challenging nature of many antibody target classes (e.g. complex membrane proteins, targets with minimal epitope space, proteins that are highly similar to orthologs, etc.) can make it difficult to raise B-cell responses in animals due to a lack of robust immunogenicity. Coupled with the extreme complexity of some antibody design goals, generating immune animals with the desired immune profiles (i.e., B-cell repertoires) can be difficult.

[0006] In view of the foregoing, more efficient antibody discovery methods are needed. For example, antibody discovery methods that can better position traditional animal immunization and B-cell methods for successful antibody discovery would greatly enhance animal-based antibody discovery.

SUMMARY

[0007] Provided for the first time are the rationale, experimental methods, and data demonstrating techniques useful in antibody discovery. In exemplary examples~~aspects~~, the methods involve the identification of antigen-specific antibodies directly from the peripheral blood of a living, non-human animal. Advantageously, such methods provided herein allow for antibody discovery without the need for animal sacrifice, unlike traditional methods which rely on animal euthanasia followed by immune organ harvest (e.g., spleen, lymph nodes, and bone marrow). Because such methods are non-terminal (e.g., do not involve the euthanasia of the antibody-producing animals), the methods may be repeated multiple times in the same animal(s) until, for instance, an antibody of interest is obtained. The ability to repeat the method in the same animal(s) has several advantages over traditional methods. For example, repeating the method in the same animals(s) reduces the overall cost of the antibody discovery process. Also, since the animals are kept alive, the presently disclosed methods allow for real-time, in-life sampling of the antibody repertoire, such that if, for example, the animals do not produce a B cell expressing the target antibody of interest, strategic adjustments to the immunization protocol (used in the next immunization) may be made based on the observed B-cell response (from the prior immunization). The methods of the present disclosure~~invention~~ thereby permit rational repertoire shaping and/or purposeful steering of the immune response to match antibody design goals. Exemplary processes of the present disclosure are illustrated in Figures 1B-1E. Figure 1B illustrates an exemplary non-terminal method for monitoring immune responses comprising screening of single cells obtained from a blood sample of immunized animals. Based on the outcome of the single cell screening, the animal may be subjected to a repeat round of immunization (e.g., an alternative immunization) followed by single cell screening of cells from the blood sample obtained from the immunized animal, or may undergo tissue harvest if the screening determines that the animal exhibits the desired phenotype. Figure 1C illustrates an exemplary non-terminal method of monitoring for the production of select antibodies, wherein antibody secreting cells (ASCs) purified from a blood sample obtained from an immunized animal are screened at the

single cell level. The process is repeated until design goals are met and/or select antibodies are produced. Figure 1D illustrates an exemplary non-

terminal method of guiding antibody production for select antibody production wherein a primary strategy is used to immunize animals, and the ASCs obtained from PBMCs isolated from the immunized animal are screened for the desired phenotype. If the screening determines that the design goal is not met, then the animal is immunized with an alternative strategy (e.g., that differs from the primary strategy) and, the ASCs obtained from PBMCs isolated from the immunized animal are screened for the desired phenotype. The process is repeated until the screening determines that the design goal is met. When and if the design goal is met, terminal tissue may be harvested for antibody rescue using hybridoma, single cell platforms or sequence-based discovery. Figure 1E illustrates an exemplary non-terminal method of screening animals and B-cell profiling wherein a series of animals is immunized with an immunogen and ASCs obtained from a blood sample obtained from each animal are screened and a B-cell repertoire is profiled. In various examples~~aspects~~ of the exemplary processes, antibody secreting cells (ASCs), e.g., plasmablasts, are purified from the peripheral blood of an immunized mouse and then screened at single cell resolution for the relevant activity or phenotype. Compared to the traditional hybridoma production process (illustrated in Figure 1A), which typically requires about 8 weeks and requires a high level of technical skill, the process of the present disclosure is less labor-intensive and requires less time.

[0008] Accordingly, the present disclosure provides methods of monitoring for the production of select antibodies in a non-human animal. In exemplary examples~~embodiments~~, the method comprises (a) immunizing a non-human animal with an immunogen; (b) obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; and (c) assaying, e.g., individually assaying, ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies. In one aspect, the present disclosure refers to a method of monitoring for the production of select antibodies in a non-human animal, said method comprising a. immunizing a non-human animal with an immunogen; b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; and c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies. In various instances, the method further comprises repeating (b) and (c) one or more times until design goals are met, e.g., until select antibodies are produced. Figure 1C illustrates this exemplary example~~aspect~~ of the present disclosure. The present disclosure also provides methods of guiding antibody production in a non-human animal for the production of select antibodies. In exemplary examples~~embodiments~~, the method comprises (a) performing an initial immunization on a non-human animal with an immunogen; (b) obtaining a blood sample comprising ASCs from said non-human animal; (c) assaying, e.g., individually assaying, ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; and (d) performing a

cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises (i) performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold, (ii) obtaining a blood sample comprising ASCs from said non-human animal, and (iii) assaying, e.g., individually assaying, ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies. In another aspect, the present disclosure refers to a method of guiding antibody production in a non-human animal for the production of select antibodies, said method comprising: a. performing an initial immunization on a non-human animal with an immunogen; b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; and d. performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises: i. performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold, ii. obtaining a blood sample comprising ASCs from said non-human animal, iii. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies.

[0009] In various examples~~aspects~~, the assaying comprises a single-cell, live-cell assay. As used herein, the phrase "individually assaying ASCs" means that the ASCs are assayed or examined at the single cell

level or at a single cell resolution. In exemplary instances, "individually assaying ASCs" provide results relevant to a single ASC. Optionally, multiple ASCs are simultaneously assayed. In various examples~~aspects~~, multiple ASCs are simultaneously individually assayed. In exemplary examples~~aspects~~, the blood sample is obtained from the non-human animal in a non-terminal manner, e.g., the non-human animal is not killed during the blood sample collection. In exemplary instances, the method comprises performing a non-terminal blood draw from the non-human animal. In various instances, the method comprises applying the blood sample, or a fraction thereof, to a matrix and assigning a unique address of the matrix to each ASC. Optionally, a result of the assaying is the identification of each ASC producing select antibodies. In certain examples~~aspects~~, the result of the assaying is the identification of the unique address of each ASC producing select antibodies. In exemplary instances, the method comprises at least one cycle of (i) performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold, (ii) obtaining a blood sample comprising ASCs from said non-human animal, (iii) assaying, e.g., individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies. Optionally, the cycle is repeated until the percentage of ASCs producing select antibodies, as assayed in (iii), is at or above the threshold. In various instances, the cycle is repeated at least two times.

[0010] The immunogen of the subsequent immunization may differ from the immunogen of the initial immunization in exemplary examples~~aspects~~. For instance, in exemplary examples~~aspects~~, each subsequent immunization differs from a prior immunization in that (A) a different immunogen, adjuvant, and/or immunomodulatory agent is administered to the non-human animal, (B) a different dose of the immunogen is administered to the non-human animal, (C) the time between each administration of the immunogen, adjuvant, immunomodulatory agent is different, and/or (D) the route of administration for each administration of immunogen, adjuvant, immunomodulatory agent is different. Optionally, a different immunogen is used each time the non-human animal is immunized. Figure 1D illustrates an exemplary method of guiding antibody production for select antibody production.

[0011] The present disclosure further provides methods of producing select antibodies in a non-human animal. In exemplary examples~~embodiments~~, the method comprises guiding antibody production in a non-human animal for the production of select antibodies in accordance with the presently disclosed methods of guiding antibody production and then isolating the select antibodies and/or an ASC producing the select antibodies. In exemplary examples~~embodiments~~, the method comprises (a) performing an initial immunization campaign on a non-human animal with an immunogen; (b) obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; (c) assaying, e.g.,

individually assaying, ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; (d) performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises (i) performing a subsequent

immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold, (ii) obtaining a blood sample comprising ASCs from said non-human animal, and (iii) assaying, e.g., individually assaying, ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; and (e) isolating the select antibodies and/or an ASC producing the select antibodies. In another aspect, the present disclosure refers to a method of producing select antibodies in a non-human animal, comprising a. performing an initial immunization on a non-human animal with an immunogen; b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; d. performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises: i. performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold, ii. obtaining a blood sample comprising ASCs from said non-human animal, iii. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies, and e. isolating the select antibodies and/or an ASC producing the select antibodies. In various examples~~aspects~~, the method comprises (f) determining the nucleotide sequence encoding the heavy chain variable region of the select antibodies produced by an ASC (e.g., the isolated ASC producing the select antibodies) and the nucleotide sequence encoding the light chain variable region of the select antibodies produced by the ASC, (g) introducing into a host cell a first vector comprising the nucleotide sequence encoding the heavy chain variable region of the select antibodies and a second vector comprising the nucleotide sequence encoding the light chain variable region of the select antibodies, and (h) isolating the antibodies produced by the host cell.

[0012] In exemplary examples~~aspects~~, the assaying of the presently disclosed methods comprises (a) combining the ASCs within the matrix with reagents that bind to the select antibodies and produce a detectable signal, e.g., a fluorescent signal, upon binding to the select antibodies. In various examples~~aspects~~, the assaying of the presently disclosed methods comprises (a) combining the ASCs within the matrix with at least one reagent which binds to the Fc domain of the select antibodies and at least one reagent to which select antibodies bind (e.g., a reagent which binds to the antigen-binding domain of the select antibodies), wherein at least one of these reagents is attached to a detectable label. In exemplary instances, the ASCs are combined with a detection reagent which binds to the Fc domain of the select antibodies and comprises a first detectable label and a target to which select antibodies bind (e.g., a reagent which binds to the antigen-binding domain of the select antibodies). Figures 2A-2C illustrate exemplary assaying in the context of the presently disclosed methods. In various instances, the target is labeled by a second detectable label which is different from the first

detectable label. In some instances, a capture reagent which binds to the Fc domain of the select antibodies and comprises a solid support is further combined with the ASCs, detection reagent and labeled target. In various instances, the method further comprises (b) assaying for the first detectable label and the second detectable label; and (c) identifying the positions within the matrix at which the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies. Figures 2A and 2B illustrate such exemplary assaying with a labeled target and a capture reagent. Figure 2A illustrates the matrix as a well. Figure 2B illustrates the matrix as a multi-pen chip or multi-well plate and each ASCs is positioned into a single pen or well. In various instances, the target is expressed by cells and the cells expressing the target are combined with the ASCs and the detection reagent. In exemplary examples~~aspects~~, the method further comprises (b) assaying for the first detectable label; and (c) identifying the positions within the matrix at which the first detectable label is detected, wherein each identified position locates an individual ASC producing select antibodies. Figure 2C illustrates such exemplary assaying with a cell

expressing the target. In exemplary instances, the assaying of the presently disclosed methods comprises (a) combining the ASCs within the matrix with (i) a capture reagent which binds to the select antibodies and comprises a solid support, (ii) a detection reagent which binds to the select antibodies and comprises a first detectable label, and (iii) a labeled target to which the select antibodies bind, wherein the labeled target comprises a second detectable label distinct from the first detectable label; (b) assaying for the first detectable label and for the second detectable label; and (c) identifying the positions within the matrix at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies. Optionally, the capture agent comprises an antibody that binds to an antibody Fc domain attached to a solid support. The detection agent, in exemplary instances, comprises an antibody that binds to an antibody Fc domain attached to a first detectable label. In various examples~~aspects~~, the antibody that binds to an antibody Fc domain of the capture agent is the same antibody of the detection agent. In exemplary instances, the combining takes place in a well and the capture agent forms a monolayer in the well. In various examples~~aspects~~, the method comprises identifying the positions within the well at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies.

[0013] The present disclosure additionally provides single-cell assays for identifying ASCs producing select antibodies. The present disclosure provides methods of assaying for ASCs producing select antibodies. In exemplary examples~~embodiments~~, the assay or method comprises (a) combining in a well (i) a blood sample obtained from a non-human animal immunized with an immunogen, or a fraction thereof, wherein the blood sample comprises ASCs, (ii) a detection reagent which binds to the select antibodies and comprises a first detectable label, and (iii) a target to which the select antibodies bind, wherein (A) the target is a labeled target comprising a second detectable label distinct from the first detectable label and a capture reagent which binds to the select antibodies and comprises a solid support is further combined in the well to form a monolayer in the well or (B) the target is expressed on the surface of cells and the cells are combined in the well to form a monolayer in the well; (b) assaying for the first detectable label and optionally assaying for the second detectable label, when the target is a labeled target; and (c) identifying the positions within the well at which the first detectable label is detected or the first and second detectable labels are detected, wherein each identified position locates an individual ASC producing select antibodies. In various examples~~aspects~~, the assay or method comprises (a) combining in a well (i) a blood sample obtained from a non-human animal immunized with an immunogen, or a fraction thereof, (ii) a capture reagent comprising an antibody that binds to an Fc of an antibody

attached to a solid support, (iii) a detection reagent comprising an antibody that binds to an Fc of an antibody attached to a first detectable label, and (iv) a labeled target comprising the immunogen, or a portion thereof, attached to a second detectable label distinct from the first detectable label, wherein the capture agent forms a monolayer

in the well; (b) assaying for the first detectable label; (c) assaying for the second detectable label; and (d) identifying the positions within the well at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies. In various examplesaspects, the assay or method comprises (a) combining in a well (i) a blood sample obtained from a non-human animal immunized with an immunogen, or a fraction thereof, (ii) a detection reagent which binds to the select antibodies, and (iii) cells expressing on the cell surface a target to which the select antibodies bind, wherein the cells are combined in the well to form a monolayer in the well, (b) assaying for the first detectable label; and (c) identifying the positions within the well at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies. In another aspect, the present disclosure refers to a method of assaying for ASCs producing select antibodies, said method comprising: a. combining in a well (i) a blood sample obtained from a non-human animal immunized with an immunogen, or a fraction thereof, wherein the blood sample comprises antibody secreting cells (ASCs), (ii) a detection reagent which binds to the select antibodies and comprises a first detectable label, and (iii) a target to which the select antibodies bind, wherein: (A) the target is a labeled target comprising a second detectable label distinct from the first detectable label and a capture reagent which binds to the select antibodies and comprises a solid support is further combined in the well to form a monolayer in the well, or (B) the target is expressed on the surface of cells and the cells are combined in the well to form a monolayer in the well, b. assaying for the first detectable label and optionally assaying for the second detectable label, when the target is a labeled target; c. identifying the positions within the well at which the first detectable label is detected or the first and second detectable labels are detected, wherein each identified position locates an individual ASC producing select antibodies.

[0014] In various examplesaspects of the presently disclosed methods, the non-human animal is subjected to neither removal of one or more secondary lymphoid organs nor euthanasia. Also, in various instances, ASCs from the blood sample are not used in making hybridomas. In exemplary examplesaspects, the non-human animal is one of a series of non-human animals, and a result of the assaying is the identification of the non-human animals having a percentage of ASCs producing select antibodies below the threshold and/or requiring further immunization. In alternative examplesaspects, the method comprises sacrificing the non-human animal and harvesting tissues from the non-human animal, when the percentage of ASCs producing select antibodies is at or above a threshold. In various instances, the steps of the method are carried out on a series of non-human animals and the method comprises profiling the B-cell repertoire of the blood sample for each non-

human animal of the series and selecting a subset of the series having a target B-cell profile. Figure 1E illustrates such steps.

[0015] Rational immune repertoire generation and selection is a critical component in animal-based antibody discovery technologies. Despite the advancement from traditional B-cell immortalization to direct B-cell platforms such as (but not limited to) NanOBLAST (an antibody discovery process on a nanofluidic Beacon® device) and microencapsulation, the diversity and quality of the input B-cells continues to be an essential determining factor in meeting antibody design goals. Traditional approaches to evaluate immune animals rely on the interrogation of the polyclonal secreted antibody (serum) to evaluate immune responses and select animals for B-cell harvest and antibody generation. The "serum titer" approach is less than ideal since it measures the total reactivity of all the secreted antibodies and not the quality of the individual B-cell source of the detected antibodies. The lack of a direct connection between the antibody titer measurement and the responsible B-cell source makes interpreting the quality of the B- cell response difficult. Aside from determining whether there is soluble, antigen-specific antibody in the serum, it is difficult to obtain additional useful information from this polyclonal analysis that can aid animal selection or immune steering strategies. Provided herein are ASC assays to interrogate the B-cell response of an immune animal using samples derived from non-terminal peripheral blood that would address these challenges. Accordingly, the present

disclosure provides a method of screening non-human animals for antibody secreting cells (ASCs) producing select antibodies. The method in exemplary examples~~embodiments~~ comprises (a) immunizing a series of non-human animals with an immunogen; (b) obtaining a blood sample comprising ASCs from each non-human animal of the series; and (c) individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies, wherein, for each non-human animal of the series, a percentage of ASCs producing select antibodies is determined. In various examples~~aspects~~, the screening method further comprises selecting the non-human animal(s) for sacrifice and/or tissue harvest, when the percentage of ASCs producing select antibodies is at or above a threshold. In various examples~~aspects~~, the screening method further comprises selecting the non-human animal(s) for subsequent immunization, when the percentage of ASCs producing select antibodies is below a threshold. Accordingly, in various examples~~embodiments~~, the screening method identifies animals for sacrifice vs. animals for subsequent immunization based on the percentage of ASCs producing select antibodies.

[0016] Consistent with the foregoing, methods of selecting immunized non-human animals for subsequent immunization are provided. In exemplary examples~~embodiments~~, the method comprises monitoring for the production of select antibodies in a non-human animal in accordance with any one of the presently disclosed methods, wherein the method is carried out on a series of non-human animals, wherein for each non-human animal of the series the number of ASCs producing the select antibodies is identified, and selecting the animal for subsequent immunization when the percentage of ASCs producing select antibodies for an animal is below a threshold. In another aspect, the present disclosure refers to a method of screening a series of non-human animals for antibody secreting cells (ASCs) producing select antibodies, said method comprising: monitoring for the production of select antibodies in a non-human animal in a series of non-human animals in accordance with the method as described herein, wherein for each non-human animal of the series the number of ASCs producing the select antibodies is identified. Also provided herein are methods of selecting immunized non-human animals for euthanasia and secondary lymphoid harvest. In exemplary examples~~embodiments~~, the method comprises monitoring for the production of select antibodies in a non-human animal in accordance with any one of the presently disclosed methods, wherein the method is carried out on a series of non-human animals, wherein for each non-human animal of the series the number of ASCs producing the select antibodies is identified, and selecting the animal for euthanasia and secondary lymphoid harvest, when the percentage of ASCs producing select antibodies for an animal is at or above a threshold. In another aspect, the present disclosure refers to a method of selecting immunized non-human animals for subsequent immunization,

said method comprising: monitoring for the production of select antibodies in a non-human animal in accordance with the method as described herein, wherein for each non-human animal, the number of ASCs producing the select antibodies is identified, and selecting the animal for subsequent immunization when the percentage of ASCs producing select antibodies for an animal is below a threshold. In another aspect, the present disclosure refers to a method of selecting immunized non-human animals for euthanasia and secondary lymphoid harvest, said method comprising: monitoring for the production of select antibodies in a non-human animal in accordance with the method as described herein, wherein for each non-human animal, the number of ASCs producing the select antibodies is identified, and selecting the animal for euthanasia and secondary lymphoid harvest, when the percentage of ASCs producing select antibodies for an animal is at or above a threshold.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1A is an illustration of a traditional transgenic mouse hybridoma generation method. Figure 1B is an illustration of a non-terminal method for monitoring immune response. Figure 1C is an illustration of a non-terminal method of monitoring for the production of select antibodies. Figure 1D is an illustration of a non-terminal method of guiding antibody production for select antibody production. Figure 1E is an illustration of a non-terminal method of screening animals and B-cell profiling.

WHAT IS CLAIMED IS:

1. A method of guiding antibody production in a non-human animal for the production of select antibodies, said method comprising:

a. performing an initial immunization on a non-human animal with an immunogen;

b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal;

c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; and

d. performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises:

i. performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold,

ii. obtaining a blood sample comprising ASCs from said non-human animal,

iii. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies.

2~~1~~. A method of monitoring for the production of select antibodies in a non-human animal, said method comprising

a. immunizing a non-human animal with an immunogen;

b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal; and

c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies.

~~2. A method of guiding antibody production in a non-human animal for the production of select antibodies, said method comprising:~~

~~a. performing an initial immunization on a non-human animal with an immunogen;~~

~~b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal;~~

~~c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies; and~~

~~d. performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises:~~

~~i. performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold,~~

~~ii. obtaining a blood sample comprising ASCs from said non-human animal,~~

~~iii. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select a~~

3. The method of claim 1 or 2, wherein:

(A) the assaying comprises a single-cell, live-cell assay, optionally~~.~~

~~4. The method of claim 3~~, wherein multiple ASCs are simultaneously assayed; and/or

(B) ~~.5. The method of any one of~~ the method comprises~~preceding claims, comprising~~ applying the blood sample, or a fraction thereof, to a matrix and assigning a unique address of the matrix to each ASC, optionally~~.~~

~~6. The method of claim 5~~, wherein a result of the assaying is the identification of each ASC producing select antibodies, and further optionally~~.~~

~~7. The method of claim 6~~, wherein the result comprises the identification of the unique address of each ASC producing select antibodies.

4~~8~~. The method of any one of claims 1 and 3~~2 7~~, wherein:

(A) the cycle is carried out at least one time~~.~~, optionally

~~9. The method of claim 8~~, wherein the cycle is repeated until the number of ASCs producing select antibodies, as assayed in (iii), is at or above the threshold, and further optionally~~.~~

~~10. The method of claim 9~~, wherein the cycle is repeated at least two times~~.~~; and/or

(B) ~~11. The method of any one of claims 2-10, wherein~~ the immunogen of the subsequent immunization is different from the immunogen of the initial immunization.

5~~12~~. The method of any one of claims 1, 3, and 4 ~~2-11~~, wherein:
(I) ~~-~~each subsequent immunization differs from a prior immunization in that (A) a different immunogen, adjuvant, and/or immunomodulatory agent is administered to the non-human animal, (B) a different dose of the immunogen is administered to the non-human animal, (C) the time between each administration of the immunogen, adjuvant, immunomodulatory agent is different, and/or (D) the route of administration for each administration of immunogen, adjuvant, immunomodulatory agent is different and/or
~~.~~

(II)~~13. The method of any one of claims 2-12, wherein~~ a different immunogen is used each time the non-human animal is immunized.

6~~14~~. A method of producing select antibodies in a non-human animal, comprising

a. performing an initial immunization on a non-human animal with an immunogen;

b. obtaining a blood sample comprising antibody secreting cells (ASCs) from said non-human animal;

c. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies;

d. performing a cycle of steps when the percentage of ASCs producing select antibodies is below a threshold, wherein the cycle comprises:

i. performing a subsequent immunization on the non-human animal with an immunogen when the percentage of ASCs producing select antibodies is below a threshold,

ii. obtaining a blood sample comprising ASCs from said non-human animal,

iii. individually assaying ASCs present in the blood sample, or a fraction thereof, for the production of select antibodies, and

e. isolating the select antibodies and/or an ASC producing the select antibodies.

7~~15~~. The method of claim 6~~14~~, comprising determining the nucleotide sequence encoding the heavy chain variable region of the select antibodies produced by an ASC and the nucleotide sequence encoding the light chain variable region of the select antibodies produced by the ASC, introducing into a host cell a first vector comprising the nucleotide sequence encoding the heavy chain variable region of the select antibodies and a second vector comprising the nucleotide sequence encoding the light chain variable region of the select antibodies, and isolating the antibodies produced by the host cell.

8~~16~~. The method of any one of the preceding claims, wherein the assaying comprises:

a. combining the ASCs within the matrix with (i) a capture reagent which binds to the select antibodies and comprises a solid support, (ii) a detection reagent which binds to the select antibodies and comprises a first detectable label, and (iii) a labeled target to which the select antibodies bind, wherein the labeled target comprises a second detectable label distinct from the first detectable label;

b. assaying for the first detectable label and for the second detectable label; and;

c. identifying the positions within the matrix at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies.

9~~17~~. The method of claim 8~~16~~, wherein

(A) the capture agent comprises an antibody that binds to an antibody Fc domain attached to a solid support~~.~~;

~~18.~~ (B) ~~The method of claim 16 or 17, wherein~~ the detection agent comprises an antibody that binds to an antibody Fc domain attached to a first detectable label, optionally~~.~~

~~19. The method of claim 18~~, wherein the antibody that binds to an antibody Fc domain of the capture agent is the same antibody of the detection agent~~.~~; and/or

(C) ~~20. The method of any one of claims 16-19, wherein~~ the combining takes place in a well and the capture agent forms a monolayer in the well, optionally, wherein the ASCs are first exposed to the capture reagent, detection reagent, and/or labeled target in the well or immediately prior to being added to the well, optionally~~.~~

~~21. The method of claim 20~~, wherein the method comprises identifying the positions within the well at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies.

10~~22~~. The method of ~~any one of~~ claim~~s~~ 8~~16~~ or ~~19~~9, wherein the combining takes place in a microfluidic or nanofluidic chamber, a microwell or nanowell device, a microcapillary or nanocapillary tube, or a nanopen of a nanofluidic chip~~.~~, optionally

~~23. The method of claim 22~~, wherein the combining takes place in a nanopen of a nanofluidic chip.

11~~24~~. The method of claim 10~~23~~, wherein:

(A) the method comprises identifying the position of each pen within the nanofluidic chip at which both the first detectable label and the second detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies~~.~~; and/or

(B) ~~25. The method of claim 23 or 24, wherein~~ a single ASC of the blood sample is moved into a pen of the nanofluidic chip through optoelectro positioning (OEP).

12~~26~~. The method of any one of the preceding claims, wherein:
(A) the select antibodies bind to a target which is the same as or similar to the immunogen used to immunize the non-human animal, optionally~~.~~

~~27. The method of claim 26~~, wherein the select antibodies bind to the target in the presence of one or more competitive binding agents and further optionally~~.~~

~~28. The method of claim 27~~, wherein the competitive binding agents are combined with the ASCs, capture reagent, detection reagent, and labeled target during the assaying~~.~~;

(B)~~29. The method of any one of the preceding claims, wherein~~ the select antibodies bind to a target with a target affinity, optionally, wherein the K_D of the select antibodies for the target is about 10^{-11} M to about 10^{-9} M, optionally~~.~~

~~30. The method of claim 29~~, wherein the assaying is carried out in a first round with a first amount of the labeled target and a second round with a second amount of the labeled target, wherein the first amount is greater than the second amount, optionally, wherein the assaying is further carried out in a third round with a third amount of the labeled target and the third amount is less than the second amount, wherein when the ASC binds to the labeled target in each round, the ASC produces select antibodies~~.~~;

(C)~~31. The method of any one of the preceding claims, wherein~~ the select antibodies bind to a target and to an ortholog or paralog thereof, optionally, wherein the target is a human protein and the ortholog is a cynomolgus monkey protein, optionally~~.~~

~~32. The method of claim 31~~, wherein a second labeled target is combined with the ASCs, capture reagent, detection reagent, and labeled target, wherein the second labeled target comprises the ortholog attached to a third detectable label which is distinct from the first detectable label and the second detectable label, wherein the method further comprises assaying for the third detectable label and identifying the position(s) at which the first detectable label, the second detectable label, and the third detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies~~.~~;

(D) ~~33. The method of any one of the preceding claims, wherein~~ the select antibodies bind to a target and not to an ortholog or paralog thereof, optionally~~.~~

~~34. The method of claim 33~~, wherein a second labeled target is combined with the ASCs, capture reagent, detection reagent, and labeled target, wherein the second labeled target comprises the ortholog attached to a third detectable label which is distinct from the first detectable label and the second detectable label, wherein the method further comprises assaying for the third detectable label and identifying the position(s) at which only the first detectable label and the second detectable label, but not the third detectable label, are detected, wherein each identified position locates an individual ASC producing select antibodies~~.~~;

(E) ~~35. The method of any one of the preceding claims, wherein~~ the select antibodies bind to a portion of the target, optionally~~.~~

~~36. The method of claim 35~~, wherein a second labeled target is combined with the ASCs, capture reagent, detection reagent, and labeled target, wherein the ~~second~~ labeled target comprises the portion of the target attached to a third detectable label which is distinct from the first detectable label and the second detectable label, 6565erein~~and wherein~~ the method further comprises assaying for the third detectable label and identifying the position(s) at which the first detectable label, the second detectable label, and the third detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies~~.~~; and/or

~~37. The method of claim 36, wherein the target is a protein comprising multiple domains and the select antibodies bind to only one domain of the target, wherein the labeled target comprises the extracellular domain of the target attached to the second detectable label and the second labeled target comprises the one domain attached to third detectable label.~~

(F) ~~38. The method of any one of the preceding claims, wherein~~ the select antibodies bind to a conformational epitope formed upon dimerization or multimerization of the target and the target comprises a dimerization domain or multimerization domain, optionally~~.~~

~~39. The method of claim 38~~, wherein the labeled target comprises the extracellular domain of the immunogen attached to the second detectable label, wherein a second labeled target is combined with the ASCs, capture reagent, detection reagent, and labeled target, wherein the second labeled target comprises the dimerization domain or multimerization domain of the immunogen attached to the third detectable label which is distinct from the first detectable label and the second detectable label, and wherein the method further comprises assaying for the third detectable label and identifying the position(s) at which the first detectable label, the second detectable label, and the third detectable label are detected, wherein each identified position locates an individual ASC producing select antibodies.

13. The method of claim 12(E), wherein the target is a protein comprising multiple domains and the select antibodies bind to only one domain of the target, wherein the labeled target comprises the extracellular domain of the target attached to the second detectable label and the second labeled target comprises the one domain attached to third detectable label.

14~~40~~. The method of any one of the preceding claims, wherein the blood sample is obtained from the non-human animal about 3 to about 7 days after the immunizing step.

15~~41~~. The method of any one of the preceding claims, wherein:

(A) the blood sample obtained from the non-human animal is less than or about 500 µL~~.~~;

(B) the blood sample obtained from the non-human animal is less than or about 500 µL; optionally~~42. The method of claim 41~~, wherein the blood sample is about 100 µL to about 250 µL~~.~~;

(C) ~~43. The method of any one of the preceding claims, wherein~~ the ASCs are CD138+ B cells~~.~~;

(D) ~~44. The method of any one of the preceding claims, wherein~~ the ASCs comprise migratory plasmablasts~~.~~;

(E) ~~45. The method of any one of~~ the method ~~preceding claims,~~ further comprises~~comprising~~ removing one or more components of the blood sample obtained from the non-human animal prior to assaying, optionally~~.~~

~~46. The method of claim 45~~, wherein red blood cells, plasma, and/or platelets are removed from the blood sample and/or~~.~~

~~47. The method of claim 45 or 46, wherein~~ the fraction of the blood sample is prepared by selecting for $CD138^+$ cells~~.~~;

(F) ~~48. The method of any one of the preceding claims, wherein~~ the non-human animal is subjected to neither removal of one or more secondary lymphoid organs nor euthanasia~~.~~; and/or

(G) ~~49. The method of any one of the preceding claims, wherein~~ ASCs from the blood sample are not used in making hybridomas.

16~~50~~. The method of any one of claims ~~2~~1 and 3 to ~~49~~13, wherein:

(A) the non-human animal is one of a series of non-human animals, and an outcome of the assaying is the identification of the non-human animals having a number of ASCs producing select antibodies below the threshold and/or requiring further immunization~~.~~; and/or

(B) ~~51. The method of any one of claims 2 to 50, wherein~~ the steps of the method are carried out on a series of non-human animals and the method comprises profiling the B-cell repertoire of the blood sample for each non-human animal of the series and selecting a subset of the series having a target B-cell profile.

17~~52~~. The method of any one of the preceding claims, comprising sacrificing the non-human animal and harvesting tissues from the non-human animal, when the percentage of ASCs producing select antibodies is at or above a threshold~~.~~, optionally, wherein

~~53. The method of claim 52,~~ the method comprises~~comprising~~ harvesting the spleen from the non-human animal~~.~~ and, optionally

~~54. The method of claim 53,~~ further comprises~~comprising~~ screening for B-cells of the spleen and/or generating hybridomas from cells of the spleen.

18~~55~~. A method of screening a series of non-human animals for antibody secreting cells (ASCs) producing select antibodies, said method comprising:

monitoring for the production of select antibodies in a non-human animal in a series of non-human animals in accordance with the method of any one of the preceding claims,

wherein for each non-human animal of the series the number of ASCs producing the select antibodies is identified.

19~~56~~. The method of claim 16~~55~~, wherein when the percentage of ASCs producing select antibodies for an animal is below a threshold, the method comprises performing a subsequent immunization.

20~~57~~. The method of claim 17~~56~~, wherein when the percentage of ASCs producing select antibodies for an animal is at or above a threshold, the method further comprises harvesting secondary lymphoid organs from the animal.

21~~58~~. A method of selecting immunized non-human animals for subsequent immunization, said method comprising:

monitoring for the production of select antibodies in a non-human animal in accordance with the method of any one of the preceding claims,

wherein for each non-human animal, the number of ASCs producing the select antibodies is identified, and

selecting the animal for subsequent immunization when the percentage of ASCs producing select antibodies for an animal is below a threshold.

22~~59~~. A method of selecting immunized non-human animals for euthanasia and secondary lymphoid harvest, said method comprising:

monitoring for the production of select antibodies in a non-human animal in accordance with the method of any one of the preceding claims,

wherein for each non-human animal, the number of ASCs producing the select antibodies is identified, and

selecting the animal for euthanasia and secondary lymphoid harvest, when the percentage of ASCs producing select antibodies for an animal is at or above a threshold.

23~~60~~. A method of assaying for ASCs producing select antibodies, said method comprising:

a. combining in a well (i) a blood sample obtained from a non-human animal immunized with an immunogen, or a fraction thereof, wherein the blood sample comprises antibody secreting cells (ASCs), (ii) a detection reagent which binds to the select antibodies and comprises a first detectable label, and (iii) a target to which the select antibodies bind,

wherein:

(A) the target is a labeled target comprising a second detectable label distinct from the first detectable label and a capture reagent which binds to the select antibodies and comprises a solid support is further combined in the well to form a monolayer in the well,

or

(B) the target is expressed on the surface of cells and the cells are combined in the well to form a monolayer in the well,

b. assaying for the first detectable label and optionally assaying for the second detectable label, when the target is a labeled target;

c. identifying the positions within the well at which the first detectable label is detected or the first and second detectable labels are detected, wherein each identified position locates an individual ASC producing select antibodies.

~~61. The method of claim 60, wherein the ASCs are first exposed to the detection reagent and/or target in the well or immediately prior to being added to the well.~~

~~62. The method of claim 60 or 61 wherein the select antibodies bind to a target which is the same as or similar to the immunogen used to immunize the non-human animal.~~

~~63. The method of any one of claims 60-62, wherein the detection reagent comprises an antibody that binds to an antibody Fc domain attached to a solid support and/or the detection agent comprises an antibody that binds to an antibody Fc domain attached to a first detectable label~~

~~64. The method of claim 63, wherein the antibody that binds to an antibody Fc domain of the capture agent is the same antibody of the detection agent.~~

~~65. The method of any one of claims 60-64, wherein the blood sample is obtained from the non-human animal about 3 to about 7 days after the immunizing step.~~

~~66. The method of any one of the preceding claims, wherein the blood sample obtained from the non-human animal is less than or about 500 µL, optionally, about 100 µL to about 250 µL67.The method of any one of claims 60-66, wherein the ASCs are CD138+ B cells.~~

68. The method of any one of claims 60-67, wherein the ASCs comprise migratory plasmablasts.

69. The method of any one of claims 60-68, further comprising removing one or more components of the blood sample obtained from the non-human animal prior to combining in the well.

70. The method of claim 69, wherein red blood cells, plasma, and/or platelets are removed from the blood sample.

71. The method of claim 69 or 70, wherein the fraction of the blood sample is prepared by selecting for CD138+ cells.

72. The method of any one of claims 60-71, wherein the select antibodies bind to the target in the presence of one or more competitive binding agents.

73. The method of claim 72, wherein the competitive binding agents are combined with the ASCs, detection reagent, and cells expressing the target during the assaying.

74. The method of any one of claims 60-73, wherein the select antibodies bind to a target with a target affinity, optionally, wherein the KD of the select antibodies for the target is about 10-11 M to about 10-9 M.

75. The method of claim 74, wherein the assaying is carried out in a first round with a first amount of cells expressing the target and a second round with a second amount of the cells expressing the target, wherein the first amount is greater than the second amount, optionally, wherein the assaying is further carried out in a third round with a third amount of the cells expressing the target and the third amount is less than the second amount, wherein when the ASC binds to the labeled target in each round, the ASC produces select antibodies.

76. The method of any one of claims 60-75, wherein the select antibodies bind to a target and to an ortholog or paralog thereof, optionally, wherein the target is a human protein and the ortholog is a cynomolgus monkey protein.

77. The method of claim 76, wherein the cells express the target and the ortholog or paralog thereof.

78. The method of any one of claims 60-77, wherein the select antibodies bind to a target and not to an ortholog or paralog thereof.

79. The method of any one of claims 60-78, wherein the select antibodies bind to a portion of the target.

80. The method of claim 79, wherein the target is a protein comprising multiple domains and the select antibodies bind to only one domain of the target, wherein the labeled target comprises the extracellular domain of the target attached to the second

~~detectable label and the second labeled target comprises the one domain attached to third detectable label.~~

~~81. The method of any one of claims 60-80, wherein the select antibodies bind to a conformational epitope formed upon dimerization or multimerization of the target and the target comprises a dimerization domain or multimerization domain.~~