

HIGH AFFINITY T CELL RECEPTOR FOR RECOGNIZING AFP

ANTIGEN

Technical field

5 The present invention relates to the field of biotechnology, and in particular to a T cell receptor (TCR) capable of recognizing a polypeptide derived from AFP protein. The invention also relates to the preparation and uses of such receptors.

Background

10 There are only two types of molecules that can recognize antigens in a specific manner. One is immunoglobulin or antibody; and the other is T cell receptor (TCR), which is a α/β or γ/δ heterodimeric glycoprotein present on cell membrane. The physical repertoire of TCR of immune system is generated in thymus through V(D)J recombination, followed by positive and negative selections. In peripheral environment,
15 TCRs mediate the recognition of specific Major Histocompatibility Complex-peptide complexes (pMHC) by T cells and, as such, are essential to the immunological functioning of cells in the immune system.

TCR is the only receptor for presenting specific peptide antigens in Major Histocompatibility Complex (MHC). The exogenous or endogenous peptides may be
20 the only sign of abnormality in a cell. In the immune system, direct physical contact of a T-cell and an antigen presenting cell (APC) will be initiated by the binding of antigen-specific TCRs to pMHC complexes. Then, the interaction of other membrane molecules in T cell and APC occurs and the subsequent cell signaling and other physiological responses are initiated so that a range of different antigen-specific T cells
25 exert immune effects on their target cells.

Molecular ligands of MHC class I and class II corresponding to TCR are also proteins of the immunoglobulin superfamily, but are specific for antigen presentation, and different individuals have different MHCs, thereby presenting different short peptides in one protein antigen to the surface of respective APC cells. Human MHC is
30 commonly referred to as HLA gene or HLA complex.

AFP, also known as α fetoprotein, is a protein expressed during embryonic development and the main component of embryonic serum. During development, AFP is relatively highly expressed in the yolk sac and liver, and subsequently inhibited. In hepatocellular carcinoma, the expression of AFP is activated (Butterfield et al. J
35 Immunol., 2001, Apr 15; 166(8): 5300-8). After AFP is produced in a cell, it is

degraded into small molecule polypeptides, and combined with MHC (major histocompatibility complex) molecules to form a complex, which is presented to the cell surface. FMNKFIYEI is a short peptide derived from AFP antigen and is a target for treating AFP-related diseases.

5 Therefore, a marker, by which TCR can target tumor cells is provided from FMNKFIYEI-HLA A0201 complex. There is a high application value for the TCR that can bind to FMNKFIYEI-HLA A0201 complex for treating tumors. For example, the TCR that can target the tumor cell marker can be used to deliver cytotoxic agents or immunostimulants to target cells, or be transformed into T cells, so that the T cells
10 expressing the TCR can destroy tumor cells and be given to patients during the course of treatment called adoptive immunotherapy. For the former purpose, an ideal TCR will possess a high affinity, so that the TCR can reside on the targeted cells for a long time. For the latter purpose, it is preferable to use a TCR with medium affinity. Therefore, skilled persons in the art devote themselves for developing TCRs targeting tumor cell
15 markers that can be used for different purposes.

Summary of the invention

It is an object of the present invention to provide a TCR having a higher affinity for FMNKFIYEI-HLA A0201 complex.

20 It is another object of the present invention to provide a method for preparing the TCR of the above type and uses of the TCR of the above type.

In a first aspect of the invention, a T cell receptor (TCR) is provided, which has an activity of binding to FMNKFIYEI-HLA A0201 complex.

In another preferred embodiment, the T cell receptor (TCR) has an activity of
25 binding to FMNKFIYEI-HLA A0201 complex, and the T cell receptor comprises a TCR α chain variable domain and a TCR β chain variable domain, the TCR α chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR α chain variable domain are listed as follows,

CDR1 α : TSINN

30 CDR2 α : IRSNERE

CDR3 α : ATDPRTGANSKLT, and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 2 of CDR1 α	D or P or A or E
I at position 3 of CDR1 α	V
N at position 4 of CDR1 α	S or Y or D
N at position 5 of CDR1 α	S or K

R at position 5 of CDR3 α	A or S or V or D or E or Q or T
T at position 6 of CDR3 α	M or R or L or A or N or S
G at position 7 of CDR3 α	A or Q or S or E or N
A at position 8 of CDR3 α	S or G or P or W
N at position 9 of CDR3 α	S or T
S at position 10 of CDR3 α	A or N or T or P

and/or, the TCR β chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR β chain variable domain are listed as follows,

CDR1 β : SEHNR

5 CDR2 β : FQNEAQ

CDR3 β : ASEGLAYEQY, and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 1 of CDR1 β	A or Q or G
E at position 2 of CDR1 β	G or T or R or L
H at position 3 of CDR1 β	W or R
N at position 4 of CDR1 β	A or P
Q at position 2 of CDR2 β	H
N at position 3 of CDR2 β	G
E at position 4 of CDR2 β	D
A at position 5 of CDR2 β	M or L
Q at position 6 of CDR2 β	E.

In another preferred embodiment, there are 1-8 mutations in the 3 CDR regions of the TCR α chain variable domain and/or 1-8 mutations in the 3 CDR regions of the TCR β chain variable domain.

10 In another preferred embodiment, the number of mutations in the CDR regions of the TCR α chain may be 1, 2, 3, 4, 5, 6, 7 or 8.

In another preferred embodiment, the number of mutations in the CDR regions of the TCR β chain may be 4, 5, 6, 7 or 8.

In another preferred embodiment, the affinity of the TCR for FMNKFIYEI-HLA A0201 complex is at least 2 times of that of the wild type TCR.

15 In another preferred embodiment, the TCR can also bind to FMNKFIYEI-HLA A0207 complex.

In another preferred embodiment, the α chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 2.

In another preferred embodiment, the mutated position in the α chain variable domain of the TCR includes position 7 and / or position 8 of CDR3 α .

In another preferred embodiment, the mutation in the α chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
G at position 7 of CDR3 α	S
A at position 8 of CDR3 α	P

and / or the mutation in the β chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
N at position 3 of CDR2 β	G
E at position 4 of CDR2 β	D
Q at position 6 of CDR2 β	E.

5 In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α .

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain
10 comprises CDR1 α , CDR2 α and CDR3 α , wherein the amino acid sequence of CDR1 α is selected from the group consisting of: TSINN, TPISS, TDIYK, TAVDS, TEISS and TDVSS.

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain
15 comprises CDR1 α , CDR2 α and CDR3 α , wherein the amino acid sequence of CDR2 α is IRSNERE.

In another preferred embodiment, the CDR1 α comprises a sequence:

T[1 α X1][1 α X2][1 α X3][1 α X4], wherein [1 α X1], [1 α X2], [1 α X3], [1 α X4] are independently selected from any natural amino acid residue, respectively.

20 In another preferred embodiment, the [1 α X1] is S or D or P or A or E.

In another preferred embodiment, the [1 α X2] is I or V.

In another preferred embodiment, the [1 α X3] is N or S or Y or D.

In another preferred embodiment, the [1 α X4] is N or S or K.

In another preferred embodiment, the [1 α X1] is S or D or P or A or E, [1 α X2] is I
25 or V, [1 α X3] is N or S or Y or D, and [1 α X4] is N or S or K.

In another preferred embodiment, the CDR1 α comprises a sequence selected from the group consisting of: TSINN, TPISS, TDIYK, TAVDS, TEISS and TDVSS.

In another preferred embodiment, the CDR3 α comprises a sequence:

ATDP[3 α X1][3 α X2][3 α X3][3 α X4][3 α X5][3 α X6]KLT, wherein [3 α X1], [3 α X2],

[3 α X3], [3 α X4], [3 α X5], [3 α X6] are independently selected from any natural amino acid residue, respectively.

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , wherein the CDR3 α comprises a sequence:
5 ATDP[3 α X1][3 α X2][3 α X3][3 α X4][3 α X5][3 α X6]KLT, wherein [3 α X1], [3 α X2], [3 α X3], [3 α X4], [3 α X5] and [3 α X6] are independently selected from any natural amino acid residue, respectively.

In another preferred embodiment, the [3 α X1] is R or A or S or D or E or Q or V
10 or T.

In another preferred embodiment, the [3 α X2] is T or M or R or L or A or N or S.

In another preferred embodiment, the [3 α X3] is G or A or Q or S or E or N.

In another preferred embodiment, the [3 α X4] is A or S or G or P or W.

In another preferred embodiment, the [3 α X5] is N or S or T.

15 In another preferred embodiment, the [3 α X6] is S or A or N or T or P.

In another preferred embodiment, the [3 α X1] is R or A or S or D or E or Q or V or T, [3 α X2] is T or M or R or L or A or N or S, [3 α X3] is G or A or Q or S or E or N, [3 α X4] is A or S or G or P or W, [3 α X5] is N or S or T, and [3 α X6] is S or A or N or T or P.

20 In another preferred embodiment, the CDR3 α comprises a sequence selected from the group consisting of: ATDPSMSPNSKLT, ATDPSLSPNSKLT and ATDPSLQSNKLT.

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR β chain variable domain
25 comprises CDR1 β , CDR2 β and CDR3 β , wherein the CDR3 β comprises a sequence: ASEGLAYEQY.

In another preferred embodiment, the CDR1 β comprises a sequence:

[1 β X1][1 β X2][1 β X3][1 β X4]R,

wherein [1 β X1], [1 β X2], [1 β X3], [1 β X4] are independently selected from any
30 natural amino acid residue, respectively.

In another preferred embodiment, the [1 β X1] is S or A or Q or G.

In another preferred embodiment, the [1 β X2] is E or G or T or R or L.

In another preferred embodiment, the [1 β X3] is H or W or R.

In another preferred embodiment, the [1 β X4] is N or A or P.

35 In another preferred embodiment, the CDR2 β comprises a sequence:

F[2 β X1][2 β X2][2 β X3][2 β X4][2 β X5], wherein [2 β X1], [2 β X2], [2 β X3], [2 β X4], [2 β X5] are independently selected from any natural amino acid residue, respectively.

In another preferred embodiment, the [2 β X1] is Q or H.

In another preferred embodiment, the [2 β X2] is N or G.

5 In another preferred embodiment, the [2 β X3] is E or D.

In another preferred embodiment, the [2 β X4] is A or M or L.

In another preferred embodiment, the [2 β X5] is Q or E.

In another preferred embodiment, the CDR2 β comprises a sequence selected from the group consisting of: FQGDME, FQGDLE and FHGDLE.

10 In another preferred embodiment, the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR β chain variable domain comprises CDR1 β , CDR2 β and CDR3 β , wherein the amino acid sequence of CDR2 β is selected from the group consisting of: FQGDME, FQGDLE and FHGDLE.

In another preferred embodiment, the TCR α chain variable domain of the TCR
15 does not simultaneously comprise following CDRs: CDR1 α : TSINN; CDR2 α : IRSNERE; and CDR3 α : ATDPRTGANSKLT.

In another preferred embodiment, the TCR β chain variable domain of the TCR does not simultaneously comprise following CDRs: CDR1 β : SEHNR; CDR2 β : FQNEAQ; and CDR3 β : ASEGLAYEQY.

20 In another preferred embodiment, the mutation occurs in one or more CDR regions of the α chain and/or β chain variable domain.

In another preferred embodiment, the mutation occurs in CDR1 and/or CDR3 of the α chain, and/or the mutation occurs in CDR1 and/or CDR2 of the β chain.

In a preferred embodiment of the present invention, the affinity of the TCR for
25 FMNKFIYEI-HLA A0201 complex is at least 2 times of that of the wild type TCR; preferably at least 5 times; and more preferably at least 10 times.

In another preferred embodiment, the affinity of the TCR for FMNKFIYEI-HLA A0201 complex is at least 50 times of that of the wild type TCR; preferably, at least 100 times; more preferably, at least 500 times; and most preferably, at least 1000 times.

30 In another preferred embodiment, the affinity of the TCR for FMNKFIYEI-HLA A0201 complex is at least 10^4 times of that of the wild type TCR; preferably, at least 10^5 times; and more preferably, at least 10^6 times.

In particular, the dissociation equilibrium constant K_D of the TCR to FMNKFIYEI-HLA A0201 complex is $\leq 20 \mu\text{M}$;

35 In another preferred embodiment, the dissociation equilibrium constant of the

TCR to FMNKFIYEI-HLA A0201 complex is $5 \mu\text{M} \leq K_D \leq 10 \mu\text{M}$; preferably, $0.1 \mu\text{M} \leq K_D \leq 1 \mu\text{M}$; and more preferably, $1 \text{nM} \leq K_D \leq 100 \text{nM}$;

In another preferred embodiment, the dissociation equilibrium constant of the TCR to FMNKFIYEI-HLA A0201 complex is $100 \text{pM} \leq K_D \leq 1000 \text{pM}$; and more preferably, $10 \text{pM} \leq K_D \leq 100 \text{pM}$.

In another preferred embodiment, the TCR has CDRs selected from the group consisting of:

CDR No.	CDR1 α	CDR2 α	CDR3 α	CDR1 β	CDR2 β	CDR3 β
1	TSINN	IRSNERE	ATDPSRQSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
2	TSINN	IRSNERE	ATDPRTGSSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
3	TSINN	IRSNERE	ATDPRTASSAKLT	SEHNR	FQNEAQ	ASEGLAYEQY
4	TSINN	IRSNERE	ATDPAMQANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
5	TSINN	IRSNERE	ATDPVLSANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
6	TSINN	IRSNERE	ATDPRTSGSTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
7	TSINN	IRSNERE	ATDPDRSPNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
8	TSINN	IRSNERE	ATDPRTASTPKLT	SEHNR	FQNEAQ	ASEGLAYEQY
9	TSINN	IRSNERE	ATDPEAQWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
10	TSINN	IRSNERE	ATDPRTSTTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
11	TSINN	IRSNERE	ATDPSLQSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
12	TSINN	IRSNERE	ATDPQNSWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
13	TSINN	IRSNERE	ATDPASNWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
14	TSINN	IRSNERE	ATDPRTGANSKLT	SEHNR	FQGDME	ASEGLAYEQY
15	TDVSS	IRSNERE	ATDPAAQWNSKLT	AGHAR	FQGDME	ASEGLAYEQY
16	TPISS	IRSNERE	ATDPAAQWNSKLT	QGHAR	FQGDME	ASEGLAYEQY
17	TPISS	IRSNERE	ATDPATSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
18	TDIYK	IRSNERE	ATDPRTASSPKLT	GTWPR	FQGDME	ASEGLAYEQY
19	TAVDS	IRSNERE	ATDPSLSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
20	TAVDS	IRSNERE	ATDPSLSPNSKLT	QGHAR	FQGDME	ASEGLAYEQY
21	TEISS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
22	TAVDS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
23	TEISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
24	TEISS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
25	TDVSS	IRSNERE	ATDPSMSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY
26	TDVSS	IRSNERE	ATDPTMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
27	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGHAR	FQGDLE	ASEGLAYEQY
28	TAVDS	IRSNERE	ATDPSLSPNSKLT	ARHAR	FQGDLE	ASEGLAYEQY
29	TEISS	IRSNERE	ATDPATSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
30	TPISS	IRSNERE	ATDPATSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY
31	TAVDS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
32	TDVSS	IRSNERE	ATDPSMSPNSKLT	GLWPR	FHGDLE	ASEGLAYEQY
33	TPISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
34	TDVSS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
35	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGRAR	FQGDME	ASEGLAYEQY
36	TAVDS	IRSNERE	ATDPTSSWNSKLT	GRWPR	FQGDLE	ASEGLAYEQY。

In another preferred embodiment, the TCR is soluble.

In another preferred embodiment, the TCR is an $\alpha\beta$ heterodimeric TCR or a single chain TCR.

In another preferred embodiment, the TCR of the present invention is an $\alpha\beta$ heterodimeric TCR, and preferably, the TCR comprises α chain constant region

sequence TRAC*01 and β chain constant region sequence TRBC1*01 or TRBC2*01. The α chain variable domain of the TCR comprises an amino acid sequence having at least 90%, preferably at least 92%; more preferably, at least 94% (e.g., may be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90%, preferably at least 92%; more preferably, at least 94% (e.g., may be at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with the amino acid sequence shown in SEQ ID NO: 2.

10 In another preferred embodiment, the TCR comprises (i) all or part of the TCR α chain except for its transmembrane domain, and (ii) all or part of the TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of the constant domain of the TCR chain.

15 In another preferred embodiment, the TCR is an $\alpha\beta$ heterodimeric TCR, and an artificial interchain disulfide bond is contained between the α chain variable region and the β chain constant region of the TCR.

In another preferred embodiment, cysteine residues forming an artificial interchain disulfide bond between the α chain variable region and the β chain constant region of the TCR are substituted for one or more groups of amino acids selected from the following:

amino acid at position 46 of TRAV and amino acid at position 60 of TRBC1*01 or TRBC2*01 exon 1;

amino acid at position 47 of TRAV and amino acid at position 61 of TRBC1*01 or TRBC2*01 exon 1;

25 amino acid at position 46 of TRAV and amino acid at position 61 of TRBC1*01 or TRBC2*01 exon 1; or

amino acid at position 47 of TRAV and amino acid at position 60 of TRBC1*01 or TRBC2*01 exon 1.

30 In another preferred embodiment, the TCR comprising an artificial interchain disulfide bond between α chain variable region and β chain constant region comprises α chain variable domain and β chain variable domain as well as all or part of β chain constant domains except for its transmembrane domain, however it does not comprise α chain constant domain, and α chain variable domain and β chain of the TCR form a heterodimer.

35 In another preferred embodiment, the TCR comprising an artificial interchain

disulfide bond between α chain variable region and β chain constant region comprises (i) all or part of TCR α chain except for its transmembrane domain, and (ii) all or part of TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of constant domains of the TCR chain.

5 In another preferred embodiment, the TCR is an $\alpha\beta$ heterodimeric TCR comprising (i) all or part of TCR α chain except for its transmembrane domain, and (ii) all or part of TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of the constant domain of the TCR chain, and an artificial interchain disulfide bond is contained between α chain
10 constant region and β chain constant region.

In another preferred embodiment, an artificial interchain disulfide bond is contained between α chain constant region and β chain constant region of the TCR.

In another preferred embodiment, cysteine residues forming an artificial interchain disulfide bond between the TCR α chain constant region and β chain
15 constant region are substituted for one or more groups of amino acids selected from the following:

Thr48 of TRAC*01 exon 1 and Ser57 of TRBC1*01 or TRBC2*01 exon 1;
Thr45 of TRAC*01 exon 1 and Ser77 of TRBC1*01 or TRBC2*01 exon 1;
Tyr10 of TRAC*01 exon 1 and Ser17 of TRBC1*01 or TRBC2*01 exon 1;
20 Thr45 of TRAC*01 exon 1 and Asp59 of TRBC1*01 or TRBC2*01 exon 1;
Ser15 of TRAC*01 exon 1 and Glu15 of TRBC1*01 or TRBC2*01 exon 1;
Arg53 of TRAC*01 exon 1 and Ser54 of TRBC1*01 or TRBC2*01 exon 1;
Pro89 of TRAC*01 exon 1 and Ala19 of TRBC1*01 or TRBC2*01 exon 1;
and

25 Tyr10 of TRAC*01 exon 1 and Glu20 of TRBC1*01 or TRBC2*01 exon 1.

In another preferred embodiment, the TCR is a single chain TCR.

In another preferred embodiment, the TCR is a single-chain TCR consisting of an α chain variable domain and a β chain variable domain, and the α chain variable domain and the β chain variable domain are connected by a flexible short peptide
30 sequence (linker).

In another preferred embodiment, the hydrophobic core of the TCR α chain variable domain and / or β chain variable domain is mutated.

In another preferred embodiment, the TCR, in which the hydrophobic core is mutated, is a single-chain TCR consisting of an α variable domain and a β variable
35 domain, and the α variable domain and the β variable domain are connected by a

flexible short peptide sequence (linker).

In another preferred embodiment, the TCR of the present invention is a single-chain TCR, and the α chain variable domain of the TCR comprises an amino acid sequence having at least 85%, preferably at least 90%; more preferably, at least 92%;
5 most preferably at least 94% (e.g., may be at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with the amino acid sequence shown in SEQ ID NO: 3; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 85%, preferably at least 90%; more preferably, at least 92%; most preferably at least 94% (e.g., may be at least 91%,
10 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with the amino acid sequence shown in SEQ ID NO: 4.

In another preferred embodiment, the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 9-35, 51-77 and 91-92; and / or the amino acid sequence of the β chain variable domain of the TCR
15 is selected from the group consisting of: SEQ ID NO: 36-46, 78-88 and 93-101.

In another preferred embodiment, the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
s-1	12	4
s-2	10	4
s-3	9	4
s-4	11	4
s-5	13	4
s-6	14	4
s-7	15	4
s-8	16	4
s-9	17	4
s-10	18	4
s-11	19	4
s-12	20	4
s-13	21	4
s-14	3	36
s-15	22	37
s-16	23	38
s-17	24	39
s-18	25	39
s-19	26	39
s-20	26	38
s-21	27	37
s-22	28	40
s-23	29	37
s-24	29	41
s-25	30	42
s-26	31	41

s-27	32	43
s-28	26	44
s-29	33	40
s-30	24	42
s-31	26	37
s-32	30	45
s-33	34	37
s-34	30	39
s-35	32	46
s-36	35	42。

In another preferred embodiment, the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
1	54	2
2	52	2
3	51	2
4	53	2
5	55	2
6	56	2
7	57	2
8	58	2
9	59	2
10	60	2
11	61	2
12	62	2
13	63	2
14	1	78
15	64	79
16	65	80
17	66	81
18	67	81
19	68	81
20	68	80
21	69	79
22	70	82
23	71	79
24	71	83
25	72	84
26	73	83
27	74	85
28	68	86
29	75	82
30	66	84
31	68	79
32	72	87
33	76	79
34	72	81
35	74	88
36	77	84
37	1	93
38	1	94
39	1	95
40	1	96

41	1	97
42	1	98
43	1	99
44	1	100
45	91	101
46	92	101。

In another preferred embodiment, a conjugate binds to the α chain and/or β chain of the TCR at C- or N-terminal.

In another preferred embodiment, the conjugate that binds to the TCR is a detectable label, a therapeutic agent, a PK modified moiety, or a combination thereof.

In another preferred embodiment, the therapeutic agent that binds to the TCR is an anti-CD3 antibody linked to the α or β chain of the TCR at C- or N-terminal.

In a preferred embodiment of the invention, the T cell receptor (TCR) has the activity of binding to FMNKFIYEI-HLA A2 complex and comprises a TCR α chain variable domain and a TCR β chain variable domain, there is a mutation in the α chain variable domain of the TCR as shown in SEQ ID NO: 1, and the mutated amino acid residue site includes one or more of 28S, 29I, 30N, 31N, 94R, 95T, 96G, 97A, 98N and 99S, wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or there is a mutation in the β chain variable domain of the TCR as shown in SEQ ID NO: 2, and the mutated amino acid residue site includes one or more of 27S, 28E, 29H, 30N, 50Q, 51N, 52E, 53A and 54Q, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2;

preferably, upon mutation, the TCR α chain variable domain comprises one or more amino acid residues selected from the group consisting of: 28D or 28P or 28A or 28E; 29V; 30S or 30Y or 30D; 31S or 31K; 94A or 94S or 94V or 94D or 94E or 94Q or 94T; 95M or 95R or 95L or 95A or 95N or 95S; 96A or 96Q or 96S or 96E or 96N; 97S or 97G or 97P or 97W; 98S or 98T; 和 99A or 99N or 99T or 99P; wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or upon mutation, the TCR β chain variable domain comprises one or more amino acid residues selected from the group consisting of: 27A or 27Q or 27G; 28G or 28T or 28R or 28L; 29W or 29R; 30A or 30P; 50H; 51G; 52D; 53M or 53L; and 54E, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2.

In a second aspect of the invention, a multivalent TCR complex comprising at least two TCR molecules is provided, and at least one TCR molecule is the TCR of the first aspect of the invention.

In a third aspect of the invention, a nucleic acid molecule is provided, comprising a nucleic acid sequence encoding the TCR molecule of the first aspect of the invention or the multivalent TCR complex of the second aspect of the invention, or a complement sequence thereof.

5

In a fourth aspect of the invention, a vector is provided, comprising the nucleic acid molecule of the third aspect of the invention.

10 In a fifth aspect of the present invention, a host cell is provided, comprising the vector of the fourth aspect of the present invention or having the exogenous nucleic acid molecule of the third aspect of the present invention integrated into its genome.

15 In a sixth aspect of the invention, an isolated cell is provided, expressing the TCR of the first aspect of the invention.

20 In a seventh aspect of the invention, a pharmaceutical composition is provided, comprising a pharmaceutically acceptable carrier, and a TCR of the first aspect of the invention, or a TCR complex of the second aspect of the invention, or the cell of the sixth aspect of the invention.

25 In an eighth aspect of the present invention, a method for treating a disease is provided, comprising administering an appropriate amount of the TCR of the first aspect of the present invention, or the TCR complex of the second aspect of the present invention, or the cell of the sixth aspect of the invention, or the pharmaceutical composition of the seventh aspect of the invention to a subject in need thereof.

30 In a ninth aspect of the invention, use of the TCR of the first aspect of the invention, or the TCR complex of the second aspect of the invention, or the cell of the sixth aspect of the invention is provided for preparing a medicament for treating tumor; and preferably, the tumor is liver cancer.

35 The TCR of the first aspect of the invention, or the TCR complex of the second aspect of the invention, or the cell of the sixth aspect of the invention, for use as a medicament for treating tumor; and preferably, the tumor is liver cancer.

In a eleventh aspect of the invention, a method for preparing the T cell receptor of the first aspect of the invention is provided, comprising the steps of:

- 5 (i) culturing the host cell of the fifth aspect of the invention to express the T cell receptor of the first aspect of the invention;
- (ii) isolating or purifying the T cell receptor.

10 It is to be understood that within the scope of the present invention, the various technical features of the present invention and the technical features specifically described hereinafter (as in the embodiments) may be combined with each other to constitute a new or preferred technical solution, which will not be repeated herein one by one.

Description of drawings

15 Figure 1a and Figure 1b show the amino acid sequences of wild-type TCR α and β chain variable domain that are capable of specifically binding to FMNKFIYEI-HLA A0201 complex, respectively.

Figure 2a and Figure 2b show the amino acid sequences of the α chain variable domain and the β chain variable domain of the single-chain template TCR constructed
20 in the present invention, respectively.

Figure 3a and Figure 3b show the DNA sequences of the α chain variable domain and the β chain variable domain of the single-chain template TCR constructed in the present invention, respectively.

25 Figure 4a and Figure 4b are the amino acid sequence and nucleotide sequence of the linking short peptide (linker) of the single-chain template TCR constructed in the present invention, respectively.

Figures 5(1)-(27) show the amino acid sequences of α chain variable domain of single chain TCRs with high affinity for FMNKFIYEI-HLA A0201 complex, respectively, and the mutated residues are underlined.

30 Figures 6(1)-(11) show the amino acid sequences of β chain variable domain of single chain TCRs with high affinity for FMNKFIYEI-HLA A0201 complex, respectively, and the mutated residues are underlined.

Figure 7a and Figure 7b show the amino acid sequence and DNA sequence of the single chain template TCR constructed in the present invention, respectively.

35 Figures 8a and 8b show the amino acid sequences of the α and β chains of a

soluble reference TCR in the present invention, respectively, and the mutated cysteines are underlined.

Figures 9(1)-(29) show the amino acid sequences of α chain variable domain of a heterodimeric TCR with high affinity for FMNKFIYEI-HLA A0201 complex,
5 respectively, and the mutated residues are underlined.

Figures 10(1)-(20) show the amino acid sequences of β chain variable domain of a heterodimeric TCR with high affinity for FMNKFIYEI-HLA A0201 complex, respectively, and the mutated residues are underlined.

Figure 11a and Figure 11b show the amino acid sequences of wild-type TCR α
10 and β chain that are capable of specifically binding to FMNKFIYEI-HLA A0201 complex, respectively.

Figure 12 is a binding curve of a soluble reference TCR, that is, wild-type TCR and FMNKFIYEI-HLA A0201 complex.

Figures 13a and 13b show results of activation function experiment of effector
15 cells transfected with the high affinity TCR of the present invention.

Figures 14a, 14b show results of killing function experiment of effector cells transfected with the high affinity TCR of the present invention.

Figures 15a, 15b, 15c, 15d, 15e, 15f and 15g show the results of the function verification-activation experiment of a fusion protein formed from the high-affinity
20 TCR of the present invention and anti-CD3 antibody.

Figure 16 shows the result of the function verification-killing experiment of a fusion protein formed from the high-affinity TCR of the present invention and anti-CD3 antibody.

Figure 17a and Figure 17b show extracellular amino acid sequences of wild-type
25 TCR α and β chains which can specifically bind to FMNKFIYEI-HLA A0201 complex, respectively.

Modes for carrying out the invention

Through extensive and intensive research, a high affinity T cell receptor (TCR)
30 recognizing FMNKFIYEI short peptide (derived from AFP protein) was obtained, and the FMNKFIYEI short peptide is presented in a form of peptide-HLA A2 complexe. The high affinity TCR has a mutation in three CDR regions of its α chain variable domain:

CDR1 α : TSINN

35 CDR2 α : IRSNERE

CDR3 α : ATDPRTGANSKLT; and / or has a mutation in three CDR regions of its β chain variable domain:

CDR1 β : SEHNR

CDR2 β : FQNEAQ

- 5 CDR3 β : ASEGLAYEQY; and after mutation, the affinity and/or binding half-life of the TCR of the present invention for the above FMNKFIYEI-HLA A0201 complex is at least 2-fold greater than that of the wild-type TCR.

10 Before the present invention is described, it is to be understood that the invention is not limited to the specific methods and experimental conditions described, as such methods and conditions may vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting, and the scope of the present invention shall be only limited by the attached claim set.

15 All technical and scientific terms used herein have the same meaning as commonly understood by a skilled person in the art to which this invention belongs, unless otherwise defined.

Although any methods and materials similar or equivalent to those described in the present invention can be used in the practice or testing of the present invention, the
20 preferred methods and materials are exemplified herein.

Terms

T cell receptor (TCR)

International Immunogenetics Information System (IMGT) can be used to
25 describe a TCR. A native $\alpha\beta$ heterodimeric TCR has an α chain and β chain. Generally speaking, each chain comprises a variable region, a junction region and a constant region, and the β chain typically also contains a short hypervariable region between the variable region and junction region, which however is often considered as a part of the junction region. The TCR junction region is determined by the unique TRAJ and TRBJ
30 of IMGT, and the constant region of a TCR is determined by TACT and TRBC of IMGT.

Each variable region comprises three CDRs (complementarity determining regions), CDR1, CDR2 and CDR3, which are chimeric in the framework sequence. In IMGT nomenclature, the different numbers of TRAV and TRBV refer to different V α
35 types and V β types, respectively. In IMGT system, there are following symbols for α

chain constant domain: TRAC*01, where "TR" represents T cell receptor gene; "A" represents α chain gene; C represents the constant region; "*01" represents allele gene 1. There are following symbols for β -chain constant domain: TRBC1*01 or TRBC2*01, where "TR" represents T cell receptor gene; "B" represents β -chain gene; C represents constant region; "*01" represents allele gene 1. The constant region of α chain is uniquely defined, and in the form of β chain, there are two possible constant region genes "C1" and "C2". A skilled person in the art can obtain constant region gene sequences of TCR α and β chains through the disclosed IMGT database.

The α and β chains of TCR are generally considered as having two "domains" respectively, i.e., variable domain and constant domain. The variable domain consists of a connected variable region and a connection region. Therefore, in the specification and claims of the present application, "TCR α chain variable domain" refers to a connected TRAV and TRAJ region, and likewise, "TCR β chain variable domain" refers to a connected TRBV and TRBD/TRBJ region. The three CDRs of TCR α chain variable domain are CDR1 α , CDR2 α and CDR3 α , respectively; and the three CDRs of TCR β chain variable domain are CDR1 β , CDR2 β and CDR3 β , respectively. The framework sequences of TCR variable domains of the invention may be of murine or human origin, preferably of human origin. The constant domain of TCR comprises an intracellular portion, transmembrane region, and extracellular portion. To obtain a soluble TCR for determining the affinity between TCR and FMNKFIYEI-HLA A2 complex, TCR of the invention preferably does not comprise a transmembrane region. More preferably, the amino acid sequence of the TCR of the present invention refers to the extracellular amino acid sequence of the TCR.

The α chain amino acid sequence and β chain amino acid sequence of the "wild type TCR" described in the present invention are SEQ ID NO: 89 and SEQ ID NO: 90, respectively, as shown in Figs. 11a and 11b. The extracellular amino acid sequences of α chain and β chain of the "wild type TCR" described in the present invention are SEQ ID NO: 102 and SEQ ID NO: 103, respectively, as shown in Figs. 17a and 17b. In the present invention, the α chain amino acid sequence and β chain amino acid sequence of the "reference TCR" are SEQ ID NO: 49 and SEQ ID NO: 50, respectively, as shown in Figs. 8a and 8b. In the present invention, the α and β chain variable domain amino acid sequences of the wild type TCR capable of binding to FMNKFIYEI-HLA A0201 complex are SEQ ID NO: 1 and SEQ ID NO: 2, respectively, as shown in Figures 1a and 1b. In the present invention, the terms "polypeptide of the present invention", "TCR of the present invention" and "T cell receptor of the present invention" are used

interchangeably.

Natural inter-chain disulfide bond and artificial inter-chain disulfide bond

A group of disulfide bonds is present between the C α and C β chains in the membrane proximal region of a native TCR, which is named herein as "natural inter-chain disulfide bond". In the present invention, an inter-chain covalent disulfide bond which is artificially introduced and the position of which is different from the position of a natural inter-chain disulfide bond is named as "artificial inter-chain disulfide bond".

For convenience of description, in the present invention, the positions of the amino acid sequences of TRAC*01 and TRBC1*01 or TRBC2*01 are sequentially numbered in order from N-terminal to C-terminal. For example, the 60th amino acid in the order from N-terminal to C-terminal in TRBC1*01 or TRBC2*01 is P (valine), which can be described as Pro60 of TRBC1*01 or TRBC2*01 exon 1 in the present invention, and can also be expressed as the amino acid at position 60 of TRBC1* 01 or TRBC2*01 exon 1. For another example, the 61st amino acid in the order from N-terminal to C-terminal in TRBC1*01 or TRBC2*01 is Q (glutamine), which can be described as Gln61 of TRBC1*01 or TRBC2*01 exon 1 in the invention, and can also be expressed as the amino acid at position 61 of TRBC1*01 or TRBC2*01 exon 1, and so on. In the present invention, the positions of the amino acid sequences of variable regions TRAV and TRBV are numbered according to the positions listed in IMGT. As for an amino acid in TRAV, the position is numbered as 46 in IMGT, which is described in the present invention as the amino acid at position 46 of TRAV, and so on. In the present invention, if the sequence positions of other amino acids are specifically described, the special description shall prevail.

Tumor

The term "tumor" refers to include all types of cancer cell growth or carcinogenic processes, metastatic tissues or malignant transformed cells, tissues or organs, regardless of pathological type or stage of infection. Examples of tumors include, without limitation, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include: malignant tumors of different organ systems, such as sarcoma, lung squamous cell carcinoma, and cancer. For example: infected prostate, lung, breast, lymph, gastrointestinal (e.g., colon) and genitourinary tract (e.g., kidney, epithelial cells), pharynx. Squamous cell carcinoma of lung includes malignant tumors, for

example, most of colon cancer, rectal cancer, renal cell carcinoma, liver cancer, non-small cell cancer of lung, small intestine cancer and esophageal cancer. Metastatic lesions of the above cancers can likewise be treated and prevented using the methods and compositions of the invention.

5

Detailed description of the invention

It is well known that the α chain variable domain and the β chain variable domain of a TCR contain three CDRs (similar to the complementarity determining regions of antibodies), respectively. CDR3 interacts with the antigen short peptide, and CDR1 and CDR2 interact with HLA. Therefore, the CDRs of a TCR molecule determine its interaction with the antigen short peptide-HLA complex. The amino acid sequences of α chain variable domain and β chain variable domain of a wild type TCR capable of binding the complex of antigen short peptide FMNKFIYEI and HLA-A0201 (i.e., FMNKFIYEI-HLA-A0201 complex) are SEQ ID NO: 1 and SEQ ID NO: 2, respectively. These sequences were firstly discovered by the inventors. It has the following CDR regions:

α chain variable domain CDR CDR1 α : TSINN
CDR2 α : IRSNERE
CDR3 α : ATDPRTGANSKLT
and β chain variable domain CDR CDR1 β : SEHNR
CDR2 β : FQNEAQ
CDR3 β : ASEGLAYEQY.

In the present invention, a high affinity TCR is obtained by subjecting mutation and screen in the above CDR regions, which has an affinity for FMNKFIYEI-HLA-A0201 complex that is at least 2 times greater than that of a wild type TCR for FMNKFIYEI-HLA-A0201 complex.

In the present invention, a T cell receptor (TCR) is provided, which has an activity in binding to FMNKFIYEI-HLA-A0201 complex.

The T cell receptor comprises a TCR α chain variable domain and a TCR β chain variable domain, the TCR α chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR α chain variable domain are listed as follows,

CDR1 α : TSINN
CDR2 α : IRSNERE
CDR3 α : ATDPRTGANSKLT, and contains at least one of the following

mutations:

Residue before mutation	Residue after mutation
S at position 2 of CDR1 α	D or P or A or E
I at position 3 of CDR1 α	V
N at position 4 of CDR1 α	S or Y or D
N at position 5 of CDR1 α	S or K
R at position 5 of CDR3 α	A or S or V or D or E or Q or T
T at position 6 of CDR3 α	M or R or L or A or N or S
G at position 7 of CDR3 α	A or Q or S or E or N
A at position 8 of CDR3 α	S or G or P or W
N at position 9 of CDR3 α	S or T
S at position 10 of CDR3 α	A or N or T or P

and/or, the TCR β chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR β chain variable domain are listed as follows,

5 CDR1 β : SEHNR

CDR2 β : FQNEAQ

CDR3 β : ASEGLAYEQY, and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 1 of CDR1 β	A or Q or G
E at position 2 of CDR1 β	G or T or R or L
H at position 3 of CDR1 β	W or R
N at position 4 of CDR1 β	A or P
Q at position 2 of CDR2 β	H
N at position 3 of CDR2 β	G
E at position 4 of CDR2 β	D
A at position 5 of CDR2 β	M or L
Q at position 6 of CDR2 β	E.

In a preferred embodiment of the present invention, the T cell receptor of the present invention comprises a TCR α chain variable domain and TCR β chain variable domain, and the TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α .

In another preferred embodiment, the CDR1 α comprises a sequence:

T[1 α X1][1 α X2][1 α X3][1 α X4], wherein [1 α X1], [1 α X2], [1 α X3], [1 α X4] are independently selected from any natural amino acid residue.

In another preferred embodiment, the [1 α X1] is S or D or P or A or E.

15 In another preferred embodiment, the [1 α X2] is I or V.

In another preferred embodiment, the [1 α X3] is N or S or Y or D.

In another preferred embodiment, the [1 α X4] is N or S or K.

In another preferred embodiment, the [1 α X1] is S or D or P or A or E, [1 α X2] is I or V, [1 α X3] is N or S or Y or D, and [1 α X4] is N or S or K.

20 In another preferred embodiment, the CDR1 α comprises a sequence selected from

the group consisting of: TSINN, TPISS, TDIYK, TAVDS, TEISS and TDVSS.

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and TCR β chain variable domain, and the TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , wherein the amino acid sequence of CDR2 α is
5 IRSNERE.

In another preferred embodiment, the TCR comprises a TCR α chain variable domain and TCR β chain variable domain, the TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , and the CDR3 α comprises a sequence:
10 ATDP[3 α X1][3 α X2][3 α X3][3 α X4][3 α X5][3 α X6]KLT, wherein [3 α X1], [3 α X2], [3 α X3], [3 α X4], [3 α X5] and [3 α X6] are independently selected from any natural amino acid residue.

In another preferred embodiment, the [3 α X1] is R or A or S or D or E or Q or V or T.

15 In another preferred embodiment, the [3 α X2] is T or M or R or L or A or N or S.

In another preferred embodiment, the [3 α X3] is G or A or Q or S or E or N.

In another preferred embodiment, the [3 α X4] is A or S or G or P or W.

In another preferred embodiment, the [3 α X5] is N or S or T.

In another preferred embodiment, the [3 α X6] is S or A or N or T or P.

20 In another preferred embodiment, the [3 α X1] is R or A or S or D or E or Q or V or T, [3 α X2] is T or M or R or L or A or N or S, [3 α X3] is G or A or Q or S or E or N, [3 α X4] is A or S or G or P or W, [3 α X5] is N or S or T, and [3 α X6] is S or A or N or T or P.

In another preferred embodiment, the CDR3 α comprises a sequence selected from
25 the group consisting of: ATDPSMSPNSKLT, ATDPSLSPNSKLT and ATDPSLQSNKLT.

In a preferred embodiment of the present invention, the TCR comprises a TCR α chain variable domain and TCR β chain variable domain, the TCR β chain variable
30 domain comprises CDR1 β , CDR2 β and CDR3 β , and the CDR3 β comprises a sequence: ASEGLAYEQY.

In another preferred embodiment, the CDR1 β comprises a sequence:
[1 β X1][1 β X2][1 β X3][1 β X4]R, wherein [1 β X1], [1 β X2], [1 β X3], [1 β X4] are
35 independently selected from any natural amino acid residue.

In another preferred embodiment, the [1 β X1] is S or A or Q or G.

In another preferred embodiment, the [1 β X2] is E or G or T or R or L.

In another preferred embodiment, the [1 β X3] is H or W or R.

In another preferred embodiment, the [1 β X4] is N or A or P.

5

In another preferred embodiment, the CDR2 β comprises a sequence:

F[2 β X1][2 β X2][2 β X3][2 β X4][2 β X5], wherein [2 β X1], [2 β X2], [2 β X3], [2 β X4], [2 β X5] are independently selected from any natural amino acid residue.

In another preferred embodiment, the [2 β X1] is Q or H.

10

In another preferred embodiment, the [2 β X2] is N or G.

In another preferred embodiment, the [2 β X3] is E or D.

In another preferred embodiment, the [2 β X4] is A or M or L.

In another preferred embodiment, the [2 β X5] is Q or E.

15 In another preferred embodiment, the CDR2 β comprises a sequence selected from the group consisting of: FQGDME, FQGDLE and FHGDLE.

20 In another preferred embodiment, the TCR comprises a TCR α chain variable domain and TCR β chain variable domain, the TCR β chain variable domain comprises CDR1 β , CDR2 β and CDR3 β , and the amino acid sequence of CDR2 β is selected from the group consisting of: FQGDME, FQGDLE and FHGDLE.

In another preferred embodiment, the TCR α chain variable domain of the TCR does not comprise the following CDRs simultaneously:

CDR1 α : TSINN; CDR2 α : IRSNERE; and CDR3 α : ATDPRTGANSKLT.

25

In another preferred embodiment, the TCR β chain variable domain of the TCR does not comprise the following CDRs simultaneously:

CDR1 β : SEHNR; CDR2 β : FQNEAQ; and CDR3 β : ASEGLAYEQY.

30 In particular, the number of mutations in the CDR region of the TCR α chain may be 3, 4, 5, 6, 7 or 8; and / or the number of mutations in the CDR region of the TCR β chain may be 4, 5, 6, 7 or 8.

Moreover, the TCR of the present invention is an $\alpha\beta$ heterodimeric TCR, and the α chain variable domain of the TCR comprises an amino acid sequence having at least 90%, preferably at least 92%; more preferably, at least 94% (e.g., may be at least 90%, 35 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence

homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90%, preferably at least 92%; more preferably, at least 94% (e.g., may be at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with
5 the amino acid sequence shown in SEQ ID NO: 2.

Additionally, the TCR of the present invention is a single-chain TCR, and the α chain variable domain of the TCR comprises an amino acid sequence having at least 85%, preferably at least 90%; more preferably, at least 92%; most preferably at least 94% (e.g., may be at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
10 98%, 99% sequence homology) sequence homology with the amino acid sequence shown in SEQ ID NO: 3; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 85%, preferably at least 90%; more preferably, at least 92%; most preferably at least 94% (e.g., may be at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence homology) sequence homology with the amino
15 acid sequence shown in SEQ ID NO: 4.

Preferably, the TCR comprises (i) all or part of TCR α chain except for its transmembrane domain, and (ii) all or part of TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of constant domains of the TCR chain.

20 In the present invention, the three CDRs of α chain variable domain SEQ ID NO: 1 of the wild type TCR, i.e., CDR1, CDR2 and CDR3 are located at positions 27-31, 49-55 and 90-102 of SEQ ID NO: 1, respectively. Accordingly, the amino acid residue is numbered as shown in SEQ ID NO: 1, 28S is S at the 2nd position of CDR1 α , 29I is I at the 3rd position of CDR1 α , 30N is N at the 4th position of CDR1 α , 31N is N at the 5th
25 position of CDR1 α , 94R is R at the 5th position of CDR3 α , 95T is T at the 6th position of CDR3 α , 96G is G at the 7th position of CDR3 α , 97A is A at the 8th position of CDR3 α , 98N is N at the 9th position of CDR3 α , and 99S is S at the 10th position of CDR3 α .

Similarly, in the present invention, the three CDRs of β chain variable domain SEQ ID NO: 2 of the wild type TCR, i.e., CDR1, CDR2 and CDR3 are located at
30 positions 27-31, 49-54 and 93-102 of SEQ ID NO: 2, respectively. Accordingly, the amino acid residue is numbered as shown in SEQ ID NO: 2, 27S is S at the 1st position of CDR1 β , 28E is E at the 2nd position of CDR1 β , 29H is H at the 3rd position of CDR1 β , 30N is N at the 4th position of CDR1 β , 50Q is Q at the 2nd position of CDR2 β , 51N is N at the 3rd position of CDR2 β , 52E is E at the 4th position of CDR2 β , 53A is A
35 at the 5th position of CDR2 β , 54Q is Q at the 6th position of CDR2 β .

The present invention provides a TCR having the property of binding to FMNKFIYEI-HLA-A0201 complex, and comprises an α chain variable domain and a β chain variable domain, wherein the TCR has a mutation in the α chain variable domain shown in SEQ ID NO: 1, and the site of the mutated amino acid residue includes one or more of 28S, 29I, 30N, 31N, 94R, 95T, 96G, 97A, 98N and 99S, wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or the TCR has a mutation in the β chain variable domain shown in SEQ ID NO: 2, and the site of the mutated amino acid residue includes one or more of 27S, 28E, 29H, 30N, 50Q, 51N, 52E, 53A and 54Q, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2;

Preferably, the mutated TCR α chain variable domain comprises one or more amino acid residues selected from the group consisting of: 28D or 28P or 28A or 28E; 29V; 30S or 30Y or 30D; 31S or 31K, 94A or 94S or 94V or 94D or 94E or 94Q or 94T, 95M or 95R or 95L or 95A or 95N or 95S, 96A or 96Q or 96S or 96E or 96N, 97S or 97G or 97P or 97W, 98S or 98T, 99A or 99N or 99T or 99P, wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or the mutated TCR β chain variable domain comprises one or more amino acid residues selected from the group consisting of: 27A or 27Q or 27G, 28G or 28T or 28R or 28L, 29W or 29R, 30A or 30P, 50H, 51G, 52D, 53M or 53L, 54E, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2.

More specifically, in the α chain variable domain, specific forms of the mutation include one or more groups of S28D/P/A/E, I29V, N30S/Y/D, N31S/K, R94A/S/V/D/E/Q/T, T95M/R/L/A/N/S, G96A/Q/S/E/N, A97S/G/P/W, N98S/T, S99A/N/T/P; and in the β chain variable domain, specific forms of the mutation include one or more groups of S27A/Q/G, E28G/T/R/L, H29W/R, N30A/P, Q50H, N51G, E52D, A53M/L, Q54E.

Thr48 of the wild type TCR α chain constant region TRAC*01 exon 1 was mutated to cysteine, and Ser57 of the β chain constant region TRBC1*01 or TRBC2*01 exon 1 was mutated to cysteine according to the site-directed mutagenesis method well known to a skilled person in the art, so as to obtain a reference TCR, the amino acid sequences of which are shown in Figures 8a and 8b, respectively, and the mutated cysteine residues are indicated by bold letters. The above cysteine substitutions can form an artificial interchain disulfide bond between the constant regions of α and β chain of the reference TCR to form a more stable soluble TCR, so that it is easier to evaluate the binding affinity and/or binding half-life between TCR and FMNKFIYEI-HLA-A2 complex. It will be appreciated that the CDR regions of the TCR variable region determine its affinity for

pMHC complex, therefore, the above cysteine substitutions in the TCR constant region won't affect the binding affinity and/or binding half-life of TCR. Therefore, in the present invention, the measured binding affinity between the reference TCR and FMNKFIYEI-HLA-A0201 complex is considered to be the binding affinity between the wild-type TCR and FMNKFIYEI-HLA-A0201 complex. Similarly, if the binding affinity between the TCR of the invention and FMNKFIYEI-HLA-A0201 complex is determined to be at least 10 times the binding affinity between the reference TCR and FMNKFIYEI-HLA-A0201 complex, the binding affinity between the TCR of the present invention and FMNKFIYEI-HLA-A0201 complex is at least 10 times the binding affinity between the wild type TCR and FMNKFIYEI-HLA-A0201 complex.

The binding affinity (in inverse proportion to the dissociation equilibrium constant K_D) and the binding half-life (expressed as $T_{1/2}$) can be determined by any suitable method, such as Surface Plasmon Resonance. It should be understood that doubling of the affinity of the TCR will halve K_D . $T_{1/2}$ is calculated as $\ln 2$ divided by dissociation rate (K_{off}). Therefore, doubling of $T_{1/2}$ will halve K_{off} . Preferably, the binding affinity or binding half-life of a given TCR is detected for several times by using the same test protocol, for example 3 or more times, and the average of the results is taken. In a preferred embodiment, the affinity of soluble TCR is detected by the surface plasmon resonance (BIAcore) method in the Examples herein with conditions: a temperature of 25°C and pH value of 7.1-7.5. The dissociation equilibrium constant K_D of the reference TCR to FMNKFIYEI-HLA-A2 complex is detected as 4.3E-05M, that is, 43 μ M by the method, and in the present invention, the dissociation equilibrium constant K_D of the wild type TCR to FMNKFIYEI-HLA-A2 complex is also considered as 43 μ M. Since doubling of the affinity of TCR will halve K_D , if the dissociation equilibrium constant K_D of the high affinity TCR to FMNKFIYEI-HLA-A2 complex is detected as 4.3E-06M, i.e., 4.3 μ M, the affinity of the high affinity TCR for FMNKFIYEI-HLA-A2 complex is 10 times that of the wild type TCR for FMNKFIYEI-HLA-A2 complex. A skilled person is familiar with the conversion relationship between K_D value units, i.e., 1 M = 10⁶ μ M, 1 μ M = 1000 nM, and 1 nM = 1000 pM.

In a preferred embodiment of the invention, the affinity of the TCR for FMNKFIYEI-HLA-A0201 complex is at least 2 times that of the wild type TCR; preferably at least 5 times; more preferably at least 10 times.

In another preferred embodiment, the affinity of the TCR for FMNKFIYEI-HLA-A0201 complex is at least 50 times that of the wild type TCR; preferably, at least 100 times; more preferably, at least 500 times; most preferably, at

least 1000 times.

In another preferred embodiment, the affinity of the TCR for FMNKFIYEI-HLA-A0201 complex is at least 10^4 times; preferably, at least 10^5 times ; more preferably, at least 10^6 times that of the wild type TCR.

5 Specifically, the dissociation equilibrium constant K_D of the TCR for FMNKFIYEI-HLA-A0201 complex is $\leq 20 \mu\text{M}$;

In another preferred embodiment, the dissociation equilibrium constant of the TCR for FMNKFIYEI-HLA-A0201 complex is $5 \mu\text{M} \leq K_D \leq 10 \mu\text{M}$; preferably, $0.1 \mu\text{M} \leq K_D \leq 1 \mu\text{M}$; and more preferably, $1 \text{ nM} \leq K_D \leq 100 \text{ nM}$;

10 In another preferred embodiment, the dissociation equilibrium constant of the TCR for FMNKFIYEI-HLA-A0201 complex is $100 \text{ pM} \leq K_D \leq 1000 \text{ pM}$; and more preferably, $10 \text{ pM} \leq K_D \leq 100 \text{ pM}$.

Mutations can be carried out by any suitable method including, but not limited to, those based on polymerase chain reaction (PCR), restriction enzyme-based cloning or
15 linkage-independent cloning (LIC) methods. Many standard molecular biology textbooks describe these methods in detail. More details about polymerase chain reaction (PCR) mutagenesis and cloning based on restriction enzymes can be found in Sambrook and Russell, (2001) Molecular Cloning-A Laboratory Manual (Third Edition) CSHL Publishing house. More information about LIC method can be found in
20 Rashtchian, (1995) Curr Opin Biotechnol 6(1): 30-6.

The method for producing the TCR of the present invention may be, but not limited to, screening for a TCR having high affinity for FMNKFIYEI-HLA-A2 complex from a diverse library of phage particles displaying such TCRs, as described in a literature (Li, et al). (2005) Nature Biotech 23(3): 349-354).

25 It will be appreciated that genes expressing amino acid of α and β chain variable domain of a wild-type TCR or genes expressing amino acid of α and β chain variable domain of a slightly modified wild-type TCR can be used to prepare template TCRs. Changes necessary to produce the high affinity TCR of the invention are then introduced into the DNA encoding the variable domain of the template TCR.

30 The high affinity TCR of the present invention comprises one of α chain variable domain amino acid sequences of SEQ ID NO: 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 91, 92 and / or one of β chain variable domain amino acid sequences of SEQ ID NO: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 95, 96, 97, 98, 99, 100, 101. Therefore, a TCR α chain
35 comprising α chain variable domain amino acid sequence of the wild-type TCR (SEQ

ID NO: 1) can bind to a TCR β chain comprising one of SEQ ID NO: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 95, 96, 97, 98, 99, 100, 101 to form a heterodimeric TCR or a single-chain TCR molecule. Alternatively, a TCR β chain comprising β variable domain amino acid sequence of the wild type TCR (SEQ ID NO: 2) can bind to a TCR α chain comprising one of SEQ ID NO: 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 91, 92 to form a heterodimeric TCR or single-chain TCR molecule. Alternatively, a TCR α chain comprising one of TCR α chain variable domain amino acid sequences SEQ ID NO: 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 91, 92 can bind to a TCR β chain comprising one of TCR β chain variable domain amino acid sequences SEQ ID NO: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 95, 96, 97, 98, 99, 100, 101 to form a heterodimeric TCR or a single-chain TCR molecule. In the present invention, the amino acid sequences of the α chain variable domain and β chain variable domain which form the heterodimeric TCR molecule are preferably selected from the following Table 1:

Table 1

TCR No.	α chain variable domain sequence SEQ ID NO:	β chain variable domain sequence SEQ ID NO:
1	54	2
2	52	2
3	51	2
4	53	2
5	55	2
6	56	2
7	57	2
8	58	2
9	59	2
10	60	2
11	61	2
12	62	2
13	63	2
14	1	78
15	64	79
16	65	80
17	66	81
18	67	81
19	68	81
20	68	80
21	69	79
22	70	82
23	71	79
24	71	83

25	72	84
26	73	83
27	74	85
28	68	86
29	75	82
30	66	84
31	68	79
32	72	87
33	76	79
34	72	81
35	74	88
36	77	84
37	1	93
38	1	94
39	1	95
40	1	96
41	1	97
42	1	98
43	1	99
44	1	100
45	91	101
46	92	101

For the purposes of the present invention, the TCR of the invention is a moiety having at least one TCR α and/or TCR β chain variable domain. They usually comprise both of TCR α chain variable domain and TCR β chain variable domain. They may be $\alpha\beta$ heterodimers or single-chain forms or any other stable forms. In adoptive immunotherapy, the full length chain of the $\alpha\beta$ heterodimeric TCR (including the cytoplasmic and transmembrane domains) can be transfected. The TCR of the present invention can be used as a targeting agent for delivering a therapeutic agent to an antigen presenting cell or in combination with other molecules to prepare a bifunctional polypeptide to direct effector cells, when the TCR is preferably in a soluble form.

For stability, it is disclosed in the prior art that a soluble and stable TCR molecule can be obtained by introducing an artificial interchain disulfide bond between the α and β chain constant domains of a TCR, as described in PCT/CN2015/093806. Therefore, the TCR of the invention may be a TCR that an artificial interchain disulfide bond is introduced between the residues of its α and β chain constant domains. Cysteine residues form an artificial interchain disulfide bond between the α and β chain constant domains of the TCR. A cysteine residue can replace other amino acid residue at a suitable position in a native TCR to form an artificial interchain disulfide bond. For example, Thr48 of TRAC*01 exon 1 and Ser57 of TRBC1*01 or TRBC2*01 exon 1 can be replaced to form a disulfide bond. Other sites for introducing a cysteine residue to form a disulfide bond may be: Thr45 of TRAC*01 exon 1 and Ser77 of TRBC1*01

or TRBC2*01 exon 1; Tyr10 of TRAC*01 exon 1 and Ser17 of TRBC1*01 or TRBC2*01 exon 1; Thr45 of TRAC*01 exon 1 and Asp59 of TRBC1*01 or TRBC2*01 exon 1; Ser15 of TRAC*01 exon 1 and Glu15 of TRBC1*01 or TRBC2*01 exon 1; Arg53 of TRAC*01 exon 1 and Ser54 of TRBC1*01 or TRBC2*01 exon 1; Pro89 of TRAC*01 exon 1 and Ala19 of TRBC1*01 or TRBC2*01 exon 1; or Tyr10 of TRAC*01 exon 1 and Glu20 of TRBC1*01 or TRBC2*01 exon 1. That is, cysteine residues replace any group of the above-mentioned sites in α and β chain constant domains. A maximum of 15, or a maximum of 10, or a maximum of 8 or fewer amino acids may be truncated at one or more C-termini of the constant domain of the TCR of the invention such that it does not include cysteine residues to achieve the purpose of deleting native interchain disulfide bonds, or the cysteine residues forming a natural interchain disulfide bond can also be mutated to another amino acid for achieving the above purpose.

As described above, the TCR of the present invention may comprise an artificial interchain disulfide bond introduced between residues of its α and β chain constant domains. It should be noted that the introduced artificial disulfide bond as described above can be contained or not contained between the constant domains, and the TCR of the present invention may contain a TRAC constant domain sequence and a TRBC1 or TRBC2 constant domain sequence. The TRAC constant domain sequence and the TRBC1 or TRBC2 constant domain sequence of the TCR can be joined by a natural interchain disulfide bond present in the TCR.

Additionally, as for stability, it was also disclosed in a patent literature PCT/CN2016/077680 that the introduction of an artificial interchain disulfide bond between α chain variable region and β chain constant region of a TCR can significantly improve the stability of the TCR. Therefore, an artificial interchain disulfide bond may be contained between α chain variable region and β chain constant region of a high affinity TCR of the present invention. Specifically, cysteine residues forming an artificial interchain disulfide bond between α chain variable region and β chain constant region of the TCR is substituted for: an amino acid at position 46 of TRAV and amino acid at position 60 of TRBC1*01 or TRBC2*01 exon 1; an amino acid at position 47 of TRAV and amino acid at position 61 of TRBC1*01 or TRBC2*01 exon 1; amino acid at position 46 of TRAV and amino acid at position 61 of TRBC1*01 or TRBC2*01 exon 1; or an amino acid at position 47 of TRAV and amino acid at position 60 of TRBC1*01 or TRBC2*01 exon 1. Preferably, such a TCR may comprises (i) all or part of TCR α chain other than its transmembrane domain, and (ii) all or part of TCR β chain

other than its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of constant domains of the TCR chain, and the α chain and β chain form a heterodimer. More preferably, such TCR may comprise α chain variable domain and β chain variable domain and all or part of β chain constant domain other than the transmembrane domain, which, however, does not comprise α chain constant domain, and the α chain variable domain of the TCR and the β chain form a heterodimer.

For stability, in another aspect, the TCR of the present invention also includes a TCR having a mutation in its hydrophobic core region, and these mutations in hydrophobic core region are preferably mutations capable of increasing the stability of the TCR of the present invention, as described in WO 2014/206304. Such a TCR can have mutations at following positions in the variable domain hydrophobic core: (α and/or β chain) variable region amino acids at position 11, 13, 19, 21, 53, 76, 89, 91, 94, and / or α chain J gene (TRAJ) short peptide amino acid at reciprocal positions 3, 5, 7 and/or β chain J gene (TRBJ) short peptide amino acid at reciprocal positions 2, 4, 6, wherein the positions in amino acid sequence are numbered according to the position numbers listed in the International Immunogenetics Information System (IMGT). A skilled person in the art will know the above-described international immunogenetic information system and can obtain the position numbers of the amino acid residues of different TCRs in the IMGT based on the database.

More specifically, in the present invention, a TCR in which there is a mutation in the hydrophobic core region may be a high-stability single-chain TCR consisting of TCR α and β chain variable domains that linked by a flexible peptide chain. The CDR regions of TCR variable region determine its affinity for the short peptide-HLA complex, and mutations in hydrophobic core can increase the stability of the TCR, but won't affect its affinity for the short peptide-HLA complex. It should be noted that the flexible peptide chain in the present invention may be any peptide chain suitable for linking TCR α and β chain variable domains. The template chain constructed in Example 1 of the present invention for screening high-affinity TCRs is a high-stability single-chain TCR containing mutations in hydrophobic core as described above. The affinity between a TCR and FMNKFIYEI-HLA-A0201 complex can be easily evaluated by using a TCR with higher stability.

The CDR regions of α chain variable domain and β chain variable domain of the single chain template TCR are identical to the CDR regions of the wild type TCR. That is, the three CDRs of α chain variable domain are CDR1 α : TSINN, CDR2 α : IRSNERE,

and CDR3 α : ATDPRTGANSKLT and the three CDRs of β chain variable domains are CDR1 β : SEHNR, CDR2 β : FQNEAQ, and CDR3 β : ASEGLAYEQY, respectively. The amino acid sequence (SEQ ID NO: 47) and nucleotide sequence (SEQ ID NO: 48) of the single-chain template TCR are shown in Figures 7a and 7b, respectively, thereby screening a single-chain TCR consisting of α -chain variable domain and β -chain variable domain and having high affinity for FMNKFIYEI-HLA-A0201 complex.

In the present invention, the three CDRs of α chain variable domain of the single-chain template TCR (SEQ ID NO: 3), i.e., CDR1, CDR2 and CDR3 are located at positions 27-31, 49-55 and 90-102 of SEQ ID NO: 3, respectively. Accordingly, the amino acid residues are numbered according to the number as shown in SEQ ID NO: 3. 28S is S at the 2nd position of CDR1 α , 29I is I at the 3rd position of CDR1 α , 30N is N at the 4th position of CDR1 α , 31N is N at the 5th position of CDR1 α , 94R is R at the 5th position of CDR3 α , 95T is T at the 6th position of CDR3 α , 96G is G at the 7th position of CDR3 α , 97A is A at the 8th position of CDR3 α , 98N is N at the 9th position of CDR3 α , and 99S is S at the 10th position of CDR3 α .

Similarly, in the present invention, the three CDRs of β chain variable domain of the single-chain template TCR (SEQ ID NO: 4), i.e., CDR1, CDR2 and CDR3 are located at positions 27-31, 49-54 and 93-102 of SEQ ID NO: 2, respectively. Therefore, the amino acid residues are numbered according to the number as shown in SEQ ID NO: 4. 27S is S at the 1st position of CDR1 β , 28E is E at the 2nd position of CDR1 β , 29H is H at the 3rd position of CDR1 β , 30N is N at the 4th position of CDR1 β , 50Q is Q at the 2nd position of CDR2 β , 51N is N at the 3rd position of CDR2 β , 52E is E at the 4th position of CDR2 β , 53A is A at the 5th position of CDR2 β , and 54Q is Q at the 6th position of CDR2 β .

The $\alpha\beta$ heterodimer of the present invention having high affinity for FMNKFIYEI-HLA-A0201 complex was obtained by transferring the CDR regions of α and β chain variable domains of the selected high affinity single-chain TCR to the corresponding positions of α chain variable domain (SEQ ID NO: 1) and β chain variable domain (SEQ ID NO: 2) of a wild type TCR. Another part was obtained by artificial combination based on the mutation sites of the CDR regions obtained by screening.

The high affinity TCR of the present invention further comprises one of α chain variable domain amino acid sequences of SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35 and/or one of β chain variable domain amino acid sequences of SEQ ID NO: 36, 37, 38, 39, 40, 41, 42,

43, 44, 45 和 46. Therefore, the α chain variable domain (SEQ ID NO: 3) of the above described high-stability single-chain TCR as a template chain can be combined with TCR β chain variable domain, the amino acid sequence of which is SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46, to form the single-chain TCR molecule.

- 5 Alternatively, the β chain variable domain (SEQ ID NO: 4) of the above described high-stability single-chain TCR as a template chain can be combined with TCR α chain variable domain, the amino acid sequence of which is SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35, to form the single-chain TCR molecule. Alternatively, one of the TCR α chain variable domains
- 10 SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35 can be combined with one of the TCR β chain variable domains SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46 to form the single-chain TCR molecule. In the present invention, the amino acid sequences of α chain variable domain and β chain variable domain of the high-affinity single-chain
- 15 TCR molecule are preferably selected from the following Table 2:

Table 2

TCR No.	α chain variable domain sequence SEQ ID NO:	β chain variable domain sequence SEQ ID NO:
s-1	12	4
s-2	10	4
s-3	9	4
s-4	11	4
s-5	13	4
s-6	14	4
s-7	15	4
s-8	16	4
s-9	17	4
s-10	18	4
s-11	19	4
s-12	20	4
s-13	21	4
s-14	3	36
s-15	22	37
s-16	23	38
s-17	24	39
s-18	25	39
s-19	26	39
s-20	26	38
s-21	27	37
s-22	28	40
s-23	29	37
s-24	29	41

s-25	30	42
s-26	31	41
s-27	32	43
s-28	26	44
s-29	33	40
s-30	24	42
s-31	26	37
s-32	30	45
s-33	34	37
s-34	30	39
s-35	32	46
s-36	35	42

The TCR of the present invention can be provided in a form of multivalent complex. The multivalent TCR complex of the present invention comprises a polymer formed by combining two, three, four or more TCRs of the present invention, for example, a tetrameric domain of p53 can be used to produce a tetramer. Alternatively, more TCRs of the invention can be combined with another molecule to form a complex. The TCR complexes of the invention can be used to track or target cells that present a particular antigen *in vitro* or *in vivo*, or produce intermediates of other multivalent TCR complexes with such uses.

The TCR of the present invention may be used alone or combined with a conjugate in a covalent manner or other manner, preferably in a covalent manner. The conjugate includes a detectable label (for diagnostic purposes, wherein the TCR is used to detect the presence of a cell presenting FMNKFIYEI-HLA-A2 complex), a therapeutic agent, a PK (protein kinase) modifying moiety, or combination of any of the above described substances.

Detectable labels for diagnostic purposes include, but are not limited to, fluorescent or luminescent labels, radioactive labels, MRI (magnetic resonance imaging) or CT (electron computed tomography) contrast agents, or enzymes capable of producing detectable products.

Therapeutic agents that can be combined with or coupled to the TCRs of the invention include, but are not limited to: 1. Radionuclides (Koppe et al., 2005, Cancer metastasis reviews 24, 539); 2. Biotoxin (Chaudhary et al., 1989, Nature 339, 394; Epel et al., 2002, Cancer Immunology and Immunotherapy 51, 565); 3. Cytokines, such as IL-2, etc. (Gillies et al., 1992, National Academy of Sciences (PNAS) 89, 1428; Card et al., 2004, Cancer Immunology and Immunotherapy 53, 345; Halin et al., 2003, Cancer Research 63, 3202); 4. Antibody Fc fragment (Mosquera et al., 2005, The Journal Of Immunology 174, 4381); 5. Antibody scFv fragments (Zhu et al., 1995, International Journal of Cancer 62, 319); 6. Gold nanoparticles / Nanorods (Lapotko et al., 2005,

Cancer letters 239, 36; Huang et al., 2006, Journal of the American Chemical Society 128, 2115); 7. Viral particles (Peng et al., 2004, Gene therapy 11, 1234); 8. Liposomes (Mamot et al., 2005, Cancer research 65, 11631); 9. Nanomagnetic particles; 10. Prodrug activating enzymes (e.g., DT-diaphorase (DTD) or biphenyl hydrolase-like protein (BPHL); 11. chemotherapeutic agent (e.g., cisplatin) or any form of nanoparticles, and the like.

An antibody to which the TCR of the present invention binds or a fragment thereof includes an anti-T cell or an NK-cell determining antibody, such as an anti-CD3 or anti-CD28 or anti-CD16 antibody, and the above antibody or a fragment thereof binds to a TCR, thereby better directing effector cells to target cells. In a preferred embodiment, the TCR of the invention binds to an anti-CD3 antibody or a functional fragment or variant thereof. Specifically, a fusion molecule of the TCR of the present invention and an anti-CD3 single-chain antibody comprises a TCR α chain variable domain, the amino acid sequence of which is selected from the group consisting of SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 91, 92, and a TCR β chain variable domain, the amino acid sequence of which is selected from the group consisting of SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 95, 96, 97, 98, 99, 100, 101.

The invention also relates to a nucleic acid molecule encoding the TCR of the invention. The nucleic acid molecule of the invention may be in a form of DNA or RNA. DNA can be a coding strand or a non-coding strand. For example, a nucleic acid sequence encoding the TCR of the invention may be the same as the nucleic acid sequence set forth in the Figures of the invention or a degenerate variant thereof. By way of example, "degenerate variant", as used herein, refers to a nucleic acid sequence which encodes a protein with a sequence of SEQ ID NO: 47, but is differences from the sequence of SEQ ID NO: 48.

The full length sequence of the nucleic acid molecule of the present invention or a fragment thereof can generally be obtained by, but not limited to, PCR amplification, recombinant methods or synthetic methods. At present, it is possible to obtain a DNA sequence encoding the TCR (or a fragment thereof, or a derivative thereof) of the present invention completely by chemical synthesis. And then the DNA sequence can be introduced into various existing DNA molecules (or vectors) and cells known in the art.

The invention also relates to vectors comprising the nucleic acid molecules of the invention, as well as host cells genetically engineered using the vectors or coding sequences of the invention.

5 The invention also encompasses isolated cells, particularly T cells, which express the TCR of the invention. There are a number of methods suitable for T cell transfection with DNA or RNA encoding the high affinity TCR of the invention (e.g., Robbins et al., (2008) J. Immunol. 180: 6116-6131). T cells expressing the high affinity TCR of the invention can be used in adoptive immunotherapy. A skilled person in the art can know many suitable methods for performing adoptive therapy (e.g., Rosenberg et al., (2008)
10 Nat Rev Cancer 8(4): 299-308).

The invention also provides a pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a TCR of the invention, or a TCR complex of the invention, or cells presenting the TCR of the invention.

The invention also provides a method for treating a disease, comprising
15 administering to a subject in need thereof an appropriate amount of a TCR of the invention, or a TCR complex of the invention, or cells presenting a TCR of the invention, or a pharmaceutical composition of the invention.

It should be understood that the amino acid names herein are identified by internationally accepted single English letters, and the corresponding three-letter
20 abbreviated names of an amino acid are: Ala (A), Arg (R), Asn (N), Asp (D), Cys (C), Gln (Q), Glu (E), Gly (G), His (H), Ile (I), Leu (L), Lys (K), Met (M), Phe (F), Pro (P), Ser (S), Thr (T), Trp (W), Tyr (Y), Val (V).

In the present invention, both of Pro60 or 60P represent proline at position 60. Further, regarding the expression of the specific form of mutation in the present
25 invention, such as "T27G" represents that T at the 27th position is substituted by G. Similarly, "I29A/V" means that I at the 29th position is substituted by A or substituted by V, and so on.

In the art, when an amino acid with similar properties is used for substitution, the function of the protein is usually not altered. The addition of one or several amino acids
30 at C-terminus and/or N-terminus generally does not alter the structure and function of the protein. Therefore, the TCR of the invention further includes a TCR, wherein up to 5, preferably up to 3, more preferably up to 2, the most preferably 1 amino acid (especially an amino acid located outside CDR regions) of the TCR of the invention is replaced by an amino acid with similar properties and still be able to maintain its
35 function.

The present invention also includes a TCR obtained from the TCR of the present invention by slight modification. Form of modification (usually without altering the primary structure) includes: chemically derived forms of the TCR of the invention, such as acetylation or carboxylation. Modifications also include glycosylation, such as those TCRs produced by glycosylation modifications in the synthesis and processing or in further processing steps of the TCR of the invention. Such modification can be accomplished by exposing the TCR to an enzyme performing glycosylation (such as a mammalian glycosylation enzyme or a deglycosylation enzyme). Modification forms also include sequences having phosphorylated amino acid residues (such as phosphotyrosine, phosphoserine, phosphothreonine). Also included are TCRs that have been modified to enhance their antiproteolytic properties or optimize solubility properties.

The TCR, TCR complexes of the invention or T cells transfected by the TCRs of the invention can be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier. The TCR, multivalent TCR complex or cell of the invention is typically provided as part of a sterile pharmaceutical composition, which typically comprises a pharmaceutically acceptable carrier. The pharmaceutical composition can be of any suitable form (depending on the desired method for administration to a patient). It can be provided in a unit dosage form, usually in a sealed container, and can be provided as part of a kit. Such kit (but not necessary) includes instructions. It can include a plurality of said unit dosage form.

Furthermore, the TCR of the invention may be used alone or in combination with other therapeutic agents (e.g., formulated in the same pharmaceutical composition).

The pharmaceutical composition may also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for the administration of a therapeutic agent. The term refers to such pharmaceutical carriers which themselves do not induce the production of antibodies harmful to the individual receiving the composition and which are not excessively toxic after administration. These carriers are well known to a skilled person in the art. A full discussion of pharmaceutically acceptable excipients can be found in Remington's Pharmaceutical Sciences (Mack Pub. Co., N. J. 1991). Such carriers include, but are not limited to, saline, buffer, dextrose, water, glycerol, ethanol, adjuvants, and combinations thereof.

The pharmaceutically acceptable carrier in the therapeutic composition may

contain a liquid such as water, saline, glycerol and ethanol. In addition, auxiliary substances such as wetting or emulsifying agents, pH buffering substances and the like may also be present in these carriers.

5 In general, the therapeutic compositions can be formulated as injectables, such as liquid solutions or suspensions; and solid forms such as liquid carriers, which may be suitable for being formulated in solution or suspension prior to injection.

10 Once a composition of the invention is formulated, it can be administered by conventional routes including, but not limited to, intraocular, intramuscular, intravenous, subcutaneous, intradermal, or topical administration, preferably parenteral, including subcutaneous, intramuscular or intravenous administration. A subject to be prevented or treated may be an animal; especially a human.

15 When the pharmaceutical composition of the present invention is used for actual treatment, pharmaceutical compositions of various dosage forms may be employed depending on the uses, preferably, an injection, an oral preparation, or the like.

20 These pharmaceutical compositions can be formulated by mixing, diluting or dissolving according to conventional methods, occasionally, suitable pharmaceutical additives can be added such as excipients, disintegrating agents, binders, lubricants, diluents, buffers, isotonicity Isotonicities, preservatives, wetting agents, emulsifiers, dispersing agents, stabilizers and co-solvents, and the formulation process can be carried out in a customary manner depending on the dosage form.

25 The pharmaceutical composition of the present invention can also be administered in the form of a sustained release preparation. For example, the TCR of the present invention can be incorporated into a pill or microcapsule in which the sustained release polymer is used as a carrier, and then the pill or microcapsule is surgically implanted into the tissue to be treated. Examples of the sustained-release polymer include ethylene-vinyl acetate copolymer, 30 polyhydrometaacrylate, polyacrylamide, polyvinylpyrrolidone, methylcellulose, lactic acid polymer, lactic acid-glycolic acid copolymer or the like, preferably biodegradable polymer, such as lactic acid polymer and lactic acid-glycolic acid copolymer.

35 When the pharmaceutical composition of the present invention is used for actual treatment, the amount of the TCR or TCR complex of the present invention

or the cell presenting the TCR of the present invention as an active ingredient may be reasonably determined based on the body weight, age, sex, and degree of symptoms of each patient to be treated, and ultimately by a doctor.

5 **Main advantages of the invention:**

(1) The affinity and/or binding half-life of the TCR of the present invention for FMNKFIYEI-HLA-A2 complex is at least 2 times, preferably at least 10 times that of a wild type TCR.

10 (2) The affinity and/or binding half-life of the TCR of the present invention for FMNKFIYEI-HLA-A2 complex is at least 100 times, preferably at least 1000 times, and more preferably up to 10^4 - 10^7 times that of a wild type TCR.

(3) Effector cells transduced with the high-affinity TCR of the present invention exhibit a strong killing effect on target cells.

15 The invention is further illustrated by the following specific examples. It is to be understood that these examples are for illustrative purposes only and are not intended to limit the scope of the invention. The experimental methods in the following examples which do not specify the specific conditions are usually performed under conventional conditions, for example, conditions described in Sambrook and Russell et al., Molecular Cloning-A Laboratory Manual (Third Edition) (2001) CSHL Publishing company, or in
20 accordance with the conditions recommended by the manufacturer. Percentages and parts are by weight unless otherwise stated.

Materials and Method

25 The experimental materials used in the examples of the present invention can be commercially available, unless otherwise specified, among which *E. coli* DH5 α was purchased from Tiangen, *E. coli* BL21 (DE3) was purchased from Tiangen, *E. coli* Tuner (DE3) was purchased from Novagen, and plasmid pET28a was purchased from Novagen.

30

Example 1. Generation of stable single-chain TCR template chains with mutations in hydrophobic core

In the present invention, a method of site-directed mutagenesis was used according to a patent literature WO2014/206304 to construct a stable single-chain TCR molecule
35 consisting of TCR α and β -chain variable domain connected by a flexible short peptide, and

the amino acid and DNA sequences of which are SEQ ID NO: 47 and SEQ ID NO: 48, respectively, as shown in Figures 7a and 7b. The single-chain TCR molecule was used as a template for screening high-affinity TCR molecules. The amino acid sequences of α variable domain (SEQ ID NO: 3) and β variable domain (SEQ ID NO: 4) of the template chain are shown in Figures 2a and 2b; the corresponding DNA sequences are SEQ ID NO: 5 and 6, respectively, as shown in Figures 3a and 3b; and the amino acid sequence and DNA sequence of the flexible short linker are SEQ ID NOS: 7 and 8, respectively, as shown in Figures 4a and 4b.

The target gene carrying the template chain was digested with NcoI and NotI, and ligated with pET28a vector digested with NcoI and NotI. The ligation product was transformed into *E. coli* DH5 α , plated on a kanamycin-containing LB plate, inverted and cultured at 37°C overnight, and the positive clones were picked for PCR screening. Positive recombinants were sequenced to determine the correct sequence and the recombinant plasmid was extracted and transferred into *E. coli* BL21 (DE3) for expression.

Example 2. Expression, renaturation and purification of the stable single-chain TCR constructed in Example 1.

All of BL21(DE 3) colonies containing the recombinant plasmid pET28a-template chain prepared in Example 1 were inoculated into LB medium containing kanamycin, and cultured at 37°C until OD600 was 0.6-0.8. IPTG was added to a final concentration of 0.5 mM, and cultured at 37°C for another 4 hrs. The cell pellets were harvested by centrifugation at 5000 rpm for 15 mins, and the cell pellets were lysed with Bugbuster Master Mix (Merck). The inclusion bodies were recovered by centrifugation at 6000 rpm for 15 min, followed by washing with Bugbuster (Merck) to remove cell debris and membrane fraction. The inclusion bodies were collected by centrifugation at 6000 rpm for 15 min, and dissolved in a buffer (20 mM Tris-HCl pH 8.0, 8 M urea), and the insoluble matters were removed by high-speed centrifugation. The supernatant was quantitatively determined by BCA method, and then dispensed and stored at -80°C until use.

To 5 mg of dissolved single-chain TCR inclusion body protein, 2.5 mL of buffer (6 M Gua-HCl, 50 mM Tris-HCl pH 8.1, 100 mM NaCl, 10 mM EDTA) was added, then DTT was added to a final concentration of 10 mM, and incubated at 37°C for 30 min. The single-chain TCRs as treated above was added dropwise to a 125 mL of refolding buffer (100 mM Tris-HCl pH 8.1, 0.4 M L-arginine, 5 M urea,

2 mM EDTA, 6.5 mM β -mercapthoethylamine, 1.87 mM Cystamine) with a syringe, and stirred at 4°C for 10 min. Then the refolded solution was loaded into a cellulose membrane dialysis bag with a cut-off of 4 kDa, and the dialysis bag was placed in 1 L of pre-cooled water, and stirred slowly at 4°C overnight. After 17 hours, the dialysis liquid was changed to 1 L of pre-chilled buffer (20 mM Tris-HCl pH 8.0) and dialysis was continued for 8 h at 4°C. The dialysis liquid was then replaced with the same fresh buffer and dialysis was continued overnight. After 17 hours, the sample was filtered through a 0.45 μ m filter, vacuum degassed and purified through an anion exchange column (HiTrap Q HP, GE Healthcare) with a linear gradient elution of 0-1 M NaCl prepared with 20 mM Tris-HCl pH 8.0. The collected fractions were subjected to SDS-PAGE analysis, and the fractions containing single-chain TCRs were concentrated and further purified by a gel filtration column (Superdex 75 10/300, GE Healthcare), and the target components were also subjected to SDS-PAGE analysis.

The eluted fractions for BIAcore analysis was further tested for purity using gel filtration. The conditions were as follows: chromatographic column Agilent Bio SEC-3 (300 Å, ϕ 7.8×300 mm), mobile phase 150 mM phosphate buffer, flow rate 0.5 mL/min, column temperature 25°C, and UV detection wavelength 214 nm.

Example 3. Binding characterization

BIAcore analysis

The binding activity of the TCR molecule to FMNKFIYEI-HLA-A0201 complex was detected using BIAcore T200 real-time analysis system. The anti-streptavidin antibody (GenScript) was added to a coupling buffer (10 mM sodium acetate buffer, pH 4.77), and then the antibody was passed through a CM5 chip pre-activated with EDC and NHS to immobilize the antibody on the surface of the chip. The unreacted activated surface was finally blocked with a solution of ethanolamine in hydrochloric acid to complete the coupling process at a coupling level of about 15,000 RU under a conditions of 25°C, pH 7.1-7.5.

A low concentration of streptavidin flowed over the surface of the antibody-coated chip, then FMNKFIYEI-HLA-A0201 complex flowed through the detection channel with another channel being used as a reference channel. 0.05 mM biotin flowed over the chip for 2 min at a flow rate of 10 μ L/min, thereby blocking the remaining binding sites for streptavidin. The affinity was determined by single-cycle kinetic analysis. TCR was diluted to several different concentrations with HEPES-EP

buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% P20, pH 7.4), and flowed over the the surface of the chip in turn at a flow rate of 30 μ L/min, with a binding time of 120 s per injection. After the last injection, the chip was placed for dissociation for 600 s. At the end of each round of assay, the chip was regenerated with 10 mM Gly-HCl, pH 1.75. Kinetic parameters were calculated using BIAcore Evaluation software.

The preparation process for the above FMNKFIYEI-HLA-A0201 complex is described as follows:

a. Purification

100 ml of E. coli liquid induced to express heavy or light chain was collected, and centrifuged at 8000 g for 10 min at 4°C, and the cells were washed once with 10 ml of PBS, and then vigorously shaken in 5 ml of BugBuster Master Mix Extraction Reagents (Merck) for resuspending the cells. The suspension was incubated for 20 min at room temperature, and then centrifuged at 6000 g for 15 min at 4°C. The supernatant was discarded to collect inclusion bodies.

The above inclusion bodies was resuspended in 5 ml BugBuster Master Mix and incubated vortically at room temperature for 5 min. 30 ml of 10 time-diluted BugBuster was added, mixed, and centrifuged at 6000 g for 15 min at 4°C. The supernatant was discarded, 30 ml of 10 time-diluted BugBuster was added to resuspend the inclusion body, mixed, and centrifuged twice at 6000 g at 4°C for 15 min. 30 ml of 20 mM Tris-HCl pH 8.0 was added to resuspend the inclusion bodies, mixed, and centrifuged at 6000 g at 4°C for 15 min. Finally, inclusion bodies were dissolved in 20 mM Tris-HCl 8M urea, and the purity of inclusion bodies was determined by SDS-PAGE and the concentration was measured by BCA kit.

b. Refolding

Synthesized short peptide FMNKFIYEI (Beijing Saibaisheng Gene Technology Co., Ltd.) were dissolved in DMSO to a concentration of 20 mg/ml. Inclusion bodies of light and heavy chains were solubilized in 8 M urea, 20 mM Tris pH 8.0, 10 mM DTT, and further denatured by adding 3 M guanidine hydrochloride, 10 mM sodium acetate, 10 mM EDTA before refolding. FMNKFIYEI peptide was added to a refolding buffer (0.4 M L-arginine, 100 mM Tris pH 8.3, 2 mM EDTA, 0.5 mM oxidized glutathione, 5 mM reduced glutathione, 0.2 mM PMSF, cooled to 4°C) at 25 mg/L (final concentration). Then 20 mg/L of light chain and 90 mg/L of heavy chain (final concentration, heavy chain was added in three portions, 8 h/portion) were successively added, and refolded at 4°C for at least 3 days to completion of refolding, and SDS-PAGE was

used to confirm refolding.

c. Purification upon refolding

The refolding buffer was replaced with 10 volumes of 20 mM Tris pH 8.0 for dialysis, and the buffer was exchanged for at least two times to substantially
5 reduce the ionic strength of the solution. After dialysis, the protein solution was filtered through a 0.45 µm cellulose acetate filter and loaded onto a HiTrap Q HP (GE, General Electric Company) anion exchange column (5 ml bed volume). The protein was eluted with a linear gradient of 0-400 mM NaCl prepared in 20 mM
10 250 mM NaCl. Peak fractions were collected and the purity thereof was detected by SDS-PAGE.

d. Biotinylation

Purified pMHC molecules were concentrated in a Millipore ultrafiltration tube, while the buffer was replaced with 20 mM Tris pH 8.0, and then biotinylation
15 reagent 0.05 M Bicine pH 8.3, 10 mM ATP, 10 mM MgOAc, 50 µM D-Biotin, 100 µg/ml BirA enzyme (GST-BirA) was added. The resulting mixture was incubated at room temperature overnight, and SDS-PAGE was used to detect the completion of biotinylation.

e. Purification of biotinylated complex

20 The biotinylated and labeled pMHC molecules were concentrated to 1 ml in a Millipore ultrafiltration tube. The biotinylated pMHC was purified by gel filtration chromatography. 1 ml of concentrated biotinylated pMHC molecules was loaded on a HiPrepTM 16/60 S200 HR column (GE) pre-equilibrated with filtered PBS using an Akta Purifier (GE) and eluted with PBS at a flow rate of 1 ml/min. The
25 biotinylated pMHC molecules were eluted as a single peak at about 55 ml. The protein-containing fractions were combined and concentrated in a Millipore ultrafiltration tube. The concentration of protein was determined by BCA method (Thermo), protease inhibitor cocktail (Roche) was added and the biotinylated pMHC molecules were dispensed and stored at -80°C.

30

Example 4. Generation of high-affinity single-chain TCR

Phage display technology is a means to generate high affinity TCR variant libraries for screening high affinity variants. The TCR phage display and screening method described by Li et al. ((2005) Nature Biotech 23(3): 349-354) was applied to the
35 single-chain TCR template of Example 1. A library of high affinity TCRs was

established by mutating CDR regions of the template chain and panned. After several rounds of panning, the phage library can specifically bind to the corresponding antigen, the monoclonal was picked and sequence analysis was performed.

BIAcore method of Example 3 was used to analyze the interaction between a TCR molecule and FMNKFIYEI-HLA-A0201 complex, and a high affinity TCR with affinity and/or binding half-life of at least 2 times that of the wild-type TCR was screened out, that is, the dissociation equilibrium constant K_D of the screened high affinity TCR for binding FMNKFIYEI-HLA-A0201 complex is less than or equal to one-half of the dissociation equilibrium constant K_D of the wild type TCR for binding FMNKFIYEI-HLA-A0201 complex, and the results are shown in Table 3 below. K_D value of the interaction between the soluble reference TCR and FMNKFIYEI-HLA-A0201 complex was detected to be 43 μ M by using the above method, and the interaction curve is shown in Fig. 12, that is, K_D value of the wild type TCR interacting with FMNKFIYEI-HLA-A0201 complex is also 43 μ M (4.3E-05M).

Specifically, when using the numbering shown in SEQ ID NO: 1, an amino acid at one or more of the following sites in the α chain variable domain of these high-affinity TCR mutants mutated: 28S, 29I, 30N, 31N, 94R, 95T, 96G, 97A, 98N and 99S and/or when using the numbering shown in SEQ ID NO: 2, an amino acid at one or more of the following sites in the β chain variable domain of these high-affinity TCR mutants mutated: 27S, 28E, 29H, 30N, 50Q, 51N, 52E, 53A and 54Q.

More specifically, when using the numbering shown in SEQ ID NO: 1, the α chain variable domains of these high-affinity TCRs comprise one or more amino acid residues selected from the group consisting of 28D or 28P or 28A or 28E, 29V, 30S or 30Y or 30D, 31S or 31K, 94A or 94S or 94V or 94D or 94E or 94Q or 94T, 95M or 95R or 95L or 95A or 95N or 95S, 96A or 96Q or 96S or 96E or 96N, 97S or 97G or 97P or 97W, 98S or 98T, 99A or 99N or 99T or 99P; and/or when using the numbering shown in SEQ ID NO: 2, the β chain variable domains of these high-affinity TCRs comprise one or more amino acid residues selected from the group consisting of 27A or 27Q or 27G, 28G or 28T or 28R or 28L, 29W or 29R, 30A or 30P, 50H, 51G, 52D, 53M or 53L, 54E.

The specific amino acid sequences of α chain variable domains (SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35) and β chain variable domains (SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46) of the high-affinity single-chain TCRs are shown in Figures 5(1)-(27) and Figures 6(1)-(11), respectively.

Table 3

Single chain TCR No.	Single chain TCR variable domain (SEQ ID NO.)		K _D (M)
	α	β	
s-1	12	4	2.53E-08
s-2	10	4	2.11E-05
s-3	9	4	8.87E-08
s-4	11	4	2.04E-06
s-5	13	4	5.46E-06
s-6	14	4	1.90E-07
s-7	15	4	1.01E-06
s-8	16	4	4.11E-08
s-9	17	4	2.57E-07
s-10	18	4	1.56E-06
s-11	19	4	2.96E-08
s-12	20	4	8.60E-09
s-13	21	4	5.72E-08
s-14	3	36	2.26E-06
s-15	22	37	2.01E-09
s-16	23	38	3.14E-09
s-17	24	39	3.88E-09
s-18	25	39	2.82E-09
s-19	26	39	2.35E-09
s-20	26	38	1.91E-09
s-21	27	37	1.75E-09
s-22	28	40	1.44E-09
s-23	29	37	1.59E-09
s-24	29	41	3.65E-09
s-25	30	42	2.43E-09
s-26	31	41	2.72E-09
s-27	32	43	2.33E-09
s-28	26	44	1.95E-09
s-29	33	40	2.07E-09
s-30	24	42	3.46E-09
s-31	26	37	1.91E-09
s-32	30	45	3.51E-09
s-33	34	37	2.47E-09
s-34	30	39	2.40E-09
s-35	32	46	2.12E-09
s-36	35	42	3.40E-09

Example 5. Generation of high-affinity $\alpha\beta$ heterodimeric TCR

The mutations in CDR regions of the high-affinity single-chain TCRs screened in Example 4 were introduced into the corresponding sites of the variable domain of the $\alpha\beta$ heterodimeric TCR, and its affinity for FMNKFIYEI-HLA-A0201 complex was detected by BIAcore. The mutated sites of high-affinity can be introduced in the above CDR regions by a method of site-directed mutagenesis well known to a skilled person in the art. The amino acid sequences of α chain and β chain variable domain of the above wild type TCR are shown in Figures 1a (SEQ ID NO: 1) and 1b (SEQ ID NO: 2), respectively.

It should be noted that in order to obtain a more stable soluble TCR for easier evaluation of the binding affinity and/or binding half-life between the TCR and FMNKFIYEI-HLA A0201 complex, the $\alpha\beta$ heterodimeric TCR may be such a TCR in which a cysteine residue is respectively introduced into α and β chain constant domain to form an artificial interchain disulfide bond. In this example, the amino acid sequences of TCR α and β chains after introducing a cysteine residue are shown in Figure 8a (SEQ ID NO: 49) and 8b (SEQ ID NO: 50), and the introduced cysteine residues are indicated by bold letters.

According to standard methods described in Molecular Cloning a Laboratory Manual (3rd edition, Sambrook and Russell), genes of extracellular sequences of the TCR α and β chains to be expressed are synthesized and inserted into an expression vector pET28a+ (Novagene), in which the upstream and downstream cloning sites are NcoI and NotI, respectively. Mutations in the CDR regions are introduced by overlap PCR well known to a skilled person in the art. The inserted fragment was sequenced to confirm that it was correct.

Example 6. Expression, refolding and purification of $\alpha\beta$ heterodimeric TCR

Expression vectors for TCR α and β chain were transformed into the expression bacteria BL21 (DE3) by chemical transformation, respectively. The bacteria were grown in LB medium and induced with a final concentration of 0.5 mM IPTG at $OD_{600} = 0.6$. The inclusion bodies formed after the TCR α and β chains were expressed were extracted by BugBuster Mix (Novagene) and repeatedly washed with BugBuster solution. The inclusion bodies were finally dissolved in 6 M guanidine hydrochloride, 10 mM dithiothreitol (DTT), 10 mM ethylenediaminetetraacetic acid (EDTA) and 20 mM Tris (pH 8.1).

The dissolved TCR α and β chains were rapidly mixed in 5 M urea, 0.4 M arginine, 20 mM Tris (pH 8.1), 3.7 mM cystamine, and 6.6 mM β -mercapoethylamine (4°C) at a mass ratio of 1:1. The final concentration is 60 mg/mL. After mixing, the solution was dialyzed against 10 volumes of deionized water (4°C), and after 12 hours, deionized water was exchanged with a buffer (20 mM Tris, pH 8.0) and dialysis was continued at 4°C for 12 hours. After completion of the dialysis, the solution was filtered through a 0.45 μ m filter and purified through an anion exchange column (HiTrap Q HP, 5 ml, GE Healthcare). The elution peak of TCR containing successfully refolded α and β dimers was confirmed by SDS-PAGE gel. The TCR was then further purified by gel filtration chromatography (HiPrep 16/60, Sephacryl S-100 HR, GE Healthcare). The

purity of the purified TCR was determined by SDS-PAGE to be greater than 90%, and the concentration thereof was determined by BCA method.

Example 7. Results of BIAcore analysis

5 The affinity of the $\alpha\beta$ heterodimeric TCR, in which a high affinity CDR region was introduced, for FMNKFIYEI-HLA-A0201 complex was detected by using the method described in Example 3.

The CDR regions selected from the high-affinity single-chain TCR α and β chain were transferred into the corresponding positions of the wild-type TCR α chain variable domain SEQ ID NO: 1 and β chain variable domain SEQ ID NO: 2, respectively, to form an $\alpha\beta$ heterodimeric TCR. Additionally, $\alpha\beta$ heterodimeric TCRs were formed through artificial combination based on the mutation sites screened and obtained in the CDR regions. The amino acid sequences of resulting new TCR α and β chain variable domains are shown in Figures 9(1)-(29) and Figures 10(1)-(20), respectively. Since the CDR regions of a TCR molecule determine their affinity for the corresponding pMHC complex, a skilled person in the art can anticipate that an $\alpha\beta$ heterodimeric TCR, in which a high affinity mutation site is introduced also has a high affinity for FMNKFIYEI-HLA-A0201 complex. The expression vector was constructed by the method described in Example 5, the above-mentioned $\alpha\beta$ heterodimeric TCR with a high-affinity mutation being introduced was expressed, refolded and purified by the method described in Example 6, and then the affinity of the TCR for FMNKFIYEI-HLA-A0201 complex is determined by BIAcore T200, as shown in Table 4 below.

Table 4

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:	K _D (M)
4	54	2	6.3E-07
2	52	2	1.6E-06
1	51	2	6.2E-07
3	53	2	3.5E-07
5	55	2	1.4E-06
6	56	2	9.04E-07
7	57	2	4.65E-07
8	58	2	1.41E-06
9	59	2	2.34E-07
10	60	2	3.99E-07
11	61	2	7.87E-07
12	62	2	5.39E-07
13	63	2	9.99E-07
14	1	78	2.96E-06

15	64	79	1.66E-10
16	65	80	1.61E-10
17	66	81	1.67E-10
18	67	81	1.18E-10
19	68	81	1.70E-10
20	68	80	1.38E-10
21	69	79	8.22E-11
22	70	82	8.67E-11
23	71	79	7.26E-11
24	71	83	7.89E-11
25	72	84	7.46E-11
26	73	83	8.98E-11
27	74	85	1.50E-10
28	68	86	7.67E-11
29	75	82	8.54E-11
30	66	84	9.74E-11
31	68	79	7.45E-11
32	72	87	4.99E-11
33	76	79	3.42E-11
34	72	81	4.47E-11
35	74	88	1.31E-09
36	77	84	1.68E-10
37	1	93	1.90E-05
38	1	94	5.50E-06
39	1	95	3.30E-07
40	1	96	1.10E-05
41	1	97	1.40E-05
42	1	98	1.20E-05
43	1	99	1.6E-06
44	1	100	2.3E-06
45	91	101	6.20E-11
46	92	101	1.70E-10

As can be seen from Table 4 above, the $\alpha\beta$ heterodimeric TCR with mutation sites introduced into CDR regions maintains high affinity for FMNKFIYEI-HLA-A0201 complex. The affinity of the heterodimeric TCR for FMNKFIYEI-HLA-A0201 complex is at least 2 times of that of the wild-type TCR.

5

Example 8. Expression, refolding and purification of fusions of anti-CD3 antibodies with high-affinity single-chain TCR

The high-affinity single-chain TCR molecule of the present invention is fused with a single-chain molecule (scFv) of an anti-CD3 antibody to construct a fusion molecule.

10 Primers were designed by overlapping PCR, and the genes of anti-CD3 antibodies and high-affinity single-chain TCR molecule were ligated. The intermediate linker was designed as GGGGS, and the gene fragment of the fusion molecule had restriction enzyme sites NcoI and NotI. The PCR amplification product was digested with NcoI and NotI and ligated with pET28a vector digested with NcoI and NotI. The ligation
15 product was transformed into *E. coli* DH5 α competent cells, and plated on LB plate

containing kanamycin and inverted and cultured overnight at 37°C. Positive clones were picked for PCR screening, and the positive recombinants were sequenced to determine the correct sequence. The recombinant plasmids were extracted and transformed into *E. coli* BL21 (DE3) competent cells for expression.

5 Expression of fusion protein

The expression plasmid containing the gene of interest was transformed into *E. coli* strain BL21 (DE3), plated on a LB plate (kanamycin, 50 µg/ml) and cultured at 37°C overnight. On the next day, the clones were picked and inoculated into 10 ml of LB liquid medium (kanamycin, 50 µg/ml), cultured for 2-3 h, then inoculated to 1 L of LB medium (kanamycin, 50 µg/ml) at a volume ratio of 1:100, cultured until the OD₆₀₀ was 0.5-0.8, and then induced to express the protein of interest using IPTG at a final concentration of 0.5 mM. 4 hours after induction, the cells were harvested by centrifugation at 6000 rpm for 10 min. The cells were washed once in PBS buffer, and dispensed. Cells corresponding to 200 ml of the bacterial culture were taken and lysed with 5 ml of BugBuster Master Mix (Novagen), and the inclusion bodies were collected by centrifugation at 6000 g for 15 minutes. The inclusion bodies were washed for 4 times with detergent to remove cell debris and membrane components. The inclusion bodies are then washed with a buffer such as PBS to remove detergent and salt. Finally, the inclusion bodies were dissolved in Tris buffer solution containing 8 M urea, the concentration of inclusion bodies was measured, and inclusion bodies were dispensed and cryopreserved at -80°C.

Refolding of fusion proteins

About 10 mg of inclusion bodies were taken from a -80°C ultra-low temperature freezer and thawed, and dithiothreitol (DTT) was added to a final concentration of 10 mM, and incubated at 37°C for 30 min to 1 hour to ensure complete opening of the disulfide bond. The solution of inclusion body sample was then added dropwise into 200 ml of 4°C pre-cooled refolding buffer (100 mM Tris pH 8.1, 400 mM L-arginine, 2 mM EDTA, 5 M urea, 6.5 mM β-mercapthoethylamine, 1.87 mM Cystamine), respectively, and stirred slowly at 4°C for about 30 minutes. The refolding solution was dialyzed against 8 volumes of pre-cooled H₂O for 16-20 hours. It was further dialyzed twice with 8 volumes of 10 mM Tris pH 8.0, and dialysis was continued at 4°C for about 8 hours. After dialysis, the sample was filtered and subjected to the following purification process.

35 First step purification of fusion protein

The dialyzed and refolded material (in 10 mM Tris pH 8.0) was eluted on a POROS HQ/20 anion exchange chromatography preppacked column (Applied Biosystems) with a gradient of 0-600 mM NaCl using AKTA Purifier (GE Healthcare). Each component was analyzed by Coomassie brilliant blue stained SDS-PAGE and then combined.

Second step purification of the fusion protein

The purified and combined sample solution from the first step was concentrated for purification in this step, and the fusion protein was purified by Superdex 75 10/300 GL gel filtration chromatography preppacked column (GE Healthcare) pre-equilibrated in PBS buffer. The components of the peaks were analyzed by Coomassie Brilliant Blue-stained SDS-PAGE and then combined.

Example 9. Expression, refolding and purification of fusions of anti-CD3 antibody with high-affinity $\alpha\beta$ heterodimeric TCR

A fusion molecule was prepared by fusing an anti-CD3 single-chain antibody (scFv) with an $\alpha\beta$ heterodimeric TCR. The anti-CD3 scFv was fused with β chain of the TCR, and the TCR β chain may comprise β chain variable domain of any of the above high-affinity $\alpha\beta$ heterodimeric TCRs, and the TCR α chain of the fusion molecule may comprise α chain variable domain of any of the above high-affinity $\alpha\beta$ heterodimeric TCR.

Construction of expression vector for fusion molecule

1. Construction of expression vector for α chain

The target gene carrying α chain of the $\alpha\beta$ heterodimeric TCR was digested with NcoI and NotI, and ligated with pET28a vector digested with NcoI and NotI. The ligation product was transformed into E. coli DH5 α , plated on a LB plate containing kanamycin, and inverted and cultured overnight at 37°C. Positive clones were picked for PCR screening, and the positive recombinants were sequenced to determine the correct sequence. The recombinant plasmids were extracted and transformed into E. coli Tuner (DE3) for expression.

2. Construction of expression vector for anti-CD3 (scFv)- β chain

Primers were designed by overlapping PCR to connect genes of the anti-CD3 scFv and high-affinity heterodimeric TCR β chain.

The anti-CD3 scFv can be connected to the N-terminal or C terminal of the TCR β chain. The intermediate linker was GGGGS, and the gene fragment of the fusion protein of anti-CD3 scFv and the high-affinity heterodimeric TCR β chain had the

restriction endonuclease sites NcoI (CCATGG) and NotI (GCGGCCGC). The PCR amplification product was digested with NcoI and NotI and ligated with pET28a vector digested with NcoI and NotI. The ligation product was transformed into *E. coli* DH5 α competent cells, plated on a kanamycin-containing LB plate, and inverted and cultured overnight at 37°C. Positive clones were picked for PCR screening, and the positive recombinants were sequenced to determine the correct sequence. The recombinant plasmids were extracted and transformed into *E. coli* Tuner (DE3) competent cells for expression.

Expression, refolding and purification of fusion protein

The expression plasmids were separately transformed into *E. coli* Tuner (DE3) competent cells, plated on LB plates (kanamycin 50 μ g/mL) and cultured overnight at 37°C. On the next day, clones were picked and inoculated into 10 mL LB liquid medium (kanamycin 50 μ g/mL) for 2-3 h, and inoculated into 1 L LB medium at a volume ratio of 1: 100, the culture was continued until the OD600 was 0.5- 0.8, and a final concentration of 1 mM IPTG was added to induce expression of the protein of interest. After 4 hours, cells were harvested by centrifugation at 6000 rpm for 10 mins. The cells were washed once in PBS buffer and were dispensed, and cells corresponding to 200 mL of the bacterial culture were taken and lysed with 5 mL of BugBuster Master Mix (Merck), inclusion bodies were collected by centrifugation at 6000 g for 15 min and then washed with detergent for 4 times to remove cell debris and membrane components. The inclusion bodies were then washed with a buffer such as PBS to remove detergent and salt. Finally, the inclusion bodies were dissolved in 6M guanidine hydrochloride, 10 mM dithiothreitol (DTT), 10 mM ethylenediaminetetraacetic acid (EDTA), 20 mM Tris, pH 8.1 buffer solution, and the concentration of inclusion bodies was determined. The inclusion bodies were dispensed and cryopreserved at -80°C.

The dissolved TCR α chain and anti-CD3 (scFv)- β chain were rapidly mixed in a mass ratio of 2: 5 in 5 M urea (urea), 0.4 M L-arginine (L-arginine), 20 mM Tris pH 8.1, 3.7 mM cystamine, and 6.6 mM β -mercapoethylamine (4°C), and the final concentrations of α chain and anti-CD3 (scFv)- β chain were 0.1 mg/mL, 0.25 mg/mL, respectively.

After mixing, the solution was dialyzed against 10 volumes of deionized water (4°C), and after 12 hours, deionized water was exchanged with buffer (10 mM Tris, pH 8.0) for another 12 hours at 4°C. After completion of dialysis, the solution was filtered through a 0.45 μ m filter and purified by an anion exchange column

(HiTrap Q HP 5 ml, GE healthcare). The eluted peaks containing the reconstituted TCR α chain and anti-CD3 (scFv)- β chain dimer TCR were confirmed by SDS-PAGE gel. The TCR fusion molecule was then purified by size exclusion chromatography (S-100 16/60, GE healthcare) and further purified by an anion exchange column (HiTrap Q HP 5 ml, GE healthcare). The purity of the purified TCR fusion molecule was determined by SDS-PAGE to be greater than 90%, and the concentration was determined by BCA method.

Example 10. Activation function assay of effector cells transfected with high affinity TCR of the present invention

This example demonstrates that effector cells transfected with the high affinity TCR of the present invention have a good specific activation effect on target cells.

The function and specificity of the high affinity TCR of the present invention in cells were examined by ELISPOT assay. Methods for detecting cellular function using ELISPOT assays are well known to a skilled person in the art. In the IFN- γ ELISPOT assay of this example, CD8⁺ T cells isolated from the blood of healthy volunteers and transfected with the high affinity TCR of the present invention using lentivirus were used as effector cells,

The TCRs of the present invention were randomly selected, and divided into two groups for the experiment. The TCRs and numbers thereof are listed in Table 4:

TCR3 (α chain variable domain SEQ ID NO: 51, β chain variable domain SEQ ID NO: 2), TCR2 (α chain variable domain SEQ ID NO: 52, β chain variable domain SEQ ID NO: 2), TCR4 (α chain variable domain SEQ ID NO: 53, β chain variable domain SEQ ID NO: 2) and TCR1 (α chain variable domain SEQ ID NO: 54, β chain variable domain SEQ ID NO: 2), were the first group; and effector cells in the control group were labeled as WT-TCR (transfected with wild-type TCR) and A6 (transfected with other TCR, which is not of the present invention). The target cell lines were HepG2, Huh-7, Huh-1, HCCC9810 and SK-HEP-1 cells, among which, the target cell line HepG2 expresses relevant antigens and its genotype is also consistent with positive cell lines. Huh-7, Huh-1, HCCC9810 and SK-HEP-1 are negative cell lines and used as controls.

TCR7 (α chain variable domain SEQ ID NO: 57, β chain variable domain SEQ ID NO: 2), TCR11 (α chain variable domain SEQ ID NO: 61, β chain variable domain SEQ ID NO: 2) and TCR13 (α chain variable domain SEQ ID NO: 63, β chain variable domain SEQ ID NO: 2), were the second group. Effector cells in the control group were labeled as WT-TCR (transfected with wild-type TCR) and A6 (transfected with other

TCR, which is not of the present invention). The target cell lines were HCCC9810-AFP, PLCPRF5-A2, SNU398 and HCCC9810, among which, the target cell lines HCCC9810-AFP and PLCPRF5-A2 expresses relevant antigens and its genotype is also consistent with positive cell lines. SNU398 and HCCC9810 are negative cell lines and used as controls.

Firstly, a ELISPOT plate was prepared. The ELISPOT plate was activated and coated with ethanol overnight at 4°C. On the first day of the experiment, the coating solution was removed, and the plate was washed, blocked and incubated at room temperature for 2 hrs, and the blocking solution was removed. Components of the assay were added to the ELISPOT plate in the following order: the medium for adjusting effector cells to 1×10^4 cells/ml, and the medium for adjusting each target cell line to 2×10^5 cells/ml. After homogeneously mixing, 100 μ L of target cell line (i.e., 20,000 cells/well) and 100 μ L of effector cells (i.e., 1,000 cells/well) were added to the corresponding wells in duplicate, and incubate overnight (37°C, 5% CO₂). On the second day of the experiment, the plate was washed, subjected to a secondary detection and development, and dried, and the spots formed on the film were counted using an immunospot plate reader (ELISPOT READER system; AID20 company).

Experimental results from the first group and the second group are shown in Figure 13a and Figure 13b, in which the effector cells transfected with the high affinity TCR of the present invention exhibit no activation effects on negative target cells, while exhibit excellent specific activation effects on positive target cells, which is significantly superior to those of the effector cells transfected with WT TCR.

Example 11. Killing function assay of effector cells transfected with high affinity TCR of the present invention

In this example, the release of LDH was determined by non-radioactive cytotoxicity assay to verify the killing function of cells transducing the TCR of the present invention. The assay is a colorimetric substitution assay for 51Cr release cytotoxicity assay to quantify lactate dehydrogenase (LDH) released after cell lysis. LDH released in the medium was detected using a 30 minute-coupled enzyme reaction, in which LDH converts tetrazolium salt (INT) into red formazan. The amount of produced red product is directly proportional to the number of lysed cells. Absorbance data of visible light at 490 nm can be collected using a standard 96-well plate reader.

Methods for detecting cellular function using LDH release assay are well known to a skilled person in the art. In the LDH experiment of this example, PBL cells isolated

from the blood of healthy volunteers transfected with TCR by lentivirus were used as effector cells. The target cell lines were HepG2, HCCC9810 and SNU-398A375 (3525), among which, HepG2 was positive target cell line; and HCCC9810 and SNU-398 were negative target cell lines as a control.

5 The TCRs of the present invention were randomly selected, and divided into two groups for the experiment. The TCRs and numbers thereof are listed in Table 4: TCR3 (α chain variable domain SEQ ID NO: 51, β chain variable domain SEQ ID NO: 2), TCR2 (α chain variable domain SEQ ID NO: 52, β chain variable domain SEQ ID NO: 2) and TCR1 (α chain variable domain SEQ ID NO: 54, β chain variable domain SEQ ID NO: 2), were the first group; and TCR7 (α chain variable domain SEQ ID NO: 57, β chain variable domain SEQ ID NO: 2) and TCR13 (α chain variable domain SEQ ID NO: 63, β chain variable domain SEQ ID NO: 2), were the second group. Effector cells in the control group were labeled as WT-TCR (transfected with wild-type TCR) and A6 (transfected with other TCR, which is not of the present invention).

15 Firstly, a LDH plate was prepared. On the first day of the experiment, components of the assay were added to the plate in the following order: the medium for adjusting effect cells to 3×10^5 cells/ml, and the medium for adjusting each target cell line to 3×10^5 cells/ml. After homogeneously mixing, 100 μ L of target cell line (i.e., 30,000 cells/well) and 100 μ L of effector cells (i.e., 30,000 cells/well) were added to the corresponding wells in triplicate. Wells for spontaneous effector cells, for spontaneous target cells, for maximum target cells, for volume-corrected control and for medium background control were simultaneously set, each containing 200 μ L of liquid. The plate was incubated overnight (37°C, 5% CO₂). On the second day of the experiment, color development was detected, and after the reaction was terminated, the absorbance at 490 nm was recorded using a microplate reader (Bioteck).

20 Experimental results from the first group and the second group are shown in Figure 14a and Figure 14b, in which the effector cells transfected with the high affinity TCR of the present invention exhibit strong killing effects on target cells expressing relevant antigens, while substantially exhibit no killing effects on target cells not expressing relevant antigens; and the effector cells transfected with other TCR exhibit no killing effects on target cells expressing relevant antigens.

Example 12. Functional verification-activation assay of fusion protein of high affinity TCR of the present invention and anti-CD3 antibody

35 This example demonstrates that the fusion protein of the high affinity TCR of the

present invention and anti-CD3 antibody is capable of redirecting effector cells and exhibits good activation effects.

Methods for detecting cellular function using ELISPOT assays are well known to a skilled person in the art. The effector cells used in IFN- γ ELISPOT assay of this example were CD8⁺ T cells isolated from the blood of healthy volunteers. The target cell lines were HEPG2, HCCC9810 and SNU-398, wherein HEPG2 was positive target cell line, and HCCC9810 and SNU-398 were negative target cell lines. The high affinity TCR of the present invention was randomly selected and the fusion protein was prepared as described in Example 9. Specifically, the selected TCRs and numbers thereof are listed in Table 4, which are TCR21 (α chain variable domain SEQ ID NO: 69, β chain variable domain SEQ ID NO: 79), TCR24 (α chain variable domain SEQ ID NO: 71, β chain variable domain SEQ ID NO: 83), TCR25 (α chain variable domain SEQ ID NO: 72, β chain variable domain SEQ ID NO: 84), TCR17 (α chain variable domain SEQ ID NO: 66, β chain variable domain SEQ ID NO: 81), TCR23 (α chain variable domain SEQ ID NO: 71, β chain variable domain SEQ ID NO: 79), TCR33 (α chain variable domain SEQ ID NO: 76, β chain variable domain SEQ ID NO: 79) and TCR34 (α chain variable domain SEQ ID NO: 72, β chain variable domain SEQ ID NO: 81), respectively.

Firstly, a ELISPOT plate was prepared. ELISPOT plate was activated and coated with ethanol, and incubated overnight at 4°C. On the first day of the experiment, the coating solution was removed, and the plate was washed, blocked and incubated at room temperature for two hours, and the blocking solution was removed. Components of the assay were added to the ELISPOT plate. Target cells were added at 20000 cells/well, effector cells were added at 4000 cells/well, and the concentration of the fusion protein was 0.04 μ M (serially diluted by 10 times, for a total of 6 concentration gradients). Duplicate wells were set. The plate was incubated overnight (37°C, 5% CO₂). On the second day of the experiment, the plate was washed and subjected to secondary detection and color development. The plate was dried, and the spots formed on the film were counted using an immunospot plate reader (ELISPOT READER system; AID20 company).

Experiment results are shown in Figures 15a, 15b and 15c, 15d, 15e, 15f and 15g. The results show that the fusion protein of the high affinity TCR of the present invention and anti-CD3 antibody is capable of redirecting effector cells, the effector cells exhibits strong activation effects on the positive target cells, while substantially exhibits no activation effects on the negative target cells, so that the effector cells

exhibits good specificity.

Example 13. Functional verification-killing assay of fusion protein of high affinity TCR of the present invention and anti-CD3 antibody

5 In this example, the killing function was verified by non-radioactive cytotoxicity assay. The assay is a colorimetric substitution assay for ⁵¹Cr release cytotoxicity assay to quantify lactate dehydrogenase (LDH) released after cell lysis. LDH released in the medium was detected using a 30 minute-coupled enzyme reaction, in which LDH converts tetrazolium salt (INT) into red formazan. The amount of produced red product
10 is directly proportional to the number of lysed cells. Absorbance data of visible light at 490 nm can be collected using a standard 96-well plate reader.

 The target cell lines were HepG2, HCCC9810 and SNU-398, among which, HepG2 was positive target cell line; and HCCC9810 and SNU-398 were negative target cell lines. Effector cells were CD8⁺ T cells. The high-affinity TCRs of the present
15 invention were randomly selected. The TCRs and numbers thereof can be known from Table 4, which are TCR23 (α chain variable domain SEQ ID NO: 71, β chain variable domain SEQ ID NO: 79). The fusion protein was prepared according to Example 9, and diluted in the medium for the experiment to different concentrations (10^{-13} - 10^{-8} M). Components of the assay were added to the ELISPOT plate in the following order: 50 μ l
20 of target cells, 5×10^4 cells/well; 100 μ l of effector cells, 5×10^4 cells/well; and 50 μ l of the fusion protein into each well.

 Several controls were prepared as follows: spontaneous release of target cells: only 200 μ l of target cells; maximum release of target cells: only 200 μ l of target cells. Medium control group: only 200 μ l of medium; volume correction control group: 200 μ l
25 of medium and 20 μ l of cell lysate. All wells were prepared in triplicate with a final volume of 200 μ l. The plate was centrifuged at 250 x g for 4 minutes and then incubated at 37°C for 20 hours. 20 μ l of lysate liquid was added to the control wells of maximum release of target cells and volume correction control wells, and the supernatant was collected after 45 minutes. The plate was centrifuged at 250 x g for 4 minutes. 50 μ l of
30 the supernatant was transferred from each well of the test plate to the corresponding well of a flat bottom 96-well Nunc Vlnxisorb plate. The substrate mixture was reconstituted with test buffer (12 ml). Afterwards, 50 μ l of the reconstituted substrate mixture was added to each well of the plate. The plate was covered with an aluminum foil and incubated at room temperature for 30 minutes. 50 μ l of quenching solution was
35 added to each well of the plate to quench the reaction. The absorbance was recorded at

490nm with an Elisa plate reader within 1 hour after the quenching solution was added.

The experimental results are shown in FIG. 16. The fusion protein of the high-affinity TCR of the present invention and anti-CD3 antibody can redirect effector cells and kill positive target cells, but have no killing effects on negative target cells,
5 thereby exhibiting good specificities.

All documents mentioned in the present application are hereby incorporated by reference in their entireties, as if each is incorporated by reference. In addition, it should be understood that after reading the teachings of the present invention described above,
10 a skilled person in the art can make various changes or modifications of the invention, and these equivalent forms also fall into the scope as defined by the appended claims of the present application.

Sequence listing

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Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Glu

85 90 95

Ser Thr Thr Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 19

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 19

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Gln

85 90 95

Ser Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 20

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 20

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Gln Asn Ser

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 21

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 21

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50

55

60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65

70

75

80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ser Asn

85

90

95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 22

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 22

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ala Gln

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 23

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 23

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ala Gln

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 24

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 24

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Thr Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 25

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 25

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Asp Ile Tyr Lys Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Ala

85 90 95

Ser Ser Pro Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 26

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 26

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Ser
85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg
100 105 110

Pro

<210> 27

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 27

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly
1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu
20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50

55

60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65

70

75

80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Ser

85

90

95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 28

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 28

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 29

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 29

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 30

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 30

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 31

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 31

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 32

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 32

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 33

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 33

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Thr Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 34

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 34

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 35

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 35

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Ser Ser

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 36

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 36

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 37

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 37

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Ala Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 38

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 38

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gln Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 39

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 39

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gly Thr Trp Pro Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 40

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 40

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Ala Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 41

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 41

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gly Thr Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 42

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 42

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gly Arg Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 43

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 43

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gln Gly His Ala Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln
65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly
 85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr
 100 105 110

<210> 44

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 44

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly
1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Ala Arg His Ala Arg Leu
 20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 45

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 45

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gly Leu Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 46

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 46

Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu Ser Val Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Gln Gly Arg Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 47

<211> 245

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 47

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Val Thr Ile Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Gly

85 90 95

Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

115 120 125

Gly Gly Gly Gly Ser Asp Thr Gly Val Ser Gln Asp Pro Arg His Leu

130 135 140

Ser Val Lys Arg Gly Gln Asn Val Thr Leu Arg Cys Asp Pro Ile Ser

145 150 155 160

Glu His Asn Arg Leu Tyr Trp Tyr Arg Gln Thr Pro Gly Gln Gly Pro

165 170 175

Glu Phe Leu Thr Tyr Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg

180 185 190

Leu Leu Ser Asp Arg Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser

195 200 205

Thr Leu Glu Ile Gln Arg Val Glu Pro Gly Asp Ser Ala Met Tyr Leu

210 215 220

Cys Ala Ser Glu Gly Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr

225 230 235 240

Arg Leu Thr Val Thr

245

<210> 48

<211> 735

<212> DNA

<213> Artificial sequence

<220>

<223> Synthesized polynucleotide

<400> 48

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attaattgta gctataagac cagtattaac aacctgcagt ggtatcgcca gaatagcggc 120

cgcggtctgg ttcatctgat tctgattcgt agcaatgaac gtgaaaaaca tagcggccgt 180

ctgcgtgtga ccctggatac cagtaaaaaa ttagcagcc tggaaattac cgatgtgcgc 240

ccgagcgata ccgcaagtta tttttgtgca accgatccgc gcaccggcgc aaatagcaaa 300

ctgacctttg gtaaaggcat taccctgagt gttcgtccgg gcggtggtgg cagcggcggt 360

ggtggttcag gcggtggtgg aagcgggtgt ggcggtagt ataccggtgt tagtcaggat 420

ccgcgccatc tgagcgtaa acgtggccag aatgttacc tgcgtgtga tccgattagt 480

gaacataatc gtctgtattg gtatcgtcag accccgggtc aggttccgga atttctgacc 540

tattttcaga atgaagcaca gctggaaaaa agccgtctgc tgagtgatcg ttttagcgcc 600

gaacgtccga aaggcagttt tagtaccctg gaaattcagc gcgtggaacc gggtgacagc 660

gcaatgtatc tgtgcgccag cgaaggtctg gcctatgaac agtatatttg cccgggtacc 720

cgctgaccg ttacc 735

<210> 49

<211> 208

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 49

Met Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu

1 5 10 15

Gly Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn

20 25 30

Leu Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile

35 40 45

Leu Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val

50 55 60

Thr Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser

65 70 75 80

Arg Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr

85 90 95

Gly Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val

100 105 110

Arg Pro Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp

115 120 125

Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser

130

135

140

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp

145

150

155

160

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala

165

170

175

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn

180

185

190

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser

195

200

205

<210> 50

<211> 243

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 50

Met Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg

1 5 10 15

Gly Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg

20 25 30

Leu Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr

35 40 45

Tyr Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp

50 55 60

Arg Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile

65 70 75 80

Gln Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu

85 90 95

Gly Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val

100 105 110

Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu

115 120 125

Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys

130

135

140

Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val

145

150

155

160

Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu

165

170

175

Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg

180

185

190

Leu Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe Arg

195

200

205

Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln

210

215

220

Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly

225

230

235

240

Arg Ala Asp

<210> 51

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 51

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Ala

85

90

95

Ser Ser Ala Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 52

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 52

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Gly

85 90 95

Ser Ser Asn Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 53

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 53

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Met Gln

85 90 95

Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 54

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 54

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Arg Gln

85

90

95

Ser Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 55

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 55

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu
35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr
50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg
65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Val Leu Ser
85 90 95

Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg
100 105 110

Pro

<210> 56

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 56

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Ser

85 90 95

Gly Ser Thr Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 57

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 57

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Asp Arg Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 58

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 58

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Ala

85 90 95

Ser Thr Pro Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 59

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 59

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Glu Ala Gln

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 60

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 60

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Glu

85 90 95

Ser Thr Thr Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 61

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 61

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu
20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu
35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr
50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg
65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Gln
85 90 95

Ser Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg
100 105 110

Pro

<210> 62

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 62

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Gln Asn Ser

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 63

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 63

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50

55

60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65

70

75

80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ser Asn

85

90

95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 64

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 64

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ala Gln

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 65

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 65

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Ala Gln

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 66

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 66

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Thr Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 67

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 67

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Asp Ile Tyr Lys Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Ala

85 90 95

Ser Ser Pro Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 68

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 68

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Ser
85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg
100 105 110

Pro

<210> 69

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 69

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly
1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu
20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50

55

60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65

70

75

80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Leu Ser

85

90

95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 70

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 70

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 71

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 71

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 72

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 72

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 73

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 73

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Asp Val Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 74

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 74

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 75

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 75

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Glu Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ala Thr Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 76

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 76

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Pro Ile Ser Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Ser Met Ser

85 90 95

Pro Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100

105

110

Pro

<210> 77

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 77

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ala Val Asp Ser Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Glu Ile Thr Asp Val Arg

65 70 75 80

Pro Ser Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Thr Ser Ser

85 90 95

Trp Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 78

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 78

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 79

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 79

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ala Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 80

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 80

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gln Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

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90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

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105

110

<210> 81

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 81

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

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15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Thr Trp Pro Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

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<210> 82

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 82

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

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Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ala Gly His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 83

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 83

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Thr Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 84

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 84

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Arg Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 85

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 85

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gln Gly His Ala Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 86

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 86

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ala Arg His Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 87

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 87

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Leu Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 88

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 88

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gln Gly Arg Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Glu Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 89

<211> 254

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 89

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser Arg

65 70 75 80

Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Gly

85 90 95

Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser

115 120 125

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln

130 135 140

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys

145 150 155 160

Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val

165 170 175

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn

180 185 190

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys

195 200 205

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn

210 215 220

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val

225 230 235 240

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser

245 250

<210> 90

<211> 291

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 90

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro

115

120

125

Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu

130

135

140

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn

145 150 155 160

Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys

165 170 175

Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu

180 185 190

Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys

195 200 205

Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp

210 215 220

Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg

225 230 235 240

Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser

245 250 255

Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala

260 265 270

Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp

275

280

285

Ser Arg Gly

290

<210> 91

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 91

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1

5

10

15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20

25

30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35

40

45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser Arg

65 70 75 80

Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro His Trp Ser

85 90 95

Thr Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 92

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 92

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Asp Ile Ala Ser Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser Arg

65 70 75 80

Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro His Trp Ser

85 90 95

Thr Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro

<210> 93

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 93

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Val Asp Asn Ala Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 94

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 94

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Leu Gly His Ala Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 95

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 95

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Trp Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

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110

<210> 96

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 96

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Glu Leu Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 97

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 97

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Met Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 98

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 98

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Asp Leu Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 99

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 99

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

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10

15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20

25

30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35

40

45

Phe His Tyr Thr Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50

55

60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65

70

75

80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85

90

95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100

105

110

<210> 100

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 100

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1

5

10

15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Ala Trp Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 101

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 101

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Gly Trp Trp Pro Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Gly Glu Leu Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

<210> 102

<211> 207

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 102

Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu Gly

1 5 10 15

Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn Leu

20 25 30

Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile Leu

35 40 45

Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val Thr

50 55 60

Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser Arg

65 70 75 80

Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr Asp Pro Arg Thr Gly

85 90 95

Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val Arg

100 105 110

Pro Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser

115 120 125

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln

130 135 140

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys

145 150 155 160

Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val

165 170 175

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn

180 185 190

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser

195 200 205

<210> 103

<211> 242

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesized polypeptide

<400> 103

Asp Thr Gly Val Ser Gln Asp Pro Arg His Lys Ile Thr Lys Arg Gly

1 5 10 15

Gln Asn Val Thr Phe Arg Cys Asp Pro Ile Ser Glu His Asn Arg Leu

20 25 30

Tyr Trp Tyr Arg Gln Thr Leu Gly Gln Gly Pro Glu Phe Leu Thr Tyr

35 40 45

Phe Gln Asn Glu Ala Gln Leu Glu Lys Ser Arg Leu Leu Ser Asp Arg

50 55 60

Phe Ser Ala Glu Arg Pro Lys Gly Ser Phe Ser Thr Leu Glu Ile Gln

65 70 75 80

Arg Thr Glu Gln Gly Asp Ser Ala Met Tyr Leu Cys Ala Ser Glu Gly

85 90 95

Leu Ala Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr

100 105 110

Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro

115 120 125

Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu

130 135 140

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn

145 150 155 160

Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys

165 170 175

Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu

180 185 190

Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys

195 200 205

Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp

210

215

220

Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg

225

230

235

240

Ala Asp

Claims

1. A T cell receptor (TCR) having an activity of binding to FMNKFIYEI-HLA A0201 complex, and the T cell receptor comprises a TCR α chain variable domain and a TCR β chain variable domain, the TCR α chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR α chain variable domain are listed as follows,

CDR1 α : TSINN

CDR2 α : IRSNERE

- 10 CDR3 α : ATDPRTGANSKLT, and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 2 of CDR1 α	D or P or A or E
I at position 3 of CDR1 α	V
N at position 4 of CDR1 α	S or Y or D
N at position 5 of CDR1 α	S or K
R at position 5 of CDR3 α	A or S or V or D or E or Q or T
T at position 6 of CDR3 α	M or R or L or A or N or S
G at position 7 of CDR3 α	A or Q or S or E or N
A at position 8 of CDR3 α	S or G or P or W
N at position 9 of CDR3 α	S or T
S at position 10 of CDR3 α	A or N or T or P

and/or, the TCR β chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR β chain variable domain are listed as follows,

- 15 CDR1 β : SEHNR

CDR2 β : FQNEAQ

CDR3 β : ASEGLAYEQY, and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 1 of CDR1 β	A or Q or G
E at position 2 of CDR1 β	G or T or R or L
H at position 3 of CDR1 β	W or R
N at position 4 of CDR1 β	A or P
Q at position 2 of CDR2 β	H
N at position 3 of CDR2 β	G
E at position 4 of CDR2 β	D
A at position 5 of CDR2 β	M or L
Q at position 6 of CDR2 β	E.

2. The TCR of claim 1, wherein there are 3-8 mutations in the 3 CDR regions of the TCR α chain variable domain and/or 4-8 mutations in the 3 CDR regions of the TCR β chain variable domain.

3. The TCR of claim 1, wherein the affinity of the TCR for FMNKFIYEI-HLA A0201 complex is at least 2 times of that of the wild type TCR.

4. The TCR of claim 1, wherein the α chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 2.

5. The TCR of claim 1, wherein the mutated position in the α chain variable domain of the TCR includes position 7 and / or position 8 of CDR3 α .

6. The TCR of claim 1, wherein the mutation in the α chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
G at position 7 of CDR3 α	S
A at position 8 of CDR3 α	P

and / or the mutation in the β chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
N at position 3 of CDR2 β	G
E at position 4 of CDR2 β	D
Q at position 6 of CDR2 β	E.

7. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , wherein the amino acid sequence of CDR1 α is selected from the group consisting of: TSINN, TPISS, TDIYK, TAVDS, TEISS and TDVSS.

8. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , wherein the amino acid sequence of CDR2 α is IRSNERE.

9. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1 α , CDR2 α and CDR3 α , wherein the CDR3 α comprises a sequence: ATDP[3 α X1][3 α X2][3 α X3][3 α X4]NSKLT, wherein [3 α X1], [3 α X2], [3 α X3] and [3 α X4] are independently selected from any natural amino acid residue, respectively.

10. The TCR of claim 9, wherein the [3 α X1] is R or A or S or D or E or Q or V or T.

11. The TCR of claim 9, wherein the [3 α X2] is T or M or R or L or A or N or S.

12. The TCR of claim 9, wherein the [3 α X3] is G or A or Q or S or E or N.

13. The TCR of claim 9, wherein the [3 α X4] is A or S or G or P or W.

14. The TCR of claim 9, wherein the CDR3 α comprises a sequence selected from
5 the group consisting of: ATDPSMSPNSKLT, ATDPSLSPNSKLT and
ATDPSLQSNKLT.

15. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable
domain and a TCR β chain variable domain, and said TCR β chain variable domain
comprises CDR1 β , CDR2 β and CDR3 β , wherein the CDR2 β comprises a sequence
10 selected from the group consisting of: FQGDME, FQGDLE and FHGDLE.

16. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable
domain and a TCR β chain variable domain, said TCR β chain variable domain
comprises CDR1 β , CDR2 β and CDR3 β , and the amino acid sequence of CDR3 β is
ASEGLAYEQY.

15 17. The TCR of claim 1, wherein the TCR has CDRs selected from the group
consisting of:

CDR No.	CDR1 α	CDR2 α	CDR3 α	CDR1 β	CDR2 β	CDR3 β
1	TSINN	IRSNERE	ATDPSRQSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
2	TSINN	IRSNERE	ATDPRTGSSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
3	TSINN	IRSNERE	ATDPRTASSAKLT	SEHNR	FQNEAQ	ASEGLAYEQY
4	TSINN	IRSNERE	ATDPAMQANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
5	TSINN	IRSNERE	ATDPVLSANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
6	TSINN	IRSNERE	ATDPRTSGSTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
7	TSINN	IRSNERE	ATDPDRSPNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
8	TSINN	IRSNERE	ATDPRTASTPKLT	SEHNR	FQNEAQ	ASEGLAYEQY
9	TSINN	IRSNERE	ATDPEAQWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
10	TSINN	IRSNERE	ATDPRTSTTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
11	TSINN	IRSNERE	ATDPSLQSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
12	TSINN	IRSNERE	ATDPQNSWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
13	TSINN	IRSNERE	ATDPASNWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
14	TSINN	IRSNERE	ATDPRTGANSKLT	SEHNR	FQGDME	ASEGLAYEQY
15	TDVSS	IRSNERE	ATDPAAQWNSKLT	AGHAR	FQGDME	ASEGLAYEQY
16	TPISS	IRSNERE	ATDPAAQWNSKLT	QGHAR	FQGDME	ASEGLAYEQY
17	TPISS	IRSNERE	ATDPATSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
18	TDIYK	IRSNERE	ATDPRTASSPKLT	GTWPR	FQGDME	ASEGLAYEQY
19	TAVDS	IRSNERE	ATDPSLSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
20	TAVDS	IRSNERE	ATDPSLSPNSKLT	QGHAR	FQGDME	ASEGLAYEQY
21	TEISS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
22	TAVDS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
23	TEISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
24	TEISS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
25	TDVSS	IRSNERE	ATDPSMSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY
26	TDVSS	IRSNERE	ATDPTMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
27	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGHAR	FQGDLE	ASEGLAYEQY
28	TAVDS	IRSNERE	ATDPSLSPNSKLT	ARHAR	FQGDLE	ASEGLAYEQY
29	TEISS	IRSNERE	ATDPATSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
30	TPISS	IRSNERE	ATDPATSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY

31	TAVDS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
32	TDVSS	IRSNERE	ATDPSMSPNSKLT	GLWPR	FHGDLE	ASEGLAYEQY
33	TPISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
34	TDVSS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
35	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGRAR	FQGDME	ASEGLAYEQY
36	TAVDS	IRSNERE	ATDPTSSWNSKLT	GRWPR	FQGDLE	ASEGLAYEQY.

18. The TCR of claim 1, wherein the TCR is soluble.

19. The TCR of claim 1, wherein the TCR is an $\alpha\beta$ heterodimeric TCR or a single chain TCR; and preferably, the TCR comprises α chain constant region sequence TRAC*01 and β chain constant region sequence TRBC1*01 or TRBC2*01.

5 20. The TCR of claim 19, wherein the TCR comprises (i) all or part of the TCR α chain except for its transmembrane domain, and (ii) all or part of the TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of the constant domain of the TCR chain.

10 21. The TCR of claim 20, wherein an artificial interchain disulfide bond is contained between the α chain variable region and the β chain constant region of the TCR.

15 22. The TCR of claim 21, wherein cysteine residues forming an artificial interchain disulfide bond between the α chain variable region and the β chain constant region of the TCR are substituted for one or more groups of amino acids selected from the following:

Thr48 of TRAC*01 exon 1 and Ser57 of TRBC1*01 or TRBC2*01 exon 1;
 Thr45 of TRAC*01 exon 1 and Ser77 of TRBC1*01 or TRBC2*01 exon 1;
 Tyr10 of TRAC*01 exon 1 and Ser17 of TRBC1*01 or TRBC2*01 exon 1;
 Thr45 of TRAC*01 exon 1 and Asp59 of TRBC1*01 or TRBC2*01 exon 1;
 20 Ser15 of TRAC*01 exon 1 and Glu15 of TRBC1*01 or TRBC2*01 exon 1;
 Arg53 of TRAC*01 exon 1 and Ser54 of TRBC1*01 or TRBC2*01 exon 1;
 Pro89 of TRAC*01 exon 1 and Ala19 of TRBC1*01 or TRBC2*01 exon 1;

and

Tyr10 of TRAC*01 exon 1 and Glu20 of TRBC1*01 or TRBC2*01 exon 1.

25 23. The TCR of claim 1, wherein the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 51-77 and 91-92; and / or the amino acid sequence of the β chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 78-88 and 93-101.

30 24. The TCR of claim 1, wherein the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:

1	54	2
2	52	2
3	51	2
4	53	2
5	55	2
6	56	2
7	57	2
8	58	2
9	59	2
10	60	2
11	61	2
12	62	2
13	63	2
14	1	78
15	64	79
16	65	80
17	66	81
18	67	81
19	68	81
20	68	80
21	69	79
22	70	82
23	71	79
24	71	83
25	72	84
26	73	83
27	74	85
28	68	86
29	75	82
30	66	84
31	68	79
32	72	87
33	76	79
34	72	81
35	74	88
36	77	84
37	1	93
38	1	94
39	1	95
40	1	96
41	1	97
42	1	98
43	1	99
44	1	100
45	91	101
46	92	101.

25. The TCR of claim 1, wherein the TCR is a single-chain TCR.

26. The TCR of claim 1, wherein the TCR is a single-chain TCR consisting of an α variable domain and a β variable domain, and the α variable domain and the β variable domain are connected by a flexible short peptide sequence (linker).

5 27. The TCR of claim 1, wherein the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 9-35; and / or

the amino acid sequence of the β chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 36-46.

28. The TCR of claim 1, wherein the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
s-1	12	4
s-2	10	4
s-3	9	4
s-4	11	4
s-5	13	4
s-6	14	4
s-7	15	4
s-8	16	4
s-9	17	4
s-10	18	4
s-11	19	4
s-12	20	4
s-13	21	4
s-14	3	36
s-15	22	37
s-16	23	38
s-17	24	39
s-18	25	39
s-19	26	39
s-20	26	38
s-21	27	37
s-22	28	40
s-23	29	37
s-24	29	41
s-25	30	42
s-26	31	41
s-27	32	43
s-28	26	44
s-29	33	40
s-30	24	42
s-31	26	37
s-32	30	45
s-33	34	37
s-34	30	39
s-35	32	46
s-36	35	42.

5 29. The TCR of any one of the preceding claims, wherein a conjugate binds to the α chain and/or β chain of the TCR at C- or N-terminal.

30. The TCR of claim 29, wherein the conjugate that binds to the TCR is a detectable label, a therapeutic agent, a PK modified moiety, or a combination thereof.

10 31. The TCR of claim 30, wherein the therapeutic agent that binds to the TCR is an anti-CD3 antibody linked to the α or β chain of the TCR at C- or

N-terminal.

32. A multivalent TCR complex comprising at least two TCR molecules, and at least one TCR molecule is the TCR of any one of the preceding claims.

5 33. A nucleic acid molecule, comprising a nucleic acid sequence encoding the TCR of any one of claims 1-31, or a complement sequence thereof.

34. A vector, comprising the nucleic acid molecule of claim 33.

35. A host cell, comprising the vector of claim 34 or having the exogenous nucleic acid molecule of claim 33 integrated into its genome.

36. An isolated cell, expressing the TCR of any one of claims 1-31.

10 37. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier, and the TCR of any one of claims 1-31, or the TCR complex of claim 32, or the cell of claim 36.

15 38. A method for treating a disease, comprising administering the TCR of any one of claims 1-31, or the TCR complex of claim 32, or the cell of claim 36, or the pharmaceutical composition of claim 37 to a subject in need thereof.

39. Use of the TCR of any one of claims 1-31, or the TCR complex of claim 32, or the cell of claim 36 for preparing a medicament for treating tumor; and preferably, the tumor is liver cancer.

20 40. The TCR of any one of claims 1-31, or the TCR complex of claim 32, or the cell of claim 36 for use as a medicament for treating tumor; and preferably, the tumor is liver cancer.

41. A method for preparing the T cell receptor of any one of claims 1-31, comprising the steps of:

25 (i) culturing the host cell of claim 35 to express the T cell receptor of any one of claims 1-31;

(ii) isolating or purifying the T cell receptor.