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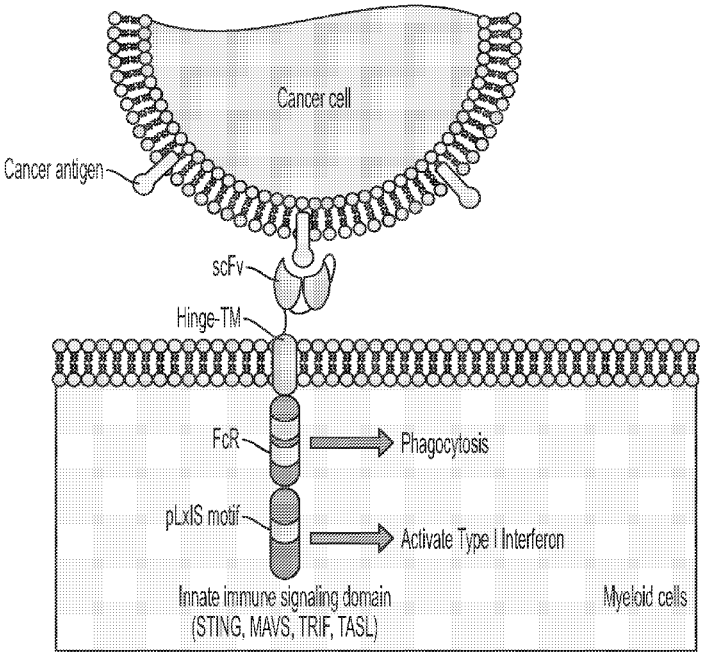


FIG. 1C

(57) Abstract: Compositions and methods for making and using engineered cells, such as, engineered myeloid cells that express a chimeric fusion protein that has a binding domain capable to binding surface molecules on target cells such as diseased cells.

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[Continued on next page]

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ENGINEERED CHIMERIC FUSION PROTEIN COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/162,352 filed on March 17, 2021, U.S. Provisional Application No. 63/172,922, filed on April 9, 2021, U.S. Provisional Application No. 63/243,947, filed on September 14, 2021, and U.S. Provisional Application No. 63/255,540, filed on October 14, 2021, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Cellular immunotherapy is a promising new technology for fighting difficult to treat diseases, such as cancer, and persistent infections and also certain diseases that are refractory to other forms of treatment. A major breakthrough has come across with the discovery of CAR-T cell and their potential use in immunotherapy. CAR-T cells are T lymphocytes expressing a chimeric antigen receptor which helps target the T cell to specific diseased cells such as cancer cells, and can induce cytotoxic responses intended to kill the target cancer cell or immunosuppression and/or tolerance depending on the intracellular domain employed and co-expressed immunosuppressive cytokines. Although CAR T cells continue to remain prospective tools for cancer therapy, several limitations along the way has slowed the progress on CAR-T cells and dampened its promise in clinical trials.

[0003] Understanding the limitations of CAR-T cells is the key to leveraging the technology and continue innovations towards better immunotherapy models. Specifically, in T cell malignancies, CAR-T cells appear to have faced a major problem. CAR-T cells and malignant T cells share surface antigen in most T cell lymphomas (TCL), therefore, CAR-T cells are subject to cytotoxicity in the same way as cancer cells. In some instances, the CAR-T products may be contaminated by malignant T cells. Additionally, T cell aplasia is a potential problem due to prolonged persistence of the CAR-T cells. Other limitations include the poor ability for CAR-T cells to penetrate into solid tumors and the potent tumor microenvironment which acts to downregulate their anti-tumor potential. CAR-T cell function is also negatively influenced by the immunosuppressive tumor microenvironment (TME) that leads to endogenous T cell inactivation and exhaustion.

[0004] Myeloid cells, including macrophages, are cells derived from the myeloid lineage and belong to the innate immune system. They are derived from bone marrow stem cells which egress into the blood and can migrate into tissues. Some of their main functions include phagocytosis, the activation of T cell responses, and clearance of cellular debris and extracellular matrices. They also play an important role in maintaining homeostasis, and initiating and resolving inflammation. Moreover, myeloid cells can differentiate into numerous downstream cells, including macrophages, which can display different responses ranging from pro-inflammatory to anti-inflammatory depending on the type of stimuli they receive from the surrounding microenvironment. Furthermore, tissue macrophages

have been shown to play a broad regulatory and activating role on other immune cell types including CD8+ and CD4+ T effector cells, NK cells and T regulatory cells. Macrophages have been shown to be a main immune infiltrate in malignant tumors and have been shown to have a broad immunosuppressive influence on effector immune infiltration and function.

SUMMARY

[0005] The diverse functionality of myeloid cells makes them an ideal cell therapy candidate that can be engineered to have numerous therapeutic effects. The present disclosure is related to immunotherapy using myeloid cells (e.g., CD14+ cells) of the immune system, particularly phagocytic cells. A number of therapeutic indications could be contemplated using myeloid cells. For example, myeloid cell immunotherapy could be exceedingly important in treating cancer, autoimmunity, fibrotic diseases and infections. The present disclosure is related to immunotherapy using myeloid cells, including phagocytic cells of the immune system, particularly monocytes. It is an object of the invention disclosed herein to harness one or more of these functions of myeloid cells for therapeutic uses. For example, it is an object of the invention disclosed herein to harness the phagocytic activity of myeloid cells, including engineered myeloid cells, for therapeutic uses. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote T cell activation. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote secretion of tumoricidal molecules. For example, it is an object of the invention disclosed herein to harness the ability of myeloid cells, including engineered myeloid cells, to promote recruitment and trafficking of immune cells and molecules. In one aspect the disclosure provides new and useful chimeric constructs that, when expressed in a myeloid cell, the myeloid cell can drive targeted attack and phagocytosis of the molecule, molecular assembly, object or a cell that comprises the target, e.g., a target antigen on its surface. One of the many facets of the present disclosure is to (i) enhance the phagocytic ability of the myeloid cells (e.g., the engineered myeloid cells expressing the new and improved chimeric constructs); help initiate a coordinated and sustained immune response against the target (e.g., target antigen). The present disclosure provides innovative methods and compositions that can successfully transfect or transduce a myeloid cell, or otherwise induce a genetic modification in a myeloid cell, with the purpose of augmenting a functional aspect of a myeloid cell, additionally, without compromising the cell's differentiation capability, maturation potential, and/or its plasticity. The resultant cells may be termed therapeutically effective engineered myeloid cells, or effector myeloid cells. One strategy for improvement described herein is to induce an inflammatory phenotype of the myeloid cells to develop effector myeloid cells. One strategy is to generate effector myeloid cells capable of mounting an inflammatory phenotype upon engagement with the target. In one aspect, the effector myeloid cell is capable of inducing or activating an interferon type I response within the myeloid cells upon engagement to its target. Another strategy is to potentiate the interferon type I

response in the myeloid cells to develop these cells into effector myeloid cells. One strategy for inducing an inflammatory phenotype is to induce or activate an NF-kB response in the effector myeloid cell upon engagement to its target. One strategy to develop myeloid cells isolated from a biological sample into effector myeloid cells for immunotherapy described herein is to induce and/or potentiate interferon type I response or NF-kappa B response or both in the myeloid cells.

[0006] The present disclosure involves making and using engineered myeloid cells (e.g., CD14+ cells, such as macrophages or other phagocytic cells, which can attack and kill (ATAK) diseased cells directly and/or indirectly, such as cancer cells and infected cells. Engineered myeloid cells, such as macrophages and other phagocytic cells, can be prepared by incorporating nucleic acid sequences (e.g., mRNA, DNA, plasmids, viral constructs) encoding a chimeric fusion protein (CFP), that has an extracellular binding domain specific to disease associated antigens (e.g., cancer antigens), into the cells using, for example, recombinant nucleic acid technology, synthetic nucleic acids, gene editing techniques (e.g., CRISPR), transduction (e.g., using viral constructs), electroporation, or nucleofection. It has been found that myeloid cells can be engineered to have a broad and diverse range of activities. For example, it has been found that myeloid cells can be engineered to express a chimeric fusion protein (CFP) containing an antigen binding domain to have a broad and diverse range of activities. For example, it has been found that myeloid cells can be engineered to have enhanced phagocytic activity such that upon binding of the CFP to an antigen on a target cell, the cell exhibits increased phagocytosis of the target cell. It has also been found that myeloid cells can be engineered to promote T cell activation such that upon binding of the CFP to an antigen on a target cell, the cell promotes activation of T cells, such as T cells in the tumor microenvironment. The engineered myeloid cells can be engineered to promote secretion of tumoricidal molecules such that upon binding of the CFP to an antigen on a target cell, the cell promotes secretion of tumoricidal molecules from nearby cells. The engineered myeloid cells can be engineered to promote recruitment and trafficking of immune cells and molecules such that upon binding of the CFP to an antigen on a target cell, the cell promotes recruitment and trafficking of immune cells and molecules to the target cell or a tumor microenvironment.

[0007] The present disclosure is based on the important finding that engineered myeloid cells overcome at least some of the limitations of CAR-T cells, including being readily recruited to solid tumors; having an engineerable duration of survival, therefore lowering the risk of prolonged persistence resulting in aplasia and immunodeficiency; myeloid cells cannot be contaminated with T cells; myeloid cells can avoid fratricide, for example, because they do not express the same antigens as malignant T cells; and myeloid cells have a plethora of anti-tumor functions that can be deployed. In some respects, engineered myeloid derived cells can be safer immunotherapy tools to target and destroy diseased cells.

[0008] Moreover, myeloid cells, such as macrophages, have been ubiquitously found in the tumor environment (TME) and are notably the most abundant cells in some tumor types. As part of their role in the immune system, myeloid cells, such as macrophages, are naturally engaged in clearing diseased cells. The present invention relates too harnessing myeloid cell function and specifically for targeting, killing and directly and/or indirectly clearing diseased cells as well as the delivery payloads such as antigens and cytokines.

[0009] Engineered myeloid cells can also be short-lived *in vivo*, phenotypically diverse, sensitive, plastic, and are often found to be difficult to manipulate *in vitro*. For example, exogenous gene expression in monocytes has been difficult compared to exogenous gene expression in non-hematopoietic cells. There are significant technical difficulties associated with transfecting myeloid cells (e.g., monocytes/macrophages). As professional phagocytes, myeloid cells, such as monocytes/macrophages, comprise many potent degradative enzymes that can disrupt nucleic acid integrity and make gene transfer into these cells an inefficient process. This is especially true of activated macrophages which undergo a dramatic change in their physiology following exposure to immune or inflammatory stimuli. Viral transduction of these cells are not ideal because macrophages are end-stage cells that generally do not divide; therefore, some of the vectors that depend on integration into a replicative genome have met with limited success. Furthermore, macrophages are quite responsive to "danger signals," and therefore several of the original viral vectors that were used for gene transfer induced potent anti-viral responses in these cells making these vectors inappropriate for gene delivery. Additionally, myeloid cells have potential to differentiate into phenotypic variants that have distinct functionality, and under certain circumstances may be ineffective for the purpose intended for in vivo use. For example, a myeloid cell necessary for active migration to tumor and effective tumoricidal activity may be required to be delivered in vivo in a cellular stage that retains its plasticity, based on identification of certain cell surface markers. Myeloid cell plasticity can be affected by the nature of isolation, handling, mode of introduction of nucleic acid material into the cell ex vivo in order to engineer the cell, and so forth. While myeloid cells of various cellular stages (e.g., myeloid cells at high, or low plasticity stages, or myeloid cells with phenotypes conferring to inflammatory or else immunomodulatory function) are each of particular value for the purpose intended for in vivo use, and that all of which are within the purview of the instant disclosure, at least in one aspect, the methods discussed herein focus on generation of a therapeutic myeloid cell population with the retention of a high degree myeloid cell plasticity prior to in vivo administration. In one aspect, the present disclosure provides innovative methods and compositions that can successfully transfect or transduce a myeloid cell, or otherwise induce a genetic modification in a myeloid cell, with the purpose of augmenting a functional aspect of a myeloid cell, additionally, without compromising the cell's differentiation capability, maturation potential, and/or its plasticity.

[0010] In one aspect, provided herein, are therapeutic agents that bind to an antigen expressed on a target cell, such as a diseased cell, for example, a cancer cell. Binding of the therapeutic agents to a target antigen on a target cell can initiate the process of destruction of the target cell. In some embodiments, the therapeutic agent is a recombinant nucleic acid that can be expressed in a cell of a subject, such as a mammalian cell, a human cell, a myeloid cell or a monocyte. In some embodiments, the therapeutic agent is a recombinant protein that can bind to a target antigen on a target cell. In some embodiments, the therapeutic agent is a cell, for example, a mammalian cell, a human cell, a myeloid cell or a monocyte, wherein the cell comprises a recombinant nucleic acid, and/or expresses a recombinant protein, such that the myeloid cell can be targeted to a diseased cell, expressing the target antigen on the surface of the cell; and the myeloid cell lyses or phagocytizes the diseased cell.

[0011] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the chimeric fusion protein responds to an extracellular cue, wherein the intracellular domain influences the intracellular mechanism of action and activation of the myeloid cell upon receiving the extracellular cue. In one embodiment, the chimeric fusion protein is a chimeric receptor, having an extracellular antigen binding domain in addition to (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, and engagement of the extracellular antigen binding domain of the chimeric fusion protein to the target antigen that the extracellular binding domain binds to provides the extracellular cue to the receptor and for the receptor mediated activation of the myeloid cell.

[0012] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain with a Xaa1-Leu-Xaa3-Iso-Ser motif, wherein Xaa1 is a hydrophilic amino acid, and Xaa3 is any amino acid. In some embodiments, the Ser of Xaa1-Leu-Xaa3-Iso-Ser is a phosphorylation site.

[0013] In some embodiments, Xaa1 is serine, threonine, tyrosine, histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is serine. In some embodiments, Xaa1 is threonine. In some embodiments, Xaa1 is tyrosine. In some embodiments, Xaa1 is histidine. In some embodiments, Xaa1 is asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is asparagine. In some embodiments, Xaa1 is aspartic acid. In some embodiments, Xaa1 is glutamine. In some embodiments, Xaa1 is glutamic acid. In some embodiments, Xaa1 is lysine. In some embodiments, Xaa1 is arginine.

[0014] In some embodiments, Xaa1 is histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is histidine. In some embodiments, Xaa1 is asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is asparagine. In some embodiments, Xaa1 is aspartic acid. In some embodiments, Xaa1 is glutamine. In some

embodiments, Xaa1 is glutamic acid. In some embodiments, Xaa1 is lysine. In some embodiments, Xaa1 is arginine. In some embodiments, Xaa1 is not serine. In some embodiments, Xaa1 is not threonine. In some embodiments, Xaa1 is not tyrosine.

[0015] In some embodiments, Xaa1 is serine, asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is serine. In some embodiments, Xaa1 is asparagine. In some embodiments, Xaa1 is aspartic acid. In some embodiments, Xaa1 is glutamine. In some embodiments, Xaa1 is glutamic acid. In some embodiments, Xaa1 is lysine. In some embodiments, Xaa1 is arginine. In some embodiments, Xaa1 is not histidine. In some embodiments, Xaa1 is not threonine. In some embodiments, Xaa1 is not tyrosine.

[0016] In some embodiments, Xaa1 is asparagine, aspartic acid, glutamine, glutamic acid, lysine or arginine. In some embodiments, Xaa1 is asparagine. In some embodiments, Xaa1 is aspartic acid. In some embodiments, Xaa1 is glutamine. In some embodiments, Xaa1 is glutamic acid. In some embodiments, Xaa1 is lysine. In some embodiments, Xaa1 is arginine. In some embodiments, Xaa1 is not serine. In some embodiments, Xaa1 is not threonine. In some embodiments, Xaa1 is not tyrosine. In some embodiments, Xaa1 is not histidine.

[0017] In some embodiments, Xaa1-Leu-Xaa3-Iso-Ser is SLHIS.

[0018] In some embodiments, Xaa1-Leu-Xaa3-Iso-Ser is NLEIS.

[0019] In some embodiments, Xaa1-Leu-Xaa3-Iso-Ser is DLAIS.

[0020] In some embodiments, Xaa1-Leu-Xaa3-Iso-Ser is ELLIS.

[0021] In some aspects, the chimeric fusion protein further comprises an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain. In some embodiments, the intracellular domain comprises at least one additional intracellular signaling domain. In some embodiments, the at least one additional intracellular signaling domain is derived form an intracellular PI3-kinase recruitment domain, a phagocytosis receptor intracellular domain, a pattern recognition receptor intracellular domain, a CD40 intracellular domain, an FcR intracellular domain, or a cytokine or chemokine receptor intracellular domain. In some embodiments, intracellular signaling domain comprises an amino acid sequence derived from a cytosolic adaptor protein, a mitochondrial membrane protein or an endoplasmic reticulum membrane protein. In one aspect, the intracellular signaling domain of the chimeric fusion protein comprises an amino acid sequence derived from a TRIF protein. In some embodiments, intracellular signaling domain comprises a truncated TRIF intracellular domain. In some embodiments, the sequence of the truncated TRIF intracellular domain is about 150 to 300 amino acids in length, about 150 to 270 amino acids in length, or about 150-250 amino acids in length. In some embodiments, intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 36. In some embodiments, intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 37. In some

embodiments, intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 38. In some embodiments, wherein intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 41.

[0022] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises a signaling domain from G protein-coupled receptor 84 (GPR84), G protein subunit beta 2 (GNB2) or Phosphatidylinositol-3,4,5-Trisphosphate Dependent Rac Exchange Factor 1 (PREX1). The intracellular signaling domains of GPR84, GNB2 or PREX1 can have pro-phagocytic activity. In some embodiments, the chimeric fusion protein further comprises an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain. In some embodiments, the signaling domain from GPR84, GNB2 or PREX1 is an intracellular signaling domain from GPR84, GNB2 or PREX1.

[0023] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 39.

[0024] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 40.

[0025] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 42.

[0026] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 43.

[0027] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 45.

[0028] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain,

wherein the transmembrane domain is derived from CD68 and comprises a mutation that inhibits or prevents homodimerization.

[0029] In some embodiments, the chimeric fusion protein further comprises an intracellular domain operably linked to the transmembrane domain. In some embodiments, the transmembrane domain comprises SEQ ID NO: 47.

[0030] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain, wherein the transmembrane domain is derived from CD64 or CD89; and wherein the chimeric fusion protein lacks an intracellular signaling domain. In some embodiments, the extracellular domain, wherein the transmembrane domain is derived from CD16 protein, e.g., CD16a protein. In some embodiments, the chimeric fusion protein forms a complex with an FcRγ chain when expressed in a cell.

[0031] In one aspect, provided herein is a recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 48.

[0032] In some embodiments, the chimeric fusion protein further comprises a second intracellular signaling domain that comprises a PI3 kinase recruitment domain. In some embodiments, the CFP further comprises an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 39 and the sequence of an IRF activation enhancing motif. In some embodiments, the CFP further comprising an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 40 and the sequence of an IRF activation enhancing motif.

[0033] In one embodiment, the CFP further comprising an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 39 and SEQ ID NO: 40.

[0034] In some embodiments, the intracellular domain binds to an IRF protein. In some embodiments, the intracellular domain comprises a TRAF protein binding domain. In some embodiments, the intracellular domain comprises a TBK1 phosphorylation site. In some embodiments, the CFP further comprises a second, third, or fourth intracellular signaling domain. In some embodiments, the CFP further comprises an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain.

[0035] In some embodiments, the antigen binding domain comprises a domain having an affinity for a tumor antigen. In some embodiments, the extracellular domain comprises a T lymphocyte antigen binding domain. In some embodiments, the extracellular domain comprises a B lymphocyte antigen binding domain. In some embodiments, the antigen to which the antigen binding domain binds is selected from the group consisting of an antigen from CD2, CD3, CD4, CD5, CD7, CCR4, CD8,

CD30, CD45, CD56, Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD70, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin, an integrin receptor, Claudin 3.0, Claudin 18.2, Trop-2, PRSS21, VEGFR2, PDGFRβ, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, Dsg1, Dsg3, and IGLL1. In some embodiments, the antigen binding domain binds to CD5. In some embodiments, the antigen binding domain binds to HER2. In some embodiments, the antigen binding domain binds to CD137, CD70, Claudin 3.0, Claudin 18.2 or Trop-2. In some embodiments, the extracellular domain comprises a first target antigen binding domain, and a second target antigen binding domain that is non-identical to the first target binding domain. In some embodiments, the second target antigen binding domain binds to CD47. In some embodiments, the antigen binding domain comprises a domain having an affinity for a microbial antigen. In some embodiments, the antigen binding domain comprises a domain having an affinity for a viral antigen. In some embodiments, the domain comprises an antibody or a fragment thereof. In some embodiments, the domain comprises an scFv. In some embodiments, the transmembrane domain is derived from the transmembrane domain of CD8a, CD28, CD68, CD2, FcRγ, FcRα, FcRβ, FcRε, syntaxin 3, syntaxin 4, or syntaxin 5. In some embodiments, the extracellular domain comprises a hinge domain connecting the antigen binding domain and the transmembrane domain. In some embodiments, when expressed in a cell, the chimeric fusion protein upon binding to its target antigen activates the intracellular domain to induce IRF activation. In some embodiments, the recombinant nucleic acid is an RNA. In some embodiments, the recombinant nucleic acid is an mRNA. In some embodiments, the recombinant nucleic acid is associated with one or more lipids. In some embodiments, the recombinant nucleic acid is encapsulated in a liposome. In some embodiments, the liposome is a lipid nanoparticle.

[0036] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 52. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 52. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 52. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 52. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid

encoding a sequence of SEQ ID NO: 52 or a sequence having at least 80% sequence identity to SEQ ID NO: 52.

[0037] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 53. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 53. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 53. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 53. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 53, or a sequence having at least 80% sequence identity to SEQ ID NO: 53.

[0038] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 54. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 54. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 54. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 54. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 54, or a sequence having at least 80% sequence identity to SEQ ID NO: 54.

[0039] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 55. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 55. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 55. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 55. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 55, or a sequence having at least 80% sequence identity to SEQ ID NO: 55.

[0040] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 56. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 56. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 56. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 56. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 56, or a sequence having at least 80% sequence identity to SEQ ID NO: 56.

[0041] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 57. In one aspect, provided herein is a recombinant mRNA

construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 57. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 57. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 57. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 57, or a sequence having at least 80% sequence identity to SEQ ID NO: 57.

[0042] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 58. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 58. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 58. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 58. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 58, or a sequence having at least 80% sequence identity to SEQ ID NO: 58.

[0043] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 59. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 59. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 59. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 59. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 59, or a sequence having at least 80% sequence identity to SEQ ID NO: 59.

[0044] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 60. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 60. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 60. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 60. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 60, or a sequence having at least 80% sequence identity to SEQ ID NO: 60.

[0045] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 61. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 61. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 61. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 61. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid

encoding a sequence of SEQ ID NO: 61, or a sequence having at least 80% sequence identity to SEQ ID NO: 61.

[0046] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 62. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 62. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 62. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 62. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 62, or a sequence having at least 80% sequence identity to SEQ ID NO: 62.

[0047] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 63. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 63. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 63. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 63. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 63, or a sequence having at least 80% sequence identity to SEQ ID NO: 63.

[0048] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 64. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 64. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 64. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 64. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 64, or a sequence having at least 80% sequence identity to SEQ ID NO: 64.

[0049] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 65. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 65. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 65. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 65. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 65, or a sequence having at least 80% sequence identity to SEQ ID NO: 65.

[0050] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 66. In one aspect, provided herein is a recombinant mRNA

construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 66. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 66. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 66. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 66, or a sequence having at least 80% sequence identity to SEQ ID NO: 66.

[0051] In one aspect, provided herein is a recombinant nucleic acid encoding a sequence that is at least 80% identical to SEQ ID NO: 67. In one aspect, provided herein is a recombinant mRNA construct, encoding a sequence that is at least 80% identical to SEQ ID NO: 67. Provided herein is a recombinant mRNA construct, encoding a sequence that is at least 90% identical to SEQ ID NO: 67. Provided herein is a recombinant mRNA construct, encoding a sequence that is identical to SEQ ID NO: 67. In some embodiments, provided herein is a cell comprising a recombinant nucleic acid encoding a sequence of SEQ ID NO: 67, or a sequence having at least 80% sequence identity to SEQ ID NO: 67.

[0052] In some embodiments, the recombinant nucleic acid is a plasmid or a vector.

[0053] In one aspect, provided herein is a cell comprising the recombinant nucleic acid of any one of the embodiments described above. In some embodiments, the cell is an immune cell. In some embodiments, the cell is a myeloid cell, a lymphoid cell, a precursor cell, a stem cell or an induced pluripotent cell. In some embodiments, the cell is CD14+/CD16-.

[0054] In one aspect, provided herein is an engineered cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular signaling domain comprises an intracellular signaling domain that binds to an IFN transcription factor when activated.

[0055] In one aspect, provided herein is an engineered cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular signaling domain comprises an intracellular signaling domain derived from a mitochondrial receptor.

[0056] In some embodiments, the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 39. In some embodiments, the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 40.

[0057] In some embodiments, the intracellular domain comprises an intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR 9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, MAVS, TRIF, TASL, NLRP1, NLRP2, NLRP3, NLRP4, NLRP5, NLRP6, NLRP7,

NLRP89, NLRP9, NLRP10, NLRP11, NLRP12, NLRP13, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, CD40, Tank1-binding kinase (TBK), TNFR1, a chemokine, MHC Class II transactivator (CIITA), IPAF, BIRC1, a RIG-I-like receptor (RLR) protein, macrophage galactose-type lectin (MGL), DC-SIGN (CLEC4L), Langerin (CLEC4K), Myeloid DAP12 associating lectin (MDL)-1 (CLEC5A), a DC associated C type lectin 1 (Dectin1) subfamily protein, dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C type lectin like receptor (MICL) (CLEC12A), CLEC2 (CLEC1B), CLEC12B, a DC immunoreceptor (DCIR) subfamily protein, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), and Mincle (macrophage inducible C type lectin) (CLEC4E).

[0058] In some embodiments, the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 42.

[0059] In some embodiments, the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 43.

[0060] In some embodiments, the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 45.

[0061] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain that binds to a IFN transcription factor when activated.

[0062] In some embodiments, the IFN transcription factor is any one of the proteins IRF1-IRF9.

[0063] In some embodiments, the intracellular domain comprises a TRAF recruitment domain.

[0064] In some embodiments, the intracellular domain that induces an IKK phosphorylation.

[0065] In some embodiments, the intracellular domain induces a TBK phosphorylation.

[0066] In some embodiments, the antigen binding domain binds to a tumor antigen or a microbial antigen.

[0067] In some embodiments, the antigen binding domain binds to one or more of CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, CD56, Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD70, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin, an integrin receptor, Claudin 3.0, Claudin 18.2, Trop-2, PRSS21,

VEGFR2, PDGFRβ, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, Dsg1, Dsg3, and IGLL1.

[0068] In some embodiments, the antigen binding domain binds to a viral antigen.

[0069] In some embodiments, the transmembrane domain is derived from the transmembrane domain of CD8a, CD28, CD68, CD2, FcRγ, FcRα, FcRβ, FcRε, syntaxin 3, syntaxin 4, or syntaxin 5.

[0070] In some embodiments, the intracellular domain further comprises an intracellular signaling domain derived from an FcRγ, FcRα, or FcRε. In some embodiments, the intracellular domain further comprises PI3-kinase recruitment domain.

[0071] In some embodiments, the intracellular signaling domain binds to an IRF transcription factor and activates it upon binding of the antigen binding domain to its cognate antigen.

[0072] In some embodiments, the cell induces pro-inflammatory cytokines upon binding of the antigen binding domain to its cognate antigen.

[0073] In some embodiments, the pro-inflammatory cytokines comprise one or more of IL-1, IL-6, IL-12, IL-23, TNF, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27 and interferons.

[0074] In some embodiments, the cell is a myeloid cell, a lymphocyte or a stem cell.

[0075] In some embodiments, the cell is a myeloid cell.

[0076] Also provided herein, in one aspect of the disclosure is a pharmaceutical composition comprising the recombinant nucleic acid of any one of the embodiments described herein, the cell of any one of the embodiments described, or the engineered cell of any one of the embodiments described; and a pharmaceutically acceptable excipient

[0077] In some embodiments, the engineered cell is CD14+/CD16-.

[0078] The pharmaceutical composition of the embodiments described above, comprising a population of cells wherein at least 50% of the cells are CD14+/CD16-, and less than 10% cells are dendritic cells. In some embodiments, the cells exhibit high expression of CCR2. In some embodiments, the cells do not exhibit tonal signaling and activation de novo, and exhibit M0, M1 or M2 differentiation upon activation.

[0079] In some aspects, provided herein is a method of treating a cancer or a viral infection in a subject comprising: administering to the subject the pharmaceutical composition of any one of the embodiments above.

[0080] In some aspects, provided herein is an engineered CD14+/CD16- cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular signaling domain comprises a domain derived from TRIF.

[0081] In some embodiments, the intracellular domain comprising a domain derived from TRIF is a truncated TRIF domain.

[0082] In some embodiments, the intracellular domain comprising a domain derived from TRIF comprises less than 300 amino acids.

[0083] In some embodiments, the intracellular domain comprising a domain derived from TRIF comprises less than 250 amino acids.

[0084] In some embodiments, the intracellular domain comprising a domain derived from TRIF exhibits IFN response upon engagement of the extracellular binding domain with the target.

[0085] In one aspect, provided herein is a method of treating a cancer in a subject, comprising administering to the subject a pharmaceutical composition of any one of the embodiments described above.

[0086] In one aspect, provided herein is a method of treating a cancer in a subject, comprising administering to the subject the pharmaceutical composition of any one of the embodiments described above.

[0087] In some embodiments, the cancer is selected from a group consisting of gastric cancer, ovarian cancer, renal cancer, breast cancer, prostate cancer, liver cancer, brain cancer, lymphoma, leukemia, skin cancer, pancreatic cancer, colorectal cancer, glioblastoma and lung cancer.

INCORPORATION BY REFERENCE

[0088] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings.

[0090] **FIG. 1A** depicts a schematic showing exemplary CFPs, containing an extracellular binding domain, a transmembrane domain. Left two figures represent CFP contain multiple intracellular signaling domains for augmenting intracellular signaling related to phagocytosis or other domains for intracellular signaling for augmenting immune response or inflammatory signaling inside the cell that expresses the CFP. The figure on the right exemplifies another exemplary chimeric fusion protein, that comprises a transmembrane domain derived from an Fc alpha R1 a chain (FcaR1 a chain or CD89) or an Fc gamma R1 a chain (FcgR1 a chain or CD64). Such a CFP as shown in the figure on the right can lack an intracellular domain of its own, and intracellular signaling will occur if and only if the CFP binds to an endogenous protein in the cell in which it is expressed that has an intracellular domain or otherwise trigger an intracellular signaling. As described elsewhere in the disclosure, an exemplary

CFP as shown in the figure on the right side can bind to an endogenous Fc gamma receptor expressed on a myeloid cell, and thereafter it is functional in intracellular signaling and activation of the myeloid cell via the Fc gamma receptor activation. The extracellular domains are target binding domains.

[0091] FIG. 1B is a graphical representation of an exemplary myeloid cell expressing CFP receptors and engaging a cancer cell by binding to target antigens on the cancer cell. Activation of intracellular signaling domain upon target engagement potentiates phagocytosis. TM, transmembrane domain.

[0092] FIG. 1C shows a graphical representation of an exemplary CFP expressed on a myeloid cell, the CFP targets a cancer antigen, and has an intracellular domain capable for activating Type 1 interferon. FcR, Fc receptor.

[0093] FIG. 1D shows a schematic of an exemplary nucleic acid encoding an exemplary chimeric antigen receptor construct comprising an intracellular signaling domain (ICD) having a pLxIS motif to trigger type I interferon response.

[0094] FIG. 2A upper panel depicts a schematic showing exemplary CFP construct designs with indicated domains. Each construct is generated and used for functional characterization. Each have a hinge and TM domain derived from CD8 molecule. FIG. 2A lower panel show flow cytometry data demonstrating expression of the respective constructs on the upper panel in a THP-1 cell.

[0095] FIG. 2B upper panel depicts a schematic showing exemplary CFP construct designs with indicated domains. Each construct is generated and used for functional characterization. Each have a hinge and TM domain derived from CD68 molecule. FIG. 2B lower panel show flow cytometry data demonstrating expression of the respective constructs shown in the upper panel in a THP-1 cell.

[0096] FIG. 2C upper panel depicts a schematic showing exemplary CFP construct designs with indicated domains. The construct on the left have a CD64 transmembrane domain, and the construct on the right has a CD89 transmembrane domain . FIG. 2C lower panel show flow cytometry data demonstrating expression of the respective constructs shown in the upper panel in a THP-1 cell.

[0097] FIG. 2D upper panel depicts a schematic showing exemplary CFP construct designs with indicated domains. The two construct on the left each has a CD64 transmembrane domain and additional intracellular signaling domains as follows: the first CFP from left contains a PI3K recruitment domain and a signaling domain derived from MDA5; second from left contains a CD40 intracellular signaling domain and a PI3K recruitment domain. The two construct on the right has a CD89 transmembrane domain and additional intracellular signaling domains as follows: the third from left CFP contains a PI3K recruitment domain and a signaling domain derived from MDA5; fourth from left contains a CD40 intracellular signaling domain and a PI3K recruitment domain.. FIG. 2D lower panel show flow cytometry data demonstrating expression of the respective constructs shown in the upper panel in a THP-1 cell.

[0098] FIG. 2E shows quantitative data of relative expression levels of each of the constructs indicated in the figure in THP-1 cells from analysis of flow cytometry results.

[0099] FIG. 2F shows exemplary CFP design (upper panel), and expression data (lower panel) of the constructs in THP-1 cells. The respective domains are as indicated in the figure. HER-FcR-PI3K construct has a CD8 TM domain, others have a CD68 TM domain.

[00100] FIG. 2G shows exemplary CFP design (upper panel), and expression data (lower panel) of the constructs in THP-1 cells. The respective domains are as indicated in the figure.

[00101] FIG. 3 shows exemplary CFP designs with extracellular domains containing one or more scFvs, directed towards binding to a single target antigen or to multiple targets, with each scFv having a different antigen specificity.

[00102] FIG. 4A shows exemplary assay set up for quantitative assessment of phagocytosis of target cells by myeloid cells expressing a CFP. SKOV3 tumor cell lines are the exemplary target cells, labeled with a cytoplasmic dye. Phagocytosis of the target cell leads to release of the dye in the myeloid cell which is then analyzed by flow cytometry. Exemplary flow cytometry data from a representative experiment are also shown.

[00103] FIG 4B depicts representative quantitative data showing phagocytosis index of myeloid cells expressing each construct as indicated. E:T ratio, effector (myeloid) cell : target cell ratio. Experimental results were analyzed according to the method of FIG. 4A.

[00104] FIG. 4C depicts representative data showing phagocytosis index of myeloid cells expressing each construct as indicated.

[00105] FIG. 4D depicts representative data showing phagocytosis index of myeloid cells expressing each construct as indicated.

[00106] FIG. 4E depicts representative data showing phagocytosis index of myeloid cells expressing each construct as indicated. Exemplary flow cytometry plots are shown in the upper panel, and quantitative data shown in lower panel. The data demonstrated cells expressing HER2-FcR-PI3K-TRIF (short) construct show high phagocytic index compared to other constructs.

[00107] FIG. 5A shows exemplary assay set up for quantitative assessment of phagocytic lysis of target cells by myeloid cells expressing a CFP and multiplexed assay for analyzing cytokines and chemokines. Target cells express a luciferase gene, and upon engulfment and lysis of the target cell the luciferase is released in the myeloid cell which can be used to detect and quantitate direct target cell lysis.

[00108] FIG. 5B shows representative data of target cell lysis by myeloid cells each set expressing a CFP construct as indicated. Left, results from experiment using effector:target cell ratio of 1:1; Right, results from experiment using effector:target cell ratio of 5:1.

[00109] FIG. 5C shows data demonstrating NF-kappa B activation in THP-1 cells that were either mock electroporated or electroporated with the indicated CFP construct and assayed after 24 hours of transfection, in the presence or absence of target SKOV3 cells. Cells expressing constructs having CD64 intracellular domain alone show high NF-kappa B activation in presence of target cells.

Constructs having both PI3 kinase recruitment domain and an innate immune response signaling domain from CD40 exhibit tonic signaling (graph at the right) for NF-kappa B activation. Positive control is shown on the left graph using NF-kappa B agonist.

[00110] FIG. 5D shows data demonstrating IFN activation in THP-1 cells 24 hours after transfection with the CFP construct as in FIG. 5C or mock transfected. HER2-CD64 constructs do not activate IFN response. Multi-domain constructs (right graph) do not activate IFN in absence or in presence of the target cell. Positive controls are shown on the left graph using a STING agonist.

[00111] FIG. 5E shows data demonstrating NF-kappa B activation in THP-1 cells that were either mock electroporated or electroporated with the indicated CFP construct and assayed after 24 hours of transfection, in presence or absence of target SKOV3 cells. Each of the constructs has a CD89 intracellular signaling domain. CD89 intracellular domain alone is capable of activating NF-kappa B, however additional intracellular domains did not induce NF-kappa B activation.

[00112] FIG. 5F shows data demonstrating IFN activation in THP-1 cells 24 hours after transfection with the CFP construct as in FIG. 5E or mock transfected.

[00113] FIG. 5G shows data demonstrating NF-kappa B activation in THP-1 cells expressing individual constructs as indicated. Cells expressing constructs having FcR domain or FcR domain along with additional intracellular domains were tested and results shown.

[00114] FIG. 5H shows flow cytometry data demonstrating IFN activation in THP-1 cells 24 hours after electroporation with the CFP construct as indicated.

[00115] FIG. 5I shows data demonstrating expression of the different HER2-CFP constructs having different intracellular domains comprising TLR domains. Flow cytometry analysis of the cell surface expression of HER2 binder CFP using labeled antibody, anti-HER2-AF647 in mRNA electroporated THP-1 cells. Percentage of HER2-binder expressing cells are indicated in the figure.

[00116] FIG. 5J shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in THP-1 cells (top panel) and NF-kappa B activation 24 hours after electroporation with the CFP construct as indicated (bottom panel). Expression of HER2-FcR-PI3Kinase-MyD88 construct led to tonic NF-kappa B signaling (signaling in absence of the target cell SKOV3, that is, in absence of engagement of the extracellular binding domain of the CFP to the antigen on SKOV3 cells).

[00117] FIG. 5K shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in THP-1 cells and NF-kappa B activation and IFN activation 24 hours after electroporation with the HER2-CFP construct as indicated. The data demonstrated that tonic NF-kappa B and IFN gamma signaling was seen in the multi-domain constructs having FcR-PI3K-RIG1 intracellular domains, or FcR-PI3K-MDA5 intracellular domains.

[00118] FIG. 5L shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in THP-1 cells and IFN activation in THP-1 cells 24 hours after electroporation with the CFP constructs as indicated.

[00119] FIG. 5M shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs and NF-kappa B activation in THP-1 cells 24 hours after electroporation with the CFP construct as indicated.

[00120] FIG. 5N shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs and cytokine expression by THP-1 cells 24 hours after electroporation with the CFP mRNA construct as indicated.

[00121] FIG. 6A shows data demonstrating baseline cytokine (IL6 and TNFalpha) and chemokine (CCL3) expression by THP-1 cells expressing the indicated constructs, compared to mock transfected cells. Negative control (mock transfected) graphs appear on the left side of each CFP transfected graph per experimental set.

[00122] FIG. 6B shows data demonstrating baseline cytokine (IL6 and TNFalpha) expression by THP-1 cells expressing the indicated constructs, compared to mock transfected cells. Negative control (mock transfected) graphs appear on the left side of each CFP transfected graph per experimental set.

[00123] FIG. 6C shows data demonstrating baseline cytokine (IL6 and TNFalpha) expression by THP-1 cells expressing the indicated constructs, compared to mock transfected cells. Negative control (mock transfected) graphs appear on the left side of each CFP transfected graph per experimental set.

[00124] FIG. 6D shows data demonstrating baseline cytokine (IL6, TNFalpha and IFN-gamma as indicated on the respective graphs) and chemokine (CCL3, CCL5 and IP10 as indicated) expression by THP-1 cells expressing the indicated constructs, compared to mock transfected cells. Negative control (mock transfected) graphs appear on the left side of each CFP transfected graph per experimental set.

[00125] FIG. 6E shows data demonstrating cytokine and chemokine expression in cells transfected with the indicated constructs compared to mock transfected cells.

[00126] FIG. 6F shows data demonstrating cytokine and chemokine expression in cells transfected with the indicated constructs compared to mock transfected cells.

[00127] FIG. 7A shows diagrammatic representation of exemplary chimeric antigen receptor constructs with CD68 transmembrane domains.

[00128] FIG. 7B shows exemplary chimeric antigen receptors with the indicated domains. Top row, middle and right figures show exemplary CFPs with CD64 transmembrane domains that oligomerize with endogenous Fc gamma, and lack intracellular domains of their own. Middle row shows exemplary intracellular domains that were paired with various combinations of domains in the recombinant CFP construct designs. Bottom row shows exemplary CFPs comprising multiple domains.

[00129] FIG. 8A shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a first donor after electroporation of the indicated HER2-CFP constructs. For example, HER2-CD8hTM-CD40-FcR-Pi3K signifies a construct that has anti-

HER2-binding extracellular domain; CD8 hinge and transmembrane domains; and three separate intracellular domains that are derived from CD40, FcR and PI3kinase molecules.

[00130] FIG. 8B shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a second donor after electroporation of the indicated HER2-CFP constructs.

[00131] FIG. 8C shows graphs of the percentage of cells positive for the indicated HER2-CFP constructs and the mean fluorescence intensity using the data from **FIG. 8A** and **FIG. 8B**. Similar expression of the HER2-CFP constructs was observed in both donors.

[00132] FIG. 9A shows a graph of SKOV3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs. 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKOV3-GFP-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity.

[00133] FIG. 9B shows a graph of SKBR3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs. 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKBR3-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity.

[00134] FIG. 10A shows graphs of pro-inflammatory cytokine IL-6 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). For antigen stimulated HER2-CFP primary monocyte samples, a 96-well plate coated with 2.5 μg/mL of HER2-his protein and 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were added per well. Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. For tumor cell stimulated HER2-CFP primary monocyte samples, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 tumor cells (SKOV3-GFP-Luciferase cells or SKBR3-Luciferase cells). Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. Statistical significance was determined between HER2-CFP electroporated primary monocyte samples co-cultured with tumor cells and mock electroporated primary monocyte samples co-cultured with tumor cells.

[00135] FIG. 10B shows graphs of pro-inflammatory cytokine IL-1β induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 10A**.

[00136] FIG. 10C shows graphs of TNF-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 10A**.

[00137] FIG. 10D shows graphs of IFN-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 10A**.

[00138] FIG. 10E shows graphs of IP10 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 10A**.

[00139] FIG. 10F shows graphs of IL-12 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 10A**.

[00140] FIG. 11A shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a first donor after electroporation of the indicated HER2-CFP constructs.

[00141] FIG. 11B shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a second donor after electroporation of the indicated HER2-CFP constructs.

[00142] FIG. 11C shows graphs of the percentage of cells positive for the indicated HER2-CFP constructs and the mean fluorescence intensity using the data from **FIG. 11A** and **FIG. 11B**.

[00143] FIG. 12A shows a graph of SKOV3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs. 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKOV3-GFP-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity.

[00144] FIG. 12B shows a graph of SKBR3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs. 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKBR3-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity.

[00145] FIG. 13A shows graphs of pro-inflammatory cytokine IL-6 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and

stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). For antigen stimulated HER2-CFP primary monocyte samples, a 96-well plate coated with 2.5 μg/mL of HER2-his protein and 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were added per well. Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. For tumor cell stimulated HER2-CFP primary monocyte samples, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 tumor cells (SKOV3-GFP-Luciferase cells or SKBR3-Luciferase cells). Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. Statistical significance was determined between HER2-CFP electroporated primary monocyte samples co-cultured with tumor cells and mock electroporated primary monocyte samples co-cultured with tumor cells.

[00146] FIG. 13B shows graphs of pro-inflammatory cytokine IL-1β induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 13A**.

[00147] FIG. 13C shows graphs of TNF-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 13A**.

[00148] FIG. 13D shows graphs of IFN-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 13A**.

[00149] FIG. 13E shows graphs of IP10 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 13A**.

[00150] FIG. 13F shows graphs of IL-12 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). The experiments and analyses were performed as in **FIG. 13A**.

[00151] FIG. 14 shows graphs indicating tumor regression (left) and survival (right) comparisons in mice after administering CFP constructs having indicated intracellular domains.

DETAILED DESCRIPTION

[00152] T cells therapies have revolutionized cancer treatment for many patients. However, for the majority of patients with advanced solid tumors, sustained clinical benefit has not been achieved.

Unlike T cells, myeloid cells readily accumulate in tumors, in some cases contributing up to 50% of the tumor mass. Myeloid cells can be specifically engineered to become highly effective anti-tumor cells, referred to as Activate, Target, Attack & Kill (ATAK) cells, that specifically target, phagocytize and lyse tumor cells, and orchestrate an immune activation in vivo against the tumor cells.

[00153] The present application is based on an approach towards augmenting engineered myeloid cells to evade the immunosuppressive tumor microenvironment and exhibit high tumor cell lytic/immunogenic potency. Engaging innate immune signaling sensors such as Toll-like receptors and STING-cGAS have been investigated as important pathways to upregulate pro-inflammatory anti-tumor immune response and are associated with anti-tumor immunity. These approaches, using synthetic agonists to activate these pathways, can be potent but delivering a localized and tumor specific activation of innate immune signaling is difficult to achieve. Many conventional chemotherapeutics, targeted anticancer agents, immunological adjuvants and oncolytic viruses are only fully efficient in the presence of intact type I IFN signaling. Immunotherapy using recombinant type I interferons and IFN encoding vectors have been attempted. For example, TLR-9 agonist has been combined with chemotherapy to treat previously untreated stage IIIb or stage IV non–small cell lung carcinoma (NSCLC) (Manegold, C., et al. Addition of PF-3512676 (CpG 7909) to a taxane/platinum regimen for first-line treatment of unresectable non-small cell lung cancer (NSCLC) improves objective response - phase II clinical trial. *Eur. J. Cancer.* **3**:326, 2005). Another study suggested that administration of IFN-a producing-iPSC-derived proliferating myeloid cells in combination with immune checkpoint blockade might be useful to overcome resistance to single-treatment modalities, e.g., in cancers refractory to checkpoint blockade (Tsuchiya, N., et al., Cell Reports 29, 162–175, October, 2019)

[00154] The present approach disclosed herein is to generate a new class of chimeric antigen receptors (ATAK receptors) that couple tumor recognition with innate immune signaling. Such recombinant chimeric receptors are expressed in myeloid cells described herein, such that via the chimeric receptor, the myeloid cell is targeted to a cancer cell, and then further activate innate immune response in the myeloid cell upon tumor recognition, leading to phagocytic lysis of the tumor cell, and further activation of the lymphocytic immune response. By combining cancer recognition domains with intracellular signaling domains from innate immune receptors such as FcRg, TLR and cytokine receptors, the chimeric antigen receptors as designed and described herein demonstrate that myeloid cells can be programmed to recognize cancer and elicit a broad and tunable immune response. The data show the versatility of building ATAK receptors by harnessing innate immune pathways and support their clinical development in cell and direct in vivo therapies.

[00155] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[00156] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[00157] Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the disclosure can also be implemented in a single embodiment.

[00158] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosure.

[00159] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the disclosure, and vice versa. Furthermore, compositions of the disclosure can be used to achieve methods of the disclosure.

[00160] The term "about" or "approximately" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-30% or less, +/-20% or less, +/-10% or less, +/-5% or less, or +/-1% or less of and from the specified value, insofar such variations are appropriate to perform in the present disclosure. It is to be understood that the value to which the modifier "about" or "approximately" refers is itself also specifically disclosed.

[00161] An "agent" can refer to any cell, small molecule chemical compound, antibody or fragment thereof, nucleic acid molecule, or polypeptide.

[00162] An "alteration" or "change" can refer to an increase or decrease. For example, an alteration can be an increase or decrease of 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, or by 40%, 50%, 60%, or even by as much as 70%, 75%, 80%, 90%, or 100%. For example, an alteration can be an increase or decrease of 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, or by 40-fold, 50-fold, 60-fold, or even by as much as 70-fold, 75-fold, 80-fold, 90-fold, or 100-fold.

[00163] An "antigen presenting cell" or "APC" as used herein includes professional antigen presenting cells (e.g., B lymphocytes, macrophages, monocytes, dendritic cells, Langerhans cells), as well as other antigen presenting cells (e.g., keratinocytes, endothelial cells, astrocytes, fibroblasts, oligodendrocytes, thymic epithelial cells, thyroid epithelial cells, glial cells (brain), pancreatic beta cells, and vascular endothelial cells). An APC can express Major Histocompatibility complex (MHC)

molecules and can display antigens complexed with MHC on its surface which can be recognized by T cells and trigger T cell activation and an immune response. Professional antigen-presenting cells, notably dendritic cells, play a key role in stimulating naive T cells. Nonprofessional antigen-presenting cells, such as fibroblasts, may also contribute to this process. APCs can also cross-present peptide antigens by processing exogenous antigens and presenting the processed antigens on class I MHC molecules. Antigens that give rise to proteins that are recognized in association with class I MHC molecules are generally proteins that are produced within the cells, and these antigens are processed and associate with class I MHC molecules.

[00164] A "biological sample" can refer to any tissue, cell, fluid, or other material derived from an organism.

[00165] The term "epitope" can refer to any protein determinant, such as a sequence or structure or amino acid residues, capable of binding to an antibody or binding fragment thereof, a T cell receptor, and/or an antibody-like molecule. Epitopic determinants typically consist of chemically active surface groups of molecules such as amino acids or sugar side chains and generally have specific three dimensional structural characteristics as well as specific charge characteristics. A "T cell epitope" can refer to peptide or peptide-MHC complex recognized by a T cell receptor.

[00166] An engineered cell, such as an engineered myeloid cell, can refer to a cell that has at least one exogenous nucleic acid sequence in the cell, even if transiently expressed. Expressing an exogenous nucleic acid may be performed by various methods described elsewhere, and encompasses methods known in the art. The present disclosure relates to preparing and using engineered cells, for example, engineered myeloid cells, such as engineered phagocytic cells. The present disclosure relates to, *inter alia*, an engineered cell comprising an exogenous nucleic acid encoding, for example, a chimeric fusion protein (CFP).

[00167] The term "immune response" includes, but is not limited to, T cell mediated, NK cell mediated and/or B cell mediated immune responses. These responses may be influenced by modulation of T cell costimulation and NK cell costimulation. Exemplary immune responses include T cell responses, e.g., cytokine production, and cellular cytotoxicity. In addition, immune responses include immune responses that are indirectly affected by NK cell activation, B cell activation and/or T cell activation, e.g., antibody production (humoral responses) and activation of cytokine responsive cells, e.g., macrophages. Immune responses include adaptive immune responses. The adaptive immune system can react to foreign molecular structures, such as antigens of an intruding organism. Unlike the innate immune system, the adaptive immune system is highly specific to a pathogen. Adaptive immunity can also provide long-lasting protection. Adaptive immune reactions include humoral immune reactions and cell-mediated immune reactions. In humoral immune reactions, antibodies secreted by B cells into bodily fluids bind to pathogen-derived antigens leading to elimination of the pathogen through a variety of mechanisms, e.g. complement-mediated lysis. In cell-mediated immune reactions, T cells

capable of destroying other cells are activated. For example, if proteins associated with a disease are present in a cell, they can be fragmented proteolytically to peptides within the cell. Specific cell proteins can then attach themselves to the antigen or a peptide formed in this manner, and transport them to the surface of the cell, where they can be presented to molecular defense mechanisms, such as T cells. Cytotoxic T cells can recognize these antigens and kill cells that harbor these antigens.

[00168] A "ligand" can refer to a molecule which is capable of binding or forming a complex with another molecule, such as a receptor. A ligand can include, but is not limited to, a protein, a glycoprotein, a carbohydrate, a lipoprotein, a hormone, a fatty acid, a phospholipid, or any component that binds to a receptor. In some embodiments, a receptor has a specific ligand. In some embodiments, a receptor may have promiscuous binding to a ligand, in which case it can bind to several ligands that share at least a similarity in structural configuration, charge distribution or any other physicochemical characteristic. A ligand may be a biomolecule. A ligand may be an abiotic material. For example, a ligand may be a negative charged particle that is a ligand for scavenger receptor MARCO. For example, a ligand may be TiO_2, which is a ligand for the scavenger receptor SRA1.In the context of a CFP described herein, the extracellular binding domain may bind to a ligand, which is a also designated as a target of the binding domain. In some embodiments, the target is an antigen expressed on a diseased cell, such as a cancer cell, which in this case is a target cell, in the sense that the target cell expresses on its cell surface a target antigen to which the extracellular antigen binding domain of the CFP binds. Anti-(target) binding domain or anti-(target) binding extracellular domain or anti-(target) CFP are often interchangeably used with terms such as (target) binding domain or (target) binding extracellular domain or (target) CFP respectively in the disclosure. For example, HER2 expressed on cancer cells is an antigen (ligand) to which the anti-HER2 binding extracellular domain of a CFP binds; or alternatively stated as, a HER2-binding extracellular domain of a CFP binds.

[00169] The term "major histocompatibility complex (MHC)", "MHC molecule", or "MHC protein" refers to a protein capable of binding an antigenic peptide and present the antigenic peptide to T lymphocytes. Such antigenic peptides can represent T cell epitopes. The human MHC is also called the HLA complex. Thus, the terms "human leukocyte antigen (HLA)", "HLA molecule" or "HLA protein" are used interchangeably with the terms "major histocompatibility complex (MHC)", "MHC molecule", and "MHC protein". HLA proteins can be classified as HLA class I or HLA class II. The structures of the proteins of the two HLA classes are very similar; however, they have very different functions. Class I HLA proteins are present on the surface of almost all cells of the body, including most tumor cells. Class I HLA proteins are loaded with antigens that usually originate from endogenous proteins or from pathogens present inside cells, and are then presented to naïve or cytotoxic T-lymphocytes (CTLs). HLA class II proteins are present on antigen presenting cells (APCs), including but not limited to dendritic cells, B cells, and macrophages. They mainly present peptides which are processed from external antigen sources, e.g. outside of cells, to helper T cells.

[00170] In the HLA class II system, phagocytes such as macrophages and immature dendritic cells can take up entities by phagocytosis into phagosomes – though B cells exhibit the more general endocytosis into endosomes – which fuse with lysosomes whose acidic enzymes cleave the uptaken protein into many different peptides. Autophagy is another source of HLA class II peptides. The most studied subclass II HLA genes are: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1.

[00171] Presentation of peptides by HLA class II molecules to CD4+ helper T cells can lead to immune responses to foreign antigens. Once activated, CD4+ T cells can promote B cell differentiation and antibody production, as well as CD8+ T cell (CTL) responses. CD4+ T cells can also secrete cytokines and chemokines that activate and induce differentiation of other immune cells. HLA class II molecules are typically heterodimers of α-and β-chains that interact to form a peptide-binding groove that is more open than class I peptide-binding grooves.

[00172] HLA alleles are typically expressed in codominant fashion. For example, each person carries 2 alleles of each of the 3 class I genes, (HLA-A, HLA-B and HLA-C) and so can express six different types of class II HLA. In the class II HLA locus, each person inherits a pair of HLA-DP genes (DPA1 and DPB1, which encode α and β chains), HLA-DQ (DQA1 and DQB1, for α and β chains), one gene HLA-DRα (DRA1), and one or more genes HLA-DRβ (DRB1 and DRB3, -4 or-5). HLA-DRB1, for example, has more than nearly 400 known alleles. That means that one heterozygous individual can inherit six or eight functioning class II HLA alleles: three or more from each parent. Thus, the HLA genes are highly polymorphic; many different alleles exist in the different individuals inside a population. Genes encoding HLA proteins have many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles), each of which is given a particular number. In some embodiments, the class I HLA alleles are HLA-A*02:01, HLA-B*14:02, HLA-A*23:01, HLA-E*01:01 (non-classical). In some embodiments, class II HLA alleles are HLA-DRB*01:01, HLA-DRB*01:02, HLA-DRB*11:01, HLA-DRB*15:01, and HLA-DRB*07:01.

[00173] A "myeloid cell" can refer broadly to cells of the myeloid lineage of the hematopoietic cell system, and can exclude, for example, the lymphocytic lineage. Myeloid cells comprise, for example, cells of the granulocyte lineage and monocyte lineages. Myeloid cells are a major cellular compartment of the immune system comprising monocytes, dendritic cells, tissue macrophages, and granulocytes. Models of cellular ontogeny, activation, differentiation, and tissue-specific functions of myeloid cells have been revisited during the last years with surprising results. However, their enormous plasticity and heterogeneity, during both homeostasis and disease, are far from understood. Although myeloid cells have many functions, including phagocytosis and their ability to activate T cells, harnessing these functions for therapeutic uses has remained elusive. Newer avenues are

therefore sought for using other cell types towards development of improved therapeutics, including but not limited to T cell malignancies.

[00174] Myeloid cells are differentiated from common progenitors derived from the hematopoietic stem cells in the bone marrow. Commitment to myeloid cell lineages may be governed by activation of distinct transcription factors, and accordingly myeloid cells may be characterized as cells having a level of plasticity, which may be described as the ability to further differentiate into terminal cell types based on extracellular and intracellular stimuli. Myeloid cells can be rapidly recruited into local tissues via various chemokine receptors on their surface. Myeloid cells are responsive to various cytokines and chemokines.

[00175] A myeloid cell, for example, may be a cell that originates in the bone marrow from a hematopoietic stem cell under the influence of one or more cytokines and chemokines, such as G-CSF, GM-CSF, Flt3L, CCL2, VEGF and S100A8/9. In some embodiments, the myeloid cell is a precursor cell. In some embodiments, the myeloid cell may be a cell having characteristics of a common myeloid progenitor, or a granulocyte progenitor, a myeloblast cell, or a monocyte-dendritic cell progenitor or a combination thereof. A myeloid can include a granulocyte or a monocyte or a precursor cell thereof. A myeloid can include an immature granulocyte, an immature monocyte, an immature macrophage, an immature neutrophil, and an immature dendritic cell. A myeloid can include a monocyte or a pre-monocytic cell or a monocyte precursor. In some cases, a myeloid cell as used herein may refer to a monocyte having an M0 phenotype, an M1 phenotype or an M2 phenotype. A myeloid can include a dendritic cell (DC), a mature DC, a monocyte derived DC, a plasmacytoid DC, a pre-dendritic cell, or a precursor of a DC. A myeloid can include a neutrophil, which may be a mature neutrophil, a neutrophil precursor, or a polymorphonucleocyte (PMN). A myeloid can include a macrophage, a monocyte-derived macrophage, a tissue macrophage, a macrophage of an M0, an M1 or an M2 phenotype. A monocyte or a macrophage exhibit polarization. "Polarization" as used herein may refer to a process by which macrophages exhibit distinct functional phenotypes in response to specific microenvironmental stimuli and signals, often referred to as physiological states. In some cases, macrophages can pass from one polarization state to another. For example, macrophages can be polarized into classically activated (M1) and alternatively activated (M2) macrophages. M2 macrophages are divided into M2a, M2b, M2c, and M2d subcategories. These macrophages differ in their cell surface markers, secreted cytokines and biological functions. M1 macrophages are typically characterized by phenotypes in which the cells express TLR-2, TLR-4, CD80, CD86, iNOS, and MHC-II on the surface. These cells release various cytokines and chemokines e.g., TNF-α, IL-1α, IL-1β, IL-6, IL-12, CXCL9, and CXCL10, and typically exhibit activation of transcription factors, such as NF-kB, STAT1, STAT5, IRF3, and IRF5 that regulate the expression of M1 genes. It is believed that NF-κB and STAT1 are the two major pathways involved in M1 macrophage polarization. The M1 phenotype is associated with microbicidal and tumoricidal functions of macrophages, exhibiting high

phagocytic and inflammatory function. On the other hand, tumor associated macrophages subject to immunosuppressive environment become generally more M2 polarized. A myeloid can include a tumor infiltrating monocyte (TIM). A myeloid can include a tumor associated monocyte (TAM). A myeloid can include a myeloid derived suppressor cell (MDSC). A myeloid can include a tissue resident macrophage. A myeloid can include a tumor associated DC (TADC). Accordingly, a myeloid cell may express one or more cell surface markers, for example, CD11b, CD14, CD15, CD16, CD38, CCR5, CD66, Lox-1, CD11c, CD64, CD68, CD163, CCR2, CCR5, HLA-DR, CD1c, CD83, CD141, CD209, MHC-II, CD123, CD303, CD304, a SIGLEC family protein and a CLEC family protein. In some cases, a myeloid cell may be characterized by a high or a low expression of one or more of cell surface markers, for example, CD11b, CD14, CD15, CD16, CD66, Lox-1, CD11c, CD64, CD68, CD163, CCR2, CCR5, HLA-DR, CD1c, CD83, CD141, CD209, MHC-II, CD123, CD303, CD304 or a combination thereof. In one embodiment, activating the M1 polarization of macrophages are desirable using the methods described herein.

[00176] "Phagocytosis" is used interchangeably with "engulfment" and can refer to a process by which a cell engulfs a particle, such as a cancer cell or an infected cell. This process can give rise to an internal compartment (phagosome) containing the particle. This process can be used to ingest and or remove a particle, such as a cancer cell or an infected cell from the body. A phagocytic receptor may be involved in the process of phagocytosis. The process of phagocytosis can be closely coupled with an immune response and antigen presentation. The processing of exogenous antigens follows their uptake into professional antigen presenting cells by some type of endocytic event. Phagocytosis can also facilitate antigen presentation. For example, antigens from phagocytosed cells or pathogens, including cancer antigens, can be processed and presented on the cell surface of APCs.

[00177] A "polypeptide" can refer to a molecule containing amino acids linked together via a peptide bond, such as a glycoprotein, a lipoprotein, a cellular protein or a membrane protein. A polypeptide may comprise one or more subunits of a protein. A polypeptide may be encoded by a recombinant nucleic acid. In some embodiments, polypeptide may comprise more than one peptide sequence in a single amino acid chain, which may be separated by a spacer, a linker or peptide cleavage sequence. A polypeptide may be a fused polypeptide. A polypeptide may comprise one or more domains, modules or moieties.

[00178] A "receptor" can refer to a chemical structure composed of a polypeptide, which transduces a signal, such as a polypeptide that transduces an extracellular signal to a cell. A receptor can serve to transmit information in a cell, a cell formation or an organism. A receptor comprises at least one receptor unit and can contain two or more receptor units, where each receptor unit comprises a protein molecule, e.g., a glycoprotein molecule. A receptor can contain a structure that binds to a ligand and can form a complex with the ligand. Signaling information can be transmitted by a conformational change of the receptor following binding with the ligand on the surface of a cell.

[00179] The term "antibody" refers to a class of proteins that are generally known as immunoglobulins, including, but not limited to IgGl, IgG2, IgG3, and IgG4), IgA (including IgAl and IgA2), IgD, IgE, IgM, and IgY, The term "antibody" includes, but is not limited to, full length antibodies, single-chain antibodies, single domain antibodies (sdAb) and antigen-binding fragments thereof. Antigen-binding antibody fragments include, but are not limited to, Fab, Fab' and F(ab')2, Fd (consisting of V_H and C_H1), single-chain variable fragment (scFv), single-chain antibodies, disulfide-linked variable fragment (dsFv) and fragments comprising a V_L and/or a V_H domain. Antibodies can be from any animal origin. Antigen-binding antibody fragments, including single-chain antibodies, can comprise variable region(s) alone or in combination with tone or more of a hinge region, a CH1 domain, a CH2 domain, and a CH3 domain. Also included are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. Antibodies can be monoclonal, polyclonal, chimeric, humanized, and human monoclonal and polyclonal antibodies which, e.g., specifically bind an HLA-associated polypeptide or an HLA-peptide complex.

[00180] The term "recombinant nucleic acid" refers a nucleic acid prepared, expressed, created or isolated by recombinant means. A recombinant nucleic acid can contain a nucleotide sequence that is not naturally occurring. A recombinant nucleic acid may be synthesized in the laboratory. A recombinant nucleic acid may be prepared by using recombinant DNA technology, for example, enzymatic modification of DNA, such as enzymatic restriction digestion, ligation, and DNA cloning. A recombinant nucleic acid can be DNA, RNA, analogues thereof, or a combination thereof. A recombinant DNA may be transcribed ex vivo or in vitro, such as to generate a messenger RNA (mRNA). A recombinant mRNA may be isolated, purified and used to transfect a cell. A recombinant nucleic acid may encode a protein or a polypeptide. Throughout the specification, nucleic acid sequences are described which may comprise deoxyribonucleotides (DNA), ribonucleotides (RNA), or in some embodiments, modified deoxyribonucleotides, or modified ribonucleotides. For example, a modified nucleotide may be a 5-hydroxymethylcytosine (5hmC), a 5-formylacytosine (5fC), a 7-methylguanosine, a pseudouridine, a dihydrouridine etc. One of skill in the art can determine an RNA sequence, e.g., an mRNA sequence from a given polynucleotide sequence without difficulty. Sequences may be codon optimized.

[00181] The process of introducing or incorporating a nucleic acid into a cell can be via transformation, transfection or transduction. Transformation is the process of uptake of foreign nucleic acid by a bacterial cell. This process is adapted for propagation of plasmid DNA, protein production, and other applications. Transformation introduces recombinant plasmid DNA into competent bacterial cells that take up extracellular DNA from the environment. Some bacterial species are naturally competent under certain environmental conditions, but competence is artificially induced in a laboratory setting. Transfection is the introduction of small molecules such as DNA, RNA, or antibodies into eukaryotic cells. Transfection may also refer to the introduction of bacteriophage into bacterial cells.

'Transduction' is mostly used to describe the introduction of recombinant viral vector particles into target cells, while 'infection' refers to natural infections of humans or animals with wild-type viruses.

[00182] The term "vector", can refer to a nucleic acid molecule capable of autonomous replication in a host cell, and which allow for cloning of nucleic acid molecules. As known to those skilled in the art, a vector includes, but is not limited to, a plasmid, cosmid, phagemid, viral vectors, phage vectors, yeast vectors, mammalian vectors and the like. For example, a vector for exogenous gene transformation may be a plasmid. In certain embodiments, a vector comprises a nucleic acid sequence containing an origin of replication and other elements necessary for replication and/or maintenance of the nucleic acid sequence in a host cell. In some embodiments, a vector or a plasmid provided herein is an expression vector. Expression vectors are capable of directing the expression of genes and/or nucleic acid sequence to which they are operatively linked. In some embodiments, an expression vector or plasmid is in the form of circular double stranded DNA molecules. A vector or plasmid may or may not be integrated into the genome of a host cell. In some embodiments, nucleic acid sequences of a plasmid are not integrated in a genome or chromosome of the host cell after introduction. For example, the plasmid may comprise elements for transient expression or stable expression of the nucleic acid sequences, e.g. genes or open reading frames harbored by the plasmid, in a host cell. In some embodiments, a vector is a transient expression vector. In some embodiments, a vector is a stably expressed vector that replicates autonomously in a host cell. In some embodiments, nucleic acid sequences of a plasmid are integrated into a genome or chromosome of a host cell upon introduction into the host cell. Expression vectors that can be used in the methods as disclosed herein include, but are not limited to, plasmids, episomes, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages or viral vectors. A vector can be a DNA or RNA vector. In some embodiments, a vector provide herein is a RNA vector that is capable of integrating into a host cell's genome upon introduction into the host cell (e.g., via reverse transcription), for example, a retroviral vector or a lentiviral vector. Other forms of expression vectors known by those skilled in the art which serve the equivalent functions can also be used, for example, self-replicating extrachromosomal vectors or vectors capable of integrating into a host genome. Exemplary vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked.

[00183] In some embodiments, nucleic acid may be delivered into a living system in the form of nanoparticles. Nucleic acid sequences disclosed herein may be delivered in vivo via suitable nanoparticles, e.g., liposomes, lipid nanoparticles, or polymeric nanoparticles. A lipid nanoparticle may comprise a polar lipid. In some embodiments, the lipid nanoparticle comprises a cationic lipid. In some embodiments, the lipid nanoparticle comprises a cationic lipid and a non-cationic lipid. In some embodiments, the lipid nanoparticle comprises a neutral lipid. In some embodiments, the lipid nanoparticle comprises a PEGylated lipid.

[00184] Alternatively, in some embodiments, the nucleic acid can be electroporated in a living cell ex vivo for preparation of a cellular therapy, wherein the cell is a myeloid cell.

[00185] The terms "spacer" or "linker" as used in reference to a fusion protein refers to a peptide sequence that joins two other peptide sequences of the fusion protein. In some embodiments, a linker or spacer has no specific biological activity other than to join or to preserve some minimum distance or other spatial relationship between the proteins or RNA sequences. In some embodiments, the constituent amino acids of a spacer can be selected to influence some property of the molecule such as the folding, flexibility, net charge, or hydrophobicity of the molecule. Suitable linkers for use in an embodiment of the present disclosure are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. In some embodiments, a linker is used to separate two or more polypeptides, e.g. two antigenic peptides by a distance sufficient to ensure that each antigenic peptide properly folds. Exemplary peptide linker sequences adopt a flexible extended conformation and do not exhibit a propensity for developing an ordered secondary structure. Amino acids in flexible linker protein region may include Gly, Asn and Ser, or any permutation of amino acid sequences containing Gly, Asn and Ser. Other near neutral amino acids, such as Thr and Ala, also can be used in the linker sequence.

[00186] The terms "treat," "treated," "treating," "treatment," and the like are meant to refer to reducing, preventing, or ameliorating a disorder and/or symptoms associated therewith (e.g., a neoplasia or tumor or infectious agent or an autoimmune disease). "Treating" can refer to administration of the therapy to a subject after the onset, or suspected onset, of a disease (e.g., cancer or infection by an infectious agent or an autoimmune disease). "Treating" includes the concepts of "alleviating", which can refer to lessening the frequency of occurrence or recurrence, or the severity, of any symptoms or other ill effects related to the disease and/or the side effects associated with therapy. The term "treating" also encompasses the concept of "managing" which refers to reducing the severity of a disease or disorder in a patient, e.g., extending the life or prolonging the survivability of a patient with the disease, or delaying its recurrence, e.g., lengthening the period of remission in a patient who had suffered from the disease. It is appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition, or symptoms associated therewith be completely eliminated. The term "prevent", "preventing", "prevention" and their grammatical equivalents as used herein, can refer to avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences. In certain embodiments, treating a subject or a patient as described herein comprises administering a therapeutic composition, such as a drug, a metabolite, a preventive component, a nucleic acid, a peptide, or a protein that encodes or otherwise forms a drug, a metabolite or a preventive component. In some embodiments, treating comprises administering a cell or a population of cells to a subject in need thereof. In some embodiments, treating comprises

administering to the subject one or more of engineered cells described herein, e.g. one or more engineered myeloid cells, such as phagocytic cells. Treating comprises treating a disease or a condition or a syndrome, which may be a pathological disease, condition or syndrome, or a latent disease, condition or syndrome. In some cases, treating, as used herein may comprise administering a therapeutic vaccine. In some embodiments, the engineered phagocytic cell is administered to a patient or a subject. In some embodiments, a cell administered to a human subject results in reduced immunogenicity. For example, an engineered phagocytic cell may lead to no or reduced graft versus host disease (GVHD) or fratricide effect. In some embodiments, an engineered cell administered to a human subject is immunocompatible to the subject (i.e. having a matching HLA subtype that is naturally expressed in the subject). Subject specific HLA alleles or HLA genotype of a subject can be determined by any method known in the art. In exemplary embodiments, the methods include determining polymorphic gene types that can comprise generating an alignment of reads extracted from a sequencing data set to a gene reference set comprising allele variants of the polymorphic gene, determining a first posterior probability or a posterior probability derived score for each allele variant in the alignment, identifying the allele variant with a maximum first posterior probability or posterior probability derived score as a first allele variant, identifying one or more overlapping reads that aligned with the first allele variant and one or more other allele variants, determining a second posterior probability or posterior probability derived score for the one or more other allele variants using a weighting factor, identifying a second allele variant by selecting the allele variant with a maximum second posterior probability or posterior probability derived score, the first and second allele variant defining the gene type for the polymorphic gene, and providing an output of the first and second allele variant.

[00187] A "fragment" can refer to a portion of a protein or nucleic acid. In some embodiments, a fragment retains at least 50%, 75%, or 80%, or 90%, 95%, or even 99% of the biological activity of a reference protein or nucleic acid.

[00188] The terms "isolated," "purified", "biologically pure" and their grammatical equivalents refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of the present disclosure is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially

one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications can give rise to different isolated proteins, which can be separately purified.

[00189] The terms "neoplasia" or "cancer" refers to any disease that is caused by or results in inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. Glioblastoma is one non-limiting example of a neoplasia or cancer. The terms "cancer" or "tumor" or "hyperproliferative disorder" refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells can exist alone within an animal, or can be a non-tumorigenic cancer cell, such as a leukemia cell.

[00190] The term "vaccine" is to be understood as meaning a composition for generating immunity for the prophylaxis and/or treatment of diseases (e.g., neoplasia/tumor/infectious agents/autoimmune diseases). Accordingly, vaccines as used herein are medicaments which comprise recombinant nucleic acids, or cells comprising and expressing a recombinant nucleic acid and are intended to be used in humans or animals for generating specific defense and protective substance by vaccination. A "vaccine composition" can include a pharmaceutically acceptable excipient, carrier or diluent. Aspects of the present disclosure relate to use of the technology in preparing a phagocytic cell-based vaccine.

[00191] The term "pharmaceutically acceptable" refers to approved or approvable by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans. A "pharmaceutically acceptable excipient, carrier or diluent" refers to an excipient, carrier or diluent that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

[00192] Nucleic acid molecules useful in the methods of the disclosure include, but are not limited to, any nucleic acid molecule with activity or that encodes a polypeptide. Polynucleotides having substantial identity to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. "Hybridize" refers to when nucleic acid molecules pair to form a double-stranded molecule between complementary polynucleotide sequences, or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507). For example, stringent salt concentration can ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, or at least about 50% formamide. Stringent temperature conditions can

ordinarily include temperatures of at least about 30° C, at least about 37°C, or at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In an exemplary embodiment, hybridization can occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In another exemplary embodiment, hybridization can occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In another exemplary embodiment, hybridization can occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art. For most applications, washing steps that follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps can be less than about 30 mM NaCl and 3 mM trisodium citrate, or less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps can include a temperature of at least about 25°C, of at least about 42°C, or at least about 68°C. In exemplary embodiments, wash steps can occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In other exemplary embodiments, wash steps can occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In another exemplary embodiment, wash steps can occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

[00193] "Substantially identical" refers to a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Such a sequence can be at least 60%, 80% or 85%, 90%, 95%, 96%, 97%, 98%, or even 99% or more identical at the amino acid level or nucleic acid to the sequence used for comparison. Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative

substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program can be used, with a probability score between e-3 and e-m° indicating a closely related sequence. A "reference" is a standard of comparison. It will be understood that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used. Numbering might be different, e.g., in precursors of a mature protein and the mature protein itself, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment to a reference sequence and determination of homologous residues.

[00194] The term "subject" or "patient" refers to an organism, such as an animal (e.g., a human) which is the object of treatment, observation, or experiment. By way of example only, a subject includes, but is not limited to, a mammal, including, but not limited to, a human or a non-human mammal, such as a non-human primate, murine, bovine, equine, canine, ovine, or feline.

[00195] The term "therapeutic effect" refers to some extent of relief of one or more of the symptoms of a disorder (e.g., a neoplasia, tumor, or infection by an infectious agent or an autoimmune disease) or its associated pathology. On one hand it may indicate a reduction of a symptom of the disease, e.g., a 10%, 20%, 30% and so on reduction in tumor mass following administration of the therapeutic composition. On another embodiment, it can relate to partial or complete remission of one or more symptoms, or amelioration of the disease. "Therapeutically effective amount" as used herein refers to an amount of an agent which is effective, upon single or multiple dose administration to the cell or subject, in prolonging the survivability of the patient with such a disorder, reducing one or more signs or symptoms of the disorder, preventing or delaying, and the like beyond that expected in the absence of such treatment. "Therapeutically effective amount" is intended to qualify the amount required to achieve a therapeutic effect. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the "therapeutically effective amount" (e.g., ED50) of the pharmaceutical composition required.

[00196] Provided herein are engineered myeloid cells (including, but not limited to, neutrophils, monocytes, myeloid dendritic cells (mDCs), mast cells and macrophages), designed to specifically bind a target antigen. The target antigen may be expressed only on a target cell, such as an infected cell, a damaged cell, a malignant cell, a leukemia cell or a tumor cell. The engineered myeloid cells can attack and kill target cells directly (e.g., by phagocytosis) and/or indirectly (e.g., by activating T cells). In some embodiments, the target cell is a cancer cell.

[00197] While cancer is one exemplary embodiment described in detail in the instant disclosure, the methods and technologies described herein are contemplated to be useful in targeting an infected or

otherwise diseased cell inside the body. Similarly, therapeutic and vaccine compositions using the engineered cells are described herein.

[00198] Myeloid effector cells may be generated from the isolated myeloid cells from a human biological sample and modified ex vivo to prepare cells of therapeutic interest using methods to engineer such cells and such that the modifications do not alter the plasticity of these cells. Monocytic lineage cells are phagocytic and are efficient antigen presenter cells. In one aspect, the present invention stems from an important finding that engineered myeloid cells can be a highly efficient therapeutic modality in treating a number of diseases including cancer. Myeloid cells may be engineered to express a chimeric antigen receptor that enhances a myeloid cell's immune function in which the cells are highly phagocytic and can attack and kill a diseased cell or an infected cell in the body. The chimeric antigen receptor is a recombinant construct that is designed, and specifically modified as described herein, to be (a) highly target specific, specifically directed to bind to a target antigen, having an extracellular antigen binding domain, and (b) an intracellular domain that is highly specialized to activate a myeloid cell to attain an activate phagocytic cell phenotype. For example, highly specialized intracellular domains are designed to generate chimeric receptors that, upon activation by binding of the extracellular region of the receptor to the target, can generate signaling cues inside the cell that activate intracellular interferon signaling cascade, and transcription factors, namely, directs the activation of transcription factor IRFs (IFN regulatory factors). In addition, the methods and compositions described herein are also useful in gene therapy in which a recombinant nucleic acid encoding a chimeric antigen receptor is administered locally or systemically in a subject in need thereof, such that the recombinant nucleic acid is specifically expressed in a myeloid cell in vivo, and thereby generates activated myeloid cells having the therapeutic ability. In some embodiments, the nucleic acid is mRNA. In some embodiments the mRNA is delivered in an LNP.

[00199] Phagocytes are the natural sentinels of the immune system and form the first line of defense in the body. They engulf a pathogen, a pathogen infected cell, a foreign body, or a cancerous cell and remove it from the body. Most potential pathogens are rapidly neutralized by this system before they can cause, for example, a noticeable infection or a disease. This can involve receptor-mediated uptake through the clathrin-coated pit system, pinocytosis, particularly macropinocytosis as a consequence of membrane ruffling and phagocytosis. The phagocytes therefore can be activated by a variety of non-self (and self) elements and exhibit a level of plasticity in recognition of their "targets". Most phagocytes express scavenger receptors on their surface which are pattern recognition molecules and can bind to a wide range of foreign particles as well as dead cell, debris and unwanted particles within the body. In one aspect, recombinant nucleic acids encoding chimeric antigen receptors (CAR) may be expressed in the cells. The CARs may be variously designed to attack specific tumor cells, and myeloid effector cells expressing CARs can be activated to

phagocytose and kill tumor cells. The CARs may be designed to generate phagocytic receptors that are activated specifically in response to the target engagement, and the phagocytic potential of a macrophage is enhanced by specifically engineered intracellular domains of the receptor. The CAR platform for myeloid cells as described herein is designed such that no tonic signaling is detected in the myeloid cells at the time of administering in the body, or any time before the myeloid cell engages with its target via the CAR. This is often tested ex vivo. At the same time, the myeloid cells expressing the CAR can be further differentiated into M0, M1 or M2 phenotypes in presence of the suitable stimulus, and retain the cellular plasticity to do so at least at the time of administration. In addition, CAR-expressing myeloid effector cells can migrate to lymph nodes and cross-present antigens to naïve T cells in the lymph node thereby activating the adaptive response.

[00200] In some embodiments, disclosed herein are compositions and methods for generating myeloid cells that are isolated from a biological sample and engineered *ex vivo* to express a recombinant protein, and formulated into a pharmaceutical composition, such that the myeloid cells of the composition are "effector" myeloid cells efficient in induction of immune activation *in vivo*. In some embodiments, the myeloid cells of the composition are termed 'ATAK' myeloid cells where the cells are myeloid efficient in attacking and destroying target cells. The ATAK myeloid cells disclosed herein is an engineered myeloid cell that expresses a recombinant protein, e.g., a chimeric receptor, e.g., a chimeric antigen receptor, comprising at least one intracellular signaling domain that is derived from an interferon inducing protein in an immune cell. In some embodiments, the methods and compositions described herein are directed to render an engineered myeloid cell to exhibit the effector phenotype. In some embodiments, the engineered myeloid cells, e. g., monocytes are M0 or M1 phenotype monocytes, and the activation of the chimeric antigen receptor expressed in the myeloid cell renders the cells to exhibit the M1 phenotype. The M1 phenotype exhibited by the engineered cell renders the cell to be highly tumoricidal when designed to be targeted to a tumor cell.

[00201] Provided herein are compositions and methods for treating diseases or conditions, such as cancer. The compositions and methods provided herein utilize human myeloid cells, including, but not limited to, neutrophils, monocytes, myeloid dendritic cells (mDCs), mast cells and macrophages, to target diseased cells, such as cancer cells. The compositions and methods provided herein can be used to eliminate diseased cells, such as cancer cells and or diseased tissue, by a variety of mechanisms, including T cell activation and recruitment, effector immune cell activation (e.g., CD8 T cell and NK cell activation), antigen cross presentation, enhanced inflammatory responses, reduction of regulatory T cells and phagocytosis. For example, the myeloid cells can be used to sustain immunological responses against cancer cells.

[00202] Applicants previously described compositions comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), such as a phagocytic receptor (PR) fusion protein (PFP), a

scavenger receptor (SR) fusion protein (SFP), an integrin receptor (IR) fusion protein (IFP) or a caspase-recruiting receptor (caspase-CAR) fusion protein. A CFP encoded by the recombinant nucleic acid can comprise an extracellular domain (ECD) comprising an antigen binding domain that binds to an antigen of a target cell. The extracellular domain can be fused to a hinge domain or an extracellular domain derived from a receptor, such as CD2, CD8, CD28, CD68, a phagocytic receptor, a scavenger receptor or an integrin receptor. The CFP encoded by the recombinant nucleic acid can further comprise a transmembrane domain, such as a transmembrane domain derived from CD2, CD8, CD28, CD68, a phagocytic receptor, a scavenger receptor or an integrin receptor. In some embodiments, a CFP encoded by the recombinant nucleic acid further comprises an intracellular domain comprising an intracellular signaling domain, such as an intracellular signaling domain derived from a phagocytic receptor, a scavenger receptor or an integrin receptor. For example, the intracellular domain can comprise one or more intracellular signaling domains derived from a phagocytic receptor, a scavenger receptor or an integrin receptor. For example, the intracellular domain can comprise one or more intracellular signaling domains that promote phagocytic activity, inflammatory response, nitric oxide production, integrin activation, enhanced effector cell migration (e.g., via chemokine receptor expression), antigen presentation, and/or enhanced cross presentation. In some embodiments, the CFP is a phagocytic receptor fusion protein (PFP). In some embodiments, the CFP is a phagocytic scavenger receptor fusion protein (PFP). In some embodiments, the CFP is an integrin receptor fusion protein (IFP). In some embodiments, the CFP is an inflammatory receptor fusion protein. In some embodiments, a CFP encoded by the recombinant nucleic acid further comprises an intracellular domain comprising a recruitment domain. For example, the intracellular domain can comprise one or more PI3K recruitment domains, caspase recruitment domains or caspase activation and recruitment domains (CARDs).

[00203] Provided herein are improved immunogenic CAR compositions, for example, recombinant nucleic acid encoding a chimeric fusion protein (CFP, interchangeably termed chimeric antigen receptor, CAR) comprising an intracellular domain that activates interferon response in a cell expressing the CAR. Provided herein are immunogenic CFPs that comprise at least one intracellular domain comprising a pLxIS motif. The recombinant nucleic acid may be DNA or RNA. The recombinant nucleic acid encoding the CAR may be comprised in a vector. The recombinant CAR when expressed in a cell activates Type I interferon production in the cell. Such a cell is a mammalian cell, that is capable of Type 1 Interferon response. Such cell is an immune cell, e.g. a lymphocyte cell or a myeloid cell.

[00204] In some embodiments the recombinant nucleic acid encoding the chimeric receptor comprises a specific sequence therein that encodes a pro-inflammatory intracellular domain of the chimeric receptor. In some embodiments, the chimeric receptor protein described herein comprises an intracellular domain capable of activating an interferon response gene or a signaling cascade

leading to induction of Type I interferon production in the cell that expresses the chimeric antigen receptor upon engagement with its target at the extracellular domain. In some embodiments, the chimeric receptor protein described herein comprises a domain from an innate immune pathway adaptor protein, e.g., Mitochondrial antiviral-signaling protein (MAVS), Stimulator of interferon genes (STING), Toll/IL-1R domain-containing adaptor inducing IFN (TRIF), and TLR adaptor interacting with endolysosomal SLC15A4 protein (TASL), or a portion thereof. In some embodiments, a domain or fragment of an innate immune pathway adaptor protein e.g., MAVS, STING, TRIF or TASL proteins may be incorporated by recombinant DNA technology in the intracellular domain of the CFP or CAR as described herein, wherein the domain or fragment comprises a pLxIS motif (in which *p* represents the hydrophilic residue, *x* represents any residue, and S represents the phosphorylation site), that is phosphorylated by TBK1 or IKKε and mediates the recruitment of IRF-3 to the signaling complexes.

[00205] In some embodiments, the chimeric receptor protein described herein comprises an intracellular domain capable of activating nuclear factor kappa B responsive gene or a signaling cascade leading to induction of NF-kappa B response in the cell that expresses the chimeric antigen receptor upon engagement with its target at the extracellular domain.

Effector myeloid cells and interferon activation

[00206] Type I and Type II interferons (IFNs) play important roles in regulating immune responses during infections and cancer. Type I is represented by multiple subtypes including numerous IFNα family members, IFNβ, IFNδ, IFNε, IFNκ, IFNτ and IFNω, and all these utilize the same cell surface receptor, IFNαR, which is a heterodimer comprised of IFNαR1 and IFNαR2 proteins. Type II IFN is represented by IFNγ. These two IFN types bind to distinct cell surface receptors that are expressed by nearly all cells to trigger signal transduction events and elicit diverse cellular responses. Myeloid cells are key targets of interferons. During early immune responses to intracellular bacterial infections. Activated natural killer (NK) and T cells are the sources of IFNγ production. During early stages of infection, production of the cytokines interleukin (IL)-12 and IL-18 drives antigen-nonspecific IFNγ production by these lymphocyte populations. Antigen-specific $CD4^+$ and $CD8^+$ T cells also can produce IFNγ in response to these pathogens. There are a large number of individual type I IFNs, including ~20 IFNα proteins and a single IFNβ. Each of these type I IFNs signals to host cells by binding the conserved cell surface type I IFN receptor, IFNαR. Ligation of cell surface IFNαR induces expression of numerous antiviral immune stimulated gene (ISG) products and thus protects the host from certain viral infections (*Sadler AJ,* Interferon-inducible antiviral effectors. (Review) *Nat Rev Immunol. 2008 Jul; 8(7):559-68*). However, responsiveness to type I IFNs also correlates dramatically with increased susceptibility to a number of intracellular bacterial infections (*Rayamajhi M., et al.,* Antagonistic crosstalk between type I and II interferons and increased host susceptibility to bacterial infections. *Virulence. 2010 Sep-Oct; 1(5):418-22*), including *Listeria*

monocytogenes, *Mycobacterium tuberculosis*, *Fransicella tularensis*, and others. IFNγ is secreted as a homodimer and acts on host cells by ligating cell surface receptors. Each IFNγ receptor is a heterodimer comprised of two type I integral membrane subunits, IFNγR1 and IFNγR2. Binding of an IFNγ homodimer to the cell causes the aggregation of two receptor complexes, such that there are two IFNγR1 subunits and two IFNγR2 subunits, as well as additional signaling components. While both subunits are required for signal transduction, the actual binding site for IFNγ is located on IFNγR1 (*Kearney S. et al., Differential effects of type I and II interferons on myeloid cells and resistance to intracellular bacterial infections. Immunol Res. 2013 Mar; 55(0): 187–200*). When IFNγ interacts with an IFNγR1 subunit, it induces a conformational change that permits a closer association of the IFNγR1 and IFNγR2 subunits. These rearrangements in the receptor induce auto- and cross-phosphorylation of Janus-associated kinases (JAKs) that are constitutively associated with the receptor. IFNγR1 contains a binding motif for JAK1, and IFNγR2 contains a binding motif for JAK2. Phosphorylation of the JAK proteins stimulates their catalytic activity and they then phosphorylate a tyrosine residue (Y_{440}) at the C-terminus of IFNγR1. This phosphorylated tyrosine residue provides a docking site for the SH2 domain on the Signal Transducer and Activator of Transcription-1 (STAT-1) protein. Because each receptor complex contains two IFNγR1 subunits, two STAT-1 proteins are able to bind to the receptor. JAK1 and JAK2 remain receptor-associated and phosphorylate each recruited STAT-1 protein at tyrosine residue 701 (Y_{701}). This phosphorylation allows release of the STAT-1 monomers from the receptor and their formation of homodimers. STAT-1 homodimers translocate to the nucleus and bind Gamma-Activated Sequences (GAS) in the promoter DNA of IFN-stimulated genes (ISGs), resulting in their increased transcription. Type I IFNs signal through a canonical JAK/STAT pathway, similar to that activated by IFNγ. Ligand binding to the IFNαR initiates dimerization of the two receptor subunits and trans-phosphorylation of their associated TYK2 and JAK1 kinases. The kinases phosphorylate residues in the cytoplasmic tails of IFNαR1 and IFNαR2 to recruit STAT1 and STAT2 proteins via their SH2 domains. Docking of these STAT proteins to the receptor subunits allows their phosphorylation by the activated JAK proteins at Y_{701} on STAT-1 and Y_{690} on STAT-2. Phosphorylation of the STAT monomers releases them from their docking site, allowing them to dimerize and combine in a homodimeric or heterodimeric form with IRF9 to produce the transcription factor ISG factor 3 (ISGF3). ISGF3 translocates into the nucleus to identify ISGs and induces their transcription. ISGs induced by type I IFN signaling typically contain interferon stimulated response element (ISRE) or a gamma activated sequence (GAS) elements within their promoters, although there is a clear preference for genes containing an ISRE. Some examples of ISGs transcribed as a result of type I IFNs are ISRE containing genes ISG15, IP-10, IRF-7 and PKR [66], and GAS containing genes IRF-1, IRF-2, IRF-8 and IRF-9 (*Kearney S. et al., Differential effects of type I and II interferons on*

myeloid cells and resistance to intracellular bacterial infections. Immunol Res. 2013 Mar; 55(0): 187–200).

Recombinant Chimeric Receptor Proteins

[00207] Provided herein is a class of phagocytic or tethering receptor (PR) subunit (e.g., a phagocytic receptor fusion protein (PFP)) comprising: (i) a transmembrane domain, and (ii) an intracellular domain comprising a phagocytic receptor intracellular signaling domain; and an antigen binding domain specific to an antigen, e.g., an antigen of or presented on a target cell; wherein the transmembrane domain and the antigen binding domain are operatively linked such that antigen binding to the target by the antigen binding domain of the fused receptor activated in the intracellular signaling domain of the phagocytic receptor.

[00208] In some embodiments, the extracellular domain of a CFP comprises an Ig binding domain. In some embodiments, the extracellular domain comprises an IgA, IgD, IgE, IgG, IgM, FcRγI, FcRγIIA, FcRγIIB, FcRγIIC, FcRγIIIA, FcRγIIIB, FcRn, TRIM21, FcRL5 binding domain. In some embodiments, the extracellular domain of a CFP comprises an FcR extracellular domain. In some embodiments, the extracellular domain of a CFP comprises an FcRα, FcRβ, FcRε or FcRγ extracellular domain. In some embodiments, the extracellular domain comprises an FcRα (FCAR) extracellular domain. In some embodiments, the extracellular domain comprises an FcRβ extracellular domain. In some embodiments, the extracellular domain comprises an FCER1A extracellular domain. In some embodiments, the extracellular domain comprises an FDGR1A, FCGR2A, FCGR2B, FCGR2C, FCGR3A, or FCGR3B extracellular domain. In some embodiments, the extracellular domain comprises an integrin domain or an integrin receptor domain. In some embodiments, the extracellular domain comprises one or more integrin α1, α2, αIIb, α3, α4, α5, α6, α7, α8, α9, α10, α11, αD, αE, αL, αM, αV, αX, β1, β2, β3, β4, β5, β6, β7, or β8 domains.

[00209] In some embodiments, the CFP further comprises an extracellular domain comprising an antigen binding domain operatively linked to the transmembrane domain. In some embodiments, the extracellular domain further comprises an extracellular domain of a receptor, a hinge, a spacer and/or a linker. In some embodiments, the extracellular domain comprises an extracellular portion of a phagocytic receptor. In some embodiments, the extracellular portion of the CFP is derived from the same receptor as the receptor from which the intracellular signaling domain is derived. In some embodiments, the extracellular domain comprises an extracellular domain of a scavenger receptor. In some embodiments, the extracellular domain comprises an immunoglobulin domain. In some embodiments, the immunoglobulin domain comprises an extracellular domain of an immunoglobulin or an immunoglobulin hinge region. In some embodiments, the extracellular domain comprises a phagocytic engulfment domain. In some embodiments, the extracellular domain comprises a structure capable of multimeric assembly. In some embodiments, the extracellular domain comprises a scaffold for multimerization. In some embodiments, the extracellular domain is at least 10, 20, 30, 40, 50, 60,

70, 80, 90, 100, 150, 200, 300, 300, 400, or 500 amino acids in length. In some embodiments, the extracellular domain is at most 500, 400, 300, 200, or 100 amino acids in length. In some embodiments, the antigen binding domain specifically binds to the antigen of a target cell. In some embodiments, the antigen binding domain comprises an antibody domain. In some embodiments, the antigen binding domain comprises a receptor domain, antibody domain, wherein the antibody domain comprises a functional antibody fragment, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a bispecific antibody, a diabody, or a functional fragment or a combination thereof. In some embodiments, the antigen binding domain comprises a ligand, an extracellular domain of a receptor or an adaptor. In some embodiments, the antigen binding domain comprises a single antigen binding domain that is specific for a single antigen. In some embodiments, the antigen binding domain comprises at least two antigen binding domains, wherein each of the at least two antigen binding domains is specific for a different antigen.

[00210] In some embodiments, the antigen is a cancer associated antigen, a lineage associated antigen, a pathogenic antigen or an autoimmune antigen. In some embodiments, the antigen comprises a viral antigen. In some embodiments, the antigen is a T lymphocyte antigen. In some embodiments, the antigen is an extracellular antigen. In some embodiments, the antigen is an intracellular antigen. In some embodiments, the antigen is selected from the group consisting of an antigen from Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin receptor, PRSS21, VEGFR2, PDGFRβ, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, Dsg1, Dsg3, IGLL1 and combinations thereof. In some embodiments, the antigen is an antigen of a protein selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, and CD56. In some embodiments, the antigen is an ovarian cancer antigen or a T lymphoma antigen. In some embodiments, the antigen is an antigen of an integrin receptor. In some embodiments, the antigen is an antigen of an integrin receptor or integrin selected from the group consisting of α1, α2, αIIb, α3, α4, α5, α6, α7, α8, α9, α10, α11, αD, αE, αL, αM, αV, αX, β 1, β 2, β 3, β 4, β 5, β 6, β 7, and β8. In some embodiment, the antigen is an antigen of an integrin receptor ligand.

In some embodiments, the antigen is an antigen of fibronectin, vitronectin, collagen, or laminin. In some embodiments, the antigen binding domain can bind to two or more different antigens.

[00211] In some embodiments, the antigen binding domain comprises a sequence of an antigen binding domain provided herein, such as a sequence of an antigen binding domain in a Table provided herein.

[00212] In some embodiments, the target protein is CD70. In some embodiments, the antigen binding domain comprises an anti-CD70 antibody or binding fragment thereof, wherein the antigen binding domain comprises a heavy chain variable domain (VH) comprising a heavy chain complementarity determining region 3 (HC CDR3) that is the HC CDR3 of any one of the VH sequences selected from the group consisting of QVQLQESGGGLVQAGGSLRLSCAAPRSIFSINAMGWYRQAPGKQRELVAAITSGGSPTYADSVK GRFTISRDNAKNTVYLQMNSLKAEDTAVYYCATGPYGLDNALDAWGQGTQVTVSS and QVQLQESGGGLVQTGGSLRLACTASGFTFDDYAIAWFRQAPGKEREFVAAISWSGGTTHYADS VKGRFTISRDNAKNTLYLQMSSLKPEDTAVYFCAKSLRSSPSSRWFGSRGQGTQVTVSS. In some embodiments, the VH of the anti-CD70 antibody or binding fragment thereof further comprises a heavy chain complementarity determining region 1 (HC CDR1) that is the HC CDR1 of any one of the VH sequences selected from the group consisting of QVQLQESGGGLVQAGGSLRLSCAAPRSIFSINAMGWYRQAPGKQRELVAAITSGGSPTYADSVK GRFTISRDNAKNTVYLQMNSLKAEDTAVYYCATGPYGLDNALDAWGQGTQVTVSS and QVQLQESGGGLVQTGGSLRLACTASGFTFDDYAIAWFRQAPGKEREFVAAISWSGGTTHYADS VKGRFTISRDNAKNTLYLQMSSLKPEDTAVYFCAKSLRSSPSSRWFGSRGQGTQVTVSS. In some embodiments, the VH of the anti-CD70 antibody or binding fragment thereof further comprises a heavy chain complementarity determining region 2 (HC CDR2) that is the HC CDR2 of any one of the VH sequences selected from the group consisting of QVQLQESGGGLVQAGGSLRLSCAAPRSIFSINAMGWYRQAPGKQRELVAAITSGGSPTYADSVK GRFTISRDNAKNTVYLQMNSLKAEDTAVYYCATGPYGLDNALDAWGQGTQVTVSS and QVQLQESGGGLVQTGGSLRLACTASGFTFDDYAIAWFRQAPGKEREFVAAISWSGGTTHYADS VKGRFTISRDNAKNTLYLQMSSLKPEDTAVYFCAKSLRSSPSSRWFGSRGQGTQVTVSS. In some embodiments, the VH of the anti-CD70 antibody or binding fragment thereof comprises with 70-100% sequence identity to any one of the sequences selected from the group consisting of QVQLQESGGGLVQAGGSLRLSCAAPRSIFSINAMGWYRQAPGKQRELVAAITSGGSPTYADSVK GRFTISRDNAKNTVYLQMNSLKAEDTAVYYCATGPYGLDNALDAWGQGTQVTVSS and QVQLQESGGGLVQTGGSLRLACTASGFTFDDYAIAWFRQAPGKEREFVAAISWSGGTTHYADS VKGRFTISRDNAKNTLYLQMSSLKPEDTAVYFCAKSLRSSPSSRWFGSRGQGTQVTVSS In some embodiments, the VH is a single domain antibody domain. In some embodiments, the VH is a VHH.

[00213] In some embodiments the target protein is GPC3. In some embodiments, the antigen binding domain comprises an anti-GPC3 antibody or binding fragment thereof, wherein the antigen binding domain comprises a heavy chain variable domain (VH) comprising a heavy chain complementarity

determining region 3 (HC CDR3) of any one of the sequences selected from the group consisting of ATACADTTQYAYDY, ATACADTTLYEYDY, ATACVDTTQYEYDY, ATACADATQHEYDY, ATACADTTQYDYDY, ATACADTTQYEYDY, ATACADTTHYEYDY, ATACVITTLYEYDY, ATACAETTLYEYDY, ATACADTTQHEYDY, ATACVDTTHYEYDY, ATACASTTLYEYDY, ATACVVTTLYEYDY, ATACGGATGPYDY, ATACAGAIGPYDY, ATACVVVGDQNDY, ATACVVVGDRNDY, ATDCAGGTSTPYDY, ATDCAGGTATPYDY, ATACVVADRNEYDY, ATSCVVVTKNEYDY, ATACSGLTHEYDY, ATTCSGLTHEYDY, ATACANWSSLGPYDY, ATACANWSTLGPYDY, ATACSDPRVYEYDY, ATTCASPEKYEYDY, ATHCGGTSWGTSYDY, ATHCGGSSWSNEYDY, YARYSGRTY, ASSAWPAGPKHQVEYDY, ATACGSLVGMYDY, ATACGSAVHEYDY, ATDCVGFGSNWFDY, ATACASPVIYEYDY, ATDCAGGVGHEYDY, ATDCSLHGSDYPYDY and AVRIYSGSFDNTLAYDY. In some embodiments, the VH of the anti-GPC3 antibody or binding fragment thereof further comprises a heavy chain complementarity determining region 1 (HC CDR1) of any one of the sequences selected from the group consisting of SEQ ID NOs: GFPLAYYA, GFSLDYYA, GFPLDYYA, GFTLDYYA, GFSLNYYA, GFTLAYYA, GFTLGYYA, GFPLNYYA, GFPLHYYA, GFSLGYYA, GFPLGYYA, GFPLEYYA, GSDFRADA, GRTFSSYG, GFSLAYYA and GLTFRSVG. In some embodiments, the VH of the anti-GPC3 antibody or binding fragment thereof further comprises a heavy chain complementarity determining region 2 (HC CDR2) of any one of the sequences selected from the group consisting of SEQ ID NOs: ISNSDGST, ISASDGST, ISSSDGST, ISSSDGNT, ISSADGST, ISSSGGST, ISSGDGST, ISAGDGNT, ISSSDDST, ISSNDGST, ISSPDGST, ISSRTGGT, ISAGDGSST, ISSSDGSSSDGNT, ISSGDGNT, ISSGDGKT, ISSSDGGT, ISSRTGST, ISSRTGNT, ISSSDGHSST, ISSSSDGNT, ISASNGNT, ISSGSDGNT, ISASDGNT, IDSITSI, ISWSGGSTIAASVGST, ISSSDGSDGNT and ASPSGVIT. In some embodiments, the VH of the anti-GPC3 antibody or binding fragment thereof comprises with 70-100% sequence identity to any one of the sequences selected from the group consisting of QVQLQESGGGLVHSGGSLRLSCAASGFPLAYYAIGWFRQAPGKEREGVSCISSSDGNTYYADAV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATACADTTQHEYDYWGQGTQVTVSS, QVQLQESGGGLVHSGGSLRLSCAASGFPLDYYAIGWFRQAPGKEREGVSCISSADGSTYYADSV KGRFTISRDNAKNTVYLQMNSLGPEDTAVYYCATACADTTQYDYDYWGQGTQVTVSS, QVQLQESGGGLVHSGGSLRLSCAASGFTLDYYAIGWFRRAPGKEREGVSCISSGDGKTYYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATACAGAIGPYDYWGQGTQVTVSS, QVQLQESGGGLVPPGGSLRLSCAASGFPLDYYAIGWFRQAPGKEREGVSCISSADGSTYYADSV KGRFTISRDNAKNTVYLQMNSLGPEDTAVYYCATACADTTQYDYDYWGQGTQVTVSS, QVQLQESGGGLVQAGGSLRLSCAASGFSLGYYAIGWFRQAPGKEREGVSCISSSDGHSSTYYAD SVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCATDCAGGTATPYDYWGQGTQVTVSS, QVQLQESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVAAISWSGGSTYYADS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCASSAWPAGPKHQVEYDYWGQGTQVTVSS, QVQLQESGGGLVQAGGSLRLSCTASGFSLDYYAIGWFRQAPGKEREGVACISSRTGSTYYADSV KGRFTISRDNAKNTVALQMNSLKPEDTAVYYCATACVVVGDQNDYWGQGTQVTVSS,

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QVQLQESGGGLVQPGGSLRLSCAASGFPLAYYAIGWFRQAPGKEREGVSCISSSDGSTYYADSV
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KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATACGGATGPYDYWGQGTQVTVSS,
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KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATACADTTLYEYDYWGQGTQVTVSS,
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VKGRFTISRDNAKNTVYLEMNSLKPEDTAVYYCAVRIYSGSFDNTLAYDYWGQGTQVTVSS,
QVQLQESGGGVVQPGGSLRLSCTASGFSLGYYAIGWFRQAPGKEREGVSCISSRTGSTYYADSV
KGRFTVSRDDAKNTVYLQMNSLKPEDTAVYYCATACVVVGDQNDYWGQGTQVTVSS, and
QVQLQESGGGVVQSGGSLRLSCTASGFSLDYYAIGWFRQAPGKEREGVSCISSRTGSTYYADSV
KGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCATACVVVGDQNDYWGQGTQVTVSS. In some embodiments, the VH is a single domain antibody domain. In some embodiments, the VH is a VHH.

[00214] In some embodiments, the antigen binding domain comprises an autoantigen or fragment thereof, such as Dsg1 or Dsg3. In some embodiments, the antigen binding domain comprises a receptor domain or an antibody domain wherein the antibody domain binds to an auto antigen, such as Dsg1 or Dsg3.

[00215] In some embodiments, the transmembrane domain and the antigen binding domain are operatively linked through a linker. In some embodiments, the transmembrane domain and the antigen binding domain are operatively linked through a linker such as a hinge region of CD8α, IgG1 or IgG4.

[00216] In some embodiments, the extracellular domain comprises a multimerization scaffold.

[00217] In some embodiments, the transmembrane domain comprises a CD8 transmembrane domain. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain. In some embodiments, the transmembrane domain comprises a CD68 transmembrane domain. In some embodiments, the transmembrane domain comprises a CD2 transmembrane domain. In some embodiments, the transmembrane domain comprises an FcR transmembrane domain. In some embodiments, the transmembrane domain comprises an FcRγ transmembrane domain. In some embodiments, the transmembrane domain comprises an FcRα transmembrane domain. In some embodiments, the transmembrane domain comprises an FcRβ transmembrane domain. In some embodiments, the transmembrane domain comprises an FcRε transmembrane domain. In some embodiments, the transmembrane domain comprises a transmembrane domain from a syntaxin, such as syntaxin 3 or syntaxin 4 or syntaxin 5. In some embodiments, the transmembrane domain

oligomerizes with a transmembrane domain of an endogenous receptor when the CFP is expressed in a cell. In some embodiments, the transmembrane domain oligomerizes with a transmembrane domain of an exogenous receptor when the CFP is expressed in a cell. In some embodiments, the transmembrane domain dimerizes with a transmembrane domain of an endogenous receptor when the CFP is expressed in a cell. In some embodiments, the transmembrane domain dimerizes with a transmembrane domain of an exogenous receptor when the CFP is expressed in a cell. In some embodiments, the transmembrane domain is derived from a protein that is different than the protein from which the intracellular signaling domain is derived. In some embodiments, the transmembrane domain is derived from a protein that is different than the protein from which the extracellular domain is derived. In some embodiments, the transmembrane domain comprises a transmembrane domain of a phagocytic receptor. In some embodiments, the transmembrane domain and the extracellular domain are derived from the same protein. In some embodiments, the transmembrane domain is derived from the same protein as the intracellular signaling domain. In some embodiments, the recombinant nucleic acid encodes a DAP12 recruitment domain. In some embodiments, the transmembrane domain comprises a transmembrane domain that oligomerizes with DAP12.

[00218] In some embodiments, the transmembrane domain is at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 amino acids in length. In some embodiments, the transmembrane domain is at most 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 amino acids in length.

[00219] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a phagocytic receptor. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a phagocytic receptor other than a phagocytic receptor selected from Megf10, MerTk, FcRα, or Bai1. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a phagocytic receptor selected from the group consisting of TNFR1, MDA5, CD40, lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fc-alpha receptor I, CR1, CD35, CD3ζ, CR3, CR4, Tim-1, Tim-4 and CD169. In some embodiments, the intracellular signaling domain comprises a PI3K recruitment domain. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a scavenger receptor. In some embodiments, the intracellular domain comprises a CD47 inhibition domain. In some embodiments, the intracellular domain comprises a Rac inhibition domain, a Cdc42 inhibition domain or a GTPase inhibition domain. In some embodiments, the Rac inhibition domain, the Cdc42 inhibition domain or the GTPase inhibition domain inhibits Rac, Cdc42 or GTPase at a phagocytic cup of a cell expressing the PFP. In

some embodiments, the intracellular domain comprises an F-actin disassembly activation domain, a ARHGAP12 activation domain, a ARHGAP25 activation domain or a SH3BP1 activation domain. In some embodiments, the intracellular domain comprises a phosphatase inhibition domain. In some embodiments, the intracellular domain comprises an ARP2/3 inhibition domain. In some embodiments, the intracellular domain comprises at least one ITAM domain. In some embodiments, the intracellular domain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more ITAM domains. In some embodiments, the intracellular domain comprises at least one ITAM domain select from an ITAM domain of CD3 zeta, CD3 epsilon, CD3 gamma, CD3 delta, Fc epsilon receptor 1 chain, Fc epsilon receptor 2 chain, Fc gamma receptor 1 chain, Fc gamma receptor 2a chain, Fc gamma receptor 2b 1 chain, Fc gamma receptor 2b2 chain, Fc gamma receptor 3a chain, Fc gamma receptor 3b chain, Fc beta receptor 1 chain, TYROBP (DAP12), CD5, CD16a, CD16b, CD22, CD23, CD32, CD64, CD79a, CD79b, CD89, CD278, CD66d, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications thereto. In some embodiments, the at least one ITAM domain comprises a Src-family kinase phosphorylation site. In some embodiments, the at least one ITAM domain comprises a Syk recruitment domain. In some embodiments, the intracellular domain comprises an F-actin depolymerization activation domain. In some embodiments, the intracellular domain lacks enzymatic activity.

[00220] In some embodiments, the intracellular domain does not comprise a domain derived from a CD3 zeta intracellular domain. In some embodiments, the intracellular domain does not comprise a domain derived from a MerTK intracellular domain. In some embodiments, the intracellular domain does not comprise a domain derived from a TLR4 intracellular domain. In some embodiments, the intracellular domain comprises a CD47 inhibition domain. In some embodiments, the intracellular signaling domain comprises a domain that activates integrin, such as the intracellular region of PSGL-1

[00221] In some embodiments, the intracellular signaling domain comprises a domain that activates Rap1 GTPase, such as that from EPAC and C3G. In some embodiments, the intracellular signaling domain is derived from paxillin. In some embodiments, the intracellular signaling domain activates focal adhesion kinase. In some embodiments, the intracellular signaling domain is derived from a single phagocytic receptor. In some embodiments, the intracellular signaling domain is derived from a single scavenger receptor. In some embodiments, the intracellular domain comprises a phagocytosis enhancing domain.

[00222] In some embodiments, the intracellular domain comprises a pro-inflammatory signaling domain. In some embodiments, the pro-inflammatory signaling domain comprises a kinase activation domain or a kinase binding domain. In some embodiments, the pro-inflammatory signaling domain comprises an IL-1 signaling cascade activation domain. In some embodiments, the pro-inflammatory signaling domain comprises an intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR

9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, an NLRP family member, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, CD40, Tank1-binding kinase (TBK), a caspase domain, a procaspase binding domain or any combination thereof.

[00223] In some embodiments, the intracellular domain comprises a signaling domain, such as an intracellular signaling domain, derived from a connexin (Cx) protein. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from Cx43, Cx46, Cx37, Cx40, Cx33, Cx50, Cx59, Cx62, Cx32, Cx26, Cx31, Cx30.3, Cx31.1, Cx30, Cx25, Cx45, Cx47, Cx31.3, Cx36, Cx31.9, Cx39, Cx40.1 or Cx23. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from Cx43.

[00224] In some embodiments, the intracellular domain comprises a signaling domain, such as an intracellular signaling domain, derived from a SIGLEC protein. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from Siglec-1 (Sialoadhesin), Siglec-2 (CD22), Siglec-3 (CD33), Siglec-4 (MAG), Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-12, Siglec-13, Siglec-14, Siglec-15, Siglec-16 or Siglec-17.

[00225] In some embodiments, the intracellular domain comprises a signaling domain, such as an intracellular signaling domain, derived from a TLR protein. In some embodiments, the intracellular domain may comprise an intracellular signaling domain of the endo-lysosomal TLR, e.g., TLR3, TLR7, TLR8, or TLR9. In some embodiments, the intracellular signaling domain may be derived from a TLR3 protein. In some embodiments, the intracellular signaling domain may be derived from a TLR7, 8, or 9 protein. In some embodiments, the intracellular domain may comprise an intracellular signaling domain of the cell surface TLRs 1, 2, 4, 5, 6, and 10.

[00226] In some embodiments, an intracellular signaling domain is specifically paired with another intracellular domain or a transmembrane domain for maximizing the efficiency and phagocytic potential of the myeloid cell expressing the construct. For example, in some embodiments, a TM domain comprising CD64 TM or a part thereof may be specifically paired with an intracellular signaling domain comprising an innate immune adaptor protein ICD or a PI3kinase recruitment domain, or both. In some embodiments, the combination of domains of the chimeric receptor intracellular domain(s) and/or the transmembrane domains are directed towards maximizing the phagocytosis index of the cell expression the construct, e.g., a myeloid cell. In some embodiments, the combination of domains of the chimeric receptor intracellular domain(s) and/or the transmembrane domains are directed towards maximizing the inflammatory potential of the cell expression the construct such that the cell is capable of lysing the target cell, and activating an immune response pathway for rendering long term immune responsiveness. In some embodiments, the combination of domains of the chimeric receptor intracellular domain(s) and/or the transmembrane domains are directed towards minimizing or obliterating any tonic signaling by the cell expressing the chimeric

protein. In some embodiments, the combination of domains of the chimeric receptor intracellular domain(s) and/or the transmembrane domains are directed towards maximizing specificity of the immune response.

[00227] In some embodiments, the intracellular domain comprises a signaling domain, such as an intracellular signaling domain, derived from a C-type lectin protein. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from a mannose receptor protein. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from an asialoglycoprotein receptor protein. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from macrophage galactose-type lectin (MGL), DC-SIGN (CLEC4L), Langerin (CLEC4K), Myeloid DAP12 associating lectin (MDL)-1 (CLEC5A), a DC associated C type lectin 1 (Dectin1) subfamily protein, dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C type lectin like receptor (MICL) (CLEC12A), CLEC2 (CLEC1B), CLEC12B, a DC immunoreceptor (DCIR) subfamily protein, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), Mincle (macrophage inducible C type lectin) (CLEC4E), a NOD-like receptor protein, NOD-like receptor MHC Class II transactivator (CIITA), IPAF, BIRC1, a RIG-I-like receptor (RLR) protein, RIG-I, MDA5, LGP2, NAIP5/Birc1e, an NLRP protein, NLRP1, NLRP2, NLRP3, NLRP4, NLRP5, NLRP6, NLRP7, NLRP89, NLRP9, NLRP10, NLRP11, NLRP12, NLRP13, NLRP14, an NLR protein, NOD1 or NOD2, or any combination thereof.

[00228] In some embodiments, the intracellular domain comprises a signaling domain, such as an intracellular signaling domain, derived from a cell adhesion molecule. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from an IgCAMs, a cadherin, an integrin, a C-type of lectin-like domains protein (CTLD) and/or a proteoglycan molecule. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from an E-cadherin, a P-cadherin, an N-cadherin, an R-cadherin, a B-cadherin, a T-cadherin, or an M-cadherin. For example, the intracellular domain can comprise a signaling domain, such as an intracellular signaling domain, derived from a selectin, such as an E-selectin, an L-selectin or a P-selectin.

[00229] In some embodiments, the CFP does not comprise a full length intracellular signaling domain. In some embodiments, the intracellular domain is at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 300, 400, or 500 amino acids in length. In some embodiments, the intracellular domain is at most 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 300, 400, or 500 amino acids in length.

[00230] In some embodiments, the recombinant nucleic acid encodes an FcRα chain extracellular domain, an FcRα chain transmembrane domain and/or an FcRα chain intracellular domain. In some embodiments, the recombinant nucleic acid encodes an FcRβ chain extracellular domain, an FcRβ

chain transmembrane domain and/or an FcRβ chain intracellular domain. In some embodiments, the FcRα chain or the FcRβ chain forms a complex with FcRγ when expressed in a cell. In some embodiments, the FcRα chain or FcRβ chain forms a complex with endogenous FcRγ when expressed in a cell. In some embodiments, the FcRα chain or the FcRβ chain does not incorporate into a cell membrane of a cell that does not express FcRγ. In some embodiments, the CFP does not comprise an FcRα chain intracellular signaling domain. In some embodiments, the CFP does not comprise an FcRβ chain intracellular signaling domain. In some embodiments, the recombinant nucleic acid encodes a TREM extracellular domain, a TREM transmembrane domain and/or a TREM intracellular domain. In some embodiments, the TREM is TREM1, TREM 2 or TREM 3.

[00231] In some embodiments, the recombinant nucleic acid comprises a sequence encoding a pro-inflammatory polypeptide. In some embodiments, the composition further comprises a proinflammatory nucleotide or a nucleotide in the recombinant nucleic acid, for example, an ATP, ADP, UTP, UDP, and/or UDP-glucose.

Intracellular interferon responsive domains

[00232] Most TLRs activate an adaptor protein called MyD88 activates the transcription-factor protein NF-κB, which drives expression of pro-inflammatory genes as part of the immune response. A subgroup of TLRs (TLR3 and TLR4) can engage the protein TRIF, which acts as a scaffold enabling a kinase enzyme to add a phosphate group to the transcription factor IRF3. This phosphorylation activates IRF3, a member of a family of transcription factors termed interferon regulatory factors (IRFs), which activate broad gene-expression programs. A hallmark of these programs is the production of type I interferon molecules Interferons are potent drivers of a branch of the immune system termed the adaptive immune response, and their presence therefore runs the risk of contributing to autoimmunity. To prevent such an attack by the host's own immune system, an interferon response must be tightly regulated. As a safeguard, a particular sequence of amino-acid residues in TRIF, the pLxIS motif, must be phosphorylated before IRF3 can be activated. This control mechanism provides a 'licensing step' that is not specific just for TRIF as an adaptor protein for TLR signaling, but is a general hallmark of sensing pathways that engage IRF3, or the related protein IRF7, to drive interferon expression. Every identified innate sensing pathway connecting the recognition of nucleic acids to the production of type I interferons, with one exception, had been shown previously to signal through one of the three adaptor proteins known so far to contain a pLxIS motif: TRIF, MAVS and STING. Thus, pLxIS-motif-containing adaptor proteins specifically hardwire nucleic-acid recognition to antiviral defenses. In some embodiments, the intracellular signaling domain of the CFP comprises an ICD of an innate immune response protein. In some embodiments, the innate immune response protein is selected from a an intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR 9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, MAVS, TRIF, TASL, NLRP1, NLRP2, NLRP3, NLRP4, NLRP5, NLRP6, NLRP7, NLRP89, NLRP9, NLRP10, NLRP11, NLRP12,

NLRP13, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, CD40, Tank1-binding kinase (TBK), TNFR1, a chemokine, MHC Class II transactivator (CIITA), IPAF, BIRC1, a RIG-I-like receptor (RLR) protein, macrophage galactose-type lectin (MGL), DC-SIGN (CLEC4L), Langerin (CLEC4K), Myeloid DAP12 associating lectin (MDL)-1 (CLEC5A), a DC associated C type lectin 1 (Dectin1) subfamily protein, dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C type lectin like receptor (MICL) (CLEC12A), CLEC2 (CLEC1B), CLEC12B, a DC immunoreceptor (DCIR) subfamily protein, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), and Mincle (macrophage inducible C type lectin) (CLEC4E). In some embodiments, the CFP comprises at least one intracellular signaling domain that comprises an amino acid sequence motif, pLxIS. In some embodiments, the intracellular interferon inducing moiety comprises an amino acid sequence selected from SEQ ID NOs. 36-41. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an MDA5 intracellular signaling domain of SEQ ID NO: 30. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an RIG1 intracellular signaling domain of SEQ ID NO: 36. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an Myd88 intracellular signaling domain of SEQ ID NO: 37. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an intracellular signaling domain comprising a STING moiety of SEQ ID NO: 38. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an intracellular signaling domain comprising a MAVS moiety of SEQ ID NO: 39. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an intracellular signaling domain comprising a TRIF moiety of SEQ ID NO: 40. Provided herein is a chimeric antigen receptor fusion protein for enhancing phagocytosis, comprising an antigen binding domain and an intracellular signaling domain comprising a TASL moiety of SEQ ID NO: 41.

[00233] In some embodiments, the composition further comprises a pro-inflammatory polypeptide. In some embodiments, the pro-inflammatory polypeptide is a chemokine, cytokine. In some embodiments, the chemokine is selected from the group consisting of IL-1, IL3, IL5, IL-6, il8, IL-12, IL-13, IL-23, TNF, CCL2, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, RANTES, and interferon. In some embodiments, the cytokine is selected from the group consisting of IL-1, IL3, IL5, IL-6, IL-12, IL-13, IL-23, TNF, CCL2, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, RANTES, and interferon.

[00234] In some embodiments, the intracellular signaling domains from intracellular adaptor proteins known to be highly active in innate immune defense are incorporated in the chimeric receptor protein. In some embodiments, on or more mutations are introduced in one or more intracellular domains to reduce responsiveness of the intracellular domain to intracellular stimulus that is characteristic of the

native intracellular adaptor protein domain, without compromising the effectiveness of the chimeric protein. In some embodiments, such effectiveness is referred to as the enhanced phagocytic potential compared to an identical cell that does not express a chimeric protein. In some embodiments, such effectiveness is referred to as the enhanced inflammatory potential compared to an identical cell that does not express a chimeric protein. In some embodiments, such effectiveness is referred to as the enhanced NF-kappa B activation, or interferon activation in the cell expressing the chimeric protein, compared to an identical cell that does not express a chimeric protein.

[00235] In some embodiments, the myeloid cells are specifically targeted for delivery. Myeloid cells can be targeted using specialized biodegradable polymers, such as PLGA (poly(lactic-co-glycolic) acid and/or polyvinyl alcohol (PVA). In some embodiments, one or more compounds can be selectively incorporated in such polymeric structures to affect the myeloid cell function. In some embodiments, the targeting structures are multilayered, e.g., of one or more PLGA and one or more PVA layers. In some embodiments, the targeting structures are assembled in an order for a layered activity. In some embodiments, the targeted polymeric structures are organized in specific shaped components, such as labile structures that can adhere to a myeloid cell surface and deliver one or more components such as growth factors and cytokines, such as to maintain the myeloid cell in a microenvironment that endows a specific polarization. In some embodiments, the polymeric structures are such that they are not phagocytosed by the myeloid cell, but they can remain adhered on the surface. In some embodiments the one or more growth factors may be M1 polarization factors, such as a cytokine. In some embodiments the one or more growth factors may be an M2 polarization factor, such as a cytokine. In some embodiments, the one or more growth factors may be a macrophage activating cytokine, such as IFNγ. In some embodiments the polymeric structures are capable of sustained release of the one or more growth factors in an in vivo environment, such as in a solid tumor.

[00236] In some embodiments, the recombinant nucleic acid comprises a sequence encoding a homeostatic regulator of inflammation. In some embodiments, the homeostatic regulator of inflammation is a sequence in an untranslated region (UTR) of an mRNA. In some embodiments, the sequence in the UTR is a sequence that binds to an RNA binding protein. In some embodiments, translation is inhibited or prevented upon binding of the RNA binding protein to the sequence in an untranslated region (UTR). In some embodiments, the sequence in the UTR comprises a consensus sequence of WWWU(AUUUA)UUUW, wherein W is A or U. In some embodiments, the recombinant nucleic acid is expressed on a bicistronic vector.

[00237] In some embodiments, the target cell is a mammalian cell. In some embodiments, the target cell is a human cell. In some embodiments, the target cell comprises a cell infected with a pathogen. In some embodiments, the target cell is a cancer cell. In some embodiments, the target cell is a cancer cell that is a lymphocyte. In some embodiments, the target cell is a cancer cell that is an ovarian cancer cell. In some embodiments, the target cell is a cancer cell that is a breast cell. In some embodiments,

the target cell is a cancer cell that is a pancreatic cell. In some embodiments, the target cell is a cancer cell that is a glioblastoma cell.

[00238] In some embodiments, the recombinant nucleic acid is DNA. In some embodiments, the recombinant nucleic acid is RNA. In some embodiments, the recombinant nucleic acid is mRNA. In some embodiments, the recombinant nucleic acid is an unmodified mRNA. In some embodiments, the recombinant nucleic acid is a modified mRNA. In some embodiments, the recombinant nucleic acid is a circRNA. In some embodiments, the recombinant nucleic acid is a tRNA. In some embodiments, the recombinant nucleic acid is a microRNA.

[00239] Also provided herein is a vector comprising a recombinant nucleic acid sequence encoding a CFP described herein. In some embodiments, the vector is viral vector. In some embodiments, the viral vector is retroviral vector or a lentiviral vector. In some embodiments, the vector further comprises a promoter operably linked to at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector is polycistronic. In some embodiments, each of the at least one nucleic acid sequence is operably linked to a separate promoter. In some embodiments, the vector further comprises one or more internal ribosome entry sites (IRESs). In some embodiments, the vector further comprises a 5'UTR and/or a 3'UTR flanking the at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector further comprises one or more regulatory regions.

[00240] Also provided herein is a polypeptide encoded by the recombinant nucleic acid of a composition described herein.

[00241] Provided herein is a composition comprising a recombinant nucleic acid sequence encoding a CFP comprising a phagocytic or tethering receptor (PR) subunit (e.g., a phagocytic receptor fusion protein (PFP)) comprising: a PR subunit comprising: a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain; and an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein upon binding of the CFP to the antigen of the target cell, the killing or phagocytosis activity of a myeloid cell, such as a neutrophil, monocyte, myeloid dendritic cell (mDC), mast cell or macrophage expressing the CFP is increased by at least greater than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, or 1000% compared to a cell not expressing the CFP.

[00242] Table 1 shows exemplary sequences of chimeric fusion protein domains and/or fragments thereof that are meant to be non-limiting for the disclosure. Underlines denote the CDR sequences in sequential order of CDR1, CDR2 and CDR3 for the respective heavy and light chains in accordance to the Kabat numbering system.

TABLE 1. Exemplary Chimeric Fusion Proteins and Receptor Domains

SEQ ID NO	CFP/ Domain	Sequence
1	*CD5-FcR-PI3K*	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTV*SSGGGGSGGGGSGGGGS*DIQMTQSPSSLSASVGDRVTI TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRF*SGS GSG*TDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIK*SG GGGSG*ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLY CRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ *GSGS*YEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM
2	*HER2-FcR-PI3K*	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQ DVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDF TLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTGSTSGSG KPGSGEGSEVQLVE*SGGG*LVQPGGSLRLSCAASGFNIKDTYIHW VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTA YLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTV*SS SGGGGSG*ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVIT LYCRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKP PQ*GSGS*YEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM
3	*CD5-FcR-CD40*	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTV*SSGGGGSGGGGSGGGGS*DIQMTQSPSSLSASVGDRVTI TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGS GSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIK*SG GGGSG*ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLY CRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQK KVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQP VTQEDGKESRISVQERQ

SEQ ID NO	CFP/ Domain	Sequence
4	*CD5-FcR-MDA5*	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGS GSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIKSG GGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLY CRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQG SGSMSNGYSTDENFRYLISCFRARVKMYIQVEPVLDYLTFLPAE VKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFVEAL RRTGSPLAARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVD KLLVRDVLDKCMEEELLTIEDRNRIAAAENNGNESGVRELLKRI VQKENWFSAFLNVLRQTGNNELVQELTGSDCSESNAEIEN
5	CD5-FcR-TNFR1	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGS GSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIKSG GGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLY CRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQG SGSQRWKSKLYSIVCGKSTPEKEGELEGTTTKPLAPNPSFSPTPG FTPTLGFSPVPSSTFTSSSTYTPGDCPNFAAPRREVAPPYQGADPI LATALASDPIPNPLQKWEDSAHKPQSLDTDDPATLYAVVENVP PLRWKEFVRRLGLSDHEIDRLELQNGRCLREAQYSMLATWRRR TPRREATLELLGRVLRDMDLLGCLEDIEEALCGPAALPPAPSLL R
6	CD5-FcR-TNFR2	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI

SEQ ID NO	CFP/ Domain	Sequence
		TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGS GSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIKSG GGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLY CRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQG SGSPLCLQREAKVPHLPADKARGTQGPEQQHLLITAPSSSSSSLE SSASALDRRAPTRNQPQAPGVEASGAGEARASTGSSDSSPGGH GTQVNVTCIVNVCSSSDHSSQCSSQASSTMGDTDSSPSESPKDE QVPFSKEECAFRSQLETPETLLGSTEEKPLPLGVPDAGMKPS
7	GMCSF Signal peptide	MWLQSLLLLGTVACSIS
8	Anti-CD5 heavy chain variable domain	EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSL RAEDTAVYFCTRRGYDWYFDVWGQGTTVTV
91	Anti-CD5 heavy chain variable domain	EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSL RAEDTAVYFCTRRGYDWYFDVWGQGTTVTVSS
9	Anti-CD5 light chain variable domain	DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQKPGKAP KTLIYRANRLESGVPSRFSGSGSGTDYTLTISSLQYEDFGIYYCQ QYDESPWTFGGGTKLEIK
10	Anti-CD5 scFv	EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSL RAEDTAVYFCTRRGYDWYFDVWGQGTTVTV*SSGGGGSGGGGS GGGGS*DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQK PGKAPKTLIYRANRLESGVPSRFSGSGSGTDYTLTISSLQYEDFGI YYCQQYDESPWTFGGGTKLEIK
11	Anti-CD5 scFv with leader sequence	MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGY TFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTYADSFKGRF TFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQ GTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGS GSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIK

SEQ ID NO	CFP/ Domain	Sequence
12	Anti-HER2 light chain variable domain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ QHYTTPPTFGQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVE
92	Anti-HER2 light chain variable domain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ QHYTTPPTFGQGTKVEIK
13	Anti-HER2 heavy chain variable domain	LVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYP TNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRWGGDGFYAMDVWGQGTLVTV
93	Anti-HER2 heavy chain variable domain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGK GLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS
14	Anti-HER2 scFv	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ QHYTTPPTFGQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVE*SSG GGGSGGGGSGGGGS*LVQPGGSLRLSCAASGFNIKDTYIHWVRQ APGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQ MNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTV
94	Anti-HER2 scFv	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ QHYTTPPTFGQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGG GLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDVWGQGTLVTVSS
105	Anti-HER2 scFv	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ QHYTTPPTFGQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGG GLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDVWGQGTLVTVSS
31	Anti-CD137 binding domain	MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLLIDGP LSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLEL RRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEA

SEQ ID NO	CFP/ Domain	Sequence
		RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV LGLFRVTPEIPAGLPSPRSE
32	Anti-CD70 binding domain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAP GQGLEWMGWINTYTGEPTYADAFKGRVTMTTDTSTSTAYMEL RSLRSDDTAVYYCARDYGDYGMDYWGQGTTVTVSSGSTSGSG KPGSSEGSTKGDIVMTQSPDSLAVSLGERATINCRASKSVSTSG YSFMHWYQQKPGQPPKLLIYLASNLESGVPDRFSGSGSGTDFTL TISSLQAEDVAVYYCQHSREVPWTFGQGTKVEIK
33	Anti-Claudin18.2 binding domain (scFv)	QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGYNWHWIRQPPGK GLEWIGYIHYTGSTNYNPALRSRVTISVDTSKNQFSLKLSSVTA ADTAVYYCARIYNGNSFPYWGQGTTVTVSSGGGGSGGGGSGG GGSDIVMTQSPDSLAYSLGERATINCKSSQSLFNSGNQKNYLTW YQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDIFITISSLQAE DVAVYYCQNAYSFPYTFGGGTKLEIKR
34	Anti-Trop-2 binding domain (scFv)	DIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAP KLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQ QHYITPLTFGAGTKVEIKRGGGGSGGGGSGGGGSQVQLQQSGS ELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMG WINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAV YFCARGGFGSSYWYFDVWGQGSLVTVSS
35	Anti-Trop-2 binding domain (scFv)	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPG QGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISS LKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSSGGGGSG GGGSGGGGSDIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAW YQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQP EDFAVYYCQQHYITPLTFGAGTKVEIKR
95	Anti-Trop-2 VH domain	QVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPG QGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISS LKADDTAVYFCARGGFGSSYWYFDVWGQGSLVTVSS
96	Anti-TROP2 VL domain	DIQLTQSPSSLSASVGDRVSITCKASQDVSIAVAWYQQKPGKAP KLLIYSASYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQ QHYITPLTFGAGTKVEIKR

SEQ ID NO	CFP/ Domain	Sequence
97	Anti-GPC3 binding domain variable heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAP GQGLEWMGALDPKTGDTAYSQKFKGKATLTADKSTSTAYMEL SSLTSEDTAVYYCTRFYSYTYWGQGTLVTVSS
98	Anti-GPC3 binding domain variable light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNRNTYLHWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVG VYYCSQNTHVPPTFGQGTKLEIK
106	Anti-GPC3 binding domain (scFv)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAP GQGLEWMGALDPKTGDTAYSQKFKGKATLTADKSTSTAYMEL SSLTSEDTAVYYCTRFYSYTYWGQGTLVTVSSGGGGSGGGGSG GGGSDVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNRNTYLHW YLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEA EDVGVYYCSQNTHVPPTFGQGTKLEIK
	Anti-GPC3 VHH binding domains	
15	CD8a transmembrane domain	IYIWAPLAGTCGVLLLSLVIT
16	CD8a transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYC
17	CD2 Transmembrane domain	IYLIIGICGGGSLLMVFVALLVFYIT
18	CD28 transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWV
19	CD68 transmembrane domain	ILLPLIIGLILLGLLALVLIAFCII

SEQ ID NO	CFP/ Domain	Sequence
46	Mutant CD68 transmembrane domain	ILLPLIIGLILLGLLALVLIAFAII
20	CD8α hinge domain + transmembrane domain	ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLYC
21	CD8α hinge domain + transmembrane domain	ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVIT
22	FcRγ-chain intracellular signaling domain	LYCRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKP PQ
23	FcRγ-chain intracellular signaling domain	LYCRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPP Q
24	FcRγ-chain intracellular signaling domain	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
25	FcRγ-chain intracellular signaling domain	RRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
26	PI3K recruitment domain	YEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM
27	CD40 intracellular domain	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGC QPVTQEDGKESRISVQERQ

SEQ ID NO	CFP/ Domain	Sequence
28	TNFR1 intracellular domain	QRWKSKLYSIVCGKSTPEKEGELEGTTTKPLAPNPSFSPTPGFTP TLGFSPVPSSTFTSSSTYTPGDCPNFAAPRREVAPPYQGADPILA TALASDPIPNPLQKWEDSAHKPQSLDTDDPATLYAVVENVPPLR WKEFVRRLGLSDHEIDRLELQNGRCLREAQYSMLATWRRRTPR REATLELLGRVLRDMDLLGCLEDIEEALCGPAALPPAPSLLR
29	TNFR2 intracellular domain	PLCLQREAKVPHLPADKARGTQGPEQQHLLITAPSSSSSSLESSA SALDRRAPTRNQPQAPGVEASGAGEARASTGSSDSSPGGHGTQ VNVTCIVNVCSSSDHSSQCSSQASSTMGDTDSSPSESPKDEQVPF SKEECAFRSQLETPETLLGSTEEKPLPLGVPDAGMKPS
90	CLEC9A (1-35) intracellular signaling domain	MHEEEIYTSLQWDSPAPDTYQKCLSSNKCSGACCL

[00243] Provided herein is a composition comprising a recombinant nucleic acid sequence encoding a CFP comprising a phagocytic or tethering receptor (PR) subunit (e.g., a phagocytic receptor fusion protein (PFP)) comprising: an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; a transmembrane domain; and an intracellular domain comprising an intracellular signaling domain; and wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein upon binding of the CFP to the antigen on the target cell, the killing or phagocytosis activity of the myeloid cell, such as a neutrophil, monocyte, myeloid dendritic cell (mDC), mast cell or macrophage cell expressing the CFP is increased by at least 1.1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold,-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 75-fold, or 100-fold compared to a cell not expressing the CFP.

[00244] Provided herein is a recombinant nucleic acid sequence encoding a CFP as described in the immediate above paragraph, wherein the intracellular domain comprises at least one innate immune activating intracellular domain, e.g., a pattern recognition receptor intracellular signaling domain, a TLR intracellular signaling domain, an FcR intracellular signaling domain, an intracellular adapter protein signaling domain, or fragments thereof, that is capable of activating innate immune response of the myeloid cell, activating its phagocytic potential, activating inflammatory cytokine and chemokine response, antigen presentation and T cell activation of the myeloid cell that expresses the CFP, and upon contact with its target antigen, e.g., upon engagement of the antigen binding domain with the target antigen.

[00245] In some embodiments, the pro-inflammatory signaling domain comprises an intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR 9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, MAVS, TRIF or TASL intracellular domains, an NLRP family member, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, IL-1, IL3, IL5, IL-6, IL-12, IL-13, IL-23, TNF, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, or RANTES.

[00246] In some embodiments, the CFP comprises an intracellular signaling domain comprising a sequence derived from protein that activates interferon responsive transcription factors IRF1, IRF2, IRF3, IRF4, IRF5, IRF6, IRF7, IRF8, or IRF9.

[00247] In some embodiments, the CFP comprises intracellular signaling domain comprising a sequence derived from an intracellular adaptor protein. In some embodiments, the adaptor protein may comprise a transmembrane that anchors it to a organelle, such as mitochondria, endoplasmic reticulum or a lysosomal compartment. In some embodiments, the intracellular adaptor protein is a cytosolic protein.

TABLE 2. Interferon Regulatory Factor (IRF)-activating intracellular domains

SEQ ID NO	**CFP/ Domain**	**Sequence**
30	MDA5 intracellular domain (CARD domains)	MSNGYSTDENFRYLISCFRARVKMYIQVEPVLDYLTFLPA EVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREF VEALRRTGSPLAARYMNPELTDLPSPSFENAHDEYLQLLN LLQPTLVDKLLVRDVLDKCMEEELLTIEDRNRIAAAENNG NESGVRELLKRIVQKENWFSAFLNVLRQTGNNELVQELTG SDCSESNAEIEN
36	RIG-1 intracellular domain (CARD domains)	MTTEQRRSLQAFQDYIRKTLDPTYILSYMAPWFREEEVQY IQAEKNNKGPMEAATLFLKFLLELQEEGWFRGFLDALDH AGYSGLYEAIESWDFKKIEKLEEYRLLLKRLQPEFKTRIIPT DIISDLSECLINQECEEILQICSTKGMMAGAEKLVECLLRSD KENWPKTLKLALEKERNKFSELW
37	Myd88 intracellular domain	MAAGGPGAGSAAPVSSTSSLPLAALNMRVRRRLSLFLNV RTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAW QGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILK QQQEEAEKPLQVAAVDSSVPRTAELAGITTLDDPLGHMPE RFDAFICYCPSDIQFVQEMIRQLEQTNYRLKLCVSDRDVLP GTCVWSIASELIEKRCRRMVVVVSDDYLQSKECDFQTKFA LSLSPGAHQKRLIPIKYKAMKKEFPSILRFITVCDYTNPCTK SWFWTRLAKALSLP

38	STING (341-379)	VTVGSLKTSAVPSTSTMSQEPELLISGMEKPLPLRTDFS
39	MAVS (138-152)	PSYPMPVQETQAPES
40	MAVS (401-450)	SSAWLDSSSENRGLGSELSKPGVLASQVDSPFSGCFEDLAI SASTSLGMG
45	MAVS (138-152)-gggs linker- MAVS (401-450)	PSYPMPVQETQAPESgggsSSAWLDSSSENRGLGSELSKPG VLASQVDSPFSGCFEDLAISASTSLGMG
41	TRIF (1-255)	MACTGPSLPSAFDILGAAGQDKLLYLKHKLKTPRPGCQGQ DLLHAMVLLKLGQETEARISLEALKADAVARLVARQWA GVDSTEDPEEPPDVSWAVARLYHLLAEEKLCPASLRDVA YQEAVRTLSSRDDHRLGELQDEARNRCGWDIAGDPGSIRT LQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSLRSTG SPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQASLVP EPVPGGCQEPEEMSW
43	TRIF (short) (153-387)	GSIRTLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSL RSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQA SLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEV APDATSTGLPDTPAAPETSTNYPVECTEGSAGPQSLPLPILE PVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTPTTPETSPPP PPPPPSSTPCSAHLTPSSLFPSSLE
44	TRIF (long) (153-545)	GSIRTLQSNLGCLPPSSALPSGTRSLPRPIDGVSDWSQGCSL RSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCDDPQA SLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEV APDATSTGLPDTPAAPETSTNYPVECTEGSAGPQSLPLPILE PVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTPTTPETSPPP PPPPPSSTPCSAHLTPSSLFPSSLESSSEQKFYNFVILHARAD EHIALRVREKLEALGVPDGATFCEDFQVPGRGELSCLQDA IDHSAFIILLLTSNFDCRLSLHQVNQAMMSNLTRQGSPDCV IPFLPLESSPAQLSSDTASLLSGLVRLDEHSQIFARKVANTF KPHRLQARKAMWRKEQD
42	TASL (279-301)	MSTEITEISTPSLHISQYSNVNP

[00248] A CFP, as described herein may comprise any one of the sequences listed in Table 1, in combination with an intracellular domain listed in Table 2.

[00249] In some embodiments, for example, the intracellular signaling domain of a CFP described herein comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having an amino acid sequence of SEQ ID NO: 30 or at least 85% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 86%, or at least 87%, or at least 88% or at least 89% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 91% , or at least 92%, or at least 93% or at least 94% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 96%, or at least 97%, or at least 98% or at least 99% sequence identity to SEQ ID NO: 30. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 30; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 30; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MDA5 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 30; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule

on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 30.

[00250] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an RIG-1 intracellular domain having an amino acid sequence of SEQ ID NO: 36 or at least 85% sequence identity to SEQ ID NO: 36. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an RIG-1 intracellular domain intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 36. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an RIG-1 intracellular domain intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 36. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an RIG-1 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 36; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an RIG-1 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 36; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an RIG-1 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 36; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19

molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 36.

[00251] In some embodiments, the intracellular signaling domain of a CFP comprises an intracellular signaling domain derived from an MyD88 intracellular domain having an amino acid sequence of SEQ ID NO: 37 or at least 85% sequence identity to SEQ ID NO: 37. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an MyD88 intracellular domain intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 37. In some embodiments, the intracellular signaling domain comprises a sequence having at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 37. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an MyD88 intracellular domain intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 37. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MyD88 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 37; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MyD88 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 37; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain,

and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MyD88 intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 37; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 37.

[00252] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a STING intracellular domain having an amino acid sequence of SEQ ID NO: 38 or at least 85% sequence identity to SEQ ID NO:38. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from STING intracellular domain intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 38. In some embodiments, the intracellular signaling domain comprises a sequence having at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 38. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from STING intracellular domain intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 38. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an STING intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 38; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an STING intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 38; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on

a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an STING intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 38; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 38.

[00253] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a MAVS intracellular domain having an amino acid sequence of SEQ ID NO: 39 or 40 or an amino acid sequence with at least 85% sequence identity to SEQ ID NO: 39 or 40. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from MAVS intracellular domain intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 39 or 40. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from MAVS intracellular domain intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 39 or 40. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 39 or 40; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 39 or 40; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule

on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 39 or 40; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 39 or 40.

[00254] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a MAVS intracellular domain having an amino acid sequence of SEQ ID NO: 45 or a sequence with at least 85% sequence identity to SEQ ID NO: 45. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from MAVS intracellular domain intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 45. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from MAVS intracellular domain intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 45. In some embodiments, the intracellular signaling domain comprises a sequence having at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 45. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 45; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule

on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 45; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an MAVS intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 45; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 45.

[00255] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a TRIF intracellular domain having an amino acid sequence of SEQ ID NO: 41 or at least 85% sequence identity to SEQ ID NO: 41. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 86%, or at least 87%, or at least 88% or at least 89% sequence identity to SEQ ID NO: 41. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 41. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 91% , or at least 92%, or at least 93% or at least 94% sequence identity to SEQ ID NO: 41. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 41. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF

intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 41; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 41; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 41; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 41.

[00256] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a TRIF intracellular domain having an amino acid sequence of SEQ ID NO: 43 or at least 85% sequence identity to SEQ ID NO: 43. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 86%, or at least 87%, or at least 88% or at least 89% sequence identity to SEQ ID NO: 43. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 43. In some embodiments, the intracellular

signaling domain comprises a sequences that has at least at least 91% , or at least 92%, or at least 93% or at least 94% sequence identity to SEQ ID NO: 43. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 43. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 43; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 43; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 43; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 43.

[00257] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a TRIF intracellular domain having an amino acid sequence of SEQ ID NO: 44

or at least 85% sequence identity to SEQ ID NO: 44. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 86%, or at least 87%, or at least 88% or at least 89% sequence identity to SEQ ID NO: 44. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 44. In some embodiments, the intracellular signaling domain comprises a sequences that has at least at least 91% , or at least 92%, or at least 93% or at least 94% sequence identity to SEQ ID NO: 44. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 95% sequence identity to SEQ ID NO: 44.

[00258] In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 44; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 44; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TRIF intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 44; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid

sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 44.

[00259] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a TASL intracellular domain having an amino acid sequence of SEQ ID NO: 42 or at least 85% sequence identity to SEQ ID NO: 42. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TASL intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 42; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TASL intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 42; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD68 transmembrane domain or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and a hinge domain. In some embodiments, the intracellular domain of a CFP comprises an intracellular signaling domain derived from an TASL intracellular signaling domain having at least 90% sequence identity to SEQ ID NO: 42; wherein the CFP comprises and extracellular binding domain capable of binding a CD5 molecule on a target cell, a HER2 molecule on a target cell, a CD19 molecule on a target cell, a TROP2 molecule on a target cell, a GPC3 molecule on a target cell, a CD70 molecule on a target cell, a CD137 molecule on a target cell, a CD7 molecule on a target cell, a Claudin molecule on a target cell, a CD22 molecule on a target cell or GP75 molecule on a target cell; with a CD8 transmembrane domain or a CD28 transmembrane domain, or a CD64 transmembrane domain or a CD16 transmembrane domain, or a CD89 transmembrane domain, and one or more additional intracellular signaling domains such as PI3kinase recruitment domain or a CD40 intracellular signaling domain. For example, an exemplary CFP molecule disclosed herein comprises an extracellular CD5 binding domain having an amino acid sequence comprising the sequence of SEQ ID NO: 8, or SEQ ID NO: 9 or both, or that of SEQ ID NO: 10 or SEQ ID NO: 11; and an intracellular domain comprising a sequence having at least 90% sequence identity to SEQ ID NO: 42.

[00260] In some embodiments, an exemplary anti-CD5-binding CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a heavy chain variable domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a light chain variable domain comprising a CDR3 sequence of QQYDESPWT (SEQ ID NO: 100) and further comprises an intracellular domain of SEQ ID NO: 41. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 43. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 44. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 30. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 36. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 37. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 38. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 39. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO:

100; and further comprises an intracellular domain of SEQ ID NO: 40. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 10, or SEQ ID NO: 11, or at least a VH domain comprising a CDR3 sequence of RGYDWYFDV (SEQ ID NO: 99), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 100; and further comprises an intracellular domain of SEQ ID NO: 45.

[00261] In some embodiments, an exemplary anti-HER2-binding CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a heavy chain variable domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a light chain variable domain comprising a CDR3 sequence of QQHYTTPPT (SEQ ID NO: 102) and further comprises an intracellular domain of SEQ ID NO: 41. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 43. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 44. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 30. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 36. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 37. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 38. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence

of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 39. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 40. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 14, or SEQ ID NO: 94, or at least a VH domain comprising a CDR3 sequence of WGGDGFYAMDV (SEQ ID NO: 101), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 102; and further comprises an intracellular domain of SEQ ID NO: 45.

[00262] In some embodiments, an exemplary anti-TROP2-binding CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a heavy chain variable domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a light chain variable domain comprising a CDR3 sequence of QQHYITPLT (SEQ ID NO: 104) and further comprises an intracellular domain of SEQ ID NO: 41. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 43. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 44. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 30. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 36. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 37. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a

sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 38. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 39. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 40. In some embodiments, an exemplary CFP described herein comprises an extracellular antigen binding domain having a sequence of SEQ ID NO: 34, or SEQ ID NO: 35, or at least a VH domain comprising a CDR3 sequence of GGFGSSYWYFDV (SEQ ID NO: 103), and/or a VL domain comprising a CDR3 sequence of SEQ ID NO: 104; and further comprises an intracellular domain of SEQ ID NO: 45.

IRF inducible proteins and IRF activation pathways

[00263] Type I IFNs are key cytokines mediating innate antiviral immunity and therefore drive a pro-inflammatory response. Type I IFNs are readily induced by cGMP-AMP synthase, retinoic acid-inducible protein 1 (RIG-I)–like receptors, and Toll-like receptors that recognize microbial double-stranded (ds)DNA, dsRNA, and LPS. These signaling pathways converge at the recruitment and activation of the transcription factor IRF-3 (IFN regulatory factor 3). The adaptor proteins STING (stimulator of IFN genes), MAVS (mitochondrial antiviral signaling), and TRIF (TIR domain-containing adaptor inducing IFN-β) mediate the recruitment of IRF-3 through a conserved *p*L*x*IS motif. The *p*L*x*IS motif of phosphorylated STING, MAVS, and TRIF generally binds to IRF-3 in a similar manner, whereas residues upstream of the motif confer specificity. Type I IFNs, such as IFN-α and -β, are a major family of cytokines mediating antiviral immunity. Microbial dsDNA in the cytosol binds to and activates the enzyme cGAS (cGMP–AMP synthase), which catalyzes the synthesis of a cyclic dinucleotide, cGAMP (cyclic [G(2′,5′)pA(3′,5′)p]). As a second messenger, cGAMP binds to the adaptor protein STING (stimulator of IFN genes) located on the endoplasmic reticulum (ER) membrane and directs the activation of transcription factor IRF-3 (IFN regulatory factor 3) through the protein kinase TBK1 (TANK-binding kinase 1). Phosphorylated IRF-3 dimerizes and translocates to the nucleus to initiate the transcription of the IFN-β gene. In contrast, viral dsRNA in the cytosol is sensed by the RLRs [retinoic acid-inducible protein 1 (RIG-I)–like receptors] to activate IRF-3 via the adaptor protein MAVS (mitochondrial antiviral signaling). Moreover, the TLRs (Toll-like receptors) TLR3 and TLR4, which recognize viral dsRNA in the endosome and bacterial cell wall component LPS, respectively, also mediate the induction of type I

IFNs and inflammatory cytokines (**1**). These two TLRs use the adaptor protein TRIF (TIR domain-containing adaptor inducing IFN-β) to mediate the recruitment and activation of IRF-3. Strikingly, the signaling pathways of these three families of innate immune sensors converge at the activation of TBK1 and IRF-3. Mechanistically, the adaptor proteins STING, MAVS, and TRIF contain a conserved motif, *p*L*x*IS (in which *p* represents the hydrophilic residue, *x* represents any residue, and S represents the phosphorylation site), that is phosphorylated by TBK1 or IKKε and mediates the recruitment of IRF-3 to the signaling complexes. The induced proximity between TBK1 and IRF-3 results in IRF-3 phosphorylation and activation. Moreover, IRF-3 itself also contains a *p*L*x*IS motif that is crucial for phosphorylation-induced dimerization and activation of IRF-3. Mutations of the phosphorylation site serine in the *p*L*x*IS motif of STING, MAVS, and TRIF abolish the induction of type I IFNs in their respective signaling pathways. However, the exact molecular mechanisms of IRF-3 recruitment and activation remain unknown. To elucidate the structural bases of IRF-3 recruitment by phosphorylated STING (pSTING), MAVS (pMAVS), and TRIF (pTRIF), we expressed peptides containing the *p*L*x*IS motif from the three adaptor proteins, phosphorylated them in vitro with TBK1, and determined the crystal structures of their complexes with the IRF-3 C-terminal domain (CTD).

Mechanisms of IRF-3 Recruitment by pMAVS and pTRIF.

[00264] In contrast to dsDNA sensing through the cGAS–STING pathway, the RLRs sense dsRNA in the cytosol and activate IRF-3 via the adaptor MAVS, and TLR3 and TLR4 use the adaptor TRIF to recruit IRF-3. Phosphorylation of the *p*L*x*IS motif of MAVS or TRIF is required for the recruitment and activation of IRF-3.

TRAF interacting proteins:

[00265] The presence of the TRAF domain, a ~180 amino acid protein-interacting domain, is a distinct feature of TRAF family proteins and six TRAF proteins (TRAF1–TRAF6) among the seven in the family, in accordance with this criterion, have been identified as the TRAF family in mammals. The TRAF domain can be subdivided into two distinct regions: the TRAF-N domain and TRAF-C domain. Various receptors bind to the TRAF-C domain, while various intracellular signaling molecules bind to the TRAF-N domain. Despite the structural similarity of TRAF domains, each TRAF protein has specific biological functions with specificity to the interacting partners: upstream receptors and downstream effector molecules. The structure of the TRAF domain of TRAF2 was first reported by Dr. Wu's group around 1999, and the structure of TRAF6's TRAF domain was reported 3 years later by the same group. Since then, the structures of the TRAF domain of TRAF3, TRAF5, TRAF4, and TRAF1 have been reported. The TRAF structures revealed that the TRAF-N domain is a coiled-coil structure, and TRAF-C is composed of seven to eight anti-parallel β-sheet folds. Structural alignment of all six TRAF family members shows that the TRAF-C domain is well-aligned, while the location and length of TRAF-N varies among TRAF family members. Sequence analysis indicates that the length of TRAF-N varies in the family, whereas that of the TRAF-C domain is conserved: the length

of TRAF-N of TRAF4 and TRAF6 is relatively shorter, while TRAF3 and TRAF5 are relatively longer. Although the overall structures are nearly identical, obvious structural differences have been observed. For example, the length and position of some loops in the TRAF domain of TRAF4 and TRAF6 differ from those of other TRAF family members. TRAF4 contains a more negatively charged surface in the middle of the receptor-binding region, whereas TRAF6 contains a more positively charged surface in the receptor-binding region. Because the surface features often determine their mode of interactions with partners, the similar electrostatic surface of the TRAF domain among TRAF1, TRAF2, TRAF3, and TRAF5, namely, diversely charged surfaces, has been shown to be important for accommodating diverse receptors in the same binding pocket with similar modes of interaction. In contrast, different features on the binding surface of functionally different TRAFs, TRAF4, and TRAF6, indicate that TRAF4 and TRAF6 can accommodate different receptors with different modes of interactions.

[00266] For the purpose of this disclosure, any pathway, signaling intermediate, or activating moiety discussed in the paragraphs above may be considered as activable or functional as it applies, upon induction of the CFP disclosed herein. Likewise, the CFP disclosed herein may be useful in targeting any of the applicable targets that are described in the pathways discussed. Any pathway or part thereof readily known to one of skill in the art as of date from existing literature that relates to the signaling domain, signaling pathways, signaling intermediates, transcription factors of activated genes is understood to be within the prevue of this disclosure.

Chimeric proteins with TLR intracellular domains, TLR intracellular signaling pathways and NF-kappa B activation:

[0267] In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain derived from a TLR protein. In some embodiments, the CFP designed to comprise an intracellular signaling domain derived from a TLR intracellular signaling domains can activate NF-kappa B upon engagement of the receptor's extracellular domain to its target. In some embodiments, the intracellular domain may comprise an intracellular signaling domain of the endolysosomal TLR, e.g., TLR3, TLR7, TLR8, or TLR9. In some embodiments, the intracellular signaling domain may be derived from a TLR3 protein. In some embodiments, the intracellular signaling domain may be derived from a TLR7, 8, or 9 protein. In some embodiments, the intracellular domain may comprise an intracellular signaling domain of the cell surface TLRs 1, 2, 4, 5, 6, and 10. In some embodiments, the cytoplasmic domain for inflammatory response comprises an intracellular signaling domain of TLR3, TLR4, TLR9, MYD88, TRIF, RIG-1, MDA5, CD40, IFN receptor, NLRP-1, NLRP-2, NLRP-3, NLRP-4, NLRP-5, NLRP-6, NLRP-7, NLRP-8, NLRP-9, NLRP-10, NLRP-11, NLRP-12, NLRP-13, NLRP-14, NOD1, NOD2, Pyrin, AIM2, NLRC4 and/or CD40.

[0268] In some embodiments, the phagocytic scavenger receptor (PR) fusion protein (PFP) comprises a pro-inflammatory cytoplasmic domain for activation of IL-1 signaling cascade.

[0269] In some embodiments, the cytoplasmic portion of the chimeric receptor (for example, phagocytic receptor (PR) fusion protein (PFP)) comprises a cytoplasmic domain from a toll-like receptor, such as the intracellular signaling domains of toll-like receptor 3 (TLR3), toll-like receptor 4 (TLR4), toll-like receptor 7 (TLR7), toll-like receptor 8 (TLR8), toll-like receptor 9 (TLR9).

[0270] In general TLRs have a diverse cellular localization, pathways of generation, activation, recognition and mode of action. TLRs are expressed in innate immune cells such as dendritic cells (DCs) and macrophages as well as non-immune cells such as fibroblast cells and epithelial cells. Cell surface TLRs mainly recognize microbial membrane components such as lipids, lipoproteins, and proteins. TLR4 recognizes bacterial lipopolysaccharide (LPS). TLR2 along with TLR1 or TLR6 recognizes a wide variety of PAMPs including lipoproteins, peptidoglycans, lipoteichoic acids, zymosan, mannan, and tGPI-mucin. TLR5 recognizes bacterial flagellin. TLR10 is pseudogene in mouse due to an insertion of a stop codon, but human TLR10 collaborates with TLR2 to recognize ligands from listeria. TLR10 can also sense influenza A virus infection.

[0271] Intracellular TLRs recognize nucleic acids derived from bacteria and viruses, and also recognize self-nucleic acids in disease conditions such as autoimmunity. TLR3 recognizes viral double-stranded RNA (dsRNA), small interfering RNAs, and self-RNAs derived from damaged cells. TLR7 is predominantly expressed in plasmacytoid DCs (pDCs) and recognizes single-stranded (ss)RNA from viruses. It also recognizes RNA from streptococcus B bacteria in conventional DCs (cDCs). Human TLR8 responds to viral and bacterial RNA. Structural analysis revealed that unstimulated human TLR8 exists as a preformed dimer, and although the Z-loop between LRR14 and LRR15 is cleaved, the N- and C-terminal halves remain associated with each other and participate in ligand recognition and dimerization. Ligand binding induces reorganization of the dimer to bring the two C termini into close proximity. TLR13 recognizes bacterial 23S rRNA and unknown components of vesicular stomatitis virus. TLR9 recognizes bacterial and viral DNA that is rich in unmethylated CpG-DNA motifs; it also recognizes hemozoin, an insoluble crystalline byproduct generated by *Plasmodium falciparum* during the process of detoxification after host hemoglobin is digested. TLR11 is localized in the endolysosome and recognizes flagellin or an unknown proteinaceous component of uropathogenic *Escherichia coli* (UPEC) as well as a profilin-like molecule derived from *Toxoplasma gondii*. TLR12 is predominantly expressed in myeloid cells and is highly similar to TLR11 and recognizes profilin from *T. gondii*. TLR12 functions either as a homodimer or a heterodimer with TLR11. All TLRs are synthesized in the ER, traffic to the Golgi, and are recruited to the cell surface or to intracellular compartments such as endosomes. Intracellular localization of TLRs is thought to be critical for ligand recognition as well as for preventing TLRs from coming into contact with self-nucleic acids, which could cause autoimmunity.

[0272] Individual TLRs differentially recruit members of a set of TIR domain-containing adaptors such as MyD88, TRIF, TIRAP/MAL, or TRAM. MyD88 is utilized by all TLRs and activates NF-κB

and MAPKs for the induction of inflammatory cytokine genes. TIRAP is a sorting adaptor that recruits MyD88 to cell surface TLRs such as TLR2 and TLR4. TIRAP also participates in signaling through endosomal TLRs such as TLR9. The lipid-binding domain of TIRAP binds to $PI(4,5)P_2$ at the plasma membrane and to PI(3)P on endosomes, which mediates the formation of functional TLR4 and TLR9 signaling complexes at their respective sites. Thus, TIRAP associates with both cell surface and endosomal TLRs by binding to different lipids. TRIF is recruited to TLR3 and TLR4 and promotes an alternative pathway that leads to the activation of IRF3, NF-κB, and MAPKs for induction of type I IFN and inflammatory cytokine genes. TRAM is selectively recruited to TLR4 but not TLR3 to link between TRIF and TLR4. TLR3 directly interacts with TRIF, and this interaction requires phosphorylation of the two tyrosine residues in the cytoplasmic domain of TLR3 by the epidermal growth factor ErbB1 and Btk. After TLR engagement, MyD88 forms a complex with IRAK kinase family members. IRAK4 activates IRAK1, which is then autophosphorylated at several sites and released from MyD88. IRAK1 associates with the RING-domain E3 ubiquitin ligase TRAF6. TRAF6, along with ubiquitin-conjugating enzyme UBC13 and UEV1A, promotes K63-linked polyubiquitination of both TRAF6 itself and the TAK1 protein kinase complex. TAK1 is a member of the MAPKKK family and forms a complex with the regulatory subunits TAB1, TAB2, and TAB3, which interact with polyubiquitin chains generated by TRAF6 to drive TAK1 activation. TAK1 then activates two different pathways that lead to activation of the IKK complex-NF-κB pathway and -MAPK pathway. The IKK complex is composed of the catalytic subunits IKKα and IKKβ and the regulatory subunit NEMO (also called IKKγ). TAK1 binds to the IKK complex through ubiquitin chains, which allows it to phosphorylate and activate IKKβ. The IKK complex phosphorylates the NF-κB inhibitory protein IκBα, which undergoes proteasome degradation, allowing NF-κB to translocate into the nucleus to induce proinflammatory gene expression. TAK1 activation also results in activation of MAPK family members such as ERK1/2, p38 and JNK, which mediates activation of AP-1 family transcription factors or stabilization of mRNA to regulate inflammatory responses.

[0273] In some embodiments, an intracellular domain described herein may be specifically paired with another domain, e.g., a structural domain such as another intracellular domain, a transmembrane domain or an extracellular domain; a functional domain such as a signaling domain. In some embodiments, the intracellular domain described in the section herein, such as a MyD88, TRIF, TIRAP/MAL, TLR, MAVS, MDA5, STING, RIG1, TASL or any other domain mentioned herein may be adjusted or modified for pairing with another domain or component thereof. By pairing it is intended to mean inclusion of the part of the domain or domains in consideration, or a fragment or component thereof within the CFP design, as any part of the CFP protein molecular structure. In some embodiments, the adjustment may be structural alignment or placement of the domain within the CFP molecule, for example two domains in consideration are juxtaposed, or separated by one or more domains in between or separated by one or more amino acids in between. The one or more amino acids

may be linkers. The one or more amino acids may provide structural distancing between two adjacent domains, flexibility between two adjacent domains or confer a three dimensional orientation than a molecular structure comprising the domains without the one or more amino acids. In some embodiments, the adjustment or modification may comprise a modification within the domain, such as a mutation.

[0274] In some embodiments, the intracellular domain described in the section herein, such as a MyD88, TRIF, TIRAP/MAL, TLR, MAVS, MDA5, STING, RIG1, TASL or any other domain mentioned herein may be adjusted or modified for pairing or inclusion with another intracellular domain within the CFP, such as a kinase recruitment domain, such as a PI3 kinase recruitment domain. In some embodiments, the PI3kinase recruitment domain is modified to mask tonic signaling. In some embodiments one of the other intracellular domains, such as any one or more of the MyD88, TRIF, TIRAP/MAL, TLR, MAVS, MDA5, STING, RIG1, TASL domains or fragments thereof are modified to reduce or eliminate tonic signaling.

[0275] In some embodiments, the intracellular domain described in the section herein, such as a MyD88, TRIF, TIRAP/MAL, TLR, MAVS, MDA5, STING, RIG1, TASL or any other domain mentioned herein may be adjusted or modified for pairing or inclusion with another structural domain such as a transmembrane domain. In some embodiments, the transmembrane domain is a CD68 domain. In some embodiments, the transmembrane domain is a CD64 domain. In some embodiments the transmembrane domain is a CD89 domain. In some embodiments, a mutated CD68 domain may be used, e.g., SEQ ID NO: 46.

[00276] For the purpose of this disclosure, any pathway, signaling intermediate, or activating moiety discussed in the paragraphs above may be considered as activable or functional as it applies, upon induction of the CFP disclosed that comprises a TLR intracellular signaling domain as disclosed herein. Likewise, the CFP disclosed herein may be useful in targeting any of the applicable targets that are described in the pathways discussed. Any pathway or part thereof readily known to one of skill in the art as of date from existing literature that relates to the signaling domain, signaling pathways, signaling intermediates, transcription factors of activated genes is understood to be within the prevue of this disclosure.

Therapeutic compositions

[00277] Provided herein, in one aspect, is a myeloid cell, such as a CD14+ cell, a CD14+/CD16- cell, a CD14+/CD16+ cell, a CD14-/CD16+ cell, CD14-/CD16- cell, a dendritic cell, an M0 macrophage, an M2 macrophage, an M1 macrophage or a mosaic myeloid cell/macrophage/dendritic cell. In some embodiments, provided herein is a therapeutic composition comprising at least 20%, at least 30%, at least 40% or at least 50% CD14+ cells. In some embodiments, the therapeutic composition comprises at least 20%, at least 30%, at least 40% or at least 50% CD14+/CD16- cells. In some embodiments, provided herein is a therapeutic composition comprising less than 20%, less than 15%, less than 10%

or less than 5% dendritic cells. The myeloid cell for the therapeutic composition as described herein, comprises a recombinant nucleic acid that encodes a chimeric fusion protein encoding a CFP receptor protein or an engager protein as described herein. The myeloid cell for the therapeutic composition as described herein, expresses the CFP encoded by the recombinant nucleic acid or expresses an engager protein encoded by the recombinant nucleic acid as described herein.

[00278] In some embodiments, provided herein is a therapeutic composition comprising a chimeric fusion protein, such as a chimeric fusion receptor protein (CFP), the CFP comprises: (a) an extracellular domain comprising: (i) a scFv that specifically binds any one of the targets disclosed herein, and (ii) a hinge domain derived from CD8, a hinge domain derived from CD28 or at least a portion of an extracellular domain from CD68; (b) a CD8 transmembrane domain, a CD28 transmembrane domain, a CD2 transmembrane domain or a CD68 transmembrane domain; and (c) an intracellular domain comprising at least two intracellular signaling domains, wherein the at least two intracellular signaling domains comprise: (i) a first intracellular signaling domain derived from FcRγ or FcRε, (ii) a second intracellular signaling domain e.g., intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR 9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, MAVS, TRIF or TASL intracellular domains, an NLRP family member, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, IL-1, IL3, IL5, IL-6, IL-12, IL-13, IL-23, TNF, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, or RANTES and a second intracellular signaling domain, that comprises a PI3K recruitment domain, or a domain that is derived from CD40.

[00279] In some embodiments, the pharmaceutical composition comprises a population of cells comprising therapeutically effective dose of the myeloid cells. In some embodiments, the population of cells: differentiate into effector cells in the subject after administration; infiltrate into a diseased site of the subject after administration or migrate to a diseased site of the subject after administration; and/or have a life-span of at least 5 days in the subject after administration.

[00280] In some embodiments, myeloid cells may be further modified or manipulated to develop a therapeutically effective myeloid cells. Isolated cells can be manipulated by expressing a gene or a fragment thereof in the cell, without altering its functional and developmental plasticity, differential potential and cell viability.

[00281] In some embodiments, myeloid cells may be further modified or manipulated to develop a therapeutically effective myeloid cells by expressing a non-endogenous polynucleotide into the cell. A non-endogenous polynucleotide may encode for a protein or a peptide. Alternatively, a non-endogenous polypeptide may be a non-coding sequence, such as an inhibitory RNA, or a morpholino.

[00282] In some embodiments, myeloid cells may be further modified or manipulated to develop a therapeutically effective myeloid cells by stably altering the genomic sequence of the cell. In some embodiments, the myeloid cell is manipulated by editing the myeloid cell genome using a CRISPR-CAS system. In some embodiments, one or more genes may be edited to silence the gene expression.

In some embodiments, the myeloid cell is manipulated to delete a gene. In some embodiments, one or more genes may be edited to enhance the gene expression. In some embodiments, the genetic material is introduced into a myeloid cell in the form of a messenger RNA, wherein the messenger RNA encodes a protein or a peptide, thereby rendering the myeloid cell therapeutically effective. In some embodiments, naked DNA or messenger RNA (mRNA) may be used to introduce the nucleic acid inside the myeloid cell. In some embodiments, DNA or mRNA encoding the chimeric antigen receptor is introduced into the phagocytic cell by lipid nanoparticle (LNP) encapsulation. mRNA is single stranded and may be codon optimized. In some embodiments the mRNA may comprise one or more modified or unnatural bases such as 5'-Methylcytosine, or Pseudouridine or methyl pseudouridine. In some embodiments greater than or about 50% uridine ('U') residues of the mRNA may be converted to methyl-pseudouridine. In some embodiments, the mRNA may be 50-10,000 bases long. In one aspect the transgene is delivered as an mRNA. The mRNA may comprise greater than about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000 bases. In some embodiments, the mRNA may be more than 10,000 bases long. In some embodiments, the mRNA may be about 11,000 bases long. In some embodiments, the mRNA may be about 12,000 bases long. In some embodiments, the mRNA comprises a transgene sequence that encodes a fusion protein. LNP encapsulated DNA or RNA can be used for transfecting a macrophage or can be administered to a subject. In some embodiments, the mRNA is incorporated into an effector myeloid cell population by transient transfection. In some embodiments the transient transfection method comprises electroporation of the mRNA. In some embodiments, the transient transfection comprises chemical transfection. In some embodiments, 1-5,000 micrograms/ml of the mRNA may be used for transfection using a suitable protocol for the methods described above. In some embodiments, 1-2,000 micrograms/ml of the mRNA may be used for transfection. In some embodiments, 1-1,000 micrograms/ml of the mRNA may be used for transfection. In some embodiments, 1-1,000 micrograms/ml of the mRNA may be used for transfection. In some embodiments, 1-500 micrograms/ml of the mRNA may be used for transfection. In some embodiments, 1-250 micrograms/ml of the mRNA may be used for transfection. In some embodiments, about 500 micrograms/ml of the mRNA or less may be used for transfection. In some embodiments, about 250 micrograms/ml of the mRNA or less may be used for transfection. In some embodiments, about 10 micrograms/ml of the mRNA is used. In some embodiments, about 20 micrograms/ml of the mRNA is used. In some embodiments, about 30 micrograms/ml of the mRNA is used. In some embodiments, about 40 micrograms/ml of the mRNA is used. In some embodiments, about 50 micrograms/ml of the mRNA is used. In some embodiments, about 60 micrograms/ml of the mRNA is used. In some embodiments, about 80 micrograms/ml of the mRNA is used. In some embodiments, about 100 micrograms/ml of the mRNA is used. In some embodiments, about 150 micrograms/ml of the mRNA is used. In some embodiments, about 200 micrograms/ml of the mRNA

is used. In some embodiments, 20, 50, 100, 150, 200, 250, 300, 400, 500 or about 1000 micrograms/ml of the mRNA is used. A suitable cell density is selected for a transfection, based on the method and instrument and/or reagent manufacturer's instructions, or as is well-known to one of skill in the art.

[00283] In some embodiments the recombinant nucleic acid is an mRNA. mRNA constructs may be thawed on ice and gently pipetted to monocytes and pre-mixed. In some embodiments, the mRNA is electroporated into the cells. Cells following elutriation may be pooled, centrifuged and may be subjected to electroporation with mRNA using MaxCyte ATX system optimized for the said purpose. In some embodiments, optimized electroporation buffer, cell density, and/or mRNA concentration is used for each protocol for each construct.

[00284] In some embodiments, a polynucleotide may be introduced into a myeloid cell in the form of a circular RNA (circRNAs). In circular RNAs (circRNAs) the 3′ and 5′ ends are covalently linked. CircRNA may be delivered inside a cell using LNPs.

[00285] In some embodiments, a stable integration of transgenes into macrophages and other phagocytic cells may be accomplished via the use of a transposase and transposable elements, in particular, mRNA-encoded transposase. In one embodiment, Long Interspersed Element-1 (L1) RNAs may be contemplated for retrotransposition of the transgene and stable integration into a macrophage or a phagocytic cell. Retrotransposon may be used for stable integration of a recombinant nucleic acid encoding a phagocytic or tethering receptor (PR) fusion protein (PFP).

[00286] In some embodiments, the myeloid cell may be modified by expressing a transgene via incorporation of the transgene in a transient expression vector. In some embodiments expression of the transgene may be temporally regulated by a regulator from outside the cell. Examples include the Tet-on Tet-off system, where the expression of the transgene is regulated via presence or absence of tetracycline.

[00287] In some embodiments, the myeloid cell may be modified to develop a therapeutically effective cell by contacting the cell with a compound, which compound may be an inhibitor or an activator of a protein or enzyme within the myeloid cell.

[00288] In some embodiments, a polynucleotide encoding a chimeric antigen receptor may be introduced into an isolated myeloid cell that is obtained by the method described in the preceding section, where the chimeric antigen receptor upon expression in the myeloid cell augments an innate immune response function of the myeloid cell. In some embodiments, the chimeric antigen receptor expression can direct a myeloid cell to a specific target *in vivo* or *in vitro*. In some embodiments, the chimeric antigen receptor may increase the phagocytic potential of the myeloid cell. In some embodiments, the chimeric antigen receptor increases the immunogenicity of the myeloid cell. In some embodiments, the chimeric antigen receptor may increase augment intracellular signaling. In some embodiments, the chimeric antigen receptor may function cooperatively with one or more proteins within the cell. In some embodiments, the chimeric antigen receptor may dimerize or multimerize with

a second receptor or transmembrane protein inside the myeloid cell, where the second receptor or transmembrane protein is an endogenous protein. In some embodiments, the cells are cultured *ex vivo* briefly after thawing or after incorporation of the nucleic acid. In some embodiments, the *ex vivo* culture is performed in presence of a suitable medium, that may comprise a regulated serum component, e.g., human serum albumin (HSA). In some embodiments, the *ex vivo* culture and manipulation may be performed in low serum containing media. In some embodiment, the serum is specifically treated for compliment deactivation. In some embodiments, the myeloid cells may be cultured *ex vivo* as described above, in the presence of M-CSF. In some embodiments, the myeloid cells may be cultured *ex vivo* as described above, in the presence of GM-CSF. In some embodiments, the myeloid cells may be cultured in the presence of one or more cytokines. In some embodiments, the myeloid cells may be cultured or manipulated *ex vivo* in the absence of growth factor or cytokines for a period. In some embodiments, the method provided herein comprises isolation or enrichment and manipulation of a myeloid cell in less than 72 hours, 70 hours, 65 hours, 60 hours, 55 hours, 50 hours, 45 hours, 40 hours, or 35 hours, or 30 hours, or 28 hours, or 26 hours or 24 hours. In some embodiments, the myeloid cell may be culture for less than 24 hours, or less than 20 hours or less than 16 hours, or less than 14 hours, or less than 12 hours, or less than 10 hours, or less than 8 hours, or less than 6 hours or less than about 4 hours. The myeloid cell following isolation or enrichment and manipulation may be cultured briefly and frozen till further use. In some embodiments, the myeloid cell is thawed once or at the most twice.

[00289] In some embodiments, the therapeutically competent cells are cells that have been electroporated with a recombinant nucleic acid encoding a polypeptide, frozen and thawed, culture stabilized for less than 24 hours, and wherein the cells in the cell population at the time of administration exhibit (i) greater than at least 70% viability, (ii) greater than at least 50% CD14+ and CD16- cells; and/or greater than 50% CD11b+/CD14+/CD16- cells; (iii) less than 5% CD3+ cells, less than 5% CD19+ cells, less than about 10% CD56+ cells, less than about 10% CD42b+ cells (iv) greater than 50% cells express the polypeptide encoded by the electroporated nucleic acid. In some embodiments, the therapeutically competent cells are cells that have been electroporated with a recombinant nucleic acid encoding a polypeptide, culture stabilized for less than 24 hours, frozen and thawed, and wherein the cells in the cell population at the time of administration exhibit (i) greater than at least 70% viability, (ii) greater than at least 50% CD14+ and CD16- cells; and/or greater than 50% CD11b+/CD14+/CD16- cells; (iii) less than 5% CD3+ cells, less than 5% CD19+ cells, less than about 10% CD56+ cells, less than about 10% CD42b+ cells (iv) greater than 50% cells express the polypeptide encoded by the electroporated nucleic acid. In some embodiments, the therapeutically competent cells are cells that have been culture stabilized for less than 24 hours, that have been electroporated with a recombinant nucleic acid encoding a polypeptide, frozen and thawed, and wherein the cells in the cell population at the time of administration exhibit (i) greater than at least

70% viability, (ii) greater than at least 50% CD14+ and CD16- cells; and/or greater than 50% CD11b+/CD14+/CD16- cells; (iii) less than 5% CD3+ cells, less than 5% CD19+ cells, less than about 10% CD56+ cells, less than about 10% CD42b+ cells (iv) greater than 50% cells express the polypeptide encoded by the electroporated nucleic acid. Cells must be pathogen free. In the above embodiments, the therapeutically competent cells may have been frozen and thawed not more than twice, preferably once, and may be administered within 24 hours of thawing, within 18 hours of thawing, within 8 hours of thawing, or within 2 hours of thawing. Cells are tested for quality assurance to meet the standards as described herein in the disclosure prior to administering.

[00290] Provided herein are methods for treating cancer in a subject using a pharmaceutical composition comprising engineered phagocytic cells, particularly macrophages, expressing recombinant nucleic acid encoding a phagocytic receptor (PR) fusion protein (PFP), which is specifically designed to target, attack and kill cancer cells. The PFP is also designated as a chimeric antigenic receptor for phagocytosis (CAR-P), and both the terms may be used interchangeably herein. The engineered phagocytic cells are also designated as CAR-P cells in the descriptions herein.

[00291] Cancers include, but are not limited to, T cell lymphoma, cutaneous lymphoma, B cell cancer (e.g., multiple myeloma, Waldenstrom's macroglobulinemia), the heavy chain diseases (such as, for example, alpha chain disease, gamma chain disease, and mu chain disease), benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer (e.g., metastatic, hormone refractory prostate cancer), pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Other non-limiting examples of types of cancers applicable to the methods encompassed by the present disclosure include human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, colorectal cancer, pancreatic cancer, breast cancer, ovarian cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, liver cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, bone cancer, brain tumor, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma,

neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In some embodiments, the cancer is an epithelial cancer such as, but not limited to, bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer. In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (e.g., serous ovarian carcinoma), or breast carcinoma. The epithelial cancers can be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, or undifferentiated. In some embodiments, the present disclosure is used in the treatment, diagnosis, and/or prognosis of lymphoma or its subtypes, including, but not limited to, mantle cell lymphoma. Lymphoproliferative disorders are also considered to be proliferative diseases.

[00292] In general, cellular immunotherapy comprises providing the patient a medicament comprising live cells. In some aspects a patient or a subject having cancer, is treated with autologous cells, the method comprising, isolation or enrichment of PBMC-derived macrophages, modifying the macrophages *ex vivo* to generate highly phagocytic macrophages capable of tumor lysis by introducing into the macrophages a recombinant nucleic acid encoding chimeric antigenic receptor for phagocytosis which is a phagocytic receptor fusion protein (PFP), and administering the modified macrophages into the patient or the subject.

[00293] In one aspect, a subject is administered one or more doses of a pharmaceutical composition comprising therapeutic phagocytic cells, wherein the cells are allogeneic. An HLA may be matched for compatibility with the subject, and such that the cells do not lead to graft versus Host Disease, GVHD. A subject arriving at the clinic is HLA typed for determining the HLA antigens expressed by the subject, prior to determining a therapeutic or therapeutic regimen.

[00294] In some embodiments a therapeutically effective dose ranges between 10^7 cells to 10^12 myeloid cells for one infusion. The cell number may vary according to the age, body weight and other subject-related parameters and can be determined by a medical practitioner. In some embodiments, a therapeutically effective dose is about 10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 2 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 3 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 4 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 5 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 6 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 7 x10^7 myeloid cells. In some embodiments,

a therapeutically effective dose is about 8 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 9 x10^7 myeloid cells. In some embodiments, a therapeutically effective dose is about 10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 2 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 3 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 4 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 5 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 6 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 7 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 8 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 9 x10^8 myeloid cells. In some embodiments, a therapeutically effective dose is about 10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 2 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 3 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 4 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 5 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 6 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 7 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 8 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 9 x10^9 myeloid cells. In some embodiments, a therapeutically effective dose is about 10^10 myeloid cells. In some embodiments, a therapeutically effective dose is about 5 x 10^10 myeloid cells. In some embodiments a therapeutically effective dose is about 10^11 myeloid cells. In some embodiments a therapeutically effective dose is about 5 x 10^11 myeloid cells. In some embodiments a therapeutically effective dose is about 10^12 myeloid cells.

[00295] Provided herein, in one aspect, one or more recombinant polynucleic acid(s) encoding one or more recombinant proteins that can be a chimeric fusion protein such as a receptor, or an engager as described herein. In some embodiments, the recombinant polynucleic acid(s) is an mRNA. In some embodiments, the recombinant polynucleic acid comprises a circRNA. In some embodiments, the recombinant polynucleic acid is encompassed in a viral vector. In some embodiments, the recombinant polynucleic acid is delivered via a viral vector.

[00296] In some embodiments, provided herein is a therapeutic composition comprising a recombinant nucleic acid encoding a chimeric fusion protein, such as a chimeric fusion receptor protein (CFP), the CFP comprises: (a) an extracellular domain comprising: (i) a scFv that specifically binds any one of the targets disclosed herein, and (ii) a hinge domain derived from CD8, a hinge domain derived from CD28 or at least a portion of an extracellular domain from CD68; (b) a CD8 transmembrane domain, a CD28 transmembrane domain, a CD2 transmembrane domain or a CD68 transmembrane domain; and (c) an intracellular domain comprising at least two intracellular signaling domains, wherein the at least two intracellular signaling domains comprise: (i) a first intracellular signaling domain derived

from FcRγ or FcRε, an interferon inducing domain, and/or (ii) a third intracellular signaling domain that: (A) comprises a PI3K recruitment domain, or (B) is derived from CD40.

[00297] In some embodiments, provided herein is therapeutic composition comprising a recombinant nucleic acid encoding a bispecific or trispecific engager as disclosed herein.

Other therapeutic compositions for co-administration

[00298] In some embodiments, the therapeutic composition further comprises an additional therapeutic agent selected from the group consisting of a CD47 agonist, an agent that inhibits Rac, an agent that inhibits Cdc42, an agent that inhibits a GTPase, an agent that promotes F-actin disassembly, an agent that promotes PI3K recruitment to the PFP, an agent that promotes PI3K activity, an agent that promotes production of phosphatidylinositol 3,4,5-trisphosphate, an agent that promotes ARHGAP12 activity, an agent that promotes ARHGAP25 activity, an agent that promotes SH3BP1 activity, an agent that promotes sequestration of lymphocytes in primary and/or secondary lymphoid organs, an agent that increases concentration of naïve T cells and central memory T cells in secondary lymphoid organs, and any combination thereof.

[00299] In some embodiments, the myeloid cell further comprises: (a) an endogenous peptide or protein that dimerizes with the CFP, (b) a non-endogenous peptide or protein that dimerizes with the CFP; and/or (c) a second recombinant polynucleic acid sequence, wherein the second recombinant polynucleic acid sequence comprises a sequence encoding a peptide or protein that interacts with the CFP; wherein the dimerization or the interaction potentiates phagocytosis by the myeloid cell expressing the CFP as compared to a myeloid cell that does not express the CFP.

[00300] In some embodiments, the myeloid cell exhibits (i) an increase in effector activity, cross-presentation, respiratory burst, ROS production, iNOS production, inflammatory mediators, extra-cellular vesicle production, phosphatidylinositol 3,4,5-trisphosphate production, trogocytosis with the target cell expressing the antigen, resistance to CD47 mediated inhibition of phagocytosis, resistance to LILRB1 mediated inhibition of phagocytosis, or any combination thereof; and/or (ii) an increase in expression of a IL-1, IL3, IL-6, IL-10, IL-12, IL-13, IL-23, TNFα, a TNF family of cytokines, CCL2, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL-17, IP-10, RANTES, an interferon, MHC class I protein, MHC class II protein, CD40, CD48, CD58, CD80, CD86, CD112, CD155, a TRAIL/TNF Family death receptor, TGFβ, B7-DC, B7-H2, LIGHT, HVEM, TL1A, 41BBL, OX40L, GITRL, CD30L, TIM1, TIM4, SLAM, PDL1, an MMP (e.g., MMP2, MMP7 and MMP9) or any combination thereof.

[00301] In some embodiments, the intracellular signaling domain is derived from a phagocytic or tethering receptor or wherein the intracellular signaling domain comprises a phagocytosis activation domain. In some embodiments, the intracellular signaling domain is derived from a receptor other than a phagocytic receptor selected from Megf10, MerTk, FcR-alpha, or Bai1. In some embodiments, the intracellular signaling domain is derived from a protein, such as receptor (e.g., a phagocytic receptor),

selected from the group consisting of TNFR1, MDA5, CD40, lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fcα receptor I, CR1, CD35, CD3ζ, a complement receptor, CR3, CR4, Tim-1, Tim-4 and CD169. In some embodiments, the intracellular signaling domain comprises a pro-inflammatory signaling domain. In some embodiments, the intracellular signaling domain comprises a pro-inflammatory signaling domain that is not a PI3K recruitment domain.

[00302] In some embodiments, the intracellular signaling domain is derived from an ITAM domain containing receptor.

[00303] Provided herein is a composition comprising a recombinant nucleic acid encoding a CFP, such as a phagocytic or tethering receptor (PR) fusion protein (PFP), comprising: a PR subunit comprising: a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain; and an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein the intracellular signaling domain is derived from a phagocytic receptor other than a phagocytic receptor selected from Megf10, MerTk, FcRα, or Bai1.

[00304] In some embodiments, upon binding of the CFP to the antigen of the target cell, the killing activity of a cell expressing the CFP is increased by at least greater than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, or 1000% compared to a cell not expressing the CFP. In some embodiments, the CFP functionally incorporates into a cell membrane of a cell when the CFP is expressed in the cell. In some embodiments, upon binding of the CFP to the antigen of the target cell, the killing activity of a cell expressing the CFP is increased by at least 1.1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold,-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 75-fold, or 100-fold compared to a cell not expressing the CFP.

[00305] In some embodiments, the intracellular signaling domain is derived from a receptor, such as a phagocytic receptor, selected from the group consisting of TNFR1, MDA5, CD40, lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fcα receptor I, CR1, CD35, CD3ζ, CR3, CR4, Tim-

1, Tim-4 and CD169. In some embodiments, the intracellular signaling domain comprises a pro-inflammatory signaling domain.

[00306] Provided herein is a composition comprising a recombinant nucleic acid encoding a CFP, such as a phagocytic or tethering receptor (PR) fusion protein (PFP), comprising: a PR subunit comprising: a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain; and an extracellular domain comprising an antigen binding domain specific to an antigen of a target cell; wherein the transmembrane domain and the extracellular domain are operatively linked; and wherein the intracellular signaling domain is derived from a receptor, such as a phagocytic receptor, selected from the group consisting of TNFR1, MDA5, CD40, lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fcα receptor I, CR1, CD35, CD3ζ, CR3, CR4, Tim-1, Tim-4 and CD169.

[00307] In some embodiments, upon binding of the CFP to the antigen of the target cell, the killing activity of a cell expressing the CFP is increased by at least greater than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, or 1000% compared to a cell not expressing the CFP. In some embodiments, the intracellular signaling domain is derived from a phagocytic receptor other than a phagocytic receptor selected from Megf10, MerTk, FcRα, or Bai1. In some embodiments, the intracellular signaling domain comprises a pro-inflammatory signaling domain. In some embodiments, the intracellular signaling domain comprises a PI3K recruitment domain, such as a PI3K recruitment domain derived from CD19. In some embodiments, the intracellular signaling domain comprises a pro-inflammatory signaling domain that is not a PI3K recruitment domain.

[00308] In some embodiments, a cell expressing the CFP exhibits an increase in phagocytosis of a target cell expressing the antigen compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits at least a 1.1-fold increase in phagocytosis of a target cell expressing the antigen compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits at least a 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold or 50-fold increase in phagocytosis of a target cell expressing the antigen compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in production of a cytokine compared to a cell not expressing the CFP. In some embodiments, the cytokine is selected from the group consisting of IL-1, IL3, IL-6, IL-12, IL-13, IL-23, TNF, CCL2, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, RANTES, an

interferon and combinations thereof. In some embodiments, a cell expressing the CFP exhibits an increase in effector activity compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in cross-presentation compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of an MHC class II protein compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD80 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD86 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of MHC class I protein compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of TRAIL/TNF Family death receptors compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of B7-H2 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of LIGHT compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of HVEM compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD40 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of TL1A compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of 41BBL compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of OX40L compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of GITRL death receptors compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD30L compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of TIM4 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of TIM1 ligand compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of SLAM compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD48 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD58 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD155 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of CD112 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of PDL1 compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in expression of B7-DC compared to a cell not expressing the

CFP. In some embodiments, a cell expressing the CFP exhibits an increase in respiratory burst compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in ROS production compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in iNOS production compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in iNOS production compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in extra-cellular vesicle production compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in trogocytosis with a target cell expressing the antigen compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in resistance to CD47 mediated inhibition of phagocytosis compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in resistance to LILRB1 mediated inhibition of phagocytosis compared to a cell not expressing the CFP. In some embodiments, a cell expressing the CFP exhibits an increase in phosphatidylinositol 3,4,5-trisphosphate production.

[00309] Also provided herein is a pharmaceutical composition comprising a composition described herein, such as a recombinant nucleic acid described herein, a vector described herein, a polypeptide described herein or a cell described herein; and a pharmaceutically acceptable excipient. The engineered cell is a myeloid cell. In one aspect, a pharmaceutical composition is disclosed, comprising a recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51, or a cell comprising the recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51, or an engineered cell comprising the recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51; and a pharmaceutically acceptable excipient. In one embodiment the cell comprises a recombinant nucleic acid encoding an amino acid sequence comprising at least one of the sequences selected from SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44 and 45. In one embodiment the cell is a myeloid cell. In one embodiment, the cell is a mammalian cell. In one embodiment the cell is a primary human cell. In one embodiment, the cell is a human primary immune cell. In some embodiments the cell is a precursor cell or a stem cell, or an undifferentiated cell. In some embodiments the cell is obtained from a biological sample of a human subject. In some embodiments the cell is isolated from a biological sample of a human subject, and is selected for a phenotype, such as expression of cell surface markers. In one embodiment, the isolated cell is a precursor cell, a precursor myeloid cell, a cell characterized as CD14+/CD16-.

[00310] In one embodiment, the isolated cell or the engineered cell is CD14+/CD16-.

[00311] In one aspect, a pharmaceutical composition comprises an engineered cell, wherein the engineered cell is CD14+/CD16-.

[00312] In one aspect the pharmaceutical composition comprises a population of cells wherein at least 50% of the cells are CD14+/CD16-, and less than 10% cells are dendritic cells. In one embodiment,

the cells exhibit high expression of CCR2. In one embodiment, the cells do not exhibit tonal signaling and activation de novo, and exhibit M0, M1 or M2 differentiation upon activation.

[00313] Provided herein is a method of treating a cancer or a viral infection in a subject comprising: administering to the subject the pharmaceutical composition comprising the recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51, or a cell comprising the recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51, or an engineered cell comprising the recombinant nucleic acid of encoding or comprising any one of the sequences of SEQ ID NOs: 1-51; and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition comprises a population of cells wherein at least 50% of the cells are CD14+/CD16-, with less than 10% cells that are dendritic cells; and the cells exhibit high expression of CCR2. In one embodiment, the cells do not exhibit tonal signaling and activation de novo, and exhibit M0, M1 or M2 differentiation upon activation.

[00314] In some embodiments, provided herein is a therapeutic composition comprising a cell, the cell comprising a recombinant nucleic acid as described anywhere within the specification. In some embodiments, the therapeutic composition comprising a recombinant nucleic acid expressing a chimeric protein as described anywhere herein. In some embodiments, the myeloid cell is a CD14+ cell, a CD14+/CD16- cell, a CD14+/CD16+ cell, a CD14-/CD16+ cell, CD14-/CD16- cell, a dendritic cell, an M0 macrophage, an M2 macrophage, an M1 macrophage or a mosaic myeloid cell/macrophage/dendritic cell.

[00315] In some embodiments, the pharmaceutical composition further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of a CD47 agonist, an agent that inhibits Rac, an agent that inhibits Cdc42, an agent that inhibits a GTPase, an agent that promotes F-actin disassembly, an agent that promotes PI3K recruitment to the PFP, an agent that promotes PI3K activity, an agent that promotes production of phosphatidylinositol 3,4,5-trisphosphate, an agent that promotes ARHGAP12 activity, an agent that promotes ARHGAP25 activity, an agent that promotes SH3BP1 activity and any combination thereof. In some embodiments, the pharmaceutically acceptable excipient comprises serum free media, a lipid, or a nanoparticle.

[00316] In some embodiments, the therapeutic is a recombinant nucleic acid encoding or comprising any one of the sequences of SEQ ID NOs: 1-51, that is injected systemically or topically in a subject. In some embodiments the recombinant nucleic acid comprises at least one of the sequences encoding sequences selected from SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44 and 45. In some embodiments the recombinant nucleic acid is mRNA or circRNA. In some embodiments the recombinant nucleic acid is associated with one or more lipid components in the pharmaceutical composition. The lipid components may be associated in the form of a liposome or a lipid nanoparticle. The lipid components

may comprise at least one cationic lipid. One or more lipids of the pharmaceutical composition may be conjugated or modified.

Methods for generation of novel chimeric receptors fusion proteins (CFP) constructs

[00317] In one aspect, provided herein is a method for generating novel chimeric receptor proteins, including, for example, identification of novel domains that can be useful in augmenting a myeloid cell function such that when the fusion receptor is expressed in a myeloid cell, it functions as an effector myeloid cell of the specifications described herein. Generation of fusion proteins as described herein can be performed using well known molecular cloning techniques, and the sequences can be verified after generating of the recombinant nucleic acid.

[00318] *Preparation of Recombinant Nucleic Acid Encoding A Chimeric Antigen Receptor*: Recombinant nucleic acid constructs are prepared that encode chimeric antigen receptor (CAR) designed for expression in a myeloid cell and are incorporated in plasmid vectors for amplification and/or testing expression in an eukaryotic cell. The recombinant CARs are constructed using molecular cloning techniques known in the art. A recombinant CAR protein comprises an intracellular domain, a transmembrane domain and an extracellular domain. Each domain or subsection of a domain can be encoded by a nucleic acid sequence that is generated by PCR from heterologous source sequences, and pieced together by cloning individually into the vector, or ligated into a longer nucleic acid that is then inserted into the multi-cloning sites of a suitable plasmid or vector with appropriate promoter and 3'-regulatory elements for amplification. Briefly, an exemplary CAR is prepared by incorporating a nucleic sequence encoding one or more signaling domains, (e.g., a PI3Kinase recruiting domain), a nucleic acid sequence encoding the CD8 hinge and transmembrane domain, a nucleic acid sequence encoding an extracellular domain, having a sequence encoding a target antigen binding scFv at the extracellular end. Certain constructs include a FLAG peptide sequence at the extracellular end designed such that it does not pose hindrance to the scFv binding to its target antigen. These components are ligated together into a sequence that encode a fully functional transmembrane CAR. The nucleic acid subunits encoding individual domains of the recombinant protein is designed to include intervening short flexible linker sequences between two domains. The construct is ligated in a plasmid having a promoter and 3' stabilizing structural units. In one variation, the construct is placed within an Alu retrotransposon element that encodes ORF2p and has the respective 5'- and 3'-UTR sequences, a CMV promoter. The plasmid is amplified in E. coli, validated by sequencing or stored in (-) 80°C.

mRNA Preparation: mRNA can be prepared by *in vitro* transcription using the digested plasmid as template and purified to remove contaminant DNA and polyadenylated. The RNA product is purified, resuspended to 1mg/ml in RNase free water and stored in cryovials.

[00319] Identification of useful CFP ECD, TM, ICD and antigen binding domains for the generation of novel CFPs can be done using the method described herein. Briefly, a large number of potential

candidate proteins can be screened for enhanced phagocytic properties and their respective phagocytosis related intracellular signaling. The useful domains can be then used for generation of novel CFPs. The screen can be divided in two parts: *A. Screening for the phagocytic receptor (PR) domains*; *B. Screening for the antigen binding domains.*

Screening for the PR domains:

[00320] In one embodiment, about 5,800 plasma membrane proteins were screened for their phagocytic potential following the general method described herein. J774 macrophage cells can be transiently transfected with the library of 5800 plasma proteins. High-throughput multiplex assays (ranging from 6-well plate assay set up to up to 384-well plate assay with robotic handling) can be set up to evaluate various potential functions of the plasma membranes. Exemplary assays include, but are not limited to phagocytosis assay, cytokine production assay, inflammasome activation assay, and iNOS activation assay. Exemplary simplified methods can be described in the following paragraphs. Variations of each method can be also used and can be understood by a skilled artisan. Variations of each method can be also used and can be understood by a skilled artisan. Exemplary intracellular signaling domains tested for include but are not limited to CD40-FcRγ; FcRγ-CD40; NLRP3; FcRγ-SH2-Procaspase; FcRγ-Myd88; FcRγ-IFN receptor; FcR-TNFR1; FcRγ-TNFR2; FcR-AIM2; FcRγ-TRIFN; FcRγ-Procaspase; TRIFC; RIG1; MDA5; TBK; CD64; CD16A; CD89; FcRε; SIRPβ; (two consecutive intracellular domains can be represented as hyphenated terms, for example, FcRγ-Myd88 refers to an intracellular domain comprising an FcRγ intracellular signaling domain as signaling domain 1; and an Myd88 intracellular signaling domain as signaling domain 2). The extracellular linker domains screened include but are not limited to CD64, CD16A, CD89, SIRPα, FcRε, CD8 hinge. The transmembrane domains tested include but are not limited to CD8, CD64, CD16A, CD89, FcRε, SIRPα, TNFR1 and CD40. MDA5 domains were also screened.

Phagocytosis assay:

[00321] Antigen-linked silica or polysterene beads ranging in diameters 1 nm, 5 nm or 10 nm were used for a screen of macrophages. Inert beads can be coated in a supported lipid bilayer and the antigens can be ligated to the lipid bilayer. J774 macrophage cell lines can be prepare d, each cell line expressing a cloned recombinant plasma membrane protein. The recombinant plasma membrane protein may also express a fluorescent tag. The cell lines can be maintained and propagated in complete RPMI media with heat inactivated serum and antibiotics (Penicillin/Streptomycin). On the day of the assay, cells can be plated at a density of 1x10^6 cells/ml per well in 6 well plates or in a relative proportion in 12 or 24 well plates, and incubated for 2-6 hours. The cells can be then washed once in Phosphate Buffer Saline, and the beads can be added in serum depleted or complement depleted nutrient media. Cells can be visualized by light microscopy at 30 minutes and 2 hours after addition of the beads. Immunofluorescence reaction may be performed using tagged antibody, and fluorescent confocal microscopy is used to detect the interaction and co-localization of cellular

proteins at engulfment. Confidence levels can be determined by Kruskal-Wallis test with Dunn's multiple comparison correction.

[00322] In some examples, dye loaded tumor cells can be fed to macrophage cell lines and phagocytosis is assessed by microscopy.

Cytokine production:

[00323] Macrophage cell lines can be cultured as described above. In one assay, each J774 cell line expressing a plasma membrane protein is plated in multi-wells and challenged with antigen-linked beads and cytokine production was assayed by collecting the supernatants at 4 hours and 24 hours. Cytokines can be assayed from the supernatant by ELISA. In another fraction, cells can be collected at 4 and 24 hours after incubation with the beads and flow cytometry is performed for detection of cytokines. In each case, multiple cytokines can be assayed in a multiplex format, which can be selected from: IL-1α, IL-1β, IL-6, IL-12, IL-23, TNF-α, GMCSF, CXCL1, CXCL3, CXCL9, CXCL-10, MIP1-α and MIP-2. Macrophage inflammatory cytokine array kit (R&D Systems) is used.

[00324] Intracellular signaling pathway for inflammatory gene and cytokine activation can be identified by western blot analysis for phosphorylation of MAP kinases, JNK, Akt signaling pathway, Interferon activation pathway including phosphorylation and activation of STAT-1.

Functional Assays

Inflammasome activation assay:

[00325] Activation of NLRP3 inflammasome is assayed by ELISA detection of increased IL-1 production and detection caspase-1 activation by western blot, detecting cleavage of procaspase to generate the shorter caspase. In a microwell plate multiplex setting, Caspase-Glo (Promega Corporation) is used for faster readout of Caspase 1 activation.

iNOS activation assay:

[00326] Activation of the oxidative burst potential can be measured by iNOS activation and NO production using a fluorimetric assay NOS activity assay kit (AbCAM).

Cancer cell killing assay:

[00327] Raji B cells can be used as cancer antigen presenting cells. Raji cells can be incubated with whole cell crude extract of cancer cells, and co-incubated with J774 macrophage cell lines. The macrophages can destroy the cells after 1 hour of infection, which can be detected by microscopy or detected by cell death assay.

Screening for high affinity antigen binding domains:

[00328] Cancer ligands can be subjected to screening for antibody light chain and heavy chain variable domains to generate extracellular binding domains for the CFPs. Human full length antibodies or scFv libraries can be screened. Also potential ligands can be used for immunizing llama for development of novel immunoglobulin binding domains in llama, and preparation of single domain antibodies.

[00329] Specific useful domains identified from the screens can be then reverse transcribed, and cloned into lentiviral expression vectors to generate the CFP constructs. A recombinant nucleic acid encoding a CFP can generated using one or more domains from the extracellular, TM and cytoplasmic regions of the highly phagocytic receptors generated from the screen. Briefly plasma membrane receptors showing high activators of pro-inflammatory cytokine production and inflammasome activation can be identified. Bioinformatics studies can be performed to identify functional domains including extracellular activation domains, transmembrane domains and intracellular signaling domains, for example, specific kinase activation sites, SH2 recruitment sites. These screened functional domains can be then cloned in modular constructions for generating novel CFPs. These can be candidate CFPs, and each of these chimeric construct is tested for phagocytic enhancement, production of cytokines and chemokines, and/or tumor cell killing in vitro and/or in vivo. A microparticle based phagocytosis assay was used to examine changes in phagocytosis. Briefly, streptavidin coupled fluorescent polystyrene microparticles (6 μm diameter) can be conjugated with biotinylated recombinantly expressed and purified cancer ligand. Myeloid cells expressing the novel CFP can be incubated with the ligand coated microparticles for 1-4 h and the amount of phagocytosis was analyzed and quantified using flow cytometry. Plasmid or lentiviral constructions of the designer CFPs can be then prepared and tested in macrophage cells for cancer cell lysis.

Method of manufacturing myeloid cells from a subject

Myeloid cell isolation from PBMCs:

[00330] Peripheral blood mononuclear cells can be separated from normal donor buffy coats by density centrifugation using Histopaque 1077 (Sigma). After washing, CD14+ monocytes can be isolated from the mononuclear cell fraction using CliniMACS GMP grade CD14 microbeads and LS separation magnetic columns (Miltenyi Biotec). Briefly, cells can be resuspended to appropriate concentration in PEA buffer (phosphate-buffered saline [PBS] plus 2.5 mmol/L ethylenediaminetetraacetic acid [EDTA] and human serum albumin [0.5% final volume of Alburex 20%, Octopharma]), incubated with CliniMACS CD14 beads per manufacturer's instructions, then washed and passed through a magnetized LS column. After washing, the purified monocytes can be eluted from the demagnetized column, washed and re-suspended in relevant medium for culture. Isolation of CD14+ cells from leukapheresis: PBMCs can be collected by leukapheresis from cirrhotic donors who gave informed consent to participate in the study. Leukapheresis of peripheral blood for mononuclear cells (MNCs) is carried out using an Optia apheresis system by sterile collection. A standard collection program for MNC is used, processing 2.5 blood volumes. Isolation of CD14 cells is carried out using a GMP-compliant functionally closed system (CliniMACS Prodigy system, Miltenyi Biotec). Briefly, the leukapheresis product is sampled for cell count and an aliquot taken for pre-separation flow cytometry. The percentage of monocytes (CD14+) and absolute cell number can be determined, and, if required, the volume is adjusted to meet the required criteria for selection ($\leq 20 \times 10^9$ total white blood cells; <

400 x 10^6 white blood cells/mL; ≤ 3.5 x 10^9 CD14 cells, volume 50–300 mL). CD14 cell isolation and separation is carried out using the CliniMACS Prodigy with CliniMACS CD14 microbeads (medical device class III), TS510 tubing set and LP-14 program. At the end of the process, the selected CD14+ positive monocytes can be washed in PBS/EDTA buffer (CliniMACS buffer, Miltenyi) containing pharmaceutical grade 0.5% human albumin (Alburex), then re-suspended in TexMACS (or comparator) medium for culture.

Cell count and purity:

[00331] Cell counts of total MNCs and isolated monocyte fractions can be performed using a Sysmex XP-300 automated analyzer (Sysmex). Assessment of macrophage numbers is carried out by flow cytometry with TruCount tubes (Becton Dickinson) to determine absolute cell number, as the Sysmex consistently underestimated the number of monocytes. The purity of the separation is assessed using flow cytometry (FACSCanto II, BD Biosciences) with a panel of antibodies against human leukocytes (CD45-VioBlue, CD15-FITC, CD14-PE, CD16-APC), and product quality is assessed by determining the amount of neutrophil contamination (CD45int, CD15pos).

Cell culture—development of cultures with healthy donor samples

[00332] Optimal culture medium for macrophage differentiation is investigated, and three candidates can be tested using for the cell product. In addition, the effect of monocyte cryopreservation on deriving myeloid cells and macrophages for therapeutic use is examined. Functional assays can be conducted to quantify the phagocytic capacity of myeloid cells and macrophages and their capacity for further polarization, and phagocytic potential as described elsewhere in the disclosure.

Full-scale process validation with subject samples

[00333] Monocytes cultured from leukapheresis from Prodigy isolation can be cultured at 2 x 10^6 monocytes per cm^2 and per mL in culture bags (MACS GMP differentiation bags, Miltenyi) with GMP-grade TexMACS (Miltenyi) and 100 ng/mL M-CSF. Monocytes can be cultured with 100 ng/mL GMP-compliant recombinant human M-CSF (R&D Systems). Cells can be cultured in a humidified atmosphere at 37°C, with 5% CO_2 for 7 days. A 50% volume media replenishment is carried out twice during culture (days 2 and 4) with 50% of the culture medium removed, then fed with fresh medium supplemented with 200 ng/mL M-CSF (to restore a final concentration of 100 ng/mL).

Cell harvesting:

[00334] For normal donor-derived macrophages, cells can be removed from the wells at day 7 using Cell Dissociation Buffer (Gibco, Thermo Fisher) and a pastette. Cells can be resuspended in PEA buffer and counted, then approximately 1x10^6 cells per test can be stained for flow cytometry. Leukapheresis-derived macrophages can be removed from the culture bags at day 7 using PBS/EDTA buffer (CliniMACS buffer, Miltenyi) containing pharmaceutical grade 0.5% human albumin from

serum (HAS; Alburex). Harvested cells can be resuspended in excipient composed of two licensed products: 0.9% saline for infusion (Baxter) with 0.5% human albumin (Alburex).

Flow cytometry characterization:

[00335] Monocyte and macrophage cell surface marker expression can be analyzed using either a FACSCanto II (BD Biosciences) or MACSQuant 10 (Miltenyi) flow cytometer. Typically, approximately 20,000 events can be acquired for each sample. Cell surface expression of leukocyte markers in freshly isolated and day 7 matured cells is carried out by incubating cells with specific antibodies (final dilution 1:100). Cells are incubated for 5 min with FcR block (Miltenyi) then incubated at 4°C for 20 min with antibody cocktails. Cells can be washed in PEA, and dead cell exclusion dye DRAQ7 (BioLegend) is added at 1:100. Cells can be stained for a range of surface markers as follows: CD45-VioBlue, CD14-PE or CD14-PerCP-Vio700, CD163-FITC, CD169-PE and CD16-APC (all Miltenyi), CCR2-BV421, CD206-FITC, CXCR4-PE and CD115-APC (all BioLegend), and 25F9-APC and CD115-APC (eBioscience). Both monocytes and macrophages can be gated to exclude debris, doublets and dead cells using forward and side scatter and DRAQ7 dead cell discriminator (BioLegend) and analyzed using FlowJo softwcan be (Tree Star). From the initial detailed phenotyping, a panel is developed as Release Criteria (CD45-VB/CD206-FITC/CD14-PE/25F9 APC/DRAQ7) that defined the development of a functional macrophage from monocytes. Macrophages can be determined as having mean fluorescence intensity (MFI) five times higher than the level on day 0 monocytes for both 25F9 and CD206. A second panel is developed which assessed other markers as part of an Extended Panel, composed of CCR2-BV421/CD163-FITC/CD169-PE/CD14-PerCP-Vio700/CD16-APC/DRAQ7), but is not used as part of the Release Criteria for the cell product.

[00336] Monocytes and macrophages can be isolated from withdrawing a buffy coat layer formed in a sucrose gradient centrifugation sample of isolated peripheral blood cells. CD14 cells can be tested for phagocytic uptake using pHRodo beads, which fluoresce only when taken into acidic endosomes. Briefly, monocytes or macrophages can be cultured with 1–2 uL of pHRodo *Escherichia coli* bioparticles (Life Technologies, Thermo Fisher) for 1 h, then the medium is taken off and cells are washed to remove non-phagocytosed particles. Phagocytosis is assessed using an EVOS microscope (Thermo Fisher), images captured and cellular uptake of beads quantified using ImageJ software (NIH). The capacity to polarize toward defined differentiated macrophages is examined by treating day 7 macrophages with IFNγ (50 ng/mL) or IL-4 (20 ng/mL) for 48 h to induce polarization to M1 or M2 phenotype (or M[IFNγ] versus M[IL-4], respectively). After 48 h, the cells can be visualized by EVOS bright-field microscopy, then harvested and phenotyped as before. Further analysis is performed on the cytokine and growth factor secretion profile of macrophages after generation and in response to inflammatory stimuli. Macrophages can be generated from healthy donor buffy coats as before, and either left untreated or stimulated with TNFα (50 ng/mL, Peprotech) and

polyinosinic:polycytidylic acid (poly I:C, a viral homolog which binds TLR3, 1 g/mL, Sigma) to mimic the conditions present in the inflamed liver, or lipopolysaccharide (LPS, 100 ng/mL, Sigma) plus IFNγ (50 IU/mL, Peprotech) to produce a maximal macrophage activation. Day 7 macrophages can be incubated overnight and supernatants collected and spun down to remove debris, then stored at −80°C until testing. Secretome analysis is performed using a 27-plex human cytokine kit and a 9-plex matrix metalloprotease kit run on a Magpix multiplex enzyme linked immunoassay plate reader (BioRad).

Product stability:

[00337] Various excipients can be tested during process development including PBS/EDTA buffer; PBS/EDTA buffer with 0.5% HAS (Alburex), 0.9% saline alone or saline with 0.5% HAS. The 0.9% saline (Baxter) with 0.5% HAS excipient is found to maintain optimal cell viability and phenotype (data not shown). The stability of the macrophages from cirrhotic donors after harvest is investigated in three process optimization runs, and a more limited range of time points assessed in the process validation runs (n = 3). After harvest and re-suspension in excipient (0.9% saline for infusion, 0.5% human serum albumin), the bags can be stored at ambient temperature (21–22°C) and samples taken at 0, 2, 4, 6, 8, 12, 24, 30 and 48 h postharvest. The release criteria antibody panel is run on each sample, and viability and mean fold change from day 0 is measured from geometric MFI of 25F9 and CD206.

Statistical analysis:

[00338] Results can be expressed as mean ± SD. The statistical significance of differences is assessed where possible with the unpaired two-tailed *t*-test using GraphPad Prism 6. Results can be considered statistically significant when the *P* value is < 0.05.

[00339] Also provided herein is a cell comprising a composition described herein, a vector described herein or a polypeptide described herein. In some embodiments, the cell is a phagocytic cell. In some embodiments, the cell is a stem cell derived cell, a myeloid cell, a macrophage, a dendritic cell, a lymphocyte, a mast cell, a monocyte, a neutrophil, a microglia, or an astrocyte. In some embodiments, the cell is an autologous cell. In some embodiments, the cell is an allogeneic cell. In some embodiments, the cell is an M1 cell. In some embodiments, the cell is an M2 cell. In some embodiments, the cell is an M1 macrophage cell. In some embodiments, the cell is an M2 macrophage cell. In some embodiments, the cell is an M1 myeloid cell. In some embodiments, the cell is an M2 myeloid cell.

[00340] Also provided herein is a method of treating a disease in a subject in need thereof comprising administering to the subject a pharmaceutical composition described herein. In some embodiments, the disease is cancer. In some embodiments, the cancer is a solid cancer. In some embodiments, the solid cancer is selected from the group consisting of ovarian cancer, suitable cancers include ovarian cancer, renal cancer, breast cancer, prostate cancer, liver cancer, brain cancer, lymphoma, leukemia,

skin cancer, pancreatic cancer, colorectal cancer, lung cancer. In some embodiments, the cancer is a liquid cancer. In some embodiments, the liquid cancer is leukemia or a lymphoma. In some embodiments, the liquid cancer is a T cell lymphoma. In some embodiments, the disease is a T cell malignancy.

[00341] In some embodiments, the method further comprises administering an additional therapeutic agent to the subject. In some embodiments, the additional therapeutic agent is selected from the group consisting of a CD47 agonist, an agent that inhibits Rac, an agent that inhibits Cdc42, an agent that inhibits a GTPase, an agent that promotes F-actin disassembly, an agent that promotes PI3K recruitment to the PFP, an agent that promotes PI3K activity, an agent that promotes production of phosphatidylinositol 3,4,5-trisphosphate, an agent that promotes ARHGAP12 activity, an agent that promotes ARHGAP25 activity, an agent that promotes SH3BP1 activity and any combination thereof.

[00342] In some embodiments, administering comprises infusing or injecting. In some embodiments, administering comprises administering directly to the solid cancer. In some embodiments, administering comprises a circRNA-based delivery procedure, anon-particle encapsulated mRNA-based delivery procedure, an mRNA-based delivery procedure, viral-based delivery procedure, particle-based delivery procedure, liposome-based delivery procedure, or an exosome-based delivery procedure. In some embodiments, a CD4+ T cell response or a CD8+ T cell response is elicited in the subject.

[00343] Also provided herein is a method of preparing a cell, the method comprising contacting a cell with a composition described herein, a vector described herein or a polypeptide described herein. In some embodiments, contacting comprises transducing. In some embodiments, contacting comprises chemical transfection, electroporation, nucleofection, or viral infection or transduction.

[00344] Provided herein is a method for administering a therapeutic comprising any one of the compositions described above. In some embodiments, the therapeutic is administered via a parenteral administration route.

[00345] In some embodiments, the therapeutic is administered via intramuscular administration route. In some embodiments, the therapeutic is administered via intravenous administration route. In some embodiments, the therapeutic is administered via subcutaneous administration route.

[00346] Also provided herein is a method of preparing a pharmaceutical composition comprising the one or more recombinant nucleic acids described herein and a lipid in an aqueous composition described herein. In some embodiments, the composition comprises a vector described herein. In some embodiments, the lipid comprises forming a lipid nanoparticle.

EXAMPLES

Example 1. Chimeric antigen receptor protein constructs with intracellular interferon activating domains:

[00347] In this example, amino acid and nucleic acid sequences of the various CFP constructs described herein having inflammatory signal transducing intracellular domains are disclosed. Nucleic acid sequences as detailed below can be easily interpreted by one of skill in the art for DNA and mRNA sequences in order to provide guidance in making and using a suitable constructs or variants therefrom using commonly used molecular cloning techniques.

TABLE 3. Amino acid sequence of exemplary CFP constructs used herein are provided below

Name	Amino Acid Sequence
HER2-CD8-FcR-PI3K	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSGALSNSIMYFSHFVP VFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDIYIWAPLAGT CGVLLLSLVITLYCRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ GSGSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM (**SEQ ID NO: 52**).
HER2-CD8-FcR-41BB	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAADYKDDDDKSGGGGSGAL SNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DIYIWAPLAGTCGVLLLSLVITLYCRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETY

	ETLKHEKPPQGSGSKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (**SEQ ID NO: 53**).
HER2-CD8-41BB-FcR	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAADYKDDDDKSGGGGSG AL SNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GL DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFP EEEEGGCELGSGSLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (**SEQ ID NO: 54**).
HER2-CD68-CD40-FcR	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAAILLPLIIGLILLGLLALV LIA FAIIKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDG KES RISVQERQGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ (**SEQ ID NO: 55**).
HER2-CD68-FcR-MDA5	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF

	GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAAILLPLIIGLILLGLLALV LIA FAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSGSGSMSN GY STDENFRYLISCFRARVKMYIQVEPVLDYLTFLPAEVKEQIQRTVATSGNMQAVE LLL STLEKGVWHLGWTREFVEALRRTGSPLAARYMNPELTDLPSPSFENAHDEYLQL LNL LQPTLVDKLLVRDVLDKCMEEELLTIEDRNRIAAAENNGNESGVRELLKRIVQK ENW FSAFLNVLRQTGNNELVQELTGSDCSESNAEIEN (**SEQ ID NO: 56**).
HER2-CD64	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAAGSQLPTPVWFHVLFYL AV GIMFLVNTVLWVTIRKELKRKKKWDLEISLDSGHEKKVISSLQEDRHLEEELKCQ EQ KEEQLQEGVHRKEPQGAT (**SEQ ID NO: 57**).
HER2-CD89	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR

	AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSAAAGSDSIHQDYTTQNLIR MAV AGLVLVALLAILVENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPSVC K **(SEQ ID NO: 58)**.
HER2- CD68- CD40- FcR- PI3K	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SL RAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAGSDSIHQDY TT QNLIRMAVAGLVLVALLAILVENWHSHTALNKEASADVAEPSWSQQMCQPGLT FA RTPSVCKGTKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQP VT QEDGKESRISVQERQGSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENM **(SEQ ID NO: 59)**.
HER2- CD68- FcR- PI3K- MDA5	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSMSNGYSTDENFRYLISCFR ARV

	KMYIQVEPVLDYLTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGW TRE FVEALRRTGSPLAARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVDKLLVR DVLD KCMEEELLTIEDRNRIAAAENNGNESGVRELLKRIVQKENWFSAFLNVLRQTGN NEL VQELTGSDCSESNAEIEN (**SEQ ID NO: 60**).
HER2-CD68-FcR-PI3K-RIGI	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSMTTEQRRSLQAFQDYIRKT LDP TYILSYMAPWFREEEVQYIQAEKNNKGPMEAATLFLKFLLELQEEGWFRGFLDA LDH AGYSGLYEAIESWDFKKIEKLEEYRLLLKRLQPEFKTRIIPTDIISDLSECLINQECE EILQ ICSTKGMMAGAEKLVECLLRSDKENWPKTLKLALEKERNKFSELW (**SEQ ID NO: 61**).
HER2-CD68-FcR-PI3K-Myd88	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR

	AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSAAGGPGAGSAAPVSSTSSL PLA ALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLD AWQ GRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQVAA VDSS VPRTAELAGITTLDDPLG (**SEQ ID NO: 62**).
HER2-CD68tm-FcR-PI3K-STING	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSVTVGSLKTSAVPSTSTMSQ EPEL LISGMEKPLPLRTDFS (**SEQ ID NO: 63**).
HER2-CD68tm-FcR-PI3K-MAVS	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SL RAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLIL LG

	LLALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQG SG SYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSPSYPMPVQETQAPESG GG SSSAWLDSSSENRGLGSELSKPGVLASQVDSPFSGCFEDLAISASTSLGMG (**SEQ ID NO: 64**).
HER2- CD68t m- FcR- PI3K- TRIF long	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSGSIRTLQSNLGCLPPSSALP SGTR SLPRPIDGVSDWSQGCSLRSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCD DPQA SLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEVAPDATSTGLPDTPAA PETS TNYPVECTEGSAGPQSLPLPILEPVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTP TTPETSPPPPPPPPSSTPCSAHLTPSSLFPSSLESSSEQKFYNFVILHARADEHIALRV REKLEALGVPDGATFCEDFQVPGRGELSCLQDAIDHSAFIILLLTSNFDCRLSLHQ VNQAMMSNLTRQGSPDCVIPFLPLESSPAQLSSDTASLLSGLVRLDEHSQIFARK VANTFKPHRLQARKAMWRKEQD (**SEQ ID NO: 65**).
HER2- CD68t m- FcR- PI3K- TRIF short	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD

	TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSGSIRTLQSNLGCLPPSSALP SGTR SLPRPIDGVSDWSQGCSLRSTGSPASLASNLEISQSPTMPFLSLHRSPHGPSKLCD DPQA SLVPEPVPGGCQEPEEMSWPPSGEIASPPELPSSPPPGLPEVAPDATSTGLPDTPAA PETS TNYPVECTEGSAGPQSLPLPILEPVKNPCSVKDQTPLQLSVEDTTSPNTKPCPPTP TTPETSPPPPPPPPSSTPCSAHLTPSSLFPSSLE (**SEQ ID NO: 66**).
HER2-CD68tm-FcR-PI3K-TASL	MWLQSLLLLGTVACSISDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ QK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP TF GQGTKVEIKRTGSTSGSGKPGSGEGSEVQLVESGGGLVQPGGSLRLSCAASGFNI KD TYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN SLR AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSSSGGGGSAAAILLPLIIGLILL GLL ALVLIAFAIIRRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQGSG SYE DMRGILYAAPQLRSIRGQPGPNHEEDADSYENMGSMSTEITEISTPSLHISQYSNV NP (**SEQ ID NO: 67**).

[00348] HER2-targeted PFP was designed and constructed using recombinant DNA techniques. The PFP has an extracellular domain comprising a signal peptide fused to an scFv containing a heavy chain variable domain linked to a light chain variable domain that binds to HER2 on a target cell, attached to a CD8 alpha chain hinge and CD8 alpha chain TM domain via a short linker. The TM domain is fused at the cytosolic end with an FcγR gamma intracellular domain, and a MDA5 recruitment domain separated by a short linker. Linkers are marked as italicized. The construct was sequenced. The sequence is provided below. The intracellular signaling domain of MDA5 is marked as bold.

[00349] HER2-FcR-MDA5 chimeric fused protein (CFP) amino acid sequence (CDR sequences are underlined):

MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDW YFDVWGQGTTVTVS*SGGGGSGGGGSGGGGS*DIQMTQSPSSLSASVGDRVTITCRASQDINS YLSWFQQKPGKAPKTLIYRANRLESGVPSRF*SGSGS*GTDYTLTISSLQYEDFGIYYCQQYDES PWTFGGGTKLEIKSGGGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLR PEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLYCRLKIQVRKAAITSYEKSDGV YTGLSTRNQETYETLKHEKPPQ*GSGS***MSNGYSTDENFRYLISCFRARVKMYIQVEPVLDY LTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFVEALRRTGSPLA ARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVDKLLVRDVLDKCMEEELLTIEDR NRIAAAENNGNESGVRELLKRIVQKENWFSAFLNVLRQTGNNELVQELTGSDCSESNA EIEN (SEQ ID NO: 47).**

[00350] An exemplary polynucleotide sequence encoding SEQ ID NO: 47 may comprise a sequence: atgtggctgcagagcctgctgctgctgggcaccgtggcgtgcagcattagcgaaattcagctggtgcagagcggcggcggcctggtgaaaccg ggcggcagcgtgcgcattagctgcgcggcgagcggctatacctttaccaactatggcatgaactgggtgcgccaggcgccgggcaaaggcct ggaatggatgggctggattaacacccataccggcgaaccgacctatgcggatagctttaaaggccgctttacctttagcctggatgatagcaaaa acaccgcgtatctgcagattaacagcctgcgcgcggaagataccgcggtgtatttttgcacccgccgcggctatgattggtattttgatgtgtgggg ccagggcaccaccgtgaccgtgagcagcggcggcggcggcagcggcggcggcggcggcagcggcggcggcggcagcgatattcagatgacc cagagcccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgccgcgcgagccaggatattaacagctatctgagctggttt cagcagaaaccgggcaaagcgccgaaaaccctgatttatcgcgcgaaccgcctggaaagcggcgtgccgagccgctttagcggcagcggca gcggcaccgattataccctgaccattagcagcctgcagtatgaagattttggcatttattattgccagcagtatgatgaaagcccgtggacctttggc ggcggcaccaaactggaaattaaaagcggcggcggcggcagcggcgcgctgagcaacagcattatgtattttagccattttgtgccggtgtttct gccggcgaaaccgaccaccaccccggcgccgcgcccgccgaccccggcgccgaccattgcgagccagccgctgagcctgcgcccggaag cgtgccgcccggcggcgggcggcgcggtgcatacccgcggcctggatatttatatttgggcgccgctggcgggcacctgcggcgtgctgctg ctgagcctggtgattaccctgtattgccgcctgaaaattcaggtgcgcaaagcggcgattaccagctatgaaaaaagcgatggcgtgtataccgg cctgagcacccgcaaccaggaaacctatgaaaccctgaaacatgaaaaaccgccgcagggcagcggcagcatgagcaacggctatagcacc gatgaaaactttcgctatctgattagctgctttcgcgcgcgcgtgaaaatgtatattcaggtggaaccggtgctggattatctgacctttctgccggcg gaagtgaaagaacagattcagcgcaccgtggcgaccagcggcaacatgcaggcggtggaactgctgctgagcaccctggaaaaaggcgtgt ggcatctgggctggacccgcgaatttgtggaagcgctgcgccgcaccggcagcccgctggcggcgcgctatatgaacccggaactgaccgat ctgccgagcccgagctttgaaaacgcgcatgatgaatatctgcagctgctgaacctgctgcagccgaccctggtggataaactgctggtgcgcg atgtgctggataaatgcatggaagaagaactgctgaccattgaagatcgcaaccgcattgcggcggcggaaaacaacggcaacgaaagcggc gtgcgcgaactgctgaaacgcattgtgcagaaagaaaactggtttagcgcgtttctgaacgtgctgcgccagaccggcaacaacgaactggtgc aggaactgaccggcagcgattgcagcgaaagcaacgcggaaattgaaaac (**SEQ ID NO: 48**).

[00351] An exemplary nucleic acid sequence encoding a HER2-CD8hinge-FcR-MDA5 as disclosed herein, comprises a sequence as follows:

ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCATCAGCGACATCCA
GATGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAGAGTGACCATCACCT
GTAGAGCCAGCCAGGATGTGAATACCGCCGTGGCCTGGTATCAGCAGAAGCCTGGAAA
AGCCCCTAAGCTGCTGATCTACAGCGCCAGCTTTCTGTACAGCGGCGTGCCAAGCAGAT
TCAGCGGCAGCAGATCTGGCACCGACTTCACCCTGACCATCTCTAGCCTGCAGCCTGAG
GACTTCGCCACCTACTACTGCCAGCAGCACTACACCACACCTCCAACCTTTGGCCAGGG
CACCAAGGTGGAAATCAAGAGAACAGGCAGCACCAGCGGCTCTGGAAAGCCTGGATCT
GGCGAAGGATCTGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTTGTTCAGCCTGGCG
GCTCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCAACATCAAGGACACCTACATCCAC
TGGGTCCGACAGGCCCCTGGAAAGGGACTTGAATGGGTCGCCAGAATCTACCCCACCA
ACGGCTACACCAGATACGCCGATAGCGTGAAGGGCAGATTCACCATCAGCGCCGACAC
CAGCAAGAACACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTG
TACTACTGTTCTAGATGGGGAGGCGACGGCTTCTACGCCATGGATGTTTGGGGACAGGG
CACCCTGGTCACAGTTTCTTCTAGCGGAGGCGGAGGAAGCGGAGCCCTGAGCAATAGC
ATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAACAACACCC
GCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACC
TGAAGCCTGTAGACCTGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATATCTACA
TTTGGGCCCCTCTGGCTGGCACATGTGGCGTTCTGCTGCTCTCTCTGGTCATCACCCTGT
ACTGCAGACGGCTGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACGAGAAATC
TGATGGCGTGTACACCGGCCTGAGCACCCGGAATCAAGAAACCTACGAGACACTGAAG
CACGAGAAGCCTCCACAAATGAGTAACGGTTACAGCACGGACGAGAACTTCCGCTATC
TGATTAGCTGTTTCCGGGCTCGCGTAAAGATGTATATCCAGGTAGAGCCAGTGCTGGAT
TACCTGACGTTCCTCCCTGCCGAGGTGAAGGAACAGATTCAGCGAACAGTAGCGACATC
AGGAAATATGCAAGCGGTGGAGTTGCTGCTCTCTACCCTCGAAAAAGGTGTTTGGCACC
TGGGATGGACACGGGAATTCGTCGAAGCTCTCAGGCGAACTGGATCTCCTCTTGCCGCT
AGGTACATGAACCCGGAACTCACTGATTTGCCGTCACCGTCTTTCGAGAACGCCCATGA
TGAGTATCTCCAGCTTCTGAATTTGCTTCAGCCTACCTTGGTCGACAAACTGTTGGTTCG
GGACGTTTTGGACAAGTGTATGGAGGAGGAGCTGCTGACCATCGAGGACAGAAACCGG
ATAGCTGCGGCAGAGAACAATGGCAACGAGTCAGGAGTTCGGGAGTTGTTGAAGAGGA
TAGTGCAAAAGGAGAATTGGTTCAGCGCTTTCCTTAACGTACTCCGACAGACAGGCAAC
AATGAACTCGTACAAGAGTTGACAGGGTCAGATTGCAGTGAATCCAACGCCGAAATTG
AAAAT (**SEQ ID NO: 68**).

TABLE 4. Partial nucleotide sequences denoting component sequences encoding the individual domains of the CFP: HER2-CD8hinge-FcR-MDA5 (e.g., from full length sequence SEQ ID NO: 68).

Sequence encoding	Nucleic Acid Sequence

GMCSF signal peptide	ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCAT CAGC **(SEQ ID NO: 69)**
HER2 scFv	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGG CGATAGAGTGACCATCACCTGTAGAGCCAGCCAGGATGTGAATACCG CCGTGGCCTGGTATCAGCAGAAGCCTGGAAAAGCCCCTAAGCTGCTG ATCTACAGCGCCAGCTTTCTGTACAGCGGCGTGCCAAGCAGATTCAG CGGCAGCAGATCTGGCACCGACTTCACCCTGACCATCTCTAGCCTGC AGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGCACTACACCACA CCTCCAACCTTTGGCCAGGGCACCAAGGTGGAAATCAAGAGAACAG GCAGCACCAGCGGCTCTGGAAAGCCTGGATCTGGCGAAGGATCTGA GGTGCAGCTGGTTGAATCTGGCGGAGGACTTGTTCAGCCTGGCGGCT CTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCAACATCAAGGACACC TACATCCACTGGGTCCGACAGGCCCCTGGAAAGGGACTTGAATGGGT CGCCAGAATCTACCCCACCAACGGCTACACCAGATACGCCGATAGCG TGAAGGGCAGATTCACCATCAGCGCCGACACCAGCAAGAACACCGC CTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACT ACTGTTCTAGATGGGGAGGCGACGGCTTCTACGCCATGGATGTTTGG GGACAGGGCACCCTGGTCACAGTTTCTTCT **(SEQ ID NO: 70)**
Linker	AGCGGAGGCGGAGGAAGCGGA **(SEQ ID NO: 71)**
CD8 hinge and trans-membrane region	GCCCTGAGCAATAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTT CTGCCCGCCAAGCCTACAACAACACCCGCTCCTAGACCACCTACACC AGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACCTGAAGCCT GTAGACCTGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATATC TACATTTGGGCCCCTCTGGCTGGCACATGTGGCGTTCTGCTGCTCTCT CTGGTCATCACCCTGTACTGC **(SEQ ID NO: 72)**
FcR ICD	AGACGGCTGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACG AGAAATCTGATGGCGTGTACACCGGCCTGAGCACCCGGAATCAAGA AACCTACGAGACACTGAAGCACGAGAAGCCTCCACAA **(SEQ ID NO: 73)**
MDA5 ICD	ATGAGTAACGGTTACAGCACGGACGAGAACTTCCGCTATCTGATTAG CTGTTTCCGGGCTCGCGTAAAGATGTATATCCAGGTAGAGCCAGTGC TGGATTACCTGACGTTCCTCCCTGCCGAGGTGAAGGAACAGATTCAG CGAACAGTAGCGACATCAGGAAATATGCAAGCGGTGGAGTTGCTGC TCTCTACCCTCGAAAAAGGTGTTTGGCACCTGGGATGGACACGGGAA TTCGTCGAAGCTCTCAGGCGAACTGGATCTCCTCTTGCCGCTAGGTAC ATGAACCCGGAACTCACTGATTTGCCGTCACCGTCTTTCGAGAACGC

	CCATGATGAGTATCTCCAGCTTCTGAATTTGCTTCAGCCTACCTTGGT CGACAAACTGTTGGTTCGGGACGTTTTGGACAAGTGTATGGAGGAGG AGCTGCTGACCATCGAGGACAGAAACCGGATAGCTGCGGCAGAGAA CAATGGCAACGAGTCAGGAGTTCGGGAGTTGTTGAAGAGGATAGTG CAAAAGGAGAATTGGTTCAGCGCTTTCCTTAACGTACTCCGACAGAC AGGCAACAATGAACTCGTACAAGAGTTGACAGGGTCAGATTGCAGT GAATCCAACGCCGAAATTGAAAAT **(SEQ ID NO: 74)**

[00352] Additional anti-HER2 binding sequence may comprise a VHH domain comprising a sequence: EVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTQVTVSS (SEQ ID NO: 107).

[00353] An additional exemplary CFP construct using MDA5 intracellular domain can be a CD5-FcR-MDA5 construct (SEQ ID NO: 4), having the amino acid sequence:

MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDW YFDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDINS YLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGSGSGTDYTLTISSLQYEDFGIYYCQQYDE SPWTFGGGTKLEIKSGGGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLYCRLKIQVRKAAITSYEKSDG VYTGLSTRNQETYETLKHEKPPQGSGS**MSNGYSTDENFRYLISCFRARVKMYIQVEPVLD YLTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFVEALRRTGSPL AARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVDKLLVRDVLDKCMEEELLTIED RNRIAAAENNGNESGVRELLKRIVQKENWFSAFLNVLRQTGNNELVQELTGSDCSESN AEIEN (SEQ ID NO: 4)**

[00354] In this example, a CD5-targeted CFP was constructed using known molecular biology techniques having an intracellular domain comprising two caspase activation (CARD) domains of MDA-5 intracellular domain sequence (Tandem CARD ICD sequence shown in bold letters).

[00355] An exemplary polynucleotide sequence encoding a CD5-FcR-MDA5 may comprise a sequence:

atgtggctgcagagcctgctgctgctgggcaccgtggcgtgcagcattagcgaaattcagctggtgcagagcggcggcggcctggtgaaaccg ggcggcagcgtgcgcattagctgcgcggcgagcggctataccttaccaactatggcatgaactgggtgcgccaggcgccgggcaaaggcct ggaatggatgggctggattaacacccataccggcgaaccgacctatgcggatagctttaaaggccgctttacctttagcctggatgatagcaaaa acaccgcgtatctgcagattaacagcctgcgcgcggaagataccgcggtgtatttttgcacccgccgcggctatgattggtattttgatgtgtgggg ccagggcaccaccgtgaccgtgagcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcgatattcagatgacc cagagcccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgccgcgcgagccaggatattaacagctatctgagctggttt

cagcagaaaccgggcaaagcgccgaaaaccctgatttatcgcgcgaaccgcctggaaagcggcgtgccgagccgctttagcggcagcggca
gcggcaccgattataccctgaccattagcagcctgcagtatgaagattttggcatttattattgccagcagtatgatgaaagcccgtggacctttggc
ggcggcaccaaactggaaattaaaagcggcggcggcggcagcggcgcgctgagcaacagcattatgtattttagccattttgtgccggtgtttct
gccggcgaaaccgaccaccaccccggcgccgcgcccgccgaccccggcgccgaccattgcgagccagccgctgagcctgcgcccggaag
cgtgccgcccggcggcgggcggcgcggtgcatacccgcggcctggatatttatatttgggcgccgctggcgggcacctgcggcgtgctgctg
ctgagcctggtgattaccctgtattgccgcctgaaaattcaggtgcgcaaagcggcgattaccagctatgaaaaaagcgatggcgtgtataccgg
cctgagcacccgcaaccaggaaacctatgaaaccctgaaacatgaaaaaccgccgcagggcagcggcagcatgagcaacggctatagcacc
gatgaaaactttcgctatctgattagctgctttcgcgcgcgcgtgaaaatgtatattcaggtggaaccggtgctggattatctgacctttctgccggcg
gaagtgaaagaacagattcagcgcaccgtggcgaccagcggcaacatgcaggcggtggaactgctgctgagcaccctggaaaaaggcgtgt
ggcatctgggctggacccgcgaatttgtggaagcgctgcgccgcaccggcagcccgctggcggcgcgctatatgaacccggaactgaccgat
ctgccgagcccgagctttgaaaacgcgcatgatgaatatctgcagctgctgaacctgctgcagccgaccctggtggataaactgctggtgcgcg
atgtgctggataaatgcatggaagaagaactgctgaccattgaagatcgcaaccgcattgcggcggcggaaaacaacggcaacgaaagcggc
gtgcgcgaactgctgaaacgcattgtgcagaaagaaaactggtttagcgcgtttctgaacgtgctgcgccagaccggcaacaacgaactggtgc
aggaactgaccggcagcgattgcagcgaaagcaacgcggaaattgaaaac (**SEQ ID NO: 49**).

[00356] An exemplary nucleic acid sequence encoding a CD5-CD8hinge-CD8TM-FcR-MDA5 as disclosed herein, comprises a sequence as follows:

ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCATCAGCGAGATCCA
GCTGGTTCAGTCTGGCGGCGGACTTGTGAAACCTGGCGGATCTGTCAGAATCAGCTGTG
CCGCCAGCGGCTACACCTTCACCAACTACGGCATGAACTGGGTCCGACAGGCCCCTGGA
AAAGGCCTTGAGTGGATGGGCTGGATCAATACCCACACCGGCGAGCCAACCTACGCCG
ATAGCTTTAAGGGCAGATTCACCTTCAGCCTGGACGACAGCAAGAACACCGCCTACCTG
CAGATCAACAGCCTGAGAGCCGAGGATACCGCCGTGTACTTCTGCACCAGAAGAGGCT
ACGACTGGTACTTCGATGTGTGGGGCCAGGGCACCACAGTGACAGTTTCTAGCGGAGGC
GGAGGATCAGGTGGCGGTGGATCTGGCGGTGGTGGCTCTGATATCCAGATGACACAGA
GCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAGAGTGACCATCACCTGTAGAGCCAGC
CAGGACATCAACAGCTACCTGAGCTGGTTCCAGCAGAAGCCTGGCAAGGCCCCTAAGA
CACTGATCTACCGGGCCAACAGACTGGAAAGCGGCGTGCCAAGCAGATTTTCTGGCAG
CGGCTCTGGCACCGACTACACCCTGACAATCAGCAGCCTGCAGTACGAGGACTTCGGCA
TCTACTACTGCCAGCAGTACGACGAGAGCCCTTGGACATTTGGCGGAGGCACCAAGCTG
GAAATCAAAAGCGGAGGCGGAGGAAGCGGAGCCCTGAGCAATAGCATCATGTACTTCA
GCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAACAACACCCGCTCCTAGACCA
CCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACCTGAAGCCTGTAG
ACCTGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATATCTACATTTGGGCCCCTC
TGGCTGGCACATGTGGCGTTCTGCTGCTCTCTCTGGTCATCACCCTGTACTGCAGACGGC
TGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACGAGAAATCTGATGGCGTGTA
CACCGGCCTGAGCACCCGGAATCAAGAAACCTACGAGACACTGAAGCACGAGAAGCCT

CCACAAATGAGTAACGGTTACAGCACGGACGAGAACTTCCGCTATCTGATTAGCTGTTT
CCGGGCTCGCGTAAAGATGTATATCCAGGTAGAGCCAGTGCTGGATTACCTGACGTTCC
TCCCTGCCGAGGTGAAGGAACAGATTCAGCGAACAGTAGCGACATCAGGAAATATGCA
AGCGGTGGAGTTGCTGCTCTCTACCCTCGAAAAAGGTGTTTGGCACCTGGGATGGACAC
GGGAATTCGTCGAAGCTCTCAGGCGAACTGGATCTCCTCTTGCCGCTAGGTACATGAAC
CCGGAACTCACTGATTTGCCGTCACCGTCTTTCGAGAACGCCCATGATGAGTATCTCCA
GCTTCTGAATTTGCTTCAGCCTACCTTGGTCGACAAACTGTTGGTTCGGGACGTTTTGGA
CAAGTGTATGGAGGAGGAGCTGCTGACCATCGAGGACAGAAACCGGATAGCTGCGGCA
GAGAACAATGGCAACGAGTCAGGAGTTCGGGAGTTGTTGAAGAGGATAGTGCAAAAGG
AGAATTGGTTCAGCGCTTTCCTTAACGTACTCCGACAGACAGGCAACAATGAACTCGTA
CAAGAGTTGACAGGGTCAGATTGCAGTGAATCCAACGCCGAAATTGAAAAT **(SEQ ID NO: 75)**

[00357] TABLE 5. Partial nucleotide sequences denoting component sequences encoding the individual domains of the CFP: CD5-CD8hinge-CD8TM-FcR-MDA5 (e.g., SEQ ID NO: 75).

Sequence encoding	Nucleic Acid Sequence
GMCSF signal peptide	ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCATC AGC **(SEQ ID NO: 76)**
CD5 scFv	GAGATCCAGCTGGTTCAGTCTGGCGGCGGACTTGTGAAACCTGGCGGA TCTGTCAGAATCAGCTGTGCCGCCAGCGGCTACACCTTCACCAACTACG GCATGAACTGGGTCCGACAGGCCCCTGGAAAAGGCCTTGAGTGGATGG GCTGGATCAATACCCACACCGGCGAGCCAACCTACGCCGATAGCTTTA AGGGCAGATTCACCTTCAGCCTGGACGACAGCAAGAACACCGCCTACC TGCAGATCAACAGCCTGAGAGCCGAGGATACCGCCGTGTACTTCTGCA CCAGAAGAGGCTACGACTGGTACTTCGATGTGTGGGGCCAGGGCACCA CAGTGACAGTTTCTAGCGGAGGCGGAGGATCAGGTGGCGGTGGATCTG GCGGTGGTGGCTCTGATATCCAGATGACACAGAGCCCTAGCAGCCTGT CTGCCTCTGTGGGCGATAGAGTGACCATCACCTGTAGAGCCAGCCAGG ACATCAACAGCTACCTGAGCTGGTTCCAGCAGAAGCCTGGCAAGGCCC CTAAGACACTGATCTACCGGGCCAACAGACTGGAAAGCGGCGTGCCAA GCAGATTTTCTGGCAGCGGCTCTGGCACCGACTACACCCTGACAATCAG CAGCCTGCAGTACGAGGACTTCGGCATCTACTACTGCCAGCAGTACGAC GAGAGCCCTTGGACATTTGGCGGAGGCACCAAGCTGGAAATCAAA **(SEQ ID NO: 77)**
Linker	AGCGGAGGCGGAGGAAGCGGA **(SEQ ID NO: 78)**

CD8 hinge and trans-membrane region	GCCCTGAGCAATAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCT GCCCGCCAAGCCTACAACAACACCCGCTCCTAGACCACCTACACCAGCT CCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACCTGAAGCCTGTAGACC TGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATATCTACATTTGGG CCCCTCTGGCTGGCACATGTGGCGTTCTGCTGCTCTCTCTGGTCATCACCC TGTACTGC **(SEQ ID NO: 79)**
FcR	AGACGGCTGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACGAG AAATCTGATGGCGTGTACACCGGCCTGAGCACCCGGAATCAAGAAACCT ACGAGACACTGAAGCACGAGAAGCCTCCACAA **(SEQ ID NO: 80)**
MDA5	ATGAGTAACGGTTACAGCACGGACGAGAACTTCCGCTATCTGATTAGCT GTTTCCGGGCTCGCGTAAAGATGTATATCCAGGTAGAGCCAGTGCTGGA TTACCTGACGTTCCTCCCTGCCGAGGTGAAGGAACAGATTCAGCGAACA GTAGCGACATCAGGAAATATGCAAGCGGTGGAGTTGCTGCTCTCTACCC TCGAAAAAGGTGTTTGGCACCTGGGATGGACACGGGAATTCGTCGAAG CTCTCAGGCGAACTGGATCTCCTCTTGCCGCTAGGTACATGAACCCGGA ACTCACTGATTTGCCGTCACCGTCTTTCGAGAACGCCCATGATGAGTAT CTCCAGCTTCTGAATTTGCTTCAGCCTACCTTGGTCGACAAACTGTTGGT TCGGGACGTTTTGGACAAGTGTATGGAGGAGGAGCTGCTGACCATCGAG GACAGAAACCGGATAGCTGCGGCAGAGAACAATGGCAACGAGTCAGGA GTTCGGGAGTTGTTGAAGAGGATAGTGCAAAAGGAGAATTGGTTCAGCG CTTTCCTTAACGTACTCCGACAGACAGGCAACAATGAACTCGTACAAGA GTTGACAGGGTCAGATTGCAGTGAATCCAACGCCGAAATTGAAAAT **(SEQ ID NO: 81)**

[00358] An additional exemplary CFP construct is CD5-FcR-TNFR1 construct, having an amino acid sequence (CDR sequences are underlined):

MWLQSLLLLGTVACSISEIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGK GLEWMGWINTHTGEPTYADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDW YFDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDINS YLSWFQQKPGKAPKTLIYRANRLESGVPSRFSGSGSGTDYTLTISSLQYEDFGIYYCQQYDE SPWTFGGGTKLEIKSGGGGSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLDIYIWAPLAGTCGVLLLSLVITLYCRLKIQVRKAAITSYEKSDG VYTGLSTRNQETYETLKHEKPPQGSGSQRWKSKLYSIVCGKSTPEKEGELEGTTTKPLAPNP SFSPTPGFTPTLGFSPVPSSTFTSSSTYTPGDCPNFAAPRREVAPPYQGADPILATALASDPIPN PLQKWEDSAHKPQSLDTDDPATLYAVVENVPPLRWKEFVRRLGLSDHEIDRLELQNGRCLR

EAQYSMLATWRRRTPRREATLELLGRVLRDMDLLGCLEDIEEALCGPAALPPAPSLLR **(SEQ ID NO: 50)**.

[00359] An exemplary polynucleotide sequence encoding a CD5-FcR-TNFR1 may comprise a sequence:

atgtggctgcagagcctgctgctgctgggcaccgtggcgtgcagcattagcgaaattcagctggtgcagagcggcggcggcctggtgaaaccg
ggcggcagcgtgcgcattagctgcgcggcgagcggctatacctttaccaactatggcatgaactgggtgcgccaggcgccgggcaaaggcct
ggaatggatgggctggattaacacccataccggcgaaccgacctatgcggatagctttaaaggccgctttacctttagcctggatgatagcaaaa
acaccgcgtatctgcagattaacagcctgcgcgcggaagataccgcggtgtatttttgcacccgccgcggctatgattggtattttgatgtgtgggg
ccagggcaccaccgtgaccgtgagcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcgatattcagatgacc
cagagcccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgccgcgcgagccaggatattaacagctatctgagctggttt
cagcagaaaccgggcaaagcgccgaaaaccctgatttatcgcgcgaaccgcctggaaagcggcgtgccgagccgctttagcggcagcggca
gcggcaccgattataccctgaccattagcagcctgcagtatgaagattttggcatttattattgccagcagtatgatgaaagcccgtggacctttggc
ggcggcaccaaactggaaattaaaagcggcggcggcggcagcggcgcgctgagcaacagcattatgtattttagccattttgtgccggtgtttct
gccggcgaaaccgaccaccaccccggcgccgcgcccgccgaccccggcgccgaccattgcgagccagccgctgagcctgcgcccggaag
cgtgccgcccggcggcgggcggcgcggtgcatacccgcggcctggatatttatatttgggcgccgctggcgggcacctgcggcgtgctgctg
ctgagcctggtgattaccctgtattgccgcctgaaaattcaggtgcgcaaagcggcgattaccagctatgaaaaaagcgatggcgtgtataccgg
cctgagcacccgcaaccaggaaacctatgaaaccctgaaacatgaaaaaccgccgcagggcagcggcagccagcgctggaaaagcaaact
gtatagcattgtgtgcggcaaaagcaccccggaaaaagaaggcgaactggaaggcaccaccaccaaaccgctggcgccgaacccgagcttta
gcccgaccccgggctttaccccgaccctgggctttagcccggtgccgagcagcacctttaccagcagcagcacctataccccgggcgattgcc
cgaactttgcggcgccgcgccgcgaagtggcgccgccgtatcagggcgcggatccgattctggcgaccgcgctggcgagcgatccgattccg
aacccgctgcagaaatgggaagatagcgcgcataaaccgcagagcctggataccgatgatccggcgaccctgtatgcggtggtggaaaacgt
gccgccgctgcgctggaaagaatttgtgcgccgcctgggcctgagcgatcatgaaattgatcgcctggaactgcagaacggccgctgcctgcg
cgaagcgcagtatagcatgctggcgacctggcgccgccgcaccccgcgccgcgaagcgaccctggaactgctgggccgcgtgctgcgcgat
atggatctgctgggctgcctggaagatattgaagaagcgctgtgcggcccggcggcgctgccgccggcgccgagcctgctgcgc **(SEQ ID NO: 51)**.

[00360] An exemplary nucleic acid sequence encoding CD5-CD8hinge-CD8TM-FcR-TNFR1 is as follows:

ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCATCAGCGAGATCCA
GCTGGTTCAGTCTGGCGGCGGACTTGTGAAACCTGGCGGATCTGTCAGAATCAGCTGTG
CCGCCAGCGGCTACACCTTCACCAACTACGGCATGAACTGGGTCCGACAGGCCCCTGGA
AAAGGCCTTGAGTGGATGGGCTGGATCAATACCCACACCGGCGAGCCAACCTACGCCG
ATAGCTTTAAGGGCAGATTCACCTTCAGCCTGGACGACAGCAAGAACACCGCCTACCTG
CAGATCAACAGCCTGAGAGCCGAGGATACCGCCGTGTACTTCTGCACCAGAAGAGGCT
ACGACTGGTACTTCGATGTGTGGGGCCAGGGCACCACAGTGACAGTTTCTAGCGGAGGC
GGAGGATCAGGTGGCGGTGGATCTGGCGGTGGTGGCTCTGATATCCAGATGACACAGA
GCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAGAGTGACCATCACCTGTAGAGCCAGC

CAGGACATCAACAGCTACCTGAGCTGGTTCCAGCAGAAGCCTGGCAAGGCCCCTAAGA
CACTGATCTACCGGGCCAACAGACTGGAAAGCGGCGTGCCAAGCAGATTTTCTGGCAG
CGGCTCTGGCACCGACTACACCCTGACAATCAGCAGCCTGCAGTACGAGGACTTCGGCA
TCTACTACTGCCAGCAGTACGACGAGAGCCCTTGGACATTTGGCGGAGGCACCAAGCTG
GAAATCAAAAGCGGAGGCGGAGGAAGCGGAGCCCTGAGCAATAGCATCATGTACTTCA
GCCACTTCGTGCCCGTGTTTCTGCCCGCCAAGCCTACAACAACACCCGCTCCTAGACCA
CCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACCTGAAGCCTGTAG
ACCTGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATATCTACATTTGGGCCCCTC
TGGCTGGCACATGTGGCGTTCTGCTGCTCTCTCTGGTCATCACCCTGTACTGCAGACGGC
TGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACGAGAAATCTGATGGCGTGTA
CACCGGCCTGAGCACCCGGAATCAAGAAACCTACGAGACACTGAAGCACGAGAAGCCT
CCACAAGGCAGCGGCAGCCAAAGATGGAAGAGCAAGCTGTATAGCATCGTGTGCGGCA
AGTCCACCCCTGAGAAGGAAGGAGAGCTGGAAGGCACCACAACAAAGCCTCTGGCCCC
TAACCCCTCATTCAGCCCTACCCCCGGCTTCACCCCCACCCTGGGATTTAGCCCCGTGCC
CAGCAGCACCTTCACCAGCTCTAGCACCTACACCCCTGGCGACTGCCCCAACTTCGCCG
CCCCTAGACGCGAGGTGGCCCCTCCTTACCAGGGCGCCGACCCTATCCTGGCCACAGCC
CTGGCTTCTGATCCGATTCCTAATCCTCTGCAGAAGTGGGAGGACAGCGCCCACAAGCC
CCAGAGCCTGGACACCGACGACCCCGCCACCCTGTACGCCGTGGTGGAAAACGTGCCT
CCACTGCGGTGGAAAGAGTTCGTGCGGCGGCTGGGCCTGAGCGACCACGAGATCGACA
GACTGGAACTGCAGAACGGCCGTTGCCTGAGAGAGGCCCAGTACAGCATGCTGGCAAC
ATGGCGGAGAAGAACACCCAGAAGAGAGGCCACCCTGGAACTGCTGGGCAGAGTGCTG
AGAGATATGGACCTGCTGGGTTGTCTGGAAGATATCGAGGAAGCCCTGTGCGGTCCTGC
CGCTCTGCCTCCTGCTCCATCTCTGCTGAGA (**SEQ ID NO: 82**)

[00361] TABLE 6. Partial nucleotide sequences denoting component sequences encoding the individual domains of the CFP: CD5-CD8hinge-CD8TM-FcR-TNFR1 (e.g., SEQ ID NO: 82).

Sequence encoding	Nucleic Acid Sequence
GMCSF signal peptide	ATGTGGCTGCAGTCTCTGCTGCTGCTGGGAACAGTGGCCTGCAGCATCAG C **(SEQ ID NO: 83)**
CD5 scFv	GAGATCCAGCTGGTTCAGTCTGGCGGCGGACTTGTGAAACCTGGCGGATC TGTCAGAATCAGCTGTGCCGCCAGCGGCTACACCTTCACCAACTACGGCA T GAACTGGGTCCGACAGGCCCCTGGAAAAGGCCTTGAGTGGATGGGCTGG A

	TCAATACCCACACCGGCGAGCCAACCTACGCCGATAGCTTTAAGGGCAGA TTCACCTTCAGCCTGGACGACAGCAAGAACACCGCCTACCTGCAGATCAA C AGCCTGAGAGCCGAGGATACCGCCGTGTACTTCTGCACCAGAAGAGGCT AC GACTGGTACTTCGATGTGTGGGGCCAGGGCACCACAGTGACAGTTTCTAG C GGAGGCGGAGGATCAGGTGGCGGTGGATCTGGCGGTGGTGGCTCTGATA TC CAGATGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAGAGT G ACCATCACCTGTAGAGCCAGCCAGGACATCAACAGCTACCTGAGCTGGTT C CAGCAGAAGCCTGGCAAGGCCCCTAAGACACTGATCTACCGGGCCAACA G ACTGGAAAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTGGCACCG A CTACACCCTGACAATCAGCAGCCTGCAGTACGAGGACTTCGGCATCTACT A CTGCCAGCAGTACGACGAGAGCCCTTGGACATTTGGCGGAGGCACCAAG CT GGAAATCAAA (**SEQ ID NO: 84**)
Linker	AGCGGAGGCGGAGGAAGCGGA (**SEQ ID NO: 85**)
CD8 hinge and trans membran e region	GCCCTGAGCAATAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTT TCTGCCCGCCAAGCCTACAACAACACCCGCTCCTAGACCACCTACAC CAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTCAGACCTGAAGCC TGTAGACCTGCAGCTGGCGGAGCTGTGCATACCAGAGGCCTGGATA TCTACATTTGGGCCCCTCTGGCTGGCACATGTGGCGTTCTGCTGCTC TCTCTGGTCATCACCCTGTACTGC (**SEQ ID NO: 86**)
FcR	AGACGGCTGAAGATCCAAGTGCGGAAGGCCGCCATCACCAGCTACG AGAAATCTGATGGCGTGTACACCGGCCTGAGCACCCGGAATCAAGAA ACCTACGAGACACTGAAGCACGAGAAGCCTCCACAA (**SEQ ID NO: 87**)
Linker	GGCAGCGGCAGC (**SEQ ID NO: 88**)
TNFR1	CAAAGATGGAAGAGCAAGCTGTATAGCATCGTGTGCGGCAAGTCCA CCCCTGAGAAGGAAGGAGAGCTGGAAGGCACCACAACAAAGCCTC TGGCCCCTAACCCCTCATTCAGCCCTACCCCCGGCTTCACCCCCACC

	CTGGGATTTAGCCCCGTGCCCAGCAGCACCTTCACCAGCTCTAGCAC CTACACCCCTGGCGACTGCCCCAACTTCGCCGCCCCTAGACGCGAGG TGGCCCCTCCTTACCAGGGCGCCGACCCTATCCTGGCCACAGCCCTG GCTTCTGATCCGATTCCTAATCCTCTGCAGAAGTGGGAGGACAGCGC CCACAAGCCCCAGAGCCTGGACACCGACGACCCCGCCACCCTGTACG CCGTGGTGGAAAACGTGCCTCCACTGCGGTGGAAAGAGTTCGTGCGG CGGCTGGGCCTGAGCGACCACGAGATCGACAGACTGGAACTGCAGAA CGGCCGTTGCCTGAGAGAGGCCCAGTACAGCATGCTGGCAACATGGC GGAGAAGAACACCCAGAAGAGAGGCCACCCTGGAACTGCTGGGCAG AGTGCTGAGAGATATGGACCTGCTGGGTTGTCTGGAAGATATCGAGGA AGCCCTGTGCGGTCCTGCCGCTCTGCCTCCTGCTCCATCTCTGCTGAGA **(SEQ ID NO: 89)**

Example 2. Functional Assays for testing CFP constructs

[00362] The targeted constructs are tested for functional properties. THP-1 cells or CD14+/CD16- monocytes isolated from leukapheresis samples are transfected with the polynucleotide constructs encoding respecting CFPs.

[00363] Method for cell transfection and detection of transfection efficiency: THP-1 cells are harvested and washed once with MaxCyte Electroporation buffer. The cells are resuspended at 10 million/ml density and added to 100ug of ATAK-receptor RNA in an Eppendorf tube, mixed twice and loaded into a MaxCyte processing assembly (OC-25x3). The cells are electroporated using the THP-1 program on MaxCyte. After the electroporation, cells are incubated at 37°C for 10 mins to recover in the processing assembly and then transferred to plates containing pre-warmed media at 0.5 million cells/ml density.

[00364] After overnight incubation, the expression of ATAK-receptor in electroporated monocytes is assessed by Flow cytometry. Anti-Fab-Alexa Fluor-647 antibody (1:50 dilution) is used to detect the expression of scFv of ATAK receptor. The stained samples are acquired on Cytek Northern lights cytometer and percent of binder positive cells calculated based on increase in Anti-Fab intensity over mock-transfected control.

[00365] Method for phagocytosis assay: Target tumor cells (SKOV3) are labeled with pHrodo-Red dye (700ng/ml final concentration) following the Sartorius Incucyte pHrodo-Red labeling kit protocol. After labeling, the SKOV3 cells are resuspended at 0.5 million cells/ml density using the culture medium. CD14-positive monocytes are isolated from donor leukopak and electroporated with 100ug/ml of ATAK-receptor RNA using MaxCyte. After electroporation, cells are recovered overnight at 2 million cells/mL density in culture medium at 37°C. Next day, cells are counted using NC-200 and are resuspended at 2.5 million cells/ml density using the culture medium. In a low

adhesion U-bottom 96-well plate, 50uL of tumor cells (50,000 cells total) are added to 50uL of ATAK-receptor transfected monocytes (125,000 cells total) at 5:1 E:T ratio. The cells are mixed and incubated at 37°C overnight.

[00366] The following day, cells are stained with CD45-Alexa Fluor 700 which labels monocytes specifically. The samples are then acquired on Cytek Northern lights to detect pHrodo-Red and CD45 signal intensities. Phagocytosis is measured as percent phagocytosis index as well as specific increase in pHrodo-Red intensity in monocytes. The phagocytosis index is calculated as the percent of monocytes with high pHrodo-red signal normalized to total number of monocytes. Phagocytic activity of ATAK-monocytes is compared with the mock-transfected control to determine the efficacy of ATAK-receptor.

[00367] Phagocytosis can be tested using labeled tumor cells as shown in FIGs. 4A and 5A.

[00368] Method for SKOV3 cell killing assay: The tumoricidal activity of ATAK receptor-transfected monocytes is tested using SKOV3-Luciferase cells. CD14-positive monocytes are isolated from donor leukopak and electroporated with 100ug/ml of ATAK-receptor RNA using MaxCyte. After electroporation, cells are recovered for 2 hours in culture medium at 37°C. After recovery, cells are resuspended at 2.5 million cells/ml density using the culture medium. SKOV3-Luciferase cells are harvested and resuspended at density of 0.25 million cells/mL and 100ul of cell suspension (total 25,000 cells per well) is added in a 96 well flat-bottom plate. 100uL of ATAK-receptor transfected monocytes are added to the same well at E:T ratio of 1:1 and 10:1. The cells are mixed and incubated at 37°C for 3 days.

[00369] On day 3, supernatant is collected and frozen to measure the cytokines and chemokines secreted by the ATAK-monocytes. The cells are lysed, and SKOV3 luciferase levels are measured using luminescence plate reader. A decrease in Luciferase level for samples containing ATAK-monocytes, compared to mock transfected control, is indicative of SKOV3 killing activity of the ATAK cells.

[00370] Polarization potential of myeloid cells is tested using the following method. These effector myeloid cells electroporated with the polynucleic acid construct and frozen for later use and testing. Upon thawing, the cells were then subject to culture in polarizing stimuli, for example in separate aliquot cultures, with (i) GMCSF (ii) IL4, IL10, and TGFbeta (M2 stimuli), (iii) activated T cell conditioned media (TCM) and (iv) MCSF. Cells were analyzed at 24, 48 and 72 hours by flow cytometry, and cytokine analysis was performed by Luminex.

[00371] Method for detecting NF-κB and IFN pathway activation using THP1-Dual cells: THP1-Dual cells have NF-κB response elements upstream of secreted Alkaline phosphatase and IFN stimulated response elements upstream of secreted Luciferase. Measuring the levels of Alkaline phosphatase in the supernatant indicates the activation of NF-κB signaling pathway, while levels of Luciferase in the supernatant indicates IFN signaling pathway activation. THP1-Dual cells are

electroporated with 100ug/ml of ATAK-receptor RNA using MaxCyte. After electroporation, cells are recovered for 2 hours in culture medium at 37°C. Post recovery, cells are resuspended at 0.5 million cells/ml density using the culture medium. SKOV3 cells are harvested and resuspended at density of 0.5 million cells/mL. In a 96-well plate, 100ul of SKOV3 cell suspension (total 50,000 cells per well) and 100uL of ATAK-THP1-Dual cells (50,000 cells total) are added in a 96 well flat-bottom plate at E:T ratio of 1:1. The cells are mixed and incubated for 24 hours at 37°C. After 24 hours, the cells are centrifuged, and supernatant is collected.

[00372] To detect NF-κB pathway activation, QUANTI-Blue solution (Invivogen) is added to the supernatant and incubated at 37°C for 2 hours, after which OD is measured using an absorbance plate reader. Increase in absorbance is indicative of activation of NF-κB signaling and the OD values for ATAK-transfected THP1-Dual cells can be compared to that of mock transfected controls to determine activity of ATAK-receptors.

[00373] To detect IFN pathway activation, QUANTI-Luc solution (Invivogen) is added to the supernatant and luciferase levels are measured using luminescence plate reader. Increase in luciferase levels implies activation of IFN signaling and can be compared between the ATAK-transfected THP1-Dual cells compared to mock transfected controls.

Example 3. NF-kappa B activation and Inflammatory cytokine induction in myeloid cells expressing CFP constructs

[00374] In this example, individual constructs are electroporated into THP-1 cells and expression and functional analysis were performed. Expression of various constructs (**FIGs. 2A-2D**) were high, other than the construct having a MDA5 intracellular domain (**FIG. 2E**). Likewise, constructs encoding a HER2-FcR-PI3K-MDA5, HER2-CD68-FcR-PI3K-RIG1, and HER2-CD68-FcR-PI3K-MyD88 showed poor expression in THP-1 myeloid cells (**FIG. 2E, 2F**). In cases where the CFP construct comprised a CD64 TM domain, or a CD89 TM domain, high CFP expression was observed, but not in otherwise identical constructs that contained additional dual or multiple intracellular signaling domains, particularly, for example a PI3K signaling ICD and a TLR signaling ICD. It was observed that fusion of additional domains to CD64 or CD89 apparently resulted in loss of function (e.g., phagocytosis, shown in **FIG. 4C**). Constructs having CD40 ICD with another signaling ICD, such as a PI3Kinase recruitment domain show slightly higher expression levels in parallel assays, compared to the CFP constructs with multi-ICDs that include a TLR signaling domain instead of CD40 ICD. Phagocytosis potential as indicated by phagocytic index was higher in CD40 ICD constructs relative to the TLR-ICD containing multi-ICDs (**FIG. 2F, FIG. 4D**). Constructs containing an MDA5 ICD with an FcR ICD (HER2-FcR-MDA5) showed higher or comparable phagocytic index with HER2-CD40-FcR CFPs as shown in FIG. 4B. When a PI3 kinase recruitment domain was placed in the intracellular region, along with an MDA5, RIG-1 or MyD88 signaling intracellular domains both

expression (**FIG. 2F, FIG. 2G**) and phagocytosis (**FIG. 4D and FIG. 4E**) were lower than a construct containing FcR and PI3 kinase recruitment domains.

[00375] THP-1 cells expressing some of the constructs mentioned in the preceding paragraph exhibit tonic signaling upon expression, especially when a PI3 kinase recruitment domain was placed in the intracellular region, along with an MDA5, RIG-1 or MyD88 signaling intracellular domains. Shown in **FIG. 5C**, HER2-CD64-CD40-PI3K construct exhibited tonic signaling. NF-kappa B activation was induced in HER2-CD64 constructs, but when CD40 ICD and PI3kinase recruitment intracellular domains were added, tonic NF-kappa B induction was observed (**FIG. 5C**). As is hereby understood, expression of HER2-CD64 or HER2-CD89 do not induce IFN (**FIG. 5D**). Similarly, with HER2-CD89 construct alone, without additional ICD, NF-kappa B was induced, but addition of CD40 and PI3K signaling ICDs to the construct decreased its ability to induce NF-kappa B upon contact with a target cell (e.g., HER2- expressing SKOV3). Addition of PI3kinase recruitment domain (PI3K) and MDA5 domains had a similar effect in HER2-CD89 construct. Likewise, HER2-FcR-PI3K-MAVS exhibited tonic NF-kappa B or IFN-gamma signaling (**FIG. 5G**, and **FIG. 5H**). These results revealed considerable unpredictability of the effects of the intracellular ICD function in the combined recombinant protein, even though the ICDs may individually, or in their native protein, function in a certain predictable manner. Therefore success in designing the constructs does not depend merely upon routine molecular cloning with the expectation that any assortment of fragments or functional domains can create a CAR with a desired function. The compatibility and functional ability of different domains with respect to each other when structured into a CAR is the crucial aspect in generating a successful CAR construct designed for improved phagocytosis of myeloid cells. Preliminary data exemplifying some ongoing findings that are not yet comprehensive are tabulated below (Table 7):

TABLE 7.

Construct Name	**CD64 constructs**	**CD64-PI3K-MDA5**	**CD64-CD40-PI3K**	**CD89**	**CD89-PI3K-MDA5**	**CD89-CD40-PI3K**
Expression	+++	+	++	++	+	++
Phagocytosis	+++	+	+	++	-	-
NF-kB Activation	+++	+++ (Tonic signaling)	+++ (Tonic signaling)	++	-	-
IFN-1 Activation	-	+++ (Tonic signaling)		-	-	-

[00376] Without wishing to be bound by a theory, it is plausible that the intracellular adaptor protein domains, e.g. pLxIS motif containing domains can be triggered by a host of intracellular triggers when overexpressed, and which may be augmented by a PI3kinase recruitment domain in the intracellular region of the chimeric protein.

Example 4. NF-kappa B activation and IFN response in cells expressing TRIF long and short constructs

[00377] In order to design constructs that exhibit sufficient level of expression, avoid tonic signaling, and would show NF-kappa B activation and IFN response upon activation of the extracellular domains several other constructs were designed and tested. **FIG. 2G** and **FIG. 5I** show representative construct designs and expression data in THP-1 cells. mRNA constructs were electroporated in THP-1 cells and expression of the HER2-CFP constructs were determined by flow cytometry. Phagocytosis was assayed following the protocol demonstrated in the schematic of FIG. 5A, NF-kappa B activation, interferon gamma activation, and cytokine expression levels were determined in these cells. Surprisingly, a construct that comprises both a PI3kinase recruitment domain and a truncated version of TRIF, called TRIF (short) (TRIF domain of SEQ ID NO: 43), demonstrated higher expression levels than in the constructs described in the previous paragraph, including the TRIF long (SEQ ID NO: 44) (FIG. 5I). THP-1 cells expressing TRIP (short) lacked tonic signaling and showed NF-kappa B and IFN activation only in presence of the target SKOV3 tumor cells (FIG. **5G-5H, FIG. 5J**), showed improved phagocytosis index **(FIG. 4E)** of the TRIF (short) expressing cells than cells expressing other innate immune adaptor domains with PI3kinase recruitment domain. Despite 30-fold lower expression (FIG. 2G, right), TRIF short construct phagocytosis is comparable to baseline construct (HER2-FcR-PI3K) (**FIG. 4E**). Expression levels and NF-kappa B activation for other constructs were demonstrated in **FIGs. 5J-5L**. **FIG. 5N** shows inflammatory cytokine expression of CD40 and TRIF constructs.

[00378] Preliminary findings are tabulated below (Table 8):

TABLE 8.

Construct Name	**Binder-FcR-PI3K-MAVS**	**Binder-FcR-PI3K-TRIF(short)**	**Binder-FcR-PI3K-TRIF(long)**
Expression	9%	74%	11%
Phagocytosis	-	++	-
NF-kB Activation	+	++	-
IFN-1 Activation	+++ (Tonic signaling)	+++	-

[00379] Monocytes expressing the above constructs demonstrate high inflammatory response in presence of a target cell. Data shown in **FIGs. 6A-6F** demonstrate the above. For example, monocytes expressing HER2-CD68TM-CD40-FcR construct shows elevated CCL3, IL6 and TNF-alpha response when co-cultured with target SKOV3 cells (**FIG. 6A**). Monocytes expressing a HER2-CD64 construct show greater than 2-fold increase in IL6 and TNF-alpha production compared to mock-transfected monocytes in the presence of the target SKOV3 cells (**FIG. 6B**); and similar results are observed in monocytes expressing HER2-CD89 (**FIG. 6C**). Monocytes expressing HER2-CD68TM-FcR-MDA5

construct show high induction of chemokines CCL3 and IP10, and cytokines IL6, TNF-alpha and interferon alpha (**FIG. 6D**). Similar results were obtained with MyD88 and RIG-1 intracellular domains (**FIGs. 6E** and **6F**). These results clearly demonstrate that the TM and intracellular domains described here are highly functional in inducing an inflammatory response when in monocytes, in the presence of the target cell.

Example 5. Testing of primary human monocytes with various HER2-CD40 CFP constructs

[00380] Based on the results above, receptor enhancements using specific combinations of intracellular signaling domains and/or TM domains were tested in human primary monocytes, testing for expression, phagocytosis and inflammatory cytokine/chemokine release in presence of the respective CFP target. ICD signaling domains comprising domains from (i) CD40 and (ii) TRIF were leading candidates for CFP enhancements. Although high expression and activity of CD40 and TRIF constructs as demonstrated in the previous section in THP-1 cells held great promise, the expression of these constructs were lower in human primary monocytes. Therefore to improve expression and stability of the constructs, the CD68TM domains were swapped with CD64TM and CD8hinge and TM domains. To test surface expression of HER2-CD40 CFP constructs in primary monocytes, primary monocytes were harvested from two healthy human donors and electroporated with the constructs in the table below (Table 9).

TABLE 9. List of HER2-CD40 CFP Constructs tested

HER2-CD40 CFP Constructs tested
HER2-CD8h-CD8TM
HER2-CD8h-CD8TM-FcR-PI3K
HER2-CD8h-CD8TM-CD40-FcR
HER2-CD8h-CD8TM-CD40-FcR-PI3K
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-PI3K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-PI3K

[00381] **FIG. 8A** shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a first donor after electroporation of the indicated HER2-CFP constructs. **FIG. 8B** shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a second donor after electroporation of the indicated HER2-CFP constructs. **FIG. 8C** shows graphs of the percentage of cells positive for the

indicated HER2-CFP constructs and the mean fluorescence intensity using the data from **FIG. 8A** and **FIG. 8B**. Similar expression of the HER2-CFP constructs was observed in both donors.

[00382] To test SKOV3 tumor killing activity of human primary monocytes expressing the constructs above, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKOV3-GFP-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity. **FIG. 9A** shows a graph of SKOV3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs.

[00383] To test SKBR3 tumor killing activity of human primary monocytes expressing the constructs above, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKBR3-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity. **FIG. 9B** shows a graph of SKBR3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs.

[00384] To test the cytokine/chemokine induction ability of human primary monocytes expressing the constructs above, antigen stimulated and tumor cell stimulated human primary monocytes expressing the constructs above were tested. For antigen stimulated HER2-CFP primary monocyte samples, a 96-well plate coated with 2.5 μg/mL of HER2-his protein and 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were added per well. Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. For tumor cell stimulated HER2-CFP primary monocyte samples, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 tumor cells (SKOV3-GFP-Luciferase cells or SKBR3-Luciferase cells). Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. Statistical significance was determined between HER2-CFP electroporated primary monocyte samples co-cultured with tumor cells and mock electroporated primary monocyte samples co-cultured with tumor cells. **FIG. 10A** shows graphs of pro-inflammatory cytokine IL-6 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 10B** shows graphs of pro-inflammatory cytokine IL-1β induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 10C** shows graphs of TNF-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 10D** shows graphs of IFN-α induction by primary

human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 10E** shows graphs of IP10 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 10F** shows graphs of IL-12 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). It was seen from the studies above that the constructs induced high cytokine and chemokine response (e.g., FIG. 10A, FIG. 10B; specifically, a combination of CD40, FcR and PI3K intracellular domains with the CD64TM domains induced high levels of cytokines, e.g., IL-1b and IL6). This indicates that the monocytes were highly activated upon engagement of these chimeric receptor to target antigen, and were capable of enhanced inflammatory response. In addition to phagocytosis of the target cell by the activated cells themselves, the activated CFP-mediated induction of NF-kappa B and IFN responsive genes, as well as release of inflammatory cytokines and chemokines by the myeloid cell are indications that these cells could potentially lead to an enhanced immune activation in vivo. To further characterize human primary monocytes expressing the constructs above, Nanostring gene expression analysis will be employed using the Human nCounter Myeloid Innate Immunity V2 Panel (770 genes).

Example 6. Testing of primary human monocytes with various HER2-TRIF CFP constructs

[00385] To test surface expression of HER2-TRIF CFP constructs in primary monocytes, primary monocytes were harvested from two healthy human donors and electroporated with the constructs in the table below (Table 10).

[00386] TABLE 10. List of HER2-TRIF CFP Constructs tested

HER2-TRIF CFP Constructs tested
HER2-CD8h-CD8TM
HER2-CD8h-CD8TM-FcR-PI3K
HER2-CD8h-CD8TM-FcR-TRIFshort
HER2-CD8h-CD8TM-FcR-PI3K-TRIFshort
HER2-CD64TM-FcR-TRIFshort
HER2-CD64TM-FcR-PI3K-TRIFshort
HER2-CD8h-CD64TM-FcR-TRIFshort
HER2-CD8h-CD64TM-FcR-PI3K-TRIFshort

[00387] **FIG. 11A** shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a first donor after electroporation of the indicated HER2-CFP constructs. **FIG. 11B** shows flow cytometry data demonstrating expression of the indicated HER2-CFP constructs in primary human monocytes from a second donor after electroporation of the indicated HER2-CFP constructs. **FIG. 11C** shows graphs of the percentage of cells positive for the indicated HER2-CFP constructs and the mean fluorescence intensity using the data from **FIG. 11A** and **FIG. 11B**.

[00388] To test SKOV3 tumor killing activity of human primary monocytes expressing the constructs above, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKOV3-GFP-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity. **FIG. 12A** shows a graph of SKOV3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs.

[00389] To test SKBR3 tumor killing activity of human primary monocytes expressing the constructs above, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 SKBR3-Luciferase tumor cells. Luciferase activity of the tumor cells was detected after 72 hours of co-culture and killing was determined by a decrease in luciferase signal intensity. **FIG. 12B** shows a graph of SKBR3 killing activity by primary human monocytes from two different donors that were electroporated with the indicated HER2-CFP constructs.

[00390] To test the cytokine/chemokine induction ability of human primary monocytes expressing the constructs above, antigen stimulated and tumor cell stimulated human primary monocytes expressing the constructs above were tested. For antigen stimulated HER2-CFP primary monocyte samples, a 96-well plate coated with 2.5 μg/mL of HER2-his protein and 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were added per well. Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. For tumor cell stimulated HER2-CFP primary monocyte samples, 100,000 primary human monocytes electroporated with the indicated HER2-CFP constructs were co-cultured with 20,000 tumor cells (SKOV3-GFP-Luciferase cells or SKBR3-Luciferase cells). Supernatant was collected after 48 hours of stimulation and secreted cytokine was analyzed by Luminex. Statistical significance was determined between HER2-CFP electroporated primary monocyte samples co-cultured with tumor cells and mock electroporated primary monocyte samples co-cultured with tumor cells. **FIG. 13A** shows graphs of pro-inflammatory cytokine IL-6 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 13B** shows graphs of pro-inflammatory cytokine IL-1β induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP

constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 13C** shows graphs of TNF-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 13D** shows graphs of IFN-α induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 13E** shows graphs of IP10 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). **FIG. 13F** shows graphs of IL-12 induction by primary human monocytes from a human donor that were electroporated with the indicated HER2-CFP constructs and stimulated with HER-2 antigen (top) or co-cultured with SKOV3 tumor cells (bottom, left) or SKBR3 tumor cells (bottom, right). Nanostring gene expression analysis will be employed using the Human nCounter Myeloid Innate Immunity V2 Panel (770 genes).

[00391] Additionally, in a preliminary in vivo study, a CFP comprising TRIF and CD40 intracellular domains indicate that the ICDs were indeed conferring protective immunity, as shown in **FIG. 14**. Primary monocytes armed with TRIF or CD40 based ATAK receptors showed strong anti-tumor response in B16 melanoma tumor model (Left) Posttreatment melanoma tumor volume in mice treated with ATAK-monocytes. (Right) Survival of tumor-bearing mice treated with FcR-PI3K ATAK receptor or ATAK receptors with TRIF/CD40 domains.

CLAIMS

What is claimed is:

1. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain with a Xaa1-Leu-Xaa3-Iso-Ser motif, wherein Xaa1 is a hydrophilic amino acid, and Xaa3 is any amino acid.
2. The recombinant nucleic acid of claim 1, wherein the chimeric fusion protein further comprises an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain.
3. The recombinant nucleic acid of claim 1 or 2, wherein the intracellular domain comprises at least one additional intracellular signaling domain.
4. The recombinant nucleic acid of claim 3, wherein the at least one additional intracellular signaling domain is derived form an intracellular PI3-kinase recruitment domain, a phagocytosis receptor intracellular domain, a pattern recognition receptor intracellular domain, a CD40 intracellular domain, an FcR intracellular domain, or a cytokine or chemokine receptor intracellular domain.
5. The recombinant nucleic acid of any one of claims 1-4, wherein intracellular signaling domain comprises an amino acid sequence derived from a cytosolic adaptor protein, a mitochondrial membrane protein or an endoplasmic reticulum membrane protein.
6. The recombinant nucleic acid of any one of claims 1-5, wherein intracellular signaling domain comprises an amino acid sequence derived from a TRIF protein.
7. The recombinant nucleic acid of any one of claims 1-6, wherein intracellular signaling domain comprises a truncated TRIF intracellular domain.
8. The recombinant nucleic acid of any one of claims 1-7, wherein the sequence of the truncated TRIF intracellular domain is about 150 to 300 amino acids in length, about 150 to 270 amino acids in length, or about 150-250 amino acids in length.
9. The recombinant nucleic acid of any one of claims 1-5, wherein intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 36.
10. The recombinant nucleic acid of any one of claims 1-5, wherein intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 37.
11. The recombinant nucleic acid of any one of claims 1-5, wherein intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 38.
12. The recombinant nucleic acid of any one of claims 1-5, wherein intracellular signaling domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 41.
13. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein

the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 39.

14. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 40.
15. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 42.
16. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 43.
17. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 45.
18. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain, wherein the transmembrane domain is derived from CD68 and comprises a mutation that inhibits or prevents homodimerization.
19. The recombinant nucleic acid of claim 15, wherein the chimeric fusion protein further comprises an intracellular domain operably linked to the transmembrane domain.
20. The recombinant nucleic acid of claim 15 or 16, wherein the transmembrane domain comprises SEQ ID NO: 47.
21. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain, wherein the transmembrane domain is derived from CD64 or CD89; and wherein the chimeric fusion protein lacks an intracellular signaling domain.
22. The recombinant nucleic acid of claim 18 or 21, wherein the chimeric fusion protein forms a complex with an FcRγ chain when expressed in a cell.
23. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein

the intracellular domain comprises an intracellular signaling domain comprising a sequence having at least 80% amino acid sequence identity to SEQ ID NO: 48.

24. The recombinant nucleic acid of any one of claims 1-23, further comprising a second intracellular signaling domain that comprises a PI3 kinase recruitment domain.
25. The recombinant nucleic acid of claim 10, further comprising an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 39 and the sequence of an IRF activation enhancing motif.
26. The recombinant nucleic acid of claim 10, further comprising an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 40 and the sequence of an IRF activation enhancing motif.
27. The recombinant nucleic acid of claim 10, further comprising an intracellular signaling domain comprising fused amino acid sequence comprising SEQ ID NO: 39 and SEQ ID NO: 40.
28. The recombinant nucleic acid of any one of claims of 1-27, wherein the intracellular domain binds to an IRF protein.
29. The recombinant nucleic acid of any one of claims of 1-28, wherein the intracellular domain comprises a TRAF protein binding domain.
30. The recombinant nucleic acid of any one of claims 1-29, wherein the intracellular domain comprises a TBK1 phosphorylation site.
31. The recombinant nucleic acid of any one of claims 1-30, further comprising a second, third, or fourth intracellular signaling domain.
32. The recombinant nucleic acid of any one of claims of 10-31, further comprising an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain.
33. The recombinant nucleic acid of any one of claims of 1-32, wherein the antigen binding domain comprises a domain having an affinity for a tumor antigen.
34. The recombinant nucleic acid of any one of claims of 1-33, wherein the extracellular domain comprises a T lymphocyte antigen binding domain.
35. The recombinant nucleic acid of any one of claims of 1-33, wherein the extracellular domain comprises a B lymphocyte antigen binding domain.
36. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) a transmembrane domain and (b) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises a signaling domain from GPR84, GNB2 or PREX1.
37. The recombinant nucleic acid of claim 36, wherein the chimeric fusion protein further comprises an extracellular domain comprising an antigen binding domain, wherein the transmembrane domain is operably linked with the extracellular domain.

38. The recombinant nucleic acid of claim 36, wherein the signaling domain from GPR84, GNB2 or PREX1 is an intracellular signaling domain from GPR84, GNB2 or PREX1.
39. The recombinant nucleic acid of any one of claims of 1-38, wherein the antigen to which the antigen binding domain binds is selected from the group consisting of an antigen from CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, CD56, Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD70, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin, an integrin receptor, Claudin 3.0, Claudin 18.2, Trop-2, PRSS21, VEGFR2, PDGFRβ, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, Dsg1, Dsg3, and IGLL1.
40. The recombinant nucleic acid of any one of claims of 1-39, wherein the extracellular domain comprises a first target antigen binding domain, and a second target antigen binding domain that is non-identical to the first target binding domain.
41. The composition of claim 40, wherein the second target antigen binding domain binds to CD47.
42. The recombinant nucleic acid of any one of claims of 1-38, wherein the antigen binding domain comprises a domain having an affinity for a microbial antigen.
43. The recombinant nucleic acid of claim 42, wherein the antigen binding domain comprises a domain having an affinity for a viral antigen.
44. The recombinant nucleic acid of any one of claims of 1-43, wherein the domain comprises an antibody or a fragment thereof.
45. The recombinant nucleic acid of any one of claims of 1-43, wherein the domain comprises an scFv.
46. The recombinant nucleic acid of any one of claims of 1-45, wherein the transmembrane domain is derived from the transmembrane domain of CD8a, CD28, CD68, CD2, FcRγ, FcRα, FcRβ, FcRε, syntaxin 3, syntaxin 4, or syntaxin 5.
47. The recombinant nucleic acid of any one of claims of 1-46, wherein the extracellular domain comprises a hinge domain connecting the antigen binding domain and the transmembrane domain.

48. The recombinant nucleic acid of any one of claims of 1-47, wherein when expressed in a cell, the chimeric fusion protein upon binding to its target antigen activates the intracellular domain to induce IRF activation.
49. The recombinant nucleic acid of any one of claims of 1-48, wherein the recombinant nucleic acid is an RNA.
50. The recombinant nucleic acid of claim 49, wherein the recombinant nucleic acid is an mRNA.
51. The recombinant nucleic acid of any one of claims 1-50, wherein the recombinant nucleic acid is associated with one or more lipids.
52. The recombinant nucleic acid of claim 51, wherein the recombinant nucleic acid is encapsulated in a liposome.
53. The recombinant nucleic acid of claim 52, wherein the liposome is a lipid nanoparticle.
54. The recombinant nucleic acid of any one of claims 1-53, wherein the recombinant nucleic acid is a vector.
55. A cell comprising the recombinant nucleic acid of any one of claims 1-54.
56. The cell of claim 55, wherein the cell is an immune cell.
57. The cell of claim 55 or 56, wherein the cell is a myeloid cell, a lymphoid cell, a precursor cell, a stem cell or an induced pluripotent cell.
58. The cell of claim 57, wherein the cell is CD14+/CD16-.
59. An engineered cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular signaling domain comprises an intracellular signaling domain that binds to an IFN transcription factor when activated.
60. An engineered cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular signaling domain comprises an intracellular signaling domain derived from a mitochondrial receptor.
61. The composition of claim 59 or 60, wherein the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 39.
62. The composition of claim 59 or 60, wherein the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 40.
63. The engineered cell of claim 59, wherein the intracellular domain comprises an intracellular signaling domain derived from TLR3, TLR4, TLR7, TLR 9, TRIF, RIG-1, MYD88, MAL, IRAK1, MDA-5, an IFN-receptor, STING, MAVS, TRIF, TASL, NLRP1, NLRP2, NLRP3,

NLRP4, NLRP5, NLRP6, NLRP7, NLRP89, NLRP9, NLRP10, NLRP11, NLRP12, NLRP13, NLRP1-14, NOD1, NOD2, Pyrin, AIM2, NLRC4, FCGR3A, FCERIG, CD40, Tank1-binding kinase (TBK), TNFR1, a chemokine, MHC Class II transactivator (CIITA), IPAF, BIRC1, a RIG-I-like receptor (RLR) protein, macrophage galactose-type lectin (MGL), DC-SIGN (CLEC4L), Langerin (CLEC4K), Myeloid DAP12 associating lectin (MDL)-1 (CLEC5A), a DC associated C type lectin 1 (Dectin1) subfamily protein, dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C type lectin like receptor (MICL) (CLEC12A), CLEC2 (CLEC1B), CLEC12B, a DC immunoreceptor (DCIR) subfamily protein, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), and Mincle (macrophage inducible C type lectin) (CLEC4E).

64. The engineered cell of claim 59, wherein the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 42.

65. The engineered cell of claim 59 or 60, wherein the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 43.

66. The engineered cell of claim 59 or 60, wherein the intracellular domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 45.

67. The engineered cell of claim 60, wherein the intracellular signaling domain comprises an intracellular signaling domain that binds to a IFN transcription factor when activated.

68. The engineered cell of claim 59 or 67, wherein the IFN transcription factor is any one of the proteins IRF1-IRF9.

69. The engineered cell of claim 59, wherein the intracellular domain comprises a TRAF recruitment domain.

70. The engineered cell of claim 59, wherein the intracellular domain that induces an IKK phosphorylation.

71. The engineered cell of claim 59, wherein the intracellular domain induces a TBK phosphorylation.

72. The engineered cell of any one of claims 59-71, wherein the antigen binding domain binds to a tumor antigen or a microbial antigen.

73. The engineered cell of any one of claims 59-71, wherein the antigen binding domain binds to one or more of CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, CD56, Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand,

Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD70, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin, an integrin receptor, Claudin 3.0, Claudin 18.2, Trop-2, PRSS21, VEGFR2, PDGFRβ, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, Dsg1, Dsg3, and IGLL1.

74. The engineered cell of any one of claims 59-71, wherein the antigen binding domain binds to a viral antigen.
75. The engineered cell of any one of claims 59-74, wherein the transmembrane domain is derived from the transmembrane domain of CD8a, CD28, CD68, CD2, FcRγ, FcRα, FcRβ, FcRε, syntaxin 3, syntaxin 4, or syntaxin 5.
76. The engineered cell of any one of claims 59-75, wherein the intracellular domain further comprises an intracellular signaling domain derived from an FcRγ, FcRα, or FcRε.
77. The engineered cell of any one of claims 59-76, wherein the intracellular domain further comprises PI3-kinase recruitment domain.
78. The engineered cell of any one of claims 59-77, wherein the intracellular signaling domain binds to an IRF transcription factor and activates it upon binding of the antigen binding domain to its cognate antigen.
79. The engineered cell of any one of claims 59-78, wherein the cell induces pro-inflammatory cytokines upon binding of the antigen binding domain to its cognate antigen.
80. The engineered cell of claim 79, wherein the pro-inflammatory cytokines comprise one or more of IL-1, IL-6, IL-12, IL-23, TNF, CXCL9, CXCL10, CXCL11, IL-18, IL-23, IL-27 and interferons.
81. The engineered cell of any one of claims 59-80, wherein the cell is a myeloid cell, a lymphocyte or a stem cell.
82. The engineered cell of claim 81, wherein the cell is a myeloid cell.
83. A pharmaceutical composition comprising the recombinant nucleic acid of any one of claims 1-54, the cell of any one of claims 55-58, or the engineered cell of any one of claims 59-82; and a pharmaceutically acceptable excipient.
84. The pharmaceutical composition of claim 3 wherein the engineered cell is CD14+ and/or CD16-.
85. The pharmaceutical composition of claim 83 or 84, comprising a population of cells wherein at least 50% of the cells are CD14+/CD16-, and less than 10% cells are dendritic cells.
86. The pharmaceutical composition of claim 85, wherein the cells exhibit high expression of CCR2.
87. The pharmaceutical composition of claim 85 or 86, wherein the cells do not exhibit tonal signaling and activation de novo, and exhibit M0, M1 or M2 differentiation upon activation.

88. A method of treating a cancer or a viral infection in a subject comprising: administering to the subject the pharmaceutical composition of any one of claims 83-87.
89. An engineered CD14+/CD16- cell comprising a recombinant nucleic acid encoding a chimeric fusion protein (CFP), the CFP comprising: (a) an extracellular domain comprising an antigen binding domain; (b) a transmembrane domain operably linked to the extracellular domain, and (c) an intracellular domain operably linked to the transmembrane domain, wherein the intracellular domain comprises a domain derived from TRIF.
90. The engineered cell of claim 89, wherein the intracellular domain comprising a domain derived from TRIF is a truncated TRIF domain.
91. The engineered cell of claim 89, wherein the intracellular domain comprising a domain derived from TRIF comprises less than 300 amino acids.
92. The engineered cell of claim 89, wherein the intracellular domain comprising a domain derived from TRIF comprises less than 250 amino acids.
93. The engineered cell of claim 89, wherein the intracellular domain comprising a domain derived from TRIF exhibits IFN response upon engagement of the extracellular binding domain with the target.
94. The recombinant nucleic acid of any one of claims 1-5 or 15, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 66, or a sequence having at least 80% sequence identity to SEQ ID NO: 66.
95. A pharmaceutical composition, comprising a recombinant polynucleotide comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 66, or a sequence having at least 80% sequence identity to SEQ ID NO: 66, and a suitable excipient.
96. The pharmaceutical composition of claim 95, wherein the recombinant polynucleotide is an mRNA, associated with one or more lipids.
97. A pharmaceutical composition comprising a population of engineered cells expressing a recombinant protein having a sequence of SEQ ID NO: 66, or a sequence having at least 80% sequence identity to SEQ ID NO: 66, wherein at least 50% of the cells are CD14+/CD16-, and less than 10% cells are dendritic cells; and a pharmaceutically acceptable excipient.
98. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 56, or a sequence having at least 80% sequence identity to SEQ ID NO: 56.
99. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 59, or a sequence having at least 80% sequence identity to SEQ ID NO: 59.

100. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 60, or a sequence having at least 80% sequence identity to SEQ ID NO: 60.
101. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 61, or a sequence having at least 80% sequence identity to SEQ ID NO: 61.
102. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 62, or a sequence having at least 80% sequence identity to SEQ ID NO: 62.
103. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 63, or a sequence having at least 80% sequence identity to SEQ ID NO: 63.
104. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 64, or a sequence having at least 80% sequence identity to SEQ ID NO: 64.
105. The recombinant nucleic acid of any one of claims 1-5, comprising a sequence encoding a polypeptide having an amino acid sequences of SEQ ID NO: 67, or a sequence having at least 80% sequence identity to SEQ ID NO: 67.
106. An engineered myeloid cell comprising a recombinant nucleic acid of any one of claims 98-105.
107. A recombinant nucleic acid encoding a chimeric fusion protein comprising (a) an extracellular domain comprising an antigen binding domain, (b) a transmembrane domain operably linked to the extracellular domain and (c) an intracellular domain operably linked to the transmembrane domain, wherein the transmembrane domain is a transmembrane domain derived from CD68, CD64 or CD8, and

wherein the intracellular domain comprises a domain derived from TRIF or an intracellular signaling domain derived from CD40.
108. The recombinant nucleic acid of claim 107, wherein the intracellular domain comprises a domain derived from TRIF.
109. The recombinant nucleic acid of claim 108, wherein the domain derived from TRIF is a truncated TRIF domain.
110. The recombinant nucleic acid of claim 107, wherein the intracellular domain comprises an intracellular signaling domain derived from CD40.
111. The recombinant nucleic acid of any one of claims 107-110, wherein the intracellular domain further comprises intracellular signaling domain derived from an Fc receptor (FcR).

112. The recombinant nucleic acid of any one of claims 107-110, wherein the intracellular domain further comprises intracellular signaling domain derived from Fc gamma.
113. The recombinant nucleic acid of any one of claims 107-112, wherein the intracellular domain further comprises a PI3 kinase recruitment domain.
114. The recombinant nucleic acid of any one of claims 107-113, wherein the extracellular domain comprises a hinge domain derived from CD8-alpha.
115. The recombinant nucleic acid of any one of claims 107-114, wherein the antigen binding domain is a HER2 binding domain.
116. The recombinant nucleic acid of any one of claims 107-115, wherein the transmembrane domain is a transmembrane domain derived from CD64.
117. The recombinant nucleic acid of any one of claims 107-115, wherein the transmembrane domain is a transmembrane domain derived from CD8-alpha.
118. The recombinant nucleic acid of claim 107, wherein the antigen binding domain is a HER2 binding domain, the extracellular domain comprises a hinge domain derived from CD8-alpha, the transmembrane domain is a transmembrane domain derived from CD8-alpha; and wherein the intracellular domain comprises an intracellular signaling domain derived from CD40, an intracellular signaling domain derived from Fcγ (Fc gamma), and a PI3 kinase recruitment domain.
119. The recombinant nucleic acid of claim 118, wherein intracellular signaling domain derived from CD40 is linked to the transmembrane domain, the intracellular signaling domain derived from Fcγ is linked to the intracellular signaling domain derived from CD40 and the PI3 kinase recruitment domain is linked to the intracellular signaling domain derived from Fcγ.
120. The recombinant nucleic acid of claim 107, wherein the antigen binding domain is a HER2 binding domain, the transmembrane domain is a transmembrane domain derived from CD64; and wherein the intracellular domain comprises an intracellular signaling domain derived from Fcγ, a PI3 kinase recruitment domain, and an intracellular domain derived TRIF.
121. The recombinant nucleic acid of claim 120, wherein the extracellular domain does not comprise a hinge domain derived from CD8-alpha.
122. The recombinant nucleic acid of claim 120, wherein the extracellular domain does not comprise a hinge domain.
123. The recombinant nucleic acid of any one of claims 120-122, wherein the intracellular signaling domain derived from Fcγ is linked to the transmembrane domain, the PI3 kinase recruitment domain is linked to the intracellular signaling domain derived from Fcγ and the intracellular domain derived TRIF is linked to the PI3 kinase recruitment domain.
124. A cell comprising the recombinant nucleic acid of any one of claims 107-123.
125. The cell of claim 124, wherein the cell is a myeloid cell.

126. A pharmaceutical composition comprising the cell of claim 124 or 125.

127. An mRNA comprising the sequence according to SEQ ID NOs: 68, 75, or 82.

128. The mRNA of claim 127, for use in treating cancer.

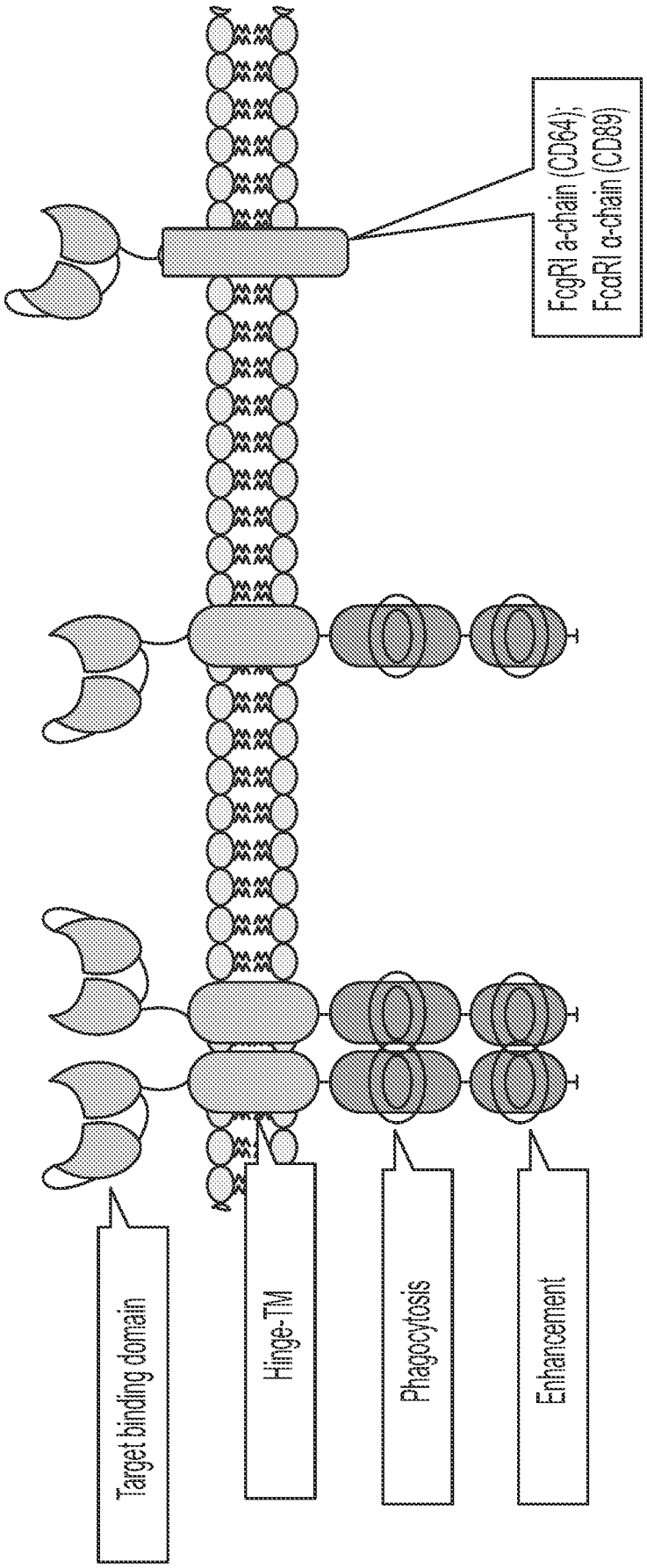

FcgRI a-chain (CD64);
FcaRI a-chain (CD89)
Target binding domain
Hinge-TM
Phagocytosis
Enhancement

FIG. 1A

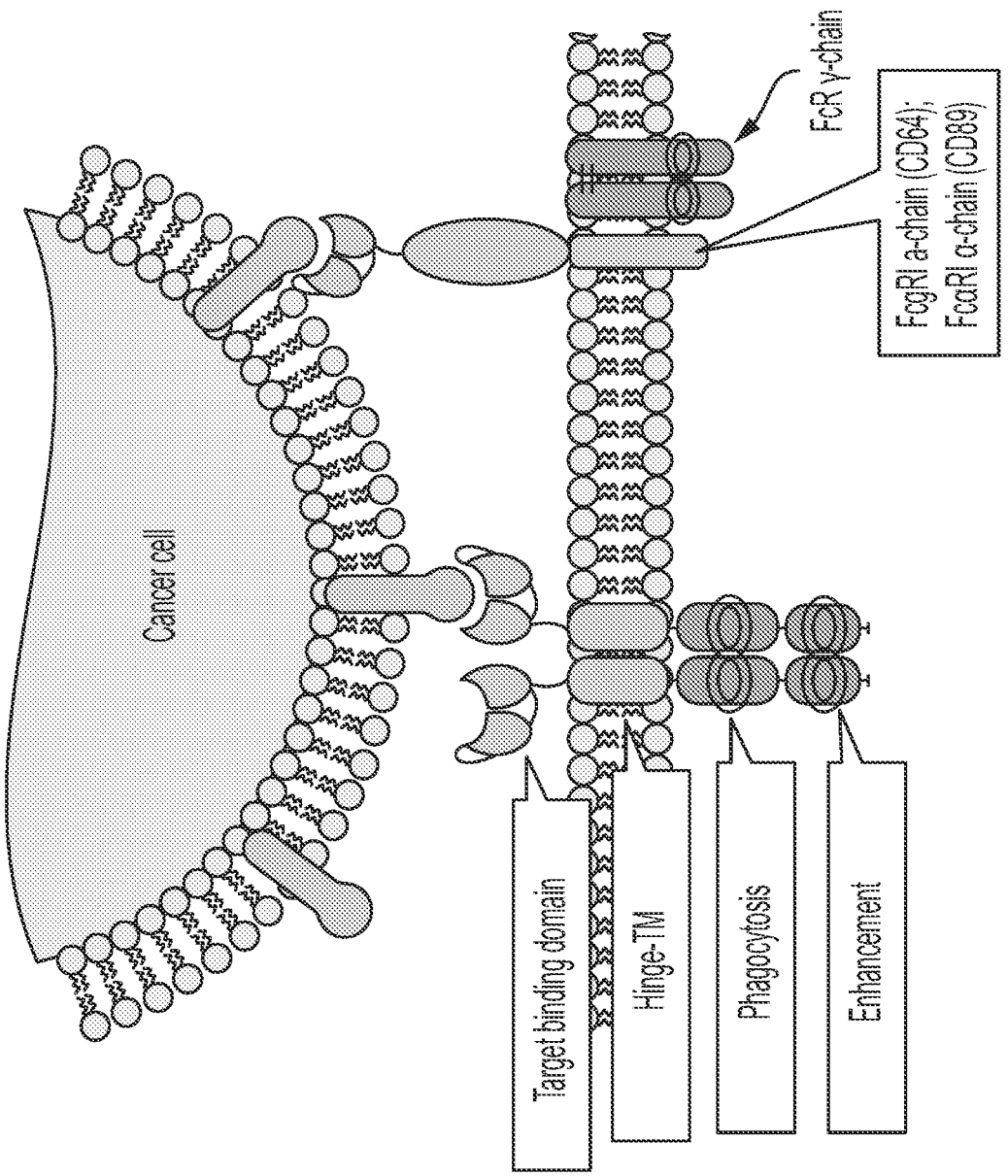

Cancer cell
Target binding domain
Hinge-TM
Phagocytosis
Enhancement
FcR γ-chain
FcgRI a-chain (CD64);
FcαRI α-chain (CD89)

FIG. 1B

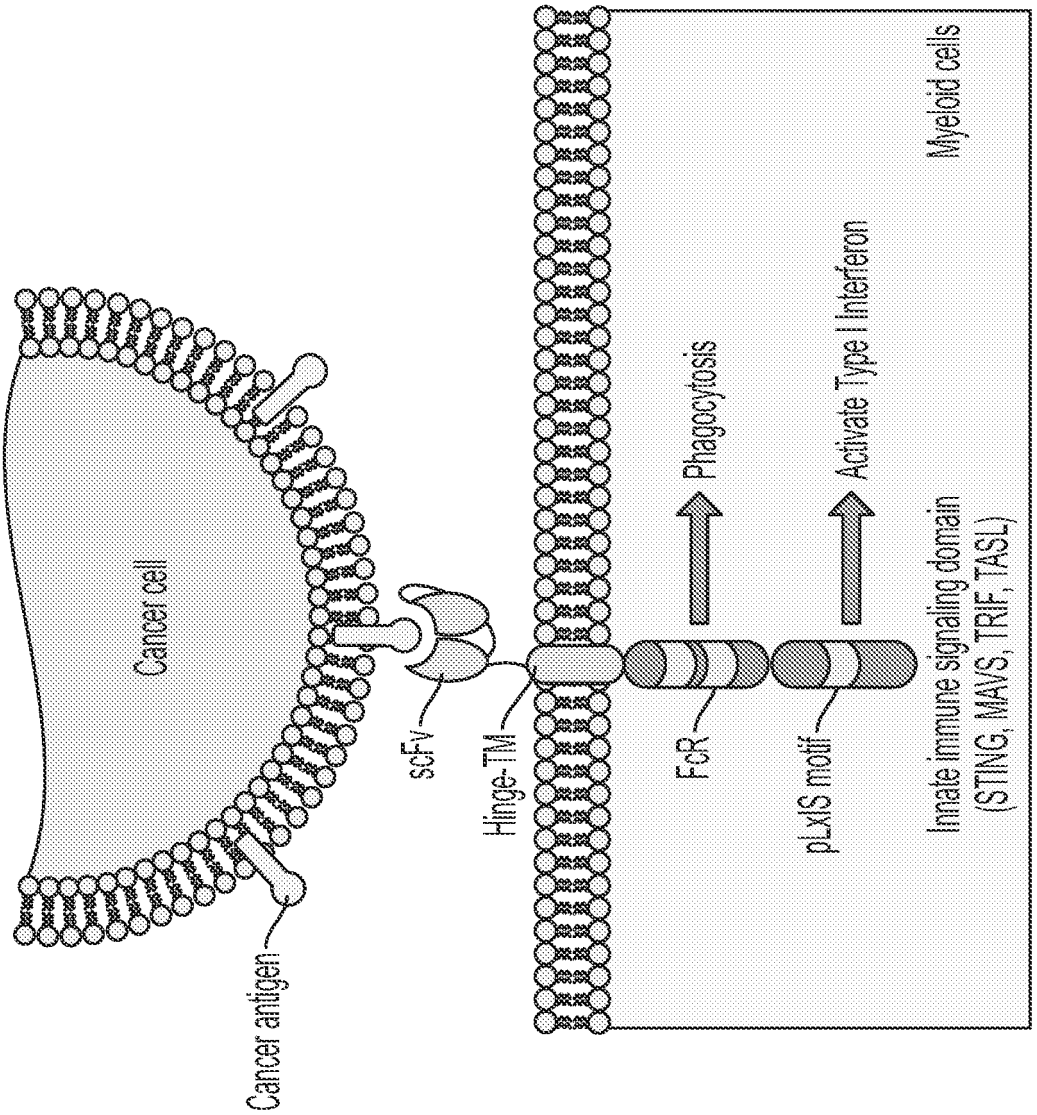

Cancer cell
Cancer antigen
scFv
Hinge-TM
FcR
pLxIS motif
Innate immune signaling domain
(STING, MAVS, TRIF, TASL)
Phagocytosis
Activate Type I Interferon
Myeloid cells

FIG. 1C

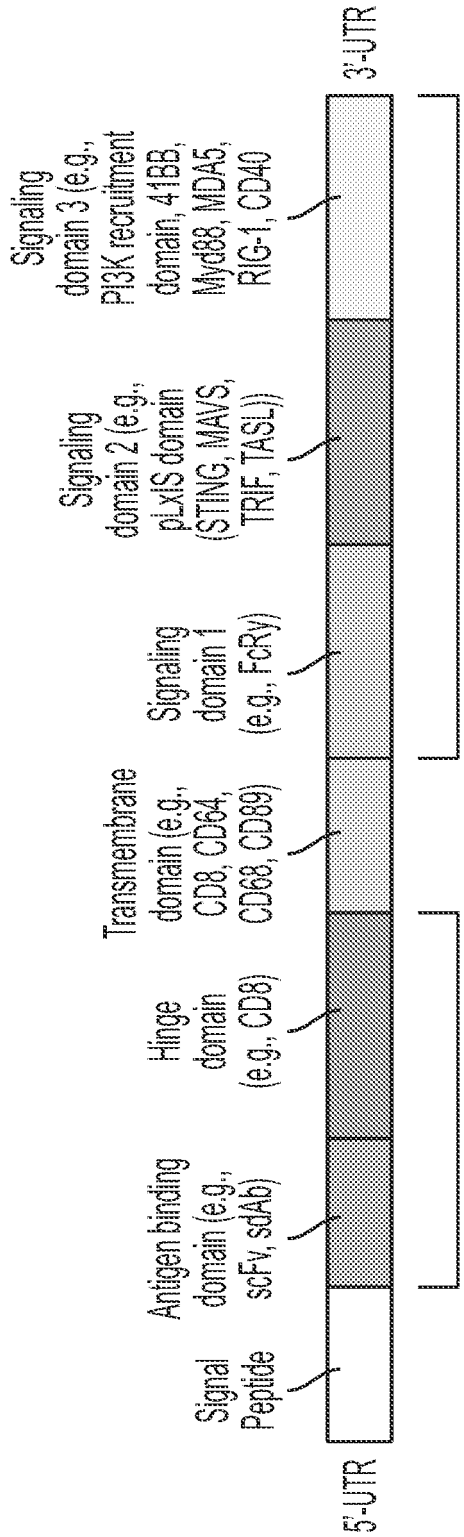

5'-UTR
Signal Peptide
Antigen binding domain (e.g., scFv, sdAb)
Hinge domain (e.g., CD8)
Transmembrane domain (e.g., CD8, CD64, CD68, CD89)
Signaling domain 1 (e.g., FcRγ)
Signaling domain 2 (e.g., pLxIS domain (STING, MAVS, TRIF, TASL))
Signaling domain 3 (e.g., PI3K recruitment domain, 41BB, Myd88, MDA5, RIG-1, CD40
3'-UTR
Extracellular domain (ECD)
Intracellular domain (ICD)

FIG. 1D

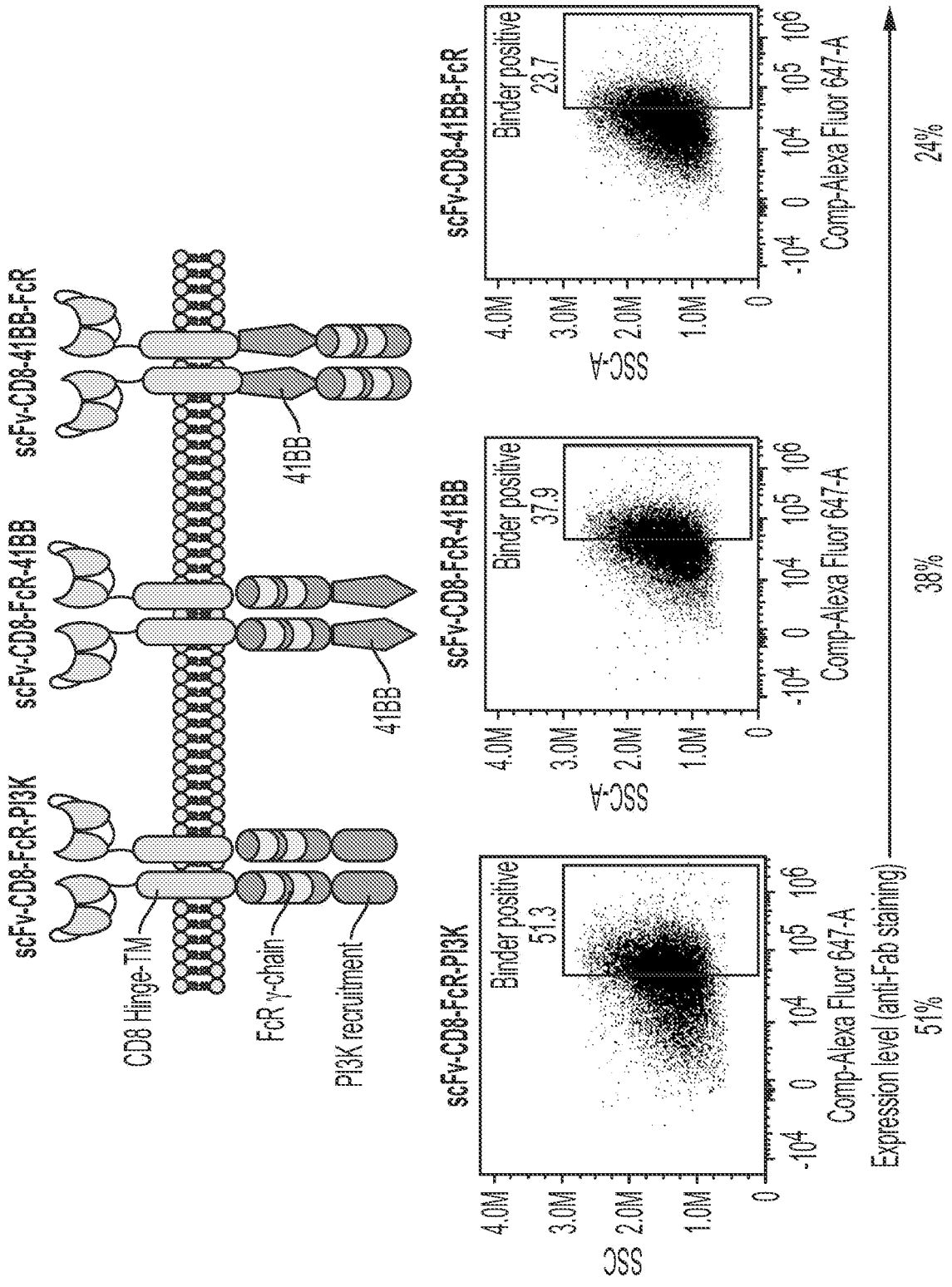

scFv-CD8-FcR-PI3K
scFv-CD8-FcR-41BB
scFv-CD8-41BB-FcR
CD8 Hinge-TM
FcR γ-chain
PI3K recruitment
41BB
41BB
scFv-CD8-FcR-PI3K
Binder positive
51.3
scFv-CD8-FcR-41BB
Binder positive
37.9
scFv-CD8-41BB-FcR
Binder positive
23.7
SSC-A
4.0M
3.0M
2.0M
1.0M
0
-10^4
0
10^4
10^5
10^6
Comp-Alexa Fluor 647-A
Expression level (anti-Fab staining)
51%
38%
24%

FIG. 2A

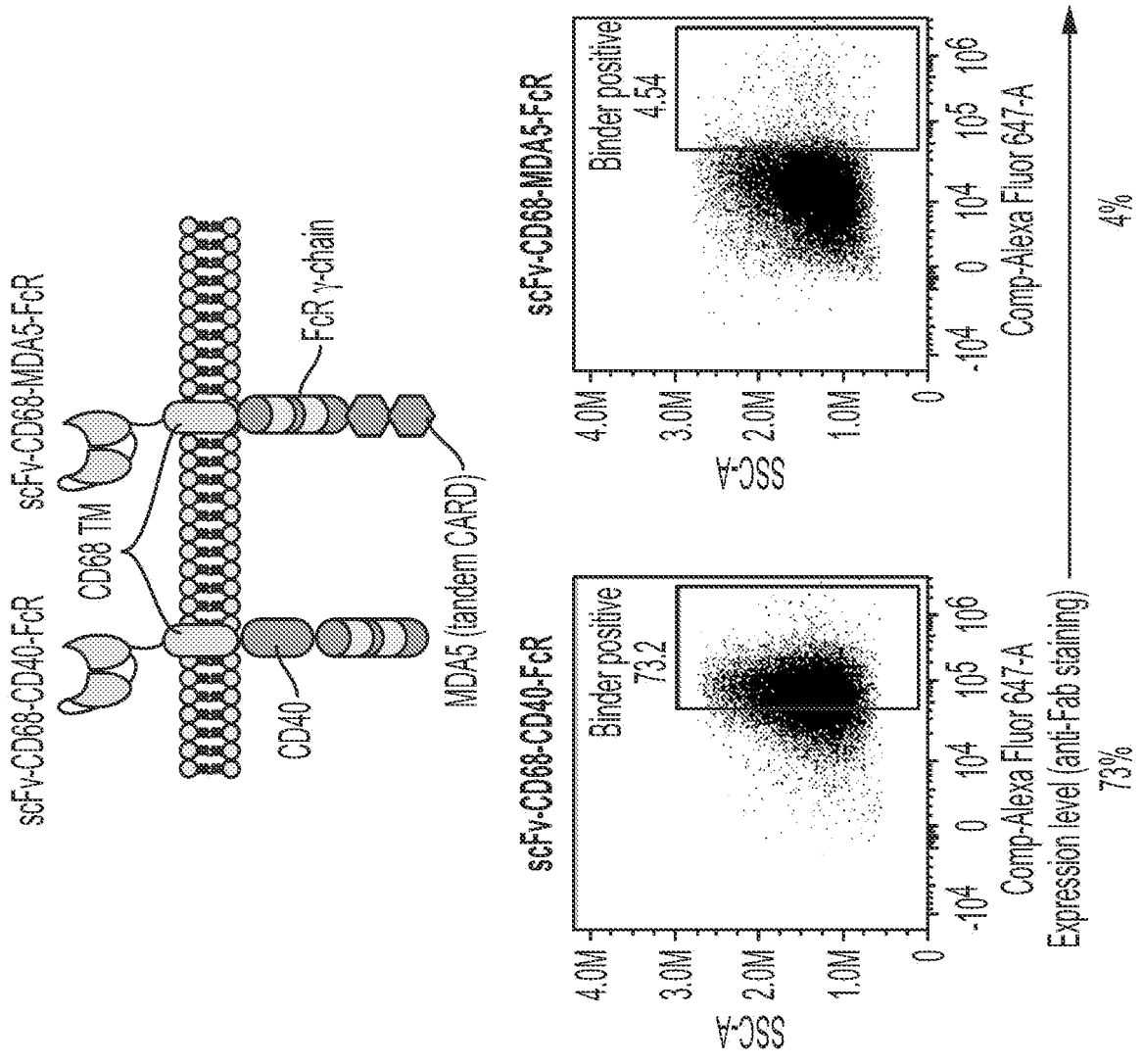

scFv-CD68-CD40-FcR
scFv-CD68-MDA5-FcR
CD68 TM
FcR γ-chain
CD40
MDA5 (tandem CARD)
scFv-CD68-MDA5-FcR
Binder positive
4.54
scFv-CD68-CD40-FcR
Binder positive
73.2
SSC-A
4.0M
3.0M
2.0M
1.0M
0
-10^4
0
10^4
10^5
10^6
Comp-Alexa Fluor 647-A
Expression level (anti-Fab staining)
73%
4%

FIG. 2B

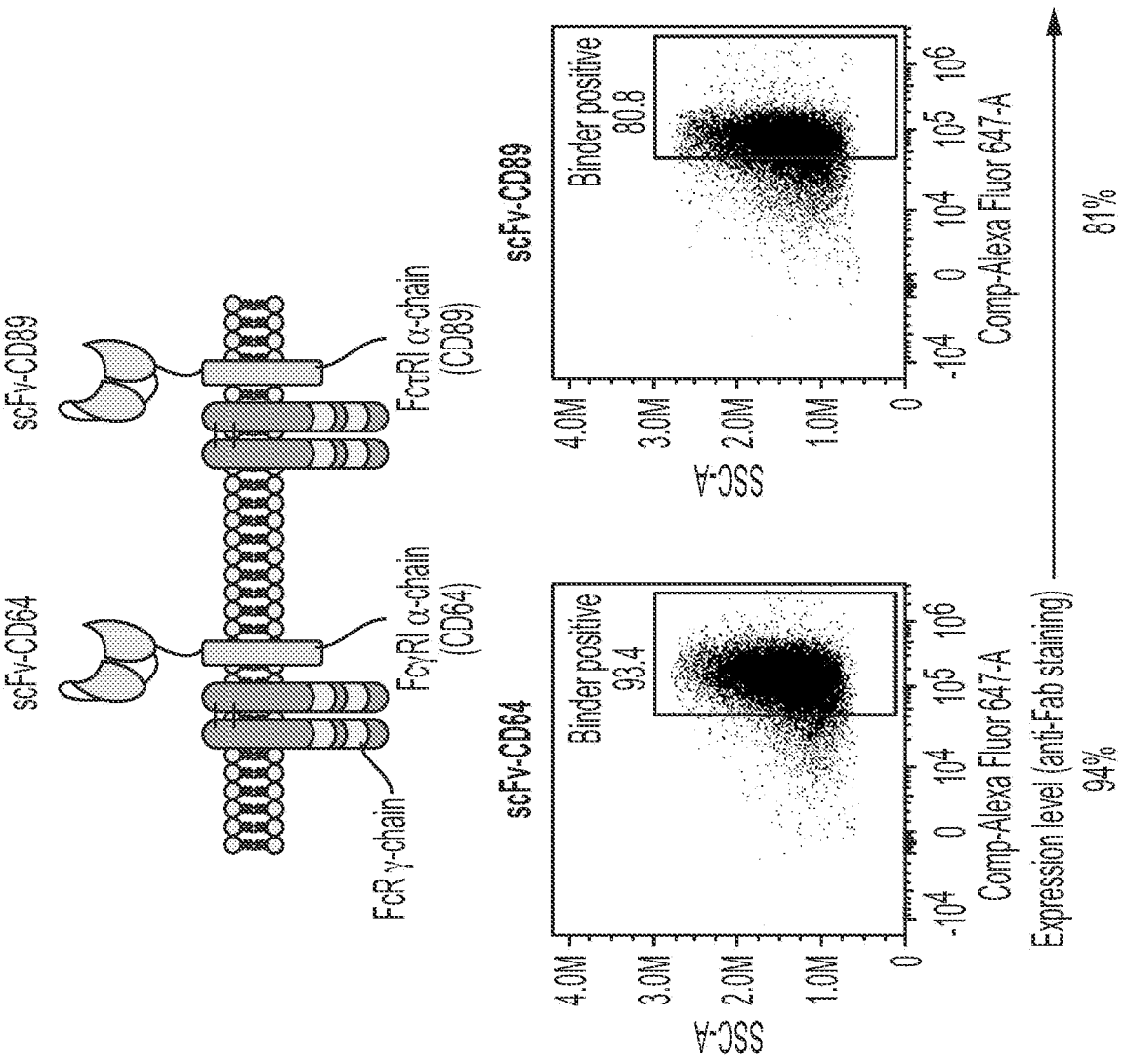

scFv-CD89
scFv-CD64
FcεRI α-chain (CD89)
FcγRI α-chain (CD64)
FcR γ-chain
scFv-CD89
Binder positive 80.8
SSC-A
4.0M
3.0M
2.0M
1.0M
0
-10⁴ 0 10⁴ 10⁵ 10⁶
Comp-Alexa Fluor 647-A
scFv-CD64
Binder positive 93.4
Expression level (anti-Fab staining)
94%
81%

FIG. 2C

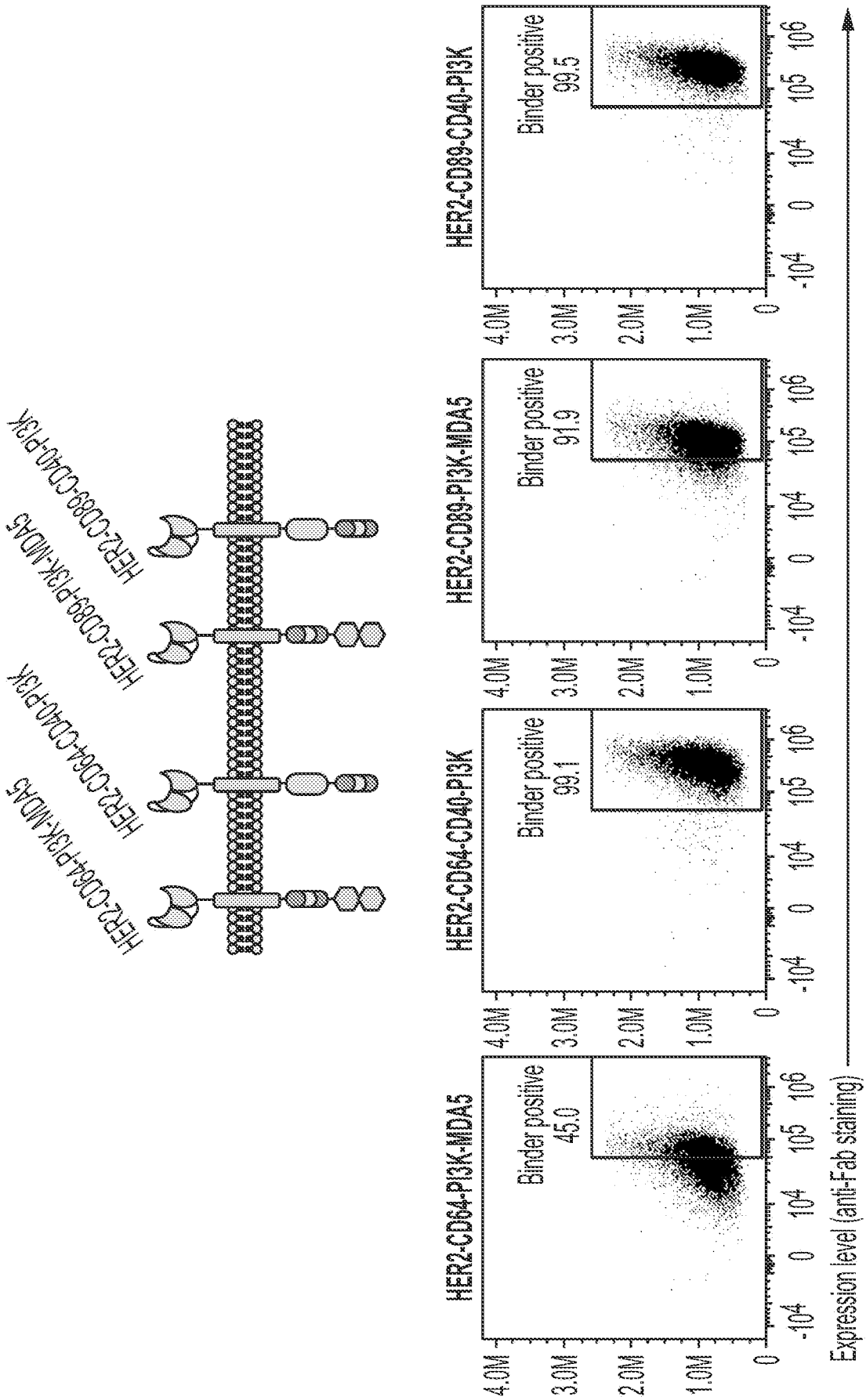

HER2-CD64-PI3K-MDA5
HER2-CD64-CD40-PI3K
HER2-CD89-PI3K-MDA5
HER2-CD89-CD40-PI3K
Binder positive
45.0
Binder positive
99.1
Binder positive
91.9
Binder positive
99.5
4.0M
3.0M
2.0M
1.0M
0
-10^4
0
10^4
10^5
10^6
Expression level (anti-Fab staining)
HER2-CD64-PI3K-MDA5
HER2-CD64-CD40-PI3K
HER2-CD89-PI3K-MDA5
HER2-CD89-CD40-PI3K

FIG. 2D

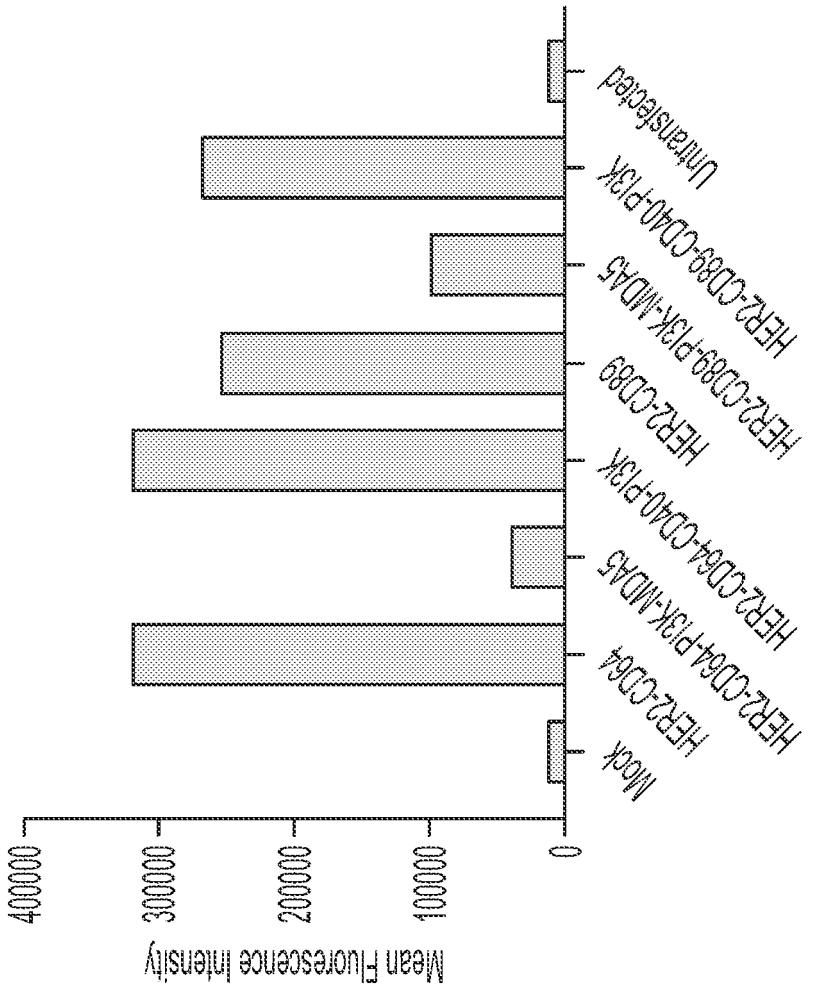

Mean Fluorescence Intensity
400000
300000
200000
100000
0
Mock
HER2-CD64
HER2-CD64-P13K-MD45
HER2-CD64-CD40-P13K
HER2-CD89
HER2-CD89-P13K-MD45
HER2-CD89-CD40-P13K
Untranstected

FIG. 2E

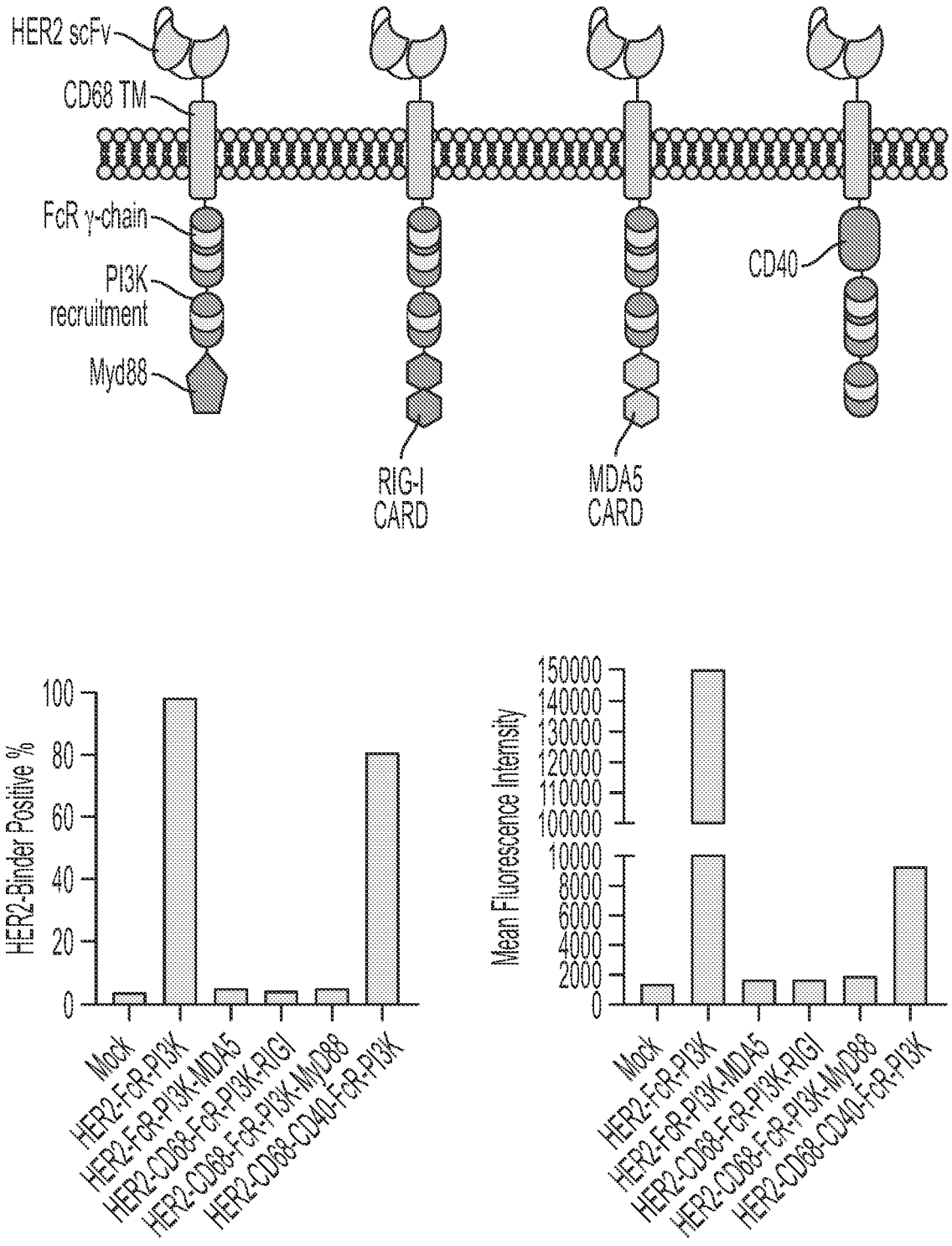

HER2 scFv
CD68 TM
FcR γ-chain
PI3K recruitment
Myd88
RIG-I CARD
MDA5 CARD
CD40
HER2-Binder Positive %
100
80
60
40
20
0
Mock
HER2-FcR-PI3K
HER2-FcR-PI3K-MDA5
HER2-CD68-FcR-PI3K-RIGI
HER2-CD68-FcR-PI3K-MyD88
HER2-CD68-CD40-FcR-PI3K
Mean Fluorescence Intensity
150000
140000
130000
120000
110000
100000
10000
8000
6000
4000
2000
0
Mock
HER2-FcR-PI3K
HER2-FcR-PI3K-MDA5
HER2-CD68-FcR-PI3K-RIGI
HER2-CD68-FcR-PI3K-MyD88
HER2-CD68-CD40-FcR-PI3K

FIG. 2F

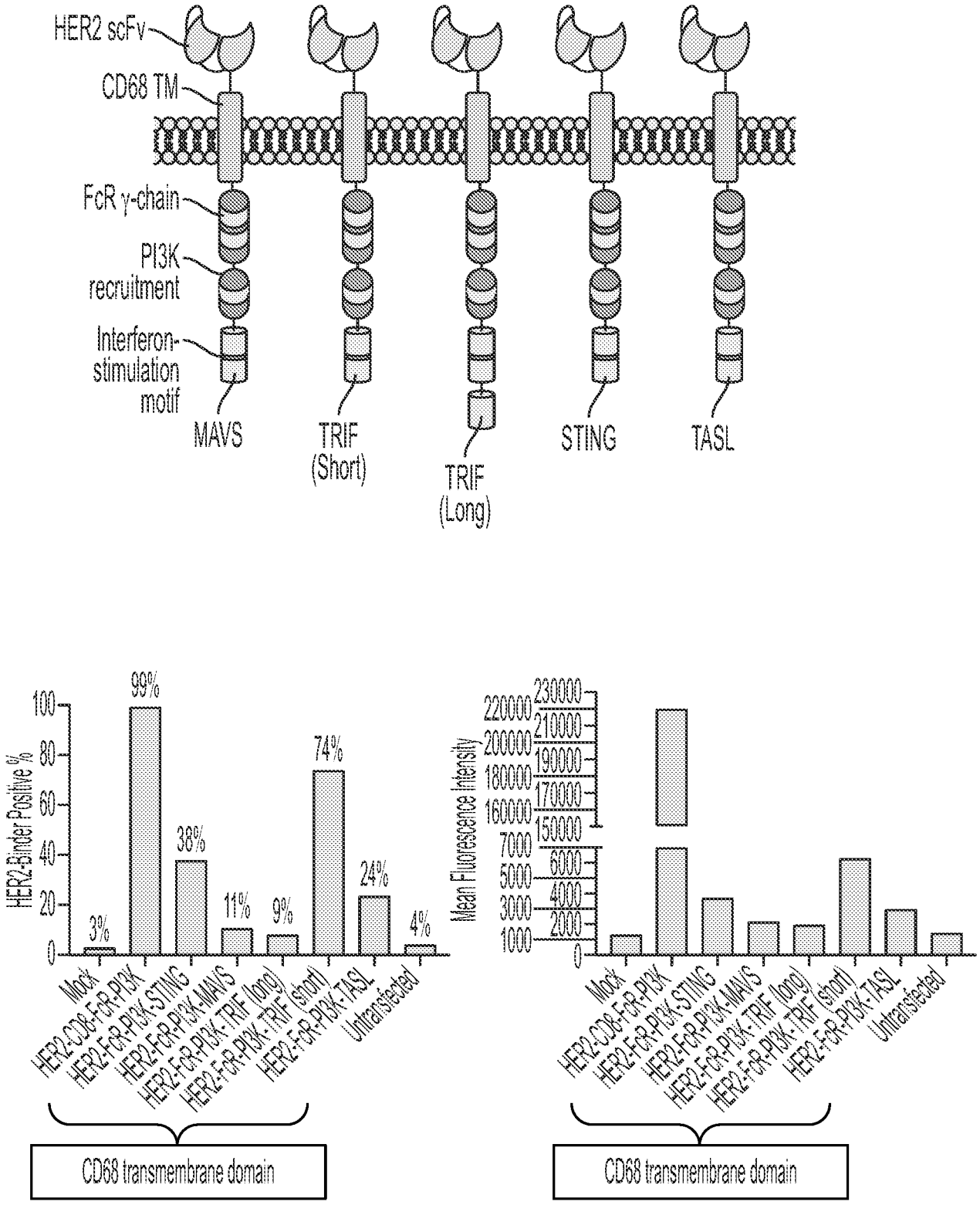

HER2 scFv
CD68 TM
FcR γ-chain
PI3K recruitment
Interferon-stimulation motif
MAVS
TRIF (Short)
TRIF (Long)
STING
TASL
HER2-Binder Positive %
Mean Fluorescence Intensity
99%
38%
11%
9%
74%
24%
4%
3%
Mock
HER2-CD68-FcR-PI3K
HER2-FcR-PI3K-STING
HER2-FcR-PI3K-MAVS
HER2-FcR-PI3K-TRIF (long)
HER2-FcR-PI3K-TRIF (short)
HER2-FcR-PI3K-TASL
Untransfected
CD68 transmembrane domain
CD68 transmembrane domain

FIG. 2G

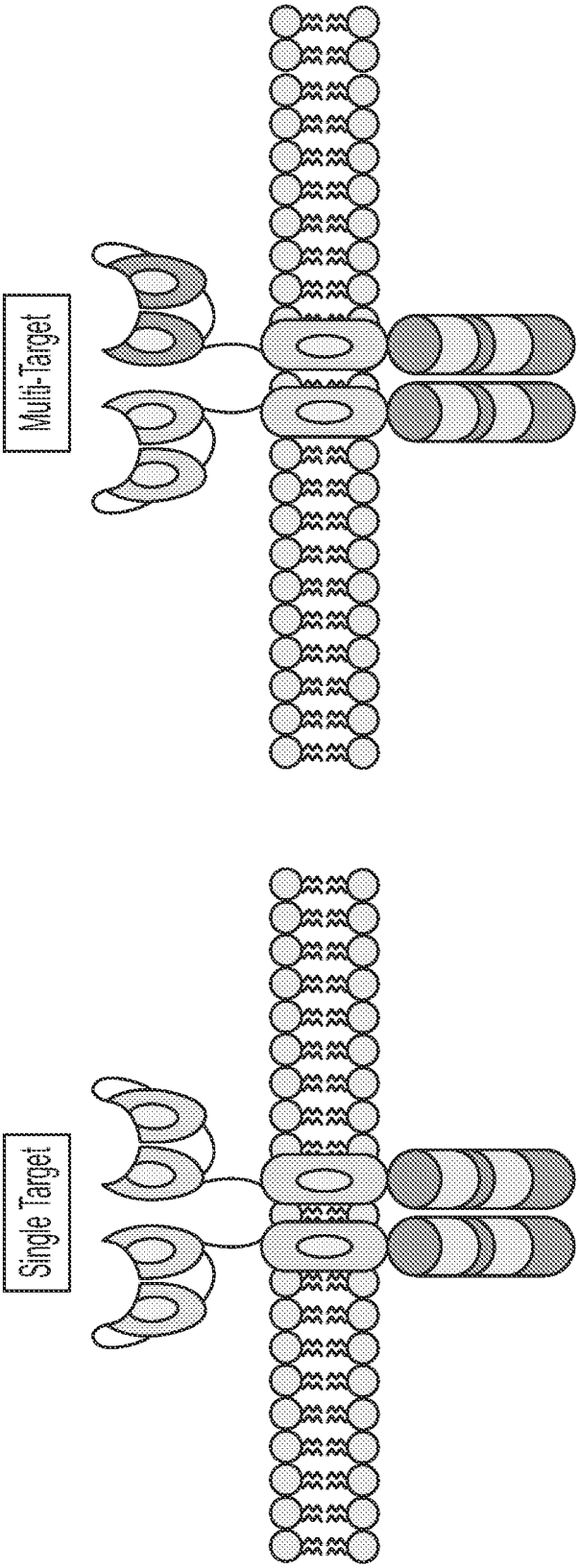


FIG. 3

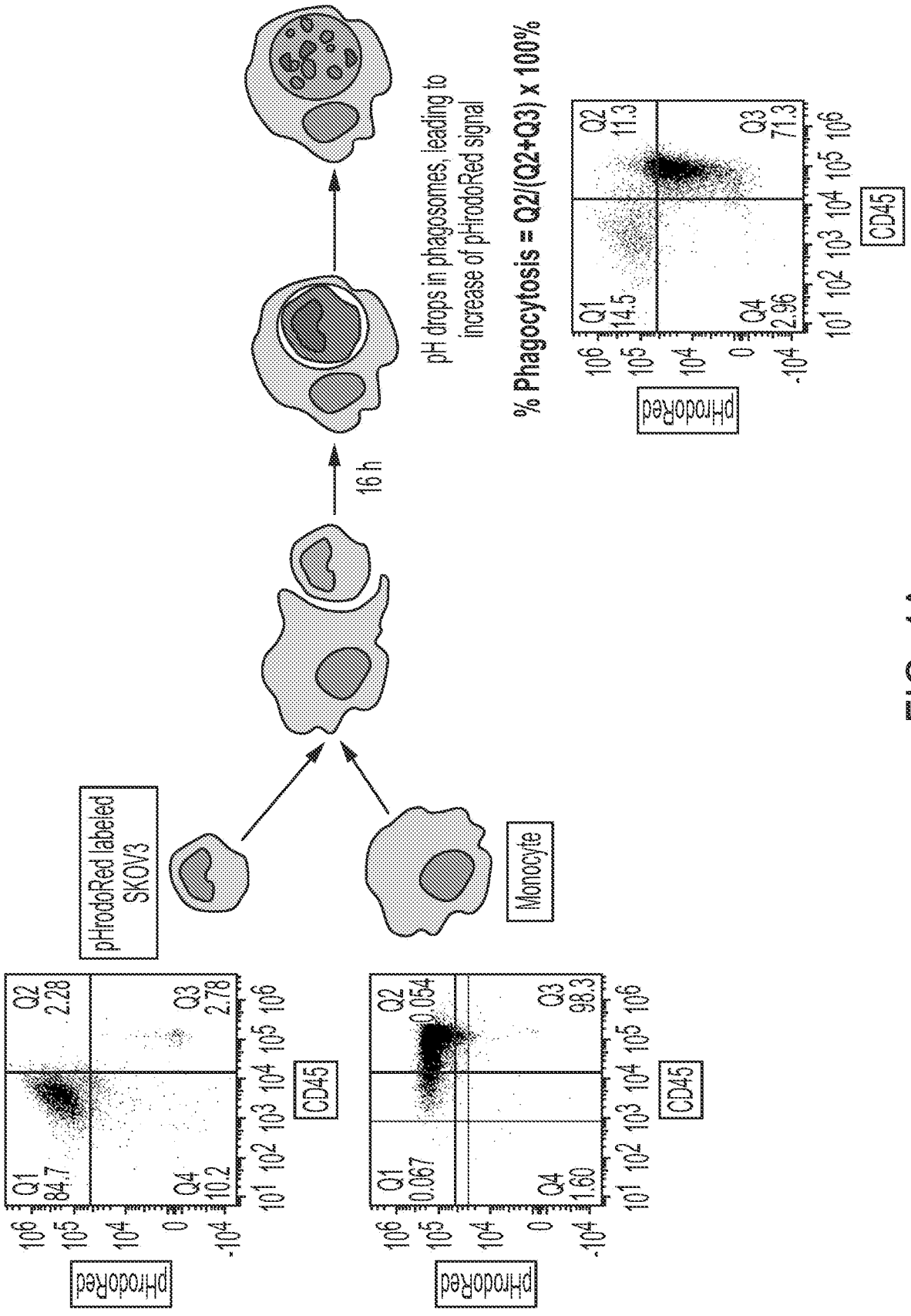

pHrodoRed labeled SKOV3
Monocyte
16 h
pH drops in phagosomes, leading to increase of pHrodoRed signal
% Phagocytosis = Q2/(Q2+Q3) x 100%
pHrodoRed
CD45
Q1 84.7
Q2 2.28
Q3 2.78
Q4 10.2
Q1 0.067
Q2 0.054
Q3 98.3
Q4 1.60
Q1 14.5
Q2 11.3
Q3 71.3
Q4 2.96

FIG. 4A

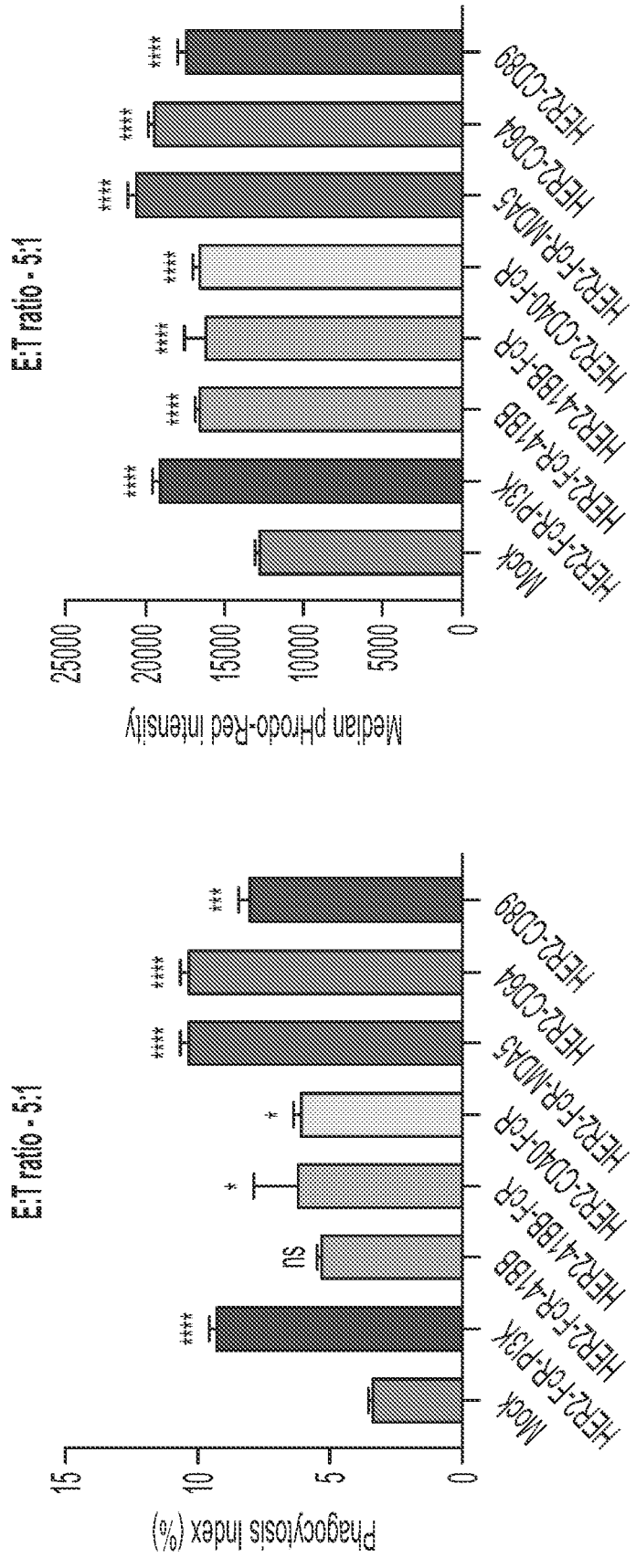

E:T ratio - 5:1
Median pHrodo-Red intensity
25000
20000
15000
10000
5000
0
Mock
HER2-FCR-P13K
HER2-FCR-4/1BB
HER2-4/1BB-FCR
HER2-CD40-FCR
HER2-FCR-MDA5
HER2-CD64
HER2-CD89
****
E:T ratio - 5:1
Phagocytosis Index (%)
15
10
5
0
Mock
HER2-FCR-P13K
HER2-FCR-4/1BB
HER2-4/1BB-FCR
HER2-CD40-FCR
HER2-FCR-MDA5
HER2-CD64
HER2-CD89
ns
*
****
***

FIG. 4B

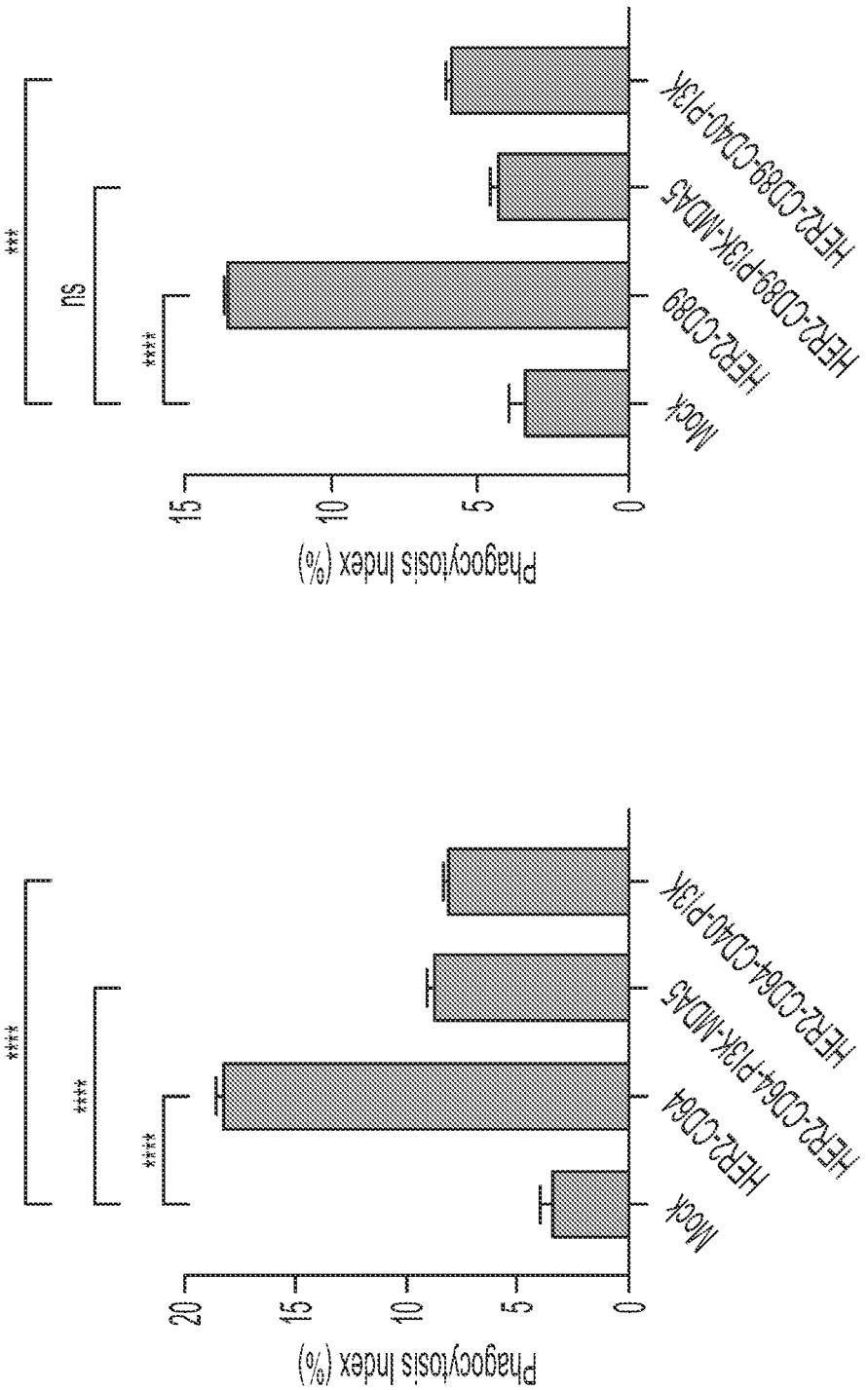

Phagocytosis Index (%)
0
5
10
15
20
Mock
HER2-CD64
HER2-CD64-PI3K-AID45
HER2-CD64-CD40-PI3K
****
****
****
Phagocytosis Index (%)
0
5
10
15
Mock
HER2-CD89
HER2-CD89-PI3K-AID45
HER2-CD89-CD40-PI3K
****
ns
***

FIG. 4C

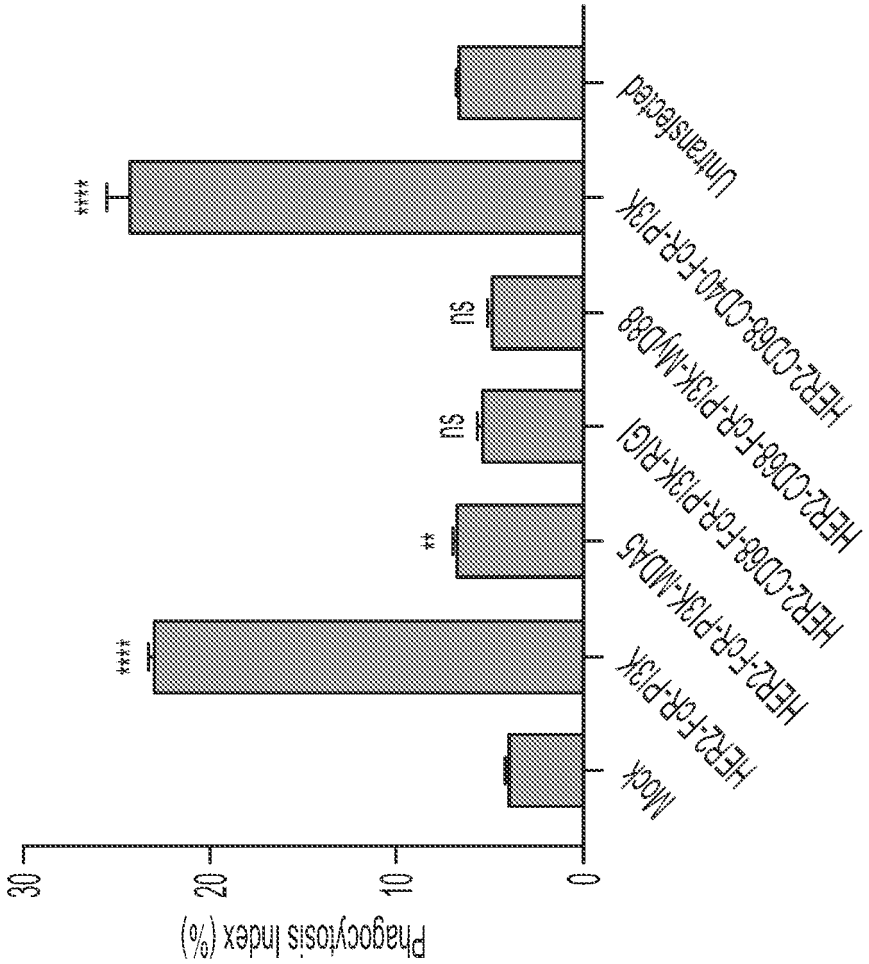
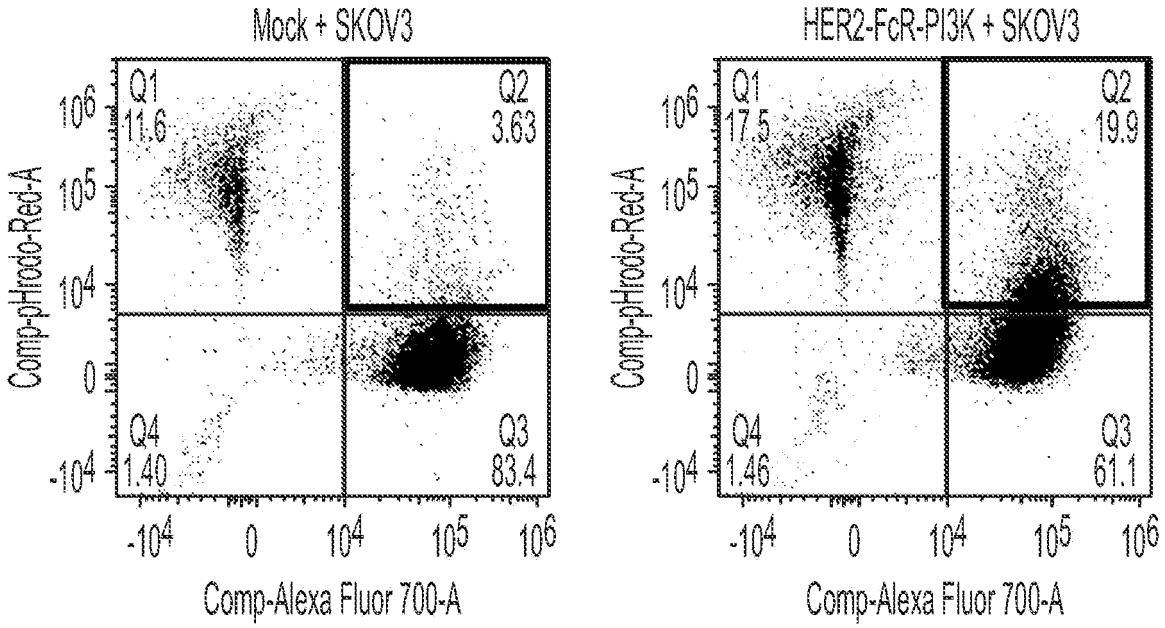

Phagocytosis Index (%)
0
10
20
30
Mock
HER2-FCR-PI3K
HER2-FCR-PI3K-MD45
HER2-CD68-FCR-PI3K-RIGI
HER2-CD68-FCR-PI3K-MyD88
HER2-CD68-CD40-FCR-PI3K
Untransfected
****
**
ns
ns
****

FIG. 4D


Mock + SKOV3
HER2-FcR-PI3K + SKOV3
Q1
11.6
Q2
3.63
Q4
1.40
Q3
83.4
Q1
17.5
Q2
19.9
Q4
1.46
Q3
61.1
Comp-pHrodo-Red-A
Comp-Alexa Fluor 700-A
10^6
10^5
10^4
0
-10^4

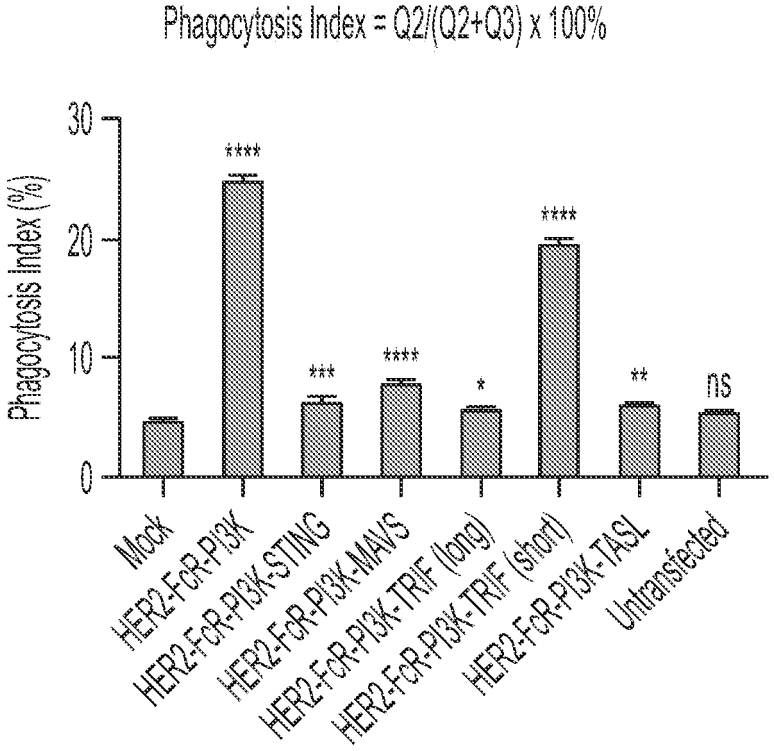

Phagocytosis Index = Q2/(Q2+Q3) x 100%
Phagocytosis Index (%)
30
20
10
0
****
***
****
*
****
**
ns
Mock
HER2-FcR-PI3K
HER2-FcR-PI3K-STING
HER2-FcR-PI3K-MAVS
HER2-FcR-PI3K-TRIF (long)
HER2-FcR-PI3K-TRIF (short)
HER2-FcR-PI3K-TASL
Untransfected

FIG. 4E

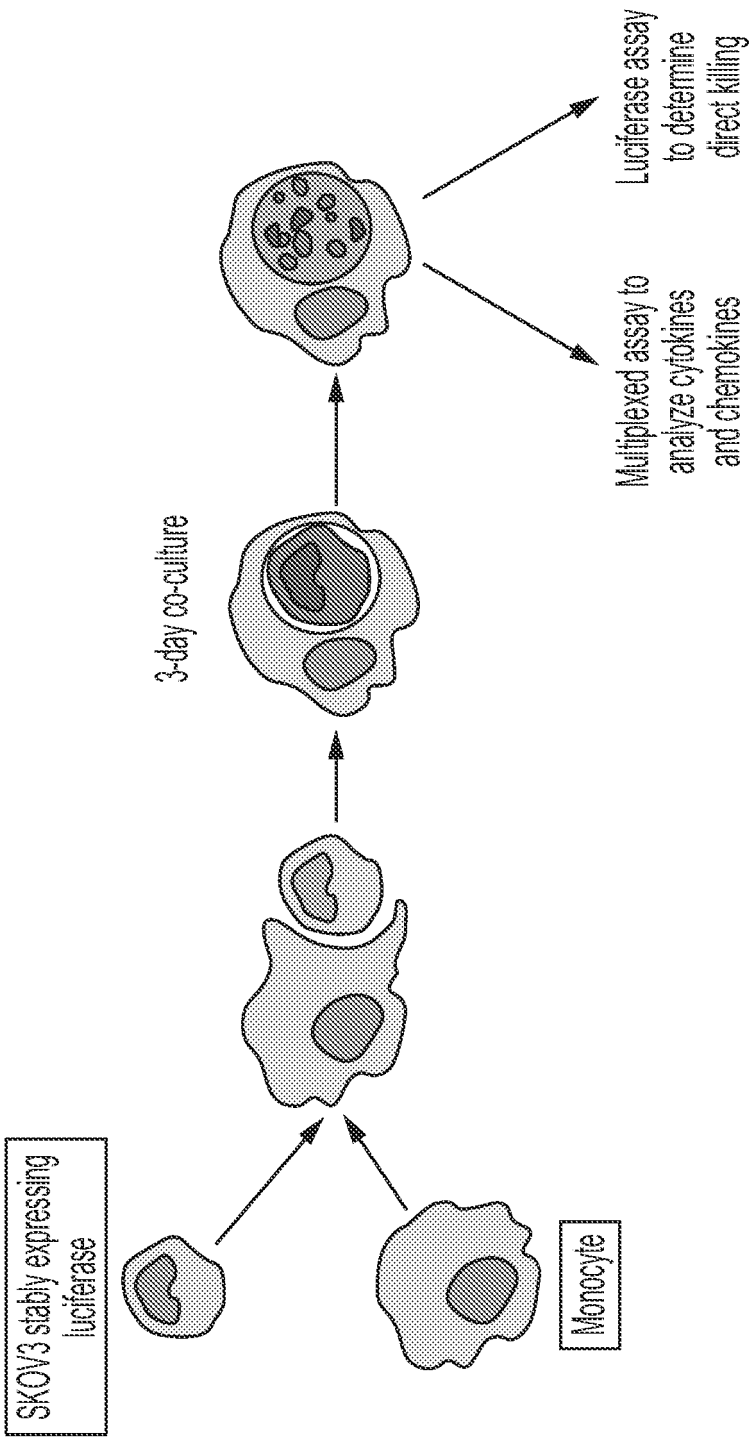

SKOV3 stably expressing luciferase
Monocyte
3-day co-culture
Multiplexed assay to analyze cytokines and chemokines
Luciferase assay to determine direct killing

FIG. 5A

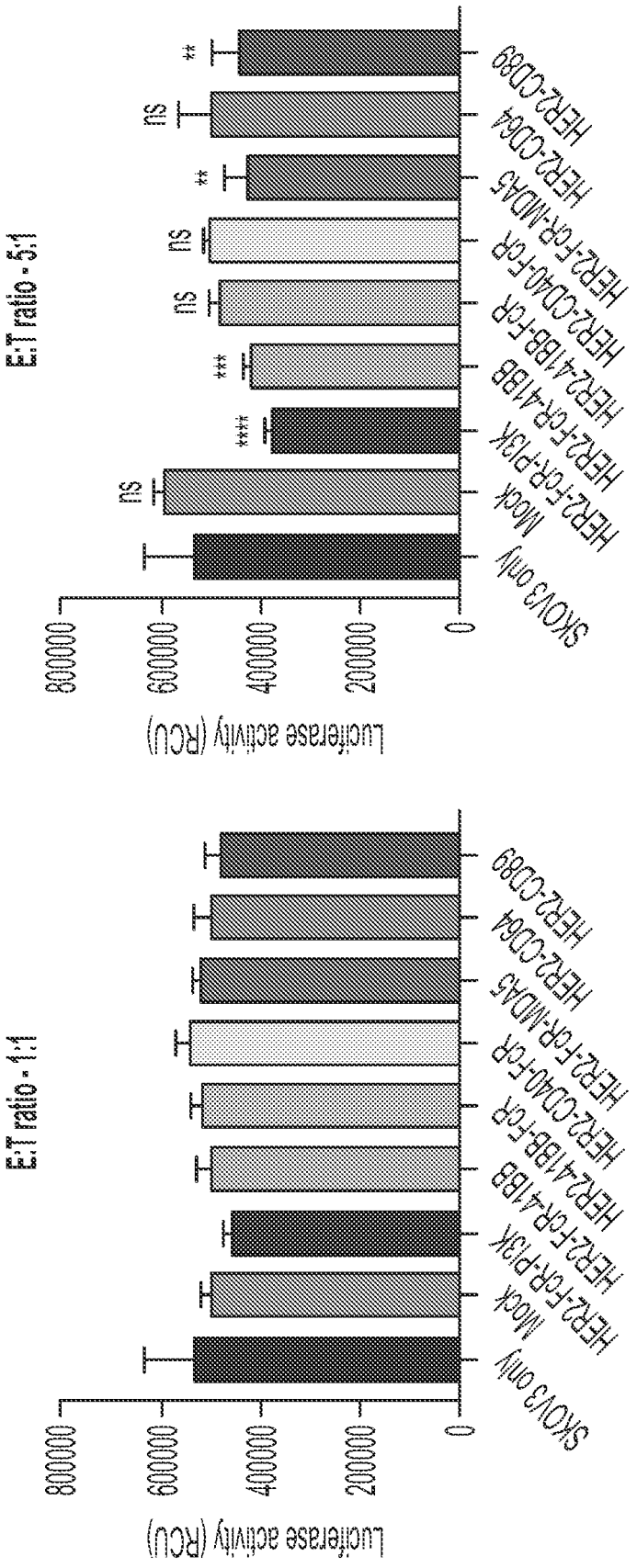

E:T ratio - 5:1
Luciferase activity (RCU)
800000
600000
400000
200000
0
SKOV3 only
Mock
HER2-FCR-P13K
HER2-FCR-4-1BB
HER2-4-1BB-FCR
HER2-CD40-FCR
HER2-FCR-MDA5
HER2-CD64
HER2-CD89
ns
ns
***
****
ns
ns
**
ns
**
E:T ratio - 1:1
Luciferase activity (RCU)
800000
600000
400000
200000
0
SKOV3 only
Mock
HER2-FCR-P13K
HER2-FCR-4-1BB
HER2-4-1BB-FCR
HER2-CD40-FCR
HER2-FCR-MDA5
HER2-CD64
HER2-CD89

FIG. 5B

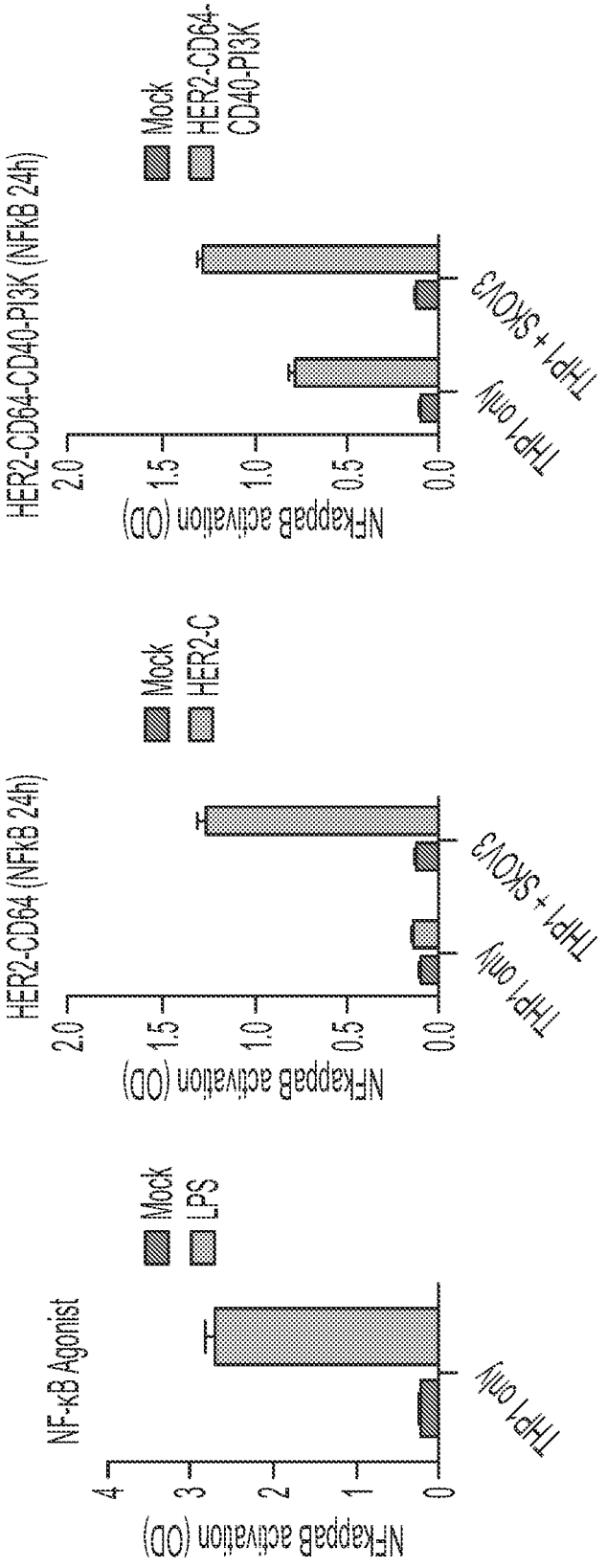

NF-κB Agonist
Mock
LPS
NFkappaB activation (OD)
0
1
2
3
4
THP1 only
HER2-CD64 (NFkB 24h)
Mock
HER2-C
NFkappaB activation (OD)
0.0
0.5
1.0
1.5
2.0
THP1 only
THP1 + SKOV3
HER2-CD64-CD40-PI3K (NFkB 24h)
Mock
HER2-CD64-CD40-PI3K
NFkappaB activation (OD)
0.0
0.5
1.0
1.5
2.0
THP1 only
THP1 + SKOV3

FIG. 5C

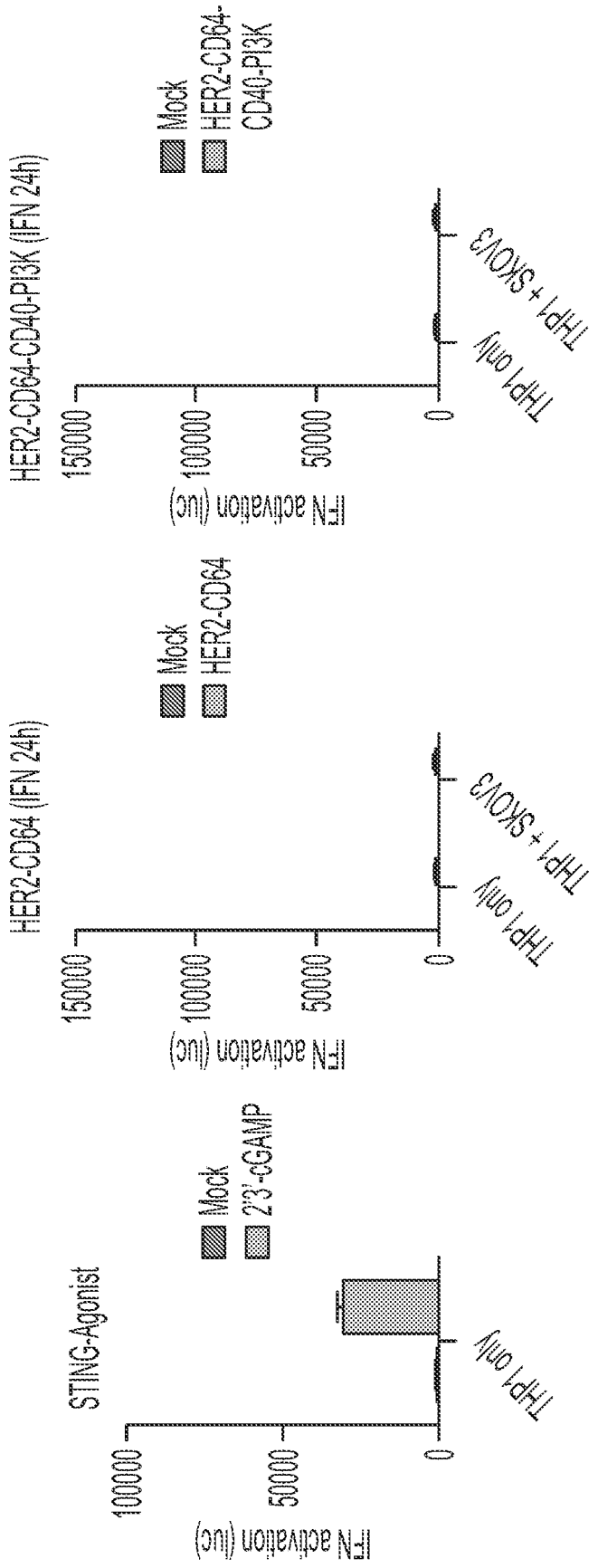
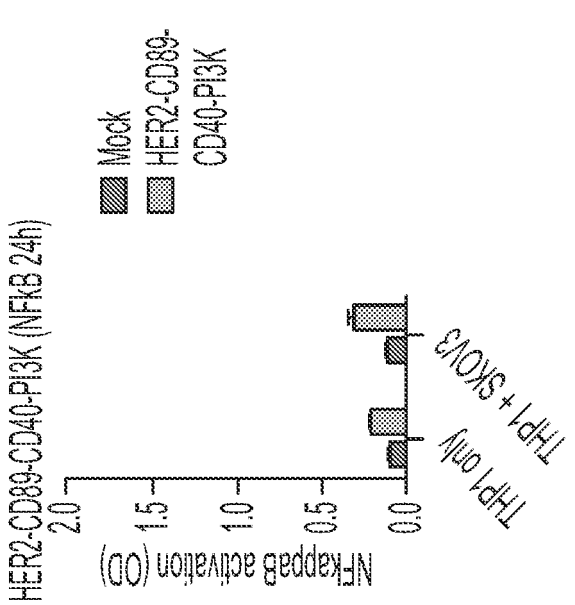

STING-Agonist
IFN activation (luc)
100000
50000
0
THP1 only
Mock
2'3'-cGAMP
HER2-CD64 (IFN 24h)
IFN activation (luc)
150000
100000
50000
0
THP1 only
THP1 + SKOV3
Mock
HER2-CD64
HER2-CD64-CD40-PI3K (IFN 24h)
IFN activation (luc)
150000
100000
50000
0
THP1 only
THP1 + SKOV3
Mock
HER2-CD64-CD40-PI3K

FIG. 5D


HER2-CD89-CD40-PI3K (NFkB 24h)
NFkappaB activation (OD)
2.0
1.5
1.0
0.5
0.0
THP1 only
THP1 + SKOV3
Mock
HER2-CD89-CD40-PI3K

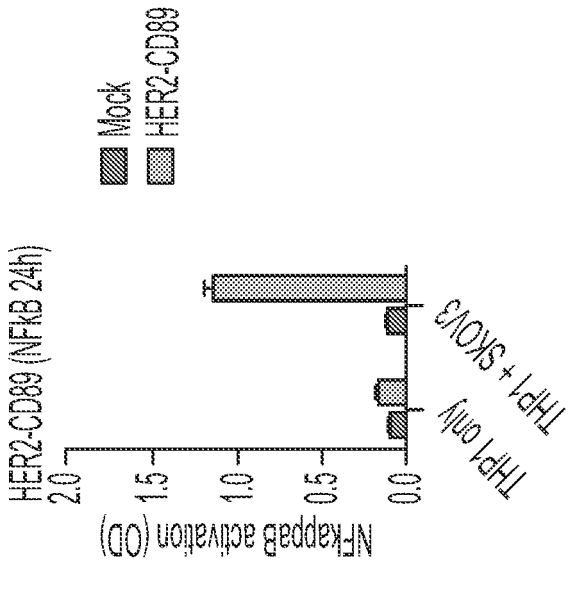
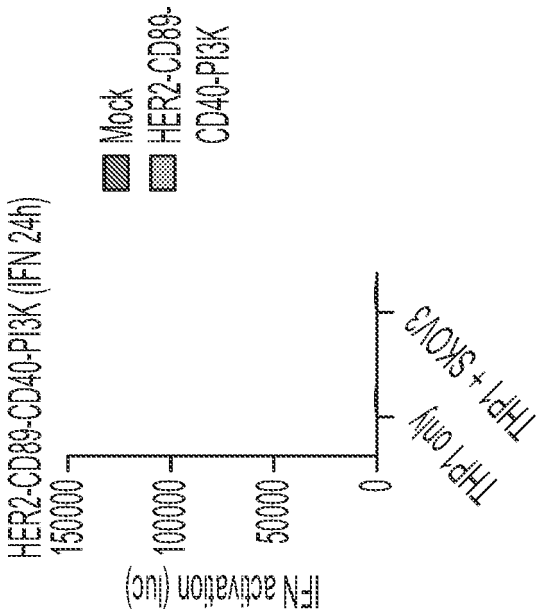

HER2-CD89 (NFkB 24h)
NFkappaB activation (OD)
2.0
1.5
1.0
0.5
0.0
THP1 only
THP1 + SKOV3
Mock
HER2-CD89

FIG. 5E


HER2-CD89-CD40-PI3K (IFN 24h)
IFN activation (luc)
150000
100000
50000
0
THP1 only
THP1 + SKOV3
Mock
HER2-CD89-CD40-PI3K

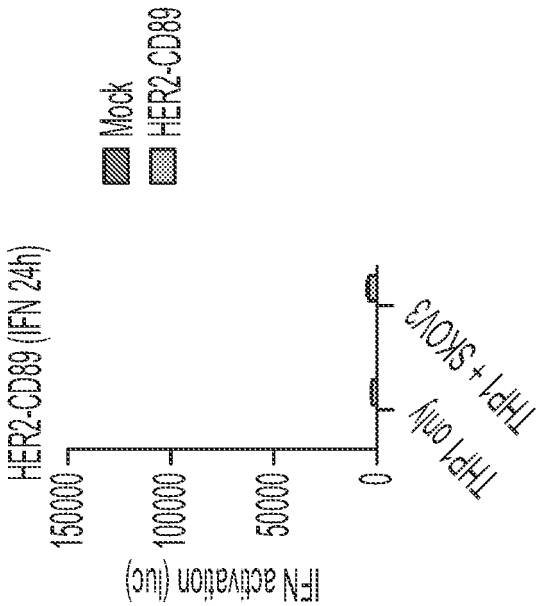

HER2-CD89 (IFN 24h)
IFN activation (luc)
150000
100000
50000
0
THP1 only
THP1 + SKOV3
Mock
HER2-CD89

FIG. 5F

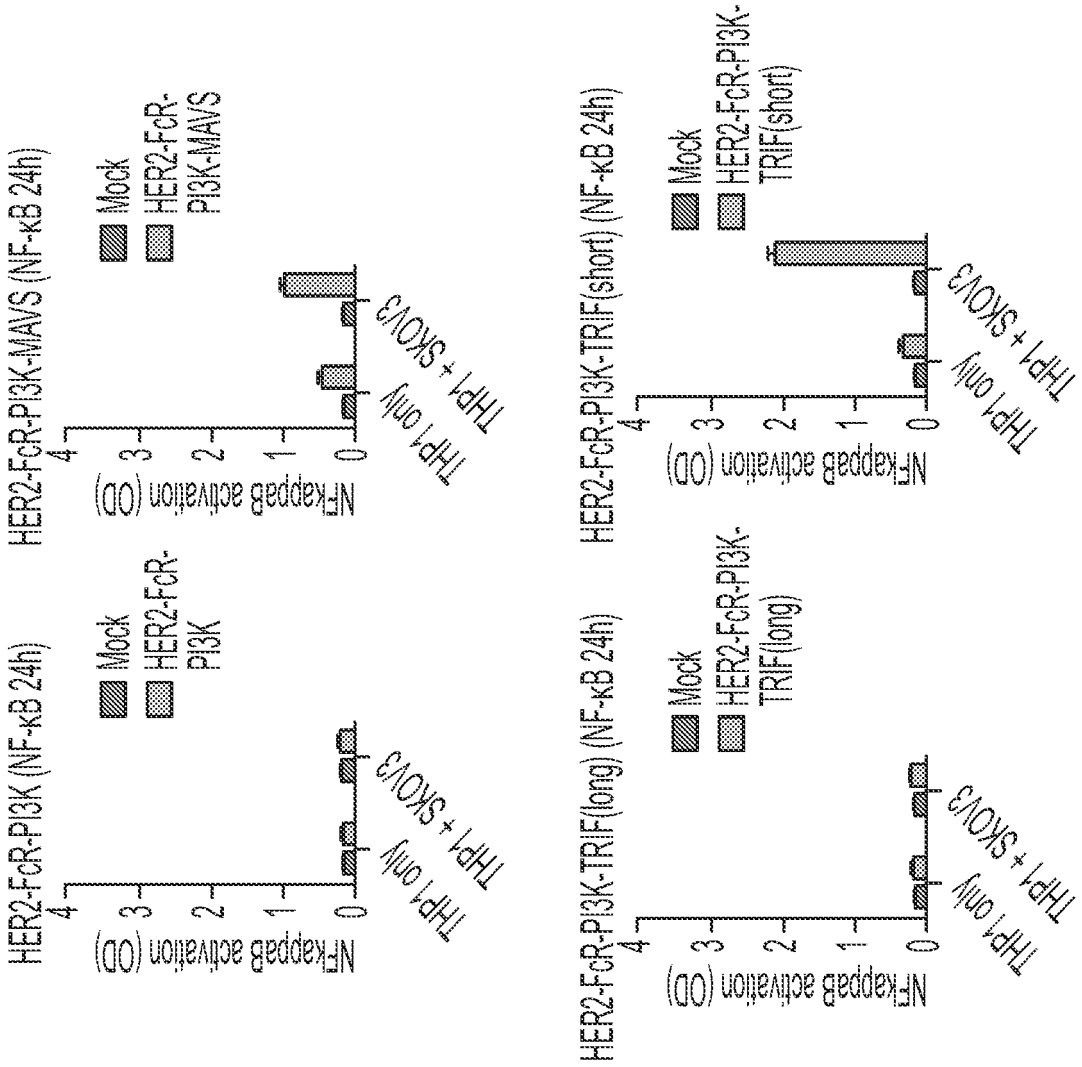

HER2-FcR-PI3K (NF-κB 24h)
HER2-FcR-PI3K-MAVS (NF-κB 24h)
HER2-FcR-PI3K-TRIF(long) (NF-κB 24h)
HER2-FcR-PI3K-TRIF(short) (NF-κB 24h)
Mock
HER2-FcR-PI3K
HER2-FcR-PI3K-MAVS
HER2-FcR-PI3K-TRIF(long)
HER2-FcR-PI3K-TRIF(short)
NFkappaB activation (OD)
0
1
2
3
4
THP1 only
THP1 + SKOV3

FIG. 5G

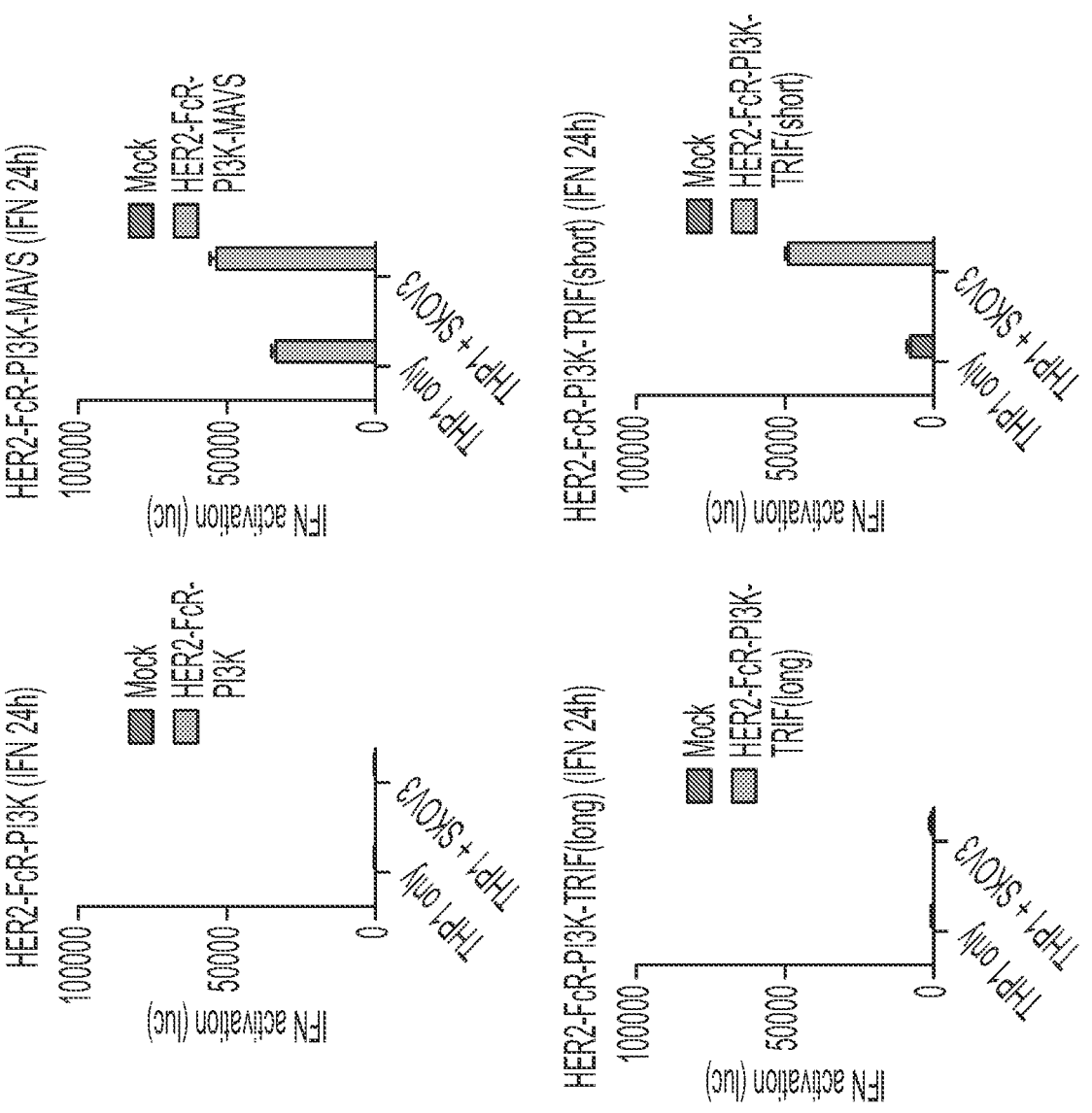

HER2-FcR-PI3K (IFN 24h)
HER2-FcR-PI3K-MAVS (IFN 24h)
HER2-FcR-PI3K-TRIF(long) (IFN 24h)
HER2-FcR-PI3K-TRIF(short) (IFN 24h)
Mock
HER2-FcR-PI3K
HER2-FcR-PI3K-MAVS
HER2-FcR-PI3K-TRIF(long)
HER2-FcR-PI3K-TRIF(short)
IFN activation (luc)
100000
50000
0
THP1 only
THP1 + SKOV3

FIG. 5H

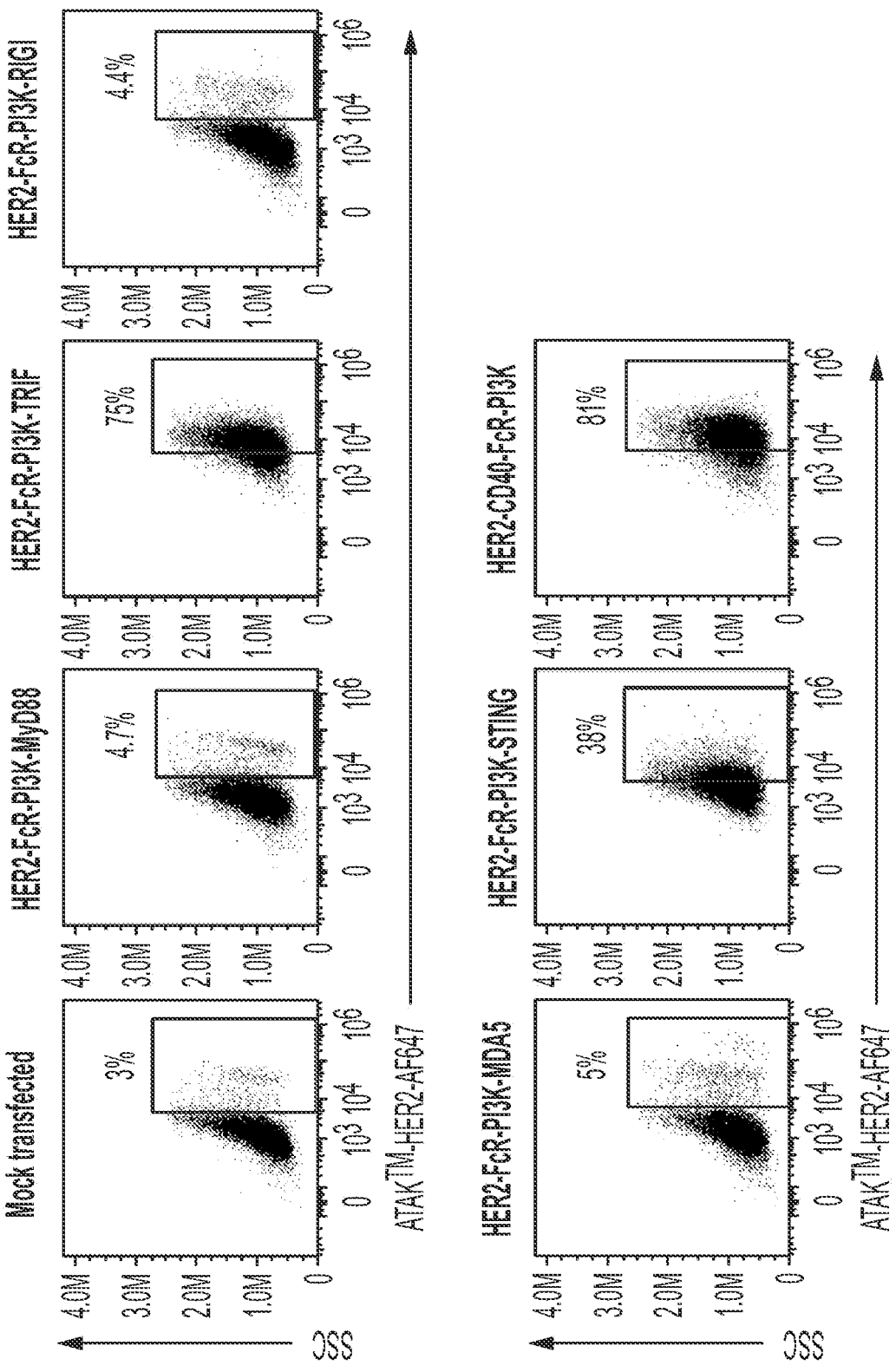

Mock transfected
HER2-FcR-PI3K-MyD88
HER2-FcR-PI3K-TRIF
HER2-FcR-PI3K-RIGI
HER2-FcR-PI3K-MDA5
HER2-FcR-PI3K-STING
HER2-CD40-FcR-PI3K
3%
4.7%
75%
4.4%
5%
38%
81%
SSC
ATAK™-HER2-AF647
4.0M
3.0M
2.0M
1.0M
0
10^3
10^4
10^6

FIG. 5I

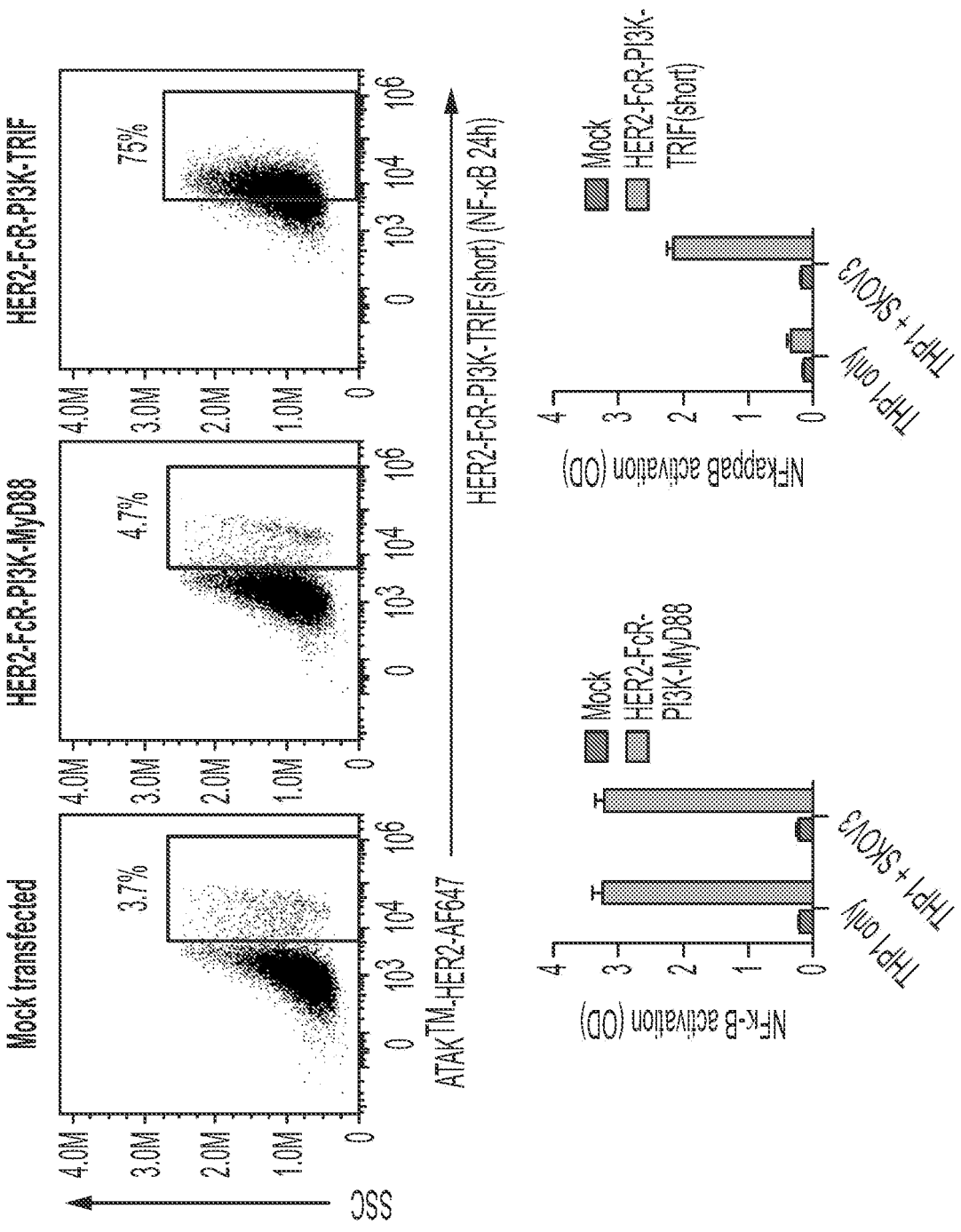

Mock transfected
HER2-FcR-PI3K-MyD88
HER2-FcR-PI3K-TRIF
3.7%
4.7%
75%
SSC
ATAK TM-HER2-AF647
HER2-FcR-PI3K-TRIF(short) (NF-κB 24h)
Mock
HER2-FcR-PI3K-MyD88
HER2-FcR-PI3K-TRIF(short)
NF-κ-B activation (OD)
NFkappaB activation (OD)
THP1 only
THP1 + SKOV3

FIG. 5J

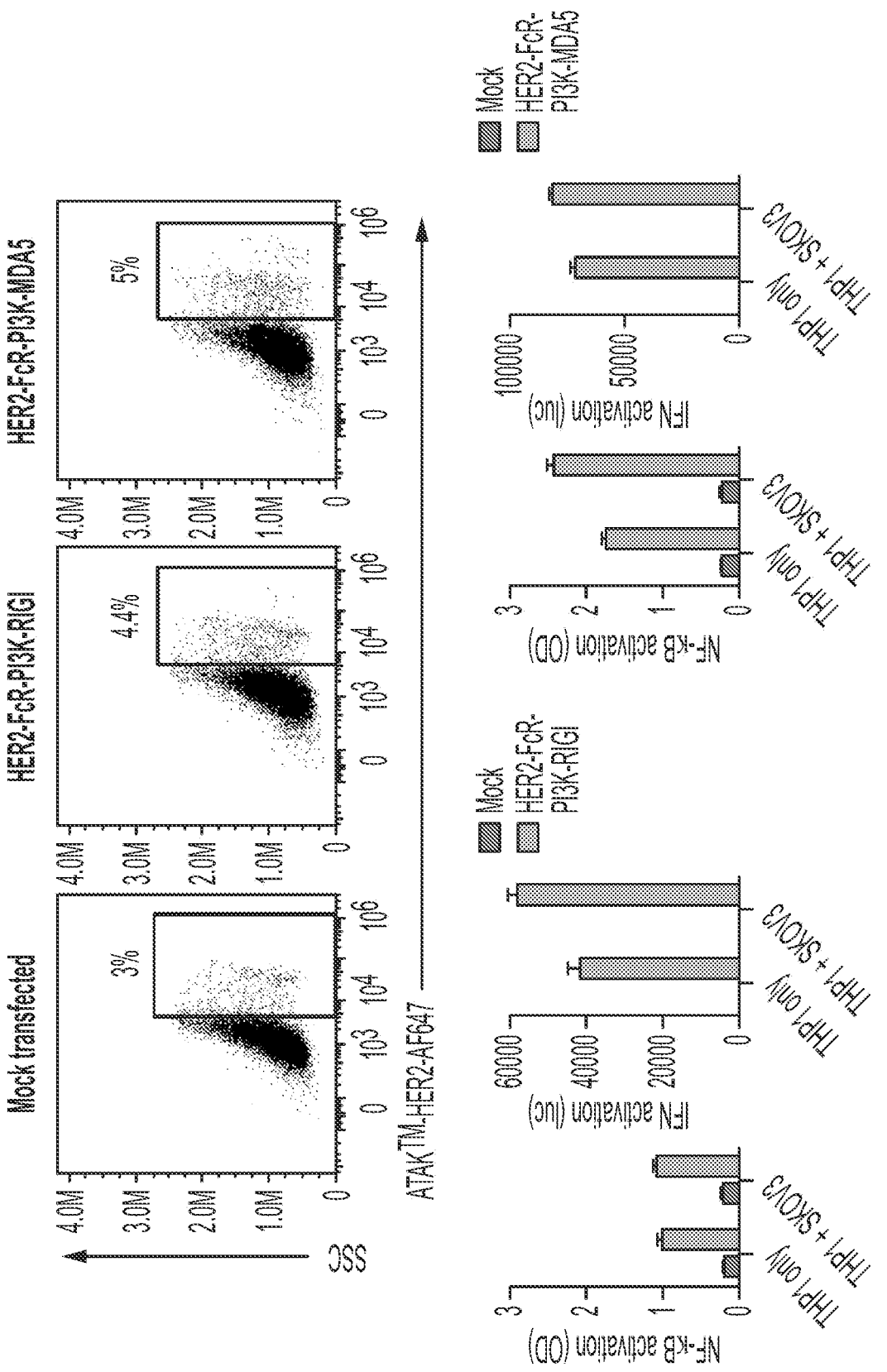

Mock transfected
HER2-FcR-PI3K-RIGI
HER2-FcR-PI3K-MDA5
SSC
ATAK™ HER2-AF647
3%
4.4%
5%
Mock
HER2-FcR-PI3K-RIGI
HER2-FcR-PI3K-MDA5
NF-κB activation (OD)
IFN activation (luc)
THP1 only
THP1 + SKOV3

FIG. 5K

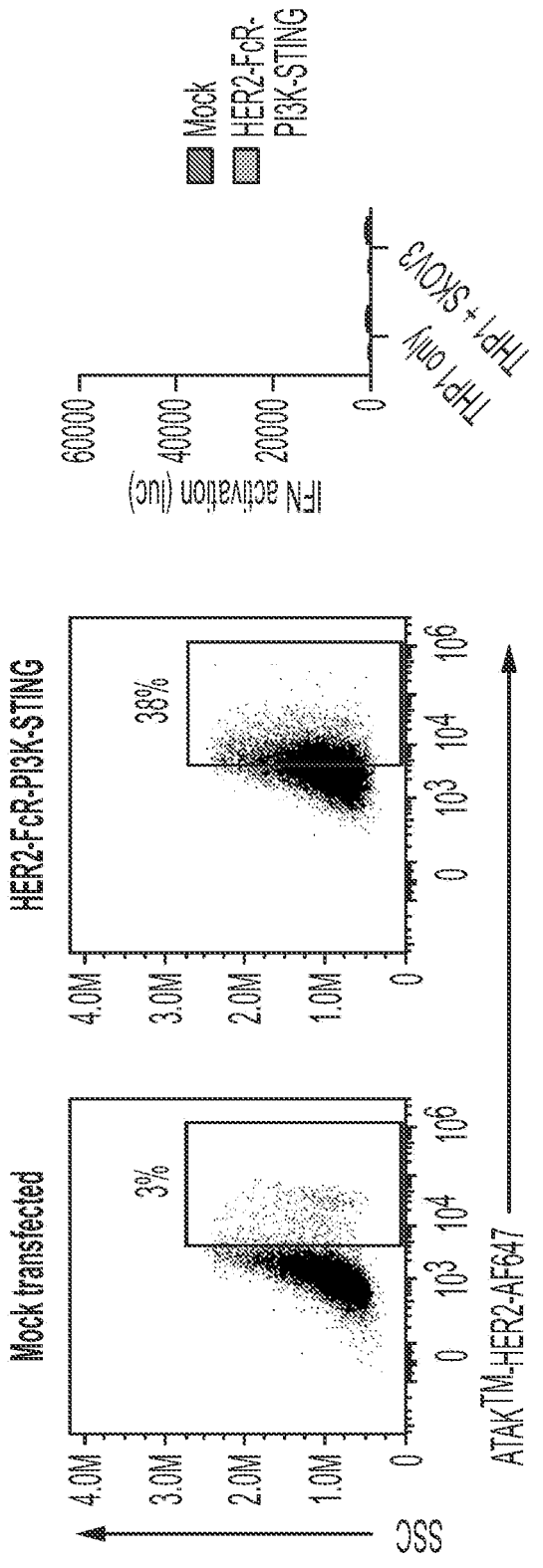

Mock transfected
HER2-FcR-PI3K-STING
SSC
ATAK™ HER2-AF647
3%
38%
4.0M
3.0M
2.0M
1.0M
0
10^3
10^4
10^6
IFN activation (luc)
60000
40000
20000
0
THP1 only
THP1 + SKOV3
Mock
HER2-FcR-PI3K-STING

FIG. 5L

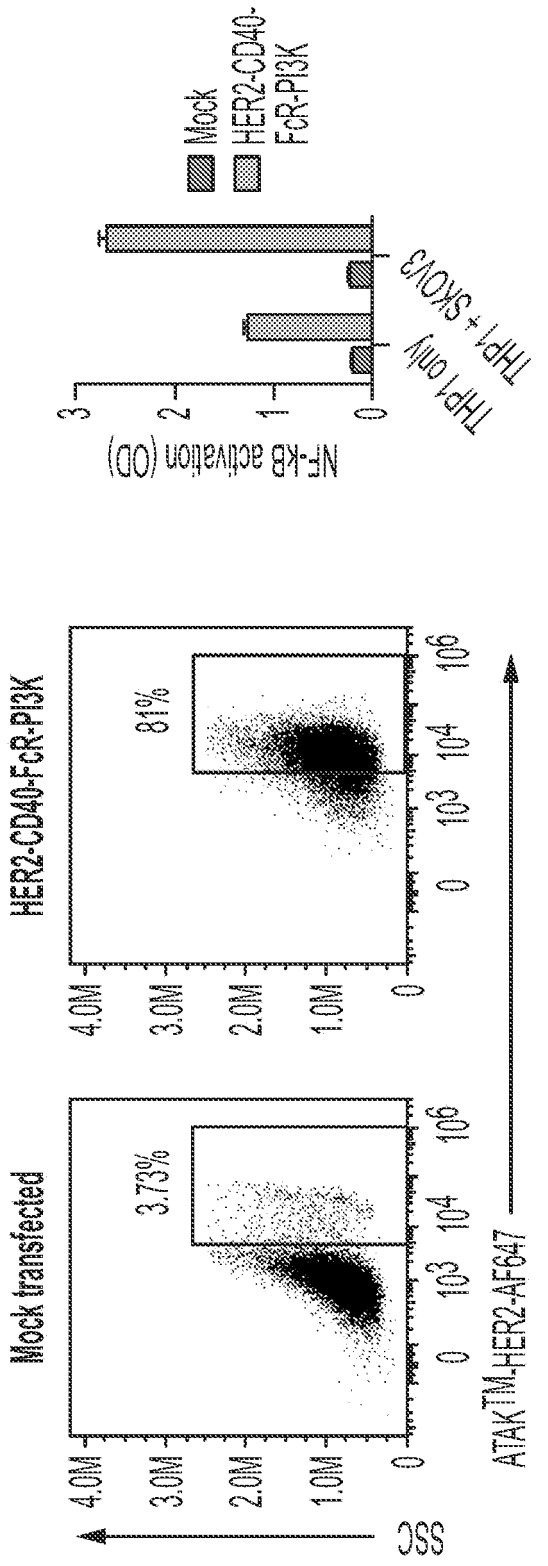

Mock transfected
HER2-CD40-FcR-PI3K
SSC
4.0M
3.0M
2.0M
1.0M
0
3.73%
81%
0
10^3
10^4
10^6
ATAK™-HER2-AF647
NF-κB activation (OD)
3
2
1
0
THP1 only
THP1 + SKOV3
Mock
HER2-CD40-FcR-PI3K

FIG. 5M

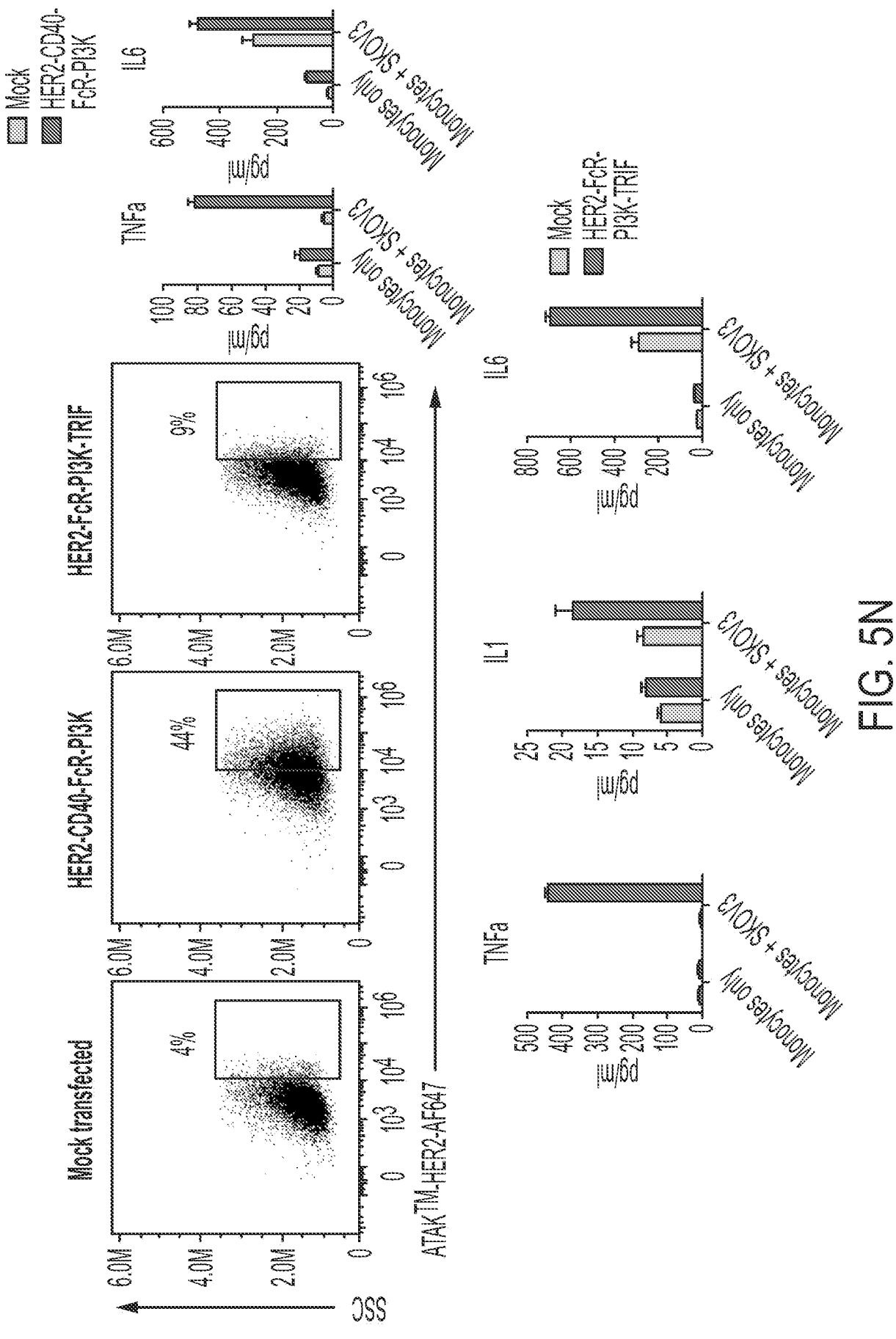

Mock transfected
HER2-CD40-FcR-PI3K
HER2-FcR-PI3K-TRIF
SSC
ATAK TM-HER2-AF647
4%
44%
9%
6.0M
4.0M
2.0M
0
10^3
10^4
10^6
Mock
HER2-CD40-FcR-PI3K
HER2-FcR-PI3K-TRIF
IL6
TNFa
IL1
pg/ml
Monocytes only
Monocytes + SKOV3

FIG. 5N

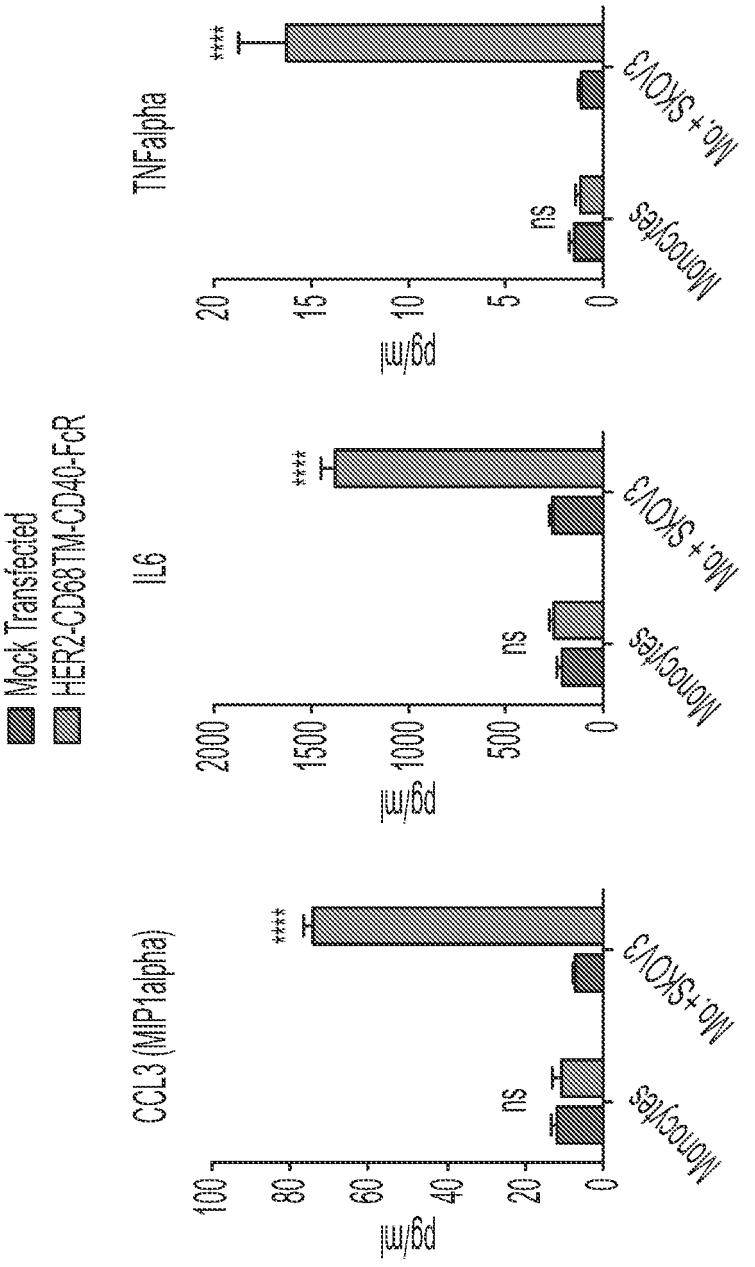

Mock Transfected
HER2-CD68TM-CD40-FcR
CCL3 (MIP1alpha)
IL6
TNFalpha
pg/ml
100
80
60
40
20
0
2000
1500
1000
500
15
10
5
ns
****
Monocytes
Mo.+SKOV3

FIG. 6A

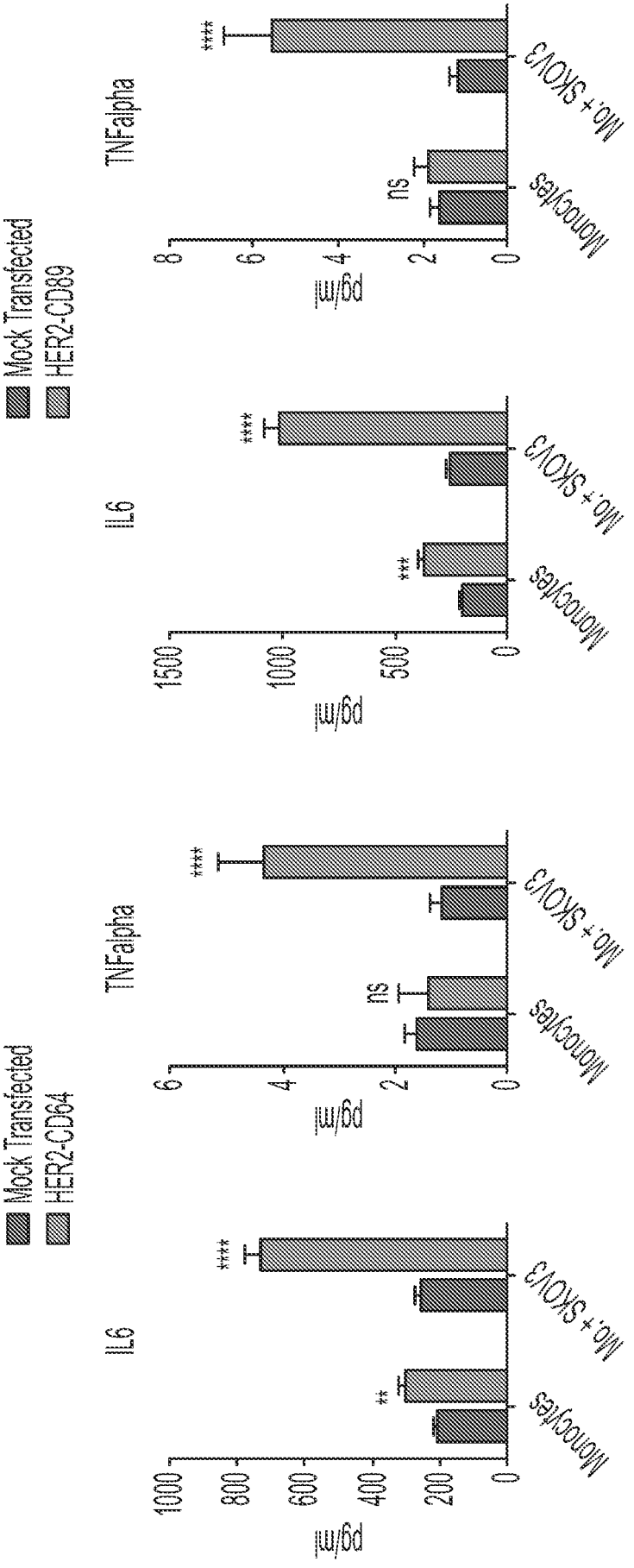

Mock Transfected
HER2-CD64
IL6
TNFalpha
pg/ml
Monocytes
Mo.+SKOV3
ns
**
***
****

FIG. 6B

Mock Transfected
HER2-CD89
IL6
TNFalpha
pg/ml
Monocytes
Mo.+SKOV3
ns
***
****

FIG. 6C

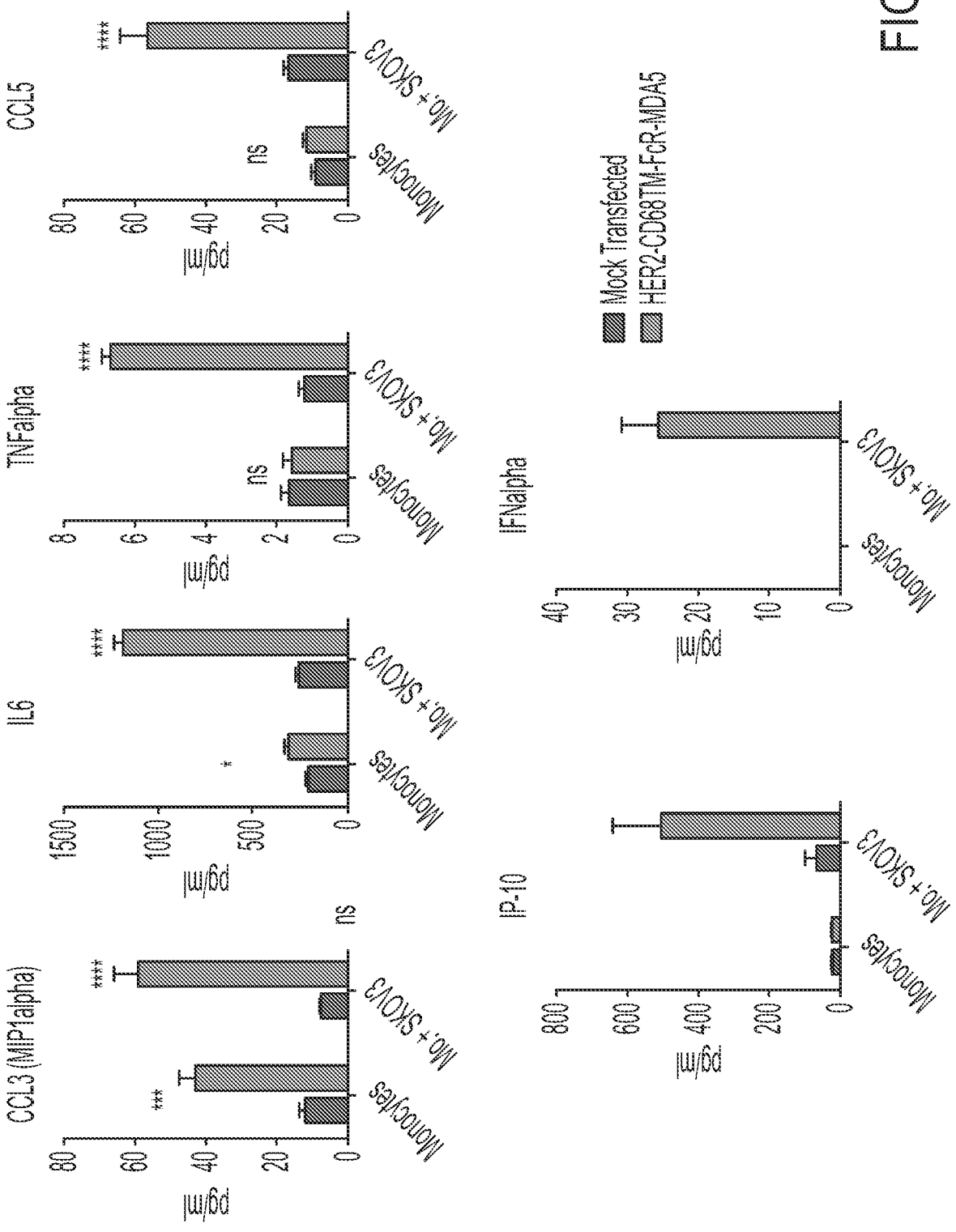

CCL3 (MIP1alpha)
IL6
TNFalpha
CCL5
IFNalpha
IP-10
pg/ml
Monocytes
Mo.+SKOV3
Mock Transfected
HER2-CD68TM-FcR-MDA5
ns
*
***
****

FIG. 6D

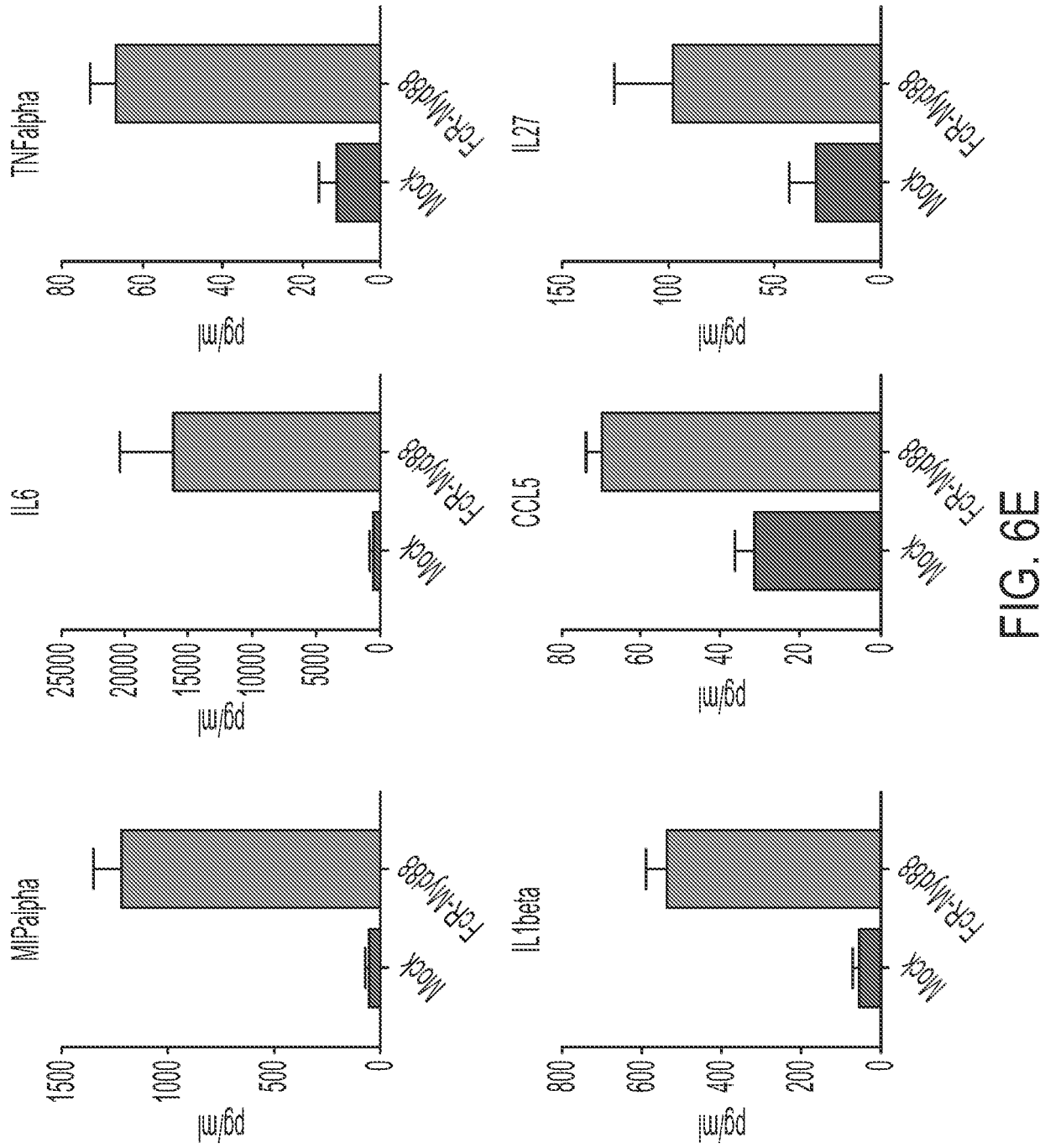

TNFalpha
IL27
IL6
CCL5
MIPalpha
IL1beta
pg/ml
Mock
FCR-Myd88
0
20
40
60
80
50
100
150
5000
10000
15000
20000
25000
200
400
600
800
500
1000
1500

FIG. 6E

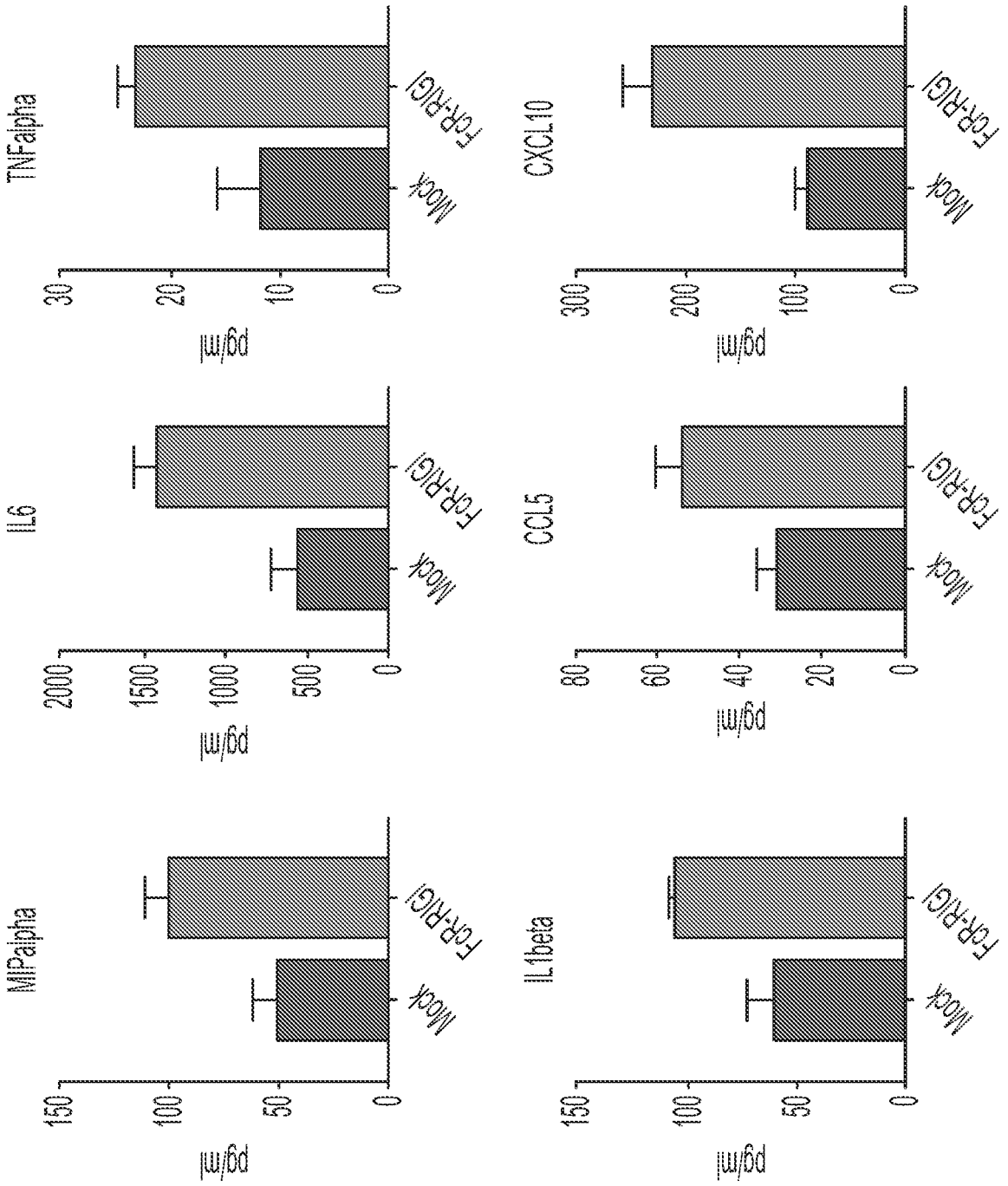
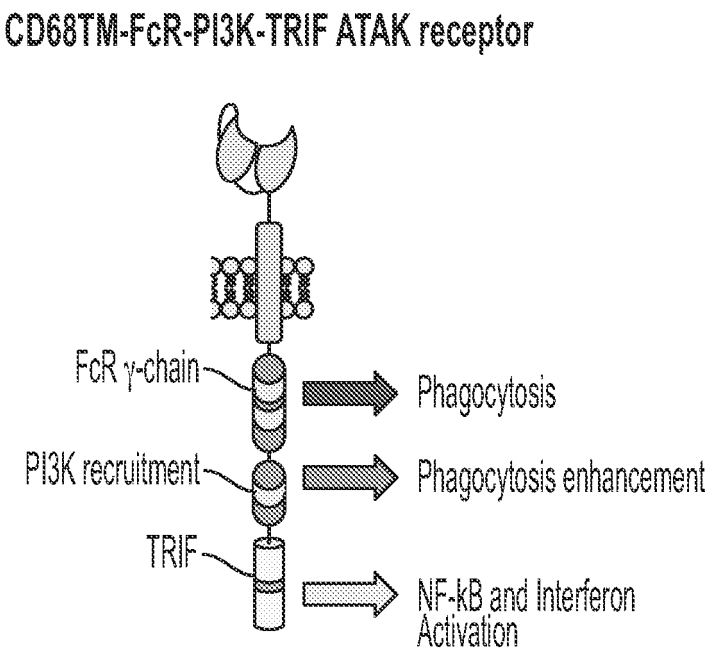

TNFalpha
CXCL10
IL6
CCL5
MIPalpha
IL1beta
pg/ml
Mock
FCR-RIG1
0
10
20
30
100
200
300
500
1000
1500
2000
20
40
60
80
50
100
150

FIG. 6F


CD68TM-FcR-PI3K-TRIF ATAK receptor
FcR γ-chain
PI3K recruitment
TRIF
Phagocytosis
Phagocytosis enhancement
NF-kB and Interferon Activation

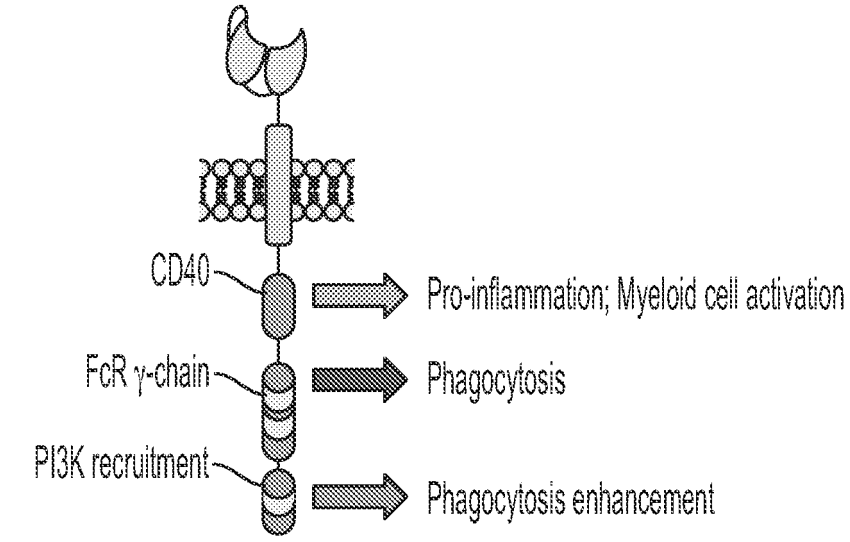

CD68TM-CD40-FcR-PI3K ATAK receptor
CD40
FcR γ-chain
PI3K recruitment
Pro-inflammation; Myeloid cell activation
Phagocytosis
Phagocytosis enhancement

FIG. 7A

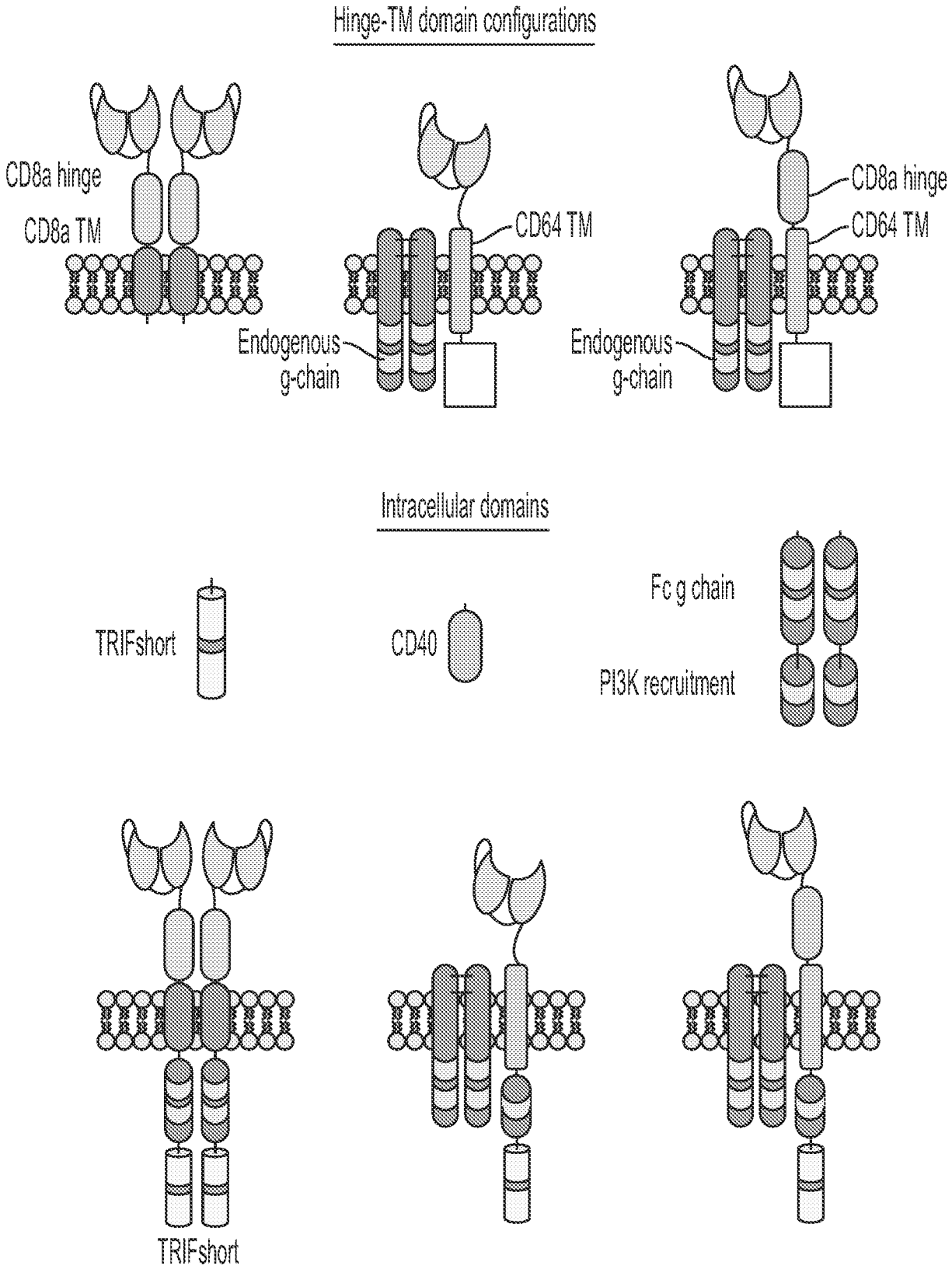

Hinge-TM domain configurations
CD8a hinge
CD8a TM
CD64 TM
Endogenous g-chain
CD8a hinge
CD64 TM
Endogenous g-chain
Intracellular domains
TRIFshort
CD40
Fc g chain
PI3K recruitment
TRIFshort

FIG. 7B

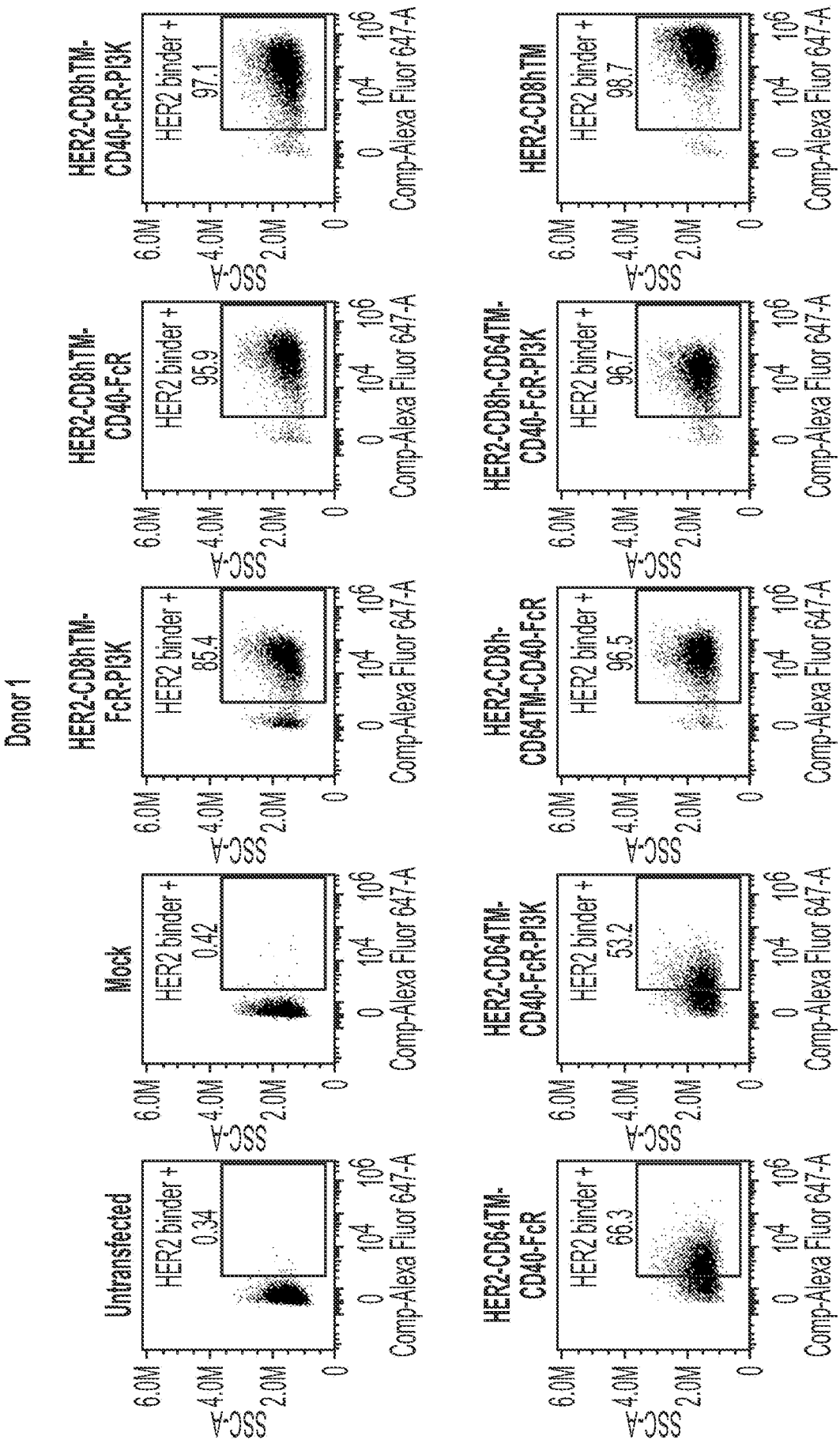

Donor 1
Untransfected
Mock
HER2-CD8hTM-FcR-PI3K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-PI3K
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-PI3K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-PI3K
HER2-CD8hTM
HER2 binder +
0.34
0.42
85.4
95.9
97.1
66.3
53.2
96.5
96.7
98.7
SSC-A
Comp-Alexa Fluor 647-A
6.0M
4.0M
2.0M
0
10^4
10^5
10^6

FIG. 8A

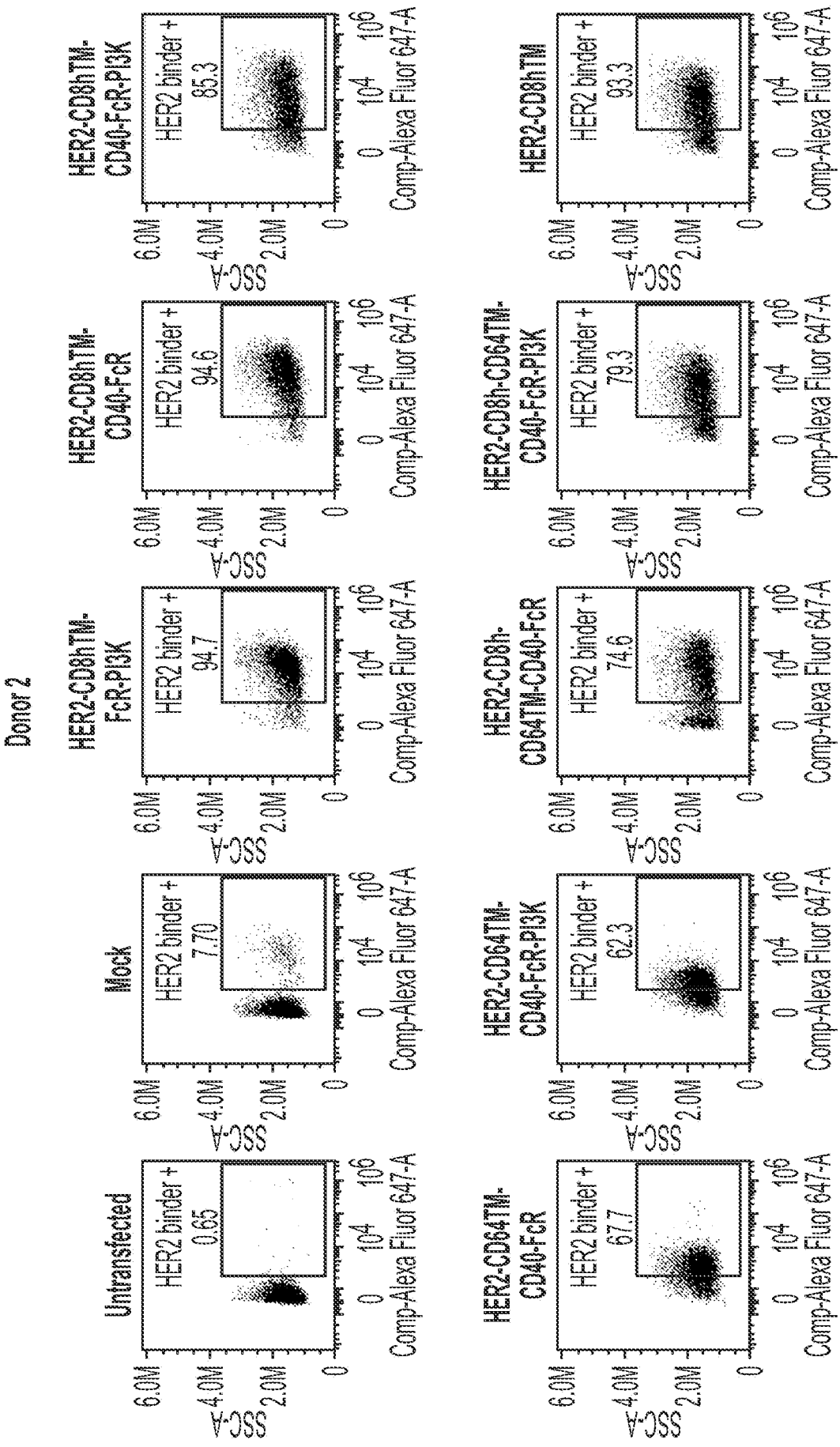

Donor 2
Untransfected
HER2 binder +
0.65
Mock
HER2 binder +
7.70
HER2-CD8hTM-FcR-PI3K
HER2 binder +
94.7
HER2-CD8hTM-CD40-FcR
HER2 binder +
94.6
HER2-CD8hTM-CD40-FcR-PI3K
HER2 binder +
85.3
HER2-CD64TM-CD40-FcR
HER2 binder +
67.7
HER2-CD64TM-CD40-FcR-PI3K
HER2 binder +
62.3
HER2-CD8h-CD64TM-CD40-FcR
HER2 binder +
74.6
HER2-CD8h-CD64TM-CD40-FcR-PI3K
HER2 binder +
79.3
HER2-CD8hTM
HER2 binder +
93.3
SSC-A
6.0M
4.0M
2.0M
0
0
10^4
10^5
10^6
Comp-Alexa Fluor 647-A

FIG. 8B

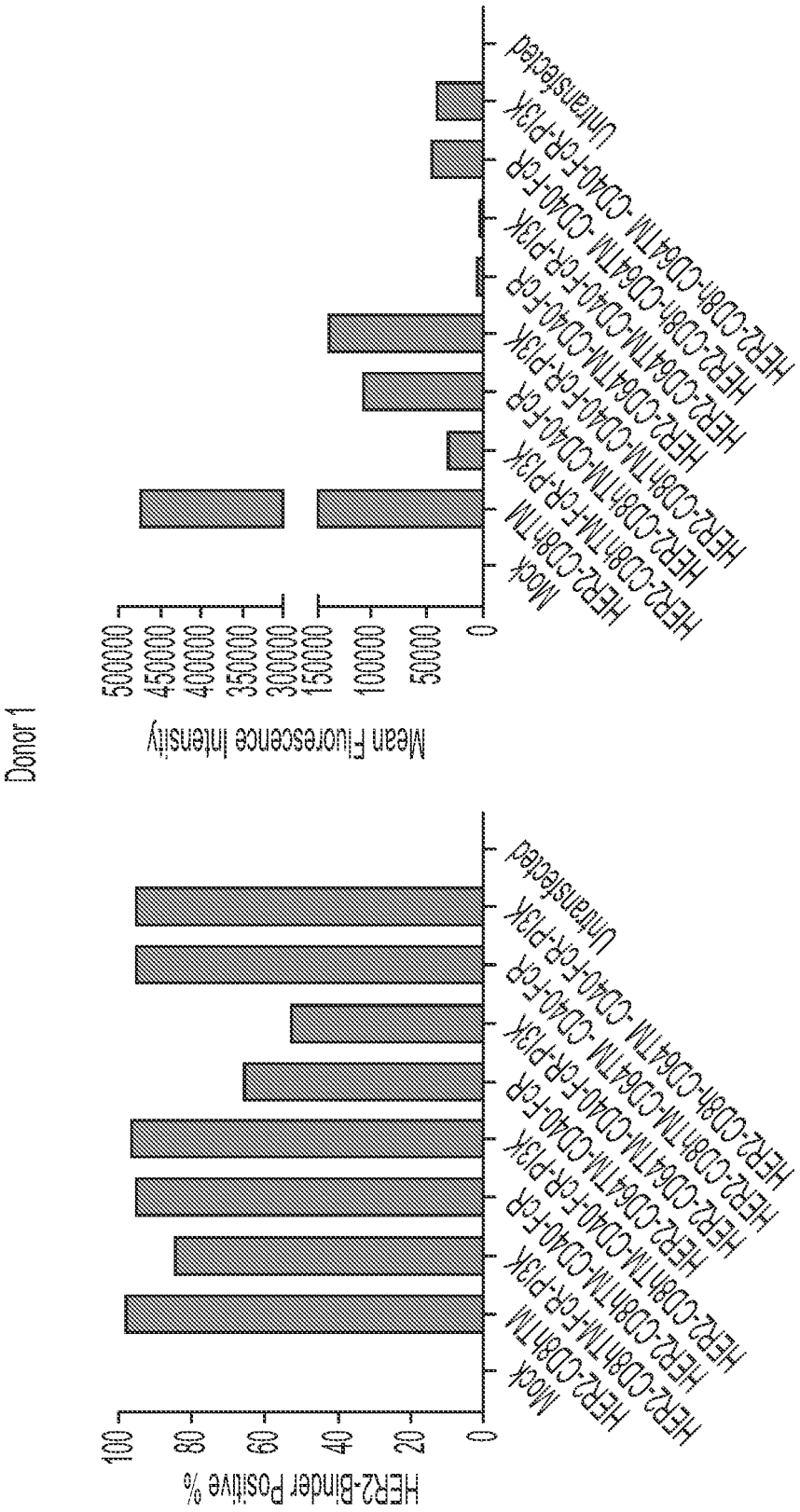

Donor 1
Mean Fluorescence Intensity
500000
450000
400000
350000
300000
150000
100000
50000
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FcR-P13K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-P13K
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-P13K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-P13K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-P13K
Untranstected
HER2-Binder Positive %
100
80
60
40
20
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FcR-P13K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-P13K
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-P13K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-P13K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-P13K
Untranstected

FIG. 8C

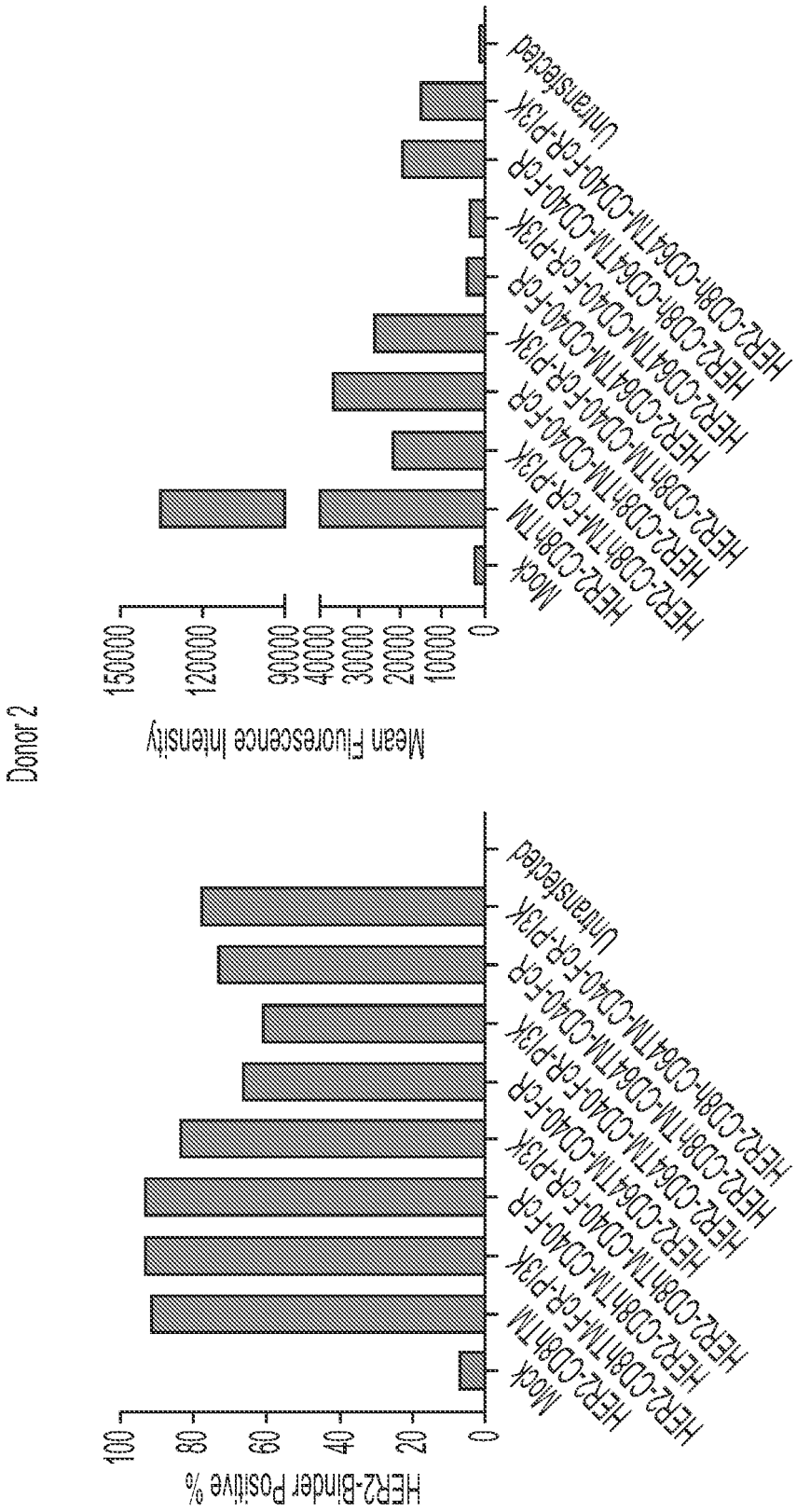
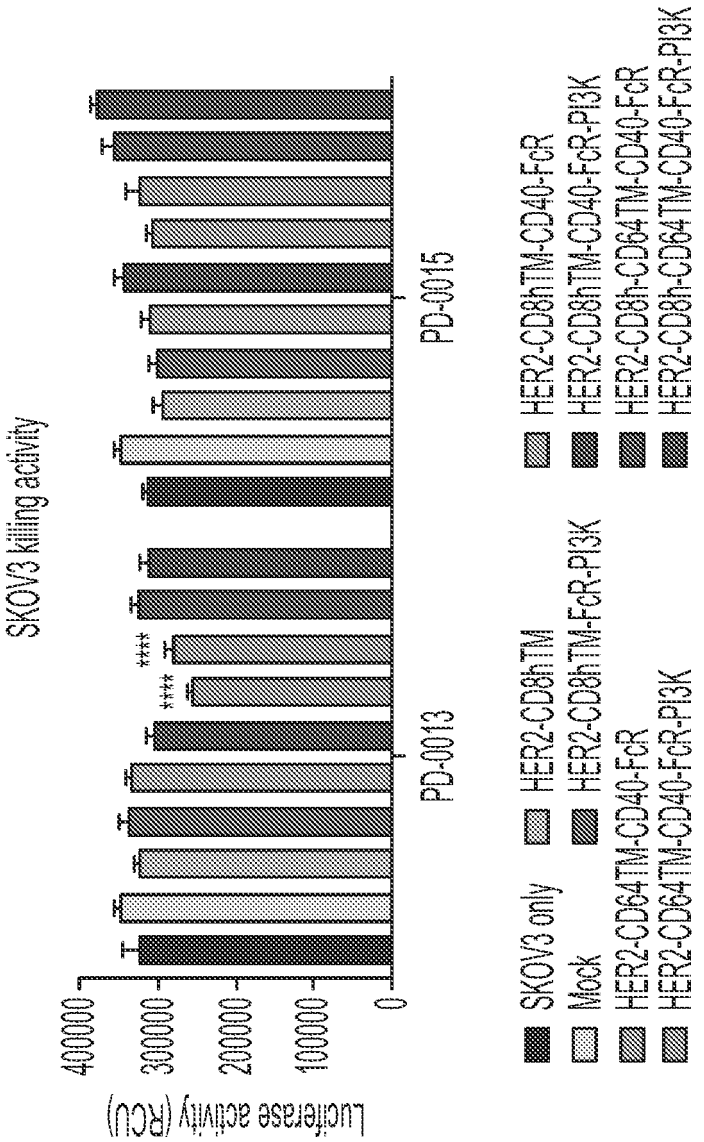


FIG. 8C
CONTINUED


SKOV3 killing activity
Luciferase activity (RCU)
400000
300000
200000
100000
0
PD-0013
PD-0015
****
****
SKOV3 only
Mock
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-PI3K
HER2-CD8hTM
HER2-CD8hTM-FcR-PI3K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-PI3K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-PI3K

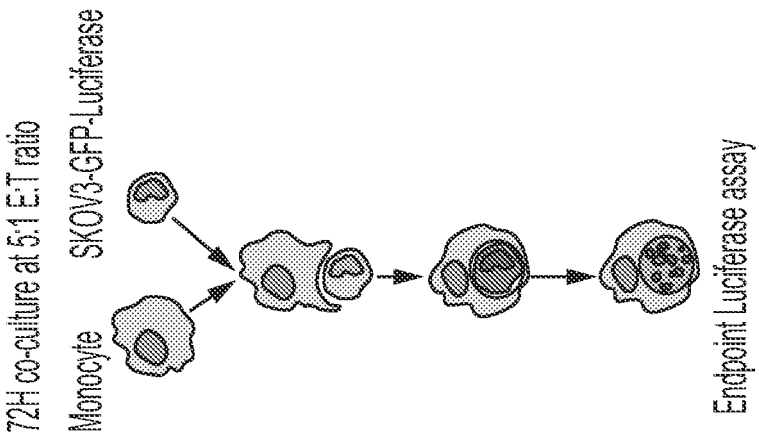

72H co-culture at 5:1 E:T ratio
Monocyte
SKOV3-GFP-Luciferase
Endpoint Luciferase assay

FIG. 9A

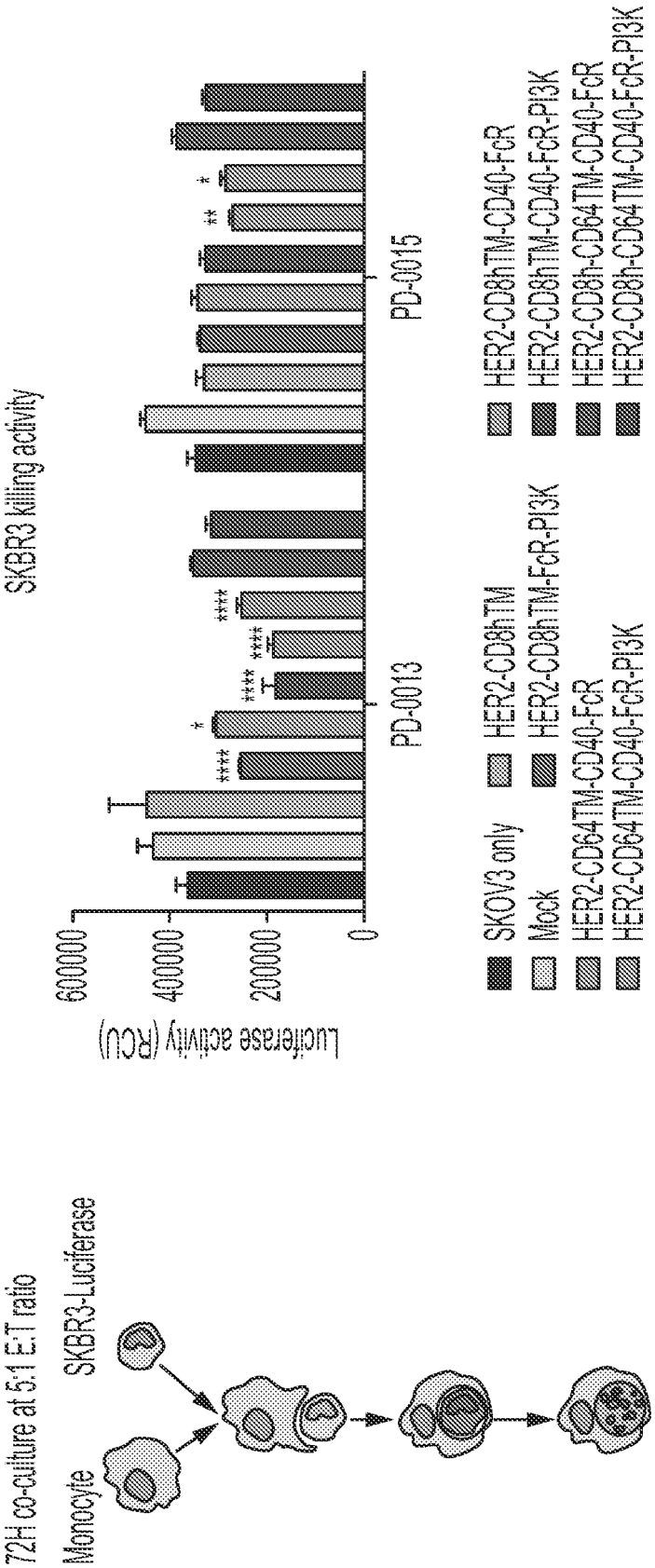

72H co-culture at 5:1 E:T ratio
Monocyte
SKBR3-Luciferase
Endpoint Luciferase assay
SKBR3 killing activity
Luciferase activity (RCU)
600000
400000
200000
0
PD-0013
PD-0015
SKOV3 only
Mock
HER2-CD64TM-CD40-FcR
HER2-CD64TM-CD40-FcR-PI3K
HER2-CD8hTM
HER2-CD8hTM-FcR-PI3K
HER2-CD8hTM-CD40-FcR
HER2-CD8hTM-CD40-FcR-PI3K
HER2-CD8h-CD64TM-CD40-FcR
HER2-CD8h-CD64TM-CD40-FcR-PI3K

FIG. 9B

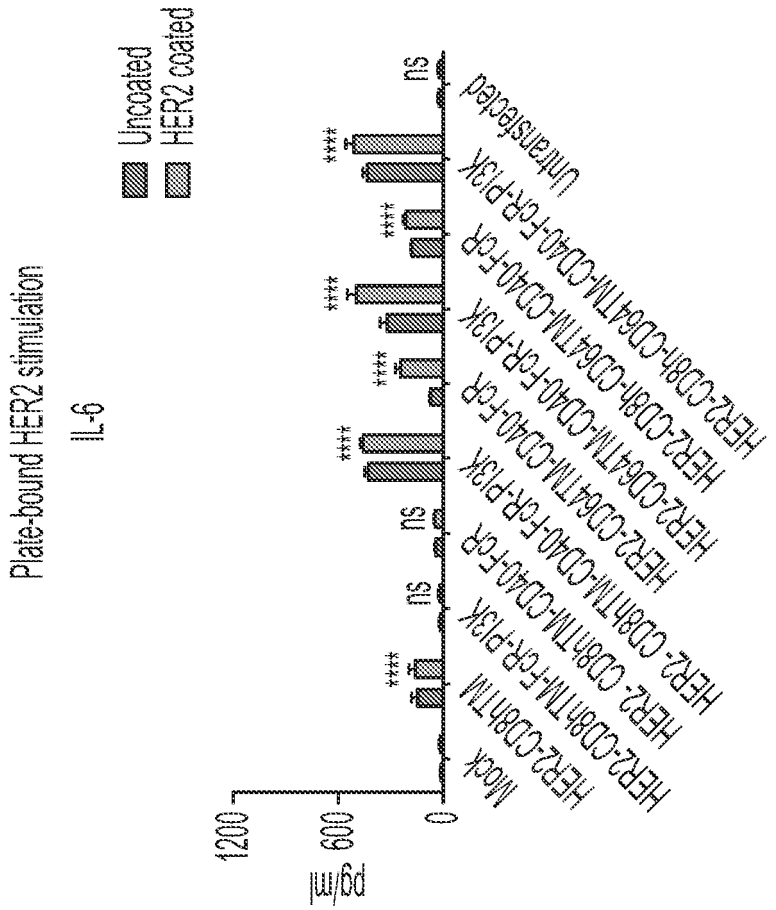

Plate-bound HER2 stimulation
IL-6
Uncoated
HER2 coated
pg/ml
1200
600
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected
ns
ns
ns
****
****
****
****
****

FIG. 10A

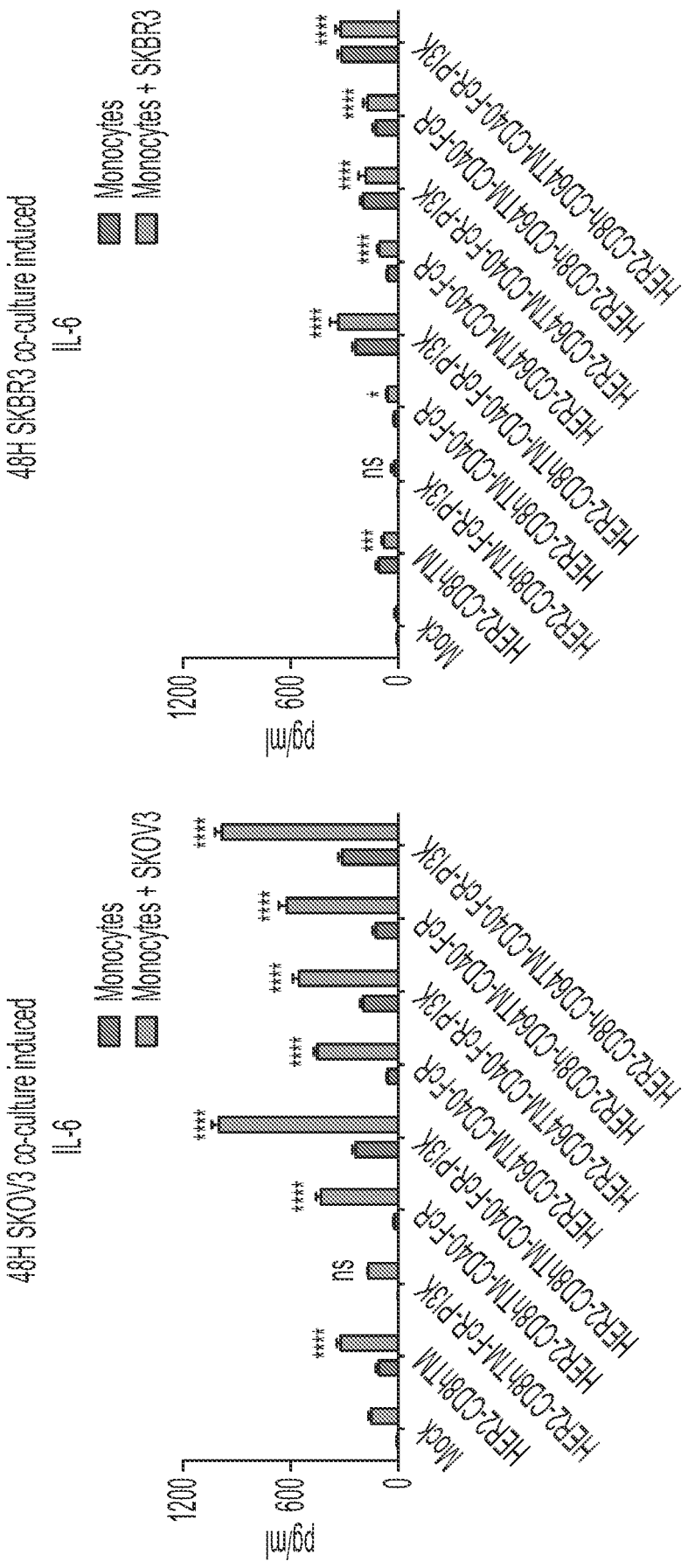


FIG. 10A
CONTINUED

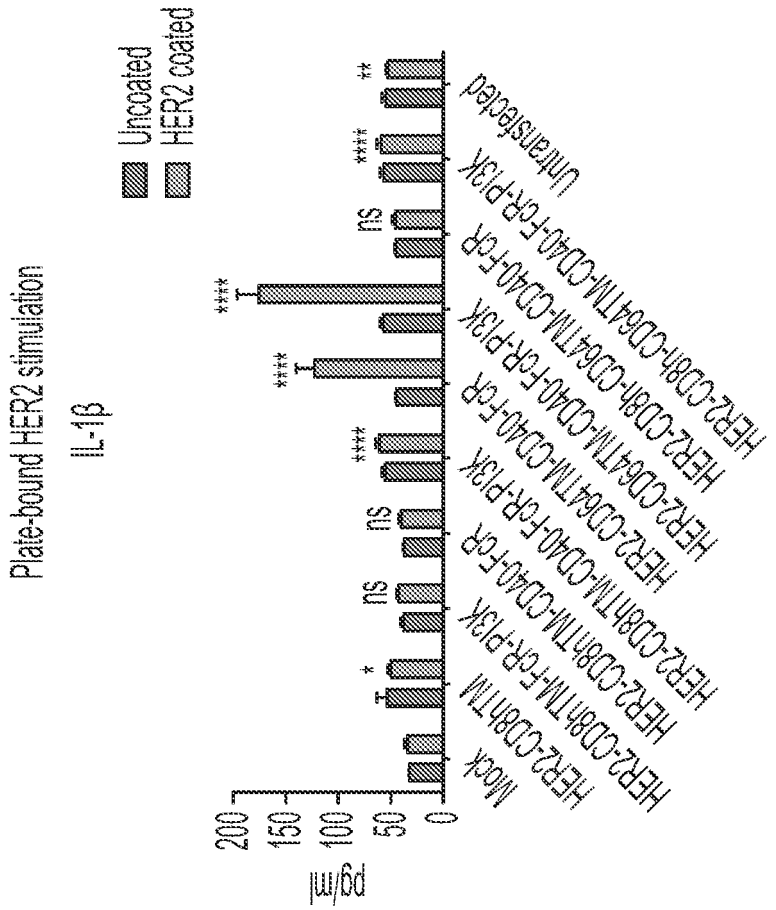

Plate-bound HER2 stimulation
IL-1β
Uncoated
HER2 coated
pg/ml
200
150
100
50
0
*
ns
ns
****
****
****
ns
****
**
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected

FIG. 10B

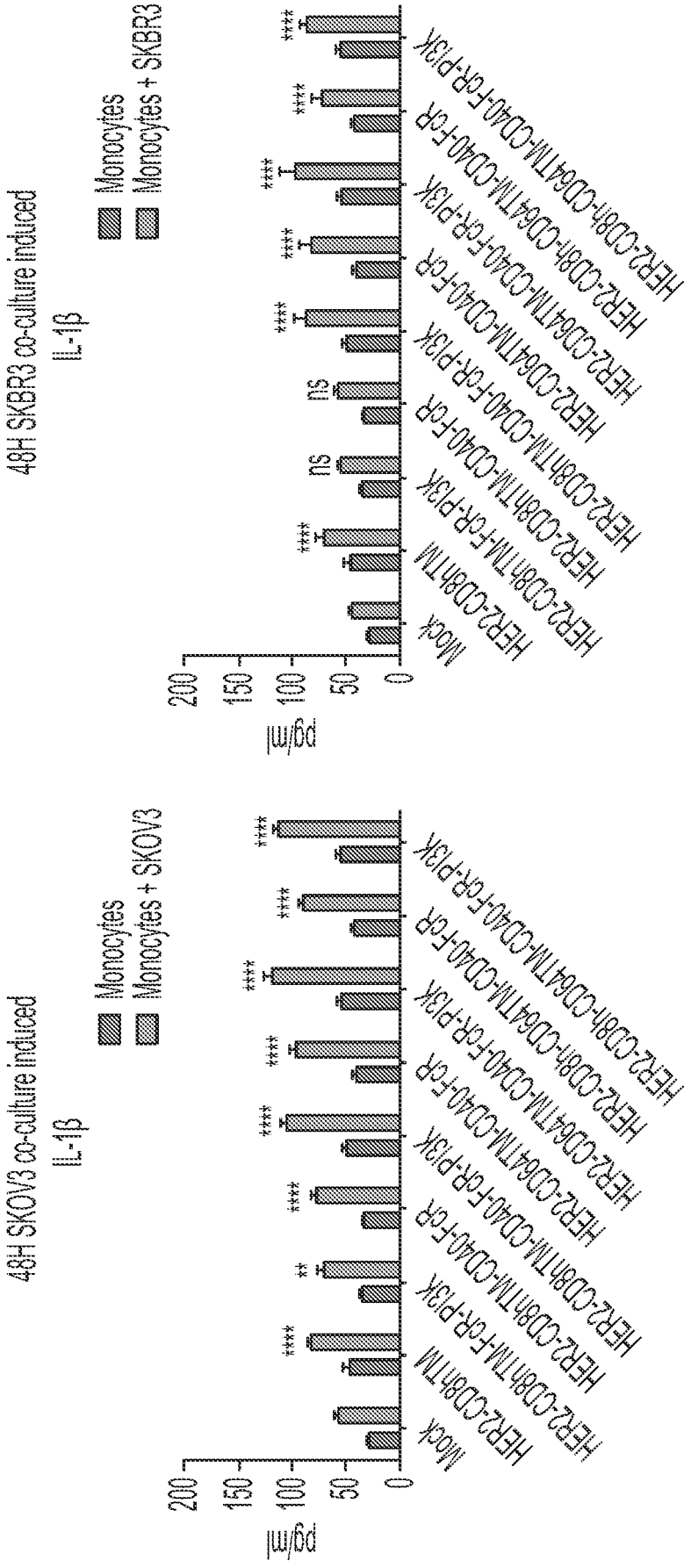


FIG. 10B
CONTINUED

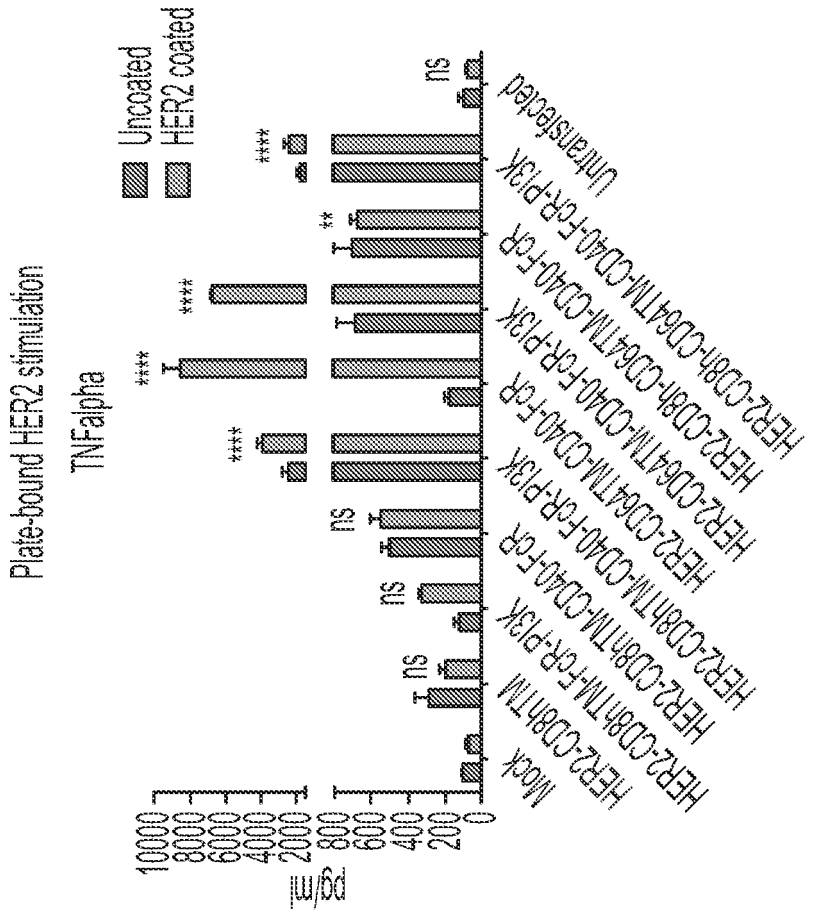

Plate-bound HER2 stimulation
TNFalpha
Uncoated
HER2 coated
pg/ml
10000
8000
6000
4000
2000
800
600
400
200
0
****
****
ns
ns
ns
****
**
ns
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected

FIG. 10C

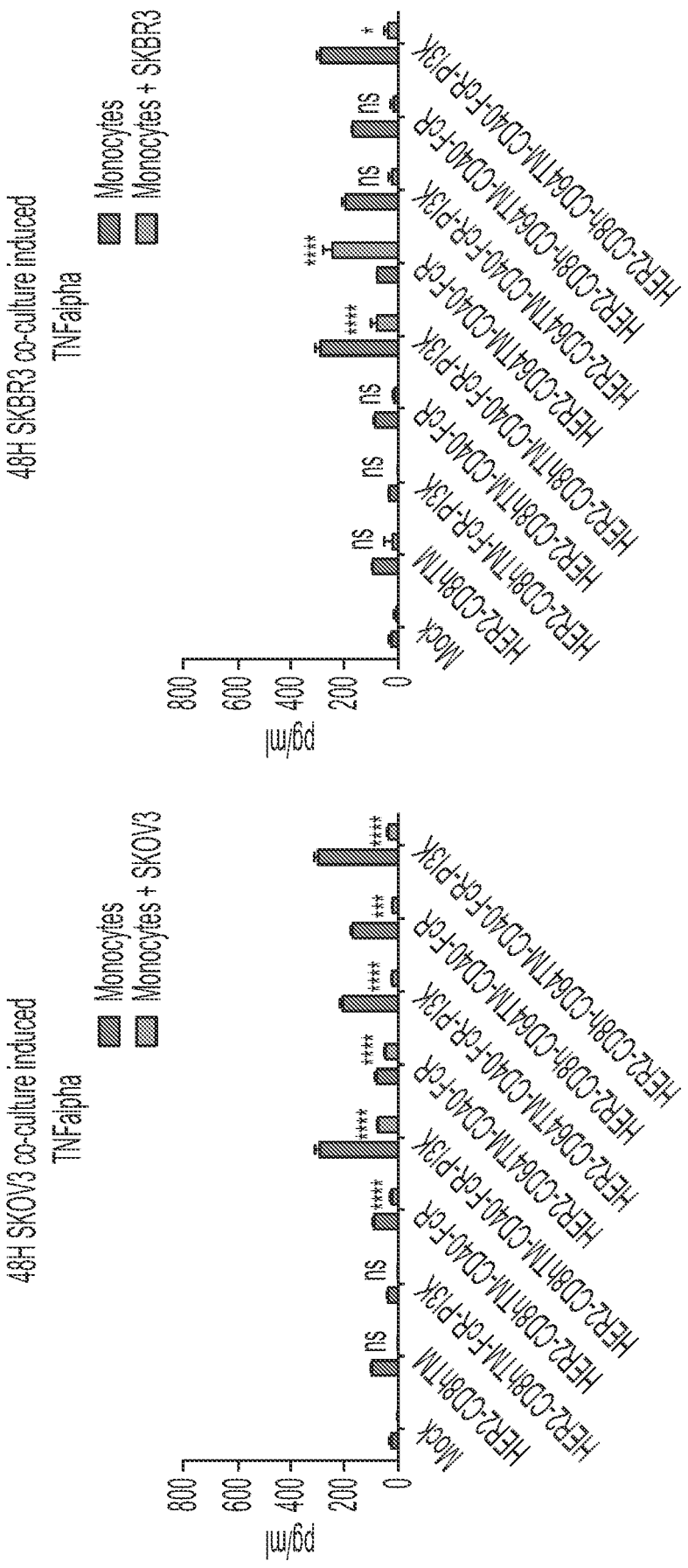


FIG. 10C
CONTINUED

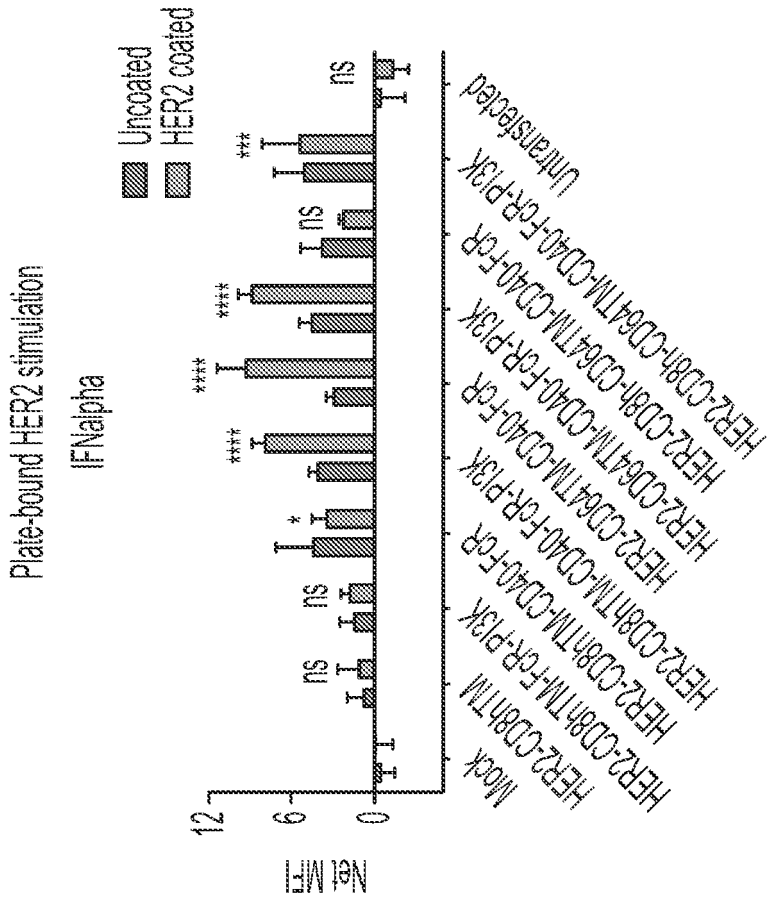

Plate-bound HER2 stimulation
IFNalpha
Uncoated
HER2 coated
Net MFI
12
6
0
ns
ns
*
****
****
****
ns
***
ns
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected

FIG. 10D

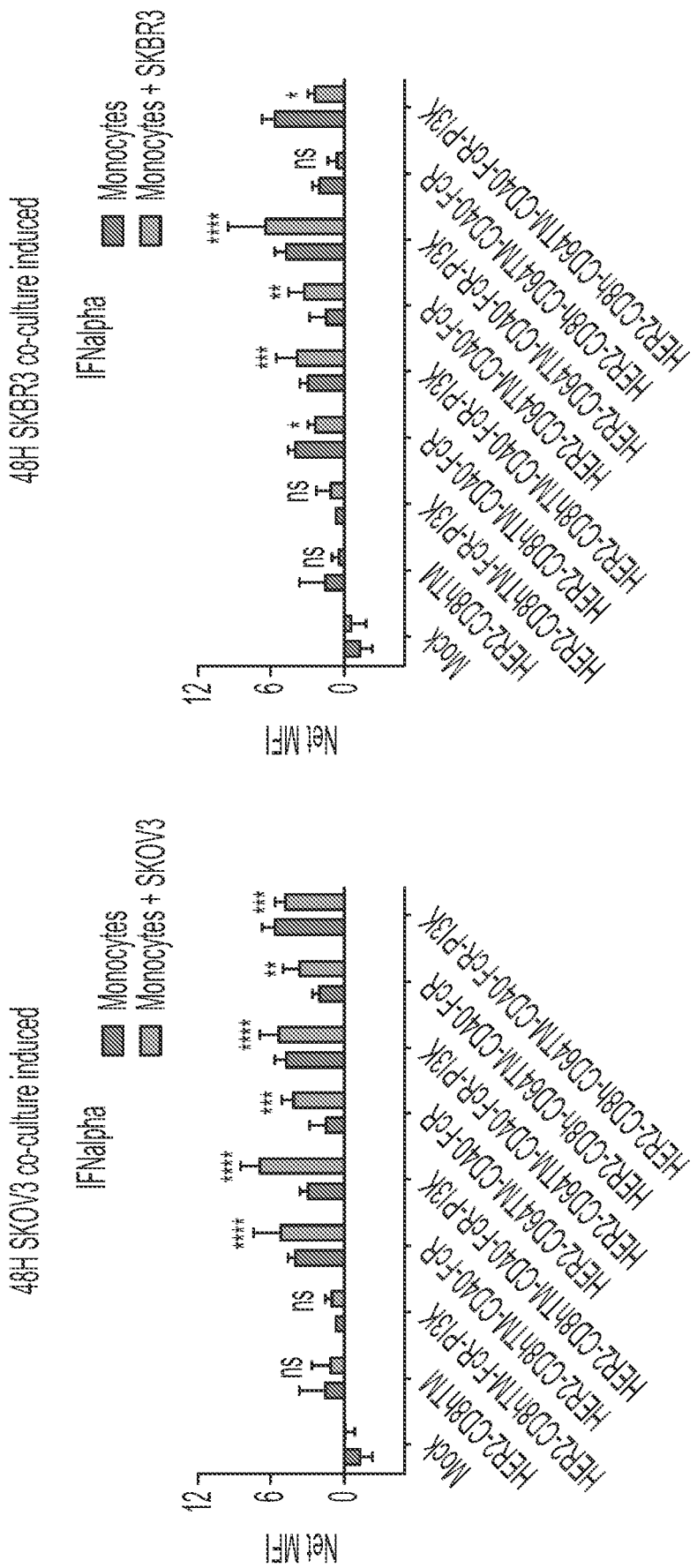


FIG. 10D
CONTINUED

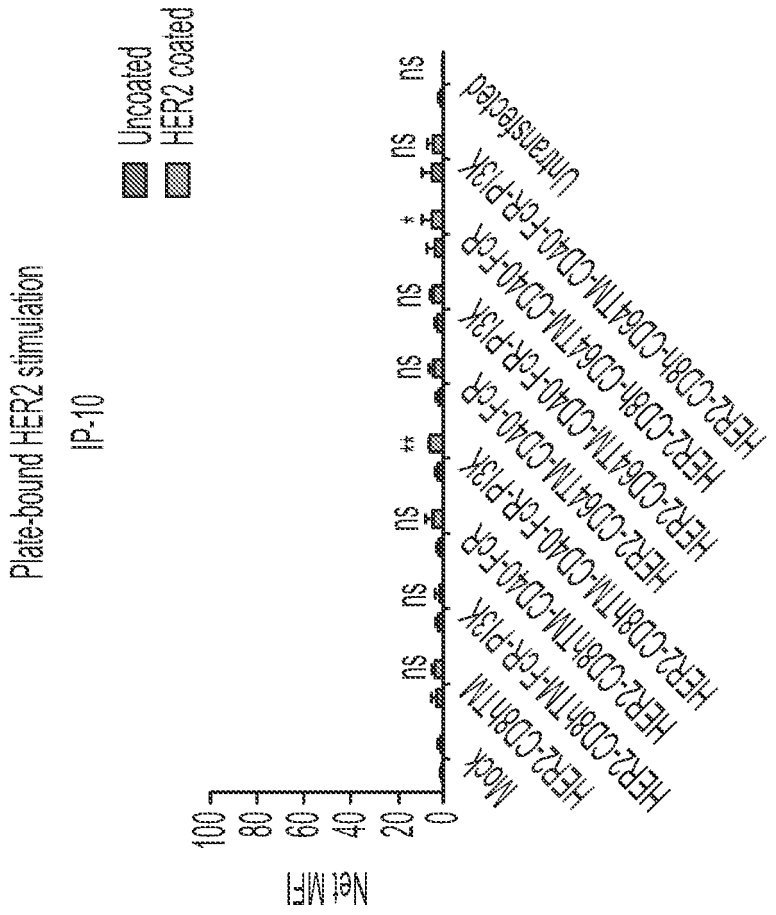

Plate-bound HER2 stimulation
IP-10
Uncoated
HER2 coated
Net MFI
100
80
60
40
20
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected
ns
ns
ns
**
ns
ns
*
ns
ns

FIG. 10E

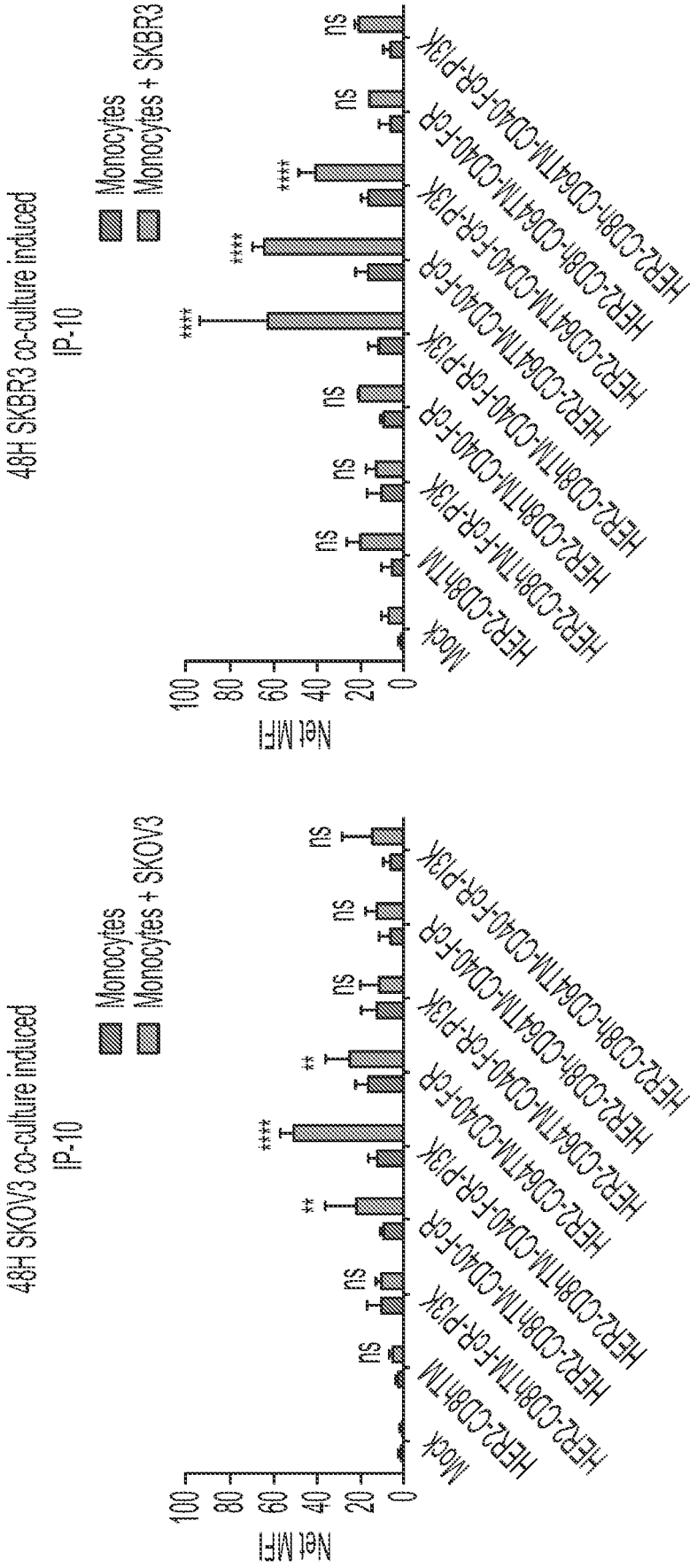


FIG. 10E
CONTINUED

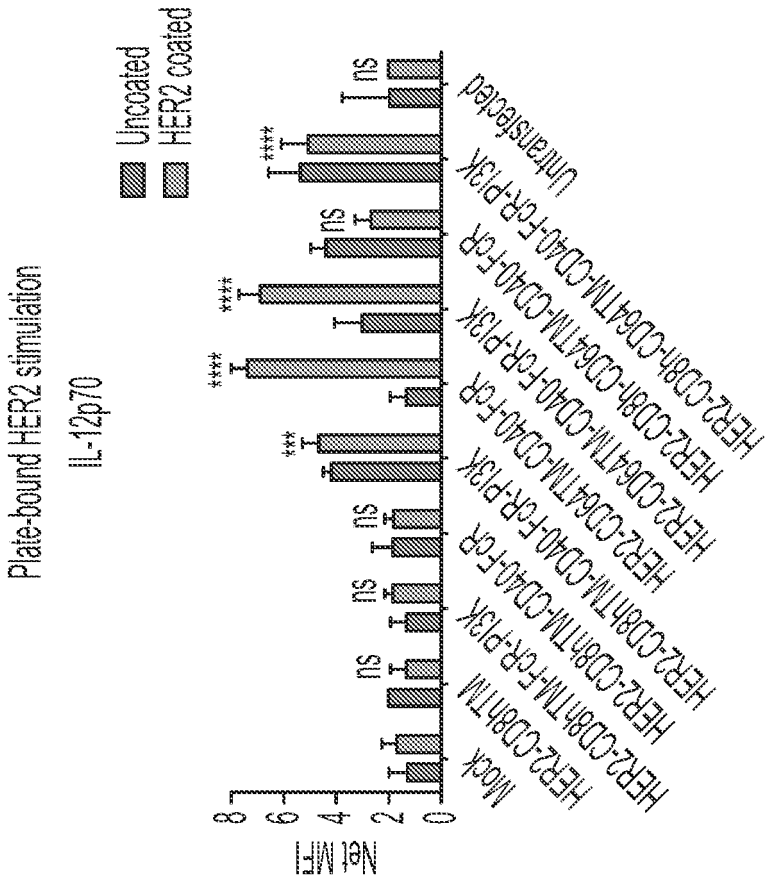

Plate-bound HER2 stimulation
IL-12p70
Uncoated
HER2 coated
Net MFI
0
2
4
6
8
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-CD40-FCR
HER2-CD8hTM-CD40-FCR-P13K
HER2-CD64TM-CD40-FCR
HER2-CD64TM-CD40-FCR-P13K
HER2-CD8h-CD64TM-CD40-FCR
HER2-CD8h-CD64TM-CD40-FCR-P13K
Untransfected
ns
****
***

FIG. 10F

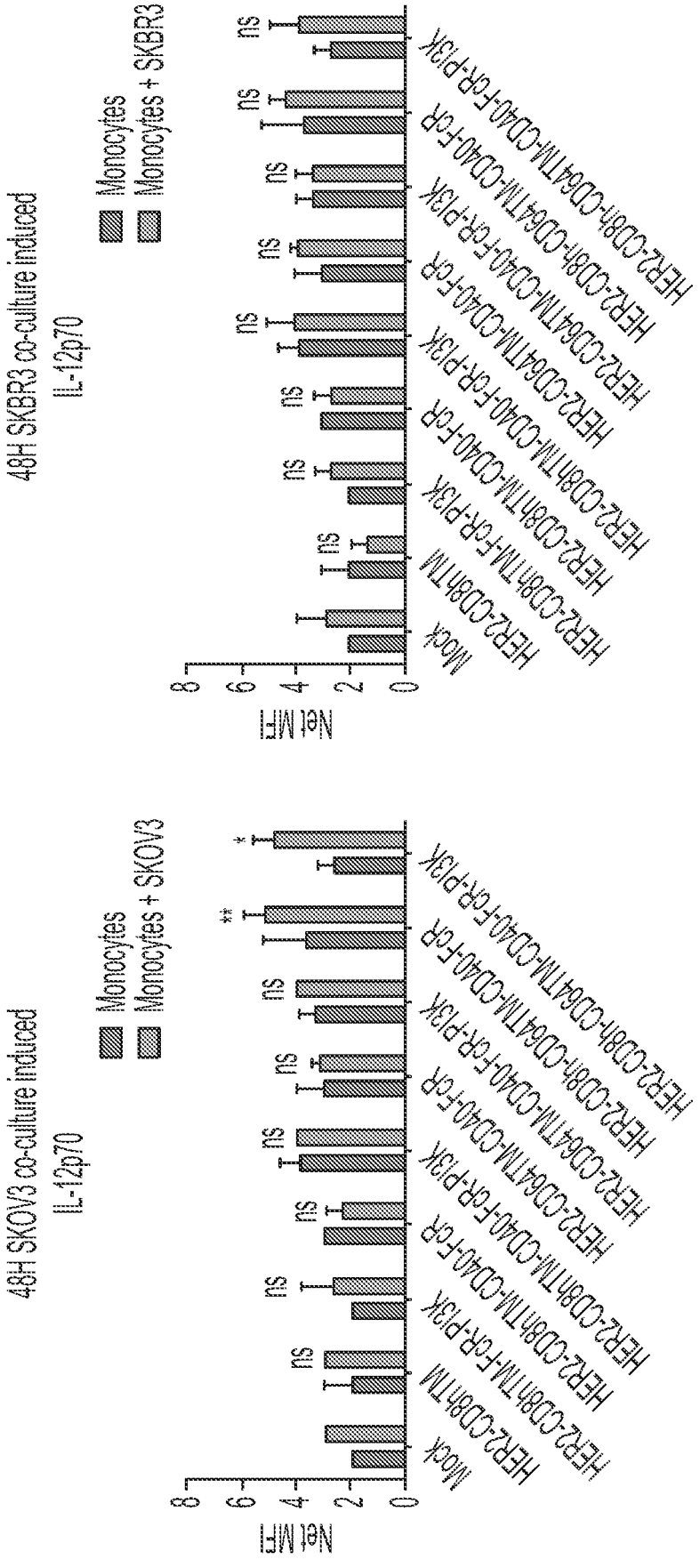


FIG. 10F
CONTINUED

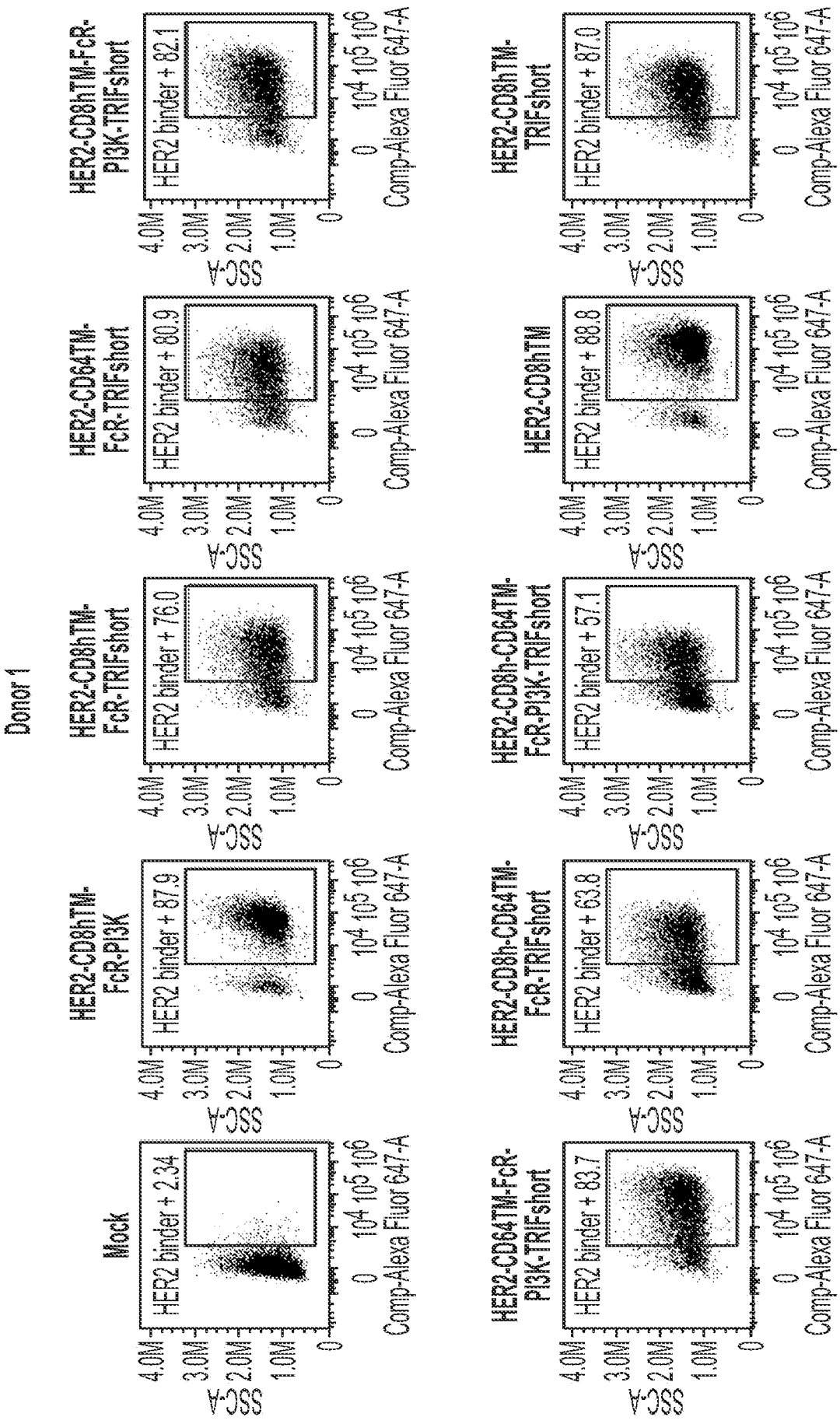

Donor 1
Mock
HER2-CD8hTM-FcR-P13K
HER2-CD8hTM-FcR-TRIFshort
HER2-CD64TM-FcR-TRIFshort
HER2-CD8hTM-FcR-P13K-TRIFshort
HER2-CD64TM-FcR-P13K-TRIFshort
HER2-CD8h-CD64TM-FcR-TRIFshort
HER2-CD8h-CD64TM-FcR-P13K-TRIFshort
HER2-CD8hTM
HER2-CD8hTM-TRIFshort
HER2 binder + 2.34
HER2 binder + 87.9
HER2 binder + 76.0
HER2 binder + 80.9
HER2 binder + 82.1
HER2 binder + 63.8
HER2 binder + 57.1
HER2 binder + 88.8
HER2 binder + 87.0
HER2 binder + 83.7
SSC-A
Comp-Alexa Fluor 647-A
4.0M
3.0M
2.0M
1.0M
0
10^4 10^5 10^6

FIG. 11A

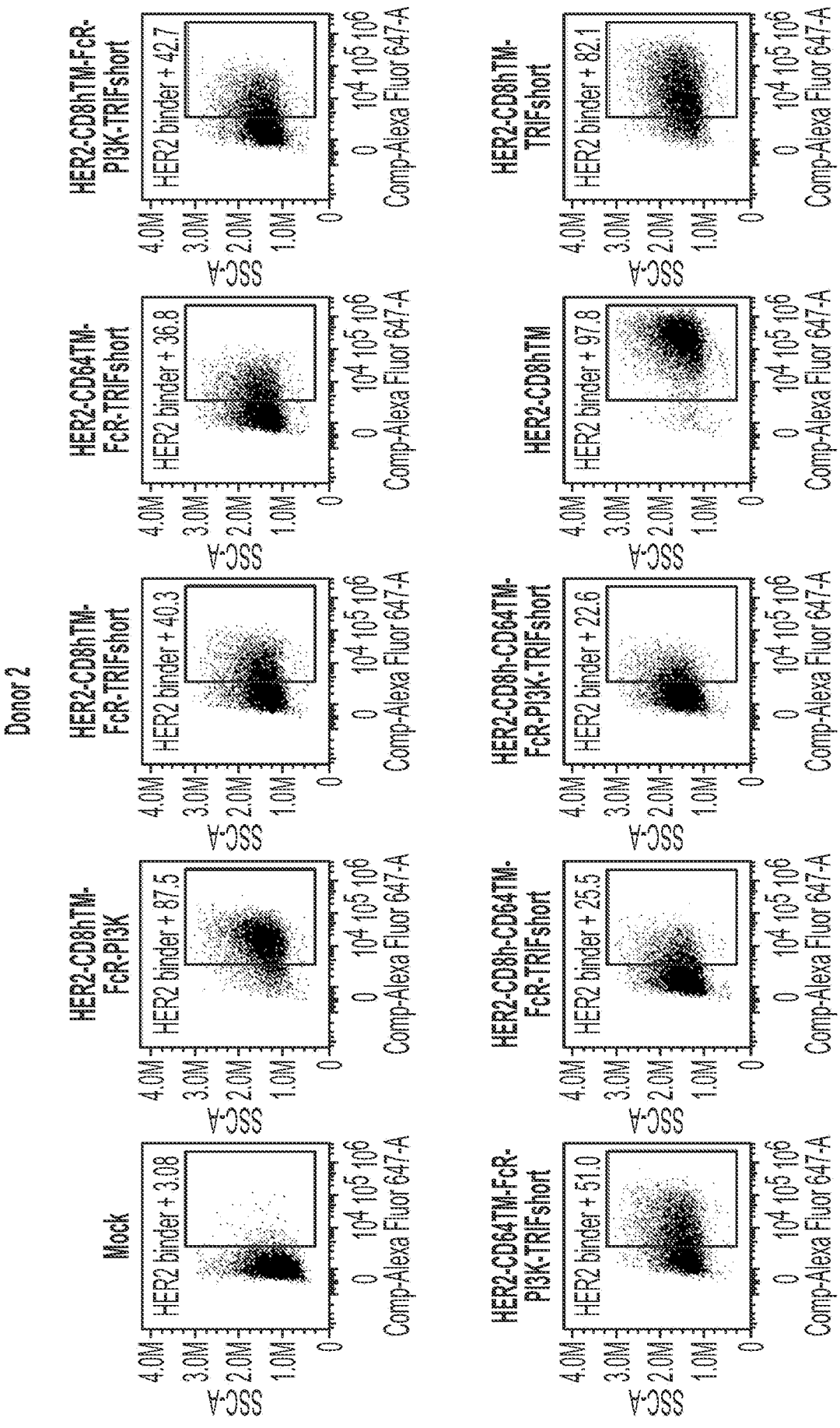

Donor 2
Mock
HER2 binder + 3.08
HER2-CD8hTM-FcR-P13K
HER2 binder + 87.5
HER2-CD8hTM-FcR-TRIFshort
HER2 binder + 40.3
HER2-CD64TM-FcR-TRIFshort
HER2 binder + 36.8
HER2-CD64TM-FcR-P13K-TRIFshort
HER2 binder + 42.7
HER2-CD64TM-FcR-P13K-TRIFshort
HER2 binder + 51.0
HER2-CD8h-CD64TM-FcR-TRIFshort
HER2 binder + 25.5
HER2-CD8h-CD64TM-FcR-P13K-TRIFshort
HER2 binder + 22.6
HER2-CD8hTM
HER2 binder + 97.8
HER2-CD8hTM-TRIFshort
HER2 binder + 82.1
SSC-A
Comp-Alexa Fluor 647-A
4.0M
3.0M
2.0M
1.0M
0
10^4 10^5 10^6

FIG. 11B

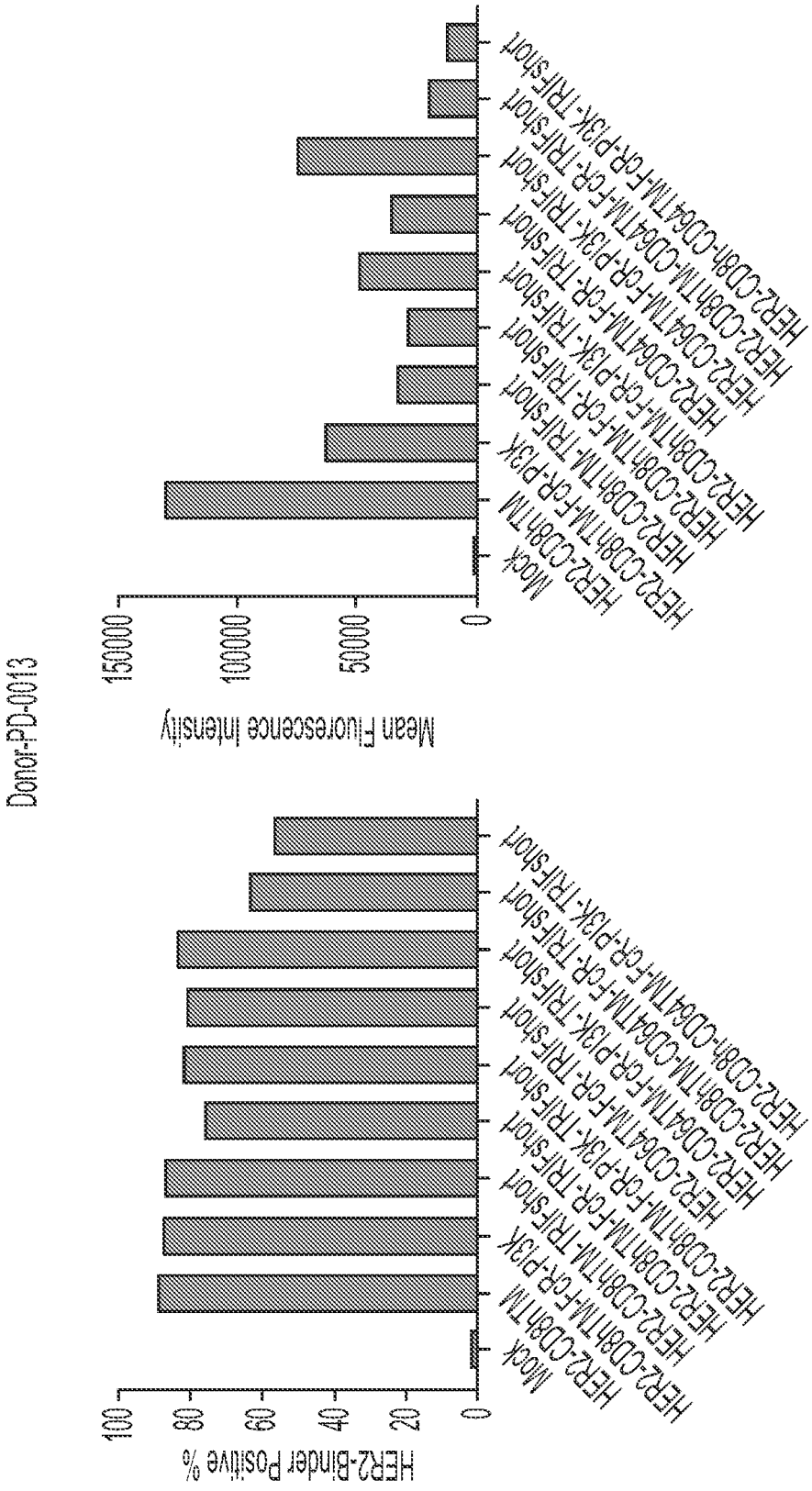

Donor-PD-0013
Mean Fluorescence Intensity
150000
100000
50000
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-FCR-TRIFshort
HER2-CD8hTM-FCR-P13K-TRIFshort
HER2-CD64TM-FCR-TRIFshort
HER2-CD64TM-FCR-P13K-TRIFshort
HER2-CD8hTM-CD64TM-FCR-TRIFshort
HER2-CD8hTM-CD64TM-FCR-P13K-TRIFshort
HER2-Binder Positive %
100
80
60
40
20
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-FCR-TRIFshort
HER2-CD8hTM-FCR-P13K-TRIFshort
HER2-CD64TM-FCR-TRIFshort
HER2-CD64TM-FCR-P13K-TRIFshort
HER2-CD8hTM-CD64TM-FCR-TRIFshort
HER2-CD8hTM-CD64TM-FCR-P13K-TRIFshort

FIG. 11C

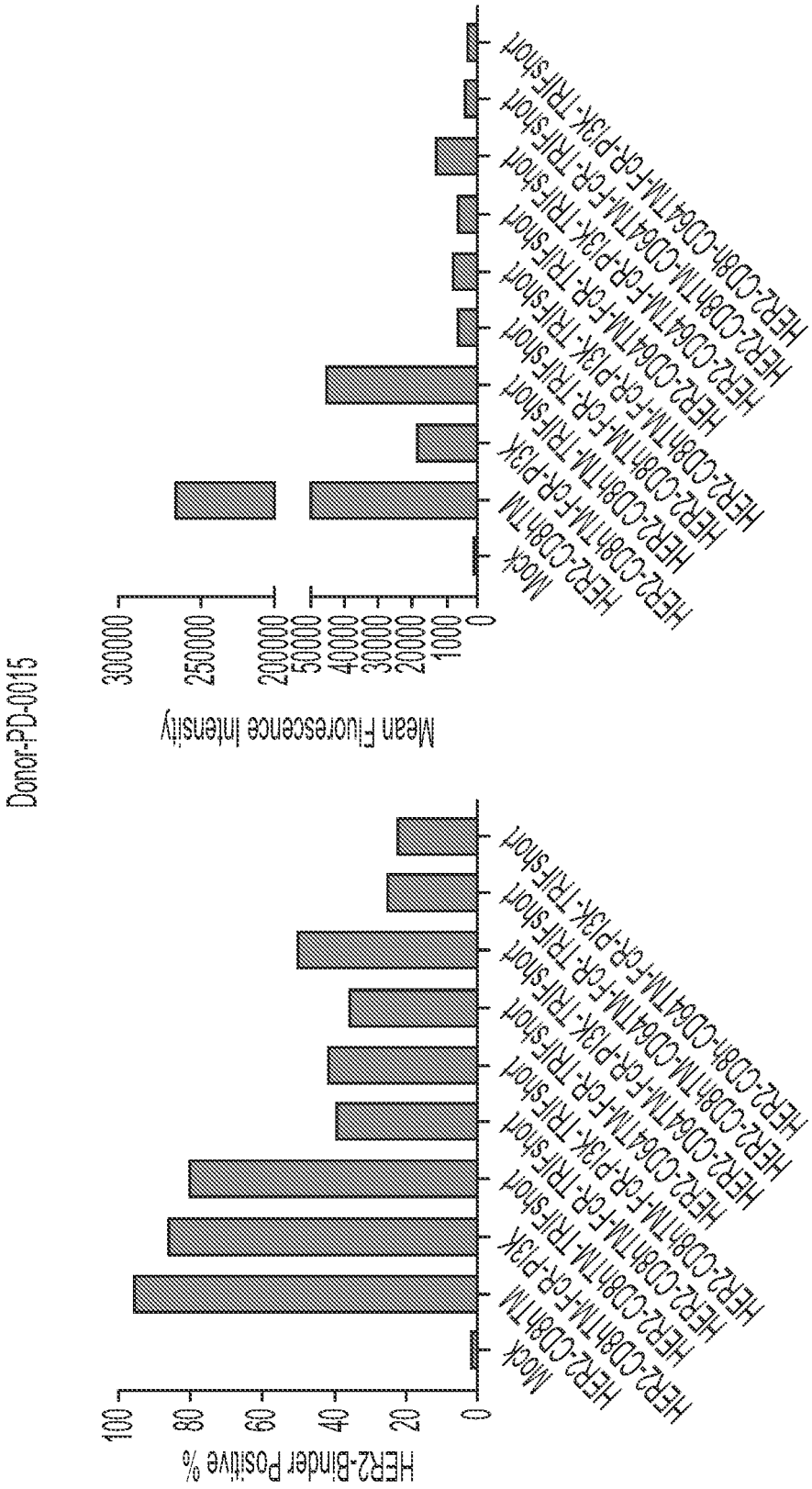


FIG. 11C
CONTINUED

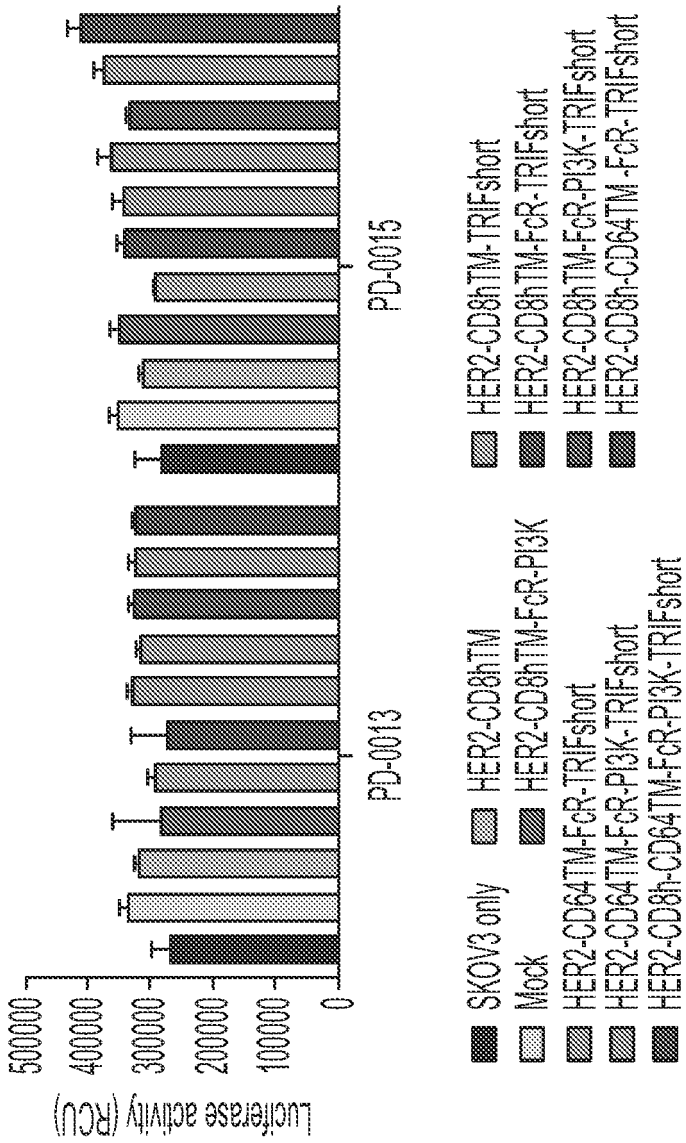

SKOV3 killing activity
Luciferase activity (RCU)
500000
400000
300000
200000
100000
0
PD-0013
PD-0015
SKOV3 only
Mock
HER2-CD64TM-FcR-TRIFshort
HER2-CD64TM-FcR-PI3K-TRIFshort
HER2-CD8h-CD64TM-FcR-PI3K-TRIFshort
HER2-CD8hTM
HER2-CD8hTM-FcR-PI3K
HER2-CD8hTM-TRIFshort
HER2-CD8hTM-FcR-TRIFshort
HER2-CD8hTM-FcR-PI3K-TRIFshort
HER2-CD8h-CD64TM-FcR-TRIFshort

FIG. 12A

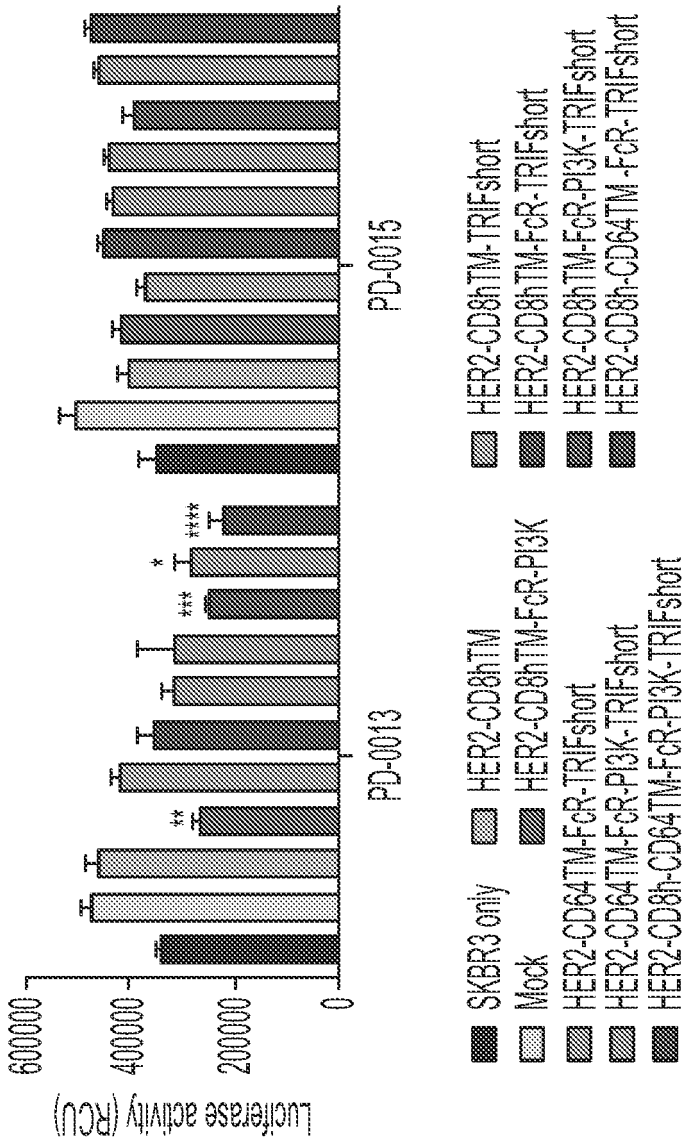

SKBR3 killing activity
Luciferase activity (RCU)
600000
400000
200000
0
PD-0013
PD-0015
**
***
*
****
SKBR3 only
Mock
HER2-CD64TM-FcR-TRIFshort
HER2-CD64TM-FcR-PI3K-TRIFshort
HER2-CD8h-CD64TM-FcR-PI3K-TRIFshort
HER2-CD8hTM
HER2-CD8hTM-FcR-PI3K
HER2-CD8hTM-TRIFshort
HER2-CD8hTM-FcR-TRIFshort
HER2-CD8hTM-FcR-PI3K-TRIFshort
HER2-CD8h-CD64TM-FcR-TRIFshort

FIG. 12B

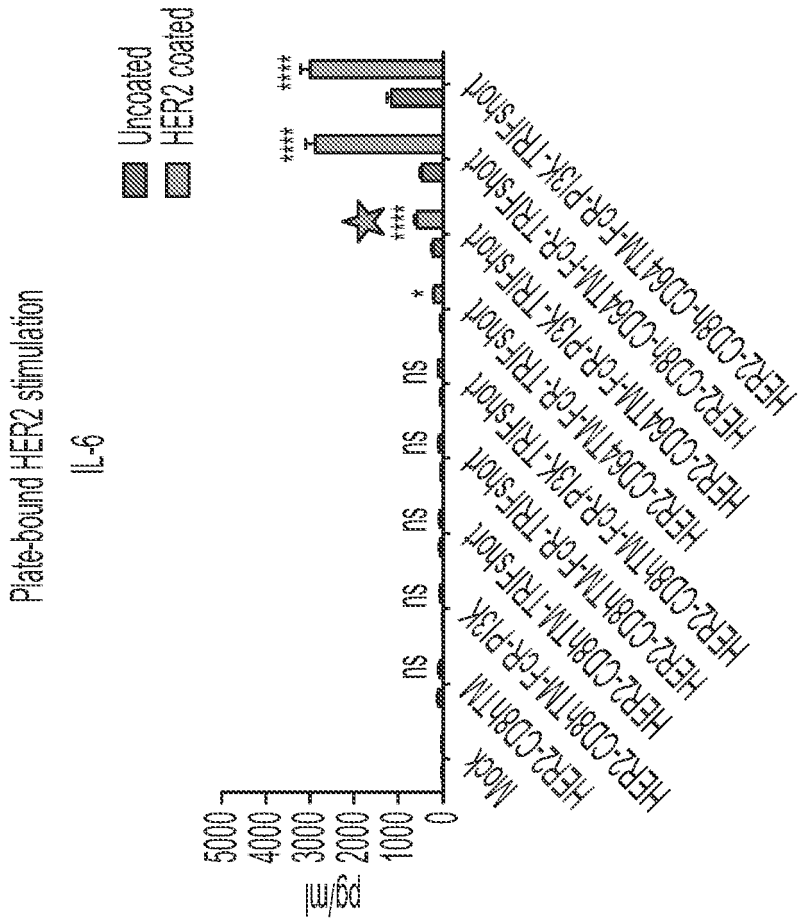

Plate-bound HER2 stimulation
IL-6
Uncoated
HER2 coated
pg/ml
5000
4000
3000
2000
1000
0
ns
ns
ns
ns
*
****
****
****
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIF-short
HER2-CD8hTM-FCR-TRIF-short
HER2-CD8hTM-FCR-P13K-TRIF-short
HER2-CD64TM-FCR-TRIF-short
HER2-CD64TM-FCR-P13K-TRIF-short
HER2-CD8h-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-P13K-TRIF-short

FIG. 13A

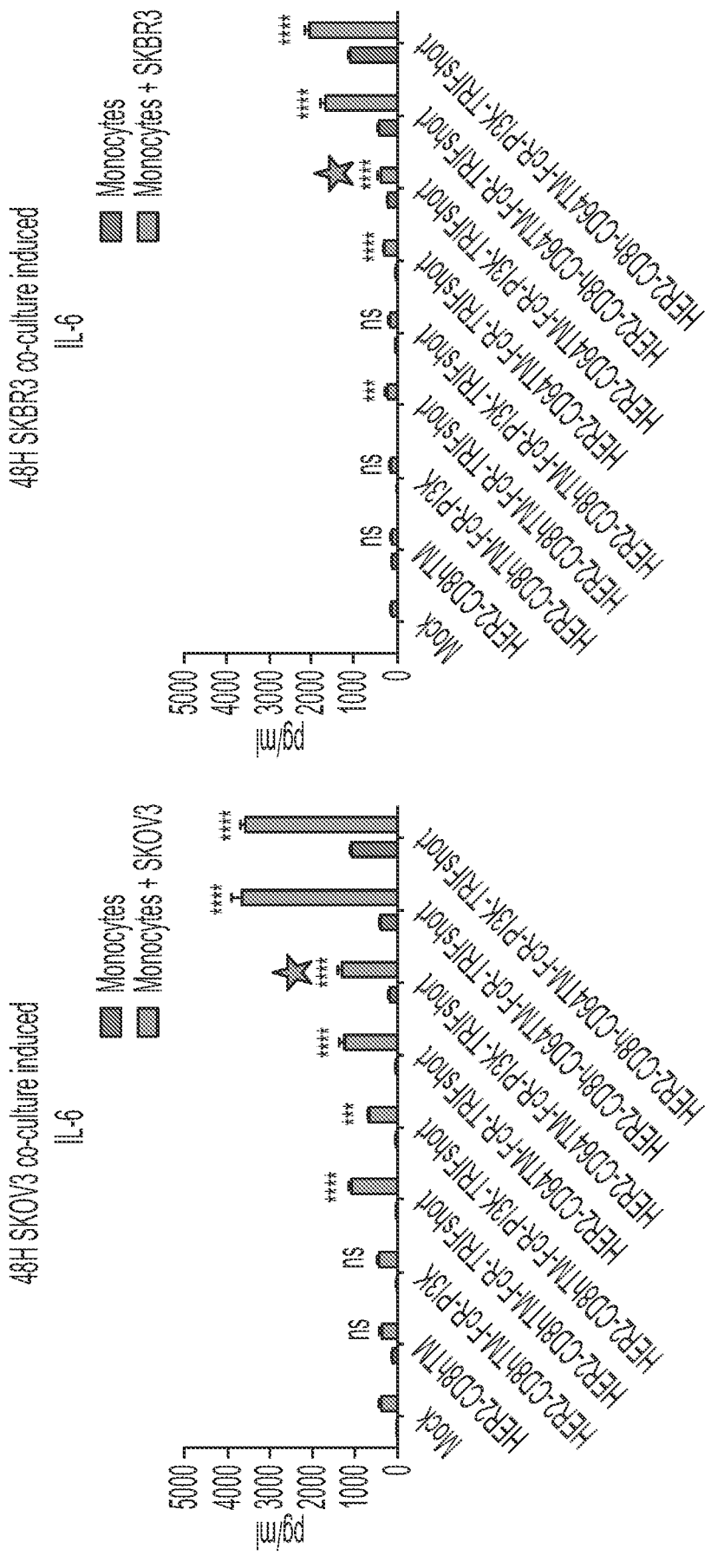


FIG. 13A
CONTINUED

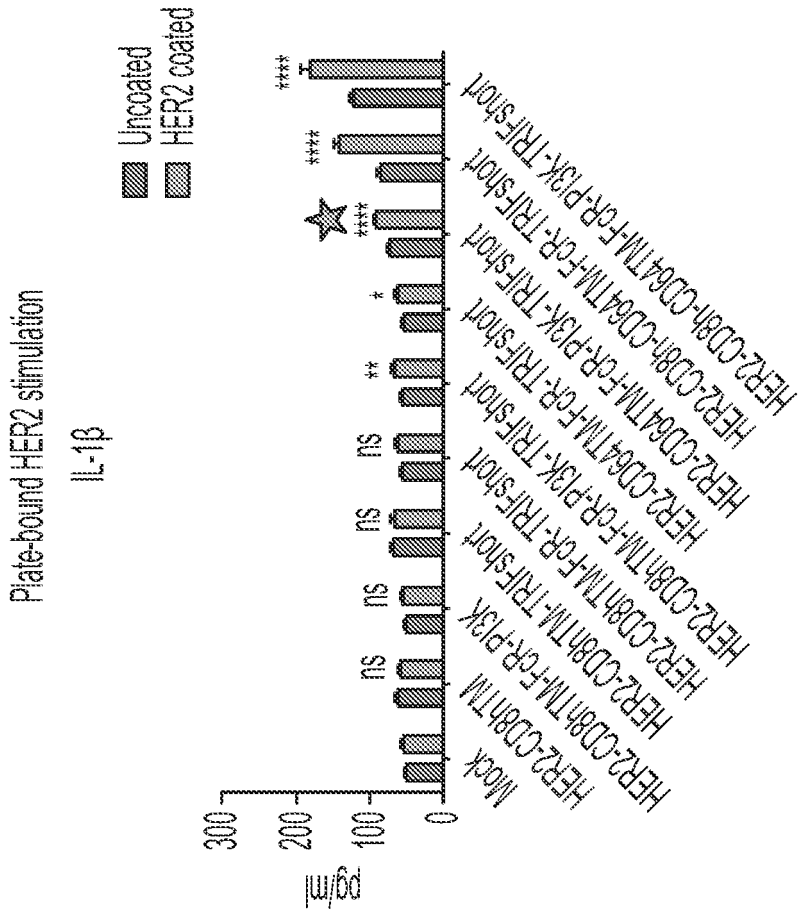

Plate-bound HER2 stimulation
IL-1β
Uncoated
HER2 coated
pg/ml
300
200
100
0
ns
ns
ns
ns
**
*
****
****
****
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIFshort
HER2-CD8hTM-FCR-TRIFshort
HER2-CD8hTM-FCR-P13K-TRIFshort
HER2-CD64TM-FCR-TRIFshort
HER2-CD64TM-FCR-P13K-TRIFshort
HER2-CD8h-CD64TM-FCR-TRIFshort
HER2-CD8h-CD64TM-FCR-P13K-TRIFshort

FIG. 13B

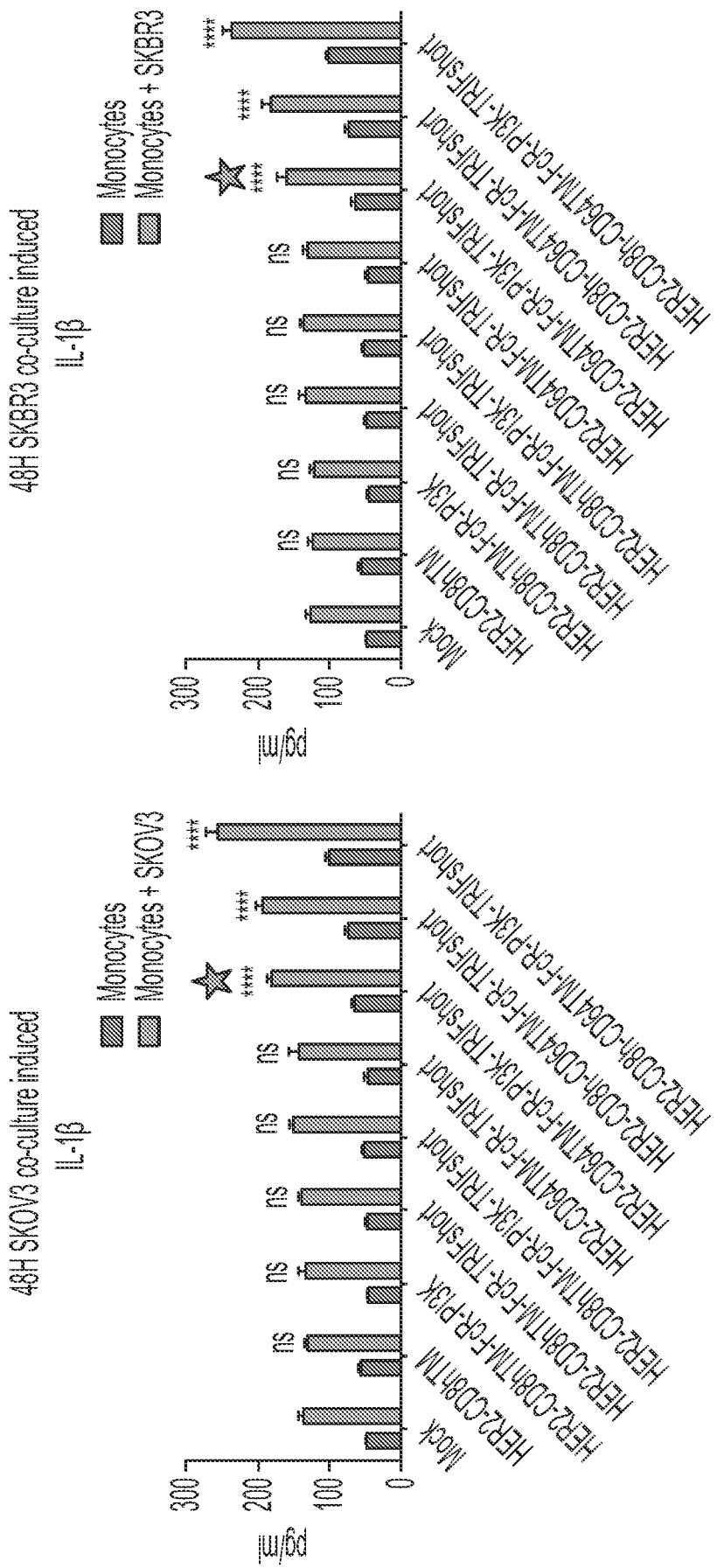


FIG. 13B
CONTINUED

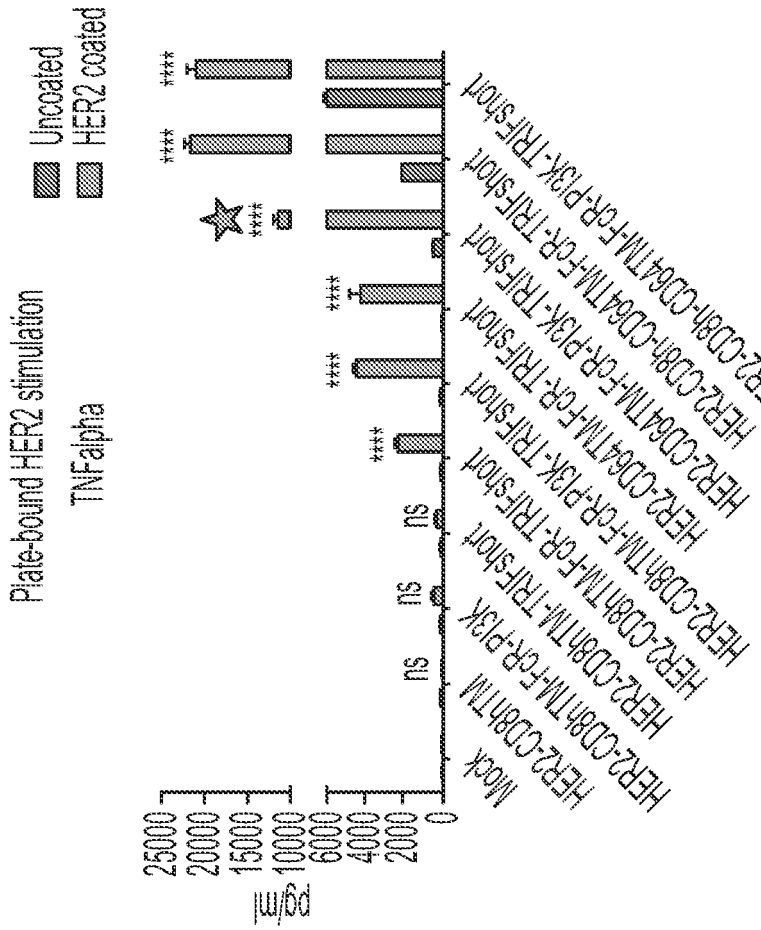

Plate-bound HER2 stimulation
TNFalpha
Uncoated
HER2 coated
pg/ml
25000
20000
15000
10000
6000
4000
2000
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIFshort
HER2-CD8hTM-FCR-TRIFshort
HER2-CD64TM-FCR-P13K-TRIFshort
HER2-CD64TM-FCR-TRIFshort
HER2-CD8h-CD64TM-FCR-P13K-TRIFshort
HER2-CD8h-CD64TM-FCR-TRIFshort
ns
****

FIG. 13C

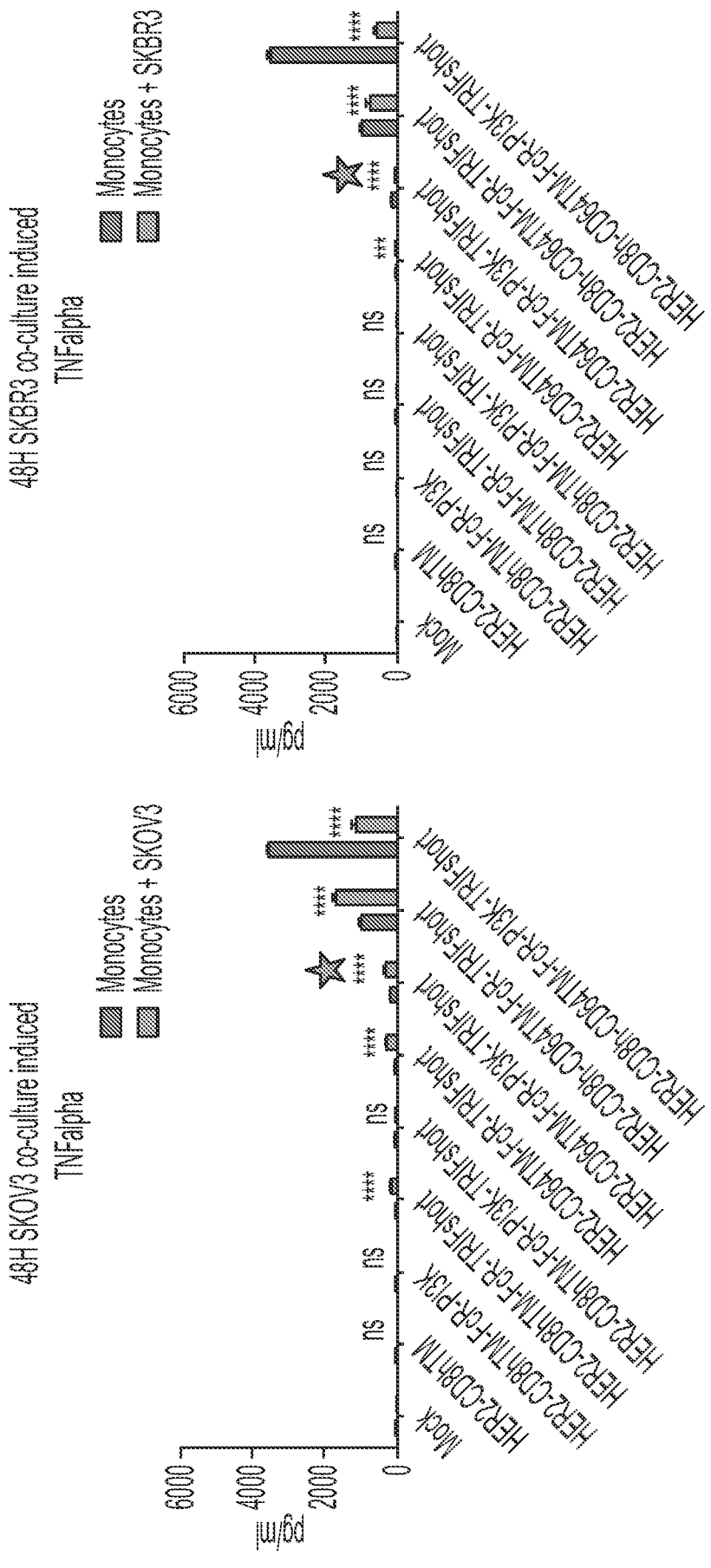


FIG. 13C
CONTINUED

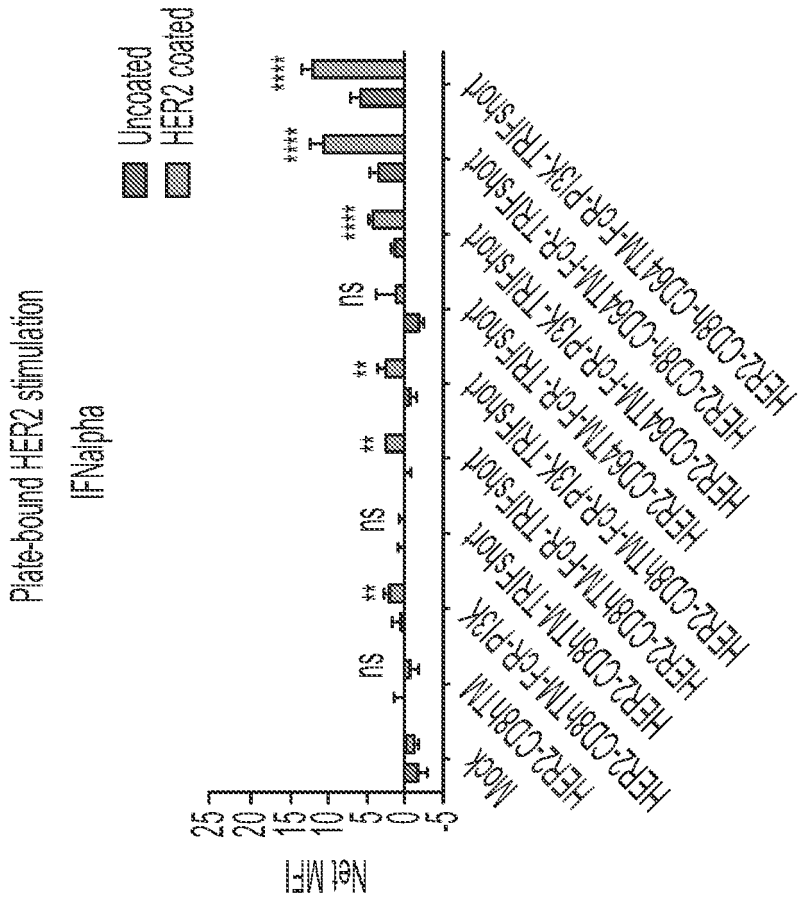

Plate-bound HER2 stimulation
IFNalpha
Uncoated
HER2 coated
Net MFI
25
20
15
10
5
0
-5
ns
**
ns
**
ns
****
****
****
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIF-short
HER2-CD8hTM-FCR-TRIF-short
HER2-CD8hTM-FCR-P13K-TRIF-short
HER2-CD64TM-FCR-TRIF-short
HER2-CD64TM-FCR-P13K-TRIF-short
HER2-CD8h-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-P13K-TRIF-short

FIG. 13D

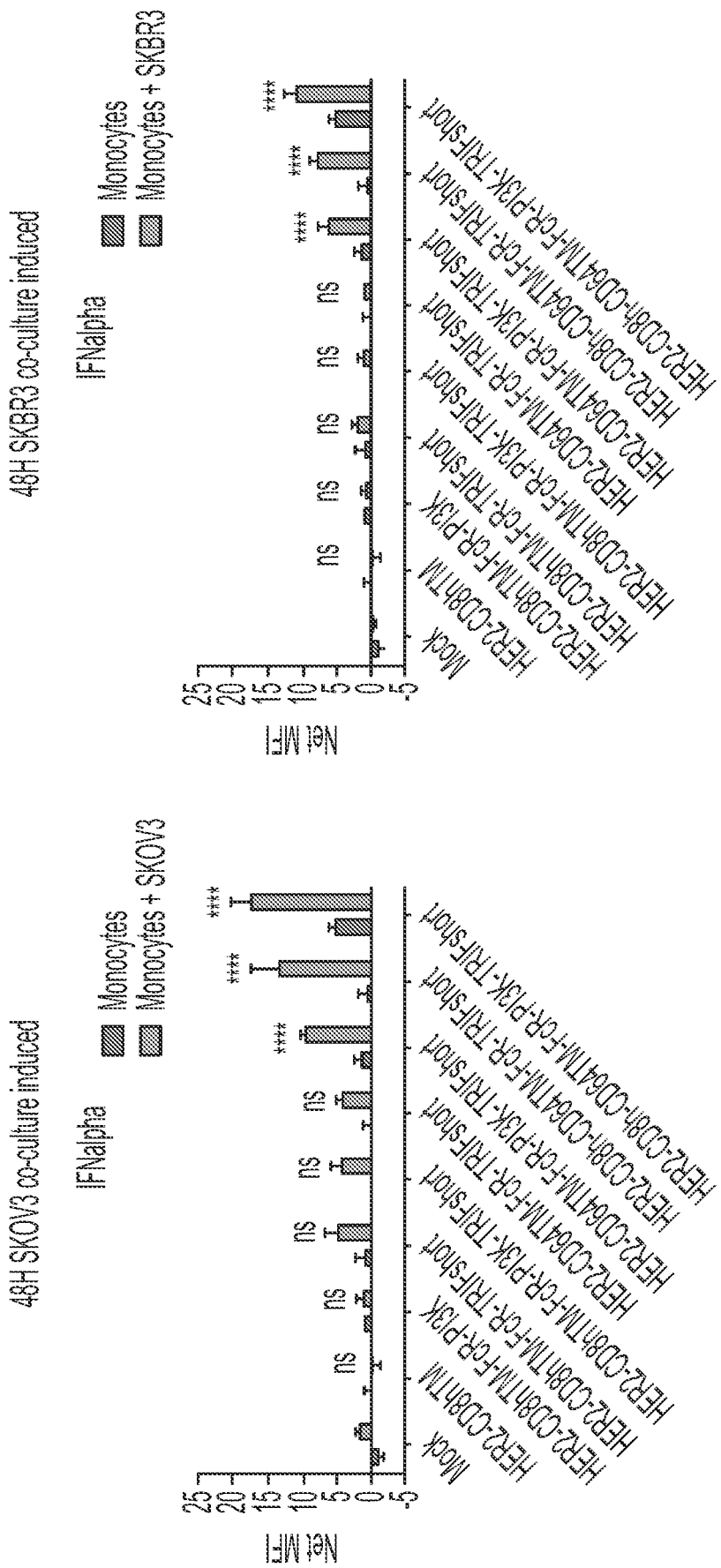


FIG. 13D
CONTINUED

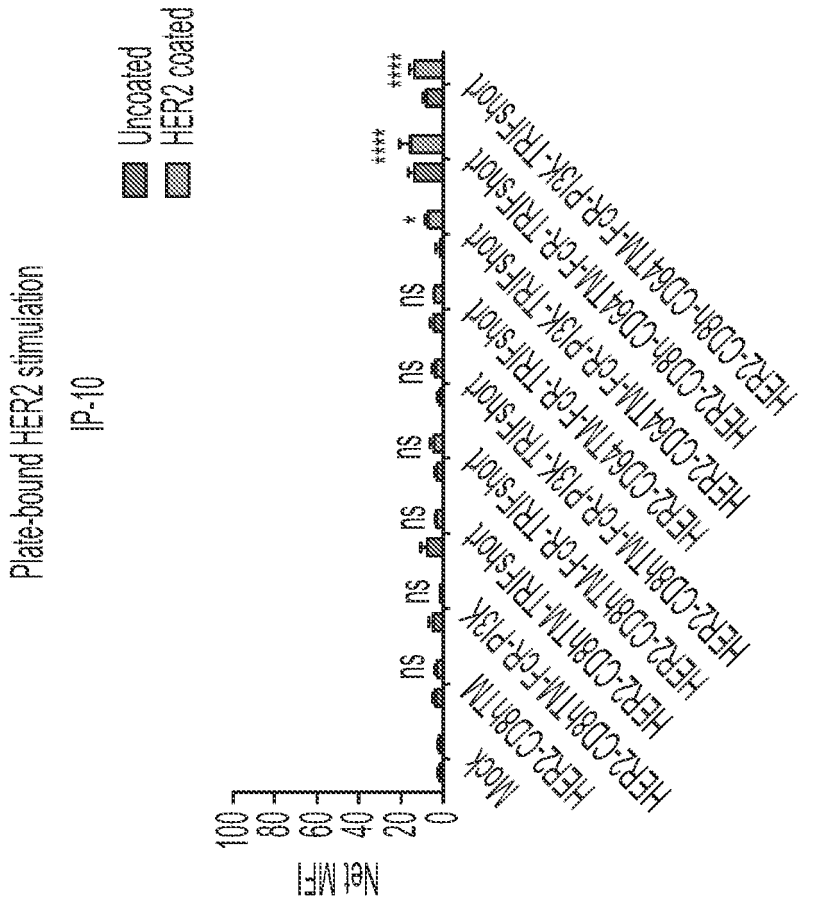

Plate-bound HER2 stimulation
IP-10
Uncoated
HER2 coated
Net MFI
100
80
60
40
20
0
ns
ns
ns
ns
ns
*
****
****
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIF-short
HER2-CD8hTM-FCR-TRIF-short
HER2-CD64TM-FCR-P13K-TRIF-short
HER2-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-P13K-TRIF-short

FIG. 13E

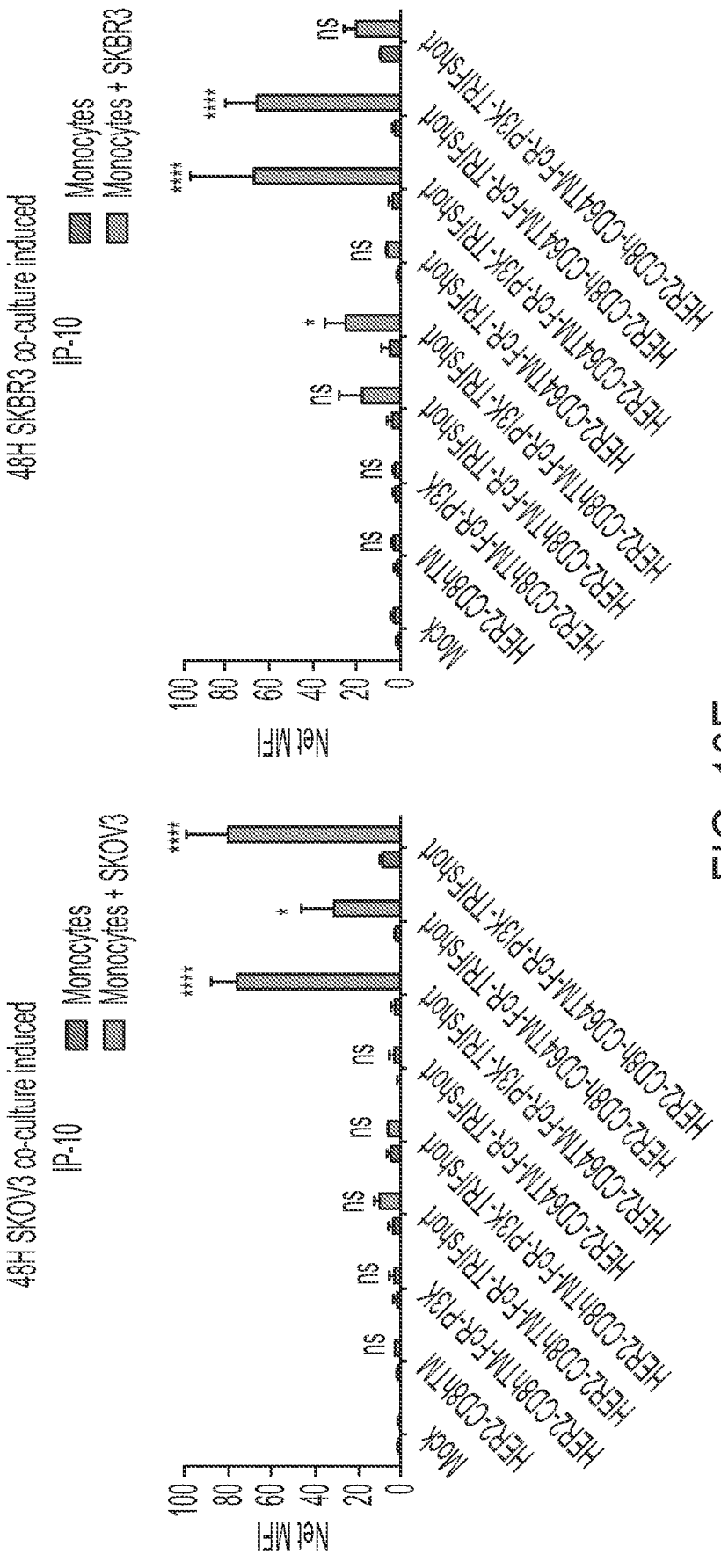


FIG. 13E
CONTINUED

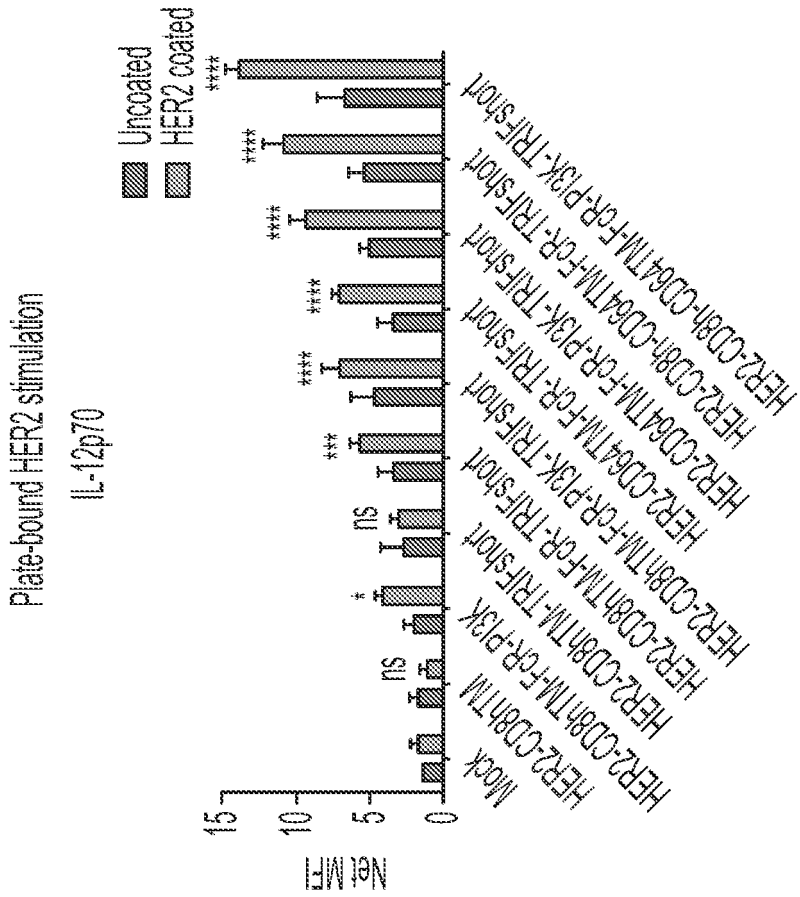

Plate-bound HER2 stimulation
IL-12p70
Uncoated
HER2 coated
Net MFI
15
10
5
0
Mock
HER2-CD8hTM
HER2-CD8hTM-FCR-P13K
HER2-CD8hTM-TRIF-short
HER2-CD8hTM-FCR-TRIF-short
HER2-CD64TM-FCR-P13K-TRIF-short
HER2-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-TRIF-short
HER2-CD8h-CD64TM-FCR-P13K-TRIF-short
ns
*
ns
***
****
****
****
****

FIG. 13F

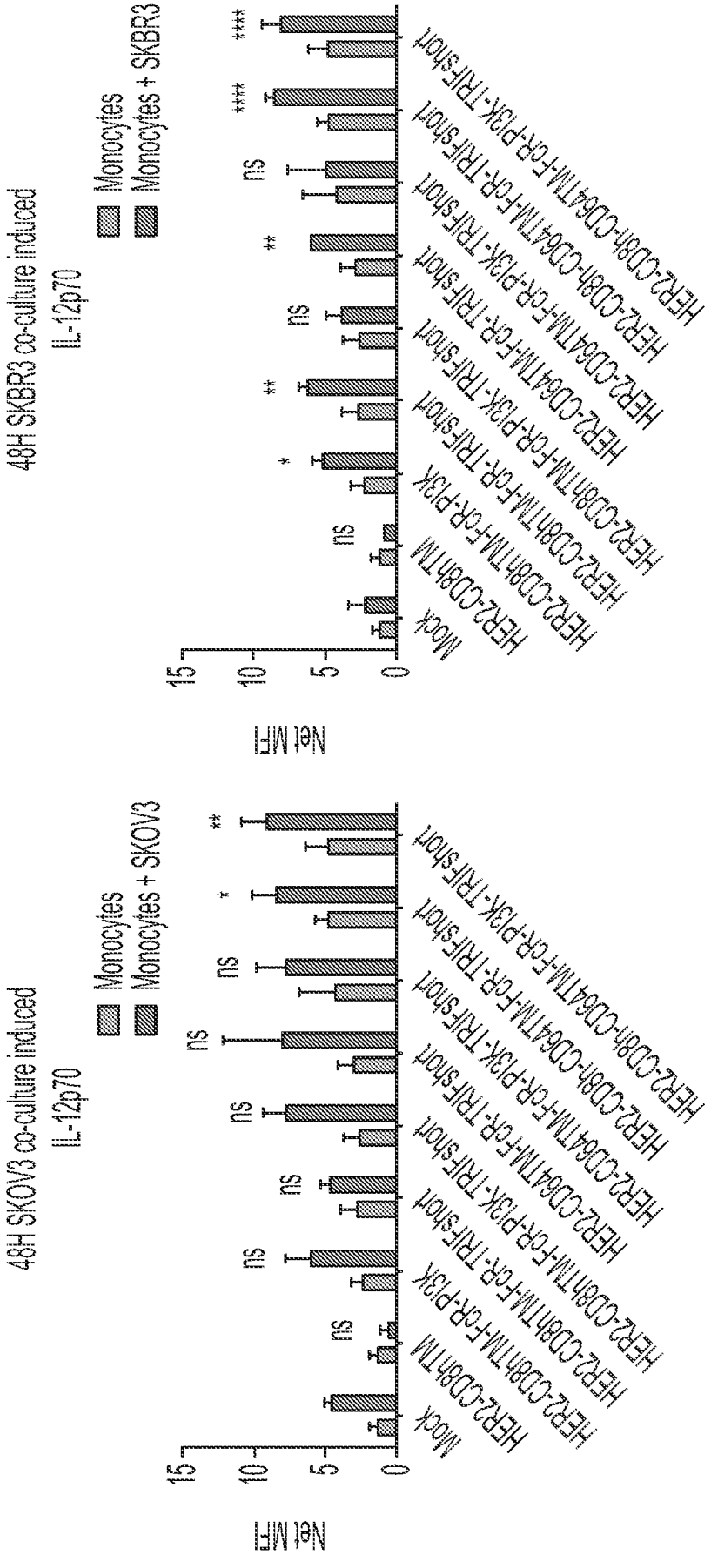


FIG. 13F
CONTINUED

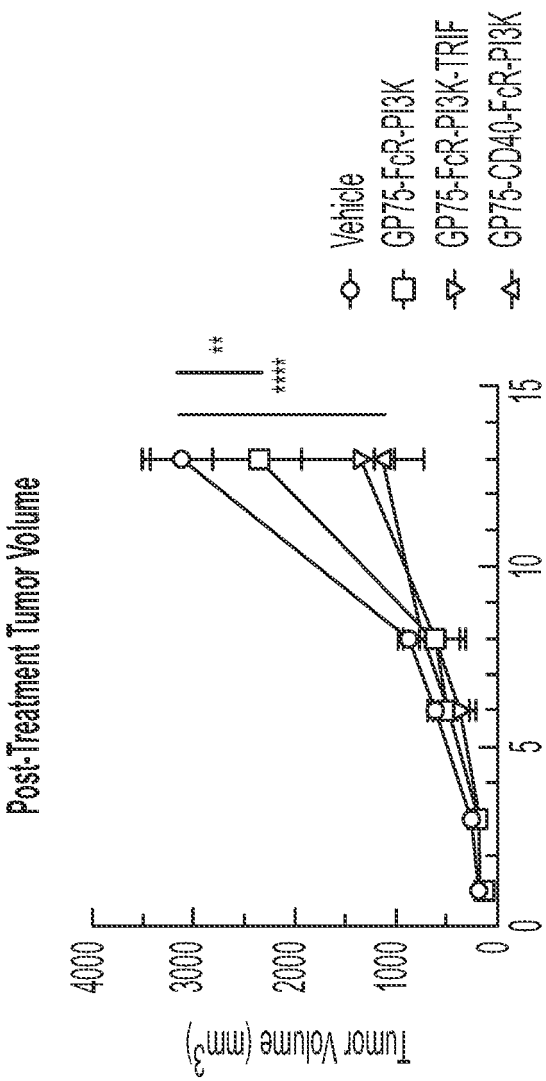

Post-Treatment Tumor Volume
Tumor Volume (mm3)
4000
3000
2000
1000
0
5
10
15
Days Post Treatment
**
****
Vehicle
GP75-FcR-PI3K
GP75-FcR-PI3K-TRIF
GP75-CD40-FcR-PI3K

FIG. 14

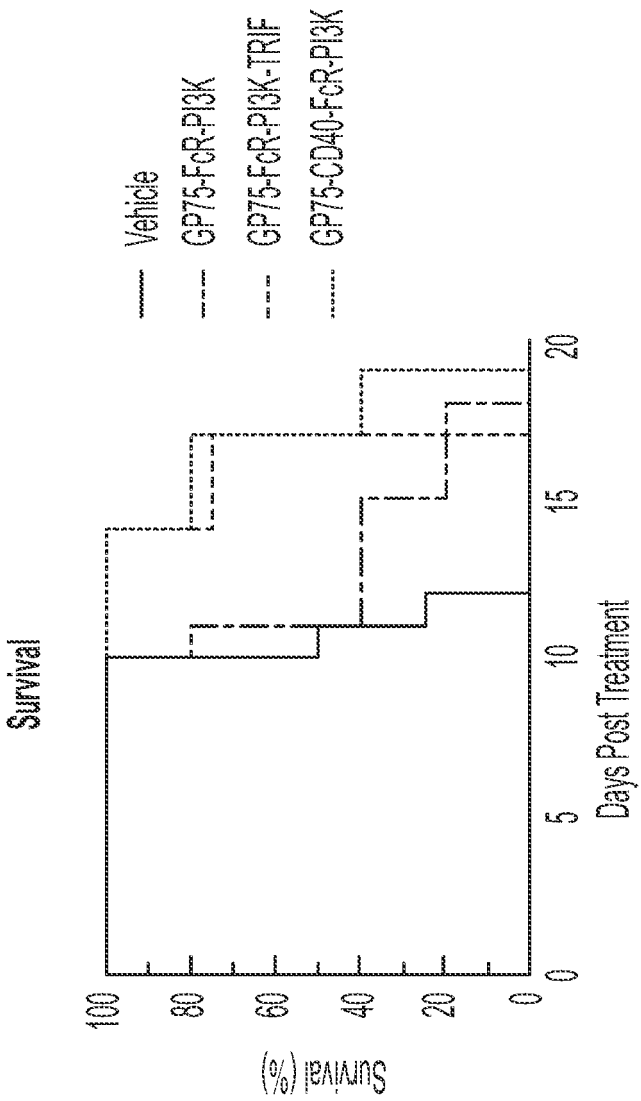


FIG. 14
CONTINUED