Summary

The present disclosure relates to a polypeptide comprising a modified Fc region and a modified Fcγ receptor that binds specifically to this polypeptide that can be used as an immunotherapy. It is an object of the present disclosure to provide an immunotherapy in which endogenous molecules do not diminish drug efficacy.

The present disclosure is based, at least in part, on the discovery of combinations of non-naturally occurring Fcγ receptor mutants that do not bind to endogenous immunoglobulins and non-naturally occurring Fc region mutants that do not bind to endogenous Fcγ receptors, the use of these combinations to specifically bind the non-naturally occurring Fcγ receptor mutants and non-naturally occurring Fc region mutants as an immunotherapy for treating a subject, and the methods for making these combinations. For example, the present inventors extracted the amino acid site that affects the binding activity between CD16A and the Fc region of an antibody in silico, prepared mutants in which mutations were introduced to CD16A and the Fc region of the antibody, and evaluated the change in binding activity to the wild type, discovering that binding activity to the wild type had decreased (Examples 1-4). Based on this discovery, the present inventors identfied non-naturally occurring Fc region mutants showing no binding activity to wild type CD16A, but maintaining high binding activity to non-naturally occurring mutated CD16A. The inventors also identified combinations of non-naturally occurring modified Fc regions that did not bind to wild type CD16A and non-naturally occurring mutated CD16A that did not bind to the wild type antibody Fc region. Because similar results were obtained using a plurality of antibodies against different antigens, it was also determined that these characteristics do not depend on the antigen (Examples 5-8). The present inventors further established natural killer (NK) cell lines expressing wild type CD16A or non-naturally occurring mutated CD16A, and confirmed that the antibody-dependent cellular cytotoxicity (ADCC) reflects the binding activity confirmed in Example 6 (Examples 10, 11). Furthermore, the present inventors confirmed the binding activity characteristics in Example 6 in the presence of excess IgG1 antibodies (Example 9).

The present disclosure provides the following aspects as compositions and methods expected to be useful in medicine and industry.

In one aspect, the present disclosure provides a polypeptide comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to an Fc region of a wild type or naturally occurring

IgG. The polypeptide has essentially no binding activity to a wild type or naturally occurring Fcγ receptor and is capable of binding to a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to the wild type or naturally occurring Fcγ receptor.

In some examples, the wild type or naturally occurring Fcγ receptor is a wild type or naturally occurring CD16A, and the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation is a non-naturally occurring CD16A comprising at least one amino acid mutation.

In some examples, the wild type or naturally occurring CD16A comprises the amino acid sequence shown in SEQ ID NO: 78.

In some examples, the CD16A comprising at least one amino acid mutation comprises at least one mutation selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation).

In some examples, the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation. In other examples, the CD16A comprising at least one amino acid mutation comprises one or both of the K131E mutation and the K128E mutation. In some examples, the CD16A comprising at least one amino acid mutation comprises the K131D mutation, and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation). In other examples, the CD16A comprising at least one amino acid mutation comprises the amino acid sequence shown in SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88.

In some examples, the polypeptide comprises a modified Fc region of human Igγ1, and the modified Fc region comprises (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (ii) at least one mutation selected from (a) a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R mutation) and (b) a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation).

In some examples, the polypeptide is an antibody. In other examples, the polypeptide is an antibody that binds to a cancer antigen.

In another aspect, the present disclosure provides the use of the polypeptide as disclosed herein and a cell expressing the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor in the manufacture of a medicament for treating or preventing a disease or a disorder in a patient using immunotherapy, wherein the polypeptide is capable of binding to said non-naturally occurring Fcγ receptor comprising at least one amino acid mutation. In another example, the present disclosure provides a method of treating or preventing a disease or a disorder in a patient using immunotherapy. The method comprises administering to a patient a polypeptide as described herein and a cell expressing the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor, wherein the polypeptide is capable of binding to said non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

In some examples, the cell is a human immune cell. In other examples, the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell. NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell. In some examples, the cell is derived from a stem cell. In other examples, the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell. In some examples, the stem cell is a pluripotent stem cell. In other examples, the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

In some examples, the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene.

In some examples, the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain. In some examples, the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G.

In some examples, the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene. In some examples, the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB.

In some examples, the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

In some examples, the method is a method for treating or preventing cancer.

In one aspect, the present disclosure provides a pharmaceutical composition comprising a polypeptide as described herein and a pharmaceutically acceptable excipient.

In some examples, the pharmaceutical composition is for combined use with a cell for immunotherapy, wherein the cell expresses a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor, wherein the polypeptide is capable of binding to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

In some examples, the cell is a human immune cell. In some examples, the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell. In some examples, the cell is derived from a stem cell. In some examples, the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell. In some examples, the stem cell is a pluripotent stem cell. In some examples, the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

In some examples, the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene.

In some examples, the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain. In some examples, the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G.

In some examples, the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene. In some examples, the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB.

In some examples, the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

In another aspect, the present disclosure provides a kit for treatment or prevention of a disease or disorder in a patient using immunotherapy. The kit comprises (i) a polypeptide as described herein and (ii) a cell expressing a non-naturally occurring Fcγ receptor comprising

at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor, wherein the polypeptide is capable of binding to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

In some examples, the cell is a human immune cell. In some examples, the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell. In some examples, the cell is derived from a stem cell. In some examples, the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell. In some examples, the stem cell is a pluripotent stem cell. In some examples, the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

In some examples, the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene.

In some examples, the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain. In some examples, the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G.

In some examples, the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene. In some examples, the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB.

In some examples, the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

In one aspect, the present disclosure provides a cell expressing a non-naturally occurring CD16A comprising at least one amino acid mutation compared to a wild type or naturally occurring CD16A, wherein the at least one amino acid mutation is selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation), and wherein the non-

naturally occurring CD16A comprises an amino acid sequence having 90% or more amino acid sequence identity with SEQ ID NO: 78.

In some examples, the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation.

In some examples, the CD16A comprising at least one amino acid mutation comprises one or both of the K131E mutation and the K128E mutation.

In some examples, the CD16A comprising at least one amino acid mutation comprises the K131D mutation, and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation).

In some examples, the CD16A comprising at least one amino acid mutation comprises the amino acid sequence shown in SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88.

In some examples, the cell is a human immune cell. In some examples, the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell. In some examples, the cell is derived from a stem cell. In some examples, the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell. In some examples, the stem cell is a pluripotent stem cell. In some examples, the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

In some examples, the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene.

In some examples, the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain. In some examples, the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G.

In some examples, the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene. In some examples, the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP),

class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB.

In some examples, the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

In one aspect, the present disclosure provides a pharmaceutical composition comprising a cell as described herein and a pharmaceutically acceptable excipient.

In some examples, the pharmaceutical composition is for combined use with a polypeptide comprising a modified Fc region of IgG for immunotherapy, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to an Fc region of a wild type or naturally occurring IgG, and the polypeptide has essentially no binding activity to a wild type or a naturally occurring CD16A and is capable of binding to a non-naturally occurring CD16A comprising at least one amino acid mutation expressed by the cell.

In some examples, the polypeptide comprises a modified Fc region of human Igγ1, and the modified Fc region comprises (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (ii) at least one mutation selected from (a) a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R mutation) and (b) a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation).

In some examples, the polypeptide is an antibody. In some examples, the polypeptide is an antibody that binds to a cancer antigen.

In some examples, the pharmaceutical composition is for treating cancer.

In one aspect, the present disclosure provides a method for preparing a polypeptide containing a modified Fc region of IgG. The method comprises the steps of: 1) providing polypeptides comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to a wild type or naturally occurring IgG; 2) measuring the binding activity of the polypeptides obtained in 1) to a wild type or naturally occurring Fcγ receptor; 3) measuring the binding activity of the polypeptides obtained in 1) to a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor; and 4) selecting from the polypeptides obtained in 1) a polypeptide having essentially no binding activity to the wild type or naturally occurring Fcγ receptor and which binds to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

In some examples, the wild type or naturally occurring Fcγ receptor is a wild type or naturally occurring CD16A and the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation is a non-naturally occurring CD16A comprising at least one amino acid mutation.

In some examples, the wild type or naturally occurring CD16A comprises the amino acid sequence shown in SEQ ID NO: 78.

In some examples, the non-naturally occurring CD16A comprising at least one amino acid mutation comprises at least one mutation selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation).

In some examples, the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation.

In some examples, the CD16A comprising at least one amino acid mutation contains one or both of the K131E mutation and the K128E mutation.

In some examples, the CD16A comprising at least one amino acid mutation comprises the K131D mutation and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation).

In some examples, the non-naturally occurring polypeptide comprising a modified Fc region of IgG is an antibody. In some examples, the antibody is an antibody that binds to a cancer antigen.

In some examples, the polypeptide comprising a modified Fc region of IgG is an antibody, and the method further comprises a step of contacting the antibody with an immune cell expressing the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.

In another aspect, the present disclosure provides a method for preparing a non-naturally occurring Fcγ receptor. The method comprises the steps of: 1) providing non-naturally occurring Fcγ receptors comprising at least one amino acid mutation compared with a wild type or naturally occurring Fcγ receptor; 2) providing a polypeptide comprising an Fc region of wild type or naturally occurring IgG and a polypeptide comprising an Fc region of

IgG comprising at least one amino acid mutation compared to the wild type or naturally occurring IgG; 3) measuring the binding activity of the non-naturally occurring Fcγ receptors obtained in 1) to the polypeptide comprising the Fc region of the wild type or naturally occurring IgG; 4) measuring the binding activity of the non-naturally occurring Fcγ receptors obtained in 1) to the polypeptide comprising the Fc region of IgG comprising at least one amino acid mutation; and 5) selecting from the non-naturally occurring Fcγ receptors obtained in 1) a non-naturally occurring Fcγ receptor having essentially no binding activity to the polypeptide comprising the Fc region of wild type or naturally occurring IgG and which binds to the polypeptide comprising the Fc region of the IgG comprising at least one amino acid mutation.

In some examples, the Fcγ receptor is CD16A.

In some examples, wild type or naturally occurring Fcγ receptor is CD16A comprising the amino acid sequence shown in SEQ ID NO: 78.

In some examples, the Fc region of IgG comprising at least one amino acid mutation is an Fc region of human Igγ1 comprising at least one amino acid mutation compared to a wild type or naturally occurring human Igγ1 and comprises (a) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (b) at least one mutation selected from (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R mutation) and (ii) a mutation from a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation).

In some examples, the polypeptide comprising an Fc region of IgG comprising at least one amino acid mutation is an antibody. In some examples, the antibody is an antibody that binds to a cancer antigen.

In some examples, the polypeptide comprising an Fc region of IgG comprising at least one amino acid mutation is an antibody, and the method further comprises a step of contacting the antibody comprising an Fc region of IgG comprising at least one amino acid mutation obtained in 2) with an immune cell expressing the Fcγ receptor comprising at least one amino acid mutation obtained in 1) and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.

In another aspect, the present disclosure provides a method for preparing a binding pair comprising (a) a polypeptide comprising a modified Fc region of IgG and (b) a non-naturally occurring modified Fcγ receptor. The method comprises the steps of: 1) providing a

polypeptide comprising an Fc region of wild type or naturally occurring IgG and polypeptides comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to the Fc region of the wild type or naturally occurring IgG; 2) providing a wild type or naturally occurring Fcγ receptor and non-naturally occurring modified Fcγ receptors, wherein the modified Fcγ receptor comprises at least one amino acid mutation compared to the wild type or naturally occurring Fcγ receptor; 3) measuring the binding activity of each Fcγ receptor obtained in 2) to each polypeptide obtained in 1); and 4) selecting (a) a polypeptide comprising a modified Fc region that binds to the modified Fcγ receptor and has essentially no binding activity to the wild type or naturally occurring Fcγ receptor, and (b) a modified Fcγ receptor that binds to the polypeptide comprising the modified Fc region and that does not bind to the Fc region of the wild type or naturally occurring IgG.

In some examples, the Fcγ receptor is CD16A.

In some examples, wild type or naturally occurring CD16A contains the amino acid sequence shown in SEQ ID NO: 78.

In some examples, the polypeptide comprising the modified Fc of IgG is an antibody. In some examples, the antibody is an antibody that binds to a cancer antigen.

In some examples, the polypeptide comprising a modified Fc region of IgG selected in 4) is an antibody, and the method further comprises a step of contacting the antibody with an immune cell expressing the modified Fcγ receptor selected in 4) and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.

It is expected that the present disclosure can provide combinations of mutagenized Fcγ receptors and mutagenized Fc regions showing specific binding patterns and an immunotherapy using these combinations in which endogenous molecules do not diminish drug efficacy.

Brief Description of the Drawings

FIG. 1 depicts the binding activity of wild type Fc or mutant Fc type anti-HER2 antibody to CD16V or CD16V mutants. The vertical axis represents the difference between the absorbance at 450 nm and the absorbance at reference wavelength 570 nm, and the horizontal axis represents the CD16V or CD16V mutant concentration (ng/mL).

We claim:

1. A polypeptide comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to an Fc region of a wild type or naturally occurring IgG, and the polypeptide has essentially no binding activity to a wild type or naturally occurring Fcγ receptor and is capable of binding to a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to the wild type or naturally occurring Fcγ receptor.

2. The polypeptide according to claim 1, wherein the wild type or naturally occurring Fcγ receptor is a wild type or naturally occurring CD16A and said non-naturally occurring Fcγ receptor comprising at least one amino acid mutation is a non-naturally occurring CD16A comprising at least one amino acid mutation; optionally, wherein the wild type or naturally occurring CD16A comprises an amino acid sequence shown in SEQ ID NO: 78; and/or
wherein the CD16A comprising at least one amino acid mutation comprises at least one mutation selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation).

3. The polypeptide according to claim 2,
(a) wherein the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation;

(b) wherein the CD16A comprising at least one amino acid mutation comprises one or both of the K131E mutation and the K128E mutation;

(c) wherein the CD16A comprising at least one amino acid mutation comprises the K131D mutation, and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation); or

(d) wherein the CD16A comprising at least one amino acid mutation comprises an amino acid sequence shown in SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88.

4. The polypeptide according to any one of claims 1 to 3,
(a) wherein the polypeptide comprises a modified Fc region of human Igγ1, and the modified Fc region comprises (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (ii) at least one mutation selected from (a) a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R mutation) and (b) a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation); and/or

(b) wherein the polypeptide is an antibody; optionally,

wherein the polypeptide is an antibody that binds to a cancer antigen.

5. Use of the polypeptide of any one of claims 1-4 and a cell expressing the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor in the manufacture of a medicament for treating or preventing a disease or a disorder in a patient using immunotherapy, wherein the polypeptide is capable of binding to said non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

6. The use according to claim 5,
(a) wherein the cell is a human immune cell;

optionally wherein the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell;

(b) wherein the cell is derived from a stem cell;

optionally wherein the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell; and/or

(c) wherein the stem cell is a pluripotent stem cell;

optionally wherein the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

7. The use of claim 5 or 6,
(a) wherein the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene;

(b) wherein the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain;

optionally, wherein the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G;

(c) wherein the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene;

optionally, wherein the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB; and/or

(d) wherein the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

8. The use according to any one of claims 5-7, wherein the use is for treating or preventing cancer.

9. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 4 and a pharmaceutically acceptable excipient.

10. The pharmaceutical composition according to claim 9 for combined use with a cell for immunotherapy, wherein the cell expresses a non-naturally occurring Fcγ receptor

comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor, wherein the polypeptide is capable of binding to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

11. The pharmaceutical composition according to claim 10,
(a) wherein the cell is a human immune cell;

optionally, wherein the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell;

(b) wherein the cell is derived from a stem cell;

optionally, wherein the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell; and/or

(c) wherein the stem cell is a pluripotent stem cell;

optionally, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

12. The pharmaceutical composition of any one of claims 9-11,
(a) wherein the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene;

(b) wherein the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain;

optionally, wherein the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G;

(c) wherein the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene;

optionally, wherein the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB; and/or

(d) wherein the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

13. A kit for treatment or prevention of a disease or disorder in a patient using immunotherapy, the kit comprising (i) a polypeptide according to any one of claims 1 to 4 and (ii) a cell expressing a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor, wherein the polypeptide is capable of binding to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

14. The kit according to claim 13,
(a) wherein the cell is a human immune cell;

optionally, wherein the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell;

(b) wherein the cell is derived from a stem cell;

optionally, wherein the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell; and/or

(d) wherein the stem cell is a pluripotent stem cell;

optionally, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

15. The kit of claim 13 or 14,
(a) wherein the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene;

(b) wherein the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain;

optionally, wherein the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G;

(c) wherein the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene;

optionally, wherein the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB; and/or

(d) wherein the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

16. A cell expressing a non-naturally occurring CD16A comprising at least one amino acid mutation compared to a wild type or naturally occurring CD16A, wherein the at least one amino acid mutation is selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation), and wherein the non-naturally occurring CD16A comprises an amino acid sequence having 90% or more amino acid sequence identity with SEQ ID NO: 78.

17. The cell according to claim 16,

(a) wherein the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation;

(b) wherein the CD16A comprising at least one amino acid mutation comprises one or both of the K131E mutation and the K128E mutation;

(c) wherein the CD16A comprising at least one amino acid mutation comprises the K131D mutation, and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation); or

(d) wherein the CD16A comprising at least one amino acid mutation comprises the amino acid sequence shown in SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88.

18. The cell according to claim 16 or 17,

(a) wherein the cell is a human immune cell;

optionally, wherein the human immune cell is a cell selected from a T cell, macrophage, dendritic cell, NKT-cell, NK cell, microglia, osteoclast, granulocyte, monocyte, and innate immune cell;

(b) wherein the cell is derived from a stem cell;

optionally, wherein the stem cell is selected from a pluripotent stem cell, hematopoietic stem cell, adult stem cell, fetal stem cell, mesenchymal stem cell, postpartum stem cell, multipotent stem cell, and embryonic germ cell; and/or

(c) wherein the stem cell is a pluripotent stem cell;

optionally, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPS cell) or an embryonic stem cell (ES cell).

19. The cell of any one of claims 16-18,

(a) wherein the cell comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene;

(b) wherein the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the

B2M protein covalently linked, either directly or via a linker sequence, to at least a portion of an HLA-1α chain;

optionally, wherein the HLA-1α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G;

(c) wherein the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene;

optionally, wherein the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB; and/or

(d) wherein the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein.

20. A pharmaceutical composition comprising a cell according to any one of claims 16-19 and a pharmaceutically acceptable excipient.

21. The pharmaceutical composition according to claim 20 for combined use with a polypeptide comprising a modified Fc region of IgG for immunotherapy, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to an Fc region of a wild type or naturally occurring IgG, and the polypeptide has essentially no binding activity to a wild type or a naturally occurring CD16A and is capable of binding to a non-naturally occurring CD16A comprising at least one amino acid mutation expressed by the cell.

22. The pharmaceutical composition according to claim 21,
(a) wherein the polypeptide comprises a modified Fc region of human Igγ1, and the modified Fc region comprises (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (ii) at least one mutation selected from (I) a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R

mutation) and (II) a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation); and/or

(b) wherein the polypeptide is an antibody;
optionally,wherein the polypeptide is an antibody that binds to a cancer antigen.

23. The pharmaceutical composition according to claim 21 or 22, wherein the pharmaceutical composition is for treating cancer.

24. A method for preparing a polypeptide comprising a modified Fc region of IgG, the method comprising the steps of:

1) providing polypeptides comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to a wild type or naturally occurring IgG;

2) measuring the binding activity of the polypeptides obtained in 1) to a wild type or naturally occurring Fcγ receptor;

3) measuring the binding activity of the polypeptides obtained in 1) to a non-naturally occurring Fcγ receptor comprising at least one amino acid mutation compared to a wild type or naturally occurring Fcγ receptor; and

4) selecting from the polypeptides obtained in 1) a polypeptide having essentially no binding activity to the wild type or naturally occurring Fcγ receptor and which binds to the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation.

25. The method according to claim 24, wherein the wild type or naturally occurring Fcγ receptor is a wild type or naturally occurring CD16A and the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation is a non-naturally occurring CD16A comprising at least one amino acid mutation; optionally,

wherein the wild type or naturally occurring CD16A comprises the amino acid sequence shown in SEQ ID NO: 78; and/or

wherein the non-naturally occurring CD16A comprising at least one amino acid mutation comprises at least one mutation selected from (i) a lysine to an aspartic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131D mutation), (ii) a lysine to a glutamic acid at a position corresponding to position 128 in SEQ ID NO: 78 (K128E

mutation), and (iii) a lysine to a glutamic acid at a position corresponding to position 131 in SEQ ID NO: 78 (K131E mutation).

26. The method according to claim 25,

(a) wherein the CD16A comprising at least one amino acid mutation comprises one or both of the K131D mutation and the K128E mutation;

(b) wherein the CD16A comprising at least one amino acid mutation contains one or both of the K131E mutation and the K128E mutation;

(c) wherein the CD16A comprising at least one amino acid mutation comprises the K131D mutation and further comprises at least one mutation selected from (iv) an asparagine to a glutamine at a position corresponding to position 38 in SEQ ID NO: 78 (N38Q mutation) and (v) an asparagine to a glutamine at a position corresponding to position 74 in SEQ ID NO: 78 (N74Q mutation); or

(d) wherein the CD16A comprising at least one amino acid mutation comprises an amino acid sequence shown in SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88.

27. The method according to any one of claims 24-26,

(a) wherein the non-naturally occurring polypeptide comprising a modified Fc region of IgG is an antibody; optionally

wherein the antibody is an antibody that binds to a cancer antigen; and/or

(b) wherein the polypeptide comprising a modified Fc region of IgG is an antibody, and the method further comprises a step of contacting the antibody with an immune cell expressing the non-naturally occurring Fcγ receptor comprising at least one amino acid mutation and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.

28. A method for preparing a non-naturally occurring Fcγ receptor, the method comprising the steps of:

1) providing non-naturally occurring Fcγ receptors comprising at least one amino acid mutation compared with a wild type or naturally occurring Fcγ receptor;

2) providing a polypeptide comprising an Fc region of wild type or naturally occurring IgG and a polypeptide comprising an Fc region of IgG comprising at least one amino acid mutation compared to the wild type or naturally occurring IgG;

3) measuring the binding activity of the non-naturally occurring Fcγ receptors obtained in 1) to the polypeptide comprising the Fc region of the wild type or naturally occurring IgG;

4) measuring the binding activity of the non-naturally occurring Fcγ receptors obtained in 1) to the polypeptide comprising the Fc region of IgG comprising at least one amino acid mutation; and

5) selecting from the non-naturally occurring Fcγ receptors obtained in 1) a non-naturally occurring Fcγ receptor having essentially no binding activity to the polypeptide comprising the Fc region of wild type or naturally occurring IgG and which binds to the polypeptide comprising the Fc region of the IgG comprising at least one amino acid mutation.

29. The method according to claim 28, wherein the Fcγ receptor is CD16A; optionally,

wherein the wild type or naturally occurring Fcγ receptor is CD16A comprising the amino acid sequence shown in SEQ ID NO: 78.

30. The method according to claim 28 or 29, wherein the Fc region of IgG comprising at least one amino acid mutation is an Fc region of human Igγ1 comprising at least one amino acid mutation compared to a wild type or naturally occurring human Igγ1 and comprises (a) a mutation from a glutamic acid to an arginine at a position corresponding to position 269 according to EU index numbering (E269R mutation) and (b) at least one mutation selected from (i) a mutation from a glutamic acid to an arginine at a position corresponding to position 294 according to EU index numbering (E294R mutation) and (ii) a mutation from a glutamic acid to a lysine at a position corresponding to position 294 according to EU index numbering (E294K mutation).

31. The method according to any one of claims 28-30,
(a) wherein the polypeptide comprising an Fc region of IgG comprising at least one amino acid mutation is an antibody; optionally

wherein the antibody is an antibody that binds to a cancer antigen; and/or

(b) wherein the polypeptide comprising an Fc region of IgG comprising at least one amino acid mutation is an antibody, and the method further comprises a step of contacting the antibody comprising an Fc region of IgG comprising at least one amino acid mutation obtained in 2) with an immune cell expressing the Fcγ receptor comprising at least one amino acid mutation obtained in 1) and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.

32. A method for preparing a binding pair comprising

(a) a polypeptide comprising a modified Fc region of IgG and (b) a non-naturally occurring modified Fcγ receptor, the method comprising the steps of:

1) providing a polypeptide comprising an Fc region of wild type or naturally occurring IgG and polypeptides comprising a modified Fc region of IgG, wherein the modified Fc region is non-naturally occurring and comprises at least one amino acid mutation compared to the Fc region of the wild type or naturally occurring IgG;

2) providing a wild type or naturally occurring Fcγ receptor and non-naturally occurring modified Fcγ receptors, wherein the modified Fcγ receptor comprises at least one amino acid mutation compared to the wild type or naturally occurring Fcγ receptor;

3) measuring the binding activity of each Fcγ receptor obtained in 2) to each polypeptide obtained in 1); and

4) selecting (a) a polypeptide comprising a modified Fc region that binds to the modified Fcγ receptor and has essentially no binding activity to the wild type or naturally occurring Fcγ receptor, and

(b) a modified Fcγ receptor that binds to the polypeptide comprising the modified Fc region and that does not bind to the Fc region of the wild type or naturally occurring IgG.

33. The method according to claim 32, wherein the Fcγ receptor is CD16A; optionally,

wherein wild type or naturally occurring CD16A contains the amino acid sequence shown in SEQ ID NO: 78.

34. The method according to claim 32 or 33,

(a) wherein the polypeptide comprising the modified Fc region of IgG is an antibody;

optionally wherein the antibody is an antibody that binds to a cancer antigen; and/or

(b) wherein the polypeptide comprising a modified Fc region of IgG selected in 4) is an antibody, and the method further comprises a step of contacting the antibody with an immune cell expressing the modified Fcγ receptor selected in 4) and a cell expressing an antigen to which the antibody binds, and measuring antibody-dependent cellular cytotoxicity (ADCC) activity.