bias and sequencing error, with the consequences that clonotype abundances can be drastically distorted and that non-existent clonotypes can be recorded, thus falsely increasing the observed diversity. Moreover, selection and/or synthesis of cognate pairs of the TCR chains from the sequencing data for downstream applications such as cloning, functional study and therapeutic use can be time consuming. And the library of selected and synthesized cognate pairs of the TCR chains is small or has low diversity.

SUMMARY ~~OF THE INVENTION~~

[0003a] In one aspect, provided herein is a method of identifying a plurality of target-reactive T-cell receptors (TCRs), comprising:

(a) providing a mixture comprising a plurality of cells expressing a plurality of TCRs, each cell of the plurality of cells expressing a TCR of the plurality of TCRs, wherein the plurality of TCRs comprises at least 50 different cognate pairs from a sample from a subject and comprises V regions from a plurality of V genes, wherein each cognate pair of the at least 50 different cognate pairs comprises a TCR alpha (TCRα) chain paired with a TCR beta (TCRβ) chain, and wherein the plurality of TCRs are exogenous to the plurality of cells;

(b) contacting the mixture comprising the plurality of cells from (a) with one or more target peptide antigens in complex with a major histocompatibility complex (MHC) molecule, wherein a subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target peptide antigens in complex with the MHC molecule; and

(c) identifying at least two cells of the subset of the plurality of cells from (b) that express at least two target-reactive TCRs of the plurality of target-reactive TCRs, thereby identifying the at least two target-reactive TCRs of the plurality of target-reactive TCRs.

[0003b] In another aspect, provided herein is use of at least one of the at least two cells identified in (c) as described herein or a cell expressing a TCR of at least one of the at least two cells identified in (c) as described herein for the manufacture of a medicament for the treatment of cancer or auto-immune disease in a subject.

[0003c] In another aspect, provided herein is at least one of the at least two cells identified in (c) as described herein or a cell expressing a TCR of at least one of the at least two cells identified in (c) as described herein for use in therapy.

[0004] Provided herein are compositions and methods for high throughput cloning of natively paired TCRs which can be used for various applications. The compositions and methods provided herein can surpass sequencing and synthesis and provide fused nucleic acid molecules encoding cognate pairs of TCRs for direct expression in host cells. The compositions and methods can also be applied for other bipartite immunoreceptors, such as B-cell receptors (BCRs).

[0005] According to an ~~aspect~~example, provided herein is a composition comprising a plurality of fused T-cell receptor (TCR) polynucleotides, wherein each fused TCR polynucleotide of the plurality comprises: a first nucleic acid sequence and a second nucleic acid sequence, wherein (1)

the first nucleic acid sequence encodes a first variable domain of a first TCR peptide chain, wherein the first variable domain comprises a CDR2 and a CDR3, and (2) the second nucleic acid sequence encodes a second variable domain of a second TCR peptide chain, wherein the second variable domain comprises a CDR2 and a CDR3; wherein the first and the second nucleic acid sequence of each fused TCR polynucleotide encode a cognate pair of the first and the second TCR peptide chain from an immune cell; wherein the plurality of fused TCR polynucleotides encode at least 50 different cognate pairs; and wherein the plurality of fused TCR polynucleotides comprise V regions from at least 5, 10, or 20 different V genes. In some ~~embodiment~~examples, the first TCR peptide chain is a T-cell receptor (TCR) alpha peptide chain, and the second TCR peptide chain is a TCR beta peptide chain. In some ~~embodiment~~examples, the first TCR peptide chain is a TCR gamma peptide chain, and the second TCR peptide chain is a TCR delta peptide chain. In some ~~embodiment~~examples, the first variable domain further comprises a CDR1, and/or the second variable domain further comprises a CDR1. In some ~~embodiment~~examples, the first variable domain of the first TCR peptide chain is a first full-length variable domain comprising FR1, CDR1, FR2, CDR2, FR3, and CDR3, and/or the second variable domain of the second TCR peptide chain is a second full-length variable domain comprising FR1, CDR1, FR2, CDR2, FR3, and CDR3. In some ~~embodiment~~examples, the first nucleic acid sequence further encodes a first constant domain or a portion thereof of the first TCR peptide chain and/or the second nucleic acid sequence further encodes a second constant domain or a portion thereof of the second TCR peptide chain. In some ~~embodiment~~examples, the first constant

domain is a first extracellular domain, and/or the second constant domain is a second extracellular domain. In some ~~embodiment~~examples, the first constant domain comprises a first extracellular domain, a first hinge region, a first transmembrane region and a first cytoplasmic tail of the first TCR chain, and/or the second constant domain comprises a second extracellular domain, a second hinge region, a second transmembrane region and a second cytoplasmic tail of the second TCR peptide chain. In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality is at least 800, at least 900, at least 1000, or at least 1500 base pairs in length. In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality is at least 1000, at least 1500, or at least 2000 base pairs in length. In some ~~embodiment~~examples, the first nucleic acid sequence and the second nucleic acid sequence are obtained or released from an immune cell.

[0006] In some ~~embodiment~~examples, the immune cell is isolated from a sample. In some ~~embodiment~~examples, the sample is obtained from a subject. In some ~~embodiment~~examples, the immune cell is a lymphocyte. In some ~~embodiment~~examples, the lymphocyte is a T cell. In some ~~embodiment~~examples, the T cell is an inflammatory T cell, a cytotoxic T cell, a regulatory T cell, a helper T cell, a natural killer T cell, or a combination thereof. In some ~~embodiment~~examples, the T cell is a CD4+ T cell or a CD8+ T cell. In some ~~embodiment~~examples, the immune cell is expanded in vitro. In some ~~embodiment~~examples, the sample is a blood cell sample, a bone marrow sample, a cord blood sample, an ascites sample, a pleural effusion sample, a cerebrospinal sample, a seminal fluid sample, a sputum sample, a urine sample, a stool sample, or a combination thereof. In some ~~embodiment~~examples, the sample is a tissue sample obtained from brain, liver, lung, kidney, prostate, ovary, spleen, lymph node, tonsil, thyroid, pancreas, heart, skeletal muscle, intestine, larynx, esophagus, thymus, stomach, tumor, site of infection, or a combination thereof. In some ~~embodiment~~examples, the subject is a mammal. In some ~~embodiment~~examples, the mammal is a human, a dog, a cat, a mouse, or a rat. In some ~~embodiment~~examples, the subject is a healthy subject or a diseased subject. In some ~~embodiment~~examples, the immune cell is isolated from the sample by a marker. In some ~~embodiment~~examples, the marker is a cell surface marker. In some ~~embodiment~~examples, the cell surface marker is CD39, CD69, CD103, CD25, PD-1, TIM-3, OX-40, 4-1BB, CD137, CD3, CD28, CD4, CD8, CD45RA, and CD45RO, GITR, FoxP3, or a combination thereof. In some ~~embodiment~~examples, the marker is a cytokine. In some ~~embodiment~~examples, the cytokine is IFN-γ, TNF-alpha, IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, perforin, or a combination thereof.

[0007] In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality further comprises a promoter. In some ~~embodiment~~examples, the promoter is constitutive or inducible. In some ~~embodiment~~examples, the promoter is a tetracycline-responsive promoter. In some ~~embodiment~~examples, the promoter is a viral promoter. In some ~~embodiment~~examples, the promoter is a β-actin promoter, a SV40

early promoter, a SV40 late promoter, an immunoglobulin promoter, a cytomegalovirus promoter, a retrovirus promoter, a Friend spleen focus-forming virus promoter, a Herpes virus TK promoter, a Rous sarcoma virus promoter; a mouse mammary tumor virus promoter, a metallothionein promoter, an adenovirus late promoter, a vaccinia 7.5K promoter, or an enolase promoter. In some ~~embodiment~~examples, the first nucleic acid and the second nucleic acid are fused in-frame such that expression of the first variable domain and the second variable domain is under control of one promoter. In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality further comprises a sequence encoding a protease cleavage site. In some ~~embodiment~~examples, the protease cleavage site is a cellular protease cleavage site or a viral protease cleavage site. In some ~~embodiment~~examples, the protease cleavage site is an enterokinase cleavage site, a factor Xa cleavage site, a thrombin cleavage site, a renin cleavage site, a collagenase cleavage site, a trypsin cleavage site, a caspase protease cleavage site, a furin cleavage site, a PC5/6 protease cleavage site; a PACE protease cleavage site, a LPC/PC7 protease cleavage site, a Factor Xa protease cleavage site, a genenase I cleavage site, a MMP protease cleavage site, or a KEX2 protease cleavage site. In some ~~embodiment~~examples, the protease cleavage site is a viral 2A protease cleavage site, a viral 3C protease cleavage site, an infectious pancreatic necrosis virus (IPNV) VP4 protease cleavage site, a tobacco etch virus (TEV) protease cleavage site, or a nuclear inclusion protein a (N1a) of turnip mosaic potyvirus cleavage site. In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality comprises a sequence encoding a self-cleaving peptide. In some ~~embodiment~~examples, the self-cleaving peptide is an intein peptide, a hedgehog peptide, or a 2A peptide. In some ~~embodiment~~examples, the at least 20 different V genes comprise at least 10 different TRAV genes and/or at least 10 different TRBV genes. In some ~~embodiment~~examples, the TRAV genes and TRBV genes are human or mouse TRAV genes and TRBV genes.

[0008] In some ~~embodiment~~examples, the at least 10 different TRAV genes are selected from the group consisting of human TRAV1-1, TRAV1-2, TRAV2, TRAV3, TRAV4, TRAV5, TRAV6, TRAV7, TRAV8-1, TRAV8-2, TRAV8-3, TRAV8-4, TRAV8-6, TRAV9-1, TRAV9-2, TRAV10, TRAV12-1, TRAV12-2, TRAV12-3, TRAV13-1, TRAV13-2, TRAV14, TRAV16, TRAV17, TRAV18, TRAV19, TRAV20, TRAV21, TRAV22, TRAV23, TRAV24, TRAV25, TRAV26-1, TRAV26-2, TRAV27, TRAV29, TRAV30, TRAV34, TRAV35, TRAV36, TRAV38-1, TRAV38-2, TRAV39, TRAV40, TRAV41. In some ~~embodiment~~examples, the at least 10 different TRBV genes are selected from the group consisting of human TRBV2, TRBV3-1, TRBV4-1, TRBV4-2, TRBV4-3, TRBV5-1, TRBV5-4, TRBV5-5, TRBV5-6, TRBV5-8, TRBV6-1, TRBV6-2, TRBV6-3, TRBV6-4, TRBV6-5, TRBV6-6, TRBV6-8, TRBV6-9, TRBV7-2, TRBV7-3, TRBV7-4, TRBV7-6, TRBV7-7, TRBV7-8, TRBV7-9, TRBV9, TRBV10-

1, TRBV10-2, TRBV10-3, TRBV11-1, TRBV11-2, TRBV11-3, TRBV12-3, TRBV12-4, TRBV12-5, TRBV13, TRBV14, TRBV15, TRBV16, TRBV18, TRBV19, TRBV20-1, TRBV24-1, TRBV25-1, TRBV27, TRBV28, TRBV29-1, and TRBV30.

[0009] In some ~~embodiment~~examples, the at least 20 different V genes comprise at least 20 different V gene subgroups. In some ~~embodiment~~examples, the at least 20 different V gene subgroups comprise at least 10 different TRAV gene subgroups and/or at least 10 different TRBV gene subgroups. In some ~~embodiment~~examples, the at least 10 different TRAV gene subgroups are selected from the group consisting of human TRAV1, TRAV2, TRAV3, TRAV4, TRAV5, TRAV6, TRAV7, TRAV8, TRAV9, TRAV10, TRAV12, TRAV13, TRAV14, TRAV16, TRAV17, TRAV18, TRAV19, TRAV20, TRAV21, TRAV22, TRAV23, TRAV24, TRAV25, TRAV26, TRAV27, TRAV29, TRAV30, TRAV34, TRAV35, TRAV36, TRAV38, TRAV39, TRAV40, and TRAV41 subgroup. In some ~~embodiment~~examples, the at least 10 different TRBV gene subgroups are selected from the group consisting of human TRBV2, TRBV3, TRBV4, TRBV5, TRBV6, TRBV7, TRBV9, TRBV10, TRBV11, TRBV12, TRBV13, TRBV14, TRBV15, TRBV16, TRBV18, TRBV19, TRBV20, TRBV24, TRBV25, TRBV27, TRBV28, TRBV29, and TRBV30 subgroup.

[0010] In some ~~embodiment~~examples, the at least 10 different TRAV genes are selected from the group consisting of mouse TRAV1, TRAV2, TRAV3-1, TRAV3-3, TRAV3-4, TRAV3D-3, TRAV3N-3, TRAV4-2, TRAV4-3, TRAV4-4, TRAV4D-2, TRAV4D-3, TRAV4D-4, TRAV4N-3, TRAV4N-4, TRAV5-1, TRAV5-2, TRAV5-4, TRAV5D-2, TRAV5D-4, TRAV5N-2, TRAV5N-4, TRAV6-1, TRAV6-2, TRAV6-3, TRAV6-4, TRAV6-5, TRAV6-6, TRAV6-7, TRAV6D-3, TRAV6D-4, TRAV6D-5, TRAV6D-6, TRAV6D-7, TRAV6N-5, TRAV6N-6, TRAV6N-7, TRAV7-1, TRAV7-2, TRAV7-3, TRAV7-4, TRAV7-5, TRAV7-6, TRAV7D-2, TRAV7D-3, TRAV7D-4, TRAV7D-5, TRAV7D-6, TRAV7N-4, TRAV7N-5, TRAV7N-6, TRAV8-1, TRAV8-2, TRAV8D-1, TRAV8D-2, TRAV8N-2, TRAV9-1, TRAV9-2, TRAV9-3, TRAV9-4, TRAV9D-1, TRAV9D-2, TRAV9D-3, TRAV9D-4, TRAV9N-2, TRAV9N-3, TRAV9N-4, TRAV10, TRAV10D, TRAV10N, TRAV11, TRAV11D, TRAV11N, TRAV12-1, TRAV12-2, TRAV12-3, TRAV12D-1, TRAV12D-2, TRAV12D-3, TRAV12N-1, TRAV12N-2, TRAV12N-3, TRAV13-1, TRAV13-2, TRAV13-3, TRAV13-4, TRAV13-5, TRAV13D-1, TRAV13D-2, TRAV13D-3, TRAV13D-4, TRAV13N-1, TRAV13N-2, TRAV13N-3, TRAV13N-4, TRAV14-1, TRAV14-2, TRAV14-3, TRAV14D-1, TRAV14D-2, TRAV14D-3, TRAV14N-1, TRAV14N-2, TRAV14N-3, TRAV15-1, TRAV15-2, TRAV15D-1, TRAV15D-2, TRAV15N-1, TRAV15N-2, TRAV16, TRAV16D, TRAV16N, TRAV17, TRAV18, TRAV19, TRAV20, and TRAV21.

[0011] In some ~~embodiment~~examples, the at least 10 different TRBV genes are selected from the group consisting of mouse TRBV1, TRBV2, TRBV3, TRBV4, TRBV5, TRBV8, TRBV9, TRBV10,

TRBV12-1, TRBV12-2, TRBV13-1, TRBV13-2, TRBV13-3, TRBV14, TRBV15, TRBV16, TRBV17, TRBV19, TRBV20, TRBV21, TRBV23, TRBV24, TRBV26, TRBV29, TRBV30, and TRBV31. In some ~~embodiment~~examples, the at least 10 different TRAV gene subgroups are selected from the group consisting of TRAV1, TRAV2, TRAV3, TRAV4, TRAV5, TRAV6, TRAV7, TRAV8, TRAV9, TRAV10, TRAV11, TRAV12, TRAV13, TRAV14, TRAV15, TRAV16, TRAV17, TRAV18, TRAV19, TRAV20, and TRAV21 subgroup. In some ~~embodiment~~examples, the at least 10 different TRBV gene subgroups are selected from the group consisting of mouse TRBV1, TRBV2, TRBV3, TRBV4, TRBV5, TRBV8, TRBV9, TRBV10, TRBV12, TRBV13, TRBV14, TRBV15, TRBV16, TRBV17, TRBV19, TRBV20, TRBV21, TRBV23, TRBV24, TRBV26, TRBV29, TRBV30, and TRBV31 subgroup.

[0012] In some ~~embodiment~~examples, each fused TCR polynucleotide of the plurality is circularized. In some ~~embodiment~~examples, the plurality of fused TCR polynucleotides comprise at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 different sequences.

[0013] According to another ~~aspect~~example, provided herein is a plurality of vectors, each comprising a different fused TCR polynucleotide from an immune cell. In some ~~embodiment~~examples, the plurality of vectors comprises at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 vectors. In some ~~embodiment~~examples, the plurality of vectors is self-amplifying RNA replicons, plasmids, phages, transposons, cosmids, viruses, or virions. In some ~~embodiment~~examples, the plurality of vectors are derivatives of the TC-83 alphavirus replicon that have been selected or engineered to reduce host cell type I interferon production, prolong the duration of expression, increase the levels of protein production, and/or express additional agent(s) of therapeutic benefit in addition to the bipartite immunoreceptor. In some ~~embodiment~~examples, the plurality of vectors is viral vectors. In some ~~embodiment~~examples, the viral vectors are derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus, a herpes virus, a pox virus, an alpha virus, a vaccina virus, a hepatitis B virus, a human papillomavirus or a pseudotype thereof. In some ~~embodiment~~examples, the plurality of vectors is non-viral vectors. In some ~~embodiment~~examples, the non-viral vectors are nanoparticles, cationic lipids, cationic polymers, metallic nanopolymers, nanorods, liposomes, micelles, microbubbles, cell-penetrating peptides, or lipospheres.

[0014] Also provided herein is a plurality of TCRs, each encoded by a different fused TCR polynucleotide from the composition described herein, or a different fused TCR polynucleotide from the plurality of vectors of described herein, wherein the plurality of TCRs comprises at least

100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 TCRs.

[0015] Also provided herein is a plurality of host cells, each comprising a different fused TCR polynucleotide from the composition described herein, a different vector of the plurality of vectors described herein, or a different TCR of the plurality of TCRs described herein. In some ~~embodiment~~examples, the plurality of host cells is T cells or B cells. In some ~~embodiment~~examples, the T cells are an inflammatory T cell, a cytotoxic T cell, a regulatory T cell, a helper T cell, a natural killer T cell, or a combination thereof. In some ~~embodiment~~examples, the T cells are CD4+ T cells or CD8+ T cells. In some ~~embodiment~~examples, the plurality of host cells is autologous cells. In some ~~embodiment~~examples, the plurality of host cells is allogeneic cells. In some ~~embodiment~~examples, the plurality of host cells is obtained from a donor. In some ~~embodiment~~examples, the donor is a human. In some ~~embodiment~~examples, the donor is a healthy donor or a diseased donor. In some ~~embodiment~~examples, the plurality of host cells is obtained from a sample. In some ~~embodiment~~examples, the sample is a blood sample, a bone marrow sample, a cord blood sample, a ascites sample, a pleural effusion sample, a cerebrospinal sample, a seminal fluid sample, a sputum sample, a urine sample, a stool sample, or a combination thereof. In some ~~embodiment~~examples, the sample is a tissue sample obtained from brain, liver, lung, kidney, prostate, ovary, spleen, lymph node, tonsil, thyroid, pancreas, heart, skeletal muscle, intestine, larynx, esophagus, thymus, stomach, tumor, site of infection, or a combination thereof. In some ~~embodiment~~examples, the plurality of host cells is cell line cells. In some ~~embodiment~~examples, the cell line cells are CHO-K1 cells; HEK293 cells; Caco2 cells; U2-OS cells; NIH 3T3 cells; NSO cells; SP2 cells; CHO-S cells; DG44 cells; K-562 cells, U-937 cells; MRC5 cells; IMR90 cells; Jurkat cells; HepG2 cells; HeLa cells; HT-1080 cells; HCT-116 cells; Hu-h7 cells; Huvec cells; or Molt 4 cells. In some ~~embodiment~~examples, the plurality of host cells are genetically modified cells.

[0016] In some ~~embodiment~~examples, an endogenous gene encoding a TCR alpha peptide chain, a TCR beta peptide chain, a TCR gamma peptide chain, a TCR delta peptide chain, a BCR heavy peptide chain, or a BCR light peptide chain is downregulated or inactivated. In some ~~embodiment~~examples, an additional endogenous gene is downregulated or inactivated, wherein the additional endogenous gene is selected from the group consisting of PD1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, and any combination thereof. In some ~~embodiment~~examples, each of the plurality of host cells is engineered to express an additional agent to enhance a function of the host cell. In some ~~embodiment~~examples, the function is a cytotoxic function, a pro-inflammatory function, or an anti-inflammatory function. In some ~~embodiment~~examples, the additional agent is a cytokine. In some ~~embodiment~~examples, the cytokine is a pro-inflammatory cytokine. In some ~~embodiment~~examples, the cytokine is an anti-inflammatory cytokine. In some ~~embodiment~~examples, the cytokine is tumor necrosis factor alpha

(TNFα); interleukin (IL)-1α; IL-1β; IL-2; IL-5; IL-6; IL-8; IL-15; IL-18; interferon (IFN-γ); platelet-activating factor (PAF); Monocyte chemotactic proteins 1 and 2 (MCP-1, MCP-2); macrophage migration inhibitory factor (MIF); CXCL8; CXCL9; CXCL10; high mobility group box protein 1 (HMGB-1), IL-1ra, IL-4, IL-10, IL-11, IL-13, transforming growth factor beta (TGF-β), IL-16, or any combination thereof.

[0017] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of vectors, each vector of the plurality comprises a fused T-cell receptor (TCR) polynucleotide having a first nucleic acid sequence and a second nucleic acid sequence, wherein (1) the first nucleic acid sequence encodes a first variable domain of a first TCR peptide chain, wherein the first variable domain comprises a CDR1, a CDR2 and a CDR3, and (2) the second nucleic acid sequence encodes a second variable domain of a second TCR peptide chain, wherein the second variable domain comprises a CDR1, a CDR2, and a CDR3; wherein the first and the second nucleic acid sequence of each fused TCR polynucleotide encode a cognate pair of the first and the second TCR peptide chain from an immune cell; and wherein the plurality of fused TCR polynucleotides comprises V regions from at least 20 different V genes. In some ~~embodiment~~examples, the plurality of vectors comprises at least 50, at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, or at least 1,000,000 different cognate pairs. In some ~~embodiment~~examples, the plurality of vectors comprises at least about 5, 10, 50 ,100, 250, 500, 750, 1000, 1500, 2000, 2500, 5000, 7500, or 10000, 20000, 30000, 40000, 50000, 60000, 70000, 80000, 90000, 100000, 200000, 300000, 400000, 500000, 600000, 700000, 800000, 900000, 1000000, 2000000, 3000000, 4000000, 5000000, 10000000, 20000000, or more different cognate pairs. In some ~~embodiment~~examples, the plurality of vectors comprises at least 50, at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 different sequences. In some ~~embodiment~~examples, the at least 20 different V genes comprise at least 10 different TRAV gene subgroups and/or at least 10 different TRBV gene subgroups.

[0018] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of hydrogel particles or beads, each hydrogel particle or bead of the plurality comprising: a first nucleic acid molecule and a first amplification product thereof encoding a first variable domain of a first immunoreceptor peptide chain, wherein the first variable domain comprises a CDR3, a second nucleic acid molecule and a second amplification product thereof encoding a second variable domain of a second immunoreceptor peptide chain, wherein the second variable domain comprises a CDR3, wherein the first amplification product and the second amplification product are embedded or entrapped within a matrix having a polymerized or gelled plurality of polymers

and/or monomers, wherein diffusion of the first amplification product and the second amplification product are restricted.

[0019] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of hydrogel particles or beads, each hydrogel particle or bead of the plurality comprising: a first nucleic acid molecule and a first primer extension product thereof encoding a first variable domain of a first immunoreceptor peptide chain, wherein the first variable domain comprises a CDR3, and a second nucleic acid molecule and a second primer extension product thereof encoding a second variable domain of a second immunoreceptor peptide chain, wherein the second variable domain comprises a CDR3, wherein the first primer extension product and the second primer extension product are embedded or entrapped within a matrix having a polymerized or gelled plurality of polymers and/or monomers, wherein diffusion of the first primer extension product and the second primer extension product are restricted.

[0020] In some ~~embodiment~~examples, the first and the second primer extension product comprise an adaptor having a pre-designed sequence. In some ~~embodiment~~examples, the adaptor is not hybridizable or complementary to the first or the second nucleic acid. In some ~~embodiment~~examples, the adaptor comprises a sequence or a reverse complement sequence of a template-switch oligonucleotide. In some ~~embodiment~~examples, the first and the second primer extension product is a reverse transcription (RT) product. In some ~~embodiment~~examples, the first and the second primer extension product is a second strand synthesis (SSS) product. In some ~~embodiment~~examples, the RT product is linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the SSS product is linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the SSS product is indirectly linked to the diffusion restricting agent. In some ~~embodiment~~examples, the first and the second primer extension product are a first and a second amplification product. In some ~~embodiment~~examples, the first amplification product and/or the second amplification product is linked to a diffusion restricting agent. In some ~~embodiment~~examples, the first amplification product and/or the second amplification product is linked to a diffusion restricting agent through a capture agent. In some ~~embodiment~~examples, the capture agent comprises an oligonucleotide having a complementary sequence to an adaptor sequence of the first amplification product and/or the second amplification product.

[0021] In some ~~embodiment~~examples, the diffusion restricting agent is a polymer. In some ~~embodiment~~examples, the polymer is a polyacrylamide, a polyethylene glycol, or a polysaccharide. In some ~~embodiment~~examples, the diffusion restricting agent is a particle. In some ~~embodiment~~examples, the particle has a diameter that is larger than a pore size of the matrix. In some ~~embodiment~~examples, the diffusion restricting agent is the matrix. In some ~~embodiment~~examples, the first nucleic acid molecule and the second nucleic acid molecule are released from a cell. In some ~~embodiment~~examples, the cell is a single

cell. In some ~~embodiment~~examples, the cell is a lymphocyte. In some ~~embodiment~~examples, the cell is a T cell or a B cell. In some ~~embodiment~~examples, the T cell is a CD3+ T cell, a CD28+ T cell, a CD4+ T cell, a CD8+ T cell, a CD45RA+ T cell, a CD45RO+ T cell, or any combination thereof. In some ~~embodiment~~examples, the B cell is a plasmablast cell, a plasma cell, a lymphoplasmacytoid cell, a memory B cell, a follicular B cell, a marginal zone B cell, a B-1 cell, a B-2 cell, or a regulatory B cell. In some ~~embodiment~~examples, the first immunoreceptor peptide chain is a TCR alpha peptide chain and the second immunoreceptor peptide chain is a TCR beta peptide chain. In some ~~embodiment~~examples, the first immunoreceptor peptide chain is a TCR gamma peptide chain and the second immunoreceptor peptide chain is a TCR delta peptide chain. In some ~~embodiment~~examples, the first immunoreceptor peptide chain is an immunoglobulin heavy peptide chain and the second immunoreceptor peptide chain is an immunoglobulin light peptide chain. In some ~~embodiment~~examples, the first immunoreceptor peptide chain and the second immunoreceptor peptide chain is a cognate pair of a bipartite immunoreceptor. In some ~~embodiment~~examples, the first amplification product and the second amplification product are linked to form a continuous polynucleotide strand. In some ~~embodiment~~examples, the first amplification product and/or the second amplification product comprises at least 100, at least 500, at least 1000, at least 10000, or more copies of the first nucleic acid molecule and/or the second nucleic acid molecule. In some ~~embodiment~~examples, the first or the second nucleic acid is diffusion restricted. In some ~~embodiment~~examples, the first nucleic acid molecule and/or the second nucleic acid molecule is a deoxyribonucleic acid or a ribonucleic acid. In some ~~embodiment~~examples, the first nucleic acid molecule and/or the second nucleic acid molecule is a single-stranded nucleic acid or a double-stranded nucleic acid. In some ~~embodiment~~examples, the first nucleic acid molecule further encodes a first constant domain and/or the second nucleic acid molecule further encodes a second constant domain. In some ~~embodiment~~examples, the first constant domain is a first extracellular constant domain, and/or the second constant domain is a second extracellular constant domain. In some ~~embodiment~~examples, the first constant domain comprises a first extracellular constant domain, a first hinge region, a first transmembrane domain, and a first cytoplasmic tail, and/or the second constant domain comprises a second extracellular constant domain, a second hinge region, a second transmembrane domain, and a second cytoplasmic tail.

[0022] In some ~~embodiment~~examples, the plurality of hydrogel particles or beads comprises at least 50, at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 hydrogel particles or beads. In some ~~embodiment~~examples, the plurality of hydrogel particles or beads comprises at least 50, at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 different cognate pairs of a bipartite immunoreceptor. In some ~~embodiment~~examples, the polymers are polysaccharides,

polyacrylamides, or a combination thereof. In some ~~embodiment~~examples, the polysaccharides are agarose, hyaluronic acids, carboxymethycellose, chitosan, starch, dextran, or alginate. In some ~~embodiment~~examples, the monomers are acrylamide or methacrylamide monomers. In some ~~embodiment~~examples, the polymerized or gelled plurality of polymers and/or monomers comprises a mixture of agarose and polyacrylamides. In some ~~embodiment~~examples, the polymerized or gelled plurality of polymers and/or monomers is cross-linked. In some ~~embodiment~~examples, the first variable domain and/or the second variable domain further comprises a CDR1, a CDR2, or a combination thereof. In some ~~embodiment~~examples, each hydrogel particle or bead is an agarose gel particle.

[0023] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least five hydrogel particles, wherein each of the at least five hydrogel particles comprises polynucleotides comprising (a) a first polynucleotide comprising a sequence encoding a first immunoreceptor peptide chain and (b) a second polynucleotide comprising a sequence encoding a second immunoreceptor peptide chain, wherein each of the first and second immunoreceptor peptide chains comprise unique cognate immunoreceptor paired chains, wherein the first polynucleotide and the second polynucleotide of an individual hydrogel particle of the at least five hydrogel particles are from a single cell, and (ii) linked to each other; and wherein diffusion of the first polynucleotide and the second polynucleotide from the hydrogel particle is restricted. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is a DNA. In some ~~embodiment~~examples, the DNA is an amplification product. In some ~~embodiment~~examples, the first polynucleotide and the second polynucleotide are covalently linked. In some ~~embodiment~~examples, the first polynucleotide and the second polynucleotide are linked by a phosphodiester bond. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is linked to a diffusion-restricting agent.

[0024] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least five hydrogel particles, wherein each of the at least five hydrogel particles comprises (a) a first RNA comprising a sequence encoding a first immunoreceptor peptide chain and (b) a second RNA comprising a sequence encoding a second immunoreceptor peptide chain, wherein each of the first and second immunoreceptor peptide chains comprise unique cognate immunoreceptor paired chains, wherein each first RNA and second RNA of an individual hydrogel particle of the at least five hydrogel particles is from a single cell, and wherein (1) each first RNA is hybridized to a first cDNA comprising a reverse complement sequence of the first RNA and (2) each second RNA is hybridized to a second cDNA comprising a reverse complement sequence of the second RNA; and wherein diffusion of the first cDNA and the second cDNA from the hydrogel particle is restricted. In some ~~embodiment~~examples, the first cDNA or the second cDNA further comprises a

sequence that is not hybridizable or complementary to the first RNA or the second RNA. In some ~~embodiment~~examples, the first cDNA or the second cDNA further comprises reverse complement sequence of a template-switch oligonucleotide. In some ~~embodiment~~examples, the first cDNA or the second cDNA is linked to a diffusion-restricting agent.

[0025] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least five hydrogel particles, wherein each of the at least five hydrogel particles comprises (a) a first polynucleotide comprising a sequence encoding a first immunoreceptor peptide chain and (b) a second polynucleotide comprising a sequence encoding a second immunoreceptor peptide chain, wherein each of the first and second immunoreceptor peptide chains comprise unique cognate immunoreceptor paired chains, wherein each first polynucleotide and second polynucleotide of an individual hydrogel particle of the at least five hydrogel particles is from a single cell, and wherein (1) each first polynucleotide is hybridized to a first primer and (2) each second polynucleotide is hybridized to a second primer; and wherein diffusion of the first primer and the second primer from the hydrogel particle is restricted.

[0026] In some ~~embodiment~~examples, the first primer or the second primer is a reverse transcription primer. In some ~~embodiment~~examples, the first primer or the second primer is an amplification primer. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is RNA. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is DNA. In some ~~embodiment~~examples, the first primer or the second primer is linked to a diffusion-restricting agent.

[0027] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least five hydrogel particles, wherein each of the at least five hydrogel particles comprises (a) a first DNA comprising a sequence encoding a first immunoreceptor peptide chain and (b) a second DNA comprising a sequence encoding a second immunoreceptor peptide chain, wherein each of the first and second immunoreceptor peptide chains comprise unique cognate immunoreceptor paired chains, wherein each first DNA and second DNA of an individual hydrogel particle of the at least five hydrogel particles is from a single cell, and wherein (1) each first DNA is hybridized to a first polynucleotide comprising a reverse complement sequence of the sequence encoding the first immunoreceptor chain and (2) each second DNA is hybridized to a second polynucleotide comprising a reverse complement sequence of the sequence encoding the second immunoreceptor chain; and wherein diffusion of the first polynucleotide and the second polynucleotide from the hydrogel particle is restricted.

[0028] In some ~~embodiment~~examples, the first DNA or the second DNA is cDNA. In some ~~embodiment~~examples, the first DNA or the second DNA is genomic DNA. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is RNA. In some ~~embodiment~~examples, the RNA is a messenger RNA. In

some ~~embodiment~~examples, diffusion of the first DNA or the second DNA from the hydrogel particle is restricted. In some ~~embodiment~~examples, the first polynucleotide or the second polynucleotide is an amplification product. In some ~~embodiment~~examples, the amplification product comprises an adaptor that is not hybridizable or complementary to the first or the second DNA. In some ~~embodiment~~examples, the adaptor further hybridizes to a capture agent. In some ~~embodiment~~examples, the capture agent is linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the diffusion-restricting agent is a polymer or a particle. In some ~~embodiment~~examples, the first and the second immunoreceptor peptide chains are TCR alpha and TCR beta peptide chains, TCR gamma and TCR delta peptide chains, or BCR heavy and light peptide chains. In some ~~embodiment~~examples, the single cell is an immune cell. In some ~~embodiment~~examples, the immune cell is a T cell or a B cell.

[0029] According to another ~~aspect~~example, provided herein is a method for preparing a fused bipartite immunoreceptor polynucleotide library, comprising: (a) generating a plurality of vessels, each comprising (1) a cell, wherein the cell comprises a first nucleic acid encoding a first peptide chain of a bipartite immunoreceptor and a second nucleic acid encoding a second peptide chain of the bipartite immunoreceptor, and (2) a plurality of polymerizable or gellable polymers and/or monomers; and (b) polymerizing or gelling the plurality of polymerizable or gellable polymers and/or monomers to form a plurality of hardened particles, each hardened particle of the plurality having a matrix composed of the polymerized or gelled plurality of polymers and/or monomers, wherein each hardened particle of the plurality comprises a first primer extension product of the first nucleic acid and a second primer extension product of the second nucleic acid; wherein the first primer extension product and the second primer extension product are embedded or entrapped within the matrix, and wherein diffusion of the first primer extension product and the second primer extension product are restricted.

[0030] In some ~~embodiment~~examples, the first and the second primer extension product is a reverse transcription (RT) product, a second strand synthesis (SSS) product, or an amplification product. In some ~~embodiment~~examples, the first and/or the second primer extension product comprise an adaptor sequence. In some ~~embodiment~~examples, the adaptor sequence is not hybridizable or complementary to the first or the second nucleic acid molecule. In some ~~embodiment~~examples, the first and the second primer extension product encode a variable domain. In some ~~embodiment~~examples, the variable domain comprises CDR1, CDR2, and CDR3. In some ~~embodiment~~examples, the first and/or the second primer extension product further encodes a constant domain.

[0031] In some ~~embodiment~~examples, the method further comprises lysing the cell to release the first nucleic acid and the second nucleic acid. In some ~~embodiment~~examples, the method further comprises reverse transcribing the first nucleic acid and the second nucleic acid. In some ~~embodiment~~examples, the

reverse transcribing is performed by using a RT primer. In some ~~embodiment~~examples, the RT primer is linked to a diffusion-restricting agent, wherein the diffusion-restricting agent restricts diffusion of the RT primer within the matrix. In some ~~embodiment~~examples, the method further comprises performing a template-switch reaction or a SSS reaction. In some ~~embodiment~~examples, the method further comprises amplifying the first nucleic acid and the second nucleic acid to generate a first and a second amplification product. In some ~~embodiment~~examples, for each of the first or the second nucleic acid, the amplifying is performed by using a first amplification primer and a second amplification primer. In some ~~embodiment~~examples, the first amplification primer is linked to a diffusion-restricting agent, wherein the diffusion-restricting agent restricts diffusion of the first amplification primer within the matrix.

[0032] In some ~~embodiment~~examples, the method further comprises washing the plurality of hardened particles. In some ~~embodiment~~examples, the method further comprises washing the plurality of hardened particles to allow a reagent to diffuse out from the plurality of hardened particles. In some ~~embodiment~~examples, the reagent comprises a RT primer, an amplification primer, a template-switch primer, a SSS primer, or any combination thereof. In some ~~embodiment~~examples, the method further comprises repeatedly washing the plurality of hardened particles. In some ~~embodiment~~examples, the method further comprises emulsifying the plurality of hardened particles in oil after a washing step, thereby forming an additional plurality of vessels, each vessel of the additional plurality of vessels comprising a single hardened particle of the plurality of hardened particles. In some ~~embodiment~~examples, the first and the second primer extension product are linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the diffusion-restricting agent is a polymer. In some ~~embodiment~~examples, the polymer is a polyacrylamide, a polyethylene glycol, or a polysaccharide. In some ~~embodiment~~examples, the diffusion restricting agent is a particle. In some ~~embodiment~~examples, the particle has a diameter that is larger than a pore size of the matrix. In some ~~embodiment~~examples, the diffusion restricting agent is the matrix. In some ~~embodiment~~examples, the first and the second primer extension product is linked to the diffusion-restricting agent through a capture agent. In some ~~embodiment~~examples, the capture agent comprises an immobilization moiety. In some ~~embodiment~~examples, the immobilization moiety links the capture agent to the diffusion-restricting agent.

[0033] In some ~~embodiment~~examples, the immobilization moiety comprises a reactive group. In some ~~embodiment~~examples, the capture agent further comprises a targeting moiety. In some ~~embodiment~~examples, the targeting moiety is a capture oligonucleotide. In some ~~embodiment~~examples, the first amplification primer comprises an oligonucleotide sequence that hybridizes to the capture oligonucleotide. In some ~~embodiment~~examples, the first and the second amplification product comprise the oligonucleotide sequence that hybridizes to the capture oligonucleotide, thereby linking the first and the second

amplification product to the capture agent and thereby linking to the diffusion-restricting agent. In some ~~embodiment~~examples, the reactive group is a succinimidyl ester, an amide, an acrylamide, an acyl azide, an acyl halide, an acyl nitrile, an aldehyde, a ketone, an alkyl halide, an alkyl sulfonate, an anhydride, an aryl halide, an aziridine, a boronate, a carbodiimide, a diazoalkane, an epoxide, a haloacetamide, a haloplatinate, a halotriazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a silyl halide, a sulfonate ester, a sulfonyl halide, an amine, an aniline, a thiol, an alcohol, a phenol, a hyrazine, a hydroxylamine, a carboxylic acid, a glycol, or a heterocycle. In some ~~embodiment~~examples, the method further comprises linking the first amplification product and the second amplification product to form a fused bipartite immunoreceptor polynucleotide within each vessel of the additional plurality of vessels, thereby generating the fused bipartite immunoreceptor polynucleotide library having a plurality of fused bipartite immunoreceptor polynucleotides. In some ~~embodiment~~examples, the first amplification product and the second amplification product are linked by ligation or PCR. In some ~~embodiment~~examples, the first amplification product and the second amplification product are linked by a phosphodiester bond to form a continuous polynucleotide. In some ~~embodiment~~examples, the first amplification product and the second amplification product are linked in-frame.

[0034] In some ~~embodiment~~examples, the method further comprises releasing the plurality of fused bipartite immunoreceptor polynucleotides from the additional plurality of vessels.

[0035] In some ~~embodiment~~examples, the method further comprises circularizing each fused bipartite immunoreceptor polynucleotide of the plurality. In some ~~embodiment~~examples, the method further comprises inserting each fused bipartite immunoreceptor polynucleotide of the plurality into a vector. In some ~~embodiment~~examples, the vector is a self-amplifying RNA replicon, a plasmid, a phage, a transposon, a cosmid, a virus, or a virion. In some ~~embodiment~~examples, the vector is a viral vector. In some ~~embodiment~~examples, the viral vector is derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus, a herpes virus, a pox virus, an alpha virus, a vaccina virus, a hepatitis B virus, a human papillomavirus or a pseudotype thereof. In some ~~embodiment~~examples, the vector is a non-viral vector. In some ~~embodiment~~examples, the non-viral vector is a nanoparticle, a cationic lipid, a cationic polymer, a metallic nanopolymer, a nanorod, a liposome, a micelle, a microbubble, a cell-penetrating peptide, or a liposphere. In some ~~embodiment~~examples, the bipartite immunoreceptor is a T-cell receptor (TCR) or a B-cell receptor (BCR). In some ~~embodiment~~examples, the TCR comprises a TCR alpha peptide chain and a TCR beta peptide chain, or a TCR gamma peptide chain and a TCR delta peptide chain; the BCR comprises a heavy peptide chain and a light peptide chain. In some ~~embodiment~~examples, the cell is an immune cell. In some ~~embodiment~~examples, the immune cell is a lymphocyte. In some ~~embodiment~~examples, the lymphocyte is a T cell or a B cell. In some ~~embodiment~~examples, the T cell is

an inflammatory T cell, a cytotoxic T cell, a regulatory T cell, a helper T cell, a natural killer T cell, or a combination thereof. In some ~~embodiment~~examples, the T cell is a CD4+ T cell or a CD8+ T cell. In some ~~embodiment~~examples, the B cell is a plasmablast cell, a plasma cell, a lymphoplasmacytoid cell, a memory B cell, a follicular B cell, a marginal zone B cell, a B-1 cell, a B-2 cell, or a regulatory B cell. In some ~~embodiment~~examples, the immune cell is isolated from a tumor tissue or a blood sample. In some ~~embodiment~~examples, the method further comprises delivering the fused bipartite immunoreceptor polynucleotide into a host cell. In some ~~embodiment~~examples, the fused bipartite immunoreceptor polynucleotide library comprise at least 50, at least 100, at least 200, at least 500, at least 1,000, at least 10,000, at least 100,000, at least 1,000,000, or at least 10,000,000 different fused bipartite immunoreceptor sequences. In some ~~embodiment~~examples, the first peptide chain and the second peptide chain are a cognate pair of the bipartite immunoreceptor. In some ~~embodiment~~examples, the vessel is a droplet. In some ~~embodiment~~examples, the droplet is a water-in-oil droplet. In some ~~embodiment~~examples, the hardened particle is a hydrogel particle. In some ~~embodiment~~examples, the polymers are polysaccharides, polyacrylamides, or a combination thereof. In some ~~embodiment~~examples, the polysaccharides are agarose, hyaluronic acids, carboxymethycellose, chitosan, or alginate. In some ~~embodiment~~examples, the monomers are acrylamide or methacrylamide monomers. In some ~~embodiment~~examples, the polymerized or gelled plurality of polymers and/or monomers comprises a mixture of agarose and polyacrylamides. In some ~~embodiment~~examples, the polymerized or gelled plurality of polymers and/or monomers is cross-linked. In some ~~embodiment~~examples, polymerizing or gelling the plurality of polymerizable or gellable polymers and/or monomers comprises using an initiator. In some ~~embodiment~~examples, the initiator is a UV light or a chemical. In some ~~embodiment~~examples, polymerizing or gelling the plurality of polymerizable or gellable polymers and/or monomers comprises reducing temperature of the vessel.

[0036] According to another ~~aspect~~example, provided herein is a method performed in a liquid comprising: (a) extending a first oligonucleotide hybridized to a nucleic acid molecule, thereby forming a first extension product; (b) amplifying the first extension product or a reverse complement strand thereof with a primer set comprising a first primer and a second primer, thereby forming a first amplification product; (c) generating a polymer matrix in the liquid to form a hydrogel particle, thereby restricting diffusion of the first amplification product; and (d) washing the hydrogel particle, thereby depleting the second primer from the hydrogel particle. In some ~~embodiment~~examples, the first primer or the first amplification product is linked to a diffusion-restricting agent.

[0037] According to another ~~aspect~~example, provided herein is a method performed in a liquid comprising: (a) extending a first oligonucleotide hybridized to a nucleic acid molecule, thereby forming a first extension product; (b) generating a polymer matrix in the liquid to form a hydrogel particle,

thereby restricting diffusion of the first extension product or a reverse complement strand thereof; (c) washing the hydrogel particle; and (d) amplifying the first extension product or the reverse complement strand thereof with a primer set comprising a first primer and a second primer, thereby forming a first amplification product.

[0038] In some ~~embodiment~~examples, the first oligonucleotide or the first extension product is linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the method further comprises extending a second oligonucleotide hybridized to an additional nuclei acid molecule. In some ~~embodiment~~examples, the nucleic acid molecule and the additional nucleic acid molecule encode a first peptide chain and a second peptide chain of an immunoreceptor, wherein the first peptide chain and the second peptide chain are a cognate pair of the immunoreceptor. In some ~~embodiment~~examples, the diffusion-restricting agent is a polymer or a particle. In some ~~embodiment~~examples, the polymer is a polyacrylamide, a polyethylene glycol, or a polysaccharide. In some ~~embodiment~~examples, the particle has a diameter that is larger than a pore size of the polymer matrix. In some ~~embodiment~~examples, the diffusion-restricting agent is the polymer matrix. In some ~~embodiment~~examples, the nucleic acid molecule is DNA or RNA. In some ~~embodiment~~examples, the nucleic acid molecule is a genomic DNA. In some ~~embodiment~~examples, the nucleic acid molecule is a messenger RNA. In some ~~embodiment~~examples, the first oligonucleotide is a reverse transcription (RT) primer. In some ~~embodiment~~examples, the method further comprises extending the RT primer with a template-switch oligonucleotide, thereby generating the first extension product having a reverse complement sequence of the template-switch oligonucleotide. In some ~~embodiment~~examples, the method further comprises using a second strand synthesis (SSS) primer having an adaptor sequence to synthesize the reverse complement strand of the first extension product. In some ~~embodiment~~examples, the adaptor sequence is not hybridizable or complementary to the nucleic acid molecule or the first extension product. In some ~~embodiment~~examples, the first extension product comprises the adaptor sequence. In some ~~embodiment~~examples, the nucleic acid molecule encodes a peptide chain of an immunoreceptor. In some ~~embodiment~~examples, the method further comprises, after or during washing the hydrogel particle, contacting a reagent with the hydrogel particle such that the reagent diffuses into the hydrogel particle. In some ~~embodiment~~examples, the reagent is an oligonucleotide or an enzyme. In some ~~embodiment~~examples, the enzyme is a polymerase. In some ~~embodiment~~examples, the method further comprises emulsifying the hydrogel particle in oil after washing.

[0039] According to another ~~aspect~~example, provided herein is a method performed in a liquid comprising: (a) forming a plurality of droplets, wherein at least two droplets of the plurality comprise a single cell; (b) extending a first oligonucleotide hybridized to a first nucleic acid molecule from the single cell, thereby forming a first extension product; and extending a second oligonucleotide hybridized to a second nucleic acid molecule from the single cell, thereby forming a second

extension product; (c) amplifying the first extension product or a reverse complement strand thereof with a first primer set comprising a first primer and a second primer, thereby forming a first set of amplification products; and amplifying the second extension product or a reverse complement strand thereof with a second primer set comprising a third primer and a fourth primer, thereby forming a second set of amplification products; and (d) linking an amplification product of the first set of amplification products to an amplification product of the second set of amplification products, wherein linking comprises linking in the liquid in the absence of the second and the fourth primer.

[0040] According to another ~~aspect~~example, provided herein is a method performed in a liquid comprising: (a) forming a plurality of droplets, wherein at least two droplets of the plurality comprise a single cell; (b) extending a first oligonucleotide hybridized to a first nucleic acid molecule from the single cell, thereby forming a first extension product; and extending a second oligonucleotide hybridized to a second nucleic acid molecule from the single cell, thereby forming a second extension product; (c) amplifying the first extension product or a reverse complement strand thereof with a first primer set comprising a first primer and a second primer, thereby forming a first set of amplification products; and amplifying the second extension product or a reverse complement strand thereof with a second primer set comprising a third primer and a fourth primer, thereby forming a second set of amplification products; (d) removing the second and the fourth primer; and linking an amplification product of the first set of amplification products to an amplification product of the second set of amplification products.

[0041] In some ~~embodiment~~examples, each droplet comprises a plurality of polymerizable or gellable polymers and/or monomers. In some ~~embodiment~~examples, the method further comprises generating a polymer matrix in the liquid to form a hydrogel particle, thereby restricting diffusion of the first set of amplification products and the second set of amplification products. In some ~~embodiment~~examples, the method further comprises washing the hydrogel particle, thereby depleting the second primer and the fourth primer from the hydrogel particle. In some ~~embodiment~~examples, linking comprises generating a sticky end on the amplification product of the first and the second set. In some ~~embodiment~~examples, generating the sticky end on the amplification product comprises using a USER enzyme. In some ~~embodiment~~examples, linking comprises hybridizing the amplification product of the first and the second set. In some ~~embodiment~~examples, linking comprises ligating the amplification product of the first and the second set. In some ~~embodiment~~examples, the first primer and the third primer are the same primer. In some ~~embodiment~~examples, the first primer, the third primer, the first set of amplification products, or the second set of amplification products is linked to a diffusion-restricting agent.

[0042] According to another ~~aspect~~example, provided herein is a method performed in a liquid comprising: (a) forming a plurality of droplets, wherein at least two droplets of the plurality comprise a single cell; (b) extending a first oligonucleotide hybridized to a first nucleic acid molecule from the single cell, thereby forming a first extension product; and extending a second oligonucleotide hybridized to a second nucleic acid molecule from the single cell, thereby forming a second extension product; (c) generating a polymer matrix in the liquid to form a hydrogel particle, thereby restricting the diffusion of the first extension product and the second extension product are restricted; (d) amplifying the first extension product or a reverse complement strand thereof with a first primer set comprising a first primer and a second primer, thereby forming a first set of amplification products; and amplifying the second extension product or a reverse complement strand thereof with a second primer set comprising a third primer and a fourth primer, thereby forming a second set of amplification products; and (e) linking an amplification product of the first set of amplification products to an amplification product of the second set of amplification products.

[0043] In some ~~embodiment~~examples, the method further comprises washing the hydrogel particle after (c). In some ~~embodiment~~examples, the method further comprises contacting a reagent with the hydrogel particle such that the reagent diffuses into the hydrogel particle. In some ~~embodiment~~examples, the reagent comprises an enzyme or an oligonucleotide. In some ~~embodiment~~examples, the oligonucleotide comprises the first primer set and/or the second primer set. In some ~~embodiment~~examples, the enzyme is a polymerase, a ligase, a USER enzyme, or a combination thereof. In some ~~embodiment~~examples, the method further comprises emulsifying the hydrogel particle in oil after washing. In some ~~embodiment~~examples, the first oligonucleotide or the second oligonucleotide is linked to a diffusion-restricting agent. In some ~~embodiment~~examples, the first oligonucleotide or the second oligonucleotide is a RT primer. In some ~~embodiment~~examples, the method further comprises using a second strand synthesis (SSS) primer to synthesize the reverse complement strand of the first and/or the second extension product. In some ~~embodiment~~examples, the SSS primer comprises an adaptor sequence. In some ~~embodiment~~examples, the adaptor sequence is not hybridizable or complementary with the first and/or the second extension product. In some ~~embodiment~~examples, the method further comprises extending the RT primer with a template-switch oligonucleotide. In some ~~embodiment~~examples, the single cell is an immune cell. In some ~~embodiment~~examples, the immune cell is a T cell or a B cell. In some ~~embodiment~~examples, the first nucleic acid molecule and the second nucleic acid molecule are DNA or RNA. In some ~~embodiment~~examples, the DNA is a genomic DNA. In some ~~embodiment~~examples, the RNA is a messenger RNA. In some ~~embodiment~~examples, the first nucleic acid molecule encodes a first peptide chain of an immunoreceptor and the second nucleic acid molecule encodes a second peptide chain of the

immunoreceptor. In some ~~embodiment~~examples, the first peptide chain and the second peptide chain are a cognate pair of the immunoreceptor. In some ~~embodiment~~examples, the first peptide chain or the second peptide chain comprises a variable domain. In some ~~embodiment~~examples, the variable domain comprises a CDR1, CDR2, CDR3, or a combination thereof. In some ~~embodiment~~examples, the first peptide chain or the second peptide chain comprises a constant domain. In some ~~embodiment~~examples, the first peptide chain or the second peptide chain comprises a transmembrane region and/or a cytoplasmic tail. In some ~~embodiment~~examples, the immunoreceptor is a B-cell receptor. In some ~~embodiment~~examples, the immunoreceptor is a T-cell receptor. In some ~~embodiment~~examples, the diffusion-restricting agent is a polymer or a particle. In some ~~embodiment~~examples, the polymer is a polyacrylamide, a polysaccharide, or a polyethylene glycol. In some ~~embodiment~~examples, the particle has a diameter that is larger than a pore size of the hydrogel particle. In some ~~embodiment~~examples, the diffusion-restricting agent is the polymer matrix. In some ~~embodiment~~examples, linking comprises generating a sticky end on the amplification product of the first and the second set. In some ~~embodiment~~examples, generating the sticky end on the amplification product comprises using a USER enzyme. In some ~~embodiment~~examples, linking comprises hybridizing the amplification product of the first and the second set. In some ~~embodiment~~examples, linking comprises ligating the amplification product of the first and the second set.

[0044] According to another ~~aspect~~example, provided herein is a method comprising: (a) obtaining a population of host cells, each host cell in the population expressing a TCR having a natively paired TCR alpha and beta peptide chains or a BCR having a natively paired BCR heavy and light peptide chain; enriching (i) a subpopulation of host cells from the population, or (ii) expressed TCRs or BCRs of a subpopulation of host cells from the population, wherein the subpopulation of host cells or the expressed TCRs or BCRs of the subpopulation of host cell bind to a target antigen or a target MHC-antigen complex; and (b) administering the subpopulation of host cells or the expressed TCRs or BCRs of the subpopulation enriched from step (b) to a subject expressing the target antigen or the target MHC-antigen complex.

[0045] In some ~~embodiment~~examples, obtaining comprises using any of the methods described herein. In some ~~embodiment~~examples, (b) comprises contacting the population of host cells or the expressed TCRs or BCRs with the target antigen or the target MHC-antigen complex. In some ~~embodiment~~examples, the MHC is an MHC tetramer. In some ~~embodiment~~examples, (c) comprises administering by injection. In some ~~embodiment~~examples, the injection comprises injecting intravenously, subcutaneously, intradermally, or intramuscularly. In some ~~embodiment~~examples, the target antigen is a neoantigen or a tumor-associated antigen.

[0046] According to another ~~aspect~~example, provided herein is a method comprising: (1) providing a plurality of at least 1,000 cells, each cell of the at least 1,000 cells comprising a TCR alpha chain

and a TCR beta chain; (2) providing a plurality of at least 1,000 compartments, each compartment of the at least 1,000 compartments comprising a solid support, wherein the solid support comprises: (a) a first polynucleotide, comprising a first common sequence, a second common sequence, and a protein-coding sequence encoding a TCR alpha chain between the first and the second common sequence, (b) a second polynucleotide, comprising a third common sequence, a fourth common sequence, and a protein-coding sequence encoding a TCR beta chain between the third and the fourth common sequence, wherein, the TCR alpha chain and the TCR beta chain in each compartment is a cognate pair present in at least one of the plurality of cells, thereby providing a first plurality of protein-coding sequences each encoding a TCR alpha chain and a second plurality of protein-coding sequences each encoding a TCR beta chain; and (3) physically linking the first polynucleotide and the second polynucleotide in each compartment. In some ~~embodiment~~examples, the first plurality of protein-coding sequences comprises at least 10 TRAV subgroups and the second plurality of protein-coding sequences comprises at least 10 TRBV subgroups. In some ~~embodiment~~examples, each compartment of the at least 1,000 compartments comprise a cell from the plurality of at least 1,000 cells. In some ~~embodiment~~examples, the compartment is a well, a microwell, or a droplet. In some ~~embodiment~~examples, the solid support is a bead, a hydrogel particle, or a surface of the well or microwell. In some ~~embodiment~~examples, the first common sequence, the second common sequence, the third common sequence, or the fourth common sequence is the same in the plurality of at least 1,000 compartments.

[0047] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least 1,000 compartments, each compartment of the at least 1,000 compartments comprising a solid support, wherein the solid support comprises: (a) a first polynucleotide, comprising a first common sequence, a second common sequence, and a protein-coding sequence encoding a TCR alpha chain between the first and the second common sequence, and (b) a second polynucleotide, comprising a third common sequence, a fourth common sequence, and a protein-coding sequence encoding a TCR beta chain between the third and the fourth common sequence, wherein, (i) the TCR alpha chain and the TCR beta chain in each compartment is a cognate pair, (ii) a plurality of first common sequences in the plurality of compartments has the same sequence and is hybridizable or complementary to a first primer, (iii) a plurality of second common sequences in the plurality of compartments has the same sequence and is hybridizable or complementary to a second primer, (iv) a plurality of third common sequences in the plurality of compartments has the same sequence and is hybridizable or complementary to a third primer, and (v) a plurality of fourth common sequences in the plurality of compartments has the same sequence and is hybridizable or complementary to a fourth primer.

[0048] In some ~~embodiment~~examples, each compartment further comprises the first primer, the second primer, the third primer, and the fourth primer. In some ~~embodiment~~examples, the concentration of the first primer is at least 1 nM, the concentration of the second primer is at least 1 nM, the concentration of the third primer is at least 1 nM, and concentration of the fourth primer is at least 1 nM. In some ~~embodiment~~examples, the second common sequence is hybridizable or complementary to the fourth common sequence or a reverse complement sequence thereof in each compartment.

[0049] According to another ~~aspect~~example, provided herein is a composition comprising a plurality of at least 1,000 compartments, each compartment of the at least 1,000 compartments comprising: (a) a first fully or partially single-stranded polynucleotide, comprising a first common sequence at the 5' end, a second common sequence at the 3' end, and a protein-coding sequence encoding a TCR alpha chain between the first and the second common sequence, and (b) a second fully or partially single-stranded polynucleotide, comprising a third common sequence at the 5' end, a fourth common sequence at the 3' end, and a protein-coding sequence encoding a TCR beta chain between the third and the fourth common sequence, wherein, (i) the TCR alpha chain and the TCR beta chain is a cognate pair, and (ii) the second common sequence is hybridized to the fourth common sequence. In some ~~embodiment~~examples, the first common sequence, the second common sequence, the third common sequence, or the fourth common sequence is the same in the plurality of at least 1,000 compartments. In some ~~embodiment~~examples, each compartment further comprises a solid support. In some ~~embodiment~~examples, the solid support is a bead or a hydrogel particle.

[0050] According to another ~~aspect~~example, provided herein is a method of identifying a target-reactive T-cell receptor (TCR), comprising: (a) providing a plurality of T cells expressing a plurality of TCRs, wherein each T cell of the plurality of T cells expresses a cognate pair of a TCR of the plurality of TCRs; (b) partitioning the plurality of T cells into a plurality of compartments, wherein each compartment comprises an individual T cell of the plurality of T cells; (c) within each compartment, linking a first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of the cognate pair of the TCR of the individual T cell, thereby generating a plurality of fused polynucleotides, wherein (i) the first polynucleotide and the second polynucleotide are transcribed or amplified products of endogenous nucleic acids of the individual T cell or (ii) the first polynucleotide and the second polynucleotide are not chemically synthesized using phosphoramidite; (d) generating a plurality of vectors comprising the plurality of fused polynucleotides, each vector of the plurality of vectors comprising a fused polynucleotide of the plurality of fused polynucleotides; (e) delivering the plurality of vectors into a plurality of cells, wherein each cell of the plurality of cells comprises at least one vector of the plurality of vectors; (f) expressing the plurality of fused polynucleotides from the plurality of

vectors in the plurality of cells, wherein a subset of the plurality of cells expresses a plurality of target-reactive TCRs; (g) contacting the plurality of cells with one or more target antigens, wherein the subset of the plurality of cells expressing the plurality of target-reactive TCRs binds to the one or more target antigens; and (h) identifying a target-reactive TCR of the plurality of target-reactive TCRs of the subset of the plurality of cells.

[0051] According to another ~~aspect~~example, provided herein is a method of identifying a target-reactive T-cell receptor (TCR), comprising: (a) providing a plurality of T cells expressing a plurality of TCRs, wherein each T cell of the plurality of T cells expresses a cognate pair of a TCR of the plurality of TCRs; (b) partitioning the plurality of T cells into a plurality of compartments, wherein each compartment comprises an individual T cell of the plurality of T cells; (c) within each compartment, linking a first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of the cognate pair of the TCR of the individual T cell, thereby generating a plurality of fused polynucleotides, wherein the first polynucleotide and the second polynucleotide are transcribed or amplified products of endogenous nucleic acids of the individual T cell or (ii) the first polynucleotide and the second polynucleotide are not chemically synthesized using phosphoramidite; (d) delivering the plurality of fused polynucleotides into a plurality of cells, wherein each cell of the plurality of cells comprises at least one fused polynucleotide of the plurality of fused polynucleotides; (e) expressing the plurality of fused polynucleotides from the plurality of vectors in the plurality of cells, wherein a subset of the plurality of cells expresses a plurality of target-reactive TCRs; (f) contacting the plurality of cells with one or more target antigens, wherein the subset of the plurality of cells expressing the plurality of target-reactive TCRs binds to the one or more target antigens; and (g) identifying a target-reactive TCR of the plurality of target-reactive TCRs of the subset of the plurality of cells.

[0052] In some cases, the method further comprises, prior to delivering, generating a plurality of vectors comprising the plurality of fused polynucleotides, each vector of the plurality of vectors comprising a fused polynucleotide of the plurality of fused polynucleotides. In some cases, the plurality of cells is a plurality of recipient cells. In some cases, the endogenous nucleic acids are deoxyribonucleic acids (DNAs) or ribonucleic acids (RNAs). In some cases, the DNAs are genomic DNAs. In some cases, the RNAs are messenger RNAs. In some cases, contacting further comprises contacting the population of cells with one or more cells presenting the one or more target antigens. In some cases, the one or more cells are one or more tumor cells, tumorspheres, tumor lysate-pulsed antigen-presenting cells (APCs) or APCs engineered to present the one or more target antigens. In some cases, the one or more APCs engineered to present the one or more

target antigens are delivered (e.g., transfected or electroporated) with a target antigen coding DNA or RNA. In some cases, contacting further comprises contacting the population of cells with a tumor tissue. In some cases, the one or more target antigens are in complex with a major histocompatibility complex (MHC). In some cases, the MHC is a MHC tetramer. In some cases, the first TCR chain is a TCR alpha chain and the second TCR chain is a TCR beta chain. In some cases, the first TCR chain is a TCR gamma chain and the second TCR chain is a TCR delta chain. In some cases, the plurality of cells is cell line cells. In some cases, the cell line cells are CHO-K1 cells; HEK293 cells; Caco2 cells; U2-OS cells; NIH 3T3 cells; NSO cells; SP2 cells; CHO-S cells; DG44 cells; K-562 cells, U-937 cells; MRC5 cells; IMR90 cells; Jurkat cells; HepG2 cells; HeLa cells; HT-1080 cells; HCT-116 cells; Hu-h7 cells; Huvec cells; or Molt 4 cells. In some cases, the plurality of cells is isolated from a sample from a subject. In some cases, the plurality of T cells is isolated from a sample from a subject. In some cases, the sample is a tumor tissue, a blood sample, a peripheral blood mononuclear cell (PBMC) sample, or a combination thereof. In some cases, the tumor tissue is at most about 2000 mm^3. In some cases, the blood sample comprises peripheral blood mononuclear cells (PBMCs). In some cases, the plurality of T cells is tumor-infiltrating T cells or peripheral T cells. In some cases, the plurality of T cells comprises CD8+ T cells, CD4+ T cells, exhausted T cells, regulatory T cells, or any combinations thereof. In some cases, the method further comprises isolating at least one cell of the subset of the plurality of cells. In some cases, the at least one cell of the subset of the plurality of cells is isolated by FACS. In some cases, the at least one cells of the subset of the plurality of cells is isolated based on a marker. In some cases, the marker is a cell surface marker or a cytokine. In some cases, the cell surface marker is CD39, CD69, CD103, CD25, PD-1, TIM-3, OX-40, 4-1BB, CD137, CD3, CD28, CD4, CD8, CD45RA, CD45RO, GITR, FoxP3, or a combination thereof. In some cases, the cytokine is IFN-γ, TNF-alpha, IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, perforin, or a combination thereof. In some cases, the method further comprises administering (i) at least one cell of the subset of the plurality of cells into the subject or (ii) an autologous or allogeneic cell comprising an identified target-reactive TCR into the subject. In some cases, the autologous or the allogeneic cell comprises a polynucleotide encoding the identified target-reactive TCR. In some cases, the polynucleotide encoding the identified target-reactive TCR is the fused polynucleotide or an amplified product thereof, or comprises a sequence encoding the first TCR chain and the second TCR chain of the fused polynucleotide.

[0053] According to another ~~aspect~~example, provided herein is a method of identifying a plurality of target-reactive T-cell receptors (TCRs), comprising: (a) providing a plurality of cells expressing a plurality of TCRs, each cell of the plurality of cells expressing a TCR of the plurality of TCRs,

wherein the plurality of TCRs comprises at least 50 different cognate pairs and comprises V regions from a plurality of V genes, and wherein the plurality of TCRs are exogenous to the plurality of cells; (b) contacting the plurality of cells with one or more target antigens, wherein a subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target antigens; and (c) identifying at least two cells of the subset of the plurality of cells, which at least two cells express at least two target-reactive TCRs of the plurality of target-reactive TCRs, thereby identifying the at least two target-reactive TCRs of the plurality of target-reactive TCRs.

[0054] In some cases, the plurality of V genes comprises at least 10 different V genes. In some cases, the plurality of cells is a plurality of genetically engineered cells. In some cases, the plurality of cells is not isolated from a patient. In some cases, the plurality of cells is isolated from a sample from a subject. In some cases, the sample is a tissue sample, a blood sample, a PBMC sample, or a combination thereof. In some cases, the plurality of cells does not comprise exhausted T cells. In some cases, the plurality of TCRs comprises at least 100 different cognate pairs. In some cases, the method further comprises isolating the at least two cells of the subset of the plurality of cells. In some cases, (b) comprises contacting the plurality of cells with one or more cells presenting the one or more target antigens. In some cases, the one or more cells are one or more tumor cells, tumorspheres, tumor lysate-pulsed antigen-presenting cells (APCs) or APCs engineered to present the one or more target antigens. In some cases, the one or more APCs engineered to present the one or more target antigens comprise a target antigen coding DNA or RNA. In some cases, (b) comprises contacting the plurality of cells with a tumor tissue. In some cases, (b) comprises contacting the plurality of cells with the one or more target antigens in complex with a major histocompatibility complex (MHC). In some cases, the MHC is a MHC tetramer. In some cases, sequence or identity of the one or more target antigens is unknown. In some cases, the method further comprises administering at least one of the at least two cells of the subset of the plurality of cells into a subject. In some cases, each cell of the plurality of cells comprises a reporter gene, which reporter gene is regulated to send a signal when a TCR of the cell binds to a target antigen of the one or more target antigens. In some cases, the plurality of cells is cell line cells. In some cases, the plurality of TCRs comprises at least 100 different VJ combinations.

[0055] According to another ~~aspect~~example, provided herein is a method of treating a cancer in a subject, comprising: (a) providing a plurality of T cells expressing a plurality of TCRs, wherein each T cell of the plurality of T cells expresses a cognate pair of a TCR of the plurality of TCRs; (b) partitioning the plurality of T cells into a plurality of compartments, wherein each compartment comprises an individual T cell of the plurality of T cells; (c) within each compartment, linking a

first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of the cognate pair of the TCR of the individual T cell, thereby generating a plurality of fused polynucleotides, wherein (i) the first polynucleotide and the second polynucleotide are transcribed or amplified products of endogenous nucleic acids of the individual T cell or (ii) the first polynucleotide and the second polynucleotide are not chemically synthesized using phosphoramidite; (d) delivering the plurality of fused polynucleotides into a plurality of cells, wherein each cell of the plurality of cells comprises at least one fused polynucleotide of the plurality of fused polynucleotides; (e) expressing the plurality of fused polynucleotides in the plurality of cells, wherein a subset of the plurality of cells expresses a plurality of target-reactive TCRs from a subset of the plurality of fused polynucleotides; (f) identifying the plurality of target-reactive TCRs from the subset of the plurality of fused polynucleotides; (g) delivering one or more fused polynucleotides or a derivative thereof of the subset of the plurality of fused polynucleotides into a plurality of recipient cells, wherein each cell of the plurality of recipient cells comprises at least one of the one or more fused polynucleotides or a derivative thereof of the subset of the plurality of fused polynucleotides; and (h) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs.

[0056] In some cases, the plurality of T cells is tumor-infiltrating T cells or peripheral T cells. In some cases, the plurality of T cells comprises CD8+ T cells, CD4+ T cells, exhausted T cells, regulatory T cells, or any combinations thereof. In some cases, the plurality of T cells are activated and/or expanded in vitro. In some cases, the plurality of fused polynucleotides is delivered in a plurality of vectors, wherein each vector of the plurality of vectors comprises a fused polynucleotide of the plurality of fused polynucleotides. In some cases, the method further comprises, prior to delivering in (d), generating the plurality of vectors. In some cases, identifying in (f) comprises contacting the plurality of cells with one or more target antigens, wherein the subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target antigens. In some cases, the one or more target antigens are presented by one or more tumor cells or antigen-presenting cells (APCs). In some cases, the one or more APCs (i) are pulsed by the one or more target antigens or (ii) comprise a target antigen coding DNA or RNA. In some cases, each antigen of the one or more antigens is in complex with a major histocompatibility complex (MHC). In some cases, the MHC is a MHC tetramer. In some cases, the method further comprises, prior to delivering in (g), isolating one or more cells of the subset of the plurality of cells. In some cases, the method further comprises isolating the plurality of T cells expressing the plurality of TCRs from the subject. In some cases, administering is performed at most about 60

days, 50 days, 40 days, 30 days, 20 days or less after isolating the plurality of T cells. In some cases, the plurality of recipient cells is allogeneic cells, autologous cells, or cell line cells. In some cases, the plurality of cells is genetically engineered cells or cell line cells. In some cases, the method further comprises, prior to (h), expanding the plurality of recipient cells. In some cases, the derivative comprises a sequence of the one or more fused polynucleotides. In some cases, the derivative is an amplified product or a synthesized product of the one or more fused polynucleotides.

[0057] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a plurality of T cells from the subject expressing a plurality of T-cell receptors (TCRs), wherein each T cell of the plurality of T cells expresses a cognate pair of TCR of the plurality of TCRs, wherein the plurality of TCRs comprises a plurality of tumor-reactive TCRs; (b) identifying the plurality of tumor-reactive TCRs from the plurality of TCRs; (c) delivering polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof into a plurality of recipient cells, wherein each recipient cell of the plurality of recipient cells comprises at least one polynucleotide of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof; (d) expressing the plurality of tumor-reactive TCRs or a subset thereof in the plurality of recipient cells; and (e) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs, wherein administering is performed at most about 60 days, 50 days, 40 days, 30 days, 20 days or less after isolating the plurality of T cells in (a).

[0058] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a plurality of T cells from the subject expressing a plurality of T-cell receptors (TCRs), wherein each T cell of the plurality of T cells expresses a cognate pair of TCR of the plurality of TCRs, wherein the plurality of TCRs comprises a plurality of tumor-reactive TCRs; (b) identifying the plurality of tumor-reactive TCRs from the plurality of TCRs; (c) delivering polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof into a plurality of recipient cells, wherein each recipient cell of the plurality of recipient cells comprises at least one polynucleotide of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof; (d) expressing the plurality of tumor-reactive TCRs or a subset thereof in the plurality of recipient cells; and (e) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs, wherein the tumor of the subject has not progressed for more than about 60 days, 50 days, 40 days, 30 days, 20 days

or less from isolating the plurality of T cells from the subject to administering the at least one or the at least two recipient cells of the plurality of recipient cell into the subject.

[0059] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a plurality of T cells from the subject expressing a plurality of T-cell receptors (TCRs), wherein each T cell of the plurality of T cells expresses a cognate pair of TCR of the plurality of TCRs, wherein the plurality of TCRs comprises a plurality of tumor-reactive TCRs; (b) identifying the plurality of tumor-reactive TCRs from the plurality of TCRs; (c) delivering polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof into a plurality of recipient cells, wherein each recipient cell of the plurality of recipient cells comprises at least one polynucleotide of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof; (d) expressing the plurality of tumor-reactive TCRs or a subset thereof in the plurality of recipient cells; and (e) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs, wherein (i) a size of the tumor has increased by less than about 50%, 30%, 40%, 20%, 15%, 10%, 5% or 2%, or (ii) number of tumor cells in the subject has not increased by about 2 fold, 3 fold, 4 fold, 5 fold or more, from isolating the plurality T cells to administering the at least one or the at least two recipient cells of the plurality of recipient cells.

[0060] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a plurality of T cells from the subject expressing a plurality of T-cell receptors (TCRs), wherein each T cell of the plurality of T cells expresses a cognate pair of TCR of the plurality of TCRs, wherein the plurality of TCRs comprises a plurality of tumor-reactive TCRs; (b) identifying the plurality of tumor-reactive TCRs from the plurality of TCRs; (c) delivering polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof into a plurality of recipient cells, wherein each recipient cell of the plurality of recipient cells comprises at least one polynucleotide of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof; (d) expressing the plurality of tumor-reactive TCRs or a subset thereof in the plurality of recipient cells; and (e) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs, wherein the tumor has not progressed to a new stage from isolating the plurality of T cells to administering the at least one or the at least two recipient cells of the plurality of recipient cells.

[0061] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a plurality of T cells from the subject expressing a plurality of T-cell

receptors (TCRs), wherein each T cell of the plurality of T cells expresses a cognate pair of TCR of the plurality of TCRs from endogenous nucleic acids, wherein the plurality of TCRs comprises a plurality of tumor-reactive TCRs; (b) identifying the plurality of tumor-reactive TCRs from the plurality of TCRs; (c) delivering polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof into a plurality of recipient cells, wherein each recipient cell of the plurality of recipient cells comprises at least one polynucleotide of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof, wherein (i) the polynucleotides are transcribed or amplified products of the endogenous nucleic acids or (ii) the polynucleotides are not chemically synthesized using phosphoramidite; (d) expressing the plurality of tumor-reactive TCRs or a subset thereof in the plurality of recipient cells; and (e) administering (i) at least one recipient cell of the plurality of recipient cells into the subject or (ii) at least two recipient cells of the plurality of recipient cells into the subject, wherein the at least two recipient cells express different TCRs.

[0062] In some cases, the method does not comprise chemical synthesis of the polynucleotides encoding the plurality of tumor-reactive TCRs or a subset thereof using phosphoramidite. In some cases, the plurality of T cells is tumor-infiltrating T cells or peripheral T cells. In some cases, the plurality of T cells comprises CD8+ T cells, CD4+ T cells, exhausted T cells, regulatory T cells, or any combinations thereof. In some cases, the plurality of recipient cells is allogeneic T cells, autologous T cells, or cell line cells. In some cases, the method further comprises, prior to (b), expressing the plurality of TCRs in a plurality of reporter cells. In some cases, expressing comprises delivering nucleic acid sequences encoding the plurality of TCRs by a virus vector. In some cases, the virus vector is a lentivirus vector. In some cases, each reporter cell of the plurality of reporter cells comprises a reporter gene. In some cases, in (b), identifying comprises contacting the plurality of TCRs with one or more target antigens, or a cell or a tissue presenting one or more target antigens. In some cases, the one or more target antigens are presented by one or more tumor cells or antigen presenting cells (APCs). In some cases, the one or more APCs comprise a target antigen coding DNA or RNA. In some cases, the one or more target antigens are in complex with a MHC. In some cases, the MHC is a MHC tetramer. In some cases, the plurality of tumor-reactive TCRs of the plurality of TCRs comprises at least 2, 5, 10, 15, or 20 different cognate pairs of TCRs. In some cases, each TCR of the plurality of tumor-reactive TCRs is specific to a different epitope or a different protein. In some cases, each TCR of the plurality of tumor-reactive TCRs comprises a different (i) TCR alpha CDR3 sequence, (ii) TCR beta CDR3 variable domain sequence, (iii) TCR alpha variable domain sequence, (iv) TCR beta variable domain sequence, or (v) TCR alpha and TCR beta variable domain sequence in combination. In some cases, the plurality of tumor-reactive TCRs binds to a tumor cell from the subject but does not bind to a

healthy cell from the subject or bind to the healthy cell from the subject with at least 10-fold less affinity than to the tumor cell. In some cases, the plurality of recipient cells administered in (e) comprises at least 10, at least 20, at least 50, at least 100, at least 500, at least 1,000, at least 5,000, at least 10,000, at least 100,000, or at least 1,000,000 times more cells than the plurality of T cells isolated in (a).

[0063] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) isolating a population of T cells from the subject that expresses a population of T-cell receptors (TCRs), wherein the population of T cells comprises at most about 10,000 cells; (b) identifying a plurality of tumor-reactive TCRs from the population of TCRs; and (c) administering a plurality of cells expressing the plurality of tumor-reactive TCRs or a subset thereof into the subject, wherein the plurality of tumor-reactive TCRs or a subset thereof comprises at least 2 different cognate pairs.

[0064] According to another ~~aspect~~example, provided herein is a method of treating a tumor in a subject, comprising: (a) identifying a plurality of tumor-reactive T-cell receptors (TCRs) from a population of TCRs, wherein the population of TCRs comprise at least 50 different cognate pairs of TCRs; and (b) administering a plurality of cells expressing the plurality of tumor-reactive TCRs or a subset thereof into the subject, wherein the plurality of tumor-reactive TCRs or a subset thereof comprises at least 5, at least 10, at least 15, or at least 20 different cognate pairs of the at least 50 different cognate pairs, wherein the plurality of tumor-reactive TCRs are exogenous to the plurality of cells. In some cases, the plurality of tumor-reactive TCRs or a subset thereof comprises at least 5 TCRs, and wherein each TCR of the at least 5 TCRs (1) is specific to a different epitope or a different protein or (2) comprises a different (i) TCR alpha CDR3 sequence, (ii) TCR beta CDR3 variable domain sequence, (iii) TCR alpha variable domain sequence, (iv) TCR beta variable domain sequence, or (v) TCR alpha and TCR beta variable domain sequence in combination.

[0065] In some cases, the method further comprises, prior to (a), isolating a population of T cells expressing the population of TCRs from the subject. In some cases, the at least 50 different cognate pairs comprise V regions from at least 5, 10, 15, or 20 different V genes. In some cases, identifying comprises isolating the plurality of tumor-reactive TCRs by a marker. In some cases, the plurality of cells expressing the plurality of tumor-reactive TCRs or a subset thereof is a plurality of allogeneic cells, autologous cells or cell line cells. In some cases, the plurality of allogeneic cells expresses a protein that binds to an inhibitory Natural Killer (NK) cell receptor. In some cases, the protein is a B2M-HLA-E or B2M-HLA-G fusion protein.

[0066] According to another ~~aspect~~example, provided herein is a method for identifying a target-reactive T-cell receptor (TCR), comprising: (a) contacting a plurality of T cells from a first sample with a second sample comprising tumor cells from a subject or a third sample, wherein the third sample is derived from the second sample, and wherein the third sample comprises: (i) a target antigen from the tumor cells of the second sample or a nucleic acid encoding the target antigen, and an MHC, (ii) a cell presenting the target antigen in an MHC, or (iii) a cell comprising an MHC and a protein product encoded by the nucleic acid, and wherein a subset of the plurality of T cells binds to the target antigen in complex with the MHC; (b) isolating the subset of the plurality of T cells or a portion thereof of the first sample; (c) partitioning the subset of the plurality of T cells or a portion thereof into a plurality of compartments, wherein each compartment comprises an individual T cell of the subset of the plurality of T cells or a portion thereof; and (d) within each compartment, identifying a first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of a cognate pair of TCR of the individual T cell, thereby generating one or more paired polynucleotides.

[0067] In some cases, identifying comprises physically linking the first polynucleotide encoding the first TCR chain and the second polynucleotide encoding the second TCR chain of a cognate pair of TCR of the individual T cell. In some cases, the one or more paired polynucleotides are one or more fused polynucleotides. In some cases, identifying comprises sequencing the first polynucleotide encoding the first TCR chain and the second polynucleotide encoding the second TCR chain of a cognate pair of TCR of the individual T cell.

[0068] In some cases, the first polynucleotide and the second polynucleotide are not chemically synthesized using phosphoramidite. In some cases, the first polynucleotide and the second polynucleotide are transcribed or amplified products of endogenous nucleic acids of the individual T cell. In some cases, the first sample or the second sample is isolated from a subject. In some cases, the first sample and the second sample are isolated from a same subject. In some cases, the first sample and the second sample are isolated from a different subject. In some cases, the first sample or the second sample is a tissue sample, a blood sample, a PBMC sample, or a combination thereof. In some cases, the tissue sample is a tumor tissue or a healthy tissue. In some cases, the first sample or the second sample is isolated from a subject by core biopsy, fine-needle biopsy, or apheresis. In some cases, isolating comprises isolating the subset of the plurality of T cells or a portion thereof by a marker. In some cases, the marker is a cell surface marker or a cytokine. In some cases, the cell surface marker is CD39, CD69, CD103, CD25, PD-1, TIM-3, OX-40, 4-1BB, CD137, CD3, CD28, CD4, CD8, CD45RA, CD45RO, GITR, FoxP3, or a combination thereof. In some cases, the cell presenting the target antigen is a tumor cell, an antigen-presenting cell (APC),

an artificial APC, or any combinations thereof. In some cases, the APC or the aAPC is pulsed with the target antigen. In some cases, the cell comprising a protein product encoded by the nucleic acid is an APC or an aAPC delivered (e.g., transfected or electroporated) with the nucleic acid or a derivative thereof. In some cases, the APC or the aAPC is further delivered an additional nucleic acid encoding an MHC. In some cases, the nucleic acid or a derivative thereof is a DNA or a RNA. In some cases, the cell presenting the target antigen or the cell comprising a protein product encoded by the nucleic acid is isolated from a subject or is a cell line cell. In some cases, the cell presenting the target antigen or the cell comprising a protein product encoded by the nucleic acid is isolated from the same subject from which the first sample and the second sample are isolated. In some cases, the method further comprises generating one or more vectors comprising the one or more fused polynucleotides, each vector of the one or more vectors comprising a fused polynucleotide of the one or more fused polynucleotides. In some cases, the method further comprises delivering the one or more vectors into a plurality of cells, wherein each cell of the plurality of cells comprises at least one vector of the one or more vectors. In some cases, the method further comprises expressing the one or more fused polynucleotides in the plurality of cells, wherein a subset of the plurality of cells expresses a plurality of target-reactive TCRs. In some cases, the method further comprises contacting the plurality of cells with one or more target antigens, wherein the subset of the plurality of cells expressing the plurality of target-reactive TCRs binds to the one or more target antigens. In some cases, the method further comprises identifying one or more target-reactive TCR of the plurality of target-reactive TCRs. In some cases, the method further comprises delivering a polynuclotide encoding the one or more target-reactive TCR into a plurality of recipient cells. In some cases, the method further comprises administering one or more cells of the plurality of recipient cells into the subject.

[0069] According to another ~~aspect~~example, provided herein is a pharmaceutical composition comprising a recipient cell comprising a sequence encoding a target-reactive or tumor-reactive TCR identified by a method described herein.

[0070] According to another ~~aspect~~example, provided herein is a method comprising administering the pharmaceutical composition described herein into a subject in need thereof. In some cases, the recipient cell is administered at a dose from about 1 x 10^9 cells to about 1 x 10^{11} cells.

[0071] Additional ~~aspect~~examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative ~~embodiment~~examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different ~~embodiment~~examples, and its several details are capable of

CLAIMS

What is claimed is:

1. A method of identifying a plurality of target-reactive T-cell receptors (TCRs), comprising:

(a) providing a mixture comprising a plurality of cells expressing a plurality of TCRs, each cell of the plurality of cells expressing a TCR of the plurality of TCRs, wherein the plurality of TCRs comprises at least 50 different cognate pairs from a sample from a subject and comprises V regions from a plurality of V genes, wherein each cognate pair of the at least 50 different cognate pairs comprises a TCR alpha (TCRα) chain paired with a TCR beta (TCRβ) chain, and wherein the plurality of TCRs are exogenous to the plurality of cells;

(b) contacting the mixture comprising the plurality of cells from (a) with one or more target peptide antigens in complex with a major histocompatibility complex (MHC) molecule, wherein a subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target peptide antigens in complex with the MHC molecule; and

(c) identifying at least two cells of the subset of the plurality of cells from (b), ~~which at least two cells~~ that express at least two target-reactive TCRs of the plurality of target-reactive TCRs, thereby identifying the at least two target-reactive TCRs of the plurality of target-reactive TCRs.

2. The method of claim 1, wherein the plurality of V genes comprises at least 10 different V genes.

3. The method of claim 1 or 2, wherein the plurality of cells is isolated from a sample from a subject, and wherein the sample is a tissue sample, a blood sample, a peripheral blood mononuclear cell (PBMC) sample, or a combination thereof.

4. The method of any one of claims 1-3, wherein the plurality of TCRs comprises at least 100 different cognate pairs, wherein each cognate pair of the at least 100 different cognate pairs comprises a TCRα chain paired with a TCRβ chain.

5. The method of any one of claims 1-4, wherein (b) comprises contacting the plurality of cells with one or more cells presenting the one or more target peptide antigens in complex with the MHC molecule.

6. The method of claim 5, wherein the one or more cells are one or more tumor cells, tumorspheres, tumor lysate-pulsed antigen-presenting cells (APCs), or APCs engineered to present the one or more target peptide antigens.

7. The method of claim 6, wherein the one or more APCs engineered to present the one or more target peptide antigens comprise a ~~target antigen coding~~ DNA or RNA encoding the one or more target peptide antigens.

8. The method of claim 6, wherein the one or more APCs engineered to present the one or more target peptide antigens exogenously expresses an ~~major histocompatibility complex (~~MHC~~)~~ molecule from a subject.

9. The method of any one of claims 1-8, wherein the plurality of TCRs comprises a TCR from an exhausted T cell~~(b) comprises contacting the plurality of cells with the one or more target antigens in complex with a MHC molecule~~.

10. The method of any one of claims ~~9~~1-4, wherein the MHC molecule is an MHC tetramer.

11. The method of any one of claims 1-10, wherein the sequence or identity of the one or more target peptide antigens is unknown.

~~12. The method of any one of claims 1-11, further comprising administering at least one of the at least two cells of the subset of the plurality of cells or a cell expressing a TCR of at least one of the at least two cells of the subset of the plurality of cells into a subject.~~

~~13.~~12. The method of any one of claims 1-1~~2~~, wherein each cell of the plurality of cells comprises a reporter gene, which reporter gene is regulated to send a signal when a TCR of the cell binds to a target peptide antigen of the one or more target peptide antigens, and wherein identifying in (c) comprises selecting the at least two cells based on the reporter gene.

~~14.~~13. The method of any one of claims 1-1~~23~~, wherein identifying in (c) comprises selecting the at least two cells based on expression of a cell surface marker.

~~15.~~14. The method of any one of claims 1-1~~34~~, wherein identifying in (c) comprises selecting the at least two cells based on calcium influx.

~~16.~~15. The method of any one of claims 1-1~~45~~, wherein the plurality of cells is cell line cells.

~~17.~~16. The method of any one of claims 1-1~~56~~, wherein the plurality of TCRs comprises at least 100 different VJ combinations.

~~18.~~17. The method of any one of claims 1-1~~6~~7, wherein the plurality of TCRs comprises TCRs from a sample from a subject~~, and wherein the subject has~~ with a disease or a condition.

~~19.~~18. The method of any one of claims 1-1~~7~~8, wherein the plurality of TCRs comprises TCRs from a sample comprising tumor-infiltrating lymphocytes from a subject.

~~20.~~19. The method of any one of claims 1-1~~8~~9, further comprising, prior to (a), physically linking a first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of each TCR of the plurality of TCRs, thereby generating a plurality of fused polynucleotides; and delivering the plurality of fused polynucleotides or ~~a derivative~~ copies thereof into the plurality of cells.

20. Use of at least one of the at least two cells identified in (c) of any one of claims 1-19 or a cell expressing a TCR of at least one of the at least two cells identified in (c) of any one of claims 1-19 for the manufacture of a medicament for the treatment of cancer or auto-immune disease in a subject.

21. At least one of the at least two cells identified in (c) of any one of claims 1-19 or a cell expressing a TCR of at least one of the at least two cells identified in (c) of any one of claims 1-19 for use in therapy.