



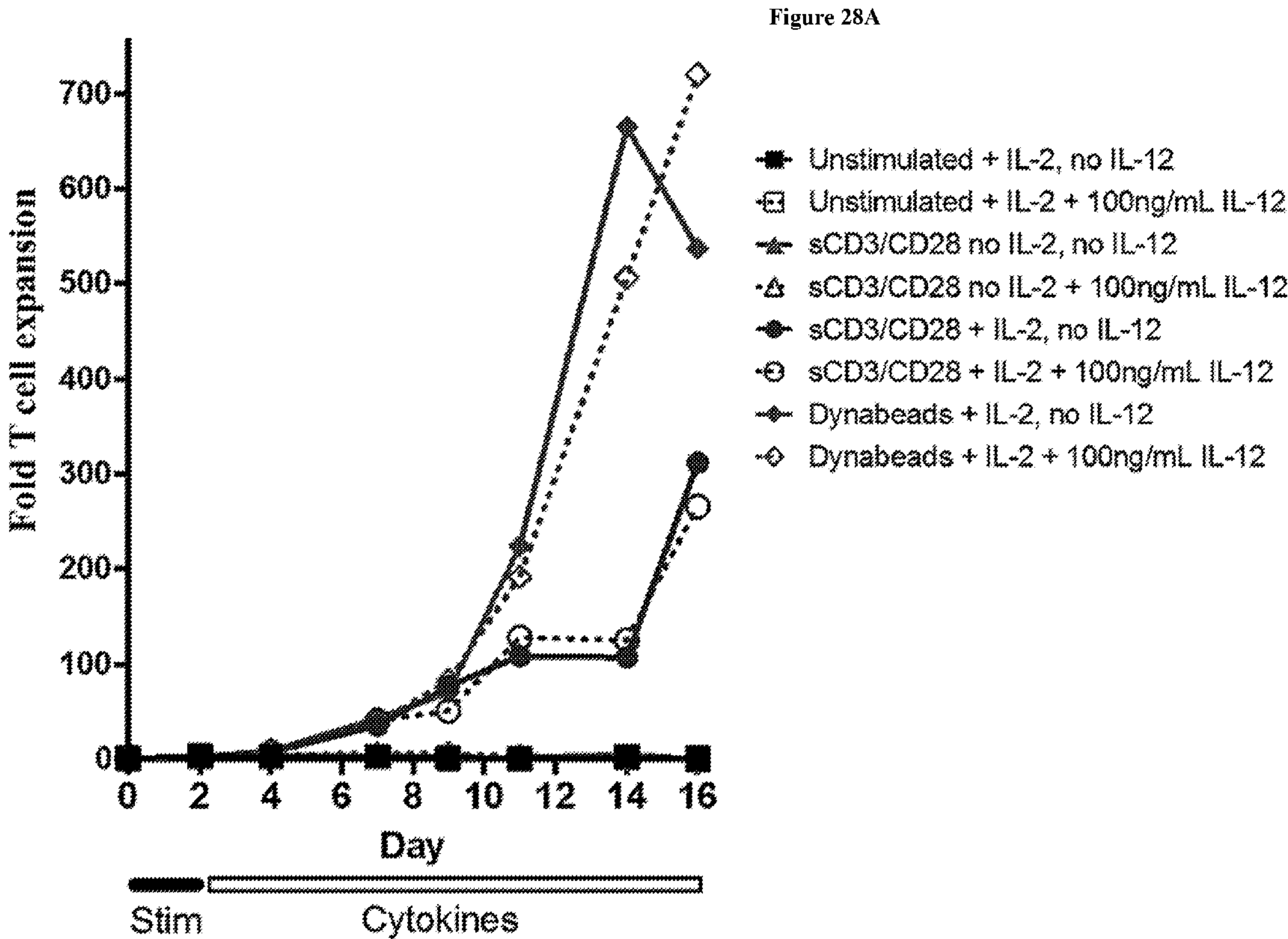
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(54) Titre : COMPOSITIONS A BASE DE CD19 ET METHODES POUR L'IMMUNOTHERAPIE
(54) Title: CD19 COMPOSITIONS AND METHODS FOR IMMUNOTHERAPY



(57) Abrégé/Abstract:
The present invention provides biocircuit systems, effector modules and compositions for cancer immunotherapy. Methods for inducing anti-cancer immune responses in a subject are also provided.

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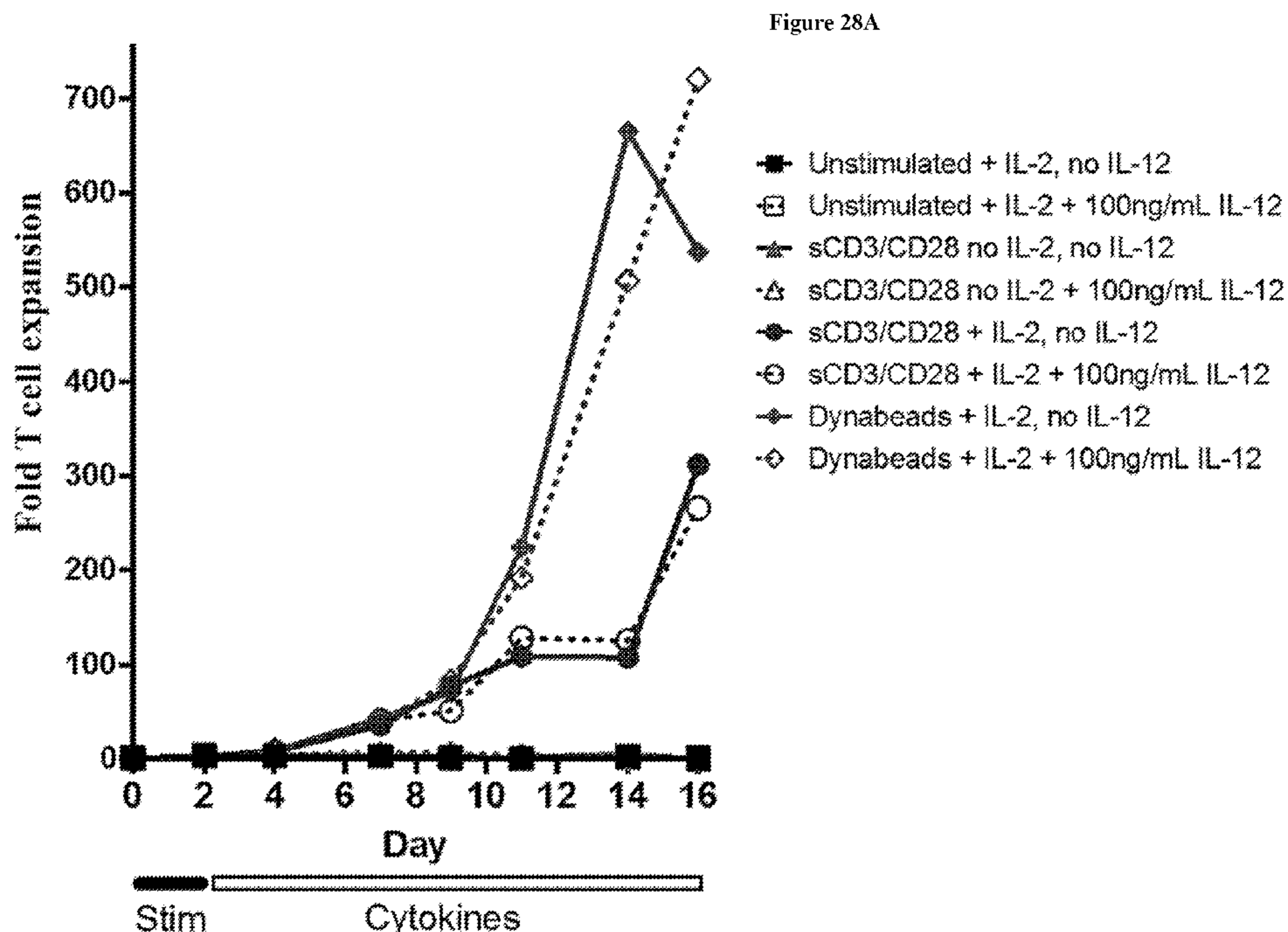
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JUMBO APPLICATIONS/PATENTS

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CONTAINING PAGES 1 TO 153

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CD19 COMPOSITIONS AND METHODS FOR IMMUNOTHERAPY**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to the US Provisional Patent Application No. 62/466,601, filed on March 3, 2017 entitled Compositions and Methods for Immunotherapy and the US Provisional Patent Application No. 62/484,052, filed on April 11, 2017 entitled Anti CD19 compositions and methods for immunotherapy, the contents of each of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 2095_1201PCT_SL.txt, created on March 2, 2018, which is 2,116,001 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to compositions and methods for immunotherapy. Provided in the present invention include polypeptides of biocircuit systems, effector modules, stimulus response elements (SREs) and immunotherapeutic agents, polynucleotides encoding the same, vectors and cells containing the polypeptides and/or polynucleotides for use in cancer immunotherapy. In one embodiment, the compositions comprise destabilizing domains (DDs) which tune protein stability.

BACKGROUND OF THE INVENTION

[0004] Cancer immunotherapy aims to eradicate cancer cells by rejuvenating the tumoricidal functions of tumor-reactive immune cells, predominantly T cells. Strategies of cancer immunotherapy including the recent development of checkpoint blockade, adoptive cell transfer (ACT) and cancer vaccines which can increase the anti-tumor immune effector cells have produced remarkable results in several tumors.

[0005] The impact of host anti-tumor immunity and cancer immunotherapy is impeded by three major hurdles: 1) low number of tumor antigen-specific T cells due to clonal deletion; 2) poor activation of innate immune cells and accumulation of tolerogenic antigen-presenting cells in the tumor microenvironment; and 3) formation of an immunosuppressive tumor microenvironment. Particularly, in solid tumors the therapeutic efficacy of immunotherapeutic regimens remains unsatisfactory due to lack of an effective anti-tumor response in the immunosuppressive tumor microenvironment. Tumor cells often induce immune tolerance or suppression and such tolerance is acquired because even truly foreign tumor antigens will

become tolerated. Such tolerance is also active and dominant because cancer vaccines and adoptive transfer of pre-activated immune effector cells (e.g., T cells), are subject to suppression by inhibitory factors in the tumor microenvironment (TME).

[0006] In addition, administration of engineered T cells could result in on/off target toxicities as well as a cytokine release syndrome (reviewed by Tey *Clin. Transl. Immunol.*, 2014, 3: e1710.1038).

[0007] Development of a tunable switch that can turn on or off the transgenic immunotherapeutic agent expression is needed in case of adverse events. For example, adoptive cell therapies may have a very long and an indefinite half-life. Since toxicity can be progressive, a safety switch is desired to eliminate the infused cells. Systems and methods that can tune the transgenic protein level and expression window with high flexibility can enhance therapeutic benefit, and reduce potential side effects.

[0008] To develop regulatable therapeutic agents for disease therapy, in particular cancer immunotherapy, the present invention provides biocircuit systems to control the expression of immunotherapeutic agents. The biocircuit system comprises a stimulus and at least one effector module that responds to the stimulus. The effector module may include a stimulus response element (SRE) that binds and is responsive to a stimulus and an immunotherapeutic agent operably linked to the SRE. In one example, a SRE is a destabilizing domain (DD) which is destabilized in the absence of its specific ligand and can be stabilized by binding to its specific ligand.

SUMMARY OF THE INVENTION

[0009] The present invention provides compositions and methods for immunotherapy. The compositions relate to tunable systems and agents that induce anti-cancer immune responses in a cell or in a subject. The tunable system and agent may be a biocircuit system comprising at least one effector module that is responsive to at least one stimulus. The biocircuit system may be, but is not limited to, a destabilizing domain (DD) biocircuit system, a dimerization biocircuit system, a receptor biocircuit system, and a cell biocircuit system. These systems are further taught in co-owned U.S. Provisional Patent Application No. 62/320,864 filed April 11, 2016, 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587 (the contents of each of which are herein incorporated by reference in their entirety).

[0010] In some embodiments, the composition for inducing an immune response may comprise an effector module. In some embodiments, the effector module may comprise a stimulus response element (SRE) operably linked to at least one payload. In one aspect, the payload may be an immunotherapeutic agent.

[0011] In some embodiments, the immunotherapeutic agent may be selected from, but is not limited to a chimeric antigen receptor (CAR) and an antibody.

[0012] In one aspect, the SRE of the composition may be responsive to or interact with at least one stimulus.

[0013] In some embodiments, the SRE may comprise a destabilizing domain (DD). The DD may be derived from a parent protein or from a mutant protein having one, two, three, or more amino acid mutations compared to the parent protein. In some embodiments, the parent protein may be selected from, but is not limited to, human protein FKBP, comprising the amino acid sequence of SEQ ID NO. 3; human DHFR (hDHFR), comprising the amino acid sequence of SEQ ID NO. 2; E. Coli DHFR, comprising the amino acid sequence of SEQ ID NO. 1; PDE5, comprising the amino acid sequence of SEQ ID NO. 4; PPAR, gamma comprising the amino acid sequence of SEQ ID NO. 5; CA2, comprising the amino acid sequence of SEQ ID NO. 6; or NQO2, comprising the amino acid sequence of SEQ ID NO. 7.

[0014] In one aspect, the parent protein is hDHFR and the DD comprises a mutant protein. The mutant protein may comprise a single mutation and may be selected from, but not limited to hDHFR (I17V), hDHFR (F59S), hDHFR (N65D), hDHFR (K81R), hDHFR (A107V), hDHFR (Y122I), hDHFR (N127Y), hDHFR (M140I), hDHFR (K185E), hDHFR (N186D), and hDHFR (M140I), hDHFR (Amino acid 2-187 of WT; N127Y), hDHFR (Amino acid 2-187 of WT; I17V), hDHFR (Amino acid 2-187 of WT; Y122I), and hDHFR (Amino acid 2-187 of WT; K185E). In some embodiments, the mutant protein may comprise two mutations and may be selected from, but not limited to, hDHFR (C7R, Y163C), hDHFR (A10V, H88Y), hDHFR (Q36K, Y122I), hDHFR (M53T, R138I), hDHFR (T57A, I72A), hDHFR (E63G, I176F), hDHFR (G21T, Y122I), hDHFR (L74N, Y122I), hDHFR (V75F, Y122I), hDHFR (L94A, T147A), DHFR (V121A, Y22I), hDHFR (Y122I, A125F), hDHFR (H131R, E144G), hDHFR (T137R, F143L), hDHFR (Y178H, E181G), and hDHFR (Y183H, K185E), hDHFR (E162G, I176F) hDHFR (Amino acid 2-187 of WT; I17V, Y122I), hDHFR (Amino acid 2-187 of WT; Y122I, M140I), hDHFR (Amino acid 2-187 of WT; N127Y, Y122I), hDHFR (Amino acid 2-187 of WT; E162G, I176F), and hDHFR (Amino acid 2-187 of WT; H131R, E144G), and hDHFR (Amino acid 2-187 of WT; Y122I, A125F). In some embodiments, the mutant may comprise three mutations and the mutant may be selected from hDHFR (V9A, S93R, P150L), hDHFR (I8V, K133E, Y163C), hDHFR (L23S, V121A, Y157C), hDHFR (K19E, F89L, E181G), hDHFR (Q36F, N65F, Y122I), hDHFR (G54R, M140V, S168C), hDHFR (V110A, V136M, K177R), hDHFR (Q36F, Y122I, A125F), hDHFR (N49D, F59S, D153G), and hDHFR (G21E, I72V, I176T), hDHFR (Amino acid 2-187 of WT; Q36F, Y122I, A125F), hDHFR (Amino acid

2-187 of WT; Y122I, H131R, E144G), hDHFR (Amino acid 2-187 of WT; E31D, F32M, V116I), and hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I). In some embodiments, the mutant may comprise four or more mutations and the mutant may be selected from hDHFR (V2A, R33G, Q36R, L100P, K185R), hDHFR (Amino acid 2-187 of WT; D22S, F32M, R33S, Q36S, N65S), hDHFR (I17N, L98S, K99R, M112T, E151G, E162G, E172G), hDHFR (G16S, I17V, F89L, D96G, K123E, M140V, D146G, K156R), hDHFR (K81R, K99R, L100P, E102G, N108D, K123R, H128R, D142G, F180L, K185E), hDHFR (R138G, D142G, F143S, K156R, K158E, E162G, V166A, K177E, Y178C, K185E, N186S), hDHFR (N14S, P24S, F35L, M53T, K56E, R92G, S93G, N127S, H128Y, F135L, F143S, L159P, L160P, E173A, F180L), hDHFR (F35L, R37G, N65A, L68S, K69E, R71G, L80P, K99G, G117D, L132P, I139V, M140I, D142G, D146G, E173G, D187G), hDHFR (L28P, N30H, M38V, V44A, L68S, N73G, R78G, A97T, K99R, A107T, K109R, D111N, L134P, F135V, T147A, I152V, K158R, E172G, V182A, E184R), hDHFR (V2A, I17V, N30D, E31G, Q36R, F59S, K69E, I72T, H88Y, F89L, N108D, K109E, V110A, I115V, Y122D, L132P, F135S, M140V, E144G, T147A, Y157C, V170A, K174R, N186S), hDHFR (L100P, E102G, Q103R, P104S, E105G, N108D, V113A, W114R, Y122C, M126I, N127R, H128Y, L132P, F135P, I139T, F148S, F149L, I152V, D153A, D169G, V170A, I176A, K177R, V182A, K185R, N186S), and hDHFR (A10T, Q13R, N14S, N20D, P24S, N30S, M38T, T40A, K47R, N49S, K56R, I61T, K64R, K69R, I72A, R78G, E82G, F89L, D96G, N108D, M112V, W114R, Y122D, K123E, I139V, Q141R, D142G, F148L, E151G, E155G, Y157R, Q171R, Y183C, E184G, K185del, D187N).

[0015] In one aspect, the stimulus of the SRE may be Trimethoprim or Methotrexate.

[0016] In some embodiments, the immunotherapeutic agent of the effector module is a chimeric antigen receptor (CAR). The chimeric antigen may comprise an extracellular target moiety; a transmembrane domain; an intracellular signaling domain; and optionally, one or more co-stimulatory domains.

[0017] In one aspect, the CAR may be selected from, but is not limited to a standard CAR, a split CAR, an off-switch CAR, an on-switch CAR, a first-generation CAR, a second-generation CAR, a third-generation CAR, or a fourth-generation CAR.

[0018] In some embodiments, the extracellular target moiety of the CAR may be selected from, but is not limited to an Ig NAR, a Fab fragment, a Fab' fragment, a F(ab)'2 fragment, a F(ab)'3 fragment, an Fv, a single chain variable fragment (scFv), a bis-scFv, a (scFv)2, a minibody, a diabody, a triabody, a tetrabody, an intrabody, a disulfide stabilized Fv protein (dsFv), a unibody, a nanobody, and an antigen binding region derived from an antibody that may

specifically bind to any of a protein of interest, a ligand, a receptor, a receptor fragment or a peptide aptamer.

[0019] In one aspect, the extracellular target moiety may be an scFv derived from an antibody.

In one aspect, the scFv may specifically bind to a CD19 antigen

[0020] In one aspect, the scFv of the CAR may be a CD19 scFv. In some embodiments, the CD19 scFv may comprise a heavy chain variable region having an amino acid sequence independently selected from the group consisting of SEQ ID NO: 49-80, and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122. In some embodiments, the CD19 scFv may comprise an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267 and 624.

[0021] In some embodiments, the intracellular signaling domain of the CAR may be a signaling domain derived from T cell receptor CD3zeta. In some embodiments, the intracellular signaling domain may be selected from a cell surface molecule selected from the group consisting of FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. In one aspect, the CAR may include a co-stimulatory domain. In some embodiments, the co-stimulatory domain may be selected from the group consisting of 2B4, HVEM, ICOS, LAG3, DAP10, DAP12, CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, ICOS (CD278), glucocorticoid-induced tumor necrosis factor receptor (GITR), lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3.

[0022] (b) the co-stimulatory domain is present and is selected from the group consisting of 2B4, HVEM, ICOS, LAG3, DAP10, DAP12, CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, ICOS (CD278), glucocorticoid-induced tumor necrosis factor receptor (GITR), lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3.

[0023] In some embodiments, the intracellular signaling domain of the CAR may be a T cell receptor CD3zeta signaling domain, which may comprise the amino acid sequence of SEQ ID NO: 339.

[0024] In some embodiments, T cell receptor CD3zeta signaling domain of the CAR, comprising the amino acid sequence of SEQ ID NO: 626 may further comprise at least one co-stimulatory domain. The co-stimulatory domain may comprise an amino acid sequence of SEQ ID NOs: 268-374.

[0025] In one embodiment, the transmembrane domain of the CAR may be derived from a transmembrane region of an alpha, beta or zeta chain of a T-cell receptor. In one aspect, the transmembrane domain may be derived from the CD3 epsilon chain of a T-cell receptor. In one embodiment, the transmembrane domain may be derived from a molecule selected from CD4,

CD5, CD8, CD8 α , CD9, CD16, CD22, CD33, CD28, CD37, CD45, CD64, CD80, CD86, CD148, DAP 10, EpoRI, GITR, LAG3, ICOS, Her2, OX40 (CD134), 4-1BB (CD137), CD152, CD154, PD-1, or CTLA-4. In another embodiment, the transmembrane domain may be derived from an immunoglobulin selected from IgG1, IgD, IgG4, and an IgG4 Fc region. In one aspect, the transmembrane domain may comprise an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 375-425 and 897-907.

[0026] In some embodiments, the CAR of the effector module may further comprise a hinge region near the transmembrane domain. In one aspect, the hinge region may comprise an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 426-504.

[0027] In some embodiments, the immunotherapeutic agent may be an antibody that is specifically immunoreactive to an antigen selected from a tumor specific antigen (TSA), a tumor associated antigen (TAA), or an antigenic epitope.

[0028] In one aspect, the antigen may be an antigenic epitope. In some embodiments, the antigenic epitope may be CD19.

[0029] In some embodiments, the antibody may comprise a heavy chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 49-80 and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122. In one aspect, the antibody may comprise an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267 and 624.

[0030] In one aspect, the first effector module may comprise the amino acid sequence of any of SEQ ID NO: 635-649, 1005-1010, 1015-1018 and 1215-1231.

[0031] In some embodiments, the first SRE of the effector module may stabilize the immunotherapeutic agent by a stabilization ratio of 1 or more, wherein the stabilization ratio may comprise the ratio of expression, function or level of the immunotherapeutic agent in the presence of the stimulus to the expression, function or level of the immunotherapeutic agent in the absence of the stimulus.

[0032] In some embodiments, the SRE may destabilize the immunotherapeutic agent by a destabilization ratio between 0, and 0.09, wherein the destabilization ratio may comprise the ratio of expression, function or level of the immunotherapeutic agent in the absence of the stimulus specific to the SRE to the expression, function or level of the immunotherapeutic agent that is expressed constitutively, and in the absence of the stimulus specific to the SRE.

[0033] The present invention also provides polynucleotides comprising the compositions of the invention.

[0034] In one aspect, the polynucleotides may be a DNA or RNA molecule. In one aspect, the polynucleotides may comprise spatiotemporally selected codons. In one aspect, the polynucleotides of the invention may be a DNA molecule. In some embodiments, the polynucleotides may be an RNA molecule. In one aspect, the RNA molecule may be a messenger molecule. In some embodiments, the RNA molecule may be chemically modified.

[0035] In some embodiments, the polynucleotides may further comprise, at least one additional feature selected from, but not limited to, a promoter, a linker, a signal peptide, a tag, a cleavage site and a targeting peptide.

[0036] The present invention also provides vectors comprising polynucleotides described herein. In one aspect, the vector may be a viral vector. In some embodiments, the viral vector may be a retroviral vector, a lentiviral vector, a gamma retroviral vector, a recombinant AAV vector, an adeno viral vector, and an oncolytic viral vector.

[0037] The present invention also provides immune cells for adoptive cell transfer (ACT) which may express the compositions of the invention, the polynucleotides described herein. In one aspect, the immune cells may be infected or transfected with the vectors described herein. The immune cells for ACT may be selected from, but not limited to a CD8⁺ T cell, a CD4⁺ T cell, a helper T cell, a natural killer (NK) cell, a NKT cell, a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), a memory T cell, a regulatory T (Treg) cell, a cytokine-induced killer (CIK) cell, a dendritic cell, a human embryonic stem cell, a mesenchymal stem cell, a hematopoietic stem cell, or a mixture thereof.

[0038] In some embodiments, the immune cells may be autologous, allogeneic, syngeneic, or xenogeneic in relation to a particular individual subject.

[0039] In some embodiments, the immune cell may further express a composition comprising a second effector module, said second effector module comprising a second SRE linked to a second immunotherapeutic agent. In one aspect, the second immunotherapeutic agent may be selected from a cytokine, and a cytokine- cytokine receptor fusion.

[0040] In one aspect, the second immunotherapeutic agent may be a cytokine. In one aspect, the cytokine may be IL12 or IL15.

[0041] In one aspect, the second immunotherapeutic agent may be a cytokine- cytokine receptor fusion polypeptide.

[0042] In some embodiments, the cytokine-cytokine receptor fusion polypeptide may be selected from, but is not limited to a IL12-IL12 receptor fusion polypeptide, a IL15-IL15 receptor fusion polypeptide, and a IL15-IL15 receptor sushi domain fusion polypeptide.

[0043] The present invention provides methods for reducing a tumor volume or burden in a subject comprising contacting the subject with the immune cells of the invention. Also provided herein, is a method for inducing an anti-tumor immune response in a subject, comprising administering the immune cells of the system to the subject.

[0044] The present invention also provides methods for enhancing the expansion and/or survival of immune cells, comprising contacting the immune cells with the compositions of the invention, the polynucleotides of the invention, and/or the vectors of the invention.

[0045] Also provided herein, is a method for inducing an immune response in a subject, administering the compositions of the invention, the polynucleotides of the invention, and/or the immune cells of the invention to the subject.

[0046] The present invention also provides a method of identifying a domain of a CD19 antigen which will not bind the FMC63 antibody (FMC63-distinct CD19 binding domain). The method may comprise (a) preparing a composition comprising a CD19 antigen, (b) contacting the composition in (a) with saturating levels of FMC63 antibody, (c) contacting the composition of step (b) with one or more selected members of a library of potential CD19 binders; and (d) identifying a binding domain on the CD19 antigen based on the differential binding of the selected members of the library of CD19 binders compared to the binding of FMC63. In some embodiments, the binding domains of the library may be generated using phage display techniques with the CD19 antigen as the seed sequence. In one aspect, the binding domain may be selected from a Fab fragment, a Fab' fragment, a F(ab)'₂ fragment, a F(ab)'₃ fragment, Fv, a single chain variable fragment (scFv), a bis-scFv, a (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein (dsFv), a unibody, a nanobody, or an antigen binding region of an antibody, and an antibody fragment. In one aspect, the CD19 antigen may be selected from a whole or a portion of a human CD19 antigen, and a whole or a portion of a Rhesus CD19 antigen.

[0047] The present invention also provides chimeric antigen receptors that may comprise the FMC63-distinct CD19 binding domain obtained according to the methods described herein. Also, provided herein is a stimulus response element (SRE) operably linked to the chimeric antigen receptors that include the FMC63-distinct CD19 binding domain.

[0048] In some embodiments, the effector module comprises a stimulus response element (SRE) and at least one payload comprising a protein of interest (POI).

[0049] In some embodiments, the SRE may be a destabilizing domain (DD). In some examples, the DD is a mutant domain derived from a protein such as FKBP (FK506 binding

protein), *E. coli* DHFR (Dihydrofolate reductase) (ecDHFR), human DHFR (hDHFR), or any protein of interest. In this context, the biocircuit system is a DD biocircuit system.

[0050] The payload may be any immunotherapeutic agent used for cancer immunotherapy such as a chimeric antigen receptor (CAR) such as CD19 CAR that targets any molecule of tumor cells, an antibody, an antigen binding domain or combination of antigen binding domains, a cytokine such as IL12, IL15 or IL15/IL15Ra fusion, or any agent that can induce an immune response. The SRE and payload may be operably linked through one or more linkers and the positions of components may vary within the effector module.

[0051] In some embodiments, the effector module may further comprise of one or more additional features such as linker sequences (with specific sequences and lengths), cleavage sites, regulatory elements (that regulate expression of the protein of interest such as microRNA targeting sites), signal sequences that lead the effector module to a specific cellular or subcellular location, penetrating sequences, or tags and biomarkers for tracking the effector module.

[0052] In some embodiments, the DD may stabilize the immunotherapeutic agent with a stabilization ratio of at least one in the presence of the stimulus. According to the present invention, the DD may destabilize the immunotherapeutic agent in the absence of ligand with a destabilization ratio between 0, and 0.99.

[0053] The invention provides isolated biocircuit polypeptides, effector modules, stimulus response elements (SREs) and payloads, as well as polynucleotides encoding any of the foregoing; vectors comprising polynucleotides of the invention; and cells expressing polypeptides, polynucleotides and vectors of the invention. The polypeptides, polynucleotides, viral vectors and cells are useful for inducing anti-tumor immune responses in a subject.

[0054] In some embodiments, the vector of the invention is a viral vector. The viral vector may include, but is not limited to a retroviral vector, an adenoviral vector, an adeno-associated viral vector, or a lentiviral vector.

[0055] In some embodiments, the vector of the invention may be a non-viral vector, such as a nanoparticles and liposomes.

[0056] The present invention also provides immune cells engineered to include one or more polypeptides, polynucleotides, or vectors of the present invention. The cells may be immune effector cells, including T cells such as cytotoxic T cells, helper T cells, memory T cells, regulatory T cells, natural killer (NK) cells, NK T cells, cytokine-induced killer (CIK) cells, cytotoxic T lymphocytes (CTLs), and tumor infiltrating lymphocytes (TILs). The engineered cell may be used for adoptive cell transfer for treating a disease (e.g., a cancer).

[0057] The present invention also provides methods for inducing immune responses in a subject using the compositions of the invention. Also provided are methods for reducing a tumor burden in a subject using the compositions of the invention.

[0058] Also provided herein are methods for identifying FMC63-distinct binding domains and using CD19 antigens in which the FMC63 binding epitope is masked or absent. In some embodiments, the FMC63 binding domain may be included in the payloads and effector modules of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] Figure 1 shows an overview diagram of a biocircuit system of the invention. The biocircuit comprises a stimulus and at least one effector module responsive to a stimulus, where the response to the stimulus produces a signal or outcome. The effector module comprises at least one stimulus response element (SRE) and one payload.

[0060] Figure 2 shows representative effector modules carrying one payload. The signal sequence (SS), SRE and payload may be located or positioned in various arrangements without (A to F) or with (G to Z, and AA to DD) a cleavage site. An optional linker may be inserted between each component of the effector module.

[0061] Figure 3 shows representative effector modules carrying two payloads without a cleavage site. The two payloads may be either directly linked to each other or separated.

[0062] Figure 4 shows representative effector modules carrying two payloads with a cleavage site. In one embodiment, an SS is positioned at the N-terminus of the construct, while other components: SRE, two payloads and the cleavage site may be located at different positions (A to L). In another embodiment, the cleavage site is positioned at the N-terminus of the construct (M to X). An optional linker may be inserted between each component of the effector module.

[0063] Figure 5 shows effector modules of the invention carrying two payloads, where an SRE is positioned at the N-terminus of the construct (A to L), while SS, two payloads and the cleavage site can be in any configuration. An optional linker may be inserted between each component of the effector module.

[0064] Figure 6 shows effector modules of the invention carrying two payloads, where either the two payloads (A to F) or one of the two payloads (G to X) is positioned at the N-terminus of the construct (A to L), while SS, SRE and the cleavage site can be in any configuration. An optional linker may be inserted between each component of the effector module.

[0065] Figure 7 depicts representative configurations of the stimulus and effector module within a biocircuit system. A trans-membrane effector module is activated either by a free stimulus (Figure 7A) or a membrane bound stimulus (Figure 7B) which binds to SRE. The

response to the stimulus causes the cleavage of the intracellular signal/payload, which activates down-stream effector/payload.

[0066] Figure 8 depicts a dual stimulus-dual presenter biocircuit system, where two bound stimuli (A and B) from two different presenters (e.g., different cells) bind to two different effector modules in a single receiver (e.g., another single cell) simultaneously and create a dual-signal to downstream payloads.

[0067] Figure 9 depicts a dual stimulus-single presenter biocircuit system, where two bound stimuli (A and B) from the same presenter (e.g., a single cell) bind to two different effector modules in another single cell simultaneously and create a dual-signal.

[0068] Figure 10 depicts a single-stimulus-bridged receiver biocircuit system. In this configuration, a bound stimulus (A) binds to an effector module in the bridge cell and creates a signal to activate a payload which is a stimulus (B) for another effector module in the final receiver (e.g., another cell).

[0069] Figure 11 depicts a single stimulus-single receiver biocircuit system, wherein the single receiver contains the two effector modules which are sequentially activated by a single stimulus.

[0070] Figure 12 depicts a biocircuit system which requires a dual activation. In this embodiment, one stimulus must bind the transmembrane effector module first to prime the receiver cell being activated by the other stimulus. The receiver only activates when it senses both stimuli (B).

[0071] Figure 13 depicts a standard effector module of a chimeric antigen receptor (CAR) system which comprises an antigen binding domain as an SRE, and signaling domain(s) as payload.

[0072] Figure 14 depicts the structure design of a regulatable CAR system, where the trans-membrane effector modules comprise antigen binding domains sensing an antigen and a first switch domain and the intracellular module comprises a second switch domain and signaling domains. A stimulus (e.g., a dimerization small molecule) can dimerize the first and second switch domains and assemble an activated CAR system.

[0073] Figure 15 shows schematic representation of CAR systems having one (A) or two (B and C) SREs incorporated into the effector module.

[0074] Figure 16 depicts a split CAR design to control T cell activation by a dual stimulus (e.g., an antigen and small molecule). Figure 16A shows normal T cell activation which entails a dual activation of TCR and co-stimulatory receptor. The regular CAR design (Figure 16B) combines the antigen recognition domain with TCR signaling motif and co-stimulatory motif in a single molecule. The split CAR system separates the components of the regular CAR into two

separate effector modules which can be reassembled when a heterodimerizing small molecule (stimulus) is present.

[0075] Figure 17 depicts the positive and negative regulation of CAR engineered T cell activation. The absence or presence of a second stimulus can negatively (A) or positively (B) control T cell activation.

[0076] Figure 18 shows schematic representation of gated activation of CAR engineered T cells. If a normal cell that has no stimulus (e.g., an antigen) (Figure 18A) or an antigen that cannot bind to the trans-membrane effector module (Figure 18B), or only an antigen that activates the trans-membrane effector module and primes the receiver T cell to express the second effector (Fig 18C), the receiver T cell remains inactive. When both stimuli (e.g. two antigens) that bind the trans-membrane effector module and the primed effector, are present on the presenter cell (e.g. a cancer cell), the T cell is activated (Figure 18D).

[0077] Figure 19A is a bar graph depicting IL12 levels in the various dilutions of media derived from cells expressing DD-IL12. Figure 19B is a bar graph depicting the Shield-1 dose responsive induction of DD- IL12. Figure 19C depicts plasma IL12 levels in mice implanted with SKOV3 cells. Figure 19D depicts plasma IL12 levels in mice in response to different Shield-1 dosing regimens.

[0078] Figure 20A is a western blot of IL15 protein levels in 293 cells. Figure 20B and 20C are histograms depicting surface expression of IL15 and IL15Ra. Figure 20 D is a western blot of IL15 and hDHFR in HCT116 cells.

[0079] Figure 21A and Figure 21B are western blots of depicting the protein levels of CD3 Zeta of the DD- CD19 CAR construct and actin. Figure 21C shows the expression of CD19 chimeric antigen receptors in a western blot using 4-1BB antibody. Figure 21D is a bar graph depicting the surface expression of CD19 CAR.

[0080] Figure 22 denotes the frequency of IFNgamma positive T cells.

[0081] Figure 23A depicts IFN gamma production in T cells. Figure 23B depicts T cell expansion with IL15/IL15Ra treatment. Figure 23C is a dot plot depicting percentage human cells after *in vivo* cell transfer. Figure 23D is scatter plot depicting CD4+/CD8+ T cells.

[0082] Figure 24A depicts T cell subpopulations expressing CD19 CAR. Figure 28B depicts cell death caused by CD19 CAR expressing T cells.

[0083] Figure 25A is a bar graph depicting IL15Ra positive cells with 24 hour TMP treatment. Figure 25B is a bar graph depicting IL15Ra positive cells with 48 hour TMP treatment. Figure 25C is a bar graph depicting IL15Ra positive cells in response to varying concentrations of TMP.

[0084] Figure 26 is a western blot of IL15Ra protein levels in HCT116 cells.

[0085] Figure 27A represents percentage of human T cells blood with respect to mouse T cells. Figure 27B represents the number of T cells in blood. Figure 27C represents ratio of CD4 to CD8 cells in the blood. Figure 27D represents the percentage of IL15Ra positive CD4 and CD8 T cells in the blood.

[0086] Figure 28A depicts the expansion of T cells in response to cytokine treatment. Figure 28B, Figure 28C and Figure 28D depict the frequency of IFN gamma positive cells with IL12 treatment.

[0087] Figure 29 is a bar graph representing the effect of promoters on transgene expression.

[0088] Figure 30A shows the expression of CD19 in parental K562 cells and K562-CD19 cells. Figure 30B shows the proliferation of K562 cells cocultured with T cells expressing DD regulated CAR constructs, in the presence or absence of ligand. Figure 30C shows the area of target cells killed by T cells expressing DD regulated CAR constructs, in the presence of ligand.

[0089] Figure 31A shows IFNgamma concentration. Figure 31B shows IL2 concentration.

[0090] Figure 32A provides the final IL12 concentration for each of the four groups tested. Figure 32B shows that IL12 is detectable in kidney and Figure 32C shows that IL12 is detectable in tumor.

[0091] Figure 33A shows the regulation of IL12 over 24 hours. Figure 33B shows the regulation in the plasma and Figure 33C shows the detection of flexi-IL12 in the kidneys.

[0092] Figure 34A shows that restimulation increased the expression of IL12. Figure 34B and Figure 34C show that ligand increased production of IL12.

[0093] Figure 35A shows the concentration-dependent induction of IL12 secretion of IL12 secretion from primary human T cells. Figure 35B shows the time course induction of IL12 secretion from primary human T cells.

[0094] Figure 36A shows the dose response of Aquashield-Induced DD-IL12 regulation *in vivo*. Figure 36B shows that plasma levels of IL12 remain high in animals transplanted with constitutive IL12 transduced T cells.

[0095] Figure 37A and 37B show the expression of IL12 *in vivo* over 7 days. Figure 37C and 37D show the expression of IL12 *in vivo* over 11 days. Figure 37E shows the Geometric MFI (GeoMFI) of Granzyme B (GrB) after 7 days in CD8+ T cells. Figure 37F shows the GeoMFI of Perforin at day 7 in CD8+ T cells.

[0096] Figure 38A shows the regulation of IL12 with PGK and EF1a promoters and FKBP domains. Figure 38B shows the relative expression of IL12.

[0097] Figure 39 depicts the kinetics of IL15Ra surface expression on CD4 T cells after TMP treatment.

[0098] Figure 40 represents a western blot of IL15-IL15Ra protein in HCT116 tumors from mice treated with TMP for 17 days in xenograft assays.

[0099] Figure 41 is a graph of the results of the MSD assay of IL15 protein levels in HEK293 cells.

[00100] Figure 42A provides FACS plots showing the expression of membrane bound IL15 after a dose response study of TMP. Figure 42B is two graphs showing the dose and time of exposure of TMP *in vitro* influences membrane bound IL15 expression.

[00101] Figures 43A- 43C show the regulation of membrane bound IL15 using IL15 (Figure 43A), IL15Ra (Figure 43B), or IL15/IL15Ra double ++ staining (Figure 43C). Figure 43D shows FACS plots of the expression of IL15. Figure 43E is a graph of the regulation of IL15 in blood and Figure 43F is a graph of the plasma TMP levels.

[00102] Figure 44 represents the regulation of membrane bound IL15 with PO or IP dosing of TMP.

DETAILED DESCRIPTION OF THE INVENTION

[00103] The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred materials and methods are now described. Other features, objects and advantages of the invention will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present description will control.

I. INTRODUCTION

[00104] Cancer immunotherapy aims' at the induction or restoration of the reactivity of the immune system towards cancer. Significant advances in immunotherapy research have led to the development of various strategies which may broadly be classified into active immunotherapy and passive immunotherapy. In general, these strategies may be utilized to directly kill cancer cells or to counter the immunosuppressive tumor microenvironment. Active immunotherapy aims at induction of an endogenous, long-lasting tumor-antigen specific immune response. The response can further be enhanced by non-specific stimulation of immune response modifiers such as cytokines. In contrast, passive immunotherapy includes approaches where immune effector molecules such as tumor-antigen specific cytotoxic T cells or antibodies are administered to the host. This approach is short lived and requires multiple applications.

[00105] Despite significant advances, the efficacy of current immunotherapy strategies is limited by associated toxicities. These are often related to the narrow therapeutic window associated with immunotherapy, which in part, emerges from the need to push therapy dose to the edge of potentially fatal toxicity to get a clinically meaningful treatment effect. Further, dose expands *in vivo* since adoptively transferred immune cells continue to proliferate within the patient, often unpredictably.

[00106] A major risk involved in immunotherapy is the on-target but off tumor side effects resulting from T-cell activation in response to normal tissue expression of the tumor associated antigen (TAA). Clinical trials utilizing T cells expressing T-cell receptor against specific TAA reported skin rash, colitis and hearing loss in response to immunotherapy.

[00107] Immunotherapy may also produce on target, on-tumor toxicities that emerge when tumor cells are killed in response to the immunotherapy. The adverse effects include tumor lysis syndrome, cytokine release syndrome and the related macrophage activation syndrome. Importantly, these adverse effects may occur during the destruction of tumors, and thus even a successful on-tumor immunotherapy might result in toxicity. Approaches to regulatably control immunotherapy are thus highly desirable since they have the potential to reduce toxicity and maximize efficacy.

[00108] The present invention provides systems, compositions, immunotherapeutic agents and methods for cancer immunotherapy. These compositions provide tunable regulation of gene expression and function in immunotherapy. The present invention also provides biocircuit systems, effector modules, stimulus response elements (SREs) and payloads, as well as polynucleotides encoding any of the foregoing. In one aspect, the systems, compositions, immunotherapeutic agents and other components of the invention can be controlled by a separately added stimulus, which provides a significant flexibility to regulate cancer immunotherapy. Further, the systems, compositions and the methods of the present invention may also be combined with therapeutic agents such as chemotherapeutic agents, small molecules, gene therapy, and antibodies.

[00109] The tunable nature of the systems and compositions of the invention has the potential to improve the potency and duration of the efficacy of immunotherapies. Reversibly silencing the biological activity of adoptively transferred cells using compositions of the present invention allows maximizing the potential of cell therapy without irretrievably killing and terminating the therapy.

[00110] The present invention provides methods for fine tuning of immunotherapy after administration to patients. This in turn improves the safety and efficacy of immunotherapy and increases the subject population that may benefit from immunotherapy.

II. COMPOSITIONS OF THE INVENTION

[00111] According to the present invention, biocircuit systems are provided which comprise, at their core, at least one effector module system. Such effector module systems comprise at least one effector module having associated, or integral therewith, one or more stimulus response elements (SREs). The overall architecture of a biocircuit system of the invention is illustrated in Figure 1. In general, a stimulus response element (SRE) may be operably linked to a payload construct which could be any protein of interest (POI) (e.g., an immunotherapeutic agent), to form an effector module. The SRE, when activated by a particular stimulus, e.g., a small molecule, can produce a signal or outcome, to regulate transcription and/or protein levels of the linked payload either up or down by perpetuating a stabilizing signal or destabilizing signal, or any other types of regulation. A much-detailed description of a biocircuit system can be found in U.S. Provisional Patent Application No. 62/320,864 filed April 11, 2016 or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587 (the contents of each of which are herein incorporated by reference in their entirety). In accordance with the present invention, biocircuit systems, effector modules, SREs and components that tune expression levels and activities of any agents used for immunotherapy are provided.

[00112] As used herein, a “biocircuit” or “biocircuit system” is defined as a circuit within or useful in biologic systems comprising a stimulus and at least one effector module responsive to a stimulus, where the response to the stimulus produces at least one signal or outcome within, between, as an indicator of, or on a biologic system. Biologic systems are generally understood to be any cell, tissue, organ, organ system or organism, whether animal, plant, fungi, bacterial, or viral. It is also understood that biocircuits may be artificial circuits which employ the stimuli or effector modules taught by the present invention and effect signals or outcomes in acellular environments such as with diagnostic, reporter systems, devices, assays or kits. The artificial circuits may be associated with one or more electronic, magnetic, or radioactive components or parts.

[00113] In accordance with the present invention, a biocircuit system may be a destabilizing domain (DD) biocircuit system, a dimerization biocircuit system, a receptor biocircuit system, and a cell biocircuit system. Any of these systems may act as a signal to any other of these biocircuit systems.

Effector modules and SREs for immunotherapy

[00114] In accordance with the present invention, biocircuit systems, effector modules, SREs, and components that tune expression levels and activities of any agents used for immunotherapy are provided. As non-limiting examples, an immunotherapeutic agent may be an antibody and fragments and variants thereof, a cancer specific T cell receptor (TCR) and variants thereof, an anti-tumor specific chimeric antigen receptor (CAR), a chimeric switch receptor, an inhibitor of a co-inhibitory receptor or ligand, an agonist of a co-stimulatory receptor and ligand, a cytokine, chemokine, a cytokine receptor, a chemokine receptor, a soluble growth factor, a metabolic factor, a suicide gene, a homing receptor, or any agent that induces an immune response in a cell and a subject.

[00115] As stated, the biocircuits of the invention include at least one effector module as a component of an effector module system. As used herein, an “effector module” is a single or multi-component construct or complex comprising at least (a) one or more stimulus response elements (i.e. proteins of interest (POIs)). As used herein a “stimulus response element (SRE)” is a component of an effector module which is joined, attached, linked to or associated with one or more payloads of the effector module and in some instances, is responsible for the responsive nature of the effector module to one or more stimuli. As used herein, the “responsive” nature of an SRE to a stimulus may be characterized by a covalent or non-covalent interaction, a direct or indirect association or a structural or chemical reaction to the stimulus. Further, the response of any SRE to a stimulus may be a matter of degree or kind. The response may be a partial response. The response may be a reversible response. The response may ultimately lead to a regulated signal or output. Such output signal may be of a relative nature to the stimulus, e.g., producing a modulatory effect of between 1% and 100% or a factored increase or decrease such as 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more.

[00116] In some embodiments, the present invention provides methods for modulating protein expression, function or level. In some aspects, the modulation of protein expression, function or level refers to modulation of expression, function or level by at least about 20%, such as by at least about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%.

[00117] In some embodiments, the present invention provides methods for modulating protein, expression, function or level by measuring the stabilization ratio and destabilization ratio. As used herein, the stabilization ratio may be defined as the ratio of expression, function or level of a protein of interest in response to the stimulus to the expression, function or level of the protein of interest in the absence of the stimulus specific to the SRE. In some aspects, the stabilization ratio is at least 1, such as by at least 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, 1-100, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-95, 20-100, 30-40, 30-50, 30-60, 30-70, 30-80, 30-90, 30-95, 30-100, 40-50, 40-60, 40-70, 40-80, 40-90, 40-95, 40-100, 50-60, 50-70, 50-80, 50-90, 50-95, 50-100, 60-70, 60-80, 60-90, 60-95, 60-100, 70-80, 70-90, 70-95, 70-100, 80-90, 80-95, 80-100, 90-95, 90-100 or 95-100. As used herein, the destabilization ratio may be defined as the ratio of expression, function or level of a protein of interest in the absence of the stimulus specific to the effector module to the expression, function or level of the protein of interest, that is expressed constitutively and in the absence of the stimulus specific to the SRE. As used herein “constitutively” refers to the expression, function or level of a protein of interest that is not linked to an SRE, and is therefore expressed both in the presence and absence of the stimulus. In some aspects, the destabilization ratio is at least 0, such as by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or at least, 0-0.1, 0-0.2, 0-0.3, 0-0.4, 0-0.5, 0-0.6, 0-0.7, 0-0.8, 0-0.9, 0.1-0.2, 0.1-0.3, 0.1-0.4, 0.1-0.5, 0.1-0.6, 0.1-0.7, 0.1-0.8, 0.1-0.9, 0.2-0.3, 0.2-0.4, 0.2-0.5, 0.2-0.6, 0.2-0.7, 0.2-0.8, 0.2-0.9, 0.3-0.4, 0.3-0.5, 0.3-0.6, 0.3-0.7, 0.3-0.8, 0.3-0.9, 0.4-0.5, 0.4-0.6, 0.4-0.7, 0.4-0.8, 0.4-0.9, 0.5-0.6, 0.5-0.7, 0.5-0.8, 0.5-0.9, 0.6-0.7, 0.6-0.8, 0.6-0.9, 0.7-0.8, 0.7-0.9 or 0.8-0.9.

[00118] In some embodiments, the stimulus of the present invention may be ultrasound stimulation. In some embodiments, the SREs of the present invention may be derived from mechanosensitive proteins. In one embodiment, the SRE of the present invention may be the mechanically sensitive ion channel, Piezo1.

[00119] Expression of the payload of interest in such instances is tuned by providing focused ultrasound stimulation. In other embodiments, the SREs of the present invention may be derived from calcium biosensors, and the stimulus of the present invention may be calcium. The calcium may be generated by the ultrasound induced mechanical stimulation of mechanosensitive ion channels. The ultrasound activation of the ion channel causes a calcium influx thereby generating the stimulus. In one embodiment, the mechanosensitive ion channel is Piezo 1. Mechanosensors may be advantageous to use since they provide spatial control to a specific location in the body.

[00120] The SRE of the effector module may be selected from, but is not limited to, a peptide, peptide complex, peptide-protein complex, protein, fusion protein, protein complex, protein-

protein complex. The SRE may comprise one or more regions derived from any natural or mutated protein, or antibody. In this aspect, the SRE is an element, when responding to a stimulus, can tune intracellular localization, intramolecular activation, and/or degradation of payloads.

[00121] In some embodiments, effector modules of the present invention may comprise additional features that facilitate the expression and regulation of the effector module, such as one or more signal sequences (SSs), one or more cleavage and/or processing sites, one or more targeting and/or penetrating peptides, one or more tags, and/or one or more linkers. Additionally, effector modules of the present invention may further comprise other regulatory moieties such as inducible promoters, enhancer sequences, microRNA sites, and/or microRNA targeting sites. Each aspect or tuned modality may bring to the effector module or biocircuit a differentially tuned feature. For example, an SRE may represent a destabilizing domain, while mutations in the protein payload may alter its cleavage sites or dimerization properties or half-life and the inclusion of one or more microRNA or microRNA binding site may impart cellular detargeting or trafficking features. Consequently, the present invention embraces biocircuits which are multifactorial in their tenability. Such biocircuits may be engineered to contain one, two, three, four or more tuned features.

[00122] In some embodiments, effector modules of the present invention may include one or more degrons to tune expression. As used herein, a "degron" refers to a minimal sequence within a protein that is sufficient for the recognition and the degradation by the proteolytic system. An important property of degrons is that they are transferrable, that is, appending a degron to a sequence confers degradation upon the sequence. In some embodiments, the degron may be appended to the destabilizing domains, the payload or both. Incorporation of the degron within the effector module of the invention, confers additional protein instability to the effector module and may be used to minimize basal expression. In some embodiments, the degron may be an N-degron, a phospho degron, a heat inducible degron, a photosensitive degron, an oxygen dependent degron. As a non-limiting example, the degron may be an Ornithine decarboxylase degron as described by Takeuchi et al. (Takeuchi J et al. (2008). *Biochem J.* 2008 Mar 1;410(2):401-7; the contents of which are incorporated by reference in their entirety). Other examples of degrons useful in the present invention include degrons described in International patent publication Nos. WO2017004022, WO2016210343, and WO2011062962; the contents of each of which are incorporated by reference in their entirety.

[00123] As shown in Figure 2, representative effector module embodiments comprising one payload, i.e. one immunotherapeutic agent are illustrated. Each components of the effector

module may be located or positioned in various arrangements without (A to F) or with (G to Z, and AA to DD) a cleavage site. An optional linker may be inserted between each component of the effector module.

[00124] Figures 3 to 6 illustrate representative effector module embodiments comprising two payloads, i.e. two immunotherapeutic agents. In some aspects, more than two immunotherapeutic agents (payloads) may be included in the effector module under the regulation of the same SRE (e.g., the same DD). The two or more agents may be either directly linked to each other or separated (Figure 3). The SRE may be positioned at the N-terminus of the construct, or the C-terminus of the construct, or in the internal location.

[00125] In some aspects, the two or more immunotherapeutic agents may be the same type such as two antibodies, or different types such as a CAR construct and a cytokine IL12. Biocircuits and components utilizing such effector molecules are given in Figures 7-12.

[00126] In some embodiments, biocircuits of the invention may be modified to reduce their immunogenicity. Immunogenicity is the result of a complex series of responses to a substance that is perceived as foreign and may include the production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, hypersensitivity responses, and anaphylaxis. Several factors can contribute to protein immunogenicity, including, but not limited to protein sequence, route and frequency of administration and patient population. In a preferred embodiment, protein engineering may be used to reduce the immunogenicity of the compositions of the invention. In some embodiments, modifications to reduce immunogenicity may include modifications that reduce binding of the processed peptides derived from the parent sequence to MHC proteins. For example, amino acid modifications may be engineered such that there are no or a minimal of number of immune epitopes that are predicted to bind with high affinity, to any prevalent MHC alleles. Several methods of identifying MHC binding epitopes of known protein sequences are known in the art and may be used to score epitopes in the compositions of the present invention. Such methods are disclosed in US Patent Publication No. US 20020119492, US20040230380, and US 20060148009; the contents of each of which are incorporated by reference in their entirety.

[00127] Epitope identification and subsequent sequence modification may be applied to reduce immunogenicity. The identification of immunogenic epitopes may be achieved either physically or computationally. Physical methods of epitope identification may include, for example, mass spectrometry and tissue culture/cellular techniques. Computational approaches that utilize information obtained on antigen processing, loading and display, structural and/or proteomic data toward identifying non-self-peptides that may result from antigen processing, and that are

likely to have good binding characteristics in the groove of the MHC may also be utilized. One or more mutations may be introduced into the biocircuits of the invention directing the expression of the protein, to maintain its functionality while simultaneously rendering the identified epitope less or non-immunogenic.

[00128] In some embodiments, protein modifications engineered into the structure of the compositions of the invention to interfere with antigen processing and peptide loading such as glycosylation and PEGylation, may also be useful in the present invention. Compositions of the invention may also be engineered to include non-classical amino acid sidechains to design less immunogenic compositions. Any of the methods discussed in International Patent Publication No. WO2005051975 for reducing immunogenicity may be useful in the present invention (the contents of which are incorporated by reference in their entirety).

[00129] In one embodiment, patients may also be stratified according to the immunogenic peptides presented by their immune cells and may be utilized as a parameter to determine suitable patient cohorts that may therapeutically benefit for the compositions of the invention.

[00130] In some embodiments, reduced immunogenicity may be achieved by limiting immunoproteasome processing. The proteasome is an important cellular protease that is found in two forms: the constitutive proteasome, which is expressed in all cell types and which contains active e.g. catalytic subunits and the immunoproteasome that is expressed in cell of the hematopoietic lineage, and which contains different active subunits termed low molecular weight proteins (LMP) namely LMP-2, LMP- 7 and LMP-10. Immunoproteasomes exhibit altered peptidase activities and cleavage site preferences that result in more efficient liberation of many MHC class I epitopes. A well described function of the immunoproteasome is to generate peptides with hydrophobic C terminus that can be processed to fit in the groove of MHC class I molecules. Deol P et al. have shown that immunoproteasomes may lead to a frequent cleavage of specific peptide bonds and thereby to a faster appearance of a certain peptide on the surface of the antigen presenting cells; and enhanced peptide quantities (Deol P et al. (2007) *J Immunol* 178 (12) 7557-7562; the contents of which are incorporated herein reference in its entirety). This study indicates that reduced immunoproteasome processing may be accompanied by reduced immunogenicity. In some embodiments, immunogenicity of the compositions of the invention may be reduced by modifying the sequence encoding the compositions of the invention to prevent immunoproteasome processing. Biocircuits of the present invention may also be combined with immunoproteasome-selective inhibitors to achieve the same effects. Examples of inhibitors useful in the present invention include UK-101 (Bli selective compound), IPSI-001, ONX 0914 (PR-957), and PR-924 (IPSI).

1. Destabilizing domains (DDs)

[00131] In some embodiments, biocircuit systems, effector modules, and compositions of the present invention relate to post-translational regulation of protein (payload) function anti-tumor immune responses of immunotherapeutic agents. In one embodiment, the SRE is a stabilizing/destabilizing domain (DD). The presence, absence or an amount of a small molecule ligand that binds to or interacts with the DD, can, upon such binding or interaction modulate the stability of the payload(s) and consequently the function of the payload. Depending on the degree of binding and/or interaction the altered function of the payload may vary, hence providing a “tuning” of the payload function.

[00132] In some embodiments, destabilizing domains described herein or known in the art may be used as SREs in the biocircuit systems of the present invention in association with any of the immunotherapeutic agents (payloads) taught herein. Destabilizing domains (DDs) are small protein domains that can be appended to a target protein of interest. DDs render the attached protein of interest unstable in the absence of a DD-binding ligand such that the protein is rapidly degraded by the ubiquitin-proteasome system of the cell (Stankunas, K., et al., *Mol. Cell*, 2003, 12: 1615–1624; Banaszynski, *et al.*, *Cell*; 2006, 126(5): 995–1004; reviewed in Banaszynski, L.A., and Wandless, T.J. *Chem. Biol.*; 2006, 13:11–21 and Rakhit R et al., *Chem Biol.* 2014; 21(9):1238-1252). However, when a specific small molecule ligand binds its intended DD as a ligand binding partner, the instability is reversed and protein function is restored. The conditional nature of DD stability allows a rapid and non-perturbing switch from stable protein to unstable substrate for degradation. Moreover, its dependency on the concentration of its ligand further provides tunable control of degradation rates.

[00133] In some embodiments, the desired characteristics of the DDs may include, but are not limited to, low protein levels in the absence of a ligand of the DD (i.e. low basal stability), large dynamic range, robust and predictable dose-response behavior, and rapid kinetics of degradation. DDs that bind to a desired ligand but not endogenous molecules may be preferred.

[00134] Several protein domains with destabilizing properties and their paired small molecules have been identified and used to control protein expression, including FKBP/shield-1 system (Egeler et al., *J Biol. Chem.* 2011, 286(36): 32328-31336; the contents of which are incorporated herein by reference in their entirety), ccDHFR and its ligand trimethoprim (TMP); estrogen receptor domains which can be regulated by several estrogen receptor antagonists (Miyazaki et al., *J Am Chem. Soc.*, 2012, 134(9): 3942-3945; the contents of which are incorporated by reference herein in their entirety); and fluorescent destabilizing domain (FDD) derived from

bilirubin-inducible fluorescent protein, UnaG and its cognate ligand bilirubin (BR) (Navarro et al., *ACS Chem Biol.*, 2016, June 6; the contents of which are incorporated herein by reference in their entirety).

[00135] Known DDs also include those described in U.S. Pat. NO. 8,173,792 and U.S. Pat. NO. 8,530,636, the contents of which are each incorporated herein by reference in their entirety.

[00136] In some embodiments, the DDs of the present invention may be derived from some known sequences that have been approved to be capable of post-translational regulation of proteins. For example, Xiong et al., have demonstrated that the non-catalytic N-terminal domain (54-residues) of ACS7 (1-aminocyclopropane-1-carboxylate synthase) in *Arabidopsis*, when fused to the β -glucuronidase (GUS) reporter, can significantly decrease the accumulation of the GUS fusion protein (Xiong et al., *J. Exp. Bot.*, 2014, 65(15): 4397-4408). Xiong et al. further demonstrated that both exogenous 1-aminocyclopropane-1-carboxylic acid (ACC) treatment and salt can rescue the levels of accumulation of the ACS N-terminal and GUS fusion protein. The ACS N-terminus mediates the regulation of ACS7 stability through the ubiquitin–26S proteasome pathway.

[00137] Another non-limiting example is the stability control region (SCR, residues 97-118) of Tropomyosin (Tm), which controls protein stability. A destabilizing mutation L110A, and a stabilizing mutation A109L dramatically affect Tropomyosin protein dynamics (Kirwan and Hodges, *J. Biol. Chem.*, 2014, 289: 4356-4366). Such sequences can be screened for ligands that bind them and regulate their stability. The identified sequence and ligand pairs may be used as components of the present invention.

[00138] In some embodiments, the DDs of the present invention may be developed from known proteins. Regions or portions or domains of wild type proteins may be utilized as SREs/DDs in whole or in part. They may be combined or rearranged to create new peptides, proteins, regions or domains of which any may be used as SREs/DDs or the starting point for the design of further SREs and/or DDs.

[00139] Ligands such as small molecules that are well known to bind candidate proteins can be tested for their regulation in protein responses. The small molecules may be clinically approved to be safe and have appropriate pharmaceutical kinetics and distribution. In some embodiments, the stimulus is a ligand of a destabilizing domain (DD), for example, a small molecule that binds a destabilizing domain and stabilizes the POI fused to the destabilizing domain. In some embodiments, ligands, DDs and SREs of the present invention, include without limitation, any of those taught in Tables 2-4 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed

March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety. Some examples of the proteins that may be used to develop DDs and their ligands are listed in Table 1.

Table 1: Proteins and their binding ligands

Protein	Protein Sequence	Protein SEQ ID NO.:	Ligands
E. coli Dihydrofolate reductase (ecDHFR) (Uniprot ID: P0ABQ4)	MISLIAALAVDRVIGMENAMPWNLPADL AWFKRNTLNKPVIMGRHTWESIGRPLPGR KNILSSQPGTDDRVTWVKSVDIAAACG DVPEIMVIGGGRVYEQFLPKAQLYLTHI DAEVEGDTHFPDYEPDDWESVFSEFHDA DAQNSHSYCFEILERR	1	Methotrexate (MTX) Trimethoprim (TMP)
Human Dihydrofolate reductase (hDHFR) (Uniprot ID: P00374)	MVGSLNCIVAVSQNMGIGKNGDLPWPPL RNEFRYFQRM TTTSSVEGKQNLVIMGKK TWFSIPEKNRPLKGRINLVLSRELKEPPQG AHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDF ESDTFFPEIDLEKYKLLPEYPGVLSDVQEE KGIKYKFEVYEKND	2	Methotrexate (MTX) Trimethoprim (TMP)
FK506 binding protein (FKBP) (Uniprot ID: P62942)	GVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRDNRNPKFKMLGKQEV RGWEEGVAQMSVGQRAKLTISPDYAYGA TGHPGIIPPHATLVFDVELLKLE	3	Shield-1
Phosphodiesterase 5 (PDE5), ligand binding domain (Uniprot ID: Uniprot ID O76074)	MEETRELQSLAAAVVPSAQT KITDFSF DFELSDLETALCTIRMTDLNLVQNFQMK HEVLCRWILSVKKNYRKNAVYHNWRHA FNTAQCMFAALKAGKIQNKLTDLLEILALL IAALSHDLDRGVNNSYIQRSEHPLAQLY CHSIMEHHHFDQCLMILNSPGNQILSGLSI EEYKTTLKIIKQAILATDLALYIKRRGEFFE LIRKNQFNLEDPHQKELFLAMLMTACDLS AITKPWPIQQRIAE LVATEFFDQGDREKE LNIPTDLMNREKKNKIPSMQVGFIDAICL QLYEALTHVSEDCFPLLDGCRKNRQKWQ ALAEQQ	4	Sildenafil; Vardenafil; Tadalafil
PPAR gamma, ligand binding domain (Uniprot ID: P37231; amino acids 317-505)	SVEAVQEITEYAKSIPGFVNLDLNDQVTL LKYGVHEIITYTMLASLMNKDGVLISEGQG FMTREFLKSRLRKPFGDFMEPKFEFAVKFN ALELDDSDLAIFIAVILSGDRPGLLNVPKI EDIQDNLLQALELQLKLNHPSSQLFAKL LQKMTDLRQIVTEHVQLLQVIKKTETDMS LHPLLQEIKDLY	5	Posiglitazone Pioglitazone
Carbonic anhydrase II (CA2) (Uniprot ID: P00918)	MSHHWGYGKHNGPEHWHKDFPIAKGER QSPVDIDHTAKYDPSLKPLSVSYDQATS LRILNNGHAFNVEFDDSDKAVLKGGPL DGTYRLIQFHFHWGSLDGQSGSEHTVDKK KYAAELHLVHWNTKYGDFGKAVQQPDG LAVLGIFLKVGSAPGLQKVVDVLD SIKT KGKSADFTNFDPRGLLPESLDYWTYPGSL TTPPLLECVTWIVLKEPISVSSEQVLKFRK LNFNGEGEPEELMVDNWRPAQPLKNRQI KASFK	6	Celecoxib Acetazolamide
NRH: Quinone oxidoreductase 2 (NQO2) (Uniprot ID: P16083)	MAGKKVLIVYAHQEPKSFNGSLKNVAVD ELSRQGCTVTVSDLYAMNLEPRATDKDIT GTLSNPEVFNYGVETHEAYKQRSASDIT DEQKKVREADLVIFQFPLYWFSVPAILKG	7	Imatinib Melatonin

	WMDRVLCQGFAFDIPGFYDSGLLQGKLA LLSVTTGGTAEMYTKTGVNGDSRYFLWP LQHGT LHFCGFKVLAPQISFAPEIASSEER KGMVAAWSQRLQTIWKEEPICTAHWHF GQ		
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[00140] In some embodiments, DDs of the invention may be FKBP DD or ecDHFR DDs such as those listed in Table 2. The position of the mutated amino acid listed in Table 2 is relative to the ecDHFR (Uniprot ID: P0ABQ4) of SEQ ID NO. 1 for ecDHFR DDs and relative to FKBP (Uniprot ID: P62942) of SEQ ID NO. 3 for FKBP DDs.

Table 2: ecDHFR DDs and FKBP DDs

DD	Sequence	SEQ ID NO:
ecDHFR (R12Y, Y100I)	MISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTL NKPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTW VKSVD EAIACGDVPEIMVIGGGRVIEQFLPKAQKLY LTHIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNS HSYCFEILERR	8
ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I)	ISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTLN KPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWV KSVDEAIAACGDVPEIMVIGGGRVIEQFLPKAQKLYLT HIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHS YCFEILERR	9
ecDHFR (Amino acid 2-159 of WT) (R12H, E129K)	ISLIAALAVDHVIGMENAMPWNLPADLAWFKRNTLN KPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWV KSVDEAIAACGDVPEIMVIGGGRVYEQFLPKAQKLYL THIDAEVEGDTHFPDYKPDDWESVFSEFHDADAQNSH SYCFEILERR	10
FKBP (F36V, L106P)	GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKV DSSRDRNKPFFKFMLGKQEVIRGWEEGVAQMSVGQRA KLTISPDYAYGATGHPGIIPPHATLVFDVELLKPE	11
FKBP (E31G, F36V, R71G, K105E)	GVQVETISPGDGRTFPKRGQTCVVHYTGMLGDGKKV DSSRDRNKPFFKFMLGKQEVIRGWEEGVAQMSVGQGA KLTISPDYAYGATGHPGIIPPHATLVFDVELLELE	12

[00141] Inventors of the present invention have tested and identified several candidate human proteins that may be used to develop destabilizing domains. As show in Table 2, these candidates include human DHFR (hDHFR), PDE5 (phosphodiesterase 5), PPAR gamma (peroxisome proliferator-activated receptor gamma), CA2 (Carbonic anhydrase II) and NQO2 (NRH: Quinone oxidoreductase 2). Candidate destabilizing domain sequence identified from protein domains of these proteins (as a template) may be mutated to generate libraries of mutants based on the template candidate domain sequence. Mutagenesis strategies used to generate DD libraries may include site-directed mutagenesis e.g. by using structure guided information; or random mutagenesis e.g. using error-prone PCR, or a combination of both. In some embodiments, destabilizing domains identified using random mutagenesis may be used to identify structural

properties of the candidate DDs that may be required for destabilization, which may then be used to further generate libraries of mutations using site directed mutagenesis.

[00142] In some embodiments, novel DDs derived from E.coli DHFR (ecDHFR) may comprise amino acids 2-159 of the wild type ecDHFR sequence. This may be referred to as an M1del mutation.

[00143] In some embodiments, novel DDs derived from ecDHFR may comprise amino acids 2-159 of the wild type ecDHFR sequence (also referred to as an M1del mutation), and may include one, two, three, four, five or more mutations including, but not limited to, M1del, R12Y, R12H, Y100I, and E129K.

[00144] In some embodiments, novel DDs derived from FKBP may comprise amino acids 2-107 of the wild type FKBP sequence. This may be referred to as an M1del mutation.

[00145] In some embodiments, novel DDs derived from FKBP may comprise amino acids 2-107 of the wild type FKBP sequence (also referred to as an M1del mutation), and may include one, two, three, four, five or more mutations including, but not limited to, M1del, E31G, F36V, R71G, K105E, and L106P.

[00146] In some embodiments, DD mutant libraries may be screened for mutations with altered, preferably higher binding affinity to the ligand, as compared to the wild type protein. DD libraries may also be screened using two or more ligands and DD mutations that are stabilized by some ligands but not others may be preferentially selected. DD mutations that bind preferentially to the ligand compared to a naturally occurring protein may also be selected. Such methods may be used to optimize ligand selection and ligand binding affinity of the DD. Additionally, such approaches can be used to minimize deleterious effects caused by off-target ligand binding.

[00147] In some embodiments, suitable DDs may be identified by screening mutant libraries using barcodes. Such methods may be used to detect, identify and quantify individual mutant clones within the heterogeneous mutant library. Each DD mutant within the library may have distinct barcode sequences (with respect to each other). In other instances, the polynucleotides can also have different barcode sequences with respect to 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleic acid bases. Each DD mutant within the library may also comprise a plurality of barcode sequences. When used in plurality may be used such that each barcode is unique to any other barcode. Alternatively, each barcode used may not be unique, but the combination of barcodes used may create a unique sequence that can be individually tracked. The barcode sequence may be placed upstream of the SRE, downstream of the SRE, or in some instances may be placed within the SRE. DD mutants may be identified by barcodes using sequencing approaches such as Sanger sequencing, and next generation sequencing, but also by polymerase chain reaction and

quantitative polymerase chain reaction. In some embodiments, polymerase chain reaction primers that amplify a different size product for each barcode may be used to identify each barcode on an agarose gel. In other instances, each barcode may have a unique quantitative polymerase chain reaction probe sequence that enables targeted amplification of each barcode.

[00148] In some embodiments, DDs of the invention may be derived from human dihydrofolate reductase (hDHFR). hDHFR is a small (18 kDa) enzyme that catalyzes the reduction of dihydrofolate and plays a vital role in variety of anabolic pathway. Dihydrofolate reductase (DHFR) is an essential enzyme that converts 7,8-dihydrofolate (DHF) to 5,6,7,8, tetrahydrofolate (THF) in the presence of nicotinamide adenine dihydrogen phosphate (NADPH). Anti-folate drugs such as methotrexate (MTX), a structural analogue of folic acid, which bind to DHFR more strongly than the natural substrate DHF, interferes with folate metabolism, mainly by inhibition of dihydrofolate reductase, resulting in the suppression of purine and pyrimidine precursor synthesis. Other inhibitors of hDHFR such as folate, TQD, Trimethoprim (TMP), epigallocatechin gallate (EGCG) and ECG (epicatechin gallate) can also bind to hDHFR mutants and regulates its stability. In one aspect of the invention, the DDs of the invention may be hDHFR mutants including the single mutation hDHFR (Y122I), hDHFR (K81R), hDHFR (F59S), hDHFR (I17V), hDHFR (N65D), hDHFR (A107V), hDHFR (N127Y), hDHFR (K185E), hDHFR (N186D), and hDHFR (M140I); double mutations: hDHFR (M53T, R138I), hDHFR (V75F, Y122I), hDHFR (A125F, Y122I), hDHFR (L74N, Y122I), hDHFR (L94A, T147A), hDHFR (G21T, Y122I), hDHFR (V121A, Y122I), hDHFR (Q36K, Y122I), hDHFR (C7R, Y163C), hDHFR (Y178H, E181G), hDHFR (A10V, H88Y), hDHFR (T137R, F143L), hDHFR (E63G, I176F), hDHFR (T57A, I72A), hDHFR (H131R, E144G), and hDHFR (Y183H, K185E); and triple mutations: hDHFR (Q36F, N65F, Y122I), hDHFR (G21E, I72V, I176T), hDHFR (I8V, K133E, Y163C), hDHFR (V9A, S93R, P150L), hDHFR (K19E, F89L, E181G), hDHFR (G54R, M140V, S168C), hDHFR (L23S, V121A, Y157C), hDHFR (V110A, V136M, K177R), and hDHFR (N49D, F59S, D153G).

[00149] In one embodiment, the stimulus is a small molecule that binds to a SRE to post-translationally regulate protein levels. In one aspect, DHFR ligands: trimethoprim (TMP) and methotrexate (MTX) are used to stabilize hDHFR mutants. The hDHFR based destabilizing domains are listed in Table 3. The position of the mutated amino acid listed in Table 3 is relative to the human DHFR (Uniprot ID: P00374) of SEQ ID NO. 2 for human DHFR. In Table 3, “del” means that the mutation is the deletion of the amino acid at that position relative to the wild type sequence.

Table 3: Human DHFR mutants and novel destabilizing domains

Mutants	Amino acid Sequence	SEQ ID NO
hDHFR (I17V)	MVGSLNCIVAVSQNMGVGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	13
hDHFR (F59S)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWSSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	14
hDHFR (N65D)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKDRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	15
hDHFR (K81R)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELREPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	16
hDHFR (A107V)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELVNKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	17
hDHFR (Y122I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	18
hDHFR (N127Y)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMYHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	19
hDHFR (M140I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIIQDFESDTFFPEIDL EKYKLLPEYPGVLSVQEEKGIKYKFEVYEKND	20
hDHFR (K185E)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEEND	21
hDHFR (N186D)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSVQEEKGIKYKFEVYEKDD	22
hDHFR (C7R, Y163C)	MVGSLNRIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPECVPGVLSVQEEKGIKYKFEVYEKND	23

hDHFR (A10V, H88Y)	MVGSLNCIVVVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGA YFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	24
hDHFR (Q36K, Y122I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	25
hDHFR (M53T, R138I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVITGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTIIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	26
hDHFR (T57A, I72A)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKA WFSIPEKNRPLKGRANLV LSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMV WIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEI DLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	27
hDHFR (E63G, I176F)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPGKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSDVQEEKGFKYKFEVYEKND	28
hDHFR (G21T, Y122I)	MVGSLNCIVAVSQNMGIGKNTDLPWPPLRNEFRYFORM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	29
hDHFR (L74N, Y122I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINNVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	30
hDHFR (V75F, Y122I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLFL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	31
hDHFR (L94A, T147A)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSR SADDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDAFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	32
DHFR (V121A, Y22I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSAIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	33
hDHFR (Y122I, A125F)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVIKEFMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	34
hDHFR (H131R, E144G)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGRLKLFVTRIMQDFGSDTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	35

hDHFR (T137R, F143L)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVRRIMQDLESdTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	36
hDHFR (Y178H, E181G)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESdTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKHKFGVYEKND	37
hDHFR (Y183H, K185E)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESdTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVHEEND	38
hDHFR (V9A, S93R, P150L)	MVGSLNCIAAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRRLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESdTFFLEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	39
hDHFR (I8V, K133E, Y163C)	MVGSLNCVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLELFVTRIMQDFESdTFFPEID LEKYKLLPECPGVLSDVQEEKGIKYKFEVYEKND	40
hDHFR (L23S, V121A, Y157C)	MVGSLNCIVAVSQNMGIGKNGDSPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSAYKEAMNHPGHLKLFVTRIMQDFESdTFFPEID LEKCKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	41
hDHFR (K19E, F89L, E181G)	MVGSLNCIVAVSQNMGIGENGDLWPWPPLRNEFRYFORM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLS RELKEPPQGAHLLSRSLDDALKLTEQPELANKVDMVWI VGGSSVYKEAMNHPGHLKLFVTRIMQDFESdTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFGVYEKND	42
hDHFR (Q36F, N65F, Y122I)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFFRM TTTSSVEGKQNLVIMGKKTWFSIPEKFRPLKGRINLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLFVTRIMQDFESdTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	43
hDHFR (G54R, M140V, S168C)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMRKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIVQDFESdTFFPEIDL EKYKLLPEYPGVLCDVQEEKGIKYKFEVYEKND	44
hDHFR (V110A, V136M, K177R)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKADMVW IVGGSSVYKEAMNHPGHLKLFMTRIMQDFESdTFFPEID LEKYKLLPEYPGVLSDVQEEKGIRYKFEVYEKND	45
hDHFR (Amino acid 2-187 of WT; Q36F, Y122I, A125F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFFRMT TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEFMNHPGHLKLFVTRIMQDFESdTFFPEIDLEK YKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	46
hDHFR (N49D, F59S, D153G)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQDLVIMGKKTWSSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESdTFFPEIG LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	47

hDHFR (G21E, I72V, I176T)	MVGSLNCIVAVSQNMGIGKNEDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRVNLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGTKYKFEVYEKND	48
hDHFR (L100P, E102G, Q103R, P104S, E105G, N108D, V113A, W114R, Y122C, M126I, N127R, H128Y, L132P, F135P, I139T, F148S, F149L, I152V, D153A, D169G, V170A, I176A, K177R, V182A, K185R, N186S)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKPTGRSGLADKVDMAR IVGGSSVCKEAIRYPGHPKLPVTRTMQDFESDTSLPEVA LEKYKLLPEYPGVLSGAQEEKGARYKFEAYERSD	871
hDHFR (V2A, R33G, Q36R, L100P, K185R)	MAGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFGYFRR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKPTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVYERND	872
hDHFR (G16S, I17V, F89L, D96G, K123E, M140V, D146G, K156R)	MVGSLNCIVAVSQNM SVGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHLLSRSLDGALKLTEQPELANKVDMVW IVGGSSVYEEAMNHPGHLKLFVTRIVQDFESGTFFPEIDL ERYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	873
hDHFR (F35L, R37G, N65A, L68S, K69E, R71G, L80P, K99G, G117D, L132P, I139V, M140I, D142G, D146G, E173G, D187G)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYLQG MTTSSVEGKQNLVIMGKKTWFSIPEKARPSEGGINLVL SREPKEPPQGAHFLSRSLDDALGLTEQPELANKVDMVW IVDGSSVYKEAMNHPGHPKLFVTRVIQGFESGTFFPEIDL EKYKLLPEYPGVLSDVQEGKGIKYKFEVYEKNG	874
hDHFR (I17N, L98S, K99R, M112T, E151G, E162G, E172G)	MVGSLNCIVAVSQNMGNKGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDASRLTEQPELANKVDTVWI VGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPGIDL EKYKLLPGYPGVLSDVQGEKGIKYKFEVYEKND	875
hDHFR (R138G, D142G, F143S, K156R, K158E, E162G, V166A, K177E, Y178C, K185E, N186S)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTGIMQGSSESDTFFPEID LERYELLPGYPGALS DVQEEKGIECKFEVYEEESD	876
hDHFR (K81R, K99R, L100P, E102G, N108D, K123R, H128R, D142G, F180L, K185E)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVL SRELREPPQGAHFLSRSLDDALRPTGQPELADKVDMVW IVGGSSVYREAMNRPGHLKLFVTRIMQGFESDTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKLEVYEEND	877
hDHFR (N14S, P24S, F35L, M53T, K56E, R92G, S93G, N127S, H128Y, F135L, F143S, L159P, L160P, E173A, F180L)	MVGSLNCIVAVSQSMGIGKNGDLSWPPLRNEFRYLORM TTTSSVEGKQNLVITGKETWFSIPEKNRPLKGRINLVL SRELKEPPQGAHFLSGGLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMSYPGHLKLLVTRIMQDSESDTFFPEID LEKYKPPPEYPGVLSDVQEAAGIKYKLEVYEKND	878
hDHFR (V2A, I17V, N30D, E31G, Q36R, F59S, K69E, I72T, H88Y, F89L, N108D, K109E, V110A, I115V, Y122D, L132P, F135S, M140V, E144G, T147A, Y157C, V170A, K174R, N186S)	MAGSLNCIVAVSQNMGVGKNGDLPWPPLRDGFRYFRR MTTSSVEGKQNLVIMGKKTWSSIPEKNRPLEGRTNLV LSRELKEPPQGAAYLLSRSLDDALKLTEQPELADEAGMV WVVGSSVDKEAMNHPGHPKLSVTRIVQDFGSDAFFPE IDLEKCKLLPEYPGVLSDAQEERGIKYKFEVYEKSD	879
hDHFR (L28P, N30H, M38V, V44A, L68S, N73G, R78G, A97T, K99R, A107T, K109R, D111N, L134P, F135V, T147A, I152V, K158R, E172G, V182A, E184R)	MVGSLNCIVAVSQNMGIGKNGDLPWPPRHEFRYFQRV TTTSSAEGKQNLVIMGKKTWFSIPEKNRPSKGRIGLVLS GELKEPPQGAHFLSRSLDDTLRLTEQPELTNRVNMVWI VGGSSVYKEAMNHPGHLRPVVTRIMQDFESDAFFPEVD LEKYRLLPEYPGVLSDVQGEKGIKYKFEAYRKND	880
hDHFR (A10T, Q13R, N14S, N20D, P24S, N30S, M38T, T40A,	MVGSLNCIVTVSRSMGIGKDGDLSWPPLRSEFRYFQRTT ATSSVEGRQSLVIMGKRTWFS TPERNRPLRGRANLVLS	881

K47R, N49S, K56R, I61T, K64R, K69R, I72A, R78G, E82G, F89L, D96G, N108D, M112V, W114R, Y122D, K123E, I139V, Q141R, D142G, F148L, E151G, E155G, Y157R, Q171R, Y183C, E184G, K185del, D187N)	GELKGPPQGAHLLSRSLDGALKLTEQPELADKVDVVRI VGGSSVDEEAMNHPGHLKLFVTRVMRGFESDTLFP LGKRKLLPEYPGVLSDVREEKGIKYKLEVCGNN	
hDHFR (Amino acid 2-187 of WT; I17V, Y122I)	VGSLNCIVAVSQNMGVGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV VGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	882
hDHFR (Amino acid 2-187 of WT; Y122I, M140I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE YKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	883
hDHFR (Amino acid 2-187 of WT; N127Y, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVIKEAMYHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	884
hDHFR (Amino acid 2-187 of WT; Y122I, H131R, E144G)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVIKEAMNHPGRLKLFVTRIMQDFGSDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	885
hDHFR (Amino acid 2-187 of WT; D22S, F32M, R33S, Q36S, N65S)	VGSLNCIVAVSQNMGIGKNGSLPWPPLRNEMSYFSRMT TTSSVEGKQNLVIMGKKTWFSIPEKSRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	886
hDHFR (Amino acid 2-187 of WT; E31D, F32M, V116I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNDMRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	887
hDHFR (Amino acid 2-187 of WT; E162G, I176F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPGYPGVLSDVQEEKGFKYKFEVYEKND	888
hDHFR (Amino acid 2-187 of WT; K185E)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEEND	889
hDHFR (Amino acid 2-187 of WT; Y122I, A125F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVIKEFMNHPGHLKLFVTRIMQDFESDTFFPEIDLE YKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	890
hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFFRMT TTSSVEGKQNLVIMGKKTWFSIPEKFRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV GGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	891
hDHFR (Amino acid 2-187 of WT; N127Y)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWV	892

	GGSSVYKEAMYHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	
hDHFR (Amino acid 2-187 of WT; H131R, E144G)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVYKEAMNHPGRLKLFVTRIMQDFGSDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	893
hDHFR (Amino acid 2-187 of WT; I17V)	VGSLNCIVAVSQNMGVGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLS RELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDL EKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	894
hDHFR (Amino acid 2-187 of WT; Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRM TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	895
hDHFR (E162G, I176F)	MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLV SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPGYPGVLSDVQEEKGFKYKFEVYEKND	896
hDHFR (Amino acid 2-187 of WT; Q36K, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFKRMT TTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSR ELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLE KYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	981

[00150] In some embodiments, DD mutations that do not inhibit ligand binding may be preferentially selected. In some embodiments, ligand binding may be improved by mutation of residues in DHFR. Amino acid positions selected for mutation include aspartic acid at position 22 of SEQ ID NO. 2, glutamic acid at position 31 of SEQ ID NO. 2; phenyl alanine at position 32 of SEQ ID NO. 2; arginine at position 33 of SEQ ID NO. 2; glutamine at position 36 of SEQ ID NO. 2; asparagine at position 65 of SEQ ID NO. 2; and valine at position 115 of SEQ ID NO. 2. In some embodiments, one or more of the following mutations may be utilized in the DDs of the present invention to improve TMP binding, including but not limited to, D22S, E31D, F32M, R33S, Q36S, N65S, and V116I. The position of the mutated amino acids is relative to the wildtype human DHFR (Uniprot ID: P00374) of SEQ ID NO. 2.

[00151] In some embodiments, novel DDs derived from human DHFR may include one, two, three, four, five or more mutations including, but not limited to, M1del, V2A, C7R, I8V, V9A, A10T, A10V, Q13R, N14S, G16S, I17N, I17V, K19E, N20D, G21T, G21E, D22S, L23S, P24S, L28P, N30D, N30H, N30S, E31G, E31D, F32M, R33G, R33S, F35L, Q36R, Q36S, Q36K, Q36F, R37G, M38V, M38T, T40A, V44A, K47R, N49S, N49D, M53T, G54R, K56E, K56R, T57A, F59S, I61T, K64R, N65A, N65S, N65D, N65F, L68S, K69E, K69R, R71G, I72T, I72A, I72V, N73G, L74N, V75F, R78G, L80P, K81R, E82G, H88Y, F89L, R92G, S93G, S93R, L94A, D96G, A97T, L98S, K99G, K99R, L100P, E102G, Q103R, P104S, E105G, A107T, A107V,

N108D, K109E, K109R, V110A, D111N, M112T, M112V, V113A, W114R, I115V, I115L, V116I, G117D, V121A, Y122C, Y122D, Y122I, K123R, K123E, A125F, M126I, N127R, N127S, N127Y, H128R, H128Y, H131R, L132P, K133E, L134P, F135P, F135L, F135S, F135V, V136M, T137R, R138G, R138I, I139T, I139V, M140I, M140V, Q141R, D142G, F143S, F143L, E144G, D146G, T147A, F148S, F148L, F149L, P150L, E151G, I152V, D153A, D153G, E155G, K156R, Y157R, Y157C, K158E, K158R, L159P, L160P, E162G, Y163C, V166A, S168C, D169G, V170A, Q171R, E172G, E173G, E173A, K174R, I176A, I176F, I176T, K177E, K177R, Y178C, Y178H, F180L, E181G, V182A, Y183C, Y183H, E184R, E184G, K185R, K185del, K185E, N186S, N186D, D187G, and D187N.

[00152] In some embodiments, novel DDs derived from human DHFR may comprise amino acids 2-187 of the wild type human DHFR sequence. This may be referred to as an M1del mutation.

[00153] In some embodiments, novel DDs derived from human DHFR may comprise amino acids 2-187 of the wild type human DHFR sequence (also referred to as an M1del mutation), and may include one, two, three, four, five or more mutations including, but not limited to, M1del, V2A, C7R, I8V, V9A, A10T, A10V, Q13R, N14S, G16S, I17N, I17V, K19E, N20D, G21T, G21E, D22S, L23S, P24S, L28P, N30D, N30H, N30S, E31G, E31D, F32M, R33G, R33S, F35L, Q36R, Q36S, Q36K, Q36F, R37G, M38V, M38T, T40A, V44A, K47R, N49S, N49D, M53T, G54R, K56E, K56R, T57A, F59S, I61T, K64R, N65A, N65S, N65D, N65F, L68S, K69E, K69R, R71G, I72T, I72A, I72V, N73G, L74N, V75F, R78G, L80P, K81R, E82G, H88Y, F89L, R92G, S93G, S93R, L94A, D96G, A97T, L98S, K99G, K99R, L100P, E102G, Q103R, P104S, E105G, A107T, A107V, N108D, K109E, K109R, V110A, D111N, M112T, M112V, V113A, W114R, I115V, I115L, V116I, G117D, V121A, Y122C, Y122D, Y122I, K123R, K123E, A125F, M126I, N127R, N127S, N127Y, H128R, H128Y, H131R, L132P, K133E, L134P, F135P, F135L, F135S, F135V, V136M, T137R, R138G, R138I, I139T, I139V, M140I, M140V, Q141R, D142G, F143S, F143L, E144G, D146G, T147A, F148S, F148L, F149L, P150L, E151G, I152V, D153A, D153G, E155G, K156R, Y157R, Y157C, K158E, K158R, L159P, L160P, E162G, Y163C, V166A, S168C, D169G, V170A, Q171R, E172G, E173G, E173A, K174R, I176A, I176F, I176T, K177E, K177R, Y178C, Y178H, F180L, E181G, V182A, Y183C, Y183H, E184R, E184G, K185R, K185del, K185E, N186S, N186D, D187G, and D187N.

2. Payloads: Immunotherapeutic agents

[00154] In some embodiments, payloads of the present invention may be immunotherapeutic agents that induce immune responses in an organism. The immunotherapeutic agent may be, but is not limited to, an antibody and fragments and variants thereof, a chimeric antigen receptor

(CAR), a chimeric switch receptor, a cytokine, chemokine, a cytokine receptor, a chemokine receptor, a cytokine-cytokine receptor fusion polypeptide, or any agent that induces an immune response. In one embodiment, the immunotherapeutic agent induces an anti-cancer immune response in a cell, or in a subject.

Antibodies

[00155] In some embodiments, antibodies, fragments and variants thereof are payloads of the present invention.

[00156] In some embodiments, antibodies of the present invention, include without limitation, any of those taught in Table 5 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

Antibody fragments and variants

[00157] In some embodiments, antibody fragments and variants may comprise antigen binding regions from intact antibodies. Examples of antibody fragments and variants may include, but are not limited to Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules such as single chain variable fragment (scFv); and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site. Also produced is a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking with the antigen. Pharmaceutical compositions, biocircuits, biocircuit components, effector modules including their SREs or payloads of the present invention may comprise one or more of these fragments.

[00158] For the purposes herein, an "antibody" may comprise a heavy and light variable domain as well as an Fc region. As used herein, the term "native antibody" usually refers to a heterotetrameric glycoprotein of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Genes encoding antibody heavy and light chains are known and segments making up each have been well characterized and described (Matsuda et al., *The Journal of Experimental Medicine*, 1998, 188(11): 2151-62 and Li et al., *Blood*, 2004, 103(12): 4602-4609; the content of each of which are herein incorporated by reference in their entirety). Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each

heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[00159] As used herein, the term "variable domain" refers to specific antibody domains found on both the antibody heavy and light chains that differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. Variable domains comprise hypervariable regions. As used herein, the term "hypervariable region" refers to a region within a variable domain comprising amino acid residues responsible for antigen binding. The amino acids present within the hypervariable regions determine the structure of the complementarity determining regions (CDRs) that become part of the antigen-binding site of the antibody. As used herein, the term "CDR" refers to a region of an antibody comprising a structure that is complimentary to its target antigen or epitope. Other portions of the variable domain, not interacting with the antigen, are referred to as framework (FW) regions. The antigen-binding site (also known as the antigen combining site or paratope) comprises the amino acid residues necessary to interact with a particular antigen. The exact residues making up the antigen-binding site are typically elucidated by co-crystallography with bound antigen, however computational assessments based on comparisons with other antibodies can also be used (Strohl, W.R. *Therapeutic Antibody Engineering*. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p47-54, the contents of which are herein incorporated by reference in their entirety). Determining residues that make up CDRs may include the use of numbering schemes including, but not limited to, those taught by Kabat (Wu et al., *JEM*, 1970, 132(2):211-250 and Johnson et al., *Nucleic Acids Res.* 2000, 28(1): 214-218, the contents of each of which are herein incorporated by reference in their entirety), Chothia (Chothia and Lesk, *J. Mol. Biol.* 1987, 196, 901, Chothia et al., *Nature*, 1989, 342, 877, and Al-Lazikani et al., *J. Mol. Biol.* 1997, 273(4): 927-948, the contents of each of which are herein incorporated by reference in their entirety), Lefranc (Lefranc et al., *Immunome Res.* 2005, 1:3) and Honegger (Honegger and Pluckthun, *J. Mol. Biol.* 2001, 309(3): 657-70, the contents of which are herein incorporated by reference in their entirety).

[00160] VH and VL domains have three CDRs each. VL CDRs are referred to herein as CDR-L1, CDR-L2 and CDR-L3, in order of occurrence when moving from N- to C- terminus along the variable domain polypeptide. VH CDRs are referred to herein as CDR-H1, CDR-H2 and CDR-H3, in order of occurrence when moving from N- to C- terminus along the variable domain polypeptide. Each of CDRs has favored canonical structures with the exception of the CDR-H3,

which comprises amino acid sequences that may be highly variable in sequence and length between antibodies resulting in a variety of three-dimensional structures in antigen-binding domains (Nikoloudis, et al., *PeerJ*. 2014, 2: e456). In some cases, CDR-H3s may be analyzed among a panel of related antibodies to assess antibody diversity. Various methods of determining CDR sequences are known in the art and may be applied to known antibody sequences (Strohl, W.R. *Therapeutic Antibody Engineering*. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p47-54, the contents of which are herein incorporated by reference in their entirety).

[00161] As used herein, the term “Fv” refers to an antibody fragment comprising the minimum fragment on an antibody needed to form a complete antigen-binding site. These regions consist of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. Fv fragments can be generated by proteolytic cleavage, but are largely unstable. Recombinant methods are known in the art for generating stable Fv fragments, typically through insertion of a flexible linker between the light chain variable domain and the heavy chain variable domain (to form a single chain Fv (scFv)) or through the introduction of a disulfide bridge between heavy and light chain variable domains (Strohl, W.R. *Therapeutic Antibody Engineering*. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p46-47, the contents of which are herein incorporated by reference in their entirety).

[00162] As used herein, the term “light chain” refers to a component of an antibody from any vertebrate species assigned to one of two clearly distinct types, called kappa and lambda based on amino acid sequences of constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

[00163] As used herein, the term “single chain Fv” or “scFv” refers to a fusion protein of VH and VL antibody domains, wherein these domains are linked together into a single polypeptide chain by a flexible peptide linker. In some embodiments, the Fv polypeptide linker enables the scFv to form the desired structure for antigen binding. In some embodiments, scFvs are utilized in conjunction with phage display, yeast display or other display methods where they may be expressed in association with a surface member (e.g. phage coat protein) and used in the identification of high affinity peptides for a given antigen.

[00164] Using molecular genetics, two scFvs can be engineered in tandem into a single polypeptide, separated by a linker domain, called a “tandem scFv” (tascFv). Construction of a tascFv with genes for two different scFvs yields a “bispecific single-chain variable fragments” (bis-scFvs). Only two tascFvs have been developed clinically by commercial firms; both are

bispecific agents in active early phase development by Micromet for oncologic indications, and are described as “Bispecific T-cell Engagers (BiTE).” Blinatumomab is an anti-CD19/anti-CD3 bispecific tascFv that potentiates T-cell responses to B-cell non-Hodgkin lymphoma in Phase 2. MT110 is an anti-EP-CAM/anti-CD3 bispecific tascFv that potentiates T-cell responses to solid tumors in Phase 1. Bispecific, tetravalent “TandAbs” are also being researched by Affimed (Nelson, A. L., *MAbs.*, 2010, Jan-Feb; 2(1):77–83). maxibodies (bivalent scFv fused to the amino terminus of the Fc (CH2-CH3 domains) of IgG may also be included.

[00165] As used herein, the term “bispecific antibody” refers to an antibody capable of binding two different antigens. Such antibodies typically comprise regions from at least two different antibodies. Bispecific antibodies may include any of those described in Riethmuller, G. *Cancer Immunity*. 2012, 12:12-18, Marvin et al., 2005. *Acta Pharmacologica Sinica*. 2005, 26(6): 649-658 and Schaefer et al., *PNAS*. 2011, 108(27):11187-11192, the contents of each of which are herein incorporated by reference in their entirety.

[00166] As used herein, the term “diabody” refers to a small antibody fragment with two antigen-binding sites. Diabodies are functional bispecific single-chain antibodies (bscAb). Diabodies comprise a heavy chain variable domain VH connected to a light chain variable domain VL in the same polypeptide chain. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al. (Hollinger, P. et al., “Diabodies”: Small bivalent and bispecific antibody fragments. *PNAS*, 1993. 90: 6444-6448); the contents of each of which are incorporated herein by reference in their entirety.

[00167] The term “intrabody” refers to a form of antibody that is not secreted from a cell in which it is produced, but instead targets one or more intracellular proteins. Intrabodies may be used to affect a multitude of cellular processes including, but not limited to intracellular trafficking, transcription, translation, metabolic processes, proliferative signaling and cell division. In some embodiments, methods of the present invention may include intrabody-based therapies. In some such embodiments, variable domain sequences and/or CDR sequences disclosed herein may be incorporated into one or more constructs for intrabody-based therapy.

[00168] As used herein, the term “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous cells (or clones), i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibodies, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include

different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

[00169] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. The monoclonal antibodies herein include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies.

[00170] As used herein, the term "humanized antibody" refers to a chimeric antibody comprising a minimal portion from one or more non-human (e.g., murine) antibody source(s) with the remainder derived from one or more human immunoglobulin sources. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the hypervariable region from an antibody of the recipient are replaced by residues from the hypervariable region from an antibody of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and/or capacity. In one embodiment, the antibody may be a humanized full-length antibody. As a non-limiting example, the antibody may have been humanized using the methods taught in US Patent Publication NO. US20130303399, the contents of which are herein incorporated by reference in its entirety.

[00171] As used herein, the term "antibody variant" refers to a modified antibody (in relation to a native or starting antibody) or a biomolecule resembling a native or starting antibody in structure and/or function (e.g., an antibody mimetic). Antibody variants may be altered in their amino acid sequence, composition or structure as compared to a native antibody. Antibody variants may include, but are not limited to, antibodies with altered isotypes (e.g., IgA, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM), humanized variants, optimized variants, multispecific antibody variants (e.g., bispecific variants), and antibody fragments.

[00172] In some embodiments, pharmaceutical compositions, biocircuits, biocircuit components, effector modules including their SREs or payloads of the present invention may be antibody mimetics. As used herein, the term "antibody mimetic" refers to any molecule which mimics the function or effect of an antibody and which binds specifically and with high affinity to their molecular targets. In some embodiments, antibody mimetics may be monobodies, designed to incorporate the fibronectin type III domain (Fn3) as a protein scaffold (US

6,673,901; US 6,348,584). In some embodiments, antibody mimetics may be those known in the art including, but are not limited to affibody molecules, affilins, affitins, anticalins, avimers, Centyrins, DARPINSTM, Fynomers and Kunitz and domain peptides. In other embodiments, antibody mimetics may include one or more non-peptide regions.

[00173] In one embodiment, the antibody may comprise a modified Fc region. As a non-limiting example, the modified Fc region may be made by the methods or may be any of the regions described in US Patent Publication NO. US20150065690, the contents of which are herein incorporated by reference in its entirety.

[00174] In some embodiments, payloads of the invention may encode multispecific antibodies that bind more than one epitope. As used herein, the terms “multibody” or “multispecific antibody” refer to an antibody wherein two or more variable regions bind to different epitopes. The epitopes may be on the same or different targets. In one embodiment, the multispecific antibody may be generated and optimized by the methods described in International Patent Publication NO. WO2011109726 and US Patent Publication NO. US20150252119, the contents of which each of which are herein incorporated by reference in their entirety. These antibodies are able to bind to multiple antigens with high specificity and high affinity.

[00175] In certain embodiments, a multi-specific antibody is a “bispecific antibody” which recognizes two different epitopes on the same or different antigens. In one aspect, bispecific antibodies are capable of binding two different antigens. Such antibodies typically comprise antigen-binding regions from at least two different antibodies. For example, a bispecific monoclonal antibody (BsMAb, BsAb) is an artificial protein composed of fragments of two different monoclonal antibodies, thus allowing the BsAb to bind to two different types of antigen. Bispecific antibody frameworks may include any of those described in Riethmuller, G., 2012, *Cancer Immunity*, 2012, 12:12-18; Marvin et al., *Acta Pharmacologica Sinica*. 2005, 26(6):649-658; and Schaefer et al., *PNAS*. 2011, 108(27): 11187-11192, the contents of each of which are herein incorporated by reference in their entirety. New generations of BsMAb, called “trifunctional bispecific” antibodies, have been developed. These consist of two heavy and two light chains, one each from two different antibodies, where the two Fab regions (the arms) are directed against two antigens, and the Fc region (the foot) comprises the two heavy chains and forms the third binding site.

[00176] In some embodiments, payloads may encode antibodies comprising a single antigen-binding domain. These molecules are extremely small, with molecular weights approximately one-tenth of those observed for full-sized mAbs. Further antibodies may include “nanobodies” derived from the antigen-binding variable heavy chain regions (VHHs) of heavy chain antibodies

found in camels and llamas, which lack light chains (Nelson, A. L., MABs.2010. Jan-Feb; 2(1):77-83).

[00177] In some embodiments, the antibody may be “miniaturized”. Among the best examples of mAb miniaturization are the small modular immunopharmaceuticals (SMIPs) from Trubion Pharmaceuticals. These molecules, which can be monovalent or bivalent, are recombinant single-chain molecules containing one VL, one VH antigen-binding domain, and one or two constant “effector” domains, all connected by linker domains. Presumably, such a molecule might offer the advantages of increased tissue or tumor penetration claimed by fragments while retaining the immune effector functions conferred by constant domains. At least three “miniaturized” SMIPs have entered clinical development. TRU-015, an anti-CD20 SMIP developed in collaboration with Wyeth, is the most advanced project, having progressed to Phase 2 for rheumatoid arthritis (RA). Earlier attempts in systemic lupus erythematosus (SLE) and B cell lymphomas were ultimately discontinued. Trubion and Facet Biotechnology are collaborating in the development of TRU-016, an anti-CD37 SMIP, for the treatment of CLL and other lymphoid neoplasias, a project that has reached Phase 2. Wyeth has licensed the anti-CD20 SMIP SBI-087 for the treatment of autoimmune diseases, including RA, SLE and possibly multiple sclerosis, although these projects remain in the earliest stages of clinical testing. (Nelson, A. L., MABs, 2010. Jan-Feb; 2(1):77-83).

[00178] One example of miniaturized antibodies is called “unibody” in which the hinge region has been removed from IgG4 molecules. While IgG4 molecules are unstable and can exchange light-heavy chain heterodimers with one another, deletion of the hinge region prevents heavy chain-heavy chain pairing entirely, leaving highly specific monovalent light/heavy heterodimers, while retaining the Fc region to ensure stability and half-life in vivo. This configuration may minimize the risk of immune activation or oncogenic growth, as IgG4 interacts poorly with FcRs and monovalent unibodies fail to promote intracellular signaling complex formation (see, e.g., Nelson, A. L., MABs, 2010. Jan-Feb; 2(1):77-83).

[00179] In some embodiments, payloads of the invention may encode single-domain antibodies (sdAbs, or nanobodies) which are antibody fragment consisting of a single monomeric variable antibody domain. Like a whole antibody, it is able to bind selectively to a specific antigen. In one aspect, a sdAb may be a “Camel Ig or “camelid VHH”. As used herein, the term “camel Ig” refers to the smallest known antigen-binding unit of a heavy chain antibody (Koch-No lte, et al, *FASEB J.*, 2007, 21: 3490- 3498). A “heavy chain antibody” or a “camelid antibody” refers to an antibody that contains two VH domains and no light chains (Riechmann L. et al, *J. Immunol. Methods*, 1999, 231: 25-38; International patent publication NOs. WO1994/04678 and

W01994/025591; and U.S. Patent No. 6,005,079). In another aspect, a sdAb may be a "immunoglobulin new antigen receptor" (IgNAR). As used herein, the term "immunoglobulin new antigen receptor" refers to class of antibodies from the shark immune repertoire that consist of homodimers of one variable new antigen receptor (VNAR) domain and five constant new antigen receptor (CNAR) domains. IgNARs represent some of the smallest known immunoglobulin-based protein scaffolds and are highly stable and possess efficient binding characteristics. The inherent stability can be attributed to both (i) the underlying Ig scaffold, which presents a considerable number of charged and hydrophilic surface exposed residues compared to the conventional antibody VH and VL domains found in murine antibodies; and (ii) stabilizing structural features in the complementary determining region (CDR) loops including inter-loop disulphide bridges, and patterns of intra-loop hydrogen bonds.

[00180] In some embodiments, payloads of the invention may encode intrabodies. Intrabodies are a form of antibody that is not secreted from a cell in which it is produced, but instead targets one or more intracellular proteins. Intrabodies are expressed and function intracellularly, and may be used to affect a multitude of cellular processes including, but not limited to intracellular trafficking, transcription, translation, metabolic processes, proliferative signaling and cell division. In some embodiments, methods described herein include intrabody-based therapies. In some such embodiments, variable domain sequences and/or CDR sequences disclosed herein are incorporated into one or more constructs for intrabody-based therapy. For example, intrabodies may target one or more glycosylated intracellular proteins or may modulate the interaction between one or more glycosylated intracellular proteins and an alternative protein.

[00181] The intracellular expression of intrabodies in different compartments of mammalian cells allows blocking or modulation of the function of endogenous molecules (Biocca, et al., *EMBO J.* 1990, 9: 101-108; Colby et al., *Proc. Natl. Acad. Sci. U.S.A.* 2004, 101: 17616-17621). Intrabodies can alter protein folding, protein-protein, protein-DNA, protein-RNA interactions and protein modification. They can induce a phenotypic knockout and work as neutralizing agents by direct binding to the target antigen, by diverting its intracellular trafficking or by inhibiting its association with binding partners. With high specificity and affinity to target antigens, intrabodies have advantages to block certain binding interactions of a particular target molecule, while sparing others.

[00182] Sequences from donor antibodies may be used to develop intrabodies. Intrabodies are often recombinantly expressed as single domain fragments such as isolated VH and VL domains or as a single chain variable fragment (scFv) antibody within the cell. For example, intrabodies are often expressed as a single polypeptide to form a single chain antibody comprising the

variable domains of the heavy and light chains joined by a flexible linker polypeptide. Intrabodies typically lack disulfide bonds and are capable of modulating the expression or activity of target genes through their specific binding activity. Single chain intrabodies are often expressed from a recombinant nucleic acid molecule and engineered to be retained intracellularly (e.g., retained in the cytoplasm, endoplasmic reticulum, or periplasm). Intrabodies may be produced using methods known in the art, such as those disclosed and reviewed in: (Marasco et al., *PNAS*, 1993, 90: 7889-7893; Chen et al., *Hum. Gene Ther.* 1994, 5:595-601; Chen et al., 1994, *PNAS*, 91: 5932-5936; Maciejewski et al., 1995, *Nature Med.*, 1: 667-673; Marasco, 1995, *Immunotech*, 1: 1-19; Mhashilkar, et al., 1995, *EMBO J.* 14: 1542-51; Chen et al., 1996, *Hum. Gene Therap.*, 7: 1515-1525; Marasco, *Gene Ther.* 4:11-15, 1997; Rondon and Marasco, 1997, *Annu. Rev. Microbiol.* 51:257-283; Cohen, et al., 1998, *Oncogene* 17:2445-56; Proba et al., 1998, *J. Mol. Biol.* 275:245-253; Cohen et al., 1998, *Oncogene* 17:2445-2456; Hassanzadeh, et al., 1998, *FEBS Lett.* 437:81-6; Richardson et al., 1998, *Gene Ther.* 5:635-44; Ohage and Steipe, 1999, *J. Mol. Biol.* 291:1119-1128; Ohage et al., 1999, *J. Mol. Biol.* 291:1129-1134; Wirtz and Steipe, 1999, *Protein Sci.* 8:2245-2250; Zhu et al., 1999, *J. Immunol. Methods* 231:207-222; Arafat et al., 2000, *Cancer Gene Ther.* 7:1250-6; der Maur et al., 2002, *J. Biol. Chem.* 277:45075-85; Mhashilkar et al., 2002, *Gene Ther.* 9:307-19; and Wheeler et al., 2003, *FASEB J.* 17: 1733-5; and references cited therein).

[00183] In some aspects, payloads of the invention may encode biosynthetic antibodies as described in U.S. Patent No. 5,091,513, the contents of which are herein incorporated by reference in their entirety. Such antibody may include one or more sequences of amino acids constituting a region which behaves as a biosynthetic antibody binding site (BABS). The sites comprise 1) non-covalently associated or disulfide bonded synthetic VH and VL dimers, 2) VH-VL or VL-VH single chains wherein the VH and VL are attached by a polypeptide linker, or 3) individuals VH or VL domains. The binding domains comprise linked CDR and FR regions, which may be derived from separate immunoglobulins. The biosynthetic antibodies may also include other polypeptide sequences which function, e.g., as an enzyme, toxin, binding site, or site of attachment to an immobilization media or radioactive atom. Methods are disclosed for producing the biosynthetic antibodies, for designing BABS having any specificity that can be elicited by in vivo generation of antibody, and for producing analogs thereof.

[00184] In some embodiments, payloads may encode antibodies with antibody acceptor frameworks taught in U.S. Patent No. 8,399,625. Such antibody acceptor frameworks may be particularly well suited accepting CDRs from an antibody of interest.

[00185] In one embodiment, the antibody may be a conditionally active biologic protein. An antibody may be used to generate a conditionally active biologic protein which are reversibly or irreversibly inactivated at the wild type normal physiological conditions as well as to such conditionally active biologic proteins and uses of such conditional active biologic proteins are provided. Such methods and conditionally active proteins are taught in, for example, International Publication No. WO2015175375 and WO2016036916 and US Patent Publication No. US20140378660, the contents of each of which are incorporated herein by reference in their entirety.

Antibody preparations

[00186] The preparation of antibodies, whether monoclonal or polyclonal, is known in the art. Techniques for the production of antibodies are well known in the art and described, e.g. in Harlow and Lane "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1988; Harlow and Lane "Using Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, 1999 and "Therapeutic Antibody Engineering: Current and Future Advances Driving the Strongest Growth Area in the Pharmaceutical Industry" Woodhead Publishing, 2012.

[00187] The antibodies and fragments and variants thereof as described herein can be produced using recombinant polynucleotides. In one embodiment, the polynucleotides have a modular design to encode at least one of the antibodies, fragments or variants thereof. As a non-limiting example, the polynucleotide construct may encode any of the following designs: (1) the heavy chain of an antibody, (2) the light chain of an antibody, (3) the heavy and light chain of the antibody, (4) the heavy chain and light chain separated by a linker, (5) the VH1, CH1, CH2, CH3 domains, a linker and the light chain or (6) the VH1, CH1, CH2, CH3 domains, VL region, and the light chain. Any of these designs may also comprise optional linkers between any domain and/or region. The polynucleotides of the present invention may be engineered to produce any standard class of immunoglobulins using an antibody described herein or any of its component parts as a starting molecule.

[00188] Recombinant antibody fragments may also be isolated from phage antibody libraries using techniques well known in the art and described in e.g. Clackson et al., 1991, Nature 352: 624-628; Marks et al., 1991, J. Mol. Biol. 222: 581-597. Recombinant antibody fragments may be derived from large phage antibody libraries generated by recombination in bacteria (Sblattero and Bradbury, 2000, Nature Biotechnology 18:75-80; the contents of which are incorporated herein by reference in its entirety).

Antibodies used for immunotherapy

[00189] In some embodiments, payloads of the present invention may be antibodies, fragments and variants thereof which are specific to tumor specific antigens (TSAs) and tumor associated antigens (TAAs). Antibodies circulate throughout the body until they find and attach to the TSA/TAA. Once attached, they recruit other parts of the immune system, increasing ADCC (antibody dependent cell-mediated cytotoxicity) and ADCP (antibody dependent cell-mediated phagocytosis) to destroy tumor cells. As used herein, the term “tumor specific antigen (TSA)” means an antigenic substance produced in tumor cells, which can trigger an anti-tumor immune response in a host organism. In one embodiment, a TSA may be a tumor neoantigen. The tumor antigen specific antibody mediates complement-dependent cytotoxic response against tumor cells expressing the same antigen.

[00190] In some embodiments, the tumor specific antigens (TSAs), tumor associated antigens (TAAs), pathogen associated antigens, or fragments thereof can be expressed as a peptide or as an intact protein or portion thereof. The intact protein or a portion thereof can be native or mutagenized. Antigens associated with cancers or virus-induced cancers as described herein are well-known in the art. Such a TSA or TAA may be previously associated with a cancer or may be identified by any method known in the art.

[00191] In one embodiment, the antigen is CD19, a B-cell surface protein expressed throughout B-cell development. CD19 is a well-known B cell surface molecule, which upon B cell receptor activation enhances B-cell antigen receptor induced signaling and expansion of B cell populations. CD19 is broadly expressed in both normal and neoplastic B cells. Malignancies derived from B cells such as chronic lymphocytic leukemia, acute lymphocytic leukemia and many non-Hodgkin lymphomas frequently retain CD19 expression. This near universal expression and specificity for a single cell lineage has made CD19 an attractive target for immunotherapies. Human CD19 has 14 exons wherein exon 1-4 encode the extracellular portion of the CD19, exon 5 encodes the transmembrane portion of CD19 and exons 6-14 encode the cytoplasmic tail.

[00192] In one embodiment, payloads of the present invention may be antibodies, fragments and variants thereof which are specific to CD19 antigen.

[00193] In one embodiment, the payload of the invention may be a FMC63 antibody, antibody fragment or variant. FMC63 is an IgG2a mouse monoclonal antibody clone specific to the CD19 antigen that reacts with CD19 antigen on cells of the B cell lineage. The epitope of CD19 recognized by the FMC63 antibody is in exon 2 (Sotillo et al (2015) Cancer Discov ;5(12):1282-95; the contents of which are incorporated by reference in their entirety). In some embodiments,

the payload of the invention may be other CD19 monoclonal antibody clones including but not limited to 4G7, SJ25C1, CVID3/429, CVID3/155, HIB19, and J3-119.

[00194] In some embodiments, the payloads of the present invention may include variable heavy chain and variable light chain comprising the amino acid sequences selected from those in Table 4.

Table 4: Variable Heavy and Light Chain Sequences

<u>Target</u>	<u>Antibody chain</u>	<u>SEQ ID NO</u>	<u>Source</u>
CD19	VH	49	SEQ ID NO: 28 in WO2016168773A3
CD19	VH	50	SEQ ID NO: 29 in WO2016168773A3
CD19	VH	51	SEQ ID NO: 32 in WO2016168773A3
CD19	VH	52	SEQ ID NO: 33 in WO2016168773A3
CD19	VH	53	SEQ ID NO: 34 in WO2016168773A3
CD19	VH	54	SEQ ID NO: 35 in WO2016168773A3
CD19	VH	55	SEQ ID NO: 51 in WO2016187349A1
CD19	VH	56	SEQ ID NO: 20 in US20160039942
CD19	VH	57	SEQ ID NO: 1 in WO2014184143
CD19	VH	58	SEQ ID NO: 5 in US20160145337A1
CD19	VH	59	SEQ ID NO: 15 in US20160319020
CD19	VH	60	SEQ ID NO: 166 in US20160152723
CD19	VH	61	SEQ ID NO: 167 in US20160152723
CD19	VH	62	SEQ ID NO: 168 in US20160152723
CD19	VH	63	SEQ ID NO: 17 in EP3057991A1
CD19	VH	64	SEQ ID NO: 172 in US20160152723
CD19	VH	65	SEQ ID NO: 176 in US20160152723
CD19	VH	66	SEQ ID NO: 177 in US20160152723
CD19	VH	67	SEQ ID NO: 181 in US20160152723
CD19	VH	68	SEQ ID NO: 183 in US20160152723
CD19	VH	69	SEQ ID NO: 184 in US20160152723
CD19	VH	70	SEQ ID NO: 185 in US20160152723
CD19	VH	71	SEQ ID NO: 62 in US20160152723
CD19	VH	72	SEQ ID NO: 62 in WO2016097231
CD19	VH	73	SEQ ID NO: 12 in WO2016134284
CD19	VH	74	SEQ ID NO: 111 in US20160333114A1
CD19	VH	75	SEQ ID NO: 113 in US20160333114A1
CD19	VH	76	SEQ ID NO: 33 in EP3057994A1
CD19	VH	77	SEQ ID NO: 34 in EP3057994A1
CD19	VH	78	SEQ ID NO: 35 in EP3057994A1
CD19	VH	79	SEQ ID NO: 53 in WO2016120216
CD19	VH	80	SEQ ID NO: 55 in WO2016120216
CD19	VK	81	SEQ ID NO: 13 in US20160319020

CD19	VK	82	SEQ ID NO: 6 in US20160319020
CD19	VL	83	SEQ ID NO: 27 in WO2016168773A3
CD19	VL	84	SEQ ID NO: 31 in WO2016168773A3
CD19	VL	85	SEQ ID NO: 49 in WO2016187349A1
CD19	VL	86	SEQ ID NO: 11 in WO2016134284
CD19	VL	87	SEQ ID NO: 194 in US20140134142A1
CD19	VL	88	SEQ ID NO: 54 in WO2016120216
CD19	VL	89	SEQ ID NO: 56 in WO2016120216
CD19	VL	90	SEQ ID NO: 13 in US20160152723
CD19	VL	91	SEQ ID NO: 14 in US20160152723
CD19	VL	92	SEQ ID NO: 15 in US20160152723
CD19	VL	93	SEQ ID NO: 16 in US20160152723
CD19	VL	94	SEQ ID NO: 17 in US20160152723
CD19	VL	95	SEQ ID NO: 186 in US20160152723
CD19	VL	96	SEQ ID NO: 187 in US20160152723
CD19	VL	97	SEQ ID NO: 188 US20160152723
CD19	VL	98	SEQ ID NO: 189 in US20160152723
CD19	VL	99	SEQ ID NO: 192 in US20160152723
CD19	VL	100	SEQ ID NO: 196 in US20160152723
CD19	VL	101	SEQ ID NO: 197 in US20160152723
CD19	VL	102	SEQ ID NO: 198 in US20160152723
CD19	VL	103	SEQ ID NO: 199 in US20160152723
CD19	VL	104	SEQ ID NO: 200 in US20160152723
CD19	VL	105	SEQ ID NO: 201 in US20160152723
CD19	VL	106	SEQ ID NO: 202 in US20160152723
CD19	VL	107	SEQ ID NO: 203 in US20160152723
CD19	VL	108	SEQ ID NO: 204 in US20160152723
CD19	VL	109	SEQ ID NO: 205 in US20160152723
CD19	VL	110	SEQ ID NO: 22 in US20160039942
CD19	VL	111	SEQ ID NO: 63 in WO2016097231
CD19	VL	112	SEQ ID NO: 64 in US20160152723
CD19	VL	113	SEQ ID NO: 66 in US20160152723
CD19	VL	114	SEQ ID NO: 67 in US20160152723
CD19	VL	115	SEQ ID NO: 68 in US20160152723
CD19	VL	116	SEQ ID NO: 69 in US20160152723
CD19	VL	117	SEQ ID NO: 70 in US20160152723
CD19	VL	118	SEQ ID NO: 71 in US20160152723
CD19	VL	119	SEQ ID NO: 91 in US20160152723
CD19	VL	120	SEQ ID NO: 3 in US20160145337A1
CD19	VL	121	SEQ ID NO: 112 in US20160333114A1
CD19	VL	122	SEQ ID NO: 114 in US20160333114A1

[00195] A tumor specific antigen (TSA) may be a tumor neoantigen. A neoantigen is a mutated antigen that is only expressed by tumor cells because of genetic mutations or alterations in transcription which alter protein coding sequences, therefore creating novel, foreign antigens. The genetic changes result from genetic substitution, insertion, deletion or any other genetic changes of a native cognate protein (i.e. a molecule that is expressed in normal cells). In the context of CD19, neoantigens such as a transcript variant of CD19 lacking exon 2 or lacking exon 5-6 or both have been described (see International patent publication No. WO2016061368; the contents of which are incorporated herein by reference in their entirety). Since FMC63 binding epitope is in exon 2, CD19 neoantigen lacking exon 2 is not recognized by FMC63 antibody. Thus, in some embodiments, payloads of the invention may include FMC63-distinct antibodies, or fragments thereof. As used herein "FMC63-distinct" refers, to an antibody or fragment thereof that is immunologically specific and binds to an epitope of the CD19 antigen that is different or unlike the epitope of CD19 antigen that is bound by FMC63. In some instances, antibodies of the invention may include CD19 antibodies, antibody fragments or variants that recognize CD19 neoantigens including the CD19 neoantigen lacking exon2. In one embodiment, the antibody or fragment thereof is immunologically specific to the CD19 encoded by exon 1, 3 and/or 4. In one example, the antibody or fragment thereof is specific to the epitope that bridges the portion of CD19 encoded by exon 1 and the portion of CD19 encoded by exon 3.

[00196] Chimeric antigen receptors (CARs)

[00197] In some embodiments, payloads of the present invention may be a chimeric antigen receptors (CARs) which when transduced into immune cells (e.g., T cells and NK cells), can re-direct the immune cells against the target (e.g., a tumor cell) which expresses a molecule recognized by the extracellular target moiety of the CAR.

[00198] As used herein, the term "chimeric antigen receptor (CAR)" refers to a synthetic receptor that mimics TCR on the surface of T cells. In general, a CAR is composed of an extracellular targeting domain, a transmembrane domain/region and an intracellular signaling/activation domain. In a standard CAR receptor, the components: the extracellular targeting domain, transmembrane domain and intracellular signaling/activation domain, are linearly constructed as a single fusion protein. The extracellular region comprises a targeting domain/moiety (e.g., a scFv) that recognizes a specific tumor antigen or other tumor cell-surface molecules. The intracellular region may contain a signaling domain of TCR complex (e.g., the signal region of CD3 ζ), and/or one or more costimulatory signaling domains, such as those from CD28, 4-1BB (CD137) and OX-40 (CD134). For example, a "first-generation CAR" only has the CD3 ζ signaling domain. In an effort to augment T-cell persistence and proliferation,

costimulatory intracellular domains are added, giving rise to second generation CARs having a CD3 ζ signal domain plus one costimulatory signaling domain, and third generation CARs having CD3 ζ signal domain plus two or more costimulatory signaling domains. A CAR, when expressed by a T cell, endows the T cell with antigen specificity determined by the extracellular targeting moiety of the CAR. Recently, it is also desirable to add one or more elements such as homing and suicide genes to develop a more competent and safer architecture of CAR, so called the fourth-generation CAR.

[00199] In some embodiments, the extracellular targeting domain is joined through the hinge (also called space domain or spacer) and transmembrane regions to an intracellular signaling domain. The hinge connects the extracellular targeting domain to the transmembrane domain which transverses the cell membrane and connects to the intracellular signaling domain. The hinge may need to be varied to optimize the potency of CAR transformed cells toward cancer cells due to the size of the target protein where the targeting moiety binds, and the size and affinity of the targeting domain itself. Upon recognition and binding of the targeting moiety to the target cell, the intracellular signaling domain leads to an activation signal to the CAR T cell, which is further amplified by the “second signal” from one or more intracellular costimulatory domains. The CAR T cell, once activated, can destroy the target cell.

[00200] In some embodiments, the CAR of the present invention may be split into two parts, each part is linked a dimerizing domain, such that an input that triggers the dimerization promotes assembly of the intact functional receptor. Wu and Lim recently reported a split CAR in which the extracellular CD19 binding domain and the intracellular signaling element are separated and linked to the FKBP domain and the FRB* (T2089L mutant of FKBP-rapamycin binding) domain that heterodimerize in the presence of the rapamycin analog AP21967. The split receptor is assembled in the presence of AP21967 and together with the specific antigen binding, activates T cells (Wu et al., *Science*, 2015, 625(6258): aab4077).

[00201] In some embodiments, the CAR of the present invention may be designed as an inducible CAR. Sakemura et al recently reported the incorporation of a Tet-On inducible system to the CD19 CAR construct. The CD19 CAR is activated only in the presence of doxycycline (Dox). Sakemura reported that Tet-CD19CAR T cells in the presence of Dox were equivalently cytotoxic against CD19⁺ cell lines and had equivalent cytokine production and proliferation upon CD19 stimulation, compared with conventional CD19CAR T cells (Sakemura et al., *Cancer Immuno. Res.*, 2016, Jun 21, Epub ahead of print). In one example, this Tet-CAR may be the payload of the effector module under the control of SREs (e.g., DDs) of the invention. The dual systems provide more flexibility to turn-on and off of the CAR expression in transduced T cells.

[00202] According to the present invention, the payload of the present invention may be a first-generation CAR, or a second-generation CAR, or a third-generation CAR, or a fourth-generation CAR. Representative effector module embodiments comprising CAR constructs are illustrated in Figures 13-18. In some embodiments, the payload of the present invention may be a full CAR construct composed of the extracellular domain, the hinge and transmembrane domain and the intracellular signaling region. In other embodiments, the payload of the present invention may be a component of the full CAR construct including an extracellular targeting moiety, a hinge region, a transmembrane domain, an intracellular signaling domain, one or more co-stimulatory domain, and other additional elements that improve CAR architecture and functionality including but not limited to a leader sequence, a homing element and a safety switch, or the combination of such components.

[00203] CARs regulated by biocircuits and compositions of the present invention are tunable and thereby offer several advantages. The reversible on-off switch mechanism allows management of acute toxicity caused by excessive CAR-T cell expansion. Pulsatile CAR expression using SREs of the present invention may be achieved by cycling ligand level. The ligand conferred regulation of the CAR may be effective in offsetting tumor escape induced by antigen loss, avoiding functional exhaustion caused by tonic signaling due to chronic antigen exposure and improving the persistence of CAR expressing cells *in vivo*.

[00204] In some embodiments, biocircuits and compositions of the invention may be utilized to down regulate CAR expression to limit on target on tissue toxicity caused by tumor lysis syndrome. Down regulating the expression of the CARs of the present invention following anti-tumor efficacy may prevent (1) On target off tumor toxicity caused by antigen expression in normal tissue, (2) antigen independent activation *in vivo*.

[00205] In one embodiment, selection of a CAR with a lower affinity may provide more T cell signaling and less toxicity.

Extracellular targeting domain/moiety

[00206] In accordance with the invention, the extracellular target moiety of a CAR may be any agent that recognizes and binds to a given target molecule, for example, a neoantigen on tumor cells, with high specificity and affinity. The target moiety may be an antibody and variants thereof that specifically binds to a target molecule on tumor cells, or a peptide aptamer selected from a random sequence pool based on its ability to bind to the target molecule on tumor cells, or a variant or fragment thereof that can bind to the target molecule on tumor cells, or an antigen recognition domain from native T- cell receptor (TCR) (e.g. CD4 extracellular domain to

recognize HIV infected cells), or exotic recognition components such as a linked cytokine that leads to recognition of target cells bearing the cytokine receptor, or a natural ligand of a receptor.

[00207] In some embodiments, the targeting domain of a CAR may be a Ig NAR, a Fab fragment, a Fab' fragment, a F(ab)'2 fragment, a F(ab)'3 fragment, Fv, a single chain variable fragment (scFv), a bis-scFv, a (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein (dsFv), a unitbody, a nanobody, or an antigen binding region derived from an antibody that specifically recognizes a target molecule, for example a tumor specific antigen (TSA). In one embodiment, the targeting moiety is a scFv antibody. The scFv domain, when it is expressed on the surface of a CAR T cell and subsequently binds to a target protein on a cancer cell, is able to maintain the CAR T cell in proximity to the cancer cell and to trigger the activation of the T cell. A scFv can be generated using routine recombinant DNA technology techniques and is discussed in the present invention.

[00208] In one embodiment, the targeting moiety of the CAR may recognize CD19. CD19 is a well-known B cell surface molecule, which upon B cell receptor activation enhances B-cell antigen receptor induced signaling and expansion of B cell populations. CD19 is broadly expressed in both normal and neoplastic B cells. Malignancies derived from B cells such as chronic lymphocytic leukemia, acute lymphocytic leukemia and many non-Hodgkin lymphomas frequently retain CD19 expression. This near universal expression and specificity for a single cell lineage has made CD19 an attractive target for immunotherapies. Human CD19 has 14 exons wherein exon 1-4 encode the extracellular portion of the CD19, exon 5 encodes the transmembrane portion of CD19 and exons 6-14 encode the cytoplasmic tail. In one embodiment, the targeting moiety may comprise scFvs derived from the variable regions of the FMC63 antibody. FMC63 is an IgG2a mouse monoclonal antibody clone specific to the CD19 antigen that reacts with CD19 antigen on cells of the B lineage. The epitope of CD19 recognized by the FMC63 antibody is in exon 2 (Sotillo et al (2015) Cancer Discov ;5(12):1282-95; the contents of which are incorporated by reference in their entirety). In some embodiments, the targeting moiety of the CAR may be derived from the variable regions of other CD19 monoclonal antibody clones including but not limited to 4G7, SJ25C1, CVID3/429, CVID3/155, HIB19, and J3-119.

[00209] In some embodiments, the targeting moiety of a CAR may recognize a tumor specific antigen (TSA), for example a cancer neoantigen that is only expressed by tumor cells because of genetic mutations or alterations in transcription which alter protein coding sequences, therefore creating novel, foreign antigens. The genetic changes result from genetic substitution, insertion, deletion or any other genetic changes of a native cognate protein (i.e. a molecule that is

expressed in normal cells). In the context of CD19, TSAs may include a transcript variant of human CD19 lacking exon 2 or lacking exon 5-6 or both (see International patent publication No. WO2016061368; the contents of which are incorporated herein by reference in their entirety). Since FMC63 binding epitope is in exon 2, CD19 lacking exon 2 is not recognized by FMC63 antibody. Thus, in some embodiments, the targeting moiety of the CAR may be an FMC63-distinct scFv. As used herein "FMC63-distinct" refers, to an antibody, scFv or a fragment thereof that is immunologically specific and binds to an epitope of the CD19 antigen that is different or unlike the epitope of CD19 antigen that is bound by FMC63. In some instances, targeting moiety may recognize a CD19 antigen lacking exon2. In one embodiment, the targeting moiety recognizes a fragment of CD19 encoded by exon 1, 3 and/or 4. In one example, the targeting moiety recognizes the epitope that bridges the portion of CD19 encoded by exon 1 and the portion of CD19 encoded by exon 3.

[00210] In some embodiments, the targeting moieties of the present invention may be scFv comprising the amino acid sequences in Table 5.

Table 5: scFv sequences

Target	Description	SEQ ID NO	Source
CD19	scFv	123	SEQ ID NO. 53 in EP3083671A1
CD19	scFv	124	SEQ ID NO. 54 in EP3083671A1
CD19	scFv	125	SEQ ID NO. 1 in WO2015157252
CD19	scFv	126	SEQ ID NO. 10 in WO2015157252
CD19	scFv	127	SEQ ID NO. 10 in WO2016033570
CD19	scFv	128	SEQ ID NO. 11 in WO2015157252
CD19	scFv	129	SEQ ID NO. 12 in WO2015157252
CD19	scFv	130	SEQ ID NO. 2 in WO2015157252
CD19	scFv	131	SEQ ID NO. 2 in WO2016033570
CD19	scFv	132	SEQ ID NO. 206 in WO2016033570
CD19	scFv	133	SEQ ID NO. 207 in WO2016033570
CD19	scFv	134	SEQ ID NO. 208 in WO2016033570
CD19	scFv	135	SEQ ID NO. 209 in WO2016033570
CD19	scFv	136	SEQ ID NO. 210 in WO2016033570
CD19	scFv	137	SEQ ID NO. 211 in WO2016033570
CD19	scFv	138	SEQ ID NO. 213 in WO2016033570
CD19	scFv	139	SEQ ID NO. 214 in WO2016033570
CD19	scFv	140	SEQ ID NO. 215 in WO2016033570
CD19	scFv	141	SEQ ID NO. 216 in WO2016033570
CD19	scFv	142	SEQ ID NO. 217 in WO2016033570
CD19	scFv	143	SEQ ID NO. 218 in WO2016033570
CD19	scFv	144	SEQ ID NO. 219 in WO2016033570
CD19	scFv	145	SEQ ID NO. 220 in WO2016033570
CD19	scFv	146	SEQ ID NO. 221 in WO2016033570
CD19	scFv	147	SEQ ID NO. 222 in WO2016033570
CD19	scFv	148	SEQ ID NO. 223 in WO2016033570
CD19	scFv	149	SEQ ID NO. 224 in WO2016033570
CD19	scFv	150	SEQ ID NO. 225 in WO2016033570
CD19	scFv	151	SEQ ID NO. 3 in WO2015157252
CD19	scFv	152	SEQ ID NO. 4 in WO2015157252

CD19	scFv	153	SEQ ID NO. 4 in WO2016033570
CD19	scFv	154	SEQ ID NO. 45 in WO2016033570
CD19	scFv	155	SEQ ID NO. 47 in WO2016033570
CD19	scFv	156	SEQ ID NO. 49 in WO2016033570
CD19	scFv	157	SEQ ID NO. 5 in WO2015155341A1
CD19	scFv	158	SEQ ID NO. 5 in WO2015157252
CD19	scFv	159	SEQ ID NO. 51 in WO2016033570
CD19	scFv	160	SEQ ID NO. 53 in WO2016033570
CD19	scFv	161	SEQ ID NO. 55 in WO2016033570
CD19	scFv	162	SEQ ID NO. 57 in WO2016033570
CD19	scFv	163	SEQ ID NO. 59 in WO2015157252
CD19	scFv	164	SEQ ID NO. 59 in WO2016033570
CD19	scFv	165	SEQ ID NO. 6 in WO2015157252
CD19	scFv	166	SEQ ID NO. 6 in WO2016033570
CD19	scFv	167	SEQ ID NO. 7 in WO2014184143
CD19	scFv	168	SEQ ID NO. 7 in WO2015157252
CD19	scFv	169	SEQ ID NO. 8 in WO2015157252
CD19	scFv	170	SEQ ID NO. 8 in WO2016033570
CD19	scFv	171	SEQ ID NO. 87 in WO2016033570
CD19	scFv	172	SEQ ID NO. 9 in WO2015157252
CD19	scFv	173	SEQ ID NO. 9 in WO2016139487
CD19	scFv	174	SEQ ID NO. 10 in US20160152723
CD19	scFv	175	SEQ ID NO. 2 in US20160152723
CD19	scFv	176	SEQ ID NO. 206 in US20160152723
CD19	scFv	177	SEQ ID NO. 207 in US20160152723
CD19	scFv	178	SEQ ID NO. 208 in US20160152723
CD19	scFv	179	SEQ ID NO. 209 in US20160152723
CD19	scFv	180	SEQ ID NO. 210 in US20160152723
CD19	scFv	181	SEQ ID NO. 211 in US20160152723
CD19	scFv	182	SEQ ID NO. 212 in US20160152723
CD19	scFv	183	SEQ ID NO. 213 in US20160152723
CD19	scFv	184	SEQ ID NO. 214 in US20160152723
CD19	scFv	185	SEQ ID NO. 215 in US20160152723
CD19	scFv	186	SEQ ID NO. 216 in US20160152723
CD19	scFv	187	SEQ ID NO. 217 in US20160152723
CD19	scFv	188	SEQ ID NO. 218 in US20160152723
CD19	scFv	189	SEQ ID NO. 219 in US20160152723
CD19	scFv	190	SEQ ID NO. 220 in US20160152723
CD19	scFv	191	SEQ ID NO. 221 in US20160152723
CD19	scFv	192	SEQ ID NO. 222 in US20160152723
CD19	scFv	193	SEQ ID NO. 223 in US20160152723
CD19	scFv	194	SEQ ID NO. 224 in US20160152723
CD19	scFv	195	SEQ ID NO. 225 in US20160152723
CD19	scFv	196	SEQ ID NO. 32 in EP3083691A2
CD19	scFv	197	SEQ ID NO. 35 in EP3083691A2
CD19	scFv	198	SEQ ID NO. 38 in EP3083691A2
CD19	scFv	199	SEQ ID NO. 4 in US20160152723
CD19	scFv	200	SEQ ID NO. 45 in US20160152723
CD19	scFv	201	SEQ ID NO. 47 in US20160152723
CD19	scFv	202	SEQ ID NO. 49 in US20160152723
CD19	scFv	203	SEQ ID NO. 51 in US20160152723
CD19	scFv	204	SEQ ID NO. 53 in US20160152723
CD19	scFv	205	SEQ ID NO. 55 in US20160152723
CD19	scFv	206	SEQ ID NO. 57 in US20160152723
CD19	scFv	207	SEQ ID NO. 59 in US20160152723
CD19	scFv	208	SEQ ID NO. 6 in US20160152723
CD19	scFv	209	SEQ ID NO. 8 in US20160152723
CD19	scFv	210	SEQ ID NO. 87 in US20160152723

CD19	scFv	211	SEQ ID NO. 89 in US20160152723
CD19	scFv	212	SEQ ID NO. 39 in WO2016109410
CD19	scFv	213	SEQ ID NO. 37 in EP3083671A1
CD19	scFv	214	SEQ ID NO. 174 in WO2016115482
CD19	scFv	215	SEQ ID NO. 20 in WO2012079000
CD19	scFv	216	SEQ ID NO. 32 in WO2015092024
CD19	scFv	217	SEQ ID NO. 33 in WO2015092024A2
CD19	scFv	218	SEQ ID NO. 35 in WO2015092024A2
CD19	scFv	219	SEQ ID NO. 38 in WO2015092024A2
CD19	scFv	220	SEQ ID NO. 40 in WO2016109410
CD19	scFv	221	SEQ ID NO. 41 in WO2016109410
CD19	scFv	222	SEQ ID NO. 42 in WO2016109410
CD19	scFv	223	SEQ ID NO. 43 in WO2016109410
CD19	scFv	224	SEQ ID NO. 44 in WO2016109410
CD19	scFv	225	SEQ ID NO. 45 in WO2016109410
CD19	scFv	226	SEQ ID NO. 46 in WO2016109410
CD19	scFv	227	SEQ ID NO. 47 in WO2016109410
CD19	scFv	228	SEQ ID NO. 48 in WO2016109410
CD19	scFv	229	SEQ ID NO. 49 in WO2016109410
CD19	scFv	230	SEQ ID NO. 5 in WO2015155341A1
CD19	scFv	231	SEQ ID NO. 50 in WO2016109410
CD19	scFv	232	SEQ ID NO. 51 in WO2016109410
CD19	scFv	233	SEQ ID NO. 7 in US20160145337A1
CD19	scFv	234	SEQ ID NO. 9 in US20160145337A1
CD19	scFv	235	SEQ ID NO.20 in US9499629B2
CD19	scFv	236	SEQ ID NO.6 in WO2015155341A1
CD19	scFv	237	SEQ ID NO.73 in WO2016164580
CD19	scFv	238	SEQ ID NO. 10 US20160152723
CD19	scFv	239	SEQ ID NO. 2 in US20160152723
CD19	scFv	240	SEQ ID NO. 206 in US20160152723
CD19	scFv	241	SEQ ID NO. 207 in US20160152723
CD19	scFv	242	SEQ ID NO. 209 in US20160152723
CD19	scFv	243	SEQ ID NO. 210 in US20160152723
CD19	scFv	244	SEQ ID NO. 212 in US20160152723
CD19	scFv	245	SEQ ID NO. 216 in US20160152723
CD19	scFv	246	SEQ ID NO. 218 in US20160152723
CD19	scFv	247	SEQ ID NO. 219 in US20160152723
CD19	scFv	248	SEQ ID NO. 220 in US20160152723
CD19	scFv	249	SEQ ID NO. 221 in US20160152723
CD19	scFv	250	SEQ ID NO. 222 in US20160152723
CD19	scFv	251	SEQ ID NO. 223 in US20160152723
CD19	scFv	252	SEQ ID NO. 224 in US20160152723
CD19	scFv	253	SEQ ID NO. 225 in US20160152723
CD19	scFv	254	SEQ ID NO. 4 in US20160152723
CD19	scFv	255	SEQ ID NO. 45 in US20160152723
CD19	scFv	256	SEQ ID NO. 47 in US20160152723
CD19	scFv	257	SEQ ID NO. 49 in US20160152723
CD19	scFv	258	SEQ ID NO. 51 in US20160152723
CD19	scFv	259	SEQ ID NO. 53 in US20160152723
CD19	scFv	260	SEQ ID NO. 55 in US20160152723
CD19	scFv	261	SEQ ID NO. 57 in US20160152723
CD19	scFv	262	SEQ ID NO. 59 in US20160152723
CD19	scFv	263	SEQ ID NO. 6 in US20160152723
CD19	scFv	264	SEQ ID NO. 8 in US20160152723
CD19	scFv	265	SEQ ID NO. 87 in US20160152723
CD19	scFv	266	SEQ ID NO. 89 in US20160152723
CD19	scFv	267	SEQ ID NO. 5 in WO2016055551

Intracellular signaling domains

[00211] The intracellular domain of a CAR fusion polypeptide, after binding to its target molecule, transmits a signal to the immune effector cell, activating at least one of the normal effector functions of immune effector cells, including cytolytic activity (e.g., cytokine secretion) or helper activity. Therefore, the intracellular domain comprises an "intracellular signaling domain" of a T cell receptor (TCR).

[00212] In some aspects, the entire intracellular signaling domain can be employed. In other aspects, a truncated portion of the intracellular signaling domain may be used in place of the intact chain as long as it transduces the effector function signal.

[00213] In some embodiments, the intracellular signaling domain of the present invention may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs (ITAMs). Examples of ITAM containing cytoplasmic signaling sequences include those derived from TCR CD3zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. In one example, the intracellular signaling domain is a CD3 zeta (CD3ζ) signaling domain.

[00214] In some embodiments, the intracellular region of the present invention further comprises one or more costimulatory signaling domains which provide additional signals to the immune effector cells. These costimulatory signaling domains, in combination with the signaling domain can further improve expansion, activation, memory, persistence, and tumor-eradicating efficiency of CAR engineered immune cells (e.g., CAR T cells). In some cases, the costimulatory signaling region contains 1, 2, 3, or 4 cytoplasmic domains of one or more intracellular signaling and /or costimulatory molecules. The costimulatory signaling domain may be the intracellular/cytoplasmic domain of a costimulatory molecule, including but not limited to CD2, CD7, CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, ICOS (CD278), GITR (glucocorticoid-induced tumor necrosis factor receptor), LFA-1 (lymphocyte function-associated antigen- 1), LIGHT, NKG2C, B7-H3. In one example, the costimulatory signaling domain is derived from the cytoplasmic domain of CD28. In another example, the costimulatory signaling domain is derived from the cytoplasmic domain of 4-1BB (CD137). In another example, the costimulatory signaling domain may be an intracellular domain of GITR as taught in U.S. Pat. NO.: 9, 175, 308; the contents of which are incorporated herein by reference in its entirety.

[00215] In some embodiments, the intracellular region of the present invention may comprise a functional signaling domain from a protein selected from the group consisting of an MHC class I molecule, a TNF receptor protein, an immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation protein (SLAM) such as CD48, CD229, 2B4, CD84,

NTB-A, CRACC, BLAME, CD2F-10, SLAMF6, SLAMF7, an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFRR, LIGHT, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, IL15Ra, ITGA4, 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, NKG2D, NKG2C, NKD2C SLP76, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, CD270 (HVEM), GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, DAP 10, TRIM, ZAP70, Killer immunoglobulin receptors (KIRs) such as KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, and KIR2DP1; lectin related NK cell receptors such as Ly49, Ly49A, and Ly49C.

[00216] In some embodiments, the intracellular signaling domain of the present invention may contain signaling domains derived from JAK-STAT. In other embodiments, the intracellular signaling domain of the present invention may contain signaling domains derived from DAP-12 (Death associated protein 12) (Topfer et al., *Immunol.*, 2015, 194: 3201-3212; and Wang et al., *Cancer Immunol.*, 2015, 3: 815-826). DAP-12 is a key signal transduction receptor in NK cells. The activating signals mediated by DAP-12 play important roles in triggering NK cell cytotoxicity responses toward certain tumor cells and virally infected cells. The cytoplasmic domain of DAP12 contains an Immunoreceptor Tyrosine-based Activation Motif (ITAM). Accordingly, a CAR containing a DAP12-derived signaling domain may be used for adoptive transfer of NK cells.

[00217] In some embodiments, T cells engineered with two or more CARs incorporating distinct co-stimulatory domains and regulated by distinct DD may be used to provide kinetic control of downstream signaling.

[00218] In some embodiments, the intracellular domain of the present invention may comprise amino acid sequences of Table 6.

Table 6: Intracellular signaling and co-stimulatory domains

Description	Amino Acid Sequence	Amino Acid SEQ ID
2B4 co-stimulatory domain	WRRKRKEKQSETSPKEFLTIYEDVKDLKTRRNHEQEQTFF GGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRN HSPSFNSTIYEVIGKSQPKAQNPAPRLSRKELENFDVYS	268
CD27 co-stimulatory domain	HQRRKYRSNKGESPVPAEPCRYSCPREEEGSTIPIQEDYR KPEPACSP	269
CD272 (BTLA1) co-stimulatory domain	RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQ VLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGVYA SLNHSVIGPNSRLARNVKEAPTEYASICVRS	270
CD272 (BTLA1) co-stimulatory domain	CCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQ NSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPG IVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS	271
CD28 co-stimulatory	FWVLVVVGVLACYSLLVTVAFIIFWV	272
CD28 co-stimulatory domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC EL	273
CD28 co-stimulatory domain	FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDF AAYRS	274
CD28 co-stimulatory domain	RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY RS	275
CD28 co-stimulatory domain	RSKRSRGGHSDYIVINMTPRRPGPTRKHYPYAPPRDFAA YRS	276
CD28 co-stimulatory signaling region	MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLS KYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQQLQVY SKTGFNC DGKLGNESVTFYLQNLVYNQTDIYFCKIEVMYP PPYLDNEK SNGTIIHVKGKHLCPSPLPFGPSKPFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRS	277
CD30 co-stimulatory domain	RRACRKRIKQLHLCPVQTSQPKLELVDSRPRRSSTQLR SGASVTEPVAEERGLMSQPLMETCHSVGAAYLESPLQD ASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVG TVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPL GSCSDVMLSVEEEGKEDPLPTAASGK	278
CD30 co-stimulatory domain	RRACRKRIKQLHLCPVQTSQPKLELVDSRPRRSSTQLR SGASVTEPVAEERGLMSQPLMETCHSVGAAYLESPLQD ASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVG TVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPL GSCSDVMLSVEEEGKEDPLPTAASGK	279
GITR co-stimulatory domain	HIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPPEERGE RSAEEKGRLGDLWV	280
HVEM co-stimulatory domain	CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPP DVTTVAVEETIPSFTGRSPNH	281
ICOS co-stimulatory domain	TKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL	282
ICOS co-stimulatory signaling domain	CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL	283
LAG-3 co-stimulatory region	HLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEP EPEPEPEPEPEQL	284
OX40 co-stimulatory domain	ALYLLRRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLA KI	285
OX40 co-stimulatory domain	RRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKI	286
4-1BB intracellular domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE L	287
4-1BB signaling domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGY EL	288

4-1BB-CD3Zeta intracellular domain	TGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSL VITL YCKRGRKKLLYIF KQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	289
4-1BB-Z endodomain fusion	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DK RRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	290
CD127 intracellular domain	KRIKPIVWPSLPDHKKLTLEHLCKKPRKNLNVSFNPESFLDC QIHRVDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQS PNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL DCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLN PVAQQQPILTSLGSNQEEAYVTMSSFYQNNQ	291
CD137 intracellular domain	RFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL	292
CD148 intracellular domain	RKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQAD SNCGFAEEYEDLKL VGISQPKYAAELAENRGKNRYNNVL PYDISRVKLSVQTHSTDDYINANYMPGYHKKDFIATQGP LPNTLKDFWRMVWEKNVYAIIMLT KCVEQGRTKCEEYW PSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESH LRQFHFTSWPDHGVDPD TDLINFRYLVRDYMKQSPPE SPI LVHCSAGVGRTGT FIAIDRLIYQIENENTVDVYGIVYDLR MHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNT TAMTIYENLAPVTTFGKTNGYIA	293
CD27 intracellular domain	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRK PEPACSP	294
CD28 intracellular domain	FAAYRS	295
CD28 signaling chain	FWVLVVVGGLACYSLLVTVAFIIFWVRSKRSRLLHSDY MNMTPRRPGPTRKHYPYAPPRDFAAYRS	296
CD28 signaling domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY RS	297
CD28 signaling domain	SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYR S	298
CD28 signaling domain	IEVMYPPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSK PFWVLVVVGGLACYSLLVTVAFIIFWVRSKRSRLLHSDY MNMTPRRPGPTRKHYPYAPPRDFAAYRS	299
CD28, 4-1BB, and/or CD3ζ signaling domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY RSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF EEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVL DKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR	300
CD28/CD3C	AAAEIVMYPPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSK PFWVLVVVGGLACYSLLVTVAFIIFWVRSKRSRLLHSDY MNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	301
CD28-0XZ intracellular domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS RDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR	302
CD28-4-1BB intracellular domain	MFWVLVVVGGLACYSLLVTVAFIIFWVRGRKKLLYIF KQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL	303

CD28-4-1BB intracellular domain	IEVMYPPPPYLDNEKSNGTHHVKGKHLCPSPLPGPSKPFW VLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFEEEEGGCEL	304
CD28-CD3 Zeta intracellular domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY RSRVKFSRSADAPAYQOGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	305
CD28-CD3Zeta intracellular domain	KRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS RVKFSRSADAPAYQOGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	306
CD3 delta chain intracellular signaling domain	MEHSTFLSGLVLATLLSQVSPFKIPIEELEDVRFVNCNTSIT WVEGTVGTLTSDITRLDLGKRILDPRGYRCNGTDIYKDK ESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLALGV FCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYS HLGGNWARNK	307
CD3 delta chain intracellular signaling domain	MEHSTFLSGLVLATLLSQVSPFKIPIEELEDVRFVNCNTSIT WVEGTVGTLTSDITRLDLGKRILDPRGYRCNGTDIYKDK ESTVQVHYRTADTQALLRNDQVYQPLRDRDDAQYSHLG GNWARNK	308
CD3 delta chain intracellular signaling domain	DQVYQPLRDRDDAQYSHLGGN	309
CD3 delta intracellular domain	MEHSTFLSGLVLATLLSQVSPFKIPIEELEDVRFVNCNTSIT WVEGTVGTLTSDITRLDLGKRILDPRGYRCNGTDIYKDK ESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLALGV FCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYS HLGGNWARNK	310
CD3 delta intracellular domain	MEHSTFLSGLVLATLLSQVSPFKIPIEELEDVRFVNCNTSIT WVEGTVGTLTSDITRLDLGKRILDPRGYRCNGTDIYKDK ESTVQVHYRTADTQALLRNDQVYQPLRDRDDAQYSHLG GNWARNK	311
CD3 delta intracellular domain	DQVYQPLRDRDDAQYSHLGGN	312
CD3 epsilon intracellular domain	MQSGTHWRVLGLCLLSVGWVGQDGNEEMGGITQTPYKV SISGTTVILTCPQYPGSEILWQHNDKNIGGEDDDKNIGSDE DHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVC ENCMEMDVMSVATIVIVDITGGLLLL VYYWSKNRKAK AKPVTRGAGAGGRQRGQNKERPPPVNPDYEPKRGQRD LYSGLNQRRI	313
CD3 epsilon intracellular domain	NPDYEPKRGQRDLYSGLNQR	314
CD3 gamma intracellular domain	MEQGKGLAVLILAILLQGTLAQSIKGNHLVKVYDYQEDG SVLLTCDAAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAK DPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGF LFAEIVSIFVLA VGVYFIAGQDGVRSRASDKQTLLPNDQ LYQPLKDREDDQYSHLQGNQLRRN,	315
CD3 gamma intracellular domain	DQLYQPLKDREDDQYSHLQGN	316
CD3 gamma intracellular domain	DQLYQPLKDREDDQYSHLQGN	317
CD3 gamma intracellular domain	MEQGKGLAVLILAILLQGTLAQSIKGNHLVKVYDYQEDG SVLLTCDAAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAK DPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGF LFAEIVSIFVLA VGVYFIAGQDGVRSRASDKQTLLPNDQ LYQPLKDREDDQYSHLQGNQLRRN	318
CD3 zeta intracellular domain	MKWKALFTAAILQAQLPTEAQSFGLLDPKLCYLLDGILFI YGVILTALFLRVKFSRSADAPAYQOGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM	319

	AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
CD3 zeta intracellular domain	MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	320
CD3 zeta intracellular domain	MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	321
CD3 zeta intracellular domain	NQLYNELNLGRREEYDVLDKR	322
CD3 zeta domain 2 (NM_000734.3)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	323
CD3 zeta intracellular domain	DGLYQGLSTATKDTYDALHMQ	324
CD3 zeta intracellular domain	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	325
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	326
CD3 zeta intracellular domain	RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	327
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	328
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	329
CD3 zeta intracellular domain	MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLVGIVLTLVLCRLKIQVRKAAITSYEKSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAVSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	330
CD3 zeta intracellular domain	LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	331
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLY	332
CD3 zeta intracellular domain	LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	333
CD3 zeta intracellular domain	RRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	334
CD3 zeta intracellular domain	NQLYNELNLGRREEYDVLDKR	335
CD3 zeta intracellular domain	EGLYNELQKDKMAEAYSEIGMK	336
CD3 zeta intracellular domain	DGLYQGLSTATKDTYDALHMQ	337
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	338

CD3 zeta intracellular domain	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	339
CD3 zeta intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALP	340
CD3 zeta intracellular domain	DPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR	341
CD3 zeta intracellular domain	MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFI YGVILTALFLRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR	342
CD40 intracellular domain	RSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI	343
CD79A intracellular domain	MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVP ASLMVSLGEDAHFQCPHNSSNNANVTWWRVLHGNYTWP PEFLGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQ SCGTYLRVRQPPRPFLDMGEGTKNRIITAEGIILLFCAVVP GTLLLFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCS MYEDISRGLQGTYQDVGSLNIGDVQLEKP	344
CD79A intracellular domain	MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVP ASLMVSLGEDAHFQCPHNSSNNANVTWWRVLHGNYTWP PEFLGPGEDPNEPPRPFLDMGEGTKNRIITAEGIILLFCAV VPGTLLLFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDC SMYEDISRGLQGTYQDVGSLNIGDVQLEKP	345
CD79A intracellular domain	MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVP ASLMVSLGEDAHFQCPHNSSNNANVTWWRVLHGNYTWP PEFLGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQ SCGTYLRVRQPPRPFLDMGEGTKNRIITAEGIILLFCAVVP GTLLLFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCS MYEDISRGLQGTYQDVGSLNIGDVQLEKP	346
CD79A intracellular domain	ENLYEGLNLDDCSMYEDISRG	347
CD8 intracellular domain	FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHR NR	348
CD8 intracellular domain	FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHR NR	349
CD8a intracellular domain	PTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDI	350
CTLA4 intracellular domain	AVSLSKMLKKRSPLTTGVFVKMAPTEAECEKQFQPYFIPI N	351
CTLA4 intracellular domain	AVSLSKMLKKRSPLTTGVYMNMTPRRPECEKQFQPYAPP RDFAAAYS	352
DAPI0 intracellular domain	RPRRSPAQDGKVYINMPGRG	353
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSP GVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAAEAAT RKQRITETESPYQELQGQQRSDVYSDLNTQRPYYK	354
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSP GVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAAEAAT RKQRITETESPYQELQGQQRSDVYSDLNTQRPYYK	355
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEAATRKQRITETESPY QELQGQQRSDVYSDLNTQRPYYK	356

DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEATRKQRITETESPYQ ELQGQRSDVYSDLNTQRPYYK	357
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSP GVLGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEAAT RKQRITETESPYQELQGQRSDVYSDLNTQRPYYK	358
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSP GVLGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEATR KQRITETESPYQELQGQRSDVYSDLNTQRPYYK;	359
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEAATRKQRITETESPY QELQGQRSDVYSDLNTQRPYYK	360
DAPI2 intracellular domain	MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLGIVMGDL VLTVLIALAVYFLGRLVPRGRGAAEATRKQRITETESPYQ ELQGQRSDVYSDLNTQRPYYK	361
DAPI2 intracellular domain	ESPYQELQGQRSDVYSDLNTQ	362
DAPI2 intracellular domain	ESPYQELQGQRSDVYSDLNTQ	363
GITR intracellular domain	RSQCMWPRETQLLLEVPPSTEDARSCQFPPEERGERSAEE KGRLGDLWV	364
ICOS intracellular domain	TKKKYSSSVHDPNGEFMFMRVNTAKKSRLTDVTL	365
IL15Ra intracellular domain	KSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL	366
OX40-CD3 Zeta intracellular domain	RRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVK FRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPENMGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	367
ZAP70 intracellular domain	MPDPAAHLPPFFYGSISRAEAEHLKLAGMADGLFLLRQCL RSLGGYVLSLVHDVRFHFPIERQLNGTYAIAGGKAHCGP AELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLRD AMVRDYYRQTWKLEGEALEQAISQAPQVEKLIATTAHE RMPWYHSSLTREEAERKLYSGAQTGDKFLLRPRKEQGTY ALSLIYGKTVYHYLISQDKAGKYCIPEGTKFDTLWQLVEY LKLKADGLIYCLKEACPNSSASNASGAAAPTLPAPHPSTLT HPQRRIDTLNSDGYTPEPARITSPDKPRPMPMDTSVYESPY SDPEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRM RKKQIDVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYI VRLIGVCQAEALMLVMEMAGGGPLHKFLVGKREEIPVSN VAELLHQVSMGMKYLEEKNFVHRDLAARNVLLVNRHYA KISDFGLSKALGADDSYYTARSAGKWPLKWYAPECINFR KFSSRSDVWSYGVMTWEALSYGQKPYKKMKGPVMAFI EQGKRMECPPECPELYALMSDCWIYKWEDRPDFTVEQ RMRACYYSLASKVEGPPGSTQKAEACA	368
CD28 intracellular domain	MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSK KYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQLQVY SKTGFNCDGKLGNESVTFYLQNLVYNQTDIYFCKIEVMYP PPYLDNEKSNGTIIHVKGKHLCPSPLPFGPSKPFWVLVVVG GVLACYSLLVTVAFIIFWVR	369
4-1BB intracellular domain	MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNN RNQICSPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSST SNAECDCTPGFHCLGAGCSMCEQDCKQGOELTKKGCKD CCFGTENDQKRIGICRPWTNCSLDGKSVLVNGTKERDVVC GPSPADLSPGASSVTPAPAREPGHSPQIISFFLALTSTALLF LLFFLTLRPSVVKRGRKLLYIFKQPFMRPVQTTQEEDG	370
Fc epsilon Receptor I gamma chain intracellular domain	MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTL LYCRLKIQVRKAAITSYEKSDGVYTGGLSTRNQETIYETLKH EKPPQ	371

Fc epsilon Receptor I gamma chain intracellular domain	DGVYTGLSTRNQETYETLKHE	372
Fc epsilon Receptor I gamma chain intracellular domain	DPKLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYEKSD GVYTGLSTRNQETYETLKHEKPPQ	373
Fc epsilon Receptor I gamma chain intracellular domain	DGVYTGLSTRNQETYETLKHE	374

Transmembrane domains

[00219] In some embodiments, the CAR of the present invention may comprise a transmembrane domain. As used herein, the term “Transmembrane domain (TM)” refers broadly to an amino acid sequence of about 15 residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 amino acid residues and spans the plasma membrane. In some embodiments, the transmembrane domain of the present invention may be derived either from a natural or from a synthetic source. The transmembrane domain of a CAR may be derived from any naturally membrane-bound or transmembrane protein. For example, the transmembrane region may be derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD3 epsilon, CD4, CD5, CD8, CD8 α , CD9, CD16, CD22, CD33, CD28, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, or CD154.

[00220] Alternatively, the transmembrane domain of the present invention may be synthetic. In some aspects, the synthetic sequence may comprise predominantly hydrophobic residues such as leucine and valine.

[00221] In some embodiments, the transmembrane domain of the present invention may be selected from the group consisting of a CD8 α transmembrane domain, a CD4 transmembrane domain, a CD 28 transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, and a human IgG₄ Fc region. As non-limiting examples, the transmembrane domain may be a CTLA-4 transmembrane domain comprising the amino acid sequences of SEQ ID NOs.: 1-5 of International Patent Publication NO.: WO2014/100385; and a PD-1 transmembrane domain comprising the amino acid sequences of SEQ ID NOs.: 6-8 of International Patent Publication NO.: WO2014/100385; the contents of each of which are incorporated herein by reference in their entirety.

[00222] In some embodiments, the CAR of the present invention may comprise an optional hinge region (also called spacer). A hinge sequence is a short sequence of amino acids that facilitates flexibility of the extracellular targeting domain that moves the target binding domain

away from the effector cell surface to enable proper cell/cell contact, target binding and effector cell activation (Patel et al., *Gene Therapy*, 1999; 6: 412-419). The hinge sequence may be positioned between the targeting moiety and the transmembrane domain. The hinge sequence can be any suitable sequence derived or obtained from any suitable molecule. The hinge sequence may be derived from all or part of an immunoglobulin (e.g., IgG1, IgG2, IgG3, IgG4) hinge region, i.e., the sequence that falls between the CH1 and CH2 domains of an immunoglobulin, e.g., an IgG4 Fc hinge, the extracellular regions of type 1 membrane proteins such as CD8 α , CD4, CD28 and CD7, which may be a wild type sequence or a derivative. Some hinge regions include an immunoglobulin CH3 domain or both a CH3 domain and a CH2 domain. In certain embodiments, the hinge region may be modified from an IgG1, IgG2, IgG3, or IgG4 that includes one or more amino acid residues, for example, 1, 2, 3, 4 or 5 residues, substituted with an amino acid residue different from that present in an unmodified hinge. Table 7 provides various transmembrane regions that can be used in the CARs described herein.

Table 7: Transmembrane domains

Transmembrane domain	Amino Acid Sequence	SEQ ID NO.
CD8 Transmembrane domain	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI	375
2B4 Transmembrane domain	FLVIIILSALFLGTLACFCV	424
4-1BB Transmembrane domain	IISFFLALTSTALLFLLFFLTLRFSVVKRGR	376
4-1BB Transmembrane domain	IISFFLALTSTALLFLLFFLTLRFSVV	377
CD134 (OX40) Transmembrane domain	VAAILGLGLVLGLLGPLAILLALYLL	378
CD148 Transmembrane and intracellular domain	AVFGCIFGALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHKKDFIATQGPLPNTLKDFWRMVWEKNVYAILMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEVVTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVDPDITDLLINFRYLVRDYMKQSPPEPILVHCSAGVGRGTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA	379
CD148 Transmembrane domain	AVFGCIFGALVIVTVGGFIFW	380
CD2 Transmembrane domain	KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSDDKKIAQFRKEKETFEKDTYKLFKNGTLKIKHLKTDDQDIYKVSIYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCPEKGLD	381
CD28 Transmembrane and intracellular domain	IEVMYPPPYLDNEKSNGTITHVKGKHLCPSPFLPGPSKPFWVLVVVGGLVACYSLLVTVAHIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	382
CD28 Transmembrane domain	FWVLVVVGGLVACYSLLVTVAHIFWV	383
CD28 Transmembrane domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFWVLVVVGGLVACYSLLVTVAHIFWV	384

CD28 Transmembrane domain	IFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRR	385
CD28 Transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQP YAPPRDFAAYRS	386
CD28 Transmembrane domain	MFWVLVVVGGVLACYSLLVTVAFIIFWV	387
CD28 Transmembrane domain	FWVLVVVGGVLACYSLLVTVAFHFWV	388
CD28 Transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQAYAAARDFAAYRS	425
CD28 Transmembrane domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWV LWVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMT PRPGPTRKHYQPYAPPRDFAAYRS	897
CD28 Transmembrane domain	MFWVLVVVGGVLACYSGGVTVAFIIFWV	389
CD28 Transmembrane domain	WVLVVVGGVLACYSLLVTVAFIIFWV	390
CD28 Transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWVR	898
CD28 Transmembrane domain	PFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDY MNMTPRRPGPTRKHYQPYAPPRDFAAYRS	391
CD28 Transmembrane domain and CD28 and CD3 Zeta intracellular domain	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPA YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	392
CD28 Transmembrane domain and CD28, OX40, and CD3 Zeta intracellular domain	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKP PGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR	393
CD28 Transmembrane domain and CD3 Zeta intracellular domain	FWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPA YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	394
CD28 transmembrane-CD3 zeta signaling domain ("28z")	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSK FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPA YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	395
CD3 zeta Transmembrane domain	LCYLLDGILFIYGVILTALFLRV	396
CD3 zeta Transmembrane domain	MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIY GVILTALFL	397
CD3 zeta Transmembrane domain	LCYLLDGILFIYGVILTALFL	398
CD4 Transmembrane domain	ALIVLGGVAGLLLFIGLGIFFCVRC	399
CD4 Transmembrane domain	MALIVLGGVAGLLLFIGLGIFF	400
CD45 Transmembrane and intracellular domain	ALIAFLAFLIIVTSIALLVVLYKIYDLHKRSCNLDEQQELV ERDDEKQLMNVPIHADILLETYKRKIADEGRLFLAEFQSIP RVFSKFPIKEARKPFNQKNRYVDILPYDYNRVELSEINGD AGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWE QKATVIVMVTRCEEGRNKNCAEYWPSMEEGTRAFGDVVV KINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDH GVPEDPHLLLKLRRRVNAFSNFFSGPIWHCSAGVGRGTGYI	401

	GIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYI LIHQALVEYNQFGETE VNLSELHPYLHNMKKRDPPSEPSPL EAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNR VPLKHELEMSKESEHDSDESSDDSDSEEPSKYINASFIMSY WKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKH GDQEICAQYWGEKGQTYGDIÉVDLKD TDKSSTYT LRVFEL RHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQWKQK LPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLES AETEEWDIFQWKALRKARPGMVSTFEQYQFLYDVIASSTYP AQNGQVKKNNHQEDKIEFDNEVDKVKQDANCVNPLGAPE KLPEAKEQAE GSEPTSGTEGPEHSVNGPASPALNQGS	
CD62L Transmembrane domain	PLFIPVAVMVTAFSGLAFIWL A	402
CD7 Transmembrane domain	ALPAALAVISFLLGLGLGVACVLA	403
CD8 Transmembrane domain	MALPVTALLPLALLLHAARP	404
CD8 Transmembrane domain and CD28 signaling domain	AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCN HRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA AYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR	405
CD8 transmembrane domain-CD137 (4-1BB) signaling domain and CD3 zeta signaling domain ("BBz")	AAATTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSAD APAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	406
CD8a Transmembrane domain	FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN	407
CD8a Transmembrane domain	IWAPLAGTCGVLLLSLVITLYC	408
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYC	409
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYCR	410
CD8a Transmembrane domain	PTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCN	411
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLVCR	412
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVIT	413
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLY	414
CD8a Transmembrane domain (NP 001139345.1)	TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAWTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCNHRNRRR	899
CD8b Transmembrane domain	LGLLVAGVLVLLVSLGVAIHLCC	900
DAP10 Transmembrane domain	ILLAGLVAADAVASLLIVGAVFLCARR	901
EpoR Transmembrane domain	APVGLVARLADESGHVVLRLWPPPETPMTSHIRYEVDVSA GNGAGSVQRVEILEGRTECVLSNLRGRTRYTF AVRARMAE PSFGGFWSAWSEPVSLTTPSD	415
FcERI a Transmembrane domain	FFIPLLVLVILFAVD TGLFISTQQQVTFLLKIKRTRKGFRLNLP HPKPNPKNN	416

FcERI a- Transmembrane domain	MAPAMESPTLLCVALLFFAPDGVLA VPQKPKVSLNPPWNR IFKGENVTLCNGNNFFEVSSTKWFHNGSLSEETNSSLNIV NAKFEDSGEYKCOHQQVNESEPVYLEVFSDWLLLQASAEV VMEGQPLFLRCHGWRNWDVYKVIYYKDGEALKYWYENH NISITNATVEDSGTYICTGK VWQLDYESEPLNITVIKAPRE KYWLQFFIPLL VVILFAVDTGLFISTQQQVTFLLKIKRTRKG FRLLNPHPKPNPKNN	417
FcERI b- Transmembrane region	MDTESNRRANLALPQEPSSVPAFEVLEISPQEVSSGRLLKSA SSPPLHTWLTVLKKEQEFLGVTQILTAMICLCFGTVVCSVL DISHIEGDIFSSFKAGYPFWGAIFFSISGMLSISERRNATYLV RGS LGANTASSIAGGTGITILINLKKSLAYIHHSQCQFFETK CFMASFSTEIVVMMLFLTILGLGSAVSLTICGAGEELKGNK VPEDRVYEELNIYSATYSELEDPGEMSPPIDL	418
FcERI g- Transmembrane region	MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLL YCRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEK PPQ	419
FceRIa Transmembrane domain	DIFIPLL VVILFAVDTGLFISTQQQVTFLLKIKRTRKGFRLN PHPKPNPKNNR	420
GITR Transmembrane domain	PLGWLTVVLLAVAACVLLLTSAQLGLHIWQL	421
Her2 Transmembrane domain	SIISAVVGILLVVVLGVVFGILII	422
Her2 Transmembrane domain	CHPECQPQNGSVTCFGPEADQCVACAHYKDPFPCVARCPS GVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGC PAEQRASPLTSIISAVVGILLVVVLGVVFGILI	423
ICOS Transmembrane domain	FWLPIGCAAFVVVCILGCILI	902
IgG1 Transmembrane domain	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSQSVMEALHNHYTQKSLSLSPGKKD	903
LAG-3 Transmembrane domain	LLFLILGVLSLLLLVTGAFFG	904
OX40 Transmembrane domain	VAAILGLGLVLGLLGPLAILL	905
PD-1 Transmembrane domain	VGWGGLLGLSVLLVWVLAVI	906
Transmembrane domain	FWALVVVAGVLFYCYGLLVTVALCVIWT	907

[00223] Hinge region sequences useful in the present invention are provided in Table 8A.

Table 8A: Hinge regions

Hinge Domain	Amino Acid Sequence	SEQ ID NO.
Hinge	DKTHT	426
Hinge	CPPC	427
Hinge	CPEPKSCDTPPPCPR	428
Hinge	ELKTPLGDTTHT	429
Hinge	KSCDKTHTCP	430
Hinge	KCCVDCP	431
Hinge	KYGPPCP	432
C233P Hinge	VEPKSPDKTHTCPPCP	433
C233S Hinge	LDPKSSDKTHTCPPCP	434

CD28 Hinge	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP	435
CD8a Hinge	GGAVHTRGLDFA	436
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACD	437
CD8a Hinge	AKPTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACD	438
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACD	439
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACD	440
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDT	441
CD8a Hinge	PAKPTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIY	442
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYC	443
CD8a Hinge	TTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACD	444
CD8a Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIY	445
Delta5 Hinge	LDKTHTCPPCP	446
EpoR Hinge	APVGLVARLADESGHVLRWLPPETPMTSHIRYEVDVS AGNGAGSVQRVEILEGRTECVLSNLRGRTRYTFVRARM AEPSFGGFWSAWSEPVSLLTPSD	447
FCRII α Hinge	GLAVSTISSFFPPGYQ	448
Fc γ RIII α Hinge	GLAVSTISSFFPPGYQ	449
Hinge	RWPESPKAQASSVPTAQQAEGSLAKATTAPATTRNTGR GGEEKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	450
Hinge	YVTVSSQDPAEPKSPDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSL SPGKKDPK	451
Hinge	KPTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFA	452
Hinge	LEPKSCDKTHTCPPCP	453
Hinge	KPTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LD	454
Hinge	EPKSCDKTHTCPPCP	455
Hinge	ELKTPLGDTHTCPRCP	456
Hinge	EPKSCDTPPPCPRCP	457
Hinge	ESKYGPPCPSCP	458
Hinge	ERKCCVECPCP	459
Hinge (CH2- CH3)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA	460

	VEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK	
Hinge (CH3)	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	461
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	462
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLHPSL PPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEV SGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWA WSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSY VTDH	463
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMVVLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	464
IgD Hinge	ESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGRGGEE KKKEKEKEEQEERETKTP	465
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	466
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	467
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVS YVTDH	468
IgD Hinge	RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGR GGEEKKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAV QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGV EEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHP LPPQRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCE	469

	VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW AWSVLRVPAPSPQPATYTCVVSHEDSRTLNASRSLEVS YVTDH	
IgG1 (CH2CH3) Hinge domain	AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVUTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKD	470
IgG1 (CH2CH3) Hinge domain	AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKD	471
IgG1 Hinge	AEPKSPDKTHTCPPCPKDPK	472
IgG1 Hinge	EPKSCDKTHTCPPCP	473
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKD	474
IgG1 Hinge	SVFLFPPKPKDTL	475
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	476
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPK	477
IgG1 Hinge	VECPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK	478
IgG1 Hinge (CH2CH3 domain)	DPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMI ARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKK	479
IgG2 Hinge	ERKCCVECPPCP	480
IgG3 Hinge	ELKTPLGDTHTCPRCP	481
IgG3 Hinge	ELKTPLGDTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPC PRCPEPKSCDTPPPCPRCP	482
IgG4 (CH2 and CH3)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSPGKM	483

IgG4 (CH2 and CH3)	ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGKM	484
IgG4 Hinge	SPNMVPHAHHAQ	485
IgG4 Hinge	GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMEALHNHYTQKSLSLGLGK	486
IgG4 Hinge	ESKYGPPCPPCPGGGSSGGSGGQPREPQVYTLPPSQEEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYT QKSLSLGLGK	487
IgG4 Hinge	ESKYGPPCPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHQAATKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFVPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGK	488
IgG4 Hinge	ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHQAATKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFVPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGK	489
IgG4 Hinge	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGKM	490
IgG4 Hinge	GAATCTAAGTACGGACCGCCCTGCCCCCTTGCCCT	491
IgG4 Hinge	ESKYGPPCPPCP	492
IgG4 Hinge	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK	493
IgG4 Hinge	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGK	494
IgG4 Hinge	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLGLGK	495
IgG4 Hinge	ESKYGPPCPPCP	496
IgG4 Hinge	YGPPCPPCP	497
IgG4 Hinge	KYGPPCPPCP	498
IgG4 Hinge	EVVKYGPPCPPCP	499
IgG4 Hinge	ESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA	500

	VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDLRW QEGNVFSCSVMHEALHNHYTQKSLSLSPGK	
IgG4 Hinge and Linker	ESKYGPPCPPCPGGGSSGGGSG	501
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	502
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	503
IgG1 Hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	504

[00224] Hinge and transmembrane region sequences useful in the present invention are provided in Table 8B.

Table 8B: Hinge and Transmembrane regions

Hinge Domain	Amino Acid Sequence	SEQ ID NO.
CD8a Transmembrane and Hinge	TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYC	505
CD8a Transmembrane and Hinge	DIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQK PGNAPRLISGATSLETGVPSRFSGSGSGKDYTLSTSLQTE DVATYYCQQYWSTPFTFGSGTKLEIKGGGGSGGGGSGGG GSQVQLKESGPGLVAPSQSLSITSTVSGFSLRYSVHWVR QPPGKGLEWLGMIWGGGSTDYNSALKSRLSISKDNSKSQ VFLKMNSLQTTDDTAMYYCARNEGDTTAGTWFAFWGQG TLVTVSS	506
CD8a Transmembrane and Hinge	ALNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLD	507
CD8a Transmembrane and Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLY	508
CD8a Transmembrane and Hinge	KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLY	509

[00225] In some embodiments, the CAR of the present invention may comprise one or more linkers between any of the domains of the CAR. The linker may be between 1-30 amino acids long. In this regard, the linker may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acids in length. In other embodiments, the linker may be flexible.

[00226] In some embodiments, the components including the targeting moiety, transmembrane domain and intracellular signaling domains of the present invention may be constructed in a

single fusion polypeptide. The fusion polypeptide may be the payload of an effector module of the invention. In some embodiments, more than one CAR fusion polypeptides may be included in an effector module, for example, two, three or more CARs may be included in the effector module under the control of a single SRE (e.g., a DD). Representative effector modules comprising the CAR payload are illustrated in Figures 2-6.

[00227] In some embodiments, the CAR sequences may be selected from Table 9.

Table 9: CAR sequences

Description	SEQ ID NO	Source
CD19 CAR	510	SEQ ID NO: 12 in US9499629B2
CD19 CAR	511	SEQ ID NO: 24 in US20160333108A1
CD19 CAR	512	SEQ ID NO: 25 in US20160333108A1
CD19 CAR	513	SEQ ID NO: 26 in US20160333108A1
CD19 CAR	514	SEQ ID NO: 27 in US20160333108A1
CD19 CAR	515	SEQ ID NO: 1 in EP2997134A4
CD19 CAR	516	SEQ ID NO: 19 in EP3071687A1
CD19 CAR	517	SEQ ID NO: 20 in EP3071687A1
CD19 CAR	518	SEQ ID NO: 181 in WO2016168773A3
CD19 CAR	519	SEQ ID NO: 2 in WO2015157399A9
CD19 CAR	520	SEQ ID NO: 56 in WO2016174409A1
CD19 CAR	521	SEQ ID NO: 62 in WO2016174409A1
CD19 CAR	522	SEQ ID NO: 145 in WO2016179319A1
CD19 CAR	523	SEQ ID NO: 293 in US20160311907A1
CD19 CAR	524	SEQ ID NO: 294 in US20160311907A1
CD19 CAR	525	SEQ ID NO: 295 in US20160311907A1
CD19 CAR	526	SEQ ID NO: 296 in US20160311907A1
CD19 CAR	527	SEQ ID NO: 297 in US20160311907A1
CD19 CAR	528	SEQ ID NO: 298 in US20160311907A1
CD19 CAR	529	SEQ ID NO: 73 in WO2013176915A1
CD19 CAR	530	SEQ ID NO: 73 in WO2013176916A1
CD19 CAR	531	SEQ ID NO: 73 in US20130315884A1
CD19 CAR	532	SEQ ID NO: 73 in US20140134142A1
CD19 CAR	533	SEQ ID NO: 73 in US20150017136A1
CD19 CAR	534	SEQ ID NO: 73 in US20150203817A1
CD19 CAR	535	SEQ ID NO: 73 in US20160120905A1
CD19 CAR	536	SEQ ID NO: 73 in US20160120906A1
CD19 CAR	537	SEQ ID NO: 8 in WO2015124715
CD19 CAR	538	SEQ ID NO: 5 in WO2015124715
CD19 CAR	539	SEQ ID NO: 73 in WO2014184744
CD19 CAR	540	SEQ ID NO: 73 in WO2014184741
CD19 CAR	541	SEQ ID NO: 14 in US20160145337A1
CD19 CAR	542	SEQ ID NO: 15 in US20160145337A1
CD19 CAR	543	SEQ ID NO: 14 in WO2014184143
CD19 CAR	544	SEQ ID NO: 15 in WO2014184143
CD19 CAR	545	SEQ ID NO: 15 in WO2015075175
CD19 CAR	546	SEQ ID NO: 16 in WO2015075175
CD19 CAR	547	SEQ ID NO: 16 in US20160145337A1
CD19 CAR	548	SEQ ID NO: 16 in WO2014184143
CD19 CAR	549	SEQ ID NO 12 in WO2012079000
CD19 CAR	550	SEQ ID NO.31 in WO2016164580
CD19 CAR	551	SEQ ID NO.32 in WO2016164580
CD19 CAR	552	SEQ ID NO.33 in WO2016164580
CD19 CAR	553	SEQ ID NO.34 in WO2016164580

CD19 CAR	554	SEQ ID NO.35 in WO2016164580
CD19 CAR	555	SEQ ID NO.36 in WO2016164580
CD19 CAR	556	SEQ ID NO.37 in WO2016164580
CD19 CAR	557	SEQ ID NO.38 in WO2016164580
CD19 CAR	558	SEQ ID NO.39 in WO2016164580
CD19 CAR	559	SEQ ID NO.40 in WO2016164580
CD19 CAR	560	SEQ ID NO.41 in WO2016164580
CD19 CAR	561	SEQ ID NO.42 in WO2016164580
CD19 CAR	562	SEQ ID NO.58 in WO2016164580
CD19 CAR	563	SEQ ID NO: 14 in US20160296563A1
CD19 CAR	564	SEQ ID NO: 15 in US20160296563A1
CD19 CAR	565	SEQ ID NO.31 in WO2015157252
CD19 CAR	566	SEQ ID NO.32 in WO2015157252
CD19 CAR	567	SEQ ID NO.33 in WO2015157252
CD19 CAR	568	SEQ ID NO.34 in WO2015157252
CD19 CAR	569	SEQ ID NO.35 in WO2015157252
CD19 CAR	570	SEQ ID NO.36 in WO2015157252
CD19 CAR	571	SEQ ID NO.37 in WO2015157252
CD19 CAR	572	SEQ ID NO.38 in WO2015157252
CD19 CAR	573	SEQ ID NO.39 in WO2015157252
CD19 CAR	574	SEQ ID NO.40 in WO2015157252
CD19 CAR	575	SEQ ID NO.41 in WO2015157252
CD19 CAR	576	SEQ ID NO.42 in WO2015157252
CD19 CAR	577	SEQ ID NO. 14 in WO2016139487
CD19 CAR	578	SEQ ID NO.15 in WO2016139487
CD19 CAR	579	SEQ ID NO: 53 in US20160250258A1
CD19 CAR	580	SEQ ID NO: 54 in US20160250258A1
CD19 CAR	581	SEQ ID NO: 55 in US20160250258A1
CD19 CAR	582	SEQ ID NO: 56 in US20160250258A1
CD19 CAR	583	SEQ ID NO: 57 in US20160250258A1
CD19 CAR	584	SEQ ID NO: 58 in US20160250258A1
CD19 CAR	585	SEQ ID NO. 1 in WO2015187528
CD19 CAR	586	SEQ ID NO. 2 in WO2015187528
CD19 CAR	587	SEQ ID NO. 3 in WO2015187528
CD19 CAR	588	SEQ ID NO. 4 in WO2015187528
CD19 CAR	589	SEQ ID NO. 5 in WO2015187528
CD19 CAR	590	SEQ ID NO. 6 in WO2015187528
CD19 CAR	591	SEQ ID NO. 7 in WO2015187528
CD19 CAR	592	SEQ ID NO. 8 in WO2015187528
CD19 CAR	593	SEQ ID NO. 9 in WO2015187528
CD19 CAR	594	SEQ ID NO. 10 in WO2015187528
CD19 CAR	595	SEQ ID NO. 11 in WO2015187528
CD19 CAR	596	SEQ ID NO. 12 in WO2015187528
CD19 CAR	597	SEQ ID NO. 13 in WO2015187528
CD19 CAR	598	SEQ ID. NO. 31 in WO2015157252
CD19 CAR	599	SEQ ID. NO. 32 in WO2015157252
CD19 CAR	600	SEQ ID. NO. 33 in WO2015157252
CD19 CAR	601	SEQ ID. NO. 34 in WO2015157252
CD19 CAR	602	SEQ ID. NO. 35 in WO2015157252
CD19 CAR	603	SEQ ID. NO. 36 in WO2015157252
CD19 CAR	604	SEQ ID. NO. 37 in WO2015157252
CD19 CAR	605	SEQ ID. NO. 38 in WO2015157252
CD19 CAR	606	SEQ ID. NO. 39 in WO2015157252
CD19 CAR	607	SEQ ID. NO. 40 in WO2015157252
CD19 CAR	608	SEQ ID. NO. 41 in WO2015157252
CD19 CAR	609	SEQ ID. NO. 42 in WO2015157252
CD19 CAR	610	SEQ ID. NO. 58 in WO2015157252
CD19 CAR	611	SEQ ID NO. 31 in WO2014153270

CD19 CAR	612	SEQ ID NO. 32 in WO2014153270
CD19 CAR	613	SEQ ID NO. 33 in WO2014153270
CD19 CAR	614	SEQ ID NO. 34 in WO2014153270
CD19 CAR	615	SEQ ID NO. 35 in WO2014153270
CD19 CAR	616	SEQ ID NO. 36 in WO2014153270
CD19 CAR	617	SEQ ID NO. 37 in WO2014153270
CD19 CAR	618	SEQ ID NO. 38 in WO2014153270
CD19 CAR	619	SEQ ID NO. 39 in WO2014153270
CD19 CAR	620	SEQ ID NO. 40 in WO2014153270
CD19 CAR	621	SEQ ID NO. 41 in WO2014153270
CD19 CAR	622	SEQ ID NO. 42 in WO2014153270
CD19 CAR (Third generation)	623	SEQ ID NO.13 in WO2016139487

[00228] In one embodiment of the present invention, the payload of the invention is a CD19 specific CAR targeting different B cell. In the context of the invention, an effector module may comprise a hDHFR DD, ecDHFR DD, or FKBP DD operably linked to a CD19 CAR fusion construct. In some instances, the promoter utilized to drive the expression of the effector module in the vector may be a CMV promoter or an EF1a. The efficiency of the promoter in driving the expression of the same construct may be compared. For example, two constructs that differ only by their promoter, CMV (in OT-CD19N-001) or EF1a promoter (in OT-CD19N-017) may be compared. The amino acid sequences of CD19 CAR constructs and its components are presented in Table 10a and Table 10b. The amino acid sequences in Table 10a and/or Table 10b may comprise a stop codon which is denoted in the table with a “*” at the end of the amino acid sequence.

Table 10a: Sequences of components of CD19 CARs

Description	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
CD19 scFv	DIQMTQTTSLSASLGDRVTISCRASQDISKYLNW YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNLTPYTFGGGKLEI TGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSL SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW GSETTYYNLSALKSRLTIKDNSKSQVFLKMNSLQT DDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVS S	624	626, 650-654
CD8 α hinge--TM	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC	625	627, 655, 982-984
CD8 α hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACD	440	656-660
CD3 zeta signaling domain	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR	339	661-665, 986
4-1BB (41BB) intracellular signaling domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL	273	666-670, 985
CD8a Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYC	409	990, 992

CD8 α leader	MALPVTALLLPLALLLHAARP	628	671-675
p40 signal sequence	MCHQQLVISWFSLVFLASPLVA	719	736-744
p40	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEE DGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEP KNKTFLRCEAKNYSGRFTCWWLTITISTDLTFSVK SSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSV ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTS SFFIRDIKPDPPKNLQKPLKNSRQVEVSWEYPDT WSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTS ATVICRKNASISVRAQDRYYSSSWSEWASVPCS	723	632-634, 752-761
p35	RNLPVATPDPMFPCLLHHSQNLLRAVSNMLQKA RQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELT KNESCLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS	724	762-771, 1012
IL15	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC KVTAMKCFLELQVISLESGDASIHTVENLIILAN NSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIV QMFINTS	785	794-797, 1001
IL15Ra	ITCPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPA VHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASSPS SNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESS HGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVSLLACYLKSRTPLASVE MEAMEALPVTWGTSSRDEDLNCSHHL	803	812-813, 1003
mCherry (MIL)	LSKGEEDNMAIIEFMRFKVHMEGSVNGHEFEIE GEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQ FMYGSKAYVKHPADIPDYLKLSFPEGFKWERVM NFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPS DGPVMQKKTMGWEASSERMYPEDGALKGEIKQR LKLKDDGGHYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK	1029	1030
IRES	-	-	999
Linker (GGSGG)	GGSGG	629	676-680
Linker (SG)	SG	-	AGTGGA
Linker ((G4S)3)	GGGGSGGGGSGGGGS	720	910-915
Linker (GGSG)	GGSG	822	823
Linker	MLLLVTSLLLCELPHPAFLLIIP	1031	1032
Linker (SG3-(SG4)3-SG3-SLQ)	SGGGSGGGGSGGGGSGGGGSGGGGSLQ	802	811, 916- 920, 1002
Modified Furin	ESRRVRRNKRSK	630	681-683
BamHI	-	-	GGATCC
Spacer	-	-	1000
HA Tag	YPYDVPDYA	1024	1025-1027
FKBP (F36V, L106P)	GVQVETISPGDGRTFPKRGQTCVVHYTGMLDGG KVDSSDRNKPFFKMLGKQEVIRGWEEGVAQMS VGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVE LLKPE	11	684-686, 987, 989
FKBP (E31G, F36V, R71G, K105E)	GVQVETISPGDGRTFPKRGQTCVVHYTGMLDGG KKVDSSDRNKPFFKMLGKQEVIRGWEEGVAQM SVGQGAKLITSPDYAYGATGHPGIIPPHATLVFDV ELLELE	12	688-691, 994, 1013, 1028
ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I)	ISLIAALVDYVIGMENAMPWNLPADLAWFKRN TLNKPVIMGRHTWESIGRPLPGRKNILSSQPGTDD RVTWVKSVDIAAACGDVPEIMVIGGGRVIEQFLP	9	692, 772, 814, 687, 988, 991

	KAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSE FHDADAQNSHSYCFEILERR		
ecDHFR (Amino acid 2-159 of WT) (R12H, E129K)	ISLIAALAVDHVIGMENAMPWNLPA DLAWFKRN TLNKPVIMGRHTWESIGRPLPGRKNILSSQPGTDD RVTWVKSVD EAIACGDVPEIMVIGGGRVYEQFL PKAQKLYLTHIDAEVEGDTHFPDYKPDWESVFS EFHDADAQNSHSYCFEILERR	10	798, 815, 993
hDHFR (Amino acid 2-187 of WT; Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF QRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEAMNHPGHLKLFVT RIMQDFESD TFFPEIDLEKYKLLPEYPGVLSDVQE EKG I KYKFEVYEKND	895	694, 995
hDHFR (Amino acid 2-187 of WT; Y122I, A125F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF QRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEFMNHPGHLKLFVT RIMQDFESD TFFPEIDLEKYKLLPEYPGVLSDVQE EKG I KYKFEVYEKND	890	696, 973, 974, 996
hDHFR (Amino acid 2-187 of WT; Q36K, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF KRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEAMNHPGHLKLFVT RIMQDFESD TFFPEIDLEKYKLLPEYPGVLSDVQE EKG I KYKFEVYEKND	981	698, 997
hDHFR (Q36F, N65F, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF FRM TTTSSVEGKQNLVIMGKKTWFSIPEKFRPLKG RINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPE LANKVDMVWIVGGSSVIKEAMNHPGHLKLFVTRI MQDFESD TFFPEIDLEKYKLLPEYPGVLSDVQEEK GIKYKFEVYEKND	891	700, 975, 976, 998
Description	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
CD19 scFv	DIQMTQT TSSLSASLGDRVTISCRASQDISKYLNW YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEI TGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSL SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVW GSETTYYN SALKSRLTIKD NSKSQVFLKMNSLQT DDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVS S	624	626, 650- 654
CD8α hinge--TM	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC	625	627, 655, 982-984
CD8α hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACD	440	656-660
CD3 zeta signaling domain	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR	339	661-665, 986
4-1BB (41BB) intracellular signaling domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL	273	666-670, 985
CD8α Transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYC	409	990, 992
CD8α leader	MALPVTALLLPLALLLHAARP	628	671-675
p40 signal sequence	MCHQQLVISWFSLVFLASPLVA	719	736-744
p40	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEE DGITWTL DQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEP	723	632-634, 752-761

	KNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVK SSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSV ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTS SFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPTD WSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTS ATVICRKNASISVRAQDRYYSSSWSEWASVPCS		
p35	RNLPVATPDPMFPCLLHHSQNLLRAVSNMLQKA RQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELT KNESCLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS	724	762-771, 1012
IL15	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC KVTAMKCFLELQVISLESGDASIHDTVENLIILAN NSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIV QMFINTS	785	794-797, 1001
IL15Ra	ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAL VHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASSPS SNNTAATTAIVPGSQLMPSKSPSTGTTEISSHESS HGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVSLACYLKSRQTPPLASVE MEAMEALPVTWGTSSRDEDELENCSHHL	803	812-813, 1003
mCherry (MIL)	LSKGEEDNMAIIEKFMRFKVHMEGSVNGHEFEIE GEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQ FMYGSKAYVKHPADIPDYLLKLSFPEGFKWERVM NFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFP DGPVMQKKTMGWEASSERMYPEDGALKGEIKQR LKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNI KLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK	1029	1030
IRES	-	-	999
Linker (GGSGG)	GGSGG	629	676-680
Linker (SG)	SG	-	AGTGGA
Linker ((G4S)3)	GGGGSGGGGSGGGGS	720	910-915
Linker (GGSG)	GGSG	822	823
Linker	MLLLVTSLLLCELPHPAFLIP	1031	1032
Linker (SG3-(SG4)3-SG3-SLQ)	SGGGSGGGGSGGGGSGGGGSGGGGSLQ	802	811, 916- 920, 1002
Modified Furin	ESRRVRRNKRSK	630	681-683
BamHI	-	-	GGATCC
Spacer	-	-	1000
HA Tag	YPYDVPDYA	1024	1025-1027
FKBP (F36V, L106P)	GVQVETISPGDGRTFPKRGGQTCVVHYTGMLDGG KVDSSRDNRNPKFKFMLGKQEVIRGWEEGVAQMS VGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVE LLKPE	11	684-686, 987, 989
FKBP (E31G, F36V, R71G, K105E)	GVQVETISPGDGRTFPKRGGQTCVVHYTGMLDGG KKVDSSRDNRNPKFKFMLGKQEVIRGWEEGVAQM SVGQGAKLITSPDYAYGATGHPGIIPPHATLVFDV ELLELE	12	688-691, 994, 1013, 1028
ecDHFR (Amino acid 2- 159 of WT) (R12Y, Y100I)	ISLIAALAVDYVIGMENAMPWNLPADLAWFKRN TLNKPVIMGRHTWESIGRPLPGRKNILSSQPGTDD RVTWVKSVDEAIAACGDVPEIMVIGGGRVIEQFLP KAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSE FHDADAQNSHSHSYCFEILERR	9	692, 772, 814, 687, 988, 991
ecDHFR (Amino acid 2- 159 of WT) (R12H, E129K)	ISLIAALAVDHVIGMENAMPWNLPADLAWFKRN TLNKPVIMGRHTWESIGRPLPGRKNILSSQPGTDD RVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQFL	10	798, 815, 993

	PKAQKLYLTHIDAEVEGDTHFPDYKPDWESVFS EFHDADAQNSHSYCFEILERR		
hDHFR (Amino acid 2-187 of WT; Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF QRMTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEAMNHPGHLKLFVT RIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQE EKGIKYKFEVYEKND	895	694, 995
hDHFR (Amino acid 2-187 of WT; Y122I, A125F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF QRMTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEFMNHPGHLKLFVT RIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQE EKGIKYKFEVYEKND	890	696, 973, 974, 996
hDHFR (Amino acid 2-187 of WT; Q36K, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF KRMTTSSVEGKQNLVIMGKKTWFSIPEKNRPLK GRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQP ELANKVDMVWIVGGSSVIKEAMNHPGHLKLFVT RIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQE EKGIKYKFEVYEKND	981	698, 997
hDHFR (Q36F, N65F, Y122I)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYF FRMTTSSVEGKQNLVIMGKKTWFSIPEKFRPLKG RINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPE LANKVDMVWIVGGSSVIKEAMNHPGHLKLFVTRI MQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQEEK GIKYKFEVYEKND	891	700, 975, 976, 998

Table 10b: Sequences of CD19 CARs

Description	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
OT-CD19 CAR-001 (OT-CD19c-001) (CD8a leader -CD19 scFV - CD8a-Tm -41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPDQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTVSSTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGGHDGLYQGLSTATKDTYDALHM QALPPR*	635	701
OT-CD19 CAR-002 (OT-CD19c-002) (CD8a leader - CD19 scFV - FKBP (F36V, L106P) - CD8a-Tm - 41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPDQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTVSSGVQVETISPGDG RTFPKRGQTCVVHYTGMLEDGKKVDSSRDKNP FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPD YAYGATGHPGIIPPHATLVFDVELLKPETTTAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA	636	702

	CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLY IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYKQGGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR*		
OT-CD19 CAR-003 (OT- CD19c-003) (CD8a leader - CD19 scFV - ecDHFR - CD8a-Tm - 41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTVSSISLIAALAVDYVI GMENAMPWNLPADLAWFKRNTLNKPVIMGRHT WESIGRPLPGRKNIILSSQPGTDDRVTWVKSVDIA IAACGDVPEIMVIGGGRVIEQFLPKAQKLYLTHID AEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHS YCFEILERRTTTPAPRPPTPAPTASQPLSLRPEACR PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR*	637	703
OT-CD19 CAR-004 (OT- CD19c-004) (CD8a leader - CD19 scFV - CD8a Hinge - FKBP (F36V, L106P) -CD8a Transmembrane domain- 41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTVSSSTTPAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDGV QVETISPGDGRFTFPRKGQTCVVHYTGMLDGGKV DSSRDRNKPFFKMLGKQEVIRGWEEGVAQMSVG QRAKLTISPDYAYGATGHPGIIPPHATLVFDVELL KPEIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYKQGGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR*	638	704
OT-CD19 CAR-005 (OT- CD19c-005) (CD8a leader - CD19 scFV - CD8a Hinge - ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I) - CD8a Transmembrane domain -41BB -CD3zeta - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTVSSSTTPAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDISLI AALAVDYVIGMENAMPWNLPADLAWFKRNTLN KPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVT WVKSVDIAIAACGDVPEIMVIGGGRVIEQFLPK AQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSEF HDADAQNSHSYCFEILERRIYIWAPLAGTCGVLLL	639	705

	SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEEGGCEL RVKFSRSADAPAYKQGQNNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR*		
OT-CD19c-006 (CD8a leader-CD19 scFV - CD8a-Tm - 41BB - CD3zeta -linker (GGSGG) - ecDHFR (Amino acid 2-159 of WT) (R12H, E129K) - stop)	MALPVTALLLPLALLLHAARPDQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGSLIAALAVDHVIGMENAMPWNL PADLA WFKRNTLNKPVIMGRHTWESIGRPLPGRK NIILSSQPGTDDRVTWVKSVD EAIACGDVPEIMV IGGGRVYEQFLPKAQKLYLTHIDAEVEGDTHFPD YKPDDWESVFSEFHDADAQNSHSYCFEILERR*	640	706
OT-CD19c-007 (CD8a leader - CD19 scFV- CD8a-Tm - 41BB - CD3zeta - linker (GGSGG) -FKBP (E31G, F36V, R71G, K105E) - stop)	MALPVTALLLPLALLLHAARPDQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGGVQVETISPGDGRTPKRGQTCV VHYTGMLGDGKKVDSSRDRNKPFFKMLGKQEV RGWEEGVAQMSVGQGA KLTISPDYAYGATGHPG IIPPHATLVFDVELLELE*	641	707
OT-CD19c-008 (CD8a leader - CD19 scFV - CD8a-Tm -41BB - CD3zeta - linker (GGSGG) - hDHFR (Amino acid 2-187 of WT; Y122I) - stop)	MALPVTALLLPLALLLHAARPDQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGVGSLNCIVAVSQNMGIGKNGDLP	642	708

	WPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRS LDDALKLTEQPELANKVDMVWIVGGSSVIKEAM NHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLP EYPGVLSDVQEEKGIKYKFEVYEKND*		
OT-CD19c-009 (CD8a leader - CD19 scFV - CD8a-Tm - 41BB - CD3zeta -linker (GGSGG) - hDHFR (Amino acid 2-187 of WT; Y122I, A125F) - stop)	MALPVTALLLPLALLLHAARPD IQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGG TKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVVSSTTTAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGVGSLNCIVAVSQNMGIGKNGDLP WPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRS LDDALKLTEQPELANKVDMVWIVGGSSVIKEFM NHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLP EYPGVLSDVQEEKGIKYKFEVYEKND*	643	709
OT-CD19c-010 (CD8a leader - CD19 scFV - CD8a-Tm - 41BB - CD3zeta - linker (GGSGG) -hDHFR (Amino acid 2-187 of WT; Q36K, Y122I) - stop)	MALPVTALLLPLALLLHAARPD IQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGG TKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVVSSTTTAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGVGSLNCIVAVSQNMGIGKNGDLP WPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRS LDDALKLTEQPELANKVDMVWIVGGSSVIKEAM NHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLP EYPGVLSDVQEEKGIKYKFEVYEKND*	644	710
OT-CD19c-011 (CD8a leader -CD19 scFV - CD8a-Tm - 41BB - CD3zeta -linker (GGSGG) - hDHFR (Amino acid 2-187 of WT; Q36K,N65F, Y122I) -stop)	MALPVTALLLPLALLLHAARPD IQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGG TKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVVSSTTTAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG	645	711

	RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGVGSLNCIVAVSQNMGIGKNGDLP WPPLRNEFRYFFRMTTSSVEGKQNLVIMGKKTW FSIPEKFRPLKGRINLVLSRELKEPPQGAHFLSRSL DDALKLTEQPELANKVDMVWIVGGSSVIKEAMN HPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPE YPGVLSDVQEEKGIKYKFEVYEKND*		
OT-CD19n-012 (CD8a leader - Linker (SG)- FKBP (F36V, L106P) - Furin Site -CD19 scFV - CD8a-Tm - 41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPSGGVQVETISPGDG RTFPKRGGQTCVVHYTGMLEDGKKVDSSRDKNP FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPD YAYGATGHPGIIPPHATLVFDVELLKPEESRRVRR NKRSKDIQMTQTSSLSASLGDRVTISCRASQDIS KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSG SGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGL VAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLE WLGVIWGSETTYYN SALKSRLTHKDNSKSQVFLK MNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQG TSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLV ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYN ELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR*	646	712
OT-CD19n-013 (CD8a leader - Linker (SG)- ecDHFR (Amino acid 2- 159 of WT) (R12Y, Y100I) - Furin Site - CD19 scFV -CD8a-Tm - 41BB - CD3zeta - stop)	MALPVTALLLPLALLLHAARPSGISLIAALAVDYVI GMENAMPWNL PADLAWFKRNTLNKPVIMGRHT WESIGRPLPGRKNILSSQPGTDDRVTWVKS VDEA IAACGDVPEIMVIGGGRVIEQFLPKAQKLYLTHID AEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHS YCFEILERRESRRVRRNKRSKDIQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGG TKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKH YYGGSYAMDYWGQGT SVTVSSTTTPAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVL DKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR*	647	713
OT-CD19n-014 (CD8a leader - Linker (SG)- hDHFR (Amino acid 2- 187 of WT; Y122I, A125F) - Furin Site - CD19 scFV - CD8a-Tm - 41BB -CD3zeta - stop)	MALPVTALLLPLALLLHAARPSGVGSLNCIVAVSQ NMGIGKNGDLPWPPLRNEFRYFQRM TTTSSVEGK QNLVIMGKKTWFSIPEKNRPLKGRINLVLSRELKE PPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEFMNHPGHLKLFVTRIMQDFESDTFFPEI DLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKN DESRRVRRNKRSKDIQMTQTSSLSASLGDRVTIS CRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHS GVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQG NTLPYTFGGG TKLEITGGGGSGGGGSGGGGSEVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR	648	714

	QPPRKGLEWLGVIWGSETTYYN SALKSRLTIKDN SKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA MDYWGQGTSTVVSSTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLDFACDIYWAPLAGT CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR *		
OT-CD19n-015 (CD8a leader - Linker (SG)- hDHFR (Amino acid 2- 187 of WT; Q36K, Y122I) -Furin Site - CD19 scFV - CD8a-Tm - 4IBB - CD3zeta -stop)	MALPVTALLLPLALLLHAARPSGVGSLNCIVAVSQ NMGIGKNGDLPWPPLRNEFRYFKRMTTSSVEGK QNLVIMGKKTWFSIPEKNRPLKGRINLVLSRELKE PPQGAHFLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPE IDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKN DESRRVRRNKRSKDIQMTQTSSLSASLGDRVTIS CRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHS GVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQG NTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR QPPRKGLEWLGVIWGSETTYYN SALKSRLTIKDN SKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA MDYWGQGTSTVVSSTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLDFACDIYWAPLAGT CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR *	649	715
OT-CD19-056 (CD8a leader - CD19 scFV - CD8a-Tm - 4IBB - CD3zeta - linker (GGSGG) - hDHFR (Amino acid 2-187 of WT; Y122I) -stop)	MALPVTALLLPLALLLHAARPD IQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKH YYGGSYAMDYWGQGTSTVVSSTTTPAPRPPTPAP TASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQ FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGGVGSLNCIVAVSQNMGIGKNGDLP WPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRS LDDALKLTEQPELANKVDMVWIVGGSSVIKEAM NHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLP EYPGVLSDVQEEKGIKYKFEVYEKND*	1005	1022
OT-CD19-057 (CD8a leader -CD19 scFV - CD8a-Tm - 4IBB - CD3zeta - BamHI (GS)- stop)	MALPVTALLLPLALLLHAARPD IQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS	1006	1023

	RLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVL DKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGs*		
OT-CD19-058 (CD8a leader -CD19 scFV - CD8a-Tm - 41BB - CD3zeta - p2A - BamHI (GS)- stop)	MALPVTALLLPLALLLHAARPDIQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVL DKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGATNFSLLKQAGDVEENPGPGS*	1007	1033
OT-CD19-059 (CD8a leader - HA Tag - CD19 scFV - CD8a-Tm - 41BB - CD3zeta - BamHI (GS)- stop)	MALPVTALLLPLALLLHAARPYPYDVPDYADIQM TQTSSLSASLGDRVTISCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTI SNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGG GSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTC TVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSET TYYN SALKSRLTIHKDNSKSQVFLKMNSLQTDDTA IYYCAKHYYYGGSYAMDYWGQGTSTVTSSTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPRGs*	1008	1034
OT-CD19-060 (CD8a leader - CD19 scFV- CD8a-Tm- 41BB - CD3zeta - Linker (SG)- Furin - BamHI (GS)- stop)	MALPVTALLLPLALLLHAARPDIQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVL DKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRSGESRRVRRNKRSGS*	1009	1035
OT-CD19-063 (CD8a leader - CD19 scFV -	MALPVTALLLPLALLLHAARPDIQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI	1010	1036

CD8a-Tm - 41BB - CD3zeta - stop)	YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR*		
OT-CD19-064 (CD8a leader - CD19 scFV - CD8a-Tm - 41BB - CD3zeta - Linker (TR)- HA Tag - FKBP (E31G, F36V, R71G, K105E) - stop-IRES- mCherry)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRTRYPDYAGVQVETISPGDGRTPFK RGQTCVVHYTGMLGDGKKVDSSRDNRNPKPFK FML GKQEVIRGWEEGVAQMSVGQGAKLTISPDYAYG ATGHPGIIPPHATLVFDVELLELE*MHRSAAAAT*I PPPPPLSLPPP*RYWPKPLGIRPVCVCLYVIFHHIAV FWQCEGPETWPCLLDEHS*GSFPSRQRNARSVEC REGSSSSGSFLKTNNVCSDPLQAAEPPTWRQVPLR PKATCIRYTCKGGTTPVPRCELDSCGKSQMALLK RIQQGAEGCPEGTPLYGI*SGASVHMLYMCLVEV KKTSRPPEPRGRGFPLKNTMIIWPQP**ARARRIT WPSSRSSCASRCTWRAP*TATSSRSRARARAAPTR APRPPS*R*PRVAPCPSPGTSCPLSSCTAPRPT*STP PTSPTT*SCPSPRASSGSA**TSRTAAW*P*PRTPPC RTASSSTR*SCAAPTSPPTAP*CRRRPWAGRPPPSG CTPRTAP*RARSSRG*S*RTAATTTLSRPPTRPRS PCSCPAPTSTSSWTSPPTTRTTPSWNSTNAPRAA TPPAAWTSCTS*	1215-1231	1037
OT-CD19-066 (CD8a leader - CD19 scFV - CD8a-Tm - 41BB - CD3zeta - Linker (GS)- P2A peptide -mCherry (MIL) - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM	1015	1039

	QALPPRGSGATNFSLLKQAGDVEENPGPLSKGEE DNMAIIEFMRFKVHMEGSVNGHEFEIEGEGEGR PYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGG VVTVTQDSSLQDGEFIYKVKLRTNFPDGPVMQ KKTMGWEASSERMYPEDGALKGEIKQRLKLKDG GHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSH NEDYTIVEQYERAEGRHSTGGMDELYK*		
OT-CAR19-IL15-001 (CD8a leader - CD19 scFV -CD8a-Tm - 41BB - CD3zeta - Linker (GS)- P2A - IL15 -Linker (SG3- (SG4)3-SG3-SLQ) - IL15Ra - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVWGSSETTYNSALKS RLTHKDNSKSQVFLKMNSLQTDDETAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLLSLVITLYCKRGRKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGATNFSLLKQAGDVEENPGPNWVNV SDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK CFLLELQVISLESGDASIHDVTENLIILANNSLSSNG NVTESGCKECELEEKNIKEFLQSFVHIVQMFINTS SGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMS VEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT ECVLNKATNVAHWTTPSLKCIRDPALVHQRPAAP STVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATT AAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTT AKNWELTASASHQPPGVYPQGHSDTTVAISTSTV LLCGLSAVSLLACYLKSRQTPPLASVEMEAMEAL PVTWGTSSRDEDLNCSSHL*	1016	1040
OT-CAR19-IL15-002 (CD8a leader -CD19 scFV -CD8a-Tm -41BB - CD3zeta - Linker (GS)- P2A - Linker (MLLLVTSLLLCELPH AFLIP) (SEQ ID NO: 1031) - IL15 - Linker (SG3-(SG4)3-SG3-SLQ) - IL15Ra - stop)	MALPVTALLLPLALLLHAARPDQMTQTTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVWGSSETTYNSALKS RLTHKDNSKSQVFLKMNSLQTDDETAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLLSLVITLYCKRGRKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRS ADAPAYKQGGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGATNFSLLKQAGDVEENPGPMLLLVT SLLLCELPHPAFLIPNWVNVISDLKKIEDLIQSMH IDATLYTESDVHPSCKVTAMKCFLELQVISLESG DASIHDVTENLIILANNSLSSNGNVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADIWVKSY LYSRERYICNSGFKRKAGTSSLTECVLNKATNVA HWTTPSLKCIRDPALVHQRPAAPSTVTTAGVTPQP ESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPS KSPSTGTTEISSHESSHGTPSQTTAKNWELTASAS	1017	1041

	HQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSQRQTPPLASVEMEAMEALPVTWGTSSRDED LENC SHHL*		
OT-CD19-IL15-006 (CD8a leader -CD19 scFV - CD8a-Tm - 41BB - CD3zeta - Linker (GS)- P2A - IgE Leader - IL15 - Linker (SG3-(SG4)3- SG3-SLQ) - IL15Ra - stop)	MALPVTALLLPLALLLHAARPDIQMTQTTSSLSAS LGDRV TISCRA SQDISKYLNWYQQKPDGTVKLLI YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIA TYFCQQGNTLPYTFGGG TKLEITGGGSGGGGSG GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKS RLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSTVTSSTTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVL DKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPRGSGATNFSLLKQAGDVEENPGPMDWTW ILFLVAAATR VHSNWVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMK CFLLELQVISLESGDA SIHDTVENLIILANNSLSSNGNVTESGCKECEEELEE KNIKEFLQSFVHIVQMFINTSSGGGSGGGGSGGGG SGGGGSGGGSLQITCPPPMSVEHADIWVKSYSLYS RERYICNSGFKRKAGTSSLTECVLNKATNV AHWT TPSLK CIRDPALVHQR PAPPSTVTTAGVTPQPESLS PSGKEPAASSPSSNNTAATTAAIVPGSQLMP SKSPS TGTTEISSHESHGTPSQTTAKNWELTASASHQPP GVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLK SRQTPPLASVEMEAMEALPVTWGTSSRDEDLENC SHHL*	1018	1042

[00229] Constructs disclosed in Table 10 which are transcriptionally controlled by a CMV promoter, in some instances may be placed under the transcriptional control of a different promoter to test the role of promoters in CD19 CAR expression. In one embodiment, the CMV promoter may be replaced by an EF1a promoter. In one embodiment, the CMV promoter of the, OT-CD19-001 construct, may be replaced to generate OT-CD19N-017 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-002 construct, may be replaced to generate OT-CD19N-018 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-003 construct, may be replaced to generate OT-CD19N-019 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-004 construct, may be replaced to generate OT-CD19N-020 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-005 construct, may be replaced to generate OT-CD19N-021 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-006 construct, may be replaced to generate OT-CD19N-022 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the

CD19 CAR, OT-CD19 CAR-007 construct, may be replaced to generate OT-CD19N-023 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-008 construct, may be replaced to generate OT-CD19N-024 construct, with a EF1a promoter. In another embodiment, the CMV promoter of the CD19 CAR, OT-CD19 CAR-009 construct, may be replaced to generate OT-CD19N-025 construct, with a EF1a promoter.

[00230] In one embodiment, the CAR construct comprises a CD19 scFV (e.g., CAT13.1E10 or FMC63), a CD8 α spacer or transmembrane domain, and a 4-1BB and CD3 ζ endodomain. These constructs with CAT13.1E10 may have increased proliferation after stimulation *in vitro*, increased cytotoxicity against the CD19+ targets, and increased effector and target interactions as compared to constructs with FMC63.

[00231] In some embodiments, the payloads of the present invention may be tuned using the catalytic domains of the E3 ubiquitin ligases. The catalytic domains of E3 ligases may be fused to an antibody or a fragment of the antibody. The payload is fused to the antigen recognized by the antibody or a fragment of the antibody that is fused to the E3 ligases catalytic domain. The E3 ligases useful in the present invention include, but are not limited to Ring E3 ligase, HECT E3 ligases and RBR E3 ligases. Any of the methods taught by Kanner SA et al. (2017) eLife; 6: e29744 may be useful in the present invention (the contents of which are incorporated by reference in their entirety).

[00232] In some embodiments, the payloads described herein, may be regulated by E3 ubiquitin ligases constructs. The E3 ligases constructs may comprise the catalytic domain of E3 ligases fused to an SRE and an antibody or a fragment of an antibody. The payloads are fused to the antigen recognized by the antibody or a fragment of an antibody, that is appended to the catalytic domain of E3 ligases. In the absence of the stimulus corresponding to the SRE, the E3 ubiquitin ligases constructs are destabilized, which in turn, allows the expression of the payloads fused to the antigen. In the presence of ligand corresponding to the SRE, the E3 ubiquitin ligases constructs are stabilized and available to bind to the antigen fused to the payloads. Binding of the E3 ligases constructs to the antigens, targets the protein for degradation. The E3 ubiquitin ligases constructs may be used to regulate any payload described herein, provided the payload is fused to an antigen recognized by the antibody or the fragment of the antibody in the E3 ubiquitin ligases construct. In some embodiments, the payload is a chimeric antigen receptor. The E3 ubiquitin ligases constructs may be used to design logic gates. In one embodiment, the E3 ubiquitin ligases constructs may be used to generate a NOT gate, wherein one ligand induces the expression of the payload, while another inhibits the expression of the payload. In some embodiments, the NOT gate may be generated using the E3 ubiquitin ligases constructs and by

fusing the payloads-antigen fusion protein to a second a SRE that is distinct from the SRE in the E3 ubiquitin ligase construct.

[00233] In some embodiments, the payload of the invention may be any of the co-stimulatory molecules and/or intracellular domains described herein. In some embodiments, one or more co-stimulatory molecules, each under the control of different SRE may be used in the present invention. SRE regulated co-stimulatory molecules may also be expressed in conjunction with a first generation CAR, a second generation CAR, a third generation CAR, a fourth generation, or any other CAR design described herein.

Tandem CAR (TanCAR)

[00234] In some embodiments, the CAR of the present invention may be a tandem chimeric antigen receptor (TanCAR) which is able to target two, three, four, or more tumor specific antigens. In some aspects, The CAR is a bispecific TanCAR including two targeting domains which recognize two different TSAs on tumor cells. The bispecific CAR may be further defined as comprising an extracellular region comprising a targeting domain (e.g., an antigen recognition domain) specific for a first tumor antigen and a targeting domain (e.g., an antigen recognition domain) specific for a second tumor antigen. In other aspects, the CAR is a multispecific TanCAR that includes three or more targeting domains configured in a tandem arrangement. The space between the targeting domains in the TanCAR may be between about 5 and about 30 amino acids in length, for example, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30 amino acids.

Split CAR

[00235] In some embodiments, the components including the targeting moiety, transmembrane domain and intracellular signaling domains of the present invention may be split into two or more parts such that it is dependent on multiple inputs that promote assembly of the intact functional receptor. In one embodiment, the split synthetic CAR system can be constructed in which the assembly of an activated CAR receptor is dependent on the binding of a ligand to the SRE (e.g. a small molecule) and a specific antigen to the targeting moiety. As a non-limiting example, the split CAR consists of two parts that assemble in a small molecule-dependent manner; one part of the receptor features an extracellular antigen binding domain (e.g. scFv) and the other part has the intracellular signaling domains, such as the CD3 ζ intracellular domain.

[00236] In other aspects, the split parts of the CAR system can be further modified to increase signal. In one example, the second part of cytoplasmic fragment may be anchored to the plasma membrane by incorporating a transmembrane domain (e.g., CD8 α transmembrane domain) to the construct. An additional extracellular domain may also be added to the second part of the CAR

system, for instance an extracellular domain that mediates homo-dimerization. These modifications may increase receptor output activity, i.e., T cell activation.

[00237] In some aspects, the two parts of the split CAR system contain heterodimerization domains that conditionally interact upon binding of a heterodimerizing small molecule. As such, the receptor components are assembled in the presence of the small molecule, to form an intact system which can then be activated by antigen engagement. Any known heterodimerizing components can be incorporated into a split CAR system. Other small molecule dependent heterodimerization domains may also be used, including, but not limited to, gibberellin-induced dimerization system (GID1-GAI), trimethoprim-SLF induced ecDHFR and FKBP dimerization (Czlapinski et al., *J Am Chem Soc.*, 2008, 130(40): 13186-13187) and ABA (abscisic acid) induced dimerization of PP2C and PYL domains (Cutler et al., *Annu Rev Plant Biol.* 2010, 61: 651-679). The dual regulation using inducible assembly (e.g., ligand dependent dimerization) and degradation (e.g., destabilizing domain induced CAR degradation) of the split CAR system may provide more flexibility to control the activity of the CAR modified T cells.

Switchable CAR

[00238] In some embodiments, the CAR of the invention may be a switchable CAR. Juillerat et al (Juillerat et al., *Sci. Rep.*, 2016, 6: 18950; the contents of which are incorporated herein by reference in their entirety) recently reported controllable CARs that can be transiently switched on in response to a stimulus (e.g. a small molecule). In this CAR design, a system is directly integrated in the hinge domain that separate the scFv domain from the cell membrane domain in the CAR. Such system is possible to split or combine different key functions of a CAR such as activation and costimulation within different chains of a receptor complex, mimicking the complexity of the TCR native architecture. This integrated system can switch the scFv and antigen interaction between on/off states controlled by the absence/presence of the stimulus.

Reversible CAR

[00239] In other embodiments, the CAR of the invention may be a reversible CAR system. In this CAR architecture, a LID domain (ligand-induced degradation) is incorporated into the CAR system. The CAR can be temporarily down-regulated by adding a ligand of the LID domain. The combination of LID and DD mediated regulation provides tunable control of continually activated CAR T cells, thereby reducing CAR mediated tissue toxicity.

Activation-conditional CAR

[00240] In some embodiments, payloads of the invention may be an activation-conditional chimeric antigen receptor, which is only expressed in an activated immune cell. The expression of the CAR may be coupled to activation conditional control region which refers to one or more

nucleic acid sequences that induce the transcription and/or expression of a sequence e.g. a CAR under its control. Such activation conditional control regions may be promoters of genes that are upregulated during the activation of the immune effector cell e.g. IL2 promoter or NFAT binding sites. In some embodiments, activation of the immune cell may be achieved by a constitutively expressed CAR (International Publication No: WO2016126608; the contents of which are incorporated herein by reference in their entirety).

Cytokines, chemokines and other soluble factors

[00241] In accordance with the present invention, CARs of the present invention may be utilized along with other payloads of the present invention may be cytokines, chemokines, growth factors, and soluble proteins produced by immune cells, cancer cells and other cell types, which act as chemical communicators between cells and tissues within the body. These proteins mediate a wide range of physiological functions, from effects on cell growth, differentiation, migration and survival, to a number of effector activities. For example, activated T cells produce a variety of cytokines for cytotoxic function to eliminate tumor cells.

[00242] In some embodiments, payloads of the present invention may be cytokines, and fragments, variants, analogs and derivatives thereof, including but not limited to interleukins, tumor necrosis factors (TNFs), interferons (IFNs), TGF beta and chemokines. In some embodiments, payloads of the present invention may be cytokines that stimulate immune responses. In other embodiments, payloads of the invention may be antagonists of cytokines that negatively impact anti-cancer immune responses.

[00243] In some embodiments, payloads of the present invention may be cytokine receptors, recombinant receptors, variants, analogs and derivatives thereof; or signal components of cytokines.

[00244] In some embodiments, cytokines of the present invention may be utilized to improve expansion, survival, persistence, and potency of immune cells such as CD8+T_{EM}, natural killer cells and tumor infiltrating lymphocytes (TIL) cells used for immunotherapy. In other embodiments, T cells engineered with two or more DD regulated cytokines are utilized to provide kinetic control of T cell activation and tumor microenvironment remodeling. In one aspect, the present invention provides biocircuits and compositions to minimize toxicity related to cytokine therapy. Despite its success in mitigating tumor burden, systemic cytokine therapy often results in the development of severe dose limiting side effects. Two factors contribute to the observed toxicity (a) Pleiotropism, wherein cytokines affect different cells types and sometimes produce opposing effects on the same cells depending on the context (b) Cytokines have short serum half-life and thus need to be administered at high doses to achieve therapeutic

effects, which exacerbates the pleiotropic effects. In one aspect, cytokines of the present invention may be utilized to modulate cytokine expression in the event of adverse effects. In some embodiments, cytokines of the present invention may be designed to have prolonged life span or enhanced specificity to minimize toxicity.

[00245] In some embodiments, the payload of the present invention may be an interleukin (IL) cytokine. Interleukins (ILs) are a class of glycoproteins produced by leukocytes for regulating immune responses. As used herein, the term “interleukin (IL)” refers to an interleukin polypeptide from any species or source and includes the full-length protein as well as fragments or portions of the protein. In some aspects, the interleukin payload is selected from IL1, IL1alpha (also called hematopoietin-1), IL1beta (catabolin), IL1 delta, IL1epsilon, IL1eta, IL1 zeta, interleukin-1 family member 1 to 11 (IL1F1 to IL1F11), interleukin-1 homolog 1 to 4 (IL1H1 to IL1H4), IL1 related protein 1 to 3 (IL1RP1 to IL1RP3), IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL10C, IL10D, IL11, IL11a, IL11b, IL12, IL13, IL14, IL15, IL16, IL17, IL17A, IL17B, IL17C, IL17E, IL17F, IL18, IL19, IL20, IL20 like (IL20L), IL21, IL22, IL23, IL23A, IL23-p19, IL23-p40, IL24, IL25, IL26, IL27, IL28A, IL28B, IL29, IL30, IL31, IL32, IL33, IL34, IL35, IL36 alpha, IL36 beta, IL36 gamma, IL36RN, IL37, IL37a, IL37b, IL37c, IL37d, IL37e and IL38. In other aspects, the payload of the present invention may be an interleukin receptor selected from CD121a, CDw121b, IL2R α /CD25, IL2R β /CD122, IL2R γ /CD132, CDw131, CD124, CD131, CDw125, CD126, CD130, CD127, CDw210, IL8RA, IL11R α , CD212, CD213 α 1, CD213 α 2, IL14R, IL15R α , CDw217, IL18R α , IL18R β , IL20R α , and IL20R β .

[00246] In one embodiment, the payload of the invention may comprise IL12. IL12 is a heterodimeric protein of two subunits (p35, p40) that is secreted by antigen presenting cells, such as macrophages and dendritic cells. IL12 is type 1 cytokine that acts on natural killer (NK) cells, macrophages, CD8⁺ Cytotoxic T cells, and CD4⁺ T helper cells through STAT4 pathway to induce IFN- γ production in these effector immune cells (reviewed by Trinchieri G, *Nat Rev Immunol.* 2003; 3(2): 133–146). IL12 can promote the cytotoxic activity of NK cells and CD8⁺ T cells, therefore has anti-tumor function. Intravenous injection of recombinant IL12 exhibited modest clinical efficacy in a handful of patients with advanced melanoma and renal cell carcinoma (Gollob et al., *Clin. Cancer Res.* 2000; 6(5):1678–1692). IL12 has been used as an adjuvant to enhance cytotoxic immunity using a melanoma antigen vaccine, or using peptide pulsed peripheral blood mononuclear cells; and to promote NK cell activity in breast cancer with trastuzumab treatment. Local delivery of IL12 to the tumor microenvironment promotes tumor regression in several tumor models. These studies all indicate that locally increased IL12 level can promote anti-tumor immunity. One major obstacle of systemic or local administration of

recombinant IL12 protein, or through oncolytic viral vectors is the severe side effects when IL12 is presented at high level. Developing a system that tightly controls IL12 level may provide a safe use of IL12 in cancer treatment.

[00247] It is understood in the art that certain gene and/or protein nomenclature for the same gene or protein may be inclusive or exclusive of punctuation such as a dash “-” or symbolic such as Greek letters. Whether these are included or excluded herein, the meaning is not meant to be changed as would be understood by one of skill in the art. For example, IL2, IL2 and IL 2 refer to the same interleukin. Likewise, TNFalpha, TNF α , TNF-alpha, TNF- α , TNF alpha and TNF α all refer to the same protein.

[00248] In one aspect, the effector module of the invention may be a DD-IL12 fusion polypeptide. This regulatable DD-IL12 fusion polypeptide may be directly used as an immunotherapeutic agent or be transduced into an immune effector cell (T cells and TIL cells) to generate modified T cells with greater *in vivo* expansion and survival capabilities for adoptive cell transfer. The need for harsh preconditioning regimens in current adoptive cell therapies may be minimized using regulated IL12 DD-IL12 may be utilized to modify tumor microenvironment and increase persistence in solid tumors that are currently refractory to tumor antigen targeted therapy. In some embodiments, CAR expressing T cells may be armored with DD regulated IL12 to relieve immunosuppression without systemic toxicity.

[00249] In some embodiments, the IL12 may be a Flexi IL12, wherein both p35 and p40 subunits, are encoded by a single cDNA that produces a single chain polypeptide. The single chain polypeptide may be generated by placing p35 subunit at the N terminus or the c terminus of the single chain polypeptide. Similarly, the p40 subunit may be at the N terminus or C terminus of the single chain polypeptide. In some embodiments, the IL12 constructs of the invention may be placed under the transcriptional control of the CMV promoter (SEQ ID NO. 716), an EF1a promoter (SEQ ID NO. 717, SEQ ID NO. 908) or a PGK promoter (SEQ ID NO. 718). Any portion of IL12 that retains one or more functions of full length or mature IL12 may be useful in the present invention. In some aspects, the DD-IL12 comprises the amino acid sequences listed in Table 11. The amino acid sequences in Table 11 may comprise a stop codon which is denoted in the table with a “*” at the end of the amino acid sequence.

Table 11: DD-IL12 constructs

Description	Promoter	Amino acid Sequence	Amino acid SEQ ID NO	Nucleic Acid SEQ ID NO
p40 signal sequence	-	MCHQQLVISWFSLVFLASPLVA	719	736-744

Linker	-	GGSGG	629	679-680
Linker	-	GGGGSGGGGSGGGGS	720	910-915
Linker		GS	-	GGATCC
Spacer		ATNFSLLKQAGDVEENPGP	745	746
Furin cleavage site	-	SARNRQKRS	721	750
Furin cleavage site	-	ARNRQKRS	722	751
Modified Furin	-	ESRRVRRNKRSK	630	681-683
P2A Cleavable Peptide	-	GATNFSLLKQAGDVEENPGP	925	926
p40	-	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLTQSSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS	723	752-761, 632-634
p40 (K217N)	-	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLTQSSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIKPDPPNNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS	747	748
p35	-	RNLPAVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFIRI RAVTIDRVMSYLNAS	724	762-771, 1012
ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I)	-	ISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNII LSSQPGTDDRVTWVKSVDIAAACGDVPEI MVIGGGRVIEQFLPKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHSYCFEILERR	9	692, 772, 814, 687, 988, 991
FKBP (F36V, L106P)	-	GVQVETISPGDGRTPPKRGQTCVVHYTGML EDGKKVDSSRDRNKPFFKMLGKQEVIRGW EEGVAQMSVGQRAKL TISPDYAYGATGHP GIIPPHATLVFDVELLKPE	11	684-686, 987, 989
FKBP (F36V, E31G, R71G, K105E)	-	GVQVETISPGDGRTPPKRGQTCVVHYTGML GDGKKVDSSRDRNKPFFKMLGKQEVIRGW EEGVAQMSVGQGA KL TISPDYAYGATGHP GIIPPHATLVFDVELLELE	12	688-691, 994, 1013, 1028
hDHFR (Amino acid 2-187 of WT; Q36F, Y122I, A125F)	-	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNE FRYFFRMTTSSVEGKQNLVIMGKKTWFSI PEKNRPLKGRINLVLSRELKEPPQGAHFLSR SLDDALKLTEQPELANKVDMVWIVGGSSVI KEFMNHPGHLKLFVTRIMQDFESDTFFPEID	46	773

		LEKYKLLPEYPGVLSDVQEEKGIKYKFEVY EKND		
hDHFR (Amino acid 2- 187 of WT) (I17V)	-	VGSLNCIVAVSQNMGVGKNGDLPWPPLRN EFRYFQRM TTTSSVEGKQNLVIMGKKTWFS IPEKNRPLKGRINLVLSRELKEPPQGAHFLS RSLDDALKLTEQPELANKVDMVWIVGGSS VYKEAMNHPGHLKLFVTRIMQDFESDTFFP EIDLEKYKLLPEYPGVLSDVQEEKGIKYKFE VYEKND	894	979
hDHFR (Amino acid 2- 187 of WT) (Y122I)	-	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNE FRYFQRM TTTSSVEGKQNLVIMGKKTWFSI PEKNRPLKGRINLVLSRELKEPPQGAHFLSR SLDDALKLTEQPELANKVDMVWIVGGSSVI KEAMNHPGHLKLFVTRIMQDFESDTFFPEID LEKYKLLPEYPGVLSDVQEEKGIKYKFEVY EKND	895	694, 995
OT-IL12-001 (p40 signal sequence - FKBP (F36V, L106P) - linker (GGSGG) - p40 - linker2 (G4S)3 - p35- stop)	CMV	MCHQQLVISWFSLVFLASPLVAGVQVETISP GDGRTPFKRGQTCVVHYTG MLEDGKKVDS SRDRNKPFKFM LGKQEVIRGWEEGVAQMS VGQRAKLTISPDYAYGATGHPGIIPPHATLV FDVELLKPEGGSGGIWELKKDVYVVELDW YPDAPGEMVVL TCDTPEEDGITWTLDQSSE VLGSGKTLTIQVKEFGDAGQYTCHKGGEV L SHSLLLLHKKEDGIWSTDILKDQKEPKNKT FLRCEAKNYSGRFTCWWLT TISTDLTFSVKS SRGSSDPQGVTCGAATLSAERVGRDNKEYE YSVEQCQEDSACPAAEESLPIEVMVDAVHKL KYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR QVEVSWEYPDTWSTPHSYFSLTFCVQVQG KSKREKKDRVFTDKTSATVICRKNASISVR AQDRYYSSSWSEWASVPCSGGGGSGGGGS GGGGSRNLPVATPDPMFPCLHHSQNL LRA VSNMLQKARQTLEFYPTCTSEEIDHEDITKDK TSTVEACLPLELTKNESCLNSRETSFITNGSC LASRKTSFMMALCLSSIYEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQA LNFNSETVPQKSSLEEPDFYKTKIKLCILLH AFRIRAVTIDRVMSYLNAS*	727	774
OT-IL12-002 (Met - FKBP (F36V, L106P) - linker (GGSGG) - p40 signal sequence - p40 - linker ((G4S)3) - p35 - stop)	CMV	MGVQVETISPGDGRTPFKRGQTCVVHYTG MLEDGKKVDSSRDRNKPFKFM LGKQEVIR GWEEGVAQMSVGQRAKLTISPDYAYGATG HPGIIPPHATLVFDVELLKPEGGSGGMCHQ QLVISWFSLVFLASPLVAIWELKKDVYVVE LDWYPDAPGEMVVL TCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKG GEVLSHSLLLLHKKEDGIWSTDILKDQKEP KNKTFLRCEAKNYSGRFTCWWLT TISTDLT FSVKSSRGSSDPQGVTCGAATLSAERVGRD NKEYEYSVEQCQEDSACPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSATVICRKN ASISVRAQDRYYSSSWSEWASVPCSGGGGS GGGGSGGGGSRNLPVATPDPMFPCLHHS QNLLRAVSNMLQKARQTLEFYPTCTSEEIDH EDITKDKTSTVEACLPLELTKNESCLNSRET SFITNGSCLASRKTSFMMALCLSSIYEDLKM YQVEFKTMNAKLLMDPKRQIFLDQNMLAV IDELMQALNFNSETVPQKSSLEEPDFYKTKI KLCILLHAFRIRAVTIDRVMSYLNAS*	728	775

OT-IL12-003 (p40 signal sequence - FKBP (F36V, L106P)- furin (SARNRQKRS) - p40- linker ((G4S)3)- p35 - stop)	CMV	MCHQQLVISWFSLVFLASPLVAGVQVETISP GDGRTPFKRGQTCVVHYTGMLDGGKKVDS SRDRNKPFKFM LGKQEVIRGWEEGVAQMS VGQRAKLTISPDYAYGATGHPGHPHATLV FDVELLKPEARNRQKRSIWELKKDVYVVE LDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKG GEVLSHSLLLLHKKEDGIWSTDILKDQKEP KNKTFLRCEAKNYSGRFTCWWLTTISTDLT FSVKSSRGSSDPQGVTCGAATLSAERVRGD NKEYEYSVEQCEDSACPAAEESLPVEMVD AVHKLKYENYTSSFFIRDIKPDPPKNLQK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSATVICRKN ASISVRAQDRYYSSSWSEWASVPCSGGGGS GGGGSGGGGSRNLPVATPDPGMFPCLHHS QNLLRAVSNMLQKARQTLEFYPTSEEIDH EDITKDKTSTVEACLPLELTKNESCLNSRET SFITNGSCLASRKTSFMMALCLSSIYEDLKM YQVEFKTMNAKLLMDPKRQIFLDQNMLAV IDELMQALNFNSETVPOKSSLEEPDFYKTKI KLCILLHAFRIRAVTIDRVMSYLNAS*	729	776
OT-IL12-004 (p40 signal sequence - p40 - linker ((G4S)3) - p35 - furin (ARNRQKRS) - FKBP (E31G, F36V, R71G, K105E) -stop)	CMV	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPV VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPOKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS ARNRQKRSGVQVETISPGDGRTPFKRGQTC VVHYTGMLDGGKKVDSSDRNKPFKFM GKQEVIRGWEEGVAQMSVGQGAKLTI SPD YAYGATGHPGHPHATLVFDVELLELE*	730	777
OT-IL12-005 (p40 signal sequence- p40 -- linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	CMV	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPV VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN	731	778

		MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSRDNRNPKFKFMLGKQEV IRGWEEGVAQMSVGQGAKLITSPDYAYGA TGHPGIIPPHATLVFDVELLELE*		
OT-IL12-006 (p40 signal sequence- p40 -- linker ((G4S)3)- p35 - stop)	CMV	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLHKKEDGIWSTDILKD QKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGSRNLPVATPDPMFPCL HHSQNLLRAVS NMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS *	732	779
OT-IL12-007 (p40 signal sequence; ecDHFR (Amino acid 2- 159 of WT) (R12Y, 100I) - furin site (ESRRVRRNK RSK) - p40 -- linker ((G4S)3) - p35)	CMV	MCHQQLVISWFSLVFLASPLVAISLIAALAV DYVIGMENAMPWNLPADLAWFKRNTLNK PVIMGRHTWESIGRPLPGRKNILSSQPGTD DRVTWVKSVDEAIAACGDVPEIMVIGGGR VIEQFLPKAQKLYLTHIDAEVEGDTHFPDYE PDDWESVFSEFHDADAQNSHSYCFEILERR ESRRVRRNKRSKIWELKKDVYVVELDWYP DAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSH SLLLHKKEDGIWSTDILKDQKEPKNKTFLR CEAKNYSGRFTCWWLTTISTDLTFSVKSSR GSSDPQGVTCGAATLSAERVRGDNKEYEY SVEQCEDSACPAAEESLPIEVMVDAVHKLK YENYTSSFFIRDIKPDPPKNLQLKPLKNSRQ VEVSWEYPDTWSTPHSYFSLTFCVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQ DRYYSSSWSEWASVPCSGGGGGSGGGSGG GGSRNLPVATPDPMFPCLHHSQNLLRAVS NMLQKARQTLEFYPTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCL ASRKTSFMMALCLSSIYEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQA LNFNSETVPQKSSLEEPDFYKTKIKLCILLH AFRIRAVTIDRVMSYLNAS	733	780
OT-IL12-008 (p40 signal sequence; hDHFR (Amino acid 2- 187 of WT) (Q36K, Y122I, A125F) - furin site (ESRRVRRNK RSK)- p40 -	CMV	MCHQQLVISWFSLVFLASPLVAVGSLNCIV AVSQNMGIGKNGDLPWPPLRNEFRYFFRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPL KGRINLVLSRELKEPPQGAHFLSRSLDDALK LTEQPELANKVDMVWIVGGSSVIKEFMNHP GHLKLFVTRIMQDFESDTFFPEIDLEKYKLL PEYPGVLSVQEEKGIKYKFEVYEKNDES RVRNRNKRSKIWELKKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDQSSEVLGSG KTLTIQVKEFGDAGQYTCHKGGEVLSHSL LLHKKEDGIWSTDILKDQKEPKNKTFLRCE	734	781

linker((G4S)3) - p35)		AKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAVHKLKYE NYTSSFFIRDIKPDPPKNLQKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCSGGGGGSGGGGGSGGGGSRNLPVATPDPMFPCLHHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNA KLLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS		
OT-IL12-009 (p40 signal sequence- p40 - linker ((G4S)3) - p35 - furin (ESRRVRRNK RSK) - FKBP (E31G, F36V, R71G, K105E)- stop)	CMV	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYE NYTSSFFIRDIKPDPPKNLQKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGGSRNLPVATPDPMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNA KLLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS ESRRVRRNKRSKGVQVETISPGDGRTFPCR GQTCVVHYTGMLGDGKKVDSSRDRNKPFFK FMLGKQEVIRGWEEGVAQMSVGQGAKLTI SPDYAYGATGHPGHPHATLVFDVELLELE *	735	782
OT-IL12-019 (p40 signal sequence- p40- linker((G4S)3)- p35-stop)	PGK	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYE NYTSSFFIRDIKPDPPKNLQKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGGSRNLPVATPDPMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNA KLLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS *	732	779
OT-IL12-020 (p40 signal sequence- p40-	EF1a	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKD	732	779

linker((G4S)3)- p35-stop)		QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQVGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS *		
OT-IL12-021 (p40 signal sequence- p40- linker((G4S)3)- p35-stop)	No promoter	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQVGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS *	732	779
OT-IL12-022 (p40 signal sequence- p40 – linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	PGK	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQVGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSDRNKPFFKMLGKQEV IRGWEEGVAQMSVGQGAKLTI SPDYAYGA TGHPGIIPPHATLVFDVELLELE*	731	778
OT-IL12-023 (p40 signal sequence- p40 – linker-	EF1a	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD	731	778

((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)		QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSDRNKPFFKMLGKQEV IRGWEEGVAQMSVGQGAKL TISPDYAYGA TGHPGIIPPHATLVFDVELLELE*		
OT-IL12-024 (p40 signal sequence- p40 – linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	No promoter	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSDRNKPFFKMLGKQEV IRGWEEGVAQMSVGQGAKL TISPDYAYGA TGHPGIIPPHATLVFDVELLELE*	731	778
OT-IL12-025 (p40 signal sequence- p40 – linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	PGK	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSDRNKPFFKMLGKQEV	731	778

		IRGWEEGVAQMSVGQGAKLTISPDYAYGA TGHPGIIPPHATLVFDVELLELE*		
OT-IL12-026 (p40 signal sequence- p40 – linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	EF1a	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSSHLLLLHKKEDGIWSTDILKD QKEPKNKTLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSRDRNKPFFMLGKQEV IRGWEEGVAQMSVGQGAKLTISPDYAYGA TGHPGIIPPHATLVFDVELLELE*	731	778
OT-IL12-027 (p40 signal sequence- p40 – linker- ((G4S)3) - p35 - linker (GGSG) - FKBP (E31G, F36V, R71G, K105E) - stop)	No promoter	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSSHLLLLHKKEDGIWSTDILKD QKEPKNKTLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS GGSGGVQVETISPGDGRTFPKRGQTCVVHY TGMLGDGKKVDSSRDRNKPFFMLGKQEV IRGWEEGVAQMSVGQGAKLTISPDYAYGA TGHPGIIPPHATLVFDVELLELE*	731	778
OT-IL12-028 (p40 signal sequence - p40 - linker ((G4S)3) - p35 - furin (ARNRQKRS) - FKBP (E31G, F36V, R71G, K105E) -stop)	PGK	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSSHLLLLHKKEDGIWSTDILKD QKEPKNKTLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIHKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS	730	777

		RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS ARNRQKRSGVQVETISPGDGRTFPKRGQTC VVHYTGMLGDGKKVDSSRDRNKPFKFML GKQEVIRGWEEGVAQMSVGQGAKLTISPD YAYGATGHPGIIPPHATLVFDVELLELE*		
OT-IL12-029 (p40 signal sequence- p40 - linker ((G4S)3) - p35 - furin (ESRRVRRNK RSK) - FKBP (E31G, F36V, R71G, K105E)- stop)	EF1a	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDOSSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSSHLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFQVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS ESRRVRRNKRSKGVQVETISPGDGRTFPKR GQTCVVHYTGMLGDGKKVDSSRDRNKPFK FMLGKQEVIRGWEEGVAQMSVGQGAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLELE *	735	782
OT-IL12-030 (p40 signal sequence- p40 - linker ((G4S)3) - p35 - furin (ESRRVRRNK RSK) - FKBP (E31G, F36V, R71G, K105E)- stop)	No promoter	MCHQQLVISWFSLVFLASPLVAIWELKKDV YVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDOSSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSSHLLLLHKKEDGIWSTDILKD QKEPKNKTFRLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFS LTFQVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGG GGSGGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNMLQKARQTLEFYPTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDF YKTKIKLCILLHAFRIRAVTIDRVMSYLNAS ESRRVRRNKRSKGVQVETISPGDGRTFPKR GQTCVVHYTGMLGDGKKVDSSRDRNKPFK FMLGKQEVIRGWEEGVAQMSVGQGAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLELE *	735	782
OT-IL12-046 (p40 signal sequence -- FKBP (F36V, L106P) -- Linker	EF1a	MCHQQLVISWFSLVFLASPLVAGVQVETISP GDGRTFPKRGQTCVVHYTGMLDGKKVDS SRDRNKPFKFMLGKQEVIRGWEEGVAQMS VGQRAKLTISPDYAYGATGHPGIIPPHATLV FDVELLKPEGGSGGIWELKKDVYVVELDW YPDAPGEMVVLTCDTPEEDGITWTLDOSSSE	727	774

(GGSGG) – p40 – Linker ((G4S)3) – p35 - stop)		VLGSGKTLTIQVKEFGDAGQYTCHKGGEVL SHSLLLLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWLTTISTDLTFSVKSS RGSSDPQGVTCGAATLSAERVGRDNKEYE YSVEQCEDSACPAAEESLPIEVMVDAVHKL KYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR QVEVSWEYPDTWSTPHSYFSLTFCVQVQGK SKREKKDRVFTDKTSATVICRKNASISVRAQ DRYYSSSWSEWASVPCSGGGGSGGGGSGG GGSRLNPVATPDPGMFPC LHHSQNLLRAVS NMLQKARQTLEFYPC TSEEIDHEDITKDKTS TVEACLPLELTKNESCLNSRETSFITNGSCLA SRKTSFMMALCLSSIYEDLKMYQVEFKTMN AKLLMDPKRQIFLDQNMLAVIDELMQALNF NSETVPQKSSLEEPDFYKTKIKLCILLHAFRI RAVTIDRVMSYLNAS*		
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[00250] In one embodiment, the payload of the invention may comprise IL15. Interleukin 15 is a potent immune stimulatory cytokine and an essential survival factor for T cells, and Natural Killer cells. Preclinical studies comparing IL2 and IL15, have shown that IL15 is associated with less toxicity than IL2. In some embodiments, the effector module of the invention may be a DD-IL15 fusion polypeptide. IL15 polypeptide may also be modified to increase its binding affinity for the IL15 receptor. For example, the asparagine may be replaced by aspartic acid at position 72 of IL15 (SEQ ID NO. 2 of US patent publication US20140134128A1; the contents of which are incorporated by reference in their entirety). In some embodiments, the IL15 constructs of the invention may be placed under the transcriptional control of the CMV promoter (SEQ ID NO. 716), an EF1a promoter (SEQ ID NO. 717, SEQ ID NO. 908) or a PGK promoter (SEQ ID NO. 718). In some aspects, the DD-IL15 comprises the amino acid sequences listed in Table 12. The amino acid sequences in Table 12 may comprise a stop codon which is denoted in the table with a “*” at the end of the amino acid sequence.

Table 12: DD IL15 constructs

Description/ Construct ID	Promoter	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
IL2 signal sequence	-	MYRMQLLSIALSLALVTNS	783	788-791
IgE Leader	-	MDWTWILFLVAAATRVHS	801	810, 930, 931
Linker	-	EFSTEF	784	792-793
Linker	-	GGSGG	629	676-680
HA Tag	-	YPYDVPDYA	1024	1025-1027
BamHI	-	GS	-	GGATCC
P2A Cleavable Peptide	-	GATNFSLKQAGDVEENPGP	925	926
mCherry (MIL)	-	LSKGEEDNMAIIEFMRFKVHMEGSVNG HEFEIEGEGEGRPYEGTQTAKLKVTGGP LPFAWDILSPQFMYGSKAYVKHPADIPDY LKLSFPEGFKWERVMNFEDGGVVTVTQD SSLQDGEFIYKVKLRGTNFPSDGPVMQKK	1029	1030

		TMGWEASSERMYPEDGALKGEIKQRLKL KDGGHYDAEVKTTYKAKKPVQLPGAYN VNIKLDITSHNEDYTIVEQYERAEGRHSTG GMDELYK		
IL15	-	NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINTS*	785	794-797, 1001
ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I)	-	ISLIAALAVDYVIGMENAMPWNLPADLA WFKRNTLNKPVIMGRHTWESIGRPLPGRK NIILSSQPGTDDRVTWVKSVDIAAAGD VPEIMVIGGGRVIEQFLPKAQKLYLTHIDA EVEGDTHFPDYEPDDWESVFSEFHDADA QNSHSYCFEILERR*	9	692, 772, 814, 687, 988, 991
hDHFR (Amino acid 2-187 of WT) (Y122I)	-	VGSLNCIVAVSQNMGIGKNGDLPWPPLR NEFRYFQRMTTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINLVLSRELKEPPQGA HFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLFVTRIMQDFE SDTFFPEIDLEKYKLLPEYPGVLSDVQEEK GIKYKFEVYEKND	895	694, 995
OT-IL15-001 (IL2 signal sequence- IL15-stop)	CMV	MYRMQLLSIALSLALVTNSNWVNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDTVENLIIL ANNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFINTS*	786	799
OT-IL15-002 (IL2 signal sequence- linker[EFSTEF]- ecDHFR (amino acid 2-159 of WT, R12Y, 100I)- linker [GGSGG]- IL15- stop)	CMV	MYRMQLLSIALSLALVTNSEFSTEFISLIA ALAVDYVIGMENAMPWNLPADLAWFKR NTLNKPVIMGRHTWESIGRPLPGRKNIILS SQPGTDDRVTWVKSVDIAAAGDVPEI MVIGGGRVIEQFLPKAQKLYLTHIDAEVE GDTHFPDYEPDDWESVFSEFHDADAQNS HSYCFEILERRGGSGGNWVNVISDLKKIE DLIQSMHIDATLYTESDVHPSCKVTAMKC FLELQVISLESGDASIHDTVENLIILANNS LSSNGNVTESGCKECEELEEKNIKEFLQSF VHIVQMFINTS*	787	800
OT-IL15-062 (IgE leader -- HA Tag -- IL15 -- BamHI (GS) -- stop)	EF1a	MDWTWILFLVAAATRVHSYPYDVPDYA NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINTSGS*	631	749
OT-IL15-132 (IgE leader --IL15 -- BamHI (GS) -- P2A cleavable peptide -- mCherry (MIL) - stop)	EF1a	MDWTWILFLVAAATRVHSNWVNVISDLK KIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLESGDASIHDTVENLIIL ANNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFINTSGSGATNFSLLKQAGD VEENPGPLSKGEEDNMAIIEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQAKLK VTKGGPLPFAWDILSPQFMYGSKAYVKH PADIPDYLLKLSFPEGFKWERVMNFEDGGV VTVTQDSSLQDGEFIYKVKLRGTNFPDGD PVMQKKTMGWEASSERMYPEDGALKGEI KQRLKLDGGHYDAEVKTTYKAKKPVQ LPGAYNVNIKLDITSHNEDYTIVEQYERA EGRHSTGGMDELYK*	725	1055
OT-IL15-134 (IgE leader --IL15 -- Linker (GS) -- hDHFR (WT 2-187, Y122I) --	EF1a	MDWTWILFLVAAATRVHSNWVNVISDLK KIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLESGDASIHDTVENLIIL ANNSLSSNGNVTESGCKECEELEEKNIKEFL	726	1056

BamHI (GS) -- P2A cleavable peptide -- mCherry (MIL) - stop)		QSFVHIVQMFIN TSGSVGSLNCIVAVSQN MGIGKNGDLPWPPLRNEFRYFQRM TTTSS VEGKQNLVIMGKKTWFSIPEKNRPLKGRI NLVLSRELKEPPQGAHFLSRSLDDALKLT EQPELANKVDMVWIVGGSSVIKEAMNHP GHLKLFVTRIMQDFESDTFFPEIDLEKYKL LPEYPGVLSDVQEEKGIKYKFEVYEKNDG SGATNFSLLKQAGDVEENPGPLSKGEEDN MAIIEFMRFKVHMEGSVNGHEFEIEGEG EGRPYEGTQTAKLKVTGGPLPFAWDILS PQFMYGSKAYVKHPADIPDYKLKLSFPEGF KWERVMNFEDGGVVTVTQDSSLQDGEFI YKVKLRGTNFPDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLDGGHYD AEVKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDELYK *		
Description/ Construct ID	Promoter	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
IL2 signal sequence	-	MYRMQLLSIALSLALVTNS	783	788-791
IgE Leader	-	MDWTWILFLVAAATRVHS	801	810, 930, 931
Linker	-	EFSTEF	784	792-793
Linker	-	GGSGG	629	676-680
HA Tag	-	YPYDVPDYA	1024	1025-1027
BamHI	-	GS	-	GGATCC
P2A Cleavable Peptide	-	GATNFSLLKQAGDVEENPGP	925	926
mCherry (MIL)	-	LSKGEEDNMAIIEFMRFKVHMEGSVNG HEFEIEGEGEGRPHYEGTQTAKLKVTGGP LPFAWDILSPQFMYGSKAYVKHPADIPDY LKLSFPEGFKWERVMNFEDGGVVTVTQD SSLQDGEFIYKVKLRGTNFPDGPVMQKKT TMGWEASSERMYPEDGALKGEIKQRLKL KDGGHYDAEVTYKAKKPVQLPGAYN VNIKLDITSHNEDYTIVEQYERAEGRHSTG GMDELYK	1029	1030
IL15	-	NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEE LEEKNIKEFLQSFVHIVQMFIN TSGSVGSLNCIVAVSQN	785	794-797, 1001
ecDHFR (Amino acid 2-159 of WT) (R12Y, Y100I)	-	ISLIAALAVDYVIGMENAMPWNLPADLA WFKRNTLNKPVIMGRHTWESIGRPLPGRK NIILSSQPGTDDRVTWVKSVDIAAACGD VPEIMVIGGGRVIEQFLPKAQLYLTHIDA EVEGDTHFPDYEPDDWESVFSEFHDADA QNSHSYCFEILERR*	9	692, 772, 814, 687, 988, 991
hDHFR (Amino acid 2-187 of WT) (Y122I)	-	VGSLNCIVAVSQNMGIGKNGDLPWPPLR NEFRYFQRM TTTSSVEGKQNLVIMGKKT WFSIPEKNRPLKGRINL VLSRELKEPPQGA HFLSRSLDDALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLFVTRIMQDFE SDTFFPEIDLEKYKLLPEYPGVLSDVQEEK GIKYKFEVYEKND	895	694, 995
OT-IL15-001 (IL2 signal sequence-IL15-stop)	CMV	MYRMQLLSIALSLALVTNSNWVNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLLLELQVISLESGDASIHDTVENLI ILANNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFIN TSGSVGSLNCIVAVSQN	786	799

OT-IL15-002 (IL2 signal sequence-linker[EFSTEF]-ecDHFR (amino acid 2-159 of WT, R12Y, 100I)- linker [GGSGG]- IL15-stop)	CMV	MYRMQLLSIALSLALVTNSEFSTEFISLIA ALAVDYVIGMENAMPWNLPA DLAWFKR NTLNKPVIMGRHTWESIGRPLPGRKNIILS SQPGTDDRVTWVKSVD EAIACGDVPEI MVIGGGRVIEQFLPKAQKLYLTHIDAEVE GDTHFPDYEPDDWESVFSEFHDADAQNS HSYCFEILERRGGSGGNWVNVISDLKKIE DLIQSMHIDATLYTESDVHPSCKVTAMKC FLLELQVISLES GDASIHDTVENLIILANNS LSSNGNVTESGCKECEELEEKNIKEFLQSF VHIVQMFINTS*	787	800
OT-IL15-062 (IgE leader – HA Tag – IL15 – BamHI (GS) – stop)	EF1a	MDWTWILFLVAAATRVHSYPYDVPDYA NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLELQVISLES GDASIH DTVENLIILANNSLSSNGNVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINTSGS*	631	749
OT-IL15-132 (IgE leader –IL15 – BamHI (GS) – P2A cleavable peptide – mCherry (MIL) - stop)	EF1a	MDWTWILFLVAAATRVHSNWVNVISDLK KIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLES GDASIHDTVENLIILA NNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFINTSGSGATNFSLLKQAGD VEENPGPLSKGEEDNMAIIEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQTAKLK VTKGGPLPFAWDILSPQFMYGSKAYVKH PADIPDYLKLSFPEGFKWERVMNFEDGGV VTVTQDSSLQDGEFIYKVKLRGTNFPSDG PVMQKKTMGWEASSERMYPEDGALKGEI KQRLKLKDGGHYDAEVKTTYKAKKPVQ LPGAYNVNIKLDITSHNEDYTIVEQYERAE GRHSTGGMDELYK*	725	1055
OT-IL15-134 (IgE leader –IL15 – Linker (GS) – hDHFR (WT 2-187, Y122I) – BamHI (GS) – P2A cleavable peptide – mCherry (MIL) - stop)	EF1a	MDWTWILFLVAAATRVHSNWVNVISDLK KIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLES GDASIHDTVENLIILA NNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFINTSGSVGSLNCIVAVSQN MGIGKNGDLPWPPLRNEFRYFQRM TTTSS VEGKQNLVIMGKKTWFSIPEKNRPLKGRI NLVLSRELKEPPQGAHFLSRSLDDALKLT EQPELANKVDMVWIVGGSSVIKEAMNHP GHLKLFVTRIMQDFESDTFFPEIDLEKYKL LPEYPGVLSDVQEEKGIKYKFEVYEKNDG SGATNFSLLKQAGDVEENPGPLSKGEEDN MAIIEFMRFKVHMEGSVNGHEFEIEGEG EGRPYEGTQTAKLKVTKGGPLPFAWDILS PQFMYGSKAYVKHPADIPDYLKLSFPEGF KWERVMNFEDGGVVVTVTQDSSLQDGEFI YKVKLRGTNFPSDGPVMQKKTMGWEAS SERMYPEDGALKGEIKQRLKLKDGGHYD AEVKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDELYK *	726	1056

[00251] A unique feature of IL15 mediated activation is the mechanism of trans-presentation in which IL15 is presented as a complex with the alpha subunit of IL15 receptor (IL15Ra) that binds to and activates membrane bound IL15 beta/gamma receptor, either on the same cell or a different cell. The IL15/IL15Ra complex is more effective in activating IL15 signaling, than

[00254] In some aspects, the DD-IL115/IL15Ra comprises the amino acid sequences provided in Table 13a, 13b, and 13c. The amino acid sequences in Tables 13a, 13b and 13c may comprise a stop codon which is denoted in the table with a “*” at the end of the amino acid sequence.

Description/ Construct ID	Amino Acid Sequence	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
IgE leader	MDWTWILFLVAAATRVHS	801	810, 930, 931
IL15RA Leader	MAPRRARGCRTLGLPALLLLLLLLRPPATRG	932	933
Linker (SG3- (SG4)3-SG3-SLQ)	SGGGSGGGGSGGGGSGGGGSGGGSLQ	802	811, 916-920, 1002
Linker (SG3S)	SGGGS	827	828, 844, 909
Linker (SG3(SG4)5SG3S)	SGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGS	921	923
Linker	SGGGSGGGGSGGGGSGGGGS	922	924
Linker	GS	-	GGTTCC
Linker	SG	-	AGCGGC
Linker	GSG	-	GGATCCGG A or GGATCCGG T
Spacer	-	-	927-929, 1000, TCGCGAAT G, TCGCA

IL15	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHTVENLILANNSLSSN GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS*	785	794-797, 1001
IL15Ra	ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAG TSSLTECVLNKATNVAHWTTPSLKCIRDPAALVHQRPA PPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATT AAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKN WELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLS AVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSS RDEDLENCSHHL*	803	812-813, 1003
IL15Ra (31-205 of Uniprot ID: Q13261.1)	ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAG TSSLTECVLNKATNVAHWTTPSLKCIRDPAALVHQRPA PPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTA AIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKN WELTASASHQPPGVYPQGHSDTT	1057	1058
mCherry	MSKGEEDNMAIHEFMRFKVHMEGSVNGHEFEIEGEG EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVT VTQDSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMG WEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVK TTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYE RAEGRHSTGGMDELYK*	1059	1060
mCherry (M1L)	LSKGEEDNMAIHEFMRFKVHMEGSVNGHEFEIEGEG EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSK AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVT VTQDSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMG WEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVK TTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYE RAEGRHSTGGMDELYK	1029	1030
HA Tag	YPYDVPDYA	1024	1025-1027
Flag	DYKDDDDK	1232	-
BamHI	GS	-	GGATCC
P2A Cleavable Peptide	GATNFSLLKQAGDVEENPGP	925	926
ecDHFR (Amino acid 2-159 of WT R12Y, Y100I)	ISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTLN KPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWV KSVDEAIAACGDVPEIMVIGGGRVIEQFLPKAQKLYLT HIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHS YCFEILERR*	9	692, 772, 814, 687, 988, 991
ecDHFR (Amino acid 2-159 of WT R12H, E129K)	ISLIAALAVDHVIGMENAMPWNLPADLAWFKRNTLN KPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWV KSVDEAIAACGDVPEIMVIGGGRVYEQFLPKAQKLYL THIDAEVEGDTHFPDYKPDWESVFSEFHDADAQNS HSYCFEILERR*	10	798, 815, 993
FKBP (E31G, F36V, R71G, K105E)	GVQVETISPGDGRTPFKRGQTCVVHYTGMLGDGKKV DSSRDRNKPFFKFMLGKQEVIRGWEEGVAQMSVGQG AKLTISPDYAYGATGHPGIIPPHATLVFDVELLELE*	12	688-691, 994, 1013, 1028
hDHFR (Amino acid 2-187 of WT; Y122I, A125F)	VGSLNCIVAVSQNMIGKNGDLPWPPLRNEFRYFQR MTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVIKEFMNHPGHLKLFVTRIMQDFESDTF FPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKN D*	890	696, 973, 974, 996
hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I)	VGSLNCIVAVSQNMIGKNGDLPWPPLRNEFRYFFRM TTTSSVEGKQNLVIMGKKTWFSIPEKFRPLKGRINLVL SRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMV WIVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDTFFPE IDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND	891	700, 975, 976, 998

hDHFR (Amino acid 2-187 of WT; K185E)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDT FFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEE ND*	889	972
hDHFR (Amino acid 2-187 of WT; E162G, I176F)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDT FFPEIDLEKYKLLPGYPGVLSDVQEEKGFKYKFEVYE KND*	888	970-972
hDHFR (Amino acid 2-187 of WT; N127Y)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVYKEAMYHPGHLKLFVTRIMQDFESDT FFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEK ND	892	977
hDHFR (Amino acid 2-187 of WT; I17V)	VGSLNCIVAVSQNMGVGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDT FFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEK ND	894	979
hDHFR (Amino acid 2-187 of WT; I17V, Y122D)	VGSLNCIVAVSQNMGVGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVIKEAMNHPGHLKLFVTRIMQDFESDT FFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEK ND	882	969
hDHFR (Amino acid 2-187 of WT; H131R, E144G)	VGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQR MTTTSSEVGKQNLVIMGKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVD MVWIVGGSSVYKEAMNHPGRLKLFVTRIMQDFGSDT FFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEK ND	893	978

Table 13b: DD-IL15/IL15Ra constructs

Description	Promoter	Amino acid sequences	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO
OT-IL15-006 (IgE signal sequence; IL15; linker1 (SG3- (SG4)5-SG3); IL15Ra; linker2 (GGSGG); ecDHFR (R12H, E129K))	EF1a	MDWTWILFLVAAATRVHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLLLELQVISLESGDASIHDVTENLII LANNSSLSSNGNVTESGCKECEEELEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPAV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGISLI AALAVDHVIGMENAMPWNLPADLAWF KRNTLNKPVIMGRHTWESIGRPLPGRKN IILSSQPGTDDRVTWVKSVDIAAACGD	804	816

		VPEIMVIGGGRVYEQFLPKAQKLYLTHI DAEVEGDTHFPDYKPDDWESVFSEFHD ADAQNSHSYCFEILERR*		
OT-IL15-007 (IgE signal sequence; IL15; linker1 (SG3- (SG4)5-SG3); IL15Ra; linker2 (GGSGG); FKBP (E31G,F36V, R71G, K105E))	EF1a	MDWTWILFLVAAATRVHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLLACYLKSRTTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGGVQ VETISPGDGRTFPKRGQTCVVHYTGMLG DGKKVDSSRDRNKPFFKMLGKQEVIRG WEEGVAQMSVGQGAKLTISPDYAYGAT GHPGIIPPHATLVFDVELLELE*	805	817
OT-IL15-008 (IgE signal sequence- IL15- linker (SG3- (SG4)3-SG3- SLQ)- IL15Ra- stop)	EF1a	MDWTWILFLVAAATRVHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLLACYLKSRTTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL*	806	818
OT-IL15-009 (IgE signal sequence- IL15- linker (SG3- (SG4)3-SG3- SLQ)- IL15Ra- linker (SG)- ecDHFR (Amino acid 2-159 of WT; R12Y, Y100I)-stop)	EF1a	MDWTWILFLVAAATRVHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLLACYLKSRTTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGISLI AALAVDYVIGMENAMPWNLPADLAWF KRNTLNKPVIMGRHTWESIGRPLPGRKN IILSSQPGTDDRVTWVKSVDIAAACGD VPEIMVIGGGRVIEQFLPKAQKLYLTHID AEVEGDTHFPDYEPDDWESVFSEFHDAD AQNSHSYCFEILERR*	807	819
OT-IL15-010 (IgE signal sequence- IL15- linker (SG3- (SG4)3-SG3-	EF1a	MDWTWILFLVAAATRVHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG	808	820

SLQ)- IL15Ra-linker (SG)-hDHFR (Y122I, A125F)-stop)		GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPA LHQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGVGS LNCIVAVSQNMIGKNGDLPWPPLRNEF RYFQRM TTTSSVEGKQNLVIMGKKTWF SIPEKNRPLKGRINLVLSRELKEPPQGAH FLSRSLDDALKLTEQPELANKVDMVWIV GGSSVIKEFMNHPGHLKLFVTRIMQDFE SDTFFPEIDLEKYKLLPEYPGVLSDVQEE KGIKYKFEVYEKND*		
OT-IL15-011 (IgE signal sequence- IL15- linker (SG3- (SG4)3-SG3- SLQ)- IL15Ra; linker (SG)- hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I)- stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPA LHQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGVGS LNCIVAVSQNMIGKNGDLPWPPLRNEF RYFFRM TTTSSVEGKQNLVIMGKKTWFS IPEKFRPLKGRINLVLSRELKEPPQGAHFL SRSLDDALKLTEQPELANKVDMVWIVG GSSVIKEAMNHPGHLKLFVTRIMQDFES DTFFPEIDLEKYKLLPEYPGVLSDVQEEK GIKYKFEVYEKND*	809	821
OT-IL15-017 (IgE signal sequence- IL15- linker (SG3- (SG4)3-SG3- SLQ)- IL15Ra- linker (SG)- hDHFR (Amino acid 2-187 of WT; K185E)- stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPA LHQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLSGVGS LNCIVAVSQNMIGKNGDLPWPPLRNEF RYFQRM TTTSSVEGKQNLVIMGKKTWF SIPEKNRPLKGRINLVLSRELKEPPQGAH FLSRSLDDALKLTEQPELANKVDMVWIV GGSSVYKEAMNHPGHLKLFVTRIMQDF ESDTFFPEIDLEKYKLLPEYPGVLSDVQE EKGIKYKFEVYEEND*	1061	1086
OT-IL15-018 (IgE signal	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT	1062	1087

sequence- IL15-linker (SG3-(SG4)3-SG3-SLQ)- IL15Ra-linker (SG)-hDHFR (Amino acid 2-187 of WT; E162G, I176F)-stop)		AMKCFLELELQVISLES GDASIHTVENLII LANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSL LACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL SGVGS LNCIVAVSQNMGIGKNGDLPWPPLRNEF RYFQRM TTTSSVEGKQNLVIMGKKTWF SIPEKNRPLKGRINL VLSRELKEPPQGAH FLSRSLDDALKLTEQPELANKVDMVWIV GGSSVYKEAMNHPGHLKLFVTRIMQDF ESDTFFPEIDLEKYKLLPGYPGVLSDVQE EKGFKYKFEVYEKND*		
OT-IL15-038 (IgE leader – IL15 – Linker (SG3-(SG4)3- SG3-SLQ) – IL15Ra – Linker (SG) – hDHFR (Amino acid 2- 187 of WT; N127Y)-stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELELQVISLES GDASIHTVENLII LANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSL LACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL SGVGS LNCIVAVSQNMGIGKNGDLPWPPLRNEF RYFQRM TTTSSVEGKQNLVIMGKKTWF SIPEKNRPLKGRINL VLSRELKEPPQGAH FLSRSLDDALKLTEQPELANKVDMVWIV GGSSVYKEAMYHPGHLKLFVTRIMQDF ESDTFFPEIDLEKYKLLPEYPGVLSDVQE EKGIKYKFEVYEKND*	1063	1088
OT-IL15-051 (IgE leader – IL15 – Linker (SG3-(SG4)3- SG3-SLQ) – HA Tag – IL15Ra – stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELELQVISLES GDASIHTVENLII LANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQYPYDVPDYAITCPP PMSVEHADIWVKSYSLYSRERYICNSGF KRKAGTSSLTECVLNKATNVAHWTTPSL KCIRDPALVHQRPAAPPSTVTTAGVTPQPE SLSPSGKEPAASSPSSNNTAATTAAIVPG SQLMPSKSPSTGTTEISSHESSHGTPSQTT AKNWELTASASHQPPGVYPQGHSDTTV AISTSTVLLCGLSAVSL LACYLKSRQTPP LASVEMEAMEALPVTWGTSSRDEDLN CSHHL*	1064	1089
OT-IL15-053 (IgE leader – IL15 – Linker (SG3(SG4)3SG3	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELELQVISLES GDASIHTVENLII LANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG	1066	1091

S) – IL15Ra – Stop)		GGSGGGGSGGGGSGGGGSGGGGSITCPPP MSVEHADIWVKSYSLYSRERYICNSGFK RKAGTSSLTECVLNKATNVAHWTTPSLK CIRDPALVHQRPAAPPSTVTTAGVTPQPES LSPSGKEPAASSPSSNNTAATTAAIVPGS QLMPSKSPSTGTTEISSHESSHGTPSQTTA KNWELTASASHQPPGVYPQGHSDTTVAI STSTVLLCGLSAVSLACYLKSRQTPPLA SVEMEAMEALPVTWGTSSRDEDLENC SHHL*		
OT-IL15-054 (IgE leader – IL15 – Linker (SG3(SG4)3S) – HA Tag – Linker (SG3S) – IL15Ra – Stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSYPYDVPDYASGGGSITCPPP MSVEHADIWVKSYSLYSRERYICNSGFK RKAGTSSLTECVLNKATNVAHWTTPSLK CIRDPALVHQRPAAPPSTVTTAGVTPQPES LSPSGKEPAASSPSSNNTAATTAAIVPGS QLMPSKSPSTGTTEISSHESSHGTPSQTTA KNWELTASASHQPPGVYPQGHSDTTVAI STSTVLLCGLSAVSLACYLKSRQTPPLA SVEMEAMEALPVTWGTSSRDEDLENC SHHL*	1067	1092
OT-IL15-055 (IgE leader – IL15 – Linker (SG) - IL15Ra – Stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECELEEKNIK EFLQSFVHIVQMFINTSSGITCPPPMSVEH ADIWVKSYSLYSRERYICNSGFKRKAGT SSLTECVLNKATNVAHWTTPSLK CIRDP ALVHQRPAAPPSTVTTAGVTPQPESLSPSG KEPAASSPSSNNTAATTAAIVPGSQLMPS KSPSTGTTEISSHESSHGTPSQTTAKNWE LTASASHQPPGVYPQGHSDTTVAISTSTV LLCGLSAVSLACYLKSRQTPPLASVEM EAMEALPVTWGTSSRDEDLENC SHHL*	1068	1093
OT-IL15-060 (IL15Ra signal peptide – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – IL15Ra – stop)	EF1a	MAPRRARGCRTLGLPALLLLLLLRPPAT RGNWNVISDLKKIEDLIQSMHIDATLYT ESDVHPSCKVTAMKCFLELQVISLESG DASIHDVTENLIILANNLSNNGNVTESG CKECELEEKNIKEFLQSFVHIVQMFINT SSGGGSGGGGSGGGGSGGGGSGGGSLQI TCPPPMSVEHADIWVKSYSLYSRERYIC NSGFKRKAGTSSLTECVLNKATNVAHW TTPSLK CIRDPALVHQRPAAPPSTVTTAGV TPQPESLSPSGKEPAASSPSSNNTAATTAA IVPGSQLMPSKSPSTGTTEISSHESSHGT PSQTTAKNWELTASASHQPPGVYPQGHSD TTVAISTSTVLLCGLSAVSLACYLKSR QTPPLASVEMEAMEALPVTWGTSSRDE DLENC SHHL*	1069	1094
OT-IL15-063 (IgE leader – IL15 – Linker (SG3-(SG4)3- SG3-SLQ) – IL15Ra –	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNLSNNGNVTESGCKECELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL	1070	1095

BamHI (GS) - stop)		TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLGS*		
OT-IL15-064 and OT-IL15-071 (IgE leader -- IL15 -- Linker (SG3-(SG4)3-SG3-SLQ) -- IL15Ra -- stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNSSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL*	806	818
OT-IL15-066 (IgE leader -- IL15 -- Linker (SG3-(SG4)3-SG3-SLQ) -- IL15Ra -- Linker (SG) ecDHFR (Amino acid 2-159 of WT, R12Y, Y100I) - stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNSSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL SGISLI AALAVDYVIGMENAMPWNLPADLAWF KRNTLNKPVIMGRHTWESIGRPLPGRKN IILSSQPGTDDRVTWVKSVDIAAACGD VPEIMVIGGGRVIEQFLPKAQKLYLTHID AEVEGDTHFPDYEPDDWESVFSEFHDAD AQNSHSYCFEILERR*	807	819
OT-IL15-067 (IgE leader -- IL15 -- Linker (SG3-(SG4)3-SG3-SLQ) -- IL15Ra -- Linker (SG) - ecDHFR (Amino acid 2-159 of WT, R12Y, Y100I) - stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDVTENLII LANNSSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL SGISLI AALAVDYVIGMENAMPWNLPADLAWF KRNTLNKPVIMGRHTWESIGRPLPGRKN IILSSQPGTDDRVTWVKSVDIAAACGD VPEIMVIGGGRVIEQFLPKAQKLYLTHID	1071	1098

		AEVEGDTHFPDYEPDDWESVFSEFHDAD AQNSHSYC FEILERR*		
OT-IL15-070 (IL15Ra signal peptide – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – IL15Ra – stop)	EF1a	MAPRRARGCRTLGLPALLLLLLLRPPAT RGNWVNVISDLKKIEDLIQSMHIDATLYT ESDVHPSCKVTAMKCFLELQVISLESG DASIHDTVENLIILANNSLSSNGNVTESG CKECEEELEEKNIKEFLQSFVHIVQMFINT SSGGGSGGGGSGGGGSGGGGSGGGSLQI TCPPPMMSVEHADIWVKSYSLSRERYIC NSGFKRKAGTSSLTECVLNKATNVAHW TTPSLKCIRDPALVHQRPAAPPSTVTTAGV TPQPESLSPSGKEPAASSPSSNNTAATTA AIVPGSQLMPSKSPSTGTTEISSHESHG PSQTTAKNWELTASASHQPPGVYPQGH SDTTVAISTSTVLLCGLSAVSLACYLKSR QTPPLASVEMEAMEALPVTWGTSSRDE DLENC SHHL*	1072	1101
OT-IL15-072 (IgE leader – IL15 – Linker (SG3-(SG4)3- SG3-SLQ) – IL15Ra – stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDTVENLI LANNSLSSNGNVTESGCKECEEELEEKNI KEFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMMSVEHADI WVKSYSLSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTA AIVPGSQLMPSKSP STGTTEISSHESHGTPSQTTAKNWELTAS ASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDE DLENC SHHL*	806	1096
OT-IL15-089 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – linker (GSG) – ecDHFR (Amino acid 2-159 of WT, R12Y, Y100I) - stop)	EF1a	MDWTWILFLVAAATR VHS DYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLIILANNSLSSNGNVTESGCK ECEEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVPDYAITCPPPMMSVEHADIWVKSYS LSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTTPSLKCIRDPALVHQRPAAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTA AIVPGSQLMPSKSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLA CYLKSRQTPPLASVEMEAMEALPVTWG TSSRDE DLENC SHHLGSGISLIAALAVDY VIGMENAMPWNL PADLAWFKRNTLNKP VIMGRHTWESIGRPLPGRKNILSSQPGT DDRVTWVKSVD EAIACGDVPEIMVIGG GRVIEQFLPKAQKLYLTHIDAEVEGDTH FPDYEPDDWESVFSEFHDADAQNSHSYC FEILERR*	1074	1102
OT-IL15-109 (IgE leader – IL15 – Linker (SG3-(SG4)3- SG3-SLQ) – IL15Ra – BamHI (GS) - stop)	EF1a	MDWTWILFLVAAATR VHSNWNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDTVENLI LANNSLSSNGNVTESGCKECEEELEEKNI KEFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMMSVEHADI WVKSYSLSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTTPSLKCIRDPALV	1070	1095

		HQRPAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLLACYLKSQRQTPPLASVEMEAM EALPVTWGTSSRDEDELENCSHHLGS*		
OT-IL15-110 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – BamHI (GS) - stop)	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVDPYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSKSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSQRQTPPLASVEMEAMEALPVTWG TSSRDEDELENC SHHLGS*	1075	1103
OT-IL15-114 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – Linker (GSG) – hDHFR (Amino acid 2-187 of WT; K185E) - stop)	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVDPYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSKSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSQRQTPPLASVEMEAMEALPVTWG TSSRDEDELENC SHHLGSGVGS LNCIVAV SQNMGIGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVY KEAMNHPGHLKLFVTRIMQDFESDTFFP EIDLEKYKLLPEYPGVLSDVQEEKGIKYK FEVYEEND*	1076	1104
OT-IL15-115 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – Linker (GSG) – hDHFR (Amino acid 2-187 of WT; E162G, I176F) - stop)	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVDPYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSKSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSQRQTPPLASVEMEAMEALPVTWG TSSRDEDELENC SHHLGSGVGS LNCIVAV SQNMGIGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR	1077	1105

		PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVY KEAMNHPGHLKLFVTRIMQDFESDTFFP EIDLEKYKLLPGYPGVLSDVQEEKGFKY KFEVYEKND*		
OT-IL15-116 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – Linker (GSG) – hDHFR (Amino acid 2-187 of WT; H131R, E144G) - stop)	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVPDYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSRTTPPLASVEMEAMEALPVTWG TSSRDEDLNCSHHLGSGVGSLNCIVAV SQNMGIGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVY KEAMNHPGRLKLFVTRIMQDFGSDTFFP EIDLEKYKLLPEYPGVLSDVQEEKGIKYK FEVYEKND*	1078	1106
OT-IL15-117 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – Linker (GSG) – hDHFR(Amino acid 2-187 of WT; I17V) - stop)	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVPDYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSRTTPPLASVEMEAMEALPVTWG TSSRDEDLNCSHHLGSGVGSLNCIVAV SQNMGVGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVY KEAMNHPGHLKLFVTRIMQDFESDTFFP EIDLEKYKLLPEYPGVLSDVQEEKGIKYK FEVYEKND*	1079	1107
OT-IL15-118 (IgE leader – FLAG – IL15 – Linker (SG3- (SG4)3-SG3- SLQ) – HA Tag – IL15Ra – Linker (GSG) – hDHFR (Amino acid 2-187 of	EF1a	MDWTWILFLVAAATRVHSDYKDDDDK NWNVISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILANNSLSSNGNVTESGCK ECEEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVPDYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRDPA LVHQRPAAPPST VTTAGVTPQPESLSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSPSTGTTEISSH	1080	1108

WT, N127Y) - stop)		ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSRTTPPLASVEMEAMEALPVTWG TSSRDEDLNCSHHLGSGVGSLNCIVAV SQNMGIGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVY KEAMYHPGHLKLFVTRIMQDFESDTFFP EIDLEKYKLLPEYPGVLSDVQEEKGIKYK FEVYEKND*		
OT-IL15-119 (IgE leader -- FLAG -- IL15 -- Linker (SG3- (SG4)3-SG3- SLQ) -- HA Tag -- IL15Ra -- Linker (GSG) -- hDHFR (Amino acid 2-187 of WT, I17V, Y122I) - stop)	EF1a	MDWTWILFLVAAATR VHSDYKDDDDK NWNVNISDLKKIEDLIQSMHIDATLYTES DVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLIILANNSLSSNGNVTESGCK ECEEELEEKNIKEFLQSFVHIVQMFINTSSG GGSGGGGSGGGGSGGGGSGGGSLQYPY DVPDYAITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIRD PALVHQR PAPPST VTTAGVTPQPELSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPSKSPSTGTTEISSH ESSHGTPSQTTAKNWELTASASHQPPGV YPQGHSDTTVAISTSTVLLCGLSAVSLLA CYLKSRTTPPLASVEMEAMEALPVTWG TSSRDEDLNCSHHLGSGVGSLNCIVAV SQNMGVGKNGDLPWPPLRNEFRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNR PLKGRINLVLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWIVGGSSVIK EAMNHPGHLKLFVTRIMQDFESDTFFPEI DLEKYKLLPEYPGVLSDVQEEKGIKYKF EVYEKND*	1081	1109
OT-IL15-128 (IgE leader -- IL15 -- Linker (SG3-(SG4)3- SG3-SLQ) -- IL15Ra-- Spacer -- Flagx3 - Spacer - BamHI (GS) -- P2A cleavable peptide -- mCherry (M1L) - stop)	EF1a	MDWTWILFLVAAATR VHSNWNVNISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLELQVISLESGDASIHDTVENLII LANNSLSSNGNVTESGCKECEEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRD PALV HQR PAPPSTVTTAGVTPQPELSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLLA CYLKSRTTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHL SRMD YKDDDDKDYKDDDDKDYKDDDDKSRG SGATNFSLLKQAGDVEENPGPLSKGEED NMAIIEFMRFKVHMEGSVNGHEFEIEG EGEGRPYEGTQTAKLK VTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYKLKLSF PEGFKWERVMNFEDGGVVTVTQDSSLQ DGEFIYKVKLRGTNFPDGPVMQKKT GWEASSERMYPEDGALKGEIKQRLKLK DGGHYDAEVKTTYKAKKPVLPGAYN VNIKLDITSHNEDYTIVEQYERAEGRHST GGMDELYK*	1082	1110
OT-IL15-129 (IgE leader --	EF1a	MDWTWILFLVAAATR VHSNWNVNISDL KKIEDLIQSMHIDATLYTESDVHPSCKVT	1083	1111

IL15 -- Linker (SG3-(SG4)3-SG3-SLQ) -- IL15Ra-BamHI (GS) -- P2A cleavable peptide -- mCherry (MIL)-stop)		AMKCFLLELQVISLESGDASIHTVENLII LANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTSSGGGSGGGGSGG GGSGGGGSGGGSLQITCPPPMSEHADI WVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALV HQRPAAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSP STGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLC GLSAVSLACYLKSRQTPPLASVEMEAM EALPVTWGTSSRDEDLNCSHHLGSGAT NFSLLKQAGDVEENPGPLSKGEEDNMAI IKEFMRFKVHMEGSVNGHEFEIEGEGEG RPYEGTQTAKLKVTKGGPLPFAWDILSP QFMYGSKAYVKHPADIPDYLKLSFPEGF KWERVMNFEDGGVVTVTQDSSLQDGEF IYKVKLRGTNFPDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGH YDAEVKTTYKAKKPVQLPGAYNVNIKL DITSHNEDYTIVEQYERAEGRHSTGGMD ELYK*		
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Table 13c: IL15/IL15Ra constructs

Construct Description	Sequence Description	Promoter	Amino acid sequences	Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO/ Sequence
OT-IL15-122 (IgE leader -- IL15 -- linker (GS) -- hDHFR (Amino acid 2-187 of WT, Y122I) -- stop -- spacer -- IRES -- spacer -- mCherry -- stop)	Full construct	EF1a	MDWTWILFLVAAATRVHSN WVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKC FLLELQVISLESGDASIHTVE NLII LANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIV QMFINTSGSVGSLNCIVAVSQ NMGIGKNGDLPWPPLRNEFR YFQRM TTTSSVEGKQNLVIM GKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLF VTRIMQDFESDTFFPEIDLEKY KLLPEYPGVLSDVQEEKGIKY KFEVYEKND*SR*YDSLEIPPL SLPPP*RYWPKPLGIRPVCVC LYVIFHHIAVFWQCEGPETWP CLLDEHS*GSFPSRQRNARSV ECREGSSSSGSFLKTNNVCSD PLQAAEPPTWRQVPLRPKAT CIRYTCKGGTTPVPRCELDSC GKSQMA LLKRIQQGAEGCPE GTPLYGI*SGASVHMLYMCL VEVKKTSRPPEPRGRGFPLKN TMIIWPQP**ARARRITWPSSR SSCASRCTWRAP*TATSSRSR ARARAAPTRAPRPPS*R*PRV APCPSPGTSCPLSSCTAPRPT*	1114, 1126- 1140	1120

			STPPTSPTT*SCPSPRASSGSA* *TSRTAAW*P*PRTPPCRTASS STR*SCAAPTSPPTAP*CRRRP WAGRPPPSGCTPRTAP*RARS SRG*S*RTAATTTLRSRPPTRP RSPCSCPAPTTSTSSWTSPPTT RTTPSWNSTNAPRAATPPAA WTSCTS		
	IgE leader -- IL15 -- linker (GS) -- hDHFR (Amino acid 2-187 of WT, Y122I) -- stop	-	MDWTWILFLVAAATR VHSN WVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKC FLLELQVISLESGDASIHTVE NLILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIV QMFINTSGSVGSLNCIVAVSQ NMGIGKNGDLPWPPLRNEFR YFQRM TTTSSVEGKQNLVIM GKKTWFSIPEKNRPLKGRINL VLSRELKEPPQGAHFLSRSLD DALKLTEQPELANKVDMVWI VGGSSVIKEAMNHPGHLKLF VTRIMQDFESDTFFPEIDLEKY KLLPEYPGVLSDVQEEKGIKY KFEVYEKND*	1115	1121
	mCherry - stop	-	MSKGEEDNMAIIEKFMRFKV HMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPA DIPDYLKLSFPEGFKWERVM NFEDGGVVTVTQDSSLQDGE FIYKVKLRGTNFPSDGPVMQ KKTMGWEASSERMYPEDGA LKGEIKQRLKLKDGGHYDAE VKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYER AEGRHSTGGMDELYK*	1116	1122
OT-IL15- 123 and OT- IL15- 127(IgE leader -- IL15 -- BamHI (GS) -- stop -- spacer -- IRES -- spacer -- mCherry - stop)	Full construct	EF1a	MDWTWILFLVAAATR VHSN WVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKC FLLELQVISLESGDASIHTVE NLILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIV QMFINTSGS*NLDNTTH*RSR PSPSPPPNVTGRSRLE*GRCAF VYMLFSTILPSFGNVRARKPG PVFLT SIPRGLSPLAKGMQGL LNVVKEAVPLEAS*RQTTSVA TLCRQRNPPPGDRCLCGQKP RV*DTPAKAAQPQCHVVS WI VVERVKWLSSSVFNKGLKDA QKVPHCMGSDLGPRCTCFTC V*SRLKKRLGPPNHGDVVFL* KTR**YGHNHDEQGRGG*HG HHQGVHALQGAHGGLRERP RVRDRGRGRGPPLRGHPDRQ AEGDQGWPPALRLGHPVPSV HVRLOQLREAPRRHPRLLEA VLPRGLQVGARDELRGRRRG DRDPGLLPAGRRVHLQGEAA RHQLPLRRPRNAEEDHGLGG	1117, 1141- 1149	1123

			LLRADVPRGRRPEGRDQAEA EAEGRRPLRR*GQDHLQGQE ARAAARRLQRQHGVGHHL QRGLHHRGTVRTRRGPP LHR RHGRAVQV		
	IgE leader – IL15 – BamHI (GS) -- stop	-	MDWTWILFLVAAATRVHSN WVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKC FLELQVISLES GDASIHDTVE NLILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIV QMFINTSGS*	1118	1124
	mCherry - stop	-	MSKGEEDNMAIIEFMRFKV HMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTGGPLPFA WDILSPQFMYGSKAYVKHPA DIPDYLKLSFPEGFKWERVM NFEDGGVVTVTQDSSLQDGE FIYKVKLRGTNFPDGPVMQ KKTMGWEASSERMYPEDGA LKGEIKQRLKLDGGHYDAE VKTTYKAKKPVQLPGAYNV NIKLDITSHNEDYTIVEQYER AEGRHSTGGMDELYK*	1119	1125

[00255] In one embodiment, the payload of the present invention may comprise IL18. IL18 is a proinflammatory and immune regulatory cytokine that promotes IFN- γ production by T and NK cells. IL18 belongs to the IL1 family. Secreted IL18 binds to a heterodimer receptor complex, consisting of IL18R α and β -chains and initiates signal transduction. IL18 acts in concert with other cytokines to modulate immune system functions, including induction of IFN- γ production, Th1 responses, and NK cell activation in response to pathogen products. IL18 showed anti-cancer effects in several tumors. Administration of recombinant IL18 protein or IL18 transgene induces melanoma or sarcoma regression through the activation of CD4⁺ T and/or NK cell-mediated responses (reviewed by Srivastava et al., *Curr. Med. Chem.*, 2010, 17: 3353–3357). The combination of IL18 with other cytokines, such as IL12 or co-stimulatory molecules (e.g., CD80) increases IL18 anti-tumor effects. For example, IL18 and IL12A/B or CD80 genes have been integrated successfully in the genome of oncolytic viruses, with the aim to trigger synergistically T cell-mediated anti-tumor immune responses (Choi et al., *Gene Ther.*, 2011, 18: 898-909). IL2/IL18 fusion proteins also display enhanced anti-tumor properties relative to either cytokine alone and low toxicity in preclinical models (Acres et al., *Cancer Res.*, 2005, 65:9536–9546).

[00256] IL18 alone, or in combination of IL12 and IL15, activates NK cells. Preclinical studies have demonstrated that adoptively transferred IL12, IL15 and IL18 pre-activated NK cells display enhanced effector function against established tumors *in vivo* (Ni et al., *J Exp Med.* 2012, 209: 2351–2365; and Romee et al., *Blood.* 2012,120:4751–4760). Human IL12/IL15/IL18

activated NK cells also display memory-like features and secrete more IFN- γ in response to cytokines (e.g., low concentration of IL2). In one embodiment, the effector module of the present invention may be a DD-IL18 fusion polypeptide.

[00257] In one embodiment, the payload of the present invention may comprise IL21. IL21 is another pleiotropic type I cytokine that is produced mainly by T cells and natural killer T (NKT) cells. IL21 has diverse effects on a variety of cell types including but not limited to CD4⁺ and CD8⁺ T cells, B cells, macrophages, monocytes, and dendritic cells (DCs). The functional receptor for IL21 is composed of IL21 receptor (IL21R) and the common cytokine receptor gamma chain, which is also a subunit of the receptors for IL2, IL4, IL7, IL9 and IL15. Studies provide compelling evidence that IL21 is a promising immunotherapeutic agent for cancer immunotherapy. IL21 promotes maturation, enhances cytotoxicity, and induces production of IFN- γ and perforin by NK cells. These effector functions inhibit the growth of B16 melanoma (Kasaian et al., *Immunity*. 2002, 16(4):559–569; and Brady et al., *J Immunol.* 2004, 172(4):2048–2058). IL21 together with IL15 expands antigen-specific CD8⁺ T-cell numbers and their effector function, resulting in tumor regression (Zeng et al., *J Exp Med.* 2005, 201(1):139–148). IL21 may also be used to rejuvenate multiple immune effector cells in the tumor microenvironment. IL21 may also directly induce apoptosis in certain types of lymphoma such as diffuse large B-cell lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia cells, via activation of STAT3 or STAT1 signal pathway. IL21, alone or in combination with anti-CD20 mAb (rituximab) can activate NK cell-dependent cytotoxic effects. Interestingly, discovery of the immunosuppressive actions of IL21 suggests that this cytokine is a “double-edged sword”- IL21 stimulation may lead to either the induction or suppression of immune responses. Both stimulatory and suppressive effects of IL21 must be considered when using IL21-related immunotherapeutic agents. The level of IL21 needs to be tightly controlled by regulatory elements. In one aspect, the effector module of the present invention may be a DD-IL21 fusion polypeptide.

[00258] In some embodiments, payloads of the present invention may comprise type I interferons. Type I interferons (IFNs-I) are soluble proteins important for fighting viral infection in humans. IFNs-I include IFN-alpha subtypes (IFN- α 1, IFN- α 1b, IFN- α 1c), IFN-beta, IFN-delta subtypes (IFN-delta 1, IFN-delta 2, IFN-delta 8), IFN-gamma, IFN-kappa, and IFN-epsilon, IFN-lambda, IFN-omega, IFN-tau and IFN-zeta. IFN- α and IFN- β are the main IFN-I subtypes in immune responses. All subtypes of IFN-I signal through a unique heterodimeric receptor, interferon alpha receptor (IFNAR), composed of 2 subunits, IFNAR1 and IFNAR2. IFNAR activation regulates the host response to viral infections and in adaptive immunity. Several

signaling cascades can be activated by IFNR, including the Janus activated kinase--signal transducer and activation of transcription (JAK-STAT) pathway, the mitogen activated protein kinase (MAPK) pathway, the phosphoinositide 3-kinase (PI3K) pathway, the v-erk sarcoma virus CT10 oncogene homolog (avian)-like (CRKL) pathway, and NF- κ B cascade. It has long been established that type I IFNs directly inhibit the proliferation of tumor cells and virus-infected cells, and increase MHC class I expression, enhancing antigen recognition. IFNs-I have also proven to be involved in immune system regulation. IFNs can either directly, through interferon receptor (IFNR), or indirectly by the induction of chemokines and cytokines, regulate the immune system. Type I IFNs enhance NK cell functions and promote survival of NK cells. Type I IFNs also affect monocytes, supporting the differentiation of monocytes into DC with high capacity for antigen presentation, and stimulate macrophage function and differentiation. Several studies also demonstrate that IFNs-I promote CD8⁺ T cell survival and functions. In some instances, it may be desirable to tune the expression of Type I IFNs using biocircuits of the present invention to avoid immunosuppression caused by long-term treatment with IFNs.

[00259] New anticancer immunotherapies are being developed that use recombinant type I IFN proteins, type I IFN transgene, type I IFN-encoding vectors and type I IFN-expressing cells. For example, IFN- α has received approval for treatment of several neoplastic diseases, such as melanoma, RCC and multiple myeloma. Though type I IFNs are powerful tools to directly and indirectly modulate the functions of the immune system, side effects of systemic long-term treatments and lack of sufficiently high efficacy have dampened the interest of IFN- α for clinical use in oncology. It is believed that if IFN levels are tightly regulated at the malignant tissues, type I IFNs are likely more efficacious. Approaches for intermittent delivery are proposed according to the observation that intermittency at an optimized pace may help to avoid signaling desensitizing mechanisms (negative feedback mechanisms) induced by IFNs-I (i.e., because of SOCS1 induction) in the responding immune cells. In accordance with the present invention, the effector module may comprise a DD-IFN fusion polypeptide. The DD and its ligand control the expression of IFN to induce an antiviral and antitumor immune responses and in the meantime, to minimize the side effects caused by long-term exposure of IFN.

[00260] In some embodiments, payloads of the present invention may comprise members of tumor necrosis factor (TNF) superfamily. The term "TNF superfamily" as used herein refers to a group of cytokines that can induce apoptosis. Members of TNF family include TNF- α , TNF- β (also known as lymphotoxin- α (LT- α)), lymphotoxin- β (LT- β), CD40L(CD154), CD27L (CD70), CD30L(CD153), FASL(CD178), 4-1BBL (CD137L), OX40L, TRAIL (TNF-related apoptosis inducing ligand), APRIL (a proliferation-inducing ligand), TWEAK,

TRANCE, TALL-1, GITRL, LIGHT and TNFSF1 to TNFSF20 (TNF ligand superfamily member 1 to 20). In one embodiment, the payload of the invention may be TNF-alpha. TNF-alpha can cause cytolysis of tumor cells, and induce cell proliferation differentiation as well. In one aspect, the effector module of the present invention may comprise a DD-TNF alpha fusion polypeptide.

[00261] In some embodiments, payloads of the present invention may comprise inhibitory molecules that block inhibitory cytokines. The inhibitors may be blocking antibodies specific to an inhibitory cytokine, and antagonists against an inhibitory cytokine, or the like.

[00262] In some aspects, payloads of the present invention may comprise an inhibitor of a secondary cytokine IL35. IL35 belongs to the interleukin-12 (IL12) cytokine family, and is a heterodimer composed of the IL27 β chain Ebi3 and the IL12 α chain p35. Secretion of bioactive IL35 has been described only in forkhead box protein 3 (Foxp3)⁺ regulatory T cells (Tregs) (resting and activated Tregs). Unlike other membranes in the family, IL35 appears to function solely in an anti-inflammatory fashion by inhibiting effector T cell proliferation and perhaps other parameters (Collison et al., *Nature*, 2007, 450(7169): 566–569).

[00263] In some embodiments, payloads of the present invention may comprise inhibitors that block the transforming growth factor beta (TGF- β) subtypes (TGF- β 1, TGF- β 2 and TGF- β 3). TGF- β is secreted by many cell types, including macrophages and is often complexed with two proteins LTBP and LAP. Serum proteinases such as plasmin catalyze the release of active TGF- β from the complex from the activated macrophages. It has been shown that an increase in expression of TGF- β correlates with the malignancy of many cancers. The immunosuppressive activity of TGF- β in the tumor microenvironment contributes to oncogenesis.

[00264] In some embodiments, payloads of the present invention may comprise inhibitors of IDO enzyme.

[00265] In some embodiments, payloads of the present invention may comprise chemokines and chemokine receptors. Chemokines are a family of secreted small cytokines, or signaling proteins that can induce directed chemotaxis in nearby responsive cells. The chemokine may be a SCY (small cytokine) selected from the group consisting of SCYA1-28 (CCL1-28), SCYB1-16 (CXCL1-16), SCYC1-2 (XCL1-2), SCYD-1 and SCYE-1; or a C chemokine selected from XCL1 and XCL2; or a CC chemokine selected from CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27 and CCL28; or a CXC chemokine selected from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14,

CXCL15, CXCL16 and CXCL17; or a CX3C chemokine CX3CL1. In some aspects, the chemokine receptor may be a receptor for the C chemokines including XCR1; or a receptor for the CC chemokines including CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9 and CCR10; or a receptor for the CXC chemokines including CXCR1, CXCR2, CXCR3, CXCR4 and CXCR5; or a CX3C chemokine receptor CX3CR1.

[00266] In some embodiments, payloads of the present invention may comprise other immunomodulators that play a critical role in immunotherapy, such as GM-CSF (Granulocyte-macrophage colony stimulating factor), erythropoietin (EPO), MIP3a, monocyte chemotactic protein (MCP)-1, intracellular adhesion molecule (ICAM), macrophage colony stimulating factor (M-CSF), Interleukin-1 receptor activating kinase (IRAK-1), lactotransferrin, and granulocyte colony stimulating factor (G-CSF).

[00267] In some embodiments, the payload of the present invention may comprise Amphiregulin. Amphiregulin (AREG) is an EGF-like growth factor which binds to the EGFR receptor and enhances CD4⁺ regulatory T cells (Tregs) function. AREG promotes immune suppression in the tumor environment. Thus, in some embodiment, the payloads of the present invention may comprise Amphiregulin to dampen immune response during immunotherapy.

[00268] In some embodiments, payloads of the present invention may comprise fusion proteins wherein a cytokine, chemokine and/or other soluble factor may be fused to other biological molecules such as antibodies and or ligands for a receptor. Such fusion molecules may increase the half-life of the cytokines, reduce systemic toxicity, and increase local concentration of the cytokines at the tumor site. Fusion proteins containing two or more cytokines, chemokines and or other soluble factors may be utilized to obtain synergistic therapeutic benefits. In one embodiment, payload may be a GM-CSF/IL2 fusion protein.

3. Additional effector module features

[00269] The effector module of the present invention may further comprise a signal sequence which regulates the distribution of the payload of interest, a cleavage and/or processing feature which facilitate cleavage of the payload from the effector module construct, a targeting and/or penetrating signal which can regulate the cellular localization of the effector module, a tag, and/or one or more linker sequences which link different components of the effector module.

Signal sequences

[00270] In addition to the SRE (e.g., DD) and payload region, effector modules of the invention may further comprise one or more signal sequences. Signal sequences (sometimes referred to as signal peptides, targeting signals, target peptides, localization sequences, transit peptides, leader sequences or leader peptides) direct proteins (e.g., the effector module of the present invention)

to their designated cellular and/or extracellular locations. Protein signal sequences play a central role in the targeting and translocation of nearly all secreted proteins and many integral membrane proteins.

[00271] A signal sequence is a short (5-30 amino acids long) peptide present at the N-terminus of the majority of newly synthesized proteins that are destined towards a particular location. Signal sequences can be recognized by signal recognition particles (SRPs) and cleaved using type I and type II signal peptide peptidases. Signal sequences derived from human proteins can be incorporated as a regulatory module of the effector module to direct the effector module to a particular cellular and/or extracellular location. These signal sequences are experimentally verified and can be cleaved (Zhang et al., *Protein Sci.* 2004, 13:2819-2824).

[00272] In some embodiments, a signal sequence may be, although not necessarily, located at the N-terminus or C-terminus of the effector module, and may be, although not necessarily, cleaved off the desired effector module to yield a “mature” payload, i.e., an immunotherapeutic agent as discussed herein.

[00273] In some examples, a signal sequence may be a secreted signal sequence derived from a naturally secreted protein, and its variant thereof. In some instances, the secreted signal sequences may be cytokine signal sequences such as, but not limited to, IL2 signal sequence comprising amino acid of SEQ ID NO: 783, encoded by the nucleotide of SEQ ID NO: 788-791 and/or p40 signal sequence comprising the amino acid sequence of SEQ ID NO: 719, encoded by the nucleotide of SEQ ID NO: 736-744.

[00274] In some instances, signal sequences directing the payload of interest to the surface membrane of the target cell may be used. Expression of the payload on the surface of the target cell may be useful to limit the diffusion of the payload to non-target *in vivo* environments, thereby potentially improving the safety profile of the payloads. Additionally, the membrane presentation of the payload may allow for physiologically and qualitative signaling as well as stabilization and recycling of the payload for a longer half-life. Membrane sequences may be the endogenous signal sequence of the N terminal component of the payload of interest. Optionally, it may be desirable to exchange this sequence for a different signal sequence. Signal sequences may be selected based on their compatibility with the secretory pathway of the cell type of interest so that the payload is presented on the surface of the T cell. In some embodiments, the signal sequence may be IgE signal sequence comprising amino acid SEQ ID NO: 801 and nucleotide sequence of SEQ ID NO: 810, 930, or 931, CD8a signal sequence (also referred to as CD8a leader) comprising amino acid SEQ ID NO: 628 and nucleotide sequence of SEQ ID NO:

671-675, or IL15Ra signal sequence (also referred to as IL15Ra leader) comprising amino acid SEQ ID NO: 932 and nucleotide sequence of SEQ ID NO: 933.

[00275] Other examples of signal sequences include, a variant may be a modified signal sequence discussed in U.S. Pat. NOs.: 8, 148, 494; 8,258,102; 9,133,265; 9,279,007; and U.S. patent application publication NO.: 20070141666; and International patent application publication NO.: WO1993018181; the contents of each of which are incorporated herein by reference in their entirety.

[00276] In other examples, a signal sequence may be a heterogeneous signal sequence from other organisms such as virus, yeast and bacteria, which can direct an effector module to a particular cellular site, such as a nucleus (e.g., EP 1209450). Other examples may include Aspartic Protease (NSP24) signal sequences from *Trichoderma* that can increase secretion of fused protein such as enzymes (e.g., U. S. Pat. NO.: 8,093,016 to Cervin and Kim), bacterial lipoprotein signal sequences (e.g., PCT application publication NO.: WO199109952 to Lau and Rioux), *E.coli* enterotoxin II signal peptides (e.g., U.S. Pat. NO.: 6,605,697 to Kwon et al.), *E.coli* secretion signal sequence (e.g., U.S. patent publication NO.: US2016090404 to Malley et al.), a lipase signal sequence from a methylotrophic yeast (e.g., U.S. Pat. NO.: 8,975,041), and signal peptides for DNases derived from *Coryneform bacteria* (e.g., U.S. Pat. NO.: 4,965,197); the contents of each of which are incorporated herein by reference in their entirety.

[00277] Signal sequences may also include nuclear localization signals (NLSs), nuclear export signals (NESs), polarized cell tubulo-vesicular structure localization signals (See, e.g., U.S. Pat. NO.: 8, 993,742; Cour et al., *Nucleic Acids Res.* 2003, 31(1): 393-396; the contents of each of which are incorporated herein by reference in their entirety), extracellular localization signals, signals to subcellular locations (e.g. lysosome, endoplasmic reticulum, golgi, mitochondria, plasma membrane and peroxisomes, etc.) (See, e.g., U.S. Pat. NO.: 7,396,811; and Negi et al., *Database*, 2015, 1-7; the contents of each of which are incorporated herein by reference in their entirety).

[00278] In some embodiments, signal sequences of the present invention, include without limitation, any of those taught in Table 6 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

Cleavage sites

[00279] In some embodiments, the effector module comprises a cleavage and/or processing feature. The effector module of the present invention may include at least one protein cleavage

signal/site. The protein cleavage signal/site may be located at the N-terminus, the C-terminus, at any space between the N- and the C- termini such as, but not limited to, half-way between the N- and C-termini, between the N-terminus and the half-way point, between the half-way point and the C-terminus, and combinations thereof.

[00280] The effector module may include one or more cleavage signal(s)/site(s) of any proteinases. The proteinases may be a serine proteinase, a cysteine proteinase, an endopeptidase, a dipeptidase, a metalloproteinase, a glutamic proteinase, a threonine proteinase and an aspartic proteinase. In some aspects, the cleavage site may be a signal sequence of furin, actinidain, calpain-1, carboxypeptidase A, carboxypeptidase P, carboxypeptidase Y, caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, cathepsin B, cathepsin C, cathepsin G, cathepsin H, cathepsin K, cathepsin L, cathepsin S, cathepsin V, clostripain, chymase, chymotrypsin, elastase, endoproteinase, enterokinase, factor Xa, formic acid, granzyme B, Matrix metalloproteinase-2, Matrix metalloproteinase-3, pepsin, proteinase K, SUMO protease, subtilisin, TEV protease, thermolysin, thrombin, trypsin and TAGZyme.

[00281] In one embodiment, the cleavage site is a furin cleavage site comprising the amino acid sequence SARNRQKRS (SEQ ID NO: 721), encoded by nucleotide sequence of SEQ ID NO: 750; or a revised furin cleavage site comprising the amino acid sequence ARNRQKRS (SEQ ID NO: 722), encoded by nucleotide sequence of SEQ ID NO: 751; or a modified furin site comprising the amino acid sequence ESRRVRRNKRSK (SEQ ID NO: 630), encoded by nucleotide sequence of SEQ ID NO: 681-683.

[00282] In some embodiments, cleavage sites of the present invention, include without limitation, any of those taught in Table 7 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

Protein tags

[00283] In some embodiments, the effector module of the invention may comprise a protein tag. The protein tag may be used for detecting and monitoring the process of the effector module. The effector module may include one or more tags such as an epitope tag (e.g., a FLAG or hemagglutinin (HA) tag). A large number of protein tags may be used for the present effector modules. They include, but are not limited to, self-labeling polypeptide tags (e.g., haloalkane dehalogenase (halotag2 or halotag7), ACP tag, clip tag, MCP tag, snap tag), epitope tags (e.g., FLAG, HA, His, and Myc), fluorescent tags (e.g., green fluorescent protein (GFP), red

fluorescent protein (RFP), yellow fluorescent protein (YFP), and its variants), bioluminescent tags (e.g. luciferase and its variants), affinity tags (e.g., maltose-binding protein (MBP) tag, glutathione-S-transferase (GST) tag), immunogenic affinity tags (e.g., protein A/G, IRS, AU1, AU5, glu-glu, KT3, S-tag, HSV, VSV-G, Xpress and V5), and other tags (e.g., biotin (small molecule), StrepTag (StrepII), SBP, biotin carboxyl carrier protein (BCCP), eXact, CBP, CYD, HPC, CBD intein-chitin binding domain, Trx, NorpA, and NusA.

[00284] In other embodiments, a tag may also be selected from those disclosed in U.S. Pat. NOs.: 8,999,897; 8,357,511; 7,094, 568; 5,011,912; 4,851,341; and 4,703,004; U.S. patent application publication NOs.: US2013115635 and US2013012687; and International application publication NO.: WO2013091661; the contents of each of which are incorporated herein by reference in their entirety.

[00285] In some aspects, a multiplicity of protein tags, either the same or different tags, may be used; each of the tags may be located at the same N- or C-terminus, whereas in other cases these tags may be located at each terminus.

[00286] In some embodiments, protein tags of the present invention, include without limitation, any of those taught in Table 8 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

Targeting peptides

[00287] In some embodiments, the effector module of the invention may further comprise a targeting and/or penetrating peptide. Small targeting and/or penetrating peptides that selectively recognize cell surface markers (e.g. receptors, trans-membrane proteins, and extra-cellular matrix molecules) can be employed to target the effector module to the desired organs, tissues or cells. Short peptides (5-50 amino acid residues) synthesized *in vitro* and naturally occurring peptides, or analogs, variants, derivatives thereof, may be incorporated into the effector module for homing the effector module to the desired organs, tissues and cells, and/or subcellular locations inside the cells.

[00288] In some embodiments, a targeting sequence and/or penetrating peptide may be included in the effector module to drive the effector module to a target organ, or a tissue, or a cell (e.g., a cancer cell). In other embodiments, a targeting and/or penetrating peptide may direct the effector module to a specific subcellular location inside a cell.

[00289] A targeting peptide has any number of amino acids from about 6 to about 30 inclusive. The peptide may have 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26,

27, 28, 29 or 30 amino acids. Generally, a targeting peptide may have 25 or fewer amino acids, for example, 20 or fewer, for example 15 or fewer.

[00290] Exemplary targeting peptides may include, but are not limited to, those disclosed in the art, e.g., U.S. Pat. NOs.: 9,206,231; 9,110,059; 8,706,219; and 8,772,449; and U.S. application publication NOs.: 2016089447; 2016060296; 2016060314; 2016060312; 2016060311; 2016009772; 2016002613; 2015314011 and 2015166621; and International application publication NOs.: WO2015179691 and WO2015183044; the contents of each of which are incorporated herein by reference in their entirety.

[00291] In some embodiments, targeting peptides of the present invention, include without limitation, any of those taught in Table 9 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

Linkers

[00292] In some embodiments, the effector module of the invention may further comprise a linker sequence. The linker region serves primarily as a spacer between two or more polypeptides within the effector module. The "linker" or "spacer", as used herein, refers to a molecule or group of molecules that connects two molecules, or two parts of a molecule such as two domains of a recombinant protein.

[00293] In some embodiments, "Linker" (L) or "linker domain" or "linker region" or "linker module" or "peptide linker" as used herein refers to an oligo- or polypeptide region of from about 1 to 100 amino acids in length, which links together any of the domains/regions of the effector module (also called peptide linker). The peptide linker may be 1-40 amino acids in length, or 2-30 amino acids in length, or 20-80 amino acids in length, or 50-100 amino acids in length. Linker length may also be optimized depending on the type of payload utilized and based on the crystal structure of the payload. In some instances, a shorter linker length may be preferably selected. In some aspects, the peptide linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I), Serine (S), Cysteine (C), Threonine (T), Methionine (M), Proline (P), Phenylalanine (F), Tyrosine (Y), Tryptophan (W), Histidine (H), Lysine (K), Arginine (R), Aspartate (D), Glutamic acid (E), Asparagine (N), and Glutamine (Q). One or more of these amino acids may be glycosylated, as is understood by those in the art. In some

[00295] In other examples, a peptide linker may be made up of a majority of amino acids that are sterically unhindered, such as Glycine (G) and Alanine (A). Exemplary linkers are polyglycines (such as (G)₄ (SEQ ID NO: 1233), (G)₅ (SEQ ID NO: 831), (G)₈ (SEQ ID NO: 1234), poly(GA), and polyalanines. The linkers described herein are exemplary, and linkers that are much longer and which include other residues are contemplated by the present invention.

[00296] A linker sequence may be a natural linker derived from a multi-domain protein. A natural linker is a short peptide sequence that separates two different domains or motifs within a protein.

[00297] In some aspects, linkers may be flexible or rigid. In other aspects, linkers may be cleavable or non-cleavable. As used herein, the terms “cleavable linker domain or region” or “cleavable peptide linker” are used interchangeably. In some embodiments, the linker sequence may be cleaved enzymatically and/or chemically. Examples of enzymes (e.g., proteinase/peptidase) useful for cleaving the peptide linker include, but are not limited, to Arg-C proteinase, Asp-N endopeptidase, chymotrypsin, clostripain, enterokinase, Factor Xa, glutamyl endopeptidase, Granzyme B, *Achromobacter* proteinase I, pepsin, proline endopeptidase, proteinase K, *Staphylococcal* peptidase I, thermolysin, thrombin, trypsin, and members of the Caspase family of proteolytic enzymes (e.g. Caspases 1-10). Chemical sensitive cleavage sites may also be included in a linker sequence. Examples of chemical cleavage reagents include, but are not limited to, cyanogen bromide, which cleaves methionine residues; N-chloro succinimide, iodobenzoic acid or BNPS-skatole (2-(2-nitrophenylsulfenyl)-3-methylindole), which cleaves tryptophan residues; dilute acids, which cleave at aspartyl-prolyl bonds; and e aspartic acid-proline acid cleavable recognition sites (i.e., a cleavable peptide linker comprising one or more D-P dipeptide moieties). The fusion module may include multiple regions encoding peptides of interest separated by one or more cleavable peptide linkers.

[00298] In other embodiments, a cleavable linker may be a “self-cleaving” linker peptide, such as 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), *Thosea asigna* virus (T2A) or combinations, variants and functional equivalents thereof. Other linkers will be apparent to those skilled in the art and may be used in connection with alternate embodiments of the invention. In some embodiments, the biocircuits of the present invention may include 2A peptides. The 2A peptide is a sequence of about 20 amino acid residues from a virus that is recognized by a protease (2A peptidases) endogenous to the cell. The 2A peptide was identified among picornaviruses, a typical example of which is the Foot-and Mouth disease virus (Robertson BH, et. al., *J Virol* 1985, 54:651-660). 2A-like sequences have also been found in Picornaviridae like equine rhinitis A virus, as well as unrelated viruses such as porcine teschovirus-1 and the insect *Thosea asigna* virus (TaV). In such viruses, multiple proteins are derived from a large polyprotein encoded by an open reading frame. The 2A peptide mediates the co-translational cleavage of this polyprotein at a single site that forms the junction between the virus capsid and replication polyprotein

domains. The 2A sequences contain the consensus motif D-V/I-E-X-N-P-G-P (SEQ ID NO: 1235). These sequences are thought to act co-translationally, preventing the formation of a normal peptide bond between the glycine and last proline, resulting in the ribosome skipping of the next codon (Donnelly ML et al. (2001). *J Gen Virol*, 82:1013-1025). After cleavage, the short peptide remains fused to the C -terminus of the protein upstream of the cleavage site, while the proline is added to the N-terminus of the protein downstream of the cleavage site. Of the 2A peptides identified to date, four have been widely used namely FMDV 2A (abbreviated herein as F2A); equine rhinitis A virus (ERAV) 2A (E2A); porcine teschovirus-1 2A (P2A) and *Thoseaasigna* virus 2A (T2A). In some embodiments, the 2A peptide sequences useful in the present invention are selected from SEQ ID NO.8-11 of International Patent Publication WO2010042490, the contents of which are incorporated by reference in its entirety.

[00299] As a non-limiting example, the P2A cleavable peptide may be GATNFSLLKQAGDVEENPGP (SEQ ID NO: 925; encoded by SEQ ID NO: 926).

[00300] The linkers of the present invention may also be non-peptide linkers. For example, alkyl linkers such as —NH—(CH₂)_a—C(O)—, wherein a=2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C₁-C₆) lower acyl, halogen (e.g., Cl, Br), CN, NH₂, phenyl, etc.

[00301] In some aspects, the linker may be an artificial linker from U.S. Pat. NOs.: 4,946,778; 5, 525, 491; 5,856,456; and International patent publication NOs.: WO2012/083424; the contents of each of which are incorporated herein by reference in their entirety.

[00302] In some embodiments, linkers of the present invention, include without limitation, any of those taught in Table 11 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

[00303] In one embodiment, the linker may be a spacer region of one or more nucleotides. Non-limiting examples of spacers are TCTAGATAATACGACTCACTAGAGATCC (SEQ ID NO: 927), TATGGCCACAACCATG (SEQ ID NO: 928), AATCTAGATAATACGACTCACTAGAGATCC (SEQ ID NO: 929), GCTTGCCACAACCCACAAGGAGACGACCTTCC (SEQ ID NO: 1000), TCGCGAATG, or TCGCGA.

[00304] In one embodiment, the linker may be a BamHI site. As a non-limiting example, the BamHI site has the amino acid sequence GS and/or the DNA sequence GGATCC.

Embedded stimulus, signals and other regulatory features

[00305] In some embodiments, the effector module of the present invention may further comprise one or more microRNAs, microRNA binding sites, promoters and tunable elements. In one embodiment, microRNA may be used in support of the creation of tunable biocircuits. Each aspect or tuned modality may bring to the effector module or biocircuit a differentially tuned feature. For example, a destabilizing domain may alter cleavage sites or dimerization properties or half-life of the payload, and the inclusion of one or more microRNA or microRNA binding site may impart cellular detargeting or trafficking features. Consequently, the present invention embraces biocircuits which are multifactorial in their tenability. Such biocircuits and effector modules may be engineered to contain one, two, three, four or more tuned features.

[00306] In some embodiments, micro RNA sequences of the present invention, include without limitation, any of those taught in Table 13 of copending commonly owned U.S. Provisional Patent Application No. 62/320,864 filed on 4/11/2016, or in US Provisional Application No. 62/466,596 filed March 3, 2017 and the International Publication WO2017/180587, the contents of each of which are incorporated herein by reference in their entirety.

[00307] In some embodiments, compositions of the invention may include optional proteasome adaptors. As used herein, the term "proteasome adaptor" refers to any nucleotide/ amino acid sequence that targets the appended payload for degradation. In some aspects, the adaptors target the payload for degradation directly thereby circumventing the need for ubiquitination reactions. Proteasome adaptors may be used in conjunction with destabilizing domains to reduce the basal expression of the payload. Exemplary proteasome adaptors include the UbL domain of Rad23 or hHR23b, HPV E7 which binds to both the target protein Rb and the S4 subunit of the proteasome with high affinity, which allows direct proteasome targeting, bypassing the ubiquitination machinery; the protein gankyrin which binds to Rb and the proteasome subunit S6.

Polynucleotides

[00308] The term "polynucleotide" or "nucleic acid molecule" in its broadest sense, includes any compound and/or substance that comprise a polymer of nucleotides, e.g., linked nucleosides. These polymers are often referred to as polynucleotides. Exemplary nucleic acids or polynucleotides of the invention include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β -D-ribo configuration, α -LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization) or hybrids thereof.

[00309] In some embodiments, polynucleotides of the invention may be a messenger RNA (mRNA) or any nucleic acid molecule and may or may not be chemically modified. In one aspect, the nucleic acid molecule is a mRNA. As used herein, the term “messenger RNA (mRNA)” refers to any polynucleotide which encodes a polypeptide of interest and which is capable of being translated to produce the encoded polypeptide of interest *in vitro*, *in vivo*, *in situ* or *ex vivo*.

[00310] Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5'UTR, a 3'UTR, a 5' cap and a poly-A tail. Building on this wild type modular structure, the present invention expands the scope of functionality of traditional mRNA molecules by providing payload constructs which maintain a modular organization, but which comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the polynucleotide, for example tenability of function. As used herein, a “structural” feature or modification is one in which two or more linked nucleosides are inserted, deleted, duplicated, inverted or randomized in a polynucleotide without significant chemical modification to the nucleosides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. For example, the polynucleotide “ATCG” may be chemically modified to “AT-5meC-G”. The same polynucleotide may be structurally modified from “ATCG” to “ATCCCG”. Here, the dinucleotide “CC” has been inserted, resulting in a structural modification to the polynucleotide.

[00311] In some embodiments, polynucleotides of the present invention may harbor 5'UTR sequences which play a role in translation initiation. 5'UTR sequences may include features such as Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of genes, Kozak sequences have the consensus XCCR(A/G)CCAUG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG) and X is any nucleotide. In one embodiment, the Kozak sequence is ACCGCC. By engineering the features that are typically found in abundantly expressed genes of target cells or tissues, the stability and protein production of the polynucleotides of the invention can be enhanced.

[00312] Further provided are polynucleotides, which may contain an internal ribosome entry site (IRES) which play an important role in initiating protein synthesis in the absence of 5' cap structure in the polynucleotide. An IRES may act as the sole ribosome binding site, or may serve as one of the multiple binding sites. Polynucleotides of the invention containing more than one

functional ribosome binding site may encode several peptides or polypeptides that are translated independently by the ribosomes giving rise to bicistronic and/or multicistronic nucleic acid molecules.

[00313] In some embodiments, polynucleotides encoding biocircuits, effector modules, SREs and payloads of interest such as immunotherapeutic agents may include from about 30 to about 100,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 1,000, from 30 to 1,500, from 30 to 3,000, from 30 to 5,000, from 30 to 7,000, from 30 to 10,000, from 30 to 25,000, from 30 to 50,000, from 30 to 70,000, from 100 to 250, from 100 to 500, from 100 to 1,000, from 100 to 1,500, from 100 to 3,000, from 100 to 5,000, from 100 to 7,000, from 100 to 10,000, from 100 to 25,000, from 100 to 50,000, from 100 to 70,000, from 100 to 100,000, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 3,000, from 500 to 5,000, from 500 to 7,000, from 500 to 10,000, from 500 to 25,000, from 500 to 50,000, from 500 to 70,000, from 500 to 100,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 3,000, from 1,000 to 5,000, from 1,000 to 7,000, from 1,000 to 10,000, from 1,000 to 25,000, from 1,000 to 50,000, from 1,000 to 70,000, from 1,000 to 100,000, from 1,500 to 3,000, from 1,500 to 5,000, from 1,500 to 7,000, from 1,500 to 10,000, from 1,500 to 25,000, from 1,500 to 50,000, from 1,500 to 70,000, from 1,500 to 100,000, from 2,000 to 3,000, from 2,000 to 5,000, from 2,000 to 7,000, from 2,000 to 10,000, from 2,000 to 25,000, from 2,000 to 50,000, from 2,000 to 70,000, and from 2,000 to 100,000 nucleotides). In some aspects, polynucleotides of the invention may include more than 10,000 nucleotides.

[00314] Regions of the polynucleotides which encode certain features such as cleavage sites, linkers, trafficking signals, tags or other features may range independently from 10-1,000 nucleotides in length (e.g., greater than 20, 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 nucleotides or at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1,000 nucleotides).

[00315] In some embodiments, polynucleotides of the present invention may further comprise embedded regulatory moieties such as microRNA binding sites within the 3'UTR of nucleic acid molecules which when bind to microRNA molecules, down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. Conversely, for the purposes of the polynucleotides of the present invention, microRNA binding sites can be engineered out of (i.e. removed from) sequences in which they naturally occur in order to increase protein expression in specific tissues. For example, miR-142 and miR-146 binding sites may be removed to improve protein expression in the immune cells. In some embodiments, any of the encoded

payloads may be regulated by an SRE and then combined with one or more regulatory sequences to generate a dual or multi-tuned effector module or biocircuit system.

[00316] In some embodiments, polynucleotides of the present invention may encode fragments, variants, derivatives of polypeptides of the inventions. In some aspects, the variant sequence may keep the same or a similar activity. Alternatively, the variant may have an altered activity (e.g., increased or decreased) relative to the start sequence. Generally, variants of a particular polynucleotide or polypeptide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res.*, 1997, 25:3389-3402.)

[00317] In some embodiments, polynucleotides of the present invention may be modified. As used herein, the terms “modified”, or as appropriate, “modification” refers to chemical modification with respect to A, G, U (T in DNA) or C nucleotides. Modifications may be on the nucleoside base and/or sugar portion of the nucleosides which comprise the polynucleotide. In some embodiments, multiple modifications are included in the modified nucleic acid or in one or more individual nucleoside or nucleotide. For example, modifications to a nucleoside may include one or more modifications to the nucleobase and the sugar. Modifications to the polynucleotides of the present invention may include any of those taught in, for example, International Publication NO: WO2013052523, the contents of which are incorporated herein by reference in its entirety.

[00318] As described herein “nucleoside” is defined as a compound containing a sugar molecule (e.g., a pentose or ribose) or a derivative thereof in combination with an organic base (e.g., a purine or pyrimidine) or a derivative thereof (also referred to herein as “nucleobase”). As described herein, “nucleotide” is defined as a nucleoside including a phosphate group.

[00319] In some embodiments, the modification may be on the internucleoside linkage (e.g., phosphate backbone). Herein, in the context of the polynucleotide backbone, the phrases “phosphate” and “phosphodiester” are used interchangeably. Backbone phosphate groups can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the wholesale replacement of an unmodified phosphate moiety with another internucleoside linkage. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates,

boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates). Other modifications which may be used are taught in, for example, International Application NO: WO2013052523, the contents of which are incorporated herein by reference in their entirety.

[00320] Chemical modifications and/or substitution of the nucleotides or nucleobases of the polynucleotides of the invention which are useful in the present invention include any modified substitutes known in the art, for example, ($\pm$)-1-(2-Hydroxypropyl)pseudouridine TP, (2R)-1-(2-Hydroxypropyl)pseudouridine TP, 1-(4-Methoxy-phenyl)pseudo-UTP, 2'-O-dimethyladenosine, 1,2'-O-dimethylguanosine, 1,2'-O-dimethylinosine, 1-Hexyl-pseudo-UTP, 1-Homoallylpseudouridine TP, 1-Hydroxymethylpseudouridine TP, 1-iso-propyl-pseudo-UTP, 1-Me-2-thio-pseudo-UTP, 1-Me-4-thio-pseudo-UTP, 1-Me-alpha-thio-pseudo-UTP, 1-Me-GTP, 2'-Amino-2'-deoxy-ATP, 2'-Amino-2'-deoxy-CTP, 2'-Amino-2'-deoxy-GTP, 2'-Amino-2'-deoxy-UTP, 2'-Azido-2'-deoxy-ATP, tubercidine, under modified hydroxywybutosine, uridine 5-oxyacetic acid, uridine 5-oxyacetic acid methyl ester, wybutosine, wyosine, xanthine, Xanthosine-5'-TP, xylo-adenosine, zebularine, α -thio-adenosine, α -thio-cytidine, α -thio-guanosine, and/or α -thio-uridine.

[00321] Polynucleotides of the present invention may comprise one or more of the modifications taught herein. Different sugar modifications, base modifications, nucleotide modifications, and/or internucleoside linkages (e.g., backbone structures) may exist at various positions in the polynucleotide of the invention. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a polynucleotide such that the function of the polynucleotide is not substantially decreased. A modification may also be a 5' or 3' terminal modification. The polynucleotide may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, i.e. any one or more of A, G, U or C) or any intervening percentage (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%,

from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%).

[00322] In some embodiments, one or more codons of the polynucleotides of the present invention may be replaced with other codons encoding the native amino acid sequence to tune the expression of the SREs, through a process referred to as codon selection. Since mRNA codon, and tRNA anticodon pools tend to vary among organisms, cell types, sub cellular locations and over time, the codon selection described herein is a spatiotemporal (ST) codon selection.

[00323] In some embodiments of the invention, certain polynucleotide features may be codon optimized. Codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cell by replacing at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 50 or more codons of the native sequence with codons that are most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Codon usage may be measured using the Codon Adaptation Index (CAI) which measures the deviation of a coding polynucleotide sequence from a reference gene set. Codon usage tables are available at the Codon Usage Database (<http://www.kazusa.or.jp/codon/>) and the CAI can be calculated by EMBOSS CAI program (<http://emboss.sourceforge.net/>). Codon optimization methods are known in the art and may be useful in efforts to achieve one or more of several goals. These goals include to match codon frequencies in target and host organisms to ensure proper folding, bias nucleotide content to alter stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove protein signaling sequences, remove/add post translation modification sites in encoded protein (e.g. glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and degradation sites, to adjust translational rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art, and non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA), OptimumGene (GenScript, Piscataway, NJ), algorithms such as but not limited to, DNAWorks v3.2.3 and/or proprietary methods. In one embodiment, a polynucleotide sequence or portion thereof is codon optimized using optimization algorithms. Codon options for each amino acid are well-known in the art as are various species table for optimizing for expression in that particular species.

[00324] In some embodiments of the invention, certain polynucleotide features may be codon optimized. For example, a preferred region for codon optimization may be upstream (5') or downstream (3') to a region which encodes a polypeptide. These regions may be incorporated into the polynucleotide before and/or after codon optimization of the payload encoding region or open reading frame (ORF).

[00325] After optimization (if desired), the polynucleotide components are reconstituted and transformed into a vector such as, but not limited to, plasmids, viruses, cosmids, and artificial chromosomes.

[00326] Spatiotemporal codon selection may impact the expression of the polynucleotides of the invention, since codon composition determines the rate of translation of the mRNA species and its stability. For example, tRNA anticodons to optimized codons are abundant, and thus translation may be enhanced. In contrast, tRNA anticodons to less common codons are fewer and thus translation may proceed at a slower rate. Presnyak et al. have shown that the stability of an mRNA species is dependent on the codon content, and higher stability and thus higher protein expression may be achieved by utilizing optimized codons (Presnyak et al. (2015) Cell 160, 1111-1124; the contents of which are incorporated herein by reference in their entirety). Thus, in some embodiments, ST codon selection may include the selection of optimized codons to enhance the expression of the SRES, effector modules and biocircuits of the invention. In other embodiments, spatiotemporal codon selection may involve the selection of codons that are less commonly used in the genes of the host cell to decrease the expression of the compositions of the invention. The ratio of optimized codons to codons less commonly used in the genes of the host cell may also be varied to tune expression.

[00327] In some embodiments, certain regions of the polynucleotide may be preferred for codon selection. For example, a preferred region for codon selection may be upstream (5') or downstream (3') to a region which encodes a polypeptide. These regions may be incorporated into the polynucleotide before and/or after codon selection of the payload encoding region or open reading frame (ORF).

[00328] The stop codon of the polynucleotides of the present invention may be modified to include sequences and motifs to alter the expression levels of the SREs, payloads and effector modules of the present invention. Such sequences may be incorporated to induce stop codon readthrough, wherein the stop codon may specify amino acids e.g. selenocysteine or pyrrolysine. In other instances, stop codons may be skipped altogether to resume translation through an alternate open reading frame. Stop codon read through may be utilized to tune the expression of components of the effector modules at a specific ratio (e.g. as dictated by the stop codon context).

Examples of preferred stop codon motifs include UGAN, UAAN, and UAGN, where N is either C or U. Polynucleotide modifications and manipulations can be accomplished by methods known in the art such as, but not limited to, site directed mutagenesis and recombinant technology. The resulting modified molecules may then be tested for activity using *in vitro* or *in vivo* assays such as those described herein or any other suitable screening assay known in the art.

[00329] In some embodiments, polynucleotides of the invention may comprise two or more effector module sequences, or two or more payloads of interest sequences, which are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different effector module component.

[00330] In yet another embodiment, polynucleotides of the invention may comprise two or more effector module component sequences with each component having one or more SRE sequences (DD sequences), or two or more payload sequences. As a non-limiting example, the sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times in each of the regions. As another non-limiting example, the sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times across the entire polynucleotide. In these patterns, each letter, A, B, or C represent a different sequence or component.

[00331] According to the present invention, polynucleotides encoding distinct biocircuits, effector modules, SREs and payload constructs may be linked together through the 3'-end using nucleotides which are modified at the 3'-terminus. Chemical conjugation may be used to control the stoichiometry of delivery into cells. Polynucleotides can be designed to be conjugated to other polynucleotides, dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g. EDTA), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, (MPEG)₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g. biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases, proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell, hormones and hormone receptors, non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, or a drug. As non-limiting examples, they may be conjugates with other immune conjugates.

[00332] In some embodiments, the compositions of the polynucleotides of the invention may be generated by combining the various components of the effector modules using the Gibson assembly method. The Gibson assembly reaction consists of three isothermal reactions, each relying on a different enzymatic activity including a 5' exonuclease which generates long overhangs, a polymerase which fills in the gaps of the annealed single strand regions and a DNA ligase which seals the nicks of the annealed and filled-in gaps. Polymerase chain reactions are performed prior to Gibson assembly which may be used to generate PCR products with overlapping sequence. These methods can be repeated sequentially, to assemble larger and larger molecules. For example, the method can comprise repeating a method as above to join a second set of two or more DNA molecules of interest to one another, and then repeating the method again to join the first and second set DNA molecules of interest, and so on. At any stage during these multiple rounds of assembly, the assembled DNA can be amplified by transforming it into a suitable microorganism, or it can be amplified in vitro (e.g., with PCR).

[00333] In some embodiments, polynucleotides of the present invention may encode a fusion polypeptide comprising a destabilizing domain (DD) and at least one immunotherapeutic agent taught herein. The DD domain may be a FKBP mutant encoded by nucleotide sequence of SEQ ID NO: 684-686, 688-691, 987-989, 994, 1013, and/or 1028, an ecDHFR mutant encoded by nucleotide sequence of SEQ ID NO: 687, 692, 772, 798, 814-815, 988, 991, and/or 993, hDHFR mutant encoded by nucleotide sequence of SEQ ID NO: 693-700, 773, 852-857 and/or 934-980, and/or 995-998.

[00334] In some embodiments, the polynucleotides of the invention may encode effector modules comprising the CD19 CAR as the payload comprising the nucleotide sequence of SEQ ID NO: 701-715 and/or 1019-1042, or IL12 as the payload comprising the nucleotide sequence of SEQ ID NO: 774-782, or IL15 as the payload comprising the nucleotide sequence of SEQ ID NO: 749, 799-800, and/or 1055-1056, or IL15/IL15Ra fusion polypeptide as the payload comprising the nucleotide sequence of SEQ ID NO: 816-821, 1086-1089, 1091-1095, 1098-1111, 1120, and/or 1123.

Cells

[00335] In accordance with the present invention, cells genetically modified to express at least one biocircuit, SRE (e.g., DD), effector module and immunotherapeutic agent of the invention, are provided. Cells of the invention may include, without limitation, immune cells, stem cells and tumor cells. In some embodiments, immune cells are immune effector cells, including, but not limiting to, T cells such as CD8⁺ T cells and CD4⁺ T cells (e.g., Th1, Th2, Th17, Foxp3⁺ cells), memory T cells such as T memory stem cells, central T memory cells, and effector

memory T cells, terminally differentiated effector T cells, natural killer (NK) cells, NK T cells, tumor infiltrating lymphocytes (TILs), cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), and dendritic cells (DCs), other immune cells that can elicit an effector function, or the mixture thereof. T cells may be $T\alpha\beta$ cells and $T\gamma\delta$ cells. In some embodiments, stem cells may be from human embryonic stem cells, mesenchymal stem cells, and neural stem cells. In some embodiments, T cells may be depleted endogenous T cell receptors (See US Pat. NOs.: 9, 273, 283; 9, 181, 527; and 9,028, 812; the contents of each of which are incorporated herein by reference in their entirety).

[00336] In some embodiments, cells of the invention may be autologous, allogeneic, syngeneic, or xenogeneic in relation to a particular individual subject.

[00337] In some embodiments, cells of the invention may be mammalian cells, particularly human cells. Cells of the invention may be primary cells or immortalized cell lines.

[00338] In some embodiments, cells of the invention may include expansion factors as payload to trigger proliferation and expansion of the cells. Exemplary payloads include RAS such as KRAS, NRAS, RRAS, RRAS2, MRAS, ERAS, and HRAS, DIRAS such as DIRAS1, DIRAS2, and DIRAS3, NKIRAS such as NKIRAS1, and NKIRAS2, RAL such as RALA, and RALB, RAP such as RAP1A, RAP1B, RAP2A, RAP2B, and RAP2C, RASD such as RASD1, and RASD2, RASL such as RASL10A, RASL10B, RASL11A, RASL11B, and RASL12, REM such as REM1, and REM2, GEM, RERG, RERGL, and RRAD.

[00339] Engineered immune cells can be accomplished by transducing a cell compositions with a polypeptide of a biocircuit, an effector module, a SRE and/or a payload of interest (i.e., immunotherapeutic agent), or a polynucleotide encoding said polypeptide, or a vector comprising said polynucleotide. The vector may be a viral vector such as a lentiviral vector, a gamma-retroviral vector, a recombinant AAV, an adenoviral vector and an oncolytic viral vector. In other aspects, non-viral vectors for example, nanoparticles and liposomes may also be used. In some embodiments, immune cells of the invention are genetically modified to express at least one immunotherapeutic agent of the invention which is tunable using a stimulus. In some examples, two, three or more immunotherapeutic agents constructed in the same biocircuit and effector module are introduced into a cell. In other examples, two, three, or more biocircuits, effector modules, each of which comprises an immunotherapeutic agent, may be introduced into a cell.

[00340] In some embodiments, immune cells of the invention may be T cells modified to express an antigen-specific T cell receptor (TCR), or an antigen specific chimeric antigen receptor (CAR) taught herein (known as CAR T cells). Accordingly, at least one polynucleotide

encoding a CAR system (or a TCR) described herein, or a vector comprising the polynucleotide is introduced into a T cell. The T cell expressing the CAR or TCR binds to a specific antigen via the extracellular targeting moiety of the CAR or TCR, thereby a signal via the intracellular signaling domain (s) is transmitted into the T cell, and as a result, the T cell is activated. The activated CAR T cell changes its behavior including release of a cytotoxic cytokine (e.g., a tumor necrosis factor, and lymphotoxin, etc.), improvement of a cell proliferation rate, change in a cell surface molecule, or the like. Such changes cause destruction of a target cell expressing the antigen recognized by the CAR or TCR. In addition, release of a cytokine or change in a cell surface molecule stimulates other immune cells, for example, a B cell, a dendritic cell, a NK cell, and a macrophage.

[00341] The CAR introduced into a T cell may be a first-generation CAR including only the intracellular signaling domain from TCR CD3zeta, or a second-generation CAR including the intracellular signaling domain from TCR CD3zeta and a costimulatory signaling domain, or a third-generation CAR including the intracellular signaling domain from TCR CD3zeta and two or more costimulatory signaling domains, or a split CAR system, or an on/off switch CAR system. In one example, the expression of the CAR or TCR is controlled by a destabilizing domain (DD) such as a hDHFR mutant, in the effector module of the invention. The presence or absence of hDHFR binding ligand such as TMP is used to tune the CAR or TCR expression in transduced T cells or NK cells.

[00342] In some embodiments, CAR T cells of the invention may be further modified to express another one, two, three or more immunotherapeutic agents. The immunotherapeutic agents may be another CAR or TCR specific to a different target molecule; a cytokine such as IL2, IL12, IL15 and IL18, or a cytokine receptor such as IL15Ra; a chimeric switch receptor that converts an inhibitory signal to a stimulatory signal; a homing receptor that guides adoptively transferred cells to a target site such as the tumor tissue; an agent that optimizes the metabolism of the immune cell; or a safety switch gene (e.g., a suicide gene) that kills activated T cells when a severe event is observed after adoptive cell transfer or when the transferred immune cells are no longer needed. These molecules may be included in the same effector module or in separate effector modules.

[00343] In one embodiment, the CAR T cell (including TCR T cell) of the invention may be an “armed” CAR T cell which is transformed with an effector module comprising a CAR and an effector module comprising a cytokine. The inducible or constitutively secrete active cytokines further armor CAR T cells to improve efficacy and persistence. In this context, such CAR T cell is also referred to as “armored CAR T cell”. The “armor” molecule may be selected based on the

tumor microenvironment and other elements of the innate and adaptive immune systems. In some embodiments, the molecule may be a stimulatory factor such as IL2, IL12, IL15, IL18, type I IFN, CD40L and 4-1BBL which have been shown to further enhance CAR T cell efficacy and persistence in the face of a hostile tumor microenvironment via different mechanisms (Yeku et al., *Biochem Soc Trans.*, 2016, 44(2): 412-418).

[00344] In some aspects, the armed CAR T cell of the invention is modified to express a CD19 CAR and IL12. Such T cells, after CAR mediated activation in the tumor, release inducible IL12 which augments T-cell activation and attracts and activates innate immune cells to eliminate CD19-negative cancer cells.

[00345] In one embodiment, T cells of the invention may be modified to express an effector module comprising a CAR and an effector module comprising a suicide gene.

[00346] In one embodiment, the CAR T cell (including TCR T cell) of the invention may be transformed with effector modules comprising a cytokine and a safety switch gene (e.g., suicide gene). The suicide gene may be an inducible caspase such as caspase 9 which induces apoptosis, when activated by an extracellular stimulus of a biocircuit system. Such induced apoptosis eliminates transferred cell as required to decrease the risk of direct toxicity and uncontrolled cell proliferation.

[00347] In some embodiments, immune cells of the invention may be NK cells modified to express an antigen-specific T cell receptor (TCR), or an antigen specific chimeric antigen receptor (CAR) taught herein.

[00348] Natural killer (NK) cells are members of the innate lymphoid cell family and characterized in humans by expression of the phenotypic marker CD56 (neural cell adhesion molecule) in the absence of CD3 (T-cell co-receptor). NK cells are potent effector cells of the innate immune system which mediate cytotoxic attack without the requirement of prior antigen priming, forming the first line of defense against diseases including cancer malignancies and viral infection.

[00349] Several pre-clinical and clinical trials have demonstrated that adoptive transfer of NK cells is a promising treatment approach against cancers such as acute myeloid leukemia (Ruggeri et al., *Science*, 2002, 295: 2097-2100; and Geller et al., *Immunotherapy*, 2011, 3: 1445-1459). Adoptive transfer of NK cells expressing CAR such as DAP12-Based Activating CAR revealed improved eradication of tumor cells (Topfer et al., *J Immunol.* 2015; 194:3201-3212). NK cell engineered to express a CS-1 specific CAR also displayed enhanced cytotoxicity and interferon- γ (IFN- γ) production in multiple myeloma (Chu et al., *Leukemia*, 2014, 28(4): 917-927).

[00350] NK cell activation is characterized by an array of receptors with activating and inhibitory functions. The important activation receptors on NK cells include CD94/NKG2C and NKG2D (the C-type lectin-like receptors), and the natural cytotoxicity receptors (NCR) NKp30, NKp44 and NKp46, which recognize ligands on tumor cells or virally infected cells. NK cell inhibition is essentially mediated by interactions of the polymorphic inhibitory killer cell immunoglobulin-like receptors (KIRs) with their cognate human–leukocyte–antigen (HLA) ligands via the alpha-1 helix of the HLA molecule. The balance between signals that are generated from activating receptors and inhibitory receptors mainly determines the immediate cytotoxic activation.

[00351] NK cells may be isolated from peripheral blood mononuclear cells (PBMCs), or derived from human embryonic stem (ES) cells and induced pluripotent stem cells (iPSCs). The primary NK cells isolated from PBMCs may be further expanded for adoptive immunotherapy. Strategies and protocols useful for the expansion of NK cells may include interleukin 2 (IL2) stimulation and the use of autologous feeder cells, or the use of genetically modified allogeneic feeder cells. In some aspects, NK cells can be selectively expanded with a combination of stimulating ligands including IL15, IL21, IL2, 41BBL, IL12, IL18, MICA, 2B4, LFA-1, and BCM1/SLAMF2 (e.g., US patent publication NO: US20150190471).

[00352] Immune cells expressing effector modules comprising a CAR and/or other immunotherapeutic agents can be used as cancer immunotherapy. The immunotherapy comprises the cells expressing a CAR and/or other immunotherapeutic agents as an active ingredient, and may further comprise a suitable excipient. Examples of the excipient may include the aforementioned pharmaceutically acceptable excipients, including various cell culture media, and isotonic sodium chloride.

[00353] In some embodiments, cells of the present invention may be dendritic cells that are genetically modified to express the compositions of the invention. Such cells may be used as cancer vaccines.

Methods of CD19 antibody development and characterization

[00354] In some embodiments, the present invention provides methods of producing CD19 antibodies, antibody fragments or variants. Such methods may include the steps of: (1) preparing a composition with CD19, (2) contacting a library of antibodies or antibody fragments or variable with the composition, and (3) identifying one or more CD19 antibodies. Also, provided herein are methods for identifying FMC63-distinct CD19 antibodies, antibody fragments or variable.

[00355] In some embodiments, the present invention provides methods of identifying CD19 scFvs. Such methods may involve screening phagemid libraries for CD19 scFvs. Phagemid libraries expressing recombinant scFvs associated with the surface of bacteria or bacteriophages are useful in the present inventions. Phagemid libraries may be generated by PCR implication of the polynucleotides encoding the heavy chain and the kappa light chain of the immunoglobulin IgM and infecting Cre recombinase positive bacteria with the vectors containing the PCR products at a high multiplicity of infection (MOI). The high MOI results in bacteria containing multiple phagemids, each of which encodes a different VH and VL genes, which can be recombined by the Cre recombinase. The resulting library that may be generated by recombination is approximately 10^8 unique scFvs. In some instances, libraries of CD19 scFvs formatted into chimeric antigen receptor constructs may be screened to identify CD19scFvs useful in the present invention.

[00356] In some embodiments, scFvs immunologically specific to CD19 may be identified using cells that ectopically express full length, a fragment or a portion of CD19. Cell lines with low endogenous CD19 expression may be selected for ectopic expression. In some embodiments, the CD19 may be a naturally occurring isoform of human CD19.

[00357] In some embodiments, fusion proteins comprising the extracellular domains of CD19 (i.e. exon 1- exon 4) fused to the Fc region of human IgG1 (CD19sIg) are utilized to identify CD19 specific scFvs. Such fusion proteins have been described by Oliveira et al (2013) Journal of Translational Medicine 11:23; the contents of which are incorporated herein by reference in their entirety.

[00358] Also, provided herein are methods to identify FMC63-distinct scFvs, which include scFvs that are immunologically specific to and bind to an epitope of the CD19 antigen that is different or unlike the epitope of CD19 antigen that is bound by FMC63. In some embodiments, FMC63-distinct scFvs are identified by screening the scFv library with a complex consisting of human CD19 bound to FMC63. The CD19 of Rhesus macaque (*Macaca mulatta*) herein referred to as Rhesus CD19, bears 88% homology to the human CD19. Despite this high degree of homology, the Rhesus CD19 is not recognized by FMC63, indicating that the FMC63 epitope is in the region of human CD19 that is non-homologous to Rhesus CD19. Thus, in some embodiments, Rhesus CD19 may be used to screen scFv libraries for FMC63-distinct scFvs. Mutations in the region of Rhesus CD19 that is non-homologous to the human CD19 have been previously utilized to identify residues of human CD19 that confer binding to FMC63 (Sommermeier et al. (2017) Leukemia Feb 16. doi: 10.1038/leu.2017.57). In some embodiments, the mutational analysis described by Sommermeier et al. may be utilized to

design human CD19 mutants that are unable to bind to FMC63. Such mutants may include human CD19 (H218R, A237D, M243V, E244D, P250T) and human CD19 (H218R, A237D) and may be utilized to screen scFv libraries for FMC63-distinct scFvs. Sotillo et al have identified a splice variant of human CD19 lacking exon 2 in cancer patients (Sotillo et al. (2015) Cancer Discov. 2015 Dec;5(12):1282-95). The splice variant lacking exon 2 is not recognized by FMC63 and may also be used to screen scFv libraries for FMC63-distinct scFvs.

[00359] CD19 IgG fusion molecules generated by fusing the Fc region of human IgG1 with the human CD19-complete extracellular domains, i.e., exons 1-4 (CD19sIgG1-4) or extracellular domains lacking exon 2, i.e., exons 1, 3 and 4 (CD19sIgG1,3,4) may also be utilized to screen scFv libraries for FMC63-distinct scFvs.

[00360] CD19 proteins, variants and mutants useful in the invention are provided in Table 14.

Table 14: CD19 proteins, variants and mutants

Description	Sequence	SEQ ID NO
Human CD19, Isoform 1 (NCBI Reference No. NP_001171569.1)	MPPRLLFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLK GTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIW LFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGE LFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVW AKDRPEIWEGEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARD MWVMETGLLLPRATAQDAGKYCHRGNTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQR ALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLP TPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSR SPPGVGPEEEEGEGYEEDSEEDSEFYENDSNLGQDQLSQ DGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVAR TMDFLSPHGSAWDPSREATSLAGSQSYEDMRGILYAAPQ LRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRM GTWSTR	858
Human CD19, Isoform 2 (NCBI Reference No. NP_001761.3)	MPPRLLFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLK GTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIW LFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGE LFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVW AKDRPEIWEGEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARD MWVMETGLLLPRATAQDAGKYCHRGNTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQR ALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLP TPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSR SPPGVGPEEEEGEGYEEDSEEDSEFYENDSNLGQDQLSQ DGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVAR TMDFLSPHGSAWDPSREATSLAGSQSYEDMRGILYAAPQLR SIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRMGT WSTR	859
Rhesus CD19 (Uniprot ID: F7F486)	MPPPCLLFFLLFLTPMEVRPQEPLVVKVEEGDNAVLQCLE GTSDGPTQQLVWCRDSPFEPFLNLSLGLPGMGIRMGPLGI WLLIFNVSNTGGFYLCQGPLPSEKAWQPGWTVSVEGSG ELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLNSSQLYV WAKDRPEMWEGEPCGPPRDSLNSQLSQDLTMAPGSTL WLSCGVPPDSVSRGPLSWTHVRPKGPKSLLSLELKDDRP	860

	DRDMWVVDTGLLLTRATAQDAGKYYCHRGNWTKSFYL EITARPALWHWLLRIGGWKVPAVTLTYLIFCLCSLVGILQ LQRALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNV LSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQVDGA VGSRSPPGAGPEEEEEGEGYEEDSEEGSEFYENDSNFGQD QLSQDGSYENPEDEPLGPEDEDSFSNAESYENEDEELTQ PVARTMDFLSPHGSAWDPSREATSLGSQSYEDMRGLLYA APQLRTIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGG RMGTWSAR	
Human CD19 (H218R, A237D, M243V, E244D, P250T)	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLK GTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIW LFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSSE LFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVW AKDRPEIWEGEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVRPKGPKSLLSLELKDDRPDRD MWVVDTGLLLTRATAQDAGKYYCHRGNTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQR ALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLP TPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSR SPPGVGPEEEEEGEGYEEDSEEDSEFYENDSNLGQDQLSQ DGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVAR TMDFLSPHGSAWDPSREATSLAGSQSYEDMRGILYAAPQ LRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRM GTWSTR	861
Human CD19 (H218R, A237D)	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLK GTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIW LFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSSE LFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVW AKDRPEIWEGEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVRPKGPKSLLSLELKDDRPDRD MWVMDTGLLLPRATAQDAGKYYCHRGNTMSFHLEITA RPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQR ALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLP TPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSR SPPGVGPEEEEEGEGYEEDSEEDSEFYENDSNLGQDQLSQ DGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVAR TMDFLSPHGSAWDPSREATSLAGSQSYEDMRGILYAAPQ LRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRM GTWSTR	862
Human CD19 (Delta exon 2)	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEGELFRWNVSDL GGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWE GEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARD MWVMDTGLLLPRATAQDAGKYYCHRGNTMSFHLEITARPVLWHWLL RTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKR MTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQ RWAAGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEE EGEGYEEDSEEDSEFYENDSNLGQDQLSQDGSYENPED EPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGS AWDPSREATSLGSQSYEDMRGILYAAPQLRSIRGQPGPNH EEDADSYENMDNPDGPDPAWGGGGGRMGTWSTR	863
Human CD19 (Exon 1- 4)	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLK GTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIW LFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSSE LFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVW AKDRPEIWEGEPPCLPPRDSLNSQLSQDLTMAPGSTLWLS CGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARD MWVMDTGLLLPRATAQDAGKYYCHRGNTMSFHLEITA RP	864

Human CD19 (Exon 1,3,4)	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEGELFRWNVSDL GGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWE GEPPCLPPRDSLNQSLSQDLTMAPGSTLWLSCGVPPDSVS RGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETGL LLPRATAQDAGKYYCHRGNLTMSFHLEITARP	865
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III. PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS

[00361] The present invention further provides pharmaceutical compositions comprising one or more biocircuits, effector modules, SREs (e.g., DDs), stimuli and payloads of interest (i.e., immunotherapeutic agents), vectors, cells and other components of the invention, and optionally at least one pharmaceutically acceptable excipient or inert ingredient.

[00362] As used herein the term “pharmaceutical composition” refers to a preparation of biocircuits, SREs, stimuli and payloads of interest (i.e., immunotherapeutic agents), other components, vectors, cells and described herein, or pharmaceutically acceptable salts thereof, optionally with other chemical components such as physiologically suitable carriers and excipients. The pharmaceutical compositions of the invention comprise an effective amount of one or more active compositions of the invention. The preparation of a pharmaceutical composition that contains at least one composition of the present invention and/or an additional active ingredient will be known to those skilled in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference.

[00363] The term “excipient” or “inert ingredient” refers to an inactive substance added to a pharmaceutical composition and formulation to further facilitate administration of an active ingredient. For the purposes of the present disclosure, the phrase “active ingredient” generally refers to any one or more biocircuits, effector modules, SREs, stimuli and payloads of interest (i.e., immunotherapeutic agents), other components, vectors, and cells to be delivered as described herein. The phrases “pharmaceutically acceptable” refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate.

[00364] In some embodiments, pharmaceutical compositions and formulations are administered to humans, human patients or subjects. Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to non-human animals, e.g. non-human mammals. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, non-human mammals, including

agricultural animals such as cattle, horses, chickens and pigs, domestic animals such as cats, dogs, or research animals such as mice, rats, rabbits, dogs and non-human primates. It will be understood that, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[00365] A pharmaceutical composition and formulation in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00366] The compositions of the present invention may be formulated in any manner suitable for delivery. The formulation may be, but is not limited to, nanoparticles, poly (lactic-co-glycolic acid) (PLGA) microspheres, lipidoids, lipoplex, liposome, polymers, carbohydrates (including simple sugars), cationic lipids and combinations thereof.

[00367] In one embodiment, the formulation is a nanoparticle which may comprise at least one lipid. The lipid may be selected from, but is not limited to, DLin-DMA, DLin-K-DMA, 98N12-5, C12-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-DMG and PEGylated lipids. In another aspect, the lipid may be a cationic lipid such as, but not limited to, DLin-DMA, DLin-D-DMA, DLin-MC3-DMA, DLin-KC2-DMA and DODMA.

[00368] For polynucleotides of the invention, the formulation may be selected from any of those taught, for example, in International Application PCT/US2012/069610, the contents of which are incorporated herein by reference in its entirety.

[00369] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient or inert ingredient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1 and 100, e.g., between 0.5 and 50, between 1-30, between 5-80, at least 80 (w/w) active ingredient.

[00370] Efficacy of treatment or amelioration of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or

prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of compositions of the present invention, "effective against" for example a cancer, indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease load, reduction in tumor mass or cell numbers, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of cancer.

[00371] A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10 in a measurable parameter of disease, and preferably at least 20, 30, 40, 50 or more can be indicative of effective treatment. Efficacy for a given composition or formulation of the present invention can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant change is observed.

IV. APPLICATIONS

[00372] In one aspect of the present invention, methods for reducing a tumor volume or burden are provided. The methods comprise administering a pharmaceutically effective amount of a pharmaceutical composition comprising at least one biocircuit system, effector module, DD, and/or payload of interest (i.e., an immunotherapeutic agent), at least one vector, or cells to a subject having a tumor. The biocircuit system and effector module having any immunotherapeutic agent as described herein may be in forms of a polypeptide, or a polynucleotide such as mRNA, or a viral vector comprising the polynucleotide, or a cell modified to express the biocircuit, effector module, DD, and payload of interest (i.e., immunotherapeutic agent).

[00373] In another aspect of the present invention, methods for inducing an anti-tumor immune response in a subject are provided. The methods comprise administering a pharmaceutically effective amount of a pharmaceutical composition comprising at least one biocircuit system, effector module, DD, and/or payload of interest (i.e., an immunotherapeutic agent), at least one vector, or cells to a subject having a tumor. The biocircuit and effector module having any immunotherapeutic agent as described herein may be in forms of a polypeptide, or a polynucleotide such as mRNA, or a viral vector comprising the polynucleotide, or a cell modified to express the biocircuit, effector module, DD, and payload of interest (i.e., immunotherapeutic agent).

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CLAIMS

1. A composition for inducing an immune response in a cell or a subject comprising a first effector module, said effector module comprising a first stimulus response element (SRE) operably linked to at least one immunotherapeutic agent.
2. The composition of claim 1, wherein said at least one immunotherapeutic agent is selected from a chimeric antigen receptor (CAR) and an antibody.
3. The composition of claim 2, wherein said first SRE is responsive to or interacts with at least one stimulus.
4. The composition of claim 3, wherein said first SRE is a destabilizing domain (DD).
5. The composition of claim 4, wherein the DD is derived from a parent protein or a mutant protein having one, two, three or more amino acid mutations compared to said parent protein, wherein the parent protein is selected from:
 - (a) human protein FKBP comprising the amino acid sequence of SEQ ID NO. 3,
 - (b) human DHFR (hDHFR) comprising the amino acid sequence of SEQ ID NO. 1,
 - (c) E. coli DHFR (ecDHFR) comprising the amino acid sequence of SEQ ID NO. 2,
 - (d) PDE5 comprising the amino acid sequence of SEQ ID NO. 4,
 - (e) PPAR gamma comprising the amino acid sequence of SEQ ID NO. 5,
 - (f) CA2 comprising the amino acid sequence of SEQ ID NO. 6, and
 - (g) NQO2 comprising the amino acid sequence of SEQ ID NO. 7.
6. The composition of claim 5, wherein the parent protein is hDHFR and the DD comprises a mutant protein having:
 - (a) a single mutation selected from hDHFR (I17V), hDHFR (F59S), hDHFR (N65D), hDHFR (K81R), hDHFR (A107V), hDHFR (Y122I), hDHFR (N127Y), hDHFR (M140I), hDHFR (K185E), hDHFR (N186D), hDHFR (M140I), hDHFR (Amino acid 2-187 of WT; N127Y), hDHFR (Amino acid 2-187 of WT; I17V), hDHFR (Amino acid 2-187 of WT; Y122I), and hDHFR (Amino acid 2-187 of WT; K185E);
 - (b) a double mutation selected from hDHFR (C7R, Y163C), hDHFR (A10V, H88Y), hDHFR (Q36K, Y122I), hDHFR (M53T, R138I), hDHFR (T57A, I72A), hDHFR (E63G,

I176F), hDHFR (G21T, Y122I), hDHFR (L74N, Y122I), hDHFR (V75F, Y122I), hDHFR (L94A, T147A), DHFR (V121A, Y22I), hDHFR (Y122I, A125F), hDHFR (H131R, E144G), hDHFR (T137R, F143L), hDHFR (Y178H, E181G), hDHFR (Y183H, K185E), hDHFR (E162G, I176F) hDHFR (Amino acid 2-187 of WT; I17V, Y122I), hDHFR (Amino acid 2-187 of WT; Y122I, M140I), hDHFR (Amino acid 2-187 of WT; N127Y, Y122I), hDHFR (Amino acid 2-187 of WT; E162G, I176F), and hDHFR (Amino acid 2-187 of WT; H131R, E144G), and hDHFR (Amino acid 2-187 of WT; Y122I, A125F);

(c) a triple mutation selected from hDHFR (V9A, S93R, P150L), hDHFR (I8V, K133E, Y163C), hDHFR (L23S, V121A, Y157C), hDHFR (K19E, F89L, E181G), hDHFR (Q36F, N65F, Y122I), hDHFR (G54R, M140V, S168C), hDHFR (V110A, V136M, K177R), hDHFR (Q36F, Y122I, A125F), hDHFR (N49D, F59S, D153G), hDHFR (G21E, I72V, I176T), hDHFR (Amino acid 2-187 of WT; Q36F, Y122I, A125F), hDHFR (Amino acid 2-187 of WT; Y122I, H131R, E144G), hDHFR (Amino acid 2-187 of WT; E31D, F32M, V116I), and hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I); or

(d) a quadruple or higher mutation selected from hDHFR (V2A, R33G, Q36R, L100P, K185R), hDHFR (Amino acid 2-187 of WT; D22S, F32M, R33S, Q36S, N65S), hDHFR (I17N, L98S, K99R, M112T, E151G, E162G, E172G), hDHFR (G16S, I17V, F89L, D96G, K123E, M140V, D146G, K156R), hDHFR (K81R, K99R, L100P, E102G, N108D, K123R, H128R, D142G, F180L, K185E), hDHFR (R138G, D142G, F143S, K156R, K158E, E162G, V166A, K177E, Y178C, K185E, N186S), hDHFR (N14S, P24S, F35L, M53T, K56E, R92G, S93G, N127S, H128Y, F135L, F143S, L159P, L160P, E173A, F180L), hDHFR (F35L, R37G, N65A, L68S, K69E, R71G, L80P, K99G, G117D, L132P, I139V, M140I, D142G, D146G, E173G, D187G), hDHFR (L28P, N30H, M38V, V44A, L68S, N73G, R78G, A97T, K99R, A107T, K109R, D111N, L134P, F135V, T147A, I152V, K158R, E172G, V182A, E184R), hDHFR (V2A, I17V, N30D, E31G, Q36R, F59S, K69E, I72T, H88Y, F89L, N108D, K109E, V110A, I115V, Y122D, L132P, F135S, M140V, E144G, T147A, Y157C, V170A, K174R, N186S), hDHFR (L100P, E102G, Q103R, P104S, E105G, N108D, V113A, W114R, Y122C, M126I, N127R, H128Y, L132P, F135P, I139T, F148S, F149L, I152V, D153A, D169G, V170A, I176A, K177R, V182A, K185R, N186S), and hDHFR (A10T, Q13R, N14S, N20D, P24S, N30S, M38T, T40A, K47R, N49S, K56R, I61T, K64R, K69R, I72A,

R78G, E82G, F89L, D96G, N108D, M112V, W114R, Y122D, K123E, I139V, Q141R, D142G, F148L, E151G, E155G, Y157R, Q171R, Y183C, E184G, K185del, D187N).

7. The composition of claim 6, wherein the stimulus is selected from the group consisting of Trimethoprim (TMP) and Methotrexate (MTX).

8. The composition of claim 2, wherein the immunotherapeutic agent is a chimeric antigen receptor (CAR).

9. The composition of claim 8, wherein the chimeric antigen receptor (CAR) comprises

- (a) an extracellular target moiety;
- (b) a transmembrane domain;
- (c) an intracellular signaling domain; and
- (d) optionally, one or more co-stimulatory domains.

10. The composition of claim 9, wherein the CAR is a standard CAR, a split CAR, an off-switch CAR, an on-switch CAR, a first-generation CAR, a second-generation CAR, a third-generation CAR, or a fourth-generation CAR.

11. The composition of claim 9, wherein the extracellular target moiety recognizes a target molecule on the surface of a cancer cell, wherein said target molecule on the surface of the cancer cell is selected from a cancer antigen, a plasma membrane lipid, a receptor and a membrane bound glycoprotein.

12. The composition of any of claims 9-11, wherein the extracellular target moiety is selected from any of:

- i. an Ig NAR,
- ii. a Fab fragment,
- iii. a Fab' fragment,
- iv. a F(ab)'2 fragment,
- v. a F(ab)'3 fragment,
- vi. an Fv,
- vii. a single chain variable fragment (scFv),
- viii. a bis-scFv, a (scFv)2,

- ix. a minibody,
- x. a diabody,
- xi. a triabody,
- xii. a tetrabody,
- xiii. an intrabody,
- xiv. a disulfide stabilized Fv protein (dsFv),
- xv. a unibody,
- xvi. a nanobody, and
- xvii. an antigen binding region derived from an antibody that specifically binds to any of a protein of interest, a ligand, a receptor, a receptor fragment or a peptide aptamer.

13. The composition of claim 12, wherein the extracellular target moiety is a scFv derived from an antibody that specifically binds a CD19 antigen.

14. The composition of claim 13, wherein the scFv is a CD19 scFv is selected from one that comprises:

- (a) a heavy chain variable region having an amino acid sequence independently selected from the group consisting of SEQ ID NO: 49-80, and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122; or
- (b) an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267 and 624.

15. The composition of claim 9, wherein

- (a) the intracellular signaling domain of the CAR is the signaling domain derived from T cell receptor CD3zeta or a cell surface molecule selected from the group consisting of FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d; and
- (b) the co-stimulatory domain is present and is selected from the group consisting of 2B4, HVEM, ICOS, LAG3, DAP10, DAP12, CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, ICOS (CD278), glucocorticoid-induced tumor necrosis factor receptor (GITR), lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3.

16. The composition of claim 15, wherein the intracellular signaling domain of the CAR is a T cell receptor CD3zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 339.

17. The composition of claim 9, wherein the intracellular signaling domain of the CAR is a T cell receptor CD3zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 626 and the co-stimulatory domain is present, said co-stimulatory domain being selected from amino acid sequence of any of SEQ ID NOs: 268-374.

18. The composition of claim 9, wherein the transmembrane domain is derived from any of the members of the group consisting of:

- (a) a transmembrane region of an alpha, beta or zeta chain of a T-cell receptor;
- (b) the CD3 epsilon chain of a T-cell receptor;
- (c) a molecule selected from CD4, CD5, CD8, CD8 α , CD9, CD16, CD22, CD33, CD28, CD37, CD45, CD64, CD80, CD86, CD148, DAP 10, EpoRI, GITR, LAG3, ICOS, Her2, OX40 (CD134), 4-1BB (CD137), CD152, CD154, PD-1, or CTLA-4; and
- (d) an immunoglobulin selected from IgG1, IgD, IgG4, and an IgG4 Fc region.

19. The composition of claim 9, wherein the transmembrane domain comprises an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 375-425 and 897-907.

20. The composition of claim 9, wherein the CAR further comprises

- (e) a hinge region near the transmembrane domain, said hinge region comprising an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 426-504.

21. The composition of claim 2, wherein the immunotherapeutic agent is an antibody that is specifically immunoreactive to an antigen selected from a tumor specific antigen (TSA), a tumor associated antigen (TAA), or an antigenic epitope.

22. The composition of claim 21, wherein the antigen is an antigenic epitope and said antigenic epitope is CD19.

23. The composition of claim 22, wherein the antibody is selected from one that comprises

- (a) a heavy chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 49-80 and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122; or
- (b) an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267.

24. The composition of claim 1 wherein said first effector module comprises the amino acid sequence of any of SEQ ID NO: 635-649, 1005-1010, 1015-1018, and 1215-1231.

25. The composition of claim 24, wherein said first SRE of the effector module stabilizes the immunotherapeutic agent by a stabilization ratio of 1 or more, wherein the stabilization ratio comprises the ratio of expression, function or level of the immunotherapeutic agent in the presence of the stimulus to the expression, function or level of the immunotherapeutic agent in the absence of the stimulus.

26. The composition of any of claims 24-25, wherein the SRE destabilizes the immunotherapeutic agent by a destabilization ratio between 0, and 0.09, wherein the destabilization ratio comprises the ratio of expression, function or level of the immunotherapeutic agent in the absence of the stimulus specific to the SRE to the expression, function or level of the immunotherapeutic agent that is expressed constitutively, and in the absence of the stimulus specific to the SRE.

27. A polynucleotide encoding any of the compositions of claims 1-26.

28. The polynucleotide of claim 27, wherein the polynucleotide is a DNA molecule, or a RNA molecule.

29. The polynucleotide of claim 28, wherein the polynucleotide is an RNA molecule and said RNA molecule is a messenger RNA.

30. The polynucleotide of claim 29, which is chemically modified.

31. The polynucleotide of claim 28, which comprises spatiotemporally selected codons.

32. The polynucleotide of claim 29, further encoding a promoter, a linker, a signal peptide, a tag, a cleavage site and/or a targeting peptide.
33. A vector comprising a polynucleotide of any of claims 27-32.
34. The vector of claim 33, wherein the vector is a viral vector, or a plasmid.
35. The vector of claim 34, which is a viral vector and wherein the viral vector is a retroviral vector, a lentiviral vector, a gamma retroviral vector, a recombinant AAV vector, an adeno viral vector, or an oncolytic viral vector.
36. An immune cell for adoptive cell transfer (ACT), which expresses any of the compositions of any of claims 1-26, the polynucleotides of any of claims 27-32, and/or is infected or transfected with the vector of any of claims 33-35.
37. The immune cell of claim 36, wherein the immune cell is a CD8⁺ T cell, a CD4⁺ T cell, a helper T cell, a natural killer (NK) cell, a NKT cell, a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), a memory T cell, a regulatory T (Treg) cell, a cytokine-induced killer (CIK) cell, a dendritic cell, a human embryonic stem cell, a mesenchymal stem cell, a hematopoietic stem cell, or a mixture thereof.
38. The immune cell of claim 36, wherein the immune cell 49which further expresses a composition comprising a second effector module, said second effector module comprising a second SRE linked to a second immunotherapeutic agent wherein the second immunotherapeutic agent is selected from a cytokine, and a cytokine- cytokine receptor fusion.
39. The immune cell of claim 38, wherein the second immunotherapeutic agent is a cytokine.
40. The immune cell of claim 39, wherein the cytokine is IL12 or IL15.
41. The immune cell of claim 38, wherein the second immunotherapeutic agent is a cytokine- cytokine receptor fusion polypeptide.

42. The immune cell of claim 41, wherein the cytokine-cytokine receptor fusion polypeptide is selected from a IL12-IL12 receptor fusion polypeptide, a IL15-IL15 receptor fusion polypeptide, and a IL15-IL15 receptor sushi domain fusion polypeptide.

43. The immune cell of claim 36 or 37, wherein the immune cell is autologous, allogeneic, syngeneic, or xenogeneic in relation to a particular individual subject.

44. A method of reducing a tumor volume or burden in a subject, comprising contacting said subject with a composition of any of claims 1-26, the polynucleotides of any of claims 27-32, the vectors of any of claims 33-35 or the immune cells of any of claims 36-43, wherein the SRE responds to a stimulus and regulates the expression and function of the immunotherapeutic agent.

45. A method of inducing an immune response in a subject comprising administering to the subject an effective amount of any of the compositions of claims 1-26, the polynucleotides of any of claims 27-32, the vectors of any of claims 33-35 or the immune cells of any of claims 36-43.

46. A method of identifying a domain of a CD19 antigen which will not bind the FMC63 antibody (FMC63-distinct CD19 binding domain), said method comprising:

- (a) preparing a composition comprising a CD19 antigen,
- (b) contacting the composition in (a) with saturating levels of FMC63 antibody,
- (c) contact the composition of step (b) with one or more selected members of a library of potential CD19 binders; and
- (d) identifying a binding domain on the CD19 antigen based on the differential binding of the selected members of the library of CD19 binders compared to the binding of FMC63.

47. The method of claim 46, wherein said binding domains of the library are generated using phage display techniques with the CD19 antigen as the seed sequence.

48. The method of claim 47, wherein the binding domain is selected from a Fab fragment, a Fab' fragment, a F(ab)'2 fragment, a F(ab)'3 fragment, Fv, a single chain variable fragment (scFv), a bis-scFv, a (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv

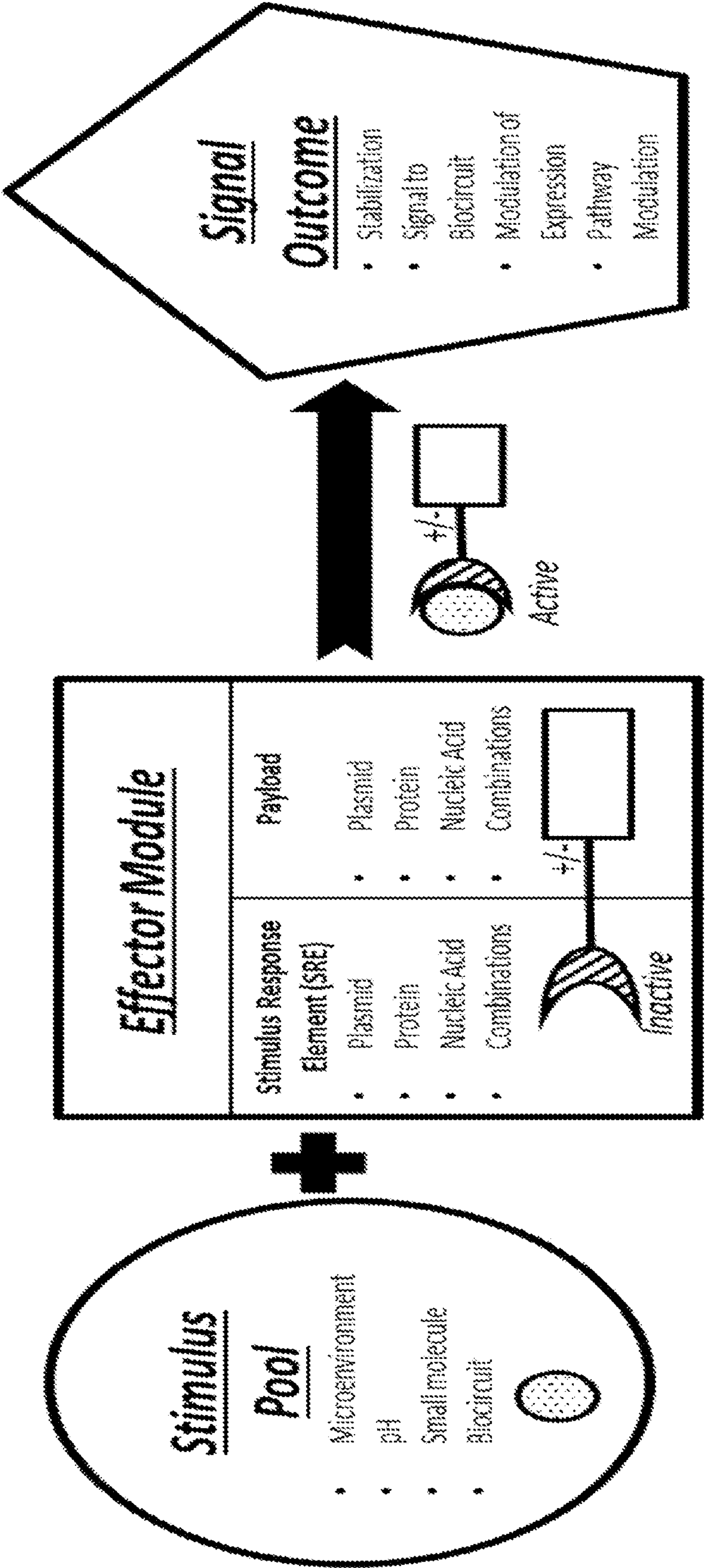
protein (dsFv), a unibody, a nanobody, or an antigen binding region of an antibody, and an antibody fragment.

49. The method of claim 48, wherein the CD19 antigen is selected from a whole or a portion of a human CD19 antigen, and a whole or a portion of a Rhesus CD19 antigen.

50. A chimeric antigen receptor comprising the FMC63-distinct CD19 binding domain obtained according to the method of any of claims 46-49.

51. An effector module comprising a stimulus response element (SRE) operably lined to the chimeric antigen receptor of claim 50.

Figure 1



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Figure 2

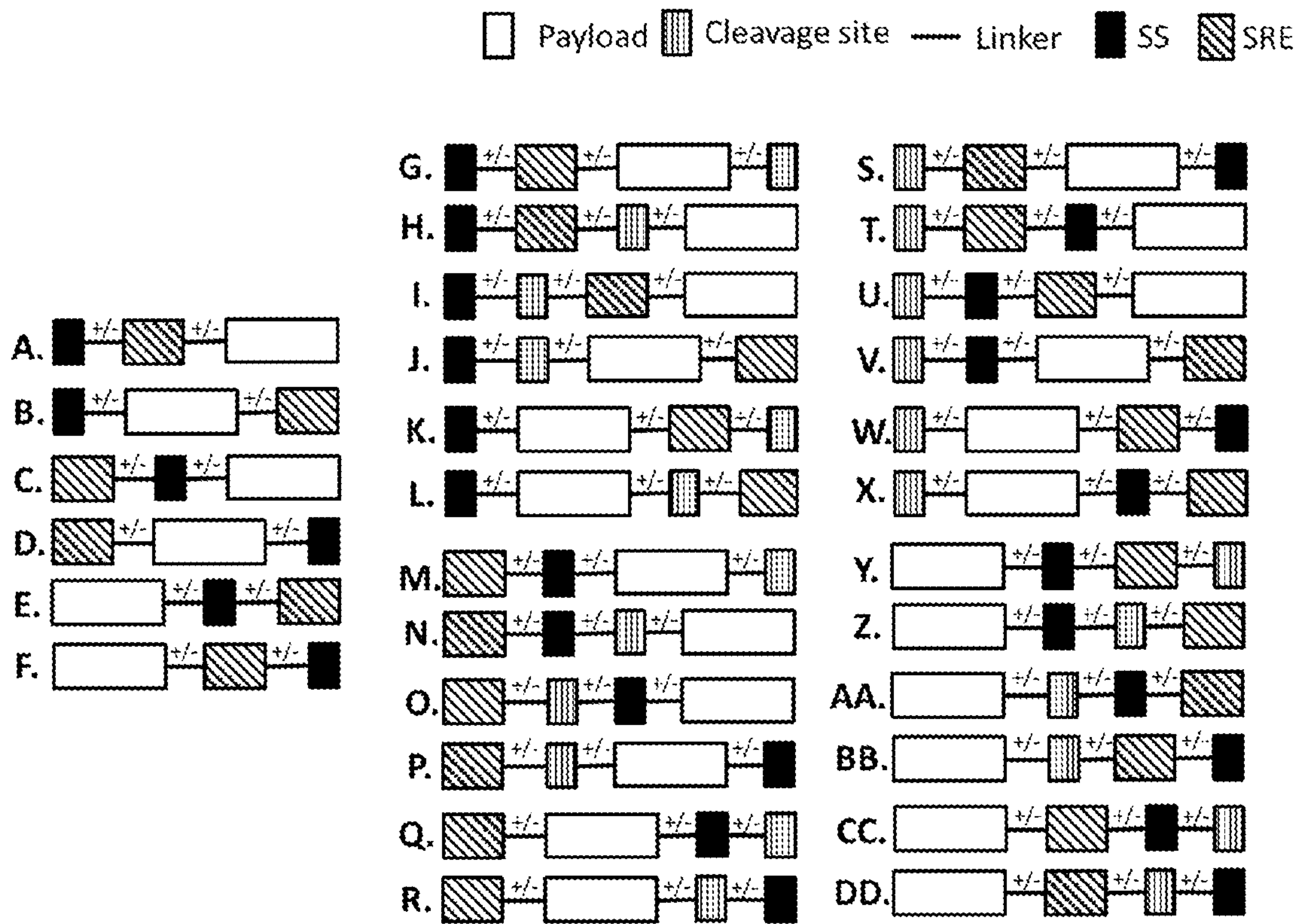
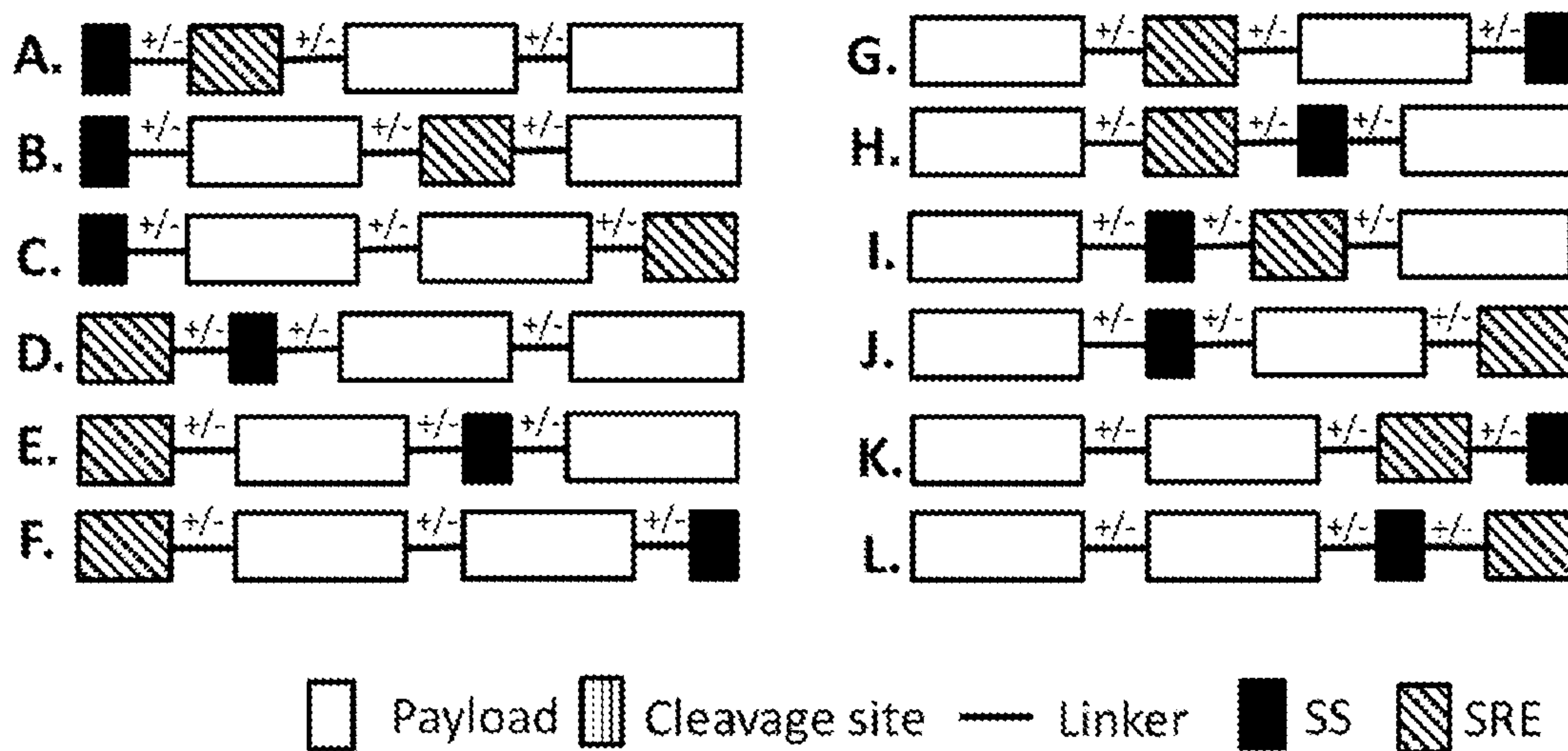


Figure 3



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Figure 4

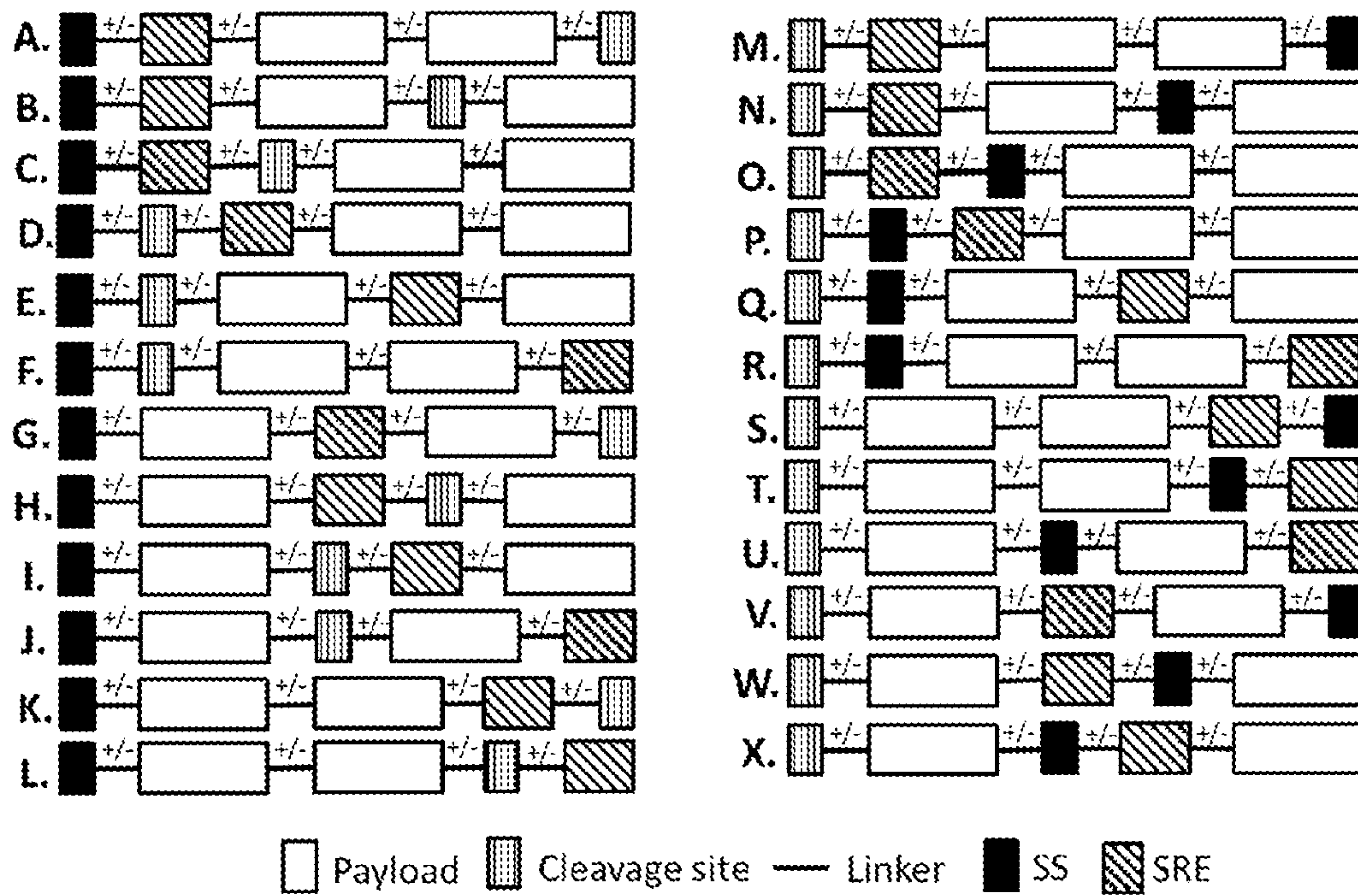
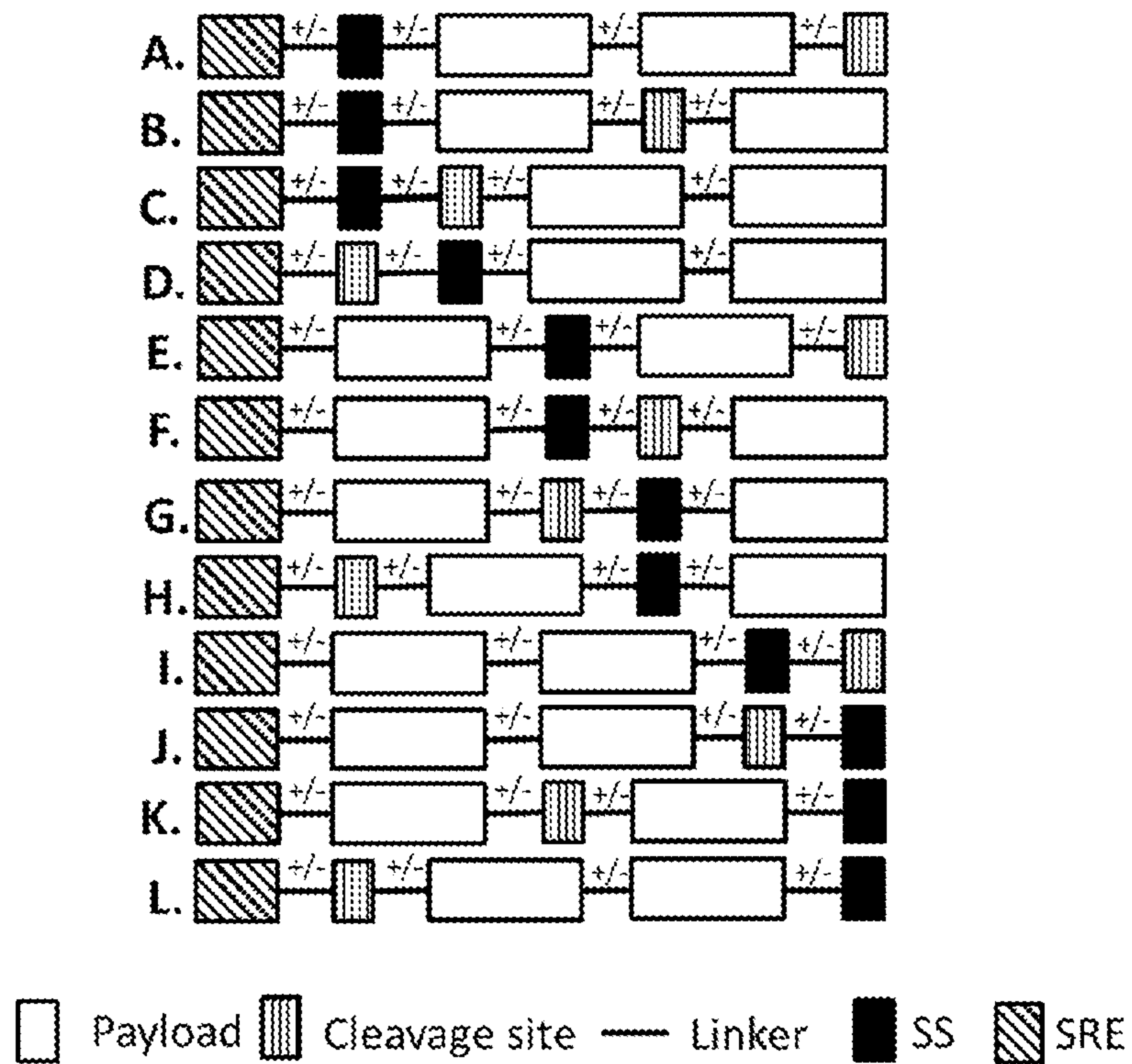


Figure 5



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Figure 6

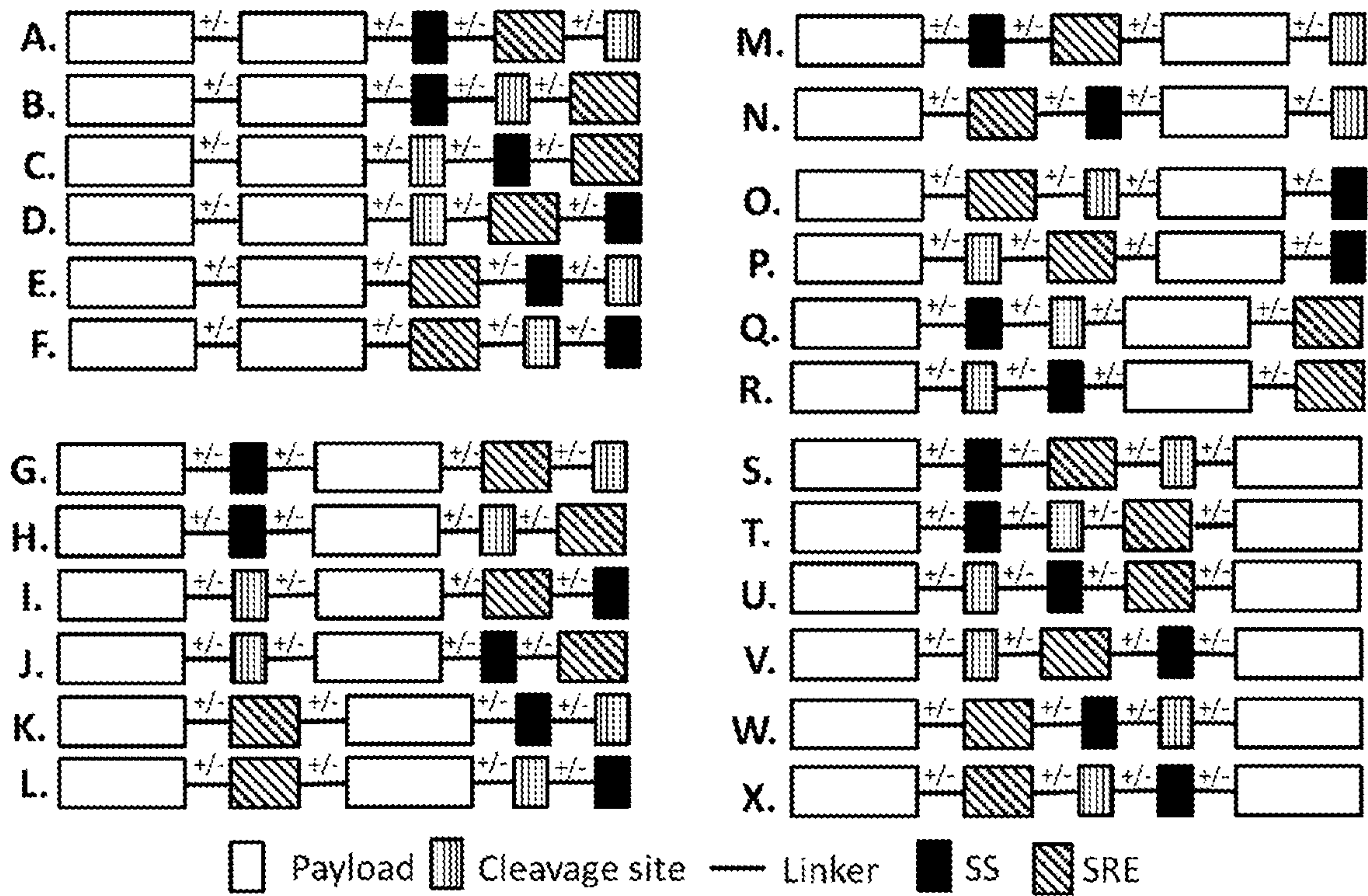
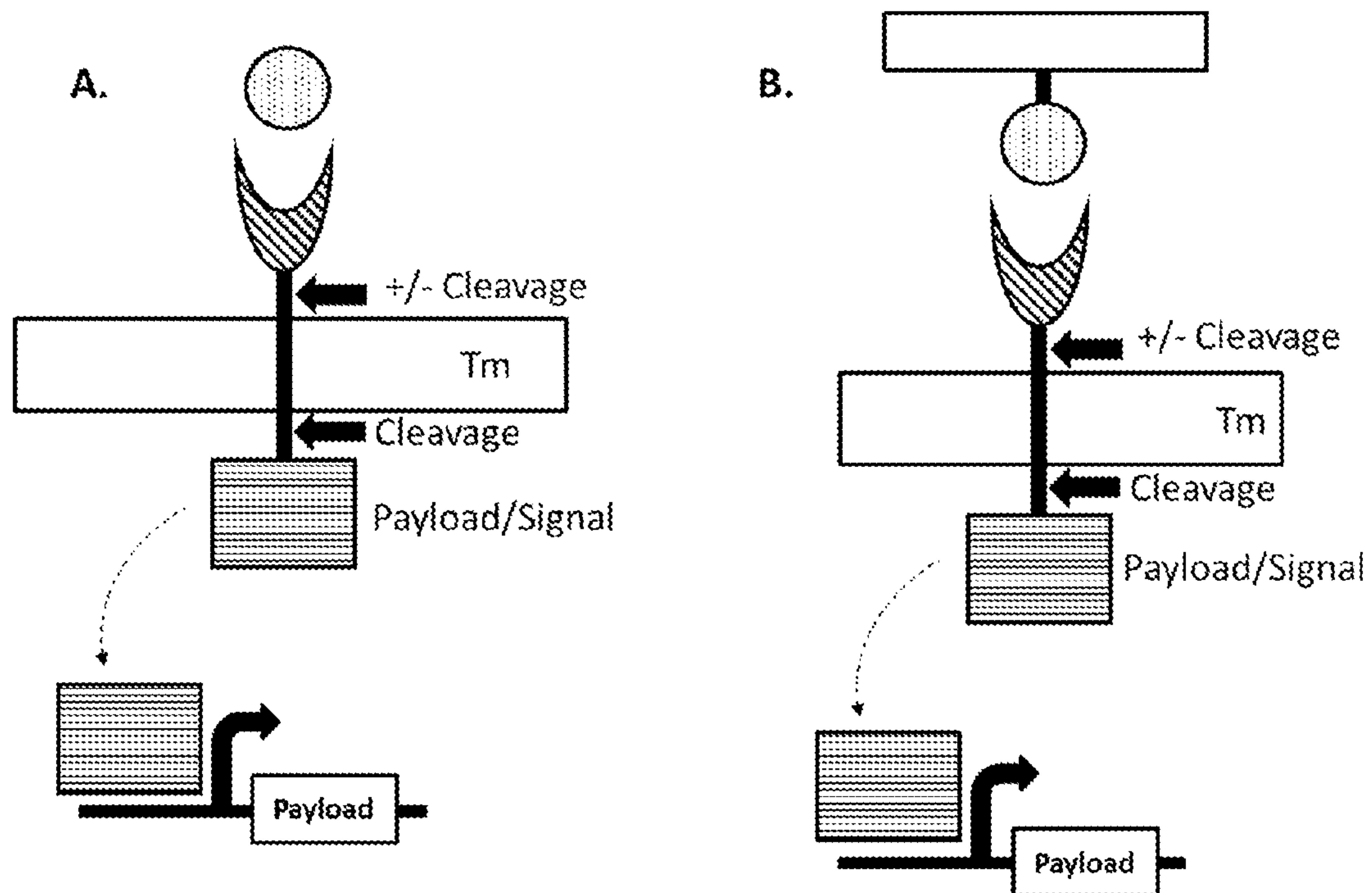


Figure 7



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Figure 8

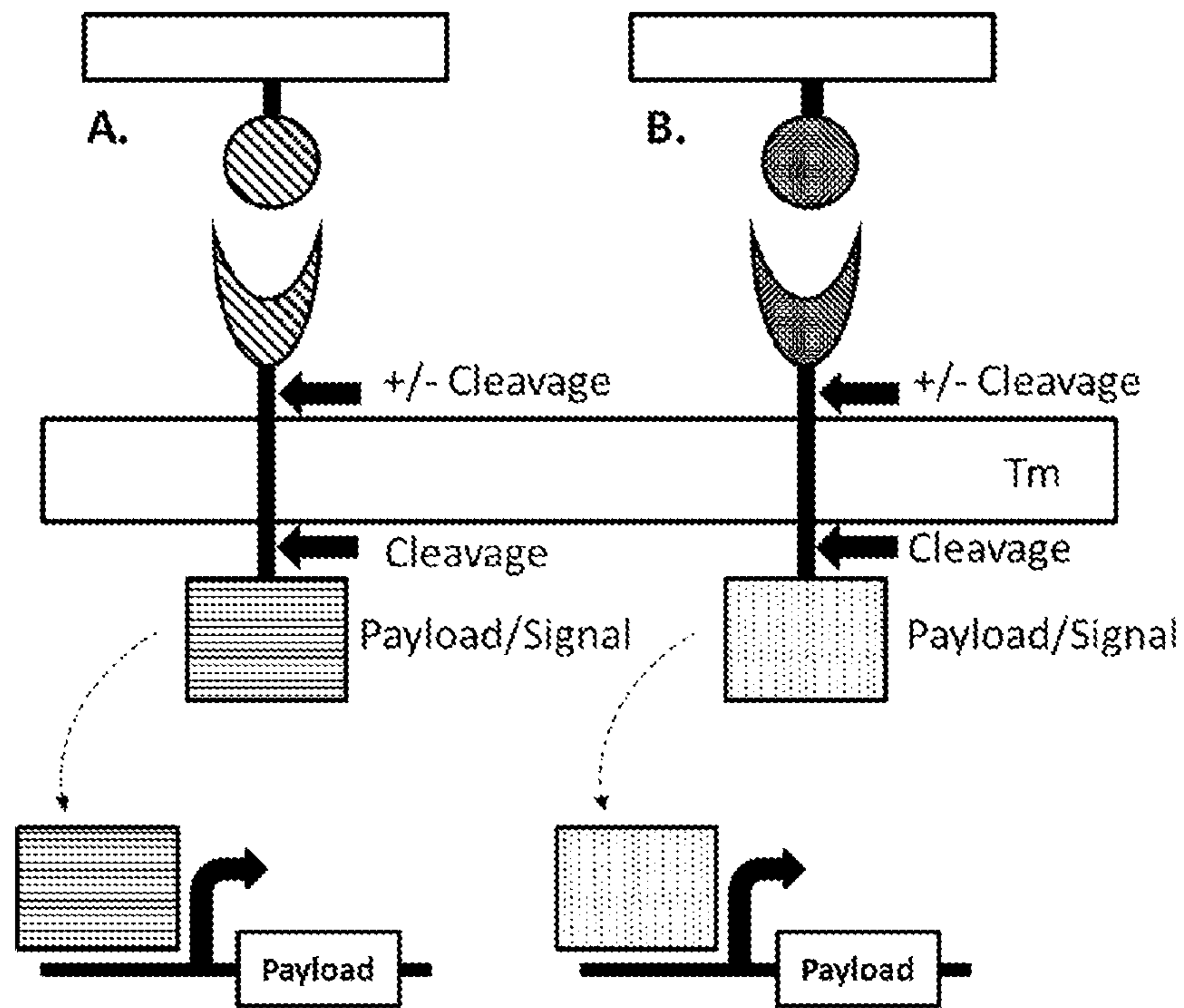
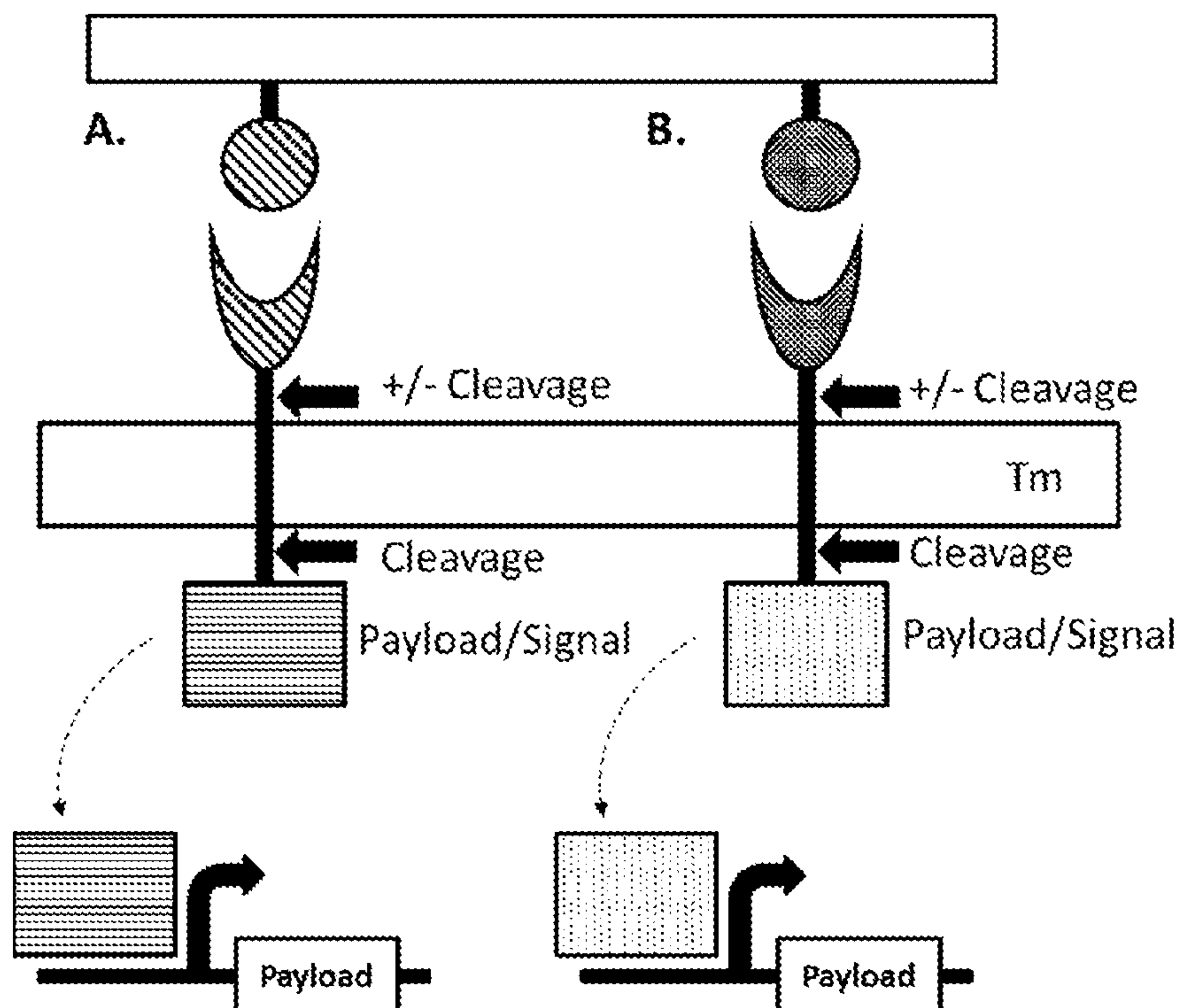


Figure 9



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Figure 10

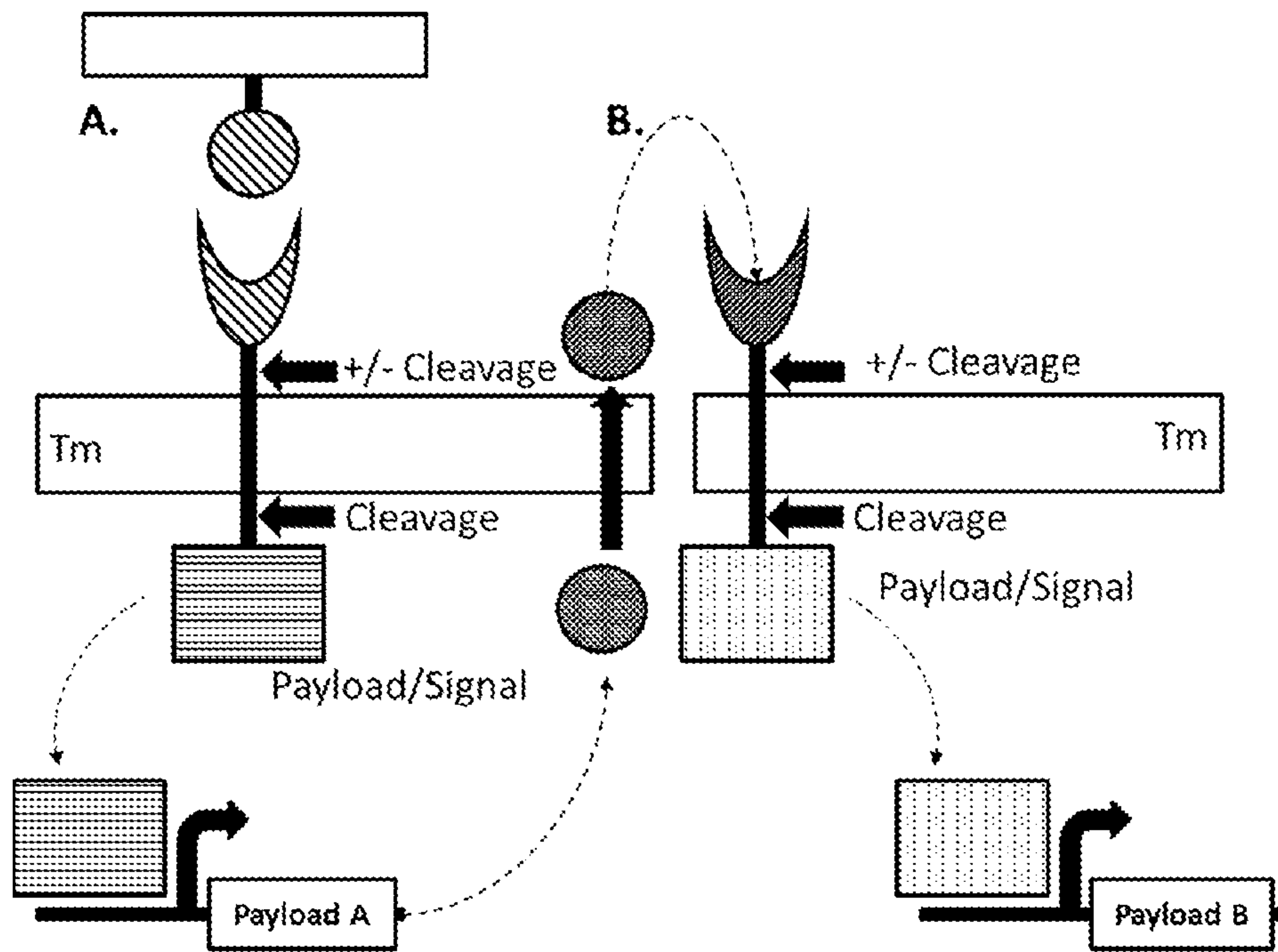
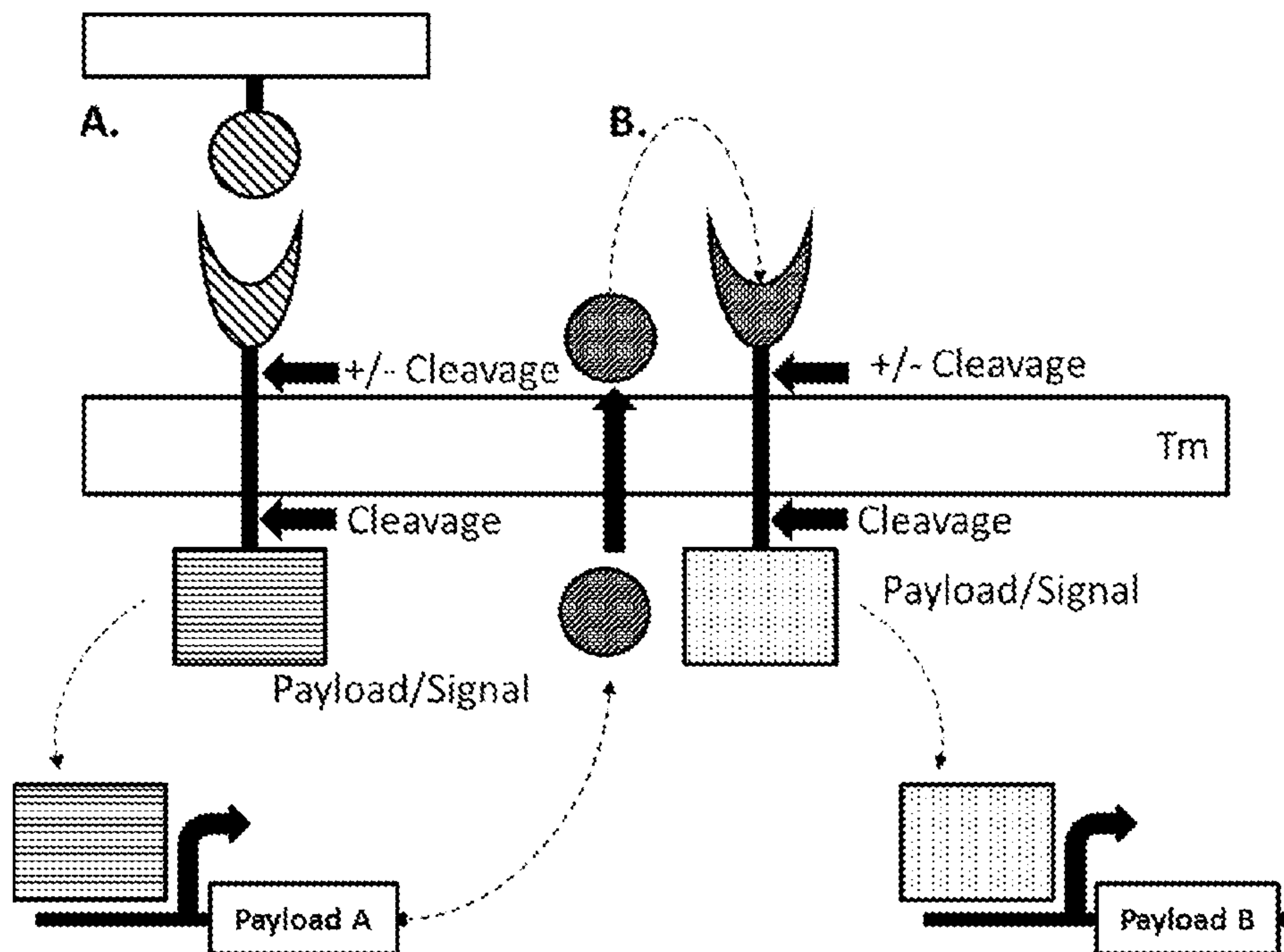


Figure 11



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Figure 12

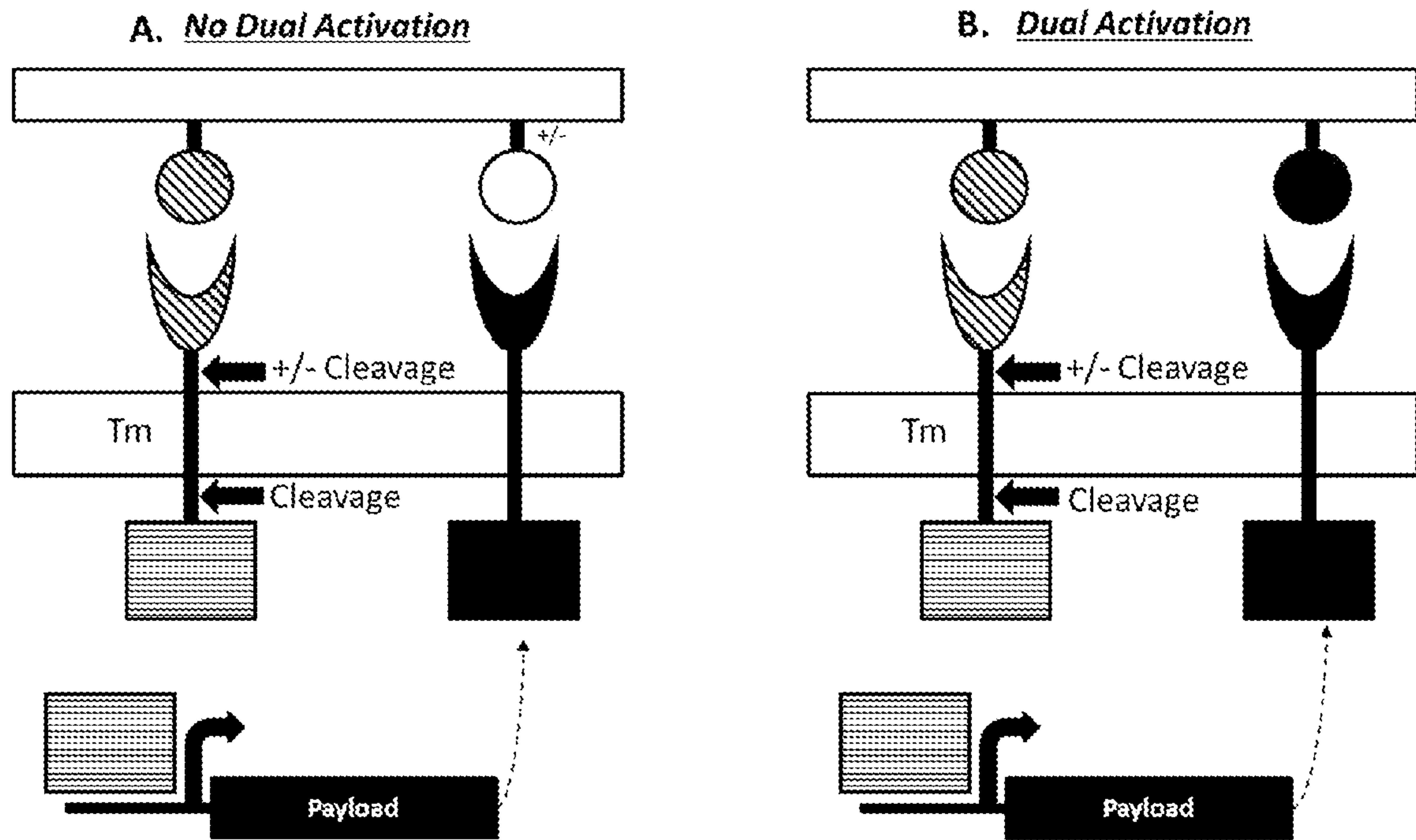
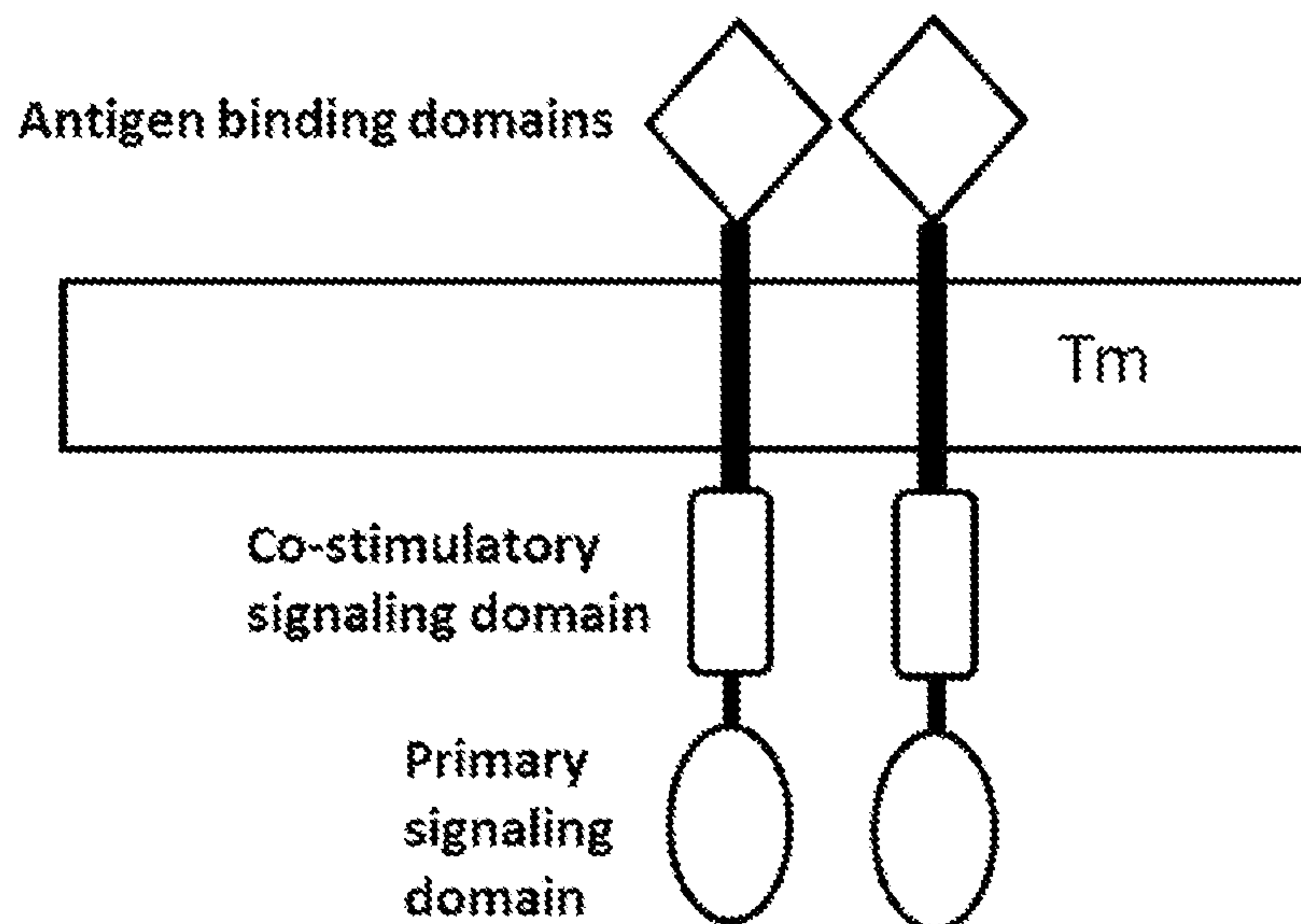


Figure 13



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Figure 14

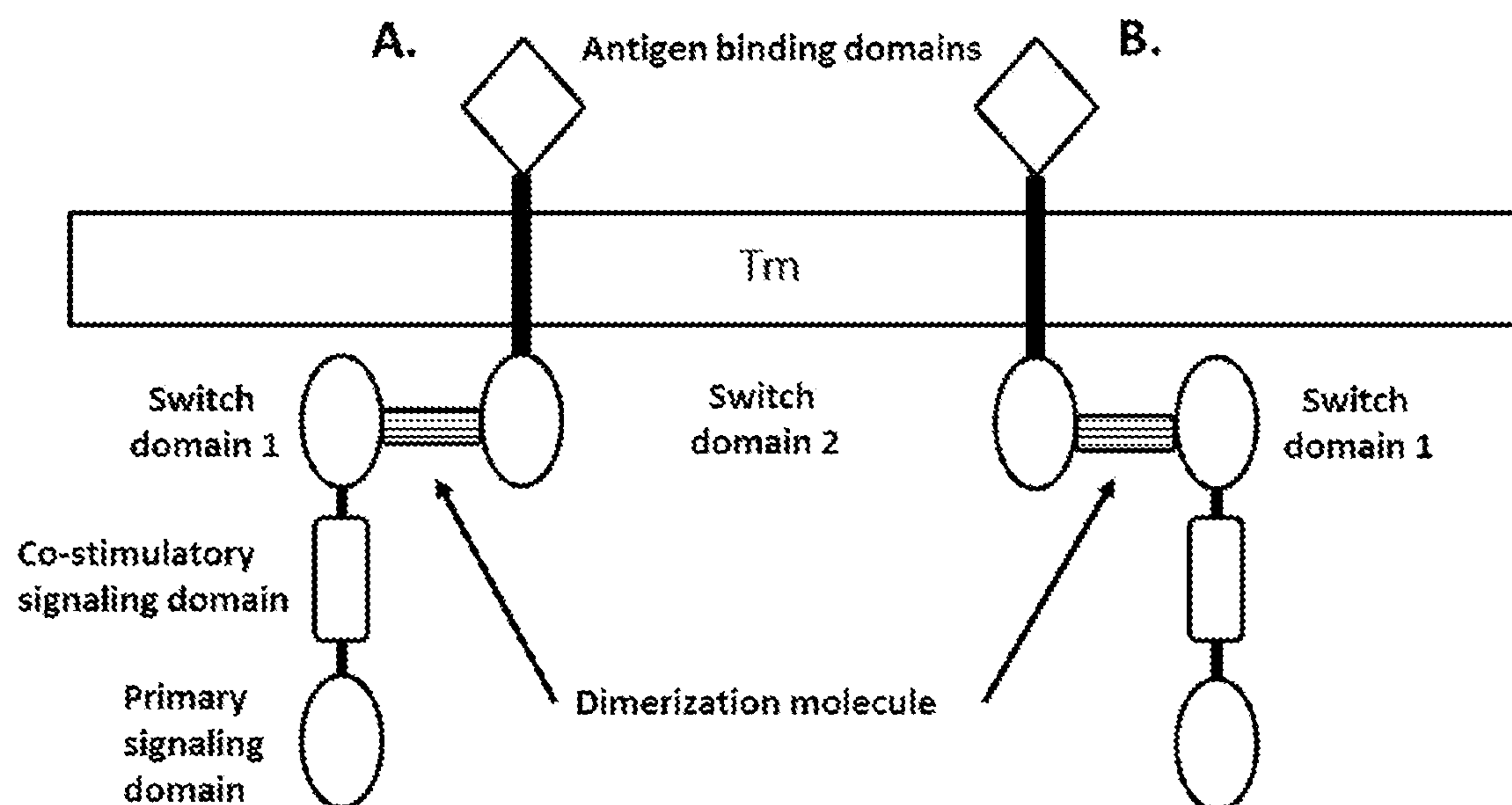
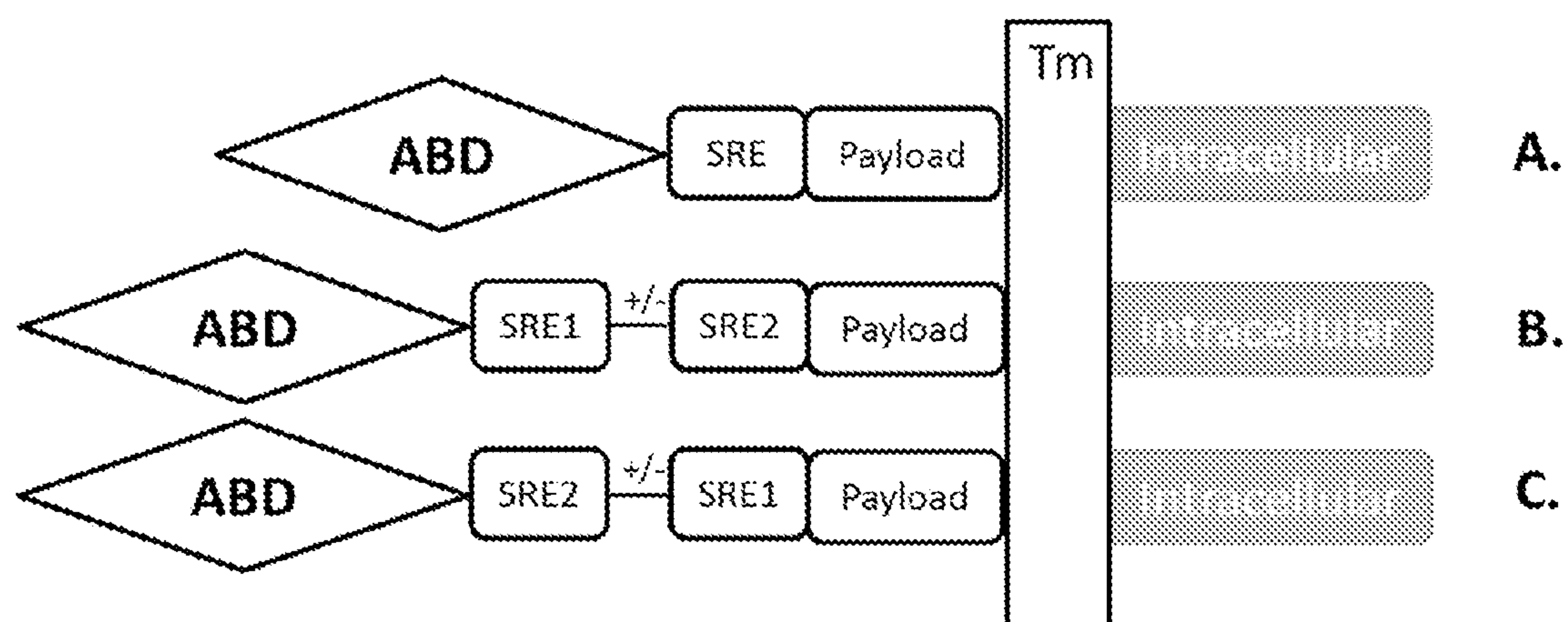


Figure 15



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Figure 16

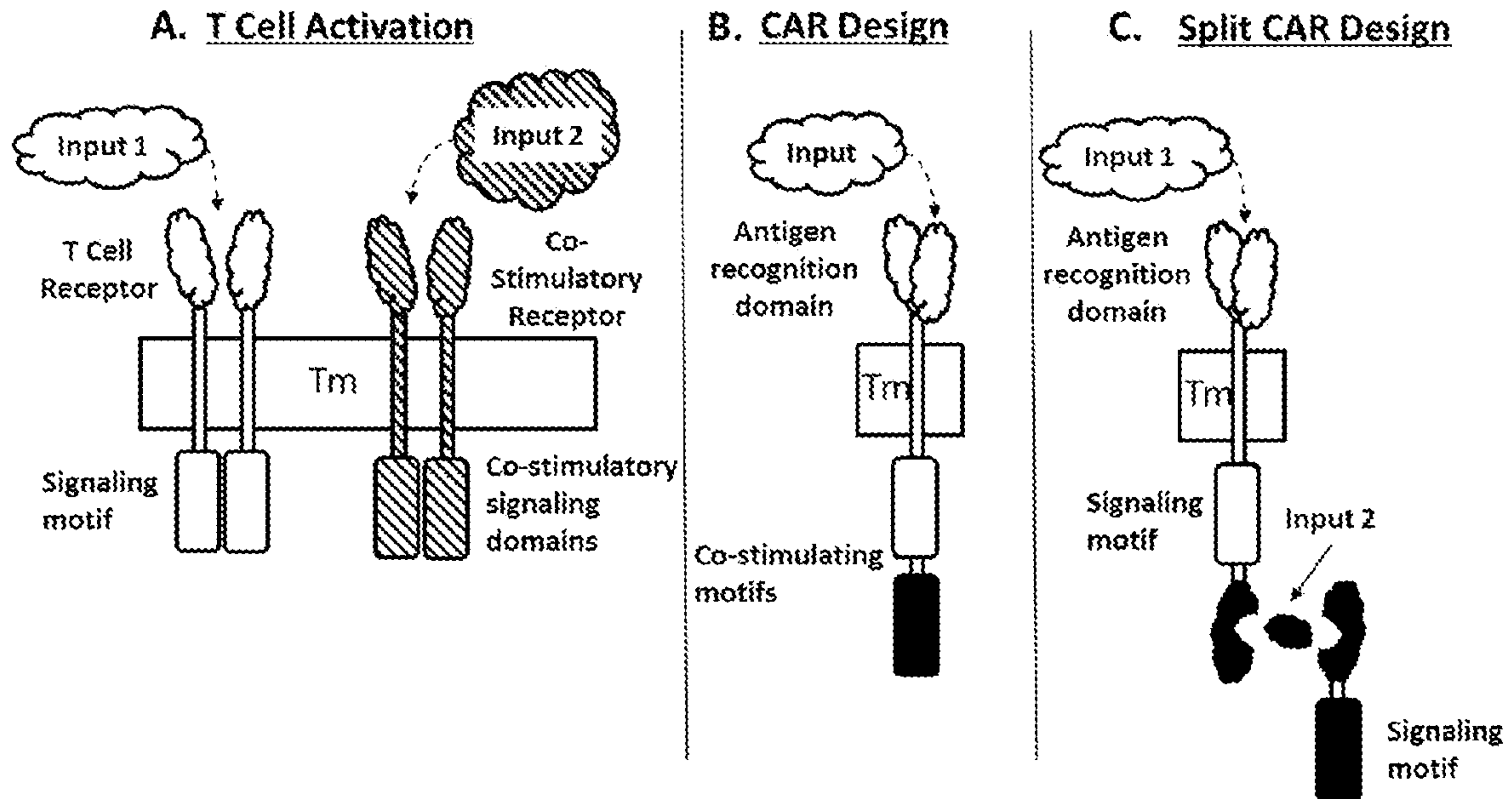
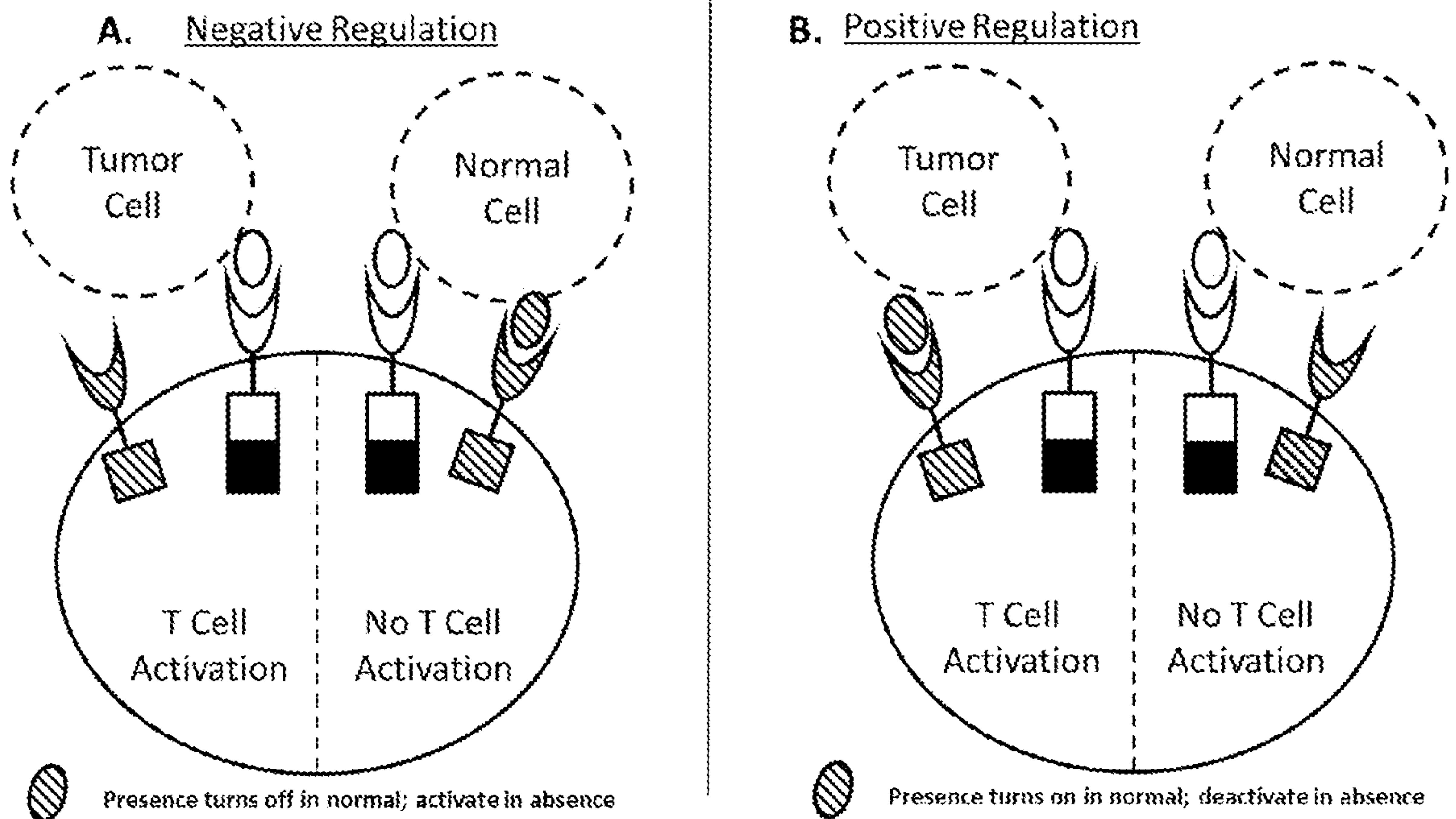


Figure 17



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Figure 18

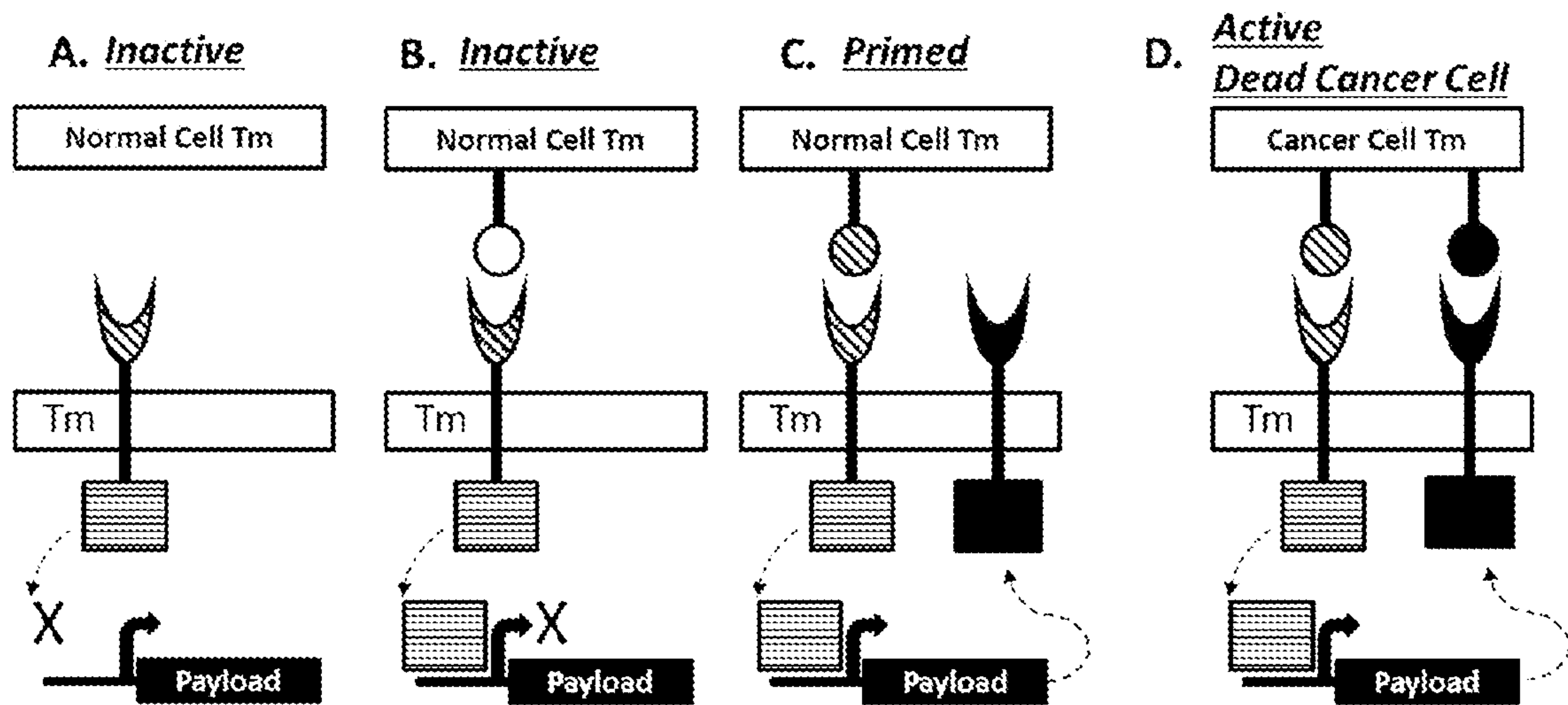
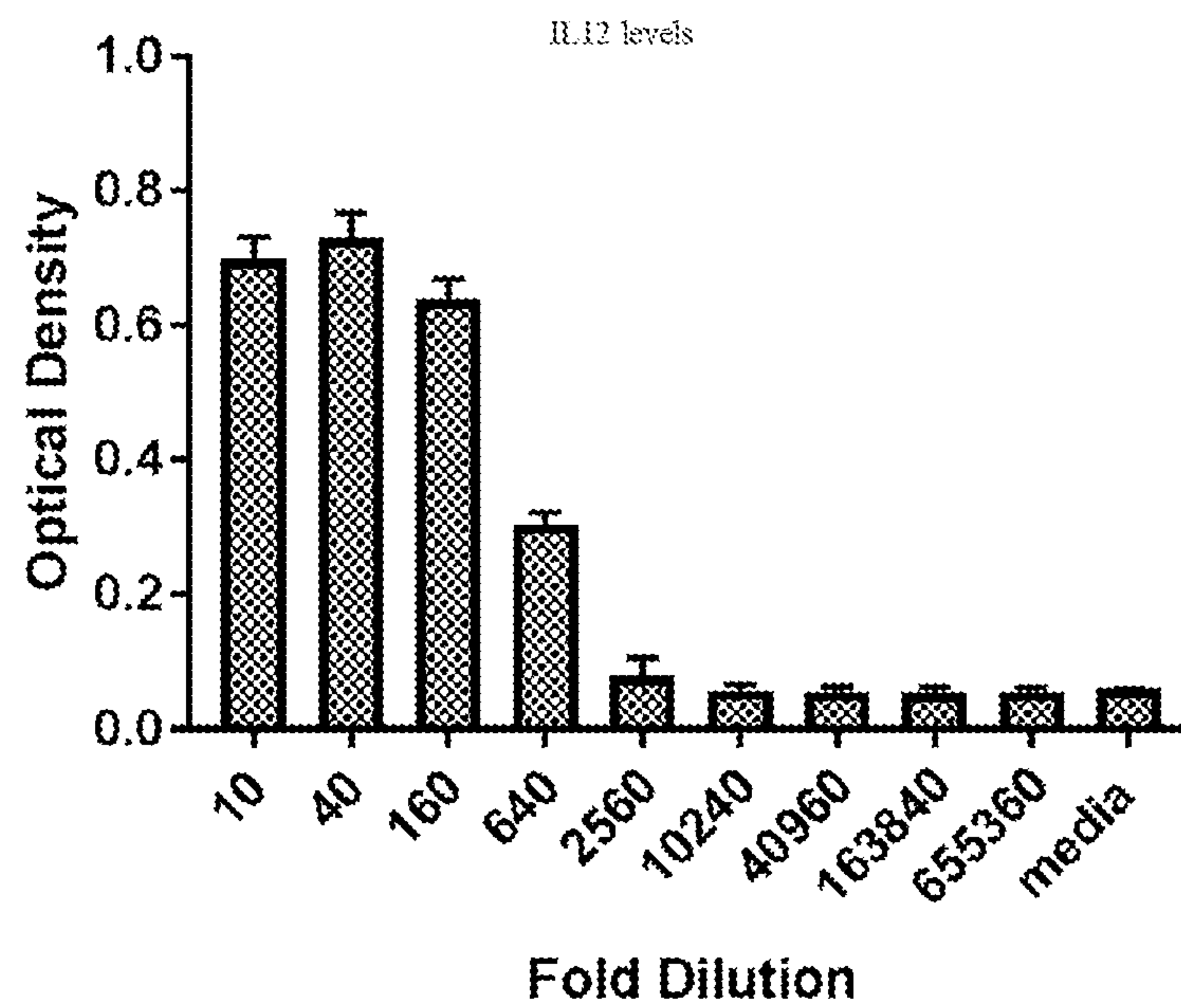
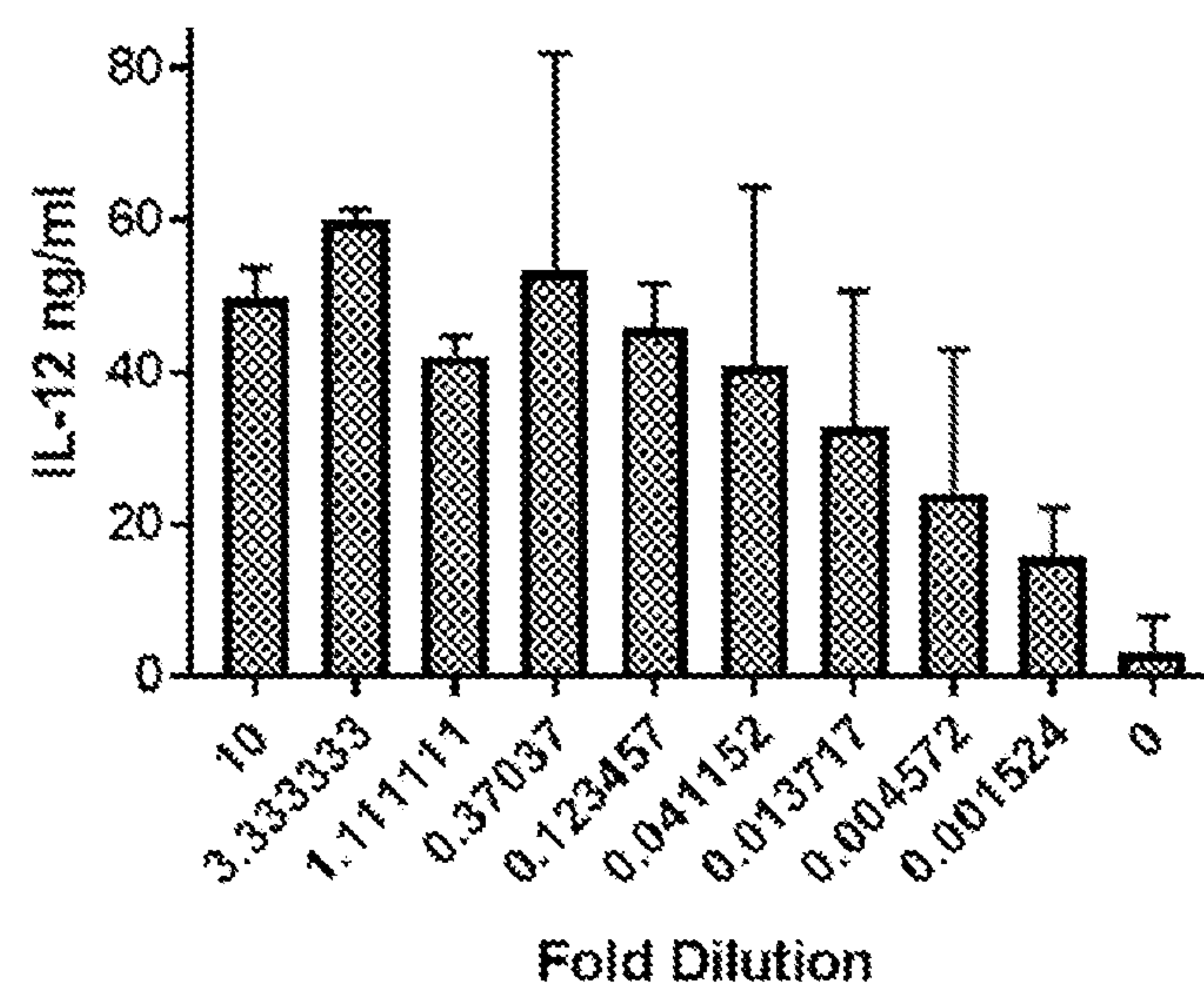


Figure 19A



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Figure 19B



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Figure 19C

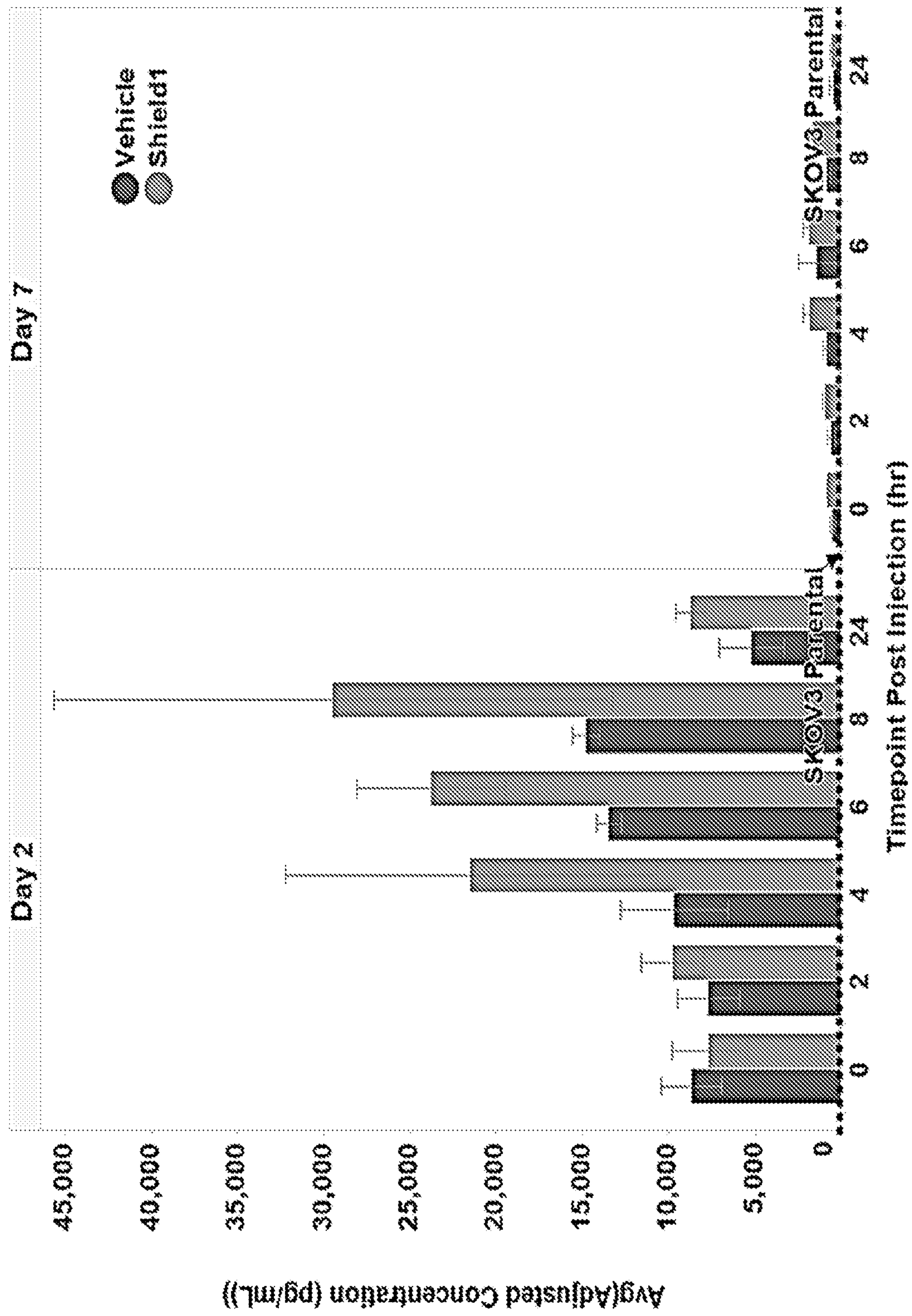


Figure 19D

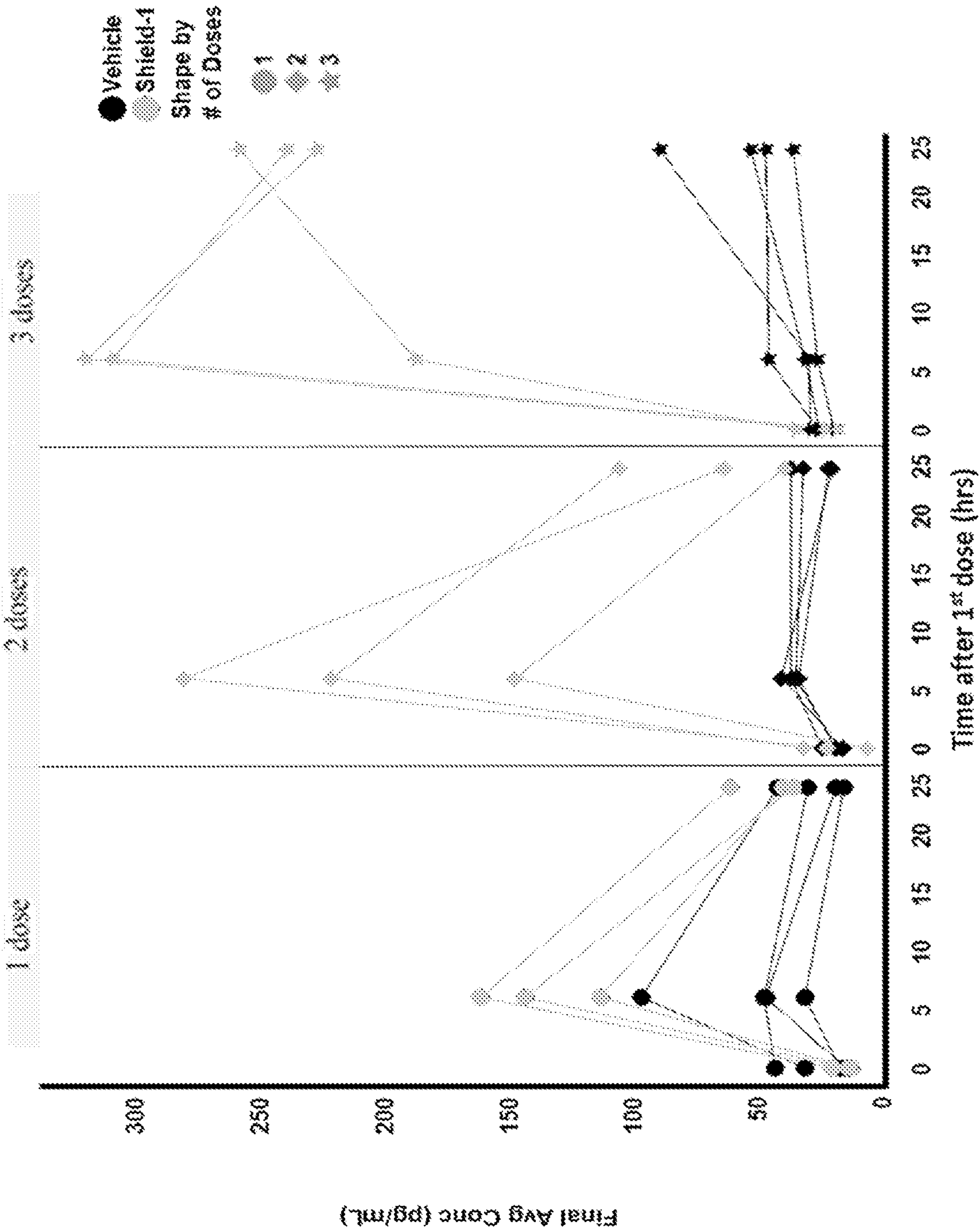


Figure 20A

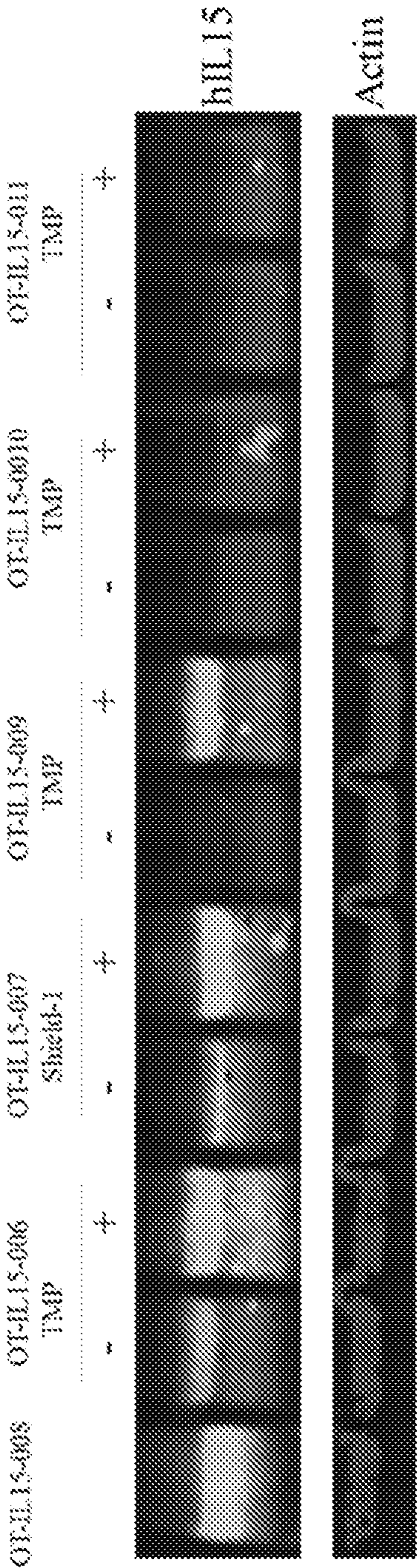
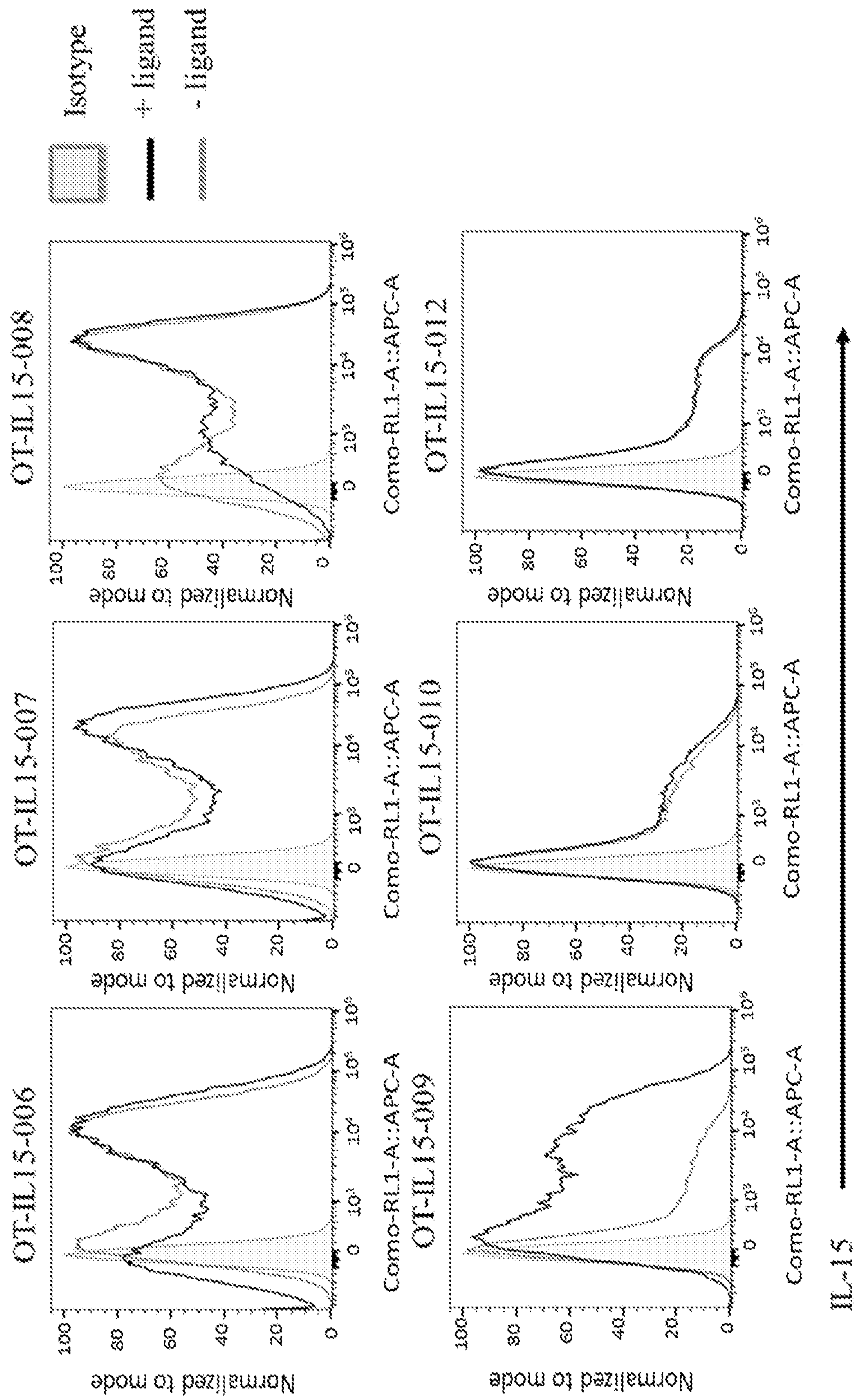


Figure 20B



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Figure 20C

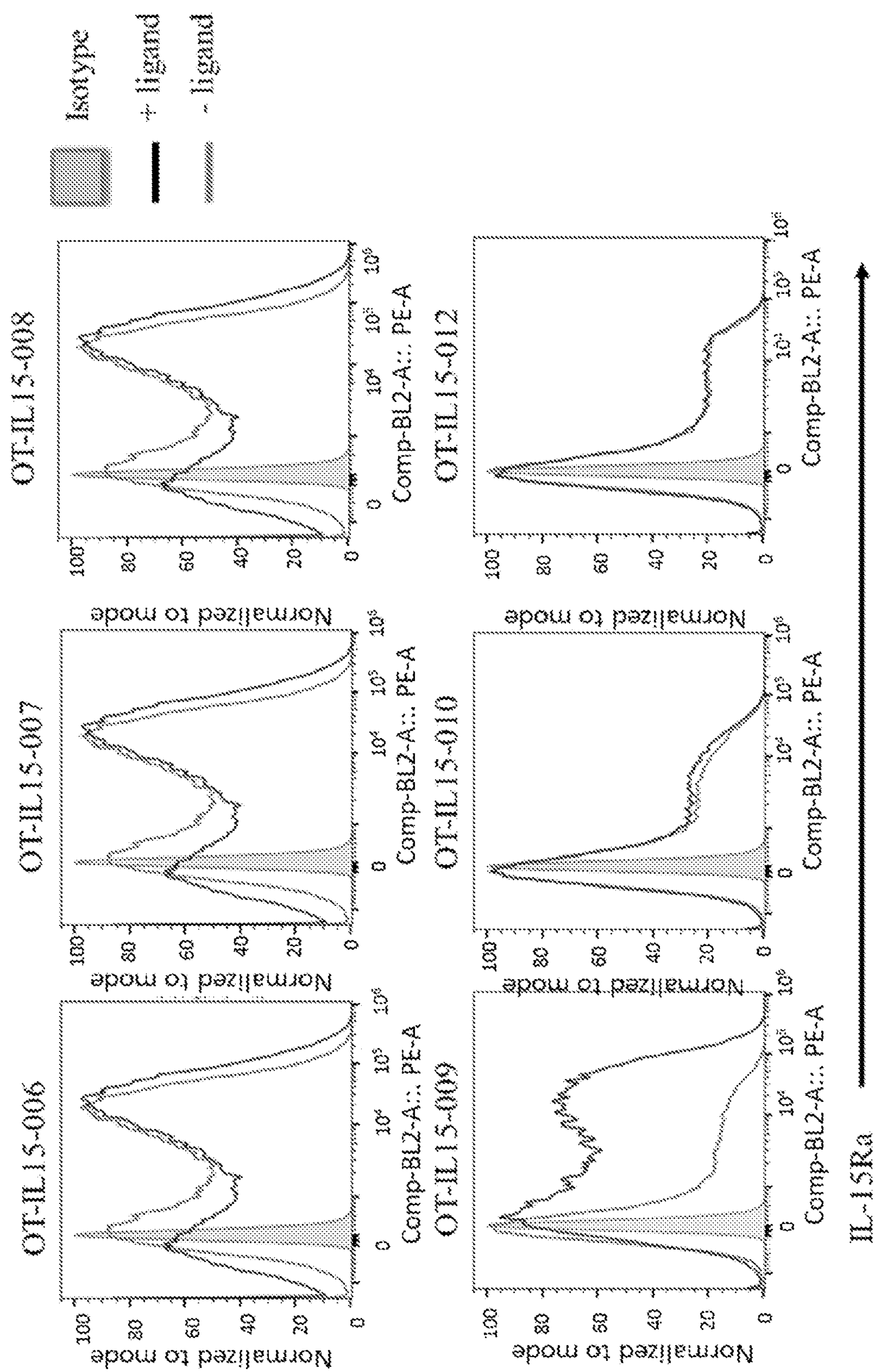
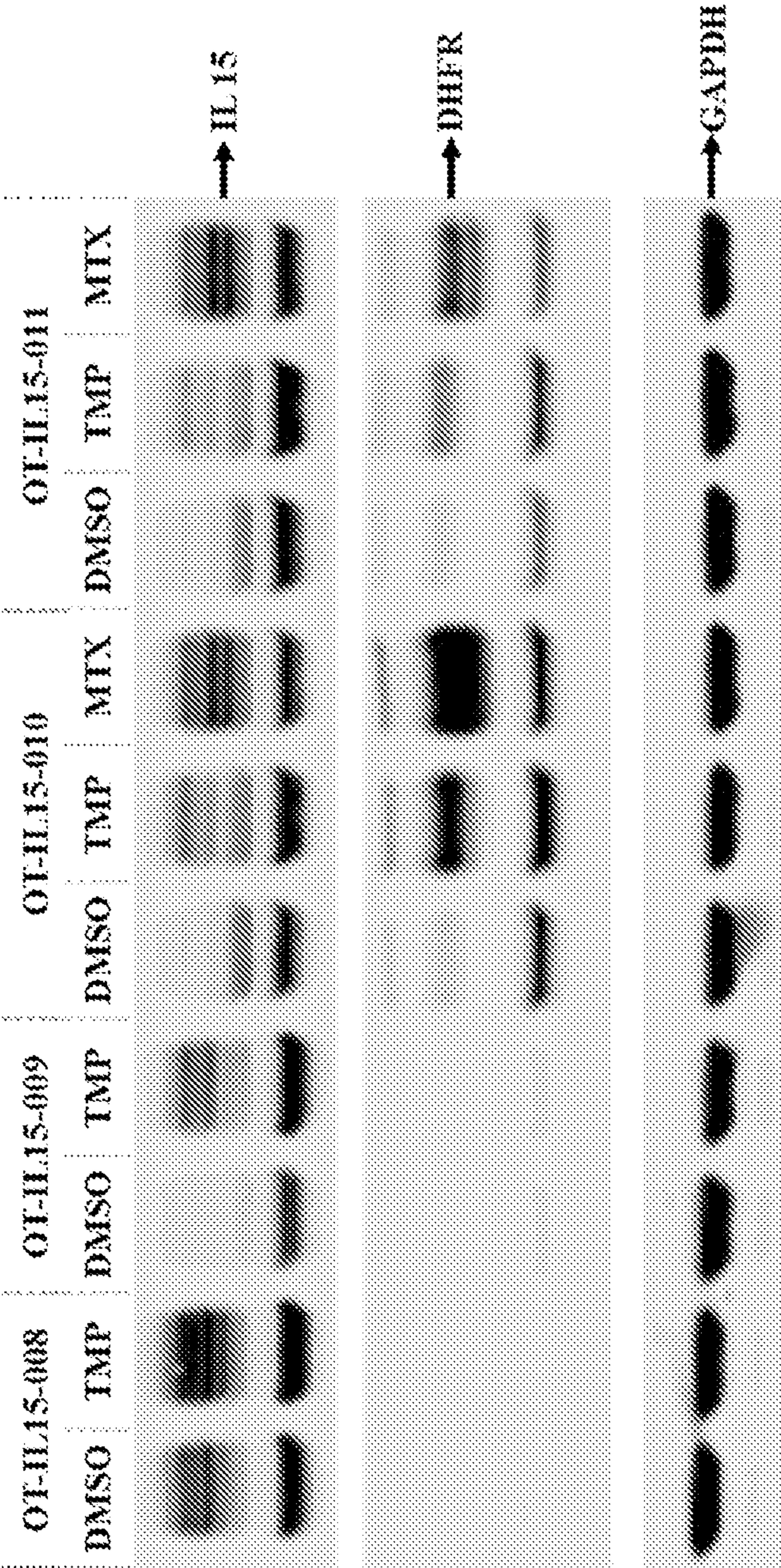


Figure 20D



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Figure 21A

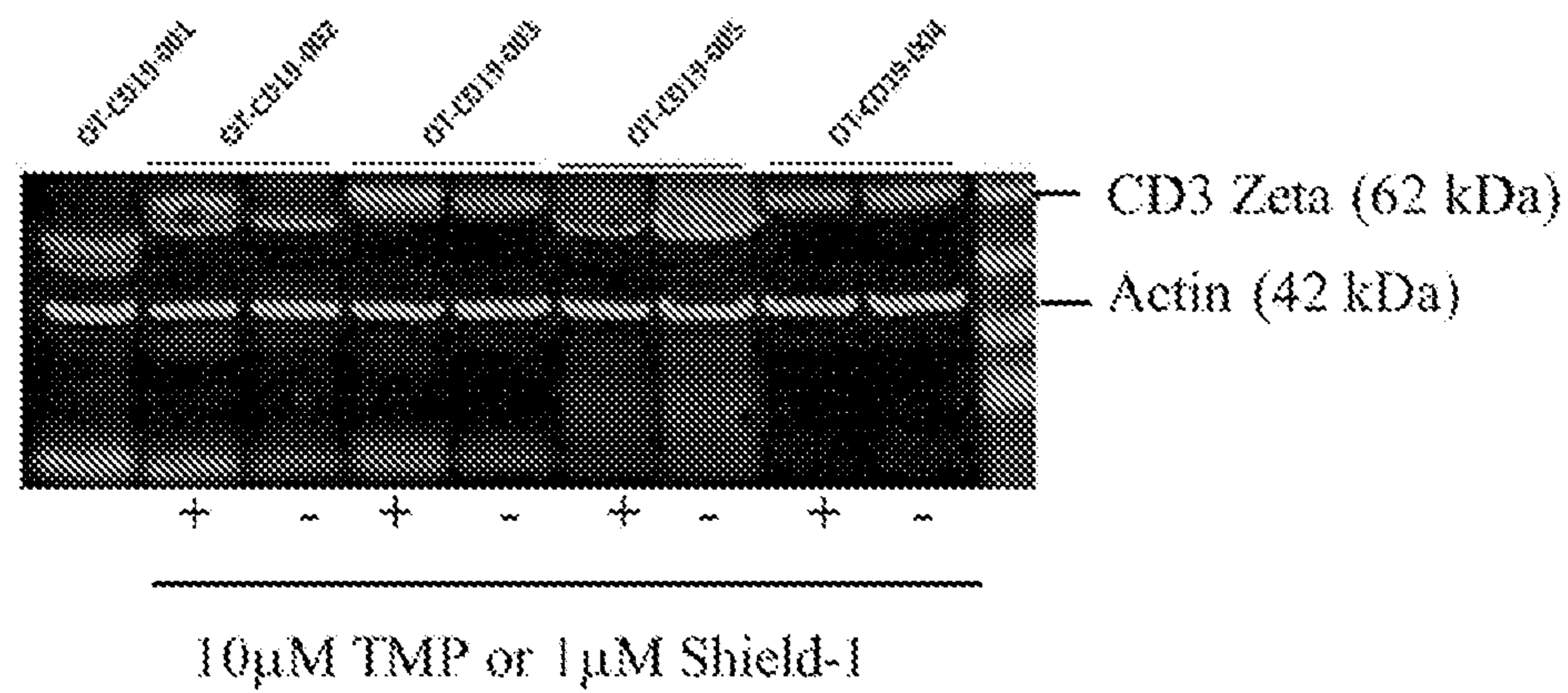


Figure 21B

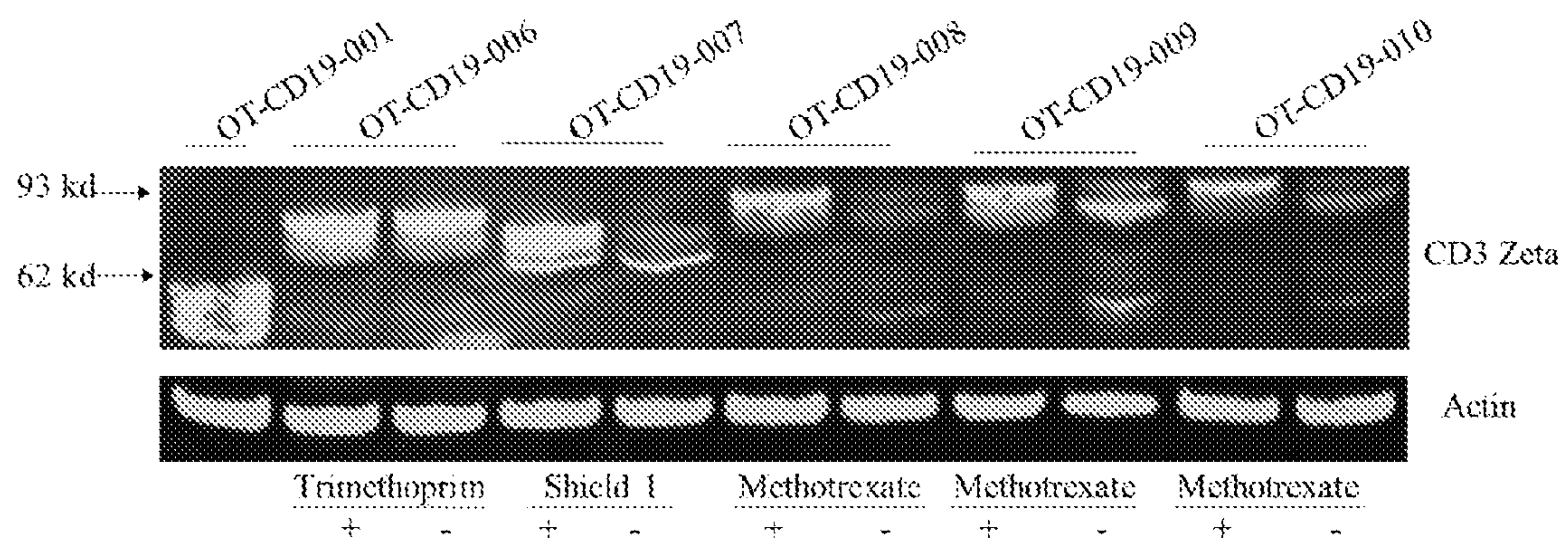
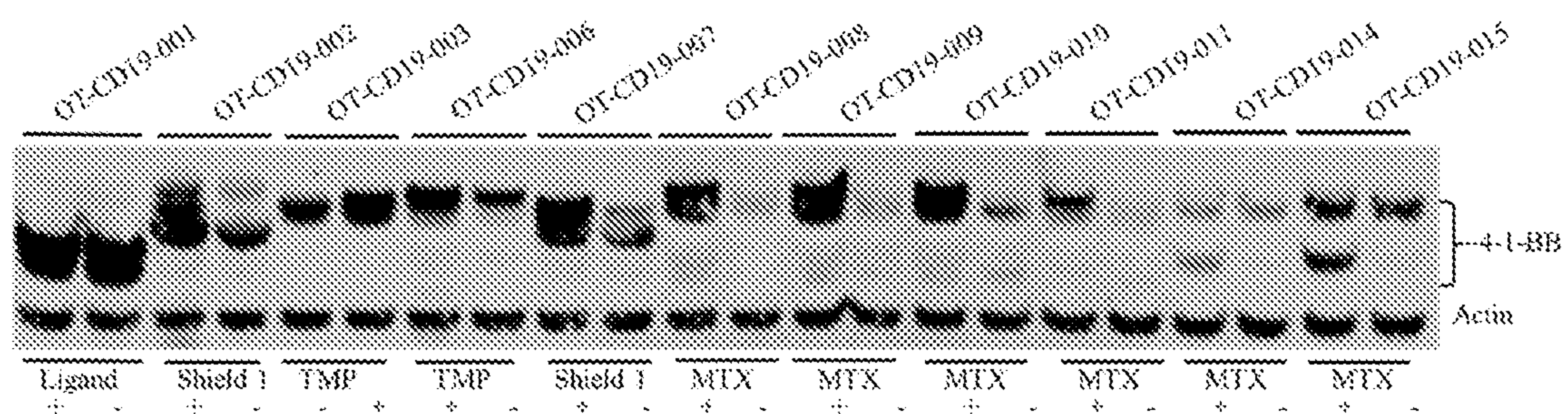


Figure 21C



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Figure 21D

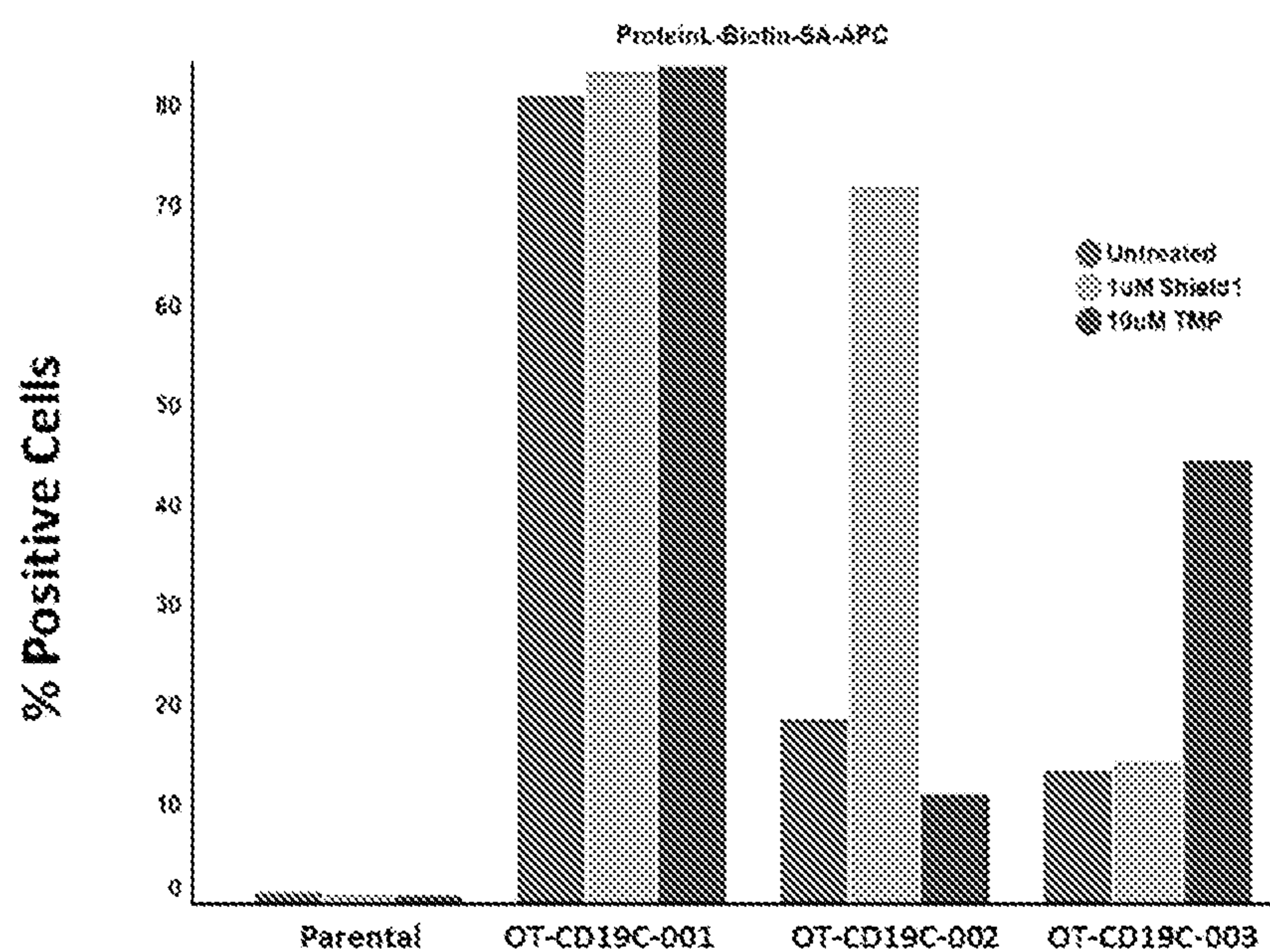
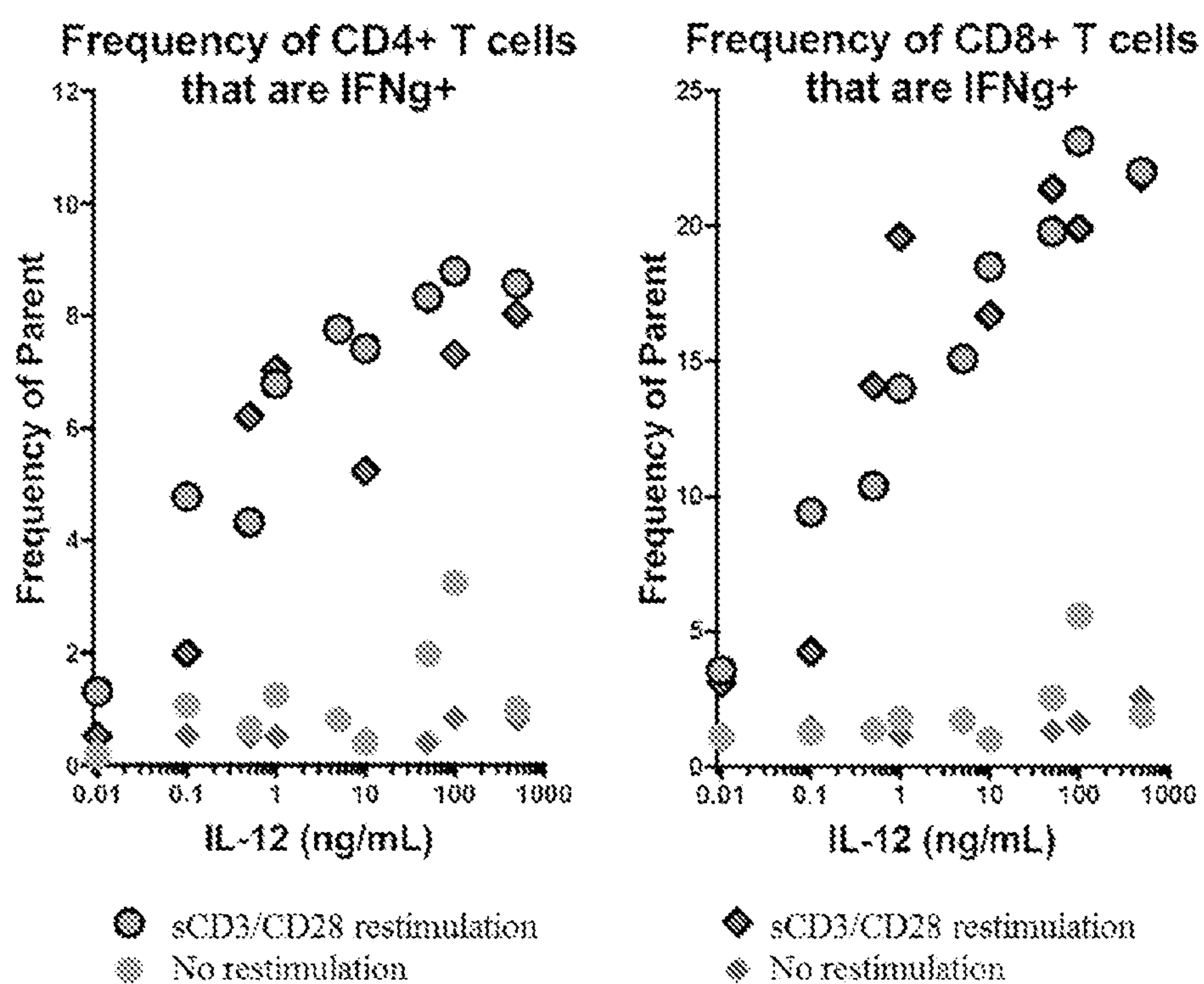


Figure 22



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Figure 23A

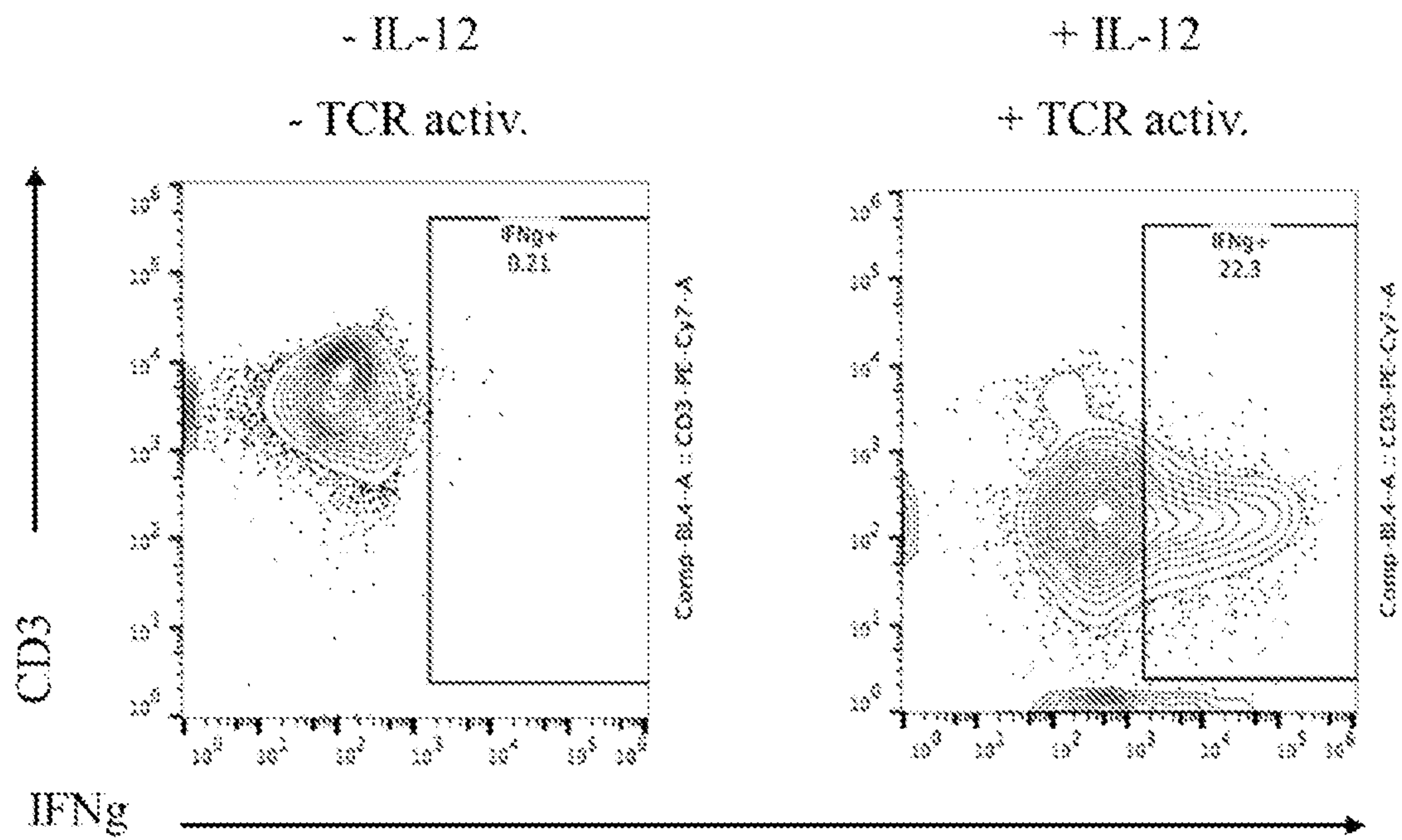
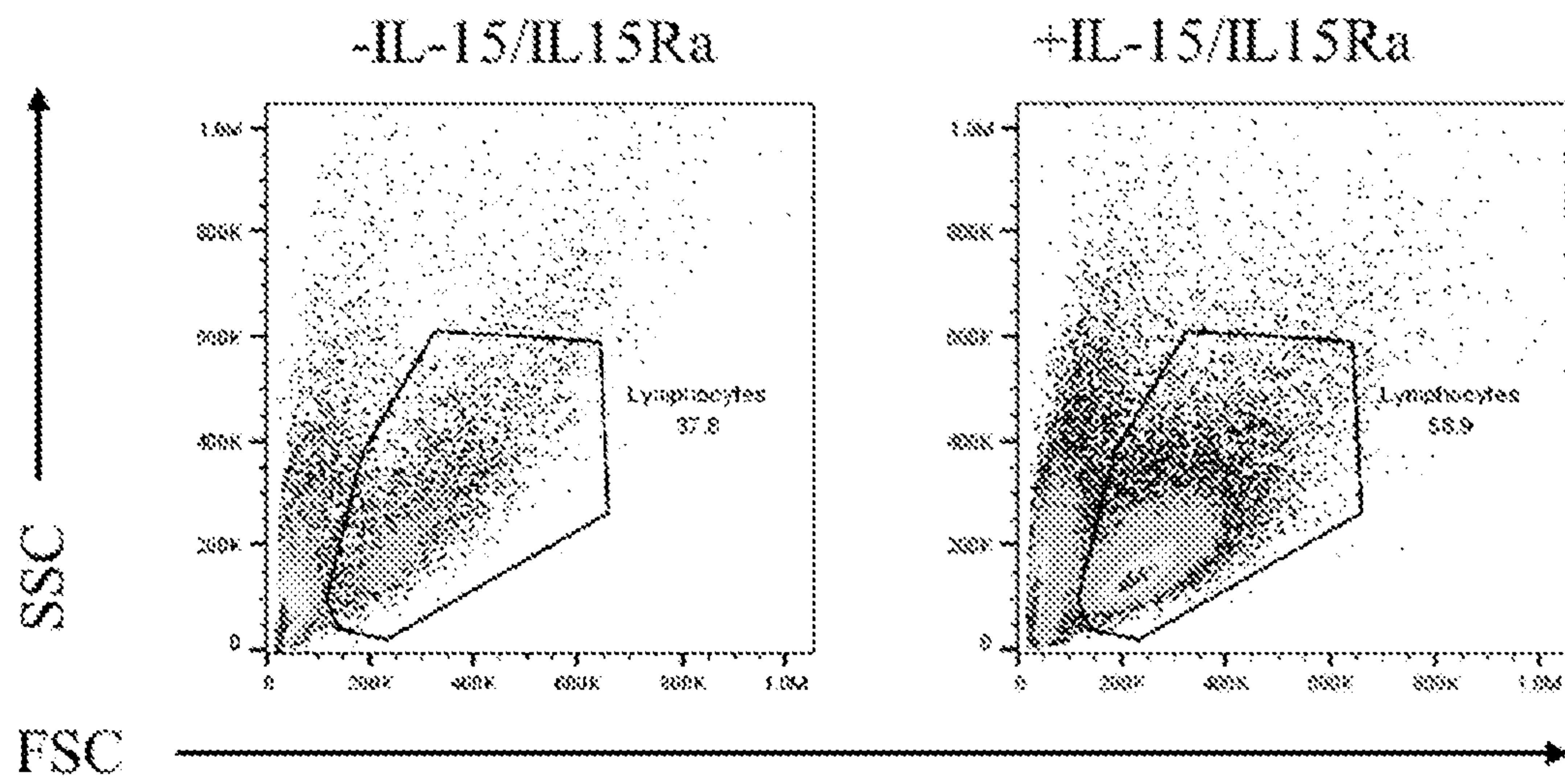


Figure 23B



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Figure 23C

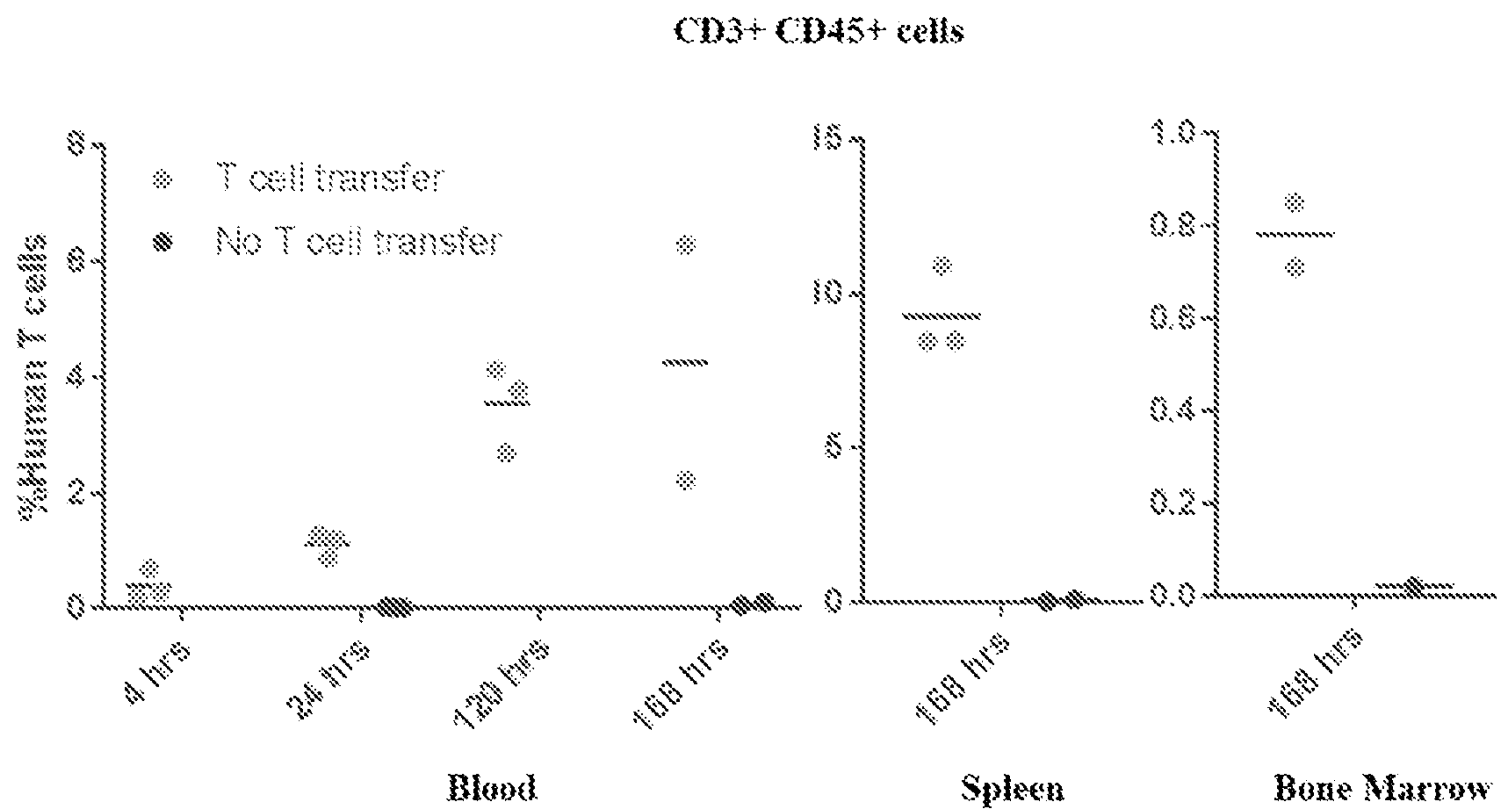
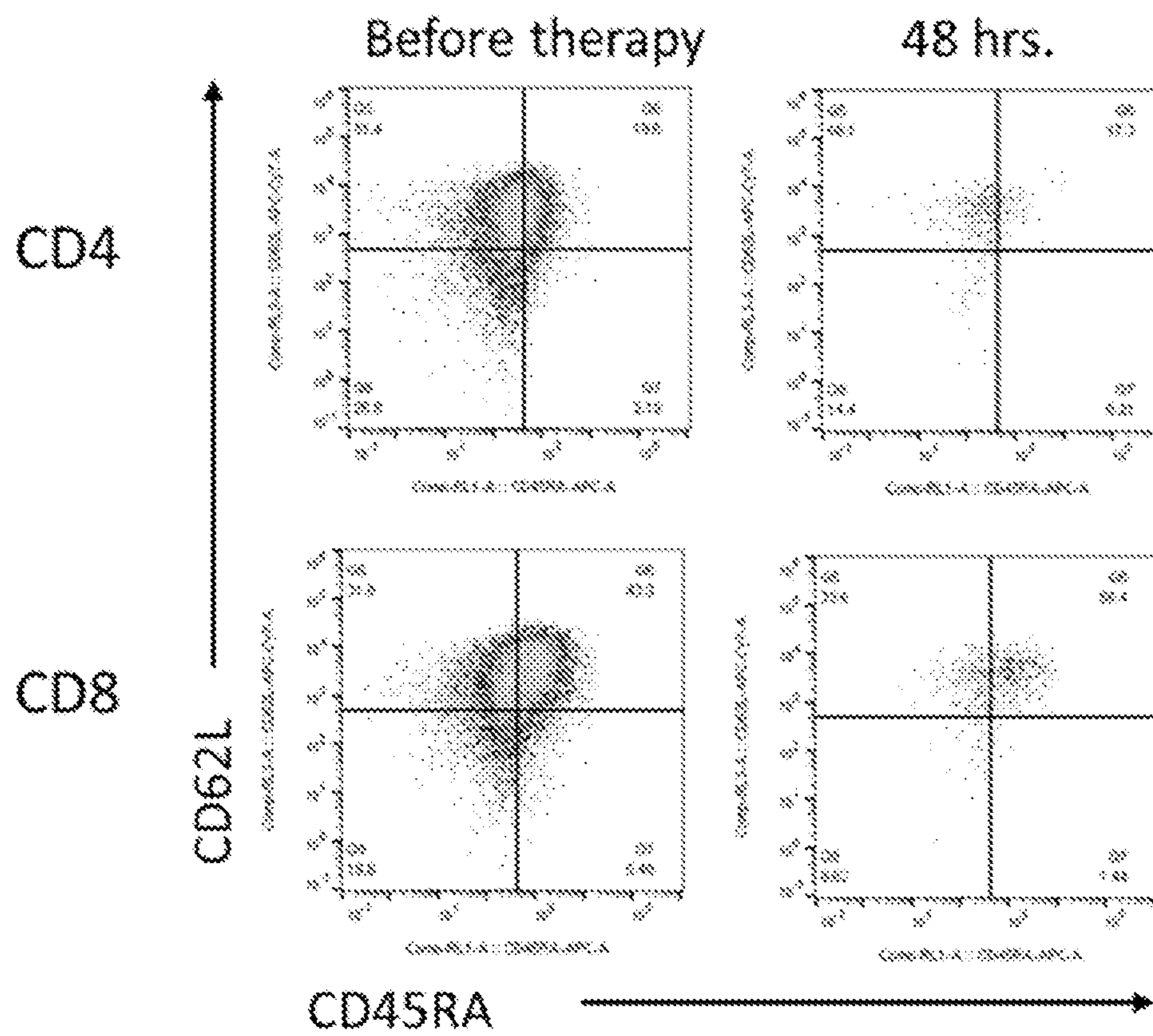


Figure 23D



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Figure 24A

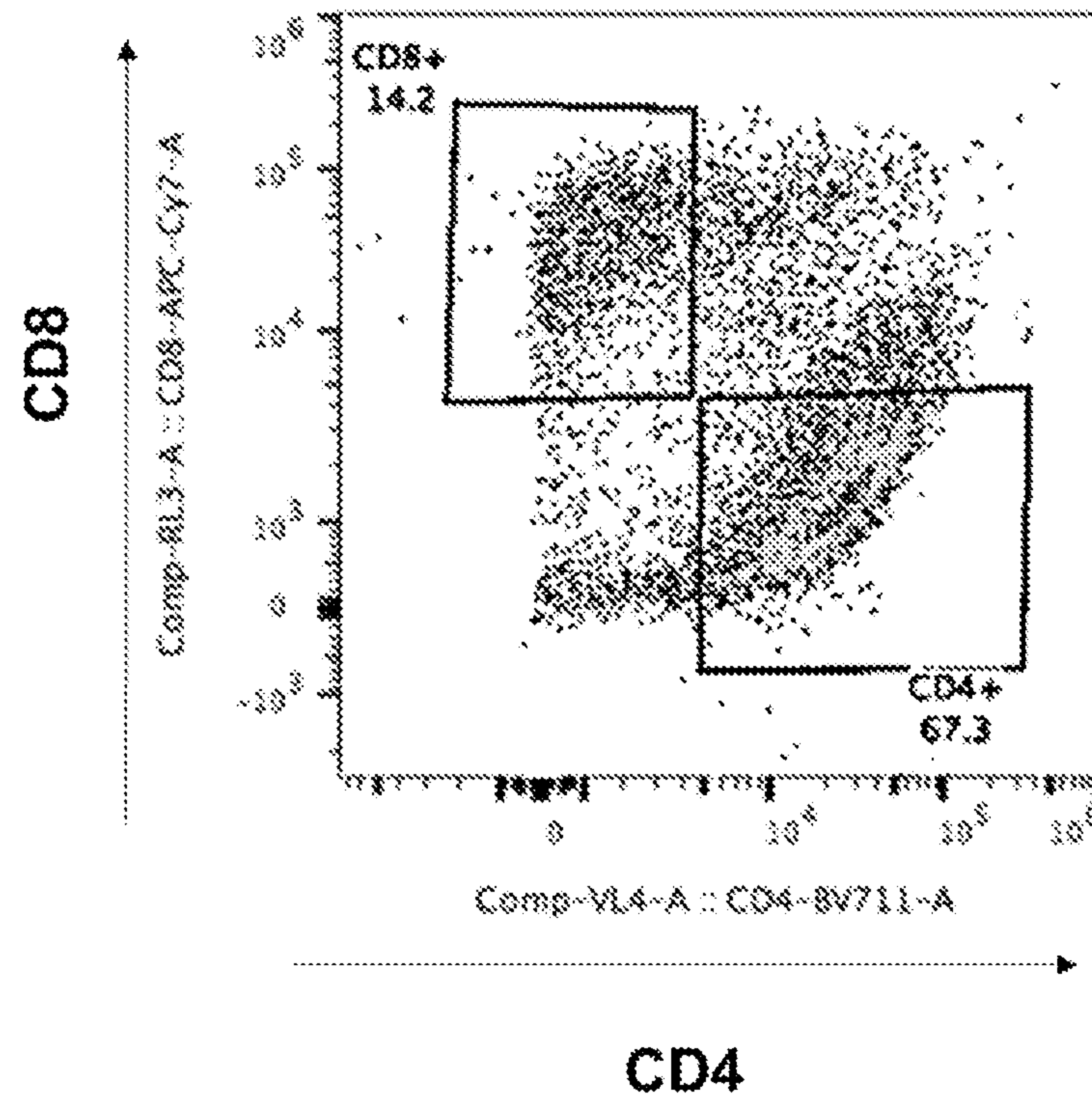
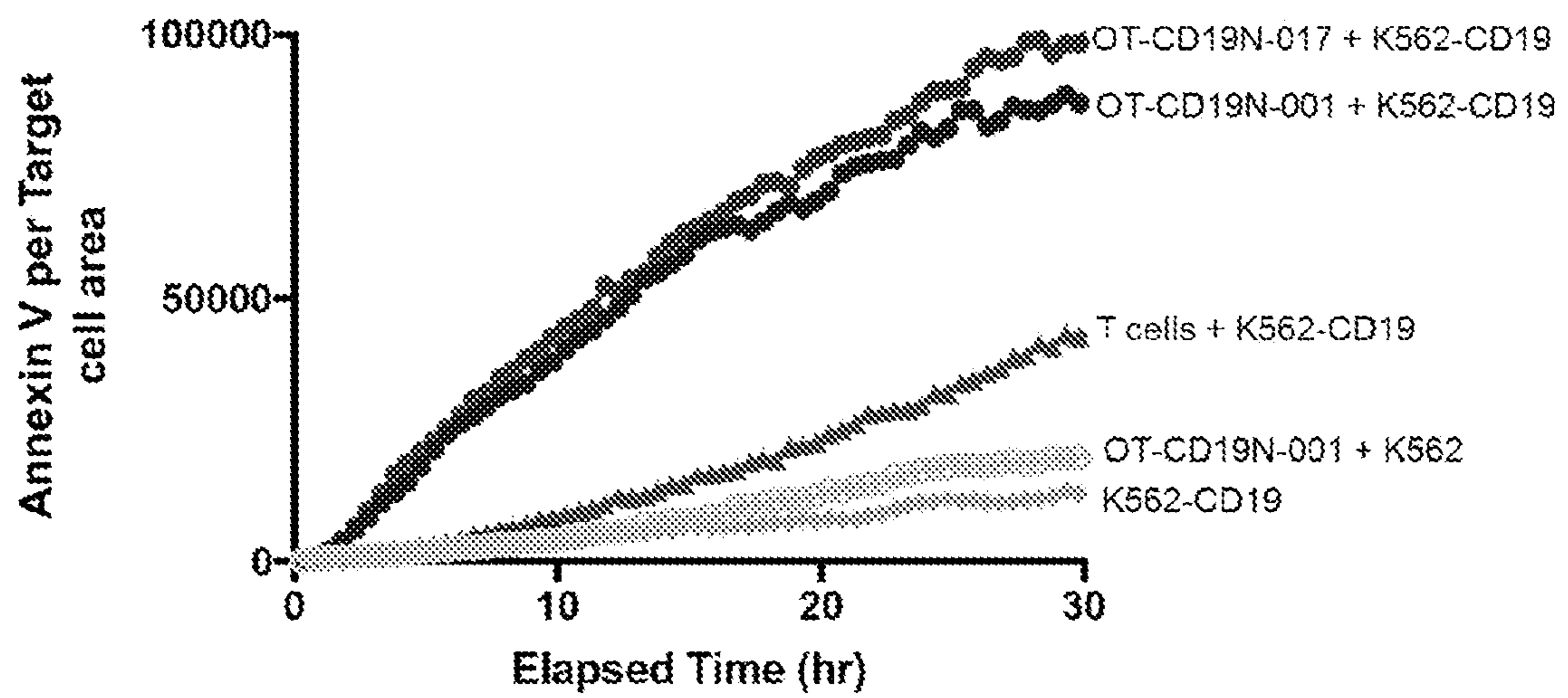


Figure 24B



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Figure 25A

24 hours

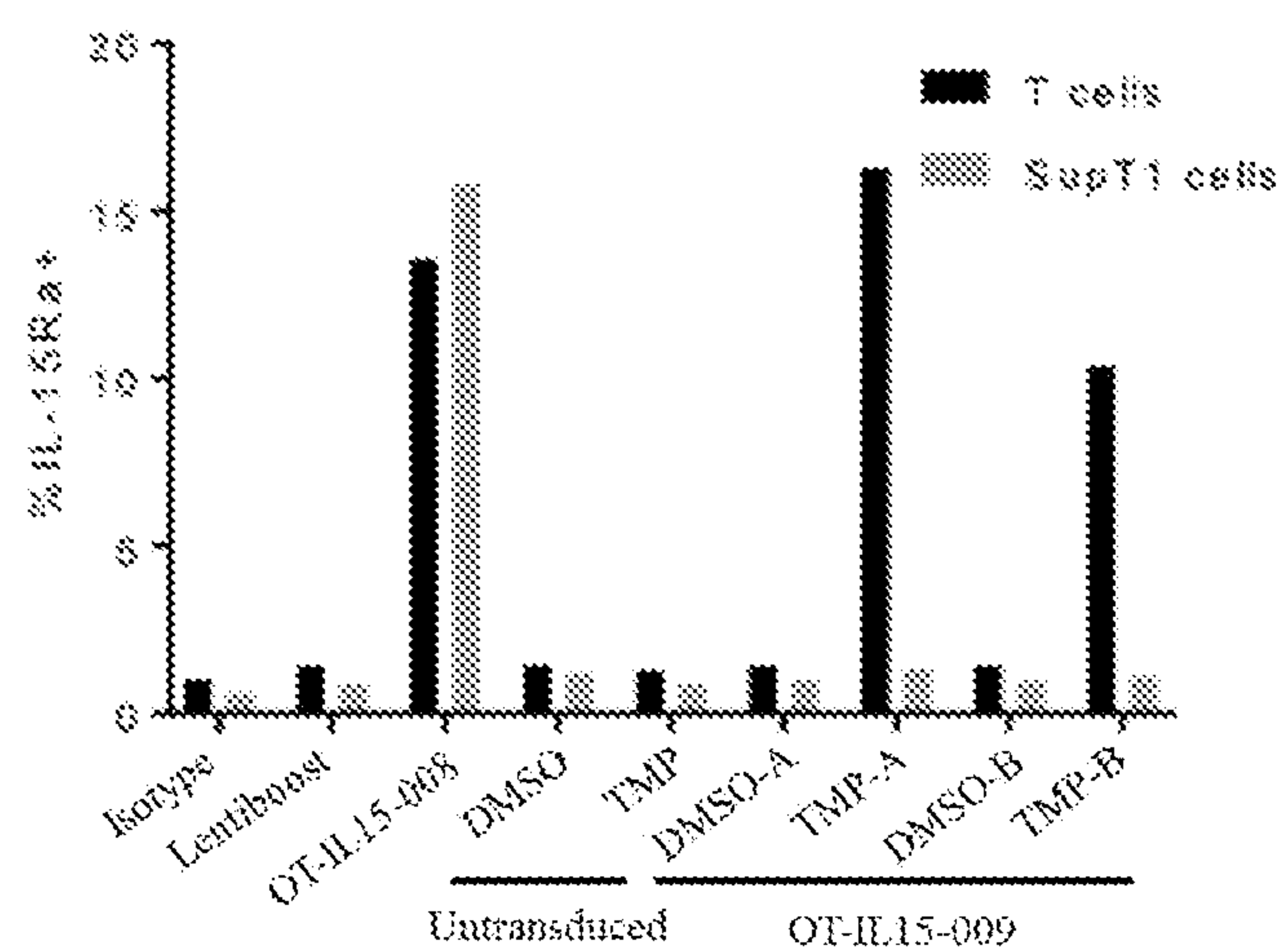


Figure 25B

48 hours

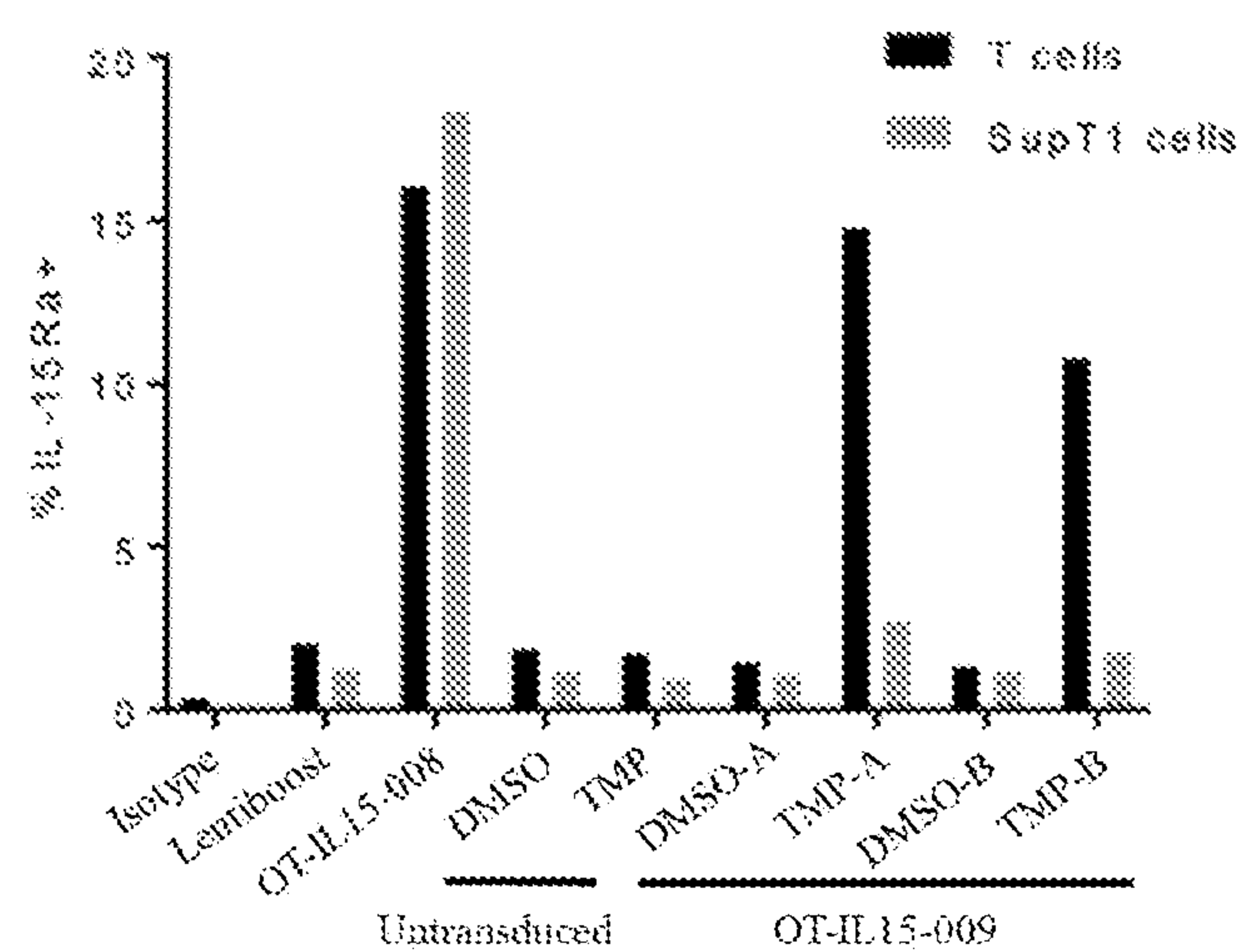
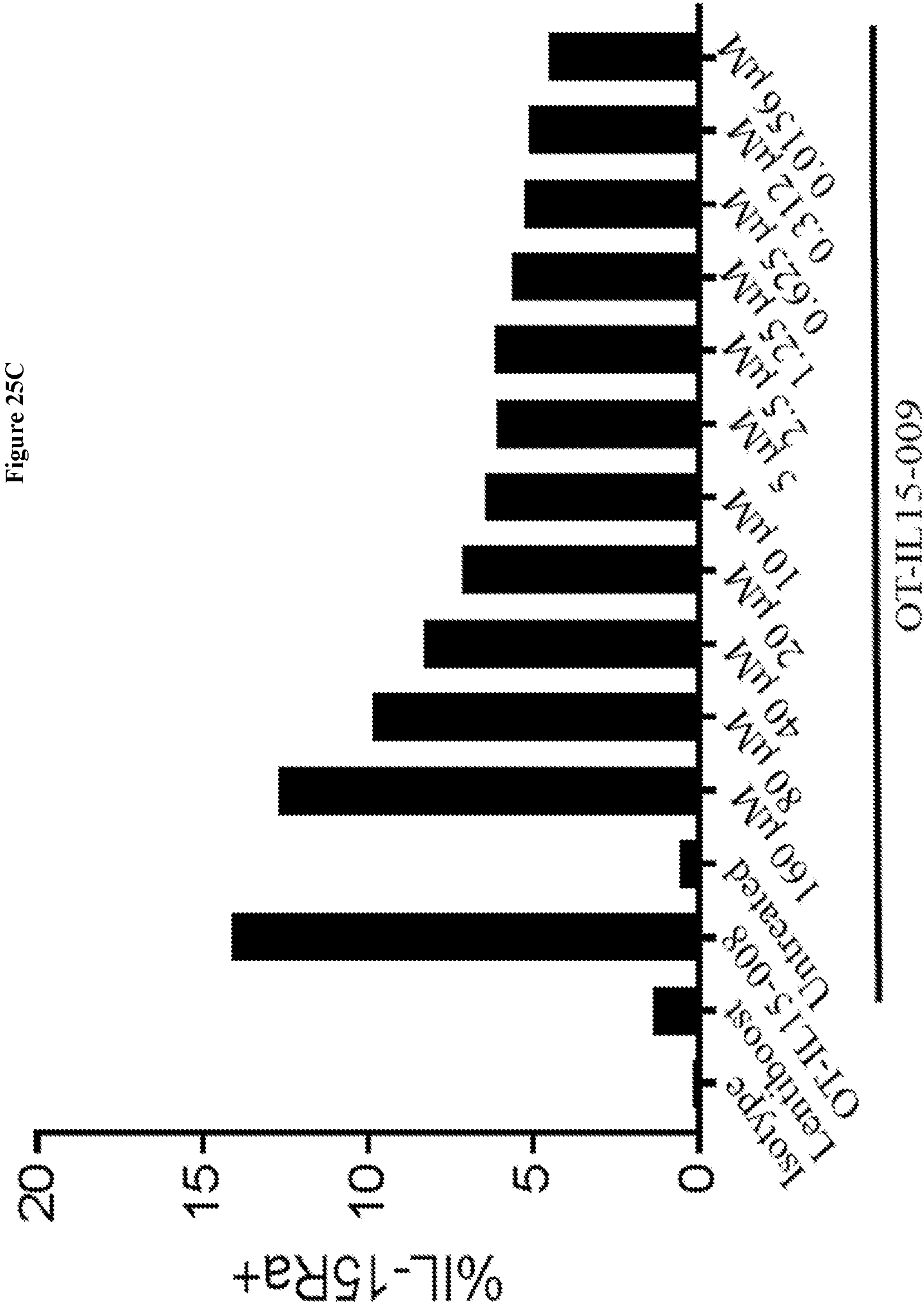
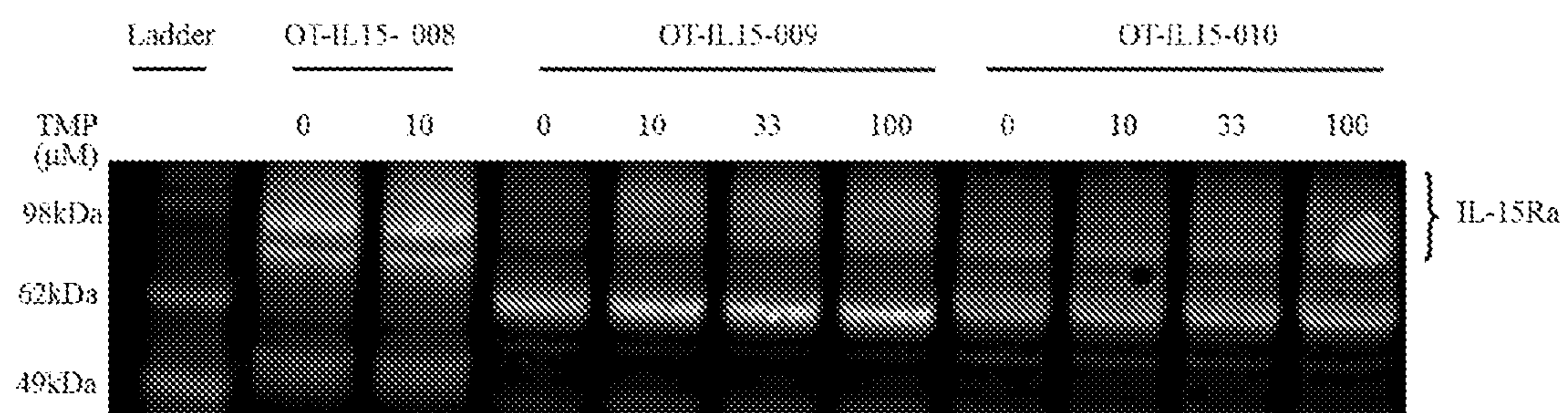


Figure 25C



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Figure 26



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Figure 27A

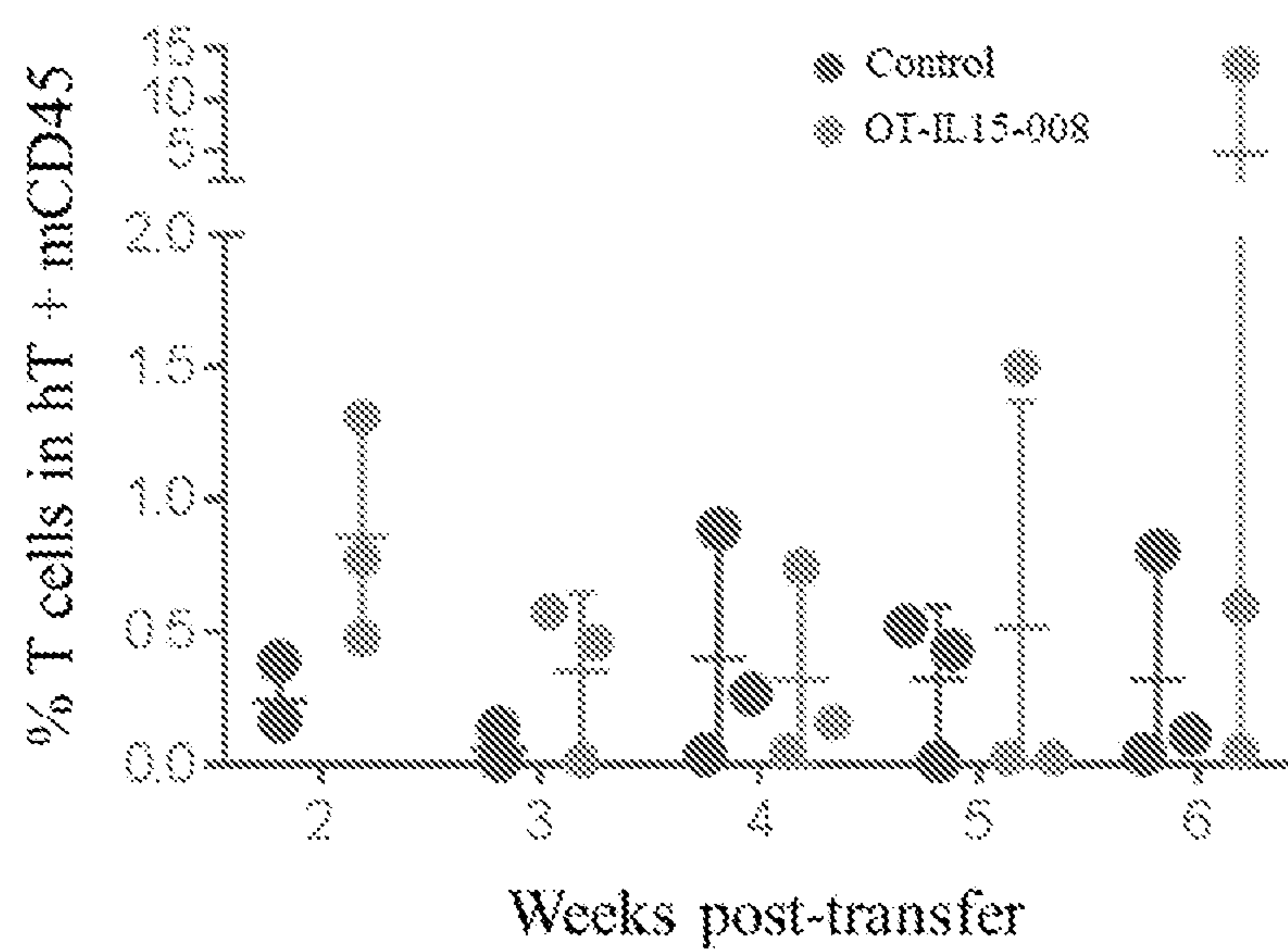
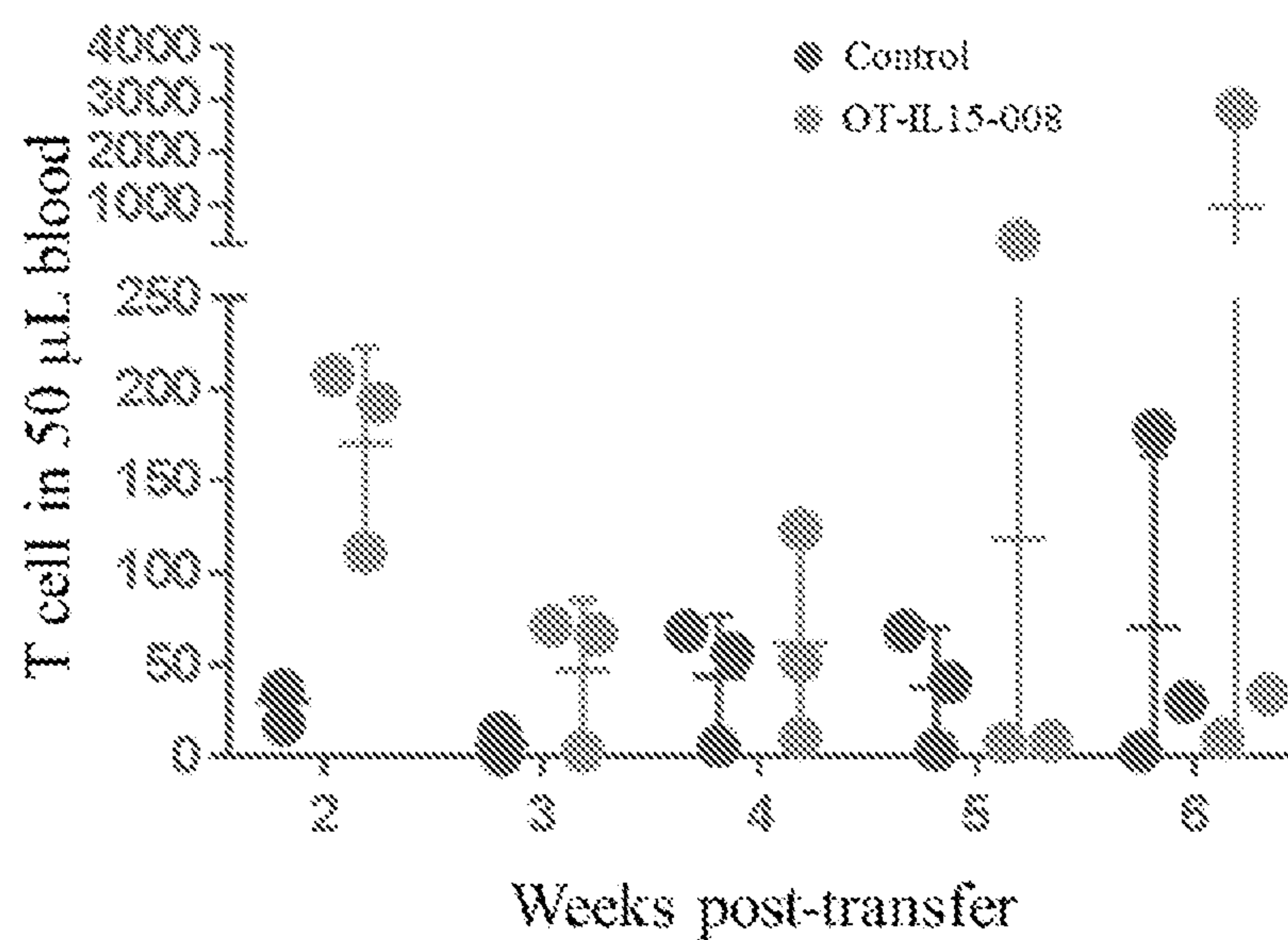


Figure 27B



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Figure 27C

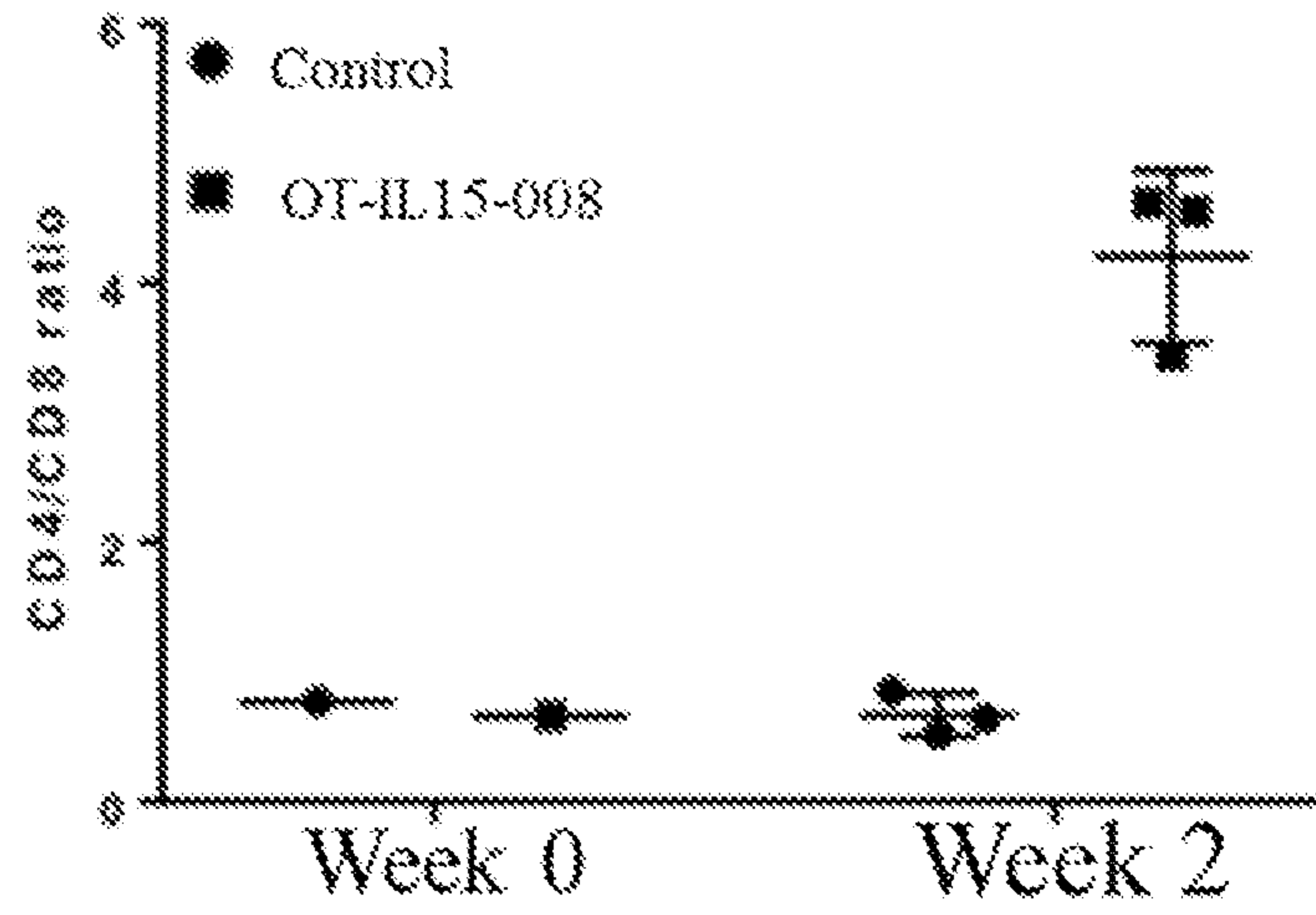
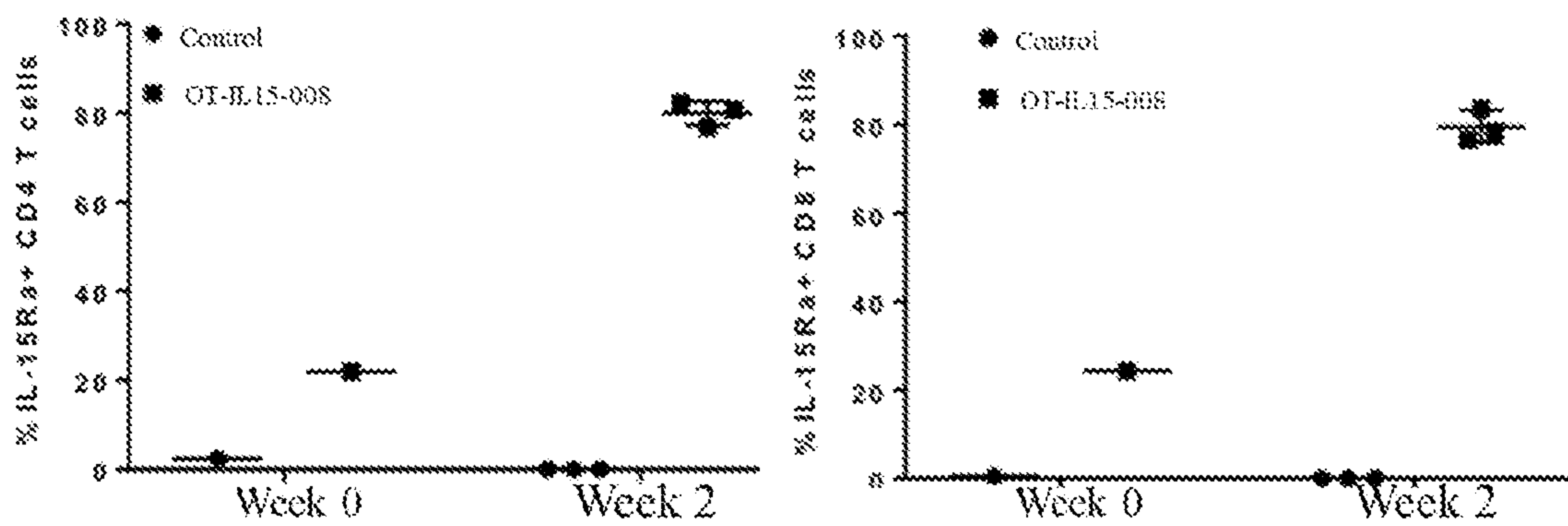
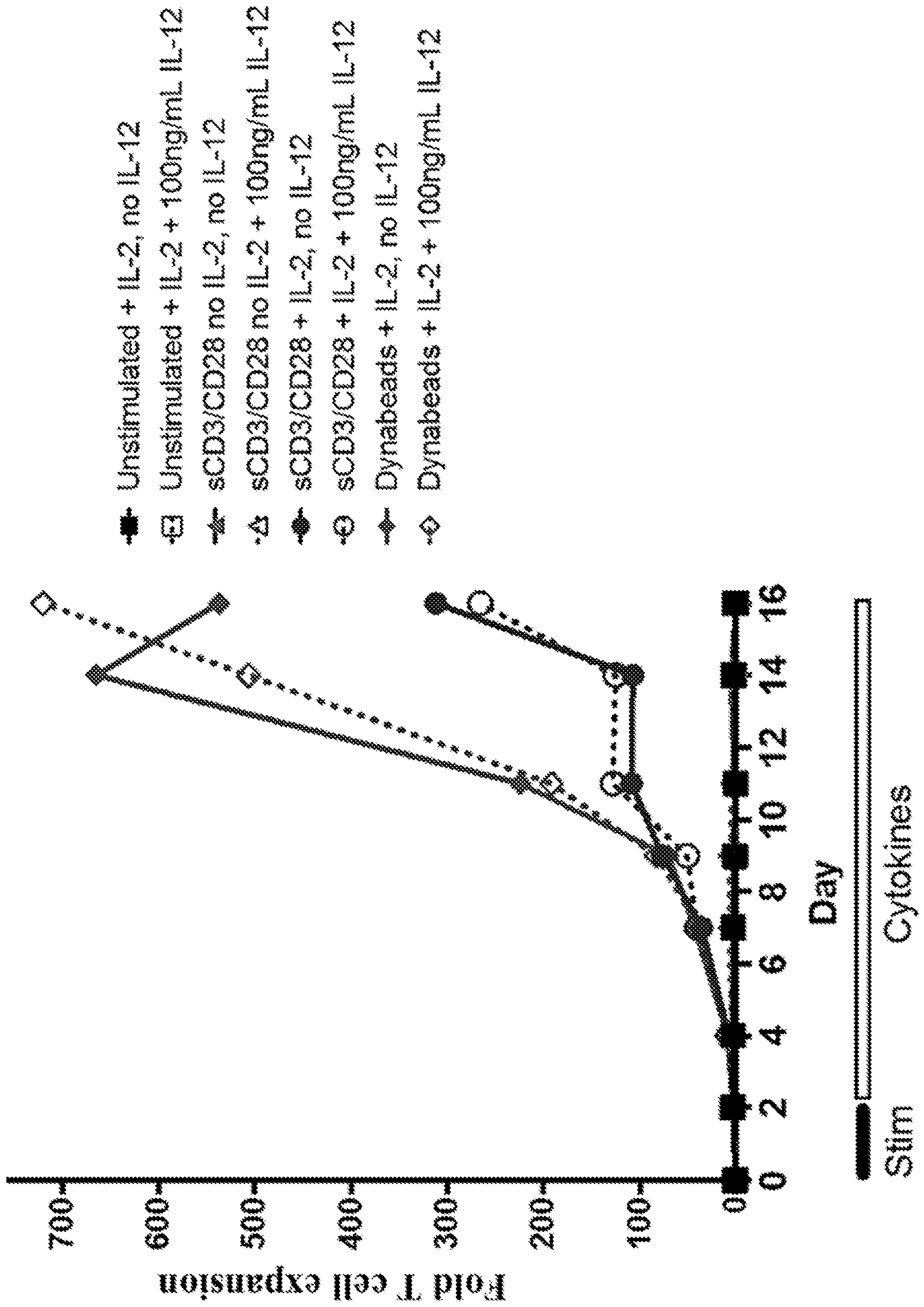


Figure 27D



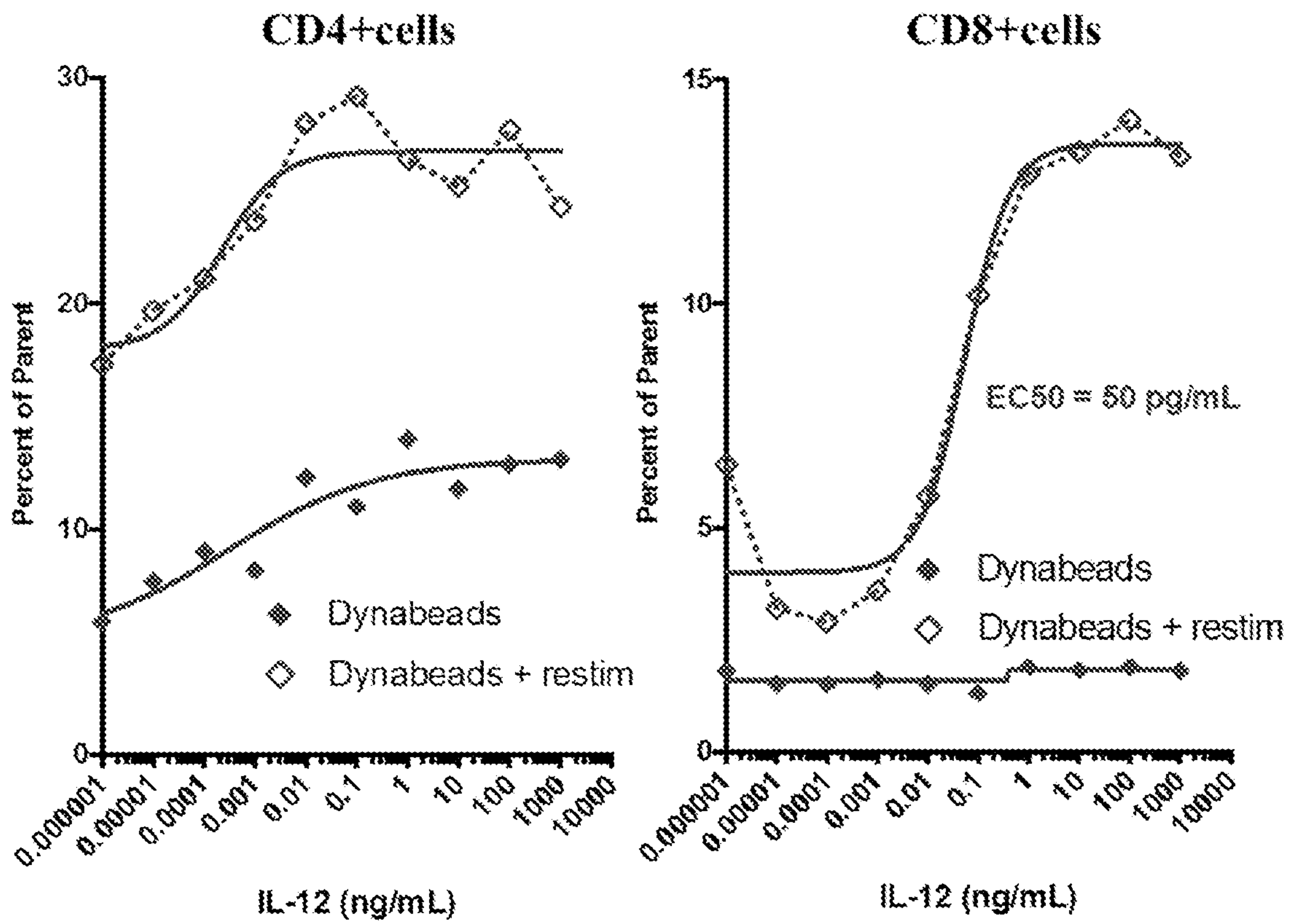
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Figure 28A



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Figure 28B



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Figure 28C

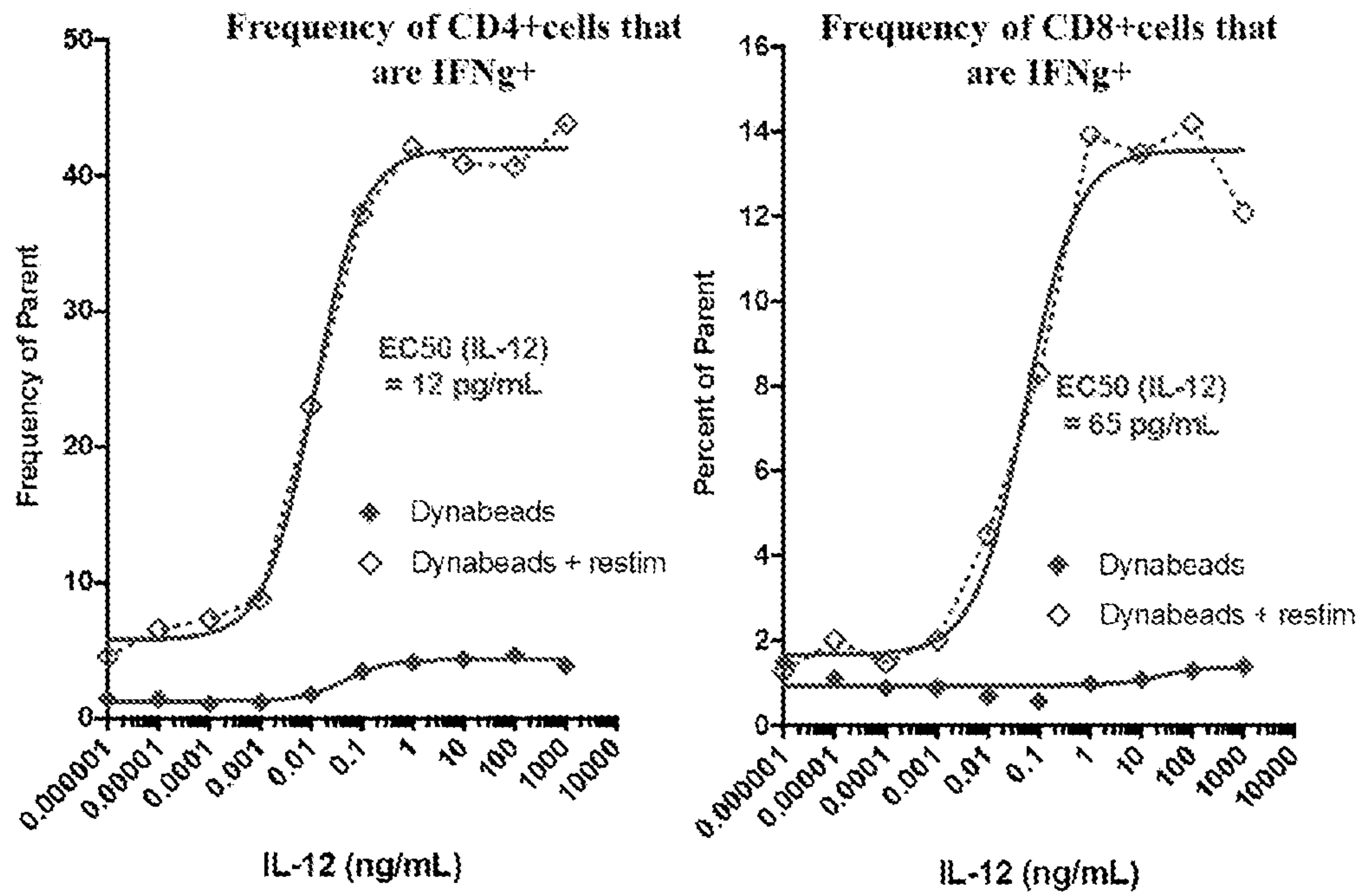
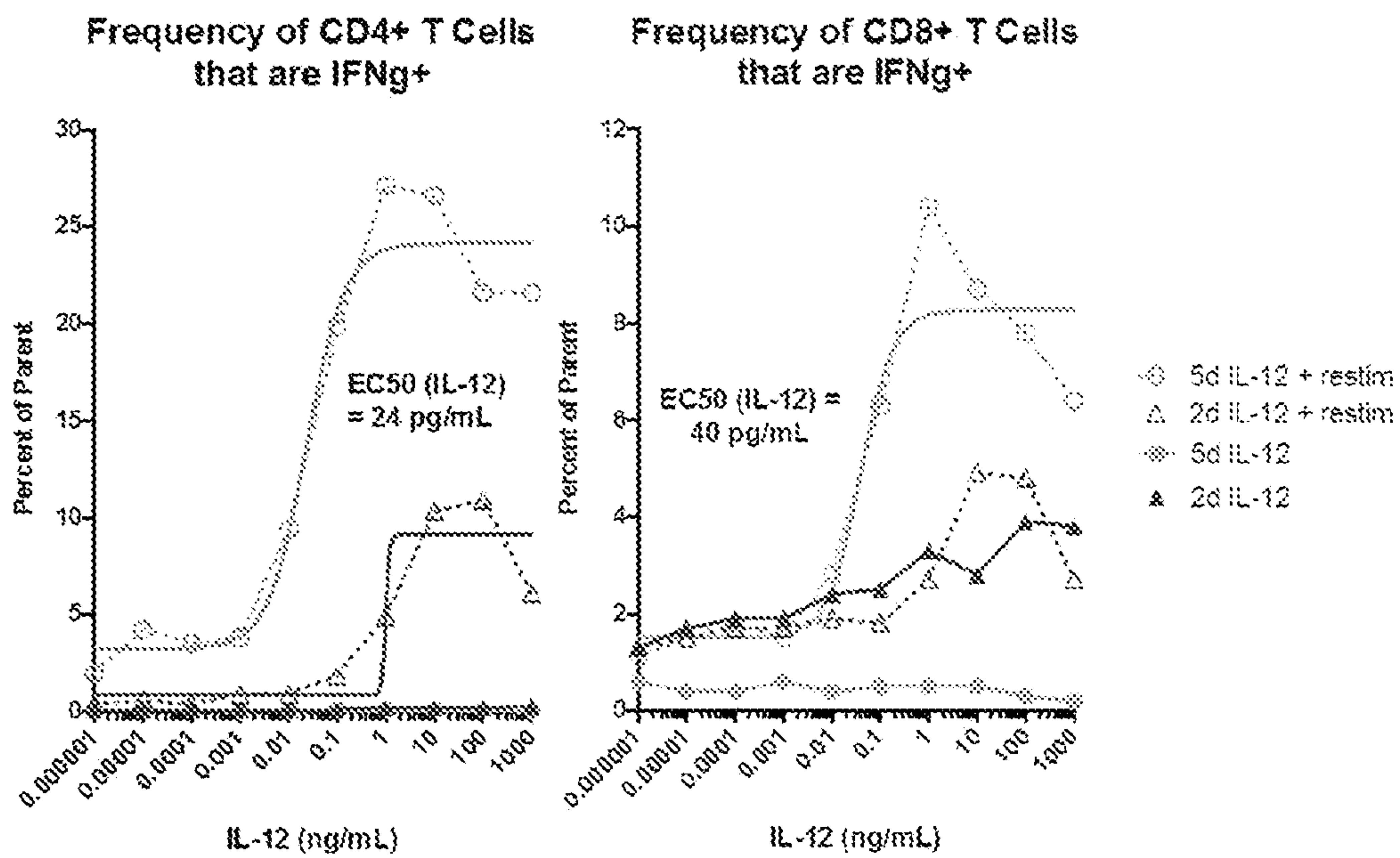


Figure 28D



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Figure 29

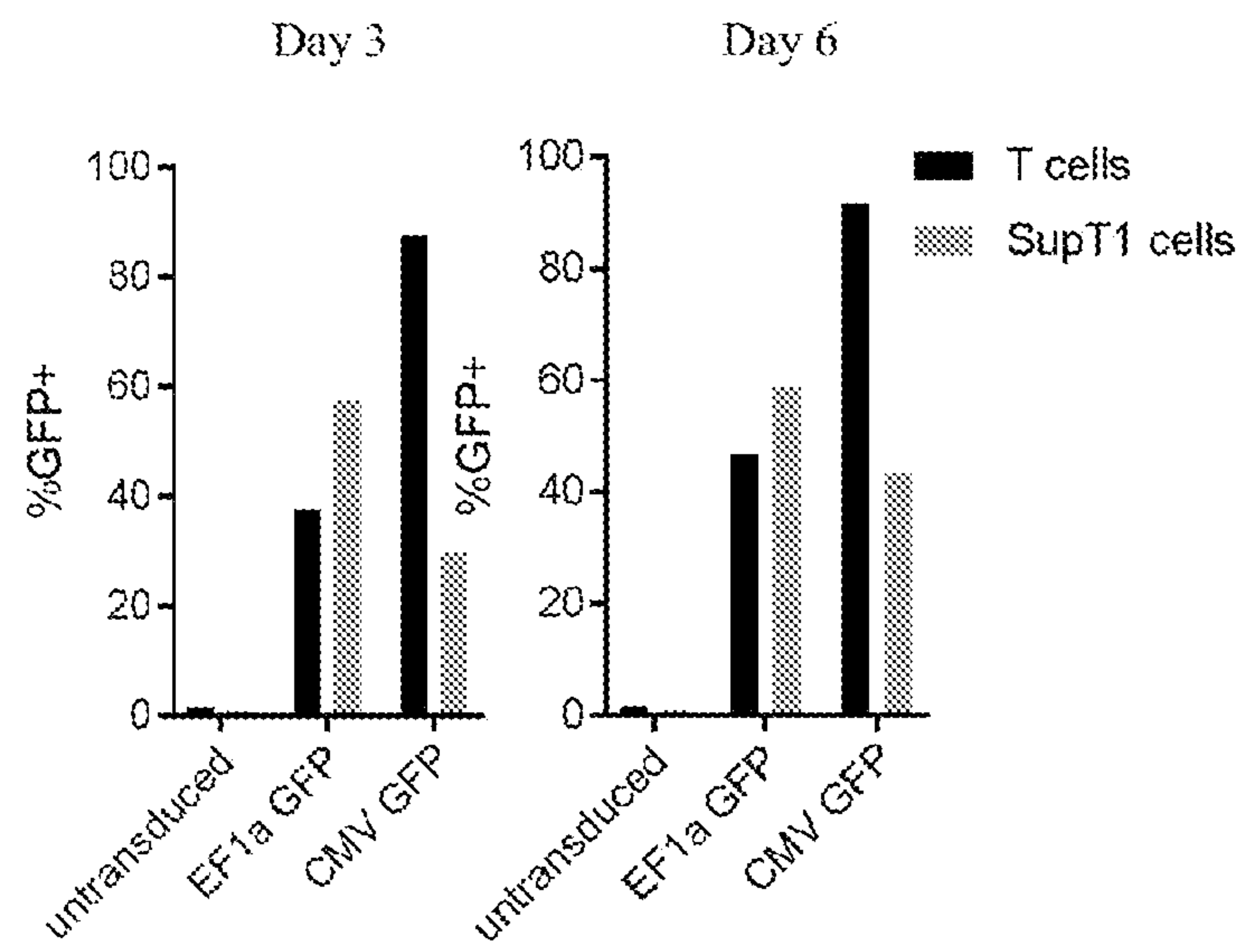
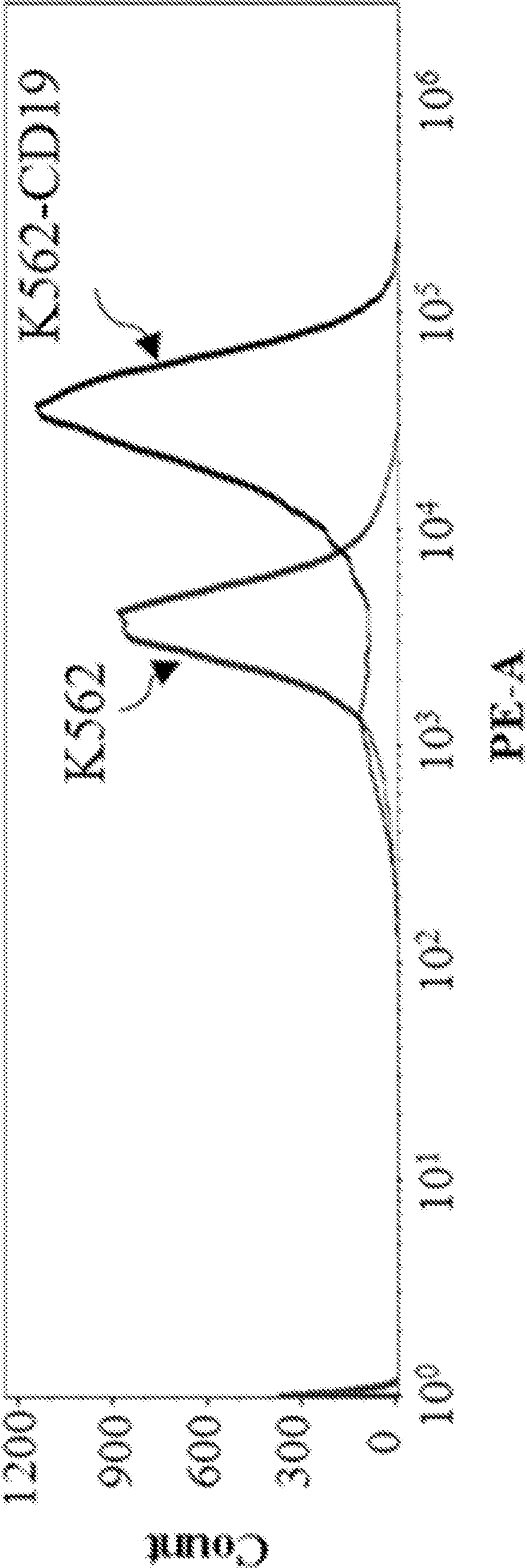


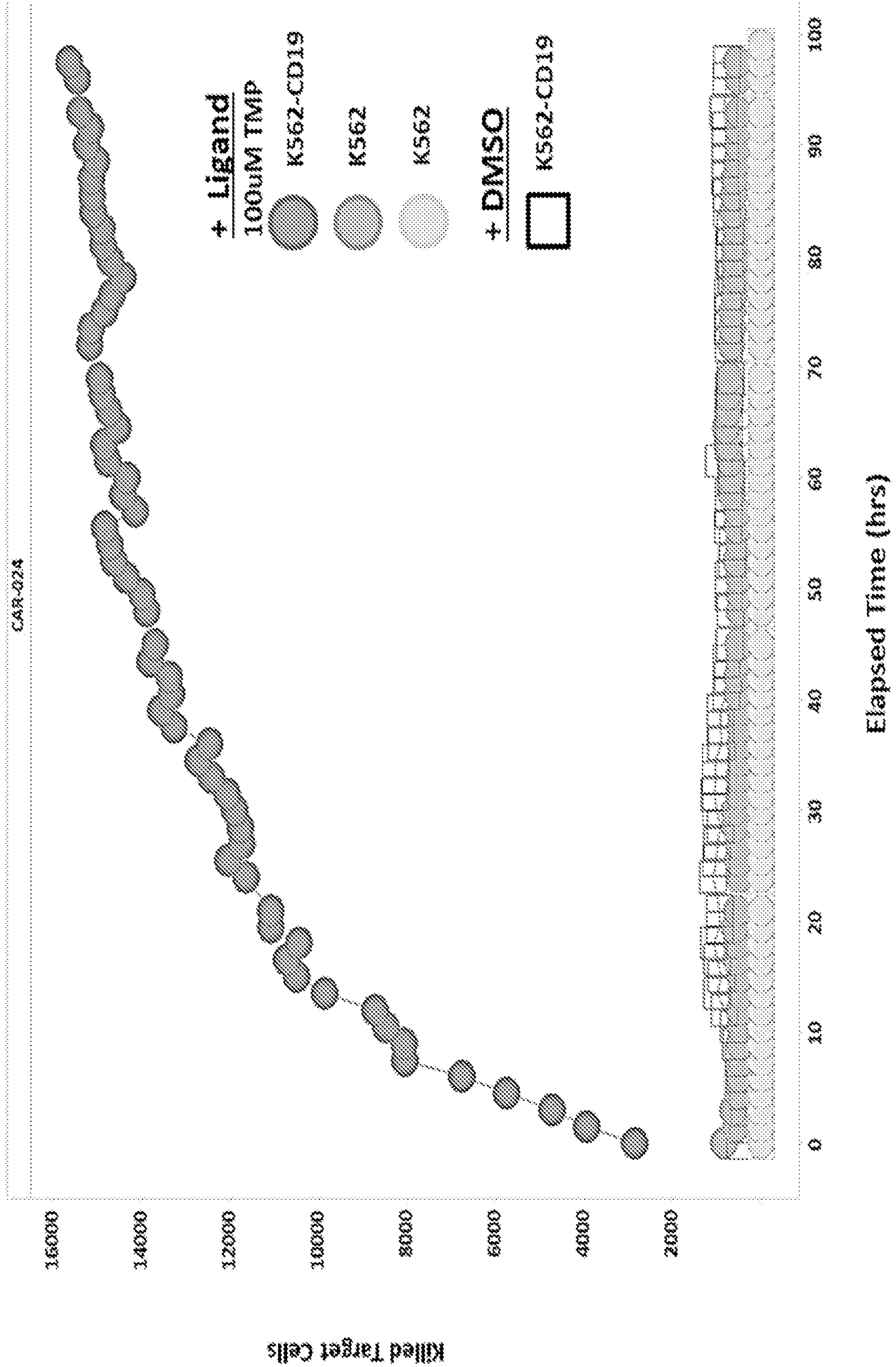
Figure 30A

CD19 expressing cells



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Figure 30B



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Figure 30C

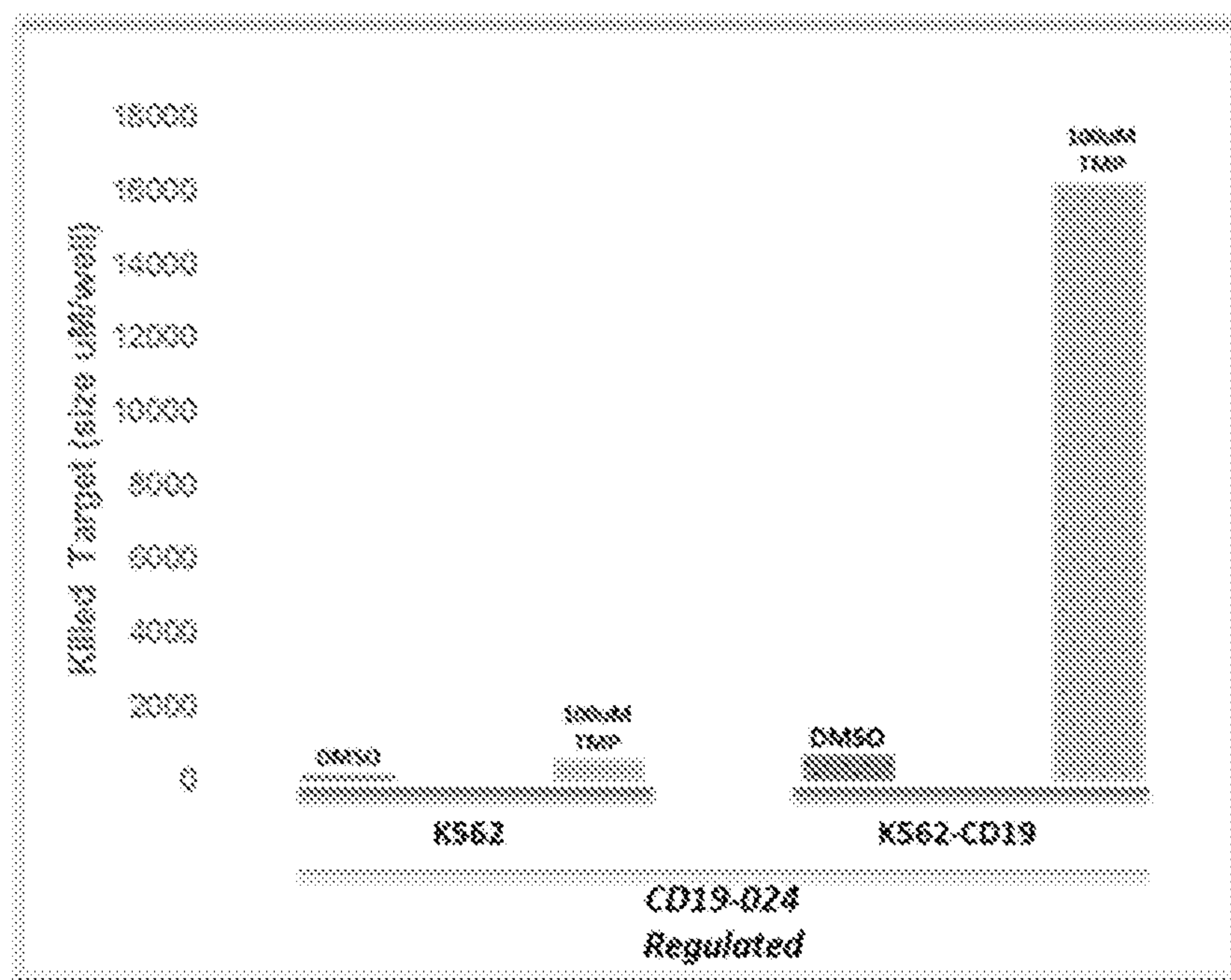


Figure 31A

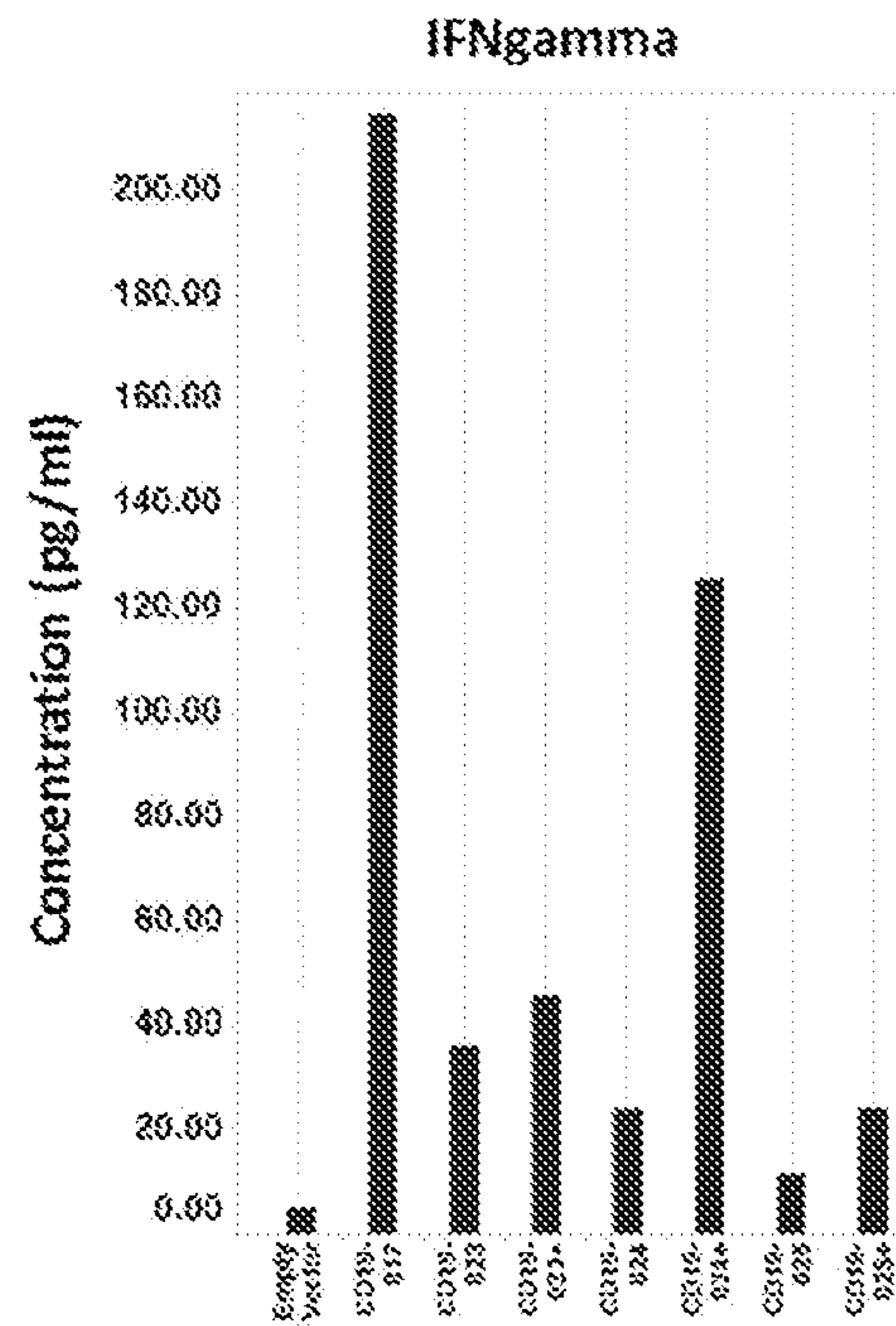


Figure 31B

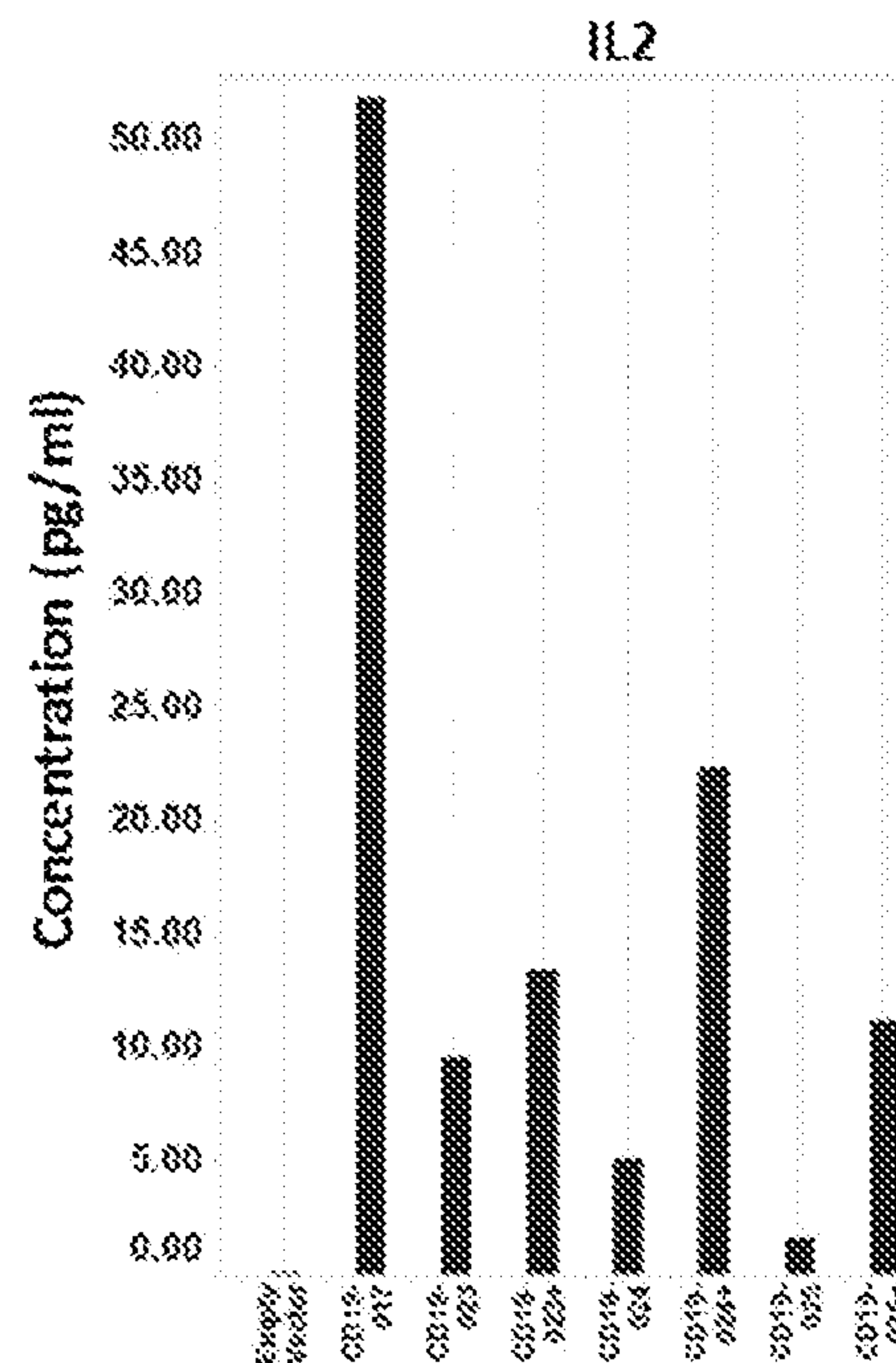


Figure 32A

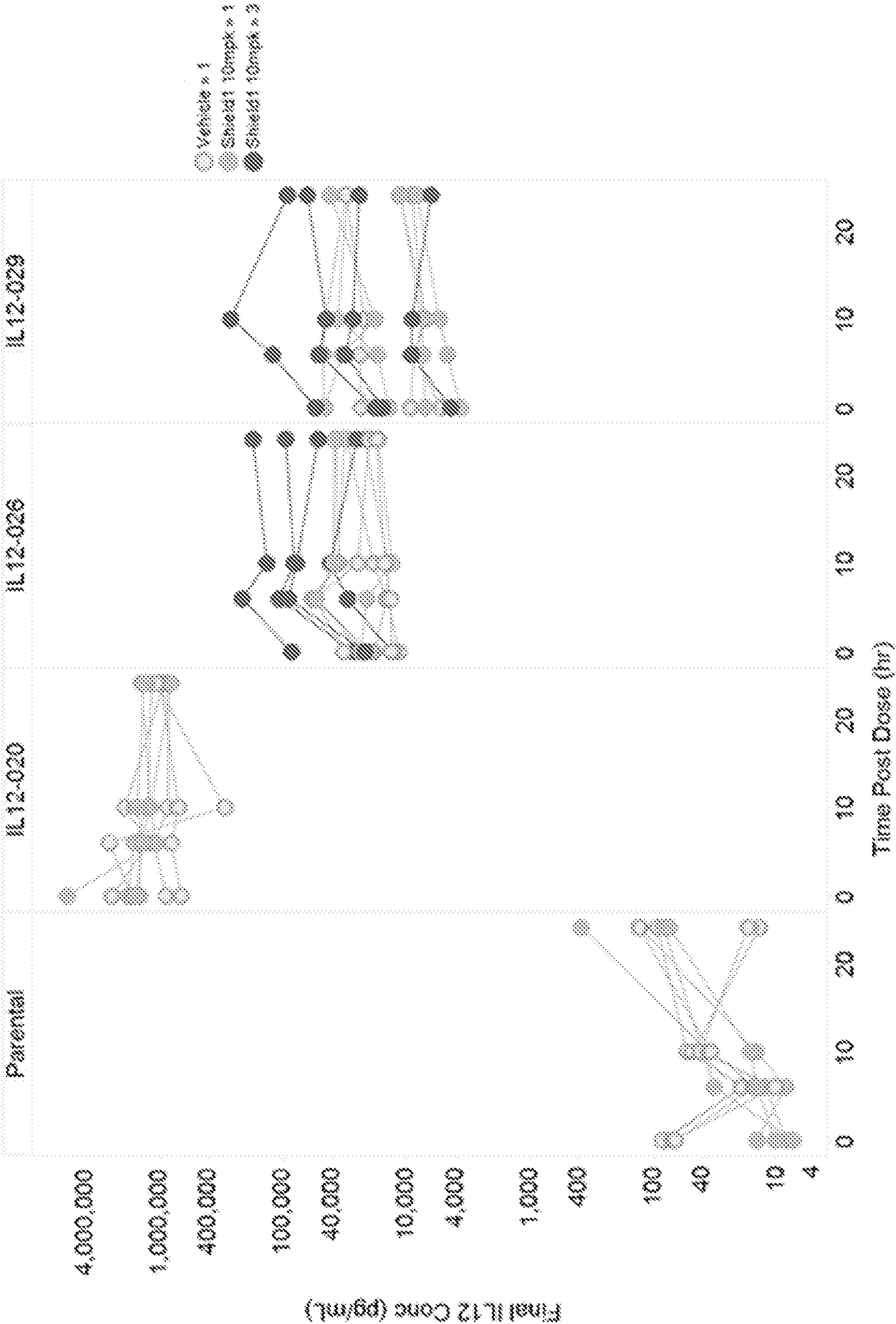


Figure 32B

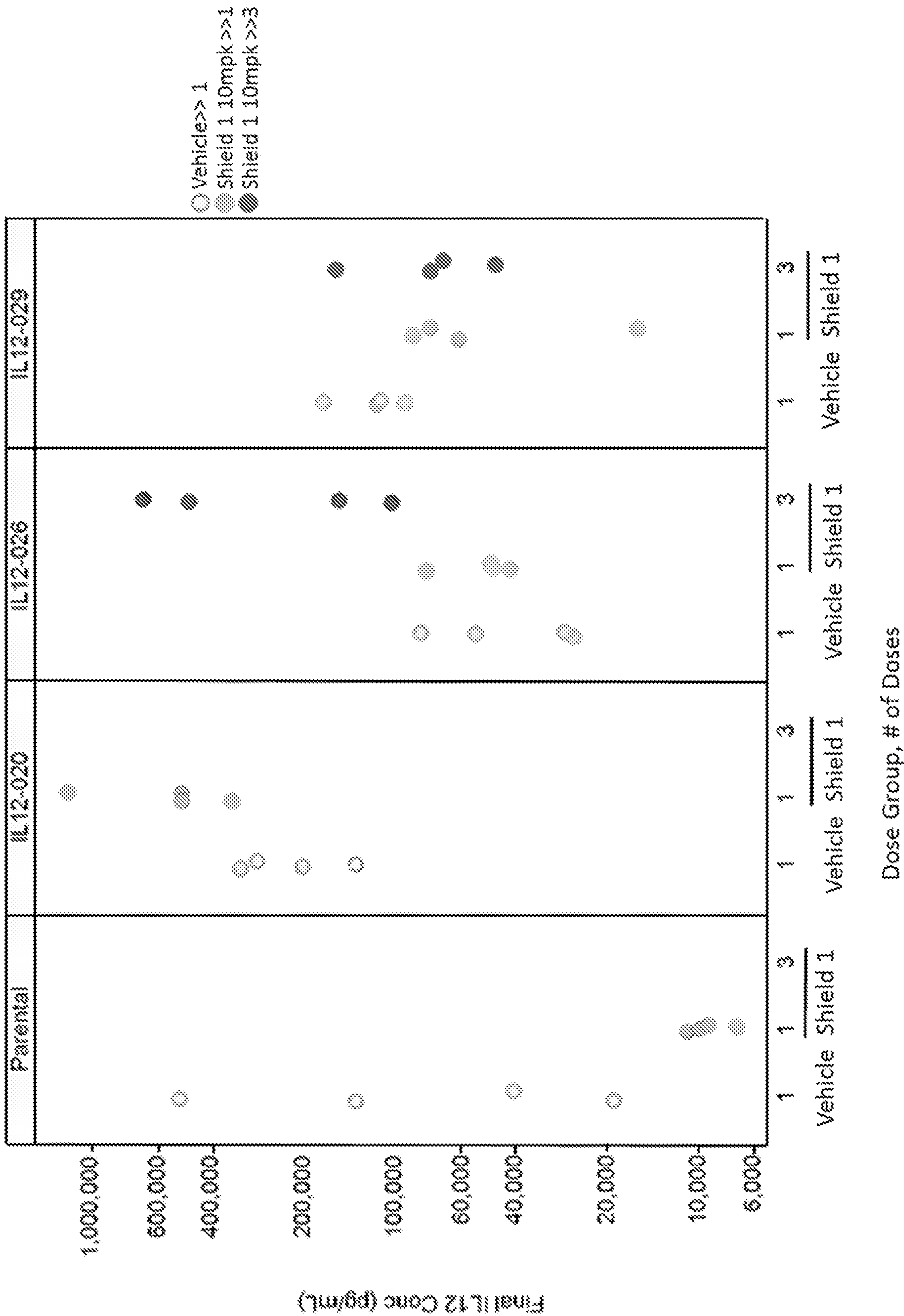
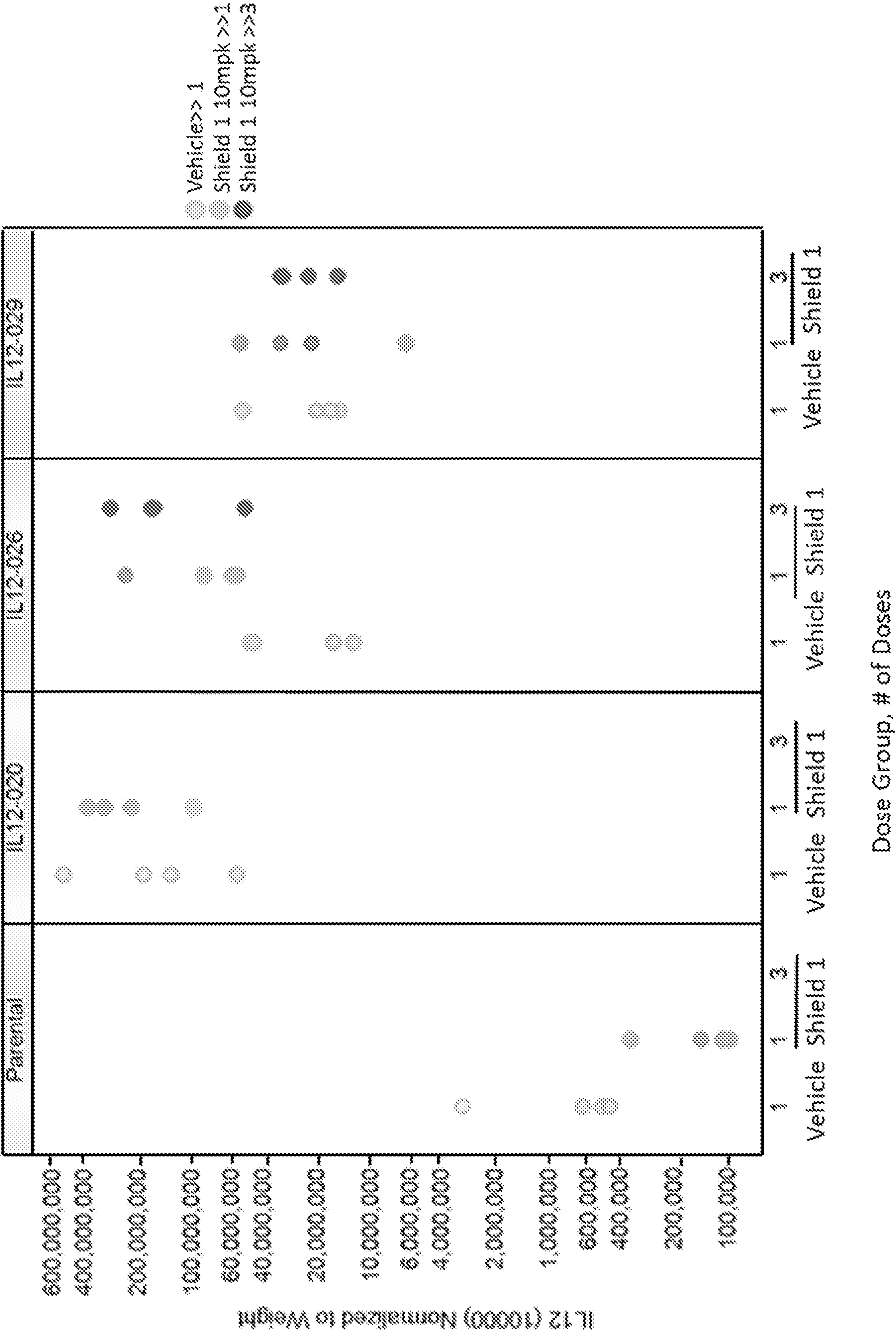
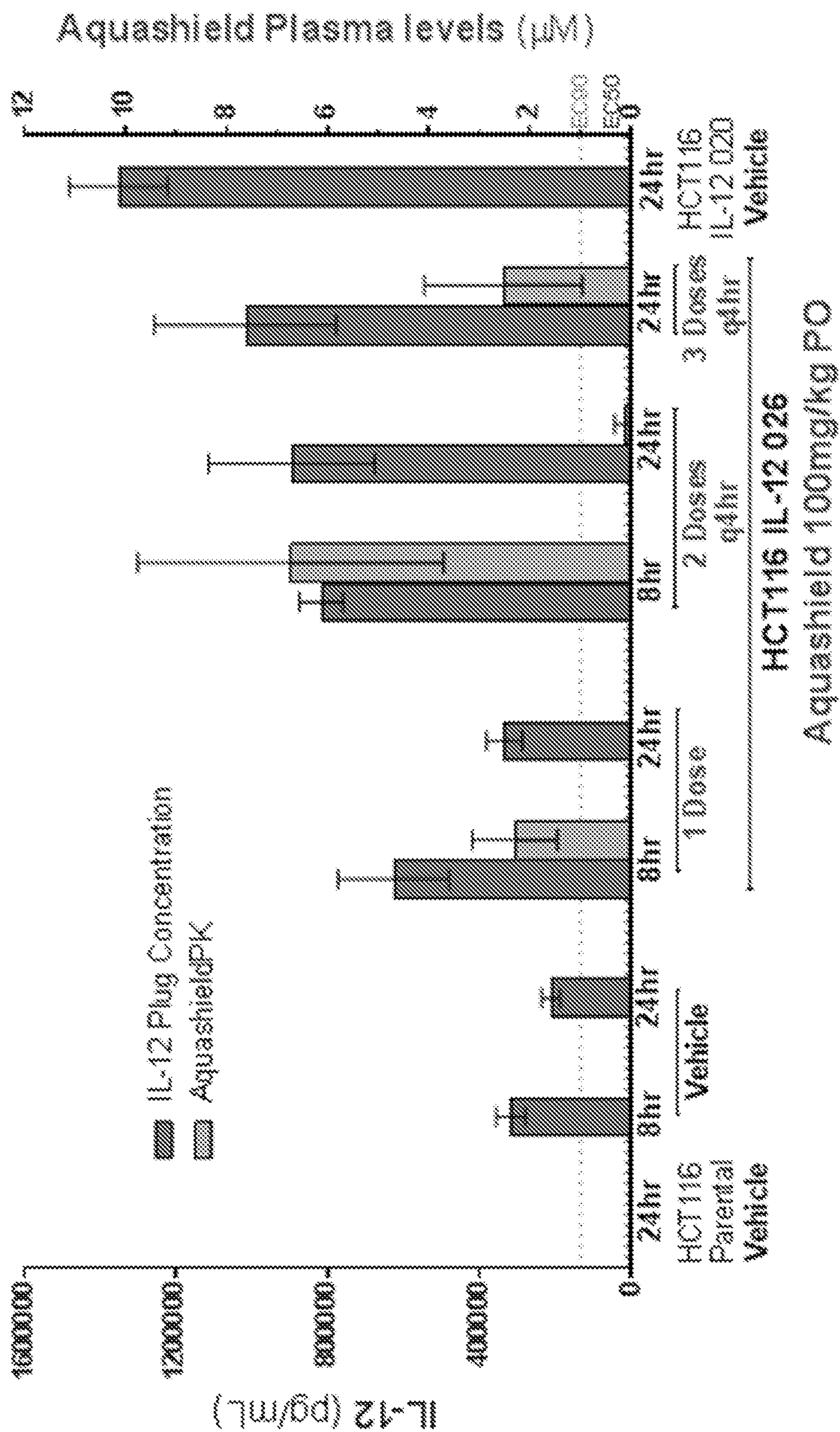


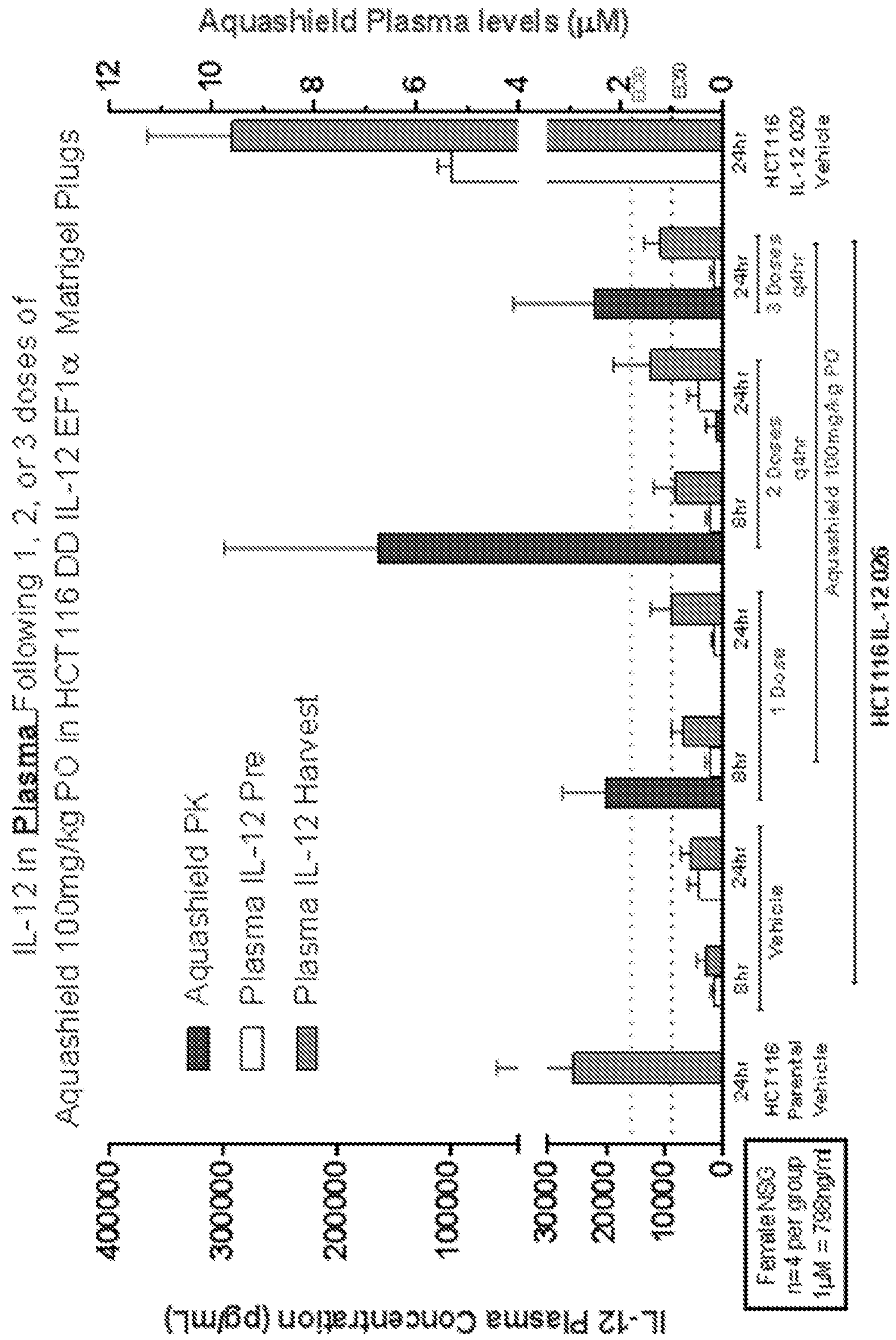
Figure 32C





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Figure 33B



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Figure 33C

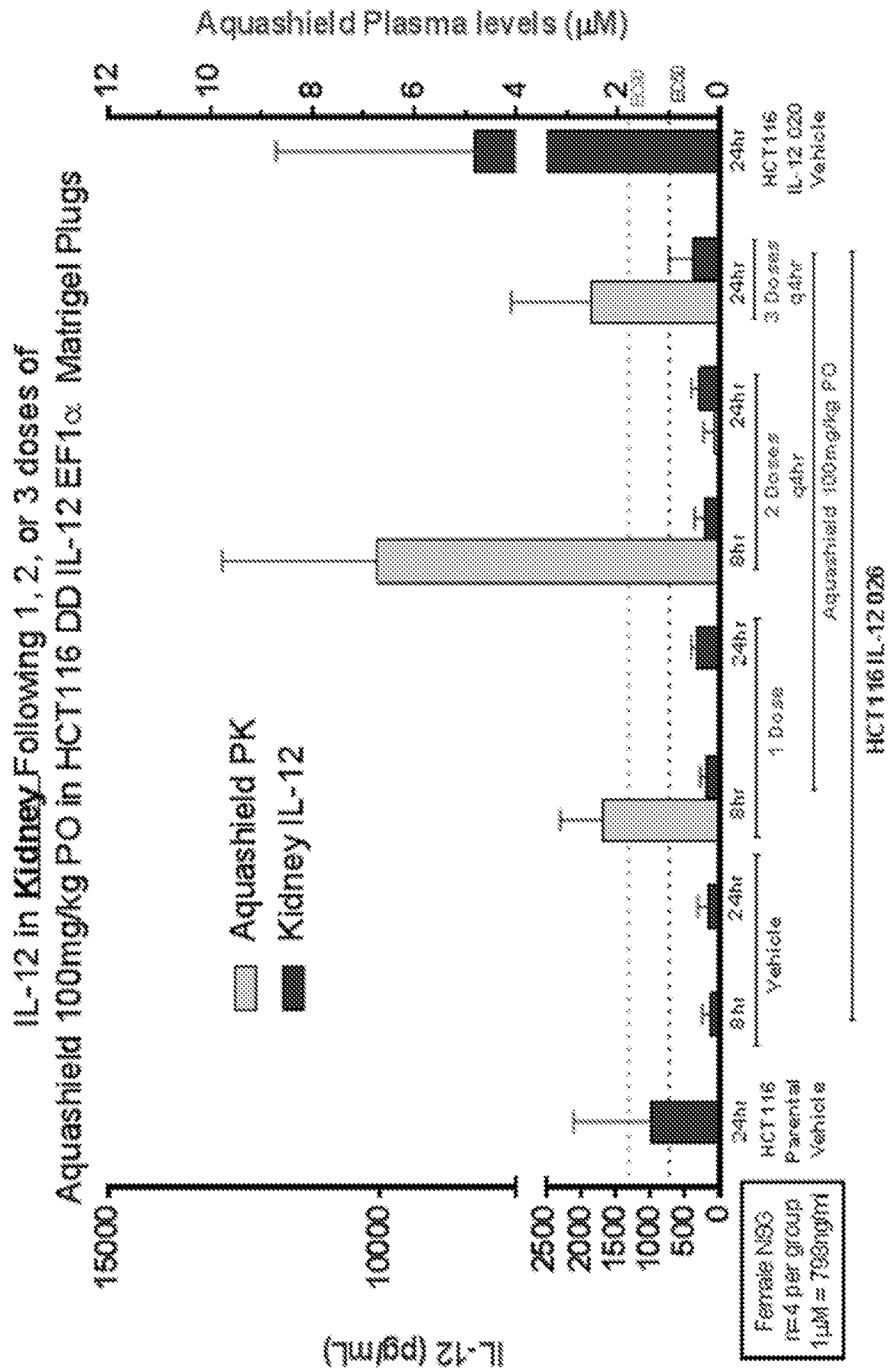


Figure 34A

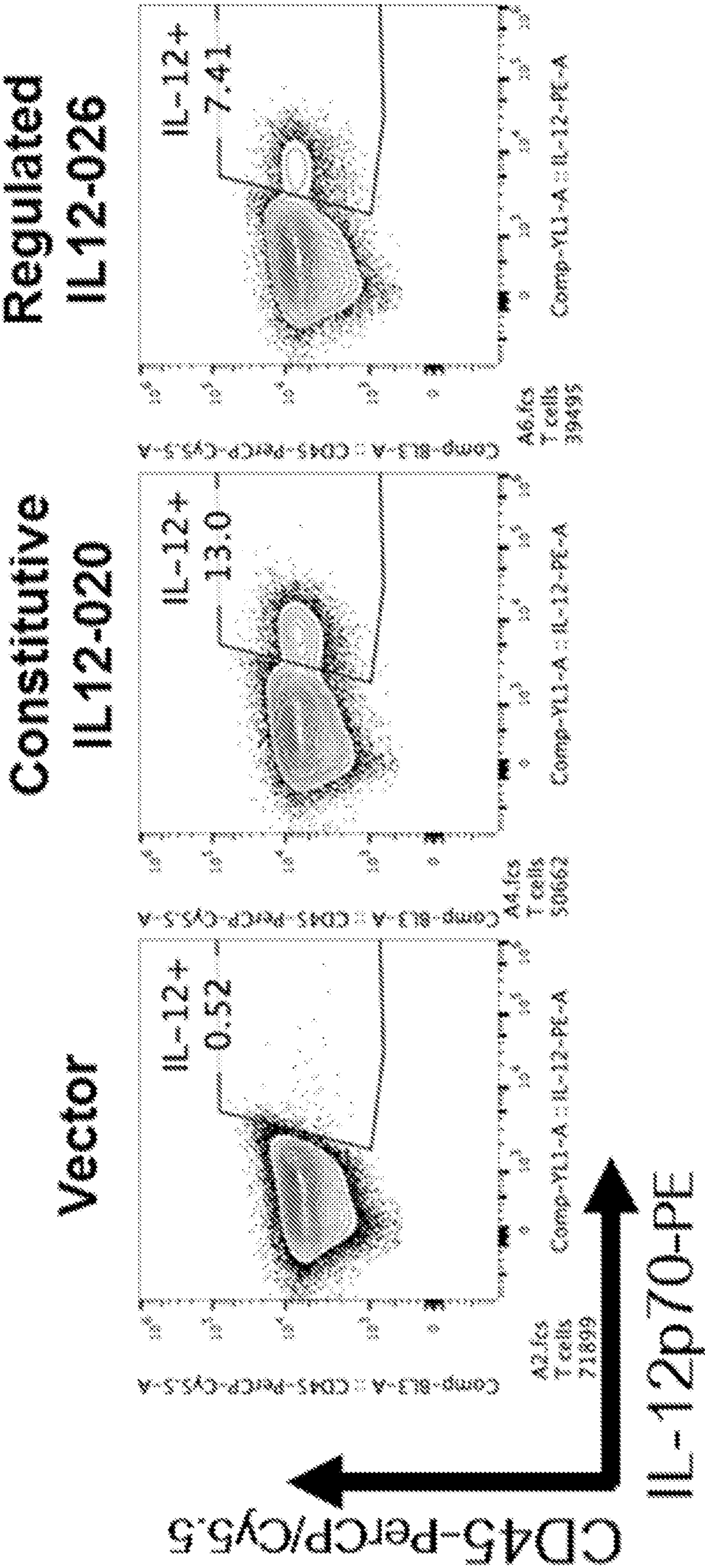


Figure 34B

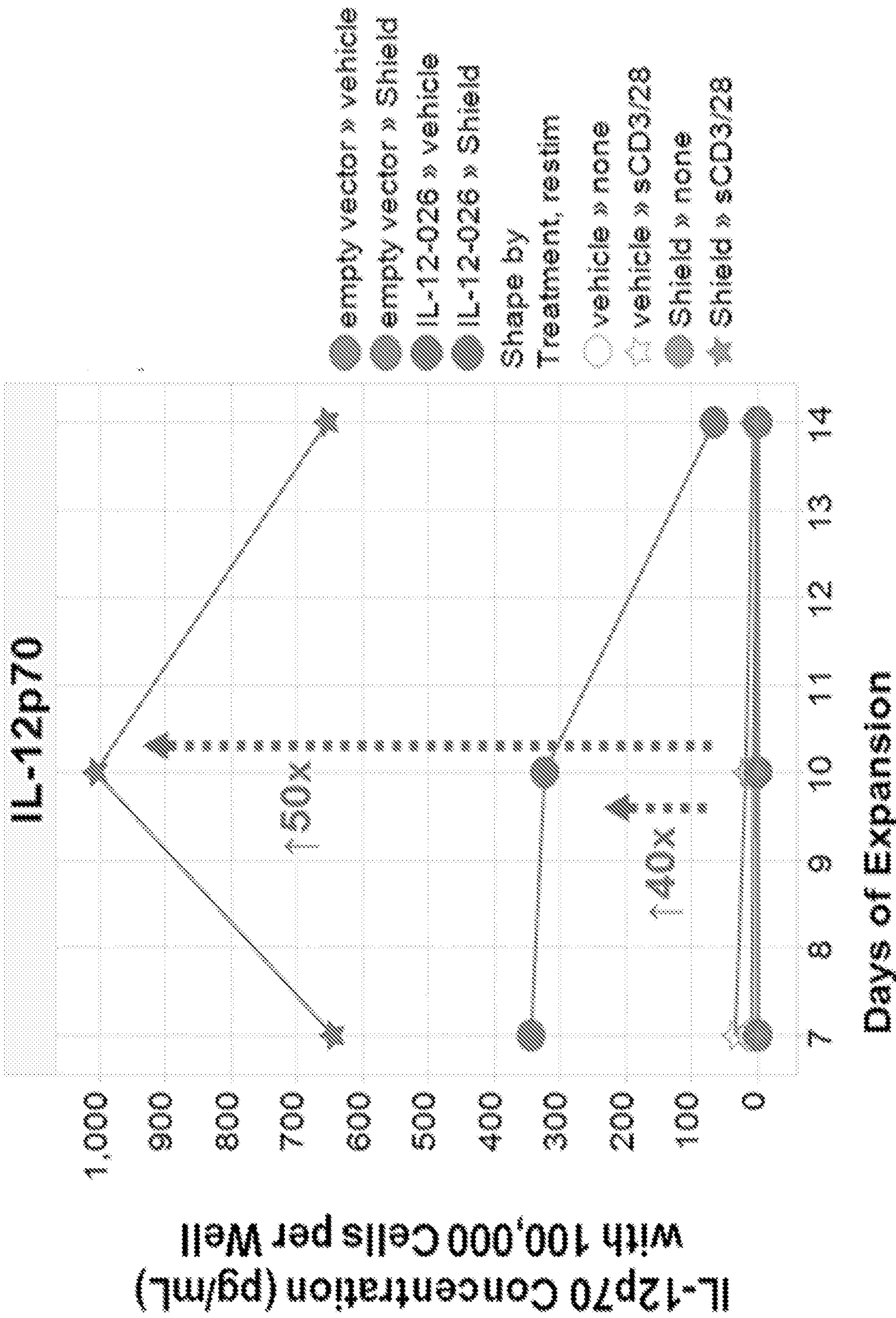
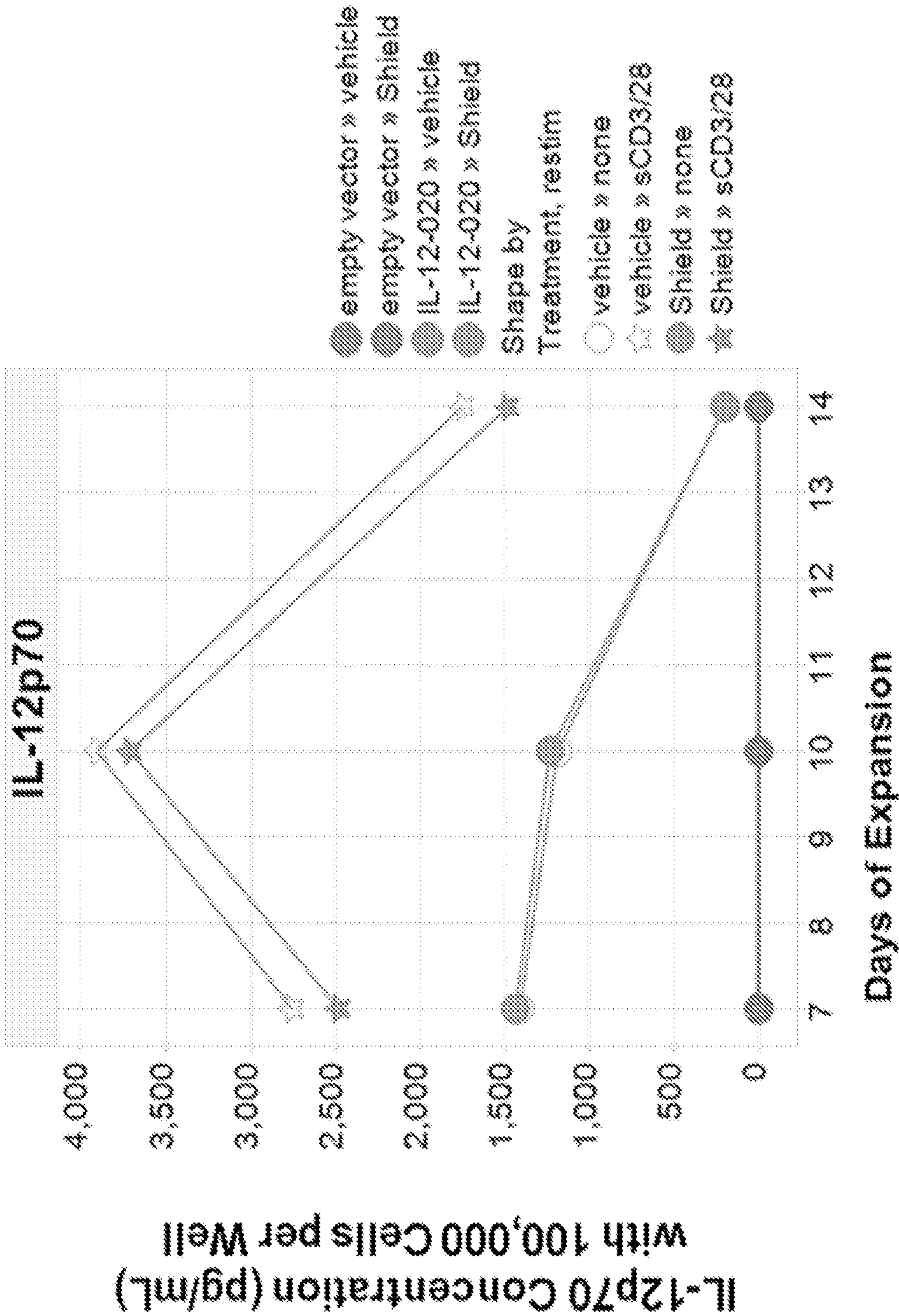
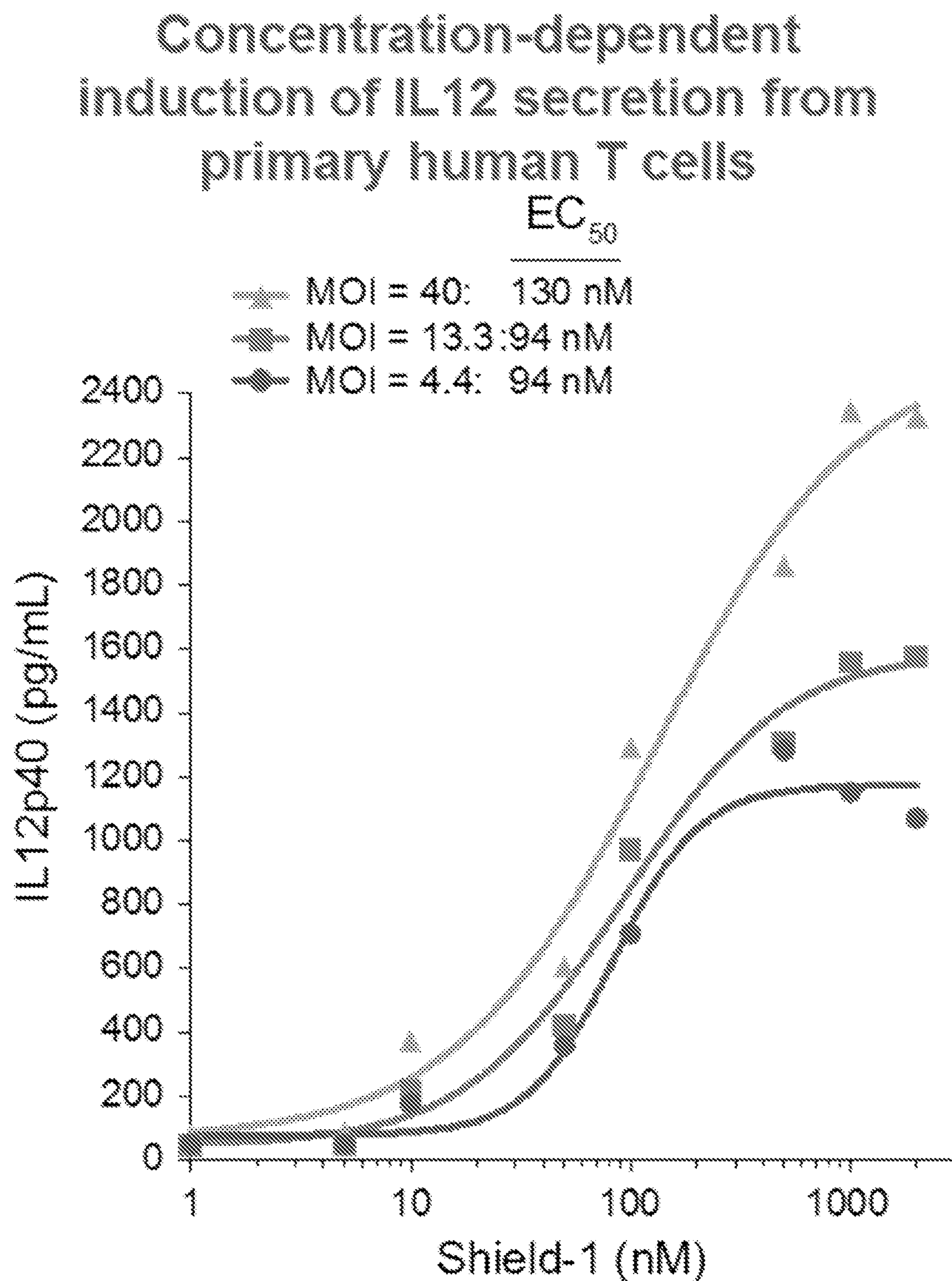


Figure 34C



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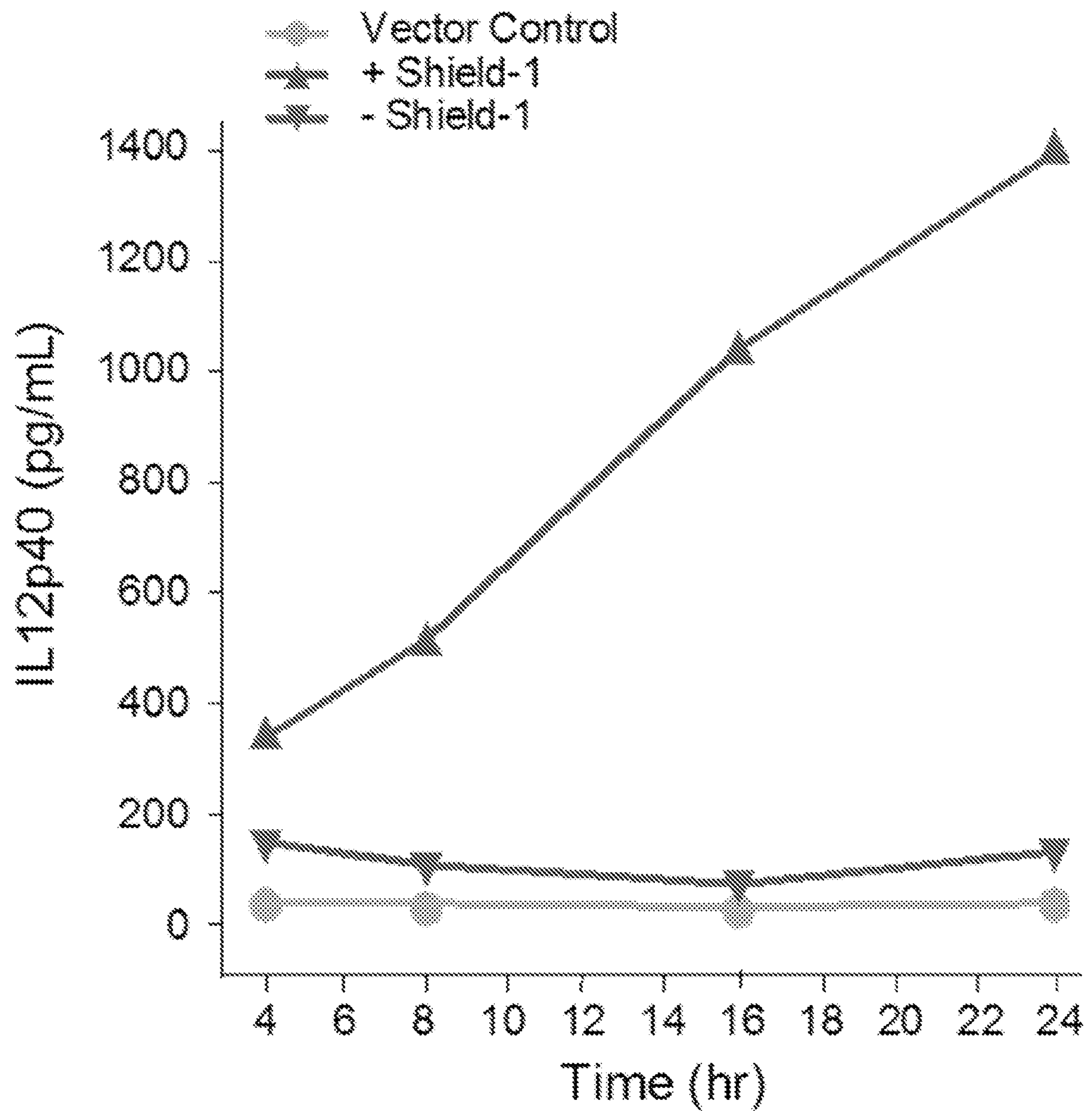
Figure 35A



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Figure 35B

Time course of induction of IL12 secretion from primary human T cells



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Figure 36A

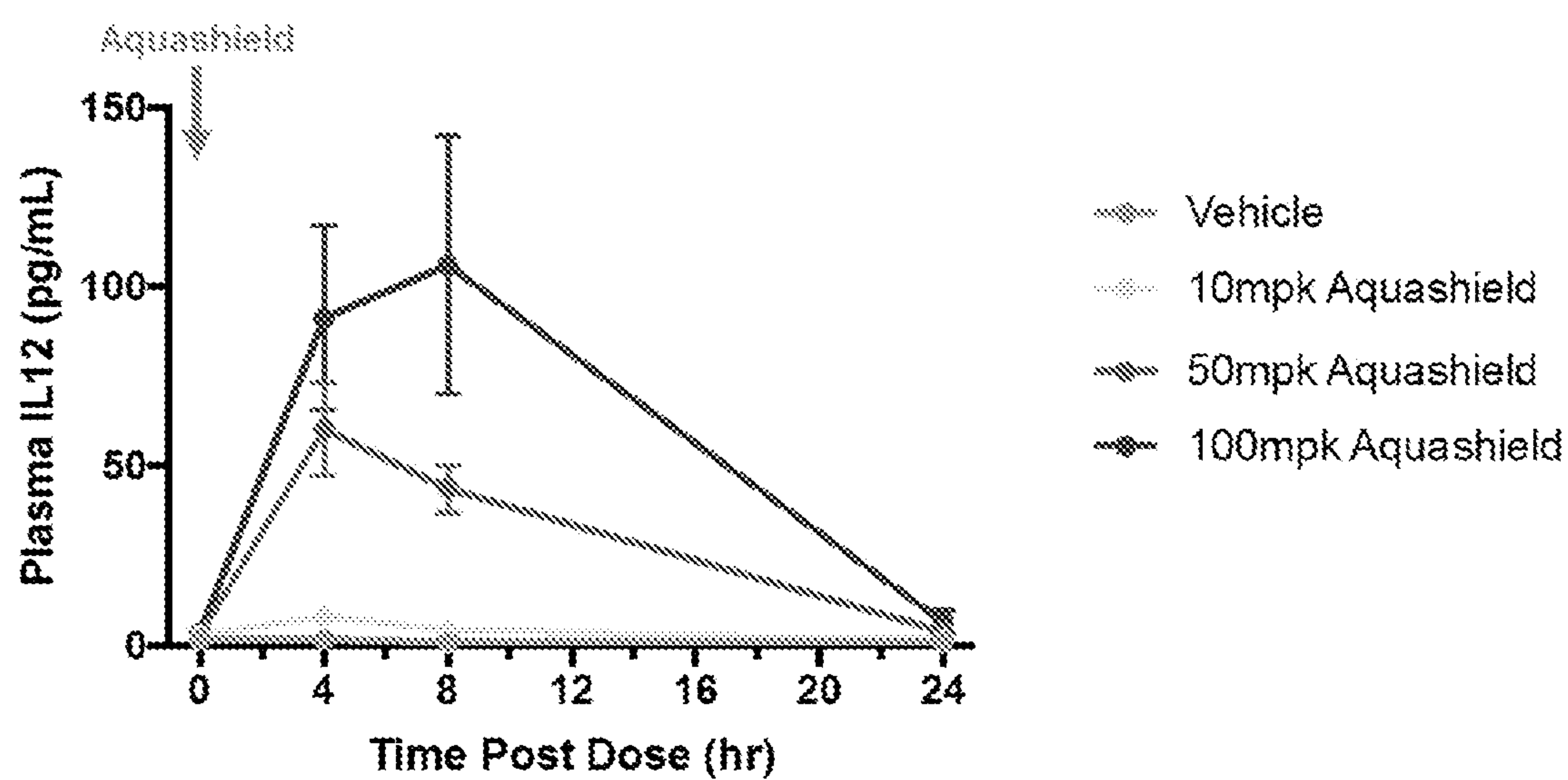
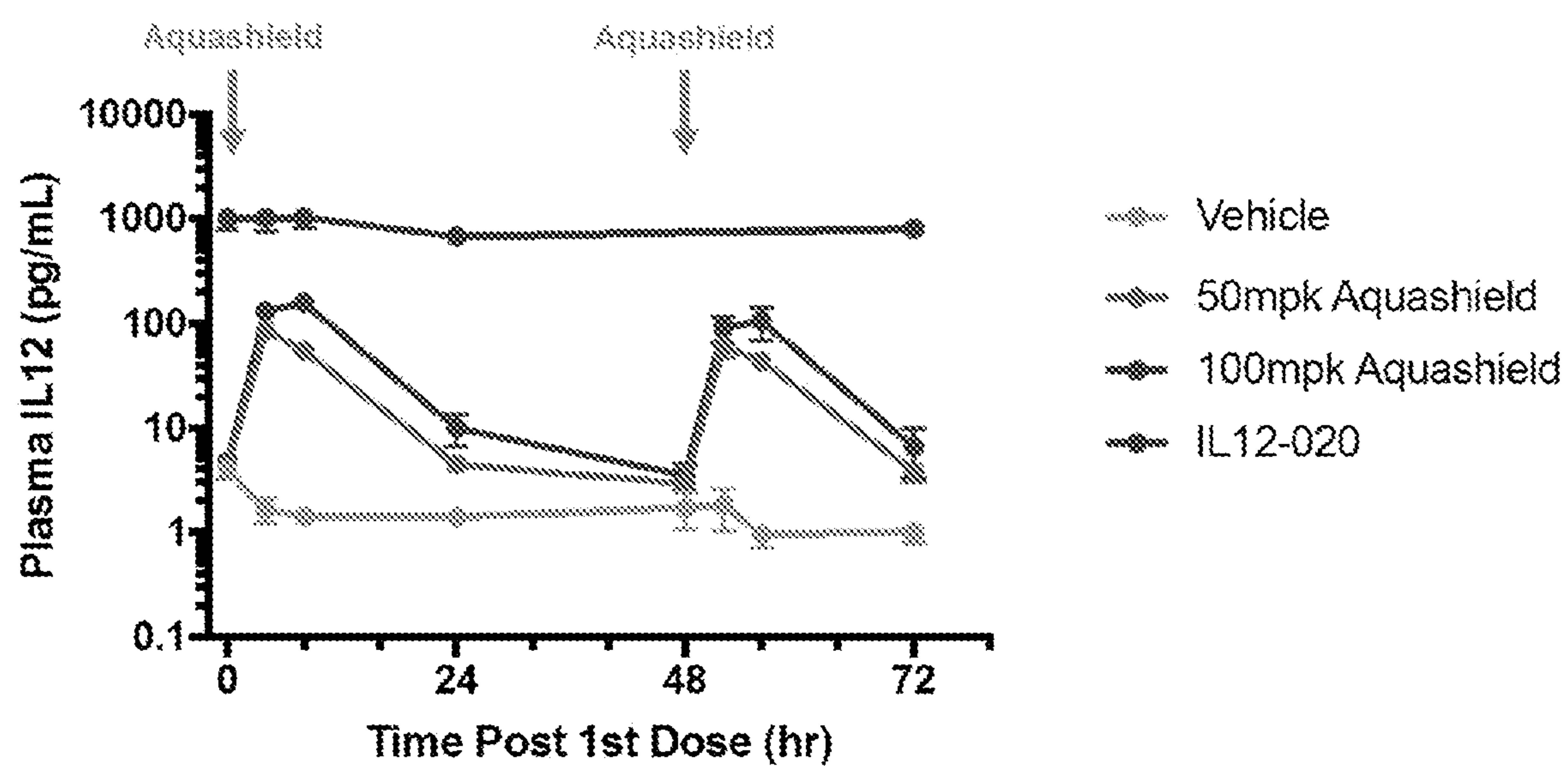


Figure 36B



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Figure 37A

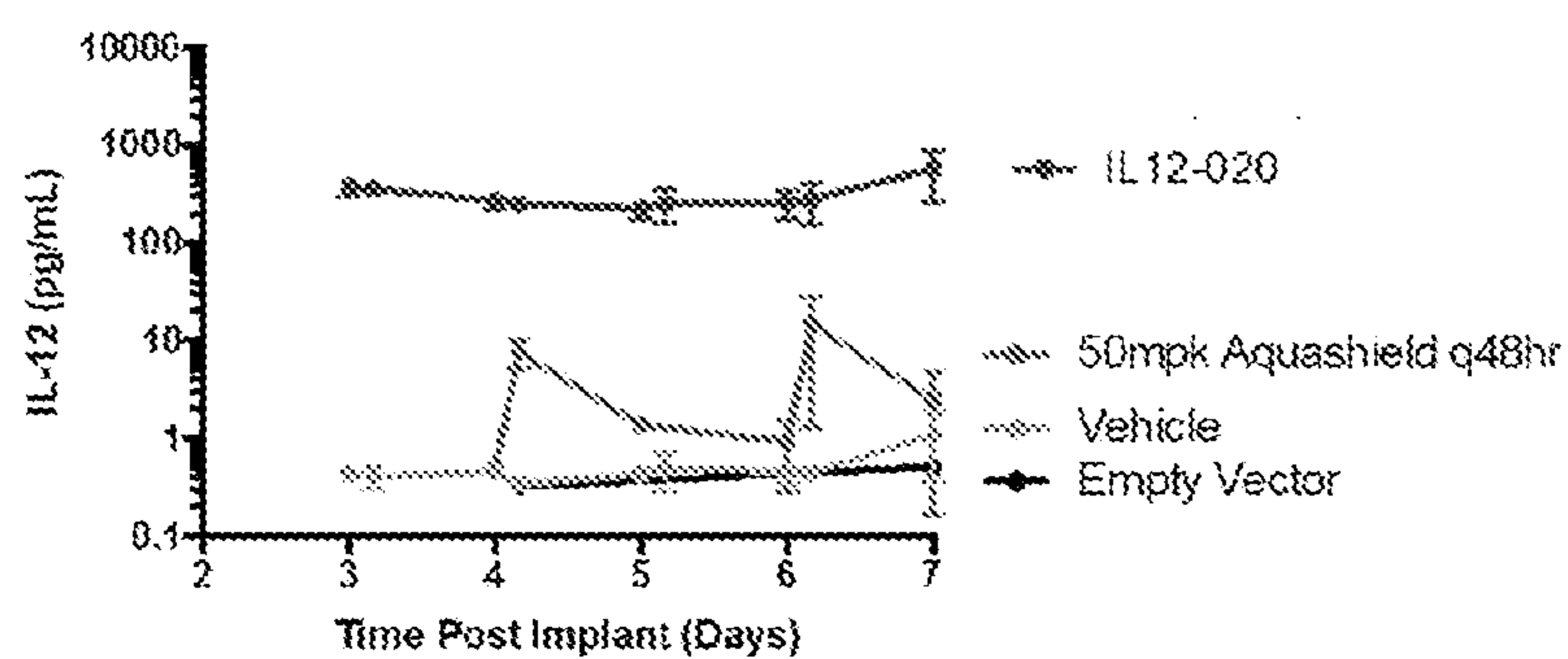


Figure 37B

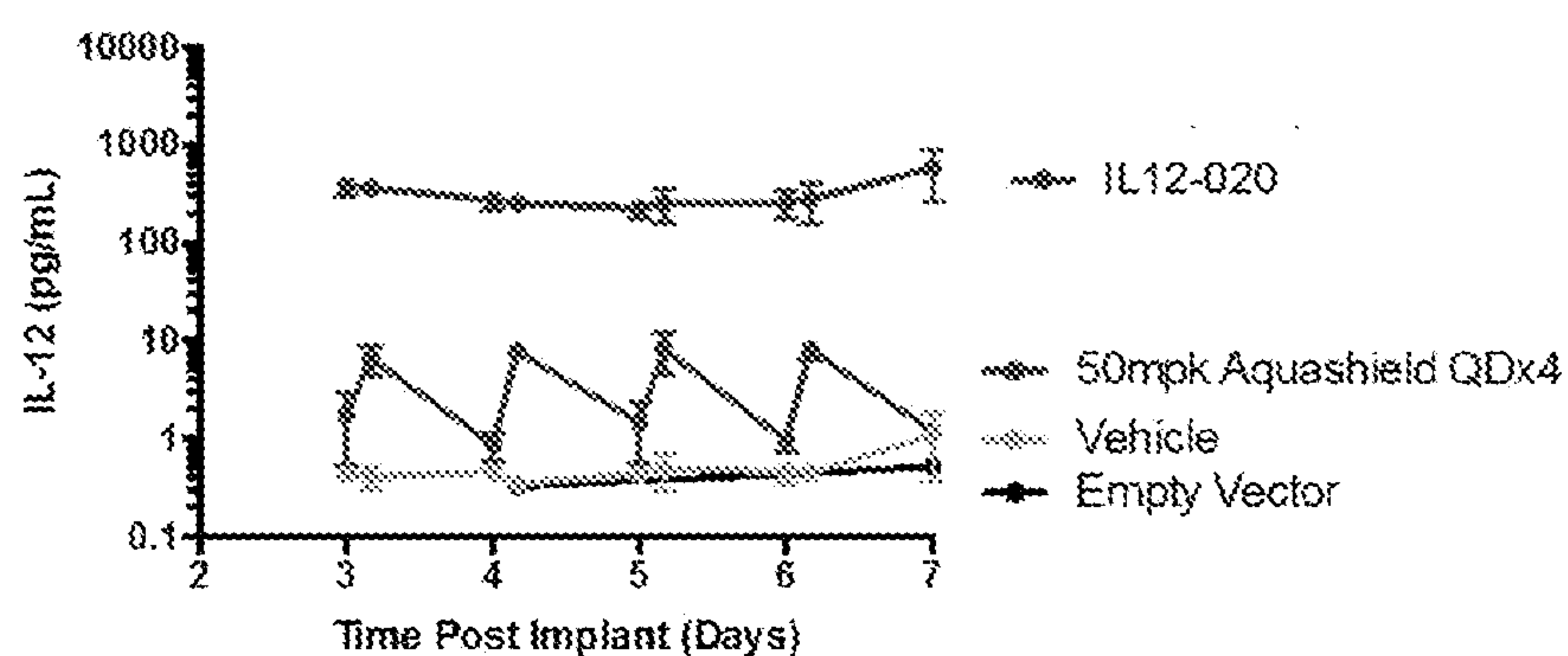


Figure 37C

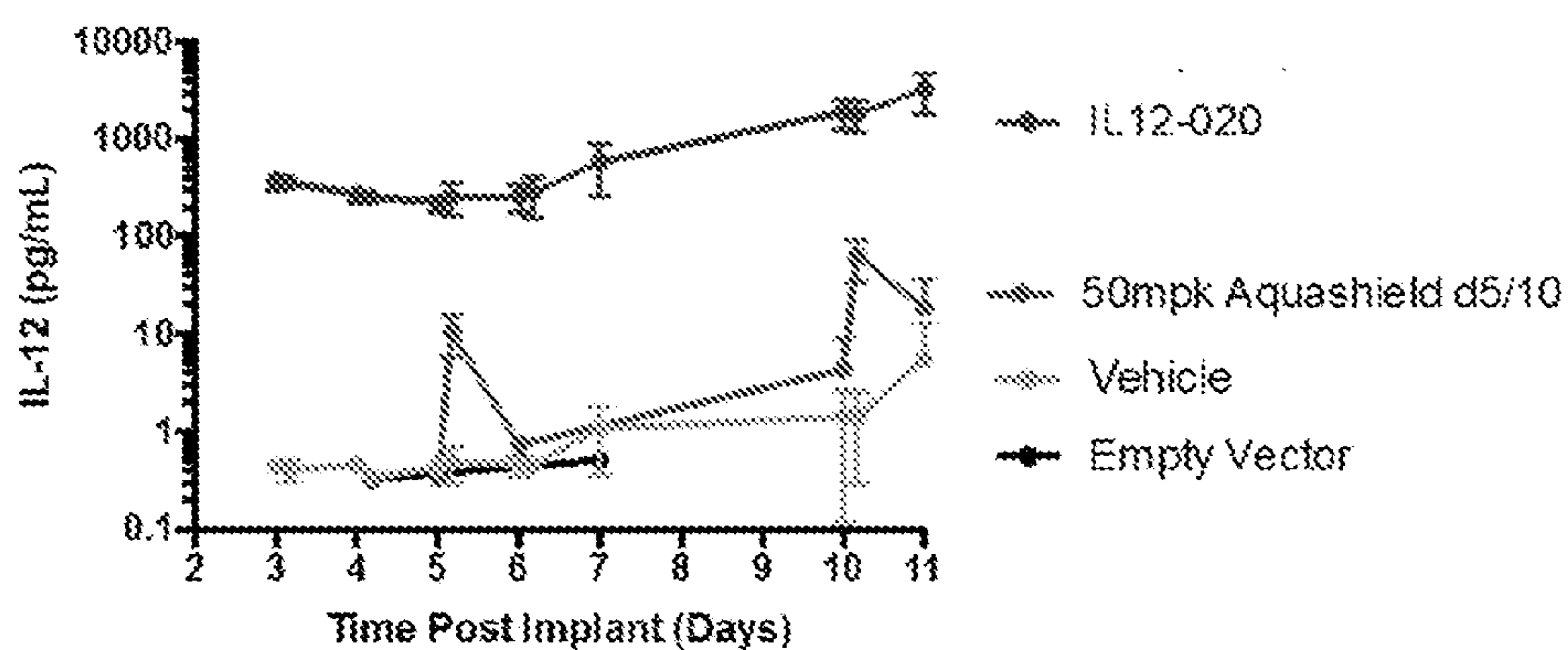
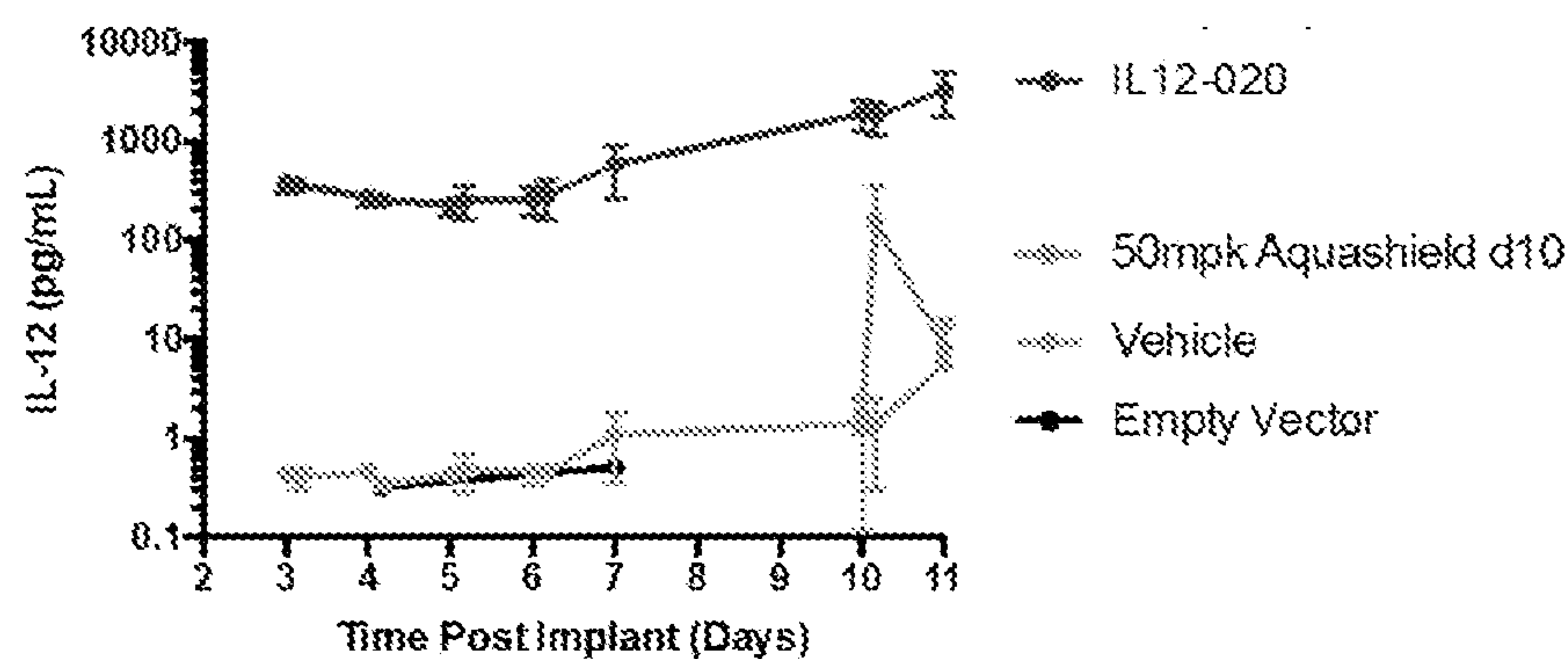


Figure 37D



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Figure 37E

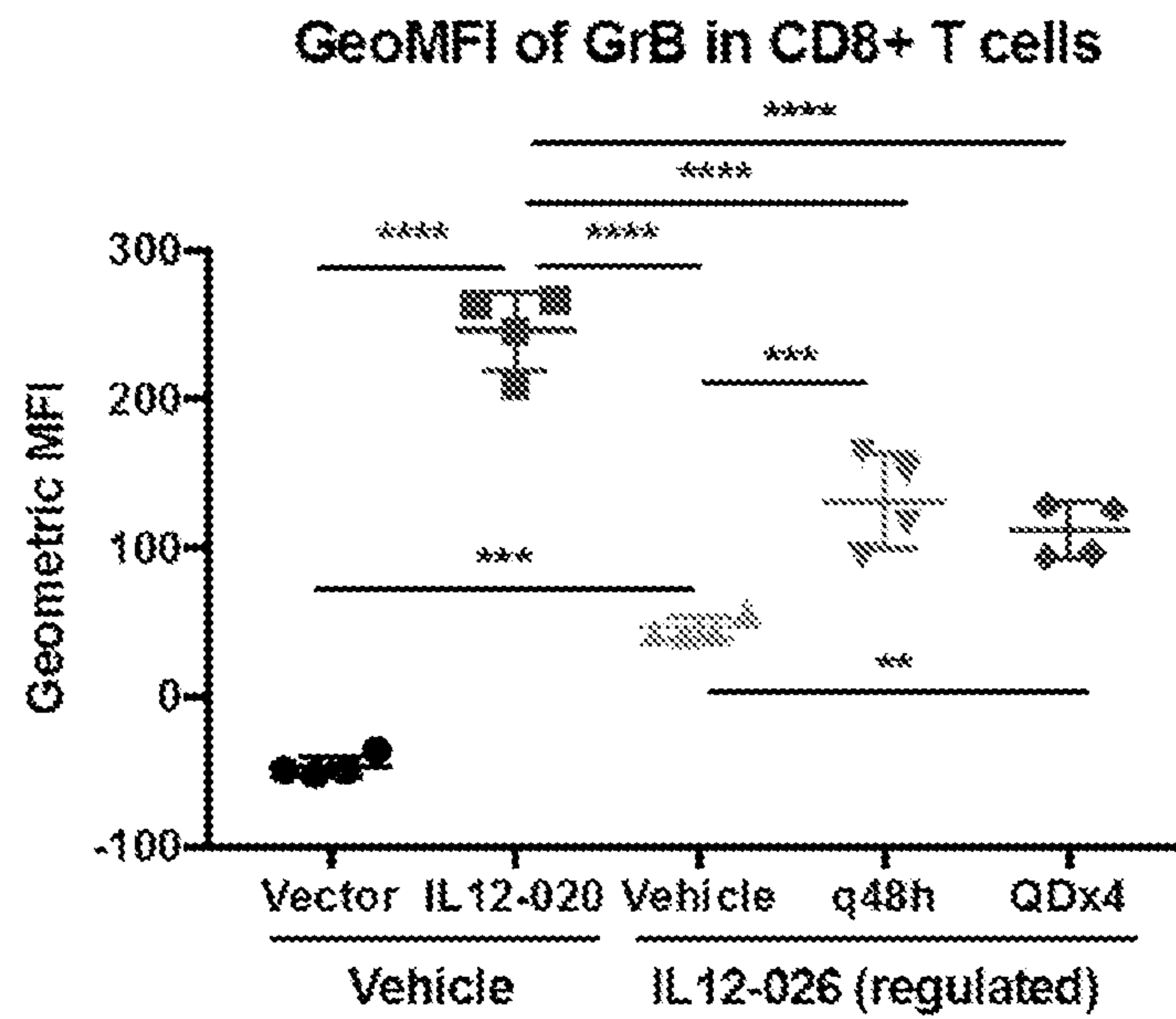
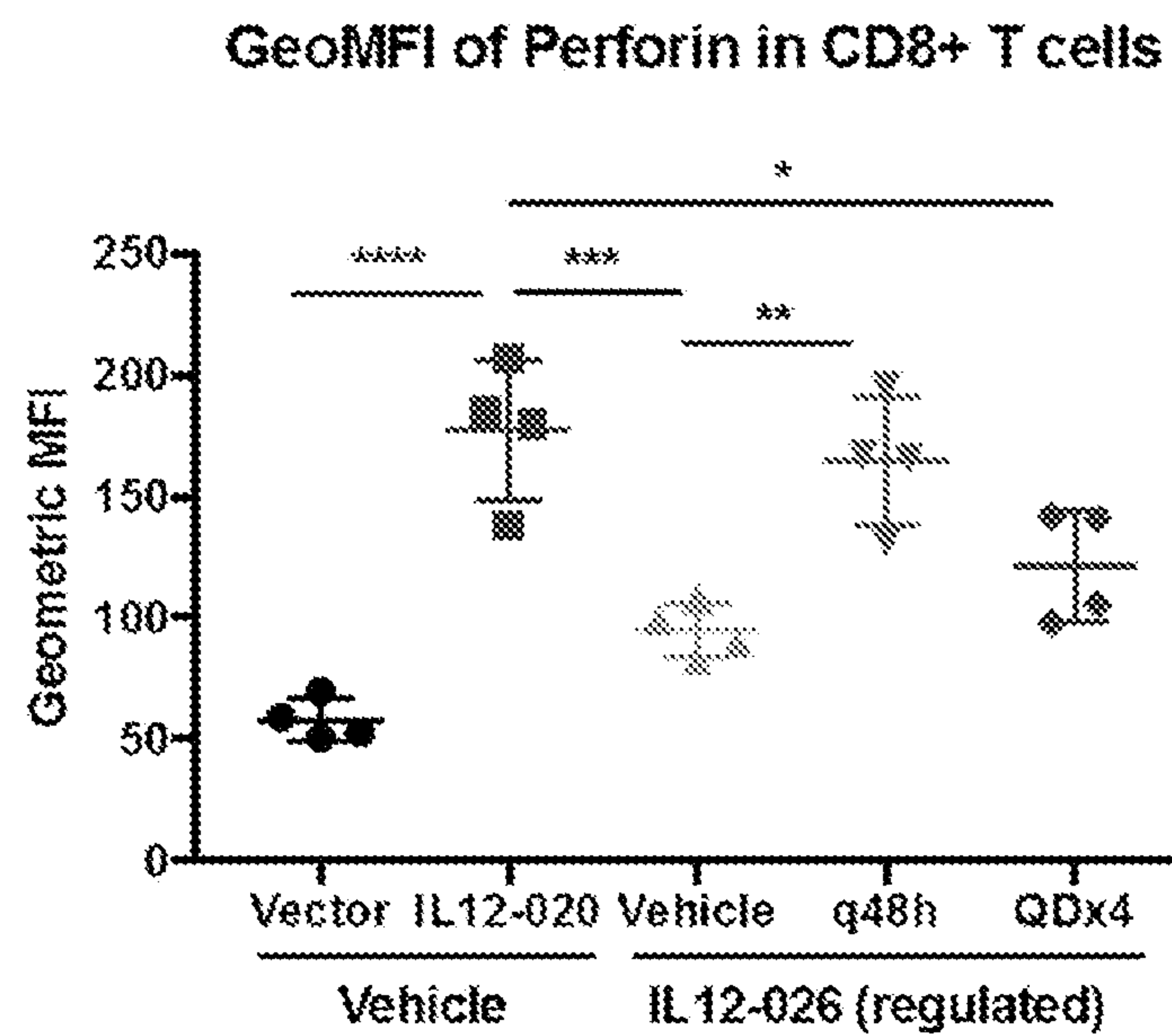


Figure 37F



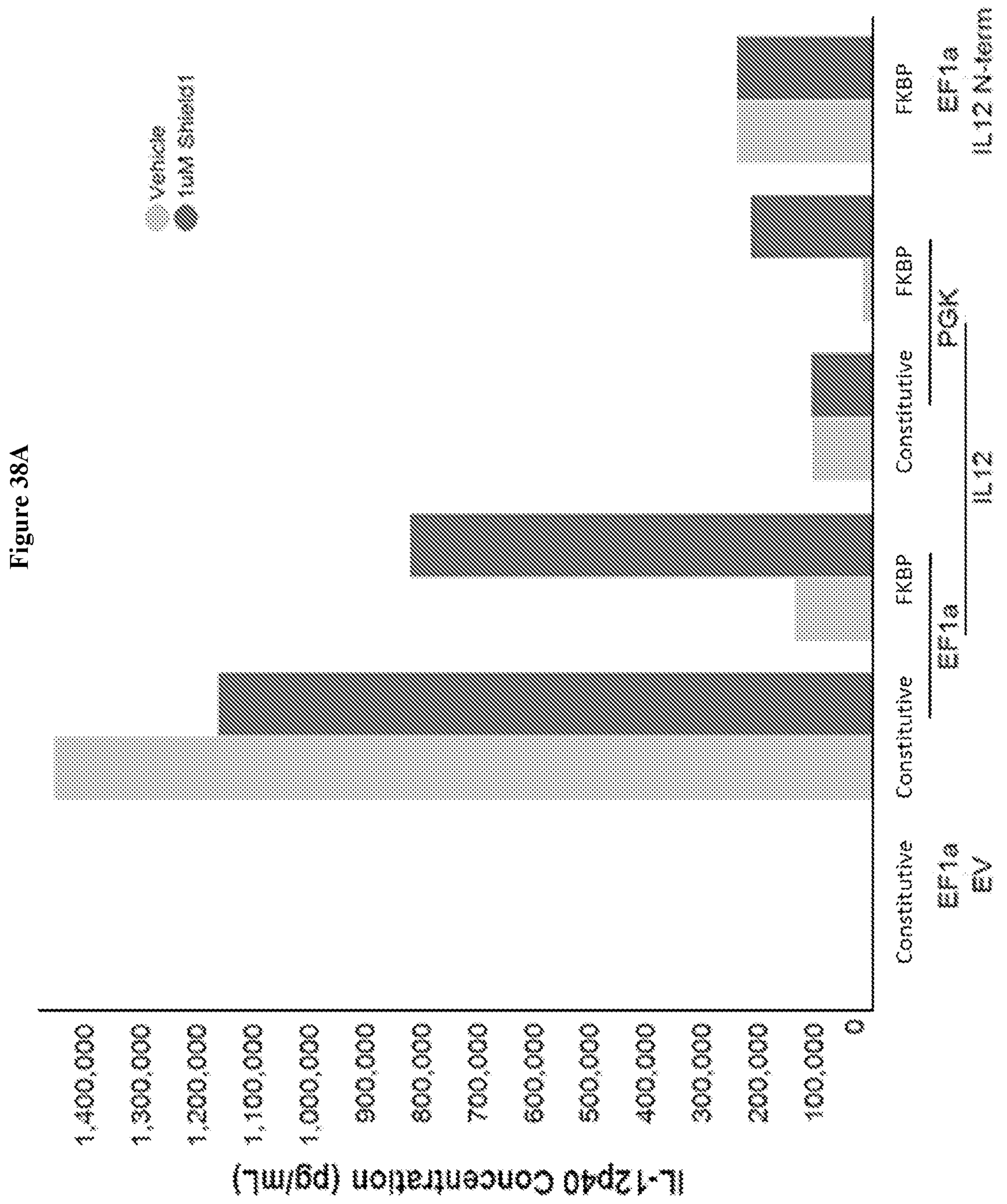
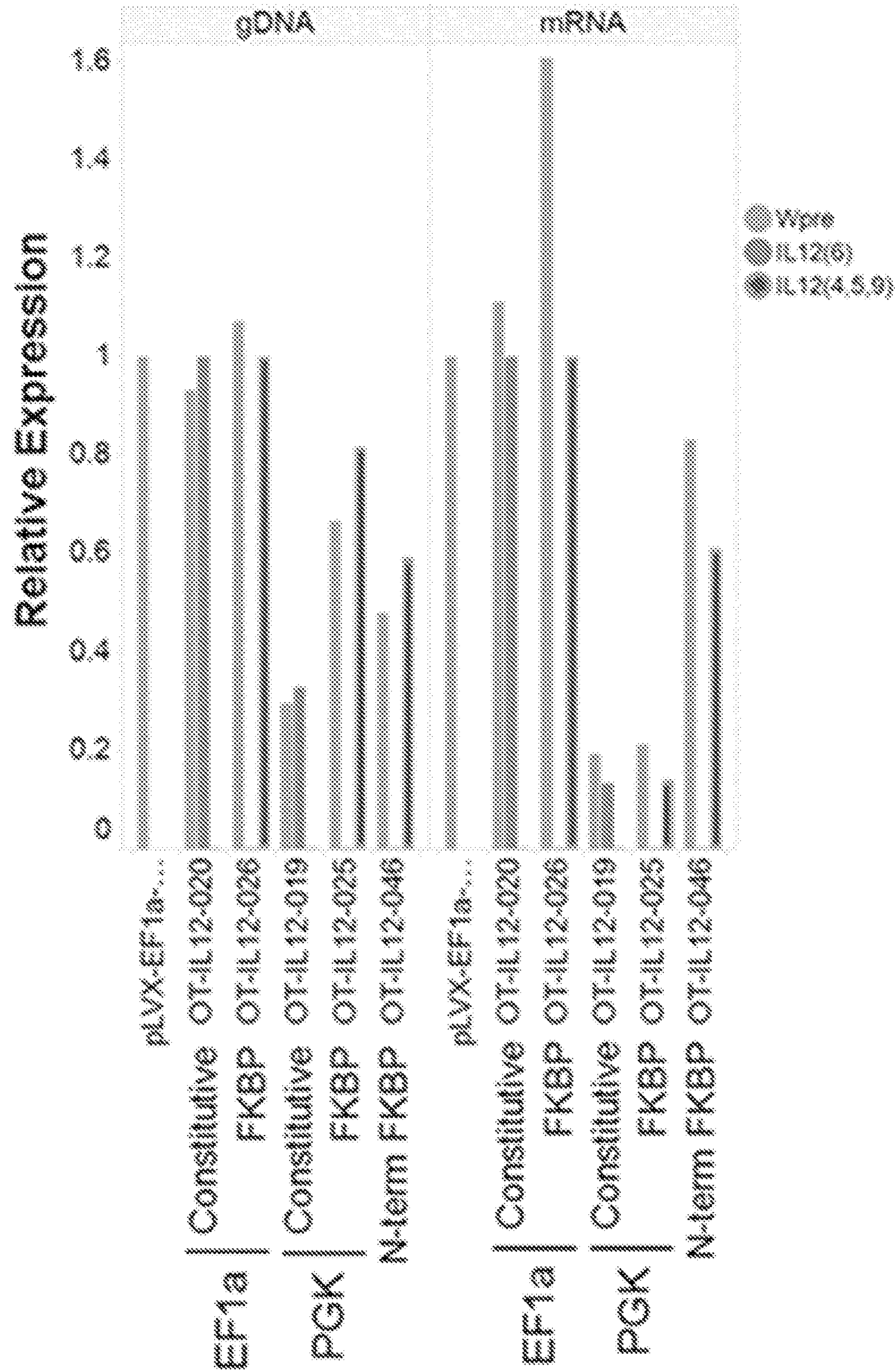


Figure 38B



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Figure 39

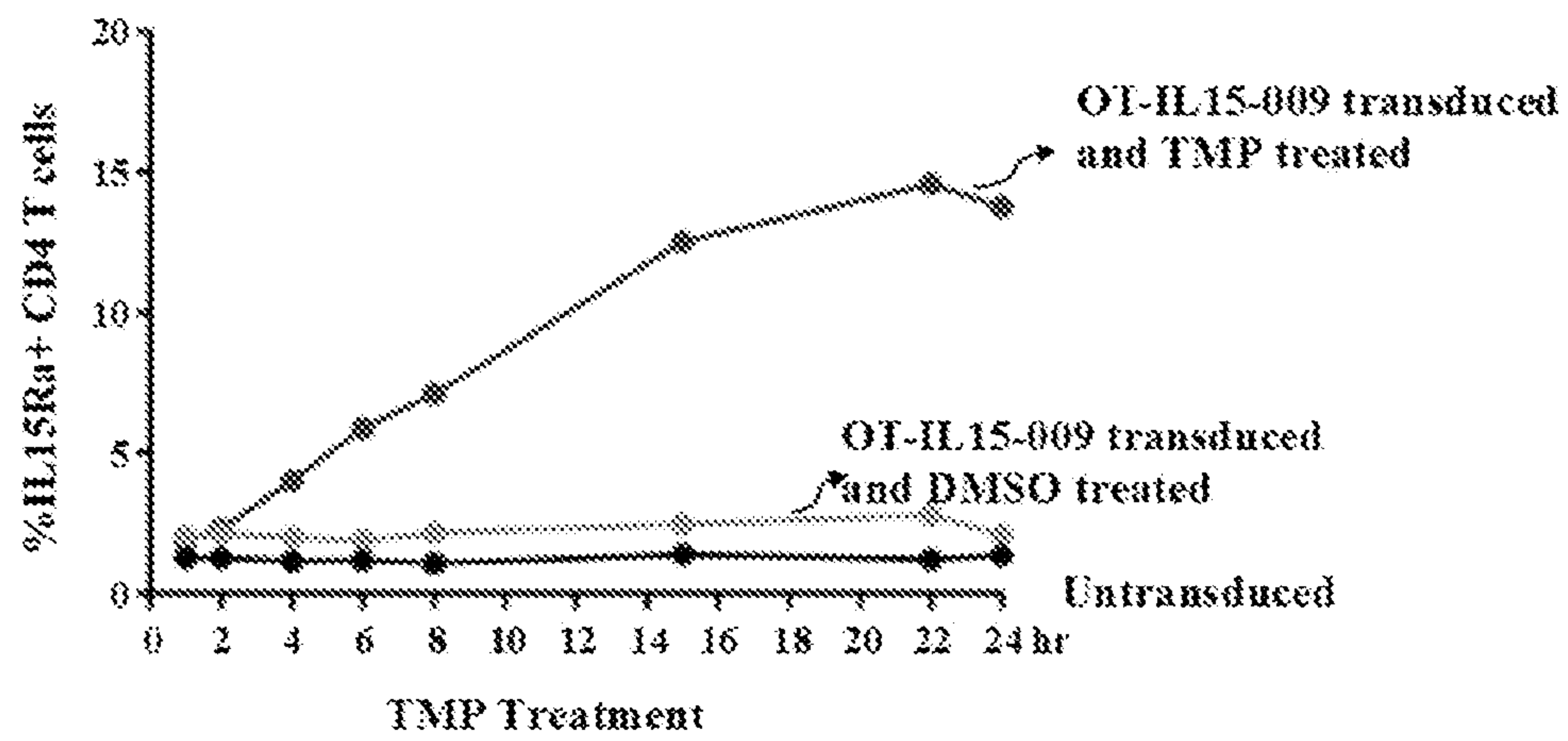


Figure 40

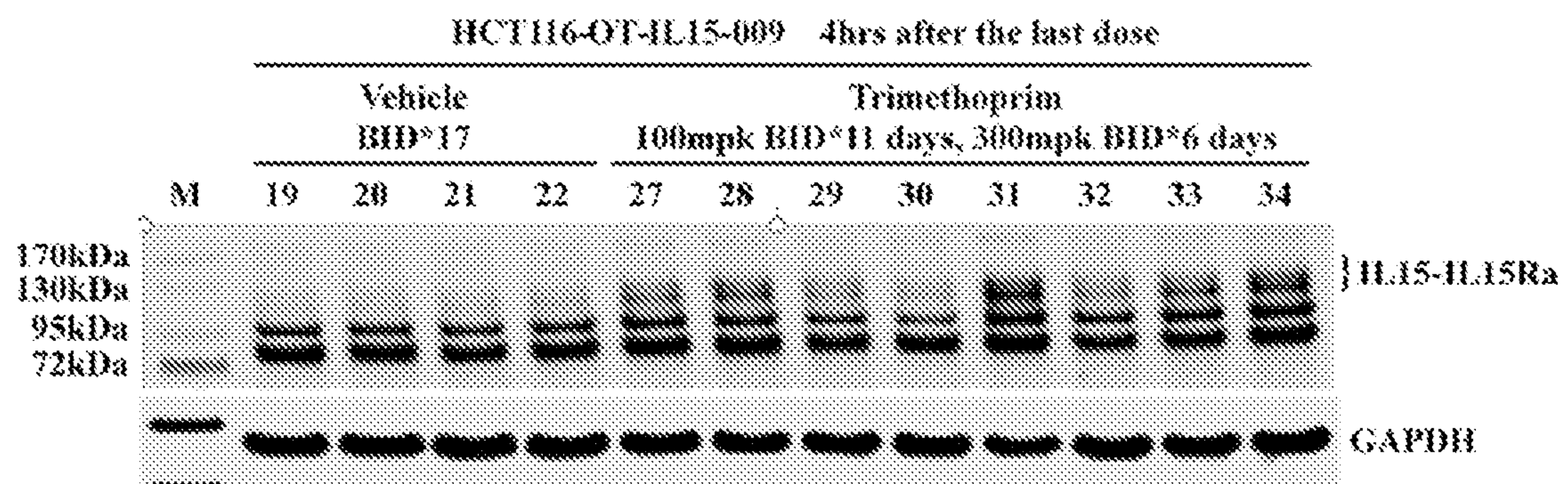
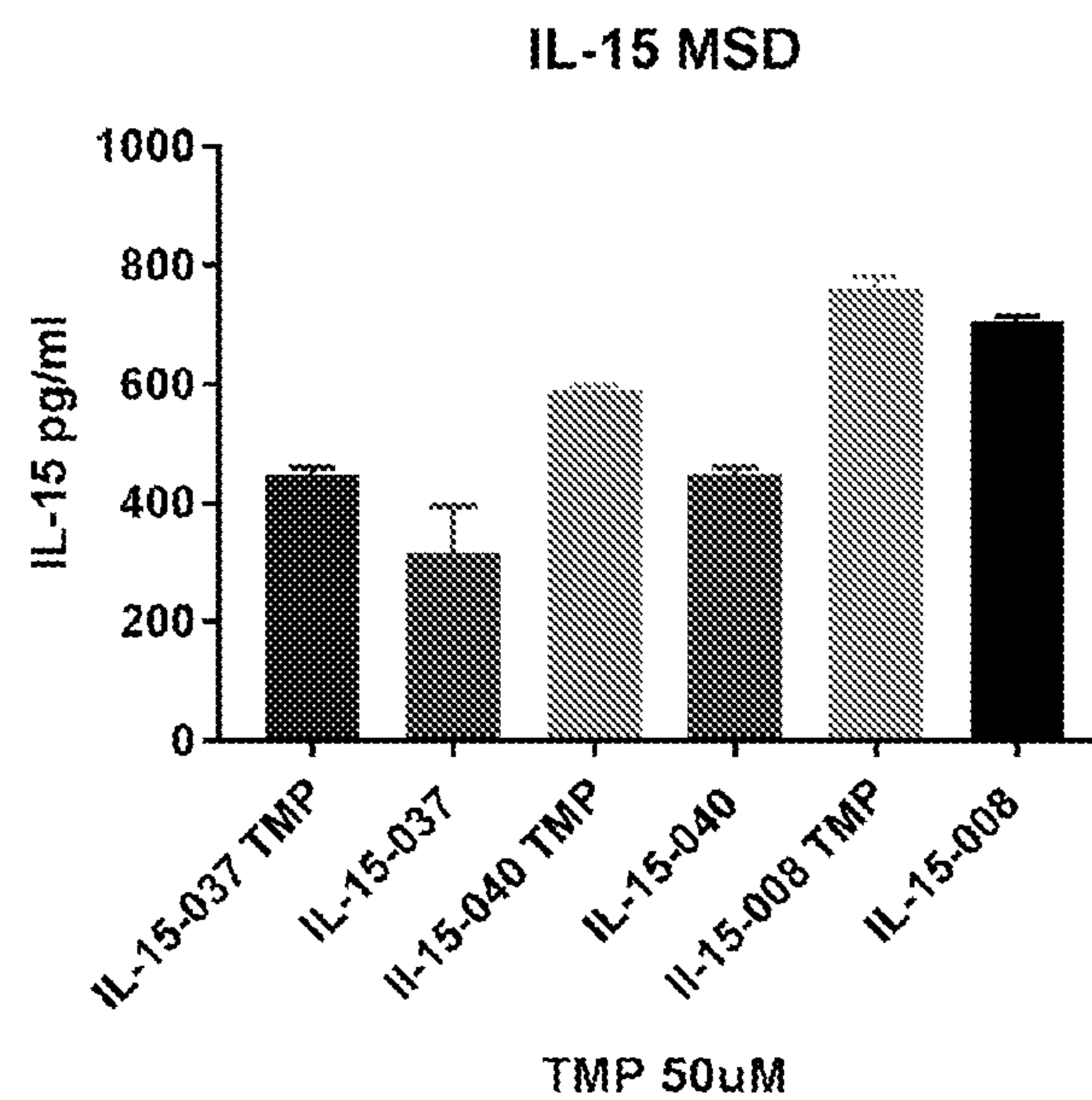


Figure 41



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Figure 42A

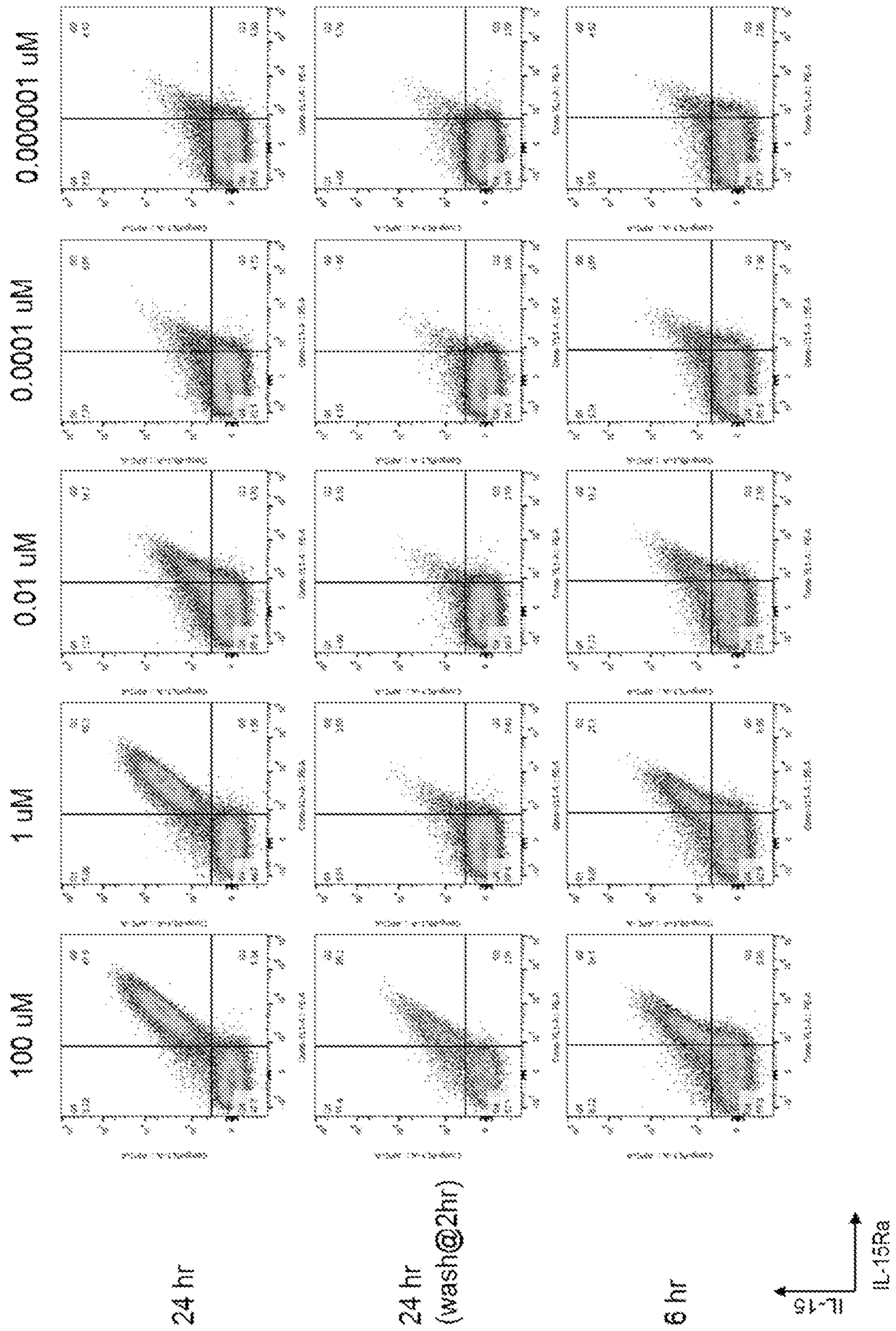
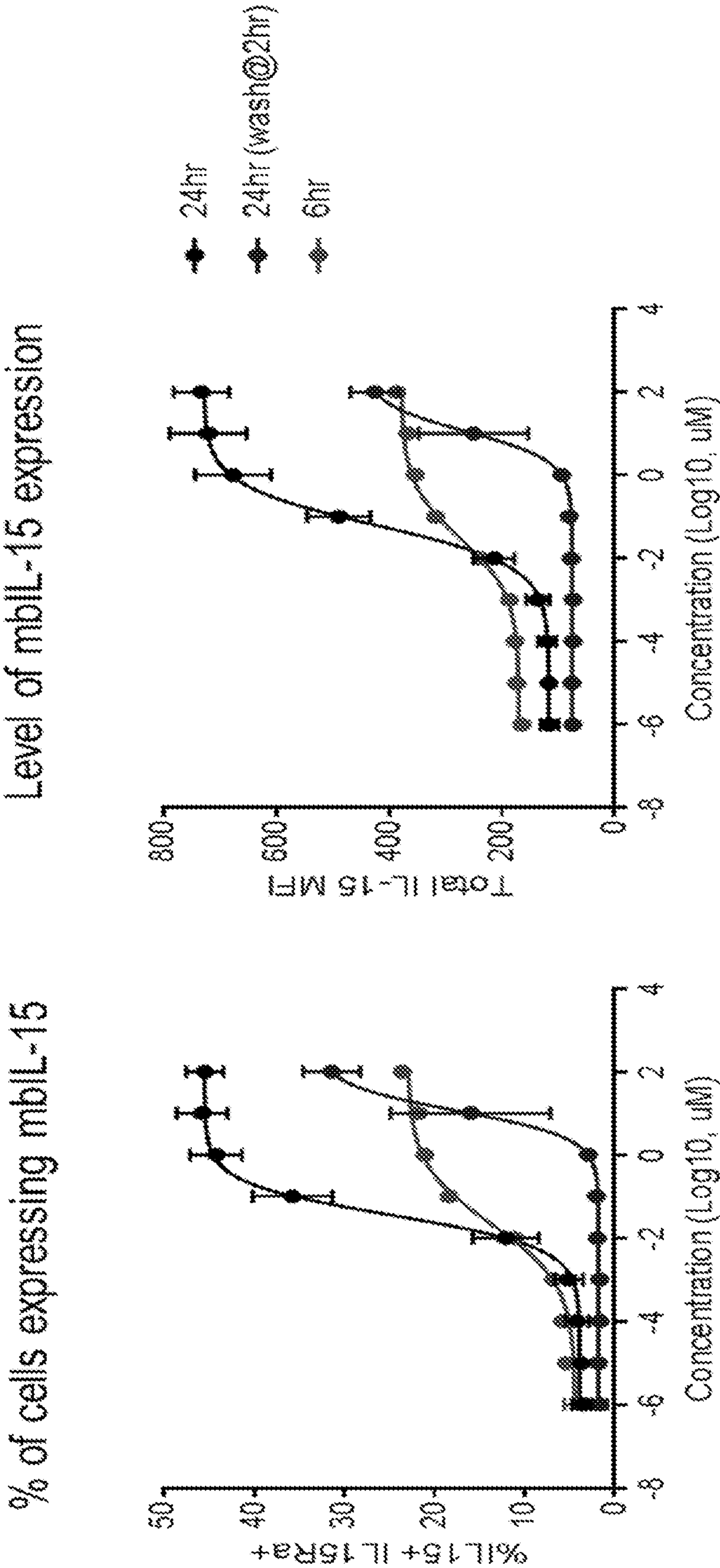


Figure 42B



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Figure 43A

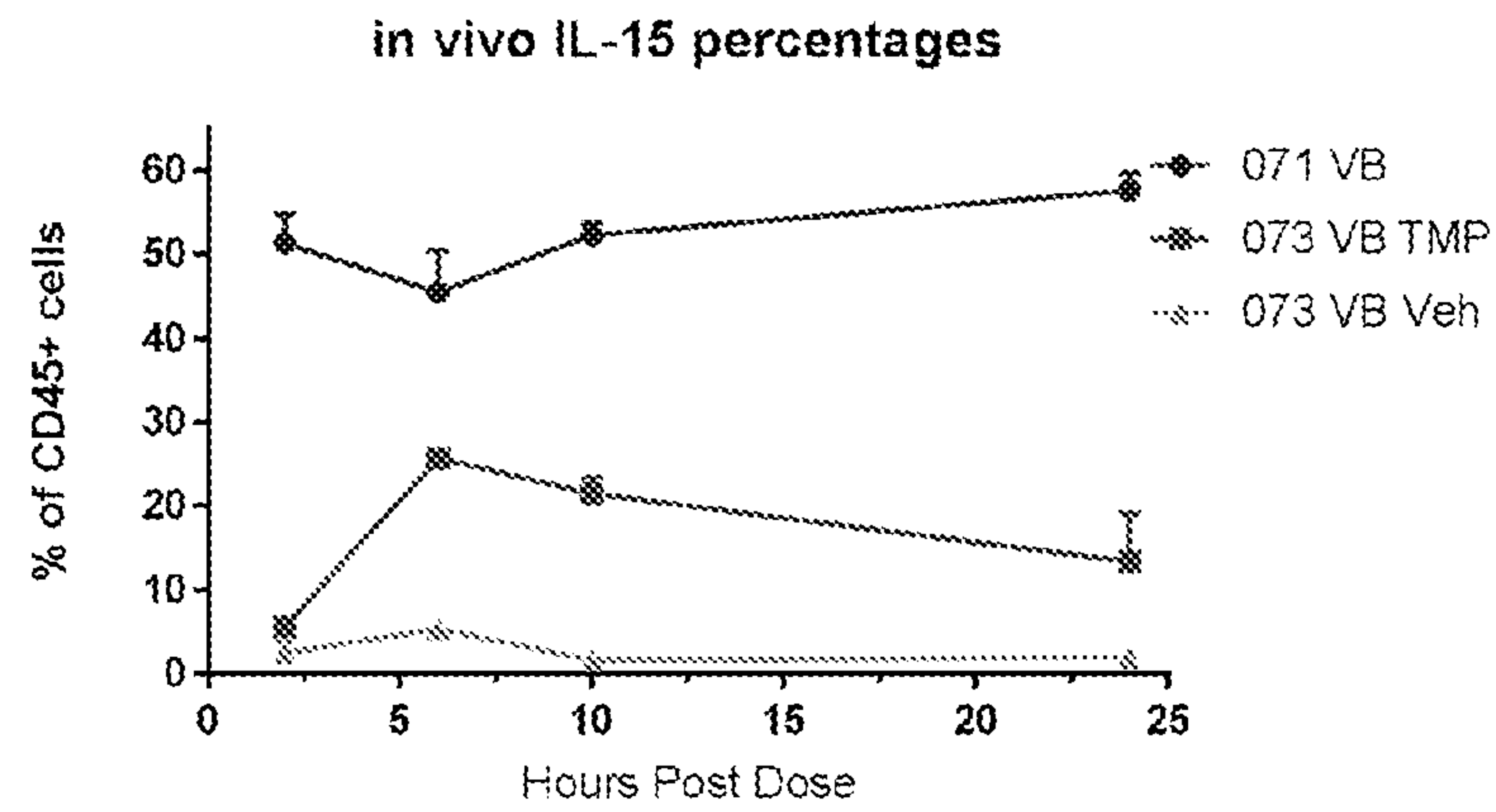
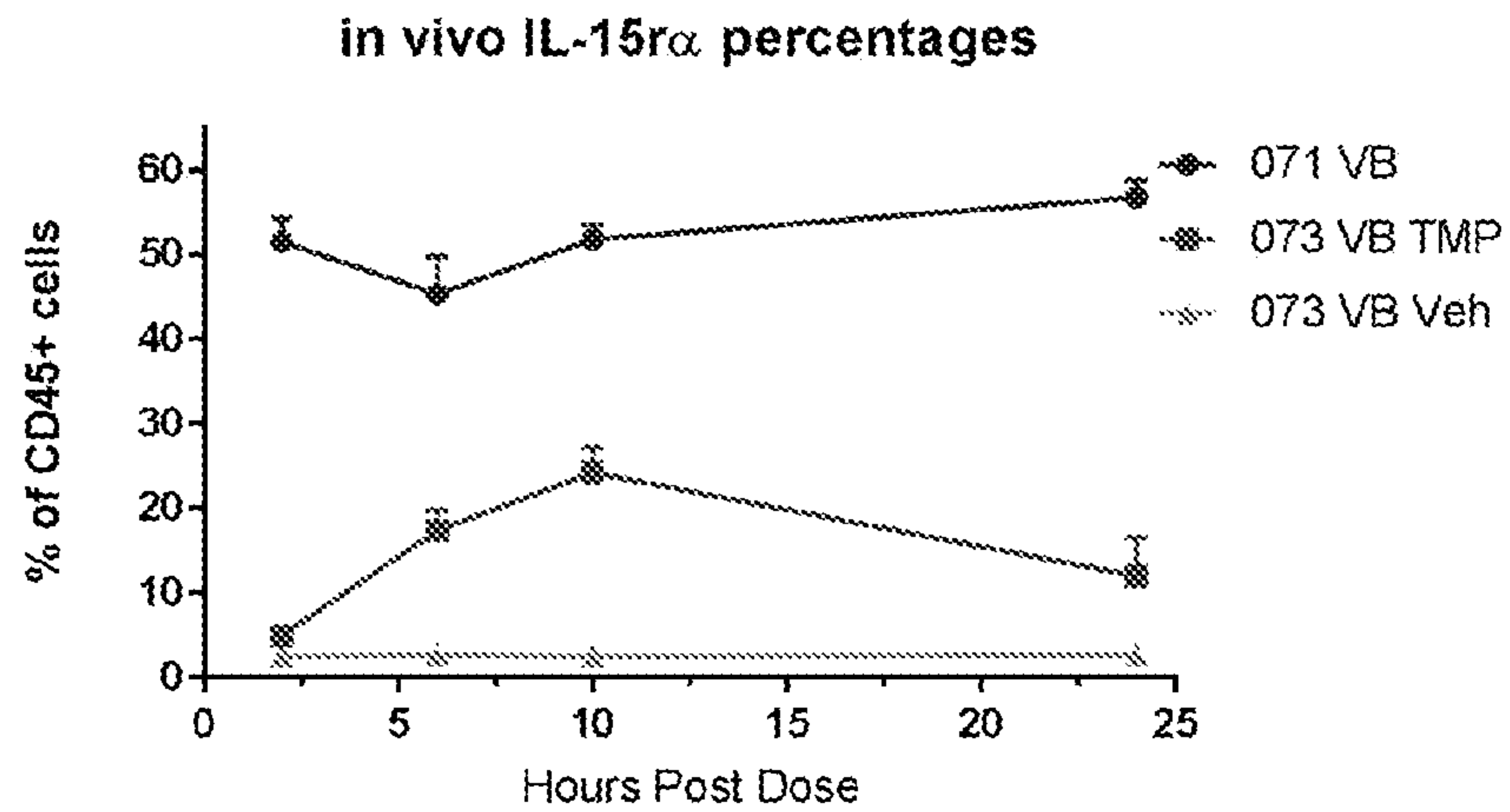
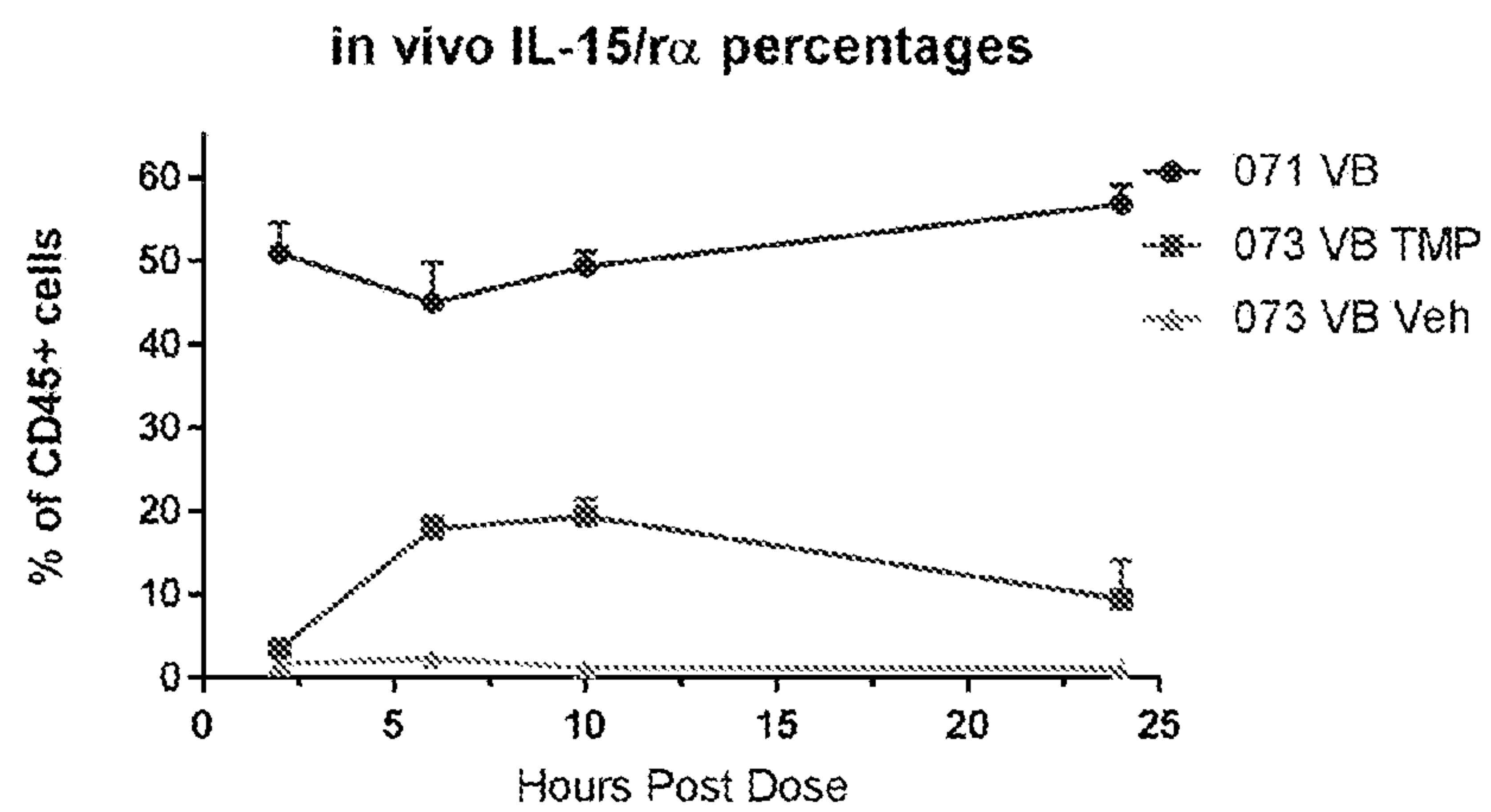


Figure 43B



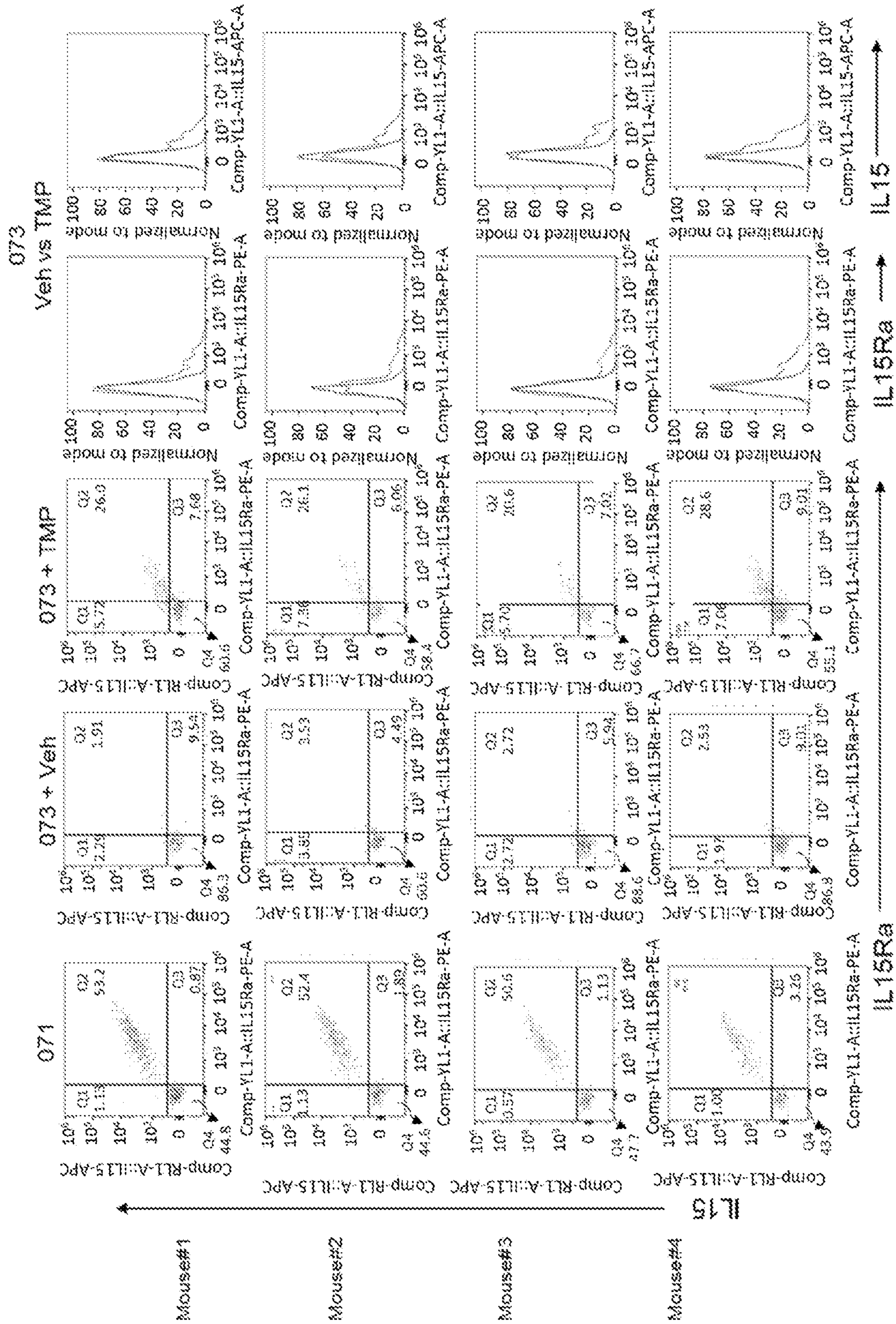
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Figure 43C



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Figure 43D



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Figure 43E

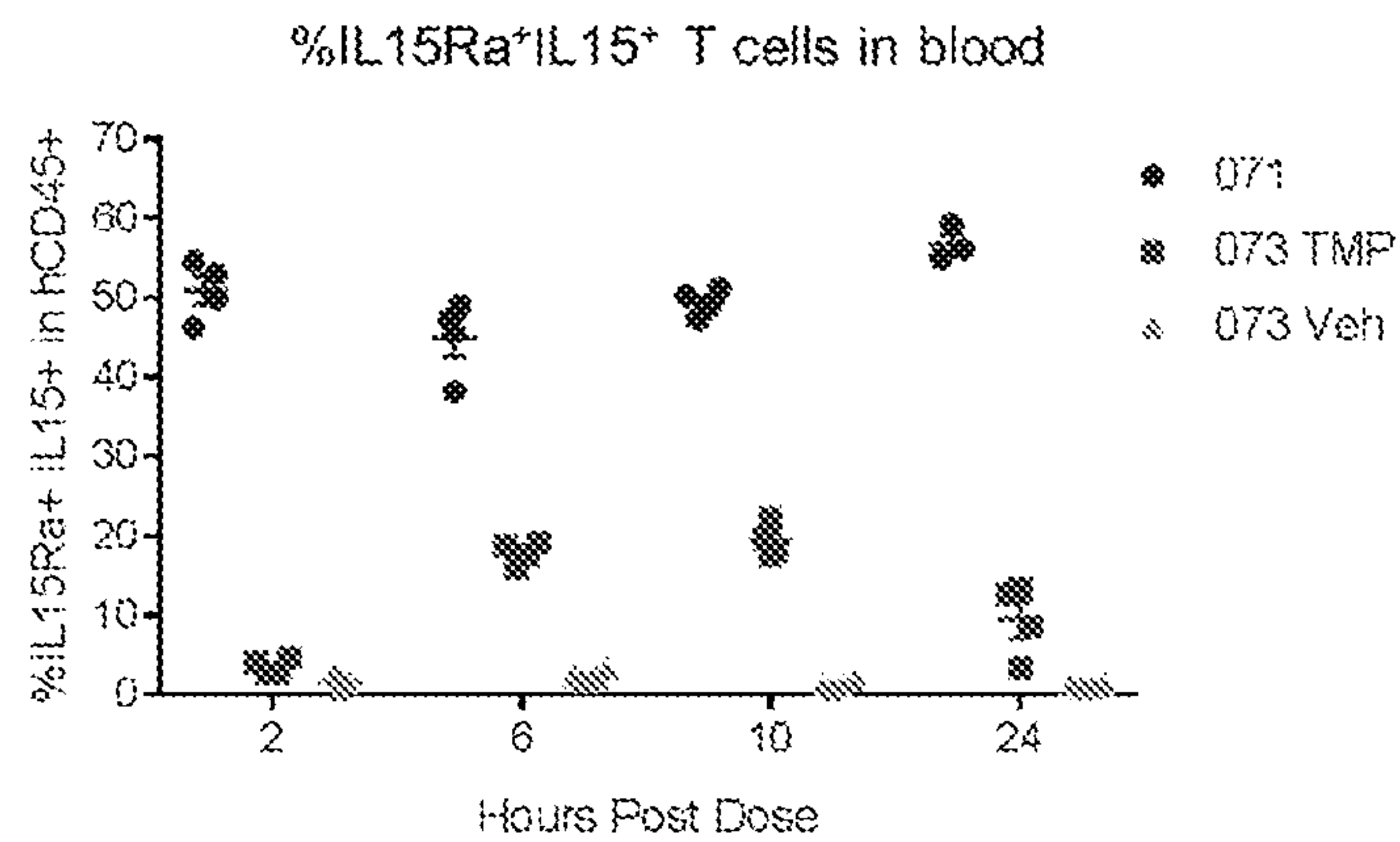


Figure 43F

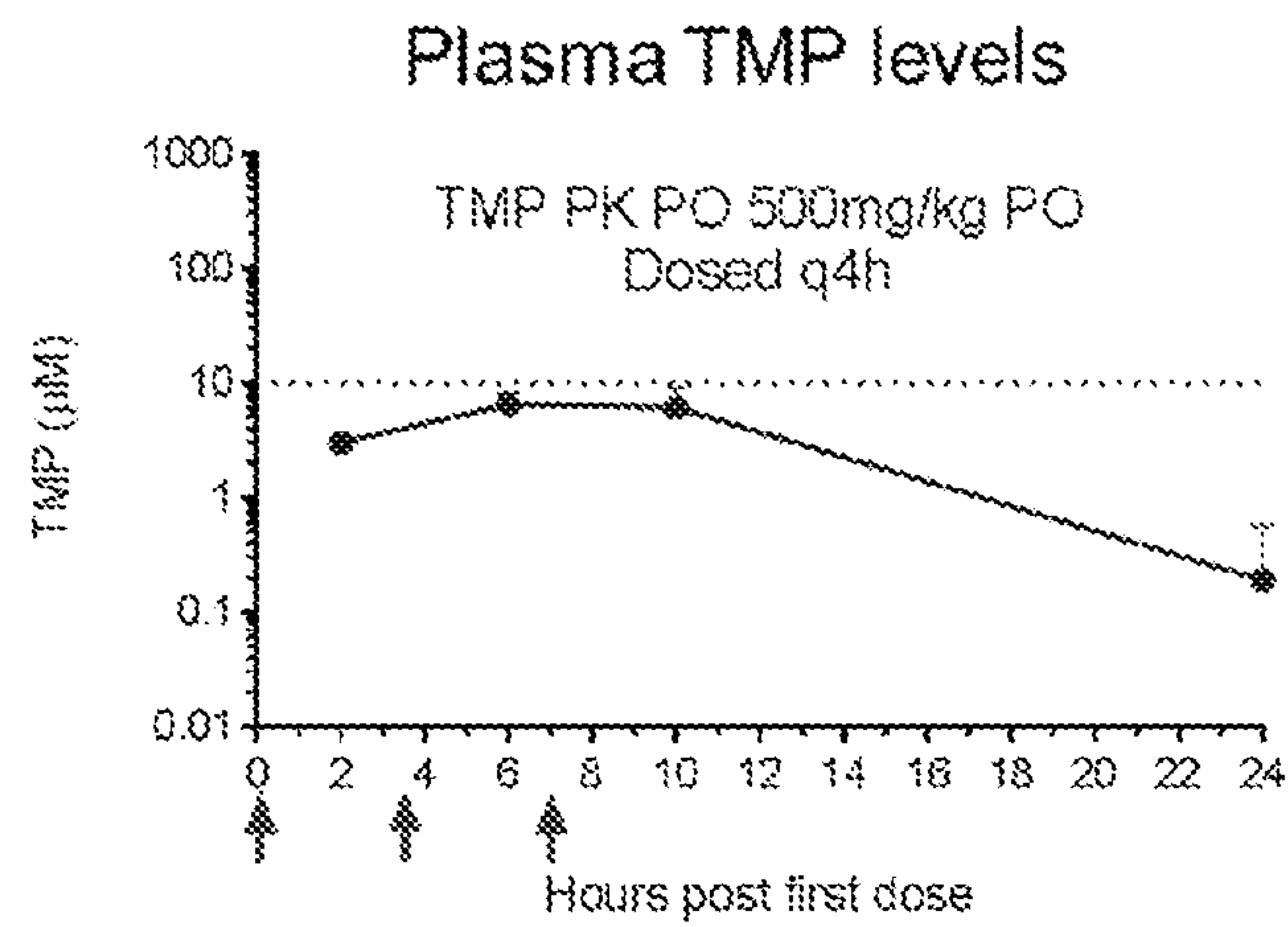


Figure 44

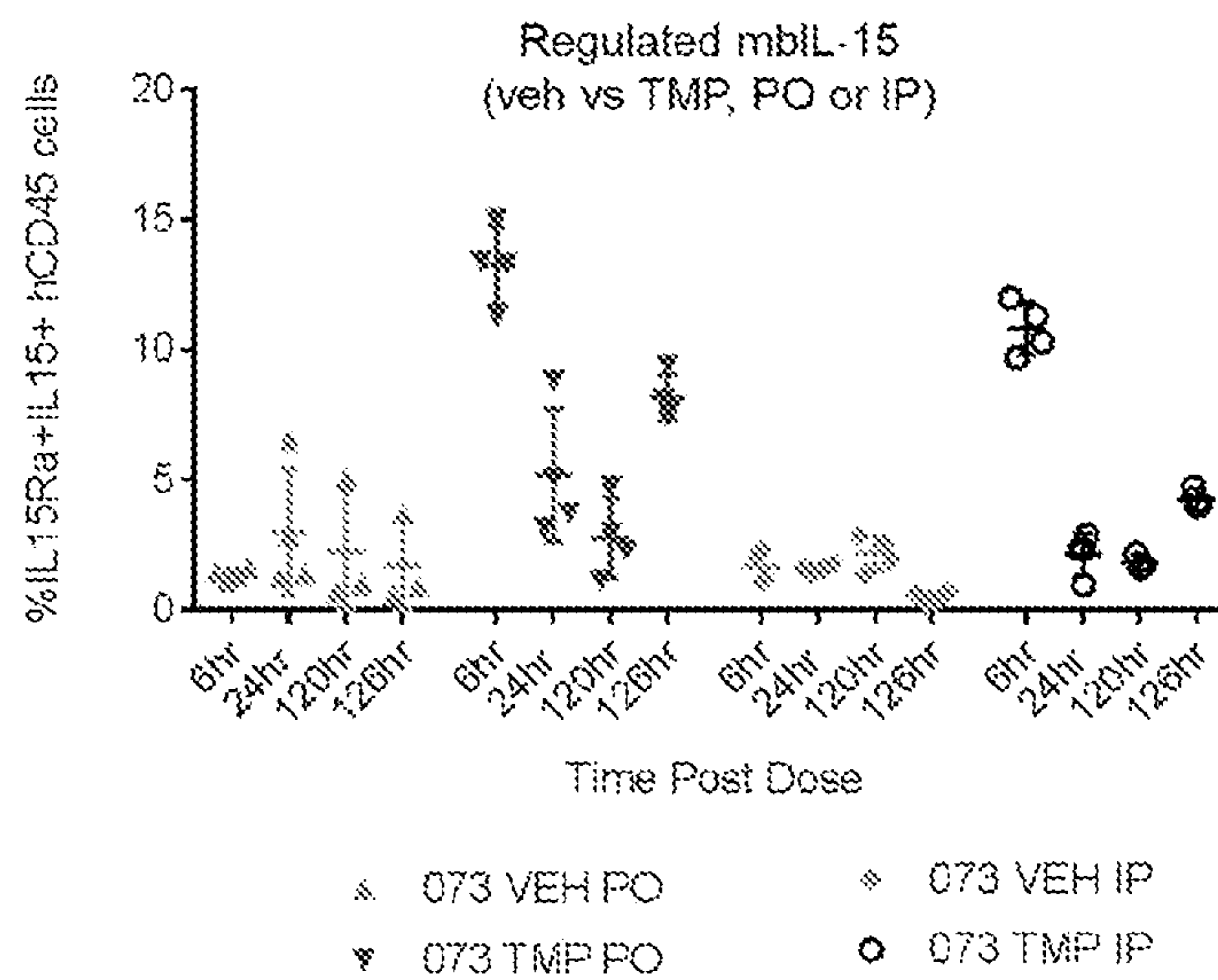


Figure 28A

