim 106, wherein the multivalent form of the antigen is provided by a multivalent molecule to which the antigen is bound or linked.
- 20 108. The method of claim 107, wherein the multivalent molecule is a streptavidin multimer such as tetramer, a dimer of an immunoglobulin Fc fragment, or a trimer of a trimerization molecule such as foldon.
109. The method according to any of claims 106-108, wherein the multivalent form of the antigen is labeled with a second detectable label.
- 25 110. The method of claim 109, wherein the second detectable label is a second fluorescent label.
111. The method of claim 100, wherein in step (c) the population of antibody secreting cells is contacted with the unlabeled form of the antigen.
112. The method of claim 111, wherein the unlabeled form of the antigen is a monovalent
30 form of the antigen.
113. The method of claim 111 or 112, wherein the antigen in the unlabeled form is at least 2 to 4 fold in molar ratio relative to the first labeled form of the antigen used in step (a).

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114. The method of claim 100, wherein in step (c) the population of antibody secreting cells is contacted with the second labeled form of the antigen.
115. The method of claim 114, wherein the second labeled form of the antigen is a multivalent form of the antigen.
- 5 116. The method of claim 114 or 115, wherein the antigen in the second labeled form is at least 2 to 4-fold in molar ratio relative to the first labeled form of the antigen used in step (a).
117. The method according to claims 111-116, wherein the contacting in step (c) and the washing in step (d) are repeated at least once before collecting the cells in step (e).
118. The method of claim 100, wherein in step (c) the population of antibody secreting cells
10 is contacted with an unlabeled form of the antigen and a second labeled form of the antigen.
119. The method of claim 118, wherein the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.
120. The method of claim 118, wherein the antigen is a monomeric protein, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a
15 multivalent form of the antigen.
121. The method of claim 118, wherein the antigen is a multimeric protein, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.
122. The method of claim 118, wherein the antigen is a protein present in both monomeric
20 and multimeric forms, the unlabeled form is a monovalent form of the antigen, and the second labeled form of the antigen is a multivalent form of the antigen.
123. The method of any one of claims 118-122, wherein the cells are contacted with the unlabeled form of the antigen and the second labeled form of the antigen at the same time.
124. The method according to any one of claims 118-123, wherein the unlabeled form of the
25 antigen is at least 2 to 4-fold in molar ratio relative to the first labeled form of the antigen used in step (a).
125. The method according to any claims 93-124, wherein the first detectable label is a fluorescent label, and wherein fluorescence-activated cell sorting is used to collect cells that remain bound to the first labeled form of the antigen.
- 30 126. The method according to any one of claims 111-124, wherein the first detectable label is a first fluorescent label, and the second detectable label is a second fluorescent label that differentiates from the first fluorescent label, wherein two-dimensional fluorescence-activated

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cell sorting is performed to collect the pool of antibody secreting cells that remain bound to the first labeled form of the antigen, and wherein the collected pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody which remains bound to the first labeled form of the antigen.

5 127. The method according to claims 93-126, wherein the target antibody has an affinity in the range of from about 0.1pM to about 25nM (KD).

128. The method of claim 127, wherein the affinity is less than about 10nM (KD).

129. The method according to any one of claims 93-128, further comprising separating the pool of antibody secreting cells collected in step (e) into single cells and isolating the antibody
10 secreting cell that secretes the target antibody.

130. The method according to claim 82, 85, 92 or 129, further comprising isolating antibody-encoding nucleic acids from the isolated antibody secreting cell that secretes the target antibody.

131. A method of identifying a region of a gene encoding an antigen-binding fragment of a
15 target antibody, wherein the target antibody is secreted by a cell comprising a modified cell surface, and wherein the target antibody is subsequently captured on the modified cell surface, the method comprising:

a) providing an antibody secreting cell comprising a modified cell surface, wherein the antibody secreting cell secretes a target antibody, wherein the target antibody is subsequently
20 captured on the modified cell surface, wherein the target antibody captured on the modified cell surface is bound to an antigen, wherein the antigen is linked to a barcode nucleic acid molecule, and wherein the antibody secreting cell further comprises a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface;

25 b) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface;

c) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface to a portion of a second nucleic acid molecule attached to the solid surface;

30 d) preparing a first library of amplicons of gene expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions

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(VDJ) in the antibody secreting cell, and a third library of amplicons of antigen barcode nucleic acid molecules;

e) sequencing each of the three libraries of amplicons; and

f) identifying the region of the gene encoding the antigen-binding fragment of the

5 target antibody captured on the modified cell surface.

132. The method of claim 131, wherein the portion of the barcode nucleic acid molecule is complementary to a first template switch oligonucleotide (TSO), wherein the portion of the barcode nucleic acid molecule terminates at the 3' terminus of the barcode nucleic acid molecule.

10 133. The method of claim 131 or claim 132, wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first template switch oligo (TSO).

134. The method of any one of claims 131-133, wherein the 5' terminus of the first nucleic acid molecule attached to the solid surface comprises a first sequence of three contiguous riboguanosine residues.

15 135. The method of any one of claims 131 to 134, wherein the first nucleic acid molecule attached to the solid surface further comprises a first unique molecular identifier (UMI).

136. The method of any one of claims 131 to 135, wherein the first nucleic acid molecule attached to the solid surface further comprises a first surface barcode.

20 137. The method of any one of claims 131 to 136, wherein the first nucleic acid molecule attached to the solid surface further comprises a first sequencing primer.

138. The method of any of claims 131 to 137, wherein the first nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the first sequence of three riboguanosine residues, the first TSO, the first UMI, the first surface barcode, and the first sequencing primer.

25 139. The method of any one of claims 131 to 138, wherein the first nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the first nucleic acid molecule attached to the solid surface.

140. The method of any one of claims 131-139, wherein the first nucleic acid molecule attached to the solid surface comprises a first DNA molecule attached to the solid surface.

30 141. The method of claim 140, wherein the first DNA molecule attached to the solid surface comprises a first single-stranded DNA molecule attached to the solid surface.

142. The method of claim 141, wherein the portion of the first single-stranded DNA molecule attached to the solid surface comprises a first TSO, wherein the portion of the

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barcode nucleic acid molecule hybridized to the first single-stranded DNA molecule is complementary to the first TSO, and wherein the method further comprises reverse transcribing the first single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the barcode nucleic acid molecule which is

5 complementary to the first TSO.

143. The method of any one of claims 131-142, wherein the barcode nucleic acid molecule is a single-stranded DNA barcode nucleic acid molecule, and wherein the portion of the first nucleic acid molecule attached to the solid surface comprises a first TSO, and the method further comprises reverse transcribing the single-stranded DNA barcode nucleic acid molecule
10 beginning from the 5' terminus of the first TSO.

144. The method of any one of claims 131-143, wherein the 3' terminus of the portion of the barcode nucleic acid molecule comprises three contiguous cytidine or ribocytidine residues.

145. The method of claim 144, further comprising reverse transcribing an mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody
15 captured on the modified cell surface, thereby generating a single-stranded cDNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface, wherein the nucleotide sequence of the single-stranded cDNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface differs from a DNA complement of the
20 mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface in that the 3' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three additional, contiguous cytidine or ribocytidine residues.

25 146. The method of claim 145, wherein the 3' terminus of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a plurality of contiguous adenine residues.

147. The method of claim 145 or claim 146, wherein the 5' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the
30 target antibody captured on the modified cell surface comprises a plurality of contiguous thymine residues.

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148. The method of any one of claims 131 to 147, wherein the 5' terminus of the second nucleic acid molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues.
149. The method of any one of claims 131 to 148, wherein the second nucleic acid molecule
5 attached to the solid surface further comprises a second UMI.
150. The method of any one of claims 131 to 149, wherein the second nucleic acid molecule attached to the solid surface further comprises a second surface barcode, wherein the nucleotide sequences of the first and second surface barcode nucleic acid molecules are the same.
- 10 151. The method of any one of claims 131 to 150, wherein the second nucleic acid molecule attached to the solid surface further comprises a second sequencing primer.
152. The method of any of claims 131 to 151, wherein the second nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the second sequence of three riboguanosine residues, the second TSO, the second UMI, the second surface barcode, and the
15 second sequencing primer, wherein the nucleotide sequences of the first and second surface barcode nucleic acid molecules are the same.
153. The method of any one of claims 131 to 152, wherein the second nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the second nucleic acid molecule attached to the solid surface.
- 20 154. The method of any one of claims 131 to 153, wherein the second nucleic acid molecule attached to the solid surface comprises a second DNA molecule attached to the solid surface.
155. The method of claim 154, wherein the second DNA molecule attached to the solid surface comprises a second single-stranded DNA molecule attached to the solid surface.
156. The method of claim 155, wherein the 5' terminus of the portion of the second single-
25 stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, and wherein the 3' terminus of the portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three contiguous cytidine or ribocytidine residues, wherein the method further comprises reverse transcribing the second
30 single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the three contiguous cytidine or ribocytidine residues.

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157. The method of any one of claims 131 to 156, wherein the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises a second single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody
5 captured on the modified cell surface.
158. The method of claim 156, wherein the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface comprises three contiguous cytidine or ribocytidine residues, and wherein the 5' terminus of the portion of the second single-stranded
10 DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, wherein the method further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.
159. The method of any one of claims 131 to 158, wherein the antibody secreting cell is disposed within a partition with the solid surface.
160. The method of any one of claims 131 to 159, wherein the solid surface comprises a bead.
161. The method of claim 159 or 160, further comprising lysing the antibody secreting cell
20 within the partition.
162. The method of any one of claims 131 to 161, wherein the barcoded nucleic acid molecule further comprises a third sequencing primer, wherein the third sequencing primer begins at the 5' terminus of the barcoded nucleic acid molecule.
163. The method of any one of claims 131 to 162, wherein each amplicon of the first library
25 of amplicons comprises a gene, wherein each amplicon does not comprise a nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody captured on the modified cell surface.
164. The method of claim 163, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the first library of amplicons.
165. The method of any one of claims 131 to 164, wherein the sequencing of the first
30 library of amplicons comprises next-generation sequencing.

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166. The method of any one of claims 131 to 165, the method further comprising mapping and aligning each amplicon of the first library of amplicons to a standard reference genome.
167. The method of claim 166, wherein the standard reference genome comprises a human standard reference genome.
- 5 168. The method of claim 167, wherein the human standard reference genome is GRCh38.
169. The method of claim 166, wherein the standard reference genome comprises a murine standard reference genome.
170. The method of claim 169, wherein the murine standard reference genome is mm10.
171. The method of any one of claims 166-170, wherein the mapping and aligning
- 10 comprises mapping each amplicon of the first library of amplicons to the standard reference genome with a splice aware aligner (STAR), wherein each amplicon of the first library of amplicons comprises a unique molecular identifier, wherein all amplicons of the first library of amplicons which map to annotated genes of the standard reference genome are binned, and wherein the binned amplicons are counted for each unique molecular identifier (UMI), thereby
- 15 generating a single cell count matrix comprising mapped count for each annotated gene and a count for each UMI.
172. The method of claim 171, the method further comprising filtering the single cell count matrix for high quality cells and extensively profiled genes by calculating the ratio of the total number of annotated genes divided by the $\log_{10}$ of the total number of UMIs counted, wherein
- 20 cells with a ratio below about 0.1 are filtered out, wherein cells with more than about four times the interquartile range of the total number of UMIs counted are filtered out, and cells with more than about 80% of reads that map to a mitochondrial gene are filtered out.
173. The method of claim 172, the method further comprising normalizing the single cell count matrix such that the total number of UMIs counted is about 10,000.
- 25 174. The method of claim 172 or 173, the method further comprising computing the PCA embeddings of the single cell count matrix, and performing iterative clustering of major cell types to identify similarities across batches, thereby generating a uniform manifold approximation and projection (UMAP).
175. The method of any of claims 172 to 174, the method further comprising using cluster-
- 30 specific centroids to determine a linear adjustment function per cell, and applying the linear adjustment function per cell to correct for differences in batches, thereby generating batch

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corrected embeddings, and using the batch corrected embeddings to generate a uniform manifold approximation and projection (UMAP) in two dimensions.

176. The method of claim 174 or 175, the method further comprising determining a weighted k-neighbor graph and applying the Leiden algorithm to the weighted k-neighbor graph with resolution values of about 0.1, about 0.2, about 0.5, and about 1.0 to determine cell type clusters in an unsupervised manner.
177. The method of claim 176, the method further comprising performing a pairwise Wilcoxon test to all of the cells in one cluster and comparing the result of the first pairwise Wilcoxon test, and performing another pairwise Wilcoxon test to all of the cells in every other cluster to quantify a p-value and fold-change for each gene in each cluster, wherein when a gene has the low p-value and the high fold-change, the cells are labeled by a common marker of antibody secreting cells and the cells are labeled by clustered subtypes that are named by the top genes from the differential expression result.
178. The method of any of claims 131 to 177, the method further comprising performing sample de-multiplexing, de novo assembly of read pairs into contigs, and aligning and annotating the contigs against germline segment V(D)J reference sequences for the second family of amplicons.
179. The method of claim 178, wherein alignment of the contigs against germline segment V(D)J reference sequences comprises aligning against a human germline reference database or a murine germline reference database.
180. The method of claim 179, wherein the human germline reference database comprises the IMGT database of human germline immunoglobulin sequences.
181. The method of claim 179, wherein the murine germline reference database comprises the IMGT germline reference database.
182. The method of any one of claims 179 to 181, the method further comprising comparing VDJ sequences against sequences in the human germline reference database or the murine germline reference database, thereby labeling the VDJ sequences for quality alignments by checking for in-frame alignments, length of the CDR3, and the absence of stop codons in the variable region sequence, and filtering the VDJ sequences based on the labels.
183. The method of claim 182, the method further comprising mapping the VDJ sequences to a reference database of immunoglobulin chains.

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184. The method of claim 179, wherein the immunoglobulin isotype, the variable region mapping, the joining region mapping, the diversity region mapping, the accuracy of full-length variable regions for both heavy and light chain sequence per cell are confirmed by the alignment.
- 5 185. The method of any of claims 131 to 184, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the third library of amplicons.
186. The method of any one of claims 131 to 185, the method further comprising mapping the third library of amplicons to a custom short-read reference which comprises the barcode nucleic acid molecule reference associated with each antigen, wherein the counts for each
- 10 uniquely mapped barcode nucleic acid molecule are summed for each cell in a barcoded antigen single cell matrix.
187. The method of claim 186, the method further comprising quantifying the barcode nucleic acid molecules across all cells and normalizing the quantification by taking the centered log-ratio of each barcode nucleic acid molecule of the plurality of barcode nucleic acid
- 15 molecules across each sample capture.
188. The method of claim 187, the method further comprising removing background antigen signal by denoising and scaling by background (DSB) for each barcode nucleic acid molecule of the plurality of barcoded nucleic acid molecules.
189. The method of claim 188, the method further comprising determining an antigen
- 20 signal distribution, wherein when there is a well separated bimodal distribution in the antigen signal distribution, z-transformed values are used to compute antigen specificity, and wherein when there is not a well separated bimodal distribution in the antigen signal distribution, a quantile value is used.
190. The method of claim 189, the method further comprising statistically modeling the
- 25 target antigen and negative control antigen distributions.
191. The method of any one of claims 131 to 190, wherein the analysis of the first library of amplicons, the second library of amplicons, and the third library of amplicons are analyzed simultaneously to obtain at least one candidate sequence of the antigen-positive target antibody captured on the modified cell surface.
- 30 192. The method of claim 191, the method further comprising subsetting antibody secreting cells with a valid antibody constant region using the analysis of the first library of amplicons and the analysis of the second library of amplicons.

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193. The method of claim 192, wherein the antibody constant region comprises an IgE constant region.
194. The method of claim 192 or 193, wherein the subsetted antibody secreting cell comprises detectable levels of IgE heavy chain and CD79a but lacks detectable levels of Ms4a1 and CD19, wherein the subsetted antibody secreting cell is an IgE⁺ antibody secreting cell.
195. The method of claim 194, the method further comprising assessing the differential expression of the IgE⁺ antibody secreting cell against an IgA-secreting cell isotype, an IgG-secreting cell isotype, and/or an IgM-secreting cell isotype.
196. The method of claim 195, wherein the assessment of differential expression further characterizes the unique transcriptional signature of the IgE⁺ antibody secreting cell.
197. The method of claim 196, the method further comprising assessing the antigen specificity of the IgE⁺ antibody secreting cell.
198. The method of claim 197, wherein the assessment comprises comparing the antigen specificity of the IgE⁺ antibody secreting cell against a plurality of antigens and a control antigen, and wherein the plurality of antigens comprises the target antigen.
199. The method of claim 198, wherein the comparing comprises calculating an empirical score for each antigen of the plurality of antigens and the control antigen by subtracting a quantile value of a signal associated with the antigen (qT) from a quantile value of a signal associated with the control antigen (qC) with a penalty factor (x) to determine an antigen specificity score where the antigen specificity score = $qT - qC^x$.
200. The method of claim 199, wherein the penalty factor is the same for all antigens.
201. The method of claim 199, wherein the penalty factor is different for different antibody isotypes.
202. The method of claim 199, wherein the penalty factor is different when other antigens are used.
203. The method of claim 199, the method further comprising determining the antigen specificity score for each of the plurality of antigens and the control antigen.
204. The method of claim 203, the method further comprising calculating a control antigen specificity score, wherein when the antigen specificity score is high and the control antigen specificity score is low, selecting the cell.
205. The method of claim 204, wherein the VDJ regions identify antigen-specific antibodies.

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206. The method of claim 204, the method further comprising selecting antibody candidates from a ranked list of antigen signals using the paired $V_H:V_L$ antibody sequence of the cell.

207. The method of any one of claims 191 to 206, the method further comprising
5 determining differential expression between isotypes, conditions, and/or antigen specificity, wherein determining the differential expression between isotypes comprises performing a pairwise Wilcoxon test for all of the cells in one isotype and performing a pairwise Wilcoxon test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster; and wherein determining the differential expression between antigen
10 specificity comprises performing a pairwise Wilcoxon test for all of the cells in one isotype and performing a pairwise Wilcoxon test for all of the cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster.

208. The method of any one of claims 191 to 207, the method further comprising clustering VDJ sequence similarity in an antigen positive target cell, wherein the clustering VDJ sequence
15 similarity comprises sequence-based alignment, grouping of similar variable regions by amino acid sequence.

209. The method of any one of claims 191 to 208, wherein the antigen positive target cell is IgE^+ .

210. The method of any one of claims 191 to 209, the method further comprising trimming
20 redundant sequences.

211. A method of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the method comprising:

a) contacting a population of antibody secreting cells with a second component of a
25 binding pair, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, whereby the second component of the binding pair binds to the cell surface of the population of antibody secreting cells;

b) contacting the population of antibody secreting cells with an antibody capture complex, wherein the antibody capture complex comprises a first component of the binding
30 pair linked to an antibody-capture molecule, whereby the first component of the binding pair binds to the second component of the binding pair on the cell surface of the population of

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antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell;

c) contacting the population of antibody secreting cells with a secondary anti-Ig antibody to allow the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind the secondary anti-Ig antibody;

d) contacting the population of antibody secreting cells with an antigen comprising a barcode nucleic acid molecule to allow the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex to bind the antigen;

e) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody;

f) separating the pool of antibody secreting cells into single antibody secreting cells, and for each single antibody secreting cell:

1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell;

2) hybridizing a portion of the nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface;

3) preparing a first library of amplicons of gene expression, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ), and a third library of amplicons of antigen barcode nucleic acid molecules; and

4) sequencing each of the three libraries of amplicons; and

g) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

212. The method of claim 211, wherein:

the first component of the binding pair comprises avidin, streptavidin, or anti-biotin and the second component of the binding pair comprises biotin;

the first component of the binding pair comprises one of jun and fos and the second component of the binding pair comprises the other of jun and fos;

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the first component of the binding pair comprises one of mad and max and the second component of the binding pair comprises the other of mad and max;

the first component of the binding pair comprises one of myc and max and the second component of the binding pair comprises the other of myc and max;

5 the first component of the binding pair comprises one of an azide and an alkyne and the second component of the binding pair comprises the other of an azide and an alkyne; or

the first component of the binding pair comprises one of an azide and a phosphine and the second component of the binding pair comprises the other of an azide and a phosphine.

213. The method of claim 211, wherein the first component of the binding pair comprises
10 avidin or streptavidin and the second component of the binding pair comprises biotin.

214. The method of claim 211, wherein:

the second component of the binding pair comprises fluorescein isothiocyanate (FITC), and the first component of the binding pair comprises an anti-FITC antibody;

the second component of the binding pair comprises phycoerythrin (PE), and the first
15 component of the binding pair comprises an anti-PE antibody;

the second component of the binding pair comprises APC, and the first component of the binding pair comprises an anti-APC antibody;

the second component of the binding pair comprises BV421, and the first component of the binding pair comprises an anti-BV421 antibody;

20 the second component of the binding pair comprises BV510, and the first component of the binding pair comprises an anti-BV510 antibody;

the second component of the binding pair comprises BV605, and the first component of the binding pair comprises an anti-BV605 antibody;

the second component of the binding pair comprises BV650, and the first component
25 of the binding pair comprises an anti-BV650 antibody;

the second component of the binding pair comprises BV711, and the first component of the binding pair comprises an anti-BV711 antibody; or

the second component of the binding pair comprises BV786, and the first component of the binding pair comprises an anti-BV786 antibody.

30 215. A method of identifying a region of a gene encoding an antigen-binding fragment of a target antibody, wherein the target antibody is secreted by an antibody secreting cell, the method comprising:

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a) contacting a population of antibody secreting cells with an antibody capture complex, wherein the population of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody, wherein the antibody capture complex comprises a first component of a binding pair linked to an antibody-capture molecule, whereby the first
5 component of the binding pair binds to a second component of the binding pair on the cell surface of the population of antibody secreting cells, and whereby the antibody-capture molecule captures the target antibody secreted by the antibody secreting cell;

b) contacting the population of antibody secreting cells with a secondary anti-Ig antibody to allow the target antibody secreted by the antibody secreting cell and captured by
10 the antibody capture complex to bind the secondary anti-Ig antibody;

c) contacting the population of antibody secreting cells with an antigen comprising a barcode nucleic acid molecule, whereby the target antibody secreted by the antibody secreting cell and captured by the antibody capture complex binds the antigen;

d) collecting a pool of antibody secreting cells, wherein each cell in the pool secretes
15 an antibody that is captured by the antibody capture complex, bound by the secondary anti-Ig antibody and by the antigen comprising the barcode nucleic acid molecule, and wherein the pool of antibody secreting cells comprises the antibody secreting cell that secretes the target antibody;

e) separating the pool of antibody secreting cells into single antibody secreting cells,
20 and for each single antibody secreting cell:

1) hybridizing a portion of the barcode nucleic acid molecule to a portion of a first nucleic acid molecule attached to a solid surface, wherein the solid surface and the first nucleic acid molecule attached thereto are unique to each single antibody secreting cell;

2) hybridizing a portion of the nucleic acid molecule encoding the region of the gene
25 encoding the antigen-binding fragment of the target antibody to a portion of a second nucleic acid molecule attached to the solid surface;

3) preparing a first library of amplicons of gene expression in the antibody secreting cell, a second library of amplicons of variable regions, diversity regions, and joining regions (VDJ) in the antibody secreting cell, and a third library of amplicons of antigen barcode nucleic
30 acid molecules; and

4) sequencing each of the three libraries of amplicons; and

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f) identifying the region of the gene encoding the antigen-binding fragment of the target antibody.

216. The method of claim 215, wherein the second component of the binding pair comprises a surface marker of the antibody secreting cell and the first component of the binding pair is an antibody that binds to the surface marker.
217. The method of claim 216, wherein the surface marker comprises CD27, CD38, CD45, CD138, CD98, CD78, CD319, CXCR4, BCMA, GPRC5D, FCRL5, CD19, Ly6D, CD52, or transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI).
218. The method of claim 215, wherein the second component of the binding pair comprises an interleukin-6 receptor (IL-6R), and the first component of the binding pair comprises an anti-IL-6R antibody or interleukin-6 (IL-6).
219. The method of claim 215, wherein the second component of the binding pair comprises CD27, and the first component of the binding pair comprises an anti-CD27 antibody or CD70.
220. The method of any one of claims 211 to 219, wherein the antibody-capture molecule comprises a capture antibody.
221. The method of claim 220, wherein the capture antibody comprises an anti-Fc antibody, an anti-light chain kappa antibody, or an anti-light chain lambda antibody.
222. The method of claim 221, wherein the anti-Fc antibody comprises an anti-Fc γ antibody, an anti-Fc α antibody, or an anti-Fc ϵ antibody.
223. The method of claim 222, wherein the anti-Fc γ antibody comprises an anti-Fc γ RI antibody, an anti-Fc γ RIIA antibody, an anti-Fc γ RIIB antibody, an anti-Fc γ RIIB1 antibody, an anti-Fc γ RIIB2 antibody, an anti-Fc γ RIIIA antibody, or an anti-Fc γ RIIIB antibody.
224. The method of claim 222, wherein the anti-Fc α antibody comprises an anti-Fc α RI antibody.
225. The method of claim 222, wherein the anti-Fc ϵ antibody comprises an anti-Fc ϵ RI antibody or an anti-Fc ϵ RII antibody.
226. The method of claim 220, wherein the capture antibody comprises an anti-IgM antibody.
227. The method of claim 220, wherein the capture antibody comprises an anti-IgG antibody.

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228. The method of claim 227, wherein the anti-IgG antibody comprises an anti-IgG1 antibody, an anti-IgG2 antibody, an anti-IgG2a antibody, an anti-IgG2b antibody, an anti-IgG3 antibody, or an anti-IgG4 antibody.
229. The method of claim 220, wherein the capture antibody comprises an anti-IgA
5 antibody.
230. The method of claim 229, wherein the anti-IgA antibody comprises an anti-IgA1 antibody, an anti-IgA2 antibody, an anti-secretory IgA antibody, or a polymeric anti-IgA antibody.
231. The method of claim 220, wherein the capture antibody comprises an anti-IgE
10 antibody.
232. The method of any one of claims 211 to 219, wherein the antibody-capture molecule comprises an Fc receptor or an ectodomain of the Fc receptor.
233. The method of claim 232, wherein the Fc receptor comprises an Fc γ receptor, an Fc α receptor, or an Fc ϵ receptor.
- 15 234. The method of claim 233, wherein the Fc γ receptor comprises an Fc γ RI receptor, an Fc γ RIIA receptor, an Fc γ RIIB receptor, an Fc γ RIIB1 receptor, an Fc γ RIIB2 receptor, an Fc γ RIIIA receptor, an Fc γ RIIIB receptor, or an FcRn receptor.
235. The method of claim 233, wherein the Fc α receptor comprises an Fc α RI receptor or an Fc α / μ R receptor.
- 20 236. The method of claim 233, wherein the Fc ϵ receptor comprises an Fc ϵ RI receptor or an Fc ϵ RII receptor.
237. The method of any one of claims 211 to 219, wherein the antibody-capture molecule comprises protein A or protein G.
238. The method of any one of claims 211 to 219, wherein the antibody-capture molecule
25 comprises protein L.
239. The method of any one of claims 211 to 238, wherein the secondary anti-Ig antibody is anti-IgE or anti-IgG.
240. The method of any one of claims 211 to 239, wherein the secondary anti-Ig antibody is detectably labeled.
- 30 241. The method of claim 240, wherein the secondary anti-Ig antibody is labeled with a radioactive compound, a fluorescent compound, or an enzyme.

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242. The method of claim 241, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
243. The method of claim 241, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
244. The method of claim 241, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
245. The method of any one of claims 211 to 244, wherein the antibody secreting cell is contacted with a plurality of barcoded antigens, wherein each barcoded antigen of the plurality of barcoded antigens comprises a unique antigen linked to a unique nucleic acid molecule.
246. The method of claim 245, wherein the unique nucleic acid molecule comprises DNA.
247. The method of claim 245, wherein the unique nucleic acid molecule comprises RNA.
248. The method of any one of claims 245 to 247, wherein each unique nucleic acid molecule comprises from about 10 nucleotides to about 500 nucleotides.
249. The method of any one of claims 245 to 248, wherein each barcoded antigen is detectably labeled.
250. The method of claim 249, wherein each barcoded antigen is labeled with a radioactive compound, a fluorescent compound, or an enzyme.
251. The method of claim 250, wherein the radioactive compound comprises ^3H , ^{14}C , ^{19}F , ^{35}S , ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc .
252. The method of claim 250, wherein the fluorescent compound comprises fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, or a fluorescent protein.
253. The method of claim 250, wherein the enzyme comprises alkaline phosphatase, horseradish peroxidase, luciferase, or glucose oxidase.
254. The method of any one of claims 211 to 253, wherein the barcoded antigen comprises an allergen.
255. The method of any one of claims 211 to 254, wherein the antibody secreting cell is a plasma cell or plasmablast.
256. The method of any one of claims 211 to 255, the method further comprising enriching a population of cells obtained from a human for immune cells prior to contacting the surface of the population of antibody secreting cells with the second component of a binding pair or prior

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to contacting the surface of the population of antibody secreting cells with the antibody capture complex.

257. The method of claim 256, wherein the population of cells obtained from the human is enriched for plasma cells or plasmablasts.

5 258. The method of claim 257, wherein the population of enriched immune cells is enriched for plasma cells or plasmablasts using pan-B cell enrichment.

259. The method of any one of claims 211 to 258, wherein the population of antibody secreting cells is obtained from the lymph node, lung, bone marrow, or blood of a human.

260. The method of any one of claims 211 to 259, the method further comprising detecting
10 the secondary anti-Ig antibody and/or one or more of the barcoded antigens.

261. The method of any one of claims 211 to 260, the method further comprising sorting the population of antibody secreting cells to obtain the pool of antibody secreting cells that are bound by secondary anti-Ig antibody and/or one or more of the barcoded antigens.

262. The method of any one of claims 211 to 261, the method further comprising
15 contacting the population of antibody secreting cells with an Fc block prior to contacting the population of antibody secreting cells with the second component of the binding pair.

263. The method of any one of claims 211 to 262, wherein the portion of the barcode nucleic acid molecule is complementary to a first template switch oligonucleotide (TSO).

264. The method of any one of claims 211 to 263, wherein the portion of the first nucleic
20 acid molecule attached to the solid surface comprises a first template switch oligo (TSO).

265. The method of any one of claims 211 to 264, wherein the 5' terminus of the first nucleic acid molecule attached to the solid surface comprises a first sequence of three contiguous riboguanosine residues.

266. The method of any one of claims 211 to 265, wherein the first nucleic acid molecule
25 attached to the solid surface further comprises a first unique molecular identifier (UMI).

267. The method of any one of claims 211 to 266, wherein the first nucleic acid molecule attached to the solid surface further comprises a first surface barcode.

268. The method of any one of claims 211 to 267, wherein the first nucleic acid molecule attached to the solid surface further comprises a first sequencing primer.

30 269. The method of any of claims 211 to 268, wherein the first nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the first sequence of three riboguanosine residues, the first TSO, the first UMI, the first surface barcode, and the first sequencing primer.

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270. The method of any one of claims 211 to 269, wherein the first nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the first nucleic acid molecule attached to the solid surface.
271. The method of any one of claims 211 to 270, wherein the first nucleic acid molecule
5 attached to the solid surface comprises a first DNA molecule attached to the solid surface.
272. The method of claim 271, wherein the first DNA molecule attached to the solid surface comprises a first single-stranded DNA molecule attached to the solid surface.
273. The method of claim 272, wherein the portion of the first single-stranded DNA molecule attached to the solid surface comprises a first TSO and the method further comprises
10 reverse transcribing the first single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the barcode nucleic acid molecule which is complementary to the first TSO.
274. The method of any one of claims 211 to 273, wherein the barcode nucleic acid molecule is a single-stranded DNA barcode nucleic acid molecule, and wherein the portion of
15 the first nucleic acid molecule attached to the solid surface comprises a first TSO, and the method further comprises reverse transcribing the single-stranded DNA barcode nucleic acid molecule beginning from the 3' terminus of the first TSO.
275. The method of any one of claims 211 to 274, wherein the 3' terminus of the portion of the barcode nucleic acid molecule comprises three contiguous cytidine residues.
- 20 276. The method of claim 275, further comprising reverse transcribing an mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody, thereby generating a single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody, and wherein the nucleotide sequence of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-
25 binding fragment of the target antibody differs from a complement of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody in that the 3' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three additional, contiguous cytidine residues.
- 30 277. The method of claim 276, wherein the 3' terminus of the mRNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises a plurality of contiguous adenine residues.

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278. The method of claim 276 or claim 277, wherein the 5' terminus of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises a plurality of contiguous thymine residues.
279. The method of any one of claims 211 to 278, wherein the 5' terminus of the second
5 nucleic acid molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues.
280. The method of any one of claims 211 to 279, wherein the second nucleic acid molecule attached to the solid surface further comprises a second UMI.
281. The method of any one of claims 211 to 280, wherein the second nucleic acid molecule
10 attached to the solid surface further comprises a second surface barcode.
282. The method of any one of claims 211 to 281, wherein the second nucleic acid molecule attached to the solid surface further comprises a second sequencing primer.
283. The method of any of claims 211 to 282, wherein the second nucleic acid molecule attached to the solid surface comprises, from 5' to 3', the second sequence of three
15 riboguanosine residues, the second TSO, the second UMI, the second surface barcode, and the second sequencing primer.
284. The method of any one of claims 211 to 283, wherein the second nucleic acid molecule attached to the solid surface is attached by the 3' terminus of the second nucleic acid molecule attached to the solid surface.
- 20 285. The method of any one of claims 211 to 284, wherein the second nucleic acid molecule attached to the solid surface comprises a second DNA molecule attached to the solid surface.
286. The method of claim 285, wherein the second DNA molecule attached to the solid surface comprises a second single-stranded DNA molecule attached to the solid surface.
287. The method of claim 286, wherein the 5' terminus of the portion of the second single-
25 stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, and wherein the 3' terminus of the portion of the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three contiguous cytidine residues, wherein the method further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid
30 surface beginning from the 3' terminus of the three contiguous cytidine residues.
288. The method of any one of claims 211 to 287, wherein the nucleic acid molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody

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comprises a second single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody.

289. The method of claim 287, wherein the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody comprises three contiguous cytidine residues, and wherein the 5' terminus of the portion of the second single-stranded DNA molecule attached to the solid surface comprises a second sequence of three contiguous riboguanosine residues, wherein the method further comprises reverse transcribing the second single-stranded DNA molecule attached to the solid surface beginning from the 3' terminus of the portion of the single-stranded DNA molecule encoding the region of the gene encoding the antigen-binding fragment of the target antibody.

290. The method of any one of claims 211 to 289, wherein the single antibody secreting cells are each disposed within a partition with a solid surface.

291. The method of any one of claims 211 to 290, wherein the solid surface comprises a bead.

292. The method of claim 290 or claim 291, further comprising lysing the single antibody secreting cells within the partitions.

293. The method of any one of claims 211 to 292, wherein the barcoded nucleic acid molecule further comprises a third sequencing primer.

294. The method of any one of claims 211 to 293, wherein each amplicon of the first library of amplicons comprises a gene, wherein each amplicon does not comprise a nucleic acid molecule comprising the region of the gene encoding the antigen-binding fragment of the target antibody.

295. The method of claim 294, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the first library of amplicons.

296. The method of any one of claims 211 to 295, wherein the sequencing of the first library of amplicons comprises next-generation sequencing.

297. The method of any one of claims 211 to 296, the method further comprising mapping and aligning each amplicon of the third library of amplicons to a standard reference genome.

298. The method of claim 297, wherein the standard reference genome comprises a human standard reference genome.

299. The method of claim 298, wherein the human standard reference genome is GRCh38.

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300. The method of claim 297, wherein the standard reference genome comprises a murine standard reference genome.

301. The method of claim 300, wherein the murine standard reference genome is mm10.

302. The method of any one of claims 297 to 301, wherein the mapping and aligning
5 comprises mapping each amplicon of the first library of amplicons to the standard reference genome with a splice aware aligner (STAR), wherein each amplicon of the first library of amplicons comprises a unique molecular identifier, wherein all amplicons of the first library of amplicons which map to annotated genes of the standard reference genome are binned, and wherein the binned amplicons are counted for each unique molecular identifier (UMI), thereby
10 generating a single cell count matrix comprising a mapped count for each annotated gene and a count for each UMI.

303. The method of claim 302, the method further comprising filtering the single cell count matrix for high quality single antibody secreting cells and extensively profiled genes by calculating the ratio of the total number of annotated genes divided by the $\log_{10}$ of the total
15 number of UMIs counted, wherein single antibody secreting cells with a ratio below about 0.1 are filtered out, wherein single antibody secreting cells with more than about four times the interquartile range of the total number of UMIs counted are filtered out, and single antibody secreting cells with more than about 80 % of reads map to a mitochondrial gene are filtered out.

20 304. The method of claim 303, the method further comprising normalizing the single cell count matrix such that the total number of UMIs counted is about 10,000.

305. The method of claim 303 or claim 304, the method further comprising computing the PCA embeddings of the single cell count matrix, performing iterative clustering of major cell types to identify similarities across batches, thereby generating a uniform manifold
25 approximation and projection (UMAP).

306. The method of any of claims 303 to 305, the method further comprising using cluster-specific centroids to determine a linear adjustment function per cell, applying the linear adjustment function per cell to correct for differences in batches, thereby generating batch corrected embeddings, and using the batch corrected embeddings to generate a uniform
30 manifold approximation and projection (UMAP) in two dimensions.

307. The method of claim 305 or claim 306, the method further comprising determining a weighted k-neighbor graph and applying the Leiden algorithm to the weighted k-neighbor

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graph with resolution values of 0.1, 0.2, 0.5, and 1.0 to determine cell type clusters in an unsupervised manner.

308. The method of claim 305, the method further comprising performing a pairwise Wilcoxon test to all of the single antibody secreting cells in one cluster and comparing the result of the first pairwise Wilcoxon test, and performing another pairwise Wilcoxon test to all of the single antibody secreting cells in every other cluster to quantify a p-value and fold-change for each gene in each cluster, wherein when a gene has the low p-value and the high fold-change, the single antibody secreting cells are labeled by a common marker of plasma cells, plasmablasts, or B cells and the single antibody secreting cells are labeled by clustered subtypes that are named by the top genes from the differential expression result.

309. The method of any of claims 211 to 308, the method further comprising performing sample de-multiplexing, de novo assembly of read pairs into contigs, and aligning and annotating the contigs against germline segment V(D)J reference sequences for the second family of amplicons.

310. The method of claim 309, wherein alignment of the contigs against germline segment V(D)J reference sequences comprises aligning against a human germline reference database or a murine germline reference database.

311. The method of claim 310, wherein the human germline reference database comprises the IMGT database of human germline immunoglobulin sequences.

312. The method of claim 310, wherein the murine germline reference database comprises the IMGT germline reference database.

313. The method of any one of claims 310 to 312, the method further comprising comparing VDJ sequences against sequences in the human germline reference database or the murine germline reference database, thereby labeling the VDJ sequences for quality alignments by checking for in-frame alignments, length of the CDR3, and the absence of stop codons in the variable region sequence, and filtering the VDJ sequences based on the labels.

314. The method of claim 313, the method further comprising mapping the VDJ sequences to a reference database of immunoglobulin chains.

315. The method of claim 310, wherein the immunoglobulin isotype, the variable region mapping, the joining region mapping, the diversity region mapping, the accuracy of full-length variable regions for both heavy and light chain sequence per cell are confirmed by the alignment.

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316. The method of any of claims 211 to 315, the method further comprising sample de-multiplexing, aligning, filtering, and UMI counting the third library of amplicons.

317. The method of any one of claims 211 to 316, the method further comprising mapping the third library of amplicons to a custom short-read reference which comprises the barcode
5 nucleic acid molecule reference associated with each antigen, wherein the counts for each uniquely mapped barcode nucleic acid molecule are summed for each cell in a barcoded antigen single cell matrix.

318. The method of claim 317, the method further comprising quantifying the barcode nucleic acid molecules across all single antibody secreting cells and normalizing the
10 quantification by taking the centered log-ratio of each barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules across each sample capture.

319. The method of claim 318, the method further comprising removing background antigen signal by denoising and scaling by background (DSB) for each barcode nucleic acid molecule of the plurality of barcoded nucleic acid molecules.

15 320. The method of claim 319, the method further comprising determining an antigen signal distribution, wherein when there is a well separated bimodal distribution in the antigen signal distribution, z-transformed values are used to compute antigen specificity, and wherein when there is not a well separated bimodal distribution in the antigen signal distribution, a quantile value is used.

20 321. The method of claim 320, the method further comprising statistically modeling the target antigen and negative control antigen distributions.

322. The method of any one of claims 211 to 321, wherein the analysis of the first library of amplicons, the second library of amplicons, and the third library of amplicons are analyzed simultaneously to obtain at least one candidate sequence of the antigen-positive target
25 antibody.

323. The method of claim 322, the method further comprising subsetting plasma cells or plasmablasts with a valid antibody constant region using the analysis of the first library of amplicons and the analysis of the second library of amplicons.

324. The method of claim 323, wherein the antibody constant region comprises an IgE
30 constant region.

325. The method of claim 323 or claim 324, wherein the subsetted plasma cell or plasmablast comprises detectable levels of IgE heavy chain and CD79a but lacking detectable

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levels of Ms4a1 and CD19, wherein the subsetted plasma cell or plasmablast is an IgE⁺ plasma cell or plasmablast.

326. The method of claim 325, the method further comprising assessing the differential expression of the IgE⁺ plasma cell or plasmablast against an IgA-secreting plasma cell or
5 plasmablast isotype, an IgG-secreting plasma cell or plasmablast isotype, and/or an IgM-secreting plasma cell or plasmablast isotype.
327. The method of claim 326, wherein the assessment of differential expression further characterizes the unique transcriptional signature of the IgE⁺ plasma cell or plasmablast.
328. The method of claim 327, the method further comprising assessing the antigen
10 specificity of the IgE⁺ plasma cell or plasmablast.
329. The method of claim 328, wherein the assessment comprises comparing the antigen specificity of the IgE⁺ plasma cell or plasmablast against a plurality of antigens and a control antigen, and wherein the plurality of antigens comprises the target antigen.
330. The method of claim 329, wherein the comparing comprises calculating an empirical
15 score for each antigen of the plurality of antigens and the control antigen by subtracting a quantile value of a signal associated with the antigen (qT) from a quantile value of a signal associated with the control antigen (qC) with a penalty factor (x) to determine an antigen specificity score where the antigen specificity score = $qT - qC^x$.
331. The method of claim 330, wherein the penalty factor is the same for all antigens.
- 20 332. The method of claim 330, wherein the penalty factor is different for different antibody isotypes.
333. The method of claim 330, wherein the penalty factor is different when other antigens are used.
334. The method of claim 331, the method further comprising determining the antigen
25 specificity score for each of the plurality of antigens and the control antigen.
335. The method of claim 334, the method further comprising calculating a control antigen specificity score, wherein when the antigen specificity score is high and the control antigen specificity score is low, selecting the cell.
336. The method of claim 335, wherein the VDJ regions identify antigen-specific antibodies.
- 30 337. The method of claim 335, the method further comprising selecting antibody candidates from a ranked list of antigen signals using the paired V_H:V_L antibody sequence of the cell.

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338. The method of any one of claims 322 to 337, the method further comprising determining differential expression between isotypes, conditions, and/or antigen specificity, wherein determining the differential expression between isotypes comprises performing a pairwise Wilcoxon test for all of the single antibody secreting cells in one isotype and performing a pairwise Wilcoxon test for all of the single antibody secreting cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster; and wherein determining the differential expression between antigen specificity comprises performing a pairwise Wilcoxon test for all of the single antibody secreting cells in one isotype and performing a pairwise Wilcoxon test for all of the single antibody secreting cells in another isotype, thereby quantifying a p-value and fold-change of each gene for each cluster.
339. The method of any one of claims 320 to 338, the method further comprising clustering VDJ sequence similarity in an antigen positive target cell, wherein the clustering VDJ sequence similarity comprises sequence-based alignment, grouping of similar variable regions by amino acid sequence.
340. The method of any one of claims 320 to 339, wherein the antigen positive target cell is IgE⁺.
341. The method of any one of claims 320 to 340, the method further comprising trimming redundant sequences.