

CHIMERIC ADAPTOR POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 63/272,613 filed on October 27, 2021.

FIELD OF DISCLOSURE

[0002] The present disclosure relates generally to cellular immunotherapy, and particularly to chimeric adaptor polypeptides that associate with particular receptor(s) in various cell types, for improved cell survival, proliferation, signaling, and the like.

BACKGROUND OF THE DISCLOSURE

[0003] Adoptive cellular therapy has undergone near constant iteration for more than thirty years, from early days focusing on basic lymphokine activation and/or tumor infiltration to more recent strategies engineering immune cells to express genetically engineered antigen receptors, such as chimeric antigen receptors (CARs). However, while there have been some hints and indications of curative potential of various approaches along the way, there are a myriad of issues that remain.

[0004] One issue relates to the issue of low or lost (i.e., antigen escape) expression of a target of an adoptive cellular therapy. Specifically, a common mechanism of resistance to adoptive (or even innate) cellular therapies is the emergence of cell types (e.g., tumor) with loss or downregulation of the target antigen. Such loss or downregulation can lead to a reduction in efficacy of an adoptive (or innate) cellular response (Majzner RG and Mackall, CL. (2018) Cancer Discovery, 8(10): 1219-26).

[0005] Another related issue is the downregulation of naturally-occurring receptor(s) capable to recognize ligands specifically present on cells associated with a particular disease, which also can lead to ineffective cellular responses to various disease conditions. As a representative example, NKG2D is an activating immune receptor found on natural killer (NK) cells, CD8⁺ $\alpha\beta$ T cells, and $\gamma\delta$ T cells in humans that regulates both innate and adoptive immune responses. The natural ligands of NKG2D include MICA and MICB and several UL16-binding

proteins (Bauer S, et al. (1999) Science, vol. 285 5428: 727-729; Burgess S.J., et al. (2008) Immunol Res, 2008, 40(1):18-34). In humans, NKG2D ligands are not expressed on normal cells, but are widely expressed at varying levels on transformed or virally infected cancer cells (see e.g., Bauer S, et al. (1999) Science, vol. 285 5428: 727-729; Burgess S.J., et al. (2008) Immunol Res, 40(1):18-34; Baugh R, et al. (2020) Cancers 12(12): 3827). Expression of NKG2D ligands on a tumor cell surface sensitizes tumor cells to immune cell-mediated destruction by engaging NKG2D to activate NK cells and costimulate effector T cells. Therefore, NKG2D receptor and its ligands are a target of interest for cancer immunotherapy.

[0006] Unfortunately, however, NKG2D can be downregulated at times when it is most needed. For example, tumor-derived tumor growth factor- β (TGF- β) can downregulate NKG2D thereby reducing tumor cell killing by NK and CD8⁺ cells (see e.g., Crane, C., et al. (2010) Neuro-Oncology, 12(1): 7–13, and Dasgupta, S., et al. (2005) Journal of Immunol, 175: 5541-50). This, in turn, is associated with poor prognosis for the treatment of tumors.

[0007] NKG2D is mentioned as an example to demonstrate that there is a need in the art for compositions and methods that can *inter alia* improve, e.g., immune cell survival and proliferation, prevent downregulation of endogenous receptors (e.g., NKG2D), and compensate for immune escape of antigens that are, for example, the target of an adoptive immunotherapy approach. Such compositions and methods would improve the prognosis for patients undergoing adoptive immunotherapy.

SUMMARY OF DISCLOSURE

[0008] The present invention addresses the foregoing shortcomings in the prior art with chimeric adaptor (CAD) constructs and polypeptides comprising human DAP10 and methods of using same. As articulated and demonstrated herein for the first time, the subject CAD constructs and polypeptides can improve the stability of receptors capable of recognizing target antigens on various cell surfaces, promote a favorable balance of cell signaling pathway(s) upon receptor-target engagement, and/or improve functional properties (e.g., enhanced cytolytic, proliferative, survival and/or costimulatory properties) elicited upon engagement with various ligands of the receptors (e.g., NKG2D).

[0009] In one aspect, the invention provides an isolated nucleic acid encoding a chimeric adaptor (CAD) polypeptide, wherein the CAD polypeptide comprises a DAP10 domain and at least one costimulatory signaling domain, and wherein the CAD polypeptide specifically lacks an ectodomain comprising a functional extracellular receptor and/or ligand-binding domain. In preferred embodiments, the DAP10 domain comprises a human DAP10 amino acid sequence.

[0010] In embodiments, the at least one costimulatory domain is selected from the group comprising or consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD3C, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD70, CD80, CD83, CD86, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), FcR, LAT, NKD2C, SLP76, TRIM, and ZAP70, or combinations thereof. In embodiments, the at least one costimulatory domain is 4-1BB. In embodiments, the at least one costimulatory domain is CD28.

[0011] In embodiments, the CAD polypeptide further comprises at least one intracellular signaling domain, wherein the at least one intracellular signaling domain is selected from the group comprising or consisting of CD3 ζ , DAP12, LFA-1, and CD3t, or combinations thereof. In embodiments, the at least one signaling domain is CD3 ζ . In embodiments, the at least one costimulatory domain is 4-1BB and the at least one intracellular signaling domain is CD3 ζ . In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD3 ζ intracellular signaling domain.

[0012] In embodiments, the CAD polypeptide comprises, N-terminus to C-terminus, the DAP10 domain, a 4-1BB costimulatory domain and a CD28 costimulatory domain. In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain. In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain.

[0013] In embodiments, the DAP10 domain comprises a human DAP10 amino acid sequence with an amino acid sequence having at least 90%, 95%, 97%, or 99% sequence identity to SEQ ID NO: 1. In embodiments, the human DAP10 amino acid sequence comprises a mutated human DAP10 amino acid sequence. In embodiments, the mutated human DAP10 amino acid sequence comprises amino acid substitutions at positions corresponding to K84 and/or Y86. In embodiments, the amino acid substitution at position K84 comprises a K84R substitution. In embodiments, the amino acid substitution at position Y86 comprises a Y86F substitution.

[0014] In embodiments, the isolated nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO: 60, or SEQ ID NO: 62, or SEQ ID NO: 64, or SEQ ID NO: 66, or SEQ ID NO: 68, or SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 82, or SEQ ID NO: 90.

[0015] In embodiments, the isolated nucleic acid is operably linked to a regulatable promoter. In embodiments, the isolated nucleic acid further encodes for a cytokine. In embodiments, the cytokine is selected from the group consisting of IL-2, IL-4, IL-7, IL-15, IL-21, IL-23. In embodiments, the isolated nucleic acid further encodes a marker protein. In embodiments, the marker protein is selected from the group consisting of truncated CD19, CD20 (Rituxumab recognition domain), truncated EGFR, and LNGFR.

[0016] In another aspect, the invention provides an expression vector comprising any of the foregoing isolated nucleic acids.

[0017] In another aspect, the invention provides a chimeric adaptor (CAD) polypeptide encoded by any of the foregoing isolated nucleic acids or expression vectors.

[0018] In another aspect, the invention provides a mammalian cell comprising any of the foregoing expression vectors or CAD polypeptides, wherein the mammalian cell expresses at least one receptor that associates with DAP10. In embodiments, the at least one receptor that associates with DAP10 is endogenous, exogenous, or over-expressed. In exemplary embodiments, the receptor is NKG2D. In embodiments, the mammalian cell is an immune cell, preferably wherein the immune cell is a cytotoxic cell.

[0019] In another aspect, the invention provides a method for activating an immune cell, comprising: expressing a CAD polypeptide according to the subject invention in the

immune cell, wherein the immune cell expresses at least one receptor that associates with DAP10; and wherein the activating occurs responsive to the receptor engaging a corresponding target molecule. In embodiments, the receptor is endogenous, exogenous, or over-expressed. In exemplary embodiments, the receptor is NKG2D. In embodiments, the immune cell, or a plurality thereof, are introduced to a subject in need thereof; and the activating occurs in the subject.

[0020] In a further aspect, the invention provides a use of the mammalian cell of the subject invention, or a plurality thereof, in the preparation of a medicament for treating a subject with a condition for which the mammalian cell, or the plurality thereof, reduces at least one symptom or sign of said condition in the subject.

[0021] In a still further aspect, pharmaceutical compositions are provided comprising a pharmaceutically acceptable excipient and a plurality of mammalian cells of the subject invention.

[0022] Other features, objects, and advantages will be apparent from the disclosure that follows.

INCORPORATION BY REFERENCE

[0023] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **FIGS. 1A-1D** are schematic depictions of exemplary chimeric DAP10 adaptor polypeptides of the present disclosure, in conjunction with a receptor with which they associate. In the depicted illustrations, the receptor is NKG2D. FIG. 1A depicts a chimeric DAP10 adaptor polypeptide with K84R and Y86F modifications; FIG. 1B depicts a chimeric DAP10 adaptor polypeptide with K84R and Y86F modifications, as well as a C-terminal fusion comprising CD3 ζ signaling domain; FIG. 1C depicts a chimeric DAP10 adaptor polypeptide with K84R and Y86F modifications, as well as a C-terminal fusion comprising a 4-1BB costimulatory domain; and FIG. 1D depicts a chimeric DAP10 adaptor polypeptide with K84R and Y86F modifications,

as well as a C-terminal fusion comprising both a 4-1BB costimulatory domain and a CD3 ζ signaling domain.

[0025] FIGS. 2A-2C are graphs showing a cytotoxicity index of various chimeric DAP10 adaptor polypeptides of the present disclosure expressed in V δ 1 cells and tested against PLC/PRF/5 cells, as compared to tumor cells alone and a control chimeric antigen receptor (CAR) construct also expressed in V δ 1 cells.

[0026] FIGS. 2D-2F are plots showing extent of V δ 1 cell proliferation seen in the assays of FIGS. 2A-2C.

[0027] FIGS. 3A-3C are graphs showing a cytotoxicity index of various chimeric DAP10 adaptor polypeptides of the present disclosure expressed in V δ 1 cells and tested against HepG2 cells, as compared to tumor cells alone and a control CAR construct also expressed in V δ 1 cells.

[0028] FIGS. 3D-3F are plots showing extent of V δ 1 cell proliferation seen in the assays of FIGS. 3A-3C.

[0029] FIG. 4 is a graph showing survival of V δ 1 cells transduced with various chimeric DAP10 adaptor constructs of the present disclosure, following 5 days of co-culture with PLC/PRF/5 cells.

[0030] FIGS. 5A-5B are graphs illustrating robust *in vivo* tumor growth via V δ 1 cells transduced with select chimeric DAP10 adaptor polypeptides of the present disclosure.

[0031] FIG. 6 is a graph illustrating NKG2D expression level on V δ 1 cells transduced with various DAP10 adaptor polypeptides of the present disclosure and following co-culture of the transduced cells with PLC cells. The greatest NKG2D expression level was observed in cells transduced with a DAP10 adaptor polypeptide that included each of K84R, Y86F, a 4-1BB costimulatory domain and a CD3 ζ signaling domain.

[0032] FIG. 7 is a western blot for CAD protein visualized by anti-DAP10 and anti-CD3 ζ antibodies that illustrates that DAP10 CAD expression is similar across different lots of V δ 1 cells.

[0033] FIGS. 8A-8D are graphs illustrating that cytotoxic activity of DAP10 CADs is mediated by NKG2D. CAD+ (FIGS. 8A-8B) or chimeric antigen receptor (CAR)+ V δ 1 cells (FIGS. 8C-8D) were preincubated with various dilutions of either anti-NKG2D antibody (clone

1D11) or isotype control (1µg/ml-0.01ng/ml) prior to co-culture with luciferase-labeled target cells (PLC/PRF/5 or HL60). Target cell killing was assessed after 18 hours by measuring luciferase signal. NKG2D-mediated cytotoxicity can be assessed by comparing % cytotoxicity with isotype pre-incubation to % cytotoxicity with NKG2D antibody pre-incubation.

[0034] FIGS. 9A-9C illustrate that DAP10 CADs of the present disclosure have consistent molecular activation signature. The data was obtained from Nanostring analysis post stimulation from multiple donors and cell lines.

[0035] FIGS. 10A-10G illustrate that Vδ1 cells transduced with DAP10 CADs of the present disclosure exhibit anti-cancer activity against various cancer types having a broad range of NKG2D ligand expression levels/patterns in an 18-hour assay. The graphs shown at FIGS. 10A-10G represent % cytotoxic activity of Vδ1 cells transduced with DAP10 CADs. DAP10 CAD+ Vδ1 cells or controls were co-cultured with a variety of luciferase-expressing target cell lines across varying E:T ratios (1:6-10:1) in a short term 18-hour cytotoxicity assay.

[0036] FIG. 10H is a table showing that tested target cell lines comprise a broad range of NKG2D ligand (MICA/B, ULBP1, ULBP2/5/6, ULBP3, ULBP4) expression levels/patterns. Data is presented as fold change mean fluorescence intensity (MFI) of NKG2D ligand over relevant isotype control.

[0037] FIGS. 10I-10J are a series of graphs showing the raw data used to populate the table shown at FIG. 10H.

[0038] FIGS. 11A-11G illustrate that Vδ1 cells transduced with DAP10 CADs of the present disclosure exhibit anti-cancer activity against various cancer types having a broad range of NKG2D ligand expression levels/patterns in an 120-hour assay. FIGS. 11A-11G are graphs illustrating cytotoxicity index of Vδ1 cells transduced with DAP10 CADs, at different effector:target ratios, compared to controls. Tested target cell lines comprise a broad range of NKG2D ligand (MICA/B, ULBP1, ULBP2/5/6, ULBP3, ULBP4) expression levels/patterns (see FIG. 10H-10J).

[0039] FIGS. 12A-12B are graphs showing that cytotoxic activity of Vδ1 cells transduced with DAP10 CADs is comparable for different lots of Vδ1 cells and DAP10 CADs. A 120 hour cytotoxicity assay is shown at FIG. 12A, where target cells were PLC/PRF/5 cells. % reduction

in cytotoxicity of tumor alone relative to treated using the final time point of the assay of FIG. 12A, is shown at FIG. 12B.

[0040] FIG. 12C is a graph showing a comparison of cytotoxicity of V δ 1 cells transduced with either DAP10.6, DAP10.16, or DAP10.17 constructs. Data obtained for each construct is an aggregate of 3 donors. PLC/PRF/5 cells were used as target cells in a 120-hour cytotoxicity assay. Controls included DAP10.0, and PLC/PRF/5 alone. Using a stringent E:T ratio, in this particular assay DAP10.6 showed improved cytotoxicity as compared to DAP10.16 and DAP10.17.

[0041] FIGS. 12D-12F are graphs illustrating donor dependence of three different DAP10 constructs (DAP10.6, FIG. 12D; DAP10.16, FIG. 12E; DAP10.17, FIG. 12F) as measured by cytotoxicity index in a 120-hour co-culture cytotoxicity assay. The DAP10 constructs were transduced into V δ 1 cells, target cells were PLC/PRF/5 cells. Controls included untransduced V δ 1 cells from the same three different donors.

[0042] FIGS. 13A-13B illustrate that DAP10 CAD stimulation results in a polyfunctional cytokine profile, that is a function of different DAP10 CAD constructs and target cell type. A cytokine profile as a function of DAP10 CAD and target cell is depicted at FIG. 13A. FIG. 13B is a graph showing interferon gamma induction as a function of DAP10 CAD and target cell type.

[0043] FIGS. 13C-13F are plots showing levels of interferon gamma secretion from DAP10 CAD+ V δ 1 cells alone (effector) and after co-culture with various target cells.

[0044] FIG. 14 illustrates cytokine profile of V δ 1 cells transduced with DAP10 CAD in presence or absence of target cell, as compared to cytokine profile of V δ 1 cells transduced with a chimeric antigen receptor (CAR).

[0045] FIG. 15 illustrates DAP10 CADs of the present disclosure drive proliferation of V δ 1 cells from multiple donors. FIG. 15 shows plots illustrating donor dependence on proliferation of V δ 1 cells obtained from two different donors (SCT29 and SCT46), transduced with either DAP10.6, DAP10.16, or DAP10.17, relying on a co-culture experiment with PLC/PRF/5 cells. Controls included prior tested V δ 1 cells obtained from a different donor (SCT06) and transduced

with DAP10.6 (positive control), and V δ 1 cells from the two different donors (SCT29 and SCT46) transduced with DAP10.0 (negative control).

[0046] FIGS. 16A-16B illustrate *in vivo* tumor control in a mouse model for V δ 1 cells transduced with a DAP10 CAD. FIG. 16A is a graph comparing *in vivo* tumor control of DAP10.6 and a DAP10 CAD including a modified (“1XX”) CD3 ζ intracellular signaling domain (DAP10.16). A schematic of the experimental procedure is depicted at FIG. 16B.

[0047] FIGS. 17A-C illustrate that anti-tumor activity of V δ 1 cells transduced with a DAP10 CAD of the present disclosure exhibits anti-tumor activity with kinetics similar to CAR V δ 1 cells. FIG. 17A is a graph showing comparison of *in vivo* tumor growth kinetics for DAP10 CAD+ V δ 1 cells compared to CAR V δ 1 cells in an HCT-15 mouse xenograft model. FIG. 17B is a graph quantifying tumor volume at day 27. FIG. 17C is a schematic illustration of the experimental procedure used to obtain the data depicted at FIGS. 17A-17B.

[0048] FIGS. 18A-18D illustrate that V δ 1 cells transduced with a DAP10 CAD of the present disclosure proliferate in tumor tissue *in vivo* in a mouse model. FIG. 18A are flow cytometry plots illustrating that proliferation of V δ 1 cells transduced with a DAP10 CAD is specific to tumor tissue, and FIG. 18B is a graph showing that proliferation in tumor tissue progresses over 14 days. FIG. 18C is a graph showing quantification of V δ 1 cells in tumor tissue or other tissues taken 4, 7, and 14 days after treatment. The experimental procedure used to obtain the data depicted in FIGS. 18A-18C is schematically shown at FIG. 18D.

[0049] FIG. 19 illustrates that treatment of mice with V δ 1 cells transduced with a DAP10 CAD of the present disclosure is not associated with significant changes in body weight.

[0050] FIGS. 20A-20B illustrate that V δ 1 cells transduced with a DAP10 CAD of the present disclosure target tumor cells while sparing non-tumor cells. V δ 1 cells transduced with a DAP10 CAD of the present disclosure significantly reduced THP1 cell viability, compared to control as depicted in the graph of FIG. 20A, and do not target healthy PBMCs, as shown in the graph of FIG. 20B.

[0051] FIG. 21 is a graph showing V δ 1 cell fold-expansion in small scale shake flask expansions for six different donors. Fold-expansion was measured on day 14. The data compares V δ 1 cells transduced with DAP10.6 and DAP10.16 CADs.

[0052] FIGS 22A-22C are graphs illustrating expansion kinetics of V δ 1 cells transduced with lead DAP10 CADs of the present disclosure (DAP10.6, DAP10.16, DAP10.17), as compared to controls. Data at FIG. 22A corresponds to V δ 1 cells obtained from a first donor (SCT06), data at FIG. 22B corresponds to V δ 1 cells obtained from a second donor (SCT29), and data at FIG. 22C corresponds to V δ 1 cells obtained from a third donor (SCT45). For each of FIGS. 22A-22C, %V δ 1 cells was measured as a function of expansion time (days).

[0053] FIG. 23A depicts a schematic representing a process for generating “off-the-shelf” allogeneic DAP10 CAD V δ 1 cells.

[0054] FIGS. 23B-23D are graphs showing independent expansions of V δ 1 cells transduced with preferred DAP10 CAD constructs of the present disclosure. V δ 1 cells used for the experiments depicted were obtained from three different donors (SCT06, SCT29, SCT45), respectively.

[0055] FIGS. 23E-23F are graphs illustrating that *ex vivo* culture of V δ 1 cells results in substantial fold expansion (FIG. 23E), and robust DAP10 CAD transduction (FIG. 23F). The data at FIG. 23E is represented as V δ 1 fold-expansion, and the data at FIG. 23F is represented as % DAP10 CAD of V δ 1 cells.

[0056] FIG. 23G depicts plots illustrating cellular composition of V δ 1 cells, V δ 2 cells, $\alpha\beta$ cells, and NK cells over time, expressed as % of culture.

DETAILED DESCRIPTION

[0057] The present invention provides chimeric adaptors, or CADs, wherein the chimeric adaptor generally includes an endodomain comprising a DAP10 domain and at least one costimulatory domain, and optionally further comprises at least one intracellular signaling domain, but specifically lacks an ectodomain comprising a ligand-binding domain. Accordingly, in some embodiments, the CAD polypeptides of the subject invention may also further comprise a transmembrane domain and/or an extracellular spacer domain, but will specifically lack a functional extracellular receptor and/or ligand-binding domain. In contrast, the prior art has typically employed DAP10 as a component of a CAR or NKG2D fusion chimera. *See, e.g.*, Zhao et al., *OncoImmunology*. 2019; 8(1): e1509173; Lynch et al., 2017, *Immunol* 152:472; US

2020/0308248; WO/2018/183385; CN109096404; CN111995689. The CAD polypeptides of the subject invention are also clearly different from that found in nature, generally comprising at least two polypeptide domains that are not naturally linked together, and optionally further including additional advantageous signaling domains and mutations as detailed herein.

[0058] The CAD polypeptides of the subject invention preferably include a DAP10 domain comprising human DAP10, optionally including one or more substitution mutations, deletion mutations, and/or addition mutations. For example, the DAP10 domain may have a Y86F mutation and/or a K84R mutation.

[0059] The “costimulatory domain” in the context of a CAD polypeptide of the present disclosure enhances cell proliferation, cell survival and development of memory cells for cytotoxic cells that express the chimeric adaptor. The CAD polypeptides of the invention may include one or more costimulatory domains selected from the costimulatory domains of proteins in the TNFR superfamily, CD28, CD137 (4-1BB), CD134 (OX40), Dapl0, CD27, CD2, CD7, CD5, ICAM-1, LFA-1 (CD1 1a/CD18), Lck, TNFR-I, PD-1, TNFR-II, Fas, CD30, CD40, ICOS LIGHT, NKG2C, B7-H3, or combinations thereof. If the CAD includes more than one costimulatory domain, these domains may be arranged in tandem, optionally separated by a linker. The costimulatory domain is an intracellular domain that may locate between the DAP10 domain and the optional intracellular signaling domain in the CAD.

[0060] In embodiments, the costimulatory domain includes a costimulatory domain of CD28, CD27, ICOS, 4-1BB, OX40, and CD40L. The term “costimulatory domain” as used herein also encompasses any modifications thereof, examples of which are described in US Patent Application No. 20200129554; US Patent Application No. 20200317777; WO2019010383; Li, W., et al., (2020) Immunity 53: 456-470; and Li, G., et al., (2017) J Immunol 198(1 Supplement): 198.4, the contents of each of which are incorporated herein in their entirety.

[0061] The “intracellular signaling domain” in the context of a CAD polypeptide of the present disclosure transduces the effector function signal and directs the cytotoxic cell to perform its specialized function, i.e., harming and/or destroying the target cells. Examples of suitable intracellular signaling domains include, *e.g.*, the ζ chain of the T cell receptor complex or any of its homologs, *e.g.*, η chain, Fc γ R γ and β chains, MB 1 (Iga) chain, B29 (Ig) chain, etc., human CD3 ζ chain, CD3 polypeptides (Δ , δ and ϵ), syk family tyrosine kinases (Syk, ZAP 70, etc.), src

family tyrosine kinases (Lck, Fyn, Lyn, etc.) and other molecules involved in T cell transduction, such as CD2, CD5 and CD28. Specifically, the intracellular signaling domain may be human CD3 ζ chain, Fc γ RIII, Fc ϵ RI, cytoplasmic tails of Fc receptors, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors and combinations thereof.

[0062] The intracellular signaling domains may include intracellular signaling domains of several types of various other immune signaling receptors, including, but not limited to, first, second, and third generation T cell signaling proteins including CD3, B7 family costimulatory, and Tumor Necrosis Factor Receptor (TNFR) superfamily receptors (Park et al., "Are all chimeric antigen receptors created equal?" J Clin Oncol., vol. 33, pp. 651-653, 2015). Additional intracellular signaling domains include signaling domains used by NK and NKT cells (Hermanson, et al., "Utilizing chimeric antigen receptors to direct natural killer cell activity," Front Immunol., vol. 6, p. 195, 2015) such as signaling domains of NKp30 (B7-H6) (Zhang et al., "An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy *in vivo*," J Immunol., vol. 189, pp. 2290-2299, 2012), and DAP12 (Topfer et al., "DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy," J Immunol., vol. 194, pp. 3201-3212, 2015), NKG2D, NKp44, NKp46, DAP10, and CD3z. Additionally intracellular signaling domains also includes signaling domains of human Immunoglobulin receptors that contain immunoreceptor tyrosine based activation motif (ITAM) such as Fc γ RI, Fc γ RIIA, Fc γ RIIC, Fc γ RIIIA, FcRL5 (Gillis et al., "Contribution of Human Fc γ Rs to Disease with Evidence from Human Polymorphisms and Transgenic Animal Studies," Front Immunol., vol. 5, p. 254, 2014).

[0063] In embodiments, the intracellular signaling domain includes a cytoplasmic signaling domain of TCR ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, or CD66d. In exemplary embodiments the intracellular signaling domain in the CAD includes a cytoplasmic signaling domain of human CD3 ζ . The term "intracellular signaling domain" as used herein also encompasses any modifications thereof, examples of which are described in US Patent Application No. 2020/0317777, as well as Combadière, B., et al., (1996) J Exp Med 183(5): 2109-17; Lowin-Kropf B., et al., (1998) J Cell Biol 140(4): 861-871; Ardouin L., et al., (1999) Immunity 10(4): 409-20; Liu H. and Vignali DAA., (1999) J Immunol 163: 599-602; Kersh EN., et al., J Exp Med (1999) 190(11): 1627-36; Chae WJ., et al., (2004) Int Immunol 16(9): 1225-36; Becker, AM., et al., (2007) J Immunol 178(7): 4120-8; Methi T., et al., (2007) Eur J Immunol

37(9): 2539-48; Baudouin SJ., et al., (2008) Mol Biol Cell 19(6): 2444-56; Zhao Y., et al., (2009) J Immunol 183(9): 5563-74; Kochenderfer JN., et al., (2010) Blood 116(19): 3875-86; Bridgeman JS., et al., (2014) Clin Exp Immunol 175(2): 258-67; Long AH., et al., (2015) Nat Med 21(6): 581-90; Hwang S., et al., (2015) Nat Commun 6: 6982; WO2019126748; Feucht J., et al., (2019) Nat Med 25(1): 82-88; Roda-Navarro, P., and Reyburn, HT., (2009) J Biol Chem 284(24): 16463-16472; Giurisato, E., et al., (2007) Mol Cell Biol 27(24): 8583-8599; and Wu, J., et al., (2000) J Exp Med 192(7): 1059-1068, the contents of each of which are incorporated herein in their entirety.

[0064] In embodiments, two or more components of the CAD of the invention may be separated by one or more linkers. Linkers are oligo- or polypeptide regions of from about 1 to 100 amino acids in length. In some embodiments, the linkers may be, for example, 5-12 amino acids in length, 5-15 amino acids in length or 5 to 20 amino acids in length. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers, for example those longer than 100 amino acids, may be used in connection with alternate embodiments of the invention, and may be selected to, for example, ensure that two adjacent domains do not sterically interfere with one another. Examples of linkers which may be used in the instant invention include but are not limited to 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof.

[0065] In an exemplary embodiment, a chimeric DAP10-4-1BB adaptor polypeptide is provided comprising a DAP10 domain and a 4-1BB costimulatory domain. In another exemplary embodiment, a chimeric DAP10-CD28 adaptor polypeptide is provided comprising a DAP10 domain and a CD28 costimulatory domain. In another exemplary embodiment, a chimeric DAP10-4-1BB-CD3 ζ adaptor polypeptide is provided comprising a DAP10 domain, a 4-1BB costimulatory domain, and a CD3 ζ intracellular signaling domain. In another exemplary embodiment, a chimeric DAP10-CD28-CD3 ζ adaptor polypeptide is provided comprising a DAP10 domain, a CD28 costimulatory domain, and a CD3 ζ intracellular signaling domain. In yet another exemplary embodiment, a chimeric DAP10-4-1BB-CD28-CD3 ζ adaptor polypeptide is provided comprising a DAP10 domain, a 4-1BB costimulatory domain, a CD28 costimulatory domain, and a CD3 ζ intracellular signaling domain.

[0066] The chimeric adaptor polypeptides of the subject invention may optionally further comprise a transmembrane domain. The transmembrane domain of the CAD is a region that is capable of spanning the plasma membrane of the cytotoxic cells. The transmembrane domain is selected from a transmembrane region of a transmembrane protein such as, for example, Type I transmembrane proteins, an artificial hydrophobic sequence or a combination thereof. Suitable examples of the transmembrane domain include the transmembrane regions of the alpha, beta or zeta chain of the T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Synthetic transmembrane domains may include a triplet of phenylalanine, tryptophan and valine. Optionally, 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 signaling domain of the CAD. A glycine-serine doublet provides a particularly suitable linker between the transmembrane domain and the intracellular signaling domain.

[0067] The chimeric adaptor polypeptides of the subject invention may optionally further comprise an extracellular spacer domain. The extracellular spacer domain of the CAD is a hydrophilic region which is typically located between a ligand-binding domain (which is absent in the present invention) and the transmembrane domain. In some embodiments, this domain facilitates proper protein folding for the CAD. The extracellular spacer domain may include a domain selected from Fc fragments of antibodies, hinge regions of antibodies, CH2 regions of antibodies, CH3 regions of antibodies, artificial spacer sequences or combinations thereof. Examples of extracellular spacer domains include CD8a hinge, artificial spacers made of polypeptides which may be as small as, three glycines (Gly), as well as CH1 and CH3 domains of IgGs (such as human IgG4).

Definitions

[0068] For purposes of interpreting this specification, the following definitions will apply, and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth conflicts with any document incorporated herein by reference, the definition set forth below shall control. Unless defined otherwise, all technical and

scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0069] “About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[0070] “Optional” or “optionally” as used herein means that the particular limitation, event, circumstance, and the like can but need not occur, and that the description includes instances where said limitation, event, or circumstance occurs and instances where it does not.

[0071] As used herein, the term “DAP10” refers to the transmembrane adaptor protein present in lymphoid and myeloid cells of mammals whose exact sequence might vary slightly based on the species, isoform and from individual to individual. Alternative names for DAP10, as recognized in the art, include Hematopoietic cell signal transducer (HCST), DNAX-activation protein 10, membrane protein DAP10, transmembrane adaptor protein KAP10 (KAP10) and PIK3AP. For example, in humans DAP10 refers to the protein represented by the predominant polypeptide sequence UniProt Q9UBK5 and NCBI accession NP_055081.1 and AF072845; however, different isoforms and variants may exist. While the name DAP10 might refer to multiple proteins with related structures and polypeptide sequences from various species, to protein members of the DAP10 protein family having high sequence identity to human DAP10 (SEQ ID NO: 1), a skilled worker will be able to identify a human DAP10 related protein in mammals, even if it differs from the sequences referenced herein.

[0072] The term “host cell” as used herein refers to a cell type selected to express a CAD polypeptide of the present disclosure. In embodiments, the host cell endogenously expresses at least one receptor that associates with DAP10, and by extension, the chimeric adaptor polypeptides of the present disclosure. In embodiments, the host cell is engineered to express at least one receptor that associates with DAP10, and by extension, the chimeric adaptor polypeptides of the present disclosure. Exemplary host cells can include, without limitation, a wide variety of immune cells, including in particular cytotoxic cells, preferable examples of which are herein disclosed (e.g., $\gamma\delta$ T-cells, $\alpha\beta$ T cells, NK cells, NKT cells, B-cells, neutrophils, monocytes/macrophages). It is also within the scope of this disclosure that “host cells” can

include non-immune cells, for example and without limitation, stem cells (e.g., embryonic stem cells, hematopoietic stem cells, stromal stem cells, induced pluripotent stem cells, and the like).

[0073] As used herein, the term “T lymphocyte” or “T cell” refers to an immune cell that expresses or has expressed CD3 (CD3+) and a T Cell Receptor (TCR+). T cells play a central role in cell-mediated immunity. A T cell that “has expressed” CD3 and a TCR has been engineered to eliminate CD3 and/or TCR cell surface expression.

[0074] The term “ $\gamma\delta$ T-cells (gamma delta T-cells)” as used herein refers to a subset of T-cells that express a distinct T-cell receptor (TCR), namely $\gamma\delta$ TCR, on their surface, composed of one γ -chain and one δ -chain. The term “ $\gamma\delta$ T-cells” specifically includes all subsets of $\gamma\delta$ T-cells, including, without limitation, V δ 1, V δ 2, and V δ 3 $\gamma\delta$ T cells, as well as naive, effector memory, central memory, and terminally differentiated $\gamma\delta$ T-cells. As a further example, the term “ $\gamma\delta$ T-cells” includes V δ 4, V δ 5, V δ 7, and V δ 8 $\gamma\delta$ T cells, as well as V γ 2, V γ 3, V γ 5, V γ 8, V γ 9, V γ 10, and V γ 11 $\gamma\delta$ T cells. In some embodiments, the $\gamma\delta$ T-cells are V δ 1⁺, V δ 2⁺, or V δ 1⁺ and V δ 2⁺. Compositions and methods for making and using engineered and non-engineered $\gamma\delta$ T cells and/or sub-types thereof include, without limitation, those described in US 2016/0175358; WO 2017/197347; US 9499788; US 2018/0169147; US 9907820; US 2018/0125889 and US 2017/0196910, the contents of each of which are incorporated by reference for all purposes, including the said compositions and methods for making and using engineered and non-engineered $\gamma\delta$ T cells and/or sub-types thereof. The present application further contemplates T cells, or other engineered leukocytes or lymphocytes, that express one γ -chain or one δ -chain, optionally in combination with a second polypeptide to form a functional TCR. Such engineered leukocytes or lymphocytes, that express one γ -chain or one δ -chain may be used in the methods or present in the compositions described herein.

[0075] The $\gamma\delta$ T cells described herein can be δ 1, δ 2, δ 3, or δ 4 $\gamma\delta$ T cells, or combinations thereof. In some cases, the $\gamma\delta$ T cells are mostly (>50%), substantially (>90%), essentially all, or entirely δ 2 $\gamma\delta$ T cells. In some cases, the $\gamma\delta$ T cells are mostly (>50%), substantially (>90%), essentially all, or entirely δ 1 $\gamma\delta$ T cells. In some cases, the $\gamma\delta$ T cells are mostly (>50%), substantially (>90%), essentially all, or entirely δ 3 $\gamma\delta$ T cells.

[0076] $\gamma\delta$ T cells for use as described herein can be obtained from an allogeneic or an autologous donor. The $\gamma\delta$ T cells can be, partially or entirely purified, or not purified, and expanded ex vivo. Methods and compositions for ex vivo expansion include, without limitation, those described in WO 2017/197347. The expansion may be performed before or after, or before and after, a chimeric adaptor polypeptide of the present disclosure is introduced into the $\gamma\delta$ T cell(s). Other additional or alternative methods of expansion include the use of, e.g., artificial antigen-presenting cells (aAPCs), aminobisphosphonates, cytokine cocktails, and feeder cells (Cortés-Selva, D et al., (2021) Trends Pharmacol Sci. 42(1): 45-59).

[0077] As used herein, the term “ $\alpha\beta$ T cell” refers to T cells expressing α and β chains of the TCR as part of a complex with CD3 chain molecules. Each α and β chain contains one variable and one constant domain. $\alpha\beta$ T cells primarily recognize peptide antigens presented by major histocompatibility complex (MHC) class I and class II molecules, where most of the receptor diversity is contained within the third complementarity determining region (CDR3) of the TCR α and β chains.

[0078] As used herein, the term “Natural killer (NK) cell” refers to CD56⁺CD3⁻ granular lymphocytes that play important roles in immunity against viruses and in the immune surveillance of tumors, and constitute a critical cellular subset of the innate immune system (Godfrey J, et al. Leuk Lymphoma 2012 53:1666-1676). NK cells express a remarkably diverse repertoire of inhibitory and activating receptors on their cell surface, which regulates their immune responses. NK cells can kill transformed or infected cells by the release of perforin and granzymes or by using effector molecules of the tumor necrosis factor (TNF) family, such as TNF, TNF-related apoptosis inducing ligand (TRAIL), and Fas ligand, which induce apoptosis in the target cells. Additionally, upon activation NK cells rapidly produce chemokines and cytokines, including interferon (IFN)- γ , GM-CSF, and IL-10, that recruit and affect the function of hematopoietic and nonhematopoietic cells in the host. Unlike cytotoxic CD8⁺ T lymphocytes, NK cells launch cytotoxicity against tumor cells without the requirement for prior sensitization, and can also eradicate MHC-I-negative cells (Narni-Mancinelli E, et al. Int Immunol 2011 23:427-431). NK cells are considered fairly safe effector cells, as they may avoid the potentially lethal complications of cytokine storms (Morgan R A, et al. Mol Ther 2010 18:843-851), tumor

lysis syndrome (Porter D L, et al. N Engl J Med 2011 365:725-733), and on-target, off-tumor effects.

[0079] NK cells can be obtained from an allogeneic or an autologous donor. The NK cells can be partially or entirely purified, or not purified, and expanded ex vivo. Methods and compositions for ex vivo expansion include, without limitation, those described in Becker et al., (2016) Cancer Immunol. Immunother. 65(4): 477-84). The expansion may be performed before or after, or before and after, a chimeric DAP10 adaptor polypeptide is introduced into the NK cell(s). Briefly, and without limitation, expansion of NK cells can include the use of engineered feeder cells, cytokine cocktails (e.g., IL-2, IL-15), and/or aAPCs (Cortés-Selva, D et al., (2021) Trends Pharmacol Sci. 42(1): 45-59).

[0080] In some examples, placental hematopoietic stem-cell derived natural killer (PNK) cells or immortalized cell lines (e.g., NK-92) may be engineered to express chimeric adaptor polypeptides of the present disclosure. In other examples, NK cells that can be used for engineering the expression of chimeric adaptor polypeptides herein can be differentiated from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). As used herein, the term “Natural killer T (NKT) cells” are T lineage cells that share morphological and functional characteristics with both T cells and NK cells. NKT cells are rapid responders of the innate immune system and mediate potent immunoregulatory and effector functions in a variety of disease settings. Ligand recognition in NKT cells leads to rapid secretion of proinflammatory cytokines (such as IFN- γ and TNF- α) and anti-inflammatory cytokines (such as IL-4, IL-10, and IL-13) that enhance the immune response to e.g., cancer by directly targeting tumor cells and by indirectly modulating the antitumor response through the release of diverse cytokines or by altering the TME. Following activation, NKT cells can immediately commence cytokine secretion without first having to differentiate into effector cells. The rapidity of their response makes NKT cells important players in the very first lines of innate defense against some types of bacterial and viral infections. In addition, many of the cytokines secreted by NKT cells have powerful effects on $\alpha\beta$ T cell differentiation and function, linking NKT cells to adaptive defense. NKT cells bridge the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. NKT cells can be obtained from an allogeneic or an autologous donor. The NKT

cells can be partially or entirely purified, or not purified, and expanded ex vivo. Briefly and without limitation, NKT cells can be expanded via the use of ex vivo IL-2, and/or monoclonal antibodies specific for the TCR α -chain CDR3 loop (Cortés-Selva, D et al., (2021) Trends Pharmacol Sci. 42(1): 45-59).

[0081] As used herein, the term “ $\gamma\delta$ natural killer T cells” or “ $\gamma\delta$ NKT cells” refers to iPSC-derived cells that express $\gamma\delta$ TCRs and NK receptors, but lack the expression of hallmark $\gamma\delta$ T cell markers (Cortés-Selva, D et al., (2021) Trends Pharmacol Sci. 42(1): 45-59). These cells have been shown to have anti-tumor activity against a broad number of cancer cell lines, but not against normal cells, and showed more potent killing than donor-derived $\gamma\delta$ T cells or donor-derived NK cells (Zeng J et al., (2019) PLoS ONE 14(5): e0216815). Chimeric adaptor polypeptides can be expressed in $\gamma\delta$ NKT cells, in embodiments herein, for use in accordance with the methods disclosed herein.

[0082] As used herein, the term “myeloid cells” refers to a subgroup of leukocytes represented by granulocytes, monocytes, macrophages, and dendritic cells (DCs). They circulate through the blood and lymphatic system and are rapidly recruited to sites of tissue damage and infection via various chemokine receptors. Within the tissues they are activated for phagocytosis as well as secretion of inflammatory cytokines, thereby playing major roles in protective immunity. Myeloid cells can also be found in tissues under steady-state condition, where they control development, homeostasis, and tissue repair.

[0083] As used herein, the term “macrophages” refers to highly plastic innate cells with functional and phenotypic signatures that can be shaped in response to various stimuli. Macrophage polarization is broadly simplified into two different states, either a M1 phenotype (classically activated) in response to factors such as lipopolysaccharide (LPS) or IFN- γ , or a M2 phenotype in response to cytokines such as IL-4, IL-5, and IL-13. An example of M1-like macrophages express iNOS and proinflammatory cytokines such as TNF- α , IL1- β , IL-6, IL-12, and IL-23. An example of M2 macrophages exhibit increased expression of CD209, CD200R, CD1a, and CD1b in humans, and have been implicated in wound healing and antitumor responses. The ability of macrophages to infiltrate solid tumors and be reprogrammed, as well as the antitumor effects associated with a switch to the M1 phenotype, render macrophages relevant to the present disclosure in terms of engineered macrophages that express a chimeric adaptor

polypeptide described herein. For example, it has been shown that macrophages can be reprogrammed towards antitumor M1 phenotype cells that are capable of producing nitric oxide and inducing IL-12-dependent NK-mediated antitumor effects by inhibiting NK- κ B signaling in a murine model of ovarian cancer (Zhang F et al., (2019) Nat Commun 10: 3974).

[0084] Macrophages can be obtained/derived from an allogeneic or an autologous donor. The macrophages can be partially or entirely purified, or not purified, and cultured ex vivo (see, *e.g.*, Davies JQ and Gordon A (2005) Methods Mol Biol 290:105016). In some embodiments, the present disclosure encompasses macrophages derived from hESCs (Karlsson, KR et al., (2008) Exp Hematol 36: 1167-1175), or iPSC-derived macrophages (Takata K. et al., (2017) Immunity 47: 183-198).

[0085] As used herein, the term “NKG2D receptor” refers to a transmembrane protein belonging to the NKG2 family of C-type lectin-like receptors. NKG2D serves as a primary activating receptor wherein ligand binding triggers cytotoxicity and cytokine production. NKG2D provides costimulation through an associated adapter molecule, DAP10, which recruits phosphatidylinositol-3 kinase. In mice, NKG2D also associates with DAP12, which recruits protein tyrosine kinases. NKG2D is encoded by KLRK1 gene which is located in the NK-gene complex (NKC) situated on chromosome 6 in mice and chromosome 12 in humans. In humans, NKG2D is expressed by NK cells, $\gamma\delta$ T cells and CD8⁺ $\alpha\beta$ T cells, and CD4⁺ T cells under certain pathological conditions (Stanjanovic A., et al. (2018) Front. Immunol. 23: 1-15). In mice, NKG2D is expressed by NK cells, NK1.1⁺ T cells, $\gamma\delta$ T cells, activated CD8⁺ $\alpha\beta$ T cells and activated macrophages. The full length human NKG2G amino acid sequence is set forth herein as SEQ ID NO: 95, the amino acid sequence of the transmembrane domain of human NKG2D is set forth herein as SEQ ID NO: 96, and the amino acid sequence of the transmembrane and extracellular ligand binding domain of NKG2D is set forth herein as SEQ ID NO: 97.

[0086] The term “recombinant mammalian cell” as used herein refers to cell or cell line derived from a mammal comprising at least one alteration brought about using genetic engineering technology. In some embodiments, a “recombinant mammalian cell” is a $\gamma\delta$ T cell, or an NK cell, or an NKT cell, or an $\alpha\beta$ T cell, etc., that comprises a nucleic acid construct that

encodes a chimeric DAP10 adaptor polypeptide. A “recombinant mammalian cell” can be derived from any mammal such as e.g., a human, a rodent, etc.

[0087] As used herein, the term “TCR” or “T cell receptor” refers to a dimeric heterologous cell surface signaling protein forming an alpha-beta or gamma-delta receptor or combinations thereof. $\alpha\beta$ TCRs recognize an antigen presented by an MHC molecule, whereas $\gamma\delta$ TCR can recognize an antigen independently of MHC presentation.

[0088] The term “MHC” (major histocompatibility complex) refers to a subset of genes that encodes cell-surface antigen-presenting proteins. In humans, these genes are referred to as human leukocyte antigen (HLA) genes. Herein, the abbreviations MHC or HLA are used interchangeably.

[0089] The term “antigen” or “Ag” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA that comprises a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present disclosure includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated, synthesized, or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

[0090] The term “antibody,” as used herein, refers to an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example,

polyclonal antibodies, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal antibodies), antibody compositions with polypeptidic specificity, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity), formed from at least two intact antibodies, diabodies, single domain antibodies (sdAbs), as long as they exhibit the desired biological or immunological activity, Fv, Fab and F(ab), as well as single chain antibodies and humanized antibodies (Harlow et al, 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al, 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, N.Y.; Houston et al, 1988, Proc. Nat Acad. Sci. USA 85:5879-5883; Bird et al, 1988, Science 242:423-426).

[0091] The term "epitope" includes any protein determinant, lipid or carbohydrate determinant capable of specific binding to an immunoglobulin or receptor, for example a T-cell receptor. Epitopic determinants usually consist of active surface groupings of molecules such as amino acids, lipids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics.

[0092] A "modification" of an amino acid residue/position, as used herein, refers to a change of a primary amino acid sequence as compared to a starting amino acid sequence, wherein the change results from a sequence alteration involving said amino acid residue/positions. A "modification" of an amino acid residue/position is synonymous with "mutation" of an amino acid residue/position. For example, typical modifications include substitution of the residue (or at said position) with another amino acid (e.g., a conservative or non-conservative substitution), insertion of one or more amino acids, and deletion of one or more amino acids. An "amino acid substitution", or variation thereof, refers to the replacement of an existing amino acid residue in a predetermined (starting) amino acid sequence with a different amino acid residue. Generally and preferably, the modification results in alteration in at least one physicochemical activity of the variant polypeptide compared to a polypeptide comprising the starting (or "wild type") amino acid sequence. A "modified" amino acid sequence, as referred to herein thus comprises an amino acid sequence in which one or more amino acids have been mutated, and/or in which any number of amino acids have been inserted, and/or in which any number of amino acids have been deleted.

[0093] The term “endogenous” as used herein, refers to substances and/or processes that originate from within a system including but not limited to an organism, tissue, or cell. For example, in the context of this disclosure, “endogenous” refers to a nucleic acid molecule or polypeptide that is normally expressed in a cell or tissue.

[0094] Conversely, the term “exogenous” as used herein refers to substances and/or processes that originate from outside of a system including but not limited to an organism, tissue, or cell. Particularly, “exogenous” in the context of the present disclosure is meant a nucleic acid molecule or polypeptide that is not naturally present in a cell. The term “exogenous” would therefore encompass any foreign or heterologous recombinant nucleic acid molecule or polypeptide expressed in a cell, including an exogenous nucleic acid having a different sequence relative to its native endogenous counterpart. As is well known in the art, these exogenous sequences may be introduced by genetic engineering into the cell itself or a progenitor thereof, and may optionally be linked to alternative control sequences, such as a non-native promoter or secretory sequence.

[0095] The term “overexpression” as used herein refers to expression at a level exceeding the endogenous expression level of the subject nucleic acid or polypeptide in the cell or tissue. In exemplary embodiments, a receptor of interest (*e.g.* NKG2D) can be overexpressed in a host cell, where the level of expression of the receptor is greater than the naturally-occurring expression level of same receptor. Methods for overexpressing a nucleic acid or polypeptide of interest are not particularly limited and are discussed in more detail herein *e.g.*, a polypeptide (*e.g.* NKG2D) can be overexpressed by the transfer of the corresponding nucleic acid using the same or a different expression vector than that encoding the CAD polypeptide. The expression vector is not particularly limited as long as the vector can be used in genetic engineering. For example, a plasmid vector, a virus vector, a cosmid vector, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), and any of other non-plasmid vectors can be used.

[0096] The term “anti-tumor effect” as used herein, refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition. An “anti-tumor

effect" can also be manifested by the ability of the polynucleotides and cells of the disclosure in prevention of the occurrence of tumor in the first place.

[0097] As used herein, the term "autologous" is meant to refer to any material derived from an individual which is later to be re-introduced into the same individual.

[0098] As used herein, the term "allogeneic" refers to material derived from an animal which is later introduced into a different animal of the same species.

[0099] As used herein, the term "syngeneic" refers to material that is genetically similar or identical and hence immunologically compatible, such that transplantation does not provoke an immune response.

[00100] As used herein, the term "agent" refers to any protein, nucleic acid molecule (including chemically modified nucleic acids), compound, antibody, small molecule, organic compound, inorganic compound, other molecule of interest, or cell (e.g., cell engineered to express a chimeric adaptor polypeptide). Agent can include a therapeutic agent, a diagnostic agent or a pharmaceutical agent. A therapeutic or pharmaceutical agent is one that alone or together with an additional agent induces the desired response (such as inducing a therapeutic or prophylactic effect when administered to a subject, including treating a subject suffering cancer, viral infection (e.g., cytomegalovirus (CMV), influenza, hepatitis B, Epstein-Barr, adenovirus, and the like), bacterial infection (e.g., E. coli, M. tuberculosis, etc.) rheumatoid arthritis (RA), or other disease/condition. Discussed herein, an agent may be referred to as a modulatory agent.

[00101] The term "diagnosis", or "diagnosing" as used herein refers to the process of identifying a disease, such as cancer, by its signs, symptoms, and/or results of various tests. A conclusion reached through such a process is a diagnosis. Forms of testing commonly performed include blood tests, medical imaging, urinalysis, biopsy, and the like.

[00102] The term "therapeutically effective amount", or simply "effective amount" refers to the amount of an agent or composition (e.g., composition comprising an agent) that will elicit a biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" includes that amount of an agent, or a composition comprising an agent, that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more

of the signs or symptoms of the disorder or disease (e.g., hematological or solid tumor) being treated. The therapeutically effective amount will vary depending on the composition, the disease and its severity and the age, weight, etc., of the subject to be treated.

[00103] To "treat" a disease as the term is used herein, means to decrease or reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

[00104] The term "decrease" as used herein means to reduce the quality, amount, or strength of something. In one example, a therapy (e.g., administration of a therapeutic agent of the present disclosure) decreases one or more signs or symptoms associated with a disease or condition, for example as compared to the response in the absence of the therapy. For example, administration of a therapeutic agent may in examples provide an anti-tumor effect that decreases one or more signs or symptoms associated with cancer.

[00105] As used herein, the term "administration" means to provide or give a subject one or more agents, such as an agent that treats one or more signs or symptoms associated with a condition/disorder or disease including but not limited to cancer, viral infection, bacterial infection, etc., by any effective route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), oral, sublingual, rectal, transdermal, intranasal, vaginal and inhalation routes.

[00106] The term "pharmaceutically acceptable", as used herein, refers to a material, including but not limited, to a salt, carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, i.e., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained. The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, Pa., 19th Edition (1995), describes compositions and formulations suitable for pharmaceutical delivery of one or more agents, such as one or more modulatory agents. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations can include injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose,

glycerol or the like as a vehicle. In addition to biologically-neutral carriers, pharmaceutical agents to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate, sodium lactate, potassium chloride, calcium chloride, and triethanolamine oleate.

[00107] The term “cytokine” as used herein refers to a diverse group of soluble proteins and peptides released from cells which act as humoral regulators at nano- to picomolar concentrations, and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Many growth factors and cytokines act as cellular survival factors by preventing programmed cell death. Cytokines include both naturally occurring peptides and variants that retain full or partial biological activity.

[00108] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[00109] "Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[00110] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and

that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[00111] The terms “patient,” “subject,” “individual,” and the like are used interchangeably herein, and refer to any animal, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

[00112] By the term “specifically binds”, as used herein with respect to a cell surface receptor, is meant a receptor which recognizes a specific molecule/ligand, but does not substantially recognize or bind other molecules in a sample. For example, a receptor that specifically binds to a molecule from one species may also bind to that molecule from one or more species. But, such cross-species reactivity does not itself alter the classification as specific. In another example, a receptor that specifically binds to a molecule may also bind to different allelic forms of the molecule. However, such cross reactivity does not itself alter the classification as specific. In some instances, the terms “specific binding” or “specifically binding,” can be used in reference to the interaction of a protein (or a peptide) with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, a receptor recognizes and binds to a specific structure rather than to proteins generally. If receptor is specific for epitope “A”, the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled “A” and the receptor, will reduce the amount of labeled A bound to the receptor.

[00113] In some embodiments, specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-8} M or less (e.g., a smaller KD denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like.

[00114] The term “cancer” as used herein refers to a physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Neoplasia, malignancy, cancer, and tumor may be used interchangeably and refer to abnormal growth of a tissue or cells that results from excessive cell division. The amount of a tumor in an individual is the “tumor burden” which can be measured as the number, volume, or weight of the tumor. A

tumor that does not metastasize is referred to as “benign.” A tumor that invades the surrounding tissue and/or can metastasize is referred to as “malignant.” A “non-cancerous tissue” is a tissue from the same organ wherein the malignant neoplasm formed, but does not have the characteristic pathology of the neoplasm. Generally, noncancerous tissue appears histologically normal. A “normal tissue” is tissue from an organ, wherein the organ is not affected by cancer or another disease or disorder of that organ. A “cancer-free” subject has not been diagnosed with a cancer of that organ and does not have detectable cancer.

[00115] Symptoms of cancer may include but are not limited to persistent cough or blood-tinged saliva, a change in bowel habits, blood in the stool, unexplained anemia (low blood count), breast lump or breast discharge, lumps in testicles, a change in urination, blood in urine, hoarseness, persistent lumps or swollen glands, obvious change of a wart or mole, indigestion, difficulty swallowing, unusual vaginal bleeding or discharge, unexpected weight loss, night sweats, or fever, continued itching in the anal or genital area, nonhealing sores, headaches, back pain, pelvic pain, and bloating, among others.

[00116] Hematologic cancers are cancers originating in the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[00117] Solid tumors are tumors that comprise a tumor mass of at least about 10 or at least about 100 tumor cells. The solid tumor can be a soft tissue tumor, a primary solid tumor, or a metastatic lesion.

[00118] Examples of solid tumors include, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (e.g., colon), genitourinary tract (e.g., renal, urothelial cells), pancreas, prostate and pharynx. Adenocarcinomas include malignancies such as most colon

cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. In one embodiment, the cancer is a melanoma, e.g., an advanced stage melanoma. In another embodiment, the cancer is a glioma. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the disclosure.

[00119] "Expression cassette" refers to a nucleic acid comprising expression control sequences operatively linked to a nucleic acid encoding a transcript or polypeptide to be expressed. An expression cassette comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an *in vitro* expression system. Expression cassettes can be a component of a vector such as a cosmid, a plasmid (e.g., naked or contained in a liposome), or a virus (e.g., lentivirus, retrovirus, adenovirus, and adeno-associated virus). An expression cassette can be in a host cell, such as an immune cell (e.g., $\gamma\delta$ T cell). Ranges: throughout this disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[00120] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with another nucleic acid (or the complementary strand of the other nucleic acid), there is nucleotide sequence identity in %, for example, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain

instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[00121] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity. In some aspects, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) *Science* 256: 1443-45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00122] Sequence identity and/or similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including

conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Sequences also can be compared using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. Another preferred algorithm when comparing a sequence disclosed herein to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, e.g., Altschul et al. (1990) *J. Mol. Biol.* 215: 403-410 and (1997) *Nucleic Acids Res.* 25:3389-3402, each of which is herein incorporated by reference.

I. Introduction

[00123] DNAX-activating protein of 10kDa (DAP10) is the adaptor molecule that associates with the cell surface, cytotoxic receptor natural killer group 2 member D (NKG2D). The NKG2D receptor is a type II transmembrane-anchored C-type lectin-like protein, which belongs to the CD94/NKG2 family of C-type lectin-like receptors (Houchins et al., (1991) *J. Exp. Med.* 173: 1017-1020). NKG2D is capable to bind numerous and highly diversified MHC class I-like self-molecules. These ligands are often poorly expressed on normal cells but can be induced on damaged, transformed, or infected cells (Zingoni, A et al. (2018) *Front. Immunol.* 9(476): 1-12). Said ligands belong to the H60(a-c), RAE (α - ϵ), and MULT1 families in mice, and to the MIC (MICA and MICB) and ULBP (ULBP1-ULBP6) families in humans, where their repertoire is more complex than in other species. In fact, MIC molecules are encoded by the most highly polymorphic human genes after the classical HLA molecules (Eagle, RA and Trowsdale, J. (*Nat. Rev. Immunol.* (2007) 7(9): 737-44).

[00124] NKG2D is an activating immune receptor which regulates both innate and adoptive immune responses. NKG2D is abundantly present on all NK cells, CD8 T cells, subsets of $\gamma\delta$ T

cells and some autoreactive CD4 T cells. NKG2D acts with other costimulatory molecules such as DAP10, to modify the strength and duration of antigen-specific responses mediated by the T cell receptor and to influence the pattern of anti-tumor reactivity by T lymphocytes (see e.g., Maccalli C, et al. (2003) *Eur. J. Immunol.* 33(7):2033-43).

[00125] NKG2D lacks a signaling motif in its cytoplasmic domain. Thus, after ligand binding, NKG2D signal transduction and cellular activation relies upon association of NKG2D with the DAP10 adaptor molecule which promotes and stabilizes NKG2D surface membrane expression (Wu, J., et al., (1999) *Science* 285: 730-732). However, it is known, for example, that TGF- β is capable of mediating down-regulation of NKG2D (and NKG2DL) surface expression (Lazarova M and Steinle. (2019) *Front. Immunol* 10(2689): 1-11), and that TGF- β can substantially decrease DAP10 expression both at mRNA and protein levels (Park, YP et al. (2011) *Blood*. 118: 3019-27; Lee, JC et al. (2011) *Tumori* 97:350-7). Accordingly, it is herein recognized that methodologies capable of modulating NKG2D and/or DAP10 expression and/or associated signaling pathways are of therapeutic interest.

[00126] In addition to NKG2D, DAP10 is known to associate with a number of other receptors. For example, Ly49H and Ly49D were co-immunoprecipitated with DAP10 from mouse NK cells, and have been shown to associate with DAP10 when co-transfected into 293T cells (Coudert JD et al. (2008) *Blood* 111: 3571-3578). By co-transfection studies, DAP10 has also been shown to associate with human Sirp-b1 in transfected rat RBL-2H3 cells (Anfossi N et al. (2003) *Eur. J. Immunol.* 33: 3514-3522). Similarly, human and mouse Siglec-15 (Angata T et al. (2007) *Glycobiology* 17: 838-846) and Cd300lb (Yamanishi Y et al. (2008) *Blood* 111: 688-698) have been shown by co-transfection and co-immunoprecipitation to pair with DAP10. As mentioned, certain DAP10-associated receptors (i.e., NKG2D) appear to recognize host-encoded molecules, including carbohydrate and protein ligands, however other DAP10-associated receptors can directly recognize microbial ligands. One example includes the recognition of the mouse CMV-encoded glycoprotein m157 by Ly49H (Lanier LL (2008) *Nat. Rev. Immunol.* 8(4): 259-68; Smith HR et al. (2002) *Proc. Natl. Acad. Sci. USA* 99(13): 8826-31). m157 is a GPI-anchored glycoprotein with homology to MHC class I, which is displayed on the surface of mouse CMV-infected cells, resulting in activation of Ly49H⁺ NK cell-mediated cytotoxicity and cytokine production.

[00127] The above-mentioned receptors are meant to be illustrative of the ability of DAP10 to pair with, and hence modulate signaling through, numerous receptors in addition to NKG2D, and is not mentioned to be exhaustive. It is to be understood that with regard to the present disclosure, the nucleic acids, polypeptides encoded thereby, cells, compositions, and methods apply to any and all receptors that DAP10 is capable of partnering with.

II. General Methods

[00128] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. In particular, this disclosure utilizes routine techniques in the field of recombinant genetics, immunology, and biochemistry. Basic texts disclosing the general terms in molecular biology and genetics include e.g., Lackie, *Dictionary of Cell and Molecular Biology*, Elsevier (5th ed. 2013). Basic texts disclosing methods in recombinant genetics and molecular biology include e.g., Sambrook et al, *Molecular Cloning—A Laboratory Manual*, Cold Spring Harbor Press 4th Edition (Cold Spring Harbor, N.Y. 2012) and *Current Protocols in Molecular Biology* Volumes 1-3, John Wiley & Sons, Inc. (1994-1998) and Supplements 1-115 (1987-2016). Basic texts disclosing the general methods and terms in biochemistry include e.g., Lehninger *Principles of Biochemistry* sixth edition, David L. Nelson and Michael M. Cox eds. W.H. Freeman (2012). Basic texts disclosing the general methods and terms immunology include *Janeway's Immunobiology* (Ninth Edition) by Kenneth M. Murphy and Casey Weaver (2017) Garland Science; *Fundamental Immunology* (Seventh Edition) by William E. Paul (2013) Lippincott, Williams and Wilkins.

DAP10

[00129] As discussed above, the NKG2D receptor, for example, lacks a signaling motif in its cytoplasmic domain. Thus, the signaling function of the NKG2D receptor, among others, is intimately tied to its association with the adaptor molecule, DAP10. The below discussion with regard to DAP10 is presented with regard to the NKG2D receptor for clarity and ease of presentation, but it should be understood that the teachings are broadly applicable to other receptors for which DAP10 can partner with.

[00130] The human NKG2D receptor assembles with the DAP10 signaling dimer, with one NKG2D homodimer paired with a DAP10 dimer by formation of two salt bridges between

conserved transmembrane (TM) arginine residues (Garrity, D. et al. (2005) PNAS USA 102(21): 7641-7646). The DAP10 dimer carries a pair of aspartic acid residues close to the center of the transmembrane (TM) domains, and these residues interact with the conserved arginine in the TM sequence of NKG2D for assembly with the DAP10 dimer. Thus, the NKG2D homodimer associates with the DAP10 adaptor molecule in its transmembrane domain to form a hexameric structure which can initiate signaling cascades (*see e.g.*, Garrity et al (2005) *supra*).

[00131] As mentioned, the DAP10 dimer is a disulfide-linked homodimer. The amino acid sequence of the wild-type human DAP10 polypeptide is shown below as SEQ ID NO: 1: MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPLLAGLVAADAVA SLLIVGAVFLCARPRRSPAQEDGKVYINMPGRG (SEQ ID NO: 1). See also Table 2, which lists a number of sequences relevant to the present disclosure.

[00132] The DAP10 cytoplasmic domain comprises a tyrosine-based motif (YINM), comprising residues 86-89 of SEQ ID NO: 1. Upon tyrosine (Y86) phosphorylation, DAP10 is capable to bind either the p85 subunit of phosphatidylinositol 3-kinase (PI3K, through YXXM) or the adaptor Grb2 (through YXNX). Because these two binding sites overlap, a single DAP10 chain will bind either p85 or Grb2, but not both (Lanier LL. (2008) Nat. Immunol. 9(5): 495-502). This YINM motif is similar to the motif in CD28, which provides for costimulatory signaling in conjunction with the immunoreceptor tyrosine-based activation motif (ITAM)-based TCR/CD3 complex in T cells.

[00133] Both recruitment of the p85 subunit of PI3K or the adaptor protein Grb2 can activate Vav1 and PLC- γ 2 and thus are essential for the activation of Ca^{2+} mobilization and cytotoxicity toward cells (Upshaw JL et al. (2006) Nat. Immunol. 7(5): 524-32).

[00134] DAP10 further comprises a ubiquitinylation site that encompasses the lysine at amino acid 84 of the DAP10 protein sequence (SEQ ID NO: 1). Ligand stimulation of NKG2D on NK cells results in the ubiquitylation of DAP10, which is required for the endocytosis and degradation of the NKG2D-DAP10 complex (*see e.g.*, Molfetta, R., et al. (2014) Eur. J. Immunol. 44, 2761-2770). Furthermore, it has been shown that ubiquitin-dependent receptor endocytosis is required for the activation of extracellular signal-regulated kinase (ERK) and NK cell functions, such as the secretion of cytotoxic granules and the inflammatory cytokine

interferon- γ . Hence, NKG2D-DAP10 endocytosis represents a means to decrease cell surface receptor abundance, as well as to control signaling in cytotoxic lymphocytes.

Chimeric Adaptor Constructs

[00135] Aspects of the present disclosure include constructs comprising nucleic acids encoding chimeric adaptor polypeptides. In embodiments, the nucleic acids encode chimeric adaptor polypeptides comprising i) a human DAP10 amino acid sequence and ii) one or more costimulatory domains (e.g., 4-1BB, OX40, ICOS, CD28), wherein the CAD polypeptide specifically lacks an ectodomain. In embodiments, the CAD polypeptides can additionally comprise one or more intracellular signaling domains (e.g., CD3 ζ) as herein described. In embodiments, the CAD polypeptides can also comprise one or more mutations, for example one or more mutations in DAP10 and/or one or more modifications (e.g., one or more mutations, additions, or deletions) in the costimulatory domain(s) and/or the intracellular signaling domain(s).

[00136] In embodiments, the CAD polypeptides function to modulate and/or mute signaling through one or more receptors with which they associate. In embodiments, the CAD polypeptides are designed with modulated/muted attributes (e.g., by way of the one or more mutations) and/or added signaling attributes (e.g., by way of the C-terminal fusion) and expressed in a host cell promote a favorable balance of signaling pathways upon receptor-target engagement (e.g., NKG2D engagement of an extracellular target ligand), which may serve to address the problem of low or lost expression of the target (i.e., antigen escape) for the primary TCR. The term “favorable balance” refers broadly to the introduction of signaling cascades that are complementary to, alternative to, or that act to modulate primary DAP10 signal transduction cascades in a desired manner. Accordingly, the CAD polypeptides disclosed herein provide for altered (e.g., improved) functional properties including but not limited to altered (e.g., enhanced) cytolytic, proliferative, survival and/or costimulatory properties that are elicited upon engagement with the ligands of receptors that partner with DAP10 (e.g., widely expressed ligands of NKG2D). The precise composition of the CAD polypeptides can be designed based on a given disease indication and in some examples on pairing with the specificity and signaling components of other receptor(s) (e.g., TCR receptor(s)) that are present on the same cells. As one illustrative example, immunosuppressive signals within a tumor microenvironment (TME)

may inhibit anti-tumor T cell responses through inhibitory receptors on T cells, and it is within the scope of this disclosure that via the use of a CAD polypeptide such an inhibitory output may be switched to an immunostimulatory one. Other relevant examples may be found in, e.g., Guo, J., et al., (2021) *Journal for ImmunoTherapy of Cancer* 9:e002628.

[00137] In embodiments, the CAD polypeptides may function at least in part to stabilize cell surface receptor(s) (e.g., NKG2D) with which they associate. To “stabilize” cell surface receptor(s) as herein disclosed means to decrease a rate at which said cell surface receptors are endocytosed or otherwise removed from the surface of the cell, as compared to the rate at which said cell surface receptors are otherwise removed under similar circumstances in absence of a CAD polypeptide as herein described. Encompassed within the scope of receptor stabilization as herein described includes positive feedback mechanisms through which CAD polypeptide signaling results in increased cell surface expression of the endogenous receptor(s) (e.g., NKG2D) with which endogenous DAP10 and the CAD polypeptides associate (Wu, J., et al., (2000) *Journal of Exp Med* 192(7): 1059-1068).

[00138] In embodiments, expression of CAD polypeptides comprising heterologous signaling domains that influence cell survival and proliferation (e.g., IL2R, 4-1BB, CD27 etc.) enables logic gating strategies (e.g., “AND” gating) that can increase stringency and/or potency of target attack when paired with endogenous receptors (e.g., endogenous TCRs) on the same host cell (e.g., T cell or NK cell) that engage targets that have low level expression in normal tissue. Accordingly, host cells with both a receptor that recognizes a primary target (e.g., primary cancer target) and an appropriate CAD polypeptide may promote sustained survival, proliferation and killing of target cells that express both the primary target(s) and ligands of a receptor (e.g., NKG2D) that associates with DAP10. Examples of logic gating strategies applicable to the present disclosure can be found, e.g., in WO2019118518, WO2020154635, WO2020223445, WO2021035093, WO2019222642A1, WO2018236825A1, WO2019164979, and Chang, ZL, and Chen YY (2017) *Trends Mol Med* 23(5): 430-450).

[00139] The disclosure also provides stably expressed CAD polypeptides. In embodiments, a CAD polypeptide is expressed at a level substantially similar to the level at which endogenous DAP10 is expressed in a host cell of interest. In embodiments, a CAD polypeptide is expressed at a level higher than the level at which endogenous DAP10 is expressed in the host cell of

interest. For example, a CAD polypeptide may be expressed at a level 10% higher, or between 10-20% higher, or between 20-30% higher, or between 30-40% higher, or between 40-50% higher, or between 50-60% higher, or between 60-70% higher, or between 70-80% higher, or between 80-90% higher or between 90-100% higher, or even higher, such as 2-fold higher, or 3-fold higher, or 4-fold higher, or 5-fold higher, or 6-fold higher, or 7-fold higher, or 8-fold higher, or 9-fold higher, or 10-fold higher, or 20-fold higher, or 30-fold higher, or 40-fold higher, or 50-fold higher or 100-fold higher, than a corresponding level at which endogenous DAP10 is expressed. Thus, it may be understood that in embodiments, the CAD polypeptides of the subject invention may compete with endogenous DAP10 binding to a receptor (e.g., NKG2D), depending on the particular host cell type.

[00140] Aspects of the disclosure include nucleic acids encoding CAD polypeptides, and constructs/vectors containing such nucleic acids. Therefore, described herein are nucleic acids encoding CAD polypeptides that incorporate select mutations and signaling domains that modulate and/or add signaling attributes that impart desired properties including but not limited to *e.g.*, sustained survival, proliferation and/or killing on the host cell expressing said CAD polypeptides.

A. Mutations

[00141] In embodiments, a CAD polypeptide carries one or both of an amino acid modification at position K84 and/or position Y86 of SEQ ID NO: 1. In embodiments, K84 is modified to comprise another positively charged amino acid, for example K84R, or K84H, although it is within the scope of this disclosure that the modification at K84 can comprise other amino acid substitutions. In one embodiment, the modification is a K84R modification. In embodiments, a CAD polypeptide with a K84R modification comprises SEQ ID NO: 18. In embodiments, Y86 is modified to another aromatic amino acid, for example Y86F or Y86W, although it is within the scope of this disclosure that the modification at Y86 can comprise other amino acid substitutions. In one embodiment, the modification is a Y86F modification. In embodiments, a CAD polypeptide with a Y86F modification comprises SEQ ID NO: 19. In embodiments, a CAD polypeptide with both a K84R modification and a Y86F modification comprises SEQ ID NO: 20.

[00142] In embodiments, the modification at position 86 of SEQ ID NO: 1 reduces or eliminates p85/PI3K binding to the CAD polypeptide, and in turn reduces or eliminates PI3K/AKT/PKC θ signaling. Hence, the modification at position 86 may serve to functionally reduce or eliminate one or more of costimulation, calcium-flux, and/or degranulation. In a preferred embodiment, the modification is Y86F.

[00143] In additional or alternative embodiments, the modification at position 86 of SEQ ID NO: 1 reduces or eliminates Grb2 binding to the CAD polypeptide, and in turn reduces or eliminates Vav1/SLP-76/PLC γ signaling. Hence, the modification at position 86 may serve to reduce or eliminate one or more of calcium-influx and/or degranulation. In a preferred embodiment, the modification is Y86F.

[00144] In additional or alternative embodiments, the modification at position 84 of SEQ ID NO: 1 reduces or completely disrupts ubiquitinylation of the CAD polypeptide, which in turn reduces or completely prevents the internalization of the chimeric DAP10 adaptor polypeptide-endogenous receptor complex at the cellular membrane of the particular host cell (see *e.g.*, Quatrini, L., et al., (2015) *Sci Signal* 8(400):ra108). In this way, via reliance on the CAD polypeptide harboring at least the K84 modification (*e.g.*, K84R), an endogenous receptor that associates with DAP10 (*e.g.*, NKG2D) can be stabilized at the cell surface. Furthermore, a modification at K84 (*e.g.*, K84R) may reduce or eliminate signaling (*e.g.*, ERK1/2) that otherwise occurs en route to lysosomal degradation. Reduction or elimination of signaling (*e.g.*, ERK1/2) may serve to reduce or eliminate one or more of exhaustion, activation-induced cell death and/or induction of cell cycle arrest upon hyperactivation, one or more of which may otherwise occur in absence of a CAD polypeptide harboring the K84 modification (*e.g.*, K84R).

[00145] In additional or alternative embodiments, a CAD polypeptide may include a modification at position 57 of SEQ ID NO: 1, for example a D57A modification, although amino acid modifications other than alanine are within the scope of this disclosure. In embodiments, a chimeric DAP10 adaptor polypeptide with a D57A modification comprises SEQ ID NO: 37. A modification at D57 may in embodiments serve to modify (*e.g.*, reduce or abolish) stable interaction with KLRK1 (Wu, J., et al., (1999) *Science* 285(5428): 730-2).

[00146] In additional or alternative embodiments, a CAD polypeptide may include a modification at position 88 of SEQ ID NO: 1, for example a N88Q modification, although amino

acid modification other than glutamine are within the scope of this disclosure. In embodiments, a CAD polypeptide with a N88Q modification comprises SEQ ID NO: 38. A modification at N88 may in embodiments serve to modify (e.g., reduce) cell killing activity and/or interaction with GRB2, while having minimal to no effect on interaction with PIK3R1 (Upshaw, JL., (2006) Nat Immunol 7:524-532).

[00147] In additional or alternative embodiments, a CAD polypeptide may include a modification at position 89 of SEQ ID NO: 1, for example a M89Q modification, although amino acid modifications other than glutamine are within the scope of this disclosure. In embodiments, a CAD polypeptide with a M89Q modification comprises SEQ ID NO: 39. A modification at M89Q may in embodiments serve to modify (e.g., reduce) cell killing activity and/or interaction with PIK3R1, while having minimal to no effect on interaction with GRB2 (Upshaw, JL., (2006) Nat Immunol 7:524-532).

[00148] It may be understood that the present disclosure encompasses chimeric adaptor polypeptides having any one or more or each of the above-mentioned modifications.

B. Costimulatory and Signaling Domains

[00149] In embodiments, the endodomain of the chimeric adaptor polypeptides of the present disclosure comprise one or more costimulatory domains, wherein the costimulatory domain comprises functional costimulatory signaling domain derived from *e.g.*, a MHC class I molecule, TNF receptor proteins, immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, and the like. For example, it is within the scope of this disclosure that the endodomain of a disclosed CAD polypeptide can include 2, 3, 4 or more costimulatory domains. It is also within the scope of this disclosure that when more than one costimulatory domain is included, the costimulatory domains may be the same, or they may be different. In embodiments, the costimulatory domains are derived from one or more of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, B7-H3, CEACAM1, CRTAM, CD2, CD3C, CD4, CD7, CD8 α , CD8 β , CD11a, CD11b, CD11c, CD11d, IL2R β , IL2 γ , IL7R α , IL4R, IL7R, IL15R, IL21R, CD18, CD19, CD19aCD27, CD28, CD29, CD30, CD40, CDS, CD49a, CD49D, CD49f, CD54 (ICAM), CD69, CD70, CD80, CD83, CD84, CD86, CD96 (Tactile), CD100 (SEMA4D), CD103, CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4),

CD160 (BY55), CD162 (SELPLG), CD244 (2B4), CD270 (HVEM), CD226 (DNAM1), CD229 (Ly9), CD278 (ICOS), ICAM-1, LFA-1 (CD11a/CD18), FcR, FcγRI, FcγRII, FcγRIII, LAT, NKG2C, SLP76, TRIM, ZAP70, GITR, BAFFR, LTBR, LAT, GADS, LIGHT, HVEM (LIGHTR), KIRDS2, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB1, ITGB2, ITGB7, NKG2C, NKG2D, IA4, VLA-1, VLA-6, SLAM (SLAMF1, CD150, IPO-3), SLAMF4, SLAMF6 (NTB-A, Ly108), SLAMF7, SLAMF8 (BLAME), SLP-76, PAG/Cbp, NKp80 (KLRF1), NKp44, NKp30, NKp46, BTLA, JAML, CD150, PSGL1, TSLP, TNFR2, and TRANCE/RANKL, or a portion thereof, and combinations thereof.

[00150] In some embodiments, the CAD construct encodes at least one 4-1BB costimulatory domain, and optionally a second costimulatory domain selected from 4-1BB, 2B4, ICOS, CD28, OX40, and CD27 costimulatory domains, or any of the above-mentioned costimulatory domains. In some embodiments, the construct encodes at least two 4-1BB costimulatory domains, or at least two 4-1BB costimulatory domains in combination with one, two, three, or four, or more, costimulatory domains selected from 4-1BB, ICOS, CD28, OX40, and CD27, or any of the above-mentioned costimulatory domains. In some embodiments, the 4-1BB costimulatory domain comprises SEQ ID NO: 2 (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL). In some embodiments, the 4-1BB costimulatory domain comprises an amino acid sequence having at least one, at least two, or at least three or more modifications of an amino acid sequence of SEQ ID NO: 2. In some embodiments, the 4-1BB costimulatory domain is substantially similar to the 4-1BB costimulatory domain comprising SEQ ID NO: 2.

[00151] In some embodiments, the CAD construct encodes at least one CD27 costimulatory domain, and optionally at least one second costimulatory domain selected from 4-1BB, ICOS, CD28, OX40, 2B4, and CD27 costimulatory domains, or any of the above-mentioned costimulatory domains. In some embodiments, the construct encodes at least one CD27 costimulatory domain, and a 4-1BB costimulatory domain. In some embodiments, the construct encodes two CD27 costimulatory domains, and at least one second costimulatory domain selected from a 4-1BB, ICOS, CD28, and CD27. In some embodiments, the CD27 costimulation domain comprises SEQ ID NO: 5 (QRRKYRSNKGESPVPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP). In some embodiments, the CD27 costimulatory domain comprises an amino acid sequence having at least one, at least

two, at least three or more modifications of an amino acid sequence of SEQ ID NO: 5. In embodiments, the CD27 costimulatory domain is substantially similar to the CD27 costimulatory domain comprising SEQ ID NO: 5.

[00152] In some embodiments, the CAD construct encodes at least one CD28 costimulatory domain, and optionally a second costimulatory domain selected from 4-1BB, 2B4, ICOS, CD28, OX40, and CD27 costimulatory domains, or any of the above-mentioned costimulatory domains. In some embodiments, the CAD construct encodes at least two CD28 costimulatory domains, or at least two CD28 costimulatory domains in combination with one, two, three, or four, or more, costimulatory domains selected from a 4-1BB, ICOS, CD28, OX40, and CD27, or any of the above-mentioned costimulatory domains. In some embodiments, the CD28 costimulatory domain comprises SEQ ID NO: 40

FWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRS). In embodiments, the CD28 costimulatory domain comprises SEQ ID NO: 41 (FWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS). Included in SEQ ID NO: 40 and SEQ ID NO: 41 are three subdomains YNMN, PRRP, and PYAP, that are capable to regulate signaling pathways. In embodiments, a disclosed CAD polypeptide comprises mutation or deletion of one or more of said subdomains (see *e.g.*, WO2019010383). In some embodiments, the CD28 costimulatory domain comprises an amino acid sequence having at least one, at least two, at least three or more modifications of an amino acid sequence of SEQ ID NO: 40, or an amino acid sequence of SEQ ID NO: 41. In some embodiments, the CD28 costimulatory domain is substantially similar to the CD28 costimulatory domain comprising SEQ ID NO: 40. In some embodiments, the CD28 costimulatory domain is substantially similar to the CD28 costimulatory domain comprising SEQ ID NO: 41.

[00153] In some embodiments, the CAD construct encodes at least one ICOS costimulatory domain, and optionally a second costimulatory domain selected from 4-1BB, 2B4, ICOS, CD28, OX40, and CD27 costimulatory domains, or any of the above-mentioned costimulatory domains. In some embodiments, the CAD construct encodes at least two ICOS costimulatory domains, or at least two ICOS costimulatory domains in combination with one, two, three, or four, or more, costimulatory domains selected from 4-1BB, ICOS, CD28, OX40, and CD27, or any of the above-mentioned costimulatory domains. In some embodiments, the ICOS costimulatory domain comprises SEQ ID NO: 42. In some embodiments, the ICOS costimulatory domain comprises

an amino acid sequence having at least one, at least two, at least three or more modifications of an amino acid sequence of SEQ ID NO: 42 (see *e.g.*, US20170209492). In some embodiments, the ICOS costimulatory domain is substantially similar to the ICOS costimulatory domain comprising SEQ ID NO: 42.

[00154] In some embodiments, the CAD construct encodes at least one OX40 costimulatory domain, and optionally a second costimulatory domain selected from 4-1BB, 2B4, ICOS, CD28, OX40, and CD27 costimulatory domains, or any of the above-mentioned costimulatory domains. In some embodiments, the CAD construct encodes at least two OX40 costimulatory domains, or at least two OX40 costimulatory domains in combination with one, two, three, or four, or more, costimulatory domains selected from 4-1BB, ICOS, CD28, OX40, and CD27, or any of the above-mentioned costimulatory domains. In some embodiments, the OX40 costimulatory domain comprises SEQ ID NO: 43 (RRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKI). In some embodiments, the OX40 costimulatory domain comprises an amino acid sequence having at least one, at least two, at least three or more modifications of an amino acid sequence of SEQ ID NO: 43. In some embodiments, the OX40 costimulatory domain is substantially similar to the OX40 costimulatory domain comprising SEQ ID NO: 43.

[00155] In embodiments, one or more intracellular signaling domains are included in the chimeric adaptor polypeptides of the subject invention. In embodiments, the one or more intracellular signaling domains are additional to one or more costimulatory domains. In embodiments, the one or more intracellular signaling domains are included to increase proliferation, persistence, and/or cytotoxic activity of the host cell (*e.g.*, NK cell, NKT cell, $\gamma\delta$ cell, etc.) harboring the CAD polypeptide as herein disclosed. For example, in some embodiments, the intracellular signaling domain(s) comprise CD3 ζ , repeat (*e.g.*, 2-5) DAP10 YINM motifs, signaling domains derived from LFA-1, DAP12, FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD79a, CD79b, CD5, CD22, Fc ϵ RI, CD66d, and the like. It is within the scope of this disclosure that the endodomain of a disclosed chimeric adaptor polypeptide can include a plurality (*e.g.*, 2, 3, 4, or more) of intracellular signaling domains. In a case where more than one intracellular signaling domain is included, the intracellular signaling domains may be the same, or they may be different.

[00156] In some embodiments, an intracellular signaling domain is or comprises a CD3 ζ signaling domain. In some embodiments, a CD3 ζ signaling domain is or comprises RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 3) or RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 4) , or RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPQRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 76), also referred to herein as “1XX” (*see e.g.*, US 2020/0317777, the contents of which is incorporated by reference herein in its entirety). Without being bound to a theory, inclusion of the 1XX signaling domain may, in some embodiments, lead to improved activation and/or survival of modified immune cells of the present disclosure by limiting overactivation.

[00157] In some embodiments the CAD construct encodes one or more costimulatory domain(s) (e.g., 4-1BB costimulation domain), and one or more intracellular signaling domain(s) (e.g., CD3 ζ signaling domain). In some embodiments, the CAD construct encodes at least one 4-1BB costimulation domain, at least one CD28 domain, and at least one CD3 ζ signaling domain. In other embodiments, the CAD construct encodes one or more first costimulation domains (e.g., 4-1BB, CD28, OX40, ICOS) and one or more second costimulation domains (e.g., 4-1BB, CD28, OX40, ICOS), and one or more intracellular signaling domains (e.g., CD3 ζ). In embodiments, the CD3 ζ signaling domain is downstream (C-terminal) to the costimulation domain(s) (e.g., 4-1BB). In some embodiments, the CD3 ζ signaling domain is upstream (N-terminal) to the costimulation domain(s) (e.g., 4-1BB).

C. Cytokine Co-Expression

[00158] In additional embodiments, a CAD construct of the subject invention can also encode for one or more multicistronic linker region(s) configured to facilitate translation of the CAD polypeptide and one or more soluble common gamma chain cytokines as separate polypeptides. In embodiments, nucleic acids encoding the cytokine and associated linker region can be

positioned at the 3' end of the isolated nucleic acid, or at the 5' end of the isolated nucleic acid, or in some examples at both the 5' end and the 3' end of the isolated nucleic acid. The one or more soluble common gamma chain cytokines can include but are not limited to IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, IL-23. In embodiments, the linker region(s) can encode a self-cleavage and/or a cleavage polypeptide sequence. In some examples, the self-cleavage sequence is a 2A self-cleaving sequence (e.g., T2A, P2A, E2A, F2A) which can induce ribosomal skipping during translation of the chimeric DAP10 adaptor polypeptide. In embodiments, the cleavage sequence is a furin sequence. In some examples, the cleavage sequence (e.g., furin cleavage sequence) is amino terminal to a self-cleavage sequence. In some embodiments, the multicistronic linker region encodes an internal ribosome entry site. In some embodiments, the multicistronic linker region comprises a sequence of any one of SEQ ID NOs: 9-15, or SEQ ID NO: 44. In embodiments, addition of an optional linker "GSG" or "SGSG" and the like can improve cleavage efficiency. In this way, the included one or more gamma chain cytokines may be released from the chimeric DAP10 adaptor polypeptide, and secreted by the host cell.

[00159] For example, in some embodiments, the cleavage sequence is the P2A cleavage sequence of SGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 9). In some embodiments, the P2A cleavage sequence is the P2A cleavage sequence of GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 44). In some embodiments, the cleavage sequence is a furin cleavage sequence of RAKR (SEQ ID NO: 10). In some embodiments, the cleavage sequence is a P2A+furin cleavage (FP2A) sequence of RAKRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 11).

[00160] In some embodiments, the cleavage sequence is or comprises a P2A cleavage sequence of ATNFSLLKQAGDVEENPGP (SEQ ID NO: 12). In some embodiments, the cleavage sequence is or comprises an F2A cleavage sequence of VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 13). In some embodiments, the cleavage sequence is or comprises an E2A cleavage sequence of QCTNYALLKLAGDVESNPGP (SEQ ID NO: 14). In some embodiments, the cleavage sequence is or comprises a T2A cleavage sequence of EGRSLLTCGDVEENPGP (SEQ ID NO: 15). In certain aspects, multiple self-cleavage sequences can be encoded carboxy-terminal to a signaling and/or costimulatory domain and amino-terminal to an encoded secreted cytokine (e.g., common gamma chain cytokine such as IL-15), preferably wherein the multiple self-cleavage sequences are independently selected from the group consisting of a P2A cleavage sequence, a T2A cleavage sequence, an E2A

cleavage sequence, and an F2A cleavage sequence. In certain aspects, one or more self-cleavage sequences and one or more sequences cleaved by an endogenous protease are encoded in a construct described herein. In certain embodiments, an endogenous protease recognition site is encoded amino terminal to a self-cleavage sequence.

[00161] In some embodiments, the multi-cistronic linker region encodes an internal ribosome entry site. An exemplary internal ribosome entry site is encoded by

CTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGT
TATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGT
CTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCT
GTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTC
TGTAAGCGACCCCTTTCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCG
GCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCAC
GTTGTGAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAAC
AAGGGGCTGAAGGATGCCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCC
TCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCC
GAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATA (SEQ ID NO: 16).

[00162] Another exemplary internal ribosome entry site is encoded by

AGCAGGTTTCCCCAACTGACACAAAACGTGCAACTTGAACTCCGCCTGGTCTTTCC
AGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCG
AGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCT
CCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCCTCATGT
GTGCAACCCACGACGCGGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAA
CTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAA
CACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTT
TAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCTTTGCAATT
ACTGACCAC (SEQ ID NO: 17).

[00163] Further suitable internal ribosome entry sites include, but are not limited to, those disclosed e.g., in Nucleic Acids Res. 2010 Jan;38(Database issue):D131-6. doi:

10.1093/nar/gkp981. Epub 2009 Nov 16, those described at iresite.org, those described in WO 2018/215787, the sequence described in GenBank accession No. KP019382.1, and the IRES

element disclosed in GenBank accession No. LT727339.1. Additional multi-cistronic linker regions, including cleavage self-cleavage, and IRES elements, are disclosed in US 2018/0360992 and U.S. 8,865,467.

[00164] In some embodiments, the construct encodes a secretion signal, e.g., (MALPVTALLLPLALLLHAARP (SEQ ID NO: 6)) operably linked to facilitate secretion of a C-terminal polypeptide, such as a cytokine. In some embodiments, the secretion signal is a secretion signal of MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCF SAGLPKTEA (SEQ ID NO: 7). In some embodiments, the construct encodes a secretion signal, e.g., SEQ ID NO: 7 operably linked to facilitate secretion of a common gamma chain cytokine such as IL-15 or an active fragment thereof, e.g.,

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCELLELQVISLESGDASIH DTVENLILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 8); Other IL-15 sequences, including codon optimized nucleic acid sequences encoding sIL15, are disclosed in WO 2007/037780. Exemplary common gamma chain cytokines include IL-4, IL-7, IL-9, IL-15, IL-21, IL-23. In some embodiments, the common gamma chain cytokine is selected from IL-2, IL-7, and IL-15. In some embodiments, the common gamma chain cytokine is IL-15. IL-15 sequences, including codon optimized nucleic acid sequences encoding sIL15, are disclosed herein and in WO 2007/037780.

[00165] Thus, the CAD constructs of the present disclosure encode a CAD polypeptide including at least one costimulatory domain and, optionally, one or more intracellular signaling domains. In embodiments, the CAD constructs can encode for one or more common gamma chain cytokines that are released from the CAD polypeptide (e.g., during translation). As discussed above in some embodiments, the CAD constructs of the present disclosure may further comprise a mutated DAP10, for example DAP10 mutated at K84 and/or Y86, among others, of SEQ ID NO: 1.

[00166] In embodiments, the one or more costimulatory domains may be 5' to the one or more signaling domains. In embodiments, the one or more costimulatory domains may be 3' to the one or more signaling domains. In some embodiments, the one or more costimulatory domains may be 5' to the one or more signaling domains, and additionally one or more costimulatory domains may be 3' to the one or more signaling domains. In some embodiments, one or more

signaling domains may be 5' to one or more costimulatory domains, and additionally one or more signaling domains may be 3' to one or more costimulatory domains. In some embodiments, the C-terminal fusion may include alternating one or more costimulatory domains and one or more signaling domains.

[00167] For reference, FIGS. 1A-1D depict exemplary illustrations of various chimeric adaptor polypeptides and their association with a receptor (e.g., illustrated as NKG2D). The renderings are for illustrative purposes only, and are not representative of all of the various permutations of chimeric adaptor polypeptides disclosed herein. FIG. 1A illustratively depicts a chimeric adaptor polypeptide that includes both K84R and Y86F modifications. FIG. 1B illustratively depicts a chimeric adaptor polypeptide that includes both K84R and Y86F modifications, in addition to a C-terminal fusion comprising CD3 ζ . FIG. 1C illustratively depicts a chimeric adaptor polypeptide that includes both K84R and Y86F modifications, in addition to a C-terminal fusion comprising 4-1BB. FIG. 1D illustratively depicts a chimeric adaptor polypeptide that includes both K84R and Y86F modifications, in addition to a C-terminal fusion comprising both 4-1BB and CD3 ζ .

D. Marker Co-Expression

[00168] In additional embodiments, a CAD construct of the subject invention can encode for one or more labels or markers, for example to facilitate an ability to monitor CAD expression level, serve as an internal control, and the like. In some embodiments, a CAD construct can encode for a fluorescent protein, examples of which include but are not limited to green fluorescent protein (GFP), red fluorescent protein (RFP), enhanced GFP (EGFP), enhanced cyan fluorescent protein (ECFP), enhanced yellow fluorescent protein (EYFP), and the like. Other examples can include but are not limited to chloramphenicol acetyltransferase, beta-galactosidase, beta-glucuronidase, beta-lactamase, luciferase, and the like.

[00169] In other embodiments, the CAD construct can encode for a protein that is expressed on a cell surface to facilitate detection and/or isolation of cells expressing said protein, e.g., via fluorescent activated cell sorting (FACS); or for enrichment through positive selection using an antibody specific to the encoded protein, e.g., use of an antibody to purify or enrich the cells product on a column or apparatus; or for *in vivo* binding of an antibody to the protein to enhance or eliminate activity, e.g., to facilitate removal of cells expressing the protein in patients as a

safety consideration. Exemplary proteins useful for these purposes include, e.g., CD19, CD20 (Rituxumab recognition domain), LNGFR (amino acid sequence as set forth as SEQ ID NO: 100, encoded by SEQ ID NO: 101), a truncated form of the human epidermal growth factor receptor (EGFRt) (amino acid sequence as set forth as SEQ ID NO: 74, encoded by SEQ ID NO: 75), and the like. By way of example, in embodiments a marker protein can be targeted by a clinical stage antibody, where such treatment of a patient with said antibody results in elimination of cells containing an isolated nucleic acid encoding a CAD polypeptide as disclosed herein (Philip B, et al., (2014) Blood, 124(8): 1277-1287; Wang X, et al., (2011) Blood, 118(5): 1255-1263; Smith J, et al., (2015) Meeting Abstract, ASCO Annual Meeting I; 3069; Gouble A, et al., (2014) Blood, 124(21): 4689).

[00170] In embodiments, a linker region, examples of which are described herein, can be used to facilitate translation of the CAD polypeptide and the desired marker. For example, a furin and P2A linker gene may be included in an isolated nucleic acid construct of the present disclosure to facilitate CAD expression and a desired marker. Discussed below with regard to Example 1, a furin and P2A linker gene can be used to express a desired CAD polypeptide along with truncated CD19 that serves as the marker. Also exemplified herein are CAD polypeptides comprising other markers, e.g., EGFRt. Such examples are meant to be illustrative, and non-limiting.

Chimeric Adaptor Expression

[00171] As used herein, an isolated nucleic acid is intended to mean a DNA molecule which can be transformed or introduced into a host cell (e.g., a T cell, NK cell, NKT cell, etc.) and be transcribed and translated to produce a product (e.g., a chimeric adaptor polypeptide as herein described). In the isolated nucleic acids of the present invention, a promoter is operably linked to the nucleic acid sequence encoding the chimeric adaptor polypeptide of the present invention, i.e., they are positioned so as to promote transcription of the messenger RNA from the DNA encoding the chimeric adaptor polypeptide. The term “operatively linked” refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner.

[00172] The promoter can be of genomic origin or synthetically generated. A variety of promoters for use in host cells relevant to the present disclosure are well-known in the art (e.g.,

the CD4 promoter disclosed by Marodon, et al. (2003) *Blood* 101(9):3416-23). The promoter can be constitutive or inducible, where induction is associated with the specific cell type or a specific level of maturation. Alternatively, a number of well-known viral promoters may also be suitable. Promoters of interest include the β -actin promoter, SV40 early and late promoters, immunoglobulin promoter, human cytomegalovirus promoter, retrovirus promoter, and the Friend spleen focus-forming virus promoter. The promoters may or may not be associated with enhancers, wherein the enhancers may be naturally associated with the particular promoter or associated with a different promoter. In embodiments, expression of a chimeric adaptor polypeptide is under the control of an inducible promoter, for example a promoter that is inducible by a molecule present in a tumor microenvironment (e.g., TGF β).

[00173] The sequence of the open reading frame encoding the various segments of the chimeric adaptor polypeptides of the present disclosure can be obtained from a genomic DNA source, a cDNA source, or can be synthesized (e.g., via PCR), or combinations thereof.

[00174] In embodiments, for expression of a chimeric adaptor polypeptide of the present invention, the naturally occurring or endogenous transcriptional initiation region of the nucleic acid sequence encoding N-terminal component of DAP10 can be used to generate the chimeric adaptor polypeptide in the host cell. Alternatively, an exogenous transcriptional initiation region can be used which allows for constitutive or inducible expression, wherein expression can optionally be controlled depending upon the host cell, the level of expression desired, the nature of the host cell, and the like.

[00175] A termination region encoding a C-terminal component of the chimeric adaptor polypeptide can be included. Generally speaking, the source of the termination region is not considered to be critical to the expression of a recombinant protein and a wide variety of termination regions can be employed without adversely affecting expression.

[00176] The isolated nucleic acid, which encodes the chimeric adaptor polypeptide according to this invention can be prepared in conventional ways. Sequences (natural or synthetic) are isolated and manipulated, as appropriate, so as to allow for the proper joining of the various components. Thus, various nucleic acid sequences encoding for the various segments of the chimeric adaptor polypeptide can be isolated, e.g., by employing the polymerase chain reaction (PCR), using appropriate primers. If necessary, specific primers can be designed which result in

deletion of undesired portions of a nucleic acid sequence being used as a template. Additionally or alternatively, restriction digests of cloned genes can be used to generate the isolated nucleic acid constructs of the present disclosure. In either case, the sequences can be selected to provide for restriction sites which are blunt-ended, or have complementary overlaps for facilitating incorporation into various vectors. In examples, modification of a nucleic acid sequence (e.g., to introduce one or more point mutations, insertions or deletions), is performed. The modification can, for example, comprise an amino acid change at position Y86 and/or K84 of SEQ ID NO: 1. Methods for introducing modifications into nucleic acid sequences are known in the art and can include the use of various kits available for purchase (e.g., QuickChange Site Directed Mutagenesis Kit, Agilent, Santa Clara, CA).

[00177] The various manipulations for preparing the isolated nucleic acid encoding a chimeric adaptor polypeptide of the present disclosure can be carried out *in vitro*. In particular embodiments a sequence encoding a chimeric adaptor polypeptide is introduced into vectors for cloning and expression in an appropriate host cell using standard transformation or transfection methods. Thus, after each manipulation, the resulting construct from joining of the DNA sequences is cloned, the vector isolated, and the sequence screened to insure that the sequence encodes the desired chimeric adaptor polypeptide. The sequence can be screened by restriction analysis, sequencing, or the like.

[00178] It is contemplated that the isolated nucleic acid can be introduced into host cells as naked DNA or in a suitable vector. Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods that are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (1989) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors of the disclosure can be reconstituted into liposomes for delivery to target cells.

[00179] Methods of stably transfecting host cells by electroporation using naked DNA are known in the art (see for example U.S. Pat. No. 6,410,319 disclosing T cell transfection). Naked DNA generally refers to the DNA encoding a chimeric adaptor of the present invention

contained in a plasmid expression vector in proper orientation for expression. Advantageously, the use of naked DNA reduces the time required to produce host cells expressing the chimeric DAP10 adaptor polypeptide of the present invention.

[00180] Alternatively, a viral vector (e.g., a retroviral vector, adenoviral vector, adeno-associated viral vector, or lentiviral vector) can be used to introduce the isolated nucleic acid encoding a chimeric adaptor polypeptide of the present invention into host cells. Suitable vectors for use in accordance with the method of the present invention are non-replicating in the T cells. A large number of vectors are known which are based on viruses, where the copy number of the virus maintained in the cell is low enough to maintain the viability of the cell. Illustrative vectors include the pFB-neo vectors (STRATAGENE®) as well as vectors based on HIV, SV40, EBV, HSV or BPV.

[00181] Thus, it may be understood that in some embodiments, the isolated nucleic acid is a circular nucleic acid. In some embodiments, the isolated nucleic acid is a vector, such as a plasmid vector, an adenoviral vector, an adeno-associated viral vector, a viral vector, a retroviral vector, (e.g. a gamma retroviral vector), or a lentiviral vector. In some embodiments, the isolated nucleic acid, or an, e.g., contiguous, portion thereof containing a DAP10 sequence (e.g., at least a portion of SEQ ID NO:1, modified or not), and one or more signaling and/or costimulatory domains is integrated into the genome of a host cell, such as a host $\gamma\delta$ T cell. In an exemplary embodiment, the isolated nucleic acid is retroviral vector.

Host Cells

[00182] Chimeric adaptor polypeptides of the present disclosure may be expressed via their corresponding chimeric nucleic acid constructs in a wide variety of host cells. In embodiments, the host cells are mammalian cells. In embodiments, the CAD polypeptides are expressed in a host cell type that exhibits endogenous expression of a receptor that associates with DAP10. For example, the CAD polypeptide may be expressed in a host cell that expressed NKG2D. In embodiments, the CAD polypeptides are expressed in a host cell type that exhibits some level of endogenous expression of a receptor that associates with DAP10 (e.g., NKG2D). In embodiments, the host cell type can also be engineered to express the same receptor (e.g., NKG2D), for example to increase expression level of the receptor over the naturally-occurring endogenous expression level. In embodiments, the CAD polypeptides are expressed in a cell

type that does not exhibit endogenous expression of a receptor that associates with DAP10, in which case the host cell is engineered to express such a receptor (e.g., expression of NKG2D in a cell type that does not otherwise express NKG2D).

[00183] In embodiments, expression of a CAD polypeptide of the present disclosure in a host cell results in the CAD polypeptide competing with endogenous cellular DAP10 (e.g., WT DAP10). Via the competing, intracellular signaling through a receptor that associates with DAP10 can be redirected through the chimeric DAP10 adaptor polypeptide. To be effective for a desired outcome (e.g., tumor cell killing), the signaling need not be redirected 100% through the CAD polypeptide, although such a percentage is within the scope of this disclosure. Signaling through the CAD polypeptide may comprise anywhere from 20% to 100% of signaling through the DAP10, e.g., between 90-100%, between 80-100%, between 70-100%, between 60-100%, between 50-100%, etc. As a representative example for illustrative purposes, a host cell in which 80% of signaling via a receptor that associates with DAP10 is re-routed through a CAD polypeptide means that just 20% of such signaling remains through the endogenous DAP10, while 80% of signaling is through the CAD polypeptide.

[00184] Host cells, as described herein, can be stored, e.g., cryopreserved, for use in adoptive cell transfer. In embodiments, the host cells are stored prior to engineering the cells to express a chimeric DAP10 adaptor polypeptide. In embodiments, the cells are engineered to express a chimeric DAP10 adaptor polypeptide and then the cells are stored.

[00185] Preferred host cells for use with the chimeric DAP10 adaptor polypeptides of the present disclosure comprise immune cells. Such cells may be obtained from the subject to be treated (i.e. are autologous) or, alternatively, immune cell lines or donor immune cells (allogeneic, syngeneic) can be used. Immune cells can be obtained from a number of sources, including from peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. Immune cells can be obtained from blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. For example, cells from the circulating blood of an individual may be obtained by apheresis. In some embodiments, immune cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by

counterflow centrifugal elutriation. A specific subpopulation of immune cells can be further isolated by positive or negative selection techniques. For example, immune cells can be isolated using a combination of antibodies directed to surface markers unique to the positively selected cells, *e.g.*, by incubation with antibody-conjugated beads for a time period sufficient for positive selection of the desired immune cells. Alternatively, enrichment of immune cell populations can be accomplished by negative selection using a combination of antibodies directed to surface markers unique to the negatively selected cells. Other specific manners of isolation and/or enrichment are disclosed herein.

[00186] In some embodiments, the immune cells comprise any leukocyte involved in defending the body against infectious disease and foreign materials. For example, the immune cells can comprise lymphocytes, monocytes, macrophages, dendritic cells, mast cells, neutrophils, basophils, eosinophils, or any combinations thereof. For example, immune cells relevant to the present disclosure can include but are not limited to $\alpha\beta$ T cells, $\gamma\delta$ T cells, NK cells, NKT cells, $\gamma\delta$ NKT cells, B cells, innate lymphoid cells (ILCs), cytokine induced killer (CIK) cells, cytotoxic T lymphocytes (CTLs), lymphokine activated killer (LAK) cells, regulatory T cells, and the like. In embodiments, preferred immune cells comprise $\alpha\beta$ T cells, $\gamma\delta$ T cells, NK cells, NKT cells, $\gamma\delta$ NKT cells, and, in some examples, macrophages. In embodiments, the immune cells relevant to the present disclosure comprise allogeneic cells, autologous cells, or syngeneic cells.

[00187] Aspects of the disclosure include immune cells having *in vitro* or *in vivo* cytotoxic activity against a hematological or solid tumor cell that exhibits cell surface expression of a tumor associated antigen (TAA), virally infected cells displaying a virally-derived antigen, bacterial cells, etc. In embodiments, the cytotoxic activity is innate activity. In embodiments, the immune cells that functionally express a CAD polypeptide exhibit cell killing activity greater than the level of *in vitro* and/or *in vivo* cell killing activity in a control immune cell that does not comprise a CAD polypeptide of the present disclosure.

[00188] In embodiments, cytotoxicity is significantly (> about 25%) enhanced or improved by the presence of a CAD polypeptide, as compared to cytotoxicity in absence of the CAD polypeptide. In some cases, the cytotoxicity is at least in part, significantly (> about 25%), or entirely, due to the presence of the CAD polypeptide.

[00189] In embodiments, engineered immune cells relevant to the present disclosure can exhibit robust and/or persistent cell killing activity (e.g., tumor cell, virally-infected cell) through direct and/or indirect mechanisms. In some cases, the cell killing activity persists for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a target cell. In some cases, the cell killing activity of an immune cell disclosed herein, engineered to express a CAD polypeptide, or a progeny thereof, persists for at least about 6 days to 120 days, or for at least about 6 days to 180 days, from first contact with a target cell, or from administration of the engineered immune cells disclosed herein. This persistent cell killing activity may be exhibited *in vitro*, *in vivo*, or both *in vitro* and *in vivo*.

[00190] Aspects of the disclosure, in embodiments, include immune cells that proliferate in response to contact with cells that exhibit cell surface expression of a ligand that is recognized by a receptor that associates with DAP10, and specifically, associates with CAD polypeptides of the present disclosure. One example of such a receptor is NKG2D, although the present disclosure is not limited to CAD polypeptides interacting with NKG2D, and can include other receptor(s) that also associate with DAP10. In embodiments, the proliferation is at least in part, significantly (> about 20% or > about 25%, or > about 50%, or > about 80%), or entirely (e.g., 100%), due to the presence of a CAD polypeptide construct that associates with a receptor (e.g., NKG2D) expressed on the host cell. In some cases, the immune cells exhibit a greater level of *in vitro* and/or *in vivo* proliferation as compared to a control immune cell (e.g., same type of immune cell) that does not comprise a CAD polypeptide. The greater level of proliferation may comprise a 20-50% increase, a 50-80% increase, an 80-100% increase, or even a 2-fold increase, 3-fold increase, 4-fold increase, 5-fold increase, 5-10-fold increase, 10-20-fold increase, or even greater than a 20-fold increase, such as e.g., a 50-100-fold increase or more as compared to a control immune cell that lacks a CAD polypeptide as herein described.

[00191] In some embodiments, the immune cells engineered to comprise a CAD polypeptide described herein express and secrete, or persistently express and secrete, one or more pro-inflammatory cytokines, for example after contact with a cell that expresses a ligand that is recognized by a cell surface receptor on the immune cell that associates with DAP10 (e.g., NKG2D). In embodiments, the expression and secretion associated with an immune cell engineered to express a CAD polypeptide is at least in part, significantly (> about 20%, or > about 25%, or > about 50%, or > about 80%), or entirely due to the CAD polypeptide. In

embodiments, the expression and/or secretion is greater by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or even greater than 100%, for example 2-fold higher, 3-fold higher, 4-fold higher, 5-fold higher, 5-10-fold higher, 10-20-fold higher, or even greater than 20-fold higher, such as e.g., 50-100-fold higher or more as compared to expression and/or secretion otherwise observed in control immune cells (e.g., same type of immune cells) lacking expression of a CAD polypeptide.

[00192] In embodiments, engineered immune cells of the present disclosure may function to alter a cellular microenvironment (e.g., TME) in favor of, for example, an anti-tumor response. For example, solid tumors can recruit inhibitory cells such as myeloid-derived suppressor cells (MDSCs), which can strengthen a suppressive TME. Frequency of circulating or intratumoral MDSCs correlates with cancer stage, disease progression, and resistance to standard chemotherapy and radiotherapy. Certain ligands (e.g., NKG2D ligands) are expressed at high levels on several solid tumors and on tumor-infiltrating MDSCs, hence engineered immune cells of the present disclosure may in embodiments be used to alter a TME in favor of an anti-tumor response by reducing or eliminating suppressive molecules of the TME, such as TGF- β . In embodiments, engineered immune cells of the present disclosure are cytotoxic against MDSCs, but spare (i.e., are non-toxic to) NKG2D ligand-expressing normal tissues (see *e.g.*, Parihar, R., et al., (2019) *Cancer Immunol Res* 7(3): 363-375). In some embodiments, cell killing activity pertaining to killing of *e.g.*, MDSCs, by engineered host cells of the present disclosure decreases a suppressive effect of a TME. For example, the suppressive effect may be decreased by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more, as compared to a suppressive effect of a TME in absence of host cells engineered to express a CAD polypeptide as herein described.

[00193] In embodiments, engineered immune cells of the present disclosure may function to decrease growth and/or proliferation of a target cell. For example, an immune cell engineered to express a CAD polypeptide as herein described may decrease growth and/or proliferation of a target cell by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or even about 100%, or any percentage there between, as compared to growth and/or proliferation of the target cell in absence of the engineered immune cell. In embodiments, the target cell expresses a cell-surface ligand that is recognized by a receptor (e.g., NKG2D) on the

cell surface of the engineered immune cell, where said receptor associates, at least in part, with the CAD polypeptide.

[00194] Hence, in embodiments a method of making a cell comprising a CAD polypeptide are provided comprising introducing into the cell an isolated nucleic acid that encodes the CAD polypeptide, such that the cell expresses the CAD polypeptide. In embodiments, the cell is an immune cell as herein described, for example an NK cell, an NKT cell, a $\gamma\delta$ T cell, an $\alpha\beta$ T cell, or an $\gamma\delta$ NKT cell. In some embodiments, the method of making the cell further comprises introducing into the cell another isolated nucleic acid that encodes for a receptor capable of associating with the CAD polypeptide.

Methods of Use

[00195] In one aspect, the present disclosure provides a method of modulating a signal transduced through a receptor of a host cell, the host cell comprising an immune cell engineered to express a CAD polypeptide as described herein that associates with the receptor, and preferably wherein the immune cell is cytotoxic. In embodiments, the receptor is endogenous to the immune cell, and is endogenously expressed therein. In additional or alternative embodiments, the receptor is expressed by way of introducing into the immune cell an isolated nucleic acid encoding the receptor.

[00196] In embodiments, the method of modulating the signal transduced through the receptor results in stimulation of the immune cell and/or activation of the immune cell.

[00197] In embodiments, the method of modulating the signal transduced through the receptor results in an increased level of proliferation of the immune cell as compared to a level of proliferation of a control immune cell lacking the CAD polypeptide.

[00198] In embodiments, the method of modulating the signal transduced through the receptor results in increased expression and secretion of one or more cytokines, as compared to a level of expression and secretion of said one or more cytokines in a control immune cell lacking the CAD polypeptide.

[00199] In embodiments, modulating the signal transduced through the receptor comprises routing at least a portion of the signal through the CAD polypeptide, as opposed to endogenous

DAP10. In embodiments, the portion of the signal routed through the CAD polypeptide is 80% or higher, for example 90-95% or higher, for example 99% or 100%.

Methods of Treatment

[00200] Pharmaceutical compositions comprising engineered host cells that express a CAD polypeptide, and/or admixtures thereof, as described herein may be administered for prophylactic and/or therapeutic treatments. An admixture may comprise different types of host cells engineered to express a same or a different CAD polypeptide as herein described. For example, and without limitation, an admixture may comprise a population of NK cells expressing a first CAD polypeptide, and a population of $\gamma\delta$ cells engineered to express a second CAD polypeptide. As another example and without limitation, an admixture may comprise a population of NK cells and a population of $\gamma\delta$ cells each engineered to express a same CAD polypeptide. As yet another example, an admixture may comprise a population of engineered host cells, and may additionally comprise a non-engineered cell population. For example, and without limitation, an admixture may comprise a population of NK cells engineered to express a CAD polypeptide as herein described, and another non-engineered population of cells e.g., NK cells, NKT cells, $\gamma\delta$ cells, $\alpha\beta$ cells, etc. In therapeutic applications, the compositions can be administered to a subject already suffering from a disease or condition in an amount sufficient to decrease at least one sign or symptom associated with the disease or condition. In some embodiments, the amount is sufficient to cure the disease or condition.

[00201] An engineered host cell population and/or admixtures thereof can also be administered to lessen a likelihood of developing, contracting, or worsening a condition. Effective amount of a population of engineered host cells, non-engineered host cell, and/or admixtures thereof, for therapeutic use can vary based on the severity and course of the disease or condition, previous therapy, the subject's health status, weight, and/or response to various drugs, and/or the judgement of a treating physician.

[00202] In embodiments, the one or multiple engineered host cell populations, non-engineered cells and/or admixtures thereof, of the present disclosure can be used to treat a subject in need of treatment for a condition. Examples of such conditions include but are not limited to cancer, infectious disease, and autoimmune disorder. Subject can be humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep,

goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. A subject can be of any age. Subjects can be, for example, elderly adults, adults, adolescents, pre-adolescents, children, toddlers, infants.

[00203] A method of treating a condition (e.g., ailment) in a subject may comprise administering to the subject a therapeutically-effective amount of one or more engineered host cell populations (e.g., to express a CAD polypeptide), non-engineered cells and/or admixtures thereof. The one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof can be administered at various regimens (e.g., timing, concentration, dosage, spacing between treatment, and/or formulation). A subject can also be preconditioned with, for example, chemotherapy, radiation, or a combination of both, prior to receiving the therapeutically-effective amount of one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof. As part of a treatment, one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof may be administered to a subject at a first regimen and the subject may be monitored to determine whether the treatment at the first regimen meets a given level of therapeutic efficacy. In some cases, the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof may be administered to the subject at a second regimen, based on information gleaned from providing the subject with the first regimen.

[00204] In embodiments, a pharmaceutical composition comprising at least one host cell engineered to express a CAD polypeptide may be administered in a first regimen. The subject may be monitored, for example by a healthcare provider (e.g., treating physician or nurse). In some examples, the subject is monitored to determine or gauge an efficacy of the engineered host cell in treating the condition of the subject. In some situations, the subject may also be monitored to determine the *in vivo* expansion of an engineered host cell population in the subject. Another pharmaceutical composition comprising at least one host cell engineered to express a CAD polypeptide may be administered to the subject in a second regimen. The pharmaceutical composition administered in the second regimen may comprise a same type of host cell expressing a same CAD polypeptide as that administered to the subject in the first regimen. However, it is within the scope of this disclosure that the pharmaceutical composition administered in the second regimen may comprise a different type of host cell, optionally

expressing a different CAD polypeptide (e.g., a CAD polypeptide with different mutations and/or costimulatory or signaling domains). In some examples, the second regimen is not performed, for example if the first regimen is found to be effective (e.g., a single round of administration may be sufficient to treat the condition). In some embodiments, a population of engineered host cells can be administered to various subjects (e.g., where the host cell has universal donor characteristics).

[00205] A therapeutically-effective amount of one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof may be used to treat various conditions. In some cases, a therapeutically-effective amount of one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof may be used to treat cancer, including solid tumors and hematologic malignancies. In some cases, a therapeutically-effective amount of one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof may be used to treat an infectious disease caused, for example, by a pathogenic bacterium or by a virus.

[00206] Treatment with one or multiple engineered host cell populations (expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure may be provided to the subject before, during, and after the clinical onset of the condition. Treatment may be provided to the subject after 1 day, 1 week, 6 months, 12 months, or 2 years or more after clinical onset of the disease. Treatment may be provided to the subject for more than 1 day, 1 week, 1 month, 6 months, 12 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more after clinical onset of disease. Treatment may be provided to the subject for less than 1 day, 1 week, 1 month, 6 months, 12 months, or 2 years after clinical onset of the disease. Treatment may also include treating a human in a clinical trial. A treatment can comprise administering to a subject a pharmaceutical composition comprising one or multiple engineered host cell populations (e.g. expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure.

[00207] In some cases, administration of the one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure, modulates the activity of endogenous lymphocytes in a subject's body. In some

cases, administration of the one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure, results in activation of cytotoxicity of another immune cell. In some cases, the other immune cell is a CD8⁺ T-cell. In some cases, the other immune cell is a Natural Killer T-cell. Other examples of other immune cells are encompassed by the present disclosure. In some cases, administration of the one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure, suppresses a regulatory T-cell. In some cases, the regulatory T-cell is a Fox3⁺ Treg cell. In some cases, the regulatory T-cell is a Fox3⁻ Treg cell. Non-limiting examples of cells whose activity can be modulated by administration of the one or multiple engineered host cell populations (e.g., expressing a CAD polypeptide), non-engineered cells and/or admixtures thereof, of the disclosure, include hematopoietic stem cells; B cells; CD4⁺ cells; CD8⁺ cells; red blood cells; white blood cells; dendritic cells, including dendritic antigen presenting cells; leukocytes; macrophages; memory B cells; memory T-cells; monocytes; natural killer cells; neutrophil granulocytes; T-helper cells; and T-killer cells.

[00208] One or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, having cytotoxic activity against, for example and without limitation, a hematological or solid tumor cell, or a virally-infected cell, or a bacterial cell, can be administered to a subject in any order or simultaneously. If simultaneously, the engineered host cell(s), and/or admixtures thereof, of the disclosure can be provided in a single, unified form, such as an intravenous injection, or in multiple forms, for example, as multiple intravenous infusions, s.c. injections or pills. The one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof of the disclosure can be packed together or separately, in a single package or in a plurality of packages. One or all of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof of the disclosure can be given in multiple doses. If not simultaneous, the timing between the multiple doses may vary to as much as about a week, a month, two months, three months, four months, five months, six months, or about a year. In some cases, an engineered host cell of the disclosure can proliferate within a subject's body, *in vivo*, after administration to a subject. The one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof of the present disclosure can be frozen to provide cells for multiple treatments with the same cell

preparation. The one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, and pharmaceutical compositions comprising the same, can be packaged as a kit. A kit may include instructions (e.g., written instructions) on the use of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, and compositions comprising the same.

[00209] In some cases, a method of treating a subject in need thereof comprises administering to the subject a therapeutically-effective amount of one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the disclosure, wherein the administration treats a particular condition (e.g., cancer, viral or bacterial infection, autoinflammatory disease). In some embodiments the therapeutically-effective amount of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, is administered for at least about 10 seconds, 30 seconds, 1 minute, 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or 1 year. In some embodiments the therapeutically-effective amount of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, is administered for at least one week. In some embodiments the therapeutically-effective amount of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the disclosure, is administered for at least two weeks.

[00210] The one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, described herein can be administered before, during, or after the occurrence of a disease or condition, and the timing of administering a pharmaceutical composition containing the engineered host cell population can vary. For example, the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof can be used as a prophylactic and can be administered continuously to subjects with a propensity to conditions or diseases in order to lessen a likelihood of the occurrence of the disease or condition. The initial administration can be via any route practical, such as by any route described herein using any formulation described herein. In some examples, the administration of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof of the disclosure is an intravenous administration. One or multiple dosages of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof can be

administered as soon as is practicable after the onset of a particular condition (e.g., hematological or solid cancer, viral infection, bacterial infection, autoimmune disorder, etc.) and for a length of time necessary for the treatment of the disease/condition, such as, for example, from about 24 hours to about 48 hours, from about 48 hours to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, from about 1 month to about 3 months. In some embodiments, one or multiple dosages of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof can be administered years after onset of the disease/condition (e.g., cancer) and before or after other treatments.

[00211] In some embodiments, the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the disclosure, is administered simultaneously or sequentially with one or more methods to elevate common gamma chain cytokine(s). As used herein, "one or more methods to elevate common gamma chain cytokine(s): refers to a method, or combination of methods, that alters the physiological state of a subject, such that at least one common gamma chain cytokine level is elevated in the subject. In some embodiments, the method elevates the level of one or more common gamma chain cytokine(s) selected from the group consisting of IL-2, IL-4, IL-7, and IL-15 in the subject. In some embodiments, the method comprises lymphodepletion. In some embodiments, the method comprises administering one or more common gamma chain cytokine(s) to the subject. In some cases, IL-2, IL-4, IL-7, and/or IL-15, are administered. In some embodiments, the method comprises secreting common gamma chain cytokine(s) from an administered engineered host cell. In some cases, IL-2, IL-4, IL-7, and/or IL-15 are secreted.

[00212] In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before introducing the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the disclosure. In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises administering simultaneously with introducing the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, or sequentially an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof. The amount of administered common gamma chain cytokine(s) can be an amount effective to increase

proliferation, cytotoxic activity, persistence, or the combination thereof of the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof. Exemplary amounts of IL-15 include, without limitation between 0.01 — 10 µg/kg/dose every 24 hours for IL-15. Exemplary amounts of IL-2 include, without limitation, between about 3×10^6 and about 22×10^6 units every 8 - 48 hours. For example, the dosing regimen for IL2 in RCC is 600,000 International Units/kg (0.037 mg/kg) IV 48hr infused over 15 minutes for a maximum 14 doses.

[00213] In some embodiments, the administering one or more methods to elevate common gamma chain cytokine(s) comprises lymphodepletion before administering the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof and administering simultaneously with introducing the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, or sequentially, an amount of common gamma chain cytokine(s) effective to increase proliferation, cytotoxic activity, persistence, or the combination thereof of the introduced one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof.

[00214] In some embodiments, elevating common gamma chain cytokine(s) is accomplished, at least in part, via the engineered host cell(s), where the common gamma chain cytokine(s) are expressed from a CAD construct as disclosed herein. In such an example, it is within the scope of this disclosure that one or more additional gamma chain cytokine(s) are additionally administered in a manner to elevate said additional gamma chain cytokine(s).

Dosages

[00215] One or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the present disclosure, may be formulated in unit dosage forms suitable for single administration of precise dosages. In some cases, the unit dosage forms comprise additional lymphocytes. In unit dosage form, the formulation is divided into unit doses containing appropriate quantities of one or more compounds. The unit dosage can be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are packaged tablets or capsules, and powders in vials or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Multiple-dose reclosable containers can be used, for example, in combination with a preservative or without a

preservative. In some examples, the pharmaceutical composition does not comprise a preservative. Formulations for parenteral injection can be presented in unit dosage form, for example, in ampoules, or in multi-dose containers with a preservative.

[00216] One or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the present disclosure, may be present in a composition in an amount of at least 5 cells, at least 10 cells, at least 20 cells, at least 30 cells, at least 40 cells, at least 50 cells, at least 60 cells, at least 70 cells, at least 80 cells, at least 90 cells, at least 100 cells, at least 200 cells, at least 300 cells, at least 400 cells, at least 500 cells, at least 600 cells, at least 700 cells, at least 800 cells, at least 900 cells, at least 1×10^3 cells, at least 2×10^3 cells, at least 3×10^3 cells, at least 4×10^3 cells, at least 5×10^3 cells, at least 6×10^3 cells, at least 7×10^3 cells, at least 8×10^3 cells, at least 9×10^3 cells, at least 1×10^4 cells, at least 2×10^4 cells, at least 3×10^4 cells, at least 4×10^4 cells, at least 5×10^4 cells, at least 6×10^4 cells, at least 7×10^4 cells, at least 8×10^4 cells, at least 9×10^4 cells, at least 1×10^5 cells, at least 2×10^5 cells, at least 3×10^5 cells, at least 4×10^5 cells, at least 5×10^5 cells, at least 6×10^5 cells, at least 7×10^5 cells, at least 8×10^5 cells, at least 9×10^5 cells, at least 1×10^6 cells, at least 2×10^6 cells, at least 3×10^6 cells, at least 4×10^6 cells, at least 5×10^6 cells, at least 6×10^6 cells, at least 7×10^6 cells, at least 8×10^6 cells, at least 9×10^6 cells, at least 1×10^7 cells, at least 2×10^7 cells, at least 3×10^7 cells, at least 4×10^7 cells, at least 5×10^7 cells, at least 6×10^7 cells, at least 7×10^7 cells, at least 8×10^7 cells, at least 9×10^7 cells, at least 1×10^8 cells, at least 2×10^8 cells, at least 3×10^8 cells, at least 4×10^8 cells, at least 5×10^8 cells, at least 6×10^8 cells, at least 7×10^8 cells, at least 8×10^8 cells, at least 9×10^8 cells, at least 1×10^9 cells, or more.

[00217] The therapeutically effective dose of one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the invention can be from about 1 cell to about 10 cells, from about 1 cell to about 100 cells, from about 1 cell to about 10 cells, from about 1 cell to about 20 cells, from about 1 cell to about 30 cells, from about 1 cell to about 40 cells, from about 1 cell to about 50 cells, from about 1 cell to about 60 cells, from about 1 cell to about 70 cells, from about 1 cell to about 80 cells, from about 1 cell to about 90 cells, from about 1 cell to about 100 cells, from about 1 cell to about 1×10^3 cells, from about 1 cell to about 2×10^3 cells, from about 1 cell to about 3×10^3 cells, from about 1 cell to about 4×10^3 cells, from about 1 cell to about 5×10^3 cells, from about 1 cell to about 6×10^3 cells, from about 1 cell to about 7×10^3 cells, from about 1 cell to about 8×10^3 cells, from about 1 cell to about $9 \times$

10^3 cells, from about 1 cell to about 1×10^4 cells, from about 1 cell to about 2×10^4 cells, from about 1 cell to about 3×10^4 cells, from about 1 cell to about 4×10^4 cells, from about 1 cell to about 5×10^4 cells, from about 1 cell to about 6×10^4 cells, from about 1 cell to about 7×10^4 cells, from about 1 cell to about 8×10^4 cells, from about 1 cell to about 9×10^4 cells, from about 1 cell to about 1×10^5 cells, from about 1 cell to about 2×10^5 cells, from about 1 cell to about 3×10^5 cells, from about 1 cell to about 4×10^5 cells, from about 1 cell to about 5×10^5 cells, from about 1 cell to about 6×10^5 cells, from about 1 cell to about 7×10^5 cells, from about 1 cell to about 8×10^5 cells, from about 1 cell to about 9×10^5 cells, from about 1 cell to about 1×10^6 cells, from about 1 cell to about 2×10^6 cells, from about 1 cell to about 3×10^6 cells, from about 1 cell to about 4×10^6 cells, from about 1 cell to about 5×10^6 cells, from about 1 cell to about 6×10^6 cells, from about 1 cell to about 7×10^6 cells, from about 1 cell to about 8×10^6 cells, from about 1 cell to about 9×10^6 cells, from about 1 cell to about 1×10^7 cells, from about 1 cell to about 2×10^7 cells, from about 1 cell to about 3×10^7 cells, from about 1 cell to about 4×10^7 cells, from about 1 cell to about 5×10^7 cells, from about 1 cell to about 6×10^7 cells, from about 1 cell to about 7×10^7 cells, from about 1 cell to about 8×10^7 cells, from about 1 cell to about 9×10^7 cells, from about 1 cell to about 1×10^8 cells, from about 1 cell to about 2×10^8 cells, from about 1 cell to about 3×10^8 cells, from about 1 cell to about 4×10^8 cells, from about 1 cell to about 5×10^8 cells, from about 1 cell to about 6×10^8 cells, from about 1 cell to about 7×10^8 cells, from about 1 cell to about 8×10^8 cells, from about 1 cell to about 9×10^8 cells, or from about 1 cell to about 1×10^9 cells.

[00218] In some cases, the therapeutically effective dose of therapeutically effective dose of one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the invention can be from about 1×10^3 cells to about 2×10^3 cells, from about 1×10^3 cells to about 3×10^3 cells, from about 1×10^3 cells to about 4×10^3 cells, from about 1×10^3 cells to about 5×10^3 cells, from about 1×10^3 cells to about 6×10^3 cells, from about 1×10^3 cells to about 7×10^3 cells, from about 1×10^3 cells to about 8×10^3 cells, from about 1×10^3 cells to about 9×10^3 cells, from about 1×10^3 cells to about 1×10^4 cells, from about 1×10^3 cells to about 2×10^4 cells, from about 1×10^3 cells to about 3×10^4 cells, from about 1×10^3 cells to about 4×10^4 cells, from about 1×10^3 cells to about 5×10^4 cells, from about 1×10^3 cells to about 6×10^4 cells, from about 1×10^3 cells to about 7×10^4 cells, from about 1×10^3 cells to about 8×10^4 cells, from about 1×10^3 cells to about 9×10^4 cells, from about 1×10^3 cells to

about 1×10^5 cells, from about 1×10^3 cells to about 2×10^5 cells, from about 1×10^3 cells to about 3×10^5 cells, from about 1×10^3 cells to about 4×10^5 cells, from about 1×10^3 cells to about 5×10^5 cells, from about 1×10^3 cells to about 6×10^5 cells, from about 1×10^3 cells to about 7×10^5 cells, from about 1×10^3 cells to about 8×10^5 cells, from about 1×10^3 cells to about 9×10^5 cells, from about 1×10^3 cells to about 1×10^6 cells, from about 1×10^3 cells to about 2×10^6 cells, from about 1×10^3 cells to about 3×10^6 cells, from about 1×10^3 cells to about 4×10^6 cells, from about 1×10^3 cells to about 5×10^6 cells, from about 1×10^3 cells to about 6×10^6 cells, from about 1×10^3 cells to about 7×10^6 cells, from about 1×10^3 cells to about 8×10^6 cells, from about 1×10^3 cells to about 9×10^6 cells, from about 1×10^3 cells to about 1×10^7 cells, from about 1×10^3 cells to about 2×10^7 cells, from about 1×10^3 cells to about 3×10^7 cells, from about 1×10^3 cells to about 4×10^7 cells, from about 1×10^3 cells to about 5×10^7 cells, from about 1×10^3 cells to about 6×10^7 cells, from about 1×10^3 cells to about 7×10^7 cells, from about 1×10^3 cells to about 8×10^7 cells, from about 1×10^3 cells to about 9×10^7 cells, from about 1×10^3 cells to about 1×10^8 cells, from about 1×10^3 cells to about 2×10^8 cells, from about 1×10^3 cells to about 3×10^8 cells, from about 1×10^3 cells to about 4×10^8 cells, from about 1×10^3 cells to about 5×10^8 cells, from about 1×10^3 cells to about 6×10^8 cells, from about 1×10^3 cells to about 7×10^8 cells, from about 1×10^3 cells to about 8×10^8 cells, from about 1×10^3 cells to about 9×10^8 cells, or from about 1×10^3 cells to about 1×10^9 cells.

[00219] In some cases, the therapeutically effective dose of therapeutically effective dose of one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the invention can be from about 1×10^6 cells to about 2×10^6 cells, from about 1×10^6 cells to about 3×10^6 cells, from about 1×10^6 cells to about 4×10^6 cells, from about 1×10^6 cells to about 5×10^6 cells, from about 1×10^6 cells to about 6×10^6 cells, from about 1×10^6 cells to about 7×10^6 cells, from about 1×10^6 cells to about 8×10^6 cells, from about 1×10^6 cells to about 9×10^6 cells, from about 1×10^6 cells to about 1×10^7 cells, from about 1×10^6 cells to about 2×10^7 cells, from about 1×10^6 cells to about 3×10^7 cells, from about 1×10^6 cells to about 4×10^7 cells, from about 1×10^6 cells to about 5×10^7 cells, from about 1×10^6 cells to about 6×10^7 cells, from about 1×10^6 cells to about 7×10^7 cells, from about 1×10^6 cells to about 8×10^7 cells, from about 1×10^6 cells to about 9×10^7 cells, from about 1×10^6 cells to about 1×10^8 cells, from about 1×10^6 cells to about 2×10^8 cells, from about $1 \times$

10^6 cells to about 3×10^8 cells, from about 1×10^6 cells to about 4×10^8 cells, from about 1×10^6 cells to about 5×10^8 cells, from about 1×10^6 cells to about 6×10^8 cells, from about 1×10^6 cells to about 7×10^8 cells, from about 1×10^6 cells to about 8×10^8 cells, from about 1×10^6 cells to about 9×10^8 cells, from about 1×10^6 cells to about 1×10^9 cells, from about 1×10^6 cells to about 2×10^9 cells, from about 1×10^6 cells to about 3×10^9 cells, from about 1×10^6 cells to about 4×10^9 cells, from about 1×10^6 cells to about 5×10^9 cells, from about 1×10^6 cells to about 6×10^9 cells, from about 1×10^6 cells to about 7×10^9 cells, from about 1×10^6 cells to about 8×10^9 cells, from about 1×10^6 cells to about 9×10^9 cells, from about 1×10^7 cells to about 1×10^9 cells, from about 1×10^7 cells to about 2×10^9 cells, from about 1×10^7 cells to about 3×10^9 cells, from about 1×10^7 cells to about 4×10^9 cells, from about 1×10^7 cells to about 5×10^9 cells, from about 1×10^7 cells to about 6×10^9 cells, from about 1×10^7 cells to about 7×10^9 cells, from about 1×10^7 cells to about 8×10^9 cells, from about 1×10^7 cells to about 9×10^9 cells, from about 1×10^8 cells to about 1×10^9 cells, from about 1×10^8 cells to about 2×10^9 cells, from about 1×10^8 cells to about 3×10^9 cells, from about 1×10^8 cells to about 4×10^9 cells, from about 1×10^8 cells to about 5×10^9 cells, from about 1×10^8 cells to about 6×10^9 cells, from about 1×10^8 cells to about 7×10^9 cells, from about 1×10^8 cells to about 8×10^9 cells, from about 1×10^8 cells to about 9×10^9 cells, or from about 1×10^8 cells to about 1×10^{10} cells.

Preservation

[00220] In some embodiments, the one or multiple engineered host cell populations, non-engineered cells, and/or admixtures thereof, of the invention may be formulated in freezing media and placed in cryogenic storage units such as liquid nitrogen freezers (-195°C) or ultra-low temperature freezers (-65°C , -80°C or -120°C) for long-term storage of at least about 1 month, 2 months, 3 months, 4 months, 5 months, δ months, 1 year, 2 years, 3 years, or at least 5 years. The freeze media can contain dimethyl sulfoxide (DMSO), and/or sodium chloride (NaCl), and/or dextrose, and/or dextran sulfate and/or hydroxyethyl starch (HES) with physiological pH buffering agents to maintain pH between about 6.0 to about 6.5, about 6.5 to about 7.0, about 7.0 to about 7.5, about 7.5 to about 8.0 or about 6.5 to about 7.5. In embodiments, the cryopreserved cells can be thawed and further processed, for example by stimulation with antibodies, proteins, peptides, and/or cytokines as mentioned herein. The cryopreserved cells can be thawed and genetically modified with viral vectors (including retroviral and lentiviral vectors) or non-viral

means (including RNA, DNA, and proteins) as described herein. Alternatively, host cells as described herein can be, e.g., optionally expanded by the methods described herein, genetically modified, and then cryopreserved.

[00221] Thus, genetically engineered and/or non-engineered cells as disclosed herein can be cryopreserved to generate cell banks in quantities of at least about 1, 5, 10, 100, 150, 200, 500 vials at about at least 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or at least about 10^{10} cells per mL in freeze media. The cryopreserved cell banks may retain their functionality and can be thawed and, optionally, be activated/stimulated and/or expanded. In some aspects, thawed cells can be stimulated and expanded in suitable closed vessels such as cell culture bags and/or bioreactors to generate quantities of cells as allogeneic cell product. In other examples, the cryopreserved cells comprise an autologous cell product. Cryopreserved cells can maintain their biological functions for at least about 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 15 months, 18 months, 20 months, 24 months, 30 months, 36 months, 40 months, 50 months, or at least about 60 months under cryogenic storage condition. In some aspects, no preservatives are used in the formulation. In some embodiments, the cryopreserved cells can be thawed and infused into multiple patients as allogeneic off-the-shelf cell product.

EXAMPLES

[00222] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Construction of DAP10 constructs

[00223] DAP10 CAD constructs were constructed with 0-4 elements selected from Y86F mutation, K84R mutation, 4-1BB costimulatory domain, CD3 ζ signaling domain. For construction, the pSIN vector (Hariharan, MJ et al., (1998) Journal of Virology 72(2): 950-958) was used. Briefly, the pSIN vector backbone was fully synthesized by Genewiz® (South Plainfield, NJ) from sequence provided by EUFETS (Germany) a subsidiary of BioNTech

(Germany). The base plasmid used for all constructs is referred to as pL077 pRetroSIN-GFP, which has a green fluorescent protein (GFP) cassette that is replaced by a gene-of-interest. The various constructs are depicted in Table 1 below. With regard to the following constructs, FP2A refers to a furin and P2A linker gene, and CD19t refers to a truncated CD19 marker. SEQ ID corresponds to amino acid sequence. Tables 3-15 below annotate the nucleic acid sequences that encode the amino acid sequences for the constructs depicted at Table 1, and select sequences from Table 2. Although Table 1 depicts DAP10 constructs including CD19t, other variations are within the scope of this disclosure, for example similar DAP10 constructs incorporating EGFRt, and the like. Examples of such sequences are provided in Table 2.

Table 1: DAP10 CAD Constructs					
Long Name	Short Name	Signal Null? (Y86F)	Ubiquitin mutant (K84R)	4-1BB	CD3 ζ
pSIN- DAP10.0- FP2A-CD19t	DAP10.0 (wt) (SEQ ID NO: 21)	No	No	No	No
pSIN- DAP10.3- F2PA-CD19t	DAP10.3 (SEQ ID NO: 23)	No	No	No	Yes
pSIN- DAP10.4- FP2A-CD19t	DAP10.4 (SEQ ID NO: 25)	No	No	Yes	Yes
pSIN- DAP10.5- FP2A-CD19t	DAP10.5 (SEQ ID NO: 27)	Yes	No	Yes	Yes
pSIN- DAP10.6-	DAP10.6	Yes	Yes	Yes	Yes

FP2A-CD19t	(SEQ ID NO: 29)				
pSIN- DAP10.13- FP2A-CD19t	DAP10.13 (SEQ ID NO: 31)	No	Yes	No	No
pSIN- DAP10.14- FP2A-CD19t	DAP10.14 (SEQ ID NO: 33)	No	Yes	No	Yes
pSIN- DAP10.15- FP2A-CD19t	DAP10.15 (SEQ ID NO: 35)	No	Yes	Yes	Yes
pSIN- DAP10.16- FP2A-CD19t	DAP10.16 (SEQ ID NO: 79)	Yes	Yes	Yes	Yes (1XX modification)
pSIN- DAP10.17- FP2A-CD19t	DAP10.17 (SEQ ID NO: 87)	Yes	Yes	No	Yes (1XX modification)

Example 2. Tumor Control of Various DAP10 CAD Constructs in PLC/PRF/5 Assay

[00224] Various DAP10 CAD constructs from Table 1 above were tested in a PLC/PRF/5 cell lysis assay. FIG. 2A illustrates that DAP10.0 (DAP10 wild-type) and DAP10.13 (DAP10-K84R) do not display good tumor control in the PLC/PRF/5 assay. FIG. 2B illustrates some amount of tumor control for the DAP10.3 (DAP10-CD3 ζ) and DAP10.14 (DAP10-CD3 ζ -K84R) constructs. FIG. 2C illustrates that DAP10 CAD constructs that include both the 4-1BB costimulatory domain and the CD3 ζ signaling domain show good tumor control in absence of

additional mutation (i.e., DAP10.4), and that addition of just the Y86F mutation (i.e., DAP10.5) or just the K84R mutation (i.e., DAP10.15) does not substantially further improve tumor control over the DAP10.4 construct. However, tumor control was substantially improved in the DAP10 CAD construct DAP10.6 that incorporated each of the CD3 ζ signaling domain, the 4-1BB costimulatory domain, and both mutations (i.e., Y86F and K84R) (FIG. 2C). At each of FIGS. 2A-2C, also depicted is tumor control tested with a positive control CAR, as well as a plot showing cytotoxicity index of the tumor cells alone.

[00225] V δ 1 $\gamma\delta$ cell proliferation was also examined for the test conditions corresponding to FIGS. 2A-2C, and the results are shown at FIGS. 2D-2F, respectively. Good proliferation was observed from most DAP10 CAD constructs, as shown.

Example 3. Tumor Control of Various DAP10 CAD Constructs in HepG2 Assay

[00226] Various DAP10 CAD constructs from Table 1 above were tested in a HepG2 assay. FIG. 3A illustrates data obtained using the DAP10.0 (DAP10 wild-type) and DAP10.13 (DAP10-K84R) constructs, FIG. 3B illustrates data obtained using the DAP10.3 (DAP10-CD3 ζ) and DAP10.14 (DAP10-CD3 ζ -K84R) constructs, and FIG. 3C illustrates data obtained using the DAP10.4, DAP10.5, DAP10.6, and DAP10.15 constructs. At each of FIGS. 3A-3C, also depicted is tumor control tested with a positive control CAR, as well as a plot showing cytotoxicity index of the tumor cells alone.

[00227] V δ 1 $\gamma\delta$ cell proliferation was also examined for the test conditions corresponding to FIGS. 3A-3C, and the results are shown at FIGS. 3D-3F, respectively. In the Hep2G assay, good proliferation and cytotoxicity was observed in constructs that included both the CD3 ζ and 4-1BB domains, as well as at least the Y86F mutation (i.e., DAP10.15 and DAP10.6), with the DAP10.6 construct (which includes each of CD3 ζ , 4-1BB, Y86F, and K84R) showing the best performance in terms of cytotoxicity and V δ 1 $\gamma\delta$ cell proliferation.

Example 4. Survival of V δ 1 Cells Transduced with DAP10 Constructs after Tumor Co-culture

[00228] In this Example, survival of V δ 1 cells transduced with various DAP10 constructs of Table 1 was assessed. Specifically, survival was assessed following 5 days of PLC/PRF/5 co-

culture. As shown at FIG. 4, cells transduced with DAP10.6 showed the best V δ 1 survival after 5 days of co-culture with PLC/PRF/5.

Example 5. *In Vivo* Tumor Control of DAP10 CAD Constructs in a Mouse Model

[00229] In this Example DAP10 CAD constructs (DAP10.6 and DAP10.15, refer to Table 1) were examined for their effectiveness at controlling PLC/PRF/5 tumor growth in a mouse model. As shown at FIG. 5A, mean tumor volume steadily increased over the course of 35 days in mice harboring tumor alone (group A). Similar results were observed in mice harboring the tumor and injected with 5×10^6 V δ 1 T cells (untransduced, group B). Tumor growth was significantly reduced when mice were injected with V δ 1 T cells transduced with either DAP10.6 (group C) or DAP10.15 (group D), with the most robust efficacy in terms of tumor control observed for the DAP10.6 CAD. FIG. 5B shows the data obtained in FIG. 5A at day 35, as analyzed via Kruskal-Wallis test with Dunn's multiple comparisons and plotted as a function of tumor volume as shown. For each of groups A-D at FIGS. 5A and 5B, N=5, and the number of tumor cells dosed was 4×10^6 .

Example 6. NKG2D Expression in V δ 1 T cells transduced with DAP10 CAD

[00230] This Example demonstrates increased NKG2D expression levels on cells transduced with a particular DAP10 CAD (*e.g.*, DAP10.6, refer to Table 1). Briefly, V δ 1 T cells were transduced with one of DAP10.6, DAP10.13, DAP10.15, DAP10.5, and a control CAR, and said transduced cells were then co-cultured with PLC cells. Fluorescent labeling of NKG2D in conjunction with FACS analysis was used to assess V δ 1 NKG2D expression level. At FIG. 6, expression levels for the cells containing different DAP10 CADs or CAR control are plotted as geometric mean fluorescence intensity (gMFI), illustrating that the DAP10.6 CAD construct expressed in V δ 1 T cells results in significantly higher NKG2D expression levels as compared to other DAP10 CADs (10.13, 10.15, 10.5) and the CAR control. Notably, the DAP10.6 CAD construct includes each of the K84R mutation, the Y86F mutation, a 4-1BB costimulatory domain and a CD3 ζ signaling domain. A smaller but still statistically significant increase in NKG2D expression over the CAR control was observed with DAP10.15 (K84R + 4-1BB + CD3 ζ) and with DAP10.5 (Y86F + 4-1BB + CD3 ζ).

Example 7. DAP10 CAD expression

[00231] This Example demonstrates that DAP10 CAD expression is similar across different lots of Vδ1 cells.

[00232] The DAP10.6.3 construct (SEQ ID NO: 75) includes truncated EGFR as a marker, as compared to, e.g., the DAP10.6 construct (SEQ ID NO: 29) that includes CD19. A DAP10 construct referred to herein as DAP10.16 (SEQ ID NO: 77) is the same as the DAP10.6.3 construct, but includes the 1XX mutation in the CD3ζ signaling domain. Vδ1 cells transfected with DAP10.6 or DAP10.16 constructs were found to exhibit substantially similar expression levels. CAD protein was directly detected using anti-DAP10 and anti-CD3ζ antibodies by western blot analysis (n=2 donors) (FIG. 7).

Example 8. Cytotoxic activity of DAP10 CAD is mediated by NKG2D

[00233] This Example demonstrates that blocking NKG2D eliminates cytotoxic activity. Specifically, blocking NKG2D via the use of an NKG2D blocking antibody eliminated cytotoxic activity of DAP10 CAD expressing Vδ1 cells in three donors across 2 cell lines (FIGS. 8A-8B). Cytotoxic activity of control Vδ1 cells transfected with a CAR was unaffected (FIGS. 8C-8D). For FIGS. 8A, 8C, PLC target cells were used, 5:1 E:T ratio. For FIGS. 8B, 8D, HL60 target cells were used, 2.5:1 E:T ratio. The NKG2D blocking antibody also had no effect on cells expressing the DAP10.0 construct (i.e., lacking costimulatory and intracellular signaling domain) (data not shown).

Example 9. DAP10 CAD molecular activation signature

[00234] This Example demonstrates consistent DAP10 CAD activation signature across multiple donors and cell lines. For this Example, Nanostring analysis (Nanostring Technologies, Seattle, WA) was conducted post-stimulation to assess molecular activation signature. Cell lines used for stimulation included PLC (HCC), HL60, THP1 (AML), and HCT15 (CRC). Data illustrated consistent activation signature mediated through interferon gamma, 4-1BB, and Granzyme B. FIG. 9A depicts data for DAP10.6 vs innate control (DAP10.0), and FIG. 9B depicts data for DAP10.16 vs innate control (DAP10.0). No consistently detectable differences between DAP10.6 and DAP10.16 were observed (FIG. 9C).

Example 10. Broad anti-cancer activity of Vδ1 cells transduced with DAP10 CAD

[00235] This Example demonstrates that V δ 1 cells transduced with DAP10 CADs of the present disclosure exhibit anti-cancer activity against various cancer types having a broad range of NKG2D ligand expression levels/patterns.

[00236] FIGS. 10A-10E are graphs illustrating % cytotoxic activity as a function of E:T ratio for V δ 1 cells transduced with DAP10.6 as compared to V δ 1 cells transduced with DAP10.0, or cells not transduced with a DAP10 CAD, in an 18-hour assay. Target cell lines included HCT116 (FIG. 10A), SKMEL5 (FIG. 10B), Mino D2 (FIG. 10C), ScaBER (FIG. 10D), and Raji B4 (FIG. 10E). FIGS. 10F-10G are graphs showing % cytotoxicity of V δ 1 cells transduced with DAP10.6 as compared to innate control (V δ 1 cells transduced with DAP10.0), or an irrelevant CAR control, in an 18-hour assay. Target cell lines for FIGS. 10F-10G were NCI-H1581 and NCI-H2172, respectively. As shown, cytotoxic potency was significantly increased relative to controls. FIG. 10H illustrates that selected cell lines used for assays represent a broad range of NKG2D ligand expression levels/patterns. To obtain the data at FIG. 10H, different cancer cell lines as indicated, derived from a variety of hematologic and solid tumors, were assessed for NKG2D ligands by flow cytometry. 5 antibodies used for staining detected MICA/MICB, ULBP1, ULBP2/5/6, ULBP3, and ULBP4. Data is presented as fold change mean fluorescence intensity (MFI) of NKG2D ligand over relevant isotype control. Raw data is shown at FIGS. 10I-10J (cancer cell lines were stained in triplicate).

[00237] FIGS. 11A-11G are graphs illustrating cytotoxicity index as a function of target and effector co-culture time. Target cells included 22Rv1, Mino, HCT116, and HCT-15. Effectors included V δ 1 cells transduced with DAP10 CAR, CAR control, or untransduced cells. Enhanced cytotoxicity was observed when V δ 1 cells were transduced with a DAP10 CAD of the present disclosure, as compared to controls. Degree of cytotoxic potential was found to be dependent on cell line and E:T ratio. Cell lines tested represent various levels/patterns of NKG2D ligand expression (see FIG. 10H-10J). Effector cells were co-cultured with NucRed-expressing target cells at submaximal E:T ratios of 5:1 or 1.5:1 depending on the cell line. Cytotoxicity index was calculated by dividing total NucRed object area (mm²/well) of each time point by the value at time = 0.

Example 11. Comparable cytotoxic activity across multiple lots of V δ 1 cells transduced with DAP10 CADs

[00238] This Example demonstrates that cytotoxic activity of V δ 1 cells transduced with DAP10 CADs is comparable for different lots of V δ 1 cells and DAP10 CADs. FIG. 12A is a graph showing data for a representative 120 hour cytotoxicity assay. Target cells were PLC/PRF/5. V δ 1 cells used in the assay were transduced with DAP10.6, DAP10.16, DAP10 reference lot (i.e., positive control batch of expanded V δ 1 cells), and DAP10.0 (control). Also shown is data from PLC/PRF/5 cells alone (i.e., no co-culture with V δ 1 cells). FIG. 12B is a graph depicting % reduction in cytotoxicity of tumor alone relative to tumor treated with V δ 1 cells transduced with DAP10 CAD using the final time point of the 120-hour assay.

[00239] Cytotoxicity of DAP10.6 vs DAP10.16 vs DAP10.17 constructions incorporated into V δ 1 cells was examined in a 120-hour cytotoxicity assay. V δ 1 cells from three donors were tested with the DAP10 constructs, using PLC/PRF/5 target cells. Each construct shown at FIG. 12C is an aggregate of all three donors. Using a stringent E:T ratio, in this assay the DAP10.6 construct exhibited slightly better average cytotoxicity than DAP10.16 and DAP10.17.

[00240] Efficacy of DAP10.6 vs DAP10.16 vs. DAP10.17 showed some donor dependence. Cytotoxicity index was measured in a co-culture experiment with V δ 1 cells from three different donors (SCT06, SCT29, SCT46) transduced with either DAP10.6, DAP10.16, or DAP10.17, or non-transduced cells from the same donors. Target cells in the co-culture experiments were PLC/PRF/5 cells. Co-culture time was 120 hours. As shown in FIGS. 12D-12E, the DAP10.6 and DAP10.16 show very similar profiles. DAP10.17 was found to be the most donor dependent (FIG. 12F). A reference lot of V δ 1 cells transduced with DAP10.6 was used in all assays and served as a reproducible control (data not shown).

Example 12. Cytokine profile corresponding to DAP10 CAD stimulation

[00241] This Example demonstrates that DAP10 CAD stimulation results in a polyfunctional cytokine profile.

[00242] FIG. 13A illustrates cytokine profile as a function of different DAP10 constructs of the present disclosure. As illustrated, degree of cytokine activation was cell line specific. Cell lines tested included PLC, Mino, and T cells alone. Importantly, no detection of potentially problematic cytokines (e.g., IL-6 and IL-17) was observed. FIG. 13B is a graph showing interferon gamma induction (pg/ml/1E⁶ CAD⁺ cells) in PLC, Mino, and T cells alone, for various DAP10 CADs (from various donors, e.g., SCT06, SCT 46). FIGS. 13C-13F are

representative experiments illustrating interferon gamma secretion from CAD⁺ Vδ1 cells alone and after ~18-hour co-culture at submaximal E:T ratios with PLC/PRF/5, HL60, THP1, and PC3 target cells, respectively.

Example 13. Cytokine profile of DAP10 CAD compared to chimeric antigen receptor (CAR)

[00243] This Example demonstrates a high degree of similarity in cytokine profiles from DAP10 CADs of the present disclosure, as compared to CARs (FIG. 14). Conditions examined included Vδ1 cells transduced with a DAP10 CAD plus PLC cells, or alone (i.e., minus target cell), and Vδ1 cells transduced with a CAR plus target cell (HepG2, PLC, Raji), or alone (i.e., minus target cell). Notably, Vδ1 cells transduced with a DAP10 CAD in absence of target cell show less background cytokine secretion than Vδ1 cells transduced with a CAR in absence of target cell.

Example 14. DAP10 CAD stimulation drives proliferation across multiple donors

[00244] This Example demonstrates that DAP10 CAD stimulation drives Vδ1 cell proliferation in all donors tested. Specifically, for this Example, Vδ1 cell proliferation was assessed following transduction of DAP10 CAD constructs of the present disclosure (DAP10.6, DAP10.16, DAP10.17), by co-culture with PLC/PRF/5 (5:1 E:T ratio). Controls included Vδ1 cells transduced with DAP10 control batch, or DAP10.0). Less/slower proliferation of Vδ1 cells transduced with DAP10.16 is likely the result of the 1XX CD3ζ signaling domain. In this regard, enhanced regulation of activation/proliferation may be beneficial to long term efficacy/survival of engineered Vδ1 cells, due to reduced oversimulation/exhaustion.

[00245] Vδ1 cells obtained from two different donors (SCT29 and SCT46) were used in the co-culture experiment. As shown in FIG. 15, robust proliferation of Vδ1 cells transduced with DAP10.6 and DAP10.16 was observed for both donors, whereas proliferation was somewhat more donor dependent for Vδ1 cells transduced with DAP10.17.

Example 15. *In vivo* tumor control by Vδ1 cells transduced with a DAP10 CAD containing a 1XX CD3ζ intracellular signaling domain

[00246] This Example demonstrates that incorporation of a 1XX CD3ζ intracellular signaling domain may improve tumor control *in vivo*.

[00247] In this Example, DAP10 CAD constructs DAP10.6 and DAP10.16 were examined for effectiveness at controlling PLC/PRF/5 tumor grown in a mouse model. As shown in FIG. 16A, tumor control in mice treated with V δ 1 cells transduced with DAP10.16 CAD (in which the CD3 ζ intracellular signaling domain includes the 1XX mutation) is improved upon direct comparison to mice treated with V δ 1 cells transduced with DAP10.6 CAD ($p=0.0079$, Mann-Whitney test (two-tailed)). A schematic of the experimental procedure used for this Example is depicted at FIG. 16B.

Example 16. Comparison of *in vivo* anti-tumor activity of DAP10 CAD V δ 1 cells with CAR V δ 1 cells

[00248] This Example demonstrates that DAP10 CAD+ V δ 1 cells exhibit anti-tumor activity with kinetics similar to CAR V δ 1 cells

[00249] *In vivo* tumor growth kinetics for DAP10 CAD+ V δ 1 cells (5×10^6 cells/dose and 15×10^6 cells/dose as compared to control CAR V δ 1 cells and a tumor alone condition in an HCT-15 mouse xenograft model is shown at FIG. 17A. Tumor volumes as quantified at day 27 are shown at FIG. 17B. A schematic of the experimental procedure used for this Example is depicted at FIG. 17C. Data is shown as mean $\pm$ SEM for 5 mice/group. A Kruskal-Wallis test with Dunn's multiple comparisons was used to assess final statistical significance amongst complete cohorts for each treatment (ns = not significant).

Example 17. *In vivo* proliferation, persistence and targeting of engineered V δ 1 cells

[00250] This Example demonstrates that V δ 1 cells transduced with a DAP10 CAD of the present disclosure proliferates in tumors *in vivo* in a mouse model, but not in other organs. For this Example, two separate studies were conducted. As shown at FIG. 18A, proliferation of V δ 1 cells transduced with DAP10.6 was observed in tumor tissue (subcutaneous PLC/PRF/5 cells), but not in spleen, lung, liver, bone marrow, or blood on day 7 post treatment. V δ 1 cells used in Study 1 were obtained from a different donor than V δ 1 cells used in Study 2. For FIG. 18A, HuCD45+, V δ 1+ population is shown. FIG. 18B is a graph quantifying engineered V δ 1 cells per mg tumor tissue in respective studies on day 4, day 7, and day 14 (Study 1) and on day 7 and day 14 (Study 2). For each study, 5×10^6 engineered V δ 1 cells were used. Notably, an increase in total V δ 1 cells within the tumor was observed throughout each study. FIG. 18C is a graph showing quantification of V δ 1 cells in tumor tissue, or other tissues (lung, liver, spleen, bone marrow,

blood) taken 4, 7, or 14 days after treatment as assessed by flow cytometry, and represents cumulative analysis across the two independent studies presented in this Example. FIG. 18D is a schematic showing the experimental procedure corresponding to this Example for reference. No significant changes in body weight or acute clinical signs of off toxicity or xenogeneic graft vs. host disease (GvHD) was observed among treated mice (FIG. 19). Data shown at FIG. 19 is for n=4 independent efficacy studies.

[00251] It was found that V δ 1 cells transduced with a DAP10 CAD of the present disclosure efficiently target tumor cells while sparing normal (i.e., non-tumor) cells. Specifically a short-term cytotoxicity assay was adapted to an annexin/DAPI flow based method for analysis of primary cell targets. FIG. 20A is a graph showing that both V δ 1 cells transduced with a DAP10 CAD of the present disclosure (DAP10.6, DAP10.16), and V δ 1 cells transduced with an NKG2D CAR significantly reduced THP1 cell viability, as compared to THP1 alone and V δ 1 cells transduced with an innate control (DAP10.0). FIG. 20B is a graph showing a substantial lack of targeting of healthy PBMCs by V δ 1 cells transduced with a DAP10 CAD of the present disclosure (DAP10.6, DAP10.16), similar to innate control (V δ 1 cells transduced with DAP10.0). Notably, V δ 1 cells transduced with a DAP10 CAD consistently demonstrated lower PBMC targeting than an NKG2D CAR reference (FIG. 20B).

Example 18. Small scale donor screens

[00252] This Example demonstrates that expansion of V δ 1 cells transduced with a DAP10 CAD that includes a CD3 ζ 1XX modification may be improved over similar constructs in which the CD3 ζ intracellular signaling domain does not include the 1XX.

[00253] For this Example, V δ 1 cells from 6 donors (SE001, ARC007, HC45, DLS003, SE015, and SCT029) were tested over two experiments in small scale shake flask expansions. Specifically, V δ 1 cells from the different donors were transduced with either DAP10.6 or DAP10.16. 50-135% more V δ 1 cells were measured from DAP10.16 transduced cells as compared to DAP10.6 transduced cells across all donors (FIG. 21). Expansion was measured on day 14, of day 15.

Example 19. DAP10 CADV δ 1 cell growth kinetics

[00254] This Example demonstrates that there is a distinct shift in cultures to higher % Vδ1 cells transduced with lead DAP10 CAD constructs (DAP10.6, DAP10.16, DAP10.17) as compared to controls (DAP10.0, CAR control).

[00255] For this Example, Vδ1 cells from three different donors (SCT06, FIG. 22A, SCT29, FIG. 22B, SCT45, FIG. 22C) were transduced with lead DAP10 constructs or controls, and % Vδ1 of total cells was expanded as a function of expansion time. As shown in each of FIGS. 22A-22C, a distinct shift to higher Vδ1 percentages was observed for the lead DAP10 constructs as compared to controls.

Example 20. Expansion of Cells transduced with DAP10 CAD constructs

[00256] This Example demonstrates Vδ1 cells can be efficiently expanded and transduced with DAP10 CADs of the present disclosure. FIG. 23A depicts a schematic representing a process for generating “off-the-shelf” allogeneic CAD Vδ1 cells. Data showing independent expansions of lead DAP10 CAD constructs (DAP10.6, DAP10.16, DAP10.17) transduced into Vδ1 cells obtained from three different donors (SCT06, SCT29, SCT45) is depicted at FIGS. 23B, 23C, 23D, respectively. Independent expansions (blue vs red) show similar trends in growth profiles of DAP10 CAD constructs. In two of the three donors, growth was seen to be construct dependent.

[00257] A representative experiment illustrates that *ex vivo* culture of Vδ1 cells results in substantial fold-expansion (FIG. 23E) and robust DAP10 CAD transduction (FIG. 23F) Vδ1 cells. The data of FIGS. 23E-23F was obtained from 12 independent cultures using PBMCs from 7 different donors. Shown at FIG. 23G is a series of graphs showing cellular composition (Vδ1 cells, Vδ2 cells, αβ cells, and NK cells) over time (day 0, pre-αβ T cell depletion, and post-αβ T cell depletion) expressed as % of culture. Certain modifications and improvements will occur to those skilled in the art upon a reading of the foregoing description. It should be understood that all such modifications and improvements have been deleted herein for the sake of conciseness and readability but are properly within the scope of the following claims.

Table 2: Sequence information		
SEQ ID No.	Name	Sequence
1	DAP10 polypeptide	MIHLGHILFLLLLPVAAQTPGERSSLPAFYPGTSGSCSGCGSLSL PLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGKVYINMPG

		RG
2	4-1BB costimulation endodomain polypeptide	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
3	CD3z signaling domain polypeptide 1	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
4	CD3z signaling domain polypeptide 2	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
5	CD27 costimulatory domain polypeptide	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPAC SP
6	secretion signal 1 polypeptide	MALPVTALLLPLALLLHAARP
7	secretion signal 2 polypeptide	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCF SAGLPKTEA
8	IL-5 polypeptide	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCELL ELQVISLES GDASIHTVENLIILANNSLSSNGNVTESGCKECEELE EKNIKEFLQSFVHIVQMFINITS
9	P2A cleavage motif 1 polypeptide	SGSGATNFSLLKQAGDVEENPGP
10	furin cleavage site polypeptide	RAKR
11	cleavable linker polypeptide	RAKRSGSGATNFSLLKQAGDVEENPGP
12	P2A cleavage motif 2 polypeptide	ATNFSLLKQAGDVEENPGP
13	F2A cleavage motif polypeptide	VKQTLNFDLLKLAGDVESNPGP
14	E2A cleavage motif polypeptide	QCTNYALLKLAGDVESNPGP
15	T2A cleavage motif polypeptide	EGRSLLTCGDVEENPGP
16	IRES 1 nucleotide	CTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTG CGTTTGTCTATATGTTATTTCCACCATATTGCCGTCTTTTGGC AATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCA TTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCT

		GTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGA AGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAAC CCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGT GTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCAC GTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCT CAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTAC CCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTT TACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCG AACCACGGGGACGTGGTTTTTCCTTTGAAAAACACGATGATA
17	IRES 2 nucleotide	AGCAGGTTTCCCCAACTGACACAAAACGTGCAACTTGAAACT CCGCTGGTCTTTCCAGGTCTAGAGGGGTAAACACTTTGTACTG CGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAG CACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCT CCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACA CGGGCCCCGTCATGTGTGCAACCCCAGCACGGCGACTTTACTGC GAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACT GGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACA CGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAA AAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCG GAGGTCGGCACCTTTCCTTTGCAATT ACTGACCAC
18	DAP10-K84R polypeptide	MIHLGHILFLLLLPVAAAQTPGERSSLP AFYPGTSGSCSGCGLSL PLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRVYINMPG RG
19	DAP10-Y86F polypeptide	MIHLGHILFLLLLPVAAAQTPGERSSLP AFYPGTSGSCSGCGLSL PLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGKVFINMPG RG
20	DAP10-K84R- Y86F polypeptide	MIHLGHILFLLLLPVAAAQTPGERSSLP AFYPGTSGSCSGCGLSL PLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRVFINMPGR G
21	DAP10.0 Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTP GERSSLP AFYPGTSGSCSGCGLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGKVFYINMPGRGRAKRSGSGATNFS LLKQAGDVEENPGPMPPPRLLFFLLFLTPMEVRPEEPLVVKV EEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPG LGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGW TVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLM SPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGS TLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPA RDMWVMETGLLLPRATAQDAGKYCHRGNLTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRAL VLRRKRKRMTDPTRRF
22	DAP10.0 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgtggctgaccgacgccagatgc atgatccacctggccacatcctgtttctgctgctgcttctgtggccgctgctcagaca acacctggcgagagatctagcctgcctgcttctatcctggcaccagcggtctgtgtt ggctgtggatctctgagcctgcctctgctggctggactggttctgctgctgctgtggcc

		tctctgctgattgtggcgccgtgttctgtgtgcccgccctagaagatctcccgtcaa gaggatggcaaggtgtacatcaacatgcccgccagaggacgcgcgaagcgtacaggcagc ggggcgacaaatttcagccttctgaacaagcaggcgacgtggaagaaaacccgggtcca atgcctcctcctcggtgtgttcttctgtgtttctgacctatggaaagtgcggccc gaggaaacctctggtgtcaagtgaagagggcgacaacgccgtgctgagtgctgaag ggcacatctgatggccccacacagcagctgacctggtctagagagacccctgaagccc ttcctgaagctgtctctgggactgcctggactgggcatccatagaggcctctggccatc tggctgttcatcttcaacgtgtcccagcagatggcggtcttacctgtgtcaacctgga cctccaagcgagaaggcttggcagcctggctggaccgtgaatgtggaaggatccggcgag ctgttccgggtggaatgtgtctgatctcgccggcctcgatgcggcctgaagaatagatct agcgagggccctagcagccccagcggaactgatgagccccaaagctgtacgtgtggggc aaagacagacccgagatttgggagggcgagcctcctgtctgcctcctagagacagcctg aaccagagcctgagccaggacctgacaatggccctggatctacactgtggtctgagctgt ggcgtgccacctgacagtgtgtctagaggcctctgtcttgaccacgtgcacctaaag ggccctaagtctctgtgagcctggaactgaaggacgacagggcccgagagatatgtgg gtcatggaaacaggcctgtgtgctgctagagccacagcacaggatgcccggcaagtactac tgccacagaggcaacctgacctgagcttccacctggaaatcacgccagacctgtcctg tggcactggctgcttagaacggcggtggaaagtgtctgccgtgactctggcctacctg atcttctgcctgtgtagcctctgtgggcatcctgcatctgcagagagcactggtctgcgg cggaagcggaagagaatgacctatctaccagacgggtctga
23	DAP10.3 Amino Acid	MSVPTQVLGLLLLWLTARCMHILGHILFLLLLPVAAAQTTP GERSSLPAFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGKVYINMPGRGRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLKRRGRDPGEMGGKPKQRRK NPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRAKRSGSGATNFSLLKQAGDVEENPGMPMPPLLLFF LLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLT WSRESPLKPFLLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGF YLCQPGPPSEKAW QPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPS GKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLT MAPGSTLWLSGVPDPSVSRGPLSWTHVHPKGPKSLLSLELK DDRPARDMWVMEGTGLLLPRATAQDAGKYYCHRGNTMSF HLEITARPVLWHLLRT GGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKRKRMTD PTRRF
24	DAP10.3 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctgggccacatcctgttctgctgctgcttctgctggccgctgctcagaca acacctggcgagagatctagcctgctgcttctatctggcaccagcggtcttgttctggtgt ggatctctgagcctgcctctgctgctggactggttctgctgctgctgctgctgctgattgt gggcccgtgttctgtgtgcccgccctagaagatctcccgtcaa gaggatggcaaggtgtacatcaacatgcccgccagaggaagagtgaagttcagcagaagcgc cgatgcccctgcctatcagcagggccagaaccagctgtacaacgagctgaacctggcgagac gcgaggaatacagctgtgtgacaagcgccggggacgggaccccgagatggcgggc

		aagcctcaaagaaggaagaacccccaggagggcctgtataatgagctgcagaaagataagat ggccgagggcctacagcgagatcgccatgaaaggcgagagacggcggggcaagggccacga cggcctgtaccagggcctgagcaccgccacaaaggacacctacgacgccctgcatg caggccctgccacctagacgcgcgaagcgatcaggcagcggggcgacaaatttcagccttctg aaacaagcagggcgacgtggaagaaaaccccggtccaatgcctcctcctcggtgctgttcttct gctgttctgacccctatggaagtgcggcccaggaacctctggtgttcaaa gttgaagagggcgacaacgccgtgctgcagtgtctgaagggcacatctgatggcccacacag cagctgacctggtctagagagagccctctgaagcccttctgaagctgtctctgggactgcctgg actgggcatccatatgaggcctctggccatctggctgttcatcttaacgtg tccagcagatgggcggcttctacctgtgtcaacctggacctccaagcgagaaggctggcagc ctggctggaccgtgaatgtggaaggatccggcgagctgtccggtggaatgtgtctgatctcggc ggcctcggatgcggcctgaagaatagatctagcgagggccctagcagccccagcggaaaact gatgagcccaagctgtacgtgtgggccaagacagaccgagatttgggagggcgagcctc ctgtctgcctctagagacagcctgaaccagagcctgagccaggacctgacaatggcccctgg atctacactgtggctgagctgtggcgtgccacctgacagtgtgtctagaggccctctgtcttgac ccagctgcaccctaaggcgccctaagtctctgctgagcctggaactgaaggacgacaggccgc cagagatatgtgggtcatggaacaggcctgctgctgcctagagccacagcacaggatgcccgg caagtactactgccacagaggcaacctgacctgagcttccacctggaatcaccgccagacct gtcctgtggcactggctgcttagaaccggcggtggaagtgtctgccgtgactctggcctacct gatcttctgcctgtgtagcctcgtgggcacctctgcatctgcagagagcactggctcctgcggcgga agcgggaagagaatgaccgatcctaccagacgggtctga
25	DAP10.4 Amino Acid	MSVPTQVLGLLLLWLT DARCMHILGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGKVYINMPGRGKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAY QQGQNQLYNELN LGRREEYDVL DKRRGRDPEMGGKPQRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPRAKRSGSGATNFSLLKQAGDVEENPGMPPPRL LFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQ QLTWSRESPLKPFLLS LGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAW QPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPS GKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLT MAPGSTLWLS CGVPPDSVSRGPLSWTHVHPKGP KSLLSLELK DDRPARDMWV METG LLLPRATAQDAGKY YCHRGNL TMSFHLEITARPVLWHWLLR TGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKRMT DPTRRF
26	DAP10.4 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctgggccacatctgttctgctgctgctgctgtggccgccgctcaacaacccctggcg agagatctagcctgccagccttctaccccggtacaagcgatcttcagc ggctgcccagcctgtctctgcctctgctggccggcctggtgctgctgatgctgtggccagcct gctgatcgtggcgccgtgttctgtgctgcagacctagaaggccccctgcccaggaggatgg aaaggtgtacatcaacatgcctggcagaggcaagagaggcagaaagaagctg

		ctgtacatcttcaagcagcctttcatgagaccagtgcagaccaccaggaggaggacggatgta gctgcagattccccgaggaagaagaaggcggctgtgaactgagagtgaagttcagcagaagc gccgacgcccctgcttatcagcagggccagaaccagctgtacaacgagctgaac ctgggcagaagggaagagtacgacgtgctggacaagcggcggggacgggaccccgagatg ggcggaaaacctcaaagacggaagaacccccaggaggcctttataatgagctgcagaaga taagatggccgaggcctacagcgagatcgcatgaaaggagaaagacggcggggcaaa ggccacgacggcctgtaccaggcctgagcaccgccacaaaggacacctacgacgcctgc acatgcaggccctgccccccagacgcgcgaagcgatcaggcagcggggcgacaaatttcagc cttctgaaacaagcaggcgacgtggaagaaaaccccggtccaatgcctcctcctcgg ctgctgttctcctgctgtttctgaccttatggaagtgcggcccgaggaaaccttggtggtcaaa gtgaagaggggcgacaacgccgtgctgcagtgtctgaaggcgacatctgatggccccacag cagctgacctggtctagagagagccctctgaagcccttctgaagctgtct ctgggactgcctggactgggcatccatatgaggcctctggccatctggctgttcatcttaacgtg tcccagcagatggcggtcttacctgtgtcaacctggacctccaagcgagaaggctggcagc ctggctggaccgtgaatgtggaaggatccggcgagctgttccggtggaat gtgtctgatctcggcgccctcgatgcggcctgaagaatagatctagcaggggccctagcagc cccagcggaaaactgatgagcccaagctgtacgtgtgggccaagacagaccgagatttgg gaggcgagcctcctgtctgcctcctagagacagcctgaaccagagcctgagc caggacctgacaatggcccctggatctacactgtggtgagctgtggcgtgccacctgacagtg tgtctagaggccctctgtcttgaccacgtgcaccctaaggccctaagtctctgctgagcctg gaactgaaggacgacaggcccgccagagatatgtgggtcatggaacaggc ctgctgctgctagagccacagcagagatgcccggcaagtactactgccacagaggcaacctg accatgagcttccacctggaaatcaccgccagacctgtcctgtggcactggctgcttagaacgg cggctggaagtgtctgcccgtgactctggcctacctgatcttctgctgtgt agcctcgtgggcatcctgcatctgcagagagcactggtcctgcggcggaagcggaagagaatg accgatcctaccagacgggtctga
27	DAP10.5 Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTTP GERSSLPAFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGKVFINMPGRGKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPQRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQUALPPRAKRSGSGATNFSLLKQAGDVE ENPGPMPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQ CLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAI WLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGEL FRWNVSDLGGLGCLKNRSSEGPSSPSGKLMSPKLYVWAKD RPEIWEGEPPCLPPRDSL NQSLSQDLTMAPGSTLWLSCGVPP DSVSRGPLSWTHVHPKGP KSLLSLELKDDRPARDMWVMET GLLLPRATAQDAGKY YCHRGNL TMSFHLEITARPVLWHWLL RTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKRKM TDPTRRF
28	DAP10.5 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctgggacacatcctgttctgctgctgcttccagtggcggccgctcaaaacacctggcg agagaagcagcctgcccgccttctaccccggtacatctggcagctgcagc ggctgcggcagctgtctgctgctgctgctggcggactggtgctgctgatgccgtggccagcct

		gctgatcgtggcgccgtgttctgtgcccagaccagaaggtcccctgccaggaggacgg caaggtgttcatcaacatgcctggcagaggcaaaagaggcagaagaagctg ctgtacatcttcaagcagcctttcatgcgccctgtgcagaccacccaggaggaagatggctgctc ttgtcgatttccagaagagggaagaaggcggctgtgaactgagagtgaagttcagcagatccgcc gacgccccctgcttatcagcagggccagaaccagctgtataatgagctgaac ctgggcccggcggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatgg gcggaacacctaagaaggaagaaaccccaggaggactgtacaacgagctgcagaag gataagatggccgaggcctacagcgagatcgcatgaaaggagaaagaagaggaaaag gccacgacggcctgtaccaggcctgagcaccgccaccaaggacacctacgacgacctgcac atgcaggccctgccacatgacgcgcgaagcgatcaggcagcggggcgacaaatttcagcctt ctgaaacaagcaggcgacgtggagaaaaccccgggtccaatgcctcctcctcggtgctgttctt cctgctgtttctgacccctatggaagtgcggcccagggaacctctggtggtaagttgaagagg gcgacaacgcctgctgagtgctgaagggcacatctgatggcccacacagcagctgacct ggtctagagagagccctctgaagccctcctgaagctgtctctgggactgctgactgggcatc catatgaggcctctggccatctggctgttcatcttcaacgtgtcccagcagatggcggttctac ctgtgtcaacctggacctccaagcgagaaggcttgccagcctggctggaccgtgaatgtgga ggatccggcgagctgttccgggtggaatgtgtctgatctcgccggcctcgatgcggcctgaaga atagatctagcggaggccctagcagccccagcggaactgatgagcccaagctgtacgtgt gggccaagacagaccagagatttggaggggcagcctcctgtctgctccttagagacagcc tgaaccagagcctgagc caggacctgacaatggcccctggatctacactgtggctgagctgtggcgtgccacctgacagtg tgtctagaggccctctgtcttgaccacgtgcaccctaaggccctaagtctctgctgagcctg gaactgaaggacgacaggcccgccagagatatgtgggtcatggaacaggc ctgctgctgcttagagccacagcagagatgcccggcaagtactactgccacagaggcaacctg accatgagcttccacctggaaatcacggcagacctgtcctgtggcactggctgcttagaacgg cggctggaagtgtctgcccgtgactctggcctacctgatcttctgctgtgt agcctcgtgggcatcctgcatctgcagagagcactggtcctgcggcggaagcggaagagaatg accgatcctaccagacggttctga
29	DAP10.6 Amino Acid	MSVPTQVLGLLLLWLTDA RCMIH LGHILFLLLLPVAAAQTTP GERSSLPAFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGRVFINMPGRGKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPQRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQUALPPRAKRSGSGATNFSLLKQAGDVE ENPGMPMPRLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQ CLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAI WLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGEL FRWNVSDLGGLGCLKNRSSEGPPSPSGKLMSPKLYVWAKD RPEIWEGEPPCLPPRDSL NQSLSQDLTMAPGSTLWLSCGVPP DSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVME TGLLLPRATAQDAGKY YCHRGNTMSFHLEITARPVLWHWLL RTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKRKM TDPTRRF

30	DAP10.6 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctggtgaccgacgccagatgcatgat ccacctgggccacatcctgttctgctgctcctgctggtggcctgctcagaccacctggag aaagatctagcctgcccgtttctacccgggaccagcggcagctgcagc ggatgcggctctctgagcctgctgctggtggcctgctggtggcctgctggtggcagcc tgctgctggtggcgcctgttctgctggtggcagaccagaagaagcctgcccaggaggtg gcaagagtgttcacacatgcctggcagaggcaaaagaggcagaagaagctg ctgtacatcttcaagcagccttcatgcggccctgagacaaccaggagggagcagcgtgta gctgtgattccccgaggaagaagaaggcggctgagcgttagagtgaagttcagcagaagcg ccgacgcccctgctaccagcaggggcagaaccagctgtataatgagctgaacctgggaaga gggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatggcggaacc tcaaagaaggagaacctcagggagggcctgtacaacgagctgcagaagacaagatggcgc aggcctacagcagatcgcatgaaaggcagagacggcggggcaaggggccacgacggcc tgtaccaaggcctgtcaacagccaccaaggacacctacgacgcccctgcacatgcaggccctg cacctagacgcgcgaagcgtcagggcagcggggcgacaaatttcagccttctgaaacaagca ggcgagctggaagaaaaccccggtccaatgcctcctcctcggctgctgttcttctgctgttctg accctatggaagtgcggcccgaggaaacctctggtggtcaaaagtgaagaggcgacaacgc cgtgctgcagtgctgaagggcacatctgatggccccacagcagctgacctggtctagagag agccctctgaagcccttctgaagctgtcttgggactgctgactgggactccatagaggcc tctggccatctggtgttcatcttcaacgtgtcccagcagatggcgcgcttctacctgtgtcaact ggacctccaagcgagaaggccttgagcagcctggtgaccgtgaatgtggaaggatccggcga gctgttccggtggaatgtgtctgctcggcgccctcgatgcggcctgaagaatagatctagcg aggccctagcagccccagcggaactgatgagccccagctgtacgtgtggccaaagac agaccgagatttggaggggcagcctcctgtctgctccttagagacagcctgaaccagagc ctgagccaggacctgacaatggccctggtatcactgtggtgagctgtggcgtgccacctg acagtggtctagaggcctctgtcttgaccacgtgcaccctaaggcgccctaagtctgtgta gcctggaactgaaggacgacagggcccgagagatatgtgggtcatggaacaggcctgctg ctgctagagccacagcagatgcccggcaagtactactgccacagaggcaacctgacctg agcttccacctggaatcacggcagacctgtcctgtggcactggctgctt agaaccggcggtggaaagtgtctgctgctgactctggcctacctgatcttctgctgtgtagcctc gtgggcatcctgcatctgcagagagcactggtcctgcgggcgaagcgggaagagaatgacctg cctaccagacgggtctga
31	DAP10.13 Amino Acid	MSVPTQVLGLLLLWLTDA RCMIH LGHILFLLLLPVAAAQTTP GERSSLPAFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGRVYINMPGRGRAKRSGSGATNFS LLKQAGDVEENPGMPPPRLLFFLLFLTPMEVRPEEPLVVKV EEGDNAV LQCLKGTS DGPTQQLTWSRESPLK PFLKLSLGLPG LGIHMRPLAIWLFIFNV SQMGGFYLCQPGPPSEKAWQPGW TVNVEGS GELFRWNVSDLGGLGCLKNRSSEGPPSSPSGKLM SPKLYVWAKDRPEIWE GEPCLPPRDSL NQSLSQDLTMAPGS TLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPA RDMWVMETGLLLPRATAQDAGKY YCHRG NLTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRAL VLRKRKRMTDPTRRF
32	DAP10.13 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctggtgaccgacgccagatgcatgat ccacctgggccacatcctgttctgctgctcctgctggtggcctgctcagacaacctggcga gagatctagcctgctgcttctatcctggcaccagcggctcttcttct

		ggctgtggatctctgagcctgcctctgctggctggactggttgctgctgatgctgtggcctctctgc tgattgtggcgccgtgttctgtgtgcccggcctagaagatctcccgtcaagaggatggcag agtgtacatcaacatgcccggcagaggacgcgcgaagcgatcaggcagcggggcgacaaatt tcagccttctgaaacaagcaggcgacgtggaagaaaacccgggtccaatgcctcctctcggct gctgttctctgctgtttctgacccctatggaagtgcggcccaggaacctctggtgtgtaagt gaagaggcgacaaacccgtgctgagtgctgaagggcacatctgatggcccacacagca gctgacctggtctagagagagccctctgaagccctcctgaagctgtctctgggactgcctggac tggcatccatagaggcctctggccatctggctgttcatttcaacgtgtccagcagatggcg gcttctacctgtgtcaacctggacctcaagcgagaaggcttgccagcctggtggacctggaat gtggaaggatccggcgagctgttccggtggaatgtgtctgatctcggcgccctcgatgcggcc tgaagaatagatctagcagggccctagcagcccagcggaaaactgatgagcccaagctgt acgtgtgggccaagacagaccgagatttgggagggcgagcctcctgtctgcctcctagaga cagcctg aaccagagcctgagccaggacctgacaatggcccctggatctacactgtggtgagctgtggc gtgccacctgacagtgtgtctagaggccctctgtcttgaccacgtgcaccctaaggccctaa gtctctgctgagcctggaaactgaaggacgacaggcccgccagagatatgtgggtcatgaaac aggcctgctgctgctagagccacagcagcaggtgccggcaagtactactgccacagaggca acctgacctgagcttccacctggaatcaccgccagacctgtcctgtggcactggctgcttaga accggcggtggaagtgtctgcccgtgactctggcctacctg atcttctgcctgtgtagcctcgtgggcatcctgcacatgcagagagcactggtcctgcggcgaa gcggagagaatgaccgatcctaccagacgggtctga
33	DAP10.14 Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTTP GERSSLPAFYPGTSGSCSGCSLSLPLLAGLVAADAVASLLIV GAVFLCARPRRSPAQEDGRVYINMPGRGRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPENMGKPKQRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPRAKRSGSGATNFSLLKQAGDV EENPGMPMPRLFFLLFLTPMEVRPEEPLVVKVEEGDNAVL QCLKGTS DGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPL AIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSG ELFRWNVSDLGGLGCLKNRSSEGPSSPSGKLMSPKLYVWA KDRPEIWEGEPPCLPPRDSL NQSLSQDLTMAPGSTLWLSCGV PPDSVSRGPLSWTHVHPKGP KSLLSLELKDDRPARDMWVME TGLLLPRATAQDAGKYYCHRG NLTMSFHLEITARPVLWHWL LRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKR MTDPTRRF
34	DAP10.14 Nucleic Acid	atgtccgtgcctaccagtgctgggcctgctgctgctgtggtgacctgacgccagatgc atgatccacctgggccacatcctgttctgctgctgctgctgctggtgcccagacc accctggagaaagatctagccttccagccttctacccgggaccagcggagctgcagc ggctgcccgcagctgtctctgctgctgctgctgctgctgctgctgctgctgctgctgca tctctgctgatcgtggcgccgtcttctgtgctgctgctgctgctgctgctgctgctgctgctgca gaagatggcagaggtgtacatcaacatgcctggcagaggcagagtgaagttcagcagaagc gccgatgcccctgcctatcagcagggccagaaccagctgtacaacagctgaacctgggc agacgcgaggaatacagctgctggacaagcggcggggacgggaccccgagatggcgccg aagcctcaaagaaggaagaacccccagggagggcctgtataatgagctgcagaaagataag

		atggccgaggcctacagcgagatcggcatgaaaggcgagagacggcggggcaaggccac gacggcctgtaccaggcctgagcaccgccacaaaggacacctacgacgcctgcacatg caggccctgccacctagacgcgcgaagcgatcaggcagcggggcgacaaatttcagcctt ctgaaacaagcagcgacgtggaagaaaaccccggtccaatgcctcctcctcggtgctg ttcttctgctgtttctgaccctatggaagtggcgccgaggaacctctggtggtcaaa gttgaagaggcgacaaacgccgtgctgcagtgtctgaagggcacatctgatggcccaca cagcagctgacctggtctagagagagccctctgaagccctcctgaagctgtctctggga ctgcctggactgggcatccatatgaggcctctggccatctggctgttcatcttcaacgtg tccagcagatggcggttctacctgtgtcaacctggacctccaagcgagaaggctgg cagcctggctggaccgtgaatgtggaaggatccggcgagctgttccggtggaatgtgtct gatctcggcggcctcggtgcggcctgaagaatagatctagcagggccctagcagcccc agcggaaaactgatgagccccagctgtacgtgtgggccaagacagacccgagattgg gaggcgagcctcctgtgtcctcctagagacagcctgaaccagagcctgagccaggac ctgacaatggccccctggtctacactgtgtgctgagctgtggcgtgccacctgacagtgtg tctagaggccctctgtcttgaccacgtgcacctaaaggccctaagtctctgtgagc ctggaactgaaggacgacaggcccgccagagatatgtgggtcatggaacaggcctgtg ctgcctagagccacagcacaggtgcccgaagtactactgccacagaggcaacctgacc atgagcttcacctggaatcacccgcagacctgtcctgtggcactggctgcttagaacc ggcggctggaagtgtctgctgactctggcctacctgatcttctgctgtgtgacctc gtgggcatcctgcatctgcagagagcactggtcctcgggcggaagcggaagagaatgacc gatcctaccagacggttctga
35	DAP10.15 Amino Acid	MSVPTQVLGLLLLWLTDARCMIHLLGHILFLLLLPVAAAQTTPGER SSLPAFYPTSGSCSGCSLSLPLLAGLVAADAVASLLIVGAVFLC ARPRRSPAQEDGRVYINMPGRGKRGRKKLLYIFKQPFMRPVQTT QEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDRRRGRDPEMGGKPQRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQA LPPRRAKRSGSGATNFSLLKQAGDVEENPGPMPPPRLLFFLLFLTP MEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLK PFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEK AWQPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPS GKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSL NQSLSQDLTMAP GSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPAR DMWVMETGLLLPRATAQDAGKY YCHRGNL TMSFHLEITARPVL WHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRK RMTDPTRRF
36	DAP10.15 Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacccagatgc atgatccacctgggccacatcctgttctgctgctgctgctgctggtggccgctcaaca accctggcgagagatctagcctgccagccttctaccccggtacaagcggtcttcagc ggctgcggcagcctgtctctgctctgctggtggcctggtggtgctgatgctgtggcc agcctgctgatctgtggcgccgtgttctgtgcgcagacctagaaggtccccgtcccag gaggatggaagagtgtacatcaacatgcctggcagaggcaagagaggcagaagaagctg ctgtacatcttcaagcagcctttcatgagaccagtgcagaccaccaggaggaggacgga ttagctgcagattccccgaggaagaagaaggcggtgtgaactgagagtgaagttcagc agaagcgccgacgccctgcttatcagcagggccagaaccagctgtacaacgagctgaac

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37	DAP10-D57A	MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSL PLLAGLVAAAAVASLLIVGAVFLCARPRRSPAQEDGKVYINMPG RG
38	DAP10-N88Q	MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSL PLLAGLVAAADAVASLLIVGAVFLCARPRRSPAQEDGKVYIQMPG RG
39	DAP10-M89Q	MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSL PLLAGLVAAADAVASLLIVGAVFLCARPRRSPAQEDGKVYINQPG RG
40	CD28 costimulatory domain	FWVLVVVGGVLACYSLVTVAFIIFWVRSKRSRLLHSDYMN MTPRRPGPTRKHYQPYAPPRDFAAYRS
41	CD28 costimulatory domain	FWVRSKRSRLLHSDYMNMTTPRRPGPTRKHYQPYAPPRDFAA YRS
42	ICOS costimulatory domain	TKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL
43	OX40	RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI
44	P2A cleavage motif 2 polypeptide	GSGATNFSLLKQAGDVEENPGP

45	Signal polypeptide	MSVPTQVLGLLLLWLTDARC
46	DAP10.3.1 (+sig domain, -FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgttctgctgctgctcttctgtggccgctgctcagacaacacctggcga gagatctagcctgctgcttctatcttgccaccagcggtcttgttctggtgtggatctctgagc ctgctctgctggtgctgactggtgctgctgatgctgtggcctctctgctgattgtgggcgccgtgt tcctgtgtgcccggcctagaagatctcccgtcaagaggatggcaaggtgtacatcaacatgcc. cggcagaggaagagtgaagttcagcagaagcgcgcatgccctgacctatcagcagggccaga accagctgtacaacgagctgaacctgggcagacgcgaggaatacgcagctgctggacaagcgg cggggacgggaccccgagatgggcggcaagcctcaaagaaggaagaacccccaggaggg cctgtataatgagctgcagaaagataagatggccgaggcctacagcgagatcggcagaaagg cgagagacggcggggcaagggccacgacggcctgtaccagggcctgagcaccgccacaaa ggacacctacgacgacctgcacatgcaggccctgccacctaga
47	DAP10.3.1 (+sig domain, -FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLTDARCMIH LGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGK VYINMPGRGRVKF SRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPQRRK NPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
48	DAP10.4.1 (+sig domain, -FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgttctgctgctgctgctgtggccgctgctcaacaacccctggcg agagatctagcctgccagccttctaccccggtacaagcggatcttgcagc ggctgcggcagcctgtctgctgctgctgctggccggcctgggtgctgctgatgctgtggccagcct gctgatcgtgggcgcctgttctgtgctgcccagacctagaaggtccctgcccaggaggatgg aaaggtgtacatcaacatgcctggcagaggcaagagaggcagaaaagaagctg ctgtacatcttcaagcagccttcatgagaccagtgcagaccaccaggaggaggacggatgta gctgcagattccccgaggaagaagaaggcggctgtgaactgagagtgaagttcagcagaagc gccgacgcccctgcttatcagcagggccagaaccagctgtacaacgagctgaac ctgggcagaaaggaaggtacgacgtgctggacaagcggcggggacgggaccccgagatg ggcggaacacctcaaagacggaagaacccccaggaggccttataatgagctgcagaaaga taagatggccgagggcctacagcgagatcgcatgaaaggagaaagacggcgggggcaaa ggccacgacggcctgtaccagggcctgagcaccgccacaaaaggacacctacgacgacctgc acatgcaggccctgccccccaga
49	DAP10.4.1 (+sig domain, -FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLTDARCMIH LGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGK VYINMPGRGKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGK PQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
50	DAP10.5.1 (+sig domain, -FP2A, -CD19t)	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctggggacacatcctgttctgctgctgcttccagtggccgctgctcaacaacccctggcg agagaagcagcctgcccgcttctaccccggtacatctggcagctgcagc

	Nucleic Acid	ggctgcggcagctgtctctgctctgctggccggactggtggtgctgatgccgtggccagcct gctgatcgtgggcgcctgttctgtgcccagaccagaaggtcccctgccaggaggacgg caaggtg ttt catcaacatgcctggcagaggcaaaagaggcagaagaagactg ctgtacatcttcaagcagcctttcatgcggcctgtgcagaccaccaggaggaagatggctgctc ttgtcgatttccagaagaggaagaaggcggctgtgaactgagagtgaagttcagcagatccgcc gacgcccctgcttatcagcagggccagaaccagctgtataatgagctgaac ctgggcccggcggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatgg gcggaaaacctcaaagaaggaagaacccccaggagggactgtacaacgagctgcagaagga taagatggccgaggcctacagcgagatcgccatgaaaggagaaagaagagga ggccacgacggcctgtaccagggcctgagcaccgccaccaaggacacctacgacgccctgc acatgcaggccctgccacctaga
51	DAP10.5.1 (+sig domain, - FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLT DARCMIH LGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGKVF INMPGRGKRGRKKL LYIFKQPFMRPVQTTQEEDGC SCRFEEEEGGCEL RVKFSRSA DAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGK PQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
52	DAP10.6.1 (+sig domain, - FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgtgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgttctgctgctctgctgtggccgctgctcagaccaccctggag aaagatctagcctgcccgttctacccgggaccagcggcagctgcagc ggatgcggctctctgagcctgcctctgctggccggcctgctggccgccgatgctgtggccagcc tgctgatcgtgggcgcccgttctgtgctgcgcagaccagaagaagccctgccaggaggatg gc agagtgttt catcaacatgcctggcagaggcaaaagaggcagaagaagactg ctgtacatcttcaagcagcctttcatgcggcccgtgcagacaaccaggaggaggacggctgta gctgtagattccccgaggaagaagaaggcggctgcgagcttagagtgaagttcagcagaagcg ccgacgcccctgcttaccagcagggccagaaccagctgtataatgagctgaac ctggggaagaagggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatgg gcggaaaacctcaaagaaggaagaacctcaggagggcctgtacaacgagctgcagaaga caagatggccgaggcctacagcgagatcggcataaaggcgagagacggcggggcaag ggccacgacggcctgtaccaaggcctgtcaacagccaccaaggacacctacgacgccctgca catgcaggccctgccacctaga
53	DAP10.6.1 (+sig domain, - FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLT DARCMIH LGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGRVF INMPGRGKRGRKKL LYIFKQPFMRPVQTTQEEDGC SCRFEEEEGGCEL RVKFSRSA DAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGK PQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
54	DAP10.13.1 (+sig domain, - FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgtgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgtttctgctgctgcttctgtggccgctgctcagacaacacctggcga gagatctagcctgctgcttctatctggcaccagcggctctgttct ggctgtggatctctgagcctgcctctgctggctggactggttgcgtgctgatgctgtggcctctctgc tgattgtgggcgcccgttctgtgtgcccggcctagaagatctcccgtcaagaggatggc ag agt gtacatcaacatgcccggcagagga

55	DAP10.13.1 (+sig domain, - FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGRVYINMPGRG
56	DAP10.14.1 (+sig domain, - FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgttctgctgctgctgctgctgcccagaccaccctgga gaaagatctagccttcacgcttctaccccgggaccagcggaagctgcagc ggctgcggcagcttctctgctgctgctgcccggcctggtggcgcgcgacgctgttgcattct gctgatcgtgggcgcgccttctgtgctgcccagaccagacggagccctgctcaggaagatgg cagag gtgtacatcaacatgcctggcagaggcagagtgaagttcagcagaagc gccgatgcccctgctatcagcagggccagaaccagctgtacaacgagctgaacctgggcag acgcgaggaatacgcgtgctggacaagcggcggggacgggaccccgagatgggcggcaa gcctcaaagaaggaagaacccccaggaggcctgtataatgagctgcagaagataag atggccgaggcctacagcgagatcggcatgaaaggcgagagacggcggggcaagggccac gacggcctgtaccagggcctgagcaccgccacaagacacctacgcgcctgcacatgca ggcctgccacctaga
57	DAP10.14.1 (+sig domain, - FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTTP GERSSLP AFYPGTSGSCSGCSLSLPLL AGLVAADAVASLLIV GAVFLCARPRRSPA QEDGRVYINMPGRGRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGG KPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
58	DAP10.15.1 (+sig domain, - FP2A, -CD19t) Nucleic Acid	atgtccgtgcctaccaggtgctgggcctgctgctgctgtggctgaccgacgccagatgcatgat ccacctggggccacatcctgttctgctgctgctgctgcccgcgcgtcaaacaccctggcg agagatctagcctgccagccttctaccccggtacaagcggatcttgcagcggctgcggcagcct gtctctgctgctgctggcggcctggtgctgctgctgctgctgcccagcctgctgatcgtggcg ccgtgttctgtgcccagacctagaaggtccccgcccaggaggatgga agag gtgtacatcaa catgcctggcagaggcaagagaggcagaagaagctgctgtacatcttcaagcagccttcatg agaccagtgcagaccaccagaggaggacggatgtagctgcagattccccgaggaagaag aaggcggctgtgaactgagagtgaagttcagcagaagcgccgacgccctgcttatcagcagg gccagaaccagctgtacaacgagctgaacctgggcagaagggaaggtacgcagctgctggac aaggcggcggggacgggaccccgagatgggcggaaaacctcaagacggaagaacccccag gagggcctttataatgagctgcagaagataagatggccgaggcctacagcgagatcggcatg aaaggagaagacggcggggcaaaggccacgacggcctgtaccagggcctgagcaccgcc acaaaggacacctacgcgcctgcacatgcaggccctgccccccaga
59	DAP10.15.1 (+sig domain, - FP2A, -CD19t) Amino Acid	MSVPTQVLGLLLLWLT DARCMIHLGHILFLLLLPVAAAQTTPGER SSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPA QEDGRV YINMPGRGKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADA PAYQQGQNQLYNELN LGRREEYDVL DKRRGRDP EMGGKPQRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMOALPPR

60	DAPI0.3.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggcccacatcctgtttctgctgctgcttctgtggccgctgctcagacaacacctg gagagagatctagcctgccttctctcctggcaccagcggtcttctgttctggctgtggtatctct gagcctgcctctgctgctgactggttctgctgctgatgctgtggcc tctctgctgattgtggcgccgtgttctgtgtgcccggcctagaagatctcccgtcaagaggat ggcaaggtgtacatcaacatgcccggcagagggaagagtgaagttcagcagaagcgccgatgc ccctgcctatcagcagggccagaaccagctgtacaacgagctgaacctggggc agacgcgagggaatacagacgtgctggacaagcgcggggacgggaccccagatggcgggc aagcctcaaagaaggaagaacccccaggaggcctgtataatgagctgcagaaagataagat ggccgaggcctacagcagatcgccatgaaaggcagagacggcggggcaaggggccac gacggcctgtaccaggcctgagcaccgcccacaaggacacctacgacgcctgcacatgca ggcctgccacctaga
61	DAPI0.3.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLP LLAGLVAADAVASLLIVGAVFLCARPRRSPAQED GKVYINMPGRGRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQA LPPR
62	DAPI0.4.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggcccacatcctgttctgctgctgctgcttctgtggccgctcaacaacccct ggcgagagatctagcctgccagccttctaccccggtacaagcggtatctgcagcggtgctggc agcctgtctctgctgctgctggccgctggtggtgctgctgatgctgtggcc agcctgctgatcgtggcgccgtgttctgtgctgagacctaagaaggtcccctgcccaggag gatggaaaggtgtacatcaacatgctggcagaggcaagagaggcagaaagaagctgctgta catcttcaagcagcctttcatgagaccagtgcagaccaccaggaggagggacgga ttagctgcagattccccgagggaagaagaaggcgctgtgaactgagagtgaagttcagcaga agcgccgacgcccctgttatcagcaggccagaaccagctgtacaacgagctgaacctggg cagaagggaagagtagcagctgtggacaagcgcggggacgggaccccagatg ggcggaacacctaagaacggaagaacccccaggaggcctttataatgagctgcagaaaga taagatggcgaggcctacagcgagatcgccatgaaaggagaaagacggcggggcaaaaggc cacgacggcctgtaccaggcctgagcaccgcccacaaggacacctacgacgcctg cacatgcaggcctgtccccccaga
63	DAPI0.4.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLP LLAGLVAADAVASLLIVGAVFLCARPRRSPAQED GKVYINMPGRGKRGRKLLYIFKQPFMRPVQTTEEDGCSC RFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
64	DAPI0.5.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggacacatcctgttctgctgctgcttccagtggccgctcaacaacccct ggcgagagaagcagcctgcccgccttctaccccggtacatctggcagctgcagcggtgctgg cagcttgtctctgctctgctgctggcggactggtggctgctgatgccgtggcc agcctgctgatcgtggcgccgtgttctgtgctgagaccagaaaggtcccctgcccaggag gacggcaaggtgttcatcaacatgctggcagaggcaaaagaggcagaaagaagctgctgta catcttcaagcagcctttcatgcccctgtgagaccaccaggagggaagatggc tgctcttgcgatttcagaagaggaagaaggcgctgtgaactgagagtgaagttcagcagatc cgccgacgcccctgttatcagcagggccagaaccagctgtataatgagctgaacctgggccc

		gcggggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatg ggcggaaaacctcaaagaaggaagaacccccaggagggactgtacaacgagctgcagaagg ataagatggccgaggcctacagcagatcggcatgaaaggagaaaagaagaggaaaagg ccacgacggcctgtaccagggcctgagcaccgccaccaaggacacctacgacgcctg cacatgcaggccctgccacctaga
65	DAP10.5.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQED GKVFINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVL DKRRGRDP EMGGKPQRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
66	DAP10.6.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggccacatcctgttctctgctgctcctgctgcccgtgctcagaccacccct ggagaaagatctagcctgcccgtttctacccccgggaccagcggcagctgcagcggatgccc ctctctgagcctgcctctgctggccggcctctgggccgccgatgctgtggcc agcctgctgatcgtgggcgccgtgttctctgtgcgccagaccagaagaagccctgccaggag gatggc cagagtgtt catcaacatgcttgccagaggcaaaagaggcagaaagaagctgctgta catctcaagcagcctttcatgcccgtgctgagacaaccaggaggaggacggc tgtagctgtagattccccgaggaagaagaagcggctgctgagcttagagtgaagttcagcaga agcgcggacgcccctgcttaccagcaggggcagaaccagctgtataatgagctgaacctggga agaaggggaagagtacgacgtgctggacaagcggcggggcagagatcctgagatg ggcggaaaacctcaaagaaggaagaacctcaggagggcctgtacaacgagctgcagaaag acaagatggccgaggcctacagcagatcggcatgaaaggcgagagacggcggggcaagg gccacgacggcctgtaccaaggcctgtcaacagccaccaaggacacctacgacgcctg cacatgcaggccctgccacctaga
67	DAP10.6.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQED GRVFINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC R FPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVL DKRRGRDP EMGGKPQRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
68	DAP10.13.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggccacatcctgtttctgctgctgcttctgtggccgctgctcagacaacacctg gcgagagatctagcctgctgcttctatcctggcaccagcggtctgttctggctgtggatctct gagcctgctctgctggctggactggttctgctgctgatgctgtggcc tctctgctgattgtgggcgccgtgttctgtgtgcccgccctagaagatctcccgtcaagaggat ggc cagagt gtacatcaacatgcccggcagagga
69	DAP10.13.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQED GRVYINMPGRG
70	DAP10.14.2 (-sig domain, -	atgatccacctgggccacatcctgttctctgctgctgctgctgcccgtgcccagaccacccc tgagagaagatctagccttccagccttctacccccgggaccagcggaagctgcagcggctgcgg

	FP2A, -CD19t) Nucleic Acid	cagcttgctctgcctctgctggccggcctgggtggccgccgacgctgttgca tctctgctgatcgtggcgccgcttcttctgtgcgccagaccagacggagccctgctcaggaag atggc agag tgtacatcaacatgccttggcagaggcagagtgaagttcagcagaagcgccgatg cccctgcctatcagcaggggccagaaccagctgtacaacgagctgaacctgggcagacgcgag gaatacgacgtgctggacaagcggcggggacgggaccccgagatggcgcgcaagcctcaa gaaggaagaacccccaggagggcctgtataatgagctgcagaaagataagatggccgaggcc tacagcgagatcgccatgaaaggcgagagacggcggggcaagggccacgacggcctgtacc agggcctgagcacccgccacaaaggacacctacgacgcctgcacatgcaggccctgccacct aga
71	DAP10.14.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQED GRVYINMPGRGRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVL DKRRGRDPEMGGKPQRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
72	DAP10.15.2 (-sig domain, - FP2A, -CD19t) Nucleic Acid	atgatccacctgggcccacatcctgttctctgctgctgctgcctgtggccgccgctcaaaacaacctt ggcgagagatctagcctgccagccttctaccccggtacaagcggatcttgacggcgtgcggc agcctgtctctgcctctgctggccggcctggtggctgctgatgctgtggcc agcctgctgatcgtgggcccgtgttctgtgcccagacacctagaaggtcccctgcccaggag gatgg aa gagtgtacatcaacatgccttggcagaggcagaagagaggcagaagaagctgtgta catcttaagcagccttcatgagaccagtgcagaccaccaggaggaggacgga tgtagctgcagattccccgaggaagaagaaggcggctgtgaactgagagtgaagttcagcaga agcggccgacgcccctgtctatcagcaggggccagaaccagctgtacaacgagctgaacctggg cagaagggaagagtacgacgtgctggacaagcggcggggacgggaccccgagatg ggcggaaaacctcaaagacggaagaacccccaggagggccttataatgagctgcagaaaga taagatggccgaggcctacagcgagatcggcctgaaaggagaaagacggcgggggcaaaggc cacgacggcctgtaccagggcctgagcaccgccacaaaggacacctacgacgcctg cacatgcaggccctgccccccaga
73	DAP10.15.2 (-sig domain, - FP2A, -CD19t) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCS GCGSLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV YINMPGRGRGRKRL LYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSADA PAYQQGQNQLYNELN LGRREEYDVL DKRRGRDPEMGGKPQRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
74	Truncated EGFR (EGFRt) Amino Acid	RKVCNGIGIGIEFKDLSINATNIKHFKNCTSSIGDLHILPVAFR GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAF ENLEIIRGR TKQHGGQFSLAVVSLNITSLGLRSLKEISDGDV IISG NKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVC HALCSPEGCWGPEPRDCVSCRNVSRGRCV DKCNLLEGEPR EFVENSEC IOCHPECLPOAMNITCTGRGPDNCIOCAHYIDGPH

		CVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCT GPGLEGCP TNGPKIPSIATGMV GALLLLLVVALGIGLFM
75	Truncated EGFR (EGFRt) Nucleic acid	cgcaaaagtgtgaacggaataggattggtgaattaaagactcactctccataaatgctacgaata ttaaacttcaaaaactgcacccatcagtgccgatctccacatcctgccggtggcatttaggg gtgactcctcacacatactcctcctggtatccacaggaaactggatattctgaaaaccgtaaagg aaatcacagggttttctgattcaggcttggcctgaaaacaggacggacctccatgccttgaga acctagaatcatacgccgagcaggaccaagcaacatggtcagtttctcttcagtcgtcagcctg aacataacatccttgggattacgctccctcaaggagataagtgtatggagatgtgataattcagga aacaaaaatttgctatgcaaatacaataaactggaaaaaactgttgggacctccggtcagaaa accaaaattataagcaacagagggtgaaaacagctgcaaggccacaggccaggtctgccatgcc ttgtgctccccgaggggtgctggggcccgagccagggactgcgtctcttgcgggaatgtca gccgagggcagggaatgcgtggacaagtgcaccttctggagggtgagccaaggagttgtg gagaactctgagtgacatacagtgccaccagagtgccctcagggccatgaacatcacctgca caggacggggaccagacaactgtatccagtgtgcccactacattgacggccccactgcgtca agacctgccccgagggagtcagtggaagaaacaacacctggtctggaagtacgcagacgcc ggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggccaggtcttgaag gctgtccaacgaatgggcctaagatcccgctccatcgccactgggatggtgggggcccctctctt gctgctggtggtggccctggggatcgccctcttcagTGATAA
76	CD3z signaling domain polypeptide 3 (1XX)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPQRRKNPQEGLFNEQLQDKMAEAFSEIGMKGER RRGKGHDGLFQGLSTATKDTFDALHMQALPPR
77	DAP10.6.3 (+sig domain, +FP2A, +EGFRt) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLPAYPGTSGSCSGCG SLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPQRRKNPQEGLYNELQDKMAEAYS EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR RAKRS GSGATNFSLLKQAGDVEENPGPMLLLVTSLLCELPH PAFLIPRKVCNGIGIGEFKDSL SINATNIKHFNCT SISGDLHI LPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENR TDLHAFENLEIRGR TKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNK NLCYANTINWKKLFGTSGQKTKIISNRGENSCK ATGQVCHALCSPEGCWGPEDRCVSCRNVSRGRECVDKCNL LEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAH YIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCT TYGCTGPGLEGCP TNGPKIPSIATGMV GALLLLLVVALGIGLF M
78	DAP10.6.3 (+sig domain, +FP2A, +EGFRt) Nucleic Acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGGCGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCAGG

		AGGATGGCAGAGTGTTCATCAACATGCCTGGCAGAGGCA AAAGAGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGC CTTTCATGCGGCCCGTGCAGACAACCCAGGAGGAGGACG GCTGTAGCTGTAGATTCCCCGAGGAAGAAGAAGGCGGCT GCGAGCTTAGAGTGAAGTTCAGCAGAAGCGCCGACGCC CTGCTTACCAGCAGGGCCAGAACCAGCTGTATAATGAGCT GAACCTGGGAAGAAGGGAAGAGTACGACGTGCTGGACAA GCGGCGGGGCAGAGATCCTGAGATGGGCGGAAAACCTCA AAGAAGGAAGAACCCTCAGGAGGGCCTGTACAACGAGCT GCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGG CATGAAAGGCGAGAGACGGCGGGGCAAGGGCCACGACGG CCTGTACCAAGGCCTGTCAACAGCCACCAAGGACACCTAC GACGCCCTGCACATGCAGGCCCTGCCACCTAGACGCGCGA AGCGATCAGGCAGCGGGGCGACAAATTTACGCCTTCTGAA ACAAGCAGGCGACGTGGAAGAAAACCCCGGTCCAATGCT TCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACC CAGCATTCCTCCTGATCCCACGCAAAGTGTGTAACGGAAT AGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCT ACGAATATTAACACTTCAAAAACCTGCACCTCCATCAGTG GCGATCTCCACATCCTGCCGGTGGCATTAGGGGTGACTC CTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGAT ATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGA TTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCCTT TGAGAACCTAGAAATCATAACGCGGCAGGACCAAGCAACA TGGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACAT CCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATGGAGA TGTGATAATTTACAGGAAACAAAAATTTGTGCTATGCAAAT ACAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGA AAACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCA AGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGA GGGCTGCTGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGC CGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGC AACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACT CTGAGTGCATACAGTGCCACCCAGAGTGCTGCCTCAGGC CATGAACATCACCTGCACAGGACGGGGACCAGACAACCTG TATCCAGTGTGCCCCTACATTGACGGCCCCCACTGCGTC AAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACC CTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACC TGTGCCATCCAACTGCACCTACGGATGCACTGGGCCAGG TCTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCC ATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGG TGGTGGCCCTGGGGATCGGCCTCTTCATGTGATAA
79	DAP10.16 Amino acid	MIHLGHILFLLLLPVAAAQTTPGERSSLPAYPGTSGSCSGCG SLSLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE

		EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPENMGKPKRRKNPQEGLFNELQKDKMAEAFS EIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPRR AKRSGSGATNFSLLKQAGDVEENPGMPPPRLLFFLLFLTPM EVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESP LKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQP GPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCKLNR SSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLN QSLSQDLTMAPGSTLWLSGCVPPDSVSRGPLSWTHVHPKGP KSLLSLELKDDRPARDMWVWVMTGLLLPRATAQDAGKYCH RGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFC LCSLVGILHLQRALVLRKRKRMTDPTRRF
80	DAP10.16 Nucleic acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGCGGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCAGG AGGATGGCAGAGTGTTTCATCAACATGCCTGGCAGAGGCA AAAGAGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGC CTTTCATGCGGCCCGTGCAGACAACCCAGGAGGAGGACG GCTGTAGCTGTAGATTCCCCGAGGAAGAAGAAGGCGGCT GCGAGCTTCGAGTGAAGTTCAGTAGAAGCGCCGACGCCCC TGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCAGACGCGAGGAATACGACGTGCTGGACAAG CGGCGGGGCAGAGATCCTGAGATGGGCGGAAAACCTCAA AGAAGAAAGAACCCCCAGGAGGGCCTGTTCAACGAGCTG CAGAAAGATAAGATGGCCGAGGCCTTCAGCGAGATCGGC ATGAAAGGCGAGAGACGACGTGGAAAGGGCCACGACGGC TTGTTTCAGGGCCTGAGCACCGCCACAAAGGACACCTTCG ACGCCCTGCACATGCAGGCCCTCCCCCAGACGCGCGAA GCGATCAGGCAGCGGGGCGACAAATTCAGCCTTCTGAAA CAAGCAGGCGACGTGGAAGAAAACCCCGGTCCAATGCCT CCTCCTCGGCTGCTGTTCTTCCTGCTGTTTCTGACCCCTAT GGAAGTGCGGCCCGAGGAACCTCTGGTGGTCAAAGTTGA AGAGGGCGACAACGCCGTGCTGCAGTGTCTGAAGGGCAC ATCTGATGGCCCCACACAGCAGCTGACCTGGTCTAGAGAG AGCCCTCTGAAGCCCTTCCTGAAGCTGTCTCTGGGACTGC CTGGACTGGGCATCCATATGAGGCCTCTGGCCATCTGGCT GTTTCATCTTCAACGTGTCCAGCAGATGGGCGGCTTCTAC CTGTGTCAACCTGGACCTCCAAGCGAGAAGGCTTGGCAGC CTGGCTGGACCGTGAATGTGGAAGGATCCGGCGAGCTGTT CCGGTGGAATGTGTCTGATCTCGGCGGCCTCGGATGCGGC CTGAAGAATAGATCTAGCGAGGGCCCTAGCAGCCCCAGC GGAAAACCTGATGAGCCCCAAGCTGTACGTGTGGGCCAAA GACAGACCCGAGATTTGGGAGGGCGAGCCTCCTTGTCTGC

		CTCCTAGAGACAGCCTGAACCAGAGCCTGAGCCAGGACCT GACAATGGCCCCTGGATCTACACTGTGGCTGAGCTGTGGC GTGCCACCTGACAGTGTGTCTAGAGGCCCTCTGTCTTGA CCCACGTGCACCCTAAGGGCCCTAAGTCTCTGCTGAGCCT GGAAGTGAAGGACGACAGGCCCGCCAGAGATATGTGGGT CATGGAAACAGGCCTGCTGCTGCCTAGAGCCACAGCACAG GATGCCGGCAAGTACTACTGCCACAGAGGCAACCTGACCA TGAGCTTCCACCTGGAAATCACCGCCAGACCTGTCCTGTG GCACTGGCTGCTTAGAACCGGCGGCTGGAAAGTGTCTGCC GTGACTCTGGCCTACCTGATCTTCTGCCTGTGTAGCCTCGT GGGCATCCTGCATCTGCAGAGAGCACTGGTCCTGCGGCGG AAGCGGAAGAGAATGACCGATCCTACCAGACGGTTCTGAT AA
81	DAP10.16.1 (+sig domain, - FP2A, -CD19) Amino acid	MIHLGHILFLLLLPVAAAQTTPGERSSLP AFYPGTSGSCSGCG SLSLPLL AGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDP EMGGKPQRRKNPQEGLFNELQKDKMAEAFS EIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR
82	DAP10.16.1 (+sig domain, - FP2A, -CD19) Nucleic acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGGCGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCCAGG AGGATGGCAGAGTGTTCATCAACATGCCTGGCAGAGGCA AAAGAGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGC CTTTCATGCGGCCCGTGCAGACAACCCAGGAGGAGGACG GCTGTAGCTGTAGATTCCCCGAGGAAGAAGAAGGCGGCT GCGAGCTTCGAGTGAAGTTCAGTAGAAGCGCCGACGCCCC TGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCAGACGCGAGGAATACGACGTGCTGGACAAG CGGCGGGGCAGAGATCCTGAGATGGGCGGAAAACCTCAA AGAAGAAAGAACCCCCAGGAGGGCCTGTTCAACGAGCTG CAGAAAGATAAGATGGCCGAGGCCTTCAGCGAGATCGGC ATGAAAGGCGAGAGACGACGTGGAAAGGGCCACGACGGC TTGTTTCAGGGCCTGAGCACCGCCACAAAGGACACCTTCG ACGCCCTGCACATGCAGGCCCTCCCCCCCAGA
83	DAP10.16.2 (- sig domain, - FP2A, -CD19) Amino acid	QTTPGERSSLP AFYPGTSGSCSGCGSLSLPLL AGLVAADAVAS LLIVGAVFLCARPRRSPAQEDGRVFINMPGRGKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPQ RRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLF QGLSTATKDTFDALHMQALPPR
84	DAP10.16.2 (-	CAGACCACCCCTGGAGAAAGATCTAGCCTGCCCCGCTTTCT

	sig domain, - FP2A, -CD19) Nucleic acid	ACCCCGGGACCAGCGGCAGCTGCAGCGGATGCGGCTCTCT GAGCCTGCCTCTGCTGGCCGGCCTCGTGGCCGCCGATGCT GTGGCCAGCCTGCTGATCGTGGGCGCCGTGTTCTGTGCG CCAGACCCAGAAGAAGCCCTGCCAGGAGGATGGCAGAG TGTTTCATCAACATGCCTGGCAGAGGCAAAAAGAGGCAGAA AGAAGCTGCTGTACATCTTCAAGCAGCCTTTCATGCGGCC CGTGCAGACAACCCAGGAGGAGGACGGCTGTAGCTGTAG ATTCCCCGAGGAAGAAGAAGGCGGCTGCGAGCTTCGAGT GAAGTTCAGTAGAAGCGCCGACGCCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCAGA CGCGAGGAATACGACGTGCTGGACAAGCGGCGGGGCAGA GATCCTGAGATGGGCGGAAAACCTCAAAGAAGAAAGAAC CCCCAGGAGGGCCTGTTCAACGAGCTGCAGAAAGATAAG ATGGCCGAGGCCTTCAGCGAGATCGGCATGAAAGGCGAG AGACGACGTGGAAGGGGCCACGACGGCTTGTTCAGGGC CTGAGCACCGCCACAAAGGACACCTTCGACGCCCTGCACA TGCAGGCCCTCCCCCCCAGA
85	DAP10.16.3 (+sig domain, +FP2A, +EGFRt) Amino Acid	MIHLGHILFLLLLPVAAAQTTPGERSSLPAYPGTSGSCSGCG SLSPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPGEMGGKPQRRKNPQEGLFNELQDKMAEAFS EIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPRR AKRSGSGATNFSLLKQAGDVEENPGPMLLLVTSLLLCELPH AFLIPRKVCNGIGIGEFKDSLSINATNIHKFNCTSSGDLHIL PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRT DLHAFENLEIIRGRTKQHGFSLAVVSLNITSLGLRSLKEISDG DVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKAT GQVCHALCSPEGCWGPEPRDCVSCRNVSRGECVDKCNLLE GEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYI DGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCT YGCTGPGLEGCPNTPGPKIPSIATGMV GALLLLL VVALGIGLF M
86	DAP10.16.3 (+sig domain, +FP2A, +EGFRt) Nucleic Acid	ATGATCCACCTGGGCCACATCCTGTTCTGTGCTCCTGCC TGTGGCCGCTGCTCAGACCAACCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGGCGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCAGG AGGATGGCAGAGTGTTTCATCAACATGCCTGGCAGAGGCA AAAGAGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGC CTTTCATGCGGCCCCGTGCAGACAACCCAGGAGGAGGACG GCTGTAGCTGTAGATTCCCCGAGGAAGAAGAAGGCGGCT GCGAGCTTCGAGTGAAGTTCAGTAGAAGCGCCGACGCCCC TGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCAGACGCGAGGAATACGACGTGCTGGACAAG

		CGGCGGGGCAGAGATCCTGAGATGGGCGGAAAACCTCAA AGAAGAAAGAACCCCCAGGAGGGCCTGTTCAACGAGCTG CAGAAAGATAAGATGGCCGAGGCCTTCAGCGAGATCGGC ATGAAAGGCGAGAGACGACGTGGAAAGGGCCACGACGGC TTGTTTCAGGGCCTGAGCACCGCCACAAAGGACACCTTCG ACGCCCTGCACATGCAGGCCCTCCCCCAGACGCGCGAA GCGATCAGGCAGCGGGGCGACAAATTTTCAGCCTTCTGAAA CAAGCAGGCGACGTGGAAGAAAACCCCGGTCCAATGCTT CTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCC AGCATTCCCTCCTGATCCCACGCAAAGTGTGTAACGGAATA GGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTA CGAATATTAACACTTCAAAAAGTGCACCTCCATCAGTGG CGATCTCCACATCCTGCCGGTGGCATTAGGGGTGACTCC TTCACACATACTCCTCCTCTGGATCCACAGGAAGTGGATA TTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGTGAT TCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCCTTT GAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACAT GGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACATC CTTGGGATTACGCTCCCTCAAGGAGATAAGTGATGGAGAT GTGATAATTTTCAGGAAACAAAAATTTGTGCTATGCAAATA CAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAA AACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCAA GGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCGAG GGCTGCTGGGGCCCGGAGCCAGGGACTGCGTCTCTTGCC GGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCA ACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTC TGAGTGCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCC ATGAACATCACCTGCACAGGACGGGGACCAGACAACCTGT ATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCA AGACCTGCCCCGGCAGGAGTCATGGGAGAAAACAACACCC TGGTCTGGAAGTACGCAGACGCCGCCATGTGTGCCACCT GTGCCATCCAACTGCACCTACGGATGCACTGGGCCAGGT CTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCA TCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGT GGTGGCCCTGGGGATCGGCCTCTTCATGTGATAA
87	DAP10.17 Amino acid	MIHLGHILFLLLPVAAAQTTPGERSSLPFYPGTSGSCSGCG SLSLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPGEMGGKPQRRKNPQEGLFNELQKDKMAEAFS EIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPRR AKRSGSGATNFSLLKQAGDVEENPGMPMPRLFFLLFLTPM EVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESP LKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSSQMGGFYLCQP GPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCGLKNR SSEGPPSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSL NQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGP

		KSLLSLELKDDRPARDMWVMMETGLLLPRATAQDAGKYCH RGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFC LCSLVGILHLQRALVLRRKRKRMTDPTRRF
88	DAP10.17 Nucleic acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGGCGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCCAGG AGGATGGCAGAGTGTTCATCAACATGCCTGGCAGAGGCCG AGTGAAGTTCAGTAGAAGCGCCGACGCCCTGCCTACCAG CAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGC AGACGCGAGGAATACGACGTGCTGGACAAGCGGCGGGGC AGAGATCCTGAGATGGGCGGAAAACCTCAAAGAAGAAAG AACCCCCAGGAGGGCCTGTTCAACGAGCTGCAGAAAGAT AAGATGGCCGAGGCCTTCAGCGAGATCGGCATGAAAGGC GAGAGACGACGTGGAAAGGGCCACGACGGCTTGTTTCAG GGCCTGAGCACCGCCACAAAGGACACCTTCGACGCCCTGC ACATGCAGGCCCTCCCCCCCAGACGCGCGAAGCGATCAGG CAGCGGGGCGACAAATTCAGCCTTCTGAAACAAGCAGGC GACGTGGAAGAAAACCCCGGTCCAATGCCTCCTCCTCGGC TGCTGTTCTTCTGCTGTTTCTGACCCCTATGGAAGTGCGG CCCGAGGAACCTCTGGTGGTCAAAGTTGAAGAGGGCGAC AACGCCGTGCTGCAGTGTCTGAAGGGCACATCTGATGGCC CCACACAGCAGCTGACCTGGTCTAGAGAGAGCCCTCTGAA GCCCTTCCTGAAGCTGTCTCTGGGACTGCCTGGACTGGGC ATCCATATGAGGCCTCTGGCCATCTGGCTGTTTCATCTTCAA CGTGTCCCAGCAGATGGGCGGCTTCTACCTGTGTCAACCT GGACCTCCAAGCGAGAAGGCTTGGCAGCCTGGCTGGACC GTGAATGTGGAAGGATCCGGCGAGCTGTTCCGGTGGAATG TGTCTGATCTCGGCGGCCTCGGATGCGGCCTGAAGAATAG ATCTAGCGAGGGCCCTAGCAGCCCCAGCGGAAAACCTGAT GAGCCCCAAGCTGTACGTGTGGGCCAAAGACAGACCCGA GATTTGGGAGGGCGAGCCTCCTTGTCTGCCTCCTAGAGAC AGCCTGAACCAGAGCCTGAGCCAGGACCTGACAATGGCC CCTGGATCTACACTGTGGCTGAGCTGTGGCGTGCCACCTG ACAGTGTGTCTAGAGGCCCTCTGTCTTGACCCACGTGCA CCCTAAGGGCCCTAAGTCTCTGCTGAGCCTGGAAGTGAAG GACGACAGGCCCCGCCAGAGATATGTGGGTCTATGGAAACA GGCCTGCTGCTGCCTAGAGCCACAGCACAGGATGCCGGCA AGTACTACTGCCACAGAGGCAACCTGACCATGAGCTTCCA CCTGGAAATCACCGCCAGACCTGTCCTGTGGCACTGGCTG CTTAGAACC GGCGGCTGGAAAGTGTCTGCCGTGACTCTGG CCTACCTGATCTTCTGCCTGTGTAGCCTCGTGGGCATCCTG CATCTGCAGAGAGCACTGGTCCTGCGGCGGAAGCGGAAG AGAATGACCGATCCTACCAGACGGTTCTGATAA

89	DAP10.17.1 (+sig domain, - FP2A, -CD19) Amino acid	MIHLGHILFLLLLPVAAAQTTPGERSSLPFYPGTSGSCSGCG SLSLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPQRRKNPQEGLFNELQKDKMAEAFS EIGMKGERRRRGK GHDGLFQGLSTATKDTFDALHMQALPPR
90	DAP10.17.1 (+sig domain, - FP2A, -CD19) Nucleic acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGGCGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCAGG AGGATGGCAGAGTGTTCATCAACATGCCTGGCAGAGGCCG AGTGAAGTTCAGTAGAAGCGCCGACGCCCTGCCTACCAG CAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGC AGACGCGAGGAATACGACGTGCTGGACAAGCGGCGGGGC AGAGATCCTGAGATGGGCGGAAAACCTCAAAGAAGAAAG AACCCCCAGGAGGGCCTGTTCAACGAGCTGCAGAAAGAT AAGATGGCCGAGGCCTTCAGCGAGATCGGCATGAAAGGC GAGAGACGACGTGGAAAGGGCCACGACGGCTTGTTTCAG GGCCTGAGCACCGCCACAAAGGACACCTTCGACGCCCTGC ACATGCAGGCCCTCCCCCCCAGA
91	DAP10.17.2 (- sig domain, - FP2A, -CD19) Amino acid	QTPGERSSLPFYPGTSGSCSGCGSLSLPLLAGLVAADAVAS LLIVGAVFLCARPRRSPAQEDGRVFINMPGRGRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQ RRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRRGK GHDGLF QGLSTATKDTFDALHMQALPPR
92	DAP10.17.2 (- sig domain, - FP2A, -CD19) Nucleic acid	CAGACCACCCCTGGAGAAAGATCTAGCCTGCCCCGCTTTCT ACCCCGGGACCAGCGGCAGCTGCAGCGGATGCGGCTCTCT GAGCCTGCCTCTGCTGGCCGGCCTCGTGGCCGCCGATGCT GTGGCCAGCCTGCTGATCGTGGGCGCCGTGTTCTGTGCG CCAGACCCAGAAGAAGCCCTGCCAGGAGGATGGCAGAG TGTTTCATCAACATGCCTGGCAGAGGCCGAGTGAAGTTCAG TAGAAGCGCCGACGCCCTGCCTACCAGCAGGGCCAGAA CCAGCTGTACAACGAGCTGAACCTGGGCAGACGCGAGGA ATACGACGTGCTGGACAAGCGGCGGGGCAGAGATCCTGA GATGGGCGGAAAACCTCAAAGAAGAAAGAACCCCCAGGA GGCCTGTTCAACGAGCTGCAGAAAGATAAGATGGCCGA GGCCTTCAGCGAGATCGGCATGAAAGGCGAGAGACGACG TGGAAGGGGCCACGACGGCTTGTTTCAGGGCCTGAGCACC GCCACAAAGGACACCTTCGACGCCCTGCACATGCAGGCCC TCCCCCCCAGA
93	DAP10.17.3 (+sig domain, +FP2A, +EGFRt)	MIHLGHILFLLLLPVAAAQTTPGERSSLPFYPGTSGSCSGCG SLSLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQEDGRV FINMPGRGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPQRRKNPQEGLFNELQKDKMAEAFS

	Amino Acid	EIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPRR AKRSGSGATNFSLLKQAGDVEENPGPMLLLVTSLLLCELPH AFLIPRKVCNIGIGIGEFKDSLINATNIKHFNCTSSISGDLHIL PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRT DLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDG DVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKAT GQVCHALCSPEGCWGPEPRDCVSCRNVSRGRCVDKCNLLE GEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYI DGPCHVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCT YGCTGPGLEGCPNGPKIPSIATGMVGAALLLLLVVALGIGLF M
94	DAP10.17.3 (+sig domain, +FP2A, +EGFRt) Nucleic Acid	ATGATCCACCTGGGCCACATCCTGTTCTGCTGCTCCTGCC TGTGGCCGCTGCTCAGACCACCCCTGGAGAAAGATCTAGC CTGCCCCGCTTTCTACCCCGGGACCAGCGGCAGCTGCAGCG GATGCGGCTCTCTGAGCCTGCCTCTGCTGGCCGGCCTCGT GGCCGCCGATGCTGTGGCCAGCCTGCTGATCGTGGCGGCC GTGTTCTGTGCGCCAGACCCAGAAGAAGCCCTGCCAGG AGGATGGCAGAGTGTTCATCAACATGCCTGGCAGAGGCCG AGTGAAGTTCAGTAGAAGCGCCGACGCCCTGCCTACCAG CAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGC AGACGCGAGGAATACGACGTGCTGGACAAGCGGCGGGGC AGAGATCCTGAGATGGGCGGAAAACCTCAAAGAAGAAAG AACCCCCAGGAGGGCCTGTTCAACGAGCTGCAGAAAGAT AAGATGGCCGAGGCCTTCAGCGAGATCGGCATGAAAGGC GAGAGACGACGTGGAAGGGCCACGACGGCTTGTTTCAG GGCCTGAGCACCGCCACAAAGGACACCTTCGACGCCCTGC ACATGCAGGCCCTCCCCCCCAGACGCGCGAAGCGATCAGG CAGCGGGGCGACAAATTCAGCCTTCTGAAACAAGCAGGC GACGTGGAAGAAAACCCCGGTCCAATGCTTCTCCTGGTGA CAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCTC CTGATCCCACGCAAAGTGTGTAACGGAATAGGTATTGGTG AATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAA ACACTTCAAAAACCTGCACCTCCATCAGTGGCGATCTCCAC ATCCTGCCGGTGGCATTAGGGGTGACTCCTTCACACATA CTCCTCCTCTGGATCCACAGGAACTGGATATTCTGAAAAC CGTAAAGGAAATCACAGGGTTTTTGTGATTACAGGCTTGG CCTGAAAACAGGACGGACCTCCATGCCTTTGAGAACCTAG AAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTT TCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTA CGCTCCCTCAAGGAGATAAGTGATGGAGATGTGATAATTT CAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTG GAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATT ATAAGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGC CAGGTCTGCCATGCCTTGTGCTCCCCGAGGGCTGCTGGG GCCCCGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTGAG CCGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTGGA

		GGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCCAGTGTGCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCCG GCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAA ACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCCTAAGATCCCGTCCATCGCCACTGGG ATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTGG GGATCGGCCTCTTCATGTGATAA
95	NKG2D	MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPVVKSKCRENASPFFCFIAVAMGIRFIIMVAIWSAVFLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDESKNWWYESQASCMSQNASLLKVYSKEDQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIEMQKGDICALYASSFKGYIENCSTPN TYICMQRTV
96	NKG2D transmembrane domain	PFFFCFIAVAMGIRFIIMVA
97	NKG2D ligand-binding domain and transmembrane domain	PFFFCFIAVAMGIRFIIMVAIWSAVFLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDESKNWWYESQASCMSQNASLLKVYSKEDQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIEMQKGDICALYASSFKGYIENCSTPN TYICMQRTV
98	Truncated LNGFR signal peptide Amino Acid	MGAGATGRAMDGPRLLLLLLLGVSLGGA
99	Truncated LNGFR signal peptide Nucleic Acid	ATGGGAGCTGGTGCTACAGGCAGAGCCATGGATGGACCTAGACTGCTGCTGCTCCTGCTGCTTGGAGTGTCTCTTGCGGAGCC
100	Truncated LNGFR Amino Acid	KEACPTGLYTHSGECCACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCA YGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEANHVDPLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPPEGSDSTAPSTQEPEAPPEQDLIASTVAGVVTTVM GSSQPVVTRGTTDNLPVYCSILAAVVVGLVAYIAFKR
101	Truncated LNGFR Nucleic Acid	AAAGAGGCCTGTCTACCGGCCTGTATACAACTCTGGCGAGTGCTGCAAGGCCTGCAATCTTGGAGAAGGCGTGGCAGCCTTGCGGCGCTAATCAGACAGTGTGCGAGCCTTGCCCTG GACAGCGTGACCTTTAGCGACGTGGTGTCTGCCACCGAGCATGCAAGCCTGTACCGAGTGTGTGGGCCTGCAGAGCATGTCTGCCCCTTGTGTGGAAGCCGACGATGCCGTGTGTAGATGCGCCTACGGCTACTACCAGGACGAGACAACAGGCAGA

		TCGAGAGCCTGTAGAGTGTGTGAAGCCGGCTCTGGACTGG TGTTCAGCTGCCAAGACAAGCAGAACACCGTGTGCGAGG AATGCCCCGATGGCACCTATAGCGACGAGGCCAACCATGT GGATCCCTGCCTGCCTTGTACTGTGTGCGAAGATACCGAG CGGCAGCTGCGCGAGTGTACAAGATGGGCTGATGCCGAGT GCGAAGAGATCCCCGGCAGATGGATCACCAGAAGCACAC CTCCAGAGGGCAGCGATAGCACAGCCCCCTTCTACACAAGA GCCCGAGGCTCCTCCTGAGCAGGATCTGATTGCCTCTACA GTGGCCGGCGTGGTCACAACAGTGATGGGATCTTCTCAGC CCGTGGTCACCAGAGGCACCACCGACAATCTGATCCCCGT GTACTGTAGCATCCTGGCCGCCGTGGTTGTGGGACTCGTG GCCTATATCGCCTTCAAGAGATGATAA
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Table 3: SEQ ID NO: 22 Annotation	
Description	Nucleotide numbering
Signal Peptide	1-60
DAP10.0	61-339
Furin P2A	340-420
Truncated CD19	421-1419

Table 4: SEQ ID NO: 24 Annotation	
Description	Nucleotide Numbering
Signal Peptide	1-60
DAP10.3 (DAP10.0 + CD3z)	61-678
DAP10.0	61-339
CD3z Signaling domain	340-678
Furin P2A	679-759
Truncated CD19	760-1758

Table 5: SEQ ID NO: 26 Annotation	
Description	Nucleotide Numbering
Signal Peptide	1-60
DAP10.4 (DAP10.0 + 4-1BB + CD3z)	61-804
DAP10.0	61-339
4-1BB Co-stimulatory Domain	340-465
CD3z Signaling domain	466-804
Furin P2A	805-885
Truncated CD19	886-1884

Table 6: SEQ ID NO: 28 Annotation

Description	Nucleotide Numbering
Signal Peptide	1-60
DAP10.5 (DAP10-Y86F + 4-1BB + CD3z)	61-804
DAP10-Y86F (Mutation in bold and underline)	61-339
4-1BB Co-stimulatory Domain	340-465
CD3z Signaling Domain	466-804
Furin P2A	805-885
Truncated CD19	886-1884

Table 7: SEQ ID NO: 30 Annotation	
Description	Nucleotide Numbering
Signal Peptide	1-60
DAP10.6 (DAP1-K84R-Y86F + 4-1BB + CD3z)	61-804
DAP10-K84R-Y86F (Mutations in bold and underline)	61-339
4-1BB Co-stimulatory Domain	340-465
CD3z Signaling Domain	466-804
Furin P2A	805-885
Truncated CD19	886-1884

Table 8: SEQ ID NO: 32 Annotation	
Description	Nucleotide Numbering
Signal Peptide	1-60
DAP10.13 (DAP10-K84R) (Mutation in bold and underline)	61-339
Furin P2A	340-420
Truncated CD19	421-1419

Table 9: SEQ ID NO: 34 Annotation	
Description	Numbering
Signal Peptide	1-60
DAP10.14 (DAP10-K84R + CD3z)	61-678
DAP10-K84R (Mutation in bold and underline)	61-339
CD3z Signaling domain	340-678
Furin P2A	679-759
Truncated CD19	760-1758

Table 10: SEQ ID NO: 36 Annotation

Description	Numbering
Signal Peptide	1-60
DAP10.15 (DAP10-K84R + 4-1BB + CD3z)	61-804
DAP10-K84R (Mutation in bold and underline)	61-339
4-1BB Co-stimulatory Domain	340-465
CD3z Signaling domain	466-804
Furin P2A	805-885
Truncated CD19	886-1884

Table 11: SEQ ID NO: 78 Annotation	
Description	Numbering
Signal Peptide	1-54
DAP10.6 (DAP10-K84R-Y86F-41BB-CD3z)	1-744
DAP10-K84R-Y86F	1-279
K84R	250-252
Y86F	256-258
4-1BB Co-stimulatory Domain	280-405
CD3z Signaling domain	406-744
Furin P2A	745-825
GMCSFR alpha chain signal sequence	826-891
Truncated EGFR	892-1896

Table 12: SEQ ID NO: 80 Annotation	
Description	Numbering
Signal Peptide	1-54
DAP10.16 (DAP10-K84R-Y86F-41BB-1XX mutant with Y>F in ITAMS 2&3)	1-744
DAP10-K84R-Y86F	1-279
K84R	250-252
Y86F	256-258
4-1BB Co-stimulatory Domain	280-405
1XX mutant with Y>F in ITAM 2&3	406-744
Y>F in ITAM2	583-585
Y>F in ITAM2	619-621
Y>F in ITAM3	676-678
Y>F in ITAM3	709-711
Furin P2A	745-825
CD19 signal sequence	826-885
Truncated CD19	826-1824

Table 13: SEQ ID NO: 86 Annotation

Description	Numbering
Signal Peptide	1-54
DAP10.16 (DAP10-K84R-Y86F-41BB-1XX mutant with Y>F in ITAMS 2&3)	1-744
DAP10-K84R-Y86F	1-279
K84R	250-252
Y86F	256-258
4-1BB Co-stimulatory Domain	280-405
1XX mutant with Y>F in ITAM 2&3	406-744
Y>F in ITAM2	583-585
Y>F in ITAM2	619-621
Y>F in ITAM3	676-678
Y>F in ITAM3	709-711
Furin P2A	745-825
GMCSFR alpha chain signal sequence	826-891
Truncated EGFR	892-1896

Table 14: SEQ ID NO: 88 Annotation	
Description	Numbering
Signal Peptide	1-54
DAP10.17 (DAP10-K84R-Y86F-1XX mutant with Y>F in ITAMS 2&3 without 41BB)	1-618
DAP10-K84R-Y86F	1-279
K84R	250-252
Y86F	256-258
1XX mutant with Y>F in ITAM 2&3	280-618
Y>F in ITAM2	457-459
Y>F in ITAM2	493-495
Y>F in ITAM3	550-552
Y>F in ITAM3	583-585
Furin P2A	619-699
CD19 Signal Sequence	700-759
Truncated CD19	766-1698

Table 15: SEQ ID NO: 94 Annotation	
Description	Numbering
Signal Peptide	1-54
DAP10.17 (DAP10-K84R-Y86F-1XX mutant with Y>F in ITAMS 2&3 without 41BB)	1-618
DAP10-K84R-Y86F	1-279
K84R	250-252
Y86F	256-258

IXX mutant with Y>F in ITAM 2&3	280-618
Y>F in ITAM2	457-459
Y>F in ITAM2	493-495
Y>F in ITAM3	550-552
Y>F in ITAM3	583-585
Furin P2A	619-699
GMCSFR alpha chain signal sequence	700-765
Truncated EGFR	766-1770

CLAIMS

1. An isolated nucleic acid encoding a chimeric adaptor (CAD) polypeptide, wherein the CAD polypeptide comprises a DAP10 domain comprising a human DAP10 amino acid sequence and at least one costimulatory domain, wherein said CAD polypeptide specifically lacks an ectodomain comprising a functional extracellular receptor and/or ligand-binding domain.
2. The isolated nucleic acid according to claim 1, wherein the costimulatory domain is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD3C, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD70, CD80, CD83, CD86, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), FcR, LAT, NKD2C, SLP76, TRIM, and ZAP70, or combinations thereof.
3. The isolated nucleic acid according to claim 2, wherein the at least one costimulatory domain is 4-1BB.
4. The isolated nucleic acid according to claim 2, wherein the at least one costimulatory domain is CD28.
5. The isolated nucleic acid according to any one of claims 1-4, wherein said CAD polypeptide further comprises at least one intracellular signaling domain, wherein the at least one intracellular signaling domain is selected from CD3 ζ , DAP12, LFA-1, and CD3t.
6. The isolated nucleic acid according to claim 5, wherein the at least one signaling domain is CD3 ζ , optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
7. The isolated nucleic acid according to any one of claims 1-6, wherein the at least one costimulatory domain is 4-1BB and the intracellular signaling domain is CD3 ζ .

8. The isolated nucleic acid according to claim 7, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD3 ζ intracellular signaling domain.
9. The isolated nucleic acid according to claim 1, wherein said CAD polypeptide comprises a 4-1BB costimulatory domain and a CD28 costimulatory domain.
10. The isolated nucleic acid according to claim 9, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain, optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
11. The isolated nucleic acid according to claim 9, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the CD28 costimulatory domain followed by the 4-1BB costimulatory domain, followed in turn by a CD3 ζ signaling domain, optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
12. The isolated nucleic acid according to any one of claims 1-11, wherein the human DAP10 amino acid sequence comprises an amino acid sequence having at least 90%, 95%, 97%, or 99% sequence identity to SEQ ID NO: 1.
13. The isolated nucleic acid according to claim 12, wherein the human DAP10 amino acid sequence comprises a mutated human DAP10 amino acid sequence.
14. The isolated nucleic acid according to claim 13, wherein the mutated human DAP10 amino acid sequence comprises amino acid substitutions at positions corresponding to K84 and/or Y86.
15. The isolated nucleic acid according to claim 14, wherein the amino acid substitution at position K84 comprises a K84R substitution.

16. The isolated nucleic acid according to claim 14 or claim 15, wherein the amino acid substitution at position Y86 comprises a Y86F substitution.
17. The isolated nucleic acid according to any one of claims 1-16, wherein the isolated nucleic acid is operably linked to a regulatable promoter.
18. The isolated nucleic acid according to any one of claims 1-17, wherein said isolated nucleic acid further encodes for a cytokine.
19. The isolated nucleic acid according to claim 18, wherein the cytokine is selected from the group consisting of IL-2, IL-4, IL-7, IL-15, IL-21, IL-23.
20. An expression vector comprising the isolated nucleic acid of any one of claims 1-19.
21. A chimeric adaptor (CAD) polypeptide encoded by the isolated nucleic acid according to any one of claims 1-19, or the expression vector according to claim 20.
22. A mammalian cell comprising the expression vector of claim 20, or the CAD polypeptide according to claim 21, wherein the mammalian cell expresses at least one receptor that associates with DAP10.
23. The mammalian cell according to claim 22, wherein the at least one receptor that associates with DAP10 is endogenous.
24. The mammalian cell according to claim 22, wherein the at least one receptor that associates with DAP10 is over-expressed.
25. The mammalian cell according to claim 22, wherein the at least one receptor that associates with DAP10 is exogenous.

26. The mammalian cell according to any one of claims 22-25, wherein the receptor is NKG2D.
27. The mammalian cell according to any one of claims 22-26, wherein the mammalian cell is an immune cell, preferably wherein said immune cell is a cytotoxic cell.
28. A method for activating an immune cell, comprising:
expressing the CAD polypeptide of claim 21 in the immune cell, wherein the immune cell expresses at least one receptor that associates with DAP10; and
wherein the activating occurs responsive to the receptor engaging a corresponding target molecule.
29. The method according to claim 28, wherein the receptor is endogenous.
30. The method according to claim 28, wherein the receptor is exogenous.
31. The method according to claim 28, wherein the receptor is over-expressed.
32. The method according to any one of claims 28-31, wherein the receptor is NKG2D.
33. The method according to any one of claims 28-32, wherein the immune cell, or a plurality thereof, are introduced to a subject in need thereof; and
wherein the activating occurs in the subject.
34. Use of the mammalian cell, or a plurality thereof, according to any one of claims 22-27, in the preparation of a medicament for treating a subject with a condition for which the mammalian cell, or the plurality thereof, reduces at least one symptom or sign of said condition in the subject.
35. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a plurality of mammalian cells according to any one of claims 22-27.