places where variable (V), diverse (D), and joining (J) segments join to form the complementarity determining regions, including CDR1, CDR2, and CDR3, which provide specificity to the TCRs and/or BCRs, is important in understanding the TCR and BCR biological interactions. By coupling clonal information to spatial information it is possible to understand which T-cell and B-cell clonotypes may be specifically interacting with given cell types within a biological sample.

In one aspect, the present disclosure refers to a method for determining the presence of an immune cell clonotype at a location in a biological sample, the method comprising:

(a) contacting the biological sample with an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that hybridizes to a nucleic acid encoding an immune cell receptor of the immune cell clonotype;

(b) extending the capture probe using the nucleic acid encoding the immune cell receptor as a template to generate an extended capture probe;

(c) hybridizing one or more probes to the extended capture probe, or a complement thereof, in a portion encoding a constant region of the immune cell receptor of the immune cell clonotype,

(d) enriching the extended capture probe or the complement thereof; and

(e) determining (i) the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor of the immune cell clonotype or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence of the immune cell clonotype at a location in the biological sample.

Provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample. Some examples of any of the methods described herein include capturing transcripts to identify an immune cell clonotype. Some examples of any of the methods herein include generating a nucleic acid library from captured transcripts. Some examples of any of the methods described herein include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell clonotype.

Provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample. Some examples of any of the methods described herein include capturing analytes to identify an immune cell receptor. Some examples of any of the methods described herein include generating a nucleic acid library from captured analytes. Some examples of any of the methods described here include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell receptor.

Thus provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method including: (a) contacting a biological sample with an array including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of the immune cell clonotype; and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.

In some examples, the immune cell clonotype is a T cell clonotype. In some examples, the T cell clonotype is a T cell receptor alpha chain. In some examples, the capture domain binds to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence

encoding CDR3 of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

In some examples, the immune cell receptor is a T cell receptor beta chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the T cell receptor beta chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor beta chain.

In some examples, the immune cell clonotype is a B cell clonotype. In some examples, the B cell clonotype is an immunoglobulin kappa light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain. In some examples, the B cell clonotype is an immunoglobulin lambda light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain. In some examples, the B cell clonotype is an immunoglobulin heavy chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

In some examples, the capture domain binds a poly(A) sequence of a nucleic acid encoding an immune cell clonotype. In some examples, the capture domain binds to a nucleic acid sequence encoding a T cell clonotype. In some examples, the T cell clonotype is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of the T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding a full-length variable domain of the T cell receptor alpha chain, a sequence encoding a full-length variable domain of the T cell receptor beta chain, and combinations thereof.

In some examples, the capture domain binds to a nucleic acid encoding a B cell clonotype. In some examples, the B cell clonotype is an immunoglobulin kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

In some examples, step (b) includes the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe. In some examples, step (b) includes extending a 3’ end of the capture probe.

In some examples, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some examples, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof. In some examples, the capture probe includes a functional domain.

In some examples, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some examples, the method includes enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype.

In some examples, enriching includes hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor of the immune cell clonotype, where a hybridization probe includes (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a capture moiety.

In some examples, the binding moiety includes biotin and the capture moiety includes streptavidin.

In some examples, enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype includes one or more blocking probes. In some examples, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 639. In some examples, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 640.

In some examples, the method includes amplifying the nucleic acid encoding the immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) a first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode, and (ii) a second primer including a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

In some examples, the method includes amplifying the nucleic acid encoding the immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) the first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode, and (ii) a third primer including a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, where the third primer is 5' to the second primer.

In some examples, the biological sample includes a tissue sample. In some examples, the tissue sample is a tissue section. In some examples, the tissue section is a fixed tissue section. In some examples, the fixed tissue section is a formalin-fixed paraffin-embedded tissue section. In some examples, the tissue section includes a tumor region.

In some examples, the nucleic acid encoding the immune cell receptor includes RNA. In some examples, the RNA is mRNA. In some examples, the nucleic acid encoding the immune cell receptor includes DNA. In some examples, the DNA is genomic DNA.

In some examples, the method includes imaging the biological sample.

In some examples, the determining in step (b) includes sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

In some examples, step (b) includes determining the presence of the immune cell clonotype at a location in the biological sample. In some examples, step (b) includes determining the abundance of the immune cell clonotype at a location in the biological sample. In some examples, step (b) includes determining the presence and abundance of the immune cell clonotype at a location in the biological sample. In some examples, step (b) includes determining the presence of two or more immune cell clonotypes at a location in the biological sample. In some examples, step (b) includes determining the abundance of two or more immune cell clonotypes at a location in the biological sample. In some examples, step (b) includes determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample. In some examples, the method includes comparing the two or more immune cell clonotypes. In some examples, the two or more immune cell clonotypes are each a B cell clonotype.

In some examples, the two or more immune cell clonotypes are each a T cell clonotype. In some examples, the two or more immune cell clonotypes comprise at least one T cell clonotype and at least one B cell clonotype.

Also provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method including: (a) contacting a biological sample with an array including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor;

and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the biological sample.

In some examples, the immune cell receptor is a T cell receptor alpha chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain. In some examples, the immune cell receptor is a T cell receptor beta chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the T cell receptor beta chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain. In some examples, step (b) includes determining a full-length variable domain of the T cell receptor beta chain. In some examples, the immune cell receptor is an immunoglobulin kappa light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain. In some examples, the immune cell receptor is an immunoglobulin lambda light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain. In some examples, the immune cell receptor is an

immunoglobulin heavy chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some examples, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

In some examples, the capture domain binds a poly(A) sequence of a nucleic acid encoding an immune cell receptor. In some examples, the immune cell receptor is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of the T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding a full-length variable domain of the T cell receptor alpha chain, a sequence encoding a full-length variable domain of the T cell receptor beta chain, and combinations thereof.

In some examples, the immune cell receptor is a B cell receptor an immunoglobulin kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain, and combinations thereof. In some examples, step (b) includes determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

In some examples, step (b) includes extending an end of the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an

extended capture probe. In some examples, step (b) includes extending a 3' end of the capture probe.

In some examples, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some examples, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof. In some examples, the capture probe includes a functional domain.

In some examples, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some examples, the method includes enriching the nucleic acid encoding the immune cell receptor. In some examples, enriching includes hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor, where a hybridization probe includes (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a capture moiety. In some examples, the binding moiety includes biotin and the capture moiety includes streptavidin. In some examples, enriching the nucleic acid encoding the immune cell receptor of the immune cell receptor includes one or more blocking probes. In some examples, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 639. In some examples, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 640.

In some examples, the method includes amplifying the nucleic acid encoding an immune cell receptor, or a complement thereof, using (i) a first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer including a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

In some examples, the method includes amplifying the nucleic acid encoding the immune cell receptor, or a complement thereof, using (i) the first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode,

and (ii) a third primer including a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, where the third primer is 5' to the second primer.

In some examples, the biological sample includes a tissue sample. In some examples, the tissue sample is a tissue section. In some examples, the tissue section is a fixed tissue section. In some examples, the fixed tissue section is a formalin-fixed paraffin-embedded tissue section. In some examples, the tissue section includes a tumor region.

In some examples, the nucleic acid encoding the immune cell receptor includes RNA. In some examples, the RNA is mRNA. In some examples, the nucleic acid encoding the immune cell receptor includes DNA. In some examples, the DNA is genomic DNA.

In some examples, the method includes, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

In some examples, the method includes imaging the biological sample.

In some examples, the determining in step (b) includes sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

In some examples, step (b) includes determining the presence of the immune cell receptor at a location in the biological sample. In some examples, step (b) includes determining the abundance of the immune cell receptor at a location in the biological sample. In some examples, step (b) includes determining the presence and abundance of the immune cell receptor at a location in the biological sample. In some examples, step (b) includes determining the presence of two or more immune cell receptors at a location in the biological sample. In some examples, step (b) includes determining the abundance of two or more immune cell receptors at a location in the biological sample. In some examples, step (b) includes determining the presence and abundance of two or more immune cell receptors at a location in the biological sample. In some examples, the method includes comparing the two or more immune cell receptors. In some examples, the two or more immune cell clonotypes are each an immune cell receptor of a B cell. In some examples, the two or more immune cell clonotypes are each an immune cell receptor of a T cell. In some examples, the two or more immune cell clonotypes comprise at least one immune cell receptor of a T cell and at least one immune cell receptor from a B cell.

Also provided herein are arrays including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of an immune cell clonotype.

In some examples, the immune cell clonotype is a T cell clonotype. In some examples, the immune cell receptor is a T cell receptor alpha chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some examples, the immune cell receptor is a T cell receptor beta chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

In some examples, the immune cell clonotype is a B cell clonotype. In some examples, the immune cell receptor is an immunoglobulin kappa light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some examples, the immune cell receptor is an immunoglobulin lambda light chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some examples, the immune cell receptor is an immunoglobulin heavy chain. In some examples, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

In some examples, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

Also provided herein are kits any one of the arrays described herein; one or more hybridization probes, where a hybridization probe includes (i) a sequence substantially complementary to a nucleic acid encoding an immune cell receptor and (ii) a binding moiety that interacts with a capturing moiety; and one or more blocking probes.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or item of information was specifically and individually indicated to be incorporated by reference. To the extent publications, patents, patent applications, and items of information incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

Where values are described in terms of ranges, it should be understood that the description includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

The term "each," when used in reference to a collection of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection, unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

Various examples of the features of this disclosure are described herein. However, it should be understood that such examples are provided merely by way of example, and numerous variations, changes, and substitutions can occur to those skilled in the art without departing from the scope of this disclosure. It should also be understood that various alternatives to the specific examples described herein are also within the scope of this disclosure.

DESCRIPTION OF DRAWINGS

The following drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner. Like reference symbols in the drawings indicate like elements.

FIG. 1 is a schematic diagram showing an example of a barcoded capture probe, as described herein.

FIG. 2 shows an exemplary workflow for spatial transcriptomics for antigen-receptors.

FIG. 3A shows an exemplary capture probe with a capture sequence complementary to a constant region of an analyte.

FIG. 3B shows an exemplary capture probe with a poly(dT) capture domain.

FIG. 4 shows an exemplary enrichment strategy with a Read1 primer and a primer(s) complementary to a variable region of an analyte.

FIG. 5 shows an exemplary sequencing strategy with a ligated sequencing handle (P5) and a custom sequencing primer complementary to a portion of the constant region of an analyte.

FIG. 6 shows a graph showing the number of unique clonotypes detected from lymph node (LN) and spleen (SP) tissues.