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(54) Title: CHIMERIC ANTIGEN RECEPTORS FOR PHAGOCYTOSIS

(57) Abstract: The present disclosure generally relate to novel chimeric antigen receptors (CARs) that comprise an intracellular signaling domain of an engulfment receptor expressed on the surface of a phagocytic cell and activate the endogenous phagocytic signaling pathway. Also disclosed are compositions and methods useful for producing such CARs, nucleic acids encoding same, phagocytic cells that have been modified to include a targeted effector activity directed towards a cell of interest such as, e.g., a cancer cell, as well as for modifying a cell and/or for the treatment of various health disorders such as cancer, including solid tumor and hematologic malignancy.

CHIMERIC ANTIGEN RECEPTORS FOR PHAGOCYTOSIS

STATEMENT REGARDING FEDERALLY SPONSORED R&D

[001] This invention was made with government support under grant no. F32 GM120990 awarded by the National Institutes of Health. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[002] This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 62/756,235, filed on November 6, 2018, which is herein expressly incorporated by reference in its entirety, including any drawings.

INCORPORATION OF THE SEQUENCE LISTING

[003] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled "Sequence Listing_048536-626001WO.txt", created October 6, 2019, which is approximately 90 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD

[004] Aspects of the present application generally relate to novel chimeric antigen receptors (CARs) that bind an engulfment receptor expressed on the surface of a phagocytic cell and activate the endogenous phagocytic signaling pathway. Also provided are compositions and methods useful for producing such CARs, nucleic acids encoding same, phagocytic cells that have been modified to include a targeted effector activity directed against a cell of interest such as, *e.g.*, a cancer cell, as well as methods for modifying a cell and/or for the treatment of various health disorders such as cancers.

BACKGROUND

[005] Immune system constantly patrols human body, looking to eliminate cancerous cells and harmful microbes. Under normal circumstances, immune cells can identify these threats because they can recognize certain signals present at the surface of the target cells. However,

cancer cells often find ways to evade from the immune system and proliferate. In addition, the endogenous immune system is typically non-reactive to malignant cells, or can be actively immunosuppressive with respect to the body's reaction to the presence of malignant cells.

[006] Despite many recent advances in cancer immunotherapy, which have significantly accelerated the ability to improve clinical results in the setting of numerous solid tumors and hematologic malignancies, a vast majority of current cellular therapeutics focus almost exclusively on T cells, where one way to enhance treatment of tumors is to force tumor recognition by the immune system through genetic engineering of leukocytes. This focus is largely to the fact that T cells can be engineered to express a synthetic immunoreceptor containing an extracellular targeted antibody and intracellular signaling domain, known as chimeric antigen receptor (CAR), which give the T cells expressing such CAR the new ability to target a tumor-associated antigen. For example, in recent years, T cells expressing a CAR directed against CD19 have been shown to have significant anti-leukemic efficacy, where complete remission has been achieved in 90% of acute lymphoblastic leukemia patients treated. These results are accompanied by robust T cell proliferation and clearly documented T cell infiltration into tumor sites in leukemic patients so treated. Despite the high response rates demonstrated in hematopoietic malignancies, CAR T cell efficacy in solid tumors as well as in certain lymphoid tumors may be limited. Possible explanations for this include the potentially impaired ability of T cells to infiltrate solid tumors, poor trafficking, immunosuppressive tumor microenvironment, and expression of few tumor specific antigens on solid tumor cells.

[007] There is an ongoing need for new compositions and methods of treating infections, inflammatory diseases, immune diseases, and various cancers. In particular, a need exists in the art for more effective compositions and methods that treat cancers by improving specificity for tumor cells and improving infiltration into tumor sites in both solid tumors and hematologic malignancies by such compositions and methods.

SUMMARY

[008] This section provides a general summary of the disclosure, and is not comprehensive of its full scope or all of its feature.

[009] The present disclosure generally relates to the fields of cell biology and immunology. More particularly, provided herein are novel antigen-binding molecules that

specifically bind an engulfment receptor expressed on the surface of a phagocytic cell and activate the endogenous phagocytic signaling pathway. Also provided are, *inter alia*, recombinant cells such as phagocytic cells that have been modified to include a targeted effector activity directed against a cell of interest such as, *e.g.*, a cancer cell. This novel methodology, termed “Chimeric Antigen Receptors for Phagocytosis” (CAR-P), represents a new cellular therapeutic approach targeting cancerous cells. The disclosure also provides compositions and methods useful for producing such antigen-binding molecules, as well as methods for the treatment of health disorders associated with cancer, including solid tumor and hematologic malignancy.

[0010] In one aspect, disclosed herein is a pharmaceutical composition including a pharmaceutically acceptable carrier, and one or more of the following: (a) a chimeric polypeptide including: an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigen; a transmembrane domain; at least one copy of an intracellular signaling domain from an engulfment receptor; and at least one copy of a recruitment domain; (b) a nucleic acid encoding the polypeptide of (a); and (c) a recombinant cell including the polypeptide of (a) and/or the nucleic acid of (b).

[0011] In one aspect, disclosed herein is a chimeric polypeptide including: (a) an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigen; (b) a transmembrane domain; (c) at least one copy of an intracellular signaling domain from an engulfment receptor; and (d) at least one copy of a recruitment domain.

[0012] Implementations of embodiments of the chimeric polypeptide according to the present disclosure can include one or more of the following features. In some embodiments, the chimeric polypeptide further includes a signal peptide operably linked upstream (*e.g.*, N-terminally) to the extracellular domain. In some embodiments, the antigen-binding region includes an amino acid sequence encoding an antibody or a functional fragment thereof selected from the group consisting of an antigen-binding fragment (Fab), a single-chain variable fragment (scFv), a nanobody, a VH domain, a VL domain, a single domain antibody (sdAb), a VNAR domain, and a VHH domain, a bispecific antibody, a diabody, or a functional fragment thereof. In some embodiments, the one or more cancer-associated antigens is selected from the group consisting of CD19, CD22, HER2 (ERBB2/neu), Mesothelin, PSCA, CD123, CD30, CD171,

CD138, CS-1, CLECL1, CD33, CD79b, EGFRvIII, GD2, GD3, BCMA, PSMA, ROR1, FLT3, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3 (CD276), KIT (CD 117), CD213A2, IL-1 IRa, PRSS21, VEGFR2, CD24, MUC-16, PDGFR-beta, SSEA-4, CD20, MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, FAP, EphA2, GM3, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CD97, CD179a, ALK, and IGLL1. In some embodiments, the one or more cancer-associated antigens is selected from the group consisting of CD19 and CD22. In some embodiments of the disclosure, the intracellular signaling domain of the engulfment receptor includes at least 1, at least 2, at least 3, at least 4, or at least 5 immunoreceptor tyrosine-based activation motifs (ITAMs). In some embodiments, the intracellular signaling domain from the engulfment receptor is capable of mediating endogenous phagocytic signaling pathway. In some embodiments, the engulfment receptor is selected from the group consisting of Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, and Axl.

[0013] In some embodiments, the transmembrane domain includes a stalk and/or transmembrane domain derived from CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, Axl, CD45, or CD86. In some embodiments, the transmembrane domain is operably linked downstream of the extracellular domain and upstream of the at least one copy of the intracellular signaling domain from the engulfment receptor. In some embodiments, the signal peptide includes a signal peptide derived from CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, and/or Axl.

[0014] In some embodiments, the at least one copy of a recruitment domain includes: (a) a p85-binding domain derived from CD19, Gab2, IREM-1, PDGF receptor, CSFR-1, c-Kit, ErbB3, or CD7 to recruit the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K); (b) an SH3 domain derived from Crk, Cdc25, Phospholipase, Ras, Vav, GRB2, FAK, Pyk2, TRIP10, or Gads to recruit cytosolic signaling proteins; and/or (c) a proline-rich peptide from C3G, p41, PEP, p47, HPK1, SLP-1, CD3 ϵ , PAK, AIP4, or Sos to recruit SH3 domain-containing proteins. In some embodiments, the recruitment domain is operably linked downstream to the at least one copy of the intracellular signaling domain of the engulfment receptor. In some embodiments, the recruitment domain is operably linked downstream (*e.g.*, C-terminally) to the transmembrane

domain and upstream to the at least one copy of the intracellular signaling domain of the engulfment receptor. In some embodiments of the disclosure, the chimeric polypeptide further includes one or more peptide linker sequences. In some embodiments, the one or more peptide linker sequences includes a glycine-serine linker.

[0015] In some embodiments of the present disclosure, the chimeric polypeptide includes (a) a peptide signal; (b) an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigens; (c) a transmembrane domain; (d) an intracellular signaling domain from an engulfment receptor; and (e) a p85-recruitment domain. In some embodiments, the chimeric polypeptide includes (a) a peptide signal from CD8; (b) an extracellular single chain antibody variable fragment (scFv) having specific affinity for CD19 or CD22; (c) a transmembrane domain from CD8; (d) an intracellular signaling domain from Megf10, FcR γ , Bai1, or MerTK; and (e) a p85-recruitment domain from CD19.

[0016] In some embodiments, the chimeric polypeptide of the disclosure includes an amino acid sequence having at least 80% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

[0017] In one aspect, some embodiments of the disclosure relate to an isolated, synthetic, or recombinant nucleic acid molecule including a nucleic acid sequence encoding a polypeptide that includes an amino acid sequence having at least 90% identity to the amino acid sequence of a chimeric polypeptide as disclosed herein, or a functional fragment thereof. In some embodiments, the nucleic acid sequence has at least 80% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18. In some embodiments, the nucleic acid sequence is operably linked to a heterologous nucleic acid sequence. In some embodiments, the nucleic acid molecule of the disclosure is an expression cassette or an expression vector.

[0018] In another aspect, some embodiments of the disclosure relate to a recombinant cell including an isolated, synthetic, or recombinant nucleic acid molecule as disclosed herein. In some embodiments, the recombinant cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the recombinant cell is a human cell. In some embodiments, the recombinant cell is a phagocytic cell. In some embodiments, the phagocytic cell is selected from the group

consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a bone marrow derived macrophage (BMDM) or a bone marrow derived dendritic cell (BMDC). In another aspect, some embodiments of the disclosure relate to a cell culture including at least one recombinant cell as disclosed herein and a culture medium.

[0019] In one aspect, some embodiments of the disclosure relate to a pharmaceutical composition including: (a) a chimeric polypeptide as described herein; (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein; and/or (c) a recombinant cell as described herein, and a pharmaceutically acceptable carrier. In some embodiments, the recombinant cell is a human cell. In some embodiments, the recombinant cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a bone marrow derived macrophage (BMDM) or a bone marrow derived dendritic cell (BMDC).

[0020] In another aspect, some embodiments of the disclosure relate to method for modifying a cell, including introducing into the cell (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the cell is a phagocytic cell. In some embodiments, the phagocytic cell is selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes.

[0021] In another aspect, some embodiments of the disclosure relate to method for treating an individual having cancer, which includes administering to the individual an effective numbers of phagocytic cells that have been modified to express a chimeric engulfment receptor having specific affinity for an antigen associated with a cancer cell. In some embodiments, the cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a BMDM or a BMDC. In some embodiments, the phagocytic cell is derived from the same individual having cancer.

[0022] In another aspect, some embodiments of the disclosure relate to method for treating an individual having cancer, which includes administering to the individual an effective amount or number of: (a) a chimeric polypeptide as described herein; (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein; (c) a recombinant cell as described

herein; and/or a pharmaceutical composition as described herein. In some embodiments, the administration includes a viral-, particle-, liposome-, or exosome-based delivery procedure. In some embodiments, the administration includes delivering into endogenous cells *ex vivo* one or more gene-editing enzymes targeting (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the administration includes delivering into cells *in vivo* one or more gene-editing enzymes targeting (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the administration activates phagocytosis of said cancer cell. In some embodiments, the activated phagocytosis includes whole-cell internalization and/or bite internalization. In some embodiments, the cancer is selected from the group consisting of breast cancer, ovarian cancer, lung cancer, pancreatic cancer, mesothelioma, leukemia, lymphoma, brain cancer, prostate cancer, multiple myeloma, melanoma, bladder cancer, bone sarcomas, soft tissue sarcomas, retinoblastoma, renal tumors, neuroblastoma, and carcinomas.

[0023] In yet another aspect, provided herein is a kit having any of the composition described above and further having instructions for use.

[0024] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative embodiments and features described herein, further aspects, embodiments, objects and features of the disclosure will become fully apparent from the drawings and the detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIGS. 1A-1E** summarize results from experiments performed to identify intracellular signaling region for CAR-P. **FIG. 1A** schematically depicts the structure of number of non-limiting examples of the CAR-P constructs described herein. An α CD19 (left and right constructs) or α CD22 (center) scFv directs CAR specificity. Intracellular signaling domains from Megf10 or the indicated engulfment receptor activate engulfment. CAR-P^{GFP} contains only GFP and no intracellular signaling domains (right). All constructs include a transmembrane domain from CD8 and a C-terminal GFP. **FIG. 1B:** J774A.1 macrophages expressing α CD19 CAR-P with the indicated intracellular signaling domain engulf 5 μ m silica beads covered with a supported lipid bilayer containing His-tagged CD19 extracellular domain. The beads were

visualized with atto390-labeled lipid incorporated into the supported lipid bilayer. Cells infected with the cell membrane marker, mCherry-CAAX, were used as a control (no CAR, top left). To the right of each image is a histogram depicting the frequency of cells engulfing the indicated number of beads. The average number of beads eaten per cell is quantified in **FIG. 1C**. As shown in **FIG. 1D**, bone marrow derived macrophages (BMDMs) were infected with CAR- $P^{FcR\gamma}$ or GFP-CAAX (green, left and center top; grey, center bottom) and incubated with CD19 beads (magenta) for 45 min. Images show an x-y plane through the center of the engulfed beads (left), or a cross section (center) of the z plane indicated in the inset panel (white line). The histogram depicts the number of cells engulfing the indicated number of beads. The scale bar indicates 5 μ m and $n = 78$ – 163 cells per condition, collected during three separate experiments. Error bars denote 95% confidence intervals and *** indicates $p < 0.0001$ compared to mCherry-CAAX control by Kruskal-Wallis test with Dunn's multiple comparison correction. **FIG. 1E** depicts a non-limiting example of the expression level of CAR-P constructs in macrophages. Images of macrophages infected with various α CD19 CAR- P^{GFP} constructs were acquired with identical acquisition settings and scaling to depict differences in expression levels. Fluorescent intensity at the cell cortex of 20 representative α CD19 CAR- P^{GFP} -infected macrophages was quantified using the mean intensity of a 2 pixel width linescan at the cell membrane, minus the mean intensity of a linescan immediately adjacent to the cell. The images are the same cells included in **FIG. 1B** and fluorescent intensity was measured from the same macrophages assayed in **FIG. 1C**. The scale bar indicates 5 μ m.

[0026] FIGS. 2A-2B illustrate a non-limiting example of specific engulfment of diverse beads driven by CAR-P expression. **FIG. 2A**: Macrophages infected with the α CD19 or α CD22 CAR- P^{Megf10} or mCherry-CAAX control were fed 5 μ m beads ligated with either CD19 (left panel) or CD22 (right panel). Engulfment is quantified as the mean beads eaten per cell. The fraction of phagocytic cells is as follows: 31/144 GFP-CAAX cells engulfed CD19 beads, 87/149 α CD19 CAR- P^{Megf10} engulfed CD19 beads, 20/142 α CD22 CAR- P^{Megf10} engulfed CD19 beads, 28/140 GFP-CAAX cells engulfed CD22 beads, 18/151 α CD19 CAR- P^{Megf10} engulfed CD22 beads, 103/148 α CD22 CAR- P^{Megf10} engulfed CD22 beads (pooled data was collected during three separate experiments). Error bars denote 95% confidence intervals and *** indicates $p < 0.0001$ compared to mCherry-CAAX control by Kruskal-Wallis test with Dunn's multiple comparison correction. **FIG. 2B**: J774A.1 macrophages expressing the α CD19 CAR- P^{Megf10} were

fed beads of various sizes (diameter of bead indicated below image). The beads were covered in a supported lipid bilayer ligated to His-tagged CD19 extracellular domain and the number of beads engulfed per cell is reported below each image. Dark gray bars (first bars from the left on X-axis) indicate CAR-P^{Megf10} macrophages and light gray bars indicate CAR-P^{GFP} (second bars from the left on X-axis). The α CD19 CAR-P^{Megf10} macrophages were also incubated with 10 mm beads coated in phosphatidylserine (PS) and ICAM-1 (third bar in the middle graph, 51/390 cells engulfed a bead). The fraction of cells engulfing a CD19 bead is as follows: 135/169 CAR-P^{Megf10} and 134/187 CAR-P^{GFP} cells engulfed a 2.5 μ m bead, 126/395 CAR-P^{Megf10} and 112/499 CAR-P^{GFP} cells engulfed a 5 μ m bead, 48/377 CAR-P^{Megf10} and 21/378 CAR-P^{GFP} cells engulfed a 10 μ m bead, 120/706 CAR-P^{Megf10} and 45/675 CAR-P^{GFP} cells engulfed a 15 μ m bead, 194/760 CAR-P^{Megf10} and 23/587 CAR-P^{GFP} cells engulfed a 20 μ m bead (data is pooled from at least three separate experiments). Error bars denote 95% confidence intervals of the mean. *** indicates $p < 0.0001$ respectively by Mann-Whitney test. All scale bars represent 5 μ m.

[0027] FIGS. 3A-3E illustrate a non-limiting example of engulfment driven by phosphorylated ITAM at the cell-target synapse. **FIG. 3A:** Macrophages expressing α CD19 CAR-P^{Megf10} (top) or α CD19 CAR-P^{GFP} were incubated with CD19-ligated beads (position indicated with dotted line), fixed and stained for phosphotyrosine (magenta, top; greyscale, bottom). The fold enrichment of phosphotyrosine at the cell-bead synapse compared to the cell cortex is graphed on the right ($n \geq 11$; each dot represents one cell-bead synapse; lines represent the mean $\pm$ one standard deviation). **FIG. 3B:** Schematic shows the structure of CAR-P constructs in the plot at right. An α CD19 (dark gray) scFv directs CAR specificity. The intracellular signaling domains from CD3 ζ activate engulfment. On the right is a histogram depicting the fraction of macrophages engulfing the indicated number of CD19-coated beads. **FIG. 3C:** Comparison showing the average number of beads eaten per cell in J774A.1 macrophages expressing α CD19 CAR-Ps with the indicated intracellular signaling domain. 5 mm silica beads covered with a supported lipid bilayer containing His-tagged CD19 extracellular domain were used as an engulfment target ($n = 156$ – 167 cells per condition collected during three separate experiments). Error bars denote 95% confidence intervals and *** indicates $p < 0.0001$ compared to CAR-P^{GFP} control by Kruskal-Wallis test with Dunn's multiple comparison in correction. **FIG. 3D:** Model of the liposome-based fluorescence quenching assay used to determine affinity between the Syk tSH2 domains and the receptor tails of CD3 ζ and

FcRV, two intracellular signaling domains that promote engulfment. Binding between the Syk tSH2 reporter (Syk tSH2) and a receptor tail, gray was detected by rhodamine quenching of BG505 dye on the reporter (see Example 1 below). K_d was determined by assessing mean fluorescence quenching for the last 20 timepoints collected ~45 minutes after ATP addition over a receptor titration from 0 to 500 nM. Each point represents the mean $\pm$ SD from three independent experiments. $K_d \pm$ SE was calculated by nonlinear fit assuming one site specific binding. **FIG. 3E** depicts a non-limiting example of the enrichment of F-actin at the cell-target synapse. Phalloidin staining (magenta overlayed with brightfield and DAPI, left; heatmap, right) of F-actin in a CAR-P^{FcR γ} expressing macrophage shows a 2.3 fold enrichment at the cell-bead synapse (standard deviation of 1.2). The graph depicts actin enrichment at 30 cell-bead synapses collected on three separate days. Each dot represents a cell-bead synapse. The box plot indicates the interquartile range. The cell is in contact with a second bead, but this site of contact has not initiated cup formation and no actin enrichment.

[0028] FIGS. 4A-4F illustrate a non-limiting example of CAR-P promoting trogocytosis and whole cell eating. **FIG. 4A:** J774A.1 macrophages expressing the α CD19 CAR-P^{Megf10} (top panel, left; greyscale, center) engulf pieces of CD19 +Raji B cells (labeled with mCherry-CAAX; magenta in merge, left; greyscale, right). The corresponding control α CD19 CAR-P^{GFP}-infected cells are shown below. Arrows point to pieces of ingested Raji B cell. The proportion of CAR-P expressing macrophages internalizing one or more bite within 90 minutes is quantified on the right. Bites are defined as a fully internalized mCherry-positive vesicle >1 μ m in diameter; n = 46 CAR-P^{Megf10} macrophages, n = 39 CAR-P^{FcR γ} macrophages and 102 CAR-P^{GFP} macrophages acquired during three separate experiments. **FIG. 4B:** Time course of a J774A.1 macrophage expressing CAR-P^{FcR γ} internalizing a whole Raji B cell labeled with mCherry-CAAX. **FIG. 4C:** Schematic shows the structure of CAR-P^{tandem} construct, combining the intracellular signaling domain from FcRV and a p85-recruitment domain from CD19. **FIG. 4D:** Time course of a J774A.1 macrophage expressing CAR-P^{tandem} internalizing a whole Raji B cell labeled with mCherry-CAAX. **FIG. 4E:** Macrophages and Raji B cells were incubated together at a 1:2 macrophage:Raji ratio, and the number of whole Raji B cells eaten per 100 macrophages during 4–8 hours of imaging is graphed. Graph depicts pooled data from four independent experiments; n = 921 CAR-P^{GFP}, n = 762 CAR-P^{FcR γ} , n = 638 CAR-PPI3K, n = 555 CAR-P^{tandem} cells. Sample sizes were selected for their ability to detect a 5% difference between

samples with 95% confidence. **FIG. 4F:** 10,000 macrophages and 20,000 Raji B cells were incubated together for 44 hr. The number of Rajis was then quantified by FACS. 2–3 technical replicates were acquired each day on three separate days. The number of Rajis in each replicate was normalized to the average number present in the GFP-CAAX macrophage wells on that day. * indicates $p < 0.01$, *** indicates $p < 0.0001$ by two-tailed Fisher Exact Test (a and e) or by Ordinary one way ANOVA with Dunnet's correction for multiple comparisons (f); error bars denote 95% confidence intervals.

[0029] FIG. 5 pictorially summarizes the results of experiments performed to demonstrate that CAR-P localizes with pTyr at synapse with Raji B cell. Phosphotyrosine staining (teal) of macrophages expressing CAR-P^{Megf10} in contact with Raji B cells (cell membrane visualized with mCherry-CAAX. Below, the enrichment at the synapse is quantified as the mean intensity of a five pixel width linescan at the synapse divided by the mean intensity at the adjacent cell cortex for at least 11 sites of contact. Each dot represents one cell-cell synapse, lines represent the mean $\pm$ one standard deviation, and the graph is the pooled results of three biological replicates. The scale bar indicates 5 μ m.

[0030] FIG. 6 pictorially summarizes the results of experiments demonstrating that NIH 3T3 cells internalize Raji B cell bites. NIH 3T3 cells expressing the α CD19 CAR-P^{Megf10} (left; greyscale, center) engulf pieces of CD19 +Raji B cells (labeled with mCherry-CAAX; magenta in merge, left; greyscale, right). The control α CD19 CAR-P^{GFP}-infected 3T3s are shown below. Arrows point to pieces of ingested Raji B cell. The proportion of cells taking at least one bite after 90 minutes co-incubation is graphed on the left (graphs show the pooled data of three separate experiments; $n = 111$ CAR-P^{Megf10} 3T3 cells and 121 CAR-P^{GFP} 3T3; *** indicates $p < 0.0001$ by two-tailed Fisher Exact Test; error bars denote 95% confidence intervals). Bites are defined as a fully internalized piece of mCherry-labeled material $>1 \mu$ m in diameter.

[0031] FIG. 7 graphically summarizes the results of experiments showing that opsonization by an anti-CD47 antibody enhances whole cell internalization through CAR-P. Macrophages expressing CAR-P^{FcR γ} and Raji B cells were incubated together at a 1:2 macrophage:Raji ratio (20,000 macrophages and 40,000 Rajis) without antibody addition (No ab) or in the presence of anti-CD19 or anti-CD47 antibodies as indicated. The number of whole Raji B cells eaten per 100 macrophages during 4–8 hours of imaging is graphed. Graph depicts pooled data from three independent experiments; $n = 232$ with no antibody, $n = 257$ with anti-

CD19 antibody, n = 347 with anti-CD47 antibody; * indicates $p < 0.05$ by two-tailed Fisher Exact test.

[0032] FIGS. 8A-8D summarize the results of experiments demonstrating that CAR-P promotes internalization of cancer antigen. **FIG. 8A:** Schematic of antigen internalization and cross-presentation assay. CAR-P expressing BMDC cells were differentiated using GM-CS. CAR-P BMDC were incubated with Raji B cells expressing soluble ovalbumin (OVA). DC with OVA bites (internalized antigen) were then incubated with OTI T cells (OVA specific CD8 + T cells) and OTI proliferation assessed as a measure of T cell stimulation. Results from each step of this assay are shown in sequence in **FIGS. 8B-8D**. **FIG. 8B:** Ovalbumin staining in Raji B cells infected with mCherry-CAAX-p2a-Ovalbumin lentivirus (OVA) and uninfected controls (uninfected) shows robust OVA expression in infected cells. At right the intracellular OVA signal is plotted as corrected total cell fluorescence (CTCF) for the ovalbumin channel. Each dot represents the CTCF of one cell; n = 26 cells OVA, n = 33 cells (uninfected); lines represent the mean $\pm$ one standard deviation, and the graph is the pooled results of three biological replicates. The scale bar indicates 5 μ m. **FIG. 8C:** Bone marrow-derived dendritic cells expressing the CAR-P^{FcR γ} (top panel, left; greyscale, center) engulf pieces of CD19 + Raji B cells (labeled with mCherry-CAAX; magenta in merge, left; greyscale, right). The control α CD19 CAR-P^{GFP}-infected dendritic cells are shown below. Arrows point to pieces of ingested Raji B cell. The proportion of cells taking at least one bite after 90 minutes co-incubation is graphed on the right of images. Graphs show the pooled data of two separate experiments; n = 28 CAR-P^{FcR γ} dendritic cells and n = 33 CAR-P^{GFP} dendritic cells; *** indicates $p < 0.0001$ by two-tailed Fisher Exact Test; error bars denote 95% confidence intervals. Bites are defined as a fully internalized piece of mCherry-labeled material $> 1 \mu$ m in diameter. **FIG. 8D:** OTI T cell proliferation after 72 hours incubation with CAR-P transduced CD11c + dendritic cells. $\pm$ RAJI below the x-axis indicates whether Raji-OVA B cells were added to CAR-P transduced dendritic cells prior to OTI addition. To measure proliferation, T cells were uniformly stained with eFluor670 dye on day 0, and proliferation was measured by dilution of the cell-bound dye. Graphs show the mean $\pm$ SD of three independent biological replicates. Data points are values for individual wells of differentiated CD11c + dendritic cells. Boxed data indicate the mean % T cells dividing when dendritic cells were pulsed with SL8 (OVA) peptide, which directly binds to MHC without undergoing cross presentation. If dendritic cell differentiation was successful, the

pulsed dendritic cells should be capable of inducing robust OTI proliferation. Sample sizes were selected to match previous studies that were able to detect robust T cell stimulation (Roberts *et al.*, 2016).

[0033] FIG. 9 graphically summarizes the results of experiments illustrating that certain CAR-P provided in the present disclosure are effective in mouse primary macrophages (BMDMs) as demonstrated in a whole-cell eating assay.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0034] The present disclosure relates generally to the fields of cell biology and immunology. More particularly, provided herein is a new class of engineered chimeric antigen receptors (CARs) that specifically bind an engulfment receptor expressed on the surface of a phagocytic cell, activate the endogenous phagocytic signaling pathway, and thereby generate a modified phagocytic cell with targeted effector activity directed against a cell of interest such as, *e.g.*, a cancer cell. By expressing a CAR disclosed herein in a phagocytic cell, the modified phagocytic cell is recruited to the tumor microenvironment where it acts as a potent immune effector by infiltrating the tumor and killing the target cells. This novel methodology, termed “Chimeric Antigen Receptors for Phagocytosis” (CAR-P), represents a new cellular therapeutic approach targeting cancerous cells. This is because the CAR-Ps disclosed herein represent the first synthetic receptors to engineer macrophage signaling pathways to direct the innate immune system to attack cancer. One aspect of the disclosure includes a modified phagocytic cell and pharmaceutical compositions including the modified phagocytic cell for adoptive cell therapy and treating a disease or condition associated with cancer.

[0035] Immune system constantly patrols human body, looking to eliminate cancerous cells and harmful microbes. It can spot these threats because it recognizes certain signals at the surface of dangerous cells. However, cancer cells often find ways to ‘hide’ from the immune system. Current cellular therapeutics are almost exclusively T cells. In contrast, macrophages are uniquely capable of enriching in the tumor microenvironment, where T cells are often excluded. Accordingly, a person skilled in the art will readily appreciate that this is a different and yet potentially synergistic approach with existing CAR-T therapy. In addition, the CAR-P phagocytes has potential advantages over CAR-T therapy in that CAR-Ps, and drug conjugates thereof, could present all possible cancer antigens from an individual, rather than restricting T

cell killing to a single predefined antigen. Thus, by engineering macrophage signaling rather than T cell signaling, the killed artisan will be able to open new therapeutic avenues. One skilled in the art will also appreciate that CAR-Ps can be used for cross presentation using different cell types. As discussed in greater detail below, it is also contemplated that exosomes from CAR-P expressing cells can constitute an efficient means of antigen-presentation to T cells. As described below in some embodiments of the disclosure, the macrophages can be isolated from individuals, transfected with a CAR-P and introduced back into an individual to destroy cancer cells. As a proof of principle, over two days, a lymphoma cell line co-cultured with macrophages expressing the CAR-P exhibited a 40% decrease in cell number compared to the lymphoma cells cultured with un-edited macrophages. Thus, this receptor could serve as the foundation for a macrophage-based cellular therapeutic targeting leukemia and lymphoma.

[0036] Chimeric antigen receptors, or CARs, are receptors designed in a laboratory to attach to specific proteins that are found on a cancer cell. These receptors tell immune cells, such as T cells, to attack cancers. T cells that carry CARs are already used to treat people with blood cancers. Yet, these immune cells are not good at penetrating a solid tumor to kill the cells inside, which limits their use.

[0037] CARs can be synthetic transmembrane receptors that redirect T cell activity towards clinically relevant targets (reviewed in [Lim *et al.*, 2017; Fesnak *et al.*, 2016]). The CAR-T receptor contains an extracellular single chain antibody fragment (scFv) that recognizes known tumor antigens, and intracellular signaling domains from the T Cell Receptor (TCR) and costimulatory molecules that trigger T cell activation (Fesnak *et al.*, 2016; Kochenderfer *et al.*, 2009). CAR-T cells recognizing CD19, a marker expressed at high levels on the surface of B cells and B cell-derived malignancies, have been used successfully to target hematological malignancies with 70–90% of individuals showing measurable improvement (Lim *et al.*, 2017; Engel *et al.*, 1995; Haso *et al.*, 2013). The success of CAR-T suggests that programming immune cells to target cancer might be a broadly applicable approach.

[0038] In some embodiments, a CAR-P DNA vector or mRNA could be delivered to endogenous phagocytic cells (macrophages or dendritic cells). As described in further detail below, there are no specific limitations with respect to the procedures and techniques that can be suitably employed for delivery of the CAR-P DNA vector or mRNA into the target phagocytic cell. Non-limiting delivery procedures suitable for the methods disclosed herein include stable or

transient transfection, lipofection, electroporation, microinjection, liposomes, iontophoresis, and infection with recombinant viral vectors. In some embodiments, the CAR-P DNA vector or mRNA could be delivered to endogenous phagocytic cells by using one or more viral, particle, liposome, or exosome –based strategies. Such a strategy would target phagocytic cells towards cancer cells.

[0039] Macrophages and dendritic cells are also antigen-presenting cells. Therefore, without being bound any particular theory, it is also contemplated that, in various embodiments of the disclosure, by targeting phagocytic cells towards cancer cells in one of the two strategies above, it is possible that these antigen-presenting cells could internalize and cross-present antigens to T cells, which would engage in cytotoxic killing of cancer cells.

[0040] As described above, CARs can be synthetic receptors that reprogram T cells to kill cancer. The success of CAR-T cell therapies highlights the promise of programmed immunity and suggests that applying CAR strategies to other immune cell lineages may be beneficial. In some embodiments, t provided is a family of engineered CARs for phagocytosis (CAR-Ps) that direct macrophages to engulf specific targets, including cancer cells. In some embodiments, the CAR-Ps of the disclosure include an extracellular antibody fragment, which can be modified to direct CAR-P activity towards specific antigens. As described in greater detail below, by screening a panel of engulfment receptor intracellular domains, it was found that the cytosolic domains from Megf10 and FcR γ robustly triggered engulfment independently of their native extracellular domain. It was further found that CAR-Ps drive specific engulfment of antigen-coated synthetic particles and whole human cancer cells. Furthermore, addition of a tandem PI3K recruitment domain increased cancer cell engulfment. Finally, it was also found that CAR-P expressing murine macrophages reduce cancer cell number in co-culture by over 40%.

[0041] In some embodiments, the present disclosure describes the design and construction of a family of engineered CAR-Ps, and their respective expression in modified macrophages. These modified macrophages were found to be able to recognize and attack beads covered in proteins found on cancer cells. The modified macrophages were shown to also limit the growth of live cancer cells in a dish by ‘biting’ and even ‘eating’ them. Since these results are promising in the laboratory, it is also contemplated, in various embodiments, determining whether these reprogrammed macrophages can recognize and fight cancers in living animals.

The disclosure also provides compositions and methods useful for producing such CARs, as well as methods for the treatment of health disorders associated with cancer, including solid tumor and hematologic malignancy.

[0042] In some particular embodiments, the disclosure describes engineered CAR-Ps that are capable of promoting engulfment of B cell derived cancer cells and cancer antigen-coated particles. In some embodiments, the CAR-P disclosed herein includes an antibody fragment directed against CD19 (*e.g.*, amino acid residues 23-130 Genbank AMZ04819) or CD22 (*e.g.*, amino acid residues 22-258 of translated Genbank AMZ04819) fused to the intracellular domain of either mouse Megf10 (*e.g.*, amino acid residues 879-1147 of Uniprot Q6DIB5) or FcRγ (*e.g.*, amino acid residues 19-86 of Uniprot P20491). In some embodiments, targeting of the CAR-P disclosed herein to the plasma membrane was achieved by addition of the CD8 signal peptide (*e.g.*, amino acid residues 1-21 of Uniprot Q96QR6_HUMAN) to the N terminus and insertion of the CD8 stalk and transmembrane domains (*e.g.*, amino acid residues 138-206 Uniprot Q96QR6_Human) between the antibody fragment and cytosolic signaling domain. In some embodiments, the cytosolic domains of Megf10 and FcRγ, when fused to the indicated antibody fragments, robustly triggered engulfment of antigen-coated particles, “bites” of cancer cells, and whole cancer cells. In some embodiments, addition of a PI3K recruitment domain (amino acids 500-534 mouse CD19 (Uniprot CD19_MOUSE) to the FcRγ intracellular domain increased engulfment of whole cancer cells. Remarkably, as evidence of therapeutic potential, CAR-P expressing macrophages reduce cancer cell number in co-culture by over 40%. Taken together, these results demonstrate that the CAR-Ps of the disclosure can robustly trigger the phagocytosis of cancer cell material, whole cancer cells, and cancer antigen-coated synthetic particles.

DEFINITIONS

[0043] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology

by those skilled in the art.

[0044] The singular form “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes one or more cells, comprising mixtures thereof. “A and/or B” is used herein to include all of the following alternatives: “A”, “B”, “A or B”, and “A and B”.

[0045] The term “about”, as used herein, has its ordinary meaning of approximately. As such, the term “about” is used to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. If the degree of approximation is not otherwise clear from the context, “about” means either within plus or minus 10% of the provided value, or rounded to the nearest significant figure, in all cases inclusive of the provided value. Where ranges are provided, they are inclusive of the boundary values.

[0046] The term “operably linked”, as used herein, denotes a physical or functional linkage between two or more elements, *e.g.*, polypeptide sequences or polynucleotide sequences, which permits them to operate in their intended fashion. For example, an operably linkage between a polynucleotide of interest and a regulatory sequence (for example, a promoter) is functional link that allows for expression of the polynucleotide of interest. In this sense, the term “operably linked” refers to the positioning of a regulatory region and a coding sequence to be transcribed so that the regulatory region is effective for regulating transcription or translation of the coding sequence of interest. In some embodiments disclosed herein, the term “operably linked” denotes a configuration in which a regulatory sequence is placed at an appropriate position relative to a sequence that encodes a polypeptide or functional RNA such that the control sequence directs or regulates the expression or cellular localization of the mRNA encoding the polypeptide, the polypeptide, and/or the functional RNA. Thus, a promoter is in operable linkage with a nucleic acid sequence if it can mediate transcription of the nucleic acid sequence. Operably linked elements may be contiguous or non-contiguous. In addition, in the context of a polypeptide, “operably linked” refers to a physical linkage (*e.g.*, directly or indirectly linked) between amino acid sequences (*e.g.*, different segments, regions, or domains) to provide for a described activity of the polypeptide. In the present disclosure, various segments,

regions, or domains of the chimeric polypeptides of the disclosure may be operably linked to retain proper folding, processing, targeting, expression, binding, and other functional properties of the chimeric polypeptides in the cell. Unless stated otherwise, various regions, domains, and segments of the chimeric polypeptides of the disclosure are operably linked to each other. Operably linked regions, domains, and segments of the chimeric polypeptides of the disclosure may be contiguous or non-contiguous (*e.g.*, linked to one another through a linker).

[0047] The term “recombinant” or “engineered” nucleic acid molecule as used herein, refers to a nucleic acid molecule that has been altered through human intervention. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *in vitro* polymerase reaction(s), or to which linkers have been attached, or that has been integrated into a vector, such as a cloning vector or expression vector. As non-limiting examples, a recombinant nucleic acid molecule can be one which: 1) has been synthesized or modified *in vitro*, for example, using chemical or enzymatic techniques (for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, *e.g.*, methylation), or recombination (including homologous and site-specific recombination)) of nucleic acid molecules; 2) includes conjoined nucleotide sequences that are not conjoined in nature; 3) has been engineered using molecular cloning techniques such that it lacks one or more nucleotides with respect to the naturally occurring nucleic acid molecule sequence; and/or 4) has been manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally occurring nucleic acid sequence. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *in vitro* polymerase reaction(s), or to which linkers have been attached, or that has been integrated into a vector, such as a cloning vector or expression vector.

[0048] As used herein, a “subject” or an “individual” includes animals, such as human (*e.g.*, human subjects) and non-human animals. In some embodiments, a “subject” or “individual” is a patient under the care of a physician. Thus, the subject can be a human patient or an individual who has, is at risk of having, or is suspected of having a disease of interest (*e.g.*, cancer) and/or one or more symptoms of the disease. The subject can also be an individual who is diagnosed with a risk of the condition of interest at the time of diagnosis or later. The term

“non-human animals” includes all vertebrates, *e.g.*, mammals, *e.g.*, rodents, *e.g.*, mice, and non-mammals, such as non-human primates, *e.g.*, sheep, dogs, cows, chickens, amphibians, reptiles, *etc.*

[0049] The term “vector” is used herein to refer to a nucleic acid molecule or sequence capable of transferring or transporting another nucleic acid molecule. The transferred nucleic acid molecule is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. Generally, a vector is capable of replication when associated with the proper control elements. The term “vector” includes cloning vectors and expression vectors, as well as viral vectors and integrating vectors. An “expression vector” is a vector that includes a regulatory region, thereby capable of expressing DNA sequences and fragments *in vitro* and/or *in vivo*. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, *e.g.*, replication defective retroviruses and lentiviruses. In some embodiments, a vector is a gene delivery vector. In some embodiments, a vector is used as a gene delivery vehicle to transfer a gene into a cell.

[0050] It is understood that aspects and embodiments of the disclosure described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

[0051] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0052] Headings, *e.g.*, (a), (b), (i) *etc.*, are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

PHAGOCYTOSIS AND PHAGOCYTES

[0053] Phagocytosis generally refers to an engulfment process of cells or large particles ($> 0.5 \mu\text{m}$) wherein tethering of a target cell or particle, engulfment of the target cell or particle, and degradation of the internalized target cell or particle occurs. In certain embodiments, phagocytosis includes formation of a phagosome that encompasses the internalized target cell or particle and phagosome fusion with a lysosome to form a phagolysosome, wherein the contents therein are degraded. As such, "phagocytosis" includes the process of "efferocytosis", which specifically refers to the phagocytosis of apoptotic or necrotic cells in a non-inflammatory manner.

[0054] There are two principle types of phagocytosis, which are influenced by the target, cell-type and surrounding milieu. Anti-microbe phagocytosis clears and degrades disease-causing microbes, induces pro-inflammatory signaling through cytokine and chemokine secretion, and recruits immune cells to mount an effective inflammatory response. This type of phagocytosis is often referred to as "inflammatory phagocytosis" (or "immunogenic phagocytosis"). However, in some instances, such as with certain persistent infections, anti-inflammatory responses may follow microbial uptake. Anti-microbe phagocytosis is commonly performed by professional phagocytes of the myeloid lineage, such as immature dendritic cells (DCs) and macrophages and by tissue-resident immune cells.

[0055] Phagocytosis of damaged, self-derived apoptotic cells or cell debris (*e.g.*, efferocytosis), in contrast, is typically a non-inflammatory (also referred to as a "non-immunogenic") process. Billions of damaged, dying, and unwanted cells undergo apoptosis each day. Unwanted cells include, for example, excess cells generated during development, senescent cells, infected cells (intracellular bacteria or viruses), transformed or malignant cells, and cells irreversibly damaged by cytotoxic agents. Phagocytes execute specific, swift removal of apoptotic cells without causing damage to the surrounding tissues or inducing a pro-inflammatory immune response. Steps for apoptotic cell clearance include: (1) release of "find me" signals from apoptotic cells to recruit phagocytes to the location of apoptotic cells; (2) "eat me" signals exposed on the surface of apoptotic cells are bound by phagocytes via specific receptors; (3) cytoskeletal rearrangement to engulf the apoptotic cell; and (4) the ingested apoptotic cell is digested and specific phagocytic responses are elicited (*e.g.*, secretion of anti-inflammatory cytokines).

[0056] The terms "phagocytic cells" and "phagocytes" are used interchangeably herein to refer to a cell that is capable of phagocytosis, *e.g.*, ingesting microorganisms and foreign particles, for example, capable of engulfing a large particulate mass, for example from about 0.1 μm in diameter up to about 2 mm or about 1 mm in diameter; from about 0.5 μm in diameter to about 1 mm in diameter, *etc.*, particularly including up to the size of a microbial cell or mammalian cell, *e.g.* a tumor cell. Phagocytosis, as described above, encompasses the engulfment of cells, pathogens, and various particles by surrounding it with the effector cell membrane. As such, phagocytes protect the body by ingesting harmful foreign particles, bacteria, and dead or dying cells. These cells are essential for fighting infections and for subsequent immunity.

[0057] There are several categories of phagocytes. Exemplary phagocytes include macrophages, mononuclear cells (histiocytes and monocytes), polymorph nuclear leukocytes, (neutrophils) and dendritic cells. Phagocytes of humans and other jawed vertebrates are divided into "professional" and "non-professional" groups based on the efficiency with which they participate in phagocytosis. The professional phagocytes include many types of white blood cells (such as neutrophils, monocytes, macrophages, mast cells, and dendritic cells). The main difference between professional and non-professional phagocytes is that the professional phagocytes have molecules called receptors on their surfaces that can detect harmful objects, such as bacteria, that are not normally found in the body. As such, professional phagocytes are capable of recognizing a wide variety of phagocytic targets, and of ingesting them at a higher rate than non-phagocytic cells.

[0058] Dendritic cell (DC) refers to any member of a diverse population of morphologically similar cell types found in lymphoid or non-lymphoid tissues. DCs are referred to as "professional" antigen presenting cells, and have a high capacity for sensitizing MHC-restricted T cells. DCs may be recognized by function, by phenotype and/or by gene expression pattern, particularly by cell surface phenotype. These cells are characterized by their distinctive morphology, high levels of surface MHC-class II expression and ability to present antigen to CD4⁺ and/or CD8⁺ T cells, particularly to naive T cells.

[0059] Neutrophils and macrophages are representative of fully differentiated phagocytes. While neutrophils leaving the bone marrow are fully differentiated, macrophages differentiate from circulating monocytes in extra-vascular tissues. Monocytes display a lower

phagocytic response, compared to neutrophils and macrophages, and must respond to activation and differentiation signals in order to achieve optimal phagocytic capacity. The process of monocyte-to-macrophage differentiation has been well characterized, and can be performed *in vitro* or *in vivo*.

[0060] Macrophages are of particular interest. These immune cells can make their way inside tumors and travel to cancers that the rest of the immune system cannot reach. Macrophages are critical effectors of the innate immune system, responsible for engulfing debris and pathogens. Accumulating evidence suggests that macrophages are abundant in the tumor microenvironment of numerous cancers where they can adopt a classically activated (M1, antitumor) or an alternatively activated (M2, pro-tumor) phenotype. Macrophages are potent effectors of the innate immune system and are capable of at least three distinct anti-tumor functions: phagocytosis, cellular cytotoxicity, and antigen presentation to orchestrate an adaptive immune response. While T cells require antigen-dependent activation via the T cell receptor or the chimeric immunoreceptor, macrophages can be activated in a variety of ways. Direct macrophage activation is antigen-independent, relying on mechanisms such as pathogen associated molecular pattern recognition by Toll-like receptors (TLRs). Therefore, harnessing macrophages to combat tumor growth is of longstanding interest (Alvey and Discher, 2017; Lee *et al.*, 2016). Macrophages are uniquely capable of penetrating solid tumors, while other immune cells, like T cells, are physically excluded or inactivated (Lim *et al.*, 2017; Lee *et al.*, 2016). This suggests that engineered macrophages may augment existing T cell-based therapies. Early efforts transferring healthy macrophages into cancer patients failed to inhibit tumor growth, suggesting that macrophages require additional signals to direct their activity towards tumors (Lacerna *et al.*, 1988; Andreessen *et al.*, 1990). Antibody blockade of CD47, a negative regulator of phagocytosis, reduced tumor burden, indicating that shifting the balance in favor of macrophage activation and engulfment is a promising therapeutic avenue (Majeti *et al.*, 2009; Chao *et al.*, 2010; Jaiswal *et al.*, 2009; Tseng *et al.*, 2013).

COMPOSITIONS OF THE DISCLOSURE

Chimeric Polypeptides and Chimeric Antigen Receptors

[0061] In one aspect, some embodiments of the disclosure relate to novel chimeric polypeptides which includes (a) an extracellular domain including an antigen-binding region

specific for one or more cancer-associated antigen; (b) a transmembrane domain; (c) at least one copy of an intracellular signaling domain from an engulfment receptor; and (d) at least one copy of a recruitment domain. In some aspects and embodiments of the disclosure, the chimeric polypeptides described herein are chimeric antigen receptors. The terms "binding region," "binding domain," and "binding moiety" as used herein refer to a molecule, such as a peptide, oligopeptide, polypeptide, or protein that possesses the ability to specifically and non-covalently bind, associate, unite, recognize, or combine with a target molecule, such as a cancer-associated antigen (*e.g.*, CD22, CD19, CD138, CD38, CD33, CD123, CD79b, Mesothelin, PSMA, BCMA, ROR1, MUC-16, CD22, CD19, EGFRvIII, VEGFR-2, or GD2). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. In some embodiments, the binding region is an antigen-binding region, such as an antibody or functional binding domain or antigen-binding portion thereof. The antigen-binding region can include any domain that binds to the antigen and may include, but is not limited to, a monoclonal antibody, a polyclonal antibody, a synthetic antibody, a human antibody, a humanized antibody, a non-human antibody, and any fragment thereof. Thus, in some embodiments, the antigen binding domain portion includes a mammalian antibody or a fragment thereof. Non-limiting examples of antigen-binding regions suitable for the chimeric polypeptides of this disclosure include an antigen-binding fragment (Fab), a single-chain variable fragment (scFv), a nanobody, a VH domain, a VL domain, a single domain antibody (sdAb), a VNAR domain, and a VHH domain, a bispecific antibody, a diabody, or a functional fragment of any thereof.

[0062] In some embodiments, the antigen-binding region is derived from the same cell type or the same species in which the chimeric polypeptide will ultimately be used in. For example, for use in humans, the antigen-binding region of the chimeric polypeptide includes a human antibody, a humanized antibody, or a fragment thereof.

[0063] As used herein, the term "chimeric" polypeptide refers to a polypeptide including at least two amino acid sequences, *e.g.*, polypeptide domains, that are not naturally linked in nature. The amino acid sequences may normally exist in separate proteins that are brought together in the chimeric polypeptide or they may normally exist in the same protein but are placed in a new arrangement in the chimeric polypeptide. A chimeric polypeptide may be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in

which the peptide regions are encoded in the desired relationship.

[0064] An "engulfment signaling domain" of an engulfment receptor refers to an intracellular effector domain, which, upon binding of the target molecule (*e.g.*, an cancer-associated antigen) targeted by the extracellular domain of the chimeric polypeptide expressed by a host cell, activates one or more signaling pathways in the host cell resulting in engulfment, including, in specific embodiments, cytoskeletal rearrangement of the host cell and internalization of the target cell, microbe, or particle associated with the antigen. In some embodiments, an engulfment signaling domain activates one or more signaling pathways resulting in phagocytosis of the target cell, microbe, or particle. In some embodiments, the engulfment signaling domain includes a primary engulfment signaling domain. In certain other embodiments, the engulfment signaling domain includes a primary engulfment signaling domain and a secondary engulfment signaling domain.

[0065] As described above, the chimeric polypeptides of the disclosure include at least one copy of a recruitment domain. The recruitment domain generally includes an intracellular polypeptide sequence capable of mediating protein-protein interaction with one or more cellular proteins, thereby can bind to and recruit such cellular proteins. A skilled artisan in the art will appreciate that the interaction and/or binding between a recruitment domain and a cellular protein can be direct or indirect interaction and/or binding. In some embodiments, the recruitment domain of the chimeric polypeptides disclosed herein directly binds to one or more cellular proteins. In some embodiments, the one or more cellular proteins to which the recruitment domain binds to (*e.g.*, recruited) include components of the phagocytic signaling pathway. In some embodiments, the binding of the recruitment domain to one or more phagocytic signaling components activates the endogenous phagocytic signaling pathway and/or enhances the activity of the chimeric receptor. In some embodiments, the recruitment domain binds to a cellular protein confers an increase in engulfment of the target cell, microbe, or particle associated with the antigen.

[0066] In some embodiments, the recruitment domain of the chimeric polypeptides disclosed herein includes a polypeptide sequences that is capable of interacting with, *e.g.*, binding to the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K). Generally, any polypeptide sequence capable of binding to p85 can be suitably used in a recruitment domain of the chimeric polypeptides disclosed herein. Examples of recruitment domains suitable for the

compositions and methods disclosed herein include, but are not limited to, p85-binding domains derived from CD19, Gab2, IREM-1, and PDGF receptor. Additional examples of suitable recruitment domains for the compositions and methods disclosed herein include p85-binding domains derived from CSFR-1, c-Kit, ErbB3, and CD7. In some embodiments, the recruitment domain of the chimeric polypeptides of the disclosure includes a p85-recruitment domain from CD19.

[0067] In some embodiments, the recruitment domain of the chimeric polypeptides disclosed herein includes a polypeptide sequences that is capable of interacting, *e.g.*, binding to a cytosolic signaling protein. Generally, any polypeptide sequence capable of binding to a cytosolic signaling protein can be suitably used in a recruitment domain of the chimeric polypeptides disclosed herein. Suitable examples of recruitment domains for the compositions and methods disclosed herein include, but are not limited to, SRC homology 3 domains (*e.g.*, SH3 domains). SH3 domain is generally described as a conserved sequence in the viral adaptor protein v-Crk. This domain is also present in the molecules of phospholipase and several cytoplasmic tyrosine kinases such as Abl and Src. SH3 has also been identified in several other protein families such as: PI3 Kinase, Ras GTPase-activating protein, CDC24 and CDC25. SH3 domains are found in proteins of signaling pathways regulating the cytoskeleton, the Ras protein, and the Src kinase and many others. Some SH3 proteins interact with adaptor proteins and tyrosine kinases. Approximately 300 SH3 domains are found in proteins encoded in the human genome. SH3 domain is also believed to be responsible for controlling protein-protein interactions in the signal transduction pathways and regulating the interactions of proteins involved in the cytoplasmic signaling. In some embodiments, suitable examples of SH3 domains for the compositions and methods disclosed herein include, but are not limited to, SH3 domains derived from C3G, p41, PEP, p47, HPK1, SLP-1, and CD3 ϵ . In some embodiments, additional exemplary SH3 domains suitable for the compositions and methods disclosed herein include SH3 domains derived from PAK, AIP4, and Sos.

[0068] In some embodiments, the recruitment domain of the chimeric polypeptides disclosed herein includes a proline-rich peptide sequence capable of interacting with (*e.g.*, binding to) a SH3 domain-containing protein. The term proline-rich peptide is generally used to describe peptides containing a high proportion of proline and hydrophobic amino acids, and generally has a high affinity binding to SH3 domain-containing proteins. In principle, any

proline-rich polypeptide sequence capable of interacting with, *e.g.*, binding to a SH3 domain-containing protein can be suitably included in a recruitment domain of the chimeric polypeptides disclosed herein. In some embodiments, the proline-rich peptide has 5% or more of proline in its sequence. In some embodiments, the proline-rich peptide has at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% proline in its sequence. Suitable examples of proline-rich polypeptide sequences for the compositions and methods disclosed herein include, but are not limited to, proline-rich polypeptide sequences derived from C3G, p41, PEP, p47, HPK1, SLP-1, and CD3ε. In some embodiments, additional exemplary proline-rich polypeptide sequences suitable for the compositions and methods disclosed herein proline-rich polypeptide sequences derived from PAK, AIP4, and Sos.

[0069] In some embodiments, a chimeric polypeptides disclosed herein includes at least one copy of a recruitment domain selected from the group consisting of: (a) p85-binding domains derived from CD19, Gab2, IREM-1, PDGF receptor, CSFR-1, c-Kit, ErbB3, and CD7 to recruit the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K); (b) SH3 domains derived from Crk, Cdc25, Phospholipase, Ras, Vav, GRB2, FAK, Pyk2, TRIP10, and Gads to recruit cytosolic signaling proteins; and (c) proline-rich peptides from C3G, p41, PEP, p47, HPK1, SLP-1, CD3ε, PAK, AIP4, or Sos to recruit SH3 domain-containing proteins. In some embodiments, the at least one copy of a recruitment domain includes: (a) a p85-binding domain derived from CD19, Gab2, IREM-1, PDGF receptor, CSFR-1, c-Kit, ErbB3, or CD7 to recruit the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K); (b) an SH3 domain derived from Crk, Cdc25, Phospholipase, Ras, Vav, GRB2, FAK, Pyk2, TRIP10, or Gads to recruit cytosolic signaling proteins; and/or (c) a proline-rich peptide from C3G, p41, PEP, p47, HPK1, SLP-1, CD3ε, PAK, AIP4, or Sos to recruit SH3 domain-containing proteins.

[0070] The binding activity of the chimeric polypeptides of the disclosure to their target antigens can be assayed by any suitable method known in the art. For example, a variety of assays are known for identifying and/or characterizing binding domains of the present disclosure that specifically bind a particular target antigen, as well as determining binding domain affinities, such as Western blot, ELISA, and BIACORE® analysis. An antibody or polypeptide that “preferentially binds” or “specifically binds” (used interchangeably herein) to a target protein or target epitope is a term well understood in the art, and methods to determine such specific or

preferential binding are also known in the art. An antibody or polypeptide is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular protein or epitope than it does with alternative proteins or epitopes. An antibody or polypeptide "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. Also, an antibody or polypeptide "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration to that target in a sample than it binds to other substances present in the sample. For example, an antibody or polypeptide that specifically or preferentially binds to a CD19 epitope is an antibody or polypeptide that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other CD19 epitopes or non-CD19 epitopes. It is also understood by reading this definition, for example, that an antibody or polypeptide (or moiety or epitope) which specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding.

[0071] In some embodiments, the chimeric polypeptide of the disclosure further includes one or more linkers/spacers. For example, between the antigen binding domain and the transmembrane domain of the chimeric polypeptide, or between the intracellular domain and the transmembrane domain of the chimeric polypeptide, one or more linkers/spacers may be incorporated. There is no particular limitation with respect to the linkers that can be used in the chimeric polypeptides described herein. In some embodiments, the linker includes a peptide linker/spacer sequence. In some embodiments, the linker is a synthetic compound linker such as, for example, a chemical cross-linking agent. Non-limiting examples of suitable cross-linking agents that are available on the market include N-hydroxysuccinimide (NHS), disuccinimidylsuberate (DSS), bis(sulfosuccinimidyl)suberate (BS3), dithiobis(succinimidylpropionate) (DSP), dithiobis(sulfosuccinimidylpropionate) (DTSSP), ethyleneglycol bis(succinimidylsuccinate) (EGS), ethyleneglycol bis(sulfosuccinimidylsuccinate) (sulfo-EGS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo-DST), bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone (BSOCOES), and bis[2-(sulfosuccinimidooxycarbonyloxy)ethyl]sulfone (sulfo-BSOCOES).

[0072] In some embodiments, the linker includes a peptide linker sequence. In principle, there are no particular limitations to the length and/or amino acid composition of the linker peptide sequence. In some embodiments, any arbitrary single-chain peptide including about one to 100 amino acid residues (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, *etc.* amino acid residues) can be used as a peptide linker. In some embodiments, the linker peptide sequence includes about 5 to 50, about 10 to 60, about 20 to 70, about 30 to 80, about 40 to 90, about 50 to 100, about 60 to 80, about 70 to 100, about 30 to 60, about 20 to 80, about 30 to 90 amino acid residues. In some embodiments, the linker peptide sequence includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to 25, about 20 to 40, about 30 to 50, about 40 to 60, about 50 to 70 amino acid residues. In some embodiments, the linker peptide sequence includes about 40 to 70, about 50 to 80, about 60 to 80, about 70 to 90, or about 80 to 100 amino acid residues. In some embodiments, the linker peptide sequence includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to 25 amino acid residues. In some embodiments, the linker peptide sequence may include up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. In some embodiments, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the intracellular domain of the chimeric polypeptide of the disclosure.

[0073] In some embodiments, the length and amino acid composition of the linker peptide sequence can be optimized to vary the orientation and/or proximity of the polypeptide domains to one another to achieve a desired activity of the chimeric polypeptide. In some embodiments, the orientation and/or proximity of the polypeptide domains to one another can be varied as a “tuning” tool to achieve a tuning effect that would enhance or reduce the biological activity of the chimeric polypeptide. In some embodiments, the orientation and/or proximity of the polypeptide domains to one another can be optimized to create a partial to full phagocytic versions of the chimeric polypeptide. In certain embodiments, the linker contains only glycine and/or serine residues (*e.g.*, glycine-serine linker). Examples of such peptide linkers include: Gly, Ser; Gly Ser; Gly Gly Ser; Ser Gly Gly; Gly Gly Gly Ser; Ser Gly Gly Gly; Gly Gly Gly Gly Ser; Ser Gly Gly Gly Gly; Gly Gly Gly Gly Gly Ser; Ser Gly Gly Gly Gly Gly; Gly Gly Gly Gly Gly Gly Ser; Ser Gly Gly Gly Gly Gly Gly; (Gly Gly Gly Gly Ser)*n*, wherein *n* is an integer of one or more; and (Ser Gly Gly Gly Gly)*n*, wherein *n* is an integer of one or more. In some

embodiments, the linker peptides are modified such that the amino acid sequence GSG (that occurs at the junction of traditional Gly/Ser linker peptide repeats) is not present. For example, in some embodiments, the peptide linker includes an amino acid sequence selected from the group consisting of: (GGGXX)_nGGGGS and GGGGS(XGGGS)_n, where X is any amino acid that can be inserted into the sequence and not result in a polypeptide including the sequence GSG, and n is 0 to 4. In some embodiments, the sequence of a linker peptide is (GGGX_iX₂)_nGGGGS and X_i is P and X₂ is S and n is 0 to 4. In some other embodiments, the sequence of a linker peptide is (GGGX_iX₂)_nGGGGS and X_i is G and X₂ is Q and n is 0 to 4. In some other embodiments, the sequence of a linker peptide is (GGGX_iX₂)_nGGGGS and X_i is G and X₂ is A and n is 0 to 4. In yet some other embodiments, the sequence of a linker peptide is GGGGS(XGGGS)_n, and X is P and n is 0 to 4. In some embodiments, a linker peptide of the disclosure comprises or consists of the amino acid sequence (GGGGA)₂GGGGS. In some embodiments, a linker peptide comprises or consists of the amino acid sequence (GGGGQ)₂GGGGS. In another embodiment, a linker peptide comprises or consists of the amino acid sequence (GGGPS)₂GGGGS. In another embodiment, a linker peptide comprises or consists of the amino acid sequence GGGGS(PGGGS)₂. In yet a further embodiment, a linker peptide comprises or consists of the amino acid sequence GSGGS or SGGSGS.

[0074] In some embodiments, the extracellular domain of the chimeric polypeptide includes an antigen-binding region that binds to one or more target antigens of interest. In some embodiments, the antigen-binding region binds to one or more target antigens expressed on the surface of a target cell (*e.g.*, cell surface markers). Examples of cell surface markers that can act as an antigen that binds to the antigen binding domain of the chimeric polypeptide include those associated with viral, bacterial and parasitic infections, autoimmune disease, and cancer cells. In some embodiments, the antigen-binding region binds to a cancer-associated antigen, *e.g.*, tumor antigen, such as an antigen that is specific for a tumor or cancer of interest. Accordingly, in some embodiments, the extracellular domain of the chimeric polypeptide includes an antigen-binding region that binds to one or more specific for one or more cancer-associated antigen. Generally, the cancer-associated antigen can be any cancer-associated antigen. Suitable cancer-associated antigens include, but are not limited to, CD19, CD22, HER2 (ERBB2/neu), Mesothelin, PSCA, CD123, CD30, CD171, CD138, CS-1, CLECL1, CD33, CD79b, EGFRvIII, GD2, GD3, BCMA, PSMA, ROR1, FLT3, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3 (CD276), KIT (CD 117),

CD213A2, IL-1 IRa, PRSS21, VEGFR2, CD24, MUC-16, PDGFR-beta, SSEA-4, CD20, MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, FAP, EphA2, GM3, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CD97, CD179a, ALK, and IGLL1. In some embodiments, the one or more cancer-associated antigens is selected from the group consisting of CD19 and CD22.

[0075] In some embodiments, the antigen-binding region includes one or more antigen-binding determinants (*e.g.*, epitopes) of an antibody or a functional antigen-binding fragment thereof. In some embodiments, the cancer-associated antigen of the present disclosure includes one or more antigenic cancer epitopes. The antigen-binding region can include naturally-occurring polypeptides or can be engineered, designed, or modified so as to provide desired and/or improved properties.

[0076] The term "antigen-binding fragment" as used herein refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv- dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody including one or more CDRs. Blocking antibodies and non-blocking antibodies are both suitable. As used herein, the term "blocking" antibody or an "antagonist" antibody refers to an antibody that prevents, inhibits, blocks, or reduces biological or functional activity of the antigen to which it binds. Blocking antibodies or antagonist antibodies can substantially or completely prevent, inhibit, block, or reduce the biological activity or function of the antigen. For example, a blocking anti-CD19 antibody can prevent, inhibit, block, or reduce the binding interaction between CD19 and its natural ligand (*e.g.*, CD77), thus preventing, blocking, inhibiting, or reducing the immunosuppressive functions associated with the CD19/CD77 interaction. The term "non-blocking" antibody refers to an antibody that does not interfere, inhibits, blocks, or reduces biological or functional activity of the antigen to which it binds.

[0077] Accordingly, in some embodiments, the antigen-binding region of the extracellular domain includes an amino acid sequence for an antibody selected from the group consisting of antigen-binding fragments (Fab), single-chain variable fragments (scFv), nanobodies, VH domains, VL domains, single domain antibodies (dAb), VNAR domains, and VHH domains, bispecific antibodies, diabodies, or a functional fragment of any one of the

foregoing. In some embodiments, the antigen-binding region includes a heavy chain variable region and a light chain variable region.

[0078] In some embodiments, the heavy chain variable region and the light chain variable region of the antigen-binding region are operably linked to each other via one or more intervening amino acid residues that are positioned between the heavy chain variable region and the light chain variable region. In some embodiments, the one or more intervening amino acid residues include a linker peptide sequence. In principle, there are no particular limitations to the length and/or amino acid composition of the linker peptide sequence. In some embodiments, any arbitrary single-chain peptide including about one to 100 amino acid residues (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, *etc.* amino acid residues) can be used as a peptide linker. In some embodiments, the linker peptide sequence includes about 5 to 50, about 10 to 60, about 20 to 70, about 30 to 80, about 40 to 90, about 50 to 100, about 60 to 80, about 70 to 100, about 30 to 60, about 20 to 80, about 30 to 90 amino acid residues. In some embodiments, the linker peptide sequence includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to 25, about 20 to 40, about 30 to 50, about 40 to 60, about 50 to 70 amino acid residues. In some embodiments, the linker peptide sequence includes about 40 to 70, about 50 to 80, about 60 to 80, about 70 to 90, or about 80 to 100 amino acid residues. In some embodiments, the linker peptide sequence includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to 25 amino acid residues.

[0079] In some embodiments of the disclosure, the intracellular signaling domain of the engulfment receptor includes at least 1, at least 2, at least 3, at least 4, or at least 5 immunoreceptor tyrosine-based activation motifs (ITAMs). Generally, any intracellular signaling domain including an ITAM can be suitably used for the construction of the chimeric polypeptides as described herein. An “ITAM,” as used herein, is a conserved protein motif that is generally present in the tail portion of signaling molecules expressed in many immune cells. The motif may include two repeats of the amino acid sequence YxxL/I separated by 6-8 amino acids, wherein each X is independently any amino acid, producing the conserved motif YxxL/Ix(6-8)YxxL/I. ITAMs within signaling molecules are important for signal transduction within the cell, which is mediated at least in part by phosphorylation of tyrosine residues in the ITAM following activation of the signaling molecule. ITAMs may also function as docking sites for other proteins involved in signaling pathways. In some embodiments, the cytoplasmic signaling

domain including at least 1, at least 2, at least 3, at least 4, or at least 5 ITAMs independently selected from the ITAMs derived from CD3 ζ , FcR γ , Megf10, FcR γ , and combinations thereof.

[0080] In some embodiments, the intracellular signaling domain from the engulfment receptor is capable of mediating endogenous phagocytic signaling pathway. In some embodiments, the intracellular domain of the chimeric polypeptide includes a domain responsible for signal activation and/or transduction. Non-limiting examples of an intracellular domain suitable for the chimeric polypeptides disclosed herein include, the cytoplasmic portion of a surface receptor, co-stimulatory molecule, and any molecule that acts in concert to initiate signal transduction in the phagocytic cell (*e.g.*, monocyte, macrophage or dendritic cell), as well as any derivative or variant of these elements and any synthetic sequence that has the same functional capability. In some embodiments, the chimeric polypeptide of the disclosure includes at least one intracellular domain derived from engulfment receptors such as, *e.g.*, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, and/or Axl.

[0081] In some embodiments, the chimeric polypeptide of the disclosure includes an amino acid sequence for one or more signal peptides. Generally, there are no specific limitations with respect to the position where the signal peptide is operably linked, *e.g.* fused, to the chimeric polypeptide. In some embodiments, the signal peptide is operably linked upstream (*e.g.*, N-terminally) to the extracellular domain. The signal peptide can generally be any signal peptide known in the art. Non-limiting examples of signal peptides suitable for the compositions and methods disclosed herein include signal peptides derived from CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, and Axl.

[0082] In some embodiments, the CAR-P of the disclosure includes the following components: (a) an extracellular domain consisting of single chain antibody that recognizes an specific antigen or other binding motif that targets a specific protein or lipid moiety; (b) a signal peptide, or equivalent) and transmembrane segment that allows the CAR to insert into and be delivered to the plasma membrane; (c) an intracellular motif that consists of at least one ITAM motif and potentially additional domains that increase signal potential; and (d) inducing an activity of engulfment when expressed in a professional phagocyte or a cell with phagocytic potential.

[0083] With respect to the transmembrane domain, the chimeric polypeptide of the disclosure can be designed to include a transmembrane domain that connects the antigen binding domain to the intracellular domain. In some embodiment, the transmembrane domain is naturally associated with one or more of the domains in the chimeric polypeptide. In some embodiments, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane domains of particular use in this disclosure may be derived from, *e.g.*, include at least the stalk and/or transmembrane region(s) of CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, Axl, CD45, and/or CD86. There are no specific limitations with respect to the position of the transmembrane domain within the chimeric polypeptide. In some embodiments, the transmembrane domain is operably linked downstream (*e.g.*, C-terminally) of the extracellular domain and upstream of the at least one copy of the intracellular signaling domain from the engulfment receptor.

[0084] In some embodiments, the transmembrane domain may be synthetic, in which case it will include predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be inserted at each end of a synthetic transmembrane domain.

[0085] In some embodiments of the present disclosure, the chimeric polypeptide includes (a) a peptide signal; (b) an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigens; (c) a transmembrane domain; (d) an intracellular signaling domain from an engulfment receptor; and (e) a p85-recruitment domain. In some embodiments, the chimeric polypeptide includes (a) a peptide signal from CD8; (b) an extracellular single chain antibody variable fragment (scFv) having specific affinity for CD19 or CD22; (c) a transmembrane domain from CD8; (d) an intracellular signaling domain from Megf10, FcR γ , Bai1, or MerTK; and (e) a p85-recruitment domain from CD19. In some embodiments of the present disclosure, the chimeric polypeptide includes, in the N-terminal to C-terminal direction, (a) a peptide signal; (b) an extracellular domain including an antigen-

binding region specific for one or more cancer-associated antigens; (c) a transmembrane domain; (d) an intracellular signaling domain from an engulfment receptor; and (e) a p85-recruitment domain. In some embodiments, the chimeric polypeptide includes, in the N-terminal to C-terminal direction, (a) a peptide signal from CD8; (b) an extracellular single chain antibody variable fragment (scFv) having specific affinity for CD19 or CD22; (c) a transmembrane domain from CD8; (d) an intracellular signaling domain from Megf10, FcR γ , Bai1, or MerTK; and (e) a p85-recruitment domain from CD19.

[0086] In some embodiments disclosed herein, the chimeric polypeptide includes an amino acid sequence that has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, or a functional fragment thereof. The terms "percent identity," as used herein in the context of two or more nucleic acids or proteins, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acids that are the same (*e.g.*, about 60% sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. See *e.g.*, the NCBI web site at ncbi.nlm.nih.gov/BLAST. Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. This definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Sequence identity typically exists over a region that is at least about 20 amino acids or nucleotides in length, or over a region that is 10-100 amino acids or nucleotides in length, or over the entire length of a given sequence.

[0087] If necessary, sequence identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux *et al.*, Nucleic Acids Res. 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul *et al.*, J. Molecular Biol. 215:403, 1990). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the

default parameters thereof

[0088] In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8, and SEQ ID NO: 9, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 6, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 8, or a functional fragment thereof. In some embodiments, the chimeric polypeptide includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9, or a functional fragment thereof.

[0089] One skilled in the art will appreciate that the complete amino acid sequence can be used to construct a back-translated gene. For example, a DNA oligomer containing a

nucleotide sequence coding for a given polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[0090] In addition to generating desired polypeptides via expression of nucleic acid molecules that have been altered by recombinant molecular biological techniques, a subject chimeric polypeptide in accordance with the present disclosure can be chemically synthesized. Chemically synthesized polypeptides are routinely generated by those of skill in the art.

[0091] Once assembled (by synthesis, recombinant methodology, site-directed mutagenesis or another method), the DNA sequences encoding a chimeric polypeptide as disclosed herein can be inserted into an expression vector and operably linked to an expression control sequence appropriate for expression of the chimeric polypeptide in the desired transformed host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As is known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operably linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

Nucleic Acid Molecules

[0092] In one aspect, some embodiments disclosed herein relate to isolated, synthetic, or recombinant nucleic acid molecules encoding the chimeric polypeptides of the disclosure, expression cassettes, and expression vectors containing these nucleic acid molecules. In some embodiments, an isolated, synthetic, or recombinant nucleic acid molecule of the disclosure is operably linked to regulator sequences which allow expression of the chimeric polypeptides in a host cell or *ex-vivo* cell-free expression system.

[0093] The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein, and refer to both RNA and DNA molecules, including nucleic acid molecules comprising cDNA, genomic DNA, synthetic DNA, and DNA or RNA molecules containing nucleic acid analogs. A nucleic acid molecule can be double-stranded or single-stranded (*e.g.*, a sense strand or an antisense strand). A nucleic acid molecule may contain unconventional or modified nucleotides. The terms "polynucleotide sequence" and "nucleic acid

sequence” as used herein interchangeably refer to the sequence of a polynucleotide molecule. The nomenclature for nucleotide bases as set forth in 37 CFR §1.822 is used herein.

[0094] Nucleic acid molecules of the present disclosure can be nucleic acid molecules of any length, including nucleic acid molecules that are preferably between about 1 Kb and about 50 Kb, for example between about 2 Kb and about 40 Kb, between about 5 Kb and about 50 Kb, between about 5 Kb and about 40 Kb, between about 5 Kb and about 30 Kb, between about 5 Kb and about 20 Kb, or between about 10 Kb and about 50 Kb, for example between about 15 Kb to 30 Kb, between about 20 Kb and about 50 Kb, between about 20 Kb and about 40 Kb, about 5 Kb and about 25 Kb, or about 30 Kb and about 50 Kb.

[0095] The term "recombinant" nucleic acid molecule as used herein, refers to a nucleic acid molecule that has been altered through human intervention. As non-limiting examples, a cDNA is a recombinant DNA molecule, as is any nucleic acid molecule that has been generated by *in vitro* polymerase reaction(s), or to which linkers have been attached, or that has been integrated into a vector, such as a cloning vector or expression vector. As non-limiting examples, a recombinant nucleic acid molecule: 1) has been synthesized or modified *in vitro*, for example, using chemical or enzymatic techniques (for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, *e.g.*, methylation), or recombination (including homologous and site-specific recombination)) of nucleic acid molecules; 2) includes conjoined nucleotide sequences that are not conjoined in nature, 3) has been engineered using molecular cloning techniques such that it lacks one or more nucleotides with respect to the naturally occurring nucleic acid molecule sequence, and/or 4) has been manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally occurring nucleic acid sequence.

[0096] In some embodiments disclosed herein, the nucleic acid molecules of the disclosure include a nucleic acid sequence encoding a chimeric polypeptides which includes (a) an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigen; (b) a transmembrane domain; (c) at least one copy of an intracellular signaling domain from an engulfment receptor; and (d) at least one copy of a recruitment domain.

[0097] In some embodiments disclosed herein, the nucleic acid molecules include a nucleotide sequence encoding a polypeptide that includes (i) an amino acid sequence having at least 80% sequence identity to the amino acid sequence of a chimeric polypeptide as disclosed herein or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence encoding a polypeptide that includes (i) an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of a chimeric polypeptide as disclosed herein or a functional fragment thereof.

[0098] In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a nucleotide sequence selected from the group consisting of S SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 11, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 12, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 13, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 14, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide

sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 15, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 17, or a functional fragment thereof. In some embodiments, the nucleic acid molecules include a nucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleotide sequence of SEQ ID NO: 18, or a functional fragment thereof.

[0099] In some embodiments, the nucleic acid molecule as disclosed herein is operably linked to a heterologous nucleic acid sequence. Some embodiments disclosed herein relate to vectors or expression cassettes including an isolated, synthetic, or recombinant nucleic acid molecule as disclosed herein. The expression cassette generally contains coding sequences and enough regulatory information to direct proper transcription and/or translation of the coding sequences in a recipient cell, *in vivo* and/or *ex vivo*. The expression cassette may be inserted into a vector for targeting to a desired host cell and/or into a subject. As such, the term expression cassette may be used interchangeably with the term “expression construct”. An expression cassette can be inserted into a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, as a linear or circular, single-stranded or double-stranded, DNA or RNA polynucleotide molecule, derived from any source, capable of genomic integration or autonomous replication, including a nucleic acid molecule where one or more nucleic acid sequences has been linked in a functionally operative manner, *i.e.*, operably linked.

[00100] Also provided herein are vectors, plasmids, or viruses containing one or more of the nucleic acid molecules encoding any one of the chimeric polypeptides disclosed herein. The nucleic acid molecules described above can be contained within a vector that is capable of directing their expression in, for example, a cell that has been transduced with the vector. Suitable vectors for use in eukaryotic and prokaryotic cells are known in the art and are commercially available or readily prepared by a skilled artisan. Additional vectors can also be found, for example, in Ausubel, F. M., *et al.*, *Current Protocols in Molecular Biology*, (Current Protocol, 1994) and Sambrook *et al.*, “*Molecular Cloning: A Laboratory Manual*,” 2nd ED. (1989).

[00101] It should be understood that not all vectors and expression control sequences will function equally well to express the DNA sequences described herein. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation. For example, in selecting a vector, the host must be considered because the vector must replicate in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, should also be considered. For example, vectors that can be used include those that allow the DNA encoding the chimeric polypeptides of the present disclosure to be amplified in copy number. Such amplifiable vectors are known in the art. They include, for example, vectors able to be amplified by DHFR amplification (see, *e.g.*, Kaufman, U.S. Pat. No. 4,470,461) or glutamine synthetase ("GS") amplification (see, *e.g.*, U.S. Pat. No. 5,122,464 and European published application EP 338,841).

[00102] Accordingly, in some embodiments, the chimeric polypeptides of the present disclosure can be expressed from vectors, preferably expression vectors. The vectors are, in some embodiments, useful for autonomous replication in a host cell or, in certain embodiments, may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome (*e.g.*, non-episomal mammalian vectors). Expression vectors are capable of directing the expression of coding sequences to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses, and adeno-associated viruses) are also encompassed in this disclosure.

[00103] Exemplary recombinant expression vectors can include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, operably linked to the nucleic acid sequence to be expressed.

[00104] DNA vector can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals.

[00105] The nucleic acid sequences encoding the chimeric polypeptides of the present disclosure can be optimized for expression in the host cell of interest. For example, the G-C content of the sequence can be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Methods for codon optimization are known in the art. Codon usages within the coding sequence of the chimeric polypeptides as disclosed herein can be optimized to enhance expression in the host cell, such that about 1%, about 5%, about 10%, about 25%, about 50%, about 75%, or up to 100% of the codons within the coding sequence have been optimized for expression in a particular host cell.

[00106] Non-limiting examples of vectors suitable for use include T7-based vectors for use in bacteria, the pMSXND expression vector for use in mammalian cells, and baculovirus-derived vectors for use in insect cells. In some embodiments nucleic acid inserts, which encode the subject chimeric polypeptide in such vectors, can be operably linked to a promoter, which is selected based on, for example, the cell type in which expression is sought. A non-limiting exemplification of suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, a Rous sarcoma virus promoter, the elongation factor-1 α promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the disclosure should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the disclosure. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[00107] In addition, any of a wide variety of expression control sequences can be used in these vectors. Such useful expression control sequences include the expression control sequences

associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter regions of phage lambda, for example PL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, PhoA, the promoters of the yeast α -mating system, the polyhedron promoter of Baculovirus, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

[00108] A T7 promoter can be used in bacteria, a polyhedrin promoter can be used in insect cells, and a cytomegalovirus or metallothionein promoter can be used in mammalian cells. Also, in the case of higher eukaryotes, tissue-specific and cell type-specific promoters are widely available. These promoters are so named for their ability to direct expression of a nucleic acid molecule in a given tissue or cell type within the body. Skilled artisans will readily appreciate numerous promoters and other regulatory elements which can be used to direct expression of nucleic acids.

[00109] In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the subject chimeric polypeptide, particularly as regards potential secondary structures. Hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences of this disclosure, their secretion characteristics, their ability to fold the polypeptides correctly, their fermentation or culture requirements, and the ease of purification of the products coded for by the DNA sequences.

[00110] Within these parameters one of skill in the art may select various vector/expression control sequence/host combinations that will express the desired DNA sequences on fermentation or in large scale animal culture, for example, using CHO cells or COS 7 cells.

[00111] The choice of expression control sequence and expression vector, in some embodiments, will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Non-limiting examples of useful expression vectors for eukaryotic hosts, include, for example, vectors with expression control sequences from SV40,

bovine papilloma virus, adenovirus and cytomegalovirus. Non-limiting examples of useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including col El, pCRI, pER32z, pMB9 and their derivatives, wider host range plasmids, such as RP4, phage DNAs, *e.g.*, the numerous derivatives of phage lambda, *e.g.*, NM989, and other DNA phages, such as M13 and filamentous single stranded DNA phages. Non-limiting examples of useful expression vectors for yeast cells include the 2 μ plasmid and derivatives thereof. Non-limiting examples of useful vectors for insect cells include pVL 941 and pFastBac™ 1.

[00112] In addition to sequences that facilitate transcription of the inserted nucleic acid molecule, vectors can contain origins of replication, and other genes that encode a selectable marker. For example, the neomycin-resistance (neoR) gene imparts G418 resistance to cells in which it is expressed, and thus permits phenotypic selection of the transfected cells. Those of skill in the art can readily determine whether a given regulatory element or selectable marker is suitable for use in a particular experimental context.

[00113] Viral vectors that can be used in the disclosure include, for example, retroviral, adenoviral, and adeno-associated vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors (see, for example, Gluzman (Ed.), *Eukaryotic Viral Vectors*, CSH Laboratory Press, Cold Spring Harbor, N.Y.).

[00114] Recombinant prokaryotic or eukaryotic cells that contain a chimeric polypeptide as disclosed herein, and/or contain and express a nucleic acid molecule that encodes any one of the chimeric polypeptide disclosed herein are also features of the disclosure. In some embodiments, a recombinant cell of the disclosure is a transfected cell, *e.g.*, a cell into which a nucleic acid molecule, for example a nucleic acid molecule encoding a chimeric polypeptide disclosed herein, has been introduced by means of recombinant methodologies and techniques. The progeny of such a cell are also considered within the scope of the disclosure. Cell cultures containing at least one recombinant cell as disclosed herein are also within the scope of the present disclosure.

[00115] The precise components of the expression system are not critical. For example, an chimeric polypeptide as disclosed herein can be produced in a prokaryotic host, such as the bacterium *E. coli*, or in a eukaryotic host, such as an insect cell (*e.g.*, an Sf21 cell), or mammalian cells (*e.g.*, COS cells, NIH 3T3 cells, or HeLa cells). In some embodiments, the

recombinant cell is a phagocytic cell, *e.g.*, phagocyte. Both professional phagocytes and non-professional phagocytes are suitable. In some embodiments, the phagocytic cell is a professional phagocyte. In some embodiments, the phagocytic cell is a professional phagocyte. In some embodiments, the phagocytic cell is selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a BMDM or a BMDC. In some embodiments, the phagocytic cell is a J774A.1 macrophage. These cells are available from many sources, including the American Type Culture Collection (Manassas, Va.). In selecting an expression system, it matters only that the components are compatible with one another. Artisans or ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult Ausubel *et al.* (Current Protocols in Molecular Biology, John Wiley and Sons, New York, N.Y., 1993) and Pouwels *et al.* (Cloning Vectors: A Laboratory Manual, 1985 Suppl. 1987).

[00116] The expressed polypeptides can be purified from the expression system using routine biochemical procedures, and can be used, *e.g.*, as therapeutic agents, as described herein.

[00117] In some embodiments, chimeric polypeptides obtained will be glycosylated or unglycosylated depending on the host organism used to produce the chimeric polypeptides. If bacteria are chosen as the host then the chimeric polypeptide produced will be unglycosylated. Eukaryotic cells, on the other hand, will typically glycosylate the chimeric polypeptides, although perhaps not in the same way as native polypeptides is glycosylated. The chimeric polypeptides produced by the transformed host cell can be purified according to any suitable methods known in the art. Produced chimeric polypeptides can be isolated from inclusion bodies generated in bacteria such as *E. coli*, or from conditioned medium from either mammalian or yeast cultures producing a given chimeric polypeptide using cation exchange, gel filtration, and or reverse phase liquid chromatography.

[00118] Accordingly, another exemplary method of constructing a DNA sequence encoding the chimeric polypeptides of the disclosure is by chemical synthesis. This includes direct synthesis of a peptide by chemical means of the protein sequence encoding for a chimeric polypeptide exhibiting the properties described. This method can incorporate both natural and unnatural amino acids at positions that affect the binding affinity of the chimeric polypeptide with the target antigen and/or target protein. Alternatively, a gene which encodes the desired

chimeric polypeptide can be synthesized by chemical means using an oligonucleotide synthesizer. Such oligonucleotides are designed based on the amino acid sequence of the desired chimeric polypeptide, and preferably selecting those codons that are favored in the host cell in which the recombinant chimeric polypeptide will be produced. In this regard, it is well recognized in the art that the genetic code is degenerate—that an amino acid may be coded for by more than one codon. For example, Phe (F) is coded for by two codons, TIC or TTT, Tyr (Y) is coded for by TAC or TAT and his (H) is coded for by CAC or CAT. Trp (W) is coded for by a single codon, TGG. Accordingly, it will be appreciated by those skilled in the art that for a given DNA sequence encoding a particular chimeric polypeptide, there will be many DNA degenerate sequences that will code for that chimeric polypeptide. For example, it will be appreciated that in addition to the DNA sequences for chimeric polypeptides provided in the Sequence Listing, there will be many degenerate DNA sequences that code for the chimeric polypeptides disclosed herein. These degenerate DNA sequences are considered within the scope of this disclosure. Therefore, “degenerate variants thereof” in the context of this disclosure means all DNA sequences that code for and thereby enable expression of a particular chimeric polypeptide.

[00119] The DNA sequence encoding the subject chimeric polypeptide, whether prepared by site directed mutagenesis, chemical synthesis or other methods, can also include DNA sequences that encode a signal sequence. Such signal sequence, if present, should be one recognized by the cell chosen for expression of the chimeric polypeptide. It can be prokaryotic, eukaryotic or a combination of the two. In general, the inclusion of a signal sequence depends on whether it is desired to secrete the chimeric polypeptide as disclosed herein from the recombinant cells in which it is made. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be included.

[00120] The nucleic acid molecules provided can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the nucleic acid molecules can be double-stranded or

single-stranded (*e.g.*, either a sense or an antisense strand).

[00121] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence (*e.g.*, the coding sequence of a chimeric polypeptide disclosed herein) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by *in vitro* transcription.

[00122] Exemplary isolated nucleic acid molecules of the present disclosure can include fragments not found as such in the natural state. Thus, this disclosure encompasses recombinant molecules, such as those in which a nucleic acid sequence (for example, a sequence encoding a chimeric polypeptide disclosed herein) is incorporated into a vector (*e.g.*, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location).

Pharmaceutical Compositions

[00123] In some embodiments, the chimeric polypeptides, the nucleic acid molecules, and/or the recombinant cells the present disclosure can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include one or more of the chimeric polypeptides, the nucleic acid molecules, and/or the recombinant cells the present disclosure, and a pharmaceutically acceptable carrier.

[00124] In some embodiments, provided herein is a pharmaceutical composition including a pharmaceutically acceptable carrier, and one or more of the following: (a) a chimeric polypeptide including: (i) an extracellular domain including an antigen-binding region specific for one or more cancer-associated antigen; (ii) a transmembrane domain; (iii) at least one copy of an intracellular signaling domain from an engulfment receptor; and (iv) at least one copy of a recruitment domain; (b) a nucleic acid encoding the chimeric polypeptide of (a); and (c) a recombinant cell including the polypeptide of (a) and/or the nucleic acid of (b).

[00125] In some embodiments, provided herein is a pharmaceutical composition including a pharmaceutically acceptable carrier and a chimeric polypeptide that includes (i) an

extracellular domain including an antigen-binding region specific for one or more cancer-associated antigen; (ii) a transmembrane domain; (iii) at least one copy of an intracellular signaling domain from an engulfment receptor; and (iv) at least one copy of a recruitment domain.

[00126] In some embodiments, provided herein is a pharmaceutical composition including a pharmaceutically acceptable carrier and a nucleic acid encoding a chimeric polypeptide disclosed herein. In some embodiments, provided herein is a pharmaceutical composition including a pharmaceutically acceptable carrier and a recombinant cell as disclosed herein.

[00127] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.*, sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00128] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which

contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00129] Oral compositions, if used, generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound (*e.g.*, chimeric polypeptides, nucleic acid molecules, and recombinant cells of the disclosure) can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes™; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00130] In the event of administration by inhalation, the subject chimeric polypeptides and/or nucleic acids molecules of the disclosure are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798.

[00131] Systemic administration of the subject chimeric polypeptides and/or nucleic acids molecules of the disclosure can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00132] In some embodiments, the chimeric polypeptides and nucleic acids molecules of the disclosure can also be prepared in the form of suppositories (*e.g.*, with conventional

suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00133] In some embodiments, the chimeric polypeptides and nucleic acids molecules of the disclosure can also be administered by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey *et al.* (Nature 418:6893, 2002), Xia *et al.* (Nature Biotechnol. 20: 1006-1010, 2002), or Putnam (*Am. J. Health Syst. Pharm.* 53: 151-160, 1996, erratum at *Am. J. Health Syst. Pharm.* 53:325, 1996).

[00134] In some embodiments, the subject chimeric polypeptides and nucleic acids molecules of the disclosure are prepared with carriers that will protect the chimeric polypeptides and nucleic acids molecules against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

METHODS OF THE DISCLOSURE

[00135] In one aspect, some embodiments of the disclosure relate to a method for modifying a cell including introducing into the cell (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein, to produce a recombinant cell, *e.g.*, transgenic cell. For example, a chimeric polypeptide or nucleic molecule as disclosed herein can be produced in a prokaryotic host, such as the bacterium *E. coli*, or in a eukaryotic host, such as an insect cell (*e.g.*, an Sf21 cell), or mammalian cells (*e.g.*, COS cells, NIH 3T3 cells, or HeLa cells). In some embodiments, the recombinant cell is a phagocytic cell, *e.g.*, phagocyte. Both professional phagocytes and non-professional phagocytes are suitable. In some embodiments, the phagocytic cell is a professional phagocyte. In some embodiments, the phagocytic cell is a professional phagocyte. In some embodiments, the phagocytic cell is selected from the group consisting of macrophages,

dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a BMDM or a BMDC. These cells are available from many sources, including the American Type Culture Collection (Manassas, Va.). In some embodiments, the recombinant cell expresses the chimeric polypeptide and possesses targeted effector activity. In some embodiments, introducing the chimeric polypeptide into the cell includes introducing a nucleic acid sequence encoding the chimeric polypeptide. In some embodiments, introducing the nucleic acid sequence includes electroporating an mRNA encoding the chimeric polypeptide.

[00136] Methods of introducing and expressing genes, such as the nucleic acid molecules and the chimeric polypeptides encoded thereby, into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means. Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells including vectors and/or exogenous nucleic acids are well-known in the art. For example, nucleic acids can be introduced into target cells using commercially available methods which include electroporation. Nucleic acids can also be introduced into cells using cationic liposome mediated transfection using lipofection, using polymer encapsulation, using peptide mediated transfection, or using biolistic particle delivery systems such as "gene guns".

[00137] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. RNA vectors include vectors having a RNA promoter and/ other relevant domains for production of a RNA transcript. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors may be derived from lentivirus, poxviruses, herpes simplex virus, adenoviruses and adeno-associated viruses, and the like. Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial

membrane vesicle).

[00138] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers. However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as non-uniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes

[00139] The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In some embodiments, the nucleic acid molecule or chimeric polypeptide may be associated with a lipid. The nucleic acid molecule or chimeric polypeptide associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Methods of Treatment

[00140] The chimeric polypeptides, nucleic acid molecules, and/or pharmaceutical

compositions of the disclosure can be used to treat individuals who have, who are suspected of having, or who may be at high risk for developing one or more health conditions or disorders. Exemplary health conditions and disorders of interest can include, without limitation, those associated with acute and chronic infections, inflammatory diseases, immune diseases, and various cancers. In some embodiments, the methods disclosed herein are useful in treating the one or more health conditions or disorders by enhancing the removal of infected, transformed, malignant, apoptotic, damaged or necrotic cells or particles from the individual's body.

[00141] The term "cancer" as used herein refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. The aberrant cells may form solid tumors or constitute a hematological malignancy. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. There are no specific limitations with respect to the cancers which can be treated by the compositions and methods of the present disclosure. Non-limiting examples of suitable cancers include ovarian cancer, renal cancer, breast cancer, prostate cancer, liver cancer, brain cancer, lymphoma, leukemia, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, lung cancer and the like. Other cancers that can be suitably treated with the compositions and methods of the present disclosure include, but are not limited to, AML, ALL, CML, adrenal cortical cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, bone metastasis, brain cancers, central nervous system (CNS) cancers, peripheral nervous system (PNS) cancers, breast cancer, cervical cancer, childhood Non-Hodgkin's lymphoma, colon and rectum cancer, endometrial cancer, esophagus cancer, Ewing's family of tumors (*e.g.* Ewing's sarcoma), eye cancer, transitional cell carcinoma, vaginal cancer, myeloproliferative disorders, nasal cavity and paranasal cancer, nasopharyngeal cancer, neuroblastoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumor, prostate cancer, retinoblastoma, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, liver cancer, lung cancer, lung carcinoid tumors, brain cancers, central nervous system (CNS) cancers, peripheral nervous system (PNS) cancers, breast cancer, cervical cancer, childhood Non-Hodgkin's lymphoma, colon and rectum cancer, Non-Hodgkin's lymphoma, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, pituitary tumor, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, melanoma

skin cancer, non-melanoma skin cancers, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine cancer (*e.g.*, uterine sarcoma), transitional cell carcinoma, vaginal cancer, myeloproliferative disorders, nasal cavity and paranasal cancer, nasopharyngeal cancer, neuroblastoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumor, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, melanoma skin cancer, non-melanoma skin cancers, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine cancer (*e.g.*, uterine sarcoma), transitional cell carcinoma, vaginal cancer, vulvar cancer, mesothelioma, squamous cell or epidermoid carcinoma, bronchial adenoma, choriocarcinoma, head and neck cancers, teratocarcinoma, or Waldenstrom's macroglobulinemia. Particularly suitable cancers include, but are not limited to, breast cancer, ovarian cancer, lung cancer, pancreatic cancer, mesothelioma, leukemia, lymphoma, brain cancer, prostate cancer, multiple myeloma, melanoma, bladder cancer, bone sarcomas, soft tissue sarcomas, retinoblastoma, renal tumors, neuroblastoma, and carcinomas.

[00142] In another aspect, some embodiments of the disclosure relate to method for treating an individual having cancer, which includes administering to the individual an effective numbers of a phagocytic cells that has been modified to express a chimeric engulfment receptor having specific affinity for an antigen associated with a cancer cell. In some embodiments, the cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes. In some embodiments, the phagocytic cell is a BMDM or a BMDC. In some embodiments, the phagocytic cell is derived from the same individual having cancer, where phagocytes are removed from an individual (blood, tumor or ascites fluid), and modified so that they express the CAR-P receptors specific to a particular form of antigen associated with the individual's cancer. In yet some embodiments, the method further includes modifying the phagocytic cell to deliver an agent to a target, wherein the agent is selected from the group consisting of a nucleic acid, an antibiotic, an anti-inflammatory agent, an antibody or antibody fragments thereof, a growth factor, a cytokine, an enzyme, a protein, a peptide, a fusion protein, a synthetic molecule, an organic molecule, a carbohydrate or the like, a lipid, a hormone, a microsome, a derivative or a variation thereof, and any combination thereof.

[00143] In yet another aspect, some embodiments of the disclosure relate to method for treating an individual having cancer, which includes administering to the individual an effective

amount or number of: (a) a chimeric polypeptide as described herein; (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein; (c) a recombinant cell as described herein; and/or a pharmaceutical composition as described herein. Accordingly, in some embodiments, the treatment method includes administering to the individual an effective amount of one or more chimeric polypeptides as described herein. In some embodiments, the treatment method includes administering to the individual an effective amount of one or more isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the treatment method includes administering to the individual an effective numbers of one or more recombinant phagocytic cells as described herein.

[00144] In principle, there are no specific limitations with respect to the procedures and techniques that can be suitably employed for delivery of the chimeric polypeptides, nucleic acid molecules, and/or recombinant cells as described herein into the target cell. Non-limiting delivery procedures suitable for the methods disclosed herein include stable or transient transfection, lipofection, electroporation, microinjection, liposomes, iontophoresis, and infection with recombinant viral vectors. In some embodiments, the administration includes a viral-, particle-, liposome-, or exosome-based delivery procedure. In some embodiments, the administration includes delivering into endogenous cells *ex vivo* one or more gene-editing enzymes targeting (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the administration includes delivering into cells *in vivo* one or more gene-editing enzymes targeting (a) a chimeric polypeptide as described herein; and/or (b) an isolated, synthetic, or recombinant nucleic acid molecule as described herein. In some embodiments, the administration of the chimeric polypeptides, nucleic acid molecules, and/or recombinant cells as described herein activates phagocytosis of said cancer cell. In some embodiments, the activated phagocytosis includes whole-cell internalization. In some embodiments, the activated phagocytosis includes bite internalization. In some embodiments, the activated phagocytosis includes both whole-cell internalization and bite internalization.

[00145] Typically, a pharmaceutical composition is formulated to be compatible with its intended route of administration. The chimeric polypeptides, nucleic acid molecules, and/or pharmaceutical compositions of the disclosure may be given orally or by inhalation, but it is more likely that they will be administered through a parenteral route. Examples of parenteral

routes of administration include, for example, intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (*e.g.*, to a pH of about 7.2-7.8, *e.g.*, 7.5). The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00146] Dosage, toxicity and therapeutic efficacy of such subject chimeric polypeptides, nucleic acid molecules, and/or pharmaceutical compositions of the disclosure can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00147] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such chimeric polypeptides, nucleic acid molecules, and/or pharmaceutical compositions lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any molecules or compositions used in the methods of the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*e.g.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately

determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00148] As defined herein, a “therapeutically effective amount or number” of a subject chimeric polypeptide or nucleic acid molecule or a recombinant cell of the disclosure (*e.g.*, an effective dosage) depends on the polypeptide selected. For instance, single dose amounts in the range of approximately 0.001 to 0.1 mg/kg of patient body weight can be administered; in some embodiments, about 0.005, 0.01, 0.05 mg/kg may be administered. In some embodiments, 600,000 IU/kg is administered (IU can be determined by a lymphocyte proliferation bioassay and is expressed in International Units (IU) as established by the World Health Organization International Standard). The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount or number of the subject chimeric polypeptides, nucleic acid molecules, and/or recombinant cells of the disclosure can include a single treatment or, can include a series of treatments. In some embodiments, the compositions are administered every 8 hours for five days, followed by a rest period of 2 to 14 days, *e.g.*, 9 days, followed by an additional five days of administration every 8 hours.

[00149] There are no specific limitations with respect to the cancers which can be treated by the compositions and methods of the present disclosure. Non-limiting examples of suitable cancers include ovarian cancer, renal cancer, breast cancer, prostate cancer, liver cancer, brain cancer, lymphoma, leukemia, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, lung cancer and the like. Other cancers that can be suitable treated with the compositions and methods of the present disclosure include, but are not limited to, AML, ALL, CML, adrenal cortical cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, bone metastasis, brain cancers, central nervous system (CNS) cancers, peripheral nervous system (PNS) cancers, breast cancer, cervical cancer, childhood Non-Hodgkin's lymphoma, colon and rectum cancer, endometrial cancer, esophagus cancer, Ewing's family of tumors (*e.g.* Ewing's sarcoma), eye cancer, transitional cell carcinoma, vaginal cancer, myeloproliferative disorders, nasal cavity and paranasal cancer, nasopharyngeal cancer, neuroblastoma, oral cavity and

oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumor, prostate cancer, retinoblastoma, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, liver cancer, lung cancer, lung carcinoid tumors, brain cancers, central nervous system (CNS) cancers, peripheral nervous system (PNS) cancers, breast cancer, cervical cancer, childhood Non-Hodgkin's lymphoma, colon and rectum cancer, Non-Hodgkin's lymphoma, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, pituitary tumor, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, melanoma skin cancer, non-melanoma skin cancers, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine cancer (*e.g.*, uterine sarcoma), transitional cell carcinoma, vaginal cancer, myeloproliferative disorders, nasal cavity and paranasal cancer, nasopharyngeal cancer, neuroblastoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumor, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, melanoma skin cancer, non-melanoma skin cancers, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine cancer (*e.g.*, uterine sarcoma), transitional cell carcinoma, vaginal cancer, vulvar cancer, mesothelioma, squamous cell or epidermoid carcinoma, bronchial adenoma, choriocarcinoma, head and neck cancers, teratocarcinoma, or Waldenstrom's macroglobulinemia. Particularly suitable cancers include, but are not limited to, breast cancer, ovarian cancer, lung cancer, pancreatic cancer, mesothelioma, leukemia, lymphoma, brain cancer, prostate cancer, multiple myeloma, melanoma, bladder cancer, bone sarcomas, soft tissue sarcomas, retinoblastoma, renal tumors, neuroblastoma, and carcinomas.

[00150] Accordingly, in some embodiments of the disclosure, the individual in need of treatment has or is suspected of having a cancer which is selected from the group consisting of breast cancer, ovarian cancer, lung cancer, pancreatic cancer, mesothelioma, leukemia, lymphoma, prostate cancer, multiple myeloma, melanoma, bladder cancer, bone sarcomas, soft tissue sarcomas, retinoblastoma, renal tumors, neuroblastoma, and carcinomas.

Kits

[00151] Some embodiments of the disclosure provide a kit that contains any of the above-described compositions, *e.g.* chimeric polypeptides, nucleic acid molecules, recombinant cells, and pharmaceutical compositions.

[00152] In some embodiments, a kit can further include instructions for using the components of the kit to practice the methods described herein. The instructions for practicing the methods are generally recorded on a suitable recording medium. For example, the instructions can be printed on a substrate, such as paper or plastic, *etc.* The instructions can be present in the kit as a package insert, in the labeling of the container of the kit or components thereof (*e.g.*, associated with the packaging or subpackaging), *etc.* The instructions can be present as an electronic storage data file present on a suitable computer readable storage medium, *e.g.*, CD-ROM, diskette, flash drive, *etc.* In some embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (*e.g.*, via the Internet), can be provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions can be recorded on a suitable substrate.

[00153] All publications and patent applications mentioned in this disclosure are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[00154] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[00155] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application. It is appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every

combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

EXAMPLES

[00156] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art. Such techniques are explained fully in the literature, such as Sambrook, J., & Russell, D. W. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory and Sambrook, J., & Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual* (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (jointly referred to herein as “Sambrook”); Ausubel, F. M. (1987). *Current Protocols in Molecular Biology*. New York, NY: Wiley (including supplements through 2014); Bollag, D. M. *et al.* (1996). *Protein Methods*. New York, NY: Wiley-Liss; Huang, L. *et al.* (2005). *Nonviral Vectors for Gene Therapy*. San Diego: Academic Press; Kaplitt, M. G. *et al.* (1995). *Viral Vectors: Gene Therapy and Neuroscience Applications*. San Diego, CA: Academic Press; Lefkovits, I. (1997). *The Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*. San Diego, CA: Academic Press; Doyle, A. *et al.* (1998). *Cell and Tissue Culture: Laboratory Procedures in Biotechnology*. New York, NY: Wiley; Mullis, K. B., Ferré, F. & Gibbs, R. (1994). *PCR: The Polymerase Chain Reaction*. Boston: Birkhauser Publisher; Greenfield, E. A. (2014). *Antibodies: A Laboratory Manual* (2nd ed.). New York, NY: Cold Spring Harbor Laboratory Press; Beaucage, S. L. *et al.* (2000). *Current Protocols in Nucleic Acid Chemistry*. New York, NY: Wiley, (including supplements through 2014); and Makrides, S. C. (2003). *Gene Transfer and Expression in Mammalian Cells*. Amsterdam, NL: Elsevier Sciences B.V., the disclosures of which are incorporated herein by reference. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

[00157] Additional embodiments are disclosed in further detail in the following examples, which are provided by way of illustration and are not in any way intended to limit the

scope of this disclosure or the claims.

EXAMPLE 1

General Experimental Procedures

Constructions, Antibodies, Reagents, and Cell Lines

[00158] This section describes the construction of constructs and antibodies. Detailed information for all constructs, antibodies, reagents, and cell lines can be found in TABLE 1 (Key resources table). This table also includes the following information for various receptors developed in this study: signal peptide, extracellular antibody fragment, stalk/transmembrane domain, and cytosolic tail including appropriate accession numbers.

TABLE 1: Key resource table

Reagent type (species) or resource	Designation	Source or reference	ID	Additional information
Cell line (<i>Mus musculus</i>)	J774A.1 Macrophages	UCSF Cell Culture Facility		
Cell line (<i>Homo sapiens</i>)	Raji B Cells	Other		Obtained from M. McManus, UCSF
Cell line (<i>Mus musculus</i>)	3T3 Fibroblasts	UCSF Cell Culture Facility		
Cell line (<i>Mus musculus</i>)	C57BL/6J	PMID: 21356739		Bone Marrow Derived Macrophages (BMDM)
Cell line (<i>Mus musculus</i>)	C57BL/6J	PMID: 7489412		Bone Marrow derived Dendritic Cells (BMDC)
Cell line (<i>Homo sapiens</i>)	HEK293T cells	UCSF Cell Culture Facility		Lentivirus production
Genetic Reagent (<i>Mus musculus</i>)	OTI	PMID: 8287475		E. Roberts/M. Krummel Lab UCSF
Recombinant DNA reagent	CD19-mMegf10 CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: gggtggcgggtggctcggcggtgggtgggtcgggtggcgcggtatct -- V-H chain: aa 148–267 anti-CD19 CAR

				(Genbank AMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 879–1147 Mouse Megf10 (Uniprot Q6DIB5 (MEG10_MOUSE)) Fluorophore: mGFP
Recombinant DNA reagent	CD19-FcGamma CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: gggtggcgggtggctcgggcgggtgggtggcgggtggcggcgatct -- V-H chain: aa 148–267 anti-CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 19–86 Mouse Fc ERG precursor (Uniprot P20491 (FCERG_MOUSE)) Fluorophore: mGFP
Recombinant DNA reagent	CD19-empty CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: gggtggcgggtggctcgggcgggtgggtggcgggtggcggcgatct -- V-H chain: aa 148–267 anti-CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: basic linker NHRNRRR (nucleotide AACCACAGGAACCGAAGACG T) Fluorophore: mGFP
Recombinant DNA reagent	CD22-Megf10 CAR	this disclosure		Signal peptide: aa 1–21 CSF2R (Uniprot P15509 (CSF2R_HUMAN)) Extracellular antibody sequence: aa 22–258 of translated JP 2016502512-A/1: M971 Chimeric Antigen (Genbank HZ530416.1) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 879–1147 Mouse Megf10 (Uniprot Q6DIB5 (MEG10_MOUSE)) Fluorophore: mGFP
Recombinant	CD19-MerTK CAR	this disclosure		Signal peptide: aa 1–21 CD8

DNA reagent				(Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: ggtg gcggtggctcggcggtggtgggtcgggtgg cggcggatct -- V-H chain: aa 148–267 anti-CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 519–994 Mouse MerTK (Uniprot Q60805 (MERTK_MOUSE)) Fluorophore: mGFP
Recombinant DNA reagent	CD19-Bai1 CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain:aa 23–130 anti-CD19 CAR (Genbank AMZ04819)-- GS linker: ggtggcggtggctcggcggtggtgggtcgggtggcggcggcggtatct -- V-H chain: aa 148–267 anti-CD19 CAR (GenbankAMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (UniprotQ96QR6_HUMAN) Cytosolic sequence: aa1188–1582 Mouse Bai1 (Uniprot Q3UHD1 (BAI1_MOUSE)) Fluorophore: mGFP
Recombinant DNA reagent	CD19-CD3 zeta CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: ggtggcggtgggtcggcggtggcggtgggtatct - - V-H chain: aa 148–267 anti-CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138–206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 52–164, Human TCR CD3 zeta chain (Uniprot P20963) Fluorophore: sfGFP
Recombinant DNA reagent	CD19-PI3K CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS

				linker: ggtggcgggtggct cgggcgggtgggtgggtcgggtggcggcgatc t -- V-H chain: aa 148–267 anti- CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138– 206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 500–534 Mouse CD19 (Uniprot CD19_MOUSE) Fluorophore: mCherry
Recombinant DNA reagent	CD19 tandem CAR	this disclosure		Signal peptide: aa 1–21 CD8 (Uniprot Q96QR6_ HUMAN) Extracellular antibody sequence: V-L chain: aa 23–130 anti-CD19 CAR (Genbank AMZ04819) -- GS linker: ggtggcgggtggctc gggcgggtgggtgggtcgggtggcggcgatct -- V-H chain: aa 148–267 anti- CD19 CAR (Genbank AMZ04819) Stalk/Transmembrane: aa 138– 206 CD8 (Uniprot Q96QR6_HUMAN) Cytosolic sequence: aa 500–534 Mouse CD19 (Uniprot CD19_MOUSE) fused to aa 19–86 Mouse Fc ERG precursor (FCERG_MOUSE) Fluorophore: mGFP
Recombinant DNA reagent	GFP-CaaX	this disclosure		eGFP fused to a c terminal CaaX targeting sequence: aaaatgtccaaggatggtgta agaaaaagaagaagaagtcaaaaaccaagtgt gttatcatg
Recombinant DNA reagent	mCherry-CaaX	this disclosure		mCherry fused to a c terminal CaaX targeting sequence: aaaatgtccaaggatggt aagaaaaagaagaagaagtcaaaaaccaagt gtgttatcatg
Recombinant DNA reagent	OVA/p2a/mCherry- CaaX	this disclosure		Cytoplasmic Ovalbumin (UNIPROT: SERPINB14)/p2 a site: GGAAGCGGAGCTACTAA CTTCAGCCTGCTGAAGCAGG CTGGAGA CGTGGAGGAGAACCCTGGAC CT/followed by mCherry fused to a c terminal CaaX targeting sequence: aaaatgtccaaggatggtgtaagaaaaagaag aagaagtcaaaaaccaagtgtgttatcatg
Peptide, recombinant protein	His10-CD3 zeta	Hui and Vale (2014) PMID: 24463463		aa 52–164, Human TCR CD3 zeta chain (Uniprot CD3Z_HUMAN) fused to Hisx10 tag
Peptide, recombinant	His10-FcRy	this disclosure		aa 45–85, Human FcRy (Uniprot FCERG_HUMAN) fused to

protein				Hisx10 tag
Peptide, recombinant protein	SNAP-Syk tSH2	this disclosure		aa 1–262, Mouse Syk (Uniprot KSYK_MOUSE) with N-term SNAP tag
Peptide, recombinant protein	His10-Lck Y505F	Hui and Vale (2014) PMID: 24463463		full length Human Lck with inhibitory Tyr 505 mutated to Phe (Uniprot LCK_HUMAN) fused to Hisx10 tag
Antibody	anti phospho-Tyrosine	Santa Cruz	PY20	1:100 IF primary
Antibody	anti mouse IgG coupled to Alexa Fluor 647	Thermo Fisher Scientific /Lifetech	A21236	1:200 IF secondary
Antibody	anti mouse CD11c coupled to APC	BioLegend	117313	FACS
Antibody	anti mouse F4/80 coupled to APC/Cy7	BioLegend	123117	FACS
Other	DMEM	Gibco	11965–092	
Other	Pen-Strep-Glutamine	Corning	30–009 Cl	
Other	Fetal Bovine Serum (FBS)	Atlanta Biologicals	S1150H	
Other	RPMI	Gibco	11875–093	
Other	HEPES	Gibco	1530080	
Other	2-Mercaptoethanol	Sigma	M6250-100mL	
Commercial assay or kit	MycoAlert Mycoplasma Testing Kit	Lonza	LT07-318	
Recombinant DNA reagent	pMD2.G lentiviral plasmid	other	Addgene 12259	D. Stainier, Max Planck; VSV-G envelope
Recombinant DNA reagent	pCMV-dR8.91	other	Current Addgene 8455	
Recombinant DNA reagent	pHRSIN-CSGW	other		As cited James and Vale (2012), PMID: 22763440
Other	Lipofectamine LTX	Invitrogen	15338–100	Lentivirus production
Other	Lipofectamine	Invitrogen	18324–012	Added to spin infections to improve transduction
Other	Hamilton Gastight Syringes	Hamilton	8 1100	
Other	POPC	Avanti	850457	
Other	Ni2+-DGS-NTA	Avanti	790404	
Other	PEG5000-PE	Avanti	880230	
Other	atto390 DOPE	ATTO-TEC GmbH	AD 390–161	
Other	PBS (Tissue Culture Grade)	Gibco	20012050	
Other	Bioruptor Pico	Diagenode		Used for producing SUVs
Other	5 um silica microspheres	Bangs	SS05N	
Peptide,	CD19-His8	Sino Biological	11880H08H50	

recombinant protein				
Peptide, recombinant protein	CD22-His8	Sino Biological	11958H08H50	
Other	2.5 um silica microspheres (size titration)	Corpuscular	C-SIO-2.5	
Other	5 um silica microspheres (size titration)	Corpuscular	C-SIO-5	
Other	10 um silica microspheres (size titration)	Corpuscular	C-SIO-10	
Other	15 um silica microspheres (size titration)	Corpuscular	C-SIO-15	
Other	20 um silica microspheres (size titration)	Corpuscular	C-SIO-20	
Other	Low retention tubes for microsphere cleaning	Eppendorf	22431081	
Other	MatriPlate	Brooks	MGB096-1-2-LG-L	
Peptide, recombinant protein	M-CSF	Peprtech	315-02	
Other	IMDM	Thermo Fisher Scientific	12440079	
Other	Retronectin	Clontech	T100A	
Commercial assay or kit	CD8 + T cell purification kit	Stemcell	19853	
Other	eFluor670 proliferation dye	Thermo Fisher Scientific	65-0840-85	
Chemical compound, drug	phRSIN-CSGW	Sigma	L4516	
Other	Fluorobrite DMEM	Gibco	A1896701	
Other	DMEM minus phenol red	Gibco	A14430-01	
Other	Rhodamine PE	Avanti	810150C	
Other	DOPS	Avanti	840035C	
Other	SNAP-Cell 505-Star	NEB	S9103S	
Other	PD MiniTrap G-25 column	GE Healthcare	28-9225-29 AB	
Other	6.4% Paraformaldehyde solution	Electron Microscopy Sciences	50980495	

Chemical compound, drug	AlexaFluor 647 Phalloidin	Thermo Fisher Scientific /Molecular Probes	A22284	
Software, algorithm	ImageJ	NIH		
Software, algorithm	Illustrator	Adobe	CC, CS6	
Software, algorithm	Photoshop	Adobe	CC, CS6	
Software, algorithm	Fiji	https://fiji.sc/		
Software, algorithm	Prism	GraphPad	7	
Antibody	anti human CD19 (mouse antibody)	OriGene	TA506240 Clone OT12F6	IgG2a mouse monoclonal antibody
Antibody	anti human CD47 (mouse antibody)	BD Biosciences	556044 Clone B6H12	IgG1 mouse monoclonal antibody
Antibody	anti Ovalbumin (rabbit antibody)	Pierce	PA1-196	IgG rabbit polyclonal antibody

Cell Cultures

[00159] J774A.1 macrophages and NIH 3T3 fibroblasts were obtained from the UCSF cell culture facility and cultured in DMEM (Gibco, Catalog #11965–092) supplemented with 1 × Pen-Strep-Glutamine (Corning, Catalog #30–009 Cl) and 10% fetal bovine serum (FBS) (Atlanta Biologicals, Catalog #S11150H). Raji B cells were obtained from J. Blau (McManus lab, UCSF) and cultured in RPMI (Gibco, Catalog #11875–093) supplemented with 1 × Pen-Strep-Glutamine (Corning, Catalog #30–009 Cl), 10% FBS (Atlanta Biologicals, Catalog #S11150H), 10 mM HEPES (Gibco, Catalog #1530080), and 5 μM 2-Mercaptoethanol (Sigma, Catalog #M6250-100mL). All cell lines used in this study were tested for Mycoplasma at least once per month using the Lonza MycoAlert Detection Kit (Lonza, Catalog# LT07-318) and control set (Lonza, Catalog #LT07-518).

Lentivirus Production and Infection

[00160] Lentiviral infection was used to stably express CAR-P constructs in all cell types. Lentivirus was produced by HEK293T cells transfected with pMD2.G (a gift from Didier Trono, Addgene plasmid # 12259 containing the VSV-G envelope protein), pCMV-dR8.91 (since replaced by second generation compatible pCMV-dR8.2, Addgene plasmid #8455), and a lentiviral backbone vector containing the construct of interest (derived from pHRSIN-CSGW)

using lipofectamine LTX (Invitrogen, Catalog # 15338–100). The media on the HEK293T cells was replaced with fresh media 8–16 hours post transfection to remove transfection reagent. At 50–72 hours post-transfection, the lentiviral media was filtered with a 0.45 μm filter and concentrated by centrifugation at $8000 \times g$ for 4 hours or overnight. The concentrated supernatant was applied directly to $\sim 0.5 \times 10^6$ NIH 3T3 cells in 2 ml of fresh media. For J77A4.1 macrophages and Raji B cells, the concentrated supernatant was mixed with 2 ml of media and 2 μg lipofectamine (Invitrogen, Catalog # 18324–012) and added to the cells. The cells were spun at $2200 \times g$ for 45 minutes at 37°C . Cells were analyzed a minimum of 72 hours later.

Preparation of CD19 and CD22 5 μm Silica Beads

[00161] Chloroform-suspended lipids were mixed in the following molar ratio using clean glass-tight Hamilton syringes (Hamilton, Catalog #8 1100): 97% POPC (Avanti, Catalog # 850457), 2% Ni²⁺-DGS-NTA (Avanti, Catalog # 790404), 0.5% PEG5000-PE (Avanti, Catalog # 880230, and 0.5% atto390-DOPE (ATTO-TEC GmbH, Catalog # AD 390–161). Lipid mixes were dried under argon and desiccated overnight under foil. Dried lipids were resuspended in 1 ml tissue-culture grade PBS, pH 7.2 (Gibco, Catalog # 20012050), and stored under argon gas. Small unilamellar vesicles were formed by five freeze-thaw cycles followed by 2×5 minutes of bath sonication (Bioruptor Pico, Diagenode), and cleared by ultracentrifugation (TLA120.1 rotor, 35,000 rpm / $53,227 \times g$, 35 min, 4°C) or by 33 freeze thaw cycles. Lipid mixes were used immediately for form bilayers or shock frozen in liquid nitrogen and stored under argon at -80°C . To form bilayers on silica beads, 6×10^8 5 μm silica microspheres (10% solids, Bangs Labs, Catalog # SS05N) were washed $2 \times$ in water, and $2 \times$ in PBS by sequential suspension in water and spinning at 800 rcf, followed by decanting. Cleaned beads were resuspended in 150 μl tissue-culture grade PBS, pH7.2 (Gibco, Catalog # 20012050) and briefly vortexed. 30 μl cleared SUVs prepared as above as a 10 mM stock were added to bead suspension for a 2 mM final SUV concentration. Beads were vortexed for 10 seconds, covered in foil, and rotated for 30 minutes at room temperature to form bilayers. Bilayer-coated beads were washed $3 \times$ in PBS by sequential centrifugation at 800 rcf and decanting. Beads were resuspended in PBS + 0.1% w/v BSA for blocking for 15 minutes rotating at room temperature under foil. 10 nM final concentration of CD19-his8 (Sino Biological, Catalog # 11880H08H50) or CD22-his8 (Sino Biological, Catalog # 11958H08H50) protein were added to blocked beads and proteins were allowed to bind during a 45 minutes incubation rotating under foil at room temperature. Beads were washed $3 \times$ in PBS

+ 0.1% w/v BSA by sequential centrifugation at 300 rcf and decanting. Beads were resuspended in 120 μ l PBS + 0.1% w/v BSA.

Preparation of CD19 Silica Beads Over a Range of Diameters

[00162] Prior building bilayers on Silica beads ranging from 2.5 μ m–20 μ m in diameter (Microspheres-Nanospheres, Catalog# C-SIO-2.5, 5, 10, 15, 20), beads were RCA cleaned as follows: beads were pelleted at $2000 \times g$ in low retention tubes (Eppendorf, Catalog #022431081) and resuspended in acetone. Resuspended beads were sonicated for 60 minutes in a bath sonicator. Rinse and sonication were repeated in ethanol. Finally, rinse and sonication were repeated in water. Beads were then washed $2 \times$ in water to remove all traces of ethanol and left in a small volume after decanting. All further steps were performed in a 70–80°C water bath prepared in a fume hood. Proper Personal Protective Equipment (PPE) was worn throughout the RCA cleaning protocol. Washed beads were added to 3 ml of hot 1.5 M KOH in a clean glass vial suspended in the water bath described above. 1 ml 30% H₂O₂ to bead solution and allowed to react for 10 min. Washed beads were cooled on ice, pelleted at 2000xg and rinsed $5 \times$ in ultrapure water. Used cleaning solution was saved for disposal by Environmental Health and Safety (EH and S). Cleaned beads were resuspended in 240 μ l tissue-culture grade PBS, pH 7.2 (Gibco, Catalog # 20012050) and briefly vortexed. The lipid mix used in this assay differed slightly from above. Here a mix of 93.5% POPC (Avanti, Catalog # 850457), 5% Ni²⁺-DGS-NTA (Avanti, Catalog # 790404), 1% PEG5000-PE (Avanti, Catalog # 880230, and 0.5% atto390-DOPE (ATTO-TEC GmbH, Catalog # AD 390–161. Bilayers were built and proteins coupled as described above. The concentration of CD19 was scaled appropriately to account for the increased surface area of the larger beads.

Bead Engulfment Assay

[00163] Twelve to sixteen hours prior to imaging, 2.5×10^4 J774A.1 macrophages expressing the appropriate CAR-P or control construct were plated in a 96-well glass bottom MatriPlate (Brooks, Catalog # MGB096-1-2-LG-L). To assess engulfment, 0.5×10^6 CD19 or CD22-ligated beads were added to each well. Engulfment was allowed to proceed for 45 minutes at 37°C incubator with CO₂. Cells were then imaged as described below.

Bites Assay – J774A.1 Macrophage, Dendritic Cell and NIH 3T3 Fibroblasts

[00164] On the day of imaging, 0.5×10^6 NIH 3T3 fibroblasts, dendritic cells or

macrophages and 1.5 million Raji B cells were combined in a 1.5 ml Eppendorf tube and pelleted by centrifugation (800 rpm / $68 \times g$) for 5 minutes at room temperature. Culture media was decanted to ~100 μ l volume and cells were gently resuspended, and allowed to interact in the small volume for 60 minutes in a 37°C incubator with CO₂. After incubation cells and beads were diluted to a final volume of 1000 μ l and 300 μ l of this co-culture plated for imaging in a 96-well glass bottom MatriPlate (Brooks, Catalog # MGB096-1-2-LG-L), and imaged as described below.

Eating Assay Read by FACS – J774A.1 Macrophage and Raji B Cells

[00165] 20,000 J774A.1 macrophages were plated into 96-well glass bottom MatriPlate (Brooks, Catalog # MGB096-1-2-LG-L) in a final volume of 300 μ l complete DMEM (Gibco, Catalog #11965–092) supplemented with $1 \times$ Pen-Strep-Glutamine (Corning, Catalog #30–009 Cl) and 10% fetal bovine serum (FBS) (Atlanta Biologicals, Catalog #S11150H). 52 hours prior to reading the assay macrophages were stimulated with 500 ng/ml LPS (Sigma, Catalog # L4516). 44 hours prior to imaging LPS was removed by three sequential gentle washes. After LPS removal 10,000 Rajis expressing mCherry-CAAX were added to the well containing stimulated macrophages. The co-culture was incubated for 44 hours in a 37°C tissue culture incubator with 5% CO₂. After 44 hr, the remaining number of Raji B cells remaining was analyzed by FACS as follows: 10,000 counting beads were added to the well immediately prior to reading and the cell-counting bead mixture was harvested by pipetting up and down $8 \times$ with a p200 pipet. The assay was read on an LSRII (BD Biosciences) and Rajis were identified by the presence of mCherry fluorescence.

Primary Cell Transduction and Differentiation

[00166] Bone marrow derived macrophages (BMDMs) were produced as previously described (Weischenfeldt and Porse, 2008), except that L-929 conditioned media was replaced with purified 25 ng/ml M-CSF (Peprotech, Catalog # 315–02). The BMDMs were lentivirally infected with concentrated lentivirus after 5 days of differentiation. Differentiation was confirmed by F4/80 staining on day seven and found to be >95% efficient for each replicate. Phagocytosis was measured on day nine in imaging media lacking M-CSF.

[00167] To produce CAR-P expressing dendritic cells, bone marrow-derived hematopoietic stem cells were lentivirally infected immediately after harvest by spinning with

concentrated lentivirus in GMCSF-containing media (IMDM supplemented with 10% FBS and PSG) on retronectin (Clontech, Catalog # T100A)-coated plates at $2200 \times g$ for 45 minutes at 37°C. Dendritic cells were produced as previously described (Mayordomo *et al.*, 1995) by culturing bone marrow cells for 8–11 days with GMCSF. IL-4 was added 2–3 days before use. Efficient differentiation into CD11c + dendritic cells was verified by FACS, revealing $\geq 95\%$ APC-CD11c + cells (Biolegend, Catalog #N418).

Antigen Cross-Presentation Assay

[00168] The ability of CAR-P to stimulate OTI T cell proliferation was tested using the co-culture assay shown as a schematic in **FIGS. 8A-8D** and described previously (Roberts *et al.*, 2016). 10,000 CAR-P transduced CD11c + dendritic cells transduced and differentiated as above were plated in U bottom 96 well dishes (Falcon, Catalog #353077) and stimulated with 1 $\mu\text{g/ml}$ LPS. 12 hours after LPS stimulation, 40,000 Raji B cells expressing soluble cytosolic ovalbumin (Raji B-OVA) were added to the culture. 24 hours after Raji B-OVA cell addition, 50,000 OTI CD8 + T cells isolated from lymph nodes of OTI TCR transgenic mice using a CD8 + T cell purification kit (Stemcell, Catalog #19853) and labeled with e670 proliferation dye (Thermo Fisher Scientific, Catalog #65-0840-85) were added. 72 hours after OTI addition the percent of OTI cells divided was measured by eFluor670 signal using flow cytometry.

Confocal Imaging

[00169] All imaging in this study was performed using a spinning disk confocal microscope with environmental control (Nikon Ti-Eclipse inverted microscope with a Yokogawa spinning disk unit). For bead internalization assays, images were acquired using a 40×0.95 N/A air objective and unbiased live image acquisition was performed using the High Content Screening (HCS) Site Generator plugin in $\mu\text{Manager}3$. Other images were acquired using either a 100×1.49 N/A oil immersion objective. All images were acquired using an Andor iXon EM-CCD camera. The open source $\mu\text{Manager}$ software package was used to control the microscope and acquire all images.

Quantification of Whole Cell Internalization

[00170] 20,000 J774A.1 macrophages were plated into 96-well glass bottom MatriPlate (Brooks, Catalog # MGB096-1-2-LG-L). Four hours prior to imaging, the macrophages were stimulated with 500 ng/ml LPS (Sigma, Catalog # L4516). Immediately prior to imaging the

LPS-containing media was replaced with Fluobrite DMEM (Thermo Fisher Scientific, Catalog # A1896701) containing 10% FBS. 40,000 Raji cells were added to the macrophages and the co-culture was imaged at 5 minutes intervals for 12 hr. For the antibody experiments, macrophages were washed into DMEM minus phenol red (A14430-01) containing 10% FBS just prior to addition of 40,000 Raji cells. Where indicated antibody was added to a final concentration of 20 $\mu\text{g/ml}$ immediately after Raji cell addition and prior to imaging in order to limit antibody internalization. Because cells moved in and out of the field of view, only cells present after 8 hours of imaging were selected and quantified their B cell eating if they could be followed for four hours or more. Time-lapse analysis was used to ensure that the B cell appeared viable prior to engulfment by the macrophage. Engulfment of B cells with an apoptotic morphology was not counted as a whole cell eating event.

Quantification of Bite Internalization

[00171] During live cell image acquisition GFP-positive J774A.1 macrophages or NIH 3T3 cells were selected by the presence of GFP signal. A full z-stack including the entire cell was captured using 1 μm steps. All z sections were then manually inspected for internalized Raji B cell material. Cells containing one or more bites of fully internalized Raji B cell material >1 μm in diameter were scored as positive.

Liposome FRET Assay

[00172] Experiments were carried out as previously described (Alvey CM *et al.*, Current Biology 27:2065–2077, 2017). Briefly, proteins were purified using a bacterial expression system. All protein components (1 mg/ml BSA, 100 nM tSH2-Syk SNAP-505, 0 to 500 nM His10-CD3 ζ or His10-FcR γ intracellular chain, and 7.2 nM His10-Lck Y505F) were mixed into kinase buffer (50 mM HEPES-NaOH pH 6.8, 150 mM NaCl, 10 mM MgCl₂, and 1 mM TCEP). Liposomes prepared at the following molar ratios: 74.5% POPC (Avanti, Catalog # 850457C), 10% DOGS-NTA (Nickel) (Avanti, Catalog # 790404C, 0.5% Rhodamine PE (Avanti, Catalog # 810150C), and 15% DOPS (Avanti, Catalog # 840035C) were added and the mixture was incubated for 40–60 minutes at room temperature, during which the SNAP-505 fluorescence was monitored at 8 s intervals with 504 nm excitation and 540 nm emission. 1 mM ATP was then injected to trigger Lck mediated phosphorylation of CD3 ζ or FcR γ . Injection was followed by 5 seconds of automatic shaking of the plate, and the fluorescence was further monitored at 8 s intervals for at least 1 hr. Data were normalized by setting the average fluorescence value of the

last 10 data points before ATP addition as 100% and background fluorescence as 0%. The final extent of fluorescence quenching (% fluorescence change) at each concentration of receptor was determined using the average of the last 20 data points after ensuring fully equilibrated binding. Nine reactions containing increasing concentrations of CD3 ζ and nine reactions containing increasing concentrations of FcR γ were run in parallel. The final % fluorescence change was plotted against FcR γ or CD3 ζ concentration. The apparent dissociation constants (K_d) of tSH2-Syk to FcR γ and CD3 ζ were calculated by fitting the data with Graphpad Prism 6.0, using the “one site specific binding” model.

Protein Expression, Purification, and Labeling

[00173] The intracellular portion of the FcR γ -chain (amino acid residues (aa) 45–85, Human FcR γ , Uniprot FCERG_HUMAN) was cloned into a modified pET28a vector containing a His10 upstream to the multiple cloning site using BamHI and EcoRI. The intracellular portion of CD3 ζ (aa 52–164, Human CD3 ζ , Uniprot CD3Z_HUMAN) was also cloned into the His10 modified pET28a vector. A Lys-Cys-Lys-Lys sequence, originally present for fluorescent labeling, is also present between His10 and CD3 ζ in this construct. SNAP-tSH2Syk (aa 1–262) was cloned into a pGEX6 vector using BamHI and EcoRI. His10-CD3 ζ , His10-FcR γ -chain, and GST-SNAP-tSH2Syk were bacterially expressed in BL21 (DE3) RIPL strain of *Escherichia coli* as described previously (Alvey CM *et al.* 2017, *supra*). His10-Lck Y505F was expressed in SF9 cells using the Bac-to-Bac baculovirus system as described previously (Alvey CM *et al.*, 2017). All cells were lysed in an Avestin Emulsiflex system. His10 proteins were purified by using Ni-NTA agarose (Qiagen, Catalog # 30230) and GST-SNAP-tSH2Syk was purified by using glutathione-Sepharose beads (GE Healthcare, Catalog # 17075601) as described previously (Alvey CM *et al.* 2017, *supra*). Soluble SNAP-tSH2 Syk was generated by cleaving the GST moiety via the PreScission Protease at 4°C overnight. All proteins were subjected to gel-filtration chromatography using a Superdex 200 10/300 GL column (GE Healthcare, Catalog # 17517501) in HEPES-buffered saline (HBS) containing 50 mM HEPES-NaOH (pH 6.8 for His10-CD3 ζ , His10-FcR γ -chain, and GST-SNAP-tSH2Syk and pH 7.4 for His10-Lck Y505F), 150 mM NaCl, 5% glycerol, and 1 mM TCEP. The monomer fractions were pooled, frozen in liquid nitrogen and stored at –80°C. All gel-filtered proteins were quantified by SDS-PAGE and Coomassie staining, using BSA as a standard. To prepare fluorescently labeled tSH2 Syk, 10 μ M SNAP-tSH2 Syk was incubated at a 1:2 ratio with SNAP-Cell 505-Star (NEB, Catalog # S9103S)

overnight at 4°C and run over a PD MiniTrap G-25 (GE Healthcare, Catalog # 28-9225-29 AB) column to eliminate excess dye.

Phosphotyrosine and Phalloidin Staining

[00174] To fix and stain preparations described above in bead and bites assays for quantifying enrichment of phosphotyrosine staining, half the media (~150 µl) was gently removed from the imaging well and replaced with 150 µl 6.4% paraformaldehyde solution (prepared from 32% stock, Electron Microscopy Sciences, Catalog # 50980495) in tissue culture grade PBS, pH7.2 (Gibco, Catalog # 20012050). Cells were fixed for 15 minutes in a 37°C incubator with CO₂. After fixation cells were washed 2 × with PBS and permeabilized/blocked for 60 minutes at room temperature in freshly prepared, filter sterilized PBS + 5% FBS + 0.1% w/v saponin (PFS solution). After permeabilization, cells were washed 2 × 3 minutes with PFS solution. Following block, cells were incubated with 1:100 dilution of mouse anti-phosphotyrosine (pTyr) antibody to stain pan-pTyr (Santa Cruz, Catalog # PY20) diluted in PFS solution in the dark for 60 minutes at room temperature then washed 3 × 5 minutes in PFS solution. Washed cells were incubated with a 1:500 dilution of goat anti-mouse Alexa Fluor 647 antibody (Thermo Fisher Scientific/Molecular Probes, Catalog # A21236) in PFS solution in the dark for 60 minutes at room temperature. Wells were then washed 3 × 5 minutes in PFS solution. Cells were covered in 200 µl PBS. If not imaged immediately samples were wrapped in parafilm and foil and stored at 4°C prior to microscopy. Phosphotyrosine enrichment at the synapse was calculated by dividing the mean Alexa Fluor 647 signal of a 5 pixel linescan at the synapse with bead or cell by a 5 pixel linescan on the cortex. For phalloidin staining, cells were fixed with 4% PFA for 15 minutes at room temperature, blocked and permeabilized with 5% BSA in TBS with 0.5% Triton X overnight, and incubated with AlexaFluor 647 Phalloidin (Thermo Fisher Scientific/Molecular Probes, Catalog # A22284) for 20 min. Cells were then washed with PBS, imaged and quantified using the method described above. Each data point represents a single cell, and the graphs reflect pooled results from three biological replicates.

Ovalbumin Antibody Staining

[00175] To fix and stain preparations described above for ovalbumin staining, half the media (~150 µl) was gently removed from the imaging well and replaced with 150 µl 8% paraformaldehyde solution (prepared from 32% stock, Electron Microscopy Sciences, Catalog # 50980495) in tissue culture grade PBS, pH7.2 (Gibco, Catalog # 20012050). Cells were fixed for

10 minutes in a 37°C incubator with CO₂. After fixation cells were washed 2 × with PBS and permeabilized/blocked for 60 minutes at room temperature in freshly prepared, filter sterilized PBS + 0.1% w/v casein + 0.1% w/v saponin (PCS solution). After permeabilization, cells were washed 1 × 3 minutes with PCS solution and blocked for 1 hour at room temperature in PCS. Following block, cells were incubated with 1:100 dilution of rabbit anti-ovalbumin (OVA) antibody to stain OVA (Thermo Fisher Scientific/Pierce, Catalog # PA1-196) diluted in PCS solution overnight at 4°C. Washed cells were incubated with a 1:200 dilution of goat anti-rabbit Alexa Fluor 647 antibody (Thermo Fisher Scientific/Molecular Probes, Catalog # A21235) and 3.3 nM 488 phalloidin (dissolved at 6.6 μM in methanol) in PCS solution in the dark for 60 minutes at room temperature. Wells were then washed 3 × 5 minutes in PCS solution. Cells were covered in 200 μl PBS and immediately imaged. Ovalbumin signal was quantified as the corrected total cell fluorescence (CTCF). CTCF = Integrated Density – Area of Selected Cell * Mean Fluorescence of 3 Background Readings. Each data point represents a single cell, and the graphs reflect pooled results from three biological replicates.

Image Processing and Analysis

[00176] All image quantification was done on raw, unedited images. All images in figures were first analyzed in ImageJ, where a single Z-slice at the center of the cell was extracted. The image intensities were scaled to enhance contrast and cropped in Photoshop. For movies, background was subtracted in Fiji using a rolling ball radius of 50 μm and bleach corrected using the Histogram Matching plug in.

Statistics

[00177] All statistical analysis was performed in Prism 6.0 (GraphPad, Inc.). The statistical test used is indicated in each figure legend. Error bars throughout the paper denote 95% confidence intervals of the mean. *** indicates $p < 0.0001$; ** indicates $p < 0.001$ and * indicates $p < 0.01$.

EXAMPLE 2

Results

[00178] To program engulfment towards a target antigen, a CAR strategy was created using the CAR-T design as a guide (Fesnak *et al.*, 2016). This new class of synthetic receptors is termed “Chimeric Antigen Receptors for Phagocytosis” (CAR-Ps). The CAR-P molecules

contain the extracellular single-chain antibody variable fragment (scFv) recognizing the B cell antigen CD19 (α CD19) and the CD8 transmembrane domain present in the α CD19 CAR-T (Fesnak *et al.*, 2016; Kochenderfer *et al.*, 2009). To identify cytoplasmic domains capable of promoting phagocytosis, a screen was performed on a library of known murine phagocytic receptors: Megf10 (**FIG. 1A**), the common γ subunit of Fc receptors (FcR γ), Bai1, and MerTK (Penberthy and Ravichandran, 2016). FcR triggers engulfment of antibody-bound particles, while the other receptors recognize apoptotic corpses (Freeman and Grinstein, 2014; Penberthy and Ravichandran, 2016). A receptor containing an extracellular α CD19 antibody fragment and a cytoplasmic GFP, but no signaling domain, as constructed to test whether adhesion mediated by the α CD19 antibody fragment is sufficient to induce engulfment (**FIG. 1A**; CAR-P^{GFP}).

[00179] To assay the library of CAR-Ps, each CAR-P was introduced into J774A.1 murine macrophages by lentiviral infection. As an engulfment target, 5 μ m diameter silica beads coated with a supported lipid bilayer was used. A His8-tagged extracellular domain of CD19 was bound to a NiNTA-lipid incorporated into the supported lipid bilayers. Macrophages expressing a CAR-P with the Megf10 (CAR-P^{Megf10}) or FcR γ (CAR-P^{FcR γ}) intracellular domain promoted significant engulfment of CD19 beads compared to macrophages with no CAR (**FIGs. 1B-1C**). A video clip showing a non-limiting example of engulfment of silica beads by a modified macrophage was also taken to show that a macrophage infected with α CD19 CAR-P^{Megf10} engulfed 5 μ m silica beads coated in a supported lipid bilayer (labeled with atto647) and ligated to His-tagged CD19 extracellular domain ((also see, Figure 1 - Supplemental Video 1 of Morrissey *et al.*, 2018). The field of view used for this video clip was $43 \times 43 \mu$ m. The video clip is a maximum intensity projection of 17 z-planes acquired at 0.5 μ m intervals. Z-stacks were acquired every 30 seconds for 30 minutes. Images were acquired every 20 seconds for 30 minutes and time is indicated in the bottom right.

[00180] Macrophages expressing CAR-P^{Bai1}, CAR-P^{MerTK}, and the adhesion-only CAR-P^{GFP} did not bind the CD19 beads even though these CAR-Ps are present at the cell surface (**FIGs. 1B-1C and 1E**). To confirm that the CAR-P was a viable strategy for redirecting primary macrophages, the CAR-P^{FcR γ} was expressed in BMDMs and found that these transfected primary cells also were able to trigger engulfment of CD19 beads (**FIG. 1D**).

[00181] The following experiments was performed to determine if the CAR-P strategy could target a different antigen. Because CAR-P^{Megf10} performed well in the initial screen as

described in **FIG. 1A** above, an α CD22 CAR- P^{Mefl10} was developed using a previously developed α CD22 antibody fragment (Xiao *et al.*, 2009; Haso *et al.*, 2013). Consistent with the results using α CD19-based CARs, it was observed that α CD22 CAR- P^{Mefl10} promoted engulfment of CD22 beads (**FIG. 2A**). To confirm antigen specificity of CAR-P, α CD19 CAR- P^{Mefl10} macrophages were incubated with CD22 beads, and α CD22 CAR- P^{Mefl10} macrophages with CD19 beads. CD19 beads were not eaten by α CD22 CAR- P^{Mefl10} macrophages, and CD22 beads were not eaten by α CD19 CAR- P^{Mefl10} macrophages (**FIG. 2A**). These data indicate that CAR- P^{Mefl10} specifically triggers engulfment in response to the target ligand and that the CAR-P strategy is able to target multiple cancer antigens.

[00182] To further define the capabilities of the CAR-P, the capacity of CAR-P-expressing macrophages to engulf variably sized targets was assessed. It was found that CAR- P^{Mefl10} was able to trigger specific engulfment of beads ranging from 2.5 μ m to 20 μ m in diameter, with higher specificity above background engulfment being demonstrated for the larger beads (**FIG. 2B**). The high background in this assay is due to heterogeneity in the bilayers on beads purchased from a different manufacturer (Corpuscular) than previous assays. For the 10 μ m bead condition, the phagocytic efficiency of beads containing the endogenous Mefl10 ligand, phosphatidylserine was also tested. It was found that CAR- P^{Mefl10} macrophages engulfed CD19 beads and beads containing 10% phosphatidylserine and the adhesion molecule ICAM-1 at a similar frequency (**FIG. 2B**). This indicates that the CAR-P is comparably efficient to the endogenous system.

[00183] To determine if the CAR- P^{Mefl10} initiates active signaling at the synapse between the macrophage and target, a phosphotyrosine staining experiment was conducted. Macrophages expressing CAR- P^{Mefl10} exhibited an increase in phosphotyrosine at the synapse, while macrophages expressing CAR- P^{GFP} did not show this enrichment (**FIG. 3A**). Consistent with previous reports, it was found that F-actin also was enriched at the cell bead synapse (**FIG. 3E**). Without being bound to any particular theory, this result suggests that CAR- P^{Mefl10} initiates engulfment through a localized signaling cascade involving tyrosine phosphorylation.

[00184] Both successful CAR-P intracellular domains (from FcR γ and Mefl10) have cytosolic Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) that are phosphorylated by Src family kinases. Without being bound to any particular theory, it was hypothesized, based on this observation, that the expression of an alternate ITAM-containing receptor might initiate

phagocytosis when expressed in macrophages. The CD3 ζ subunit of the T cell receptor contains three ITAM motifs. To test if the CD3 ζ chain was able to activate phagocytic signaling, macrophages with the first generation CAR-T were transduced (**FIG. 3B**). The CAR-T was able to trigger engulfment of CD19 beads to a comparable extent as CAR-P^{Megf10} (**FIG. 3C**). In T cells, phosphorylated ITAMs in CD3 ζ bind to tandem SH2 domains (tSH2) in the kinase ZAP70. Zap70 is not expressed in macrophages, but Syk, a phagocytic signaling effector and tSH2 domain containing protein, is expressed at high levels (Andreu *et al.*, 2017). Previous work suggested that Syk kinase can also bind to the CD3 ζ ITAMs (Bu *et al.*, 1995), indicating that the CAR-T may promote engulfment through a similar mechanism as CAR-P^{FcR γ} . To quantitatively compare the interaction between Syk-tSH2 and CD3 ζ or FcR γ in a membrane proximal system recapitulating physiological geometry, a liposome-based assay was used (**FIG. 3D** [Hui and Vale, 2014]). In this system, His10-CD3 ζ and His10-Lck (the kinase that phosphorylates CD3 ζ) are bound to a liposome via NiNTA-lipids and the binding of labeled tandem SH2 domains to phosphorylated CD3 ζ was measured using fluorescence quenching. The results described herein demonstrate that Syk-tSH2 binds the CD3 ζ and FcR γ with comparable affinity (~15 nM and ~30 nM respectively, **FIG. 3D**). Collectively, these results demonstrate that the TCR CD3 ζ chain can promote phagocytosis in a CAR-P, likely through the recruitment of Syk kinase.

[00185] Additional experiments were performed to program engulfment towards a cellular target. In these experiments, the CAR-P^{Megf10} and CAR-P^{FcR γ} macrophages were incubated with cancerous Raji B cells that express high levels of endogenous CD19. Strikingly, the majority of CAR-P-expressing macrophages internalized bites of the target cell (**FIG. 4A**), 78% of CAR-P^{Megf10} and 85% of CAR-P^{FcR γ} macrophages internalized bites within 90 min). The biting phenotype resembles trogocytosis, or nibbling of live cells, which has been reported previously in immune cells (Joly and Hudrisier, 2003). This process was dependent on the ITAM-bearing intracellular signaling domain, as removing the signaling domain (CAR-P^{GFP}) dramatically reduced trogocytosis (**FIG. 4A**). A video clip showing the engagement of modified macrophage with Raji B cell was taken and described in Morrissey *et al.* (ELife, June 4; 7, 2018; see Figure 4 - Supplemental Video 1), which is hereby incorporated by referenced in its entirety. This video clip shows that a macrophage infected with α CD19 CAR-P^{Megf10} engaged with a Raji B cell (labeled with mCherry-CAAX). The field of view used for this video clip was 43 $\times$ 43 μ m. The video clip was a maximum intensity projection of 7 z-planes acquired at 1 μ m intervals.

Images were acquired every 20 seconds for 30 minutes. Images were acquired every 20 seconds for 30 minutes and time is indicated in the bottom right.

[00186] Enrichment of phosphotyrosine at the cell-cell synapse further supports active signaling initiating trogocytosis (see, *e.g.*, **FIG. 5**). The CAR-P module also was able to induce trogocytosis in non-professional phagocytes, human NIH 3T3 fibroblast cells (see, *e.g.*, **FIG. 6**). This suggests that the CAR-P can promote cancer antigen-dependent engulfment by both professional and non-professional phagocytes.

[00187] The next focus involved engineering strategies to engulf whole human cancer cells. It was observed that macrophages expressing the CAR-P^{Megf10} or CAR-P^{FcRγ} were capable of engulfing whole Raji B cells (2 cancer cells eaten per 100 macrophages in a 4–8 hours window for both CAR-P^{Megf10} or CAR-P^{FcRγ}) (see, **FIGS. 4B** and **4E**). A video clip showing the engagement of modified macrophage with Raji B cell was taken and described in Morrissey *et al.*, 2018, *supra* (see Figure 4 - Supplemental Video 2). This video clip shows that a macrophage infected with αCD19 CAR-P^{FcRγ} engaged with a Raji B cell (labeled with mCherry-CAAX). The field of view used for this video clip was 53 × 53 μm. Images were acquired every 5 minutes. . Time is indicated in the bottom right.

[00188] Whole cell engulfment was infrequent but trogocytosis was robust, suggesting that productive macrophage target interactions were frequently insufficient to trigger whole cell engulfment. To determine if whole cell eating could be enhanced by further opsonization of CD19, Raji B cells were opsonized with a mouse IgG2a anti-CD19 antibody. While addition of this antibody did not trigger additional whole cell internalization, blockade of the ‘don’t eat me’ signal CD47 using the mouse IgG1 anti-human B6H12 clone resulted in a 2.5 fold increase of whole cell eating of opsonized Raji B cells (see, *e.g.*, **FIG. 7**). Both endogenous FcR recognition of the anti-CD47 antibody and blockade of CD47 signaling may contribute to this effect.

[00189] To develop a receptor to enhance whole cell eating, it was hypothesized that combining signaling motifs in a tandem array might increase the frequency of whole cell engulfment by specifically recruiting effectors required for the engulfment of large targets. Previous work demonstrated that PI3K signaling is important for internalization of large targets (Schlam *et al.*, 2015). To increase PI3K recruitment to the CAR-P, the portion of the CD19 cytoplasmic domain (amino acids 500 to 534) that recruits the p85 subunit of PI3K was fused to the CAR-P^{FcRγ} creating a ‘tandem’ CAR (CAR-P^{tandem}, **FIG. 4C**) (Tuveson *et al.*, 1993; Brooks

et al., 2004). A CAR-P containing the p85 recruitment motif alone (CAR-PPI3K) was able to induce some whole cell engulfment, comparable to the CAR-P^{FcRγ} (**FIG. 4E**). Expression of CAR-P^{tandem} tripled the ability of macrophages to ingest whole cells compared to CAR-P^{GFP} (6 cancer cells eaten per 100 macrophages) (**FIGS. 4D-4E**). These data indicate that assembling an array of motifs designed to recruit distinct phagocytic effectors can increase CAR-P activity towards whole cells. A video clip showing the engagement of modified macrophage with Raji B cell was taken and described in Morrissey *et al.*, 2018, *supra* (see Figure 4 - Supplemental Video 3). This video clip shows that a macrophage infected with αCD19 CAR-P^{tandem} engaged with a Raji B cell (labeled with mCherry-CAAX). The field of view for this video clip was 53 × 53 μm. Images were acquired every 5 minutes. Time is indicated in the bottom right.

[00190] To determine if the combination of whole cell eating and trogocytosis was sufficient to drive a noticeable reduction in cancer cell number, CAR-P macrophages were incubated with Raji B cells for two days. After 44 hours of co-culture, it was found that CAR-P macrophages significantly reduced the number of Raji cells (**FIG. 4F**). Although the CAR-P^{tandem} was much more efficient at whole cell eating, the CAR-P^{FcRγ} performed nearly as well at eliminating Rajis. Importantly, the assay described herein does not distinguish between whole cell engulfment and death following trogocytosis, so it is possible both CAR-P activities are contributing to Raji death rates. Overall, these data suggest that the CAR-P is a successful strategy for directing macrophages towards cancer targets, and can initiate whole cell eating and trogocytosis leading to cancer cell elimination.

[00191] In another demonstration, additional experiments were performed to show that the CAR-P described herein is also effective in mouse primary macrophages (BMDMs) as demonstrated in a whole-cell eating assay. The results of these experiments, which is summarized in **FIG. 9**, illustrate much higher efficacy of the tested CAR-P compared to the efficacy previously observed in cell lines. In these experiments, primary mouse BMDMs were transfected with the CAR-P^{tandem}. Macrophages and Raji B cells were incubated together at a 1:2 macrophage:Raji ratio, and the number of whole Raji B cells eaten by 100 macrophages during 4–8 hours of imaging is recorded and graphed. Data is from a single replicate of bone marrow derived macrophages.

[00192] In summary, the present disclosure provides engineered phagocytes that recognize and ingest targets through specific antibody-mediated interactions. This strategy can

be directed towards multiple extracellular ligands (CD19 and CD22) and can be used with several intracellular signaling domains that contain ITAM motifs (Megf10, FcR γ , and CD3 ζ). Previous work has suggested that spatial segregation between Src-family kinases and an inhibitory phosphatase, driven by receptor ligation, is sufficient to trigger signaling by the T cell receptor (Davis and van der Merwe, 2006; James and Vale, 2012) and FcR (Freeman *et al.*, 2016). The CAR-Ps described herein may similarly convert receptor-ligand binding into receptor phosphorylation of ITAM domains through partitioning of kinases and phosphatases at the membrane-membrane interface.

[00193] Further development of CAR-Ps will be useful on several therapeutic fronts. Targeting of tumor cells by macrophages has been suggested to cause tumor cell killing (Jaiswal *et al.*, 2009; Majeti *et al.*, 2009; Chao *et al.*, 2010; Jadus *et al.*, 1996), either through directly engulfing cancer cells or by stimulating antigen presentation and a T cell-mediated response (Liu *et al.*, 2015; Tseng *et al.*, 2013). Inhibition of the CD47-SIRPA ‘Don’t eat me’ signaling pathway has also been shown to result in engulfment of cancer cells (Chen *et al.*, 2017; Gardai *et al.*, 2005; Jaiswal *et al.*, 2009; Majeti *et al.*, 2009; Chao *et al.*, 2010). A recent study suggests that CD47 inhibition is most effective when combined with a positive signal to promote target engulfment, which raises the possibility of combining CAR-P expression with CD47 or SIRPA inhibition for an additive effect (Alvey *et al.*, 2017).

[00194] Although the experiments described herein show that whole cell engulfment could be increased by recruiting the activating subunit of PI3K to the phagocytic synapse, the engulfment of larger 20 micron beads was more frequent than the engulfment of whole cells. Without being bound to any particular theory, it was hypothesized that this is due to differing physical properties of the engulfment target. Specifically, increased target stiffness has been shown to promote engulfment, suggesting that manipulating the physical properties of the engulfment target could also be a potential strategy for increasing CAR-P efficiency (Beningo and Wang, 2002; Cross *et al.*, 2007).

[00195] Overall, the experiments described above demonstrate that the CAR approach is transferrable to biological processes beyond T cell activation and that the expression of an engineered receptor in phagocytic cells is sufficient to promote specific engulfment and elimination of cancer cells.

[00196] While particular alternatives of the present disclosure have been disclosed, it is

to be understood that various modifications and combinations are possible and are contemplated within the true spirit and scope of the appended claims. There is no intention, therefore, of limitations to the exact abstract and disclosure herein presented.

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CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, and one or more of the following:
 - a) a chimeric polypeptide comprising:
 - i. an extracellular domain comprising an antigen-binding region specific for one or more cancer-associated antigen;
 - ii. a transmembrane domain;
 - iii. at least one copy of an intracellular signaling domain from an engulfment receptor; and
 - iv. at least one copy of a recruitment domain;
 - b) a nucleic acid encoding the chimeric polypeptide of (a); and
 - c) a recombinant cell comprising the polypeptide of (a) and/or the nucleic acid of (b).
2. A chimeric polypeptide comprising:
 - a) an extracellular domain comprising an antigen-binding region specific for one or more cancer-associated antigen;
 - b) a transmembrane domain;
 - c) at least one copy of an intracellular signaling domain from an engulfment receptor; and
 - d) at least one copy of a recruitment domain.
3. The chimeric polypeptide of Claim 1, further comprising an amino acid sequence for a signal peptide operably linked upstream to the extracellular domain.
4. The chimeric polypeptide of any one of Claims 1 to 3, further comprising a cell-surface expression enhancing sequence which is operably linked downstream to the transmembrane domain.
5. The chimeric polypeptide of any one of Claims 1 to 4, wherein the antigen-binding region comprises an amino acid sequence encoding an antibody, or a functional fragment thereof, selected from the group consisting of an antigen-binding fragment (Fab), a single-chain variable fragment (scFv), a nanobody, a VH domain, a VL domain, a single domain antibody

(sdAb), a VNAR domain, and a VHH domain, a bispecific antibody, a diabody, or a functional fragment of any thereof.

6. The chimeric polypeptide of any one of Claims 1 to 4, wherein the one or more cancer-associated antigen is selected from the group consisting of CD19, CD22, HER2 (ERBB2/neu), Mesothelin, PSCA, CD123, CD30, CD171, CD138, CS-1, CLECL1, CD33, CD79b, EGFRvIII, GD2, GD3, BCMA, PSMA, ROR1, FLT3, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3 (CD276), KIT (CD 117), CD213A2, IL-1 IRa, PRSS21, VEGFR2, CD24, MUC-16, PDGFR-beta, SSEA-4, CD20, MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, FAP, EphA2, GM3, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CD97, CD179a, ALK, and IGLL1.

7. The chimeric polypeptide of any one of Claims 1 to 6, wherein the one or more cancer-associated antigen is selected from the group consisting of CD19 and CD22.

8. The chimeric polypeptide of any one of Claims 1 to 7, wherein the intracellular signaling domain of the engulfment receptor comprises at least 1, at least 2, at least 3, at least 4, or at least 5 ITAM motifs.

9. The chimeric polypeptide of any one of Claims 1 to 8, wherein the intracellular signaling domain from the engulfment receptor is capable of mediating endogenous phagocytic signaling pathway.

10. The chimeric polypeptide of any one of Claims 1 to 9, the engulfment receptor is selected from the group consisting of Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, and Axl.

11. The chimeric polypeptide of any one of Claims 1 to 10, wherein the transmembrane domain comprises a stalk and/or transmembrane domain derived from CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, Axl, CD45, or CD86.

12. The chimeric polypeptide of any one of Claims 1 to 11, wherein the transmembrane domain is operably linked downstream of the extracellular domain and upstream of the at least one copy of an intracellular signaling domain from an engulfment receptor.

13. The chimeric polypeptide of any one of Claims 3 to 12, wherein the signal peptide comprises a signal peptide derived from CD8, Megf10, FcR γ , Bai1, MerTK, TIM4, Stabilin-1, Stabilin-2, RAGE, CD300f, Integrin subunit α v, Integrin subunit β 5, CD36, LRP1, SCARF1, C1Qa, or Axl.

14. The chimeric polypeptide of any one of Claims 1 to 13, wherein the at least one copy of a recruitment domain comprises:

- a) a p85-binding domain derived from CD19, Gab2, IREM-1, PDGF receptor, CSFR-1, c-Kit, ErbB3, or CD7 to recruit the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K);
- b) an SH3 domain derived from Crk, Cdc25, Phospholipase, Ras, Vav, GRB2, FAK, Pyk2, TRIP10, or Gads to recruit cytosolic signaling proteins; and/or
- c) a proline-rich peptide from C3G, p41, PEP, p47, HPK1, SLP-1, CD3 ϵ , PAK, AIP4, or Sos to recruit SH3 domain-containing proteins.

15. The chimeric polypeptide of any one of Claims 1 to 14, wherein the recruitment domain is operably linked downstream to the at least one copy of the intracellular signaling domain of the engulfment receptor.

16. The chimeric polypeptide of any one of Claims 1 to 14, wherein the recruitment domain is operably linked downstream to the transmembrane domain and upstream to the at least one copy of the intracellular signaling domain of the engulfment receptor.

17. The chimeric polypeptide of any one of Claims 1 to 14, further comprising one or more peptide linker sequence.

18. The chimeric polypeptide of Claim 15, wherein the peptide linker sequence comprises a glycine-serine linker.

19. The chimeric polypeptide of any one of Claims 4 to 18, wherein the cell-surface expression enhancing sequence comprises one or more basic amino acid residues.

20. The chimeric polypeptide of any one of Claims 1 to 19, comprising:

- a) a peptide signal;
- b) an extracellular domain comprising an antigen-binding region specific for one or more cancer-associated antigen;
- c) a transmembrane domain;

- d) an intracellular signaling domain from an engulfment receptor; and
 - e) a p85-recruitment domain.
21. The chimeric polypeptide of any one of Claims 1 to 20, comprising:
- a) a peptide signal from CD8;
 - b) an extracellular single chain antibody variable fragment (scFv) having specific affinity for CD19 or CD22;
 - c) a transmembrane domain from CD8;
 - d) an intracellular signaling domain from Megf10, FcR γ , Bai1, or MerTK; and
 - e) a p85-recruitment domain from CD19.
22. The chimeric polypeptide of any one of Claims 1 to 21, wherein the chimeric polypeptide comprises an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.
23. An isolated, synthetic, or recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide that comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of a chimeric polypeptide according to any one of Claims 1 to 22, or a functional fragment thereof.
24. The nucleic acid molecule of Claim 23, wherein the nucleic acid sequence has at least 80% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18.
25. The nucleic acid molecule of any one of Claims 23 to 24, wherein the nucleic acid sequence is operably linked to a heterologous nucleic acid sequence.
26. The nucleic acid molecule of any one of Claims 23 to 25, wherein the nucleic acid molecule is an expression cassette or an expression vector.
27. A recombinant cell comprising:
- (i) a chimeric polypeptide according to any one of Claims 1 to 22; and/or
 - (ii) a nucleic acid molecule according to any one of Claims 23 to 26.

28. The recombinant cell of Claim 27, wherein the recombinant cell is a prokaryotic cell or a eukaryotic cell.

29. The recombinant cell of any one of Claims 27 to 28, wherein said cell is a human cell.

30. The recombinant cell of any one of Claims 27 to 29, wherein said cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes.

31. The recombinant cell of Claim 29, wherein said phagocytic cell is a bone marrow derived macrophage (BMDM) or a bone marrow derived dendritic cell (BMDC).

32. A cell culture comprising at least one recombinant of any one of Claims 27 to 31, and a culture medium.

33. A pharmaceutical composition comprising:

- a) a chimeric polypeptide according to any one of Claims 1 to 22;
 - b) a nucleic acid molecule according to any one of Claims 23 to 26; and/or
 - c) a recombinant cell according to any one of Claims 27 to 31,
- and a pharmaceutically acceptable carrier.

34. The pharmaceutical composition of Claim 33, wherein the composition comprises a recombinant cell according to any one of Claims 27 to 31, and a pharmaceutically acceptable carrier.

35. The pharmaceutical composition of Claim 34, wherein said cell is a human cell.

36. The pharmaceutical composition of any one of Claims 34 to 35 wherein said cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes.

37. The pharmaceutical composition of Claim 36, wherein said phagocytic cell is a bone marrow derived macrophage (BMDM) or a bone marrow derived dendritic cell (BMDC).

38. A method for modifying a cell, comprising introducing into said cell a chimeric polypeptide according to any one of Claims 2 to 22, and/or a nucleic acid molecule according to any one of Claims 23 to 26.

39. The method of Claim 34, wherein said cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes.

40. A method for treating an individual having cancer, the method comprising administering to the individual an effective number of phagocytic cells that have been modified to express a chimeric engulfment receptor having specific affinity for an antigen associated with a cancer cell.

41. The method of Claim 40, wherein said phagocytic cell is a phagocytic cell selected from the group consisting of macrophages, dendritic cells, mast cells, monocytes, neutrophils, microglia, and astrocytes.

42. The method of Claim 41, wherein said phagocytic cell is a BMDM or a BMDC.

43. The method of any one of Claims 40 to 42, wherein the phagocytic cell is derived from the same individual having cancer.

44. A method for treating an individual having cancer, the method comprising administering to the individual an effective amount or number of:

- a) a chimeric polypeptide according to any one of Claims 2 to 22;
- b) a nucleic acid molecule according to any one of Claims 23 to 26;
- c) a recombinant cell according to any one of Claims 27 to 31; and/or
- d) a pharmaceutical composition according to any one of Claims 1 and 33-37.

45. The method of Claim 44, wherein said administering comprises a viral-, particle-, liposome-, or exosome-based delivery procedure.

46. The method of any one of Claims 44 to 45, wherein said administering comprises delivering into endogenous cells *ex vivo* one or more gene-editing enzymes targeting the chimeric polypeptide according to any one of Claims 1 to 22, and/or targeting the nucleic acid according to any one of Claims 23 to 26.

47. The method of any one of Claims 44 to 45, wherein said administering comprises delivering into cells *in vivo* one or more gene-editing enzymes targeting the chimeric polypeptide according to any one of Claims 1 to 22, and/or targeting the nucleic acid according to any one of Claims 23 to 26.

48. The method any one of Claims 40 to 47, wherein said administration activates phagocytosis of said cancer cell.

49. The method of Claim 48, wherein the activated phagocytosis comprises whole-cell internalization and/or bite internalization.

50. The method of any one of Claims 40 to 49, wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer, lung cancer, pancreatic cancer, mesothelioma, leukemia, lymphoma, brain cancer, prostate cancer, multiple myeloma, melanoma, bladder cancer, bone sarcomas, soft tissue sarcomas, retinoblastoma, renal tumors, neuroblastoma, and carcinomas.

51. A kit comprising one or more of the following:
a chimeric polypeptide according to any one of Claims 2 to 22;
a nucleic acid molecule according to any one of Claims 23 to 26;
a recombinant cell according to any one of Claims 27 to 31; and
a pharmaceutical composition according to any one of Claims 1 and 33-37.

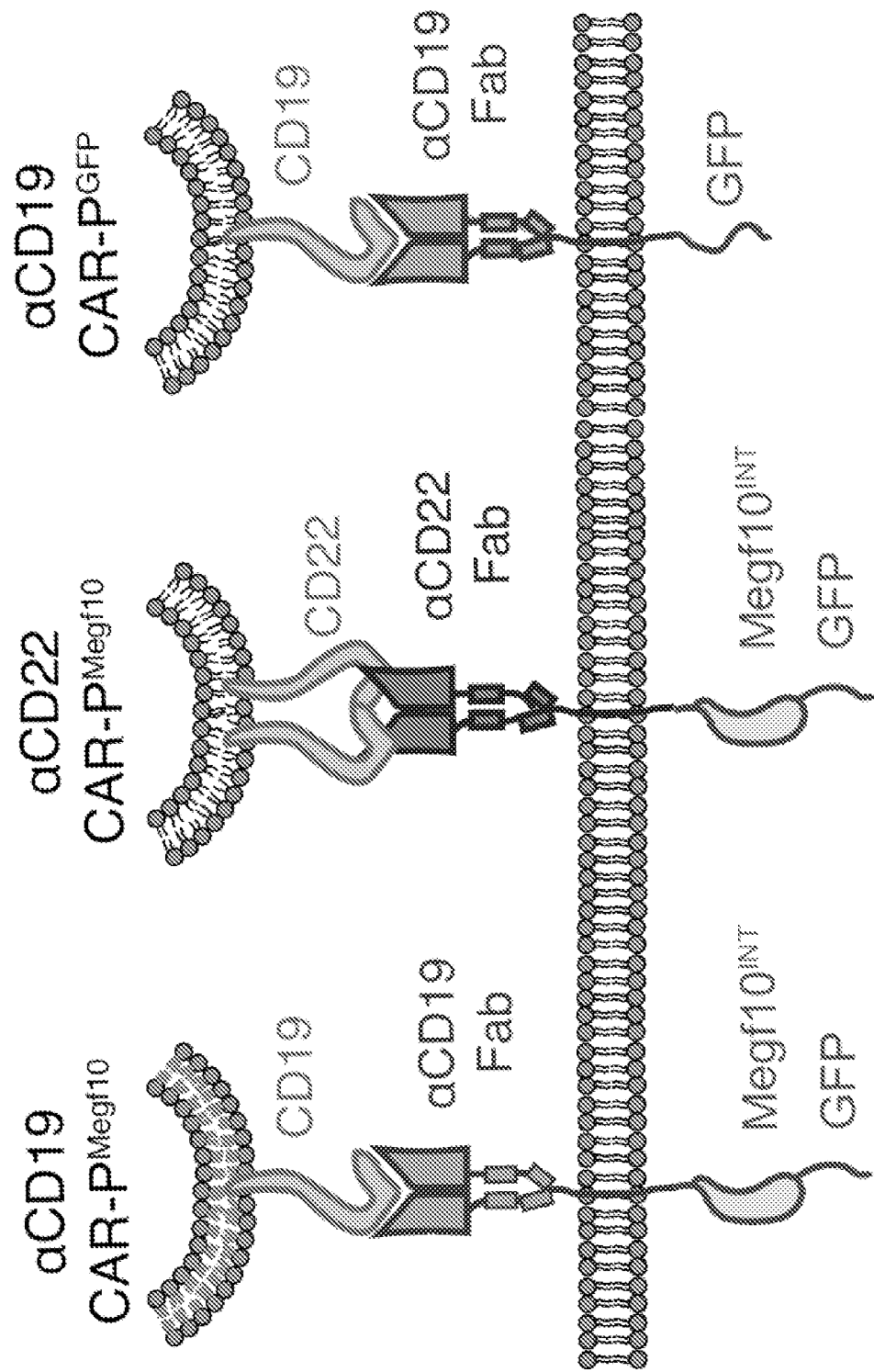


FIG. 1A

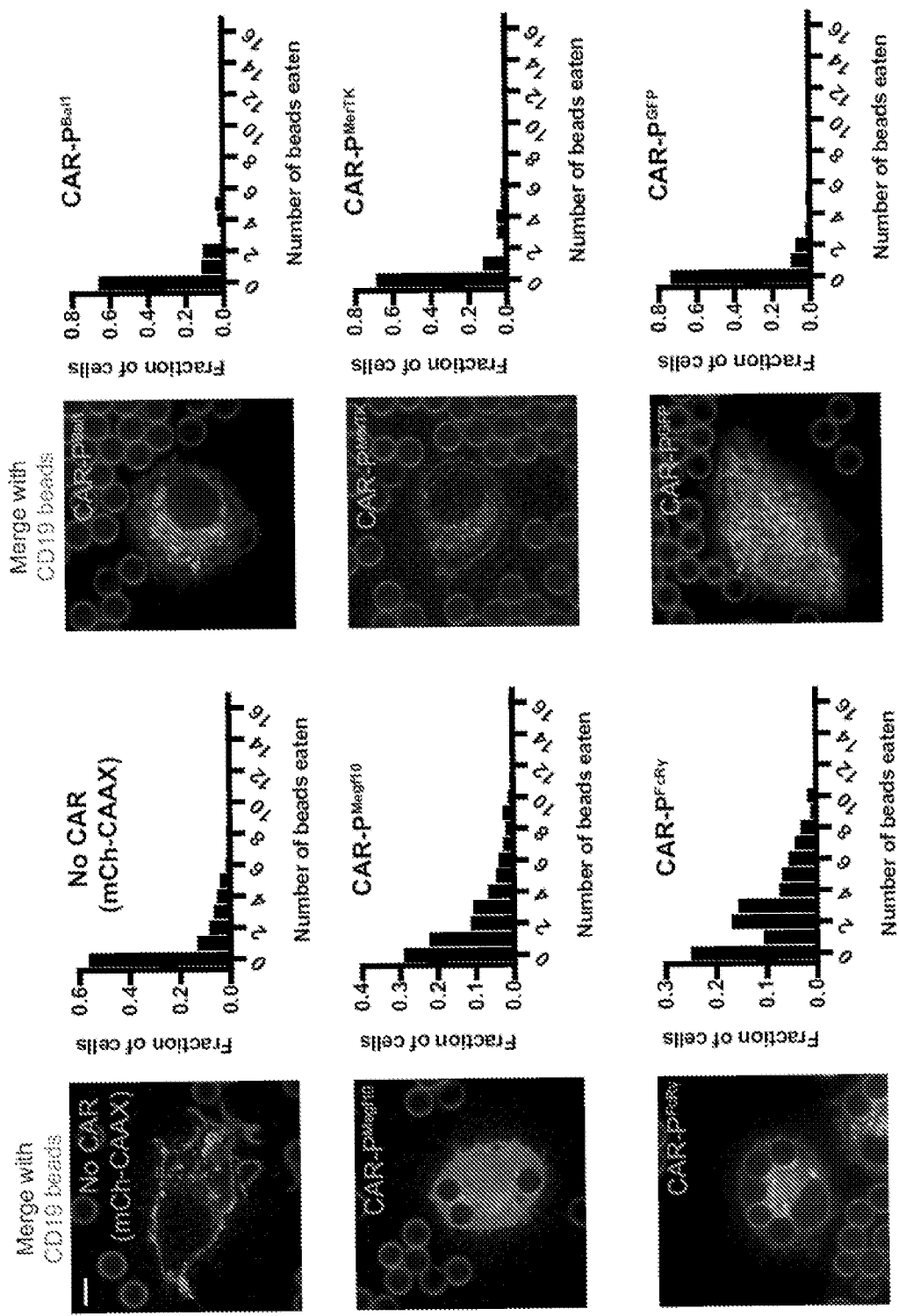


FIG. 1B

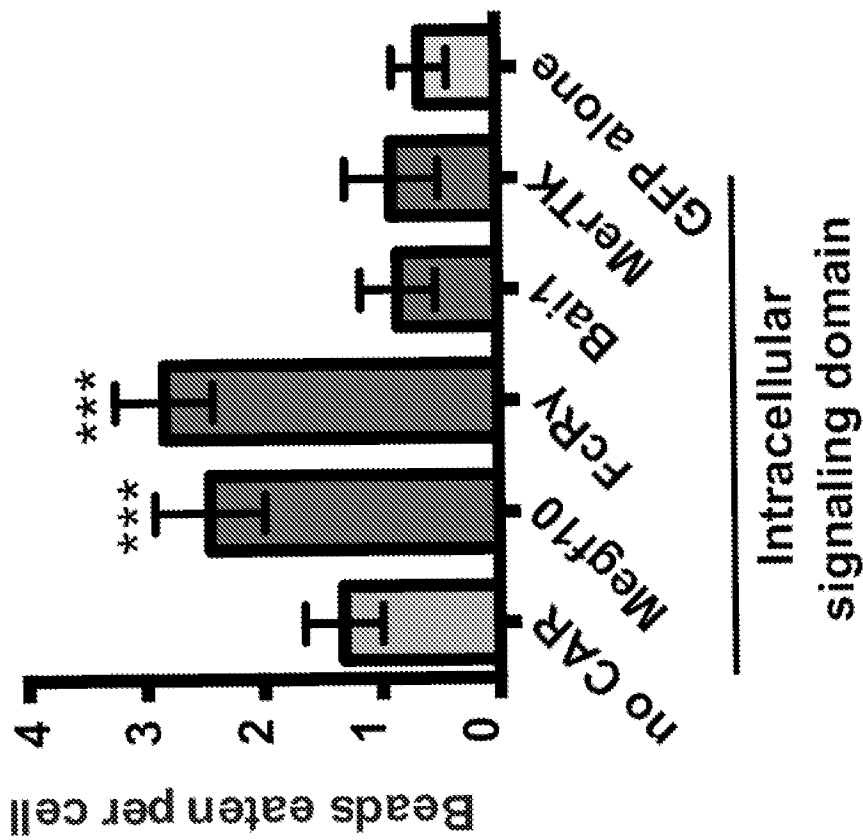


FIG. 1C

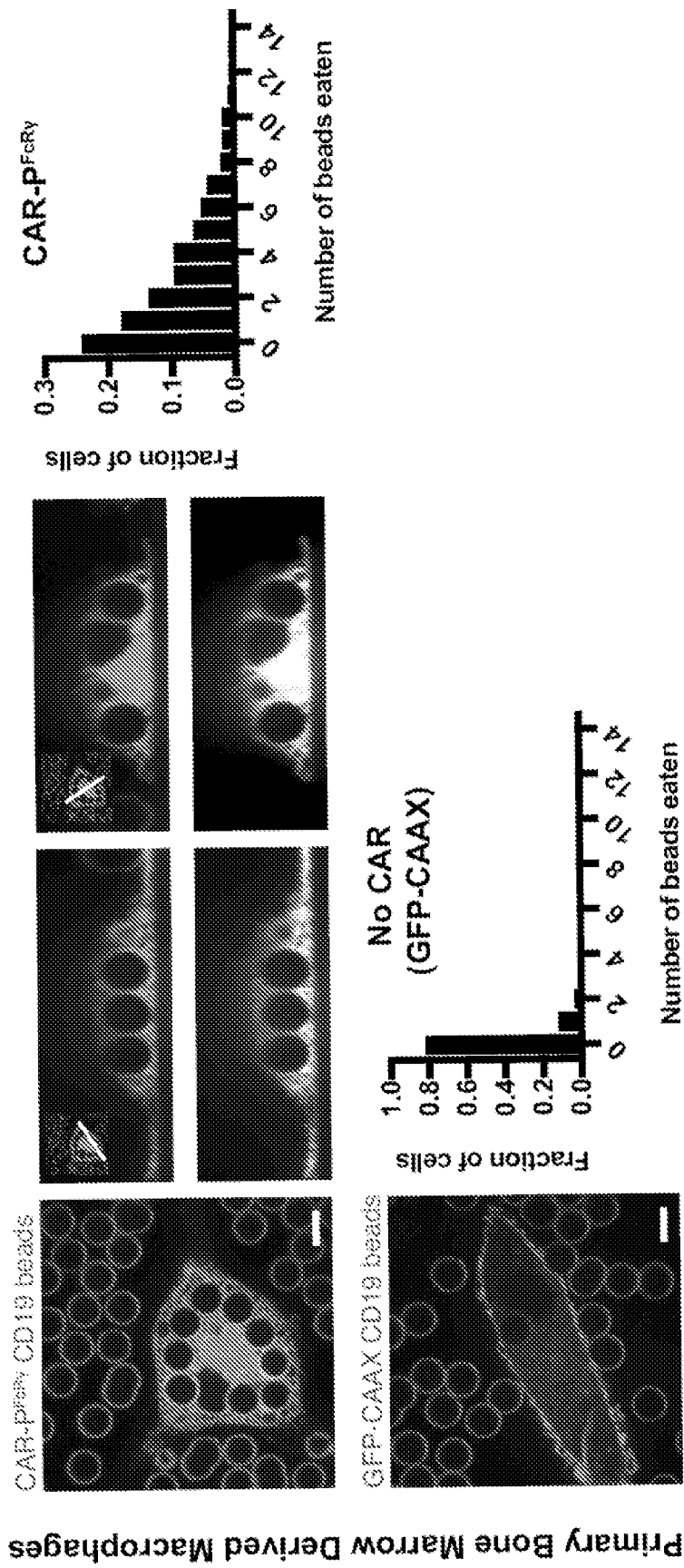
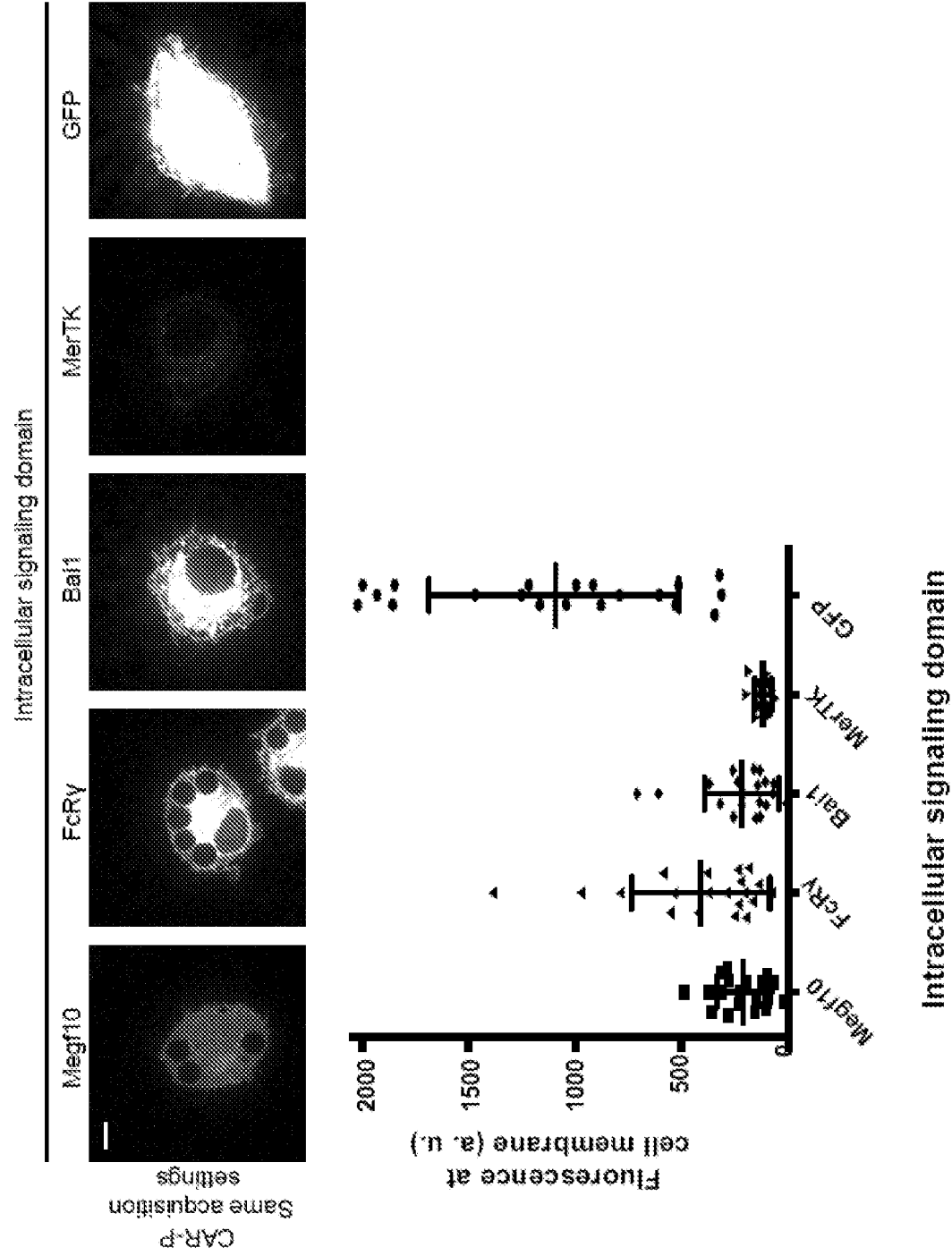


FIG. 1D



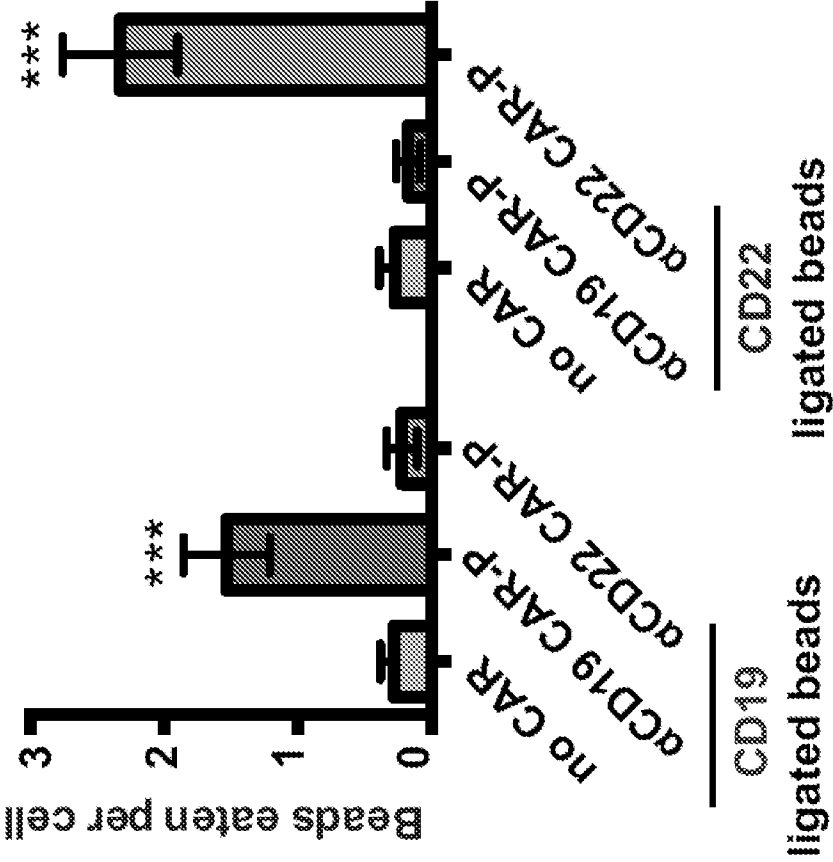


FIG. 2A

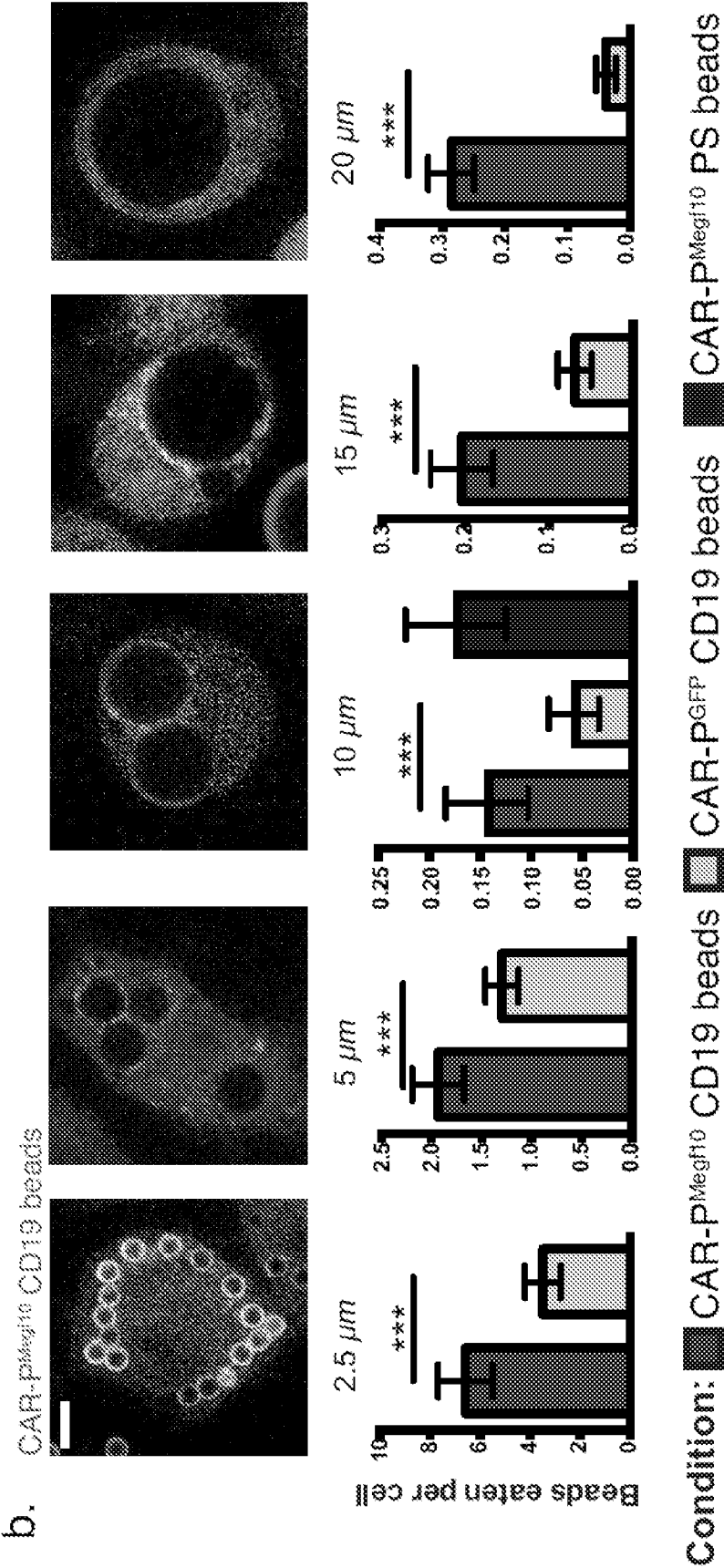


FIG. 2B

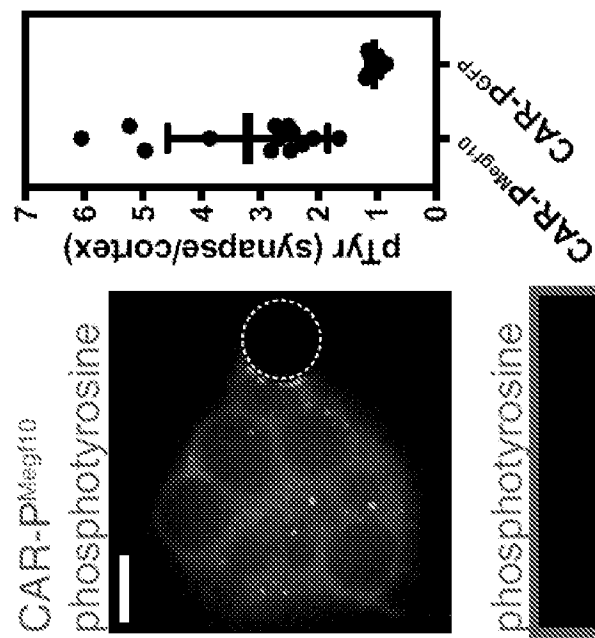


FIG. 3A

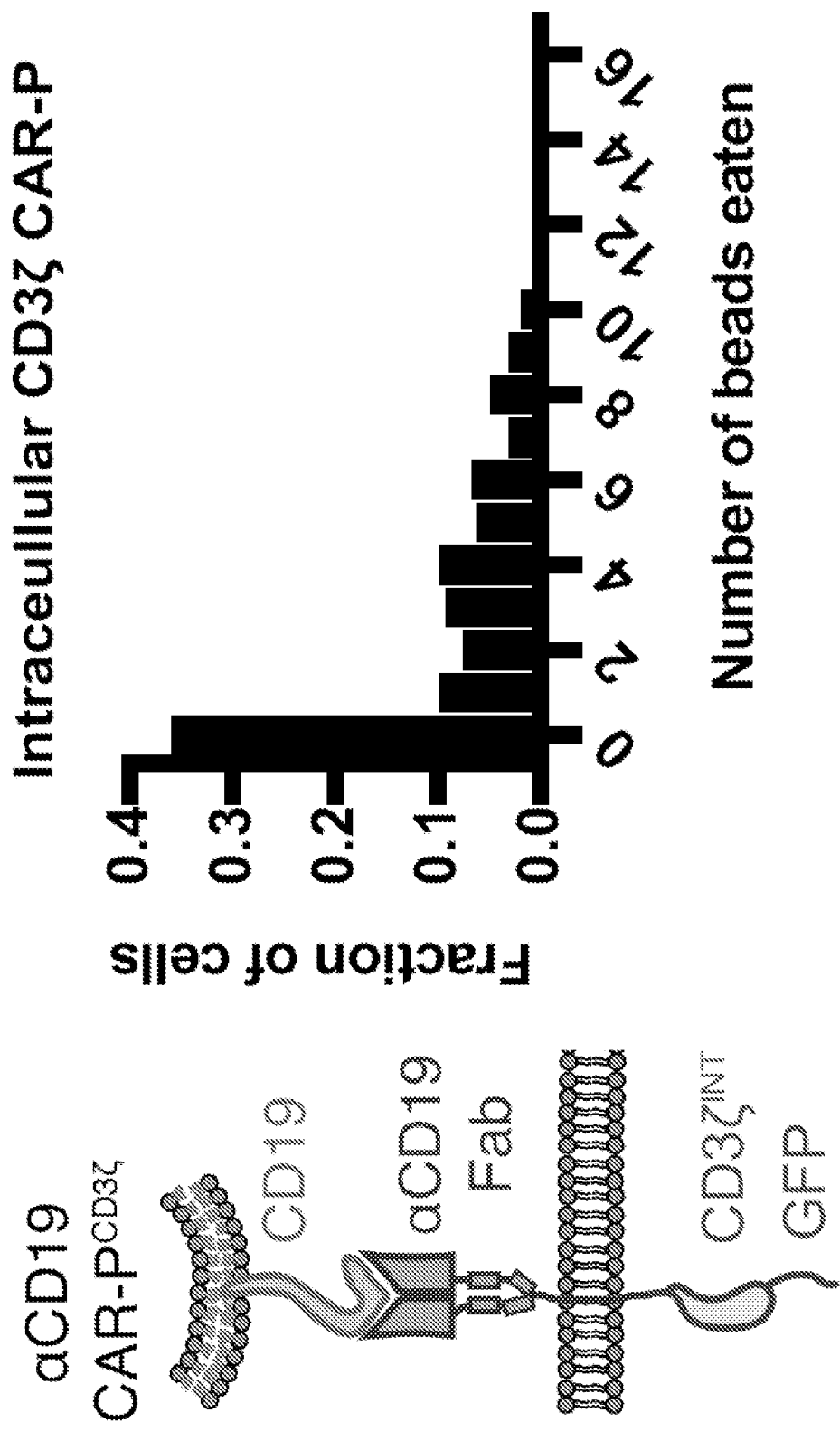


FIG. 3B

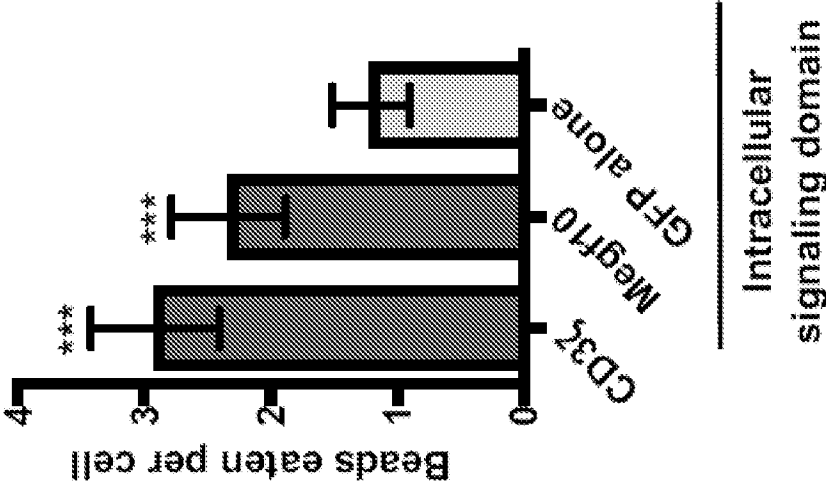


FIG. 3C

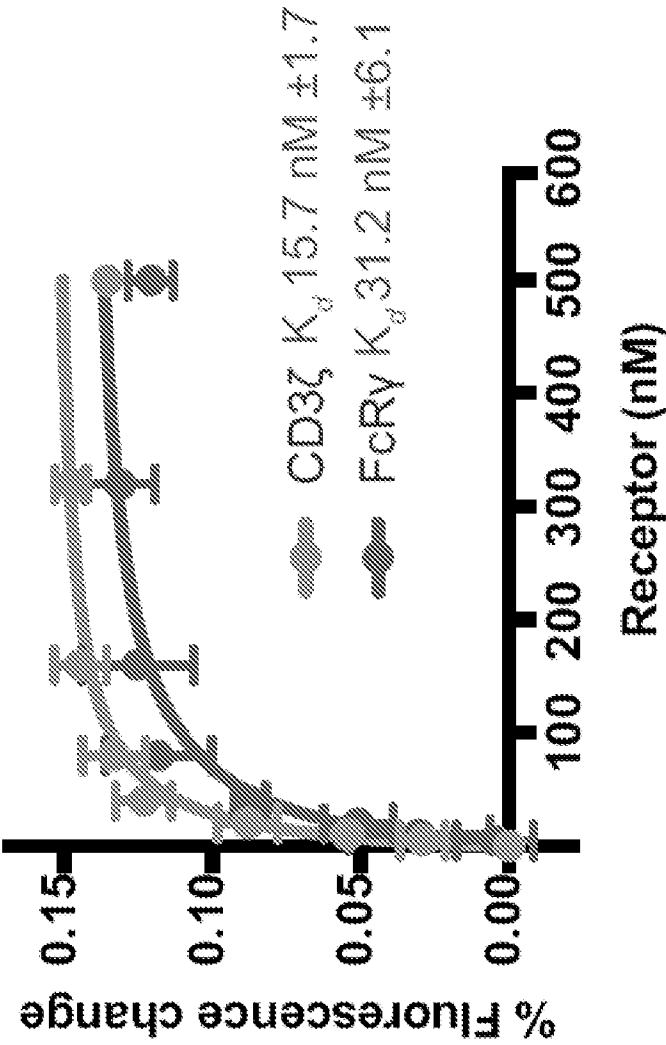
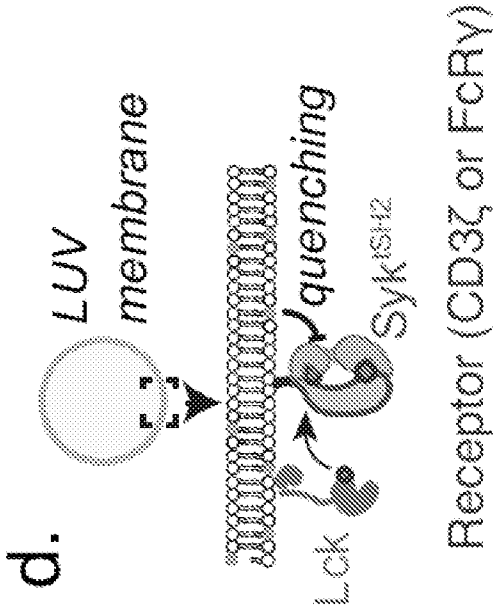


FIG. 3D



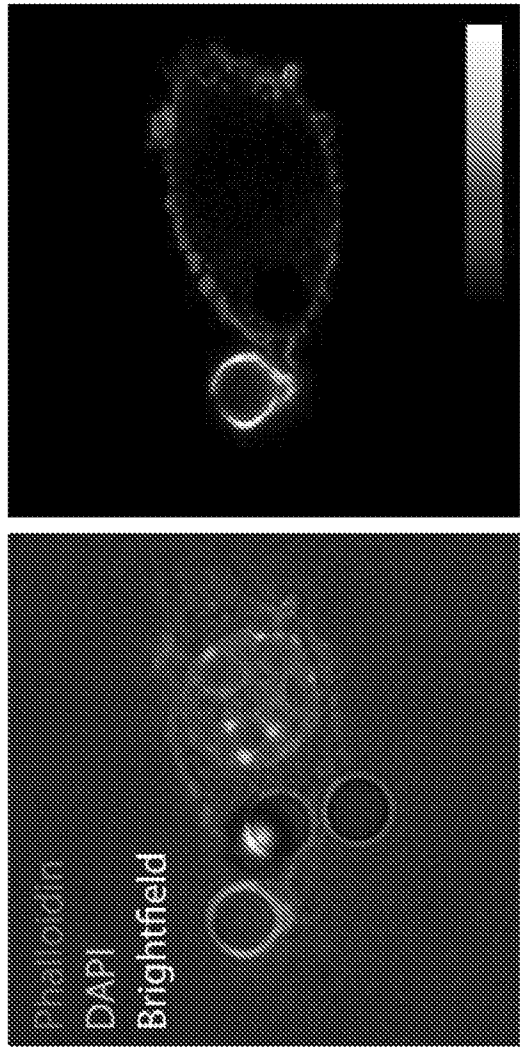
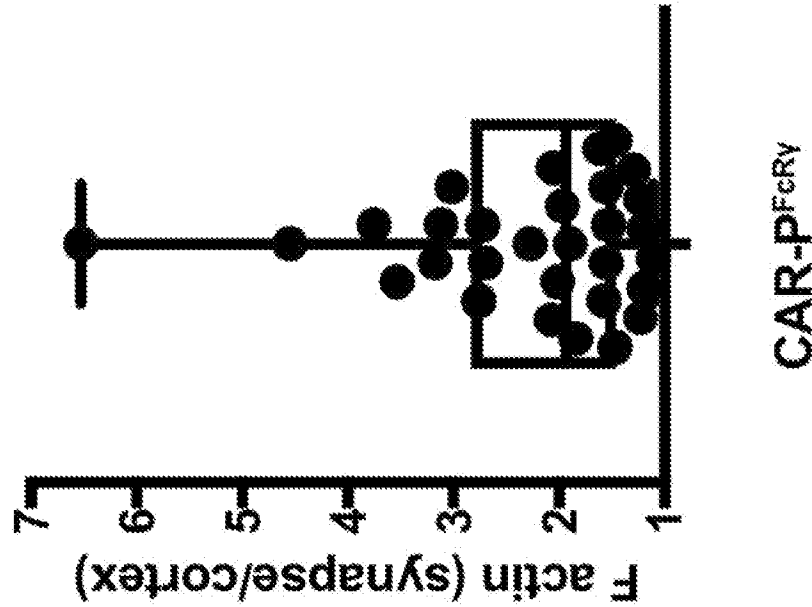


FIG. 3E

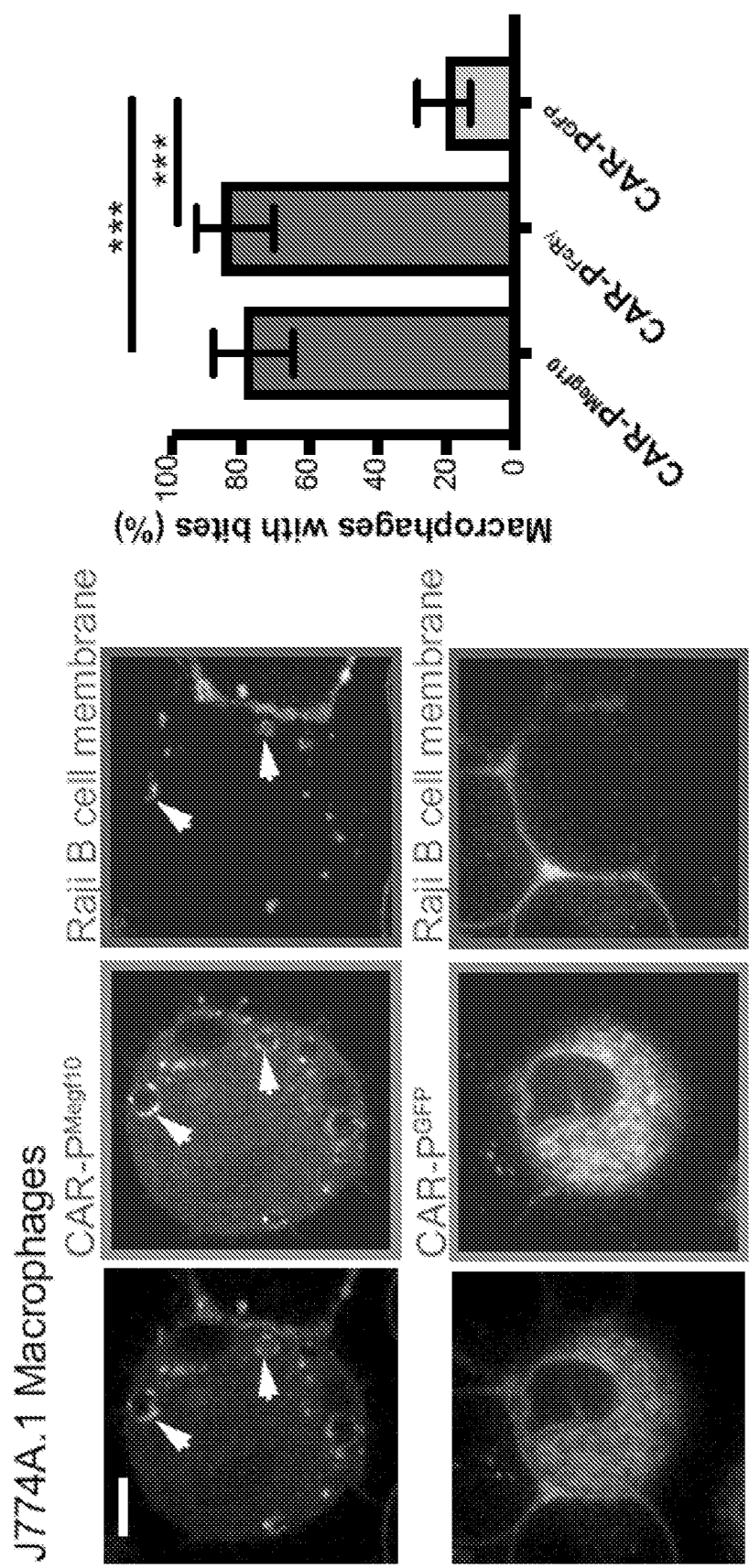


FIG. 4A

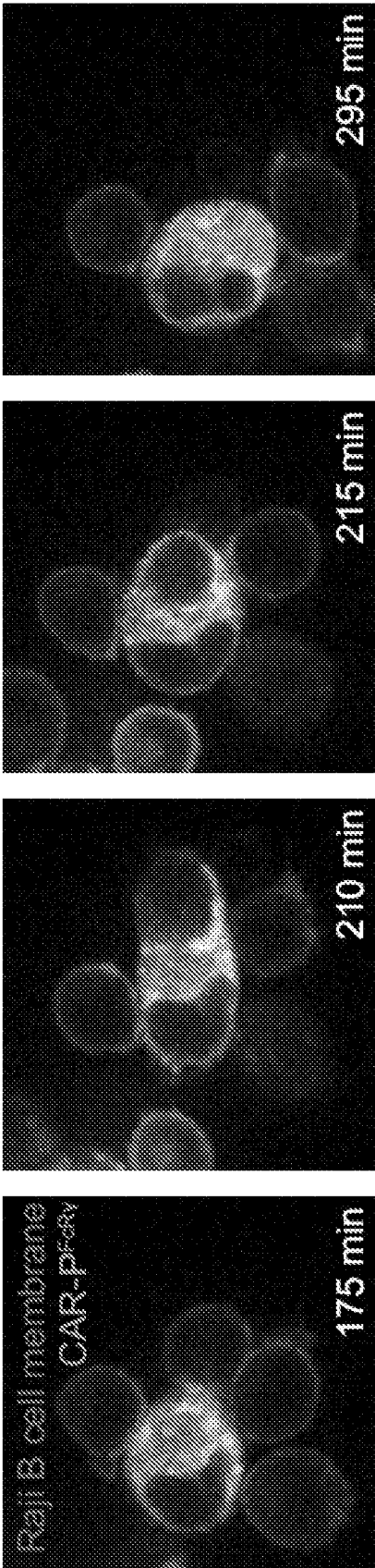


FIG. 4B

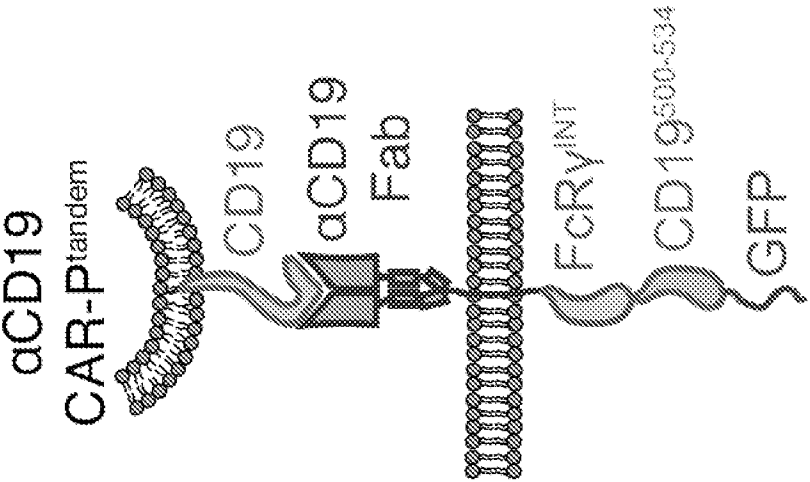


FIG. 4C

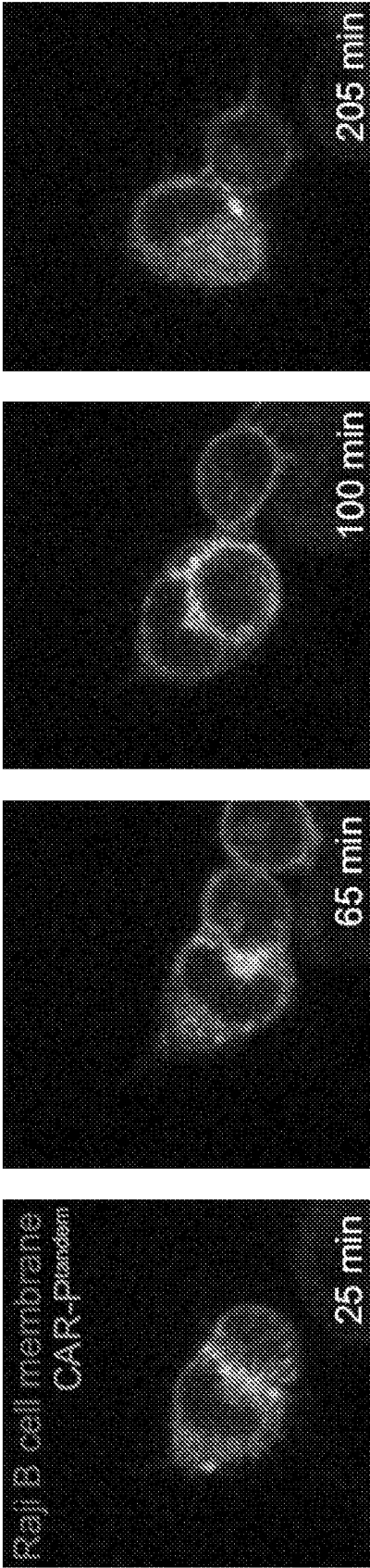


FIG. 4D

f.

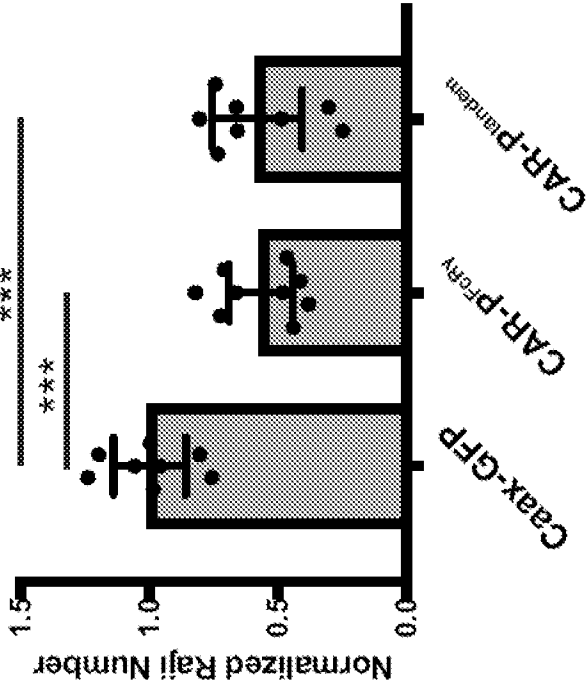


FIG. 4F

e.

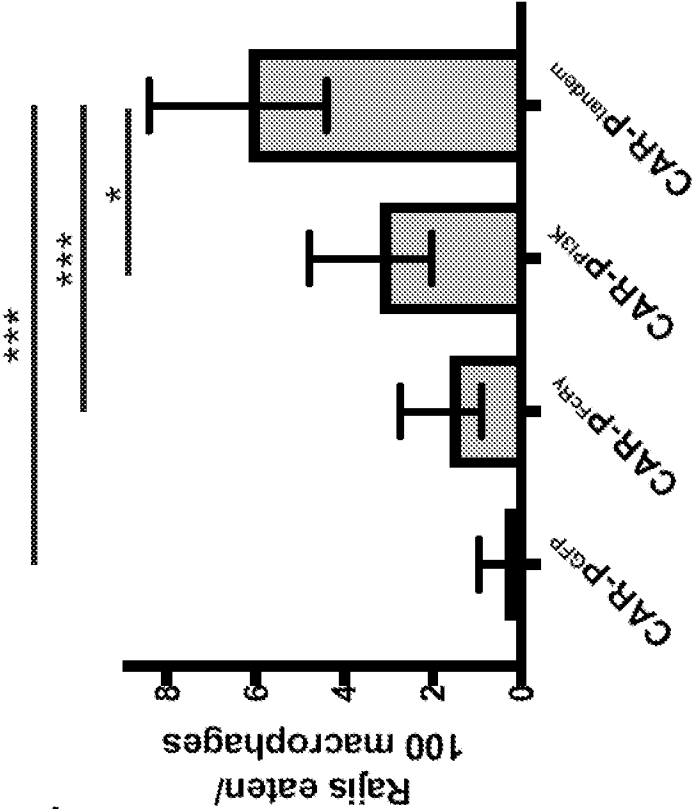


FIG. 4E

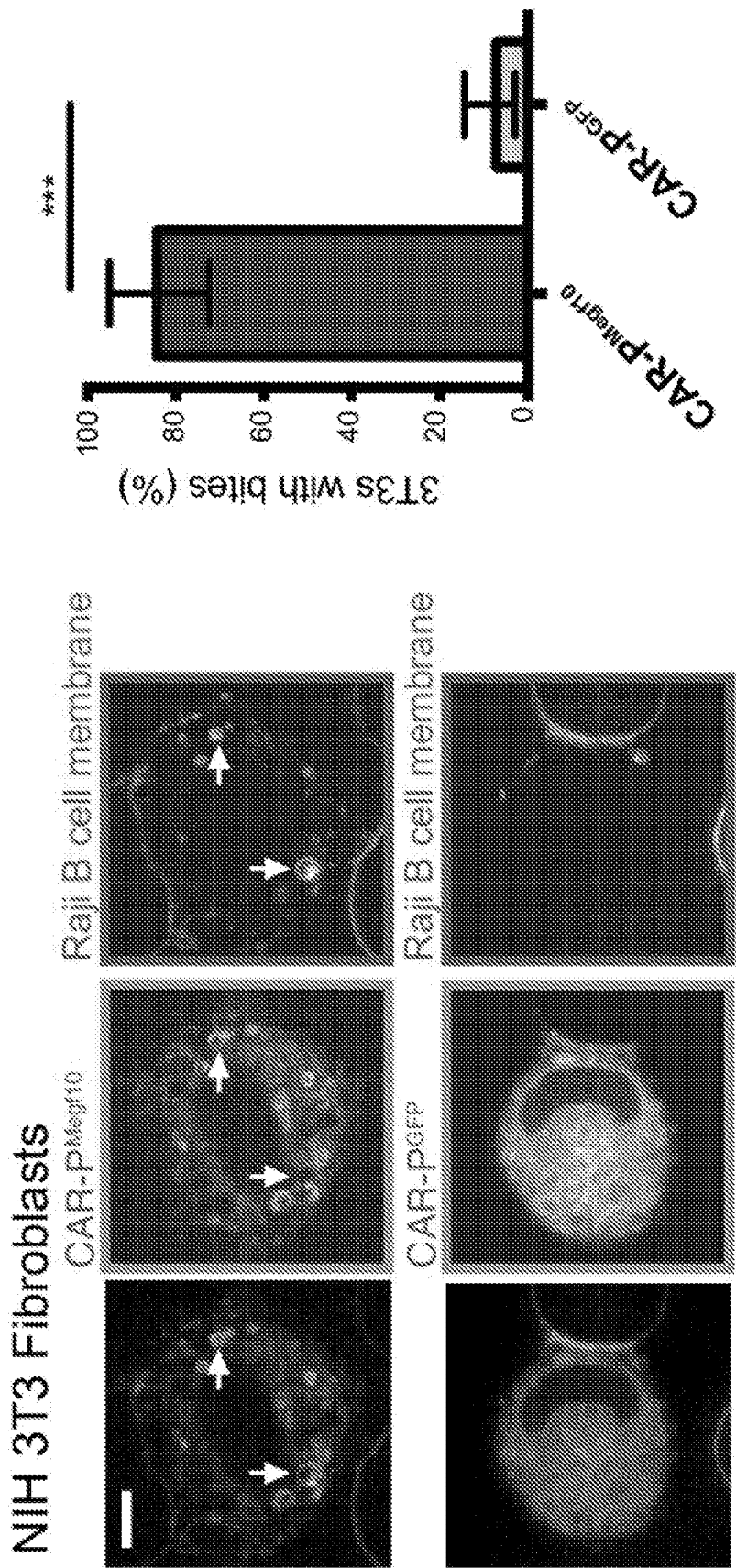


FIG. 6

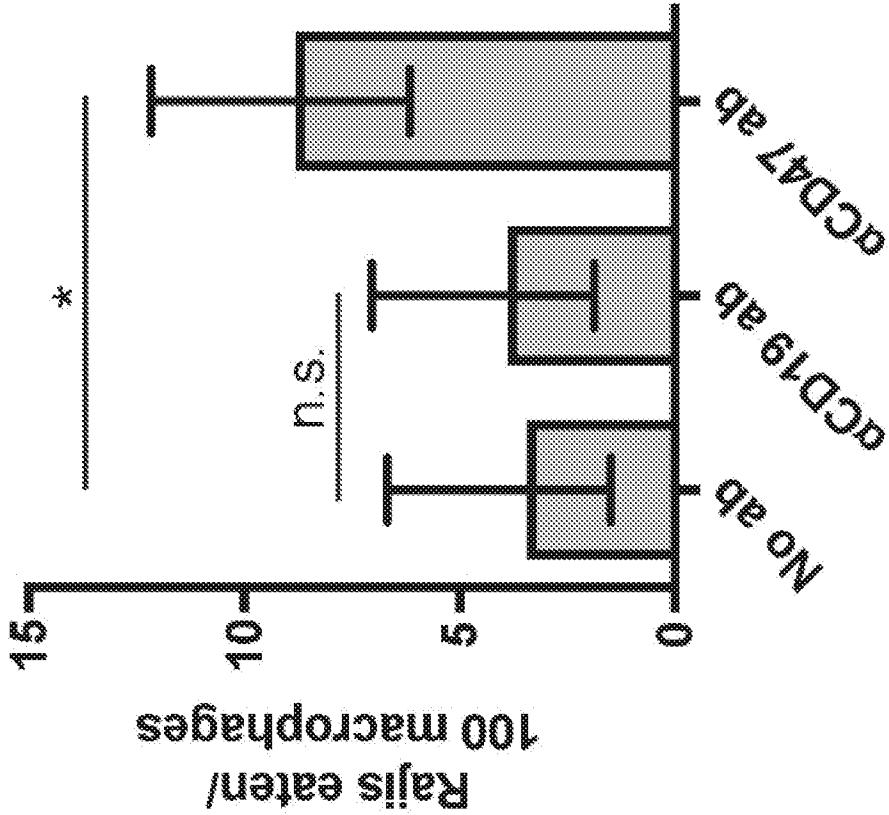


FIG. 7

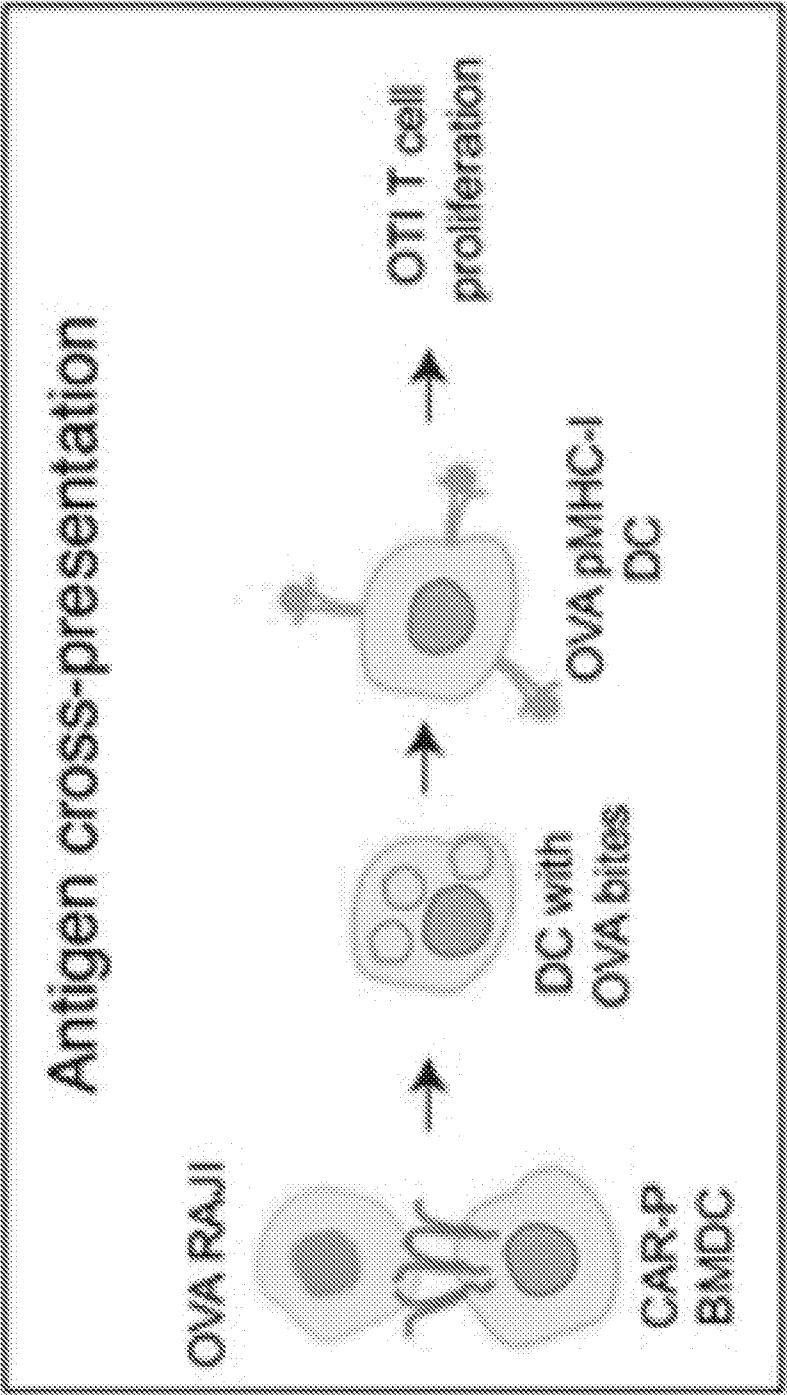


FIG. 8A

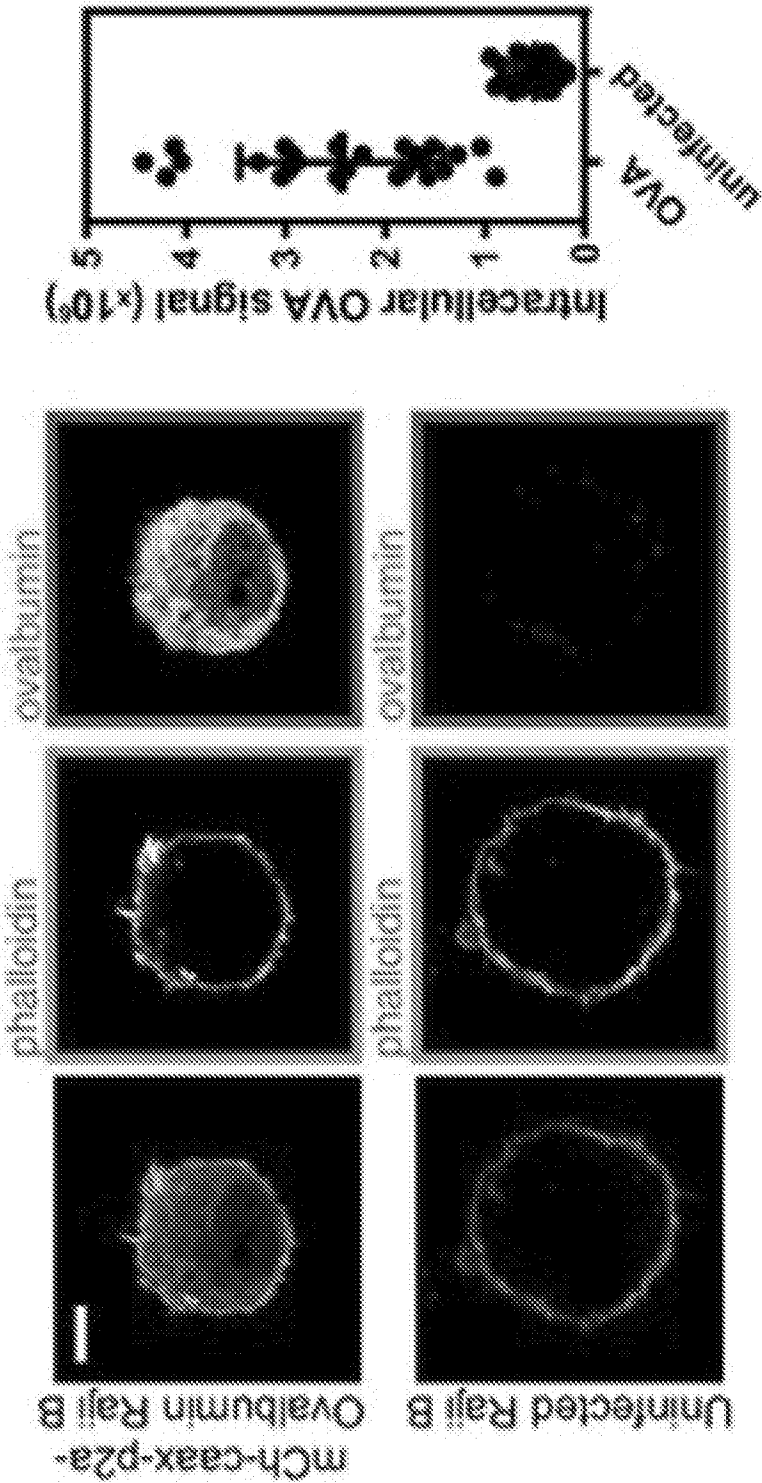


FIG. 8B

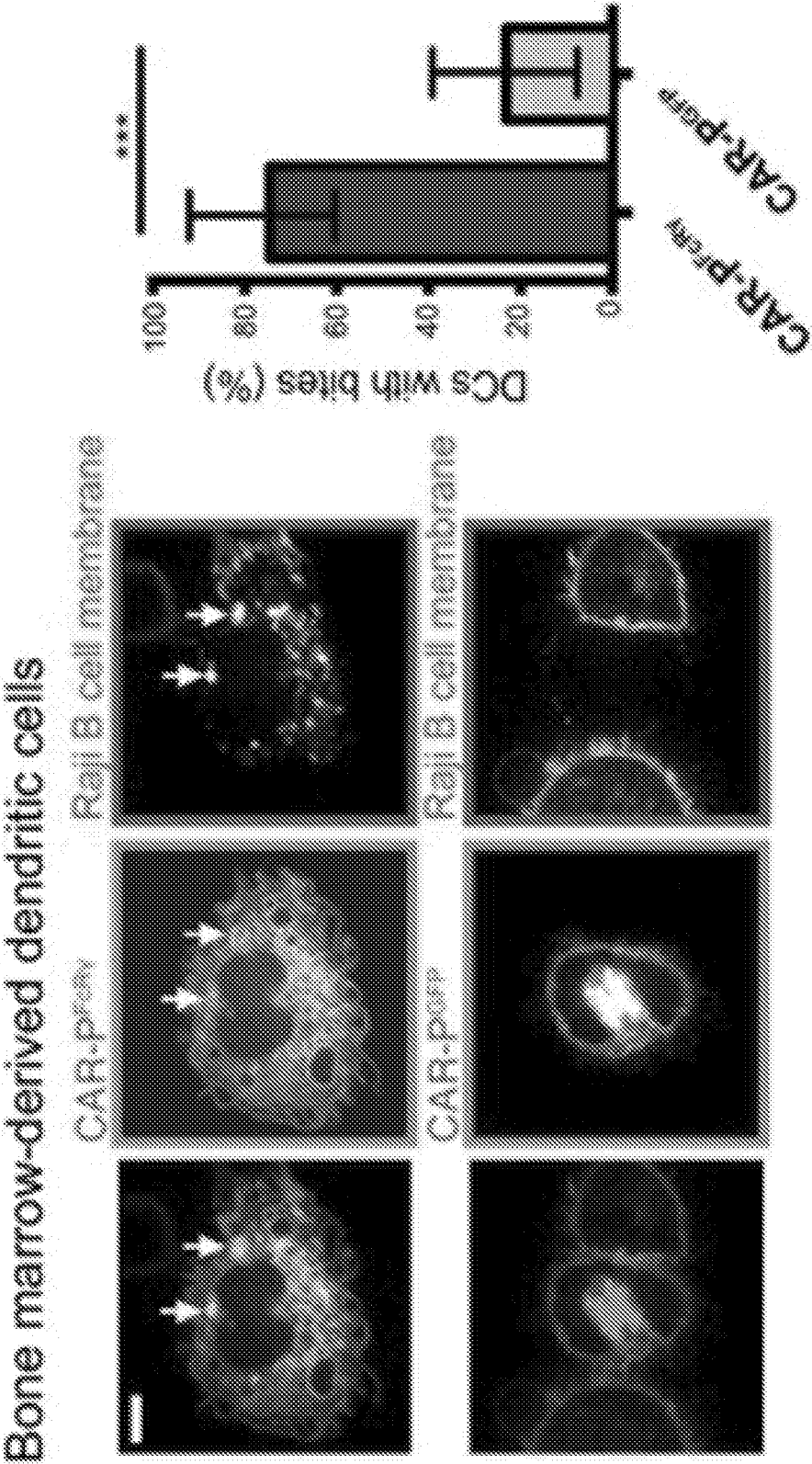


FIG. 8C

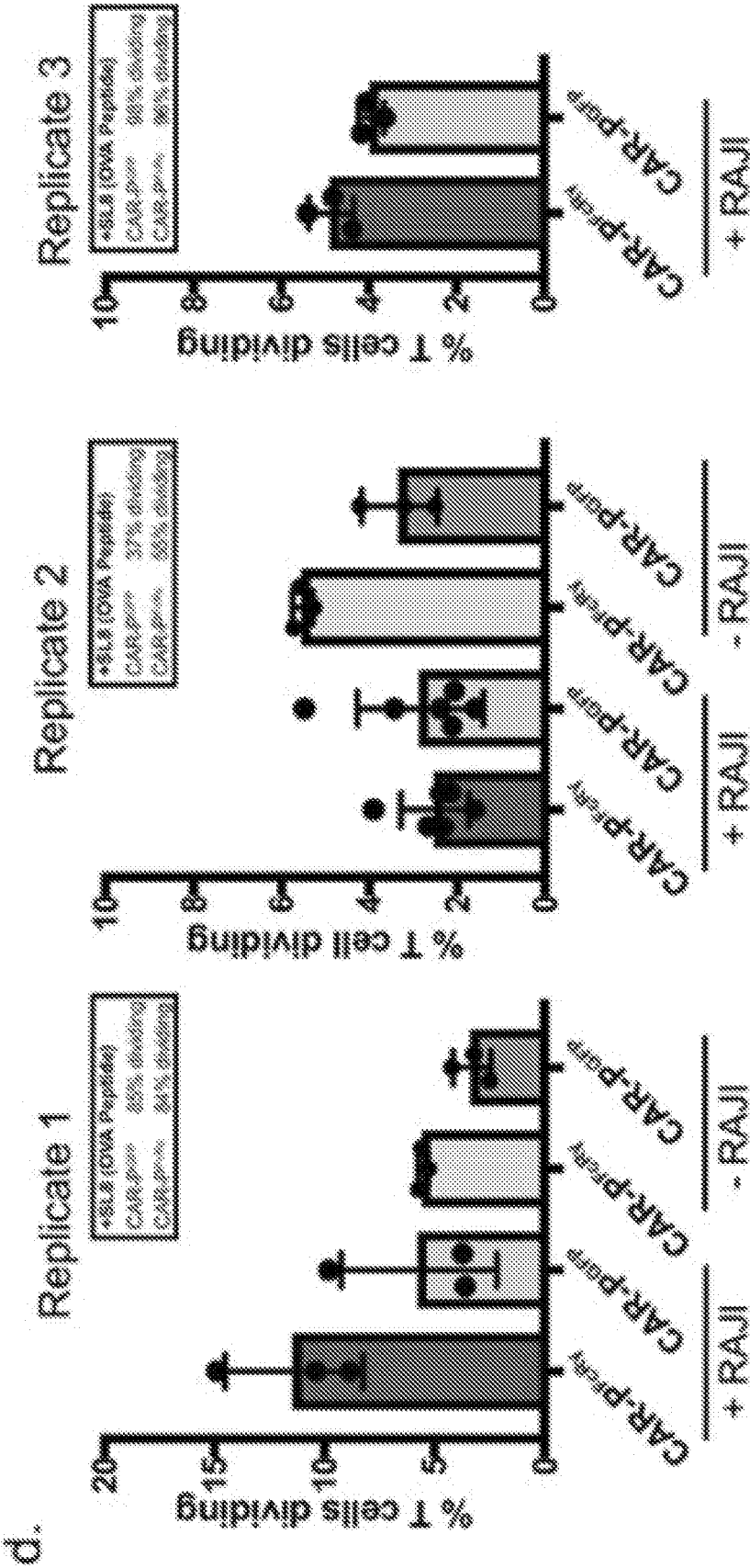


FIG. 8D

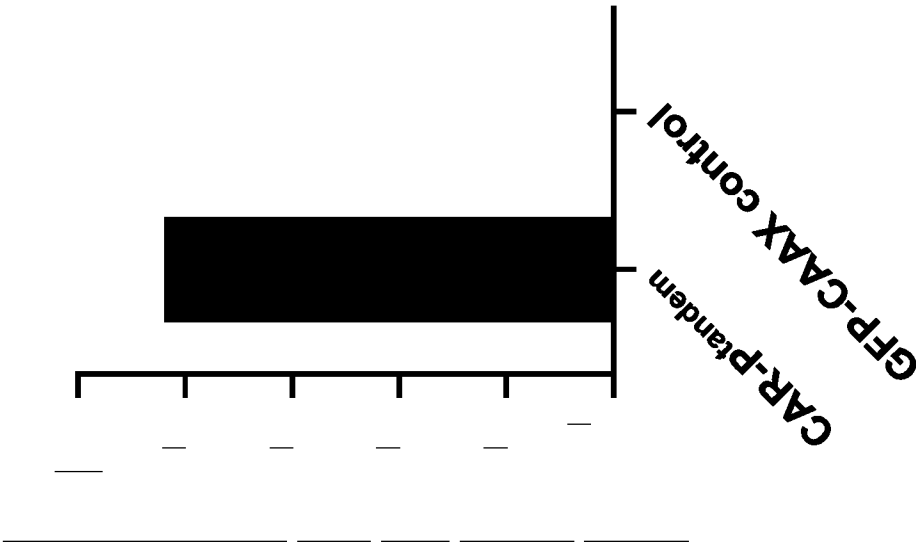


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/060052

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00 A61P35/00 C07K14/00 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEGHAN A MORRISSEY ET AL: "Chimeric antigen receptors that trigger phagocytosis", ELIFE, vol. 7, 4 June 2018 (2018-06-04), XP055683732, DOI: 10.7554/eLife.36688 page 2, page 6 - page 8; figures 4c, 4e page 11	1-51
Y	----- WO 2018/064076 A1 (CERO THERAPEUTICS INC [US]) 5 April 2018 (2018-04-05) claims 1, 11-13, 20 ----- -/--	1-51



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search

21 April 2020

Date of mailing of the international search report

30/04/2020

Name and mailing address of the ISA/

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Authorized officer

Saame, Tina

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/060052

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DANIEL SCHLAM ET AL: "Phosphoinositide 3-kinase enables phagocytosis of large particles by terminating actin assembly through Rac/Cdc42 GTPase-activating proteins", NATURE COMMUNICATIONS, vol. 6, no. 1, 14 October 2015 (2015-10-14), XP055687607, DOI: 10.1038/ncomms9623 abstract -----	1-51

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/060052

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2017335634 A1	14-03-2019
		CA 3035080 A1	05-04-2018
		CN 110036033 A	19-07-2019
		EP 3519441 A1	07-08-2019
		JP 2019536471 A	19-12-2019
		KR 20190054094 A	21-05-2019
		US 2020055917 A1	20-02-2020
		WO 2018064076 A1	05-04-2018
