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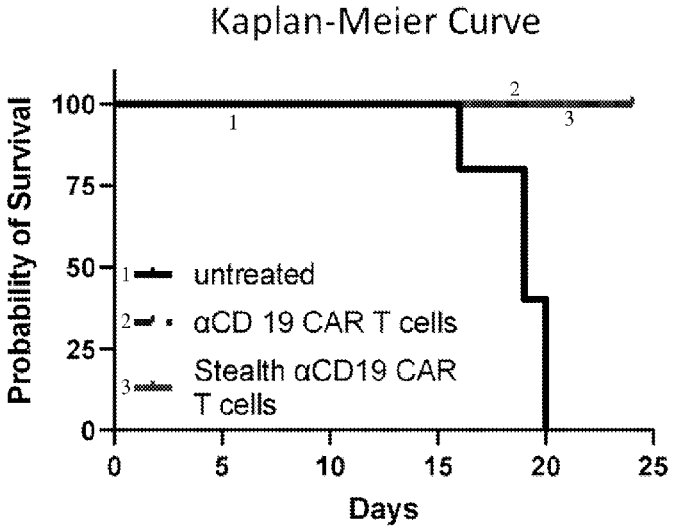

FIG. 10D

(57) Abstract: This application provides, in part, methods and compositions for decreasing the immunogenicity of cell therapies (e.g., CAR-T cell therapies) using inhibitors of transporter associated with antigen processing (TAPi) and oligonucleotides that decrease the expression of an immunogenic proteins (e.g., MHC Class I and Class II).

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COMPOSITIONS AND METHODS FOR REDUCING CELL THERAPY IMMUNOGENICITY

RELATED APPLICATION

This application claims the benefit under 35 U.S.C. §119(e) to U.S. Provisional Application No. 63/331,773, filed April 15, 2022, the entire contents of which are incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was made with government support under Grant No. 5R01CA238268-03, awarded by The National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Adoptive cell therapy, such as chimeric antigen receptor (CAR) CAR-T cell therapy, has revolutionized cancer treatment. However, clinical studies demonstrate that some patients develop humoral and cellular anti-CAR immune responses to non-self components of the CAR, limiting CAR-T cell persistence and the success of administering multiple doses. The potential for CAR-T cell rejection is even greater when using allogeneic immune effector cell products.

SUMMARY

This application discloses methods and compositions for decreasing a subject's immune response to adoptive cell therapies. In some aspects, the disclosure is directed to the discovery that a subject's immune response to adoptive cell therapies (e.g., CAR-T cells) can be reduced by engineering the cells of the adoptive cell therapy to express an inhibitor of transporter associated with antigen processing (TAPi) which decreases expression of MHC class I. The disclosure is further directed to the discovery that a subject's immune response can additionally or alternatively be reduced by decreasing the expression of MHC class II (e.g. using RNAi targeting a MHC class II transactivator protein). Methods and compositions of the disclosure based on these discoveries do not require deep host immune suppression or complex gene editing and therefore avoid the disadvantages associated with previous methods that rely on such host immune suppression and/or gene editing. Further, in some embodiments, CAR-T cells expressing a TAPi and RNAi targeting MHC class II do not have

increased susceptibility (relative to previous methods) of the therapeutic immune effector cells (IEC) to NK cell-mediated rejection, which is risk associated with current methods of β2M knockout.

In some aspects, this application discloses a cell comprising: (i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and(ii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein or variant thereof, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

In some aspects, this application discloses a cell comprising: (i) an chimeric antigen receptor (CAR); and (ii) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and/or (iii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein or variant thereof, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some embodiments, the oligonucleotide is complementary to any one of SEQ ID NOs: 7-12. In some embodiments, the oligonucleotide is complementary to SEQ ID NO: 7.

In some embodiments, the TAPi or variant thereof decreases expression of MHC class I.

In some embodiments, the TAPi is a viral TAPi. In some embodiments, the TAPi is a Herpesvirus TAPi. In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) TAPi, Human Cytomegalovirus (HCMV) TAPi, or Epstein-Barr virus (EBV) TAPi. In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) ICP47 TAPi, Human Cytomegalovirus (HCMV) US6 TAPi, or Epstein-Barr virus (EBV) BNLF2a TAPi. In some embodiments, the TAPi comprises an amino acid sequence that is at least 85% identical to any one of SEQ ID NOs: 1-3. In some embodiments, the TAPi comprises an amino acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the RNAi oligonucleotide is selected from the group consisting of a siRNA, a miRNA or a shRNA. In some embodiments, the RNAi oligonucleotide is a shRNA. In some embodiments, the shRNA comprises a nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the shRNA comprises a nucleic acid

sequence of SEQ ID NO: 13. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is a T cell.

In some embodiments, the cell further comprises a chimeric antigen receptor (CAR). In some embodiments, wherein the CAR comprises: (i) an extracellular target binding domain; (ii) a transmembrane domain; and (iii) an intracellular signaling domain. In some embodiments, the extracellular target binding domain binds to any one of CD19, CD79b, TACI, BCMA, MUC1, MUC16, B7H3, mesothelin, CD70, PSMA, PSCA, EGFRvIII, claudin6, binds to any pair of CD19/CD79b, BCMA/TACI, or is a TriPRIL antigen binding domain. In some embodiments, the extracellular target binding domain binds to CD19. In some embodiments, the extracellular target binding domain is not derived from a human polypeptide sequence. In some embodiments, the extracellular target binding domain is derived from a murine polypeptide sequence. In some embodiments, extracellular target binding domain comprises a VH amino acid sequence that has at least 85% identify to SEQ ID NO: 39 and a VL amino acid sequence that has at least 85% identify to SEQ ID NO: 40. In some embodiments, the transmembrane domain is selected from the group consisting of alpha chain of a T cell receptor, beta chain of a T cell receptor or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), 4-1BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c,ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C. In some embodiments, the intracellular signaling domain is selected from the group consisting of CD28, 4-1BB, CD27, TCR-zeta, FcR-gamma, FcR-beta, CD3-gamma, CD3-theta, CD3-sigma, CD3-eta, CD3-epsilon, CD3-zeta, CD22, CD79a, CD79b, and CD66d.

In some embodiments, the CAR comprises an amino acid sequence having at least 85% identify to SEQ ID NO: 41 and a nucleic acid sequence having at least 85% identity to SEQ ID NO: 17 or 18. In some aspects, this application discloses, a polynucleotide comprising a nucleic acid sequence encoding (i) a TAPi or variant thereof and (ii) an

oligonucleotide that is complementary to a gene encoding a MHC class II transactivator protein. In some embodiments, the TAPi is a viral TAPi. In some embodiments, the TAPi or variant thereof decreases expression of MHC class I. In some embodiments, the TAPi is a Herpes Simplex Virus (HSV) TAPi. In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) TAPi, Human Cytomegalovirus (HCMV) TAPi, or Epstein-Barr virus (EBV) TAPi. In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) ICP47 TAPi, Human Cytomegalovirus (HCMV) US6 TAPi, or Epstein-Barr virus (EBV) BNLF2a TAPi. In some embodiments, the TAPi comprises an amino acid sequence that is at least 85% identical to any one of SEQ ID NOs: 1-3. In some embodiments, the TAPi comprises an amino acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the oligonucleotide is complementary to any one of SEQ ID NOs: 7-12 or a variant thereof. In some embodiments, the oligonucleotide is complementary to SEQ ID NO: 7 or a variant thereof.

In some embodiments, the oligonucleotide is selected from the group consisting of a RNAi oligonucleotide or a CRISPR interference guide RNA. In some embodiments, the RNAi oligonucleotide is selected from the group consisting of a siRNA, a miRNA or a shRNA. In some embodiments, the RNAi oligonucleotide is an shRNA. In some embodiments, the shRNA is encoded by a nucleic acid sequence comprising of SEQ ID NO: 13.

In some embodiments, The polynucleotide further comprises a nucleic acid sequence encoding chimeric antigen receptor (CAR). In some embodiments, the CAR comprises: (i) an extracellular target binding domain; (ii) a transmembrane domain; and (iii) an intracellular signaling domain. In some embodiments, the extracellular target binding domain binds to any one of CD19, CD79b, TACI, BCMA, MUC1, MUC16, B7H3, mesothelin, CD70, PSMA, PSCA, EGFRvIII, claudin6, binds to any pair of CD19/CD79b, BCMA/TACI, or is a TriPRIL antigen binding domain. In some embodiments, the extracellular target binding domain binds to CD19. In some embodiments, the extracellular target binding domain is not derived from a human polypeptide sequence. In some embodiments, the extracellular target binding domain is derived from a murine polypeptide sequence.

In some embodiments, the transmembrane domain is selected from the group consisting of alpha, beta or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), 4-

1BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c,ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

In some embodiments, the intracellular signaling domain is selected from the group consisting of CD28, 4-1BB, CD27, TCR-zeta, FcR-gamma, FcR-beta, CD3-gamma, CD3-theta, CD3-sigma, CD3-eta, CD3-epsilon, CD3-zeta, CD22, CD79a, CD79b, and CD66d.

In some embodiments, the polynucleotide comprises a nucleic acid sequence that has at least 85% identity to SEQ ID NO: 17-18. In some embodiments, the polynucleotide comprises a nucleic acid sequence of SEQ ID NO: 19 and a nucleic acid sequence of SEQ ID NO: 20, 22 or 24.

In some embodiments, the polynucleotide is a vector, optionally a lentiviral vector. In some aspects, this application discloses a polynucleotide comprising an shRNA of SEQ ID NO: 13. In some aspects, this application discloses a cell comprising the polynucleotide described herein. In some aspects, the cell comprises the polynucleotide as described herein.

In some aspects, this application discloses a method of modifying the immunogenicity of a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a polynucleotide encoding an MHC class II complex subunit of any one of SEQ ID NOs: 7-12, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some aspects, this application discloses a method of decreasing an immune response of a subject to a cell therapy, the method comprising introducing into cells of the cell therapy an oligonucleotide that is complementary to a polynucleotide encoding class II MHC transactivator complex protein of any one of SEQ ID NOs: 7-12, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

In some embodiments, the method further comprises introducing into cells of the cell therapy a virus-derived inhibitor of transporter associated with antigen processing (TAPi) or

variant thereof. In some embodiments, the method comprises introducing into cells of the cell therapy the polynucleotide described herein. In some embodiments, the cell or cells are eukaryotic cells. In some embodiments, the cell or cells are immune cells. In some embodiments, the immune cell or immune cells are T cells. In some embodiments, the cells are allogenic to the subject. In some embodiments, the cell therapy is a CAR-T cell therapy. In some embodiments, the CAR-T cell therapy comprises an anti-CD19 CAR-T cell. In some embodiments, the subject is a human subject. In some embodiments, the method decreases natural killer cell activation. In some aspects, this application relates to a method of treating cancer in a subject, the method comprising administering the cell described herein to the subject. In some embodiments, the cancer is a hematological cancer. In some embodiments, the hematological cancer is selected from the group consisting of Leukemia, Lymphoma, and Myeloma. In some embodiments, the hematological cancer is selected from the group consisting of acute lymphoblastic leukemia or mantle cell lymphoma. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is selected from the group consisting of ovarian cancer, mesothelioma, brain cancer, liver cancer, kidney cancer, lung cancer, breast cancer, prostate cancer, throat cancer, thyroid cancer, colon cancer, testicular cancer, and skin cancer. In some embodiments, the cancer expresses CD19.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGs. 1A-1E show that lentivirus transduction of viral TAP inhibitors results in decreased cell surface expression of MHC Class I and allogeneic response in human primary T cells whilst averting an obvious NK cell and pre-existing anti-viral T cell response. FIG. 1A shows the design of the lentiviral constructs expressing viral TAPi or CRISPR-guide RNA for β2M. FIG. 1B shows reduced MHC class I cell surface expression, and, after co-incubation with NK cells, NK cell cytotoxicity and degranulation was found by flow cytometric analysis of TAPi-transduced human primary T cells (n=3 donors). FIG. 1C shows allogeneic and autologous response of T cell against human primary TAPi-expressing T cells

or β2M KO T cells was measured by proliferation of responder T cells in an MLR reaction by means of flow cytometric analysis of CellTrace labeled responder cells, which was found to be severely reduced due clearance of MHC I cell surface levels. FIG. 1D shows co-expression of viral TAPi and the HCMV pp65 in primary T cells strongly reduces NLV antigen presentation assessed by IFNγ secretion by NLV-specific CD8+ T cells upon co-incubation measured by IFNγ ELISA. FIG. 1E shows co-incubation of viral TAPi-expressing primary T cells with autologous T cells from donors with a pre-existing anti-viral cellular immunity does not elicit an T cell response as measured by IFNγ ELISPOT. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 2A-2G show lentiviral transduction of shRNA targeting CIITA results in decreased cell surface expression of MHC Class II and allogeneic response in human primary T cells, and can be combined with expression of EBV TAPi to decrease both MHC class I and II, evading allogeneic T cell responses. FIG. 2A shows the design of the lentiviral constructs expressing shRNA targeting CIITA or CRISPR-guide for CIITA. FIGs. 2B-2C show reduced MHC class II cell surface expression was found by flow cytometric analysis of human primary T cells expressing shRNA targeting CIITA whilst T cells transduction with shRNA CIITA3 maintained similar proliferation compared to UTD (n=3 donors). FIG. 2D shows allogeneic and autologous response of T cell against human primary T cells expressing shRNA targeting CIITA was measured by proliferation of responder T cells in an MLR reaction by means of flow cytometric analysis of CellTrace labeled responder cells, which was found to be severely reduced due clearance of MHC II cell surface levels. FIG. 2E is a schematic overview of the different utilized lentiviral constructs combining the EBV TAPi and the shRNA CIITA3. FIG. 2F shows MHC class I and II expression was analyzed in human primary T cells expressing the EBV TAPi and/or shRNA targeting CIITA by flow cytometric analysis (n = 3 donors). FIG. 2G shows allogeneic and autologous response of T cells against primary human T cells expressing EBV TAPi and/or shRNA CIITA3 were assessed by responder cell proliferation in an MLR assay. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001)

FIGs. 3A-3F show the stealth modification to the αCD19 CAR T cells does not alter the tumor-clearing efficacy and CAR T cell proliferation, whilst enabling the stealth αCD19 CAR T cells to evade CAR-mediated immune recognition by T cells from patients who received a single or double infusion of αCD19 CAR T cells. FIG. 3A is a schematic overview

of the different lentiviral constructs based on the αCD19 CAR w/o the combination of EBV TAPi and shRNA CIITA3. FIG. 3B shows MHC class I and II expression, NK cell cytotoxicity after co-incubation with NK cells, and T cell proliferation were analyzed in αCD19 CAR T cells w/o stealth technology. FIG. 3C shows luciferized cytotoxicity assays of αCD19 CAR T cells with or without stealth technology were performed with ALL cell line NALM-6 and Mantle cell line JeKo-1, indicating similar tumor clearance in vitro. FIGs. 3D-3E show NSG mice were engrafted with NALM6 cells and treated with αCD19 CAR T cells with or without stealth technology or left untreated. On day 7 and 14 after treatment, blood was drawn to assess CAR T cell expansion and BLI images were taken to assess the tumor burden. FIG. 3F shows IFNγ ELISpot assays were performed with T cells from patients, who had received the FMC63-based αCD19 CAR T cells (Yescarta or Kymriah) products, and autologous αCD19 CAR T cells with or without stealth technology to assess the CAR-mediated T cell immunity, indicating efficient evasion of CAR-mediated T cell immunity by the stealth technology. (Asterixes indicated statistical significances compared to the UTD - *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****: $P \leq 0.0001$)

FIGs. 4A-4C show eGFP expression in TAPi-expressing primary T cells, and assessment of CD25 and CD69 expression of responder cells in MLR assay assessing allogeneic and autologous responses towards TAPi-expressing T cells. FIG. 4A shows flow cytometric analysis of eGFP expression in TAPi-expressing T cells. FIGs. 4B-4C show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder cells in an MLR assay with TAPi-expressing T cells and β2M KO T cells show a reduced immune activation and allogeneic response.

FIGs. 5A-5F show eGFP expression primary T cells expressing shRNA targeting CIITA and/or EBV TAPi and assessment of CD25 and CD69 expression of responder cells in MLR assay assessing allogeneic and autologous responses towards T cells expressing shRNA targeting CIITA and/or EBV TAPi. FIG. 5A shows flow cytometric analysis of eGFP expression in T cells expressing shRNA targeting CIITA. FIGs. 5B-5C show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder cells in an MLR assay with T cells expressing shRNA targeting CIITA or CIITA KO T cells show a reduced immune activation and allogeneic response. FIG. 5D shows flow cytometric analysis of eGFP expression in T cells expressing shRNA targeting CIITA and/or the EBV TAPi. FIGs. 5E-5F show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder

cells in an MLR assay with T cells expressing EBV TAPi and/or shRNA targeting CIITA show a reduced immune activation and allogeneic response.

FIGs. 6A-6E show lentivirus transduction of viral TAP inhibitors results in decreased cell surface expression of MHC Class I and allogeneic response in human primary T cells whilst averting an obvious NK cell and pre-existing anti-viral T cell response. FIG. 6A shows a schematic overview of MHC class I pathway and design of the lentiviral constructs expressing viral TAPi or CRISPR-guide for β2M. FIGs. 6B-6D show reduced MHC class I cell surface expression, and, after co-incubation with NK cells, NK cell cytotoxicity and degranulation was found by flow cytometric analysis of TAPi-transduced human primary T cells (n=3 donors). FIG. 6E shows allogeneic and autologous response of T cell against human primary TAPi-expressing T cells or β2M KO T cells was measured by proliferation of responder T cells in an MLR reaction by means of flow cytometric analysis of CellTrace labeled responder cells, which was found to be severely reduced due clearance of MHC I cell surface levels.

FIGs.7A-7D show lentiviral transduction of shRNA targeting CIITA results in decreased cell surface expression of MHC Class II and allogeneic response in human primary T cells. FIG. 7A shows a schematic overview of MHC Class II pathway and design of the lentiviral constructs expressing shRNA targeting CIITA or CRISPR-guide for CIITA. FIGs. 7B-7C shows reduced MHC class II cell surface expression was found by flow cytometric analysis of human primary T cells expressing shRNA targeting CIITA whilst T cells transduction with shRNA CIITA3 maintained similar proliferation compared to UTD (n=3 donors). FIG. 7D shows allogeneic and autologous response of T cell against human primary T cells expressing shRNA targeting CIITA was measured by proliferation of responder T cells in an MLR reaction by means of flow cytometric analysis of CellTrace labeled responder cells, which was found to be severely reduced due clearance of MHC II cell surface levels. (Asterixes indicated statistical significances compared to the UTD - *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****: $P \leq 0.0001$)

FIGs. 8A-8D show lentiviral transduction of the combination of EBV TAPi and shRNA targeting CIITA decreases both MHC class I and II, evading allogeneic T cell responses. FIG. 8A shows a schematic overview of the different utilized lentiviral constructs combining the EBV TAPi and the shRNA CIITA3. FIGs. 8B-8C show MHC class I and II expression was analyzed in human primary T cells expressing the EBV TAPi and/or shRNA targeting CIITA by flow cytometric analysis (n= 3 donors). FIG. 8D shows allogeneic and

autologous response of T cells against primary human T cells expressing EBV TAPi and/or shRNA CIITA3 were assessed by responder cell proliferation in an MLR assay. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 9A-9F show the stealth modification to the αCD19 CAR T cells does not alter the in vitro characterization of CAR T cells. FIG. 9A shows a schematic overview of the different lentiviral constructs based on the αCD19 CAR w/o the combination of EBV TAPi and shRNA CIITA3. FIG. 9B shows MHC class I, MHC class II, EBV TAPi and CIITA expression, FIG. 9C shows NK cell cytotoxicity after co-incubation with NK cells, and FIG. 9D shows T cell proliferation were analyzed in αCD19 CAR T cells w/o stealth technology. FIG. 9E shows flowcytometric analysis of CAR-T cell CD4:CD8 ratios and memory phenotypes according to CD45RA and CCR7 expression. FIG. 9F luciferized cytotoxicity assays of αCD19 CAR T cells with or without stealth technology were performed with ALL cell line NALM-6 and Mantle cell line JeKo-1, indicating similar tumor clearance in vitro (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 10A-10H shows the stealth modification to the αCD19 CAR T cells does not alter the in vivo tumor-clearing efficacy and CAR T cell proliferation. In FIGs. 10A-10C and 10E-10G, NSG mice were engrafted with NALM6 or JeKo-1 cells and treated with αCD19 CAR T cells with or without stealth technology or left untreated. On day 14 after treatment, blood was drawn to assess CAR T cell expansion and BLI images were taken to assess the tumor burden. FIGs. 10D and 10H show survival as indicated by Kaplain-Meier curve (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001)

FIGs. 11A-11E show the stealth modification to the αCD19 CAR T cells enables the CAR T cells to evade CAR-mediated immune recognition by T cells from patients who received a single or double infusion of αCD19 CAR T cells. IFNγ ELISpot assays were performed with T cells from patients, who had received the FMC63-based αCD19 CAR T cells (Yescarta or Kymriah) products, and autologous αCD19 CAR T cells with or without stealth technology to assess the CAR-mediated T cell immunity, indicating efficient evasion of CAR-mediated T cell immunity by the stealth technology. FIG. 11A shows a swimmer plot of the selected patient population. FIG. 11B shows a schematic overview representing the predicted outcomes of the ELISpot assay. FIGs. 11C-11D show a heatmap and

representative wells of the ELISpot assay. FIG. 11E shows histograms depicting eGFP expression levels after sorting and graphs indicating the CAR-mediated T cell activation and anti-CAR responses from the ELISpot assay. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIG. 12A-12H show the stealth modification to the αCD19 CAR T cells enables evasion of the in vitro allogeneic response and confers increased CAR T cell proliferation in an allogeneic in vivo model. FIG. 12A shows stealth technology prevents triggering of the allogeneic response after co-incubation with allogeneic T cells as measured by a IFNγ ELIspot and flow-based cytotoxic assay. In FIGs. 12B-12C, NSG mice were engrafted with αCD3/αCD28-expanded allogeneic T cells (UTD ND2), inoculated with NALM6 and treated with αCD19 CAR T cells (ND1) with or without stealth technology or left untreated. On day 14 after treatment, blood was drawn to assess CAR T cell expansion and tumor burden. FIG. 12D shows BLI images taken to assess the tumor burden. In FIG. 12E, NSG mice were engrafted with allogeneic T cells (UTD ND2) pulsed twice with irradiated PBMCs from CAR T cell donor (ND1) and expanded by REP protocol, inoculated with NALM6 and treated with αCD19 CAR T cells (ND1) with or without stealth technology or left untreated. FIG. 12F shows the CAR T cells expansion assessed by weekly blood draws (day 7 to 28) and flow cytometry. In FIG. 12G, the tumor burden was quantified by BLI and total emission was graphed. FIG. 12H shows survival plotted in the Kaplan-Meier curve, indicating the model with REP-expanded allogeneic T cells could be followed longer before triggering severe GVhD. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 13A-13B show an assessment of CD25 and CD69 expression of responder cells in MLR assay assessing allogeneic and autologous responses towards TAPi-expressing T cells. FIG. 13A-13B show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder cells in an MLR assay with TAPi-expressing T cells and β2M KO T cells show a reduced immune activation and allogeneic response. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 14A-14B show an assessment of CD25 and CD69 expression of responder cells in MLR assay assessing allogeneic and autologous responses towards T cells expressing shRNA targeting CIITA. FIGs. 14A-14B show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder cells in an MLR assay with T cells expressing shRNA

targeting CIITA or CIITA KO T cells show a reduced immune activation and allogeneic response. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIGs. 15A-15B show an assessment of CD25 and CD69 expression of responder cells in MLR assay assessing allogeneic and autologous responses towards T cells expressing EBV TAPi and shRNA targeting CIITA. FIGs. 15A-15B show flow cytometric analysis of CD25 and CD69 of allogeneic and autologous responder cells in an MLR assay with T cells expressing EBV TAPi and shRNA targeting CIITA show a reduced immune activation and allogeneic response. (Asterixes indicated statistical significances compared to the UTD - *: P≤0.05; **: P≤0.01; ***: P≤ 0.001; ****: P≤ 0.0001).

FIG. 16 shows a survival curve of mice in the allogeneic in vivo model. NSG mice were engrafted with αCD3/αCD28-expanded allogeneic T cells (Allo T cells), inoculated with NALM6 and treated with αCD19 CAR T cells with or without stealth technology or left untreated. Survival was indicated by Kaplan-Meier curve. Mice perished early due to graft-versus-host disease as observed by fur loss and sclerosis.

DETAILED DESCRIPTION

Cell compositions

In some aspects, this application discloses a cell comprising: (i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and (ii) an oligonucleotide that is complementary to a gene encoding a subunit of MHC class II (e.g. SEQ ID NO: 6) or a gene MHC class II transactivator protein (e.g. (SEQ ID NOs: 7-12), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some aspects, this application discloses a cell comprising: (i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and (ii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein (e.g. (SEQ ID NOs: 7-12), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

A MHC class II transactivator protein, as described herein, refers to a protein that regulates MHC class II transcription or a protein that is in a protein complex that regulates MHC class II transcription. A MHC class II transactivator protein include, but are not

limited to CIITA (SEQ ID NO: 7), RFX (SEQ ID NO: 8), RFXANK (SEQ ID NO: 9), CREB (SEQ ID NO: 10), NFYA (SEQ ID NO: 11), and/or NFYC (SEQ ID NO: 12).

A "variant," or "variant thereof" as referred to herein, is a sequence (e.g., a polypeptide or polynucleotide) substantially homologous to a native or reference sequence, but which has a sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions, or substitutions. Variant polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encode a variant protein or fragment thereof that retains activity of the non-variant polypeptide. A wide variety of PCR-based site-specific mutagenesis approaches are known in the art and can be applied by the ordinarily skilled artisan.

A variant amino acid or DNA sequence can be at least 80%, at least 85%, 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%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

Inhibitor of transporter associated with antigen processing (TAPi)

A transporter associated with antigen processing (TAP) is a protein that translocates antigenic peptides and participates in loading the angiogenic peptides into MHC class I (e.g., HLA A, B, and C) for antigen presentation to the immune system, e.g., as described in Lehnert, Elisa, and Robert Tampé. Frontiers in immunology (2017): 10., which is incorporated by reference in its entirety. The term "inhibitor of transporter associated with antigen processing (TAPi)" refers to a molecule that inhibits the activity, expression or function of a TAP, e.g. as described in Matschulla et al., Scientific Reports 7.1 (2017): 1-13, which is incorporated by reference in its entirety. In some embodiments, the TAPi inhibits the activity expression or function of MHC class I. In some embodiments, TAPi decreases the expression of the MHC class I in a cell by at least 30% (e.g., at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99%). In some embodiments, TAPi decreases the expression of the MHC class I in a cell by 50-90%, 50-95% or 50-99%.

In some embodiments, the TAPi is a viral TAPi. In some embodiments, the TAPi is a Herpesvirus TAPi. In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) TAPi, Human Cytomegalovirus (HCMV) TAPi, an Epstein-Barr virus (EBV) TAPi, a varicelloviruses (TAPi), or a poxvirus TAPi, e.g., as described in Matschulla et al., Scientific Reports 7.1 (2017): 1-13. In some embodiments, the TAPi is selected from the group consisting of ICP47 (herpes simplex virus type-1, HSV-1), US6 (human cytomegalovirus, HCMV), BNLF2a (Epstein-Barr virus, EBV), UL49.5 (varicelloviruses), and CPXV12 (poxvirus). In some embodiments, the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) ICP47 TAPi, Human Cytomegalovirus (HCMV) US6 TAPi, or Epstein-Barr virus (EBV) BNLF2a TAPi. In some embodiments, the TAPi is BNFL2a (EBV). In some embodiments, the TAPi comprises an amino acid sequence that is at least 85% identical (e.g., at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical) to any one of SEQ ID NOs: 1-3. In some embodiments, the TAPi comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3 or a variant thereof. In some embodiments, the TAPi consists of an amino acid sequence selected from the group consisting of any one of SEQ ID NOs: 1-3.

Cells comprising alternative methods for decreasing MHC Class I expression

In some embodiments, the cell comprises an oligonucleotide (e.g. an RNAi oligonucleotide) that comprises a sequence which is complementary to a TAP. In some embodiments, the cell comprises an oligonucleotide that comprises a sequence which is complementary to a gene encoding a subunit of MHC Class I (e.g., the beta-2-microglobin sequence (SEQ ID NO: 5) or a variant thereof, or the HLA-B sequence (SEQ ID NO: 4) or a variant thereof). In some embodiments, the cell comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more oligonucleotides that each comprise a sequence that is complementary to a gene encoding a subunit of MHC class I. In some embodiments, the oligonucleotide is RNAi (e.g., siRNA, miRNA, or shRNA), ASO, or CRISPR sequence (e.g., a CRISPR guide RNA sequence), which are well known in the art and described below.

Oligonucleotides complementary to MHC class II

In some aspects, the cell comprises an oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II (e.g., HLA DR/DP/DQ) or variants thereof, or a nucleic acid sequence encoding a MHC class II transactivator protein. In some aspects, the

cell comprises an oligonucleotide that is complementary to a nucleic acid sequence encoding a MHC class II transactivator protein.

The term "complementary" as described herein refers to the degree of Watson-Crick base pairing between two polynucleotides (e.g. an shRNA and a target mRNA). For example, two polynucleotides may be 90% complementary if 9/10 nucleotides of each of the polynucleotides form a Watson Crick base pair. In some embodiments, complementary may refer to at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%) of nucleotides in a first polynucleotide Watson-Crick base pairing with a second polynucleotide. In some embodiments, the oligonucleotide may be sufficiently complementary to the target gene to decrease expression of the target gene. The skilled person will understand the oligonucleotide used to decrease gene expression (e.g. RNAi oligonucleotide) may comprise a first sequence that is designed to be complementary to the target gene sequence (e.g. mRNA) and other sequences that are not complementary to the target gene sequence (e.g. sequences for processing). Thus, when an oligonucleotide that is complementary to a gene (e.g. a gene encoding a subunit of MHC class II) is disclosed, the complementarity is referring to the region of oligonucleotide designed to be complementary to the gene.

In some aspects, the cell comprising a TAPi comprises an oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II (e.g., HLA DR/DP/DQ). In some embodiments, the MHC class II is mammalian MHC class II. In some embodiments, the MHC class II is human MHC class II. In some embodiments, the MHC class II is murine MHC class II.

In some embodiments, the cell comprises an oligonucleotide that is complementary to a gene encoding a MHC class II transactivator protein (e.g., any one of CIITA (SEQ ID NO: 7), RFX (SEQ ID NO: 8), RFXANK (SEQ ID NO: 9), NYFA (SEQ ID NO: 10), NYFC (SEQ ID NO: 11), and NF-gamma and CREB (SEQ ID NO: 12)), or variants thereof. In some aspects, the cell comprises an oligonucleotide that is complementary any one of SEQ ID NOs: 7-12 or a variant thereof. In some embodiments, the cell comprises an oligonucleotide that is complementary to CIITA or a variant thereof. In some aspects, the cell comprises an oligonucleotide that is complementary to SEQ ID NOs: 7 or a variant thereof. In some embodiments, the cell comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more oligonucleotides that each comprise a sequence that is complementary to a gene encoding a subunit of MHC class II and/or a MHC class II transactivator protein (e.g., SEQ ID NOs: 7-

12). In some embodiments, the cell comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more oligonucleotides that each comprise a sequence that is complementary a gene encoding CIITA (SEQ ID NO: 7).

In some embodiments, the oligonucleotide is an RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference oligonucleotide. In some embodiments, administration of the oligonucleotide decreases MHC class II expression in the cell.

In some embodiments, the RNAi oligonucleotide is selected from the group consisting of a siRNA, a miRNA, a shRNA or any other suitable RNAi oligonucleotide. Methods of constructing and using siRNAs, miRNAs and shRNA oligonucleotides to decrease the expression of a gene (e.g. MHC class I, MHC class II or a MHC class II transactivator protein) are well known in the art, e.g., as described in Agrawal et al., Microbiology and Molecular Biology Reviews 67.4 (2003): 657-685, and Taxman et al., RNA Therapeutics. Humana Press, 2010. 139-156, both of which are incorporated by reference in their entirety.

In some embodiments, the RNAi oligonucleotide is a shRNA. In some embodiments, the cell comprises an shRNA comprising a sequence that is complementary to any one of SEQ ID NOs: 7-12 or a variant thereof. In some embodiments, the cell comprises an shRNA comprising a sequence that is complementary to a gene encoding CIITA (SEQ ID NO: 7) or a variant thereof. In some embodiments, the shRNA is encoded by a nucleic acid sequence comprising SEQ ID NO: 13 or a variant thereof. In some embodiments, the shRNA is encoded by a nucleic acid sequence comprising SEQ ID NO: 13.

In some embodiments, the oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II or a MHC class II transactivator protein is an antisense oligonucleotide (ASOs). ASOs are well known in the art as e.g., as described in Quemener, Anaïs M., et al. Wiley Interdisciplinary Reviews: RNA 11.5 (2020): e1594, which is incorporated by reference in its entirety. In some embodiments, the ASO is a DNA sequence. In some embodiments, the ASO DNA sequence is modified. In some embodiments, the ASO sequence comprises one or more modification selected from the group consisting of phosphorothioate (PS) oligodeoxynucleotides, 2′ methoxyethyl (2′-MOE), 2′ constrained ethyl (2′cEt) modifications, 2′-MOE and 2′cEt PS ASOs conjugated with N-acetyl galactosamine (GalNAc).

In some embodiments, the oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II or MHC class II transactivator protein is a CRISPR gRNA

sequence (e.g., a CRISPR interference guide RNA sequence). Methods of using CRISPR interference and designing CRISPR interference guide RNA sequences are well known in the art as described in Mohr, Stephanie E., et al. The FEBS Journal 283.17 (2016): 3232-3238, which is incorporated by reference in its entirety. In some embodiments, the oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II or MHC class II transactivator protein is a CRISPR oligonucleotide. In some embodiments, CRISPR may be used to mutate the MHC class II or MHC class II transactivator protein. In some embodiments, the mutation is a loss of function mutation (e.g., a frameshift mutation or early stop codon mutation). In some embodiments, the oligonucleotide that is complementary to a nucleic acid sequence encoding MHC class II or MHC class II transactivator protein is a base editor oligonucleotide. In some embodiments, the base editor is a adenosine base editor or a cytosine base editor. In some embodiments, the base editor mutates gene encoding the MHC class II or MHC class II transactivator protein. In some embodiments, the mutation is a loss of function mutation (e.g., a frameshift mutation or early stop codon mutation).

Chimeric antigen receptor (CAR)

In some embodiments, the cell comprising a TAPi and an oligonucleotide (e.g. RNAi oligonucleotide) that is complementary to a nucleic acid sequence encoding MHC class II transactivator protein (e.g., SEQ ID NOs:7-12) or variants thereof as described herein further comprises a chimeric antigen receptor (CAR).

The terms "chimeric antigen receptor" or "CAR" or "CARs", as used herein, refer to engineered T cell receptors, which graft a ligand or antigen specificity onto T cells (for example, naive T cells, central memory T cells, effector memory T cells or combinations thereof). CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors.

A CAR places a chimeric extracellular antigen-binding domain that specifically binds a target, e.g., a polypeptide, expressed on the surface of a cell to be targeted for an immune cell response (e.g., a T cell) onto a construct including a transmembrane domain and intracellular domain(s) of a T cell receptor molecule. In some embodiments, the chimeric extracellular antigen-binding domain includes the antigen-binding domain(s) of an antibody reagent that specifically binds an antigen expressed on a cell to be targeted for a T cell response. In some embodiments, the chimeric extracellular antigen-binding domain includes

a ligand that specifically binds an antigen expressed on a cell to be targeted for a T cell response.

As used herein, a "CART cell", “CAR-T cell”, or “CAR T cell” refers to a T cell that expresses a CAR. When expressed in a T cell, CARs have the ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T-cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

In some embodiments, the CAR polypeptide comprises an amino acid sequence with at least 75%, at least 80%, at least 85%, 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% or greater sequence identity to SEQ ID NO: 17. In some embodiments, the CAR polypeptide comprises an amino acid sequence of any one of SEQ ID NO: 17. In some embodiments, the CAR polypeptide consists of an amino acid sequence of any one of SEQ ID NO: 17. As can be determined by those of skill in the art, various functionally similar or equivalent components of these CARs can be swapped or substituted with one another, as well as other similar or functionally equivalent components known in the art or listed herein.

Extracellular Antigen-Binding Domain

As used herein, the term "extracellular antigen-binding domain" refers to a polypeptide found on the outside of the cell that is sufficient to facilitate binding to a target. The extracellular target binding domain will specifically bind to its binding partner, i.e., the target. As non-limiting examples, the extracellular antigen-binding domain can include an antigen-binding domain of an antibody or antibody reagent, or a ligand, which recognizes and binds with a cognate binding partner protein. In this context, a ligand is a molecule that binds specifically to a portion of a protein and/or receptor. The cognate binding partner of a ligand useful in the methods and compositions described herein can generally be found on the surface of a cell. Ligand:cognate partner binding can result in the alteration of the ligand-bearing receptor, or activate a physiological response, for example, the activation of a signaling pathway. In some embodiments, the ligand can be non-native to the genome. In some embodiments, the ligand has a conserved function across at least two species.

Any cell-surface moiety can be targeted by a CAR. Often, the target will be a cell-surface polypeptide that may be differentially or preferentially expressed on a cell that one wishes to target for a T cell response. In some embodiments, the extracellular target binding

domain binds to any one of CD19, CD37, CD70, CD79b, TACI, BCMA, MUC1, MUC16, B7H3, mesothelin, CD70, PSMA, PSCA, EGFRvIII, claudin6, binds to any pair of CD19/CD79b, BCMA/TACI, or is a TriPRIL antigen binding domain, e.g., as described in PCT/US2020/065733, PCT/US2020/036108, PCT/US2018/013215, PCT/US2018/013213, PCT/US2018/027783, PCT/US2018/013221, PCT/US2018/022974, PCT/US2019/042268, PCT/US2019/038518, PCT/US2019/066357, PCT/US2019/013103, PCT/US2019/017727, PCT/US2020/051018, and/or PCT/US2018/013095, each of which are incorporated by reference in its entirety. In some embodiments, the extracellular target binding domain is not human. In some embodiments, the extracellular target binding domain is murine. In some embodiments, the extracellular target binding domain binds to CD19. In some embodiments, the CD19 antibody is FMC63 (VH: SEQ ID NO: 39 or VL: SEQ ID NO: 40) or a variant thereof. In some embodiments, the extracellular target binding domain comprises a VH amino acid sequence that has at least 85% identify to SEQ ID NO: 39 and a VL amino acid sequence that has at least 85% identify to SEQ ID NO: 40.

In various embodiments, the CARs described herein include an antibody reagent or an antigen-binding domain thereof as an extracellular target-binding domain. As used herein, the term “antibody reagent” refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. In some embodiments, an antibody reagent can include an antibody or a polypeptide including an antigen-binding domain of an antibody. In some embodiments of any of the aspects, an antibody reagent can include a monoclonal antibody or a polypeptide including an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In some embodiments, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. In some embodiments, the antibody reagent is a bispecific antibody reagent.

The term "antibody reagent" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab’)2, Fd fragments, Fv fragments, scFv, CDRs, and domain antibody (dAb) fragments (see, e.g., de Wildt et al., Eur. J. lmmunol. 26(3):629-639, 1996; which is incorporated by reference herein in its entirety)) as well as complete antibodies. An antibody can have the structural features of lgA, lgG, lgE, lgD, or lgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and

primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like. In some embodiments, the CAR comprises an antibody reagent. In some embodiments, the therapeutic agent comprises an antibody reagent.

Fully human antibody binding domains can be selected, for example, from phage display libraries using methods known to those of ordinary skill in the art. Furthermore, antibody reagents include single domain antibodies, such as camelid antibodies.

The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" ("FR"). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A. et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia et al., J. Mol. Biol. 196:901-917, 1987; each of which is incorporated by reference herein in its entirety). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

In some embodiments, the antibody or antibody reagent is not a human antibody or antibody reagent (i.e., the antibody or antibody reagent is mouse), but has been humanized. A "humanized antibody or antibody reagent" refers to a non-human antibody or antibody reagent that has been modified at the protein sequence level to increase its similarity to antibody or antibody reagent variants produced naturally in humans. One approach to humanizing antibodies employs the grafting of murine or other non-human CDRs onto human antibody frameworks.

In some embodiments, the extracellular target binding domain of a CAR includes or consists essentially of a single-chain Fv (scFv) fragment created by fusing the VH and VL domains of an antibody, generally a monoclonal antibody, via a flexible linker peptide. In various embodiments, the scFv is fused to a transmembrane domain and to a T cell receptor intracellular signaling domain, e.g., an engineered intracellular signaling domain as described herein. In another embodiment, the extracellular target binding domain of a CAR includes a camelid antibody.

In some embodiments, the antibody reagent binds to a tumor associated-antigen. Non-limiting examples of additional tumor antigens, tumor-associated antigens, or other antigen of interest include activated fibroblast marker, CD19, CD37, BCMA (tumor necrosis factor receptor superfamily member 17 (TNFRSF17); NCBI Gene ID: 608; NCBI Ref Seq:

NP 001183.2 and mRNA (e.g., NCBI Ref Seq: NM_001192.2)), CEA, immature laminin receptor, TAG-72, HPV E6 and E7, BING-4, calcium-activated chloride channel 2, cyclin B1, 9D7, Ep-CAM, EphA3, 15 her2/neu, telomerase, EGFR, EGFRviii SAP-1, 21urviving, BAGE family, CAGE family, GAGE family, MAGE family, SAGE family, XAGE family, NY-ESO-1/LAGE-1, PRAME, SSX-2, Melan-NMART-1, gp100/pmel17, tyrosinase, TRP-1/-2, MC1R, BRCA1/2, CDK4, MART-2, p53, Ras, MUC1, TGF-BetaRII, IL-15, IL-13Ra2, and CSF1 R. In some embodiments, the activated fibroblast marker comprises any one of αSMA (ACTA2), fibroblast activation protein (FAP), platelet derived growth factor receptor-α and -β (PDGFRA, PDGFRB), fibroblast specific protein 1 (FSP1/S100A4), endoglin (ENG), transgelin (TAGLN), tenascin C (TNC), periostin (POSTN), chondroitin sulphate proteoglycan 4 or neuron-glial antigen 2 (CSPG4/NG2), podoplanin (PDPN), or osteopontin (SPP1).

Hinge and Transmembrane Domains

Each CAR as described herein includes a transmembrane domain, e.g., a hinge/transmembrane domain, which joins the extracellular antigen-binding domain to the intracellular signaling domain. The binding domain of the CAR is optionally followed by one or more "hinge domains," which plays a role in positioning the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. A CAR optionally includes one or more hinge domains between the binding domain and the transmembrane domain (TM). The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region. Illustrative hinge domains suitable for use in the CARs described herein include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD8 (e.g., CD8alpha), CD4, CD28, 4-1BB, and CD7, which may be wild-type hinge regions from these molecules or may be altered.

In some embodiments, the hinge region is derived from the hinge region of an immunoglobulin-like protein (e.g., IgA, IgD, IgE, IgG, or IgM), CD28, or CD8. In some embodiments, the hinge domain includes a CD8a hinge region.

As used herein, "transmembrane domain" (TM domain) refers to the portion of the CAR that fuses the extracellular binding portion, optionally via a hinge domain, to the intracellular portion (e.g., the costimulatory domain and intracellular signaling domain) and anchors the CAR to the plasma membrane of the immune effector cell. The transmembrane

domain is a generally hydrophobic region of the CAR, which crosses the plasma membrane of a cell. The TM domain can be the transmembrane region or fragment thereof of a transmembrane protein (for example a Type I transmembrane protein or other transmembrane protein), an artificial hydrophobic sequence, or a combination thereof. While specific examples are provided herein and used in the Examples, other transmembrane domains will be apparent to those of skill in the art and can be used in connection with alternate embodiments of the technology. A selected transmembrane region or fragment thereof would preferably not interfere with the intended function of the CAR.

As used in relation to a transmembrane domain of a protein or polypeptide, "fragment thereof" refers to a portion of a transmembrane domain that is sufficient to anchor or attach a protein to a cell surface.

In some embodiments, the transmembrane domain or fragment thereof of the CAR described herein includes a transmembrane domain selected from the transmembrane domain of an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), 4-1BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c,
ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C. In some embodiments, the transmembrane domain is CD8 transmembrane domain.

As used herein, a "hinge/transmembrane domain" refers to a domain including both a hinge domain and a transmembrane domain. For example, a hinge/transmembrane domain can be derived from the hinge/transmembrane domain of CD8, CD28, CD7, or 4-1BB. In some embodiments, the hinge/transmembrane domain of a CAR or fragment thereof is derived from or includes the hinge/transmembrane domain of CD8. CD8 is an antigen preferentially found on the cell surface of cytotoxic T lymphocytes. CD8 mediates cell-cell interactions within the immune system, and acts as a T cell co-receptor. CD8 consists of an

alpha (CD8alpha or CD8a) and beta (CD813 or CD8b) chain. CD8a sequences are known for a number of species, e.g., human CD8a, (NCBI Gene ID: 925) polypeptide (e.g., NCBI Ref Seq NP 001139345.1) and mRNA (e.g., NCBI Ref Seq NM_ 000002.12). CD8 can refer to human CD8, including naturally occurring variants, molecules, and alleles thereof. In some embodiments of any of the aspects, e.g., in veterinary applications, CD8 can refer to the CD8 of, e.g., dog, cat, cow, horse, pig, and the like.

Homologs and/or orthologs of human CD8 are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a given species for sequence similar to a reference CD8 sequence.

In some embodiments, the hinge and transmembrane sequence corresponds to the amino acid sequence of SEQ ID NO: 25; or includes a sequence with at least 75%, at least 80%, at least 85%, 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%, or at least 100% sequence identity to the sequence of SEQ ID NO: 25 or an amino acid sequence having ≤1, ≤2, ≤3, ≤4, ≤5, ≤6, ≤7, ≤8, ≤9, or ≤10 substitutions relative to any thereof.

Co-stimulatory Domains

Each CAR described herein optionally includes the intracellular domain of one or more co-stimulatory molecule or co-stimulatory domain. As used herein, the term "co-stimulatory domain" refers to an intracellular signaling domain of a co-stimulatory molecule. Co-stimulatory molecules are cell surface molecules other than antigen receptors or Fe receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. The co-stimulatory domain can be, for example, the co-stimulatory domain of 4-1BB, CD27, CD28, or OX40. In one example, a 4-1BB intracellular domain (ICD) can be used (see, e.g., below and SEQ ID NO: 26, or variants thereof). Additional illustrative examples of such co-stimulatory molecules include CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70. In some embodiments, the intracellular domain is the intracellular domain of 4-1 BB. 4-1 BB (CD137; TNFRS9) is an activation induced costimulatory molecule, and is an important regulator of immune responses.

4-1BB is a membrane receptor protein, also known as CD137, which is a member of the tumor necrosis factor (TNF) receptor superfamily. 4-1BB is expressed on activated T

lymphocytes. 4-1BB sequences are known for a number of species, e.g., human 4-1 BB, also known as TNFRSF9 (NCBI Gene 25 ID: 3604) and mRNA (NCBI Reference Sequence: NM_001561.5). 4-1BB can refer to human 4-1BB, including naturally occurring variants, molecules, and alleles thereof. In some embodiments of any of the aspects, e.g., in veterinary applications, 4-1BB can refer to the 4-1BB of, e.g., dog, cat, cow, horse, pig, and the like. Homologs and/or orthologs of human 4-1BB are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a given species for sequence similar to a reference 4-1 BB sequence.

In some embodiments, the intracellular domain is the intracellular domain of a 4-1BB. In some embodiments, the 4-1BB intracellular domain corresponds to an amino acid sequence selected from SEQ ID NO: 26; or includes a sequence selected from SEQ ID NO: 26; or includes at least 75%, at least 80%, at least 85%, 35 at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% sequence identity to a sequence selected from SEQ ID NO: 26 or an amino acid sequence having ≤1, ≤2, ≤3, ≤4, ≤5, ≤6, ≤7, ≤8, ≤9, or ≤10 substitutions relative to SEQ ID NO: 26.

Intracellular Signaling Domains

The properties of the intracellular signaling domain(s) of the CAR can vary as known in the art and as disclosed herein, but the chimeric target/antigen-binding domains(s) render the receptor sensitive to signaling activation when the chimeric target/antigen binding domain binds the target/antigen on the surface of a targeted cell.

With respect to intracellular signaling domains, so-called "first-generation" CARs include those that solely provide CD3-zeta signals upon antigen binding. So-called "second-generation" CARs include those that provide both co-stimulation (e.g., CD28 or CD137) and activation (CD3-zeta;) domains, and so-called "third-generation" CARs include those that provide multiple costimulatory (e.g., CD28 and CD137) domains and activation domains (e.g., CD3-zeta). In various embodiments, the CAR is selected to have high affinity or avidity for the target/antigen - for example, antibody-derived target or antigen binding domains will generally have higher affinity and/or avidity for the target antigen than would a naturally occurring T cell receptor. This property, combined with the high specificity one can select for an antibody provides highly specific T cell targeting by CART cells.

CARs as described herein include an intracellular signaling domain. An "intracellular signaling domain," refers to the part of a CAR polypeptide that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector

cell to elicit effector cell function, e.g., activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited following antigen binding to the extracellular CAR domain. In various examples, the intracellular signaling domain is from CD3-zeta; (see, e.g., below). Additional non-limiting examples of immunoreceptor tyrosine-based activation motif (ITAM)-containing intracellular signaling domains that are of particular use in the technology include those derived from TCR-zeta;, FcR-gamma, FcR-beta, CD3-gamma, CD3-theta, CD3-sigma, CD3-eta, CD3-epsilon, CD3-zeta;, CD22, CD79a, CD79b, and CD66d.

CD3 is a T cell co-receptor that facilitates T lymphocyte activation when simultaneously engaged with the appropriate co-stimulation (e.g., binding of a co-stimulatory molecule). A CD3 complex consists of 4 distinct chains; mammalian CD3 consists of a CD3-gamma chain, a CD3delta chain, and two CD3-epsilon chains.

These chains associate with a molecule known as the T cell receptor (TCR) and the CD3-zeta to generate an activation signal in T lymphocytes. A complete TCR complex includes a TCR, CD3-zeta;, and the complete CD3 complex.

In some embodiments of any aspect, a CAR polypeptide described herein includes an intracellular signaling domain that includes an Immunoreceptor Tyrosine-based Activation Motif or ITAM from CD3-zeta, including variants of CD3-zeta; such as ITAM-mutated CD3-zeta, CD3-eta, or CD3-theta. In some embodiments of any aspect, the ITAM includes three motifs of ITAM of CD3-zeta; (ITAM3). In some embodiments of any aspect, the three motifs of ITAM of CD3-zeta; are not mutated and, therefore, include native or wild-type sequences. In some embodiments, the CD3-zeta; sequence includes the sequence of a CD3-zeta; as set forth in the sequences provided herein, e.g., a CD3-zeta; sequence of SEQ ID NO: 27, or variants thereof.

For example, a CAR polypeptide described herein includes the intracellular signaling domain of CD3-zeta. In some embodiments, the CD3-zeta; intracellular signaling domain corresponds to an amino acid sequence of SEQ ID NO: 27; or includes a sequence selected of SEQ ID NO: 27; or includes a sequence with at least 75%, at least 80%, at least 85%, 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%, or at least 100% sequence identity to a sequence of SEQ ID NO: 27 or an amino acid sequence having $\leq$1, $\leq$2, $\leq$3, $\leq$4, $\leq$5, $\leq$6, $\leq$7, $\leq$8, $\leq$9, or $\leq$10 substitutions relative to SEQ ID NO: 27.

In some embodiments, the intracellular signaling domain comprises a 4-1BB intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a 4-1BB intracellular signaling domain and a CD3-zeta intracellular signaling domain. In some embodiments, the 4-1BB intracellular signaling domain corresponds to an amino acid sequence of SEQ ID NO: 26; or includes a sequence selected of SEQ ID NO: 26; or includes a sequence with at least 75%, at least 80%, at least 85%, 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%, or at least 100% sequence identity to a sequence of SEQ ID NO: 26 or an amino acid sequence having ≤1, ≤2, ≤3, ≤4, ≤5, ≤6, ≤7, ≤8, ≤9, or ≤10 substitutions relative to SEQ ID NO: 26.

Individual CAR and other construct components as described herein can be used with one another and swapped in and out of various constructs described herein, as can be determined by those of skill in the art. Each of these components can include or consist of any of the corresponding sequences set forth herein, or variants thereof.

A more detailed description of CARs and CART cells can be found in Maus et al., Blood 123:2624-2635, 2014; Reardon et al., Neuro-Oncology 16:1441-1458, 2014; Hoyos et al., Haematologica 97:1622, 2012; Byrd et al., J. Clin. Oncol. 32:3039-3047, 2014; Maher et al., Cancer Res 69:4559-4562, 2009; and Tamada et al., Clin. Cancer Res. 18:6436-6445, 2012; each of which is incorporated by reference herein in its entirety.

Signal Peptide

In some embodiments, a CAR polypeptide as described herein includes a signal peptide. Signal peptides can be derived from any protein that has an extracellular domain or is secreted. A CAR polypeptide as described herein may include any signal peptides known in the art. In some embodiments, the CAR polypeptide includes a CD8 signal peptide, e.g., a CD8 signal peptide corresponding to the amino acid sequence of SEQ ID NO: 28, or including the amino acid sequence of SEQ ID NO: 28, or including an amino acid sequence having at least 75%, at least 80%, at least 85%, 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%, or at least 100% sequence identity to the sequence of SEQ ID NO: 28 or an amino acid sequence having ≤1, ≤2, ≤3, ≤4, ≤5, ≤6, ≤7, ≤8, ≤9, or ≤10 substitutions relative to SEQ ID NO: 28. In some embodiments, the CAR polypeptide includes a IgK signal peptide, e.g., a IgK signal peptide corresponding to the amino acid sequence of SEQ ID NO: 29, or including the amino acid sequence of SEQ ID NO: 29, or including an amino acid sequence having at

least 75%, at least 80%, at least 85%, 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%, or at least 100% sequence identity to the sequence of SEQ ID NO: 29 or an amino acid sequence having ≤1, ≤2, ≤3, ≤4, ≤5, ≤6, ≤7, ≤8, ≤9, or ≤10 substitutions relative to SEQ ID NO: 29.

In further embodiments, a CAR polypeptide described herein may optionally exclude one of the signal peptides described herein, e.g., a CD8 signal peptide of SEQ ID NO: 28 or an IgK signal peptide of SEQ ID NO: 29.

Linker Domain

In some embodiments, a CAR further includes a linker domain. As used herein, "linker domain" refers to an oligo- or polypeptide region from about 2 to 100 amino acids in length, which links together any of the domains/regions of the CAR as described herein. In some embodiment, linkers can include or be composed of flexible residues such as glycine and serine so that the adjacent protein domains are free to move relative to one another. Linker sequences useful for the invention can be from 2 to 100 amino acids, 5 to 50 amino acids, 10 to 15 amino acids, 15 to 20 amino acids, or 18 to 20 amino acids in length, and include any suitable linkers known in the art. For instance, linker sequences useful for the invention include, but are not limited to, glycine/serine linkers, e.g., GGGSGGGSGGGS (SEQ ID NO: 31) and Gly4Ser (G4S) (SEQ ID NO: 30) linkers such as (G4S)3 (GGGGSGGGGSGGGGS (SEQ ID NO: 32)) and (G4S)4(GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 33)); the linker sequence of GSTSGSGKPGSGEGSTKG (SEQ ID NO: 34) as described by Whitlow et al., Protein Eng. 6(8):989-95, 1993, the contents of which are incorporated herein by reference in its entirety; the linker sequence of GGSSRSSSSGGGGSGGGG (SEQ ID NO: 35) as described by Andris-Widhopf et al., Cold Spring Harb. Protoc. 2011 (9), 2011, the contents of which are incorporated herein by reference in its entirety; as well as linker sequences with added functionalities, e.g., an epitope tag or an encoding sequence containing Cre-Lox recombination site as described by Sblattero et al., Nat. Biotechnol. 18(1):75-80, 2000, the contents of which are incorporated herein by reference in its entirety. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another.

Furthermore, linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (e.g., P2A and T2A (SEQ ID NO: 36), 2A-like linkers or functional equivalents thereof and combinations thereof.

For example, a P2A linker sequence can correspond to the amino acid sequence of SEQ ID NO: 37. In various examples, linkers having sequences as set forth herein, or variants thereof, are used. It is to be understood that the indication of a particular linker in a construct in a particular location does not mean that only that linker can be used there. Rather, different linker sequences (e.g., P2A and T2A) can be swapped with one another (e.g., in the context of the constructs of the present invention), as can be determined by those of skill in the art. In some embodiments, the linker region is T2A derived from Thosea asigna virus. Non-limiting examples of linkers that can be used in this technology include T2A, P2A, E2A, BmCPV2A, and BmlFV2A. Linkers such as these can be used in the context of polyproteins, such as those described below. For example, they can be used to separate a CAR component of a polyprotein from a TAPi and/or a oligonucleotide comprising a sequence that is complementary to a gene encoding MHC class II transactivator protein (e.g. an shRNA complementary to CIITA).

Reporter Molecule

In some embodiments, a CAR as described herein optionally further includes a reporter molecule, e.g., to permit for non-invasive imaging (e.g., positron-emission tomography PET scan). In a bispecific CAR that includes a reporter molecule, the first extracellular binding domain and the second extracellular binding domain can include different or the same reporter molecule. In a bispecific CART cell, the first CAR and the second CAR can express different or the same reporter molecule. In another embodiment, a CAR as described herein further includes a reporter molecule (for example hygromycin phosphotransferase (hph)) that can be imaged alone or in combination with a substrate or chemical (for example 9-[4-[18F]fluoro-3-(hydroxymethyl)butyl]guanine ([18F]FHBG)). In another embodiment, a CAR as described herein further includes nanoparticles at can be readily imaged using non-invasive techniques (e.g., gold nanoparticles (GNP) functionalized with 64Cu2+). Labeling of CART cells for non-invasive imaging is reviewed, for example in Bhatnagar et al., lntegr. Biol. (Camb). 5(1):231-238, 2013, and Keu et al., Sci. Transl. Med. 18; 9(373), 2017, which are incorporated herein by reference in their entireties.

In some embodiments, GFP and mCherry may be used as fluorescent tags for imaging a CAR expressed on a T cell (e.g., a CART cell). It is expected that essentially any fluorescent protein known in the art can be used as a fluorescent tag for this purpose. For clinical applications, the CAR need not include a fluorescent tag or fluorescent protein. In each instance of particular constructs provided herein, therefore, any markers present in the

constructs can be removed. The invention includes the constructs with or without the markers. Accordingly, when a specific construct is referenced herein, it can be considered with or without any markers or tags (including, e.g., histidine tags, such as the histidine tag of HHHHHH (SEQ ID NO: 38)) as being included within the invention.

In some embodiments, the CAR comprises a CD8 leader sequence, an anti-CD19 antibody, a CD8 hinge/transmembrane domain, a 4-1BB intracellular signalling domain, and CD3-zeta intracellular signaling domain and a T2A peptide domain. In some embodiments, the CAR comprises a CD8 leader sequence, a FMC63 heavy chain and light chain, a CD8 hinge/transmembrane domain, a 4-1BB intracellular signaling domain, and CD3-zeta intracellular signaling domain and a T2A peptide domain. In some embodiments, the CAR comprises a CD8 leader sequence, a FMC63 heavy chain, a linker, a FMC63 light chain, a CD8 hinge/transmembrane domain, a 4-1BB intracellular signaling domain, and CD3-zeta intracellular signaling domain and a T2A peptide domain.

Cell types

In some embodiments, the cell comprising (i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and (ii) an oligonucleotide that is complementary to a polynucleotide encoding MHC class II or a MHC class II transactivator protein is a eukaryotic cell. In some embodiments, the cell comprising (i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and (ii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is an immune cell. As used herein, "immune cell" refers to a cell that plays a role in the immune response. Immune cells are of hematopoietic origin, and include lymphocytes, such as B cells and T cells; natural killer cells; myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes. In some embodiments, the immune cell is a T cell; a NK cell; a NKT cell; lymphocytes, such as B cells and T cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes. In some embodiments, the immune cell is a T cell.

In some embodiments, the immune cell is obtained from a subject having or diagnosed as having cancer, a plasma cell disorder, or autoimmune disease, modified as described herein (e.g. to comprise a TAPi, an anti-MHC class II oligonucleotide, and a CAR), and then administered to the subject.

In some embodiments, the cell is an allogenic cell. The term allogeneic cell refers to a cell that was not derived or extracted from the subject being treated (e.g., the cell is extracted or derived from another). In some embodiments, the allogenic cell is derived from an embryonic stem cell or a induced pluripotent stem cell. In some embodiments, the allogenic cell is extracted from a healthy subject. Because allogenic stem cells are from another, the immunogenicity of the stem cell in the subject may be increased. Thus, introducing the TAPi and/or the anti-MHC class II oligonucleotide may decrease the immunogenicity of the allogenic cell in the subject being treated.

In some embodiments, an immune cell, e.g., a T cell, can be engineered to include any of the TAPi or oligonucleotide complementary to MHC class I, and/or oligonucleotides complementary to MHC class II as described herein. In some embodiments, an immune cell, e.g., a T cell, can be engineered to include any of the CAR polypeptides described herein or known in the art. For example, T cells can be isolated from peripheral blood taken from a donor or patient. T cells can be isolated from a mammal. Preferably, T cells are isolated from a human.

Polynucleotides encoding TAPi and an oligonucleotide.

In some embodiments, this application discloses polynucleotides comprising a first nucleic acid sequence encoding the TAPi and a second nucleic acid sequence encoding an oligonucleotide complementary to MHC Class II (e.g., MHC class II shRNAs), as described herein. In some embodiments, this application discloses polynucleotides comprising a first nucleic acid sequence encoding the TAPi and a second nucleic acid sequence encoding an oligonucleotide complementary to a gene encoding a MHC Class II transactivator protein (e.g. CIITA), as described herein. In some embodiments, the polynucleotide further comprises and a third nucleic acid sequence encoding a CAR as described herein.

In some embodiments, the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are each operably linked to a promoter. In some embodiments, the first nucleic acid sequence is operably linked to a first promoter and the second nucleic acid sequence is operably linked to a second promoter. In some such embodiments, the third nucleic acid sequence is operably linked to the first promoter, the second promoter, or a third promoter. Promoters can be a constitutively expressed promoter (e.g., an EF1a promoter) or an inducibly expressed promoter (e.g., a NFAT promoter). In some embodiments, a promoter is induced by CAR activity or T cell receptor (TCR) activity.

In some embodiments, expression of the TAPi and CAR are driven by the same promoter, e.g., a constitutively expressed promoter (e.g., an EF1 a promoter). In other embodiments, expression of the TAPi and CAR are driven by different promoters. The polynucleotide sequence encoding the CAR can be located upstream of the polynucleotide sequence encoding the TAPi, or the polynucleotide sequence encoding the TAPi can be located upstream of the polynucleotide sequence encoding the CAR. In some embodiments, expression of the oligonucleotide complementary to a gene encoding a MHC Class II transactivator protein (e.g., CIITA) is driven by a different promoter (e.g., a U6 promoter) than expression of the TAPi or the CAR. In some embodiments, the oligonucleotide complementary to a gene encoding a MHC Class II transactivator protein is located upstream of the TAPi and the CAR. In some embodiments, the oligonucleotide complementary a gene encoding a MHC Class II transactivator protein is located downstream of the TAPi and the CAR.

In some embodiments, the nucleic acid sequence encoding the TAPi, the nucleic acid sequence encoding the oligonucleotide complementary a gene encoding a MHC Class II transactivator protein and the nucleic acid sequence encoding a CAR are encoded within the same vector. In some embodiments, the nucleic acid sequence encoding the TAPi, the nucleic acid sequence encoding the oligonucleotide complementary a gene encoding a MHC Class II transactivator protein and the nucleic acid sequence encoding a CAR are encoded on two or three vectors.

In various examples, the vectors are retroviral vectors. Retroviruses, such as lentiviruses, provide a convenient platform for delivery of nucleic acid sequences encoding a gene, or chimeric gene of interest. A selected nucleic acid sequence can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells, e.g., in vitro or ex vivo. Retroviral systems are well known in the art and are described in, for example, U.S. Patent No. 5,219,740; Kurth and Bannert (2010) "Retroviruses: Molecular Biology, Genomics and Pathogenesis" Galster Academic Press (ISBN:978-1-90455-55-4); and Hu and Pathak Pharmacological Reviews 2000 52:493-512; which are incorporated by reference herein in their entirety. Lentiviral system for efficient DNA delivery can be purchased from OriGene; Rockville, MD. In various embodiments, the protein is expressed in the T cell by transfection or electroporation of an expression vector including nucleic acid encoding the protein using vectors and methods that are known in the art. In some embodiments, the vector is a viral

vector or a non-viral vector. In some embodiments, the viral vector is a retroviral vector (e.g., a lentiviral vector), an adenovirus vector, or an adeno-associated virus vector.

In some embodiments, the cells (e.g., CAR-T cells) described herein comprises any one of the polynucleotides described above.

Transfection or electroporation methods of vectors and/ nucleic acids are known in the art.

Efficient expression of the TAPi and an oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) and/or CAR can be assessed using standard assays that detect the mRNA, DNA, or gene product of the nucleic acid encoding the TAPi, the oligonucleotide and/or CAR (and optional antibody reagent or cytokine), such as RT-PCR, FAGS, northern blotting, western blotting, ELISA, or immunohistochemistry.

Methods of use

In some embodiments, this application discloses a method of modifying a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a polynucleotide encoding MHC class II or a MHC class II transactivator protein (e.g., CIITA, RFX, RFXANK, NYFA, NYFC, NF-gamma and CREB), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

In some embodiments, this application discloses a method of modifying the immunogenicity of a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a polynucleotide encoding MHC class II or a MHC class II transactivator protein (e.g., CIITA, RFX, RFXANK, NYFA, NYFC, NF-gamma and CREB), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some embodiments, this application discloses a method of modifying the immunogenicity of a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a polynucleotide encoding MHC class II transactivator protein CIITA (SEQ ID NO: 7), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some embodiments, this application discloses a method of modifying the immunogenicity of a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a

polynucleotide encoding MHC class II transactivator protein CIITA (SEQ ID NO: 7), wherein the oligonucleotide is a RNA interference (RNAi) oligonucleotide (e.g., a shRNA).

In some embodiments, this application discloses methods of decreasing a subject's immune response to a cell therapy (e.g., an allogenic cell therapy). In some embodiments, the method comprising introducing (e.g., via electroporation) into cells of the cell therapy, prior to administration of the therapy to a subject, an oligonucleotide that is complementary to a polynucleotide encoding MHC class II or a MHC class II transactivator protein (e.g., CIITA, RFX, RFXANK, NYFA, NYFC, NF-gamma and CREB), wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide. In some embodiments, introducing the oligonucleotide comprises introducing a vector encoding the oligonucleotide (e.g., a vector encoding an shRNA). In some embodiments, introducing the oligonucleotide comprises introducing the oligonucleotide directly into the cell (e.g., transfecting an shRNA). In some embodiments, the oligonucleotide is any one of the oligonucleotides that comprise a sequence that is complementary to MHC class II as described herein. In some embodiments, the oligonucleotide is an shRNA. In some embodiments, the oligonucleotide in complementary to CIITA (SEQ ID NO: 7). In some embodiments, the oligonucleotide comprises a sequence of SEQ ID NO: 8.

In some embodiments, the method further comprises introducing into cells of the cell therapy, prior to administration to the subject, a TAPi or variant thereof, as described herein. In some embodiments, the TAPi is an EBV TAPi (e.g., SEQ ID NO: 3) or a variant thereof.

In some embodiments, the cell therapy is an immune cell therapy as described herein. In some embodiments, the cell therapy is an allogenic cell therapy as described herein. In some embodiments, the cell therapy is an allogenic immune cell therapy as described herein.

In some embodiments, the cell therapy is a CAR-T cell therapy. In some embodiments, the cell therapy is an allogenic CAR-T cell therapy. In some embodiments, the cell therapy comprises any known CAR including any CAR described herein. In some embodiments, the cell therapy comprises an anti-CD19 CAR. In some embodiments, the anti-CD19 CAR comprises an amino acid sequence that is at least 85% identical (e.g., at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical) to SEQ ID NO: 41. In some embodiments, the anti-CD19 CAR comprises an amino acid sequence of SEQ ID NO:

41. In some embodiments, the anti-CD19 CAR comprises an FMC63 VH and VL (e.g., SEQ ID NOs: 39-40), or a variant thereof.

In some embodiments, the subject is a human subject. In some embodiments, the method decreases natural killer cell activation.

In some aspects this application discloses method of treating a subject (e.g., a subject diagnosed with cancer), the method comprising administering a cell therapy comprising a oligonucleotide complementary to a gene encoding a MHC class II transactivator protein (e.g., a shRNA targeting CIITA) and/or a TAPi as described herein. In some aspects this application discloses method of treating a subject (e.g., a subject diagnosed with cancer), the method comprising administering a cell therapy comprising a oligonucleotide complementary to a gene encoding a MHC Class II transactivator protein (e.g., a shRNA targeting CIITA) and a TAPi as described herein. In some aspects this application discloses method of treating a subject (e.g., a subject diagnosed with cancer), the method comprising administering a cell therapy comprising a oligonucleotide complementary to a gene encoding a MHC class II transactivator protein (e.g., a shRNA complementary to CIITA) and a oligonucleotide that is complementary to a subunit of MHC class I (e.g., a shRNA complementary to beta-2-microglobulin).

In some embodiments, the subject is diagnosed with cancer. In some embodiments, the cancer is a hematological cancer. In some embodiments, the hematological cancer is selected from the group consisting of Leukemia, Lymphoma, and Myeloma. In some embodiments, the hematological cancer is selected from the group consisting of Acute lymphoblastic leukemia (ALL), Acute myelogenous leukemia (AML), Chronic myelogenous leukemia (CML), Chronic lymphocytic leukemia (CLL), Hairy cell leukemia, Hodgkin's disease, Non-Hodgkin lymphoma (many subtypes), Chronic lympocytic leukemia, Follicular Lymphoma, Marginal zone lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, and Multiple myeloma. In some embodiments, the hematological cancer is selected from the group consisting of acute lymphoblastic leukemia or mantle cell lymphoma. In some embodiments, the hematological cancer is B-cell acute lymphoblastic leukemia (B-ALL), acute lymphoblastic leukemia/lymphoma (ALL/LBL), or B cell lymphoma.

In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor cancer is selected from the group consisting of ovarian cancer, mesothelioma, brain cancer, liver cancer, kidney cancer, lung cancer, breast cancer, prostate cancer, throat cancer, thyroid cancer, colon cancer, testicular cancer, and skin cancer.

In some embodiments, the cancer is characterized by cells that express CD19.

Subject

As used herein, a "subject" means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include, for example, chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, e.g., rhesus. Rodents include, for example, mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include, for example, cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, "individual," "patient," and "subject" are used interchangeably herein. Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of disease, e.g., cancer. A subject can be male or female.

A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment (e.g., a pancreatic cancer, a lung cancer, an ovarian cancer, endometrial cancer, biliary cancer, gastric cancer, or mesothelioma or another type of cancer expressing mesothelin, among others) or one or more complications related to such a condition, and optionally, have already undergone treatment for the condition or the one or more complications related to the condition.

Alternatively, a subject can also be one who has not been previously diagnosed as having such condition or related complications. For example, a subject can be one who exhibits one or more risk factors for the condition or one or more complications related to the condition or a subject who does not exhibit risk factors.

A "subject in need" of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at risk of developing that condition.

Pharmaceutical Compositions

As used herein, the term "pharmaceutical composition" refers to an active agent (e.g., a cell therapy as described herein) in combination with a pharmaceutically acceptable carrier e.g., a carrier commonly used in the pharmaceutical industry.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of

sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a carrier other than water. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a cream, emulsion, gel, liposome, nanoparticle, and/or ointment. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be an artificial or engineered carrier, e.g., a carrier in
which the active ingredient would not be found to occur in nature.

In one aspect of the technology, the technology described herein relates to a pharmaceutical composition including activated CART cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein, and optionally a pharmaceutically acceptable carrier. The active ingredients of the pharmaceutical composition at a minimum include activated CART cells (e.g., comprising a CD19 CAR) comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist essentially of activated CART cells (e.g., comprising a CD19 CAR) comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist of activated CAR T cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein. Pharmaceutically acceptable carriers for cell-based therapeutic formulation include saline and aqueous buffer solutions, Ringer's solution, and serum component, such as serum albumin, HDL and LDL. The terms such as "excipient," "carrier," "pharmaceutically acceptable carrier", "pharmaceutically acceptable excipient" or the like are used interchangeably herein.

In some embodiments, the pharmaceutical composition including activated CAR T cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to

CIITA) as described herein can be a parenteral dose form. Since administration of parenteral dosage forms typically bypasses the patient's natural defenses against contaminants, the components apart from the CART cells themselves are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions. Any of these can be added to the activated CART cells preparation prior to administration. Suitable vehicles that can be used to provide parenteral dosage forms of activated CAR T cells as disclosed within are well known to those skilled in the art. Examples include, without limitation: saline solution; glucose solution; aqueous vehicles including but not limited to, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, and lactated Ringer's injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and propylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Dosage

"Unit dosage form" as the term is used herein refers to a dosage for suitable one administration. By way of example, a unit dosage form can be an amount of therapeutic disposed in a delivery device, e.g., a syringe or intravenous drip bag. In some embodiments, a unit dosage form is administered in a single administration. In another, embodiment more than one unit dosage form can be administered simultaneously.

In some embodiments, the activated CAR T cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) described herein are administered as a monotherapy, i.e., another treatment for the condition is not concurrently administered to the subject. A pharmaceutical composition including the T cells described herein can generally be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. If necessary, T cell compositions can also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. Med. 30 319:1676, 1988).

In certain aspects, it may be desired to administer activated CART cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding

a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom as described herein, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 35 10 cc to 400 cc. In certain aspects, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

Administration

In some embodiments, the methods described herein relate to treating a subject having or diagnosed as having cancer, a plasma cell disease or disorder, or an autoimmune disease or disorder with a mammalian cell including any of the CAR polypeptides described herein, or a nucleic acid encoding any of the CAR polypeptides described herein. The CART cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) described herein include mammalian cells including any of the TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein and any of the CAR polypeptides (and optional antibody reagents or cytokines) described herein or known in the art, or a nucleic acid encoding any of the TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA)s or CAR polypeptides described herein.

Subjects having a condition can be identified by a physician using current methods of diagnosing the condition. Symptoms and/or complications of the condition, which characterize these conditions and aid in diagnosis are well known in the art and include but are not limited to, fatigue, persistent infections, and persistent bleeding. Tests that may aid in a diagnosis of, e.g., the condition, but are not limited to, blood screening and bone marrow testing, and are known in the art for a given condition. A family history for a condition, or exposure to risk factors for a condition can also aid in determining if a subject is likely to have the condition or in making a diagnosis of the condition.

The compositions described herein can be administered to a subject having or diagnosed as having a condition. In some embodiments, the methods described herein include administering an effective amount of activated CAR T cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC

class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein to a subject in order to alleviate a symptom of the condition. As used herein, “alleviating a symptom of the condition” is ameliorating any condition or symptom associated with the condition. As compared with an equivalent untreated control, such reduction is by at least 5% 10% 20% 40% 50% 60% 80% 90% 95% 99% or more as measured by any standard technique. A variety of means for administering the compositions described herein to subjects are known to those of skill in the art. In some embodiments, the compositions described herein are administered systemically or locally. In a preferred embodiment, the compositions described herein are administered intravenously. In another embodiment, the compositions described herein are administered at the site of a tumor.

The term "effective amount" as used herein refers to the amount of a cell therapy (e.g., activated CAR T cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA)) described herein needed to alleviate at least one or more symptom of the disease or disorder, and relates to a sufficient amount of the cell preparation or composition to provide the desired effect. The term “therapeutically effective amount” therefore refers to an amount of a cell therapy described herein that is sufficient to provide a particular anti-condition effect when administered to a typical subject. An effective amount as used herein, in various contexts, would also include an amount sufficient to delay the development of a symptom of the disease, alter the course of a symptom disease (for example but not limited to, slowing the progression of a condition), or reverse a symptom of the condition. Thus, it is not generally practicable to specify an exact “effective amount.” However, for any given case, an appropriate “effective amount” can be determined by one of ordinary skill in the art using only routine experimentation.

Effective amounts, toxicity, and therapeutic efficacy can be evaluated by standard pharmaceutical procedures in cell cultures or experimental animals. The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of a cell therapy (e.g., activated CART cells) comprising a TAPi and a oligonucleotide comprising a sequence that

is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein, which achieves a half-maximal inhibition of symptoms) as determined in cell culture, or in an appropriate animal model. Levels in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay, e.g., assay for bone marrow testing, among others. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

Modes of Administration

Modes of administration of a cell therapy described herein can include, for example intravenous (iv) injection or infusion. The compositions described herein can be administered to a patient transarterially, intratumorally, intranodally, intraperitoneally or intramedullary. In some embodiments, the compositions of T cells may be injected directly into a tumor, lymph node, or site of infection. In some embodiments, the compositions described herein are administered into a body cavity or body fluid (e.g., ascites, pleural fluid, peritoneal fluid, or cerebrospinal fluid).

In some embodiments, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted ex vivo to select and/or isolate the cells of interest, e.g., T cells. In some embodiments, the T cells may be extracted from a healthy subject, e.g., via leukapheresis, or differentiated in vitro (e.g., using iPSC or embryonic stem cells). Any of these T cell isolates may be expanded by contact with an artificial APC, e.g., an aAPC expressing anti-CD28 and anti-CD3 CD Rs, and treated such that one or more polynucleotides of the technology (e.g., polynucleotide comprising a TAPi, an oligonucleotide that is complementary to a gene encoding CIITA and a CAR) may be introduced, thereby creating a CAR T cell. Subjects in need thereof can subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. Following or concurrent with the transplant, subjects can receive an infusion of the expanded CAR T cells. In some embodiment, expanded cells are administered before or following surgery. In some embodiments, lymphodepletion is performed on a subject prior to administering one or more CART cell as described herein. In such embodiments, the lymphodepletion can include administering one or more of melphalan, 40urvivi, cyclophosphamide, and fludarabine. The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of

the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices.

In some embodiments, a single treatment regimen is required. In others, administration of one or more subsequent doses or treatment regimens can be performed. For example, after treatment biweekly for three months, treatment can be repeated once per month, for six months or a year or longer. In some embodiments, no additional treatments are administered following the initial treatment.

The dosage of a composition as described herein can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. With respect to duration and frequency of treatment, it is typical for skilled clinicians to monitor subjects in order to determine when the treatment is providing therapeutic benefit, and to determine whether to administer further cells, discontinue treatment, resume treatment, or make other alterations to the treatment regimen. The dosage should not be so large as to cause adverse side effects, such as cytokine release syndrome. Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication.

Efficacy

The efficacy of the cell therapy (e.g., activated CART cells comprising a TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA)) described herein in, e.g., the treatment of a condition described herein, or to induce a response as described herein (e.g., a reduction in cancer cells) can be determined by the skilled clinician. However, a treatment is considered "effective treatment," as the term is used herein, if one or more of the signs or symptoms of a condition described herein is altered in a beneficial manner, other clinically accepted symptoms are improved, or even ameliorated, or a desired response is induced, e.g., by at least 10% following treatment according to the methods described herein. Efficacy can be assessed, for example, by measuring a marker, indicator, symptom, and/or the incidence of a condition treated according to the methods described herein or any other measurable parameter appropriate.

Treatment according to the methods described herein can reduce levels of a marker or symptom of a condition, e.g., by at least 10%, at least 15%, at least 20%, at least 25%, at least

30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 % or at least 90% or more.

Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization, or need for medical interventions (i.e., progression of the disease is halted). Methods of measuring these indicators are known to those of skill in the art and/or are described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human or an animal) and includes: (1) inhibiting the disease, e.g., preventing a worsening of symptoms (e.g., pain or inflammation); or (2) relieving the severity of the disease, e.g., causing regression of symptoms. An effective amount for the treatment of a disease means that amount which, when administered to a subject in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators of a condition or desired response. It is well within the ability of one skilled in the art to monitor efficacy of administration and/or treatment by measuring any one of such parameters, or any combination of parameters. Efficacy of a given approach can be assessed in animal models of a condition described herein. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant change in a marker is observed.

Cell Therapy

One aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method including: engineering a T cell to include any of the TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC Class II protein or a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) as described herein and include any CAR polypeptides described herein (e.g., a CD19 CAR) or known in the art on the T cell surface; and administering the engineered T cell to the subject. In the case of cancer, the method can be for treating diagnosed cancer, preventing recurrence of cancer, or for use in an adjuvant or neoadjuvant setting. In some embodiments, the method comprises providing a T cell engineered to include any CAR polypeptides described herein or known in the art on the T cell surface; engineering a T cell to include any of the TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) described herein; and administering the engineered T cell to the subject.

One aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method including: administering the cell of any of the mammalian cells including any of the TAPi and a oligonucleotide comprising a sequence that is complementary to a gene encoding a MHC class II transactivator protein (e.g., an shRNA complementary to CIITA) described herein, and any of the CAR polypeptides described herein or known in the art. In some embodiments of any of aspect, the engineered CAR-T cell is stimulated and/or activated prior to administration to the subject.

All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior technology or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or

amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

The technology described herein is further illustrated by the following examples, which in no way should be construed as being further limiting.

Table 1: Sequences

SEQ ID NO	Name	Sequence
1	HSV ICP47	MSWALEMADTFLDTMRVGPRTYADVRDEINKRGREDREAARTA VHDPERPLLRSPGLLPEIAPNASLGVAHRRTGGTVTDSPRNPVTR
2	HCMV US6	MDLLIRLGFLLMCALPTPGERSSRDPKTLLSLSPRQQACVPRTKSH RPVCYNDTGDCTDADDSWKQLGEDFAHQCLQAAKKRPKTHKSR PNDRNLEGRLTCQRVRRLLPCDLDIHPSHRLLTLMNNCVCDGAV WNAFRLIERHGFFAVTLYLCCGITLLVVILALLCSITYESTGRGIRR CGS
3	EBV BNLF2a	MVHVLERALLEQQSSACGLPGSSTETRPSHPCPEDPDVSRLRLLLV VLCVLFGLLCLLLI
4	MHC Class I HLA-B *39013	ATGCTGGTCATGGCGCCCCGAACCGTCCTCCTGCTGCTCTCGGC GGCCCTGGCCCTGACCGAGACCTGGGCCGGCTCCCACTCCATG AGGTATTTCTACACCTCCGTGTCCCGGCCCGGCCGCGGGGAGCC CCGCTTCATCTCAGTGGGCTACGTGGACGACACGCAGTTCGTGA GGTTCGACAGCGACGCCGCGAGTCCGAGAGAGGAGCCGCGGG CGCCGTGGATAGAGCAGGAGGGGCCGGAATATTGGGACCGGA ACACACAGATCTGCAAGACCAACACACAGACTGACCGAGAGA GCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCCGG GTCTCACACCCTCCAGAGGATGTACGGCTGCGACGTGGGGCCG GACGGGCGCCTCCTCCGCGGGCATAACCAGTTCGCCTACGACG GCAAGGATTACATCGCCCTGAACGAGGACCTGAGCTCCTGGAC CGCGGCGGACACCGCGGCTCAGATCACCCAGCGCAAGTGGGAG GCGGCCCGTGTGGCGGAGCAGCTGAGAACCTACCTGGAGGGCA CGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA GACGCTGCAGCGCGCGGACCCCCCAAAGACACATGTGACCCAC CACCCCATCTCTGACCATGAGGCCACCCTGAGGTGCTGGGCCCT GGGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGAT GGCGAGGACCAAACTCAGGACACCGAGCTTGTGGAGACCAGAC CAGCAGGAGACAGAACCTTCCAGAAGTGGGCAGCTGTGGTGGT GCCTTCTGGAGAAGAGCAGAGATACACATGCCATGTACAGCAT GAGGGGCTGCCGAAGCCCCTCACCCTGAGATGGGAGCCATCTT CCCAGTCCACCGTCCCCATCGTGGGCATTGTTGCTGGCCTGGCT GTCCTAGCAGTTGTGGTCATCGGAGCTGTGGTCGCTGCTGTGAT GTGTAGGAGGAAGAGCTCAGGTGGAAAAGGAGGGAGCTACTC TCAGGCTGCGTCCAGCGACAGTGCCCAGGGCTCTGATGTGTCTC TCACAGCTTGA

5	Beta-2-microglobulin	ATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTT TCTGGCCTGGAGGCTATCCAGCGTACTCCAAAGATTCAGGTTTA CTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCCTGAAT TGCTATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTT ACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTCAGA CTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACAC TGAATTCACCCCCACTGAAAAAGATGAGTATGCCTGCCGTGTG AACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGATC GAGACATGTAA
6	MHC Class II HLA-DQA1*010401	ATGATCCTAAACAAAGCTCTGCTGCTGGGGGCCCTCGCTCTGAC CACCATGATGAGCCCTTGTGGAGGTGAAGGCATTGTGGCTGAC CACGTTGCCTCTTGTGGTGTAAACTTGTACCAGTTTTACGGTCC CTCTGGCCAGTACACCCATGAATTTGATGGAGATGAGGAGTTCT ACGTGGACCTGGAGAGGAAGGAGACTGCCTGGCGGTGGCCTGA GTTCAGCAAATTTGGAGGTTTTGACCCGCAGGGTGCACTGAGA AACATGGCTGTGGCAAAACACAACTTGAACATCATGATTAAAC GCTACAACTCTACCGCTGCTACCAATGAGGTTCCTGAGGTCACA GTGTTTTCCAAGTCTCCCGTGACACTGGGTCAGCCCAACACCCT CATTTGTCTTGTGGACAACATCTTTCCTCCTGTGGTCAACATCA CATGGCTGAGCAATGGGCAGTCAGTCACAGAAGGTGTTTCTGA GACCAGCTTCCTCTCCAAGAGTGATCATTCCTTCTTCAAGATCA GTTACCTCACCTTCCTCCCTTCTGCTGATGAGATTTATGACTGCA AGGTGGAGCACTGGGGCCTGGACCAGCCTCTTCTGAAACACTG GGAGCCTGAGATTCCAGCCCCTATGTCAGAGCTCACAGAGACT GTGGTCTGCACCCTGGGGTTGTCTGTGGGCCTCGTGGGCATTGT GGTGGGCACTGTCTTCATCATCCAAGGCCTGCGTTCAGTTGGTG CTTCCAGACACCAAGGGCCATTGTGA
7	CIITA	ATGCGTTGCCTGGCTCCACGCCCTGCTGGGTCCTACCTGTCAGA GCCCCAAGGCAGCTCACAGTGTGCCACCATGGAGTTGGGGCCC CTAGAAGGTGGCTACCTGGAGCTTCTTAACAGCGATGCTGACC CCCTGTGCCTCTACCACTTCTATGACCAGATGGACCTGGCTGGA GAAGAAGAGATTGAGCTCTACTCAGAACCCGACACAGACACCA TCAACTGCGACCAGTTCAGCAGGCTGTTGTGTGACATGGAAGG TGATGAAGAGACCAGGGAGGCTTATGCCAATATCGCGGAACTG GACCAGTATGTCTTCCAGGACTCCCAGCTGGAGGGCCTGAGCA AGGACATTTTCATAGAGCACATAGGACCAGATGAAGTGATCGG TGAGAGTATGGAGATGCCAGCAGAAGTTGGGCAGAAAAGTCA GAAAAGACCCTTCCCAGAGGAGCTTCCGGCAGACCTGAAGCAC TGGAAGCCAGCTGAGCCCCCCACTGTGGTGACTGGCAGTCTCCT AGTGGGACCAGTGAGCGACTGCTCCACCCTGCCCTGCCTGCCA CTGCCTGCGCTGTTCAACCAGGAGCCAGCCTCCGGCCAGATGC GCCTGGAGAAAACCGACCAGATTCCCATGCCTTTCTCCAGTTCC TCGTTGAGCTGCCTGAATCTCCCTGAGGGACCCATCCAGTTTGT CCCCACCATCTCCACTCTGCCCCATGGGCTCTGGCAAATCTCTG AGGCTGGAACAGGGGTCTCCAGTATATTCATCTACCATGGTGA GGTGCCCCAGGCCAGCCAAGTACCCCCTCCCAGTGGATTCACT GTCCACGGCCTCCCAACATCTCCAGACCGGCCAGGCTCCACCA GCCCCTTCGCTCCATCAGCCACTGACCTGCCCAGCATGCCTGAA CCTGCCCTGACCTCCCGAGCAAACATGACAGAGCACAAGACGT CCCCCACCCAATGCCCGGCAGCTGGAGAGGTCTCCAACAAGCT TCCAAAATGGCCTGAGCCGGTGGAGCAGTTCTACCGCTCACTG CAGGACACGTATGGTGCCGAGCCCGCAGGCCCGGATGGCATCC TAGTGGAGGTGGATCTGGTGCAGGCCAGGCTGGAGAGGAGCAG CAGCAAGAGCCTGGAGCGGGAACTGGCCACCCCGGACTGGGCA GAACGGCAGCTGGCCCAAGGAGGCCTGGCTGAGGTGCTGTTGG CTGCCAAGGAGCACCGGCGGCCGCGTGAGACACGAGTGATTGC TGTGCTGGGCAAAGCTGGTCAGGGCAAGAGCTATTGGGCTGGG GCAGTGAGCCGGGCCTGGGCTTGTGGCCGGCTTCCCCAGTACG

		ACTTTGTCTTCTCTGTCCCCTGCCATTGCTTGAACCGTCCGGGG GATGCCTATGGCCTGCAGGATCTGCTCTTCTCCCTGGGCCCACA GCCACTCGTGGCGGCCGATGAGGTTTTCAGCCACATCTTGAAG AGACCTGACCGCGTTCTGCTCATCCTAGACGGCTTCGAGGAGCT GGAAGCGCAAGATGGCTTCCTGCACAGCACGTGCGGACCGGCA CCGGCGGAGCCCTGCTCCCTCCGGGGGCTGCTGGCCGGCCTTTT CCAGAAGAAGCTGCTCCGAGGTTGCACCCTCCTCCTCACAGCCC GGCCCCGGGGCCGCCTGGTCCAGAGCCTGAGCAAGGCCGACGC CCTATTTGAGCTGTCCGGCTTCTCCATGGAGCAGGCCCAGGCAT ACGTGATGCGCTACTTTGAGAGCTCAGGGATGACAGAGCACCA AGACAGAGCCCTGACGCTCCTCCGGGACCGGCCACTTCTTCTCA GTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTGTGCCAGCTC TCAGAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCT CCACGCTCACGGGACTCTATGTCGGCCTGCTGGGCCGTGCAGCC CTCGACAGCCCCCCCGGGGCCCTGGCAGAGCTGGCCAAGCTGG CCTGGGAGCTGGGCCGCAGACATCAAAGTACCCTACAGGAGGA CCAGTTCCCATCCGCAGACGTGAGGACCTGGGCGATGGCCAAA GGCTTAGTCCAACACCCACCGCGGGCCGCAGAGTCCGAGCTGG CCTTCCCCAGCTTCCTCCTGCAATGCTTCCTGGGGGCCCTGTGG CTGGCTCTGAGTGGCGAAATCAAGGACAAGGAGCTCCCGCAGT ACCTAGCATTGACCCCAAGGAAGAAGAGGCCCTATGACAACTG GCTGGAGGGCGTGCCACGCTTTCTGGCTGGGCTGATCTTCCAGC CTCCCGCCCGCTGCCTGGGAGCCCTACTCGGGCCATCGGCGGCT GCCTCGGTGGACAGGAAGCAGAAGGTGCTTGCGAGGTACCTGA AGCGGCTGCAGCCGGGGACACTGCGGGCGCGGCAGCTGCTGGA GCTGCTGCACTGCGCCCACGAGGCCGAGGAGGCTGGAATTTGG CAGCACGTGGTACAGGAGCTCCCCGGCCGCCTCTCTTTTCTGGG CACCCGCCTCACGCCTCCTGATGCACATGTACTGGGCAAGGCCT TGGAGGCGGCGGGCCAAGACTTCTCCCTGGACCTCCGCAGCAC TGGCATTTGCCCCTCTGGATTGGGGAGCCTCGTGGGACTCAGCT GTGTCACCCGTTTCAGGGCTGCCTTGAGCGACACGGTGGCGCTG TGGGAGTCCCTGCAGCAGCATGGGGAGACCAAGCTACTTCAGG CAGCAGAGGAGAAGTTCACCATCGAGCCTTTCAAAGCCAAGTC CCTGAAGGATGTGGAAGACCTGGGAAAGCTTGTGCAGACTCAG AGGACGAGAAGTTCCTCGGAAGACACAGCTGGGGAGCTCCCTG CTGTTCGGGACCTAAAGAAACTGGAGTTTGCGCTGGGCCCTGTC TCAGGCCCCCAGGCTTTCCCCAAACTGGTGCGGATCCTCACGGC CTTTTCCTCCCTGCAGCATCTGGACCTGGATGCGCTGAGTGAGA ACAAGATCGGGGACGAGGGTGTCTCGCAGCTCTCAGCCACCTT CCCCCAGCTGAAGTCCTTGGAAACCCTCAATCTGTCCCAGAACA ACATCACTGACCTGGGTGCCTACAAACTCGCCGAGGCCCTGCCT TCGCTCGCTGCATCCCTGCTCAGGCTAAGCTTGTACAATAACTG CATCTGCGACGTGGGAGCCGAGAGCTTGGCTCGTGTGCTTCCG GACATGGTGTCCCTCCGGGTGATGGACGTCCAGTACAACAAGT TCACGGCTGCCGGGGCCCAGCAGCTCGCTGCCAGCCTTCGGAG GTGTCCTCATGTGGAGACGCTGGCGATGTGGACGCCCACCATC CCATTCAGTGTCCAGGAACACCTGCAACAACAGGATTCACGGA TCAGCCTGAGATGA
8	RFX	ATGGCAACACAGGCGTATACTGAGCTACAGGCAGCCCCGCCAC CATCCCAGCCGCCACAGGCCCCGCCACAAGCCCAGCCCCAGCC GCCACCGCCACCACCCCCAGCGGCACCCCAGCCCCCGCAGCCA CCCACCGCTGCTGCCACCCCTCAGCCCCAATATGTCACCGAGCT GCAGAGCCCCCAGCCCCAGGCACAGCCACCGGGTGGCCAGAAG CAGTACGTGACGGAGCTCCCGGCTGTACCCGCACCCTCGCAGC CAACCGGTGCACCCACCCCTTCGCCTGCACCCCAGCAGTACATC GTGGTCACTGTCTCTGAAGGTGCCATGCGGGCCAGCGAGACAG TGTCGGAGGCCAGCCCCGGCTCCACCGCCAGCCAGACCGGCGT TCCTACTCAGGTGGTTCAGCAGGTGCAGGGCACCCAGCAGCGG CTGCTGGTCCAGACGAGCGTGCAGGCCAAGCCAGGCCACGTGT

		CGCCCCTCCAGCTGACCAACATCCAAGTGCCCCAGCAGGCTCTT CCCACGCAGCGTCTGGTGGTGCAGAGCGCAGCCCCAGGCAGCA AAGGTGGCCAGGTCTCCCTGACGGTCCATGGTACCCAGCAGGT GCACTCGCCCCCAGAGCAGTCGCCGGTGCAGGCCAACAGCTCT TCCAGCAAGACAGCCGGGGCCCCCACGGGCACAGTGCCACAGC AGCTGCAGGTCCACGGCGTCCAGCAGAGTGTCCCCGTCACCCA AGAGAGATCTGTGGTCCAGGCCACTCCACAAGCGCCCAAACCC GGCCCGGTGCAGCCGCTGACCGTGCAGGGCCTCCAGCCAGTCC ACGTGGCTCAAGAGGTGCAGCAGCTCCAGCAGGTGCCCGTCCC ACACGTGTACTCCAGCCAGGTGCAGTATGTGGAGGGCGGCGAT GCCAGCTACACGGCCAGTGCCATCCGTTCCAGCACCTACTCCTA TCCCGAGACGCCGCTGTACACGCAGACGGCAAGCACCAGCTAC TACGAGGCCGCAGGCACGGCCACCCAGGTCAGCACCCCCGCCA CCTCCCAGGCGGTGGCCAGCAGTGGCTCCATGCCCATGTACGT GTCCGGCAGCCAGGTCGTCGCCAGCTCCGCCAGCACTGGGGCT GGGGCCAGCAACAGCAGCGGAGGTGGTGGCAGTGGTGGTGGC GGCGGCGGCGGGGGAGGCGGTGGCGGGGGTGGCAGTGGCAGC ACCGGAGGCGGCGGCAGCGGAGCAGGCACCTACGTGATCCAA GGCGGCTACATGCTGGGCAGTGCCAGCCAGTCTTACTCTCACAC CACCCGTGCCTCGCCAGCCACGGTCCAGTGGCTCCTGGACAACT ATGAGACGGCTGAGGGCGTGAGTCTGCCACGGAGCACCCTCTA CTGCCACTACTTACTGCACTGCCAGGAGCAGAAGCTGGAGCCC GTCAACGCCGCCTCCTTCGGCAAGCTCATCCGCTCCGTCTTCAT GGGCCTGCGAACCCGCCGTCTGGGCACCAGGGGCAACTCCAAG TACCACTACTATGGCCTGCGCATCAAGGCCAGCTCACCCCTGCT GCGGCTGATGGAGGACCAGCAGCACATGGCCATGCGGGGCCAG CCCTTCTCGCAGAAGCAGAGGCTCAAGCCCATCCAGAAGATGG AAGGCATGACCAACGGCGTGGCGGTGGGGCAGCAGCCGAGCA CGGGGCTGTCGGACATCAGCGCCCAGGTGCAGCAGTACCAGCA ATTTTTGGATGCCTCTCGGAGCCTCCCTGACTTCACAGAGCTCG ACCTCCAGGGCAAGGTGCTGCCTGAGGGCGTCGGGCCCGGGGA CATCAAAGCCTTCCAGGTCCTGTACCGGGAACACTGTGAGGCC ATTGTCGACGTCATGGTGAACCTGCAGTTCACCCTGGTGGAGAC GCTGTGGAAGACCTTCTGGAGGTACAACCTCAGCCAGCCCAGT GAGGCGCCACCGCTGGCTGTACATGACGAGGCCGAGAAGCGAC TGCCCAAAGCCATCCTGGTGCTCCTCTCCAAGTTCGAGCCCGTG CTCCAATGGACCAAGCACTGTGACAACGTGCTGTACCAGGGCC TGGTGGAAATCCTCATTCCCGACGTGCTGCGGCCCATCCCCAGT GCCTTGACCCAAGCGATCCGGAACTTTGCCAAGAGCCTGGAGA GCTGGCTCACCCACGCCATGGTCAACATCCCCGAGGAGATGCT GCGGGTGAAGGTGGCCGCGGCTGGCGCCTTCGCGCAGACACTG CGGCGCTACACGTCGCTCAACCACCTGGCGCAGGCGGCGCGCG CTGTGCTGCAGAACACCGCACAGATCAACCAGATGCTGAGCGA CCTCAACCGCGTGGACTTCGCCAACGTGCAGGAGCAGGCCTCG TGGGTGTGCCGCTGCGAGGACCGCGTGGTGCAGCGGCTGGAGC AGGACTTCAAGGTGACGCTGCAGCAGCAGAACTCGCTGGAGCA GTGGGCGGCCTGGCTGGACGGCGTGGTGAGCCAGGTGCTCAAG CCCTACCAGGGCAGCGCCGGCTTCCCCAAGGCCGCCAAGCTCT TCCTCCTCAAGTGGTCCTTCTACAGCTCCATGGTGATCCGGGAC CTGACCCTGCGCAGCGCCGCCAGCTTCGGTTCCTTCCACCTCAT CCGGCTGCTCTACGACGAGTACATGTACTACCTGATCGAGCACC GCGTAGCCCAGGCCAAGGGCGAGACCCCCATCGCCGTCATGGG CGAGTTCGCCAATCTGGCCACCTCCCTGAACCCCCTGGACCCCG ACAAAGACGAGGAGGAAGAAGAGGAGGAGGAGAGCG AGGACGAGCTGCCGCAGGACATCTCACTGGCGGCTGGCGGCGA GTCACCCGCGCTGGGCCCGGAGACCCTGGAGCCGCCGGCCAAG CTGGCGCGGACTGACGCGCGCGGCCTCTTCGTGCAGGCGCTGC CCTCCAGCTAA

9	RFXANK	ATGGAGCTTACCCAGCCTGCAGAAGACCTCATCCAGACCCAGC AGACCCCTGCCTCAGAACTTGGGGACCCTGAAGACCCCGGAGA GGAGGCTGCAGATGGCTCAGACACTGTGGTCCTCAGTCTCTTTC CCTGCACCCCTGAGCCTGTGAATCCTGAACCGGATGCCAGTGTT TCCTCTCCACAGGCAGGCAGCTCCCTGAAGCACTCCACCACTCT CACCAACCGGCAGCGAGGGAACGAGGTGTCAGCTCTGCCGGCC ACCCTAGACTCCCTGTCCATCCACCAGCTCGCAGCACAGGGGG AGCTGGACCAGCTGAAGGAGCATTTGCGGAAAGGTGACAACCT CGTCAACAAGCCAGACGAGCGCGGCTTCACCCCCCTCATCTGG GCCTCCGCCTTTGGAGAGATTGAGACCGTTCGCTTCCTGCTGGA GTGGGGTGCCGACCCCCACATCCTGGCAAAAGAGCGAGAGAGC GCCCTGTCGCTGGCCAGCACAGGCGGCTACACAGACATTGTGG GGCTGCTGCTGGAGCGTGACGTGGACATCAACATCTATGATTG GAATGGAGGGACGCCACTGCTGTACGCTGTGCGCGGGAACCAC GTGAAATGCGTTGAGGCCTTGCTGGCCCGAGGCGCTGACCTCA CCACCGAAGCCGACTCTGGCTACACCCCGATGGACCTTGCCGT GGCCCTGGGATACCGGAAAGTGCAACAGGTGATCGAGAACCAC ATCCTCAAGCTCTTCCAGAGCAACCTGGTGCCCGCTGACCCTGA GTGA
10	CREB	ATGACCATGGAATCTGGAGCCGAGAACCAGCAGAGTGGAGATG CAGCTGTAACAGAAGCTGAAAACCAACAAATGACAGTTCAAGC CCAGCCACAGATTGCCACATTAGCCCAGGTATCTATGCCAGCA GCTCATGCAACATCATCTGCTCCCACCGTAACTCTAGTACAGCT GCCCAATGGGCAGACAGTTCAAGTCCATGGAGTCATTCAGGCG GCCCAGCCATCAGTTATTCAGTCTCCACAAGTCCAAACAGTTCA GTCTTCCTGTAAGGACTTAAAAAGACTTTTCTCCGGAACACAGA TTTCAACTATTGCAGAAAGTGAAGATTCACAGGAGTCAGTGGA TAGTGTAACTGATTCCCAAAAGCGAAGGGAAATTCTTTCAAGG AGGCCTTCCTACAGGAAAATTTTGAATGACTTATCTTCTGATGC ACCAGGAGTGCCAAGGATTGAAGAAGAGAAGTCTGAAGAGGA GACTTCAGCACTTCCTACACAGCCTGCTGAAGAAGCAGCACGA AAGAGAGAGGTCCGTCTAATGGAGAACAGGGAAGCAGCTCGA GAGTGTCGTAGAAAGAAGAAAGAATATGTGAAATGTTTAGAAA ACAGAGTGGCAGTGCTTGAAAATCAAAACAAGACATTGATTGA GGAGCTAAAAGCACTTAAGGACCTTTACTGCCACAAATCAGAT TAA
11	NFYA	ATGGAGCAGTATACAGCAAACAGCAATAGTTCGACAGAGCAGA TTGTTGTCCAGGCAGGACAGATTCAGCAGCAGCAGCAGGGTGG TGTCACTGCTGTGCAGTTGCAGACTGAGGCCCAGGTGGCATCC GCCTCAGGCCAGCAAGTCCAGACCCTCCAGGTAGTCCAAGGGC AGCCATTAATGGTGCAGGTCAGTGGAGGCCAGCTAATCACATC AACTGGCCAACCCATCATGGTCCAGGCTGTCCCTGGTGGACAA GGTCAAACCATCATGCAAGTACCTGTTTCTGGAACACAGGGTTT GCAGCAAATACAGTTGGTCCCACCTGGACAGATCCAGATCCAG GGTGGACAGGCTGTGCAGGTGCAGGGCCAGCAGGGCCAGACCC AGCAGATCATCATCCAGCAGCCCCAGACGGCTGTCACTGCTGG CCAGACTCAGACACAGCAGCAGATTGCTGTCCAGGGACAGCAA GTGGCACAGACTGCTGAAGGGCAGACCATCGTCTATCAACCAG TTAATGCAGATGGCACCATTCTCCAGCAAGTTACAGTCCCTGTT TCAGGCATGATCACTATCCCAGCAGCCAGTTTGGCAGGAGCAC AGATTGTTCAAACAGGAGCCAATACCAACACAACCAGCAGTGG GCAAGGGACTGTCACTGTGACACTACCAGTGGCAGGCAATGTG GTCAATTCAGGAGGGATGGTCATGATGGTTCCTGGGGCTGGCT CTGTGCCTGCTATCCAAAGAATCCCTCTACCTGGAGCAGAGATG CTTGAAGAAGAGCCTCTCTACGTGAATGCCAAACAATACCACC GTATTCTTAAGAGGAGGCAAGCCCGAGCTAAACTAGAGGCAGA AGGGAAAATTCCAAAGGAGAGAAGGAAATACCTGCATGAGTCT CGGCACCGTCATGCCATGGCACGGAAGCGTGGTGAAGGTGGAC GATTTTTCTCTCCAAAGGAAAAGGATAGTCCCCATATGCAGGAT

		CCAAACCAAGCCGATGAAGAAGCAATGACACAGATCATCCGAG TGTCCTAA
12	NFYC	ATGTCCACAGAAGGAGGATTTGGTGGTACTAGCAGCAGTGATG CCCAGCAAAGCCTACAGTCGTTCTGGCCTCGGGTCATGGAAGA AATCCGGAATTTAACAGTGAAAGACTTCCGAGTGCAGGAACTC CCACTGGCTCGTATTAAGAAGATTATGAAACTGGATGAAGATG TGAAGATGATCAGTGCAGAAGCGCCTGTACTCTTTGCCAAGGC AGCCCAGATTTTTATCACAGAGTTGACTCTTCGAGCCTGGATTC ACACAGAAGATAACAAGCGCCGGACTCTACAGAGAAATGATAT CGCCATGGCAATTACAAAATTTGATCAGTTTGATTTTCTCATCG ATATTGTTCCAAGAGATGAACTGAAACCTCCAAAGCGTCAGGA GGAGGTGCGCCAGTCTGTAACTCCTGCCGAGCCAGTCCAGTAC TATTTCACGCTGGCTCAGCAACCCACCGCTGTCCAAGTCCAGGG CCAGCAGCAAGGCCAGCAGACCACCAGCTCCACGACCACCATC CAGCCTGGGCAGATCATCATCGCACAGCCTCAGCAGGGCCAGA CCACACCTGTGACAATGCAGGTTGGAGAAGGTCAGCAGGTGCA GATTGTCCAGGCTCAGCCACAGGGTCAAGCCCAACAGGCCCAG AGTGGCACTGGACAGACCATGCAGGTGATGCAGCAGATCATCA CTAACACAGGAGAGATCCAGCAGATCCCGGTGCAGCTGAATGC CGGCCAGCTGCAGTATATCCGCTTAGCCCAGCCTGTATCAGGCA CTCAAGTTGTGCAGGGACAGATCCAGACACTTGCCACCAATGC TCAACAGGGGCAAAGAAATGCAAGTCAGGGGAAGCCTCGAAG GTGCCTGAAAGAAACCTTACAGATTACACAGACAGAGGTCCAG CAAGGACAGCAGCAGTTCAGCCAGTTCACAGATGGACAGCAGC TCTACCAGATCCAGCAAGTCACCATGCCTGCGGGCCAGGACCT CGCCCAGCCCATGTTCATCCAGTCAGCCAACCAGCCCTCCGACG GGCAGGCCCCCCAGGTGACCGGCGACTGA
13	CIITA shRNA	GCTTCTGCTGATGAGATTTATTTTCAAGAGAAATAAATCTCATC AGCAGAAGTTTTTT
14	EBV TAPi Construct (EF1a + EBV TAPi + T2A + eGFP + WHP)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCC CACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA TTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAG GAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGC TGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCG GGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCG TGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGC AGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCA GCCGTCGCTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGA GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA GGTGTCGTGAAGCTCTAGAGCCATGGTACACGTATTGGAACGG GCGCTTTTGGAGCAGCAAAGCTCCGCGTGCGGACTCCCAGGTT CATCCACGGAAACACGCCCATCTCATCCCTGCCCGGAGGACCC

		CGATGTATCACGACTTAGGCTCTTGCTGGTCGTACTTTGCGTGC TCTTTGGACTTCTGTGCCTCCTGCTCATCGGCTCAGGAGAGGGC AGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCG GCCCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGT GCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAG TTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCA AGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGT GCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTC AAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCT TCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGG AGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAA GCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAAC ATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCA CTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAG AAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG GGATCACTCTCGGCATGGACGAGCTGTACAAGTAAGTCGACAA TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTC TTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAA TGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCT CCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGT GGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCT GACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCT CCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGG AACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGG CTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGAC GTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCG CGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGG ACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCG CGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGC CGCCTCCCCGCCTG
15	EBV TAPi construct (EF1a + EBV TAPi + WHP) – no eGFP	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCC CACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA TTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAG GAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGC TGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCG GGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCG TGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGC AGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCA GCCGTCGCTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGA GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA

		TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA GGTGTCGTGAAGCTCTAGAGCCATGGTACACGTATTGGAACGG GCGCTTTTGGAGCAGCAAAGCTCCGCGTGCGGACTCCCAGGTT CATCCACGGAAACACGCCCATCTCATCCCTGCCCGGAGGACCC CGATGTATCACGACTTAGGCTCTTGCTGGTCGTACTTTGCGTGC TCTTTGGACTTCTGTGCCTCCTGCTCATCTAAGTCGACAATCAA CCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAA CTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCC TTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTC CTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGC CCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGAC GCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCT TTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAAC TCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTG TTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGACGTC CTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCG GGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGAC CTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCG TCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCG CCTCCCCGCCTG
16	shRNA targeting CIITA construct (hUS6 + shRNA CIITA3)	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAAC ACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT TTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGAC TATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT TTATATATCTTGTGGAAAGGACGAAACACCGAACAACAGGATT CACGGATCAGCTTCAAGAGAGCTGATCCGTGAATCCTGTTGTTT TTTTT
17	Anti-CD19 CAR construct (based on FMC63) (EF1a aCD19 CAR + T2A + eGFP + WHP)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCC CACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA TTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAG GAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGC TGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCG GGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCG TGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGC AGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCA GCCGTCGCTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGA GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA GGTGTCGTGAAGCTCTAGAGCCATGGCCCTCCCTGTCACCGCCC TGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAG GTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCAC

		AGAGCCTGTCCGTCACATGCACTGTCTCAGGGGTCTCATTACCC GACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTC TGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTA TAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACT CCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGA TGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTG GTAGCTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCAC CGTCTCCTCAGGTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGC GGTGGAGGAAGCGGCGGTGGAGGAAGCGACATCCAGATGACA CAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCAC CATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAAT TGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCT ACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAG TGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACC TGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAA TACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATA ACACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATC CAGTAATACCACTACCCCAGCACCGAGGCCACCCACCCCGGCT CCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC TTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTG CGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGC GCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATG AGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCC GGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGA AATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAACAGGGGCA GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAG TACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTAC AACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGAC GGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATG ACGCTCTTCACATGCAGGCCCTGCCGCCTCGGTCCGGAGGCGG CGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAG GAGAATCCCGGCCCTAGGATGGTGAGCAAGGGCGAGGAGCTGT TCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGT AAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCG GCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACC TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGC AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCA GGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCA TCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCT GGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTAT ATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCA AGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGA CCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTG CTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCA AAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC AAGTAAGTCGACAATCAACCTCTGGATTACAAAATTTGTGAAA GATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTG GATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTA TGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTC TTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTG TGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGC CACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCC TATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCT

		GGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTT GTCGGGGAAGCTGACGTCCTTTCCTTGGCTGCTCGCCTGTGTTG CCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCG GCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGC TCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTC GGATCTCCCTTTGGGCCGCCTCCCCGCCTG
18	Anti-CD19 CAR construct (based on FMC63) (EF1a aCD19 CAR + WHP)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCC CACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA TTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAG GAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGC TGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCG GGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCG TGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGC AGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCA GCCGTCGCTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGA GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA GGTGTCGTGAAGCTCTAGAGCCATGGCCCTCCCTGTCACCGCCC TGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAG GTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCAC AGAGCCTGTCCGTCACATGCACTGTCTCAGGGGTCTCATTACCC GACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTC TGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTA TAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACT CCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGA TGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTG GTAGCTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCAC CGTCTCCTCAGGTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGC GGTGGAGGAAGCGGCGGTGGAGGAAGCGACATCCAGATGACA CAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCAC CATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAAT TGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCT ACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAG TGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACC TGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAA TACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATA ACACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATC CAGTAATACCACTACCCCAGCACCGAGGCCACCCACCCCGGCT CCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC TTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTG CGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGC GCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATG

		AGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCC GGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGA AATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAACAGGGGCA GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAG TACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTAC AACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGAC GGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATG ACGCTCTTCACATGCAGGCCCTGCCGCCTCGGTAAGTCGACAAT CAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCT TAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAAT GCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTC CTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTG GCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTG ACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTC CTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGA ACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGC TGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGAC GTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCG CGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGG ACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCG CGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGC CGCCTCCCCGC
19	Stealth 1 construct (CIITA shRNA) hU6 + shRNA targeting CIITA	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAAC ACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT TTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGAC TATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT TTATATATCTTGTGGAAAGGACGAAACACCGAACAACAGGATT CACGGATCAGCTTCAAGAGAGCTGATCCGTGAATCCTGTTGTTT TTTTT
20	Stealth 1 construct (CAR – TAPi) EF1a + aCD19 CAR + T2A +EBV BNLF2a + P2A + eGFP + WHP	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCC CACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACC GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA TTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAG GAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGC TGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCG GGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCG TGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGC AGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGC GGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCA GCCGTCGCTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGA GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA

		GGTGTCGTGAAGCTCTAGAGCCATGGCCCTCCCTGTCACCGCCC TGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAG GTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCAC AGAGCCTGTCCGTCACATGCACTGTCTCAGGGGTCTCATTACCC GACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTC TGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTA TAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACT CCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGA TGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTG GTAGCTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCAC CGTCTCCTCAGGTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGC GGTGGAGGAAGCGGCGGTGGAGGAAGCGACATCCAGATGACA CAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCAC CATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAAT TGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCT ACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAG TGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACC TGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAA TACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATA ACACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATC CAGTAATACCACTACCCCAGCACCGAGGCCACCCACCCCGGCT CCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC TTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTG CGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGC GCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATG AGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCC GGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGA AATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAACAGGGGCA GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAG TACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTAC AACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGAC GGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATG ACGCTCTTCACATGCAGGCCCTGCCGCCTCGGGGCTCAGGAGA GGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAAT CCCGGCCCTATGGTACACGTATTGGAACGGGCGCTTTTGGAGC AGCAAAGCTCCGCGTGCGGACTCCCAGGTTCATCCACGGAAAC ACGCCCATCTCATCCCTGCCCGGAGGACCCCGATGTATCACGAC TTAGGCTCTTGCTGGTCGTACTTTGCGTGCTCTTTGGACTTCTGT GCCTCCTGCTCATCGGCTCAGGAGGTTCAGGTGCAACGAACTTC TCATTGTTGAAGCAAGCCGGTGATGTTGAGGAAAATCCGGGTC CTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCC CATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTC AGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGC TGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCC TGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTT CAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCG ACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCG AGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACAC CCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGC GCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGAT

		CACTCTCGGCATGGACGAGCTGTACAAGTAAGTCGACAATCAA CCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAA CTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCC TTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTC CTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGC CCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGAC GCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCT TTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAAC TCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTG TTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGACGTC CTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCG GGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGAC CTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCG TCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCG CCTCCCCGCCTG
21	Stealth 2 construct (CIITA shRNA) hU6 + shRNA targeting CIITA	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAAC ACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT TTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGAC TATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT TTATATATCTTGTGGAAAGGACGAAACACCGAACAACAGGATT CACGGATCAGCTTCAAGAGAGCTGATCCGTGAATCCTGTTGTTT TTTTT
22	Stealth 2 construct (CAR – TAPi) EF1a + aCD19 CAR + T2A + eGFP + P2A +EBV BNLF2a + WHP	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAG TCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGC CTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTG TACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATAT AAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTG CCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCC TGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTC CACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTG GAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCC TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCC GCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCT TTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC GACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGAC GGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGC TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCG CCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAG TCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCG CTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAGGCACCTC GATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAGGTTGGGG GGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTG GAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTT GGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCC TCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTG AAGCTCTAGAGCCATGGCCCTCCCTGTCACCGCCCTGCTGCTTC CGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAGGTGAAACTG CAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGT CCGTCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGT GTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGC TGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGC TCTCAAATCCAGACTGACCATCATCAAGGACAACTCCAAGAGC CAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAG CCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTAT

		GCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTC AGGTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGCGGTGGAGG AAGCGGCGGTGGAGGAAGCGACATCCAGATGACACAGACTAC ATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTT GCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCA GCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATACA TCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTG GGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAA GAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCC GTACACGTTCGGAGGGGGGACTAAGTTGGAAATAACACGGGCT GATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTAATAC CACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATC GCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGC AGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCG ATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTG CTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGC AGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGA GGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGC AGCGCAGATGCTCCAGCCTACCAACAGGGGCAGAACCAGCTCT ACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCT GGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCC GCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAA AAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAG GGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGG GACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACAT GCAGGCCCTGCCGCCTCGGGGCTCAGGAGAGGGCAGAGGAAGT CTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTATGG TGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCT GGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTG TCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCC TGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCC ACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCG CTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGG ACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGG CGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC AAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAA CGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGAC GGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG CACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGAT CACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCT CGGCATGGACGAGCTGTACAAGGGTTCAGGTGCAACGAACTTC TCATTGTTGAAGCAAGCCGGTGATGTTGAGGAAAATCCGGGTC CTATGGTACACGTATTGGAACGGGCGCTTTTGGAGCAGCAAAG CTCCGCGTGCGGACTCCCAGGTTCATCCACGGAAACACGCCCA TCTCATCCCTGCCCGGAGGACCCCGATGTATCACGACTTAGGCT CTTGCTGGTCGTACTTTGCGTGCTCTTTGGACTTCTGTGCCTCCT GCTCATCGGCTCAGGATAAGTCGACAATCAACCTCTGGATTAC AAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCC TTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGC TATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATC CTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGC AACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACT GGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTT CGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCT GCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGA

		CAATTCCGTGGTGTTGTCGGGGAAGCTGACGTCCTTTCCTTGGC TGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTC TGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCG CGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTC GCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCT G
23	Stealth construct no GFP (CIITA shRNA) hU6 + shRNA targeting CIITA	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAAC ACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT TTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGAC TATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT TTATATATCTTGTGGAAAGGACGAAACACCGAACAACAGGATT CACGGATCAGCTTCAAGAGAGCTGATCCGTGAATCCTGTTGTTT TTTTT
24	Stealth construct no GFP (CAR – TAPi) EF1a + aCD19 CAR + T2A +EBV BNLF2a + WHP	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAG TCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGC CTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTG TACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATAT AAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTG CCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCC TGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTC CACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTG GAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCC TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCC GCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCT TTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC GACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGAC GGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGC TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCG CCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAG TCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCG CTTCATGTGACTCCACTGAGTACCGGGCGCCGTCCAGGCACCTC GATTAGTTCTCGTGCTTTTGGAGTACGTCGTCTTTAGGTTGGGG GGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTG GAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTT GGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCC TCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTG AAGCTCTAGAGCCATGGCCCTCCCTGTCACCGCCCTGCTGCTTC CGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAGGTGAAACTG CAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGT CCGTCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGT GTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGC TGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGC TCTCAAATCCAGACTGACCATCATCAAGGACAACTCCAAGAGC CAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAG CCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTAT GCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTC AGGTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGCGGTGGAGG AAGCGGCGGTGGAGGAAGCGACATCCAGATGACACAGACTAC ATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTT GCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCA GCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATACA TCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTG GGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAA GAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCC

		GTACACGTTCGGAGGGGGGACTAAGTTGGAAATAACACGGGCT GATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTAATAC CACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATC GCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGC AGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCG ATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTG CTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGC AGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGA GGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGC AGCGCAGATGCTCCAGCCTACCAACAGGGGCAGAACCAGCTCT ACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCT GGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCC GCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAA AAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAG GGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGG GACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACAT GCAGGCCCTGCCGCCTCGGGGCTCAGGAGAGGGCAGAGGAAGT CTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTATGG TACACGTATTGGAACGGGCGCTTTTGGAGCAGCAAAGCTCCGC GTGCGGACTCCCAGGTTCATCCACGGAAACACGCCCATCTCATC CCTGCCCGGAGGACCCCGATGTATCACGACTTAGGCTCTTGCTG GTCGTACTTTGCGTGCTCTTTGGACTTCTGTGCCTCCTGCTCATC TAAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGT ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCT TTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATT TTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG TTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTT TGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTC AGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGG CGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCT CGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCT GACGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCT GCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAG CGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTT CCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTG GGCCGCCTCCCCGCCTG
25	CD8 hinge/TM domain	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY IWAPLAGTCGVLLLSLVITLYC
26	4-1BB ICD	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
27	CD3-zeta ICD	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
28	CD8 signal peptide	MALPVTALLLPLALLLHAARP
29	IgK signal sequence	METDTLLLWVLLLWVPGSTGD
30	linker-0 (G4S)	GGGGS
31	linker-1 (G3S)3	GGGSGGGSGGGS
32	linker-2 (G4S)3	GGGGSGGGGSGGGGS
33	linker-3 (G4S)4	GGGGSGGGGSGGGGSGGGGS
34	linker-4	GSTSGSGKPGSGEGSTKG
35	linker-5	GGSSRSSSSGGGGSGGGG
36	T2A	SGGGGEGRGSLLTCGDVEENPGPR
37	P2A	GSGATNFSLLKQAGDVEENPGP
38	Histidine tag	HHHHHH
39	CD19 scFv VH	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLE WLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAI YYCAKHYYYGGSYAMDYWGQGTSVTVSS

40	CD19 scFv VL	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKL LIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT LPYTFGGGTKLEITRADAAPTVSIFPPSSN
41	CD19 CAR	MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSVTCTVS GVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIK DNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGT SVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQTTSSLSASLGDRV TISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGS GSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITRADA APTVSIFPPSSNTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR

All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior technology or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or

amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

The technology described herein is further illustrated by the following examples, which in no way should be construed as being further limiting.

EXAMPLES

Example 1. Expression of viral TAP inhibitors in primary T cells results in decreased cell surface levels of MHC Class I

Herpesviruses are a class of chronic viruses that infect various human cells and manage to evade T cell immunity. Herpesvirus have convergently evolved to encode small proteins that inhibit TAP[13], a protein required for transporting cytoplasmic peptides across the endoplasmic reticulum and loading them for presentation on MHC Class I molecules at the cell surface. Cells that naturally or experimentally lack expression of functional TAP complexes show a dramatic reduction in surface MHC I levels which substantially reduces their sensitivity to $CD8^+$ T cells[14]. The disclosure is directed, in part, to the discovery that forced expression of viral TAP inhibitors (TAPi) reduces MHC I expression in gene-modified cells, thereby preventing cell-mediated immune responses to foreign transgenes. To test if expression of herpesvirus TAPi reduced surface MHC I expression in primary T cells, bicistronic lentiviral constructs were generated to express Herpes Simplex virus (HSV) ICP47, Human Cytomegalovirus (HCMV) US6, or Epstein-Barr virus (EBV) BNLF2a TAPi along with a fluorescent reporter eGFP as a marker of transduction (**FIG. 1A**). Lentiviral constructs expressing sgRNA for β-2-microglobulin (β2M) with or without electroporated with Cas9 mRNA were used as a positive control (β2M KO) or negative control (β2M--). Primary human T cells consistently expressed eGFP upon transduction with the lentiviral vectors (**FIG. 4A**) and TAPi-transduced cells had reduced levels of surface MHC I without affecting MHC Class II upregulation upon activation (**FIG. 1B**).

Since MHC I expression inhibits targeting by NK cells[15], the impact of MHC I downregulation on susceptibility to NK cell killing was investigated. Like previous reports[12], β2M KO T cells were susceptible to autologous NK cell lysis and induced NK cell degranulation, as measured by CD107a expression. Importantly, T cells expressing viral TAPi did not significantly trigger NK cell lysis or degranulation compared to untransduced (UTD) T cells (**FIG. 1B**). Similarly, MHC I expression mediates allogeneic T cell responses due to mismatch between MHC and TCR. To measure the allogeneic response of TAPi expressing T cells, a mixed lymphocyte reaction (MLR) was performed. Transduced T cells were incubated with autologous or allogeneic labeled responder T cells in the presence or absence of MHC I and II blocking antibodies. Responder T cell activation was measured by proliferation (**FIG. 1C**) and changes in CD69 and CD25 expression (**FIGs. 4B-4C**). T cells transduced with viral TAPi, especially EBV BNLF2a, induced less allogeneic responder T cell activation, which was further decreased by MHC I and/or MHC II blockade.

Next, the functional ability of TAPi-expressing T cells to present cytoplasmic antigens was tested by assessing the presentation of a peptide derived from the highly immunogenic HCMV pp65 protein. The immunogenic NLV peptide is presented on the HLA-A*02:01 allele and drives NLV-specific $CD8^+$ T cells to secrete IFNγ[16]. Lines of NLV-specific "responder T cells" were first generated by serial stimulation of PBMC derived from HLA-A*0201 healthy donors who had evidence of CMV-specific memory responses. Then, a panel of "stimulator T cells" were generated, derived from the same healthy donors, which were untransduced (UTD) or transduced with the constructs as shown, including 3 different viral TAPi. Co-cultures of "stimulator cells" with the "responder cells" demonstrated that viral TAPi expression, especially when derived from HSV or EBV, impeded antigen presentation, as shown by reduced IFNγ secretion in "responder T cells" (**FIG. 1D**). Despite reduced antigen presentation, the use of a viral protein to knock down the MHC I raises the possibility of an immune response to its sequence. To measure the immunogenicity of viral TAPi transduction in T cells, normal donors were identified with pre-existing cellular immunity to the respective TAPi viruses. PBMCs from normal donors were screened with peptides known to be immunogenic and originating from HCMV, EBV or HSV in an IFNγ ELISpot assay. T cells from normal donors with a detectable cellular response those viruses were then transduced with viral TAPi from the same virus and incubated with autologous $CD8^+$ T cells. CD8 T cell activation was measured by IFNγ ELISpot (**FIG. 1E**). While T cells from an HCMV-responsive donor were activated in response to transduction with

HCMV pp65, they did not respond to transduction with the CMV TAPi. Similarly, HSV- and EBV-responsive donors did not produce IFNγ in response to HSV or EBV TAPi, indicating that these viral TAP inhibitors do not elicit T cell responses, despite having demonstrated responsiveness to other known immunogenic sequences from the same viruses.

Taken together, the results showed that primary T cells expressing HSV, EBV, or HCMV TAPi efficiently prevented cell surface expression of MHC I molecules, thereby limiting killing by NK cells and mitigating allogeneic responses. The few remaining MHC I molecules on the cell surface were not sufficient to mount T cell activation in response to immunogenic peptides or peptides derived from the viral TAP inhibitor itself.

Expression of shRNA targeting CIITA results in decreased cell surface levels of MHC Class II and can be co-expressed with EBV TAPi to reduce both MHC class I and II at the cell surface

Activated human T cells express high levels of MHC class II molecules, which may also trigger rejection and antigen cross-presentation of gene-modified cells[4,17]. MHC Class II expression was previously reduced by targeting CIITA, the main regulatory factor that controls the transcription of MHC II genes[18].

To avoid the use of gene-editing and double-strand breaks, shRNA targeting CIITA was encoded into the lentiviral vectors (**FIG. 2A**), and a panel of shRNA sequences was used as well as a comparison of the shRNA vectors to gene knockout of CIITA with CRISPR/Cas9. Transduction efficiency was measured based on eGFP expression (**FIG. 5A**); it was noted that primary human T cells transduced with CIITA-targeting shRNA had reduced cell surface expression of MHC II, comparable to CIITA KO, without affecting MHC I expression (**FIG. 2B**). However, only shRNA CIITA3 reduced MHC II expression without compromising T cell proliferation (**FIG. 2C**). Measurements of proliferation of responder allogeneic or autologous T cells in a mixed lymphocyte reaction (MLR) demonstrated that shRNA-mediated knockdown of CIITA reduced responder T cell proliferation (**FIG. 2D FIGs. 5B-5C**). Next, the MHC I and II downregulation strategies were combined by including both EBV TAPi and shRNA CIITA3 into one lentiviral vector (**FIG. 2E**). When transduced into primary human T cells, the combined EBV-TAPi/shRNA-CIITA3 vector reduced MHC I and II expression (**FIG. 2F**) and reduced proliferative responses in MLRs (**FIG. 2G; FIG. 5D-E**). These results demonstrated that gene-modified

primary T cells can successfully evade cellular immune responses by MHC class I and II downregulation strategies as proposed herein, creating "stealth" T cells.

Stealth-enabled αCD19 CAR T cells are functional and capable of evading CAR-mediated immune recognition by T cells from patients who received a single or double infusion of αCD19 CAR T cells.

Autologous CAR T cells based on FMC63-based αCD19 single-chain scFv can elicit T cell responses against the murine scFv-fractions of the CAR in the patients[3,4]. Thus, the stealth strategy proposed herein was tested in the context of these CARs to verify retention of anti-tumor efficacy and avoidance of cellular immunity. Two stealth FMC63-based αCD19 CAR were generated, alternating the sequence position of the EBV TAPi and the eGFP marker (**FIG. 3A**). Both stealth αCD19 CAR-T cells had reduced MHC I and II molecules on their cell surface compared to the αCD19 CAR alone. Interestingly, this reduction did not increase NK cell cytotoxicity, and proliferation of the CAR-T cells was unchanged compared to the untransduced T cells (**FIG. 3B**).

The stealth αCD19 CAR-T cells also maintained their ability to target tumor cells in vitro and in vivo. When co-incubated with luciferase-expressing acute lymphoblastic leukemia (ALL) NALM6 cells or Mantle cell lymphoma JeKo-1 cells, stealth αCD19 CAR-T cells reduced tumor cell viability to the same extent as αCD19 CAR-T cells (**FIG. 3C**). Due to its slight advantage in MHC I downregulation, the configuration of stealth2 αCD19 CAR-T cells was selected and further studied in an *in vivo* NSG mouse model with ALL NALM6 cells. After tumor engraftment, mice were left untreated or injected with αCD19 CAR-T cells with or without stealth technology and assessed for CAR-T cell expansion by blood draws and tumor clearance by bioluminescence (BLI) (**FIG. 3D**). Both αCD19 CAR-T cells and stealth αCD19 CAR-T cells expanded similarly in the blood as observed on day 7 and day 14 by flow cytometric assessment of GFP+ CD3+ cells. Tumor cells, GFP+ CD3- NALM6 cells, were found to be absent on both timepoints, whilst a large expansion was found in the untreated group. This was further confirmed by BLI imaging. Treatment of engrafted NALM6 with αCD19 CAR-T cells and- stealth αCD19 CAR-T cells showed comparable tumor clearance, whilst in untreated mice the luciferase-expressing NALM6 cells vastly expanded. Kaplan-Meier survival curves demonstrated no difference in survival of mice treated with αCD19 CAR-T cells with or without the additional stealth sequences in the vectors (**FIG. 3E**). In summary, stealth αCD19 CAR-T retained their ability to recognize and clear CD19-expressing cells in both *in vitro* and *in vivo* tumor models.

Finally, the ability of the proposed stealth technology to avoid antigen presentation of immunogenic CAR sequences was tested. Eleven patients who had received autologous FMC63-based CARs either once or twice were identified, and fresh αCD19 CAR-T cells with or without the stealth technology were generated from their 3-month post-infusion PBMC. Four of the patients had initial responses of their tumor to their CAR T cell product, four had tumors that did not respond to CAR T cells, and three had received a second infusion of CAR T cells due to tumor progression after the first infusion. To assess whether T cells from these patients could be activated by their autologous T cells expressing the FMC63-based αCD19 CAR, the freshly made αCD19 CAR-T cells with or without stealth technology were used as "stimulators" and co-cultured these with autologous "responder" untransduced T cells in an IFNγ ELISpot assay (**FIG. 3F**). Responder T cells became activated in the presence of FMC63-based αCD19 CAR-T cell products, but not UTD cells or stealth αCD19 CAR-T cells. Activation of responder T cells was particularly high in those subjects who had received 2 infusions of FMC63-based CAR T cells, and in 3 of the 4 non-responders. These data provide evidence that multiple infusions can increase anti-CAR immunity in patients, and that a fraction of non-responders have robust rejection of their autologous αCD19 CAR-T cells.

Discussion

In summary, the results demonstrated that the combined expression of EBV TAPi BNLF2a and shRNA targeting CIITA effectively reduces the MHC expression and antigen presentation, and incorporation of these sequences into a lentiviral vector has potential use to evade autologous and allogeneic cellular immunity. Evasion of endogenous T cell-mediated rejection can be especially valuable in αCD19 CAR-T cell therapy where initial expansion and persistence is associated with durable remission [19,20]. Because αCD19 CAR-T cells efficiently eliminate the B cell-lineage, humoral immunity to αCD19 CAR-T cell therapy is limited, which further enhances the impact of avoiding T cell immunity in this setting [4,21]. More generally, this stealth technology can be applied in any setting that employs gene-modified cells where either the transgene, junctional sequences, or the cell types are not autologous and where avoidance of early rejection can enhance the desired therapeutic effects. See, e.g., references 4, 6, and 22.

The results further demonstrated that stealth CAR-T cells evade anti-CAR responses originating from the FMC63-based αCD19 CAR, while avoiding NK cell activation due to

loss of MHC I on the cell surface. Furthermore, an increased CAR-reactive T cell response was found in patients who received multiple FMC63-based αCD19 CAR-T cell infusions.

Methods

Mice and Cell lines

NSG mice were purchased from Jackson Laboratory and bred under pathogen-free conditions at the Center for Comparative Medicine at MGH. All experiments were performed according to protocols approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee. Where indicated, cell lines were transduced and expanded after clonal selection to express click beetle green (CBG) luciferase and enhanced GFP. HEKT cells, NALM-6 (ALL), JeKo-1 (MCL) and K562 (CML) were purchased from the American Type Culture Collection and maintained under conditions as outlined by the supplier.

(Stealth) CAR T cell production

Human T cells were purified from anonymous human healthy donor leukapheresis product (Stem Cell Technologies) purchased from the MGH blood bank under an Institutional Review Board-exempt protocol. T cell from patients treated with axicabtagene ciloleucel or tisagenlecleucel at MGH were collected on an IRB-approved protocol with written informed consent; PMBC from one subject treated at Seattle Cancer Care Alliance with two infusions of autologous FMC63-based CAR T cells were provided by Dr. Turtle and collected with written informed consent. Cells were transduced with lentivirus corresponding to various second-generation CAR-T-cell constructs. In brief, bulk human T cells were activated on day 0 using CD3/CD28 Dynabeads (Life Technologies) and cultured in RPMI 1640 medium with GlutaMAX and HEPES supplemented with 10% FBS and 20 IU ml^{-1} recombinant human IL-2. Lentiviral transduction of cells was performed on day 1 and on day 5 CD3/CD28 Dynabeads were removed and, if applicable, the T cells were electroporated with Cas9 mRNA. If the T cells needed to be sorted, the T cells were sorted to purity on day 8 using the eGFP marker and left to expand until day 14, to be subsequently transferred to storage in liquid nitrogen. When unsorted CAR T cells were used, CAR-T cells were normalized for transduction efficiency using untransduced but cultured and activated T cells from the same donor and expansion.

Cytotoxicity Assay

To assess the cytotoxicity of CAR T cells towards target cells, CAR T cells were incubated with luciferase-expressing tumor targets at indicated E/T ratios for 24h. Remaining

luciferase activity was subsequently measured with a Synergy Neo2 luminescence microplate reader (Biotek). To assess the cytotoxicity of NK cells towards stealth or CAR T cells, NK cells were purified from blood or frozen PBMCs (Stem Cell Technologies) and primed with 20 IU ml^{-1} recombinant human IL-2 before co-incubation with their respective target cells stained with CFSE (Life Technologies). After 3h of co-incubation αCD107a antibodies were added and the assay was left to incubate for another hour. After a total of 4h, cells were centrifuged, resuspended with dead/alive marker SYTOXred (Life Technologies), and assessed by flow cytometer for target cell viability and NK cell degranulation.

ELISpot Assay

Plates with Immobilon-P membrane (Millipore) were activated with 35% Ethanol for 30 seconds, washed with PBS and incubated overnight with PBS containing anti-human IFNγ antibody (Clone NIB42, Biolegend). The next day, the plate was blocked with PBS containing 1% BSA and $5x10^5$ PBMCs or $2x10^5$ T cells were co-incubated with respective peptides, antigens, or stimulants. After 24h, the plate was washed with PBS containing 0.05% Tween-20 and incubated overnight with PBS containing biotinylated anti-human IFNγ antibody (Clone 4S.B3, Biolegend) as detection antibody. After washing with PBS containing 0.05% Tween-20, the plate is incubated for 2h with avidin-HRP (Biolegend), developed using the BD Elispot AEC Substrate set and analyzed with ImmunoSpot Reader systems. All antibodies were used according to the manufacturers' recommendation.

ELISA

Interferon γ from supernatants was measured following an overnight co-incubation of NLV responder T cells with target at a E:T ratio of 1:5 using Human DuoSet ELISA kits (R&D systems).

Flow Cytometry

Generally, cells were stained in the dark for 30 min at 4°C and washed twice with RPMI before analysis. SYTOXRed or SYTOXBlue (Life Technologies) were added as dead/alive marker and singlet discrimination was performed on both the FSC and SSC detectors. The following antibodies targeting their respective antigens were used according to the manufacturers' recommendations in combination with their respective isotype control: CD4 (SK3, Biolegend), CD8 (SK1, Biolegend), CD3 (OKT3, Biolegend), CD25 (BC96, Biolegend), CD69 (FN50, Biolegend), HLA-A/B/C (W6/32, Biolegend), HLA-DR/DP/DQ (Tü39, Biolegend), CD107a (H4A3, Biolegend), murine erythroid cells (TER-119,

Biolegend), murine Ly6G/6C (RB6-8C5, Biolegend), murine CD11b (M1/70, Biolegend) and murine NK1.1 (PK136, Biolegend). Analysis was performed by FlowJo software (BD Biosciences).

Mixed Lymphocyte Reaction (MLR) assay

Stealth or CAR T cells were stained with CFSE (Life Technologies), whilst autologous or allogeneic T cells were stained with CellTrace Violet (Life Technologies) before being co-incubated at a 4:1 ratio in the presence of 20 IU ml^{-1} recombinant human IL-2 and either isotype or MHC I (W6/32, Biolegend) or MHC II (Tü39, Biolegend) or both MHC I and II blocking antibodies. Fresh IL-2 was added every other day and the T cells were pulsed with new stealth T cells and blocking antibodies on day 7 and 14. On day 16, T responder cells were stained and assessed by FCM for cell division and activation markers CD69 and CD25.

In vivo study

Luciferized NALM-6 cells were harvested in logarithmic growth phase, washed twice with PBS, and counted before injecting these tumor cells ($1x10^6$ NALM-6 cells per mouse) in NSG mice by tail vein. Presence of the tumor was confirmed 3 days later by bioluminescence, at which time the mice were treated by an injection of $2x10^6$ CAR T cells in the tail vein. Tumor progression was then longitudinally evaluated by bioluminescence emission using an Ami HT optical imaging system (Spectral Instruments) following intraperitoneal substrate injection. At day 7 and day 14, the blood of the mice was collected by cheek punch and analyzed by FCM for presence of NALM-6 and CAR T cells per microliter blood.

Stealth CAR design

DNA constructs were synthesized and cloned into a second-generation lentiviral backbone under the regulation of a human EF-1α promoter for protein translation and/or a human U6 promoter for RNA transcription. The sequences for EBV BNLF2a, HSV ICP47 and HCMV US6TAPi were synthesized and combined with eGFP by means of an 2A self-cleaving peptide. The shRNA targeting CIITA were designed with software of Dharmacon and the Whitehead institute and combined in a plasmid expressing eGFP by EF-1α promoter. Similarly, vectors with CRISPR/Cas9 guides for β2M and CIITA and eGFP expression were constructed. The lentiviral vector expressing the combination of shRNA CIITA3, EBV BNLF2a and eGFP was also constructed. For CAR constructions, plasmid expressing the

FMC63-based anti-CD19 CAR were constructed in combination with expression of EBV BNLF2a and shRNA targeting CIITA.

Statistical methods

All statistical analyses were performed with GraphPad Prism 9 software. Data were presented as means ± s.e.m. with statistically significant differences determined by tests as indicated in figure legends.

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Example 2. Additional results demonstrating expression of viral TAP inhibitors in primary T cells results in decreased cell surface levels of MHC Class I

Herpesviruses have convergently evolved to encode small proteins that inhibit TAP[29], a protein required for transporting cytoplasmic peptides across the endoplasmic reticulum and loading them for presentation on MHC Class I molecules at the cell surface. Cells that

naturally or experimentally lack expression of functional TAP complexes show a dramatic reduction in surface MHC I levels, substantially reducing their sensitivity to $CD8^+$ T cells.[30] It was hypothesized that forced expression of viral TAP inhibitors (TAPi) would reduce MHC I expression in gene-modified cells, thereby preventing cell-mediated immune responses to foreign transgenes. To test if expression of herpesvirus TAPi reduced surface MHC I expression in primary T cells, bicistronic lentiviral constructs were generated to express Herpes Simplex virus (HSV) ICP47, Human Cytomegalovirus (HCMV) US6, or Epstein-Barr virus (EBV) BNLF2a TAPi along with a fluorescent reporter eGFP as a marker of transduction (**FIG. 6A**). Lentiviral constructs expressing sgRNA for β-2-microglobulin (β2M), w/o electroporated with Cas9 mRNA, were used as a positive control (β2M KO) or negative control (β2M--). At similar transduction efficiencies, TAPi-transduced cells had reduced levels of surface MHC I without affecting MHC Class II upregulation upon activation (**FIG. 6B**). Viral TAP inhibitors reduced total surface MHC I levels by at least one log-fold, which was maintained upon additional stimulation by IFNγ or αCD3-antibody (**FIG. 6C**).

Since MHC I expression inhibits targeting by NK cells[31], the impact of MHC I downregulation on susceptibility to NK cell killing was investigated. Like previous reports[26,27], β2M KO T cells were susceptible to autologous NK cell lysis and induced NK cell degranulation, as measured by CD107a expression. Compared to β2M KO T cells, T cells expressing EBV viral TAPi triggered significantly reduced NK cell lysis or degranulation (**FIG. 6D**). Similarly, MHC I expression mediates allogeneic T cell responses due to a mismatch between the MHC and TCR. To measure the allogeneic response of TAPi-expressing T cells, a mixed lymphocyte reaction (MLR) was performed. Transduced T cells were incubated with autologous or allogeneic labeled responder T cells in the presence or absence of MHC I and II blocking antibodies. Responder T cell activation was measured by proliferation (**FIG. 6E**) and changes in CD69 and CD25 expression (**FIGs. 13A–13B**). T cells transduced with viral TAPi, especially EBV BNLF2a, induced less allogeneic responder T cell activation, comparable to MHC I and/or MHC II blockade.

Next, the ability of TAPi-expressing T cells to present cytoplasmic antigens was tested by assessing the presentation of a peptide derived from the highly immunogenic HCMV pp65 protein. The immunogenic NLV peptide is presented on the HLA-A*02:01 allele and drives NLV-specific $CD8^+$ T cells to secrete IFNγ.[32] Lines of NLV-specific "responder T cells" were first generated by serial stimulation of PBMC derived from HLA-

A*02:01 healthy donors who had evidence of CMV-specific memory responses. A panel of "stimulator T cells" derived from the same healthy donors were then generated, which were untransduced (UTD) or transduced with the constructs as shown (**FIG. 6A**), including the three different viral TAPi. Co-cultures of "stimulator cells" with "responder cells" demonstrated that viral TAPi expression, especially when derived from HSV or EBV, reduced antigen presentation, based on a reduction of IFNγ secretion in "responder T cells" (**FIG. 1D**). Despite reduced antigen presentation, using a viral protein to knock down the MHC I raises the possibility of an immune response to its sequence. To measure the immunogenicity of viral TAPi transduction in T cells, normal donors with pre-existing cellular immunity to the respective TAPi viruses were identified. PBMCs from normal donors were screened with peptides known to be immunogenic and originating from HCMV, EBV, or HSV in an IFNγ ELISpot assay.[33-36] T cells from normal donors with a detectable cellular response those viruses were then transduced with viral TAPi from the same virus and incubated with autologous $CD8^+$ T cells. CD8 T cell activation was measured by IFNγ ELISpot (**FIG. 1E**). While T cells from an HCMV-responsive donor were activated in response to transduction with HCMV pp65, they did not respond to transduction with the CMV TAPi. Similarly, HSV- and EBV-responsive donors did not produce IFNγ in response to HSV or EBV TAPi, indicating that these viral TAP inhibitors do not elicit T cell responses, despite the donors being responsive to other known immunogenic sequences from the same viruses.

Expression of shRNA targeting CIITA decreases cell surface levels of MHC Class II

Activated human T cells express high levels of MHC class II molecules. In gene-modified cells, high MCH II could trigger rejection via antigen cross-presentation of the genetic modifications.[3,37] Similar to MHC class I, direct targeting of MHC class II expression with DNA-editing techniques is highly complex and potentially patient-specific, as these genes are highly polymorphic and harbor significant allelic variation.[38] MHC Class II expression reduction was tested by targeting CIITA, the main regulatory factor that controls the transcription of MHC II genes.[39] To avoid the use of gene-editing and double-strand breaks, an shRNA targeting CIITA was encoded into the lentiviral vectors (**FIG. 7A**) using a panel of shRNA sequences. The shRNA vectors were also compared to gene knockout of CIITA with CRISPR/Cas9. It was noted that primary human T cells transduced with CIITA-targeting shRNA had reduced cell surface expression of MHC II, comparable to CIITA KO,

without affecting MHC I expression (**FIG. 7B**). Both CIITA-targeting strategies, CRISPR/Cas9 and shRNA, rendered T cells with less than 20000 MHC Class II molecules on their surface, which was unaffected by additional stimulation with IFNγ or αCD3-antibody (**FIG. 7C**). However, only shRNA CIITA3 reduced MHC II expression without compromising T cell proliferation (**FIG. 2C**). In a mixed lymphocyte reaction (MLR) using allogeneic or autologous responder T cells, shRNA-mediated knockdown of CIITA reduced responder T cell proliferation (**FIG. 7D, FIGs. 14A–14B**).

Expression of the viral TAP inhibitor EBV BNLF2a and an shRNA targeting CIITA can be combined in primary T cells to decrease cell surface levels of both MHC Class I and Class II

Both strategies to downregulate the cell surface expression of MHC I or II were effective separately, but the question remained as to whether these strategies could be combined. The TAPi EBV BNLF2a was selected to be combined with the shRNA CIITA3. This TAPi reduced sufficient MHC I at the cell surface to suppress antigen presentation, while the remaining MHC I at the cell surface can potentially suppress NK cell activation. These MHC I and II downregulation strategies were combined by including both EBV TAPi and shRNA CIITA3 into one lentiviral vector (**FIG. 8A**). When transduced into primary human T cells, the combined EBV-TAPi/shRNA-CIITA3 vector reduced MHC I and II expression (**FIGs. 8B–8C**) and reduced proliferative responses in MLRs (**FIGs. 8D, FIGs. 15A-15B**). This demonstrates that gene-modified primary T cells can successfully evade cellular immune responses by the MHC class I and II downregulation strategies presented herein, creating "stealth" T cells.

Stealth-enabled αCD19 CAR T cells are functional *in vitro* and *in vivo*

The murine scFv FMC63, which recognizes CD19 and is used in four of the six FDA-approved CAR-T cell products, has been reported to elicit autologous T cell responses in patients.[3,6] Thus, the stealth strategy was tested in the context of FMC63 CARs to verify they retain function and avoid eliciting cellular immunity. The stealth FMC63-based αCD19 CAR was generated by incorporating both the EBV TAPi and shRNA CIITA3 (**FIG. 9A**). The stealth αCD19 CAR-T cells had reduced MHC I and II molecules on their cell surface compared to the T cells transduced with the αCD19 CAR alone and had robust expression of EBV TAPi and reduced CIITA mRNA expression compared to the αCD19 CAR alone by qPCR (**FIG. 9B**). Interestingly, this reduction of MHC I molecules at the cell surface did not

increase NK cell cytotoxicity, and proliferation of the CAR-T cells was unchanged compared to the untransduced T cells (**FIGs. 9C–9D**). Additionally, phenotypic analysis by CD4, CD8, CCR7, and CD45RA further showed no differences in CD4/CD8 ratios and memory phenotypes comparing the αCD19 CAR-T cells with or without the stealth technology (**FIG. 9E**). The stealth αCD19 CAR-T cells also maintained their ability to target tumor cells *in vitro*. When co-incubated with luciferase-expressing acute lymphoblastic leukemia (ALL) NALM6 cells or mantle cell lymphoma JeKo-1 cells, stealth αCD19 CAR-T cells reduced tumor cell viability to the same extent as αCD19 CAR-T cells (**FIG. 9F**).

The *in vivo* functionality was also investigated. After tumor engraftment with NALM6 cells or JeKo-1, mice were left untreated or injected with αCD19 CAR-T cells with or without stealth technology. CAR-T cell expansion in the blood was assessed by flow cytometry, and tumor clearance was measured by bioluminescence imaging (BLI) (**FIG. 10A**). Mice treated with αCD19 CAR-T cells or stealth αCD19 CAR-T cells showed comparable tumor clearance, while tumors vastly expanded in untreated mice by BLI (**FIGs. 10B & 10F**). Both αCD19 CAR-T cells and stealth αCD19 CAR-T cells expanded similarly in the blood, as observed at day 14 by the presence of GFP+ CD3+ cells (**FIGs. 10C & 10G**). Tumor cells (GFP+ CD3- NALM6 cells) were absent or minimally present in the blood of CAR-T cell-treated mice, while a large expansion was found in the untreated group, similar to the BLI imaging. Kaplan-Meier survival curves demonstrated no difference in the survival of mice treated with αCD19 CAR-T cells with or without the additional stealth technology (**FIGs. 10D & 10H**). In summary, stealth αCD19 CAR-T cells retained their ability to recognize and clear CD19-expressing cells both *in vitro* and *in vivo*.

Stealth-enabled αCD19 CAR T cells evade CAR-mediated immune recognition by T cells from patients who received a single or second infusion of αCD19 CAR T cells.

Next, the ability of the stealth technology to avoid antigen presentation of immunogenic CAR sequences was tested. 11 patients who had received autologous FMC63-based CARs either once or twice were identified. Four of these patients had initial responses of their tumor to their CAR T cell product, while four had tumors that did not respond to CAR T cells. Three patients received a second infusion of CAR T cells due to tumor progression after the first infusion (**FIG. 11A**). To assess whether T cells from these patients could be activated by their autologous T cells expressing the FMC63-based αCD19 CAR, fresh αCD19 CAR-T cells were made with or without stealth technology from their T cells (collected 3 months post-infusion, absent of CAR) as “stimulators” and co-cultured these

with autologous, untransduced T cells as "responders" in an IFNγ ELISpot assay (**FIGs. 11B–11E**). Responder T cells became activated in the presence of FMC63-based αCD19 CAR-T cell products, but not UTD cells or stealth αCD19 CAR-T cells. Activation of responder T cells was particularly high in subjects who had received two infusions of FMC63-based CAR T cells and in 3 of the 4 non-responders. These data suggest that multiple infusions may increase anti-CAR immunity in patients and that a fraction of non-responders may robustly reject their autologous αCD19 CAR-T cells when reinfused. However, larger patient numbers would be required to establish a correlation between a lack of response and CAR T cell rejection.

Stealth αCD19 CAR T cells reduce allogeneic responses *in vitro* and *in vivo*

Finally, the evasion mechanism of stealth αCD19 CAR-T cells towards allogeneic T cells was investigated. When αCD19 CAR-T cells or stealth αCD19 CAR-T cells were co-incubated with expanded allogeneic T cells (expanded by αCD3/αCD28 beads) *in vitro*, the stealth technology reduced both IFNγ-secretion and cytotoxicity towards the CAR-T cells (**FIG. 12A**). A previously reported *in vivo* mouse model was also implemented,[40] where αCD3/αCD28-expanded allogeneic T cells were injected before NALM6 inoculation and subsequent treatment with CAR-T cells (**FIG. 12B**). Stealth CAR-T cells had expanded significantly more in the blood on day 14 (via flow cytometry) compared to αCD19 CAR-T cells, despite the presence of similar levels of allogeneic T cells and tumor burden (**FIGs. 12C–12D**). However, because the stealth system did not eliminate the large numbers of untransduced, activated T cells, the incidence and severity of xenogeneic GvHD (as indicated by fur loss and sclerosis) was early and high, resulting in no change in survival (**FIG. 16**). Therefore, a second allogeneic model was implemented using allogeneic T cells that were primed by pulsing twice with irradiated PBMC originating from the CAR T-cell donor to boost the allogeneic response. These primed cells were then expanded according to the rapid expansion protocol[28] before injecting into the mice (**FIG. 12E**). In this model, the stealth αCD19 CAR-T cells robustly expanded over course of 4 weeks compared to αCD19 CAR-T cells (**FIG. 12F**) and had comparable anti-tumor activity (**FIGs. 12G–12H**). The primed allogeneic T cells allowed for longer monitoring of the mice before the onset of severe xenogeneic GvHD. Importantly, the stealth αCD19 CAR-T cells expanded more robustly than the αCD19 CAR T cells.

Discussion

CAR-T cells targeting CD19 have provided frequent, complete responses in patients with hematological disorders deemed previously uncurable. Albeit, certain hurdles in both autologous and allogeneic settings remain.[15,16] Current clinical trials with allogeneic CAR T cells employ a means to reduce MHC I and/or class II cell surface presentation to evade one of these hurdles, the host immune response.[3,4] It is shown herein that the inclusion of stealth transgenes, EBV TAPi BNLF2a and shRNA targeting CIITA, effectively reduced MHC cell surface molecules to evade autologous and allogeneic T cell responses. These stealth transgenes were incorporated within the CAR-transduction vector to develop a one-shot transduction to produce CAR-T cells with T cell-evasive properties. This simplified approach is particularly valuable as it does not rely on CRISPR/Cas9 gene-editing technique to ablate MHC I/II from the cell surface.[4,41] CRISPR/Cas9 can introduce off-target effects through INDELs that promote aberrant mRNA or protein products, which are increased upon introducing multiple targets.[20,42] Since CRISPR/Cas9 is also being investigated for a variety of other targets in CAR-T cells, such as targets to increase CAR T cell fitness and persistence, [22,41] alternate solutions to reduce HLA from the CAR-T cell surface would enable CRISPR/Cas9 to still be used for these purposes.

Evasion of T cell immunity can be especially valuable in αCD19 CAR T cell therapy, which efficiently eliminates normal B cells in addition to the intended tumor cells, thereby naturally limiting the humoral immune response to non-self CAR components. Since anti-CAR or donor-specific antibodies and their potential interference with αCD19 CAR T cell therapy is very limited,[3,42] equipping αCD19 CAR T cells or αCD19 NK cells with a mechanism to prevent T cell immunity could have a major impact. Clinical trials with autologous CAR-T cells have shown that patients treated with CAR-T cells develop a CAR-reactive T cell response.[3,6,11] It is demonstrated herein that the stealth CAR-T cells evade anti-CAR responses originating from the FMC63-based αCD19 CAR and obtained increased proliferation in an allogeneic model. Furthermore, an increased CAR-reactive T cell response was found in patients who received multiple FMC63-based αCD19 CAR-T cell infusions.

We did not perform an exhaustive comparison of all the ways that can be used to evade immunogenicity. Indeed, CRISPR/Cas9 and TALEN gene knockouts are frequently employed to eliminate the T cell receptor and/or B2M in allogeneic T cell products. It may also be possible to use shRNA to B2M [43], or base-editing technologies to mutate B2M [44]. An advantage of the present disclosure is that it could be easily combined with other gene-editing strategies, such as CRISPR/Cas9, while economizing on the number of double-strand breaks

or possible translocation events. Furthermore, incorporation of stealth transgenes into autologous, "simple" lentiviral-transduced autologous products could be implemented quickly, without the need to develop exhaustive sequencing-based strategies to measure off-target gene editing effects or additional release assays

Besides the potential of the stealth transgenes in CAR-T cell therapy, this stealth technology may be useful in additional settings that employ gene-modified cells, where either the transgene, junctional sequences, or the cell types are not autologous and where avoidance of early rejection can enhance the desired therapeutic effects.[3,8,45]

Methods

Mice and Cell lines

NSG mice were purchased from Jackson Laboratory and bred under pathogen-free conditions at the Center for Comparative Medicine at MGH. Experiments were performed according to protocols approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee. HEKT cells, NALM-6 (ALL), JeKo-1 (MCL), and K562 (CML) were purchased from the American Type Culture Collection, maintained under conditions as outlined by the supplier and, where indicated, transduced to express click beetle green luciferase and enhanced GFP. Cell lines were periodically authenticated by STR profiling, and routinely tested to exclude mycoplasma infection.

(Stealth) CAR T cell production

Human T cells were purified (Stem Cell Technologies) from healthy donor leukapheresis products purchased from the MGH blood bank under an Institutional Review Board-exempt protocol. T cells from patients treated with axicabtagene ciloleucel or tisagenlecleucel at MGH were collected on an IRB-approved protocol (16-206) with written informed consent; PMBC from one subject treated at Seattle Cancer Care Alliance with two infusions of autologous FMC63-based CAR T cells were provided by Dr. Turtle and collected with written informed consent. In brief, bulk human T cells were activated on day 0 using CD3/CD28 Dynabeads (Life Technologies) and cultured in RPMI 1640 medium with GlutaMAX and HEPES supplemented with 10% FBS and 20 IU/ml recombinant human IL-2. Lentiviral transduction was performed on day 1, and on day 5 CD3/CD28 Dynabeads were removed. Where applicable, the T cells were electroporated with Cas9 mRNA on day 5. In cases of flow-based sorting, the T cells were sorted on day 8 using the eGFP marker and expanded until day 14 to be subsequently cryopreserved. When unsorted CAR T cells were

used, CAR-T cells were normalized for transduction efficiency using untransduced activated T cells from the same donor and expansion.

Cytotoxicity Assay

To assess the cytotoxicity of CAR T cells towards target cells, CAR T cells were incubated with luciferase-expressing tumor targets at indicated E/T ratios for 24h. Remaining luciferase activity was subsequently measured with a Synergy Neo2 luminescence microplate reader (Biotek). To assess the cytotoxicity of NK cells towards stealth or CAR T cells, NK cells were purified from blood or frozen PBMCs (Stem Cell Technologies) and primed with 20 IU/ml recombinant human IL-2 before co-incubation with their respective target cells stained with CFSE (Life Technologies). After 3h of co-incubation αCD107a antibodies were added and the assay was left to incubate for another hour. After a total of 4h, cells were centrifuged, resuspended with dead/alive marker SYTOXred (Life Technologies), and assessed by flow cytometer for target cell viability and NK cell degranulation.

ELISpot Assay

Plates with Immobilon-P membrane (Millipore) were activated with 35% Ethanol for 30 seconds, washed with PBS and incubated overnight with PBS containing anti-human IFNγ antibody (Clone NIB42, Biolegend). The next day, the plate was blocked with PBS containing 1% BSA and $5x10^5$ PBMCs or $2x10^5$ T cells were co-incubated with respective peptides, antigens, or stimulants. After 24h, the plate was washed with PBS containing 0.05% Tween-20 and incubated overnight with PBS containing biotinylated anti-human IFNγ antibody (Clone 4S.B3, Biolegend) as detection antibody. After washing with PBS containing 0.05% Tween-20, the plate is incubated for 2h with avidin-HRP (Biolegend), developed using the BD Elispot AEC Substrate set and analyzed with ImmunoSpot Reader systems. All antibodies were used according to the manufacturers' recommendation.

ELISA

Interferon ɣ from supernatants was measured following an overnight co-incubation of NLV responder T cells with target at a E:T ratio of 1:5 using Human DuoSet ELISA kits (R&D systems).

Flow Cytometry

Generally, cells were stained in the dark for 30 min at 4°C and washed twice with RPMI before analysis. SYTOXRed or SYTOXBlue (Life Technologies) were added as dead/alive marker, and singlet discrimination was performed on both the FSC and SSC

detectors. The following antibodies targeting their respective antigens were used according to the manufacturers' recommendations in combination with their respective isotype control: CD4 (SK3, Biolegend), CD8 (SK1, Biolegend), CD3 (OKT3, Biolegend), CD25 (BC96, Biolegend), CD69 (FN50, Biolegend), HLA-A/B/C (W6/32, Biolegend), HLA-DR/DP/DQ (Tü39, Biolegend), CD107a (H4A3, Biolegend), murine erythroid cells (TER-119, Biolegend), murine Ly6G/6C (RB6-8C5, Biolegend), murine CD11b (M1/70, Biolegend) and murine NK1.1 (PK136, Biolegend). When specified, antibody binding capacity was measured utilizing Quantum Simply Cellar beads (Bangs laboratories). Analysis was performed by FlowJo software (BD Biosciences).

Mixed Lymphocyte Reaction (MLR) assay

Stealth or CAR T cells were stained with CFSE (Life Technologies), whilst autologous or allogeneic T cells were stained with CellTrace Violet (Life Technologies) before being co-incubated at a 4:1 ratio in the presence of 20 IU/ml recombinant human IL-2 and either isotype or MHC I (W6/32, Biolegend) or MHC II (Tü39, Biolegend) or both MHC I and II blocking antibodies. Fresh IL-2 was added every other day and the T cells were pulsed with new stealth T cells and blocking antibodies on day 7 and 14. On day 16, T responder cells were stained with SYTOXRed (viability) and assessed by FCM for cell division. Allogeneicity of cells were assessed by PCR (American Red Cross) and a minimum of 5 out of 6 mismatched (HLA-A/B/C/DP/DQ/DR) were selected.

In vivo study

Luciferized NALM-6 or JeKo-1 cells were harvested, washed with PBS, and counted before injecting these tumor cells ($1x10^6$ NALM-6 or JeKo-1 cells per mouse) in NSG mice by tail vein. Tumor growth was confirmed 3 days later by bioluminescence, at which time the mice were treated with an injection of $2x10^6$ CAR T cells in the tail vein. Tumor progression was then longitudinally evaluated by bioluminescence emission using an Ami HT optical imaging system (Spectral Instruments) following intraperitoneal substrate injection. At day 14 (or as indicated), the blood of the mice was collected by cheek punch and analyzed by FCM for the presence of NALM-6 and CAR T cells per microliter of blood. For the allogeneic T cell mouse model, "activated" allogeneic T cells were activated with CD3/CD28 beads and mice were treated $7x10^6$ T cells per mouse. "Primed" allogeneic T cells were pulsed twice with irradiated (100 Gy) PBMC originating from the CAR T-cell donor and then expanded by a rapid expansion protocol[28]. Mice were treatd with $4x10^6$ T cells per mouse.

The allogeneic T cells were injected in NSG mice by tail vein one day prior to NALM-6 tumor cell injection.

Stealth CAR design

DNA constructs were synthesized and cloned into a second-generation lentiviral backbone under the regulation of a human EF-1α promoter for protein translation and/or a human U6 promoter for RNA transcription. The sequences for EBV BNLF2a, HSV ICP47 and HCMV US6TAPi were synthesized and combined with eGFP by means of an 2A self-cleaving peptide. The shRNA targeting CIITA were designed with software of Dharmacon and the Whitehead institute and combined in a plasmid expressing eGFP by EF-1α promoter. Similarly, vectors with CRISPR/Cas9 guides for β2M and CIITA and eGFP expression were constructed. The lentiviral vector expressing the combination of shRNA CIITA3, EBV BNLF2a and eGFP was also constructed. For CAR constructions, plasmid expressing the FMC63-based anti-CD19 CAR were constructed in combination with expression of EBV BNLF2a and shRNA targeting CIITA.

Statistical methods

All statistical analyses were performed with GraphPad Prism 9 software. Data were presented as means ± SEM with statistically significant differences determined by tests as indicated in figure legends.

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CLAIMS

1. A cell comprising:

(i) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and

(ii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein or variant thereof, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

2. A cell comprising:

(i) a chimeric antigen receptor (CAR); and

(ii) an inhibitor of transporter associated with antigen processing (TAPi) or variant thereof; and/or

(iii) an oligonucleotide that is complementary to a polynucleotide encoding a MHC class II transactivator protein or variant thereof, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

3. The cell of claim 1 or claim 2, wherein the oligonucleotide is complementary to any one of SEQ ID NOs: 7-12.

4. The cell of claim 1 or claim 2, wherein the oligonucleotide is complementary to SEQ ID NO: 7.

5. The cell of any one of claims 1-4, wherein the TAPi or variant thereof decreases expression of MHC class I.

6. The cell of any one of claims 1-5, wherein the TAPi is a viral TAPi.

7. The cell of any one of claims 1-6, wherein the TAPi is a Herpesvirus TAPi.

8. The cell of any one of claims 1-7, wherein the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) TAPi, Human Cytomegalovirus (HCMV) TAPi, or Epstein-Barr virus (EBV) TAPi.

9. The cell of any one of claims 1-8, wherein the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) ICP47 TAPi, Human Cytomegalovirus (HCMV) US6 TAPi, or Epstein-Barr virus (EBV) BNLF2a TAPi.

10. The cell of any one of claims 1-9, wherein the TAPi comprises an amino acid sequence that is at least 85% identical to any one of SEQ ID NOs: 1-3.

11. The cell of any one of claims 1-9, wherein the TAPi comprises an amino acid sequence of any one of SEQ ID NOs: 1-3.

12. The cell of any one of claims 1-11, wherein the RNAi oligonucleotide is selected from the group consisting of a siRNA, a miRNA or a shRNA.

13. The cell of claim 12, wherein the RNAi oligonucleotide is a shRNA.

14. The cell of claim 13, wherein the shRNA comprises a nucleic acid sequence of SEQ ID NO: 3.

15. The cell of any one of claims 12-14, wherein the shRNA comprises a nucleic acid sequence of SEQ ID NO: 13.

16. The cell of any one of claims 1-15, wherein the cell is a eukaryotic cell.

17. The cell of any one of claims 1-16, wherein the cell is an immune cell.

18. The cell of any one of claims 1-17, wherein the immune cell is a T cell.

19. The cell of claim 1, wherein the cell further comprises a chimeric antigen receptor (CAR).

20. The cell of claim 2-19, wherein the CAR comprises:
(i) an extracellular target binding domain;
(ii) a transmembrane domain; and
(iii) an intracellular signaling domain.

21. The cell of claim 20, wherein the extracellular target binding domain binds to any one of CD19, CD79b, TACI, BCMA, MUC1, MUC16, B7H3, mesothelin, CD70, PSMA, PSCA, EGFRvIII, claudin6, binds to any pair of CD19/CD79b, BCMA/TACI, or is a TriPRIL antigen binding domain.

22. The cell of claims 21, wherein the extracellular target binding domain binds to CD19.

23. The cell of any one of claims 20-22, wherein the extracellular target binding domain is not derived from a human polypeptide sequence.

24. The cell of claim of any one of claims 20-22, wherein the extracellular target binding domain is derived from a murine polypeptide sequence.

25. The cell of any one of claims 20-24, wherein extracellular target binding domain comprises a VH amino acid sequence that has at least 85% identify to SEQ ID NO: 39 and a VL amino acid sequence that has at least 85% identify to SEQ ID NO: 40.

26. The cell of any one of claims 20-25, wherein the transmembrane domain is selected from the group consisting of alpha chain of a T cell receptor, beta chain of a T cell receptor or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), 4-1BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c,ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160

(BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

27. The cell of any one of claims 20-26, wherein the intracellular signaling domain is selected from the group consisting of CD28, 4-1BB, CD27, TCR-zeta, FcR-gamma, FcR-beta, CD3-gamma, CD3-theta, CD3-sigma, CD3-eta, CD3-epsilon, CD3-zeta, CD22, CD79a, CD79b, and CD66d.

28. The cell of any one of claims 20-27, wherein the CAR comprises an amino acid sequence having at least 85% identify to SEQ ID NO: 41 and a nucleic acid sequence having at least 85% identity to SEQ ID NO: 17 or 18.

29. A polynucleotide comprising a nucleic acid sequence encoding (i) a TAPi or variant thereof and (ii) an oligonucleotide that is complementary to a gene encoding a MHC class II transactivator protein.

30. The polynucleotide claim 29, wherein the TAPi is a viral TAPi.

31. The polynucleotide of claim 29 or claim 30 , wherein the TAPi or variant thereof decreases expression of MHC class I.

32. The polynucleotide of any one of claims 29-31, wherein the TAPi is a Herpes Simplex Virus (HSV) TAPi.

33. The polynucleotide of any one of claims 29-32, wherein the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) TAPi, Human Cytomegalovirus (HCMV) TAPi, or Epstein-Barr virus (EBV) TAPi.

34. The polynucleotide of any one of claims 29-33, wherein the TAPi is selected from the group consisting of a Herpes Simplex virus (HSV) ICP47 TAPi, Human Cytomegalovirus (HCMV) US6 TAPi, or Epstein-Barr virus (EBV) BNLF2a TAPi.

35. The polynucleotide of any one of claims 29-34, wherein the TAPi comprises an amino acid sequence that is at least 85% identical to any one of SEQ ID NOs: 1-3.

36. The polynucleotide of any one of claims 29-34, wherein the TAPi comprises an amino acid sequence of any one of SEQ ID NOs: 1-3.

37. The polynucleotide of any one of claims 29-36, wherein the oligonucleotide is complementary to any one of SEQ ID NOs: 7-12 or a variant thereof.

38. The polynucleotide of any one of claims 29-37, wherein the oligonucleotide is complementary to SEQ ID NO: 7 or a variant thereof.

39. The polynucleotide of any one of claims 29-38, wherein the oligonucleotide is selected from the group consisting of a RNAi oligonucleotide or a CRISPR interference guide RNA.

40. The polynucleotide of any one of claims 39, wherein the RNAi oligonucleotide is selected from the group consisting of a siRNA, a miRNA or a shRNA.

41. The polynucleotide of claim 39 or claim 40, wherein the RNAi oligonucleotide is an shRNA.

42. The polynucleotide of claim 41, wherein the shRNA is encoded by a nucleic acid sequence comprising of SEQ ID NO: 13.

43. The polynucleotide of any one of claims 29-42 further comprising a nucleic acid sequence encoding chimeric antigen receptor (CAR).

44. The polynucleotide of claim 43, wherein the CAR comprises:

(i) an extracellular target binding domain;

(ii) a transmembrane domain; and

(iii) an intracellular signaling domain.

45. The polynucleotide of claim 44, wherein the extracellular target binding domain binds to any one of CD19, CD79b, TACI, BCMA, MUC1, MUC16, B7H3, mesothelin, CD70, PSMA, PSCA, EGFRvIII, claudin6, binds to any pair of CD19/CD79b, BCMA/TACI, or is a TriPRIL antigen binding domain.

46. The polynucleotide of claim 44 or claim 45, wherein the extracellular target binding domain binds to CD19.

47. The polynucleotide of any one of claims 44-46, wherein the extracellular target binding domain is not derived from a human polypeptide sequence.

48. The polynucleotide of any one of claims 44-47, wherein the extracellular target binding domain is derived from a murine polypeptide sequence.

49. The polynucleotide of any one of claims 44-48, wherein the transmembrane domain is selected from the group consisting of alpha, beta or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), 4-1BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c,ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

50. The polynucleotide of any one of claims 44-49, wherein the intracellular signaling domain is selected from the group consisting of CD28, 4-1BB, CD27, TCR-zeta, FcR-gamma, FcR-beta, CD3-gamma, CD3-theta, CD3-sigma, CD3-eta, CD3-epsilon, CD3-zeta, CD22, CD79a, CD79b, and CD66d.

51. The polynucleotide of any one of claims 44-50, comprising a nucleic acid sequence that has at least 85% identity to SEQ ID NO: 17-18.

52. The polynucleotide of any one of claims 44-50, comprising a nucleic acid sequence of SEQ ID NO: 19 and a nucleic acid sequence of SEQ ID NO: 20, 22 or 24.

53. The polynucleotide of any one of claims 29-52, wherein the polynucleotide is a vector, optionally a lentiviral vector.

54. A polynucleotide comprising an shRNA of SEQ ID NO: 13.

55. A cell comprising the polynucleotide of any one of claims 29-54.

56. A cell of any one of claims 1-27, wherein the cell comprises the polynucleotide of any one of claims 29-54.

57. A method of modifying the immunogenicity of a cell, the method comprising introducing into the cell an oligonucleotide that is complementary to a polynucleotide encoding an MHC class II complex subunit of any one of SEQ ID NOs: 7-12, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

58. A method of decreasing an immune response of a subject to a cell therapy, the method comprising introducing into cells of the cell therapy an oligonucleotide that is complementary to a polynucleotide encoding class II MHC transactivator complex protein of any one of SEQ ID NOs: 7-12, wherein the oligonucleotide is selected from the group consisting of a RNA interference (RNAi) oligonucleotide, an antisense oligonucleotide (ASO), or a CRISPR interference (CRISPRi) oligonucleotide.

59. The method of claim 57 or claim 58, further comprising introducing into cells of the cell therapy a virus-derived inhibitor of transporter associated with antigen processing (TAPi) or variant thereof.

60. The method of any one of claims 57-59, comprising introducing into cells of the cell therapy the polynucleotide of any one of claims 29-54.

61. The method of any one of claims 57-60, wherein the cell or cells are eukaryotic cells.

62. The method of any one of claims 57-61, wherein the cell or cells are immune cells.

63. The method of claim 62, wherein the immune cell or immune cells are T cells.

64. The method of any one of claims 57-63, wherein the cells are allogenic to the subject.

65. The method of any one of claims 57-64, wherein the cell therapy is a CAR-T cell therapy.

66. The method of claim 65, wherein the CAR-T cell therapy comprises an anti-CD19 CAR-T cell.

67. The method of any one of claims 58-66, wherein the subject is a human subject.

68. The method of any one of claims 58-67, wherein the method decreases natural killer cell activation.

69. A method of treating cancer in a subject, the method comprising administering the cell of any one of claims 1-28 or 55-56 to the subject.

70. The method of claim 69, wherein the cancer is a hematological cancer.

71. The method of claim 70, wherein the hematological cancer is selected from the group consisting of Leukemia, Lymphoma, and Myeloma.

72. The method of claim 70, wherein the hematological cancer is selected from the group consisting of acute lymphoblastic leukemia or mantle cell lymphoma.

73. The method of any one of claim 69, wherein the cancer is a solid tumor.

74. The method of claim 73, wherein the solid tumor is selected from the group consisting of ovarian cancer, mesothelioma, brain cancer, liver cancer, kidney cancer, lung cancer, breast cancer, prostate cancer, throat cancer, thyroid cancer, colon cancer, testicular cancer, and skin cancer.

75. The method of any one of claims 69-74, wherein the cancer expresses CD19.

76. The cell of any one of claims 1-28 or polynucleotide of any one of claims 29-54, wherein the MHC class II transactivator protein is class II MHC transactivator 3

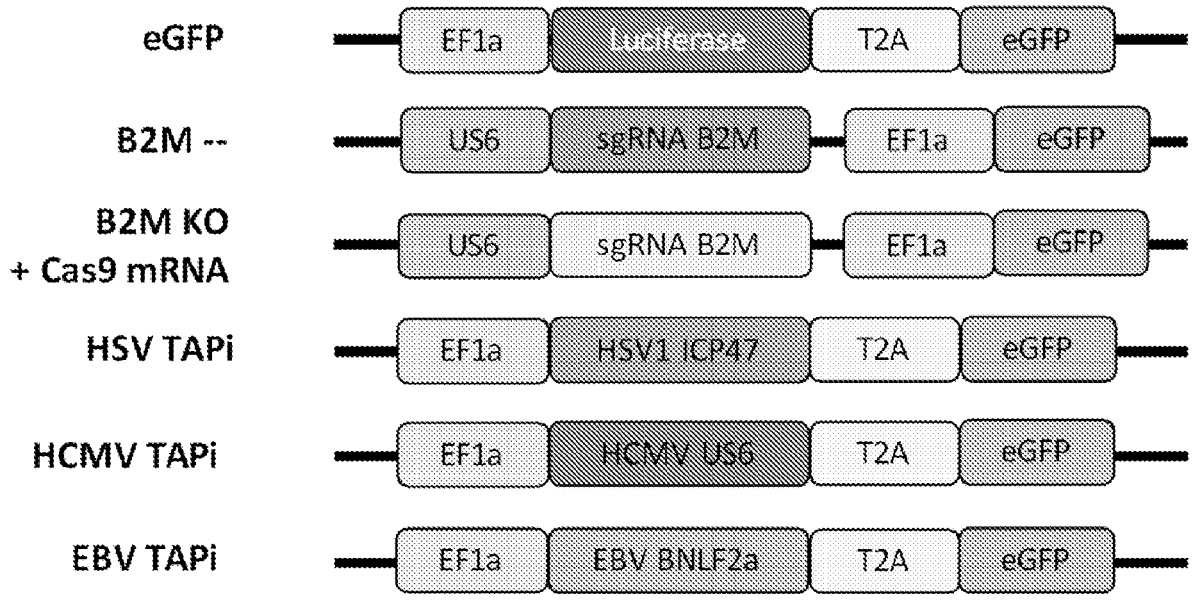
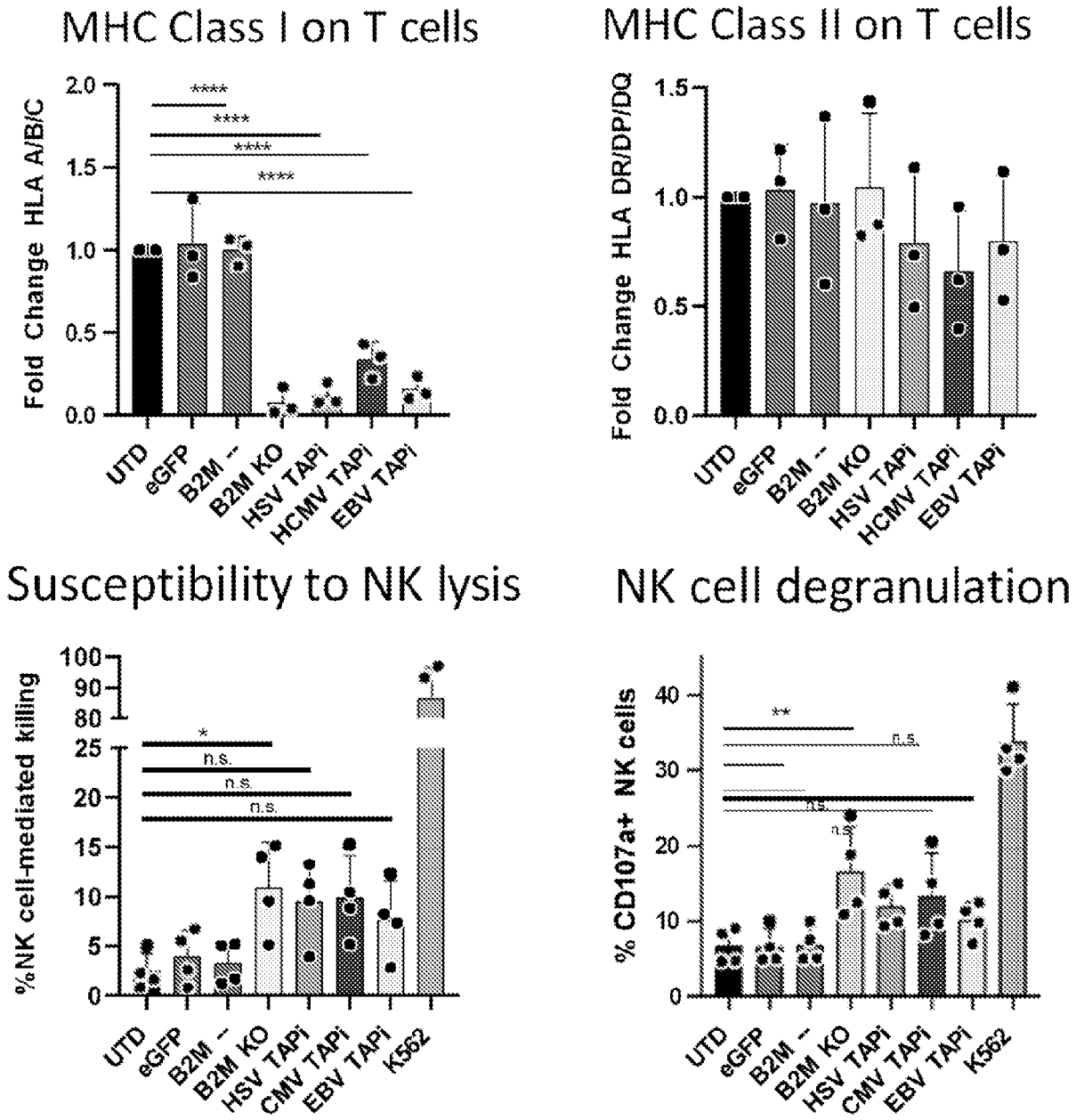

eGFP
EF1a
Luciferase
T2A
eGFP
B2M --
US6
sgRNA B2M
EF1a
eGFP
B2M KO
+ Cas9 mRNA
US6
sgRNA B2M
EF1a
eGFP
HSV TAPi
EF1a
HSV1 ICP47
T2A
eGFP
HCMV TAPi
EF1a
HCMV US6
T2A
eGFP
EBV TAPi
EF1a
EBV BNLF2a
T2A
eGFP

FIG. 1A


MHC Class I on T cells
Fold Change HLA A/B/C
2.0
1.5
1.0
0.5
0.0
****
****
****
****
UTD
eGFP
B2M --
B2M KO
HSV TAPi
HCMV TAPi
EBV TAPi
MHC Class II on T cells
Fold Change HLA DR/DP/DQ
1.5
1.0
0.5
0.0
UTD
eGFP
B2M --
B2M KO
HSV TAPi
HCMV TAPi
EBV TAPi
Susceptibility to NK lysis
%NK cell-mediated killing
100
90
80
25
20
15
10
5
0
*
n.s.
n.s.
n.s.
UTD
eGFP
B2M --
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
K562
NK cell degranulation
% CD107a+ NK cells
40
30
20
10
0
**
n.s.
n.s.
n.s.
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eGFP
B2M --
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
K562

FIG. 1B

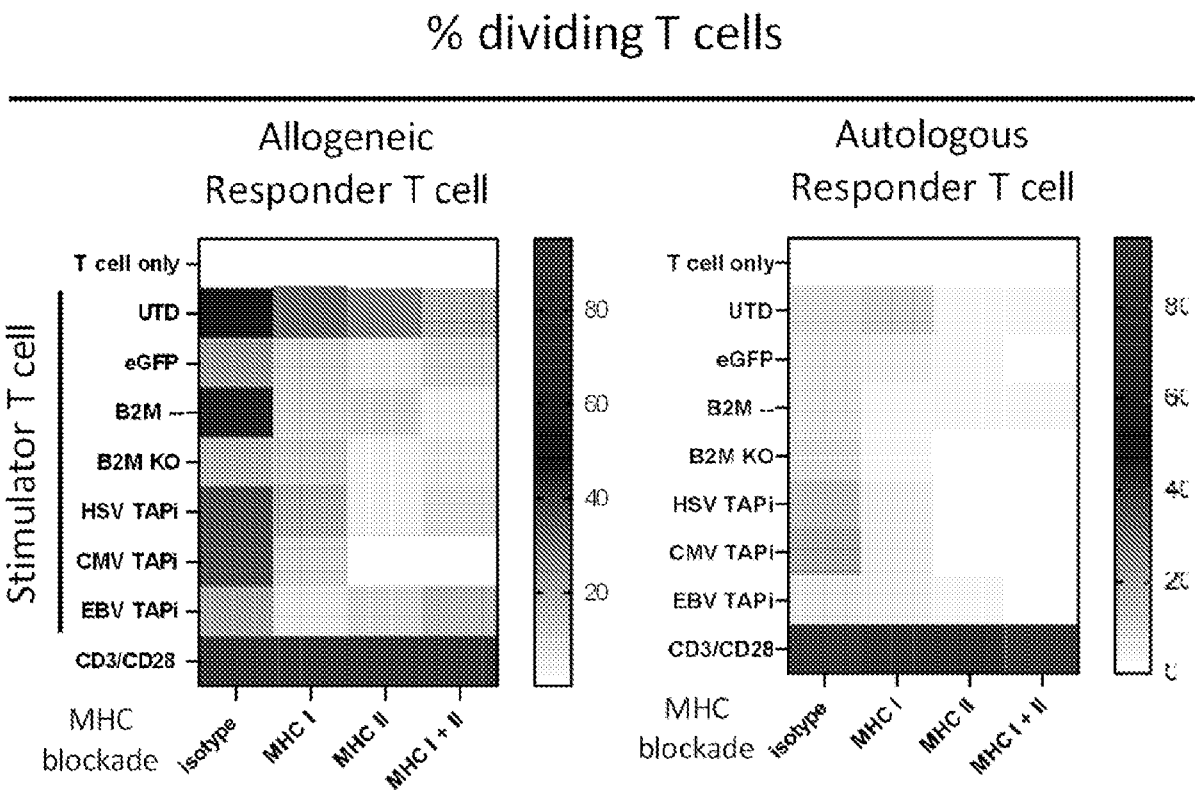
% dividing T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
B2M
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
CD3/CD28
80
60
40
20
MHC
blockade
isotype
MHC I
MHC II
MHC I + II

FIG. 1C

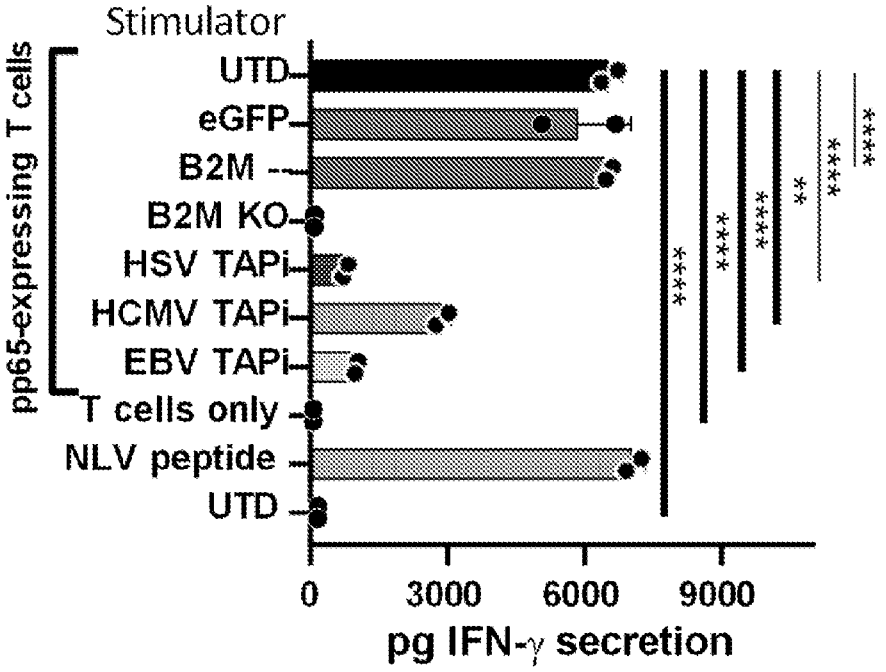
Stimulator
pp65-expressing T cells
UTD
eGFP
B2M
B2M KO
HSV TAPi
HCMV TAPi
EBV TAPi
T cells only
NLV peptide
UTD
****
****
****
**
****
****
0
3000
6000
9000
pg IFN-γ secretion

FIG. 1D

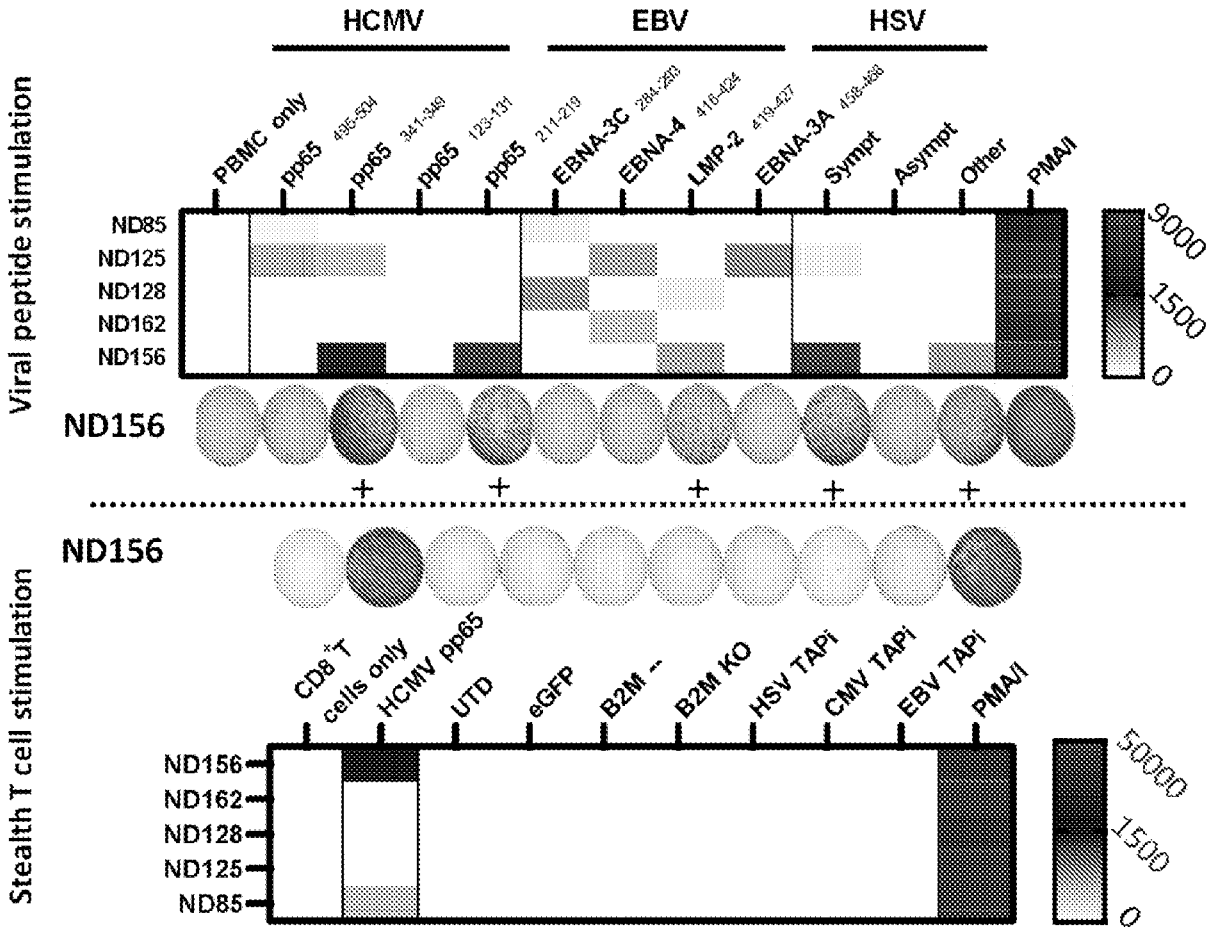
HCMV
EBV
HSV
PBMC only
pp65 496-504
pp65 341-349
pp65 123-131
pp65 211-219
EBNA-3C 284-293
EBNA-4 416-424
LMP-2 419-427
EBNA-3A 458-466
Sympt
Asympt
Other
PMA/I
Viral peptide stimulation
ND85
ND125
ND128
ND162
ND156
9000
1500
0
ND156
+
+
+
+
+
ND156
Stealth T cell stimulation
CD8+T cells only
HCMV pp65
UTD
eGFP
B2M --
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
PMA/I
ND156
ND162
ND128
ND125
ND85
50000
1500
0

FIG. 1E

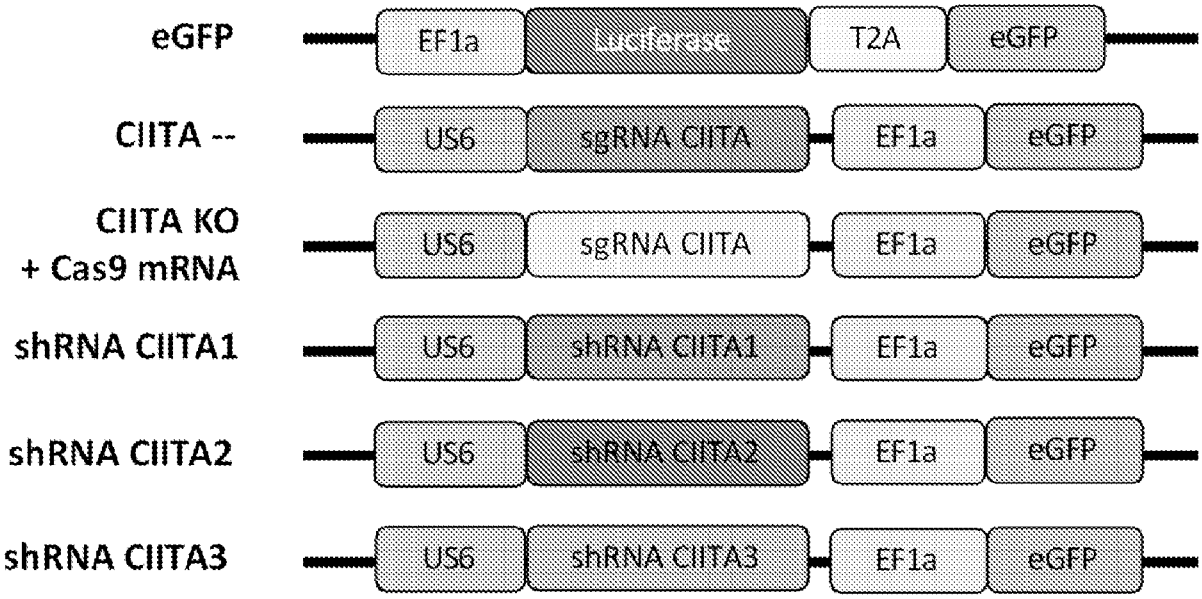
eGFP
EF1a
Luciferase
T2A
eGFP
CIITA --
U6
sgRNA CIITA
EF1a
eGFP
CIITA KO
+ Cas9 mRNA
U6
sgRNA CIITA
EF1a
eGFP
shRNA CIITA1
U6
shRNA CIITA1
EF1a
eGFP
shRNA CIITA2
U6
shRNA CIITA2
EF1a
eGFP
shRNA CIITA3
U6
shRNA CIITA3
EF1a
eGFP

FIG. 2A

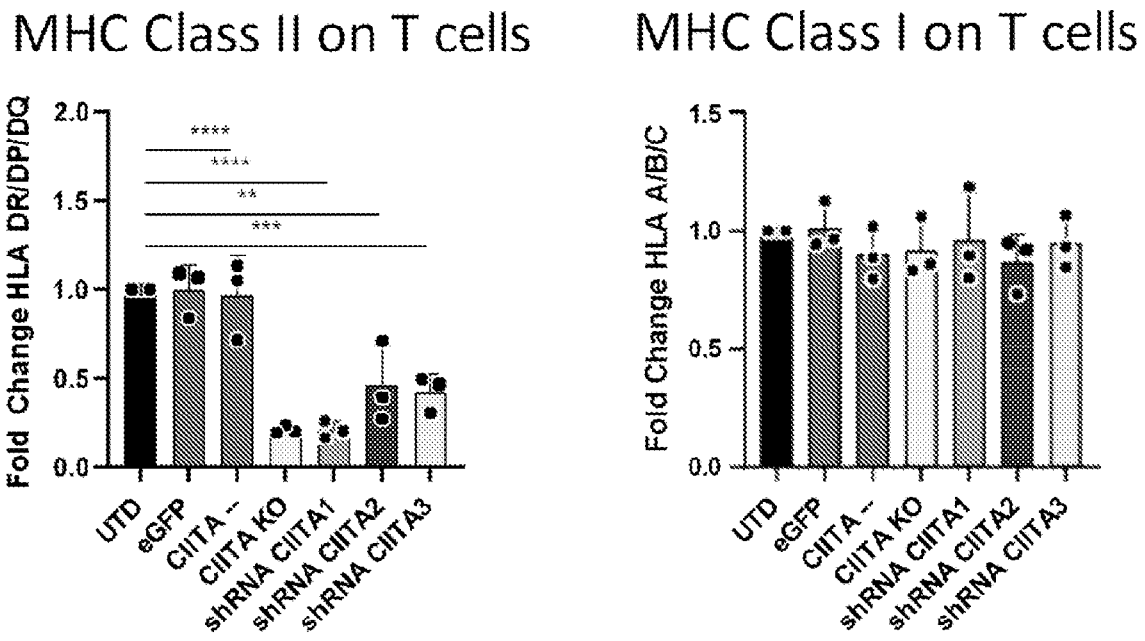
MHC Class II on T cells
Fold Change HLA DR/DP/DQ
2.0
1.5
1.0
0.5
0.0
****
****
**
***
UTD
eGFP
CIITA --
CIITA KO
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3
MHC Class I on T cells
Fold Change HLA A/B/C
1.5
1.0
0.5
0.0
UTD
eGFP
CIITA --
CIITA KO
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3

FIG. 2B

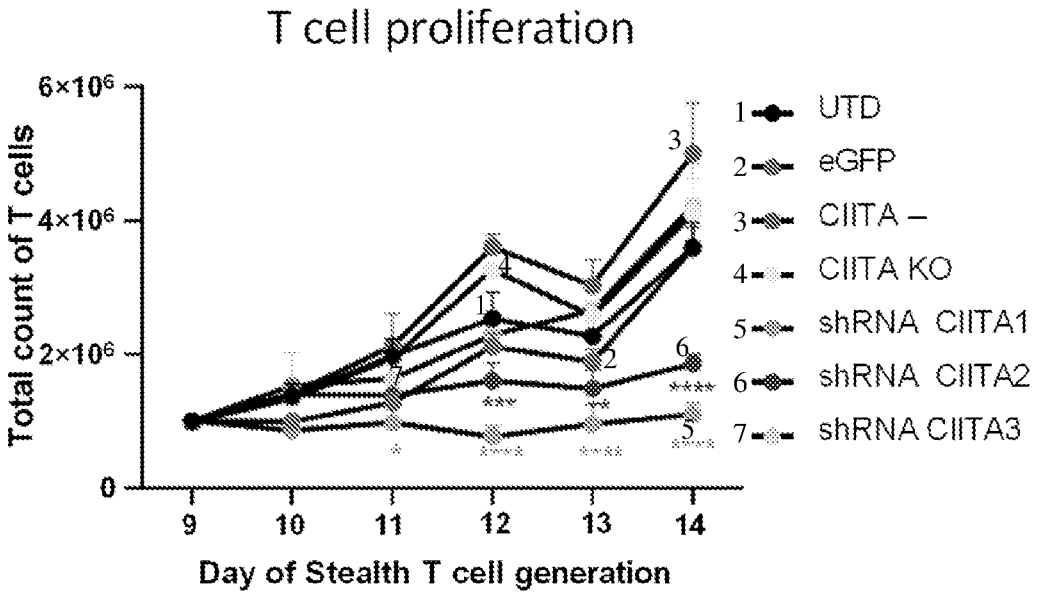
T cell proliferation
Total count of T cells
6×10^6
4×10^6
2×10^6
0
9
10
11
12
13
14
Day of Stealth T cell generation
1 UTD
2 eGFP
3 CIITA –
4 CIITA KO
5 shRNA CIITA1
6 shRNA CIITA2
7 shRNA CIITA3

FIG. 2C

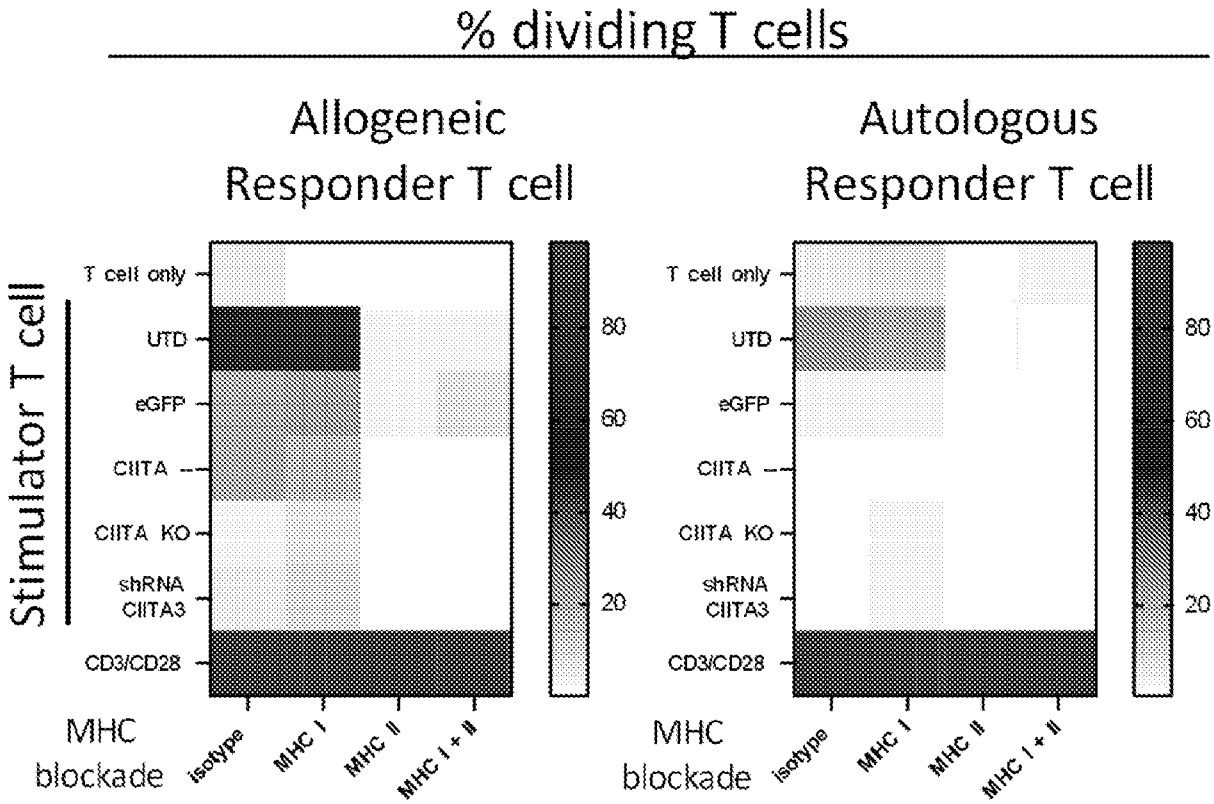
% dividing T cells
Allogeneic Responder T cell
Autologous Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
CIITA –
CIITA KO
shRNA CIITA3
CD3/CD28
80
60
40
20
MHC blockade
isotype
MHC I
MHC II
MHC I + II

FIG. 2D

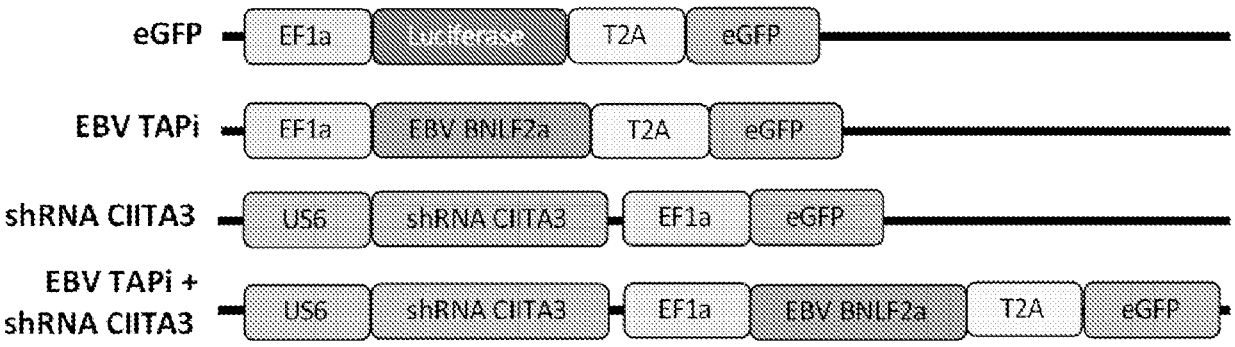
eGFP
EF1a
Luciferase
T2A
eGFP
EBV TAPi
EF1a
EBV BNLF2a
T2A
eGFP
shRNA CIITA3
U6
shRNA CIITA3
EF1a
eGFP
EBV TAPi +
shRNA CIITA3
U6
shRNA CIITA3
EF1a
EBV BNLF2a
T2A
eGFP

FIG. 2E

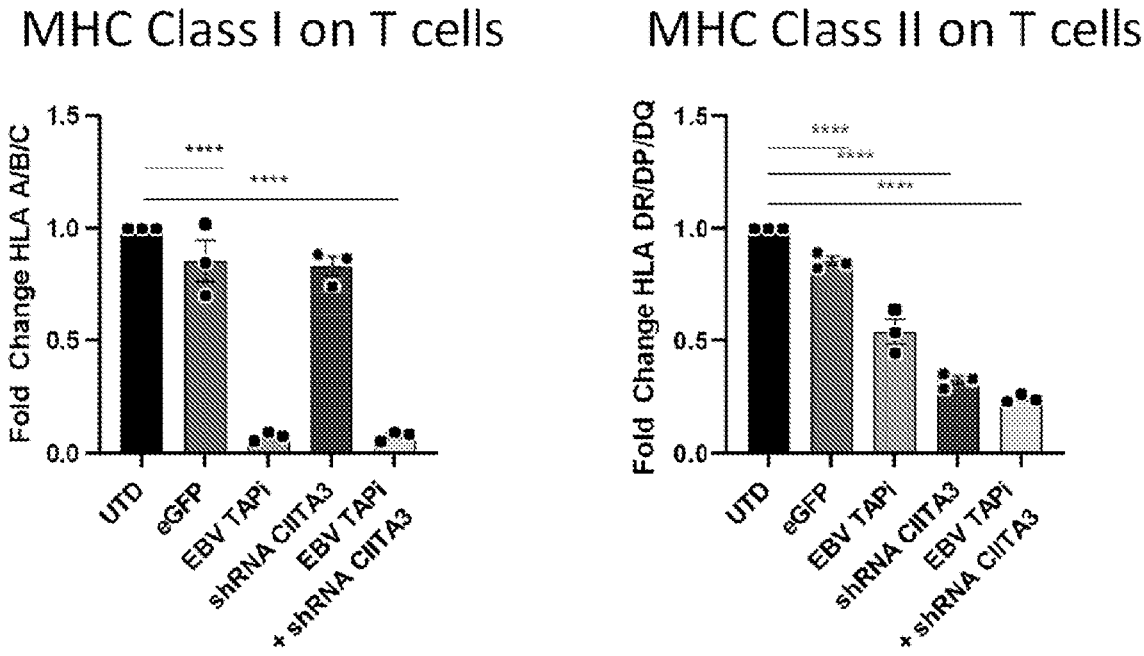
MHC Class I on T cells
Fold Change HLA A/B/C
1.5
1.0
0.5
0.0
****
****
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi
+ shRNA CIITA3
MHC Class II on T cells
Fold Change HLA DR/DP/DQ
1.5
1.0
0.5
0.0
****
****
****
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi
+ shRNA CIITA3

FIG. 2F

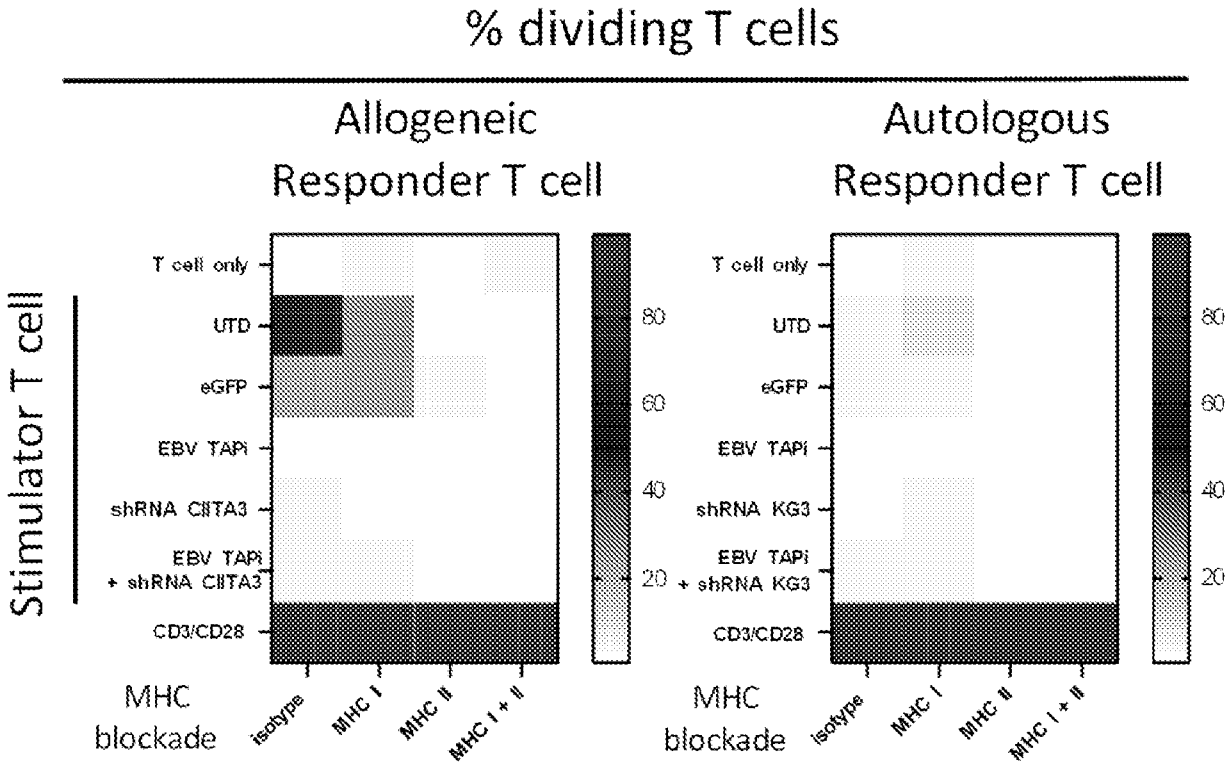
% dividing T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi
+ shRNA CIITA3
CD3/CD28
shRNA KG3
EBV TAPi
+ shRNA KG3
80
60
40
20
MHC
blockade
isotype
MHC I
MHC II
MHC I + II

FIG. 2G

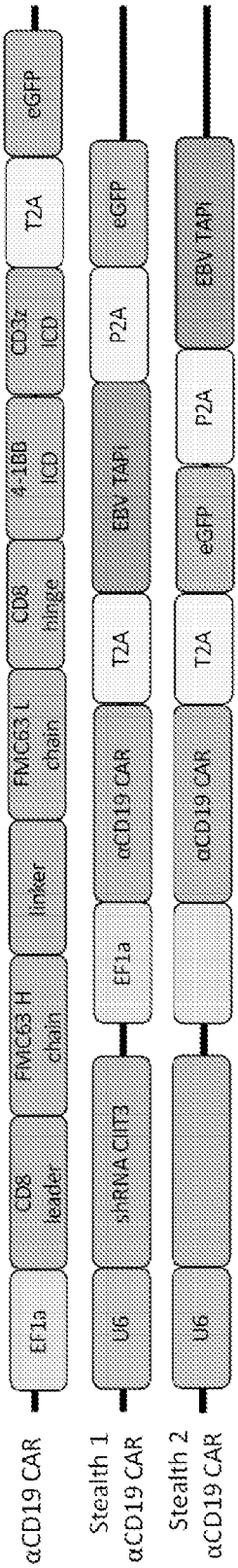
αCD19 CAR
EF1a
CD8 leader
FMC63 H chain
linker
FMC63 L chain
CD8 hinge
4-1BB ICD
CD3ζ ICD
T2A
eGFP
Stealth 1 αCD19 CAR
U6
shRNA CIITA
EF1a
αCD19 CAR
T2A
EBV TAPi
P2A
eGFP
Stealth 2 αCD19 CAR
U6
αCD19 CAR
T2A
eGFP
P2A
EBV TAPi

FIG. 3A

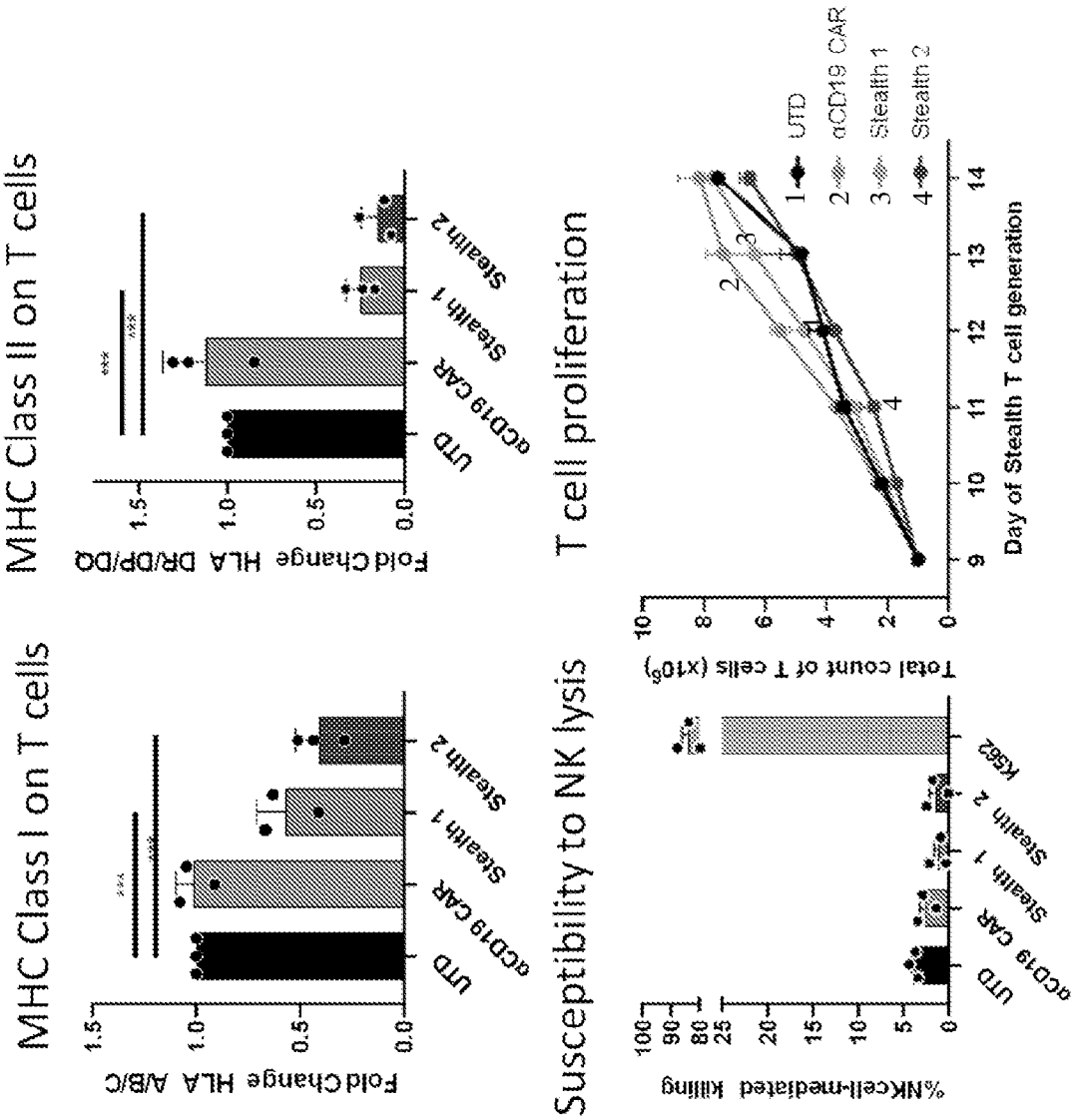
MHC Class I on T cells
Fold Change HLA A/B/C
UTD
αCD19 CAR
Stealth 1
Stealth 2
MHC Class II on T cells
Fold Change HLA DR/DP/DQ
Susceptibility to NK lysis
%NKcell-mediated killing
K562
T cell proliferation
Total count of T cells (x10^9)
Day of Stealth T cell generation
1 UTD
2 αCD19 CAR
3 Stealth 1
4 Stealth 2

FIG. 3B

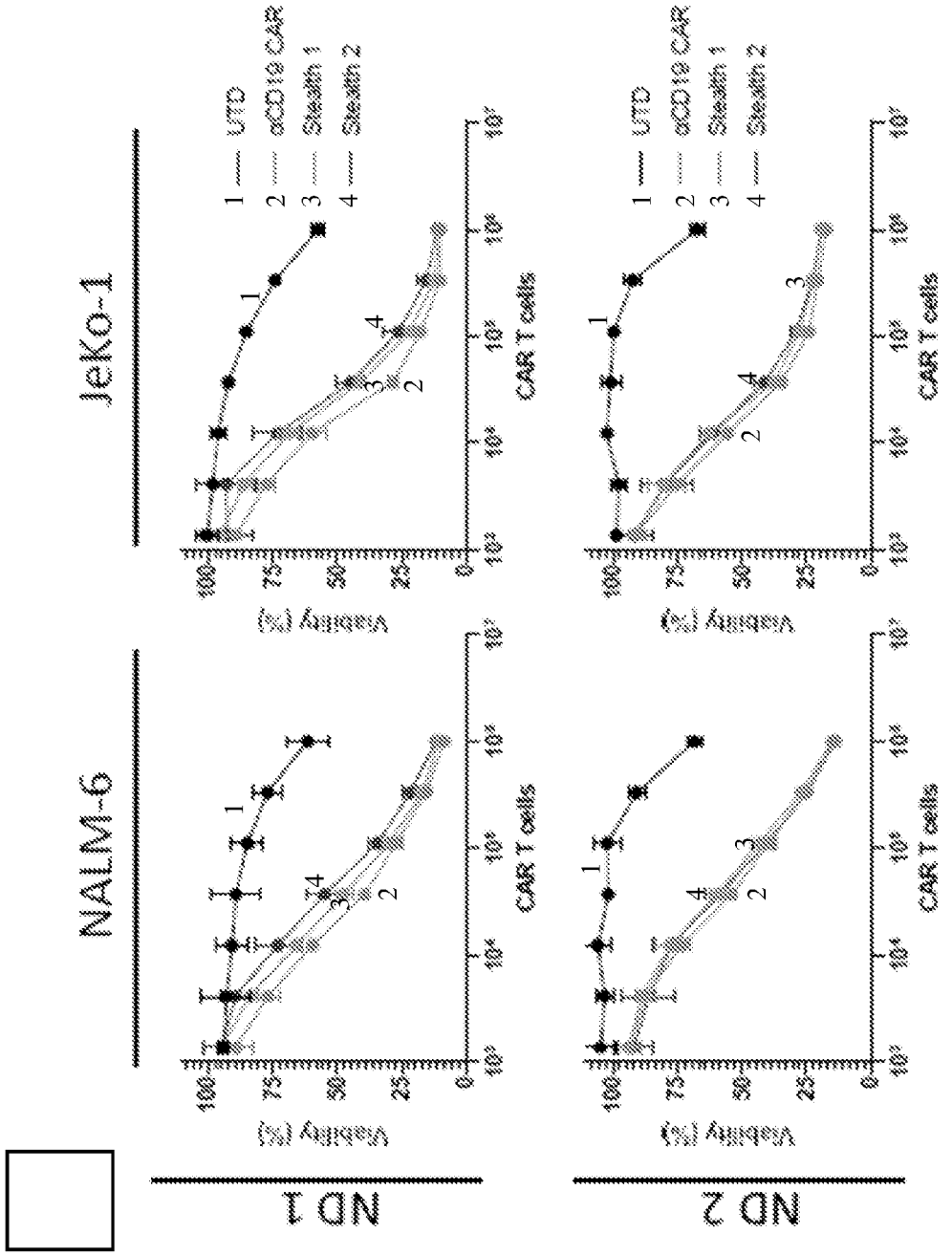
NALM-6
JeKo-1
ND 1
ND 2
Viability (%)
CAR T cells
1 — UTD
2 — αCD19 CAR
3 — Stealth 1
4 — Stealth 2

FIG. 3C

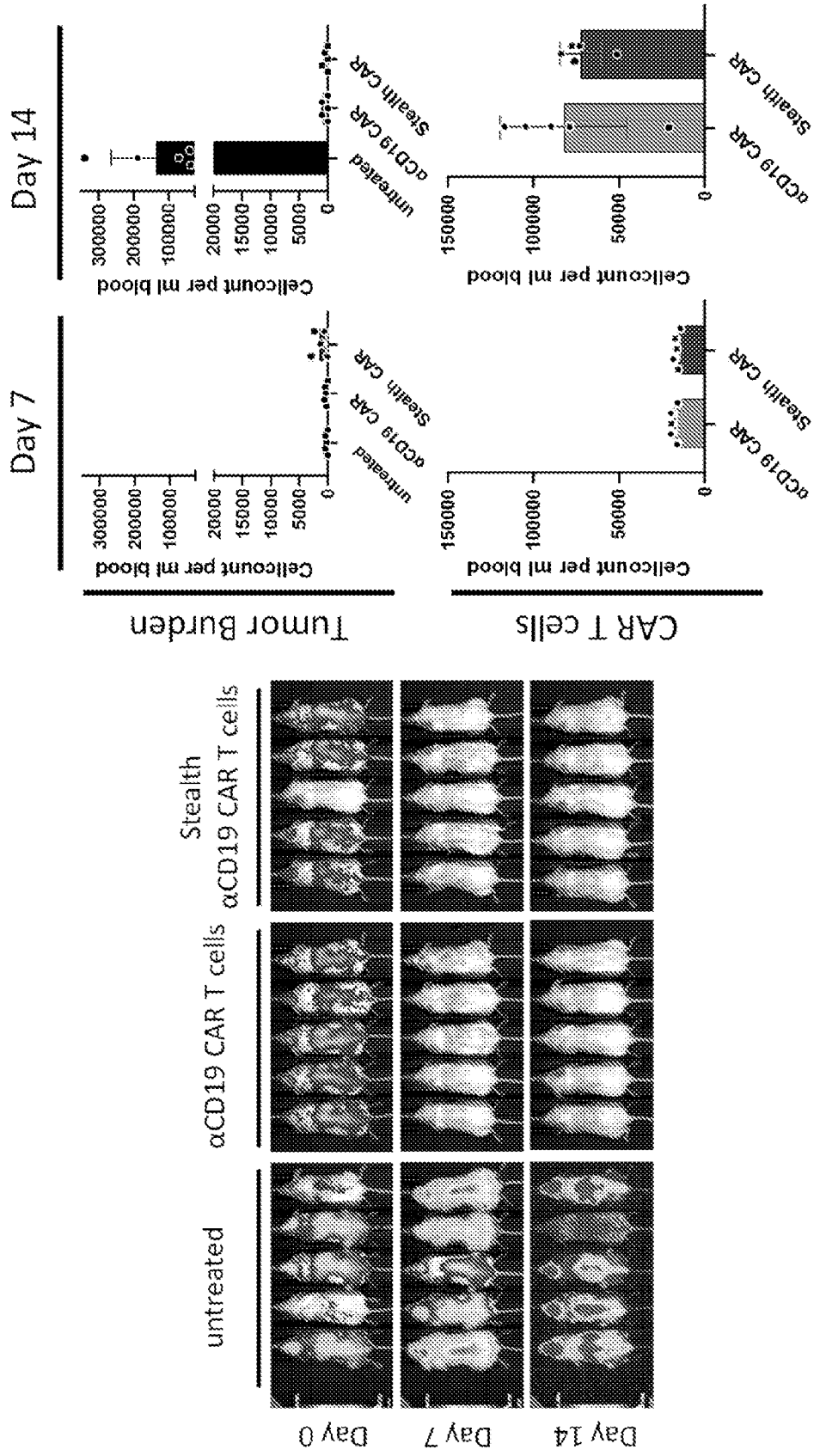
Day 14
Day 7
Tumor Burden
CAR T cells
Cellcount per ml blood
untreated
αCD19 CAR
Stealth CAR
300000
200000
100000
20000
15000
10000
5000
0
150000
100000
50000
Day 0
Day 7
Day 14
untreated
αCD19 CAR T cells
Stealth
αCD19 CAR T cells

FIG. 3D

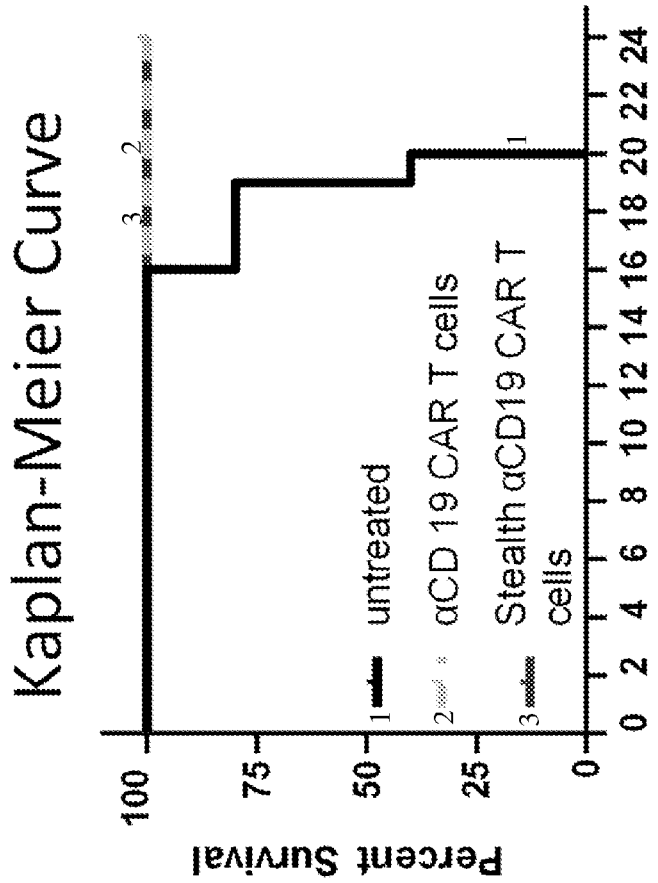
Kaplan-Meier Curve
Percent Survival
100
75
50
25
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Days
1 untreated
2 αCD 19 CAR T cells
3 Stealth αCD19 CAR T cells
1
2
3

FIG. 3E

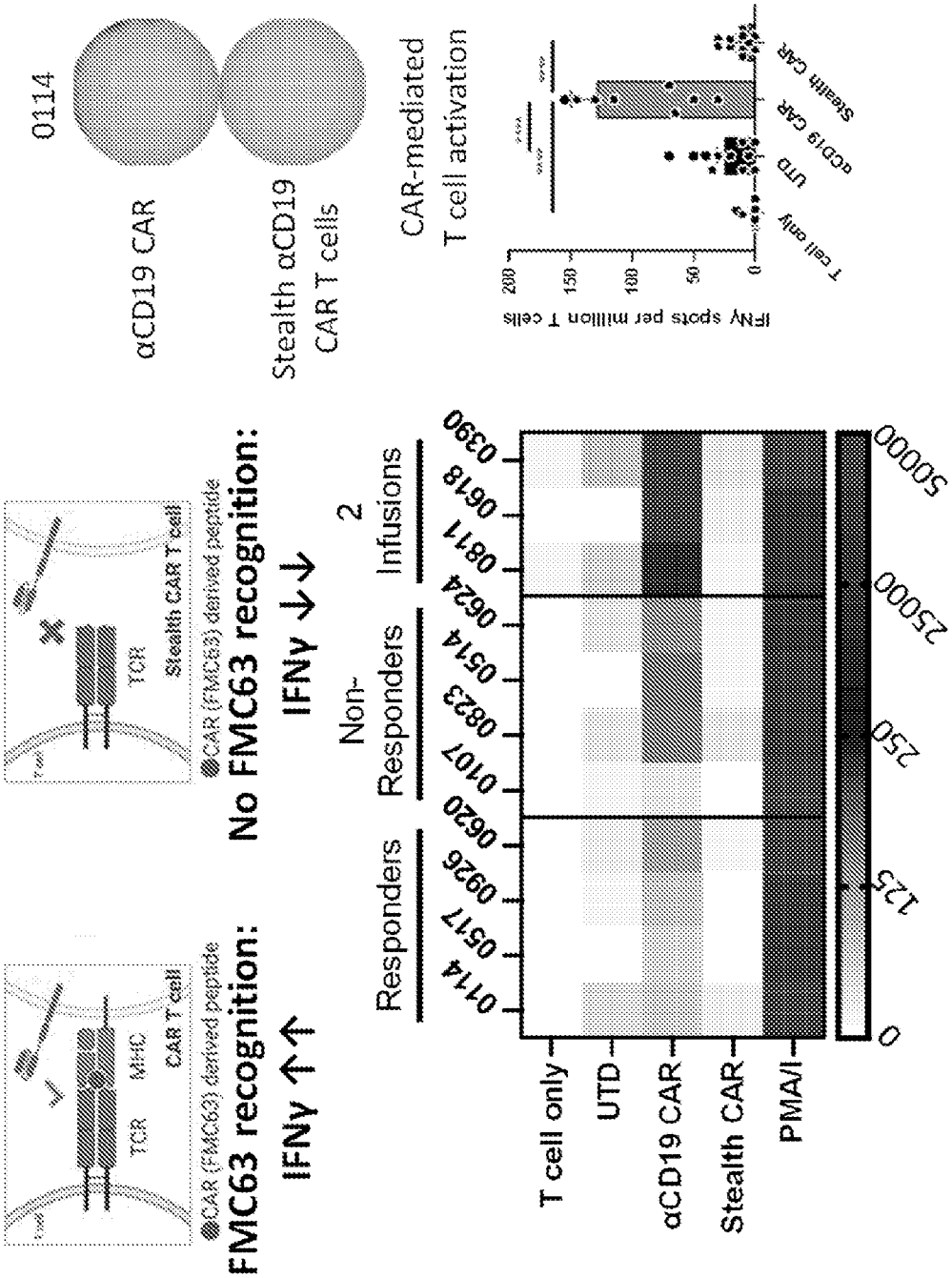
FMC63 recognition:
IFNγ ↑↑
CAR (FMC63) derived peptide
TCR
MHC
CAR T cell
No FMC63 recognition:
IFNγ ↓↓
CAR (FMC63) derived peptide
TCR
Stealth CAR T cell
Responders
Non-Responders
2 Infusions
0114
0517
0926
0620
0107
0823
0514
0624
0811
0618
0390
T cell only
UTD
αCD19 CAR
Stealth CAR
PMA/I
50000
25000
250
125
0
CAR-mediated T cell activation
IFNγ spots per million T cells
200
150
100
50
0
T cell only
UTD
αCD19 CAR
Stealth CAR
anti-CAR T cell response
αCD19 CAR IFNγ spots per million T cells
300
200
100
0
Responder
Non-Responder
2 Infusions
0114
0823
0811
αCD19 CAR
Stealth αCD19 CAR T cells

FIG. 3F

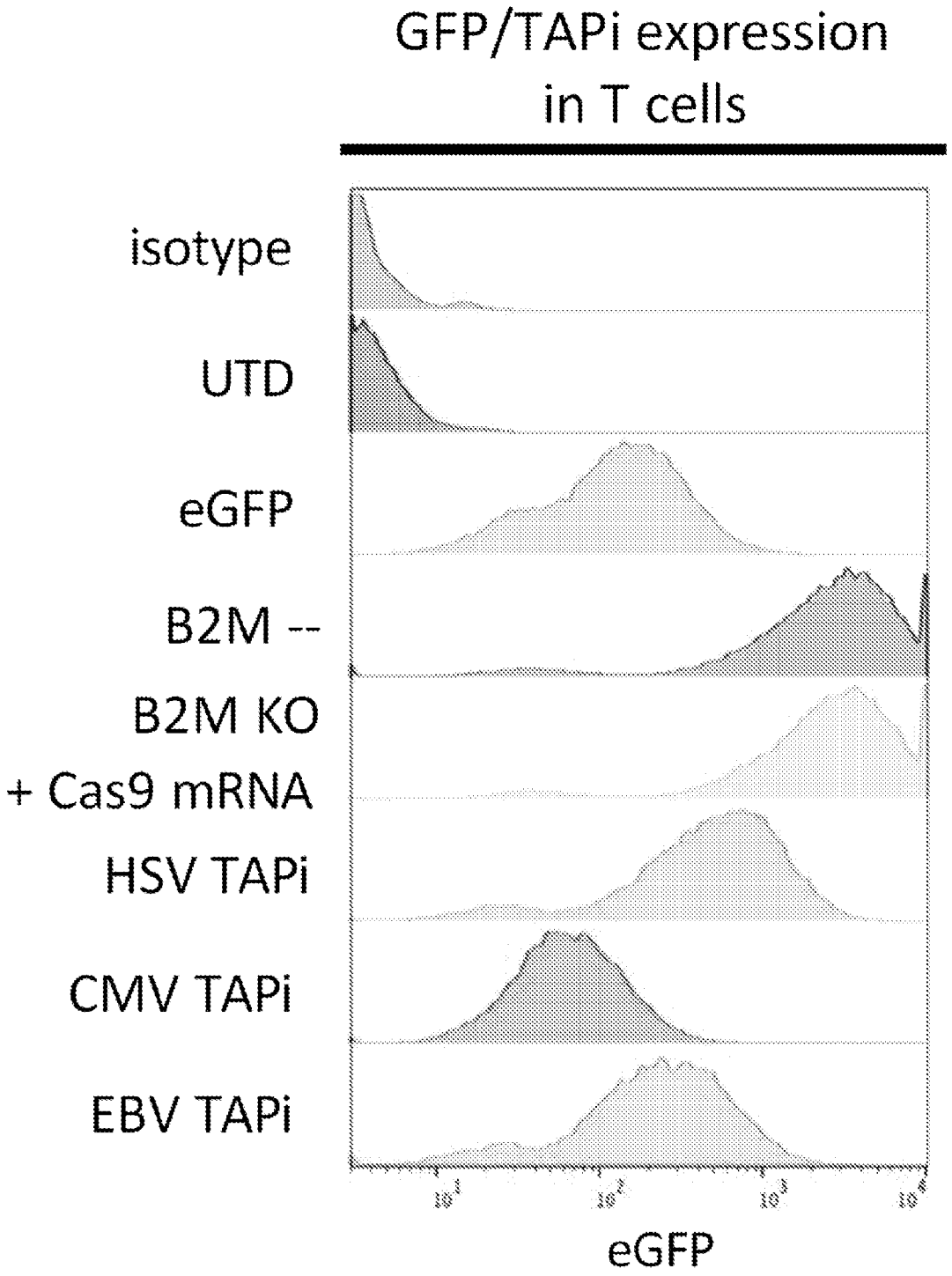
GFP/TAPi expression
in T cells
isotype
UTD
eGFP
B2M --
B2M KO
+ Cas9 mRNA
HSV TAPi
CMV TAPi
EBV TAPi
eGFP

FIG. 4A

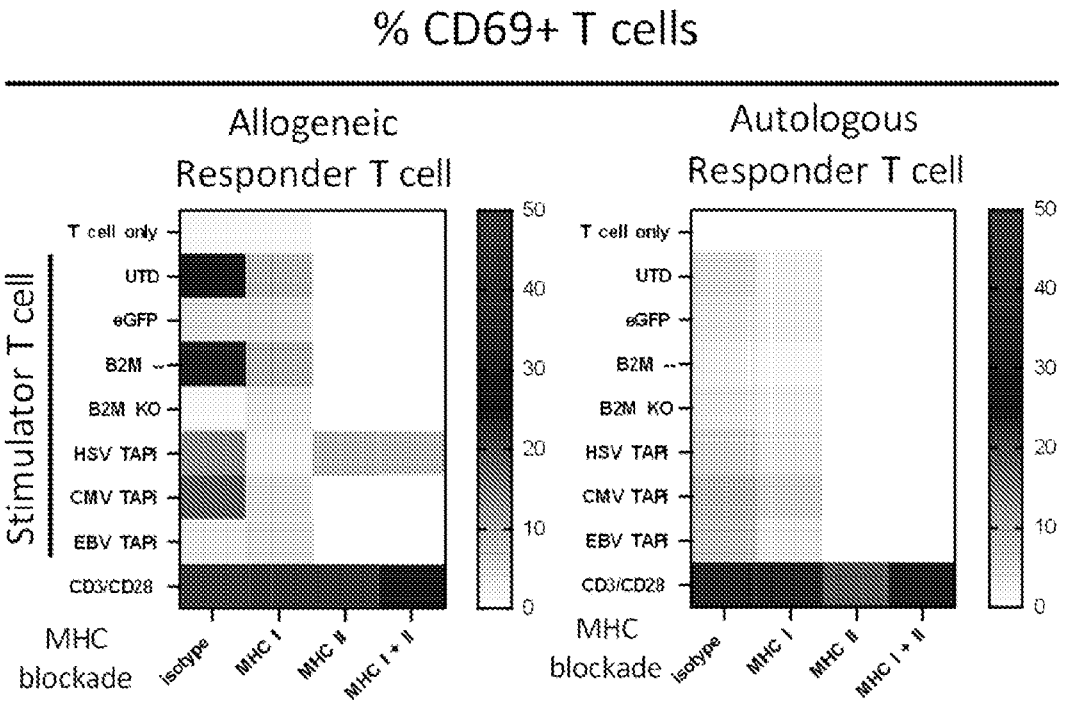
% CD69+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
B2M
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
CD3/CD28
MHC
blockade
isotype
MHC I
MHC II
MHC I + II
50
40
30
20
10
0

FIG. 4B

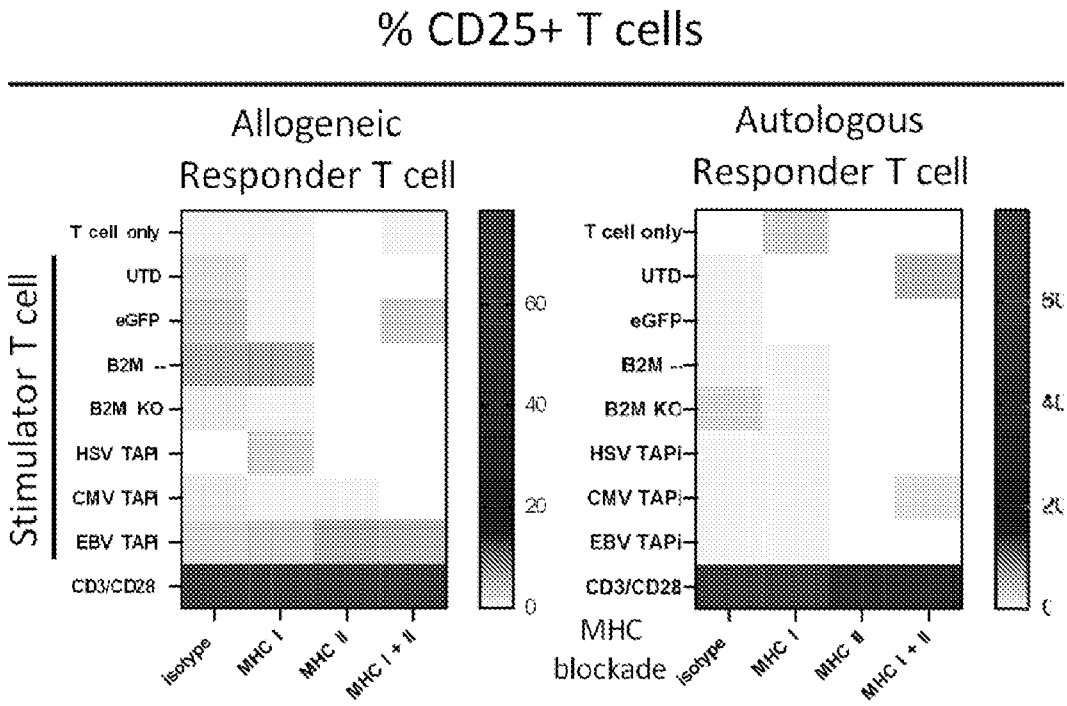
% CD25+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
B2M
B2M KO
HSV TAPi
CMV TAPi
EBV TAPi
CD3/CD28
MHC
blockade
isotype
MHC I
MHC II
MHC I + II
60
40
20
0

FIG. 4C

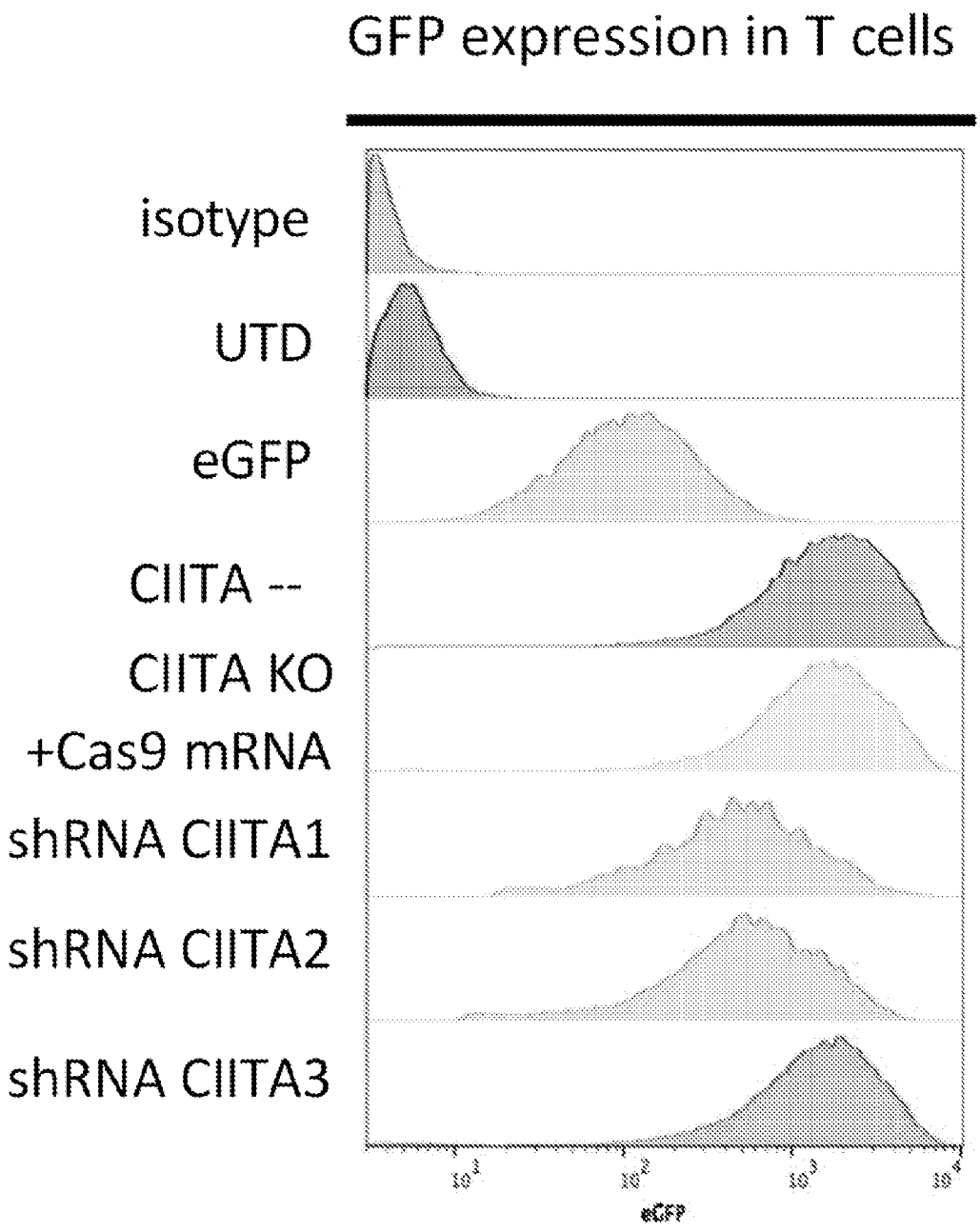
GFP expression in T cells
isotype
UTD
eGFP
CIITA --
CIITA KO
+Cas9 mRNA
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3
eGFP

FIG. 5A

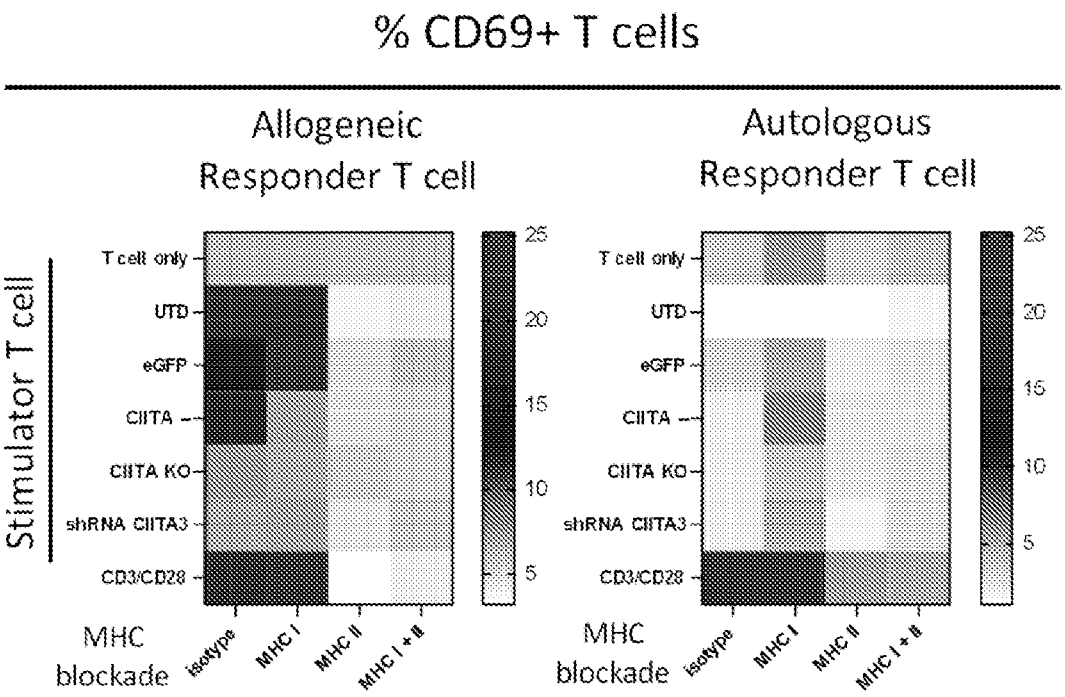
% CD69+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
CIITA
CIITA KO
shRNA CIITA3
CD3/CD28
MHC
blockade
isotype
MHC I
MHC II
MHC I + II
25
20
15
10
5

FIG. 5B

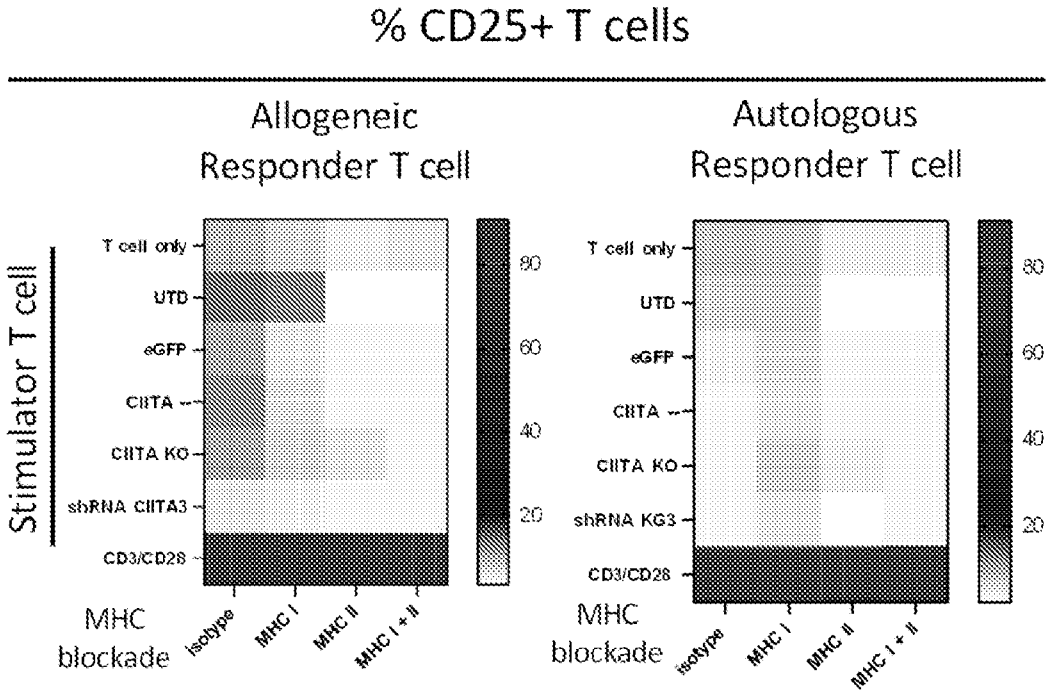
% CD25+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
CIITA
CIITA KO
shRNA CIITA3
shRNA KG3
CD3/CD28
MHC
blockade
isotype
MHC I
MHC II
MHC I + II
80
60
40
20

FIG. 5C

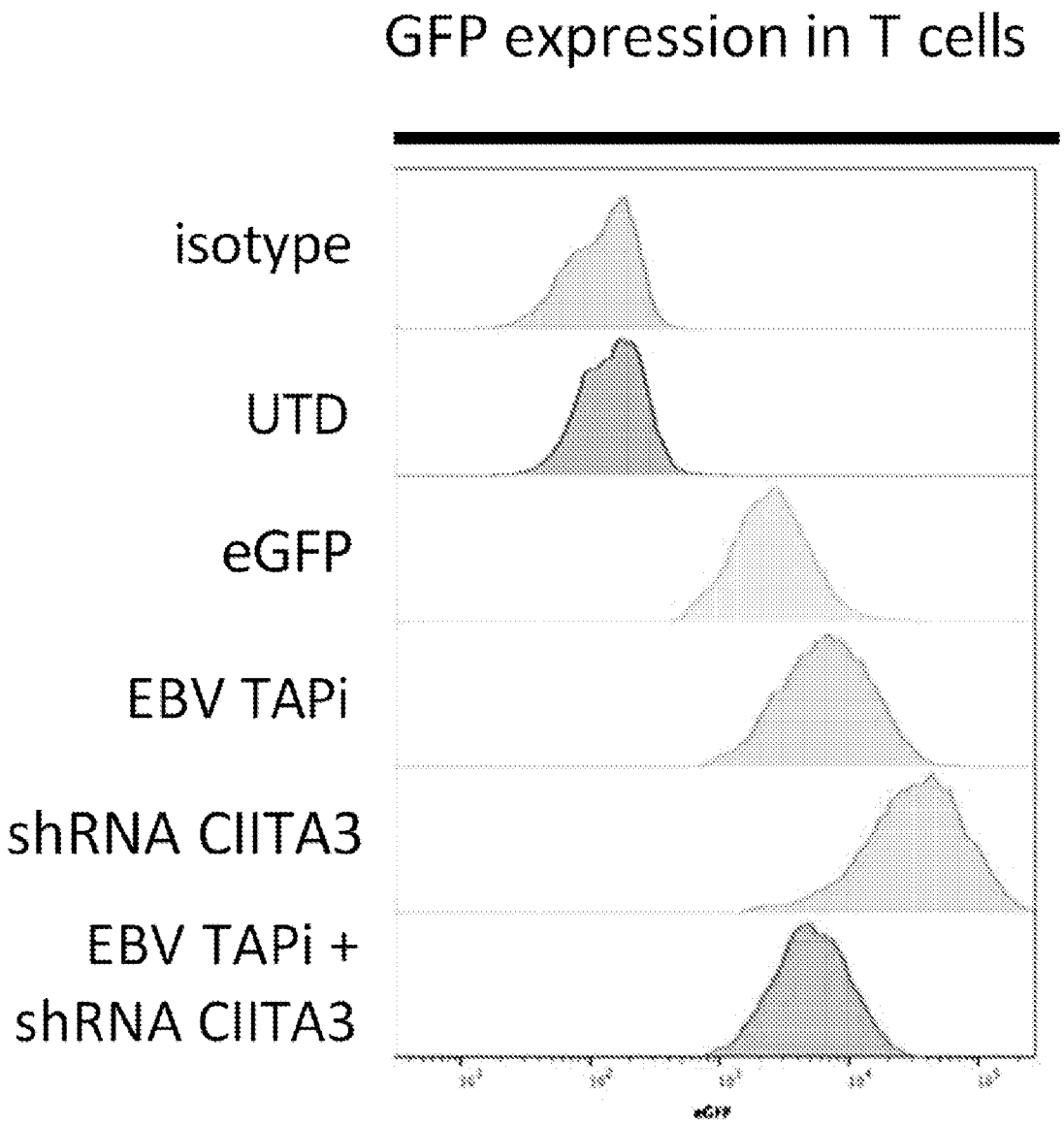
GFP expression in T cells
isotype
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi +
shRNA CIITA3

FIG. 5D

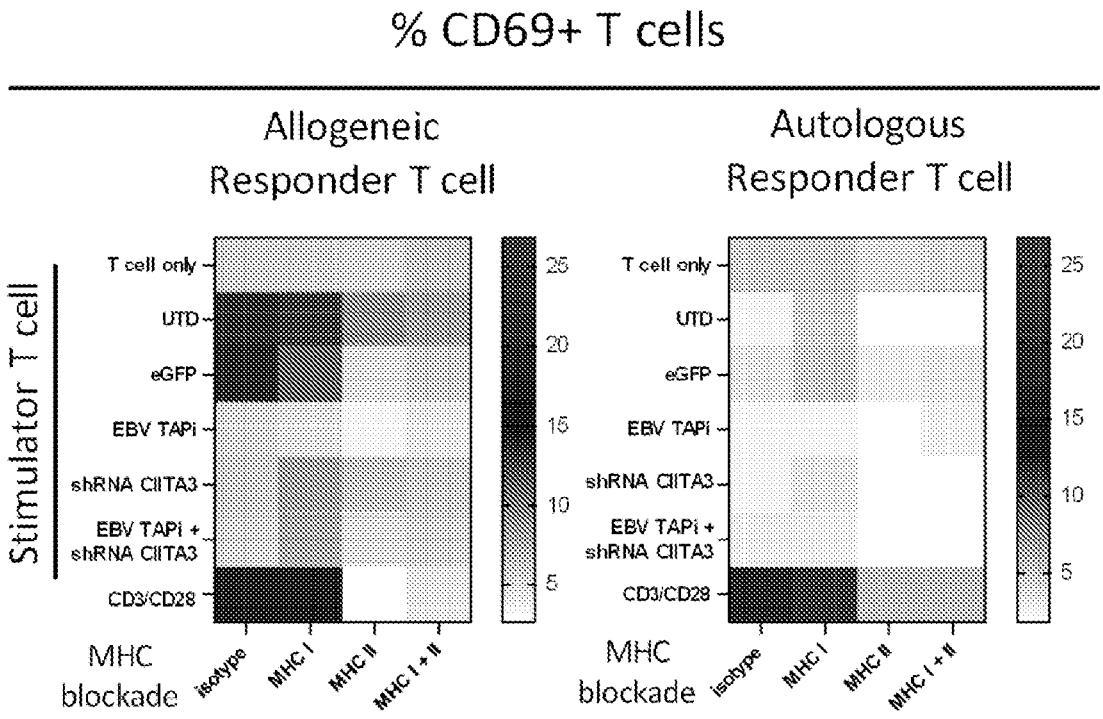
% CD69+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi +
shRNA CIITA3
CD3/CD28
25
20
15
10
5
MHC
blockade
isotype
MHC I
MHC II
MHC I + II

FIG. 5E

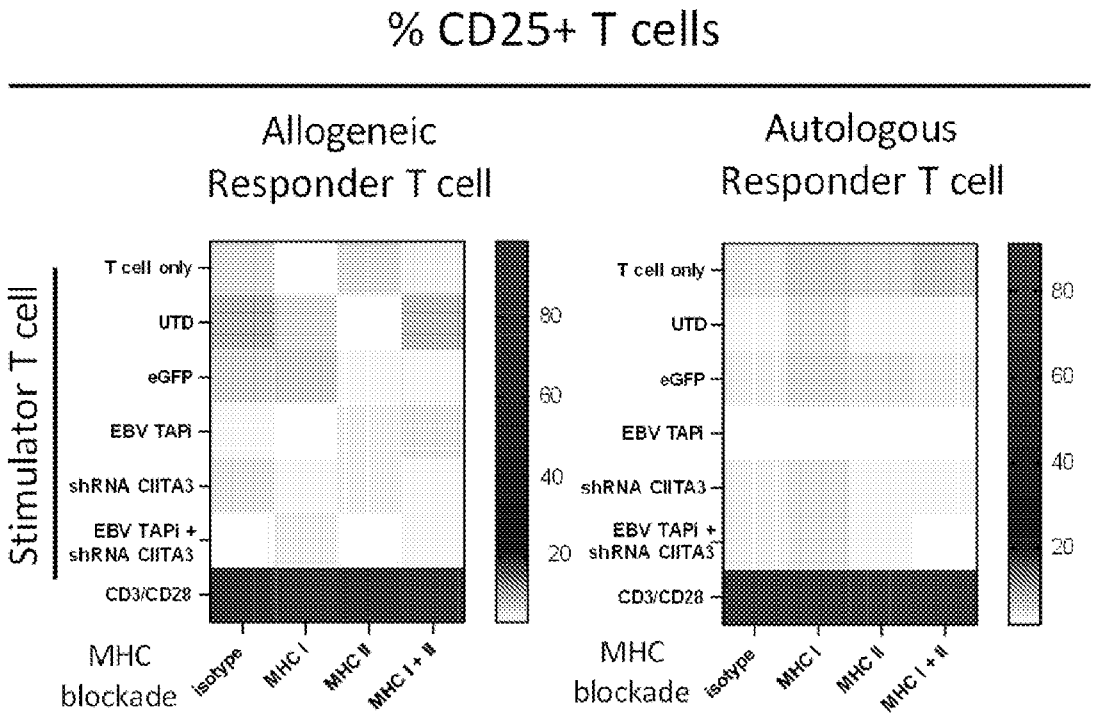
% CD25+ T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
Stimulator T cell
T cell only
UTD
eGFP
EBV TAPi
shRNA CIITA3
EBV TAPi +
shRNA CIITA3
CD3/CD28
80
60
40
20
MHC
blockade
isotype
MHC I
MHC II
MHC I + II

FIG. 5F

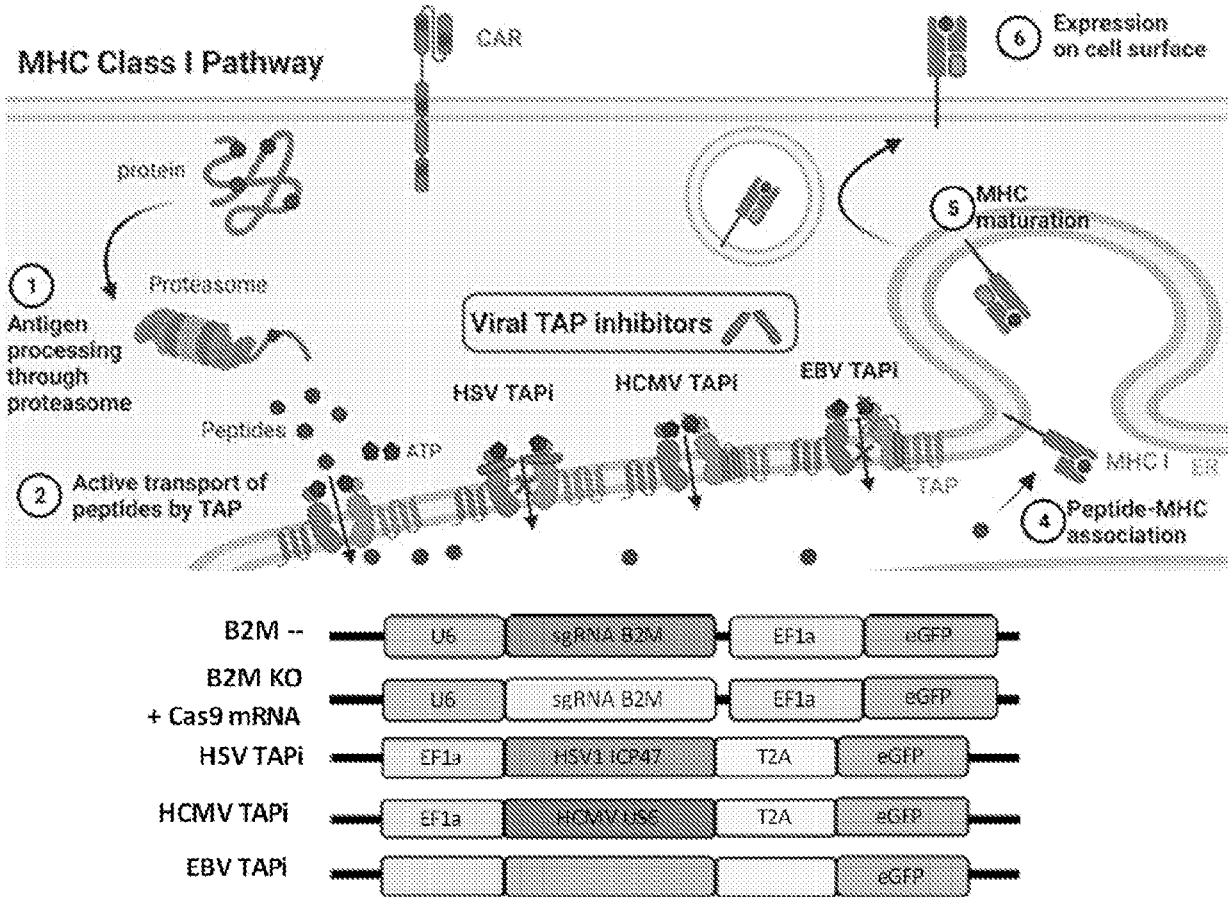
MHC Class I Pathway
CAR
6 Expression on cell surface
protein
5 MHC maturation
1 Antigen processing through proteasome
Proteasome
Viral TAP inhibitors
HSV TAPi
HCMV TAPi
EBV TAPi
Peptides
ATP
2 Active transport of peptides by TAP
TAP
MHC I
ER
4 Peptide-MHC association
B2M --
B2M KO + Cas9 mRNA
HSV TAPi
HCMV TAPi
EBV TAPi
U6
sgRNA B2M
EF1a
eGFP
HSV1 ICP47
T2A

FIG. 6A

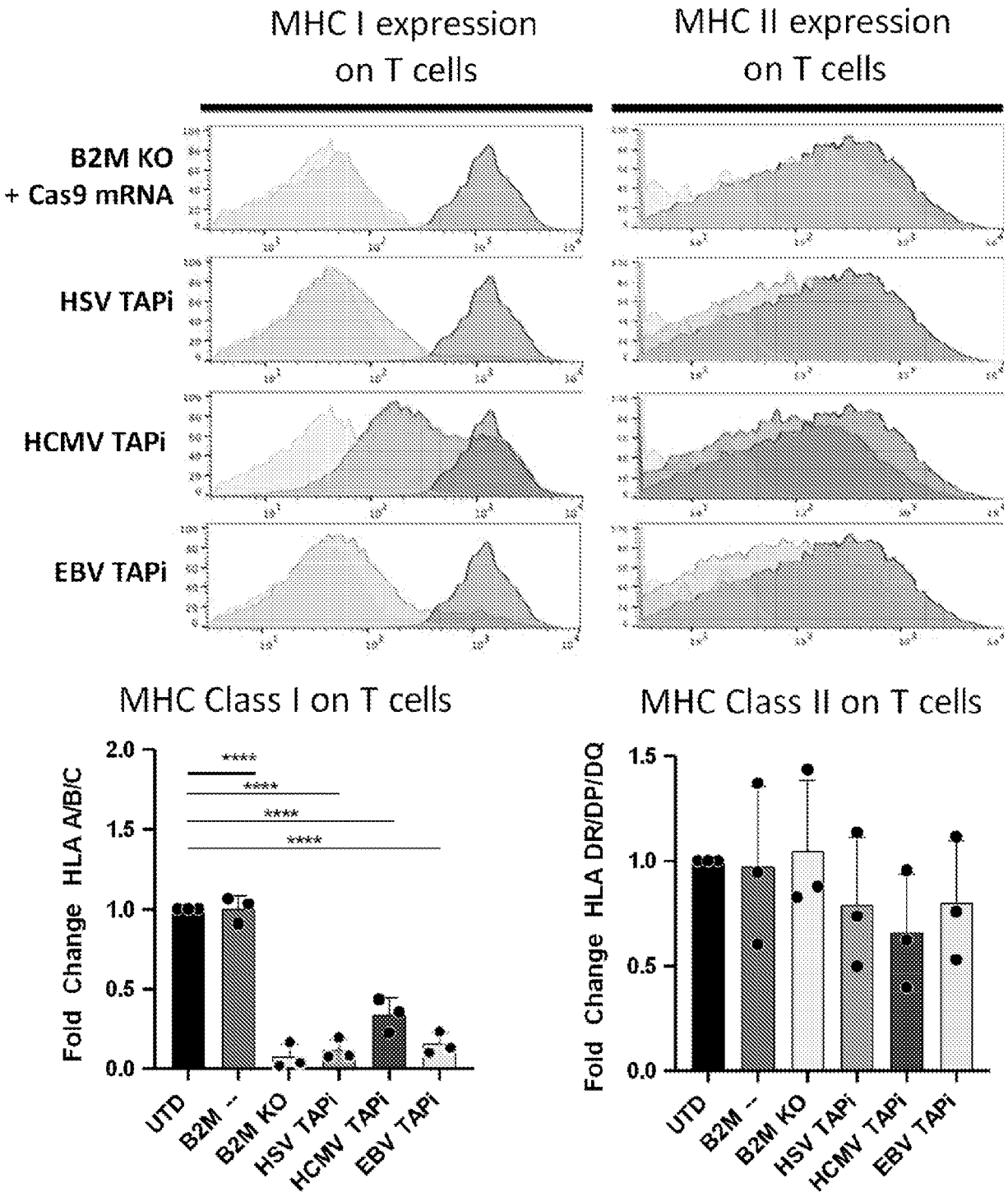
MHC I expression on T cells
MHC II expression on T cells
B2M KO + Cas9 mRNA
HSV TAPi
HCMV TAPi
EBV TAPi
MHC Class I on T cells
MHC Class II on T cells
Fold Change HLA A/B/C
Fold Change HLA DR/DP/DQ
2.0
1.5
1.0
0.5
0.0
****
UTD
B2M --
B2M KO
HSV TAPi
HCMV TAPi
EBV TAPi

FIG. 6B

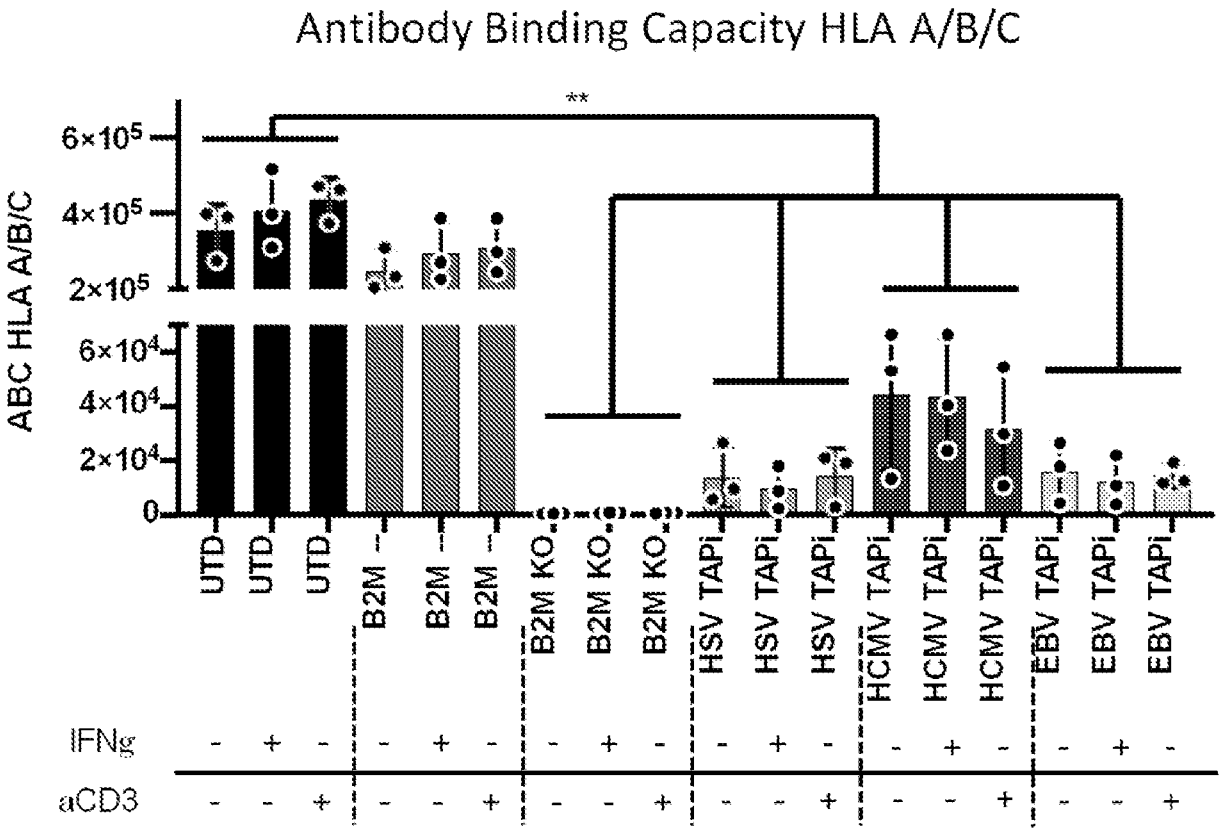
Antibody Binding Capacity HLA A/B/C
**
ABC HLA A/B/C
6×10^5
4×10^5
2×10^5
6×10^4
4×10^4
2×10^4
0
UTD
UTD
UTD
B2M
B2M
B2M
B2M KO
B2M KO
B2M KO
HSV TAPi
HSV TAPi
HSV TAPi
HCMV TAPi
HCMV TAPi
HCMV TAPi
EBV TAPi
EBV TAPi
EBV TAPi
IFNg
aCD3

FIG. 6C

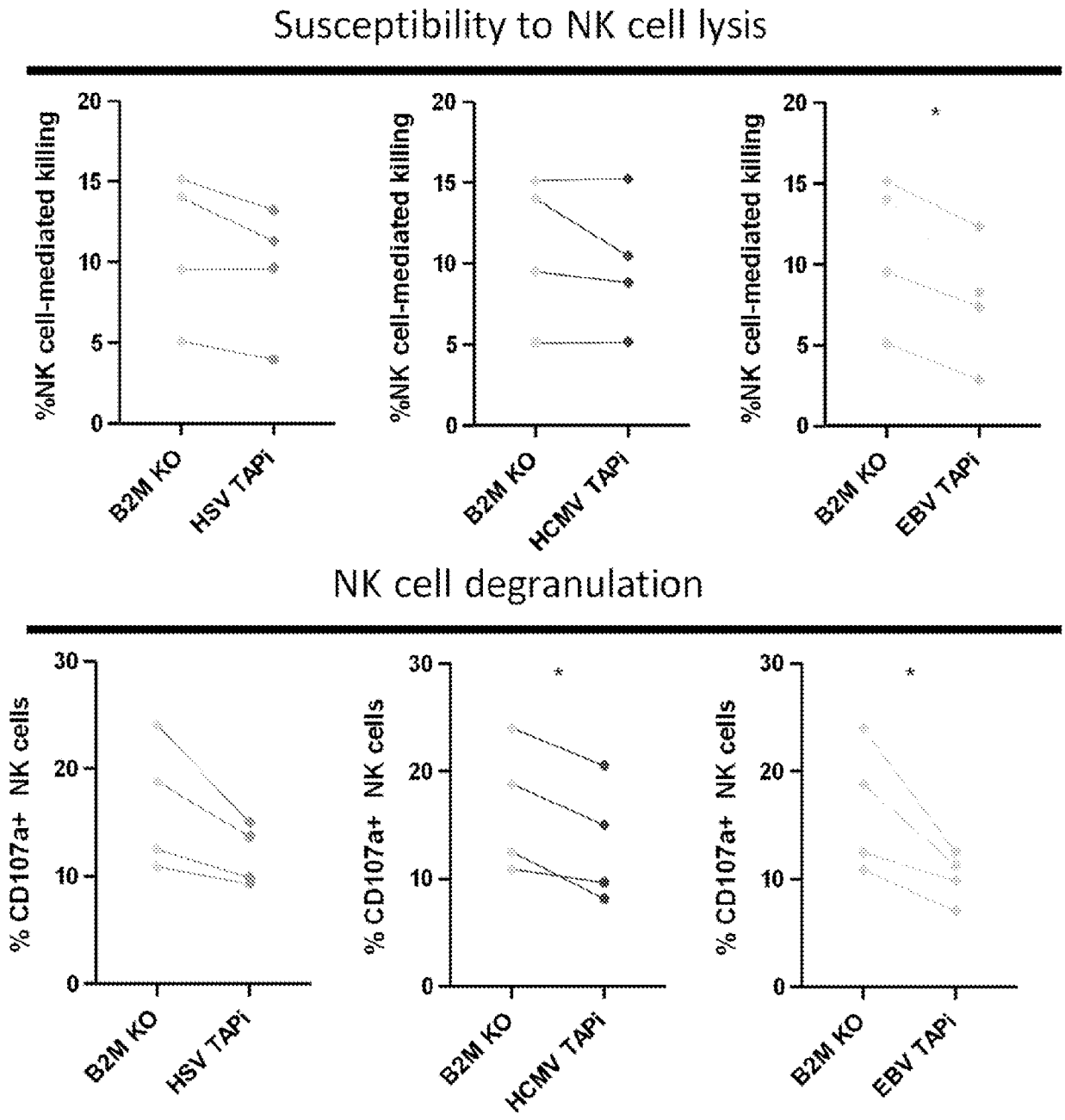
Susceptibility to NK cell lysis
%NK cell-mediated killing
20
15
10
5
0
B2M KO
HSV TAPi
B2M KO
HCMV TAPi
B2M KO
EBV TAPi
*
NK cell degranulation
% CD107a+ NK cells
30
20
10
0
B2M KO
HSV TAPi
B2M KO
HCMV TAPi
B2M KO
EBV TAPi
*

FIG. 6D

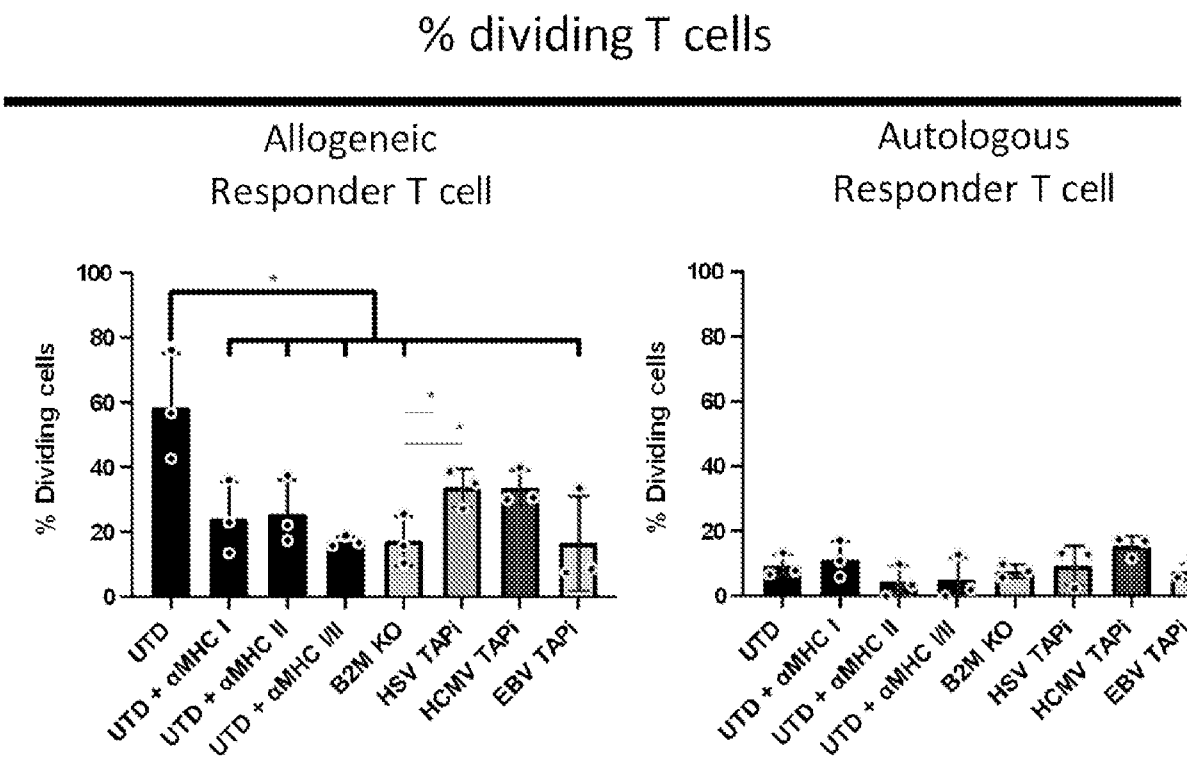
% dividing T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
% Dividing cells
100
80
60
40
20
0
UTD
UTD + αMHC I
UTD + αMHC II
UTD + αMHC I/II
B2M KO
HSV TAPi
HCMV TAPi
EBV TAPi

FIG. 6E

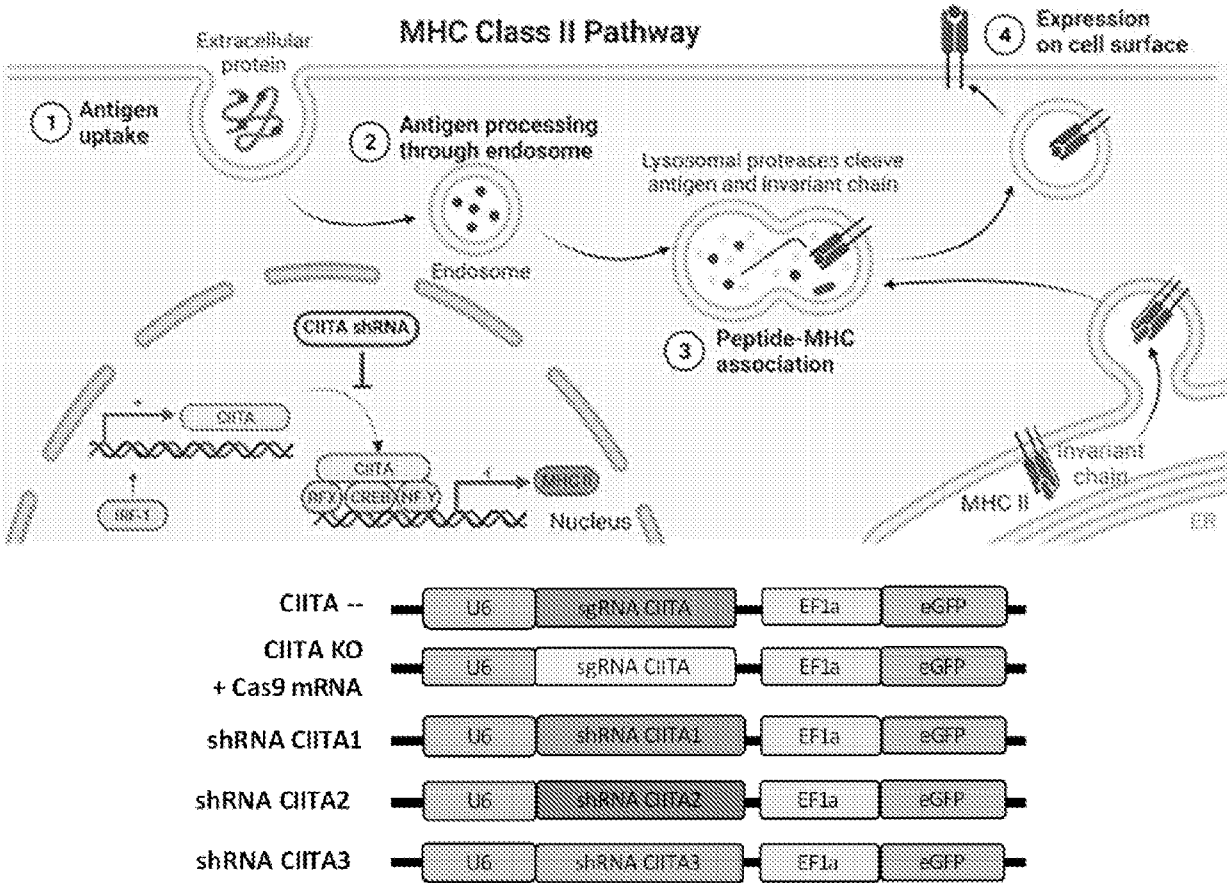
MHC Class II Pathway
Extracellular protein
1 Antigen uptake
2 Antigen processing through endosome
Endosome
Lysosomal proteases cleave antigen and invariant chain
3 Peptide-MHC association
4 Expression on cell surface
CIITA siRNA
CIITA
CIITA
Nucleus
Invariant chain
MHC II
ER
CIITA --
U6
sgRNA CIITA
EF1a
eGFP
CIITA KO + Cas9 mRNA
U6
sgRNA CIITA
EF1a
eGFP
shRNA CIITA1
U6
shRNA CIITA1
EF1a
eGFP
shRNA CIITA2
U6
shRNA CIITA2
EF1a
eGFP
shRNA CIITA3
U6
shRNA CIITA3
EF1a
eGFP

FIG. 7A

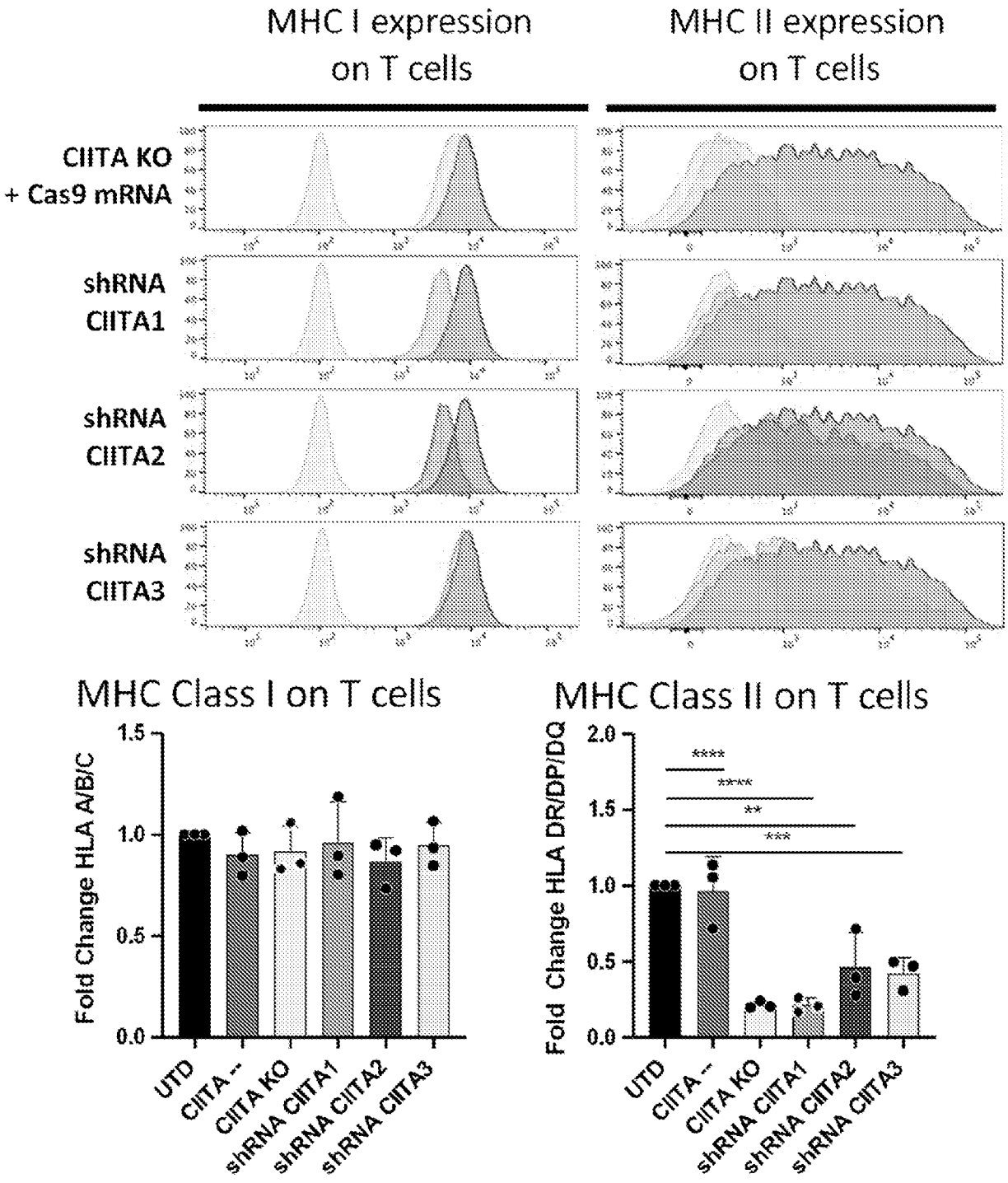
MHC I expression on T cells
MHC II expression on T cells
CIITA KO + Cas9 mRNA
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3
MHC Class I on T cells
Fold Change HLA A/B/C
1.5
1.0
0.5
0.0
UTD
CIITA --
CIITA KO
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3
MHC Class II on T cells
Fold Change HLA DR/DP/DQ
2.0
1.5
1.0
0.5
0.0
****
****
**
***
UTD
CIITA --
CIITA KO
shRNA CIITA1
shRNA CIITA2
shRNA CIITA3

FIG. 7B

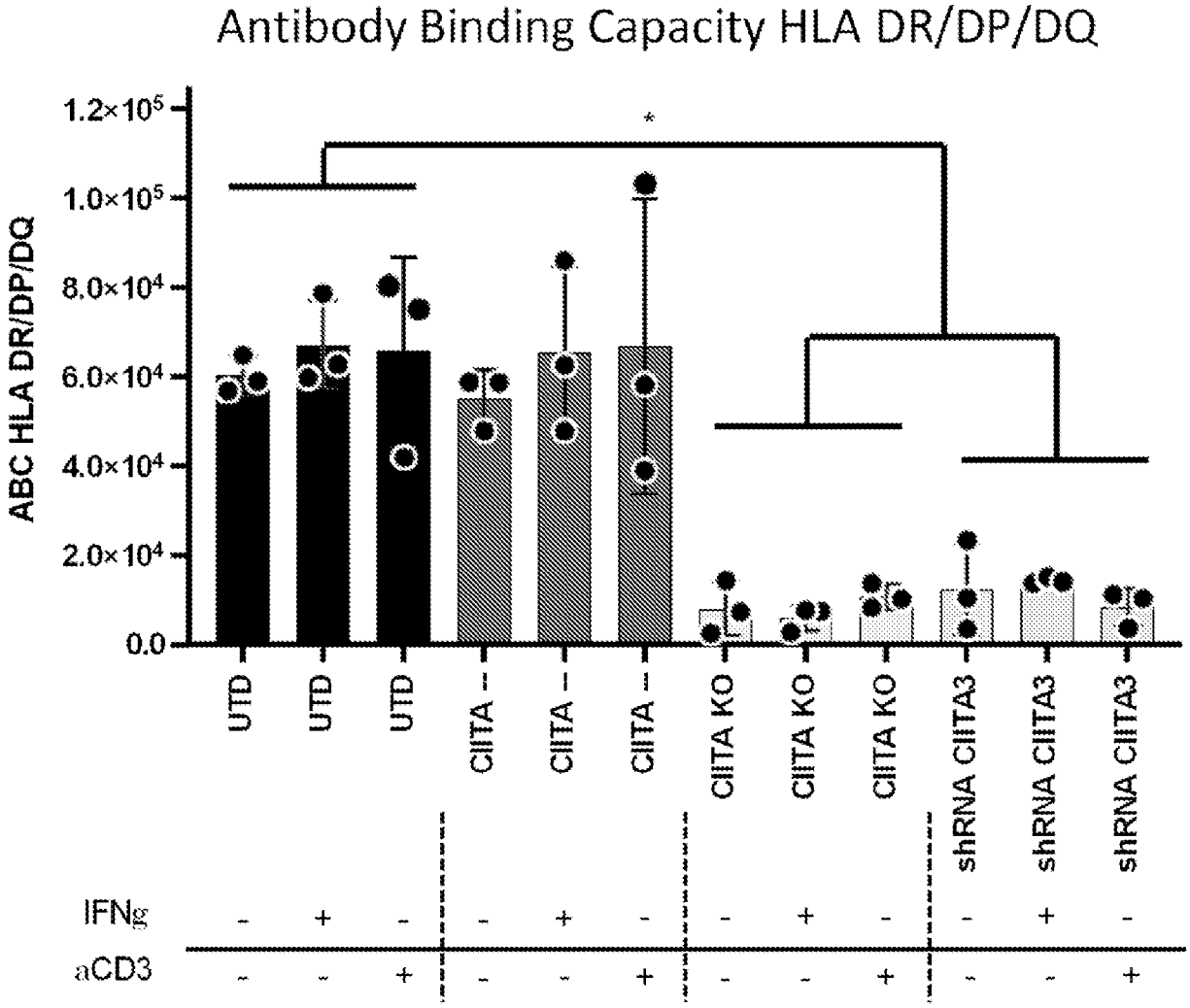
Antibody Binding Capacity HLA DR/DP/DQ
ABC HLA DR/DP/DQ
1.2×10^5
1.0×10^5
8.0×10^4
6.0×10^4
4.0×10^4
2.0×10^4
0.0
*
UTD
UTD
UTD
CIITA –
CIITA –
CIITA –
CIITA KO
CIITA KO
CIITA KO
shRNA CIITA3
shRNA CIITA3
shRNA CIITA3
IFNg
aCD3

FIG. 7C

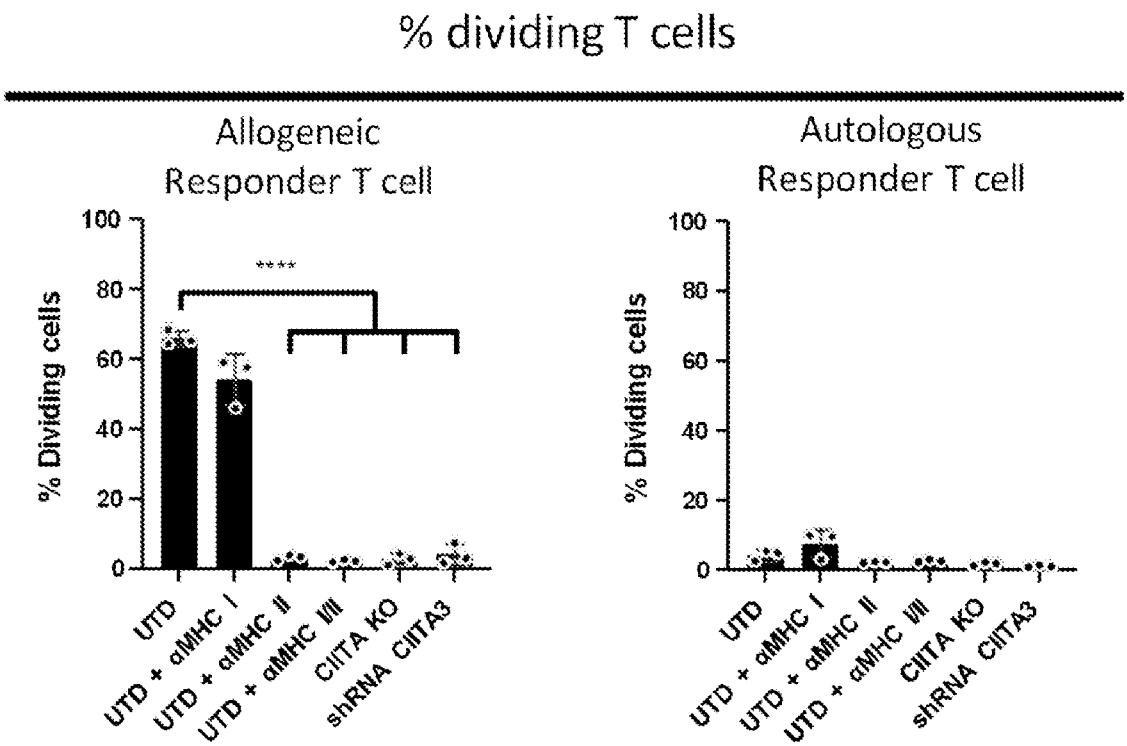
% dividing T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
% Dividing cells
100
80
60
40
20
0
****
UTD
UTD + αMHC I
UTD + αMHC II
UTD + αMHC I/II
CIITA KO
shRNA CIITA3

FIG. 7D

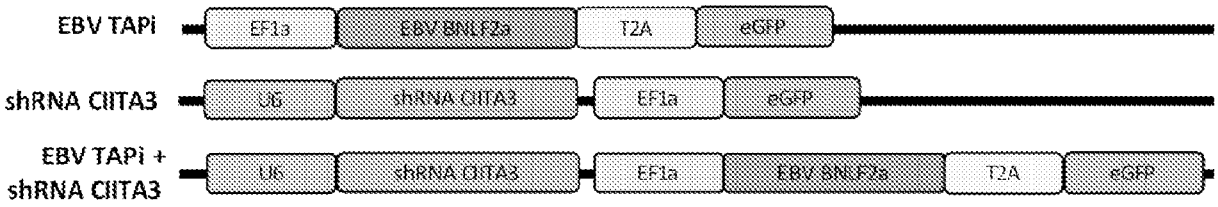
EBV TAPi
EF1a
EBV BNLF2a
T2A
eGFP
shRNA CIITA3
U6
shRNA CIITA3
EF1a
eGFP
EBV TAPi +
shRNA CIITA3
U6
shRNA CIITA3
EF1a
EBV BNLF2a
T2A
eGFP

FIG. 8A

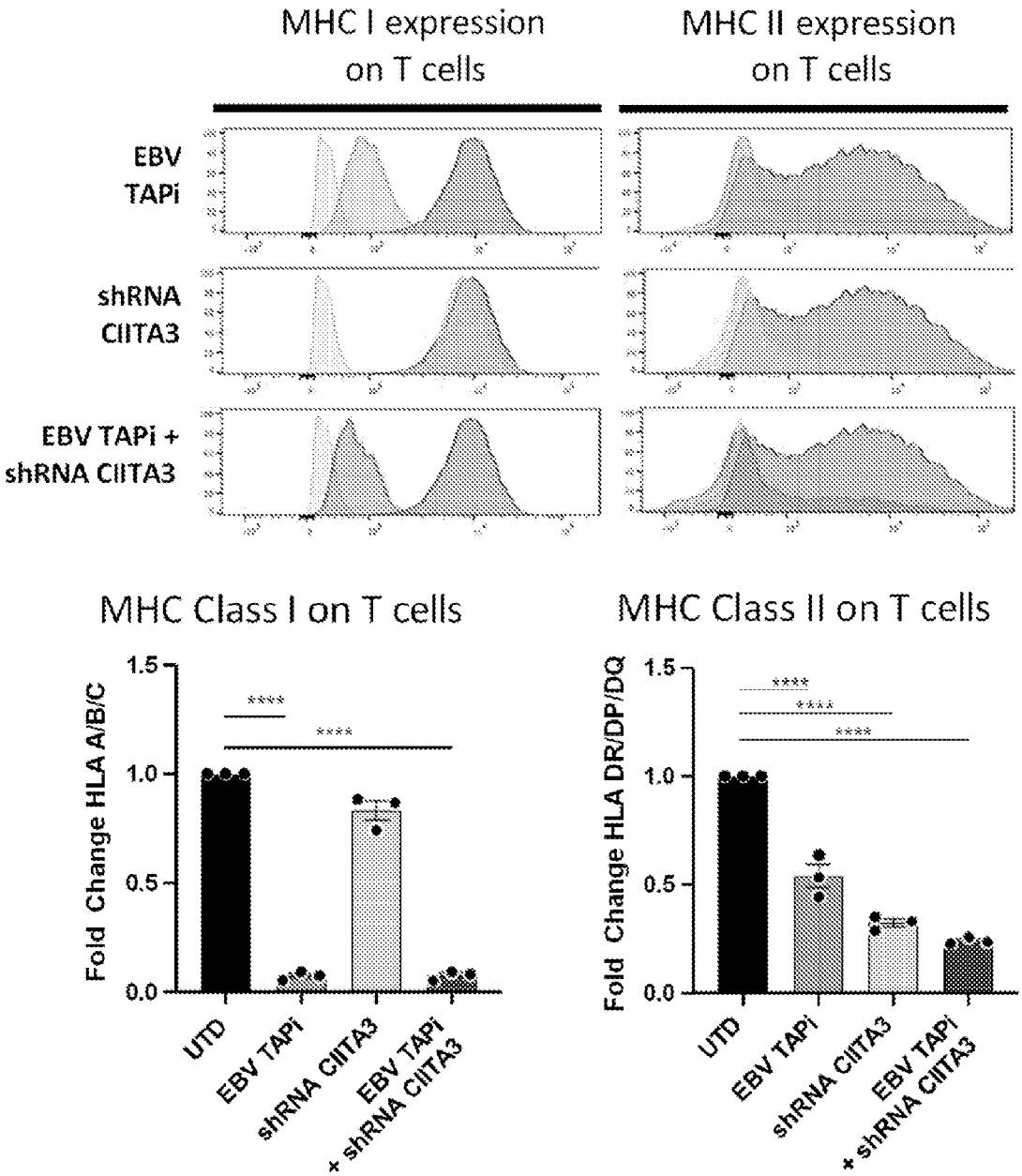
MHC I expression
on T cells
MHC II expression
on T cells
EBV
TAPi
shRNA
CIITA3
EBV TAPi +
shRNA CIITA3
MHC Class I on T cells
MHC Class II on T cells
Fold Change HLA A/B/C
Fold Change HLA DR/DP/DQ
1.5
1.0
0.5
0.0
****
UTD
EBV TAPi
shRNA CIITA3
EBV TAPi
+ shRNA CIITA3

FIG. 8B

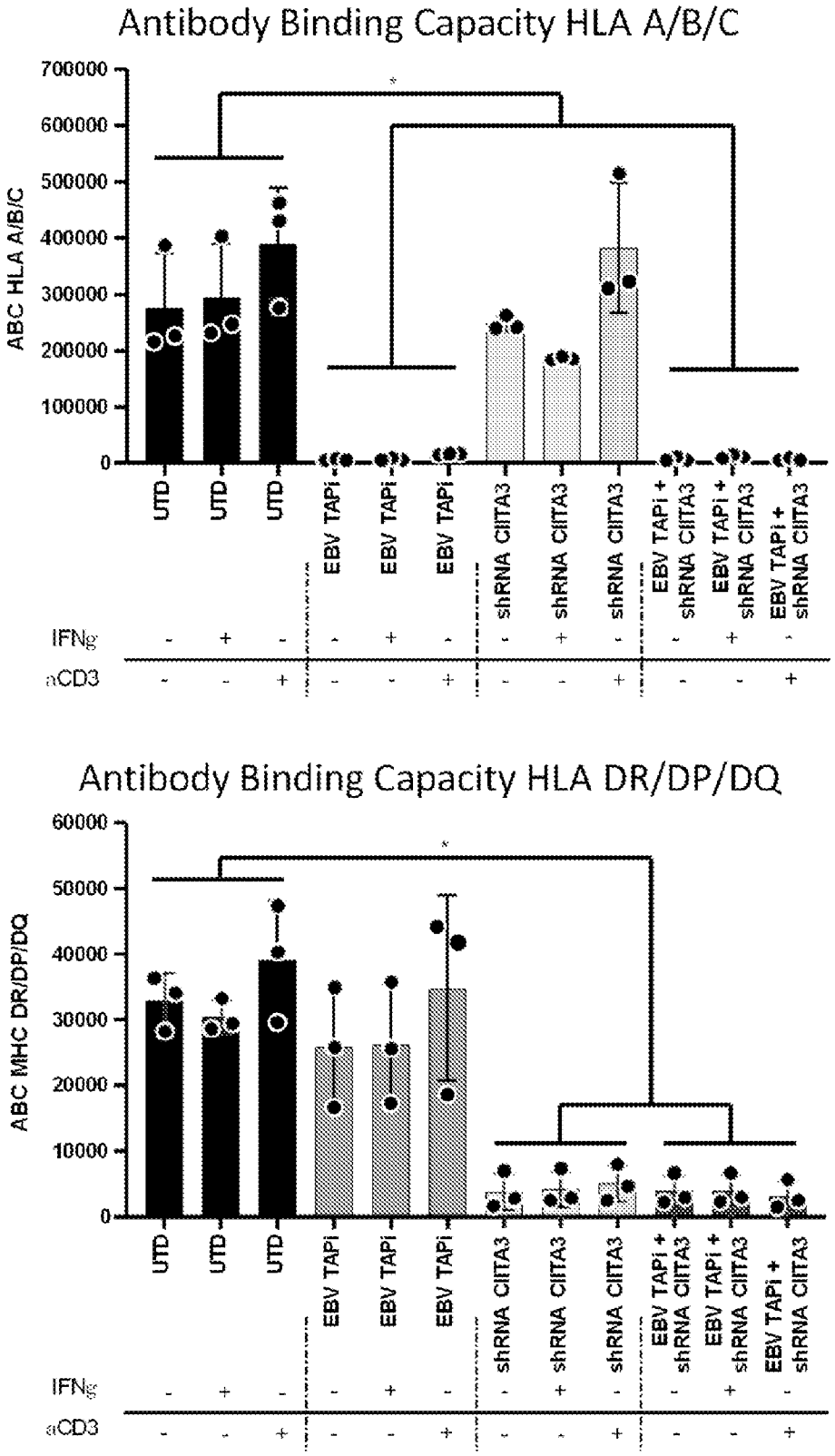
Antibody Binding Capacity HLA A/B/C
ABC HLA A/B/C
700000
600000
500000
400000
300000
200000
100000
0
UTD
UTD
UTD
EBV TAPi
EBV TAPi
EBV TAPi
shRNA CIITA3
shRNA CIITA3
shRNA CIITA3
EBV TAPi + shRNA CIITA3
EBV TAPi + shRNA CIITA3
EBV TAPi + shRNA CIITA3
IFNg
aCD3
Antibody Binding Capacity HLA DR/DP/DQ
ABC MHC DR/DP/DQ
60000
50000
40000
30000
20000
10000
0

FIG. 8C

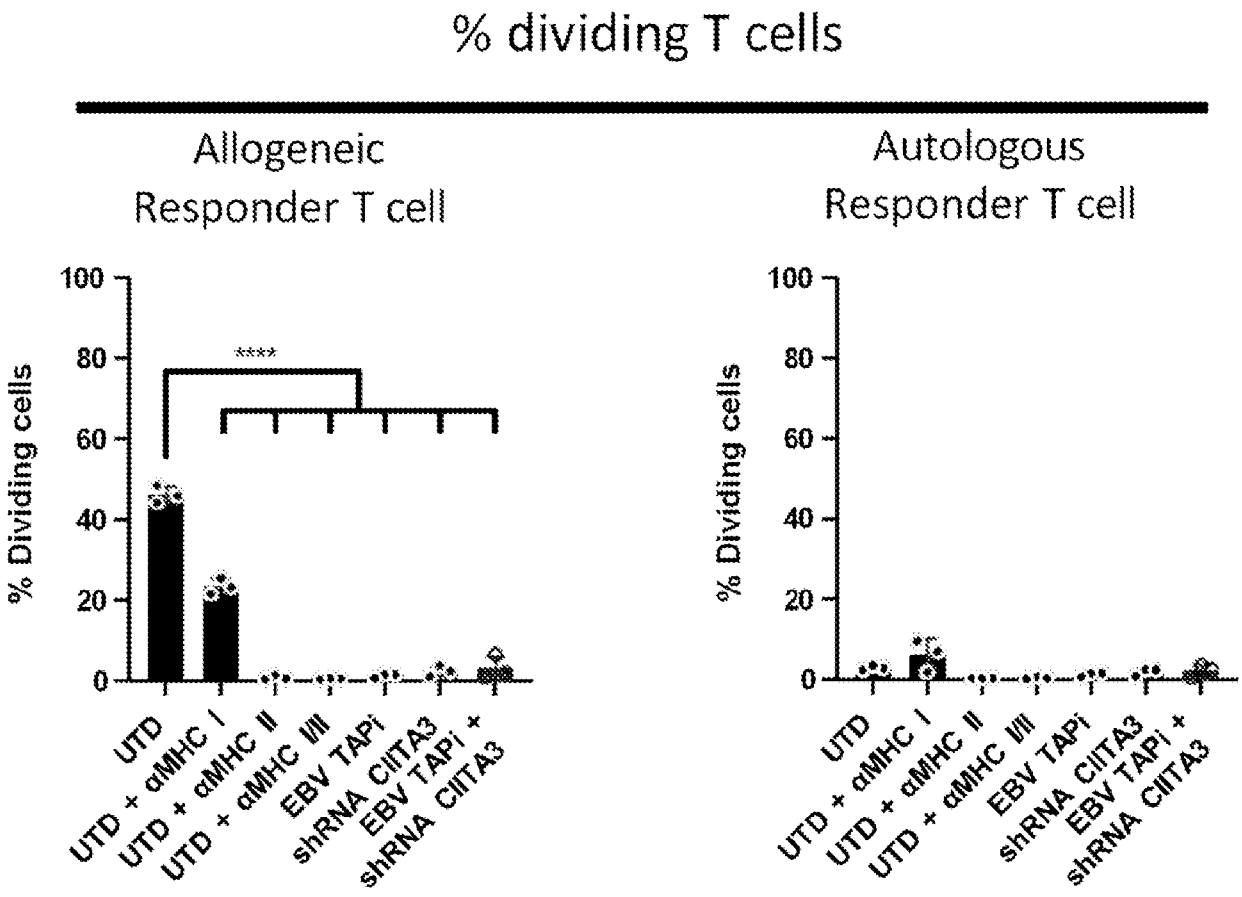
% dividing T cells
Allogeneic
Responder T cell
Autologous
Responder T cell
% Dividing cells
100
80
60
40
20
0
****
UTD
UTD + αMHC I
UTD + αMHC II
UTD + αMHC I/II
EBV TAPi
shRNA CIITA3
EBV TAPi + shRNA CIITA3

FIG. 8D

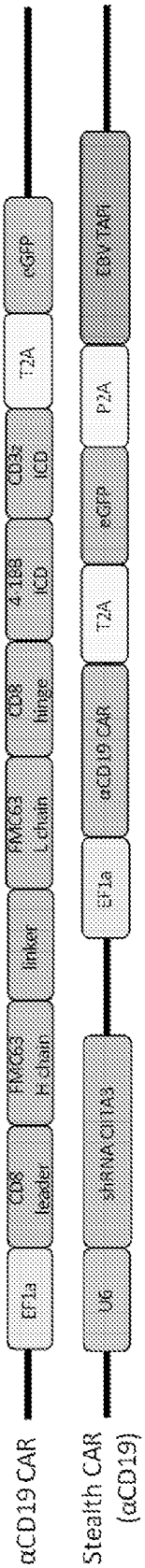
αCD19 CAR
EF1a
CD8 leader
FMC63 H chain
linker
FMC63 L chain
CD8 hinge
4-1BB ICD
CD3ζ ICD
T2A
rGFP
Stealth CAR (αCD19)
U6
shRNA CIITA
EF1a
αCD19 CAR
T2A
eGFP
P2A
EBV TAPI

FIG. 9A

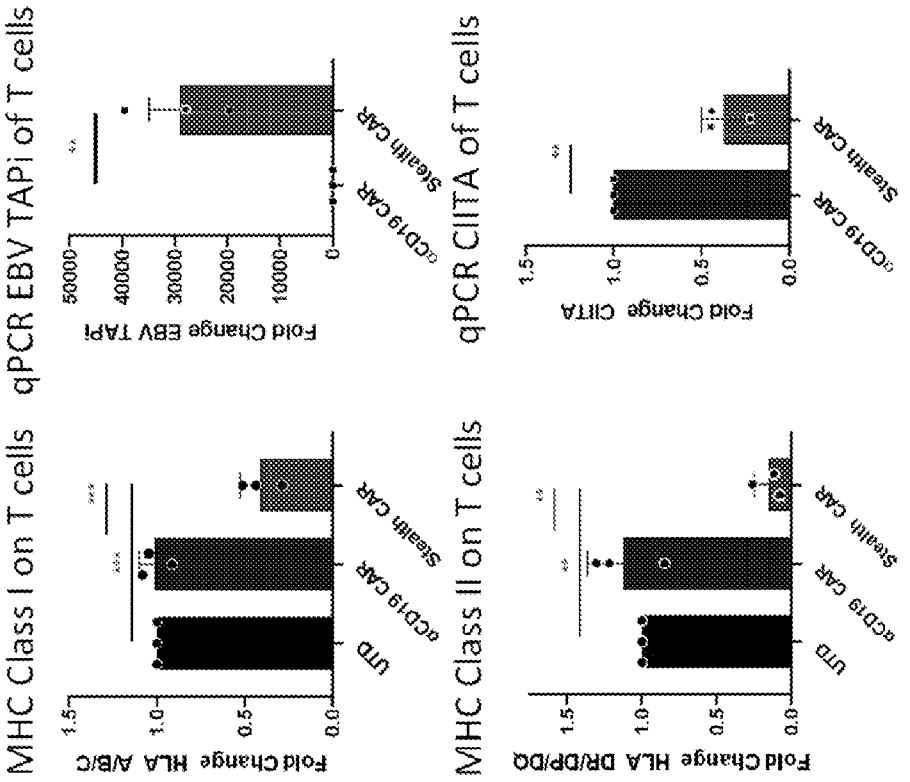
MHC Class I on T cells
qPCR EBV TAPi of T cells
Fold Change HLA A/B/C
Fold Change EBV TAPI
UTD
αCD19 CAR
Stealth CAR
MHC Class II on T cells
qPCR CIITA of T cells
Fold Change HLA DR/DP/DQ
Fold Change CIITA

FIG. 9B

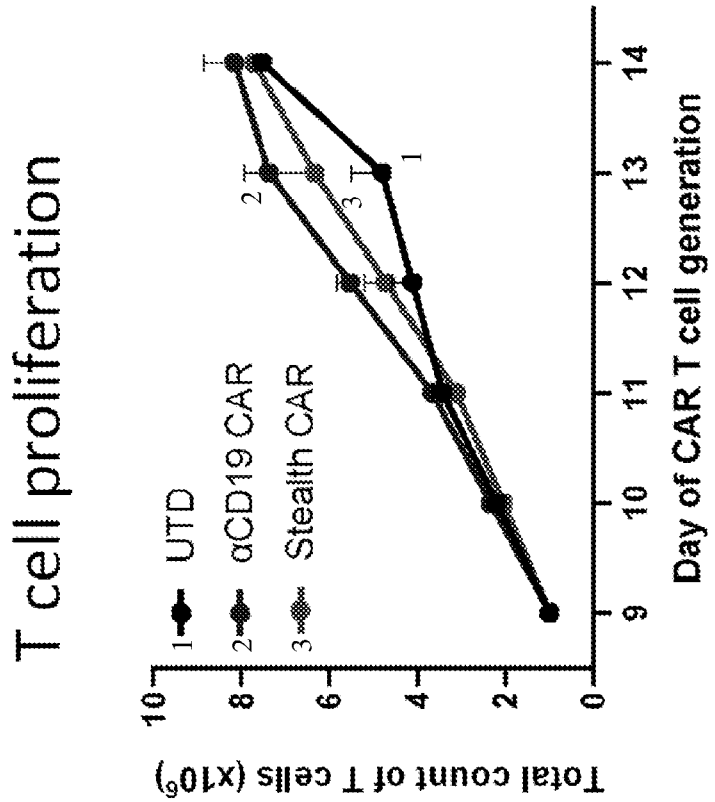
T cell proliferation
UTD
αCD19 CAR
Stealth CAR
Total count of T cells (x10⁶)
Day of CAR T cell generation

FIG. 9D

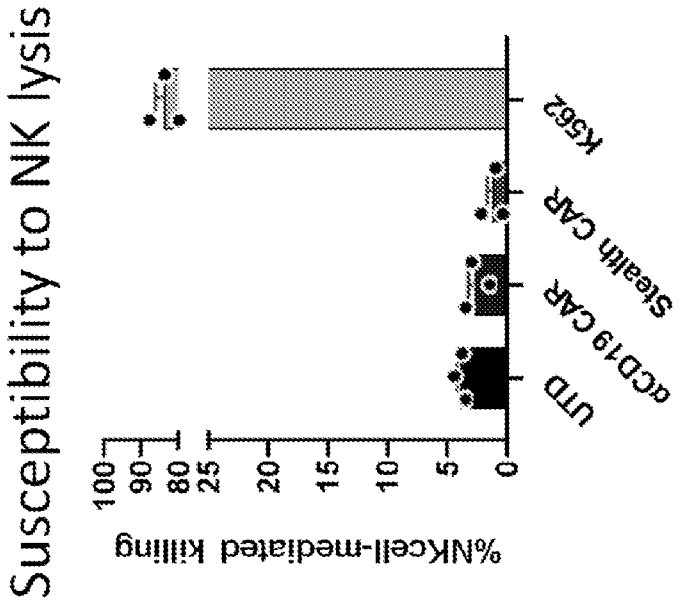
Susceptibility to NK lysis
%NKcell-mediated killing
UTD
αCD19 CAR
Stealth CAR
K562

FIG. 9C

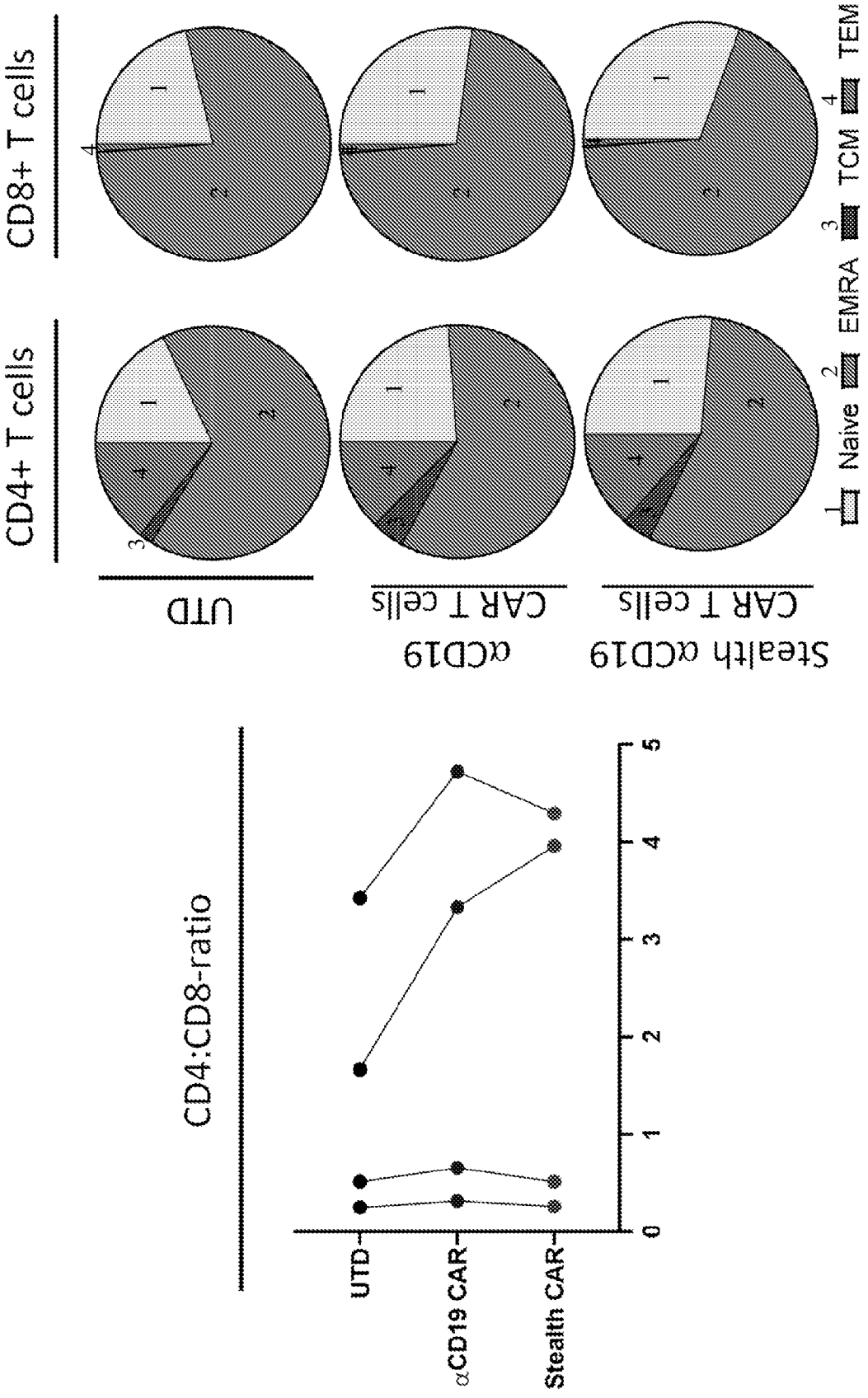
CD4+ T cells
CD8+ T cells
UTD
αCD19 CAR T cells
Stealth αCD19 CAR T cells
1 Naive
2 EMRA
3 TCM
4 TEM
CD4:CD8-ratio
UTD
αCD19 CAR
Stealth CAR
0
1
2
3
4
5

FIG. 9E

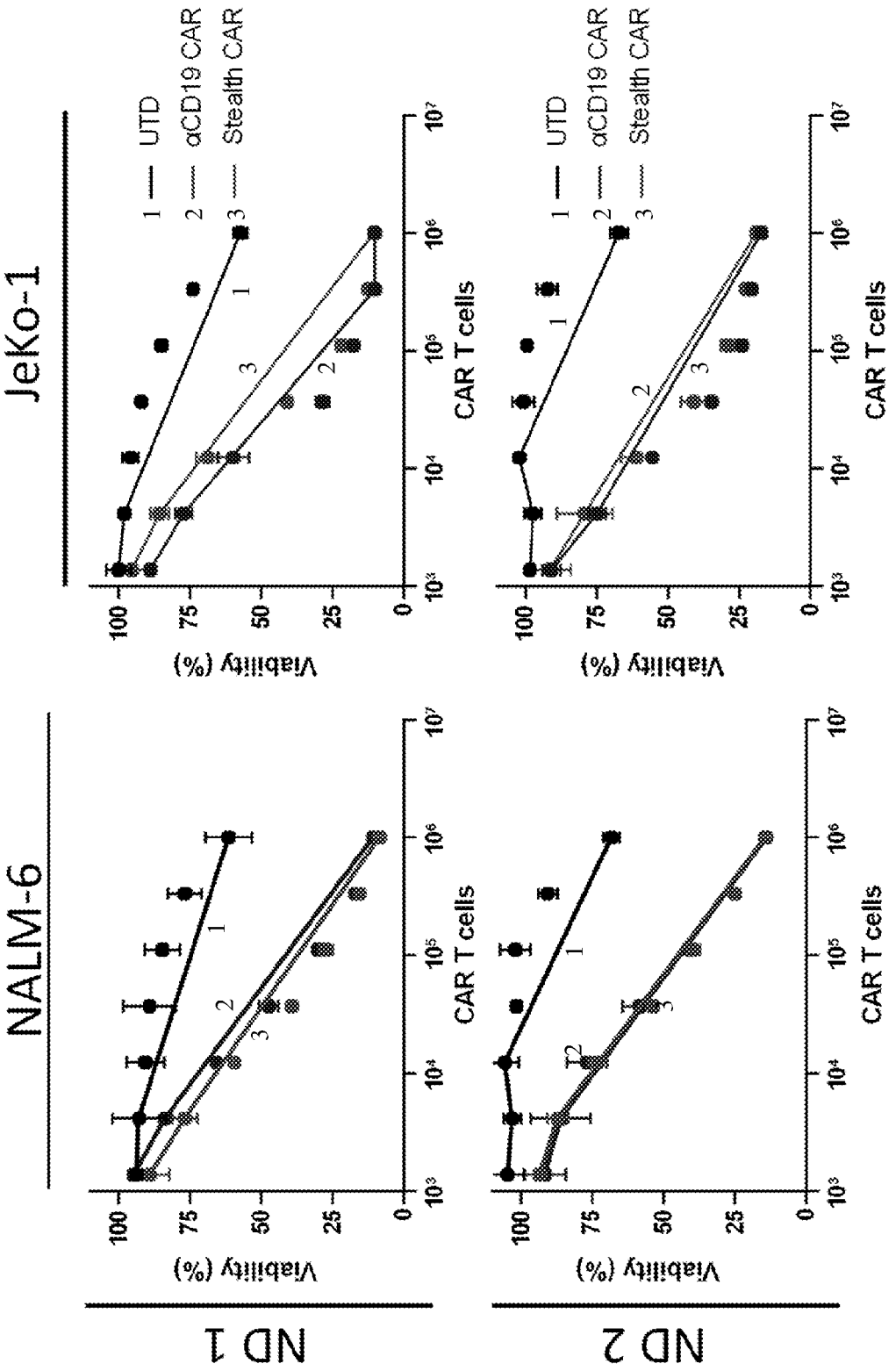
JeKo-1
NALM-6
ND 1
ND 2
UTD
αCD19 CAR
Stealth CAR
Viability (%)
CAR T cells
100
75
50
25
0
10^3
10^4
10^5
10^6
10^7
1
2
3

FIG. 9F

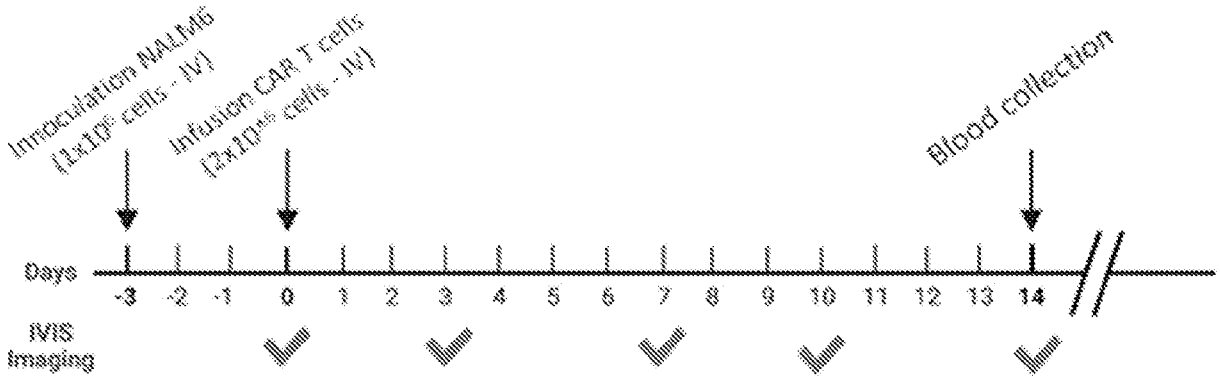

FIG. 10A

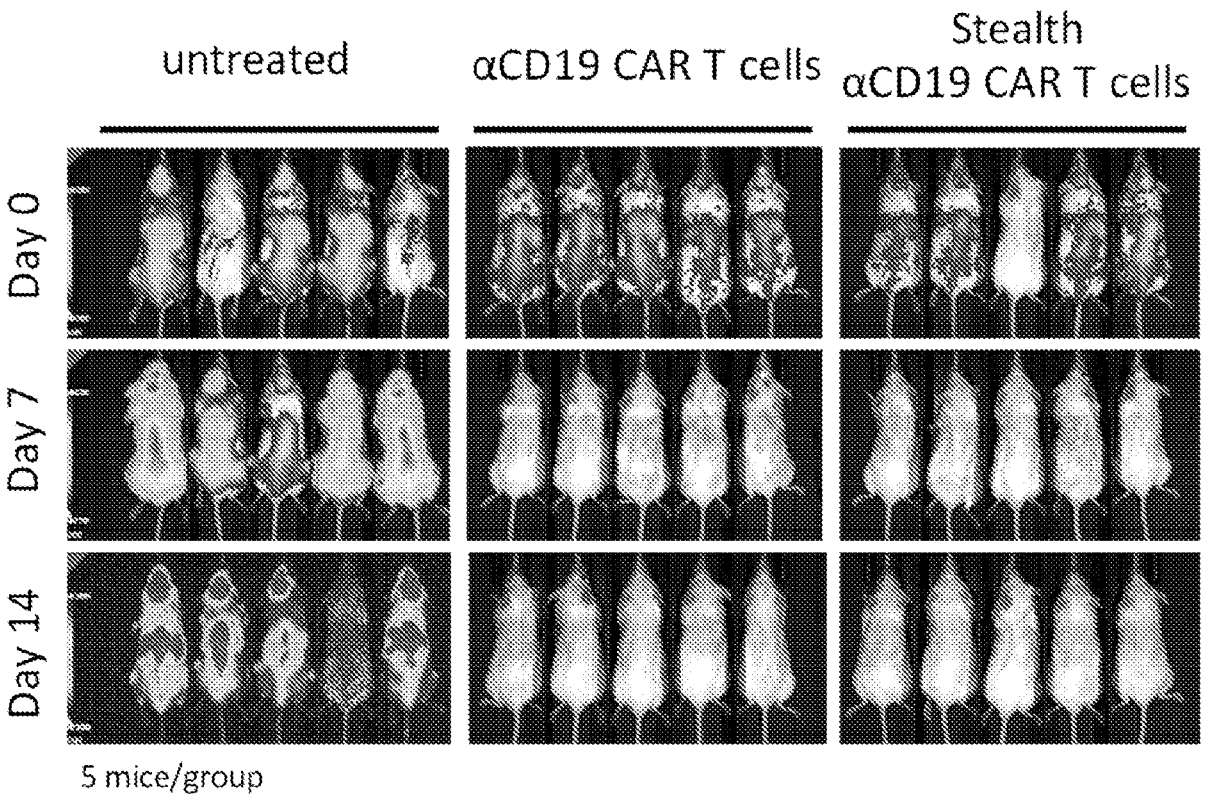

FIG. 10B

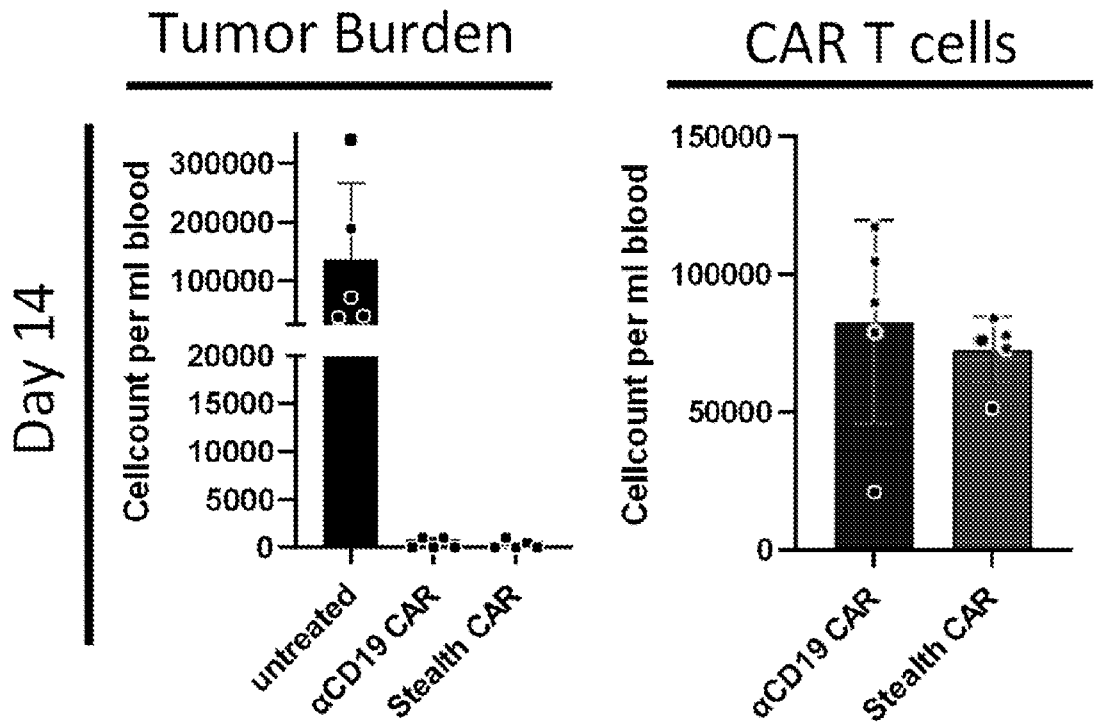

FIG. 10C

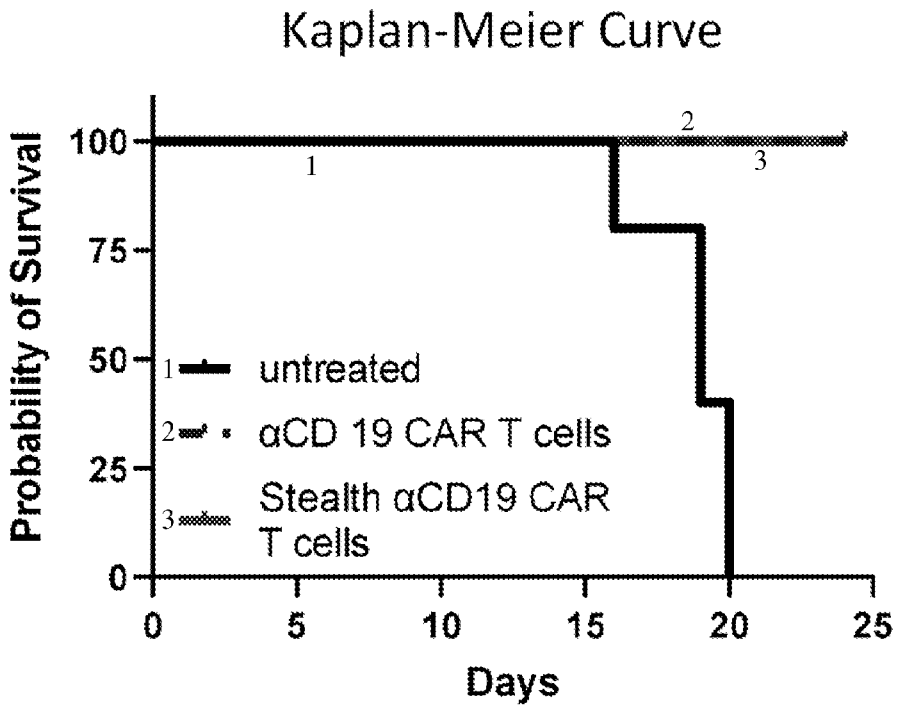

FIG. 10D

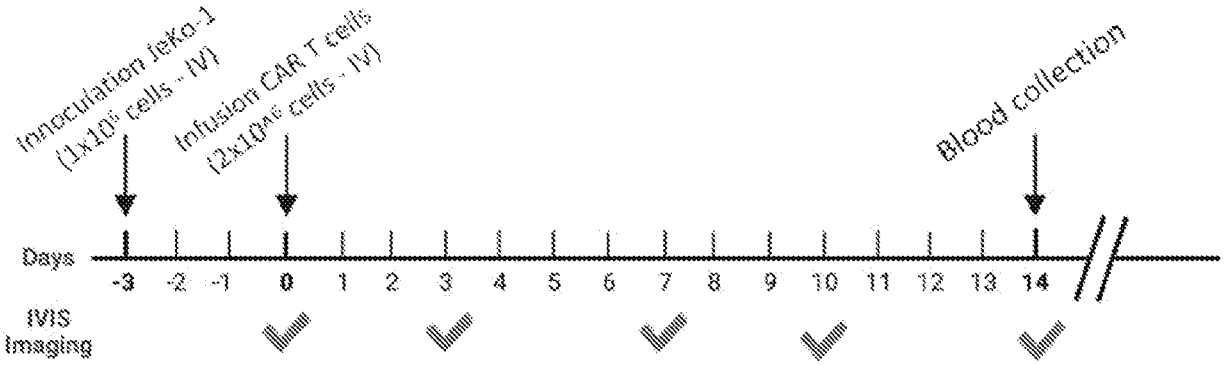

FIG. 10E

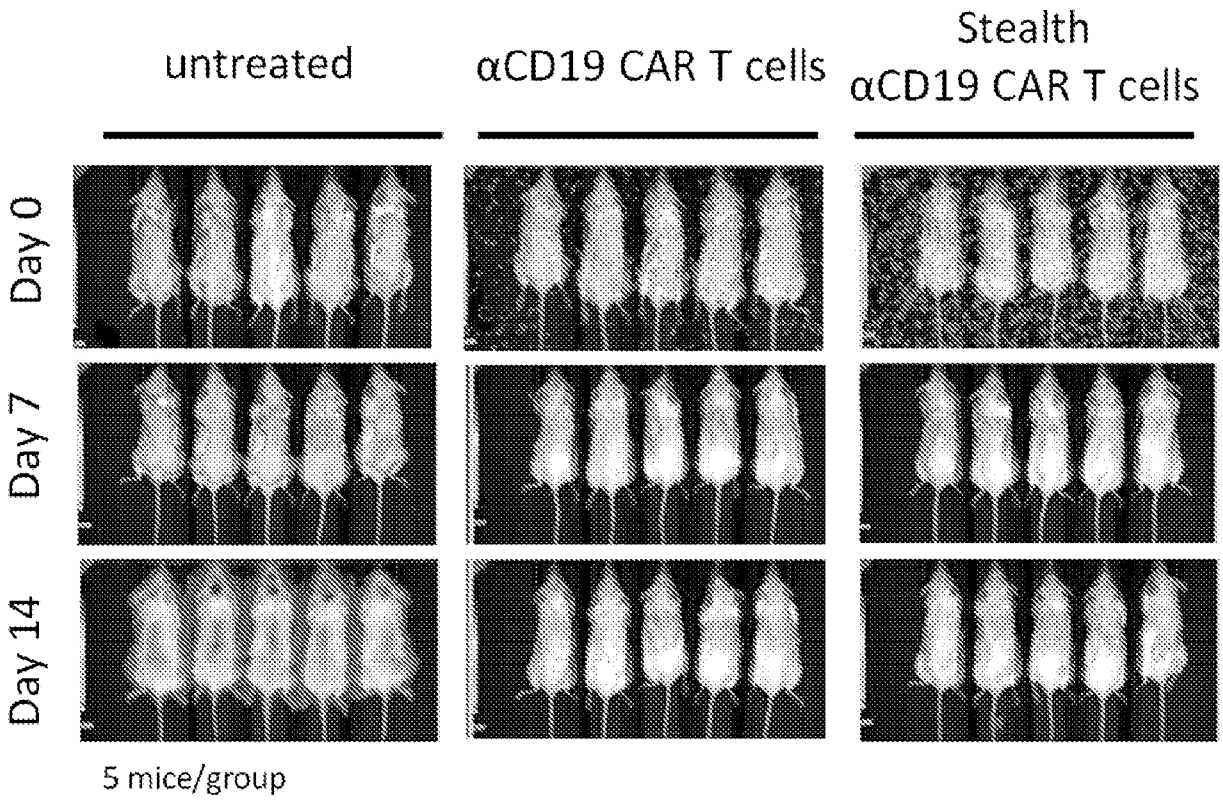

FIG. 10F

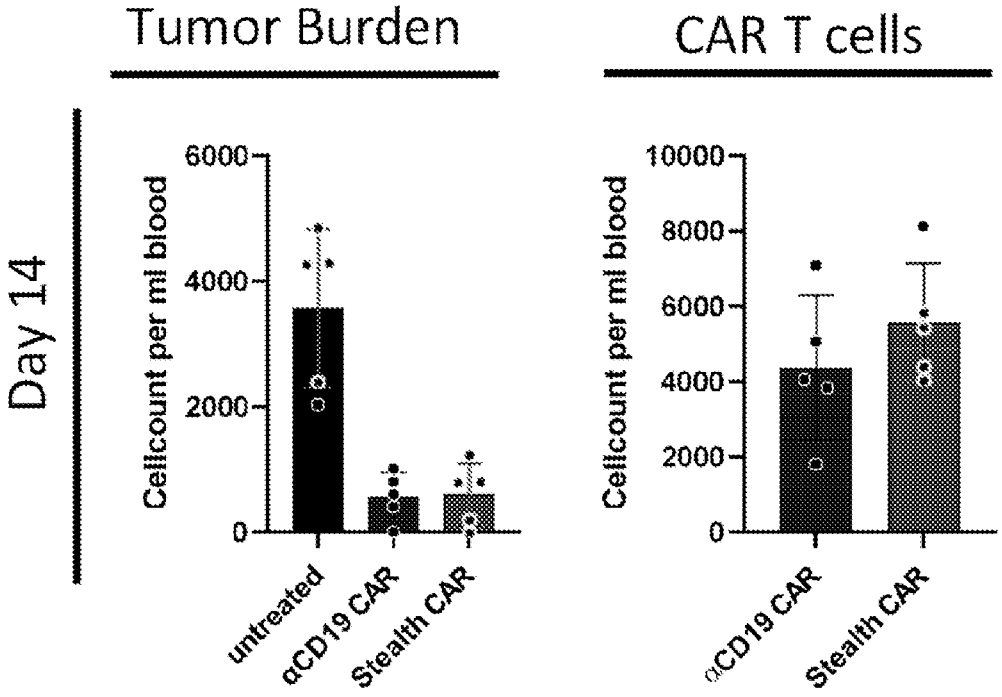

FIG. 10G

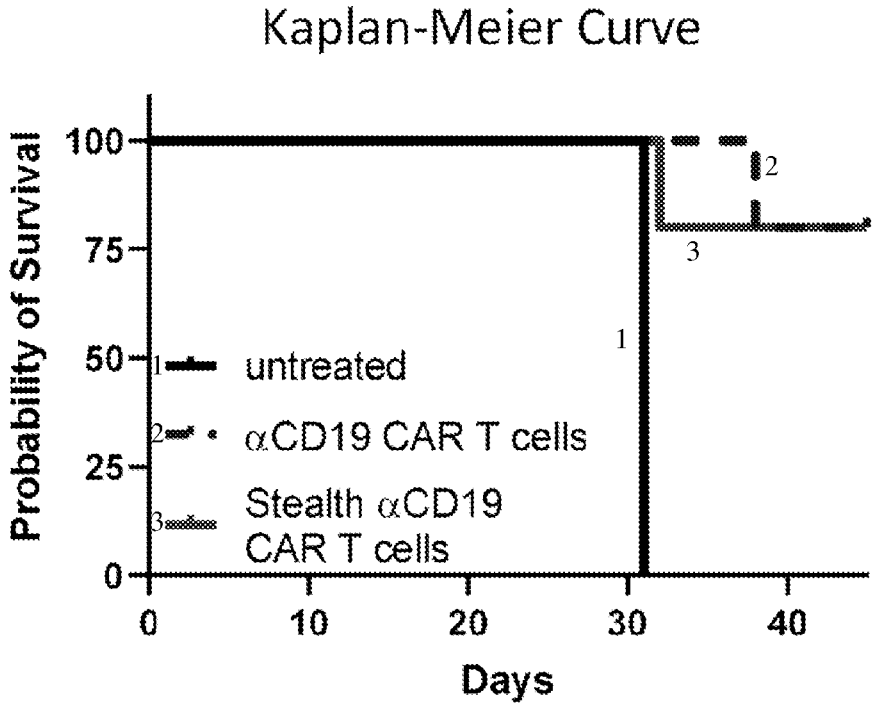

FIG. 10H

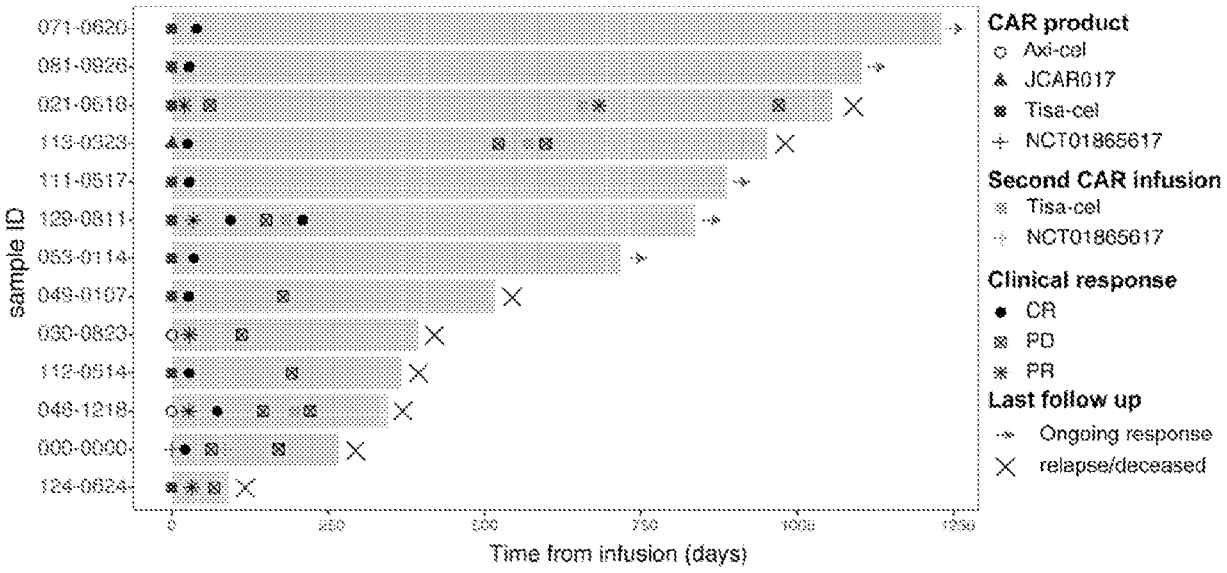

FIG. 11A

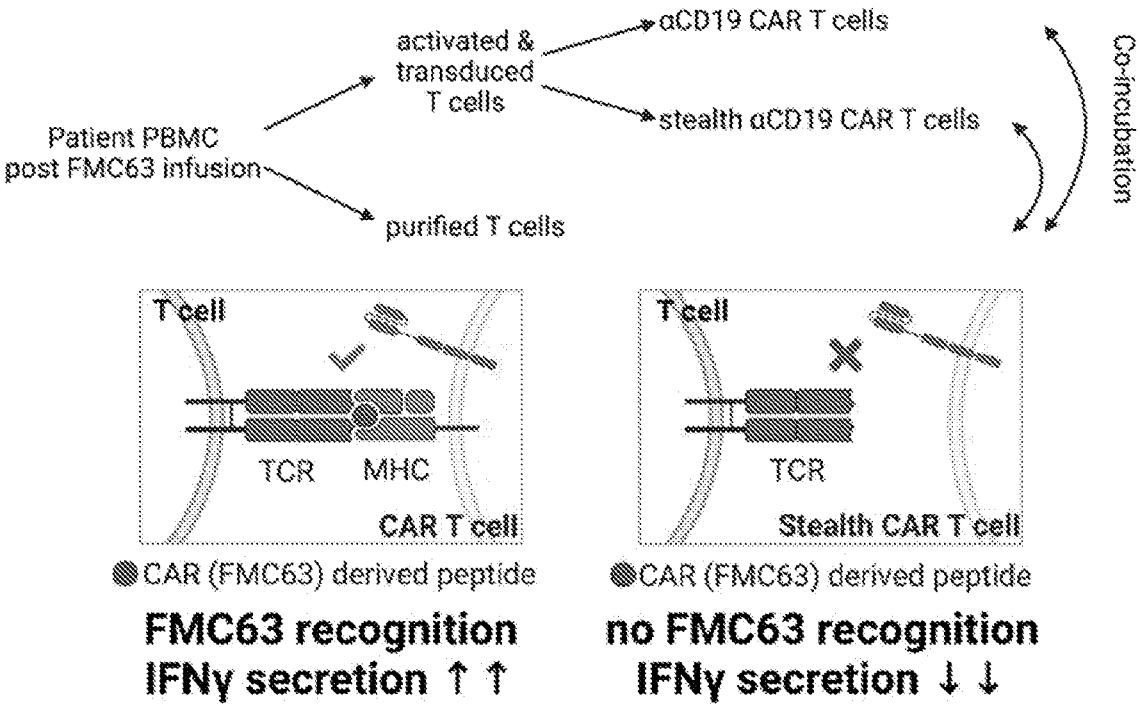

FIG. 11B

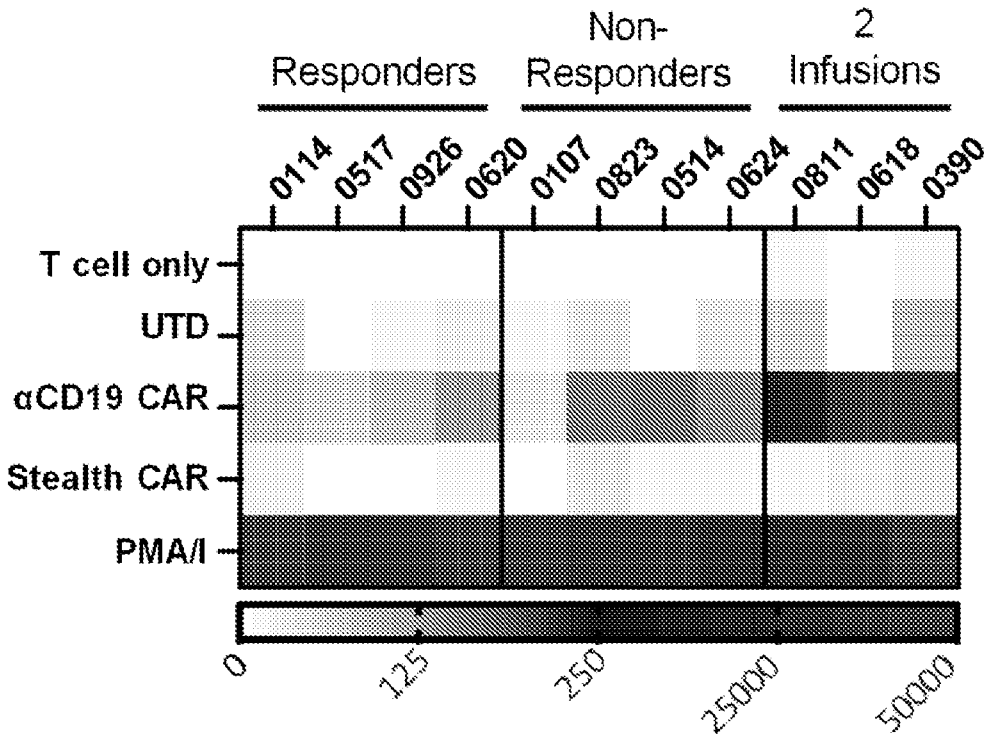

FIG. 11C

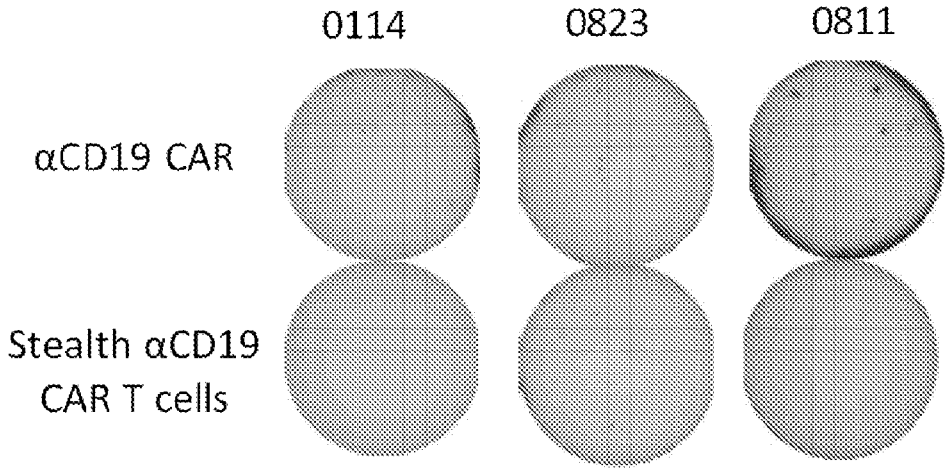

FIG. 11D

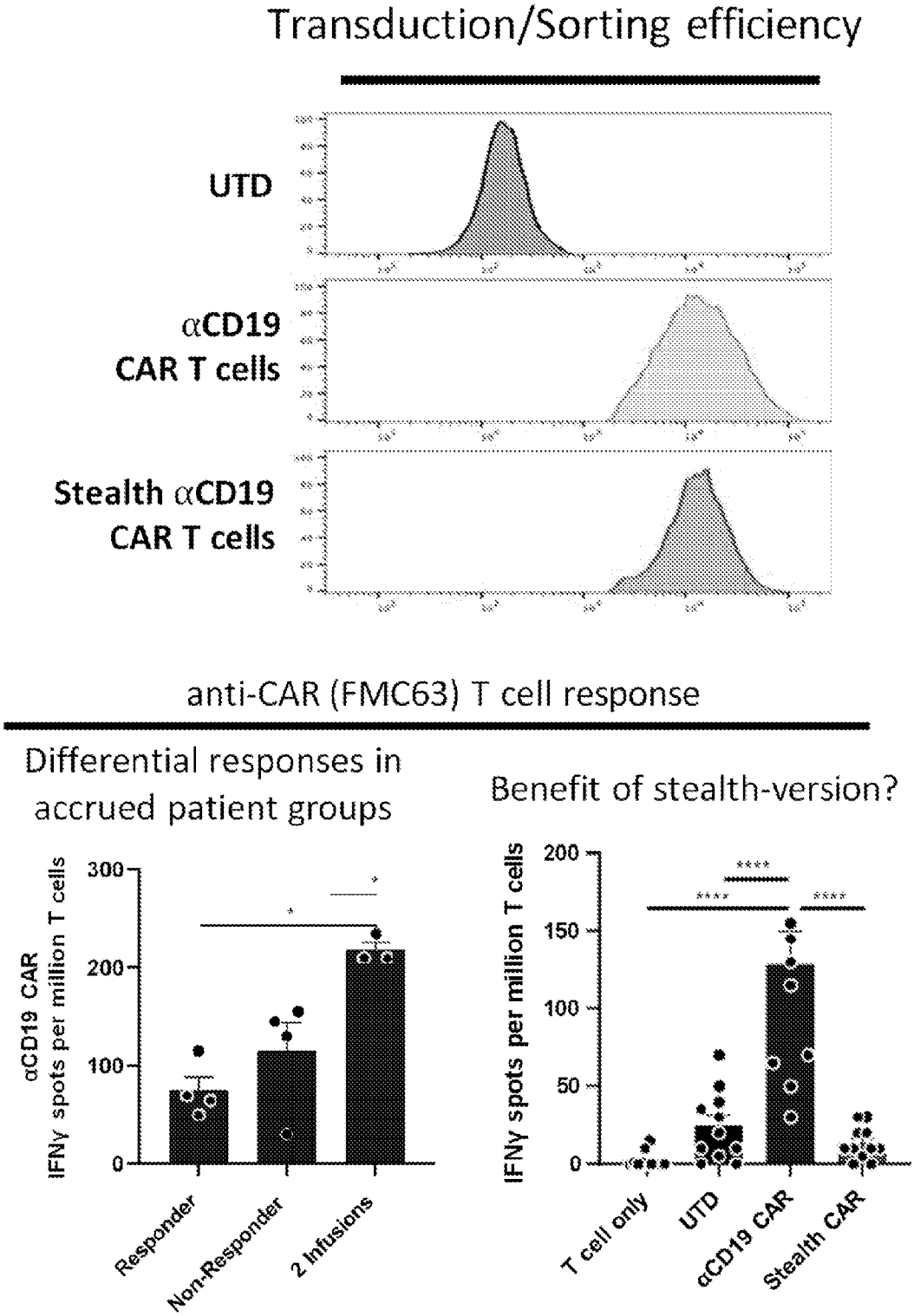
Transduction/Sorting efficiency
UTD
αCD19 CAR T cells
Stealth αCD19 CAR T cells
anti-CAR (FMC63) T cell response
Differential responses in accrued patient groups
αCD19 CAR IFNγ spots per million T cells
300
200
100
0
Responder
Non-Responder
2 Infusions
Benefit of stealth-version?
IFNγ spots per million T cells
200
150
100
50
0
****
****
****
T cell only
UTD
αCD19 CAR
Stealth CAR

FIG. 11E

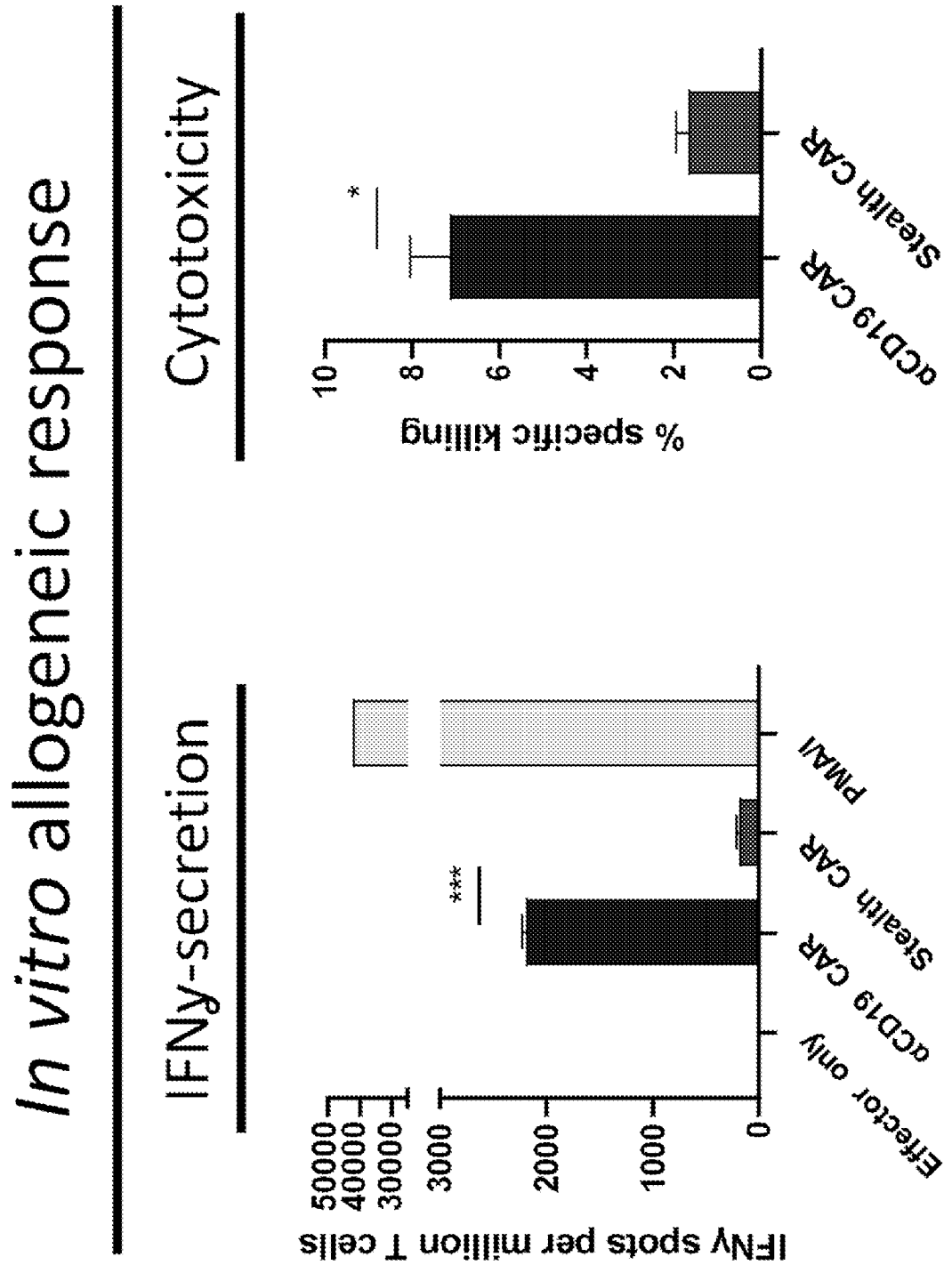
In vitro allogeneic response
IFNγ-secretion
IFNγ spots per million T cells
50000
40000
30000
3000
2000
1000
0
***
Effector only
αCD19 CAR
Stealth CAR
PMA/I
Cytotoxicity
% specific killing
10
8
6
4
2
0
*
αCD19 CAR
Stealth CAR

FIG. 12A

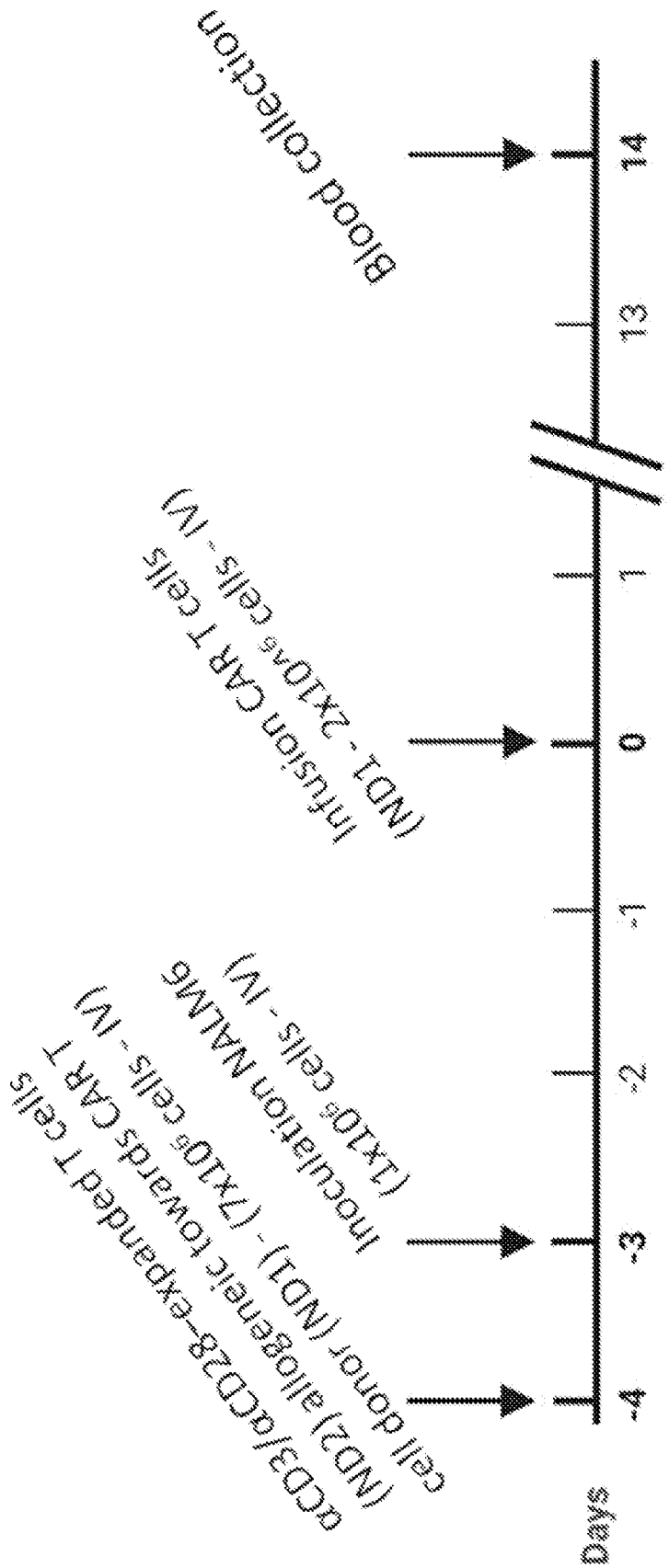
Days
-4
-3
-2
-1
0
1
13
14
10 mice/group
αCD3/αCD28–expanded T cells
(ND2) allogeneic towards CAR T
cell donor (ND1) - (7x10⁶ cells - IV)
Inoculation NALM6
(1x10⁶ cells - IV)
Infusion CAR T cells
(ND1 - 2x10⁶ cells - IV)
Blood collection

FIG. 12B

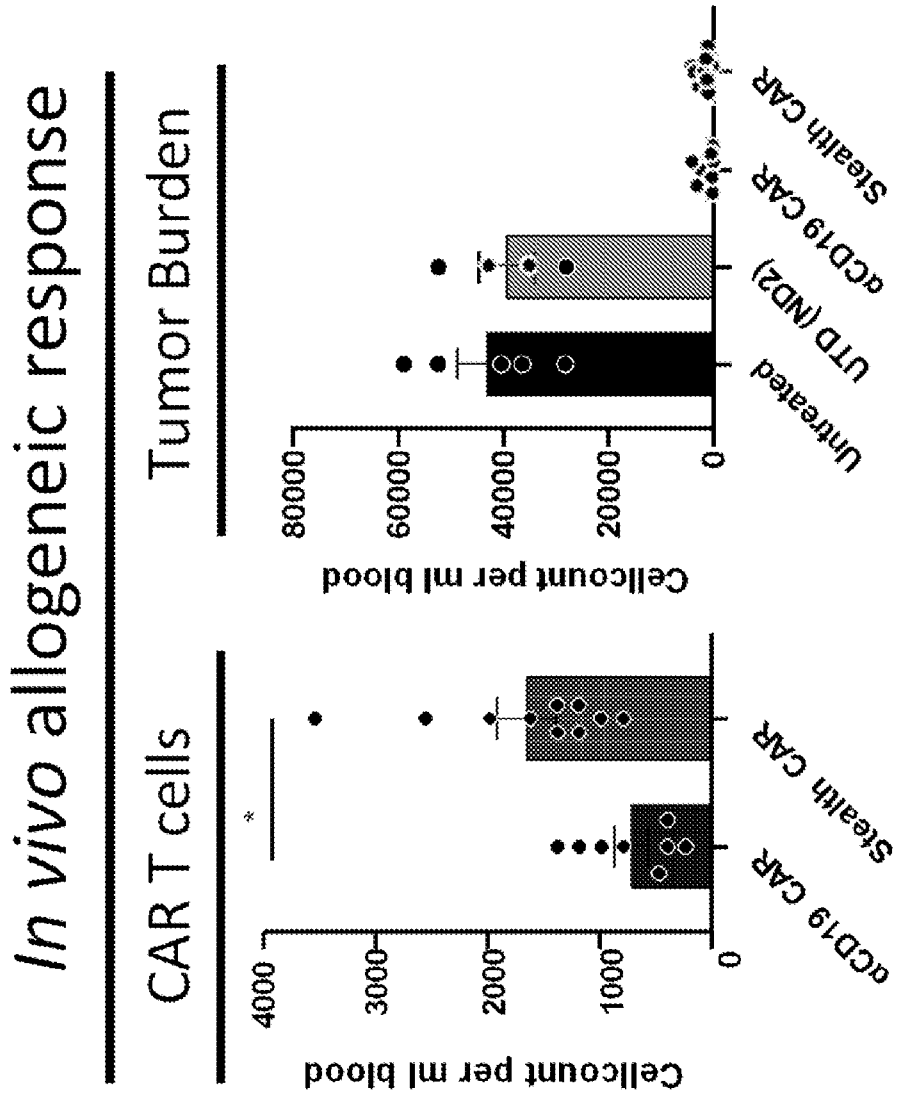
In vivo allogeneic response
CAR T cells
Tumor Burden
Cellcount per ml blood
4000
3000
2000
1000
0
*
αCD19 CAR
Stealth CAR
Cellcount per ml blood
80000
60000
40000
20000
0
Untreated
UTD (ND2)
αCD19 CAR
Stealth CAR

FIG. 12C

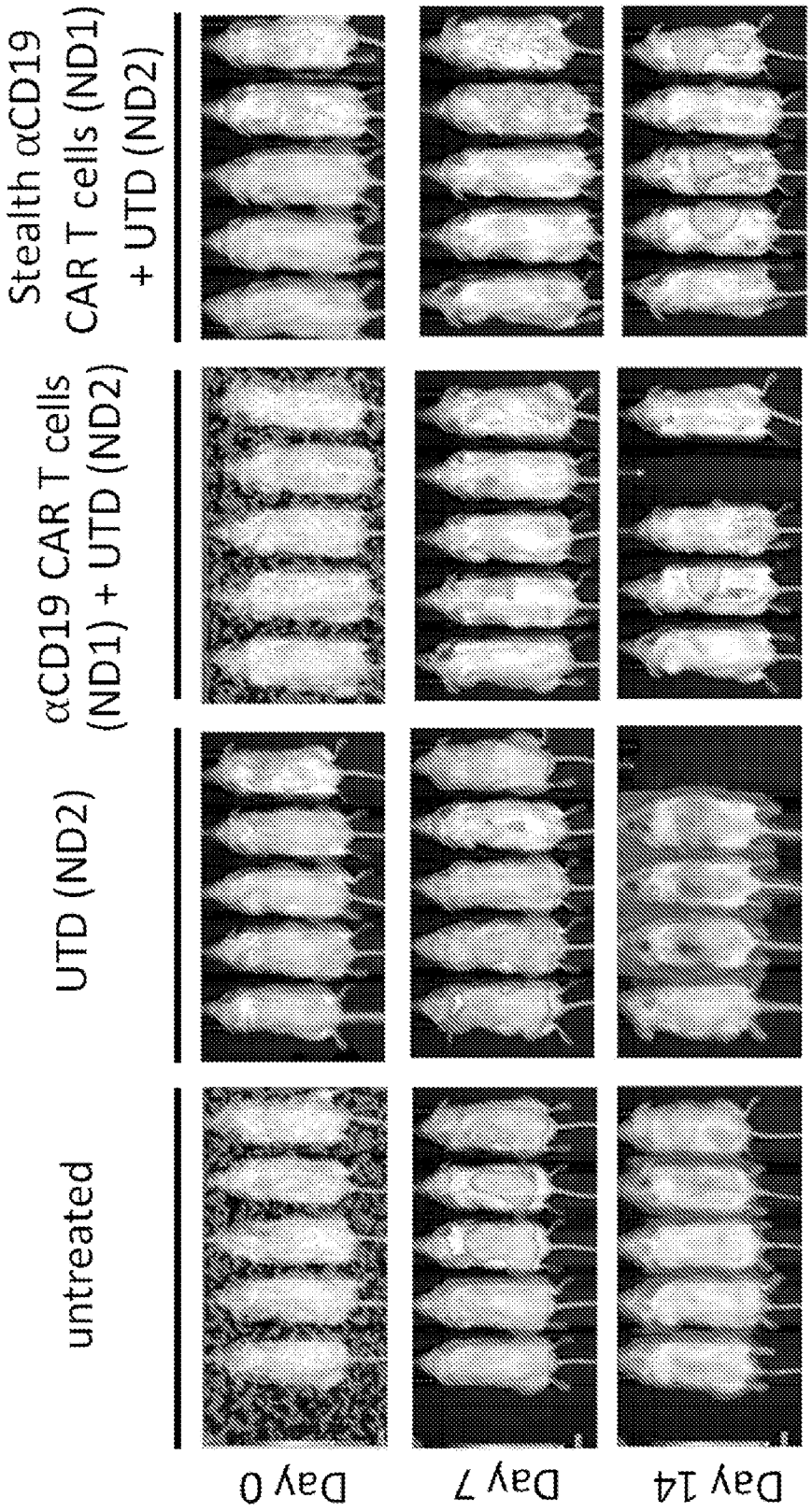
untreated
UTD (ND2)
αCD19 CAR T cells (ND1) + UTD (ND2)
Stealth αCD19 CAR T cells (ND1) + UTD (ND2)
Day 0
Day 7
Day 14

FIG. 12D

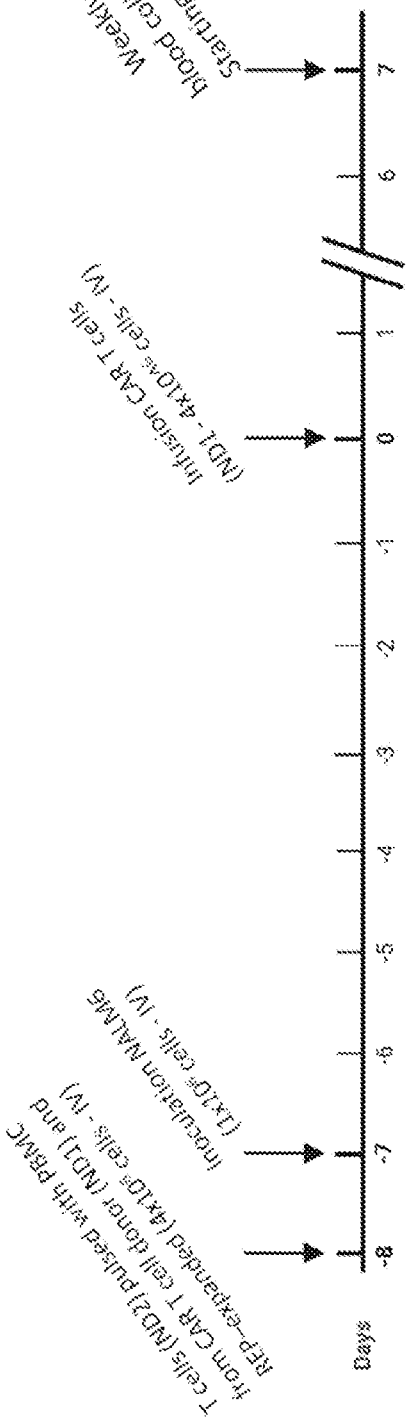

FIG. 12E

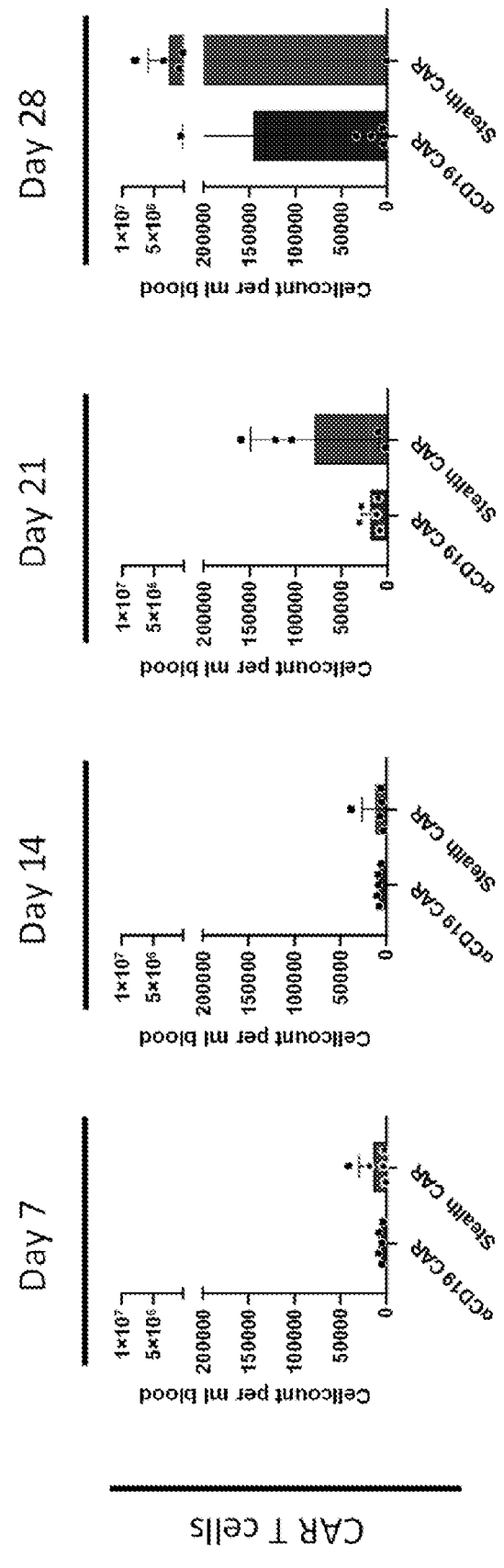

FIG. 12F

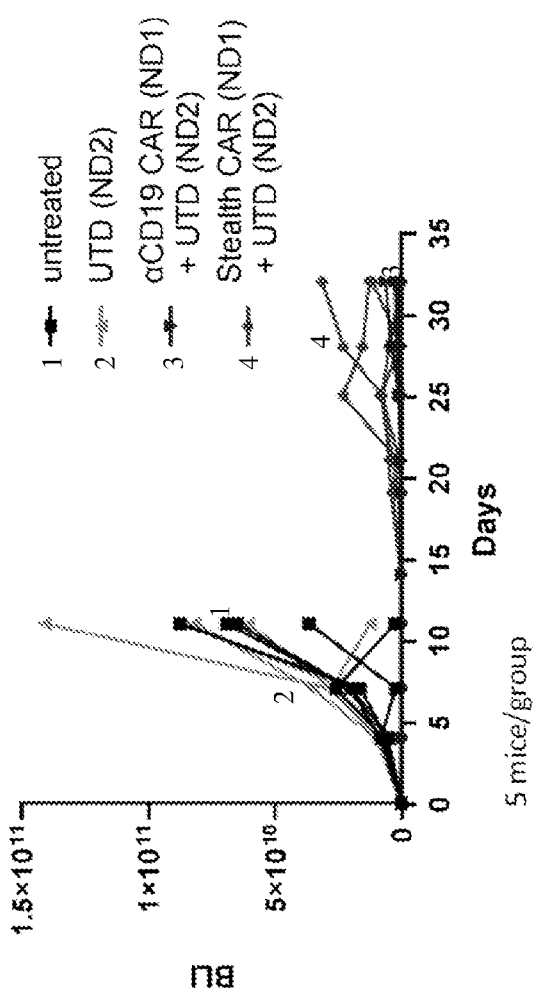

FIG. 12G

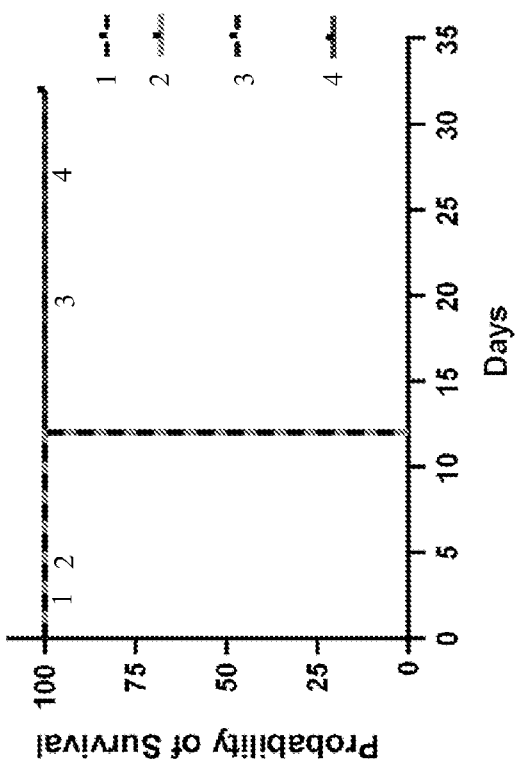

FIG. 12H

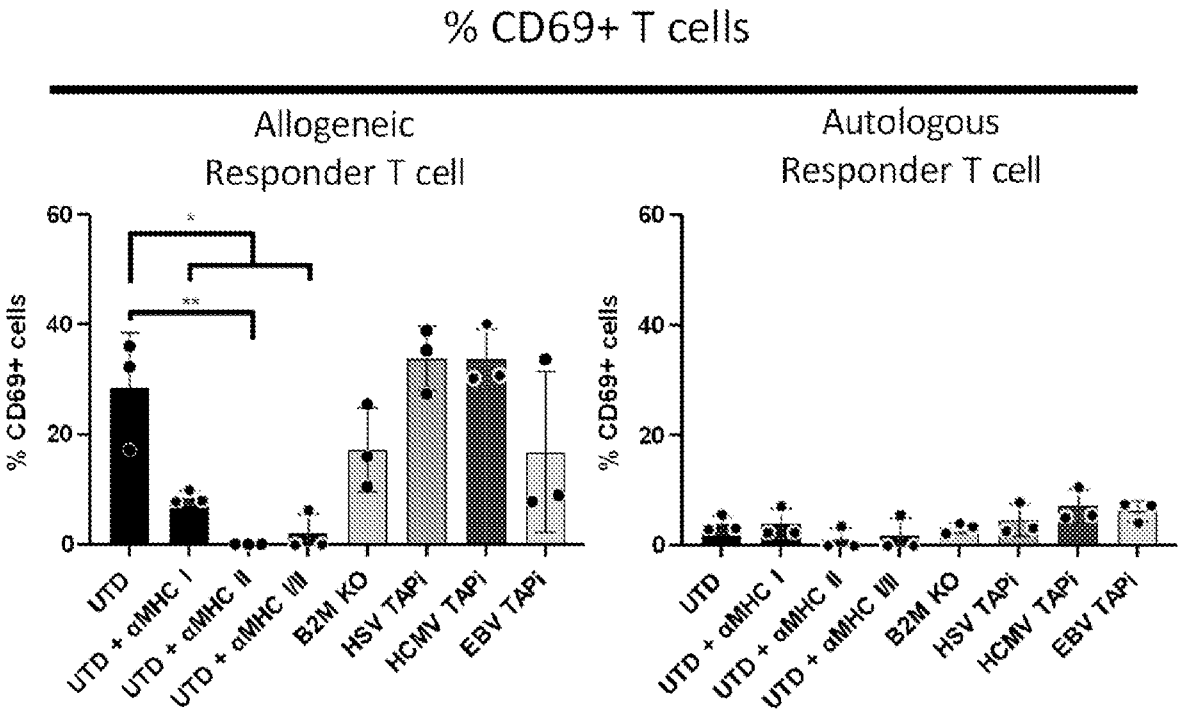

FIG. 13A

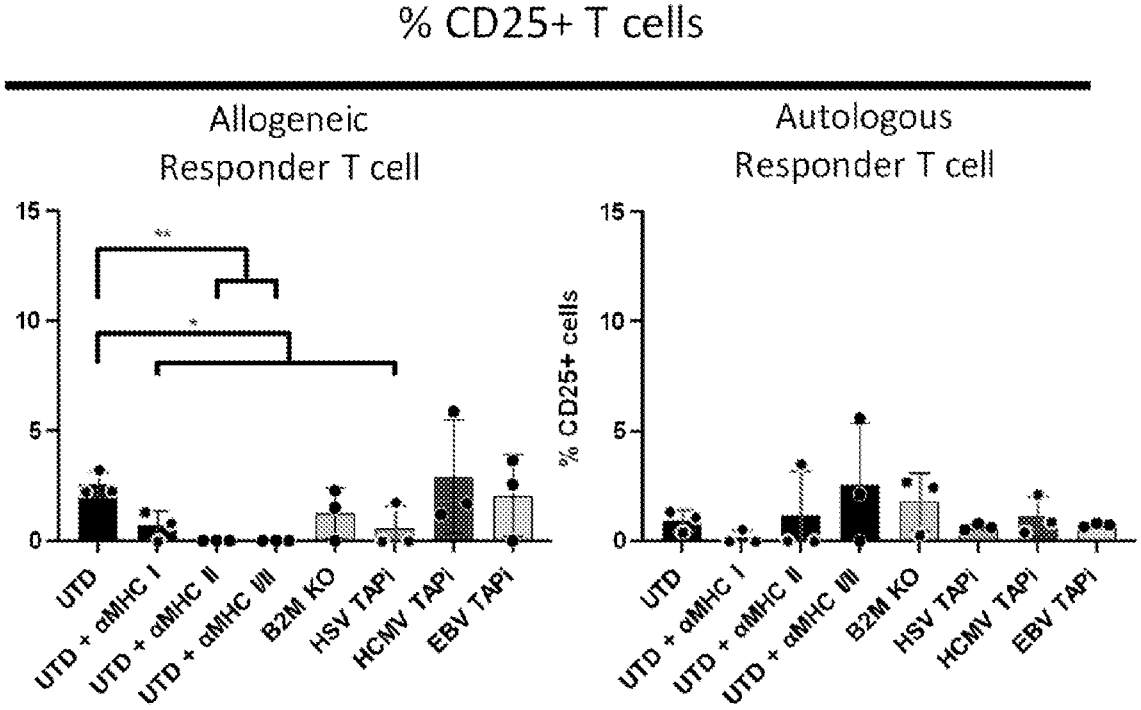

FIG. 13B

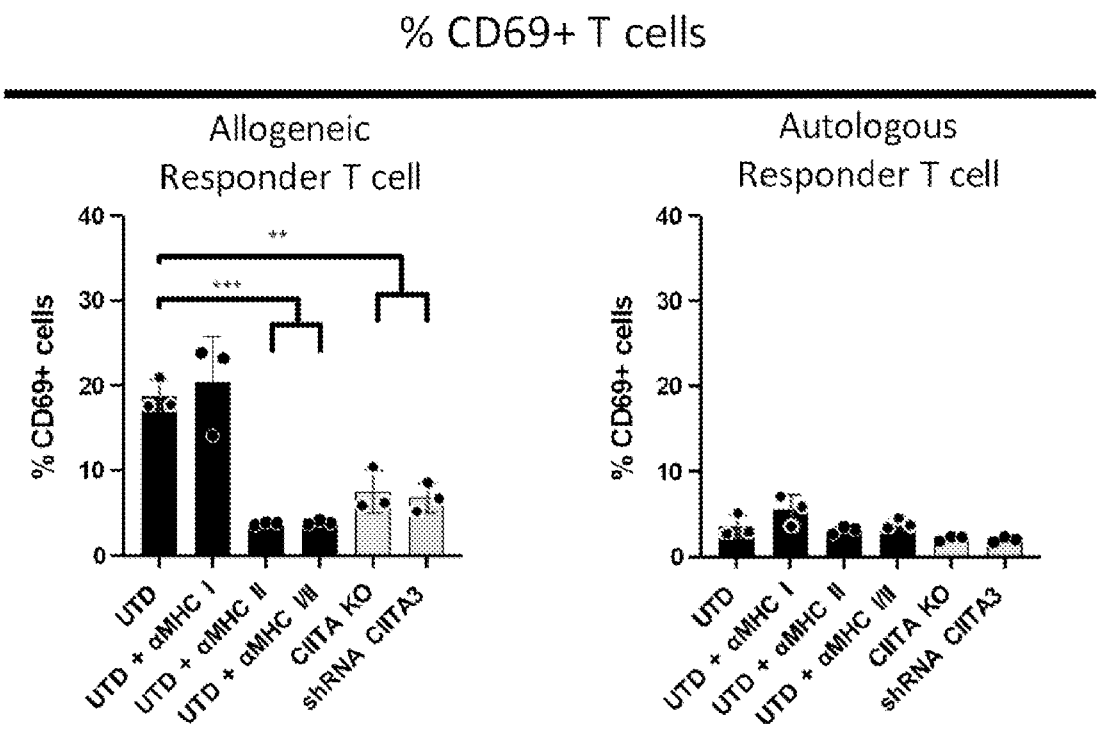

FIG. 14A

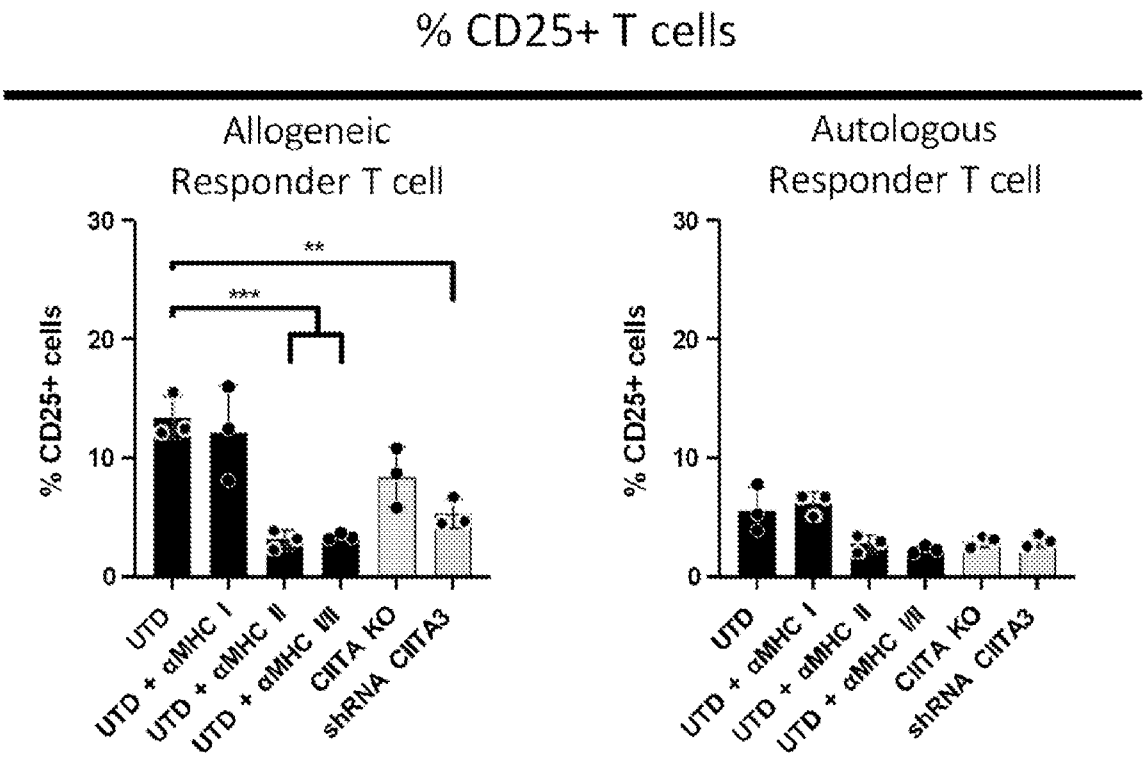

FIG. 14B

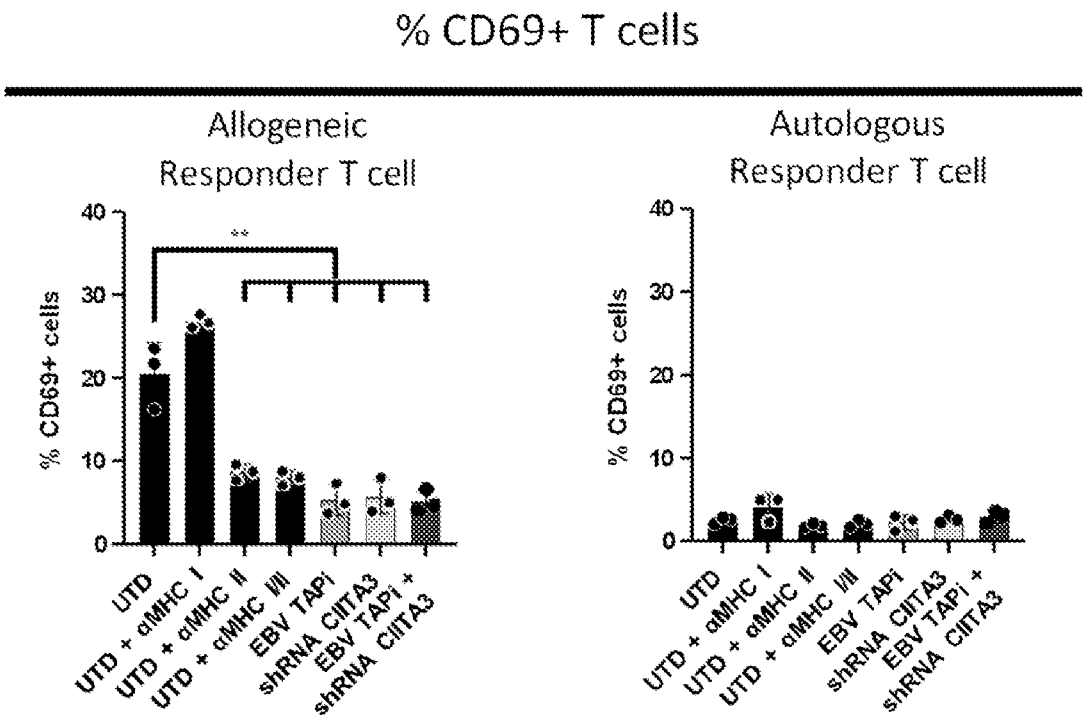

FIG. 15A

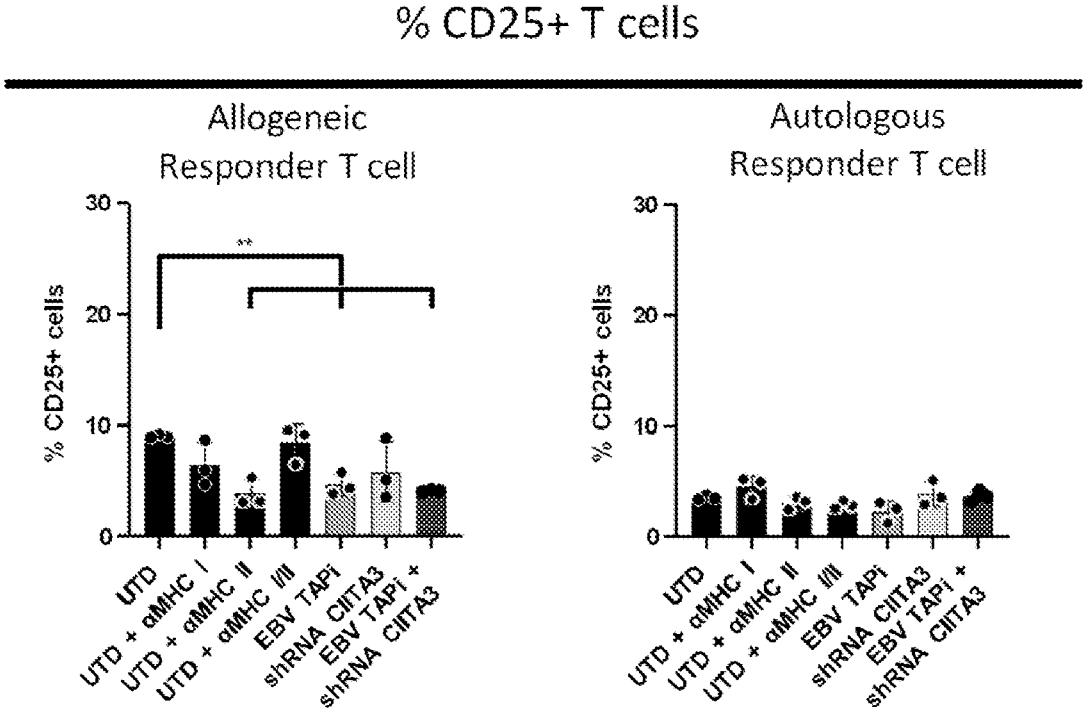

FIG. 15B

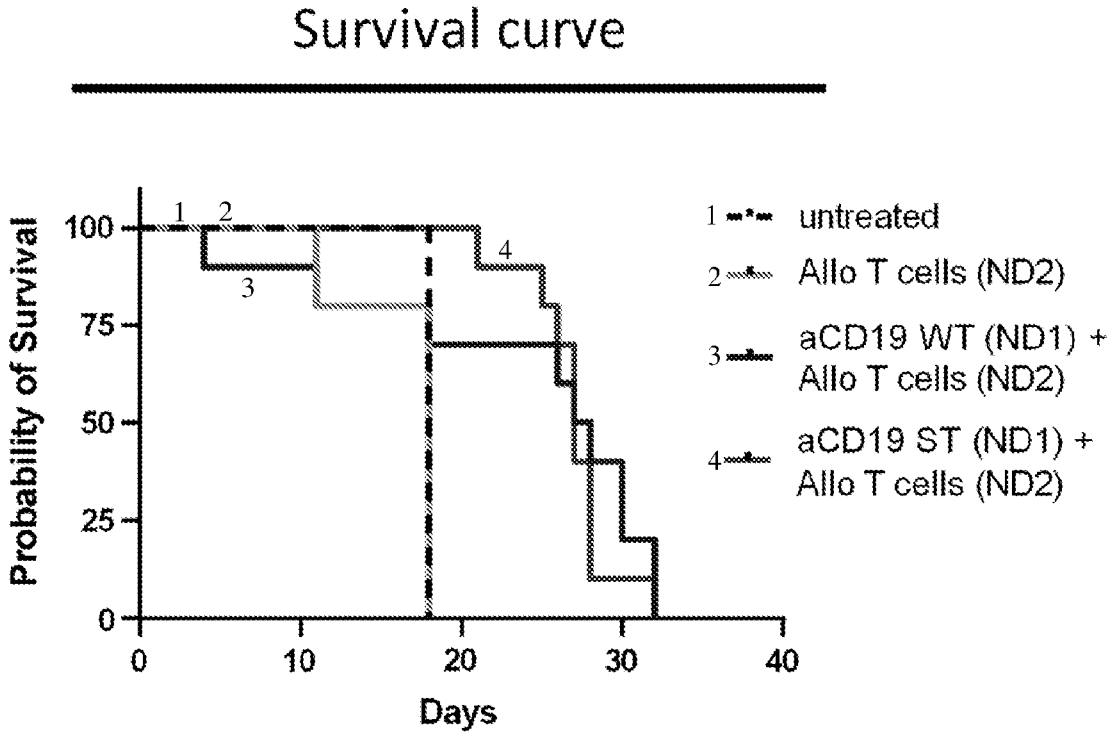
Survival curve
Probability of Survival
100
75
50
25
0
0
10
20
30
40
Days
1
2
3
4
1 untreated
2 Allo T cells (ND2)
3 aCD19 WT (ND1) + Allo T cells (ND2)
4 aCD19 ST (ND1) + Allo T cells (ND2)

FIG. 16

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	M105370038WO00-SEQ-ARM.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.2.0
1-5	Production Date	2023-04-12
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	M1053.70038WO00
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	63/331,773
2-7	Earliest priority application: Filing date	2022-04-15
2-8en	Applicant name	The General Hospital Corporation
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	COMPOSITIONS AND METHODS FOR REDUCING CELL THERAPY IMMUNOGENICITY
2-11	Sequence Total Quantity	41

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	AA
3-1-3	Length	88
3-1-4	Features Location/	**source 1..88**
	Qualifiers	mol_type=protein
		note=Herpes Simplex Virus
		organism=unidentified
	NonEnglishQualifier Value	
3-1-5	Residues	MSWALEMADT FLDTMRVGPR TYADVRDEIN KRGREDREAA RTAVHDPERP LLRSPGLLPE 60 IAPNASLGVA HRRTGGTVTD SPRNPVTR 88
3-2	**Sequences**	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	AA
3-2-3	Length	183
3-2-4	Features Location/	**source 1..183**
	Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-2-5	Residues	MDLLIRLGFL LMCALPTPGE RSSRDPKTLL SLSPRQQACV PRTKSHRPVC YNDTGDCTDA 60 DDSWKQLGED FAHQCLQAAK KRPKTHKSRP NDRNLEGRLT CQRVRRLLPC DLDIHPSHRL 120 LTLMNNCVCD GAVWNAFRLI ERHGFFAVTL YLCCGITLLV VILALLCSIT YESTGRGIRR 180 CGS 183
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	AA
3-3-3	Length	60
3-3-4	Features Location/	**source 1..60**
	Qualifiers	mol_type=protein
		note=Epstein-Barr virus
		organism=unidentified
	NonEnglishQualifier Value	
3-3-5	Residues	MVHVLERALL EQQSSACGLP GSSTETRPSH PCPEDPDVSR LRLLLVVLCV LFGLLCLLLI 60
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	DNA
3-4-3	Length	1089
3-4-4	Features Location/	**source 1..1089**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	atgctggtca tggcgccccg aaccgtcctc ctgctgctct cggcggccct ggccctgacc 60 gagacctggg ccggctccca ctccatgagg tatttctaca cctccgtgtc ccggcccggc 120 cgcggggagc cccgcttcat ctcagtgggc tacgtggacg acacgcagtt cgtgaggttc 180 gacagcgacg ccgcgagtcc gagagaggag ccgcgggcgc cgtggataga gcaggagggg 240 ccggaatatt gggaccggaa cacacagatc tgcaagacca acacacagac tgaccgagag 300 agcctgcgga acctgcgcgg ctactacaac cagagcgagg ccgggtctca caccctccag 360 aggatgtacg gctgcgacgt ggggccggac gggcgcctcc tccgcgggca taaccagttc 420 gcctacgacg gcaaggatta catcgccctg aacgaggacc tgagctcctg gaccgcggcg 480 gacaccgcgg ctcagatcac ccagcgcaag tgggaggcgg cccgtgtggc ggagcagctg 540 agaacctacc tggagggcac gtgcgtggag tggctccgca gatacctgga gaacgggaag 600 gagacgctgc agcgcgcgga ccccccaaag acacatgtga cccaccaccc catctctgac 660 catgaggcca ccctgaggtg ctgggccctg ggcttctacc ctgcggagat cacactgacc 720 tggcagcggg atggcgagga ccaaactcag gacaccgagc ttgtggagac cagaccagca 780 ggagacagaa ccttccagaa gtgggcagct gtggtggtgc cttctggaga agagcagaga 840 tacacatgcc atgtacagca tgaggggctg ccgaagcccc tcaccctgag atgggagcca 900 tcttcccagt ccaccgtccc catcgtgggc attgttgctg gcctggctgt cctagcagtt 960 gtggtcatcg gagctgtggt cgctgctgtg atgtgtagga ggaagagctc aggtggaaaa 1020 ggagggagct actctcaggc tgcgtccagc gacagtgccc agggctctga tgtgtctctc 1080 acagcttga 1089
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	DNA
3-5-3	Length	360

3-5-4	Features Location/	**source 1..360**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	atgtctcgct ccgtggcctt agctgtgctc gcgctactct ctctttctgg cctggaggct 60 atccagcgta ctccaaagat tcaggtttac tcacgtcatc cagcagagaa tggaaagtca 120 aatttcctga attgctatgt gtctgggttt catccatccg acattgaagt tgacttactg 180 aagaatggag agagaattga aaaagtggag cattcagact tgtctttcag caaggactgg 240 tctttctatc tcttgtacta cactgaattc acccccactg aaaaagatga gtatgcctgc 300 cgtgtgaacc atgtgacttt gtcacagccc aagatagtta agtgggatcg agacatgtaa 360
3-6	**Sequences**	
3-6-1	Sequence Number [ID]	6
3-6-2	Molecule Type	DNA
3-6-3	Length	768
3-6-4	Features Location/	**source 1..768**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-6-5	Residues	atgatcctaa acaaagctct gctgctgggg gccctcgctc tgaccaccat gatgagccct 60 tgtggaggtg aaggcattgt ggctgaccac gttgcctctt gtggtgtaaa cttgtaccag 120 ttttacggtc cctctggcca gtacacccat gaatttgatg gagatgagga gttctacgtg 180 gacctggaga ggaaggagac tgcctggcgg tggcctgagt tcagcaaatt tggaggtttt 240 gacccgcagg gtgcactgag aaacatggct gtggcaaaac acaacttgaa catcatgatt 300 aaacgctaca actctaccgc tgctaccaat gaggttcctg aggtcacagt gttttccaag 360 tctcccgtga cactgggtca gcccaacacc ctcatttgtc ttgtggacaa catctttcct 420 cctgtggtca acatcacatg gctgagcaat gggcagtcag tcacagaagg tgtttctgag 480 accagcttcc tctccaagag tgatcattcc ttcttcaaga tcagttacct caccttcctc 540 ccttctgctg atgagattta tgactgcaag gtggagcact ggggcctgga ccagcctctt 600 ctgaaacact gggagcctga gattccagcc cctatgtcag agctcacaga gactgtggtc 660 tgcaccctgg ggttgtctgt gggcctcgtg ggcattgtgg tgggcactgt cttcatcatc 720 caaggcctgc gttcagttgg tgcttccaga caccaagggc cattgtga 768
3-7	**Sequences**	
3-7-1	Sequence Number [ID]	7
3-7-2	Molecule Type	DNA
3-7-3	Length	3396
3-7-4	Features Location/	**source 1..3396**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-7-5	Residues	atgcgttgcc tggctccacg ccctgctggg tcctacctgt cagagcccca aggcagctca 60 cagtgtgcca ccatggagtt ggggccccta gaaggtggct acctggagct tcttaacagc 120 gatgctgacc ccctgtgcct ctaccacttc tatgaccaga tggacctggc tggagaagaa 180 gagattgagc tctactcaga acccgacaca gacaccatca actgcgacca gttcagcagg 240 ctgttgtgtg acatggaagg tgatgaagag accagggagg cttatgccaa tatcgcggaa 300 ctggaccagt atgtcttcca ggactcccag ctggagggcc tgagcaagga cattttcata 360 gagcacatag gaccagatga agtgatcggt gagagtatgg agatgccagc agaagttggg 420 cagaaaagtc agaaaagacc cttcccagag gagcttccgg cagacctgaa gcactggaag 480 ccagctgagc cccccactgt ggtgactggc agtctcctag tgggaccagt gagcgactgc 540 tccaccctgc cctgcctgcc actgcctgcg ctgttcaacc aggagccagc ctccggccag 600 atgcgcctgg agaaaaccga ccagattccc atgcctttct ccagttcctc gttgagctgc 660 ctgaatctcc ctgagggacc catccagttt gtccccacca tctccactct gccccatggg 720 ctctggcaaa tctctgaggc tggaacaggg gtctccagta tattcatcta ccatggtgag 780 gtgccccagg ccagccaagt acccctccc agtggattca ctgtccacgg cctcccaaca 840 tctccagacc ggccaggctc caccagcccc ttcgctccat cagccactga cctgcccagc 900 atgcctgaac ctgccctgac ctcccgagca aacatgacag agcacaagac gtcccccacc 960 caatgcccgg cagctggaga ggtctccaac aagcttccaa aatggcctga gccggtggag 1020 cagttctacc gctcactgca ggacacgtat ggtgccgagc ccgcaggccc ggatggcatc 1080 ctagtggagg tggatctggt gcaggccagg ctggagagga gcagcagcaa gagcctggag 1140 cgggaactgg ccaccccgga ctgggcagaa cggcagctgg cccaaggagg cctggctgag 1200 gtgctgttgg ctgccaagga gcaccggcgg ccgcgtgaga cacgagtgat tgctgtgctg 1260 ggcaaagctg gtcagggcaa gagctattgg gctggggcag tgagccgggc ctgggcttgt 1320 ggccggcttc cccagtacga ctttgtcttc tctgtcccct gccattgctt gaaccgtccg 1380 ggggatgcct atggcctgca ggatctgctc ttctccctgg gcccacagcc actcgtggcg 1440 gccgatgagg ttttcagcca catcttgaag agacctgacc gcgttctgct catcctagac 1500 ggcttcgagg agctggaagc gcaagatggc ttcctgcaca gcacgtgcgg accggcaccg 1560

		gcggagccct gctccctccg ggggctgctg gccggccttt tccagaagaa gctgctccga 1620 ggttgcaccc tcctcctcac agcccggccc cggggccgcc tggtccagag cctgagcaag 1680 gccgacgccc tatttgagct gtccggcttc tccatggagc aggcccaggc atacgtgatg 1740 cgctactttg agagctcagg gatgacagag caccaagaca gagccctgac gctcctccgg 1800 gaccggccac ttcttctcag tcacagccac agccctactt tgtgccgggc agtgtgccag 1860 ctctcagagg ccctgctgga gcttggggag gacgccaagc tgccctccac gctcacggga 1920 ctctatgtcg gcctgctggg ccgtgcagcc ctcgacagcc cccccggggc cctggcagag 1980 ctggccaagc tggcctggga gctgggccgc agacatcaaa gtaccctaca ggaggaccag 2040 ttcccatccg cagacgtgag gacctgggcg atggccaaag gcttagtcca acacccaccg 2100 cgggccgcag agtccgagct ggccttcccc agcttcctcc tgcaatgctt cctgggggcc 2160 ctgtggctgg ctctgagtgg cgaaatcaag gacaaggagc tcccgcagta cctagcattg 2220 accccaagga agaagaggcc ctatgacaac tggctggagg gcgtgccacg ctttctggct 2280 gggctgatct tccagcctcc cgcccgctgc ctgggagccc tactcgggcc atcggcggct 2340 gcctcggtgg acaggaagca gaaggtgctt gcgaggtacc tgaagcggct gcagccgggg 2400 acactgcggg cgcggcagct gctggagctg ctgcactgcg cccacgaggc cgaggaggct 2460 ggaatttggc agcacgtggt acaggagctc cccggccgcc tctcttttct gggcacccgc 2520 ctcacgcctc ctgatgcaca tgtactgggc aaggccttgg aggcggcggg ccaagacttc 2580 tccctggacc tccgcagcac tggcatttgc ccctctggat tggggagcct cgtgggactc 2640 agctgtgtca cccgtttcag ggctgccttg agcgacacgg tggcgctgtg ggagtccctg 2700 cagcagcatg gggagaccaa gctacttcag gcagcagagg agaagttcac catcgagcct 2760 ttcaaagcca agtccctgaa ggatgtggaa gacctgggaa agcttgtgca gactcagagg 2820 acgagaagtt cctcggaaga cacagctggg gagctccctg ctgttcggga cctaaagaaa 2880 ctggagtttg cgctgggccc tgtctcaggc ccccaggctt tccccaaact ggtgcggatc 2940 ctcacggcct tttcctccct gcagcatctg gacctggatg cgctgagtga gaacaagatc 3000 ggggacgagg gtgtctcgca gctctcagcc accttccccc agctgaagtc cttggaaacc 3060 ctcaatctgt cccagaacaa catcactgac ctgggtgcct acaaactcgc cgaggccctg 3120 ccttcgctcg ctgcatccct gctcaggcta agcttgtaca ataactgcat ctgcgacgtg 3180 ggagccgaga gcttggctcg tgtgcttccg gacatggtgt ccctccgggt gatggacgtc 3240 cagtacaaca agttcacggc tgccggggcc cagcagctcg ctgccagcct tcggaggtgt 3300 cctcatgtgg agacgctggc gatgtggacg cccaccatcc cattcagtgt ccaggaacac 3360 ctgcaacaac aggattcacg gatcagcctg agatga 3396
3-8	**Sequences**	
3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	DNA
3-8-3	Length	2800
3-8-4	Features Location/	**source 1..2800**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-8-5	Residues	atggcaacac aggcgtatac tgagctacag gcagccccgc caccatccca gccgccacag 60 gccccgccac aagcccagcc ccagccgcca ccgccaccac ccccagcggc accccagccc 120 ccgcagccac ccaccgctgc tgccaccccc cagccccaat atgtcaccga gctgcagagc 180 ccccagcccc aggcacagcc accgggtggc cagaagcagt acgtgacgga gctcccggct 240 gtacccgcac cctcgcagcc aaccggtgca cccacccctt cgcctgcacc ccagcagtac 300 atcgtggtca ctgtctctga aggtgccatg cgggccagcg agacagtgtc ggaggccagc 360 cccggctcca ccgccagcca gaccggcgtt cctactcagg tggttcagca ggtgcagggc 420 acccagcagc ggctgctggt ccagacgagc gtgcaggcca agccaggcca cgtgtcgccc 480 ctccagctga ccaacatcca agtgccccag caggctcttc ccacgcagcg tctggtggtg 540 cagagcgcag ccccaggcag caaaggtggc caggtctccc tgacggtcca tggtacccag 600 caggtgcact cgcccccaga gcagtcgccg gtgcaggcca acagctcttc cagcaagaca 660 gccggggccc ccacgggcac agtgccacag cagctgcagg tccacggcgt ccagcagagt 720 gtccccgtca cccaagagag atctgtggtc caggccactc cacaagcgcc caaacccggc 780 ccggtgcagc cgctgaccgt gcagggcctc cagccagtcc acgtggctca agaggtgcag 840 cagctccagc aggtgcccgt cccacacgtg tactccagcc aggtgcagta tgtggagggc 900 ggcgatgcca gctacacggc cagtgccatc cgttccagca cctactccta tcccgagacg 960 ccgctgtaca cgcagacggc aagcaccagc tactacgagg ccgcaggcac ggccacccag 1020 gtcagcaccc ccgccacctc ccaggcggtg gccagcagtg gctccatgcc catgtacgtg 1080 tccggcagcc aggtcgtcgc cagctccgcc agcactgggg ctggggccag caacagcagc 1140 ggaggtggtg gcagtggtgg tggcggcggc ggcgggggag gcggtggcgg gggtggcagt 1200 ggcagcaccg gaggcggcgg cagcggagca ggcacctacg tgatccaagg cggctacatg 1260 ctgggcagtg ccagccagtc ttactctcac accacccgtg cctcgccagc cacggtccag 1320 tggctcctgg acaactatga gacggctgag ggcgtgagtc tgccacggag caccctctac 1380 tgccactact tactgcactg ccaggagcag aagctggagc ccgtcaacgc cgcctccttc 1440 ggcaagctca tccgctccgt cttcatgggc ctgcgaaccc gccgtctggg caccaggggc 1500 aactccaagt accactacta tggcctgcgc atcaaggcca gctcacccct gctgcggctg 1560

		atggaggacc agcagcacat ggccatgcgg ggccagccct tctcgcagaa gcagaggctc 1620 aagcccatcc agaagatgga aggcatgacc aacggcgtgg cggtggggca gcagccgagc 1680 acggggctgt cggacatcag cgcccaggtg cagcagtacc agcaattttt ggatgcctct 1740 cggagcctcc ctgacttcac agagctcgac ctccagggca aggtgctgcc tgagggcgtc 1800 gggcccgggg acatcaaagc cttccaggtc ctgtaccggg aacactgtga ggccattgtc 1860 gacgtcatgg tgaacctgca gttcaccctg gtggagacgc tgtggaagac cttctggagg 1920 tacaacctca gccagcccag tgaggcgcca ccgctggctg tacatgacga ggccgagaag 1980 cgactgccca aagccatcct ggtgctcctc tccaagttcg agcccgtgct ccaatggacc 2040 aagcactgtg acaacgtgct gtaccagggc ctggtggaaa tcctcattcc cgacgtgctg 2100 cggcccatcc ccagtgcctt gacccaagcg atccggaact ttgccaagag cctggagagc 2160 tggctcaccc acgccatggt caacatcccc gaggagatgc tgcgggtgaa ggtggccgcg 2220 gctggcgcct tcgcgcagac actgcggcgc tacacgtcgc tcaaccacct ggcgcaggcg 2280 gcgcgcgctg tgctgcagaa caccgcacag atcaaccaga tgctgagcga cctcaaccgc 2340 gtggacttcg ccaacgtgca ggagcaggcc tcgtgggtgt gccgctgcga ggaccgcgtg 2400 gtgcagcggc tggagcagga cttcaaggtg acgctgcagc agcagaactc gctggagcag 2460 tgggcggcct ggctggacgg cgtggtgagc caggtgctca agccctacca gggcagcgcc 2520 ggcttcccca aggccgccaa gctcttcctc ctcaagtggt ccttctacag ctccatggtg 2580 atccgggacc tgaccctgcg cagcgccgcc agcttcggtt ccttccacct catccggctg 2640 ctctacgacg agtacatgta ctacctgatc gagcaccgcg tagcccaggc caagggcgag 2700 acccccatcg ccgtcatggg cgagttcgcc aatctggcca cctccctgaa ccccctggac 2760 cccgacaaag acgaggagga agaagaggag gaggagagcg 2800
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	DNA
3-9-3	Length	783
3-9-4	Features Location/	**source 1..783**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-9-5	Residues	atggagctta cccagcctgc agaagacctc atccagaccc agcagacccc tgcctcagaa 60 cttggggacc ctgaagaccc cggagaggag gctgcagatg gctcagacac tgtggtcctc 120 agtctctttc cctgcacccc tgagcctgtg aatcctgaac cggatgccag tgtttcctct 180 ccacaggcag gcagctccct gaagcactcc accactctca ccaaccggca gcgagggaac 240 gaggtgtcag ctctgccggc caccctagac tccctgtcca tccaccagct cgcagcacag 300 ggggagctgg accagctgaa ggagcatttg cggaaaggtg acaacctcgt caacaagcca 360 gacgagcgcg gcttcacccc cctcatctgg gcctccgcct ttggagagat tgagaccgtt 420 cgcttcctgc tggagtgggg tgccgacccc cacatcctgg caaaagagcg agagagcgcc 480 ctgtcgctgg ccagcacagg cggctacaca gacattgtgg ggctgctgct ggagcgtgac 540 gtggacatca acatctatga ttggaatgga gggacgccac tgctgtacgc tgtgcgcggg 600 aaccacgtga aatgcgttga ggccttgctg gcccgaggcg ctgacctcac caccgaagcc 660 gactctggct acaccccgat ggaccttgcc gtggccctgg gataccggaa agtgcaacag 720 gtgatcgaga accacatcct caagctcttc cagagcaacc tggtgcccgc tgaccctgag 780 tga 783
3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	DNA
3-10-3	Length	693
3-10-4	Features Location/	**source 1..693**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-10-5	Residues	atgaccatgg aatctggagc cgagaaccag cagagtggag atgcagctgt aacagaagct 60 gaaaaccaac aaatgacagt tcaagcccag ccacagattg ccacattagc ccaggtatct 120 atgccagcag ctcatgcaac atcatctgct cccaccgtaa ctctagtaca gctgcccaat 180 gggcagacag ttcaagtcca tggagtcatt caggcggccc agccatcagt tattcagtct 240 ccacaagtcc aaacagttca gtcttcctgt aaggacttaa aaagactttt ctccggaaca 300 cagatttcaa ctattgcaga aagtgaagat tcacaggagt cagtggatag tgtaactgat 360 tcccaaaagc gaagggaaat tctttcaagg aggccttcct acaggaaaat tttgaatgac 420 ttatcttctg atgcaccagg agtgccaagg attgaagaag agaagtctga agaggagact 480 tcagcacttc ctacacagcc tgctgaagaa gcagcacgaa agagagaggt ccgtctaatg 540 gagaacaggg aagcagctcg agagtgtcgt agaaagaaga aagaatatgt gaaatgttta 600 gaaaacagag tggcagtgct tgaaaatcaa aacaagacat tgattgagga gctaaaagca 660 cttaaggacc tttactgcca caaatcagat taa 693
3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11

3-11-2	Molecule Type	DNA
3-11-3	Length	1044
3-11-4	Features Location/	**source 1..1044**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-11-5	Residues	atggagcagt atacagcaaa cagcaatagt tcgacagagc agattgttgt ccaggcagga 60 cagattcagc agcagcagca gggtggtgtc actgctgtgc agttgcagac tgaggcccag 120 gtggcatccg cctcaggcca gcaagtccag accctccagg tagtccaagg gcagccatta 180 atggtgcagg tcagtggagg ccagctaatc acatcaactg gccaacccat catggtccag 240 gctgtccctg gtggacaagg tcaaaccatc atgcaagtac ctgtttctgg aacacagggt 300 ttgcagcaaa tacagttggt cccacctgga cagatccaga tccagggtgg acaggctgtg 360 caggtgcagg gccagcaggg ccagacccag cagatcatca tccagcagcc ccagacggct 420 gtcactgctg gccagactca gacacagcag cagattgctg tccagggaca gcaagtggca 480 cagactgctg aagggcagac catcgtctat caaccagtta atgcagatgg caccattctc 540 cagcaagtta cagtccctgt ttcaggcatg atcactatcc cagcagccag tttggcagga 600 gcacagattg ttcaaacagg agccaatacc aacacaacca gcagtgggca agggactgtc 660 actgtgacac taccagtggc aggcaatgtg gtcaattcag gagggatggt catgatggtt 720 cctggggctg gctctgtgcc tgctatccaa agaatccctc tacctggagc agagatgctt 780 gaagaagagc ctctctacgt gaatgccaaa caataccacc gtattcttaa gaggaggcaa 840 gcccgagcta aactagaggc agaagggaaa attccaaagg agagaaggaa atacctgcat 900 gagtctcggc accgtcatgc catggcacgg aagcgtggtg aaggtggacg atttttctct 960 ccaaaggaaa aggatagtcc ccatatgcag gatccaaacc aagccgatga agaagcaatg 1020 acacagatca tccgagtgtc ctaa 1044
3-12	**Sequences**	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	DNA
3-12-3	Length	1065
3-12-4	Features Location/	**source 1..1065**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-12-5	Residues	atgtccacag aaggaggatt tggtggtact agcagcagtg atgcccagca aagcctacag 60 tcgttctggc ctcgggtcat ggaagaaatc cggaatttaa cagtgaaaga cttccgagtg 120 caggaactcc cactggctcg tattaagaag attatgaaac tggatgaaga tgtgaagatg 180 atcagtgcag aagcgcctgt actctttgcc aaggcagccc agatttttat cacagagttg 240 actcttcgag cctggattca cacagaagat aacaagcgcc ggactctaca gagaaatgat 300 atcgccatgg caattacaaa atttgatcag tttgattttc tcatcgatat tgttccaaga 360 gatgaactga aacctccaaa gcgtcaggag gaggtgcgcc agtctgtaac tcctgccgag 420 ccagtccagt actatttcac gctggctcag caacccaccg ctgtccaagt ccagggccag 480 cagcaaggcc agcagaccac cagctccacg accaccatcc agcctgggca gatcatcatc 540 gcacagcctc agcagggcca gaccacacct gtgacaatgc aggttggaga aggtcagcag 600 gtgcagattg tccaggctca gccacagggt caagcccaac aggcccagag tggcactgga 660 cagaccatgc aggtgatgca gcagatcatc actaacacag gagagatcca gcagatcccg 720 gtgcagctga atgccggcca gctgcagtat atccgcttag cccagcctgt atcaggcact 780 caagttgtgc agggacagat ccagacactt gccaccaatg ctcaacaggg gcaaagaaat 840 gcaagtcagg ggaagcctcg aaggtgcctg aaagaaacct tacagattac acagacagag 900 gtccagcaag gacagcagca gttcagccag ttcacagatg gacagcagct ctaccagatc 960 cagcaagtca ccatgcctgc gggccaggac ctcgcccagc ccatgttcat ccagtcagcc 1020 aaccagccct ccgacgggca ggccccccag gtgaccggcg actga 1065
3-13	**Sequences**	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	DNA
3-13-3	Length	58
3-13-4	Features Location/	**source 1..58**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-13-5	Residues	gcttctgctg atgagattta ttttcaagag aaataaatct catcagcaga agtttttt 58
3-14	**Sequences**	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	DNA
3-14-3	Length	2757
3-14-4	Features Location/	**source 1..2757**
	Qualifiers	mol_type=other DNA

		organism=synthetic construct
	NonEnglishQualifier Value	
3-14-5	Residues	cgtgaggctc cggtgcccgt cagtgggcag agcgcacatc gcccacagtc cccgagaagt 60 tgggggggagg ggtcggcaat tgaaccggtg cctagagaag gtggcgcggg gtaaactggg 120 aaagtgatgt cgtgtactgg ctccgccttt ttcccgaggg tgggggagaa ccgtatataa 180 gtgcagtagt cgccgtgaac gttctttttc gcaacgggtt tgccgccaga acacaggtaa 240 gtgccgtgtg tggttcccgc gggcctggcc tctttacggg ttatggccct tgcgtgcctt 300 gaattacttc cacctggctg cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg 360 ggtgggagag ttcgaggcct tgcgcttaag gagccccttc gcctcgtgct tgagttgagg 420 cctggcctgg gcgctggggc cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg 480 ctgctttcga taagtctcta gccatttaaa atttttgatg acctgctgcg acgcttttt 540 tctggcaaga tagtcttgta aatgcgggcc aagatctgca cactggtatt tcggtttttg 600 gggccgcggg cggcgacggg gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc 660 tgcgagcgcg gccaccgaga atcggacggg ggtagtctca agctggccgg cctgctctgg 720 tgcctggcct cgcgccgccg tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg 780 caccagttgc gtgagcggaa agatggccgc ttcccggccc tgctgcaggg agctcaaaat 840 ggaggacgcg gcgctcggga gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct 900 ttccgtcctc agccgtcgct tcatgtgact ccactgagta ccgggcgccg tccaggcacc 960 tcgattagtt ctcgtgcttt tggagtacgt cgtctttagg ttgggggggag gggttttatg 1020 cgatggagtt tccccacact gagtgggtgg agactgaagt taggccagct tggcacttga 1080 tgtaattctc cttggaattt gccctttttg agtttggatc ttggttcatt ctcaagcctc 1140 agacagtggt tcaaagtttt tttcttccat ttcaggtgtc gtgaagctct agagccatgg 1200 tacacgtatt ggaacgggcg cttttggagc agcaaagctc cgcgtgcgga ctcccaggtt 1260 catccacgga aacacgccca tctcatccct gcccggagga ccccgatgta tcacgactta 1320 ggctcttgct ggtcgtactt tgcgtgctct ttggacttct gtgcctcctg ctcatcggct 1380 caggagaggg cagaggaagt cttctaacat gcggtgacgt ggaggagaat cccggcccta 1440 tggtgagcaa gggcgaggag ctgttcaccg gggtggtgcc catcctggtc gagctggacg 1500 gcgacgtaaa cggccacaag ttcagcgtgt ccggcgaggg cgagggcgat gccacctacg 1560 gcaagctgac cctgaagttc atctgcacca ccggcaagct gcccgtgccc tggcccaccc 1620 tcgtgaccac cctgacctac ggcgtgcagt gcttcagccg ctaccccgac cacatgaagc 1680 agcacgactt cttcaagtcc gccatgcccg aaggctacgt ccaggagcgc accatcttct 1740 tcaaggacga cggcaactac aagacccgcg ccgaggtgaa gttcgagggc gacaccctgg 1800 tgaaccgcat cgagctgaag ggcatcgact tcaaggagga cggcaacatc ctggggcaca 1860 agctggagta caactacaac agccacaacg tctatatcat ggccgacaag cagaagaacg 1920 gcatcaaggt gaacttcaag atccgccaca acatcgagga cggcagcgtg cagctcgccg 1980 accactacca gcagaacacc cccatcggcg acggccccgt gctgctgccc gacaaccact 2040 acctgagcac ccagtccgcc ctgagcaaag accccaacga gaagcgcgat cacatggtcc 2100 tgctggagtt cgtgaccgcc gccgggatca ctctcggcat ggacgagctg tacaagtaag 2160 tcgacaatca acctctggat tacaaaattt gtgaaagatt gactggtatt cttaactatg 2220 ttgctccttt tacgctatgt ggatacgctg ctttaatgcc tttgtatcat gctattgctt 2280 cccgtatggc tttcattttc tcctccttgt ataaatcctg gttgctgtct ctttatgagg 2340 agttgtggcc cgttgtcagg caacgtggcg tggtgtgcac tgtgtttgct gacgcaaccc 2400 ccactggttg gggcattgcc accacctgtc agctcctttc cgggactttc gctttccccc 2460 tccctattgc cacggcggaa ctcatcgccg cctgccttgc ccgctgctgg acaggggctc 2520 ggctgttggg cactgacaat tccgtggtgt tgtcggggaa gctgacgtcc tttccttggc 2580 tgctcgcctg tgttgccacc tggattctgc gcgggacgtc cttctgctac gtcccttcgg 2640 ccctcaatcc agcggacctt ccttcccgcg gcctgctgcc ggctctgcgg cctcttccgc 2700 gtcttcgcct tcgccctcag acgagtcgga tctccctttg ggccgcctcc ccgcctg 2757
3-15	**Sequences**	
3-15-1	Sequence Number [ID]	15
3-15-2	Molecule Type	DNA
3-15-3	Length	1977
3-15-4	Features Location/	**source 1..1977**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-15-5	Residues	cgtgaggctc cggtgcccgt cagtgggcag agcgcacatc gcccacagtc cccgagaagt 60 tgggggggagg ggtcggcaat tgaaccggtg cctagagaag gtggcgcggg gtaaactggg 120 aaagtgatgt cgtgtactgg ctccgccttt ttcccgaggg tgggggagaa ccgtatataa 180 gtgcagtagt cgccgtgaac gttctttttc gcaacgggtt tgccgccaga acacaggtaa 240 gtgccgtgtg tggttcccgc gggcctggcc tctttacggg ttatggccct tgcgtgcctt 300 gaattacttc cacctggctg cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg 360 ggtgggagag ttcgaggcct tgcgcttaag gagccccttc gcctcgtgct tgagttgagg 420 cctggcctgg gcgctggggc cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg 480 ctgctttcga taagtctcta gccatttaaa atttttgatg acctgctgcg acgcttttt 540

		tctggcaaga tagtcttgta aatgcgggcc aagatctgca cactggtatt tcggtttttg 600 gggccgcggg cggcgacggg gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc 660 tgcgagcgcg gccaccgaga atcggacggg ggtagtctca agctggccgg cctgctctgg 720 tgcctggcct cgcgccgccg tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg 780 caccagttgc gtgagcggaa agatggccgc ttcccggccc tgctgcaggg agctcaaaat 840 ggaggacgcg gcgctcggga gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct 900 ttccgtcctc agccgtcgct tcatgtgact ccactgagta ccgggcgccg tccaggcacc 960 tcgattagtt ctcgtgcttt tggagtacgt cgtctttagg ttggggggag gggttttatg 1020 cgatggagtt tccccacact gagtgggtgg agactgaagt taggccagct tggcacttga 1080 tgtaattctc cttggaattt gccctttttg agtttggatc ttggttcatt ctcaagcctc 1140 agacagtggt tcaaagtttt tttcttccat ttcaggtgtc gtgaagctct agagccatgg 1200 tacacgtatt ggaacgggcg cttttggagc agcaaagctc cgcgtgcgga ctcccaggtt 1260 catccacgga aacacgccca tctcatccct gcccggagga ccccgatgta tcacgactta 1320 ggctcttgct ggtcgtactt tgcgtgctct ttggacttct gtgcctcctg ctcatctaag 1380 tcgacaatca acctctggat tacaaaattt gtgaaagatt gactggtatt cttaactatg 1440 ttgctccttt tacgctatgt ggatacgctg ctttaatgcc tttgtatcat gctattgctt 1500 cccgtatggc tttcattttc tcctccttgt ataaatcctg gttgctgtct ctttatgagg 1560 agttgtggcc cgttgtcagg caacgtggcg tggtgtgcac tgtgtttgct gacgcaaccc 1620 ccactggttg gggcattgcc accacctgtc agctcctttc cgggactttc gctttccccc 1680 tccctattgc cacggcggaa ctcatcgccg cctgccttgc ccgctgctgg acaggggctc 1740 ggctgttggg cactgacaat tccgtggtgt tgtcggggaa gctgacgtcc tttccttggc 1800 tgctcgcctg tgttgccacc tggattctgc gcgggacgtc cttctgctac gtcccttcgg 1860 ccctcaatcc agcggacctt ccttcccgcg gcctgctgcc ggctctgcgg cctcttccgc 1920 gtcttcgcct tcgccctcag acgagtcgga tctccctttg ggccgcctcc ccgcctg 1977
3-16	**Sequences**	
3-16-1	Sequence Number [ID]	16
3-16-2	Molecule Type	DNA
3-16-3	Length	311
3-16-4	Features Location/	**source 1..311**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-16-5	Residues	gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgttagagag 60 ataattagaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120 aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180 atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240 cgaaacaccg aacaacagga ttcacggatc agcttcaaga gagctgatcc gtgaatcctg 300 ttgtttttt t 311
3-17	**Sequences**	
3-17-1	Sequence Number [ID]	17
3-17-2	Molecule Type	DNA
3-17-3	Length	4107
3-17-4	Features Location/	**source 1..4107**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-17-5	Residues	cgtgaggctc cggtgcccgt cagtgggcag agcgcacatc gcccacagtc cccgagaagt 60 tgggggggagg ggtcggcaat tgaaccggtg cctagagaag gtggcgcggg gtaaactggg 120 aaagtgatgt cgtgtactgg ctccgccttt ttcccgaggg tgggggagaa ccgtatataa 180 gtgcagtagt cgccgtgaac gttcttttttc gcaacgggtt tgccgccaga acacaggtaa 240 gtgccgtgtg tggttcccgc gggcctggcc tctttacggg ttatggccct tgcgtgcctt 300 gaattacttc cacctggctg cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg 360 ggtgggagag ttcgaggcct tgcgcttaag gagccccttc gcctcgtgct tgagttgagg 420 cctggcctgg gcgctggggc cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg 480 ctgctttcga taagtctcta gccatttaaa atttttgatg acctgctgcg acgcttttt 540 tctggcaaga tagtcttgta aatgcgggcc aagatctgca cactggtatt tcggtttttg 600 gggccgcggg cggcgacggg gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc 660 tgcgagcgcg gccaccgaga atcggacggg ggtagtctca agctggccgg cctgctctgg 720 tgcctggcct cgcgccgccg tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg 780 caccagttgc gtgagcggaa agatggccgc ttcccggccc tgctgcaggg agctcaaaat 840 ggaggacgcg gcgctcggga gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct 900 ttccgtcctc agccgtcgct tcatgtgact ccactgagta ccgggcgccg tccaggcacc 960 tcgattagtt ctcgtgcttt tggagtacgt cgtctttagg ttgggggggag gggttttatg 1020 cgatggagtt tccccacact gagtgggtgg agactgaagt taggccagct tggcacttga 1080 tgtaattctc cttggaattt gccctttttg agtttggatc ttggttcatt ctcaagcctc 1140

		agacagtggt tcaaagtttt tttcttccat ttcaggtgtc gtgaagctct agagccatgg 1200 ccctccctgt caccgccctg ctgcttccgc tggctcttct gctccacgcc gctcggcccg 1260 aggtgaaact gcaggagtca ggacctggcc tggtggcgcc ctcacagagc ctgtccgtca 1320 catgcactgt ctcaggggtc tcattacccg actatggtgt aagctggatt cgccagcctc 1380 cacgaaaggg tctggagtgg ctgggagtaa tatggggtag tgaaaccaca tactataatt 1440 cagctctcaa atccagactg accatcatca aggacaactc caagagccaa gttttcttaa 1500 aaatgaacag tctgcaaact gatgacacag ccatttacta ctgtgccaaa cattattact 1560 acggtggtag ctatgctatg gactactggg gtcaaggaac ctcagtcacc gtctcctcag 1620 gtggaggtgg cagcggagga ggtgggtccg gcggtggagg aagcggcggt ggaggaagcg 1680 acatccagat gacacagact acatcctccc tgtctgcctc tctgggagac agagtcacca 1740 tcagttgcag ggcaagtcag gacattagta aatatttaaa ttggtatcag cagaaaccag 1800 atggaactgt taaactcctg atctaccata catcaagatt acactcagga gtcccatcaa 1860 ggttcagtgg cagtgggtct ggaacagatt attctctcac cattagcaac ctggagcaag 1920 aagatattgc cacttacttt tgccaacagg gtaatacgct tccgtacacg ttcggagggg 1980 ggactaagtt ggaaataaca cgggctgatg ctgcaccaac tgtatccatc ttcccaccat 2040 ccagtaatac cactacccca gcaccgaggc cacccacccc ggctcctacc atcgcctccc 2100 agcctctgtc cctgcgtccg gaggcatgta gacccgcagc tggtggggcc gtgcataccc 2160 ggggtcttga cttcgcctgc gatatctaca tttgggcccc tctggctggt acttgcgggg 2220 tcctgctgct ttcactcgtg atcactcttt actgtaagcg cggtcggaag aagctgctgt 2280 acatctttaa gcaacccttc atgaggcctg tgcagactac tcaagaggag gacggctgtt 2340 catgccggtt cccagaggag gaggaaggcg gctgcgaact gcgcgtgaaa ttcagccgca 2400 gcgcagatgc tccagcctac caacaggggc agaaccagct ctacaacgaa ctcaatcttg 2460 gtcggagaga ggagtacgac gtgctggaca agcggagagg acgggaccca gaaatgggcg 2520 ggaagccgcg cagaaagaat ccccaagagg gcctgtacaa cgagctccaa aaggataaga 2580 tggcagaagc ctatagcgag attggtatga aaggggaacg cagaagaggc aaaggccacg 2640 acggactgta ccagggactc agcaccgcca ccaaggacac ctatgacgct cttcacatgc 2700 aggccctgcc gcctcggtcc ggaggcggcg gagagggcag aggaagtctt ctaacatgcg 2760 gtgacgtgga ggagaatccc ggccctagga tggtgagcaa gggcgaggag ctgttcaccg 2820 gggtggtgcc catcctggtc gagctggacg gcgacgtaaa cggccacaag ttcagcgtgt 2880 ccggcgaggg cgagggcgat gccacctacg gcaagctgac cctgaagttc atctgcacca 2940 ccggcaagct gcccgtgccc tggcccaccc tcgtgaccac cctgacctac ggcgtgcagt 3000 gcttcagccg ctaccccgac cacatgaagc agcacgactt cttcaagtcc gccatgcccg 3060 aaggctacgt ccaggagcgc accatcttct tcaaggacga cggcaactac aagacccgcg 3120 ccgaggtgaa gttcgagggc gacaccctgg tgaaccgcat cgagctgaag ggcatcgact 3180 tcaaggagga cggcaacatc ctggggcaca agctggagta caactacaac agccacaacg 3240 tctatatcat ggccgacaag cagaagaacg gcatcaaggt gaacttcaag atccgccaca 3300 acatcgagga cggcagcgtg cagctcgccg accactacca gcagaacacc cccatcggcg 3360 acggccccgt gctgctgccc gacaaccact acctgagcac ccagtccgcc ctgagcaaag 3420 accccaacga gaagcgcgat cacatggtcc tgctggagtt cgtgaccgcc gccgggatca 3480 ctctcggcat ggacgagctg tacaagtaag tcgacaatca acctctggat tacaaaattt 3540 gtgaaagatt gactggtatt cttaactatg ttgctccttt tacgctatgt ggatacgctg 3600 ctttaatgcc tttgtatcat gctattgctt cccgtatggc tttcattttc tcctccttgt 3660 ataaatcctg gttgctgtct ctttatgagg agttgtggcc cgttgtcagg caacgtggcg 3720 tggtgtgcac tgtgtttgct gacgcaaccc ccactggttg gggcattgcc accacctgtc 3780 agctcctttc cgggactttc gctttccccc tccctattgc cacggcggaa ctcatcgccg 3840 cctgccttgc ccgctgctgg acaggggctc ggctgttggg cactgacaat tccgtggtgt 3900 tgtcggggaa gctgacgtcc tttccttggc tgctcgcctg tgttgccacc tggattctgc 3960 gcgggacgtc cttctgctac gtcccttcgg ccctcaatcc agcggacctt ccttcccgcg 4020 gcctgctgcc ggctctgcgg cctcttccgc gtcttcgcct tcgccctcag acgagtcgga 4080 tctccctttg ggccgcctcc ccgcctg 4107
3-18	**Sequences**	
3-18-1	Sequence Number [ID]	18
3-18-2	Molecule Type	DNA
3-18-3	Length	3315
3-18-4	Features Location/	**source 1..3315**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-18-5	Residues	cgtgaggctc cggtgcccgt cagtgggcag agcgcacatc gcccacagtc cccgagaagt 60 tgggggggagg ggtcggcaat tgaaccggtg cctagagaag gtggcgcggg gtaaactggg 120 aaagtgatgt cgtgtactgg ctccgccttt ttcccgaggg tgggggagaa ccgtatataa 180 gtgcagtagt cgccgtgaac gttcttttтc gcaacgggtt tgccgccaga acacaggtaa 240 gtgccgtgtg tggttcccgc gggcctggcc tctttacggg ttatggccct tgcgtgcctt 300 gaattacttc cacctggctg cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg 360 ggtgggagag ttcgaggcct tgcgcttaag gagccccttc gcctcgtgct tgagttgagg 420

		cctggcctgg gcgctggggc cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg 480 ctgctttcga taagtctcta gccatttaaa atttttgatg acctgctgcg acgctttttt 540 tctggcaaga tagtcttgta aatgcgggcc aagatctgca cactggtatt tcggtttttg 600 gggccgcggg cggcgacggg gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc 660 tgcgagcgcg gccaccgaga atcggacggg ggtagtctca agctggccgg cctgctctgg 720 tgcctggcct cgcgccgccg tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg 780 caccagttgc gtgagcggaa agatggccgc ttcccggccc tgctgcaggg agctcaaaat 840 ggaggacgcg gcgctcggga gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct 900 ttccgtcctc agccgtcgct tcatgtgact ccactgagta ccgggcgccg tccaggcacc 960 tcgattagtt ctcgtgcttt tggagtacgt cgtctttagg ttgggggggag gggttttatg 1020 cgatggagtt tccccacact gagtgggtgg agactgaagt taggccagct tggcacttga 1080 tgtaattctc cttggaattt gccctttttg agtttggatc ttggttcatt ctcaagcctc 1140 agacagtggt tcaaagtttt tttcttccat ttcaggtgtc gtgaagctct agagccatgg 1200 ccctccctgt caccgccctg ctgcttccgc tggctcttct gctccacgcc gctcggcccg 1260 aggtgaaact gcaggagtca ggacctggcc tggtggcgcc ctcacagagc ctgtccgtca 1320 catgcactgt ctcaggggtc tcattacccg actatggtgt aagctggatt cgccagcctc 1380 cacgaaaggg tctggagtgg ctgggagtaa tatggggtag tgaaaccaca tactataatt 1440 cagctctcaa atccagactg accatcatca aggacaactc caagagccaa gttttcttaa 1500 aaatgaacag tctgcaaact gatgacacag ccatttacta ctgtgccaaa cattattact 1560 acggtggtag ctatgctatg gactactggg gtcaaggaac ctcagtcacc gtctcctcag 1620 gtggaggtgg cagcggagga ggtgggtccg gcggtggagg aagcggcggt ggaggaagcg 1680 acatccagat gacacagact acatcctccc tgtctgcctc tctgggagac agagtcacca 1740 tcagttgcag ggcaagtcag gacattagta aatatttaaa ttggtatcag cagaaaccag 1800 atggaactgt taaactcctg atctaccata catcaagatt acactcagga gtcccatcaa 1860 ggttcagtgg cagtgggtct ggaacagatt attctctcac cattagcaac ctggagcaag 1920 aagatattgc cacttacttt tgccaacagg gtaatacgct tccgtacacg ttcggagggg 1980 ggactaagtt ggaaataaca cgggctgatg ctgcaccaac tgtatccatc ttcccaccat 2040 ccagtaatac cactacccca gcaccgaggc cacccacccc ggctcctacc atcgcctccc 2100 agcctctgtc cctgcgtccg gaggcatgta gacccgcagc tggtggggcc gtgcataccc 2160 ggggtcttga cttcgcctgc gatatctaca tttgggcccc tctggctggt acttgcgggg 2220 tcctgctgct ttcactcgtg atcactcttt actgtaagcg cggtcggaag aagctgctgt 2280 acatctttaa gcaacccttc atgaggcctg tgcagactac tcaagaggag gacggctgtt 2340 catgccggtt cccagaggag gaggaaggcg gctgcgaact gcgcgtgaaa ttcagccgca 2400 gcgcagatgc tccagcctac caacaggggc agaaccagct ctacaacgaa ctcaatcttg 2460 gtcggagaga ggagtacgac gtgctggaca agcggagagg acgggaccca gaaatgggcg 2520 ggaagccgcg cagaaagaat ccccaagagg gcctgtacaa cgagctccaa aaggataaga 2580 tggcagaagc ctatagcgag attggtatga aaggggaacg cagaagaggc aaaggccacg 2640 acggactgta ccagggactc agcaccgcca ccaaggacac ctatgacgct cttcacatgc 2700 aggccctgcc gcctcggtaa gtcgacaatc aacctctgga ttacaaaatt tgtgaaagat 2760 tgactggtat tcttaactat gttgctcctt ttacgctatg tggatacgct gctttaatgc 2820 ctttgtatca tgctattgct tcccgtatgg ctttcatttt ctcctccttg tataaatcct 2880 ggttgctgtc tctttatgag gagttgtggc ccgttgtcag gcaacgtggc gtggtgtgca 2940 ctgtgtttgc tgacgcaacc cccactggtt ggggcattgc caccacctgt cagctccttt 3000 ccgggacttt cgctttcccc ctccctattg ccacggcgga actcatcgcc gcctgccttg 3060 cccgctgctg gacaggggct cggctgttgg gcactgacaa ttccgtggtg ttgtcgggga 3120 agctgacgtc ctttccttgg ctgctcgcct gtgttgccac ctggattctg cgcgggacgt 3180 ccttctgcta cgtcccttcg gccctcaatc cagcggacct tccttcccgc ggcctgctgc 3240 cggctctgcg gcctcttccg cgtcttcgcc ttcgccctca gacgagtcgg atctcccttt 3300 gggccgcctc cccgc 3315
3-19	**Sequences**	
3-19-1	Sequence Number [ID]	19
3-19-2	Molecule Type	DNA
3-19-3	Length	311
3-19-4	Features Location/	**source 1..311**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-19-5	Residues	gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgttagagag 60 ataattagaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120 aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180 atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240 cgaaacaccg aacaacagga ttcacggatc agcttcaaga gagctgatcc gtgaatcctg 300 ttgttttttt t 311
3-20	**Sequences**	
3-20-1	Sequence Number [ID]	20

3-20-2	Molecule Type	DNA
3-20-3	Length	4353
3-20-4	Features Location/	**source 1..4353**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-20-5	Residues	cgtgaggctc cggtgcccgt cagtgggcag agcgcacatc gcccacagtc cccgagaagt 60 tgggggggagg ggtcggcaat tgaaccggtg cctagagaag gtggcgcggg gtaaactggg 120 aaagtgatgt cgtgtactgg ctccgccttt ttcccgaggg tgggggagaa ccgtatataa 180 gtgcagtagt cgccgtgaac gttctttttc gcaacgggtt tgccgccaga acacaggtaa 240 gtgccgtgtg tggttcccgc gggcctggcc tctttacggg ttatggccct tgcgtgcctt 300 gaattacttc cacctggctg cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg 360 ggtgggagag ttcgaggcct tgcgcttaag gagccccttc gcctcgtgct tgagttgagg 420 cctggcctgg gcgctggggc cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg 480 ctgctttcga taagtctcta gccatttaaa atttttgatg acctgctgcg acgcttttt 540 tctggcaaga tagtcttgta aatgcgggcc aagatctgca cactggtatt tcggtttttg 600 gggccgcggg cggcgacggg gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc 660 tgcgagcgcg gccaccgaga atcggacggg ggtagtctca agctggccgg cctgctctgg 720 tgcctggcct cgcgccgccg tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg 780 caccagttgc gtgagcggaa agatggccgc ttcccggccc tgctgcaggg agctcaaaat 840 ggaggacgcg gcgctcggga gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct 900 ttccgtcctc agccgtcgct tcatgtgact ccactgagta ccgggcgccg tccaggcacc 960 tcgattagtt ctcgtgcttt tggagtacgt cgtctttagg ttggggggag gggttttatg 1020 cgatggagtt tccccacact gagtgggtgg agactgaagt taggccagct tggcacttga 1080 tgtaattctc cttggaattt gccctttttg agtttggatc ttggttcatt ctcaagcctc 1140 agacagtggt tcaaagtttt tttcttccat ttcaggtgtc gtgaagctct agagccatgg 1200 ccctccctgt caccgccctg ctgcttccgc tggctcttct gctccacgcc gctcggcccg 1260 aggtgaaact gcaggagtca ggacctggcc tggtggcgcc ctcacagagc ctgtccgtca 1320 catgcactgt ctcaggggtc tcattacccg actatggtgt aagctggatt cgccagcctc 1380 cacgaaaggg tctggagtgg ctgggagtaa tatggggtag tgaaaccaca tactataatt 1440 cagctctcaa atccagactg accatcatca aggacaactc caagagccaa gttttcttaa 1500 aaatgaacag tctgcaaact gatgacacag ccatttacta ctgtgccaaa cattattact 1560 acggtggtag ctatgctatg gactactggg gtcaaggaac ctcagtcacc gtctcctcag 1620 gtggaggtgg cagcggagga ggtgggtccg gcggtggagg aagcggcggt ggaggaagcg 1680 acatccagat gacacagact acatcctccc tgtctgcctc tctgggagac agagtcacca 1740 tcagttgcag ggcaagtcag gacattagta aatatttaaa ttggtatcag cagaaaccag 1800 atggaactgt taaactcctg atctaccata catcaagatt acactcagga gtcccatcaa 1860 ggttcagtgg cagtgggtct ggaacagatt attctctcac cattagcaac ctggagcaag 1920 aagatattgc cacttacttt tgccaacagg gtaatacgct tccgtacacg ttcggagggg 1980 ggactaagtt ggaaataaca cgggctgatg ctgcaccaac tgtatccatc ttcccaccat 2040 ccagtaatac cactacccca gcaccgaggc cacccacccc ggctcctacc atcgcctccc 2100 agcctctgtc cctgcgtccg gaggcatgta gacccgcagc tggtggggcc gtgcataccc 2160 ggggtcttga cttcgcctgc gatatctaca tttgggcccc tctggctggt acttgcgggg 2220 tcctgctgct ttcactcgtg atcactcttt actgtaagcg cggtcggaag aagctgctgt 2280 acatctttaa gcaacccttc atgaggcctg tgcagactac tcaagaggag gacggctgtt 2340 catgccggtt cccagaggag gaggaaggcg gctgcgaact gcgcgtgaaa ttcagccgca 2400 gcgcagatgc tccagcctac caacaggggc agaaccagct ctacaacgaa ctcaatcttg 2460 gtcggagaga ggagtacgac gtgctggaca agcggagagg acgggaccca gaaatgggcg 2520 ggaagccgcg cagaaagaat ccccaagagg gcctgtacaa cgagctccaa aaggataaga 2580 tggcagaagc ctatagcgag attggtatga aaggggaacg cagaagaggc aaaggccacg 2640 acggactgta ccagggactc agcaccgcca ccaaggacac ctatgacgct cttcacatgc 2700 aggccctgcc gcctcggggc tcaggagagg gcagaggaag tcttctaaca tgcggtgacg 2760 tggaggagaa tcccggccct atggtacacg tattggaacg ggcgcttttg gagcagcaaa 2820 gctccgcgtg cggactccca ggttcatcca cggaaacacg cccatctcat ccctgcccgg 2880 aggaccccga tgtatcacga cttaggctct tgctggtcgt actttgcgtg ctctttggac 2940 ttctgtgcct cctgctcatc ggctcaggag gttcaggtgc aacgaacttc tcattgttga 3000 agcaagccgg tgatgttgag gaaaatccgg gtcctatggt gagcaagggc gaggagctgt 3060 tcaccggggt ggtgcccatc ctggtcgagc tggacggcga cgtaaacggc cacaagttca 3120 gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg aagttcatct 3180 gcaccaccgg caagctgccc gtgccctggc ccaccctcgt gaccaccctg acctacggcg 3240 tgcagtgctt cagccgctac cccgaccaca tgaagcagca cgacttcttc aagtccgcca 3300 tgcccgaagg ctacgtccag gagcgcacca tcttcttcaa ggacgacggc aactacaaga 3360 cccgcgccga ggtgaagttc gagggcgaca ccctggtgaa ccgcatcgag ctgaagggca 3420 tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac tacaacagcc 3480 acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac ttcaagatcc 3540

		gccacaacat cgaggacggc agcgtgcagc tcgccgacca ctaccagcag aacaccccca 3600 tcggcgacgg ccccgtgctg ctgcccgaca accactacct gagcacccag tccgccctga 3660 gcaaagaccc caacgagaag cgcgatcaca tggtcctgct ggagttcgtg accgccgccg 3720 ggatcactct cggcatggac gagctgtaca agtaagtcga caatcaacct ctggattaca 3780 aaatttgtga aagattgact ggtattctta actatgttgc tccttttacg ctatgtggat 3840 acgctgcttt aatgcctttg tatcatgcta ttgcttcccg tatggctttc attttctcct 3900 ccttgtataa atcctggttg ctgtctcttt atgaggagtt gtggcccgtt gtcaggcaac 3960 gtggcgtggt gtgcactgtg tttgctgacg caacccccac tggttggggc attgccacca 4020 cctgtcagct cctttccggg actttcgctt tccccctccc tattgccacg gcggaactca 4080 tcgccgcctg ccttgcccgc tgctggacag gggctcggct gttgggcact gacaattccg 4140 tggtgttgtc ggggaagctg acgtcctttc cttggctgct cgcctgtgtt gccacctgga 4200 ttctgcgcgg gacgtccttc tgctacgtcc cttcggccct caatccagcg gaccttcctt 4260 cccgcggcct gctgccggct ctgcggcctc ttccgcgtct tcgccttcgc cctcagacga 4320 gtcggatctc cctttgggcc gcctccccgc ctg 4353
3-21	**Sequences**	
3-21-1	Sequence Number [ID]	21
3-21-2	Molecule Type	DNA
3-21-3	Length	311
3-21-4	Features Location/ Qualifiers NonEnglishQualifier Value	**source 1..311** mol_type=other DNA organism=synthetic construct
3-21-5	Residues	gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgttagagag 60 ataattagaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120 aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180 atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240 cgaaacaccg aacaacagga ttcacggatc agcttcaaga gagctgatcc gtgaatcctg 300 ttgttttttt t 311
3-22	**Sequences**	
3-22-1	Sequence Number [ID]	22
3-22-2	Molecule Type	DNA
3-22-3	Length	4348
3-22-4	Features Location/ Qualifiers NonEnglishQualifier Value	**source 1..4348** mol_type=other DNA organism=synthetic construct
3-22-5	Residues	ggctccggtg cccgtcagtg ggcagagcgc acatcgccca cagtccccga gaagttgggg 60 ggaggggtcg gcaattgaac cggtgcctag agaaggtggc gcggggtaaa ctgggaaagt 120 gatgtcgtgt actggctccg cctttttccc gagggtgggg gagaaccgta tataagtgca 180 gtagtcgccg tgaacgttct ttttcgcaac gggtttgccg ccagaacaca ggtaagtgcc 240 gtgtgtggtt cccgcgggcc tggcctcttt acgggttatg gcccttgcgt gccttgaatt 300 acttccacct ggctgcagta cgtgattctt gatcccgagc ttcgggttgg aagtgggtgg 360 gagagttcga ggccttgcgc ttaaggagcc ccttcgcctc gtgcttgagt tgaggcctgg 420 cctgggcgct ggggccgccg cgtgcgaatc tggtggcacc ttcgcgcctg tctcgctgct 480 ttcgataagt ctctagccat ttaaaatttt tgatgacctg ctgcgacgct ttttttctgg 540 caagatagtc ttgtaaatgc gggccaagat ctgcacactg gtatttcggt ttttggggcc 600 gcgggcggcg acggggcccg tgcgtcccag cgcacatgtt cggcgaggcg gggcctgcga 660 gcgcggccac cgagaatcgg acgggggtag tctcaagctg gccggcctgc tctggtgcct 720 ggcctcgcgc cgccgtgtat cgccccgccc tgggcggcaa ggctggcccg gtcggcacca 780 gttgcgtgag cggaaagatg gccgcttccc ggccctgctg cagggagctc aaaatggagg 840 acgcggcgct cgggagagcg ggcgggtgag tcacccacac aaaggaaaag ggcctttccg 900 tcctcagccg tcgcttcatg tgactccact gagtaccggg cgccgtccag gcacctcgat 960 tagttctcgt gcttttggag tacgtcgtct ttaggttggg gggaggggtt ttatgcgatg 1020 gagtttcccc acactgagtg ggtggagact gaagttaggc cagcttggca cttgatgtaa 1080 ttctccttgg aatttgccct ttttgagttt ggatcttggt tcattctcaa gcctcagaca 1140 gtggttcaaa gtttttttct tccatttcag gtgtcgtgaa gctctagagc catggccctc 1200 cctgtcaccg ccctgctgct tccgctggct cttctgctcc acgccgctcg gcccgaggtg 1260 aaactgcagg agtcaggacc tggcctggtg gcgccctcac agagcctgtc cgtcacatgc 1320 actgtctcag gggtctcatt acccgactat ggtgtaagct ggattcgcca gcctccacga 1380 aagggtctgg agtggctggg agtaatatgg ggtagtgaaa ccacatacta taattcagct 1440 ctcaaatcca gactgaccat catcaaggac aactccaaga gccaagtttt cttaaaaatg 1500 aacagtctgc aaactgatga cacagccatt tactactgtg ccaaacatta ttactacggt 1560 ggtagctatg ctatggacta ctggggtcaa ggaacctcag tcaccgtctc ctcaggtgga 1620 ggtggcagcg gaggaggtgg gtccggcggt ggaggaagcg gcggtggagg aagcgacatc 1680 cagatgacac agactacatc ctccctgtct gcctctctgg gagacagagt caccatcagt 1740

		tgcagggcaa gtcaggacat tagtaaatat ttaaattggt atcagcagaa accagatgga 1800 actgttaaac tcctgatcta ccatacatca agattacact caggagtccc atcaaggttc 1860 agtggcagtg ggtctggaac agattattct ctcaccatta gcaacctgga gcaagaagat 1920 attgccactt acttttgcca acagggtaat acgcttccgt acacgttcgg agggggact 1980 aagttggaaa taacacgggc tgatgctgca ccaactgtat ccatcttccc accatccagt 2040 aataccacta ccccagcacc gaggccaccc accccggctc ctaccatcgc ctcccagcct 2100 ctgtccctgc gtccggaggc atgtagaccc gcagctggtg gggccgtgca tacccggggt 2160 cttgacttcg cctgcgatat ctacatttgg gcccctctgg ctggtacttg cggggtcctg 2220 ctgctttcac tcgtgatcac tctttactgt aagcgcggtc ggaagaagct gctgtacatc 2280 tttaagcaac ccttcatgag gcctgtgcag actactcaag aggaggacgg ctgttcatgc 2340 cggttcccag aggaggagga aggcggctgc gaactgcgcg tgaaattcag ccgcagcgca 2400 gatgctccag cctaccaaca ggggcagaac cagctctaca acgaactcaa tcttggtcgg 2460 agagaggagt acgacgtgct ggacaagcgg agaggacggg acccagaaat gggcgggaag 2520 ccgcgcagaa agaatcccca agagggcctg tacaacgagc tccaaaagga taagatggca 2580 gaagcctata gcgagattgg tatgaaaggg gaacgcagaa gaggcaaagg ccacgacgga 2640 ctgtaccagg gactcagcac cgccaccaag gacacctatg acgctcttca catgcaggcc 2700 ctgccgcctc ggggctcagg agagggcaga ggaagtcttc taacatgcgg tgacgtggag 2760 gagaatcccg gccctatggt gagcaagggc gaggagctgt tcaccggggt ggtgcccatc 2820 ctggtcgagc tggacggcga cgtaaacggc cacaagttca gcgtgtccgg cgagggcgag 2880 ggcgatgcca cctacggcaa gctgaccctg aagttcatct gcaccaccgg caagctgccc 2940 gtgccctggc ccaccctcgt gaccaccctg acctacggcg tgcagtgctt cagccgctac 3000 cccgaccaca tgaagcagca cgacttcttc aagtccgcca tgcccgaagg ctacgtccag 3060 gagcgcacca tcttcttcaa ggacgacggc aactacaaga cccgcgccga ggtgaagttc 3120 gagggcgaca ccctggtgaa ccgcatcgag ctgaagggca tcgacttcaa ggaggacggc 3180 aacatcctgg ggcacaagct ggagtacaac tacaacagcc acaacgtcta tatcatggcc 3240 gacaagcaga agaacggcat caaggtgaac ttcaagatcc gccacaacat cgaggacggc 3300 agcgtgcagc tcgccgacca ctaccagcag aacaccccca tcggcgacgg ccccgtgctg 3360 ctgcccgaca accactacct gagcacccag tccgccctga gcaaagaccc caacgagaag 3420 cgcgatcaca tggtcctgct ggagttcgtg accgccgccg ggatcactct cggcatggac 3480 gagctgtaca agggttcagg tgcaacgaac ttctcattgt tgaagcaagc cggtgatgtt 3540 gaggaaaatc cgggtcctat ggtacacgta ttggaacggg cgcttttgga gcagcaaagc 3600 tccgcgtgcg gactcccagg ttcatccacg gaaacacgcc catctcatcc ctgcccggag 3660 gaccccgatg tatcacgact taggctcttg ctggtcgtac tttgcgtgct ctttggactt 3720 ctgtgcctcc tgctcatcgg ctcaggataa gtcgacaatc aacctctgga ttacaaaatt 3780 tgtgaaagat tgactggtat tcttaactat gttgctcctt ttacgctatg tggatacgct 3840 gctttaatgc ctttgtatca tgctattgct tcccgtatgg ctttcatttt ctcctccttg 3900 tataaatcct ggttgctgtc tctttatgag gagttgtggc ccgttgtcag gcaacgtggc 3960 gtggtgtgca ctgtgtttgc tgacgcaacc cccactggtt ggggcattgc caccacctgt 4020 cagctccttt ccgggacttt cgctttcccc ctccctattg ccacggcgga actcatcgcc 4080 gcctgccttg cccgctgctg gacaggggct cggctgttgg gcactgacaa ttccgtggtg 4140 ttgtcgggga agctgacgtc ctttccttgg ctgctcgcct gtgttgccac ctggattctg 4200 cgcgggacgt ccttctgcta cgtcccttcg gccctcaatc cagcggacct tccttcccgc 4260 ggcctgctgc cggctctgcg gcctcttccg cgtcttcgcc ttcgccctca gacgagtcgg 4320 atctcccttt gggccgcctc cccgcctg 4348
3-23	**Sequences**	
3-23-1	Sequence Number [ID]	23
3-23-2	Molecule Type	DNA
3-23-3	Length	311
3-23-4	Features Location/	**source 1..311**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-23-5	Residues	gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgttagagag 60 ataattagaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120 aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180 atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240 cgaaacaccg aacaacagga ttcacggatc agcttcaaga gagctgatcc gtgaatcctg 300 ttgttttttt t 311
3-24	**Sequences**	
3-24-1	Sequence Number [ID]	24
3-24-2	Molecule Type	DNA
3-24-3	Length	3549
3-24-4	Features Location/	**source 1..3549**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct

	NonEnglishQualifier Value	
3-24-5	Residues	ggctccggtg cccgtcagtg ggcagagcgc acatcgccca cagtccccga gaagttgggg 60 ggaggggtcg gcaattgaac cggtgcctag agaaggtggc gcggggtaaa ctgggaaagt 120 gatgtcgtgt actggctccg ccttttttccc gagggtgggg gagaaccgta tataagtgca 180 gtagtcgccg tgaacgttct ttttcgcaac gggtttgccg ccagaacaca ggtaagtgcc 240 gtgtgtggtt cccgcgggcc tggcctcttt acgggttatg gcccttgcgt gccttgaatt 300 acttccacct ggctgcagta cgtgattctt gatcccgagc ttcgggttgg aagtgggtgg 360 gagagttcga ggccttgcgc ttaaggagcc ccttcgcctc gtgcttgagt tgaggcctgg 420 cctgggcgct ggggccgccg cgtgcgaatc tggtggcacc ttcgcgcctg tctcgctgct 480 ttcgataagt ctctagccat ttaaaatttt tgatgacctg ctgcgacgct ttttttctgg 540 caagatagtc ttgtaaatgc gggccaagat ctgcacactg gtatttcggt ttttggggcc 600 gcgggcggcg acggggcccg tgcgtcccag cgcacatgtt cggcgaggcg gggcctgcga 660 gcgcggccac cgagaatcgg acgggggtag tctcaagctg gccggcctgc tctggtgcct 720 ggcctcgcgc cgccgtgtat cgccccgccc tgggcggcaa ggctggcccg gtcggcacca 780 gttgcgtgag cggaaagatg gccgcttccc ggccctgctg cagggagctc aaaatggagg 840 acgcggcgct cgggagagcg ggcgggtgag tcacccacac aaaggaaaag ggcctttccg 900 tcctcagccg tcgcttcatg tgactccact gagtaccggg cgccgtccag gcacctcgat 960 tagttctcgt gcttttggag tacgtcgtct ttaggttggg gggaggggtt ttatgcgatg 1020 gagtttcccc acactgagtg ggtggagact gaagttaggc cagcttggca cttgatgtaa 1080 ttctccttgg aatttgccct ttttgagttt ggatcttggt tcattctcaa gcctcagaca 1140 gtggttcaaa gtttttttct tccatttcag gtgtcgtgaa gctctagagc catggccctc 1200 cctgtcaccg ccctgctgct tccgctggct cttctgctcc acgccgctcg gcccgaggtg 1260 aaactgcagg agtcaggacc tggcctggtg gcgccctcac agagcctgtc cgtcacatgc 1320 actgtctcag gggtctcatt acccgactat ggtgtaagct ggattcgcca gcctccacga 1380 aagggtctgg agtggctggg agtaatatgg ggtagtgaaa ccacatacta taattcagct 1440 ctcaaatcca gactgaccat catcaaggac aactccaaga gccaagtttt cttaaaaatg 1500 aacagtctgc aaactgatga cacagccatt tactactgtg ccaaacatta ttactacggt 1560 ggtagctatg ctatggacta ctggggtcaa ggaacctcag tcaccgtctc ctcaggtgga 1620 ggtggcagcg gaggaggtgg gtccggcggt ggaggaagcg gcggtggagg aagcgacatc 1680 cagatgacac agactacatc ctccctgtct gcctctctgg gagacagagt caccatcagt 1740 tgcagggcaa gtcaggacat tagtaaatat ttaaattggt atcagcagaa accagatgga 1800 actgttaaac tcctgatcta ccatacatca agattacact caggagtccc atcaaggttc 1860 agtggcagtg ggtctggaac agattattct ctcaccatta gcaacctgga gcaagaagat 1920 attgccactt acttttgcca acagggtaat acgcttccgt acacgttcgg agggggact 1980 aagttggaaa taacacgggc tgatgctgca ccaactgtat ccatcttccc accatccagt 2040 aataccacta ccccagcacc gaggccaccc accccggctc ctaccatcgc ctcccagcct 2100 ctgtccctgc gtccggaggc atgtagaccc gcagctggtg gggccgtgca tacccggggt 2160 cttgacttcg cctgcgatat ctacatttgg gcccctctgg ctggtacttg cggggtcctg 2220 ctgctttcac tcgtgatcac tctttactgt aagcgcggtc ggaagaagct gctgtacatc 2280 tttaagcaac ccttcatgag gcctgtgcag actactcaag aggaggacgg ctgttcatgc 2340 cggttcccag aggaggagga aggcggctgc gaactgcgcg tgaaattcag ccgcagcgca 2400 gatgctccag cctaccaaca ggggcagaac cagctctaca acgaactcaa tcttggtcgg 2460 agagaggagt acgacgtgct ggacaagcgg agaggacggg acccagaaat gggcgggaag 2520 ccgcgcagaa agaatcccca agagggcctg tacaacgagc tccaaaagga taagatggca 2580 gaagcctata gcgagattgg tatgaaaggg gaacgcagaa gaggcaaagg ccacgacgga 2640 ctgtaccagg gactcagcac cgccaccaag gacacctatg acgctcttca catgcaggcc 2700 ctgccgcctc ggggctcagg agagggcaga ggaagtcttc taacatgcgg tgacgtggag 2760 gagaatcccg gccctatggt acacgtattg gaacgggcgc ttttggagca gcaaagctcc 2820 gcgtgcggac tcccaggttc atccacggaa acacgcccat ctcatccctg cccggaggac 2880 cccgatgtat cacgacttag gctcttgctg gtcgtacttt gcgtgctctt tggacttctg 2940 tgcctcctgc tcatctaaat caacctctgg attacaaaat ttgtgaaaga ttgactggta 3000 ttcttaacta tgttgctcct tttacgctat gtggatacgc tgctttaatg cctttgtatc 3060 atgctattgc ttcccgtatg gctttcattt tctcctcctt gtataaatcc tggttgctgt 3120 ctctttatga ggagttgtgg cccgttgtca ggcaacgtgg cgtggtgtgc actgtgtttg 3180 ctgacgcaac ccccactggt tggggcattg ccaccacctg tcagctcctt tccgggactt 3240 tcgctttccc cctccctatt gccacggcgg aactcatcgc cgcctgcctt gcccgctgct 3300 ggacaggggc tcggctgttg ggcactgaca attccgtggt gttgtcgggg aagctgacgt 3360 cctttccttg gctgctcgcc tgtgttgcca cctggattct gcgcgggacg tccttctgct 3420 acgtcccttc ggccctcaat ccagcggacc ttccttcccg cggcctgctg ccggctctgc 3480 ggcctcttcc gcgtcttcgc cttcgccctc agacgagtcg gatctccctt tgggccgcct 3540 ccccgcctg 3549
3-25	**Sequences**	
3-25-1	Sequence Number [ID]	25
3-25-2	Molecule Type	AA
3-25-3	Length	69

3-25-4	Features Location/	**source 1..69**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-25-5	Residues	TTTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA PLAGTCGVLL	60
		LSLVITLYC	69
3-26	**Sequences**		
3-26-1	Sequence Number [ID]	26	
3-26-2	Molecule Type	AA	
3-26-3	Length	42	
3-26-4	Features Location/	**source 1..42**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-26-5	Residues	KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL	42
3-27	**Sequences**		
3-27-1	Sequence Number [ID]	27	
3-27-2	Molecule Type	AA	
3-27-3	Length	112	
3-27-4	Features Location/	**source 1..112**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-27-5	Residues	RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN	60
		ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR	112
3-28	**Sequences**		
3-28-1	Sequence Number [ID]	28	
3-28-2	Molecule Type	AA	
3-28-3	Length	21	
3-28-4	Features Location/	**source 1..21**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-28-5	Residues	MALPVTALLL PLALLLHAAR P	21
3-29	**Sequences**		
3-29-1	Sequence Number [ID]	29	
3-29-2	Molecule Type	AA	
3-29-3	Length	21	
3-29-4	Features Location/	**source 1..21**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-29-5	Residues	METDTLLLWV LLLWVPGSTG D	21
3-30	**Sequences**		
3-30-1	Sequence Number [ID]	30	
3-30-2	Molecule Type	AA	
3-30-3	Length	5	
3-30-4	Features Location/	**source 1..5**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-30-5	Residues	GGGGS	5
3-31	**Sequences**		
3-31-1	Sequence Number [ID]	31	
3-31-2	Molecule Type	AA	
3-31-3	Length	12	
3-31-4	Features Location/	**source 1..12**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-31-5	Residues	GGGSGGGSGG GS	12
3-32	**Sequences**		
3-32-1	Sequence Number [ID]	32	
3-32-2	Molecule Type	AA	
3-32-3	Length	15	

3-32-4	Features Location/	**source 1..15**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-32-5	Residues	GGGGSGGGGS GGGGS	15
3-33	**Sequences**		
3-33-1	Sequence Number [ID]	33	
3-33-2	Molecule Type	AA	
3-33-3	Length	20	
3-33-4	Features Location/	**source 1..20**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-33-5	Residues	GGGGSGGGGS GGGGSGGGGS	20
3-34	**Sequences**		
3-34-1	Sequence Number [ID]	34	
3-34-2	Molecule Type	AA	
3-34-3	Length	18	
3-34-4	Features Location/	**source 1..18**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-34-5	Residues	GSTSGSGKPG SGEGSTKG	18
3-35	**Sequences**		
3-35-1	Sequence Number [ID]	35	
3-35-2	Molecule Type	AA	
3-35-3	Length	18	
3-35-4	Features Location/	**source 1..18**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-35-5	Residues	GGSSRSSSSG GGGSGGGG	18
3-36	**Sequences**		
3-36-1	Sequence Number [ID]	36	
3-36-2	Molecule Type	AA	
3-36-3	Length	24	
3-36-4	Features Location/	**source 1..24**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-36-5	Residues	SGGGGEGRGS LLTCGDVEEN PGPR	24
3-37	**Sequences**		
3-37-1	Sequence Number [ID]	37	
3-37-2	Molecule Type	AA	
3-37-3	Length	22	
3-37-4	Features Location/	**source 1..22**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-37-5	Residues	GSGATNFSLL KQAGDVEENP GP	22
3-38	**Sequences**		
3-38-1	Sequence Number [ID]	38	
3-38-2	Molecule Type	AA	
3-38-3	Length	6	
3-38-4	Features Location/	**source 1..6**	
	Qualifiers	mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-38-5	Residues	HHHHHH	6
3-39	**Sequences**		
3-39-1	Sequence Number [ID]	39	
3-39-2	Molecule Type	AA	
3-39-3	Length	120	
3-39-4	Features Location/	**source 1..120**	
	Qualifiers	mol_type=protein	

		organism=synthetic construct
	NonEnglishQualifier Value	
3-39-5	Residues	EVKLQESGPG LVAPSQSLSV TCTVSGVSLP DYGVSWIRQP PRKGLEWLGV IWGSETTYYN 60 SALKSRLTII KDNSKSQVFL KMNSLQTDDT AIYYCAKHYY YGGSYAMDYW GQGTSVTVSS 120
3-40	**Sequences**	
3-40-1	Sequence Number [ID]	40
3-40-2	Molecule Type	AA
3-40-3	Length	123
3-40-4	Features Location/	**source 1..123**
	Qualifiers	mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-40-5	Residues	DIQMTQTTSS LSASLGDRVT ISCRASQDIS KYLNWYQQKP DGTVKLLIYH TSRLHSGVPS 60 RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPYTFGG GTKLEITRAD AAPTVSIFPP 120 SSN 123
3-41	**Sequences**	
3-41-1	Sequence Number [ID]	41
3-41-2	Molecule Type	AA
3-41-3	Length	507
3-41-4	Features Location/	**source 1..507**
	Qualifiers	mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-41-5	Residues	MALPVTALLL PLALLLHAAR PEVKLQESGP GLVAPSQSLS VTCTVSGVSL PDYGVSWIRQ 60 PPRKGLEWLG VIWGSETTYY NSALKSRLTI IKDNSKSQVF LKMNSLQTDD TAIYYCAKHY 120 YYGGSYAMDY WGQGTSVTVS SGGGGSGGGG SGGGGSGGGG SDIQMTQTTS SLSASLGDRV 180 TISCRASQDI SKYLNWYQQK PDGTVKLLIY HTSRLHSGVP SRFSGSGSGT DYSLTISNLE 240 QEDIATYFCQ QGNTLPYTFG GGTKLEITRA DAAPTVSIFP PSSNTTTPAP RPPTPAPTIA 300 SQPLSLRPEA CRPAAGGAVH TRGLDFACDI YIWAPLAGTC GVLLLSLVIT LYCKRGRKKL 360 LYIFKQPFMR PVQTTQEEDG CSCRFPEEEE GGCELRVKFS RSADAPAYQQ GQNQLYNELN 420 LGRREEYDVL DKRRGRDPEM GGKPRRKNPQ EGLYNELQKD KMAEAYSEIG MKGERRRGKG 480 HDGLYQGLST ATKDTYDALH MQALPPR 507