



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2022/12/23
(87) **Date publication PCT/PCT Publication Date:** 2023/06/29
(85) **Entrée phase nationale/National Entry:** 2024/06/12
(86) **N° demande PCT/PCT Application No.:** EP 2022/087829
(87) **N° publication PCT/PCT Publication No.:** 2023/118608
(30) **Priorité/Priority:** 2021/12/23 (EP21217465.0)

(51) **Cl.Int./Int.Cl.** **A61K 35/00** (2006.01),
A61P 35/02 (2006.01), **C07K 14/705** (2006.01),
C07K 14/725 (2006.01), **C07K 16/28** (2006.01)

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(54) **Titre : VARIANTS DE PROTEINE DE SURFACE CELLULAIRE DISCERNABLE DE CD45 DESTINES A ETRE UTILISES EN
THERAPIE CELLULAIRE**

(54) **Title: DISCERNIBLE CELL SURFACE PROTEIN VARIANTS OF CD45 FOR USE IN CELL THERAPY**

(57) **Abrégé/Abstract:**

The present disclosure relates to the use of cells having discernible surface protein with engineered or naturally occurring mutation(s) but functional surface protein for use in therapy. The present invention also relates to the use of cells having discernible CD45 surface protein variants but functional surface protein for use in therapy, in particular adoptive cell therapy.

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Date Submitted: 2024/06/12

CA App. No.: 3240875

Abstract:

The present disclosure relates to the use of cells having discernible surface protein with engineered or naturally occurring mutation(s) but functional surface protein for use in therapy. The present invention also relates to the use of cells having discernible CD45 surface protein variants but functional surface protein for use in therapy, in particular adoptive cell therapy.

DISCERNIBLE CELL SURFACE PROTEIN VARIANTS OF CD45 FOR USE IN CELL THERAPY

TECHNICAL FIELD

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The present disclosure relates to the use of cells having discernible surface protein with engineered or naturally occurring mutation(s) but functional surface protein for use in therapy. The present invention also relates to the use of cells having discernible CD45 surface protein variants but functional surface protein for use in therapy, in particular
10 adoptive cell therapy.

STATEMENT REGARDING FUNDING

The project leading to this application has received funding from the European Research
15 Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 818806).

BACKGROUND OF THE INVENTION

20 Cell based immunotherapy is emerging as the third pillar of medicine after small molecule therapy and treatments based on biologics such as recombinant proteins including antibodies. Cellular therapy can be used in oncology for treating hematopoietic malignant diseases, but also other applications such as the treatment of genetic diseases, solid organ tumors and autoimmune diseases are under development. However, cellular therapy can
25 be associated with severe unwanted side effects. Indeed, while cancer immunotherapy with chimeric antigen receptor (CAR) T cells has been successful in targeting and

eradicating malignant cells expressing a specific antigen, it does often not discriminate between normal and malignant cells and thus induces destruction of the normal hematopoietic system. Targeted therapies, which include antibody-based therapies, such as conventional monoclonal antibodies, multispecific antibodies, such as T cell engagers (e.g. BiTE's) and cellular therapies, such as CAR cells (e.g. CAR T-cells, CAR NK cells or CAR macrophages), eliminate all cells expressing the target molecule. However, most cancer cell surface antigens are shared with normal hematopoietic or other cells. Thus, to identify targets to kill diseased cells including tumors while avoiding damage to healthy cells is a major challenge for targeted therapies (Perna et al., Cancer Cell (2017) 32:506-519). In particular, in myeloid diseases including myeloid malignancies such as myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) or Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) cell surface antigens such as CD117, CD33, or CD123 are shared with normal myeloid progenitors. Therefore, immunotherapy targeting CD117, CD33 or CD123 antigen for MDS, AML or PBDCN can be associated with depletion of normal hematopoietic cells in addition to malignant cells in patients (Gill S. I. Best practice & Research Clinical Hematology, 2019). As a consequence, targeted immunotherapy including mAbs, T cell engagers or CAR T have mostly been elusive, in part owing to the absence of truly disease-specific surface antigens (Gill S. I. Best practice & Research Clinical Hematology, 2019).

To regenerate normal hematopoiesis depleted through CD33-CAR T cell transfer, CD33 CAR T cell resistant hematopoietic cells are being engineered in such a way that the entire CD33 gene is knocked out (Kim et al. 2018. Cell. 173:1439-53). However, CD33 has a constitutive inhibitory effect on myeloid cells through its immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling domain. Thus, it remains unclear how well the loss of CD33 may be tolerated (Wißfeld et al. Glia (2021) 69:1393-1412). CD33-knock-out (CD33 KO) engineered cells transplanted in patients could present long-term functional defects (WO2018/160768, Kim et al. 2018. Cell. 173:1439-53, Borot et al. 2019. PNAS. 116:11978-87, Humbert et al. 2019. Leukemia. 33:762-808). In fact, the frequency of CD33 KO cells decreased in the two monkeys for which a long-term observation was reported. This could indicate functional impairment of CD33 KO cells, for instance through reduced engraftment of CD33 KO long-term repopulating HSC (LT-HSC) or through a competitive disadvantage

(Kim et al. 2018. Cell. 173:1439-53). In addition, the number of cell surface antigens with dispensable function is very limited and loss of said redundant cell surface antigen can induce antigen negative relapse. CD19-negative relapses are observed in approximately 30% of patients receiving CD19-targeted CAR T therapy (Orlando et al. 2018 Nat Med 24: 1504-6). Dual targeting of CD19 and CD123 can prevent antigen-loss relapses (Ruella et al. 2016 J Clin Invest 126:3814-26).

The inventors in previous patent applications showed that a single amino acid difference in surface protein variants can be genetically engineered into hematopoietic cells to change the antigenicity and be discriminated by specific and selective antibodies (WO2017/186718, WO2018/083071). Contrary to the approach where a surface protein is removed (KO cells), the surface protein variants in these cells retain their normal expression and function and enable to target surface proteins with important non-redundant functions.

CD45, also known as protein tyrosine phosphatase receptor type C (PTPRC), is an enzyme encoded by the PTPRC gene (Kaplan et al., PNAS 87:7000-7004 (1990)). CD45 is a member of the protein tyrosine phosphatase (PTP) family, which includes signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. CD45 contains an extracellular domain, a single transmembrane segment, and two tandem intracytoplasmic catalytic domains, and thus belongs to the receptor type PTP family. CD45 is a type I transmembrane protein that is present in various isoforms on differentiated hematopoietic cells (except e.g. erythrocytes) (Holmes, Immunology 7:145-55 (2006)). CD45 has been shown to be a regulator of T- and B-cell antigen receptor signaling. It functions through either direct interaction with components of the antigen receptor complexes via its extracellular domain, or by activating various Src family kinases (SFK), such as Lck, required for the antigen receptor signaling via its cytoplasmic domain. CD45 also suppresses JAK kinases, and thus functions as a negative regulator of cytokine receptor signaling.

CD45 is present on the surface of hematopoietic cells, including HSCs (hematopoietic stem cells), leukocytes, and osteoclasts, which are of hematopoietic origin (Shivtiel et al., J

Exp Med 205:2381 (2008)). Deletion mutations within CD45 in humans are associated with severe immunodeficiency. This is primarily due to the absence of CD45 on T cells, where it is typically abundant and required to modulate SFK activity during antigen responses. CD45-deficient (CD45^{-/-}) mouse bone marrow contains normal numbers of hematopoietic cells, but the most primitive HSCs are reduced in number, and their mobilization in response to G-CSF is impaired. In part, this defect is intrinsic to the HSC; without CD45-mediated downregulation of SFK activity, integrin-mediated adhesion is high and HSCs are more likely to remain in the stem cell niche. CD45^{-/-} HSCs are also deficient in G-CSF-stimulated mobilization and homing to the chemokine CXCL12/SDF-1, which negatively affects cell engraftment following transplantation. These deficiencies can be restored by supplementation with SFK inhibitors, indicating that this role is usually performed by CD45. Likewise, CD45^{-/-} recipients also show deficient engraftment and subsequent mobilization of normal HSCs, indicating a role for CD45 in the stem cell niche, as well as in the HSC (Shivtiel et al, J Exp Med 205:2381 (2008)). As CD45 is expressed, for example, on HSCs and leukocytes, it presents a target for therapies including conditioning therapies, immune reset, and treatment of diseases.

The present disclosure aimed to identify amino acid residues of CD45 that are exposed on the cell surface and that can be substituted in a manner such that a) the function of CD45 is not, or at least not substantially, altered, i.e. the variant of CD45 is functionally indistinguishable from the wild type version of CD45, and b) a moiety, such as an antibody or a CAR T cell, that binds to the wild-type version of CD45, but shows a substantially decreased or no binding to the altered version of CD45, i.e. the variant of CD45 is immunologically distinguishable from the wild type version of CD45. Most single amino acid substitution in any given target protein will only affect the binding of a moiety, if the amino acid substitution is part of, or is close to, the epitope of the binding moiety. As also will be appreciated, single amino acid substitutions that do affect binding of a binding moiety to a target antigen can also impact the functionality of the target antigen. It is therefore a highly sophisticated and unpredictable task to identify those amino acid substitutions that fulfill both requirements that do affect binding a moiety to a target antigen and which at the same time do not, or not substantially, affect its function.

CD45 is expressed on all nucleated hematopoietic cells and is therefore a target that has a broad spectrum of therapeutic applications. This includes HSC depletion, as well as autoimmune diseases since autoreactive lymphocytes (B and T cells) are depleted actively. Anti-CD45 therapies are also useful to treat antigen-negative relapses of most targeted therapies.

Several anti-CD45 moieties are known in the art. QA17A19 (Biolegend, #393411) and HI30 (Biolegend, #304001) are mouse anti-human CD45 antibodies which are commercially available. Various anti-CD45 antibodies from Magenta Therapeutics are in development, most as antibody drug conjugates (e.g. WO2017219025, WO2020092654). BC8 is a mouse hybridoma antibody commercially available from IchorBio (#ICH1155). The BC8 antibody is the basis for an anti-CD45 antibody-radioconjugate developed by Actinium Pharmaceuticals (WO2017155937, WO2019084258, WO2020159656). Other anti-CD45 antibodies are disclosed in WO2016016442, WO2019115791 and WO2020058495 (INSERM), WO2017009473 (UCB), WO2019129178 (Shanghai Baize Medical Laboratory), WO2020018580 (Fred Hutchinson) and WO2020170254 (Ramot At Tel Aviv University).

SUMMARY OF THE INVENTION

One of the objectives of the present disclosure is to develop a safer method for the treatment of malignancies, in particular cancer, hematological malignancies, and myeloid diseases. The inventors thus sought variations of the surface protein CD45, which are immunologically distinguishable while retaining or substantially retaining normal function, and where amino acid changes originate from a single or multiple amino acid or nucleotide variations. In particular, the inventors identified rationally designed and naturally occurring variants of CD45 and showed that these mutations change the antigenicity of CD45 to a specific antibody while retaining its normal expression and function, with its intracellular domain mediating the dephosphorylation of target proteins, e.g. tyrosine kinase Lck, and its extracellular region interfering with the immunological synapse, thereby modulating e.g. T- and B-cell function. CD45 is also involved in the in vivo development of hematopoietic

cells, which can for example be tested in humanized mice. Likewise, the structure of CD45 is important, especially as an extracellular spacer.

The present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of CD45 for use in a medical treatment in a patient in need thereof, said patient
5 having cells expressing a second isoform of CD45, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of CD45 and preferably wherein said first and second isoforms are functional. Alternatively said first isoform is
10 generated via RNA editing.

In a particular embodiment, the present disclosure relates to the mammalian cell or population of cells, preferably hematopoietic stem cells for use in a medical treatment in a patient in need thereof wherein said medical treatment comprises: administering a therapeutically efficient amount of said cell or population of cells expressing said first
15 isoform of CD45 to said patient in need thereof, in combination with a therapeutically efficient amount of a depleting agent comprising at least a first antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing said second isoform of CD45, preferably to restore normal hematopoiesis after immunotherapy in the treatment of hematopoietic disease, preferably malignant
20 hematopoietic disease such as acute myeloid leukemia (AML), myeloblastic syndrome (MDS), T-cell non-Hodgkin lymphomas (T-NHLs), chronic myeloid leukemia (CML), hairy cell leukemias (HCL), T-cell acute lymphoblastic leukemia (T-ALL), non-Hodgkin's lymphoma (NHL) or follicular lymphomas (FL).

In other embodiments the medical treatment relates to the restoration of the
25 hematopoietic or the immune function in genetic diseases of the hematopoietic or immune system, such as severe combined immunodeficiency syndrome (SCID), sickle cell disease (SCD), beta-thalassemia, Fanconi anemia or Diamond-Blackfan anemia.

In other embodiments the medical treatment relates to the restoration of the normal function in genetic diseases that are not originating in the hematopoietic and immune system but that can be treated by use of modified hematopoietic cells.

In other embodiments the medical treatment relates to the restoration of the normal
5 immune function in autoimmune diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc) or multiple sclerosis (MS).

In another particular embodiment, the present disclosure relates to the mammalian cell or population of cells for use in a medical treatment in a patient in need thereof, wherein said medical treatment comprises: administering a therapeutically efficient amount of said
10 cell or population of cells expressing said first isoform to said patient in need thereof in combination with a therapeutically efficient amount of a depleting agent comprising at least a second antigen-binding region that binds specifically to said first isoform to specifically deplete transferred cells expressing first isoform, preferably for use in adoptive
15 cell transfer therapy, more preferably for the treatment of malignant hematopoietic disease such as acute myeloid leukemia (AML), myeloblastic syndrome (MDS), T-cell non-Hodgkin lymphomas (T-NHLs), chronic myeloid leukemia (CML), hairy cell leukemias (HCL), T-cell acute lymphoblastic leukemia (T-ALL), non-Hodgkin's lymphoma (NHL) or follicular lymphomas (FL), again more preferably wherein said depleting agent is administered
20 subsequently to said cell or population of cells expressing said first isoform of surface protein to avoid eventual severe side effects such as graft-versus-host disease due to the transplantation.

In another aspect, the present disclosure relates to a pharmaceutical composition comprising a mammalian cell, preferably a hematopoietic stem cell or an immune cell such as a T-cell as described above and preferably a depleting agent and a pharmaceutically
25 acceptable carrier.

The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein

said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45. In certain embodiments said depleting agent binds substantially weaker to said second isoform of CD45.

- 5 The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45,
10 and wherein said first and second isoforms are substantially functionally identical. In certain embodiments said depleting agent binds substantially weaker to said second isoform of CD45.

- The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform
15 of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45, and wherein said first and second isoforms lead to the dephosphorylation of target proteins.

- 20 The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45,
25 and wherein said first and second isoforms lead to the dephosphorylation of tyrosine kinase Lck.

 The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform

of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45, and wherein said first and second isoforms lead to essentially the same modulation of T cell function and/or B cell function.

The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45, and wherein said first and second isoforms lead to the normal differentiation of hematopoietic cells.

The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45, wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1, preferably by at least one substitution of an amino acid in position E230, N257, E259, F331, K352 or E353 of SEQ ID NO: 1. In certain embodiments said depleting agents bind substantially weaker to said second isoform of CD45.

The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45, wherein said polymorphic or genetically engineered allele is characterized by at least one

substitution of an amino acid in position I328, N255, E360, E259, E364 and E269. Among these substitutions, substitutions I328V, N255G, E360G, E259G, E364K and E269G are particularly preferred. In certain embodiments said depleting agent binds substantially weaker to said second isoform of CD45.

- 5 The present disclosure also relates to a depleting agent for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45, wherein said patient's native cells express a second isoform of CD45, and wherein said depleting agent comprises at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind to said second isoform of CD45,
- 10 wherein residue E230 is substituted with K, and/or residue N257 is substituted with A, D, E, H, K, R, S, T or V, and/or residue E259 is substituted with G, N, T or Q, and/or T264 is substituted with D or E, preferably D, and/or N267 is substituted with A, H, L, S or V, and/or residue N286 is substituted with G, L or R, and/or E329 is substituted with A, and/or residue F331 is substituted with A or G, and/or Y340 is substituted with A, G, N, Q or S and/or
- 15 residue K352 is substituted with A, D, E, G, H, I, L, M, N, Q, S, T or Y and/or residue E353 is substituted with A, H, I, K, L, S, T or R, preferably A, I, K, L, S, T or R.

- The present disclosure also relates to a method for improving engraftment of hematopoietic stem cell transplants. Conditioning (depletion of HSCs) prior to hematopoietic stem cell transplantation (HSCT) is used to promote engraftment. Indeed,
- 20 conditioning efficacy is associated with improved engraftment. Avoiding toxic conditioning is an important goal that can be achieved with the present disclosure. Current methods for conditioning involve the use of intravenous busulfan. Busulfan is a DNA alkylating drug originally designed to treat hematologic diseases, such as acute myeloid leukemia (AML). However, busulfan carries the risk of significant side effects, including sterility, primary or
- 25 secondary malignancy, and additional acute and chronic toxicities.

The present disclosure also relates to human cell or a population of human cells expressing a first isoform of CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of CD45,

wherein said human cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele,

wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of CD45, and

5 wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1. Preferably said first and said second isoform are substantially functionally identical.

10 In certain embodiments, said first and said second isoforms dephosphorylate target proteins of CD45, activate the TCR signaling cascade, lead to an increase of cytokine production and/or lead to an increase of proliferation of T cells.

In certain embodiments, said first and said second isoforms of CD45 dephosphorylate tyrosine kinase Lck.

15 In certain embodiments, said medical treatment comprises administering a therapeutically efficient amount of said cell or population of cells expressing said first isoform of CD45 to said patient in need thereof, in combination with a therapeutically efficient amount of a depleting agent comprising an antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing
20 said second isoform of CD45.

In certain embodiments, said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position F331, K352 or E353 of SEQ ID NO: 1. In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position K352 of SEQ ID NO: 1. In
25 certain embodiments, said substitution in position K352 is selected from K352E, K352H, K352I, K352L, K352M, K352N, K352Q, K352S and K352T, preferably K352D, K352E and K352H, and more preferably said substitution is K352E.

In certain embodiments, the depleting agent used in conjunction with a substitution of an amino acid is in position N286, F331, K352 or E353 of SEQ ID NO: 1 is selected from

- 5 a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3; and
- 10 b) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.
- 15 20

In certain embodiments, said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid is in position E230, Y232, N257 or E259 of SEQ ID NO: 1.

25 In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain embodiments, said substitution in position N257 is selected from N257E, N257K, N257R and N257T, preferably N257R. In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position E259 of SEQ ID NO: 1. In certain embodiments, said substitution in position E259 is selected from

E259N, E259Q, E259V and E259G, preferably E259V. In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position Y232 of SEQ ID NO: 1, preferably Y232C.

In certain embodiments, the depleting agent used in conjunction with a substitution of an amino acid is in position E230, Y232, N257 or E259 of SEQ ID NO: 1 is an antigen-binding region comprising

an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24, VLCDR3 is SEQ ID NO: 25, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 18 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 19.

In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain embodiments, said substitution in position N257 is selected from N257E, N257K, N257R and N257T, preferably N257R.

In certain embodiments, the depleting agent used in conjunction with the substitution of the amino acid in position N257 of SEQ ID NO: 1 is an antigen-binding region comprising

a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and

- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16, VLCDR3 is SEQ ID NO: 17,
preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and/or
5 a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

In certain embodiments, said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, N257, E259,
10 F331, K352 or E353 of SEQ ID NO: 1. Said substitutions may be selected from CD45 variants, wherein said residue E230 is substituted with K, and/or residue N257 is substituted with A, D, E, H, K, R, S, T or V, and/or residue E259 is substituted with G, H, K, N, R, T or Q, and/or T264 is substituted with D or E, preferably with D, and/or N267 is substituted with A, H, L, S or V, and/or residue N286 is substituted with G, L or R, and/or E329 is substituted with A,
15 and/or residue F331 is substituted with A or G, and or Y340 is substituted with A, G, N, Q or S and/or residue K352 is substituted with A, D, E, G, H, I, L, M, N, Q, S, T or Y and/or residue E353 is substituted with A, H, I, K, L, S, T or R.

The present disclosure also relates to a human cell or population of human cells for use as disclosed herein, wherein said cell expressing said first isoform of CD45 has been
20 selected from a subject comprising native genomic DNA with at least one natural polymorphism allele in nucleic acid encoding said first isoform.

The present disclosure also relates to a human cell or population of human cells for use as disclosed herein, wherein said first isoform of CD45 is obtained by *ex vivo* modifying the nucleic acid sequence encoding said first isoform of CD45 by gene editing, preferably by
25 introducing into a human cell a gene editing enzyme capable of inducing site-specific mutations(s) within a target sequence encoding a surface protein region involved in the binding of agent comprising at least a first antigen-binding region.

The present disclosure also relates to a human cell or population of human cells for use as disclosed herein, wherein said medical treatment comprises administering a therapeutically efficient amount of said human cell or population of human cells expressing said first isoform of CD45 to said patient in need thereof, in combination with a
5 therapeutically efficient amount of a depleting agent comprising at least a first antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing said second isoform of CD45, preferably to restore normal haematopoiesis after immunotherapy in the treatment of hematopoietic disease, and preferably in the treatment of malignant hematopoietic disease such as acute myeloid
10 leukemia (AML), myelodysplastic syndrome (MDS), T-cell non-Hodgkin lymphomas (T-NHLs), chronic myeloid leukemia (CML), hairy cell leukemias (HCL), T-cell acute lymphoblastic leukemia (T-ALL), non-Hodgkin's lymphoma (NHL) or follicular lymphomas (FL).

The present disclosure also relates to a human cell or population of human cells for use
15 as disclosed herein, wherein said depleting agent is an antibody, antibody-drug conjugate or an immune cell, preferably a T-cell bearing a chimeric antigen receptor (CAR) comprising a first antigen-binding region which binds specifically to said second isoform and does not bind or binds substantially weaker to said first isoform.

The present disclosure also relates to pharmaceutical composition comprising a human
20 cell, preferably a hematopoietic stem cell or an immune cell such as T-cell as disclosed herein, and preferably a depleting agent as disclosed herein, and a pharmaceutically acceptable carrier.

FIGURE LEGENDS

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Figure 1 shows the binding of anti-CD45 antibodies to DF-1 cells transfected with human wild-type CD45 or with an empty vector. Serial dilutions of each antibody were tested for immunoreactivity via flow cytometry. All five antibodies in Mab format bind to human

CD45 positive cells in a concentration dependent manner. Cell transfected with the empty vector did not show any binding of the anti-CD45 antibodies.

Figure 2 shows the binding of anti-CD45 Fab fragments to DF-1 cells transfected with human wild-type CD45 or with an empty vector. Like the full-length antibodies, also the Fab fragments bind to human CD45 in a concentration dependent manner. Cells transfected with the empty vector did not show any binding of the anti-CD45 antibodies.

Figure 3 shows the result of an alanine scan on human CD45 for four antibodies tested. For each mutant clone, the mean binding value determined by flow cytometry was plotted as a function of expression. Clones harboring CD45 Ala variants that were identified as critical are circled. Secondary clones, i.e., clones that did not meet the initially set thresholds but whose decreased binding activity and proximity to critical residues suggested that the mutated residue may be part of the antibody epitope, are squared.

Figure 4 schematically depicts the location of the identified critical positions on the 3D structure of human CD45.

Figure 5 shows binding of the tested antibodies to the variants identified for Refmab #1, i.e., the variants to which Refmab #1 shows binding of less than 20% compared to wild type CD45.

Figure 6 shows binding of the tested antibodies to the variants identified for Refmab #2, i.e., the variants to which Refmab #2 shows binding of less than 20% compared to wild type CD45.

Figure 7 shows binding of the tested antibodies to the variants identified for Refmab #4, i.e., the variants to which Refmab #4 shows binding of less than 20% compared to wild type CD45.

Figure 8 shows an in-silico mutagenesis of selected variants of the present disclosure. The bars indicate the predicted Provean score (y-axis) for each variant (x-axis), predicting whether a protein sequence variation might affect protein function. The dashed horizontal

line indicates the predefined threshold (-2.5). All variants are predicted as neutral, with the exception of E259G whose predicted score is slightly below the threshold (-2.570).

Figure 9 shows the editing efficiencies of CD45 using various base editors and sgRNAs concentrations.

- 5 **Figure 10** shows human T cells engineered using base editing to express CD45 E259G. Cells were then incubated with increasing concentrations of antibody-drug-conjugate resulting in a selective depletion of unedited cells but persistence of edited cells measured by flow cytometry (A). Results were confirmed by Sanger sequencing (B).

- 10 **Figure 11** shows that variant F331del leads to a loss of binding of Refmab #1, cells are still reactive with Refmab #3.

Figure 12 shows that cell expressing wild type CD45 are killed by a Refmab #1 -Antibody Drug Conjugate. In contrast, CD45 knock-out cells, as well as cells expressing the CD45 E259G variant are protected from killing by a Refmab #1 - Antibody Drug Conjugate.

- 15 **Figure 13** shows the result of an experiment in which wild type CD45 was knocked out and cells were transfected with variant CD45 isoforms. While the CD45RO (wildtype) form of the protein did bind to both antibodies, a loss of binding was observed for Refmab #1 for the CD45 variants. All variants did retain binding to Refmab #3, demonstrating that the protein was expressed by the electroporated cells. The CD45 knock-out is shown on the top left. Wild type CD45 is shown in the top middle. Top right: Mut8 (22 aa deletion). Bottom
20 (from left to right): Mut9 (F331 deletion), Mut12 (F331S) and Mut 13 (F331I).

Figure 14 shows CD45 expression in gene-edited CD34+ cells. Dot plots show data obtained after staining of cells electroporated without gene editing reagents (EP - RNP), cells electroporated in presence of RNP complex alone (EP + RNP) or with the F331del HDR template (EP + RNP + HDRT).

- 25 **Figure 15** shows a representative chromatogram of size exclusion chromatography (panel A) and an SDS-PAGE of the recombinant purified human CD45 D1-D2 wildtype protein (panel B).

Figure 16 shows phosphorylation of Lck at position Tyr505 detected using an AlphaLISA assay. 10.000-50.000 Jurkat wildtype or Jurkat CD45 knock-out cells were incubated for 20 minutes in plates coated with anti-CD3 Antibody before cell lysis and detection of phosphorylated Lck. Activation of Jurkat cells using the anti-CD3 antibody leads to CD45
5 activation which in turn dephosphorylates Lck. Jurkat CD45 knock-out cells are not able to dephosphorylate Lck upon activation. The figure shows the acceptor signal (counts) and represents one biological experiment containing two technical replicates.

Figure 17 shows thermal unfolding curves of recombinant purified wildtype and variant human CD45 D1-D2 proteins, using DSF and Sypro Orange. Data are represented in relative
10 fluorescence units (RFU, top) and as a first derivative of RFU with respect to temperature ($d(RFU)/dT$, bottom).

Figure 18 shows conformational/thermal stability data of recombinant purified wildtype and variant human CD45 D1-D2 proteins measured by DSF. A) The onset temperature which is the temperature at which a protein starts denaturing are illustrated for elected
15 hCD45 D1-2 deglycosylated wild-type and variants, as well as hCD45 D1-2 glycosylated wild-type. The dotted line indicates the onset temperature of hCD45 D1-2 deglycosylated wild-type. B) The melting temperature of hCD45 D1-2 deglycosylated wild-type and variants, as well as of hCD45 D1-2 glycosylated wild-type, is shown. The dotted line illustrates the melting temperature of hCD45 D1-2 deglycosylated wild-type.

Figure 19 shows binding of Refmab #1 and Refmab #4 to DF-1 cells expressing wt or variant CD45 as measured by flow cytometry. The data show that a mutation of residue K352 leads to a loss of binding of Refmab #1, and a mutation of residues N257 leads to a loss of binding of Refmab #4.
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Figure 20 shows the binding of hCD45 D1-2 recombinant, purified and deglycosylated wild-type and variant CD45 D1-D2 proteins to Refmab's. A) %binding to Refmab #1 is illustrated.
25 B) %binding to Refmab #2 is illustrated. C) %binding to RefmAb #4 is illustrated. %binding was calculated by dividing the nm shift of hCD45 D1-2 variant by the nm shift of hCD45 D1-2 wt. Antibody binding to wt CD45 binding was set to 100%. For this calculation, the nm

shift of 500 nM analyte (hCD45 D1-2 variant and wt) at the end of the association was used. Marked with an asterik are calculations for which the nm shift of the 50 nM instead of 500 nM of analyte (hCD45 D1-2 variant and wt) was used. ND stands for not determined and NA stands for not analyzed.

- 5 **Figure 21 and 22** shows binding of Refmab #1 and Refmab #4 to DF-1 cells expressing wt or variant CD45 as measured by flow cytometry. The data show that mutations of residues K352 and E353 lead to a loss of binding of Refmab #1. Mutations of residue N257 lead to a loss of binding of Refmab #4, and mutations of residue E259 lead to a strong reduction of binding of Refmab #4.
- 10 **Figure 23** shows human CD34+ HSPCs engineered using base editing to express CD45 K352E. A) shows base editing using ABE8e-NG and B) shows base editing using ABE8e-SpRY and various gRNAs. Repositioning the ABE8e base editor in a more favorable editing window increased the editing efficiencies to >30% shielded HSPCs as measured by flow cytometry.

15

DETAILED DESCRIPTION OF THE INVENTION

- Immunotherapy is a promising therapy to treat cancer, genetic and autoimmune
- 20 diseases. Immunodepleting agent such as antibodies or engineered immune cells directed to tumor antigen are administered into a patient to target and kill tumor cells. However, as tumor surface proteins are also expressed at the surface of normal cells including hematopoietic cells, this strategy can induce severe side effects to the patients, e.g., by altering hematopoiesis. To restore hematopoiesis in the patient, hematopoietic cells can
- 25 be subsequently transplanted into the patient. However, the binding of the depleting agent not only to the diseased cells but also to the newly transplanted healthy cells can limit the maximal tolerated dose or limit the use to treatment before transplantation of healthy cells. Alternatively, transplanted cells need to be resistant to said immunodepleting agent

in order not to be targeted and eliminated by it. One approach is therefore to select cells resistant to said immunodepleting agent used in immunotherapy while retaining their function to restore normal hematopoiesis in the patient.

The inventors develop a method to identify functional allelic variants in the genetic
5 sequence encoding the surface protein region responsible for the binding of a specific
depleting agent. Such variants can be naturally occurring polymorphisms and/or designed
and engineered variants. Different isoforms of surface proteins can be selected or
generated. Said first isoform of a surface protein encoded by a nucleic acid with said
polymorphism is not recognized by a specific depleting agent. This variant allele particularly
10 does not alter or does not substantially alter the function of the surface protein. Thus, said
depleting agent can be used to bind specifically to the one isoform and not, or not
substantially, the other isoform thereby depleting specifically cells expressing one isoform.
For example, if the depleting agent binds specifically to the second isoform, but not the
first isoform, said depleting agent will specifically deplete cells expressing said second
15 isoform. In another embodiment, said first isoform can be recognized by a second agent
and thus this second agent can be used to deplete specifically cells expressing the first
isoform, but not second isoform. The cells expressing the first isoform of the surface
protein encoded by at least one variant allele is advantageously used in medical treatment
in a patient having cells expressing a second isoform, in particular for depleting specifically
20 transplanted or patient cells by using a second or first agent respectively.

It is impossible to predict which mutation in a surface antigen can be used in such an
approach. First, the mutations need to lie on a surface exposed stretch of the surface
antigen that is accessible for the depleting agent. Second, the depleting agent needs to
bind to this stretch on the exposed area of the surface antigen. Third, binding needs to be
25 affected sufficiently enough so that the depleting agent can discriminate the first isoform
from the second isoform. Residual binding to the other isoform should be minimal or,
better, be completely absent. Fourth, the mutation should not affect, or only marginally
affect, the function of the surface antigen. The mutated isoform should fulfill its biological
function at least to an extent that is tolerable in a given therapeutic setting. Although

certain tools exist to predict three-dimensional protein structure, only experimental testing can prove the usefulness of any given mutation.

Depleting agent

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The present disclosure relates to an agent comprising an antigen binding region which binds specifically to one isoform of CD45 on a cell and does not bind or binds substantially weaker to another isoform of CD45. Such agent is referred to herein as “**depleting agent**”. Both isoforms of CD45 are functional, i.e. CD45 is functional with respect to at least one
10 relevant property. Preferably both isoforms of CD45 have that same function, i.e., they are functionally indistinguishable.

The two isoforms of CD45 differ however with respect to binding to the depleting agent. The depleting agent only binds specifically to one of the isoforms of CD45. The isoforms can therefore be described as functional identical (or functionally substantially identical),
15 but immunologically distinguishable.

In certain embodiments, the first and the second isoform of CD45 have substantially identical biophysical properties. In certain embodiments, the first and the second isoform of CD45 have identical biophysical properties. In certain embodiments, the first and the second isoform of CD45 have substantially the same stability. In certain embodiments, the
20 first and the second isoform of CD45 have the same stability. In certain embodiments, the first and the second isoform of CD45 have substantially the same melting temperature. In certain embodiments, the first and the second isoform of CD45 have the same melting temperature. In certain embodiments, the first and the second isoform of CD45 have substantially the same aggregation propensity. In certain embodiments, the first and the
25 second isoform of CD45 have the same aggregation propensity. In certain embodiments, the first and the second isoform of CD45 have substantially the same tendency to form dimers. In certain embodiments, the first and the second isoform of CD45 have the same tendency to form dimers.

The first isoform and the second isoform of CD45 may be polymorphic alleles. Preferably, the first isoform and the second isoform of CD45 are naturally occurring polymorphic alleles. Also preferably, the first isoform and the second isoform of CD45 are single nucleotide polymorphism (SNP) alleles.

- 5 The first isoform and the second isoform of CD45 may also be genetically engineered alleles. Preferably the first isoform and the second isoform of CD45 differ by one, two, three, four or five amino acids. Most preferably the first isoform and the second isoform of CD45 differ by one amino acid.

- 10 Various methods can be used to determine the mutation that is to be introduced into CD45 to generate the second isoform. For example, mutations can be randomly inserted, followed by the functional and immunological screening of the variants generated. Alternatively, mutations can be rationally designed, for example by analysis of the secondary or tertiary protein structure of CD45.

- 15 The depleting agent comprises an antigen binding region which binds specifically to one isoform of CD45 on a cell and does not bind or binds substantially weaker to another isoform. The depleting agent of the present disclosure can be divided into two main categories.

- 20 First, the depleting agent can be a polypeptide comprising an antigen binding region. Said polypeptide may consist of one or more polypeptide chains. Preferably said polypeptide comprising an antigen binding region is an antibody. Said polypeptide comprising an antigen binding region may also be an antibody fragment, an antibody drug conjugate, or another variant of an antibody or scaffold. Exemplary antibody fragments and scaffolds include single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR, igNAR, bis-scFv, camelid antibodies, ankyrins, centyrins, 25 domain antibodies, lipocalins, small modular immuno- pharmaceuticals, maxybodyes, Protein A and affilins.

Said polypeptide comprising an antigen binding region may also be a bispecific, biparatopic or multispecific antibody. Such molecules may also contain additional

functional domains. For example, said polypeptide comprising an antigen binding region may be a T cell engager, for example a BiTE. Said polypeptide comprising an antigen binding region may also be fused to a cytokine or a chemokine, a toxin or to the extracellular domain of a cell surface receptor.

- 5 Alternative, the depleting agent can be a cell comprising an antigen binding region. For example, the depleting agent can be a chimeric antigen receptor (CAR). In certain embodiments of the present disclosure said cell comprising an antigen binding region is a CAR T-cell, CAR NK cells or CAR macrophages. In a preferred embodiment of the present disclosure said cell comprising an antigen binding region is a CAR T-cell. In another
10 preferred embodiment of the present disclosure said cell comprising an antigen binding region is a primary T cell comprising a CAR.

The depleting agent binds specifically to one isoform of CD45, but not the second isoform and thus specifically depletes cells expressing one isoform.

- In certain embodiments, the present disclosure relates to an agent comprising a first
15 antigen binding region which binds specifically to a second isoform of CD45 and does not bind a first isoform. In other embodiments, the present disclosure also relates to an agent comprising a second antigen binding region which binds specifically to the first isoform of CD45 and does not bind a second isoform. In certain embodiments said agents binds substantially weaker to said second isoform of CD45.

- 20 The first and the second isoform of CD45 may differ from each other by only one amino acid substitution. Said one amino acid difference between the first and the second isoform may also be the result of the presence of a single nucleotide polymorphism, such as a naturally occurring single nucleotide polymorphism. The first and the second isoform of CD45 may also differ from each other by more than one amino acid, such as by two, by
25 three or by more than three amino acids. The first and the second isoform of CD45 may also differ from each other in that one of the isoforms has an insertion of one, of two, of three or of more than three amino acids compared to the other isoform. The first and the second isoform of CD45 may also differ from each other in that one of the isoforms has a

deletion of one, of two, of three or of more than three amino acids compared to the other isoform. The two isoforms may also differ from each other by combinations of amino acid substitutions, insertions and/or deletions. In a preferred embodiment, said depleting agent is an antibody or an antigen-binding fragment. If the two isoforms of CD45 differ by more
5 than one amino acid, then the amino acids changed may be adjacent to each other, i.e., direct neighboring amino acids, or they may be separated.

The term "**antibody**" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term
10 antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies.

In natural antibodies of rodents and primates, two heavy chains are linked to each other by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chains, lambda (λ) and kappa (κ). There are five main heavy chain
15 classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. In typical IgG antibodies, the light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions
20 of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR).

The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and
25 consists of the variable portions of one light chain and one heavy chain variable region. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from non-hypervariable or framework regions (FR) can

participate in the antibody binding site or influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, typically includes six CDRs, comprising the CDRs set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs. Accordingly, the variable regions of the light and heavy chains typically comprise 4 framework regions and 3 CDRs of the following sequence: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (Kabat et al., 1992, hereafter "Kabat et al."). This numbering system is used in the present specification. The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues in SEQ ID sequences. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence. The CDRs of the heavy chain variable domain are located at residues 31-35 (H-CDR1), residues 50-65 (H-CDR2) and residues 95-102 (H-CDR3) according to the Kabat numbering system. The CDRs of the light chain variable domain are located at residues 24-34 (L-CDR1), residues 50-56 (L-CDR2) and residues 89-97 (L-CDR3) according to the Kabat numbering system.

In specific embodiments, an antibody provided herein is an antibody fragment, and more particularly any protein including an antigen-binding domain of an antibody as disclosed herein. The antigen-binding domain may also be integrated into another protein scaffold

Antibody fragments and scaffolds include, but are not limited to, Fv, Fab, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, diabodies, single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR, IgNAR, bis-scFv, camelid antibodies, ankyrins, centyrins, domain antibodies, lipocalins, small modular immuno-pharmaceuticals, maxybodies, Protein A and affilins.

As used herein, an **"antigen binding region"** or **"antigen-binding fragment of an antibody"** means a part of an antibody, i.e. a molecule corresponding to a portion of the structure of the antibody, that exhibits antigen-binding capacity for a specific antigen, possibly in its native form; such fragment especially exhibits the same or substantially the same antigen-binding specificity for said antigen compared to the antigen-binding specificity of the corresponding four-chain antibody. The antigen-binding capacity can be determined by measuring the affinity between the antibody and the target fragment. This antigen-binding region may also be designated as **"functional fragments"** of antibodies.

The agents of the disclosure comprise antibodies and fragments thereof but also comprise artificial proteins with the capacity to bind antigens mimicking that of antibodies, also termed herein antigen-binding antibody mimetic. Antigen-binding antibody mimetics are organic compounds that specifically bind antigens, but that are not structurally related to antibodies. They are usually artificial peptides or small proteins with a molar mass of about 3 to 20 kDa.

The phrases **"an antigen binding region recognizing an antigen"** and **"an antigen binding region having specificity for an antigen"** are used interchangeably herein with the term **"an antigen binding region which binds specifically to an antigen"**. As used herein, the term **"specificity"** refers to the ability of an agent comprising an antigen binding region such as an antibody to detectably bind an epitope presented on an antigen.

"Specific binding" or **"specifically bind to"** includes binding with a monovalent affinity of about 10⁻⁸ M (KD) or stronger. Preferably, binding is considered specific when the binding affinity is between 10⁻⁸ M (KD) and 10⁻¹² M (KD), optionally between 10⁻⁸ M (KD) and 10⁻¹⁰ M (KD), in particular at least 10⁻⁸ M (KD). The affinity can be determined by various methods

well known from the one skilled in the art. These methods include, but are not limited to, surface plasmon resonance (SPR), biolayer interferometry (BLI), microscale thermophoresis (MST) and Scatchard plot. Whether a binding domain specifically reacts with or binds to a target can be tested readily by, inter alia, comparing the reaction of said
5 binding domain with a target protein or antigen with the reaction of said binding domain with proteins or antigens other than the target protein.

As used herein, the term "**epitope**" means the part of an antigen to which the antibody or antigen binding region thereof binds. The epitopes of protein antigens can be divided into two categories, conformational epitope and linear epitope. A conformational epitope
10 corresponds to discontinuous sections of the antigen's amino acid sequence. A linear epitope corresponds to a continuous sequence of amino acids from the antigen.

In another aspect, it is further disclosed herein bispecific or multispecific molecules, such as bispecific antibodies or multispecific antibodies. For example, an antibody can be derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g.,
15 another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody may in fact be derivatized or linked to more than one other functional molecule to generate multi-specific molecules that bind to more than two different binding sites and/or target molecules; such multi-specific molecules are also intended to be encompassed by the terms "**bispecific**
20 **molecule**", "**bispecific antibody**", "**biparatopic molecule**", "**biparatopic antibody**", "**multispecific molecule**" and "**multispecific antibody**" as used herein. To create a bispecific molecule, an antibody of the disclosure can be functionally linked (e.g., by chemical coupling, genetic fusion, disulfide bonds, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment,
25 peptide or binding mimetic, cytokine, chemokine, toxin or a receptor extracellular domain, such that a bispecific molecule results. Specific bispecific and multispecific molecules contemplated by the present disclosure are T cell engagers, such as bispecific T cell engager, for example a BiTE.

As used herein, an agent which does not bind or binds substantially weaker to a particular isoform of CD45 includes an agent which is not able to bind to cells expressing said particular isoform. For experimental testing said agent may be labelled with a fluorescent marker or may be detected with a secondary antibody directed against said agent, and the percentage of cells presenting said fluorescent marker or said secondary antibody is determined by FACS analysis. Typically testing is done in cell lines expressing the recombinant target protein, i.e. CD45. The target protein may be expressed in its entirety. Alternatively, a truncated version may be used, wherein said truncated version at a minimum needs to include the extra cellular domain or the regions of the extracellular domain containing the respective antibody epitope. In order to monitor the expression of the variant isoforms, cells may be stained with two agents simultaneously, one binding the epitope where variants were introduced and a second one that binds an epitope that is different from the one bound by the first agent. The second epitope remains unaltered and thus this staining serves as an expression control. As a non-binding control, cells are used that do not express the protein of interest. As a maximum binding control, cells that normally do not express the protein of interest are transfected with the wildtype isoform. Different cell lines have different expression levels but the expression is controlled through endogenous control elements such as promoters. Such cell lines can also be used to study the mode-of-action of a depleting agent, the effective shielding against a different mode-of-action, to test cytotoxicity and shielding/resistance from cytotoxicity or to test the function of the engineered receptors. Western Blot, ELISA or FACS can be used to analyze phosphorylation of signaling molecules. Analysis of gene expression changes can serve to analyse gene expression compared to normal function. Cells can also be used to demonstrate the feasibility of editing a specific variant via different approaches, e.g. homology directed repair (HDR), base editing or prime editing.

Binding of said agent can result in depletion of the cell expressing the first isoform of CD45. Various mechanisms can lead to cell depletion. Antibody dependent cellular cytotoxicity (ADCC) results from binding of the agent to a target protein and activation of NK cells through the Fc part on the agent bound by an FcR expressed by NK cells. The Fc part of an immunoglobulin refers to the C-terminal region of an immunoglobulin heavy

chain. The Fc part can be wildtype or engineered. Mutations of enhanced, engineered Fc parts are known in the art. For certain therapeutic situations, it is desirable to reduce or abolish the normal binding of the wild-type Fc region of an antibody, such as of a wild-type IgG Fc region to one or more or all of Fc receptors and/or binding to a complement component, such as C1q in order to reduce or abolish the ability of the antibody to induce effector function. For instance, it may be desirable to reduce or abolish the binding of the Fc region of an antibody to one or more or all of the Fcγ receptors, such as: FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa. Effector function can include, but is not limited to, one or more of the following: complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen-presenting cells, binding to NK cells, binding to macrophages, binding to monocytes, binding to polymorphonuclear cells, direct signaling inducing apoptosis, crosslinking of target-bound antibodies, dendritic cell maturation, or T cell priming. Binding of said agent may also lead to the blocking of binding of the natural receptor ligand and thereby result in cell death and apoptosis without cell-mediated depletion.

A reduced or abolished binding of an Fc region to an Fc receptor and/or to C1q is typically achieved by mutating a wild-type Fc region, such as of an IgG1 Fc region, more particular a human IgG1 Fc region, resulting in a variant or engineered Fc region of said wild-type Fc region, e.g., a variant human IgG1 Fc region. Substitutions that result in reduced binding can be useful. For reducing or abolishing the binding properties of an Fc region to an Fc receptor, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties and/or charges, are preferred.

In certain embodiments of the present disclosure the Fc region of the antibody is of the IgG1 isotype, carrying the LALA or PG-LALA mutations, i.e. the constant region carries a L234A, a L235A and a P329G mutation or PA-LALA mutations, i.e. the constant region carries a L234A, a L235A and a P329A mutation, or a AEASS, i.e. the constant region carries a L234A, a L235A and a P329A mutation or a L234A, a L235E, G237A, A330S and a P331S

mutation. The skilled person will be aware of possibilities to engineer the Fc region to obtain a desired effect.

Surrogate ADCC assays constitute an industry standard to quantitate an agent's potency to mediate ADCC as described in the experimental part. Engineered Jurkat reporter cells
5 carry an NFAT-responsive luciferase gene and an Fc receptor, such as human FcγRIIIa. Binding of the Fc receptor to bound antibody results in NFAT induction through receptor clustering and therefore a luciferase signal. Absence of binding and therefore clustering does not result in a luciferase signal. Cells expressing either no target protein (e.g. HEK or DF-1 cells or human hematopoietic cancer cells such as TF-1, KG-1, KASUMI-1, K562 or
10 Jurkat engineered to be CD45-deficient (e.g. a CD45 knock-out or human T-cell cancer cells such as Jurkat cells with a CD45 knock-out), the wildtype protein (e.g. HEK-CD45 or DF-1-CD45, or TF-1, KG-1, KASUMI-1 or Jurkat cell lines) or individual variants (e.g. CD45 variants) were incubated with the test agent (e.g. antibody Refmab # 1) and mixed with the ADCC reporter cells. Then luciferase was measured to quantify the ADCC signal. The luciferase
15 luminescence signals were normalized to the maximal signal observed in HEK-CD45, DF-1-CD45 or the corresponding myeloid or T cell cancer cell line. ADCC was measured with an ADCC Reporter Assay (Promega, Cat.No. G7015).

Other potential modes-of-action in line with the present disclosure are possible as well. This includes antibody-mediated displacement of ligands of CD45, or antibody
20 internalization in conjunction with the use of an antibody drug conjugate. An alternative way of depleting target cells is through the use of T cell engager molecules. For example, a bispecific T cell engager using a CD45 binding site derived from antibody Refmab # 1 and a CD3 (OKT3) binding site may be used. The same target cells used for the ADCC assay are used. Primary human T cells and the bispecific T cell engager are added. Activation of
25 human T cells was quantified by FACS by determining the frequency of CD69 upregulation and/or cytokine release.

The depleting agent according to the present disclosure binds specifically to one isoform of CD45 and allows the depletion of cells expressing said isoform.

More preferably, in specific embodiments, said depleting agent according to the present disclosure does not bind or binds substantially weaker to a first isoform of CD45 but binds specifically to a second isoform of CD45 and allows the depletion of said cells expressing said second isoform of CD45, in particular in methods of use as disclosed herein. In particular, said depleting agent which does not bind or binds substantially weaker to a first isoform of CD45 but binds specifically to a second isoform of CD45 expressed in patient's cell is used to deplete patient's cells but not hematopoietic stem cells or their progeny expressing said first isoform of CD45 transplanted to restore hematopoiesis in said patient.

In another specific embodiments, said depleting agent according to the present disclosure does not bind or binds substantially weaker to a second isoform of CD45 but binds specifically to a first isoform of CD45 and allows the depletion of cells expressing said first isoform of CD45, in particular in methods of use as disclosed herein. In particular, said depleting agent which does not bind or binds substantially weaker to a second isoform of CD45 but binds specifically to a first isoform of CD45 expressed in transplanted cells is used to deplete specifically transplanted cells to avoid eventual severe side effects such as graft-versus-host disease due to transplantation.

Selective depletion of cells expressing a specific isoform of CD45 can be achieved without limitation by complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP).

In certain embodiments, the antigen binding region is coupled to an effector compound such as a drug or a toxin. Such conjugates are referred to herein as "**immunoconjugates**", "**antibody-drug conjugates**" or "**ADCs**". A cytotoxin or cytotoxic agent includes any agent that is detrimental to (e.g., kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, maytansinoids, calicheamicins, indolinobenzodiazepines, pyrolobenzodiazepines, pyrrolidinobenzodiazepines, camptothecins, topotecan, irinotecan,

belotecan, deruxtecan, alpha-amanitin, microcystins, auristatins and puromycin and analogs or homologs thereof.

In another particular embodiment, said depleting agent is an immune cell harboring an antigen receptor such as a chimeric antigen receptor (CAR). See for example Myburgh et al. Leukemia (2020) 34: 2688-703. Said immune cell may express a recombinant antigen binding region, also named antigen receptor on its cell surface. By "**recombinant**" is meant an antigen binding region which is not encoded by the cell in its native state, i.e. it is heterologous, non-endogenous. Expression of the recombinant antigen binding region can thus be seen to introduce a new antigen specificity to the immune cell, causing the cell to recognise and bind a previously unrecognised antigen. The antigen receptor may be isolated from any useful source. In certain embodiments of the present disclosure said cell comprising an antigen binding region is a CAR T-cell, a CAR NK cell, CAR Treg or a CAR macrophage. In a preferred embodiment of the present disclosure said cell comprising an antigen binding region is a CAR T-cell. In another preferred embodiment of the present disclosure said cell comprising an antigen binding region is a primary T cell comprising a CAR.

In a particular embodiment, said recombinant antigen receptor is a chimeric antigen receptor (CAR). CARs are fusion proteins comprising an antigen-binding region, typically derived from an antibody, linked to the signaling domain of the TCR complex. CARs can be used to direct immune cells such T-cells or NK cells against a target antigen if a suitable antigen-binding region is selected.

The antigen-binding region of a CAR is typically based on a scFv (single chain variable fragment) derived from an antibody. In addition to an N-terminal, extracellular antibody-binding region, CARs typically may comprise a hinge domain, which functions as a spacer to extend the antigen-binding region away from the plasma membrane of the immune effector cell on which it is expressed, a transmembrane (TM) domain, an intracellular signaling domain (e.g. the signaling domain from the zeta chain of the CD3 molecule (CD3ζ) of the TCR complex, or an equivalent) and optionally one or more co- stimulatory domains which may assist in signaling or functionality of the cell expressing the CAR. Signaling

domains from co-stimulatory molecules including CD28, OX-40 (CD134), CD27, ICOS and 4-1BB (CD137) can be added alone (second generation) or in combination (third generation) to enhance survival and increase proliferation of CAR modified immune cells.

The skilled person is able to select an appropriate antigen binding region as described
5 above with which to redirect an immune cell to be used according to the disclosure. In a particular embodiment, the immune cell for use in the method of the disclosure is a redirected T-cell, e.g. a redirected CD8+ T-cell or a redirected CD4+ T-cell, or a redirected NK cell.

Methods by which immune cells can be genetically modified to express a recombinant
10 antigen binding region are well known in the art. A nucleic acid molecule encoding the antigen receptor may be introduced into the cell in the form of e.g. a vector, or any other suitable nucleic acid construct, or by inserting the nucleic acid molecule into the genome using genome editing technologies. Vectors, and their required components, are well known in the art. Nucleic acid molecules encoding antigen binding region can be generated
15 using any method known in the art, e.g. molecular cloning using PCR. Antigen binding region sequences can be modified using commonly used methods, such as site-directed mutagenesis.

CD45

20

CD45 (UniProt: P08575; also known as protein tyrosine phosphatase receptor type C, PTPRC, LCA, B220 or LY5), is a member of protein tyrosine phosphatase (PTP) family, which includes signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation.

25 Human CD45 has the following amino acid sequence (SEQ ID No. 1):

MTMYLWLKLLAFGFAFLDTEVFVTIGQSP T P SPTGLTTAKMPSVPLSSDPLP THTTAFSPA
STFERENDFSETTTSLSPDNTSTQVSPDSL DNASAFNTTGVS SVQTPHLPTHADSQTPSA

GTDTQTFSGSAANAKLNPTPGSNAISDVPGERSTASTFPTDPVSPLTTTTLSTLAHHSSAAL
PARTSNTTITANTSDAYLNASETTTTSPSGSAVISTTTTIATTPSKPTCDEKYANITVDYL
YNKETKLFTAKLNVNENVECGNNTCTNNEVHNLTECKNASVSISHNSCTAPDKTLILDVP
PGVEKFQLHDCTQVEKADTTICLKWKNIETFTCDTQNIYRFQCGNMIFDNKEIKLENLE
5 PEHEYKCDSEILYNNHKFTNASKIIKTDFGSPGEPQIIFCRSEAAHQGVITWNPPQRSFH
NFTLCYIKETEKDCLNLDKNLIKYDLQNLKPYTKYVLSLHAYIIIAKVQRNGSAAMCHFTT
KSAPPSQVWNMTVSMTSDNSMHVKCRPPRDRNGPHERYHLEVEAGNTLVRNESHKNCDFR
VKDLQYSTDYTFKAYFHNGDYPGEPFILHHSTSYNSKALIAFLAFLIIVTSIALLVVLYK
IYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRFLAEFQSI
10 PRVFSKFPIKEARKPFNQKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPR
KYIAAQGPRDETVDDFWRMIWEQKATVIVMVTTRCEEGRNRNKCAEYWPMEEGTRAFGDVV
VKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKLRRRVNA
FSNFFSGPIVVHCSAGVGRGTGYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEA
QYILIHQALVEYNQFGETEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQ
15 HIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHDSDESSDDSDSEEPSKYINA
SFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWGEK
QTYGDIIEVDLKDSDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISM
IQVVKQKLPQKNSSEGKNHHKSTPLLIHCRDGSQQTGIFCALLNLLESAETEEVVDIFQV
VKALRKARPGMVSTFEQYQFLYDVIASSTYPAQNGQVKKNNHQEDKIEFDNEVDKVKQDAN
20 CVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS

In a particular embodiment, said surface protein is CD45. In other embodiments said surface protein is CD45 comprising the amino acid sequence of SEQ ID No. 1. In other embodiments said surface protein is CD45 consisting of the amino acid sequence of SEQ ID
25 No. 1.

In certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic
30 DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient

having cells expressing said second isoform of said surface protein and preferably wherein said first and second isoforms are functional. Preferably, said mammalian cell is a human cell.

In certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein, wherein said first and second isoforms are functional. Preferably, said mammalian cell is a human cell.

In certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein, wherein said first and second isoforms are substantially functionally identical. Preferably, said mammalian cell is a human cell.

Several functions are reported for CD45. In certain embodiments the present disclosure related to a first and a second isoform of CD45 wherein both isoforms are functional. In certain embodiments the present disclosure related to a first and a second isoform of CD45 wherein both isoforms are functional indistinguishable. The present invention **"functionally indistinguishable"** refers to a first and a second isoform of CD45 that are equally capable of performing the same function within a cell without significant impairment. In other words, the first and the second isoform are functionally largely indistinguishable. A slight functional impairment may be acceptable. In a preferred embodiment, said first isoform of CD45 remains functional and retain the capacity of

performing the same function as the corresponding wild-type isoform within a cell without significant impairment.

One function of CD45 is the inclusion into the immunological synapse, thereby preventing T-cell receptor engagement and T-cell activation. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform prevent T-cell receptor engagement upon inclusion into the immunological synapse. Preferably, said mammalian cell is a human cell.

One function of CD45 is the dephosphorylation of target protein. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform dephosphorylate target proteins of CD45. Preferably, said mammalian cell is a human cell.

One function of CD45 is the dephosphorylation of tyrosine kinase Lck. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or

genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform dephosphorylate tyrosine kinase Lck. Lck (UniProt: P06239) is a member of the Src family of protein tyrosine kinases (PTKs). Lck is a key signaling molecule in the selection and maturation of developing T-cells. Preferably, said mammalian cell is a human cell.

One function of CD45 is the dephosphorylation of tyrosine kinase Lck at position Y505. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform dephosphorylate tyrosine kinase Lck at position Y505. Preferably, said mammalian cell is a human cell.

One function of CD45 is the exclusion from the immunological synapse, thereby allowing activation of the TCR signaling cascade. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform activate the TCR signaling cascade. Preferably, said mammalian cell is a human cell.

One function of CD45 is the increase of cytokine production. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a

patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells
5 expressing said second isoform of said surface protein and wherein said first and said second isoform increase cytokine production. Preferably, said mammalian cell is a human cell.

One function of CD45 is the increase of the proliferation of T cells. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells
10 expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells
15 expressing said second isoform of said surface protein and wherein said first and said second isoform increase the proliferation of T cells. Preferably, said mammalian cell is a human cell.

One function of CD45 is the normal differentiation of hematopoietic cells. This can for example be tested in humanized mice, for example by implanting engineered HSCs into
20 humanized mice. Therefore, in certain embodiments the present disclosure relates to a mammalian cell or a population of cells expressing a first isoform of the surface protein CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of said surface protein, wherein said cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered
25 allele, wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of said surface protein and wherein said first and said second isoform lead to a normal differentiation of hematopoietic cells. Preferably, said mammalian cell is a human cell.

In line with the present disclosure, it is also possible to combine additional variants or isoforms of CD45 within the methods and compositions of the present disclosure. Such isoforms may for example include double mutants. Such isoforms may for example also include single and double mutants. The methods and compositions of the present disclosure may also be combined with cells carrying a CD45 knock out, e.g., a permanent knock out or a temporarily knock out (e.g. via CRISPRoff). The methods and compositions of the present disclosure may also be used in the depletion of myeloid cells in solid tumors in order to enhance tumor responses.

The methods and compositions of the present disclosure may also be combined with cells combinations, in particular when said surface protein is CD45 with knock out of other targets, such as CD117, CD123, DLL-1, CD33, CD7, CLEC12A, CD44, Flt, CD300F, EVI2B, TPO and combinations thereof.

The methods and compositions of the present disclosure may also comprise cells expressing first isoform of CD45 and other surface protein variants such as CD117 variants, CD123 variants, DLL-1 variants, CD33 variants, CD7 variants, CLEC12A (CD371) variants, CD44 variants Flt (CD135) variants, CD300F variants, EVI2B variants, TPO variants and any combination thereof.

Polymorphism of CD45

20

The cell expressing the first isoform of CD45 according to the present disclosure comprises genomic DNA with at least one polymorphic allele in the nucleic acid encoding said CD45 isoform. In particular, said polymorphism induces at least one mutation involved in the binding of a specific agent in comparison to said second isoform.

Said polymorphism is preferably within a nucleic acid sequence encoding the surface protein region of CD45 involved in binding of the first agent, preferably located in the extracellular portion of CD45, in particular in a solvent-exposed secondary structure

element. More particularly, said polymorphism is within a nucleic acid sequence encoding at least one specific amino acid residue involved in binding of the first agent. Said polymorphism can be a mutation such as a deletion, a substitution, an insertion, or a combination thereof of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 or 20 nucleotides. In a
5 particular embodiment, said polymorphism is a single nucleotide polymorphism.

The term “**isoform**” refers to a variant of a protein which differs from another variant of the same protein by at least one amino acid difference. In the context of the present disclosure such difference may be a substitution of a single amino acid, but such differences may also be double, triple or multiple amino acid substitutions, or insertions or deletions.
10 Also naturally occurring SNPs are isoforms.

The difference in the sequence of the two isoforms may also be genetically introduced. Also here the sequence difference is preferably within a nucleic acid sequence encoding the CD45 region involved in binding of the first agent, preferably located in the extracellular portion of said surface protein, in particular in a solvent-exposed secondary structure
15 element. More particularly, said sequence difference is within a nucleic acid sequence encoding at least one specific amino acid residue involved in binding of the first agent. Said sequence difference can be a mutation such as a deletion, a substitution, an insertion or a combination thereof of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 or 20 nucleotides. In a particular embodiment, said sequence difference is a single point mutation.

20 The present disclosure provides polymorphisms in CD45, including in particular polymorphisms including substitution of the residues E230, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, E329, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373. In certain embodiments, the present disclosure provides polymorphisms in CD45, including in particular polymorphisms including substitution of the residues E230, N257,
25 E259, F331, K352 or E353. Particular preferred polymorphisms include substitutions of the residue E230, wherein E230 is substituted with K. Other preferred polymorphisms include substitutions of the residue N257, wherein N257 is substituted with D, E, H, K, R, S, T or V. Other preferred polymorphisms include substitutions of the residue F331, wherein F331 is substituted with G. Other preferred polymorphisms include substitutions of the residue

K352, wherein K352 is substituted with H, I, L, M, N, Q, S or T. Yet other preferred polymorphisms include substitutions of the residue E353, wherein E353 is substituted with K or R.

- In certain embodiments the present disclosure relates to a variant CD45 polypeptide, wherein said variant CD45 polypeptide comprises at least one mutation in an amino acid selected from E230, N257, E259, T264, N267, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, Y372 and Y373 of wild type human CD45. In preferred embodiments said mutation is selected from E230, N257, E259, T264, N267, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, Y372 and Y373.
- It will be appreciated that amino acid may be designated by the 3-letter code or the 1-letter code, which both are familiar to the skilled person.

Table 1 shows the 20 natural occurring amino acids:

Amino acid	Three letter code	One letter code
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
cysteine	Cys	C
glutamic acid	Glu	E
glutamine	Gln	Q
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T

tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

In the experiments of the present disclosure certain variants of specific residues were identified. For practical reasons it is impossible to test any and all possible variants. It will however be understood that an identified variant may be substituted with a similar amino acid residue. For example, an acidic amino acid can be replaced by another acidic amino acid, since it can be expected to have the same effect. Likewise a charged amino acid can be replaced by another charged amino acid. As an example T264E is expected to be equivalent to T264D, since both, D and E are acidic amino acids.

10 Natural polymorphism

In a particular embodiment, said cell according to the present disclosure is selected from a subject comprising native genomic DNA with at least one natural polymorphism allele, preferably single nucleotide polymorphism (SNP) in the nucleic acid encoding said isoform.

15 In a particular embodiment, cells are selected from a subject that comprises native genomic DNA with at least one natural polymorphism allele, in particular SNP, in a nucleic acid sequence encoding CD45 region involved in anti- CD45 agent binding, preferably located in the extracellular portion of said surface protein, more preferably in a solvent-exposed secondary structure element.

20 Certain naturally occurring SNPs are described in the literature. These naturally SNPs may be used within the spirit of the present disclosure with a respective binding agent which is able to discriminate such SNP from another isoform of CD45.

Some naturally occurring SNPs of human CD45 are shown in Table 2. A list of natural occurring SNPs can also be found here: https://gnomad.broadinstitute.org/gene/ENSG00000081237?dataset=gnomad_r2_1

Table 2:

5

wildtype	position	mutation	rsIDs
Thr	262	Ile	rs1298769494
Asn	284	Asp	rs199673441
Ile	294	Leu	rs2230606
Ile	326	Val	rs41314039
Glu	327	Lys	rs2274367
Thr	330	Ile	rs148025707
Asn	349	Ser	rs79141749
Asp	431	Asn	rs140403368
Leu	439	Leu	rs375608198
Lys	479	Glu	rs61749266
Arg	509	Cys	rs746951007
Leu	526	Leu	rs148497366
Asp	536	Asn	rs144856406
Arg	538	His	rs140422357
Asp	558	Asn	rs760244730
Glu	562	Asp	rs774053060

10

Gene editing

15 In another particular embodiment, said cell expressing the first isoform of CD45 according to the present disclosure is obtained by gene editing, preferably by changing the sequence encoding said surface protein in the patient’s native genomic DNA.

20 The cell can be genetically engineered by introducing into the cell a gene editing system to induce said polymorphism resulting in insertion, deletion and/or substitution of amino acids of the surface protein. Said gene editing modality targets a nucleic acid sequence, named herein target sequence encoding surface protein region involved in first agent binding as described above. In particular, when said surface protein is CD45, said gene editing modality targets a nucleic acid encoding at least one amino acid residue in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331,

D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1. Preferably amino acid residue E230 is substituted with K, and/or residue Y232 is substituted with C, and/or residue N257 is substituted with A, D, E, H, K, R, S, T or V, and/or residue E259 is substituted with H, K, N, V, G, R, T or Q, and/or residue T264 is substituted with D or E, and/or residue
5 N267 is substituted with A, H, L, S or V, and/or residue N286 is substituted with D, G, L or R, and/or residue E329 is substituted with A, and/or residue F331 is substituted with A or G, and/or residue Y340 is substituted with A, G, N, Q or S, and/or residue K352 is substituted with A, D, E, G, H, I, L, M, N, Q, S, T or Y, and/or residue E353 is substituted with A, H, I, K, L, S, T or R. Gene editing enzymes may be sequence-specific nucleases, base editors, prime
10 editors or CRISPR-transposon based systems.

The term “**nuclease**” refers to a wild type or variant enzyme capable of catalyzing the hydrolysis (cleavage) of phosphodiester bonds between nucleotides of a nucleic acid (DNA or RNA) molecule, preferably a DNA molecule. By “**cleavage**” is intended a double-strand break or a single-strand break event.

15 The term “**sequence-specific nuclease**” refers to a nuclease which cleaves nucleic acid in a sequence-specific manner. Different types of site-specific nucleases can be used, such as Meganucleases, TAL-nucleases (TALEN), Zing-finger nucleases (ZFN), or RNA/DNA guided endonucleases like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system and Argonaute (Review in Li et al., Nature Signal transduction and
20 targeted Therapy, 5, 2020; Guha et al., Computational and Structural Biotechnology Journal, 2017, 15, 146-160).

According to the present disclosure, the nuclease generates a DNA cleavage within a target sequence, said target sequence encodes a surface protein region involved in first agent binding as described above. In particular embodiments, the inventors use CRISPR
25 system to induce a cleavage within a target sequence encoding surface protein region recognized by first agent as described above.

By “**target sequence**”, it is intended targeting a part of the sequence encoding the region on CD45 involved in first agent binding as described as described above and/or sequences

adjacent to said region on CD45 involved in first agent binding, in particular at least one (one or two) sequence of up to 50 nucleotides adjacent to said region on CD45 involved in first agent binding, preferably 20, 15, 10, 9, 8, 7, 6 or 5 nucleotides adjacent to said agent binding site.

- 5 CRISPR system involves two or more components, Cas protein (CRISPR-associated protein) and a guide RNA. The guide RNA can be a single guide RNA or a dual guide RNA. Cas protein is a DNA endonuclease that uses guide RNA sequence as a guide to recognize and generate double-strand cleavage in DNA that is complementary to the target sequence. Cas systems that generate single strand breaks require only one nuclease domain. Cas systems that generate double strand breaks require two nuclease domains. Cas protein may comprise two active cutting sites, such as HNH nuclease domain and RuvC-like nuclease domain.

By Cas protein is also meant an engineered endonuclease, homologue or orthologue of Cas 9 which is capable of cleaving target nucleic acid sequence. In particular embodiments, Cas protein may induce a cleavage in the nucleic acid target sequence which can correspond to either a double-stranded break or a single-stranded break. Cas protein variant may be a Cas endonuclease that does not naturally exist in nature and that is obtained by protein engineering or by random mutagenesis. The Cas protein can be one type of the Cas proteins known in the art. Non-limiting examples of Cas proteins include

15 Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as CsnI and CsxI2), SaCas9, Cas12, Cas12a (Cpf1), Cas10, CsyI, Csy2, Csy3, CseI, Cse2, CseI, Cse2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, CmrI, Cmr3, Cmr4, Cmr5, Cnrr6, CsbI, Csb2, Csb3, CsxI7, CsxM, Csx10, Csl6, CsaX, Csx3, Csl, CsxI5, CsfI, Csf2, Cso, Csf4, homologs, orthologs thereof, or modified versions thereof. Preferably Cas protein is *Streptococcus pyogenes* Cas 9 protein.

- 25 Cas is contacted with a guide RNA (gRNA) designed to comprise a complementary sequence to the target sequence to specifically induce DNA cleavage within said target sequence, in particular according to the present disclosure a complementary sequence of a part of target sequence encoding surface protein region recognized by agent as described above.

As used herein, a “**guide RNA**”, “**gRNA**”, “**sgRNA**” or “**single guide RNA**” refers to a nucleic acid that promotes the specific targeting or homing of a gRNA/Cas complex to a target nucleic acid.

5 In particular, gRNA refers to RNA that comprises a transactivating crRNA (tracrRNA) and a crRNA. Preferably, said guide RNA corresponds to a crRNA and tracrRNA which can be used separately or fused together to generate a single guide RNA. The complementary sequence pairing with the target sequence recruits Cas to bind and cleave the DNA at the target sequence.

10 According to the present disclosure, crRNA is engineered to comprise a complementary sequence to a part of a target sequence as described above encoding surface protein region recognized by agent, such that it is capable of targeting said region. In a preferred embodiment sgRNA is used to target the binding site of the said binding agent. In another preferred embodiment, the guide RNA contains chemically modifications known to the person skilled in the art.

15 In a particular embodiment, the crRNA comprises a sequence of 5 to 50 nucleotides, preferably 15 to 30 nucleotides, more preferably 20 nucleotides which is complementary to the target sequence. As used herein, the terms “**complementary sequence**” refers to the sequence part of a polynucleotide (e.g., part of crRNA or tracrRNA) that can hybridize to another part of polynucleotides under standard low stringent conditions. Preferentially, 20 the sequences are complementary to each other pursuant to the complementarity between two nucleic acid strands relying on Watson-Crick base pairing between the strands, i.e., the inherent base pairing between adenine and thymine (A-T) nucleotides and guanine and cytosine (G-C) nucleotides. Said gRNA can be designed by any methods known by one of skill in the art in view of the present disclosure.

25 According to the present disclosure said target sequence encodes surface protein region on CD45 involved in first agent binding, preferably located in the extracellular portion of CD45, more preferably in an extracellular loop in comparison to said second isoform, again more preferably comprising amino acid residues involved in agent binding.

In a preferred embodiment, when surface protein is CD45, said target sequence encodes a CD45 region involved in binding of a first agent, such as anti- CD45 agent binding as disclosed above. Preferably said target sequence encodes at least one residue in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331,
5 D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1.

The DNA strand break that is introduced by the nuclease according to the disclosure can result in mutation of the DNA at the cleavage site via non-homologous end joining (NHEJ) which often results in small insertions and/or deletions or replacement of the DNA surrounding the cleavage site via homology-directed repair (HDR).

10 In a preferred embodiment, said polymorphism within nucleic acid encoding the isoform of CD45 is induced via HDR repair following the DNA cleavage and the introduction of an exogenous nucleotide sequence, named herein HDR template.

HDR template comprises a first and a second portion of sequence which are homologous to regions 5' and 3' of the target sequence, respectively and a middle sequence portion
15 comprising polymorphism. Following cleavage of the target sequence, a homologous recombination event is achieved between the genome containing the target sequence and the HDR template and the genomic sequence containing the target sequence is replaced by the exogenous sequence.

Preferably, homologous sequences of at least 20 bp, preferably more than 30 bp, more
20 preferably more than 50 bp and most preferably less than 200 bp are used. Homologous sequences may be dsDNA or ssDNA. Preferably the homologous sequences are ds DNA. Indeed, shared DNA homologies are located in regions flanking upstream and downstream the site of the break and the exogenous sequence to be introduced should be located between the two arms. The flanking sequences may be symmetrical or asymmetrical. Both
25 strands of the target nucleic acid, i.e. the plus strand or the minus strand, may be targeted. Optionally, a PAM sequence may be used, which may be silenced to improve HDR.

In a preferred embodiment, the cell according to the present disclosure is genetically engineered by introducing into said cell said site-specific nuclease which targets the

sequence encoding the region on CD45 recognized by said first agent as described above and a HDR template.

In another particular embodiment, said gene editing enzyme is a DNA base editor as described in Komor et al., Nature 533, 420-424, and in Rees HA, Liu DR. Nat Rev Genet. 2018;19: 770-788, or a prime editor as described in Anzalone et al. Nature, 2019, 576: 149-157, Matsoukas et al., Front Genet. (2020) 11: 528, Chen et al. Cell (2021) 184: 5635-52, Koblan et al, Nat Biotechnol (2021) 39: 1414-25 and Kantor A. et al. Int. J. Mol. Sci. 2020, 21(6240). Base editor or prime editor can be used to introduce mutations at specific sites in the target sequence.

- 10 According to the present disclosure, the base editor or prime editor generates a mutation within the target sequence by sequence-specific targeting of the sequence encoding the region on CD45 involved in first agent binding.

In particular, said base editor or prime editor are CRISPR base or prime editors. Said CRISPR base or prime editor may comprise as catalytically inactive sequence specific
15 nuclease a dead Cas protein (dCas). It may also comprise Cas9 with a mutated nuclease domain. dCas refers to a modified Cas nuclease which lacks endonucleolytic activity. Nuclease activity can be inhibited or prevented in dCas proteins by one or more mutations and/or one or more deletions in the HNH and/or RuvC-like catalytic domains of the Cas protein. The resulting dCas protein lacks nuclease activity but bind to a guide RNA (gRNA)-
20 DNA complex with high specificity and efficiency to specific target sequence. In particular embodiment, said dCas may be a Cas nickase wherein one catalytic domain of the Cas is inhibited or prevented.

Said base editor is complexed with a guide RNA (gRNA) designed to comprise a complementary sequence of the target nucleic acid sequence to specifically bind said target
25 sequence as described above.

Said gRNA can be designed by any methods known by one of skill in the art in view of the present disclosure. In a particular embodiment, said gRNA may target the sequence encoding the region on CD45 recognized by said first agent as described above.

As non-limiting examples said base editor is a nucleotide deaminase domain fused to a dead Cas protein, in particular Cas nickase. Said nucleotide deaminase may be an adenosine deaminase or cytidine deaminase. Said nucleotide deaminase may be natural or engineered deaminase.

- 5 In a particular embodiment, said base editor may be as non-limiting examples selected from the group consisting of: BE1, BE2, BE3, BE4, HF-BE3, Sa-BE3, Sa-BE4, BE4-Gam, saBE4-Gam, YE1-BE3, EE-BE3, YE2-BE3, YEE-BE3, VQR-BE3, VRER-BE3, SaKKH-BE3, cas12a-BE, Target-AID, Target-AID-NG, xBE3, eA3A-BE3, A3A-BE3, BE-PLUS, TAM, CRIPS-X, ABE7.9, ABE7.10, ABE7.10* xABE, ABESa, ABEmax, ABE8e, VQR-ABE, VRER-ABE and SaKKH-ABE.
- 10 Said prime editor consists of a fusion of a catalytically inactive sequence specific nuclease as described above, particularly a Cas nickase and a catalytically active engineered reverse transcriptase (RT) enzyme. Said fusion protein is used in combination with a prime editing guide RNA (pegRNA) which contains the complementary sequence to the target sequence as described above, particularly when surface protein is CD45 comprises one of the
- 15 sequences described in the Table 13 and also an additional sequence comprising a sequence that binds to the primer binding site region on the DNA. In particular embodiment, said reverse transcriptase enzyme is a Maloney murine leukemia virus RT enzyme and variants thereof. Said prime editor may be as non-limiting examples selected from the group consisting of: PE1, PE2, PE3 and PE3b, or any of the prime editors described
- 20 in Chen et al. Cell (2021) 184: 5635-52 or Koblan et al, Nat Biotechnol (2021) 39: 1414-25.

Anti-CD45 agents

- Several anti-CD45 moieties are known in the art, some of which are currently in
- 25 development. QA17A19 (Biolegend, #393411) and HI30 (Biolegend, #304001) are mouse anti-human CD45 antibody which are commercially available. Various anti-CD45 from Magenta are in development, most as antibody drug conjugates (e.g. WO2017219025, WO2020092654). BC8 is a mouse hybridoma antibody commercially available from

IchorBio (#ICH1155). The BC8 antibody is the basis for an anti-CD45 antibody-radioconjugate developed by Actinium (WO2017155937, WO2019084258, WO2020159656). Other anti-CD45 antibodies are disclosed in WO2016016442, WO2019115791 and WO2020058495 (INSERM), WO2017009473 (UCB), WO2019129178
5 (Shanghai Baize Medical Laboratory), WO2020018580 (Fred Hutchinson) and WO2020170254 (Ramot At Tel Aviv University). These and other anti-CD45 moieties may be used in the context of the present disclosure. Several anti-CD45 antibodies were also generated in the present disclosure, in full length antibody format, as well as in Fab format. Details are provided in Example 1.

10 In a particular embodiment, said depleting agent which binds to said second isoform of CD45 and does not bind or binds substantially weaker to said first isoform of CD45 as described above binds specifically to an epitope including the amino acids E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 and/or Y373 of SEQ ID NO: 1. More preferably, said depleting
15 agents binds specifically to an epitope including the amino acids N286, F331, K352 and/or E353 of SEQ ID NO: 1. In other preferred embodiments said depleting agents binds specifically to an epitope including the amino acids E230, Y232, N257 and/or E259 of SEQ ID NO: 1.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region
20 comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1,
25 VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8 and VLCDR3 is SEQ ID NO: 9.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61 and VLCDR3 is SEQ ID NO: 9.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region derived from and retaining the binding specificity of an antibody comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8 and VLCDR3 is SEQ ID NO: 9.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region derived from and retaining the binding specificity of an antibody comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61 and VLCDR3 is SEQ ID NO: 9.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region derived competing with an antibody comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8 and VLCDR3 is SEQ ID NO: 9.

In a preferred embodiment, said anti-CD45 agent comprises an antigen binding region derived competing with an antibody comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61 and VLCDR3 is SEQ ID NO: 9.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- a) an antibody heavy chain variable domain (VH) comprising the variable heavy chain of SEQ ID NO: 2; and
- b) an antibody light chain variable domain (VL) comprising variable light chain of SEQ ID NO: 3.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- a) an antibody heavy chain variable domain (VH) comprising the variable heavy chain of SEQ ID NO: 58; and

- b) an antibody light chain variable domain (VL) comprising variable light chain of SEQ ID NO: 59.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- 5 a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 10 16 and VLCDR3 is SEQ ID NO: 17.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- a) an antibody heavy chain variable domain (VH) comprising the variable heavy chain of SEQ ID NO: 10; and
- 15 b) an antibody light chain variable domain (VL) comprising variable light chain of SEQ ID NO: 11.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- 20 a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24 and VLCDR3 is SEQ ID NO: 25.

In another preferred embodiment, said anti-CD45 agent comprises an antigen binding region comprising:

- a) an antibody heavy chain variable domain (VH) comprising the variable heavy chain of SEQ ID NO: 18; and
- 5 b) an antibody light chain variable domain (VL) comprising variable light chain of SEQ ID NO: 19.

In another preferred embodiment, said anti-CD45 agent is an antibody selected from Refmab #1, Refmab #2, Refmab #3, Refmab #4 and Refmab #5. In another preferred embodiment, said anti-CD45 agent is an antibody selected from QA17A19 (Biolegend,
10 #393411), BC8 (IchorBio; #ICH1155), AbA (WO2020092654A1, SEQ ID NO 1 & 5), HI30 (Biolegend, #304001) and 2D1 (R&D Systems, #MAB1430). In another preferred embodiment, said anti-CD45 agent is an antibody comprising the CDR of an antibody selected from QA17A19 (Biolegend, #393411), BC8 (IchorBio; #ICH1155), AbA (WO2020092654A1, SEQ ID NO 1 & 5), HI30 (Biolegend, #304001) and 2D1 (R&D Systems,
15 #MAB1430). In another preferred embodiment, said anti-CD45 agent is an antibody competing for binding to CD45 with an antibody selected from QA17A19 (Biolegend, #393411), BC8 (IchorBio; #ICH1155), AbA (WO2020092654A1, SEQ ID NO 1 & 5), HI30 (Biolegend, #304001) and 2D1 (R&D Systems, #MAB1430).

In certain preferred embodiments, said depleting agent is or is derived from QA17A19
20 (Biolegend, #393411; Refmab #1), wherein said depleting agent binds to one isoform of CD45 but not, or substantially weaker, to a second isoform of CD45, wherein one of said isoform is wild type CD45 and the other isoform has a mutation in at least one of the following amino acid residues of CD45: F331, K352 and E353. Preferably said mutation includes one or more of the following: F331G, K352H, K352E, K352D, K352I, K352L, K352M,
25 K352N, K352Q, K352S, K352T, E353K or E353R.

In certain preferred embodiments, said depleting agent is or is derived from AbA (WO2020092654A1, SEQ ID NO 1 & 5; Refmab #2), wherein said depleting agent binds to one isoform of CD45 but not, or substantially weaker, to a second isoform of CD45, wherein

one of said isoform is wild type CD45 and the other isoform has a mutation in amino acid residues N257 of CD45. Preferably said mutation is N257D, N257E, N257K, N257R or N257T.

In certain preferred embodiments, said depleting agent is or is derived from BC8 (IchorBio; #ICH1155; Refmab #4), wherein said depleting agent binds to one isoform of CD45 but not, or substantially weaker, to a second isoform of CD45, wherein one of said isoform is wild type CD45 and the other isoform has a mutation in at least one of the following amino acid residues of CD 45: E230, N257, E259, T264, N267, N286, S287, D292, F331, D334, Y340, K352, E353 and Y373. Preferably said mutation includes one or more of the following: E230K, N257T, N257H, N257R, N257S, N257V, E259G, E259N or E259Q.

It is further contemplated that the antigen-binding region of the anti-CD45 antibody may be further screened or optimized for their binding properties as above defined. In particular, it is contemplated that said antigen binding region thereof may have 1, 2, 3, 4, 5, 6, or more alterations in the amino acid sequence of 1, 2, 3, 4, 5, or 6 CDRs of monoclonal antibodies provided herein. It is contemplated that the amino acid in position 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of CDR1, CDR2, CDR3, CDR4, CDR5, or CDR6 of the VJ or VDJ region of the light or heavy variable region of antigen binding region may have an insertion, deletion, or substitution with a conserved or non-conserved amino acid. Such amino acids that can either be substituted or constitute the substitution are disclosed above.

In some embodiments, the amino acid differences are conservative substitutions, i.e., substitutions of one amino acid with another having similar chemical or physical properties (size, charge or polarity), which substitution generally does not adversely affect the biochemical, biophysical and/or biological properties of the CD45 protein. In particular, the substitution does not disrupt the interaction of the antibody with the CD45 antigen. Said conservative substitution(s) are advantageously chosen within one of the following five groups: Group 1-small aliphatic, non-polar or slightly polar residues (A, S, T, P, G); Group 2-polar, negatively charged residues and their amides (D, N, E, Q); Group 3-polar, positively charged residues (H, R, K); Group 4-large aliphatic, nonpolar residues (M, L, I, V, C); and Group 5-large, aromatic residues (F, Y, W).

In a more particular embodiment, said first antigen-binding region comprises a heavy chain variable domain comprising or consisting of any one of amino acid sequences selected from SEQ ID NO: 2, 10, 18 and 58, and/or a light chain variable domain comprising or consisting of any one of amino acid sequences selected from SEQ ID NO: 3, 11, 19 and 59.

Said first antigen binding region thereof with amino acid sequences having at least 90%, for example, at least 95%, 96%, 97%, 98%, or 99% identity to any one of the above defined amino acid sequences are also part of the present disclosure, typically first antigen binding region have at least equal or higher binding activities than said first antigen binding region consisting of heavy chain consisting of any one of amino acid sequences selected from SEQ ID NO: 2, 10, 18 and 58 and/or a light chain variable domain comprising or consisting of any one of amino acid sequences selected from SEQ ID NO: 3, 11, 19 and 59.

In a particular embodiment, said anti-CD45 agent can be a bispecific CD45 antibody, comprising at least one first binding specificity for CD45, for example, one antigen-binding region of anti-CD45 as described herein and a second binding specificity for a second target epitope or target antigen.

According to the present disclosure, said anti-CD45 agent can be an immune cell harboring an antigen receptor targeting CD45, such as a CAR targeting CD45, said antigen receptor comprising an antigen binding region as described above.

In specific embodiments, said immune cell (e.g., T cell) harboring a CAR targeting CD45 recognizes a second isoform of CD45 as expressed in a patient in need thereof, and does not recognize a first isoform of CD45. In particular said immune cell may bind specifically to an epitope including the amino acids E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1. More preferably, said immune cell binds specifically to an epitope including the amino acids N286, F331, K352 or E353 of SEQ ID NO: 1.

In specific embodiments, said anti-CD45 agent can be an immune cell (e.g., T cell) harboring a CAR, said CAR comprising an antigen-binding region, e.g. scFv, comprising

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 8, VLCDR2 is SEQ ID NO: 9, VLCDR3 is SEQ ID NO: 10.

In specific embodiments, said anti-CD45 agent can be an immune cell (e.g., T cell) harboring a CAR, said CAR comprising an antigen-binding region, e.g. scFv, comprising

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61 and VLCDR3 is SEQ ID NO: 9.

In a more particular embodiment, said anti-CD45 agent can be an immune cell (e.g., T cell) harboring a CAR comprising said first antigen-binding region e.g. scFv comprising a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3.

In another more particular embodiment, said anti-CD45 agent can be an immune cell (e.g., T cell) harboring a CAR comprising said first antigen-binding region e.g. scFv comprising a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.

According to the present disclosure, said anti-CD45 agent can be an immune cell harboring an antigen receptor targeting CD45, such as a CAR targeting a specific isoform of CD45, said antigen receptor comprising an antigen binding region as described above and

said immune cell either not expresses CD45 or expresses an isoform of CD45 which is not recognized by said CAR.

In specific embodiments, said anti-CD45 agent can be an immune cell (e.g. T cell) harboring a CAR, said CAR targeting a specific isoform of CD45 comprising an antigen-binding region, e.g. scFv, comprising:

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16 and VLCDR3 is SEQ ID NO: 17;

and said immune cell either not expresses CD45 or expresses an isoform of CD45 which is not recognized by said CAR.

In a more particular embodiment, said anti-CD45 agent can be an immune cell (e.g. T cell) harboring a CAR comprising said first antigen-binding region e.g. scFv comprising a heavy chain variable domain comprising or consisting of an amino acid sequence of SEQ ID NO: 10 and a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11, and said immune cell expresses an isoform of CD45 which is not recognized by said CAR.

In a more preferred embodiment, said anti-CD45 agent is antibody Refmab #1 as described in the examples.

In another preferred embodiment said anti-CD45 agent can be an immune cell harboring a CAR targeting a specific isoform of CD45 as described in the examples.

In particular, the disclosure also relates to depleting anti-CD45 agents as disclosed above (for example CAR cell composition or antibodies) comprising a first or a second antigen

binding region for use in selectively depleting the host cells or transferred cells respectively, in a subject in need thereof.

Cells expressing a first isoform of CD45

5

The present disclosure relates to a mammalian cell, preferably a hematopoietic cell, or a population of cells expressing a first isoform of CD45 wherein said cell or population of cells express a first isoform of CD45 comprising at least one polymorphic allele in the nucleic acid encoding said first isoform, and wherein said first isoform is not recognized by the
10 depleting agent comprising a first antigen binding region as described herein. Preferably, said mammalian cell is a human cell.

Said cell or population of cells are particularly useful in medical treatment in a patient expressing a second isoform of CD45.

In a particular embodiment, said cells (e.g. hematopoietic stem cell) encoding or
15 expressing said first isoform of CD45 not recognized by a depleting agent (e.g. hematopoietic cells) are particularly useful in medical treatment to restore normal hematopoiesis after immunotherapy, such as adoptive cell transfer in a patient expressing said second isoform, in particular wherein the treatment comprises administering a therapeutically efficient amount of said hematopoietic cells expressing said first isoform of
20 CD45 in combination with a therapeutically efficient amount of a depleting agent targeting said second isoform of CD45. In particular, said hematopoietic cells, preferably hematopoietic stem cells are administered subsequently to said depleting agent. In another particular embodiment, said hematopoietic cells, preferably hematopoietic stem cells can be administered before or concurrently to said depleting agent

25 In another particular embodiment, said cells expressing said first isoform of CD45 specifically recognized by depleting agent which does not bind or binds substantially weaker second isoform of CD45 are particularly useful in medical treatment in a patient

expressing said second isoform of CD45, in particular to avoid severe side-effect related to transplanted cells carrying the first isoform (safety switch), wherein the treatment comprises administering a therapeutically efficient amount of a depleting agent targeting said first isoform of CD45. In particular, said hematopoietic cells, preferably immune cells
5 harboring a CAR are administered prior to said depleting agent.

As used herein, the term cell relates to mammalian cells, preferably human cells.

In a particular embodiment, said cells are hematopoietic cells. Hematopoietic cells comprise immune cells including lymphocytes, such as B cells and T cells, natural killer cells, myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils,
10 granulocytes, dendritic cells (DC) and plasmacytoid dendritic cells (pDCs).

In a preferred embodiment, said immune cells are T cells. In another preferred embodiment, said immune cells are primary T cells. As used herein, the term “T cell” includes cells bearing a T cell receptor (TCR) or a cell derived from a T cell bearing a TCR. T-cells according to the disclosure can be selected from the group consisting of inflammatory
15 T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes, memory T-lymphocytes, tumor infiltrating lymphocytes and helper T- lymphocytes included both type 1 and 2 helper T cells and Th17 helper cells. In another embodiment, said cell can be derived from the group consisting of CD4+ T- lymphocytes and CD8+ T-lymphocytes or non-classical T cells such as MR1 restricted T cells, MAIT cells, NKT cells, gamma delta T cells or innate-
20 like T cells.

T-cells can be obtained from a number of non-limiting 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 embodiments, T-cells can be obtained from a unit of blood collected from a subject using
25 any number of techniques known to the skilled person. Alternatively, T cells can be differentiated from iPS cells.

In another preferred embodiment, said hematopoietic cells are hematopoietic stem cells. The stem cells can be adult stem cells, embryonic stem cells, more particularly non-human

stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human stem cells are CD34⁺ cells. Hematopoietic stem cells can be differentiated from iPS cells or can be harvested from umbilical cord blood, from bone marrow or from mobilized
5 or not mobilized peripheral blood.

In certain embodiments, the cell is an allogeneic cell which refers to a cell derived from a donor that presents with an HLA genotype that is identical, similar or different to the HLA genotype of the person receiving the cell. The donor may be a related or unrelated person. In certain embodiments, the cell is an autologous cell which refers to a cell derived from
10 the same person that is receiving the cell.

Said cells may originate from a healthy donor or from a patient, in particular from a patient diagnosed with cancer, genetic disease or an auto-immune disease or from a patient diagnosed with an infection. Hematopoietic cells can be extracted from blood, bone marrow or derived from stem cells. HSC's can for example be derived from iPS (induced
15 pluripotent stem cells.

A person skilled in the art will choose the more appropriate cells according to the patient or subject to be transplanted.

The disclosure further relates to a composition of cells or a population of cells for use in the therapy as disclosed herein.

20 CAR

For use in adoptive cell transfer therapy, said cell expressing first isoform of CD45 according to the present disclosure may be modified to display desired specificities and enhanced functionalities. In a particular embodiment, said cell may express a recombinant antigen binding region, also named antigen receptor on its cell surface as described above.
25 In a particular embodiment, said recombinant antigen receptor is a chimeric antigen receptor (CAR). According to the present disclosure, said immune cell expressing a first isoform of CD45 and a CAR can be specifically depleted by the administration of a

therapeutically efficient amount of an agent which comprises a second antigen binding region which specifically binds to said first isoform of CD45 but not to the second isoform of CD45, thereby avoiding eventual severe side effects due to transplantation of said immune cells.

- 5 In a particular embodiment, the immune cell is redirected against a cancer antigen. By “**cancer antigen**” is meant any antigen (i.e., a molecule capable of inducing an immune response) which is associated with cancer. An antigen as defined herein may be any type of molecule which induces an immune response, e.g., it may be a polysaccharide or a lipid, but most preferably it is a peptide (or protein). Human cancer antigens may be human or
- 10 human derived. A cancer antigen may be a tumor-specific antigen, by which is meant an antigen which is not found in healthy cells. Tumor-specific antigens generally result from mutations, in particular frame-shift mutations which generate a wholly new amino acid sequence not found in the healthy human proteome.

- Cancer antigens also include tumor-associated antigens, which are antigens whose
- 15 expression or production is associated with, but not limited to, tumor cells. Examples of tumor-associated antigens include for instance Her2, prostate stem cell antigen (PSCA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen- 125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD99, CD117,
- 20 CD123, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-DI, muscle-specific actin, neurofilament, neuron- specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor-1, the dimeric form of the pyruvate kinase
- 25 isoenzyme type M2 (tumor M2- PK), CD 19, CD22, CD33, CD123, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six- transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein. In another specific

embodiment, said tumor-associated antigen or tumor-specific antigen is integrin $\alpha\text{v}\beta 3$ (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene), or Ral-B.

In a particular embodiment, for use in adoptive cell transfer therapy, preferably for the treatment of malignant hematopoietic disease such as acute myeloid leukemia (AML) or B-
5 acute lymphoblastic leukemia (B-ALL), the immune cell according to the present disclosure expresses a recombinant antigen binding region such as a CAR targeting CD45. Said cell expressing the first isoform and expressing the CAR (e.g. CAR- CD45) can be further specifically depleted by administering a depleting agent comprising a second antigen-binding region which binds specifically to the first isoform of CD45, but does not bind or
10 binds substantially weaker to the second isoform of CD45, thereby avoiding eventual severe side effects such as graft-versus-host disease due to the transplantation.

In specific embodiments, said immune cell (e.g. T cell) expressing the first isoform harbors a CAR targeting CD45, said CAR comprising an antigen-binding region, e.g. scFv, comprising an antigen-binding region which binds specifically to an epitope of CD45 located within the
15 N-terminal domain, or within the polypeptide including the amino acids E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 and/or Y373 of SEQ ID NO: 1, more preferably amino acids E230, N257, E259, F331, K352 and/or E353 of SEQ ID NO: 1.

In particular, said immune cell (e.g., T cell) expressing first isoform harbors a CAR targeting
20 CD45 comprising an antigen-binding region, e.g. scFv, comprising

- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and
- b) an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1,
25 VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24 and VLCDR3 is SEQ ID NO: 25,

more preferably comprising an antigen-binding region comprising a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 18 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 19.

5

In vitro method for preparing cell expressing first isoform

The cell expressing the first isoform of CD45 according to the present disclosure can be genetically engineered by introducing into said cell a nucleic acid construct (e.g., mRNA) encoding at least one gene editing enzyme or ribonucleoprotein complex comprising gene
10 editing enzyme and/or HDR template as described above. Alternatively, the gene editing system is transduced into said cells via a viral system, such as an adenoviral system. Said cell can also be genetically engineered by further introducing into said cell a nucleic acid construct encoding a CAR as described above. In particular, said method is an *ex vivo* method performed on a culture of cells.

15 The term “**nucleic acid construct**” as used herein refers to a nucleic acid molecule resulting from the use of recombinant DNA technology. A nucleic acid construct is a nucleic acid molecule, either single- or double-stranded, which has been modified to contain segments of nucleic acid sequences, which are combined and juxtaposed in a manner, which would not otherwise exist in nature. A nucleic acid construct usually is a “**vector**”,
20 i.e., a nucleic acid molecule which is used to deliver exogenously created DNA into a host cell.

Preferably, the nucleic acid construct comprises said gene editing enzyme, HDR template and/or CAR, operably linked to one or more control sequences. Said control sequences may be a ubiquitous, tissue-specific or inducible promoter which is functional in cells of target
25 organs (i.e., hematopoietic cell). Such sequences which are well-known in the art include in particular a promoter, and further regulatory sequences capable of further controlling the expression of a transgene, such as without limitation, enhancer, terminator, intron, silencer.

The nucleic acid construct as described above may be contained in an expression vector. The vector may be an autonomously replicating vector, i.e., a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial
5 chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated.

Examples of appropriate vectors include, but are not limited to, recombinant integrating
10 or non-integrating viral vectors and vectors derived from recombinant bacteriophage DNA, plasmid DNA or cosmid DNA. Preferably, the vector is a recombinant integrating or non-integrating viral vector. Examples of recombinant viral vectors include, but not limited to, vectors derived from herpes virus, retroviruses, lentivirus, vaccinia viruses, adenoviruses, adeno-associated viruses or bovine papilloma virus.

15 The present disclosure relates to a method for expressing a first isoform of a cell surface protein in a cell by introducing into said cell a nucleic acid construct (e.g. mRNA) encoding the gene editing enzyme or ribonucleoprotein complex comprising gene editing enzyme and/or HDR template as described above. Said method may further comprise a step of introducing into said cell a nucleic acid construct encoding a CAR. Said method involves
20 introducing gene editing enzyme such as Cas protein, base editor or prime editor and guide RNA (crRNA, tracrRNA, or fusion guide RNA or pegRNA) into a cell. In particular, said gene editing enzyme is CRISPR/Cas gene editing enzyme as described above. In a more particular embodiment, said gene editing enzyme is a site-specific nuclease, more preferably CRISPR/Cas nuclease comprising a guide RNA and Cas protein, wherein said guide RNA in
25 combination with Cas protein cleaves and induces cleavage within said target sequence comprising a nucleic acid encoding surface protein region involved in agent binding as described above.

Said Cas nuclease may be a high fidelity Cas nuclease such as a high fidelity Cas9 nuclease.

Said gene editing enzyme, preferably guide RNA and/or Cas protein, base editor or prime editor as described above may be synthesized in situ in the cell as a result of the introduction of nucleic acid construct, preferably expression vector encoding said gene editing enzyme such as guide RNA and/or Cas protein, base editor or prime editor as described above into the cell. Alternatively, said gene editing enzyme such as guide RNA and/or Cas protein, base editor or prime editor may be produced outside the cell and then introduced thereto.

Said nucleic acid construct or expression vector can be introduced into cell by any methods known in the art and include, as non-limiting examples, stable transduction methods in which the nucleic acid construct or expression vector is integrated into the cell genome, transient transfection methods in which the nucleic acid construct or expression vector is not integrated into the genome of the cell and virus-mediated methods. For example, transient transformation methods include for example microinjection, electroporation, cell squeezing, particle bombardment or in vivo targeting approaches.

15

In vivo editing

The cell expressing the first isoform of CD45 according to the present disclosure may also be edited *in vivo*. Various technologies exist that enable therapeutic *in vivo* gene editing, including viral vectors, lipid nanoparticles and virus-like particles (see for example Cell (2022) 185: 2806-27. The molecular machinery to convert CD45 into a first isoform of CD45 which is not recognized by the depleting agent can be accomplished by any of these methods.

In certain embodiments, the present disclosure relates a pharmaceutical composition comprising molecular machinery capable of *in vivo* editing a gene and a depleting agent, wherein said molecular machinery capable of *in vivo* editing a gene comprises all components required to introduce a point mutation of wild type CD45 in a target cell into an isoform of CD45, and

wherein said depleting agent binds to wild type CD45, but not to said isoform of CD45
for use in a medical treatment in a patient in need thereof.

Preferably said isoform of CD45 is characterized by a substitution of the lysine at position 352 of wild type CD45 to a glutamic acid. Alternatively, said isoform of CD45 is characterized
5 by a substitution of the lysine at position 352 of wild type CD45 to an aspartic acid. Alternatively, said isoform of CD45 is characterized by a substitution of the lysine at position 352 of wild type CD45 to a histidine. Alternatively, said isoform of CD45 is characterized by a substitution of the lysine at position 353 of wild type CD45 to a lysine. Alternatively, said isoform of CD45 is characterized by a substitution of the lysine at
10 position 353 of wild type CD45 to an arginine.

Also preferably, said depleting agent comprises

- i. an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6, and
- 15 ii. an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8 and VLCDR3 is SEQ ID NO: 9.

Alternatively, said depleting agent comprises

- i. an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6, and
- 20 ii. an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61 and VLCDR3 is SEQ ID NO: 9.

25 Also preferably said isoform of CD45 is characterized by a substitution of the asparagine at position 257 of wild type CD45 to a glutamic acid. Alternatively, said isoform of CD45 is characterized by a substitution of the asparagine at position 257 of wild type CD45 to a

lysine. Alternatively, said isoform of CD45 is characterized by a substitution of the asparagine at position 257 of wild type CD45 to an arginine. Alternatively, said isoform of CD45 is characterized by a substitution of the asparagine at position 257 of wild type CD45 to a threonine. Alternatively, said isoform of CD45 is characterized by a substitution of the glutamic acid at position 259 of wild type CD45 to a valine. Alternatively, said isoform of CD45 is characterized by a substitution of the glutamic acid at position 259 of wild type CD45 to a glycine. Alternatively, said isoform of CD45 is characterized by a substitution of the tyrosine at position 232 of wild type CD45 to a cysteine. Alternatively, said isoform of CD45 is characterized by a substitution of the asparagine at position 286 of wild type CD45 to an aspartic acid.

Also preferably, said depleting agent comprises

- i. an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22, and
- 15 ii. an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24 and VLCDR3 is SEQ ID NO: 25.

Also preferably, said depleting agent comprises

- i. an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14, and
- 20 ii. an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16 and VLCDR3 is SEQ ID NO: 17.

25 In certain embodiments, the present disclosure relates to a human cell or a population of human cells expressing a first isoform of CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a second isoform of CD45,

wherein said human cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele,

wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of CD45, and

- 5 wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1. In certain embodiments said medical treatment comprises administering a therapeutically efficient amount of said cell or population
- 10 of cells expressing said first isoform of CD45 to said patient in need thereof, in combination with a therapeutically efficient amount of a depleting agent comprising an antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing said second isoform of CD45.

- In certain embodiments said polymorphic or genetically engineered allele is characterized
- 15 by at least one substitution of an amino acid in position N286, F331, K352 or E353 of SEQ ID NO: 1.

In certain embodiments said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position N286, F331, K352 or E353 of SEQ ID NO: 1, and said depleting agent comprises an antigen-binding region, selected from

- 20 a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9,
- 25 more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3; or

- b) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.
- 10 In certain embodiments said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position F331, K352 or E353 of SEQ ID NO: 1, and said depleting agent binds to the same epitope as an antigen-binding region, selected from
- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3; or
- b) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO:

58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.

In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position K352 of SEQ ID NO: 1. In certain
5 embodiments said substitution of the amino acid in position K352 of SEQ ID NO: 1 is selected from K352E, K352H, K352I, K352L, K352M, K352N, K352Q, K352S and K352T, preferably wherein said substitution is K352D, K352E and K352H, and more preferably wherein said substitution is K352E.

In certain embodiments said polymorphic or genetically engineered allele is characterized
10 by at least one substitution of an amino acid in position E230, Y232, N257, E259 or N286 of SEQ ID NO: 1.

In certain embodiments said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, Y232, N257, E259 or N286 of SEQ ID NO: 1, and said depleting agent comprises an antigen-binding region comprising
15 an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24, VLCDR3 is SEQ ID NO: 25,
20 more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 18 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 19.

In certain embodiments said polymorphic or genetically engineered allele is characterized
25 by at least one substitution of an amino acid in position E230, Y232, N257, E259 or N286 of SEQ ID NO: 1, and said depleting agent binds to the same epitope as an antigen-binding region comprising

an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24, VLCDR3 is SEQ ID NO: 25,

more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 18 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 19.

10 In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain embodiments said substitution of the amino acid in position in position N257 of SEQ ID NO: 1 is a N257E, N257K, N257R or N257T substitution, preferably wherein said substitution is a N257R substitution.

15 In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position E259 of SEQ ID NO: 1. In certain embodiments said substitution of the amino acid in position in position E259 of SEQ ID NO: 1 is a E259N, a E259Q, a E259V or a E259G substitution, preferably wherein said substitution is a E259V substitution.

20 In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position Y232 of SEQ ID NO: 1, preferably wherein said substitution is a Y232C substitution.

In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N286 of SEQ ID NO: 1, preferably wherein said substitution is N286D.

In certain embodiments said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain

embodiments said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1, and said depleting agent comprises an antigen-binding region comprising

5 an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16, VLCDR3 is SEQ ID NO: 17, preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

In certain embodiments said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1, and said depleting agent binds to the same epitope as an antigen-binding region comprising

20 an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16, VLCDR3 is SEQ ID NO: 17, preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

25 In preferred embodiments, said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1. In certain embodiments said substitution of the amino acid in position in position N257 of SEQ ID NO:

1 is a N257E, N257K, N257R or N257T substitution, preferably wherein said substitution is a N257R substitution.

Pharmaceutical composition and therapeutic use

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In a further aspect, the present disclosure also provides a pharmaceutical composition comprising cells or a population of cells expressing a first isoform of CD45 as described above with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

10 In a particular embodiment, said cell expressing the first isoform of CD45 is a hematopoietic stem cell.

In another particular embodiments, said cell expressing said first isoform of CD45 is an immune cell, preferably a T-cell, more preferably a primary T cell, bearing a chimeric antigen receptor (CAR), preferably a CAR which targets the second isoform of CD45
15 expressed by said patient's cells as described above.

The pharmaceutical composition may further comprise a depleting agent comprising a first or second antigen binding region as described above.

The pharmaceutical composition is formulated in a pharmaceutically acceptable carrier according to the route of administration. Preferably, the composition is formulated to be
20 administered by intravenous injection. Pharmaceutical compositions suitable for such administration may comprise the cells expressing first isoform as described above, in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions (e.g., balanced salt solution (BSS)), dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions
25 or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes or suspending or thickening agents.

Optionally, the composition comprising cells expressing first isoform of CD45 may be frozen for storage at any temperature appropriate for storage of the cells. For example, the cells may be frozen at about -20°C , -80°C or any other appropriate temperature. Cryogenically frozen cells may be stored in appropriate containers and prepared for storage
5 to reduce risk of cell damage and maximize the likelihood that the cells will survive thawing. Alternatively, the cells may also be maintained at room temperature or refrigerated, e.g., at about 4°C .

The present disclosure relates to the cell or population of cells expressing a first isoform of CD45 as described above for use as a medicament, in particular for use in
10 immunotherapy such as adoptive cell transfer therapy in a patient.

According to the present disclosure, said cell or population of cells (e.g., hematopoietic cells) expressing a first isoform of CD45 as described above, is used in a medical treatment in a patient in need thereof, wherein said medical treatment comprises administering a therapeutically efficient amount of cell or population of cells expressing said first isoform
15 of CD45, in combination with a therapeutically efficient amount of a depleting agent (e.g. a CAR cell or antibody) that binds specifically to the second isoform or first isoform of CD45 to specifically depleting the patients or the transplanted cells, respectively.

As used herein, the term “**in combination**” or “**in combination therapy**” means that two (or more) different treatments are delivered to the subject during the course of the
20 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
25 “**simultaneous**” or “**concurrent delivery**”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered. In one embodiment, a depleting agent that binds to a second isoform or a first isoform of CD45 is administered at a dose and/or dosing schedule described herein, and

the cells expressing the first isoform are administered at a dose and/or a dosing schedule described herein. In some embodiments, “in combination with,” is not intended to imply that the depleting agent targeting the second (e.g. CAR cells or antibody recognizing a second isoform of CD45) or the first isoform of CD45 and compositions of cells expressing

5 said first isoform of CD45, must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of this disclosure. The depleting agent (e.g. CAR cells or antibody targeting a second isoform of CD45) can be administered concurrently with, prior to or subsequent to a dose of the hematopoietic stem cells expressing the first isoform of CD45. In certain embodiments,

10 each agent will be administered at a dose and/or on a time schedule determined for that particular agent.

Adoptive cell transfer therapy according to the disclosure can be used to treat patients diagnosed with cancer, genetic disease, autoimmune disease, infectious disease, a disease requiring a hematopoietic stem cell transplantation (HSCT), the prevention of organ

15 rejection, the tumor conditioning regimen, tumor maintenance treatment, minimal residual disease, the prevention of relapse.

The present disclosure also relates to the use of cells expressing a first isoform of CD45 as described above in the manufacture of a medicament for adoptive transfer cell therapy in a patient.

20 As used herein, the term “**subject**”, or “**patient**” refers to an animal, preferably to a mammal in which an immune response can be elicited including human, pig, chimpanzee, dog, cat, cow, mouse, rabbit or rat. More preferably, the patient is a human, including adult, child and human at the prenatal stage.

As used herein, the term “**treatment**”, “**treat**” or “**treating**” refers to any act intended to

25 ameliorate the health status of patients such as therapy, prevention, prophylaxis and retardation of the disease. In certain embodiments, such term refers to the amelioration or eradication of a disease or symptoms associated with a disease. In other embodiments,

this term refers to minimizing the spread or worsening of the disease resulting from the administration of one or more therapeutic agents to a subject with such a disease.

Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-
5 solid tumors (such as hematological tumors, for example, leukemias and lymphomas including relapses and treatment-related tumors e.g. secondary malignancies after use of cytotoxic therapy and hematopoietic stem cell transplantation (HSCT)) or may comprise solid tumors.

The term "**autoimmune disease**" as used herein is defined as a disorder that results from
10 an autoimmune response. An autoimmune disease is the result of an inappropriate and excessive response to a self-antigen.

Infectious disease is a disease caused by pathogenic microorganism such as bacteria, viruses, parasites or fungi. In particular embodiments, infections according to the disclosure occur in immunosuppressed patients, such as patients after HSCT or patients
15 who received a solid organ transplantation.

In a preferred embodiment, the present disclosure relates to a cell expressing first isoform of CD45 as described above for use in hematological cancer, preferably leukemia or lymphoproliferative disorders. Said leukemia can be selected from the group consisting of: acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), blastic
20 plasmacytoid dendritic cell neoplasm (BPDCN), myeloproliferative neoplasms (MPN) including chronic myelogenous leukemia (CML), myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndromes including chronic myelomonocytic leukemia (CMML), chronic lymphoid leukemia (CLL), B- and T-cell non-hodgkin lymphomas, acute biphenotypic leukemia, hairy cell leukemia, interleukin-3
25 receptor subunit alpha positive leukemia, B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), hodgkin lymphoma (HL), systemic mastocytosis and preferably MDS, preferably AML or BPDCN.

In a particular embodiment, said cell or population of cells (e.g., hematopoietic cells) expressing a first isoform of CD45, can be used for the treatment of solid tumor, in particular for selective depletion of myeloid cells in solid tumors in a patient, to enable immunotherapy agent such as immune checkpoint inhibitors, CAR T-cells or tumor
5 infiltrating lymphocytes to access to tumors since myeloid cells in tumors can be immunosuppressive. In this situation said cell or population of cells (e.g., hematopoietic cells) expressing a first isoform CD45 as described above, can serve to replenish the hematopoietic system that might be affected by the treatment intended to deplete the myeloid cells in solid tumors.

10 In another particular embodiment, said cell or population of cells (e.g., hematopoietic cells) expressing a first isoform of CD45 as described above can be used for the treatment of autoimmune disease such as lupus, multiple sclerosis, scleroderma or systemic sclerosis.

The disclosure also relates to depleting agents (for example CAR cell composition or antibodies) comprising a first or a second antigen binding region for use in selectively
15 depleting the host cells or transferred cells respectively, in a subject in need thereof.

Method for depleting specifically patient cells and not transplanted cells

According to the present disclosure, said cell or population of cells (e.g. hematopoietic cells) expressing a first isoform of CD45 as described above, is used in a medical treatment in a patient in need thereof, wherein said medical treatment comprises administering a
20 therapeutically efficient amount of said cells or population of cells expressing said first isoform of CD45, in combination with a therapeutically efficient amount of a depleting agent (e.g. a CAR cell or antibody) that binds specifically to a second isoform of CD45.

Indeed, during immunotherapy, immunodepleting agent, such as a CAR expressing immune cells directed to CD45, can be administered to a patient to target and kill tumoral
25 cells. However, as tumoral surface proteins are also expressed at the surface of normal hematopoietic cells, this strategy can induce severe side effects to the patients by altering hematopoiesis. To restore hematopoiesis in the patient, hematopoietic cells can be subsequently transplanted into the patient. However, these cells need to be resistant to

said agent, i.e., the depleting agent for CD45 expressing cells, in order not to be targeted by it.

Thus, alternatively, according to the present disclosure, the depleting agent comprising a first antigen binding region which binds specifically to a second isoform of CD45 can be
5 administered to ablate specifically patient cells expressing said second isoform of CD45 and not transplanted cells expressing said first isoform of CD45. The selective depletion of patient cells, but not transplanted cells, allows to reconstitute the patient with a healthy hematopoietic system which will no longer be depleted by immunodepleting agent. Thus, according to the present therapeutic use, the patients have a functional immune system
10 rather than go through a prolonged phase of immunodepression. The use of cells according to the present disclosure eliminates infections as a major complication of current HSC transplantation.

In another embodiment, the present disclosure relates to a method for adoptive cell transfer therapy, preferably for hematopoietic stem cell transplantation to restore normal
15 hematopoiesis in a patient having cells expressing a second isoform of CD45 comprising:

(i) administering an effective amount of a cell (e.g. hematopoietic stem cells) expressing a first isoform of CD45 wherein said cell expressing said first isoform of CD45 comprises genomic DNA with at least one polymorphic allele, preferably single nucleotide polymorphism (SNP) allele, or a genetically engineered allele in the nucleic acid encoding
20 said first isoform and wherein said polymorphism is not present in the genome of the patient having cells expressing said second isoform of CD45 or a pharmaceutical composition thereof; and

(ii) administering a therapeutically efficient amount of an agent comprising at least a first antigen-binding region which binds specifically to said second isoform of CD45 and
25 does not bind or binds substantially weaker to said first isoform of CD45 to deplete specifically cells expressing said second isoform of CD45 (patient's cells).

Said cells expressing the first isoform of CD45 or pharmaceutical compositions thereof are administered to a subject in combination with (e.g., before, simultaneously or following) an agent comprising a first antigen binding region as described above.

In a preferred embodiment, the depleting agent (e.g., CAR cells or antibody targeting a second isoform of CD45 is administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, 12 weeks, or 16 weeks before), or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, 12 weeks, or 16 weeks after) a dose of the hematopoietic stem cells expressing a first isoform of said surface protein (e.g., a first isoform of CD45).

By a “**therapeutically efficient amount**” or “**effective amount**” is intended a number of cells, in particular hematopoietic stem cells expressing the first isoform of CD45 as described above administered to a subject that is sufficient to constitute a treatment as defined above, in particular restoration of normal hematopoiesis in a patient.

The administration of the cell or pharmaceutical composition according to the present disclosure may be carried out in any convenient manner, including injection, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermal, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In another embodiment, the cells or pharmaceutical compositions of the present disclosure are preferably administered by intravenous injection. The cells or pharmaceutical compositions of the present disclosure may be injected directly into a tumor, lymph node, or site of infection.

The administration of the cells or population of cells can consist of the administration of 10^4 - 10^9 cells per kg body weight, preferably 10^5 to 10^7 cells/kg body weight, more preferably 2×10^6 - 5×10^6 cells per kg body weight including all integer values of cell numbers

within those ranges. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. The cells or population of cells can be administered in one or more doses. Timing of administration is within the judgment of managing physician and
5 depends on the clinical condition of the subject. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art.

In particular, the disclosure also relates to depleting anti- CD45 agents as disclosed above
10 (for example CAR cell composition or antibodies) comprising a first antigen binding region for use in selectively depleting the host cells in a subject in need thereof.

Method for depleting specifically transplanted cells and not patient cells (safety switch).

According to the present disclosure, said cell or population of cells (e.g. hematopoietic cells) expressing a first isoform of CD45 as described above, is used in a medical treatment
15 in a patient in need thereof, wherein said medical treatment comprises administering a therapeutically efficient amount of a cell or a population of cells expressing said first isoform of CD45, in combination with a therapeutically efficient amount of a depleting agent (e.g. a CAR cell or antibody) that binds specifically to said first isoform CD45.

The cell or population of cells, preferably immune cells expressing the first isoform of
20 CD45 of the present disclosure is particularly used in adoptive transfer cell transfer therapy into a patient. Said transplanted cell expressing said first isoform of CD45 can be further depleted in patients by administering a therapeutically efficient amount of a depleting agent comprising a second antigen binding region which specifically binds to the first isoform of CD45 particularly and does not bind or binds substantially weaker to the second
25 isoform of CD45 expressed by patient's cells to avoid eventual severe side effects such as graft-versus-host disease due to the transplantation. In this case, said agent comprising a second antigen-binding region which binds specifically to said first isoform of CD45 (expressed by transplanted cell) is administered to deplete specifically transplanted cells

and not patient cells. Selective depletion of the transplanted cells constitutes an important safety feature by providing a “**safety switch**”.

Graft-versus-host disease (GvHD) relates to a medical complication following the receipt of transplanted tissue from a genetically different person. Immune cells in the donated
5 tissue (the graft) recognize the recipient (the host) as foreign. In certain embodiments, the medical condition is graft-versus-host disease caused by hematopoietic stem cell transplantation or adoptive cell transfer therapy wherein immune cells are transferred into patient.

Said side effects can also occur when transplanted cells, particularly immune cells
10 harboring a CAR have severe side effects such as cytokine release syndrome and/or neurotoxicity. In this case, the transplanted cells expressing the first isoform of CD45 can be eliminated when said cells become malignant or cause any type of unwanted on-target or off-target damage as a safety switch.

The present disclosure relates to a method for adoptive cell transfer therapy in a patient
15 having cells expressing a second isoform of CD45 comprising:

- (i) administering an effective amount of a cell expressing a first isoform of CD45 wherein said cell expressing said first isoform of CD45 comprises genomic DNA with at least one polymorphism allele, preferably single nucleotide polymorphism (SNP) allele, or a genetically engineered allele in the nucleic acid encoding said first isoform CD45 and
20 wherein said polymorphism is not present in the genome of the patient having cells expressing said second isoform of CD45 or a pharmaceutical composition thereof; and
- (ii) administering a therapeutically efficient amount of an agent comprising at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind or binds substantially weaker to said second isoform of CD45 to deplete
25 specifically cells expressing said first isoform of CD45.

Said cells expressing the first isoform of CD45 or pharmaceutical compositions thereof are administered to a subject in combination with (e.g., before, simultaneously or following) an agent comprising a second antigen binding region as described above.

In a preferred embodiment, the depleting agent (e.g. CAR cells or antibody targeting a second isoform of CD45) is administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, 12 weeks, or 16 weeks before), or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, 12 weeks, or 16 weeks after) a dose of the hematopoietic stem cells expressing a first isoform of CD45.

The administration of the cells or pharmaceutical composition according to the present disclosure may be carried out in any convenient manner, including injection, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermal, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In another embodiment, the cells or pharmaceutical compositions of the present disclosure are preferably administered by intravenous injection. The cells or pharmaceutical compositions of the present disclosure may be injected directly into a tumor, lymph node, or site of infection.

The administration of the cells or population of cells can consist of the administration of 10^4 - 10^9 cells per kg body weight, preferably 10^5 to 10^7 cells/kg body weight including all integer values of cell numbers within those ranges. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. The cells or population of cells can be administered in one or more doses. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the subject. The cells or population of cells may be obtained from any source, such as a blood bank or a

donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art.

Accordingly, in specific embodiments, the disclosure relates to a depleting agent (e.g. a CAR cell or an antibody) for use in preventing or reducing the risk of severe side effects in a patient having received a cell expressing a first isoform of CD45 as described above, wherein said patient have native cells expressing a second isoform of CD45, and wherein said depleting agent comprising at least a second antigen-binding region which binds specifically to said first isoform of CD45 and does not bind or binds substantially weaker to said second isoform of CD45.

- 10 In another aspect, the present disclosure relates to a kit for expressing a first isoform CD45 as describe above into a cell, said kit comprising a gene editing enzyme, such as guide RNA in combination with a Cas protein, base editor or prime editor, nucleic acid construct, expression vector as described above or isolated cell according to the present disclosure.

- 15 In another aspect, the present disclosure relates to a human cell or population of human cells according to the present disclosure for a medical use, wherein said medical use comprises administering a therapeutically efficient amount of said human cell or population of human cells expressing said first isoform of CD45 to said patient in need thereof in combination with a therapeutically efficient amount of a depleting agent comprising at least a second antigen-binding region that binds specifically to said first isoform of CD45 to specifically deplete transferred cells expressing first isoform of CD45, preferably for use in adoptive cell transfer therapy, more preferably for the treatment of malignant hematopoietic disease such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), T-cell non-Hodgkin lymphomas (T-NHLs), chronic myeloid leukemia (CML), hairy cell leukemias (HCL), T-cell acute lymphoblastic leukemia (T-ALL), non-Hodgkin's lymphoma (NHL) or follicular lymphomas (FL), again more preferably wherein said depleting agent is administered subsequently to said human cell or population of human cells expressing said first isoform of CD45 to avoid eventual severe side effects such as graft-versus-host disease due to the transplantation. In certain embodiments said human

cell or population of human cells expressing said first isoform is an immune cell, preferably a T-cell, bearing a chimeric antigen receptor (CAR).

EXAMPLES

5

Example 1: Generation of anti-CD45 Fabs and MAbs

Five different anti- CD45 antibodies were generated in Fab and MAb format based on publicly available sequence information or sources. Variable chains and CDRs (Kabat) of the antibodies (Refmab’s #1, #2 and #5) are shown in Table 5. Refmab #3 (HI30) is a mouse hybridoma antibody available from Biolegend (#304001). Refmab #5 (2D1) is a mouse hybridoma antibody available from R&D Systems (#MAB1430).

Table 5:

Refmab #1		
SEQ ID No.	Comment	Sequence
2	VH	EVQLVESGGDLVKPGGSLKLSCAASGFAFSNYDMSWVRQTPEK RLEWVAYISSGGVSTYYPDTVKG RFTISRDNAKNTLYLQMSSLKS EDTAMYYCARRYDVWWYFDVWGAGTTVTVSS
3	VL	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKP GQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY YCFQGSHVPMYTFGGGKLEIK
4	HCDR1	NYDMS
5	HCDR2	YISSGGVSTYYPDTVKG
6	HCDR3	RYDVWWYFDV
7	LCDR1	RSSQSIVHSNGNTYLE

8	LCDR2	KVSNRFS
9	LCDR3	FQGSHVPMYT
Refmab #2		
10	VH	EVQLVESGGDRVQPGRSLTSCVTSGFTFNNYWMTWIRQVPG KGLEWVASISSGGSIYYPDSVKGRFTISRDNAKNTLYLQMNSLR SEDTATYYCARTDERWAGAMDAWGQGTSVTVSS
11	VL	DIQMTQSPPVLSASVGDRTLSCKASQININKNLDWYQQKHGEA PKLLIYETNNLQTGIPSRFSGSGSGTDYTLTISSLQPEDVATYYCYQ HNSRFTFGSGTKLEIK
12	HCDR1	NYWMT
13	HCDR2	SISSGGSIYYPDSVKG
14	HCDR3	DERWAGAMDA
15	LCDR1	KASQININKNLD
16	LCDR2	ETNNLQT
17	LCDR3	YQHNSRFT
Refmab #4		
18	VH	EVKLLESGGGLVQPGGSLKLSAASGFDFSRYWMSWVRQAPGK GLEWIGEINPTSSSTINFTPSLKDVKFISRDNAKNTLYLQMSKVRSE DTALYYCARGNYYRYGDAMDYWGQGTSVTVSSAK
19	VL	DIALTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPG QPPKLLIYLASNLESGVPARFSGSGSGTDFTLNHPVEEEDAATYY CQHSRELPTFGSGTKLEIK
20	HCDR1	RYWMS
21	HCDR2	EINPTSSSTINFTPSLKD
22	HCDR3	GNYYRYGDAMDY
23	LCDR1	RASKSVSTSGYSYLH

24	LCDR2	LASNLES
25	LCDR3	QHSRELPFT

Additional features of the antibodies, as well as the formats and isotypes of the full length antibodies are shown in Table 6.

Table 6:

Antibody	Format/isotype	Source
Refmab #1	human IgG1	Biozym/BioLegend (#393411)
Refmab #2	human IgG1	WO2020092654A1, SEQ ID NO 1 & 5
Refmab #3	mouse IgG1	Biolegend (#304001)
Refmab #4	mouse IgG1	IchorBio (#ICH1155)
Refmab #5	mouse IgG1	R&D Systems (# MAB1430)

5

Example 2: Binding of MAbs to CD45 and optimization of assay conditions

DF-1 cells (ATCC number CRL-12203) were transfected with a construct containing wild-
10 type CD45 (SEQ ID No. 1) or with an empty vector. Binding of the antibodies to the
transfected cells and the optimal assay conditions were evaluated in 384-well format.
Detection of cellular expression was measured via high-throughput flow cytometry. Serial
dilutions of each antibody were tested for immunoreactivity against cells expressing CD45
or vector alone. The optimal screening concentration for each antibody was determined
15 based on the raw signal values and signal-to-background calculations. Results are shown in
Figure 1. Each point represents the mean of four replicates.

All five antibodies in Mab format bind to human CD45 in a concentration dependent manner. Cell transfected with the empty vector did not show any binding to anti-human CD45 antibodies.

Optimized assay conditions for flow cytometry are shown in Table 7.

5

Table 7:

Parameter	Test antibody	Test antibody	Test antibody	Test antibody	Test antibody
Cell Type	DF-1	DF-1	DF-1	DF-1	DF-1
Fixative	None	None	None	None	None
Blocking Buffer	10% goat serum	10% goat serum	10% goat serum	10% goat serum	10% goat serum
Primary antibody					
Name	Refmab #1	Refmab#2	Refmab#3	Refmab#4	Refmab#5
Target	CD45	CD45	CD45	CD45	CD45
Optimal conc.	0.063 µg/ml	0.25 µg/ml	0.5 µg/ml	0.125 µg/ml	1.0 µg/ml
Incubation (25° C)	60 min	60 min	60 min	60 min	60 min
Secondary antibody					
Target	Human IgG	Human IgG	Mouse IgG	Mouse IgG	Mouse IgG
Optimal conc.	1:400 (3.75 µg/ml)	1:400 (3.75 µg/ml)	1:400 (3.75 µg/ml)	1:400 (3.75 µg/ml)	1:400 (3.75 µg/ml)
Incubation (25° C)	30 min	30 min	30 min	30 min	30 min
Manufacturer	Jackson	Jackson	Jackson	Jackson	Jackson
Catalogue #	ImmunoResearch	ImmunoResearch	ImmunoResearch	ImmunoResearch	ImmunoResearch
Antibody ID	109-545-003	109-545-003	109-545-003	109-545-003	109-545-003
	AlexaFluor® 488 AffiniPure Goat anti-Human IgG (H+L)	AlexaFluor® 488 AffiniPure Goat anti-Human IgG (H+L)	AlexaFluor® 488 AffiniPure Goat anti-Human IgG (H+L)	AlexaFluor® 488 AffiniPure Goat anti-Human IgG (H+L)	AlexaFluor® 488 AffiniPure Goat anti-Human IgG (H+L)
Wash buffer	PBS (Ca2+, Mg2+ free)	PBS (Ca2+, Mg2+ free)	PBS (Ca2+, Mg2+ free)	PBS (Ca2+, Mg2+ free)	PBS (Ca2+, Mg2+ free)
Signal:Background	9:1	13:1	12:1	6:1	10:1

Example 3: Binding of Fabs to CD45 and optimization of assay conditions

Experiments were performed similar as described in Example 2, except that Fab
5 fragments were tested instead of full-length antibodies. Serial dilutions of each Fab were
tested for immunoreactivity against cells expressing wild-type CD45 or vector alone. The
optimal screening concentration for the Fabs was determined based on the raw signal
values and signal-to-background calculations. Results are shown in Figure 2. Each point
represents the mean of four replicates.

10

All four antibodies in Mab format bind to human CD45 in a concentration dependent
manner. Cell transfected with the empty vector did not show any binding to anti-human
CD45 antibodies.

Optimized assay conditions for high throughput flow cytometry are shown in Table 8.

15 Table 8:

Parameter	Test antibody	Test antibody	Test antibody	Test antibody
Cell Type	DF-1	DF-1	DF-1	DF-1
Fixative	None	None	None	None
Blocking Buffer	10% goat serum	10% goat serum	10% goat serum	10% goat serum
Primary antibody				
Name	Refmab #1	Refmab#2	Refmab#3	Refmab#4
Target	CD45	CD45	CD45	CD45
Optimal conc.	0.063 µg/ml	0.25 µg/ml	0.5 µg/ml	0.25 µg/ml
Incubation (25° C)	60 min	60 min	60 min	60 min
Secondary antibody				
Target	Human F(ab') ₂	Human F(ab') ₂	Human F(ab') ₂	Human F(ab') ₂
Optimal conc.	1:200 (7.5 µg/ml)	1:200 (7.5 µg/ml)	1:200 (7.5 µg/ml)	1:200 (7.5 µg/ml)
Incubation (25° C)	30 min	30 min	30 min	30 min
Manufacturer	Jackson	Jackson	Jackson	Jackson
Catalogue #	ImmunoResearch	ImmunoResearch	ImmunoResearch	ImmunoResearch
Antibody ID	109-546-006	109-546-006	109-546-006	109-546-006
	AlexaFluor® 488 AffiniPure Goat anti-Human IgG F(ab') ₂ fragment	AlexaFluor® 488 AffiniPure Goat anti-Human IgG F(ab') ₂ fragment	AlexaFluor® 488 AffiniPure Goat anti-mouse IgG F(ab') ₂ fragment	AlexaFluor® 488 AffiniPure Goat anti-mouse IgG F(ab') ₂ fragment
Wash buffer	PBS (Ca ²⁺ , Mg ²⁺ free)	PBS (Ca ²⁺ , Mg ²⁺ free)	PBS (Ca ²⁺ , Mg ²⁺ free)	PBS (Ca ²⁺ , Mg ²⁺ free)
Signal:Background	8:1	9:1	5:1	7:1

Example 4: Alanine scanning

An alanine scan on human CD45 was performed to determine the residues on CD45 that are involved in binding to the antibodies investigated. The alanine scan was performed via shotgun mutagenesis epitope mapping (Integral Molecular, Philadelphia/PA, USA) as described in Immunology (2014) 143, 13-20. Briefly, a mutation library of CD45 was created by high-throughput, site-directed mutagenesis. Each residue was individually mutated to alanine, with alanine codons mutated to serine. The mutant library was arrayed in 384-well microplates and transiently transfected into DF-1 cells. Following transfection, cells were incubated with the indicated antibodies (IgG or Fab) at concentrations pre-determined using an independent immunofluorescence titration curve on wild type CD45. Antibodies were detected using an Alexa Fluor 488-conjugated secondary antibody and mean cellular fluorescence was determined using Intellicyt iQue flow cytometry platform (Intellicyt/Sartorius). Mutated residues were identified as being critical to the antibody epitope if they did not support the reactivity of the test antibody but did support the reactivity of the control antibody, which was in each case another anti-CD45 RefMab, e.g., for RefMab #1 RefMab #2 was used as control antibody, and for RefMab #3 RefMab #4 was used as control antibody. This counter-screen strategy facilitates the exclusion of mutants that are locally misfolded or that have an expression defect. Binding of each antibody to each mutant clone was determined in duplicates. For each point, background fluorescence was subtracted from the raw data, which were then normalized to antibody reactivity with wild type CD45.

Since library screens of very high-affinity antibodies sometimes fail to yield critical residues for antibody binding, high-affinity antibodies were converted into Fab format to weaken binding sufficiently to allow identification of critical residues for binding. For cases where Fab screens under standard conditions are still insufficient to identify critical residues for binding, high stringency conditions were implemented. These conditions include combinations of increased pH, increased salinity, increased temperature, and/or increased wash time. Antibodies that required high stringency conditions are denoted "HS".

For each mutant clone, the mean binding value was plotted as a function of expression (represented by control reactivity). See Figure 3. To identify preliminary primary critical clones (circled), a threshold (dashed lines) of >70% wild-type binding to control antibody and <20% wild-type binding to test antibody was applied. Secondary clones (squared) are highlighted for clones that did not meet the set thresholds but whose decreased binding activity and proximity to critical residues suggested that the mutated residue may be part of the antibody epitope.

The result of the alanine scan is shown in Table 9. Mean binding reactivities (and ranges) are listed for all identified critical residues. Critical residues for antibody binding (outlined in dark grey) were residues whose mutations were negative for binding to test Abs, but positive for binding to control antibody. Additional secondary residues (outlined in light grey) were identified that did not meet the threshold guidelines, but whose decreased binding activity and proximity to critical residues suggested that they may be part of the antibody epitope.

Table 9:

Mutation	Mean binding (% compared to wild type)				
	Refmab #1	Refmab #2	Refmab #3	Refmab #4	Refmab #5
S27A	138,6	125,8	117,4	101,5	66,4
P28A	98,4	112,8	107,8	153,4	73,6
T29A	135,5	156,4	188,3	146,9	109,3
P30A	79,1	80,0	117,4	192,6	55,4
S31A	108,5	102,0	118,8	171,0	57,5
P32A	108,6	135,1	125,4	216,2	148,4
T33A	170,6	127,9	87,4	110,8	76,5
G34A	33,7	76,5	108,7	87,7	73,0
L35A	138,6	121,4	118,1	104,4	103,8
T36A	86,5	103,2	113,8	144,6	63,1
T37A	135,9	115,9	84,6	116,8	107,3
A38S	98,9	119,5	99,8	130,7	53,1
K39A	158,4	133,9	113,8	116,7	45,0
M40A	60,2	55,2	71,4	65,5	90,8
P41A	95,8	82,0	101,3	85,1	72,1
S42A	159,0	130,1	144,4	195,7	90,4
V43A	146,4	152,1	109,8	207,2	137,4

P44A	91,3	81,7	122,7	160,9	61,8
L45A	169,5	240,3	313,9	262,4	166,6
S46A	88,1	121,8	132,1	115,0	123,4
S47A	119,0	158,4	232,4	122,6	94,4
D48A	46,5	55,3	45,5	113,4	115,6
P49A	145,8	231,3	191,1	298,6	143,0
L50A	76,2	80,4	105,8	164,8	85,3
P51A	103,8	85,1	106,4	111,9	77,9
T52A	140,2	170,8	212,0	165,4	110,3
H53A	168,7	179,3	197,3	294,3	134,1
T54A	118,1	136,5	121,2	147,7	117,6
T55A	125,2	162,7	198,8	140,5	108,5
A56S	126,8	123,7	111,1	98,2	76,3
F57A	115,7	114,9	151,5	137,2	103,3
S58A	101,4	114,3	85,4	93,4	97,1
P59A	210,2	133,1	173,9	168,7	113,3
P59A	136,5	101,7	120,1	132,6	72,8
A60S	86,1	52,7	52,3	75,5	64,5
S61A	78,8	94,9	93,8	114,2	83,1
T62A	98,4	110,2	108,5	154,7	84,3
F63A	93,9	88,5	71,8	92,5	67,2
E64A	58,1	74,6	80,6	133,4	60,0
R65A	111,3	131,3	121,9	120,3	98,1
E66A	63,4	75,6	66,9	117,1	68,1
N67A	208,0	167,0	234,9	153,7	57,9
D68A	135,4	131,6	116,7	124,4	77,4
F69A	91,8	37,6	85,0	198,9	86,2
S70A	229,3	171,7	210,3	159,5	143,4
E71A	130,6	100,8	105,7	113,3	87,1
T72A	119,1	85,9	124,3	121,8	113,6
T73A	154,4	150,1	173,3	188,6	124,6
T74A	37,7	62,0	58,1	96,1	56,9
S75A	121,2	90,9	93,0	87,0	51,1
L76A	145,1	122,2	128,2	147,6	128,6
S77A	167,8	188,4	280,7	215,1	110,5
P78A	60,9	54,2	60,8	104,7	73,9
D79A	74,2	82,9	80,3	148,1	64,8
N80A	61,7	45,8	56,3	92,8	66,5
T81A	91,0	75,5	97,1	144,9	70,0
S82A	81,6	56,2	67,7	98,9	54,2
T83A	64,2	53,2	60,0	72,2	52,9
Q84A	53,6	64,5	114,4	79,9	48,8
V85A	171,1	109,1	117,5	128,2	88,3
S86A	78,3	59,6	67,0	87,9	59,1

P87A	163,7	147,7	103,7	155,4	93,2
D88A	59,8	50,7	64,4	46,6	33,1
S89A	72,5	130,9	102,0	148,1	93,6
L90A	124,5	110,7	100,6	87,1	64,9
D91A	64,2	67,6	119,5	110,3	57,2
N92A	144,0	159,8	175,2	179,4	209,0
A93S	101,7	100,7	125,4	115,6	79,7
S94A	215,5	203,1	372,0	166,5	148,2
A95S	121,2	129,6	215,8	168,1	68,1
F96A	137,0	114,7	118,8	125,0	122,4
N97A	57,7	77,8	70,5	48,6	44,1
T98A	88,2	94,9	105,8	200,6	82,6
T99A	135,5	108,3	112,4	126,2	60,9
G100A	123,8	99,4	119,3	109,6	112,0
V101A	103,9	133,3	150,0	139,6	68,6
S102A	100,5	84,6	77,6	120,9	87,6
S103A	74,6	78,8	72,5	130,2	53,8
V104A	98,1	90,0	96,2	193,0	71,5
Q105A	63,7	88,9	91,8	94,9	55,0
T106A	79,4	82,6	81,1	98,5	64,7
P107A	135,9	138,9	155,1	150,3	84,5
H108A	102,0	69,4	85,1	86,1	46,9
L109A	80,5	89,5	90,8	99,6	51,0
P110A	142,3	108,1	75,4	138,9	72,0
T111A	131,2	110,7	91,7	165,5	51,1
H112A	72,2	103,9	81,2	150,6	125,0
A113S	42,7	85,4	65,0	139,9	92,3
D114A	134,9	109,5	159,5	213,5	111,1
S115A	86,0	65,0	88,6	102,0	56,8
Q116A	159,2	159,1	190,8	226,9	87,9
T117A	79,4	107,4	112,5	105,2	66,5
P118A	137,1	182,0	170,0	192,3	80,8
S119A	161,0	157,1	148,7	179,6	56,7
A120S	122,0	155,1	161,7	335,1	60,3
G121A	99,0	99,6	105,5	106,6	50,9
T122A	88,4	107,6	110,4	121,8	54,8
D123A	118,5	103,0	142,6	85,4	52,7
T124A	154,1	127,2	155,1	186,1	82,8
Q125A	66,4	77,8	85,7	166,0	45,2
T126A	106,2	95,4	122,5	107,8	76,9
S128A	170,0	152,0	168,1	277,7	114,8
G129A	162,5	197,5	182,4	289,1	88,0
S130A	141,1	103,2	123,1	111,3	56,2
A131S	103,9	95,3	128,1	145,2	79,9

A132S	243,0	190,8	166,2	163,8	81,9
N133A	74,3	60,5	78,9	180,2	54,9
A134S	51,2	56,1	65,0	111,3	53,1
K135A	85,7	72,8	78,8	201,5	62,7
L136A	79,2	75,8	125,9	131,4	64,7
N137A	72,3	79,1	95,7	125,7	52,3
P138A	181,1	198,2	202,1	197,0	90,2
T139A	132,1	136,7	131,3	210,8	85,3
P140A	205,8	151,9	175,9	175,5	85,5
G141A	110,7	143,1	154,0	176,6	87,3
S142A	110,3	102,5	100,3	160,1	78,5
N143A	159,9	165,7	156,6	171,8	81,0
A144S	147,9	98,1	134,1	120,3	94,4
I145A	107,9	116,2	98,9	125,3	96,2
S146A	105,0	106,3	164,7	111,5	111,8
D147A	73,6	74,3	105,4	131,4	90,0
V148A	130,1	116,1	155,2	104,9	84,4
P149A	161,0	123,5	148,9	153,6	110,0
G150A	116,9	81,9	96,6	123,5	74,2
E151A	114,2	101,8	158,8	183,3	76,4
R152A	103,5	107,2	145,8	98,7	81,3
S153A	69,1	54,7	54,4	111,5	48,7
T154A	-1,0	1,6	4,7	6,9	-2,0
A155S	137,1	102,4	128,6	103,4	49,5
S156A	65,6	51,7	93,8	155,5	65,2
T157A	76,7	61,5	116,2	142,4	71,6
F158A	102,0	129,0	144,8	148,4	74,2
P159A	154,8	129,8	214,1	196,4	58,3
T160A	54,0	74,2	88,4	137,8	65,0
D161A	107,4	105,0	111,5	109,9	55,0
P162A	183,7	147,8	171,2	126,5	77,1
V163A	52,2	61,7	81,8	181,8	92,7
S164A	114,1	164,7	191,8	146,7	106,8
P165A	158,6	177,7	189,3	128,2	116,0
L166A	161,1	155,9	187,8	142,6	110,7
T167A	107,8	116,5	217,1	153,0	128,1
T168A	141,4	144,4	209,6	189,3	91,2
T169A	95,6	64,0	90,4	127,4	47,4
L170A	181,2	175,2	207,1	4,6	54,5
S171A	108,6	105,5	101,3	133,4	59,0
L172A	170,1	170,4	169,8	161,2	123,8
A173S	1,8	7,1	-3,7	-2,5	1,2
H174A	75,7	138,4	131,9	131,5	119,2
H175A	134,0	131,2	183,7	136,6	108,0

S176A	105,5	103,1	153,8	120,7	85,8
S177A	88,3	105,5	136,5	180,0	97,8
A178S	148,7	143,0	196,7	107,5	163,1
A179S	124,4	145,0	159,8	111,3	98,0
L180A	216,0	208,7	204,5	140,4	108,0
P181A	144,3	113,6	116,7	152,0	92,1
A182S	103,6	118,5	183,5	125,0	78,2
R183A	137,8	134,3	164,5	150,5	100,5
T184A	157,9	148,7	146,7	133,7	79,8
S185A	-0,5	1,8	1,2	4,6	-6,9
N186A	162,8	150,8	114,2	188,0	98,0
T187A	162,4	115,7	193,3	159,5	98,0
T188A	125,2	124,6	149,1	199,3	93,1
I189A	127,8	95,1	129,6	82,2	37,4
T190A	191,3	146,5	148,1	160,7	91,2
A191S	85,2	99,4	107,3	122,1	88,0
N192A	79,7	85,3	88,4	116,1	78,4
T193A	135,7	164,1	153,3	131,0	110,1
S194A	122,3	141,2	192,0	150,2	87,5
D195A	93,6	142,7	155,8	154,4	94,3
A196S	108,2	128,2	124,0	92,6	94,4
Y197A	94,5	105,4	124,2	148,0	77,9
L198A	113,3	115,6	176,8	150,1	78,7
N199A	114,9	120,2	127,8	122,4	73,0
A200S	119,6	119,4	98,1	148,0	65,7
S201A	111,9	124,8	108,8	111,5	61,0
E202A	129,1	96,4	163,2	115,0	80,8
T203A	120,8	131,5	187,3	144,3	76,8
T204A	102,0	82,2	108,1	103,8	52,4
T205A	104,2	89,6	136,2	124,4	98,8
L206A	113,4	138,1	145,2	158,3	90,2
S207A	128,3	127,2	116,2	186,0	86,1
P208A	75,7	81,8	100,4	153,8	59,1
S209A	103,8	94,9	133,7	91,5	101,3
G210A	104,9	136,6	143,5	94,7	77,1
S211A	131,1	152,9	163,1	141,7	81,7
A212S	99,8	105,1	121,3	88,0	68,6
I214A	121,0	79,3	101,5	91,9	73,4
T216A	104,5	94,8	93,5	105,1	83,0
T217A	115,6	110,1	165,2	240,2	77,7
T218A	105,8	108,1	148,7	137,7	68,7
I219A	117,9	149,1	128,3	175,6	76,1
A220S	114,8	100,9	26,4	17,9	57,8
T221A	128,2	110,0	139,8	216,4	75,0

T222A	54,2	112,4	92,9	182,8	72,7
P223A	118,1	93,5	176,4	96,6	73,4
S224A	115,0	103,2	105,1	105,2	104,8
K225A	139,9	114,0	237,5	79,5	128,5
P226A	109,9	79,0	123,9	103,2	99,1
T227A	95,4	98,8	88,0	144,4	35,3
C228A	1,0	-0,6	-1,9	1,3	-2,0
D229A	61,4	137,0	-2,7	149,8	-2,1
E230A	92,3	90,6	2,0	146,6	-1,8
K231A	63,0	80,8	77,5	145,2	55,8
Y232A	16,9	6,7	-1,2	5,6	-2,9
A233S	98,0	62,8	86,6	94,6	42,4
N234A	141,7	125,4	225,9	160,9	68,0
I235A	102,4	44,9	91,6	116,9	86,8
T236A	205,8	163,9	248,8	147,4	72,4
V237A	86,5	88,1	96,2	138,8	59,0
D238A	64,1	95,5	73,4	115,0	70,1
Y239A	3,1	2,2	2,3	1,5	1,4
L240A	99,3	88,6	106,7	137,1	43,2
Y241A	75,2	79,7	54,9	83,4	54,1
N242A	121,2	102,6	101,2	112,0	104,1
K243A	157,8	144,0	147,8	181,5	93,0
E244A	135,7	119,9	172,5	136,9	78,9
T245A	97,7	109,2	110,8	170,9	36,5
K246A	28,8	29,0	28,7	30,9	15,8
L247A	75,9	82,2	130,0	159,3	46,6
F248A	67,5	55,0	46,4	69,8	46,4
T249A	103,0	83,3	116,5	196,6	45,4
A250S	75,7	73,3	92,0	73,8	51,6
K251A	30,3	33,8	43,3	77,5	23,2
L252A	40,6	49,5	38,5	24,4	16,6
N253A	102,3	115,0	88,1	81,6	57,7
V254A	113,7	20,0	177,7	8,5	52,7
N255A	85,3	63,5	103,5	122,7	43,6
E256A	63,4	44,5	55,9	11,9	32,8
N257A	178,2	2,4	113,2	0,0	75,6
V258A	99,9	40,5	98,1	27,6	49,8
E259A	111,2	32,6	165,0	0,3	57,0
N262A	68,5	65,8	84,7	79,4	10,3
N263A	100,7	111,6	76,2	227,9	80,8
T264A	128,6	121,6	132,6	36,9	68,2
C265A	44,0	7,9	55,5	1,7	46,4
T266A	78,6	73,7	123,4	20,8	61,3
N267A	90,5	2,2	132,3	3,0	74,4

N268A	86,5	43,2	87,3	34,1	60,3
E269A	106,9	127,1	123,0	186,2	75,9
V270A	82,2	51,3	116,0	10,8	84,4
H271A	95,6	95,0	136,7	68,6	115,2
N272A	114,1	111,4	132,1	103,1	59,7
L273A	25,1	30,8	34,3	70,6	48,9
T274A	71,9	112,7	120,9	160,6	69,4
E275A	47,5	87,7	94,0	147,3	80,1
C276A	0,3	4,5	1,1	17,7	5,6
K277A	60,3	48,1	82,4	142,7	61,0
A279S	116,9	103,0	83,6	126,0	63,8
S280A	53,5	40,5	88,3	117,4	69,4
V281A	75,7	39,3	61,7	32,4	64,9
S282A	100,3	95,2	100,2	99,5	86,0
I283A	45,2	18,0	62,8	30,0	89,1
S284A	79,1	135,9	271,5	74,5	107,6
H285A	109,3	126,6	112,8	4,8	90,9
N286A	127,3	96,3	183,7	0,1	95,4
S287A	134,6	155,4	142,1	26,1	81,0
C288A	3,5	1,3	-3,2	-3,5	-4,0
T289A	107,3	115,2	40,3	74,7	-3,3
A290S	122,1	111,9	29,8	58,3	77,2
P291A	55,5	68,0	-3,9	54,9	-3,5
D292A	64,3	178,0	3,3	216,6	-2,9
K293A	133,3	132,7	108,4	168,4	46,0
T294A	89,3	83,2	130,2	161,4	56,2
L295A	104,2	123,6	143,2	82,7	71,8
I296A	99,6	134,4	122,0	151,1	96,3
L297A	60,0	50,7	50,3	35,2	30,7
D298A	111,8	123,1	111,5	68,1	54,0
V299A	135,8	95,8	116,1	95,7	67,9
P300A	35,5	44,1	39,7	62,1	32,3
P301A	85,3	77,3	109,3	96,8	52,9
G302A	110,6	136,2	212,9	176,6	106,5
V303A	63,6	106,9	110,2	147,2	65,8
E304A	101,4	102,8	136,2	82,8	73,0
K305A	59,8	75,9	107,7	216,1	69,9
F306A	3,9	46,4	64,6	66,4	22,6
Q307A	76,9	96,7	92,0	184,6	49,4
L308A	17,9	21,5	18,8	17,0	21,2
H309A	293,6	304,6	457,7	235,6	107,9
D310A	15,1	30,7	30,8	34,5	37,8
C311A	56,9	50,7	54,1	57,9	42,6
T312A	75,8	53,1	93,0	80,8	30,3

Q313A	16,7	9,9	12,3	8,3	10,7
V314A	101,3	88,4	90,1	137,3	69,7
E315A	90,2	90,8	70,0	67,5	49,3
K316A	128,2	152,6	194,9	197,5	113,2
A317S	128,0	150,9	201,2	190,8	96,3
D318A	118,3	114,1	171,2	171,1	86,9
T319A	104,0	67,9	133,1	109,7	78,6
T320A	103,3	122,3	170,7	99,6	69,0
I321A	152,0	124,3	192,1	146,0	63,6
C322A	111,7	65,9	71,4	53,3	53,0
L323A	69,3	86,4	87,2	47,8	68,0
K324A	33,5	111,5	96,1	117,5	57,3
W325A	5,6	32,3	51,1	44,7	43,1
N327A	91,0	144,8	105,3	120,8	59,4
I328A	12,9	40,0	39,9	59,7	57,8
E329A	4,0	20,8			3,4
F331A	0,8	132,4	170,5	202,9	84,0
T332A	58,2	78,5	96,1	135,9	61,7
T335A	7,3	139,8	74,0	109,9	61,4
Q336A	100,9	142,4	193,2	156,3	94,1
N337A	79,0	82,1	176,8	159,0	71,6
I338A	7,9	63,0	35,9	26,2	45,2
T339A	96,3	84,1	143,6	227,4	70,7
Y340A	-0,6	97,1	82,3	101,1	52,8
R341A	96,5	93,6	99,9	120,2	77,1
F342A	34,9	127,2	90,5	98,8	81,8
Q343A	159,2	124,0	100,9	129,2	42,2
C344A	22,6	32,9	32,9	41,5	35,5
G345A	126,4	88,1	104,1	90,1	47,0
N346A	118,6	92,0	93,4	92,0	69,4
I348A	157,8	149,6	117,2	104,7	69,5
F349A	50,6	94,1	102,5	81,4	55,8
D350A	85,7	83,0	73,6	50,7	52,4
N351A	144,8	162,8	228,1	265,3	68,6
K352A	0,2	163,3	185,1	196,0	53,6
E353A	2,5	108,6	135,5	202,8	72,4
I354A	26,0	119,1	202,9	150,8	87,9
K355A	135,3	120,0	143,7	110,2	64,2
L356A	186,7	87,5	126,5	127,4	72,5
N358A	99,5	136,0	146,6	159,7	69,8
L359A	113,5	98,2	114,1	72,8	40,9
E360A	114,2	105,0	94,4	75,9	58,5
P361A	149,3	105,9	113,7	111,3	60,3
E362A	81,9	83,5	80,0	118,1	67,5

H363A	143,1	109,8	171,5	178,6	85,0
E364A	98,2	63,5	85,2	117,6	57,8
Y365A	75,5	74,3	91,8	79,3	52,9
K366A	111,9	98,7	111,7	78,0	64,4
C367A	10,4	12,3	25,5	21,9	27,1
D368A	113,0	85,2	126,4	137,5	74,9
S369A	94,4	95,9	88,7	153,3	79,3
E370A	119,9	111,2	153,6	134,6	62,5
I371A	26,1	67,9	82,2	138,4	65,5
L372A	134,3	129,4	123,2	148,0	75,5
Y373A	17,6	90,6	129,7	111,6	84,6
N375A	110,6	112,4	127,4	128,0	56,4
H376A	63,6	80,9	112,0	111,5	73,4
K377A	129,3	147,6	127,0	135,2	63,7
F378A	46,8	56,2	63,7	82,3	48,2
T379A	89,4	110,1	148,2	181,2	63,8
N380A	133,5	92,8	95,4	111,9	69,4
A381S	97,0	96,9	130,9	133,8	48,9
S382A	104,5	85,3	115,8	131,6	51,6
K383A	78,1	71,0	81,3	52,7	48,0
I384A	100,7	98,3	84,9	94,7	51,2
I385A	98,2	89,8	111,8	154,6	54,7
T387A	55,3	57,0	74,3	84,7	41,3
D388A	56,1	32,7	49,3	48,7	39,8
F389A	122,6	94,5	115,8	190,8	49,4
G390A	79,4	78,3	106,0	95,4	51,5
S391A	130,2	119,9	137,5	108,7	66,1
P392A	38,0	29,2	31,9	59,9	25,2
G393A	130,3	106,5	116,2	93,1	68,2
E394A	132,4	112,0	124,1	170,5	71,8
P395A	99,9	64,4	125,1	132,6	63,3
Q396A	79,5	84,7	94,5	141,4	51,2
I397A	102,0	105,0	93,4	95,0	47,1
I398A	30,7	38,2	74,8	75,2	35,4
F399A	145,2	126,0	118,3	104,0	64,8
C400A	14,4	15,4	19,8	24,3	13,0
S402A	74,3	78,9	102,8	153,0	49,6
A404S	105,2	50,6	59,2	93,4	51,1
A405S	157,6	124,3	96,5	154,0	69,3
H406A	154,9	93,3	95,5	92,1	64,2
Q407A	24,8	22,0	21,9	31,4	8,6
G408A	94,7	88,2	115,6	103,1	58,8
V409A	125,9	128,1	79,6	76,2	54,9
I410A	2,1	4,0	10,3	20,1	4,8

T411A	129,5	126,8	116,4	115,5	91,7
W412A	0,9	4,7	0,9	6,4	-3,1
P414A	80,6	79,8	110,3	117,2	54,9
P415A	70,1	68,7	74,2	83,6	28,3
Q416A	93,4	86,1	104,4	163,4	41,9
R417A	162,7	125,6	114,2	113,9	81,0
S418A	114,1	92,3	145,7	96,5	75,4
F419A	75,4	75,2	69,9	128,8	56,9
H420A	82,3	70,6	94,5	64,2	31,4
N421A	76,0	58,0	74,5	70,1	69,6
F422A	24,3	23,7	31,6	20,5	37,3
T423A	62,5	70,5	69,5	71,6	32,0
C425A	-0,1	1,7	-3,1	99,9	-0,8
Y426A	156,5	111,2	128,9	106,0	73,4
I427A	213,9	126,9	145,1	95,8	81,4
K428A	119,0	117,3	86,0	99,3	65,3
E429A	82,0	77,9	110,7	204,0	50,9
T430A	82,3	124,7	111,2	95,7	48,1
E431A	102,8	94,0	124,9	96,6	44,0
D433A	67,6	78,7	73,3	108,6	48,3
L435A	43,0	54,8	55,9	70,7	37,8
N436A	37,8	73,6	98,3	103,4	78,3
L437A	33,5	24,8	35,5	34,1	29,4
D438A	120,5	91,5	99,1	64,4	46,2
K439A	63,0	59,6	82,0	93,7	50,0
N440A	83,8	84,7	103,8	69,4	12,6
L441A	86,1	74,5	86,0	140,6	44,5
I442A	102,6	91,0	101,4	87,5	43,8
K443A	93,9	93,5	82,3	119,5	29,3
Y444A	82,6	85,7	54,4	101,4	59,9
D445A	82,0	98,4	102,7	122,3	61,5
L446A	84,1	76,3	52,9	84,4	71,1
Q447A	75,5	66,6	59,1	92,9	43,9
N448A	69,9	79,7	88,5	79,9	52,4
K450A	140,7	114,9	132,3	73,6	51,0
P451A	96,1	78,1	118,6	81,0	59,1
Y452A	77,6	55,2	55,5	101,3	52,2
T453A	48,4	56,6	67,7	70,5	47,7
K454A	98,4	97,7	120,7	94,3	67,7
Y455A	10,7	12,4	16,1	23,8	19,8
V456A	94,1	86,0	105,1	117,4	44,0
S458A	79,2	74,9	77,0	119,1	38,7
L459A	1,9	2,3	-0,1	2,6	6,6
H460A	98,8	105,1	102,3	84,2	60,2

A461S	55,9	55,2	58,4	74,6	39,3
Y462A	98,3	78,3	70,1	80,7	48,9
I463A	128,0	106,3	119,5	83,2	73,3
I464A	24,8	49,6	48,6	109,2	46,3
A465S	146,6	181,5	243,5	201,4	59,0
K466A	115,5	84,1	92,6	81,6	39,3
V467A	74,4	66,7	83,1	94,6	39,7
Q468A	85,9	83,5	89,4	135,8	37,4
R469A	1,7	4,6	3,3	9,9	4,8
N470A	75,9	56,6	97,8	108,4	51,5
G471A	41,5	28,5	21,8	27,9	33,7
S472A	125,7	116,5	137,9	86,6	85,9
A473S	75,8	35,3	77,0	143,4	31,1
A474S	2,3	1,6	11,7	40,1	3,7
M475A	141,5	112,1	87,7	99,9	50,3
C476A	2,2	4,5	4,4	7,5	-1,9
H477A	41,1	64,2	67,1	141,4	41,2
F478A	5,0	3,6	1,9	19,1	3,1
T479A	77,7	87,9	72,7	76,4	46,2
T480A	51,9	48,2	31,8	28,2	50,5
K481A	61,6	63,7	49,7	59,2	27,8
S482A	88,0	75,0	69,4	113,8	47,2
P484A	88,9	84,7	92,9	135,0	45,0
P485A	87,2	74,5	66,4	133,3	49,3
S486A	83,0	94,5	111,1	113,4	59,7
Q487A	156,7	103,8	138,8	92,9	75,3
V488A	6,1	5,8	7,0	8,5	1,2
W489A	140,3	136,5	197,7	108,8	72,2
N490A	75,4	89,4	65,9	126,9	26,9
M491A	110,2	131,5	98,7	100,4	70,8
T492A	52,1	38,7	52,0	61,2	22,0
V493A	54,7	54,2	61,1	73,4	53,5
S494A	118,4	111,5	113,5	97,6	62,9
M495A	79,6	77,7	102,9	217,0	55,8
T496A	94,6	92,2	95,0	95,4	45,3
S497A	98,4	87,5	126,2	139,3	37,7
D498A	71,4	99,0	101,2	128,3	58,3
N499A	60,1	46,3	54,8	87,0	42,6
S500A	2,2	1,5	7,4	30,9	-3,4
M501A	119,3	98,1	135,9	88,9	29,9
H502A	61,6	87,2	77,1	83,3	49,4
V503A	51,7	43,7	31,5	58,9	34,4
K504A	110,0	131,8	108,5	113,0	61,7
R506A	87,8	73,7	110,9	117,5	47,2

P507A	136,1	109,8	119,3	127,4	76,0
P508A	51,9	30,7	69,6	51,6	32,2
R509A	91,8	121,6	153,0	117,3	77,1
D510A	103,7	118,5	142,4	166,4	62,0
R511A	88,4	109,9	121,8	115,4	48,0
N512A	70,1	63,1	56,1	109,9	64,7
G513A	2,3	2,1	3,9	3,8	-2,0
P514A	73,4	78,1	77,7	100,8	43,7
H515A	46,6	69,4	65,6	144,8	49,2
E516A	79,1	59,3	112,5	115,4	61,1
R517A	79,3	61,4	66,0	99,4	41,7
Y518A	6,2	5,5	8,2	14,1	9,2
H519A	49,4	44,7	52,1	105,9	42,7
L520A	1,0	0,7	2,0	-2,2	-4,1
E521A	62,3	41,3	89,4	79,0	51,7
V522A	27,4	30,4	37,4	44,0	33,3
E523A	86,5	69,6	113,3	104,3	59,1
A524S	26,0	15,7	27,0	65,8	27,1
G525A	76,0	58,5	97,9	69,0	57,6
N526A	74,8	102,1	61,2	188,8	55,3
T527A	62,1	52,9	84,6	89,9	59,4
L528A	51,3	64,4	75,5	131,5	49,1
V529A	63,5	83,1	69,8	119,6	50,0
R530A	83,8	58,5	102,4	76,5	62,5
N531A	89,9	86,3	135,9	141,4	64,2
E532A	99,4	105,5	146,5	158,4	63,7
S533A	106,7	87,3	85,5	84,7	53,6
H534A	112,0	94,1	135,2	166,7	54,3
K535A	87,3	82,6	92,7	171,5	60,2
N536A	82,1	62,4	99,4	164,6	58,5
C537A	0,3	-0,6	-6,0	-12,9	-3,4
D538A	60,6	58,3	49,1	97,4	62,7
F539A	3,7	3,0	-2,2	4,1	-2,1
R540A	77,0	80,5	66,8	186,4	43,8
V541A	26,9	28,8	14,1	48,3	27,4
K542A	99,5	89,3	88,6	88,1	64,5
D543A	80,5	61,1	80,9	113,7	64,9
L544A	27,2	24,9	36,4	81,9	23,7
Q545A	108,4	85,0	95,9	197,3	70,0
Y546A	59,4	52,3	114,0	55,6	54,2
S547A	123,2	95,5	99,4	86,4	72,9
T548A	130,2	86,1	62,6	2,0	46,0
D549A	102,9	80,3	127,1	96,6	64,4
Y550A	0,6	2,0	-5,3	5,7	2,2

T551A	146,8	129,0	112,1	131,7	70,3
F552A	36,0	29,2	19,7	29,6	17,9
K553A	81,8	93,8	75,5	96,5	40,6
A554S	56,1	42,5	60,8	64,7	73,8
Y555A	26,0	21,2	36,0	30,6	40,3
F556A		74,9	66,5	102,8	72,6
H557A	13,5	21,6	16,4	42,1	30,6
N558A	-2,5	0,2	-8,2	2,8	1,9
G559A	7,2	14,7	6,0	17,0	17,1
D560A	53,1	53,6	56,3	99,9	68,8
Y561A	61,5	56,9	69,6	45,9	27,3
P562A	74,3	70,7	96,4	88,2	84,2
E564A	48,6	55,7	57,3	121,3	72,2
P565A	69,0	62,1	57,4	75,0	86,1
F566A	142,2	111,9	106,3	145,6	80,1
I567A	35,3	49,2	48,2	72,5	53,8
L568A	67,1	44,6	68,7	72,0	69,1
H569A	103,3	117,3	88,1	99,0	41,8
H570A	89,3	69,8	96,1	150,3	61,5
S571A	175,6	130,8	128,2	98,8	67,5
T572A	27,5	23,3	44,4	42,1	37,3
S573A	9,3	8,8	17,5	24,0	38,0
Y574A	156,2	124,1	100,1	89,7	60,5
S576A	54,9	60,5	83,7	98,1	54,2
K577A	140,0	138,1	133,5	98,4	65,6

Table 10 summarizes the critical residues for each of the antibodies tested. Residues whose mutations gave the lowest reactivities with specific antibodies are highlighted in bold and are underlined. Validated critical residues represent amino acids whose side chains make the highest energetic contributions to the antibody-epitope interaction (J. Mol. Biol. (1998) 280, 1-9; J. Mol. Biol. (1999) 285, 2177-2198); therefore, the highlighted residues are likely the major energetic contributors to binding.

Table 10:

Antibody	Critical residues
Refmab #1	K324, <u>F331</u> , <u>T335</u> , F342, <u>K352</u> , <u>E353</u> , I354, <u>Y340</u> , Y373
Refmab #2	V254, <u>N257</u> , E259, <u>N267</u>

Refmab #3 **D229**, **E230**, **D292**

Refmab #4 **L170**, **V254**, **N257**, **E259**, T264, T266, **N267**, **H285**, **N286**, S287

Example 5: Comprehensive mutational analysis

The critical residues identified in Example 4 were investigated in more detail. First, a validation step of identified critical residues was performed considering reproducibility of binding activity, surface accessibility, structural localization and distance to other critical sites, as well as the nature and biochemical properties of the substituted amino acid (e.g. cysteine forming disulfide bridges or post-translational modification sites). After validation, each critical residue was subject to comprehensive mutagenesis to selected biophysically appropriate non-alanine amino acids, based on sequence and structure-related properties of the substituted amino acid as well as the newly introduced ones.

Antibodies were screened for binding to the human CD45 variants in IgG format. As in Example 4, binding of each test antibody to each mutant clone in the comprehensive library was determined in duplicate by high-throughput flow cytometry. For each mutation, background fluorescence was subtracted from the raw data, which were then normalized to antibody reactivity with wild-type CD45. Mean binding reactivities and ranges are listed in Table 11 for all mutant clones. Mutations that caused binding below 20% are highlighted in grey.

Table 11:

	Refmab #1		Refmab #2		Refmab #3		Refmab #4		Refmab #5	
Mutation	Mean %WT Bindin g	Range %WT Bindin g	Mean %WT Bindin g	Range %WT Bindin g	Mean %WT Bindin g	Range %WT Bindin g	Mean %WT Bindin g	Range %WT Bindin g	Mean %WT Bindin g	Range %WT Bindin g
D229G	91,2	6,8	261,4	84,4	-6,1	1,8	105,9	89,2	60,4	2,6
D229T	87,4	54,8	195,8	9,9	-0,2	6,8	147,7	12,3	7,4	6,8
D229V	91,7	60,8	150,4	51,6	0,3	1,3	115,5	34,1	5,4	4,9
E230H	90,1	6,6	186,8	16,6	-7,7	2,3	224,2	23,3	1,8	0,1
E230K	81,1	30,6	189,3	91,2	2,3	21,9	1,8	0,5	5,2	9,3

E230N	117,6	34,2	176,9	29,6	3,4	19,5	26,0	7,2	10,4	17,5
E230Q	62,6	27,9	192,8	46,4	-3,1	1,6	161,6	14,6	10,5	5,4
E230S	78,3	76,4	87,7	48,9	9,0	35,0	190,3	22,6	1,9	0,3
E230T	45,4	16,9	101,6	21,5	1,8	9,0	134,6	2,0	4,3	7,4
V254D	68,3	67,9	70,5	20,1	81,9	6,6	69,6	7,7	132,1	65,0
V254F	78,5	42,6	156,8	5,0	99,5	6,5	104,2	19,5	152,3	44,4
V254G	83,7	63,5	99,1	3,6	127,8	2,4	71,6	43,3	167,9	16,8
V254I	52,4	2,0	169,1	45,6	123,3	31,9	43,2	18,7	140,9	3,1
V254L	67,2	0,6	161,9	20,2	119,6	6,1	51,3	11,7	140,6	40,3
V254P	91,7	62,1	147,2	15,8	128,6	13,4	67,3	129,7	177,9	30,1
V254T	113,2	25,1	245,9	70,7	163,4	28,4	256,5	129,6	209,2	47,9
V254Y	93,8	17,6	52,4	4,7	109,4	7,4	178,2	64,7	130,8	11,4
N257D	78,2	8,6	3,8	0,8	126,1	15,3	140,7	36,9	170,5	22,2
N257E	78,2	5,1	2,1	2,3	93,0	1,1	134,4	15,6	126,6	9,2
N257F	29,5	18,3	11,4	7,2	24,2	16,9	78,4	6,9	32,9	9,2
N257G	67,9	12,7	12,3	3,5	66,4	18,2	49,0	25,3	104,1	9,4
N257H	53,4	12,7	14,3	11,4	22,0	7,3	52,8	31,0	50,2	7,3
N257I	84,1	53,1	10,8	0,2	133,5	75,0	163,6	50,3	191,6	38,5
N257L	78,9	8,2	16,5	6,2	90,7	20,4	80,1	5,7	148,6	42,1
N257M	91,2	38,3	19,7	5,1	127,8	17,2	-0,2	0,5	174,4	26,1
N257P	77,3	4,0	56,9	22,9	81,4	7,9	1,0	1,5	141,6	13,8
N257Q	66,5	16,0	34,0	13,1	82,6	47,7	85,6	10,5	97,8	22,8
N257R	66,4	24,4	3,4	0,3	98,8	8,0	170,4	39,6	141,0	1,0
N257S	60,5	13,8	22,7	0,6	86,0	26,9	64,0	7,3	100,8	42,6
N257T	109,5	9,8	6,3	2,2	134,8	69,3	2,5	1,1	154,9	4,3
N257V	44,7	25,5	12,5	2,2	30,6	6,9	64,2	2,4	75,3	2,7
N257Y	137,2	13,8	43,6	22,4	112,7	29,4	161,4	39,5	153,0	17,5
E259G	4,2	0,3	5,6	0,5	-2,1	2,3	6,5	5,3	22,4	36,3
E259I	116,0	10,8	71,5	17,1	141,9	36,5	176,0	10,3	156,3	43,4
E259K	149,3	52,5	91,7	8,9	114,8	11,1	150,7	17,4	137,3	2,7
E259M	67,0	2,8	108,7	13,1	86,8	4,3	153,1	10,7	102,0	12,0
E259N	72,3	17,3	98,7	8,8	104,0	30,2	2,6	1,7	105,7	10,3
E259P	62,0	7,2	26,5	4,7	83,2	30,5	19,4	40,3	143,9	6,2
E259Q	80,4	18,8	166,1	19,9	159,0	96,6	1,4	2,0	173,0	14,9
E259S	98,7	43,1	72,3	1,9	136,1	36,4	36,8	12,7	146,6	35,7
E259T	26,9	42,6	2,1	0,0	65,5	36,1	24,1	9,0	52,6	4,6
E259V	59,3	2,9	66,7	16,6	50,9	20,2	159,5	33,5	64,5	18,0
T264D	69,2	29,6	180,1	25,2	123,7	30,6	12,7	18,0	156,9	14,6
T264G	87,6	46,7	258,2	57,0	103,0	42,3	26,0	16,5	163,9	88,7
T264I	114,9	24,4	184,4	13,4	110,3	8,6	107,3	3,1	191,2	63,7
T264K	87,9	20,9	220,7	45,9	139,2	0,8	189,1	28,7	177,5	32,1
T266E	96,2	28,1	199,7	15,4	156,4	4,2	51,2	24,0	142,6	29,7
T266I	73,2	5,7	125,0	0,5	77,0	20,8	48,0	27,7	121,2	47,7
T266K	130,7	39,2	163,4	24,5	181,4	32,0	177,0	24,1	193,6	22,8
T266V	66,1	30,0	174,5	101,5	109,8	12,1	45,6	22,5	127,0	7,6
N267D	103,9	27,4	165,3	35,1	109,0	15,3	148,8	56,3	132,3	25,2

N267E	86,6	19,5	85,7	3,9	109,8	20,8	136,8	10,9	128,9	16,2
N267G	116,2	22,6	165,3	14,4	101,9	4,9	96,9	135,1	162,3	12,6
N267H	108,2	52,0	57,9	20,8	106,9	6,2	4,3	3,1	167,8	15,9
N267I	476,0	117,4	596,1	116,6	421,0	15,9	361,9	79,0	386,6	121,9
N267K	46,6	29,8	72,3	25,7	60,3	16,8	17,0	1,4	73,8	1,1
N267L	88,4	0,8	87,7	8,2	108,4	6,7	12,6	11,9	150,9	9,7
N267M	50,5	13,3	85,6	47,6	75,6	36,8	94,7	109,3	104,5	25,0
N267P	59,5	4,9	30,4	8,2	34,4	5,0	219,8	18,4	96,7	30,6
N267Q	96,0	12,3	187,0	1,0	165,5	26,4	92,7	56,8	186,5	49,0
N267R	83,7	8,5	40,1	4,6	82,7	1,1	45,5	16,0	122,8	25,1
N267S	117,9	66,6	181,4	25,3	99,5	19,4	5,3	1,5	175,6	14,8
N267T	31,8	0,9	64,9	43,1	19,3	20,2	156,3	13,7	48,1	15,4
N267V	53,5	17,2	99,7	4,2	78,8	6,4	4,4	0,7	96,9	50,9
N267Y	93,0	12,2	25,1	15,3	121,9	4,9	83,8	101,1	194,0	23,3
H285D	81,8	13,2	150,5	8,5	83,4	33,8	131,8	41,7	156,6	7,1
H285G	82,3	36,1	192,1	50,1	87,4	1,7	32,5	10,1	120,2	16,1
H285Q	117,8	10,5	148,0	39,4	122,5	22,6	178,3	42,8	166,4	8,7
H285R	104,6	41,9	148,0	2,0	151,0	23,0	176,1	4,3	158,6	34,4
H285S	94,8	25,7	243,3	110,1	115,7	6,8	139,6	25,0	156,7	1,7
N286D	142,3	48,2	114,0	16,1	108,0	29,6	584,1	3,0	170,3	10,1
N286G	14,6	0,7	20,9	1,4	-6,8	1,9	59,0	7,8	5,4	4,2
N286H	362,9	134,9	469,8	7,1	261,2	10,4	889,8	187,1	298,1	39,8
N286L	152,8	18,9	202,2	36,7	160,8	12,7	4,2	3,3	186,7	48,5
N286P	147,8	25,0	157,9	17,8	108,6	41,3	47,2	14,4	135,7	12,0
N286R	77,6	25,4	202,6	23,4	109,8	18,4	3,8	1,1	139,3	56,3
N286S	69,6	6,5	150,3	41,8	98,5	17,6	151,3	31,7	125,1	9,3
N286T	99,8	36,6	106,1	34,8	90,1	14,2	72,1	27,7	107,0	14,3
S287Q	79,3	16,7	178,0	27,6	85,5	3,3	87,6	51,3	113,8	6,5
S287T	112,0	35,4	226,0	60,6	142,8	2,2	4,8	4,3	191,3	15,1
D292F	129,4	72,3	186,4	48,0	45,7	14,6	161,8	26,8	33,7	11,3
D292H	103,9	93,7	219,7	42,9	10,1	4,4	137,1	17,5	21,4	6,8
D292K	112,0	17,6	197,4	21,1	-4,2	5,4	16,8	23,5	1,7	0,5
D292L	82,9	0,4	154,4	49,0	9,9	7,6	127,2	84,5	22,4	8,5
D292P	67,7	0,8	202,4	40,7	0,4	10,5	82,4	47,6	4,1	0,9
D292Q	104,7	8,3	226,7	63,8	86,2	46,1	10,6	17,2	15,2	8,2
D292S	42,1	18,7	117,7	21,2	-0,5	0,1	105,4	53,2	5,6	0,5
D292T	31,1	1,1	82,4	3,5	2,7	2,4	57,8	19,8	5,7	6,6
D292Y	61,4	0,5	190,9	25,9	6,0	2,2	156,8	24,0	63,5	14,9
K324D	42,1	13,5	158,2	4,0	124,9	27,2	132,2	0,1	169,4	10,4
K324E	30,8	12,3	159,9	8,2	98,6	14,8	137,5	51,6	120,2	31,2
K324M	67,7	24,8	175,5	60,3	109,9	7,5	127,9	67,9	139,8	20,0
K326D	46,4	17,5	142,6	16,1	124,6	21,8	127,7	184,3	191,0	90,0
K326E	46,1	7,6	131,1	9,4	110,1	24,6	131,9	10,0	149,3	37,6
I328F	43,3	6,6	130,6	24,8	69,9	4,5	129,5	126,9	113,3	21,1
I328V	14,4	5,7	38,1	14,9	16,9	4,5	89,5	117,4	23,4	19,9
E329K	45,9	15,1	155,1	43,0	70,2	2,0	130,5	29,8	129,0	8,3

E329V	35,8	17,4	111,7	21,3	61,5	10,1	162,0	28,3	99,6	12,6
T330D	64,4	14,0	97,2	50,0	71,8	14,9	44,6	6,5	91,8	4,3
T330F	14,4	9,5	79,1	8,7	26,2	1,0	65,5	16,0	56,4	39,0
T330R	31,1	2,6	230,5	14,8	135,0	58,9	165,5	31,9	164,3	6,5
T330Y	99,5	7,0	176,2	17,8	124,7	4,0	205,3	3,8	142,5	28,9
F331G	1,7	4,0	199,1	57,3	140,3	51,8	162,8	42,3	167,5	4,3
F331H	34,6	29,5	112,3	27,2	57,0	6,5	58,9	4,0	100,0	16,4
F331I	36,1	33,6	141,3	9,3	107,5	28,0	239,1	10,0	128,0	15,5
F331L	56,0	20,7	217,0	98,8	134,4	6,3	190,1	50,2	173,4	77,9
F331M	67,7	8,3	201,5	40,6	124,1	27,4	184,5	11,4	163,7	16,7
F331N	28,8	23,9	163,2	53,6	142,0	19,6	19,1	7,1	139,9	38,0
F331S	51,3	42,5	189,8	40,9	153,9	6,1	211,3	0,5	154,6	42,6
F331V	40,6	29,3	169,9	5,9	111,2	15,6	134,8	15,3	111,7	13,1
T332I	68,6	0,2	122,9	4,8	69,7	6,9	124,5	31,3	108,6	4,2
D334A	56,7	33,3	105,7	13,1	43,8	17,0	15,1	5,7	95,3	18,8
D334F	27,2	4,5	73,6	24,3	24,1	20,7	44,7	17,7	42,5	15,4
D334H	83,1	47,7	118,2	0,6	67,3	11,7	179,7	32,9	107,5	3,1
D334I	54,7	16,6	141,8	9,8	81,7	12,0	147,7	55,6	142,7	3,0
D334K	72,1	5,8	173,9	30,1	132,7	22,8	158,5	38,2	142,3	74,9
D334L	83,6	7,3	162,0	5,6	109,8	3,6	192,8	75,3	142,7	36,2
D334M	16,9	19,0	59,8	75,4	26,8	7,6	3,2	1,9	17,3	23,8
D334R	14,0	10,9	39,1	7,2	6,2	4,3	70,8	20,0	20,4	6,0
D334S	38,4	7,7	73,8	14,8	20,3	9,7	30,3	16,5	63,8	10,4
D334T	81,7	27,0	168,1	65,0	121,5	22,5	7,5	8,3	126,9	39,2
D334V	57,8	25,8	159,5	9,3	111,5	14,7	161,2	2,4	116,9	12,9
D334Y	91,7	57,5	146,5	18,5	100,9	13,1	169,4	46,1	137,7	12,0
T335E	25,2	3,6	57,4	5,7	15,9	6,9	52,7	28,8	43,4	7,1
T335I	74,8	2,3	189,0	35,7	77,9	21,6	95,4	4,6	109,4	0,6
T335K	73,1	3,7	208,2	56,6	135,6	38,0	145,8	50,0	164,0	40,1
T335L	81,1	19,8	157,6	25,3	118,8	27,6	155,1	69,2	132,7	20,5
T335M	80,4	6,2	215,8	28,4	122,3	12,8	247,7	16,9	155,8	6,6
T335N	71,1	13,9	128,7	22,1	90,7	1,6	69,6	43,9	116,9	42,7
T335P	39,4	12,8	54,1	4,9	34,2	21,7	1,6	1,8	35,2	18,3
T335R	99,8	72,0	187,3	34,3	142,5	1,0	224,6	72,9	158,0	39,6
T335S	31,5	44,8	75,9	44,6	21,4	17,5	52,2	48,9	41,8	30,8
T335Y	30,1	28,5	157,0	59,9	135,3	16,7	17,0	15,4	149,2	21,6
Y340F	90,0	49,6	224,2	6,7	118,2	30,1	122,5	15,9	141,5	2,6
Y340G	-0,4	1,2	9,7	5,8	1,2	3,6	60,0	31,6	9,8	5,7
Y340I	39,6	17,3	111,4	10,6	59,6	8,4	0,5	1,6	76,5	27,1
Y340M	49,8	4,1	149,4	66,3	80,7	20,1	31,9	21,2	110,4	3,1
Y340N	4,4	1,8	19,7	4,2	5,0	9,3	160,6	21,6	16,2	4,0
Y340Q	2,0	9,1	20,3	11,8	2,7	8,1	90,7	37,0	15,1	11,7
Y340S	-0,7	1,0	25,6	18,8	25,8	40,7	104,8	8,3	31,2	26,8
K352D	-0,6	4,9	184,3	41,1	116,3	3,4	57,6	6,1	179,5	34,6
K352E	1,4	0,7	255,5	136,1	148,4	7,2	153,7	28,0	207,4	57,8
K352G	2,0	8,9	190,0	33,2	135,5	3,2	6,6	1,6	152,3	5,4

K352H	5,8	0,3	147,5	45,7	154,6	44,3	77,0	35,8	125,8	11,0
K352I	3,2	4,4	259,2	54,7	134,0	33,8	160,0	1,2	156,1	23,8
K352L	4,4	7,7	275,6	153,9	213,2	48,2	182,9	37,5	245,2	7,4
K352M	9,5	7,5	216,1	59,7	119,5	9,9	142,3	23,2	192,3	11,0
K352N	4,4	3,2	277,8	82,2	140,3	13,0	173,8	95,2	169,7	14,0
K352Q	7,7	4,9	183,1	22,9	115,4	6,9	138,2	58,9	154,8	4,8
K352S	5,7	0,2	247,5	145,5	172,7	59,6	129,1	6,7	219,3	5,8
K352T	2,7	1,1	251,3	27,7	160,0	39,7	162,2	26,9	248,4	60,5
K352Y	1,3	2,4	208,9	1,2	162,9	96,6	4,8	0,5	250,5	91,5
E353G	24,0	0,1	90,3	26,0	51,4	26,9	2,2	0,2	65,7	22,5
E353I	-2,5	2,0	67,3	19,7	39,5	6,2	142,2	65,3	82,5	1,1
E353K	-1,6	0,1	81,1	22,8	36,7	2,8	8,1	1,5	51,4	13,1
E353L	3,3	1,9	100,2	74,2	31,6	39,1	22,7	16,6	45,0	27,3
E353M	21,2	41,7	154,1	7,1	67,7	2,8	156,3	98,0	123,7	21,4
E353N	39,0	31,1	134,7	26,6	67,7	5,6	181,7	16,3	95,1	24,0
E353Q	59,5	6,9	148,5	34,5	91,1	8,1	136,7	52,2	131,8	18,8
E353R	4,9	3,5	112,6	21,3	50,7	29,7	127,4	17,5	85,8	14,5
E353S	7,2	0,4	68,2	22,7	14,7	3,1	15,1	10,8	39,9	2,8
E353T	11,1	1,8	167,7	66,0	78,9	4,8	127,1	44,2	95,0	4,3
E353Y	99,8	34,4	204,1	3,7	116,3	45,7	77,4	19,7	105,4	26,9
Y372F	14,2	3,5	19,4	17,7	20,3	13,9	154,6	59,5	3,1	1,5
Y373C	15,6	7,3	40,7	19,5	26,8	10,0	155,5	26,9	39,5	9,4
Y373F	73,6	21,6	170,3	21,3	96,2	17,6	115,3	35,3	134,9	18,4
Y373T	9,3	6,2	56,0	20,5	33,2	13,4	16,7	23,0	60,9	15,0
Y373V	21,0	6,5	62,3	8,2	48,7	10,2	47,5	33,9	51,7	13,7

Table 12 summarizes the variants that reduce binding of the tested antibodies to below 20%

Table 12:

5

Residue	Mutation	Antibodies that bind to this residue
D229	G, T, V	Refmab #3 Refmab #5 (not G)
E230	H, K, N, Q, S, T	Refmab #3 Refmab #4 (only K) Refmab #5

N257	D, E, F, G, H, I, L, M, P, R, T, V	Refmab #2 (not P) Refmab #4 (M, P and T)
E259	G, N, P, Q, T	Refmab #1 (only G) Refmab #2 (G and T) Refmab #3 (only G) Refmab #4 (not T)
T264	D	Refmab #4
N267	H, K, L, S, T, V	Refmab #3 (only T) Refmab #4 (not T)
N286	G, L, R	Refmab #1 (only G) Refmab #3 (only G) Refmab #4 (L and R) Refmab #5 (only G)
S287	T	Refmab #4
D292	H, K, L, P, Q, S, T, Y	Refmab #3 (not Q) Refmab #4 (K and Q) Refmab #5 (not H, L and Y)
I328	V	Refmab #1 Refmab #3
T330	F	Refmab #1
F331	G, N	Refmab #1 (only G) Refmab #4 (only N)
D334	A, M, R, T	Refmab #1 (M and R) Refmab #3 (only R) Refmab #4 (not R) Refmab #5 (only M)
T335	E, P, Y	Refmab #3 (only E) Refmab #4 (P and Y)
Y340	G, I, N, Q, S	Refmab #1 (not I) Refmab #2 (G and N) Refmab #3 (G, N and Q) Refmab #4 (only I) Refmab #5 (G, N and Q)
K352	D, E, G, H, I, L, M, N, Q, S, T, Y	Refmab #1 Refmab #4 (G and Y)

E353	G, I, K, L, R, S, T	Refmab #1 (not G) Refmab #3 (only S) Refmab #4 (G, K and S)
Y372	F	Refmab #1 Refmab #2 Refmab #5
Y373	C, T	Refmab #1 Refmab #4 (only T)

Example 6: Analysis and comparison of the identified variants

5 Figure 4 schematically depicts the location of the identified variants on the 3D structure of human CD45.

Refmab #1 binds to a region different to that of the other tested antibodies, with the key variants identified being located between positions 328 and 373. Specifically, variants at the following position were identified for which Refmab #1 showed binding of less than
10 20% as compared to wild type CD45: residues E259, N286, I328, T330, F331, D334, Y340, K352, E353, Y372 and Y373. A comparison of binding of the antibodies tested to some of these variants is shown in Figure 5. Of the variants identified some seem to be less preferred, e.g. F331G which is part of a non-conserved loop in a low accessible side chain. Variants F331, in particular F331G, variant K352, in particular variants K352E, K352H, K352I,
15 K352L, K352M, K352N, K352Q, K352S and K352T, and variant K353, in particular variants E353H, E353K and E353R are particularly preferred variants.

Four variants were identified that inhibited binding of Refmab #2 to less than 20% as compared to CD45 wildtype: N257, E259, Y340 and Y372. A comparison of binding of the antibodies tested to some of these variants is shown in Figure 6. Variant N257, in particular
20 variants N257D, N257E, N257R and N257T are particularly preferred variants. Also variant N257K, which was not tested, by analogy is a preferred variant.

Various variants were identified that inhibited binding of Refmab #4 to less than 20% as compared to CD45 wildtype: E230, N257, E259, T264, N267, N286, S287, D292, F331, D334, Y340, K352, E353 and Y373. A comparison of binding of the antibodies tested to some of these variants is shown in Figure 7. Variant E230, in particular variants E230K and E230R, variant E259, in particular variants E259N and E259Q, and variant N257, in particular variants N257M, N257P, N257T, N257H, N257R, N257S and N257V are particularly preferred variants.

The data obtained so far were further analyzed via in silico mutagenesis. Goal was to analyze whether a protein sequence variation affects protein function. To do so, PROVEAN scores (PLoS ONE (2012); 7(10): e46688; Choy (2012), In Proceedings of the ACM Conference on Bioinformatics, Computational Biology and Biomedicine (BCB '12). ACM, New York, NY, USA, 414-417) were generated for candidate single amino acid substitutions at selected position of CD45. First, a delta alignment score is computed for each sequence belonging to the top clusters of closely related sequences, i.e., supporting sequence set. Delta scores are then averaged within and across clusters to generate the final PROVEAN score. If the predicted PROVEAN score is equal to or below(above) a given threshold (-2.5), the protein variant is predicted to have a deleterious(neutral) impact on protein function.

Results are shown in Figure 8. Most of the variants identified experimentally could be confirmed in silico. Only variant E259G is below the -2.5 threshold.

Example 7: Generation of CD45 variants by base editing

Base editing was used to test and verify that human CD45 is amenable to mutation via base editing.

To do so, we screened multiple sgRNA with NG(N) protospacer adjacent motifs designed to target selected regions of CD45 against several base editors (ABEmax-SpG, xCas9(3.7)-BE4, CBE4max-SpCas9-NG, SPACE-NG, ABEmax-SpRY, CBE4max-SpG and ABE8e-NG). For

each screening entry, we co-electroporated 5ug of plasmid encoding the base editor and 1.5ug of sgRNA plasmid in 2 millions K562 cells (ATCC CCL-243) using a Neon Transfection System 100 µL Kit (ThermoFisher Scientific) and its proprietary T buffer (Invitrogen Ref: MPK10096Tb) with a custom program: 1450V, 10ms, 3 pulses. 24h hours after co-

5 electroporation, all conditions were sorted for GFP positive cells taking advantage of the base editors plasmids' GFP cassettes using a BD FACS Aria III Cell Sorter (BD Bioscience). GFP positive cells were then expanded for 2 more days in 1mL of RPMI-1640 (Sigma-Aldrich Ref:R8758-500ML) supplemented with 10% FCS and 100X GlutaMAX (ThermoFisher Scientific Ref:35050061) and Penicillin-Streptomycin (1/1000). 72h after the initial co-

10 electroporation, we extracted the gDNA for each condition performed PCR of the corresponding screened exons (9, 10 and 11). PCR products were then sent for sanger sequencing with the correct forward primer.

Primer pairs for PCR/Sequencing of CD45 exons of interest:

hCD45_Exon9_For = ACAAGCTGAGGTCCTTGTTAG (SEQ ID No. 26)

15 hCD45_Exon9_Rev = AGCAGAAAGTTCACCCACTTG (SEQ ID No. 27)

hCD45_Exon10_For = CCATAGCAATCTCAATCCTTGCC (SEQ ID No. 28)

hCD45_Exon10_Rev = TGCCTGTGTATAACAATTGCCAAG (SEQ ID No. 29)

hCD45_Exon11_For = TGACCTCAAGCTATGTATATGAGG (SEQ ID No. 30)

hCD45_Exon11_Rev = GAGACTGTTACCTCACACCATATAC (SEQ ID No. 31)

20

Table 13 and Figure 9 display the most interesting hits from the screen (single amino-acid changes and some other relevant variant candidates).

Variants to be generated were selected by computer aided rational design. The following variants were generated with the respective base editors and sg RNA mentioned:

Table 13:

Variant	Base editor	sgRNA	Sequence
I328V	ABEmax-SpG	sg15	AATATTGAAACCTTTACTTG (SEQ ID NO. 32)
E259G	ABEmax-SpG	sg7	TGGAATGTGGAAACAATACT (SEQ ID NO. 33)
N253G	ABEmax-SpG	sg1	GCTAAATGTTAATGAGAATG (SEQ ID NO. 34)
N255G	ABEmax-SpG	sg3	GTTAATGAGAATGTGGAATG (SEQ ID NO. 35)
T264I	CBE4max-SpCas9-NG	sg10	AATACTTGCACAAACAATGA (SEQ ID NO. 36)
E364K	CBE4max-SpCas9-NG	sg40	ATACTCATGTTCTGGGTTCAA (SEQ ID NO. 37)
P361L	SPACE-NG	sg24	AACCCGAACATGAGTATAAG (SEQ ID NO. 38)
C367R	ABEmax-SpG	sg41	TCACACTTATACTCATGTTC (SEQ ID NO. 39)
E269G	ABEmax-SpG	sg13	AATGAGGTGCATAACCTTAC (SEQ ID NO. 40)
P361F	CBE4max-SpCas9-NG	sg23	TGAACCCGAACATGAGTATA (SEQ ID NO. 41)
C367Y	SPACE-NG	sg38	CACACTTATACTCATGTTCG (SEQ ID NO. 42)
I371M	ABEmax-SpG	sg27	AATACTCTATAATAACCACA (SEQ ID NO. 43)
E362G + H361R	ABEmax-SpG	sg25	CCCGAACATGAGTATAAGTG (SEQ ID NO. 44)
Y365H + C365R	ABEmax-SpRY	sg42	GTCACACTTATACTCATGTT (SEQ ID NO. 45)
N263G + T262A	ABEmax-SpG	sg11	AACAATACTTGCACAAACAA (SEQ ID NO. 46)
D368N + E368K	CBE4max-SpCas9-NG	sg43	CTGAGTCACACTTATACTCA (SEQ ID NO. 47)
C367Y +	CBE4max-SpCas9-NG	sg42	GTCACACTTATACTCATGTT (SEQ ID NO. 45)

D366H			
N253G	ABE8e-NG	sg6	AAGCTAAATGTTAATGAGAA (SEQ ID NO. 48)
E259G	ABE8e-NG	sg7	TGGAATGTGGAAACAATACT (SEQ ID NO. 33)
M247V	ABE8e-NG	sg18	AATATGATATTTGATAATAA (SEQ ID NO. 49)
E357G	ABE8e-NG	sg21	AAATTAGAAAACCTTGAACC (SEQ ID NO. 50)
E269G	ABE8e-NG	sg13	AATGAGGTGCATAACCTTAC (SEQ ID NO. 40)
I328V	ABE8e-NG	sg15	AATATTGAAACCTTTACTTG (SEQ ID NO. 32)
E360G	ABE8e-NG	sg23	TGAACCCGAACATGAGTATA (SEQ ID NO. 41)

Results are shown in Figure 9. In summary, it could be confirmed that human CD45 is amenable to base editing. In particular variants I328V, N255G, E360G, E259G, E364K, E269G, can be successfully edited via base editing. These residues can be efficiently edited
5 in K562 cells and human T cells while preserving the function of CD45. This gene editing technology is therefore compatible with the variants identified in the present disclosure and can be used in a respective clinical setting, for example for a safety switch or shielding.

10 **Example 8: Base editing can shield human T cells from antibody-drug conjugate mediated killing *in vitro***

Human primary T cells were isolated from donor PBMC using an EasySep Human T Cell Isolation Kit (Stemcell Technologies Ref:17951) following the manufacturer's recommendations. Isolated human T cells were then incubated in 200 µL of human medium
15 in a 96 well plate (1.5e6 cells/mL) for 24 hours. On the next day, cells were activated at a concentration of 1.5e6 cells/mL by addition of IL-2 (150U/mL), IL-7 (5ng/mL), IL-15 (5ng/mL) and Dynabead Human T-Activator CD3/CD28 for T Cell Expansion and Activation (Gibco

Ref:11132D) following the manufacturer's recommendation (1:1 ratio beads:cells). After 48h of incubation, cells were debeaded and were ready for electroporation.

1 million activated human T cells were electroporated with 7.5µg of ABE8e-NG mRNA (TriLink) and 7.5µg of sg7-E259G (SEQ ID NO. 33) or sg44-I283M+H285R+N286D (ATATCTCATAATTCATGTAC; SEQ ID No. 64; Synthego) using a P3 Primary Cell 4D-Nucleofector X Kit L (Lonza) following the manufacturer's recommendation. Electroporated cells were expanded for 5 days in 48 well-plates in 1 mL of human medium with supplementation of 500U/mL of IL-2 and media renewal every 48 hours.

To test if base edited human activated T cells are shielded from an antibody-toxin conjugate, 5,000 bulk base edited T cells were incubated for 3 days in 100 µL of human medium with addition of different concentrations of Refmab#4-biotin-streptavidin-saporin (1:1 Refmab#4-biotin:saporin-streptavidin; pre-incubated 30min at room temperature before addition to the wells). After 3 days of incubation, all cells from each condition were collected, stained with Refmab#4-Ax647, Refmab#1-Ax488 and for viability and were resuspended in 200uL of FACS buffer for flow cytometry analysis. The whole resuspension volume for each condition was then analyzed using a BD FACSAria III Cell Sorter (BD Biosciences). Live cells were sorted and sent for Sanger sequencing to assess the enrichment of base edits correlating with increasing concentrations of Refmab#4-biotin-streptavidin-saporin.

Results are shown in Figure 10. PBS alone and unconjugated saporin (SAP) were used as negative control groups. About a third of base edited cells lost binding to Refmab#4 (edited cells). Increasing concentrations of Refmab#4-biotin:saporin-streptavidin resulted in an increasing depletion of unedited cells. At the highest concentration (1nM), a complete depletion of unedited cells (Refmab #4+ cells) was observed when the antibody-toxin was added while edited cells persisted (Fig. 10A, Refmab #4 low cells). This was confirmed by Sanger sequencing: Increasing concentrations of Refmab#4-biotin:saporin-streptavidin resulted in an increasing % of cells harboring the A4->G4 base edit resulting in the amino acid change E259G (Fig. 10B). Similar results were obtained with sg44 (SEQ ID No. 64) resulting in I283M+H285R+N286D. Thus, base editing in human T cells can shield cells from

antibody-drug-conjugates resulting in an enrichment of edited cells as demonstrated for two independent examples.

Example 9: HDR-based gene editing renders human T cells resistant to killing by Refmab #1 conjugated to a toxin

5 Human T cells were isolated from PBMCs (peripheral blood mononuclear cells) by negative selection using the EasySep™ Human T Cell Isolation Kit (Stemcell Technologies ; Cat# 17951). Cells were rested 12h at 37C before they were activated for 2 days with Dynabeads Human-T cells Activator CD23/CD28 (Thermo Fischer; Cat. No. 111.31D) in 1:1 ratio supplemented with IL2, IL7 and IL15. Activated T cells were then electroporated with
10 60pmols of Cas9 conjugated to 120pmol of sgRNA using a Nucleofector 4D unit (Lonza) in P3 buffer and pulse EH115. PGA was added to the RNP with a ratio gRNA:PGA:Cas9 = 1 : 0.8 : 1. For knock-in's, the RNP mixture was supplemented with 50pmols of Homology Directed Repair (HDR) template. 4 days after electroporation cells were screened by FACS for knock-in efficiency analysis. Cells were stained with anti-CD4 (OKT4), anti-CD8 (RTPA-8), anti CD45
15 (Refmab #3) and anti-CD45 (Refmab #1).

The variant used in this experiment is F331del, i.e. a CD45 variant lacking the phenylalanine residue at position 331. Electroporation of the cells with the RNP only, led to a knock-out of CD45, indicated by a loss-of-binding of Refmab's #1 and #3 (Figure 11). Cells transfected with the HDR template encoding the point mutation lost binding to
20 Refmab #1, but were still reactive to Refmab #3.

gRNA used in this experiment to edit CD45: CTTACCACACTGAAATCTGT (SEQ ID No. 51)

HDRT used for human T cells engineering are shown in Table 14:

Variants	HDRT sequence
T332del	Alt-R- /HDR1/T*T*TTAAAATGGAAAAATATTGAAACCTTTTGcGAcAcTCAaAAcATcACa TAAtAGATTTCAGTGTGGTAAGAATATAACATTGACCAGAGAATTTTTTTTTGT*G* G/Alt-R-HDR2/ (SEQ ID No. 52)

T330K	/Alt-R- HDR1/T*T*TAAAATGGAAAAATATTGAAAAATTcACgTGcGAcAcTCAaAAcATcACaTAtAGATTTCAGTGTGGTAAGAATATAACATTGACCAGAGAATTTTTTTTTGT*G*G/Alt-R-HDR2/ (SEQ ID No. 53)
F331T33 2del	/Alt-R- HDR1/T*T*TAAAATGGAAAAATATTGAAACCTGcGAcAcTCAaAAcATcACaTAtAGATTTCAGTGTGGTAAGAATATAACATTGACCAGAGAATTTTTTTTTGT*G*G/Alt-R-HDR2/ (SEQ ID No. 54)
T330del	/Alt-R- HDR1/T*T*TAAAATGGAAAAATATTGAATTcACgTGcGAcAcTCAaAAcATcACaTAtAGATTTCAGTGTGGTAAGAATATAACATTGACCAGAGAATTTTTTTTTGT*G*G/Alt-R-HDR2/ (SEQ ID No. 55)
F331del	/Alt-R- HDR1/T*T*TAAAATGGAAAAATATTGAAACCACTTgGcGAcAcTCAaAAcATcACaTAtAGATTTCAGTGTGGTAAGAATATAACATTGACCAGAGAATTTTTTTTTGT*G*G/Alt-R-HDR2/ (SEQ ID No. 56)

Next, 5000 engineered and sorted human T cells were distributed in 96 well plate in 100ul of medium supplemented with 50U/ml IL2. Biotinylated Refmab #1 was conjugated to streptavidin-bound ZAP with a ratio 1:1 in PBS. Cells were incubated for 3 days at 37°C with 50nM of the Refmab #1-ZAP mixture. At the end of the incubation, 100ul of CellTiter Glow (Promega Cat Nr: G9241) was added to each well. Luminescence was read with an integration time of 1s.

Results are shown in Figure 12. Wild-type cells showed low luminescence, indicating a killing by the Antibody Drug Conjugate. In contrast, CD45 knock-out cells, as well as cells expressing the CD45 variant are protected from killing.

Similarly, CD34+ HSPCs were engineered to express F331del. The cells were then incubated with antibody *Optimus Prime*-tesirine (see Example 18). Non-edited cells were depleted while CD45 KO and CD45 F331del cells were protected.

Example 10: Knock out of CD45 in cells and re-expression of CD45 variants in cell lines

K562 cells (ATCC CCL-243) were electroporated with RNP targeting CD45 utilizing a Nucleofector 4D unit (Lonza). The gRNA was the same as used in Example 9 (SEQ ID No. 51).

5 4 days after electroporation, cells were sorted for CD45KO and separated via limiting dilution to receive single clones. Clones were grown and sequenced. A clone with all alleles showing indels in the CD45 gene was selected.

Cells propagated from the selected clone were electroporated with the Neon transfection system (Thermo Fisher). 6.5ug of plasmid encoding for the variant forms of CD45 were
10 mixed with 2 million cells. Plasmid encoding the following variants were used.

Table 15:

Variant	Plasmid	Variant
Mut1	p459_CD45RO_E329I	E329I
Mut2	p460_CD45RO_E329K	E329K
Mut 3	p461_CD45RO_T330E	T330E
Mut 4	p462_CD45RO_T330D	T330D
Mut 5	p468_CD45RO_T330R	T330R
Mut 6	p470_CD45RO_T330K	T330K
Mut 7	p469_CD45RO_T330I	T330I
Mut 8	p471_CD45RO_2aadel	2aa-del
Mut9	p511_CD45RO_F331del	F331del
Mut10	p512_CD45RO_T332del	T332del
Mut11	p552_CD45RO_T330A	T330A
Mut12	p553_CD45RO_F331S	F331S
Mut13	p554_CD45RO_F331I	F331I
Mut14	p555_CD45RO_F331L	F331L
Mut15	p598_CD45RO_T330del	T330del
Mut16	p599_CD45RO_F331A	F331A

Mut17	p600_CD45RO_T332A	T332A
WT CD45RO	p453_pCMV3_CD45RO	Wild type
Ctrl	No DNA electroporation	n/a

24h after electroporation cells were stained for FACS with anti-CD45 (Refmab #3) and anti-CD45 (Refmab #1) antibodies. While the CD45RO (wildtype) form of the protein did bind to both antibodies, a loss of binding was observed for Refmab #1 for the CD45 variants. All variants did retain binding to Refmab #3, demonstrating that the protein was expressed by the electroporated cells. Results are shown in Figure 13.

The same experiment was also performed with Jurkat cells with essentially the same results.

Example 11: Engineering of shielding CD45 variants into human CD34+ HSPCs using HDR

Human CD34 cells were isolated from G-CSF mobilized healthy donors using a CliniMACS Prodigy (Miltenyi Biotec) following the manufacturer's recommendations. Isolated human CD34 cells were then pre-stimulated for 2 days in culture (HSC Brew GMP medium supplemented with 100 ng/mL rhSCF, rhFlt3L, rhTPO and 60 ng/mL rhIL3) and electroporated with CRISPR/Cas9 gene editing reagents (SpyFi Cas9 protein + gRNA = RNP complex, and the HDR template) using a CliniMACS Prodigy Electroporator. The HDR template was the following sequence:

TTTAAATGGAAAAATATTGAAACCACTTGcGAcAcTCAaAAcATcACaTAtAGATTTCAGTGTGG
TAAGAATATAACATTGACCAGAGAATTTTTTTTGTGG (SEQ ID No. 56).

Electroporated cells were expanded for seven days and analyzed by FACS. Cells were stained with two different CD45 antibodies, one binding to the region of the mutation (Refmab #1) and the second antibody binding a different region (Refmab #3). Results shows the presence of 50% knock-out and 5-6% HDR-mediated knock-in cells (identified by the

loss of binding of the antibody targeting the mutated epitope but the retention of the binding of the second antibody). See Figure 14.

Example 12: Expression of variants in DF-1 cells

DF-1 cells (ATCC number CRL-12203) show no staining upon incubation with antibodies
5 against human CD45. Therefore, they are suitable cell lines to express human wild-type or mutant CD45 variants. DF-1 cells were transfected with a construct containing wild-type CD45 (SEQ ID No. 1) or with constructs containing mutant CD45 variants.

DF-1 cells were transfected with selected CD45 variants (K352E, K352H, N257R or N257T) or wild-type CD45 using Lipofectamine Lipofectamine™ 3000 Transfection Reagent
10 (Thermo Fischer Scientific, cat. L3000008). After 72 hours, the transfection efficiency, and the expression of CD45 were analyzed by FACS using the antibody Refmab #4 directly labeled to AlexaFluor 647 (APC) and the antibody Refmab #1 directly labeled with AlexaFluor 488 (FITC). Refmab #1 and Refmab #4 binds to different regions of CD45. Using this antibody combination, it is possible to evaluate the loss of binding of the antibody of
15 interest to a specific mutation of CD45, while measuring the retention of the binding of the second antibody shows that the variant CD45 is still expressed by the cells, and structurally folded.

Results are shown in Figure 19 (panel A: wild-type, panel B: K352E, panel C: K352H, panel D: N257R, panel E: N257T). The wild-type protein binds to both antibodies, Refmab #1 and
20 Refmab #4, whereas the K352E, K352H variants were detected with Refmab #4 only, and the N257R and N257T variants with the Refmab #1 only. This confirms that a mutation of residues K352 leads to a loss of binding of Refmab #1, and a mutation of residues N257 leads to a loss of binding of Refmab #4.

The same experiment was repeated also testing additional experiments. Tested were
25 variants K352E, K352H, K352S, K352T, N257D, N257R, N257S, N257T, E353K, E259G, E259Q and E259N. Results are shown in Figures 21 (K352E, K352H, K352S, K352T, N257D, N257R, panels A-F, respectively) and 22 (N257S, N257T, E353K, E259G, E259Q, E259N, panels A-F, respectively).

Example 13: Expression and purification of CD45 D1-D2 fragments and variants

For precise antibody-protein affinity measurements and structural characterization, ectodomains of wild-type and mutant d1-d2 CD45 were produced. The protein sequence (residues 225-394) is histidine tagged at the C-terminus and contains few N- and C-terminal added amino acids important for crystal packing (full wt sequence ETGIEGRKPTCDEKYANITVDYLYNKETKLFTAKLNVNENVECGNNTCTNNEVHNLTECKNASVSISHN SCTAPDKTLILDVPPGVEKFQLHDCTQVEKADTTICLKWKNIETFTCDTQNITYRFQCGNMIFDNKEIKL ENLEPEHEYKCDSEILYNNHKFTNASKIIKTDFGSPGEGTKHHHHHH SEQ ID No. 57/Uniprot ID P08575). Expi293F GnTI cells (Thermo Fisher; #A39240) that lack N-acetylglucosaminyltransferase I (GnTI) activity and therefore lack complex N-glycans were used for protein expression. After harvesting, protein was purified using Ni-NTA chromatography, followed by digestion of high mannose glycans with endoglycosidase H (EndoHf (New England BioLabs, P0703S)) at 37°C overnight. EndoHf was removed from the protein solution with amylose resin and the CD45 protein was further purified by size exclusion chromatography in buffer 150mM NaCl, 20mM Hepes pH7.4.. Peak monomer (c7-c10) and dimer fractions (where needed) were concentrated using 10kDa cut off Amicon centrifugal filter and protein aliquots were flash frozen in liquid nitrogen before storage at -150°C. A monomeric, CD45 D1-D2 wild type protein was produced. Variant CD45 proteins are produced using the same experimental procedure. The monomer content % for each protein was taken from the size exclusion chromatogram (fractions c5-c11). Figure 15 shows a representative chromatogram of size exclusion chromatography (panel A) and a SDS-PAGE of the purified non-glycosylated wt protein (panel B).

Example 14: Binding of CD45 variants to the Refmab's

Analysis of binding to the elected variants was performed with antibodies Refmab #1, Refmab #2 and Refmab #4. Binding of the antibodies to CD45 wildtype and variants was measured in an Octet system RED96e (Sartorius) or R8 at 25 °C with shaking at 1,000 rpm using 1x kinetic buffer (Sartorius, PN: 18-1105). The elected variants were screened for their ability to bind Refmab #1 and Refmab #2 using different concentrations of CD45 (wild

- type or variant). Antibodies were captured by Anti-Human Fc capture biosensor (AHC) (Sartorius, PN: 18-5060) for 300 s at 0.5 to 1 ug/mL. As an analyte, human CD45 wt and variants, containing only domains 1, 2 (CD45 D1-2), were titrated at 3-7 different concentrations (from 2000 nM to 1 nM). Association of the analyte to the antibodies was
- 5 monitored for 300 or 600 s and dissociation of the analyte from the antibodies was monitored for 900 or 1800 s. Reference subtraction was performed against buffer only wells. AHC tips were regenerated using 10 mM Gly-HCl pH 1.7. Data were analyzed using the Octet Data Analysis software HT 12.0. Data were fitted to a 1:1 binding model. Kinetic rates k_a and k_d were globally fitted or steady state analysis was performed.
- 10 For analyzing binding to Refmab #4, streptavidin (SA) biosensor (Sartorius, PN: 18-5020) were first coated with CaptureSelect™ biotin anti-LC-kappa (murine) conjugate (Thermo Scientific, PN: 7103152100) for 600 s at 1 ug/mL. Refmab #4 was then captured by the coated SA biosensors for 300 s at 0.5-1.0 ug/mL. Analyte titration, association and dissociation were performed as for Refmab's #1 and #2.
- 15 Results are shown in Table 16 and Figure 20. The %binding for the Refmab's was calculated by dividing the nm shift of the hCD45 variant by the hCD45 wt. The nm shift used for this calculation was for the top hCD45 concentration (500 nM) at the end of the association (300 s). Marked with an asterik are %binding results which were calculated by dividing the nm shift of 50 nM (instead of 500 nM) hCD45 variant by the nm shift of 50 nM
- 20 (instead of 500 nM) hCD45 wt. ND stands for not determined. NA stands for not analysed. Mutations in positions 230, 257, 259, and 267 did not affect binding to Refmab #1 substantially, while no binding (until 500 nM analyte concentration) was observed for K352D, K352E, K352H, K352I, K352L, K352N, K352T, E353K, E353R and the double mutant N351D K352E. No binding was also observed when position T330 and F331 were deleted.
- 25 Lower Refmab #1 binding was observed for K352S and F331A.

The single point mutations E230K, E259N, H285R, N286D as well as the triple mutation I283M H285R N286D decreased binding to Refmab #4, while no binding to Refmab #4 was observed for hCD45 D1-2 variants (N257D, N257E, N257K, N257R, N257G, N257T, E259G,

E259Q, E259V, N267S and the double mutant H285R N286D) up until 500 nM analyte concentration.

Low Refmab #2 binding was observed for N257D and N257T and no Refmab #2 binding (up to 500 nM analyte concentration) was observed for N257E, N257K, N257R, and N257G.

	Refmab #1 %binding	Refmab #2 %binding	Refmab #4 %binding	%monomer content
WT	100	100	100	55
WT glycosylated	119 (*)	NA	103	54
E230K	119	98	49	23
N257D	118	11	0	55
N257E	108	0	0	62
N257K	101	0	0	50
N257R	100	0	0	47
N257G	95	0	0	23
N257T	90	17	0	83
E259G	88	84	0	27
E259N	114	81	9	49
E259Q	110	78	0	49
E259V	110 (*)	NA	0	60
N267S	105	79	0	12
K352D	0	104	115	59
K352E	0	121	87	50
K352H	0	105	70	50
K352I	0	97	49	32
K352L	0	120	57	20
K352N	0	131	68	34
K352S	4	86	69	63
K352T	0	130	52	34
E353A	95	87	57	22
E353K	0	129	47	32
E353R	0	112	66	19
I283M	129	NA	44	21
H285R	105	NA	4	12
N286D	144	NA	7	55
H285R N286D	ND	NA	0	12
I283M H285R N286D	44	NA	5	17
N351D	119	NA	123	59
K352E	8	NA	108	43
N351D K352E	0	NA	93	40
F331A	40	NA	139	28
deletion T330 deletion F331	0	NA	104	26

Example 15: Characterization of CD45 variants by nanoDSF

Thermostability of CD45 D1-D2 variants were analyzed by differential scanning fluorimetry and monitoring tryptophane fluorescence using Nanotemper Prometheus NT.48 NanoDSF (NanoTemper Technologies). Tryptophane fluorescence was measured
5 using CD45 D1-D2 wild-type and variants at 0.25-1.0 mg/mL in 150mM NaCl, 20mM Hepes pH7.4 and the temperature was increased from 20 °C to 95 °C. The melting temperature was determined as the inflexion point of the sigmoidal curve and compared to CD45 D1-2 wt. Results are shown in Figure 17 and Figure 18.

Example 16: Functional assay

- 10 Target protein dephosphorylation by CD45 can be tested by the skilled person by any commonly used assay, such as an AlphaLISA immunoassay (Perkin Elmer). Cells expressing CD45 wild type or isoform are activated with anti-CD3 antibodies for different time points (5-20min), before cell lysis. Phosphorylation of Lck at position Tyr505 or total Lck is detected and read out by a plate reader.
- 15 Dephosphorylation of Lck at position Tyr505 by CD45 or total Lck was measured using an AlphaLISA assay. Cells e.g. Jurkat cells (wild type or variants thereof) are collected and pre-incubated with HBSS medium for 2h at 37°C. Assay plates (96-well plates) are coated for 2h at 37°C or overnight at 4°C with 10ug/mL anti-CD3 antibody in 100uL medium per well. Assay plates are washed twice with sterile PBS and cells are seeded at a concentration of
20 10.000-50.000 cells per well in 80uL of HBSS medium. The rest of the assay was performed according to the Assay Kit Protocol according to the manufacturer (Perkin Elmer) containing all the necessary reagents (PerkinElmer AlphaLISA #ALSU-PLCK-A-HV and #ALSU-TLCK-A-HC). After 5-30minutes 20uL of 5x Lysis buffer is added to the wells and incubated for 10min at 350rpm on a plate shaker. 5uL of lysate are transferred to a 384-white well plate and 5uL
25 of Acceptor Mix (according to manufacturer's protocol) added to the wells, sealed with a foil, incubated for 2min on a plate shaker and incubated at room temperature for at least 1 hour. 5uL of Donor Mix (according to the manufacturer's protocol) are added to the wells, plate sealed with a foil, mixed on a plate shaker for 2min and incubated at room

temperature for at least 1 hour. Plates are then read on the Envision plate reader (Perkin Elmer) to detect total Lck and phosphorylated Lck.

A results of an assay measuring phosphorylation of Lck at position Tyr505 via an AlphaLISA assay is shown in Figure 16. 10.000-50.000 Jurkat wildtype or Jurkat CD45 knock-out cells were incubated for 20 minutes in plates coated with anti-CD3 Antibody before cell lysis and detection of phosphorylated Lck. Activation of Jurkat cells using the anti-CD3 antibody leads to CD45 activation which in turn dephosphorylates Lck. Jurkat CD45 knock-out cells are not able to dephosphorylate Lck upon activation. The figure shows the acceptor signal (counts) and represents one biological experiment containing two technical replicates.

10 **Example 17: Internalization of antibodies into human cells**

Antibody internalization can be tested by the skilled person by any commonly used assay, such as FACS. Cells expressing CD45 are incubated with antibodies labelled with a fluorophore e.g. Alexa Fluor 488 (AF488) for different time points (1-24h), before washing and quenching with an anti-AF488 antibody for one hour. Internalized antibody is able to give a signal in the FACS readout, while the signal of Antibody bound to the cell surface is quenched and not detectable.

Antibody internalization into TF-1 or Jurkat cells expressing CD45 or variants thereof is measured by FACS. TF-1 cells are seeded at a concentration of 1 million cells/mL in 0.1mL of medium (for TF1 cells: RPMI1640 supplemented with GlutaMAX + 10% heat inactivated FBS + 2ng/mL GM-CSF; for Jurkat cells: RPMI ATCC modification + 10% heat inactivated FBS) in a 96-well plate. The next day, cells are treated with 2-20 µg/mL Antibody labelled with Alexa Fluor 488 (AF488; Alexa Fluor® 488 Conjugation Kit (Fast) - Lightning-Link®, Abcam) for 1-24hours at 37°C or 4°C, before cell collection and washing in ice cold PBS. Cells are resuspend in ice cold PBS containing 20-200ug/mL Anti-AF488 antibody (Alexa Fluor 488 Polyclonal Antibody, ThermoFisher Scientific) for one hour before acquiring data on the FACS machine (NovoCyte, Agilent). Signal of internalized antibody is measured by the FACS, while signal of antibody outside of the cell is quenched. Percentage of antibody internalization is calculated by dividing signal of cells incubated with quencher by the signal of cells incubated without quencher.

Example 18: Improved Refmab antibodies

Improved versions of antibody Refmab #1 were generated. The amino acid sequences of an exemplary improved binder, antibody *Optimus Prime*, is shown in Table 17.

Table 17:

5

Optimus Prime		
SEQ ID No.	Comment	Sequence
58	VH	QVQLVESGGGLVKPGGSLRLSCAASGFAFSNYDMSWIRQAPGK GLEWVSYISSGGVSTYYPDTVKGRFTISRDNAKNSLYLQMNSLR AEDTAVYYCARRYDVWWYFDVWGRGTLTVSS
59	VL	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNAKTYLEWYQQR PGQSPRLLIYKVSNLFSGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQGSHVPMYTFGQGTKLEIK
4	HCDR1	NYDMS
5	HCDR2	YISSGGVSTYYPDTVKG
6	HCDR3	RYDVWWYFDV
60	LCDR1	RSSQSIVHSNAKTYLE
61	LCDR2	KVSNLFS
9	LCDR3	FQGSHVPMYT

Antibody Optimus Prime demonstrated to have a binding specificity that is identical to that of Refmab #1.

Example 19: Shielding HSCs by base editing

To test shielding of human HSCs engineered by base editing to express K352E, 1 million
5 hCD34+ HSPCs were electroporated 48h post-thawing with 7.5 µg of base editor mRNA
(Trilink) and 13.6 µg of sgRNA (Synthego) (1:100 BE:sgRNA molar ratio) using a P3 Primary
Cell 4D-Nucleofector X Kit L (Lonza) following the manufacturer's recommendation with
the Lonza CA-137 pulse program. Electroporated CD34+ HSPCs were kept in culture at
0.5e6 cells per mL in 6-well flat-bottom plate (Corning #3516) in stem cell medium renewed
10 every 5 days (StemSpan SFEM II (StemCell #09655) + 1uL/mL hSCF (Miltenyi #130-096-695)
+ 1mL/mL hFlt3-Ligand (Miltenyi #130-096-479) + 1uL/mL hTPO (Miltenyi #130-095-752).
Editing was analyzed 5 days post electroporation using flow cytometry.

K352E can be engineered into CD34+ HSPCs by base editing using ABE8e-NG mRNA
(Trilink) and sgRNA-49 (SEQ ID No. 33; Synthego). This resulted in <1% Refmab#1 non-
15 staining CD34+ HSPCs (Fig. 23A). In order to increase the editing rates, we sought to
reposition the ABE8e into a more favorable position within the base editing window of the
protospacer. To this end, we took advantage of the less restrictive PAM recognition
capabilities of the SpRY Cas9 variant (Science (2020) 368: 190-6). We tiled gRNAs in the
region of interest shifting by 1 nt each. This resulted in a strongly increased desired editing
20 activity. Flow cytometry analysis showed that ABE8e-SpRY mRNA combined with sgRNA-
49.3 (SEQ ID No. 62) or sgRNA-49.4 (SEQ ID No. 63), resulted in a 33% and 26% Refmab #1
negative cell population, respectively, without altering Refmab#4 binding (Fig. 23B).
Similarly to ABE8e-NG+sgRNA-49, combining ABE8e-SpRY+sgRNA-49 resulted in <1%
shielded cells. Sanger sequencing of bulk base edited CD34+ HSPCs confirmed the improved
25 K352E base editing with sgRNA-49.3 and sgRNA-49.4.

sgRNA-49 sequence: TGGAATGTGGAAACAATACT (SEQ ID No. 33; targetable by both
ABE8e-NG and ABE8e-SpRY)

sgRNA-49.3 sequence: GGAATGTGGAAACAATACTA (SEQ ID No. 62; only targetable by ABE8e-SpRY)

sgRNA-49.4 sequence: GAATGTGGAAACAATACTAG (SEQ ID No. 63; only targetable by ABE8e-SpRY)

CLAIMS

1. A human cell or a population of human cells expressing a first isoform of CD45 for use in a medical treatment in a patient in need thereof, said patient having cells expressing a
5 second isoform of CD45,
- wherein said human cell expressing said first isoform comprises genomic DNA with at least one polymorphism or genetically engineered allele,
- wherein said polymorphism or genetically engineered allele is not present in the genome of the patient having cells expressing said second isoform of CD45, and
10 wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position E230, Y232, N255, N257, E259, T264, N267, E269, N286, S287, D292, I328, T330, F331, D334, Y340, K352, E353, E360, E364, Y372 or Y373 of SEQ ID NO: 1.
2. The human cell or population of human cells for use according to claim 1, wherein said
15 first and second isoforms are substantially functionally identical, preferably wherein said first and second isoforms have substantially identical biophysical properties, have substantially the same stability, have substantially the same melting temperature, have substantially the same aggregation propensity and/or have substantially the same tendency to form dimers.
- 20 3. The human cell or population of human cells for use according to claim 1 or 2, wherein said first and second isoforms dephosphorylate target proteins of CD45, activate the TCR signaling cascade, lead to an increase of cytokine production and/or lead to an increase of proliferation of T cells.
4. The human cell or population of human cells for use according to any one of the
25 preceding claims, wherein said first and said second isoforms of CD45 dephosphorylate tyrosine kinase Lck.

5. The human cell or population of human cells for use according to any one of the preceding claims, wherein said medical treatment comprises administering a therapeutically efficient amount of said cell or population of cells expressing said first isoform of CD45 to said patient in need thereof, in combination with a therapeutically efficient amount of a depleting agent comprising an antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing said second isoform of CD45.
6. The human cell or population of human cells for use according to claim 5, wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid in position F331, K352 or E353 of SEQ ID NO: 1, and wherein said depleting agent comprises an antigen-binding region, selected from
- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3; or
- b) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61, VLCDR3 is SEQ ID NO: 9, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO:

58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.

7. The human cell or population of human cells for use according to claim 6, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position K352 of SEQ ID NO: 1.
8. The human cell or population of human cells for use according to claim 7, wherein said substitution of the amino acid in position K352 of SEQ ID NO: 1 is selected from K352E, K352H, K352I, K352L, K352M, K352N, K352Q, K352S and K352T, preferably wherein said substitution is K352D, K352E and K352H, and more preferably wherein said substitution is K352E.
9. The human cell or population of human cells for use according to claim 6, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N286 of SEQ ID NO: 1, preferably wherein said substitution is N286D.
10. The human cell or population of human cells for use according to any one of claims 6-9, wherein said depleting agent binds to the same epitope as an antigen-binding region, selected from
- a) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9, preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3; and
- b) an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and

VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 60, VLCDR2 is SEQ ID NO: 61, VLCDR3 is SEQ ID NO: 9, preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 58 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 59.

11. The human cell or population of human cells for use according to claim 5, wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid is in position E230, Y232, N257, E259 or N286 of SEQ ID NO: 1, and

wherein said depleting agent comprises an antigen-binding region comprising an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 20, VHCDR2 is SEQ ID NO: 21 and VHCDR3 is SEQ ID NO: 22; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 23, VLCDR2 is SEQ ID NO: 24, VLCDR3 is SEQ ID NO: 25, more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 18 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 19.

12. The human cell or population of human cells for use according to claim 11, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1.

13. The human cell or population of human cells for use according to claim 12, wherein said substitution of the amino acid in position N257 of SEQ ID NO: 1 is a N257E, N257K, N257R or N257T substitution, preferably wherein said substitution is a N257R substitution.

14. The human cell or population of human cells for use according to claim 11, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position E259 of SEQ ID NO: 1.
15. The human cell or population of human cells for use according to claim 11, wherein
5 said substitution of the amino acid in position N286 of SEQ ID NO: 1 is a N286D substitution.
16. The human cell or population of human cells for use according to claim 14, wherein said substitution of the amino acid in position E259 of SEQ ID NO: 1 is a E259N, a E259Q, a E259V or a E259G substitution, preferably wherein said substitution is a E259V
10 substitution.
17. The human cell or population of human cells for use according to claim 11, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position Y232 of SEQ ID NO: 1, preferably wherein said substitution is a Y232C substitution.
- 15 18. The human cell or population of human cells for use according to any one of claims 11-17, wherein said depleting agent binds to the same epitope as an antigen-binding region comprising
- an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 4, VHCDR2 is SEQ ID NO: 5 and
20 VHCDR3 is SEQ ID NO: 6; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 7, VLCDR2 is SEQ ID NO: 8, VLCDR3 is SEQ ID NO: 9,
more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO:
25 2 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 3.

19. The human cell or population of human cells for use according to claim 5, wherein said polymorphic or genetically engineered allele is characterized by a substitution of the amino acid in position N257 of SEQ ID NO: 1, and

wherein said depleting agent comprises an antigen-binding region comprising

5 an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID NO: 15, VLCDR2 is SEQ ID NO: 16, VLCDR3 is SEQ ID NO: 17,
10 preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

20. The human cell or population of human cells for use according to claim 19, wherein
15 said substitution of the amino acid in position N257 of SEQ ID NO: 1 is a N257E, N257K, N257R or N257T substitution, preferably wherein said substitution is a N257R substitution.

21. The human cell or population of human cells for use according to claims 19 or 20,
20 wherein said depleting agent binds to the same epitope as an antigen-binding region comprising

an antibody heavy chain variable domain (VH) comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3 wherein VHCDR1 is SEQ ID NO: 12, VHCDR2 is SEQ ID NO: 13 and VHCDR3 is SEQ ID NO: 14; and an antibody light chain variable domain (VL) comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3 wherein VLCDR1 is SEQ ID
25 NO: 15, VLCDR2 is SEQ ID NO: 16, VLCDR3 is SEQ ID NO: 17,
more preferably wherein said first antigen binding region comprises a heavy chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO:

10 and/or a light chain variable domain comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

22. The human cell or population of human cells for use according to any one of claims
5 1-5 wherein said polymorphic or genetically engineered allele is characterized by at least one substitution of an amino acid is in position E230, N257, E259, F331, K352 or E353 of SEQ ID NO: 1.
23. The human cell or population of human cells for use according to claim 22 wherein
10 said residue E230 is substituted with K, and/or residue N257 is substituted with A, D, E, H, K, R, S, T or V, and/or residue E259 is substituted with G, H, K, N, R, T or Q, and/or T264 is substituted with D or E, preferably with D, and/or N267 is substituted with A, H, L, S or V, and/or residue N286 is substituted with G, L or R, and/or E329 is substituted with A, and/or residue F331 is substituted with A or G, and or Y340 is substituted with A, G, N, Q or S and/or residue K352 is substituted with A, D, E, G, H, I, L, M, N, Q, S, T or
15 Y and/or residue E353 is substituted with A, H, I, K, L, S, T or R.
24. The human cell or population of human cells for use according to anyone of the preceding claims, wherein said cell expressing said first isoform of CD45 has been selected from a subject comprising native genomic DNA with at least one natural polymorphism allele in nucleic acid encoding said first isoform.
- 20 25. The human cell or population of human cells for use according to anyone of the preceding claims, wherein said first isoform of CD45 is obtained by *ex vivo* modifying the nucleic acid sequence encoding said first isoform of CD45 by gene editing, preferably by introducing into a human cell a gene editing enzyme capable of inducing site-specific mutations(s) within a target sequence encoding a surface protein region involved in the
25 binding of agent comprising at least a first antigen-binding region.
26. The human cell or population of human cells, preferably hematopoietic stem cells for use according to anyone of the preceding claims wherein said medical treatment comprises administering a therapeutically efficient amount of said human cell or

- population of human cells expressing said first isoform of CD45 to said patient in need thereof, in combination with a therapeutically efficient amount of a depleting agent comprising at least a first antigen-binding region that binds specifically to said second isoform of CD45 to specifically deplete patient cells expressing said second isoform of
- 5 CD45, preferably to restore normal hematopoiesis after immunotherapy in the treatment of hematopoietic disease, and preferably in the treatment of malignant hematopoietic disease such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), T-cell non-Hodgkin lymphomas (T-NHLs), chronic myeloid leukemia (CML), hairy cell leukemias (HCL), T-cell acute lymphoblastic leukemia (T-ALL), non-
- 10 Hodgkin's lymphoma (NHL) or follicular lymphomas (FL).
27. The human cell or population of human cells for use according to any one of claims 5-25, wherein said depleting agent is an antibody, antibody-drug conjugate or an immune cell, preferably a T-cell bearing a chimeric antigen receptor (CAR) comprising a
- 15 first antigen-binding region which binds specifically to said second isoform and does not bind or binds substantially weaker to said first isoform.
28. A pharmaceutical composition comprising a human cell, preferably a hematopoietic stem cell or an immune cell such as T-cell as defined in any one of claims 1 to 27 and preferably a depleting agent as defined in any one of claims 5 to 27 and a pharmaceutically acceptable carrier.

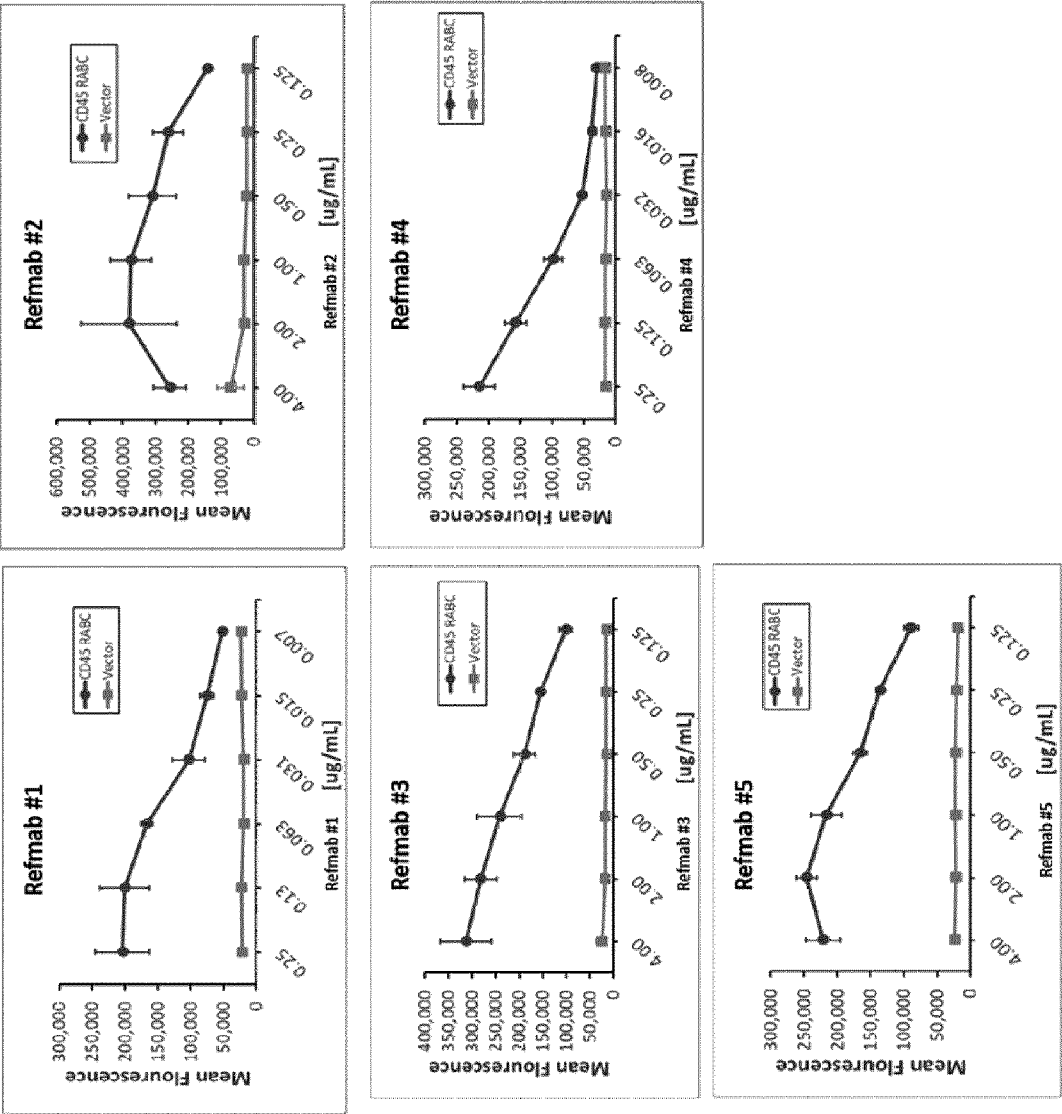


FIG. 1

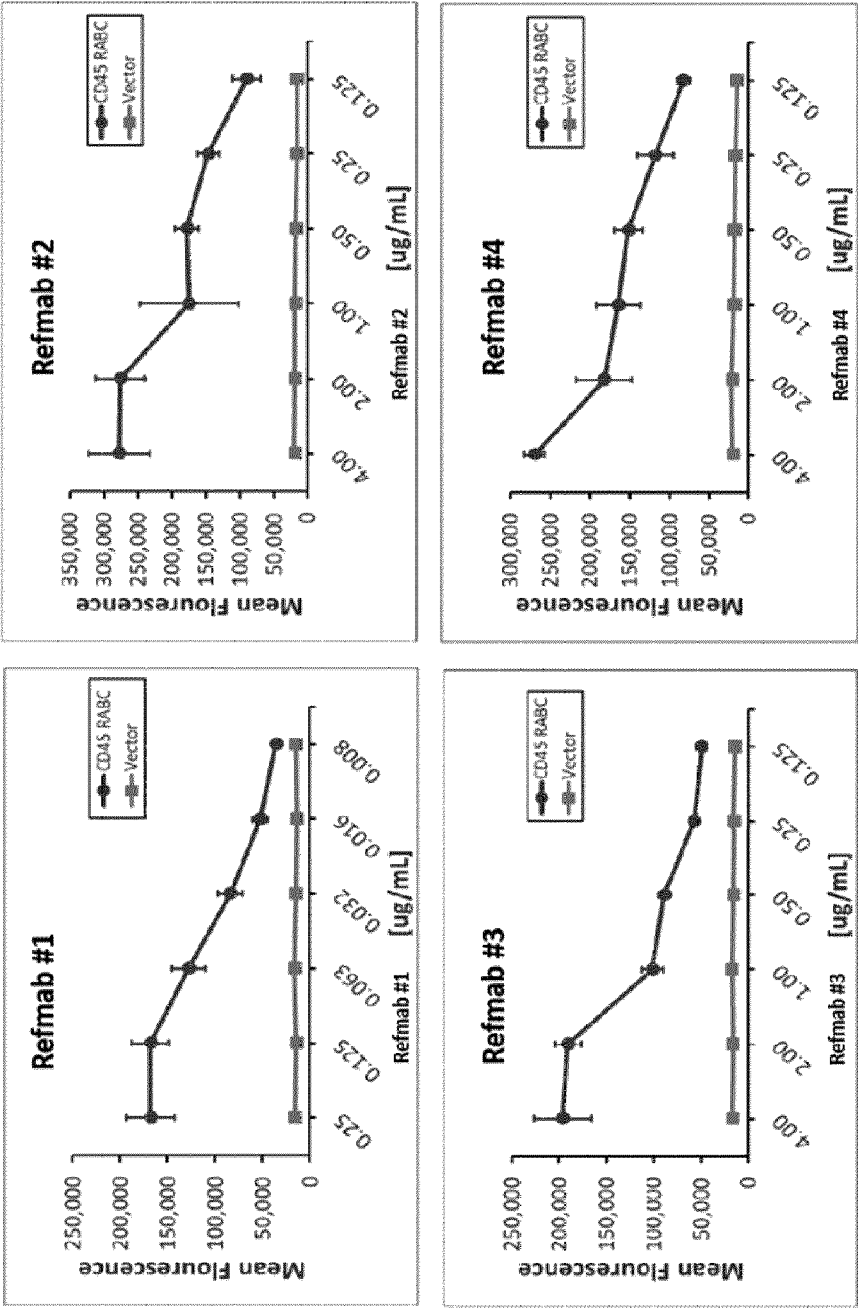


FIG. 2

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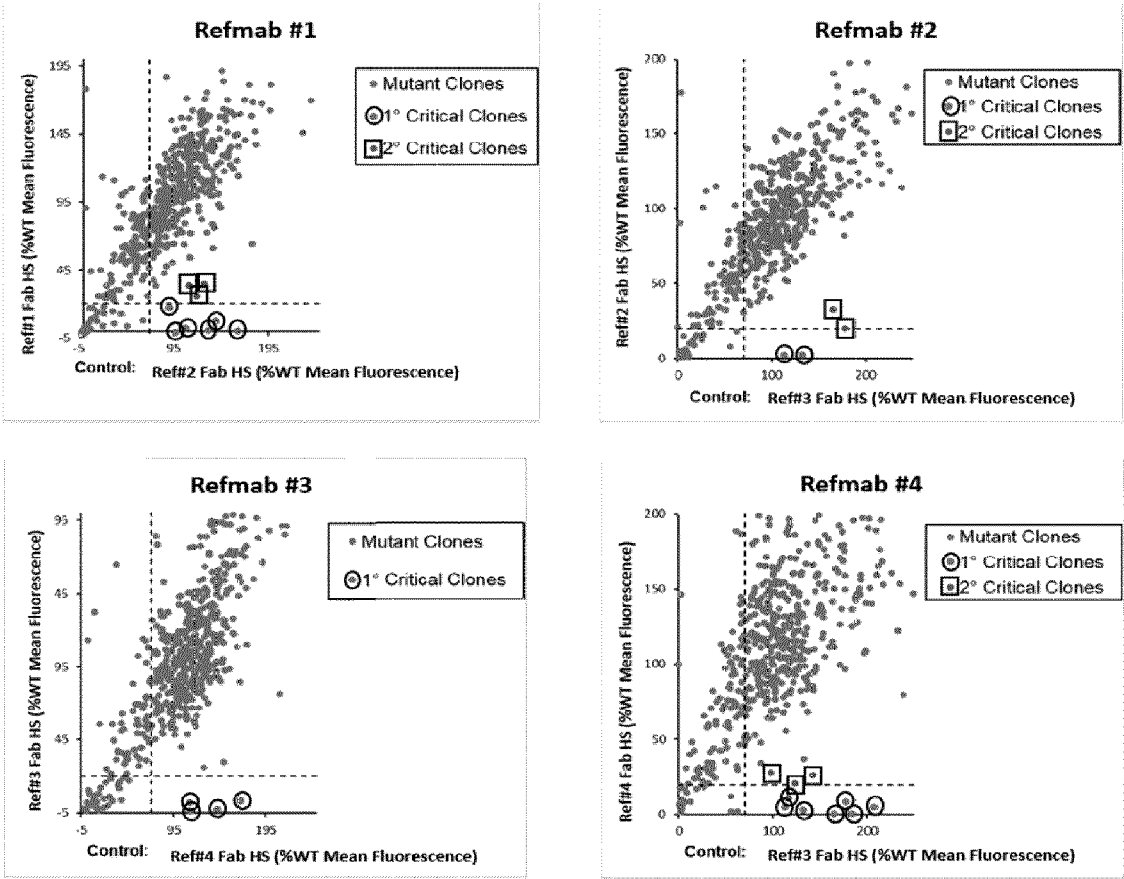


FIG. 3

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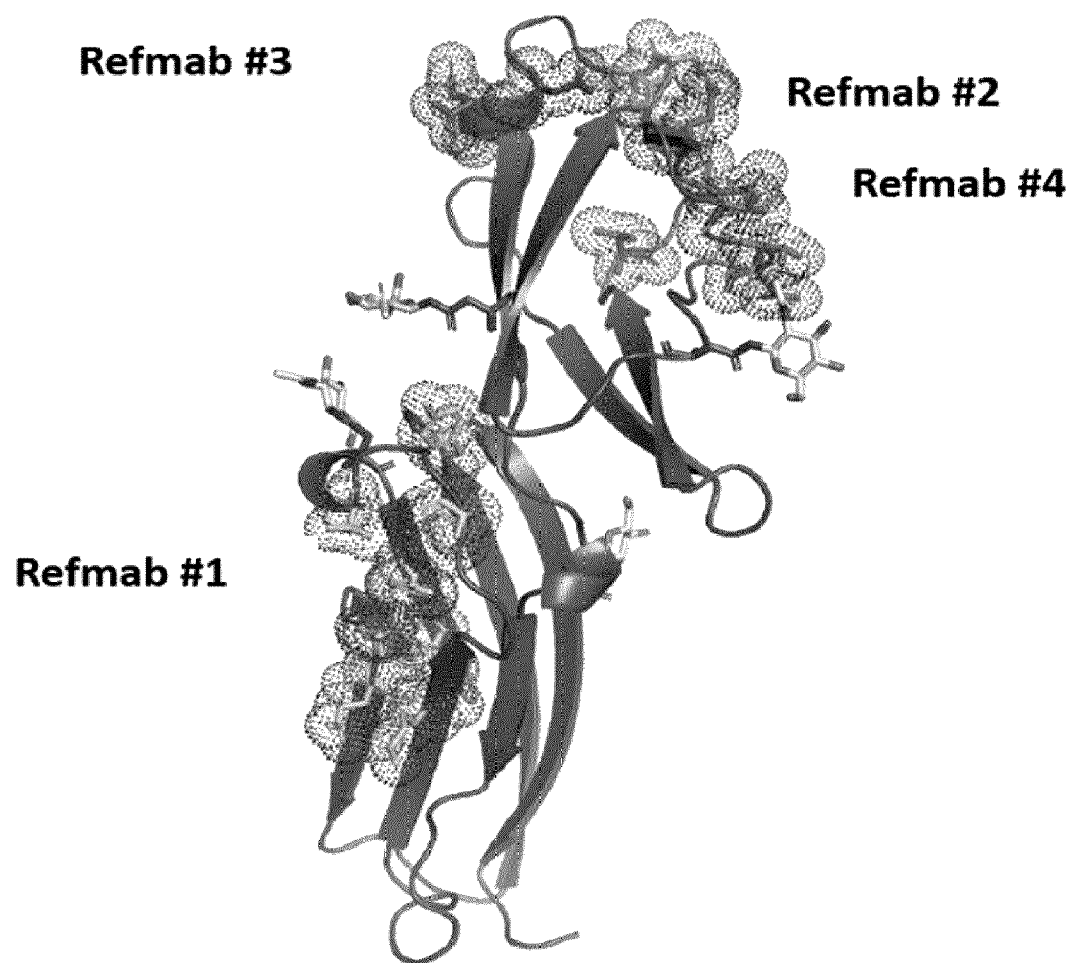


FIG. 4

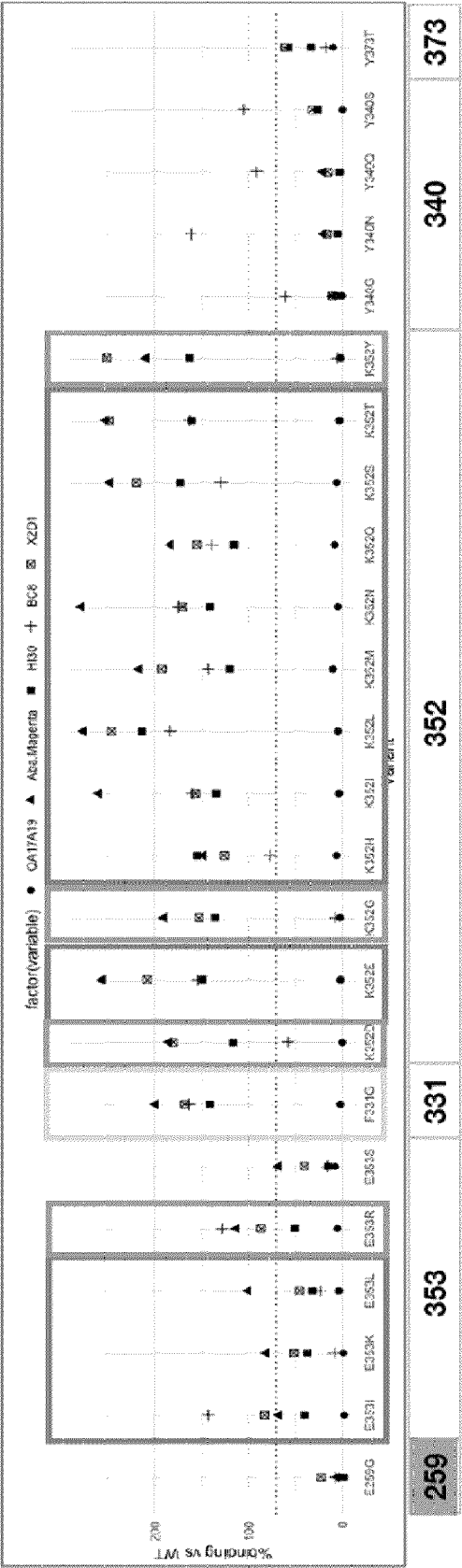


FIG. 5

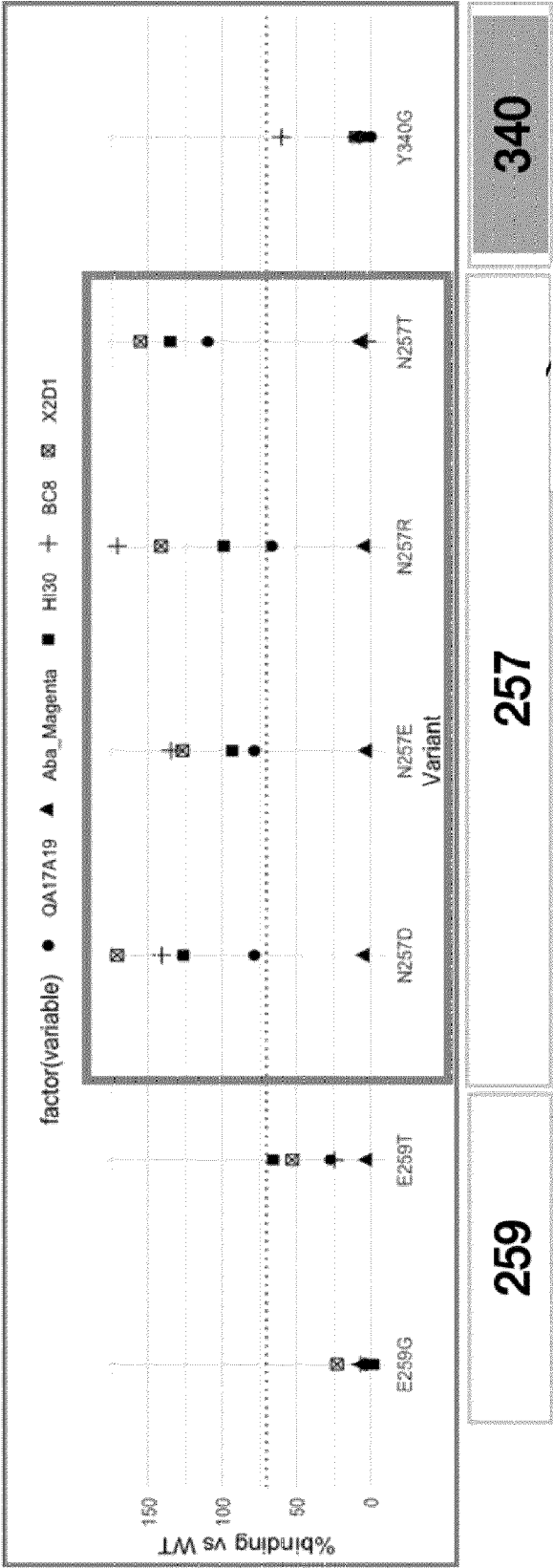


FIG. 6

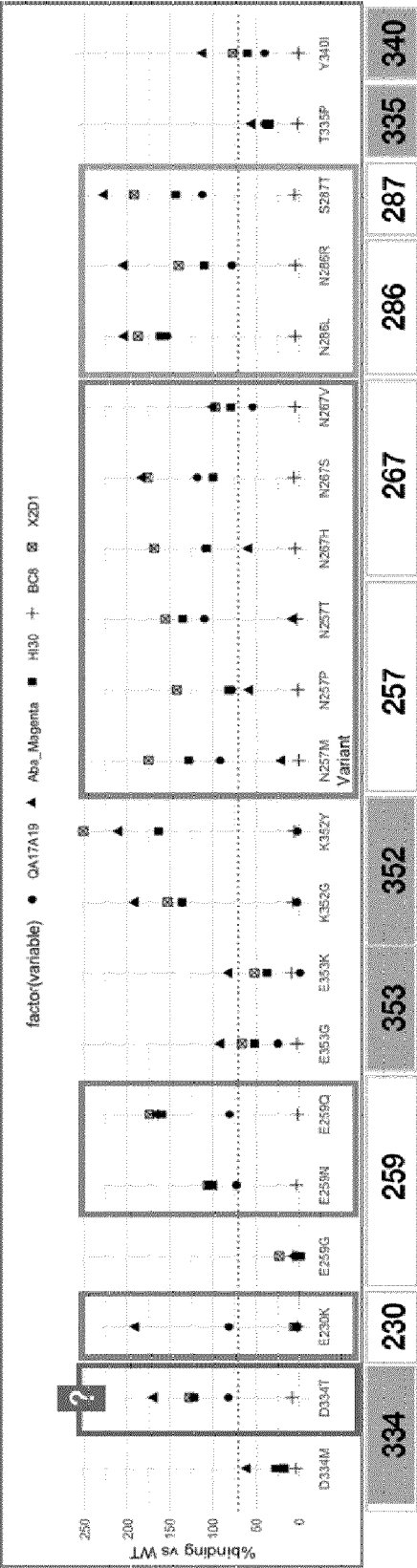


FIG. 7

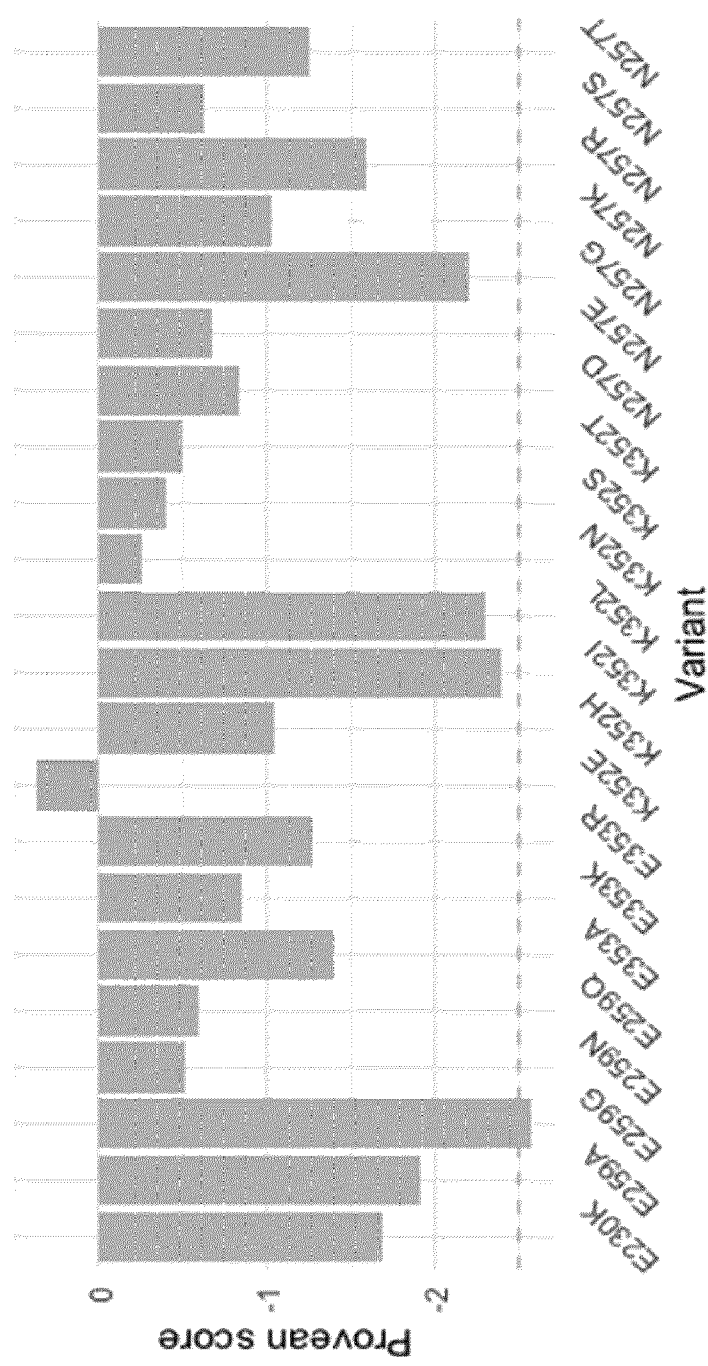


FIG. 8

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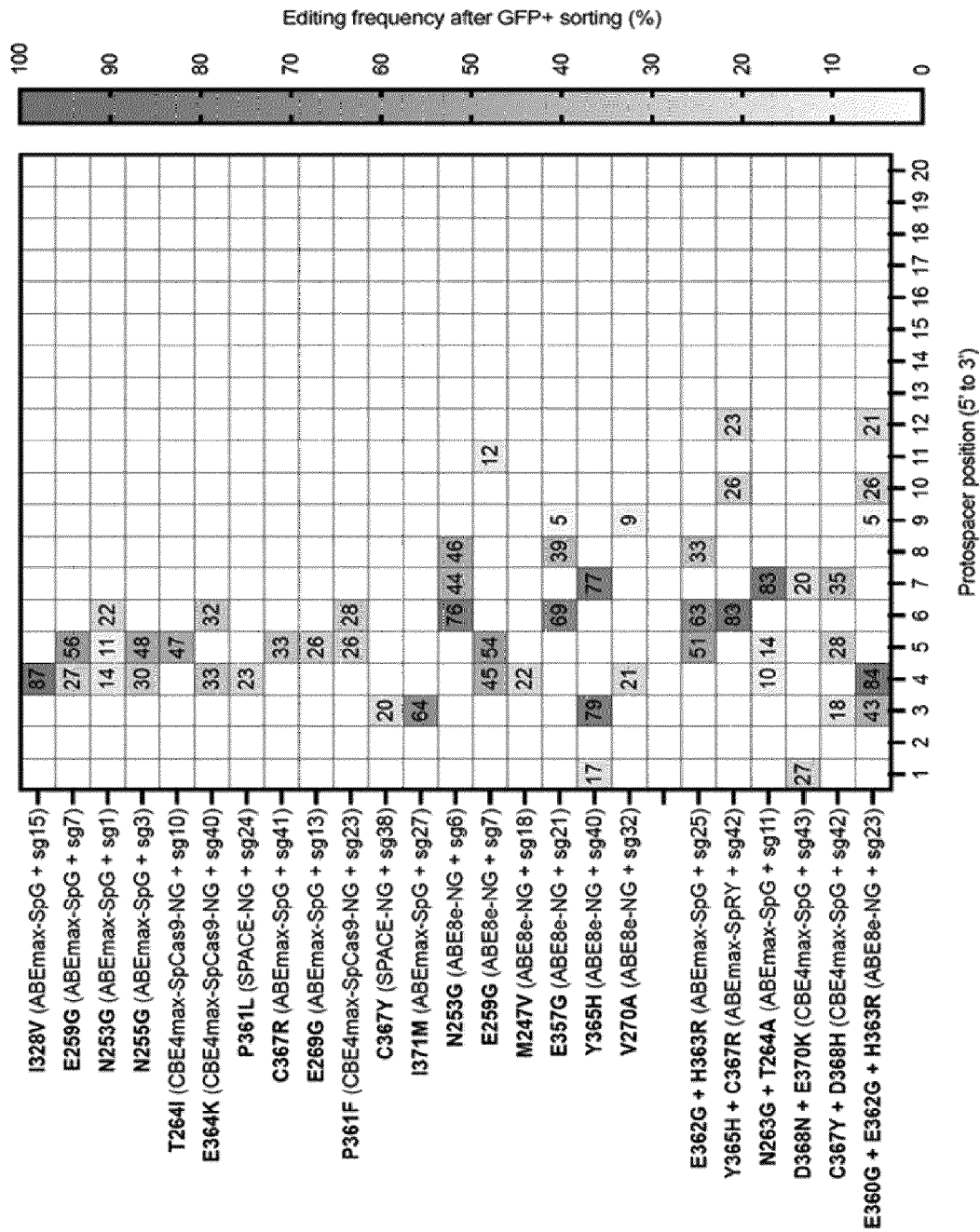
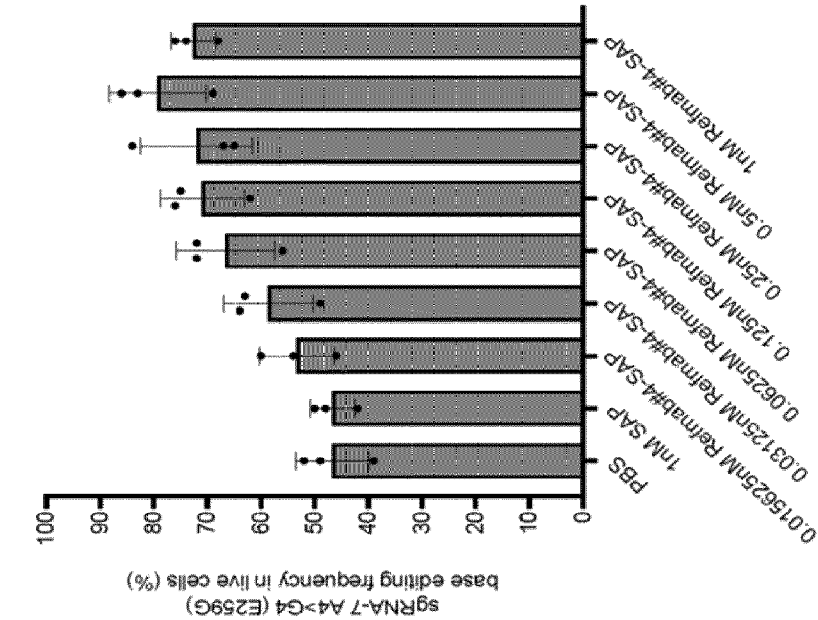
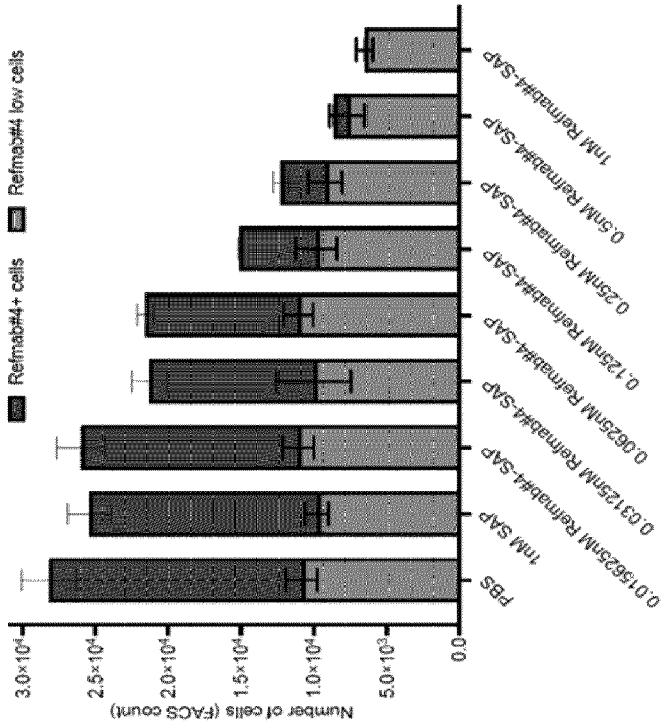


FIG. 9

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B



A

FIG. 10

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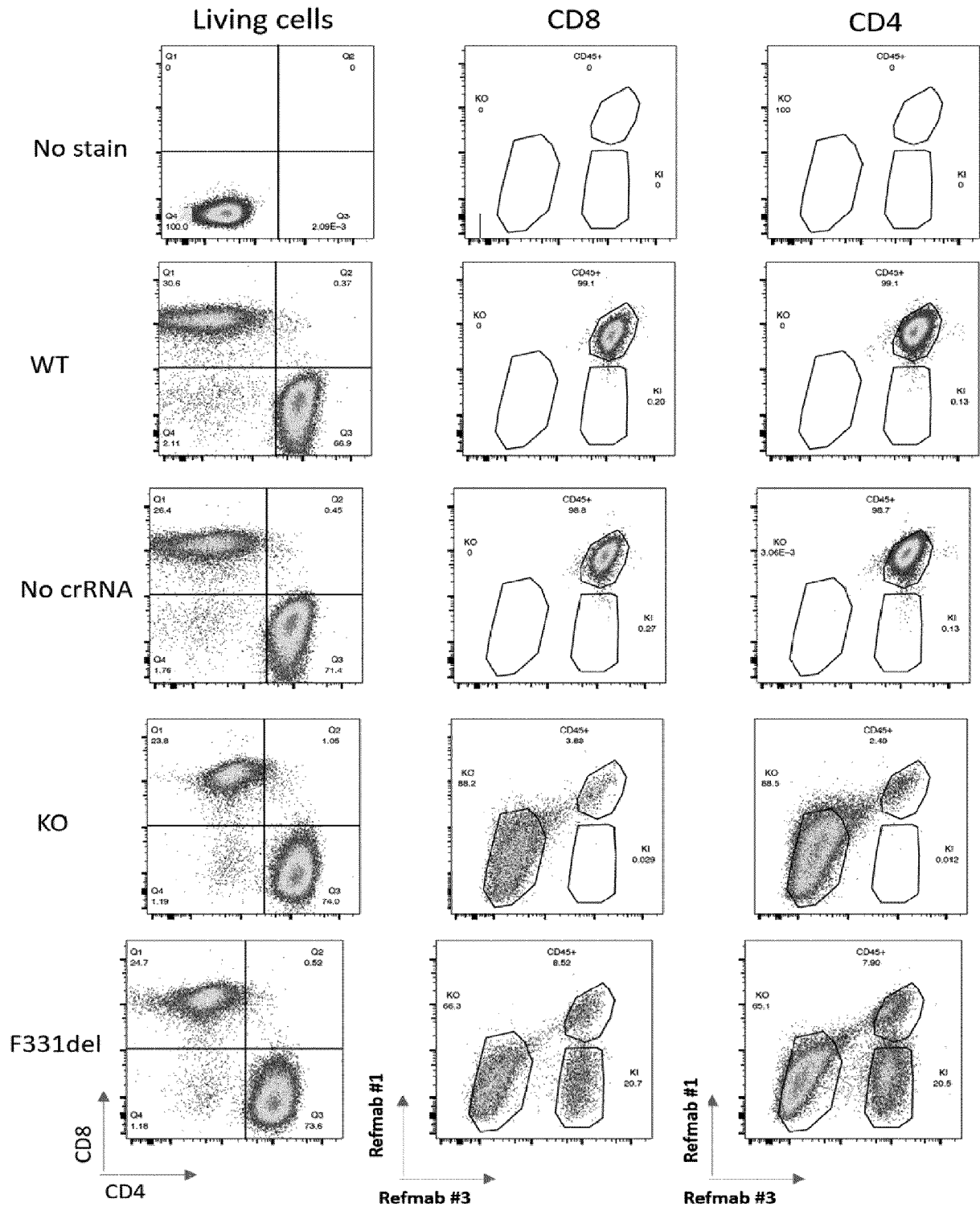


FIG. 11

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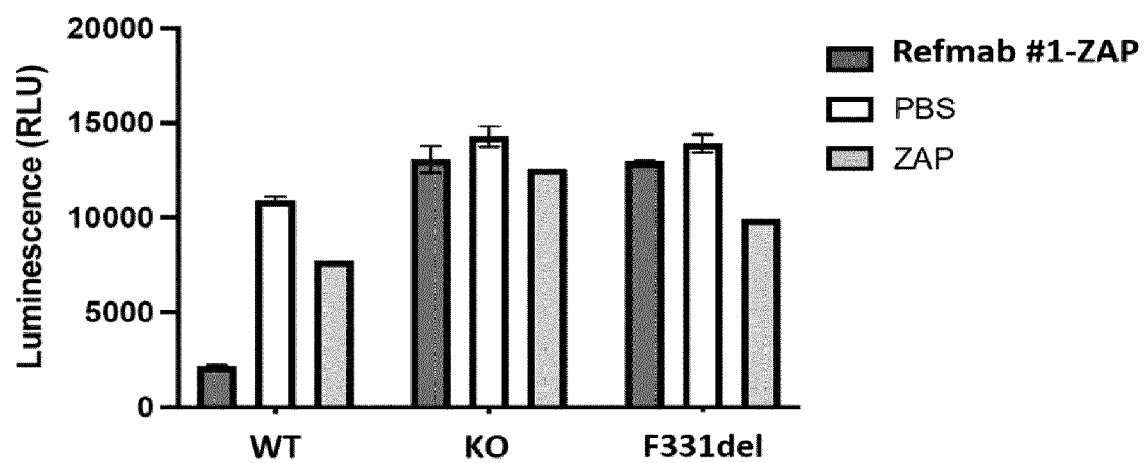


FIG. 12

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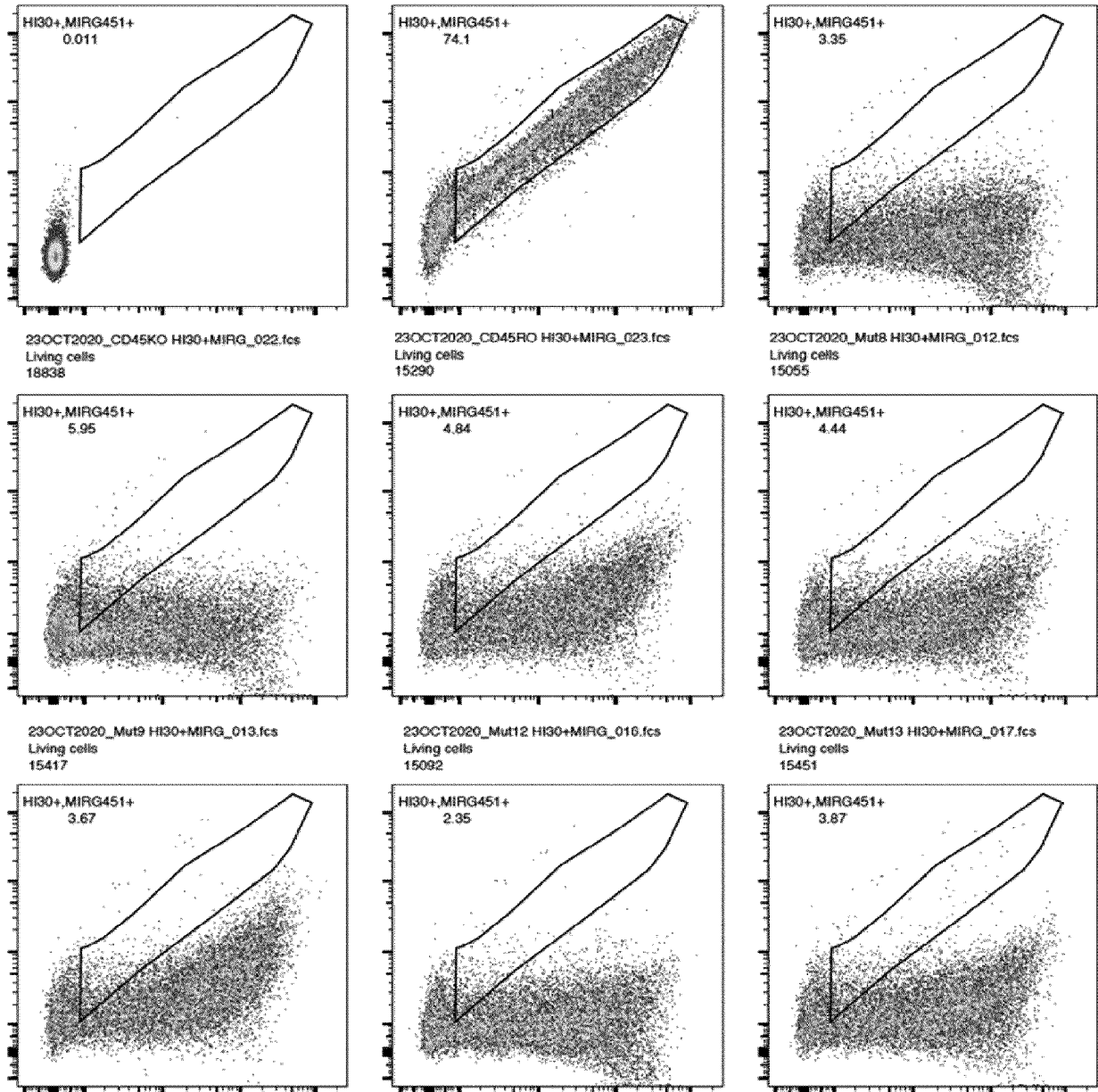


FIG. 13

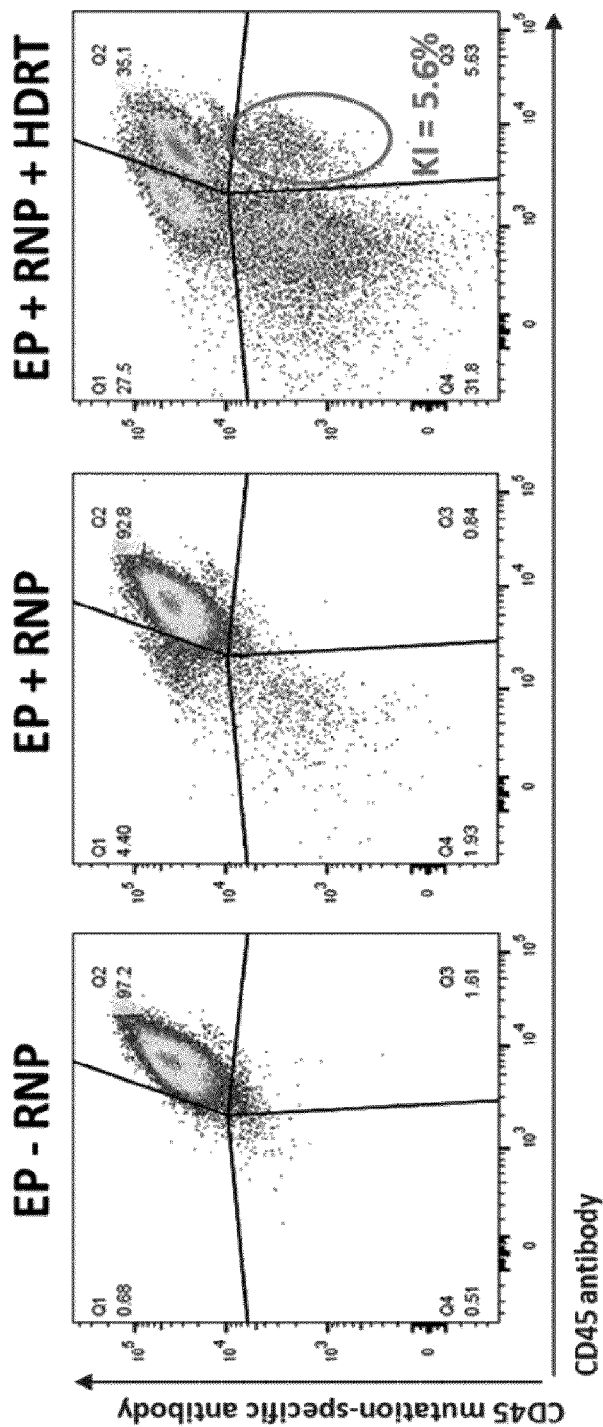


FIG. 14

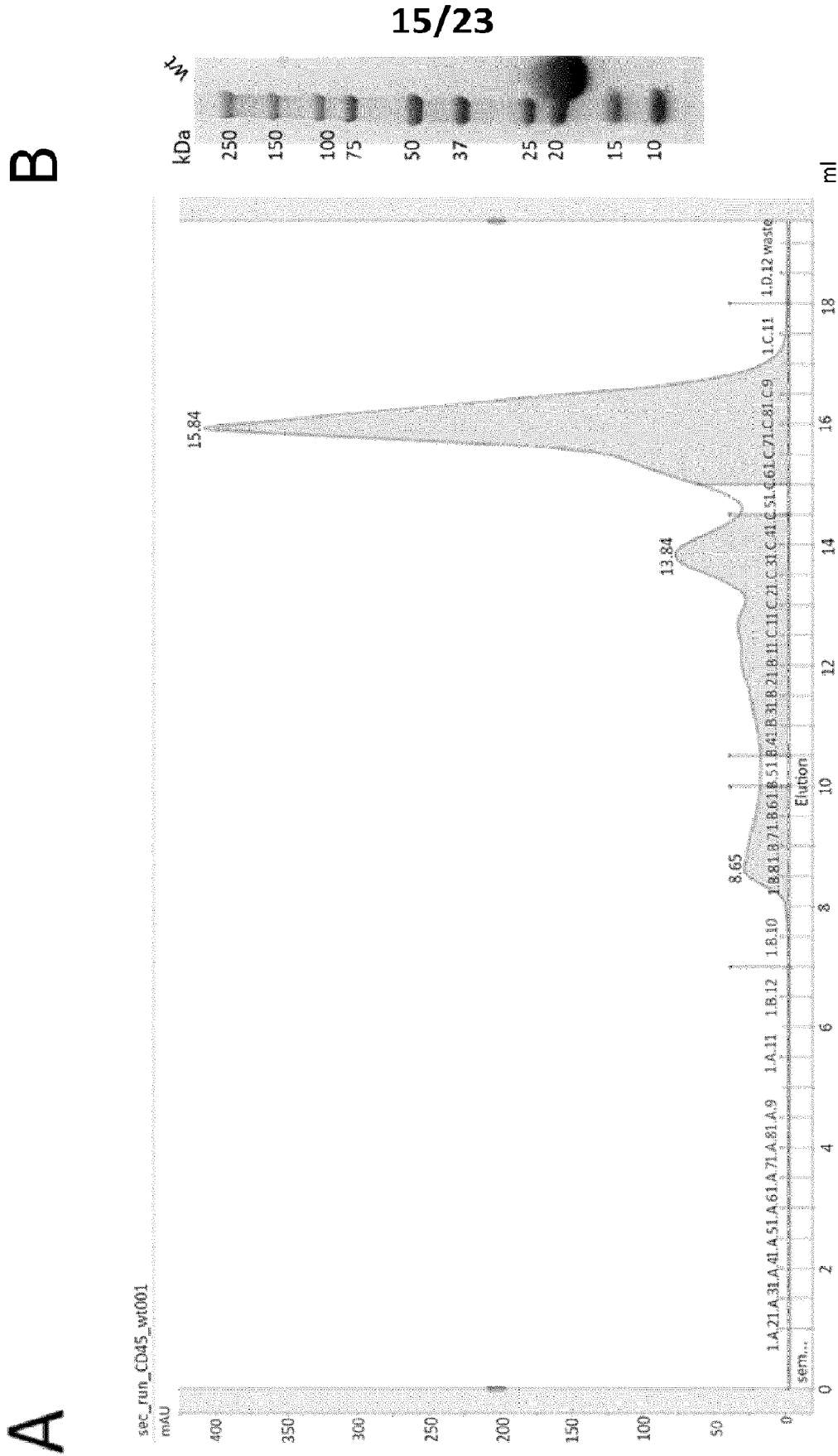
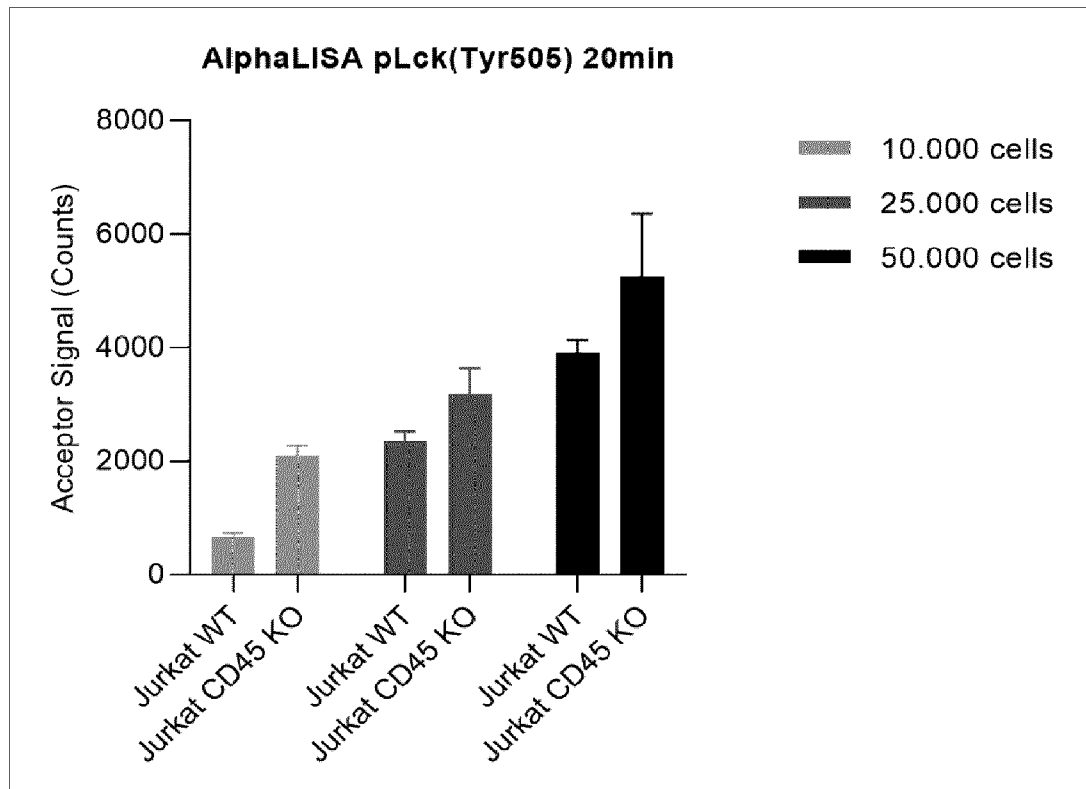


FIG. 15

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**FIG. 16**

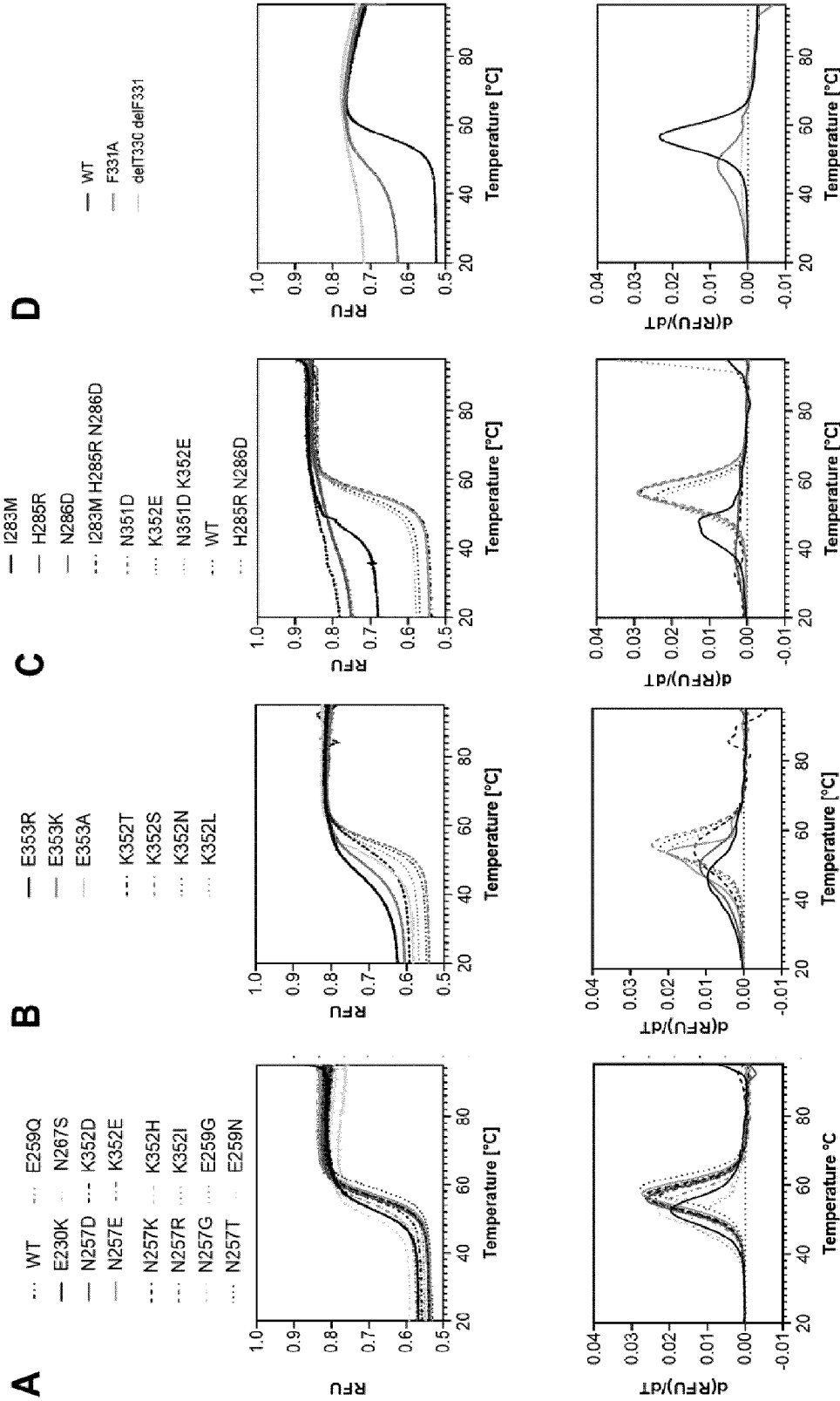


FIG. 17

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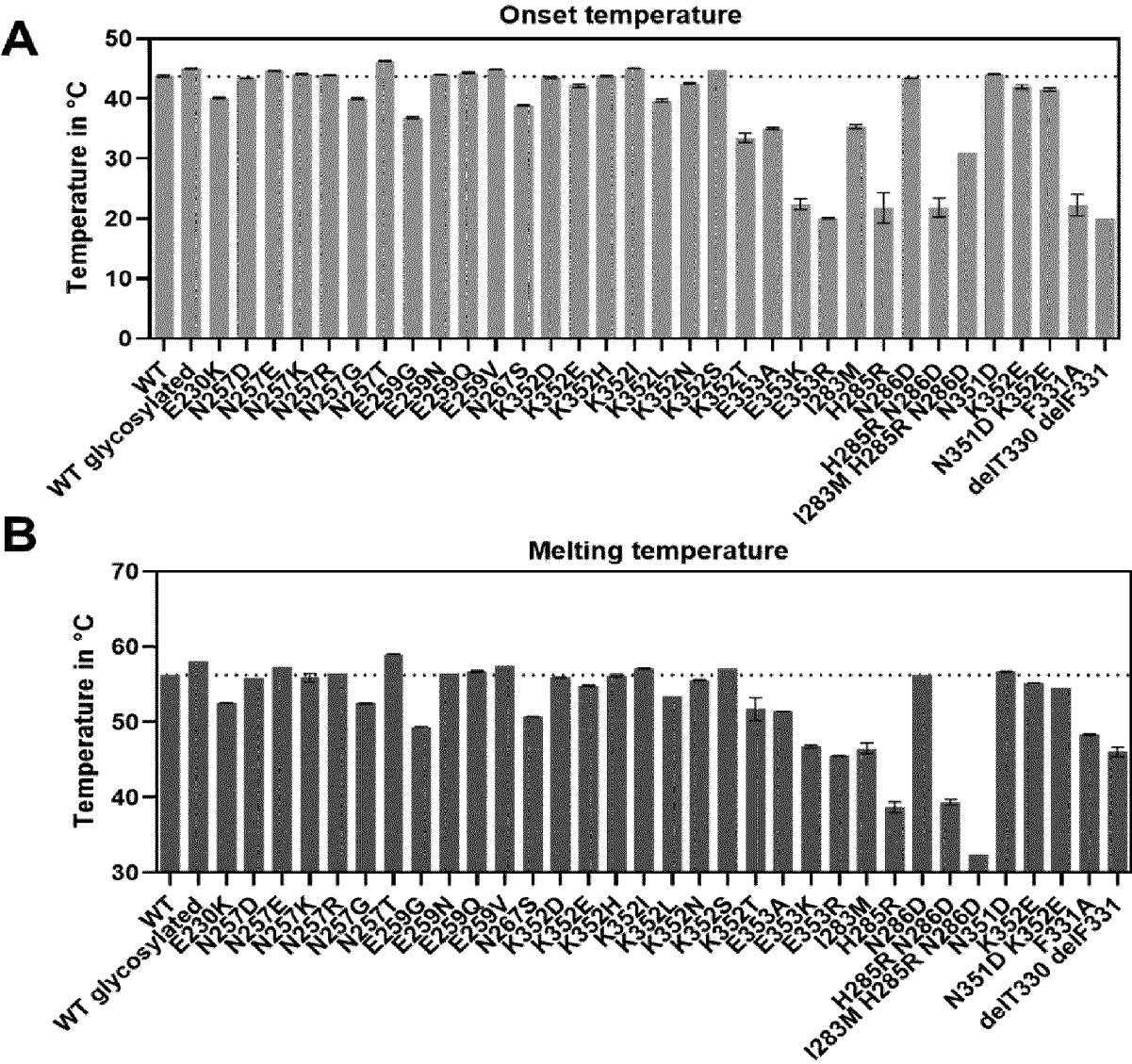
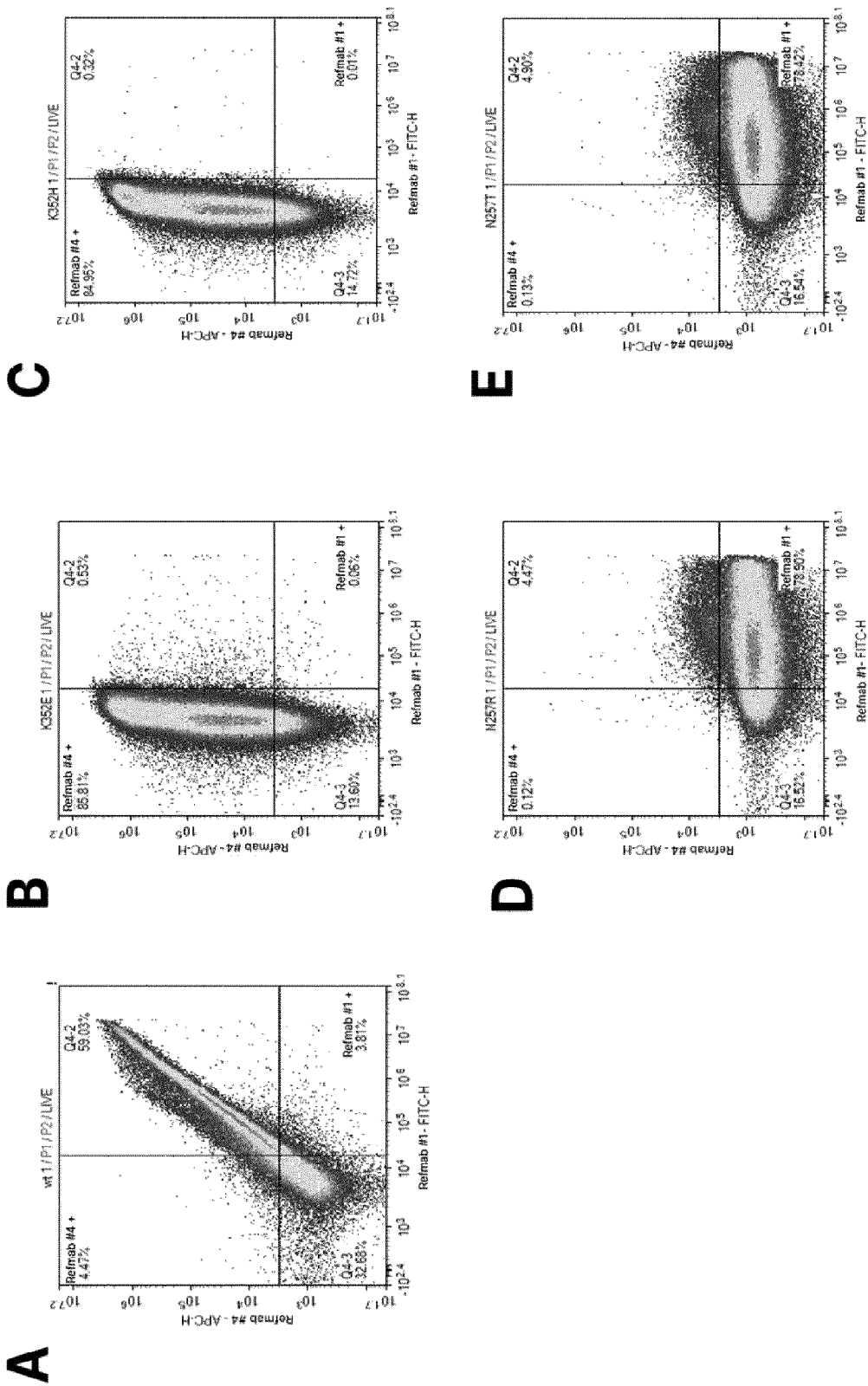


FIG. 18

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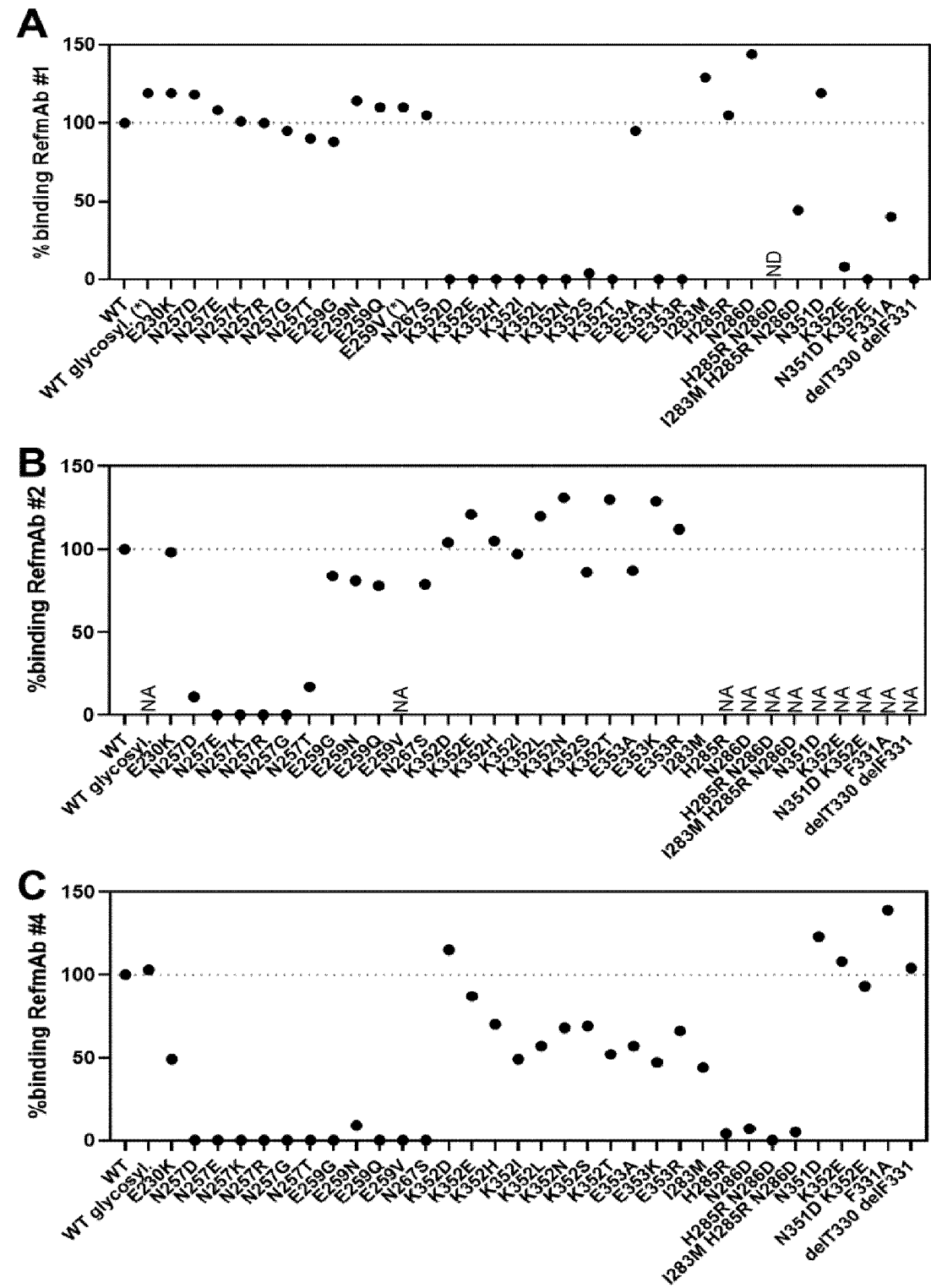


FIG. 20

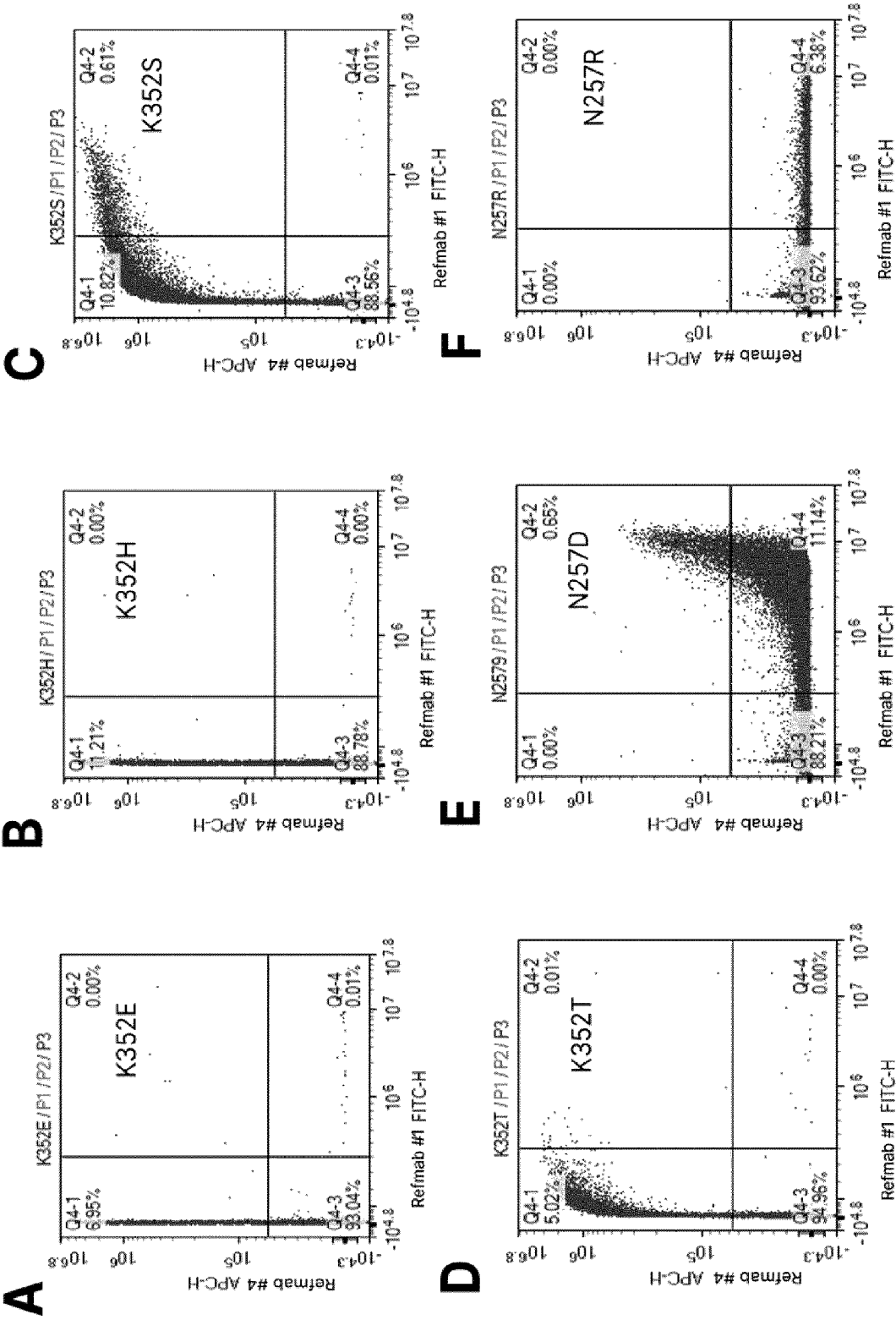


FIG. 21

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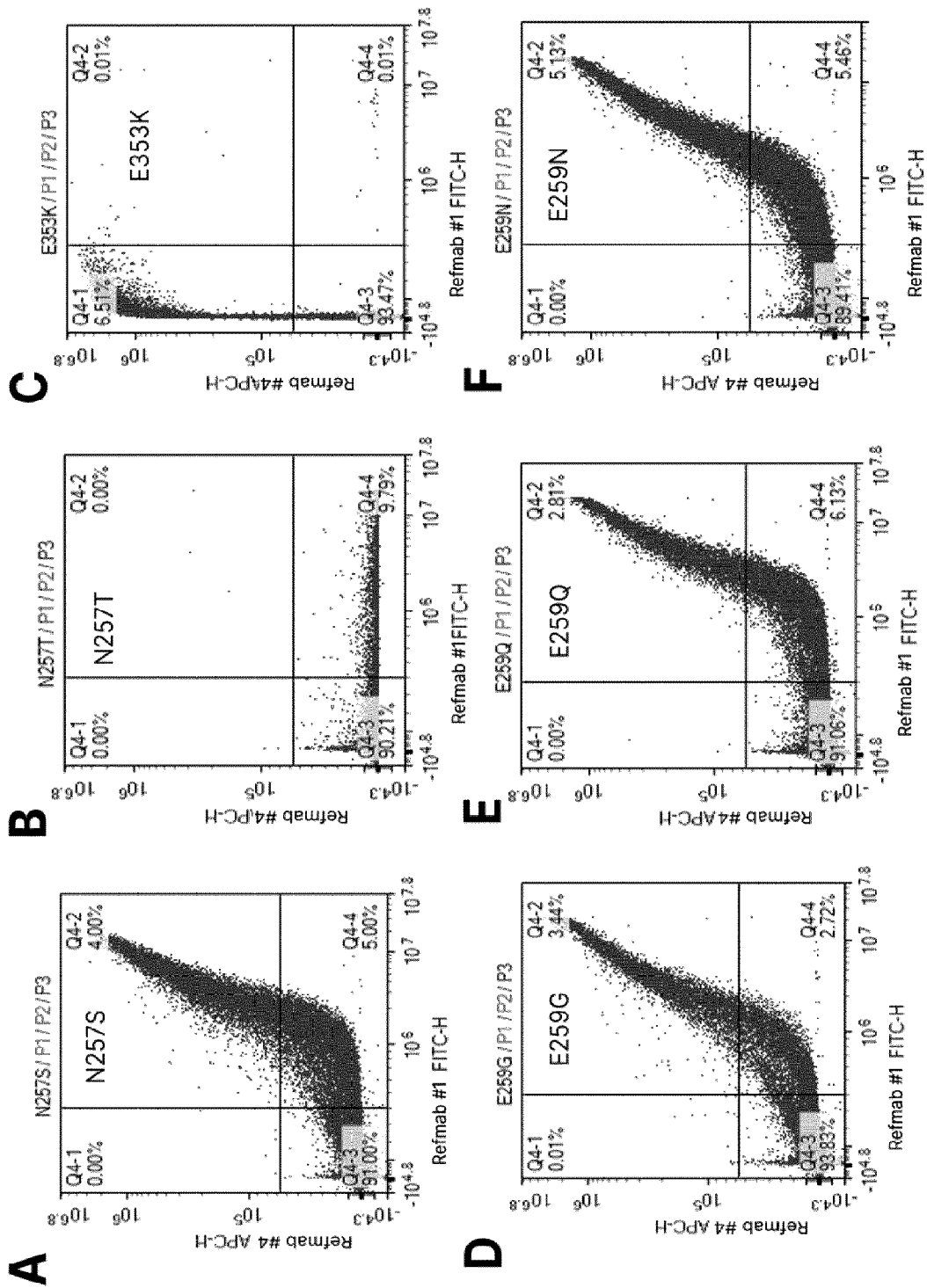


FIG. 22

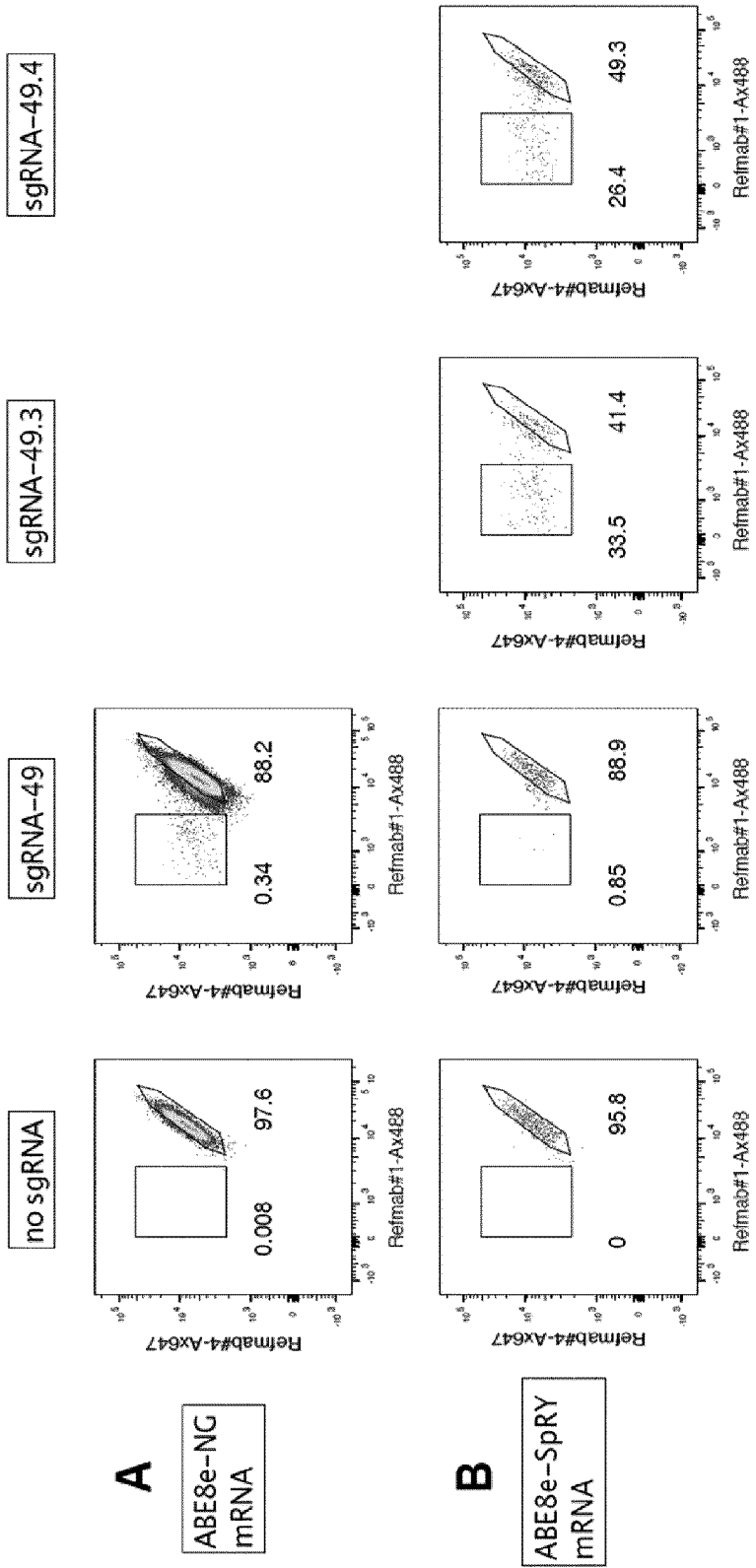


FIG. 23