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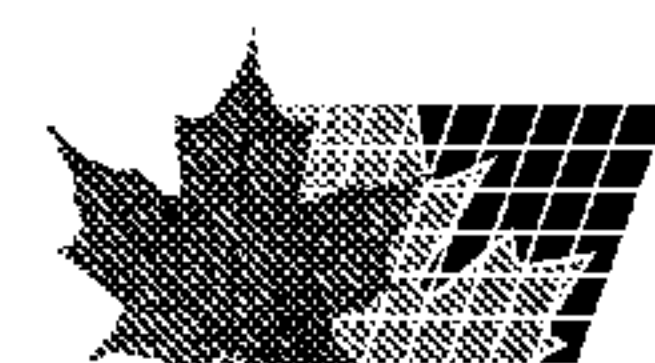
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(54) Titre : RECEPTEUR D'ANTIGENE CHIMERIQUE ANTI-CD123 (CAR) UTILISE DANS LE TRAITEMENT DU
CANCER

(54) Title: ANTI-CD123 CHIMERIC ANTIGEN RECEPTOR (CAR) FOR USE IN CANCER TREATMENT

(57) **Abrégé/Abstract:**

The invention provides compositions and methods for treating diseases associated with expression of CD 123. The invention also relates to chimeric antigen receptor (CAR) specific to CD123, vectors encoding the same, and recombinant cells comprising the CD123 CAR. The invention also includes methods of administering a genetically modified cell expressing a CAR that comprises a CD123 binding domain.



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(54) Title: ANTI-CD123 CHIMERIC ANTIGEN RECEPTOR (CAR) FOR USE IN CANCER TREATMENT

(57) Abstract: The invention provides compositions and methods for treating diseases associated with expression of CD 123. The invention also relates to chimeric antigen receptor (CAR) specific to CD123, vectors encoding the same, and recombinant cells comprising the CD123 CAR. The invention also includes methods of administering a genetically modified cell expressing a CAR that comprises a CD123 binding domain.



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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

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ANTI-CD123 CHIMERIC ANTIGEN RECEPTOR (CAR) FOR USE IN CANCER TREATMENT

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RELATED APPLICATIONS

This application claims priority to PCT Application No. PCT/CN2014/084696, filed August 19, 2014, and PCT Application No. PCT/CN2014/090508, filed November 6, 2014. The entire contents of each of these applications are incorporated herein by reference.

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SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 18, 2015, is named N2067-7064WO5_SL.txt and is 625,588 bytes in size.

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FIELD OF THE INVENTION

The present invention relates generally to the use of immune effector cells (e.g., T cells or NK cells) engineered to express a Chimeric Antigen Receptor (CAR) to treat a disease associated with expression of the Cluster of Differentiation 123 protein (CD123).

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BACKGROUND OF THE INVENTION

Most patients with acute myeloid leukemia (AML) are incurable using standard therapy (Mrozek et al, 2012, J Clin Oncol, 30:4515-23) and those with relapsed or refractory AML (RR-AML) have a particularly poor prognosis (Kern et al, 2003, Blood 2003, 101:64-70; Wheatley et al, 1999, Br J Haematol, 107:69-79).

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Genetic engineering can impart to T cells specificity toward a target of choice. T cells can be transduced with genetic material encoding a single chain variable fragment (scFv) of an antibody, in conjunction with a signaling molecule, thereby using the complementarity determining region (CDR) to recognize a cell surface antigen in a non-MHC restricted manner. These cells are termed chimeric antigen receptor (CAR) T cells. Preclinical and clinical

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attempts to target at least 20 different surface molecules in a variety of malignancies have shown some activity yet were often limited by poor persistence of the infused CAR T cell product (Sadelain et al, 2009, Curr Opin Immunol 2009, 21:215-23). Recent success with anti-CD19 redirected T cells in patients with advanced CLL and ALL (Porter et al, 2011, N Engl J Med, 365:725-33; Kalos et al, 2011, Science Transl Med, 3:95ra73; Grupp and Kalos, 2013, N Engl J Med, 368:1509-18) demonstrated that these cells can eradicate massive tumor burden after a single infusion with remission lasting up to 3 years to date, underscoring the dramatic potential of CAR T cell therapy. There have been few preclinical attempts to target AML in animal models (Marin et al, 2010, Haematologica, 95:2144-52; Tettamanti et al, 2013, Br J Haematol, 161:389-401) although a recently published small clinical trial demonstrated that it is feasible to produce and infuse T cells to patients with an aggressive malignancy (Ritchie et al, 2013, Mol Ther, 21:2122-9). Besides the ability for the chimeric antigen receptor on the genetically modified T cells to recognize and destroy the targeted cells, a successful therapeutic T cell therapy needs to have the ability to proliferate and persist over time, and to further monitor for relapse. T cells may be variably efficacious due to anergy, suppression or exhaustion but skilled practitioners have limited control over these features at this time. To be effective, CAR transformed patient T cells need to persist and maintain the ability to proliferate in response to antigen. It has been shown that ALL patient T cells perform can do this with CART19 comprising a murine scFv (see, e.g., Grupp et al., NEJM 368:1509-1518 (2013)).

SUMMARY OF THE INVENTION

In a first aspect, the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD123 binding domain (e.g., a human or humanized CD123 binding domain), a transmembrane domain, and an intracellular signaling domain (e.g., an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain). In one embodiment, the CAR comprises a CD123 binding domain described herein (e.g., a human or humanized CD123 binding domain described herein), a transmembrane domain described herein, and an intracellular signaling domain described herein (e.g., an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain).

In one embodiment, the encoded CD123 binding domain comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain

complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a CD123 binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a CD123 binding domain described herein, e.g., a CD123 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the encoded CD123 binding domain (e.g., a human or humanized CD123 binding domain) comprises a light chain variable region described herein (e.g., in Table 2, 6 or 9) and/or a heavy chain variable region described herein (e.g., in Table 2, 6 or 9). In one embodiment, the encoded CD123 binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of Table 2, 6 or 9. In an embodiment, the CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a light chain variable region provided in Table 2, 6 or 9, or a sequence with 95-99% identity with an amino acid sequence of Table 2, 6 or 9; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a heavy chain variable region provided in Table 2, 6 or 9, or a sequence with 95-99% identity to an amino acid sequence of Table 2, 6 or 9.

In other embodiments, the encoded CD123 binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9. In embodiments, the CD123 binding domain further comprises a LC CDR1, a LC CDR2, and a LC CDR3. In embodiments, the CD123 binding domain comprises a LC CDR1, a LC CDR2, and a LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2, 6 or 9.

In some embodiments, the encoded CD123 binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2 or 9, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9.

In one embodiment, the encoded CD123 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:157-160, 184-215, 478, 480, 483, and 485. In an embodiment, the encoded CD123 binding domain (e.g., an scFv) comprises an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of 157-160, 184-215, 478, 480, 483, and 485, or a sequence with 95-99% identity with an amino acid sequence of SEQ ID NO: 157-160, 184-215, 478, 480, 483, and 485.

In another embodiment, the encoded CD123 binding domain comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 216-219 or 243-274, or an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of SEQ ID NO: 216-219 or 243-274, or a sequence with 95-99% identity to SEQ ID NO: 216-219 or 243-274. In another embodiment, the encoded CD123 binding domain comprises a heavy chain variable region comprising an amino acid sequence corresponding to the heavy chain variable region of SEQ ID NO:478, 480, 483, or 485, or an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of the corresponding portion of SEQ ID NO:478, 480, 483, or 485, or a sequence with 95-99% identity to the corresponding portion of SEQ ID NO:478, 480, 483, or 485.

In another embodiment, the encoded CD123 binding domain comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 275-278 or 302-333, or an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of SEQ ID NO: 275-278 or 302-333, or a sequence with 95-99% identity to SEQ ID NO: 275-278 or 302-333. In another embodiment, the encoded CD123 binding domain comprises a light chain variable region comprising an amino acid sequence corresponding to the light chain variable region of SEQ ID NO:478, 480, 483, or 485, or an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of the corresponding

portion of SEQ ID NO:478, 480, 483, or 485, or a sequence with 95-99% identity to the corresponding portion of SEQ ID NO:478, 480, 483, or 485.

In one embodiment, the nucleic acid molecule encoding the scFv comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 479, 481, 482, 484, or a sequence
 5 with 95-99% identity thereof. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding the heavy chain variable region and/or the light chain variable region, wherein said nucleotide sequence comprises a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO: 479, 481, 482, and 484, or a sequence with 95-99% identity thereof, corresponding to the heavy chain variable region and/or the light chain variable
 10 region. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding the heavy chain variable region and/or the light chain variable region, wherein the encoded amino acid sequence is selected from the group consisting of SEQ ID NO:157-160, or a sequence with 95-99% identity thereof. In one embodiment, the nucleic acid molecule encodes an scFv comprising an amino acid sequence selected from the group consisting of SEQ
 15 ID NO:184-215, or a sequence with 95-99% identity thereof. In one embodiment, the nucleic acid molecule comprises a sequence encoding the heavy chain variable region and/or the light chain variable region, wherein the encoded amino acid sequence is selected from the group consisting of SEQ ID NO:184-215, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded CD123 binding domain includes a (Gly4-Ser)_n linker,
 20 wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO:26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

In one embodiment, the encoded CAR includes a transmembrane domain that comprises
 25 a transmembrane domain of a protein, e.g., a protein described herein, e.g., selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the encoded transmembrane domain comprises the sequence of SEQ ID NO: 6. In one embodiment, the encoded transmembrane domain
 30 comprises an amino acid sequence comprising at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:6, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the

nucleic acid sequence encoding the transmembrane domain comprises a nucleotide sequence of SEQ ID NO: 17, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded CD123 binding domain is connected to the transmembrane domain by a hinge region, e.g., a hinge region described herein. In one
 5 embodiment, the encoded hinge region comprises SEQ ID NO:2, or a sequence with 95-99% identity thereof. In one embodiment, the nucleic acid sequence encoding the hinge region comprises a nucleotide sequence of SEQ ID NO: 13, or a sequence with 95-99% identity thereof.

In one embodiment, the isolated nucleic acid molecule further comprises a sequence
 10 encoding a costimulatory domain, e.g., a costimulatory domain described herein. In embodiments, the intracellular signaling domain comprises a costimulatory domain. In embodiments, the intracellular signaling domain comprises a primary signaling domain. In embodiments, the intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

In one embodiment, the encoded costimulatory domain is a functional signaling domain from a protein, e.g., described herein, e.g., selected from the group consisting of a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1,
 20 LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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,
 25 CD18, LFA-1, ITGB7, NKG2D, NKG2C, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In one embodiment, the encoded costimulatory domain of 4-1BB comprises the amino acid sequence of SEQ ID NO:7. In one embodiment, the encoded costimulatory domain comprises an amino acid sequence having at least one, two or three modifications but not more

than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:7, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7. In one embodiment, the nucleic acid sequence encoding the costimulatory domain comprises the nucleotide sequence of SEQ ID NO:18, or a sequence with 95-99% identity thereof. In another embodiment, the encoded
5 costimulatory domain of CD28 comprises the amino acid sequence of SEQ ID NO:43. In one embodiment, the encoded costimulatory domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:43, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:43. In one embodiment, the nucleic acid sequence encoding the
10 costimulatory domain of CD28 comprises the nucleotide sequence of SEQ ID NO:44, or a sequence with 95-99% identity thereof. In another embodiment, the encoded costimulatory domain of CD27 comprises the amino acid sequence of SEQ ID NO:8. In one embodiment, the encoded costimulatory domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of
15 SEQ ID NO:8, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:8. In one embodiment, the nucleic acid sequence encoding the costimulatory domain of CD27 comprises the nucleotide sequence of SEQ ID NO:19, or a sequence with 95-99% identity thereof. In another embodiment, the encoded costimulatory domain of ICOS comprises the amino acid sequence of SEQ ID NO:45. In one embodiment, the encoded costimulatory
20 domain of ICOS comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:45, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:45. In one embodiment, the nucleic acid sequence encoding the costimulatory domain of ICOS comprises the nucleotide sequence of SEQ ID NO:46, or a sequence with 95-99% identity
25 thereof.

In one embodiment, the isolated nucleic acid molecule further comprises a sequence encoding an intracellular signaling domain, e.g., an intracellular signaling domain described herein.

In some embodiments, the encoded primary signaling domain comprises a functional
30 signaling domain of CD3 zeta. In embodiments, the functional signaling domain of CD3 zeta comprises the amino acid sequence of SEQ ID NO:9 (mutant CD3 zeta) or SEQ ID NO:10 (wild type human CD3 zeta), or a sequence with 95-99% identity thereof.

In one embodiment, the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta. In one embodiment, the encoded intracellular signaling domain of 4-1BB comprises the sequence of SEQ ID NO: 7 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

5 In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:7 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:7 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment,
10 the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:7 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the nucleic acid sequence encoding the intracellular signaling domain comprises the nucleotide sequence of SEQ ID NO:18, or a sequence with 95-99%
15 identity thereof, and/or the CD3 zeta nucleotide sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded intracellular signaling domain comprises a functional signaling domain of CD27 and/or a functional signaling domain of CD3 zeta. In one embodiment, the encoded intracellular signaling domain of CD27 comprises the amino acid
20 sequence of SEQ ID NO:8 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:8 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid
25 sequence of SEQ ID NO:8 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:8 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the nucleic acid sequence encoding the intracellular
30 signaling domain of CD27 comprises the nucleotide sequence of SEQ ID NO:19, or a sequence with 95-99% identity thereof, and/or the CD3 zeta nucleotide sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded intracellular signaling domain comprises a functional signaling domain of CD28 and/or a functional signaling domain of CD3 zeta. In one embodiment, the encoded intracellular signaling domain of CD28 comprises the amino acid sequence of SEQ ID NO:43 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:43 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:43 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:43 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the nucleic acid sequence encoding the intracellular signaling domain of CD28 comprises the nucleotide sequence of SEQ ID NO:44, or a sequence with 95-99% identity thereof, and/or the CD3 zeta nucleotide sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded intracellular signaling domain comprises a functional signaling domain of ICOS and/or a functional signaling domain of CD3 zeta. In one embodiment, the encoded intracellular signaling domain of ICOS comprises the amino acid sequence of SEQ ID NO:45 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:45 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:45 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:45 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the nucleic acid sequence encoding the intracellular signaling domain of ICOS comprises the nucleotide sequence of SEQ ID NO:46, or a sequence with 95-99% identity thereof, and/or the CD3 zeta nucleotide sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereof.

In another aspect, the invention pertains to an isolated nucleic acid molecule encoding a CAR construct comprising a leader sequence, e.g., a leader sequence described herein, e.g., the amino acid sequence of SEQ ID NO: 1; a CD123 binding domain described herein, e.g., a
 5 CD123 binding domain comprising a LC CDR1, a LC CDR2, a LC CDR3, a HC CDR1, a HC CDR2 and a HC CDR3 described herein, e.g., a human or humanized CD123 binding domain described in Table 2, 6 or 9, or a sequence with 95-99% identity thereof; a hinge region described herein, e.g., a hinge region comprising the amino acid sequence of SEQ ID NO:2; a transmembrane domain described herein, e.g., a transmembrane domain comprising the amino
 10 acid sequence of SEQ ID NO: 6; and an intracellular signaling domain, e.g., an intracellular signaling domain described herein. In one embodiment, the encoded intracellular signaling domain comprises a costimulatory domain, e.g., a costimulatory domain described herein (e.g., a 4-1BB costimulatory domain comprising the amino acid sequence of SEQ ID NO:7, or a CD27 costimulatory domain comprising the amino acid sequence of SEQ ID NO:8), and/or a
 15 primary signaling domain, e.g., a primary signaling domain described herein (e.g., a CD3 zeta stimulatory domain comprising a sequence of SEQ ID NO:9 or SEQ ID NO:10). In one embodiment, the isolated nucleic acid molecule encoding the CAR construct includes a leader sequence encoded by the nucleic acid sequence of SEQ ID NO:12, or a sequence with 95-99% identity thereto.

20 In one embodiment, the isolated nucleic acid molecule comprises (e.g., consists of) a nucleic acid encoding a CAR amino acid sequence of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID
 25 NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, or SEQ ID NO: 156; or an amino acid having one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10
 30 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID

NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID
 NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID
 NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID
 NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID
 5 NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, or SEQ ID
 NO: 156; or an amino acid sequence having 85%, 90%, 95%, 96%, 97%, 98% or 99% identity
 to an amino acid sequence of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO:
 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO:
 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO:
 10 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO:
 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:
 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO:
 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO:
 154, SEQ ID NO: 155, or SEQ ID NO: 156.

15 In one embodiment, the isolated nucleic acid molecule comprises (e.g., consists of) a
 nucleic acid sequence of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42,
 SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID
 NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ
 ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82,
 20 SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID
 NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ
 ID NO:94, SEQ ID NO:95, SEQ ID NO:96, or SEQ ID NO:97 or a nucleic acid sequence
 having 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to a nucleic acid sequence of SEQ ID
 NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:66, SEQ ID NO:67, SEQ
 25 ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
 SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID
 NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ
 ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90,
 SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID
 30 NO:96, or SEQ ID NO:97.

In one aspect, the invention pertains to an isolated nucleic acid molecule encoding a CD123 binding domain, wherein the CD123 binding domain comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a CD123 binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a CD123 binding domain described herein, e.g., a human or humanized CD123 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs.

In other embodiments, the encoded CD123 binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any CD123 heavy chain binding domain amino acid sequence listed in Table 2, 6 or 9. In embodiments, the CD123 binding domain further comprises a LC CDR1, a LC CDR2, and a LC CDR3. In embodiments, the CD123 binding domain comprises a LC CDR1, a LC CDR2, and a LC CDR3 of any CD123 light chain binding domain amino acid sequence listed in Table 2, 6 or 9.

In some embodiments, the encoded CD123 binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2, 6 or 9, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9.

In one embodiment, the encoded CD123 binding domain comprises a light chain variable region described herein (e.g., in SEQ ID NO: 275-278 or 302-333) and/or a heavy chain variable region described herein (e.g., in SEQ ID NO: 216-219 or 243-274). In one embodiment, the encoded CD123 binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of in SEQ ID NO: 157-160, 184-215, 478, 480, 483 or 485. In an embodiment, the CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a light chain variable region provided in SEQ ID NO: 275-278 or 302-333, or a sequence with 95-99% identity with an amino acid sequence of SEQ ID NO: 275-278 or 302-333; and/or a heavy chain variable region

comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a heavy chain variable region provided in SEQ ID NO: 216-219 or 243-274, or a sequence with 95-99% identity to an amino acid sequence in SEQ ID NO: 216-219 or 243-274.

In one embodiment, the encoded CD123 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:478, SEQ ID NO:480, SEQ ID NO:483 and SEQ ID NO:485, or a sequence with 95-99% identity thereof. In one embodiment, the encoded CD123 binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, is attached to a heavy chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, via a linker, e.g., a linker described herein. In one embodiment, the encoded CD123 binding domain includes a (Gly4-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

Polypeptides

In another aspect, the invention pertains to an isolated polypeptide molecule encoded by the nucleic acid molecule. In one embodiment, the isolated polypeptide molecule comprises a sequence selected from the group consisting of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO:

143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156, or an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative
 5 substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of any of the aforesaid sequences, or a sequence with 95-99% identity to any of the aforesaid sequences.

In another aspect, the invention pertains to an isolated chimeric antigen receptor (CAR)
 10 molecule (e.g., polypeptide) comprising a CD123 binding domain (e.g., a human or humanized antibody or antibody fragment that specifically binds to CD123), a transmembrane domain, and an intracellular signaling domain (e.g., an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain). In one embodiment, the CAR comprises an antibody or antibody fragment which includes a CD123 binding domain described
 15 herein (e.g., a human or humanized antibody or antibody fragment that specifically binds to CD123 as described herein), a transmembrane domain described herein, and an intracellular signaling domain described herein (e.g., an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain described herein).

In one embodiment, the CD123 binding domain comprises one or more (e.g., all three)
 20 light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a CD123 binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC
 25 CDR3) of a CD123 binding domain described herein, e.g., a CD123 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the CD123 binding domain comprises a light chain variable region described herein (e.g., in Table 2, 6 or 9) and/or a heavy chain variable region described herein (e.g., in Table 2, 6 or 9). In one embodiment, the CD123 binding domain is a scFv comprising a light
 30 chain and a heavy chain of an amino acid sequence listed in Table 2, 6 or 9. In an embodiment, the CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g.,

conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a light chain variable region provided in Table 2, 6 or 9, or a sequence with 95-99% identity with an amino acid sequence provided in Table 2, 6 or 9; and/or a heavy chain variable region comprising an amino acid
 5 sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a heavy chain variable region provided in Table 2, 6 or 9, or a sequence with 95-99% identity to an amino acid sequence provided in Table 2, 6 or 9.

10 In other embodiments, the CD123 binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any CD123 heavy chain binding domain amino acid sequences provided in Table 2, 6 or 9. In embodiments, the CD123 binding domain further comprises a LC CDR1, a LC CDR2, and a LC CDR3. In embodiments, the CD123 binding domain comprises a LC CDR1, a LC CDR2, and a LC CDR3) of any CD123 light chain binding domain amino acid
 15 sequences listed in Table 2, 6 or 9.

In some embodiments, the CD123 binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2, 6 or 9, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9.

20 In one embodiment, the CD123 binding domain comprises an amino acid sequence selected from a group consisting of SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID
 25 NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:478, SEQ ID NO:480, SEQ ID NO:483 and SEQ ID NO:485; or an amino acid sequence having at least one, two or three
 30 modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) to any of the aforesaid

sequences; or a sequence with 95-99% identity to any of the aforesaid sequences. In one embodiment, the CD123 binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, is attached to a heavy chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, via a linker, e.g., a linker described herein. In one embodiment, the CD123 binding domain includes a (Gly4-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

In one embodiment, the isolated CAR molecule comprises a transmembrane domain of a protein, e.g., a protein described herein, e.g., selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO: 6. In one embodiment, the transmembrane domain comprises an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of SEQ ID NO: 6, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 6.

In one embodiment, the CD123 binding domain is connected to the transmembrane domain by a hinge region, e.g., a hinge region described herein. In one embodiment, the encoded hinge region comprises SEQ ID NO:2, or a sequence with 95-99% identity thereof.

In one embodiment, the isolated CAR molecule further comprises a sequence encoding a costimulatory domain, e.g., a costimulatory domain described herein.

In some embodiments, the intracellular signaling domain of the isolated CAR molecule comprises a costimulatory domain. In embodiments, the intracellular signaling domain of the isolated CAR molecule comprises a primary signaling domain. In embodiments, the intracellular signaling domain of the isolated CAR molecule comprises a costimulatory domain and a primary signaling domain.

In one embodiment, the costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic

activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, 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), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, 5 CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), 10 PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In one embodiment, the costimulatory domain of 4-1BB comprises the sequence of SEQ ID NO:7. In one embodiment, the costimulatory domain comprises an amino acid sequence 15 having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of SEQ ID NO:7, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:7. In another embodiment, the costimulatory domain of CD28 comprises the amino acid sequence of SEQ ID NO:43. In one embodiment, the 20 costimulatory domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:43, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:43. In another embodiment, the costimulatory domain of CD27 comprises the amino acid sequence of SEQ ID NO:8. In one embodiment, the costimulatory domain comprises an amino acid 25 sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:8, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:8. In another embodiment, the costimulatory domain of ICOS comprises the amino acid sequence of SEQ ID NO:45. In one embodiment, the costimulatory domain comprises an amino acid sequence having at least one, two or three 30 modifications, but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:45, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:45.

In embodiments, the primary signaling domain comprises a functional signaling domain of CD3 zeta. In embodiments, the functional signaling domain of CD3 zeta comprises SEQ ID NO:9 (mutant CD3 zeta) or SEQ ID NO:10 (wild type human CD3 zeta), or a sequence with 95-99% identity thereof.

5 In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three
10 modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions, e.g., conservative substitutions) of the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10., or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular
15 signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

In one embodiment, the intracellular signaling domain comprises a functional signaling
20 domain of CD27 and/or a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain of CD27 comprises the amino acid sequence of SEQ ID NO: 8 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino
25 acid sequence of SEQ ID NO:8 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:8 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO:8 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling
30 domain are expressed in the same frame and as a single polypeptide chain.

In one embodiment, the intracellular signaling domain comprises a functional signaling domain of CD28 and/or a functional signaling domain of CD3 zeta. In one embodiment, the encoded intracellular signaling domain of CD28 comprises the amino acid sequence of SEQ ID NO:43 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one
5 embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:43 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:43 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the
10 intracellular signaling domain comprises the sequence of SEQ ID NO:43 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

In one embodiment, the intracellular signaling domain comprises a functional signaling domain of ICOS and/or a functional signaling domain of CD3 zeta. In one embodiment, the
15 intracellular signaling domain of ICOS comprises the amino acid sequence of SEQ ID NO:45 and/or the CD3 zeta amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:45 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID
20 NO:10, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:381 and/or an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:45 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide
25 chain.

In one embodiment, the isolated CAR molecule further comprises a leader sequence, e.g., a leader sequence described herein. In one embodiment, the leader sequence comprises the amino acid sequence of SEQ ID NO: 1, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:1.

30 In another aspect, the invention pertains to an isolated CAR molecule comprising a leader sequence, e.g., a leader sequence described herein, e.g., a leader sequence of SEQ ID

NO: 1, or having 95-99% identity thereof, a CD123 binding domain described herein, e.g., a CD123 binding domain comprising a LC CDR1, a LC CDR2, a LC CDR3, a HC CDR1, a HC CDR2 and a HC CDR3 described herein, e.g., a CD123 binding domain described in Table 2 or 6, or a sequence with 95-99% identify thereof, a hinge region, e.g., a hinge region described herein, e.g., a hinge region of SEQ ID NO:2, or having 95-99% identity thereof, a transmembrane domain, e.g., a transmembrane domain described herein, e.g., a transmembrane domain having a sequence of SEQ ID NO:6 or a sequence having 95-99% identity thereof, an intracellular signaling domain, e.g., an intracellular signaling domain described herein (e.g., an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain). In one embodiment, the intracellular signaling domain comprises a costimulatory domain, e.g., a costimulatory domain described herein, e.g., a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7, or having 95-99%identity thereof, and/or a primary signaling domain, e.g., a primary signaling domain described herein, e.g., a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10, or having 95-99% identity thereof. In one embodiment, the intracellular signaling domain comprises a costimulatory domain, e.g., a costimulatory domain described herein, e.g., a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7, and/or a primary signaling domain, e.g., a primary signaling domain described herein, e.g., a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10.

In one embodiment, the isolated CAR molecule comprises (e.g., consists of) an amino acid sequence of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156, or an amino acid sequence having at least one, two, three, four, five, 10, 15, 20 or 30 modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 60, 50 or 40 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129,

SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134,
 SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139,
 SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144,
 SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149,
 5 SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154,
 SEQ ID NO: 155, and SEQ ID NO: 156, or an amino acid sequence having 85%, 90%, 95%,
 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 98, SEQ ID NO:
 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127,
 SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132,
 10 SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137,
 SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142,
 SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147,
 SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152,
 SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156.

15

In one aspect, the invention pertains to a CD123 binding domain comprising one or
 more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain
 complementary determining region 2 (LC CDR2), and light chain complementary determining
 region 3 (LC CDR3) of a CD123 binding domain described herein, and/or one or more (e.g., all
 20 three) heavy chain complementary determining region 1 (HC CDR1), heavy chain
 complementary determining region 2 (HC CDR2), and heavy chain complementary
 determining region 3 (HC CDR3) of a CD123 binding domain described herein, e.g., a CD123
 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all
 three, HC CDRs (e.g., one, two or three HC CDRs according to Tables 3, 7, 10 or 12; and/or
 25 one, two or three LC CDRs according to Tables 4, 8, 11 or 13).

In other embodiments, the CD123 binding domain comprises a HC CDR1, a HC CDR2,
 and a HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in
 Table 2, 6 or 9. In embodiments, the CD123 binding domain further comprises a LC CDR1, a
 LC CDR2, and a LC CDR3. In embodiments, the CD123 binding domain comprises a LC
 30 CDR1, a LC CDR2, and a LC CDR3) of any CD123 light chain binding domain amino acid
 sequences listed in Table 2, 6 or 9.

In some embodiments, the CD123 binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2, 6 or 9, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9.

5 In one embodiment, the CD123 binding domain comprises a light chain variable region described herein (e.g., in SEQ ID NO:275-278 or 302-333) and/or a heavy chain variable region described herein (e.g. in SEQ ID NO:216-219 or 243-274). In one embodiment, the CD123 binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of SEQ ID NO:157-160, 184-215, 478, 480, 483 or 485. In an embodiment, the
10 CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a light chain variable region provided, in SEQ ID NO: 275-278 or 302-333 or a sequence with 95-99% identity with an
15 amino acid sequence in SEQ ID NO: 275-278 or 302-333; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a heavy chain variable region provided in SEQ ID NO: 216-219 or 243-274, or a sequence with 95-99%
20 identity to an amino acid sequence in SEQ ID NO: 216-219 or 243-274.

In one embodiment, the CD123 binding domain comprises a sequence selected from a group consisting of SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID
25 NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:478, SEQ ID NO:480, SEQ ID NO:483, and SEQ ID
30 NO:485, or a sequence with 95-99% identity thereof. In one embodiment, the CD123 binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, is attached to a heavy chain variable region

comprising an amino acid sequence described herein, e.g., in Table 2, 6 or 9, via a linker, e.g., a linker described herein. In one embodiment, the CD123 binding domain includes a (Gly4-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

Nucleic Acids, Vectors and Cells

The nucleic acid molecules described herein can be a DNA molecule, an RNA molecule, or a combination thereof. In one embodiment, the nucleic acid molecule is an mRNA encoding a CAR polypeptide as described herein. In other embodiments, the nucleic acid molecule is a vector that includes any of the aforesaid nucleic acid molecules.

In another aspect, the invention pertains to a vector comprising a nucleic acid molecule described herein, e.g., a nucleic acid molecule encoding a CAR described herein. In one embodiment, the vector is selected from the group consisting of a DNA molecule or an RNA molecule (e.g., a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector).

In one embodiment, the vector is a lentivirus vector. In one embodiment, the vector further comprises a promoter. In one embodiment, the promoter is an EF-1 promoter. In one embodiment, the EF-1 promoter comprises a sequence of SEQ ID NO: 11. In another embodiment, the promoter is a PGK promoter, e.g., a truncated PGK promoter as described herein.

In one embodiment, the vector is an *in vitro* transcribed vector, e.g., a vector that transcribes RNA of a nucleic acid molecule described herein. In one embodiment, the nucleic acid sequence in the vector further comprises a poly(A) tail, e.g., a poly A tail described herein, (e.g., comprising about 150 adenosine bases (SEQ ID NO: 705)). In one embodiment, the nucleic acid sequence in the vector further comprises a 3'UTR, e.g., a 3' UTR described herein, e.g., comprising at least one repeat of a 3'UTR derived from human beta-globulin. In one embodiment, the nucleic acid sequence in the vector further comprises promoter, e.g., a T2A promoter.

In another aspect, the invention pertains to a cell comprising a nucleic acid molecule or a vector, or expressing a CAR polypeptide as described herein. In one embodiment, the cell is

a cell described herein, e.g., an immune effector cell (e.g., a human T cell or NK cell, e.g., a human T cell or NK cell as described herein, or a cell population thereof). In one embodiment, the human T cell is a CD8+ T cell. In some embodiments, the cell expresses the CAR nucleic acid or polypeptide, or at some point expressed the CAR nucleic acid or polypeptide (e.g., a
5 transiently expressed CAR molecule).

In some embodiment, the cell (e.g., the CAR-expressing cell) described herein can further express another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be a chimeric molecule that comprises an inhibitory molecule or a domain thereof. Examples of inhibitory molecules include PD1, PD-
10 L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFRbeta), e.g., as described herein. In one embodiment, the chimeric molecule comprises a first polypeptide, e.g., an inhibitory molecule, associated with a
15 second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or
20 CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFRbeta), or a fragment of any of these (e.g., at least a portion of the extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described
25 herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of the extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

30 In another aspect, the invention pertains to a method of making a cell, e.g., an immune effector cell. The method includes introducing into, e.g., transducing, the immune effector cell

with a nucleic acid molecule described herein (e.g., an RNA molecule, e.g., an mRNA), or a vector comprising a nucleic acid molecule encoding a CAR, e.g., a CAR described herein.

5 The present invention also provides a method of generating a population of cells (e.g., RNA-engineered cells transiently expressing an exogenous RNA). The method includes introducing into the cell an RNA as described herein (e.g., an in vitro transcribed RNA or synthetic RNA; an mRNA sequence encoding a CAR polypeptide as described herein). In embodiments, the RNA expresses the CAR polypeptide transiently. In one embodiment, the cell is a cell as described herein, e.g., an immune effector cell (e.g., T cells or NK cells, or cell
10 population).

Therapeutic Uses

In another aspect, the invention pertains to a method of providing an anti-tumor immunity in a mammal comprising administering to the mammal an effective amount of a cell
15 expressing a CAR molecule, e.g., a cell expressing a CAR molecule described herein. In one embodiment, the cell is an autologous immune effector cell, e.g., T cell or NK cell. In one embodiment, the cell is an allogeneic immune effector cell, e.g., T cell or NK cell. In one embodiment, the mammal is a human, e.g., a patient with a hematologic cancer.

20 In another aspect, the invention pertains to a method of treating a mammal having a disease associated with expression of CD123 (e.g., a proliferative disease, a precancerous condition, or a non-cancer related indication associated with the expression of CD123). The method includes administering to the mammal an effective amount of the cells expressing a CAR molecule, e.g., a CAR molecule described herein. In one embodiment, the mammal is a
25 human, e.g., a patient with a hematologic cancer.

In one embodiment, the disease is a disease described herein. In one embodiment, the disease associated with CD123 expression is chosen from: a proliferative disease such as a cancer or a malignancy; a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a non-cancer related indication associated with expression of
30 CD123. In one embodiment, the disease is a hematologic cancer. In other embodiments, the disease is chosen from one or more acute leukemias, including but not limited to, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), acute lymphoblastic B-cell leukemia

(B-cell acute lymphoid leukemia, BALL), and acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL); myelodysplastic syndrome; a myeloproliferative neoplasm; a histiocytic disorder (e.g., a mast cell disorder or a blastic plasmacytoid dendritic cell neoplasm); a mast cell disorder, e.g., systemic mastocytosis or mast cell leukemia; a chronic
 5 myeloid leukemia (CML); and a blastic plasmacytoid dendritic cell neoplasm. In other embodiments, the disease associated with CD123 expression, includes, but is not limited to, atypical and/or non-classical cancer, a malignancy, a precancerous condition or a proliferative disease expressing CD123; and a combination thereof.

In one embodiment, the disease is chosen from one or more of acute myeloid leukemia
 10 (AML), acute lymphoblastic leukemia (ALL), acute lymphoblastic B-cell leukemia (B-cell acute lymphoid leukemia, BALL), acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL), B-cell prolymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia (CML), hairy cell leukemia, Hodgkin lymphoma, a mast cell disorder, a histiocytic disorder, a myelodysplastic syndrome, a myeloproliferative neoplasm, a plasma cell
 15 myeloma, a blastic plasmacytoid dendritic cell neoplasm, or a combination thereof. In one embodiment, the disease is a leukemia, e.g., ALL (e.g., relapsing and refractory ALL) or AML. In other embodiments, the disease is a CD19-negative cancer, e.g., a CD19-negative relapsed cancer.

In some embodiments of any of the aforesaid methods, the cell, e.g., the population of
 20 immune effector cells, comprises a vector, e.g., a lentiviral vector, comprising a nucleic acid molecule encoding the CAR polypeptide as described herein.

In other embodiments of any of the aforesaid methods, the cell, e.g., the population of immune effector cells, comprises an mRNA encoding the CAR polypeptide as described herein. In one embodiment, the cell is a CAR-expressing population of RNA-engineered cells, e.g., a
 25 population of transiently expressing cells.

In some embodiments of any of the aforesaid methods, the method further includes administering one or more doses of a cell (e.g., an immune cell containing a CAR nucleic acid or CAR polypeptide as described herein), to a mammal (e.g., a mammal having a cancer, e.g., a hematologic cancer as described herein (e.g., AML or ALL)). In some embodiments, the one
 30 or more doses of CAR cells (e.g., CD123 CAR cells) comprises at least about 1×10^6 , 5×10^6 , 1×10^7 , 2×10^7 , 5×10^7 , 1×10^8 , 2×10^8 , 5×10^8 , 1×10^9 , 2×10^9 , or 5×10^9 cells.

In one embodiment, up to 10, 9, 8, 7, 6, 5, 4, 3, or 2 doses of cells are administered. In other embodiments, one, two, three, four, five or 6 doses of the cells are administered to the mammal, e.g., in a treatment interval of one, two, three, four or more weeks. In one embodiment, up to 6 doses are administered in two weeks. The doses may be the same or different. In one embodiment, a lower dose is administered initially, followed by one or more higher doses. In one exemplary embodiment, the lower dose is about 1×10^5 to 1×10^9 cells/kg, or 1×10^6 to 1×10^8 cells/kg; and the higher dose is about 2×10^5 to 2×10^9 cells/kg or 2×10^6 to 2×10^8 cells/kg, followed by 3-6 doses of about 4×10^5 to 4×10^9 cells/kg, or 4×10^6 to 4×10^8 cells/kg.

In one embodiment, the one or more doses of the cells are administered after one or more lymphodepleting therapies, e.g., a lymphodepleting chemotherapy. In one embodiment, the lymphodepleting therapy includes a chemotherapy (e.g., cyclophosphamide).

In one embodiment, the one or more doses is followed by a cell transplant, e.g., an allogeneic hematopoietic stem cell transplant. For example, the allogeneic hematopoietic stem cell transplant occurs between about 20 to about 35 days, e.g., between about 23 and 33 days.

In some embodiments, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with one or more therapeutic agents or procedures as described herein.

In one embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with an agent that increases the efficacy of a cell expressing a CAR molecule, e.g., an agent described herein.

In one embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with a low, immune enhancing dose of an mTOR inhibitor. While not wishing to be bound by theory, it is believed that treatment with a low, immune enhancing, dose (e.g., a dose that is insufficient to completely suppress the immune system but sufficient to improve immune function) is accompanied by a decrease in PD-1 positive T cells or an increase in PD-1 negative cells. PD-1 positive T cells, but not PD-1 negative T cells, can be exhausted by engagement with cells which express a PD-1 ligand, e.g., PD-L1 or PD-L2.

In an embodiment this approach can be used to optimize the performance of CAR cells described herein in the subject. While not wishing to be bound by theory, it is believed that, in

an embodiment, the performance of endogenous, non-modified immune effector cells, e.g., T cells, is improved. While not wishing to be bound by theory, it is believed that, in an embodiment, the performance of a CD123 CAR expressing cell is improved. In other embodiments, cells, e.g., T cells, which have, or will be engineered to express a CAR, can be
5 treated ex vivo by contact with an amount of an mTOR inhibitor that increases the number of PD1 negative immune effector cells (e.g., T cells or NK cells), or increases the ratio of PD1 negative immune effector cells, e.g., T cells or NK cells/ PD1 positive immune effector cells, e.g., T cells or NK cells.

In an embodiment, administration of a low, immune enhancing, dose of an mTOR
10 inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, or a catalytic inhibitor, is initiated prior to administration of an CAR expressing cell described herein, e.g., T cells or NK cells. In an embodiment, the CAR cells are administered after a sufficient time, or sufficient dosing, of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells, or the ratio of PD1 negative immune effector cells, e.g., T cells or NK cells / PD1 positive immune
15 effector cells, e.g., T cells or NK cells, has been, at least transiently, increased.

In an embodiment, the cell, e.g., immune effector cell (e.g., T cell or NK cell), to be engineered to express a CAR, is harvested after a sufficient time, or after sufficient dosing of the low, immune enhancing, dose of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells or NK cells, or the ratio of PD1 negative immune effector
20 cells, e.g., T cells or NK cells/ PD1 positive immune effector cells, e.g., T cells or NK cells, in the subject or harvested from the subject has been, at least transiently, increased.

In an embodiment, the invention provides an mTOR inhibitor for use in the treatment of a subject, wherein said mTOR inhibitor enhances an immune response of said subject, and wherein said subject has received, is receiving or is about to receive an immune effector cell
25 that expresses a CD123 CAR as described herein.

In another embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with an agent that ameliorates one or more side effect associated with administration of a cell expressing a CAR molecule, e.g., an agent described herein.

30 In one embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein), is administered in combination with an agent that treats the disease associated with CD123, e.g., an agent described herein.

In one embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with a second therapeutic agent or procedure chosen from one or more of chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, a cytokine, surgical procedure, a radiation procedure, an agonist of a costimulatory molecule, an inhibitor of an immune checkpoint molecule, a vaccine, or a second CAR-based immunotherapy.

In one embodiment, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with an agonist of a costimulatory molecule, e.g., an agonist of a costimulatory molecule chosen from one or more of a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In other embodiments, the cell, e.g., the population of immune effector cells (e.g., cells expressing a CAR molecule described herein) is administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, TGFR (e.g., TGFR beta), or a combination thereof.

In one embodiment, the inhibitor of the immune checkpoint molecule or the agonist of the costimulatory molecule is an antibody molecule, e.g., a monospecific antibody molecule or a bispecific antibody molecule. For example, the cell, e.g., the population of immune effector

cells, can be administered in combination with a PD-1 inhibitor, a TIM-3 inhibitor, a CEACAM-1 inhibitor, or a combination thereof. In one embodiment, the PD-1 inhibitor and the TIM-3 inhibitor are administered in combination. In other embodiments, the TIM-3 inhibitor and the CEACAM-1 inhibitor are administered in combination.

5 In some embodiments, the inhibitor of the immune checkpoint molecule is administered subsequent to the administration of the cell, e.g., the population of immune effector cells, e.g., about 3-7 days after the administration of the cell, e.g., the population of immune effector cells.

As described in the Examples herein, CAR123-expressing immune effector cells have
10 been shown to be an effective treatment for CD19-negative relapses (e.g., CD19-negative B-ALL). Without being bound by theory, a combinatorial approach of CAR123- and CD19 inhibition (e.g., CAR19-expressing immune effector cells) can be used to treat CD19-positive disease, while retarding or preventing antigen-loss relapses.

Accordingly, in yet other embodiments of any of the aforesaid methods, the cell, e.g.,
15 the population of immune effector cells, is administered in combination with a CD19 inhibitor, e.g., a CD19 inhibitor as described herein.

In some embodiments, the CD19 inhibitor is a CD19 CAR-expressing cell or an anti-CD19 antibody molecule. In one embodiment, the disease is a leukemia, e.g., ALL (e.g., relapsing and refractory ALL). In other embodiments the disease is AML. In other
20 embodiments, the disease is a CD19-negative cancer, e.g., a CD19-negative relapsed cancer.

Alternatively or in combination with any of the aforesaid methods, a method of preventing a CD19-negative relapse in a mammal, e.g., a human, is provided. The method includes administering an effective amount of a cell, e.g., a population of immune effector cells
25 (e.g., cells expressing a CAR molecule described herein). In some embodiments, the method further includes administering a CD19 inhibitor, e.g., a CD19 CAR-expressing cell. In some embodiments, the disease is a leukemia, e.g., acute lymphoblastic leukemia (e.g., relapsing and refractory ALL), or AML.

In some embodiments, the cell, e.g., the population of immune effector cells, is
30 administered before, simultaneously or concurrently, or after administration of the CD19 inhibitor. In one embodiment, the cell, e.g., the population of immune effector cells, is

administered after administration of the CD19. In other embodiments, the cell, e.g., the population of immune effector cells, is administered concurrently with the CD19 inhibitor.

In some embodiments, the cell, e.g., the population of immune effector cells, expresses a CD19 CAR and the CD123 CAR (e.g., a CAR as described herein).

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Without wishing to be bound by theory, certain leukemic cells (e.g., B-ALL blasts) have been found to co-express CD19 and CD123, while hematopoietic stem cells (HSC) are typically CD123-positive (CD123+), but CD19-negative (CD19-). In order to target leukemic cells that co-express CD19 and CD123 (e.g., B-ALL blasts) while sparing HSC, a split CAR can be used where the functional intracellular domains are separated between CAR19 and CAR123. Such CAR molecules would cause full activation of the immune cell preferentially when the target cells co-express CD19 and CD123 (e.g., B-ALL blasts), as opposed to a cell that expresses one of CD19 or CD123 (e.g. HSCs).

Accordingly, in some embodiments, the CD19 CAR or CD123 CAR comprises a split intracellular signaling domain such that full activation of the cell, e.g., the population of immune effector cells, occurs when both the CD19 CAR and CD123 CAR bind to a target cell, e.g., a target CD19+CD123+ cell (e.g., a B-ALL blast cell), compared to activation when the CD19 CAR and CD123 CAR bind to a target cell that expresses one of CD19 or CD123 (e.g., a hematopoietic stem cell). In one embodiment, the CD123CAR comprises a costimulatory domain, e.g., 4-1BB signaling domain, and the CD19 CAR comprises a primary signaling domain, e.g., a CD3 zeta signaling domain. In other embodiments, the CD123CAR comprises a primary signaling domain, e.g., a CD3 zeta signaling domain, and the CD19 CAR comprises a costimulatory domain, e.g., 4-1BB signaling domain. The CD123CAR and the CD19 CAR may be optionally linked to, e.g., one or more peptide cleavage sites such as P2A.

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In yet other embodiments, the methods disclosed herein further include administering a T cell depleting agent after treatment with the cell (e.g., an immune effector cell as described herein), thereby reducing (e.g., depleting) the CAR-expressing cells (e.g., the CD123CAR-expressing cells). Such T cell depleting agents can be used to effectively deplete CAR-expressing cells (e.g., CD123CAR-expressing cells) to mitigate toxicity.

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For example, alternatively or in combination with the methods disclosed herein, a method of reducing (e.g., depleting) a CAR-expressing cell after a CAR therapy (e.g., a

CAR123 therapy disclosed herein) is disclosed. The method includes administering to a mammal a T cell depleting agent, in an amount to reduce (e.g., deplete) the CAR-expressing cells. In some embodiments, the T cell depleting agent is administered after treatment of the mammal with a cell, e.g., a population of immune effector cells (e.g., a CAR-expressing population of cells), thereby reducing (e.g., depleting) the cell (e.g., the CAR-expressing cell).

In some embodiments, the method further includes transplanting a cell, e.g., a hematopoietic stem cell, or a bone marrow, into the mammal.

In some embodiments, the mammal has a leukemia, e.g., acute lymphoblastic leukemia.

In some embodiments, the T cell depleting agent is administered one, two, three, four, or five weeks after administration of the cell, e.g., the population of immune effector cells, described herein.

In one embodiment, the T cell depleting agent is an agent that depletes CAR-expressing cells, e.g., by inducing antibody dependent cell-mediated cytotoxicity (ADCC) and/or complement-induced cell death. For example, CAR-expressing cells described herein may also express an antigen (e.g., a target antigen) that is recognized by molecules capable of inducing cell death, e.g., ADCC or complement-induced cell death. For example, CAR expressing cells described herein may also express a target protein (e.g., a receptor) capable of being targeted by an antibody or antibody fragment. Examples of such target proteins include, but are not limited to, EpCAM, VEGFR, integrins (e.g., integrins $\alpha v \beta 3$, $\alpha 4$, $\alpha I \beta 3$, $\alpha 4 \beta 7$, $\alpha 5 \beta 1$, $\alpha v \beta 3$, αv), members of the TNF receptor superfamily (e.g., TRAIL-R1, TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof (e.g., versions preserving one or more extracellular epitopes but lacking one or more regions within the cytoplasmic domain).

In some embodiments, the CAR expressing cell co-expresses the CAR and the target protein, e.g., naturally expresses the target protein or is engineered to express the target protein. For example, the cell, e.g., the population of immune effector cells, can include a nucleic acid (e.g., vector) comprising the CAR nucleic acid (e.g., a CAR nucleic acid as described herein) and a nucleic acid encoding the target protein.

In one embodiment, the T cell depleting agent is a CD52 inhibitor, e.g., an anti-CD52 antibody molecule, e.g., alemtuzumab.

In other embodiments, the cell, e.g., the population of immune effector cells, expresses a CAR molecule as described herein (e.g., CD123CAR) and the target protein recognized by the T cell depleting agent. In one embodiment, the target protein is CD20. In embodiments where the target protein is CD20, the T cell depleting agent is an anti-CD20 antibody, e.g., rituximab.

In further embodiments of any of the aforesaid methods, the methods further include transplanting a cell, e.g., a hematopoietic stem cell, or a bone marrow, into the mammal.

In another aspect, the invention features a method of conditioning a mammal prior to cell transplantation. The method includes administering to the mammal an effective amount of the cell comprising the CAR nucleic acid as described herein, or the polypeptide as described herein. In some embodiments, the cell transplantation is a stem cell transplantation, e.g., a hematopoietic stem cell transplantation, or a bone marrow transplantation. In other embodiments, conditioning a subject prior to cell transplantation includes reducing the number of CD123-expressing cells in a subject, e.g., CD123-expressing normal cells or CD123-expressing cancer cells.

In another aspect, the invention pertains to the isolated nucleic acid molecule encoding a CAR of the invention, the isolated polypeptide molecule of a CAR of the invention, the vector comprising a CAR of the invention, and the cell comprising a CAR of the invention for use as a medicament, e.g., as described herein (e.g., for use in the treatment of a disease associated with expression of CD123).

In another aspect, the invention pertains to the isolated nucleic acid molecule encoding a CAR of the invention, the isolated polypeptide molecule of a CAR of the invention, the vector comprising a CAR of the invention, and the cell comprising a CAR of the invention for use in the treatment of a disease expressing CD123, e.g., a disease expressing CD123 as described herein. In certain embodiments, the disease is a hematologic cancer, e.g., as described herein. In some embodiments, the disease is chosen from: an acute leukemia, including but not limited to, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), acute lymphoblastic

B-cell leukemia (B-cell acute lymphoid leukemia, BALL), and acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL); myelodysplastic syndrome; a myeloproliferative neoplasm; a histiocytic disorder (e.g., a mast cell disorder or a blastic plasmacytoid dendritic cell neoplasm); a mast cell disorder, e.g., systemic mastocytosis or mast
 5 cell leukemia; a chronic myeloid leukemia (CML); or a blastic plasmacytoid dendritic cell neoplasm.

Additional features and embodiments of the aforesaid compositions and methods include one or more of the following:

10 In certain embodiments, the CD123 CAR molecule (e.g., a CD123 CAR nucleic acid or a CD123 CAR polypeptide as described herein), or the CD123 binding domain as described herein, includes one, two or three CDRs from the heavy chain variable region (e.g., HC CDR1, HC CDR2 and/or HC CDR3), provided in Table 3; and/or one, two or three CDRs from the light chain variable region (e.g., LC CDR1, LC CDR2 and/or LC CDR3) provided in Table 4
 15 (e.g., one, two or three HC CDR1, HC CDR2 or HC CDR3, and/or one, two or three LC CDR1, LC CDR2 or LC CDR3, of CAR123-1, CAR123-2, CAR123-3, CAR123-4, provided in Table 3 or 4); or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

20 In certain embodiments, the CD123 CAR molecule (e.g., a CD123 CAR nucleic acid or a CD123 CAR polypeptide as described herein), or the anti-CD123 antigen binding domain as described herein, includes one, two or three CDRs from the heavy chain variable region (e.g., HC CDR1, HC CDR2 and/or HC CDR3), provided in Table 10; and/or one, two or three CDRs from the light chain variable region provided in Table 11 (e.g., one, two or three HC CDR1, HC
 25 CDR2 or HC CDR3, and/or one, two or three LC CDR1, LC CDR2 or LC CDR3, of CAR123-1, CAR123-2, CAR123-3, CAR123-4, provided in Table 10 or 11); or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

In certain embodiments, the CD123 CAR molecule, or the anti-CD123 antigen binding
 30 domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 12; and/or one, two or three CDRs from the light chain variable region provided in Table 13 (e.g., one, two or three HC CDR1, HC CDR2 or HC

CDR3, and/or one, two or three LC CDR1, LC CDR2 or LC CDR3, of CAR123-1, CAR123-2, CAR123-3, CAR123-4, provided in Table 12 or 13); or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

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In certain embodiments, the CD123 CAR molecule, or the anti-CD123 antigen binding domain, includes

(i) a LC CDR1, LC CDR2 and LC CDR3 of any CD123 light chain binding domain amino acid sequences listed in Table 2, 6 or 9, or the LC CDRs in Table 4, 8, 11 or 13; and/or.

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(ii) a HC CDR1, HC CDR2 and HC CDR3 of any CD123 heavy chain binding domain amino acid sequences listed in Table 2, 6 or 9, or the HC CDRs in Table 3, 7, 10 or 12.

In certain embodiments, the CD123 CAR molecule (e.g., a CD123 CAR nucleic acid or a CD123 CAR polypeptide as described herein), or the anti-CD123 antigen binding domain as described herein, includes:

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(1) one, two or three light chain (LC) CDRs chosen from one of the following:

(i) a LC CDR1 of SEQ ID NO:418, LC CDR2 of SEQ ID NO:446 and LC CDR3 of SEQ ID NO:474 of CAR123-1;

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(ii) a LC CDR1 of SEQ ID NO:419, LC CDR2 of SEQ ID NO:447 and LC CDR3 of SEQ ID NO:475 of CAR123-2;

(iii) a LC CDR1 of SEQ ID NO:420, LC CDR2 of SEQ ID NO:448 and LC CDR3 of SEQ ID NO:476 of CAR123-3;

(iv) a LC CDR1 of SEQ ID NO:421, LC CDR2 of SEQ ID NO:449 and LC CDR3 of SEQ ID NO:477 of CAR123-4; and/or

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(2) one, two or three heavy chain (HC) CDRs chosen from one of the following:

(i) a HC CDR1 of SEQ ID NO:334, HC CDR2 of SEQ ID NO:362 and HC CDR3 of SEQ ID NO: 390 of CAR123-1;

(ii) a HC CDR1 of SEQ ID NO: 335, HC CDR2 of SEQ ID NO: 363 and HC CDR3 of SEQ ID NO: 391 of CAR123-2;

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(iii) a HC CDR1 of SEQ ID NO: 336, HC CDR2 of SEQ ID NO: 364 and HC CDR3 of SEQ ID NO: 392 of CAR123-3;

(iv) a HC CDR1 of SEQ ID NO: 337, HC CDR2 of SEQ ID NO: 365 and HC CDR3 of SEQ ID NO: 393 of CAR123-4.

In certain embodiments, the CD123 CAR molecule (e.g., a CD123 CAR nucleic acid or a CD123 CAR polypeptide as described herein), or the anti-CD123 antigen binding domain as described herein, includes:

(1) one, two or three light chain (LC) CDRs chosen from one of the following:

(i) a LC CDR1 of SEQ ID NO:501, LC CDR2 of SEQ ID NO: 506 and LC CDR3 of SEQ ID NO: 511 of CAR123-1;

10 (ii) a LC CDR1 of SEQ ID NO: 502, LC CDR2 of SEQ ID NO: 507 and LC CDR3 of SEQ ID NO: 512 of CAR123-2;

(iii) a LC CDR1 of SEQ ID NO: 503, LC CDR2 of SEQ ID NO: 508 and LC CDR3 of SEQ ID NO: 513 of CAR123-3;

15 (iv) a LC CDR1 of SEQ ID NO: 504, LC CDR2 of SEQ ID NO: 509 and LC CDR3 of SEQ ID NO: 514 of CAR123-4; and/or

(2) one, two or three heavy chain (HC) CDRs chosen from one of the following:

(i) a HC CDR1 of SEQ ID NO:486, HC CDR2 of SEQ ID NO:491 and HC CDR3 of SEQ ID NO: 496 of CAR123-1;

20 (ii) a HC CDR1 of SEQ ID NO: 487, HC CDR2 of SEQ ID NO:492 and HC CDR3 of SEQ ID NO: 497 of CAR123-2;

(iii) a HC CDR1 of SEQ ID NO: 488, HC CDR2 of SEQ ID NO:493 and HC CDR3 of SEQ ID NO: 498 of CAR123-3;

(iv) a HC CDR1 of SEQ ID NO: 489, HC CDR2 of SEQ ID NO:494 and HC CDR3 of SEQ ID NO: 499 of CAR123-4.

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In certain embodiments, the CD123 CAR molecule (e.g., a CD123 CAR nucleic acid or a CD123 CAR polypeptide as described herein), or the anti-CD123 antigen binding domain as described herein, includes:

(1) one, two or three light chain (LC) CDRs chosen from one of the following:

30 (i) a LC CDR1 of SEQ ID NO:531, LC CDR2 of SEQ ID NO: 536 and LC CDR3 of SEQ ID NO: 541 of CAR123-1;

(ii) a LC CDR1 of SEQ ID NO: 532, LC CDR2 of SEQ ID NO: 537 and LC CDR3 of SEQ ID NO: 542 of CAR123-2;

(iii) a LC CDR1 of SEQ ID NO: 533, LC CDR2 of SEQ ID NO: 538 and LC CDR3 of SEQ ID NO: 543 of CAR123-3;

5 (iv) a LC CDR1 of SEQ ID NO: 534, LC CDR2 of SEQ ID NO: 539 and LC CDR3 of SEQ ID NO: 544 of CAR123-4; and/or

(2) one, two or three heavy chain (HC) CDRs chosen from one of the following:

(i) a HC CDR1 of SEQ ID NO: 516, HC CDR2 of SEQ ID NO: 521 and HC CDR3 of SEQ ID NO: 526 of CAR123-1;

10 (ii) a HC CDR1 of SEQ ID NO: 517, HC CDR2 of SEQ ID NO: 522 and HC CDR3 of SEQ ID NO: 527 of CAR123-2;

(iii) a HC CDR1 of SEQ ID NO: 518, HC CDR2 of SEQ ID NO: 523 and HC CDR3 of SEQ ID NO: 528 of CAR123-3;

15 (iv) a HC CDR1 of SEQ ID NO: 519, HC CDR2 of SEQ ID NO: 524 and HC CDR3 of SEQ ID NO: 529 of CAR123-4.

Unless otherwise defined, 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. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Headings, sub-headings or numbered or lettered elements, e.g., (a), (b), (i) etc, are presented merely for ease of reading.

25 The use of headings or numbered or lettered elements in this document does not require the steps or elements be performed in alphabetical order or that the steps or elements are necessarily discrete from one another. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1 shows a graphical representation of CAR expression in JNL cells transduced with anti-CD123 CAR constructs as evaluated by FACS and reported as the percent of cells showing signal above the level of signal in untransduced (CAR negative) cells using Protein L as a detection reagent.

Figure 2, comprising Figures 2A, 2B, and 2C, shows graphical representations of CD123 CAR activity in JNL cells. Anti-CD123 CAR constructs were evaluated for activity using a Jurkat cell line containing the luciferase reporter driven by the NFAT promoter (termed JNL cells). CAR activity is measured as activation of this NFAT-driven reporter.

Figure 3 shows a graphical representation of CD123 CAR expression in primary T-cells. Percentage of cells transduced (expressing the anti-CD123 CAR on the cell surface) and their relative fluorescence intensity of expression were determined by flow cytometric analysis on a BD LSRFortessa or BD-FACSCanto using Protein L as a detection reagent. Gating histogram plots of relative fluorescent intensity from that FACS for signal above unstained cells shows the percentage of transduced T cells. Transduction resulted in a range of CAR positive cells from 12-42%.

Figure 4 shows a graphical representation of CD123-CART-mediated cell killing. T cell killing was directed towards CD123-expressing MOLM13 acute myelogenous leukemia cells stably expressing luciferase. Untransduced T cells were used to determine non-specific background killing levels. The cytolytic activities of CART-CD123 were measured over a range of effector:target cell ratios of 4:1 and 2-fold downward dilutions of T cells where effectors were defined as T cells expressing the anti-CD123 chimeric receptor. Assays were initiated by mixing an appropriate number of T cells with a constant number of targets cells. After 20 hours luciferase signal was measured using the Bright-Glo™ Luciferase Assay on the EnVision instrument.

Figures 5A and 5B show transduction efficiency of T cells with CD123-CARs. Figure 5A shows transduction efficiency of T cells with 1172 and 1176. Figure 5B shows transduction efficiency of T cells with CD123 CARs 2-4.

Figure 6 shows flow cytometry of CD123 CARs 2-4 and 1172 and 1176 to determine the CD4:CD8 ratio.

Figure 7 (provided in Figures 7-1, 7-2, and 7-3) shows the degranulation of CD123 CARs 2-4 and 1172 and 1176 upon exposure to CD123+ tumor cells.

Figure 8 shows a graphical representation of a luciferase assay to assess cytotoxicity of CART cells (NVS 2-4, 1172 and 1176 clones) towards tumor target cells (MOLM14).

Figure 9 shows a comparison of tumor burden in NSG mice injected with luciferase expressing MOLM14 cells at D6 (before CART injection) and at day 13 (6 days post injection with NVS 2-4, 1172 or 1176 clones) or at day 20.

Figure 10 shows expression of CD30 and CD123 in the Hodgkin's Lymphoma cell line HDLM-2.

Figures 11A and 11B show CD123 CAR expression in T-cells transduced with pELNS anti-CD123-41BB-CD3 ζ via detection of dsRed, a surrogate marker of CD123 by T2A coexpression. dsRed was detected via flow cytometry.

Figure 12 shows Q-PCR on CD123 expression in 4 Hodgkin's Lymphoma cell lines (HDLM2, KMH2, L428, SUPHD1), CD123+ MOLM14 (positive control) and A375 (negative control). GUSB was used as a housekeeping gene. The Ct threshold was 40.

Figure 13 shows the effect of IL3 antibody on an in vivo model for Hodgkin's Lymphoma. NOD-SCID- γ -chain KO mice that overexpress human cytokines including IL-3 (NSG-S mice) were engrafted with the luciferase-expressing HDLM-2 cell line. After i.v. injection, the neoplastic cells progressively formed disseminated soft tissue masses. Serial injections of a neutralizing anti-IL3 antibody slowed the growth of tumor.

Figure 14 shows a CD107a degranulation assay. Untransduced T cells (UTD) or CART123 were incubated with HDLM-2 (CD123+ HL cell line) for 4 hours at a ratio of 5 target cells to 1 T cells in the presence of anti-CD28, anti-CD49d antibodies and monensin. Anti-CD30 CAR T cells were used as additional controls. CART123 but not UTD showed increase CD107a degranulation as detected by flow cytometry.

Figure 15 shows a graphical representation of the degranulation assay shown in Figure 20.

Figure 16 demonstrates that CD123 CAR expressing T cells show a significant increase in intra-cytoplasmatic cytokine production (IL-2, TNF).

Figure 17 demonstrates CART123, but not UTD, showed a dose dependent killing as shown by decrease in bioluminescence emission in a luciferase based 24 hr killing assay. T cells
5 were co-incubated with luciferase+ HDML-2 cells at different ratios (0.3:1 – 10:1).

Figure 18 shows CART123 and CART30 cells but not UTD showed robust proliferation when co-cultured with Hodgkin Lymphoma cell lines proliferation assay. T cells were incubated with HL cell lines (CD123+) or controls (Jurkat CD123- , MOLM-14 CD123+) or PMA/Ionomycin (positive control) or cell media (negative control) in a 5-day T cell
10 proliferation assay.

Figure 19, comprising Figures 19A, 19B, 19C, and 19D show CART123 inducing robust production of IFN γ (Fig. 19A), IL-2 (Fig. 19B), TNF α (Fig. 19C), and MIP1b (Fig. 19D) luminex MFI are shown.

Figure 20 shows a schematic representation of an in vivo model of Hodgkin
15 Lymphoma. 1 million luciferase+ HDLM-2 cells were injected i.v. on day 0. Serial bioluminescent imaging (BLI) demonstrated was then performed to observe tumor level. A low level of tumor was observed on day 7, which was followed by gradual increase in tumor burden over approximately 6 weeks, reproducing the indolent nature of the human disease. At day 43 when the tumor burden was 20-fold higher than baseline, mice were treated with 1.5 million
20 CART123 cells or control T cells.

Figure 21 shows BLI to detect HDLM-2 at days post-injection in mice treated with CART123 cells, control T cells, or untreated, according to the schematic in Figure 28.

Figure 22 shows survival of mice treated according to the schematic shown in Figure 28, CART123 induced complete and durable eradication of disseminated tumor within 14 days,
25 leading to 100% relapse-free and 100% overall survival at 6 months.

Figure 23 shows an association between tumor elimination and extensive CAR T cell expansion as detected by flow cytometry in serial peripheral blood bleedings. The expanded T cells were approximately 50% CD8 and 50% CD4 cells. The T cell number contracted over time as tumor burden decreased.

Figure 24, comprising Figures 24A, 24B, and 24C, are bar graphs showing the cytokine production from T cells expressing CAR123 constructs (CD123-2, CD123-3, and CD123-4) when cultured with target cells MOLM13, PL21, and U87 at the indicated ratios. Figure 24A
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shows production of IL-2; Figure 24B shows the production of IFN-gamma; and Figure 24C shows the production of TNF-alpha.

Figure 25 is a bar graph showing the proliferation capacity of lentivirally transduced T cells expressing CAR123-2 (LV CAR123-2) or control anti-GH when cultured in the presence of target cells MOLM13 (which express CD123), K562-human CD123 (which express human CD123), or K562 (which do not express CD123).

Figure 26, comprising Figures 26A, 26B, and 26C, shows the ability of lentivirally transduced T cells expressing CAR123 CAR (LV CAR123-2) or control (Anti-GH), or untransduced cells (UTD) to kill target cells at various target cell:effector cell ratios, where the target cells are CD123-expressing MOLM13 (Fig. 26A) or PL21 (Fig. 26B) cells, or CD123-negative U87 cells (Fig. 26C).

Figure 27, comprising Figures 27A and 27B, are graphs showing cytokine production from lentivirally transduced T cells expressing either a CAR123 (LV CAR123-2) or control (Anti-GH) when cultured in the present of target cells MOLM13 (which express CD123) and U87 (which do not express CD123). Figure 27A shows the cytokine production of IFN γ and TNF α ; Figure 27B shows the cytokine production of IL-2.

Figure 28 shows the tumor burden in an in vivo mouse model of Hodgkin lymphoma, where the mice bearing tumors were administered T cells expressing various CAR123 constructs: 1172, 1176, NVS2 (also referred to herein as CAR123-2), NVS3 (also referred to herein as CAR123-3), and NVS4 (also referred to herein as CAR123-4). Tumor burden at day 6, day 14, day 20, and day 27 were measured and represented by bioluminescent imaging (BLI).

Figure 29 shows the tumor burden in an in vivo mouse model, where the mice bearing tumors were administered T cells expressing CAR123-2 introduced from a lentiviral vector (CART123 LV), CAR123-2 introduced as RNA (CART123 RNA (NVS)), and a tool CAR123, introduced as RNA (CART123 RNA (Penn)). Untransduced T cells were used as control (UTD). Tumor burden is represented by bioluminescent imaging (BLI) units.

Figure 30, comprising Figures 30A, 30B, 30C, and 30D, shows bioluminescent imaging of the tumors in an in vivo mouse model. Figure 30A shows the mice administered untransduced cells; Figure 30B shows the mice administered the T cells expressing RNA CAR123-2; Figure 30C shows the mice administered lentivirally transduced T cells expressing CAR123-2; Figure 30D shows the mice administered T cells expressing RNA tool CAR123.

Figure 31, comprising Figures 31A, 31B, 31C, 31D, 31E, and 31F, shows CD123 is highly expressed in CD19-neg B-cell acute lymphoblastic leukemia relapses occurring after CART19 treatment. Figure 31A shows expression of CD123 compared to CD19 in 42 relapsing/refractory ALL samples. Figure 31B shows CD123 and CD19 co-expression in B-ALL blasts. Gated on blasts (SSC low, singlet, live, CD45dim). Figure 31C shows the gating strategy for the leukemia stem cell (LSC). CD123 is highly expressed in this subset. Figure 32D shows CD123 and CD19 co-expression and results from FISH analysis. Figures 31E and 31F show the comparison of CD19 and CD123 expression at baseline or after relapse.

Figure 32, comprising Figures 32A, 32B, 32C, 32D, 32E, and 32F, shows results from various in vitro assays using T cells expressing a CD19 CAR (CAR19) or a CD123 CAR (CAR123). Figure 32A shows CD19 and CD123 expression; Figure 32B shows a CD107a degranulation assay; Figure 32C shows the capability for targeted cell killing; Figure 32D and E shows proliferation capacity; Figure 32F shows cytokine production for the indicated cytokines.

Figure 33, comprising Figures 33A, 33B, and 33C, shows that CART cells expressing CD19 CAR (CAR19) or CD123 CAR (CAR123) had an anti-tumor effect in an in vivo mouse model. Figure 33A shows the tumor burden represented by bioluminescent imaging; Figure 33B shows the overall survival curve of mice receiving CART therapy; and Figure 33C shows the expansion of CART123 cells in the peripheral blood.

Figure 34, comprising Figures 34A, 34B, 34C, 34D, 34E, and 34F, shows that CART123 is active in an in vivo mouse model of antigen-loss relapse. Figure 34A shows the experimental schema; Figure 34B shows disease progression as represented by bioluminescent imaging in baseline and relapse disease with respect to CD19 expression (top graph) and in response to treatment with CART19 therapy (bottom graph); Figure 34C shows bioluminescent images of mice administered untransduced T cells or CART19 cells; Figure 34D shows the experimental schema for treating with CART19 or CART123; Figure 34E shows the disease progression; and Figure 34F shows the overall survival of the treated mice.

Figure 35, comprising Figures 35A, 35B, and 35C, shows ALL-CART interactions in skull bone marrow of xenograft mice. Figure 35A shows the experimental schema; Figure 35B shows representative multiphoton XY plane images of CART19 cells and CART123 cells interacting with ALL tumor engineered to express either CD19 and CD123 or CD123 alone

(motile cells are indicated in dashed circles, non-motile cells are indicated with the arrows); and Figure 35C is a graphic representation of the microscopy images.

Figure 36, comprising Figures 36A, 36B, and 36C, shows the prevention of CD19-neg relapses using CART19 and CART123. Figure 36A shows the experimental schema; Figure 36B shows the disease progression (tumor burden as represented by BLI) of mice treated with untransduced T cells (top graph), CART19 (middle graph), or the combination of CART19 and CART123 (bottom graph); and Figure 36C shows the overall survival from this experiment.

Figure 37, comprising Figures 37A and 37B, shows T cells expressing both CAR19 and CAR123 (Figure 37A) and the results from a degranulation assay (Figure 37B).

Figure 38, comprising Figures 38A and 38B, shows characterization of ALL blasts. Figure 38A shows expression of various markers CD19, CD123, CD10, CD34, and CD20; and Figure 38B shows the gating strategy for sorting CD19-CD123+ cells.

Figure 39, comprising Figures 39A, 39B, 39C, and 39D, shows anti-leukemia activity of CART123. Figure 39A shows the expression of CD19 and CD123 on the NALM6 cells; Figure 39B shows the tumor burden (as represented by BLI) in response to CART19 or CART123 therapy; Figure 39C shows the overall survival of mice administered CART19 or CART123; and Figure 39D shows the overall survival of mice administered varying doses of CART123.

Figure 40, comprising Figures 40A and 40B, shows the characterization of the in vivo model of antigen-loss relapse. Figure 40A shows the expression of CD123 in CD19 negative relapse disease; and Figure 40B shows the degranulation assay of CART19 or CART123 cells when cultured with baseline or relapse cells in vitro.

Figure 41, comprising Figures 41A, 41B and 41C, shows the expression of PD1 L1 or on primary AML cells (Fig. 41A), and PD1 (Fig. 41B) and TIM3 (Fig. 41C) on T cells.

Figure 42, comprising Figures 42A, 42B, 42C, and 42D, shows the expression of immune checkpoints PD1 (Fig. 42A), TIM3 (Fig. 42B), LAG3 (Fig. 42C), and CTLA4 (Fig. 42D) on CD8+ T cells.

Figure 43 shows flow cytometry analysis of TIM3 expression on AML baseline (left) and relapsed (right) samples.

Figure 44 shows the tumor burden (represented by bioluminescent imaging, photons/sec) after CART123 treatment compared to treatment with untransduced (UTD) cells.

Figure 45 shows the expression of TIM3 and PD1 on T cells in xenografts after CD123 treatment in remission or after relapse.

Figure 46, comprising Figures 46A and 46B, shows the characterization of bone marrow from relapsed mice after coculture with checkpoint inhibitors, e.g., inhibitors of PD1, TIM3, a combination of PD1 and TIM3 inhibitors, or a combination of TIM3 and CEACAM-1 inhibitors. The percentage of T cells expressing GMCSF, IFN γ , Ki67, and MIP1b was detected in the absence (Fig. 46A) or in the presence of PMA/IONO (Fig. 46B).

Figure 47 shows the experimental schema for treating AML xenografts with untransduced cells and PD1 inhibitors or TIM3 inhibitors.

Figure 48 shows the tumor burden (represented by BLI, photons/sec) for the experiment depicted in Figure 47.

Figure 49 shows the experimental schema for treating AML xenografts with CART123 in combination with TIM3 and/or PD1 inhibitors.

Figure 50, comprising Figures 50A, 50B, 50C, and 50D, shows the tumor burden (represented by BLI, photons/sec) for the experiment depicted in Figure 49. Fig. 50A compares CART123 with the combination of CART123 and PD1 inhibitor; Fig. 50B compares CART123 with the combination of CART123 and PD1 inhibitor and TIM3 inhibitor; Fig. 50C compares CART123 with the combination of CART123 and TIM3 inhibitor, and Fig. 50D shows a representative plot for the data presented in Figs. 50A-C.

Figure 51, comprising Figures 51A, 51B, 51C, 51D, and 51E, shows the various configurations on a single vector, e.g., where the U6 regulated shRNA is upstream or downstream of the EF1 alpha regulated CAR encoding elements. In the exemplary constructs depicted in Fig. 51A and 51B, the transcription occurs through the U6 and EF1 alpha promoters in the same direction. In the exemplary constructs depicted in Fig. 51C and 51D, the transcription occurs through the U6 and EF1 alpha promoters in different directions. In Figure 51E, the shRNA (and corresponding U6 promoter) is on a first vector, and the CAR (and corresponding EF1 alpha promoter) is on a second vector.

Figure 52 depicts the structures of two exemplary RCAR configurations. The antigen binding members comprise an antigen binding domain, a transmembrane domain, and a switch domain. The intracellular binding members comprise a switch domain, a co-stimulatory signaling domain and a primary signaling domain. The two configurations demonstrate that the first and second switch domains described herein can be in different orientations with respect to the antigen binding member and the intracellular binding member. Other RCAR configurations are further described herein.

Figure 53 shows that the proliferation of CAR-expressing, transduced T cells is enhanced by low doses of RAD001 in a cell culture system. CARTs were co-cultured with Nalm-6 cells in the presence of different concentrations of RAD001. The number of CAR-positive CD3-positive T cells (black) and total T cells (gray) was assessed after 4 days of co-culture.

Figure 54 depicts tumor growth measurements of NALM6-luc cells with daily RAD001 dosing at 0.3, 1, 3, and 10 mg/kg (mpk) or vehicle dosing. Circles denote the vehicle; squares denote the 10 mg/kg dose of RAD001; triangles denote the 3 mg/kg dose of RAD001, inverted triangles denote the 1 mg/kg dose of RAD001; and diamonds denote the 0.3 mg/kg dose of RAD001.

Figure 55, comprising Figures 55A and 55B, shows pharmacokinetic curves showing the amount of RAD001 in the blood of NSG mice with NALM6 tumors. FIG. 55A shows day 0 PK following the first dose of RAD001. FIG. 55B shows Day 14 PK following the final RAD001 dose. Diamonds denote the 10 mg/kg dose of RAD001; squares denote the 1 mg/kg dose of RAD001; triangles denote the 3 mg/kg dose of RAD001; and x's denote the 10 mg/kg dose of RAD001.

Figure 56, comprising Figures 56A and 56B, shows *in vivo* proliferation of humanized CD19 CART cells with and without RAD001 dosing. Low doses of RAD001 (0.003 mg/kg) daily lead to an enhancement in CAR T cell proliferation, above the normal level of huCAR19 proliferation. FIG. 56A shows CD4⁺ CAR T cells; FIG. 56B shows CD8⁺ CAR T cells. Circles denote PBS; squares denote huCTL019; triangles denote huCTL019 with 3 mg/kg RAD001; inverted triangles denote huCTL019 with 0.3 mg/kg RAD001; diamonds denote huCTL019 with 0.03 mg/kg RAD001; and circles denote huCTL019 with 0.003 mg/kg RAD001.

Figure 57 is a vector map of a lentiviral vector for expressing a CAR123 and CD20 in a strategy for terminating CART123 activity, where the CAR123 sequence and the CD20 sequence are linked by a P2A sequence.

Figure 58 shows the results of a CD107 degranulation assay comparing CART123 to CART123 P2A CD20 cells.

Figure 59, comprising Figures 59A, 59B, 59C, and 59D, show the production of GM-CSF (Fig. 59A), TNFalpha (Fig. 59B), IFNgamma (Fig. 59C), and IL-2 (Fig. 59D) by CART123 cells compared to CART123 P2A CD20 cells.

Figure 60 shows the cytotoxic capability (as represented by % specific lysis) of CART123 cells compared to CART123 P2A CD20 cells when incubated at the indicated effector:target cell ratios.

Figure 61, comprising Figures 61A and 61B, shows T cell depletion after treatment with the indicated doses of rituximab on CART123 cells (Fig. 61A) and CART123 P2A CD20 cells (Fig. 61B).

Figure 62 shows the T cell depletion after treatment with the indicated doses of rituximab on CART123 P2A CD20 cells in the presence of 15% complement.

Figure 63, comprising Figures 63A and 63B, demonstrates the strategy for a dual CART cell expressing both a CAR19 and a CAR123. Figure 63A is a vector map containing a CAR19 and a CAR123, linked through a P2A sequence. Figure 63B shows the percentage of cells expressing CAR19, CAR123 (Q3), and both CAR19 (Q1) and CAR123 (Q2).

Figure 64, comprising Figures 64A and 64B, demonstrates the strategy for a split CART cell that expresses both a CAR19 and a CAR123. Figure 64A is a vector map containing a CAR19 and a CAR123, linked through a P2A sequence. The CAR19 includes a CD3zeta domain while the CAR123 includes a 4-1BB domain. Figure 64B shows the percentage of cells expressing CAR19, CAR123 (Q3), and both CAR19 (Q1) and CAR123 (Q2).

Figure 65 shows the expression of CD123 on primary HSC, AML, and BPDCN samples.

Figure 66, comprising Figures 66A and 66B, shows a cytotoxicity assay for CART123 clones 32716 (Fig. 66A) and 26292 (Fig. 66A) when incubated in the presence of CD123-expressing target cells at the indicated effector:target ratios.

Figure 67 shows a CD107 degranulation assay for CART123 cells when incubated with HSCs compared to BPDCN.

Figure 68, comprising Figures 68A and 68B, shows CD123 expression on mononuclear cells from the blood of a patient with mast cell leukemia/systemic mastocytosis as detected by staining by isotype antibody (Fig. 68A) as control or CD123 antibody (Fig. 68B).

Figure 69, comprising Figures 69A and 69B, shows a CD107 degranulation assay where CART123 cells were cultured alone with PMA and ionomycin (Fig. 69A) or with mononuclear cells from the blood of a patient with mast cell leukemia/systemic mastocytosis (Fig. 69B).

Figure 70 shows the experimental schema for subject cohort 1 in a clinical study for administering RNA CART123 in subjects with refractory or relapsed AML.

Figure 71 shows the experimental schema for subject cohort 2 in a clinical study for administering RNA CART123 in subjects with refractory or relapsed AML. These patients receive doses of a lymphodepleting chemotherapy in addition to the CART therapy.

Figure 72, comprising Figures 72A, 72B, and 72C, shows the results of T cell ablation using anti-CD52 antibody alemtuzumab after treatment with lentivirally transduced CART123 cells in a MOLM14 xenograft model. CART123 cells or untransduced (UTD) cells were administered to mice at week 1. Alemtuzumab was administered at week 2, week 3, or week 4 to mice that received CART123 cells, and tumor burden was assessed for 24 weeks. A “MOLM14” rechallenge was introduced at 12 weeks. Figure 72A shows tumor burden as detected by bioluminescent imaging (photons/sec); Figure 72B shows the percent of CART cells detected in peripheral blood; and Figure 72C shows the overall survival.

Figure 73, comprising Figures 73A and 73B, shows the results of T cell ablation using alemtuzumab after treatment with lentivirally transduced CART123 cells in a pediatric AML xenograft model. CART123 cells or untransduced (UTD) cells were administered to mice 6 weeks after primary AML cell engraftment (week 0). Alemtuzumab was administered at week 4 to mice that received CART123 treatment. Figure 73A shows tumor burden as detected by bioluminescent imaging (photons/sec); Figure 73B shows the percent of CART cells detected in peripheral blood.

Figure 74, comprising Figures 74A and 74B, shows the analysis of AML cells in the spleen (Fig. 74A) and the bone marrow (Fig. 74B) after CART123 and alemtuzumab treatment, as performed in Figure 73.

Figure 75 shows the comparison of the three termination strategies: (1) CART cell ablation by alemtuzumab treatment; (2) CAR/CD20 co-expression in T cells and ablation by rituximab; and (3) RNA-electroporated CART cells, on tumor burden (represented by bioluminescent imaging; photons/sec) in the AML xenograft model.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

The term “a” and “an” refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The term “about” when referring to a measurable value such as an amount, a temporal
5 duration, and the like, is meant to encompass variations of $\pm 20\%$ or in some instances $\pm 10\%$, or in some instances $\pm 5\%$, or in some instances $\pm 1\%$, or in some instances $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “Chimeric Antigen Receptor” or alternatively a “CAR” refers to a recombinant polypeptide construct comprising at least an extracellular antigen binding domain,
10 a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as “an intracellular signaling domain”) comprising a functional signaling domain derived from a stimulatory molecule as defined below. In some embodiments, the domains in the CAR polypeptide construct are in the same polypeptide chain, e.g., comprise a chimeric fusion protein. In some embodiments, the domains in the CAR polypeptide construct are not
15 contiguous with each other, e.g., are in different polypeptide chains, e.g., as provided in an RCAR as described herein.

In one aspect, the stimulatory molecule of the CAR is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain comprises a primary
20 signaling domain (e.g., a primary signaling domain of CD3-zeta). In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is chosen from 4-1BB (i.e., CD137), CD27, ICOS, and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising
25 a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein
30 comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a

stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen recognition domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (e.g., aa scFv) during cellular processing and localization of the CAR to the cellular membrane.

A CAR that comprises an antigen binding domain (e.g., a scFv, a single domain antibody, or TCR (e.g., a TCR alpha binding domain or TCR beta binding domain)) that specifically binds a specific tumor marker X, wherein X can be a tumor marker as described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that specifically binds CD123 is referred to as CD123 CAR. The CAR can be expressed in any cell, e.g., an immune effector cell as described herein (e.g., a T cell or an NK cell).

The term “signaling domain” refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

As used herein, the terms “alpha subunit of the IL-3 receptor,” “IL3R α ,” “CD123,” “IL3R α chain” and “IL3R α subunit” refer interchangeably to an antigenic determinant known to be detectable on leukemia precursor cells. The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequence of human IL3R α can be found at Accession No. NP 002174 and the nucleotide sequence encoding of the human IL3R α can be found at Accession No. NM 005191. In one aspect the antigen-binding portion of the CAR recognizes and binds an epitope within the extracellular domain of the CD123 protein. In one aspect, the CD123 protein is expressed on a cancer cell. As used herein, “CD123” includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full length wild-type CD123.

The term “antibody,” as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule which specifically binds with an antigen.

Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

The term “antibody fragment” refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, e.g., an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, and multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3)(see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term “scFv” refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The term “complementarity determining region” or “CDR,” as used herein, refers to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed.

Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 (“Chothia” numbering scheme), or a combination thereof. Under the Kabat numbering scheme, in some embodiments, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under the Chothia numbering scheme, in some embodiments, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both. For instance, in some embodiments, the CDRs correspond to amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in a VH, e.g., a mammalian VH, e.g., a human VH; and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in a VL, e.g., a mammalian VL, e.g., a human VL.

The portion of the CAR composition of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv) and a humanized or human antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises a scFv.

As used herein, the term “binding domain” or “antibody molecule” (also referred to herein as “anti-target (e.g., CD123) binding domain”) refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term “binding domain” or “antibody molecule” encompasses antibodies and antibody fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a

first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable
5 domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope.

The term “antibody heavy chain,” refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

10 The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term “recombinant antibody” refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage
15 or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

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

antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

The term “anti-tumor effect” refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, decrease in tumor cell proliferation, decrease in tumor cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An “anti-tumor effect” can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

The term “anti-cancer effect” refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An “anti-cancer effect” can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of cancer in the first place.

The term “anti-tumor effect” refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival.

The term “autologous” refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

The term “allogeneic” refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically.

The term “xenogeneic” refers to a graft derived from an animal of a different species.

The term “apheresis” as used herein refers to the art-recognized extracorporeal process by which the blood of a donor or patient is removed from the donor or patient and passed through an apparatus that separates out selected particular constituent(s) and returns the

remainder to the circulation of the donor or patient, e.g., by retransfusion. Thus, in the context of “an apheresis sample” refers to a sample obtained using apheresis.

The term “combination” refers to either a fixed combination in one dosage unit form, or a combined administration where a compound of the present invention and a combination partner (e.g. another drug as explained below, also referred to as “therapeutic agent” or “co-agent”) may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect. The single components may be packaged in a kit or separately. One or both of the components (e.g., powders or liquids) may be reconstituted or diluted to a desired dose prior to administration. The terms “co-administration” or “combined administration” or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (e.g. a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time. The term “pharmaceutical combination” as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term “fixed combination” means that the active ingredients, e.g. a compound of the present invention and a combination partner, are both administered to a patient simultaneously in the form of a single entity or dosage. The term “non-fixed combination” means that the active ingredients, e.g. a compound of the present invention and a combination partner, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

The term “cancer” refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. The terms “tumor” and “cancer” are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating,

tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors.

“Derived from” as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connote or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an intracellular signaling domain that is derived from a CD3zeta molecule, the intracellular signaling domain retains sufficient CD3zeta structure such that it has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connote or include a limitation to a particular process of producing the intracellular signaling domain, e.g., it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The phrase “disease associated with expression of CD123” as used herein includes but is not limited to, a disease associated with expression of CD123 or condition associated with a cell which expresses CD123 (e.g., wild-type or mutant CD123) including, e.g., a proliferative disease such as a cancer or malignancy; a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a non-cancer related indication associated with a cell which expresses CD123 (e.g., wild-type or mutant CD123). In one aspect, a cancer associated with expression of CD123 (e.g., wild-type or mutant CD123) is a hematological cancer. In one aspect, the disease includes AML, ALL, hairy cell leukemia, Prolymphocytic leukemia, Chronic myeloid leukemia (CML), Hodgkin lymphoma, Blastic plasmacytoid dendritic cell neoplasm, lymphoblastic B-cell leukemia (B-cell acute lymphoid leukemia, BALL), acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL); myelodysplastic syndrome; a myeloproliferative neoplasm; a histiocytic disorder (e.g., a mast cell disorder or a blastic plasmacytoid dendritic cell neoplasm); a mast cell disorder, e.g., systemic mastocytosis or mast cell leukemia, and the like. Further disease associated with expression of CD123 expression include, but are not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of CD123. Non-cancer related indications associated with expression of CD123 may also be included.

In some embodiments, the tumor antigen (e.g., CD123- or CD19-)-expressing cell expresses, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the

tumor antigen (e.g., CD123- or CD19-)-expressing cell produces the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal levels or reduced levels. In an embodiment, the tumor antigen (e.g., CD123- or CD19-)-expressing cell produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein.

The term “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term “stimulation,” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as downregulation of TGF- β , and/or reorganization of cytoskeletal structures, and the like.

The term “stimulatory molecule,” refers to a molecule expressed by a T cell that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of the TCR complex in a stimulatory way for at least some aspect of the T cell signaling pathway. In one aspect, the primary signal is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary

cytoplasmic signaling sequence (also referred to as a “primary signaling domain”) that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing primary cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those
5 derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as “ICOS”), FcεRI, CD66d, DAP10 and DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, e.g., a primary signaling sequence of CD3-zeta. In a specific CAR of the invention, the primary signaling sequence of
10 CD3-zeta is the sequence provided as SEQ ID NO:9, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence as provided in SEQ ID NO:10, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

15 The term “antigen presenting cell” or “APC” refers to an immune system cell such as an accessory cell (e.g., a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

20 An “intracellular signaling domain,” as the term is used herein, refers to an intracellular portion of a molecule. The intracellular signaling domain can generate a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell or CAR-expressing NK cell. Examples of immune effector function, e.g., in a CART cell or CAR-expressing NK cell, include cytolytic activity and helper activity, including the secretion of cytokines. In
25 embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term
30 intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent stimulation. In an embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CAR-expressing immune effector cell, e.g., CART cell or CAR-expressing NK cell, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (“ICOS”), FcεRI, CD66d, DAP10, and DAP12.

The term “zeta” or alternatively “zeta chain”, “CD3-zeta” or “TCR-zeta” is defined as the protein provided as GenBan Acc. No. BAG36664.1, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, and a “zeta stimulatory domain” or alternatively a “CD3-zeta stimulatory domain” or a “TCR-zeta stimulatory domain” is defined as the amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO:9. In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO:10.

The term “costimulatory molecule” refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Costimulatory molecules include, but are not limited to an a MHC class I molecule,

TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

A costimulatory intracellular signaling domain refers to the intracellular portion of a costimulatory molecule. The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

The term “4-1BB” refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a “4-1BB costimulatory domain” is defined as amino acid residues 214-255 of GenBank accno. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the “4-1BB costimulatory domain” is the sequence provided as SEQ ID NO:7 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

“Immune effector cell,” as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

“Immune effector function or immune effector response,” as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack of a target cell. E.g., an immune effector function or response refers a property

of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

5 The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

10 The term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, 15 used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA 20 may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term “effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

25 The term “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

The term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

30 The term “expression” refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

The term “transfer vector” refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of

a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “transfer vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term “expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term “vector” as used herein refers to any vehicle that can be used to deliver and/or express a nucleic acid molecule. It can be a transfer vector or an expression vector as described herein.

The term “lentivirus” refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term “lentiviral vector” refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., Mol. Ther. 17(8): 1453–1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, e.g., the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term “homologous” or “identity” refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA

molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

“Humanized” forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature*, 321: 522-525, 1986; Reichmann et al., *Nature*, 332: 323-329, 1988; Presta, *Curr. Op. Struct. Biol.*, 2: 593-596, 1992.

“Fully human” refers to an immunoglobulin, such as an antibody or antibody fragment, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

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

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

The term “operably linked” or “transcriptional control” refers to functional linkage
10 between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence.

15 Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term “parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

20 The term “nucleic acid,” “polynucleotide,” or “nucleic acid molecule” refers to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or a combination of a DNA or RNA thereof, and polymers thereof in either single- or double-stranded form. The term “nucleic acid” includes a gene, cDNA or an mRNA. In one embodiment, the nucleic acid molecule is synthetic (*e.g.*, chemically synthesized) or recombinant. Unless specifically limited, the term
25 encompasses nucleic acids containing analogues or derivatives of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the
30 sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucleic Acid Res.

19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term “promoter” refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term “promoter/regulatory sequence” refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term “constitutive” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term “inducible” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term “tissue-specific” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product

to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The term “cancer associated antigen” or “tumor antigen” interchangeably refers to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface
 5 of a cancer cell, either entirely or as a fragment (e.g., MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, e.g., a lineage marker, e.g., CD19 or CD123 on B cells. In some
 10 embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some
 15 embodiments, a tumor antigen is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some
 20 embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (e.g., MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In some embodiments, the CARs of the present invention includes CARs comprising an antigen binding domain (e.g., antibody or antibody fragment) that binds to a MHC presented peptide. Normally, peptides
 25 peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting
 30 peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608 ; Dao et al., Sci Transl Med 2013 5(176) :176ra33 ; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term “flexible polypeptide linker” or “linker” as used in the context of a scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid
 5 sequence (Gly-Gly-Gly-Ser)_n (SEQ ID NO: 38), where *n* is a positive integer equal to or greater than 1. For example, *n*=1, *n*=2, *n*=3, *n*=4, *n*=5 and *n*=6, *n*=7, *n*=8, *n*=9 and *n*=10. In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly₄ Ser)₄ (SEQ ID NO:27) or (Gly₄ Ser)₃ (SEQ ID NO:28). In another embodiment, the linkers include multiple repeats of (Gly₂Ser), (GlySer) or (Gly₃Ser) (SEQ ID NO:29). Also included within
 10 the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference).

As used herein, a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m⁷G cap) is a modified guanine nucleotide that has been added to the “front” or 5' end of a eukaryotic messenger RNA shortly after the start of transcription. The 5' cap consists of a
 15 terminal group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA polymerase. This enzymatic complex catalyzes the
 20 chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, “in vitro transcribed RNA” refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro
 25 transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a “poly(A)” is a series of adenosines attached by polyadenylation to the mRNA. In the preferred embodiment of a construct for transient expression, the polyA is between 50 and 5000 (SEQ ID NO: 30), preferably greater than 64, more preferably greater
 30 than 100, most preferably greater than 300 or 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, “polyadenylation” refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, “transient” refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms “treat”, “treatment” and “treating” refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a CAR of the invention). In specific embodiments, the terms “treat”, “treatment” and “treating” refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms “treat”, “treatment” and “treating” -refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms “treat”, “treatment” and “treating” refer to the reduction or stabilization of tumor size or cancerous cell count.

The term “signal transduction pathway” refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one

portion of a cell to another portion of a cell. The phrase “cell surface receptor” includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.

5 The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

The term, a “substantially purified” cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of
10 cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term “therapeutic” as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

15 The term “prophylaxis” as used herein means the prevention of or protective treatment for a disease or disease state.

In the context of the present invention, “tumor antigen” or “hyperproliferative disorder antigen” or “antigen associated with a hyperproliferative disorder” refers to antigens that are common to specific hyperproliferative disorders. In certain aspects, the hyperproliferative
20 disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, non-Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, and the like.

25 The term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

30 The term “specifically binds,” refers to an antibody, or a ligand, which recognizes and binds with a cognate binding partner (e.g., a stimulatory and/or costimulatory molecule present

on a T cell) protein present in a sample, but which antibody or ligand does not substantially recognize or bind other molecules in the sample.

“Regulatable chimeric antigen receptor (RCAR),” as used herein, refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector
5 cell, provides the cell with specificity for a target cell, typically a cancer cell, and with regulatable intracellular signal generation. In some embodiments, an RCAR comprises at least an extracellular antigen binding domain, a transmembrane and a cytoplasmic signaling domain (also referred to herein as “an intracellular signaling domain”) comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as
10 defined herein in the context of a CAR molecule. In some embodiments, the set of polypeptides in the RCAR are not contiguous with each other, e.g., are in different polypeptide chains. In some embodiments, the RCAR includes a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. In some embodiments,
15 the RCAR is expressed in a cell (e.g., an immune effector cell) as described herein, e.g., an RCAR-expressing cell (also referred to herein as “RCARX cell”). In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell. The RCAR can provide the RCAR-expressing cell with specificity for a target cell, typically a cancer cell, and with regulatable
20 intracellular signal generation or proliferation, which can optimize an immune effector property of the RCAR-expressing cell. In embodiments, an RCAR cell relies at least in part, on an antigen binding domain to provide specificity to a target cell that comprises the antigen bound by the antigen binding domain.

“Membrane anchor” or “membrane tethering domain”, as that term is used herein,
25 refers to a polypeptide or moiety, e.g., a myristoyl group, sufficient to anchor an extracellular or intracellular domain to the plasma membrane.

“Switch domain,” as that term is used herein, e.g., when referring to an RCAR, refers to an entity, typically a polypeptide-based entity, that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a
30 first entity linked to, e.g., fused to, a first switch domain, and a second entity linked to, e.g., fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as

one another, e.g., they are polypeptides having the same primary amino acid sequence, and are referred to collectively as a homodimerization switch. In embodiments, the first and second switch domains are different from one another, e.g., they are polypeptides having different primary amino acid sequences, and are referred to collectively as a heterodimerization switch.

5 In embodiments, the switch is intracellular. In embodiments, the switch is extracellular. In embodiments, the switch domain is a polypeptide-based entity, e.g., FKBP or FRB-based, and the dimerization molecule is small molecule, e.g., a rapalogue. In embodiments, the switch domain is a polypeptide-based entity, e.g., an scFv that binds a myc peptide, and the dimerization molecule is a polypeptide, a fragment thereof, or a multimer of a polypeptide, e.g.,
10 a myc ligand or multimers of a myc ligand that bind to one or more myc scFvs. In embodiments, the switch domain is a polypeptide-based entity, e.g., myc receptor, and the dimerization molecule is an antibody or fragments thereof, e.g., myc antibody.

“Dimerization molecule,” as that term is used herein, e.g., when referring to an RCAR, refers to a molecule that promotes the association of a first switch domain with a second switch
15 domain. In embodiments, the dimerization molecule does not naturally occur in the subject, or does not occur in concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, e.g., rapamycin or a rapalogue, e.g., RAD001.

The term “bioequivalent” refers to an amount of an agent other than the reference compound (e.g., RAD001), required to produce an effect equivalent to the effect produced by
20 the reference dose or reference amount of the reference compound (e.g., RAD001). In an embodiment the effect is the level of mTOR inhibition, e.g., as measured by P70 S6 kinase inhibition, e.g., as evaluated in an *in vivo* or *in vitro* assay, e.g., as measured by an assay described herein, e.g., the Boulay assay, or measurement of phosphorylated S6 levels by western blot. In an embodiment, the effect is alteration of the ratio of PD-1 positive/PD-1
25 negative immune effector cells, e.g., T cells or NK cells, as measured by cell sorting. In an embodiment a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of P70 S6 kinase inhibition as does the reference dose or reference amount of a reference compound. In an embodiment, a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of alteration in the ratio of
30 PD-1 positive/PD-1 negative immune effector cells, e.g., T cells or NK cells as does the reference dose or reference amount of a reference compound.

The term “low, immune enhancing, dose” when used in conjunction with an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., RAD001 or rapamycin, or a catalytic mTOR inhibitor, refers to a dose of mTOR inhibitor that partially, but not fully, inhibits mTOR activity, e.g., as measured by the inhibition of P70 S6 kinase activity. Methods for evaluating mTOR activity, e.g., by inhibition of P70 S6 kinase, are discussed herein. The dose is insufficient to result in complete immune suppression but is sufficient to enhance the immune response. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in a decrease in the number of PD-1 positive immune effector cells, e.g., T cells or NK cells, and/or an increase in the number of PD-1 negative immune effector cells, e.g., T cells or NK cells, or an increase in the ratio of PD-1 negative T cells/PD-1 positive immune effector cells, e.g., T cells or NK cells.

In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in an increase in the number of naive immune effector cells, e.g., T cells or NK cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in one or more of the following:

an increase in the expression of one or more of the following markers: CD62L^{high}, CD127^{high}, CD27⁺, and BCL2, e.g., on memory T cells, e.g., memory T cell precursors;

a decrease in the expression of KLRG1, e.g., on memory T cells, e.g., memory T cell precursors; and

an increase in the number of memory T cell precursors, e.g., cells with any one or combination of the following characteristics: increased CD62L^{high}, increased CD127^{high}, increased CD27⁺, decreased KLRG1, and increased BCL2;

wherein any of the changes described above occurs, e.g., at least transiently, e.g., as compared to a non-treated subject.

“Refractory” as used herein refers to a disease, e.g., cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

“Relapsed” or “relapse” as used herein refers to the return or reappearance of a disease (e.g., cancer) or the signs and symptoms of a disease such as cancer after a period of improvement or responsiveness, e.g., after prior treatment of a therapy, e.g., cancer therapy.

The initial period of responsiveness may involve the level of cancer cells falling below a certain threshold, e.g., below 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. The reappearance may involve the level of cancer cells rising above a certain threshold, e.g., above 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. For example, e.g., in the context of B-ALL, the reappearance may involve, e.g., a reappearance of blasts in the blood, bone marrow (> 5%), or any extramedullary site, after a complete response. A complete response, in this context, may involve < 5% BM blast. More generally, in an embodiment, a response (e.g., complete response or partial response) can involve the absence of detectable MRD (minimal residual disease). In an embodiment, the initial period of responsiveness lasts at least 1, 2, 3, 4, 5, or 6 days; at least 1, 2, 3, or 4 weeks; at least 1, 2, 3, 4, 6, 8, 10, or 12 months; or at least 1, 2, 3, 4, or 5 years.

In some embodiments, a therapy that includes a CD19 inhibitor, e.g., a CD19 CAR therapy, may relapse or be refractory to treatment. The relapse or resistance can be caused by CD19 loss (e.g., an antigen loss mutation) or other CD19 alteration that reduces the level of CD19 (e.g., caused by clonal selection of CD19-negative clones). A cancer that harbors such CD19 loss or alteration is referred to herein as a “CD19-negative cancer” or a “CD19-negative relapsed cancer”). It shall be understood that a CD19-negative cancer need not have 100% loss of CD19, but a sufficient reduction to reduce the effectiveness of a CD19 therapy such that the cancer relapses or becomes refractory. In some embodiments, a CD19-negative cancer results from a CD19 CAR therapy.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

Description

Provided herein are compositions of matter and methods of use for the treatment or prevention of a disease such as cancer using CD123 chimeric antigen receptors (CAR).

5 In one aspect, the invention provides a number of chimeric antigen receptors (CAR) comprising an antibody or antibody fragment engineered for specific binding to a CD123 protein or fragments thereof. In one aspect, the invention provides a cell (e.g., an immune effector cell, e.g., a T cell or a NK cell) engineered to express a CAR, wherein the CAR-expressing cell (e.g., "CART" or CAR-expressing NK cell) exhibits an antitumor property. In
10 one aspect a cell is transformed with the CAR and the at least part of the CAR is expressed on the cell surface. In some embodiments, the cell (e.g., immune effector cell, e.g., T cell or NK cell) is transduced with a viral vector encoding a CAR. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the CAR. In another embodiment, the cell (e.g.,
15 immune effector cell, e.g., T cell or NK cell) is transfected with a nucleic acid, e.g., mRNA, cDNA, DNA, encoding a CAR. In some such embodiments, the cell may transiently express the CAR.

In one aspect, the CD123 binding domain, e.g., the human or humanized CD123 binding domain, of the CAR is a scFv antibody fragment. In one aspect, such antibody
20 fragments are functional in that they retain the equivalent binding affinity, e.g., they bind the same antigen with comparable efficacy, as the IgG antibody having the same heavy and light chain variable regions. In one aspect such antibody fragments are functional in that they provide a biological response that can include, but is not limited to, activation of an immune response, inhibition of signal-transduction origination from its target antigen, inhibition of
25 kinase activity, and the like, as will be understood by a skilled artisan.

In some aspects, the antibodies of the invention are incorporated into a chimeric antigen receptor (CAR). In one aspect, the CAR comprises the polypeptide sequence provided herein as SEQ ID NOS: 98-101, and 125-156.

In one aspect, the CD123 binding domain, e.g., humanized or human CD123 binding
30 domain, portion of a CAR of the invention is encoded by a transgene whose sequence has been codon optimized for expression in a mammalian cell. In one aspect, entire CAR construct of the invention is encoded by a transgene whose entire sequence has been codon optimized for

expression in a mammalian cell. Codon optimization refers to the discovery that the frequency of occurrence of synonymous codons (i.e., codons that code for the same amino acid) in coding DNA is biased in different species. Such codon degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. A variety of codon optimization methods is known in the art, and include, e.g., methods disclosed in at least US Patent Numbers 5,786,464 and 6,114,148.

In one aspect, the antigen binding domain of the CAR comprises a human CD123 antibody or antibody fragment. In one aspect, the antigen binding domain of the CAR comprises a humanized CD123 antibody or antibody fragment. In one aspect, the antigen binding domain of the CAR comprises human CD123 antibody fragment comprising an scFv. In one aspect, the antigen binding domain of the CAR is a human CD123 scFv. In one aspect, the antigen binding domain of the CAR comprises a humanized CD123 antibody fragment comprising an scFv. In one aspect, the antigen binding domain of the CAR is a humanized CD123 scFv.

In one aspect, the CAR123 binding domain comprises the scFv portion provided in SEQ ID NO:157-160 and 184-215. In one aspect the scFv portion is human. In one aspect, the human CAR123 binding domain comprises the scFv portion provided in SEQ ID NO:157-160. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 157. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 158. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 159. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 160. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 478. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 480. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 483. In one aspect, the human CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 485.

In one aspect the scFv portion is humanized. In one aspect, the humanized CAR123 binding domain comprises the scFv portion provided in SEQ ID NO:184-215. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 184. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 185. In one aspect, the humanized CD123 binding domain comprises the scFv

portion provided in SEQ ID NO: 186. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 187. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 188. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 189.

5 In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 190. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 191. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 192. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 193. In one aspect, the

10 humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 194. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 195. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 196. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 197. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 198. In one aspect, the

15 humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 199. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 200. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 201. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 202. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 203. In one aspect, the

20 humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 204. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 205. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 206. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 207. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 208. In one aspect, the

25 humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 209. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 210. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 211. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 212. In one aspect, the humanized CD123

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binding domain comprises the scFv portion provided in SEQ ID NO: 213. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 214. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NO: 215. In one aspect, the humanized CD123 binding domain comprises the scFv portion provided in SEQ ID NOs: 556-587.

Furthermore, the present invention provides CD123 CAR compositions and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune diseases involving cells or tissues which express CD123.

In one aspect, the CAR of the invention can be used to eradicate CD123-expressing normal cells, thereby applicable for use as a cellular conditioning therapy prior to cell transplantation. In one aspect, the CD123-expressing normal cell is a CD123-expressing myeloid progenitor cell and the cell transplantation is a stem cell transplantation.

In one aspect, the invention provides a cell (e.g., an immune effector cell, e.g., a T cell or NK cell) engineered to express a chimeric antigen receptor (e.g., CAR-expressing immune effector cell, e.g., CART or CAR-expressing NK cell) of the present invention, wherein the cell (e.g., "CART") exhibits an antitumor property. Accordingly, the invention provides a CD123-CAR that comprises a CD123 binding domain and is engineered into an immune effector cell, e.g., a T cell or a NK cell, and methods of their use for adoptive therapy.

In one aspect, the CD123-CAR comprises at least one intracellular domain, e.g., described herein, e.g., selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD3zeta signal domain, and any combination thereof. In one aspect, the CD123-CAR comprises at least one intracellular signaling domain is from one or more co-stimulatory molecule(s) other than a CD137 (4-1BB) or CD28.

Chimeric Antigen Receptor (CAR)

The present invention encompasses a recombinant DNA construct comprising sequences encoding a CAR, wherein the CAR comprises an antigen binding domain (e.g., antibody, antibody fragment) that binds specifically to CD123 or a fragment thereof, e.g., human CD123, wherein the sequence of the CD123 binding domain (e.g., antibody or antibody fragment) is, e.g., contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain. The intracellular signaling domain can comprise a

costimulatory signaling domain and/or a primary signaling domain, e.g., a zeta chain. The costimulatory signaling domain refers to a portion of the CAR comprising at least a portion of the intracellular domain of a costimulatory molecule.

In specific aspects, a CAR construct of the invention comprises a scFv domain selected
 5 from the group consisting of SEQ ID NOS:157-160,184-215, 478, 480, 483, 485, and 556-587 wherein the scFv may be preceded by an optional leader sequence such as provided in SEQ ID NO: 1, and followed by an optional hinge sequence such as provided in SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, a transmembrane region such as provided in SEQ ID NO:6, an intracellular signalling domain that includes SEQ ID NO:7 or SEQ ID NO:8 and a
 10 CD3 zeta sequence that includes SEQ ID NO:9 or SEQ ID NO:10, e.g., wherein the domains are contiguous with and in the same reading frame to form a single fusion protein. In some embodiments, the scFv domain is a human scFv domain selected from the group consisting of SEQ ID NOS: 157-160, 478, 480, 483, and 485. In some embodiments, the scFv domain is a humanized scFv domain selected from the group consisting of SEQ ID NOS: 184-215 and 556-
 15 587. Also included in the invention is a nucleotide sequence that encodes the polypeptide of each of the scFv fragments selected from the group consisting of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587. Also included in the invention is a nucleotide sequence that encodes the polypeptide of each of the scFv fragments selected from the group consisting of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587, and each of the domains
 20 of SEQ ID NOS: 1,2, and 6-9, plus the encoded CD123 CAR of the invention.

In one aspect an exemplary CD123CAR constructs comprise an optional leader sequence, an extracellular antigen binding domain, a hinge, a transmembrane domain, and an intracellular stimulatory domain. In one aspect an exemplary CD123CAR construct comprises an optional leader sequence, an extracellular antigen binding domain, a hinge, a transmembrane
 25 domain, an intracellular costimulatory domain and an intracellular stimulatory domain.

In some embodiments, full-length CD123 CAR sequences are also provided herein as SEQ ID NOS: 98-101 and 125-156, as shown in Table 2 or 6.

An exemplary leader sequence is provided as SEQ ID NO: 1. An exemplary hinge/spacer sequence is provided as SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ
 30 ID NO:5. An exemplary transmembrane domain sequence is provided as SEQ ID NO:6. An exemplary sequence of the intracellular signaling domain of the 4-1BB protein is provided as SEQ ID NO: 7. An exemplary sequence of the intracellular signaling domain of CD27 is

provided as SEQ ID NO:8. An exemplary CD3zeta domain sequence is provided as SEQ ID NO: 9 or SEQ ID NO:10. An exemplary sequence of the intracellular signaling domain of CD28 is provided as SEQ ID NO:43. An exemplary sequence of the intracellular signaling domain of ICOS is provided as SEQ ID NO:45.

5 In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises the nucleic acid sequence encoding a CD123 binding domain, e.g., described herein, e.g., that is contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain. In one aspect, a CD123 binding domain is selected from one
10 or more of SEQ ID NOS: 157-160, 184-215, 478, 480, 483, 485, and 556-587. In some embodiments, the CD123 binding domain is a human CD123 binding domain selected from the group consisting of SEQ ID NOS: 157-160, 478, 480, 483, and 485. In some embodiments, the CD123 binding domain is a humanized CD123 binding domain selected from the group consisting of SEQ ID NOS: 184-215 and 556-587. In one aspect, the CD123 binding domain is
15 SEQ ID NO: 157. In one aspect, the CD123 binding domain is SEQ ID NO: 158. In one aspect, the CD123 binding domain is SEQ ID NO: 159. In one aspect, the CD123 binding domain is SEQ ID NO: 160. In one aspect, the CD123 binding domain is SEQ ID NO: 184. In one aspect, the CD123 binding domain is SEQ ID NO: 185. In one aspect, the CD123 binding domain is SEQ ID NO: 186. In one aspect, the CD123 binding domain is SEQ ID NO: 187. In
20 one aspect, the CD123 binding domain is SEQ ID NO: 188. In one aspect, the CD123 binding domain is SEQ ID NO: 189. In one aspect, the CD123 binding domain is SEQ ID NO: 190. In one aspect, the CD123 binding domain is SEQ ID NO: 191. In one aspect, the CD123 binding domain is SEQ ID NO: 192. In one aspect, the CD123 binding domain is SEQ ID NO: 193. In one aspect, the CD123 binding domain is SEQ ID NO: 194. In one aspect, the CD123 binding domain is SEQ ID NO: 195. In one aspect, the CD123 binding domain is SEQ ID NO: 196. In one aspect, the CD123 binding domain is SEQ ID NO: 197. In one aspect, the CD123 binding domain is SEQ ID NO: 198. In one aspect, the CD123 binding domain is SEQ ID NO: 199. In one aspect, the CD123 binding domain is SEQ ID NO: 200. In one aspect, the CD123 binding domain is SEQ ID NO: 201. In one aspect, the CD123 binding domain is SEQ ID NO: 202. In
25 one aspect, the CD123 binding domain is SEQ ID NO: 203. In one aspect, the CD123 binding domain is SEQ ID NO: 204. In one aspect, the CD123 binding domain is SEQ ID NO: 205. In one aspect, the CD123 binding domain is SEQ ID NO: 206. In one aspect, the CD123 binding
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domain is SEQ ID NO: 207. In one aspect, the CD123 binding domain is SEQ ID NO: 208. In one aspect, the CD123 binding domain is SEQ ID NO: 209. In one aspect, the CD123 binding domain is SEQ ID NO: 210. In one aspect, the CD123 binding domain is SEQ ID NO: 211. In one aspect, the CD123 binding domain is SEQ ID NO: 212. In one aspect, the CD123 binding domain is SEQ ID NO: 213. In one aspect, the CD123 binding domain is SEQ ID NO: 213. In one aspect, the CD123 binding domain is SEQ ID NO: 215. In one aspect, the CD123 binding domain is SEQ ID NO: 478. In one aspect, the CD123 binding domain is SEQ ID NO: 480. In one aspect, the CD123 binding domain is SEQ ID NO: 483. In one aspect, the CD123 binding domain is SEQ ID NO: 485.

In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a CD123 binding domain, e.g., wherein the sequence is contiguous with and in the same reading frame as the nucleic acid sequence encoding an intracellular signaling domain. An exemplary intracellular signaling domain that can be used in the CAR includes, but is not limited to, one or more intracellular signaling domains of, e.g., CD3-zeta, CD28, 4-1BB, ICOS, and the like. In some instances, the CAR can comprise any combination of CD3-zeta, CD28, 4-1BB, ICOS, and the like.

In one aspect, the nucleic acid sequence of a CAR construct of the invention is selected from one or more of SEQ ID NOS:39-42 and 66-97. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:39. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:40. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:41. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:42. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:66. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:67. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:68. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:69. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:70. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:71. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:72. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID

NO:73. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:74. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:75. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:76. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:77. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:78. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:79. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:80. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:81. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:82. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:83. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:84. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:85. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:86. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:87. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:88. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:89. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:90. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:91. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:92. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:93. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:94. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:95. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:96. In one aspect, the nucleic acid sequence of a CAR construct comprises (e.g., consists of) SEQ ID NO:97. The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

The present invention includes retroviral and lentiviral vector constructs expressing a CAR that can be directly transduced into a cell.

The present invention also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves in vitro
5 transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:35). RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the CAR. In
10 an embodiment, an RNA CAR vector is transduced into a T cell by electroporation.

Antigen binding domain

In one aspect, the CAR of the invention comprises a target-specific binding element otherwise referred to as an antigen binding domain. The choice of moiety depends upon the
15 type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

In one aspect, the CAR-mediated T-cell response can be directed to an antigen of interest by way of engineering an antigen binding domain that specifically binds a desired
20 antigen into the CAR.

In one aspect, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets CD123 or a fragment thereof. In one aspect, the antigen binding domain targets human CD123 or a fragment thereof.

The antigen binding domain can be any domain that binds to the antigen including but
25 not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant
30 fibronectin domain, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For

example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment. Thus, in one aspect, the antigen binding domain comprises a human antibody or an antibody fragment. In one embodiment, the human CD123 binding domain comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a human CD123 binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a human CD123 binding domain described herein, e.g., a human CD123 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the human CD123 binding domain comprises one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a human CD123 binding domain described herein, e.g., the human CD123 binding domain has two variable heavy chain regions, each comprising a HC CDR1, a HC CDR2 and a HC CDR3 described herein. In one embodiment, the human CD123 binding domain comprises a human light chain variable region described herein (e.g., in Table 2 or 9) and/or a human heavy chain variable region described herein (e.g., in Table 2 or 9). In one embodiment, the human CD123 binding domain comprises a human heavy chain variable region described herein (e.g., in Table 2 or 9), e.g., at least two human heavy chain variable regions described herein (e.g., in Table 2 or 9). In one embodiment, the CD123 binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of Table 2 or 9. In an embodiment, the CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a light chain variable region provided in Table 2 or

9, or a sequence with 95-99% identity with an amino acid sequence of Table 2; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a heavy chain variable region provided in Table 2 or 9, or a sequence with 95-99% identity to an amino acid sequence of Table 2 or 9. In one embodiment, the human CD123 binding domain comprises a sequence selected from a group consisting of SEQ ID NO:157-160, 478, 480, 483, and 485, or a sequence with 95-99% identity thereof. In one embodiment, the human CD123 binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 2 or 9, is attached to a heavy chain variable region comprising an amino acid sequence described herein, e.g., in Table 2, via a linker, e.g., a linker described herein. In one embodiment, the human CD123 binding domain includes a (Gly₄-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO:26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

In some aspects, a non-human antibody is humanized, where specific sequences or regions of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. Thus, in one aspect, the antigen binding domain comprises a humanized antibody or an antibody fragment. In one embodiment, the humanized CD123 binding domain comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a humanized CD123 binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized CD123 binding domain described herein, e.g., a humanized CD123 binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the humanized CD123 binding domain comprises one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized CD123 binding domain described herein, e.g., the humanized CD123 binding domain has two variable heavy chain regions, each comprising a HC CDR1, a HC CDR2 and a

HC CDR3 described herein. In one embodiment, the humanized CD123 binding domain comprises a humanized light chain variable region described herein (e.g., in Table 6) and/or a humanized heavy chain variable region described herein (e.g., in Table 6). In one embodiment, the humanized CD123 binding domain comprises a humanized heavy chain variable region described herein (e.g., in Table 6), e.g., at least two humanized heavy chain variable regions described herein (e.g., in Table 6). In one embodiment, the CD123 binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of Table 6. In an embodiment, the CD123 binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a light chain variable region provided in Table 4, or a sequence with 95-99% identity with an amino acid sequence of Table 6; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions) of an amino acid sequence of a heavy chain variable region provided in Table 6, or a sequence with 95-99% identity to an amino acid sequence of Table 6. In one embodiment, the humanized CD123 binding domain comprises a sequence selected from a group consisting of SEQ ID NO:184-215 and 302-333, or a sequence with 95-99% identity thereof. In one embodiment, the humanized CD123 binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 6, is attached to a heavy chain variable region comprising an amino acid sequence described herein, e.g., in Table 6, via a linker, e.g., a linker described herein. In one embodiment, the humanized CD123 binding domain includes a (Gly4-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO:26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering,

7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., J. Immunol., 169:1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16):10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.)

A humanized antibody or antibody fragment has one or more amino acid residues remaining in it from a source which is nonhuman. These nonhuman amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. As provided herein, humanized antibodies or antibody fragments comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions wherein the amino acid residues comprising the framework are derived completely or mostly from human germline. Multiple techniques for humanization of antibodies or antibody fragments are well-known in the art and can essentially be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody, i.e., CDR-grafting (EP 239,400; PCT Publication No. WO 91/09967; and U.S. Pat. Nos. 4,816,567; 6,331,415; 5,225,539; 5,530,101; 5,585,089; 6,548,640, the contents of which are incorporated herein by reference herein in their entirety). In such humanized antibodies and antibody fragments,

substantially less than an intact human variable domain has been substituted by the corresponding sequence from a nonhuman species. Humanized antibodies are often human antibodies in which some CDR residues and possibly some framework (FR) residues are substituted by residues from analogous sites in rodent antibodies. Humanization of antibodies and antibody fragments can also be achieved by veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology*, 28(4/5):489-498; Studnicka et al., *Protein Engineering*, 7(6):805-814 (1994); and Roguska et al., *PNAS*, 91:969-973 (1994)) or chain shuffling (U.S. Pat. No. 5,565,332), the contents of which are incorporated herein by reference herein in their entirety.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987), the contents of which are incorporated herein by reference herein in their entirety). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (see, e.g., Nicholson et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997); Carter et al., *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993), the contents of which are incorporated herein by reference herein in their entirety). In some embodiments, the framework region, e.g., all four framework regions, of the heavy chain variable region are derived from a VH4_4-59 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g., from the amino acid at the corresponding murine sequence. In one embodiment, the framework region, e.g., all four framework regions of the light chain variable region are derived from a VK3_1.25 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g., from the amino acid at the corresponding murine sequence.

In some aspects, the portion of a CAR composition of the invention that comprises an antibody fragment is humanized with retention of high affinity for the target antigen and other favorable biological properties. According to one aspect of the invention, humanized antibodies

and antibody fragments are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind the target antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody or antibody fragment characteristic, such as increased affinity for the target antigen, is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

A humanized antibody or antibody fragment may retain a similar antigenic specificity as the original antibody, e.g., in the present invention, the ability to bind human CD123 or a fragment thereof. In some embodiments, a humanized antibody or antibody fragment may have improved affinity and/or specificity of binding to human CD123 or a fragment thereof.

In one aspect, the antigen binding domain portion comprises one or more sequence selected from SEQ ID NOS:157-160,184-215, 478, 480, 483, 485, and 556-587. In one aspect, the CD123 CAR that includes a human CD123 binding domain is selected from one or more sequence selected from SEQ ID NOS:157-160, 478, 480, 483, and 485. In one aspect, the CD123 CAR that includes a humanized CD123 binding domain is selected from one or more sequence selected from SEQ ID NOS:184-215 and 556-587.

In one aspect, the CD123 binding domain is characterized by particular functional features or properties of an antibody or antibody fragment. For example, in one aspect, the portion of a CAR composition of the invention that comprises an antigen binding domain specifically binds human CD123 or a fragment thereof. In one aspect, the invention relates to an antigen binding domain comprising an antibody or antibody fragment, wherein the antibody binding domain specifically binds to a CD123 protein or fragment thereof, wherein the antibody or antibody fragment comprises a variable light chain and/or a variable heavy chain that includes an amino acid sequence of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587. In one aspect, the antigen binding domain comprises an amino acid sequence of an scFv selected from SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587. In

certain aspects, the scFv is contiguous with and in the same reading frame as a leader sequence. In one aspect the leader sequence is the polypeptide sequence provided as SEQ ID NO:1.

In one aspect, the CD123 binding domain is a fragment, e.g., a single chain variable fragment (scFv). In one aspect, the CD123 binding domain is a Fv, a Fab, a (Fab')₂, or a bi-
5 functional (e.g. bi-specific) hybrid antibody (e.g., Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In one aspect, the antibodies and fragments thereof of the invention binds a CD123 protein or a fragment thereof with wild-type or enhanced affinity.

In some instances, a human scFv can be derived from a display library. A display library is a collection of entities; each entity includes an accessible polypeptide component and
10 a recoverable component that encodes or identifies the polypeptide component. The polypeptide component is varied so that different amino acid sequences are represented. The polypeptide component can be of any length, e.g. from three amino acids to over 300 amino acids. A display library entity can include more than one polypeptide component, for example, the two polypeptide chains of a Fab. In one exemplary embodiment, a display library can be
15 used to identify a human CD123 binding domain. In a selection, the polypeptide component of each member of the library is probed with CD123, or a fragment thereof, and if the polypeptide component binds to CD123, the display library member is identified, typically by retention on a support.

Retained display library members are recovered from the support and analyzed. The
20 analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can also include determining the amino acid sequence of the polypeptide component, i.e., the anti-CD123 binding domain, and purification of the polypeptide component for detailed characterization.

A variety of formats can be used for display libraries. Examples include the phage
25 display. In phage display, the protein component is typically covalently linked to a bacteriophage coat protein. The linkage results from translation of a nucleic acid encoding the protein component fused to the coat protein. The linkage can include a flexible peptide linker, a protease site, or an amino acid incorporated as a result of suppression of a stop codon. Phage
30 display is described, for example, in U.S. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; de Haard *et al.* (1999) *J. Biol. Chem* 274:18218-30; Hoogenboom *et*

al. (1998) *Immunotechnology* 4:1-20; Hoogenboom *et al.* (2000) *Immunol Today* 2:371-8 and Hoet *et al.* (2005) *Nat Biotechnol.* 23(3)344-8. Bacteriophage displaying the protein component can be grown and harvested using standard phage preparatory methods, e.g. PEG precipitation from growth media. After selection of individual display phages, the nucleic acid encoding the selected protein components can be isolated from cells infected with the selected phages or from the phage themselves, after amplification. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

Other display formats include cell based display (see, e.g., WO 03/029456), protein-nucleic acid fusions (see, e.g., US 6,207,446), ribosome display (See, e.g., Mattheakis *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9022 and Hanes *et al.* (2000) *Nat Biotechnol.* 18:1287-92; Hanes *et al.* (2000) *Methods Enzymol.* 328:404-30; and Schaffitzel *et al.* (1999) *J Immunol Methods.* 231(1-2):119-35), and *E. coli* periplasmic display (2005 Nov 22;PMID: 16337958).

In some instances, scFvs can be prepared according to method known in the art (see, for example, Bird *et al.*, (1988) *Science* 242:423-426 and Huston *et al.*, (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (e.g., a Ser-Gly linker) with an optimized length and/or amino acid composition. The linker length can greatly affect how the variable regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (e.g., between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, e.g., Hollinger *et al.* 1993 *Proc Natl Acad. Sci. U.S.A.* 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference.

An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions. The linker sequence may comprise any naturally occurring amino acid. In some embodiments, the linker sequence comprises amino acids glycine and serine. In another embodiment, the linker sequence comprises sets of glycine and serine repeats such as (Gly₄Ser)_n, where n is a positive integer equal to or greater than 1 (SEQ ID NO:25). In one embodiment, the linker can be (Gly₄Ser)₄ (SEQ ID NO:27) or (Gly₄Ser)₃ (SEQ ID NO:28).

Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

Exemplary CD123 CAR Constructs and Antigen Binding Domains

5 Exemplary CD123 CAR constructs disclose herein comprise an scFv (e.g., a human scFv as disclosed in Tables 2, 6 and 9 herein, optionally preceded with an optional leader sequence (e.g., SEQ ID NO:1 and SEQ ID NO:12 for exemplary leader amino acid and nucleotide sequences, respectively). The sequences of the human scFv fragments (amino acid sequences of SEQ ID NOs:157-160) are provided herein in Table 2. The sequences of human
10 scFv fragments, without the leader sequence, are provided herein in Table 9 (SEQ ID NOs: 479, 481, 482, and 484 for the nucleotide sequences, and SEQ ID NOs: 478, 480, 483, and 485 for the amino acid sequences). The CD123 CAR construct can further include an optional hinge domain, e.g., a CD8 hinge domain (e.g., including the amino acid sequence of SEQ ID NO: 2 or encoded by a nucleic acid sequence of SEQ ID NO:13); a transmembrane domain,
15 e.g., a CD8 transmembrane domain (e.g., including the amino acid sequence of SEQ ID NO: 6 or encoded by the nucleotide sequence of SEQ ID NO: 17); an intracellular domain, e.g., a 4-1BB intracellular domain (e.g., including the amino acid sequence of SEQ ID NO: 7 or encoded by the nucleotide sequence of SEQ ID NO: 18; and a functional signaling domain, e.g., a CD3 zeta domain (e.g., including amino acid sequence of SEQ ID NO: 9 or 10, or encoded by the
20 nucleotide sequence of SEQ ID NO: 20 or 21). In certain embodiments, the domains are contiguous with and in the same reading frame to form a single fusion protein. In other embodiments, the domain are in separate polypeptides, e.g., as in an RCAR molecule as described herein.

In certain embodiments, the full length CD123 CAR molecule includes the amino acid
25 sequence of, or is encoded by the nucleotide sequence of, CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26,
30 hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, provided in Table 2, 6, or 9, or a sequence substantially (e.g., 95-99%) identical thereto.

In certain embodiments, the CD123 CAR molecule, or the CD123 antigen binding domain, includes the scFv amino acid sequence of CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, provided in Table 2, 6 or 9; or includes the scFv amino acid sequence of, or is encoded by the nucleotide sequence of, CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the CD123 CAR molecule, or the CD123 antigen binding domain, includes the heavy chain variable region and/or the light chain variable region of CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, provided in Table 2 or 6, or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the CD123 CAR molecule, or the CD123 antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 3 or 7; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19,

hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, provided in Table 4 or 8; or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

5 In certain embodiments, the CD123 CAR molecule, or the CD123 antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 10; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of CD123-1, CD123-2, CD123-3, CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6,
10 hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32, provided in Table 11; or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4,
15 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the CD123 molecule, or the CD123 antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 12; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of CD123-1, CD123-2, CD123-3,
20 CD123-4, hzCD123-1, hzCD123-2, hzCD123-3, hzCD123-4, hzCD123-5, hzCD123-6, hzCD123-7, hzCD123-8, hzCD123-9, hzCD123-10, hzCD123-11, hzCD123-12, hzCD123-13, hzCD123-14, hzCD123-15, hzCD123-16, hzCD123-17, hzCD123-18, hzCD123-19, hzCD123-20, hzCD123-21, hzCD123-22, hzCD123-23, hzCD123-24, hzCD123-25, hzCD123-26, hzCD123-27, hzCD123-28, hzCD123-29, hzCD123-30, hzCD123-31, or hzCD123-32,
25 provided in Table 13; or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

The sequences of CDR sequences of the scFv domains are shown in Tables 3, 7, 10, and 12 for the heavy chain variable domains and in Tables 4, 8, 11, and 13 for the light chain
30 variable domains. "ID" stands for the respective SEQ ID NO for each CDR.

The CDRs provided in Tables 3, 4, 7, and 8 are according to a combination of the Kabat and Chothia numbering scheme.

Table 3. Heavy Chain Variable Domain CDRs

Candidate	HCDR1	ID	HCDR2	ID	HCDR3	ID
CAR123-2	GYTFTGYMH	335	WINPNSGGTNYAQKFQG	363	DMNILATVPFDI	391
CAR123-3	GYIFTGYIH	337	WINPNSGGTNYAQKFQG	364	DMNILATVPFDI	392
CAR123-4	GYTFTGYMH	336	WINPNSGGTNYAQKFQG	365	DMNILATVPFDI	393
CAR123-1	GYTFTDYMH	334	WINPNSGDTNYAQKFQG	362	DMNILATVPFDI	390

Table 4. Light Chain Variable Domain CDRs

Candidate	LCDR1	ID	LCDR2	ID	LCDR3	ID
CAR123-2	RASQSISSYLN	419	AAFSLQS	447	QQGDSVPLT	475
CAR123-3	RASQSISSYLN	420	AASSLQS	448	QQGDSVPLT	476
CAR123-4	RASQSISSYLN	421	AASSLQS	449	QQGDSVPLT	477
CAR123-1	RASQSISTYLN	418	AASSLQS	446	QQGDSVPLT	474

5 Table 7. Heavy Chain Variable Domain CDR

	HCDR1	ID	HCDR2	ID	HCDR3	ID
hzCAR123	GYTFTSYWMN	361	RIDPYDSETHYNQKFKD	389	GNWDDY	417

Table 8. Light Chain Variable Domain CDR

	LCDR1	ID	LCDR2	ID	LCDR3	ID
hzCAR123	RASKSISKDLA	445	SGSTLQS	473	QQHKNKYPYT	47

Table 10. Heavy Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

Candidate	HCDR1	ID	HCDR2	ID	HCDR3	ID
CAR123-2	GYMH	487	WINPNSGGTNYAQKFQG	492	DMNILATVPFDI	497
CAR123-3	GYIH	488	WINPNSGGTNYAQKFQG	493	DMNILATVPFDI	498
CAR123-4	DYMH	489	WINPNSGDTNYAQKFQG	494	DMNILATVPFDI	499
CAR123-1	GYMH	486	WINPNSGGTNYAQKFQG	491	DMNILATVPFDI	496

hzCAR123-1	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-2	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-3	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-4	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-5	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-6	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-7	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-8	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-9	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-10	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-11	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-12	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-13	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-14	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-15	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-16	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-17	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-18	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-19	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-20	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-21	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-22	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-23	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-24	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-25	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-26	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-27	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-28	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-29	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-30	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-31	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500
hzCAR123-32	SYWMN	490	RIDPYDSETHYNQKFKD	495	GNWDDY	500

Table 11. Light Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

Candidate	LCDR1	ID	LCDR2	ID	LCDR3	ID
CAR123-2	RASQSISSYLN	502	AASSLQS	507	QQGDSVPLT	512
CAR123-3	RASQSISSYLN	503	AASSLQS	508	QQGDSVPLT	513
CAR123-4	RASQSISSYLN	504	AASSLQS	509	QQGDSVPLT	514
CAR123-1	RASQSISTYLN	501	AAFSLQS	506	QQGDSVPLT	511
hzCAR123-1	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-2	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-3	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-4	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-5	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-6	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-7	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-8	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-10	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-10	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-11	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-12	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-13	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-14	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-15	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-16	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-17	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-18	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-19	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-20	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-21	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-22	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-23	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-24	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-25	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-26	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-27	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-28	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-29	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-30	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-31	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515
hzCAR123-32	RASKSISKDLA	505	SGSTLQS	510	QQHNKYPYT	515

Table 12. Heavy Chain Variable Domain CDRs according to the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948)

Candidate	HCDR1	ID	HCDR2	ID	HCDR3	ID
CAR123-2	GYTFTGY	517	NPNSGG	522	DMNILATVPFDI	527
CAR123-3	GYIFTGY	518	NPNSGG	523	DMNILATVPFDI	528
CAR123-4	GYTFTDY	519	NPNSGD	524	DMNILATVPFDI	529
CAR123-1	GYTFTGY	516	NPNSGG	521	DMNILATVPFDI	526
hzCAR123-1	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-2	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-3	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-4	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-5	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-6	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-7	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-8	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-9	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-10	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-11	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-12	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-13	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-14	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-15	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-16	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-17	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-18	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-19	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-20	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-21	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-22	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-23	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-24	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-25	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-26	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-27	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-28	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-29	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-30	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-31	GYTFTSY	520	DPYDSE	525	GNWDDY	530
hzCAR123-32	GYTFTSY	520	DPYDSE	525	GNWDDY	530

Table 13. Light Chain Variable Domain CDRs according to the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948)

Candidate	LCDR1	ID	LCDR2	ID	LCDR3	ID
CAR123-2	SQSISSY	532	AAS	537	GDSVPL	542
CAR123-3	SQSISSY	533	AAS	538	GDSVPL	543
CAR123-4	SQSISSY	534	AAS	539	GDSVPL	544
CAR123-1	SQSISTY	531	AAF	536	GDSVPL	541
hzCAR123-1	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-2	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-3	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-4	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-5	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-6	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-7	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-8	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-10	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-10	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-11	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-12	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-13	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-14	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-15	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-16	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-17	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-18	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-19	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-20	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-21	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-22	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-23	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-24	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-25	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-26	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-27	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-28	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-29	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-30	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-31	SKSISKD	535	SGS	540	HNKYPY	555
hzCAR123-32	SKSISKD	535	SGS	540	HNKYPY	555

In embodiments, CD123 single chain variable fragments are generated and cloned into lentiviral CAR expression vectors with the intracellular CD3zeta domain and the intracellular co-stimulatory domain of 4-1BB. Names of exemplary fully human CD123 scFvs are depicted in Table 1. Names of exemplary humanized CD123 scFvs are depicted in Table 5.

5 Table 1: CAR-CD123 constructs

Construct ID	CAR Nickname
EBB-C1357-F11	CAR123-1
EBB-C1358-B10	CAR123-2
EBB-C1358-D5	CAR123-3
EBB-C1357-C4	CAR123-4

Table 5: CAR-CD123 constructs

Construct ID	CAR Nickname
VH1_1-46_X_VK1_L8	hzCAR-1
VH1_1-46_X_VK3_L6	hzCAR-2
VH1_1-46_X_VK6_A14	hzCAR-3
VH1_1-46_X_VK4_B3	hzCAR-4
VK1_L8_X_VH1_1-46	hzCAR-5
VK3_L6_X_VH1_1-46	hzCAR-6
VK6_A14_X_VH1_1-46	hzCAR-7
VK4_B3_X_VH1_1-46	hzCAR-8
VH7_7-4.1_X_VK1_L8	hzCAR-9
VH7_7-4.1_X_VK3_L6	hzCAR-10
VH7_7-4.1_X_VK6_A14	hzCAR-11
VH7_7-4.1_X_VK4_B3	hzCAR-12
VK1_L8_X_VH7_7-4.1	hzCAR-13
VK3_L6_X_VH7_7-4.1	hzCAR-14
VK6_A14_X_VH7_7-4.1	hzCAR-15
VK4_B3_X_VH7_7-4.1	hzCAR-16
VH5_5-A_X_VK1_L8	hzCAR-17
VH5_5-A_X_VK3_L6	hzCAR-18
VH5_5-A_X_VK6_A14	hzCAR-19
VH5_5-A_X_VK4_B3	hzCAR-20
VK1_L8_X_VH5_5-A	hzCAR-21
VK3_L6_X_VH5_5-A	hzCAR-22
VK6_A14_X_VH5_5-A	hzCAR-23
VK4_B3_X_VH5_5-A	hzCAR-24
VH3_3-74_X_VK1_L8	hzCAR-25
VH3_3-74_X_VK3_L6	hzCAR-26

VH3_3-74_X_VK6_A14	hzCAR-27
VH3_3-74_X_VK4_B3	hzCAR-28
VK1_L8_X_VH3_3-74	hzCAR-29
VK3_L6_X_VH3_3-74	hzCAR-30
VK6_A14_X_VH3_3-74	hzCAR-31
VK4_B3_X_VH3_3-74	hzCAR-32

In embodiments, the order in which the VL and VH domains appear in the scFv is varied (i.e., VL-VH, or VH-VL orientation), and where either three or four copies of the “G4S” (SEQ ID NO:25) subunit, in which each subunit comprises the sequence GGGGS (SEQ ID NO:25) (e.g., (G4S)₃ (SEQ ID NO:28) or (G4S)₄(SEQ ID NO:27)), connect the variable domains to create the entirety of the scFv domain, as shown in Table 2, Table 6, and Table 9.

The amino acid and nucleic acid sequences of the CD123 scFv domains and CD123 CAR molecules are provided in Table 2, Table 6, and Table 9. The amino acid sequences for the variable heavy chain and variable light chain for each scFv is also provided in Table 2 and Table 6. It is noted that the scFv fragments (SEQ ID NOs: 157-160, and 184-215) with a leader sequence (e.g., the amino acid sequence of SEQ ID NO: 1 or the nucleotide sequence of SEQ ID NO: 12) and without a leader sequence (SEQ ID NOs: 478, 480, 483, 485, and 556-587) are also encompassed by the present invention.

Leader (amino acid sequence) (SEQ ID NO: 1)

MALPVTALLLPLALLLHAARP

Leader (nucleic acid sequence) (SEQ ID NO: 12)

ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTGCTGCTGCATGCC
GCTAGACCC

CD8 hinge (amino acid sequence) (SEQ ID NO: 2)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

CD8 hinge (nucleic acid sequence) (SEQ ID NO: 13)

ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA
GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGC
ACACGAGGGGGGCTGGACTTCGCCTGTGAT

CD8 transmembrane (amino acid sequence) (SEQ ID NO: 6)

IYIWAPLAGTCGVLLLSLVITLYC

CD8 transmembrane (nucleic acid sequence) (SEQ ID NO: 17)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTG
 5 GTTATCACCCCTTTACTGC

4-1BB Intracellular domain (amino acid sequence) (SEQ ID NO: 7)

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL

4-1BB Intracellular domain (nucleic acid sequence) (SEQ ID NO: 18)

10 AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTATGAGACC
 AGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCAGAGAAG
 AAGAAGGAGGATGTGAACTG

CD28 Intracellular domain (amino acid sequence) (SEQ ID NO: 43)

15 RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 43)

CD28 Intracellular domain (nucleotide sequence) (SEQ ID NO: 44)

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCG
 CCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTT
 CGCAGCCTATCGCTCC (SEQ ID NO: 44)

20

ICOS Intracellular domain (amino acid sequence) (SEQ ID NO: 45)

T K K K Y S S S V H D P N G E Y M F M R A V N T A K K S R L T D V T L
 (SEQ ID NO: 45)

ICOS Intracellular domain (nucleotide sequence) (SEQ ID NO: 46)

25 ACAAAAAGAAGTATTCATCCAGTGTGCACGACCCTAACGGTGAATACATGTT
 CATGAGAGCAGTGAACACAGCCAAAAAATCCAGACTCACAGATGTGACCCTA
 (SEQ ID NO: 46)

CD3 zeta domain (amino acid sequence) (SEQ ID NO: 9)

30 RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
 ALPPR

CD3 zeta (nucleic acid sequence) (SEQ ID NO: 20)

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGGCCAGA
 ACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTG
 GACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGGAAAGCCGAGAAGGAAGA
 5 ACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCC
 TACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGCACGATG
 GCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACA
 TGCAGGCCCTGCCCCCTCGC

10 **CD3 zeta domain (amino acid sequence; NCBI Reference Sequence NM_000734.3) (SEQ ID NO:10)**

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPENMGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQ
 ALPPR

15 **CD3 zeta (nucleic acid sequence; NCBI Reference Sequence NM_000734.3); (SEQ ID NO:21)**

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGGCCAG
 AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT
 TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGGAAAGCCGAGAAGGA
 20 AGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGG
 AGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGC
 ACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGC
 CCTTCACATGCAGGCCCTGCCCCCTCGC

25 **IgG4 Hinge (amino acid sequence) (SEQ ID NO:36)**

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
 WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS
 SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 ENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
 30 LSLGKM

IgG4 Hinge (nucleotide sequence) (SEQ ID NO:37)

5

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GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGC
GGACCCAGCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGC
CGGACCCCCGAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGA
GGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCA
AGCCCCGGGAGGAGCAGTTCAATAGCACCTACCGGGTGGTGTCCGTGCTGACC
GTGCTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTGTAAGGTGTCCAA
CAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGC
CTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAAG
AACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCCCCCC
TGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAA
GAGCCGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCC
TGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATG

In embodiments, these clones in Table 2 and 6 all contained a Q/K residue change in the signal domain of the co-stimulatory domain derived from CD3zeta chain.

Table 2. Exemplary CD123 CAR sequences

Name	SEQ ID	Sequence
CAR123-2 NT	40	atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccac gccgctcggccccaagtgcaactcgtccaaagcggagcggaagtcaagaaa cccgagcgcgagcgtgaaagtgtcctgcaaagcctccggctacacctttacg ggctactacatgcactgggtgcgccaggcaccaggacaggggtcttgaatgg atgggatggatcaaccctaattcgggcggaactaactacgcacagaagttc caggggagagtgactctgactcgggatacctccatctcaactgtctacatg gaactctcccgttgcggtcagatgatacggcagtgtactactgcgcccgc gacatgaatatcctggctaccgtgccgttcgacatctggggacaggggact atggttactgtctcatcgggcggtggaggttcaggaggaggcggctcggga ggcggaggttcggacattcagatgacccagtcccatcctctctgtcggcc agcgtcggagatagggtgaccattacctgtcgggcctcgcaaagcatctcc tcgtacctcaactggtatcagcaaaagccgggaaaggcgcctaagctgctg atctacgccgcttcgagcttgcaaagcgggggtgccatccagattctcggga tcaggctcaggaaccgacttcacctgaccgtgaacagcctccagccggag gactttgccacttactactgccagcagggagactccgtgccgcttactttc gggggggggtaccgcctggagatcaagaccactaccccagcaccgaggcca cccaccccggctcctaccatcgctcccagcctctgtccctgcgtccggag

		gcatgtagacccgcagctggtggggccgtgcatacccgggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctg ctgctttcactcgtgatcactctttactgtaagcgcggtcggaagaagctg ctgtacatctttaagcaacccttcatgaggcctgtgcagactactcaagag gaggacggctgttcatgccggttcccagaggaggaggaaggcggctgcgaa ctgcgcgtgaaattcagccgcagcgcagatgctccagcctacaagcagggg cagaaccagctctacaacgaactcaatcttggtcggagagaggagtacgac gtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgc agaaagaatccccaagaggggctgtacaacgagctccaaaaggataagatg gcagaagcctatagcgagattggtatgaaaggggaacgcagaagaggcaaa ggccacgacggactgtaccagggactcagcaccgccaccaaggacacctat gacgctcttcacatgcaggccctgccgcctcgg
CAR123-2 AA	99	MALPVTALLLP LALLLHAARPQVQLVQSGAEVKKPGASVKV SCKASGYTFT GYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTLTRDTSISTVYM ELSR LRSDDTAVYYCARDMNILATVPFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTF GGGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYD VLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
CAR123-2 scFv	158	MALPVTALLLP LALLLHAARPQVQLVQSGAEVKKPGASVKV SCKASGYTFT GYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTLTRDTSISTVYM ELSR LRSDDTAVYYCARDMNILATVPFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTF GGGTRLEIK
CAR123-2 VH	217	QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHWVRQAPGQGLEWMGWI NPNSGGTNYAQKFQGRVTLTRDTSISTVYMELSR LRSDDTAVYYCARDMNI LATVPFDIWGQGTMTVTVSS
CAR123-2 VL	276	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTFGGGT RLEIK
CAR123-3 NT	41	atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccac gccgctcggccccaagtccaactcgttcaatccggcgcagaagtcaagaag ccaggagcatcagtgaagtgctcctgcaaagcctcaggctacatcttcacg ggatactacatccactgggtgcgccaggctccgggccagggccttgagtgg atgggctggatcaaccctaactctgggggaaccaactacgctcagaagttc caggggagggtcactatgactcgcgatacctccatctccactgcgtacatg gaactctcgggactgagatccgacgatcctgccgtgtactactgcgcccgg gacatgaacatcttggcgaccgtgccgtttgacatttggggacagggcacc ctcgtcactgtgtcgagcgggtggaggaggctcgggggggtggcggatcagga gggggaggaagcgacatccagctgactcagagcccatcgtcgttgtccgcg tcgggtgggggatagagtgaccattacttgccgcgccagccagagcatctca

		tcatatctgaattggtaccagcagaagcccggaaaggccccaaaactgctg atctacgctgcaagcagcctccaatcgggagtgccgtcacggttctccggg tccggttcgggaactgactttaccctgaccgtgaattcgctgcaaccggag gatttcgccacgtactactgtcagcaaggagactccgtgccgctgaccttc ggtggaggcaccaaggtcgaaatcaagaccactaccccagcaccgaggcca cccaccccggctcctaccatcgctcccagcctctgtccctgctccggag gcatgtagacccgcagctggtggggccgtgcatacccgggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgccggggctctg ctgctttcactcgtgatcactctttactgtaagcgcggtcggaagaagctg ctgtacatctttaagcaacccttcatgaggcctgtgcagactactcaagag gaggacggctgttcatgccgggttcccagaggaggaggaaggcggtgcgaa ctgcgcgtgaaattcagccgcagcgcagatgctccagcctacaagcagggg cagaaccagctctacaacgaactcaatcttggtcggagagaggagtacgac gtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgc agaaagaatccccaagagggcctgtacaacgagctccaaaaggataagatg gcagaagcctatagcgagattggtatgaaaggggaacgcagaagaggcaa ggccacgacggactgtaccagggactcagcaccgccaccaaggacacctat gacgctcttcacatgcaggccctgccgcctcgg
CAR123-3 AA	100	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYIFT GYYIHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYM ELSGLRSDDPVYYCARDMNILATVPFDIWGQGTTLTVSSGGGGSGGGGSG GGGSDIQLTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTF GGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEEGGCELRVKFSSRSADAPAYKQGQNQLYNELNLGRREEYD VLDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
CAR123-3 scFv	159	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYIFT GYYIHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYM ELSGLRSDDPVYYCARDMNILATVPFDIWGQGTTLTVSSGGGGSGGGGSG GGGSDIQLTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTF GGGTKVEIK
CAR123-3 VH	218	QVQLVQSGAEVKKPGASVKVSCKASGYIFTGYYIHWVRQAPGQGLEWMGWI NPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSGLRSDDPVYYCARDMNI LATVPFDIWGQGTTLTVSS
CAR123-3 VL	277	DIQLTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTFGGGT KVEIK
CAR123-4 NT	42	atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccac gccgctcggccccaagtccaactccaacagt caggcgcagaagtgaaaaag agcgggtgcacgggtgaaagtgtcatgcaaagcctcgggctacaccttact gactactatatgcactggctgcggcaggcaccgggacagggacttgagtgg atgggatggatcaaccgaattcaggggacactaactacgcgcagaagttc

		caggggagagtgaccctgacgaggggacacctcaatttcgaccgtctacatg gaattgtcgcgctgagatcggacgatactgctgtgtactactgtgcccgc gacatgaacatcctcgcgactgtgccttttgatatctggggacaggggact atggtcaccgtttcctccgcttccgggtggcggaggctcgggaggccggggcc tccgggtggaggaggcagcgacatccagatgactcagagcccttcctcgctg agcgctcagtgaggagatcgcgtagccatcacttgccggggccagccagtcc atttcgtcctacctcaattggtaccagcagaagccgggaaaggcgcccaag ctcttgatctacgctgcgagctccctgcaaagcgggggtgccgagccgattc tcgggttccggctcgggaaccgacttcactctgaccatctcatccctgcaa ccagaggactttgccacctactactgccaacaaggagattctgtcccactg acgttcggcggagggaaccaaggctcgaaatcaagaccactacccagcaccg aggccacccaccccggtcctaccatcgctcccagcctctgtccctgcgt ccggaggcatgtagaccgcagctgggtggggccgtgcatacccgggggtctt gacttcgcctgcgatatctacatttggggccctctggctggtagcttgcggg gtcctgctgctttcactcgtgatcactctttactgtaagcgcgggtcggaag aagctgctgtacatctttaagcaacccttcatgaggcctgtgcagactact caagaggaggacggctgttcatgccgggttcccagaggaggaggaaggcggc tgcgaaactgcgcgtgaaattcagccgcagcgcagatgctccagcctacaag caggggcagaaccagctctacaacgaactcaatcttggtcggagagaggag tacgacgtgctggacaagcggagaggacgggacccagaaatgggcgggaag ccgcgcagaaagaatccccaagagggcctgtacaacgagctccaaaaggat aagatggcagaagcctatagcgagattggtatgaaaggggaacgcagaaga ggcaaaggccacgacggactgtaccagggactcagcaccgccaccaaggac acctatgacgctcttcacatgcaggccctgccgcctcgg
CAR123-4 AA	101	MALPVTALLLPLALLLHAARPQVQLQQSGAEVKKSGASVKVSCKASGYTFT DYMHHLRQAPGQGLEWMGWINPNSGDTNYAQKFQGRVTLTRDTSISTVYM ELSLRSDDTAVYYCARDMNILATVPFDIWGQGTMTVTVSSASGGGGSGGRA SGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGDSVPL TFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCK
CAR123-4 scFv	160	MALPVTALLLPLALLLHAARPQVQLQQSGAEVKKSGASVKVSCKASGYTFT DYMHHLRQAPGQGLEWMGWINPNSGDTNYAQKFQGRVTLTRDTSISTVYM ELSLRSDDTAVYYCARDMNILATVPFDIWGQGTMTVTVSSASGGGGSGGRA SGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGDSVPL TFGGGTKVEIK
CAR123-4 VH	219	QVQLQQSGAEVKKSGASVKVSCKASGYTFTDYMHHLRQAPGQGLEWMGWI NPNSGDTNYAQKFQGRVTLTRDTSISTVYMELSRLRSDDTAVYYCARDMNI LATVPFDIWGQGTMTVTVSS
CAR123-4 VL	278	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGDSVPLTFGGGT KVEIK
CAR123-1 NT	39	atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccac gccgctcggccccaagtccaactcgtccagt caggagcggaagtcaagaag

		cccggagcgtcagtc aaagtgtcatgcaaagcctcggggtacactttcact gggtactacatgcactgggtgcgccagggtccaggacagggactggaatgg atgggatggatcaacccgaactccggtggcaccaattacgcccagaagttc caggggaggggtgaccatgactcgcgacacgtcgatcagcacccgcatacatg gagctgtcaagactccggtccgacgatactgccgtgtactactgcgcacgg gacatgaacattctggccaccgtgccttttgacatctgggggtcagggaaact atgggtaccgtgtcctctgggtggaggcgggtccggcgggggggggaagcgga ggcgggtggaagcgacattcagatgacccagtcgccttcaccccttcggcg agcgtgggagatcgcgctcactatcacttgtcgggcctcgagtcctatctcc acctacctcaattggtaccagcagaagccaggaaaagcaccgaatctgctg atctacgccgcgttttccttgcaatcgggagtgccaagcagattcagcggga tcgggatcaggcactgatttcaccctcaccatcaactcgctgcaaccggag gatttcgctacgtactattgccaacaaggagacagcgtgccgctcaccttc ggcggaggggactaagctggaaatcaagaccactacccagcaccgaggcca cccaccccggtcctaccatcgctcccagcctctgtccctgcgtccggag gcatgtagacccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctg ctgctttcactcgatgactctttactgtaagcgcggtcggaagaagctg ctgtacatctttaagcaacccttcagaggcctgtgcagactactcaagag gaggacggctgttcagtcgggttcccagaggaggagggaaggcggctgcgaa ctgcgcgtgaaattcagccgcagcgcagatgctccagcctacaagcagggg cagaaccagctctacaacgaactcaatcttggtcggagagaggagtacgac gtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgc agaaagaatccccaagagggcctgtacaacgagctccaaaaggataagatg gcagaagcctatagcgagattggtatgaaaggggaacgcagaagaggcaaa ggccacgacggactgtaccagggtactcagcacccgccaccaaggacacctat gacgctcttcacatgcaggccctgccgcctcgg
CAR123-1 AA	98	malpvtalllplalllhaarpqvqlvqsgaevkkpgasvkvsckasgytft gyymhwvrqapggglewmgwinpnsggtnyaqkfqgrvtmtrdtsistaym elsrlrsddtavyycardmnilatvpfdiwgqgtmvtvssggggsggggsg gggsdiqmtqspsslsasvgdrvtitcrasqsistylnwyyqqkpgkapnll iyaafslqsgvpsrfs gsgsgtdftltinslqpedfatyycqqgds vpltf gggtkleiktttpaprpptpaptiasqplslrpeacrpaaggavhtrgldf acdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqp fmrpvqttqe edgcscr fpeeeeeggcelrvkfsrsadapaykqqnqlynelnlgrreeyd vldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgk ghdglyqglstatkdydalhmqalppr
CAR123-1 scFv	157	malpvtalllplalllhaarpqvqlvqsgaevkkpgasvkvsckasgytft gyymhwvrqapggglewmgwinpnsggtnyaqkfqgrvtmtrdtsistaym elsrlrsddtavyycardmnilatvpfdiwgqgtmvtvssggggsggggsg gggsdiqmtqspsslsasvgdrvtitcrasqsistylnwyyqqkpgkapnll iyaafslqsgvpsrfs gsgsgtdftltinslqpedfatyycqqgds vpltf gggtkleik
CAR123-1 VH	216	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVRQAPGQGLEWMGWI NPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARDMNI LATVPFDIWGQGTMTVSS

CAR123-1 VL	275	DIQMTQSPSSLSASVGDRVTITCRASQSISTYLNWYQQKPGKAPNLLIYAA FSLQSGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQGDSVPLTFGGGT KLEIK

Table 6: Humanized CD123 CAR Sequences

Name	SEQ ID	Sequence
hzCAR123 -1 NT	66	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGGCGCTAGCGTGAAAGT GTCCTGCAAAGCCTCCGGGTACACATTACCTCCTACTGGATGAATTGGGTGAGACAGGCG CCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACTCCGAAACCCATTACA ACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCACTTCCACCGCTTACAT GGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGCGCCCCGGGGAAACTGG GACGACTATTGGGGACAGGGAACTACCGTGACCGTGTCAAGCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGCAGCTCACCCAGTC GCCCTCATTTCTGTGCGCCTCAGTGGGAGACAGAGTGACCATTACTTGTGCGGGCCTCCAAG AGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCAGGAAAGGCGCCTAAGTTGCTCA TCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCCGGTTCTCCGGTTCGGGAAGCGG TACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGAGGACTTCGCCACCTACTACTGC CAACAGCACACAAGTACCCGTACACTTTCGGGGGTGGCACGAAGGTCGAAATCAAGACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -1 AA	125	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQA PGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDVAVYYCARGNW DDYWGQGTTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVTITCRASK SISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYC QQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
hzCAR123 -1 scFv	184	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQA PGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDVAVYYCARGNW DDYWGQGTTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVTITCRASK SISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYC QQHNKYPYTFGGGTKVEIK
hzCAR123 -1 VH	243	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVTMTVDKSTSTAYMELSSLRSEDVAVYYCARGNWDDYWGQGTTTVTVSS

hzCAR123 -1 VL	302	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTIISSSLQPEDFATYYCQQH NKYPYTFGGG TKVEIK
hzCAR123 -2 NT	67	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGGCGCTAGCGTGAAAGT GTCCTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAATTGGGTCAGACAGGCG CCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACTCCGAAACCCATTACA ACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCACTTCCACCGCTTACAT GGAGCTGTCCAGCCTGCGCTCCGAGGATACCGCAGTGTACTACTGCGCCCGGGGAAACTGG GACGACTATTGGGGACAGGGA ACTACCGTGACCGTGTCAAGCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGAAGTGGTGCTGACCCAGTC GCCCGCAACCTCTCTCTGTGCGCCGGGAGAACGCGCCACTCTTTTCTGTGCGGGCGTCCAAG AGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCTGGTCAAGCCCCGCGGCTGCTGA TCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCAGATTTTCCGGTTCGGGGTCGGG GACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGAGGACTTCGCCGTGTATTACTGC CAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGTACTAAGGTCGAGATCAAGACCA CTACCCAGCACCAGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggccccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcagggg gcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -2 AA	126	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSED TAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIISSLEPEDFA VYYCQQH NKYPYTFGGG TKVEIKTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -2 scFv	185	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSED TAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIISSLEPEDFA VYYCQQH NKYPYTFGGG TKVEIK
hzCAR123 -2 VH	244	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTMTVDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -2 VL	303	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTIISSLEPEDFAVYYCQQH NKYPYTFGGG TKVEIK
hzCAR123 -3 NT	68	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGGCGCTAGCGTGAAAGT GTCCTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAATTGGGTCAGACAGGCG

		CCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACTCCGAAACCCATTACA ACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCACTTCCACCGCTTACAT GGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGCGCCCGGGGAAACTGG GACGACTATTGGGGACAGGGAACTACCGTGACCGTGTCAAGCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTCGTGATGACCCAGTC ACCGGCATTTCCTGTCCGTGACTCCCGGAGAAAAGGTCACGATTACTTGCCGGGCGTCCAAG AGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCGGACCAGGCCCCTAAGCTGTTGA TCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGCGGTTTACGCGTTTCGGGTTCTGG GACCGACTTCACTTTTACCATCTCCTCACTGGAAGCCGAGGATGCCGCCACTTACTACTGT CAGCAGCACAAAGTATCCGTACACCTTCGGAGGCGGTACCAAAGTGAGATCAAGACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggccccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -3 AA	127	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -3 scFv	186	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -3 VH	245	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARGNWDDYWGGQTTVTVSS
hzCAR123 -3 VL	304	DVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -4 NT	69	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGGCGCTAGCGTGAAAGT GTCCTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAATTGGGTCAGACAGGCG CCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACTCCGAAACCCATTACA ACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCACTTCCACCGCTTACAT GGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGCGCCCGGGGAAACTGG GACGACTATTGGGGACAGGGAACTACCGTGACCGTGTCAAGCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGGTCATGACTCAGTC CCCGGACTCACTCGCGGTGTGCTTGGAGAGAGAGCGACCATCAACTGTCGGGCCTCAAAG

		AGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCGGGACAGCCGCCAAAGCTGCTGA TCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACCGGTTCTCCGGTTCGGGGTCGGG TACCGACTTCACGCTCACTATTTTCGTGCTGCAAGCCGAAGATGTGGCCGTGTACTATTGC CAACAGCACAACAAGTACCCCTACACTTTTGGCGGAGGACCAAGGTGGAAATCAAGACCA CTACCCACAGCACCAGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -4 AA	128	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARG NWDDYWQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVMQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -4 scFv	187	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARG NWDDYWQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVMQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -4 VH	246	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTMTVDKSTSTAYMELSSLRSEDVAVYYCARGNWDDYWQGTTVTVSS
hzCAR123 -4 VL	305	DVVMQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLTISLQAEDVAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -5 NT	70	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGCAGCTCACCCAGTCGCCCTCATTTCTGTGCGCCTCAGTGGGAGACAGAGTGAC CATTACTTGTGCGGCCTCCAAGAGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCA GGAAAGGCGCCTAAGTTGCTCATCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCC GGTTCTCCGGTTCGGGAAGCGGTACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGA GGACTTCGCCACCTACTACTGCCAACAGCACAACAAGTACCCGTACACTTTCGGGGGTGGC ACGAAGGTGCAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGG CGCTAGCGTGAAAGTGTCTTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAAT TGGGTCAGACAGGCGCCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACT CCGAAACCCATTACAACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCAC TTCCACCGCTTACATGGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGC GCCCCGGGAAACTGGGACGACTATTGGGGACAGGGAAGTACCGTGACCGTGTCAAGCACCA CTACCCACAGCACCAGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccggggtcttgacttc

		gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcattgaggcctgtgcagactactcaagaggaggacggctgttcattgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -5 AA	129	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -5 scFv	188	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -5 VH	247	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -5 VL	306	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -6 NT	71	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGGTGCTGACCCAGTCGCCCGCAACCCTCTCTCTGTCGCCGGGAGAACGCGCCAC TCTTTCCTGTCGGGCGTCCAAGAGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCT GGTCAAGCCCCGCGGCTGCTGATCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCA GATTTTCCGGTTCGGGGTCGGGGACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGA GGACTTCGCCGTGTATTACTGCCAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGT ACTAAGGTCGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGG CGCTAGCGTGAAAGTGTCTTGCAAAGCCTCCGGGTACACATTACCTCCTACTGGATGAAT TGGGTCAGACAGGCGCCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACT CCGAAACCCATTACAACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCAC TTCCACCGCTTACATGGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGC GCCCCGGGAACTGGGACGACTATTGGGGACAGGGAACCTACCGTGACCGTGTCAAGCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcattgaggcctgtgcagactactcaagaggaggacggctgttcattgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat

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hzCAR123 -6 AA	130	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -6 scFv	189	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -6 VH	248	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTVTDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -6 VL	307	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -7 NT	72	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTCGTGATGACCCAGTCACCGGCATTCCCTGTCCGTGACTCCCGGAGAAAAGGTCAC GATTACTTGCCGGGCGTCCAAGAGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCG GACCAGGCCCCCTAAGCTGTTGATCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGC GGTTTAGCGGTTTCGGGTTCTGGGACCGACTTCACTTTCACCATCTCCTCACTGGAAGCCGA GGATGCCGCCACTTACTACTGTCAGCAGCACACAAGTATCCGTACACCTTCGGAGGCGGT ACCAAAGTGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGG CGCTAGCGTGAAAGTGTCTTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAAT TGGGTCAGACAGGCGCCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACT CCGAAACCCATTACAACCAGAAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCAC TTCCACCGCTTACATGGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGC GCCCCGGGGAAACTGGGACGACTATTGGGGACAGGGAACTACCGTGACCGTGTCAAGCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -7 AA	131	MALPVTALLLPLALLLHAARPDVVMTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY

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hzCAR123 -7 scFv	190	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSGVQVLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGTTVTVS
hzCAR123 -7 VH	249	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRVMTMTVDKSTSTAYMELSSLRSEDTAVYYCARGNWDDYWGQGTTVTVS
hzCAR123 -7 VL	308	DVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -8 NT	73	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGGTCATGACTCAGTCCCCGGACTCACTCGCGGTGTCGCTTGGAGAGAGAGCGAC CATCAACTGTCGGGCCTCAAAGAGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCG GGACAGCCGCCAAAGCTGCTGATCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACC GGTTCTCCGGTTCCGGGTCGGGTACCGACTTCACGCTCACTATTTCTGTCGCTGCAAGCCGA AGATGTGGCCGTGTACTATTGCCAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGC ACCAAGGTGGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTCCAGTCGGGAGCCGAAGTCAAGAAGCCCGG CGCTAGCGTGAAAGTGTCTTGCAAAGCCTCCGGGTACACATTCACCTCCTACTGGATGAAT TGGGTCAGACAGGCGCCCGGCCAGGGACTCGAGTGGATGGGAAGGATTGATCCTTACGACT CCGAAACCCATTACAACCAGAAGTTCAAGGACCGCGTGACCATGACTGTGGATAAGTCCAC TTCCACCGCTTACATGGAGCTGTCCAGCCTGCGCTCCGAGGATAACCGCAGTGTACTACTGC GCCCCGGGAACTGGGACGACTATTGGGGACAGGGAAGTACCGTGACCGTGTCAAGCACCA CTACCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctgggtggggccgtgcatacccgggggtccttgacttc gcctgcgatatctacatcttgggcccctctgggtggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -8 AA	132	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSGVQVLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVMTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGTTVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR

hzCAR123 -8 scFv	191	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIISSSLQAEDVAVYYCQQH NKYPYTFGGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -8 VH	250	QVQLVQSGAEVKKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -8 VL	309	DVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIISSSLQAEDVAVYYCQQH NKYPYTFGGTKVEIK
hzCAR123 -9 NT	74	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCCGGAGCCTCCGTCAAAGT GTCCTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAACTGGGTCCGCCAGGCA CCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATTCCGAAACCCATTACA ATCAGAAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGTGTCCACCGCCTACCT CCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGCGCTCGCGGAAACTGG GATGACTATTGGGGCCAGGGAACCTACCGTGACTGTGTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGCAGCTCACCCAGTC GCCCTCATTTCTGTTCGGCCTCAGTGGGAGACAGAGTGACCATTACTTGTTCGGGCCTCCAAG AGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCAGGAAAGGCGCCTAAGTTGCTCA TCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCCGGTTCTCCGGTTCGGGAAGCGG TACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGAGGACTTCGCCACCTACTACTGC CAACAGCACACAAGTACCCGTACACTTTCGGGGGTGGCACGAAGGTCGAAATCAAGACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -9 AA	133	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITICRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTIISSSLQPEDFATYYCQQH NKYPYTFGGGTVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -9 scFv	192	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITICRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTIISSSLQPEDFATYYCQQH NKYPYTFGGGTVEIK
hzCAR123	251	QVQLVQSGSELKKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN

-9 VH		QKFKDRFVFSVDKSVSTAYLQISSSLKAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -10 VL	310	DVQLTQSPSFLSASVGDRVTITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTIISSSLQPEDFATYYCQQH NKYPYTFGGG TKVEIK
hzCAR123 -10 NT	75	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGGAGCCTCCGTCAAAGT GTCCTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAACTGGGTCCGCCAGGCA CCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATTCCGAAACCCATTACA ATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGTGTCCACCGCCTACCT CCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGCGCTCGCGGAACTGG GATGACTATTGGGGCCAGGGAAC TACCGTGACTGTGTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGAAGTGGTGCTGACCCAGTC GCCC GCAACCCTCTCTCTGTGCGCCGGGAGAACGCGCCACTCTTTCTGTGCGGGCGTCCAAG AGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCTGGTCAAGCCCCGCGGCTGCTGA TCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCAGATTTTCCGGTTCGGGGTCGGG GACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGAGGACTTCGCCGTGTATTACTGC CAGCAGCACAAACAAGTACCCGTACACCTTCGGAGGCGGTACTAAGGTCGAGATCAAGACCA CTACCC CAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagaggggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -10 AA	134	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIISSLEPEDFA VYYCQQH NKYPYTFGGG TKVEIKTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -10 scFv	193	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIISSLEPEDFA VYYCQQH NKYPYTFGGG TKVEIK
hzCAR123 -10 VH	252	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSSLKAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -10 VL	311	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTIISSLEPEDFAVYYCQQH NKYPYTFGGG TKVEIK
hzCAR123	76	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGGAGCCTCCGTCAAAGT

-11 NT		GTCCTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAACTGGGTCCGCCAGGCA CCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATTCCGAAACCCATTACA ATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGTGTCCACCGCCTACCT CCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGCGCTCGCGGAAACTGG GATGACTATTGGGGCCAGGGAAC TACCGTGACTGTGTCTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTCGTGATGACCCAGTC ACCGGCATTTCCTGTCCGTGACTCCCGGAGAAAAGGTCACGATTACTTGCCGGGCGTCCAAG AGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCGGACCAGGCCCCTAAGCTGTTGA TCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGCGGTTTAGCGGTTTCGGGTTCTGG GACCGACTTCACTTTTACCATCTCCTCACTGGAAGCCGAGGATGCCGCCACTTACTACTGT CAGCAGCACAAACAAGTATCCGTACACCTTCGGAGGCGGTACCAAAGTGGAGATCAAGACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -11 AA	135	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVM TQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGG TKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -11 scFv	194	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVM TQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGG TKVEIK
hzCAR123 -11 VH	253	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -11 VL	312	DVVM TQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFGGG TKVEIK
hzCAR123 -12 NT	77	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGGAGCCTCCGTCAAAGT GTCCTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAACTGGGTCCGCCAGGCA CCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATTCCGAAACCCATTACA ATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGTGTCCACCGCCTACCT CCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGCGCTCGCGGAAACTGG GATGACTATTGGGGCCAGGGAAC TACCGTGACTGTGTCTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGGT CATGACTCAGTC

		CCCGGACTCACTCGCGGTGTCGCTTGGAGAGAGAGCGACCATCAACTGTCGGGCCTCAAAG AGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCGGGACAGCCGCCAAAGCTGCTGA TCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACCGGTTCTCCGGTTCCGGGTCGGG TACCGACTTCACGCTCACTATTTTCGTGCTGCAAGCCGAAGATGTGGCCGTGTACTATTGC CAACAGCACAAACAAGTACCCCTACACTTTTGGCGGAGGGCACCAAGGTGGAAATCAAGACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccgggggtcttgacttc gcctgcgatatctacatttgggccccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagegcggctcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -12 AA	136	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKEIKTTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -12 scFv	195	MALPVTALLLPLALLLHAARPQVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKEIK
hzCAR123 -12 VH	254	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -12 VL	313	DVVMQTSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLTISLQAEDVAVYYCQQHNKYPYTFGGGTKEIK
hzCAR123 -13 NT	78	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGCAGCTCACCCAGTCGCCCTCATTTCTGTCGGCCTCAGTGGGAGACAGAGTGAC CATTACTTGTCGGGCCTCCAAGAGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCA GGAAAGGCGCCTAAGTTGCTCATCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCC GGTTCTCCGGTTCGGGAAGCGGTACCGAATTACCCCTTACTATCTCCTCCCTGCAACCGGA GGACTTCGCCACCTACTACTGCCAACAGCACAAACAAGTACCCGTACACTTTCGGGGGTGGC ACGAAGGTTCGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGG AGCCTCCGTCAAAGTGTCTTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAAC TGGGTCCGCCAGGCACCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATT CCGAAACCCATTACAATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGT GTCCACCGCCTACCTCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGC GCTCGCGGAAACTGGGATGACTATTGGGGCCAGGGAACCTACCGTGACTGTGTCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT

		GCGTCCGGAggcatgtagacccgcagctggtggggccgtgcataaccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcagggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -13 AA	137	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWQGTTVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -13 scFv	196	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWQGTTVTVSS
hzCAR123 -13 VH	255	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWQGTTVTVSS
hzCAR123 -13 VL	314	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -14 NT	79	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGGTGCTGACCCAGTCGCCCGCAACCCTCTCTCTGTCGCCGGGAGAACGCGCCAC TCTTTCCCTGTCGGGCGTCCAAGAGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCT GGTCAAGCCCCGCGGCTGCTGATCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCA GATTTTCCGGTTCGGGGTCGGGGACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGA GGACTTCGCCGTGTATTACTGCCAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGT ACTAAGGTCGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGG AGCCTCCGTCAAAGTGTCTTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAAC TGGGTCCGCCAGGCACCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATT CCGAAACCCATTACAATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGT GTCCACCGCCTACCTCCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGC GCTCGCGGAAACTGGGATGACTATTGGGGCCAGGGAACCTACCGTGACTGTGTCTCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAggcatgtagacccgcagctggtggggccgtgcataaccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcagggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga

		cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -14 AA	138	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSGVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -14 scFv	197	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSGVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -14 VH	256	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -14 VL	315	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -15 NT	80	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTCGTGATGACCCAGTCACCGGCATTCTGTCCGTGACTCCCGGAGAAAAGGTCAC GATTACTTGCCGGGCGTCCAAGAGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCG GACCAGGCCCCCTAAGCTGTTGATCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGC GGTTTAGCGGTTTCGGGTTCTGGGACCGACTTCACTTTCACCATCTCCTCACTGGAAGCCGA GGATGCCGCCACTTACTACTGTCAGCAGCACAACAAGTATCCGTACACCTTCGGAGGCGGT ACCAAAGTGGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGG AGCCTCCGTCAAAGTGTCTTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAAC TGGGTCCGCCAGGCACCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATT CCGAAACCCATTACAATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGT GTCCACCGCCTACCTCCAAATTAGCAGCCTGAAGGCGGAGGATACAGCTGTCTACTACTGC GCTCGCGGAAACTGGGATGACTATTGGGGCCAGGGAACCTACCGTGACTGTGTCTCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAaggcatgtagaccgcagctggtggggccgtgcatacccgggggtccttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccgggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggacagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123	139	MALPVTALLLPLALLLHAARPDVVMVTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG

-15 AA		GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -15 scFv	198	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTTISSELEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -15 VH	257	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYN QKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -15 VL	316	DVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTTISSELEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -16 NT	81	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGGTCATGACTCAGTCCCCGGACTCACTCGCGGTGTCGCTTGGAGAGAGAGCGAC CATCAACTGTCGGGCCTCAAAGAGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCG GGACAGCCGCCAAAGCTGCTGATCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACC GGTTCTCCGGTTCGGGGTCGGGTACCGACTTCACGCTCACTATTTCTGTCGCTGCAAGCCGA AGATGTGGCCGTGTACTATTGCCAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGC ACCAAGGTGGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGGCGGCT CAGGGGGCGGAGGAAGCCAAGTGCAGCTGGTGCAGTCAGGCAGCGAACTGAAGAAGCCCGG AGCCTCCGTCAAAGTGTCTTGCAAAGCCTCGGGATACACCTTCACCTCCTACTGGATGAAC TGGGTCCGCCAGGCACCTGGACAGGGGCTGGAGTGGATGGGAAGGATCGATCCCTACGATT CCGAAACCCATTACAATCAGAAGTTCAAGGACCGGTTTGTGTTCTCCGTGGACAAGTCCGT GTCCACCGCCTACCTCCAAATTAGCAGCCTGAAGGCGGAGGATAACAGCTGTCTACTACTGC GCTCGCGGAAACTGGGATGACTATTGGGGCCAGGGAAGTACCGTGACTGTGTCTCTCCACCA CTACCCACAGCACCAGGACCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccgggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -16 AA	140	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSELEAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM

		QALPPR
hzCAR123 -16 scFv	199	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIISSSLQAEDVAVYYCQQH NKYPYTFGGTKVEIKGGGGSGGGSGGGSGGGSGGGSGVQLVQSGSELKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -16 VH	258	QVQLVQSGSELKKPGASVKV SCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -16 VL	317	DVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIISSSLQAEDVAVYYCQQH NKYPYTFGGTKVEIK
hzCAR123 -17 NT	82	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGGAGAATCCCTGAGGAT CAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAATTGGGTCCGCCAGATG CCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACTCGGAAACCCATTACA ACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCATTTCCACTGCGTACCT CCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGCGCACGCGGAAACTGG GATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGCAGCTCACCCAGTC GCCCTCATTTCTGTTCGGCCTCAGTGGGAGACAGAGTGACCATTACTTGTTCGGGCCTCCAAG AGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCAGGAAAGGCGCCTAAGTTGCTCA TCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCCGGTTCTCCGGTTCGGGAAGCGG TACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGAGGACTTCGCCACCTACTACTGC CAACAGCACACAAGTACCCGTACACTTTCCGGGGGTGGCACGAAGGTGCAAATCAAGACCA CTACCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggccccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggacagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -17 AA	141	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMY YCARG NWDDYWGQGTITVTVSSGGGGSGGGSGGGSGGGSGGGSDVQLTQSPSFLSASVGDRVTITCR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTIISSSLQPEDFA TYYCQQH NKYPYTFGGGTKEIKTTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -17 scFv	200	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMY YCARG NWDDYWGQGTITVTVSSGGGGSGGGSGGGSGGGSGGGSDVQLTQSPSFLSASVGDRVTITCR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTIISSSLQPEDFA TYYCQQH NKYPYTFGGGTKEIK

hzCAR123 -17 VH	259	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -17 VL	318	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTITSSLPEDFATYYCQQHMKYPYTFGGGTKVEIK
hzCAR123 -18 NT	83	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGGAGAATCCCTGAGGATCAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAATTGGGTCCGCCAGATGCCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACTCGGAAACCCATTACAACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCATTTCCACTGCGTACCTCCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGCGCACGCGGAAACTGGGATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCTCCGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGAAGTGGTGCTGACCCAGTCGCCCGCAACCCTCTCTCTGTGCGCCGGGAGAACGCGCCACTCTTTCCTGTGCGGGCGTCCAAGAGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCTGGTCAAGCCCCGCGGCTGCTGATCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCAGATTTTCCGGTTCGGGGTCGGGGACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGAGGACTTCGCCGTGTATTACTGC CAGCAGCACAAACAAGTACCCGTACACCTTCGGAGGCGGTACTAAGGTGCAGATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGggcatgtagaccgcagctgggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat cccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -18 AA	142	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTTVTVSSGGGGSGGGSGGGSGGGGSEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHMKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -18 scFv	201	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTTVTVSSGGGGSGGGSGGGSGGGGSEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHMKYPYTFGGGTKVEIK
hzCAR123 -18 VH	260	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -18 VL	319	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHMKYPYTFGGGTKVEIK

hzCAR123 -19 NT	84	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGGAGAATCCCTGAGGAT CAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAATTGGGTCCGCCAGATG CCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACTCGGAAACCCATTACA ACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCATTTCCACTGCGTACCT CCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGCGCACGCGGAAACTGG GATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCTCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTCGTGATGACCCAGTC ACCGGCATTCTGTCCGTGACTCCCGGAGAAAAGGTCACGATTACTTGCCGGGCGTCCAAG AGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCGGACCAGGCCCTAAGCTGTTGA TCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGCGGTTTAGCGGTTTCGGGTTCTGG GACCGACTTCACTTTTACCATCTCCTCACTGGAAGCCGAGGATGCCGCCACTTACTACTGT CAGCAGCACAACAAGTATCCGTACACCTTCGGAGGCGGTACCAAAGTGGAGATCAAGACCA CTACCCACAGCACCAGAGGCCACCCACCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -19 AA	143	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -19 scFv	202	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -19 VH	261	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -19 VL	320	DVVMTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -20 NT	85	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGGAGAATCCCTGAGGAT CAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAATTGGGTCCGCCAGATG CCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACTCGGAAACCCATTACA ACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCATTTCCACTGCGTACCT CCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGCGCACGCGGAAACTGG

		GATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCCGGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGGTCATGACTCAGTC CCCGGACTCACTCGCGGTGTGCTTGGAGAGAGAGCGACCATCAACTGTCGGGCCTCAAAG AGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCGGGACAGCCGCCAAAGCTGCTGA TCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACCGGTTCTCCGGTTCGGGGTCGGG TACCGACTTCACGCTCACTATTTTCGTGCTGCAAGCCGAAGATGTGGCCGTGTACTATTGC CAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGGCACCAAGGTGGAAATCAAGACCA CTACCCAGCACCGAGGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -20 AA	144	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSI STAYLQWSSLKASDTAMY YCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVM TQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLT ISSLQAEDVA VYYCQQHNKYPYTFGGG TKVEIKTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -20 scFv	203	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSI STAYLQWSSLKASDTAMY YCARG NWDDYWGQGTTVTVSSGGGSGGGGSGGGGSGGGGSDVVM TQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLT ISSLQAEDVA VYYCQQHNKYPYTFGGG TKVEIK
hzCAR123 -20 VH	262	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSI STAYLQWSSLKASDTAMY YCARGNWDDYWGQGTTVTVSS
hzCAR123 -20 VL	321	DVVM TQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLT ISSLQAEDVAVYYCQQHNKYPYTFGGG TKVEIK
hzCAR123 -21 NT	86	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGCAGCTCACCCAGTCGCCCTCATTTCTGTCGGCCTCAGTGGGAGACAGAGTGAC CATTACTTGTCGGGCCTCCAAGAGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCA GGAAAGGCGCCTAAGTTGCTCATCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCC GGTTCTCCGGTTCGGGAAGCGGTACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGA GGACTTCGCCACCTACTACTGCCAACAGCACACAAGTACCCGTACACTTTCGGGGGTGGC ACGAAGGTCGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGG AGAATCCCTGAGGATCAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAAT TGGGTCCGCCAGATGCCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACT CGGAAACCCATTACAACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCAT TTCCACTGCGTACCTCCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGC

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hzCAR123 -21 AA	145	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -21 scFv	204	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -21 VH	263	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -21 VL	322	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -22 NT	87	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGGTGCTGACCCAGTCGCCCGCAACCCTCTCTCTGTCGCCGGGAGAACGCGCCAC TCTTTCCTGTCGGGCGTCCAAGAGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCT GGTCAAGCCCCGCGGCTGCTGATCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCA GATTTTCCGGTTCGGGGTCGGGGACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGA GGACTTCGCCGTGTATTACTGCCAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGT ACTAAGGTCGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGG AGAATCCCTGAGGATCAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAAT TGGGTCCGCCAGATGCCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACT CGGAAACCCATTACAACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCAT TTCCACTGCGTACCTCCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGC GCACGCGGAAACTGGGATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag

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hzCAR123 -22 AA	146	MALPVTALLLPLALLLHAARPEVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -22 scFv	205	MALPVTALLLPLALLLHAARPEVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -22 VH	264	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -22 VL	323	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -23 NT	88	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTCGTGATGACCCAGTCACCGGCATTCTGTCCGTGACTCCCGGAGAAAAGGTCAC GATTACTTGCCGGGCGTCCAAGAGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCG GACCAGGCCCCCTAAGCTGTTGATCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGC GGTTTAGCGGTTTCGGGTTCTGGGACCGACTTCACTTTCACCATCTCCTCACTGGAAGCCGA GGATGCCGCCACTTACTACTGTGTCAGCAGCACACAAGTATCCGTACACCTTCGGAGGCGGT ACCAAAGTGGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGG AGAATCCCTGAGGATCAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAAT TGGGTCCGCCAGATGCCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACT CGGAAACCCATTACAACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCAT TTCCACTGCGTACCTCCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGC GCACGCGGAAACTGGGATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagegcggctcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg

hzCAR123 -23 AA	147	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -23 scFv	206	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -23 VH	265	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSISTAYLQWSSLKASDTAMYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -23 VL	324	DVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -24 NT	89	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGGTCATGACTCAGTCCCCGGACTCACTCGCGGTGTCGCTTGGAGAGAGAGCGAC CATCAACTGTTCGGGCCTCAAAGAGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCG GGACAGCCGCCAAAGCTGCTGATCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACC GGTTCTCCGGTTCGGGTTCGGGTACCGACTTCACGCTCACTATTTTCGTCGCTGCAAGCCGA AGATGTGGCCGTGTACTATTGCCAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGC ACCAAGGTGGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTCAAGAAGCCTGG AGAATCCCTGAGGATCAGCTGCAAAGGCAGCGGGTATACCTTCACCTCCTACTGGATGAAT TGGGTCCGCCAGATGCCCGGAAAAGGCCTGGAGTGGATGGGACGGATTGACCCCTACGACT CGGAAACCCATTACAACCAGAAGTTCAAGGATCACGTGACCATCTCCGTGGACAAGTCCAT TTCCACTGCGTACCTCCAGTGGTCAAGCCTGAAGGCCTCCGACACTGCTATGTACTACTGC GCACGCGGAAACTGGGATGATTACTGGGGACAGGGAACAACCGTGACTGTGTCTCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggcccctctggtggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -24 AA	148	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC

		SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -24 scFv	207	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTLISSSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSIISTAYLQWSSLKASDTA MYYCARGNWDDYWGGQTTVTVSS
hzCAR123 -24 VH	266	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQMPGKGLEWMGRIDPYDSETHYN QKFKDHVTISVDKSIISTAYLQWSSLKASDTAMYYCARGNWDDYWGGQTTVTVSS
hzCAR123 -24 VL	325	DVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLTLISSSLQAEDVAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -25 NT	90	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGGAGGAAGCCTGAGGCT GTCCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAACTGGGTCAGACAGGCA CCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACTCCGAAACCCATTACA ATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAAGAGCACCGCGTACCT CCAAATGAACTCCCTGCGCGCTGAGGATAACAGCAGTGTACTATTGCGCCCGGGGAAACTGG GATGATTACTGGGGCCAGGGAAGTACTGTGACTGTGTGCATCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGCAGCTCACCCAGTC GCCCTCATTTCTGTGCGCCTCAGTGGGAGACAGAGTGACCATTACTTGTGCGGGCCTCCAAG AGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCAGGAAAGGCGCCTAAGTTGCTCA TCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCCGGTTCTCCGGTTCGGGAAGCGG TACCGAATTACCCTTACTATCTCCTCCCTGCAACCGGAGGACTTCGCCACCTACTACTGC CAACAGCACACAAGTACCCGTACACTTTCGGGGGTGGCACGAAGGTTCGAAATCAAGACCA CTACCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -25 AA	149	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITICR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTLISSSLQPEDFA TYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -25	208	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITICR

scFv		ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTLISSLQPEDFA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -25 VH	267	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -25 VL	326	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTLISSLQPEDFATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -26 NT	91	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGGAGGAAGCCTGAGGCT GTCCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAACTGGGTCAGACAGGCA CCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACTCCGAAACCCATTACA ATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAAGAGCACCGCGTACCT CCAAATGAACTCCCTGCGCGCTGAGGATACAGCAGTGTACTATTGCGCCCCGGGGAAACTGG GATGATTACTGGGGCCAGGGAAGTACTGTGACTGTGTGCATCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGAAGTGGTGCTGACCCAGTC GCCC GCAACCTCTCTCTGTGCGCCGGGAGAACGCGCCACTCTTTCCTGTGCGGGCGTCCAAG AGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCTGGTCAAGCCCCGCGGCTGCTGA TCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCAGATTTTCCGGTTCGGGGTCGGG GACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGAGGACTTCGCCGTGTATTACTGC CAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGTACTAAGGTGCAGATCAAGACCA CTACCC CAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctgggtggggccgtgcatacccggggtccttgacttc gcctgcgatatctacatttgggccccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttgggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -26 AA	150	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTLISSLEPEDFA VYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -26 scFv	209	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTLISSLEPEDFA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -26 VH	268	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123	327	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR

-26 VL		FSGSGSGTDFTLTISSLEPEDFAVYYCQQH NKYPYTFGGGTKVEIK
hzCAR123 -27 NT	92	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGGAGGAAGCCTGAGGCT GTCCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAACTGGGTCAGACAGGCA CCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACTCCGAAACCCATTACA ATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAAGAGCACCGCGTACCT CCAAATGAACTCCCTGCGCGCTGAGGATAACAGCAGTGTACTATTGCGCCCGGGGAAACTGG GATGATTACTGGGGCCAGGGA ACTACTGTGACTGTGTGCATCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTCGTGATGACCCAGTC ACCGGCATTCTGTCCGTGACTCCCGGAGAAAAGGTCACGATTACTTGCCGGGCGTCCAAG AGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCGGACCAGGCCCCCTAAGCTGTTGA TCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGCGGTTTACGCGTTTCGGGTTCTGG GACCGACTTCACTTTTACCATCTCCTCACTGGAAGCCGAGGATGCCGCCACTTACTACTGT CAGCAGCACAAACAAGTATCCGTACACCTTCGGAGGCGGTACCAAAGTGGAGATCAAGACCA CTACCCACAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagacccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -27 AA	151	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVM TQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTF TISSLEAEDAA TYYCQQH NKYPYTFGGGTKVEIKTTTTAPRPTPTAPT IASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -27 scFv	210	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVM TQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTF TISSLEAEDAA TYYCQQH NKYPYTFGGGTKVEIK
hzCAR123 -27 VH	269	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTTVTVSS
hzCAR123 -27 VL	328	DVVM TQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTF TISSLEAEDAATYYCQQH NKYPYTFGGGTKVEIK
hzCAR123 -28 NT	93	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGGAGGAAGCCTGAGGCT GTCCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAACTGGGTCAGACAGGCA CCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACTCCGAAACCCATTACA ATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAAGAGCACCGCGTACCT

		CCAAATGAACTCCCTGCGCGCTGAGGATACAGCAGTGTACTATTGCGCCCCGGGGAAACTGG GATGATTACTGGGGCCAGGGAACTACTGTGACTGTGTTCATCCGGGGGTGGCGGTAGCGGAG GAGGGGGCTCCGGCGGCGGCGGCTCAGGGGGCGGAGGAAGCGACGTGGTCATGACTCAGTC CCCGGACTCACTCGCGGTGTGCTTGGAGAGAGAGCGACCATCAACTGTCGGGCCTCAAAG AGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCGGGACAGCCGCCAAAGCTGCTGA TCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACCGGTTCTCCGGTTCCGGGTCGGG TACCGACTTCACGCTCACTATTTTCGTGCTGCAAGCCGAAGATGTGGCCGTGTACTATTGC CAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGGCACCAAGGTGGAAATCAAGACCA CTACCCAGCACCAGGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -28 AA	152	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -28 scFv	211	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGGQTTVTVSSGGGSGGGGSGGGGSGGGGSDVVMQTQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISLQAEDVA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -28 VH	270	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGGQTTVTVSS
hzCAR123 -28 VL	329	DVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLTISLQAEDVAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -29 NT	94	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGCAGCTCACCCAGTCGCCCTCATTTCTGTGCGGCCTCAGTGGGAGACAGAGTGAC CATTACTTGTGCGGCCTCCAAGAGCATCTCCAAGGACCTGGCCTGGTATCAGCAGAAGCCA GGAAAGGCGCCTAAGTTGCTCATCTACTCGGGGTCGACCCTGCAATCTGGCGTGCCGTCCC GGTTCTCCGGTTCGGGAAGCGGTACCGAATTCACCCTTACTATCTCCTCCCTGCAACCGGA GGACTTCGCCACCTACTACTGCCAACAGCACACAAGTACCCGTACACTTTCGGGGGTGGC ACGAAGGTGCAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGG AGGAAGCCTGAGGCTGTCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAAC TGGGTGAGACAGGCACCTGGAAAGGGACTGGTCTGGGTGTGCGGCATTGACCCCTACGACT CCGAAACCCATTACAATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAA

		GAGCACCGCGTACCTCCAAATGAACTCCCTGCGCGCTGAGGATACAGCAGTGTACTATTGC GCCCCGGGGAAACTGGGATGATTACTGGGGCCAGGGAACTACTGTGACTGTGTTCATCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggtgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -29 AA	153	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -29 scFv	212	MALPVTALLLPLALLLHAARPDVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -29 VH	271	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -29 VL	330	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSR FSGSGSGTEFTLTISLQPEDFATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -30 NT	95	ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGAAGTGGTGCTGACCCAGTCGCCCGCAACCCTCTCTCTGTGCGCCGGGAGAACGCGCCAC TCTTTCCTGTGCGGGCGTCCAAGAGCATCTCAAAGGACCTCGCCTGGTACCAGCAGAAGCCT GGTCAAGCCCCGCGGCTGCTGATCTACTCCGGCTCCACGCTGCAATCAGGAATCCCAGCCA GATTTTCCGGTTCGGGGTCGGGGACTGACTTCACCTTGACCATTAGCTCGCTGGAACCTGA GGACTTCGCCGTGTATTACTGCCAGCAGCACACAAGTACCCGTACACCTTCGGAGGCGGT ACTAAGGTCGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGCGGCT CAGGGGGCGGAGGAAGCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGG AGGAAGCCTGAGGCTGTCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAAC TGGGTCAGACAGGCACCTGGAAAGGGACTGGTCTGGGTGTGCGGCATTGACCCCTACGACT CCGAAACCCATTACAATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAA GAGCACCGCGTACCTCCAAATGAACTCCCTGCGCGCTGAGGATACAGCAGTGTACTATTGC GCCCCGGGGAAACTGGGATGATTACTGGGGCCAGGGAACTACTGTGACTGTGTTCATCCACCA CTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc

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hzCAR123 -30 AA	154	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPENG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -30 scFv	213	MALPVTALLLPLALLLHAARPEVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -30 VH	272	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -30 VL	331	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -31 NT	96	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTCGTGATGACCCAGTCACCGGCATTCTGTCCGTGACTCCCGGAGAAAAGGTCAC GATTACTTGCCGGGCGTCCAAGAGCATCTCCAAGGACCTCGCCTGGTACCAACAGAAGCCG GACCAGGCCCCTAAGCTGTTGATCTACTCGGGGTCCACCCTTCAATCGGGAGTGCCATCGC GGTTTAGCGGTTTCGGGTTCTGGGACCGACTTCACCTTTCACCATCTCCTCACTGGAAGCCGA GGATGCCGCCACTTACTACTGTCAGCAGCACACAAGTATCCGTACACCTTCGGAGGCGGT ACCAAAGTGGAGATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGGCGGT CAGGGGGCGGAGGAAGCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGG AGGAAGCCTGAGGCTGTCCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAAC TGGGTCAGACAGGCACCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACT CCGAAACCCATTACAATCAGAAATTCAAGGACCGCTTCACCATCTCCGTGGACAAAGCCAA GAGCACCGCGTACCTCCAAATGAACTCCCTGCGCGCTGAGGATAACAGCAGTGTACTATTGC GCCCCGGGAAACTGGGATGATTACTGGGGCCAGGGAAGTACTGTGACTGTGTGCATCCACCA CTACCCACGACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcataaccgggggtcttgacttc gcctgcgatatctacatcttgggccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag

		caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -31 AA	155	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -31 scFv	214	MALPVTALLLPLALLLHAARPDVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -31 VH	273	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -31 VL	332	DVVMQTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSR FSGSGSGTDFTFTTISSLEAEDAATYYCQQHNKYPYTFGGGTKVEIK
hzCAR123 -32 NT	97	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGC CCGACGTGGTCATGACTCAGTCCCCGGACTCACTCGCGGTGTCGCTTGGAGAGAGAGCGAC CATCAACTGTCGGGCCTCAAAGAGCATCAGCAAGGACCTGGCCTGGTACCAGCAGAAGCCG GGACAGCCGCCAAAGCTGCTGATCTACTCCGGGTCCACCTTGCAATCTGGTGTCCCTGACC GGTTCTCCGGTTCGGGTTCGGGTACCGACTTCACGCTCACTATTTTCGTCGCTGCAAGCCGA AGATGTGGCCGTGTACTATTGCCAACAGCACACAAGTACCCCTACACTTTTGGCGGAGGC ACCAAGGTGGAAATCAAGGGGGGTGGCGGTAGCGGAGGAGGGGGCTCCGGCGGCGGGCGGCT CAGGGGGCGGAGGAAGCGAAGTGCAGCTCGTCGAGAGCGGAGGGGGACTGGTGCAGCCCGG AGGAAGCCTGAGGCTGTCTGCGCTGCCTCCGGCTACACCTTCACCTCCTACTGGATGAAC TGGGTGAGACAGGCACCTGGAAAGGGACTGGTCTGGGTGTCGCGCATTGACCCCTACGACT CCGAAACCCATTACAATCAGAAATTCAAGGACCGCTTACCATCTCCGTGGACAAAGCCAA GAGCACCGCGTACCTCCAAATGAACTCCCTGCGCGCTGAGGATACAGCAGTGTACTATTGC GCCCCGGGAAACTGGGATGATTACTGGGGCCAGGGAAGTACTGTGACTGTGTGCATCCACCA CTACCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGgcatgtagaccgcagctggtggggccgtgcatacccggggtcttgacttc gcctgcgatatctacatttgggccctctggctggtacttgcggggtcctgctgctttcac tcgtgatcactctttactgtaagcgcggtcggagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcct acaagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaat ccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgaga ttggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcag caccgccaccaaggacacctatgacgctcttcacatgcaggccctgccgcctcgg
hzCAR123 -32 AA	156	MALPVTALLLPLALLLHAARPDVVMQTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTLISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT

		RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
hzCAR123 -32 scFv	215	MALPVTALLLPLALLLHAARPDVVMQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTITSSSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -32 VH	274	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQAPGKGLVWVSRIDPYDSETHYN QKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARGNWDDYWGQGTITVTVSS
hzCAR123 -32 VL	333	DVVMQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDR FSGSGSGTDFTLTITSSSLQAEDVAVYYCQQHNKYPYTFGGGTKVEIK

In embodiments, a CAR molecule described herein comprises a scFv that specifically binds to CD123, and does not contain a leader sequence, e.g., the amino acid sequence SEQ ID NO: 1. Table 9 below provides amino acid and nucleotide sequences for CD123 scFv sequences that do not contain a leader sequence SEQ ID NO: 1.

Table 9. CD123 CAR scFv sequences

Name	SEQ ID	Sequence
CAR123-2 scFv - NT	479	CAAGTGCAACTCGTCCAAAGCGGAGCGGAAGTCAAGAAACCCGGAGCGAGCGTGAAAGTG TCCTGCAAAGCCTCCGGCTACACCTTTACGGGCTACTACATGCACTGGGTGCGCCAGGCA CCAGGACAGGGTCTTGAATGGATGGGATGGATCAACCCTAATTCGGGCGGAACTAACTAC GCACAGAAGTTCCAGGGGAGAGTGACTCTGACTCGGGATACCTCCATCTCAACTGTCTAC ATGGAAGTCTCCCGCTTGCGGTCAGATGATACGGCAGTGTACTACTGCGCCCGCGACATG AATATCCTGGCTACCGTGCCGTTTCGACATCTGGGGACAGGGGACTATGGTTACTGTCTCA TCGGGCGGTGGAGGTTTCAGGAGGAGGCGGCTCGGGAGGCGGAGGTTTCGGACATTTCAGATG ACCCAGTCCCCATCCTCTCTGTCGGCCAGCGTCGGAGATAGGGTGACCATTACCTGTTCGG GCCTCGCAAAGCATCTCCTCGTACCTCAACTGGTATCAGCAAAAGCCGGGAAAGGCGCCT AAGCTGCTGATCTACGCCGCTTCGAGCTTGCAAAGCGGGGTGCCATCCAGATTCTCGGGA TCAGGCTCAGGAACCGACTTCACCCTGACCGTGAACAGCCTCCAGCCGGAGGACTTTGCC ACTTACTACTGCCAGCAGGGAGACTCCGTGCCGCTTACTTTTCGGGGGGGGTACCCGCCTG GAGATCAAG
CAR123-2 scFv - AA	480	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVRQAPGQGLEWMGWINPNSGGTNY AQKFQGRVTLTRDTSISTVYMELSRLRSDDTAVYYCARDMNILATVPFDIWGQGTMTVS SGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTVNSLQPEDFATYYCQQGDSVPLTFGGGTRL EIK
CAR123-2 ORF-free NT	481	atggccctccctgtcaccgccctgtgcttccgtggctcttctgtctccacgccgctcgccccaagtgaactcgtccaaagcg gagcggaagtcaagaaacccggagcgagcgtgaaagtgtcctgcaaagcctccggctacacctttacgggctactacatgcact gggtgcgccaggcaccaggacagggtcttgaatggatgggatggatcaaccctaattcgggcggaactaactacgcacagaag ttcaggggagagtgactctgactcgggatactccatctcaactgtctacatggaactctcccgttgcggtcagatgatacggc

		agtggtactactgcgccccgcacatgaatactctggctaccgtgccgttcgacatctggggacaggggactatgggttactgtctcat cgggcgggtggagggtcaggaggaggcggctcgggaggcggagggttcggacattcagatgacccagtcctccatcctctctgtcg gccagcgtcggagatagggtagaccattacctgtcgggcctcgcaaagcatctcctcgtacctcaactgggtatcagcaaaagccg ggaaaggcgcctaagctgctgatctacgccgcttcgagcttgcaaagcgggggtgccatccagattctcgggatcaggctcagga accgacttcaccctgaccgtgaacagcctccagccggaggactttgccacttactactgccagcagggagactccgtgccgctta ctttcgggggggggtaccgcctggagatcaagaccactacccagcaccgaggccaccaccccggtcctaccatgcctcc cagcctctgtccctgcgtccggaggcatgtagaccgcagctgggtggggccgtgcatacccggggtcttgacttcgctgcgat atctacatttggggccctctggctggtaacttgcggggctcctgctgctttcactcgtgatcactctttactgtaagcgcggtcggaaga agctgctgtacatctttaagcaacccttcatgaggcctgtgcagactactcaagaggaggacggctgttcttgcgggttccagag gaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagacgctccagcctacaagcaggggacagaacca gctctacaacgaactcaatcttggtcggagagaggagtacgacgtgctggacaagcggagaggacgggaccagaaaatgggc gggaagccgcgcagaaagaatccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgag attggtatgaaaggggaacgcagaagaggcaaaggccacgacggactgtaccagggtactcagcaccgccaccaaggacacc tatgacgctcttcacatgcaggccctgccgcctcggttaagtcgacagctcgccttcttctgtgtccaatttctattaaaggttctttgttc cctaagtccaactactaaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaaacatttattttcatttgcctgc gtcgagagctcgccttcttctgtgtccaatttctattaaaggttctttgttcctaagtccaactactaaactgggggatattatgaagg gccttgagcatctggattctgcctaataaaaaaacatttattttcatttgcctgcacgaattc
CAR123-3 scFv - NT	482	CAAGTCCAAC TCGTTCAATCCGGCGCAGAAAGTCAAGAAGCCAGGAGCATCAGTGAAAGTG TCCTGCAAAGCCTCAGGCTACATCTTCACGGGATACTACATCCACTGGGTGCGCCAGGCT CCGGGCCAGGGCCTTGAGTGGATGGGCTGGATCAACCCTAACTCTGGGGGAACCAACTAC GCTCAGAAGTTCCAGGGGAGGGTCACTATGACTCGCGATACCTCCATCTCCACTGCGTAC ATGGAAC TCTCGGGACTGAGATCCGACGATCCTGCCGTGTACTACTGCGCCCGGGACATG AACATCTTGCGGACCGTGCCGTTTGACATTTGGGGACAGGGCACCCCTCGTCACTGTGTCTG AGCGGTGGAGGAGGCTCGGGGGGTGGCGGATCAGGAGGGGGAGGAAGCGACATCCAGCTG ACTCAGAGCCCATCGTCGTTGTCCGCGTCGGTGGGGGATAGAGTGACCATTACTTGCCGC GCCAGCCAGAGCATCTCATCATATCTGAATTGGTACCAGCAGAAGCCCGGAAAGGCCCCA AAACTGCTGATCTACGCTGCAAGCAGCCTCCAATCGGGAGTGCCGTCACGGTTCTCCGGG TCCGGTTCCGGAACTGACTTTACCCTGACCGTGAATTCGCTGCAACCGGAGGATTTCCGCC ACGTACTACTGTCAGCAAGGAGACTCCGTGCCGCTGACCTTCGGTGGAGGCACCAAGGTC GAAATCAAG
CAR123-3 scFv - AA	483	QVQLVQSGAEVKKPGASVKV SCKASGYIFTGYI IHWVRQAPGQGLEWMGWINPNSGGTNY AQKFQGRVTMTRDTSISTAYMELSLRSDDPAVYYCARDMNILATVPFDIWGQGT LVTVS SGGGSGGGSGGGGSDIQLTQSPSSL SASVGDRVTITCRASQSISSYLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLT VNSLQPEDFATYYCQQGDSVPLTFGGG TKV EIK
CAR123-4 scFv - NT	484	CAAGTCCAAC TCCAACAGTCAGGCGCAGAAAGTGAAAAAGAGCGGTGCATCGGTGAAAGTG TCATGCAAAGCCTCGGGCTACACCTTCACTGACTACTATATGCACTGGCTGCGGCAGGCA CCGGGACAGGGACTTGAGTGGATGGGATGGATCAACCCGAATTCAGGGGACACTAACTAC GCGCAGAAGTTCCAGGGGAGAGTGACCCTGACGAGGGACACCTCAATTTGACCGTCTAC ATGGAATTGTGCGCCTGAGATCGGACGATACTGCTGTGTACTACTGTGCCCGCGACATG AACATCCTCGCGACTGTGCCTTTTGATATCTGGGGACAGGGGACTATGGTCACCGTTTCC TCCGCTTCCGGTGGCGGAGGCTCGGGAGGCCGGGCCTCCGGTGGAGGAGGCAGCGACATC CAGATGACTCAGAGCCCTTCCTCGCTGAGCGCCTCAGTGGGAGATCGCGTGACCATCACT TGCCGGGCCAGCCAGTCCATTTCTGTCCTACCTCAATTGGTACCAGCAGAAGCCGGGAAAG GCGCCCAAGCTCTTGATCTACGCTGCGAGCTCCCTGCAAAGCGGGGTGCCGAGCCGATTCT TCGGGTTCCGGCTCGGGAACCGACTTCACTCTGACCATCTCATCCCTGCAACCAGAGGAC TTTGCCACCTACTACTGCCAACAAGGAGATTCTGTCCCACTGACGTTCCGGCGGAGGAACC AAGGTCGAAATCAAG
CAR123-4	485	QVQLQQSGAEVKKSGASVKV SCKASGYTF TDYYMHWLRQAPGQGLEWMGWINPNSGDTNY AQKFQGRVTLTRDTSISTVYMELSRLRSDDTAVYYCARDMNILATVPFDIWGQGT MVTVS

scFv - AA		SASGGGSGGGRASGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGDSVPLTFGGGT KVEIK
CAR123-1 scFv - AA	478	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTTRDTSISTAYMELSRLSDDTAVYYCARDMNILATVPFDIWGQGTMTVTS SGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIISTYLNWYQQKPGKAP NLLIYAASFSLQSGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCQQGDSVPLTFGGGT EIK
hzCAR123-1 scFv	556	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGT TVTVSSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVTITCRASKSISKDLAWYQQKPGKAPK LLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNKYPYTFGGGT KVEIK
hzCAR123-2 scFv	557	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGT TVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSEVVL TQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIS SLEPEDFAVYYCQQHNKYPYTFGGGT KVEIK
hzCAR123-3 scFv	558	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGT TVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDVVM TQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTIS SLEAEDAAVYYCQQHNKYPYTFGGGT KVEIK
hzCAR123-4 scFv	559	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSED TAVYYCARGNWDDYWGQGT TVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDVVM TQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIS SLQAEDVAVYYCQQHNKYPYTFGGGT KVEIK
hzCAR123-5 scFv	560	DVQLTQSPSFLSASVGDRVTITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGT TVTVSS
hzCAR123-6 scFv	561	EVVL TQSPATLSLSPGERATLSCRASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTIS SLEPEDFAVYYCQQHNKYPYTFGGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGT TVTVSS
hzCAR123-7 scFv	562	DVVM TQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTIS SLEAEDAATYYCQQHNKYPYTFGGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGT TVTVSS
hzCAR123-8 scFv	563	DVVM TQSPDSLAVSLGERATINCRASKSISKDLAWYQQKPGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTIS SLQAEDVAVYYCQQHNKYPYTFGGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRVTMTVDKSTSTAYMELSSLRSEDTA VYYCARGNWDDYWGQGT TVTVSS
hzCAR123-9	564	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG

scFv		NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITTCR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-10 scFv	565	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTSQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISLLEPEDFA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-11 scFv	566	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPAFLSVTPGEKVTITTCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-12 scFv	567	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYWMNWVRQ APGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQPPELLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-13 scFv	568	DVQLTQSPSFLSASVGDRVITTCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTTVTVSS
hzCAR123-14 scFv	569	EVVLTSQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISLLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTTVTVSS
hzCAR123-15 scFv	570	DVVMTQSPAFLSVTPGEKVTITTCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTTVTVSS
hzCAR123-16 scFv	571	DVVMTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGSELKKPGASVKVSCKASGYTFTSY WMNWVRQAPGQGLEWMGRIDPYDSETHYNQKFKDRFVFSVDKSVSTAYLQISSLKAEDTA VYYCARGNWDDYWGQGTTVTVSS
hzCAR123-17 scFv	572	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSIISTAYLQWSSLKASDTAMYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITTCR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-18 scFv	573	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSIISTAYLQWSSLKASDTAMYYCARG NWDDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEVVLTSQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISLLEPEDFA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-	574	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSIISTAYLQWSSLKASDTAMYYCARG

19 scFv		NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123- 20 scFv	575	EVQLVQSGAEVKKPGESLRISCKGSGYTFTSYWMNWVRQ MPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTAMYVCARG NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQP KLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123- 21 scFv	576	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYVCARGNWDDYWGQGTITVTIVSS
hzCAR123- 22 scFv	577	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYVCARGNWDDYWGQGTITVTIVSS
hzCAR123- 23 scFv	578	DVVMTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYVCARGNWDDYWGQGTITVTIVSS
hzCAR123- 24 scFv	579	DVVMTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGESLRISCKGSGYTFTSY WMNWVRQMPGKGLEWMGRIDPYDSETHYNQKFKDHVTISVDKSISTAYLQWSSLKASDTA MYVCARGNWDDYWGQGTITVTIVSS
hzCAR123- 25 scFv	580	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSDVQLTQSPSFLSASVGDRVITITCR ASKSISKDLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123- 26 scFv	581	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSEVVLTQSPATLSLSPGERATLSCR ASKSISKDLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123- 27 scFv	582	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPAFLSVTPGEKVTITCR ASKSISKDLAWYQQKPDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAA TYYCQQHNKYPYTFGGGTKVEIK
hzCAR123- 28 scFv	583	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWMNWVRQ APGKGLVWVSRIDPYDSETHYNQKFKDRFTISVDKAKSTAYLQMNSLRAEDTAVYYCARG NWDDYWGQGTITVTIVSSGGGGSGGGGSGGGGSGGGGSDVVMTQSPDSLAVSLGERATINCR ASKSISKDLAWYQQKPGQP KLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQHNKYPYTFGGGTKVEIK
hzCAR123-	584	DVQLTQSPSFLSASVGDRVITITCRASKSISKDLAWYQQK PGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQHNKYPYTFG

29 scFv		GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTTTVTVSS
hzCAR123- 30 scFv	585	EVVLTQSPATLSLSPGERATLSCRASKSISKDLAWYQQK PGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTTTVTVSS
hzCAR123- 31 scFv	586	DVVMTQSPAFLSVTPGEKVTITCRASKSISKDLAWYQQK PDQAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTTTVTVSS
hzCAR123- 32 scFv	587	DVVMTQSPDSLAVSLGERATINCRASKSISKDLAWYQQK PGQPPKLLIYSGSTLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHNKYPYTFG GGTKVEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSY WMNWVRQAPGKGLVWVSRIDPYDSETHYNQFKDRFTISVDKAKSTAYLQMNSLRAEDTA VYYCARGNWDDYWGQGTTTVTVSS

In embodiments, the CAR scFv fragments were then cloned into lentiviral vectors to create a full length CAR construct in a single coding frame, and using the EF1 alpha promoter for expression (SEQ ID NO: 11).

5 EF1 alpha promoter

CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGG
CAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTT
CCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGAACGGGTTTGCCGCCA
GAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAAT
10 TACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT
TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCT
GGTGGCACCTTCGCGCCTGTCTCGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGAC
GCTTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTTGGGGCCGCG
GGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTTCGGCGAGGCGGGGCTGCGAGCGCGGCCACCGAGAATCG
15 GACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC
GGCAAGGCTGGCCCGGTCTGGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA
AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAG
CCGTCGCTTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTAC
GTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGG
20 CCAGCTTGGCACTTGATGTAATTCTCCTTGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCAATTCTCAAGCCTC
AGACAGTGGTTCAAAGTTTTTTTTCTTCCATTTTCAGGTGTCGTGA

Gly/Ser (SEQ ID NO:25)

GGGGS

Gly/Ser (SEQ ID NO:26): This sequence may encompass 1-6 "Gly Gly Gly Gly Ser" repeating units

GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS

5 **Gly/Ser (SEQ ID NO:27)**

GGGGSGGGGS GGGGSGGGGS

Gly/Ser (SEQ ID NO:28)

GGGGSGGGGS GGGGS

10

Gly/Ser (SEQ ID NO:29)

GGGS

PolyA: (A)₅₀₀₀ (SEQ ID NO:30)

15 This sequence may encompass 50-5000 adenines.

PolyA: (T)₁₀₀ (SEQ ID NO:31)

PolyA: (T)₅₀₀₀ (SEQ ID NO:32)

This sequence may encompass 50-5000 thymines.

20 **PolyA: (A)₅₀₀₀ (SEQ ID NO:33)**

This sequence may encompass 100-5000 adenines.

PolyA: (A)₄₀₀ (SEQ ID NO:34)

This sequence may encompass 100-400 adenines.

PolyA: (A)₂₀₀₀ (SEQ ID NO:35)

25 This sequence may encompass 50-2000 adenines.

Gly/Ser (SEQ ID NO:709): This sequence may encompass 1-10 "Gly Gly Gly Ser" repeating units

GGGSGGGSGG GSGGGSGGGGS GGGSGGGSGG GSGGGSGGGGS

The CAR scFv fragments can be cloned into lentiviral vectors to create a full length CAR construct in a single coding frame, and using the EF1 alpha promoter for expression (SEQ ID NO: 11).

5 The CAR construct can include a Gly/Ser linker having one or more of the following sequences: GGGGS (SEQ ID NO:25); encompassing 1-6 "Gly Gly Gly Gly Ser" repeating units, e.g., GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS (SEQ ID NO:26); GGGGSGGGGS GGGGSGGGGS (SEQ ID NO:27); GGGGSGGGGS GGGGS (SEQ ID NO:28); GGGGS (SEQ ID NO:29); or encompassing 1-10 "Gly Gly Gly Ser" repeating units,
 10 e.g., GGGSGGGSGG GSGGGSGGGGS GGGSGGGSGG GSGGGSGGGGS (SEQ ID NO:709). In embodiments, the CAR construct include a poly A sequence, e.g., a sequence encompassing 50-5000 or 100-5000 adenines (e.g., SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:34 or SEQ ID NO:35), or a sequence encompassing 50-5000 thymines (e.g., SEQ ID NO:31, SEQ ID NO:32). Alternatively, the CAR construct can include, for example, a linker including the
 15 sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 704)

Bispecific CARs

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody
 20 molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second
 25 epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, e.g., different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which
 30 have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half

antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv, or
5 fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope.

In certain embodiments, the antibody molecule is a multi-specific (*e.g.*, a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules are known in the art; including but not limited to, for example, the “knob in a hole”
10 approach described in, *e.g.*, US 5731168; the electrostatic steering Fc pairing as described in, *e.g.*, WO 09/089004, WO 06/106905 and WO 2010/129304; Strand Exchange Engineered Domains (SEED) heterodimer formation as described in, *e.g.*, WO 07/110205; Fab arm exchange as described in, *e.g.*, WO 08/119353, WO 2011/131746, and WO 2013/060867; double antibody conjugate, *e.g.*, by antibody cross-linking to generate a bi-specific structure
15 using a heterobifunctional reagent having an amine-reactive group and a sulfhydryl reactive group as described in, *e.g.*, US 4433059; bispecific antibody determinants generated by recombining half antibodies (heavy-light chain pairs or Fabs) from different antibodies through cycle of reduction and oxidation of disulfide bonds between the two heavy chains, as described in, *e.g.*, US 4444878; trifunctional antibodies, *e.g.*, three Fab' fragments cross-linked through
20 sulfhydryl reactive groups, as described in, *e.g.*, US5273743; biosynthetic binding proteins, *e.g.*, pair of scFvs cross-linked through C-terminal tails preferably through disulfide or amine-reactive chemical cross-linking, as described in, *e.g.*, US5534254; bifunctional antibodies, *e.g.*, Fab fragments with different binding specificities dimerized through leucine zippers (*e.g.*, c-fos and c-jun) that have replaced the constant domain, as described in, *e.g.*, US5582996; bispecific
25 and oligospecific mono-and oligovalent receptors, *e.g.*, VH-CH1 regions of two antibodies (two Fab fragments) linked through a polypeptide spacer between the CH1 region of one antibody and the VH region of the other antibody typically with associated light chains, as described in, *e.g.*, US5591828; bispecific DNA-antibody conjugates, *e.g.*, crosslinking of antibodies or Fab fragments through a double stranded piece of DNA, as described in, *e.g.*, US5635602;
30 bispecific fusion proteins, *e.g.*, an expression construct containing two scFvs with a hydrophilic helical peptide linker between them and a full constant region, as described in, *e.g.*, US5637481; multivalent and multispecific binding proteins, *e.g.*, dimer of polypeptides having

first domain with binding region of Ig heavy chain variable region, and second domain with binding region of Ig light chain variable region, generally termed diabodies (higher order structures are also encompassed creating for bispecific, trispecific, or tetraspecific molecules, as described in, *e.g.*, US5837242; minibody constructs with linked VL and VH chains further

5 connected with peptide spacers to an antibody hinge region and CH3 region, which can be dimerized to form bispecific/multivalent molecules, as described in, *e.g.*, US5837821; VH and VL domains linked with a short peptide linker (*e.g.*, 5 or 10 amino acids) or no linker at all in either orientation, which can form dimers to form bispecific diabodies; trimers and tetramers, as described in, *e.g.*, US5844094; String of VH domains (or VL domains in family members)

10 connected by peptide linkages with crosslinkable groups at the C-terminus further associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with both a VH and a VL domain linked through a peptide linker are combined into multivalent structures through non-covalent or chemical crosslinking to form, *e.g.*, homobivalent, heterobivalent, trivalent, and tetravalent structures using both scFV

15 or diabody type format, as described in, *e.g.*, US5869620. Additional exemplary multispecific and bispecific molecules and methods of making the same are found, for example, in US5910573, US5932448, US5959083, US5989830, US6005079, US6239259, US6294353, US6333396, US6476198, US6511663, US6670453, US6743896, US6809185, US6833441, US7129330, US7183076, US7521056, US7527787, US7534866, US7612181,

20 US2002004587A1, US2002076406A1, US2002103345A1, US2003207346A1, US2003211078A1, US2004219643A1, US2004220388A1, US2004242847A1, US2005003403A1, US2005004352A1, US2005069552A1, US2005079170A1, US2005100543A1, US2005136049A1, US2005136051A1, US2005163782A1, US2005266425A1, US2006083747A1, US2006120960A1, US2006204493A1,

25 US2006263367A1, US2007004909A1, US2007087381A1, US2007128150A1, US2007141049A1, US2007154901A1, US2007274985A1, US2008050370A1, US2008069820A1, US2008152645A1, US2008171855A1, US2008241884A1, US2008254512A1, US2008260738A1, US2009130106A1, US2009148905A1, US2009155275A1, US2009162359A1, US2009162360A1, US2009175851A1,

30 US2009175867A1, US2009232811A1, US2009234105A1, US2009263392A1, US2009274649A1, EP346087A2, WO0006605A2, WO02072635A2, WO04081051A1, WO06020258A2, WO2007044887A2, WO2007095338A2, WO2007137760A2,

WO2008119353A1, WO2009021754A2, WO2009068630A1, WO9103493A1, WO9323537A1, WO9409131A1, WO9412625A2, WO9509917A1, WO9637621A2, WO9964460A1. The contents of the above-referenced applications are incorporated herein by reference in their entireties.

5 Within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH₁) upstream of its VL (VL₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL₂) upstream of its VH (VH₂), such that the overall bispecific antibody molecule has
10 the arrangement VH₁-VL₁-VL₂-VH₂. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL₁) upstream of its VH (VH₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH₂) upstream of its VL (VL₂), such that the overall bispecific antibody molecule has the arrangement VL₁-VH₁-VH₂-VL₂. Optionally, a linker is disposed between the two antibodies or antibody
15 fragments (e.g., scFvs), e.g., between VL₁ and VL₂ if the construct is arranged as VH₁-VL₁-VL₂-VH₂, or between VH₁ and VH₂ if the construct is arranged as VL₁-VH₁-VH₂-VL₂. The linker may be a linker as described herein, e.g., a (Gly₄-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 64). In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. Optionally, a linker is
20 disposed between the VL and VH of the first scFv. Optionally, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers can be the same or different. Accordingly, in some embodiments, a bispecific CAR comprises VLs, VHs, and optionally one or more linkers in an arrangement as described herein.

 In one aspect, the bispecific antibody molecule is characterized by a first
25 immunoglobulin variable domain sequence, e.g., a scFv, which has binding specificity for CD123, e.g., comprises a scFv as described herein, e.g., as described in Table 2, Table 6, or Table 9, or comprises the light chain CDRs and/or heavy chain CDRs from a CD123 scFv described herein, and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope on a different antigen. In some aspects the second
30 immunoglobulin variable domain sequence has binding specificity for an antigen expressed on AML cells, e.g., an antigen other than CD123. For example, the second immunoglobulin

variable domain sequence has binding specificity for CLL-1. As another example, the second immunoglobulin variable domain sequence has binding specificity for CD33. As another example, the second immunoglobulin variable domain sequence has binding specificity for CD34. As another example, the second immunoglobulin variable domain sequence has binding specificity for FLT3. For example, the second immunoglobulin variable domain sequence has binding specificity for folate receptor beta. In some aspects, the second immunoglobulin variable domain sequence has binding specificity for an antigen expressed on B-cells, for example, CD19, CD20, CD22 or ROR1.

10 *Chimeric TCR*

In one aspect, the CD123 antibodies and antibody fragments of the present invention (for example, those disclosed in Tables 2,6 or 9) can be grafted to one or more constant domain of a T cell receptor (“TCR”) chain, for example, a TCR alpha or TCR beta chain, to create an chimeric TCR that binds specificity to CD123. Without being bound by theory, it is believed that chimeric TCRs will signal through the TCR complex upon antigen binding. For example, a CD123 scFv as disclosed herein, can be grafted to the constant domain, e.g., at least a portion of the extracellular constant domain, the transmembrane domain and the cytoplasmic domain, of a TCR chain, for example, the TCR alpha chain and/or the TCR beta chain. As another example, a CD123 antibody fragment, for example a VL domain as described herein, can be grafted to the constant domain of a TCR alpha chain, and a CD123 antibody fragment, for example a VH domain as described herein, can be grafted to the constant domain of a TCR beta chain (or alternatively, a VL domain may be grafted to the constant domain of the TCR beta chain and a VH domain may be grafted to a TCR alpha chain). As another example, the CDRs of a CD123 antibody or antibody fragment, e.g., the CDRs of a CD123 antibody or antibody fragment as described in Tables 3, 4, 5, 6, 7, 8, 10, 11, 12 or 13 may be grafted into a TCR alpha and/or beta chain to create a chimeric TCR that binds specifically to CD123. For example, the LCDRs disclosed herein may be grafted into the variable domain of a TCR alpha chain and the HCDRs disclosed herein may be grafted to the variable domain of a TCR beta chain, or vice versa. Such chimeric TCRs may be produced by methods known in the art (For example, Willemsen RA et al, Gene Therapy 2000; 7: 1369–1377; Zhang T et al, Cancer Gene Ther 2004; 11: 487–496; Aggen et al, Gene Ther. 2012 Apr;19(4):365-74).

Stability and Mutations

The stability of a CD123 binding domain, e.g., scFv molecules (e.g., soluble scFv) can be evaluated in reference to the biophysical properties (e.g., thermal stability) of a conventional control scFv molecule or a full length antibody. In one embodiment, the human scFv has a thermal stability that is greater than about 0.1, about 0.25, about 0.5, about 0.75, about 1, about 1.25, about 1.5, about 1.75, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10 degrees, about 11 degrees, about 12 degrees, about 13 degrees, about 14 degrees, or about 15 degrees Celsius than a control binding molecule (e.g. a conventional scFv molecule) in the described assays.

The improved thermal stability of the CD123 binding domain, e.g., scFv is subsequently conferred to the entire CART123 construct, leading to improved therapeutic properties of the CART123 construct. The thermal stability of the CD123 binding domain, e.g., scFv can be improved by at least about 2°C or 3°C as compared to a conventional antibody. In one embodiment, the CD123 binding domain, e.g., scFv has a 1°C improved thermal stability as compared to a conventional antibody. In another embodiment, the CD123 binding domain, e.g., scFv has a 2°C improved thermal stability as compared to a conventional antibody. In another embodiment, the scFv has a 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15°C improved thermal stability as compared to a conventional antibody. Comparisons can be made, for example, between the scFv molecules disclosed herein and full length antibodies. Thermal stability can be measured using methods known in the art. For example, in one embodiment, T_m can be measured. Methods for measuring T_m and other methods of determining protein stability are described in more detail below.

Mutations in scFv alter the stability of the scFv and improve the overall stability of the scFv and the CART123 construct. Stability of the humanized or human scFv is determined using measurements such as T_m, temperature denaturation and temperature aggregation.

The binding capacity of the mutant scFvs can be determined using assays described in the Examples.

In one embodiment, the CD123 binding domain, e.g., scFv comprises at least one mutation such that the mutated scFv confers improved stability to the CART123 construct. In another embodiment, the CD123 binding domain, e.g., scFv comprises at least 1, 2, 3, 4, 5, 6, 7,

8, 9, 10 mutations arising from the humanization process such that the mutated scFv confers improved stability to the CART123 construct.

Methods of Evaluating Protein Stability

5 The stability of an antigen binding domain may be assessed using, e.g., the methods described below. Such methods allow for the determination of multiple thermal unfolding transitions where the least stable domain either unfolds first or limits the overall stability threshold of a multidomain unit that unfolds cooperatively (e.g., a multidomain protein which exhibits a single unfolding transition). The least stable domain can be identified in a number of
10 additional ways. Mutagenesis can be performed to probe which domain limits the overall stability. Additionally, protease resistance of a multidomain protein can be performed under conditions where the least stable domain is known to be intrinsically unfolded via DSC or other spectroscopic methods (Fontana, *et al.*, (1997) *Fold. Des.*, 2: R17-26; Dimasi *et al.* (2009) *J. Mol. Biol.* 393: 672-692). Once the least stable domain is identified, the sequence encoding this
15 domain (or a portion thereof) may be employed as a test sequence in the methods.

a) Thermal Stability

The thermal stability of the compositions may be analyzed using a number of non-limiting biophysical or biochemical techniques known in the art. In certain embodiments, thermal stability is evaluated by analytical spectroscopy.

20 An exemplary analytical spectroscopy method is Differential Scanning Calorimetry (DSC). DSC employs a calorimeter which is sensitive to the heat absorbances that accompany the unfolding of most proteins or protein domains (see, e.g. Sanchez-Ruiz, *et al.*, *Biochemistry*, 27: 1648-52, 1988). To determine the thermal stability of a protein, a sample of the protein is inserted into the calorimeter and the temperature is raised until the Fab or scFv unfolds. The
25 temperature at which the protein unfolds is indicative of overall protein stability.

 Another exemplary analytical spectroscopy method is Circular Dichroism (CD) spectroscopy. CD spectrometry measures the optical activity of a composition as a function of increasing temperature. Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to
30 structural asymmetry. A disordered or unfolded structure results in a CD spectrum very different from that of an ordered or folded structure. The CD spectrum reflects the sensitivity of

the proteins to the denaturing effects of increasing temperature and is therefore indicative of a protein's thermal stability (see van Mierlo and Steemsma, J. Biotechnol., 79(3):281-98, 2000).

Another exemplary analytical spectroscopy method for measuring thermal stability is Fluorescence Emission Spectroscopy (see van Mierlo and Steemsma, *supra*). Yet another
 5 exemplary analytical spectroscopy method for measuring thermal stability is Nuclear Magnetic Resonance (NMR) spectroscopy (see, e.g. van Mierlo and Steemsma, *supra*).

The thermal stability of a composition can be measured biochemically. An exemplary biochemical method for assessing thermal stability is a thermal challenge assay. In a “thermal challenge assay”, a composition is subjected to a range of elevated temperatures for a set period
 10 of time. For example, in one embodiment, test scFv molecules or molecules comprising scFv molecules are subject to a range of increasing temperatures, e.g., for 1-1.5 hours. The activity of the protein is then assayed by a relevant biochemical assay. For example, if the protein is a binding protein (e.g. an scFv or scFv-containing polypeptide) the binding activity of the binding protein may be determined by a functional or quantitative ELISA.

Such an assay may be done in a high-throughput format and those disclosed in the Examples using *E. coli* and high throughput screening. A library of CD123 binding domains, e.g., scFv variants may be created using methods known in the art. CD123 binding domains, e.g., scFv expression may be induced and the CD123 binding domains, e.g., scFv may be subjected to thermal challenge. The challenged test samples may be assayed for binding and
 20 those CD123 binding domains, e.g., scFvs which are stable may be scaled up and further characterized.

Thermal stability is evaluated by measuring the melting temperature (T_m) of a composition using any of the above techniques (e.g. analytical spectroscopy techniques). The melting temperature is the temperature at the midpoint of a thermal transition curve wherein
 25 50% of molecules of a composition are in a folded state (See e.g., Dimasi *et al.* (2009) J. Mol Biol. 393: 672-692). In one embodiment, T_m values for a CD123 binding domain, e.g., scFv are about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C,
 30 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C. In one embodiment, T_m values for an IgG is about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C,

58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C. In one embodiment, T_m values for an multivalent antibody is about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C.

Thermal stability is also evaluated by measuring the specific heat or heat capacity (C_p) of a composition using an analytical calorimetric technique (e.g. DSC). The specific heat of a composition is the energy (e.g. in kcal/mol) is required to rise by 1°C, the temperature of 1 mol of water. As large C_p is a hallmark of a denatured or inactive protein composition. The change in heat capacity (ΔC_p) of a composition is measured by determining the specific heat of a composition before and after its thermal transition. Thermal stability may also be evaluated by measuring or determining other parameters of thermodynamic stability including Gibbs free energy of unfolding (ΔG), enthalpy of unfolding (ΔH), or entropy of unfolding (ΔS). One or more of the above biochemical assays (e.g. a thermal challenge assay) are used to determine the temperature (i.e. the T_C value) at which 50% of the composition retains its activity (e.g. binding activity).

In addition, mutations to the CD123 binding domain, e.g., scFv alter the thermal stability of the CD123 binding domain, e.g., scFv compared with the unmutated CD123 binding domain, e.g., scFv. When the humanized or human CD123 binding domain, e.g., scFv is incorporated into a CART123 construct, the CD123 binding domain, e.g., humanized or human scFv confers thermal stability to the overall CD123 CART construct. In one embodiment, the CD123 binding domain, e.g., scFv comprises a single mutation that confers thermal stability to the CD123 binding domain, e.g., scFv. In another embodiment, the CD123 binding domain, e.g., scFv comprises multiple mutations that confer thermal stability to the CD123 binding domain, e.g., scFv. In one embodiment, the multiple mutations in the CD123 binding domain, e.g., scFv have an additive effect on thermal stability of the CD123 binding domain, e.g., scFv.

b) % Aggregation

The stability of a composition can be determined by measuring its propensity to aggregate. Aggregation can be measured by a number of non-limiting biochemical or biophysical techniques. For example, the aggregation of a composition may be evaluated using chromatography, e.g. Size-Exclusion Chromatography (SEC). SEC separates molecules on the basis of size. A column is filled with semi-solid beads of a polymeric gel that will admit ions and small molecules into their interior but not large ones. When a protein composition is applied to the top of the column, the compact folded proteins (i.e. non-aggregated proteins) are distributed through a larger volume of solvent than is available to the large protein aggregates. Consequently, the large aggregates move more rapidly through the column, and in this way the mixture can be separated or fractionated into its components. Each fraction can be separately quantified (e.g. by light scattering) as it elutes from the gel. Accordingly, the % aggregation of a composition can be determined by comparing the concentration of a fraction with the total concentration of protein applied to the gel. Stable compositions elute from the column as essentially a single fraction and appear as essentially a single peak in the elution profile or chromatogram.

c) Binding Affinity

The stability of a composition can be assessed by determining its target binding affinity. A wide variety of methods for determining binding affinity are known in the art. An exemplary method for determining binding affinity employs surface plasmon resonance. Surface plasmon resonance is an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jonsson, U., i (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

In one aspect, the antigen binding domain of the CAR comprises an amino acid sequence that is homologous to an antigen binding domain amino acid sequence described herein, and the antigen binding domain retains the desired functional properties of the CD123 antibody fragments described herein. In one specific aspect, the CAR composition of the

invention comprises an antibody fragment. In a further aspect, that antibody fragment comprises an scFv.

In various aspects, the antigen binding domain of the CAR is engineered by modifying one or more amino acids within one or both variable regions (e.g., VH and/or VL), for example within one or more CDR regions and/or within one or more framework regions. In one specific aspect, the CAR composition of the invention comprises an antibody fragment. In a further aspect, that antibody fragment comprises an scFv.

It will be understood by one of ordinary skill in the art that the antibody or antibody fragment of the invention may further be modified such that they vary in amino acid sequence (e.g., from wild-type), but not in desired activity. For example, additional nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues may be made to the protein. For example, a nonessential amino acid residue in a molecule may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members, e.g., a conservative substitution, in which an amino acid residue is replaced with an amino acid residue having a similar side chain, may be made.

Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

Percent identity in the context of two or more nucleic acids or polypeptide sequences, refers to two or more sequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (e.g., 60% identity, optionally 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison

algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

5 For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then
10 calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman, (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Brent et al., (2003) Current Protocols in Molecular Biology).

20 Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977) Nuc. Acids Res. 25:3389-3402; and Altschul et al., (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

25 The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller, (1988) Comput. Appl. Biosci. 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch
30 (1970) J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossom 62 matrix or

a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In one aspect, the present invention contemplates modifications of the starting antibody or fragment (e.g., scFv) amino acid sequence that generate functionally equivalent molecules.

For example, the VH or VL of a CD123 binding domain, e.g., scFv, comprised in the CAR can be modified to retain at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity of the starting VH or VL framework region of the CD123 binding domain, e.g., scFv. The present invention contemplates modifications of the entire CAR construct, e.g., modifications in one or more amino acid sequences of the various domains of the CAR construct in order to generate functionally equivalent molecules. The CAR construct can be modified to retain at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity of the starting CAR construct.

Transmembrane domain

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the CAR-expressing cell, e.g., CART cell, cell surface. In a different aspect the amino acid sequence of the transmembrane domain may be modified or

substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell, e.g., CART cell.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8 (e.g., CD8 alpha, CD8 beta), CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, NKG2C, and CD19.

In some instances, the transmembrane domain can be attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8a hinge. In one embodiment, the hinge or spacer comprises (e.g., consists of) the amino acid sequence of SEQ ID NO:2. In one aspect, the transmembrane domain comprises (e.g., consists of) a transmembrane domain of SEQ ID NO: 6.

In one aspect, the hinge or spacer comprises an IgG4 hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence
 ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
 YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM

(SEQ ID NO:3). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of

GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGG
 ACCCAGCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGA
 5 CCCCCGAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCA
 GTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGG
 GAGGAGCAGTTCAATAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCA
 GGACTGGCTGAACGGCAAGGAATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCC
 AGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTCGGGAGCCCCAGG
 10 TGTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAAGAACCAGGTGTCCCTGACC
 TGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACG
 GCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAG
 CTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGGGCAAC
 GTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAG
 15 CCTGAGCCTGTCCCTGGGCAAGATG (SEQ ID NO:14).

In one aspect, the hinge or spacer comprises an IgD hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence

RWPESPKAQASSVPTAQPPAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERET
 KTPECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTG
 20 GVEEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQA
 PVKLSLNLASSDPPEAASWLLCEVSGFSPNILLMWLEDQREVNTSGFAPARPPPQPG
 STTFWAWSVLRVPAPPSPQPATYTCVVSHEDSRTLNASRSLEVSYVTDH (SEQ ID
 NO:4). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide
 sequence of

AGGTGGCCCGAAAGTCCCAAGGCCAGGCATCTAGTGTTCTACTGCACAGCCCCA
 GGCAGAAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACGCGCAATACT
 GGCCGTGGCGGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGA
 GAGGGAGACCAAGACCCCTGAATGTCCATCCCATAACCCAGCCGCTGGGCGTCTATC
 TCTTGACTCCCGCAGTACAGGACTTGTGGCTTAGAGATAAGGCCACCTTTACATGT
 30 TTCGTCGTGGGCTCTGACCTGAAGGATGCCCATTTGACTTGGGAGGTTGCCGGAAA
 GGTACCCACAGGGGGGGTTGAGGAAGGGTTGCTGGAGCGCCATTCCAATGGCTCT
 CAGAGCCAGCACTCAAGACTCACCCCTCCGAGATCCCTGTGGAACGCCGGGACCTC

TGTCACATGTACTCTAAATCATCCTAGCCTGCCCCACAGCGTCTGATGGCCCTTAG
 AGAGCCAGCCGCCCAGGCACCAGTTAAGCTTAGCCTGAATCTGCTCGCCAGTAGTG
 ATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCGAAGTGTCCGGCTTTAGCCCCGCC
 AACATCTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGAACACCAGCGGCTTCG
 5 CTCCAGCCCCGGCCCCCAGCCGGGTCTACCACATTCTGGGCCTGGAGTGTC
 TTAAGGGTCCCAGCACCTAGCCCCCAGCCAGCCACATACACCTGTGTTGTGTC
 CCATGAAGATAGCAGGACCCTGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCTACG
 TGACTGACCATT (SEQ ID NO:15).

In one aspect, the transmembrane domain may be recombinant, in which case it will
 10 comprise predominantly hydrophobic residues such as leucine and valine. In one aspect a triplet
 of phenylalanine, tryptophan and valine can be found at each end of a recombinant
 transmembrane domain.

Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length
 may form the linkage between the transmembrane domain and the cytoplasmic region of the
 15 CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in one
 aspect, the linker comprises the amino acid sequence of GGGGSGGGGS (SEQ ID NO:5). In
 some embodiments, the linker is encoded by a nucleotide sequence of
 GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC (SEQ ID NO:16).

In one aspect, the hinge or spacer comprises a KIR2DS2 hinge.
 20

Cytoplasmic domain

The cytoplasmic domain or region of the present CAR includes an intracellular
 signaling domain. An intracellular signaling domain is capable of activation of at least one of
 the normal effector functions of the immune cell in which the CAR has been introduced.

25 Examples of intracellular signaling domains for use in the CAR of the invention include
 the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to
 initiate signal transduction following antigen receptor engagement, as well as any derivative or
 variant of these sequences and any recombinant sequence that has the same functional
 capability.

30 It is known that signals generated through the TCR alone are insufficient for full
 activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus,
 T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling

sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

5 A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

10 Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d. In one embodiment, a CAR of the invention comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta.

15 In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

20 Further examples of molecules containing a primary intracellular signaling domain that are of particular use in the invention include those of DAP10, DAP12, and CD32.

The intracellular signalling domain of the CAR can comprise the primary signalling domain, e.g., CD3-zeta signaling domain, by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, 25 the intracellular signaling domain of the CAR can comprise a primary signalling domain, e.g., CD3 zeta chain portion, and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of 30 such molecules include a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706).

The intracellular signaling sequences within the cytoplasmic portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 2 and 10 amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In one aspect, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 7. In one aspect, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 9 (mutant CD3-zeta) or SEQ ID NO: 10 (wild type human CD3-zeta).

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In one aspect, the signaling domain of CD27 comprises an amino acid sequence of

QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:8). In
 5 one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of
 AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCC
 GCCCCGGGCCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCA
 GCCTATCGCTCC (SEQ ID NO:19).

In one aspect, the signaling domain of CD27 comprises an amino acid sequence of
 10 QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:8).

In one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of
 AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCC
 GCCCCGGGCCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCA
 GCCTATCGCTCC (SEQ ID NO:19).

15 In one aspect, the intracellular is designed to comprise the signaling domain of CD3-
 zeta and the signaling domain of CD28. In one aspect, the signaling domain of CD28
 comprises an amino acid sequence of SEQ ID NO: 379. In one aspect, the signaling domain of
 CD28 is encoded by a nucleic acid sequence of SEQ ID NO: 380.

In one aspect, the intracellular is designed to comprise the signaling domain of CD3-
 20 zeta and the signaling domain of ICOS. In one aspect, the signaling domain of CD28
 comprises an amino acid sequence of SEQ ID NO: 381. In one aspect, the signaling domain of
 ICOS is encoded by a nucleic acid sequence of SEQ ID NO: 382.

In one aspect, the CAR-expressing cell described herein can further comprise a second
 CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same
 25 target (CD123) or a different target (e.g., CD19, CD33, CLL-1, CD34, FLT3, or folate receptor
 beta). In one embodiment, the second CAR includes an antigen binding domain to a target
 expressed on acute myeloid leukemia cells, such as, e.g., CD19, CD33, CLL-1, CD34, FLT3, or
 folate receptor beta. In one embodiment, the CAR-expressing cell comprises a first CAR that
 targets a first antigen and includes an intracellular signaling domain having a costimulatory
 30 signaling domain but not a primary signaling domain, and a second CAR that targets a second,
 different, antigen and includes an intracellular signaling domain having a primary signaling
 domain but not a costimulatory signaling domain. While not wishing to be bound by theory,

placement of a costimulatory signaling domain, e.g., 4-1BB, CD28, CD27, ICOS or OX-40, onto the first CAR, and the primary signaling domain, e.g., CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first CD123 CAR that includes a CD123 binding domain, a
 5 transmembrane domain and a costimulatory domain and a second CAR that targets an antigen other than CD123 (e.g., an antigen expressed on AML cells, e.g., CD19, CD33, CLL-1, CD34, FLT3, or folate receptor beta) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first CD123 CAR that includes a CD123 binding domain, a transmembrane domain
 10 and a primary signaling domain and a second CAR that targets an antigen other than CD123 (e.g., an antigen expressed on AML cells, e.g., CD19, CD33, CLL-1, CD34, FLT3, or folate receptor beta) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In one embodiment, the CAR-expressing cell comprises a CD123 CAR described herein
 15 and an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express CD123. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1,
 20 PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFR beta).

In one embodiment, when the CAR-expressing cell comprises two or more different
 25 CARs, the antigen binding domains of the different CARs can be such that the antigen binding domains do not interact with one another. For example, a cell expressing a first and second CAR can have an antigen binding domain of the first CAR, e.g., as a fragment, e.g., an scFv, that does not form an association with the antigen binding domain of the second CAR, e.g., the antigen binding domain of the second CAR is a VHH.

30 In some embodiments, the antigen binding domain comprises a single domain antigen binding (SDAB) molecules include molecules whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain

variable domains, binding molecules naturally devoid of light chains, single domains derived from conventional 4-chain antibodies, engineered domains and single domain scaffolds other than those derived from antibodies. SDAB molecules may be any of the art, or any future single domain molecules. SDAB molecules may be derived from any species including, but not
5 limited to mouse, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. This term also includes naturally occurring single domain antibody molecules from species other than Camelidae and sharks.

In one aspect, an SDAB molecule can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the
10 immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909.

According to another aspect, an SDAB molecule is a naturally occurring single domain antigen binding molecule known as heavy chain devoid of light chains. Such single domain
15 molecules are disclosed in WO 9404678 and Hamers-Casterman, C. et al. (1993) Nature 363:446-448, for example. For clarity reasons, this variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco.
20 Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain; such VHHs are within the scope of the invention.

The SDAB molecules can be recombinant, CDR-grafted, humanized, camelized, de-immunized and/or in vitro generated (e.g., selected by phage display).

It has also been discovered, that cells having a plurality of chimeric membrane
25 embedded receptors comprising an antigen binding domain that interactions between the antigen binding domain of the receptors can be undesirable, e.g., because it inhibits the ability of one or more of the antigen binding domains to bind its cognate antigen. Accordingly, disclosed herein are cells having a first and a second non-naturally occurring chimeric membrane embedded receptor comprising antigen binding domains that minimize such
30 interactions. Also disclosed herein are nucleic acids encoding a first and a second non-naturally occurring chimeric membrane embedded receptor comprising a antigen binding domains that minimize such interactions, as well as methods of making and using such cells and nucleic

acids. In an embodiment the antigen binding domain of one of said first said second non-naturally occurring chimeric membrane embedded receptor, comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

5 In some embodiments, the claimed invention comprises a first and second CAR, wherein the antigen binding domain of one of said first CAR said second CAR does not comprise a variable light domain and a variable heavy domain. In some embodiments, the antigen binding domain of one of said first CAR said second CAR is an scFv, and the other is not an scFv. In some embodiments, the antigen binding domain of one of said first CAR said
10 second CAR comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a nanobody. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a camelid VHH domain.

15 In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a nanobody. In some embodiments, the antigen
20 binding domain of one of said first CAR said second CAR comprises comprises an scFv, and the other comprises a camelid VHH domain.

In some embodiments, when present on the surface of a cell, binding of the antigen binding domain of said first CAR to its cognate antigen is not substantially reduced by the presence of said second CAR. In some embodiments, binding of the antigen binding domain of
25 said first CAR to its cognate antigen in the presence of said second CAR is 85%, 90%, 95%, 96%, 97%, 98% or 99% of binding of the antigen binding domain of said first CAR to its cognate antigen in the absence of said second CAR.

In some embodiments, when present on the surface of a cell, the antigen binding domains of said first CAR said second CAR, associate with one another less than if both were
30 scFv antigen binding domains. In some embodiments, the antigen binding domains of said first CAR said second CAR, associate with one another 85%, 90%, 95%, 96%, 97%, 98% or 99% less than if both were scFv antigen binding domains.

In another aspect, the CAR-expressing cell described herein can further express another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, e.g., PD1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFR beta). In one embodiment, the agent which inhibits an inhibitory molecule, e.g., is a molecule described herein, e.g., an agent that comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFR beta), or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of an extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein). In embodiments, the CAR-expressing cell described herein comprises a switch costimulatory receptor, e.g., as described in WO 2013/019615, which is incorporated herein by reference in its entirety. PD1 is an inhibitory member of the CD28 family of receptors that also includes CD28, CTLA-4, ICOS, and BTLA. PD-1 is expressed on activated B cells, T cells and myeloid cells (Agata et al. 1996 Int. Immunol 8:765-75). Two ligands for PD1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD1 (Freeman et al. 2000 J Exp Med 192:1027-34; Latchman et al. 2001 Nat Immunol 2:261-8; Carter et al. 2002 Eur J Immunol 32:634-43). PD-L1 is abundant in human cancers (Dong et al. 2003 J Mol Med

81:281-7; Blank et al. 2005 Cancer Immunol. Immunother 54:307-314; Konishi et al. 2004 Clin Cancer Res 10:5094). Immune suppression can be reversed by inhibiting the local interaction of PD1 with PD-L1.

In one embodiment, the agent comprises the extracellular domain (ECD) of an inhibitory molecule, e.g., Programmed Death 1 (PD1), can be fused to a transmembrane domain and intracellular signaling domains such as 41BB and CD3 zeta (also referred to herein as a PD1 CAR). In one embodiment, the PD1 CAR, when used in combinations with a CD123 CAR described herein, improves the persistence of the CAR-expressing cell, e.g., T cell or NK cell. In one embodiment, the CAR is a PD1 CAR comprising the extracellular domain of PD1 indicated as underlined in SEQ ID NO: 24. In one embodiment, the PD1 CAR comprises the amino acid sequence of SEQ ID NO:24.

Malpvtalllplalllhaarppgwflsdprpwnpptfspallvvtgednatftcsfsntsesfvlnwyrmspsnqtdklaaf
pedrsqpgqdcfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqlvttt
paprptpaptiasqplslrpeacrpaaggavhtrglfacdiyiwaplagtcgvllslvitlyckrgrkklyifkqpfmrpvqttqeed
gcscrfeeeeeggcelrvkfsrsadapaykqggnqlynelnlgrreeydvldkrrgrdpemggkprknpqeglynelqkdkmae
ayseigmkgerrrgkghdglyqglstatkdydalhmqalppr (SEQ ID NO:24).

In one embodiment, the PD1 CAR comprises the amino acid sequence provided below (SEQ ID NO:22).

pgwflsdprpwnpptfspallvvtgednatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcfrvtqlp
ngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqlvttt
paprptpaptiasqplslrpeacrpaaggavhtrglfacdiyiwaplagtcgvllslvitlyckrgrkklyifkqpfmrpvqttqeedgcscrfeeeeeggcelrv
kfsrsadapaykqggnqlynelnlgrreeydvldkrrgrdpemggkprknpqeglynelqkdkmaeayseigmkgerrrgkgh
dglyqglstatkdydalhmqalppr (SEQ ID NO:22).

In one embodiment, the agent comprises a nucleic acid sequence encoding the PD1 CAR, e.g., the PD1 CAR described herein. In one embodiment, the nucleic acid sequence for the PD1 CAR is shown below, with the PD1 ECD underlined below in SEQ ID NO: 23

atggccctccctgtcactgccctgcttctccccctgcactcctgctccacgccgctagaccacccggatggttctggactctc
cggatcgcccggtggaatcccccaaccttctcaccggcactcttggttgtagctgagggcgataatgcgaccttcacgtgctcgttctccaac
acctccgaatcattcgtgctgaactggtaccgcatgagcccgtaaacagaccgacaagctcgccgcgttccggaagatcggtcgca
accgggacaggattgtcggttcgcgtgactcaactgccgaatggcagagacttcacatgagcgtggtccgcgctaggcgaaacgact
ccgggacctacctgtgcggagccatctcgctggcgccctaaggcccaaatcaaagagagcttgagggccgaactgagagtgaccgagc
gcagagctgaggtgccaaactgcacatccatccccatcgccctcgccgctgcggggcagtttcagacctggtcacgaccactccggcgcc

gcgcccaccgactccggccccaactatcgcgagccagcccctgtcgctgaggccggaagcatgccgccctgccgccggagggtgctgt
gcatacccggggattggacttcgcatgcgacatctacatttgggctcctctcgccggaacttgtggcgtgctccttctgtccctggtcatcac
cctgtactgcaagcgggggtcggaagaaagcttctgtacattttcaagcagcccttcatgaggcccggtgcaaaccacccaggaggaggacg
gttgctcctgccggttccccgaagaggaagaaggaggttgcgagctgcgcgtgaagttctcccgagcgcgcgacgccccgcctataa
5 gcagggccagaaccagctgtacaacgaactgaacctgggacggcgggaagagtacgatgtgctggacaagcggcgcggccgggac
cccgaaatgggcgggaagcctagaagaaagaaccctcaggaaggcctgtataacgagctgcagaaggacaagatggccgagggccta
ctccgaaattgggatgaaggagagcggcggaggggaaaggggcacgacggcctgtaccaaggactgtccaccgccaccaaggac
acatacgatgccctgcacatgcaggcccttccccctcgc (SEQ ID NO: 23).

In another aspect, the present invention provides a population of CAR-expressing cells,
10 e.g., CART cells or CAR-expressing NK cells. In some embodiments, the population of CAR-
expressing cells comprises a mixture of cells expressing different CARs. For example, in one
embodiment, the population of CAR-expressing cells (e.g., CART cells or CAR-expressing NK
cells) can include a first cell expressing a CAR having a CD123 binding domain described
herein, and a second cell expressing a CAR having a different CD123 binding domain, e.g., a
15 CD123 binding domain described herein that differs from the CD123 binding domain in the
CAR expressed by the first cell. As another example, the population of CAR-expressing cells
can include a first cell expressing a CAR that includes a CD123 binding domain, e.g., as
described herein, and a second cell expressing a CAR that includes an antigen binding domain
to a target other than CD123 (e.g., CD33, CD34, CLL-1, FLT3, or folate receptor beta). In one
20 embodiment, the population of CAR-expressing cells includes, e.g., a first cell expressing a
CAR that includes a primary intracellular signaling domain, and a second cell expressing a
CAR that includes a secondary signaling domain, e.g., a costimulatory signaling domain.

In another aspect, the present invention provides a population of cells wherein at least
one cell in the population expresses a CAR having a CD123 domain described herein, and a
25 second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-
expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an
inhibitory molecule. Inhibitory molecules, e.g., can, in some embodiments, decrease the ability
of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory
molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1,
30 CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80,
CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC
class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFR beta). In one embodiment,

the agent which inhibits an inhibitory molecule, e.g., is a molecule described herein, e.g., an agent that comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR (e.g., TGFR beta), or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of the extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

In one aspect, the present invention provides methods comprising administering a population of CAR-expressing cells, e.g., CART cells or CAR-expressing NK cells, e.g., a mixture of cells expressing different CARs, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein. In another aspect, the present invention provides methods comprising administering a population of cells wherein at least one cell in the population expresses a CAR having an anti-cancer associated antigen binding domain as described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein.

Natural Killer Cell Receptor (NKR) CARs

In an embodiment, the CAR molecule described herein comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component can be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any of the following natural killer cell receptors: killer cell immunoglobulin-like receptor

(KIR), e.g., KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIR3DP1; natural cytotoxicity receptor (NCR), e.g., NKp30, NKp44, NKp46; signaling lymphocyte activation molecule (SLAM) family of immune cell receptors, e.g., CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; Fc receptor (FcR), e.g., CD16, and CD64; and Ly49 receptors, e.g., LY49A, LY49C. The NKR-CAR molecules described herein may interact with an adaptor molecule or intracellular signaling domain, e.g., DAP12.

Exemplary configurations and sequences of CAR molecules comprising NKR components are described in International Publication No. WO2014/145252, the contents of which are hereby incorporated by reference.

Split CAR

In some embodiments, the CAR-expressing cell uses a split CAR. The split CAR approach is described in more detail in publications WO2014/055442 and WO2014/055657, incorporated herein by reference. Briefly, a split CAR system comprises a cell expressing a first CAR having a first antigen binding domain and a costimulatory domain (e.g., 41BB), and the cell also expresses a second CAR having a second antigen binding domain and an intracellular signaling domain (e.g., CD3 zeta). When the cell encounters the first antigen, the costimulatory domain is activated, and the cell proliferates. When the cell encounters the second antigen, the intracellular signaling domain is activated and cell-killing activity begins. Thus, the CAR-expressing cell is only fully activated in the presence of both antigens. In embodiments the first antigen binding domain recognizes CD123, e.g., comprises an antigen binding domain described herein, and the second antigen binding domain recognizes an antigen expressed on acute myeloid leukemia cells, e.g., CLL-1, CD33, CD34, FLT3, or folate receptor beta. In embodiments the first antigen binding domain recognizes CD123, e.g., comprises an antigen binding domain described herein, and the second antigen binding domain recognizes an antigen expressed on B-cells, e.g., CD19, CD20, CD22 or ROR1.

Strategies for Regulating Chimeric Antigen Receptors

There are many ways CAR activities can be regulated. In some embodiments, a regulatable CAR (RCAR) where the CAR activity can be controlled is desirable to optimize the

safety and efficacy of a CAR therapy. For example, inducing apoptosis using, e.g., a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18):1673-1683), can be used as a safety switch in the CAR therapy of the instant invention. In another example, CAR-expressing cells can also express an inducible Caspase-9 (iCaspase-9) molecule that, upon administration of a dimerizer drug (e.g., rimiducid (also called AP1903 (Bellicum Pharmaceuticals) or AP20187 (Ariad)) leads to activation of the Caspase-9 and apoptosis of the cells. The iCaspase-9 molecule contains a chemical inducer of dimerization (CID) binding domain that mediates dimerization in the presence of a CID. This results in inducible and selective depletion of CAR-expressing cells. In some cases, the iCaspase-9 molecule is encoded by a nucleic acid molecule separate from the CAR-encoding vector(s). In some cases, the iCaspase-9 molecule is encoded by the same nucleic acid molecule as the CAR-encoding vector. The iCaspase-9 can provide a safety switch to avoid any toxicity of CAR-expressing cells. See, e.g., Song et al. Cancer Gene Ther. 2008; 15(10):667-75; Clinical Trial Id. No. NCT02107963; and Di Stasi et al. N. Engl. J. Med. 2011; 365:1673-83.

Alternative strategies for regulating the CAR therapy of the instant invention include utilizing small molecules or antibodies that deactivate or turn off CAR activity, e.g., by deleting CAR-expressing cells, e.g., by inducing antibody dependent cell-mediated cytotoxicity (ADCC). For example, CAR-expressing cells described herein may also express an antigen that is recognized by molecules capable of inducing cell death, e.g., ADCC or complement-induced cell death. For example, CAR expressing cells described herein may also express a receptor capable of being targeted by an antibody or antibody fragment. Examples of such receptors include EpCAM, VEGFR, integrins (e.g., integrins $\alpha\beta3$, $\alpha4$, $\alpha13\beta3$, $\alpha4\beta7$, $\alpha5\beta1$, $\alpha\beta3$, $\alpha\gamma$), members of the TNF receptor superfamily (e.g., TRAIL-R1, TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof (e.g., versions preserving one or more extracellular epitopes but lacking one or more regions within the cytoplasmic domain).

For example, a CAR-expressing cell described herein may also express a truncated epidermal growth factor receptor (EGFR) which lacks signaling capacity but retains the epitope

that is recognized by molecules capable of inducing ADCC, e.g., cetuximab (ERBITUX®), such that administration of cetuximab induces ADCC and subsequent depletion of the CAR-expressing cells (see, e.g., WO2011/056894, and Jonnalagadda et al., Gene Ther. 2013; 20(8)853-860). Another strategy includes expressing a highly compact marker/suicide gene
5 that combines target epitopes from both CD32 and CD20 antigens in the CAR-expressing cells described herein, which binds rituximab, resulting in selective depletion of the CAR-expressing cells, e.g., by ADCC (see, e.g., Philip et al., Blood. 2014; 124(8)1277-1287). Other methods for depleting CAR-expressing cells described herein include administration of CAMPATH, a monoclonal anti-CD52 antibody that selectively binds and targets mature lymphocytes, e.g.,
10 CAR-expressing cells, for destruction, e.g., by inducing ADCC. In other embodiments, the CAR-expressing cell can be selectively targeted using a CAR ligand, e.g., an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, e.g., ADCC or ADC activities, thereby reducing the number of CAR-expressing cells. In other embodiments, the CAR ligand, e.g., the anti-idiotypic antibody, can be coupled to an agent that
15 induces cell killing, e.g., a toxin, thereby reducing the number of CAR-expressing cells. Alternatively, the CAR molecules themselves can be configured such that the activity can be regulated, e.g., turned on and off, as described below.

In other embodiments, a CAR-expressing cell described herein may also express a target protein recognized by the T cell depleting agent. In one embodiment, the target protein is
20 CD20 and the T cell depleting agent is an anti-CD20 antibody, e.g., rituximab. In such embodiment, the T cell depleting agent is administered once it is desirable to reduce or eliminate the CAR-expressing cell, e.g., to mitigate the CAR induced toxicity. In other embodiments, the T cell depleting agent is an anti-CD52 antibody, e.g., alemtuzumab, as described in the Examples herein.

25 In other embodiments, a RCAR comprises a set of polypeptides, typically two in the simplest embodiments, in which the components of a standard CAR described herein, e.g., an antigen binding domain and an intracellular signaling domain, are partitioned on separate polypeptides or members. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one
30 another, e.g., can couple an antigen binding domain to an intracellular signaling domain. Additional description and exemplary configurations of such regulatable CARs are provided

herein and in International Publication No. WO 2015/090229, hereby incorporated by reference in its entirety.

In an aspect, an RCAR comprises two polypeptides or members: 1) an intracellular signaling member comprising an intracellular signaling domain, e.g., a primary intracellular signaling domain described herein, and a first switch domain; 2) an antigen binding member comprising an antigen binding domain, e.g., that specifically binds a tumor antigen described herein, as described herein and a second switch domain. Optionally, the RCAR comprises a transmembrane domain described herein. In an embodiment, a transmembrane domain can be disposed on the intracellular signaling member, on the antigen binding member, or on both. (Unless otherwise indicated, when members or elements of an RCAR are described herein, the order can be as provided, but other orders are included as well. In other words, in an embodiment, the order is as set out in the text, but in other embodiments, the order can be different. E.g., the order of elements on one side of a transmembrane region can be different from the example, e.g., the placement of a switch domain relative to a intracellular signaling domain can be different, e.g., reversed).

In an embodiment, the first and second switch domains can form an intracellular or an extracellular dimerization switch. In an embodiment, the dimerization switch can be a homodimerization switch, e.g., where the first and second switch domain are the same, or a heterodimerization switch, e.g., where the first and second switch domain are different from one another.

In embodiments, an RCAR can comprise a “multi switch.” A multi switch can comprise heterodimerization switch domains or homodimerization switch domains. A multi switch comprises a plurality of, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10, switch domains, independently, on a first member, e.g., an antigen binding member, and a second member, e.g., an intracellular signaling member. In an embodiment, the first member can comprise a plurality of first switch domains, e.g., FKBP-based switch domains, and the second member can comprise a plurality of second switch domains, e.g., FRB-based switch domains. In an embodiment, the first member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain, and the second member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain.

In an embodiment, the intracellular signaling member comprises one or more intracellular signaling domains, e.g., a primary intracellular signaling domain and one or more costimulatory signaling domains.

In an embodiment, the antigen binding member may comprise one or more intracellular signaling domains, e.g., one or more costimulatory signaling domains. In an embodiment, the antigen binding member comprises a plurality, e.g., 2 or 3 costimulatory signaling domains described herein, e.g., selected from 4-1BB, CD28, CD27, ICOS, and OX40, and in embodiments, no primary intracellular signaling domain. In an embodiment, the antigen binding member comprises the following costimulatory signaling domains, from the extracellular to intracellular direction: 4-1BB-CD27; 4-1BB-CD27; CD27-4-1BB; 4-1BB-CD28; CD28-4-1BB; OX40-CD28; CD28-OX40; CD28-4-1BB; or 4-1BB-CD28. In such embodiments, the intracellular binding member comprises a CD3zeta domain. In one such embodiment the RCAR comprises (1) an antigen binding member comprising, an antigen binding domain, a transmembrane domain, and two costimulatory domains and a first switch domain; and (2) an intracellular signaling domain comprising a transmembrane domain or membrane tethering domain and at least one primary intracellular signaling domain, and a second switch domain.

An embodiment provides RCARs wherein the antigen binding member is not tethered to the surface of the CAR cell. This allows a cell having an intracellular signaling member to be conveniently paired with one or more antigen binding domains, without transforming the cell with a sequence that encodes the antigen binding member. In such embodiments, the RCAR comprises: 1) an intracellular signaling member comprising: a first switch domain, a transmembrane domain, an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain; and 2) an antigen binding member comprising: an antigen binding domain, and a second switch domain, wherein the antigen binding member does not comprise a transmembrane domain or membrane tethering domain, and, optionally, does not comprise an intracellular signaling domain. In some embodiments, the RCAR may further comprise 3) a second antigen binding member comprising: a second antigen binding domain, e.g., a second antigen binding domain that binds a different antigen than is bound by the antigen binding domain; and a second switch domain.

Also provided herein are RCARs wherein the antigen binding member comprises bispecific activation and targeting capacity. In this embodiment, the antigen binding member

can comprise a plurality, e.g., 2, 3, 4, or 5 antigen binding domains, e.g., scFvs, wherein each antigen binding domain binds to a target antigen, e.g. different antigens or the same antigen, e.g., the same or different epitopes on the same antigen. In an embodiment, the plurality of antigen binding domains are in tandem, and optionally, a linker or hinge region is disposed
5 between each of the antigen binding domains. Suitable linkers and hinge regions are described herein.

An embodiment provides RCARs having a configuration that allows switching of proliferation. In this embodiment, the RCAR comprises: 1) an intracellular signaling member comprising: optionally, a transmembrane domain or membrane tethering domain; one or more
10 co-stimulatory signaling domain, e.g., selected from 4-1BB, CD28, CD27, ICOS, and OX40, and a switch domain; and 2) an antigen binding member comprising: an antigen binding domain, a transmembrane domain, and a primary intracellular signaling domain, e.g., a CD3zeta domain, wherein the antigen binding member does not comprise a switch domain, or does not comprise a switch domain that dimerizes with a switch domain on the intracellular
15 signaling member. In an embodiment, the antigen binding member does not comprise a co-stimulatory signaling domain. In an embodiment, the intracellular signaling member comprises a switch domain from a homodimerization switch. In an embodiment, the intracellular signaling member comprises a first switch domain of a heterodimerization switch and the RCAR comprises a second intracellular signaling member which comprises a second switch domain of
20 the heterodimerization switch. In such embodiments, the second intracellular signaling member comprises the same intracellular signaling domains as the intracellular signaling member. In an embodiment, the dimerization switch is intracellular. In an embodiment, the dimerization switch is extracellular.

In any of the RCAR configurations described here, the first and second switch domains
25 comprise a FKBP-FRB based switch as described herein.

Also provided herein are cells comprising an RCAR described herein. Any cell that is engineered to express a RCAR can be used as a RCARX cell. In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell.

Also provided herein are nucleic acids and vectors comprising RCAR encoding
30 sequences. Sequence encoding various elements of an RCAR can be disposed on the same nucleic acid molecule, e.g., the same plasmid or vector, e.g., viral vector, e.g., lentiviral vector.

In an embodiment, (i) sequence encoding an antigen binding member and (ii) sequence encoding an intracellular signaling member, can be present on the same nucleic acid, e.g., vector. Production of the corresponding proteins can be achieved, e.g., by the use of separate promoters, or by the use of a bicistronic transcription product (which can result in the
 5 production of two proteins by cleavage of a single translation product or by the translation of two separate protein products). In an embodiment, a sequence encoding a cleavable peptide, e.g., a P2A or F2A sequence, is disposed between (i) and (ii). In an embodiment, a sequence encoding an IRES, e.g., an EMCV or EV71 IRES, is disposed between (i) and (ii). In these embodiments, (i) and (ii) are transcribed as a single RNA. In an embodiment, a first promoter
 10 is operably linked to (i) and a second promoter is operably linked to (ii), such that (i) and (ii) are transcribed as separate mRNAs.

Alternatively, the sequence encoding various elements of an RCAR can be disposed on the different nucleic acid molecules, e.g., different plasmids or vectors, e.g., viral vector, e.g., lentiviral vector. E.g., the (i) sequence encoding an antigen binding member can be present on
 15 a first nucleic acid, e.g., a first vector, and the (ii) sequence encoding an intracellular signaling member can be present on the second nucleic acid, e.g., the second vector.

Dimerization switches

Dimerization switches can be non-covalent or covalent. In a non-covalent dimerization switch, the dimerization molecule promotes a non-covalent interaction between the switch
 20 domains. In a covalent dimerization switch, the dimerization molecule promotes a covalent interaction between the switch domains.

In an embodiment, the RCAR comprises a FKBP/FRAP, or FKBP/FRB,-based dimerization switch. FKBP12 (FKBP, or FK506 binding protein) is an abundant cytoplasmic protein that serves as the initial intracellular target for the natural product immunosuppressive
 25 drug, rapamycin. Rapamycin binds to FKBP and to the large PI3K homolog FRAP (RAFT, mTOR). FRB is a 93 amino acid portion of FRAP, that is sufficient for binding the FKBP-rapamycin complex (Chen, J., Zheng, X. F., Brown, E. J. & Schreiber, S. L. (1995)

Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. Proc Natl Acad
 30 Sci U S A 92: 4947-51.)

In embodiments, an FKBP/FRAP, e.g., an FKBP/FRB, based switch can use a dimerization molecule, e.g., rapamycin or a rapamycin analog.

The amino acid sequence of FKBP is as follows:

DVPDYASLGGPSSPKKKRKVSRGVQVETISPGDGRTFPK
 RGQTCVVHYTGMLDGGKKFDSSRDRNKPFFKFM LGKQEVIRG
 WEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFD
 5 VELLKLETSY (SEQ ID NO: 588)

In embodiments, an FKBP switch domain can comprise a fragment of FKBP having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., the underlined portion of SEQ ID NO: 588, which is:

VQVETISPGDGRTFPKRGQTCVVHYTGMLDGGKKFDSSR
 10 DRNKPFFKFM LGKQEVIRG WEEGVAQMSVGQRAKL TISPDYAY
 YGATGHPGIIPPHATLVFDVELLKLETS (SEQ ID NO:589)

The amino acid sequence of FRB is as follows:

ILWHEMWHEG LEEASRLYFG ERNVKGMFEV LEPLHAMMER GPQTLKETSF
 NQAYGRDLME AQEWCRKYMK SGNVKDLTQA WDLYYHVFRR ISK (SEQ ID NO:
 15 590)

“FKBP/FRAP, e.g., an FKBP/FRB, based switch” as that term is used herein, refers to a dimerization switch comprising: a first switch domain, which comprises an FKBP fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., RAD001, and has at least 70, 75, 80, 85, 90, 95, 96,
 20 97, 98, or 99% identity with, or differs by no more than 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1 amino acid residues from, the FKBP sequence of SEQ ID NO: 54 or 55; and a second switch domain, which comprises an FRB fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, and has at least 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identity with, or differs by no more than 30, 25, 20, 15, 10,
 25 5, 4, 3, 2, or 1 amino acid residues from, the FRB sequence of SEQ ID NO: 56. In an embodiment, a RCAR described herein comprises one switch domain comprises amino acid residues disclosed in SEQ ID NO: 588 (or SEQ ID NO: 589), and one switch domain comprises amino acid residues disclosed in SEQ ID NO: 590.

In embodiments, the FKBP/FRB dimerization switch comprises a modified FRB switch
 30 domain that exhibits altered, e.g., enhanced, complex formation between an FRB-based switch

domain, e.g., the modified FRB switch domain, a FKBP-based switch domain, and the dimerization molecule, e.g., rapamycin or a rapalogue, e.g., RAD001. In an embodiment, the modified FRB switch domain comprises one or more mutations, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, selected from mutations at amino acid position(s) L2031, E2032, S2035, R2036, F2039, G2040, T2098, W2101, D2102, Y2105, and F2108, where the wild-type amino acid is mutated to any other naturally-occurring amino acid. In an embodiment, a mutant FRB comprises a mutation at E2032, where E2032 is mutated to phenylalanine (E2032F), methionine (E2032M), arginine (E2032R), valine (E2032V), tyrosine (E2032Y), isoleucine (E2032I), e.g., SEQ ID NO: 591, or leucine (E2032L), e.g., SEQ ID NO: 592. In an embodiment, a mutant FRB comprises a mutation at T2098, where T2098 is mutated to phenylalanine (T2098F) or leucine (T2098L), e.g., SEQ ID NO: 593. In an embodiment, a mutant FRB comprises a mutation at E2032 and at T2098, where E2032 is mutated to any amino acid, and where T2098 is mutated to any amino acid, e.g., SEQ ID NO: 594. In an embodiment, a mutant FRB comprises an E2032I and a T2098L mutation, e.g., SEQ ID NO: 595. In an embodiment, a mutant FRB comprises an E2032L and a T2098L mutation, e.g., SEQ ID NO: 596.

Table 14. Exemplary mutant FRB having increased affinity for a dimerization molecule.

FRB mutant	Amino Acid Sequence	SEQ ID NO:
E2032I mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKTS	591
E2032L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKTS	592
T2098L mutant	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	593
E2032, T2098 mutant	ILWHEMWHEGL X EASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKD LX QAWDLYYHVFRRISKTS	594
E2032I, T2098L mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	595
E2032L, T2098L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAY GRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	596

Other suitable dimerization switches include a GyrB-GyrB based dimerization switch, a Gibberellin-based dimerization switch, a tag/binder dimerization switch, and a halo-tag/snap-tag dimerization switch. Following the guidance provided herein, such switches and relevant dimerization molecules will be apparent to one of ordinary skill.

Dimerization molecule

Association between the switch domains is promoted by the dimerization molecule. In the presence of dimerization molecule interaction or association between switch domains
 5 allows for signal transduction between a polypeptide associated with, e.g., fused to, a first switch domain, and a polypeptide associated with, e.g., fused to, a second switch domain. In the presence of non-limiting levels of dimerization molecule signal transduction is increased by 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 5, 10, 50, 100 fold, e.g., as measured in a system described herein.

10 Rapamycin and rapamycin analogs (sometimes referred to as rapalogues), e.g., RAD001, can be used as dimerization molecules in a FKBP/FRB-based dimerization switch described herein. In an embodiment the dimerization molecule can be selected from rapamycin (sirolimus), RAD001 (everolimus), zotarolimus, temsirolimus, AP-23573 (ridaforolimus), biolimus and AP21967. Additional rapamycin analogs suitable for use with FKBP/FRB-based
 15 dimerization switches are further described in the section entitled "Combination Therapies", or in the subsection entitled "Combination with a low dose mTOR inhibitor".

Co-expression of CAR with a Chemokine Receptor

In embodiments, the CAR-expressing cell described herein further comprises a
 20 chemokine receptor molecule. Transgenic expression of chemokine receptors CCR2b or CXCR2 in T cells enhances trafficking to CCL2- or CXCL1-secreting solid tumors including melanoma and neuroblastoma (Craddock et al., *J Immunother.* 2010 Oct; 33(8):780-8 and Kershaw et al., *Hum Gene Ther.* 2002 Nov 1; 13(16):1971-80). Thus, without wishing to be bound by theory, it is believed that chemokine receptors expressed in CAR-expressing cells that
 25 recognize chemokines secreted by tumors, e.g., solid tumors, can improve homing of the CAR-expressing cell to the tumor, facilitate the infiltration of the CAR-expressing cell to the tumor, and enhances antitumor efficacy of the CAR-expressing cell. The chemokine receptor molecule can comprise a naturally occurring or recombinant chemokine receptor or a chemokine-binding fragment thereof. A chemokine receptor molecule suitable for expression in a CAR-expressing
 30 cell described herein include a CXC chemokine receptor (e.g., CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7), a CC chemokine receptor (e.g., CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11), a CX3C chemokine receptor

(e.g., CX3CR1), a XC chemokine receptor (e.g., XCR1), or a chemokine-binding fragment thereof. In one embodiment, the chemokine receptor molecule to be expressed with a CAR described herein is selected based on the chemokine(s) secreted by the tumor. In one embodiment, the CAR-expressing cell described herein further comprises, e.g., expresses, a CCR2b receptor or a CXCR2 receptor. In an embodiment, the CAR described herein and the chemokine receptor molecule are on the same vector or are on two different vectors. In embodiments where the CAR described herein and the chemokine receptor molecule are on the same vector, the CAR and the chemokine receptor molecule are each under control of two different promoters or are under the control of the same promoter.

RNA Transfection

Disclosed herein are methods for producing an in vitro transcribed RNA CAR. The present invention also includes a CAR encoding RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection can involve in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:35). RNA so produced can efficiently transfect different kinds of cells. In one aspect, the template includes sequences for the CAR.

In one aspect the CD123 CAR is encoded by a messenger RNA (mRNA). In one aspect the mRNA encoding the CD123 CAR is introduced into a T cell for production of a CART cell.

In one embodiment, the in vitro transcribed RNA CAR can be introduced to a cell as a form of transient transfection. The RNA is produced by in vitro transcription using a polymerase chain reaction (PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for in vitro mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA. The desired template for in vitro transcription is a CAR of the present invention. For example, the template for the RNA CAR comprises an extracellular region comprising a single chain variable domain of an anti-tumor antibody; a hinge region, a transmembrane domain (e.g., a transmembrane domain of CD8a); and a cytoplasmic region that includes an

intracellular signaling domain, e.g., comprising the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the nucleic acid can include some or all of the 5' and/or 3' untranslated regions (UTRs). The nucleic acid can include exons and introns. In one embodiment, the DNA to be used for PCR is a human nucleic acid sequence. In another embodiment, the DNA to be used for PCR is a human nucleic acid sequence including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

PCR is used to generate a template for in vitro transcription of mRNA which is used for transfection. Methods for performing PCR are well known in the art. Primers for use in PCR are designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. "Substantially complementary," as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary, or one or more bases are non-complementary, or mismatched. Substantially complementary sequences are able to anneal or hybridize with the intended DNA target under annealing conditions used for PCR. The primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a nucleic acid that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers can also be designed to amplify a portion of a nucleic acid that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR can be generated by synthetic methods that are well known in the art. "Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used herein to refer to a location 5' to the DNA sequence to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template

that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Any DNA polymerase useful for PCR can be used in the methods disclosed herein. The reagents and polymerase are commercially available from a number of sources.

5 Chemical structures with the ability to promote stability and/or translation efficiency may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between one and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach,
10 one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

 The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the nucleic acid of interest. Alternatively, UTR sequences that are not endogenous to the nucleic acid of interest can be added by incorporating the UTR sequences into the forward and reverse
15 primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the nucleic acid of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well
20 known in the art.

 In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous nucleic acid. Alternatively, when a 5' UTR that is not endogenous to the nucleic acid of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of
25 some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be 5'UTR of an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

30 To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added

to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one preferred embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters.

5 Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In a preferred embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product which is not suitable for expression in eukaryotic cells. The transcription
10 of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003).

15 The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However polyA/T sequence integrated into plasmid DNA can cause plasmid instability, which is why plasmid DNA templates obtained from bacterial cells are often highly contaminated with deletions and other aberrations. This makes cloning procedures not only laborious and time consuming but often not reliable. That is why a method which allows
20 construction of DNA templates with polyA/T 3' stretch without cloning highly desirable.

The polyA/T segment of the transcriptional DNA template can be produced during PCR by using a reverse primer containing a polyT tail, such as 100T tail (SEQ ID NO: 31) (size can be 50-5000 T (SEQ ID NO: 32)), or after PCR by any other method, including, but not limited to, DNA ligation or in vitro recombination. Poly(A) tails also provide stability to RNAs and
25 reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines (SEQ ID NO: 33).

Poly(A) tails of RNAs can be further extended following in vitro transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP). In one embodiment,
30 increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides (SEQ ID NO: 34) results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA

stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps on also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

The RNAs produced by the methods disclosed herein can also contain an internal ribosome entry site (IRES) sequence. The IRES sequence may be any viral, chromosomal or artificially designed sequence which initiates cap-independent ribosome binding to mRNA and facilitates the initiation of translation. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendorf, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001)).

Non-viral delivery methods

In some aspects, non-viral methods can be used to deliver a nucleic acid encoding a CAR described herein into a cell or tissue or a subject.

In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced out of a longer nucleic acid and inserted into another place in a genome. For example, a transposon comprises a DNA sequence made up of inverted repeats flanking genes for transposition.

Exemplary methods of nucleic acid delivery using a transposon include a Sleeping Beauty transposon system (SBTS) and a piggyBac (PB) transposon system. See, e.g., Aronovich et al. Hum. Mol. Genet. 20.R1(2011):R14-20; Singh et al. Cancer Res. 15(2008):2961–2971; Huang et al. Mol. Ther. 16(2008):580–589; Grabundzija et al. Mol. Ther. 18(2010):1200–1209; Kebriaei et al. Blood. 122.21(2013):166; Williams. Molecular Therapy 16.9(2008):1515–16; Bell et al. Nat. Protoc. 2.12(2007):3153-65; and Ding et al. Cell. 122.3(2005):473-83, all of which are incorporated herein by reference.

The SBTS includes two components: 1) a transposon containing a transgene and 2) a source of transposase enzyme. The transposase can transpose the transposon from a carrier plasmid (or other donor DNA) to a target DNA, such as a host cell chromosome/genome. For example, the transposase binds to the carrier plasmid/donor DNA, cuts the transposon (including transgene(s)) out of the plasmid, and inserts it into the genome of the host cell. See, e.g., Aronovich et al. *supra*.

Exemplary transposons include a pT2-based transposon. See, e.g., Grabundzija et al. Nucleic Acids Res. 41.3(2013):1829-47; and Singh et al. Cancer Res. 68.8(2008): 2961–2971, all of which are incorporated herein by reference. Exemplary transposases include a Tc1/mariner-type transposase, e.g., the SB10 transposase or the SB11 transposase (a hyperactive transposase which can be expressed, e.g., from a cytomegalovirus promoter). See, e.g., Aronovich et al.; Kebriaei et al.; and Grabundzija et al., all of which are incorporated herein by reference.

Use of the SBTS permits efficient integration and expression of a transgene, e.g., a nucleic acid encoding a CAR described herein. Provided herein are methods of generating a cell, e.g., T cell or NK cell, that stably expresses a CAR described herein, e.g., using a transposon system such as SBTS.

In accordance with methods described herein, in some embodiments, one or more nucleic acids, e.g., plasmids, containing the SBTS components are delivered to a cell (e.g., T or NK cell). For example, the nucleic acid(s) are delivered by standard methods of nucleic acid (e.g., plasmid DNA) delivery, e.g., methods described herein, e.g., electroporation, transfection, or lipofection. In some embodiments, the nucleic acid contains a transposon comprising a transgene, e.g., a nucleic acid encoding a CAR described herein. In some embodiments, the nucleic acid contains a transposon comprising a transgene (e.g., a nucleic acid encoding a CAR

described herein) as well as a nucleic acid sequence encoding a transposase enzyme. In other embodiments, a system with two nucleic acids is provided, e.g., a dual-plasmid system, e.g., where a first plasmid contains a transposon comprising a transgene, and a second plasmid contains a nucleic acid sequence encoding a transposase enzyme. For example, the first and the
5 second nucleic acids are co-delivered into a host cell.

In some embodiments, cells, e.g., T or NK cells, are generated that express a CAR described herein by using a combination of gene insertion using the SBTS and genetic editing using a nuclease (e.g., Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, or engineered meganuclease re-engineered
10 homing endonucleases).

In some embodiments, use of a non-viral method of delivery permits reprogramming of cells, e.g., T or NK cells, and direct infusion of the cells into a subject. Advantages of non-viral vectors include but are not limited to the ease and relatively low cost of producing sufficient amounts required to meet a patient population, stability during storage, and lack of
15 immunogenicity.

Nucleic Acid Constructs Encoding a CAR

The present invention also provides nucleic acid molecules encoding one or more CAR constructs described herein. In one aspect, the nucleic acid molecule is provided as a
20 messenger RNA transcript. In one aspect, the nucleic acid molecule is provided as a DNA construct.

Accordingly, in one aspect, the invention pertains to an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD123 binding domain (e.g., a humanized or human CD123 binding domain), a transmembrane domain, and an
25 intracellular signaling domain comprising a stimulatory domain, e.g., a costimulatory signaling domain and/or a primary signaling domain, e.g., zeta chain. In one embodiment, the CD123 binding domain is a CD123 binding domain described herein, e.g., an CD123 binding domain which comprises a sequence selected from a group consisting of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587, or a sequence with 95-99% identity thereof. In one
30 embodiment, the CD123 binding domain comprises a human CD123 binding domain which

comprises a sequence selected from a group consisting of SEQ ID NO: 157-160, 478, 480, 483, and 485. In one embodiment, the CD123 binding domain comprises a humanized CD123 binding domain which comprises a sequence selected from a group consisting of SEQ ID NO: 184-215, and 556-587. In one embodiment, the transmembrane domain is transmembrane domain of a protein, e.g., described herein, e.g., selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO: 6, or a sequence with 95-99% identity thereof. In one embodiment, the CD123 binding domain is connected to the transmembrane domain by a hinge region, e.g., a hinge described herein. In one embodiment, the hinge region comprises SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, or a sequence with 95-99% identity thereof. In one embodiment, the isolated nucleic acid molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the costimulatory domain is a functional signaling domain of a protein, e.g., described herein, e.g., selected from the group consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. .

In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO:7, or a sequence with 95-99% identity thereof. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain comprises the sequence of

SEQ ID NO: 7 or SEQ ID NO:8, or a sequence with 95-99% identity thereof, and the sequence of SEQ ID NO: 9 or SEQ ID NO:10, or a sequence with 95-99% identity thereof, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

5 In another aspect, the invention pertains to an isolated nucleic acid molecule encoding a CAR construct comprising a leader sequence of SEQ ID NO: 1, a scFv domain having a sequence selected from the group consisting of SEQ ID NOS: 157-160, 184-215, 478, 480, 483, 485, and 556-587, (or a sequence with 95-99% identity thereof), a hinge region of SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 (or a sequence with 95-99% identity
10 thereof), a transmembrane domain having a sequence of SEQ ID NO: 6 (or a sequence with 95-99% identity thereof), a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7 or a CD27 costimulatory domain having a sequence of SEQ ID NO:8 (or a sequence with 95-99% identity thereof),) or a CD28 costimulatory domain having a sequence of SEQ ID NO:43 (or a sequence with 95-99% identity thereof) or a ICOS costimulatory domain having a sequence of
15 SEQ ID NO: 45 (or a sequence with 95-99% identity thereof), and a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10 (or a sequence with 95-99% identity thereof).

In another aspect, the invention pertains to an isolated polypeptide molecule encoded by the nucleic acid molecule. In one embodiment, the isolated polypeptide molecule comprises a
20 sequence selected from the group consisting of SEQ ID NO:98-101 and 125-156, or a sequence with 95-99% identity thereof.

In another aspect, the invention pertains to a nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule that comprises a CD123 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein
25 said CD123 binding domain comprises a sequence selected from the group consisting of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, and 556-587, or a sequence with 95-99% identity thereof. In one embodiment, the CD123 binding domain comprises a human CD123 binding domain comprising a sequence selected from the group consisting of SEQ ID NO: 157-160, 478, 480, 483, and 485, or a sequence with 95-99% identity thereof. In one embodiment,
30 the CD123 binding domain comprises a humanized CD123 binding domain comprising a sequence selected from the group consisting of SEQ ID NO: 184-215, and 556-587, or a sequence with 95-99% identity thereof.

In one embodiment, the encoded CAR molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the costimulatory domain is a functional signaling domain of a protein selected from the group consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. . In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO:7.

In one embodiment, the transmembrane domain is a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO:6. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of zeta. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO: 7 and the sequence of SEQ ID NO: 9, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the CD123 binding domain is connected to the transmembrane domain by a hinge region. In one embodiment, the hinge region comprises SEQ ID NO:2. In one embodiment, the hinge region comprises SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5.

In another aspect, the invention pertains to an encoded CAR molecule comprising a leader sequence of SEQ ID NO: 1, a scFv domain having a sequence selected from the group consisting of SEQ ID NO: 157-160, 184-215, 478, 480, 483, 485, 556-587, or a sequence with 95-99% identity thereof, a hinge region of SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, a transmembrane domain having a sequence of SEQ ID NO: 6, a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7 or a CD27 costimulatory domain having a sequence of SEQ ID NO:8 or a CD28 costimulatory domain having a sequence of SEQ ID NO:43 or an ICOS costimulatory domain having a sequence of SEQ ID NO: 45, and a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded CAR molecule comprises a sequence selected from a group consisting of SEQ ID NO: 98-101 and 125-156, or a sequence with 95-99% identity thereof.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

The present invention also provides vectors in which a DNA of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-

proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. A retroviral vector may also be, e.g., a gammaretroviral vector. A gammaretroviral vector may include, e.g., a promoter, a packaging signal (ψ), a primer binding site (PBS), one or more (e.g., two) long terminal repeats (LTR), and a transgene of interest, e.g., a gene encoding a CAR. A gammaretroviral vector may lack viral structural genes such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, e.g., in Tobias Maetzig et al., "Gammaretroviral Vectors: Biology, Technology and Application" *Viruses*. 2011 Jun; 3(6): 677–713.

In another embodiment, the vector comprising the nucleic acid encoding the desired CAR of the invention is an adenoviral vector (A5/35). In another embodiment, the expression of nucleic acids encoding CARs can be accomplished using of transposons such as sleeping beauty, crispr, CAS9, and zinc finger nucleases. See below June et al. 2009 *Nature Reviews Immunology* 9.10: 704-716, is incorporated herein by reference.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et

al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an
5 origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A
10 selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

15 Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In
20 the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

An example of a promoter that is capable of expressing a CAR transgene in a mammalian T cell is the EF1a promoter. The native EF1a promoter drives expression of the
25 alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. The EF1a promoter has been extensively used in mammalian expression plasmids and has been shown to be effective in driving CAR expression from transgenes cloned into a lentiviral vector. See, e.g., Milone et al., Mol. Ther. 17(8): 1453-1464 (2009). In one aspect, the EF1a promoter comprises the sequence provided
30 as SEQ ID NO:11.

Another example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable

of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1 α promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

Another example of a promoter is the phosphoglycerate kinase (PGK) promoter. In embodiments, a truncated PGK promoter (e.g., a PGK promoter with one or more, e.g., 1, 2, 5, 10, 100, 200, 300, or 400, nucleotide deletions when compared to the wild-type PGK promoter sequence) may be desired. The nucleotide sequences of exemplary PGK promoters are provided below.

WT PGK Promoter

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACGTCTTAC
GCCTTGTGGCGCGCCCGTCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTGGCGGGGAAGGGC
CGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCTGGGTAGCGCCAGCCGCGCGACGGTAA
CGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAAGGCAAATAGTGCAGGCCGTGCGGC
GCTTGGCGTTTCTTGAAGGGCTGAATCCCCGCCTCGTCCTTCGCAGCGGCCCCCGGGTGTTCCTATCGCCG
CTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTACACGCTCTGGGTCCCAGCCGCGGCGACGCAAAGGGC
CTTGGTGCGGGTCTCGTCGGCGCAGGGACGCGTTTGGGTCCCGACGGAACCTTTTCCGCGTTGGGGTTGGGGC
ACCATAAGCT

(SEQ ID NO: 597)

Exemplary truncated PGK Promoters:

PGK100:

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACGTCTTAC
GCCTTGTGGCGCGCCCGTCTTGTCCCGGGTGTGATGGCGGGGTG
(SEQ ID NO: 598)

PGK200:

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACGTCTTAC
 GCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTGGCGGGGAAGGGC
 CGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCGCCAGCCGCGCGACGGTAA
 CG

5 (SEQ ID NO: 599)

PGK300:

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACGTCTTAC
 GCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTGGCGGGGAAGGGC
 CGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCGCCAGCCGCGCGACGGTAA
 CGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAAGGCAAATAGTGCAGGCCGTGCGGC
 GCTTGGCGTTTCCTTGAAGGGCTGAATCCCCG

10 (SEQ ID NO: 600)

PGK400:

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACGTCTTAC
 GCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTGGCGGGGAAGGGC
 CGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCGCCAGCCGCGCGACGGTAA
 CGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAAGGCAAATAGTGCAGGCCGTGCGGC
 GCTTGGCGTTTCCTTGAAGGGCTGAATCCCCGCTCGTCTTCGCAGCGGCCCCCGGGTGTTCCTTCGCGG
 CTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTACACGCTCTGGGTCCCAGCCG

20 (SEQ ID NO: 601)

A vector may also include, e.g., a signal sequence to facilitate secretion, a polyadenylation signal and transcription terminator (e.g., from Bovine Growth Hormone (BGH) gene), an element allowing episomal replication and replication in prokaryotes (e.g., SV40 origin and ColE1 or others known in the art) and/or elements to allow selection (e.g., ampicillin resistance gene and/or zeocin marker).

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity.

Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced

into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

In one embodiment, the vector can further comprise a nucleic acid encoding a second CAR. In one embodiment, the second CAR includes an antigen binding domain to a target expressed on acute myeloid leukemia cells, such as, e.g., CD33, CD34, CLL-1, FLT3, or folate receptor beta. In one embodiment, the vector comprises a nucleic acid sequence encoding a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a nucleic acid encoding a second CAR that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. In one embodiment, the vector comprises a nucleic acid encoding a first CD123 CAR that includes a CD123 binding domain, a transmembrane domain and a costimulatory domain and a nucleic acid encoding a second CAR that targets an antigen other than CD123 (e.g., an antigen expressed on AML cells, e.g., CD33, CD34, CLL-1, FLT3, or folate receptor beta) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the vector comprises a nucleic acid encoding a first CD123 CAR that includes a CD123 binding domain, a transmembrane domain and a primary signaling domain and a nucleic acid encoding a second CAR that targets an antigen other than CD123 (e.g., an antigen expressed on AML cells, e.g., CD33, CLL-1, CD34, FLT3, or folate receptor beta) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In one embodiment, the vector comprises a nucleic acid encoding a CD123 CAR described herein and a nucleic acid encoding an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express CD123. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an

intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM
 5 (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta.

In embodiments, the vector may comprise two or more nucleic acid sequences encoding a CAR, e.g., a CD123 CAR described herein and a second CAR, e.g., an inhibitory CAR or a CAR that specifically binds to an antigen other than CD123 (e.g., an antigen expressed on
 10 AML cells, e.g., CLL-1, CD33, CD34, FLT3, or folate receptor beta). In such embodiments, the two or more nucleic acid sequences encoding the CAR are encoded by a single nucleic molecule in the same frame and as a single polypeptide chain. In this aspect, the two or more CARs, can, e.g., be separated by one or more peptide cleavage sites. (e.g., an auto-cleavage site or a substrate for an intracellular protease). Examples of peptide cleavage sites include the
 15 following, wherein the GSG residues are optional:

T2A: (GSG) E G R G S L L T C G D V E E N P G P (SEQ ID NO: 602)

P2A: (GSG) A T N F S L L K Q A G D V E E N P G P (SEQ ID NO: 603)

E2A: (GSG) Q C T N Y A L L K L A G D V E S N P G P (SEQ ID NO: 604)

F2A: (GSG) V K Q T L N F D L L K L A G D V E S N P G P (SEQ ID NO: 605)

20 Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium
 25 phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate
 30 transfection

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized delivery system.

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

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine (“DMPC”) can be obtained from Sigma, St. Louis, MO; dicetyl

phosphate (“DCP”) can be obtained from K & K Laboratories (Plainview, NY); cholesterol (“Choi”) can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol (“DMPG”) and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C.

5 Chloroform is used as the only solvent since it is more readily evaporated than methanol.

“Liposome” is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous
10 medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a
15 micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be
20 performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

25 The present invention further provides a vector comprising a CAR encoding nucleic acid molecule. In one aspect, a CAR vector can be directly transduced into a cell, e.g., an immune effector cell, e.g., a T cell or NK cell. In one aspect, the vector is a cloning or expression vector, e.g., a vector including, but not limited to, one or more plasmids (*e.g.*, expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes),
30 retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the CAR construct in mammalian immune effector cells, e.g., mammalian T cells or mammalian NK cells. In one aspect, the mammalian T cell is a human T cell.

Sources of Cells

Prior to expansion and genetic modification, a source of cells (e.g., immune effector cells, e.g., T cells or NK cells), is obtained from a subject. The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors.

In certain aspects of the present invention, any number of immune effector cell (e.g., T cell or NK cell) lines available in the art, may be used. In certain aspects of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one aspect of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative aspect, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer’s instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media

conditions and compositions, for example those described in Smith *et al.*, “Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement” *Clinical & Translational Immunology* (2015) 4, e31; doi:10.1038/cti.2014.31.

5 In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLLTM gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3+, CD28+, CD4+, CD8+, CD45RA+, and CD45RO+T cells, can be further isolated by positive or negative selection techniques. For example, in one aspect, T cells are
10 isolated by incubation with anti-CD3/anti-CD28 (e.g., 3x28)-conjugated beads, such as DYNABEADS[®] M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one aspect, the time period is about 30 minutes. In a further aspect, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further aspect, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred aspect,
15 the time period is 10 to 24 hours. In one aspect, the incubation time period is 24 hours. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening
20 the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected
25 for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain aspects, it may be desirable to perform the selection procedure and use the “unselected” cells in the activation and expansion process. “Unselected” cells can also be subjected to further rounds of selection.

30 Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow

cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain aspects, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4⁺, CD25⁺, CD62L^{hi}, GITR⁺, and FoxP3⁺. Alternatively, in certain aspects, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

The methods described herein can include, e.g., selection of a specific subpopulation of immune effector cells, e.g., T cells, that are a T regulatory cell-depleted population, CD25⁺ depleted cells, using, e.g., a negative selection technique, e.g., described herein. Preferably, the population of T regulatory depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25⁺ cells.

In one embodiment, T regulatory cells, e.g., CD25⁺ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2.

In one embodiment, the anti-CD25 antibody, or fragment thereof, or CD25-binding ligand is conjugated to a substrate, e.g., a bead, or is otherwise coated on a substrate, e.g., a bead. In one embodiment, the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate as described herein.

In one embodiment, the T regulatory cells, e.g., CD25⁺ T cells, are removed from the population using CD25 depletion reagent from MiltenyiTM. In one embodiment, the ratio of cells to CD25 depletion reagent is 1e7 cells to 20 uL, or 1e7 cells to 15 uL, or 1e7 cells to 10 uL, or 1e7 cells to 5 uL, or 1e7 cells to 2.5 uL, or 1e7 cells to 1.25 uL. In one embodiment, e.g., for T regulatory cells, e.g., CD25⁺ depletion, greater than 500 million cells/ml is used. In a further aspect, a concentration of cells of 600, 700, 800, or 900 million cells/ml is used.

In one embodiment, the population of immune effector cells to be depleted includes about 6×10^9 CD25⁺ T cells. In other aspects, the population of immune effector cells to be depleted include about 1×10^9 to 1×10^{10} CD25⁺ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2×10^9 T regulatory cells, e.g., CD25⁺ cells, or less (e.g., 1×10^9 , 5×10^8 , 1×10^8 , 5×10^7 , 1×10^7 , or less CD25⁺ cells).

In one embodiment, the T regulatory cells, e.g., CD25⁺ cells, are removed from the population using the CliniMAC system with a depletion tubing set, such as, e.g., tubing 162-01.

In one embodiment, the CliniMAC system is run on a depletion setting such as, e.g., DEPLETION2.1.

Without wishing to be bound by a particular theory, decreasing the level of negative regulators of immune cells (e.g., decreasing the number of unwanted immune cells, e.g., T_{REG} cells), in a subject prior to apheresis or during manufacturing of a CAR-expressing cell product can reduce the risk of subject relapse. For example, methods of depleting T_{REG} cells are known in the art. Methods of decreasing T_{REG} cells include, but are not limited to, cyclophosphamide, anti-GITR antibody (an anti-GITR antibody described herein), CD25-depletion, and combinations thereof.

In some embodiments, the manufacturing methods comprise reducing the number of (e.g., depleting) T_{REG} cells prior to manufacturing of the CAR-expressing cell. For example, manufacturing methods comprise contacting the sample, e.g., the apheresis sample, with an anti-GITR antibody and/or an anti-CD25 antibody (or fragment thereof, or a CD25-binding ligand), e.g., to deplete T_{REG} cells prior to manufacturing of the CAR-expressing cell (e.g., T cell, NK cell) product.

In an embodiment, a subject is pre-treated with one or more therapies that reduce T_{REG} cells prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, methods of decreasing T_{REG} cells include, but are not limited to, administration to the subject of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof. Administration of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof, can occur before, during or after an infusion of the CAR-expressing cell product.

In an embodiment, a subject is pre-treated with cyclophosphamide prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, a subject is pre-treated with an anti-GITR antibody prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment.

In one embodiment, the population of cells to be removed are neither the regulatory T cells or tumor cells, but cells that otherwise negatively affect the expansion and/or function of CART cells, e.g. cells expressing CD14, CD11b, CD33, CD15, or other markers expressed by potentially immune suppressive cells. In one embodiment, such cells are envisioned to be

removed concurrently with regulatory T cells and/or tumor cells, or following said depletion, or in another order.

The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be
 5 accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail can include antibodies to CD14,
 10 CD20, CD11b, CD16, HLA-DR, and CD8.

The methods described herein can further include removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted, and tumor antigen depleted cells that are suitable for expression
 15 of a CAR, e.g., a CAR described herein. In one embodiment, tumor antigen expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-tumor antigen antibody, or fragment thereof, can be attached to the same substrate, e.g., bead, which can be used to remove the cells or an anti-CD25 antibody, or fragment thereof, or the anti-tumor antigen antibody, or fragment
 20 thereof, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the tumor antigen expressing cells is sequential, and can occur, e.g., in either order.

Also provided are methods that include removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more
 25 of PD1+ cells, LAG3+ cells, and TIM3+ cells, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, LAG3+ and/or TIM3+ depleted cells. Exemplary check point inhibitors include B7-H1, B7-1, CD160, P1H, 2B4, PD1, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, TIGIT, CTLA-4, BTLA and LAIR1. In one embodiment, check point inhibitor
 30 expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-check point inhibitor antibody, or fragment thereof, can be attached to the same bead which can be used to remove

the cells, or an anti-CD25 antibody, or fragment thereof, and the anti-check point inhibitor antibody, or fragment there, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the check point inhibitor expressing cells is sequential, and can occur, e.g., in either order.

In one embodiment, a T cell population can be selected that expresses one or more of IFN- γ , TNF α , IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other appropriate molecules, e.g., other cytokines. Methods for screening for cell expression can be determined, e.g., by the methods described in PCT Publication No.: WO 2013/126712.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain aspects, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (e.g., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one aspect, a concentration of 2 billion cells/ml is used. In one aspect, a concentration of 1 billion cells/ml is used. In a further aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (e.g., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In a related aspect, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations. In one aspect, the concentration of cells used is 5×10^6 /ml. In other aspects,

the concentration used can be from about $1 \times 10^5/\text{ml}$ to $1 \times 10^6/\text{ml}$, and any integer value in between.

In other aspects, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either $2-10^\circ\text{C}$ or at room temperature.

5 T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one
10 method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate
15 of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20°C or in liquid nitrogen.

In certain aspects, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the
20 present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as immune effector cells, e.g., T cells or NK cells,
25 isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from cell therapy, e.g., T cell therapy, such as those described herein. In one aspect a blood sample or an apheresis is taken from a generally healthy subject. In certain aspects, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are
30 isolated and frozen for later use. In certain aspects, the immune effector cells, e.g., T cells or NK cells, may be expanded, frozen, and used at a later time. In certain aspects, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but

prior to any treatments. In a further aspect, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

In a further aspect of the present invention, T cells are obtained from a patient directly following treatment that leaves the subject with functional T cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain aspects, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

In one embodiment, the immune effector cells expressing a CAR molecule, e.g., a CAR molecule described herein, are obtained from a subject that has received a low, immune enhancing dose of an mTOR inhibitor. In an embodiment, the population of immune effector cells, e.g., T cells, to be engineered to express a CAR, are harvested after a sufficient time, or after sufficient dosing of the low, immune enhancing, dose of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells, or the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells, in the subject or harvested from the subject has been, at least transiently, increased.

In other embodiments, population of immune effector cells, e.g., T cells, which have, or will be engineered to express a CAR, can be treated ex vivo by contact with an amount of an mTOR inhibitor that increases the number of PD1 negative immune effector cells, e.g., T cells

or increases the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells.

In one embodiment, a T cell population is diacylglycerol kinase (DGK)-deficient. DGK-deficient cells include cells that do not express DGK RNA or protein, or have reduced or inhibited DGK activity. DGK-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent DGK expression. Alternatively, DGK-deficient cells can be generated by treatment with DGK inhibitors described herein.

In one embodiment, a T cell population is Ikaros-deficient. Ikaros-deficient cells include cells that do not express Ikaros RNA or protein, or have reduced or inhibited Ikaros activity, Ikaros-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent Ikaros expression. Alternatively, Ikaros-deficient cells can be generated by treatment with Ikaros inhibitors, e.g., lenalidomide.

In embodiments, a T cell population is DGK-deficient and Ikaros-deficient, e.g., does not express DGK and Ikaros, or has reduced or inhibited DGK and Ikaros activity. Such DGK and Ikaros-deficient cells can be generated by any of the methods described herein.

In an embodiment, the NK cells are obtained from the subject. In another embodiment, the NK cells are an NK cell line, e.g., NK-92 cell line (Conkwest).

Allogeneic CAR Immune Effector Cells

In embodiments described herein, the immune effector cell can be an allogeneic immune effector cell, e.g., T cell or NK cell. For example, the cell can be an allogeneic T cell, e.g., an allogeneic T cell lacking expression of a functional T cell receptor (TCR) and/or human leukocyte antigen (HLA), e.g., HLA class I and/or HLA class II.

A T cell lacking a functional TCR can be, e.g., engineered such that it does not express any functional TCR on its surface, engineered such that it does not express one or more subunits that comprise a functional TCR (e.g., engineered such that it does not express (or exhibits reduced expression) of TCR alpha, TCR beta, TCR gamma, TCR delta, TCR epsilon, and/or TCR zeta) or engineered such that it produces very little functional TCR on its surface. Alternatively, the T cell can express a substantially impaired TCR, e.g., by expression of

mutated or truncated forms of one or more of the subunits of the TCR. The term “substantially impaired TCR” means that this TCR will not elicit an adverse immune reaction in a host.

A T cell described herein can be, e.g., engineered such that it does not express a functional HLA on its surface. For example, a T cell described herein, can be engineered such that cell surface expression HLA, e.g., HLA class I and/or HLA class II, is downregulated. In some aspects, downregulation of HLA may be accomplished by reducing or eliminating expression of beta-2 microglobulin (B2M).

In some embodiments, the T cell can lack a functional TCR and a functional HLA, e.g., HLA class I and/or HLA class II.

Modified T cells that lack expression of a functional TCR and/or HLA can be obtained by any suitable means, including a knock out or knock down of one or more subunit of TCR or HLA. For example, the T cell can include a knock down of TCR and/or HLA using siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcription-activator like effector nuclease (TALEN), or zinc finger endonuclease (ZFN).

In some embodiments, the allogeneic cell can be a cell which does not express or expresses at low levels an inhibitory molecule, e.g. by any method described herein. For example, the cell can be a cell that does not express or expresses at low levels an inhibitory molecule, e.g., that can decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta. Inhibition of an inhibitory molecule, e.g., by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used.

siRNA and shRNA to inhibit TCR or HLA

In some embodiments, TCR expression and/or HLA expression can be inhibited using siRNA or shRNA that targets a nucleic acid encoding a TCR and/or HLA, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g.,

CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta), in a cell.

Expression of siRNA and shRNAs in T cells can be achieved using any conventional expression system, e.g., such as a lentiviral expression system.

Exemplary shRNAs that downregulate expression of one or more components of the TCR are described, e.g., in US Publication No.: 2012/0321667. Exemplary siRNA and shRNA that downregulate expression of HLA class I and/or HLA class II genes are described, e.g., in U.S. publication No.: US 2007/0036773.

CRISPR to inhibit TCR or HLA

“CRISPR” or “CRISPR to TCR and/or HLA” or “CRISPR to inhibit TCR and/or HLA” as used herein refers to a set of clustered regularly interspaced short palindromic repeats, or a system comprising such a set of repeats. “Cas”, as used herein, refers to a CRISPR-associated protein. A “CRISPR/Cas” system refers to a system derived from CRISPR and Cas which can be used to silence or mutate a TCR and/or HLA gene, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta).

Naturally-occurring CRISPR/Cas systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa *et al.* (2007) *BMC Bioinformatics* 8: 172. This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou *et al.* (2007) *Science* 315: 1709-1712; Marragini *et al.* (2008) *Science* 322: 1843-1845.

The CRISPR/Cas system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice or primates. Wiedenheft *et al.* (2012) *Nature* 482: 331-8. This is accomplished by introducing into the eukaryotic cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas.

The CRISPR sequence, sometimes called a CRISPR locus, comprises alternating repeats and spacers. In a naturally-occurring CRISPR, the spacers usually comprise sequences

foreign to the bacterium such as a plasmid or phage sequence; in the TCR and/or HLA CRISPR/Cas system, the spacers are derived from the TCR or HLA gene sequence.

RNA from the CRISPR locus is constitutively expressed and processed by Cas proteins into small RNAs. These comprise a spacer flanked by a repeat sequence. The RNAs guide
 5 other Cas proteins to silence exogenous genetic elements at the RNA or DNA level. Horvath *et al.* (2010) *Science* 327: 167-170; Makarova *et al.* (2006) *Biology Direct* 1: 7. The spacers thus serve as templates for RNA molecules, analogously to siRNAs. Pennisi (2013) *Science* 341: 833-836.

As these naturally occur in many different types of bacteria, the exact arrangements of
 10 the CRISPR and structure, function and number of Cas genes and their product differ somewhat from species to species. Haft *et al.* (2005) *PLoS Comput. Biol.* 1: e60; Kunin *et al.* (2007) *Genome Biol.* 8: R61; Mojica *et al.* (2005) *J. Mol. Evol.* 60: 174-182; Bolotin *et al.* (2005) *Microbiol.* 151: 2551-2561; Pourcel *et al.* (2005) *Microbiol.* 151: 653-663; and Stern *et al.* (2010) *Trends. Genet.* 28: 335-340. For example, the Cse (Cas subtype, *E. coli*) proteins (e.g.,
 15 CasA) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. Brouns *et al.* (2008) *Science* 321: 960-964. In other prokaryotes, Cas6 processes the CRISPR transcript. The CRISPR-based phage inactivation in *E. coli* requires Cascade and Cas3, but not Cas1 or Cas2. The Cmr (Cas RAMP module) proteins in *Pyrococcus furiosus* and other prokaryotes form a functional complex with small
 20 CRISPR RNAs that recognizes and cleaves complementary target RNAs. A simpler CRISPR system relies on the protein Cas9, which is a nuclease with two active cutting sites, one for each strand of the double helix. Combining Cas9 and modified CRISPR locus RNA can be used in a system for gene editing. Pennisi (2013) *Science* 341: 833-836.

The CRISPR/Cas system can thus be used to edit a TCR and/or HLA gene (adding or
 25 deleting a basepair), or introducing a premature stop which thus decreases expression of a TCR and/or HLA. The CRISPR/Cas system can alternatively be used like RNA interference, turning off TCR and/or HLA gene in a reversible fashion. In a mammalian cell, for example, the RNA can guide the Cas protein to a TCR and/or HLA promoter, sterically blocking RNA polymerases.

30 Artificial CRISPR/Cas systems can be generated which inhibit TCR and/or HLA, using technology known in the art, e.g., that described in U.S. Publication No.20140068797 and Cong (2013) *Science* 339: 819-823. Other artificial CRISPR/Cas systems that are known in the art

may also be generated which inhibit TCR and/or HLA, e.g., that described in Tsai (2014) *Nature Biotechnol.*, 32:6 569-576, U.S. Patent No.: 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359.

TALEN to inhibit TCR and/or HLA

5 “TALEN” or “TALEN to HLA and/or TCR” or “TALEN to inhibit HLA and/or TCR” refers to a transcription activator-like effector nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-
10 H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta).

 TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effects (TALEs) can be engineered to bind any desired DNA sequence, including a portion of the HLA or TCR gene. By combining an
15 engineered TALE with a DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence, including a HLA or TCR sequence. These can then be introduced into a cell, wherein they can be used for genome editing. Boch (2011) *Nature Biotech.* 29: 135-6; and Boch et al. (2009) *Science* 326: 1509-12; Moscou et al. (2009) *Science* 326: 3501.

20 TALEs are proteins secreted by *Xanthomonas* bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12th and 13th amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide recognition. They can thus be engineered to bind to a desired DNA sequence.

25 To produce a TALEN, a TALE protein is fused to a nuclease (N), which is a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) *Nucl. Acids Res.* 39: e82; Miller et al. (2011) *Nature Biotech.* 29: 143-8; Hockemeyer et al. (2011) *Nature Biotech.* 29: 731-734; Wood et al. (2011) *Science* 333: 307; Doyon et al. (2010)
30 *Nature Methods* 8: 74-79; Szczepek et al. (2007) *Nature Biotech.* 25: 786-793; and Guo et al. (2010) *J. Mol. Biol.* 200: 96.

The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) *Nature Biotech.* 29: 143-8.

A HLA or TCR TALEN can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, foreign DNA can be introduced into the cell along with the TALEN; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to correct a defect in the HLA or TCR gene or introduce such a defect into a wt HLA or TCR gene, thus decreasing expression of HLA or TCR.

TALENs specific to sequences in HLA or TCR can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) *Nature Biotech.* 29: 149-53; Geibler et al. (2011) *PLoS ONE* 6: e19509.

Zinc finger nuclease to inhibit HLA and/or TCR

“ZFN” or “Zinc Finger Nuclease” or “ZFN to HLA and/or TCR” or “ZFN to inhibit HLA and/or TCR” refer to a zinc finger nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta).

Like a TALEN, a ZFN comprises a FokI nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) *Genetics Society of America* 188: 773-782; and Kim et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 1156-1160.

A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys2His2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and

modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are
 5 required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) *Proc. Natl. Acad. Sci. USA* 95: 10570-5.

Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and
 10 amount of HLA and/or TCR in a cell. ZFNs can also be used with homologous recombination to mutate in the HLA or TCR gene.

ZFNs specific to sequences in HLA AND/OR TCR can be constructed using any method known in the art. Cathomen et al. (2008) *Mol. Ther.* 16: 1200-7; Guo et al. (2010) *J. Mol. Biol.* 400: 96; U.S. Patent Publication 2011/0158957; and U.S. Patent Publication
 15 2012/0060230.

Telomerase expression

While not wishing to be bound by any particular theory, in some embodiments, a therapeutic T cell has short term persistence in a patient, due to shortened telomeres in the T
 20 cell; accordingly, transfection with a telomerase gene can lengthen the telomeres of the T cell and improve persistence of the T cell in the patient. See Carl June, "Adoptive T cell therapy for cancer in the clinic", *Journal of Clinical Investigation*, 117:1466-1476 (2007). Thus, in an embodiment, an immune effector cell, e.g., a T cell, ectopically expresses a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. In some aspects, this
 25 disclosure provides a method of producing a CAR-expressing cell, comprising contacting a cell with a nucleic acid encoding a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. The cell may be contacted with the nucleic acid before, simultaneous with, or after being contacted with a construct encoding a CAR.

In one aspect, the disclosure features a method of making a population of immune
 30 effector cells (e.g., T cells, NK cells). In an embodiment, the method comprises: providing a population of immune effector cells (e.g., T cells or NK cells), contacting the population of immune effector cells with a nucleic acid encoding a CAR; and contacting the population of

immune effector cells with a nucleic acid encoding a telomerase subunit, e.g., hTERT, under conditions that allow for CAR and telomerase expression.

In an embodiment, the nucleic acid encoding the telomerase subunit is DNA. In an embodiment, the nucleic acid encoding the telomerase subunit comprises a promoter capable of driving expression of the telomerase subunit.

In an embodiment, hTERT has the amino acid sequence of GenBank Protein ID AAC51724.1 (Meyerson et al., “hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization” Cell Volume 90, Issue 4, 22 August 1997, Pages 785–795) as follows:

10 MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVC
VPWDARPPPAAPSFRQVSCCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAF
TTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCG
PPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRRGGSASR
SLPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGAL
15 SGTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSS
LRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGV
LLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGF
VRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRGCAWLR
RSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKS
20 VWSKLQSIGIRQHLKR VQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPVINDY
VVGARTFRREKRAERLT SRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLR
VRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGH
VRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFM
CHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLL
25 VTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFVVEDEALGGTAFVQMPAHGLFP
WCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFL
DLQVNSLQTVCTNIYKILLQAYRFHACVLQLPFHQVWKNPTFFLRVISDTASLCYSI
LKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLCLKLTRHRVTYVPLLGSLRTAQTQL
SRKLPGTTLTALAEAAANPALPSDFKTILD (SEQ ID NO: 606)

30 In an embodiment, the hTERT has a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 606. In an embodiment, the hTERT has

a sequence of SEQ ID NO: 606. In an embodiment, the hTERT comprises a deletion (e.g., of no more than 5, 10, 15, 20, or 30 amino acids) at the N-terminus, the C-terminus, or both. In an embodiment, the hTERT comprises a transgenic amino acid sequence (e.g., of no more than 5, 10, 15, 20, or 30 amino acids) at the N-terminus, the C-terminus, or both.

5 In an embodiment, the hTERT is encoded by the nucleic acid sequence of GenBank Accession No. AF018167 (Meyerson et al., “hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization” Cell Volume 90, Issue 4, 22 August 1997, Pages 785–795):

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1 caggcagcgt ggtcctgctg cgcacgtggg aagccctggc cccggccacc cccgcgatgc
10 61 cgcgcgctcc ccgctgccga gccgtgcgct ccctgctgcg cagccactac cgcgaggtgc
121 tgccgctggc cacgttcgtg cggcgccctgg ggccccaggg ctggcggctg gtgcagcgcg
181 gggacccggc ggctttccgc gcgctggtgg ccagtgctt ggtgtgcgtg ccctgggacg
241 cacggccgcc ccccgccgcc cctccttcc gccaggtgtc ctgcctgaag gagctggtgg
301 cccgagtgtc gcagaggctg tgcgagcgcg gcgcgaagaa cgtgctggcc ttcggcttcg
15 361 cgctgctgga cggggcccg cgggggcccc ccgaggcctt caccaccagc gtgcgcagct
421 acctgcccac cacggtgacc gacgcactgc gggggagcgg ggcgtggggg ctgctgttgc
481 gccgcgtggg cgacgacgtg ctggttcacc tgctggcacg ctgcgcgctc tttgtgctgg
541 tggtcccag ctgcgcctac caggtgtgcg ggccgccgct gtaccagctc ggcgctgcca
601 ctcaggcccc gccccgcca cacgctagtg gaccccgaag gcgtctggga tgcgaacggg
20 661 cctggaacca tagcgtcagg gaggccgggg tccccctggg cctgccagcc ccgggtgcga
721 ggaggcgcg gggcagtgcc agccgaagtc tgccgttgcc caagaggccc aggcgtggcg
781 ctgcccctga gccggagcgg acgcccgtt ggagggggtc ctggggccac ccgggcagga
841 cgctgggacc gagtgaccgt ggtttctgtg tggtgtcacc tgccagacct gccgaagaag
901 ccacctctt ggagggtgcg ctctctggca cgcgccactc ccacccatcc gtgggccgcc
25 961 agcaccacgc gggcccccca tccacatcgc ggccaccacg tccctgggac acgccttgtc
1021 ccccggtgta cgccgagacc aagcacttcc tctactctc aggcgacaag gagcagctgc
1081 ggccctcctt cctactcagc tctctgagge ccagcctgac tggcgctcgg aggctcgtgg
1141 agaccatctt tctgggttcc aggccttga tgccaggac tccccgagg ttgccccgcc
1201 tgccccagcg ctactggcaa atgcggcccc tgtttctgga gctgcttggg aaccacgcgc
30 1261 agtggcccta cggggtgctc ctcaagacgc actgcccgtc gcgagctgcg gtcacccag
1321 cagccggtgt ctgtgcccgg gagaagcccc agggctctgt ggcggcccc gaggaggagg
1381 acacagacct ccgtgcctg gtgcagctgc tccgccagca cagcagcccc tggcaggtgt

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1441 acggcttcgt gcgggcctgc ctgcgccggc tgggtccccc aggcctctgg ggctccaggc
 1501 acaacgaacg ccgcttcctc aggaacacca agaagttcat ctccctgggg aagcatgcca
 1561 agctctcgct gcaggagctg acgtggaaga tgagcgtgcg gggctgcgct tggctgcgca
 1621 ggagcccagg ggttggctgt gttccggccg cagagcaccc tctgcgtgag gagatcctgg
 5 1681 ccaagttcct gcactggctg atgagtgtgt acgtcgtcga gctgctcagg tctttctttt
 1741 atgtcacgga gaccacgttt caaaagaaca ggctcttttt ctaccggaag agtgtctgga
 1801 gcaagttgca aagcattgga atcagacagc acttgaagag ggtgcagctg cgggagctgt
 1861 cggaagcaga ggtaggcag catcgggaag ccaggccccg cctgctgacg tccagactcc
 1921 gttcatccc caagcctgac gggctgcggc cgattgtgaa catggactac gtcgtgggag
 10 1981 ccagaacgtt ccgcagagaa aagagggccg agcgtctcac ctgaggggtg aaggcactgt
 2041 tcagcgtgct caactacgag cgggcgcggc gccccggcct cctgggcgcc tctgtgctgg
 2101 gcctggacga tatccacagg gcctggcgca ccttcgtgct gcgtgtgcgg gccagggacc
 2161 cgccgcctga gctgtacttt gtcaaggtgg atgtgacggg cgcgtacgac accatccccc
 2221 aggacaggct cacggaggtc atgccagca tcatcaaacc ccagaacacg tactgcgtgc
 15 2281 gtcggtatgc cgtgggtccag aaggccgcc atgggcacgt ccgcaaggcc ttcaagagcc
 2341 acgtctctac cttgacagac ctccagccgt acatgcgaca gttcgtggct cacctgcagg
 2401 agaccagccc gctgagggat gccgtcgtca tcgagcagag ctctccctg aatgaggcca
 2461 gcagtggcct ctgcagctc ttctacgct tcatgtgcca ccacgccgtg cgcatcaggg
 2521 gcaagtccta cgtccagtgc caggggatcc cgcagggtc catctctcc acgtgctct
 20 2581 gcagcctgtg ctacggcgac atggagaaca agctgtttgc ggggattcgg cgggacgggc
 2641 tgctctgcg tttggtggat gatttctgt tggtagacc tcacctacc cacgcgaaaa
 2701 ccttctcag gaccctggtc cgagggtgcc ctgagtatgg ctgcgtgggt aacttgcgga
 2761 agacagtggg gaacttcct gtagaagacg aggccctggg tggcacggct tttgttcaga
 2821 tgccggccca cggcctattc ccctggtgcg gcctgctgct ggatacccg accctggagg
 25 2881 tgcagagcga ctactccagc tatgcccga cctccatcag agccagtctc acctcaacc
 2941 gcggcttcaa ggctgggagg aacatgcgtc gcaaactct tggggtcttg cggctgaagt
 3001 gtcacagcct gtttctggat ttgcaggtga acagcctcca gacggtgtgc accaacatct
 3061 acaagatcct cctgctgcag gcgtacaggt ttacgcatg tgtgctgcag ctcccattc
 3121 atcagcaagt ttggaagaac cccacattt tctgcgcgt catctctgac acggcctccc
 30 3181 tctgctactc catcctgaaa gccaagaacg cagggatgtc gctggggggc aagggcgccg
 3241 ccggccctct gccctccgag gccgtgcagt ggctgtgcca ccaagcattc ctgctcaagc
 3301 tgactcgaca ccgtgtcacc tacgtgccac tctgggggtc actcaggaca gccagacgc

3361 agctgagtcg gaagctcccg gggacgacgc tgactgccct ggaggccgca gccaacccgg
 3421 cactgccctc agacttcaag accatcctgg actgatggcc accgcccac agccaggccg
 3481 agagcagaca ccagcagccc tgtcacgccg ggctctacgt cccagggagg gaggggcggc
 3541 ccacaccag gcccgccacg ctgggagtct gaggcctgag tgagtgtttg gccgaggcct
 5 3601 gcatgtccgg ctgaaggctg agtgtccggc tgaggcctga gcgagtgtcc agccaagggc
 3661 tgagtgtcca gcacacctgc cgtcttcaact tccccacagg ctggcgctcg gctccacccc
 3721 agggccagct ttctctacc aggagcccggt cttccactcc ccacatagga atagtccatc
 3781 cccagattcg ccattgttca cccctcgccc tgcctcctt tgccttcac ccccaccatc
 3841 caggtggaga ccctgagaag gaccctggga gctctgggaa tttggagtga ccaaaggtgt
 10 3901 gccctgtaca caggcgagga ccctgcacct ggatgggggt ccctgtgggt caaattgggg
 3961 ggaggtgctg tgggagtaaa atactgaata tatgagtttt tcagttttga aaaaaaaaaa
 4021 aaaaaaa (SEQ ID NO: 607)

In an embodiment, the hTERT is encoded by a nucleic acid having a sequence at
 least 80%, 85%, 90%, 95%, 96, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:
 15 607. In an embodiment, the hTERT is encoded by a nucleic acid of SEQ ID NO: 607.

Activation and Expansion of Cells

Cells may be activated and expanded generally using methods as described, for
 example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466;
 20 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874;
 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Generally, the T cells of the invention may be expanded by contact with a surface
 having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a
 ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T
 25 cell populations may be stimulated as described herein, such as by contact with an anti-CD3
 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a
 surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a
 calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a
 ligand that binds the accessory molecule is used. For example, a population of T cells can be
 30 contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate
 for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or

CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

In certain aspects, the primary stimulatory signal and the costimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one aspect, the agent providing the costimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain aspects, both agents can be in solution. In one aspect, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one aspect, the two agents are immobilized on beads, either on the same bead, i.e., “cis,” or to separate beads, i.e., “trans.” By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one aspect, a 1:1 ratio of each antibody bound to the beads for CD4+ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular aspect an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one aspect, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain aspects of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular aspect, a 1:100 CD3:CD28

ratio of antibody bound to beads is used. In one aspect, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further aspect, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred aspect, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet one aspect, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell.

For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain aspects the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further aspects the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one aspect, a ratio of particles to cells of 1:1 or less is used. In one particular aspect, a preferred particle: cell ratio is 1:5. In further aspects, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one aspect, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular aspect, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In one aspect, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type. In one aspect, the most typical ratios for use are in the neighborhood of 1:1, 2:1 and 3:1 on the first day.

In further aspects of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative aspect, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further aspect, the beads and cells are first concentrated
5 by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one aspect the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-
10 450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the
15 context of the present invention. In certain aspects, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one aspect, a concentration of about 10 billion cells/ml, 9 billion/ml, 8 billion/ml, 7 billion/ml, 6 billion/ml, 5 billion/ml, or 2 billion cells/ml is used. In one aspect, greater than 100 million cells/ml is used.
20 In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that
25 may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain aspects. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In one embodiment, cells transduced with a nucleic acid encoding a CAR, e.g., a CAR
30 described herein, are expanded, e.g., by a method described herein. In one embodiment, the cells are expanded in culture for a period of several hours (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, 21 hours) to about 14 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days). In

one embodiment, the cells are expanded for a period of 4 to 9 days. In one embodiment, the cells are expanded for a period of 8 days or less, e.g., 7, 6 or 5 days. In one embodiment, the cells, e.g., a CD123 CAR cell described herein, are expanded in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions. Potency can be defined, e.g., by various T cell functions, e.g. proliferation, target cell killing, cytokine production, activation, migration, or combinations thereof. In one embodiment, the cells, e.g., a CD123 CAR cell described herein, expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., the cells expressing a CD123 CAR described herein, are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., a CD123 CAR cell described herein, expanded for 5 days show at least a one, two, three, four, five, ten fold or more increase in pg/ml of proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

In one aspect of the present invention, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In one aspect, the mixture may be cultured for 21 days. In one aspect of the invention the beads and the T cells are cultured together for about eight days. In one aspect, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones,

and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and
5 atmosphere (e.g., air plus 5% CO₂).

In one embodiment, the cells are expanded in an appropriate media (e.g., media described herein) that includes one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold, 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry. In one embodiment, the cells
10 are expanded in the presence of IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

In embodiments, methods described herein, e.g., CAR-expressing cell manufacturing methods, comprise removing T regulatory cells, e.g., CD25+ T cells, from a cell population, e.g., using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. Methods of removing T regulatory cells, e.g., CD25+ T cells, from a cell population are
15 described herein. In embodiments, the methods, e.g., manufacturing methods, further comprise contacting a cell population (e.g., a cell population in which T regulatory cells, such as CD25+ T cells, have been depleted; or a cell population that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) with IL-15 and/or IL-7. For example, the cell population (e.g., that has previously contacted an anti-CD25 antibody, fragment thereof, or
20 CD25-binding ligand) is expanded in the presence of IL-15 and/or IL-7.

In some embodiments a CAR-expressing cell described herein is contacted with a composition comprising a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15, during the manufacturing of the CAR-expressing cell, e.g., ex vivo.
25 In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a IL-15 polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a combination of both a IL-15 polypeptide and a IL-15 Ra polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing
30 cell described herein is contacted with a composition comprising hetIL-15 during the manufacturing of the CAR-expressing cell, e.g., ex vivo.

In one embodiment the CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising an IL-15 polypeptide during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising both an IL-15 polypeptide and an IL-15Ra polypeptide during ex vivo expansion. In one embodiment the contacting results in the survival and proliferation of a lymphocyte subpopulation, e.g., CD8⁺ T cells.

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, CD4⁺) that is greater than the cytotoxic or suppressor T cell population (TC, CD8⁺). Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

Once a CD123 CAR is constructed, various assays can be used to evaluate the activity of the molecule, such as but not limited to, the ability to expand T cells following antigen stimulation, sustain T cell expansion in the absence of re-stimulation, and anti-cancer activities in appropriate in vitro and animal models. Assays to evaluate the effects of a CD123 CAR are described in further detail below.

Western blot analysis of CAR expression in primary T cells can be used to detect the presence of monomers and dimers. See, e.g., Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, T cells (1:1 mixture of CD4⁺ and CD8⁺ T cells) expressing the CARs are expanded *in vitro* for more than 10 days followed by lysis and SDS-PAGE under reducing conditions. CARs containing the full length TCR- ζ cytoplasmic domain and the endogenous TCR- ζ chain are detected by western blotting using an antibody to the TCR- ζ

chain. The same T cell subsets are used for SDS-PAGE analysis under non-reducing conditions to permit evaluation of covalent dimer formation.

In vitro expansion of CAR⁺ T cells following antigen stimulation can be measured by flow cytometry. For example, a mixture of CD4⁺ and CD8⁺ T cells are stimulated with
 5 α CD3/ α CD28 aAPCs followed by transduction with lentiviral vectors expressing GFP under the control of the promoters to be analyzed. Exemplary promoters include the CMV IE gene, EF-1 α , ubiquitin C, or phosphoglycerokinase (PGK) promoters. GFP fluorescence is evaluated on day 6 of culture in the CD4⁺ and/or CD8⁺ T cell subsets by flow cytometry. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Alternatively, a mixture of CD4⁺
 10 and CD8⁺ T cells are stimulated with α CD3/ α CD28 coated magnetic beads on day 0, and transduced with CAR on day 1 using a bicistronic lentiviral vector expressing CAR along with eGFP using a 2A ribosomal skipping sequence. Cultures are re-stimulated with either CD19⁺ K562 cells (K562-CD19), wild-type K562 cells (K562 wild type) or K562 cells expressing hCD32 and 4-1BBL in the presence of antiCD3 and anti-CD28 antibody (K562-BBL-3/28)
 15 following washing. Exogenous IL-2 is added to the cultures every other day at 100 IU/ml. GFP⁺ T cells are enumerated by flow cytometry using bead-based counting. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Similar assays can be performed using anti-CD123 T cells (see, *e.g.* Gill et al Blood 2014;123:2343) or with anti-CD123 CAR T cells.

Sustained CAR⁺ T cell expansion in the absence of re-stimulation can also be measured.
 20 See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, mean T cell volume (fl) is measured on day 8 of culture using a Coulter Multisizer III particle counter, a Nexcelom Cellometer Vision or Millipore Scepter, following stimulation with α CD3/ α CD28 coated magnetic beads on day 0, and transduction with the indicated CAR on day 1.

Animal models can also be used to measure a CART activity. For example, xenograft
 25 model using human CD19-specific CAR⁺ T cells to treat a primary human pre-B ALL in immunodeficient mice can be used. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, after establishment of ALL, mice are randomized as to treatment groups. Different numbers of α CD19- ζ and α CD19-BB- ζ engineered T cells are coinjected at a 1:1 ratio into NOD-SCID- $\gamma^{-/-}$ mice bearing B-ALL. The number of copies of α CD19- ζ and
 30 α CD19-BB- ζ vector in spleen DNA from mice is evaluated at various times following T cell injection. Animals are assessed for leukemia at weekly intervals. Peripheral blood CD19⁺ B-ALL blast cell counts are measured in mice that are injected with α CD19- ζ CAR⁺ T cells or

mock-transduced T cells. Survival curves for the groups are compared using the log-rank test. In addition, absolute peripheral blood CD4⁺ and CD8⁺ T cell counts 4 weeks following T cell injection in NOD-SCID- $\gamma^{-/-}$ mice can also be analyzed. Mice are injected with leukemic cells and 3 weeks later are injected with T cells engineered to express CAR by a bicistronic lentiviral vector that encodes the CAR linked to eGFP. T cells are normalized to 45–50% input GFP⁺ T cells by mixing with mock-transduced cells prior to injection, and confirmed by flow cytometry. Animals are assessed for leukemia at 1-week intervals. Survival curves for the CAR⁺ T cell groups are compared using the log-rank test. Similar experiments can be done with CD123 CARTS.

Dose dependent CAR treatment response can be evaluated. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). For example, peripheral blood is obtained 35–70 days after establishing leukemia in mice injected on day 21 with CAR T cells, an equivalent number of mock-transduced T cells, or no T cells. Mice from each group are randomly bled for determination of peripheral blood CD19⁺ ALL blast counts and then killed on days 35 and 49.

The remaining animals are evaluated on days 57 and 70. Similar experiments can be done with CD123 CARTS.

Assessment of cell proliferation and cytokine production has been previously described, *e.g.*, at Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, assessment of CAR-mediated proliferation is performed in microtiter plates by mixing washed T cells with K562 cells expressing CD19 (K19) or CD32 and CD137 (KT32-BBL) for a final T-cell:K562 ratio of 2:1. K562 cells are irradiated with gamma-radiation prior to use. Anti-CD3 (clone OKT3) and anti-CD28 (clone 9.3) monoclonal antibodies are added to cultures with KT32-BBL cells to serve as a positive control for stimulating T-cell proliferation since these signals support long-term CD8⁺ T cell expansion *ex vivo*. T cells are enumerated in cultures using CountBright™ fluorescent beads (Invitrogen, Carlsbad, CA) and flow cytometry as described by the manufacturer. CAR⁺ T cells are identified by GFP expression using T cells that are engineered with eGFP-2A linked CAR-expressing lentiviral vectors. For CAR⁺ T cells not expressing GFP, the CAR⁺ T cells are detected with biotinylated recombinant CD123 protein and a secondary avidin-PE conjugate. CD4⁺ and CD8⁺ expression on T cells are also simultaneously detected with specific monoclonal antibodies (BD Biosciences). Cytokine measurements are performed on supernatants collected 24 hours following re-stimulation using the human TH1/TH2 cytokine cytometric bead array kit (BD Biosciences, San Diego, CA)

according the manufacturer's instructions or using a Luminex 30-plex kit (Invitrogen).

Fluorescence is assessed using a BD Fortessa flow cytometer, and data is analyzed according to the manufacturer's instructions. Similar experiments can be done with CD123 CARTS.

Cytotoxicity can be assessed by a standard ⁵¹Cr-release assay. See, *e.g.*, Milone *et al.*,
5 Molecular Therapy 17(8): 1453-1464 (2009). Briefly, target cells (K562 lines and primary pro-
B-ALL cells) are loaded with ⁵¹Cr (as NaCrO₄, New England Nuclear, Boston, MA) at 37°C
for 2 hours with frequent agitation, washed twice in complete RPMI and plated into microtiter
plates. Effector T cells are mixed with target cells in the wells in complete RPMI at varying
ratios of effector cell:target cell (E:T). Additional wells containing media only (spontaneous
10 release, SR) or a 1% solution of triton-X 100 detergent (total release, TR) are also prepared.
After 4 hours of incubation at 37°C, supernatant from each well is harvested. Released ⁵¹Cr is
then measured using a gamma particle counter (Packard Instrument Co., Waltham, MA). Each
condition is performed in at least triplicate, and the percentage of lysis is calculated using the
formula: % Lysis = (ER – SR) / (TR – SR), where ER represents the average ⁵¹Cr released for
15 each experimental condition.

Imaging technologies can be used to evaluate specific trafficking and proliferation of
CARs in tumor-bearing animal models. Such assays have been described, for example, in
Barrett *et al.*, Human Gene Therapy 22:1575-1586 (2011). Briefly, NOD/SCID/γc^{-/-} (NSG)
mice are injected IV with Nalm-6 cells followed 7 days later with T cells 4 hour after
20 electroporation with the CAR constructs. The T cells are stably transfected with a lentiviral
construct to express firefly luciferase, and mice are imaged for bioluminescence. Alternatively,
therapeutic efficacy and specificity of a single injection of CAR⁺ T cells in Nalm-6 xenograft
model can be measured as the following: NSG mice are injected with Nalm-6 transduced to
stably express firefly luciferase, followed by a single tail-vein injection of T cells
25 electroporated with CD123 CAR 7 days later. Animals are imaged at various time points post
injection. For example, photon-density heat maps of firefly luciferase-positive leukemia in
representative mice at day 5 (2 days before treatment) and day 8 (24 hr post CAR⁺ PBLs) can
be generated.

Other assays, including those described in the Example section herein as well as those
30 that are known in the art can also be used to evaluate the CD123 CAR constructs of the
invention.

Alternatively, or in combination to the methods disclosed herein, methods and compositions for one or more of: detection and/or quantification of CAR-expressing cells (*e.g.*, *in vitro* or *in vivo* (*e.g.*, clinical monitoring)); immune cell expansion and/or activation; and/or CAR-specific selection, that involve the use of a CAR ligand, are disclosed. In one exemplary embodiment, the CAR ligand is an antibody that binds to the CAR molecule, *e.g.*, binds to the extracellular antigen binding domain of CAR (*e.g.*, an antibody that binds to the antigen binding domain, *e.g.*, an anti-idiotypic antibody; or an antibody that binds to a constant region of the extracellular binding domain). In other embodiments, the CAR ligand is a CAR antigen molecule (*e.g.*, a CAR antigen molecule as described herein).

In one aspect, a method for detecting and/or quantifying CAR-expressing cells is disclosed. For example, the CAR ligand can be used to detect and/or quantify CAR-expressing cells *in vitro* or *in vivo* (*e.g.*, clinical monitoring of CAR-expressing cells in a patient, or dosing a patient). The method includes:

providing the CAR ligand (optionally, a labelled CAR ligand, *e.g.*, a CAR ligand that includes a tag, a bead, a radioactive or fluorescent label);

acquiring the CAR-expressing cell (*e.g.*, acquiring a sample containing CAR-expressing cells, such as a manufacturing sample or a clinical sample);

contacting the CAR-expressing cell with the CAR ligand under conditions where binding occurs, thereby detecting the level (*e.g.*, amount) of the CAR-expressing cells present.

Binding of the CAR-expressing cell with the CAR ligand can be detected using standard techniques such as FACS, ELISA and the like.

In another aspect, a method of expanding and/or activating cells (*e.g.*, immune effector cells) is disclosed. The method includes:

providing a CAR-expressing cell (*e.g.*, a first CAR-expressing cell or a transiently expressing CAR cell);

contacting said CAR-expressing cell with a CAR ligand, *e.g.*, a CAR ligand as described herein), under conditions where immune cell expansion and/or proliferation occurs, thereby producing the activated and/or expanded cell population.

In certain embodiments, the CAR ligand is present on (*e.g.*, is immobilized or attached to a substrate, *e.g.*, a non-naturally occurring substrate). In some embodiments, the

substrate is a non-cellular substrate. The non-cellular substrate can be a solid support chosen from, e.g., a plate (e.g., a microtiter plate), a membrane (e.g., a nitrocellulose membrane), a matrix, a chip or a bead. In embodiments, the CAR ligand is present in the substrate (e.g., on the substrate surface). The CAR ligand can be immobilized, attached, or associated covalently or non-covalently (e.g., cross-linked) to the substrate. In one embodiment, the CAR ligand is attached (e.g., covalently attached) to a bead. In the aforesaid embodiments, the immune cell population can be expanded *in vitro* or *ex vivo*. The method can further include culturing the population of immune cells in the presence of the ligand of the CAR molecule, e.g., using any of the methods described herein.

In other embodiments, the method of expanding and/or activating the cells further comprises addition of a second stimulatory molecule, e.g., CD28. For example, the CAR ligand and the second stimulatory molecule can be immobilized to a substrate, e.g., one or more beads, thereby providing increased cell expansion and/or activation.

In yet another aspect, a method for selecting or enriching for a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and selecting the cell on the basis of binding of the CAR ligand.

In yet other embodiments, a method for depleting, reducing and/or killing a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and targeting the cell on the basis of binding of the CAR ligand, thereby reducing the number, and/or killing, the CAR-expressing cell. In one embodiment, the CAR ligand is coupled to a toxic agent (e.g., a toxin or a cell ablative drug). In another embodiment, the anti-idiotypic antibody can cause effector cell activity, e.g., ADCC or ADC activities.

Exemplary anti-CAR antibodies that can be used in the methods disclosed herein are described, e.g., in WO 2014/190273 and by Jena et al., “Chimeric Antigen Receptor (CAR)-Specific Monoclonal Antibody to Detect CD19-Specific T cells in Clinical Trials”, PLOS March 2013 8:3 e57838, the contents of which are incorporated by reference. In one embodiment, the anti-idiotypic antibody molecule recognizes an anti-CD19 antibody molecule, e.g., an anti-CD19 scFv. For instance, the anti-idiotypic antibody molecule can compete for binding with the CD19-specific CAR mAb clone no. 136.20.1 described in Jena et al., PLOS March 2013 8:3 e57838; may have the same CDRs (e.g., one or more of, e.g., all of, VH CDR1,

VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3, using the Kabat definition, the Chothia definition, or a combination of the Kabat and Chothia definitions) as the CD19-specific CAR mAb clone no. 136.20.1; may have one or more (e.g., 2) variable regions as the CD19-specific CAR mAb clone no. 136.20.1, or may comprise the CD19-specific CAR mAb clone no. 136.20.1. In some embodiments, the anti-idiotypic antibody was made according to a method described in Jena et al. In another embodiment, the anti-idiotypic antibody molecule is an anti-idiotypic antibody molecule described in WO 2014/190273. In some embodiments, the anti-idiotypic antibody molecule has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as an antibody molecule of WO 2014/190273 such as 136.20.1; may have one or more (e.g., 2) variable regions of an antibody molecule of WO 2014/190273, or may comprise an antibody molecule of WO 2014/190273 such as 136.20.1. In other embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., as described in WO 2014/190273. In some embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., a heavy chain constant region (e.g., a CH2-CH3 hinge region) or light chain constant region. For instance, in some embodiments the anti-CAR antibody competes for binding with the 2D3 monoclonal antibody described in WO 2014/190273, has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as 2D3, or has one or more (e.g., 2) variable regions of 2D3, or comprises 2D3 as described in WO 2014/190273.

In some aspects and embodiments, the compositions and methods herein are optimized for a specific subset of T cells, e.g., as described in US Serial No. 62/031,699 filed July 31, 2014, the contents of which are incorporated herein by reference in their entirety. In some embodiments, the optimized subsets of T cells display an enhanced persistence compared to a control T cell, e.g., a T cell of a different type (e.g., CD8⁺ or CD4⁺) expressing the same construct.

In some embodiments, a CD4⁺ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence in) a CD4⁺ T cell, e.g., an ICOS domain. In some embodiments, a CD8⁺ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence of) a CD8⁺ T cell,

e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain. In some embodiments, the CAR described herein comprises an antigen binding domain described herein, e.g., a CAR comprising an antigen binding domain that specifically binds CD123, e.g., a CAR of Table 2 or Table 6.

5 In an aspect, described herein is a method of treating a subject, e.g., a subject having cancer. The method includes administering to said subject, an effective amount of:

1) a CD4⁺ T cell comprising a CAR (the CAR^{CD4+})

comprising:

10 an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that specifically binds CD123, e.g., an antigen-binding domain of Table 2, Table 6, or Table 9;

a transmembrane domain; and

an intracellular signaling domain, e.g., a first costimulatory domain, e.g., an ICOS domain; and

15 2) a CD8⁺ T cell comprising a CAR (the CAR^{CD8+}) comprising:

an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that specifically binds CD123, e.g., an antigen-binding domain of Table 2, Table 6, or Table 9;

a transmembrane domain; and

20 an intracellular signaling domain, e.g., a second costimulatory domain, e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain; wherein the CAR^{CD4+} and the CAR^{CD8+} differ from one another.

Optionally, the method further includes administering:

3) a second CD8⁺ T cell comprising a CAR (the second CAR^{CD8+}) comprising:

25 an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that specifically binds CD123, e.g., an antigen-binding domain of Table 2, Table 6, or Table 9;

a transmembrane domain; and

30 an intracellular signaling domain, wherein the second CAR^{CD8+} comprises an intracellular signaling domain, e.g., a costimulatory signaling domain, not present on the CAR^{CD8+}, and, optionally, does not comprise an ICOS signaling domain.

Therapeutic Application

CD123 Associated Diseases and/or Disorders

The present invention provides, among other things, compositions and methods for
5 treating a disease associated with expression of CD123 or condition associated with cells which
express CD123 including, e.g., a proliferative disease such as a cancer or malignancy or a
precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a
preleukemia; or a noncancer related indication associated with cells which express CD123. In
one aspect, a cancer associated with expression of CD123 is a hematological cancer. In one
10 aspect, a hematological cancer includes but is not limited to AML, myelodysplastic syndrome,
ALL, chronic myeloid leukemia, blastic plasmacytoid dendritic cell neoplasm,
myeloproliferative neoplasms, Hodgkin lymphoma, and the like. Further disease associated
with expression of CD123 expression include, but are not limited to, e.g., atypical and/or non-
classical cancers, malignancies, precancerous conditions or proliferative diseases associated
15 with expression of CD123. Non-cancer related indications associated with expression of
CD123 may also be included.

In one aspect, the invention provides methods for treating a disease associated with
CD123 expression. In one aspect, the invention provides methods for treating a disease wherein
part of the tumor is negative for CD123 and part of the tumor is positive for CD123. For
20 example, the CAR of the invention is useful for treating subjects that have undergone treatment
for a disease associated with elevated expression of CD123, wherein the subject that has
undergone treatment for elevated levels of CD123 exhibits a disease associated with elevated
levels of CD123. In embodiments, the CAR of the invention is useful for treating subjects that
have undergone treatment for a disease associated with expression of CD123, wherein the
25 subject that has undergone treatment related to expression of CD123 exhibits a disease
associated with expression of CD123.

In one aspect, the invention pertains to a vector comprising CD123 CAR operably
linked to promoter for expression in mammalian immune effector cells, e.g., T cells or NK
cells. In one aspect, the invention provides a recombinant T cell expressing the CD123 CAR for
30 use in treating CD123-expressing tumors, wherein the recombinant immune effector cells, e.g.,
T cells or NK cellsexpressing the CD123 CAR is termed a CD123 CAR-expressing cell (e.g.,

CD123 CART or CD123 CAR-expressing NK cell). In one aspect, the CD123 CART of the invention is capable of contacting a tumor cell with at least one CD123 CAR of the invention expressed on its surface such that the CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) targets the tumor cell and growth of the tumor is inhibited.

5 In one aspect, the invention pertains to a method of inhibiting growth of a CD123-expressing tumor cell, comprising contacting the tumor cell with a CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) of the present invention such that the CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) is activated in response to the antigen and targets the cancer cell, wherein the growth of the tumor
10 is inhibited.

In one aspect, the invention pertains to a method of treating cancer in a subject. The method comprises administering to the subject a CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) of the present invention such that the cancer is treated in the subject. An example of a cancer that is treatable by the CD123 CAR-expressing
15 cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) of the invention is a cancer associated with expression of CD123. An example of a cancer that is treatable by the CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) of the invention includes but is not limited to AML, Hodgkin lymphoma, myelodysplastic syndrome, chronic myeloid leukemia and other myeloproliferative neoplasms, or Blastic plasmacytoid dendritic
20 cell neoplasm, and the like.

The invention includes a type of cellular therapy where immune effector cells, e.g., T cells or NK cells, are genetically modified to express a chimeric antigen receptor (CAR) and the CD123 CAR-expressing cell (e.g., CD123 CART or CD123 CAR-expressing NK cell) is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient.
25 Unlike antibody therapies, CAR-modified immune effector cells, e.g., the CAR-modified T cells or CAR-modified NK cells) are able to replicate in vivo resulting in long-term persistence that can lead to sustained tumor control. In various aspects, the immune effector cells, e.g., T cells or NK cells, administered to the patient, or their progeny, persist in the patient for at least four months, five months, six months, seven months, eight months, nine months, ten months,
30 eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one

months, twenty-two months, twenty-three months, two years, three years, four years, or five years after administration of the immune effector cell, e.g., T cell or NK cell, to the patient.

The invention also includes a type of cellular therapy where immune effector cells, e.g., T cells or NK cells, are modified, e.g., by in vitro transcribed RNA, to transiently express a chimeric antigen receptor (CAR) and the CAR-expressing cell, e.g., CAR T cell or CAR NK cell) is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Thus, in various aspects, the immune effector cells, e.g., T cells or NK cells, administered to the patient, is present for less than one month, e.g., three weeks, two weeks, one week, after administration of the immune effector cell, e.g., T cell or NK cell, to the patient.

Without wishing to be bound by any particular theory, the anti-tumor immunity response elicited by the CAR-modified immune effector cells, e.g., T cells or NK cells, may be an active or a passive immune response, or alternatively may be due to a direct vs indirect immune response. In one aspect, the CAR transduced immune effector cells, e.g., T cells or NK cells, exhibit specific proinflammatory cytokine secretion and potent cytolytic activity in response to human cancer cells expressing CD123, resist soluble CD123 inhibition, mediate bystander killing and mediate regression of an established human tumor. For example, antigen-less tumor cells within a heterogeneous field of CD123-expressing tumor may be susceptible to indirect destruction by CD123-redirected immune effector cell, e.g., T cells or NK cells, that has previously reacted against adjacent antigen-positive cancer cells.

In one aspect, the fully-human CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention may be a type of vaccine for ex vivo immunization and/or in vivo therapy in a mammal. In one aspect, the mammal is a human.

With respect to ex vivo immunization, at least one of the following occurs in vitro prior to administering the cell, e.g., T cell or NK cell, into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a CAR to the cells or iii) cryopreservation of the cells.

Ex vivo procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (e.g., a human) and genetically modified (i.e., transduced or transfected in vitro) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the CAR-modified cell can be autologous with

respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

The procedure for ex vivo expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the
5 cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of ex vivo expansion of the cells. Briefly, ex vivo culture and expansion of T cells comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells ex vivo. In addition to the cellular growth factors described in U.S.
10 Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

In addition to using a cell-based vaccine in terms of ex vivo immunization, the present invention also provides compositions and methods for in vivo immunization to elicit an immune response directed against an antigen in a patient.

15 Generally, the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, the CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention are used in the treatment of diseases, disorders and conditions associated with expression of CD123. In certain aspects, the cells of the invention are used in the treatment of patients at risk
20 for developing diseases, disorders and conditions associated with expression of CD123. Thus, the present invention provides methods for the treatment or prevention of diseases, disorders and conditions associated with expression of CD123 comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention.

25 In one aspect, the CAR-expressing cells (CART cells or CAR-expressing NK cells) of the inventions may be used to treat a proliferative disease such as a cancer or malignancy or is a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia. In one aspect, a cancer associated with expression of CD123 is a hematological cancer preleukemia, a hyperproliferative disorder, a hyperplasia or a dysplasia, which is
30 characterized by abnormal growth of cells.

In one aspect, the CAR-expressing cells (CART cells or CAR-expressing NK cells) of the invention are used to treat a cancer, wherein the cancer is a hematological cancer.

Hematological cancer conditions are the types of cancer such as leukemia and malignant lymphoproliferative conditions that affect blood, bone marrow and the lymphatic system.

In one aspect, the compositions and CAR-expressing cells (CAR cells or CAR-expressing NK cells) of the present invention are particularly useful for treating myeloid leukemias, AML and its subtypes, chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), histiocytic disorders, and mast cell disorders.

Leukemia can be classified as acute leukemia and chronic leukemia. Acute leukemia can be further classified as acute myelogenous leukemia (AML) and acute lymphoid leukemia (ALL). Chronic leukemia includes chronic myelogenous leukemia (CML) and chronic lymphoid leukemia (CLL). Other related conditions include myelodysplastic syndromes (MDS, formerly known as “preleukemia”) which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

Lymphoma is a group of blood cell tumors that develop from lymphocytes. Exemplary lymphomas include non-Hodgkin lymphoma and Hodgkin lymphoma.

In AML, malignant transformation and uncontrolled proliferation of an abnormally differentiated, long-lived myeloid progenitor cell results in high circulating numbers of immature blood forms and replacement of normal marrow by malignant cells. Symptoms include fatigue, pallor, easy bruising and bleeding, fever, and infection; symptoms of leukemic infiltration are present in only about 5% of patients (often as skin manifestations). Examination of peripheral blood smear and bone marrow is diagnostic. Existing treatment includes induction chemotherapy to achieve remission and post-remission chemotherapy (with or without stem cell transplantation) to avoid relapse.

AML has a number of subtypes that are distinguished from each other by morphology, immunophenotype, and cytochemistry. Five classes are described, based on predominant cell type, including myeloid, myeloid-monocytic, monocytic, erythroid, and megakaryocytic.

Remission induction rates range from 50 to 85%. Long-term disease-free survival reportedly occurs in 20 to 40% of patients and increases to 40 to 50% in younger patients treated with stem cell transplantation.

Prognostic factors help determine treatment protocol and intensity; patients with strongly negative prognostic features are usually given more intense forms of therapy, because

the potential benefits are thought to justify the increased treatment toxicity. The most important prognostic factor is the leukemia cell karyotype; favorable karyotypes include t(15;17), t(8;21), and inv16 (p13;q22). Negative factors include increasing age, a preceding myelodysplastic phase, secondary leukemia, high WBC count, and absence of Auer rods.

5 Initial therapy attempts to induce remission and differs most from ALL in that AML responds to fewer drugs. The basic induction regimen includes cytarabine by continuous IV infusion or high doses for 5 to 7 days; daunorubicin or idarubicin is given IV for 3 days during this time. Some regimens include 6-thioguanine, etoposide, vincristine, and prednisone, but their contribution is unclear. Treatment usually results in significant myelosuppression, with
10 infection or bleeding; there is significant latency before marrow recovery. During this time, meticulous preventive and supportive care is vital.

Chronic myelogenous (or myeloid) leukemia (CML) is also known as chronic granulocytic leukemia, and is characterized as a cancer of the white blood cells. Common treatment regimens for CML include Bcr-Abl tyrosine kinase inhibitors, imatinib (Gleevec®),
15 dasatinib and nilotinib. Bcr-Abl tyrosine kinase inhibitors are specifically useful for CML patients with the Philadelphia chromosome translocation.

Myelodysplastic syndromes (MDS) are hematological medical conditions characterized by disorderly and ineffective hematopoiesis, or blood production. Thus, the number and quality of blood-forming cells decline irreversibly. Some patients with MDS can develop severe
20 anemia, while others are asymptomatic. The classification scheme for MDS is known in the art, with criteria designating the ratio or frequency of particular blood cell types, e.g., myeloblasts, monocytes, and red cell precursors. MDS includes refractory anemia, refractory anemia with ring sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, chronic myelomonocytic leukemia (CML).

25 Treatments for MDS vary with the severity of the symptoms. Aggressive forms of treatment for patients experiencing severe symptoms include bone marrow transplants and supportive care with blood product support (e.g., blood transfusions) and hematopoietic growth factors (e.g., erythropoietin). Other agents are frequently used to treat MDS: 5-azacytidine, decitabine, and lenalidomide. In some cases, iron chelators deferoxamine (Desferal®) and
30 deferasirox (Exjade®) may also be administered.

In another embodiment, the CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells) of the present invention are used to treat cancers or leukemias with leukemia stem cells. For example, the leukemia stem cells are CD34⁺/CD38⁻ leukemia cells.

5 The present invention provides, among other things, compositions and methods for treating cancer. In one aspect, the cancer is a hematologic cancer including but is not limited to leukemia (such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia and myelodysplastic syndrome) and malignant lymphoproliferative conditions, including lymphoma (such as multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-
10 follicular lymphoma).

In one aspect, the CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells) of the invention may be used to treat other cancers and malignancies such as, but not limited to, e.g., acute leukemias including but not limited to, e.g., B-cell acute lymphoid leukemia ("B-ALL"), T-cell acute lymphoid leukemia ("T-ALL"), acute lymphoid leukemia
15 (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular
20 lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, Hodgkin's lymphoma, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or
25 dysplasia) of myeloid blood cells, and the like. The CAR-modified immune effector cells, e.g., T cells or NK cells, of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations.

The present invention also provides methods for inhibiting the proliferation or reducing
30 a CD123-expressing cell population, the methods comprising contacting a population of cells comprising a CD123-expressing cell with a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In a

specific aspect, the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing CD123, the methods comprising contacting the CD123-expressing cancer cell population with a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In one aspect, the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing CD123, the methods comprising contacting the CD123-expressing cancer cell population with a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In certain aspects, the CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% in a subject with or animal model for myeloid leukemia or another cancer associated with CD123-expressing cells relative to a negative control. In one aspect, the subject is a human.

The present invention also provides methods for preventing, treating and/or managing a disease associated with CD123-expressing cells (e.g., a hematologic cancer or atypical cancer expressing CD123), the methods comprising administering to a subject in need a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with CD123-expressing cells include autoimmune disorders (such as lupus), inflammatory disorders (such as allergies and asthma) and cancers (such as hematological cancers or atypical cancers expressing CD123).

The present invention also provides methods for preventing, treating and/or managing a disease associated with CD123-expressing cells, the methods comprising administering to a subject in need a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In one aspect, the subject is a human.

The present invention provides methods for preventing relapse of cancer associated with CD123-expressing cells, the methods comprising administering to a subject in need thereof a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention that binds to the CD123-expressing cell. In one aspect, the methods comprise administering to the subject in need thereof an effective amount of a CAR-expressing cell (e.g.,

CD123 CART cell or CD123 CAR-expressing NK cell) described herein that binds to the CD123-expressing cell in combination with an effective amount of another therapy.

Bone Marrow Ablation

In one aspect, the present invention provides compositions and methods for bone marrow ablation. For example, in one aspect, the invention provides compositions and methods for eradication of at least a portion of existing bone marrow in a subject. It is described herein that, in certain instances, the CART123 cells comprising a CD123 CAR of the present invention eradicates CD123 positive bone marrow myeloid progenitor cells.

In one aspect, the invention provides a method of bone marrow ablation comprising administering a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention to a subject in need of bone marrow ablation. For example, the present method may be used to eradicate some or all of the existing bone marrow of a subject having a disease or disorder in which bone marrow transplantation or bone marrow reconditioning is a beneficial treatment strategy. In one aspect, the bone marrow ablation method of the invention, comprising the administration of a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) described elsewhere herein, is performed in a subject prior to bone marrow transplantation. Thus, in one aspect, the method of the invention provides a cellular conditioning regimen prior to bone marrow or stem cell transplantation. In one aspect, bone marrow transplantation comprises transplantation of a stem cell. The bone marrow transplantation may comprise transplantation of autologous or allogeneic cells.

The present invention provides a method of treating a disease or disorder comprising administering a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention to eradicate at least a portion of existing bone marrow. The method may be used as at least a portion of a treatment regimen for treating any disease or disorder where bone marrow transplantation is beneficial. That is, the present method may be used in any subject in need of a bone marrow transplant. In one aspect, bone marrow ablation comprising administration of a CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) is useful in the treatment of AML. In certain aspects, bone marrow ablation by way of the present method is useful in treating a hematological cancer, a solid tumor, a hematologic disease, a metabolic disorder, HIV, HTLV, a lysosomal storage disorder, and an immunodeficiency.

Compositions and methods disclosed herein may be used to eradicate at least a portion of existing bone marrow to treat hematological cancers including, but not limited to, leukemia, lymphoma, myeloma, ALL, AML, CLL, CML, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, and multiple myeloma.

5 Compositions and methods disclosed herein may be used to treat hematologic diseases including, but not limited to myelodysplasia, anemia, paroxysmal nocturnal hemoglobinuria, aplastic anemia, acquired pure red cell anemia, Diamond-Blackfan anemia, Fanconi anemia, cytopenia, amegakaryotic thrombocytopenia, myeloproliferative disorders, polycythemia vera, essential thrombocytosis, myelofibrosis, hemoglobinopathies, sickle cell disease, β thalassemia
10 major, among others.

Compositions and methods disclosed herein may be used to treat lysosomal storage disorders including, but not limited to lipidoses, sphingolipidoses, leukodystrophies, mucopolysaccharidoses, glycoproteinoses, infantile neuronal ceroid lipofuscinosis, Jansky-Bielschowsky disease, Niemann-Pick disease, Gaucher disease, adrenoleukodystrophy,
15 metachromatic leukodystrophy, Krabbe disease, Hurler syndrome, Scheie syndrome, Hurler-Scheie syndrome, Hunter syndrome, Sanfilippo syndrome, Morquio syndrome, Maroteaux-Lamy syndrome, Sly syndrome, mucopolysaccharidosis, fucopolysaccharidosis, aspartylglucosaminuria, alpha-mannosidoses, and Wolman disease.

Compositions and methods disclosed herein may be used to treat immunodeficiencies
20 including, but not limited to, T-cell deficiencies, combined T-cell and B-cell deficiencies, phagocyte disorders, immune dysregulation diseases, innate immune deficiencies, ataxia telangiectasia, DiGeorge syndrome, severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome, Kostmann syndrome, Shwachman-Diamond syndrome, Griscelli syndrome, and NF-Kappa-B Essential Modulator (NEMO) deficiency.

25 In one aspect, the present invention provides a method of treating cancer comprising bone marrow conditioning, where at least a portion of bone marrow of the subject is eradicated by the CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) of the invention. For example, in certain instances, the bone marrow of the subject comprises a malignant precursor cell that can be targeted and eliminated by the activity of the CAR-
30 expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell). In one aspect, a bone marrow conditioning therapy comprises administering a bone marrow or stem cell transplant to the subject following the eradication of native bone marrow. In one aspect, the

bone marrow reconditioning therapy is combined with one or more other anti-cancer therapies, including, but not limited to anti-tumor CAR therapies, chemotherapy, radiation, and the like.

In one aspect, eradication of the administered CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) may be required prior to infusion of bone marrow or stem cell transplant. Eradication of the CAR-expressing cell (e.g., CD123 CART cell or CD123 CAR-expressing NK cell) may be accomplished using any suitable strategy or treatment, including, but not limited to, use of a suicide gene, limited CAR persistence using RNA encoded CARs, or anti-T cell modalities including antibodies or chemotherapy.

10 **Combination Therapies**

A CAR-expressing cell described herein may be used in combination with other known agents and therapies. Administered “in combination”, as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

A CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially.

For sequential administration, the CAR-expressing cell described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

5 The CAR therapy and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The CAR therapy can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

10 When administered in combination, the CAR therapy and the additional agent (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain embodiments, the administered amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, e.g., as a monotherapy. In other embodiments, the amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment of cancer) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent used individually, e.g., as a monotherapy, required to achieve the same therapeutic effect.

20 In further aspects, a CAR-expressing cell described herein may be used in a treatment regimen in combination with surgery, cytokines, radiation, or chemotherapy such as cytoxan, fludarabine, histone deacetylase inhibitors, demethylating agents, or peptide vaccine, such as that described in Izumoto et al. 2008 J Neurosurg 108:963-971.

25 In certain instances, compounds of the present invention are combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

30 In one embodiment, a CAR-expressing cell described herein can be used in combination with a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, ofatumumab, tositumomab, brentuximab), an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (e.g.,

fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

General Chemotherapeutic agents considered for use in combination therapies include

5 anastrozole (Arimidex[®]), bicalutamide (Casodex[®]), bleomycin sulfate (Blenoxane[®]), busulfan (Myleran[®]), busulfan injection (Busulfex[®]), capecitabine (Xeloda[®]), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin[®]), carmustine (BiCNU[®]), chlorambucil (Leukeran[®]), cisplatin (Platinol[®]), cladribine (Leustatin[®]), cyclophosphamide (Cytosan[®] or Neosar[®]), cytarabine, cytosine arabinoside (Cytosar-U[®]), cytarabine liposome injection

10 (DepoCyt[®]), dacarbazine (DTIC-Dome[®]), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine[®]), daunorubicin citrate liposome injection (DaunoXome[®]), dexamethasone, docetaxel (Taxotere[®]), doxorubicin hydrochloride (Adriamycin[®], Rubex[®]), etoposide (Vepesid[®]), fludarabine phosphate (Fludara[®]), 5-fluorouracil (Adrucil[®], Efudex[®]), flutamide (Eulexin[®]), tezacitibine, Gemcitabine

15 (difluorodeoxycytidine), hydroxyurea (Hydrea[®]), Idarubicin (Idamycin[®]), ifosfamide (IFEX[®]), irinotecan (Camptosar[®]), L-asparaginase (ELSPAR[®]), leucovorin calcium, melphalan (Alkeran[®]), 6-mercaptopurine (Purinethol[®]), methotrexate (Folex[®]), mitoxantrone (Novantrone[®]), mylotarg, paclitaxel (Taxol[®]), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel[®]), tamoxifen citrate (Nolvadex[®]), teniposide

20 (Vumon[®]), 6-thioguanine, thiotepa, tirapazamine (Tirazone[®]), topotecan hydrochloride for injection (Hycamptin[®]), vinblastine (Velban[®]), vincristine (Oncovin[®]), and vinorelbine (Navelbine[®]).

Anti-cancer agents of particular interest for combinations with the compounds of the present invention include: anthracyclines; alkylating agents; antimetabolites; drugs that inhibit

25 either the calcium dependent phosphatase calcineurin or the p70S6 kinase FK506) or inhibit the p70S6 kinase;; mTOR inhibitors; immunomodulators; anthracyclines; vinca alkaloids; proteasome inhibitors; GITR agonists; protein tyrosine phosphatase inhibitors; a CDK4 kinase inhibitor; a BTK inhibitor; a MKN kinase inhibitor; a DGK kinase inhibitor; or an oncolytic virus.

Exemplary antimetabolites include, without limitation, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors): methotrexate (Rheumatrex®, Trexall®), 5-fluorouracil (Adrucil®, Efudex®, Fluoroplex®), floxuridine (FUDF®), cytarabine (Cytosar-U®, Tarabine PFS), 6-mercaptopurine (Puri-Nethol®), 6-thioguanine (Thioguanine
 5 Tabloid®), fludarabine phosphate (Fludara®), pentostatin (Nipent®), pemetrexed (Alimta®), raltitrexed (Tomudex®), cladribine (Leustatin®), clofarabine (Clofarex®, Clolar®), azacitidine (Vidaza®), decitabine and gemcitabine (Gemzar®). Preferred antimetabolites include, cytarabine, clofarabine and fludarabine.

Exemplary alkylating agents include, without limitation, nitrogen mustards,
 10 ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytosan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®),
 15 melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation,
 20 Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®);
 25 Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytosan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine
 30 hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESP and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytosan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and rituximab (FCR). In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (*IgV_H*) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (*IgV_H*) gene. In embodiments, the fludarabine is administered at a dosage of about 10-50 mg/m² (e.g., about 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, or 45-50 mg/m²), e.g., intravenously. In embodiments, the cyclophosphamide is administered at a dosage of about 200-300 mg/m² (e.g., about 200-225, 225-250, 250-275, or 275-300 mg/m²), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m² (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m²), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with bendamustine and rituximab. In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (*IgV_H*) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (*IgV_H*) gene. In embodiments, the bendamustine is administered at a dosage of about 70-110 mg/m² (e.g., 70-80, 80-90, 90-100, or 100-110 mg/m²), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m² (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m²), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and/or a corticosteroid (e.g., prednisone). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and prednisone (R-CHOP). In embodiments, the subject has diffuse large B-cell

lymphoma (DLBCL). In embodiments, the subject has nonbulky limited-stage DLBCL (e.g., comprises a tumor having a size/diameter of less than 7 cm). In embodiments, the subject is treated with radiation in combination with the R-CHOP. For example, the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP), followed by radiation. In some cases, the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP) following radiation.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with dose-adjusted EPOCH-R (DA-EPOCH-R). In embodiments, the subject has a B cell lymphoma, e.g., a Myc-rearranged aggressive B cell lymphoma.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and/or lenalidomide. Lenalidomide ((*RS*)-3-(4-Amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl)piperidine-2,6-dione) is an immunomodulator. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and lenalidomide. In embodiments, the subject has follicular lymphoma (FL) or mantle cell lymphoma (MCL). In embodiments, the subject has FL and has not previously been treated with a cancer therapy. In embodiments, lenalidomide is administered at a dosage of about 10-20 mg (e.g., 10-15 or 15-20 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m² (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m²), e.g., intravenously.

Exemplary mTOR inhibitors include, e.g., temsirolimus; ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-penta-oxo-11,36-dioxo-4-azatricyclo[30.3.1.0^{4,9}] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-

methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and *N*²-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L- α -aspartyl-L-serine- (SEQ ID NO: 5 706), inner salt (SF1126, CAS 936487-67-1), and XL765.

Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ , CAS 951209-71-5, available from IRX Therapeutics).

Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (daunorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; 15 herbimycin; ravidomycin; and desacetylravidomycin.

Exemplary vinca alkaloids include, e.g., vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

Exemplary proteasome inhibitors include bortezomib (Velcade®); carfilzomib (PX- 20 171-007, (*S*)-4-Methyl-*N*-(((*S*)-1-(((*S*)-4-methyl-1-((*R*)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and *O*-Methyl-*N*-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-*O*-methyl-*N*-[(1*S*)-2-[(2*R*)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]-L-serinamide 25 (ONX-0912).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab. Brentuximab is an antibody-drug conjugate of anti-CD30 antibody and monomethyl auristatin E. In embodiments, the subject has Hodgkin's lymphoma (HL), e.g., relapsed or refractory HL. In embodiments, the subject comprises CD30+ HL. In 30 embodiments, the subject has undergone an autologous stem cell transplant (ASCT). In embodiments, the subject has not undergone an ASCT. In embodiments, brentuximab is

administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab and dacarbazine or in combination with brentuximab and bendamustine. Dacarbazine is an alkylating agent with a chemical name of 5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide. Bendamustine is an alkylating agent with a chemical name of 4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid. In embodiments, the subject has Hodgkin's lymphoma (HL). In embodiments, the subject has not previously been treated with a cancer therapy. In embodiments, the subject is at least 60 years of age, e.g., 60, 65, 70, 75, 80, 85, or older. In embodiments, dacarbazine is administered at a dosage of about 300-450 mg/m² (e.g., about 300-325, 325-350, 350-375, 375-400, 400-425, or 425-450 mg/m²), e.g., intravenously. In embodiments, bendamustine is administered at a dosage of about 75-125 mg/m² (e.g., 75-100 or 100-125 mg/m², e.g., about 90 mg/m²), e.g., intravenously. In embodiments, brentuximab is administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bispecific antibody) or a fragment thereof. Exemplary anti-CD20 antibodies include but are not limited to rituximab, ofatumumab, ocrelizumab, veltuzumab, obinutuzumab, TRU-015 (Trubion Pharmaceuticals), ocaratuzumab, and Pro131921 (Genentech). See, e.g., Lim et al. *Haematologica*. 95.1(2010):135-43.

In some embodiments, the anti-CD20 antibody comprises rituximab. Rituximab is a chimeric mouse/human monoclonal antibody IgG1 kappa that binds to CD20 and causes cytolysis of a CD20 expressing cell, e.g., as described in www.accessdata.fda.gov/drugsatfda_docs/label/2010/103705s53111bl.pdf. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab. In embodiments, the subject has CLL or SLL.

In some embodiments, rituximab is administered intravenously, e.g., as an intravenous infusion. For example, each infusion provides about 500-2000 mg (e.g., about 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, or 1900-2000 mg) of rituximab. In some embodiments, rituximab is administered at a dose of

150 mg/m² to 750 mg/m², e.g., about 150-175 mg/m², 175-200 mg/m², 200-225 mg/m², 225-250 mg/m², 250-300 mg/m², 300-325 mg/m², 325-350 mg/m², 350-375 mg/m², 375-400 mg/m², 400-425 mg/m², 425-450 mg/m², 450-475 mg/m², 475-500 mg/m², 500-525 mg/m², 525-550 mg/m², 550-575 mg/m², 575-600 mg/m², 600-625 mg/m², 625-650 mg/m², 650-675 mg/m², or 675-700 mg/m², where m² indicates the body surface area of the subject. In some embodiments, rituximab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, rituximab is administered at a dosing interval of at least 0.5 weeks, e.g., 0.5, 1, 2, 3, 4, 5, 6, 7, 8 weeks, or more. In some embodiments, rituximab is administered at a dose and dosing interval described herein for a period of time, e.g., at least 2 weeks, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 weeks, or greater. For example, rituximab is administered at a dose and dosing interval described herein for a total of at least 4 doses per treatment cycle (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more doses per treatment cycle).

In some embodiments, the anti-CD20 antibody comprises ofatumumab. Ofatumumab is an anti-CD20 IgG1κ human monoclonal antibody with a molecular weight of approximately 149 kDa. For example, ofatumumab is generated using transgenic mouse and hybridoma technology and is expressed and purified from a recombinant murine cell line (NS0). See, e.g., www.accessdata.fda.gov/drugsatfda_docs/label/2009/125326lbl.pdf; and Clinical Trial Identifier number NCT01363128, NCT01515176, NCT01626352, and NCT01397591. In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with ofatumumab. In some embodiments, the subject has CLL or SLL.

In some embodiments, ofatumumab is administered as an intravenous infusion. For example, each infusion provides about 150-3000 mg (e.g., about 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1200, 1200-1400, 1400-1600, 1600-1800, 1800-2000, 2000-2200, 2200-2400, 2400-2600, 2600-2800, or 2800-3000 mg) of ofatumumab. In some embodiments, ofatumumab is administered at a starting dosage of about 300 mg, followed by 2000 mg, e.g., for about 11 doses, e.g., for 24 weeks. In some embodiments, ofatumumab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, ofatumumab is administered at a dosing interval of at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 26, 28, 30 weeks, or more. In some embodiments, ofatumumab is administered at a dose and dosing interval described herein for a period of time,

e.g., at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 40, 50, 60 weeks or greater, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or greater, or 1, 2, 3, 4, 5 years or greater. For example, ofatumumab is administered at a dose and dosing interval described herein for a total of at least 2 doses per treatment cycle (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, or more doses per treatment cycle).

In some cases, the anti-CD20 antibody comprises ocrelizumab. Ocrelizumab is a humanized anti-CD20 monoclonal antibody, e.g., as described in Clinical Trials Identifier Nos. NCT00077870, NCT01412333, NCT00779220, NCT00673920, NCT01194570, and Kappos et al. *Lancet*. 19.378(2011):1779-87.

In some cases, the anti-CD20 antibody comprises veltuzumab. Veltuzumab is a humanized monoclonal antibody against CD20. See, e.g., Clinical Trial Identifier No. NCT00547066, NCT00546793, NCT01101581, and Goldenberg et al. *Leuk Lymphoma*. 51(5)(2010):747-55.

In some cases, the anti-CD20 antibody comprises GA101. GA101 (also called obinutuzumab or RO5072759) is a humanized and glyco-engineered anti-CD20 monoclonal antibody. See, e.g., Robak. *Curr. Opin. Investig. Drugs*. 10.6(2009):588-96; Clinical Trial Identifier Numbers: NCT01995669, NCT01889797, NCT02229422, and NCT01414205; and www.accessdata.fda.gov/drugsatfda_docs/label/2013/125486s000lbl.pdf.

In some cases, the anti-CD20 antibody comprises AME-133v. AME-133v (also called LY2469298 or ocaratuzumab) is a humanized IgG1 monoclonal antibody against CD20 with increased affinity for the FcγRIIIa receptor and an enhanced antibody dependent cellular cytotoxicity (ADCC) activity compared with rituximab. See, e.g., Robak et al. *BioDrugs* 25.1(2011):13-25; and Forero-Torres et al. *Clin Cancer Res*. 18.5(2012):1395-403.

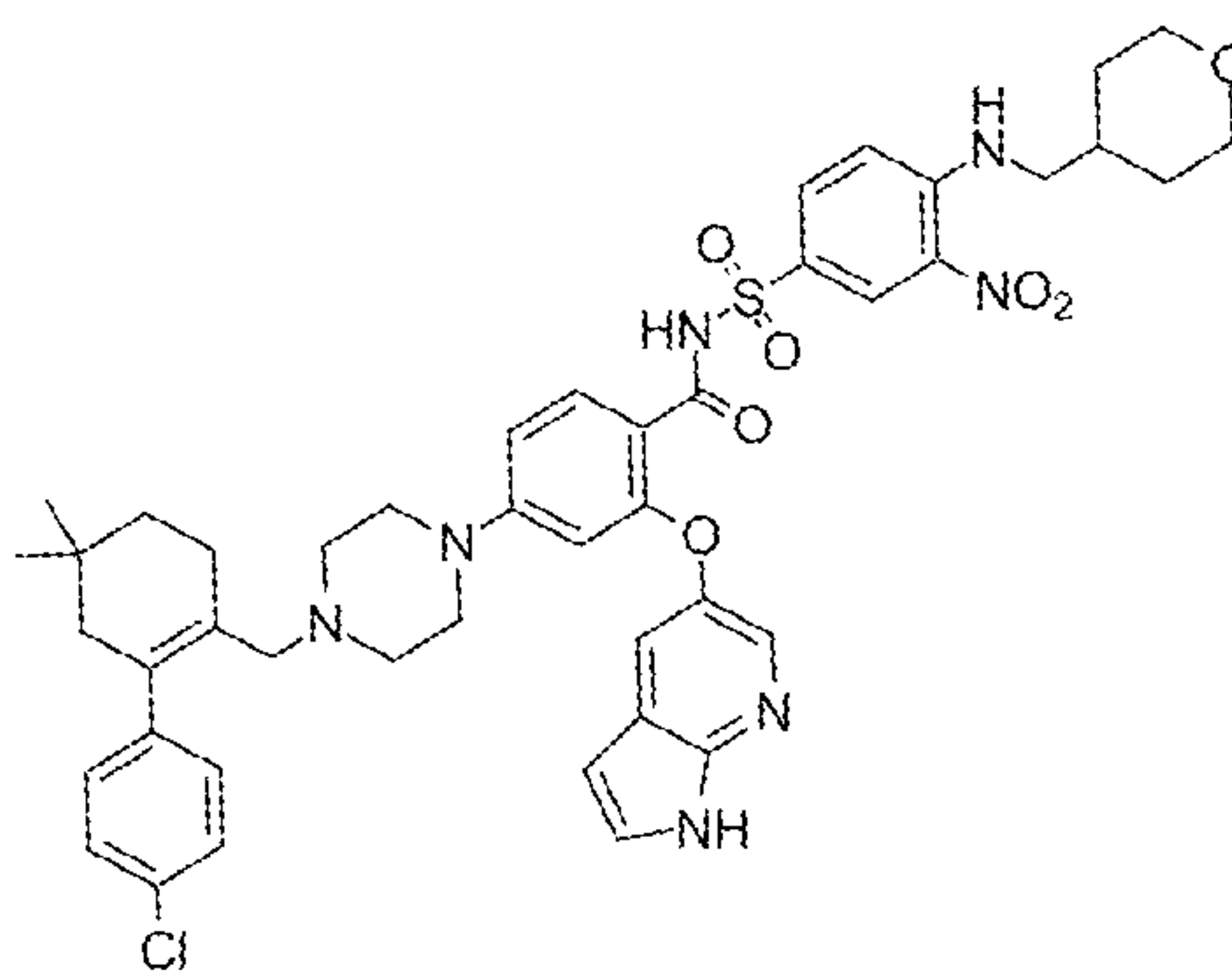
In some cases, the anti-CD20 antibody comprises PRO131921. PRO131921 is a humanized anti-CD20 monoclonal antibody engineered to have better binding to FcγRIIIa and enhanced ADCC compared with rituximab. See, e.g., Robak et al. *BioDrugs* 25.1(2011):13-25; and Casulo et al. *Clin Immunol*. 154.1(2014):37-46; and Clinical Trial Identifier No. NCT00452127.

In some cases, the anti-CD20 antibody comprises TRU-015. TRU-015 is an anti-CD20 fusion protein derived from domains of an antibody against CD20. TRU-015 is smaller than monoclonal antibodies, but retains Fc-mediated effector functions. See, e.g., Robak et al.

BioDrugs 25.1(2011):13-25. TRU-015 contains an anti-CD20 single-chain variable fragment (scFv) linked to human IgG1 hinge, CH2, and CH3 domains but lacks CH1 and CL domains.

In some embodiments, an anti-CD20 antibody described herein is conjugated or otherwise bound to a therapeutic agent, e.g., a chemotherapeutic agent (e.g., cytoxan, fludarabine, histone deacetylase inhibitor, demethylating agent, peptide vaccine, anti-tumor antibiotic, tyrosine kinase inhibitor, alkylating agent, anti-microtubule or anti-mitotic agent), anti-allergic agent, anti-nausea agent (or anti-emetic), pain reliever, or cytoprotective agent described herein.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a B-cell lymphoma 2 (BCL-2) inhibitor (e.g., venetoclax, also called ABT-199 or GDC-0199;) and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with venetoclax and rituximab. Venetoclax is a small molecule that inhibits the anti-apoptotic protein, BCL-2. The structure of venetoclax (4-([2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl)piperazin-1-yl)-N-([3-nitro-4-((tetrahydro-2H-pyran-4-ylmethyl)amino)phenyl]sulfonyl)-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide) is shown below.



In embodiments, the subject has CLL. In embodiments, the subject has relapsed CLL, e.g., the subject has previously been administered a cancer therapy. In embodiments, venetoclax is administered at a dosage of about 15-600 mg (e.g., 15-20, 20-50, 50-75, 75-100, 100-200, 200-300, 300-400, 400-500, or 500-600 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m² (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m²), e.g., intravenously, e.g., monthly.

In some embodiments, a CAR-expressing cell described herein is administered in combination with an oncolytic virus. In embodiments, oncolytic viruses are capable of

selectively replicating in and triggering the death of or slowing the growth of a cancer cell. In some cases, oncolytic viruses have no effect or a minimal effect on non-cancer cells. An oncolytic virus includes but is not limited to an oncolytic adenovirus, oncolytic Herpes Simplex Viruses, oncolytic retrovirus, oncolytic parvovirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, or oncolytic RNA virus (e.g., oncolytic reovirus, oncolytic Newcastle Disease Virus (NDV), oncolytic measles virus, or oncolytic vesicular stomatitis virus (VSV)).

In some embodiments, the oncolytic virus is a virus, e.g., recombinant oncolytic virus, described in US2010/0178684 A1, which is incorporated herein by reference in its entirety. In some embodiments, a recombinant oncolytic virus comprises a nucleic acid sequence (e.g., heterologous nucleic acid sequence) encoding an inhibitor of an immune or inflammatory response, e.g., as described in US2010/0178684 A1, incorporated herein by reference in its entirety. In embodiments, the recombinant oncolytic virus, e.g., oncolytic NDV, comprises a pro-apoptotic protein (e.g., apoptin), a cytokine (e.g., GM-CSF, interferon-gamma, interleukin-2 (IL-2), tumor necrosis factor-alpha), an immunoglobulin (e.g., an antibody against ED-B fibronectin), tumor associated antigen, a bispecific adapter protein (e.g., bispecific antibody or antibody fragment directed against NDV HN protein and a T cell co-stimulatory receptor, such as CD3 or CD28; or fusion protein between human IL-2 and single chain antibody directed against NDV HN protein). See, e.g., Zamarin et al. Future Microbiol. 7.3(2012):347-67, incorporated herein by reference in its entirety. In some embodiments, the oncolytic virus is a chimeric oncolytic NDV described in US 8591881 B2, US 2012/0122185 A1, or US 2014/0271677 A1, each of which is incorporated herein by reference in their entireties.

In some embodiments, the oncolytic virus comprises a conditionally replicative adenovirus (CRAd), which is designed to replicate exclusively in cancer cells. See, e.g., Alemany et al. Nature Biotechnol. 18(2000):723-27. In some embodiments, an oncolytic adenovirus comprises one described in Table 1 on page 725 of Alemany et al., incorporated herein by reference in its entirety.

Exemplary oncolytic viruses include but are not limited to the following:

Group B Oncolytic Adenovirus (ColoAd1) (PsiOxus Therapeutics Ltd.) (see, e.g., Clinical Trial Identifier: NCT02053220);

ONCOS-102 (previously called CGTG-102), which is an adenovirus comprising granulocyte-macrophage colony stimulating factor (GM-CSF) (Oncos Therapeutics) (see, e.g., Clinical Trial Identifier: NCT01598129);

VCN-01, which is a genetically modified oncolytic human adenovirus encoding human
5 PH20 hyaluronidase (VCN Biosciences, S.L.) (see, e.g., Clinical Trial Identifiers: NCT02045602 and NCT02045589);

Conditionally Replicative Adenovirus ICOVIR-5, which is a virus derived from wild-type human adenovirus serotype 5 (Had5) that has been modified to selectively replicate in cancer cells with a deregulated retinoblastoma/E2F pathway (Institut Català d'Oncologia) (see,
10 e.g., Clinical Trial Identifier: NCT01864759);

Celyvir, which comprises bone marrow-derived autologous mesenchymal stem cells (MSCs) infected with ICOVIR5, an oncolytic adenovirus (Hospital Infantil Universitario Niño Jesús, Madrid, Spain/ Ramon Alemany) (see, e.g., Clinical Trial Identifier: NCT01844661);

CG0070, which is a conditionally replicating oncolytic serotype 5 adenovirus (Ad5) in
15 which human E2F-1 promoter drives expression of the essential E1a viral genes, thereby restricting viral replication and cytotoxicity to Rb pathway-defective tumor cells (Cold Genesys, Inc.) (see, e.g., Clinical Trial Identifier: NCT02143804); or

DNX-2401 (formerly named Delta-24-RGD), which is an adenovirus that has been engineered to replicate selectively in retinoblastoma (Rb)-pathway deficient cells and to infect
20 cells that express certain RGD-binding integrins more efficiently (Clinica Universidad de Navarra, Universidad de Navarra/ DNAtrix, Inc.) (see, e.g., Clinical Trial Identifier: NCT01956734).

In some embodiments, an oncolytic virus described herein is administering by injection, e.g., subcutaneous, intra-arterial, intravenous, intramuscular, intrathecal, or intraperitoneal
25 injection. In embodiments, an oncolytic virus described herein is administered intratumorally, transdermally, transmucosally, orally, intranasally, or via pulmonary administration.

In an embodiment, cells expressing a CAR described herein are administered to a subject in combination with a molecule that decreases the Treg cell population. Methods that decrease the number of (e.g., deplete) Treg cells are known in the art and include, e.g., CD25
30 depletion, cyclophosphamide administration, modulating GITR function. Without wishing to be bound by theory, it is believed that reducing the number of Treg cells in a subject prior to apheresis or prior to administration of a CAR-expressing cell described herein reduces the

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What is claimed is:

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD123 binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein said CD123 binding domain comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any CD123 heavy chain binding domain amino acid sequence listed in Table 9, Table 2, or Table 6.
2. The isolated nucleic acid molecule of claim 1, wherein said CD123 binding domain further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any CD123 light chain binding domain amino acid sequence listed in Table 9, Table 2, or Table 6.
3. The isolated nucleic acid molecule of claim 2, wherein said LC CDR1, LC CDR2, and LC CDR3 are the LC CDR sequences listed in Tables 11, 13, 4 or 8.
4. The isolated nucleic acid molecules of any of claims 1-3, wherein said HC CDR1, HC CDR2, and HC CDR3 are the HC CDR sequences listed in Tables 10, 12, 3, or 7.
5. The isolated nucleic acid molecule of any of claims 1-4, which encodes a CAR comprising:
 - (i) the amino acid sequence of any light chain variable region listed in Table 9 or 2;
 - (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of any of the light chain variable regions provided in Table 9 or 2; or
 - (iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the light chain variable regions provided in Table 9 or 2.

6. The isolated nucleic acid molecule of any of claims 1-5, which encodes a CAR comprising:

- (i) the amino acid sequence of any heavy chain variable region listed in Table 9 or 2;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of any of the heavy chain variable regions provided in Table 9 or 2: or
- (iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the heavy chain variable regions provided in Table 9 or 2.

7. The isolated nucleic acid molecule of any of claims 1-6, which encodes a CAR comprising the amino acid sequence of any light chain variable region listed in Table 9 or 2, and the amino acid sequence of any heavy chain variable region listed Table 9 or 2.

8. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded CD123 binding domain comprises:

- (i) the amino acid sequence selected from a group consisting of SEQ ID NO:480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications to any of SEQ ID NO: 480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275; or
- (iii) an amino acid sequence with 95-99% identity to any of SEQ ID NO: 480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275.

9. The isolated nucleic acid molecule of any of the preceding claims, wherein the CD123 binding domain comprises a nucleotide sequence selected from a group consisting of SEQ ID NO: 479, 481, 482, 484, or a nucleotide sequence with 95-99% identity thereof.

10. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

11. The isolated nucleic acid molecule of any of the preceding claims, wherein:

(i) the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, an amino acid sequence comprises at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:6, or an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:6; or

(ii) the nucleic acid sequence encoding the transmembrane domain comprises the nucleotide sequence of SEQ ID NO:17, or a nucleotide sequence with 95-99% identity thereof.

12. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded CD123 binding domain is connected to the transmembrane domain by a hinge region.

13. The nucleic acid molecule of claim 12, wherein:

(i) the encoded hinge region comprises the amino acid sequence of SEQ ID NO:2, or an amino acid sequence comprising at least one, two, or three modifications, but no more than 20, 10, or 5 modifications, or an amino acid sequence with 95-99% identity thereof; or

(ii) the nucleic acid sequence encoding the hinge region comprises the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence with 95-99% identity thereof.

14. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded costimulatory domain is a functional signaling domain obtained from a protein selected from the group consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

15. The isolated nucleic acid molecule of claim 14, wherein the encoded costimulatory domain comprises the amino acid sequence of SEQ ID NO:7, or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7, or an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7.

16. The isolated nucleic acid molecule of claim 14, wherein the nucleic acid sequence encoding the costimulatory domain comprises the nucleotide sequence of SEQ ID NO:18, or a nucleotide sequence with 95-99% identity thereof.

17. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

18. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

19. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

20. The isolated nucleic acid molecule of any of the preceding claims, wherein the nucleic acid sequence encoding the intracellular signaling domain comprises the nucleotide sequence of SEQ ID NO:18, or a nucleotide sequence with 95-99% identity thereof, and/or the nucleotide sequence of SEQ ID NO:20 or SEQ ID NO:21, or a nucleotide sequence with 95-99% identity thereof.

21. The isolated nucleic acid molecule of any of the preceding claims, further comprising a leader sequence which encodes the amino acid sequence of SEQ ID NO:1.

22. The isolated nucleic acid molecule of any of the preceding claims, which encodes a CAR comprising:

- (i) the amino acid sequence of any of SEQ ID NOs: 99, 100, 101, or 98;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications to any of SEQ ID NOs: 99, 100, 101, or 98; or
- (iii) an amino acid sequence with 95-99% identity to any of SEQ ID NOs: 99, 100, 101, or 98.

23. The isolated nucleic acid molecule of any of the preceding claims, comprising the nucleotide sequence of any of SEQ ID NOs: 39, 40, 41, or 38, or a nucleotide sequence with 95-99% identity to any of SEQ ID NOs: 39, 40, 41, or 38.

24. An isolated polypeptide molecule encoded by the nucleic acid molecule of any one of claims 1-23.

25. An isolated chimeric antigen receptor (CAR) polypeptide, wherein the CAR comprises an antibody or antibody fragment which includes a CD123 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain, and wherein said CD123 binding domain comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any CD123 heavy chain binding domain amino acid sequences listed in Table 9, 2 or 6.

26. The isolated CAR polypeptide of claim 25, wherein said CD123 binding domain further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any CD123 light chain binding domain amino acid sequences listed in Table 9, 2 or 6.

27. The isolated CAR polypeptide of claim 26, wherein said LC CDR1, LC CDR2, and LC CDR3 are the LC CDR sequences listed in Tables 11, 13, 4 or 8.

28. The isolated CAR polypeptide of any of claims 25-27, wherein said HC CDR1, HC CDR2, and HC CDR3 are the HC CDR sequences listed in Tables 10, 12, 3, or 7.

29. The isolated CAR polypeptide of any of claims 25-28, comprising:

- (i) the amino acid sequence of any light chain variable region listed in Table 9 or 2;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of any of the light chain variable region provided in Table 9 or 2; or
- (iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the light chain variable region provided in Table 9 or 2.

30. The isolated CAR polypeptide of any of claims 25-29, comprising:

- (i) the amino acid sequence of any heavy chain variable region listed in Table 2 or 9;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of any of the heavy chain variable region provided in Table 2 or 9; or
- (iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the heavy chain variable region provided in Table 2 or 9.

31. The isolated CAR polypeptide of any of claims 25-30, comprising the amino acid sequence of any light chain variable region listed in Table 2 or 9, and the amino acid sequence of any heavy chain variable region listed Table 2 or 9.

32. The isolated CAR polypeptide of any of claims 25-31, comprising:

- (i) the amino acid sequence selected from a group consisting of SEQ ID NO: 480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275;
- (ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications to any of SEQ ID NO: 480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275; or
- (iii) an amino acid sequence with 95-99% identity to any of SEQ ID NO: 480, 483, 485, 478, 158, 159, 160, 157, 217, 218, 219, 216, 276, 277, 278, or 275.

33. The isolated CAR polypeptide of any of claims 25-32, wherein the transmembrane domain comprises a transmembrane domain from a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

34. The isolated CAR polypeptide of any of claims 25-33, wherein:

- (i) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6,
- (ii) an amino acid sequence comprises at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:6, or
- (iii) a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:6.

35. The isolated CAR polypeptide of any of claims 25-34, wherein the CD123 binding domain is connected to the transmembrane domain by a hinge region.

36. The isolated CAR polypeptide of claim 35, wherein the hinge region comprises SEQ ID NO:2, or a sequence with 95-99% identity thereof.

37. The isolated CAR polypeptide of any of claims 25-36, wherein the costimulatory domain is a functional signaling domain obtained from a protein selected from the group

consisting of a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), 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, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, 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, 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, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

38. The isolated CAR polypeptide of any of claims 25-37, wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO:7, or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7.

39. The isolated CAR polypeptide of any of claims 25-37, wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

40. The isolated CAR polypeptide of any of claims 25-39, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

41. The isolated CAR polypeptide of any of claims 25-40, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the amino acid sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

42. The isolated CAR polypeptide of any of claims 25-41, further comprising a leader sequence which comprises the amino acid sequence of SEQ ID NO:1.

43. The isolated CAR polypeptide of any of claims 25-42, comprising:
(i) the amino acid sequence of any of SEQ ID NOs: 99, 100, 101, or 98;
(ii) an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications to any of SEQ ID NOs: 99, 100, 101, or 98; or
(iii) an amino acid sequence with 95-99% identity to any of SEQ ID NOs: 99, 100, 101, or 98.

44. The nucleic acid molecule of any of claims 1-23, which is a DNA molecule, an RNA molecule, or a combination thereof.

45. The nucleic acid molecule of claim 44, which is an mRNA encoding the CAR polypeptide of claims 24-43.

46. A vector comprising the nucleic acid molecule of any of claims 1-23, or a nucleic acid encoding the CAR polypeptide of any of claims 24-43, wherein the vector is a DNA vector or an RNA vector.

47. The vector of claim 46, which is selected from the group consisting of a plasmid, a lentivirus vector, adenoviral vector, and a retrovirus vector.

48. The vector of claim 46 or 47, further comprising an EF-1 promoter comprising the sequence of SEQ ID NO: 11.

49. A cell, e.g., an immune effector cell, comprising the nucleic acid molecule of any of claims 1-23 or 44-45, the CAR polypeptide of any of claims 24-43, or the vector of any of claims 46-48.

50. A method of making a cell, e.g., an immune effector cell, comprising introducing into, e.g., transducing, an immune effector cell with the nucleic acid molecule of claims 1-23 or 44-45, or the vector of any of claims 46-48.

51. A method of generating a population of cells, comprising introducing RNA into a cell, where the RNA comprises the nucleic acid molecule of any of claims 1-23 or 44-45, or a nucleic acid encoding the CAR polypeptide of any of claims 24-43.

52. A method of providing an anti-tumor immunity in a mammal, comprising administering to the mammal an effective amount of a cell, e.g., a population of immune effector cells, comprising the nucleic acid molecule of any of claims 1-23, or the CAR polypeptide of any of claims 24-43.

53. The method of claim 52, wherein the cell is an autologous T cell or an allogeneic T cell.

54. A method of treating a mammal having a disease associated with expression of CD123, comprising administering to the mammal an effective amount of a cell, e.g., a population of immune effector cells, comprising the nucleic acid molecule of any of claims 1-23 or 44-45, or the CAR polypeptide of any of claims 24-43.

55. The method of claim 54, wherein the disease associated with CD123 expression is:

- (i) a cancer or malignancy,
- (ii) a precancerous condition, or
- (ii) a non-cancer related indication associated with expression of CD123.

56. The method of claim 54 or 55, wherein the disease is a hematologic cancer or a pre-leukemia.

57. The method of any of claims 54-56, wherein the disease is chosen from one or more of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), acute lymphoblastic B-cell leukemia (B-cell acute lymphoid leukemia, BALL), acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL), B-cell prolymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia (CML), hairy cell leukemia, Hodgkin lymphoma, a histiocytic disorder, a mast cell disorder, a myelodysplasia, a myelodysplastic syndrome, a myeloproliferative neoplasm, a plasma cell myeloma, a plasmacytoid dendritic cell neoplasm, or a combination thereof.

58. The method of any of claims 52-57, wherein the cell, e.g., the population of immune effector cells, is administered in combination with one or more of:

- (i) an agent that increases the efficacy of the cell comprising the CAR nucleic acid or CAR polypeptide;
- (ii) an agent that ameliorates one or more side effects associated with administration of the cell comprising the CAR nucleic acid or CAR polypeptide; or
- (iii) an agent that treats the disease associated with the expression of CD123.

59. The method of any of claims 52-58, wherein the cell, e.g., the population of immune effector cells, comprises an mRNA encoding the CAR polypeptide of claims 24-43.

60. The method of any of claims 52-58, wherein the cell, e.g., the population of immune effector cells, comprises a lentiviral vector comprising the nucleic acid molecule of any of claims 1-23, or encoding the CAR polypeptide of claims 24-43.

61. The method of claims 52-57, wherein the cell, e.g., the population of immune effector cells, is administered in combination with second therapeutic agent or procedure chosen from one or more of chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, a cytokine, surgical procedure, a radiation procedure, an agonist of a costimulatory molecule, an inhibitor of an immune checkpoint molecule, a vaccine, or a second CAR-based immunotherapy.

62. The method of claims 52-61, wherein the cell, e.g., the population of immune effector cells, is administered in combination with an agonist of a costimulatory molecule chosen from one or more of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3 or CD83 ligand.

63. The method of claims 52-61, wherein the cell, e.g., the population of immune effector cells, is administered in combination with an inhibitor of an immune checkpoint molecule chosen from one or more of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM-1, CEACAM-3, CEACAM-5, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, or TGFR, e.g., TGFRbeta.

64. The method of claim 61, wherein the cell, e.g., the population of immune effector cells, is administered in combination with a PD-1 inhibitor, a TIM-3 inhibitor, a CEACAM-1 inhibitor, or a combination thereof.

65. The method of claim 64, wherein the PD-1 inhibitor and the TIM-3 inhibitor are administered in combination.

66. The method of claim 64, wherein the TIM-3 inhibitor and the CEACAM-1 inhibitor are administered in combination.

67. The method of any of claims 61-66, wherein the agonist or the inhibitor is an antibody molecule, e.g., a monospecific antibody molecule or a bispecific antibody molecule.

68. The method of any of claims 61-67, wherein the inhibitor is administered subsequent to the administration of the cell, e.g., the population of immune effector cells.

69. The method of claim 68, wherein the inhibitor is administered about 3-7 days after the administration of the cell, e.g., the population of immune effector cells.

70. The method of claims 52-57, wherein the cell, e.g., the population of immune effector cells, is administered in combination with a CD19 inhibitor.

71. The method of claim 70, wherein the CD19 inhibitor is a CD19 CAR-expressing cell or an anti-CD19 antibody molecule.

72. The method of any of claims 54-71, wherein the disease is a CD19-negative cancer, e.g., a CD19-negative relapsed cancer.

73. A method of preventing a CD19-negative relapse in a mammal, comprising administering to the mammal an effective amount of a cell, e.g., a population of immune effector cells, comprising the nucleic acid of any of claims 1-23 or 44-45, or the polypeptide of any of claims 24-43.

74. The method of claim 73, further comprising administering a CD19 inhibitor, e.g., a CD19 CAR-expressing cell.

75. The method of any of claims 54-74, wherein the disease is a leukemia, e.g., acute lymphoblastic leukemia (e.g., relapsing and refractory ALL), or AML.

76. The method of any of claims 70-75, wherein the cell, e.g., the population of immune effector cells, is administered after administration of the CD19 inhibitor.

77. The method of any of claims 70-75, wherein the cell, e.g., the population of immune effector cells, is administered concurrently with the CD19 inhibitor.

78. The method of any of claims 52-75 or 77, wherein the cell, e.g., the population of immune effector cells, expresses a CD19 CAR and the CD123 CAR.

79. The method of claim 77 or 78, wherein the CD19 CAR or CD123 CAR comprises a split intracellular signaling domain such that full activation of the cell, e.g., the population of immune effector cells, occurs when both the CD19 CAR and CD123 CAR bind to a target cell,

e.g., a target CD19+CD123+ cell (e.g., a B-ALL blast cell), compared to activation when the CD19 CAR and CD123 CAR bind to a target cell that expresses one of CD19 or CD123 (e.g., a hematopoietic stem cell).

80. The method of claim 79, wherein the CD123CAR comprises a costimulatory domain, e.g., a 4-1BB signaling domain, and the CD19 CAR comprises a primary signaling domain, e.g., a CD3 zeta signaling domain.

81. The method any of claims 52-60, further comprising administering a T cell depleting agent after treatment with the cell, e.g., the population of immune effector cells, thereby reducing (e.g., depleting) the cell (e.g., a CD123CAR-expressing cell).

82. A method of reducing (e.g., depleting) CD123CAR-expressing cells after a CAR therapy, comprising administering to a mammal a T cell depleting agent, after treatment of the mammal with a cell, e.g., a population of immune effector cells, comprising the nucleic acid molecule of any of claims 1-23 or 44-45, or the CAR polypeptide of any of claims 24-43, thereby reducing (e.g., depleting) the cell (e.g., the CD123CAR-expressing cell).

83. The method of claim 81 or 82, wherein the T cell depleting agent is administered one, two, three, four, or five weeks after administration of the cell, e.g., the population of immune effector cells.

84. The method of any of claims 81-83, wherein the mammal has a leukemia, e.g., ALL or AML.

85. The method of any of claims 81-84, wherein the T cell depleting agent is a CD52 inhibitor.

86. The method of claim 85, wherein the CD52 inhibitor is an anti-CD52 antibody molecule, e.g., alemtuzumab.

87. The method of any of claims 81-86, wherein the cell, e.g., the population of immune effector cells, expresses a CD123CAR polypeptide and a target protein recognized by the T cell depleting agent.

88. The method of claim 87, wherein the target protein is CD20.

89. The method of claim 88, wherein the T cell depleting agent is an anti-CD20 antibody, e.g., rituximab.

90. The method of claim 87-89, wherein the cell, e.g., the population of immune effector cells, comprises a nucleic acid (e.g., vector) comprising the CD123CAR nucleic acid and a nucleic acid encoding the target protein.

91. The method of any of claims 52-90, further comprising transplanting a cell, e.g., a hematopoietic stem cell, or a bone marrow, into the mammal.

92. The isolated nucleic acid molecule of any of claims 1-23 or 44-45, the polypeptide of any of claims 24-43, the vector of either of any of claims 46-48, or the cell of claim 49 for use as a medicament.

93. The isolated nucleic acid molecule of any of claims 1-23 or 44-45, the polypeptide of any of claims 24-43, the vector of either of any of claims 46-48, or the cell of claim 49 for use in the treatment of a disease associated with expression of CD123.

94. The isolated nucleic acid molecule of any of claims 1-23 or 44-45, the polypeptide of any of claims 24-43, the vector of either of any of claims 46-48, or the cell of claim 49 for use in the treatment of a cancer.

95. The use of claim 93, wherein the disease is chosen from AML, ALL, B-ALL, T-ALL, B-cell prolymphocytic leukemia, chronic lymphocytic leukemia, CML, hairy cell leukemia, Hodgkin lymphoma, mast cell disorder, myelodysplastic syndrome,

myeloproliferative neoplasm, plasma cell myeloma, plasmacytoid dendritic cell neoplasm, or a combination thereof.

96. A cell, e.g., a population of immune effector cells, of claim 49, further expressing a chimeric molecule that comprises a first polypeptide that comprises at least a portion of an inhibitory molecule, associated with a second polypeptide that comprises a positive signal from an intracellular signaling domain.

97. The cell of claim 96, wherein the chimeric molecule comprise first polypeptide that comprises at least a portion of PD1 and a second polypeptide comprising a costimulatory domain and primary signaling domain.

98. The method of claim 58, wherein the agent is an mTOR inhibitor and the subject is administered a low, immune enhancing, dose of an mTOR inhibitor, e.g., RAD001 or rapamycin.

99. The method of claim 98, wherein the mTOR inhibitor is administered for an amount of time sufficient to decrease the proportion of PD-1 positive T cells, increase the proportion of PD-1 negative T cells, or increase the ratio of PD-1 negative T cells/ PD-1 positive T cells, in the peripheral blood of the subject, or in a preparation of T cells isolated from the subject.

100. A method of conditioning a subject prior to cell transplantation comprising administering to the subject an effective amount of the cell comprising the CAR nucleic acid molecule of any of claims 1-23 or 44-45, or the polypeptide of any of claims 24-43.

101. The method of claim 100, wherein the cell transplantation is a stem cell transplantation, e.g., a hematopoietic stem cell transplantation, or a bone marrow transplantation.

102. The method of either of claim 100 or 101, wherein conditioning a subject prior to cell transplantation comprises reducing the number of CD123-expressing cells in a subject, e.g., CD123-expressing normal cells or CD123-expressing cancer cells.

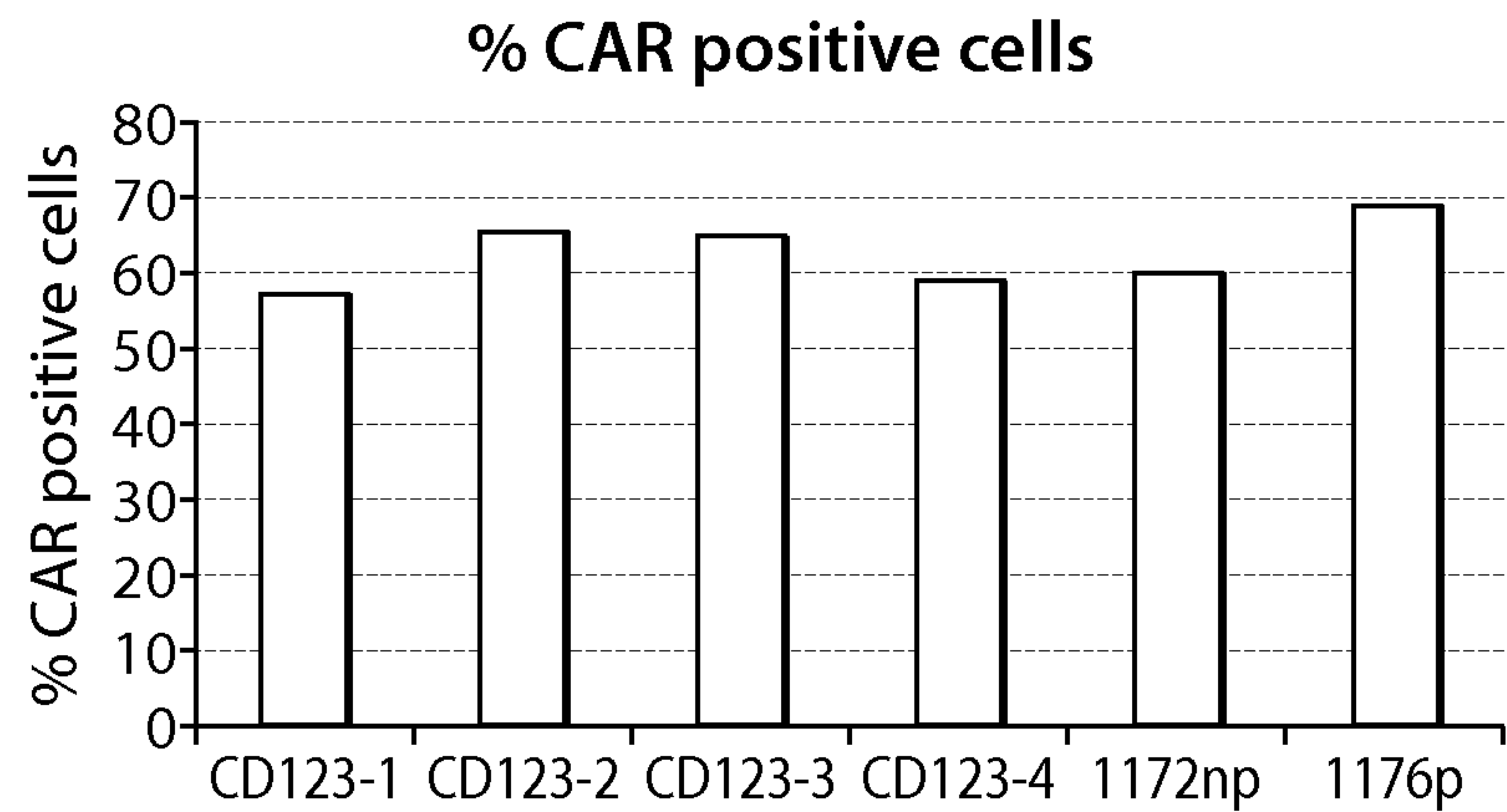


FIG. 1

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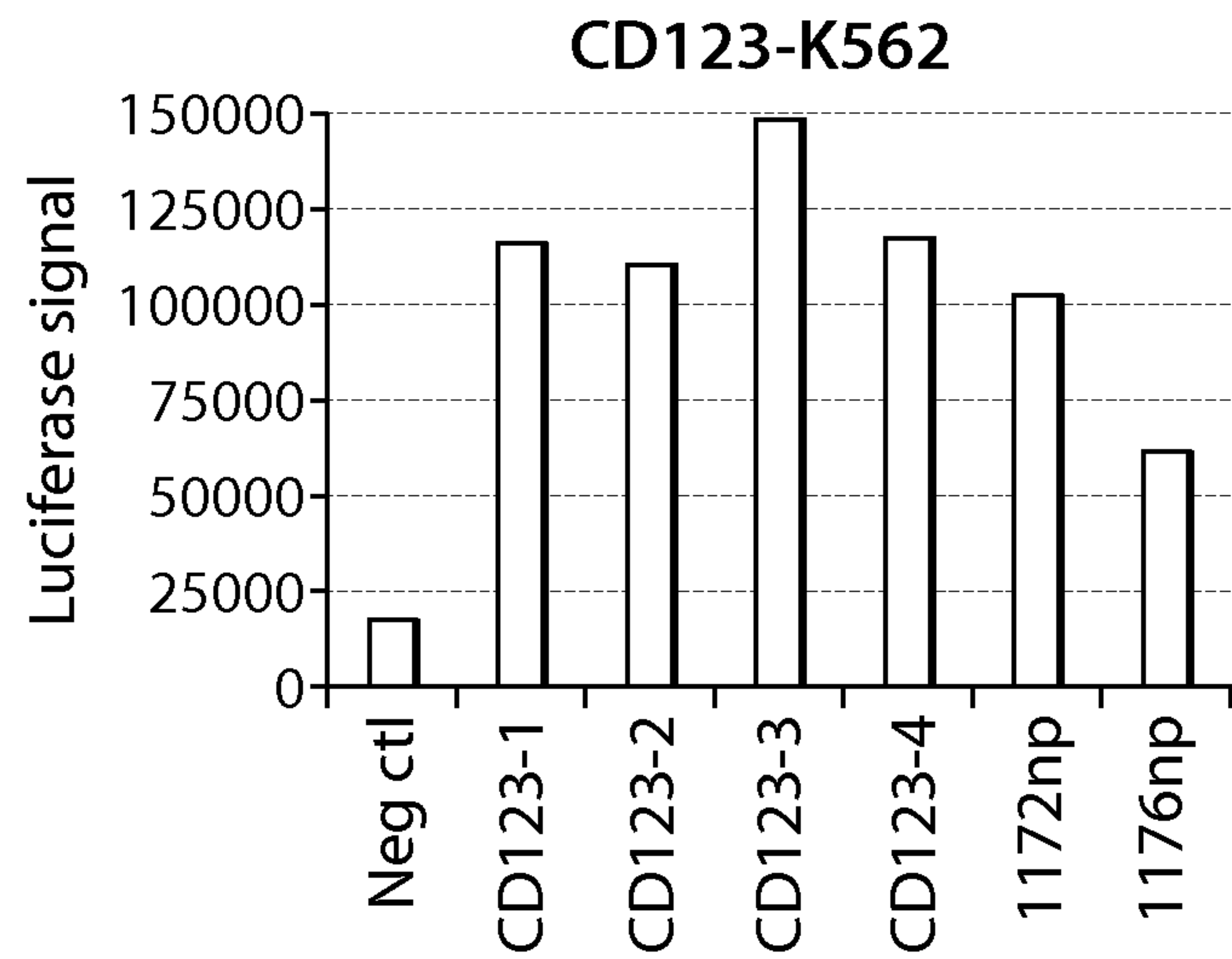


FIG. 2A

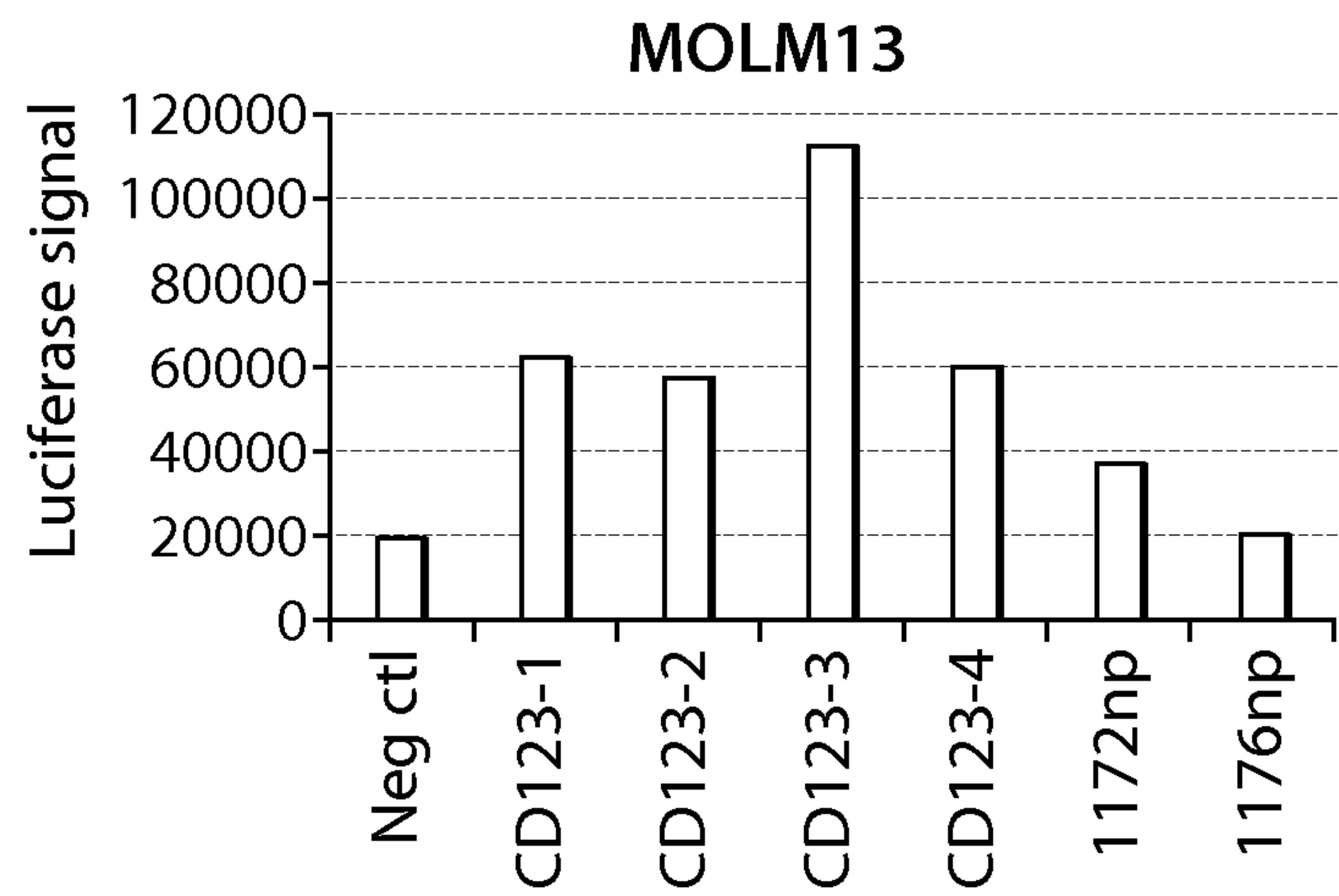


FIG. 2B

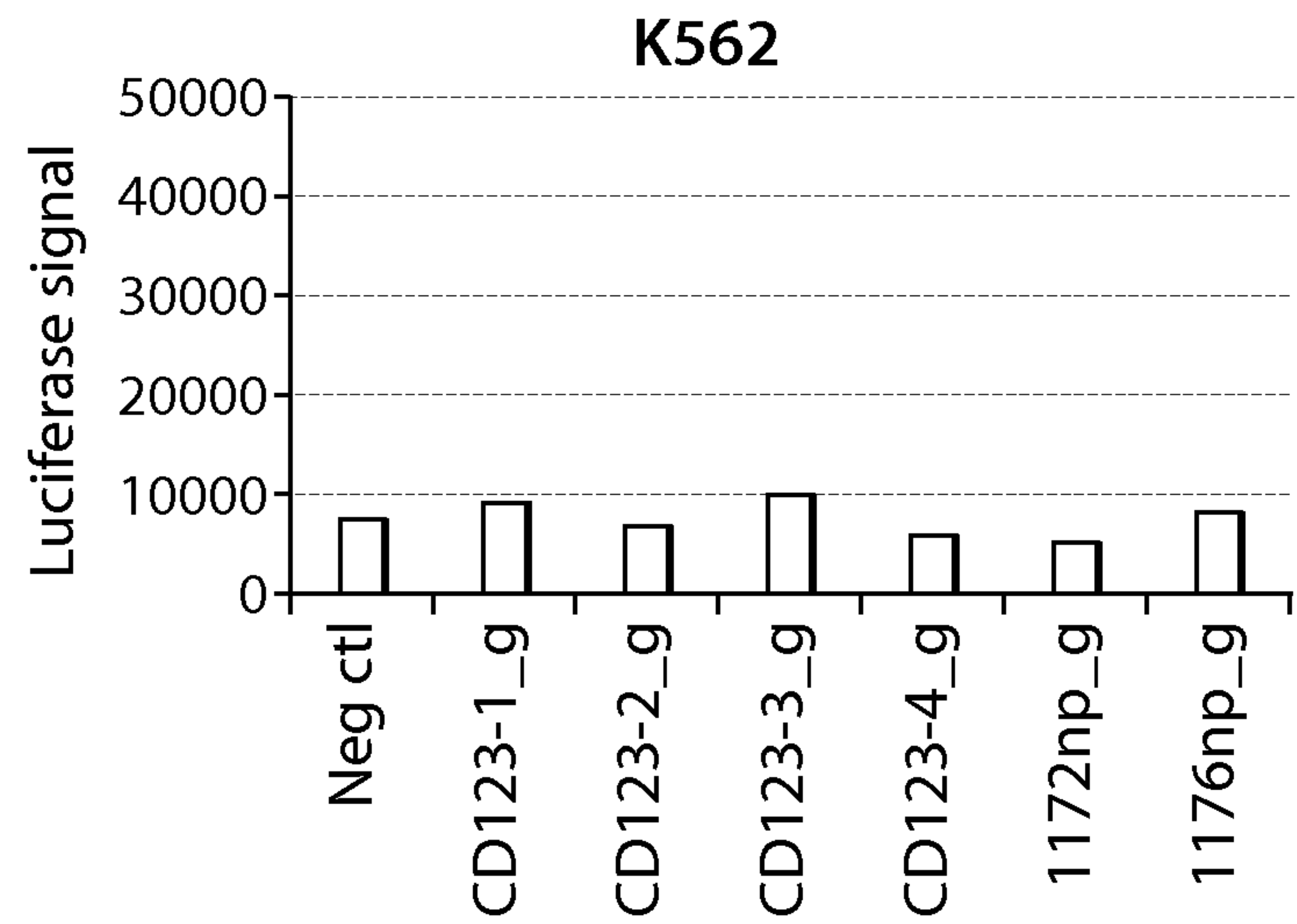


FIG. 2C

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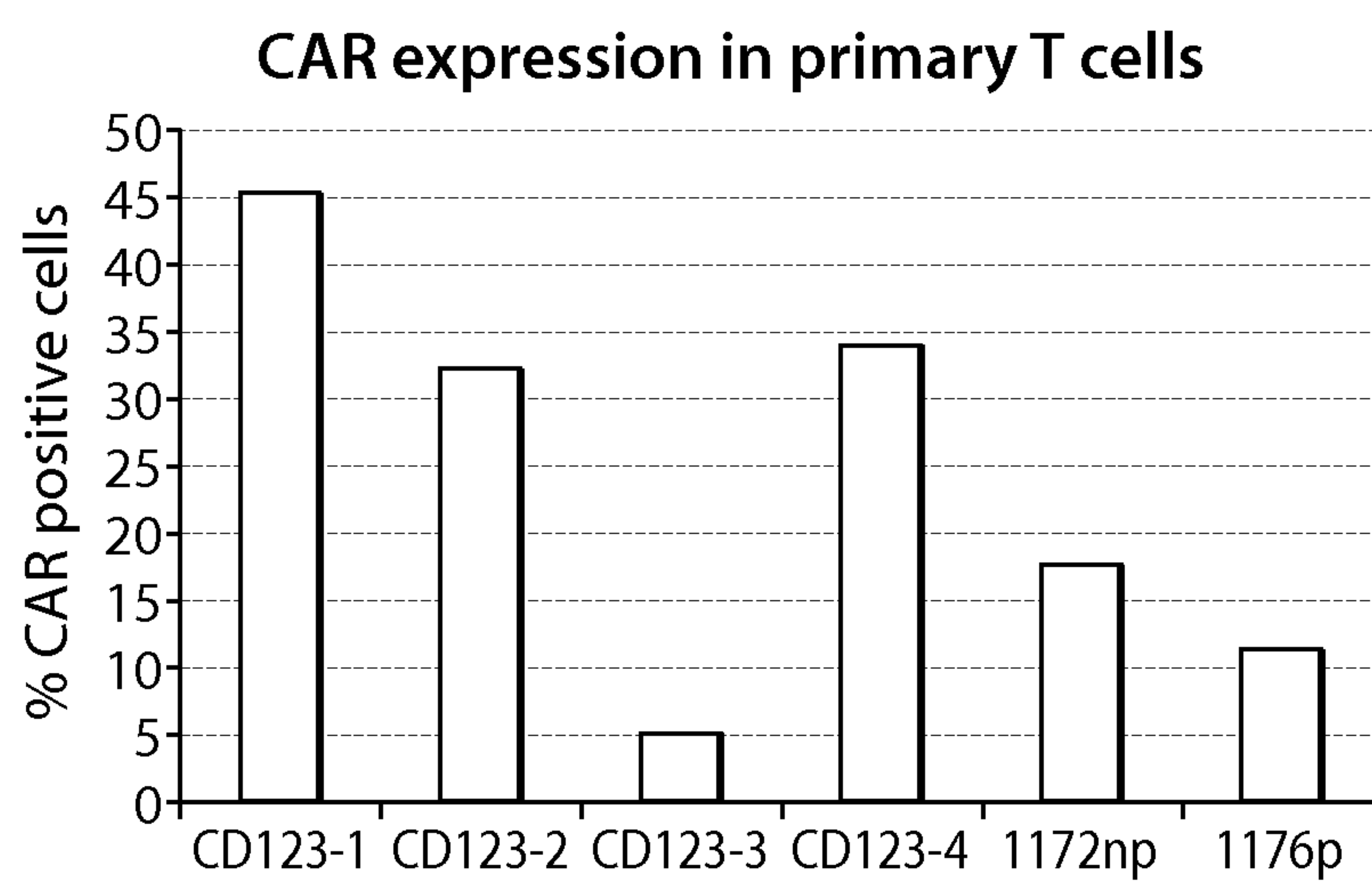


FIG. 3

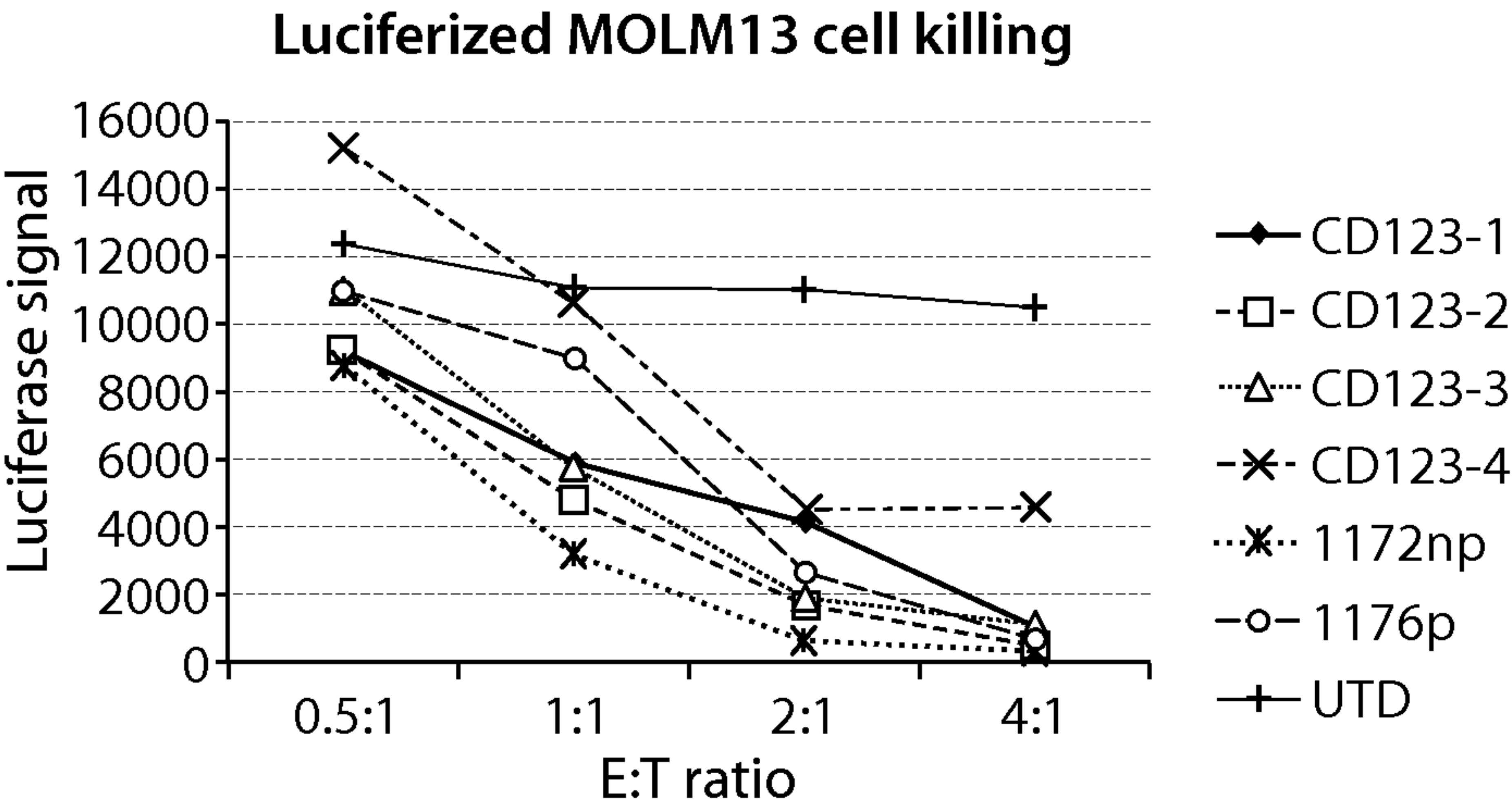


FIG. 4

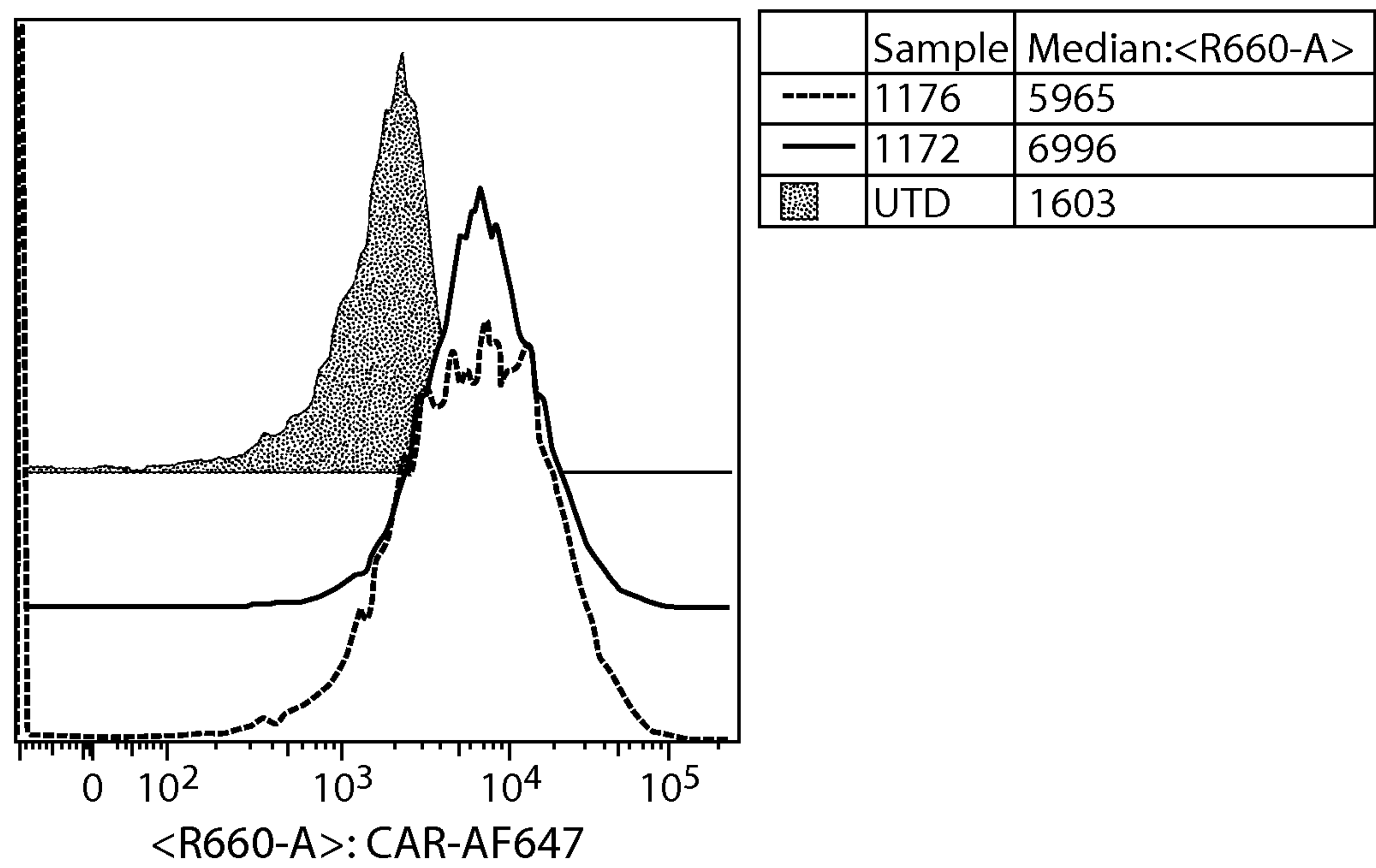


FIG. 5A

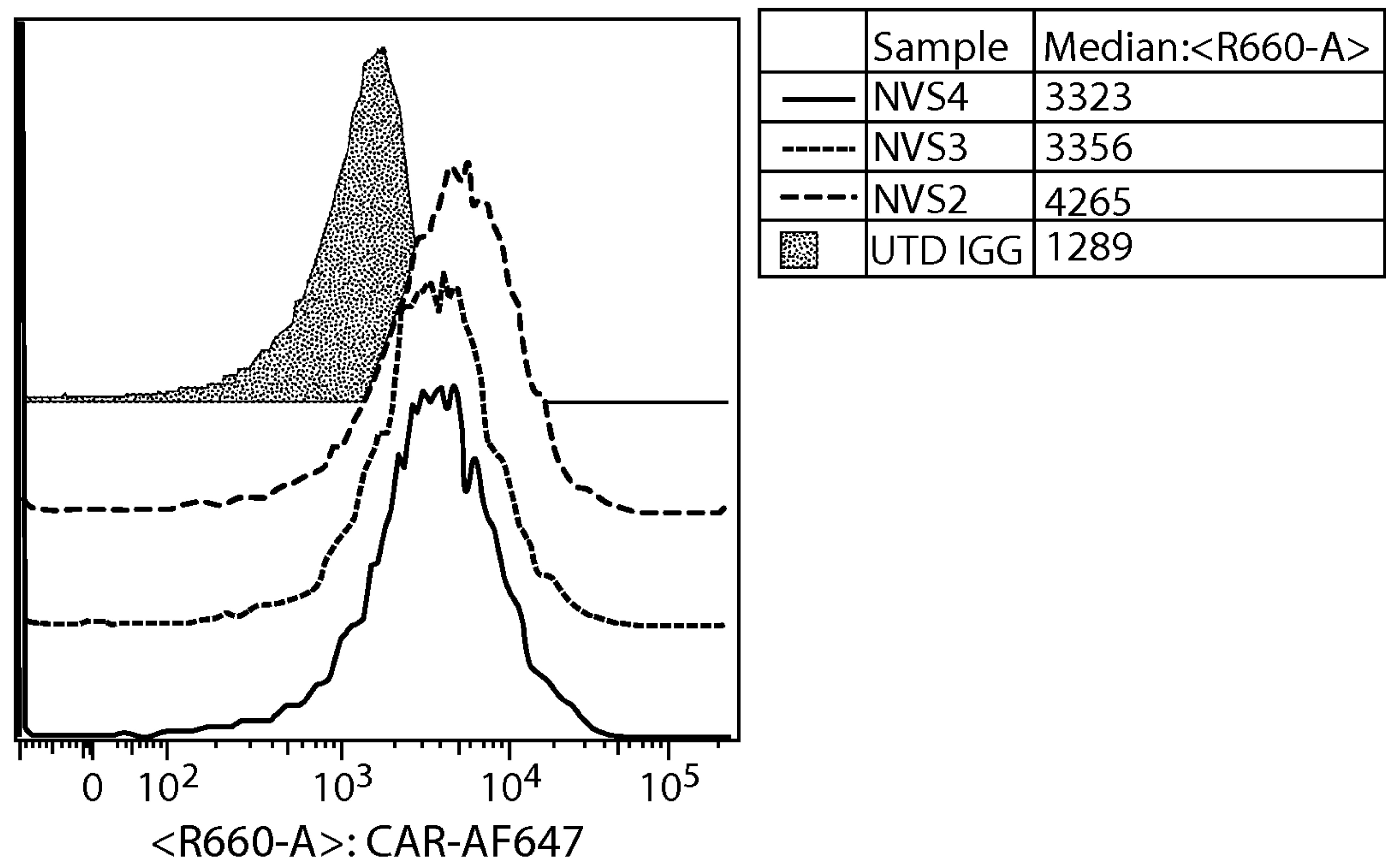


FIG. 5B

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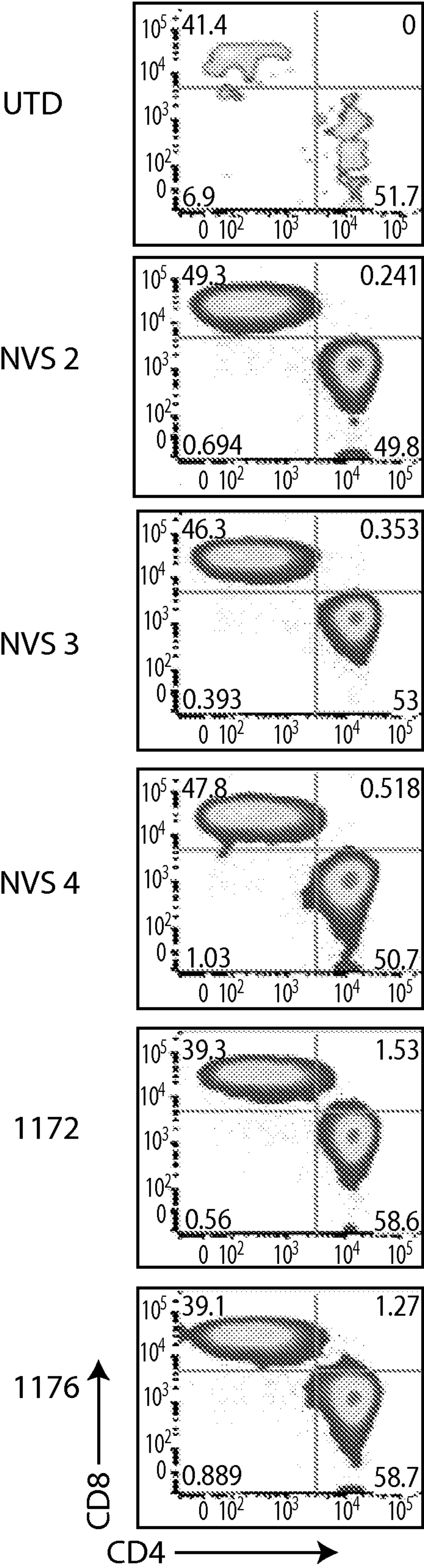


FIG. 6

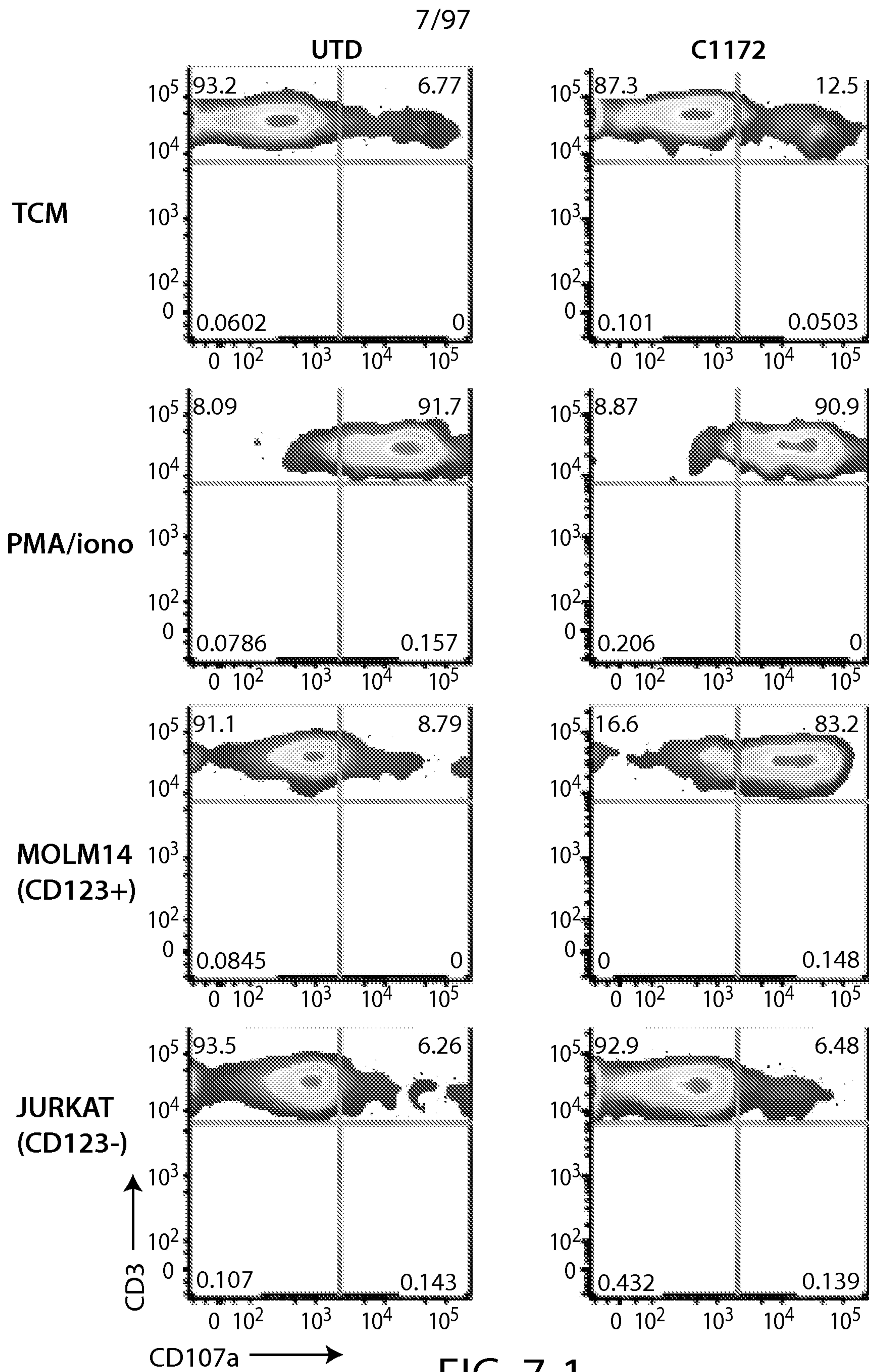


FIG. 7-1

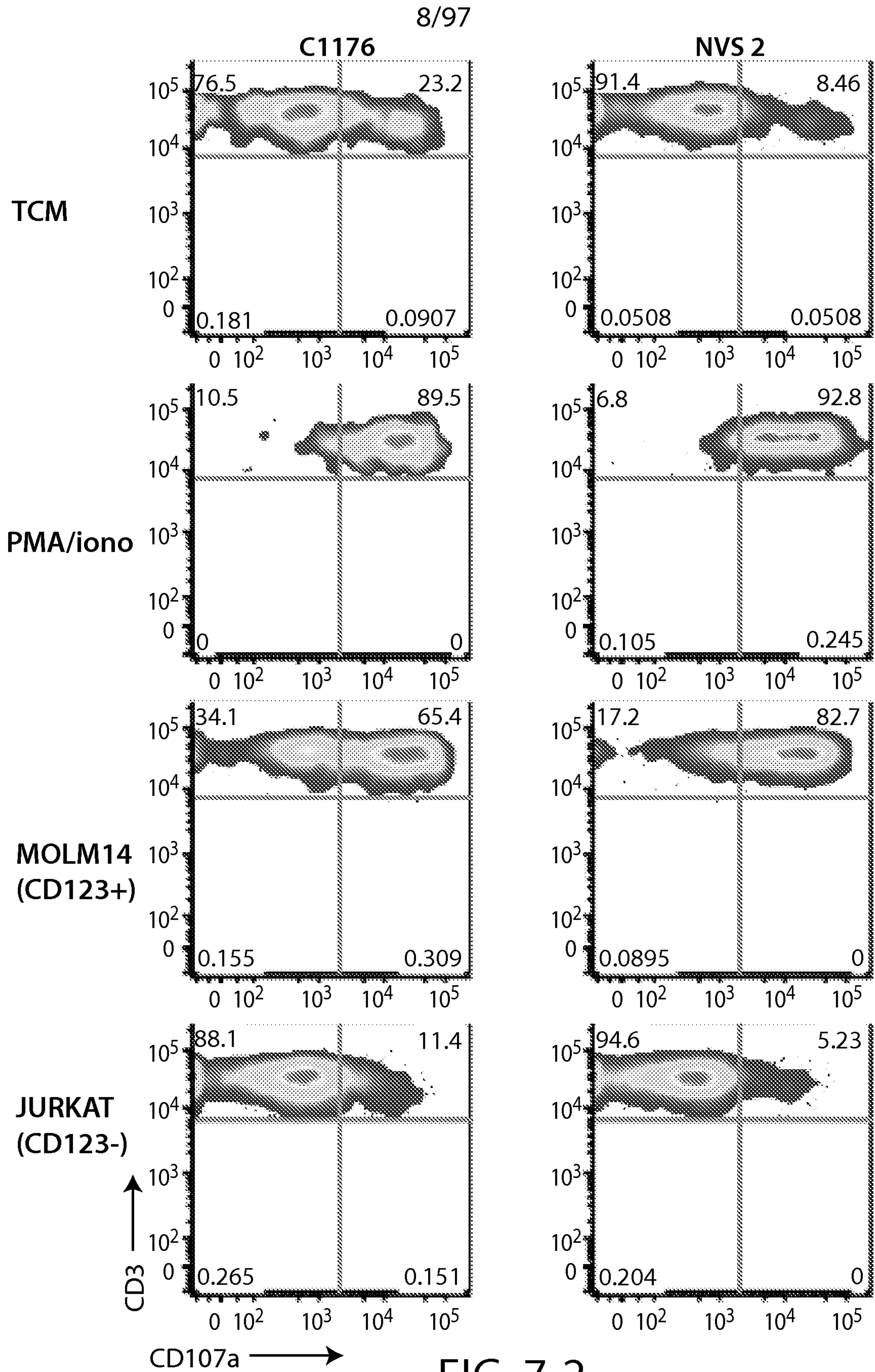


FIG. 7-2

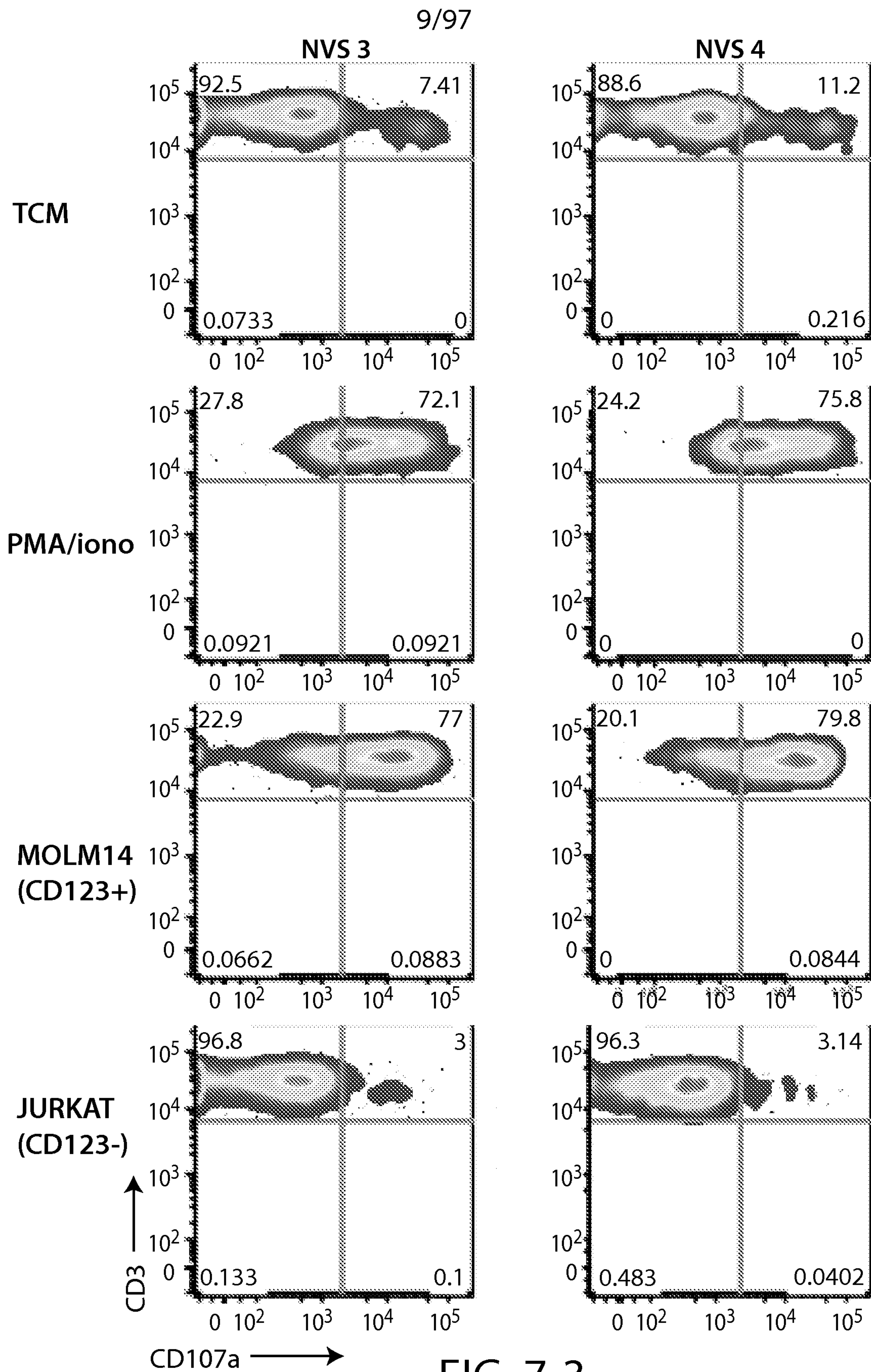


FIG. 7-3

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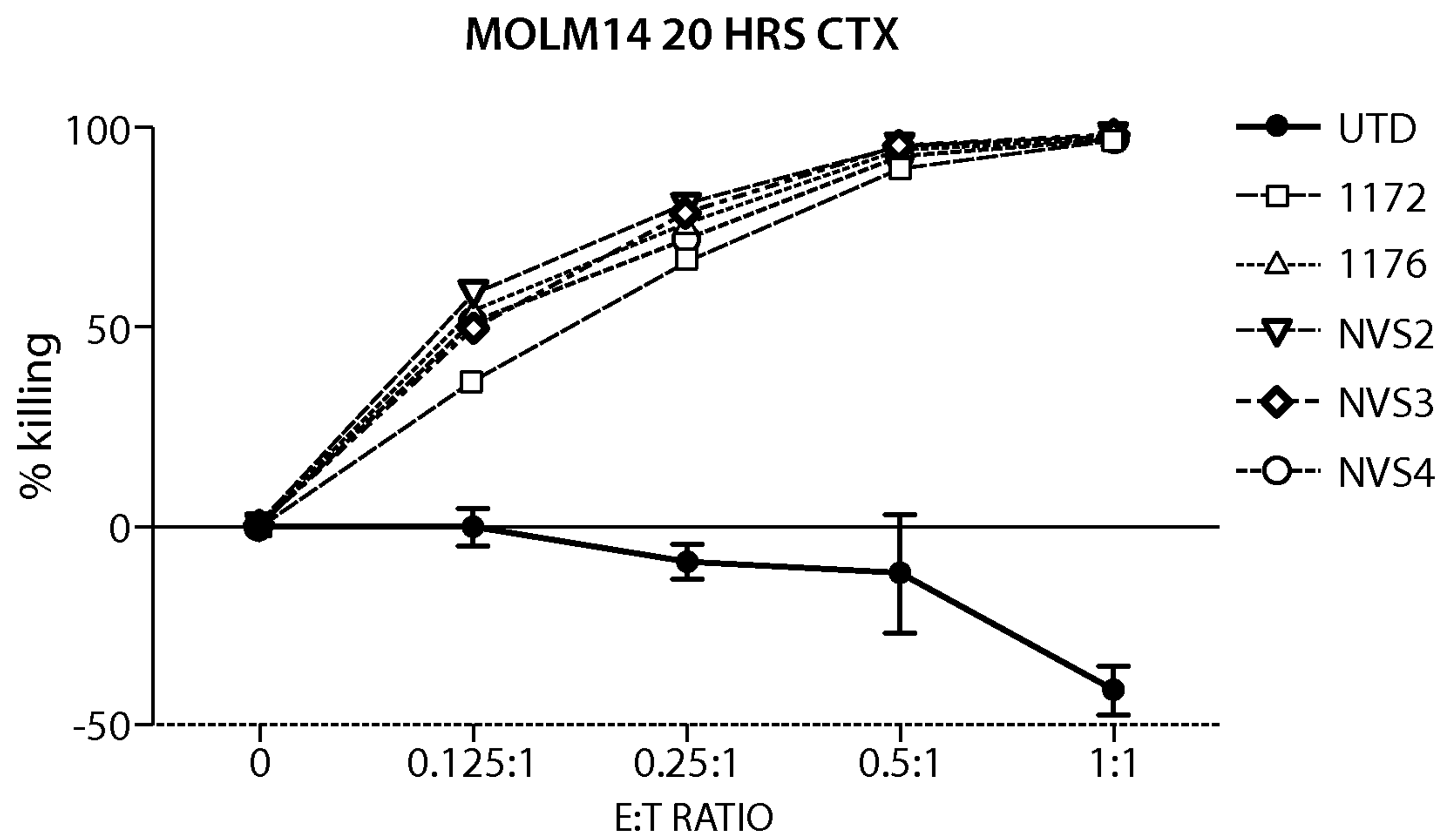


FIG. 8

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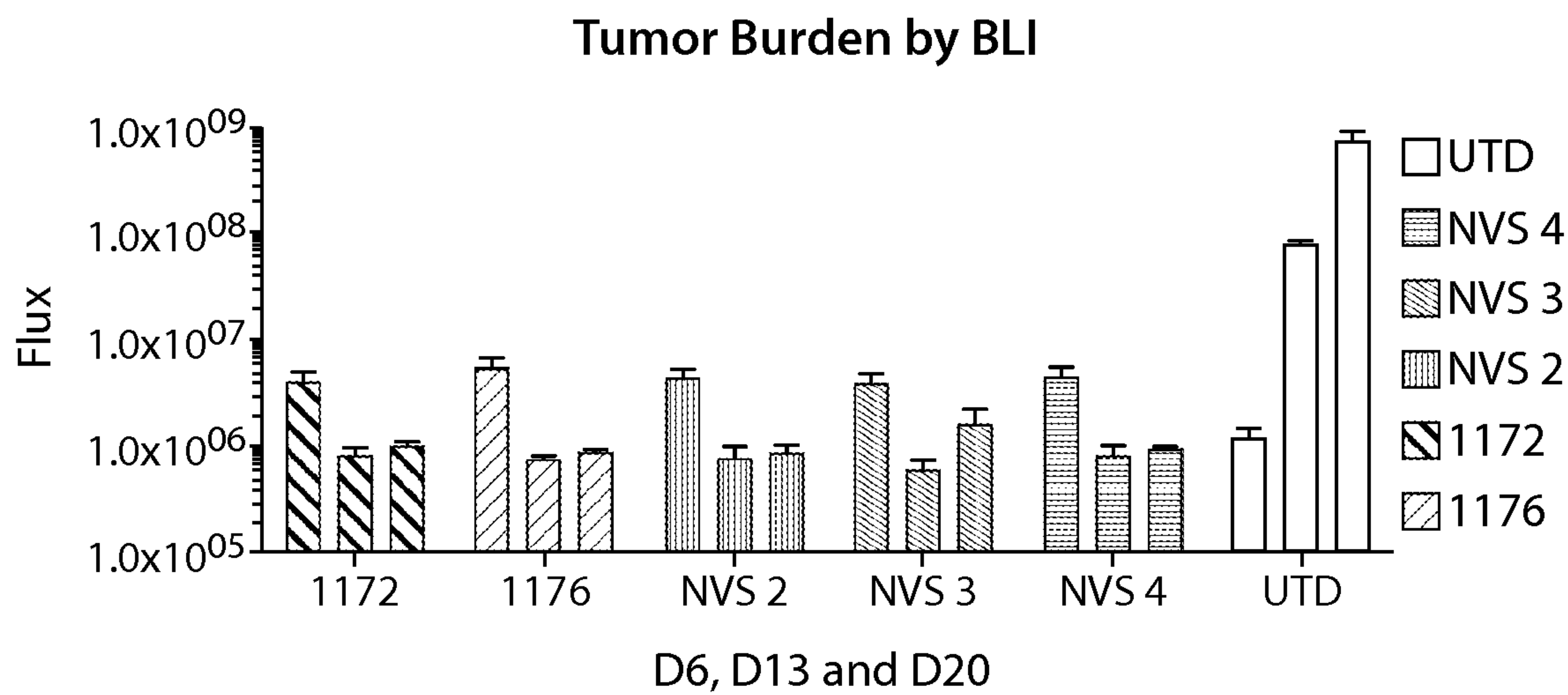


FIG. 9

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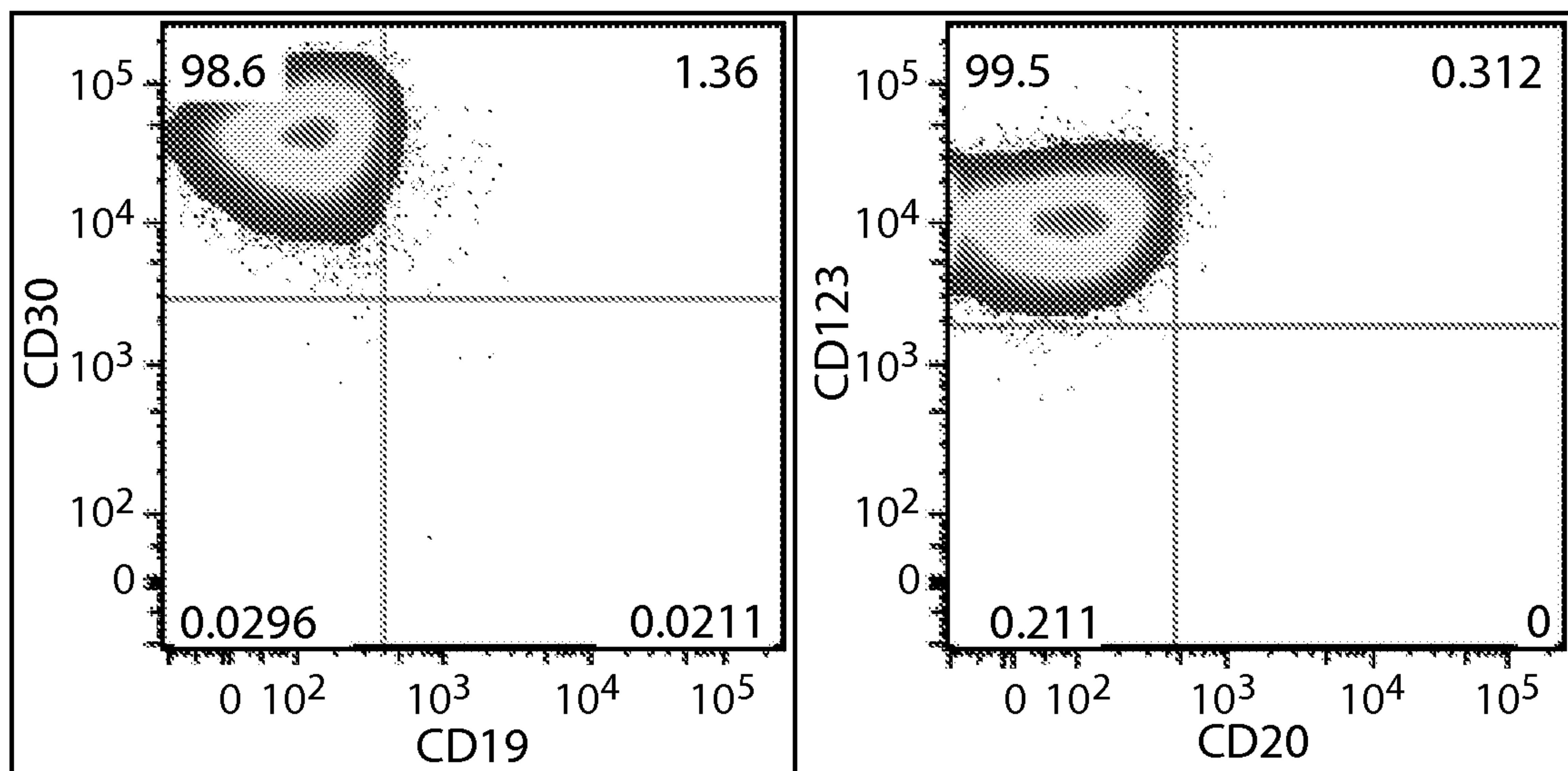
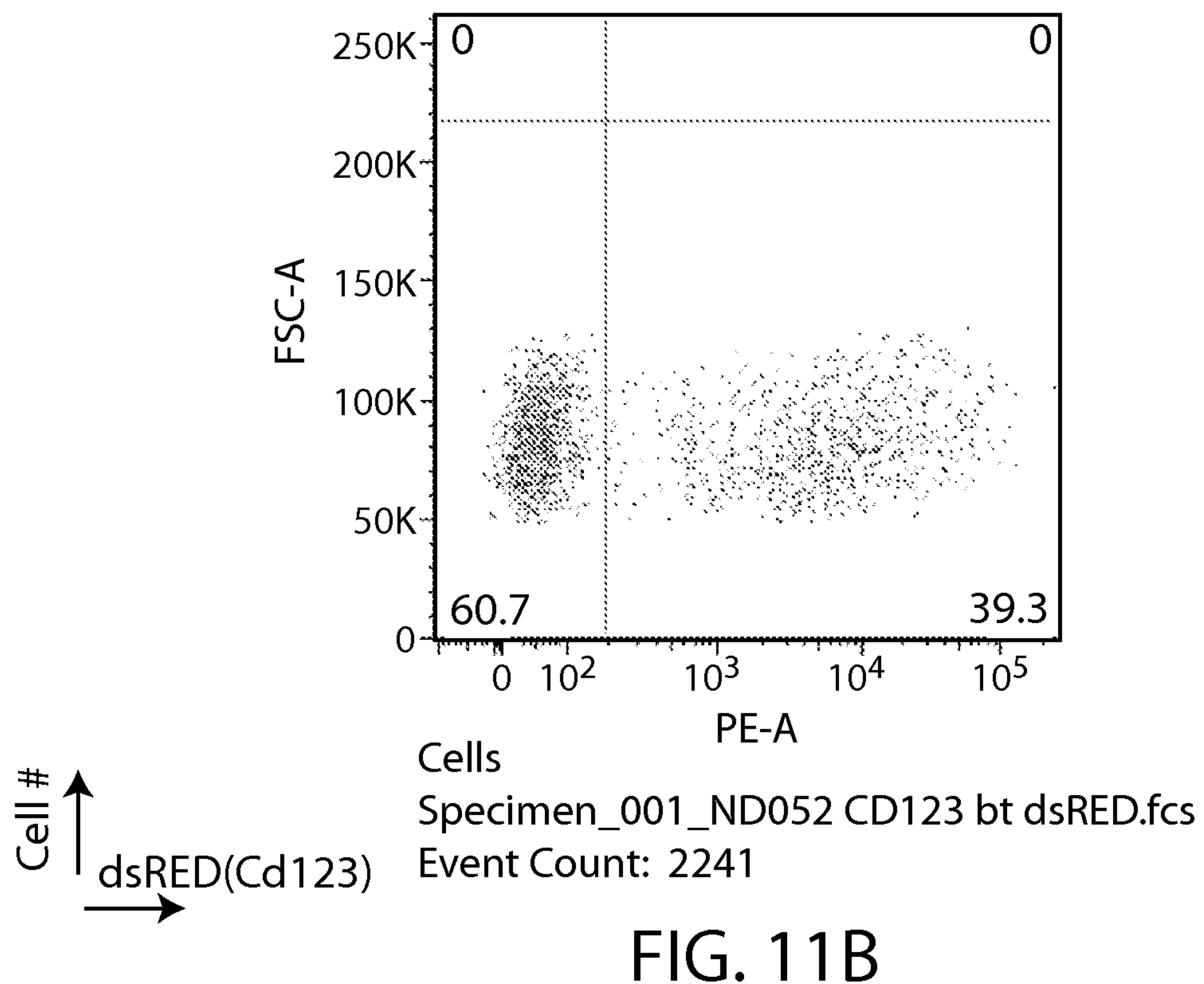
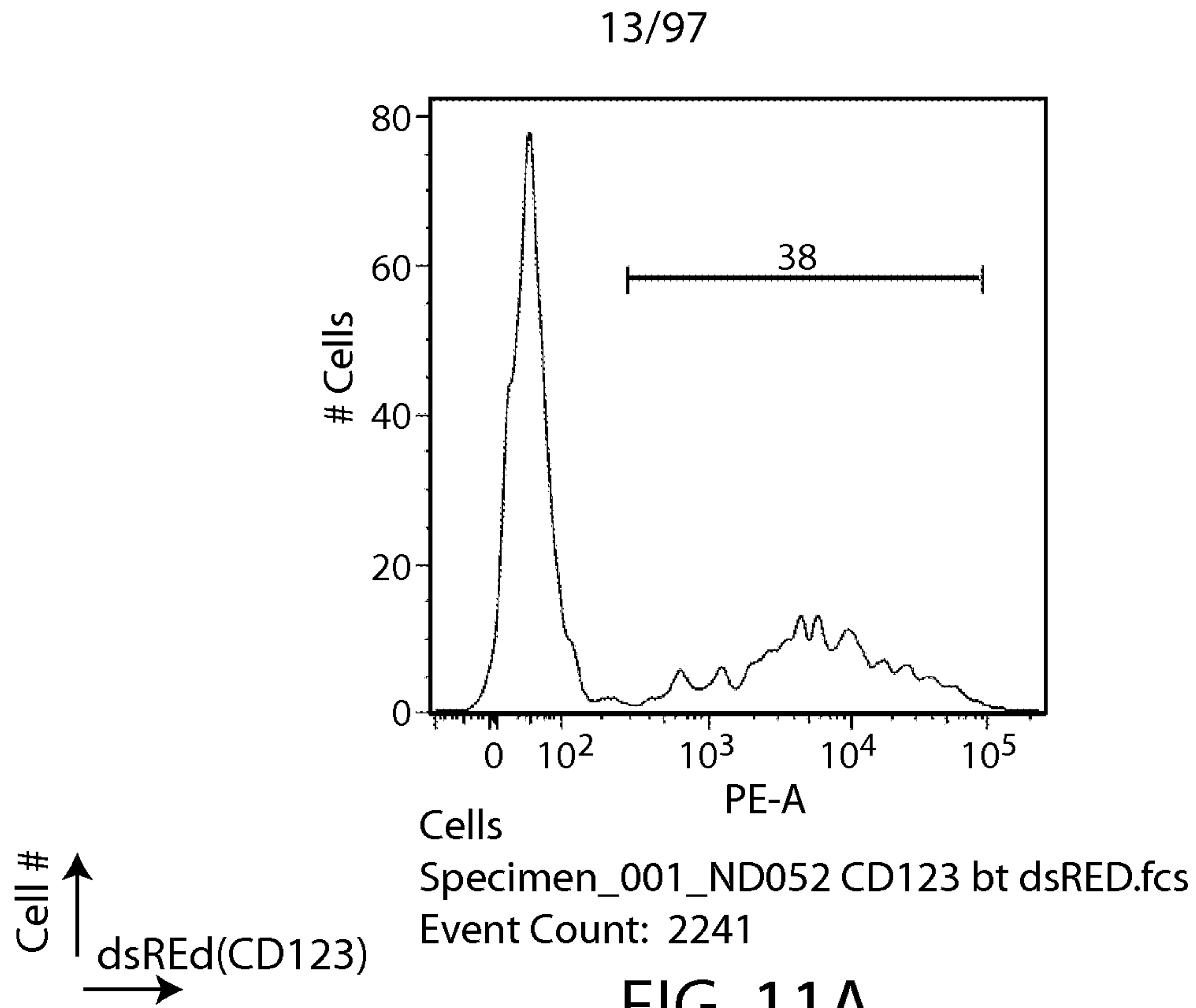


FIG. 10



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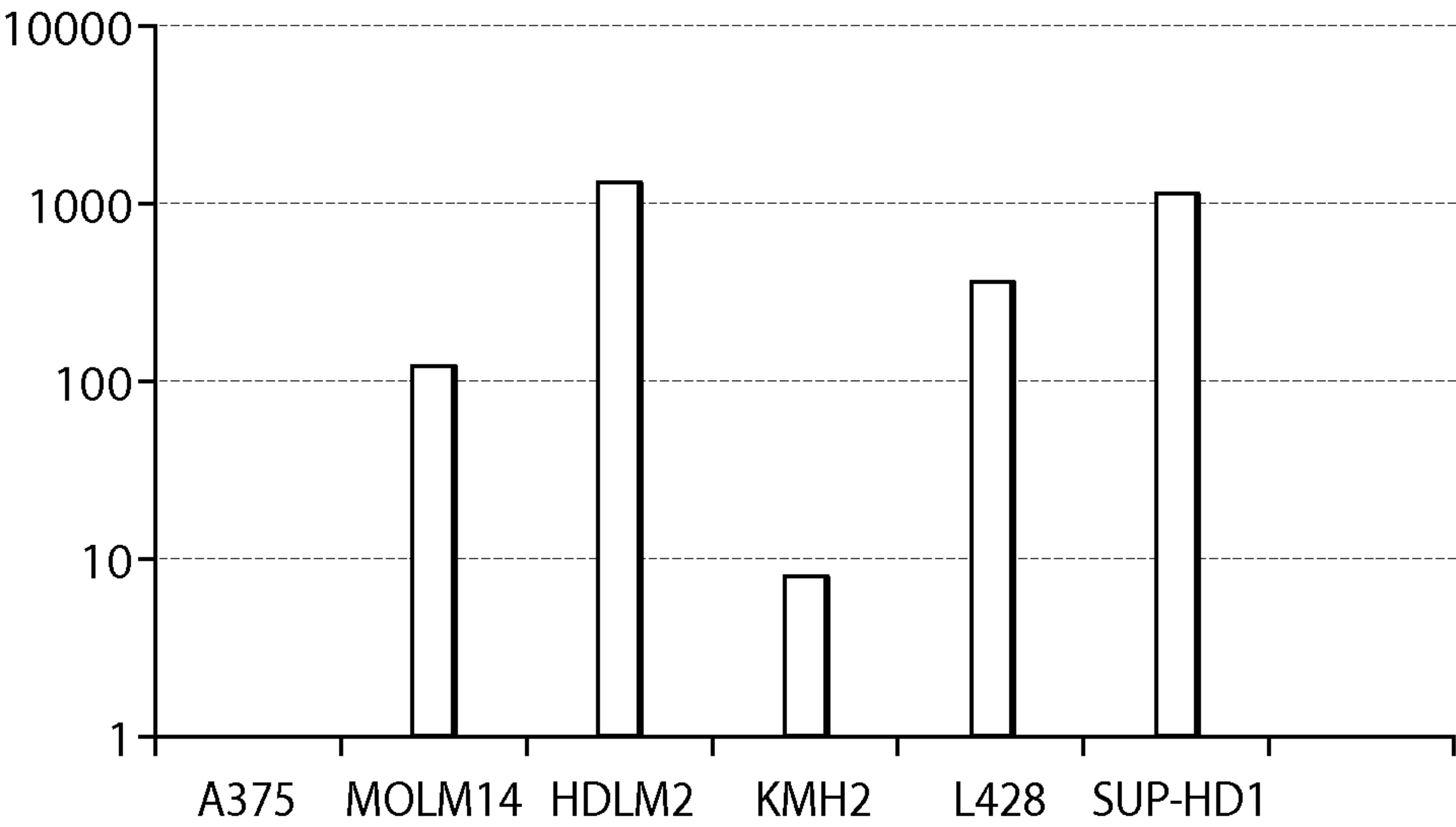


FIG. 12

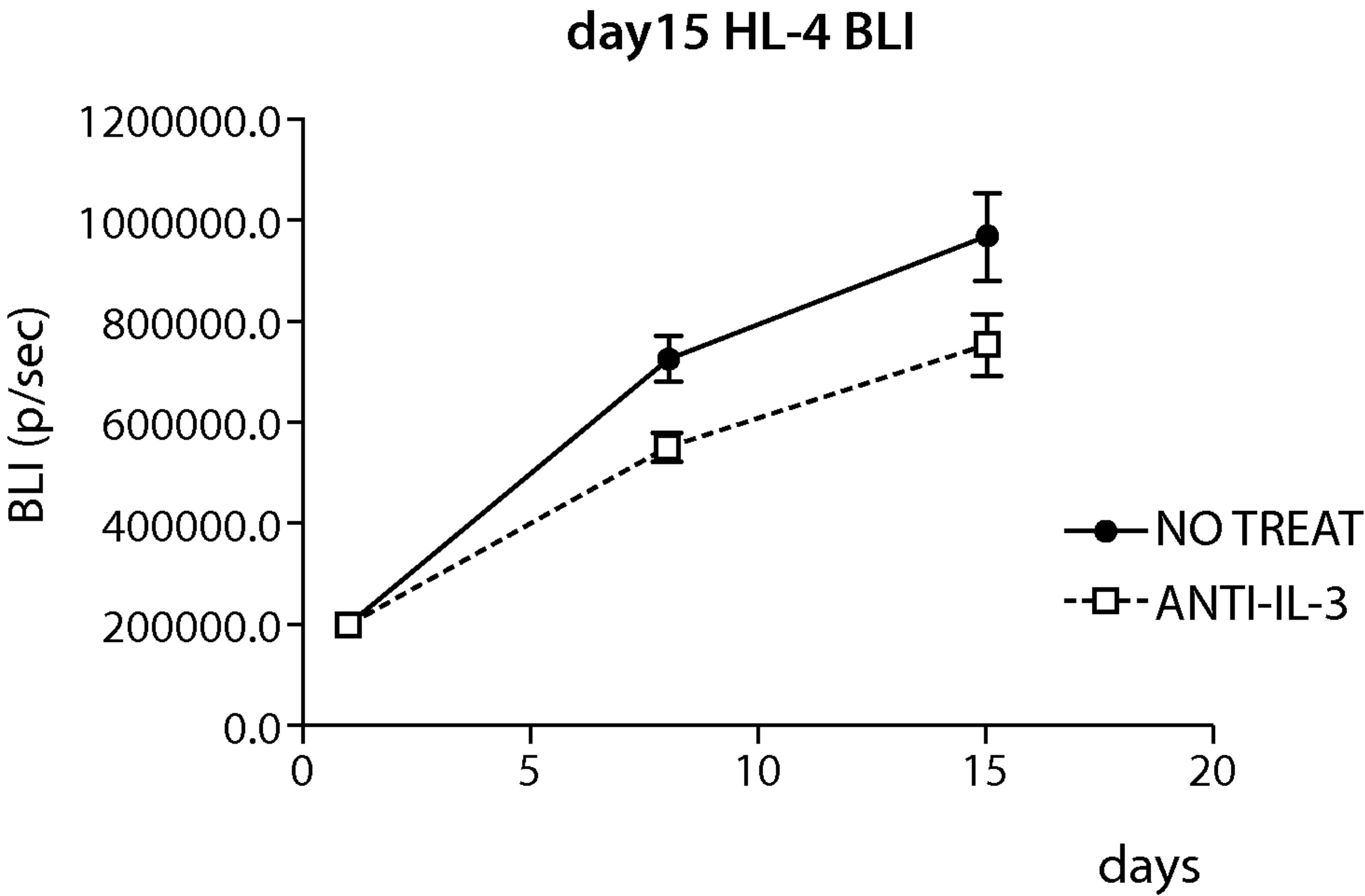


FIG. 13

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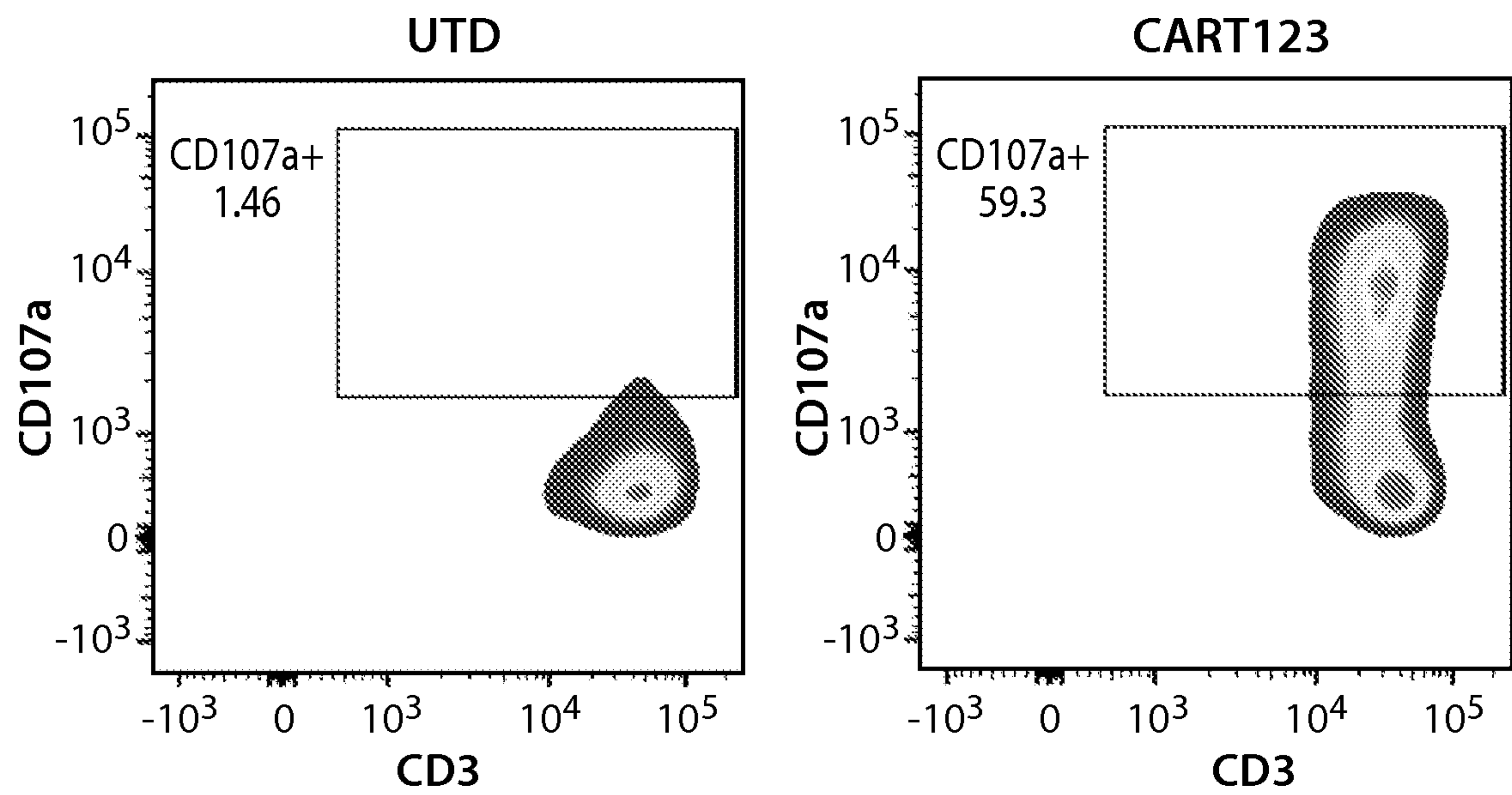


FIG. 14

FIG. 15

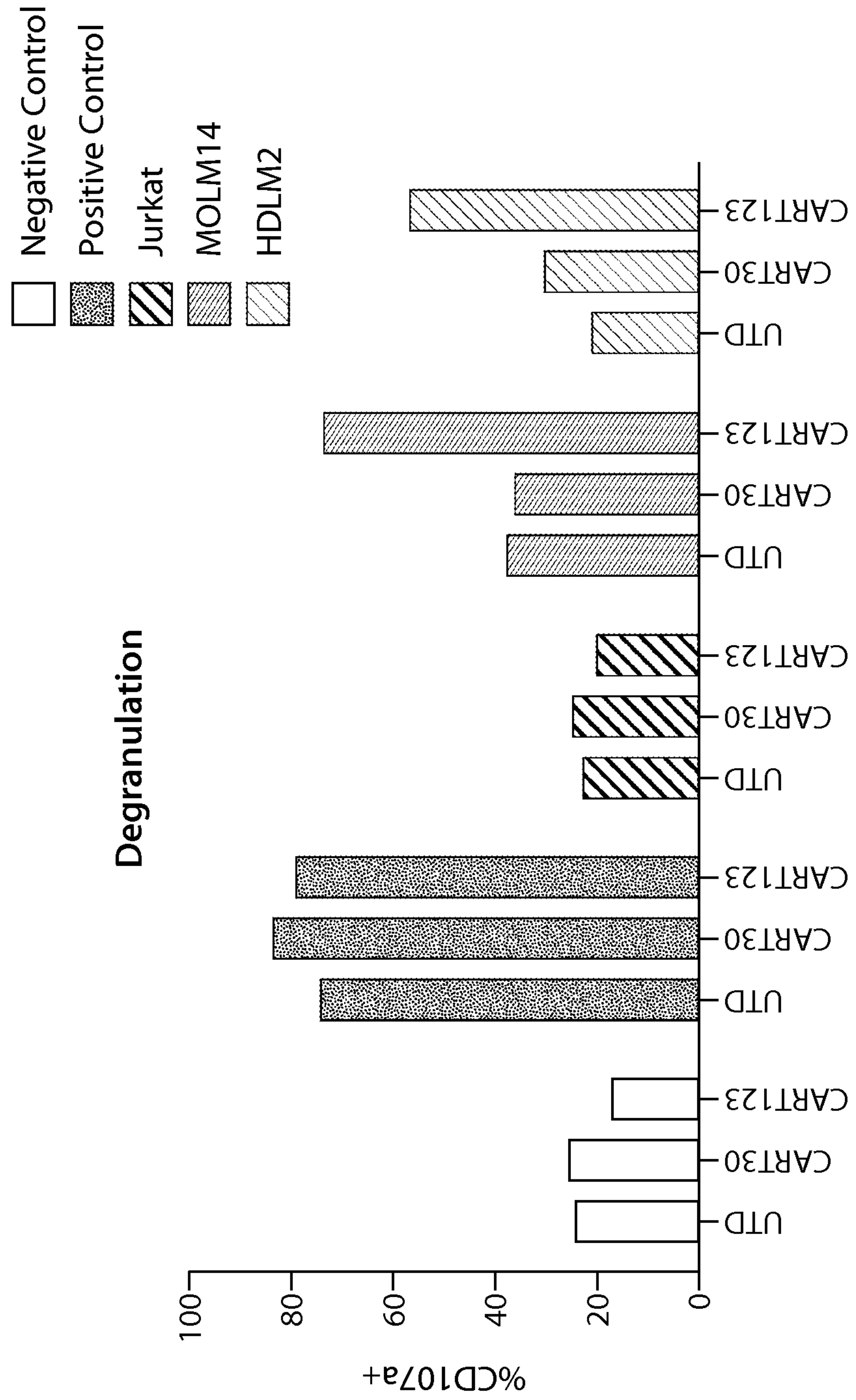




FIG. 16

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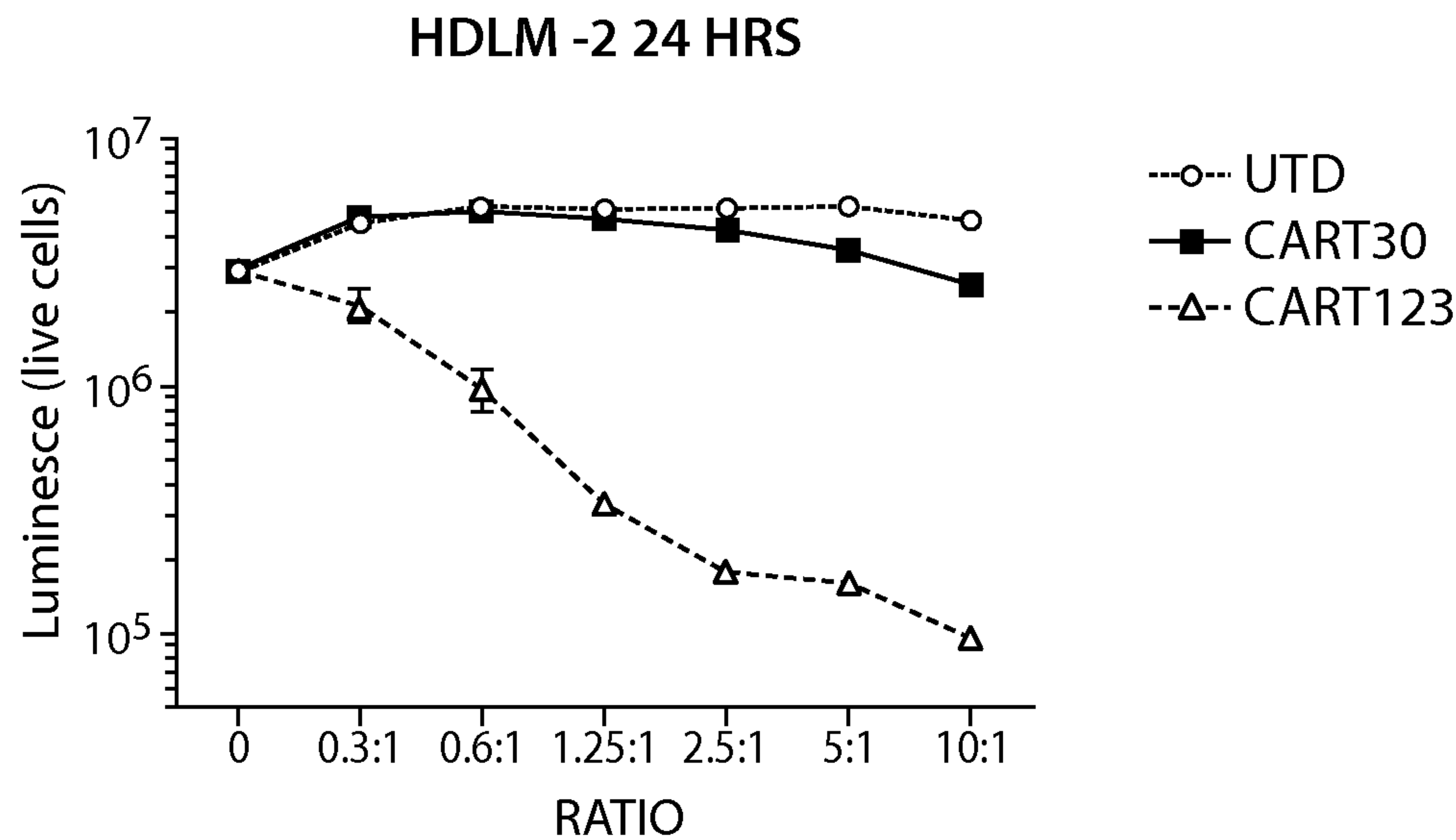


FIG. 17

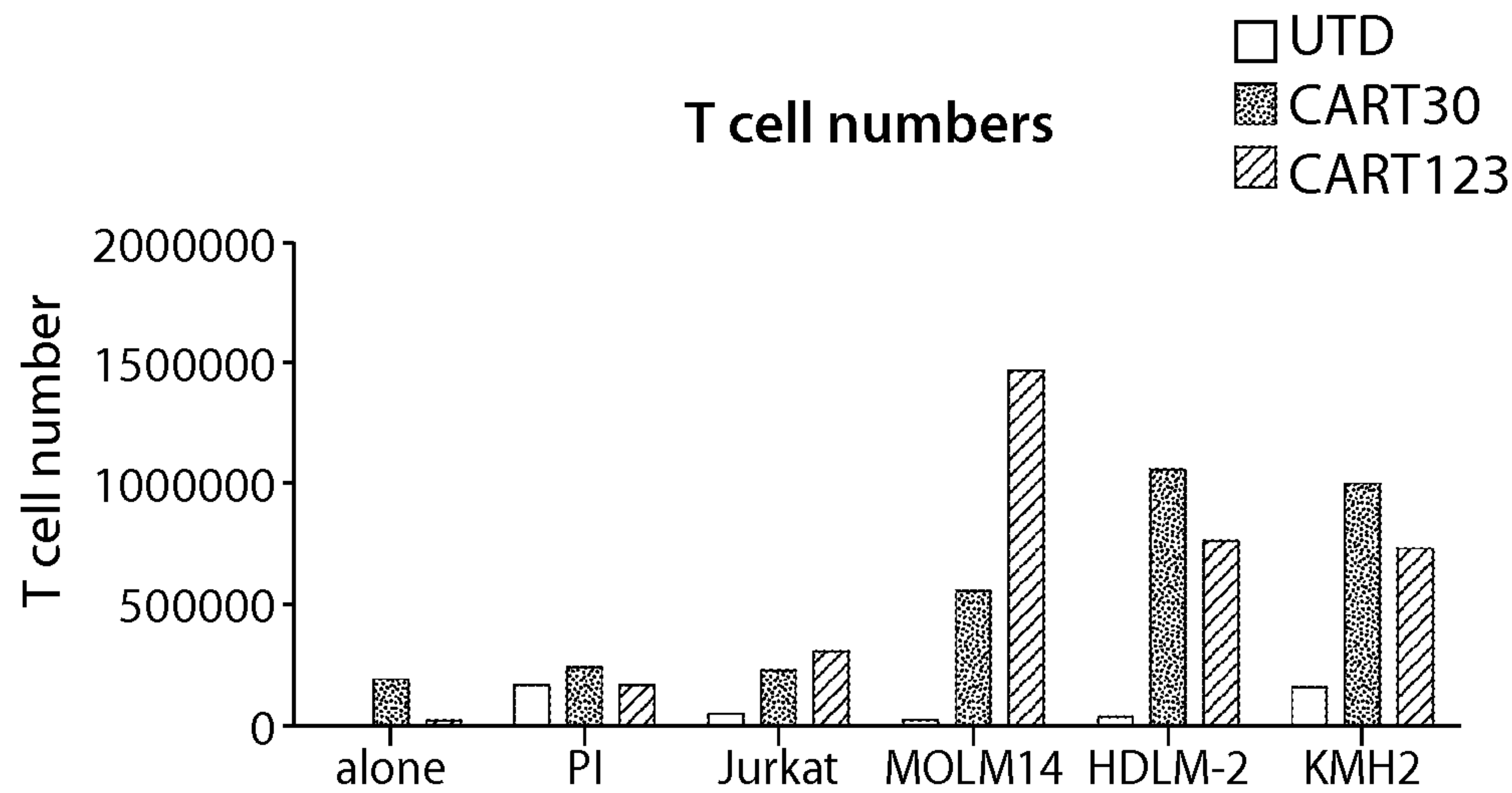


FIG. 18

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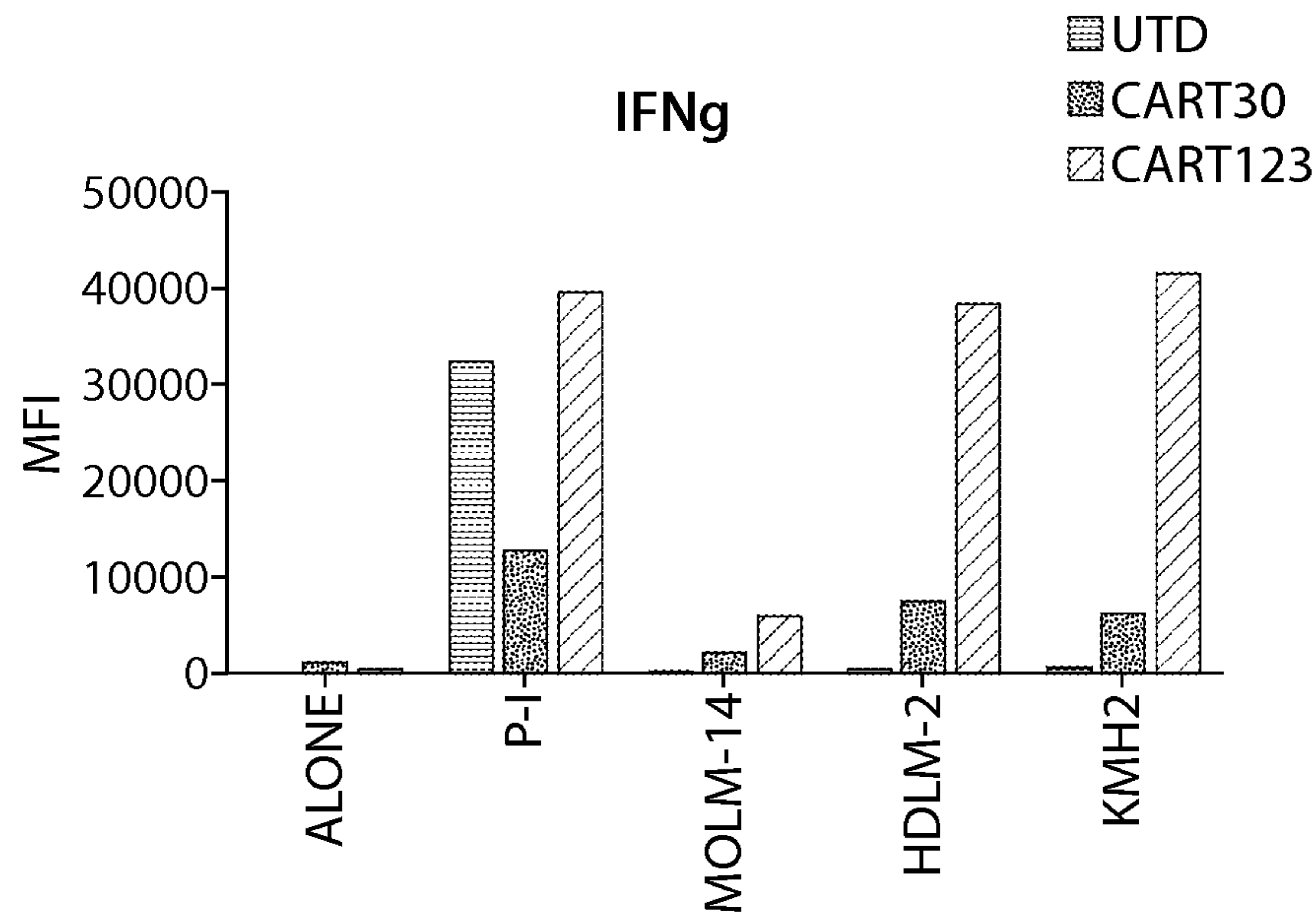


FIG. 19A

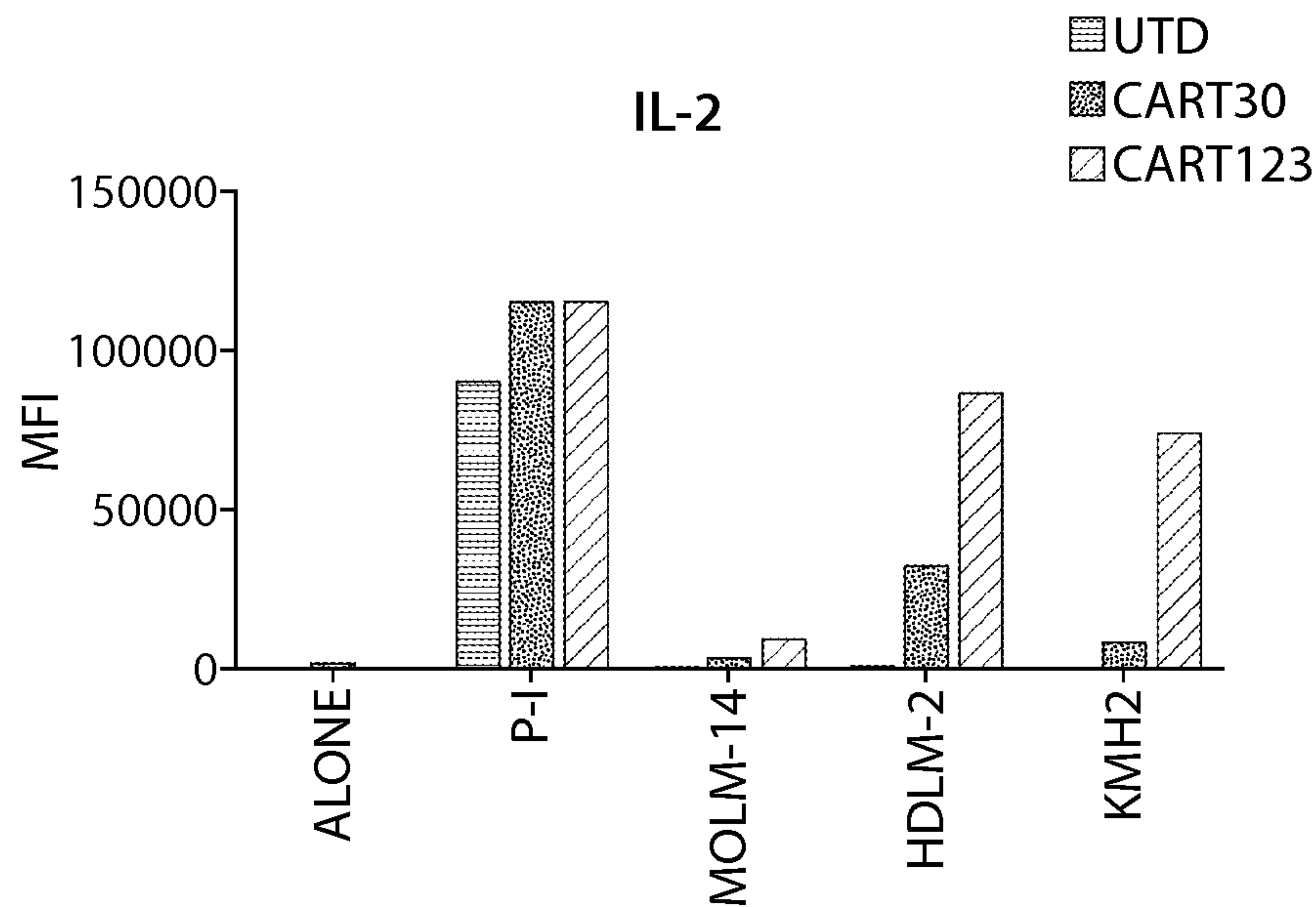


FIG. 19B

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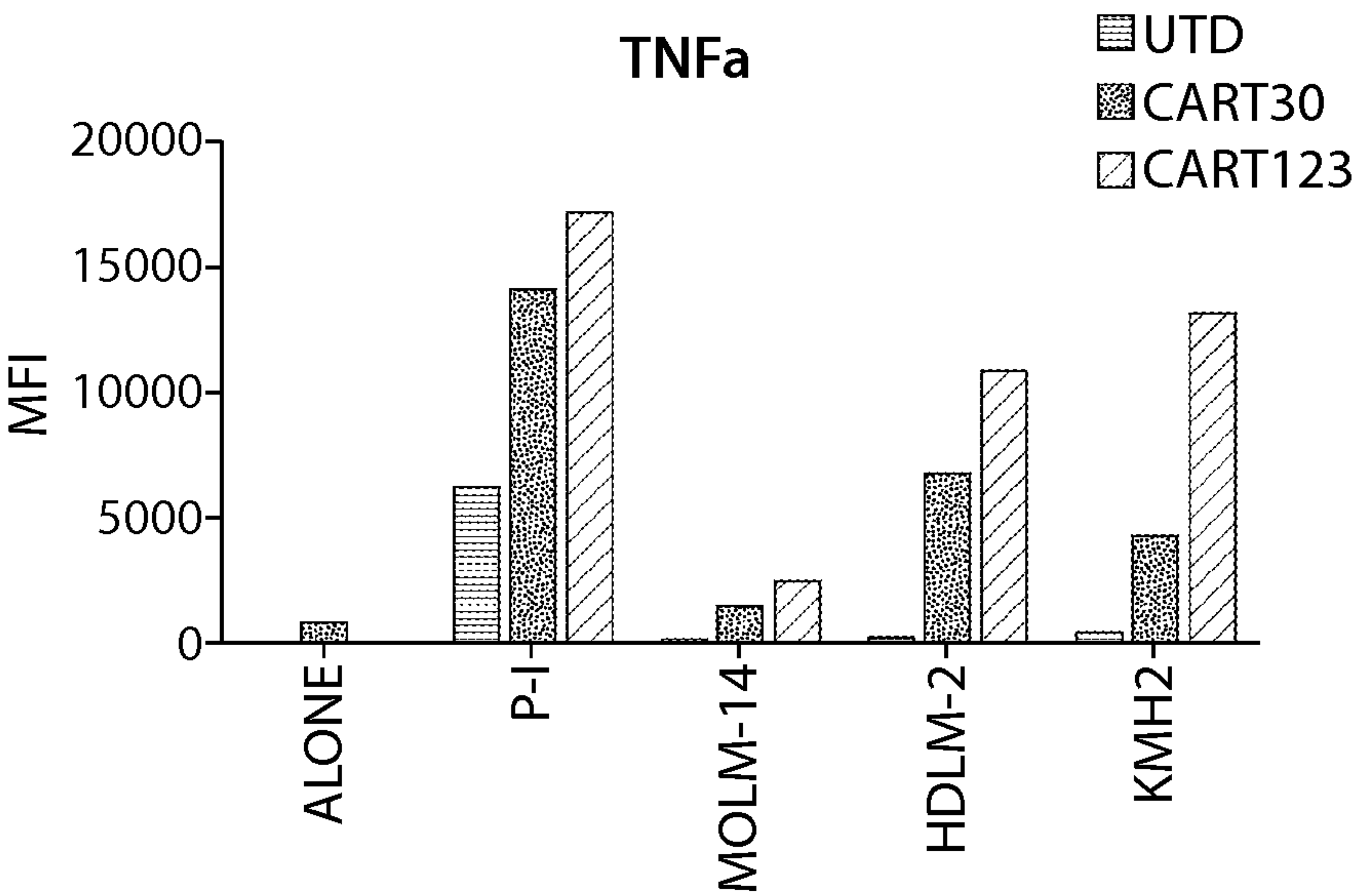


FIG. 19C

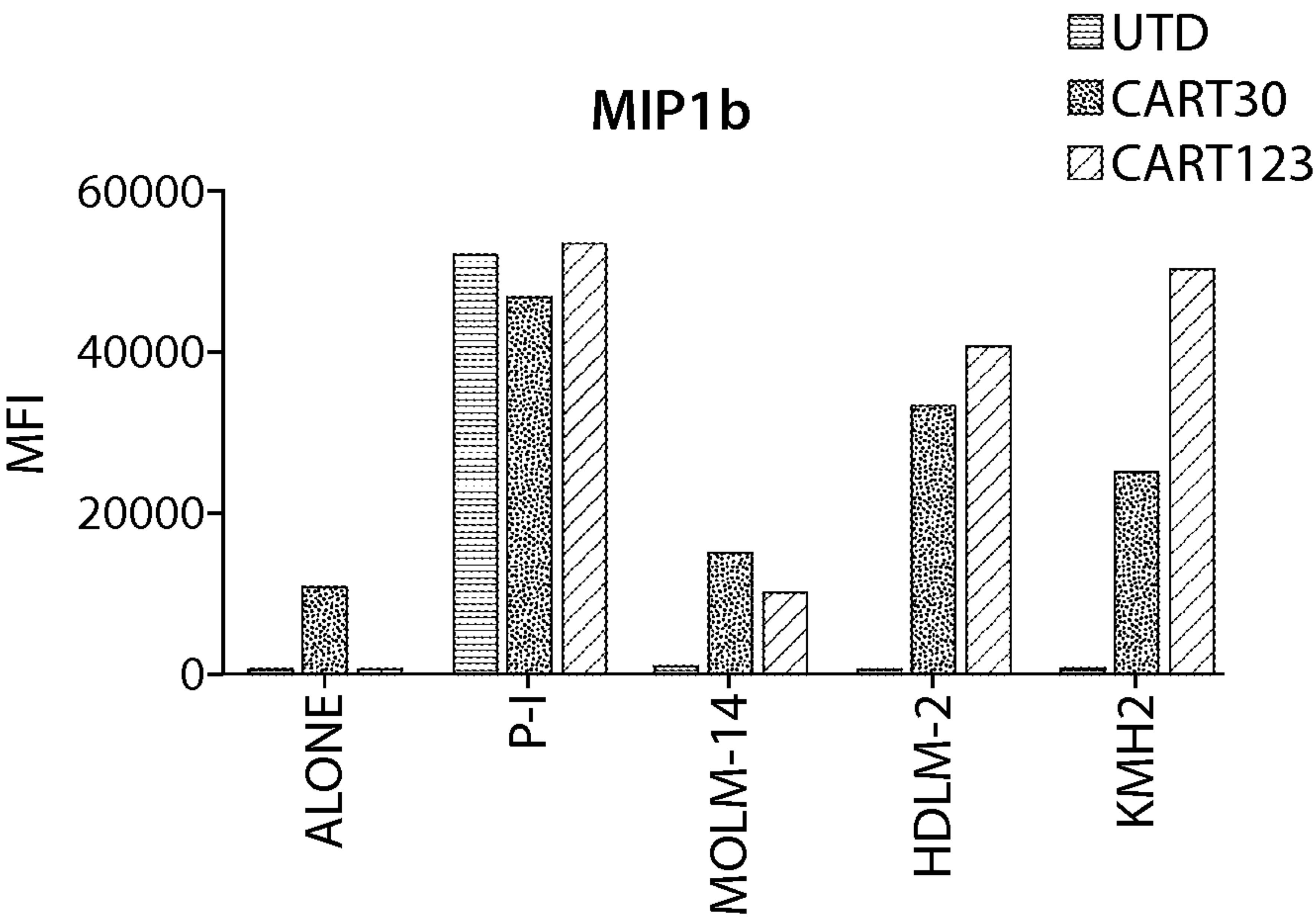


FIG. 19D

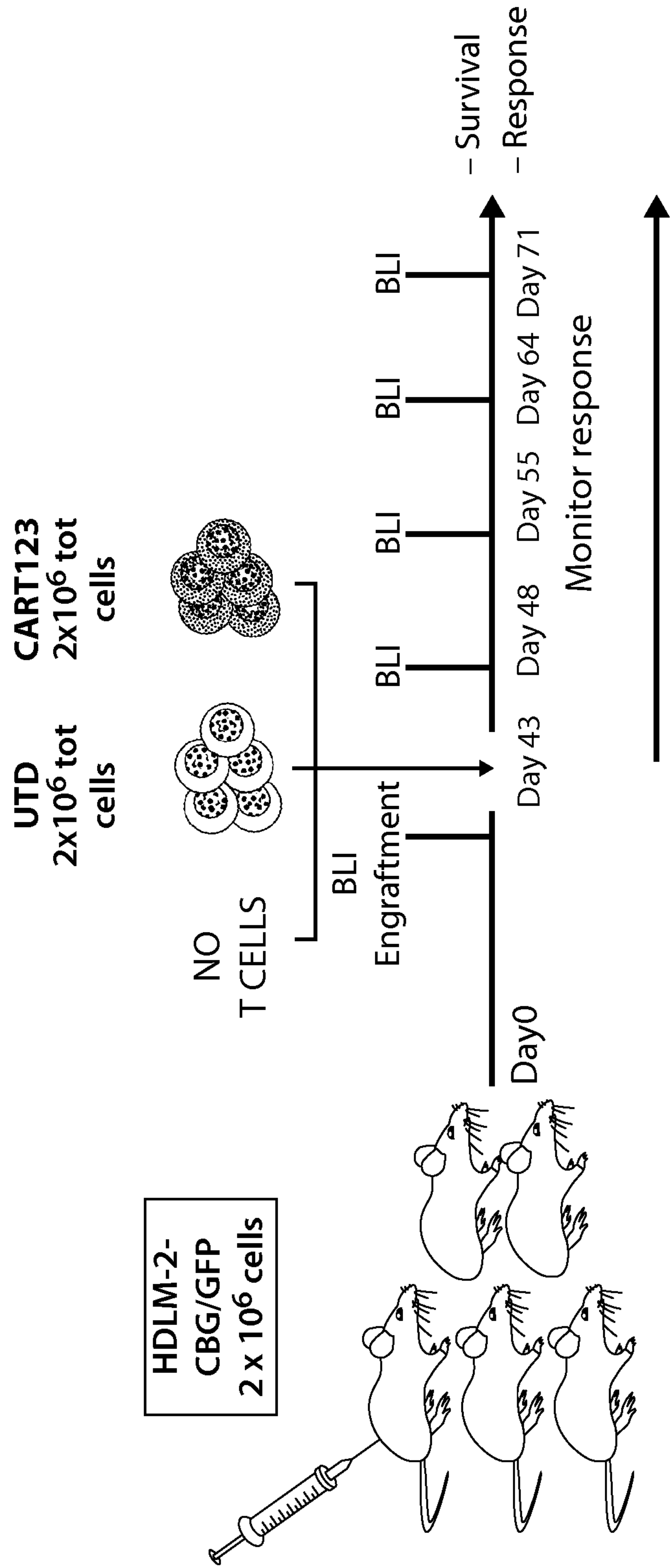


FIG. 20

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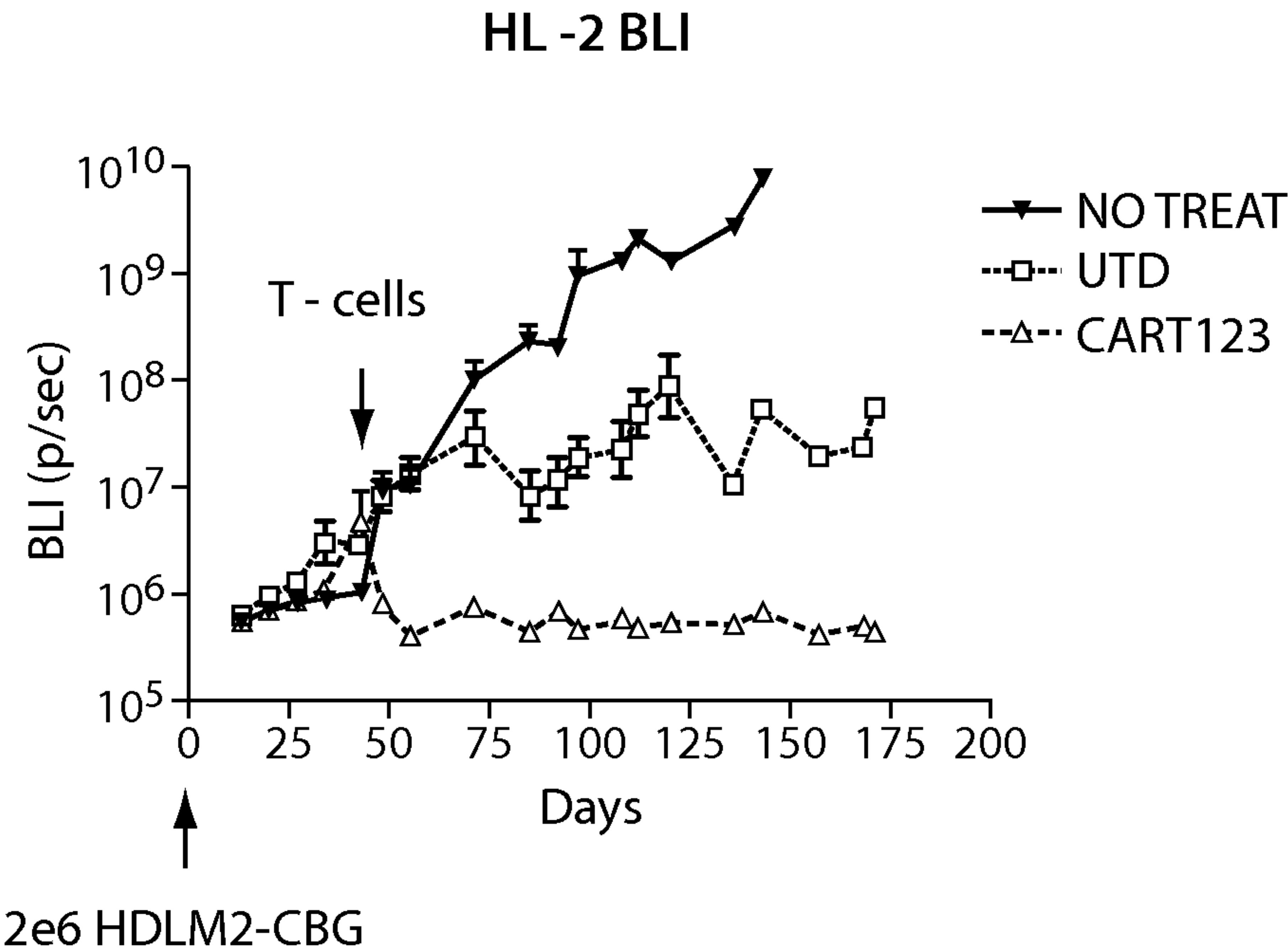


FIG. 21

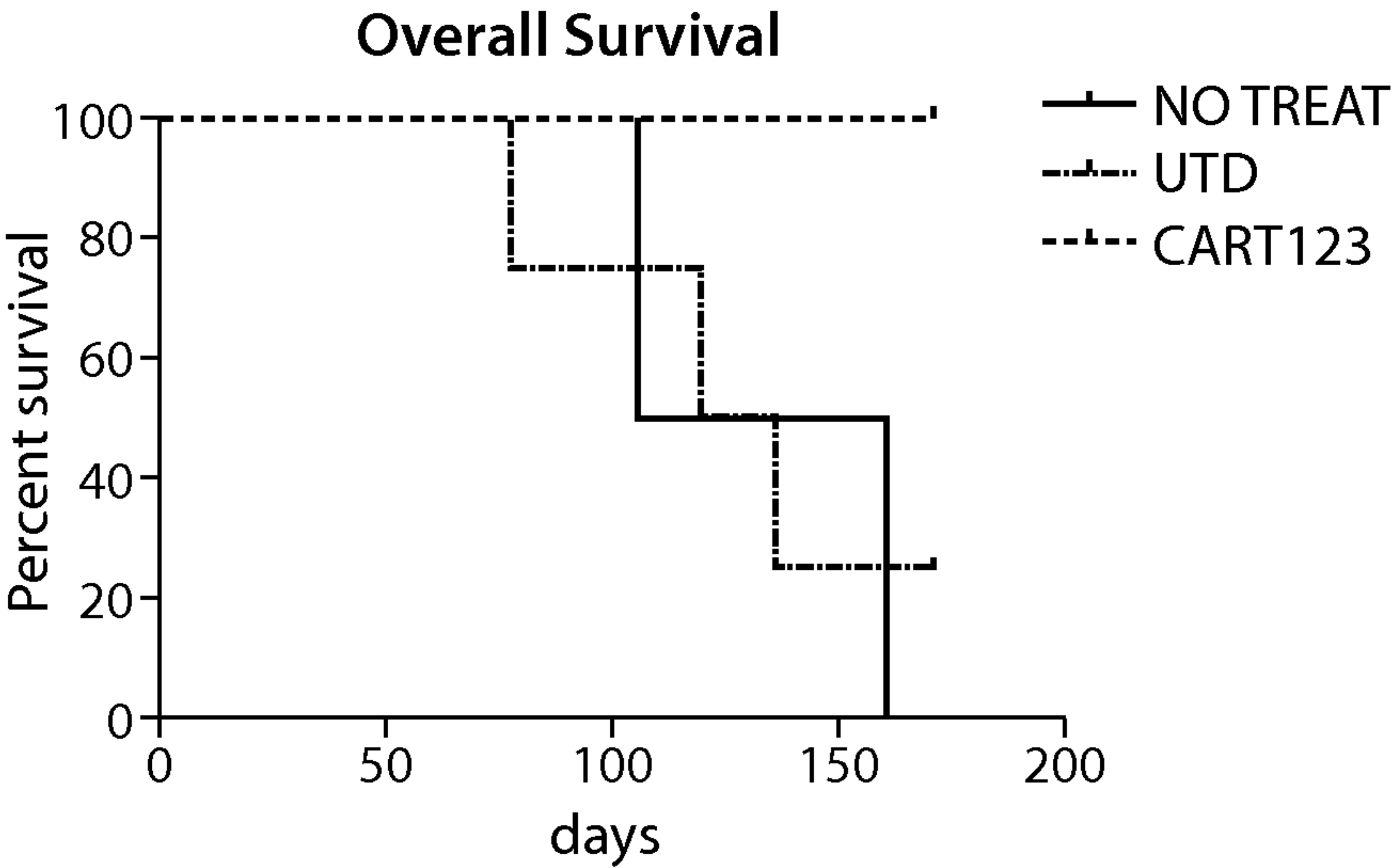
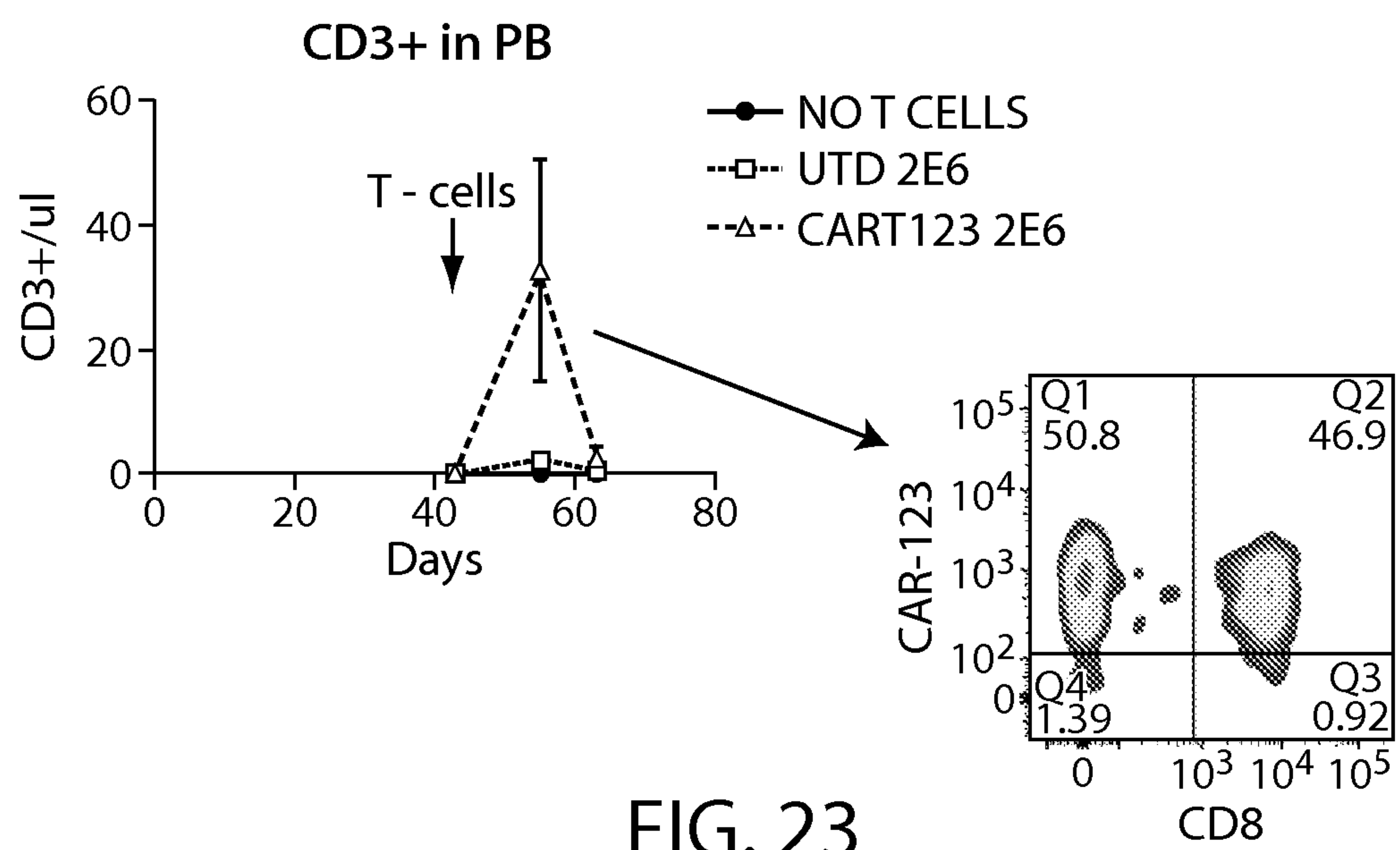


FIG. 22

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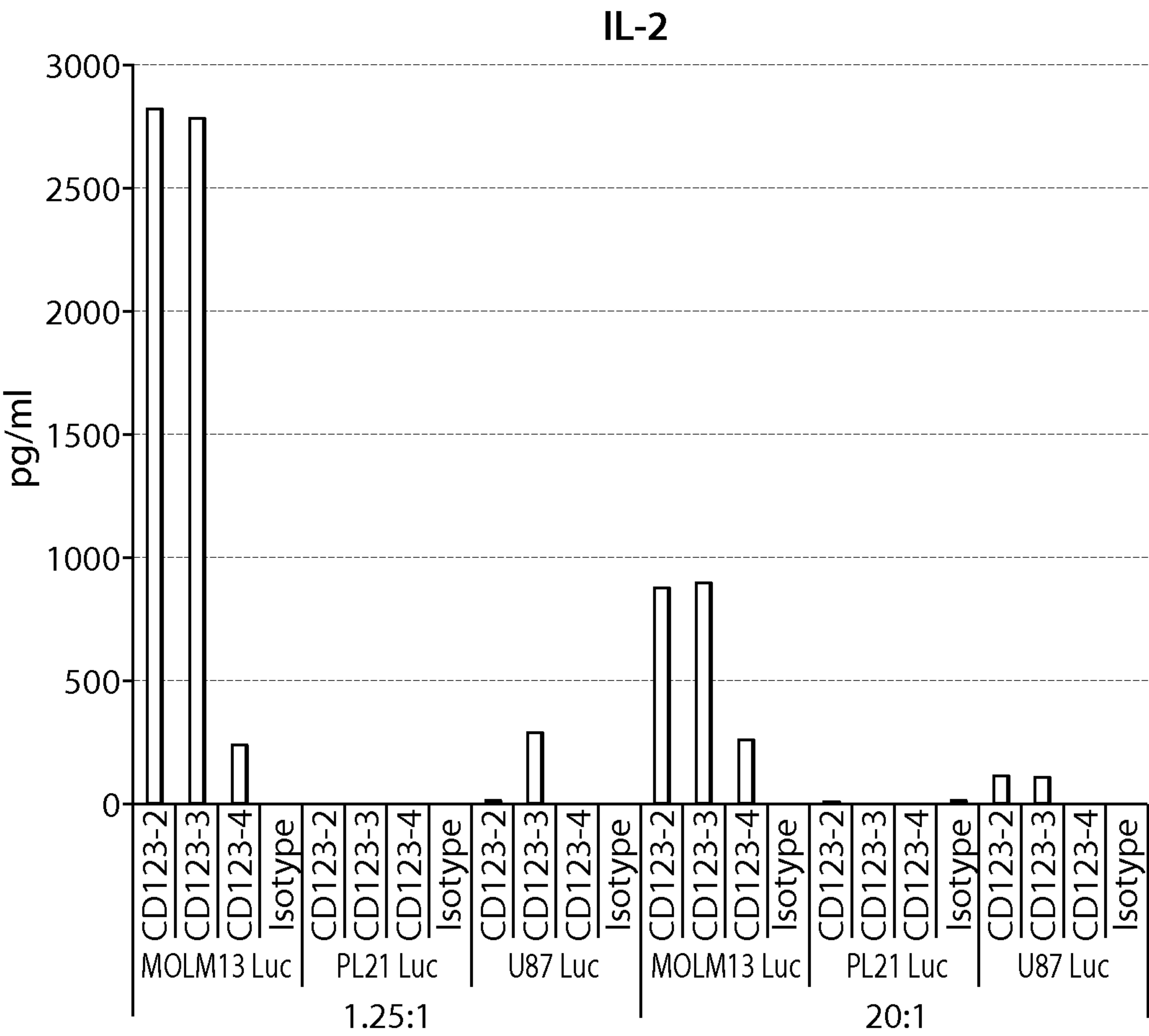


FIG. 24A

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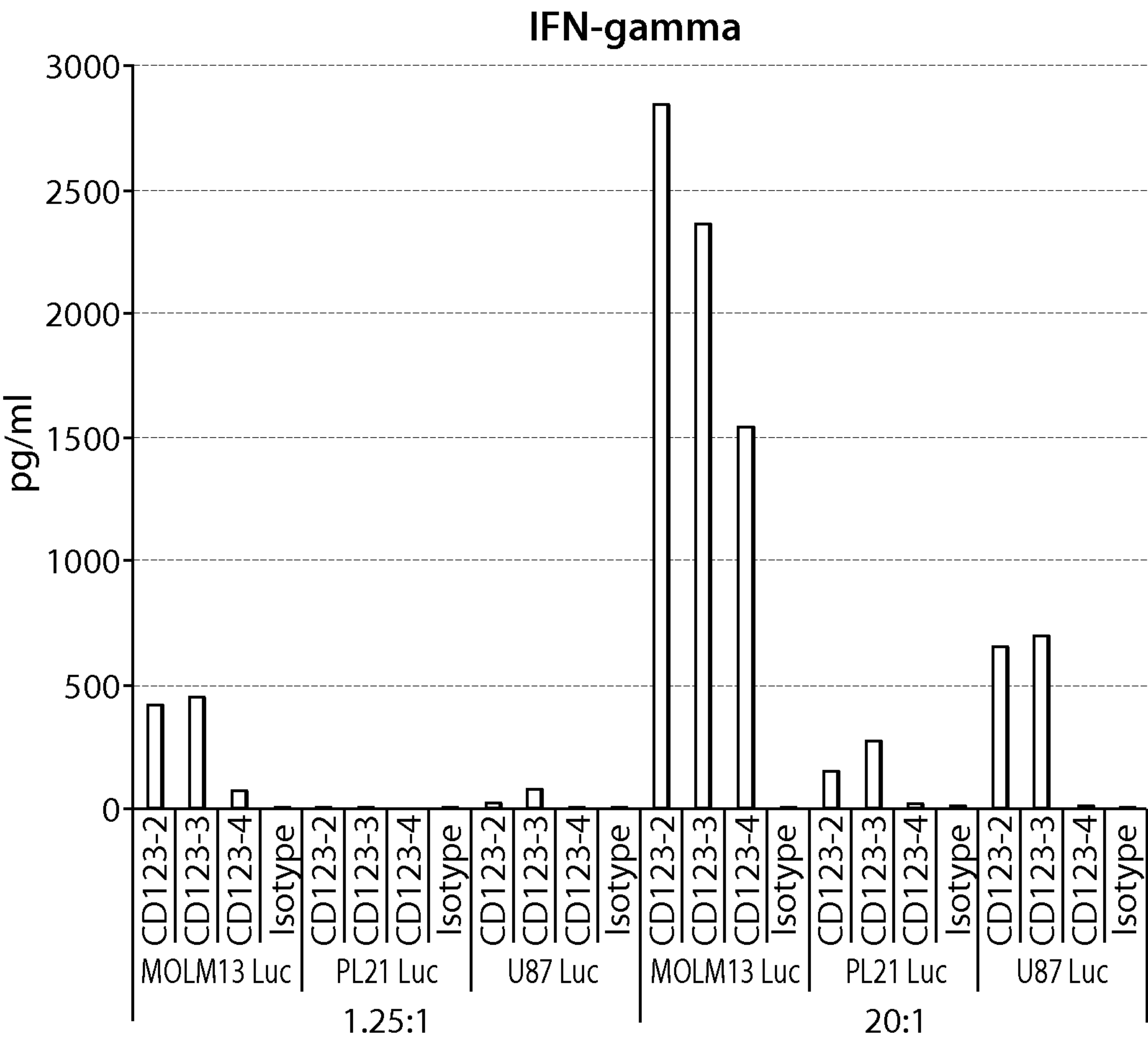


FIG. 24B

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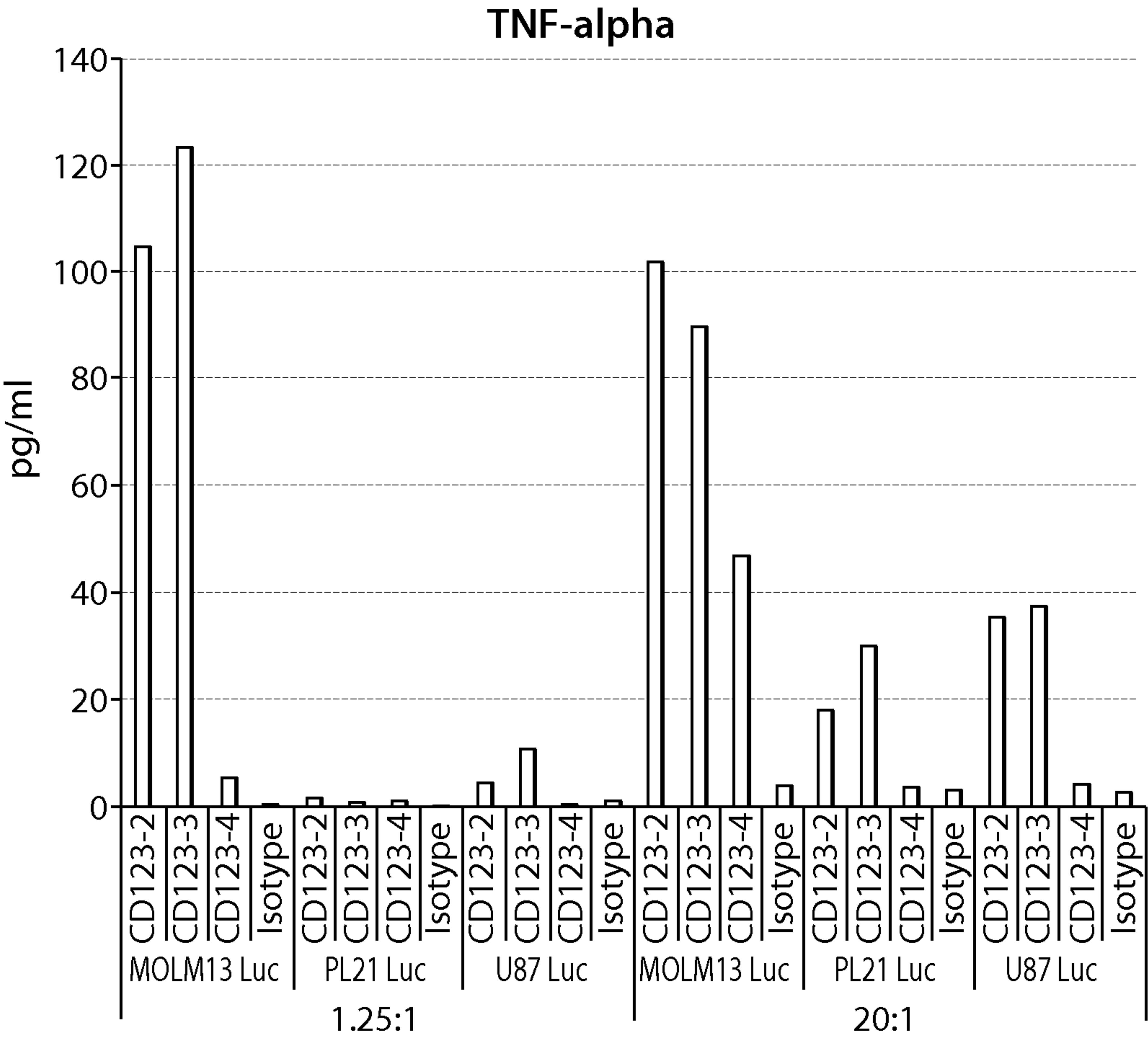


FIG. 24C

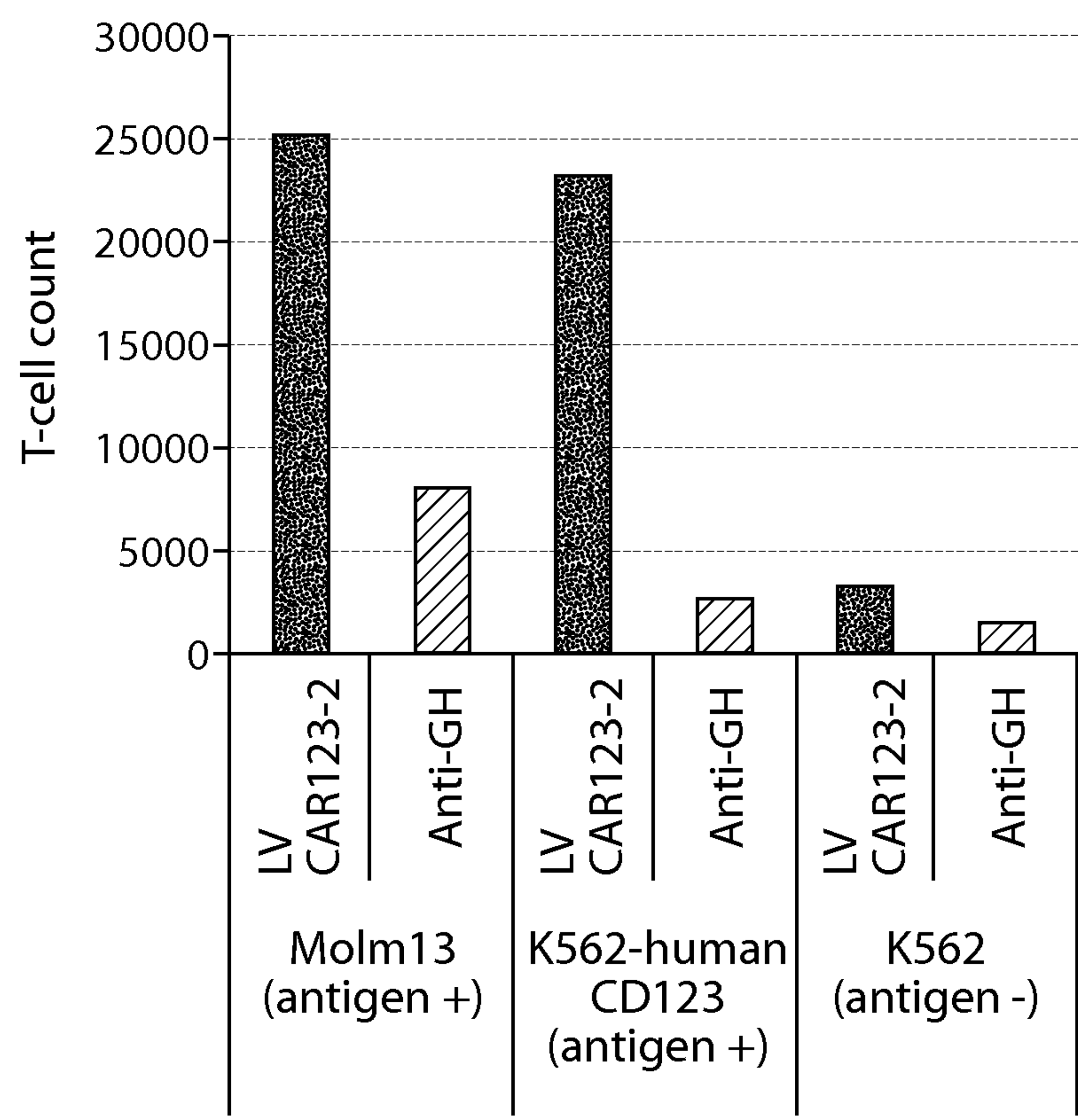


FIG. 25

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FIG. 26A

MOLM13-Luc

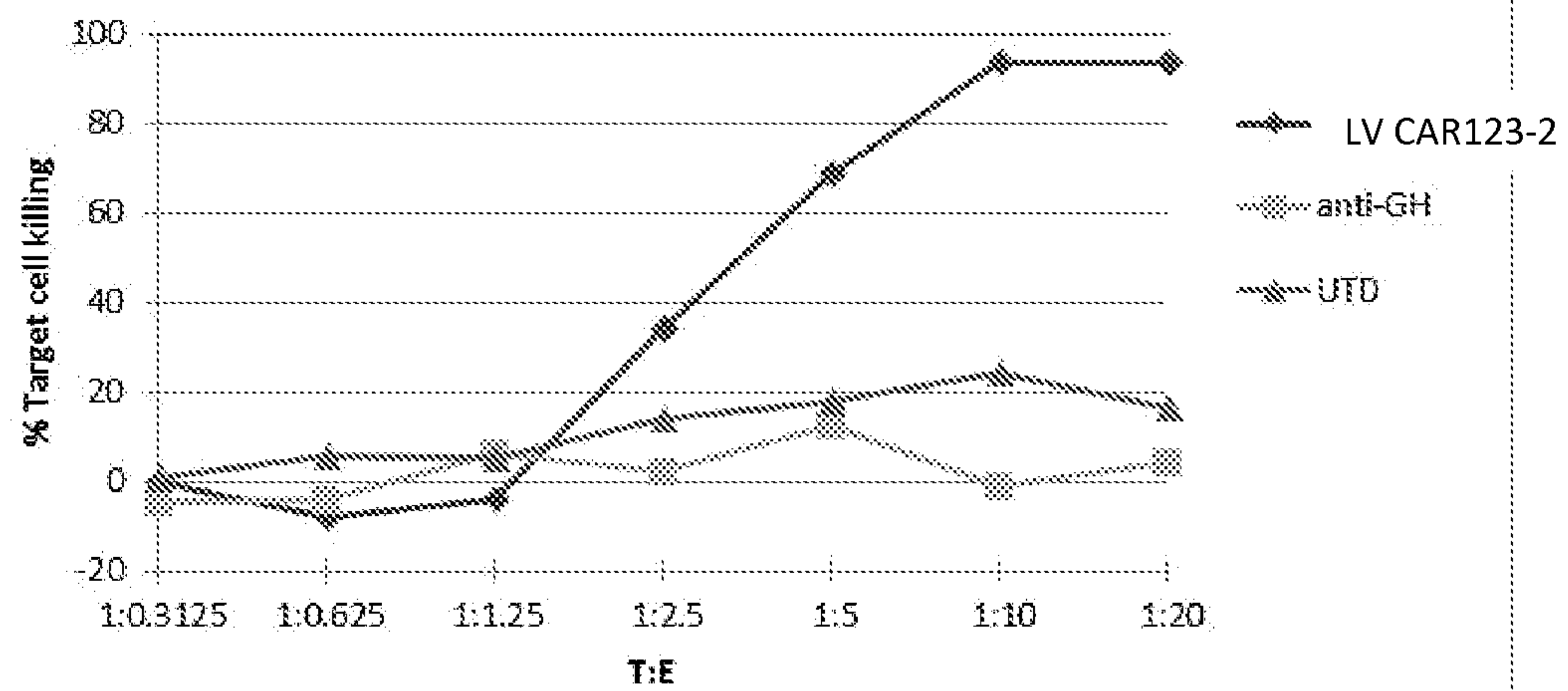


FIG. 26B

PL21-Luc

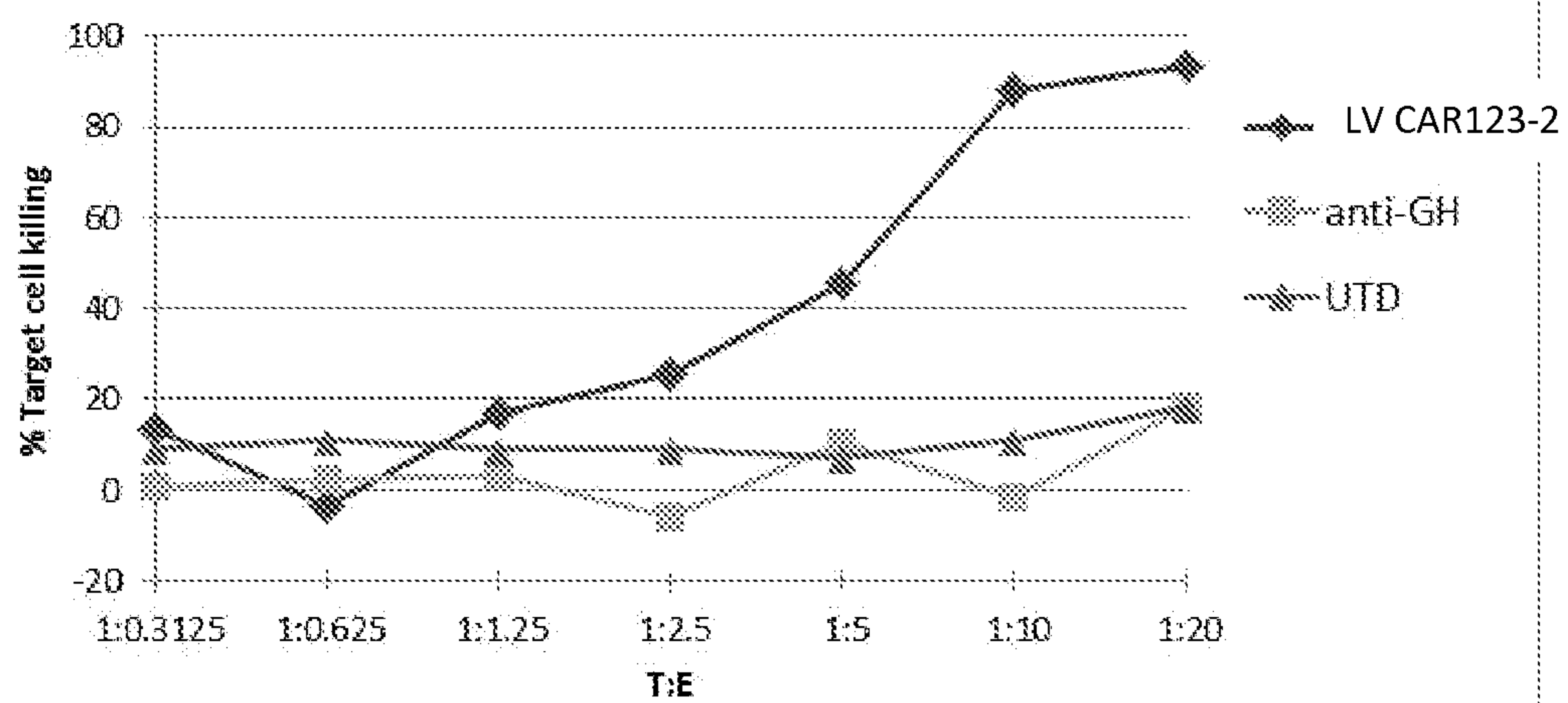
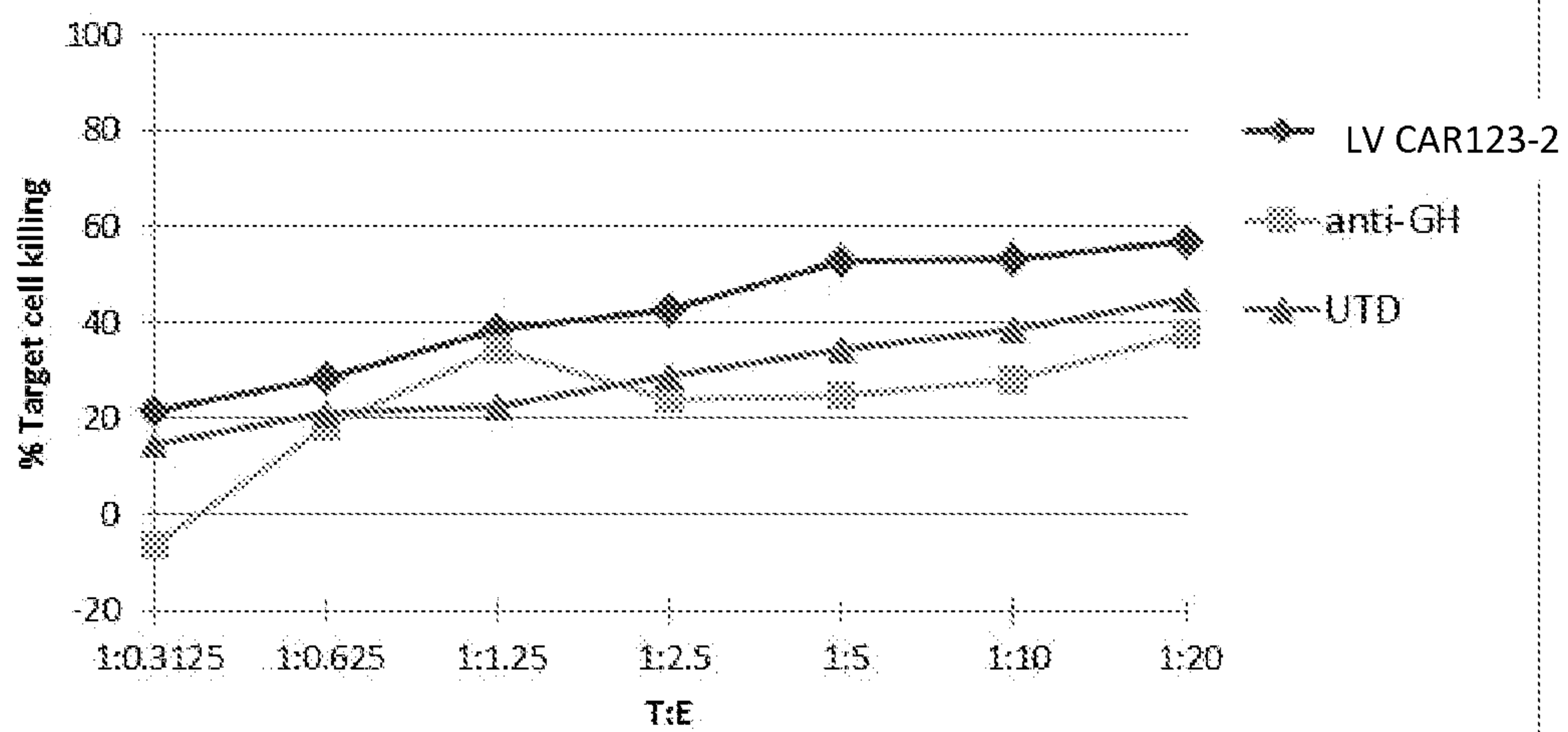


FIG. 26C

U87-Luc



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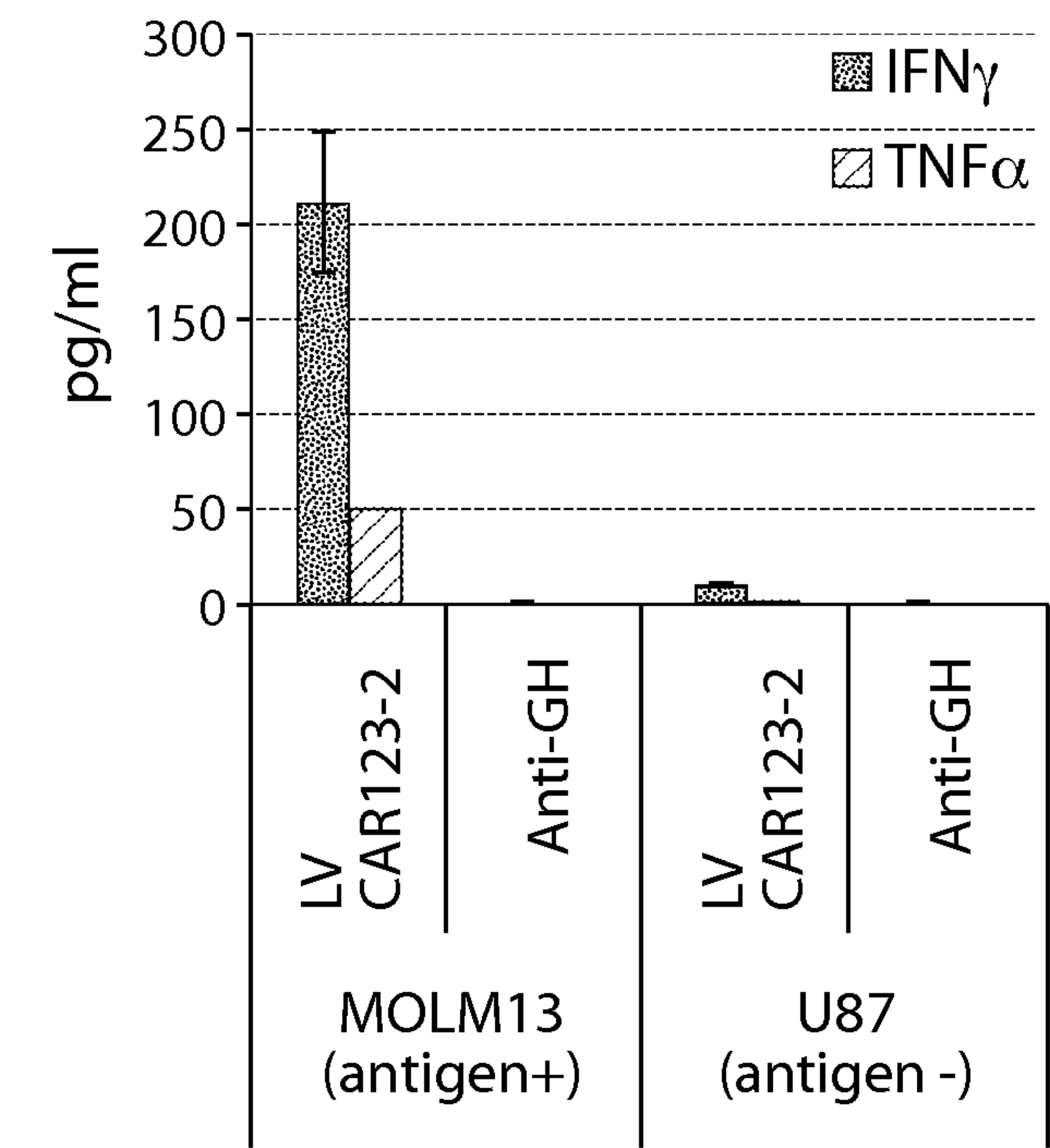


FIG. 27A

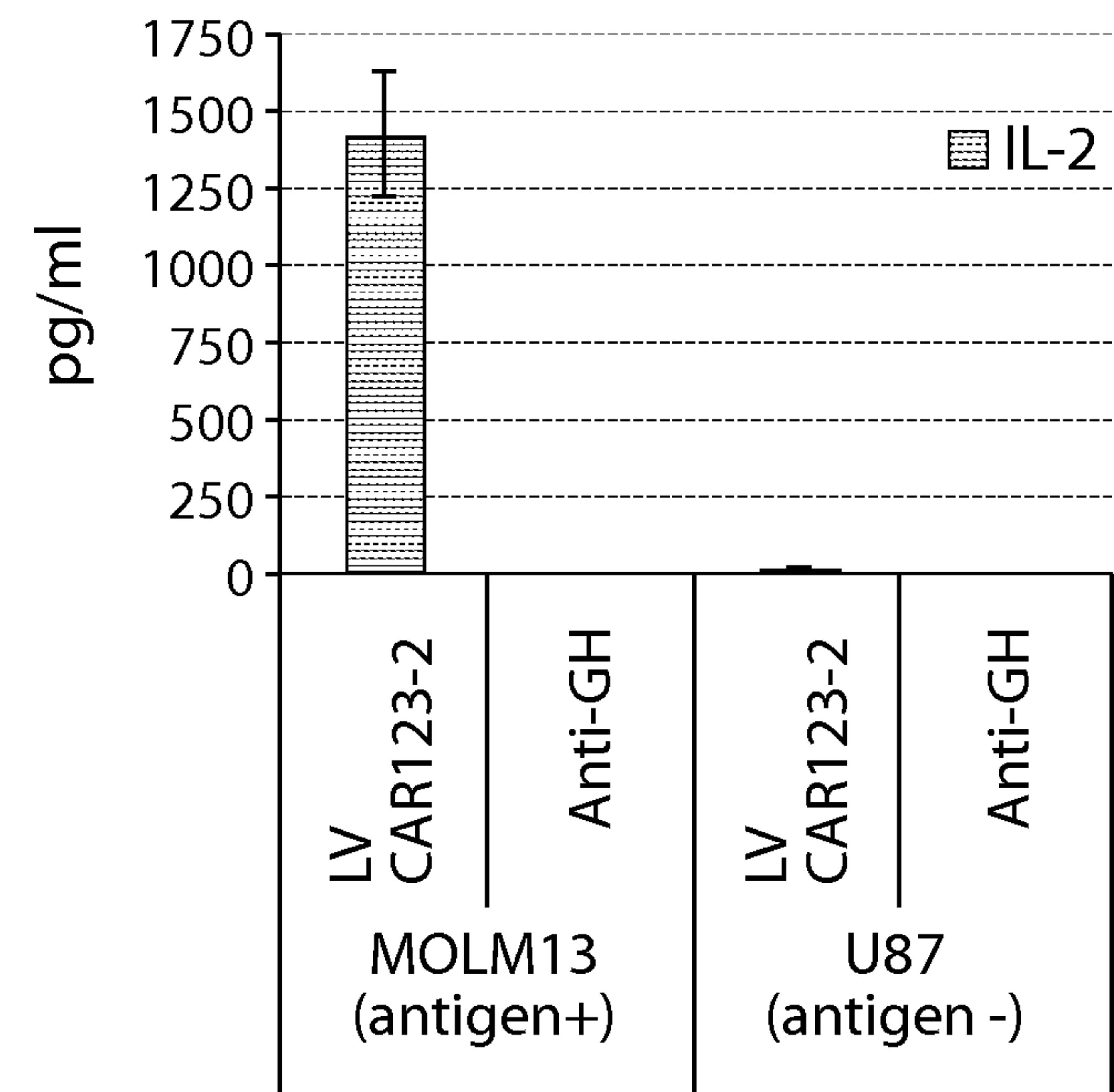


FIG. 27B

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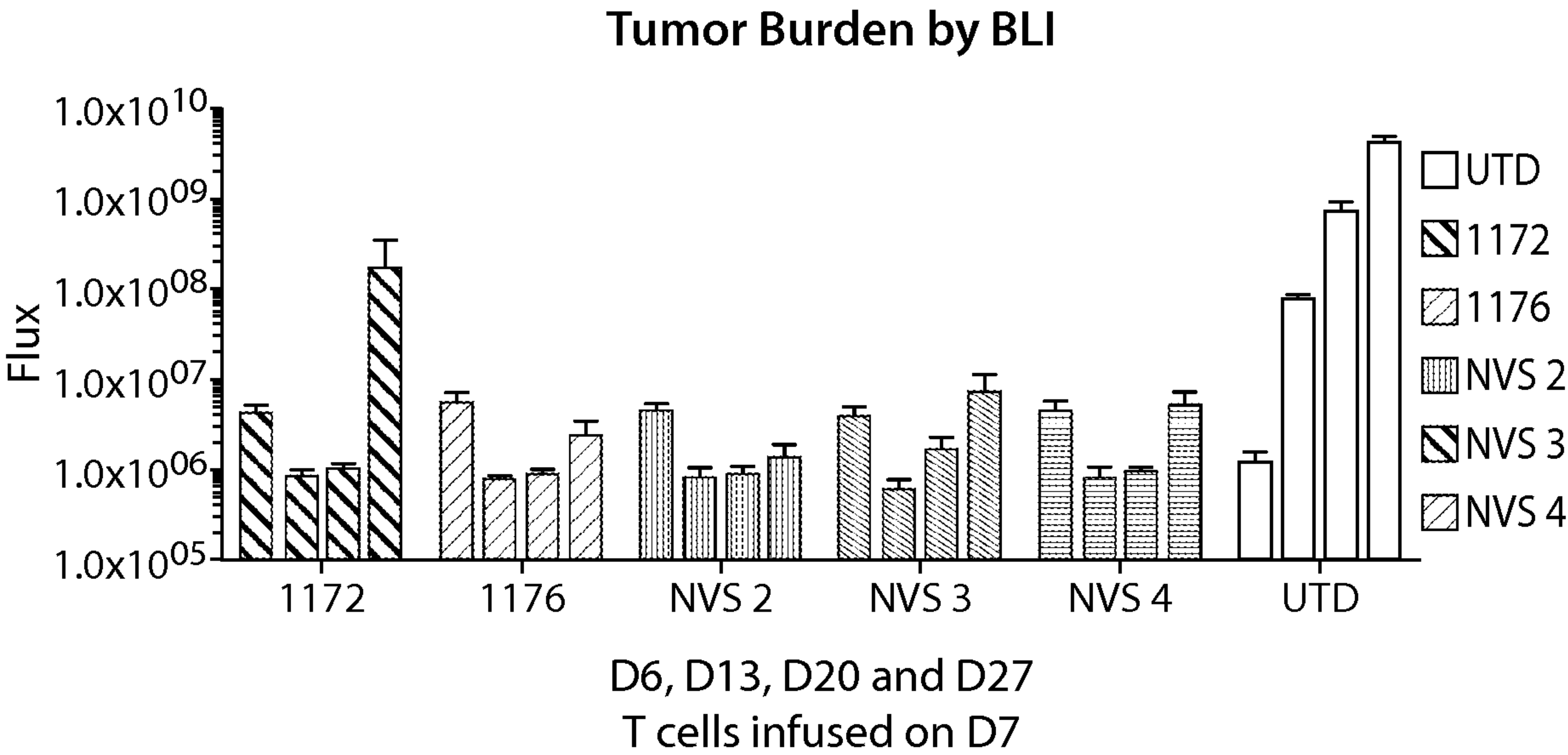


FIG. 28

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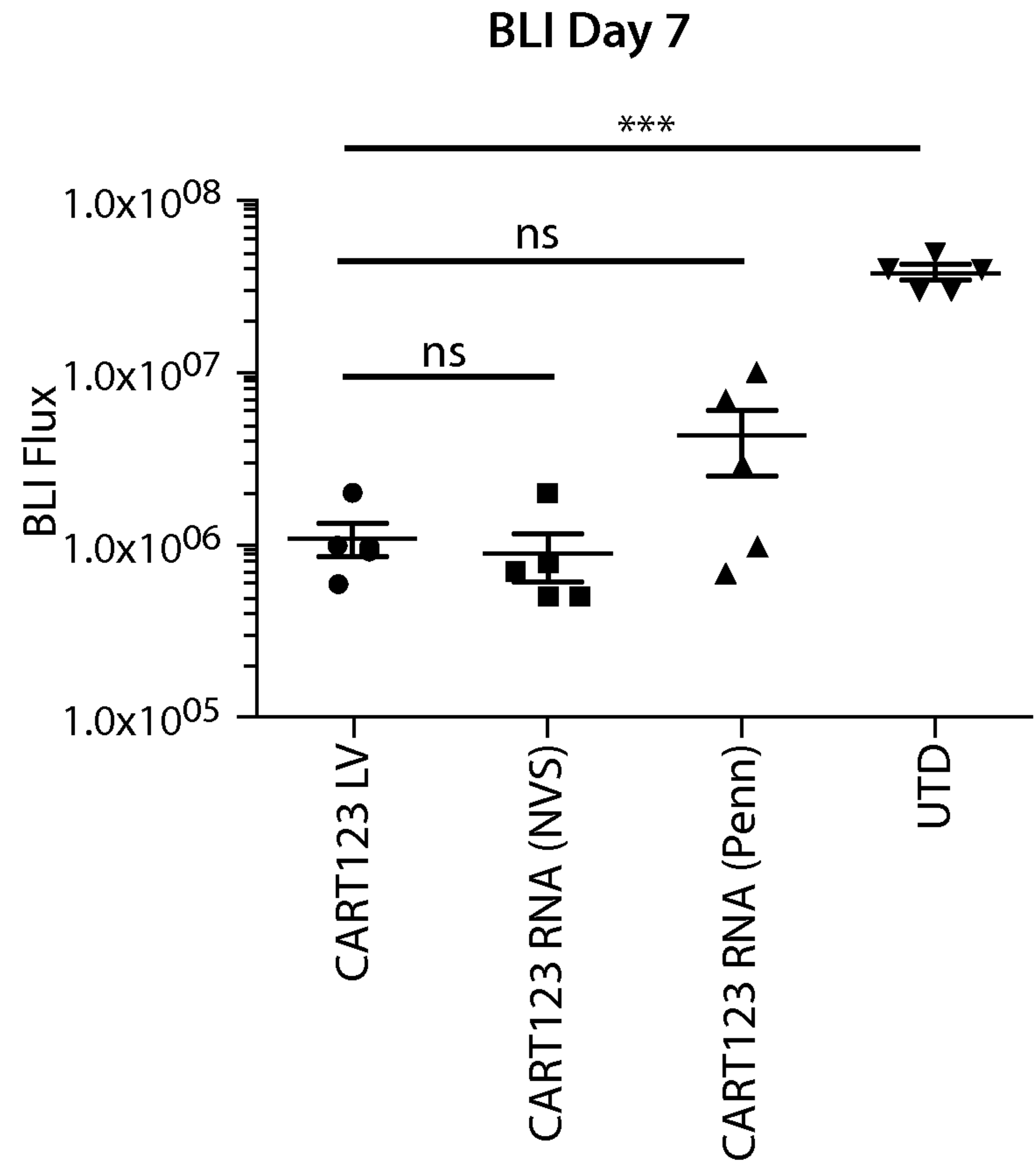
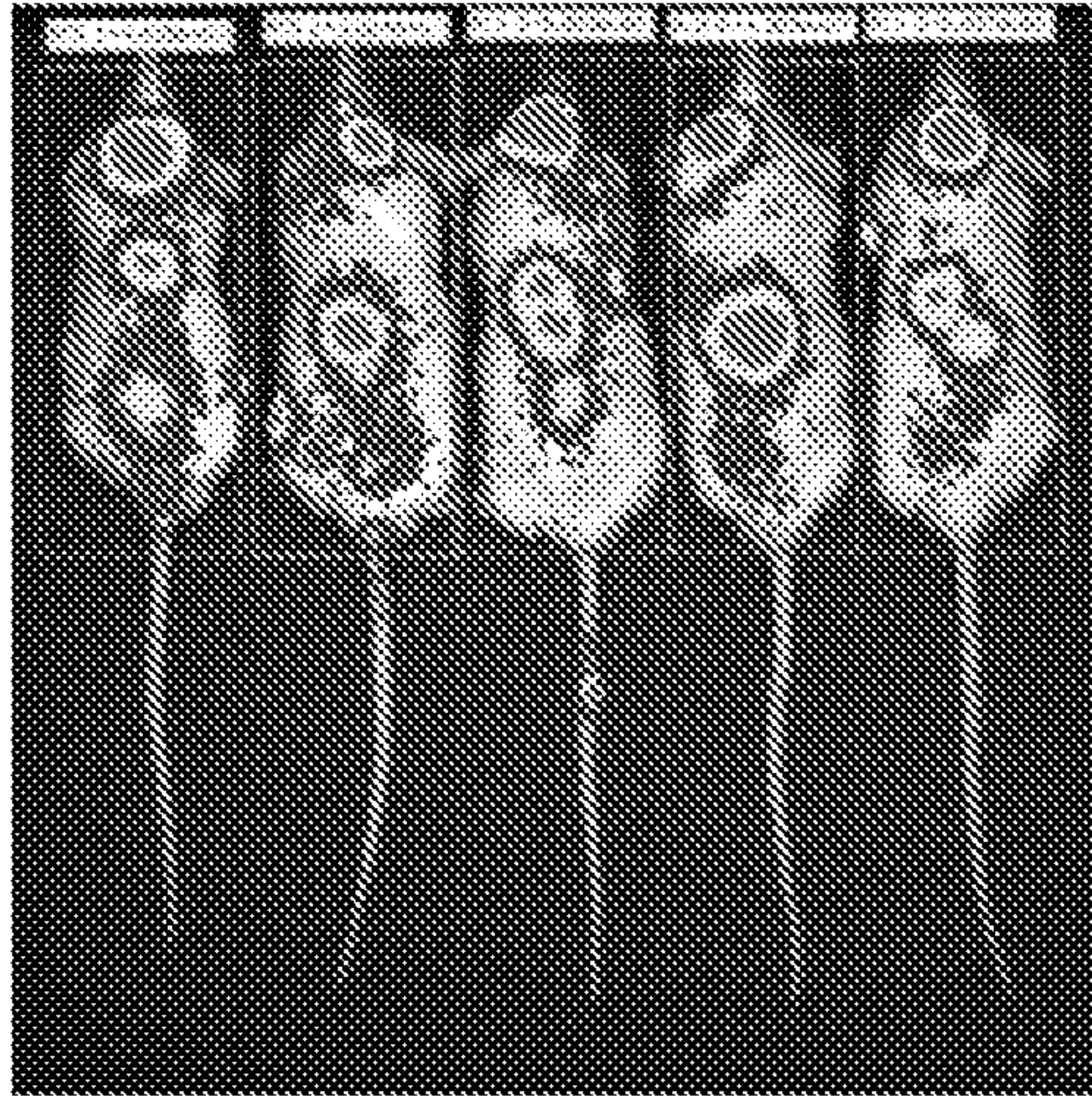
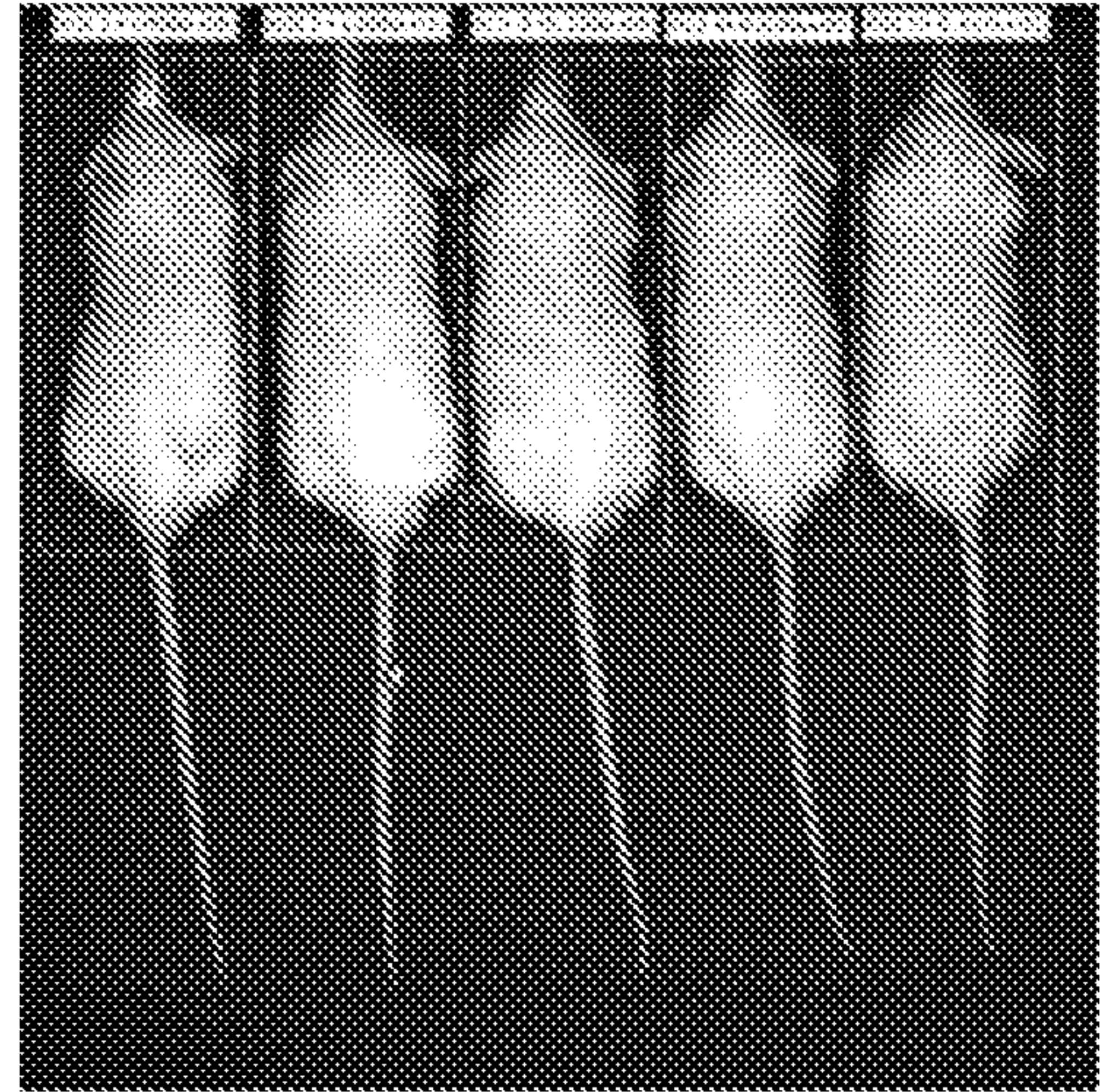


FIG. 29

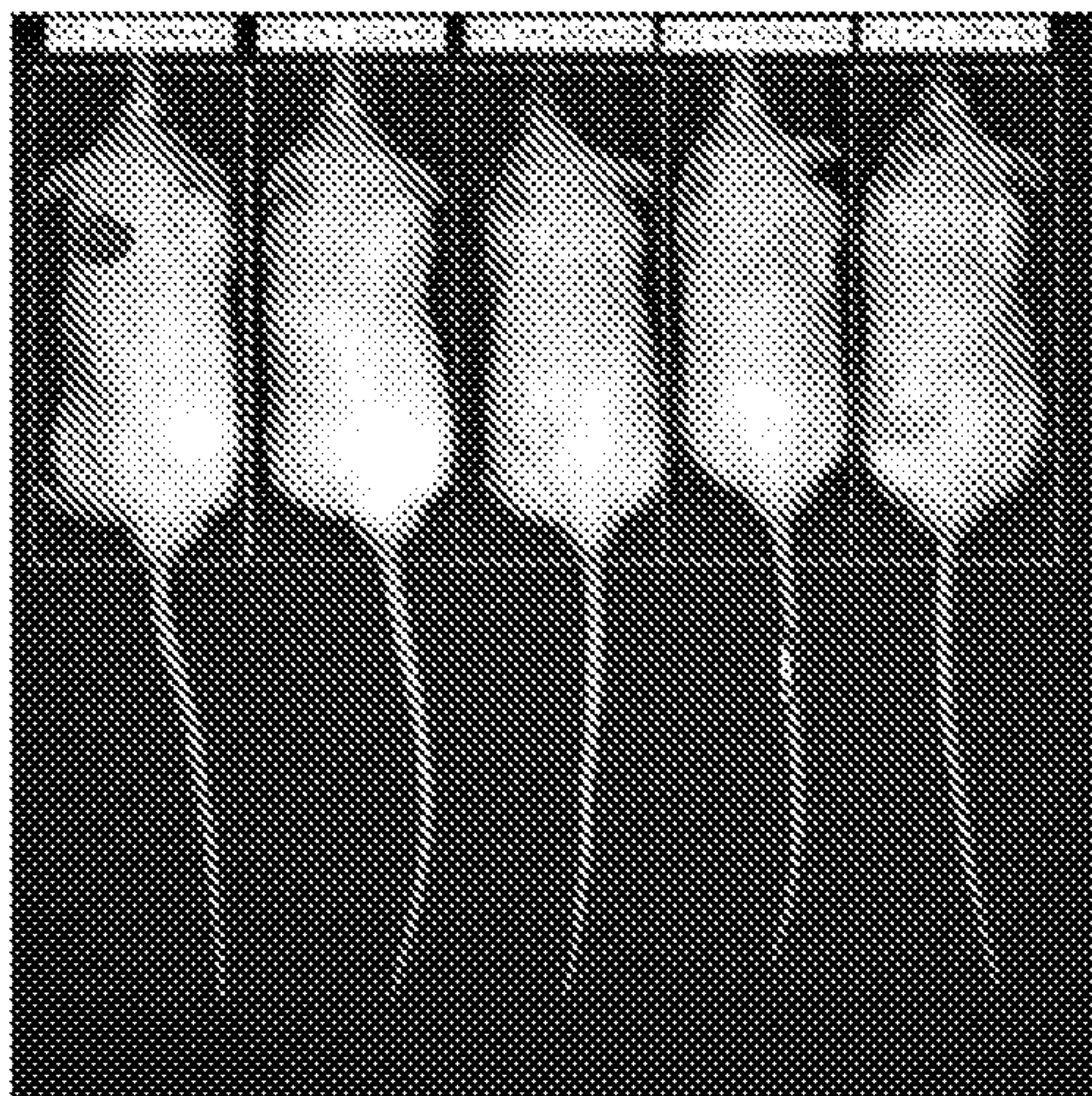
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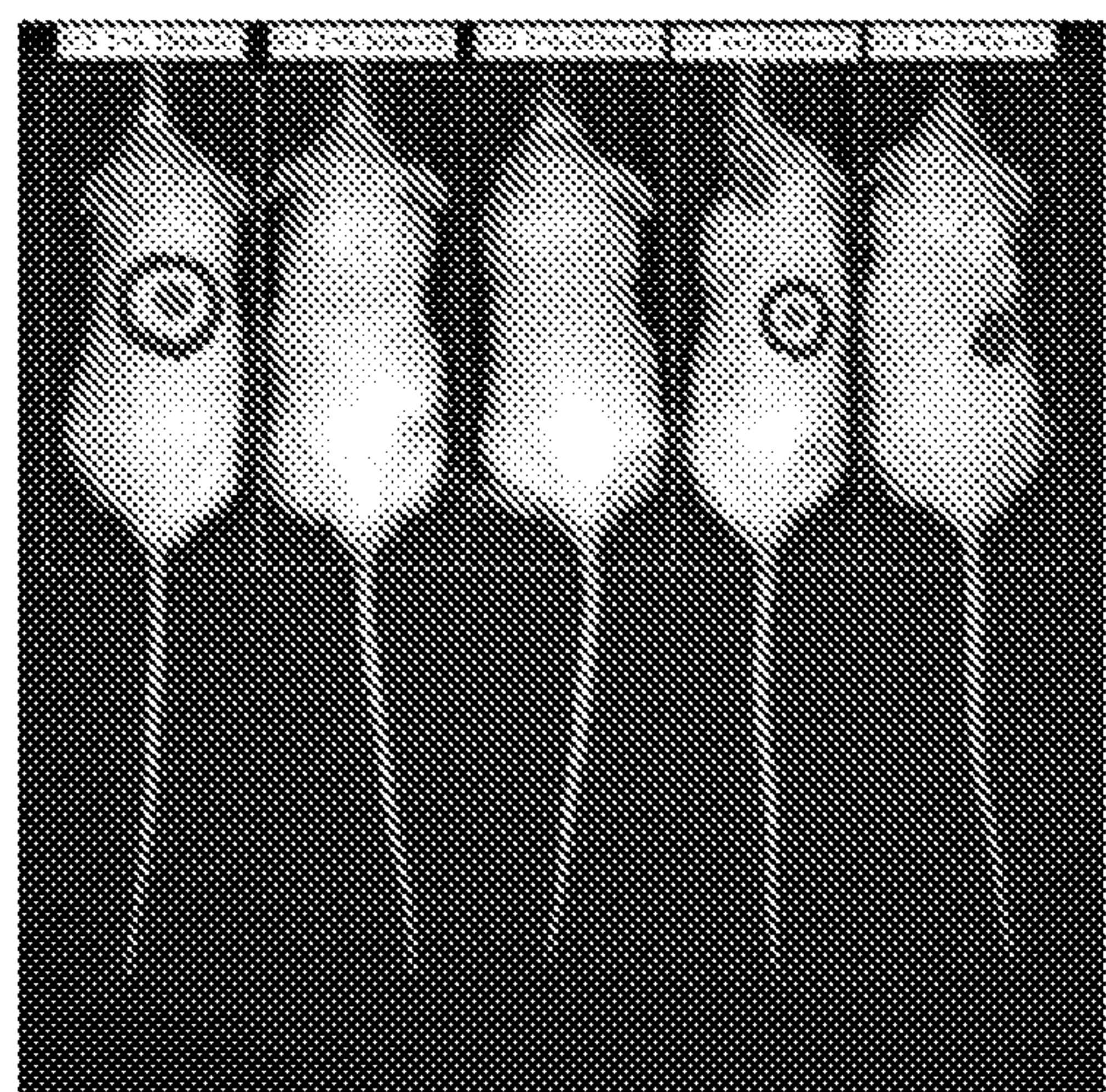
Untransduced T cells
FIG. 30A



RNA CD123-2 CART
FIG. 30B



Lentivirus CD123 tool CAR
FIG. 30C



RNA CD123 tool CAR
FIG. 30D

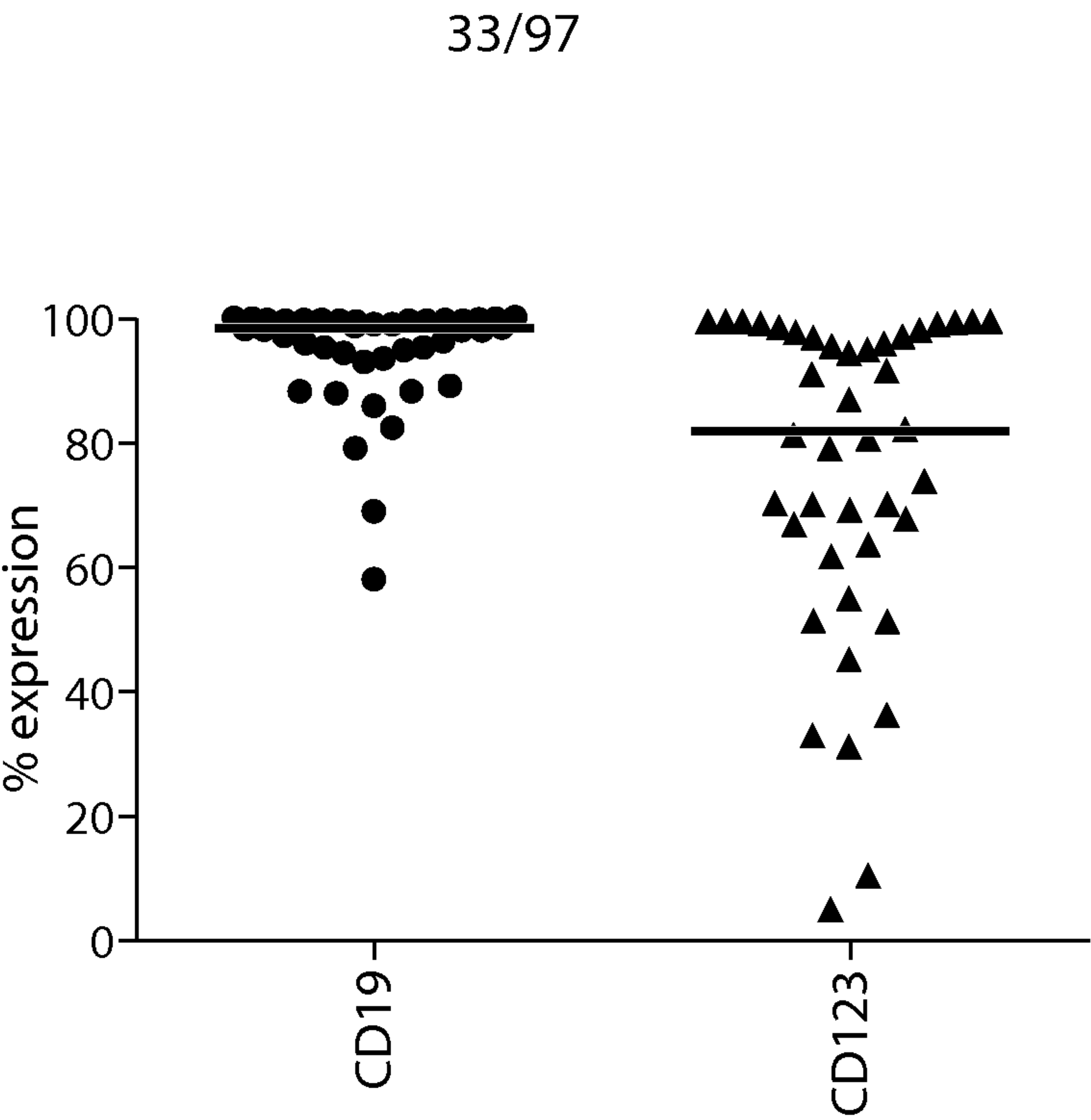


FIG. 31A

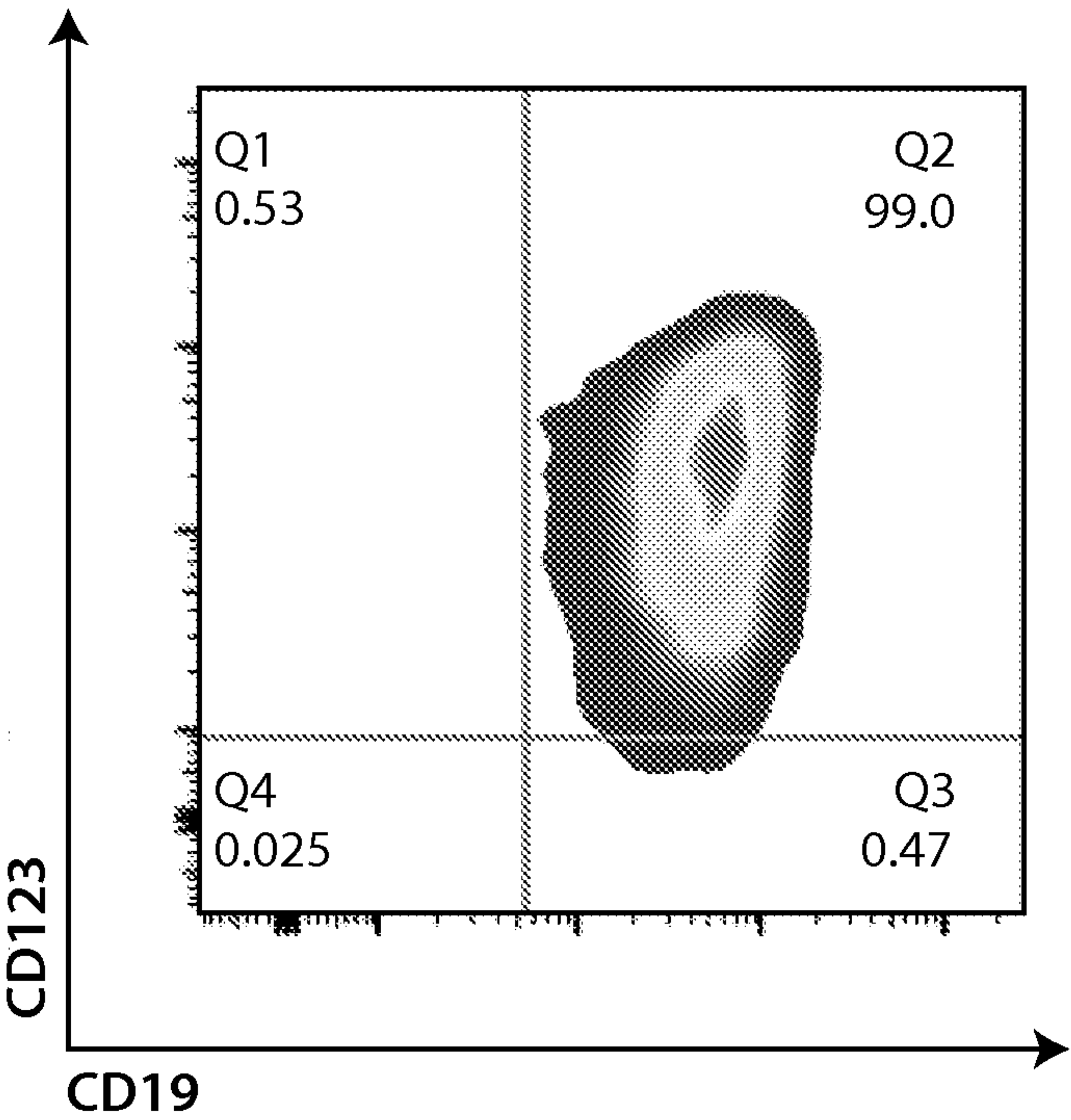


FIG. 31B

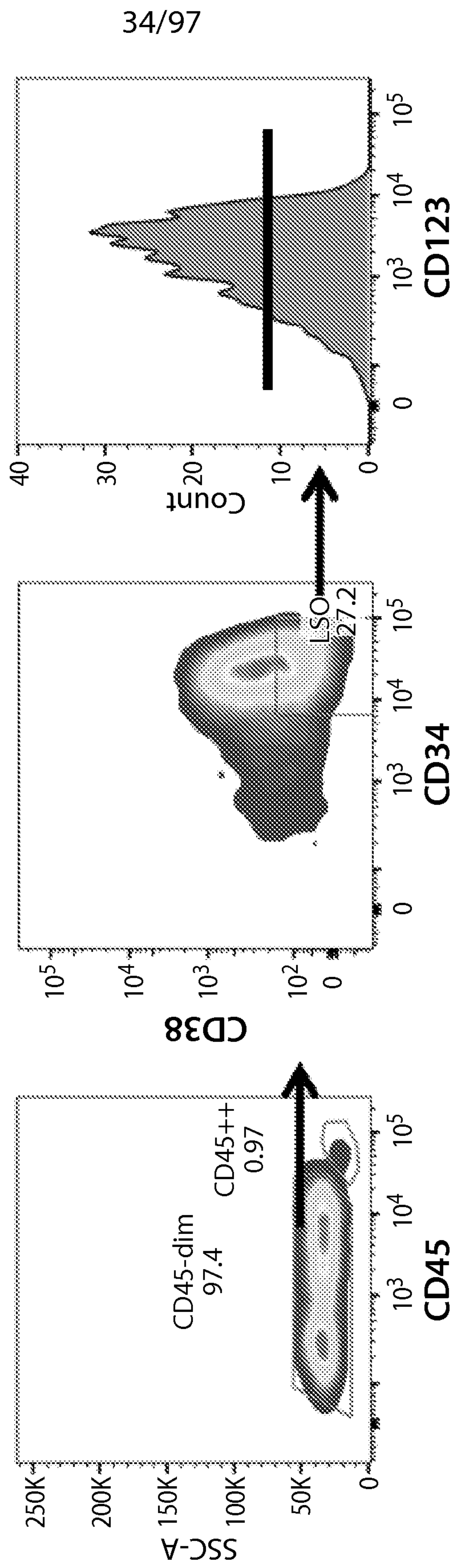


FIG. 31C

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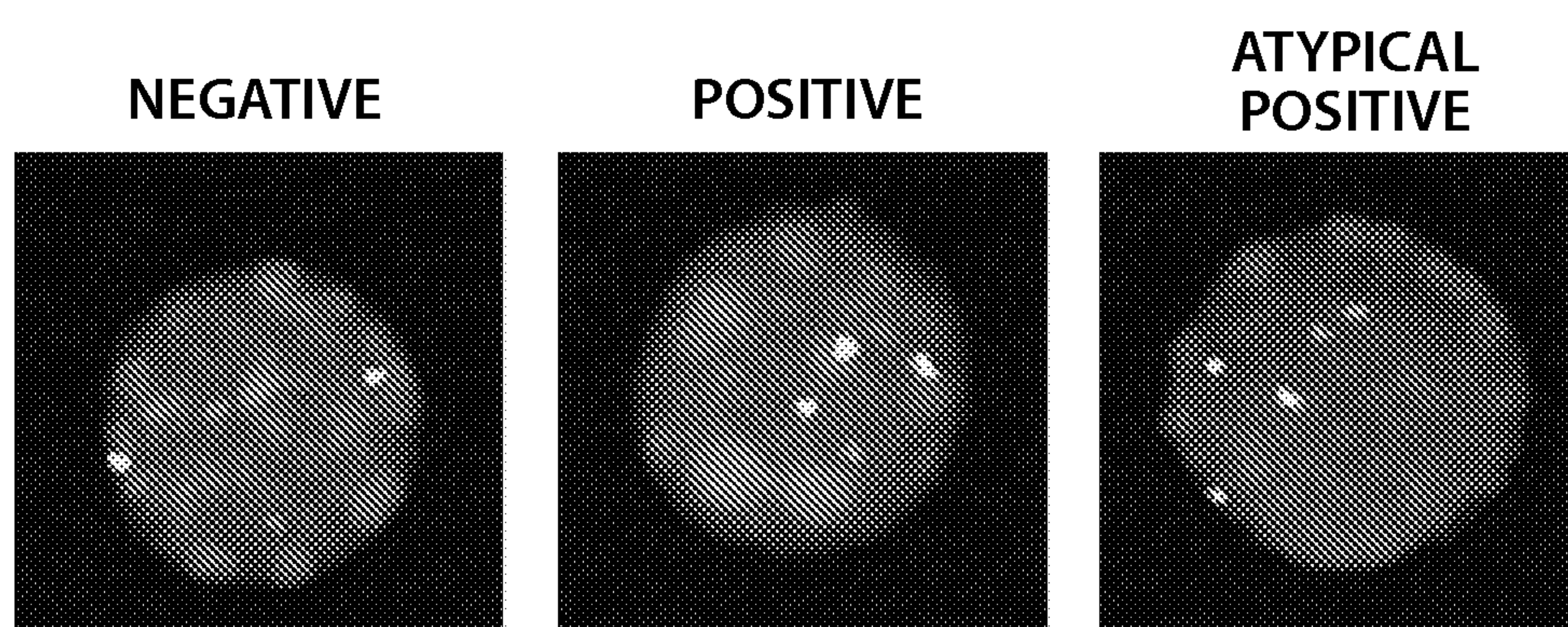
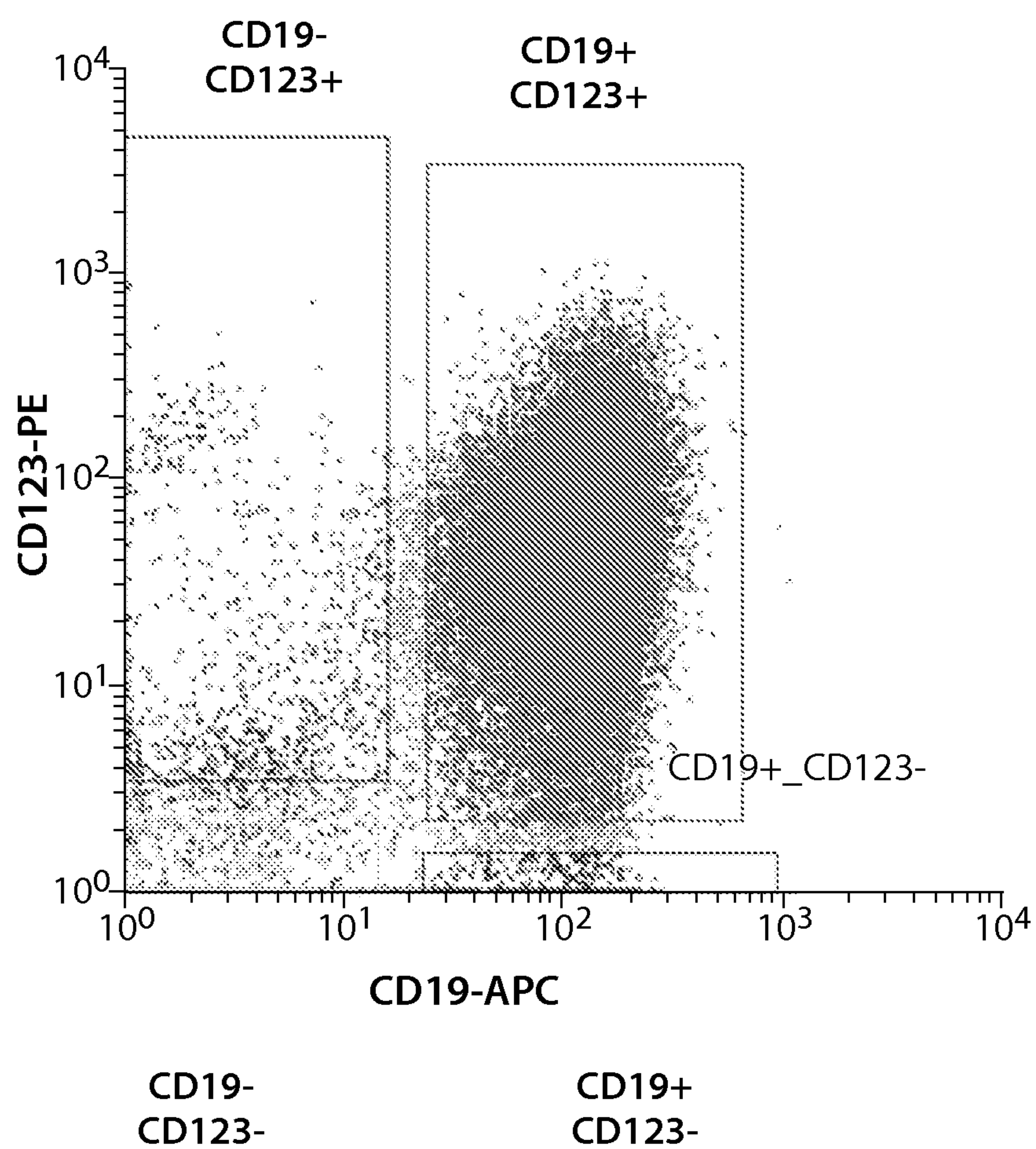


FIG. 31D

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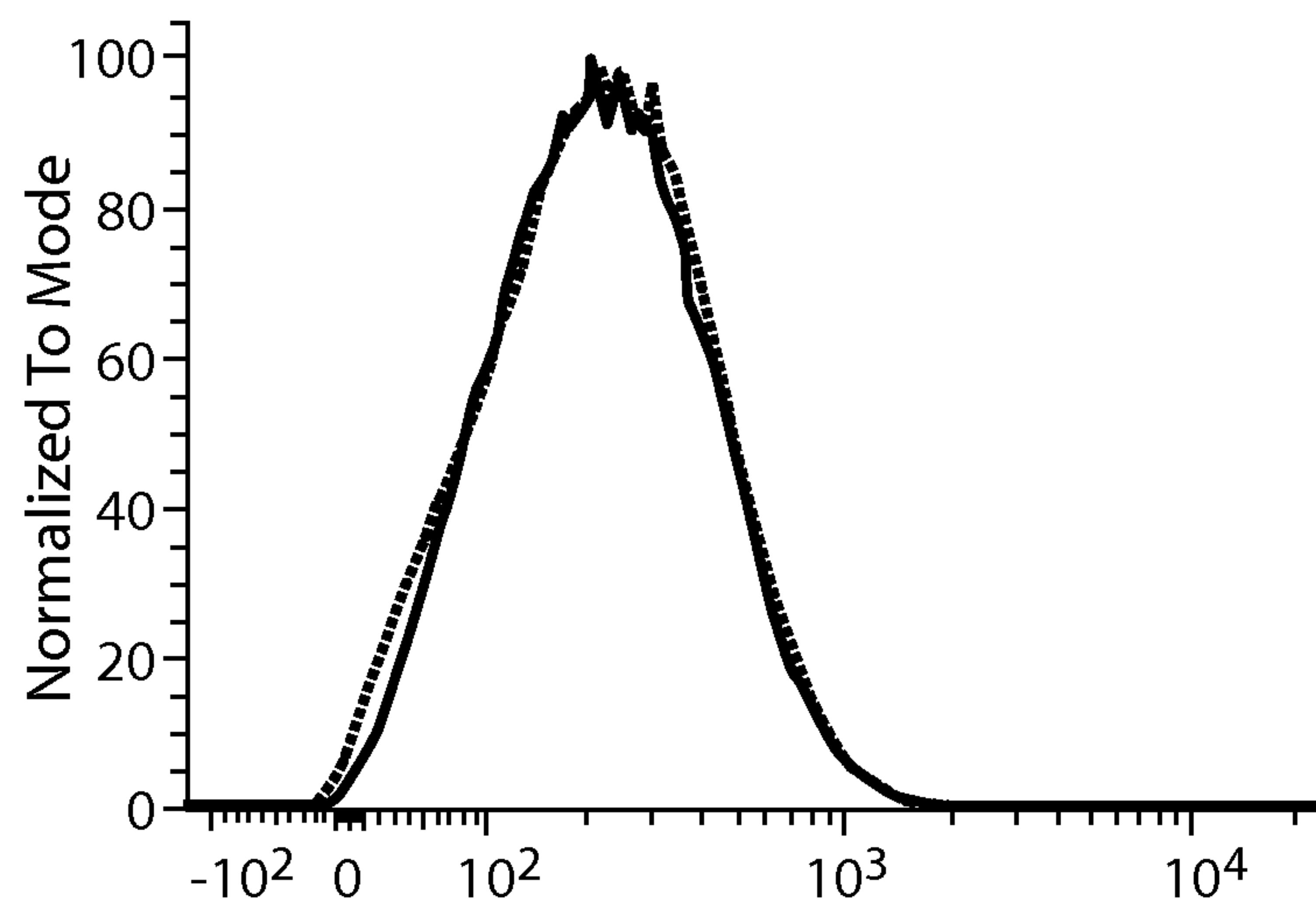
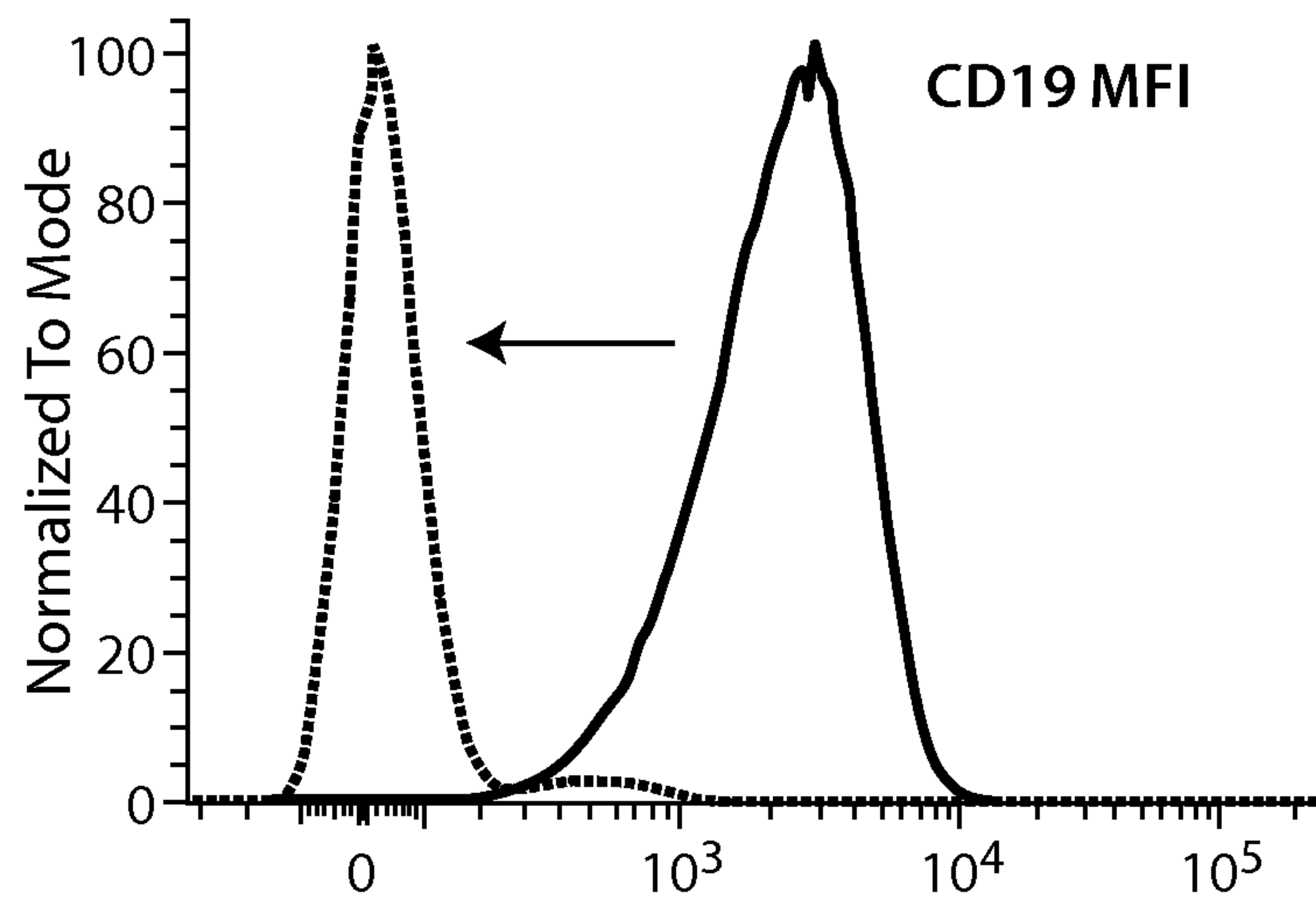


FIG. 31E

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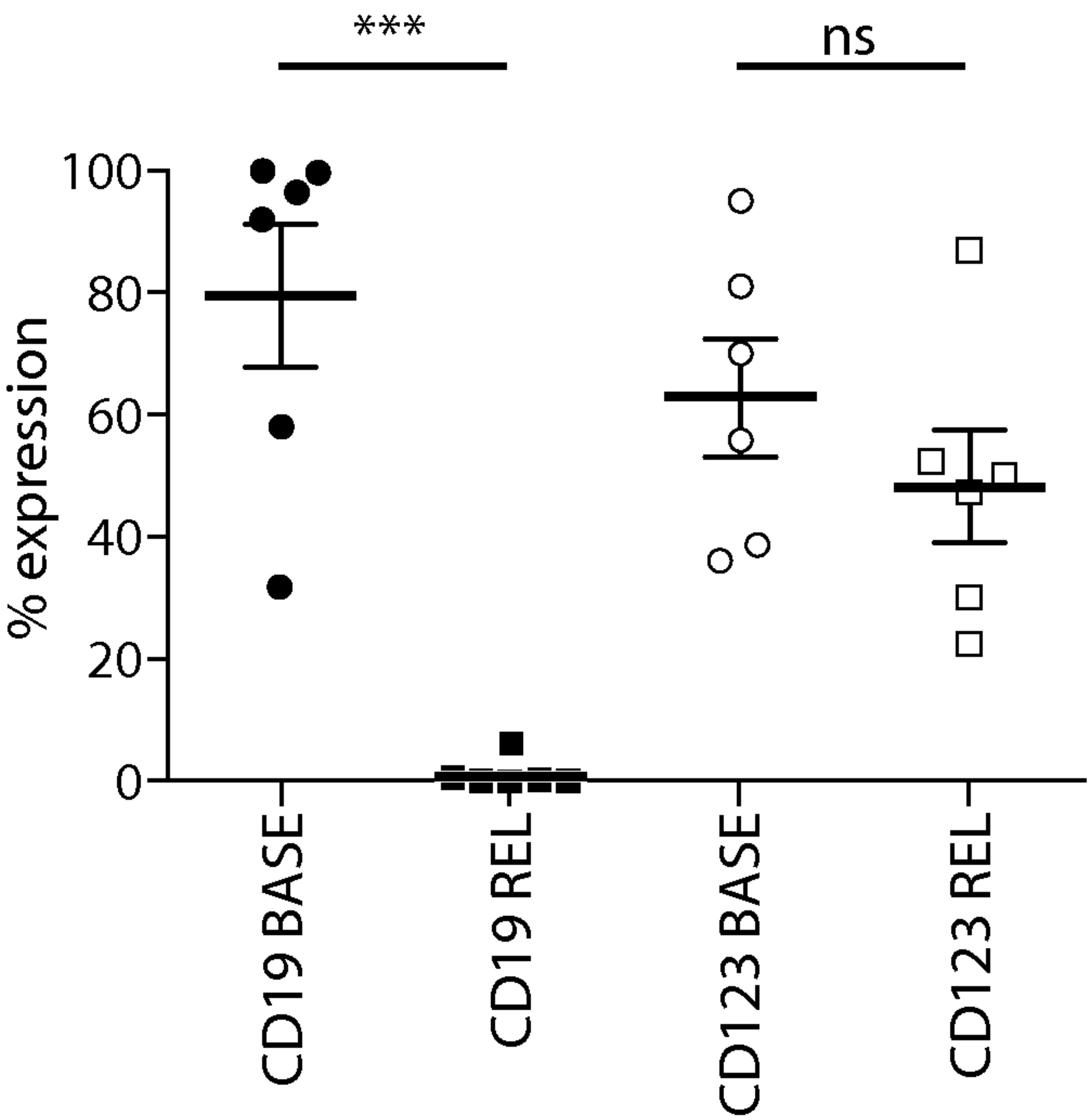


FIG. 31F

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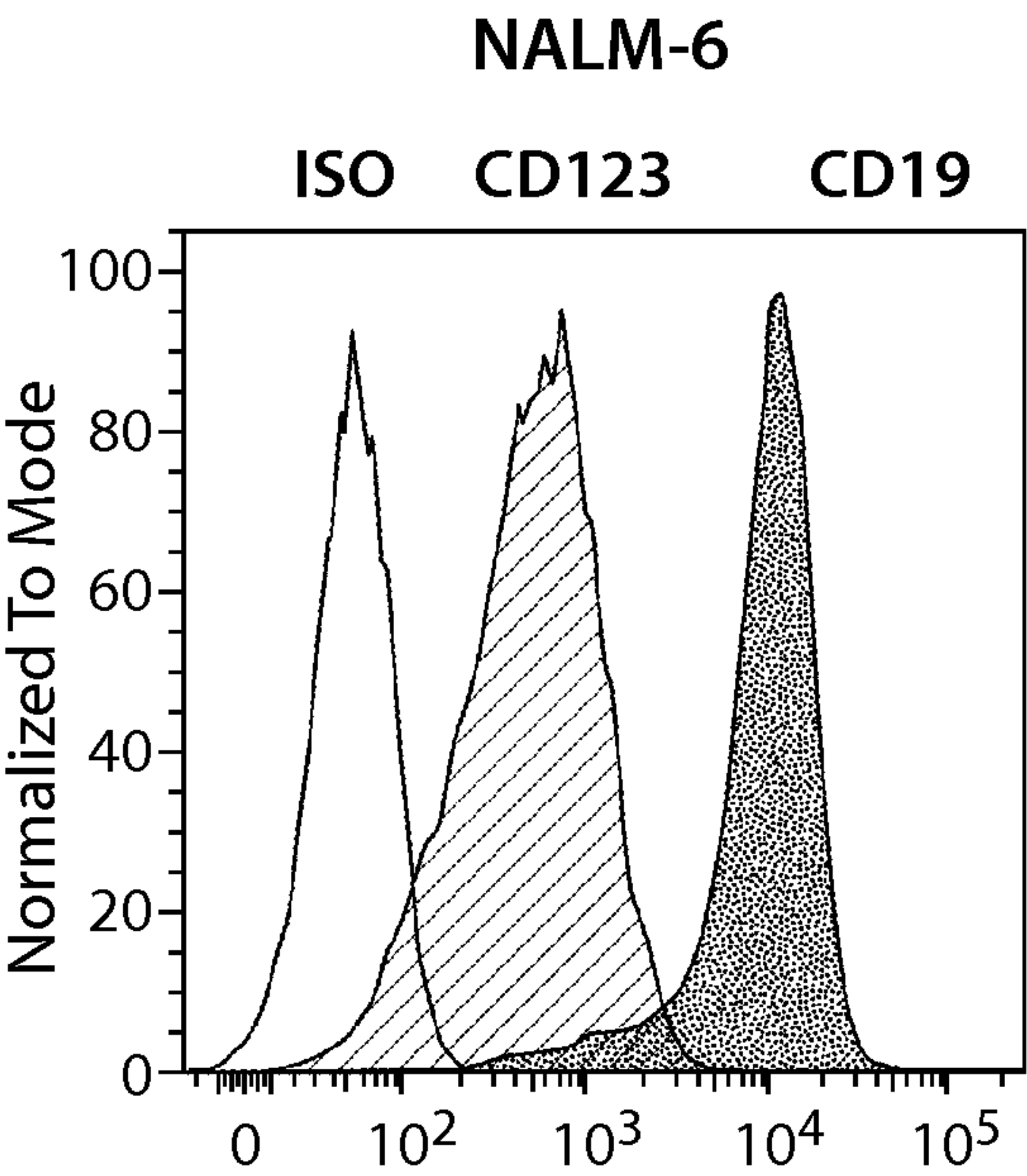


FIG. 32A

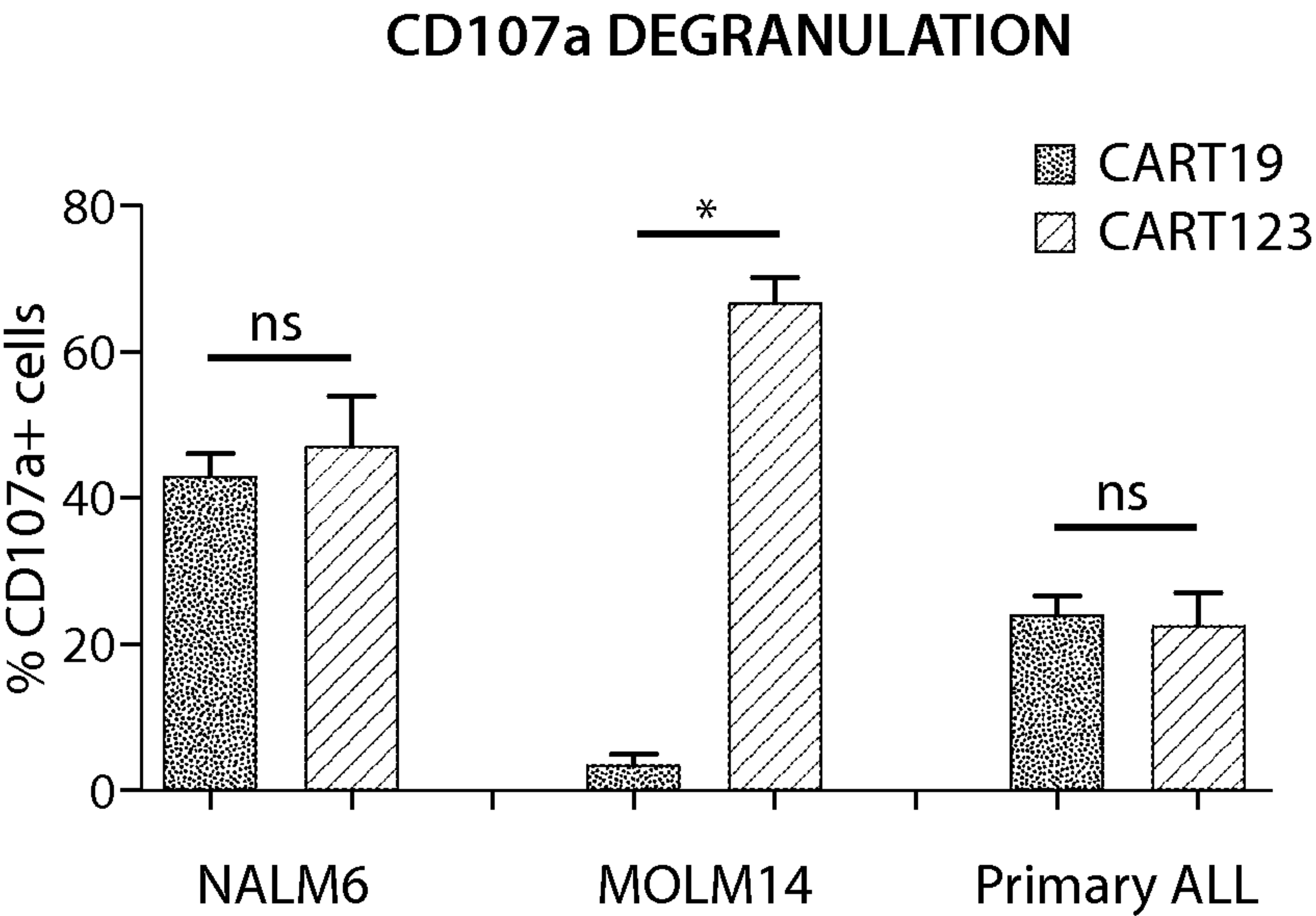


FIG. 32B

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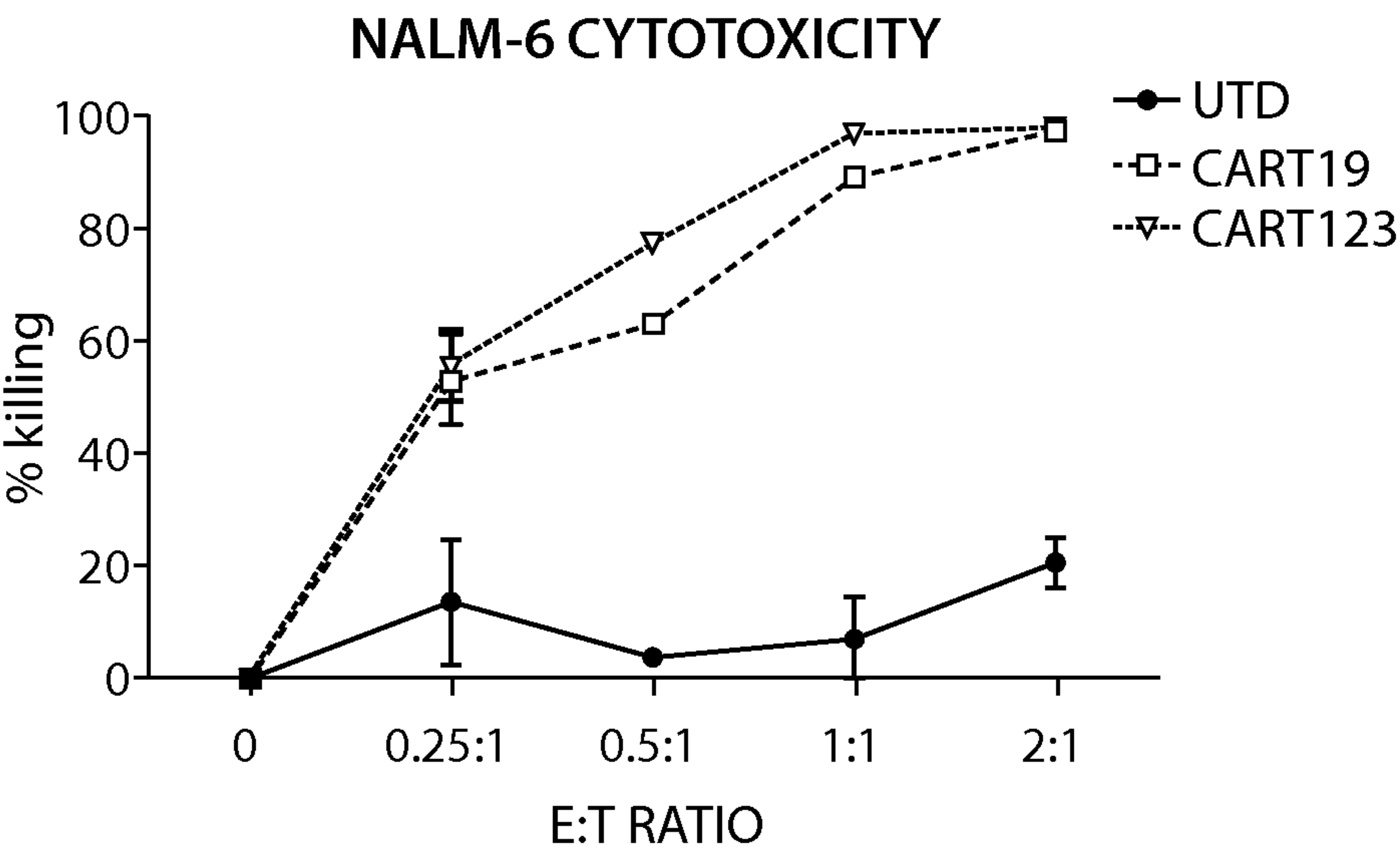


FIG. 32C

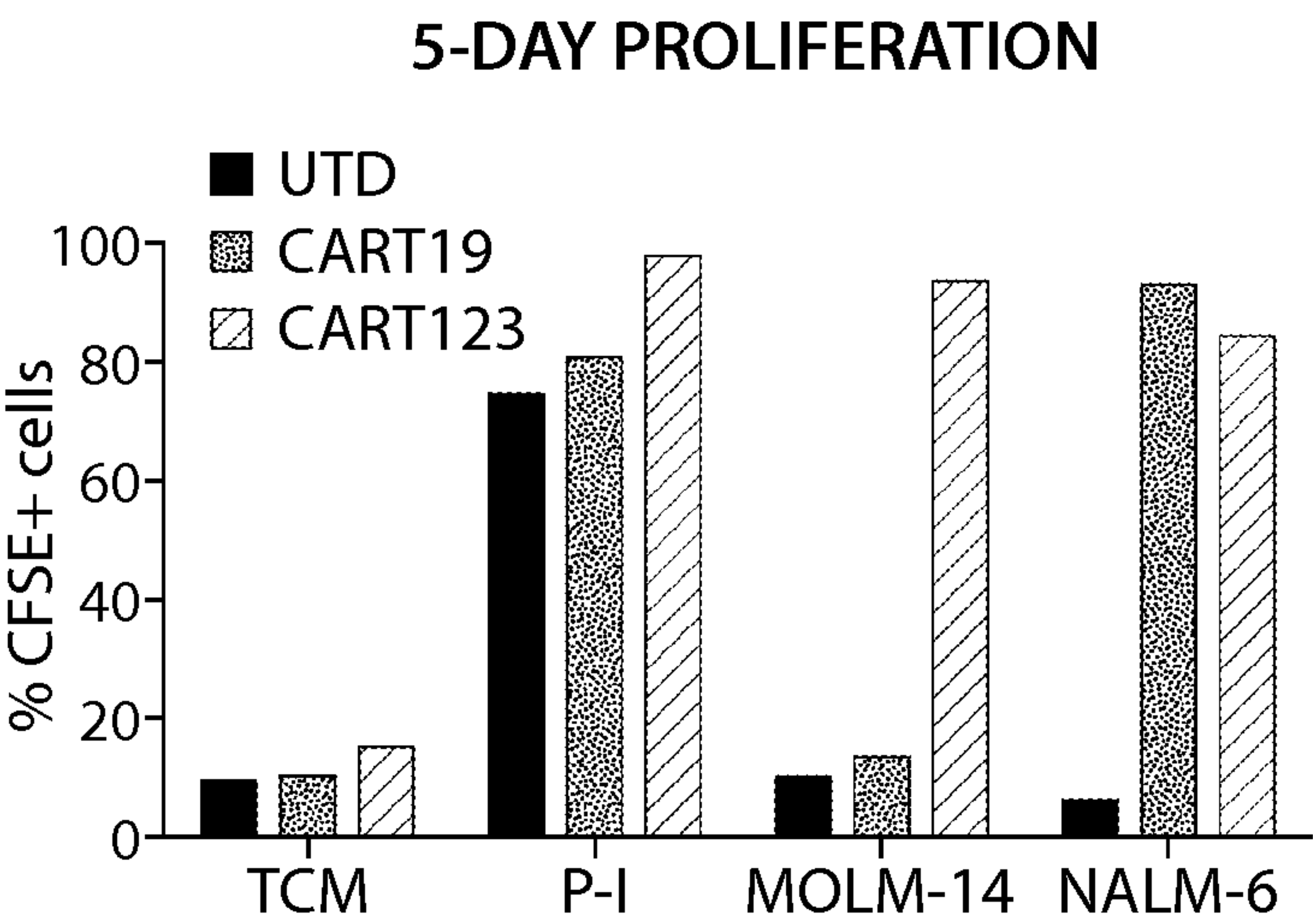
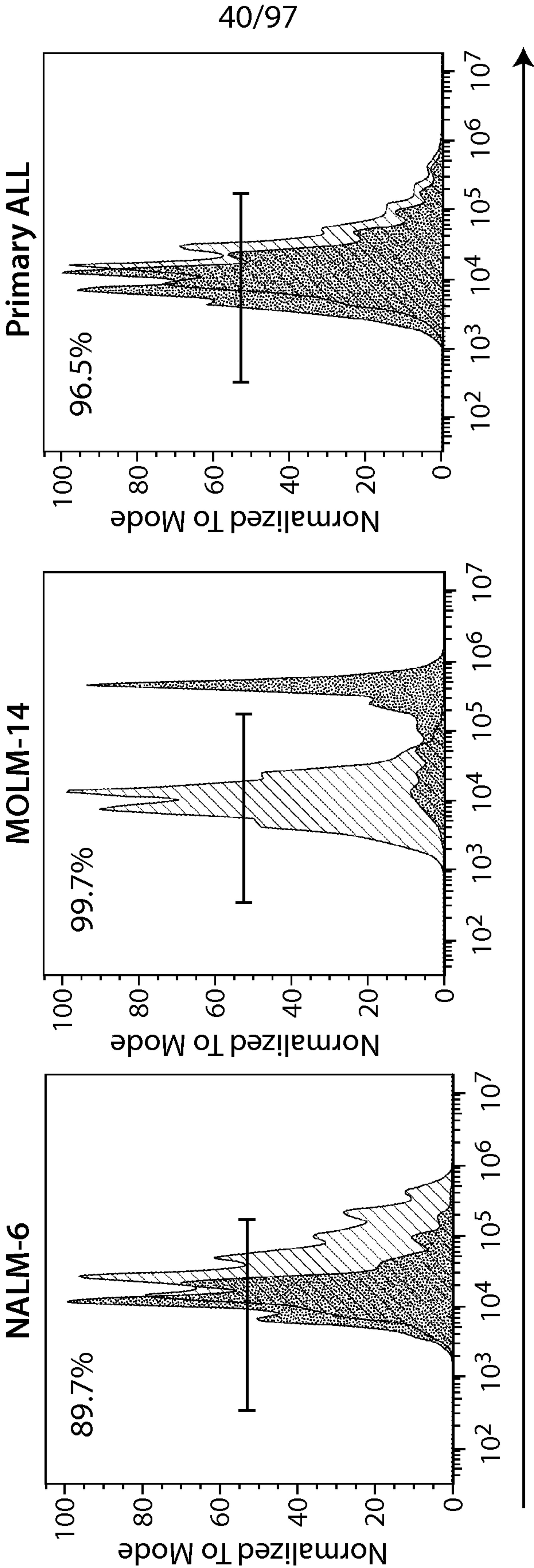


FIG. 32D



CFSE

FIG. 32E

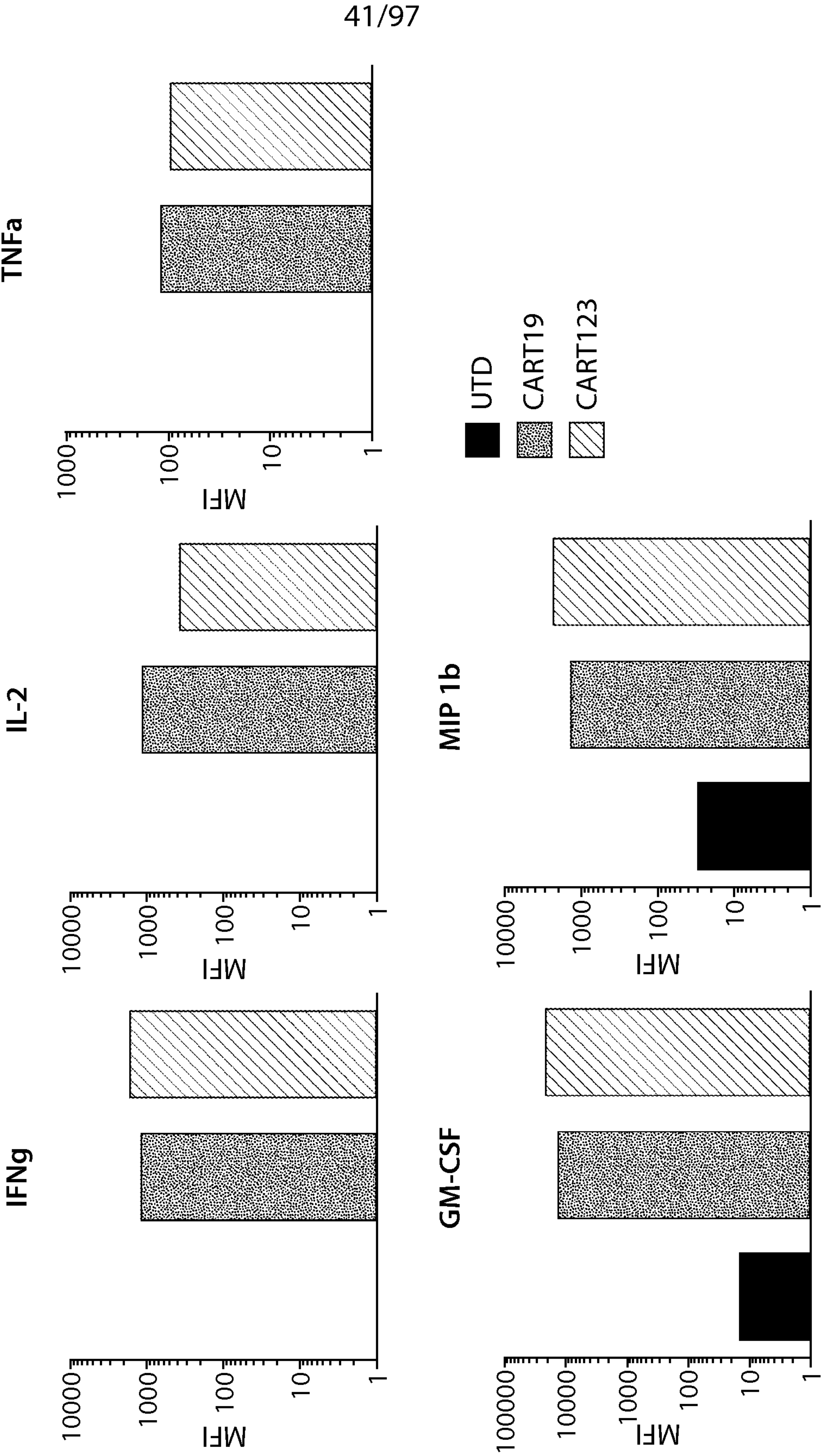


FIG. 32F

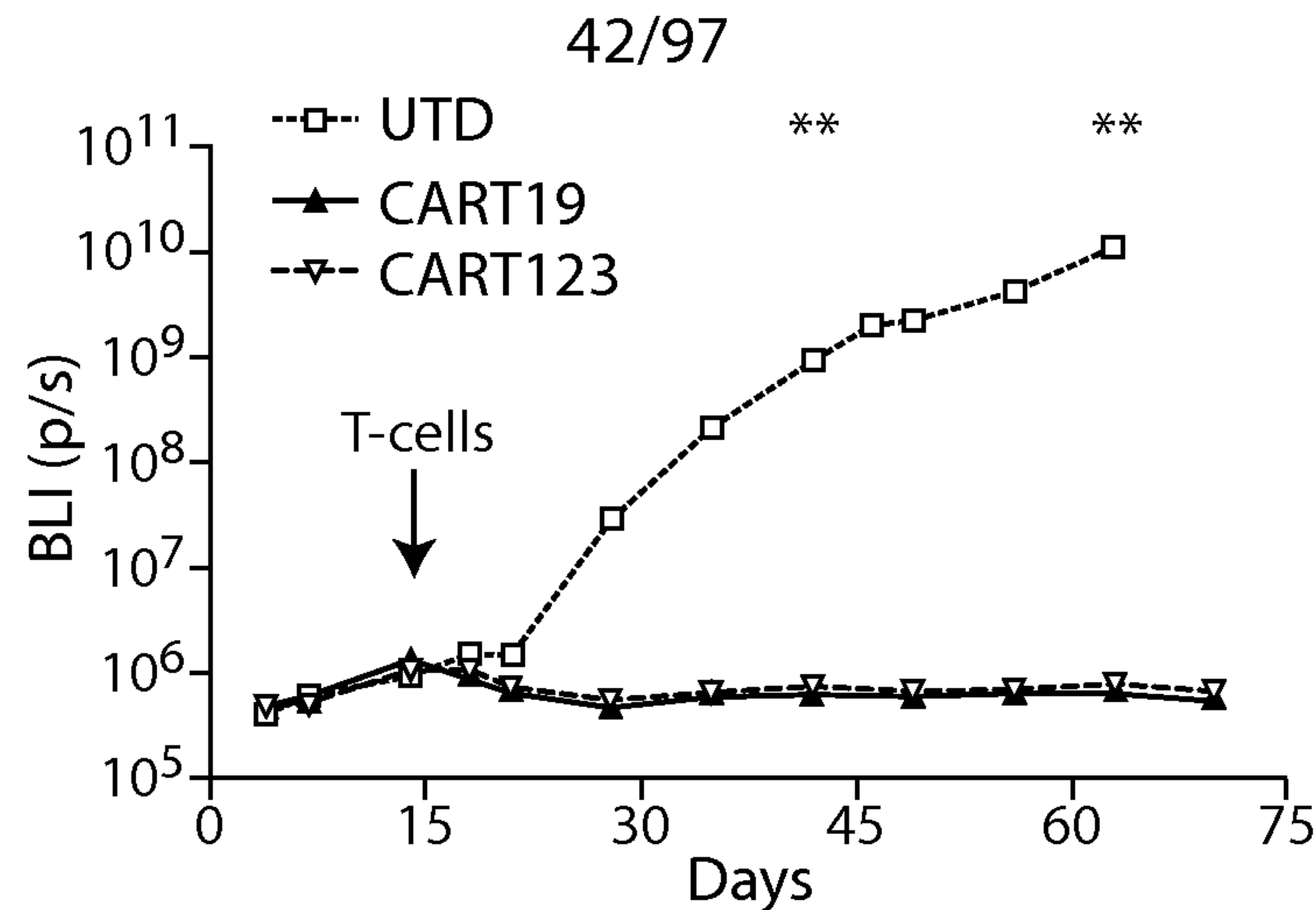


FIG. 33A

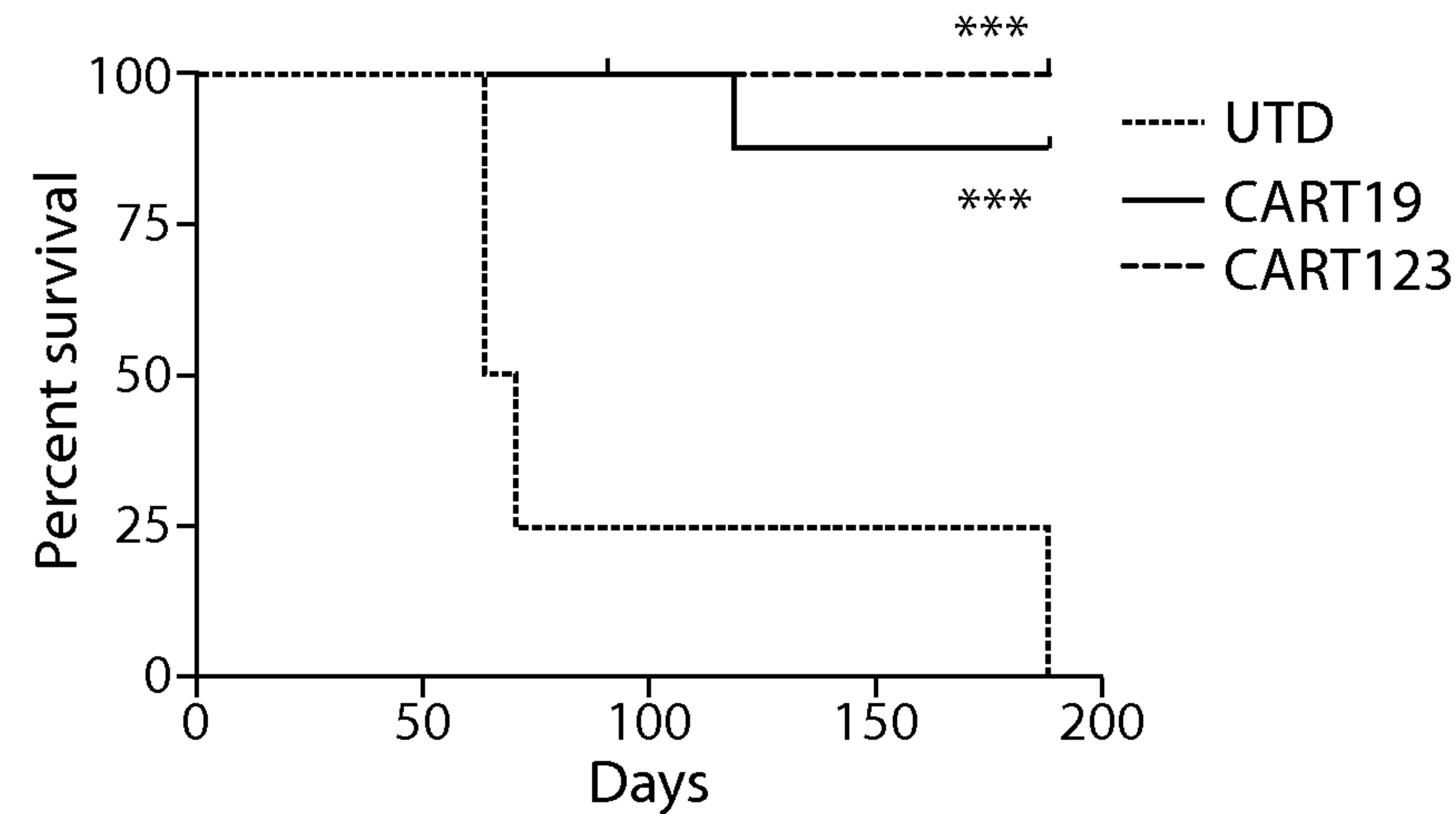


FIG. 33B

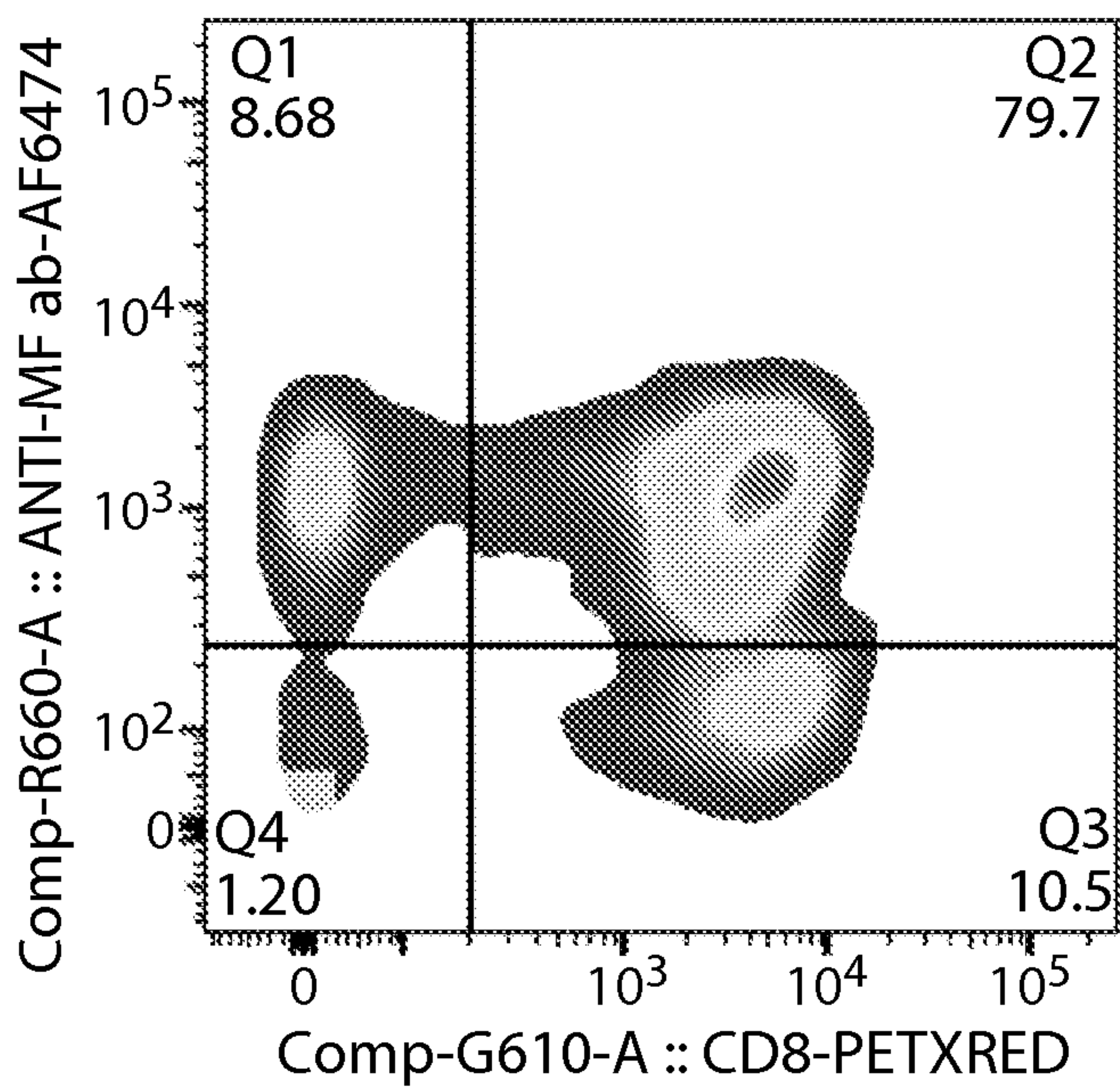
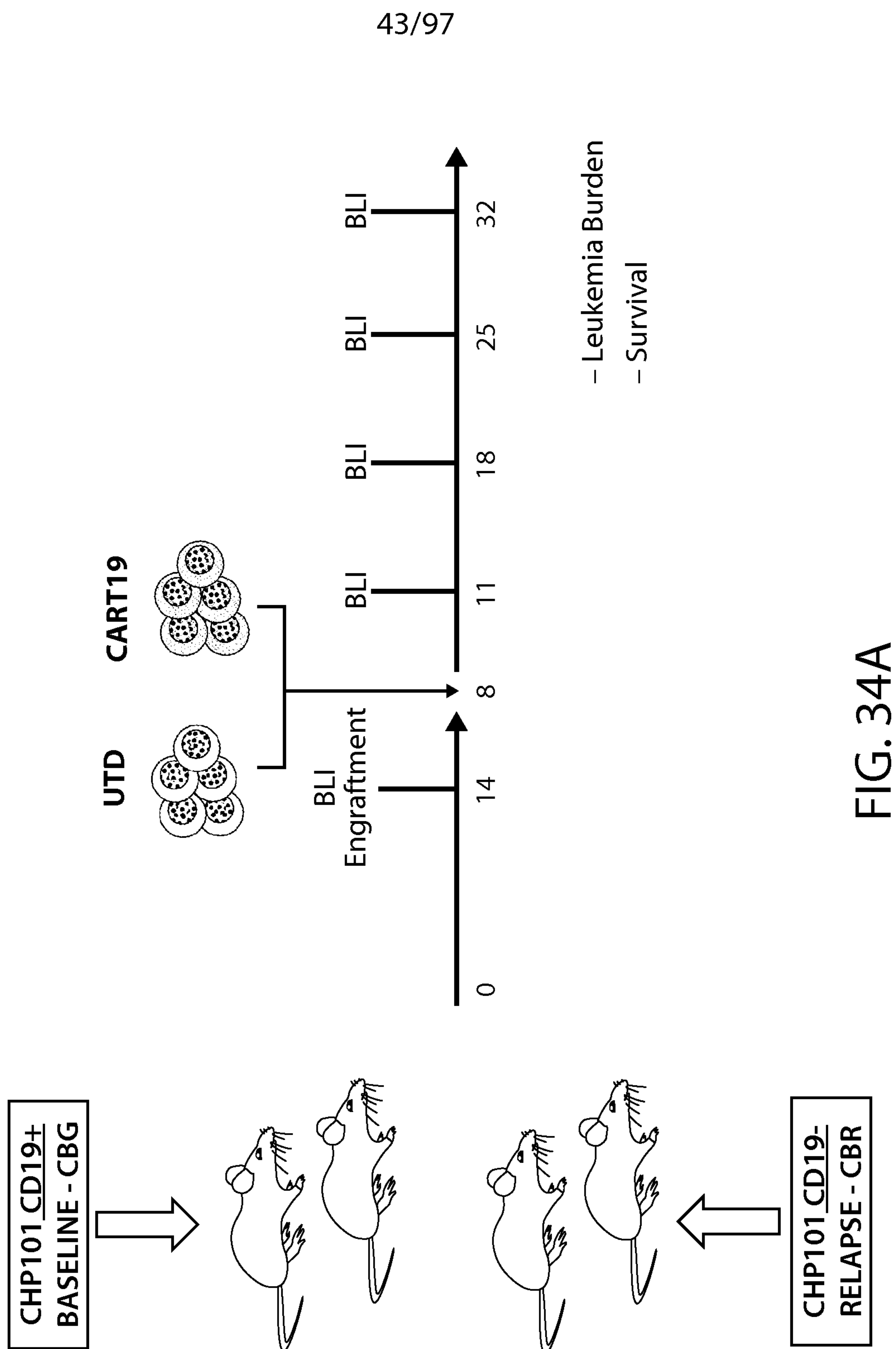


FIG. 33C



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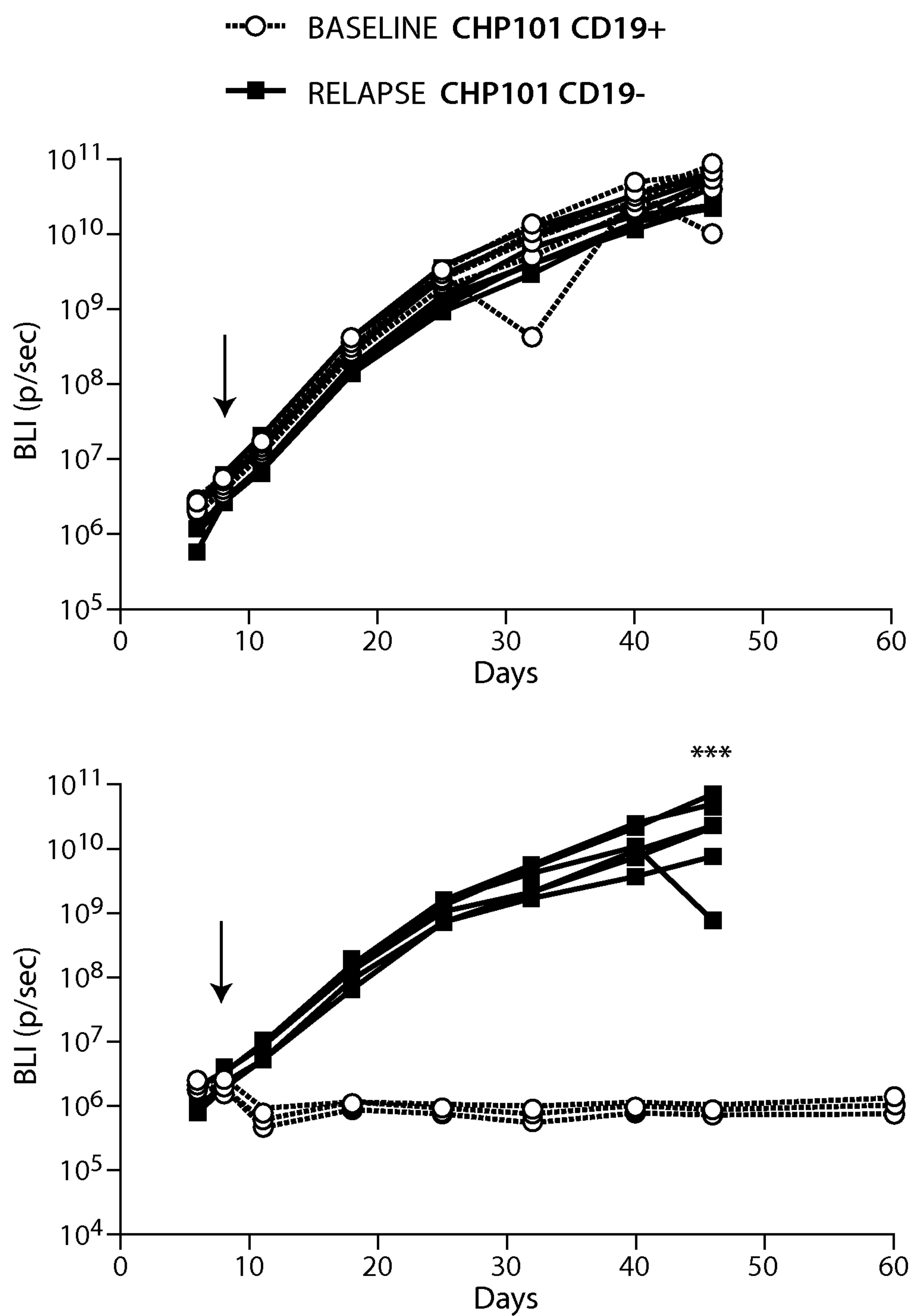


FIG. 34B

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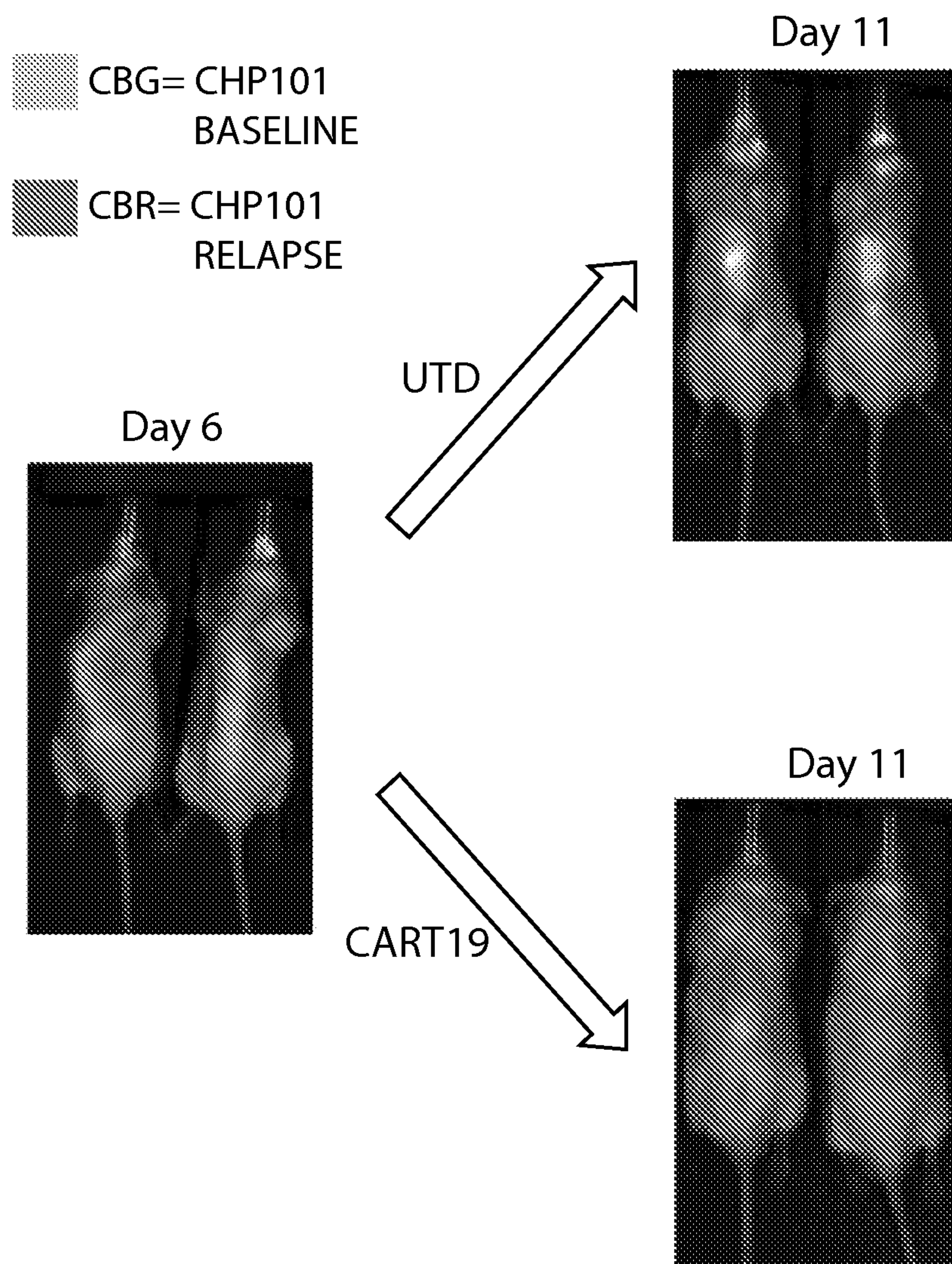
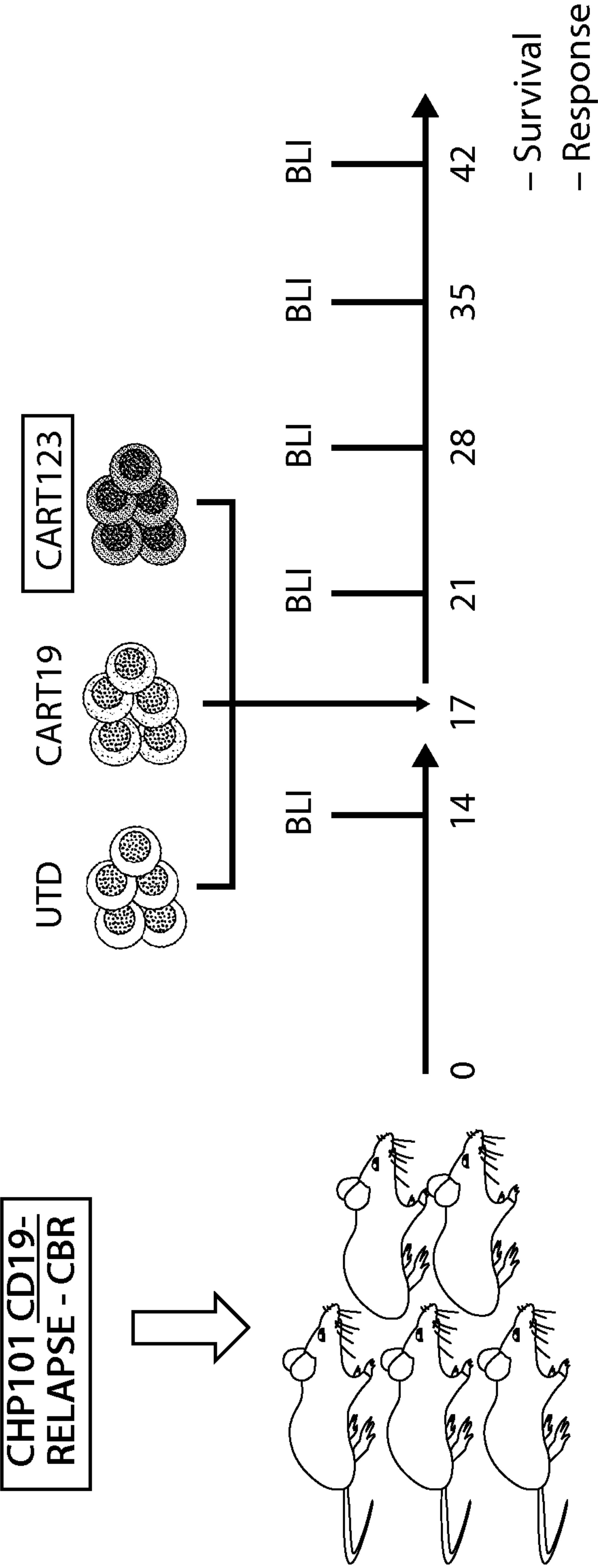


FIG. 34C



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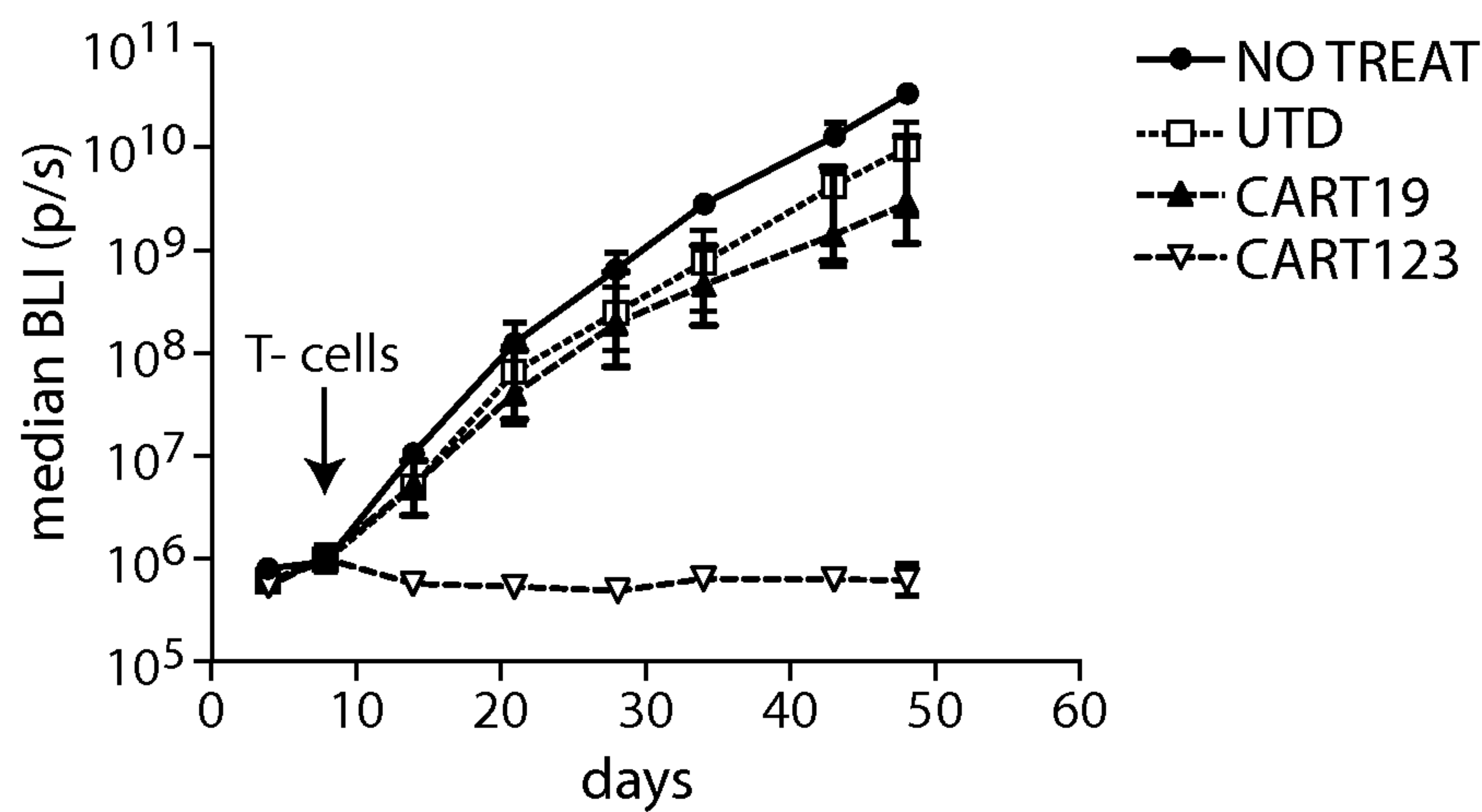


FIG. 34E

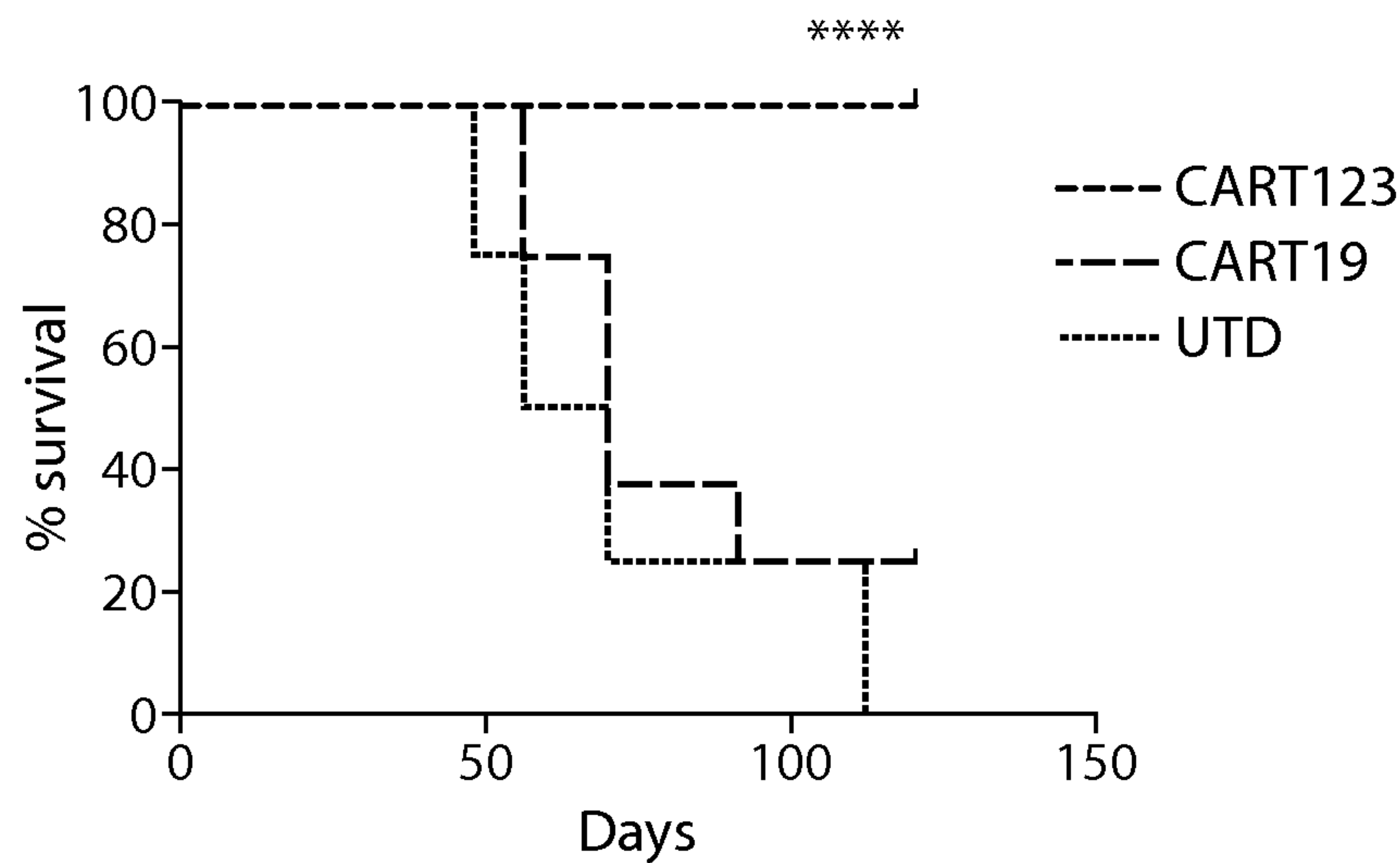
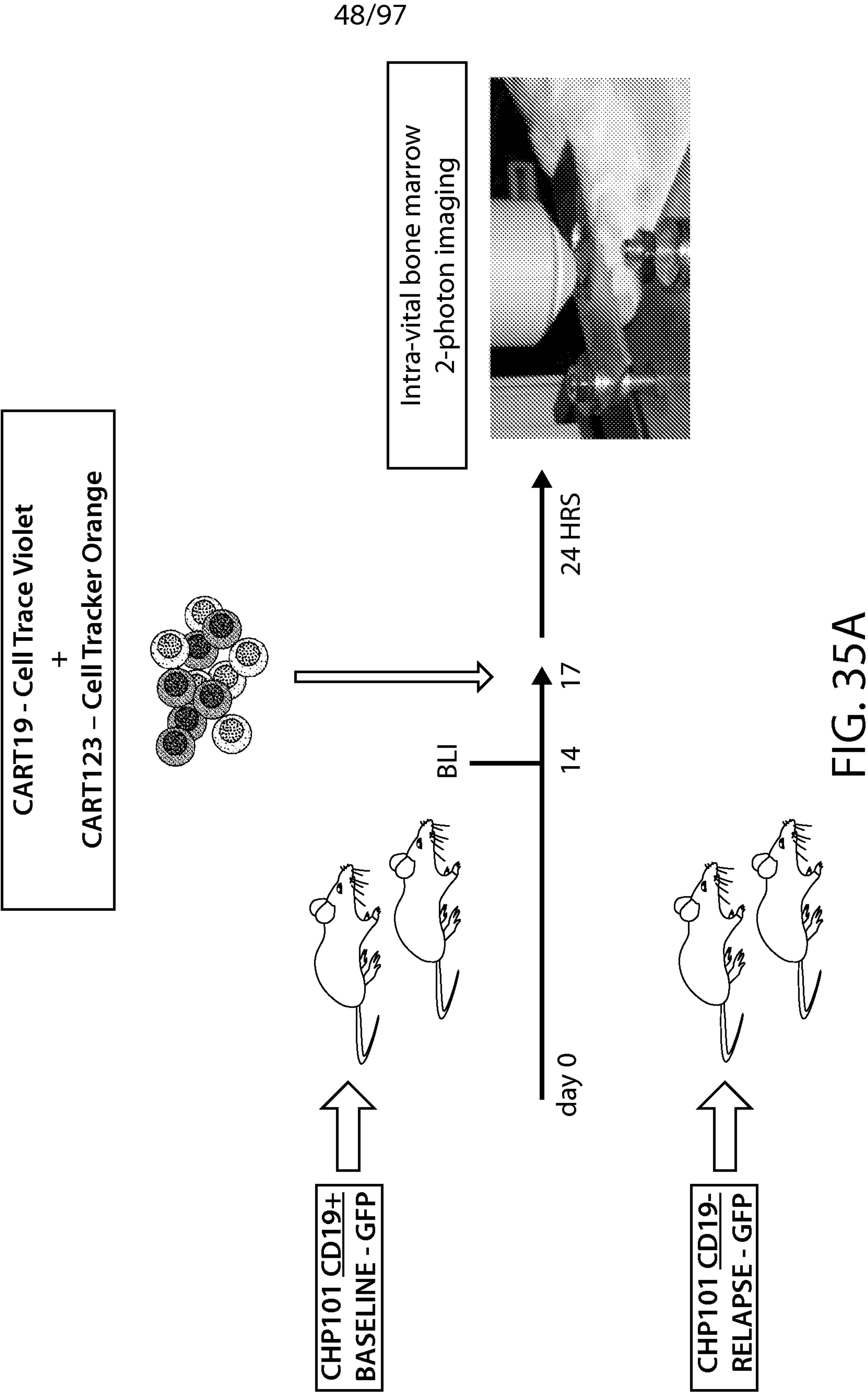


FIG. 34F



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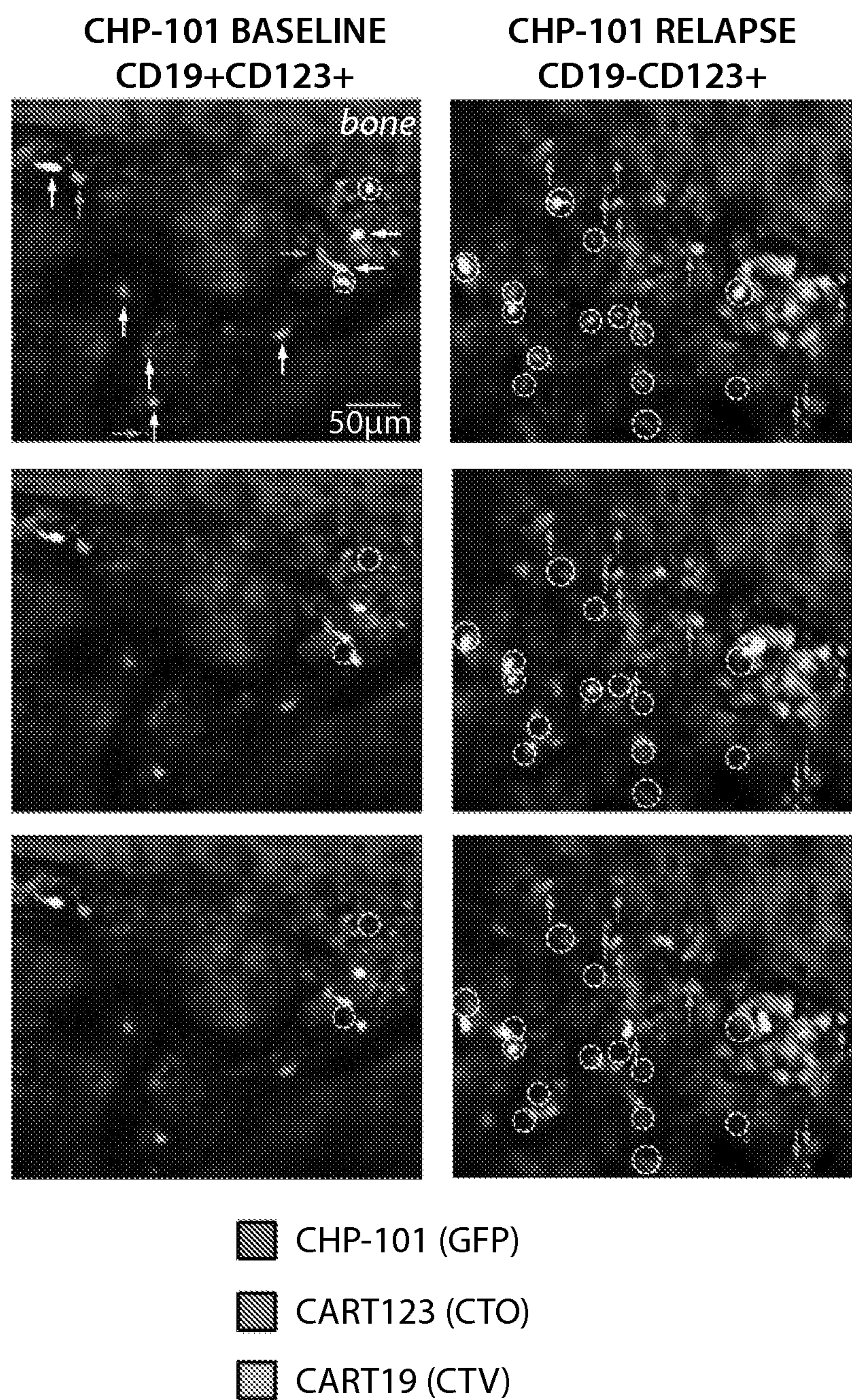


FIG. 35B

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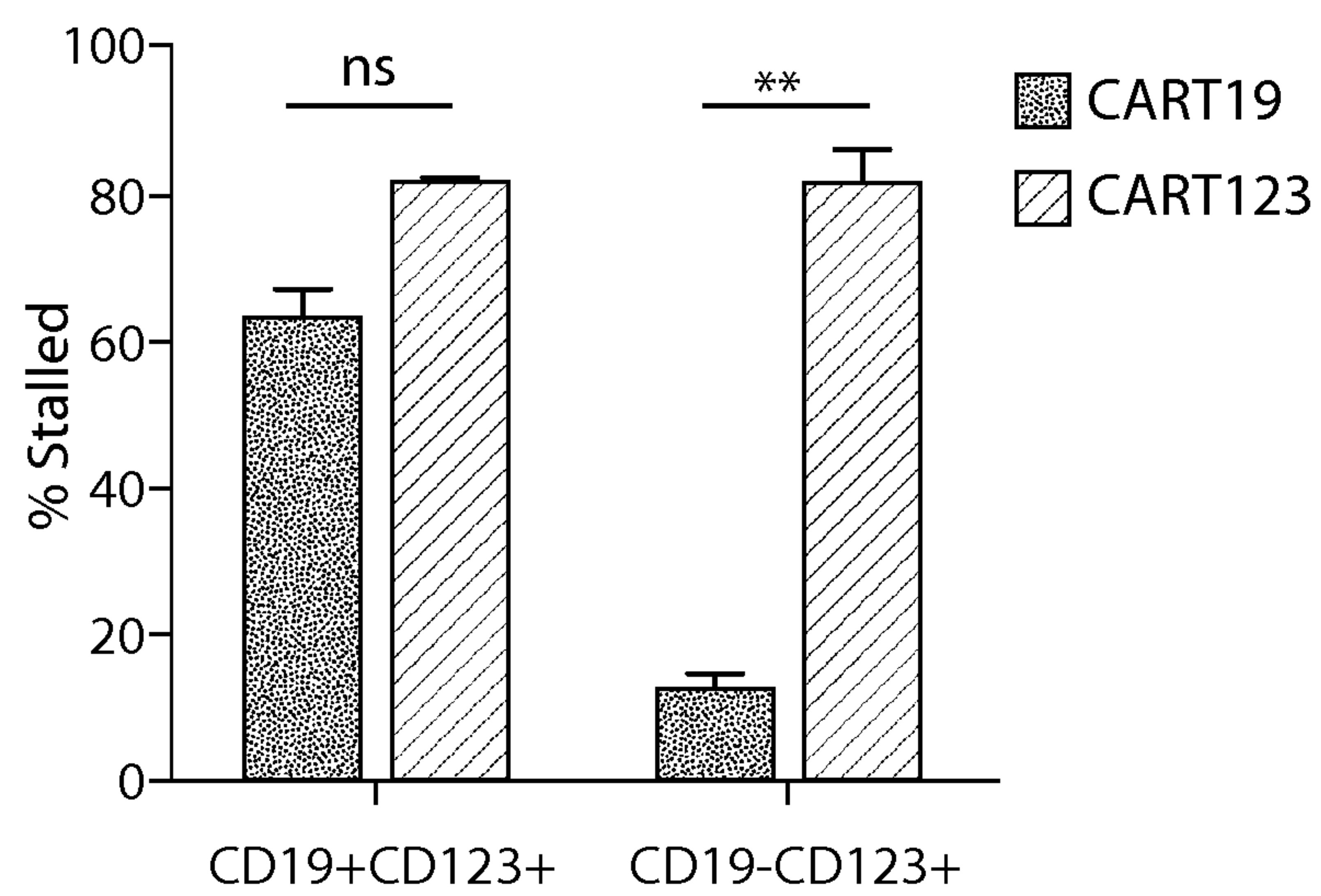


FIG. 35C

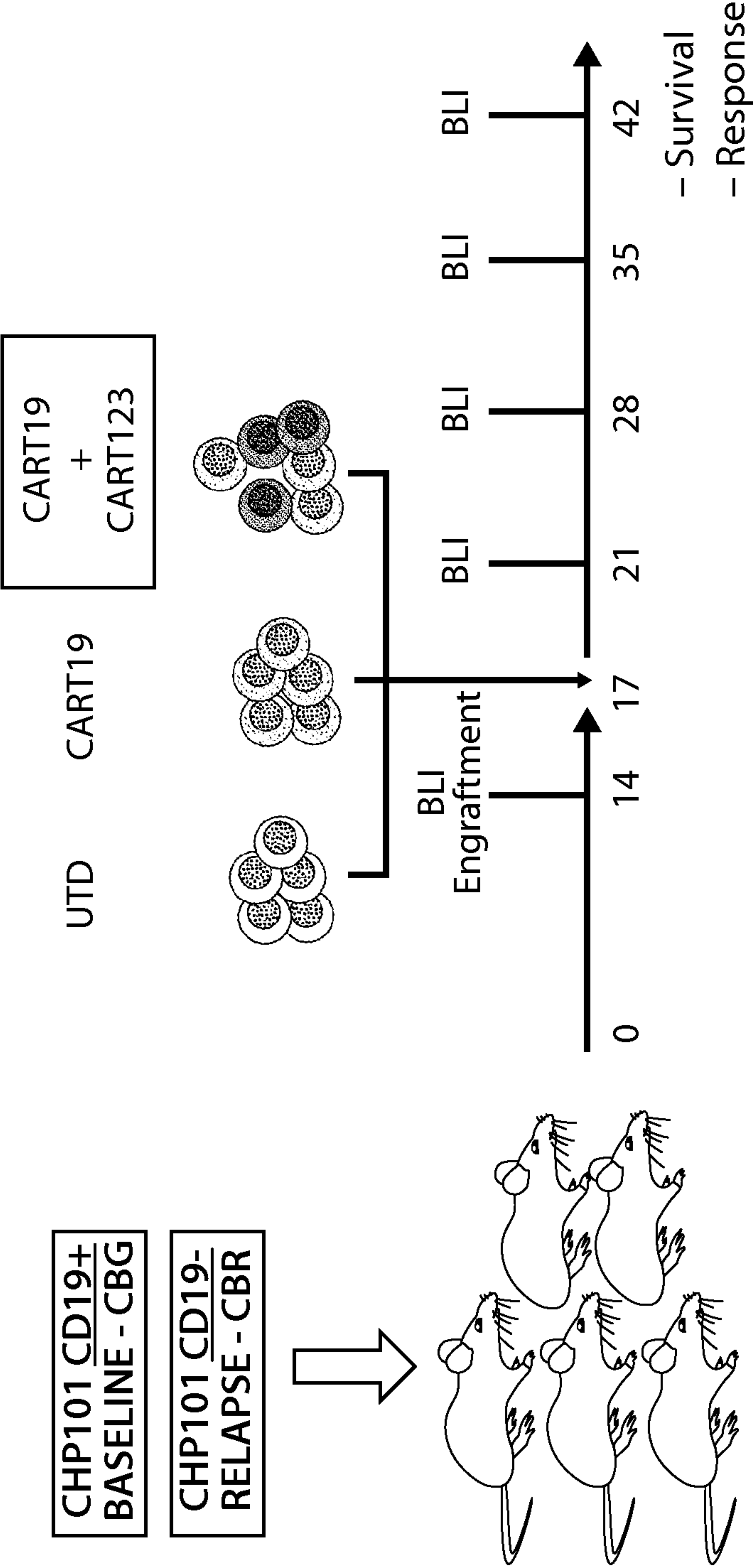


FIG. 36A

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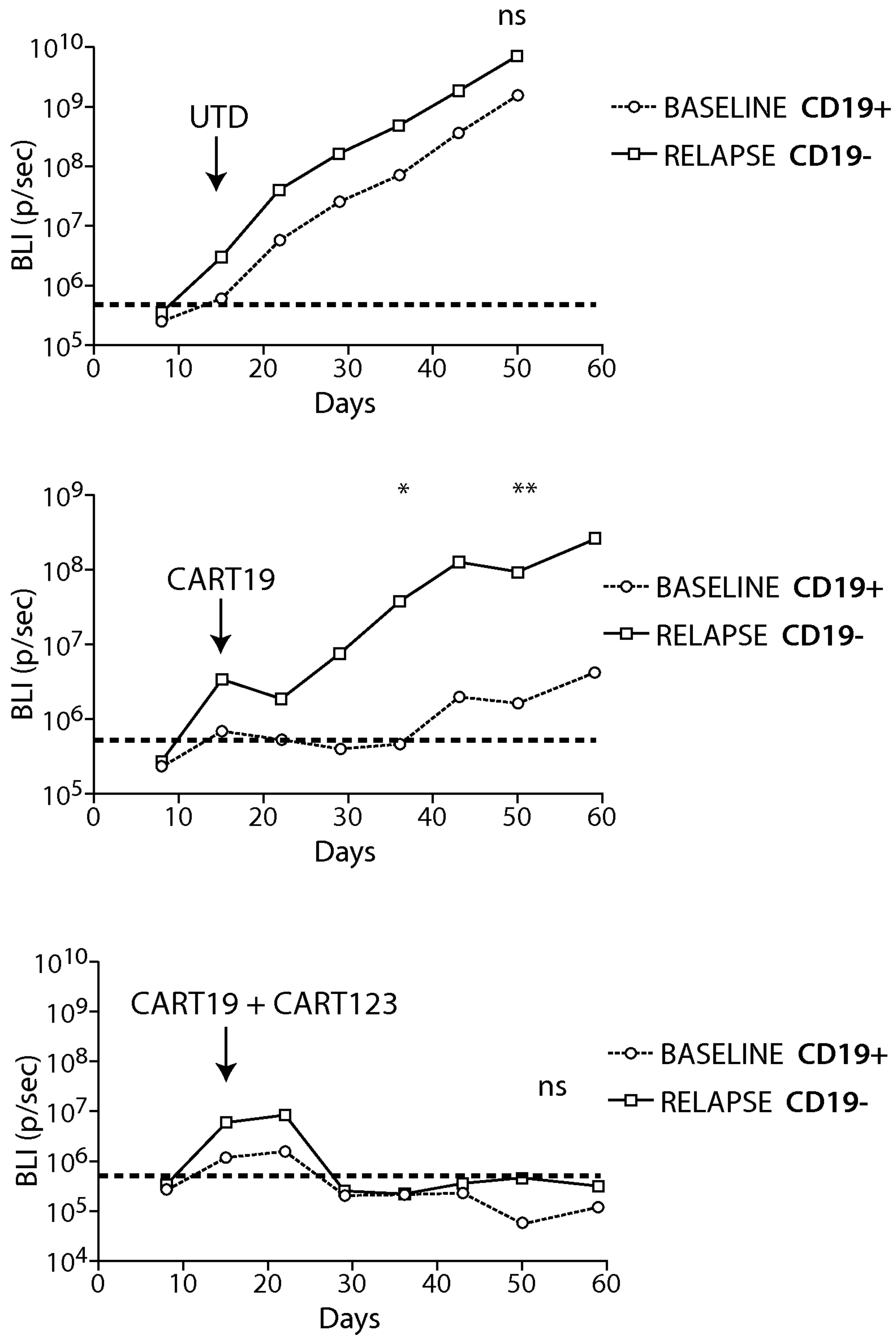


FIG. 36B

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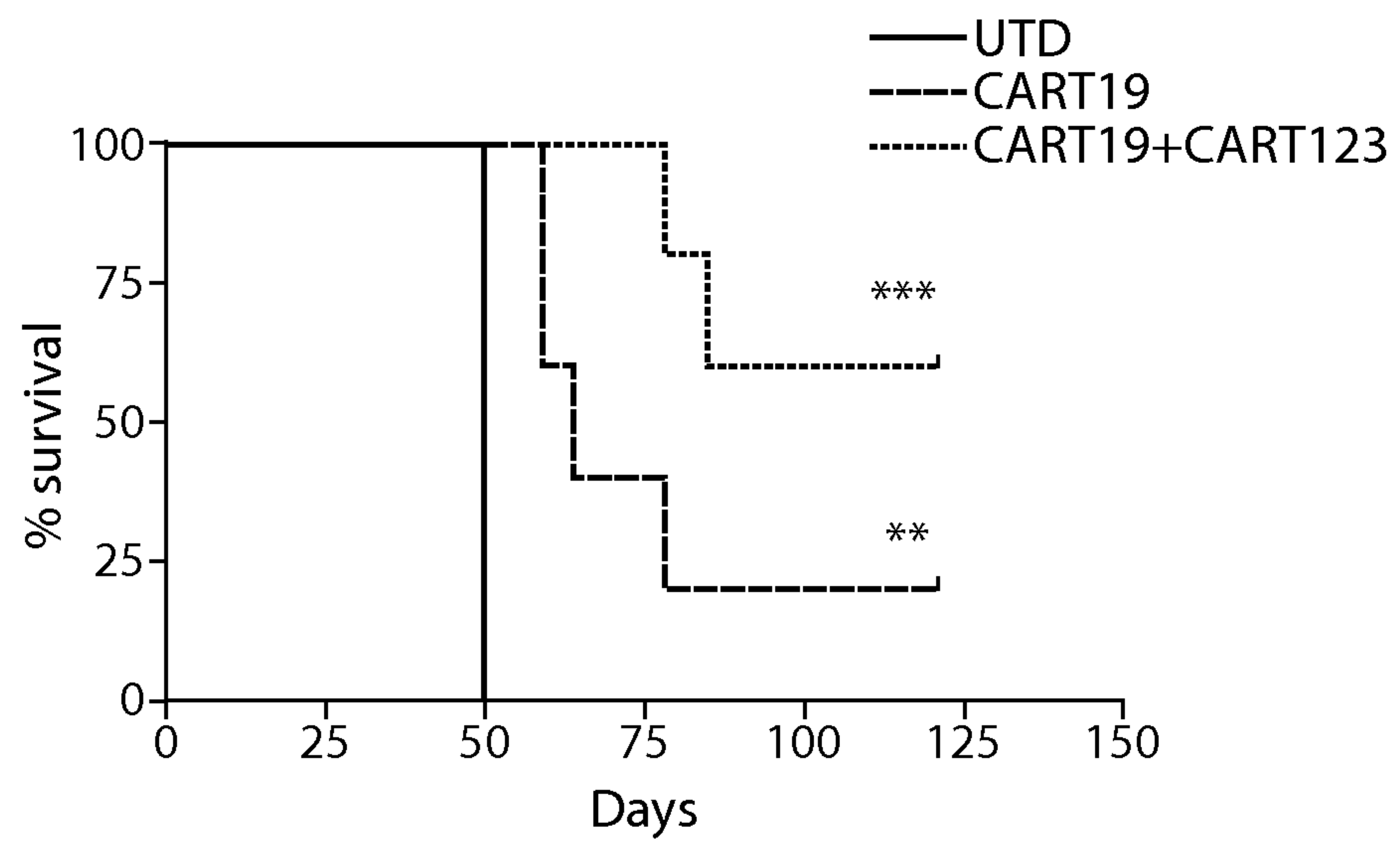


FIG. 36C

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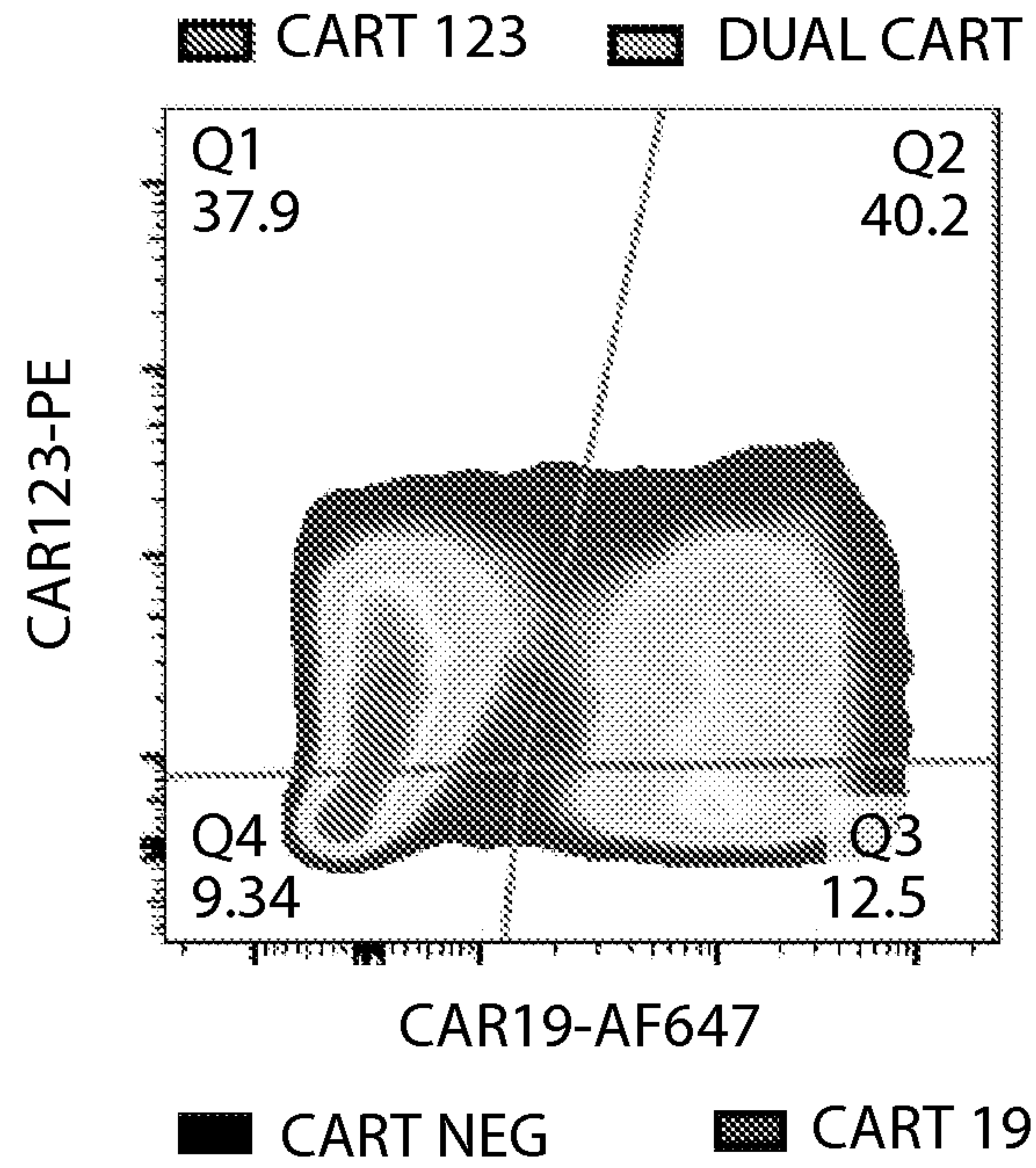


FIG. 37A

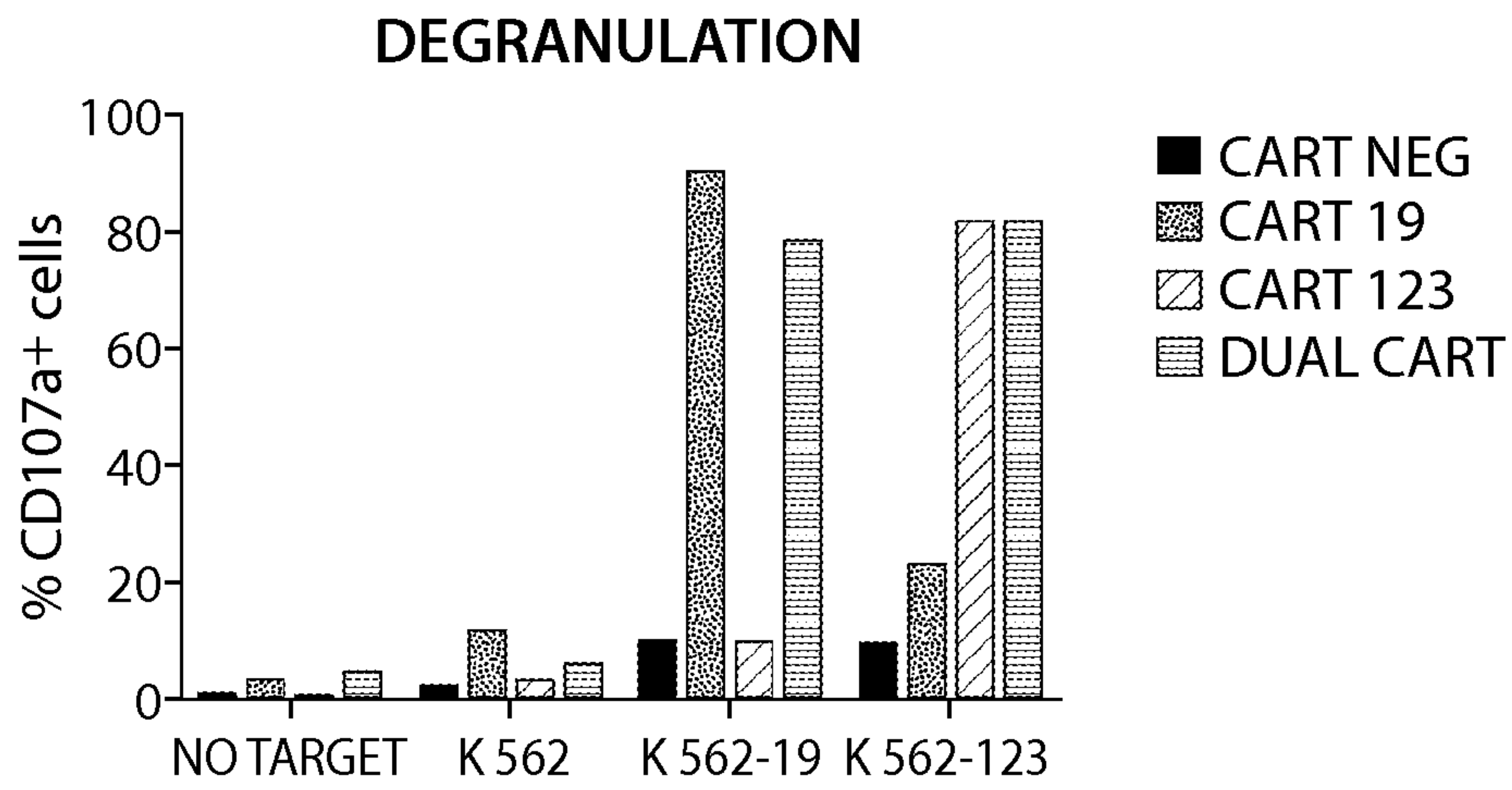


FIG. 37B

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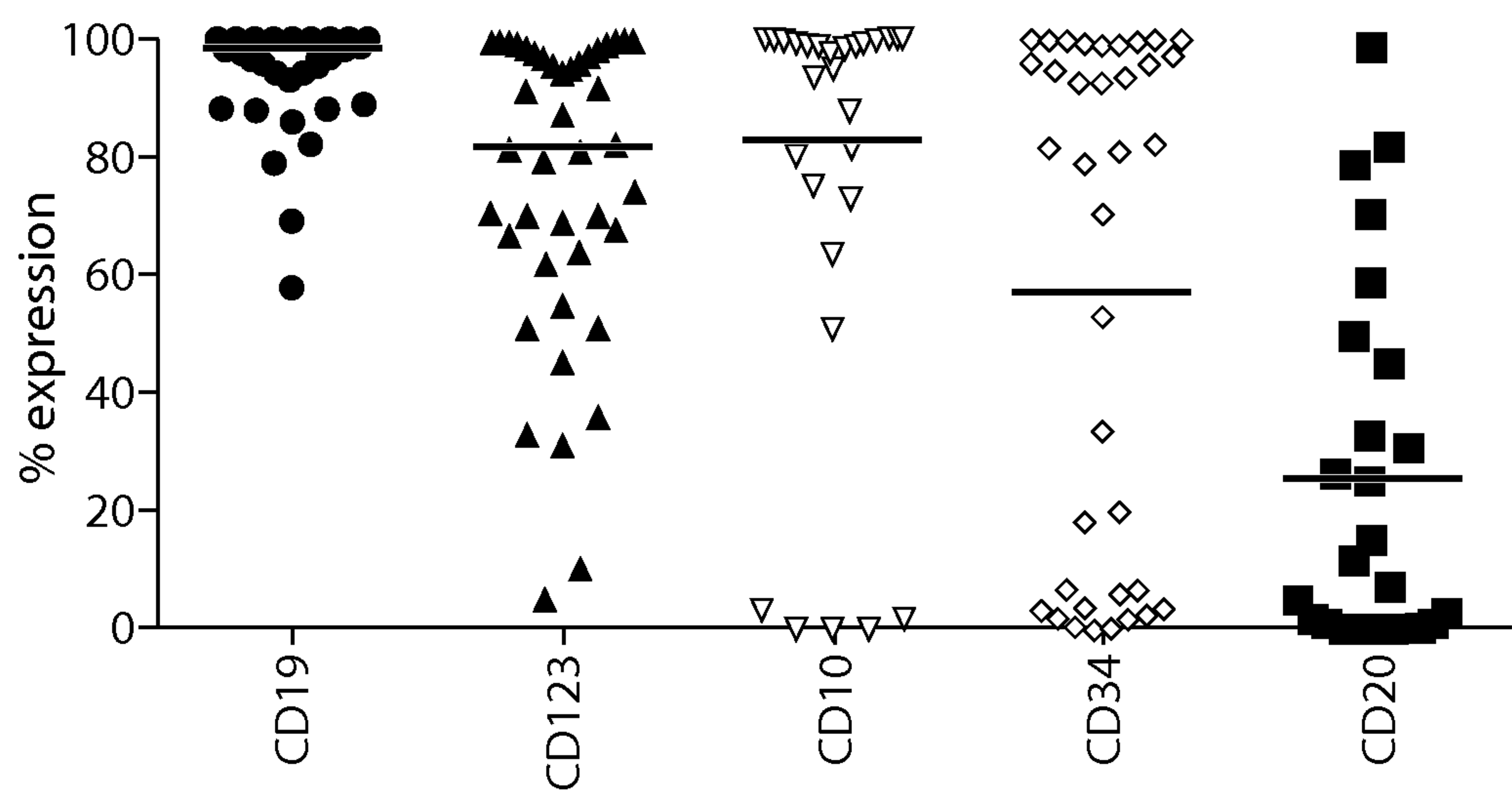


FIG. 38A

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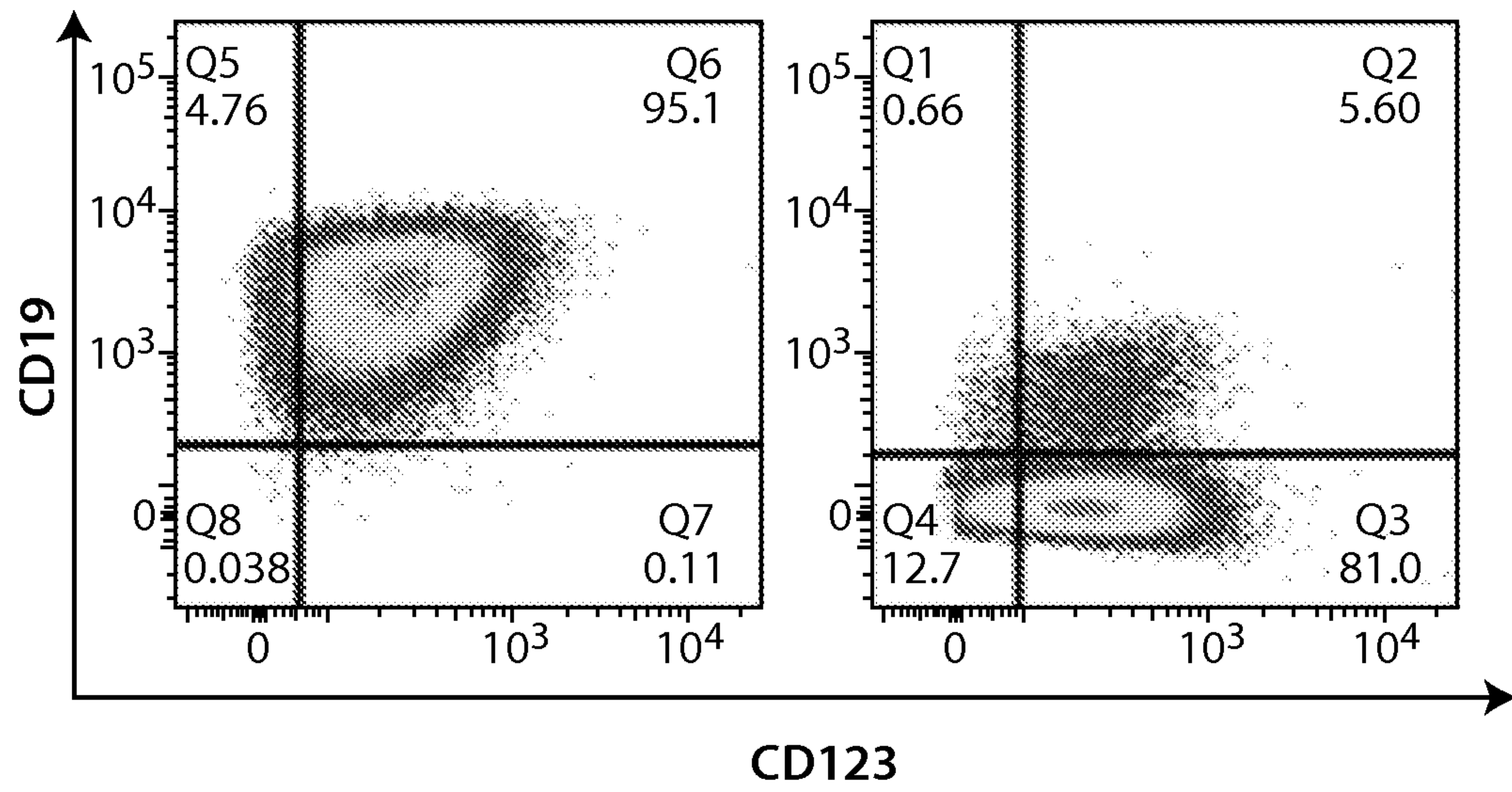
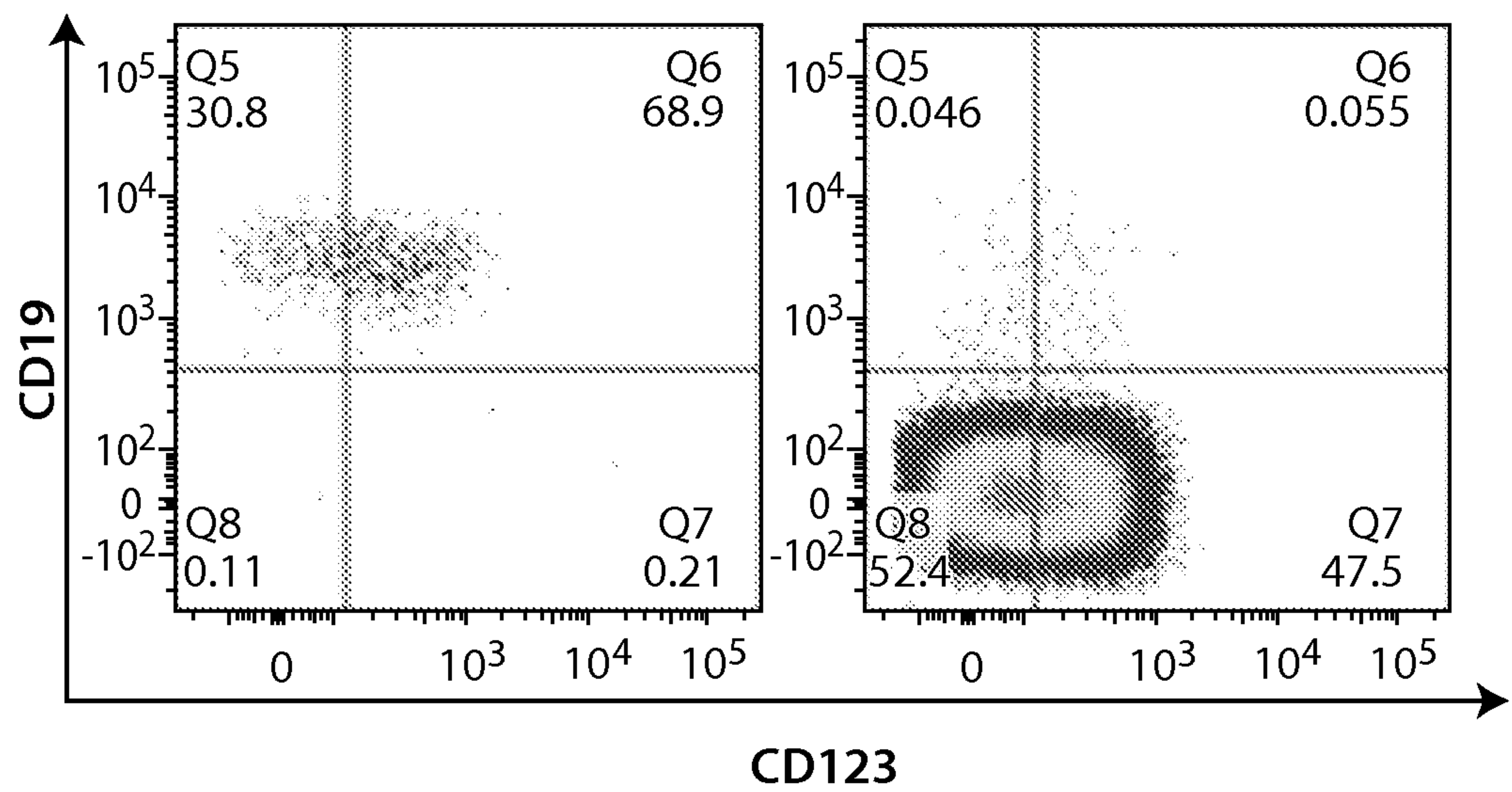
Adult UPCC-29Pediatric CHP-107

FIG. 38B

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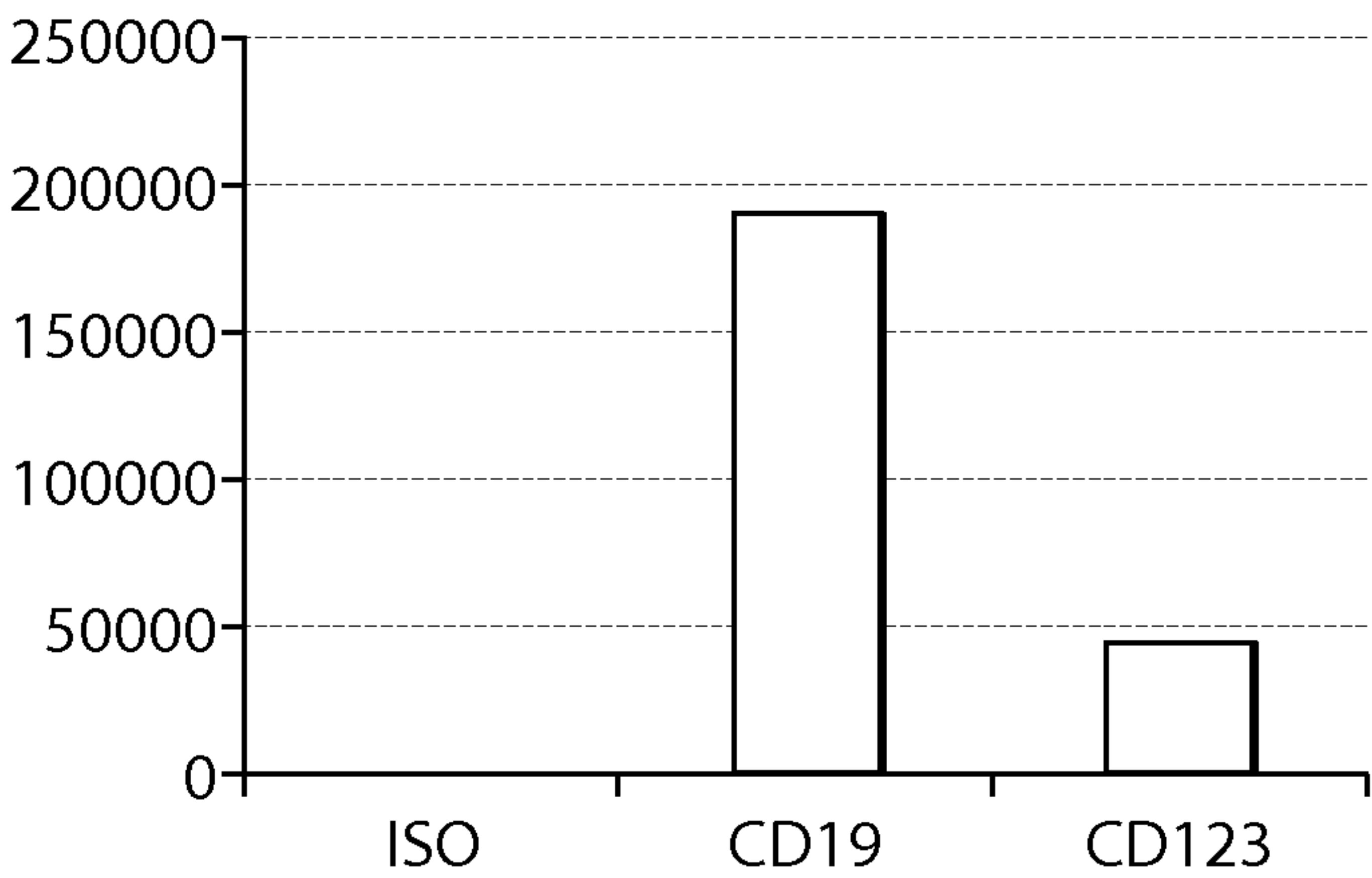


FIG. 39A

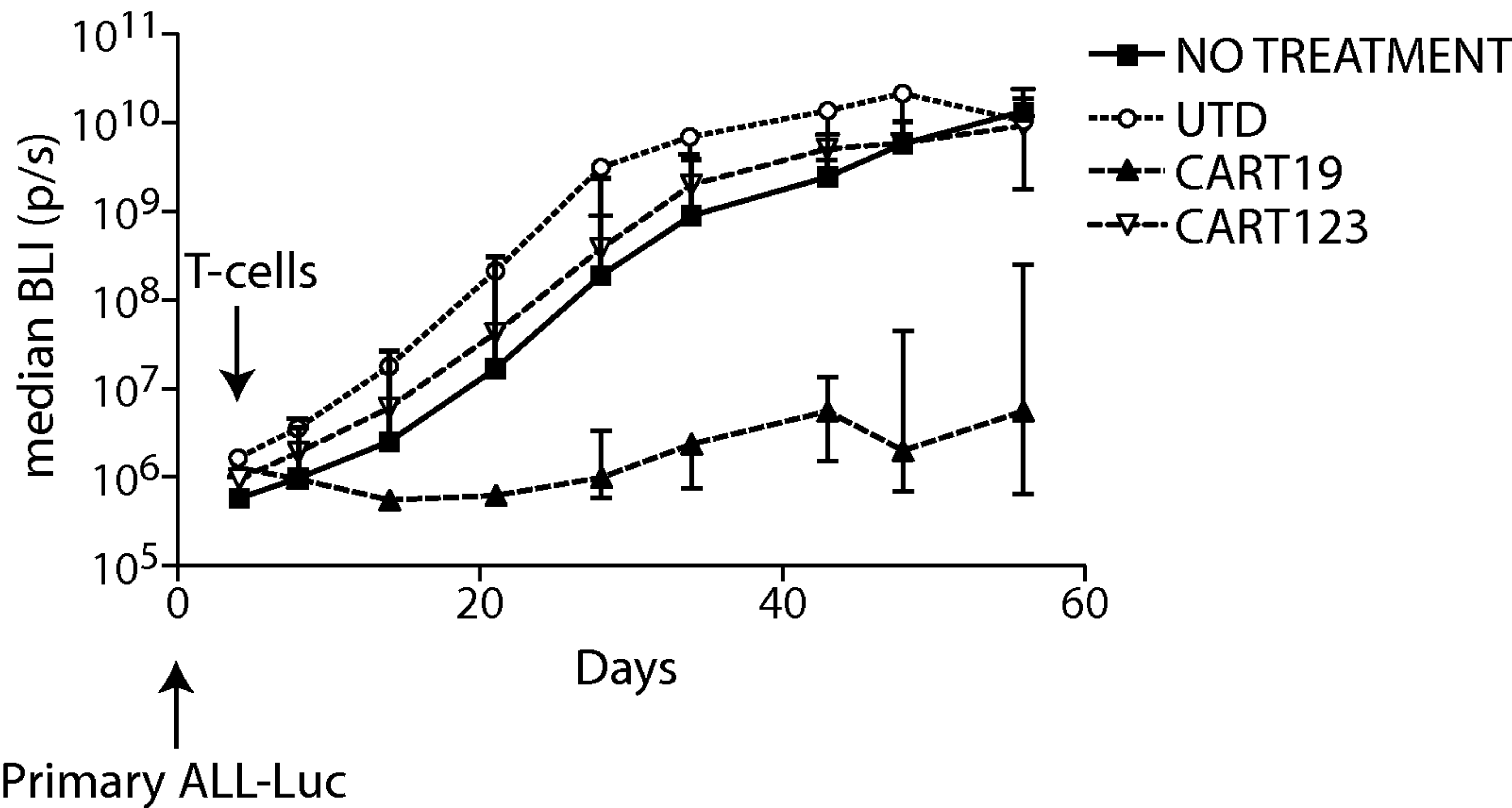


FIG. 39B

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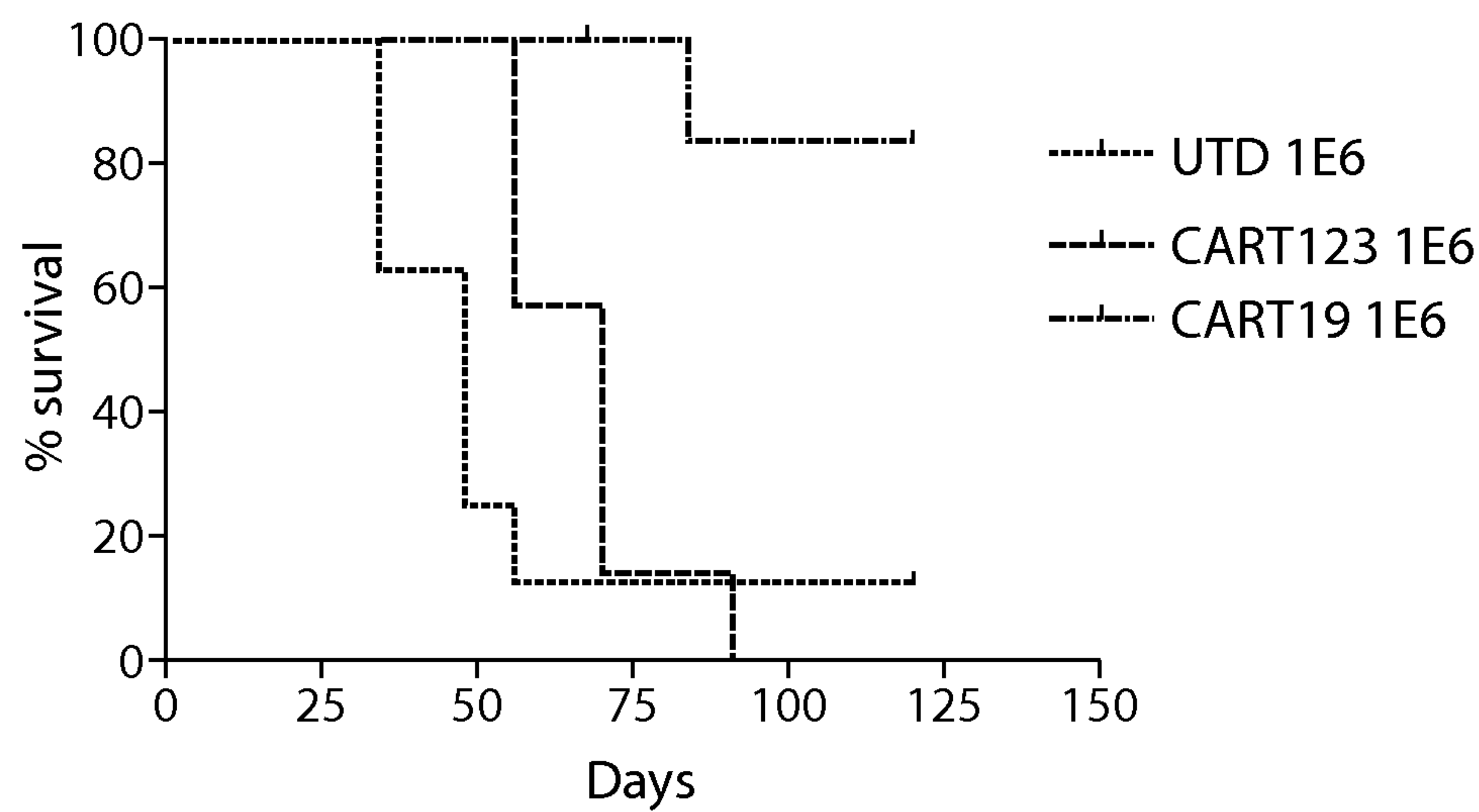


FIG. 39C

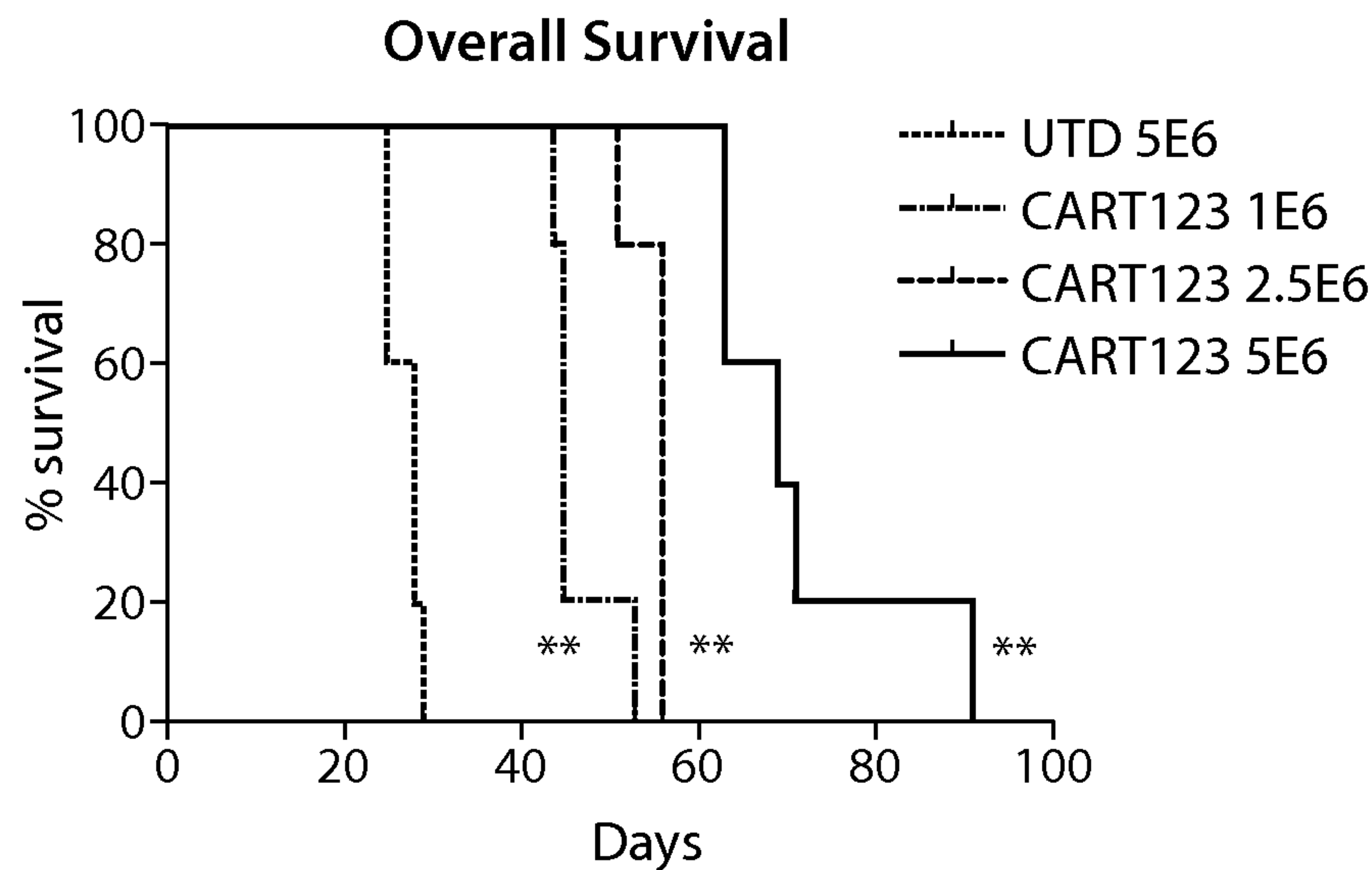


FIG. 39D

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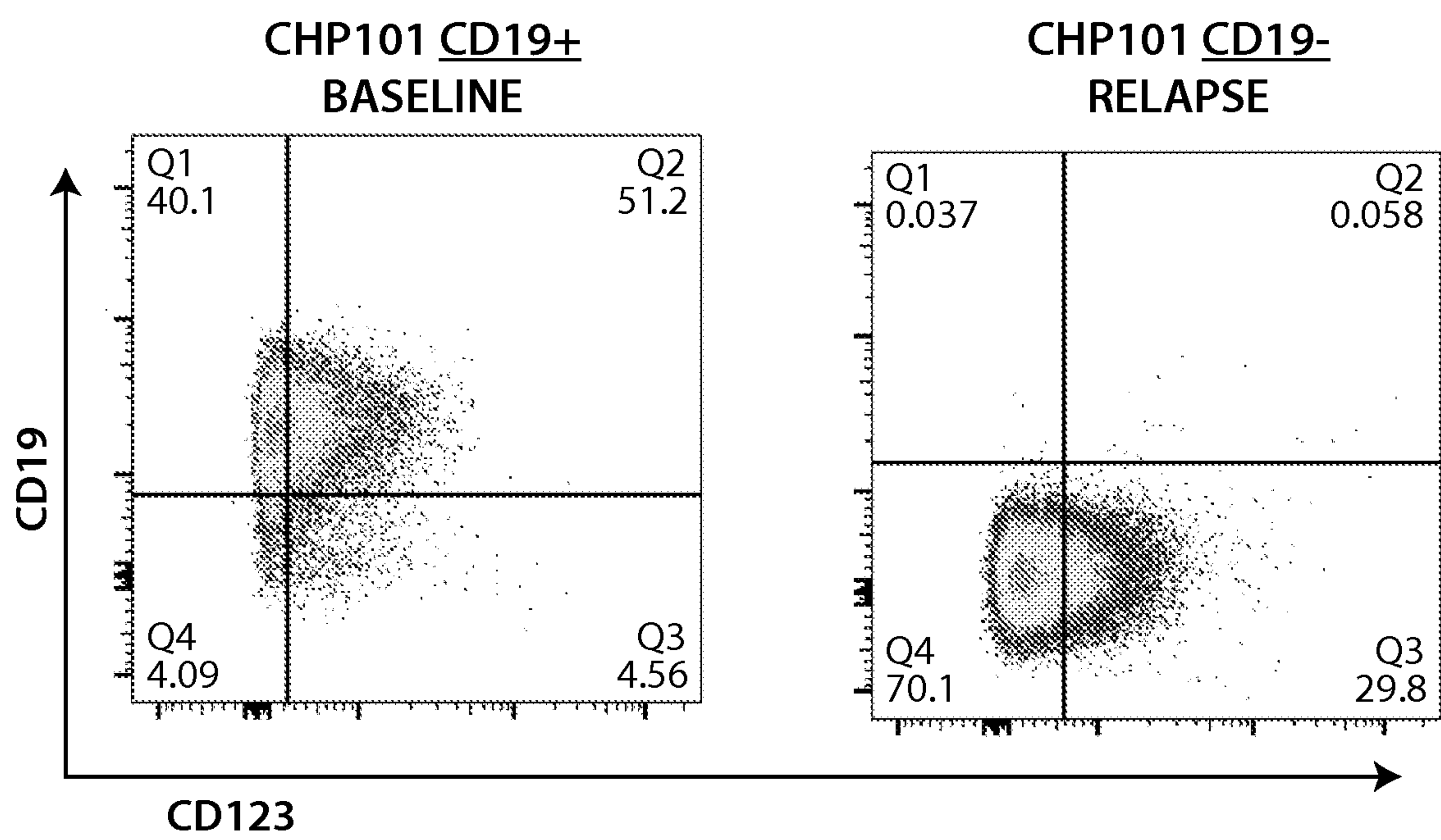


FIG. 40A

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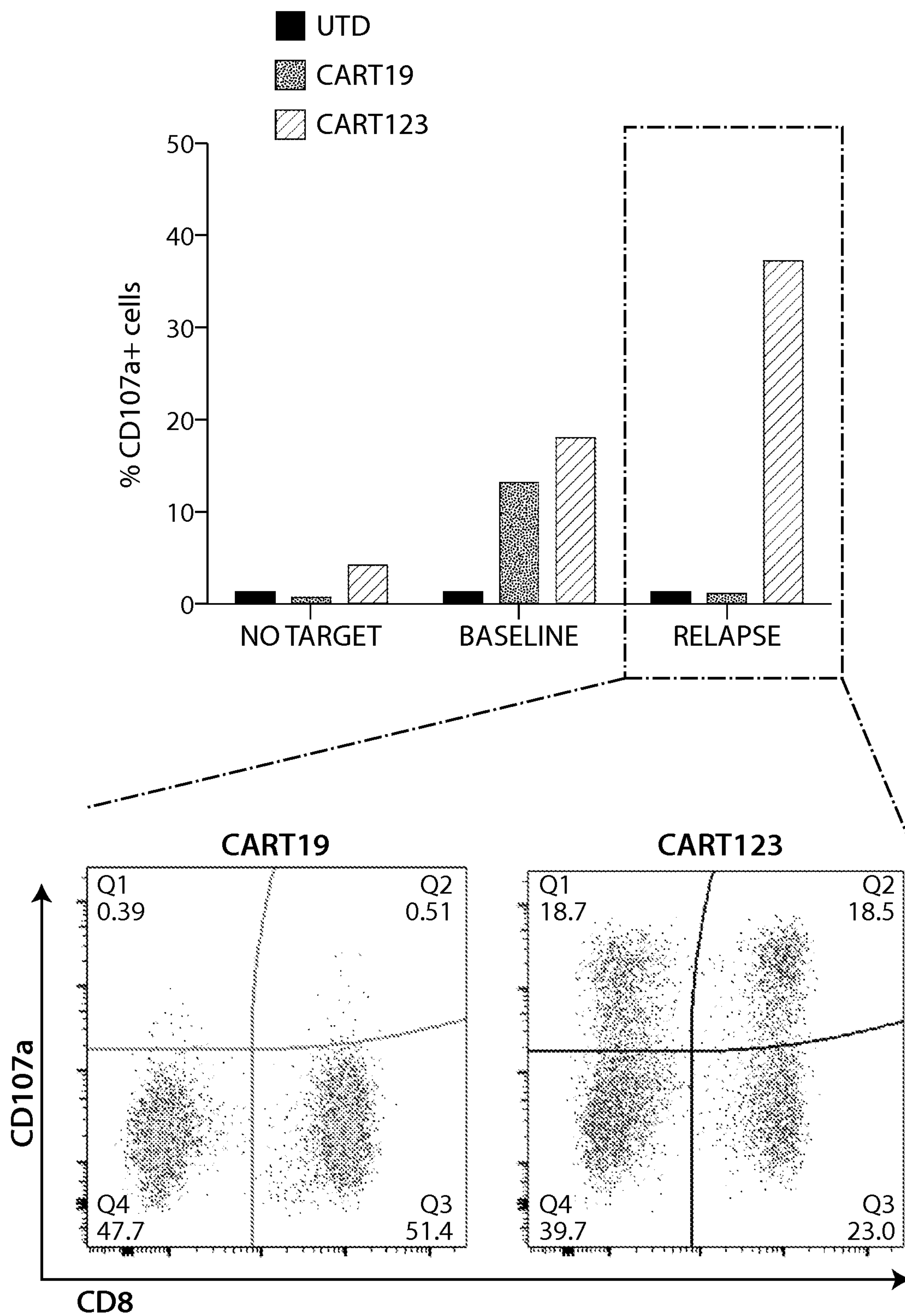


FIG. 40B

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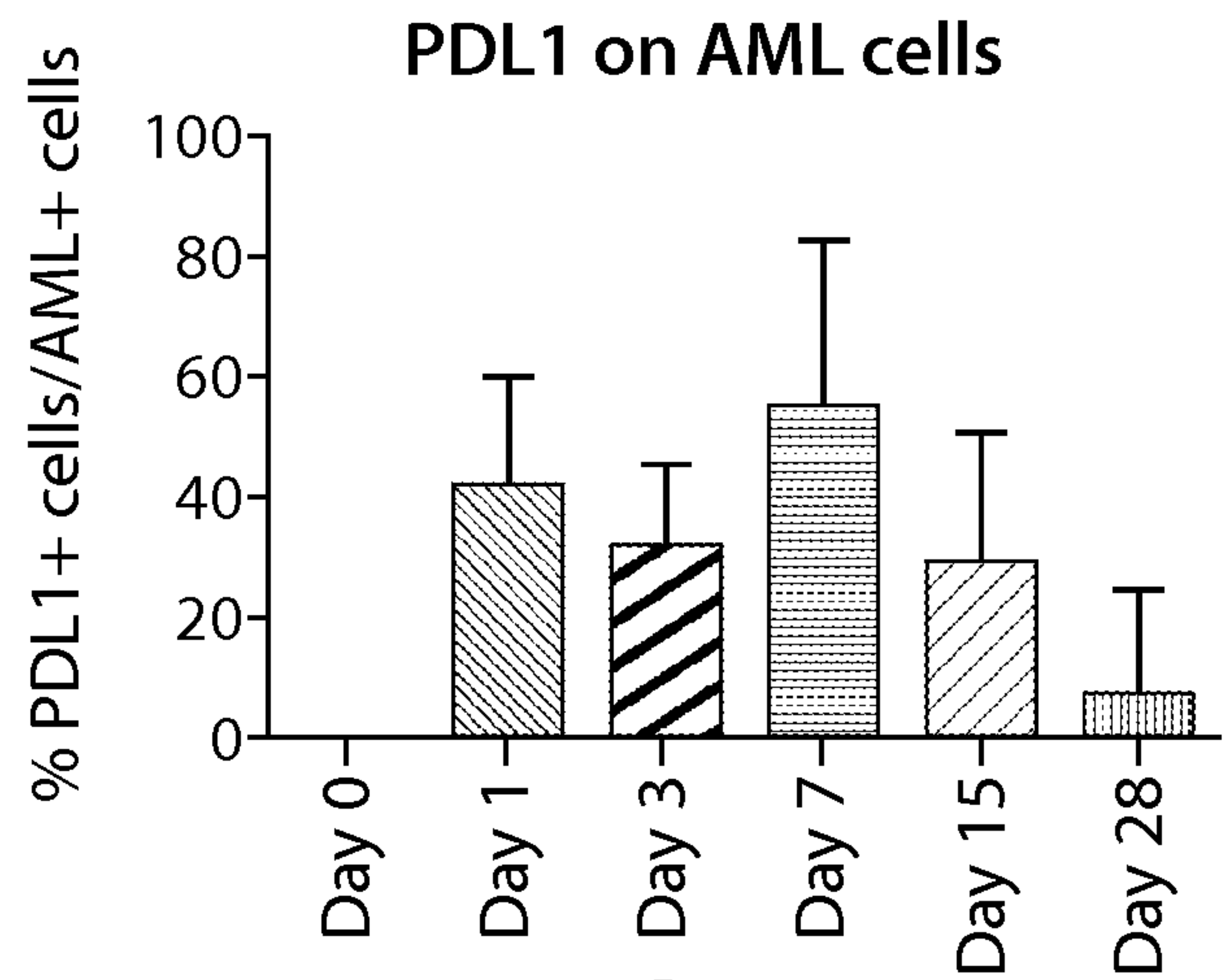


FIG. 41A

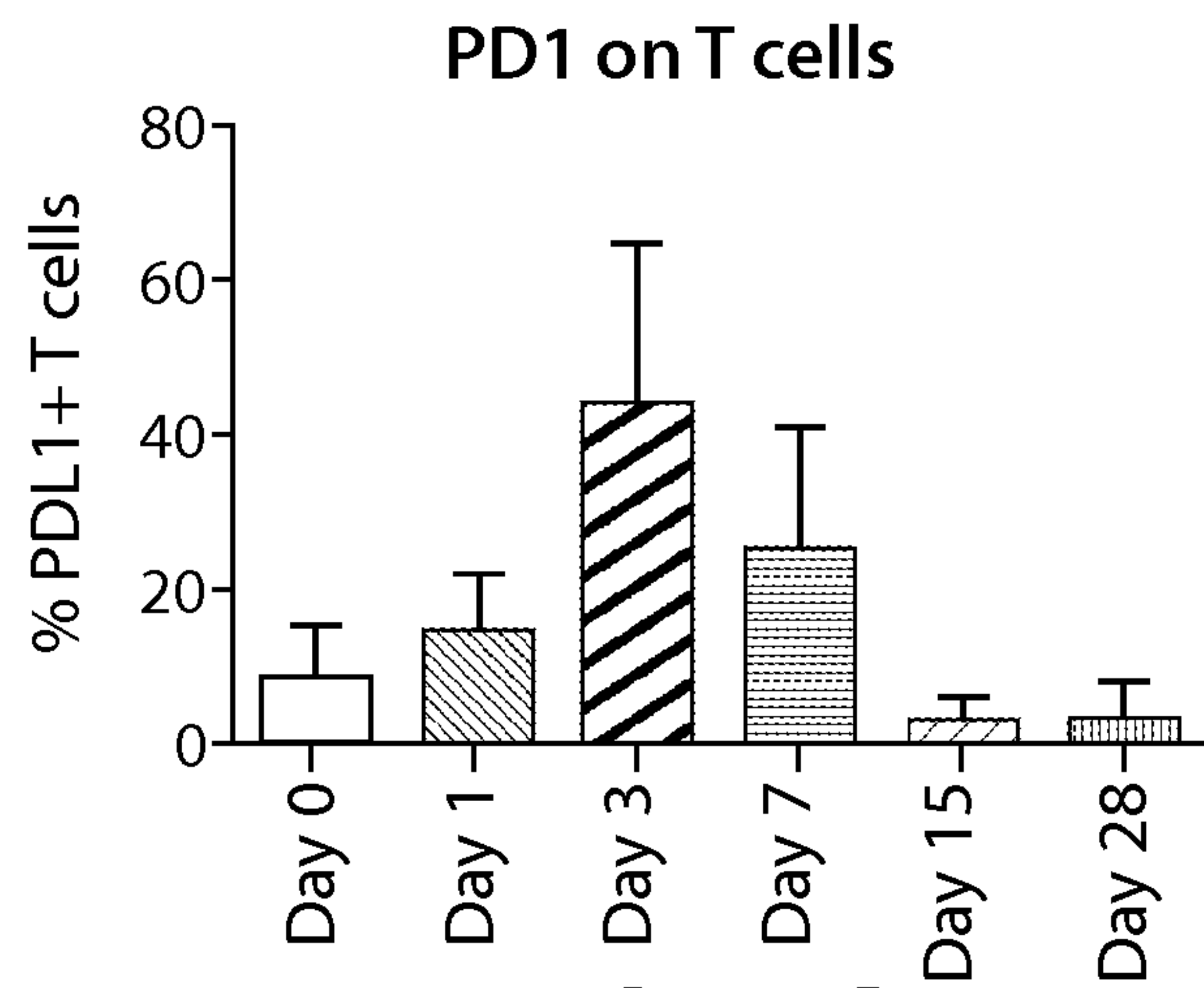


FIG. 41B

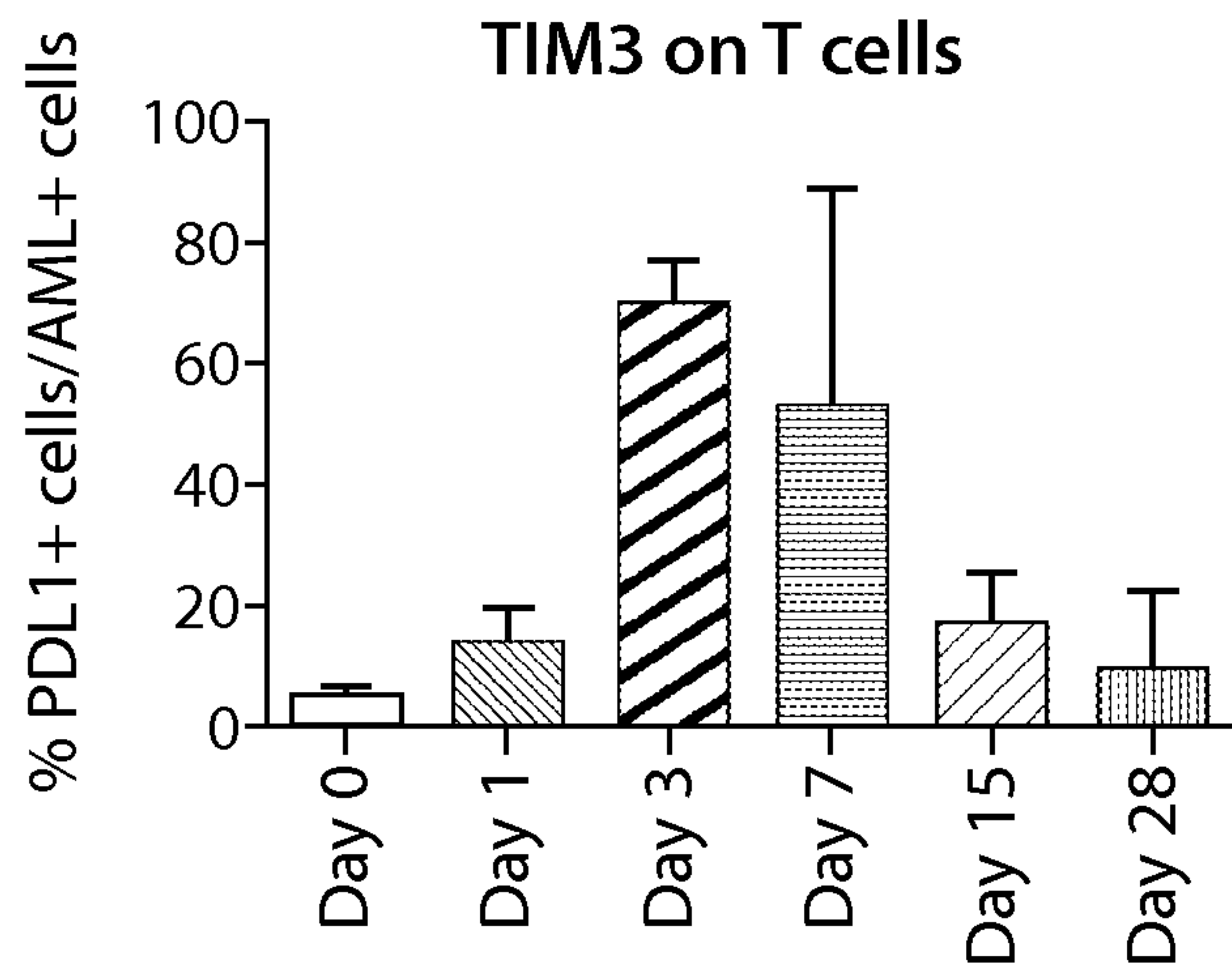


FIG. 41C

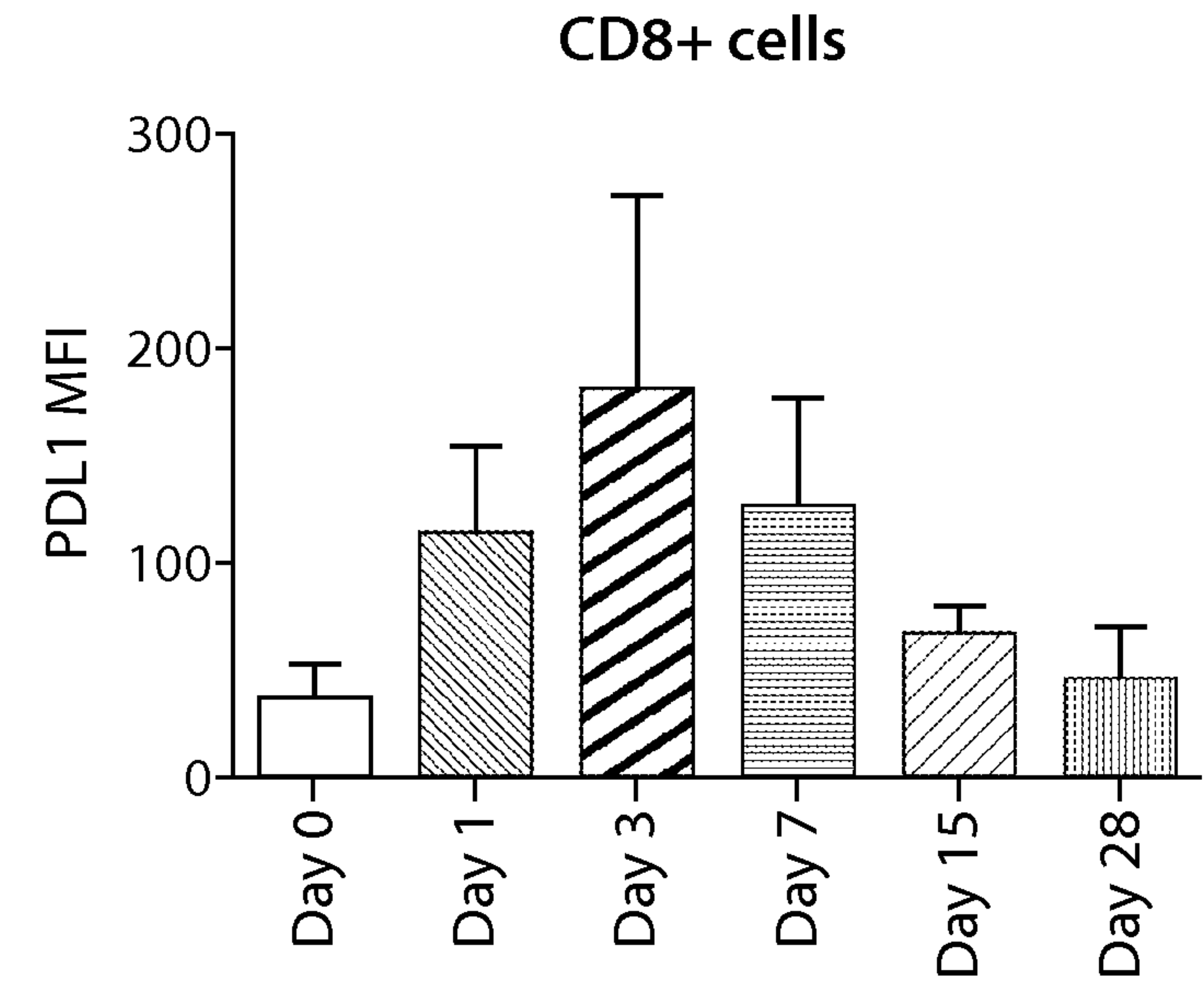


FIG. 42A

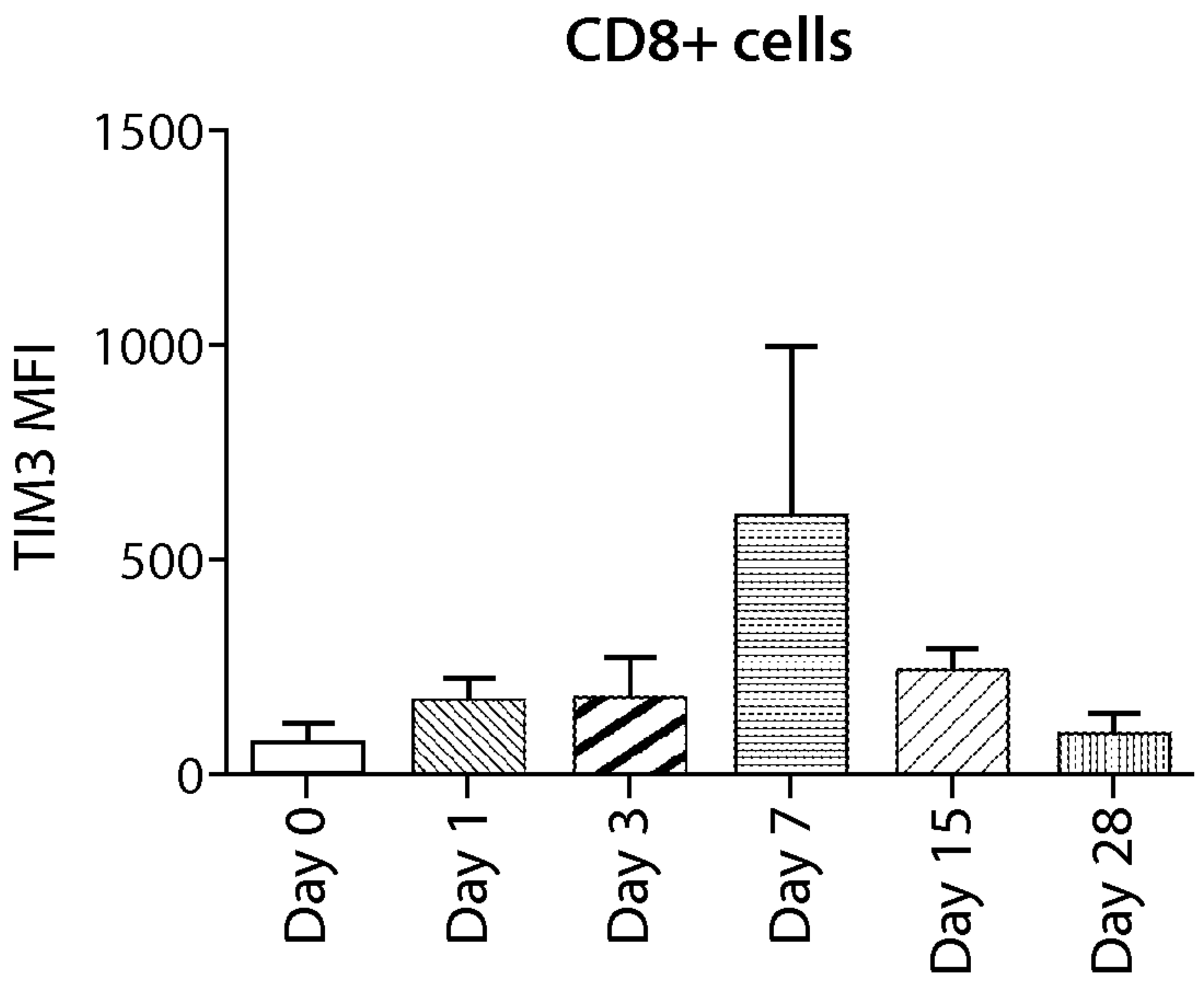


FIG. 42B

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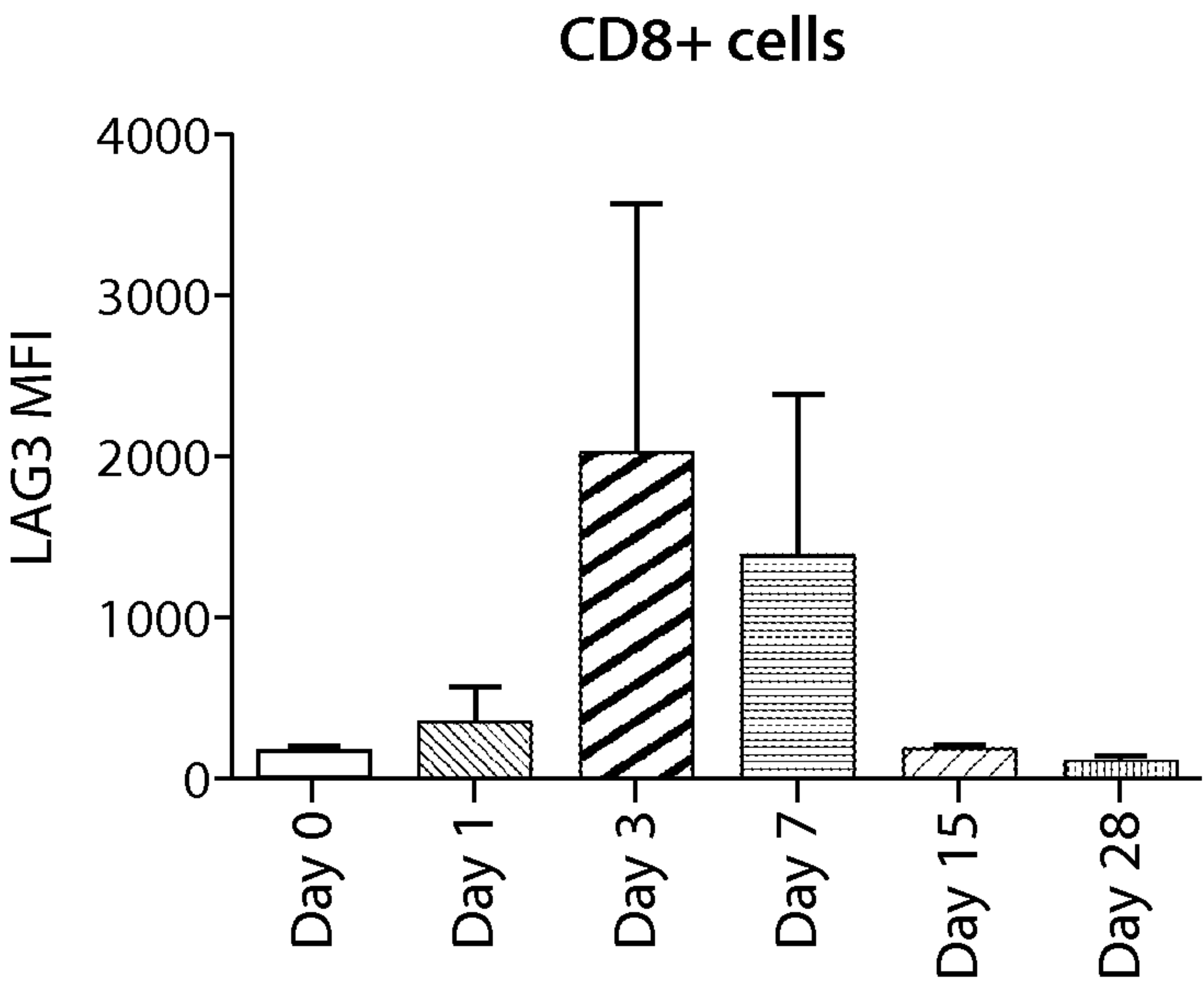


FIG. 42C

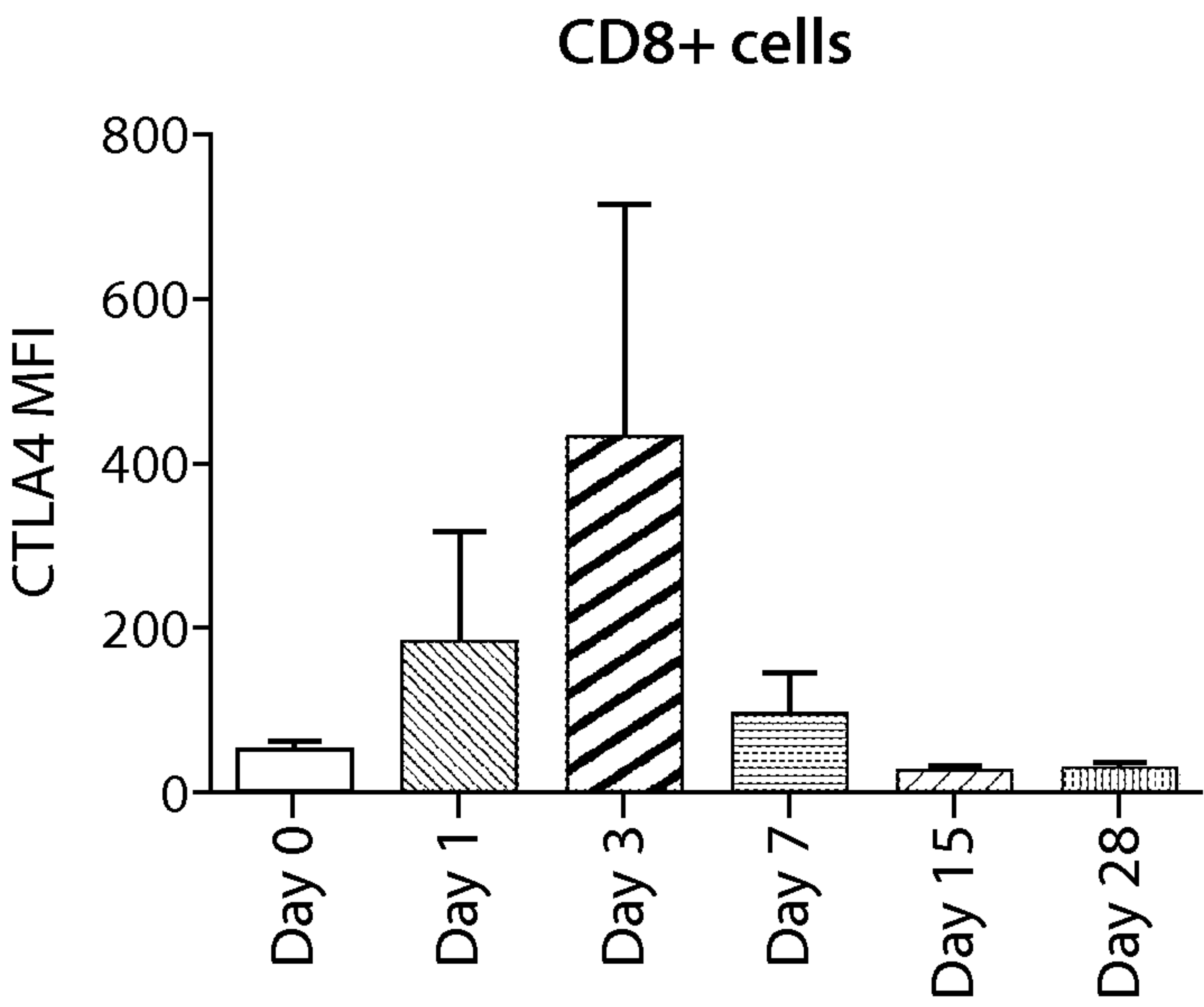


FIG. 42D

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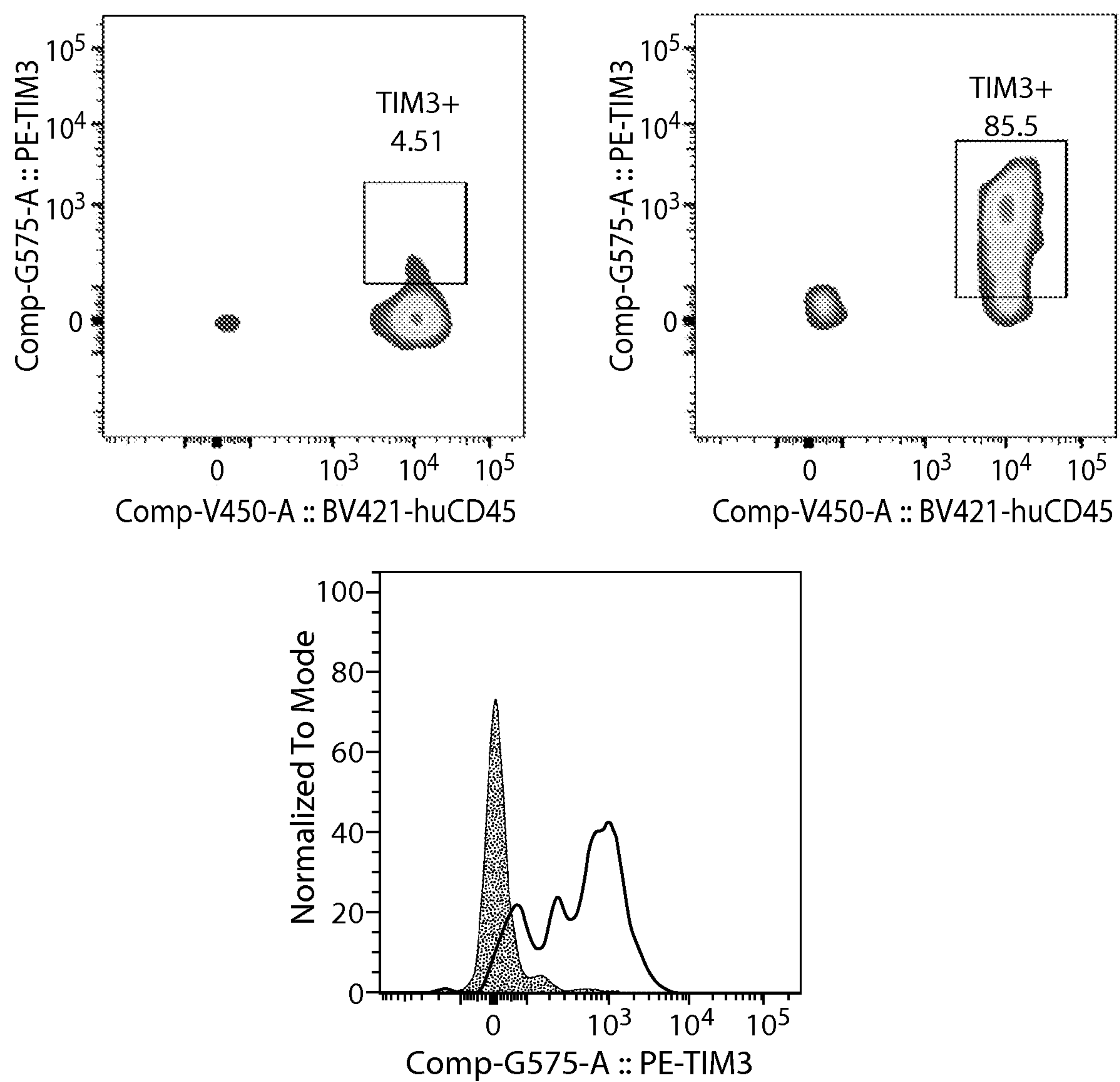


FIG. 43

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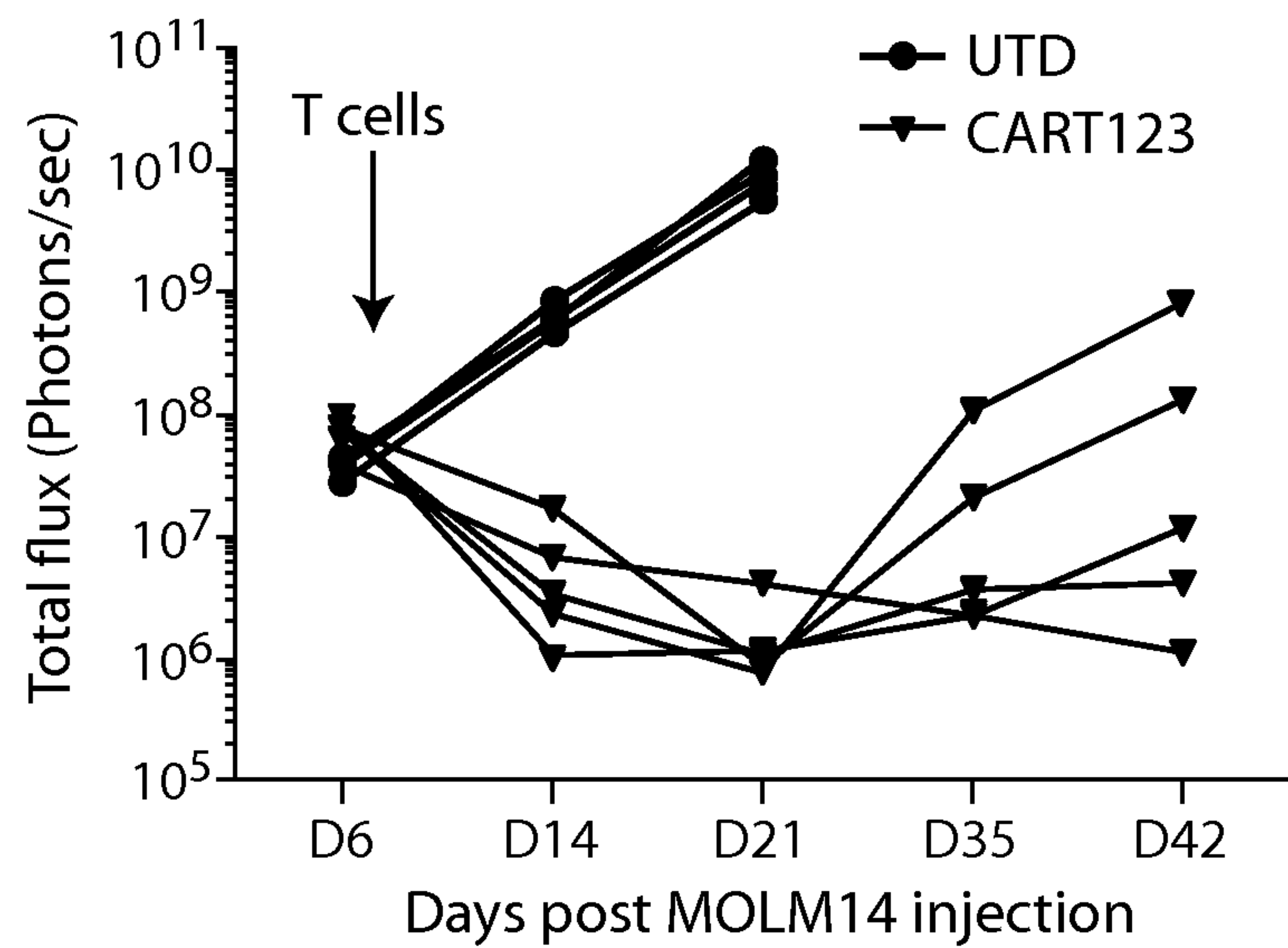


FIG. 44

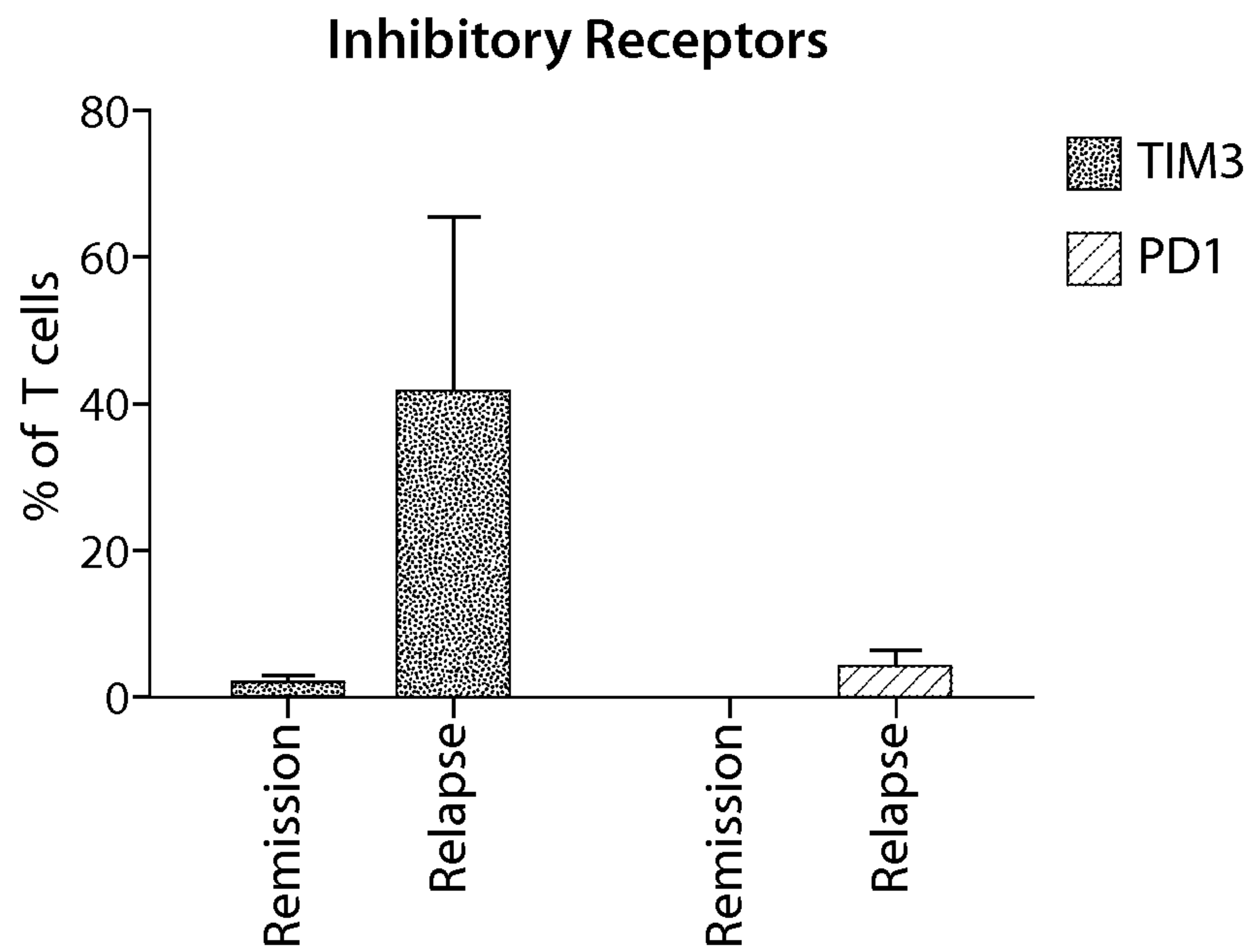


FIG. 45

BM from relapsed MOLM14 xenografts
co-cultured with checkpoint inhibitors

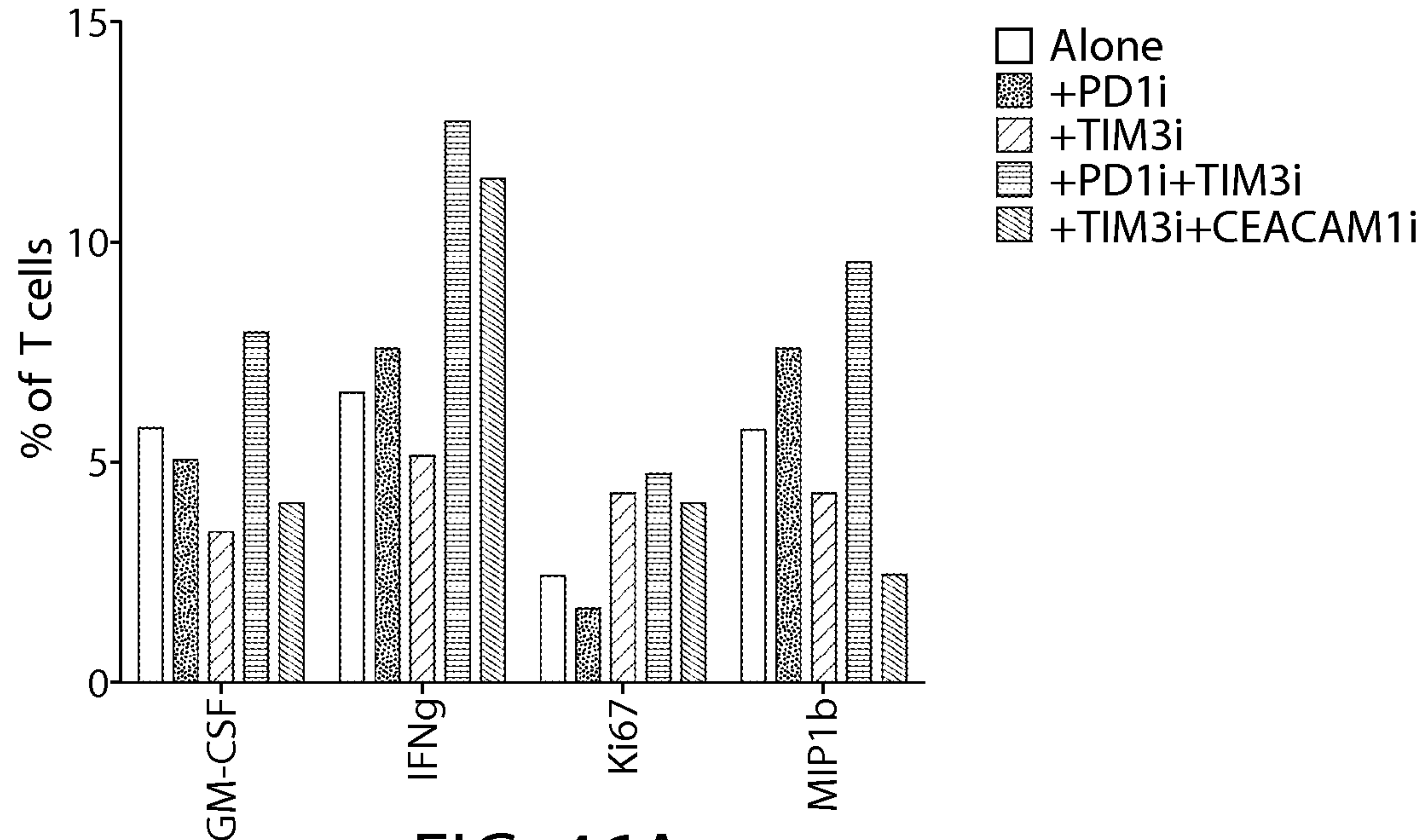


FIG. 46A

BM from relapsed MOLM14 xenografts
co-cultured with checkpoint inhibitors
(in the presence of PMA/IONO)

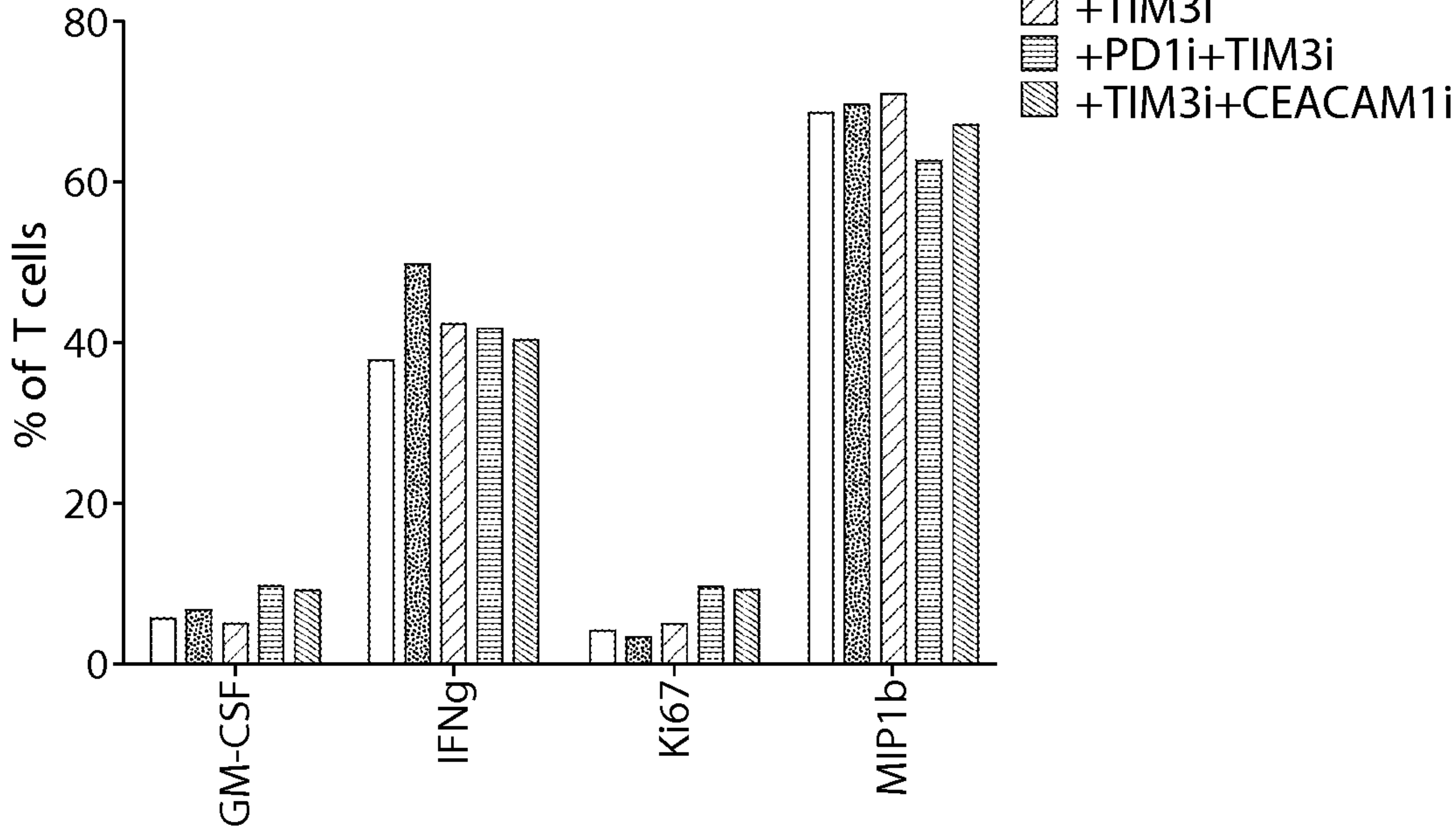


FIG. 46B

Antibody dose: 250ug/mouse IP
Early: D3,D7,D11
Late: D11,D15,D19

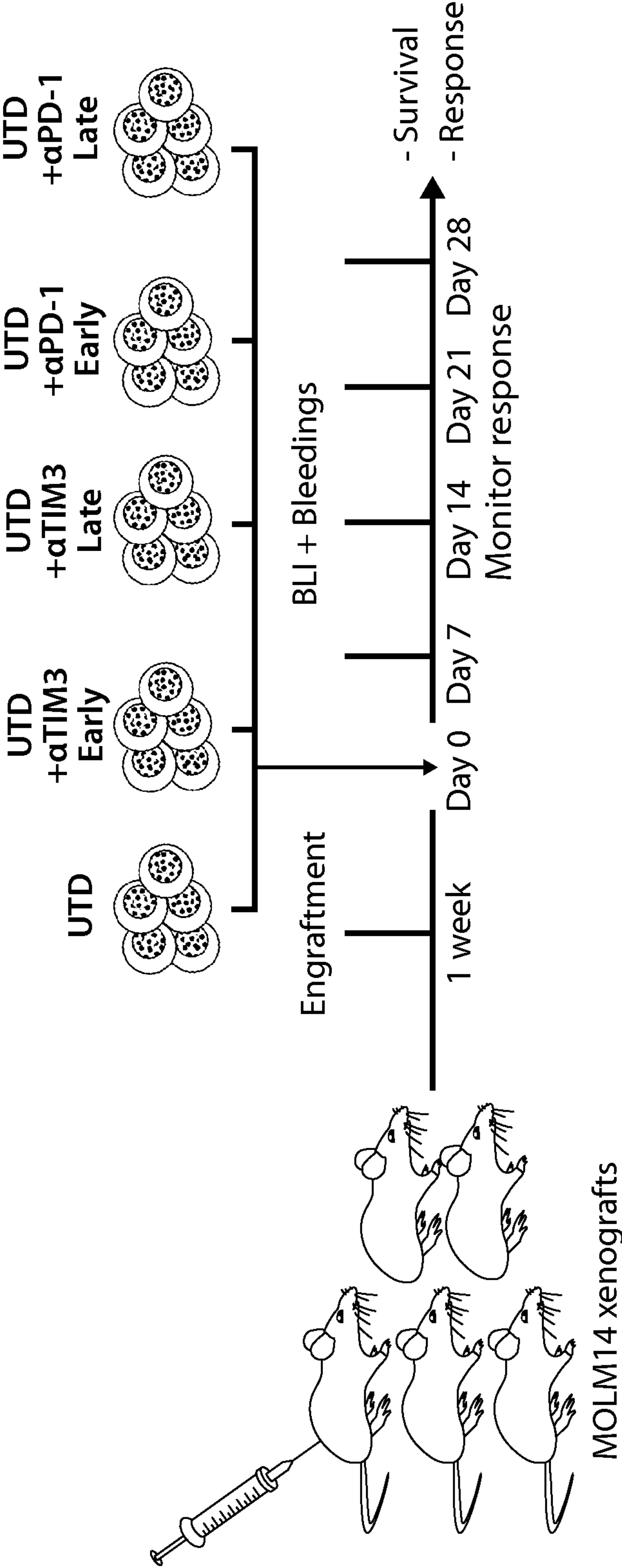


FIG. 47

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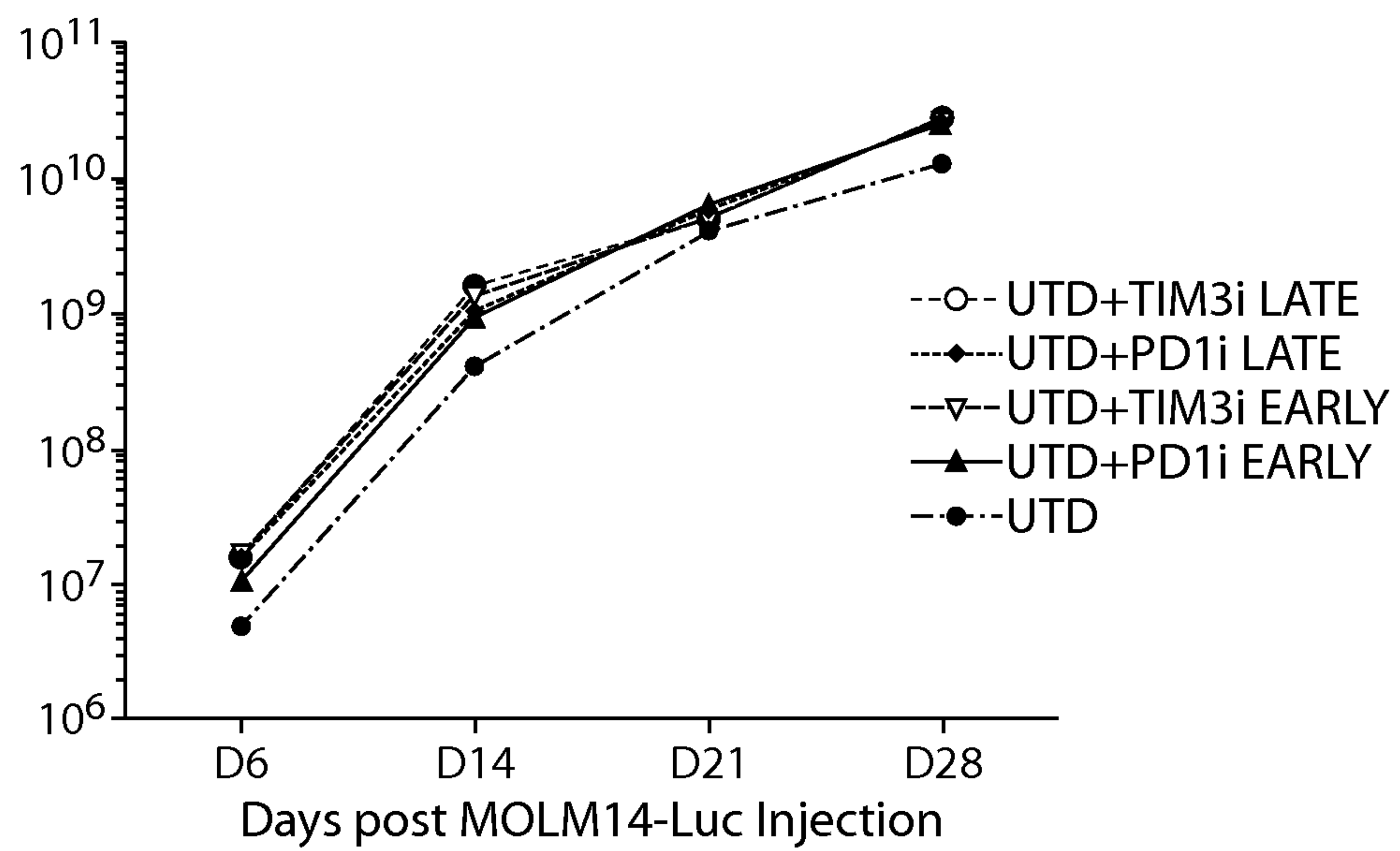
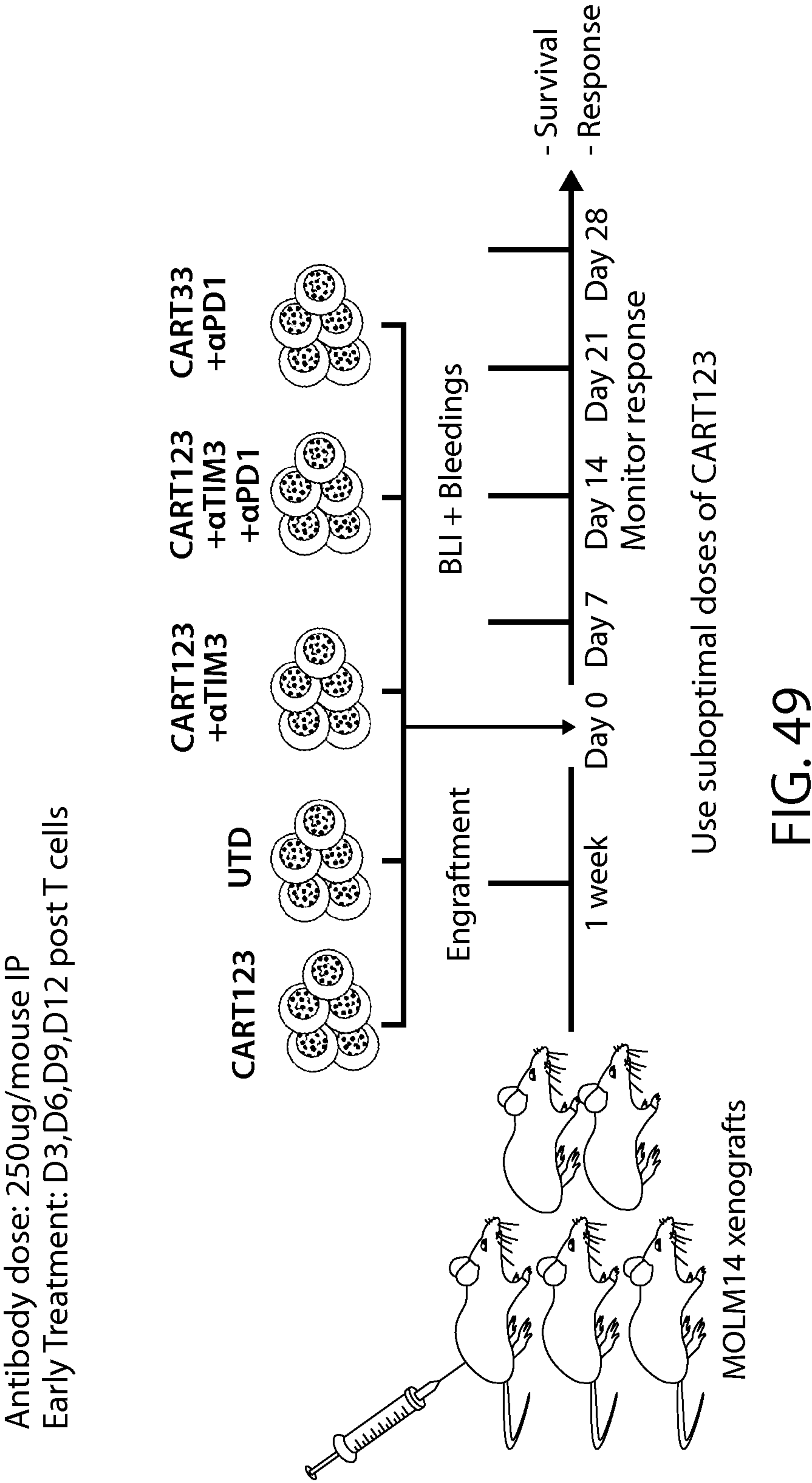


FIG. 48



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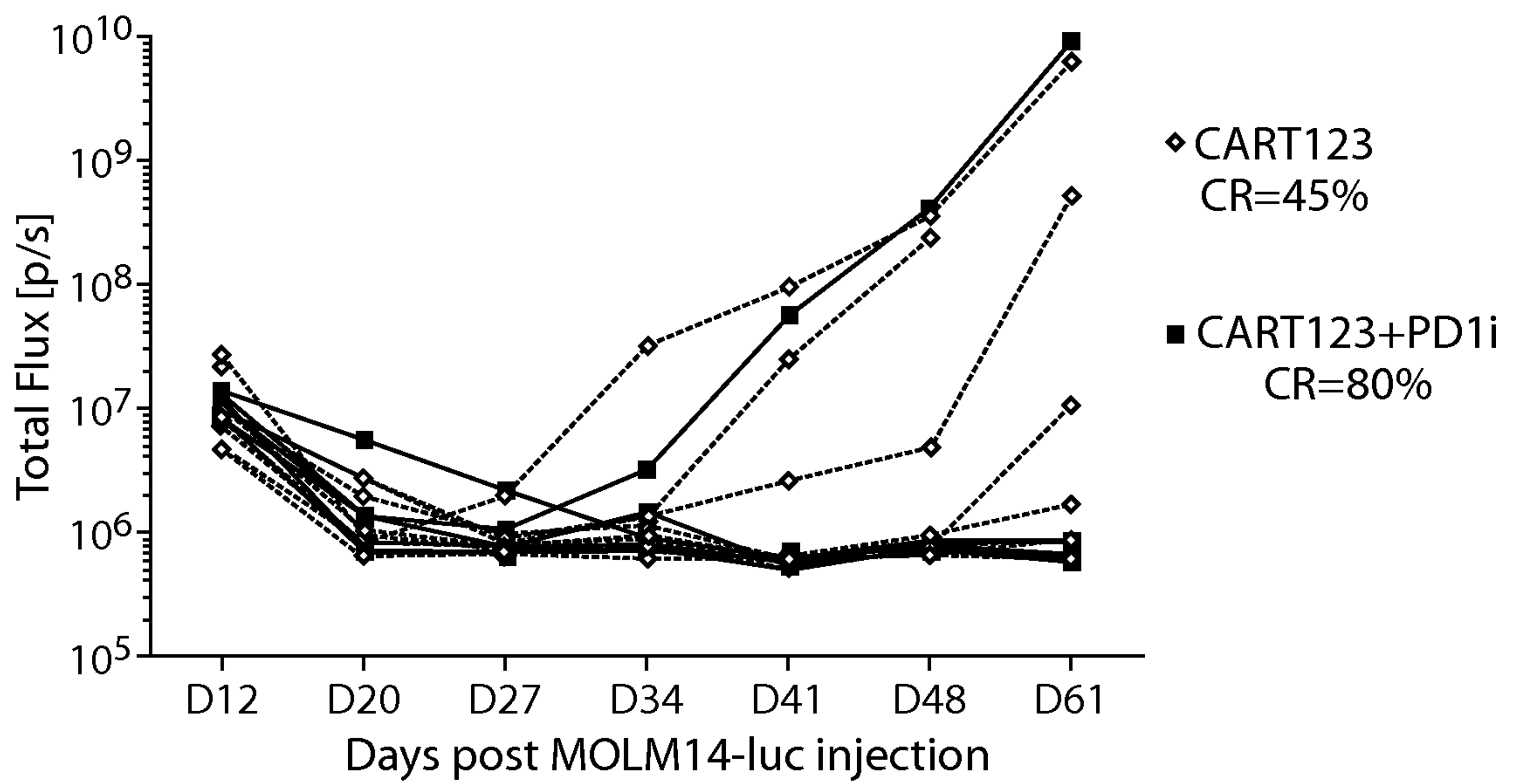


FIG. 50A

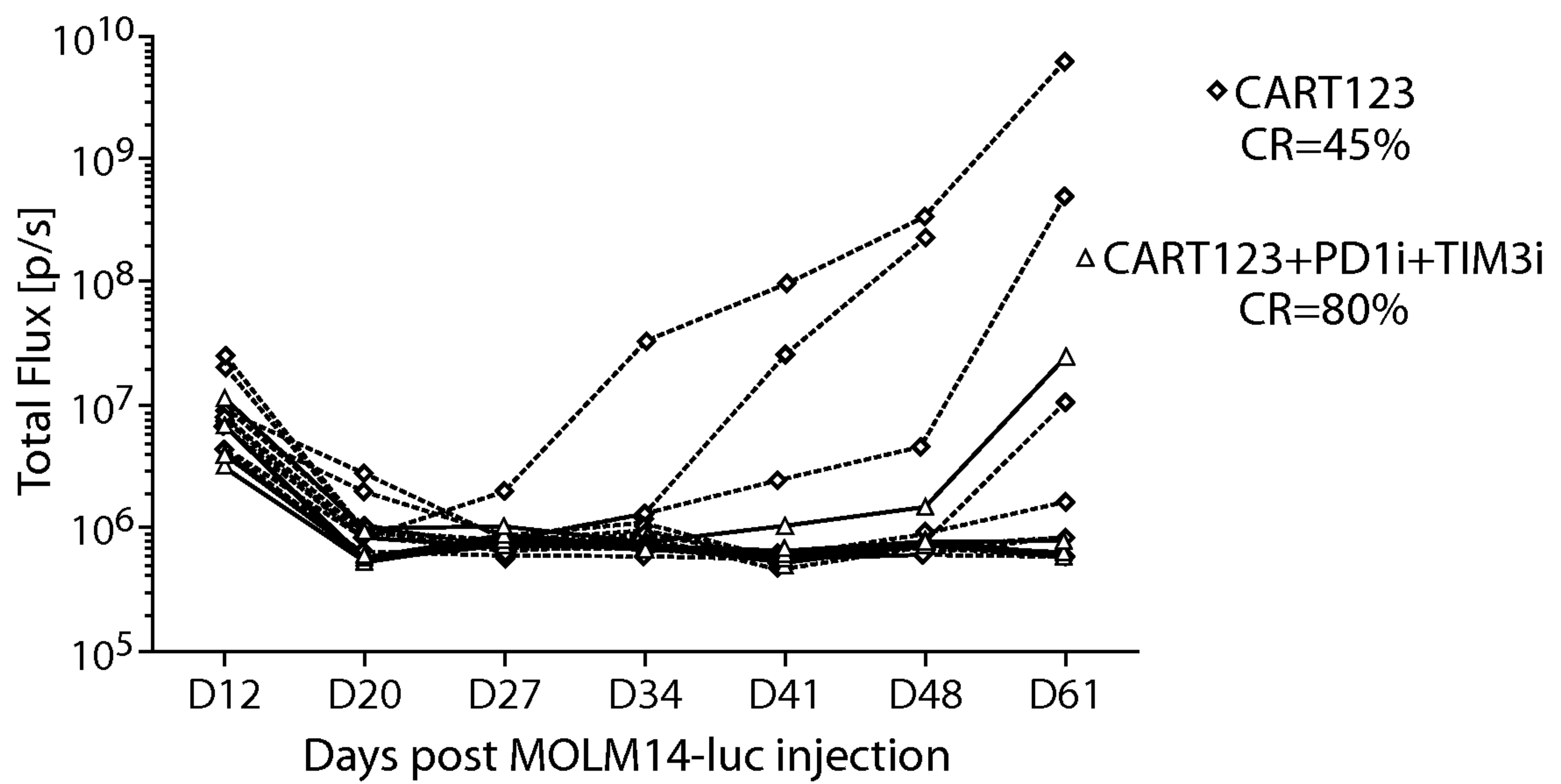


FIG. 50B

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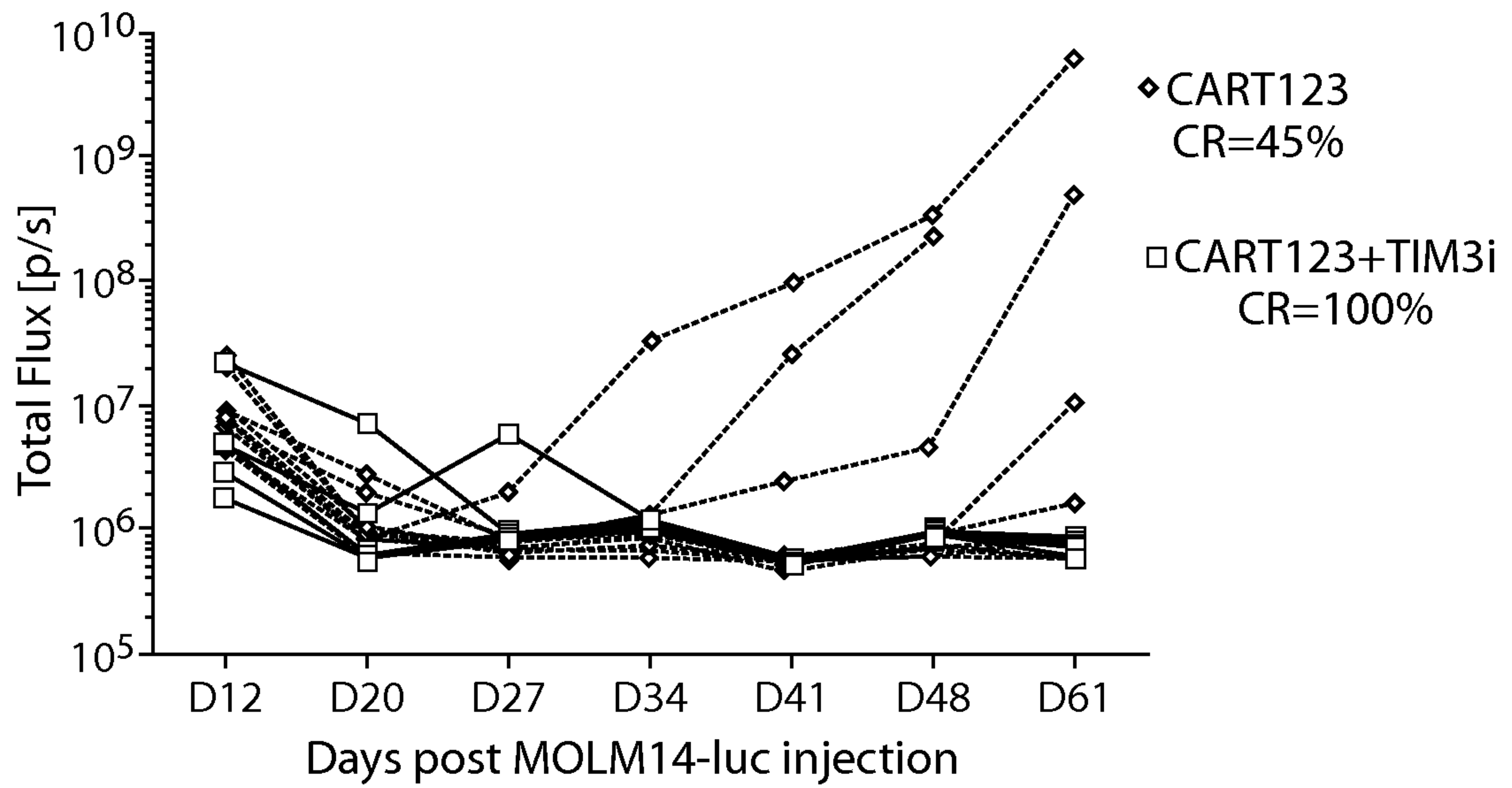


FIG. 50C

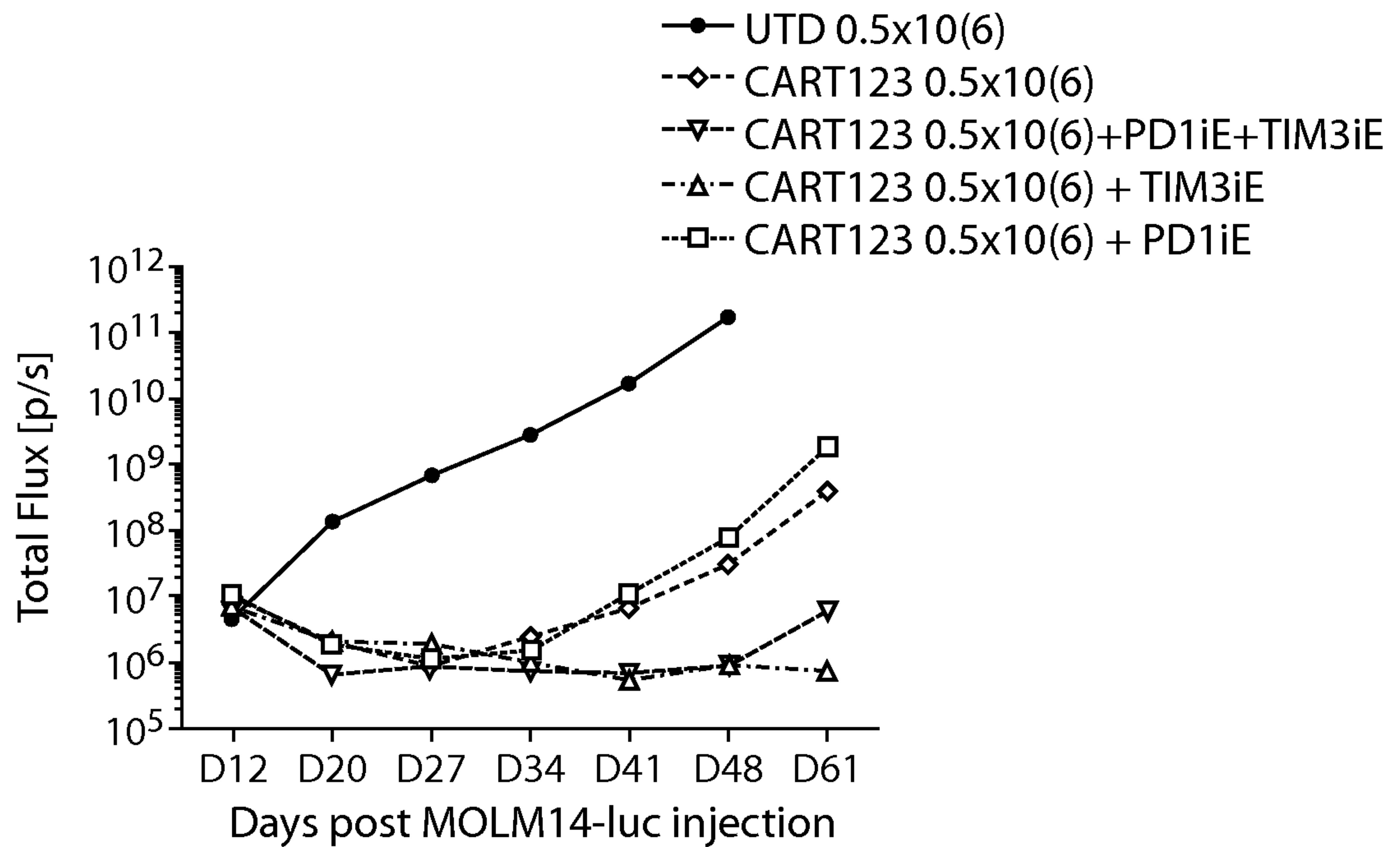
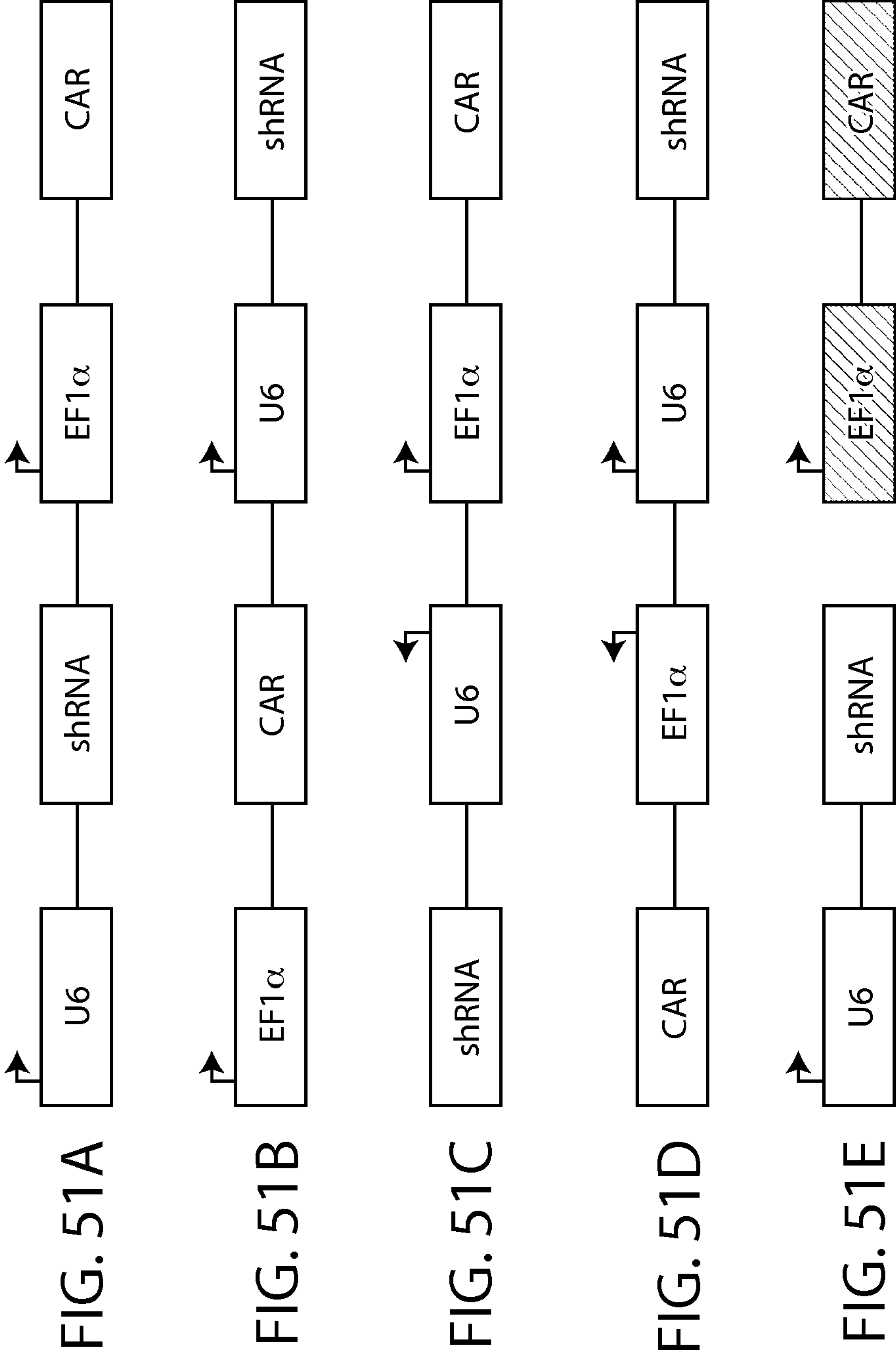


FIG. 50D

Vector constructs



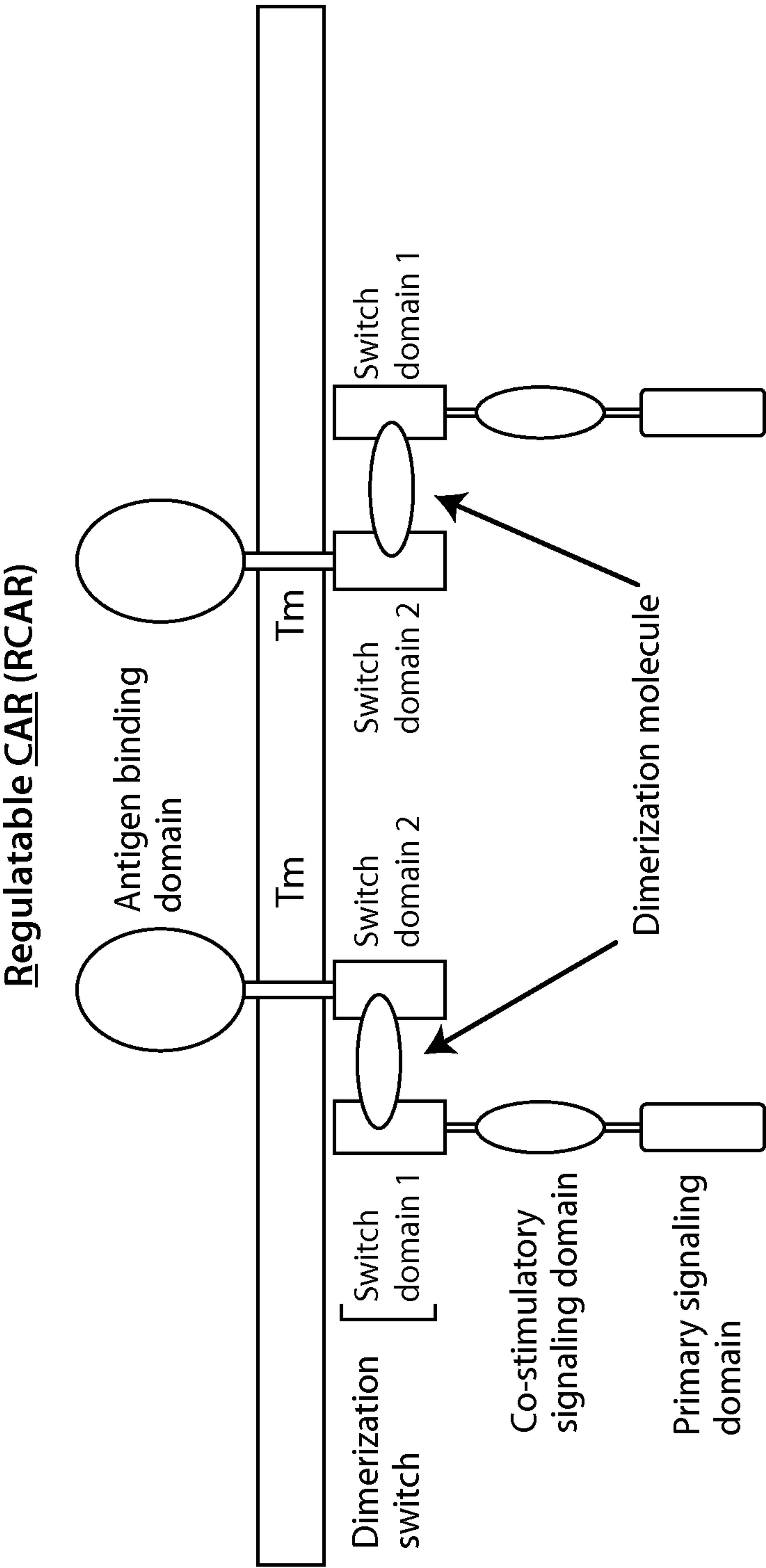


Fig. 52

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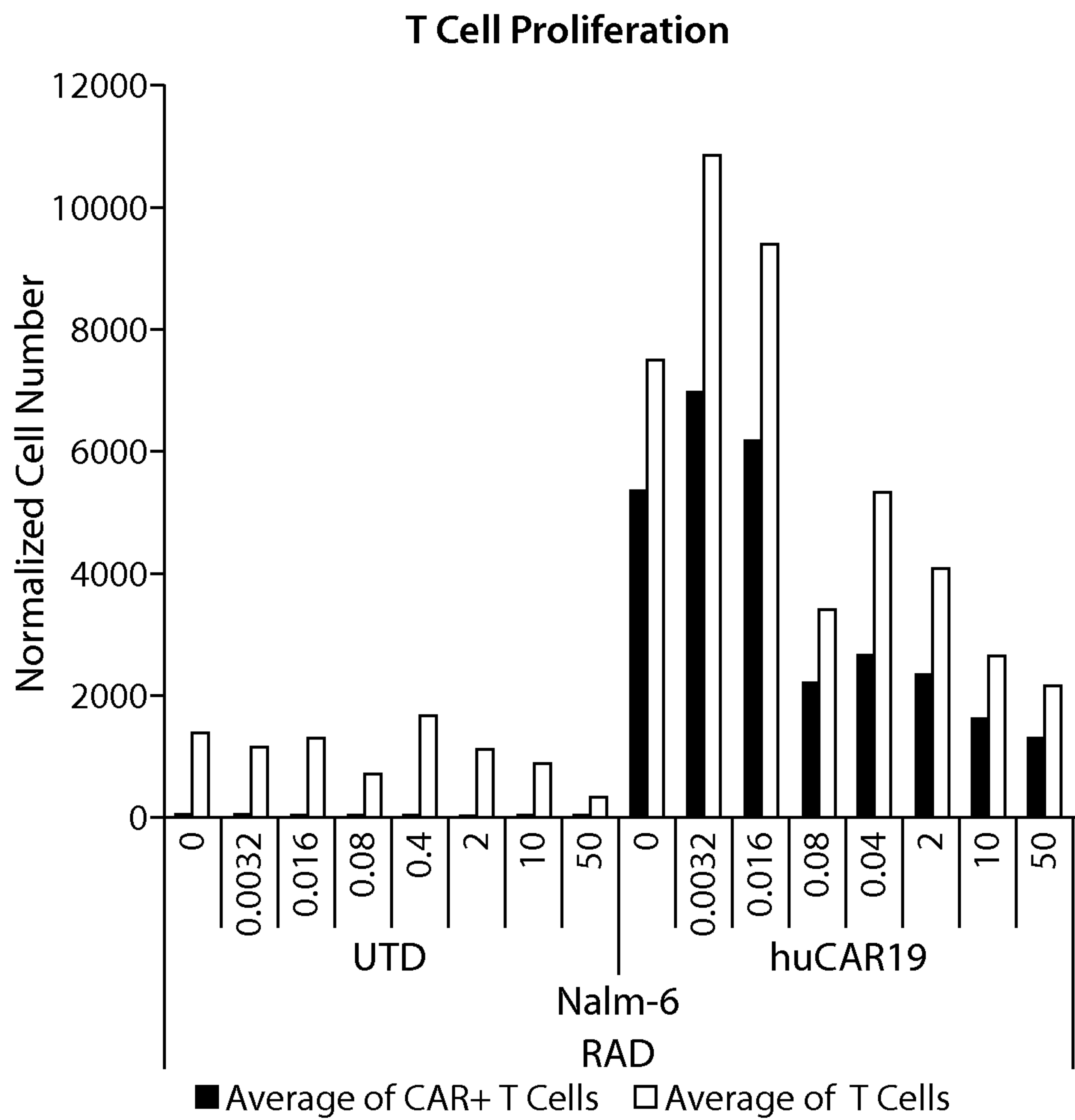


FIG. 53

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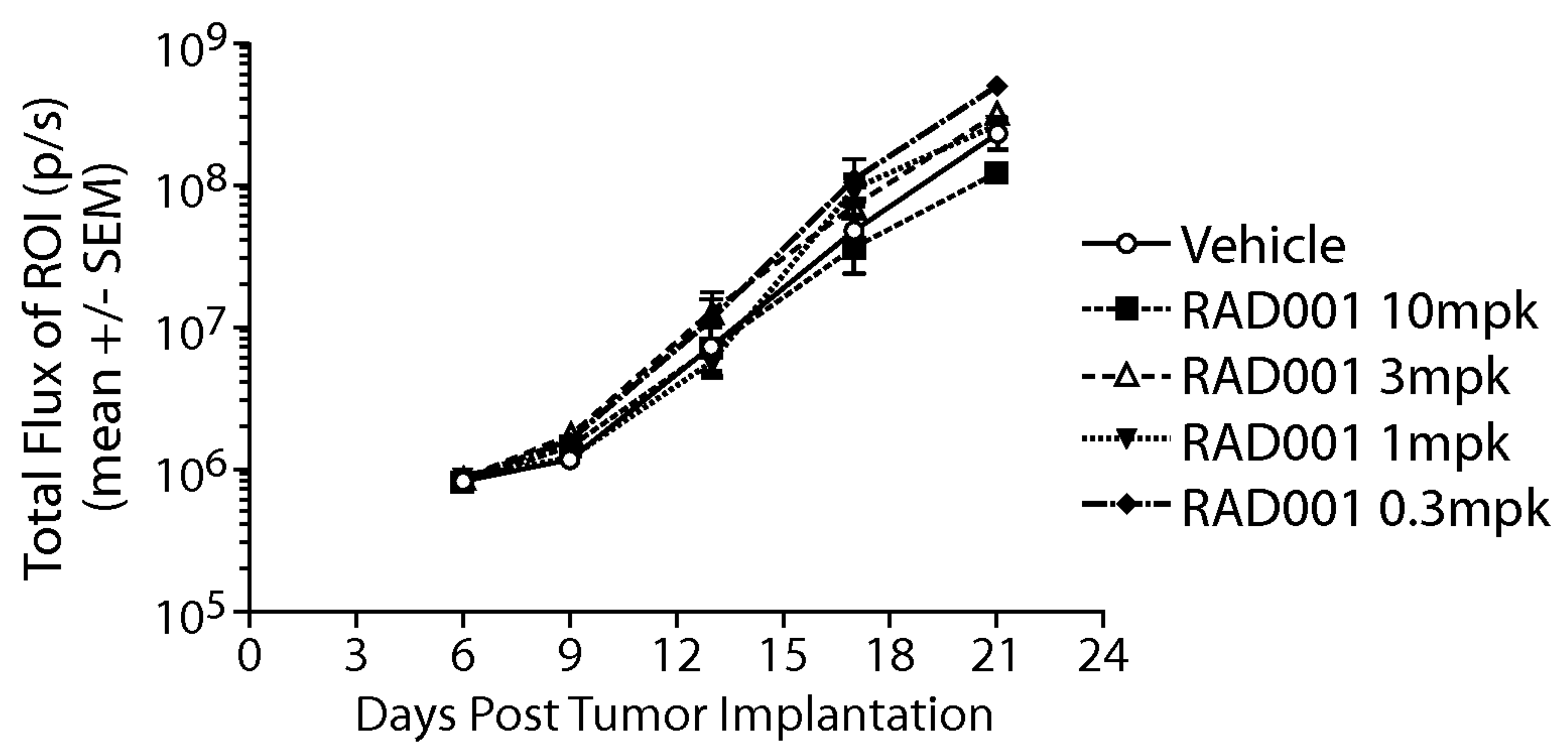


FIG. 54

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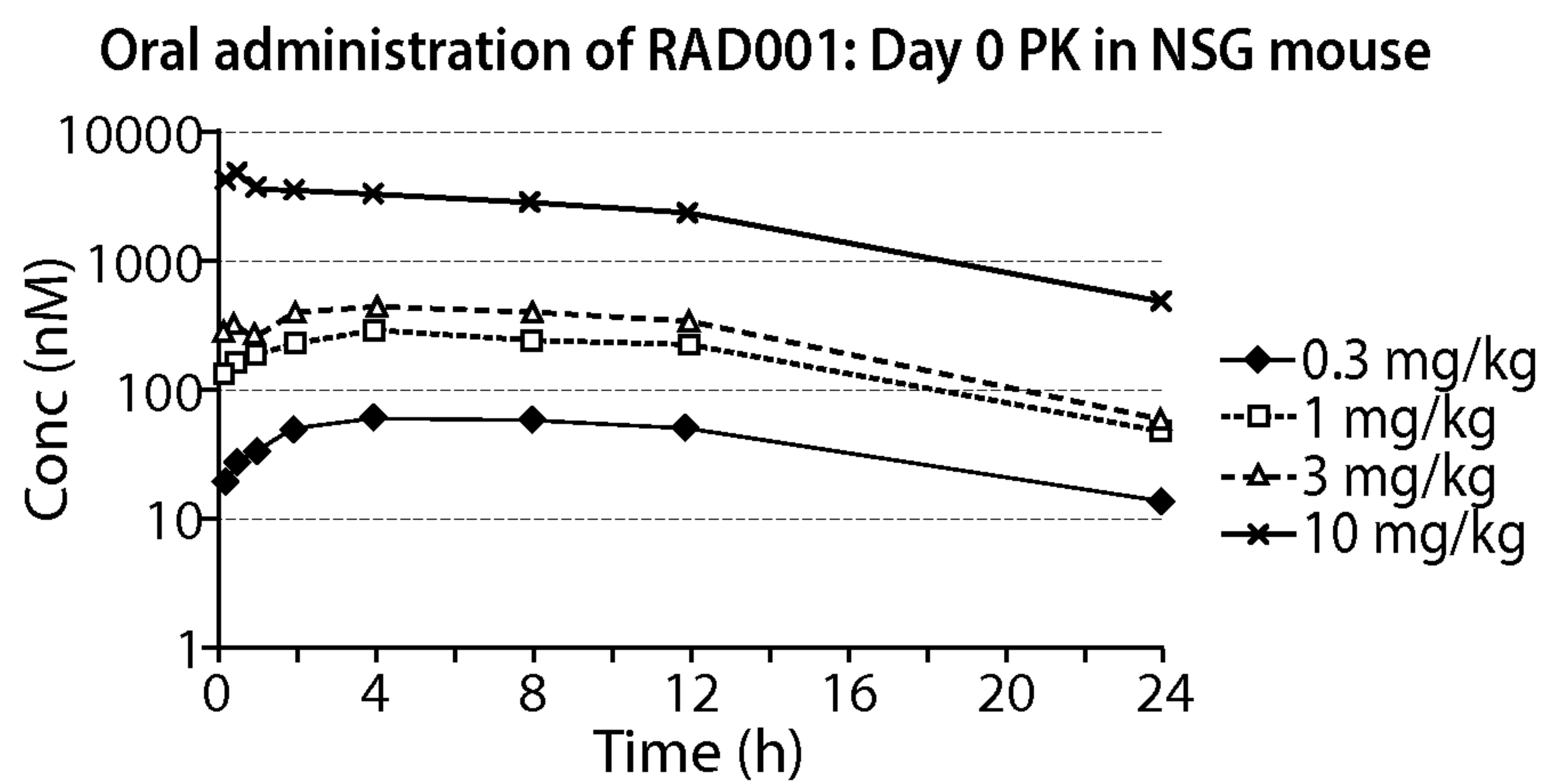


FIG. 55A

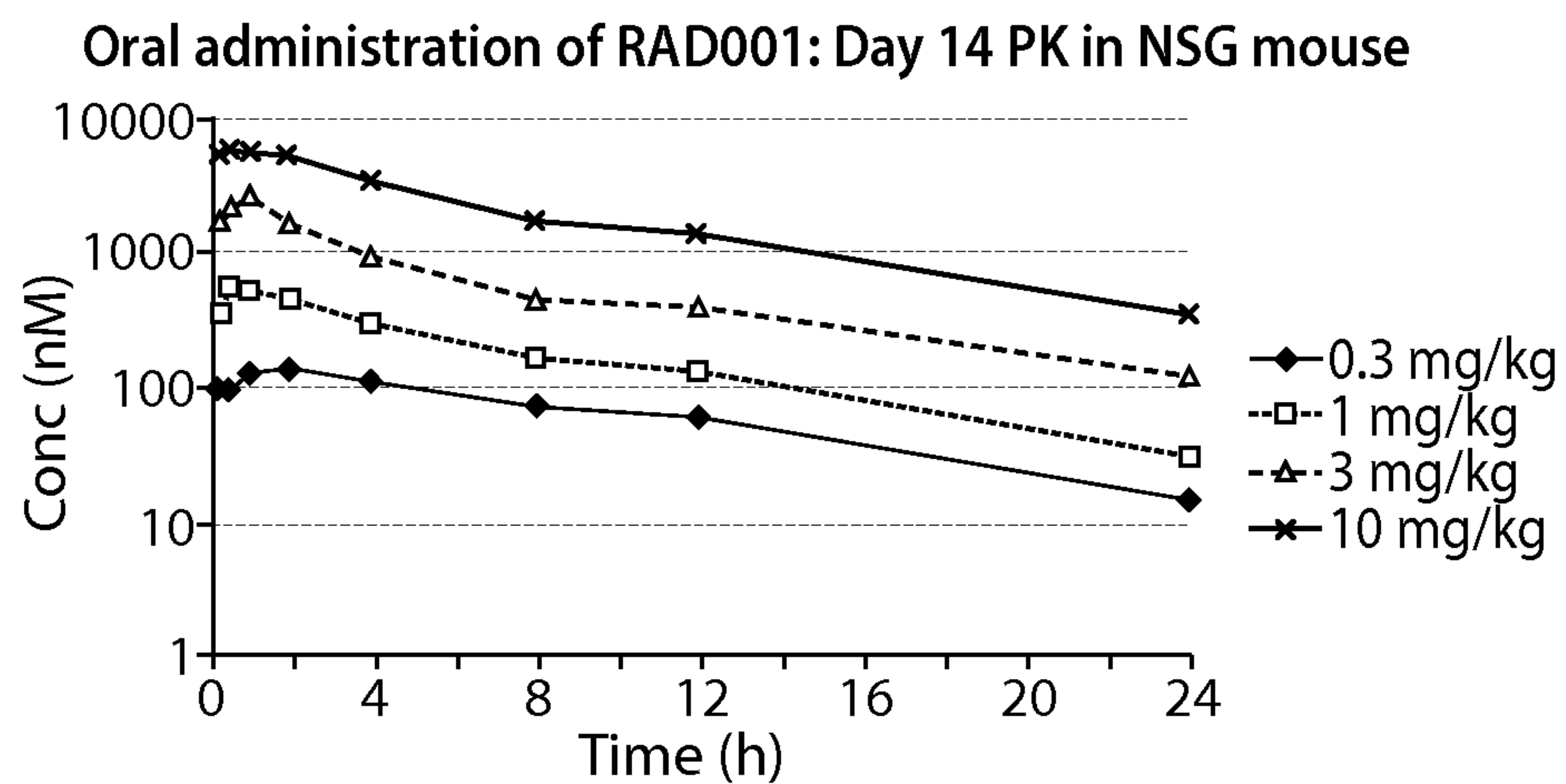


FIG. 55B

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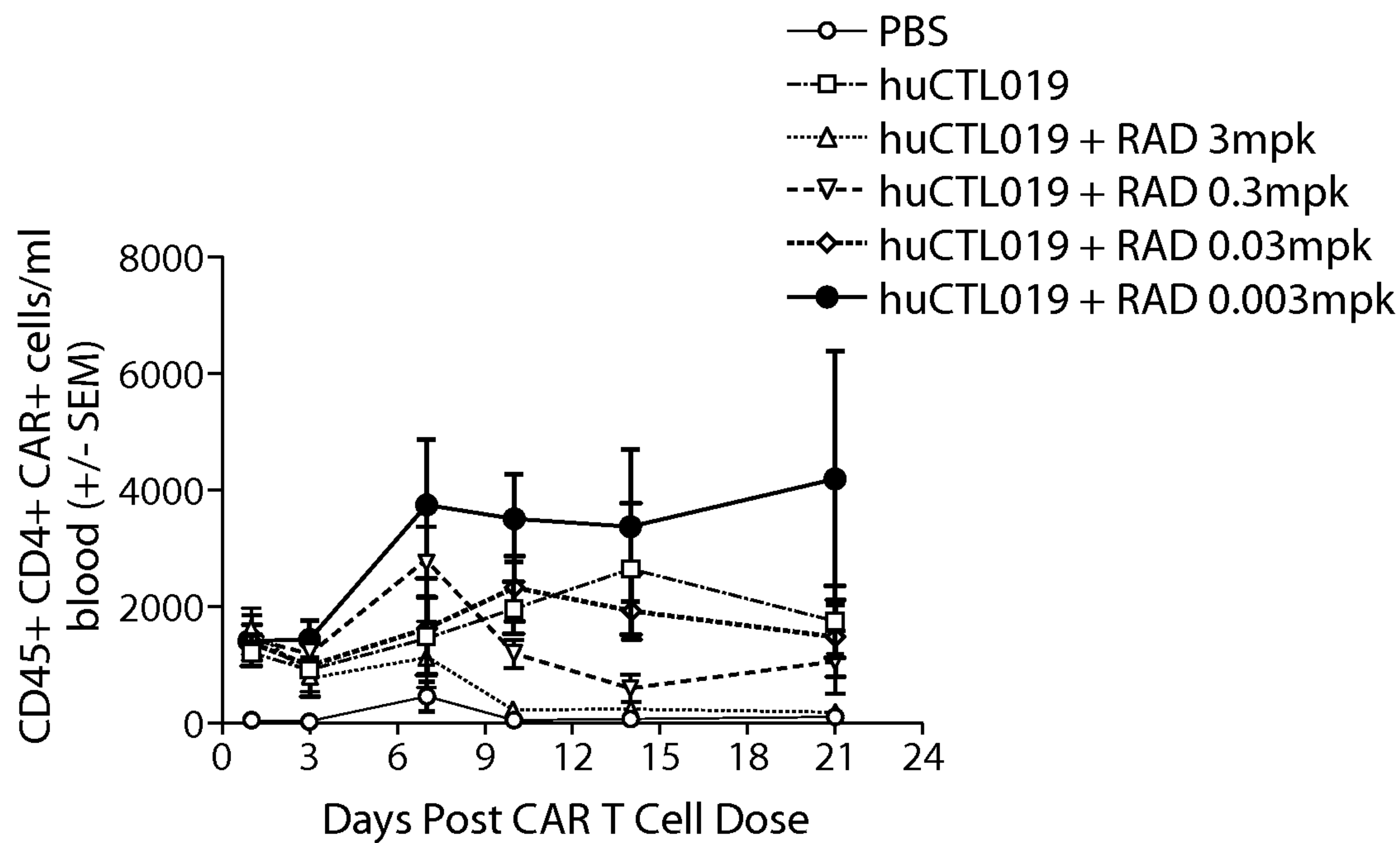


FIG. 56A

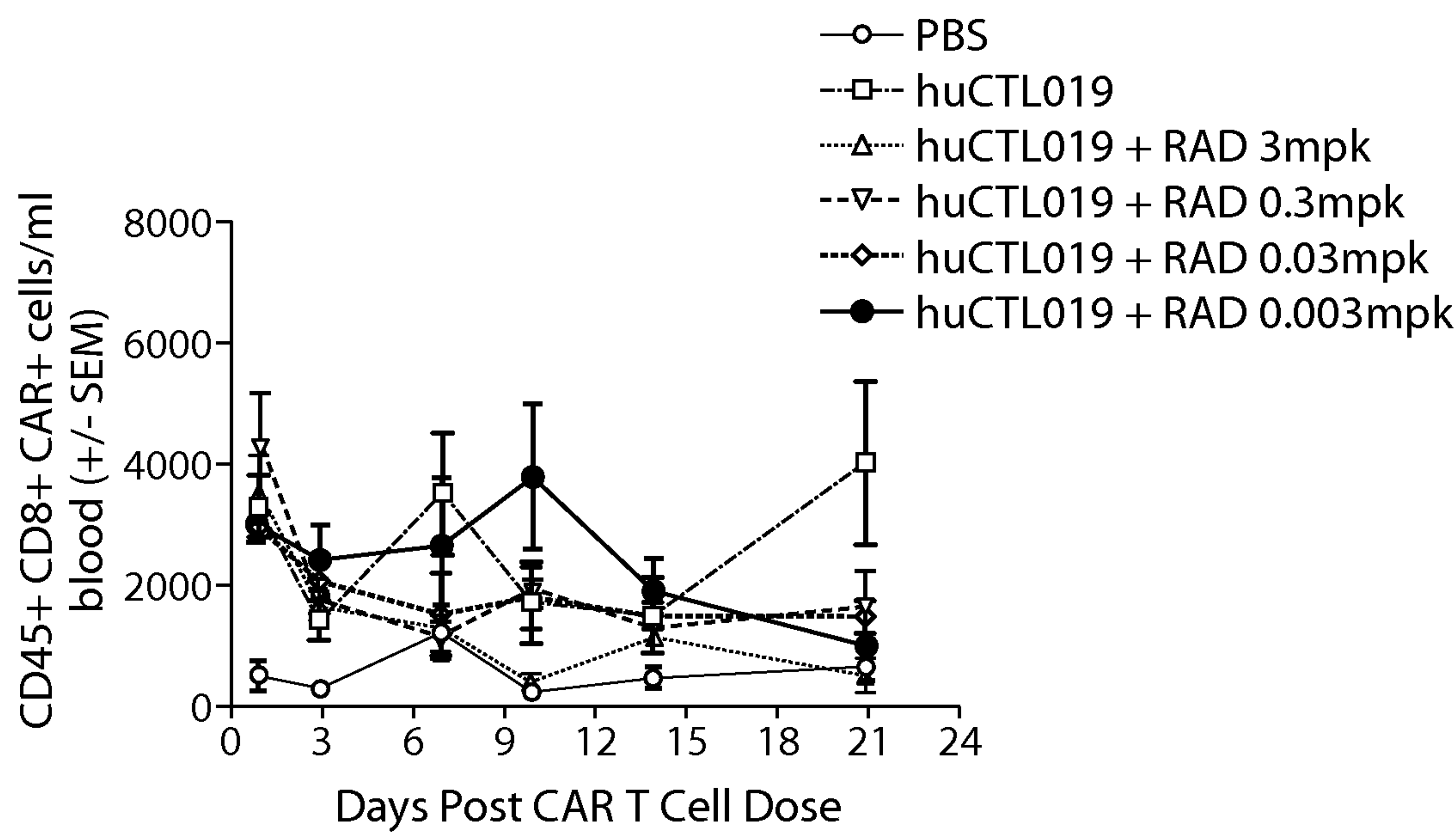


FIG. 56B

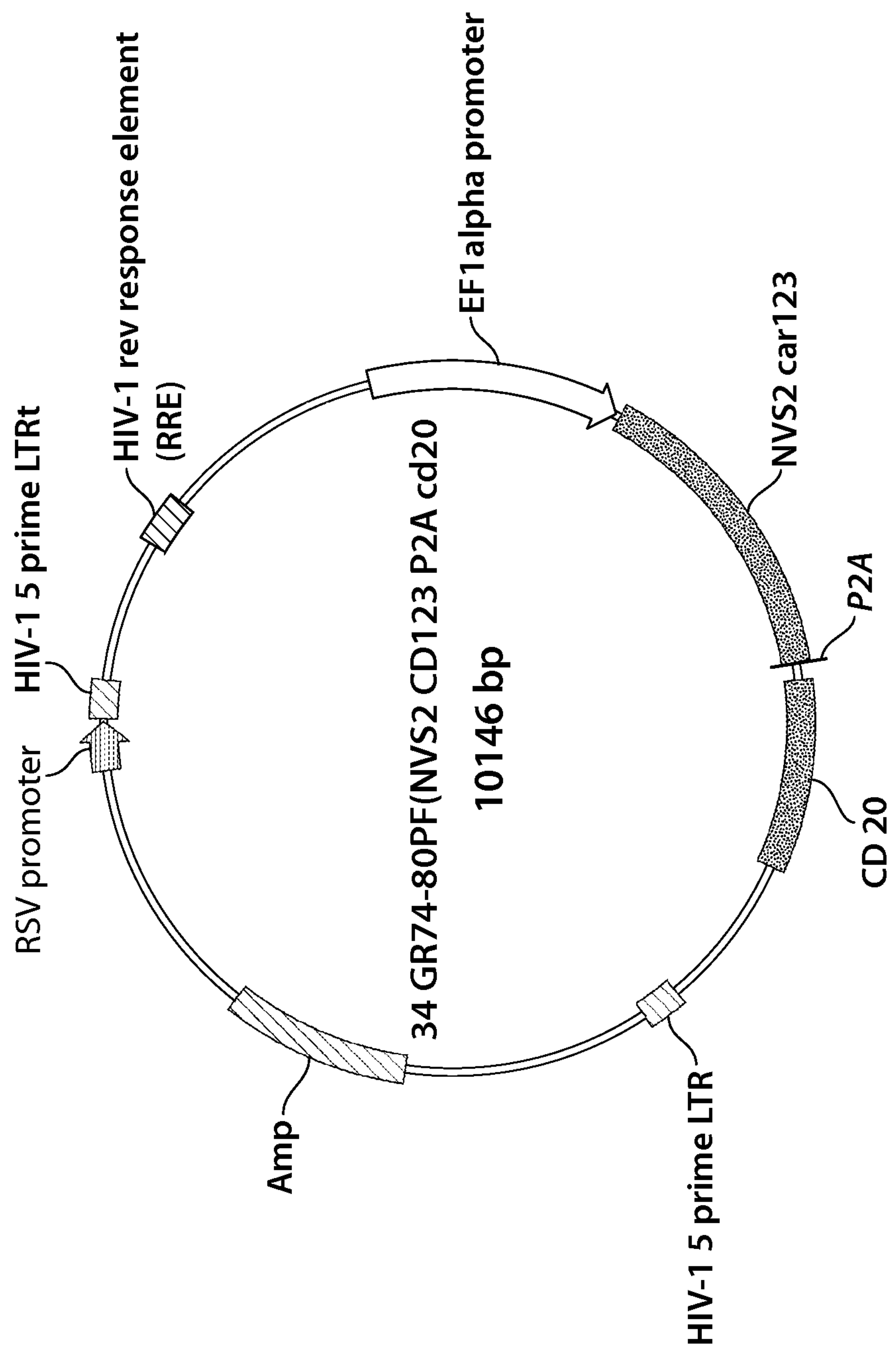


FIG. 57

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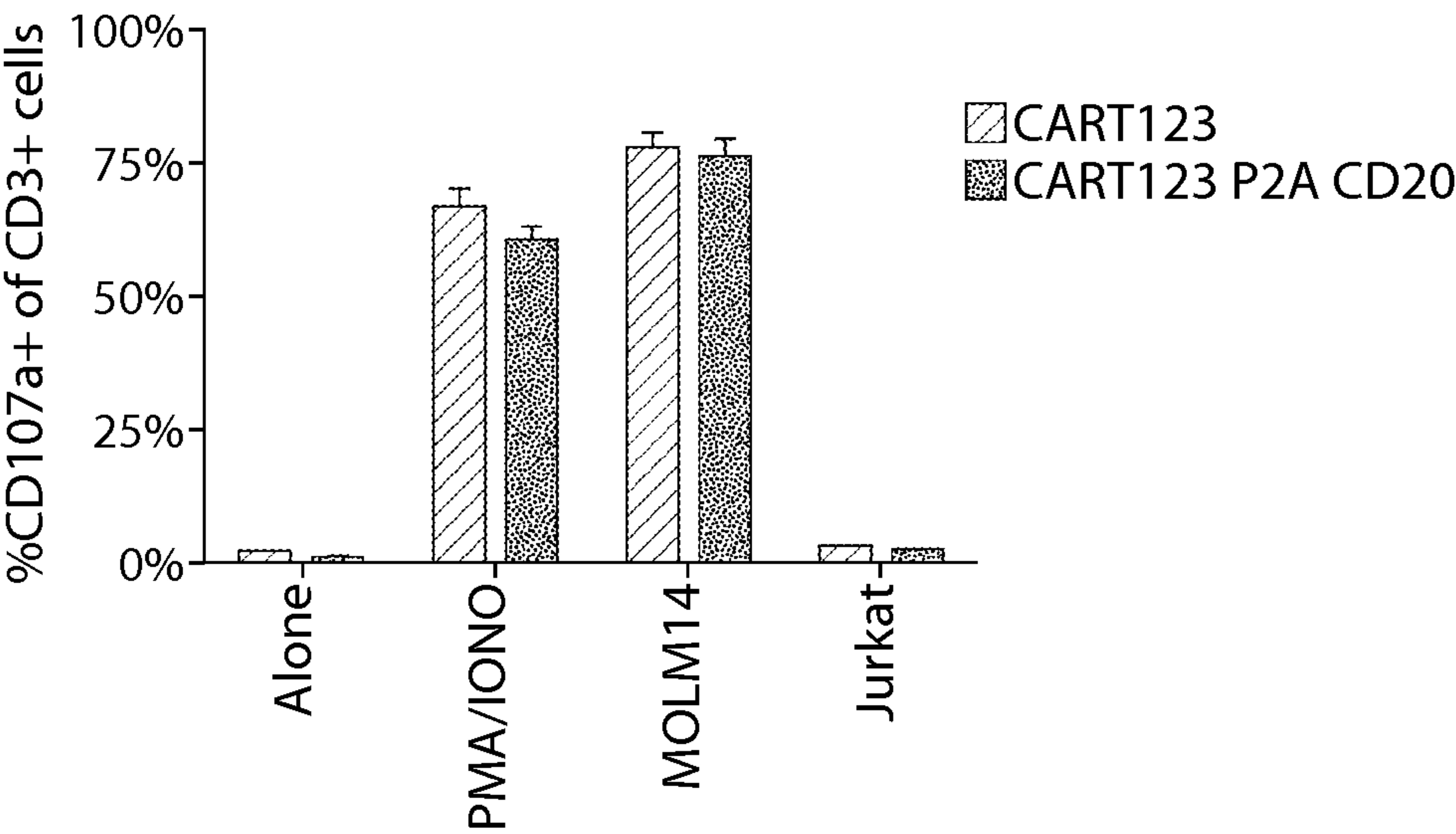


FIG. 58

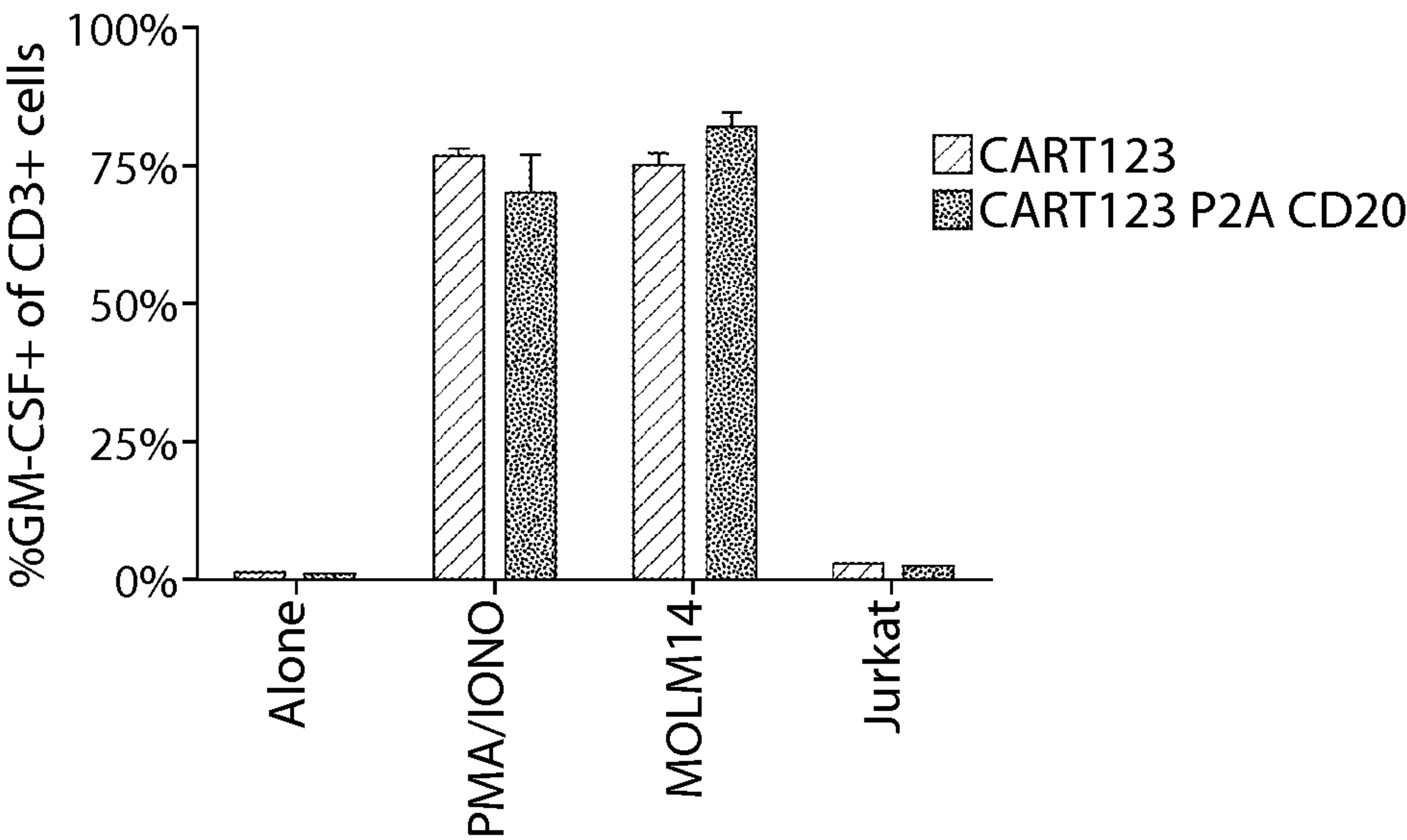


FIG. 59A

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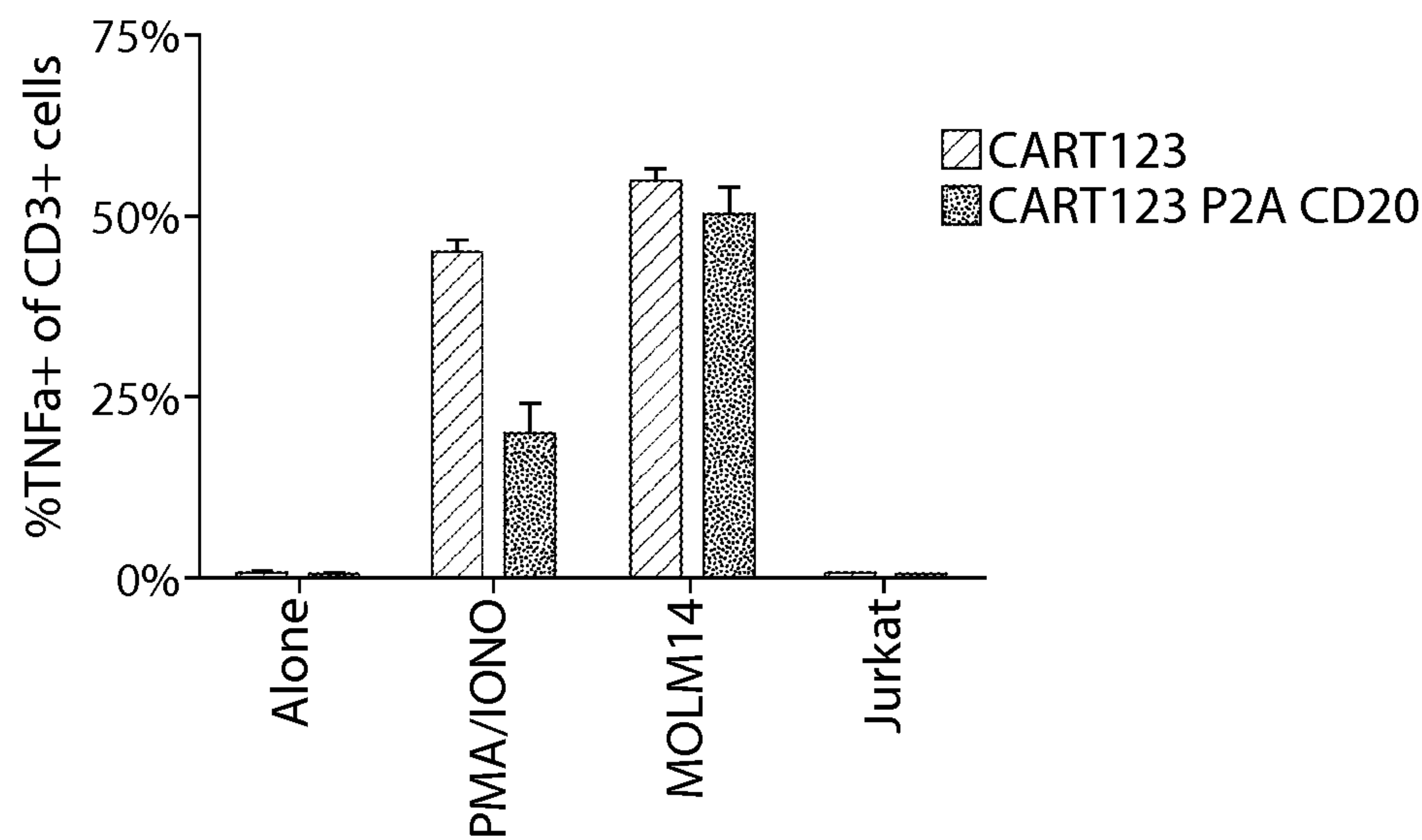


FIG. 59B

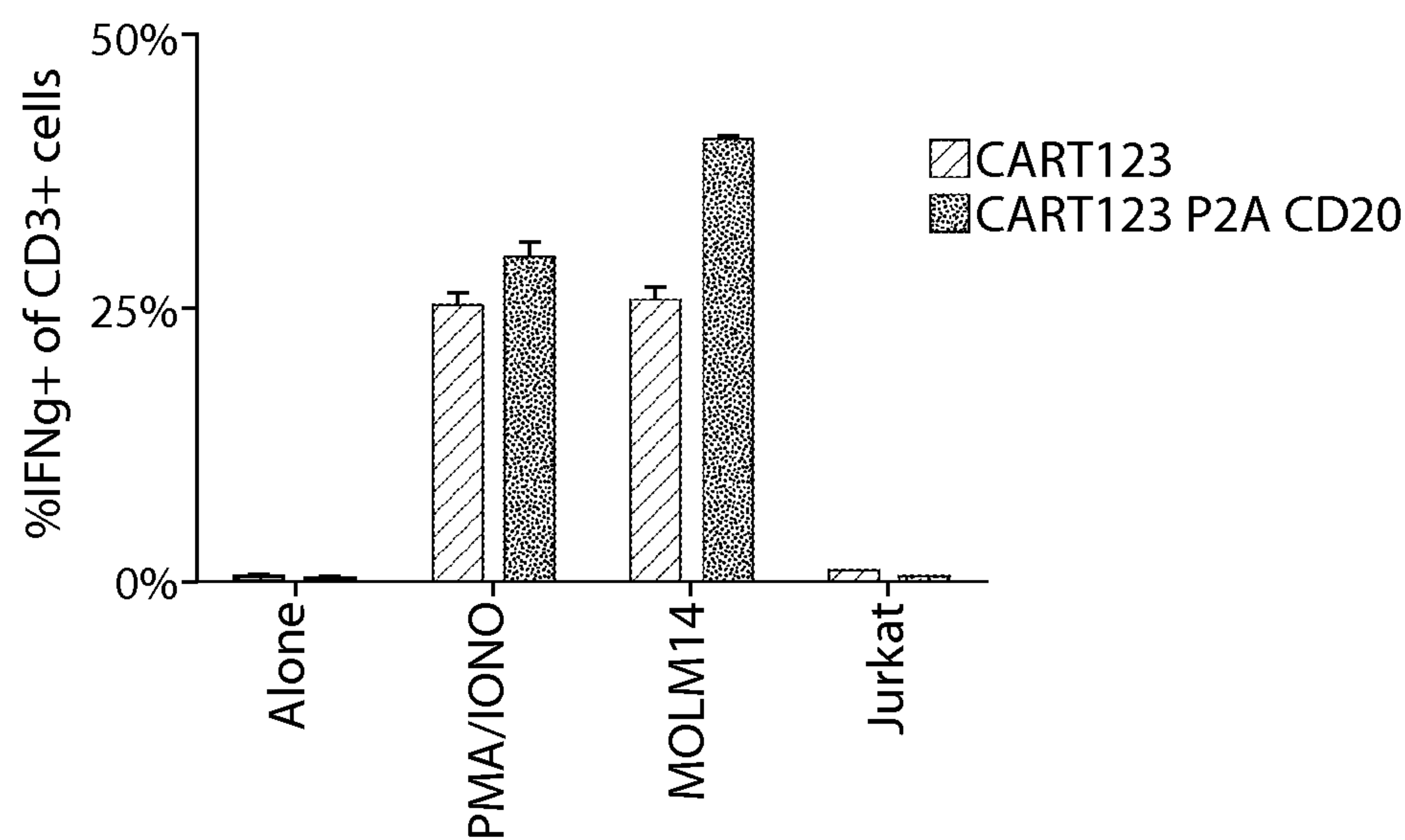


FIG. 59C

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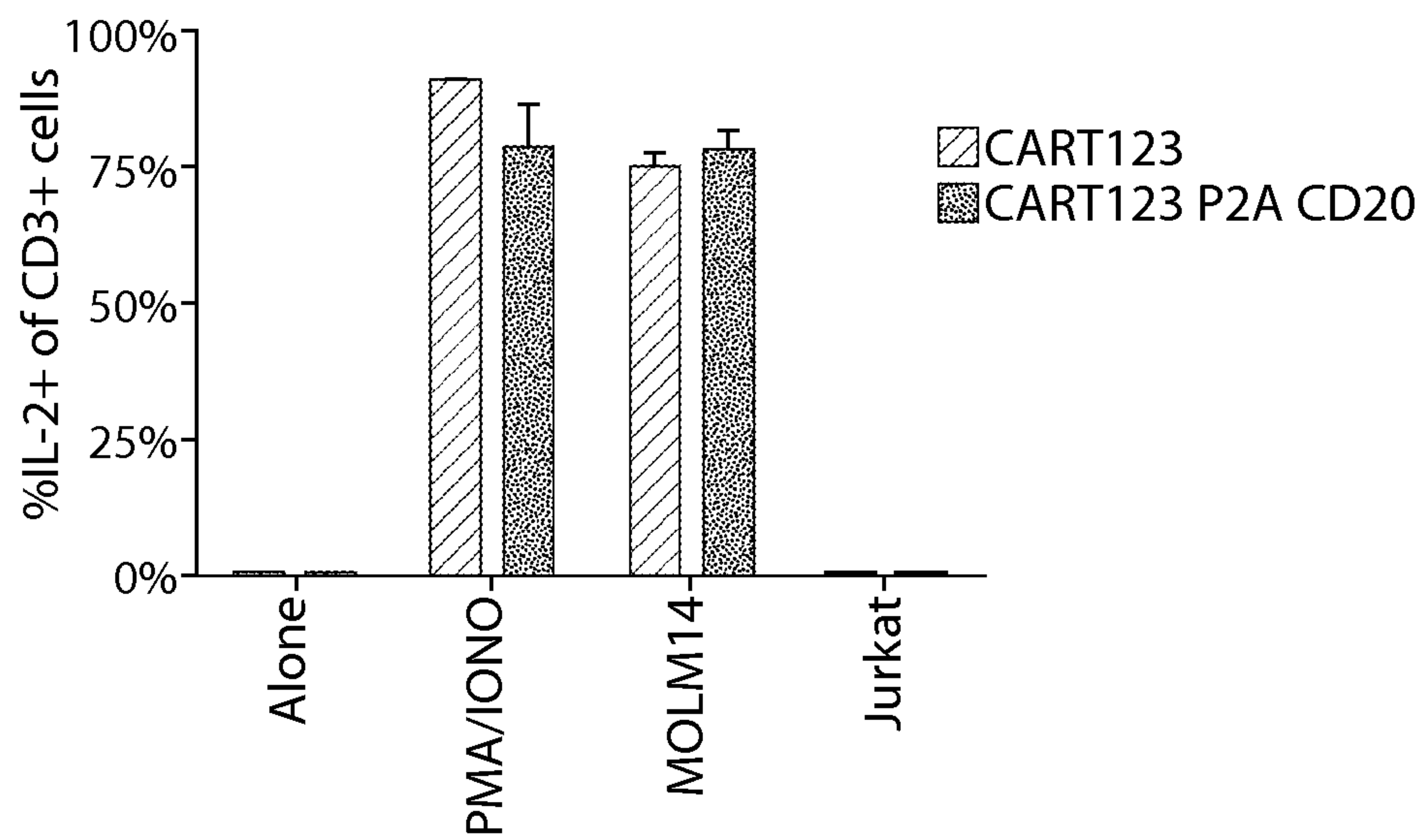


FIG. 59D

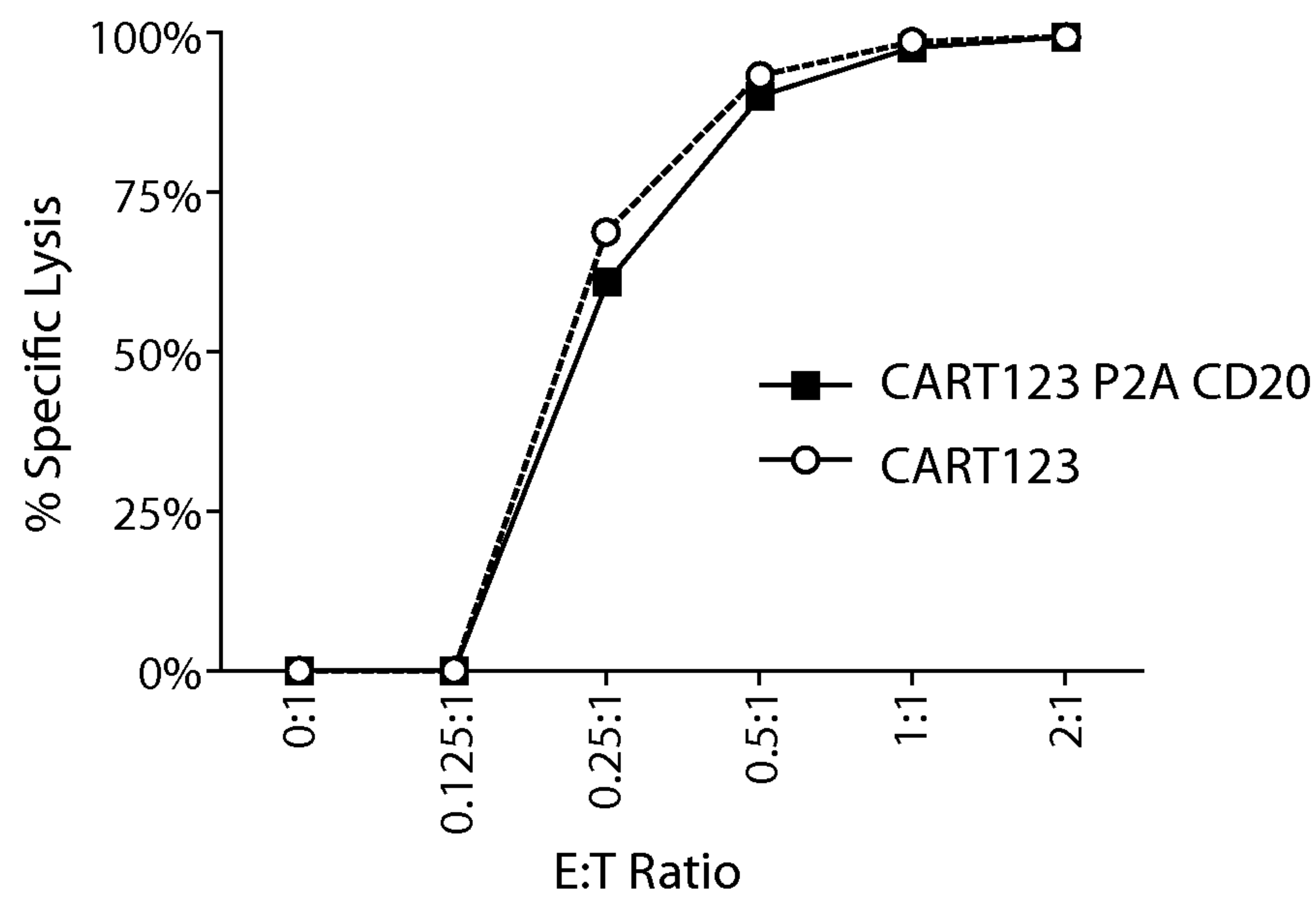


FIG. 60

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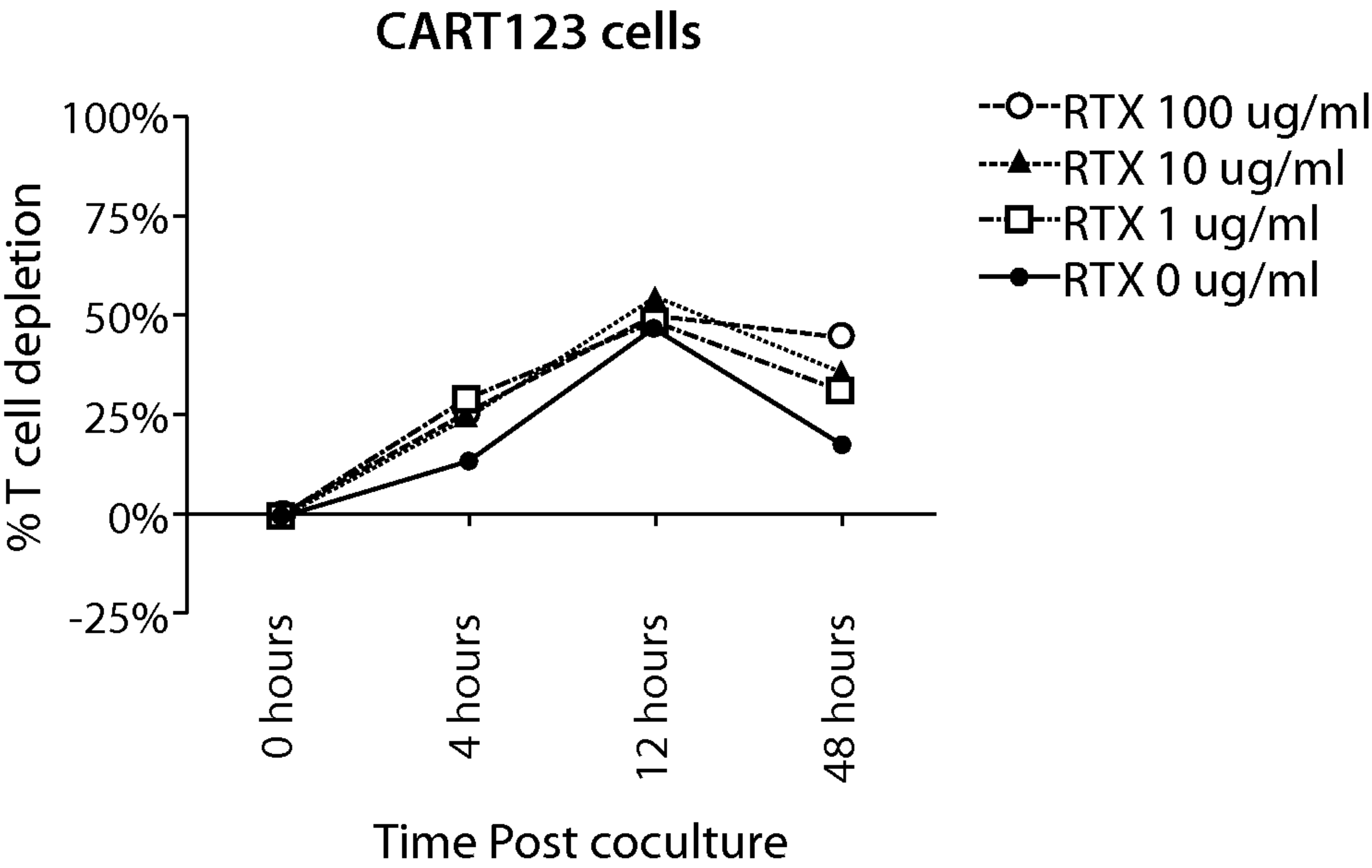


FIG. 61A

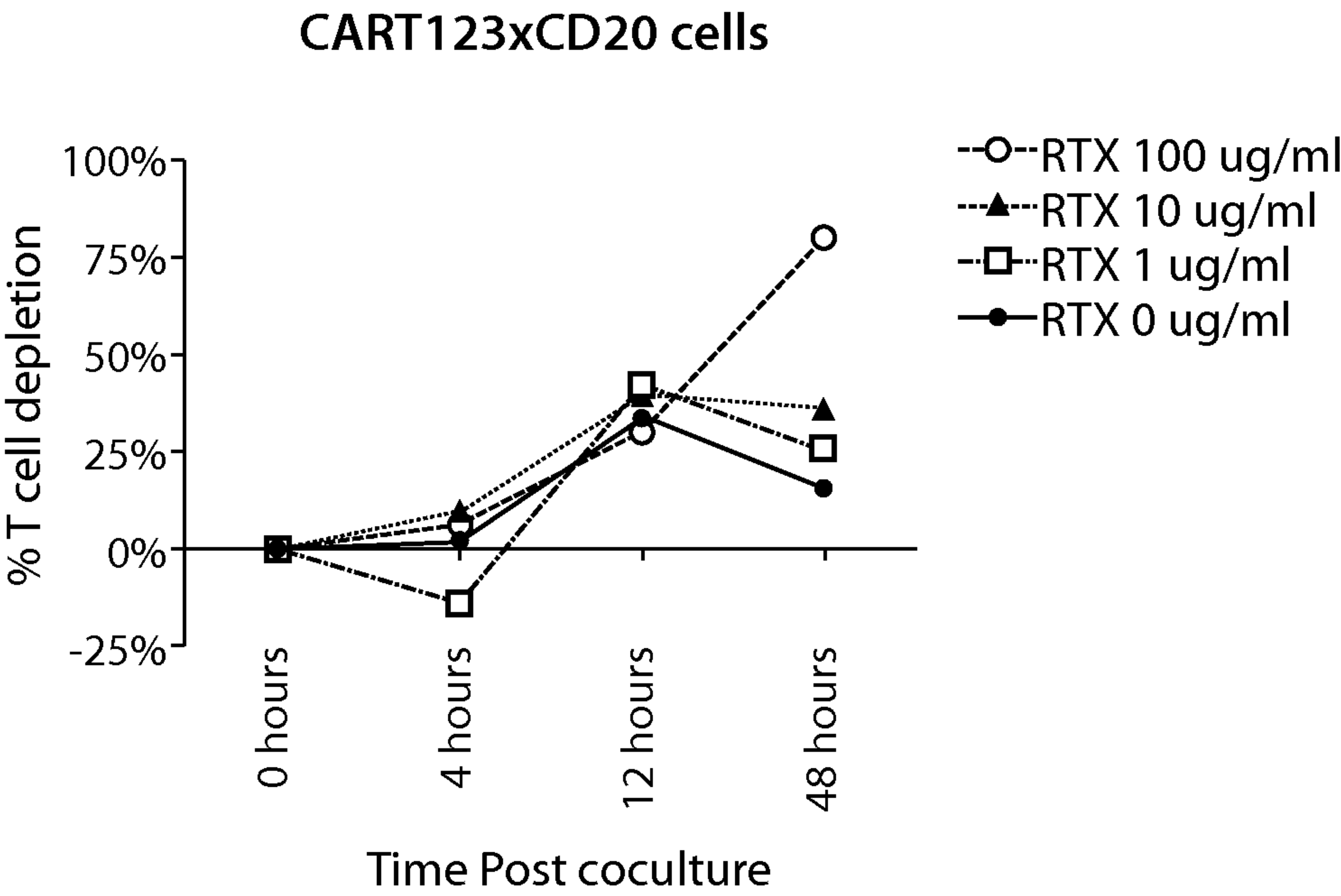


FIG. 61B

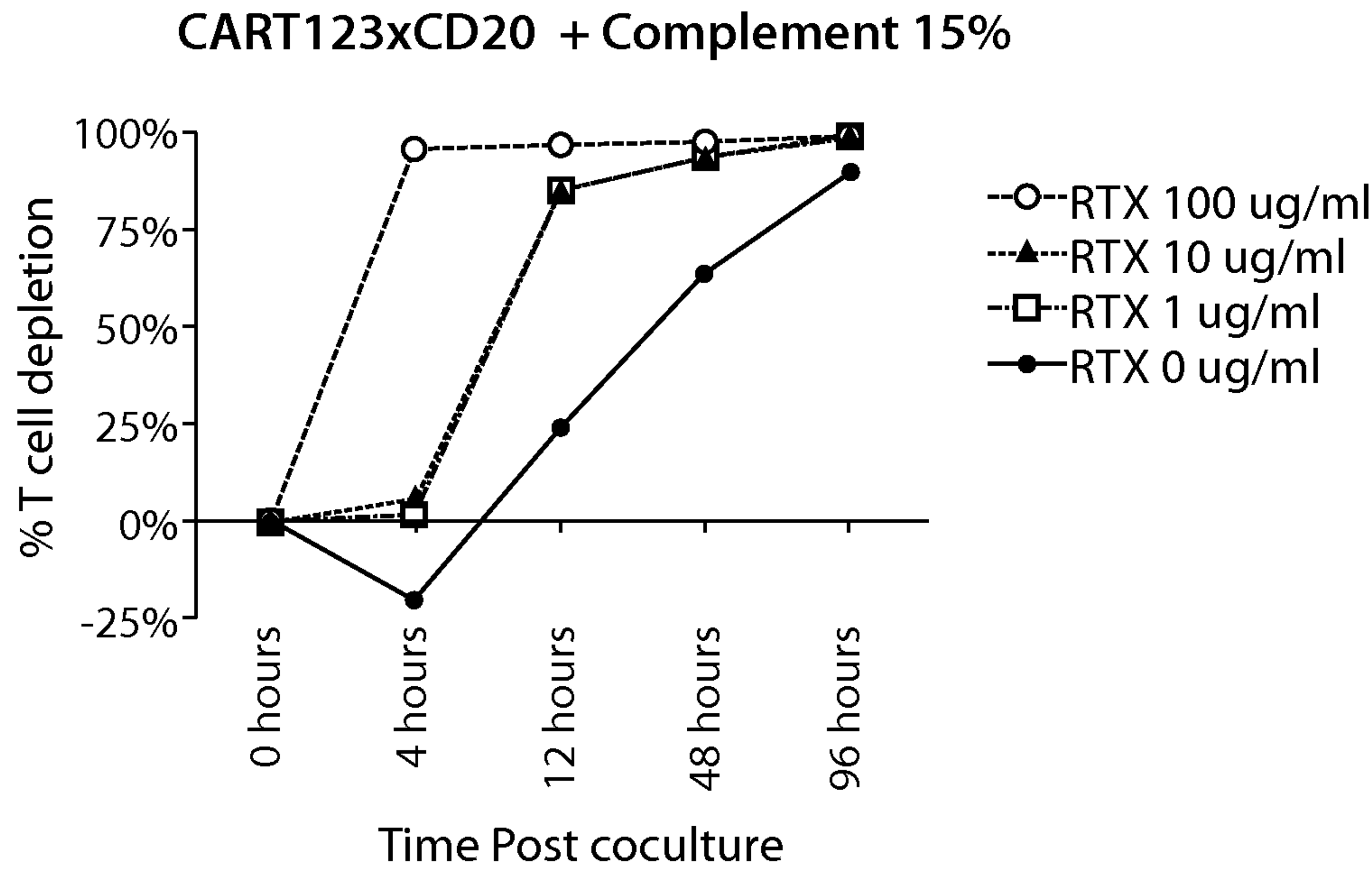


FIG. 62

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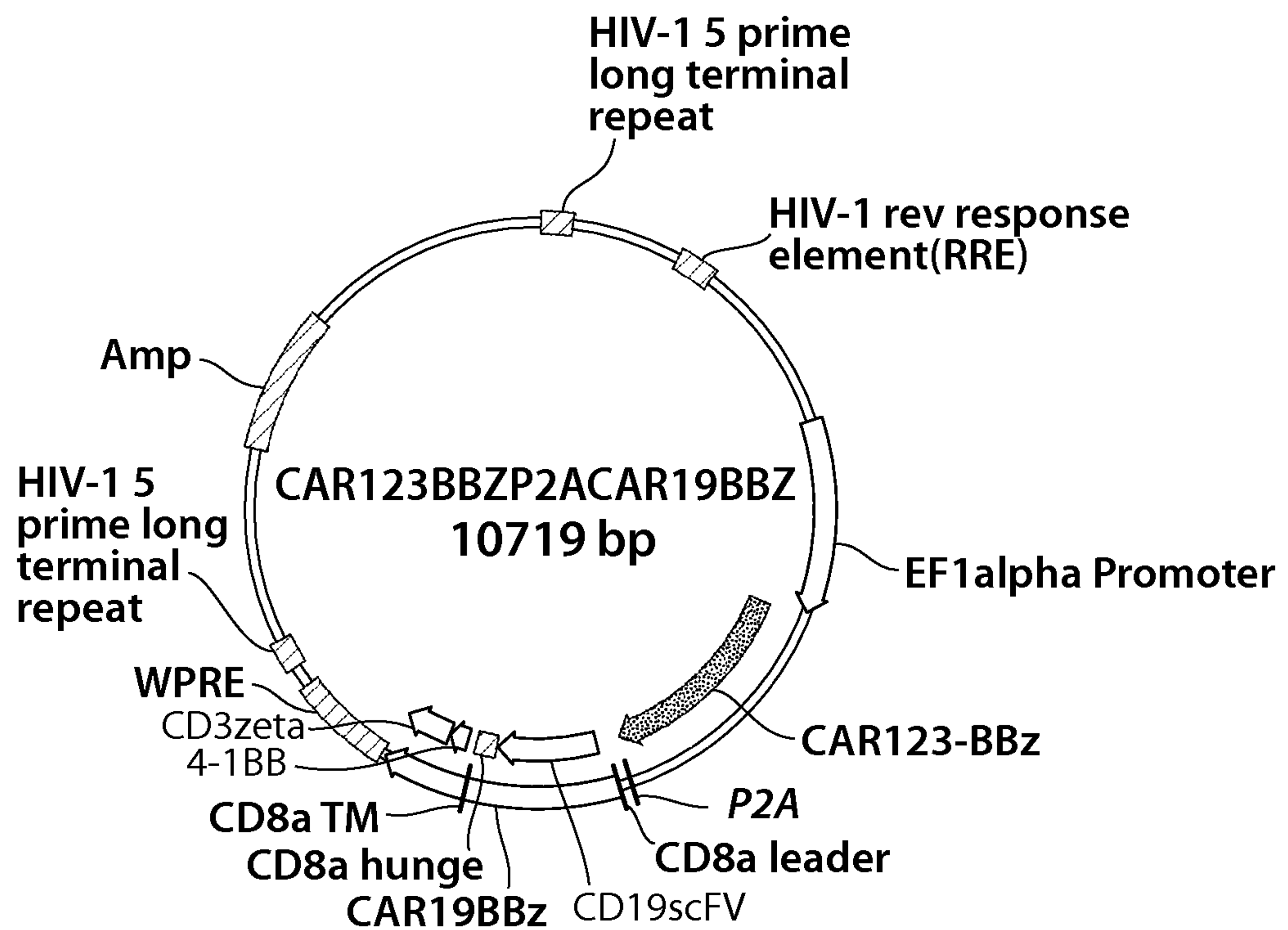


FIG. 63A

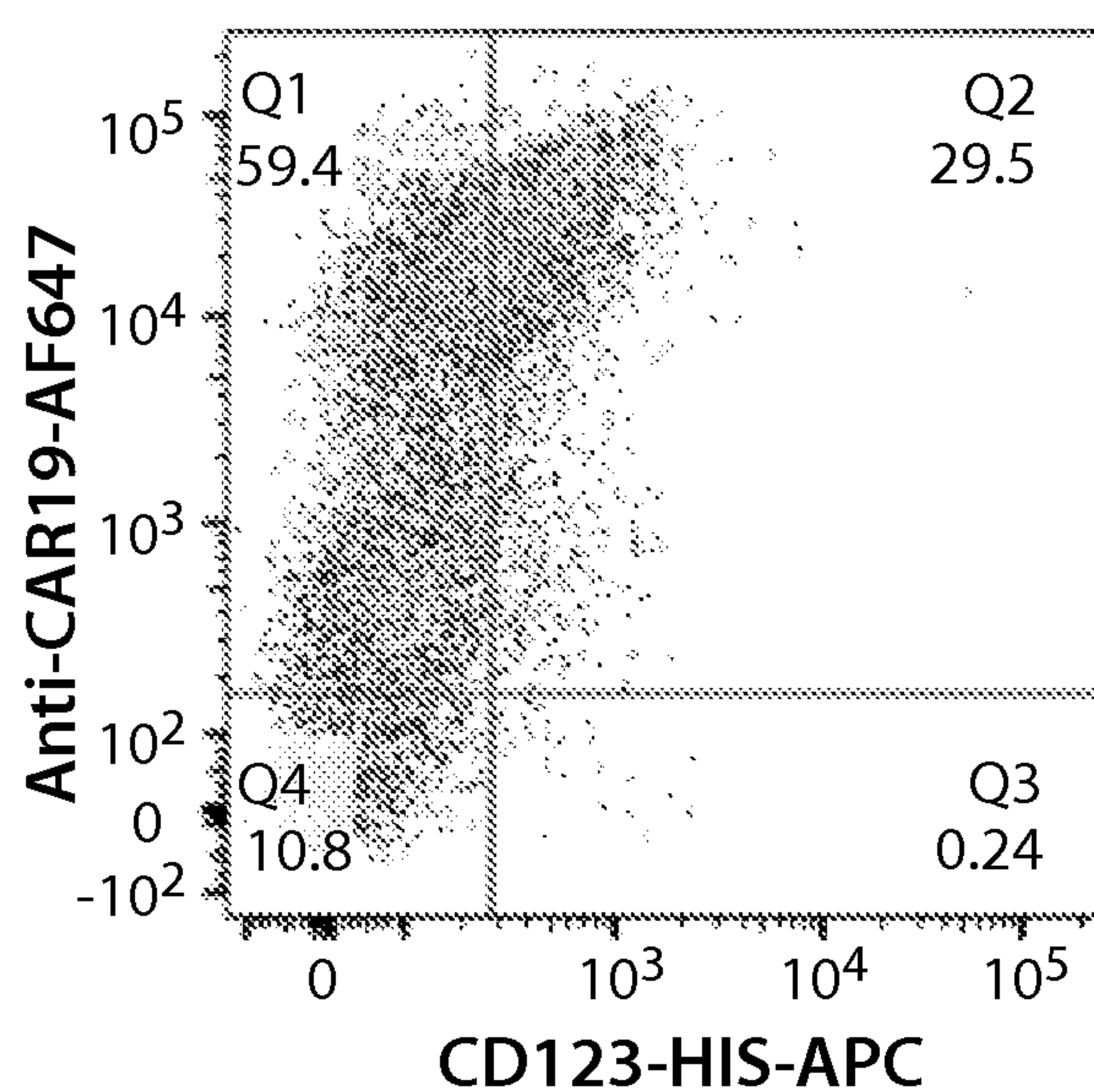


FIG. 63B

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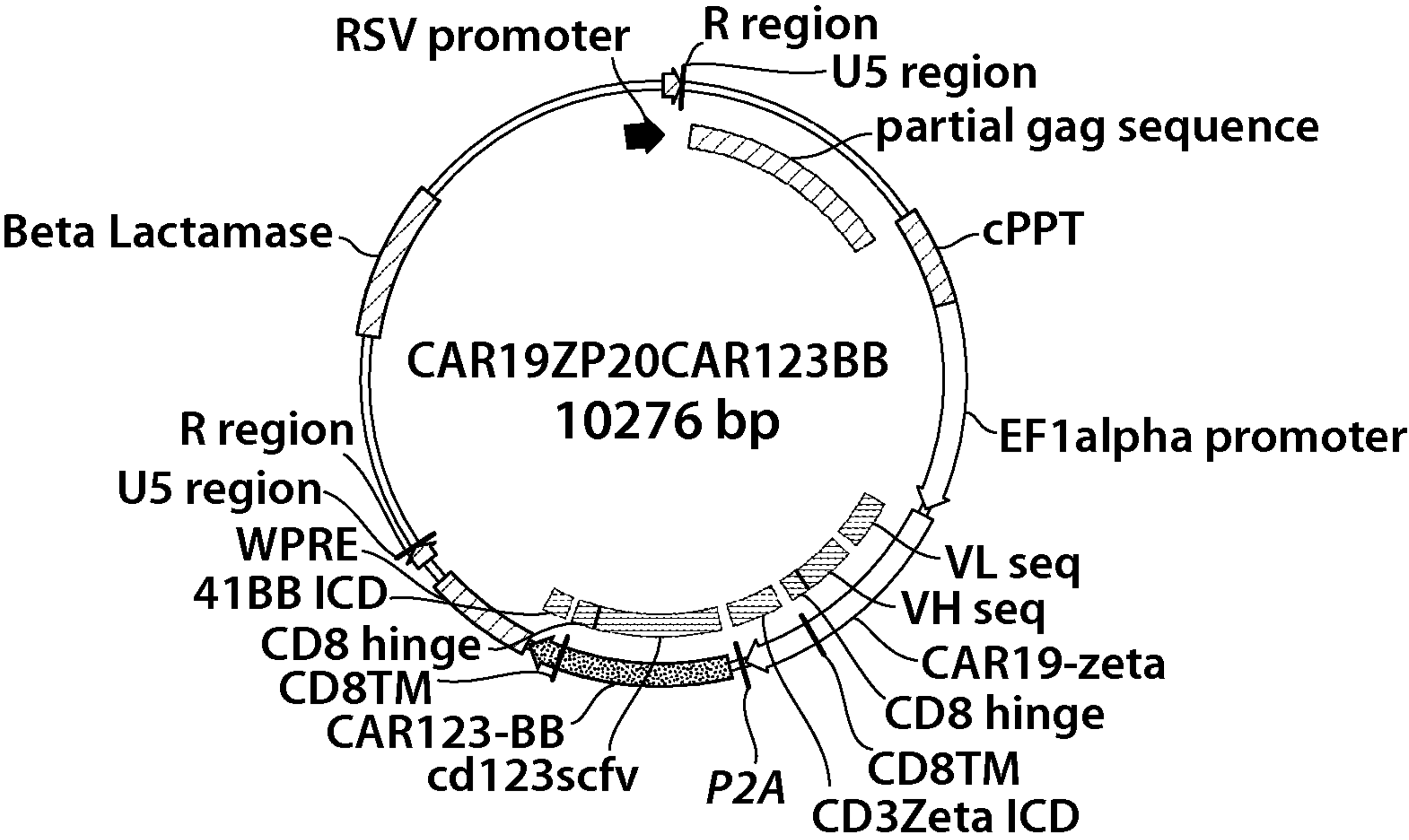


FIG. 64A

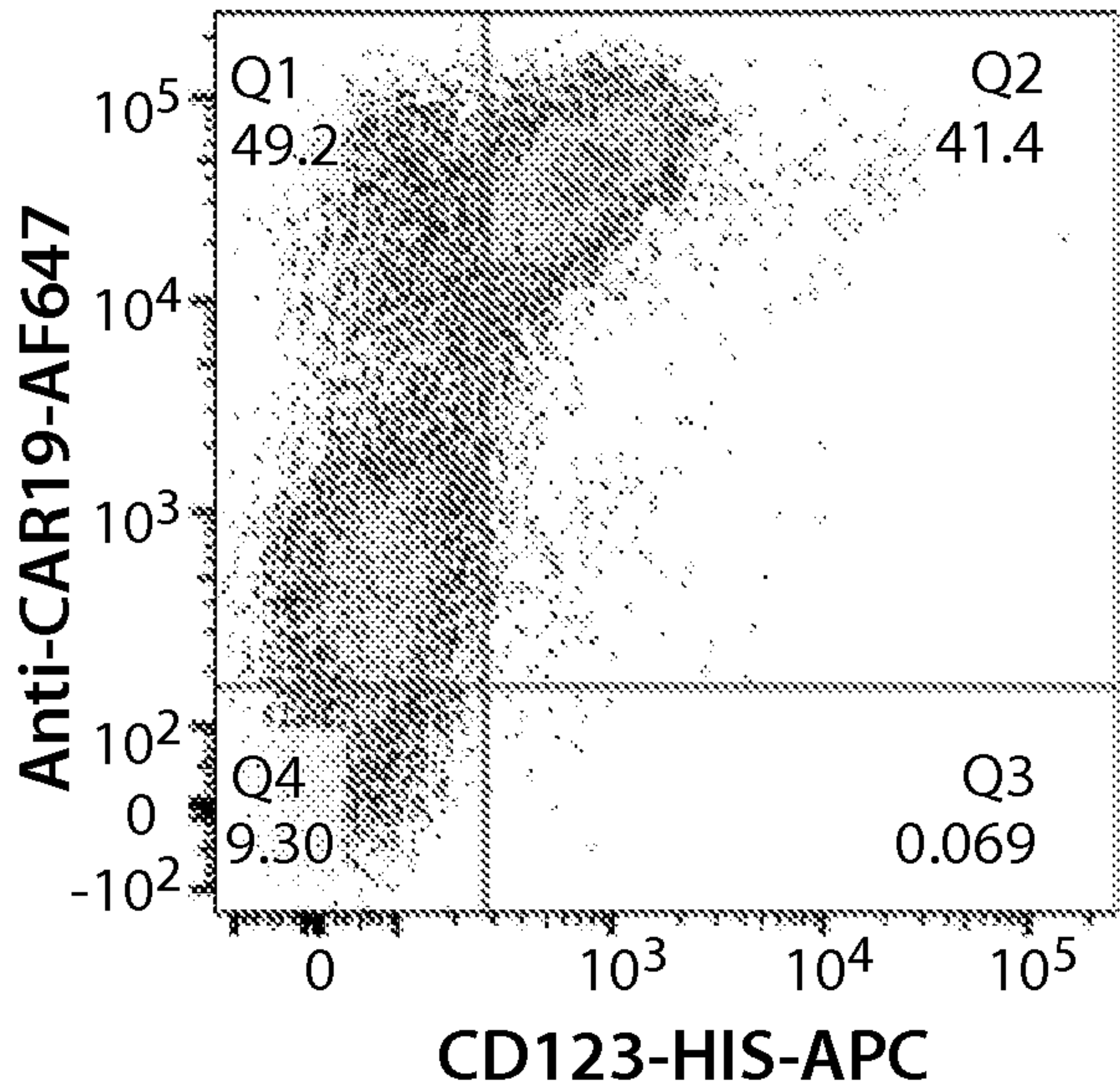


FIG. 64B

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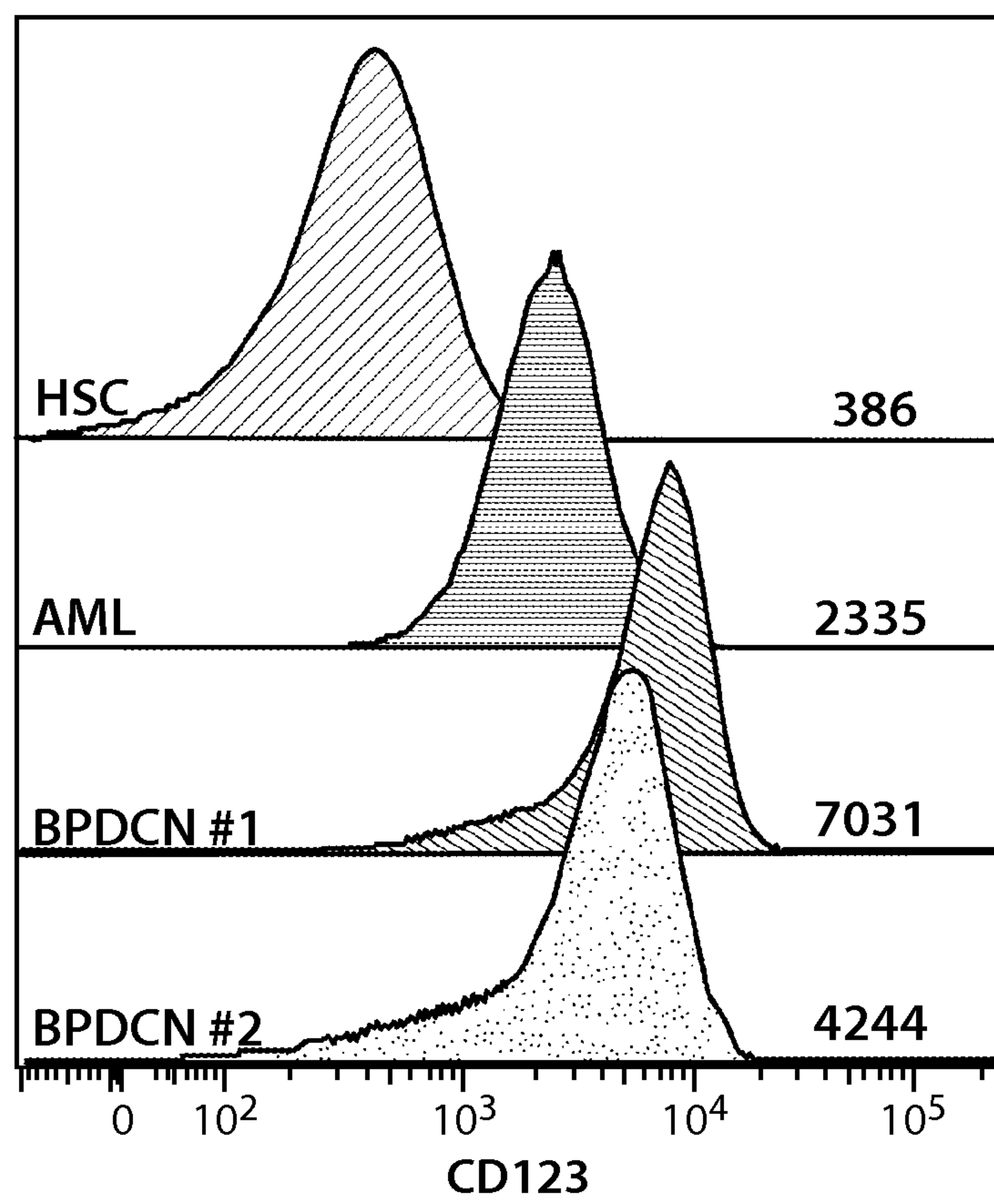


FIG. 65

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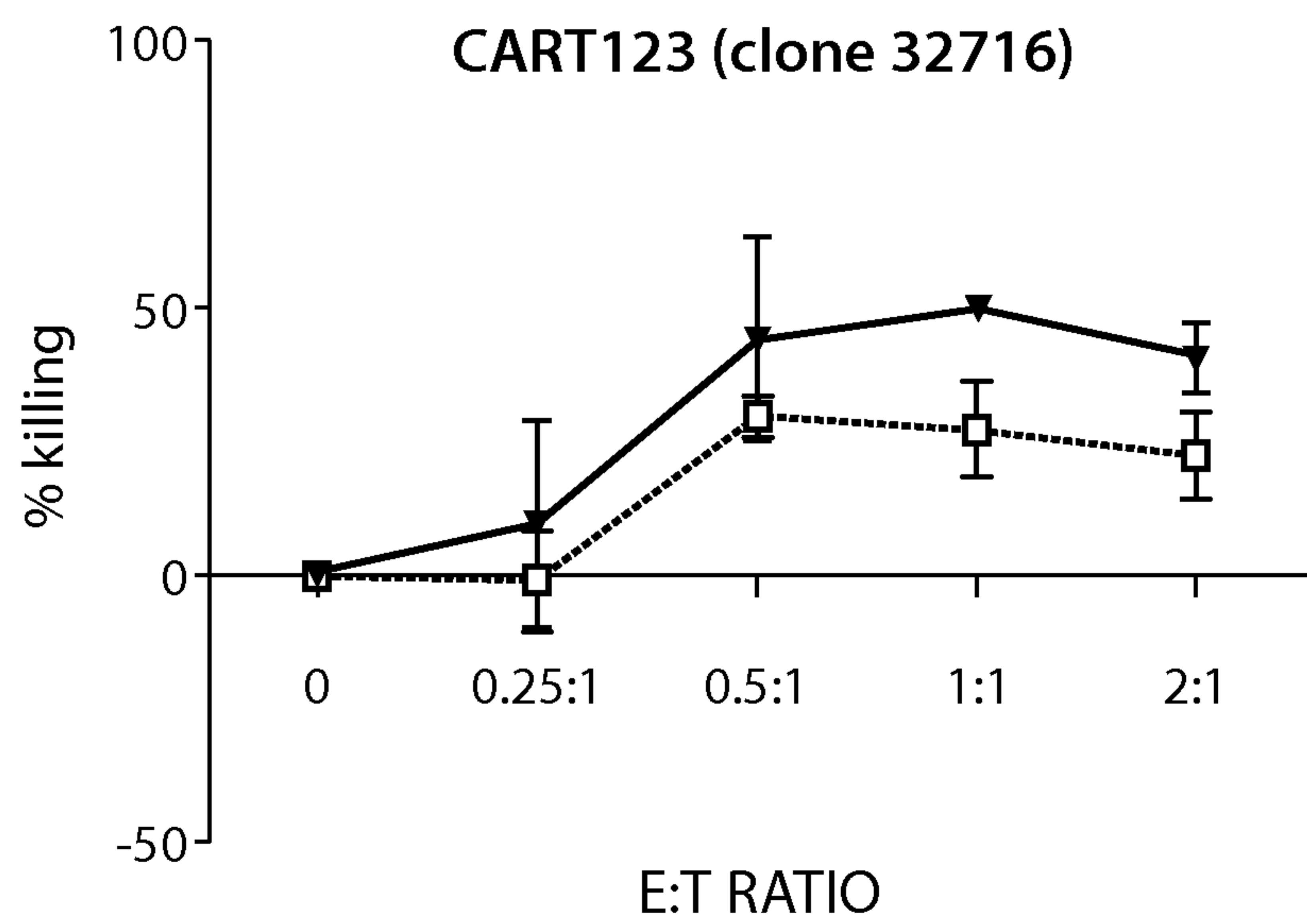


FIG. 66A

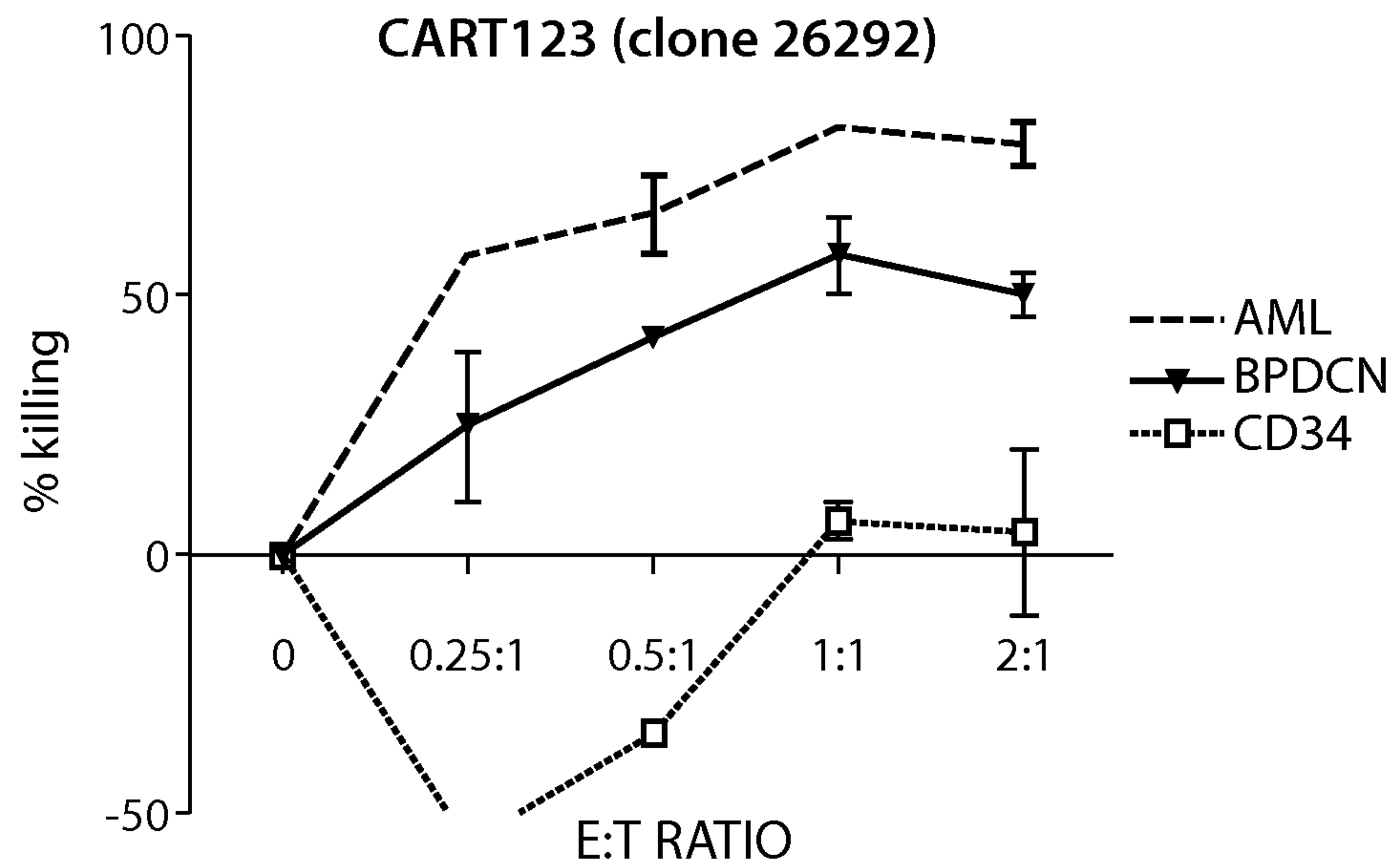


FIG. 66B

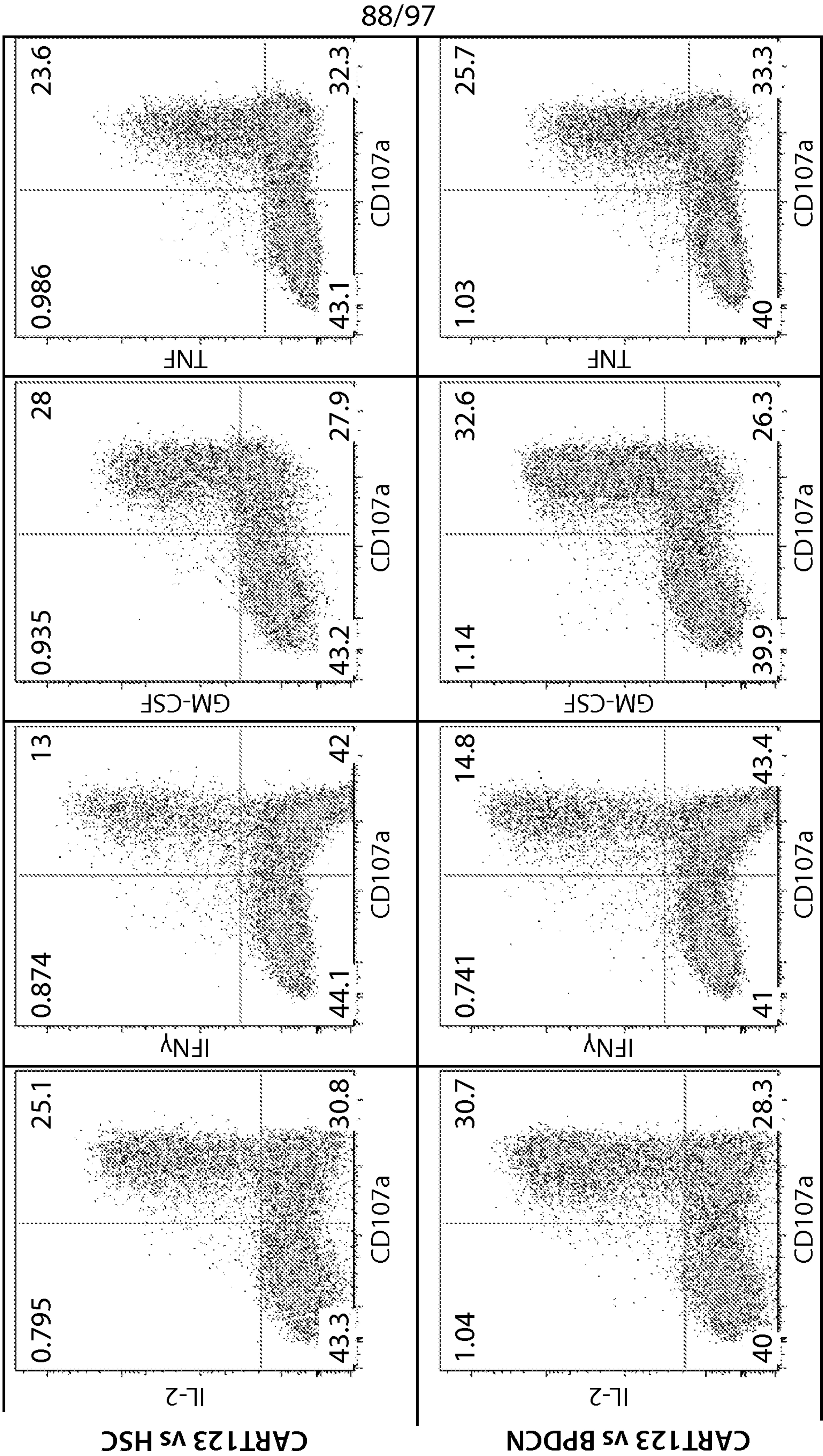


FIG. 67

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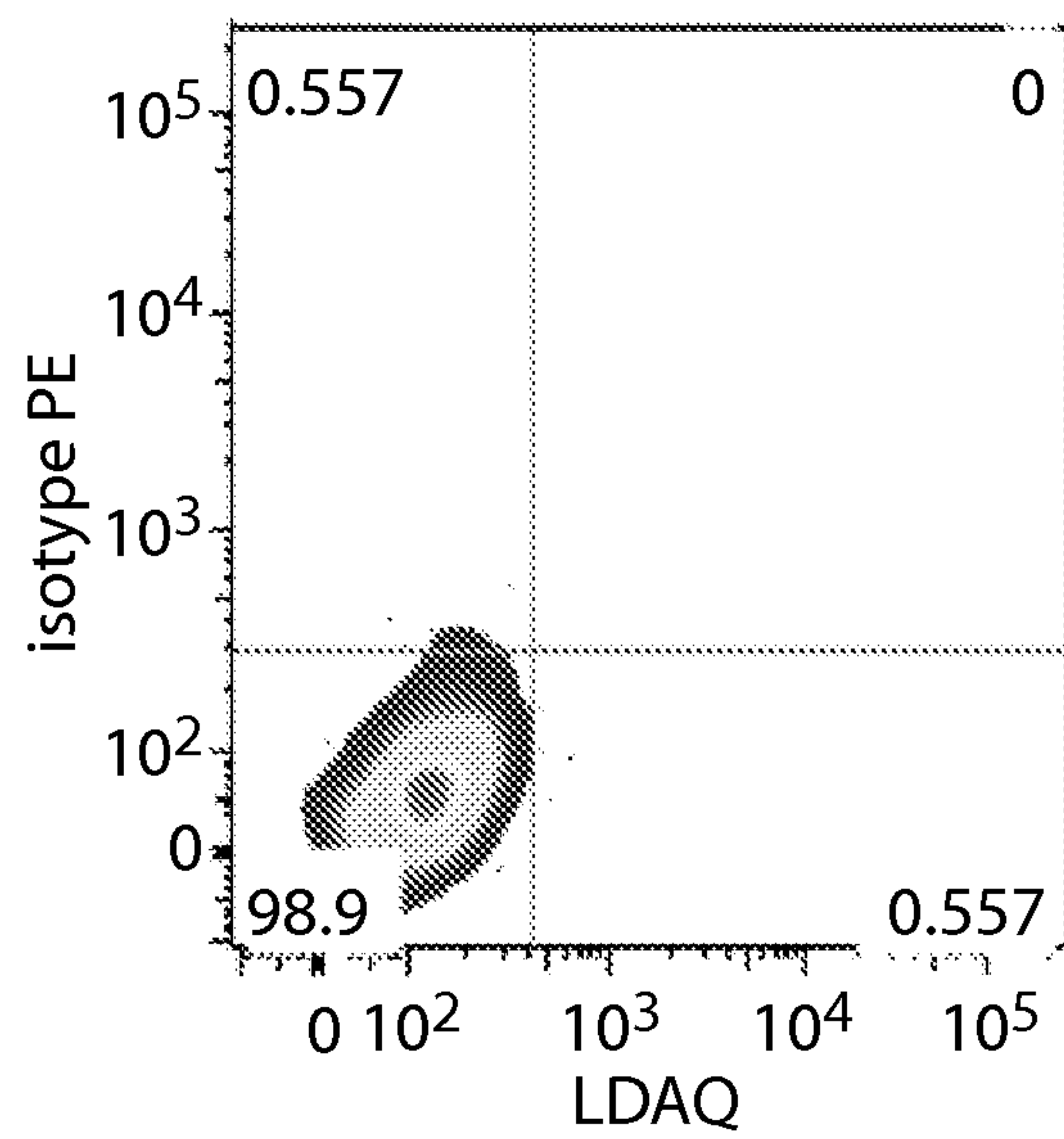


FIG. 68A

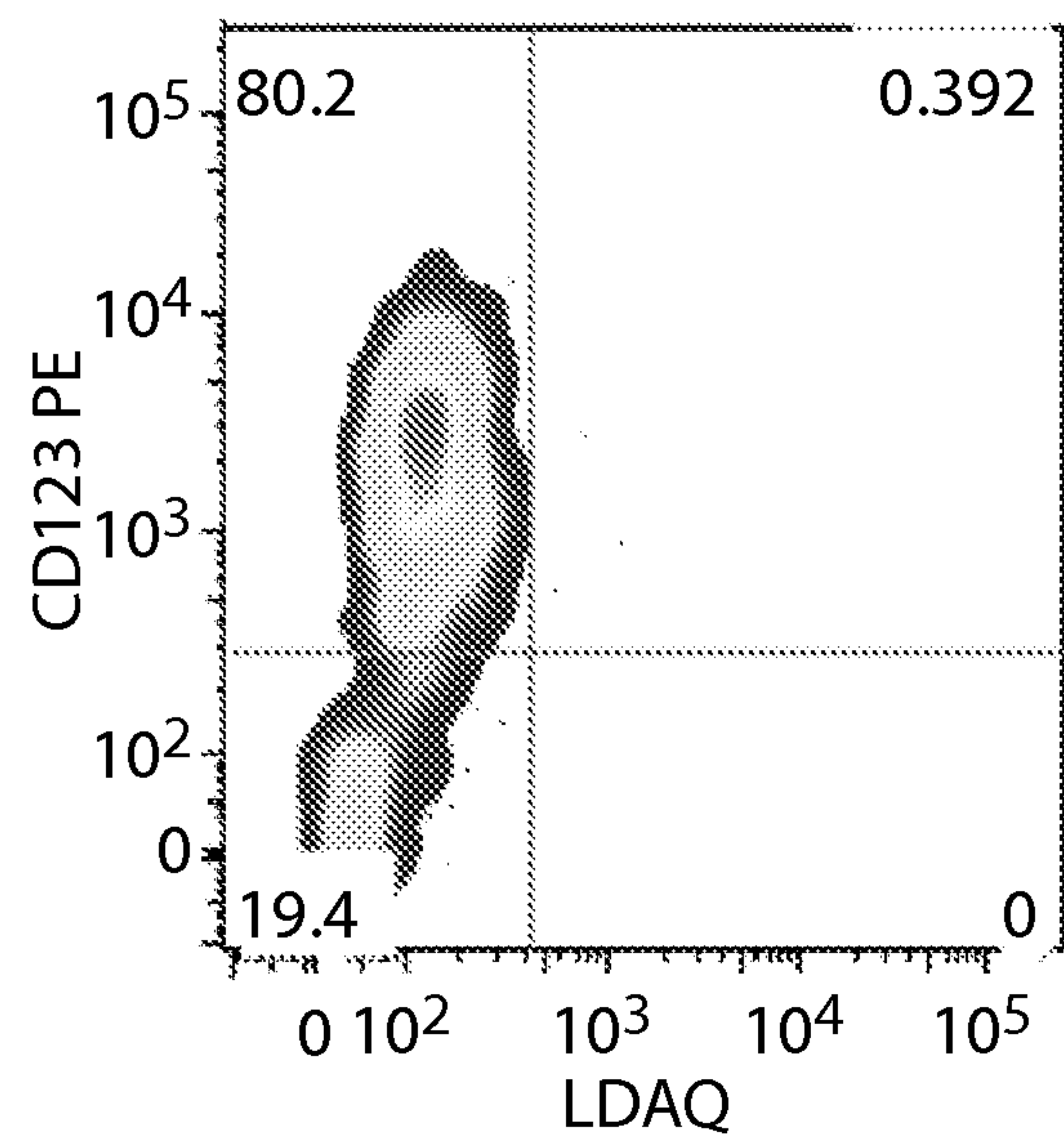


FIG. 68B

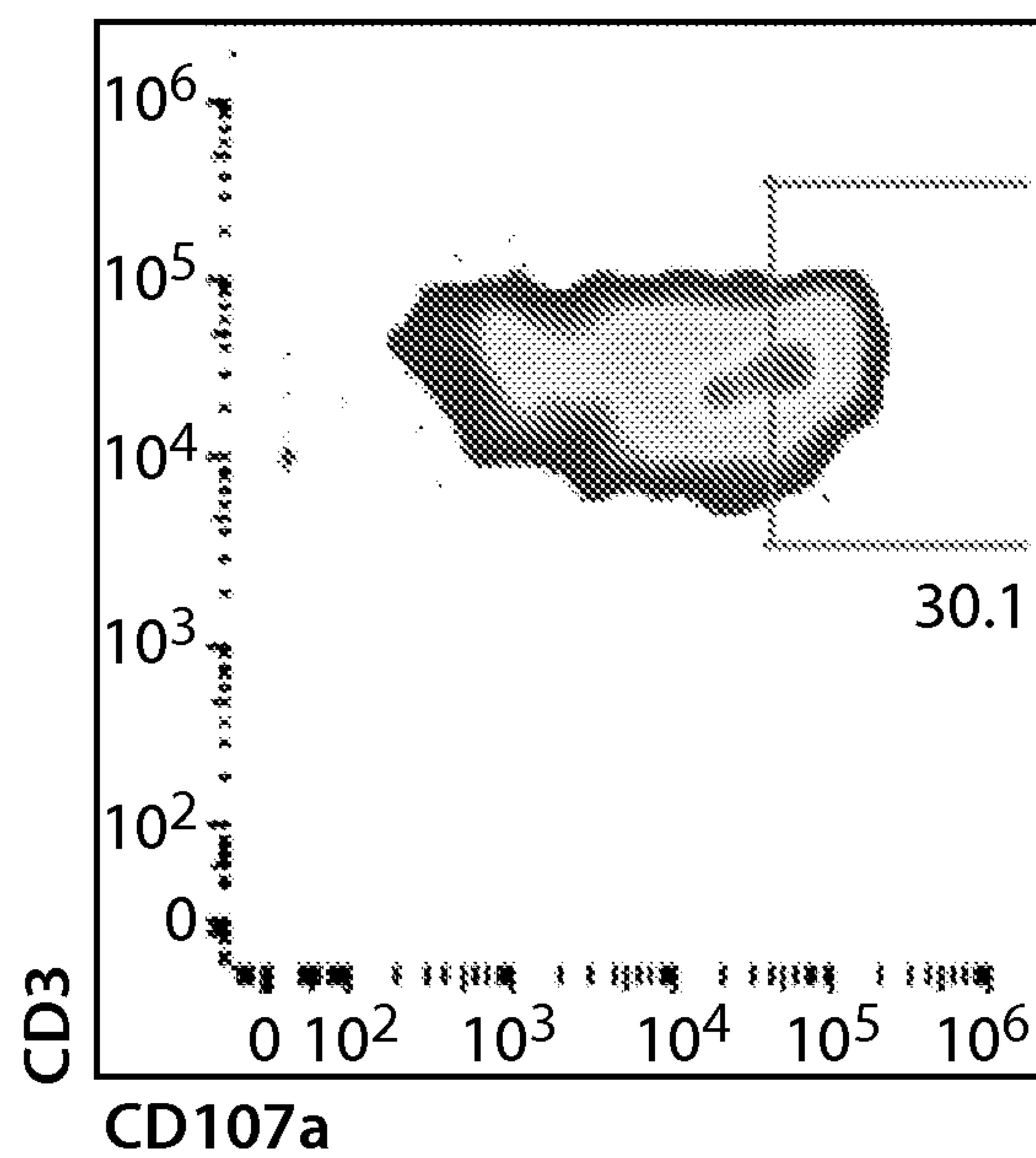


FIG. 69A

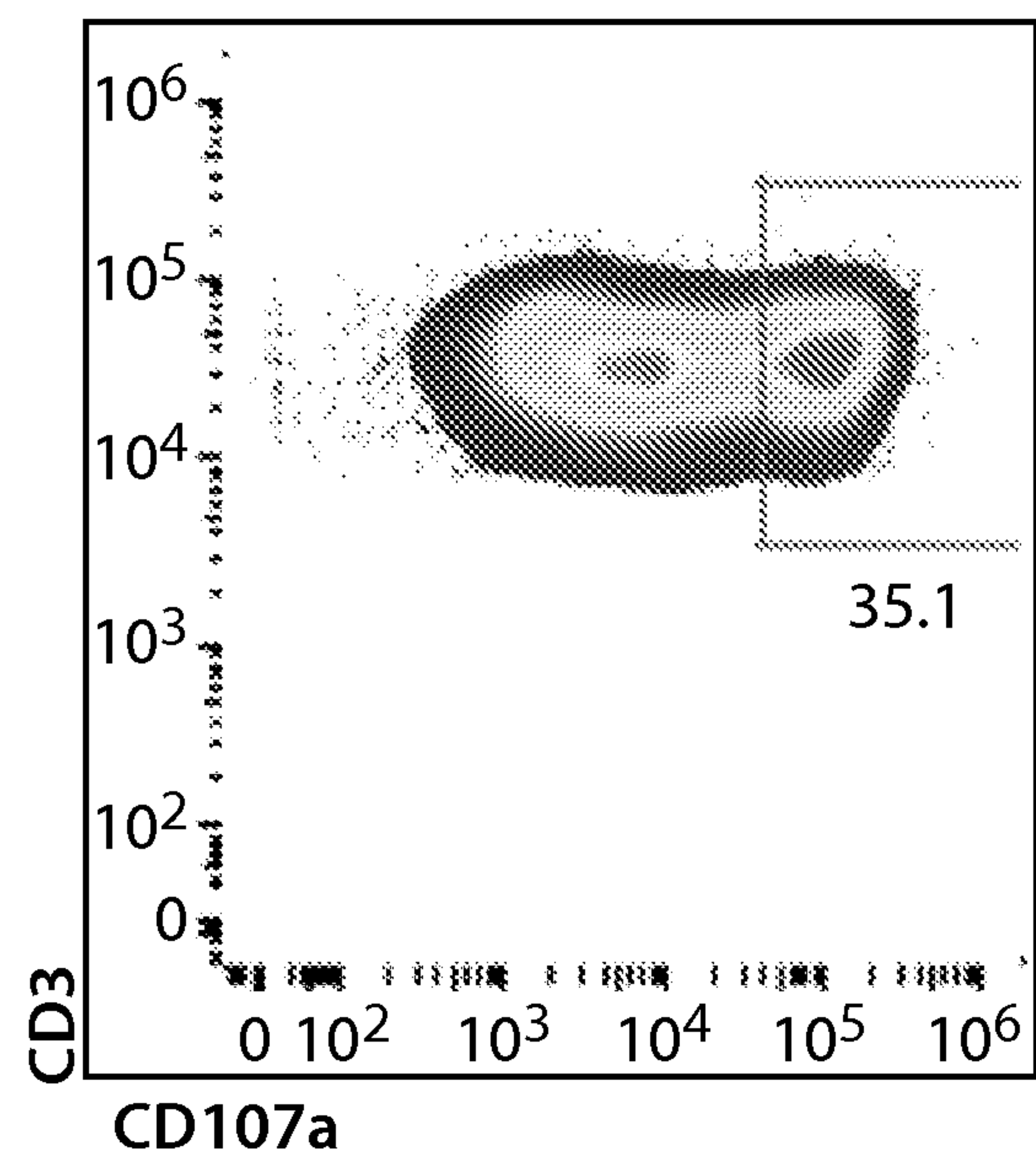


FIG. 69B

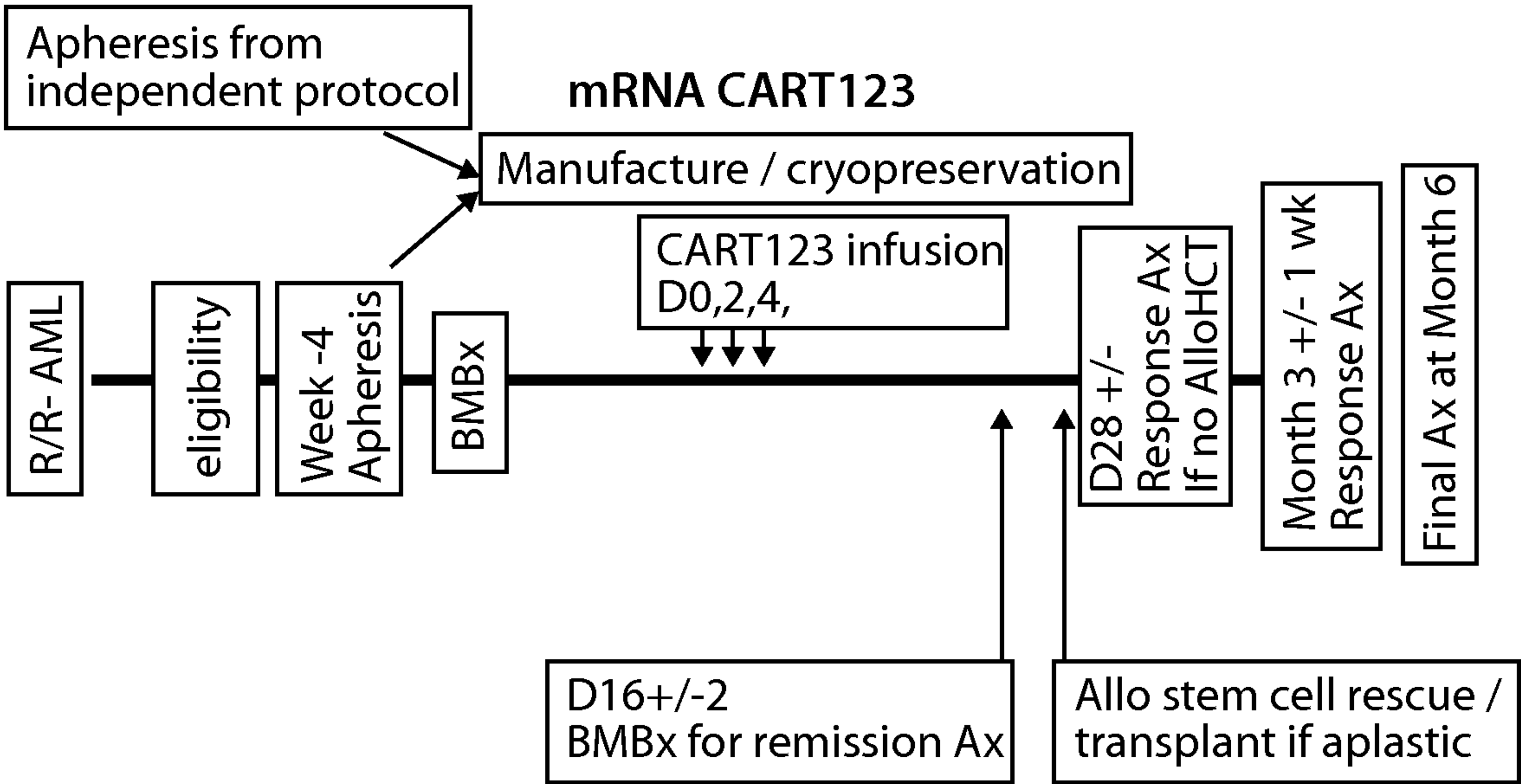


FIG. 70

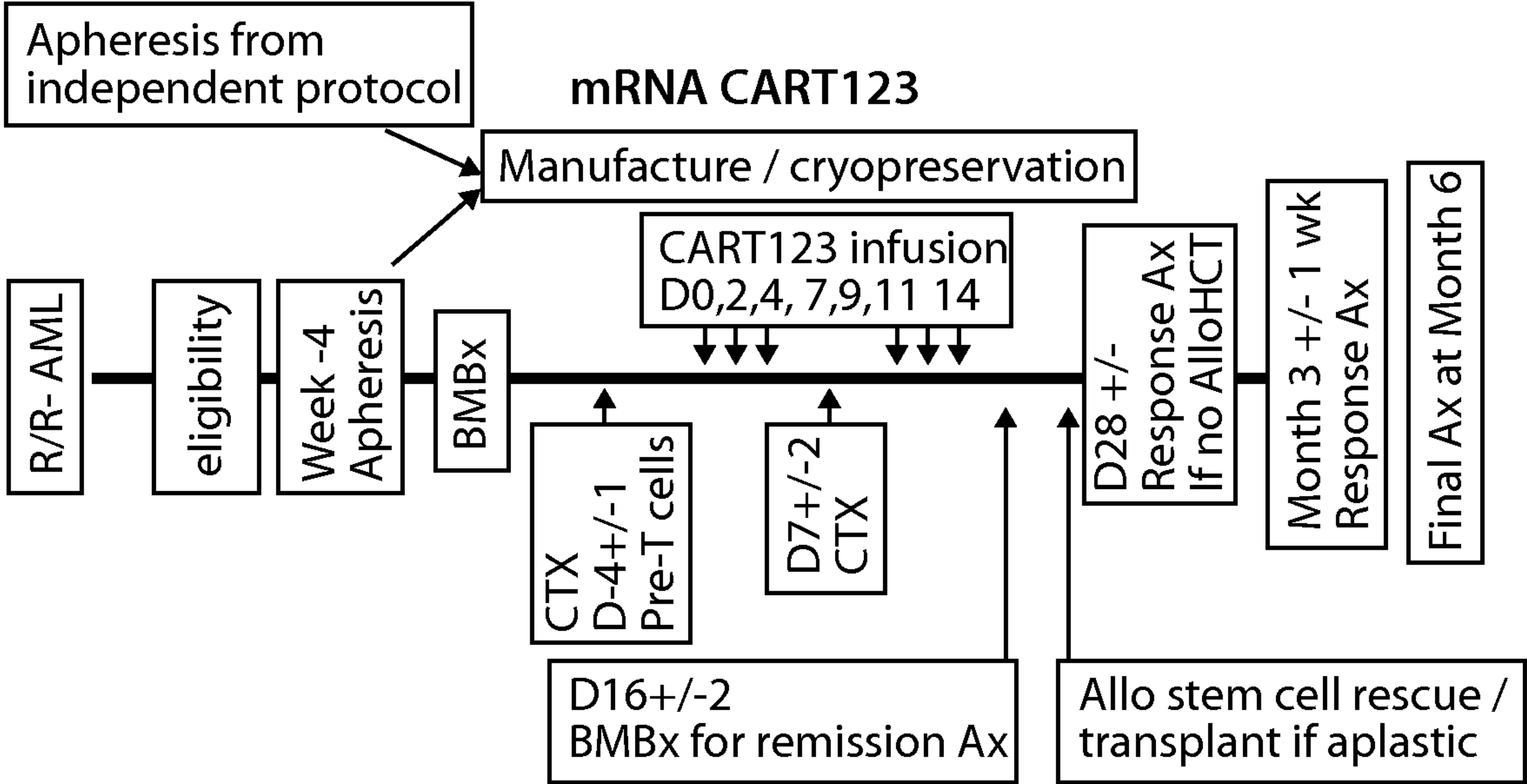


FIG. 71

FIG. 72A

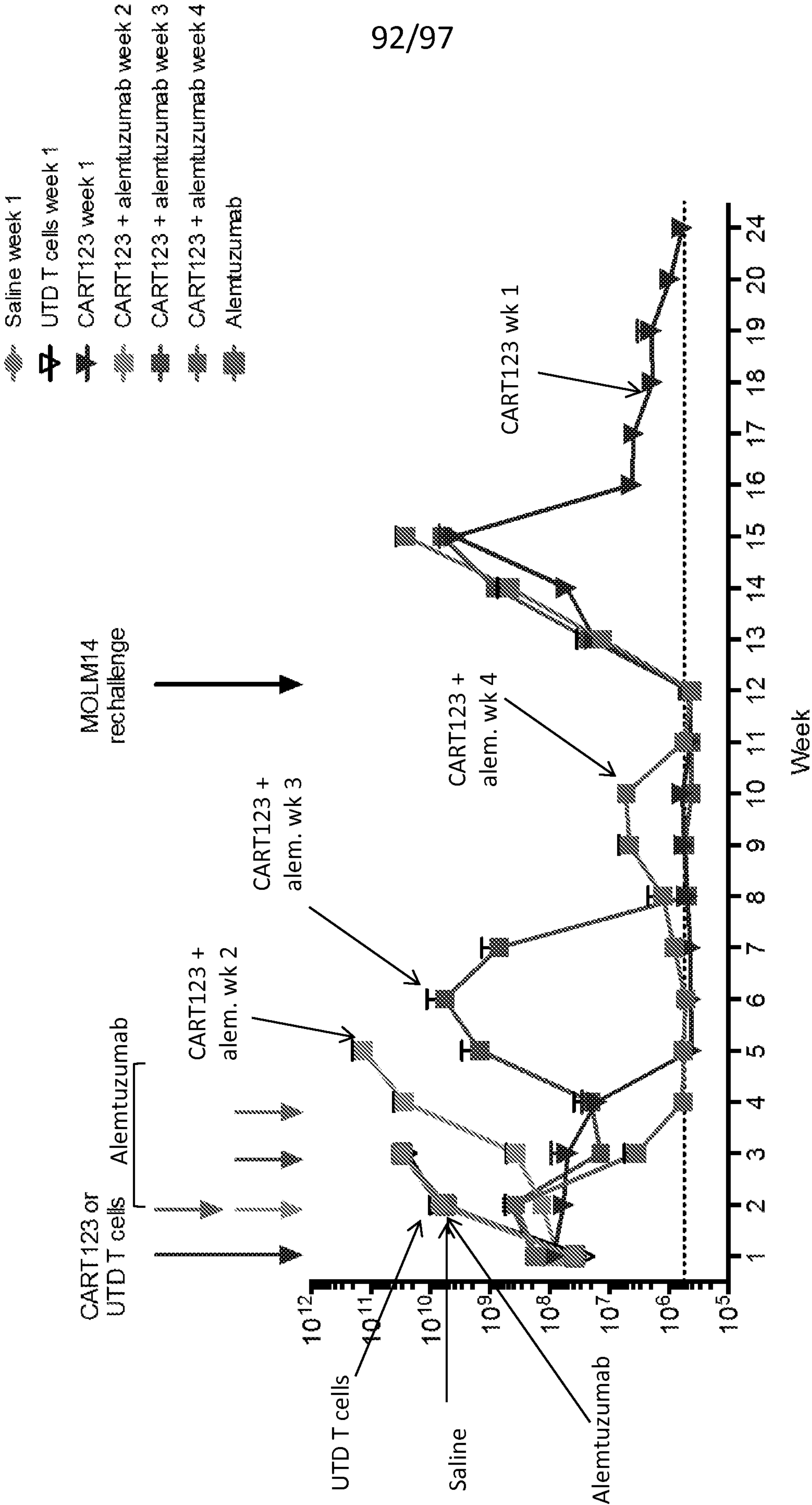


FIG. 72B

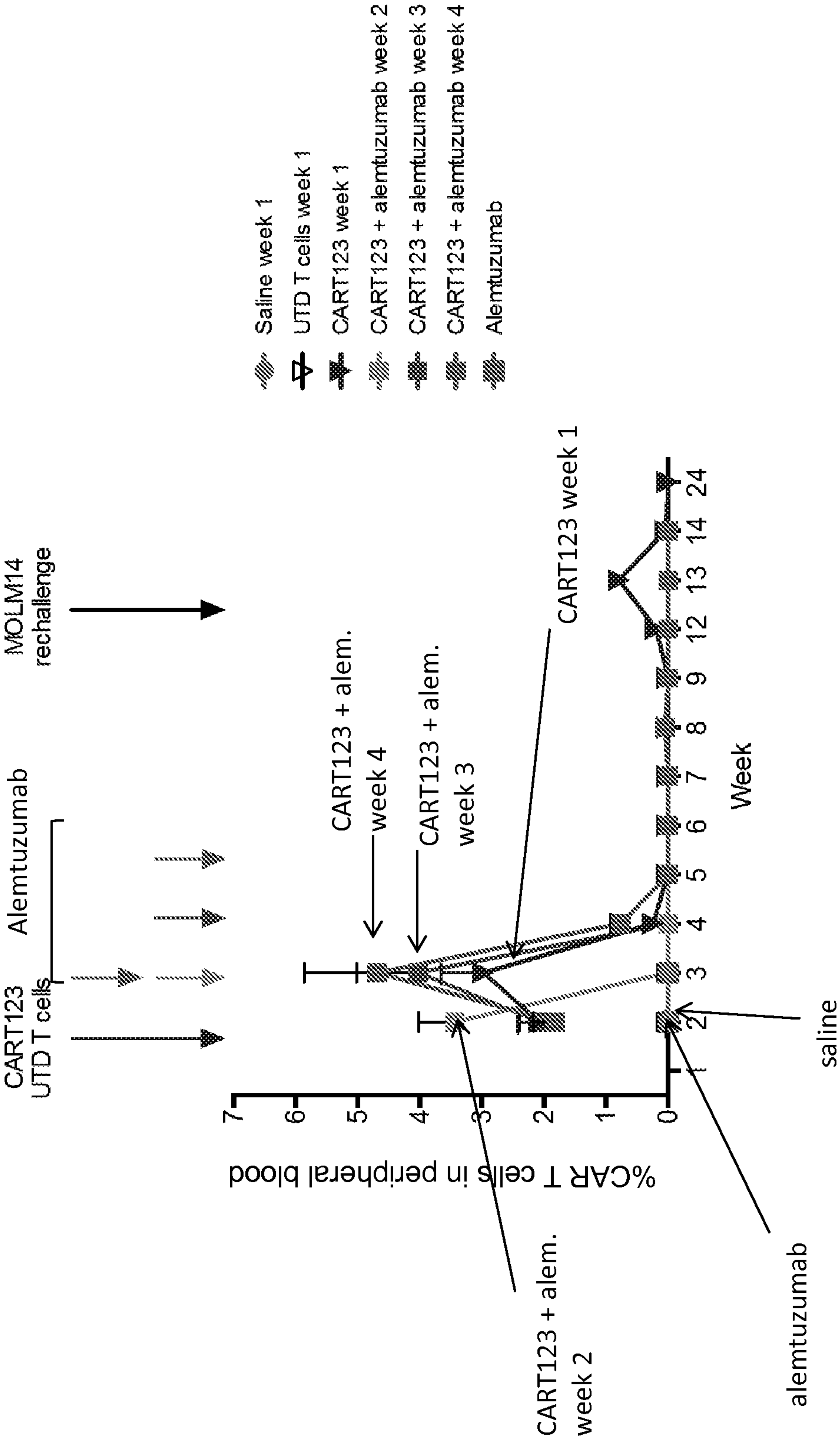


FIG. 72C

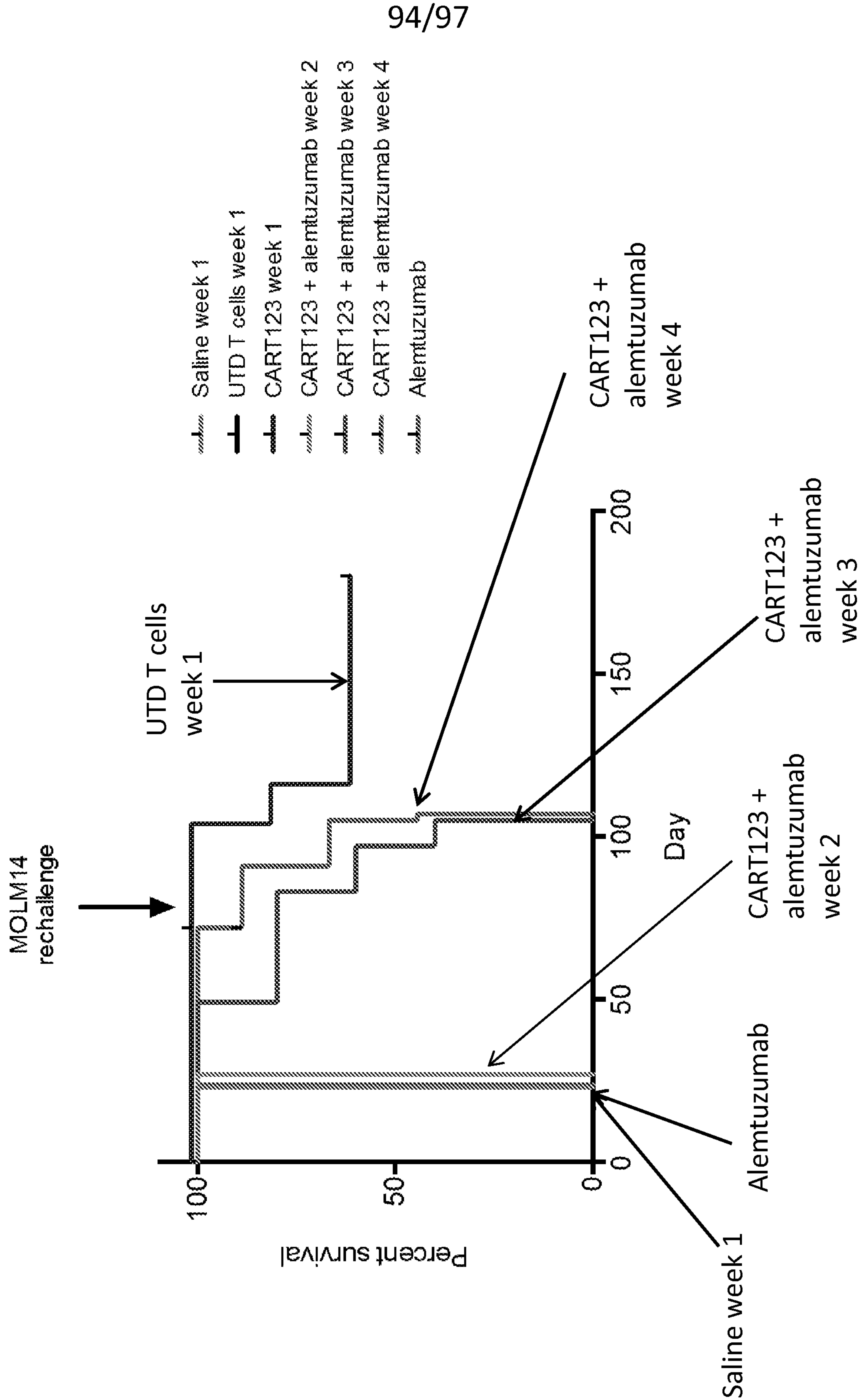


FIG. 73A

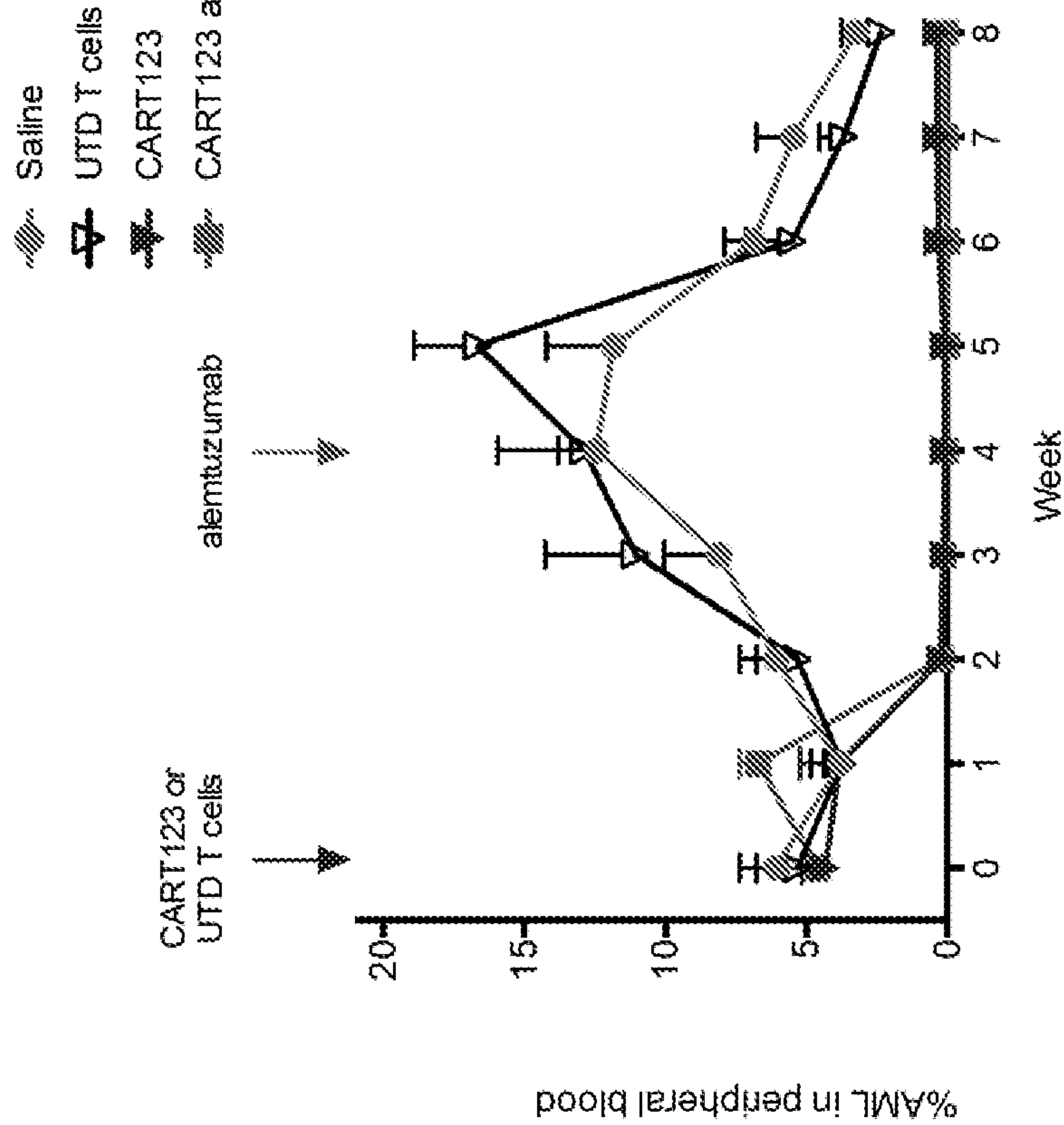
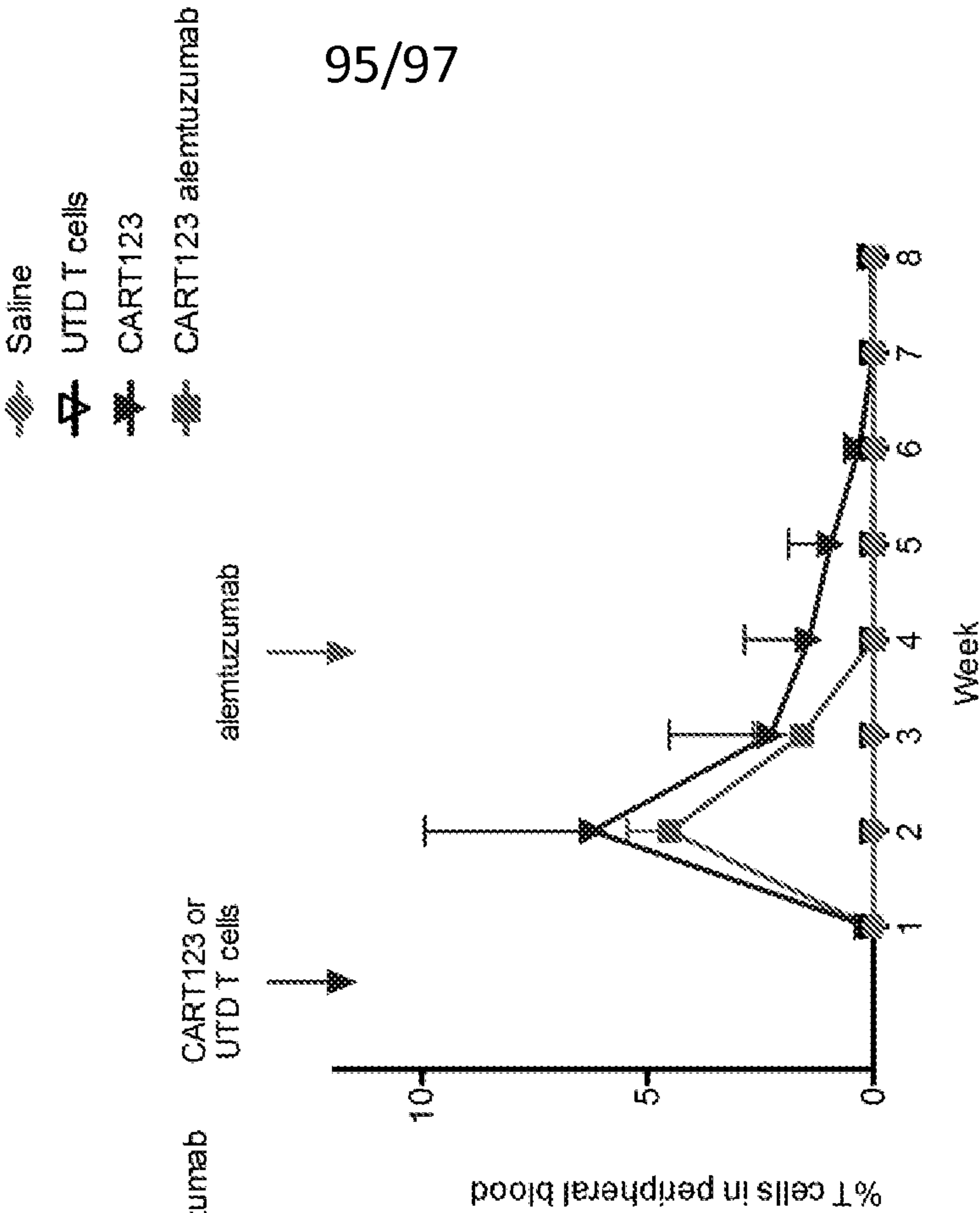


FIG. 73B



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FIG. 74A

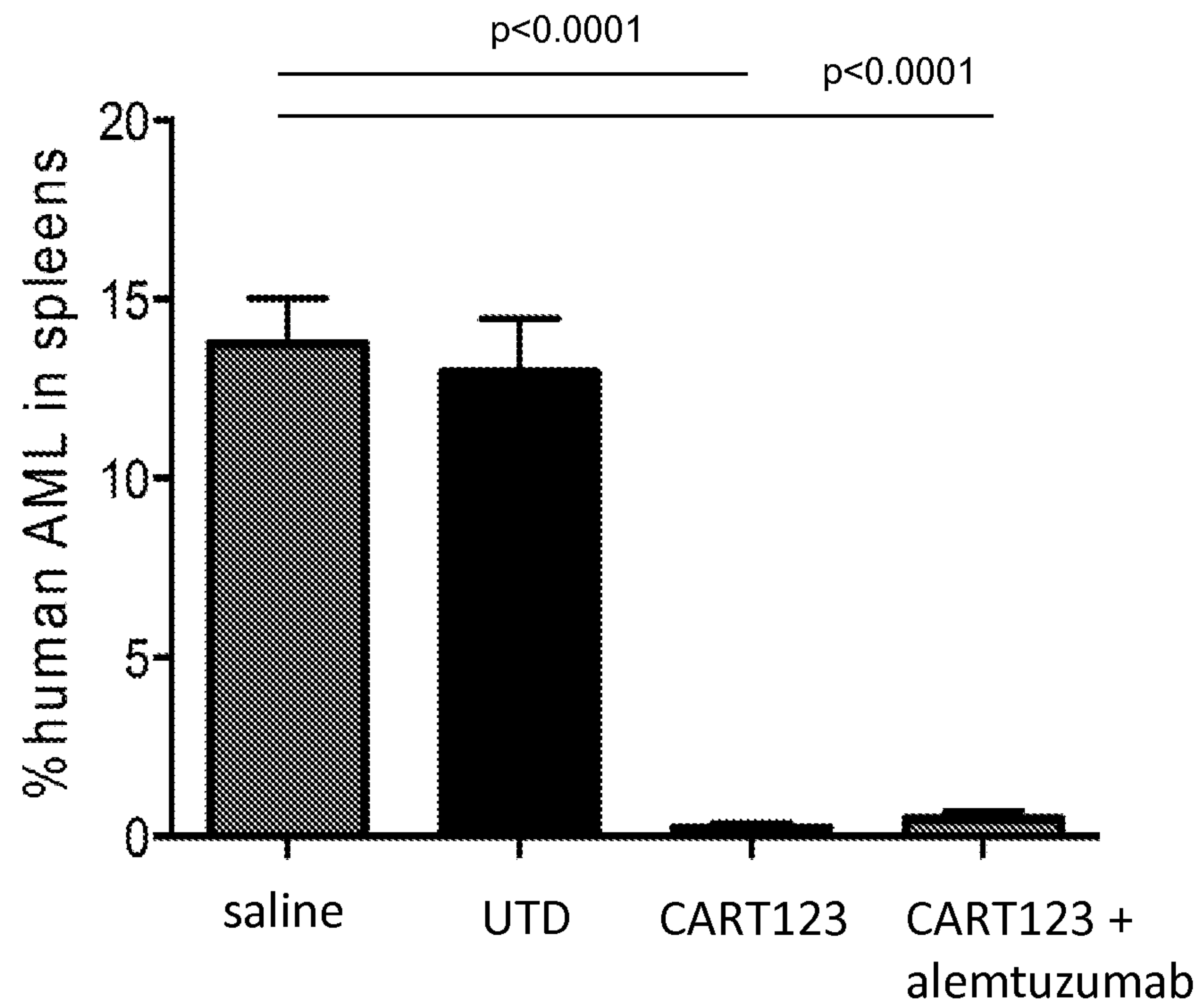
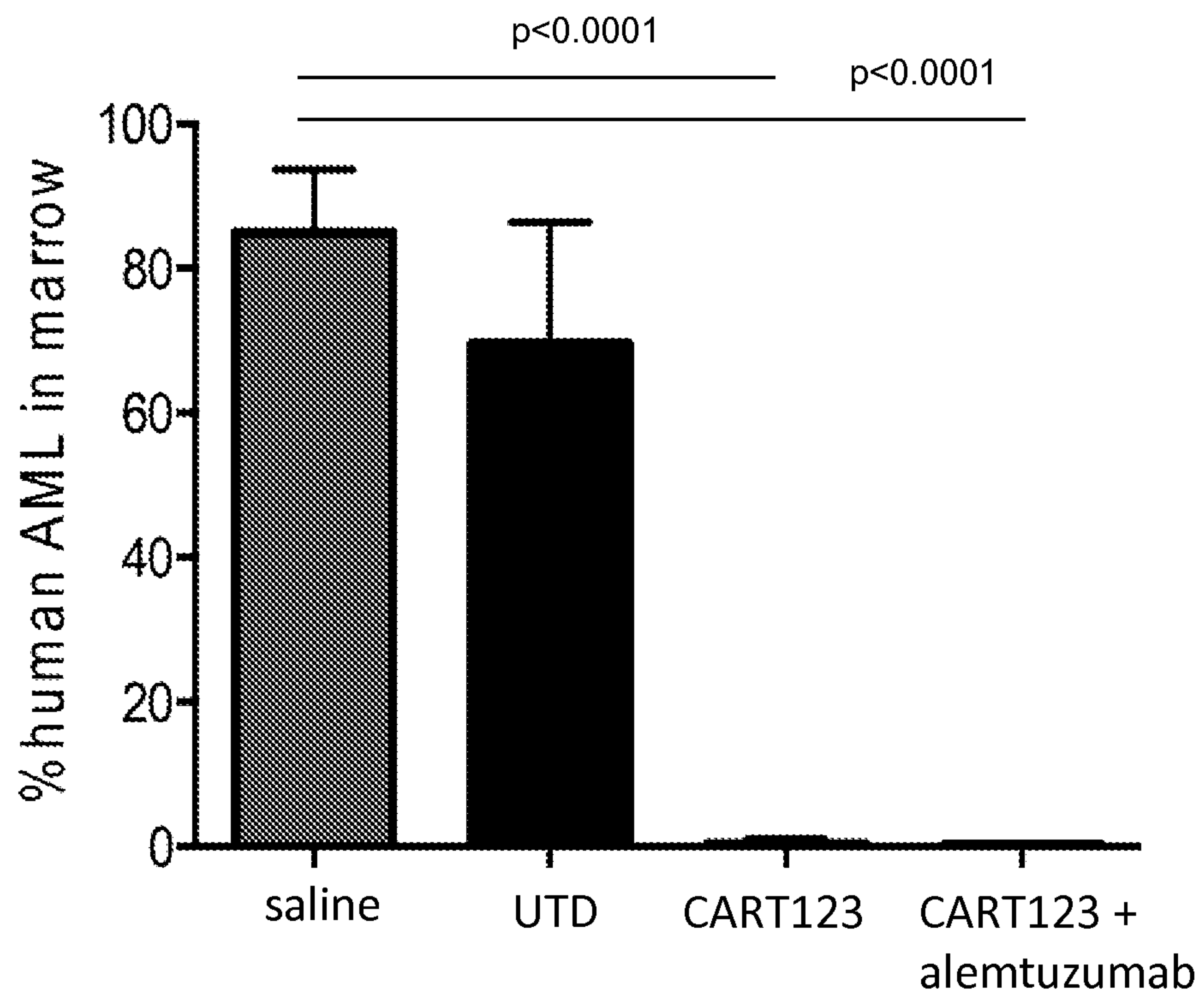


FIG. 74B



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FIG. 75

