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DESCRIPTION

Description

[0001] The present invention pertains to antigen recognizing constructs against tumor associated antigens (TAA), in particular against Preferentially Expressed Antigen of Melanoma (PRAME). The invention in particular provides novel T cell receptor (TCR) based molecules which are selective and specific for the tumor expressed antigen of the invention. The TCR of the invention, and TAA binding fragments derived therefrom, are of use for the diagnosis, treatment and prevention of TAA expressing cancerous diseases. Further provided are nucleic acids encoding the antigen recognizing constructs of the invention, vectors comprising these nucleic acids, recombinant cells expressing the antigen recognizing constructs and pharmaceutical compositions comprising the compounds of the invention.

DESCRIPTION

[0002] PRAME is encoded by the PRAME gene, which is expressed at a high level in a large proportion of tumors, including melanomas, non-small-cell lung carcinomas, ovarian carcinoma renal cell carcinoma (RCC), breast carcinoma, cervix carcinoma, colon carcinoma, sarcoma, neuroblastoma, as well as several types of leukemia. PRAME is the best characterized member of the PRAME family of leucine-rich repeat (LRR) proteins. Mammalian genomes contain multiple members of the PRAME family whereas in other vertebrate genomes only one PRAME-like LRR protein was identified. PRAME is a cancer/testis antigen that is expressed at very low levels in normal adult tissues except testis but at high levels in a variety of cancer cells.

[0003] T-cell based immunotherapy targets represent peptide epitopes derived from tumor-associated or tumor-specific proteins, which are presented by molecules of the major histocompatibility complex (MHC). These tumors associated antigens (TAAs) can be peptides derived from all protein classes, such as enzymes, receptors, transcription factors, etc. which are expressed and, as compared to unaltered cells of the same origin, usually up-regulated in cells of the respective tumor.

[0004] Specific elements of the cellular immune response are capable of specifically recognizing and destroying tumor cells. The isolation of T-cells from tumor-infiltrating cell populations or from peripheral blood suggests that such cells play an important role in natural immune defense against cancer. CD8-positive T-cells in particular, which recognize class I molecules of the major histocompatibility complex (MHC)-bearing peptides of usually 8 to 10 amino acid residues derived from proteins or defective ribosomal products (DRiPs) located in the cytosol, play an important role in this response. The MHC-molecules of the human are also designated as human leukocyte-antigens (HLA).

[0005] There are two classes of MHC-molecules, MHC class I and MHC class II. Complexes of peptide and MHC class I are recognized by CD8-positive T-cells bearing the appropriate T-cell receptor (TCR), whereas complexes of peptide and MHC class II molecules are recognized by CD4- positive-helper-T-cells bearing the appropriate TCR. Since both types of response, CD8 and CD4 dependent, contribute jointly and synergistically to the anti-tumor effect, the identification and characterization of tumor-associated antigens and corresponding T cell receptors is important in the development of cancer immunotherapies such as vaccines and cell therapies.

[0006] In the MHC class I dependent immune reaction, peptides not only have to be able to bind to certain MHC class I molecules expressed by tumor cells, they subsequently also have to be recognized by T-cells bearing specific T-cell receptors (TCR). Therefore, TAAs are a starting point for the development of a T-cell based therapy including but not limited to tumor vaccines and cell therapies.

[0007] Approximately 90 percent of peripheral blood T cells express a TCR consisting of an α poly- peptide and a β polypeptide. A small percentage of T cells (about 5% of total T cells) have been shown to express a TCR consisting of a γ polypeptide and a δ polypeptide. γδ T cells are found at their highest abundance in the gut mucosa, within a population of lymphocytes known as intraepithelial lymphocytes (IELs). The antigenic molecules that activate γδ T cells are still widely unknown. However, γδ T cells are not MHC restricted and seem to be able to recognize whole proteins rather than requiring peptides to be presented by MHC molecules on antigen presenting cells, although some recognize MHC class IB molecules.

Human Vγ9/Vδ2 T cells, which constitute the major γδ T cell population in peripheral blood, are unique in that they specifically and rapidly respond to a small non-peptidic microbial metabolite, HMB-PP, an isopentenyl pyrophosphate precursor. Estimates of the percentages of T cells that may be found in peripheral blood from healthy donors are as follows: CD3+=70.78%±4.71; CD3+CD4+=38.97%±5.66; CD3+CD8+=28.955%±7.43; CD3+CD56+=5.22%±1.74; CD3-CD56+=10.305%±4.7; CD3+CD45RA+=45.00%±7.19; and CD3+CD45RO+=27.21%±7.34.

[0008] The chains of the T cell antigen receptor of a T cell clone are each composed of a unique combination of domains designated variable (V), [diversity (D),] joining (J), and constant (C). In each T cell clone, the combination of V, D and J domains of both the alpha and the beta chains or of both the delta and gamma chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell clone and defines a unique binding site, also known as the idiotype of the T cell clone. In contrast, the C domain does not participate in antigen binding.

[0009] A TCR is a heterodimeric cell surface protein of the immunoglobulin super-family, which is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. TCRs exist in αβ and γδ forms, which are structurally similar but have quite distinct anatomical locations and probably functions. The extracellular portion of native heterodimeric αβTCR and γδTCR each contain two polypeptides, each of which has a membrane-proximal constant domain, and a membrane-distal variable domain. Each of the constant and variable domains include an intra-chain disulfide bond. The variable domains contain the highly polymorphic loops analogous to the complementarity determining regions (CDRs) of antibodies. The use of TCR gene therapy overcomes a number of current hurdles. It allows equipping patients' own T cells with desired specificities and generation of sufficient numbers of T cells in a short period of time, avoiding their exhaustion. The TCR will be transduced into potent T cells (e.g. central memory T cells or T cells with stem cell characteristics), which may ensure better persistence and function upon transfer. TCR-engineered T cells will be infused into cancer patients rendered lymphopenic by chemotherapy or irradiation, allowing efficient engraftment but inhibiting immune suppression.

[0010] While advances have been made in the development of molecular-targeting drugs for cancer therapy, there remains a need in the art to develop new anti-cancer agents that specifically target molecules highly specific to cancer cells. The present description addresses that need by providing novel PRAME TCRs, respective recombinant TCR constructs, nucleic acids, vectors and host cells that specifically bind TAA epitope(s) as disclosed; and methods of using such molecules in the treatment of cancer. The term TAA in context of the invention relates in particular to the following preferred proteins: PRAME, and fragments or analogs thereof, in particular fragments or analogs comprising or consisting of the antigenic peptide sequences shown in SEQ ID NO: 97 to 115, preferably SEQ ID NO: 97 to 106, more preferably SEQ ID NO: 97.

[0011] The object of the invention is solved in a first aspect by an antigen recognizing construct comprising at least one complementary determining region (CDR) 3 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or preferably 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 3, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 129, and 135.

[0012] In some embodiments the antigen recognizing construct of the invention specifically binds to a TAA-peptide-HLA molecule complex, wherein the TAA peptide comprises, or alternatively consists of, a variant of the TAA which is at least 66%, preferably at least 77%, and more preferably at least 88% homologous (preferably at least 77% or at least 88% identical) to the amino acid sequence of the TAA of the invention, wherein said variant binds to an HLA class I or class II molecule and/or induces T-cells cross-reacting with said peptide, or a pharmaceutically acceptable salt thereof, wherein said peptide is not the underlying full-length polypeptide.

[0013] As used herein, the terms "identical" or percent "identity", when used anywhere herein in the context of two or more nucleic acid or protein/polypeptide sequences, refer to two or more sequences or subsequences that are the same or have (or have at least) a specified percentage of amino acid residues or nucleotides that are the same (i.e., at, or at least, about 60% identity, preferably at, or at least, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93% or 94%, identity, and more preferably at, or at least, about 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region - preferably over their full length sequences - , when compared and aligned for maximum correspondence over the comparison window or designated region) as measured using a sequence comparison algorithms, or by manual alignment and visual inspection (see, e.g., NCBI web site). In a particular embodiment, for example when comparing the protein or nucleic acid sequence of an antigen recognizing construct of the invention to another protein/gene, the percentage identity can be determined by the Blast searches supported at the NCBI web site; in particular for amino acid identity, those using BLASTP with the following parameters: Expected threshold 10; Word size: 6; Matrix: BLOSUM62; Gap Costs: Existence: 11, Extension: 1; Neighboring words threshold: 11; Compositional adjustments: Conditional compositional score matrix adjustment.

[0014] In the context of the present invention it shall be understood that any embodiments referred to as "comprising"

certain features of the invention, shall be understood to include in some more preferred embodiments the more restricted description of "consisting of" or "consisting essentially of" the very same features of the present invention.

[0015] In another embodiment, the antigen recognizing construct may further comprise a CDR1 and/or a CDR2, or more preferably a CDR2bis, domain sequence. Within the variable domain, CDR1 and CDR2 or CDR2bis, are found in the variable (V) region of a polypeptide chain, and CDR3 includes some of V, all of diversity (D) and joining (J) regions. CDR3 is the most variable and is the main CDR responsible for specifically and selectively recognizing an antigen. CDR1, CDR2 and CDR2bis sequences may be selected from a CDR sequence of a human variable chain allele.

[0016] Native alpha-beta heterodimeric TCRs have an alpha chain and a beta chain. Each chain comprises variable, joining and constant regions, and the beta chain also usually contains a short diversity region between the variable and joining regions, but this diversity region is often considered as part of the joining region. Each variable region comprises three CDRs (Complementarity Determining Regions) embedded in a framework sequence, one being the hypervariable region named CDR3. There are several types of alpha chain variable (Vα) regions and several types of beta chain variable (Vβ) regions distinguished by their framework, CDR1 and CDR2 sequences, and by a partly defined CDR3 sequence. The Vα types are referred to in IMGT nomenclature by a unique TRAV number, Vβ types are referred to by a unique TRBV number. For more information on immunoglobulin antibody and TCR genes see the international ImMunoGeneTics information system®, Lefranc M-P et al (Nucleic Acids Res. 2015 Jan;43(Database issue):D413-22; and http://www.imgt.org/).

[0017] Therefore, in one embodiment the antigen recognizing construct of the invention comprises CDR1, CDR2, CDR2bis and CDR3 sequences in a combination as provided in Table 1 herein below, which display the respective variable chain allele together with the CDR3 sequence. Therefore, preferred are antigen recognizing constructs of the invention which comprise at least one, preferably, all four CDR sequences CDR1, CDR2, CDR2bis and CDR3. Preferably, an antigen recognizing construct of the invention comprises the respective CDR1, CDR2bis and CDR3 of one individual herein disclosed TCR variable region of the invention (see Table 1 herein below and the example section).

[0018] The term "specificity" or "antigen specificity" or "specific for" a given antigen, as used herein means that the antigen recognizing construct can specifically bind to said antigen, preferably a TAA antigen, more preferably with high avidity, when said antigen is presented by HLA, preferably by HLA A2. For example, a TCR, as antigen recognizing construct, may be considered to have "antigenic specificity" for the TAA, if T cells expressing the TCR and contacted with a TAA presenting HLA secrete at least about 200 pg/ml or more (e.g., 250 pg/ml or more, 300 pg/ml or more, 400 pg/ml or more, 500 pg/ml or more, 600 pg/ml or more, 700 pg/ml or more, 1000 pg ml or more, 2,000 pg/ml or more, 2,500 pg/ml or more, 5,000 pg/ml or more) of interferon γ (IFN-γ) upon co-culture with target cells pulsed with a low concentration of a TAA antigen, such as the TAA epitopes and antigens provided herein below (e.g., about 10-11 mol/l, 10-10 mol/l, 10-9 mol/l, 10-8 mol/l, 10-7 mol/l, 10-6 mol/l, 10-5 mol/l). Alternatively, or additionally, a TCR may be considered to have "antigenic specificity" for the TAA, if T cells expressing the TCR secrete at least twice as much IFN-γ as the non-transduced background level of IFN-γ upon co-culture with target cells pulsed with a low concentration of the TAA antigens. Such a "specificity" as described above can - for example - be analyzed with an ELISA.

[0019] In one embodiment of the invention, the antigen recognizing construct selectively binds to a TAA derived antigenic peptide; preferably wherein the TAA antigenic peptide is a protein epitope or peptide having an amino acid sequence shown in SEQ ID NO:97, or a variant thereof, wherein the variant is an amino acid deletion, addition, insertion or substitution of not more than three, preferably two and most preferably not more than one amino acid position.

[0020] The term "selectivity" or "selective recognizing/binding" is understood to refer to the property of an antigen recognizing construct, such as a TCR or antibody, to selectively recognize or bind to preferably only one specific epitope and preferably shows no or substantially no cross-reactivity to another epitope. Preferably "selectivity" or "selective recognizing/binding" means that the antigen recognizing construct (e.g. a TCR) selectively recognizes or binds to preferably only one specific epitope and preferably shows no or substantially no cross-reactivity to another epitope, wherein said epitope is unique for one protein, such that the antigen recognizing construct shows no or substantially no cross-reactivity to another epitope and another protein.

[0021] The antigen recognizing construct according to the invention is preferably selected from an antibody, or derivative or fragment thereof, or a T cell receptor (TCR), or derivative or fragment thereof. A derivative or fragment of an antibody or TCR of the invention shall preferably retain the antigen binding/recognizing ability of the parent molecule, in particular its specificity and/or selectivity as explained above. Such binding functionality may be retained by the presence of a CDR3 region as defined herein.

[0022] In an embodiment of the invention, the inventive TCRs are able to recognize TAA antigens in a major histocompatibility complex (MHC) class I-dependent manner. "MHC class I-dependent manner," as used herein, means that the TCR elicits an immune response upon binding to TAA antigens within the context of an MHC class I molecule. The MHC class I molecule can be any MHC class I molecule known in the art, e.g., HLA-A molecules. In a preferred embodiment of the invention, the MHC class I molecule is an HLA-A2 molecule.

[0023] The invention provides both single chain antigen recognizing construct and double chain recognizing constructs.

[0024] In an embodiment, the TCR alpha variable domain has at least one mutation relative to a TCR alpha domain shown in Table 1; and/or the TCR beta variable domain has at least one mutation relative to a TCR alpha domain shown in Table 1. In an embodiment, a TCR comprising at least one mutation in the TCR alpha variable domain and/or TCR beta variable domain has a binding affinity for, and/or a binding half-life for, a TAA peptide-HLA molecule complex, which is at least double that of a TCR comprising the unmutated TCR alpha domain and/or unmutated TCR beta variable domain.

[0025] The TCR alpha chains of the present description may further comprise a TCR alpha transmembrane domain and/or a TCR alpha intracellular domain. The TCR beta chains of the present description may further comprise a TCR beta transmembrane domain and/or a TCR beta intracellular domain.

[0026] The invention in particular provides a TCR as antigen recognizing construct, or fragment or derivative thereof. The TCR preferably is of human, which is understood as being generated from a human TCR locus and therefore comprising human TCR sequences. Furthermore, the TCR of the invention may be characterized in that it is of human origin and specifically recognizes a TAA antigen of the invention.

[0027] Another embodiment of the invention provides the antigen recognizing construct described above, which induces an immune response, preferably wherein the immune response is characterized by an increase in interferon (IFN) γ levels.

[0028] TCRs of the invention may be provided as single chain α or β, or γ and δ, molecules, or alternatively as double chain constructs composed of both the α and β chain, or γ and δ chain.

[0029] The antigen recognizing construct of the invention may comprise a TCR α or γ chain; and/or a TCR β or δ chain; wherein the TCR α or γ chain comprises a CDR3 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 3 and/or wherein the TCR β or δ chain comprises a CDR3 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 9 .

[0030] Most preferably, in some additional embodiments, wherein the disclosure refers to antigen recognizing constructs comprising any one, two, three or all of the CDR1, CDR2, CDR2bis and CDR3 regions of the herein disclosed TCR chains (see Table 1), such antigen recognizing constructs may be preferred, which comprise the respective CDR sequence of the invention with not more than three, two, and preferably only one, modified amino acid residues. A modified amino acid residue may be selected from an amino acid insertion, deletion or substitution. Most preferred is that the three, two, preferably only one modified amino acid residue is the first or last amino acid residue of the respective CDR sequence. If the modification is a substitution, then it is preferable in some embodiments that the substitution is a conservative amino acid substitution.

[0031] If the antigen recognizing construct of the invention is composed of at least two amino acid chains, such as a double chain TCR, or antigen binding fragment thereof, the antigen recognizing construct may comprises in a first polypeptide chain the amino acid sequence according to SEQ ID NO: 3, and in a second polypeptide chain the amino acid sequence according to SEQ ID NO: 9. Any one of the aforementioned double chain TCR, or antigen binding fragments thereof, are preferred TCR of the present invention. In some embodiments, the CDR3 of the double chain TCR of the invention may be mutated. Mutations of the CDR3 sequences as provided above preferably include a substitution, deletion, addition, or insertion of not more than three, preferably two, and most preferably not more than one amino acid residue. In some embodiments, the first polypeptide chain may be a TCR α or γ chain, and the second polypeptide chain may be a TCR β or δ chain. Preferred is the combination of an αβ or γδ TCR.

[0032] The TCR, or the antigen binding fragment thereof, is in some embodiments composed of a TCR α and a TCR β chain, or γ and δ chain. Such a double chain TCR comprises within each chain variable regions, and the variable regions each comprise one CDR1, one CDR2, or more preferably one CDR2bis, and one CDR3 sequence. The TCRs comprises

the CDR1, CDR2, CDR2bis and CDR3 sequences as comprised in the variable chain amino acid sequence of SEQ ID NOs: 4 and 10; or 130 and 136.

[0033] Some embodiments of the invention pertain to a TCR, or a fragment thereof, composed of a TCR α and a TCR β chain, wherein said TCR comprises the variable region sequences having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or preferably 100% sequence identity to the amino acid sequence selected from the α and β chain according to SEQ ID NOs: 4 and 10; or 130 and 136.

[0034] In a particularly preferred embodiment, the present invention provides an improved TCR, designated as R11P3D3_KE, composed of a TCR α and a TCR β chain, wherein said TCR comprises the variable region sequences having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or preferably 100% sequence identity to the amino acid sequence selected from the α and β chain according to SEQ ID NOs: 132 and 138. This TCR showed a surprisingly improved functionality in terms of tumor cell recognition when compared to its parent receptor, designated herein as R11P3D3.

[0035] The inventive TCRs may further comprise a constant region derived from any suitable species, such as any mammal, e.g., human, rat, monkey, rabbit, donkey, or mouse. In an embodiment of the invention, the inventive TCRs further comprise a human constant region. In some preferred embodiments, the constant region of the TCR of the invention may be slightly modified, for example, by the introduction of heterologous sequences, preferably mouse sequences, which may increase TCR expression and stability. In some preferred embodiments, the variable region of the TCR of the intervention may be slightly modified, for example, by the introduction of single point mutations to optimize the TCR stability and/or to enhance TCR chain pairing.

[0036] Some embodiments of the invention pertain to a TCR, or a fragment thereof, composed of a TCR α and a TCR β chain, wherein said TCR comprises the constant region having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or preferably 100% sequence identity to an amino acid sequence selected from of the α and β chain according to SEQ ID NOs: 5 and 11; or 131 and 137.

[0037] The TCR α or γ chain of the invention may further comprise a CDR1 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 1; and/or a CDR2 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 2; and/or more preferably a CDR2bis having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 196, 197, 198, 199, 200.

[0038] According to the invention the TCR β or δ chain may further comprise a CDR1 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 7; and/or a CDR2 having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 8; and/or more preferably a CDR2bis having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 8 and 134.

[0039] The antigen recognizing construct may in a further embodiment comprise a binding fragment of a TCR, and wherein said binding fragment comprises in one chain CDR1, CDR2, CDR2bis and CDR3, optionally selected from the CDR1, CDR2, CDR2bis and CDR3 sequences having the amino acid sequences of SEQ ID Nos. 1, 2, 3, 196; or 7, 8, 9.

[0040] In further embodiments of the invention the antigen recognizing construct as described herein elsewhere is a TCR, or a fragment thereof, composed of at least one TCR α and one TCR β chain sequence, wherein said TCR α chain sequence comprises the CDR1, CDR2, CDR2bis and CDR3 sequences having the amino acid sequences of SEQ ID NO: 1 to 3 and 196, and said TCR β chain sequence comprises the CDR1 to CDR3 sequences having the amino acid sequences of SEQ ID NO: 7 to 9.

[0041] In further embodiments of the invention the antigen recognizing construct as described herein before is a TCR, or a fragment thereof, comprising at least one TCR α and one TCR β chain sequence, wherein said TCR α chain sequence comprises a variable region sequence having the amino acid sequence of SEQ ID No. 4, and wherein said TCR β chain sequence comprises a variable region sequence having the amino acid sequence of SEQ ID No. 10; or wherein said TCR α chain sequence comprises a variable region sequence having the amino acid sequence of SEQ ID No. 130, and wherein said TCR β chain sequence comprises a variable region sequence having the amino acid sequence of SEQ ID No. 136.

[0042] In further embodiments of the invention the antigen recognizing construct as described herein before is a TCR, or a

fragment thereof, further comprising a TCR constant region having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 5, 11, 131, and 137 preferably wherein the TCR is composed of at least one TCR α and one TCR β chain sequence, wherein the TCR α chain sequence comprises a constant region having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 5and 131; and wherein the TCR β chain sequence comprises a constant region having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID Nos. 11 and 137.

[0043] Also disclosed are antigen recognizing constructs as described herein before comprising a first TCR chain having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID No. 6, and a second TCR chain having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID No. 12. In further embodiments, the invention provides antigen recognizing constructs which are TCR and comprise a first TCR chain having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID No. 132, and a second TCR chain having at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID No. 138.

[0044] As used herein, the term "murine" or "human," when referring to an antigen recognizing construct, or a TCR, or any component of a TCR described herein (e.g., complementarity determining region (CDR), variable region, constant region, α chain, and/or β chain), means a TCR (or component thereof), which is derived from a mouse or a human unrearranged TCR locus, respectively.

[0045] In an embodiment of the invention, chimeric TCR are provided, wherein the TCR chains comprise sequences from multiple species. Preferably, a TCR of the invention may comprise an α chain comprising a human variable region of an α chain and, for example, a murine constant region of a murine TCR α chain.

[0046] In one embodiment, the TCR of the invention is a human TCR comprising human variable regions according to the above embodiments and human constant regions.

[0047] In some embodiments, the antigen recognizing construct is murinized or humanized. These terms are used when amino acid sequences from a foreign species are introduced into a construct of the invention.

[0048] The TCR of the invention may be provided as a single chain TCR (scTCR). A scTCR according to the invention shall comprise in one polypeptide chain a full or partial alpha chain sequence and a full or partial beta chain sequence, preferably connected via a peptide linker. A scTCR can comprise a polypeptide of a variable region of a first TCR chain (e.g., an alpha chain) and a polypeptide of an entire (full-length) second TCR chain (e.g., a beta chain), or vice versa. Furthermore, the scTCR can optionally comprise one or more linkers which join the two or more polypeptides together. The linker can be, for instance, a peptide, which joins together two single chains, as described herein. Also provided is such a scTCR of the invention, which is fused to a human cytokine, such as IL-2, IL-7 or IL-15.

[0049] The antigen recognizing construct according to the invention can also be provided in the form of a multimeric complex, comprising at least two scTCR molecules, wherein said scTCR molecules are each fused to at least one biotin moiety, or other interconnecting molecule/linker, and wherein said scTCRs are interconnected by biotin-streptavidin interaction to allow the formation of said multimeric complex. Similar approaches known in the art for the generation of multimeric TCR are also possible and included in this disclosure. Also provided are multimeric complexes of a higher order, comprising more than two scTCR of the invention.

[0050] For the purposes of the present invention, a TCR is a moiety having at least one TCR alpha or gamma and/or TCR beta or delta variable domain. Generally, they comprise both a TCR alpha variable domain and a TCR beta variable domain, alternatively both a TCR gamma variable domain and a TCR delta variable domain. They may be αβ/γδ heterodimers or may be in single chain format. For use in adoptive therapy, an αβ or γδ heterodimeric TCR may, for example, be transfected as full-length chains having both cytoplasmic and transmembrane domains. If desired, an introduced disulfide bond between residues of the respective constant domains may be present.

[0051] In a preferred embodiment, the antigen recognizing construct is a human TCR, or fragment or derivative thereof. A human TCR or fragment or derivative thereof is a TCR, which comprises over 50% of the corresponding human TCR sequence. Preferably, only a small part of the TCR sequence is of artificial origin or derived from other species. It is known, however, that chimeric TCRs, e.g. derived from human origin with murine sequences in the constant domains, are

advantageous. Particularly preferred are, therefore, TCRs in accordance with the present invention, which contains murine sequences in the extracellular part of their constant domains.

[0052] Thus, it is also preferred that the inventive antigen recognizing construct is able to recognize its antigen in a human leucocyte antigen (HLA) dependent manner, preferably in a HLA-A*02 dependent manner. The term "HLA dependent manner" in the context of the present invention means that the antigen recognizing construct binds to the antigen only in the event that the antigenic peptide is presented by said HLA.

[0053] The antigen recognizing construct in accordance with the invention in one embodiment preferably induces an immune response, preferably wherein the immune response is characterized by the increase in interferon (IFN) γ levels.

[0054] Also, provided by the invention is a polypeptide comprising a functional portion of any of the TCRs (or functional variants thereof) described herein, for examples, of any one of the TCRs selected from R11P3D3 and R11P3D3_KE, as provided in the example section and Table 1. The term "polypeptide" as used herein includes oligopeptides and refers to a single chain of amino acids connected by one or more peptide bonds. With respect to the inventive polypeptides, the functional portion can be any portion comprising contiguous amino acids of the TCR (or functional variant thereof), of which it is a part, provided that the functional portion specifically binds to the TAA antigen, preferably as disclosed herein in Table 2, and peptides A1 to A9 (SEQ ID NOs:97, and 98-106, and the peptides T1 to T9 (SEQ ID NOs:107-115)). The term "functional portion" when used in reference to a TCR (or functional variant thereof) refers to any part or fragment of the TCR (or functional variant thereof) of the invention, which part or fragment retains the biological activity of the TCR (or functional variant thereof), of which it is a part (the parent TCR or parent functional variant thereof). Functional portions encompass, for example, those parts of a TCR (or functional variant thereof) that retain the ability to specifically bind to the TAA antigen (in an HLA dependent manner), or detect, treat, or prevent cancer, to a similar extent, the same extent, or to a higher extent, as the parent TCR (or functional variant thereof). In reference to the parent TCR (or functional variant thereof), the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent TCR variable sequences (or functional variant thereof).

[0055] The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, in which additional amino acids are not found in the amino acid sequence of the parent TCR or functional variant thereof. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, e.g., specifically binding to the TAA antigens; and/or having the ability to detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent TCR or functional variant thereof.

[0056] The polypeptide can comprise a functional portion of either or both of the α and β chains of the TCRs or functional variant thereof of the invention, such as a functional portion comprising one of more of CDR1, CDR2, CDR2bis and (preferably) CDR3 of the variable region(s) of the α chain and/or β chain of a TCR or functional variant thereof of the invention. In an embodiment of the invention, the polypeptide can comprise a functional portion comprising the amino acid sequence of SEQ ID NO: 3 and 9 (CDR3 of the variable regions of the TCR of the invention), or a combination thereof. In an embodiment of the invention, the inventive polypeptide can comprise, for instance, the variable region of the inventive TCR or functional variant thereof comprising a combination of the CDR regions set forth above. In this regard, the polypeptide can comprise the amino acid sequence of any of SEQ ID NO: 4, 10, 130, and 136 (the variable regions of an α or β chain of the TCR of the invention).

[0057] In some instances, the construct of the invention may comprise one or two polypeptide chains comprising a sequence according to any of the SEQ ID NO: 1 to 12 and 130-132, 136 to 138 and 196 (CDR sequences, constant and variable regions and full length sequences), or functional fragments thereof, and further comprise(s) other amino acid sequences, e.g., an amino acid sequence encoding an immunoglobulin or a portion thereof, then the inventive protein can be a fusion protein. In this regard, the invention also provides a fusion protein comprising at least one of the inventive polypeptides described herein along with at least one other polypeptide. The other polypeptide can exist as a separate polypeptide of the fusion protein, or can exist as a polypeptide, which is expressed in frame (in tandem) with one of the inventive polypeptides described herein. The other polypeptide may include any peptidic or proteinaceous molecule, or a portion thereof, including, but not limited to an immunoglobulin, CD3, CD4, CD8, an MHC molecule, a CD1 molecule, e.g., CD1a, CD1b, CD1c, CD1d, etc.

[0058] The fusion protein can comprise one or more copies of the inventive polypeptide and/or one or more copies of the other polypeptide. For instance, the fusion protein can comprise 1, 2, 3, 4, 5, or more, copies of the inventive polypeptide and/or of the other polypeptide. Suitable methods of making fusion proteins are known in the art, and include, for example,

recombinant methods. In some embodiments of the invention, the TCRs (and functional portions and functional variants thereof), polypeptides, and proteins of the invention may be expressed as a single protein comprising a linker peptide linking the α chain and the β chain, and linking the γ chain and the δ chain. In this regard, the TCRs (and functional variants and functional portions thereof), polypeptides, and proteins of the invention comprising the amino acid sequences of the variable regions of the TCR of the invention and may further comprise a linker peptide. The linker peptide may advantageously facilitate the expression of a recombinant TCR (including functional portions and functional variants thereof), polypeptide, and/or protein in a host cell. The linker peptide may comprise any suitable amino acid sequence. Linker sequences for single chain TCR constructs are well known in the art. Such a single chain construct may further comprise one, or two, constant domain sequences. Upon expression of the construct including the linker peptide by a host cell, the linker peptide may also be cleaved, resulting in separated α and β chains, and separated γ and δ chain.

[0059] As already mentioned above, the binding functionality of the TCR of the invention may be provided in the framework of an antibody. For example, CDR sequences of the TCR of the invention, possibly including additional 3, 2 or 1 N and/or C terminal framework residues, may be directly grafted into an antibody variable heavy/light chain sequence. The term "antibody" in its various grammatical forms is used herein to refer to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen-binding site or a paratope. Such molecules are also referred to as "antigen binding fragments" of immunoglobulin molecules. The invention further provides an antibody, or antigen binding portion thereof, which specifically binds to the antigens described herein. The antibody can be any type of immunoglobulin that is known in the art. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form.

[0060] The term "antibody" includes, but is not limited to, genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, chimeric antibodies, fully human antibodies, humanized antibodies (e.g. generated by "CDR-grafting"), antibody fragments, and heteroconjugate antibodies (e.g., bispecific antibodies, diabodies, triabodies, tetra-bodies, etc.). The term "antibody" includes cys-diabodies and minibodies. Thus, each and every embodiment provided herein in regard to "antibodies", or "antibody like constructs" is also envisioned as, bi-specific antibodies, diabodies, scFv fragments, chimeric antibody receptor (CAR) constructs, diabody and/or minibody embodiments, unless explicitly denoted otherwise. The term "antibody" includes a polypeptide of the immunoglobulin family or a polypeptide comprising fragments of an immunoglobulin that is capable of non-covalently, reversibly, and in a specific manner binding a corresponding antigen, preferably the TAA of the invention, as disclosed herein. An exemplary antibody structural unit comprises a tetramer. In some embodiments, a full-length antibody can be composed of two identical pairs of polypeptide chains, each pair having one "light" and one "heavy" chain (connected through a disulfide bond). Antibody structure and isotypes are well known to the skilled artisan (for example from Janeway's Immunobiology, 9th edition, 2016).

[0061] The recognized immunoglobulin genes of mammals include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes (for more information on immunoglobulin genes see the international Im-MunoGeneTics information system®, Lefranc M-P et al, Nucleic Acids Res. 2015 Jan;43(Database issue):D413-22; and http://www.imgt.org/). For full-length chains, the light chains are classified as either kappa or lambda. For full-length chains, the heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these regions of light and heavy chains respectively. As used in this invention, an "antibody" encompasses all variations of antibody and fragments thereof. Thus, within the scope of this concept are full length antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFv), Fab, Fab', and multimeric versions of these fragments (e.g., F(ab')2) with the same, essentially the same or similar binding specificity. In some embodiments, the anti-body binds specifically to a peptide TAA of the invention. Preferred antigen recognizing constructs according to the invention include an antibody heavy chain, preferably the variable domain thereof, or an antigen binding fragment thereof, and/or an antibody light chain, preferably the variable domain thereof, or an antigen binding fragment thereof. Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology, antibody fragments of the invention, however, are not limited to these exemplary types of antibody fragments. Also, the antibody, or antigen binding portion thereof, can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles). In some instances, the TCR CDR3 sequence may be slightly modified, but preferably by not more than 3 amino acid residues, preferably only two and most

preferably only one amino acid position, as compared to the CDR3 sequences provided in SEQ ID Nos: 3and 9. Preferably, the antibodies comprise the CDR3, preferably all of CDR1, CDR2, CDR2bis and CDR3 regions in the combination, as indicated for the TCR of the invention in Table 1, in each case independently, optionally with not more than three or two, preferably one, amino acid substitution(s), insertion(s) and/or deletion(s) compared to these sequences.

[0062] Suitable methods of making antibodies are known in the art. For instance, standard hybridoma methods are described in, e.g., Kohler and Milstein, Eur. J. Immunol, 5, 51 1-519 (1976), Harlow and Lane (eds.), Antibodies: A Laboratory Manual, CSH Press (1988), and C.A. Janeway et al. (eds.), Immunobiology, 8 Ed., Garland Publishing, New York, NY (201 1)). Alternatively, other methods, such as EBV-hybridoma methods (Haskard and Archer, J. Immunol. Methods, 74(2), 361-67 (1984), and Roder et al, Methods Enzymol, 121, 140-67 (1986)), and bacteriophage vector expression systems (see, e.g., Huse et al., Science, 246, 1275-81 (1989)) are known in the art. Further, methods of producing antibodies in non-human animals are described in, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, and U.S. Patent Application Publication No. 2002/0197266.

[0063] Some embodiments of the invention also pertain to TCRs, or functional fragments and polypeptides thereof, which are soluble TCRs. As used herein, the term "soluble T-cell receptor" refers to heterodimeric truncated variants of native TCRs, which comprise extracellular portions of the TCR α-chain and β-chain, for example linked by a disulfide bond, but which lack the transmembrane and cytosolic domains of the native protein. The terms "soluble T-cell receptor α-chain sequence and soluble T-cell receptor β-chain sequence" refer to TCR α-chain and β-chain sequences that lack the transmembrane and cytosolic domains. The sequence (amino acid or nucleic acid) of the soluble TCR α-chain and β-chains may be identical to the corresponding sequences in a native TCR or may comprise variant soluble TCR α-chain and β-chain sequences, as compared to the corresponding native TCR sequences. The term "soluble T-cell receptor" as used herein encompasses soluble TCRs with variant or non-variant soluble TCR α-chain and β-chain sequences. The variations may be in the variable or constant regions of the soluble TCR α-chain and β-chain sequences and can include, but are not limited to, amino acid deletion, insertion, substitution mutations as well as changes to the nucleic acid sequence, which do not alter the amino acid sequence. Soluble TCR of the invention in any case retain the binding functionality of their parent molecules.

[0064] The above problem is further solved by a nucleic acid encoding for an antigen recognizing construct of the invention, or any of the aforementioned protein or polypeptide constructs. The nucleic acid preferably (a) has a strand encoding for an antigen recognizing construct according to the invention; (b) has a strand complementary to the strand in (a); or (c) has a strand that hybridizes under stringent conditions with a molecule as described in (a) or (b). Stringent conditions are known to the person of skill in the art, specifically from Sambrook et al, "Molecular Cloning". In addition to that, the nucleic acid optionally has further sequences, which are necessary for expressing the nucleic acid sequence corresponding to the protein, specifically for expression in a mammalian/human cell. The nucleic acid used can be contained in a vector suitable for allowing expression of the nucleic acid sequence corresponding to the peptide in a cell. However, the nucleic acids can also be used to transform an antigen-presenting cell, which may not be restricted to classical antigen-presenting cells, such as dendritic cells, in such a way that they themselves produce the corresponding proteins on their cellular surface.

[0065] In some embodiments, the polypeptides of the antigen recognizing constructs can be encoded by nucleic acids and expressed *in vivo or in vitro*. Thus, in some embodiments, a nucleic acid encoding an antigen recognizing construct is provided. In some embodiments, the nucleic acid encodes one part or monomer of an antigen recognizing construct of the invention (for example one of two chains of a TCR of the invention), and/or another nucleic acid encodes another part or monomer of an antigen recognizing construct of the invention (for example the other of two chains of the TCR). In some embodiments, the nucleic acid encodes two or more antigens recognizing construct polypeptide chains, for example, at least 2 TCR chains. Nucleic acids encoding multiple antigen recognizing construct chains can include nucleic acid cleavage sites between at least two chain sequences, can encode transcription or translation start site between two or more chains sequences, and/or can encode proteolytic target sites between two or more antigen recognizing construct chains.

[0066] "Nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide.

[0067] Preferably, the nucleic acids of the invention are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

For purposes, herein, the replication can be *in vitro* replication or *in vivo* replication. The nucleic acid can comprise any nucleotide sequence, which encodes any of the TCRs, polypeptides, or proteins, or functional portions or functional variants thereof described herein.

[0068] Furthermore, the invention provides a vector comprising a nucleic acid in accordance to the invention as described above. Desirably, the vector is an expression vector or a recombinant expression vector. The term "recombinant expression vector" refers in context of the present invention to a nucleic acid construct that allows for the expression of an mRNA, protein or polypeptide in a suitable host cell. The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. Examples of animal expression vectors include pEUK-Cl, pMAM, and pMAMneo. Preferably, the recombinant expression vector is a viral vector, e.g., a retroviral vector. The recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal), into which the vector is to be introduced and in which the expression of the nucleic acid of the invention may be performed. Furthermore, the vector of the invention may include one or more marker genes, which allow for selection of transformed or transfected hosts. The recombinant expression vector can comprise a native or normative promoter operably linked to the nucleotide sequence encoding the constructs of the invention, or to the nucleotide sequence, which is complementary to or which hybridizes to the nucleotide sequence encoding the constructs of the invention. The selections of promoters include, e.g., strong, weak, inducible, tissue-specific and developmental-specific promoters. The promoter can be a non-viral promoter or a viral promoter. The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0069] The invention also pertains to a host cell comprising an antigen recognizing construct in accordance with the invention. Specifically, the host cell of the invention comprises a nucleic acid, or a vector as described herein above. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. For purposes of producing a recombinant TCR, polypeptide, or protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell preferably is a peripheral blood leukocyte (PBL) or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell. The T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal, preferably a T cell or T cell precursor from a human patient. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. Preferably, the T cell is a human T cell. More preferably, the T cell is a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4-positive and/or CD8- positive, CD4-positive helper T cells, e.g., Th1 and Th2 cells, CD8-positive T cells (e.g., cytotoxic T cells), tumor infiltrating cells (TILs), memory T cells, naive T cells, and the like. Preferably, the T cell is a CD8-positive T cell or a CD4-positive T cell.

[0070] Preferably, the host cell of the invention is a lymphocyte, preferably, a T lymphocyte, such as a CD4-positive or CD8-positive T-cell. The host cell furthermore preferably is a tumor reactive T cell specific for TAA expressing tumor cells.

[0071] The objective of the invention is also solved by a method of manufacturing a TAA specific antigen recognizing construct, or of a TAA specific antigen recognizing construct expressing cell line, comprising

1. a. Providing a suitable host cell,
2. b. Providing a genetic construct comprising a coding sequence encoding for an antigen recognizing construct according to the herein disclosed invention,
3. c. Introducing into said suitable host cell said genetic construct, and
4. d. Expressing said genetic construct by said suitable host cell.

[0072] The method may further comprise a step of cell surface presentation of said antigen recognizing construct on said suitable host cell.

[0073] In other preferred embodiments, the genetic construct is an expression construct comprising a promoter sequence

operably linked to said coding sequence. Preferably, said antigen recognizing construct is of mammalian origin, preferably of human origin. The preferred suitable host cell for use in the method of the invention is a mammalian cell, such as a human cell, in particular a human T lymphocyte. T cells for use in the invention are described in detail herein above.

[0074] Also, encompassed by the invention are embodiments, wherein said antigen recognizing construct is a modified TCR, wherein said modification is the addition of functional domains, such as a label or a therapeutically active substance. Furthermore, encompassed are TCR having alternative domains, such as an alternative membrane anchor domain instead of the endogenous transmembrane region. Also, encompassed are TCR having point mutations in the TCR variable domain or constant domain in order to improve TCR expression or stability and/or chain pairing.

[0075] Desirably, the transfection system for introducing the genetic construct into said suitable host cell is a retroviral vector system. Such systems are well known to the skilled artisan.

[0076] Also, comprised by the present invention is in one embodiment the additional method step of isolation and purification of the antigen recognizing construct from the cell and, optionally, the reconstitution of the translated antigen recognizing construct-fragments in a T-cell.

[0077] In an alternative aspect of the invention a T-cell is provided obtained or obtainable by a method for the production of a T cell receptor (TCR), which is specific for tumorous cells and has high avidity as described herein above. Such a T cell is depending on the host cell used in the method of the invention, for example, a human or non-human T-cell, preferably a human TCR.

[0078] The term "isolated" as used herein in the context of a polypeptide, such as an antigen recognizing construct (an example of which could be an antibody), refers to a polypeptide that is purified from proteins or polypeptides or other contaminants that would interfere with its therapeutic, diagnostic, prophylactic, research or other use. An antigen recognizing construct according to the invention may be a recombinant, synthetic or modified (non-natural) antigen binding construct. The term "isolated" as used herein in the context of a nucleic acid or cells refers to a nucleic acid or cells that is/are purified from DNA, RNA, proteins or polypeptides or other contaminants (such as other cells) that would interfere with its therapeutic, diagnostic, prophylactic, research or other use, or it refers to a recombinant, synthetic or modified (non-natural) nucleic acid. In this context, a "recombinant" protein/polypeptide or nucleic acid is one made using recombinant techniques. Methods and techniques for the production of recombinant nucleic acids and proteins are well known in the art.

[0079] One additional aspect of the present invention relates to the herein disclosed antigen recognizing constructs, nucleic acids, vectors, pharmaceutical compositions and/or host cell for use in medicine. The use in medicine in one preferred embodiment includes the use in the diagnosis, prevention and/or treatment of a tumor disease, such as a malignant or benign tumor disease. The tumor disease is, for example, a tumor disease characterized by the expression of the TAA, in a cancer or tumor cell of said tumor disease.

[0080] With respect to the above mentioned medical applications of the antigen recognizing constructs and other materials derived therefrom, pertaining thereto or encoding the same, in accordance of the present disclosure, the to be treated and/or to be diagnosed diseases can be any proliferative disorder, preferably characterized by the expression of the TAA or TAA epitope sequence of the invention, for example any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vagina, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, glioma, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, cancer of the oropharynx, ovarian cancer, cancer of the penis, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, cancer of the uterus, ureter cancer, and urinary bladder cancer. A preferred cancer is cancer of the uterine cervix, oropharynx, anus, anal canal, anorectum, vagina, vulva, or penis. A particularly preferred cancer is a TAA positive cancer, including preferably ovarian carcinoma, leukemia or melanoma.

[0081] The constructs, proteins, TCRs antibodies, polypeptides and nucleic acids of the invention are in particular for use in immune therapy, preferably, in adoptive T cell therapy. The administration of the compounds of the invention can, for example, involve the infusion of T cells of the invention into said patient. Preferably, such T cells are autologous T cells of the patient and *in vitro* transduced with a nucleic acid or antigen recognizing construct of the present invention.

[0082] The inventive antigen recognizing constructs, TCRs, polypeptides, proteins (including functional variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), all of which are collectively referred to as "inventive TCR materials" hereinafter, can be formulated into a composition, such as a pharmaceutical composition. In this regard, the invention provides a pharmaceutical composition comprising any of the antigen recognizing constructs, TCRs, polypeptides, proteins, functional portions, functional variants, nucleic acids, expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof) described herein, and a pharmaceutically acceptable carrier, excipient and/or stabilizer. The inventive pharmaceutical compositions containing any of the inventive TCR materials can comprise more than one inventive TCR material, e.g., a polypeptide and a nucleic acid, or two or more different TCRs (including functional portions and functional variants thereof). Alternatively, the pharmaceutical composition can comprise an inventive TCR material in combination with another pharmaceutically active agent(s) or drug(s), such as chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc. Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used for the particular inventive TCR material under consideration. Such pharmaceutically acceptable carriers are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one, which has no detrimental side effects or toxicity under the conditions of use.

[0083] Thus, also provided is a pharmaceutical composition, comprising any of the herein described products of the invention and TCR materials of the invention, specifically any proteins, nucleic acids or host cells. In a preferred embodiment, the pharmaceutical composition is suitable for use in immune therapy, preferably adoptive cell therapy.

[0084] Preferably, the inventive TCR material is administered by injection, e.g., intravenously. When the inventive TCR material is a host cell expressing the inventive TCR (or functional variant thereof), the pharmaceutically acceptable carrier for the cells for injection may include any isotonic carrier such as, for example, normal saline (about 0.90% w/v of NaCl in water, about 300 mOsm/L NaCl in water, or about 9.0 g NaCl per liter of water), NORMOSOL R electrolyte solution (Abbott, Chicago, IL), PLASMA-LYTE A (Baxter, Deerfield, IL), about 5% dextrose in water, or Ringer's lactate. In an embodiment, the pharmaceutically acceptable carrier is supplemented with human serum albumen.

[0085] For purposes of the invention, the amount or dose (e.g., numbers of cells when the inventive TCR material is one or more cells) of the inventive TCR material administered may be sufficient to affect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the inventive TCR material should be sufficient to bind to a cancer antigen, or detect, treat or prevent cancer in a period of from about 2 hours or longer, e.g., 12 to 24 or more hours, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive TCR material and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[0086] It is contemplated that the inventive pharmaceutical compositions, antigen recognizing constructs, TCRs (including functional variants thereof), polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells are suitable for use in treating or preventing cancer, or TAA-positive premalignancy. The inventive TCRs (and functional variants thereof) are believed to bind specifically to the TAA of the invention, such that the TCR (or related inventive polypeptide or protein and functional variants thereof), when expressed by or on a cell, such as a T cell, is able to mediate an immune response against a target cell expressing the TAA of the invention, preferably presenting TAA peptides via MHC I or II on the surface of said target cell. In this regard, the invention provides pharmaceutical compositions, antigen recognizing constructs, TCRs (including functional variants thereof), polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells for use in treating or preventing a condition, in particular cancer, in a mammal, comprising administering to the mammal any of the pharmaceutical compositions, antigen recognizing constructs, in particular TCRs (and functional variants thereof), polypeptides, or proteins described herein, any nucleic acid or recombinant expression vector comprising a nucleotide sequence encoding any of the TCRs (and functional variants thereof), polypeptides, proteins described herein, or any host cell or population of cells comprising a nucleic acid or recombinant vector, which encodes any of the constructs of the invention (and functional variants thereof), polypeptides, or proteins described herein, in an amount effective for use in treating or preventing the condition in the mammal, wherein the condition is preferably cancer, such as a cancer expressing the TAA of the invention.

[0087] Examples of pharmaceutically acceptable carriers or diluents useful in the present invention include stabilizers such as SPGA, carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein,

protein containing agents such as bovine serum or skimmed milk and buffers (e.g. phosphate buffer).

[0088] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of a condition in a mammal. Furthermore, the inventive use for treating or preventing can include treating or preventing of one or more conditions or symptoms of the condition, e.g., cancer. For example, treating or preventing can include promoting the regression of a tumor. Also, for purposes herein, "prevention" can encompass delaying the onset of the condition, or a symptom or condition thereof.

[0089] The present invention also relates to a TCR, a nucleic acid, or a host cell of the present description in combination with at least one chemotherapeutic agent and/or radiation therapy for use in treating cancer comprising administering said TCR, nucleic acid, or host cell in combination with at least one chemotherapeutic agent and/or radiation therapy.

[0090] Another aspect of the invention further pertains to a method for detecting a TAA protein, or a complex of MHC and the TAA protein (protein epitope of the TAA), in a (biological) sample - such as one obtained from a subject or patient comprising contacting the sample with an antigen recognizing construct specifically binding to said TAA peptide, or to the TAA peptide/MHC complex, and detecting the binding between said antigen recognizing construct and said TAA peptide, or to the TAA peptide/MHC complex. In some embodiments, the antigen recognizing construct is a TCR or antibody, or similar constructs, or preferably the antigen recognizing construct according to the herein described invention. In some embodiments, the (biological) sample is a sample of a tumor or a cancer (such as one of those described elsewhere herein) for example a sample comprising tumor or cancer cells.

[0091] Also provided is a plurality of transformed cells for use in treating cancer in a subject in need thereof, wherein the plurality of transformed cells are obtained by a method comprising the following steps:

a) isolating a cell from said subject;

b) transforming the cell with at least one vector encoding an antigen recognizing construct of the present invention to produce a transformed cell;

c expanding the transformed cell to produce a plurality of transformed cells; and

d) administering the plurality of transformed cells to said subject.

[0092] Also provided is a plurality of transformed cell for use in treating cancer in a subject in need thereof, wherein the plurality of transformed cells are obtained by a method comprising the following steps:

1. a) isolating a cell from a healthy donor;
2. b) transforming the cell with a vector encoding an antigen recognizing construct of the present invention to produce a transformed cell;
3. c) expanding the transformed cell to produce a plurality of transformed cells; and
4. d) administering the plurality of transformed cells to said subject.

[0093] Also provided is a method of detecting cancer in a biological sample comprising:

1. a) contacting the biological sample with an antigen recognizing construct of the present description;
2. b) detecting binding of the antigen recognizing construct to the biological sample.

[0094] In some embodiments, the method of detecting cancer is carried out *in vitro* or *in situ*.

[0095] Also provided is a method of detecting the presence of a condition in a mammal. The method comprises contacting a sample comprising one or more cells from the mammal with any of the inventive TCRs (and functional variants thereof), polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, antibodies, or antigen binding portions thereof, or pharmaceutical compositions described herein, thereby forming a complex, and detecting the

complex, wherein detection of the complex is indicative of the presence of the condition in the mammal, wherein the condition is cancer, such as a TAA expressing malignancy.

[0096] With respect to the inventive method of detecting a condition in a mammal, the sample of cells can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction.

[0097] For purposes of the inventive detection method, the contacting is *in vitro*.

[0098] Also, detection of the complex can occur through any number of ways known in the art. For instance, the inventive antigen recognizing constructs (and functional variants thereof), polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies or TCRs, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0099] For purposes of the inventive uses, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal.

[0100] With respect to the above mentioned medical applications of the TCR material of the invention, and their use in treating and/or diagnosing cancer, the to be treated and/or diagnosed cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vagina, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, glioma, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, cancer of the oropharynx, ovarian cancer, cancer of the penis, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, cancer of the uterus, ureter cancer, and urinary bladder cancer. A preferred cancer is cancer is cancer of the uterine cervix, oropharynx, anus, anal canal, anorectum, vagina, vulva, or penis. A particularly preferred cancer is a TAA positive cancer, such as a PRAME expressing cancer, for example ovarian carcinoma, melanoma or leukemia.

[0101] In general, the invention provides antigen recognizing constructs, nucleic acids, vectors, pharmaceutical compositions and/or host cell as disclosed by the present invention for use in treating a subject suffering from a tumor or tumor disease comprising the administration of said antigen recognizing constructs, nucleic acids, vectors, pharmaceutical compositions and/or host cell. Preferably the subject is a subject in need of such a treatment. The subject in preferred embodiments is a mammalian subject, preferably a human patient, suffering from a tumor or tumor disease, which is TAA-positive.

[0102] The present invention will now be further described in the following examples with reference to the accompanying figures and sequences. The Figures and Sequences show:

Figure 1: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R11P3D3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Several different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine- substitution variants (Thr_TCRA-0036).

Figure 2: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1C10 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Several different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine- substitution variants

(Thr_TCRA-0036).

Figure 3: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1E8 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Several different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine- substitution variants (Thr_TCRA-0036).

Figure 4: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1A9 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-106) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed with regard to alanine-substitution variants (Ala_IFN-040 and Ala_IFN-041).

Figure 5: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1D7 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine-substitution variants (Thr_TCRA-0036).

Figure 6: IFNγ release from CD8+ T- cells electroporated with alpha and beta chain RNA of TCR R17P1G3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T- cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine-substitution variants (Thr_TCRA-0036).

Figure 7: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P2B6 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or various PRAME-004 alanine- or threonine-substitution variants at positions 1-9 (X1-X9) of SEQ ID NO:97 (SEQ ID NO:98-115) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed with regard to alanine-substitution (Ala_TCRA-0017 and Ala_IFN-041) and threonine-substitution variants (Thr_TCRA-0036).

Figure 8: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R11P3D3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-040 and IFN-041.

Figure 9: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1C10 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-046 and IFN-041.

Figure 10: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1E8 (Table 1) after

co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-040 and IFN-041.

Figure 11: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1A9 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-040 and IFN-041.

Figure 12: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1D7 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-040 and IFN-041.

Figure 13: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1G3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-046 and IFN-041.

Figure 14: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P2B6 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) or similar but unrelated peptide TMED9-001 (SEQ ID NO:116), CAT-001 (SEQ ID NO:117), DDX60L-001 (SEQ ID NO:118), LRRC70-001 (SEQ ID NO:119), PTPLB-001 (SEQ ID NO:120), HDAC5-001 (SEQ ID NO: 121), VPS13B-002 (SEQ ID NO:122), ZNF318-001 (SEQ ID NO:123), CCDC51-001 (SEQ ID NO:124) or IFIT1-001 (SEQ ID NO:125) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. RNA electroporated CD8+ T-cells alone or in co-incubation with unloaded target cells served as controls. Different donors were analyzed, IFN-040 and IFN-041.

Figure 15: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R11P3D3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10μM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 16: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1C10 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10μM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 17: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1E8 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10μM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 18: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1D7 (Table 1) after

co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10µM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 19: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P1G3 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10µM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 20: IFNγ release from CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R17P2B6 (Table 1) after co-incubation with T2 target cells loaded with PRAME-004 peptide (SEQ ID NO:97) in various peptide loading concentrations from 10µM to 10pM. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors. Different donors were analyzed, TCRA-0003 and TCRA-0017.

Figure 21: HLA-A*02/PRAME-004 tetramer or HLA-A*02/NYES01-001 tetramer staining, respectively, of CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R16P1C10 (Table 1). CD8+ T-cells electroporated with RNA of 1G4 TCR (SEQ ID: 85-96) that specifically binds to the HLA-A*02/NYES01-001 complex and mock electroporated CD8+ T-cells served as controls.

Figure 22: IFNγ release from CD8+ T-cells lentivirally transduced with TCR R11P3D3 (Table 1) (D103805 and D191451) or non-transduced cells (D103805 NT and D191451 NT) after co-incubation with T2 target cells loaded with 100nM PRAME-004 peptide (SEQ ID NO:97) or similar (identical to PRAME-004 in positions 3, 5, 6 and 7) but unrelated peptides ACPL-001 (SEQ ID NO:139), HSPB3-001 (SEQ ID NO:140), UNC7-001 (SEQ ID NO: 141), SCYL2-001 (SEQ ID NO:142), RPS2P8-001 (SEQ ID NO:143), PCNXL3-003 (SEQ ID NO:144), AQP6-001 (SEQ ID NO:145), PCNX-001 (SEQ ID NO:146), AQP6-002 (SEQ ID NO:147) TRGV10-001 (SEQ ID NO:148), NECAP1-001 (SEQ ID NO:149) or FBXW2-001 (SEQ ID NO:150) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, D103805 and D191451.

Figure 23: IFNγ release from CD8+ T-cells lentivirally transduced with TCR R11 P3D3 (Table 1) after co-incubation with T2 target cells loaded with 100nM PRAME-004 peptide (SEQ ID NO:97) or similar (identical to PRAME-004 in positions 3, 5, 6 and 7) but unrelated peptides (SEQ ID NO:151-195) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, TCRA-0087 and TCRA-0088.

Figure 24: IFNγ release from CD8+ T-cells lentivirally transduced with TCR R11P3D3 (Table 1) (D103805 and D191451) or non-transduced cells (D103805 NT and D191451 NT) after co-incubation with different primary cells (HCASMC (Coronary artery smooth muscle cells), HTSMC (Tracheal smooth muscle cells), HRCEpC (Renal cortical epithelial cells), HCM (Cardiomyocytes), HCMEC (Cardiac microvascular endothelial cells), HSAEpC (Small airway epithelial cells), HCF (Cardiac fibroblasts)) and iPSC-derived cell types (HN (Neurons), iHCM (Cardiomyocytes), HH (Hepatocytes), HA (astrocytes)). Tumor cell lines UACC-257 (PRAME-004 high), Hs695T (PRAME-004 medium), U266B1 (PRAME-004 very low) and MCF-7 (no PRAME-004) present different amounts of PRAME-004 per cells. T-cells alone served as controls. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, D103805 and D191451.

Figure 25: IFNγ release from CD8+ T-cells lentivirally transduced with TCR R11P3D3 (Table 1) after co-incubation with different primary cells (NHEK (Epidermal keratinocytes), HBEpC (Bronchial epithelial cells), HDMEC (Dermal microvascular endothelial cells), HCAEC (Coronary artery endothelial cells), HAoEC (Aortic endothelial cells), HPASMC (Pulmonary artery smooth muscle cells), HAoSMC (Aortic smooth muscle cells), HPF (Pulmonary fibroblasts), SkMC (Skeletal muscle cells), HOB (osteoblasts), HCH (Chondrocytes), HWP (White preadipocytes), hMSC-BM (Mesenchymal stem cells), NHDF (Dermal fibroblasts). Tumor cell lines UACC-257 (PRAME-004 high), Hs695T (PRAME-004 medium), U266B1 (PRAME-004 very low) and MCF-7 (no PRAME-004) present different copies of PRAME-004 per cells. T-cells alone served as controls. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, TCRA-0084 and TCRA-0085.

Figure 26: IFNγ release from CD8+ T-cells lentivirally transduced with enhanced TCR R11P3D3_KE (Table 1) (D103805 and D191451) or non-transduced cells (D103805 NT and D191451 NT) after co-incubation with T2 target cells loaded with 100nM PRAME-004 peptide (SEQ ID NO:97) or similar (identical to PRAME-004 in positions 3, 5, 6 and 7) but unrelated peptide ACPL-001 (SEQ ID NO:139), HSPB3-001 (SEQ ID NO:140), UNC7-001 (SEQ ID NO: 141), SCYL2-001 (SEQ ID NO:142), RPS2P8-001 (SEQ ID NO:143), PCNXL3-003 (SEQ ID NO:144), AQP6-001 (SEQ ID NO:145), PCNX-001 (SEQ ID NO:146), AQP6-002 (SEQ ID NO:147), TRGV10-001 (SEQ ID NO:148), NECAP1-001 (SEQ ID NO:149) or FBXW2-001 (SEQ ID NO:150) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, D103805 and D191451.

Figure 27: IFNγ release from CD8+ T-cells lentivirally transduced with enhanced TCR R11 P3D3_KE (Table 1) after co-

incubation with T2 target cells loaded with 100nM PRAME-004 peptide (SEQ ID NO:97) or similar (identical to PRAME-004 in positions 3, 5, 6 and 7) but unrelated peptides (SEQ ID NO:151-195) or control peptide NYESO1-001 (SEQ ID NO:126). IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, TCRA-0087 and TCRA-0088.

Figure 28: IFNγ release from CD8+ T-cells lentivirally transduced with enhanced TCR R11P3D3_KE (Table 1) (D103805 and D191451) or non-transduced cells (D103805 NT and D191451 NT) after co-incubation with different primary cells (HCASMC (Coronary artery smooth muscle cells), HTSMC (Tracheal smooth muscle cells), HRCEpC (Renal cortical epithelial cells), HCM (Cardiomyocytes), HCMEC (Cardiac microvascular endothelial cells), HSAEpC (Small airway epithelial cells), HCF (Cardiac fibroblasts)) and iPSC-derived cell types (HN (Neurons), iHCM (Cardiomyocytes), HH (Hepatocytes), HA (astrocytes)). Tumor cell lines UACC-257 (PRAME-004 high), Hs695T (PRAME-004 medium), U266B1 (PRAME-004 very low) and MCF-7 (no PRAME-004) present different amounts of PRAME-004 per cells. T-cells alone served as controls. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, D103805 and D191451.

Figure 29: IFNγ release from CD8+ T-cells lentivirally transduced with enhanced TCR R11P3D3_KE (Table 1) after co-incubation with different primary cells (NHEK (Epidermal keratinocytes), HBEpC (Bronchial epithelial cells), HDMEC (Dermal microvascular endothelial cells), HCAEC (Coronary artery endothelial cells), HAoEC (Aortic endothelial cells), HPASMC (Pulmonary artery smooth muscle cells), HAoSMC (Aortic smooth muscle cells), HPF (Pulmonary fibroblasts), SkMC (Skeletal muscle cells), HOB (osteoblasts), HCH (Chondrocytes), HWP (White preadipocytes), hMSC-BM (Mesenchymal stem cells), NHDF (Dermal fibroblasts). Tumor cell lines UACC-257 (PRAME-004 high), Hs695T (PRAME-004 medium), U266B1 (PRAME-004 very low) and MCF-7 (no PRAME-004) present different copies of PRAME-004 per cells. T-cells alone served as controls. IFNγ release data were obtained with CD8+ T-cells derived from two different healthy donors, TCRA-0084 and TCRA-0085.

Figure 30: IFNγ release from CD8+ T-cells lentivirally transduced with TCR R11P3D3 or enhanced TCR R11P3D3_KE (Table 1) or non-transduced cells after co-incubation with tumor cell lines UACC-257 (PRAME-004 high), Hs695T (PRAME-004 medium), U266B1 (PRAME-004 very low) and MCF-7 (no PRAME-004) present different amounts of PRAME-004 per cells. T-cells alone served as controls. IFNγ release of both TCRs correlates with PRAME-004 presentation and R11P3D3_KE induces higher responses compared to R11P3D3.

Figure 31: IFNγ release from CD8+ T-cells lentivirally transduced with enhanced TCR R11P3D3_KE (Table 1) cells after co-incubation with T2 target cells loaded with various PRAME-004 alanine-substitution variants at positions 1-9 (A1-A9) of SEQ ID NO:97 (SEQ ID NO:98-106). IFNγ release data were obtained with CD8+ T-cells derived from three different healthy donors.

Figure 32: Potency assay evaluating cytolytic activity of lentivirally transduced T cells expressing TCR R11P3D3 or enhanced TCR R11P3D3_KE against PRAME-004+ tumor cells. Cytotoxic response of R11P3D3 and R11P3D3_KE transduced and non-transduced (NT) T cells measured against A-375 (PRAME-004 low) or U2OS (PRAME-004 medium) tumor cells. The assays were performed in a 72-hour fluorescence microscopy-based cytotoxicity assay. Results are shown as fold tumor growth over time.

Table 1: TCR sequences of the invention

SEQ ID NO:	TCR	Chain	Region	Sequence
1	R11P3D3	alpha	CDR1	SSNFYA
2	R11P3D3	alpha	CDR2	MTL
3	R11P3D3	alpha	CDR3	CALYNNNDMRF
4	R11P3D3	alpha	variable domain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRWETAKSPEA LFVMTLNGDEKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYNNNDMRFGAGTRLTVKP
5	R11P3D3	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
6	R11P3D3	alpha	full-length	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRWETAKSPEA LFVMTLNGDEKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYNNNDMRFGAGTRLTVKPNIQNP DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA

SEQ ID NO:	TCR	Chain	Region	Sequence
				CANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
7	R11P3D3	beta	CDR1	SGHNS
8	R11P3D3	beta	CDR2	FNNNVP
9	R11P3D3	beta	CDR3	CASSPGSTDTQYF
10	R11P3D3	beta	variable domain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRQTMMRGLELLIYF NNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPGSTDTQYFGPGTRLTVL
11	R11P3D3	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
12	R11P3D3	beta	full-length	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRQTMMRGLELLIYF NNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPGSTDTQYFGPGTRLTVLEDLK NVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALND SRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
13	R16P1C10	alpha	CDR1	DRGSQS
14	R16P1C10	alpha	CDR2	IY
15	R16P1C10	alpha	CDR3	CAAVISNFGNEKLTF
16	R16P1C10	alpha	variable domain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAVISNFGNEKLTFGTGTRLTIIP
17	R16P1C10	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
18	R16P1C10	alpha	full-length	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAVISNFGNEKLTFGTGTRLTIIPNIQN PDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDF ACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
19	R16P1C10	beta	CDR1	SGHRS
20	R16P1C10	beta	CDR2	YFSETQ
21	R16P1C10	beta	CDR3	CASSPWDSPNEQYF
22	R16P1C10	beta	variable domain	MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFE YFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSPWDSPNEQYFGPGTRLTVT
23	R16P1C10	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
24	R16P1C10	beta	full-length	MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRSVSWYQQTPGQGLQFLFE YFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSPWDSPNEQYFGPGTRLTVTE DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPAL NDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTS ESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
25	R16P1E8	alpha	CDR1	NSAFQY

SEQ ID NO:	TCR	Chain	Region	Sequence
26	R16P1E8	alpha	CDR2	TY
27	R16P1E8	alpha	CDR3	CAMSEAAGNKLTF
28	R16P1E8	alpha	variable domain	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPEGAIVSLNCTYSNSAFQYFMWYRQYSRKGP ELLMYTYSSGNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMSEAAGNKLTFGGGTRVLVKP
29	R16P1E8	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
30	R16P1 E8	alpha	full-length	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPEGAIVSLNCTYSNSAFQYFMWYRQYSRKGP ELLMYTYSSGNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMSEAAGNKLTFGGGTRVLVKPNI QNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKS DFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWS S
31	R16P1E8	beta	CDR1	SGHAT
32	R16P1E8	beta	CDR2	FQNNGV
33	R16P1E8	beta	CDR3	CASSYTNQGEAFF
34	R16P1E8	beta	variable domain	MGTRLLCWAALCLLGAELTEAGVAQSPRYKIIEKRQSVAFWCNPISGHATLYWYQQILGQGPKLLIQFQ NNGVVDDSQLPKDRFSAERLKGVDSTLKIQPAKLEDSAVYLCASSYTNQGEAFFGQGTRLTVV
35	R16P1E8	beta	constant domain	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPA LNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
36	R16P1E8	beta	full-length	MGTRLLCWAALCLLGAELTEAGVAQSPRYKIIEKRQSVAFWCNPISGHATLYWYQQILGQGPKLLIQFQ NNGVVDDSQLPKDRFSAERLKGVDSTLKIQPAKLEDSAVYLCASSYTNQGEAFFGQGTRLTVVEDLNK VFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDS RYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSY QQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
37	R17P1A9	alpha	CDR1	DRGSQS
38	R17P1A9	alpha	CDR2	IY
39	R17P1A9	alpha	CDR3	CAVLNQAGTALIF
40	R17P1A9	alpha	variable domain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMSIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVLNQAGTALIFGKGTTLSVSS
41	R17P1A9	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
42	R17P1A9	alpha	full-length	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMSIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVLNQAGTALIFGKGTTLSVSSNIQNP DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
43	R17P1A9	beta	CDR1	SGDLS
44	R17P1A9	beta	CDR2	YYNGEE
45	R17P1A9	beta	CDR3	CASSAETGPWLGNEQFF
46	R17P1A9	beta	variable	

SEQ ID NO:	TCR	Chain	Region	Sequence
			domain	MGFRLLCCVAFCLLGAGPVDSGVTQTPKHLITATGQRVTLRCSPRSGDLSVYWYQQSLDQGLQFLIQY YNGEERAKGNILERFSAQQFPDLHSELNLSSLELGDSALYFCASSAETGPWLGNEQFFGPGTRLTVL
47	R17P1A9	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
48	R17P1A9	beta	full-length	MGFRLLCCVAFCLLGAGPVDSGVTQTPKHLITATGQRVTLRCSPRSGDLSVYWYQQSLDQGLQFLIQY YNGEERAKGNILERFSAQQFPDLHSELNLSSLELGDSALYFCASSAETGPWLGNEQFFGPGTRLTVLE DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPAL NDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTS ESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
49	R17P1D7	alpha	CDR1	TSESDYY
50	R17P1D7	alpha	CDR2	QEAY
51	R17P1D7	alpha	CDR3	CAYRWAQGGSEKLVF
52	R17P1D7	alpha	variable domain	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQMILV IRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRWAQGGSEKLVFGKGTKLTVN P
53	R17P1D7	alpha	constant domain	YIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
54	R17P1D7	alpha	full-length	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQMILV IRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRWAQGGSEKLVFGKGTKLTVN PYIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWS NKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR LWSS
55	R17P1D7	beta	CDR1	MGHDK
56	R17P1D7	beta	CDR2	SYGVNS
57	R17P1D7	beta	CDR3	CATELWSSGGTGELFF
58	R17P1D7	beta	variable domain	MTIRLLCYMGFYFLGAGLMEADIYQTPRYLVIGTGKKITLECSQTMGHDKMYWYQQDPGMELHLIHYS YGVNSTEKGDLSSESTVSRIRTEHFPLTLESARPSHTSQYLCATELWSSGGTGELFFGEGSRLTVL
59	R17P1D7	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
60	R17P1D7	beta	full-length	MTIRLLCYMGFYFLGAGLMEADIYQTPRYLVIGTGKKITLECSQTMGHDKMYWYQQDPGMELHLIHYS YGVNSTEKGDLSSESTVSRIRTEHFPLTLESARPSHTSQYLCATELWSSGGTGELFFGEGSRLTVLED LKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALN DSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSE SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
61	R17P1G3	alpha	CDR1	DRGSQS
62	R17P1G3	alpha	CDR2	IY
63	R17P1G3	alpha	CDR3	CAVGPSGTYKYIF
64	R17P1G3	alpha	variable domain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMSIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVGPSGTYKYIFGTGTRLKVLA

SEQ ID NO:	TCR	Chain	Region	Sequence
65	R17P1G3	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
66	R17P1G3	alpha	full-length	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMSIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVGPSGTYKYIFGTGTRLKVLANIQNP DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
67	R17P1G3	beta	CDR1	MNHEY
68	R17P1G3	beta	CDR2	SMNVEV
69	R17P1G3	beta	CDR3	CASSPGGSGNEQFF
70	R17P1G3	beta	variable domain	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASSPGGSGNEQFFGPGTRLTVL
71	R17P1G3	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
72	R17P1G3	beta	full-length	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASSPGGSGNEQFFGPGTRLTVLEDL KNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALN DSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSE SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
73	R17P2B6	alpha	CDR1	DRGSQS
74	R17P2B6	alpha	CDR2	IY
75	R17P2B6	alpha	CDR3	CAVVSGGGADGLTF
76	R17P2B6	alpha	variable domain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVVSGGGADGLTFGKGTHLIIQP
77	R17P2B6	alpha	constant domain	YIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
78	R17P2B6	alpha	full-length	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPEL IMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVVSGGGADGLTFGKGTHLIIQPYIQK PDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDF ACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
79	R17P2B6	beta	CDR1	PRHDT
80	R17P2B6	beta	CDR2	FYEKMQ
81	R17P2B6	beta	CDR3	CASSLGRGGQPQHF
82	R17P2B6	beta	variable domain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPRHLIKEKRETATLKCYPIPRHDTVYWYQQGP GQDPQFLISFYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDSALYFCASSLGRGGQPQHFGD GTRLSIL
83	R17P2B6	beta	constant domain	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPA LNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT

SEQ ID NO:	TCR	Chain	Region	Sequence
				SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
84	R17P2B6	beta	full-length	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPRHLIKEKRETATLKCYPIPRHDTVYWYQQGP GQDPQFLISFYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDSALYFCASSLGRGGQPQHFGD GTRLSILEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWG RADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
85	1G4	alpha	CDR1	DSAIYN
86	1G4	alpha	CDR2	IQS
87	1G4	alpha	CDR3	CAVRPTSGGSYIPTF
88	1G4	alpha	variable domain	METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLI QSSQREQTSGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVRPTSGGSYIPTFGRGTSLIVHP
89	1G4	alpha	constant domain	YIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
90	1G4	alpha	full-length	METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLI QSSQREQTSGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVRPTSGGSYIPTFGRGTSLIVHPYIQNP DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
91	1G4	beta	CDR1	MNHEY
92	1G4	beta	CDR2	SVGAGI
93	1G4	beta	CDR3	CASSYVGNTGELFF
94	1G4	beta	variable domain	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIH YSVGAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVL
95	1G4	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
96	1G4	beta	full-length	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIH YSVGAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVLED LKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALN DSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSE SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
127	R11P3D3_KE	alpha	CDR1	SSNFYA
128	R11P3D3_KE	alpha	CDR2	MTL
129	R11P3D3_KE	alpha	CDR3	CALYNNNDMRF
130	R11P3D3_KE	alpha	variable domain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRKETAKSPEAL FVMTLNGDEKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYNNNDMRFGAGTRLTVKP
131	R11P3D3_KE	alpha	constant domain	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS

SEQ ID NO:	TCR	Chain	Region	Sequence
132	R11P3D3_KE	alpha	full-length	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRKETAKSPEAL FVMTLNGDEKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYNNNDMRFGAGTRLTVKPNIQNP DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
133	R11P3D3_KE	beta	CDR1	SGHNS
134	R11P3D3_KE	beta	CDR2	FNNNVP
135	R11P3D3_KE	beta	CDR3	CASSPGSTDTQYF
136	R11P3D3_KE	beta	variable domain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRETMMRGLELLIYF NNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPGSTDTQYFGPGTRLTVL
137	R11P3D3_KE	beta	constant domain	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
138	R11P3D3_KE	beta	full-length	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRETMMRGLELLIYF NNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPGSTDTQYFGPGTRLTVLEDLK NVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALND SRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
196	R11P3D3	alpha	CDR2bis	MTLNGDE
197	R16P1C10	alpha	CDR2bis	IYSNGD
198	R16P1E8	alpha	CDR2bis	TYSSGN
199	R17P1A9	alpha	CDR2bis	IYSNGD
200	R17P1D7	alpha	CDR2bis	QEAYKQQ
201	R17P1G3	alpha	CDR2bis	IYSNGD
202	R17P2B6	alpha	CDR2bis	IYSNGD
203	1G4	alpha	CDR2bis	IQSSQRE
204	R11P3D3_	alpha	CDR2bis	MTLNGDE

Table 2: Peptide sequences of the invention

Peptide Code	Sequence	SEQ ID NO:
PRAME-004	SLLQHLIGL	97
PRAME-004_A1	ALLQHLIGL	98
PRAME-004_A2	SALQHLIGL	99
PRAME-004_A3	SLAQHLIGL	100
PRAME-004_A4	SLLAHLIGL	101
PRAME-004_A5	SLLQALIGL	102
PRAME-004_A6	SLLQHAIGL	103
PRAME-004_A7	SLLQHLAGL	104
PRAME-004_A8	SLLQHLIAL	105
PRAME-004_A9	SLLQHLIGA	106
PRAME-004_T1	TLLQHLIGL	107
PRAME-004_T2	STLQHLIGL	108

Peptide Code	Sequence	SEQ ID NO:
PRAME-004_T3	SLTQHLIGL	109
PRAME-004_T4	SLLTHLIGL	110
PRAME-004_T5	SLLQTLIGL	111
PRAME-004_T6	SLLQHTIGL	112
PRAME-004_T7	SLLQHLTGL	113
PRAME-004_T8	SLLQHLITL	114
PRAME-004_T9	SLLQHLIGT	115
TMED9-001	SILQTLILV	116
CAT-001	SLIEHLQGL	117
DDX60L-001	SLIQHLEEI	118
LRRC70-001	SLLKNLIYL	119
PTPLB-001	SLLNHLPYL	120
HDAC5-001	SLLQHVLLL	121
VPS13B-002	SLLQKQIML	122
ZNF318-001	SLSQELVGV	123
CCDC51-001	SVLGALIGV	124
IFIT1-001	VLLHHQIGL	125
NYESO1-001	SLLMWITQV	126
ACPL-001	LLLVHLIPV	139
HSPB3-001	IILRHLIEI	140
UNC7-001	KILLHLIHI	141
SCYL2-001	KVLPHLIPL	142
RPS2P8-001	SALVHLIPV	143
PCNXL3-003	NALVHLIEV	144
AQP6-001	VALGHLIGI	145
PCNX-001	NALVHLIEI	146
AQP6-002	WALGHLIGI	147
TRGV10-001	QALEHLIYI	148
NECAP1-001	ISLAHLILV	149
FBXW2-001	ETLDHLISL	150
ACCSL-001	ALLSHLICR	151
ACER1-001	KELRHLIEV	152
ADAMTS14-001	IALVHLIMV	153
ARHGAP17-001	CWLCHLIKL	154
ARSE-001	GKLTHLIPV	155
ATP-009	HLLMHLIGS	156
AUNI-001	TQLDHLIPG	157
C16orf96-001	QDLWHLIKL	158
CDC7-002	IALKHLIPT	159
CDC7-003	IALKHLILT	160
CHRNA1-001	LQLIHLINV	161
FASTKD5-001	SQLVHLIYV	162
FRYL-002	CLLPHLIQH	163
FTH1-001	MVLVHLIHS	164
HERC4-002	SDLFHLIGV	165

Peptide Code	Sequence	SEQ ID NO:
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HPS5-002	KLLLHLIQS	167
HTR2C-001	SFLVHLIGL	168
IPM-001	YGLKHLISV	169
KIF16-001	SELPHLIGI	170
KLHL33-001	YALSHLIHA	171
LAMA3-001	TLLGHLISK	172
LOC100128170-001	SQLSHLIAM	173
MAP2K7-001	FFLVHLICM	174
MON2-003	VSLHHLINA	175
OR2AK2-001	IMLIHLIRL	176
OR2AK2-002	ITLIHLIRL	177
OR2B6-001	SELFHLIPL	178
OR2B6-002	SVLFHLIPL	179
OTUD7A-001	AQLAHLILS	180
OVOS2-001	FLLGHLIPR	181
PIGC-002	MLLGHLIFF	182
RAD54L2-003	VLLFHLIEE	183
RASEF-001	VFLRHLITL	184
RASGRF1-003	TLLDHLIFK	185
RPS2P20-001	SVLVHLIPA	186
SACS-001	AKLEHLIYL	187
SPATA31D5-001	SLLPHLILS	188
TPST2-001	SILGHLICS	189
TRGV10-002	QSLEHLIYI	190
UGP-001	YILNHLINP	191
USP51-001	YKLLHLIWI	192
ZNF423-002	KLLCHLIEH	193
ZNF584-001	ALLDHLITH	194
ZNF99-001	FMLSHLIQH	195

EXAMPLES

[0103] Seven PRAME-specific TCRs directed to the herein disclosed PRAME-004 peptide (R11P3D3, R16P1C10, R16P1E8, R17P1A9, R17P1D7, R17P1G3 and R17P2B6, see Table 1), each encoding tumor specific TCR-alpha and TCR-beta chains, were isolated and amplified from T-cells of healthy donors. Cells from healthy donors were in vitro stimulated according to a method previously described (Walter et al., 2003 J Immunol., Nov 15;171(10):4974-8) and target-specific cells were single-cell sorted using HLA-A*02 multimers and then used for subsequent TCR isolation. TCR sequences were isolated via 5' RACE by standard methods as described by e.g. Molecular Cloning a laboratory manual fourth edition by Green and Sambrook. The alpha and beta variable regions of TCRs R11P3D3, R16P1C10, R16P1E8, R17P1A9, R17P1D7, R17P1G3 and R17P2B6 were sequenced and cloned for further functional characterization.

[0104] R11P3D3, R16P1C10, R17P1D7 and R17P2B6 are derived from HLA-A*02 negative donor (alloreactive setting) and R16P1E8, R17P1A9 and R17P1G3 are derived from a HLA-A*02 positive donor.

[0105] Furthermore, the mutant TCR R11P3D3_KE, an enhanced variant of R11P3D3, is herein disclosed. Enhanced TCR variant R11P3D3_KE was modified from the parental TCR as described in PCT/EP2017/081745, and in example 8 below,

and the coding sequence was obtained by gene synthesis prior to the functional characterization of the TCR.

Example 1: T-cell receptor R11P3D3

[0106] TCR R11P3D3 (SEQ ID NO:1-12 and 196) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 8).

[0107] R11P3D3 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 1) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 8). NYESO1-001 peptide is used as negative control. TCR R11P3D3 has an EC50 of 0.74 nM (Figure 15) and a binding affinity (K_D) of 18 - 26μM towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97).

[0108] Re-expression of R11P3D3 in human primary CD8+ T-cells leads to selective recognition and killing of HLA-A*02/PRAME-004-presenting tumor cell lines (Figures 24, 25 30 and 32). TCR R11P3D3 does not respond to any of the 25 tested healthy, primary or iPSC-derived cell types (Figures 24 and 25) and was tested for cross-reactivity towards further 67 similar peptides (of which 57 were identical to PRAME-004 in positions 3, 5, 6 and 7) but unrelated peptides in the context of HLA-A*02 (Figures 8, 22 and 23).

Example 2: T-cell receptor R16P1C10

[0109] TCR R16P1 C10 (SEQ ID NO:13-24 and 197) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 9).

[0110] R16P1C10 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells and bind HLA-A*02 tetramers (Figure 21), respectively, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 2) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 9). NYESO1-001 peptide is used as negative control. TCR R16P1C10 has an EC50 of 9.6nM (Figure 16).

Example 3: T-cell receptor R16P1E8

[0111] TCR R16P1E8 (SEQ ID NO:25-36 and 198) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 10).

[0112] R16P1E8 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 3) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 10). NYESO1-001 peptide is used as negative control. TCR R16P1E8 has an EC50 of ~1 μM (Figure 17).

Example 4: T-cell receptor R17P1A9

[0113] TCR R17P1A9 (SEQ ID NO:37-48 and 199) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 11).

[0114] R17P1A9 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine substitution variants of PRAME-004 (Figure 4) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 11). NYESO1-001 peptide is used as negative control.

Example 5: T-cell receptor R17P1 D7

[0115] TCR R17P1D7 (SEQ ID NO:49-60 and 200) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 12).

[0116] R17P1D7 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 5) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 12). NYESO1-001 peptide is used as negative control. TCR R17P1 D7 has an EC50 of 1.83 nM (Figure 18).

Example 6: T-cell receptor R17P1G3

[0117] TCR R17P1G3 (SEQ ID NO:61-72 and 201) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 13).

[0118] R17P1G3 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 6) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 13). NYESO1-001 peptide is used as negative control. TCR R17P1G3 has an EC50 of 8.63 nM (Figure 19).

Example 7: T-cell receptor R17P2B6

[0119] TCR R17P2B6 (SEQ ID NO:73-84 and 202) is restricted towards HLA-A*02-presented PRAME-004 (SEQ ID NO:97) (see Figure 14).

[0120] R17P2B6 specifically recognizes PRAME-004, as human primary CD8+ T-cells re-expressing this TCR release IFNγ upon co-incubation with HLA-A*02+ target cells, loaded either with PRAME-004 peptide or alanine or threonine substitution variants of PRAME-004 (Figure 7) or different peptides showing high degree of sequence similarity to PRAME-004 (Figure 14). NYESO1-001 peptide is used as negative control. TCR R17P2B6 has an EC50 of 2.11 nM (Figure 20) and a binding affinity (K_D) of 13 µM towards HLA-A*02-presented PRAME-004.

Example 8: Enhanced T-cell receptor R11P3D3_KE

[0121] The mutated "enhanced pairing" TCR R11P3D3_KE is introduced as a variant of R11P3D3, where α and β variable domains, naturally bearing αW44/ βQ44, have been mutated to αK44/ βE44. The double mutation is selected among the list present in PCT/EP2017/081745. It is specifically designed to restore an optimal interaction and shape complementarity to the TCR scaffold.

[0122] Compared with the parental TCR R11P3D3 the enhanced TCR R11P3D3_KE shows superior sensitivity of PRAME-004 recognition. The response towards PRAME-004-presenting tumor cell lines are stronger with the enhanced TCR R11P3D3_KE compared to the parental TCR R11P3D3 (Figure 30). Furthermore, the cytolytic activity of R11 P3D3_KE is stronger compared to R11P3D3 (Figure 32). The observed improved functional response of the enhanced TCR R11P3D3_KE is well in line with an increased binding affinity towards PRAME-004, as described in example 1 (R11P3D3, K_D =18-26µM) and example 8 (R11P3D3_KE, K_D =5.3µM).

REFERENCES CITED IN THE DESCRIPTION

Cited references

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patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US5545806A [0062]
- US5569825A [0062]
- US5714352A [0062]
- US20020197266 [0062]
- EP2017081745W [0105] [0121]

Non-patent literature cited in the description

- **LEFRANC M-P et al.**Nucleic Acids Res., 2015, vol. 43, D413-22 [0016] [0061]
- Janeway's Immunobiology20160000 [0060]
- **KOHLERMILSTEIN**Eur. J. Immunol, 1976, vol. 5, 511-519 [0062]
- Antibodies: A Laboratory ManualCSH Press19880000 [0062]
- ImmunobiologyGarland Publishing20110000 [0062]
- **HASKARDARCHER**J. Immunol. Methods, 1984, vol. 74, 2361-67 [0062]
- **RODER et al.**Methods Enzymol, 1986, vol. 121, 140-67 [0062]
- **HUSE et al.**Science, 1989, vol. 246, 1275-81 [0062]
- **SAMBROOK et al.**Molecular Cloning, [0064]
- **WALTER et al.**J Immunol., 2003, vol. 171, 104974-8 [0103]

PATENTKRAV

1. Antigengenkendelseskonstrukt, der omfatter seks komplementaritetsbestemmende områder (CDR) 1, CDR2 og CDR3 ifølge SEQ ID NO: 1, 2, 3, 7, 8 og 9.

2. Antigengenkendelseskonstrukt ifølge krav 1, hvor antigengenkendelseskonstruktet er i stand til specifikt at binde til et PRAME- (Preferentially Expressed Antigen of Melanoma) antigent peptid vist i SEQ ID NO: 97.

3. Antigengenkendelseskonstrukt ifølge krav 1 eller 2, hvor antigengenkendelseskonstruktet er en T-cellereceptor (TCR) eller et fragment deraf.

4. Antigengenkendelseskonstrukt ifølge et hvilket som helst af krav 1 til 3, og som omfatter en TCR α- eller γ-kæde; og en TCR β- eller δ-kæde; hvor TCR α- eller γ-kæden og TCR β- eller δ-kæden omfatter sekvenserne defineret i krav 1.

5. Antigengenkendelseskonstrukt ifølge et hvilket som helst af krav 1 til 4, og som omfatter et variabelt TCR-kædeområde med mindst 80 % sekvensidentitet med en aminosyresekvens valgt blandt SEQ ID NO: 4, 10, 130 og 136.

6. Antigengenkendelseskonstrukt ifølge et hvilket som helst af krav 1 til 5, og som omfatter et bindingsfragment af en TCR, og hvor bindingsfragmentet omfatter CDR1 til CDR3 valgt blandt CDR1- til CDR3-sekvenserne med aminosyresekvenserne ifølge SEQ ID NO: 1, 2, 3; eller 7, 8, 9.

7. Antigengenkendelseskonstrukt ifølge et hvilket som helst af krav 1 til 6, hvor TCR'en er en enkeltkæde TCR (scTCR).

8. Nukleinsyre, der koder for antigengenkendelseskonstruktet ifølge et hvilket som helst af krav 1 til 7.

9. Vektor, der omfatter nukleinsyren ifølge krav 8.

10. Værtscelle, der omfatter antigengenkendelseskonstruktet ifølge et hvilket som helst af krav 1 til 7, eller nukleinsyren ifølge krav 8, eller vektoren ifølge krav 9, fortrinsvis hvor værtscellen er en lymfocyt, mere fortrinsvis en T-lymfocyt eller T-lymfocytforløber, mest fortrinsvis en CD4- eller CD8-positiv T-celle.

11. Farmaceutisk sammensætning, der omfatter antigengenkendelseskonstruktet ifølge et hvilket som helst af krav 1 til 7, eller nukleinsyren ifølge krav 8, eller vektoren ifølge krav 9, eller værtscellen ifølge krav 10, og et farmaceutisk acceptabelt bærestof, stabiliseringsmiddel og/eller hjælpestof.

12. Antigengenkendelseskonstrukt ifølge et hvilket som helst af krav 1 til 7, eller nukleinsyre ifølge krav 8, eller vektor ifølge krav 9, eller værtscelle ifølge krav 10 eller farmaceutisk sammensætning ifølge krav 11 til anvendelse i medicin, fortrinsvis til anvendelse i diagnose, forebyggelse og/eller behandling af en proliferativ sygdom.

13. Fremgangsmåde til frembringelse af et tumorassocieret antigen (TAA) specifikt antigengenkendelseskonstrukt, der udtrykker cellelinje, omfattende

a. tilvejebringelse af en egnet værtscelle,

b. tilvejebringelse af et genetisk konstrukt, der omfatter en kodende sekvens, der koder for antigengenkendelseskonstruktet ifølge et hvilket som helst af krav 1 til 7,

c. indføring i den egnede værtscelle af det genetiske konstrukt og

d. ekspression af det genetiske konstrukt ved hjælp af den egnede værtscelle,

og som fortrinsvis endvidere omfatter

e. isolering og oprensning af antigengenkendelseskonstruktet fra den egnede værtscelle og eventuelt

f. rekonstruktion af antigengenkendelseskonstruktet i en T-celle.

DRAWINGS

Drawing

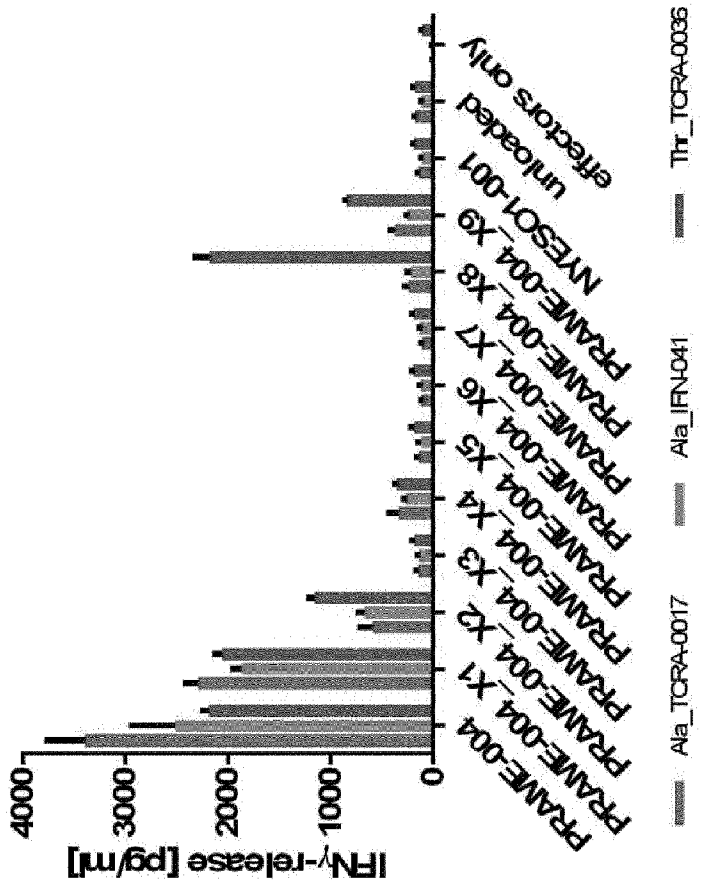
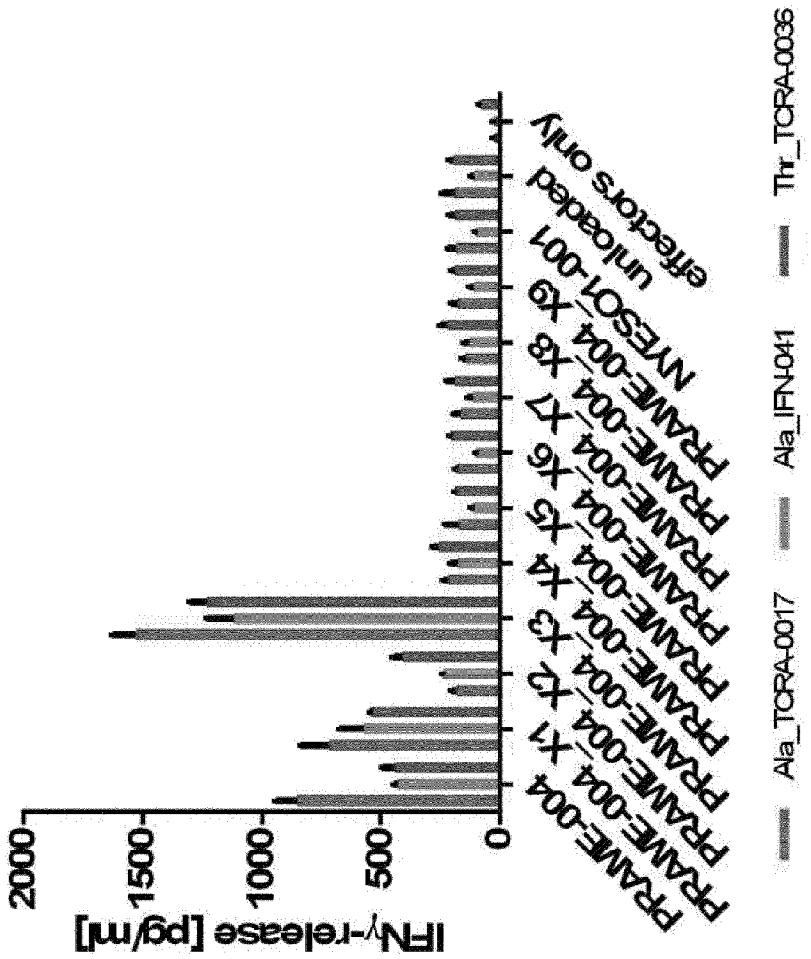


Figure 1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PRAME-004_X6
PRAME-004_X7
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unloaded
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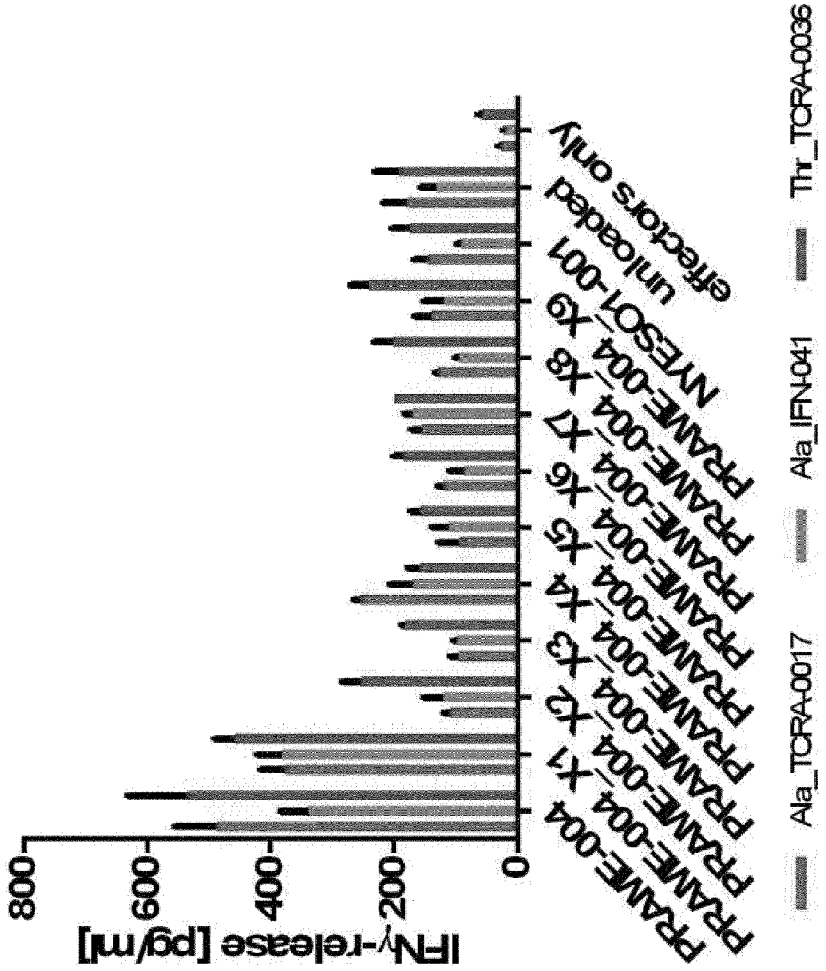
Ala_IFN-041
Thr_TCRA-0036

Figure 2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PRAME-004_x5
PRAME-004_x6
PRAME-004_x7
PRAME-004_x8
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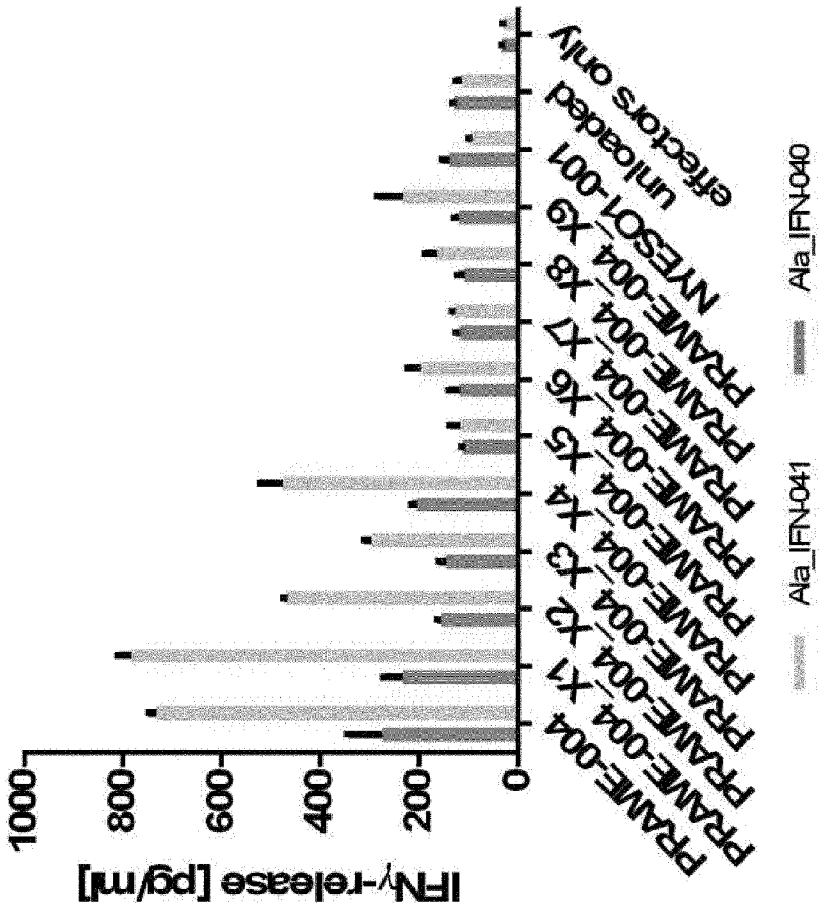
NYESO1-001
unloaded
effectors only
Ala_TCRA-0017
Ala_IFN-041
Thr_TCRA-0036

Figure 3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PRAME-004_x6
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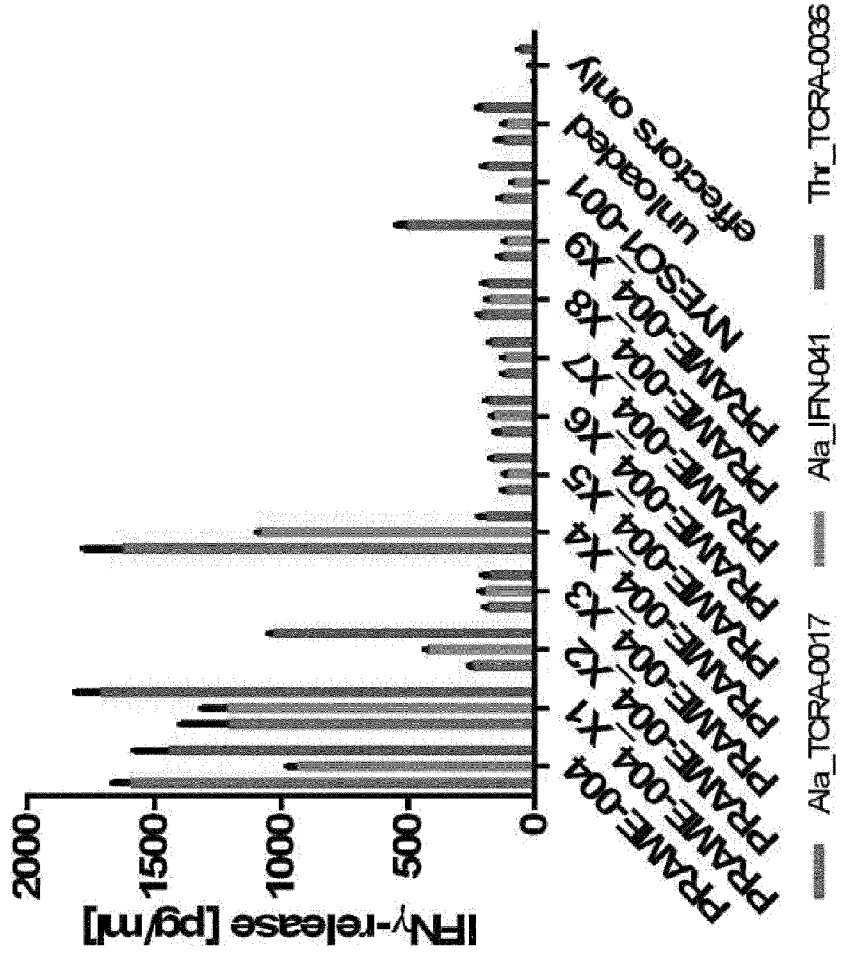
unloaded
effectors only
Ala_IFN-041
Ala_IFN-040

Figure 4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PRAME-004_X7
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unloaded
effectors only
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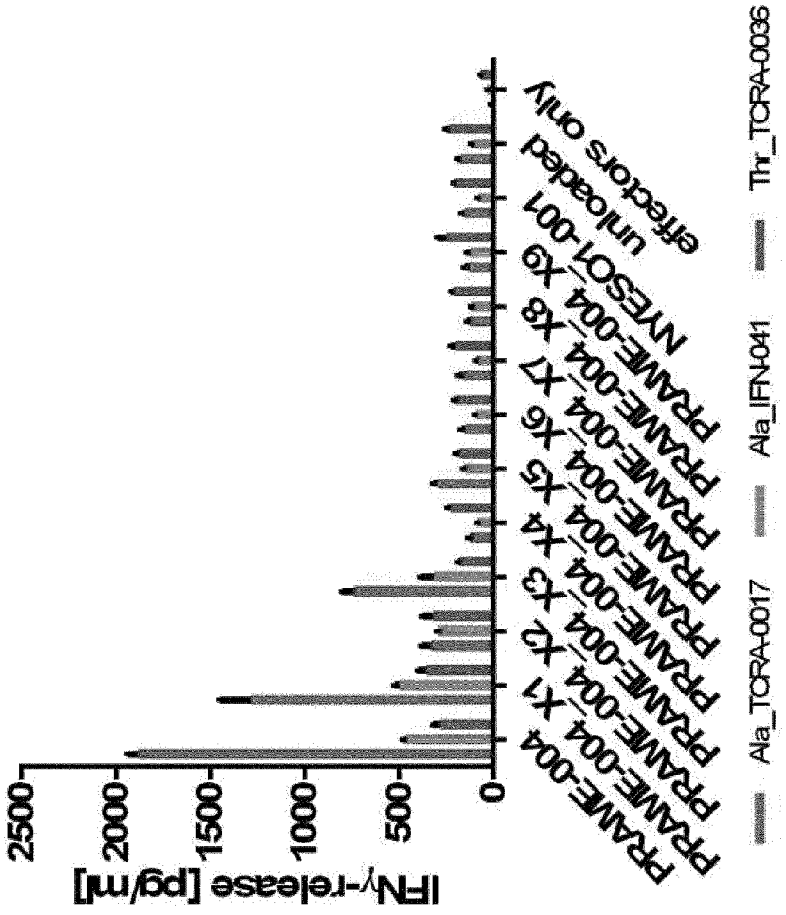
Ala_IFN-041
Thr_TCRA-0036

Figure 5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PRAME-004 X7
PRAME-004 X8
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effectors only
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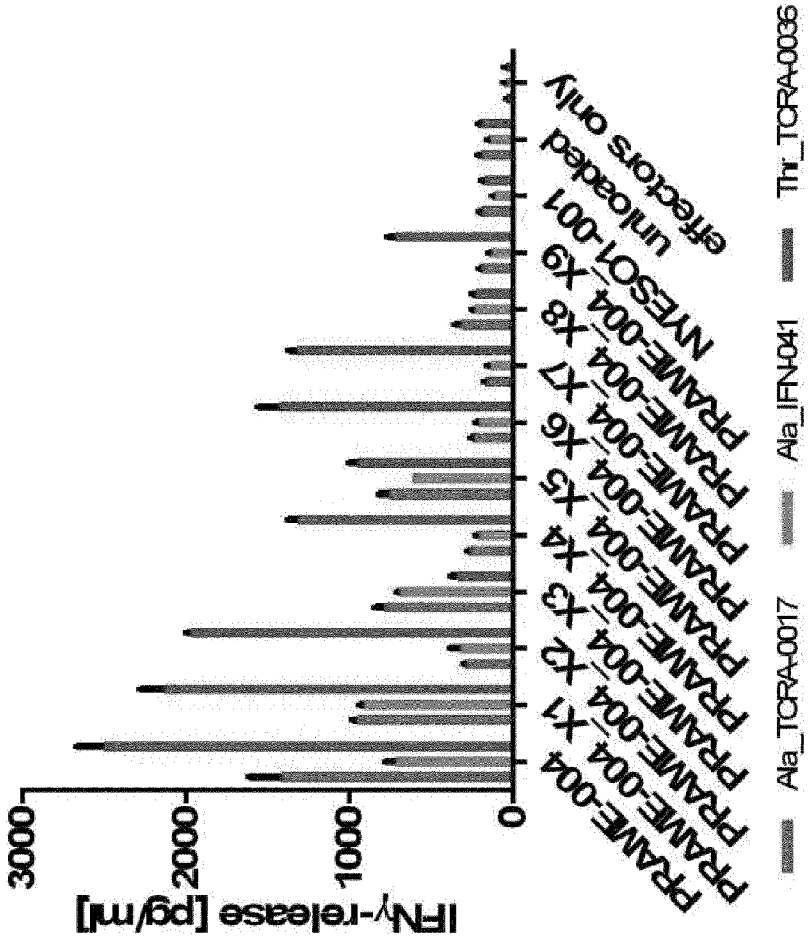
Figure 6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PRAME-004_x6
PRAME-004_x7
PRAME-004_x8
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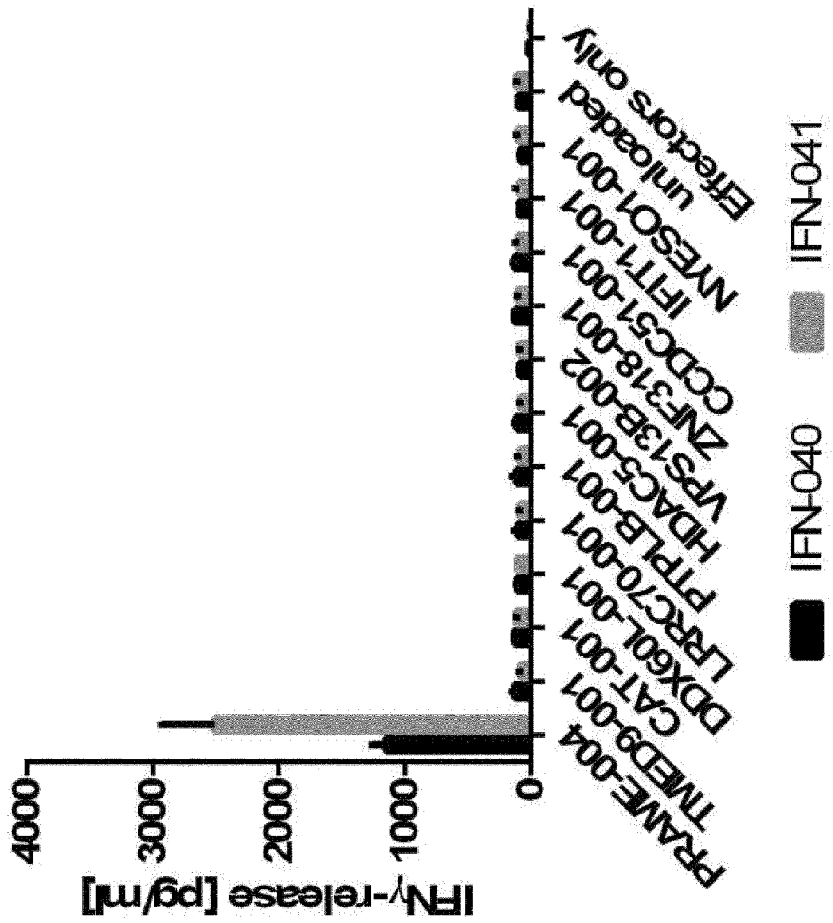
Thr_TCRA-0036

Figure 7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HDAC5-001
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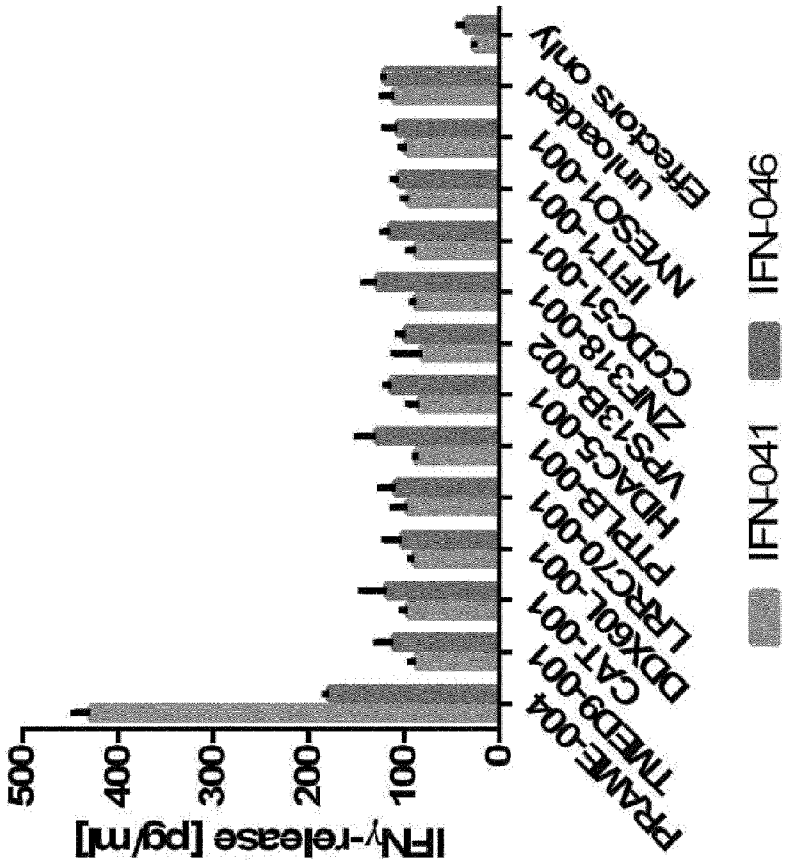
unloaded
Effectors only
IFN-040
IFN-041

Figure 8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LRRC70-001
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NYESO1-001
unloaded
Effectors only
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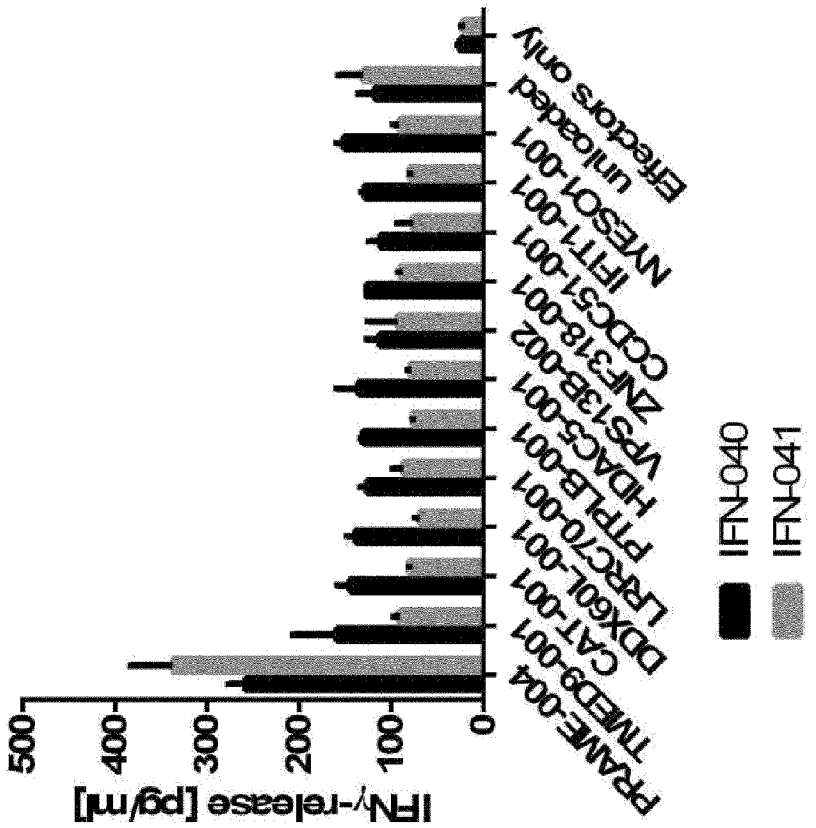
Figure 9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unloaded
Effectors only
IFN-040
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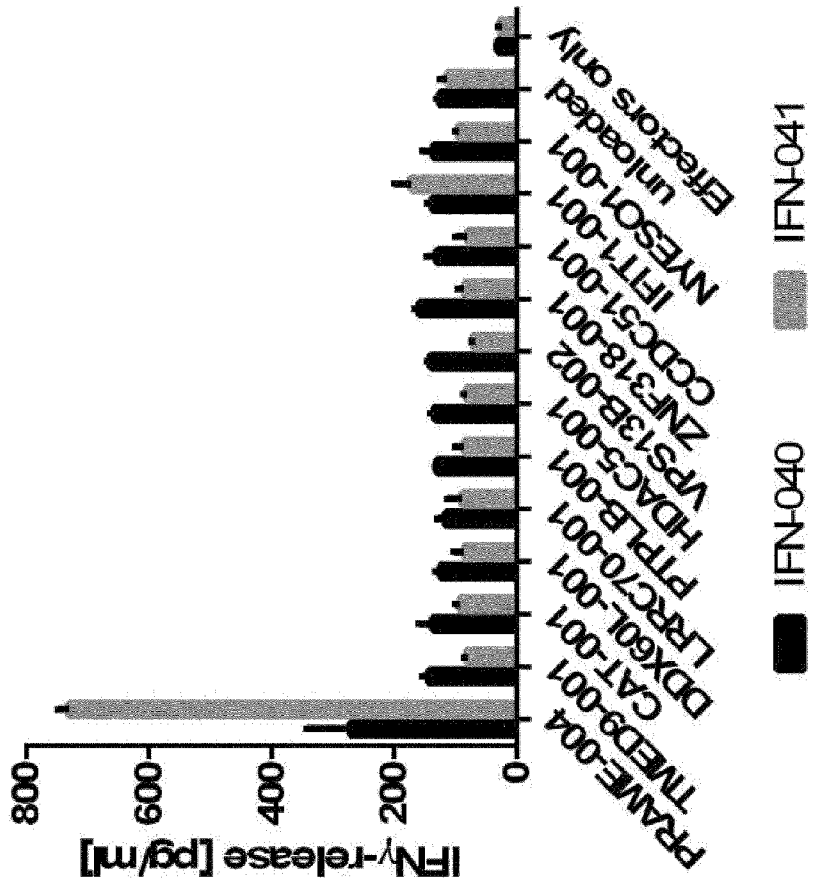
Figure 10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HDAC5-001
VPS13B-002
ZNF318-001
CCDC51-001
IFIT1-001
NYESO1-001
unloaded
Effectors only

Figure 11

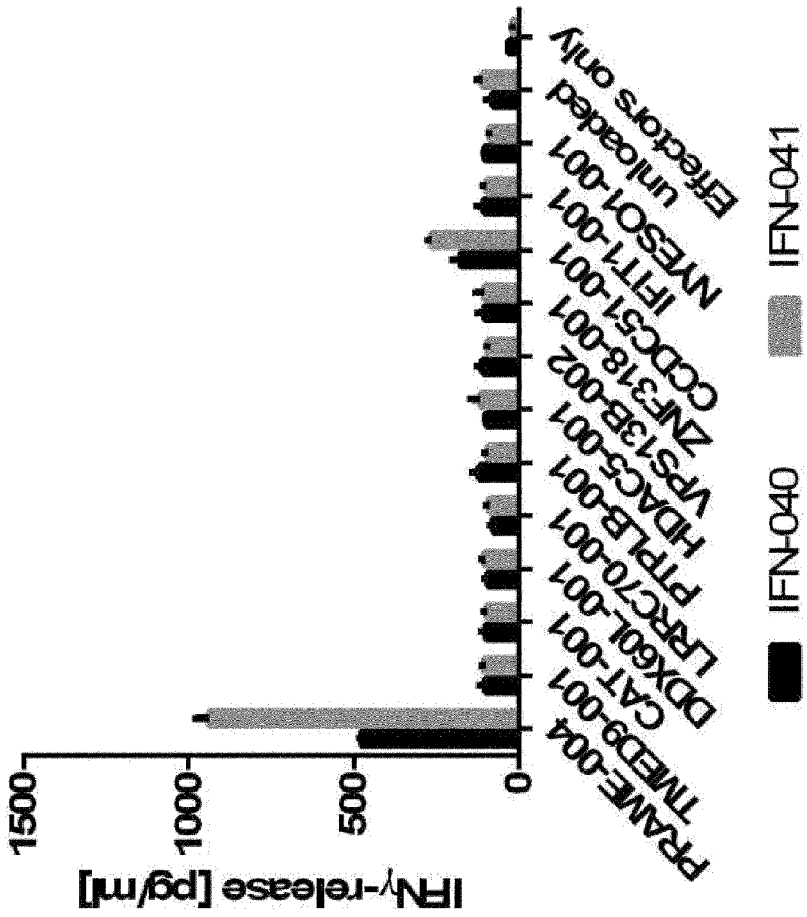

R17P1D7
IFNγ-release [pg/ml]
1500
1000
500
0
PRAME-004
TMED9-001
CAT-001
DDX60L-001
LRRC70-001
PTPLB-001
HDAC5-001
VPS13B-001
ZNF318-002
CCDC51-001
IFIT1-001
NYESO1-001
unloaded
Effectors only
IFN-040
IFN-041

Figure 12

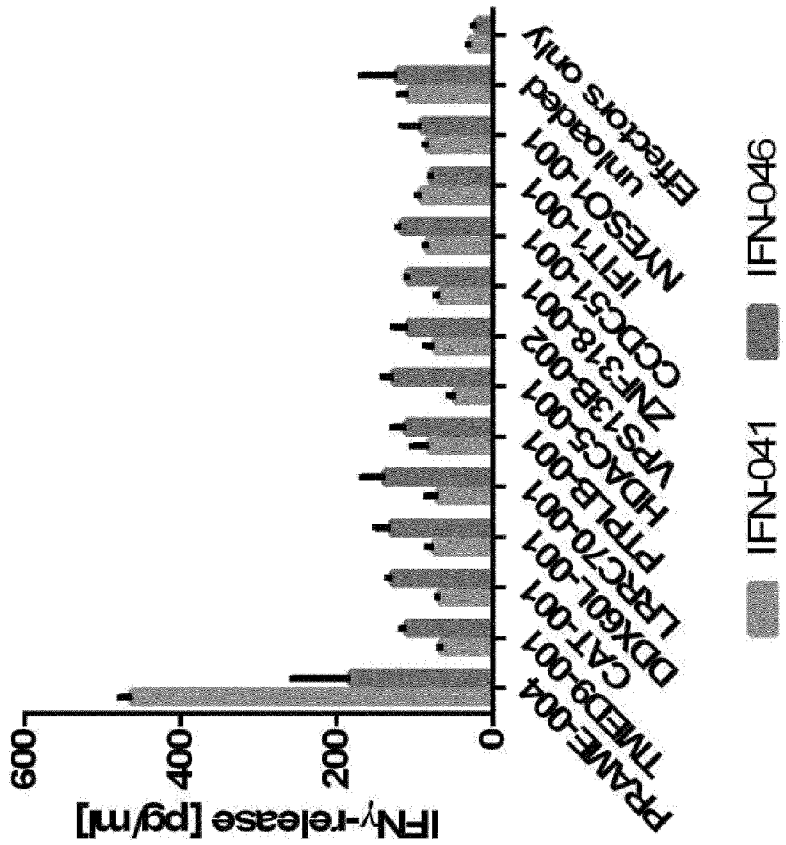

R17P1G3
IFNγ-release [pg/ml]
0
200
400
600
PRAME-004
TMED9-001
CAT-001
DDX60L-001
LRRC70-001
PTPLB-001
HDAC5-001
VPS13B-001
ZNF318-002
CCDC51-001
IFIT1-001
NYESO1-001
unloaded
Effectors only
IFN-041
IFN-046

Figure 13

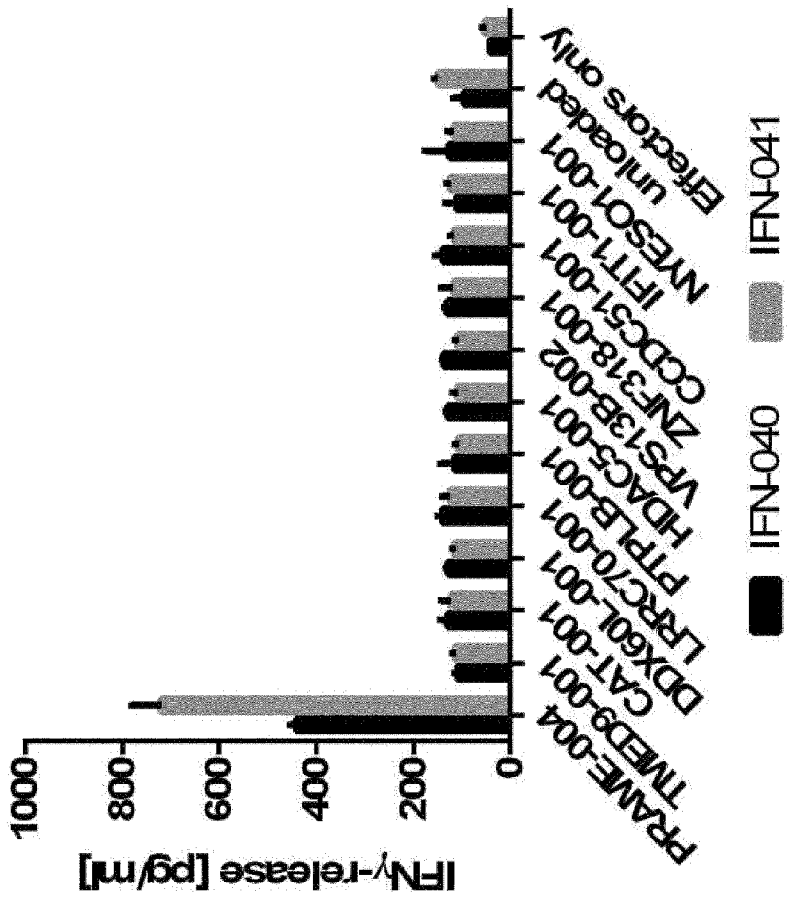

R17P2B6
IFNγ-release [pg/ml]
0
200
400
600
800
1000
PRAME-004
TMED9-001
CAT-001
DDX60L-001
LRRC70-001
PTPLB-001
HDAC5-001
VPS13B-001
ZNF318-002
CCDC51-001
IFIT1-001
NYESO1-001
unloaded
Effectors only
IFN-040
IFN-041

Figure 14

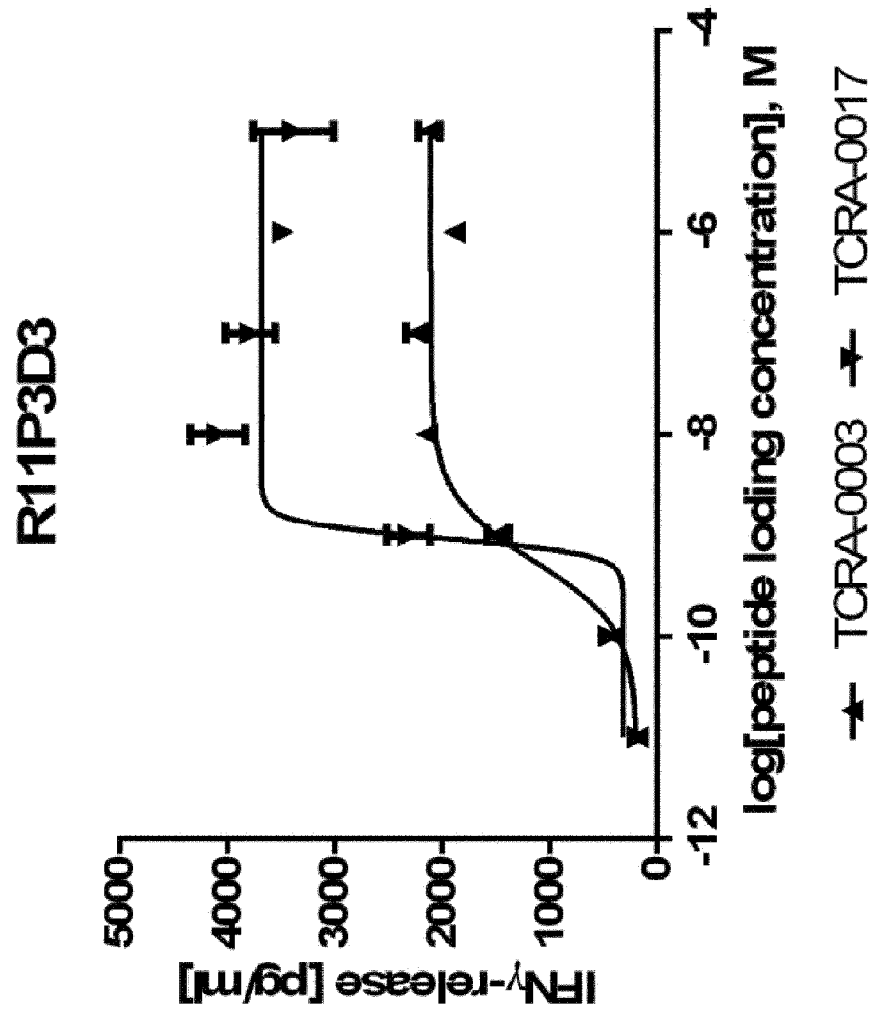

R11P3D3
IFNγ-release [pg/ml]
5000
4000
3000
2000
1000
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 15

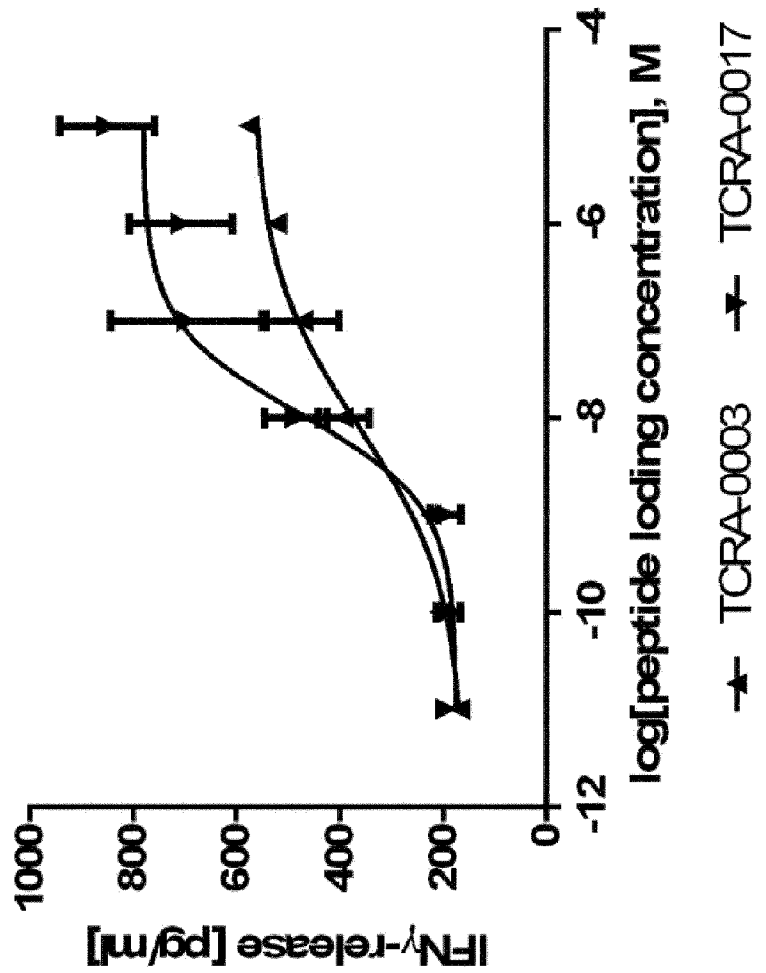

R16P1C10
IFNγ-release [pg/ml]
1000
800
600
400
200
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 16

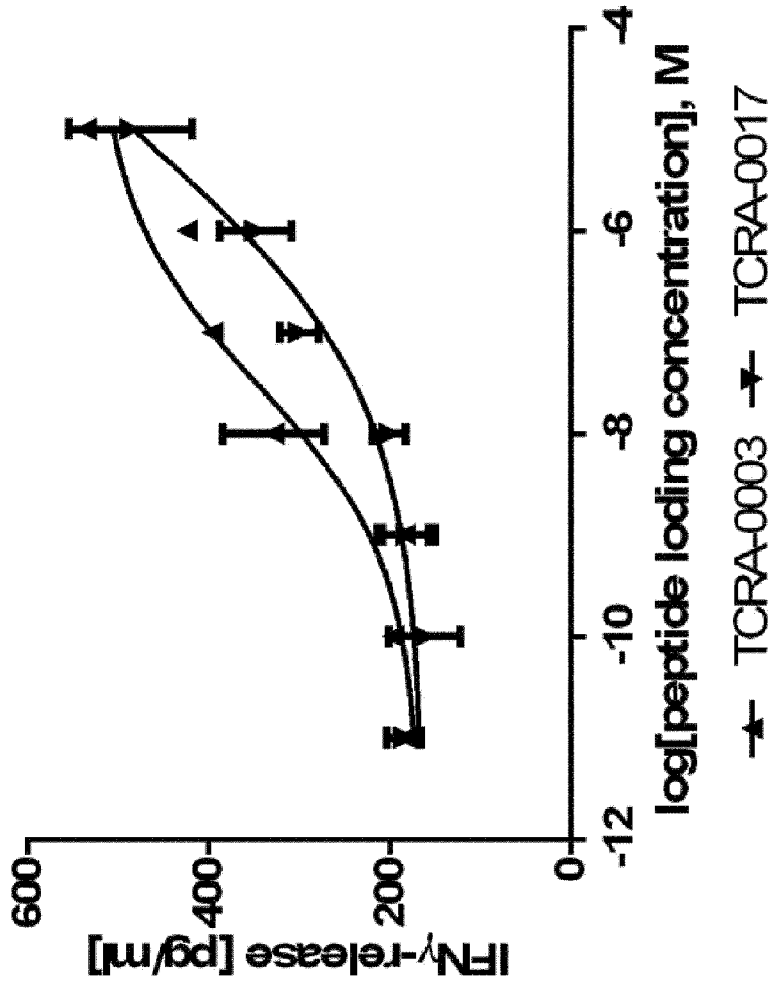

R16P1E8
IFNγ-release [pg/ml]
600
400
200
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 17

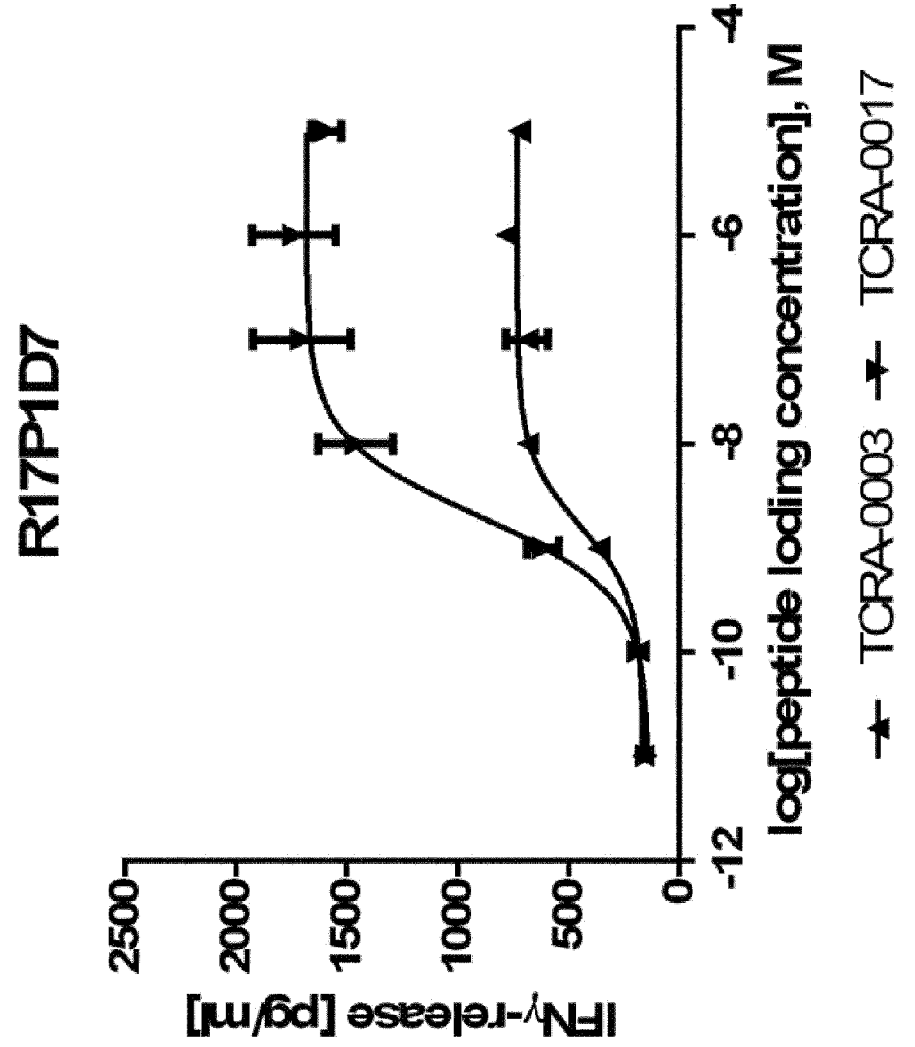

R17P1D7
IFNγ-release [pg/ml]
2500
2000
1500
1000
500
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 18

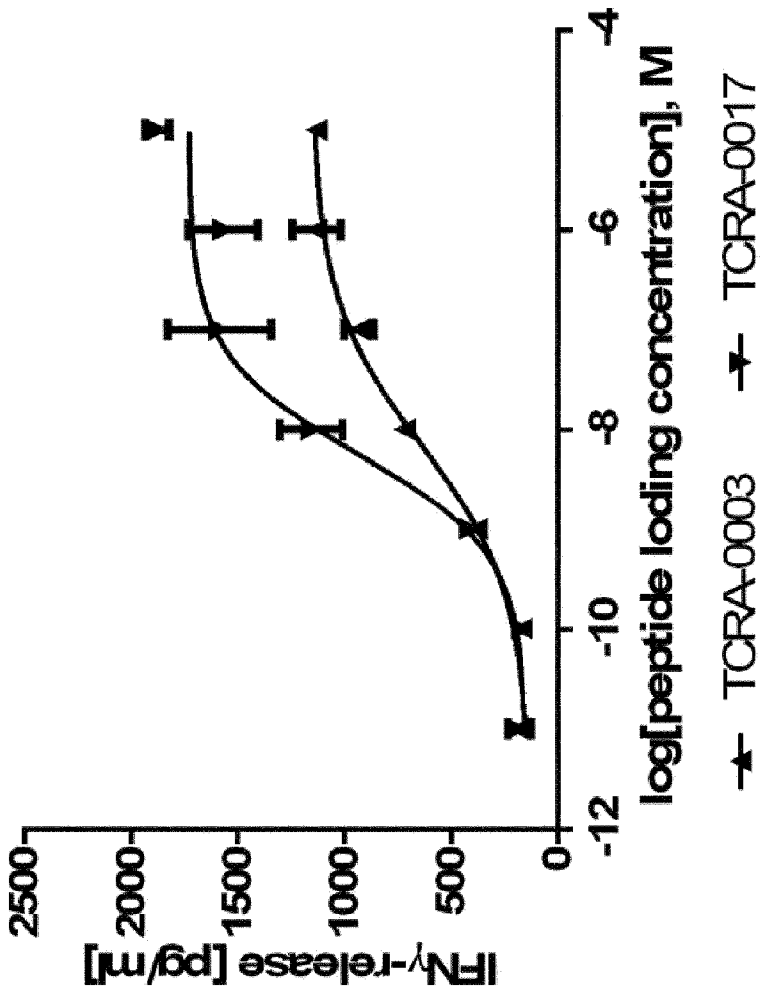

R17P1G3
IFNγ-release [pg/ml]
2500
2000
1500
1000
500
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 19

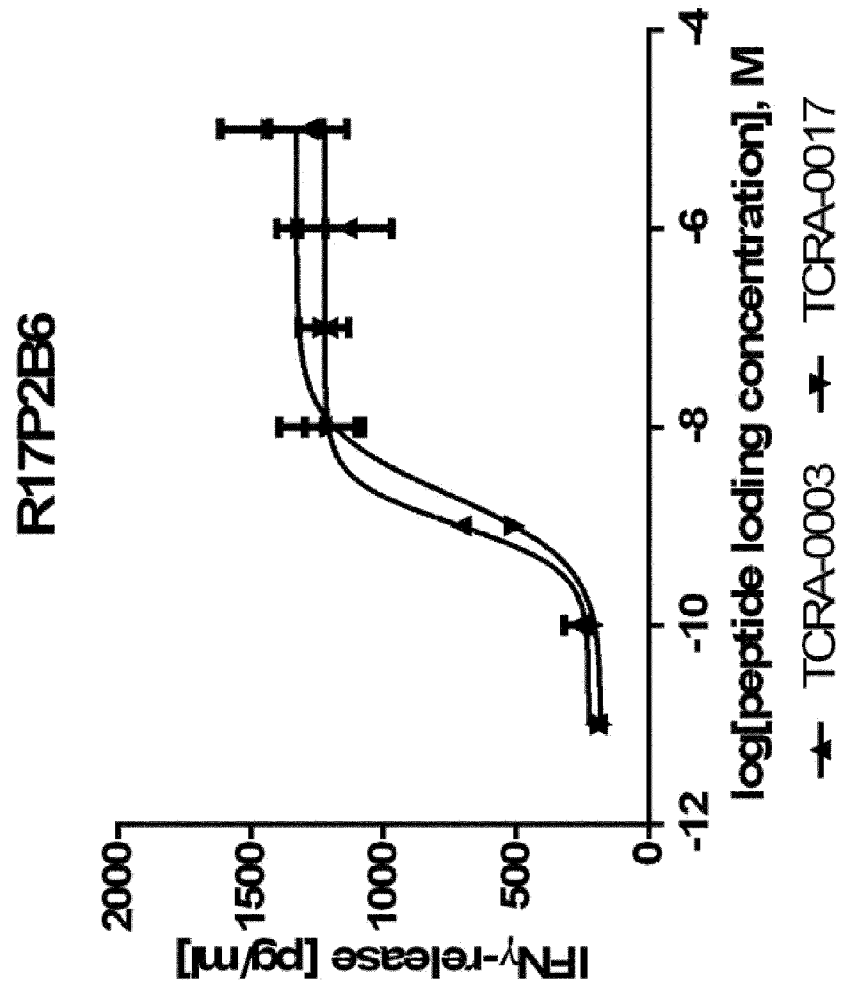

R17P2B6
IFNγ-release [pg/ml]
2000
1500
1000
500
0
-12
-10
-8
-6
-4
log[peptide loding concentration], M
TCRA-0003
TCRA-0017

Figure 20

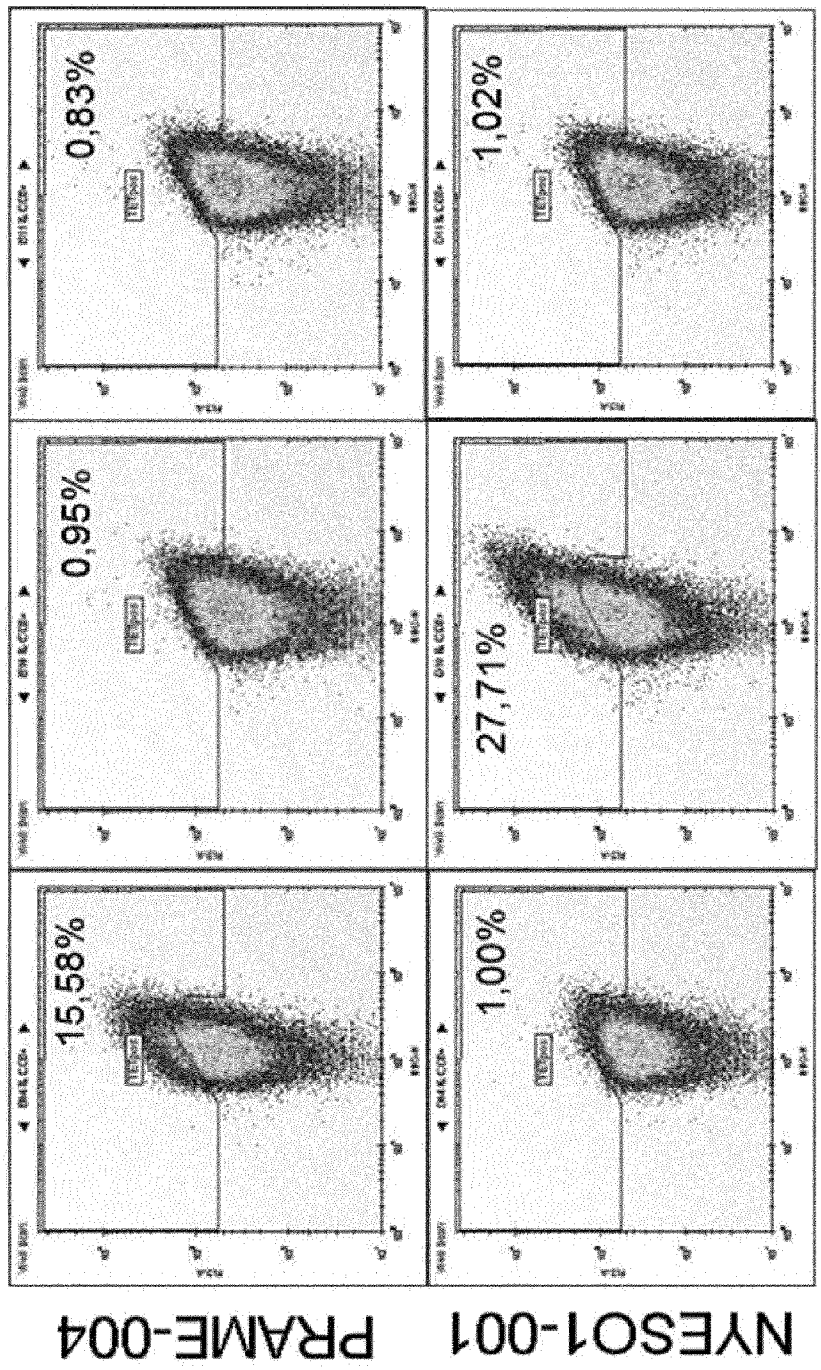

R16P1C10
1G4
mock
15,58%
0,95%
0,83%
1,00%
27,71%
1,02%
PRAME-004
NYESO1-001

Figure 21

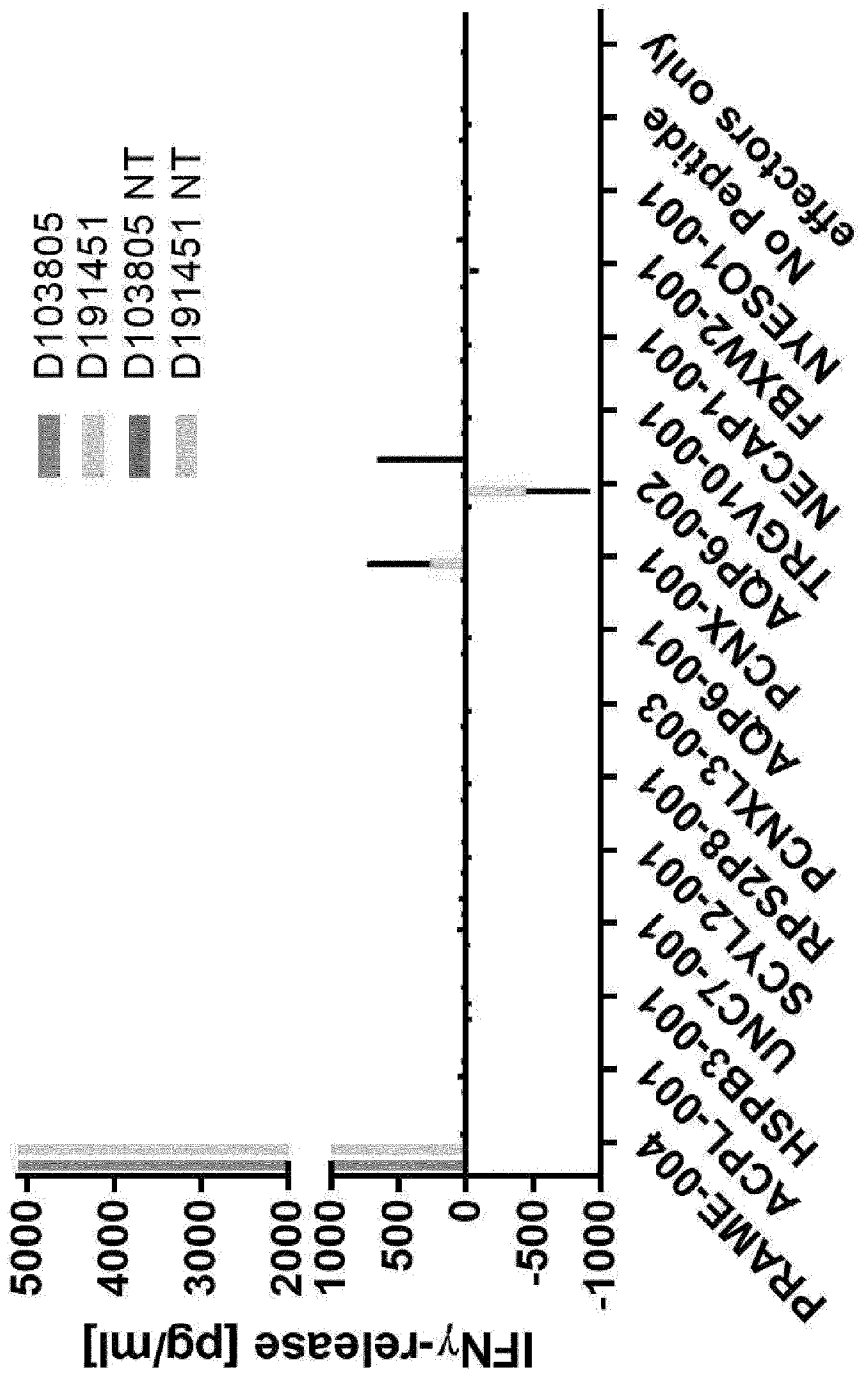

D103805
D191451
D103805 NT
D191451 NT
IFNγ-release [pg/ml]
5000
4000
3000
2000
1000
500
0
-500
-1000
PRAME-004
ACPL-001
HSPB3-001
UNC7-001
SCYL2-001
RPS2P8-001
PCNXL3-003
AQP6-001
PCNX-001
AQP6-002
TRGV10-001
NECAP1-001
FBXW2-001
NYESO1-001
No Peptide
effectors only

Figure 22

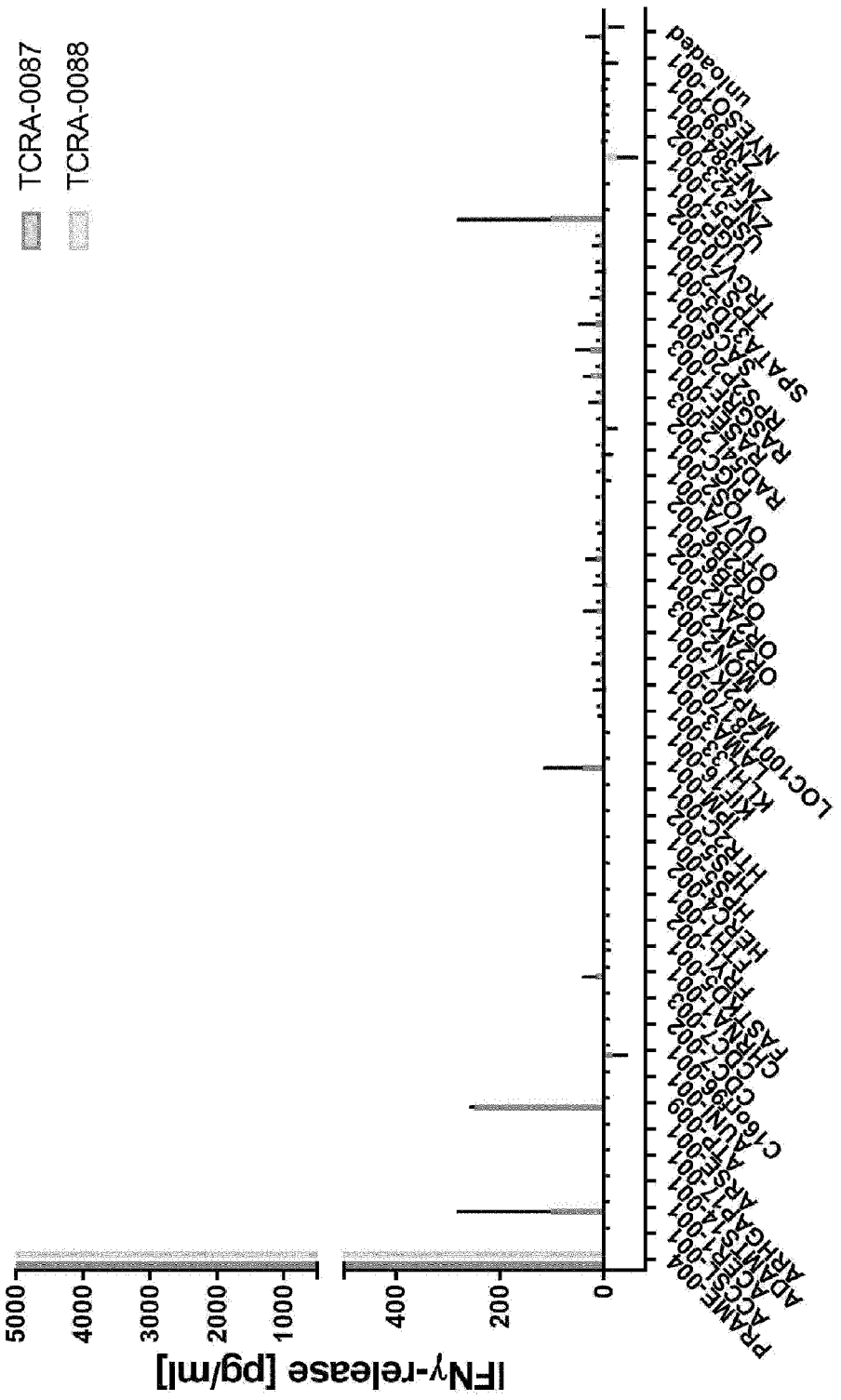

TCRA-0087
TCRA-0088
IFNγ-release [pg/ml]
5000
4000
3000
2000
1000
400
200
0
unloaded

Figure 23

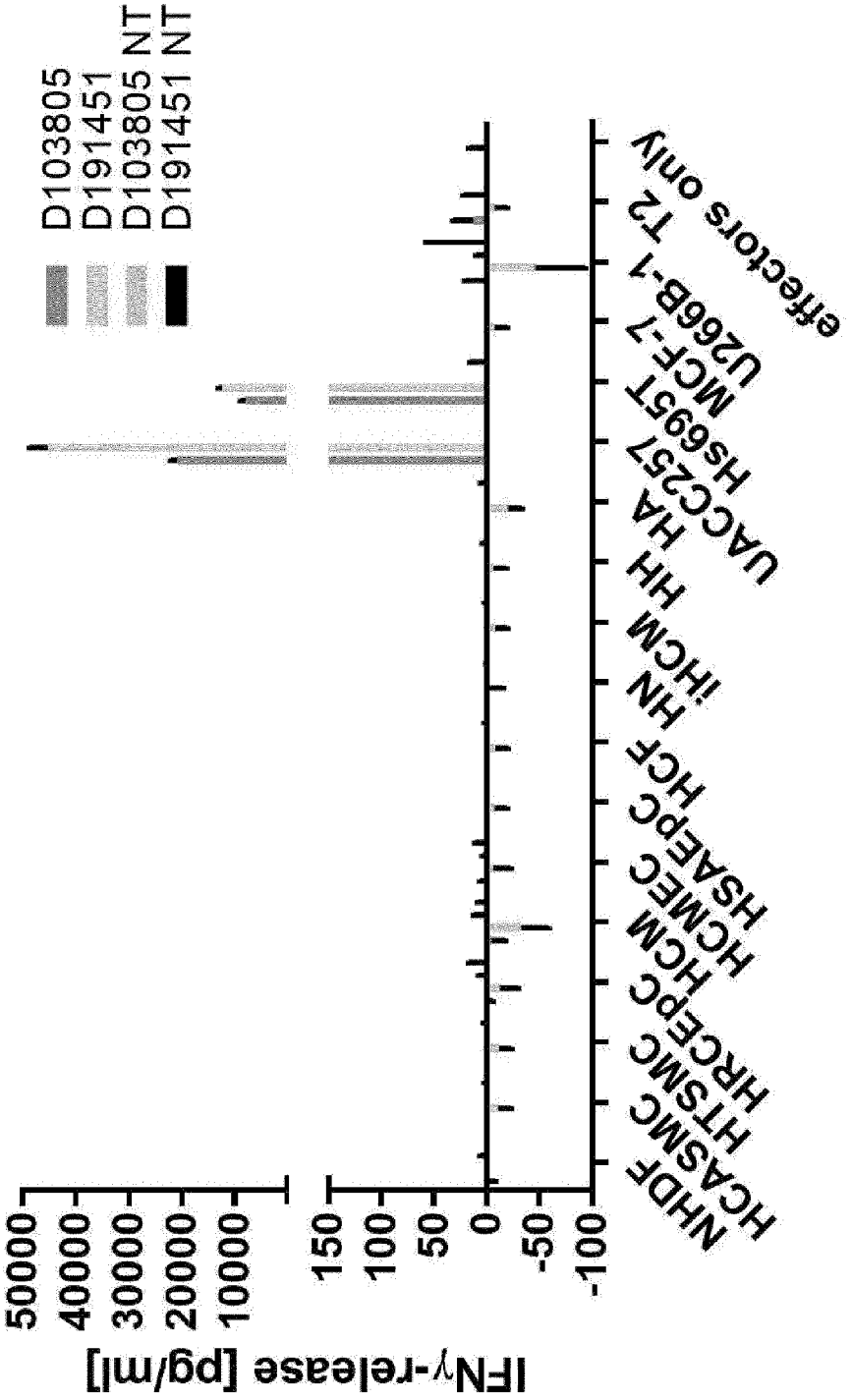

D103805
D191451
D103805 NT
D191451 NT
IFNγ-release [pg/ml]
50000
40000
30000
20000
10000
150
100
50
0
-50
-100
NHDF
HCASMC
HTSMC
HRCEpC
HCM
HCMEC
HSAEpC
HCF
HN
iHCM
HH
HA
UACC257
Hs695T
MCF-7
U266B-1
T2
effectors only

Figure 24

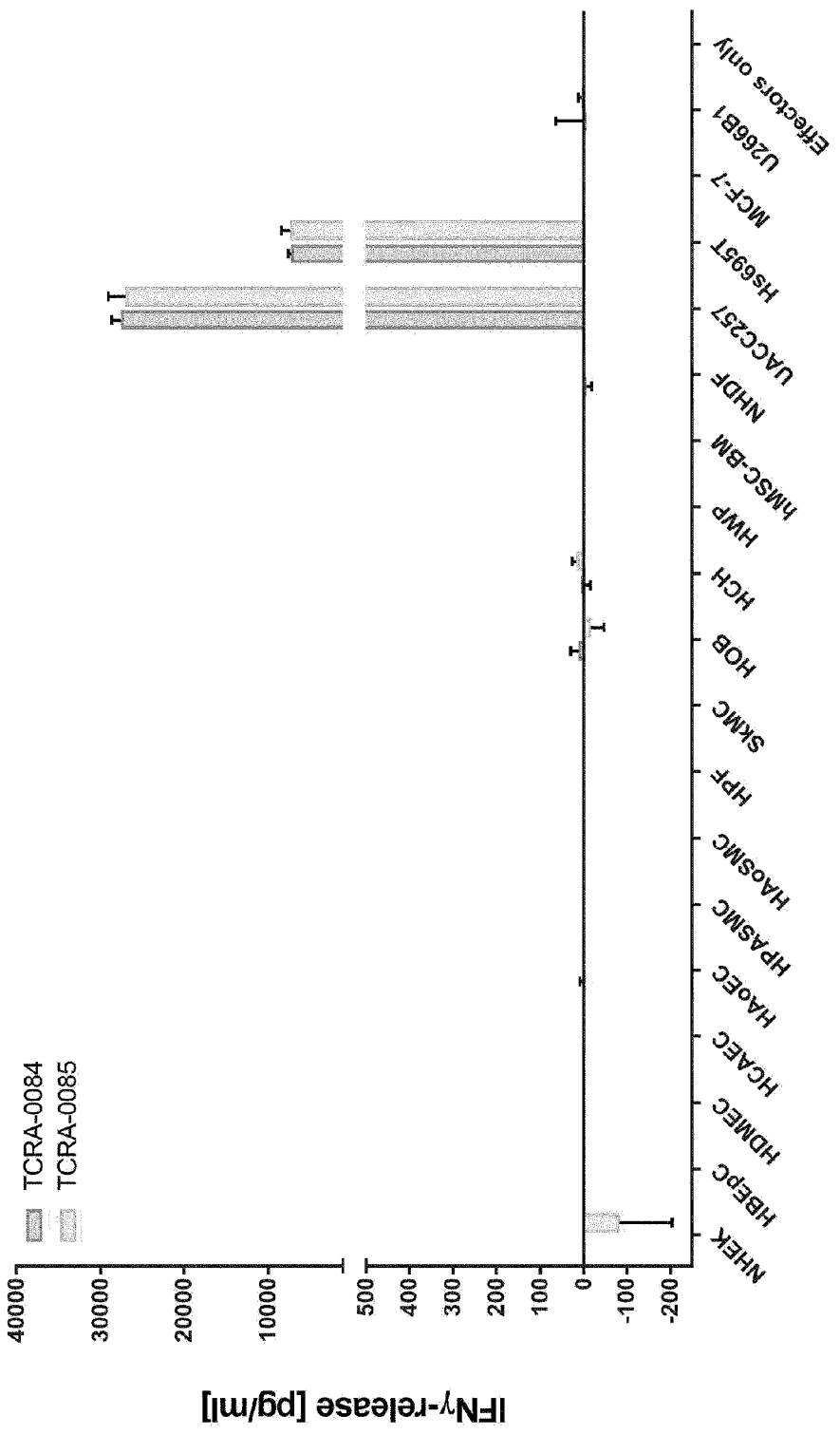

TCRA-0084
TCRA-0085
IFNγ-release [pg/ml]
40000
30000
20000
10000
500
400
300
200
100
0
-100
-200
NHEK
HBEpC
HDMEC
HCAEC
HAoEC
HPASMC
HAoSMC
HPF
SkMC
HOB
HCH
HWP
hMSC-BM
NHDF
UACC257
Hs695T
MCF-7
U266B1
Effectors only

Figure 25

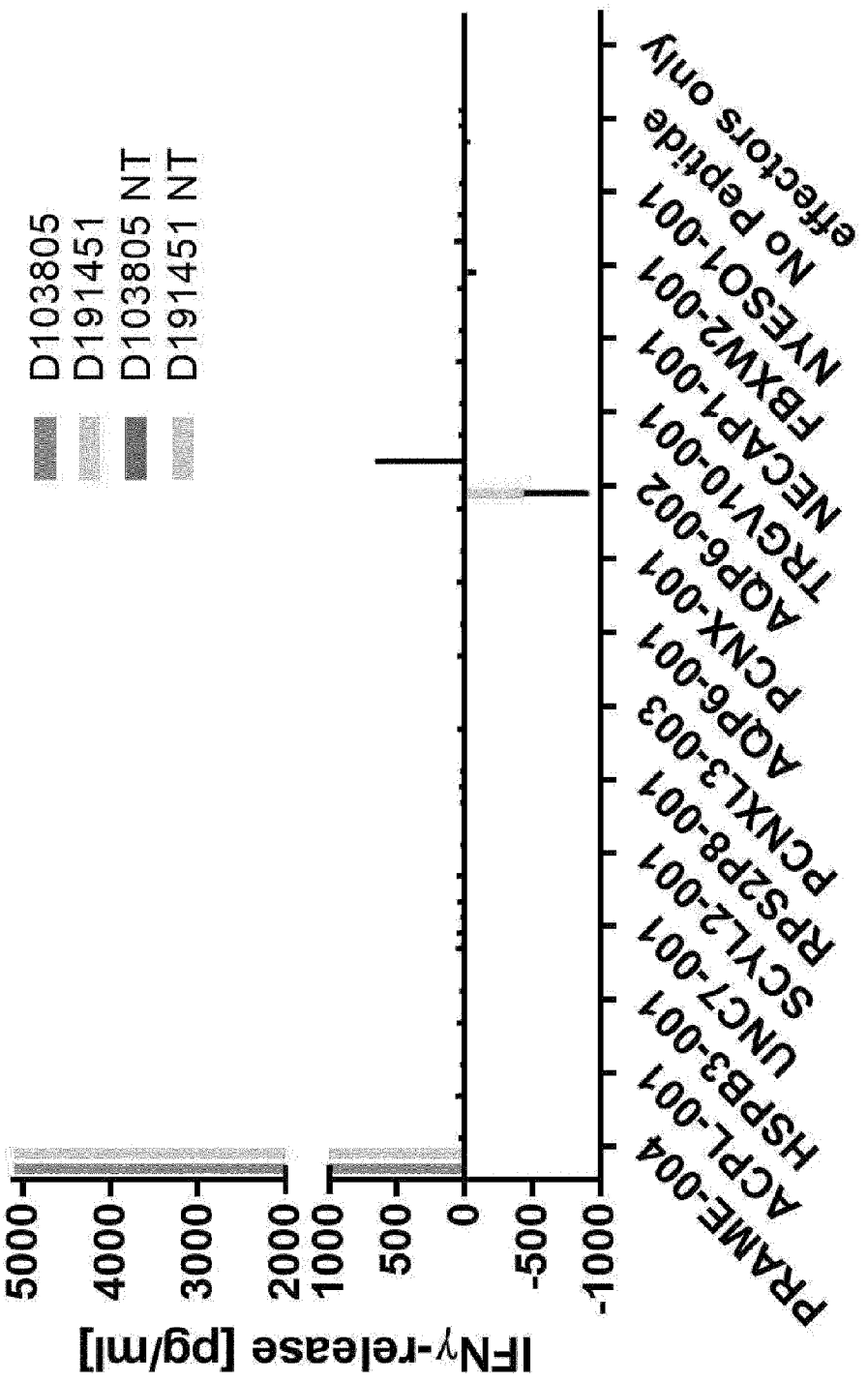

D103805
D191451
D103805 NT
D191451 NT
IFNγ-release [pg/ml]
5000
4000
3000
2000
1000
500
0
-500
-1000
PRAME-004
ACPL-001
HSPB3-001
UNC7-001
SCYL2-001
RPS2P8-001
PCNXL3-003
AQP6-001
PCNX-001
AQP6-002
TRGV10-001
NECAP1-001
FBXW2-001
NYESO1-001
No Peptide
effectors only

Figure 26

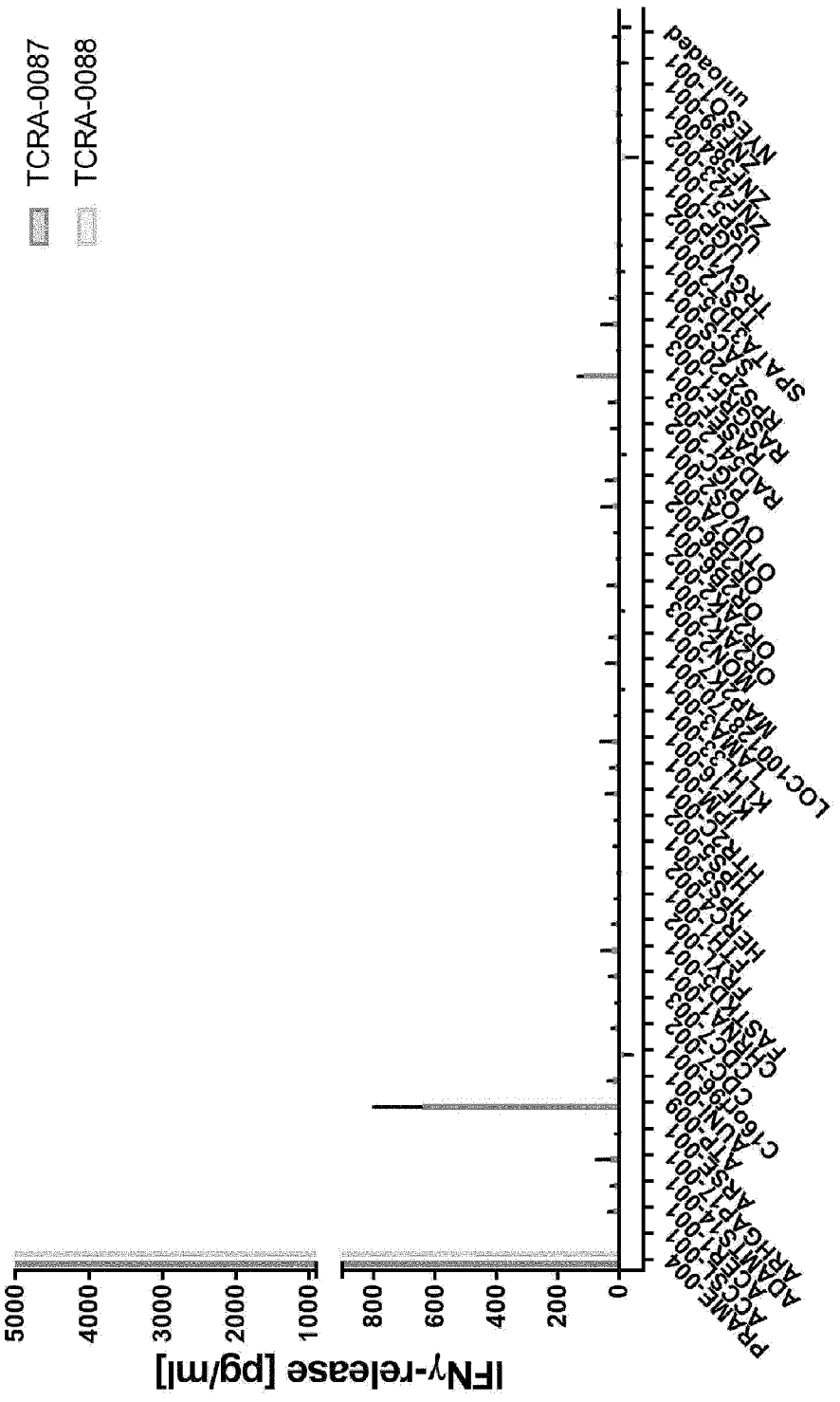

TCRA-0087
TCRA-0088
IFNγ-release [pg/ml]
5000
4000
3000
2000
1000
800
600
400
200
0
unloaded

Figure 27

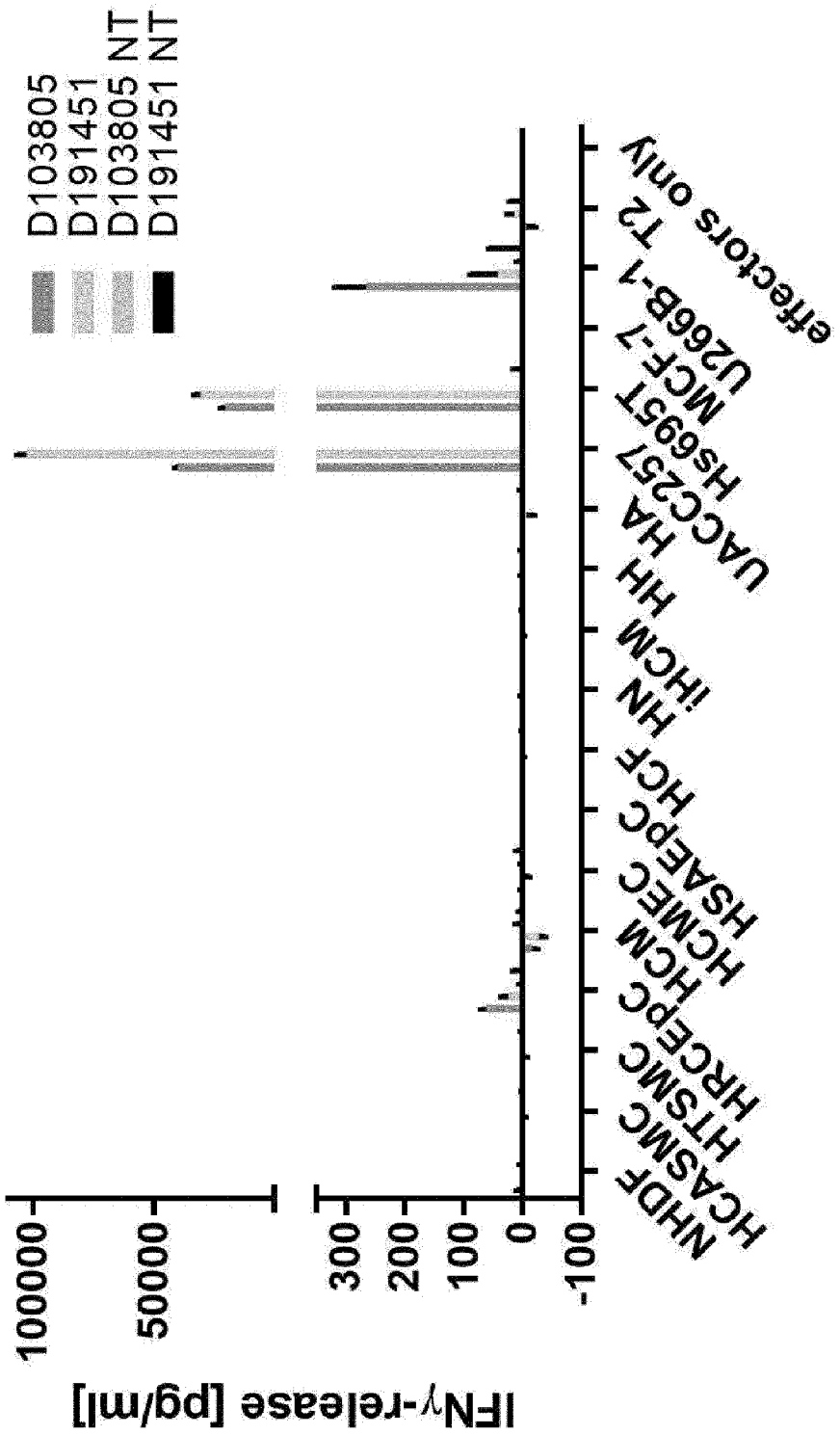

D103805
D191451
D103805 NT
D191451 NT
IFNγ-release [pg/ml]
100000
50000
300
200
100
0
-100
NHDF
HCASMC
HTSMC
HRCEpC
HCM
HCMEC
HSAEpC
HCF
HN
iHCM
HH
HA
UACC257
HS695T
MCF-7
U266B-1
T2
effectors only

Figure 28

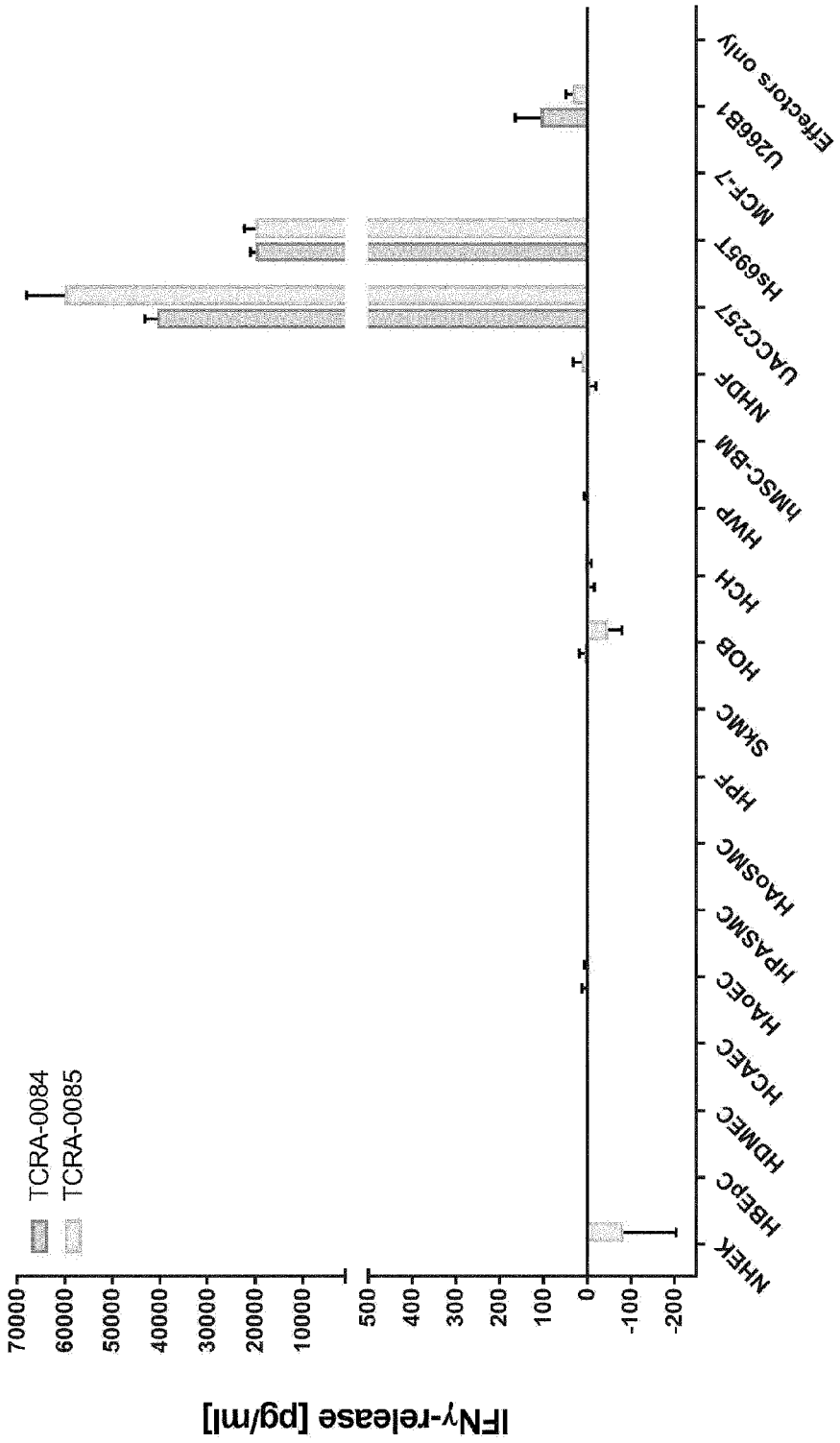

TCRA-0084
TCRA-0085
IFNγ-release [pg/ml]
70000
60000
50000
40000
30000
20000
10000
500
400
300
200
100
0
-100
-200
NHEK
HBEpC
HDMEC
HCAEC
HAoEC
HPASMC
HAoSMC
HPF
SkMC
HOB
HCH
HWP
hMSC-BM
NHDF
UACC257
Hs695T
MCF-7
U266B1
Effectors only

Figure 29

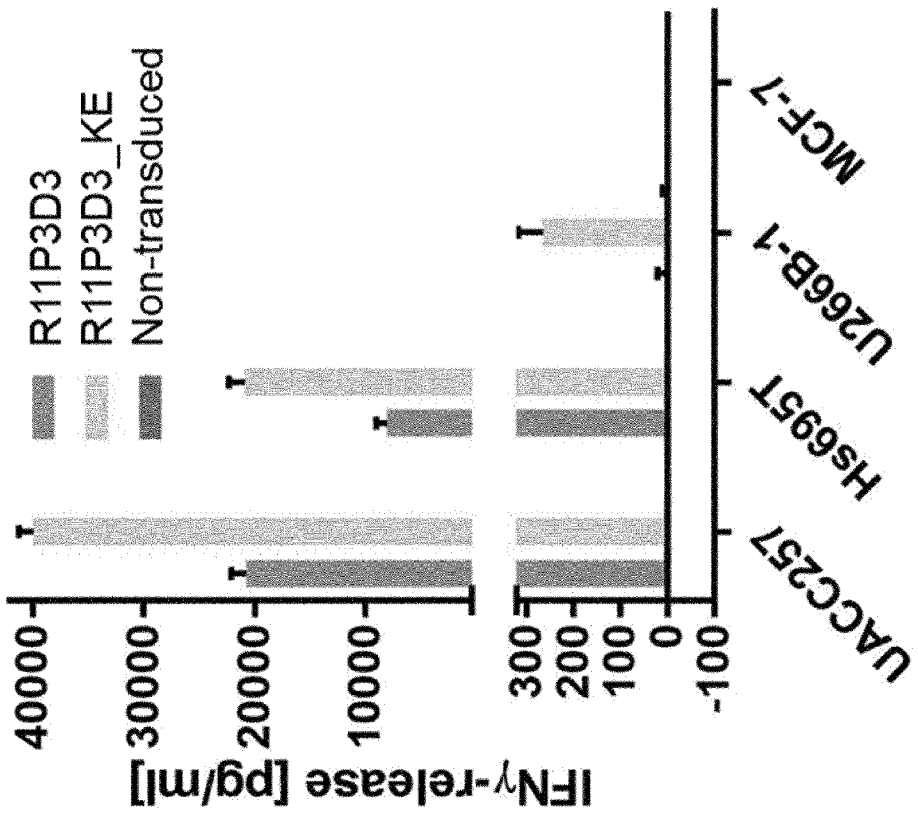

R11P3D3
R11P3D3_KE
Non-transduced
IFNγ-release [pg/ml]
40000
30000
20000
10000
300
200
100
0
-100
UACC257
Hs695T
U266B-1
MCF-7

Figure 30

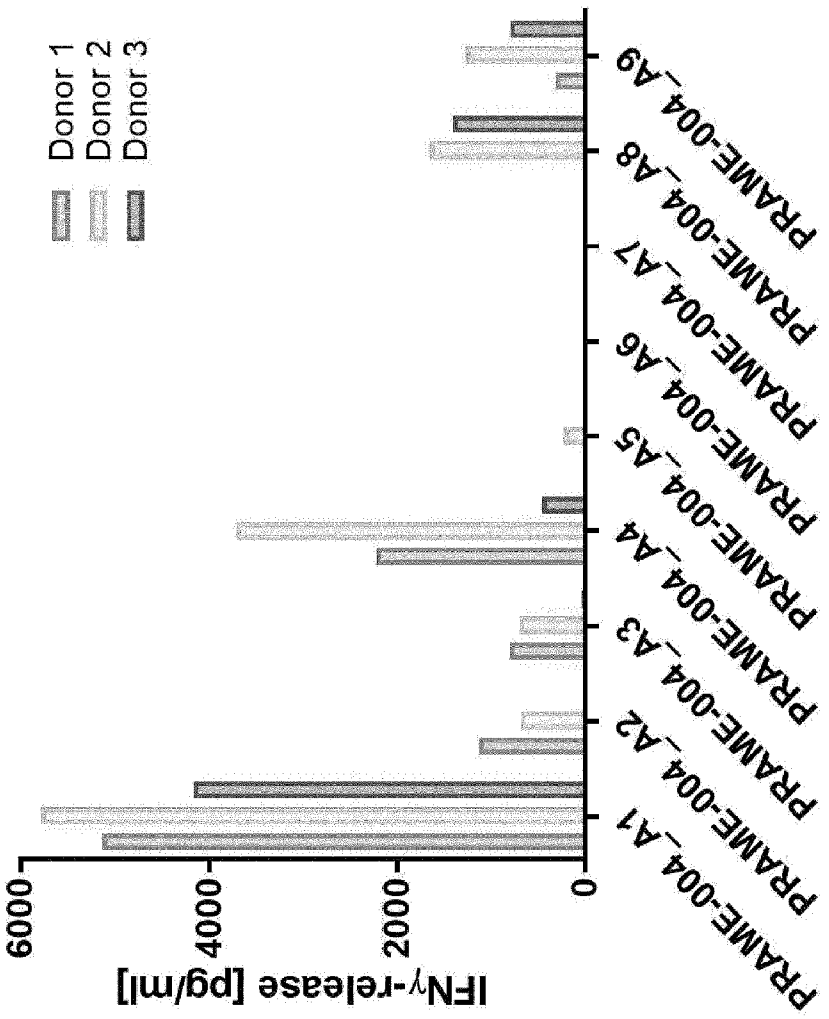
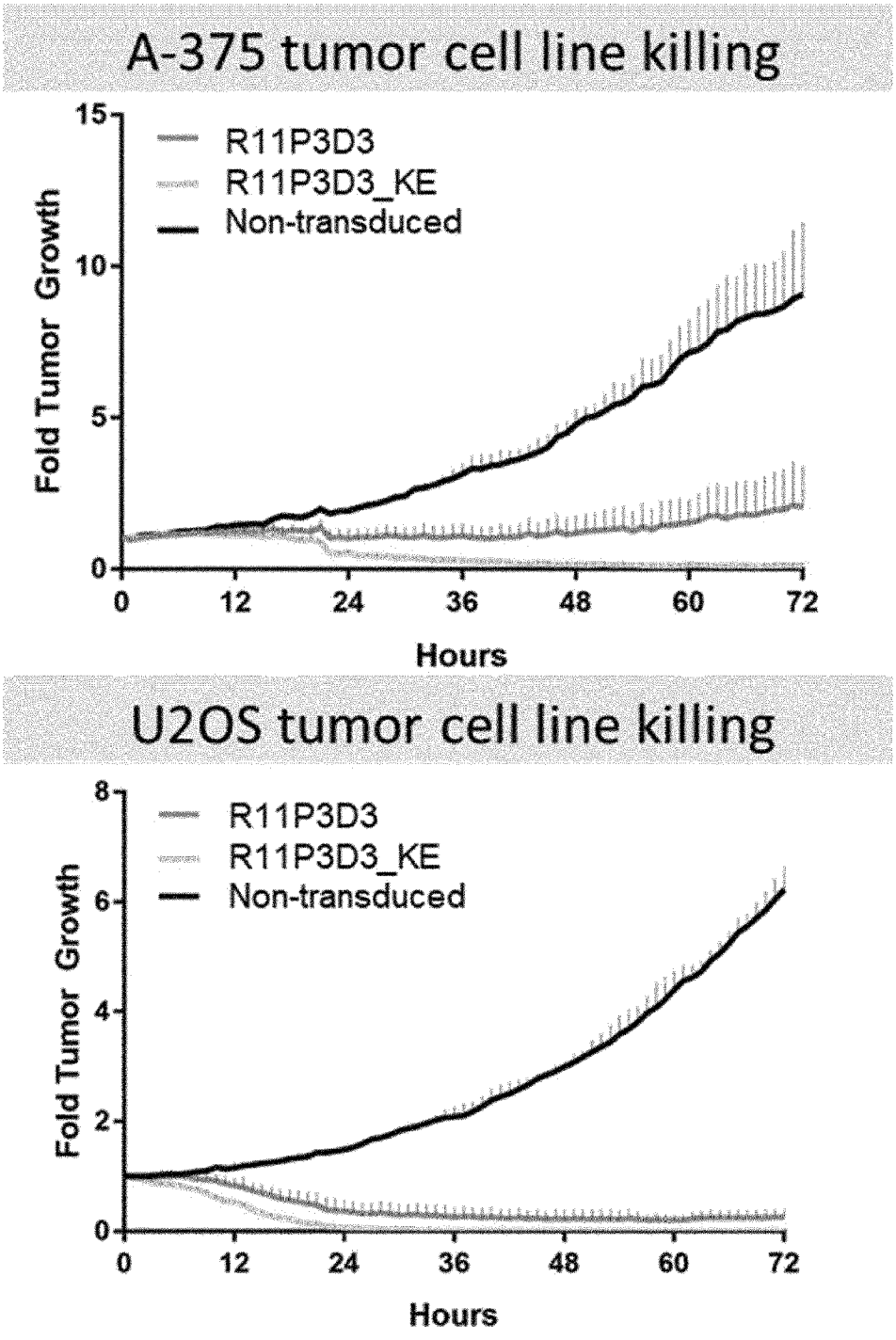

Donor 1
Donor 2
Donor 3
IFNγ-release [pg/ml]
0
2000
4000
6000
PRAME-004_A1
PRAME-004_A2
PRAME-004_A3
PRAME-004_A4
PRAME-004_A5
PRAME-004_A6
PRAME-004_A7
PRAME-004_A8
PRAME-004_A9

Figure 31

Figure 32


A-375 tumor cell line killing
R11P3D3
R11P3D3_KE
Non-transduced
Fold Tumor Growth
15
10
5
0
0
12
24
36
48
60
72
Hours
U2OS tumor cell line killing
R11P3D3
R11P3D3_KE
Non-transduced
Fold Tumor Growth
8
6
4
2
0
0
12
24
36
48
60
72
Hours

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.