



(51) International Patent Classification:

C12N 5/0783 (2010.01) C07K 14/705 (2006.01)

C07K 14/725 (2006.01) A61K 35/17 (2015.01)

C07K 14/735 (2006.01)

(21) International Application Number:

PCT/GB2019/053514

(22) International Filing Date:

11 December 2019 (11.12.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1820157.4 11 December 2018 (11.12.2018) GB

(71) Applicants: **IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE** [GB/GB]; Exhibition Road, London, SW7 2AZ (GB). **OXFORD UNIVERSITY INNOVATION LIMITED** [GB/GB]; Buxton Court, 3 West Way, Oxford OX2 0JB (GB).

(72) Inventors: **GLANVILLE, Julie**; MRC London Institute of Medical Sciences, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN (GB). **MCMICHAEL, Andrew**; Nuffield Department of Medicine, University of Oxford, Henry Wellcome Building for Molecular Physiology, Old Road Campus, Headington, Oxford OX3 7BN (GB). **DONG, Tao**; Nuffield Department of Medicine, University of Oxford, Henry Wellcome Building for Molecular Physiology, Old Road Campus, Headington, Oxford OX3 7BN (GB). **MOODY, Anne-Marie**; MRC London Institute of Medical Sciences, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN (GB).

(74) Agent: **BASSIL, Nicholas**; Kilburn & Strode LLP, Lacon London, 84 Theobalds Road, London WC1X 8NL (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: MODIFIED CELLS FOR USE IN A METHOD OF TREATMENT OF DISEASE

(57) Abstract: The present invention provides a genetically engineered cell comprising a receptor that comprises an antigen binding domain and a T-cell activating function and at least one co-receptor which modulates the activity of the receptor, wherein the cell overexpresses the co-receptor and in which the co-receptor is selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3), and uses thereof in medicine, for example in the treatment of cancer, inflammation, an auto-immune disease or viral infection. The invention also relates to nucleic acid constructs comprising co-receptor is selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) and a chimeric antigen T cell receptor, and uses thereof in medicine.



MODIFIED CELLS FOR USE IN A METHOD OF TREATMENT OF DISEASE

The present invention relates to methods for the treatment of diseases such as cancer or viral infections in which the therapy is mediated by cells, for example T cells, caused to express specific
5 cell surface receptors.

Cytotoxic T lymphocytes (CTL) that encounter antigen proliferate from a single naïve T-cell to form a population with diverse effector function and differentiation states, that control and prevent infection and cancer. Antigen-specific CTL are extremely heterogeneous in terms of clonotype, differentiation
10 state and efficacy. Factors that define CTL of superior efficacy are well explored. TCR signal strength is known to modulate the breadth and magnitude of CTL effector functions, and epigenetic marks modulate intrinsic states such as exhaustion that limit activation. It is not clear if or how TCR signal strength modulates contact-dependent killing, and TCR affinity, TCR avidity, or factors downstream of the TCR could all contribute. There is a critical period of contact when a TCR engages cognate
15 peptide- MHC class I molecules on a target cell and the signal summation may determine if the cell will be killed or survive during an encounter. Not all T-cell therapy products to treat cancer are equally efficacious despite lentiviral transduction with the same chimeric-antigen-receptor. Lack of CTL efficacy is also encountered in chronic viral infections. Indeed, CTL from HIV controllers are superior and suppress HIV replication *in vitro* unlike CTL from chronic progressors. Viral suppression
20 correlates with target cell elimination suggesting a critical role for contact-dependent killing.

CD8⁺ cytotoxic T lymphocytes (CTLs) are serial killers, polarizing the cytoskeleton to form kinapses, from which cortical actin briefly clears to permit cytotoxic granule fusion at the cell membrane (Ritter
et al. (2017) *Proc Natl Acad Sci U S A* 114(32):E6585–E6594), enabling targeted delivery of lytic
25 molecules (Halle *et al.* (2017) *Trends Immunol*). Contact-dependent killing and other effector functions are triggered at different signalling thresholds in response to antigen engagement by the T cell receptor (TCR) (Valitutti *et al.* (1996). *J Exp Med* 183(4):1917–1921) (3). As a consequence, the functional profile of any given CTL is governed by antigen dose and the efficiency of signal transduction, which in turn dictates antigen sensitivity. The protective capabilities of HIV-1-specific
30 CTLs have been variously attributed to polyfunctionality (Betts *et al.* (2006) *Blood* 107(12):4781–4789), cytotoxic activity (McMichael & Jones (2010) *Science* 330(6010):1488–1490), and *in vitro* suppression of viral replication (Saez-Cirion *et al.* (2007) *Proc Natl Acad Sci U S A* 104(16):6776–6781). For naturally processed epitopes presented at fixed densities on the target cell surface, these antiviral functions are regulated by antigen sensitivity (Almeida *et al.* (2009) *Blood* 113(25):6351–
35 636), which is known to vary among clonotypes expressing distinct TCRs. However, it is not clear if or how events downstream of the TCR contribute to the antiviral efficacy of CTLs (Flerin *et al.* (2017) *J Virol* 91(6)).

It has now been surprising found that TCR-identical CTLs isolated from individual donors can exhibit
40 similar levels of heterogeneity with respect to cytokine production, contact-dependent killing, and

suppression of viral replication *in vitro*. Expression of specific coreceptors on the cell surface of the T cells has been shown in the present invention to trigger contact-dependent killing at lower epitope densities in the more potent CTLs. The coreceptors incorporate tyrosine-based activation or inhibition motifs. The most effective coreceptor has been shown in the present invention to be FCRL3.

5

CD8⁺ CTLs protect against life-threatening viral infections and cancer. This formidable task is made possible by a process of gene recombination that can generate billions of unique T-cell receptors (TCRs). The present invention challenges the concept that the TCR is the major determinant of CTL efficacy. The inventors have observed a striking degree of functional heterogeneity among TCR-
10 identical human CTLs. It has been found that cytolytic activity and antiviral potency are dictated by TCR-mediated signal summation, and further identified co-receptors that modulate the activation threshold in response to antigen-triggered ligation of the TCR. These findings demonstrate that cell-intrinsic factors can regulate the deployment of effector functions irrespective of the TCR which enables novel approaches to optimize the protective attributes of CTLs in the treatment of cancer
15 and viral infection.

According to a first aspect of the invention there is provided a genetically engineered cell comprising a receptor that comprises an antigen binding domain and a T-cell activating function and at least one co-receptor which modulates the activity of the receptor, wherein the cell overexpresses the co-
20 receptor and in which the co-receptor is selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3).

The co-receptor is overexpressed compared to a control (i.e. an unmodified cell where expression of the co-receptor has not been altered). The cell is therefore a genetically engineered cell which
25 overexpresses the co-receptor. The cell of the invention may possess an improved function or activity such as for example an improved cytotoxic activity (i.e. cell killing activity, for example T cell mediated cell killing). Expression of one of these co-receptors can provide improved activity (i.e. cell killing activity) in transduced or transfected cells compared with control cells not expressing the co-receptor. The co-receptor may therefore be described as being a heterologous co-receptor with
30 respect to the cell in which it is overexpressed. Suitably, the cell is transfected or transduced by any convenient method of genetic engineering to overexpress the co-receptor.

The receptor may be a T cell receptor (TCR) or a chimeric antigen receptor (CAR). In a cell which expresses a CAR, the cell is also genetically engineered to express the CAR. In a cell which
35 expresses a modified T cell receptor or a heterologous T cell receptor, the cell is also genetically engineered to express the modified T cell receptor or heterologous T cell receptor.

The T cell receptor may be native to the cell it is expressed in, i.e. an autologous T cell receptor from the same individual the T cell is from. Alternatively, the T cell receptor may also be a heterologous
40 recombinant protein expressed on the surface of the cell which is transduced or transfected with a

nucleic acid sequence encoding the TCR. The TCR may therefore be an autologous TCR or a heterologous TCR.

5 The chimeric antigen receptor (CAR) is a recombinant receptor that comprises an antigen binding domain and a T-cell activating function (so-called first-generation CARs). Optionally the CAR may additionally comprise a costimulatory domain of a costimulatory receptor (so-called second-generation CARs), or the CAR may comprise 2 costimulatory domains combined with an activation domain in their cytoplasmic domain (so-called third-generation CARs). By definition, a CAR is a heterologous recombinant protein expressed on the surface of a cell which is transduced or
10 transfected with a nucleic acid sequence encoding the CAR.

The modulation of the activity of the receptor provided by the co-receptor may be activation (upregulation) or inhibition (downregulation). The activation represents enhancement of the activity of the T cell killing activity of the cell. The inhibition represents repression of the activity of the T cell
15 killing activity of the cell.

The co-receptor is a recombinant receptor comprising one or more of FCRL3, FCRL6, LRRN3 which are therefore heterologous to the cell in which the co-receptor is expressed. The co-receptor is expressed on the surface of the cell which is transduced or transfected with a nucleic acid sequence
20 or sequences encoding the co-receptor or co-receptors. The co-receptor may be co-expressed with the CAR.

FCRL3 is a glycoprotein and a member of the immunoglobulin receptor superfamily. The protein comprises cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs) and
25 immunoreceptor tyrosine-based inhibition motifs (ITIMs). The FCRL3 co-receptor may have the nucleic acid coding sequence or amino acid sequence as shown in Figure 6 or an isoform as shown in Figure 7 or a sequence substantially identical thereto, suitably 75%, 80%, 85%, 90%, 95%, 99% identical thereto. As a co-receptor, FCRL3 enhances the cytotoxic T-lymphocyte (CTL) cell mediated killing activity of the TCR or CAR.

30 FCRL6 is a glycoprotein and a member of the immunoglobulin receptor superfamily. The protein comprises cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs). The FCRL6 co-receptor may have the nucleic acid coding sequence or amino acid sequence as shown in Figure 11 or a sequence substantially identical thereto, suitably 75%, 80%, 85%, 90%, 95%, 99% identical
35 thereto. As a co-receptor, FCRL6 reduces the CTL cell mediated killing activity of the TCR or CAR.

LRRN3 is a transmembrane protein with an immunoglobulin domain and a previously unreported cytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM). The LRRN3 co-receptor may have the nucleic acid coding sequence or amino acid sequence as shown in Figure 10 or a sequence

substantially identical thereto, suitably 75%, 80%, 85%, 90%, 95%, 99% identical thereto. As a co-receptor, LRRN3 reduces the CTL cell mediated killing activity of the TCR or CAR.

5 The term "protein" in this text means, in general terms, a plurality of amino acid residues joined together by peptide bonds. It is used interchangeably and means the same as peptide, oligopeptide, oligomer or polypeptide, and includes glycoproteins and derivatives thereof. The term "protein" is also intended to include fragments, analogues and derivatives of a protein wherein the fragment, analogue or derivative retains essentially the same biological activity or function as a reference protein.

10

The fragment, analogue or derivative of the protein as defined in this text, may be at least 6, preferably 10 or 20, or up to 50 or 100 amino acids long.

15 The fragment, derivative or analogue of the protein may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably, a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv)
20 one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence which is employed for purification of the polypeptide. Such fragments, derivatives and analogues are deemed to be within the scope of those skilled in the art from the teachings herein.

25 Included within the invention are variants, analogues, derivatives and fragments having the amino acid sequence of the protein in which several e.g. 5 to 10, or 1 to 5, or 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the protein of the present invention. Conservative substitutions which do not alter the properties and
30 activities of the protein are especially preferred.

An example of a variant of the present invention is a fusion protein as defined above, apart from the substitution of one or more amino acids with one or more other amino acids. The skilled person is aware that various amino acids have similar properties. One or more such amino acids of a substance can
35 often be substituted by one or more other such amino acids without eliminating a desired activity of that substance.

The amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions, it may be preferred that
40 glycine and alanine are used to substitute for one another (since they have relatively short side chains)

and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic). Other amino acids which can often be substituted for one another include: phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains); lysine, arginine and histidine (amino acids having basic side chains); aspartate and glutamate (amino acids having acidic side chains); asparagine and glutamine (amino acids having amide side chains); and cysteine and methionine (amino acids having sulphur containing side chains). Substitutions of this nature are often referred to as “conservative” or “semi- conservative” amino acid substitutions.

Amino acid deletions or insertions may also be made relative to the amino acid sequence for the fusion protein referred to above. Thus, for example, amino acids which do not have a substantial effect on the activity of the polypeptide, or at least which do not eliminate such activity, may be deleted. Such deletions can be advantageous since the overall length and the molecular weight of a polypeptide can be reduced whilst still retaining activity. This can enable the amount of polypeptide required for a particular purpose to be reduced - for example, dosage levels can be reduced.

Amino acid insertions relative to the sequence of the fusion protein above can also be made. This may be done to alter the properties of a substance of the present invention (e.g. to assist in identification, purification or expression, as explained above in relation to fusion proteins).

Amino acid changes relative to the sequence for the fusion protein of the invention can be made using any suitable technique e.g. by using site-directed mutagenesis. It should be appreciated that amino acid substitutions or insertions within the scope of the present invention can be made using naturally occurring or non-naturally occurring amino acids. Whether or not natural or synthetic amino acids are used, it is preferred that only L- amino acids are present.

A protein according to the invention may have additional N-terminal and/or C-terminal amino acid sequences. Such sequences can be provided for various reasons, for example, glycosylation.

The term “fusion protein” in this text means, in general terms, one or more proteins joined together by chemical means, including hydrogen bonds or salt bridges, or by peptide bonds through protein synthesis or both.

“Identity” as known in the art is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness (homology) between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. While there exist a number of methods to measure identity between two polypeptides or two polynucleotide sequences, methods commonly employed to determine identity are codified in computer programs. Preferred computer programs to determine identity between two sequences

include, but are not limited to, GCG program package (Devereux, et al., Nucleic acids Research, **12**, 387 (1984), BLASTP, BLASTN, and FASTA (Atschul et al., J. Molec. Biol. **215**, 403 (1990).

5 The antigen binding domain of the CAR may recognise an epitope on an antigen presented by a disease-causing agent such as a virus or a cancerous or precancerous cell.

The virus may be a retrovirus, for example a human immunodeficiency virus, e.g. HIV-1, or HIV-2, or human lymphotropic virus (HTLV-1), human immunodeficiency virus 1.

10 The antigen may be tumour antigen such as CD52, PD-L1, CTLA4, CD20, PD-1 receptor, CD19, CD47, PD-1 (or CD279).

The T-cell activating function includes effector domains such as CD3- ζ or Fc receptor γ (FcR γ).

15 The co-stimulatory domain of a costimulatory receptor includes effector domains such as CD28, 4-1BB, DAP10, OX40, or ICOS.

The activation domain may be a CD28 domain.

20 First generation CARs include chimeric molecules between CD3- ζ or Fc receptor γ and CD8, CD4, CD25, or CD16.

Second-generation CARs include receptors encompassing the CD3- ζ chain, and the cytoplasmic domain of a costimulatory receptor such as CD28, 4-1BB, DAP10, OX40, or ICOS.

25 Third generation of CARs include receptors encompassing 2 costimulatory domains combined with an activation domain in their cytoplasmic domain, for example CD20-specific CD28/4-1BB/CD3 ζ .

30 In one embodiment of the aspect of the invention, the co-receptor may be cloned into a third-generation self-inactivating lentiviral vector such as pCCL under the control of an internal promoter (e.g. PGK or EFa) either alone or linked to chimeric antigen receptor targeting tumour antigens (e.g. CD19, CD20) via a self-cleaving peptide or IRES element. T cells can be activated using anti-Cd3/CD28 reagents, transduced, expanded and tested in functional assays. These may include cytokine release assays, proliferation and cytotoxicity experiments.

35 The genetically engineered cell may be a T cell, suitably a CD8+ T cell, including a population of such cells. The cell or population of cells may also be a CAR T cell in which the cell or cells have been modified to express a chimeric antigen T cell receptor.

For example, in one embodiment the invention may related to a population of genetically engineered cells which all express an identical receptor (e.g. a TCR and/or a CAR) and also express a co-receptor (e.g. a FCRL3, FCRL6, and/or a LRRN3 co-receptor). Such a population of cells may have a defined improved function (e.g. improved killing activity) compared with a control population lacking said co-receptor, as a population of cells with the same receptor but with a different co-receptor.

According to a second aspect of the invention, there is provided a nucleic acid sequence encoding a chimeric antigen receptor and a co-receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3).

10

The nucleic acid sequence may be natural, synthetic or recombinant. It may, for example, be cDNA, PCR product or a genomic sequence. It may be isolated, or as part of a plasmid, vector or host cell. A plasmid is a circular extrachromosomal DNA molecule with the ability to replicate independently of chromosomal DNA.

15

A plasmid may be used to introduce an expression cassette into a host cell. Plasmids may also be used to express a polypeptide in a host cell. For example, a bacterial host cell may be transfected with a plasmid capable of encoding a particular polypeptide, in order to express that polypeptide. The term also includes yeast artificial chromosomes and bacterial artificial chromosomes which are capable of accommodating longer portions of DNA.

20

A promoter is a region of DNA with a specific sequence that initiates the transcription of a particular gene or genes. Promoters used for the expression of heterologous genes include constitutive and inducible promoters. Examples of such promoters, include but are not limited to PGK or EFa.

25

In a preferred embodiment of the invention the promoter comprises a nucleotide sequence that is substantially homologous to the sequence set forth in Fig 6, 10, or 11. Nucleic acid sequences with greater than 20% identity (for example 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99%) are considered to be homologous sequences. As used herein, substantially homologous refers to sequences exhibiting at least 60% or 70%, preferably means at least 80%, more preferably at least 90%, and most preferably at least 95%, 96%, 97%, 98%, 99% or greater identity. In an embodiment of the invention the sequence has at least 80% or more (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, etc.) homology with the sequence set forth in Fig 6, 10 or 11.

30

As used herein, the terms homology and identity are interchangeable.

35

Sequence comparisons to determine homology can be carried out using readily available sequence comparison software. Examples include but are not limited to BLAST (see Ausubel et al., 1999 Short Protocols in Molecular Biology, 4th Ed - Chapter 18) and FASTA (Altschul et al., 1990 J. Mol. Biol.

403-410). Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999, Short Protocols in Molecular Biology, pages 7-58 to 7-60).

5 According to a third aspect of the invention, there is provided a vector comprising a nucleic acid sequence according to the second aspect of the invention. The vector may be a lentiviral virus, for example pCCL. The vector may comprise the nucleic acid sequence either alone or linked to chimeric antigen receptor targeting tumour antigens (e.g. CD19, CD20 etc.), via a self-cleaving peptide or IRES element.

10 An example of a suitable lentiviral vector is LeGO-iG2 (addgene) (Weber et al. (2008) Mol. Ther. 16(4), 698-706), or pCCL-c-MNDU3-X (Logan et al (2004) Hum Gene Ther 15(10), 976-988). The vector may comprise CMV promoter and optionally also an internal SFFV promoter. Other internal promoters include PGK or EFa. Additional self-cleaving peptides or IRES elements may be present also. An example of one embodiment of this aspect of the invention is shown in Figure 8 where the
15 nucleic acid sequence encoding a co-receptor is the sequence coding for FCRL3. An alternative vector may be a self-inactivating lentiviral vector such as pCCL suitably under the control of an internal promoter (e.g. PGK or EFa) either alone or additionally comprising a self-cleaving peptide or IRES element. Vectors of the invention are described in Figures 8, 9, 12 and 13.

20 Aspects of the invention relating to nucleic acid sequences and/or vectors include isolated forms of the nucleic acid and/or vector, suitably in the form of cDNA.

According to a fourth aspect of the invention, there is provided a cell comprising a vector according to the fourth aspect of the invention. The cell may be a T cell, suitably a CD8+ T cell, including a
25 population of such cells.

According to a fifth aspect of the invention, there is provided a cell according to the first aspect, a nucleic acid sequence according to the second aspect, a vector according to the third aspect or a cell according to the fourth aspect for use in medicine. Embodiments of the invention in accordance
30 with this aspect of the invention therefore include a method of treatment of a disease comprising administration of an effective amount of the cell, nucleic acid sequence, vector or cell comprising a vector to a subject in need thereof. The subject may be a human or a non-human animal, for example a non-human mammal.

35 Such uses in medicine may find greatest application in the treatment of cancer, a viral infection, an auto-immune disease or inflammation. The treatment of cancer may comprise enhanced activity in T cell mediated killing of tumour cells. This aspect of the invention therefore also extends to and includes use of a cell, nucleic acid sequence, vector or cell comprising a vector according to the invention in the manufacture of a medicament for the treatment of cancer, a viral infection, an auto-
40 immune disease or inflammation.

Cell-based treatments of cancer using genetically engineered cells of the invention may be of particular use. However, it is equally envisaged that nucleic acids and/or vectors of the invention may be used effectively also, such as to increase FCRL3/FCRL6/LRRN3 co-receptor expression for the purpose of increasing T cell killing activity in genetically engineered cells of the invention.

The cancer may be a solid tumour, for example a sarcoma, a carcinoma or a lymphoma. Alternatively, the cancer may be a haematological cancer such as a leukaemia. The viral infection may be the result of infection of a subject by a retrovirus (for example, a human T-lymphotropic virus (for example HTLV-1, -2, -3, or -4), a human immune deficiency virus (for example HIV-1 or HIV-2). The inflammation may be rheumatoid arthritis, ankylosing Spondylitis (AS), antiphospholipid antibody syndrome (APS), gout, inflammatory arthritis, myositis, scleroderma, Sjogren's syndrome, systemic lupus erythematosus (SLE or lupus), or vasculitis. The auto-immune disease may be inflammatory bowel disease (IBD), multiple sclerosis, Guillain-Barre syndrome, diabetes mellitus (type 1 diabetes), psoriasis, chronic inflammatory demyelinating polyneuropathy.

Cells for use according to the invention may be obtained from any suitable source. Typically, the cells may be autologous, i.e. isolated from the same patient to whom the genetically engineered cells are subsequently administered after modification of the cells. Such autologous cells may be derived from the patient according to any generally suitable procedure. A suitable procedure for obtaining lymphocytes, e.g. T cells from the blood of a patient is apheresis (more specifically by means of leukapheresis). Suitably, the patient is a human subject.

The cells may be obtained from the blood of a patient, subsequently genetically modified (e.g. by transduction or transfection) to express a nucleic acid sequence as defined herein which encodes a co-receptor (e.g. a FCRL3, FCRL6, and/or a LRRN3 co-receptor), optionally cultured and expanded *ex vivo*, and then returned to the circulating blood of the patient by infusion. In some embodiments, the cells may also additionally be genetically engineered to express a modified T cell receptor, or a heterologous T cell receptor, or a CAR, or a mixture thereof.

Suitably, cells obtained and prepared in this way can be stored (e.g. by cryopreservation) prior to use in suitable containers or packs, such as ready-to-use packs for infusion of the modified cells back into the patient.

In one embodiment, the present invention therefore provides CAR-T cells which recognise cancer cells using a chimeric antigen receptor that combines an antibody recognition domain and the downstream signaling machinery of a TCR which have also been transduced or transfected to co-express Fc-Receptor-Like-3 (FCRL3) to enhance killing of cancer cells.

In another embodiment, there is provided modified HTLV-1 specific CD8 T-cells which have been transduced or transfected to co-express Fc-Receptor-Like-3 (FCRL3) to reduce or eliminate HTLV-1 viral load.

- 5 Also provided is modified Tumour invasive lymphocytes (TILS) which have been transduced or transfected to co-express Fc-Receptor-Like-3 (FCRL3) to treat solid tumours, such as lymphoma.

The method of treatment may further comprise the administration of another therapeutic agent. The therapeutic agent may be administered separately, simultaneously or sequentially with the cells,
10 nucleic acid, vector or cell comprising a vector of the invention. In the treatment of cancer, the subject may also receive radiation therapy and/or chemotherapy prior to, during or after the administration of the cells, nucleic acid, vector or cell comprising a vector of the invention.

According to a sixth aspect of the invention, there is provided a kit comprising cell, nucleic acid,
15 vector or cell comprising a vector as defined above and a physiologically acceptable diluent or adjuvant.

According to a seventh aspect of the invention, there is provided a specific binding molecule which binds to a receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) for use in medicine. Embodiments
20 of the invention in accordance with this aspect of the invention therefore include a method of treatment of a disease comprising administration of an effective amount of the specific binding to a subject in need thereof. The subject may be a human or a non-human animal, for example a non-human mammal.

25 Such uses in medicine may find greatest application in the treatment of cancer, a viral infection, an autoimmune disease or inflammation. This aspect of the invention therefore also extends to and includes use of a specific binding molecule of the invention in the manufacture of a medicament for the treatment of cancer, a viral infection, an autoimmune disease, or inflammation. Examples of
30 such diseases are as described above.

It is envisaged that some particular uses concerning specific binding molecules to FCRL3 may be in the treatment of rheumatoid arthritis or T-cell lymphoma, including cutaneous T cell lymphoma. It is envisaged that some particular uses concerning specific binding molecules to FCRL6 and LRRN3
35 may be in the treatment of an autoimmune disease such as rheumatoid arthritis or multiple sclerosis. Similarly, other compounds which inhibit the function of these receptors, including approaches using CRISPR, could be used to equal effect to inhibit and down-regulate the receptors.

The specific binding molecule may be an antibody or a fragment thereof, or an aptamer. Antibodies to FCRL3 are available from BD Pharmingen (catalogue reference 565056) and R&D Systems (catalogue reference FAB3126P).

5 The terms "antibody" and "immunoglobulin" are used herein interchangeably. An antibody molecule is made up of two identical heavy (H) and two identical light (L) chains held together by disulphide bonds. Each heavy chain comprises an Fc polypeptide. The two Fc polypeptides from the two heavy chains dimerise to form the Fc region of the antibody molecule. The term "Fc region" refers to the constant region of an antibody excluding the first constant region immunoglobulin domain of the heavy chain (CH1) that interacts with the constant portion of the light chain (CL) forming a CH1-CL domain pair. Thus, an Fc region comprises the last two constant region immunoglobulin domains (CH2 and CH3) of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM (CH2, CH3 and CH4). The terms "antibody" and "antibodies" include references to engineered antibodies which include other binding domains, such as bispecific antibodies.

15

The antibodies may be monoclonal or polyclonal. Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when the substance of the present invention is injected into the animal. If necessary an adjuvant may be administered together with the substance of the present invention. The antibodies can then be purified by virtue of their binding to a substance of the present invention.

20

Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. This is the well-known Kohler & Milstein technique (*Nature* **256** 52-55 (1975)).

25

Techniques for producing monoclonal and polyclonal antibodies which bind to a particular protein are now well developed in the art. They are discussed in standard immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

30 In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to substances of the present invention. The present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* in *Tibtech* **12** 372-379 (September 1994). Antibody fragments include any portion of a full-length antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, scFv, 35 Fv, dsFv diabody and Fd fragments.

The term "single chain variable fragment" or "scFv" refers to an Fv fragment in which the heavy chain domain and the light chain domain are linked. One or more scFv fragments may be linked to other antibody fragments (such as the constant domain of a heavy chain or a light chain) to form antibody constructs having one or more antigen recognition sites.

40

Other synthetic constructs include CDR peptides. These are synthetic peptides comprising antigen binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings which mimic the structure of a CDR loop and which include antigen-interactive side chains.

Synthetic constructs include chimeric molecules. For example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Synthetic constructs also include molecules comprising a covalently linked moiety which provides the molecule with some desirable property in addition to antigen binding. For example, the moiety may be a label (e.g. a fluorescent or radioactive label) or a pharmaceutically active agent.

According to an eighth aspect of the invention, there is provided a kit comprising a specific binding molecule which binds to a receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) and a physiologically acceptable diluent or adjuvant.

The present inventors have found that FCRL3 is a novel cytotoxic (CD8) T-cell co-receptor that enhances TCR downstream signalling to increase the probability of generating sufficient signal to trigger lytic granule upon encounter with a target cell thus enhancing killing. Overexpression of this molecule in T-cell therapy products may enhance target cell killing i.e. increase killing of cancer or viral infected target cells by CD8+ T-cells. Secondly a unique population of ex vivo CD8+ T-cells is marked by expression of this receptor. The present invention therefore provides a CD8+ T-cell population characterised by expression of FCRL3 with enhanced efficacy for T-cell therapy products. The other co-receptors disclosed herein, FCRL6 and/or LRRN3, are similarly able to enhance T cell killing activity.

According to one embodiment of the invention there is therefore provided a cell comprising a receptor that comprises an antigen binding domain and a T-cell activating function and at least one co-receptor which modulates the activity of the receptor in which the co-receptor is selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3).

Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The present invention will now be described by way of illustration with reference to the following Examples. Reference is made to the following drawings in which:

Figure 1 shows functionality of TCR-identical HLA-B*0801-FL8-specific CTL clones. **(A)** Representative flow cytometry plots showing p24 antigen expression in C8166 cells after incubation with clonal CTLs from LTNP005 (A3, A9, A17, or A20) at the indicated E:T ratios. Uninfected target cells were used as the negative control. Infected target cells cultured alone were used as the positive control. The HLA-A*0201-SL9-specific clone G10 was included as a TCR-specificity control. Data represent two independent experiments. **(B)** Virus suppression by clonal CTLs from LTNP005 (A3, A9, A17, or A20) at the indicated E:T ratios. Data are shown as percent suppression relative to p24 antigen content in the supernatant of infected C8166 cells cultured alone. The HLA-A*0201-SL9-specific clone G10 was included as a TCR-specificity control. $*P = 0.0005$; $**P = <0.0001$ (Student t-test). Error bars indicate mean $\pm$ SD. Data represent two independent experiments with two generations of each clone. **(C)** Virus suppression by clonal CTLs from G713 (G713-4 and G713-20) at the indicated E:T ratios. Data are shown as percent suppression relative to p24 antigen content in the supernatant of infected C8166 cells cultured alone. $*P = 0.0039$ at an E:T ratio 1:16 (Student t-test). Error bars indicate mean $\pm$ SD. Data represent triplicate measurements from one experiment. **(D)** Antigen sensitivities of TCR-identical CTL clones from LTNP005. EC_{50} indicates the FL8 peptide concentration (μ M) required to elicit 50% maximal specific lysis of target cells in a standard 4 hr chromium release assay. $*P = 0.0443$ for A3 and A20 at 0.01 μ M (Student t-test). Error bars indicate mean $\pm$ SD. Data represent three independent experiments with three generations of each clone. **(E)** Antigen sensitivities of TCR-identical CTL clones from E833 and G713. EC_{50} values were determined as in **(D)**. $*P = 0.0286$ for E833-30 and E833-13 at 0.1 μ M (student t-test). Error bars indicate mean $\pm$ SD. Data represent three independent experiments with three generations of each clone.

Figure 2 shows characterization of effector functions in TCR-identical HLA-B*0801-FL8-specific CTL clones from LTNP005. **(A)** Pie charts indicate the percentage of clonal A3 or A20 CTLs responding to a range of cognate peptide concentrations. Three effector functions were measured simultaneously: 1 function, CCL4; 2 functions, CCL4 + CD107a; 3 functions, CCL4 + CD107a + IFN γ . **(B)** Secretion of IFN γ and TNF by clonal A3 or A20 CTLs after incubation for 24 hr with HLA-matched CD4 $^{+}$ T cells infected for 72 hr with the indicated TCID $_{50}$ doses of HIV-1. Bar height indicates the mean of triplicates. **(C)** Secretion of CCL4, IFN- γ , TNF, and IL-2 by clonal A3 or A20 CTLs after incubation for 24 hr with phytohaemagglutinin (PHA) or phorbol myristate acetate and ionomycin (PMA and IONO). Bar height indicates the mean of triplicates. **(D)** Hierarchical clustering and heatmap of differentially expressed genes in clonal A3 or A20 CTLs after stimulation for 6 hr with cognate peptide (2 μ M). The top 32 genes in either direction are shown. Each column represents a sample, and each row represents a gene. The intensity of red/blue colour indicates the relative quantity of mRNA bound to each probe (key). Data represent three independent experiments with three generations of each clone. **(E)** mRNA expression of lytic effector molecules. The graph shows tumour necrosis factor (TNF), lymphotoxin alpha (LTA),

granulysin (*GNLY*), Fas ligand (*FASL*), perforin (*PRF*), granzyme B (*GZMB*), and TRAIL (*TNFSF10*) gene expression in clonal A20 vs. A3 CTLs, expressed as mean base 2 logarithmic fold change indicated by bar height, with *P* values (LIMMA). (F) Bisulfite sequencing of the core TNF promoter. DNA isolated from clonal A3 or A20 CTLs was bisulfite converted, amplified by PCR, and sequenced to assess the frequency of methylated cytidine residues situated within CpG dinucleotides in the core TNF promoter from amino acid residues -200 to -117 (red boxes). A representative sequencing screen for residues -171 to -119 is illustrated for each population (A3, left; A20, right). Each line represents one sequence, methylated cytidines are denoted by black circles, and unmethylated cytidines are denoted as green circles.

Figure 3 shows transcriptome analysis of resting TCR-identical HLA-B*0801-FL8-specific CTL clones. (A) Tetramer avidity assay. Clonal A3, A17, or A20 CTLs were stained with the indicated concentrations of the HLA-B*0801-FL8 tetramer. Data represent three independent experiments. (B) Hierarchical clustering and heatmap of 140 genes with altered expression in clonal A20 vs. A3 CTLs (absolute fold change of at least $< 2 >$; $P < 0.01$ by ANOVA). Each column represents a sample and each row represents a probe from the bead array that maps to a gene in the Ensembl database. The colour represents the relative quantity of complementary RNA directly hybridized to each probe for each sample, scaled for each row to range from -1 (blue) to +1 (red) as indicated (key). Some genes are represented by more than one probe. Data represent three independent experiments with three generations of each clone. (C) Bar chart illustrating the mean absolute fold change (x-axis) in gene expression of selected cell surface receptors in resting clonal A20 vs. A3 CTLs ($P < 0.01$ by ANOVA). Protein name, signalling motif, and comments are shown in **Table 2**. (D) Real-time PCR analysis of mRNA extracted from resting clonal A20 vs. A3 CTLs sorted to $> 99\%$ purity by flow cytometry. Error bars indicate mean $\pm$ SD. Data represent three independent experiments with three generations of each clone. (E and F) Real-time PCR analysis of mRNA extracted from resting clonal E833-30 vs. E833-13 CTLs (E) or resting clonal G713-20 vs. G713-4 CTLs (F). In (E), clone E833-30 is the most antigen-sensitive (**Fig. 1E**). In (F), clone G713-20 is the most antigen-sensitive (**Fig. 1E**). Error bars indicate mean $\pm$ SD. Data represent two independent experiments with two generations of each clone.

Figure 4 shows FCRL3 association with TCR signal transduction and degranulation kinetics. (A) A representative flow cytometry histogram showing FCRL3 on two generations of clonal A3 CTLs and three generations of clonal A20 CTLs. MFI values were compared using a Student t-test ($**P = 0.0036$). (B) Phosphorylated forms of p44/42 MAPK in clonal A20 vs. A3 CTLs were quantified by phospho-flow cytometry after stimulation for 10 min with cognate peptide (10 μ M). $****P < 0.0001$ (Student t-test). Data represent two independent experiments with two generations of each clone. (C) CD107a mobilization on clonal A20 vs.

A3 CTLs was measured by flow cytometry at the indicated time points after stimulation with cognate peptide (10 μ M). $**P = 0.0012$ at 15 min; $*P = 0.0298$ at 30 min; $*P = 0.0152$ at 60 min (Student t-test). (D) FCRL3 was cloned into a lentiviral vector and expressed under the control of a spleen focus-forming virus (SFFV) promoter together with IRES-driven GFP to allow fluorescence-activated sorting of transduced cells and the generation of a stable Jurkat cell line expressing FCRL3. (E) A representative flow cytometry histogram showing phosphorylated p44/42 MAPK in Jurkat cell lines expressing FCRL3 or the corresponding Empty Vector after stimulation for 10 min with OKT3. (F) MFI values for phosphorylated p44/42 MAPK were compared using a Student t-test ($**P = 0.0055$).

Figure 5 shows *ex vivo* ITAM/ITIM expression in single TCR V β 13.2⁺ HLA-B*0801-FL8 tetramer-binding CD8⁺ T cells sorted directly *ex vivo* from donor LTNP005. (A) Hierarchical clustering and heatmap of ITAM/ITIM genes and housekeeping genes ACTB, GAPDH, and CD8A. Each column represents a gene, and each row represents a cell. (B) Comparison of the proportions of CD8⁺ T cells expressing the ITAM/ITIM genes *FCRL3*, *FCRL6*, or *LRRN3* in 2003 vs. 2004.

Figure 6 shows the nucleic acid sequence encoding human FCRL3 (a) with accession reference NM_052939.3 and the corresponding amino acid sequence (b) of 734 residues with accession reference NP_443171.2; showing ATG=START CODON and TAG=STOP CODON.

Figure 7 shows amino acid sequences for human FCRL3 isoforms in comparison to the canonical sequence (a) canonical sequence of 734 amino acids; (b) isoform 3; (c) isoform 6; and (d) isoform 7.

Figure 8 shows the nucleic acid sequence of the lentiviral vector comprising the human FCRL3 nucleic acid sequence with the vector residues shown in upper case and the FCRL3 encoding residues shown in lower case.

Figure 9 shows the plasmid map for the vector LeGo-iG2.

Figure 10 shows the nucleic acid sequence encoding human LRRN3 (a) with accession reference NM_001099660.1, transcript variant 1, and the corresponding amino acid sequence (b) with accession reference NP_001093130.1, and (c) a nucleic acid sequence encoding LRRN3 which has been codon optimized for expression.

Figure 11 shows the nucleic acid sequence encoding human FCRL6 (a) with accession reference NM_001004310.2, transcript variant 1, and the corresponding amino acid

sequence (b) with accession reference NP_001004310.2, and (c) a nucleic acid sequence encoding LRRN3 which has been codon optimized for expression.

5 **Figure 12** shows the nucleic acid sequence of the lentiviral vector comprising the human FCRL6 nucleic acid sequence with the vector residues shown in upper case and the FCRL6 encoding residues shown in lower case.

10 **Figure 13** shows the nucleic acid sequence of the lentiviral vector comprising the human LRRN3 nucleic acid sequence with the vector residues shown in upper case and the LRRN3 encoding residues shown in lower case.

Materials and Methods

Patients

15 Samples were obtained from one individual with chronic HIV infection (LTNP005) enrolled in the London Riverside Cohort (UK), a nested case-control study of the biological and behavioural correlates of non-progression, and two individuals (E833 and G713) with acute HIV infection recruited from the San Diego AIDS Treatment Centre (USA) (12). All three individuals were HLA-B*0801+ with known responses to the FL8 epitope derived from HIV-1 Nef (residues 90-97). Informed
20 consent was obtained in all cases. Human subjects approval for these studies was obtained from the Central Oxford Regional Ethics Committee, the Ethics Review Committee of King's College Hospital, and the University of California San Diego Institutional Review Board. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood and cryopreserved according to standard protocols.

25

Antigens and antibodies

The FLKEKGGL peptide was synthesized by FMOC to at least 90% purity confirmed by HPLC. Tetrameric HLA-B*0801-FL8 complexes were generated as described previously (12). Directly conjugated antibodies were obtained from the following vendors: (i) α -TCR V β 13.2 (PE), α -CD94 (PE), and α -p24 antigen (clone KC57 RD1; PE) (Beckman Coulter); (ii) α -CD4 (APC), α -CD8 (FITC, Pacific Blue, PE, or PE-Cy7), α -CD27 (APC), α -CD28 (FITC), α -CCL4 (PE), α -CD107a (FITC or APC), α -IFN γ (PE-Cy7), α -TNF α (APC), α -PD-1 (APC), and α -granzyme B (PE) (BD Biosciences); (iii) α -perforin (clone B-D48; FITC) (Diaclone); (iv) α -FCRL3 (PE or APC) (clone 3126P; R&D Systems); (v) α -phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling Technology); and
35 (vi) α -CD57 (FITC) (BioLegend).

Flow cytometry

Thawed PBMCs or clonal CTLs were incubated with titrated amounts of tetramer (PE) for 15 min at 37°C. Surface antibodies were then added for 20 min at 4°C. Cells were washed and, if intracellular staining was required, fixed/permeabilized using a BD Cytofix/Cytoperm Kit (BD Biosciences). Data
40

were acquired using a FACS Cyan flow cytometer (Beckman Coulter) and analyzed with FlowJo software (Tree Star).

CTL clones and cell lines

- 5 Tetramer-binding cells were sorted from thawed PBMC samples collected from HIV-1-infected individuals (Table 1) using anti-PE MicroBeads (Miltenyi Biotec) and plated at limiting dilution as described previously (Dong *et al.* (2004) *J Exp Med* 200(12):1547–1557).

- 10 Table 1 shows TCR sequences and clonotyping for TCR identical HLA B*08-restricted FL8-specific CD8+ CTL Clones. Clonotype count and frequency are shown for the ex vivo HLA B*0801-restricted FL8-specific tetramer population from donor LTNP005 (in bold) and TCR –identical CTL-clone pairs (LTNP005 A3 and A20; E833 -13 and -30; G713-4 and -20).

Table 1

Donor ID	ID	TRBV	CDR3	TRBJ	Freq (%)	Count
LTNP005	HLA B*08-FL8	6-2	CASSYLPGQGDHYSNQPQH	1-5	97.50	39
	HLA B*08-FL8	6-2	CASSYLPGQGGHYSNQPQH	1-5	2.50	1
	A3	6-2	CASSYLPGQGDHYSNQPQH	1-5	100.00	143
	A9	6-2	CASSYLPGQGDHYSNQPQH	1-5	ND	ND
	A17	6-2	CASSYLPGQGDHYSNQPQH	1-5	ND	ND
	A20	6-2	CASSYLPGQGDHYSNQPQH	1-5	100.00	164
E833	E833-10	6-2	CASSYNTPGNEQF	2-1	ND	ND
	E833-11	6-2	CASSYNTPGNEQF	2-1	ND	ND
	E833-13	6-2	CASSYNTPGNEQF	2-1	100.00	77
	E833-18	6-2	CASSYNTPGNEQF	2-1	ND	ND
	E833-30	6-2	CASSYNTPGNEQF	2-1	100.00	86
	E833-38	6-2	CASSYNTPGNEQF	2-1	ND	ND
G713	G713-4	6-2	CASSFQPGQGLFFSNQPQH	1-5	100.00	53
	G713-20	6-2	CASSFQPGQGLFFSNQPQH	1-5	100.00	50
Donor ID	ID	TRAV	CDR3	TRAJ	Freq (%)	Count
LTNP005	HLA B*08-FL8	26-1	CIVRAPGRADMR	43	100.00	18
	A3	26-1	CIVRAPGRADMR	43	100.00	79
	A9	26-1	CIVRAPGRADMR	43	ND	ND
	A17	26-1	CIVRAPGRADMR	43	ND	ND
	A20	26-1	CIVRAPGRADMR	43	100.00	76
E833	E833-10	13-1	CAASNPPDYKLS	20	ND	ND
	E833-11	13-1	CAASNPPDYKLS	20	ND	ND
	E833-13	13-1	CAASNPPDYKLS	20	100.00	75
	E833-18	13-1	CAASNPPDYKLS	20	ND	ND

Donor ID	ID	TRBV	CDR3	TRBJ	Freq (%)	Count
	E833-30	13-1	CAASNPPDYKLS	20	100.00	86
	E833-38	13-1	CAASNPPDYKLS	20	ND	ND
G713	G713-4	12-2	CAGKAAGNKLT	17	100.00	81
	G713-20	12-2	CAGKAAGNKLT	17	100.00	99

Clones were expanded by periodic restimulation using mixed irradiated allogeneic PBMCs with phytohemagglutinin (PHA; 40 µg/mL) and recombinant human IL-2 (200 IU/mL). An HLA-A*0201-restricted HIV p17 Gag-specific clone (G10) was generated as described previously (Lee *et al.* (2004) *J Exp Med* 200(11):1455–1466). The CD4⁺ T cell line C8166 was obtained from the National Institute of Health (NIH) AIDS Reference and Reagent Program and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin sulphate, and 1.7 mM sodium glutamate. Cell lines and CTL clones were negative on regular screening for mycoplasma contamination. All clones were cultured under identical conditions and transcriptome analysis was performed on day 14 post-restimulation. Day 14 was validated by gene expression studies showing that contaminating feeder cells were present at < 0.1% and CTL clones achieve a stable state at this time point.

Production of VSV-G pseudotyped lentiviral particles

FCRL3 (NCBI accession number NM_052939.3) was synthesized and cloned into the lentiviral vector Lego.iG2 (Addgene, Weber) by Blue Heron. Lentiviral particles were produced by transient transfection of HEK293T/17 cells using the calcium phosphate method. HEK293T/17 cells were transfected with lentiviral vector (Lego.iG2 or FCRL3.Lego.iG2) DNA together with the third-generation HIV-1-based packaging plasmids PMD2.G, pMDLg/pRRE, and pRSV-Rev (Addgene, Trono). Supernatants were harvested at 24 and 48 hr post-transfection, centrifuged at 400 g for 10 min at room temperature, and passed through 0.45 µm filters (Sarstedt). Viral particles were concentrated from the supernatants by ultracentrifugation at 24,000 rpm for 1.5 hr at 4°C, and the pellets were resuspended in RPMI medium and stored at -80°C.

Titration of VSV-G pseudotyped lentiviral particles

1 × 10⁵ Jurkat cells were transduced with 10-fold serial dilutions of frozen virus in 96-well plates. Polybrene (8 µg/mL) was added to the wells, and the plate was centrifuged at 2,500 rpm for 40 min at 25°C. Cells were analyzed by flow cytometry for eGFP expression on day 5. Titers were calculated from the dilution that rendered 1–20% of the cells GFP⁺.

Transduction of Jurkat cells

Jurkat cells were transduced with Lego.iG2 or FCRL3.Lego.iG2 viruses at a multiplicity of infection of 10. On day 5, GFP^{high} cells were sorted by flow cytometry and seeded at 1, 3, or 5 cells per well in a 96-well plate. FCRL3 expression was confirmed by flow cytometry. The Lego.iG2 cell line was

isolated from a well containing 3 cells, and the FCRL3.Lego.iG2 cell line was isolated from a well containing 5 cells.

TCR sequencing

- 5 RNA was extracted from CTL clones using an RNeasy Kit (Qiagen) and amplified using an RT-PCR technique with a 5' switching mechanism and 3' TRA/TRB constant region primers as described previously (Dong *et al.* (2004) *J Exp Med* 200(12):1547–1557). Gel-purified PCR products were sequenced in-house using dideoxy chain-termination on a Megabase 1000.

10 Chromium release assay

- Antigen sensitivity was determined using standard chromium release assays. In brief, ⁵¹Cr-labelled C8166 cells were plated at 5 x 10⁴ cells/well with or without clonal CTLs at an effector:target (E:T) ratio of 3:1 in the presence of peptide at a final concentration of 0–10 µg/mL. Released ⁵¹Cr in the harvested supernatant was measured using a β-plate counter (WALLAC). Specific lysis was
15 calculated using the formula: specific lysis = (experimental ⁵¹Cr release – spontaneous ⁵¹Cr release)/(maximal ⁵¹Cr release – spontaneous ⁵¹Cr release).

HIV-1 suppression assay

- C8166 cells were infected with 1 x TCID₅₀ HIV-1_{MN} (NIBSC AIDS Reagents) for 90 min at 37°C, washed three times, and re-suspended in H10 medium (RPMI 1640 medium supplemented with 10% heat-inactivated human serum) containing IL-2 (200 U/mL). Infected target cells were plated at 5 x 10⁴ cells/100 µl with 2.5 x 10³ clonal CTLs or appropriate dilutions thereof in triplicate in a 96-well plate and incubated for 96 hr at 37°C. Positive control (infected C8166 cells alone) and negative control (uninfected C8166 cells) wells were included, together with an HLA-mismatched control (the
25 HLA-A*0201-SL9-specific CTL clone G10). Additional wells were set up to allow for spectral overlap. A p24 ELISA Kit (ImmunoDiagnostics Inc.) was used to detect p24 antigen in 50 µl of neat supernatant as instructed. CTL-mediated suppression was expressed as the percentage reduction in average p24 protein at day 4 relative to the average p24 content of infected C8166 cells alone (positive control). Cellular expression of p24 in C8166 cells was determined concomitantly by flow
30 cytometry.

Polyfunctional sensitivity

- 1 x 10⁶ clonal CTLs were stimulated at an E:T ratio of 10:1 with peptide-pulsed C8166 cells. The assay was performed as described previously, with the exception that 10 µM peptide was used as a
35 positive control (Betts *et al.* (2006) *Blood* 107(12):4781–4789). Data were acquired using a FACS Cyan flow cytometer (Beckman Coulter) and analyzed with FlowJo software (Tree Star)

Measurement of cytokine production

- Clonal CTLs were stimulated for 24 hr alone in the presence of PHA (40 µg/mL) or phorbol myristate acetate (PMA; 50 ng/mL) and ionomycin (1 µg/mL), or at 10: 1 ratio with C8166 cells infected for 72
40

hr with three titrations of HIV-1_{MN}. Cytokines in the culture supernatant were measured using a Human Cytokine 17-Plex Panel Assay (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN- γ , MCP-1, CCL4, and TNF) in a suspension array system (Luminex 200; Bio-Rad Laboratories).

5

RNA extraction for gene expression analysis

RNA from three generations of two clones was isolated from 3×10^6 cells after stimulation for 24 hr at 10:1 ratio with C8166 cells. Clonal CTLs were negatively selected using anti-CD4 MicroBeads (Miltenyi Biotec). Viable cells were counted using Trypan Blue, and total RNA was extracted using either a mirVana miRNA Isolation Kit (Applied Biosystems) or an RNeasy Kit (Qiagen). RNA integrity was measured using a Bioanalyzer (Agilent).

10

Microarray analysis and quantitative RT-PCR

Genome-wide gene expression analysis was performed using a HumanHT-12 v4 Expression BeadChip containing 47,231 annotated probes (Illumina). Total RNA was amplified and labelled during *in vitro* transcription with biotin, hybridized to the array, washed, stained with Cy3-Streptavidin, and scanned for quality control and gene detection using Genome Studio (Illumina). Data files are available at the Gene Expression Omnibus data repository under accession code XXX. Quantitative real-time PCR was performed using a 7500 System (Applied Biosystems). Primers specific for listed genes were obtained from Applied Biosystems.

20

Bisulfite sequencing PCR assay

Genomic DNA was extracted from each population using a PureGene DNA Purification Kit (Gentra Systems), and 500 ng of genomic DNA was converted using a Methylcode Bisulfite Conversion Kit (Invitrogen). Fragments generated by PCR were amplified separately using the following primers: -237 amplification: 5'-TTT GTA TTT TGT TTG GAA GTT AGA AGG AAA TAG ATT A-3' and 5'-AAA ACT TCC TTA ATA AAA CCC ATA AAC TCA TC-3'; -1030 amplification: 5'-AGA GTT GTG GGG AGA ATA AAA GGA TAA GG-3' and 5'-AAA CAT TCT CCT ACC CAT TAC TAT AAT CAC ATC TC-3'. Products were subcloned into pCR4 TOPO TA (Invitrogen) and sequenced.

30

Phospho-flow cytometry

Jurkat Lego.iG2 and FCRL3.Lego.iG2 cells (1×10^6 /well) were incubated with soluble α -CD28 (BioLegend) and stimulated with plate-bound (1 mg/ml) α -CD3 (clone OKT3; BioLegend) for 10 min at 37°C. Clonal CTLs (1×10^6) were stimulated with peptide-pulsed C8166 cells at an E:T ratio of 10:1 for 10 min at 37°C. Stimulations were performed using the cognate FL8 peptide or a control peptide from the CMV pp65 protein (NLVPMVATV; residues 495-503). Cells were fixed using BD Phosflow Fix Buffer I (BD Biosciences) for 10 min at 37°C, permeabilized in chilled BD Phosflow Perm Buffer III (BD Biosciences), and stored at -80°C. Thawed cells were washed, stained for 1 hr at room temperature with phospho-specific antibodies (Cell Signaling Technology), and acquired using an LSRII flow cytometer (BD Biosciences).

40

Quantitative single cell gene expression analysis

Single tetramer-binding V β 13.2⁺ CD8⁺ T cells were sorted using a FACS Aria flow cytometer (BD Biosciences) into 96-well plates containing CellsDirect One-Shot qRT-PCR Buffer with Platinum Taq Polymerase and SuperScript III Reverse Transcriptase (Invitrogen), 0.2x TaqMan gene-specific expression assays (Applied Biosystems), and Superscript RNase inhibitor (Ambion). Immediately after cell sorting, samples were incubated for 10 min at 55°C, denatured for 2 min at 95°C, and amplified over 21 cycles of PCR (15 sec at 95°C; 4 min at 60°C). Negative control wells were run in parallel without SuperScript III. cDNA from each bulk sample was amplified from 100 cells over 18 cycles of RT-PCR. Pre-amplified cDNA was diluted 1:5 in TE buffer and then mixed with TaqMan Universal PCR Master Mix (Applied Biosystems). The sample mix and TaqMan assays were loaded separately into wells of a 192.24 Gene Expression Dynamic Array (Fluidigm). The arrays were read using a Biomark HD Analysis System (Fluidigm). Single cell expression data were initially analyzed using Fluidigm Data Collection software. Samples not expressing CD8a and the housekeeping genes were excluded from the analysis (n=8), with a Ct value for non-detected gene expression considered as 40. Δ Ct values were calculated with reference to the mean of two housekeeping genes (GAPDH and ACTB). Heatmaps were generated in R using the package *pheatmap*.

Statistical analysis

Statistical analysis was performed using Prism version 4.0b (GraphPad). Differences between groups were assessed using a non-paired Student t-test. Correlations were determined using linear regression. Statistically significant differential gene expression was identified using GeneSpring GX (Agilent) with one-way analysis of variance (ANOVA) and corrected for multiple comparisons. Significance was assumed at *P* values < 0.01. Altered expression of genes that achieved statistical significance was analyzed using the LIMMA package for R and corrected for multiple comparisons. Significance was assumed at *P* values < 0.05. The LIMMA package analyzes empirical Bayes values to determine gene-wise residual variance (significance was assumed at *B* values < 3.0). Heatmaps were generated using gplot.

Example 1: Generation of TCR-identical CTL clones by expansion of single cells ex vivo

The TCR is the key structure that mediates recognition of peptide-MHC class I complexes, yet it is not clear if TCR affinity, TCR avidity or events downstream of the TCR determine the variable anti-viral efficacy among epitope-specific CTLs. TCR-identical CTL clones were generated from three HIV-1-infected individuals with immunodominant HLA-B*0801-restricted CD8⁺ T cell responses specific for FL8. These tetramer defined populations were characterized by a dominant TCR clonotype in two individuals, LTNP005 and E833, and a codominant TCR clonotype in a third individual, G713, all of which used *TRBV6-2* in conjunction with distinct *TRBJ*, *TRAV*, and *TRAJ* genes (12). In donor, LTNP005, the TRBV 6-2⁺ (also known as V β 13.2) tetramer-binding population was dominated by the use of a single TCR (Table 1). At the point of sampling, LTNP005 had maintained a normal CD4⁺ T cell count for 14 years without anti-retroviral therapy (ART). E833 and

G713 were sampled during acute HIV-1 infection. CTL clones were generated via limiting dilution of HLA-B*0801-FL8 tetramer-binding CD8⁺ T cell populations isolated directly *ex vivo* by flow cytometry. For each clone ($n = 2-6$ per donor), the expressed TCR was characterized using a molecular approach to sequence TCR α (TRA) and TCR β (TRB) transcripts (Table 1).

5

TCR-identical CTL clones were found to have distinct antiviral efficacy. All four clones isolated from LTNP005 expressed an identical TCR (Table 1). Each clone (A3, A9, A17, and A20) had a distinct ability to suppress HIV-1 replication in an HLA-matched CD4⁺ T cell line (C8166) infected with HIV-1_{MN}. *In vitro* viral suppression required MHC class I restriction and appeared to depend on contact-dependent killing, as suppression correlated with elimination of p24⁺ cells by flow cytometry (Fig. 1A). Heterogeneity in killing capacity was more striking at lower effector: target (E:T) ratios, with suppressive activity ranging from 10-90% at an E:T ratio of 1:16 (Fig. 1B). Clone A20 particularly maintained high contact dependent killing efficiency at limiting clone concentration (Fig. 1A). Distinct viral suppression was observed for a second set of TCR-identical CTL clones from donor G713 (Fig. 1C). All TCR-identical CTL clones from E833 were potent suppressors, even at an E:T ratio of 1:128 (data not shown). Importantly, each set of clones displayed equivalent surface expression levels of the corresponding sequence-identical TCR. Thus clonal CD8⁺ T-cells can have distinct antiviral efficacy.

20 **Example 2: Antigen sensitivity and killing efficiency variation among TCR-identical CTL clones**

The peptide concentration required to trigger half-maximal killing (EC_{50}) was determined in short-term chromium release assays. Antigen sensitivity was also distinct among the four TCR-identical CTL clones from LTNP005, with EC_{50} values ranging from 0.014 to 0.045 μ M (Fig. 1D). Indeed, at low E:T ratio (1:16), virus suppression directly correlated with antigen sensitivity (antigen sensitivity ($R^2 = 0.95$, $P = 0.023$)). A clear sensitivity threshold appeared to trigger cytotoxicity at a peptide concentration of 0.01 μ M (Fig. 1D), which is likely to reflect epitope densities on the surface of naturally HIV-1-infected cells. As this result was unexpected, an unbiased molecular approach was used to confirm TCR identity for two of these clones (A3 and A20) in a different laboratory (Table 1).

30

Variable antigen sensitivity was also observed among the six TCR-identical CTL clones from E833, with EC_{50} values ranging from 0.07 to 0.02 μ M, and the two TCR-identical CTL clones from G713 (Fig. 1E). Here, the most sensitive clone, G713-20, was also the most potent suppressor. Again, TCR identity was confirmed independently for representative clones from E833 (-13 and -30) and both clones from G713 (Table 1).

35

Collectively, these data show that antigen sensitivity can vary independently of the TCR sequence and surface expression, in turn dictating cytotoxic activity and antiviral efficacy at physiological epitope densities in the presence of limiting numbers of CTLs.

40

Example 3: Study on TCR-identical CTL clones reprogramming effector functions by epigenetic modifications

CTL polyfunctionality has been associated with antigen sensitivity. Accordingly, CD107a mobilization and the production of IL-2, TNF, IFN γ , and CCL4 was assessed in peptide titration experiments. For this purpose, the experiment focused on two clones from LTNP005. Clone A20 was highly antigen sensitive and displayed potent antiviral efficacy, whereas clone A3 was moderately antigen sensitive and displayed moderate antiviral efficacy. At limiting peptide concentrations, clone A20 was more polyfunctional than clone A3 (Fig. 2A), but unexpectedly failed to secrete TNF. Moreover, TNF production was not observed in clone A20 in response to HIV-1_{MN}-infected HLA-matched CD4⁺ T cells (Fig. 2B) and could not be induced by stimulation with either phytohaemagglutinin (PHA) or phorbol myristate acetate (PMA) (Fig. 2C). Clone A20 also failed to secrete IL-2 although this could be overcome with PMA stimulation.

To determine if TNF was regulated at the level of mRNA transcription, clones A3 and A20 were activated with peptide-pulsed C8166 cells, separated by negative selection, and subjected to RNA extraction for Illumina microarray analysis (Fig. 2D). After stimulation for 6 hours, TNF (adjusted *P* value (LIMMA) 2.95×10^{-13}) was significantly less abundant in clonal A20 CTLs compared with clonal A3 CTLs. Comparison of the normalized fluorescence intensity (NFI) of the TNF transcript further indicated very low absolute expression in A20 (NFI: A20, 8.327 ± 0.048 vs. A3, 11.510 ± 0.062). Epigenetic regulation of TNF in clones A20 and A3 was therefore examined by bisulfite sequencing to determine the methylation status of the CpG islands in the TNF core promoter region. Clone A20 was highly methylated in the TNF promoter compared with clone A3 (Fig. 2F), showing that the TNF gene underwent epigenetic silencing.

Lytic granule release was measured as a correlate of CD107a mobilization on the cell surface. A higher proportion of clonal A20 CTLs were CD107a⁺ compared with clonal A3 CTLs 6 hours after stimulation with peptide-pulsed C8166 cells (27% vs. 11%) (Fig. 2A). Cytotoxic effector molecules were therefore examined for comparative transcript abundance (Fig. 2E). There was no difference in the abundance of mRNA transcripts for Fas ligand (FASL), TRAIL (TNFSF10), granzyme B (GZMB), and perforin (PRF). However, lymphotoxin- α (LTA) mRNA, which originates from the gene located adjacent to TNF on chromosome 6, was significantly less abundant in clone A20 compared with clone A3 (base 2 logarithmic fold change (Log₂ FC) -1.74, adjusted *P* value (LIMMA) = 5.41×10^{11}).

These findings show that polyfunctionality is not always directly determined by antigen sensitivity (8), but can be modified by epigenetic regulation of cytokines, such as TNF. Distinct antigen sensitivity in two TCR-identical CTL clones was confirmed by the breadth and magnitude of available effector responses. Indeed, the most antigen sensitive CTL clone had a higher probability of triggering CD107a expression per target cell encounter without inducing a higher expression of mRNA transcripts for lytic molecules at this time point.

Example 4: Functionality is not associated with memory phenotype, exhaustion state or tetramer-binding avidity

Surface expression levels of CD8a and the sequence-identical TCR were equivalent across all four clones from LTNP005. In line with these observations, clones A3, A17, and A20 also showed indistinguishable binding avidities for the HLA-B*0801-FL8 tetramer (Fig. 3A). Similar findings applied to other TCR-identical CTL clones with distinct sensitivities. Moreover, expression levels of perforin, granzyme B, CD27, and CD28, measured at the protein level by flow cytometry, were stable across generations and largely equivalent in clones A3 and A20 (data not shown). These findings are consistent with the observation that each clone was equally able to kill target cells pulsed with non-limiting concentrations of peptide (Fig. 1A and 1D). Neither clone expressed high levels of PD-1 (A3, 30%; A20, 13%; data not shown), which characterizes an exhausted phenotype associated first with loss of cytotoxicity and then with loss of cytokine secretion. There were also no significant differences in expression of basic leucine zipper transcription factor (BATF) on transcriptome analysis. Accordingly, clonal phenotype was stable across rounds of stimulation, and differences in antigen sensitivity and cytotoxic activity were not attributable to exhaustion or distinct expression levels of CD8a or the sequence-identical TCR.

Example 5: Resting TCR-identical CTL clones have differential gene regulation

To explore the molecular correlates of differential antigen sensitivity and cytotoxic activity among TCR-identical CTLs, the full transcriptomes of clones A3 and A20 (Fig. 3B) were compared. In separate experiments, mRNA was extracted from three generations of each clone under resting conditions (i.e. two weeks after the last antigen exposure). This analysis identified 140 mRNAs that were differentially expressed (absolute fold change of ≥ 2 in A20 vs. A3; $P < 0.01$ by ANOVA). Of these 140 mRNAs, 39 were identified by literature review and/or the database Uniprot to encode proteins expressed on the cell surface. Of these 39 mRNAs, three encoded Fc-receptor-like molecules, FCERIG, FCRL3, and FCRL6, which originate from one gene complex, an immunoglobulin superfamily on chromosome 1q that also encodes the TCR complex signalling protein CD3 ζ and the BCR coreceptor FCRL1. These proteins incorporate cytoplasmic domain ITAMs and/or ITIMs. Clone A20 showed higher expression of three genes, encoding three ITAMs and one ITIM (*FCERIG*, *FCRL3*, and *TYROBP*), whereas clone A3 showed higher expression of *FCRL6*, which encodes one ITIM, as well as *KIR2DL3* and *KIR2DL4*, both of which have an ITIM in their long cytoplasmic tails (Fig. 3C). Table 2 shows cell Surface Receptors differentially expressed in Clone A20 compared to Clone A3. The protein names, signalling motifs and comments related to the function of ten genes encoding the selected cell surface receptors in Fig. 3C.

Table 2

Protein Name	Signalling Motif	Comments
Chemokine (C-X-C motif) receptor 6	-	Cytolytic effector phenotype
Fc receptor-like 3	ITAM + ITIM	Unknown

Protein Name	Signalling Motif	Comments
Fc fragment of IgE, high affinity I, receptor for gamma polypeptide	ITAM	Adaptor molecule
TYRO protein tyrosine kinase binding protein	ITAM	Adaptor molecule
Leucine rich repeat neuronal 3	ITIM	Unknown
Killer cell lectin-like receptor subfamily D, member 1	-	NK receptor
Fc receptor-like 6	ITIM	Ligand HLA-DR
Killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 3	ITIM	NK receptor
Chemokine (C-X-C motif) receptor 3	-	Lymph node homing
Killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	ITIM + ITAM	Framework KIR

Clone A3 also expressed significantly higher levels of *LRRN3*, a gene first isolated from neuronal tissue that encodes a potential cytoplasmic domain ITIM. As expected from the antibody staining data, there was no differential expression of genes encoding the two TCR chains or *CD8 α* or *CD8 β* .

- 5 Real-time PCR analysis confirmed differential expression for 13 of 15 selected genes, including *FCRL3*, *FCRL6*, *TYROBP*, and *LRRN3*. To ensure that these genes were indeed differentially expressed and not artifacts of contamination by feeder cells or C8166 target cells, the two clones were sorted to > 99% purity by flow cytometry. Repeat real-time PCR analysis using these ultra-pure templates confirmed differential mRNA expression for *FCRL3*, *FCRL6*, *FCER1G*, and *LRRN3* (Fig. 10 3D).

- Thus, striking differences in gene expression were apparent between clones A3 and A20, which vary with respect to antigen sensitivity and cytotoxic activity despite identical TCR usage. Clone A20 expressed higher levels of signalling molecules with ITAMs, including cell surface and adaptor 15 proteins, whereas clone A3 expressed higher levels of receptors with ITIMs. Similar data were obtained with the two pairs of TCR-identical CTL clones from E833 and G713, confirming a correlation between high expression of molecules with ITAMs (*FCRL3*, *FCER1G*, and *TYROBP*) in the most antigen-sensitive clones (Figs. 3E and 3F).

20 **Example 6: FCRL3 enhances signalling triggered via the TCR**

- To confirm these data at the protein level, flow cytometry was used to measure surface expression of FCRL3 across successive generations of clones A3 and A20. As expected from the transcriptome analysis, clone A20 expressed higher levels of FCRL3 than clone A3 ($P = 0.0036$) (Fig. 4A). FCRL3 contains both an ITAM and an ITIM. The effects of FCRL3 on TCR signal transduction were 25 investigated using phospho-flow cytometry to assess the phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) extracellular signal-related kinase 1/2 (ERK1/2). After stimulation for 10 minutes with an HLA-B*0801+ FL8 peptide-pulsed CD4+ T cell line at an E:T ratio of 10:1, a

higher proportion of clonal A20 CTLs had phosphorylated p44/42 MAPK (mean: A20, 5.91% vs. A3, 2.05%; $P = 0.0001$), and the overall population MFI was higher compared with clonal A3 CTLs ($P < 0.0001$) (Fig. 4B). To determine if these differences in TCR signalling kinetics affected contact-dependent killing, surface mobilization of CD107a on clonal A3 and A20 CTLs was measured at intervals of 5 minutes under the same conditions. A greater proportion of clonal A20 CTLs had degranulated after 15 minutes (mean: A20, 4.79% vs. A3, 1.35%; $P = 0.0012$), and marked differences were apparent compared with clonal A3 CTLs after 60 minutes (mean: A20, 31.6% vs. A20, 22.83%; $P = 0.0152$). These results show that differential expression of FCRL3 is associated with distinct signal transduction and degranulation kinetics in TCR-identical CTLs.

To establish a direct role for FCRL3 in TCR signal transduction, FCRL3 was engineered into a lentiviral vector (Fig. 4D) encoding an internal ribosome entry site (IRES)-driven green fluorescent protein (GFP) to enable fluorescence-activated cell sorting of transduced cells and the generation of two Jurkat cell lines, one that stably expressed the empty vector and one that stably expressed FCRL3. Activation with a plate-bound CD3-specific antibody (OKT3) induced higher levels of phosphorylated p44/42 MAPK in the FCRL3⁺ Jurkat cell line (Fig. 4F). Thus, FCRL3 enhances signalling triggered via the TCR.

Example 7: *Ex vivo* expression of ITAM/ITIM receptors in TCR-identical CTLs

To confirm that differential expression of ITAM/ITIM receptors was not an artefact of prolonged *in vitro* culture, flow cytometry was used to sort individual TCR V β 13.2⁺ HLA-B*0801-FL8 tetramer-binding CD8⁺ T cells directly *ex vivo* from LTNP005. In parallel, the corresponding TCR α (TRA) and TCR β (TRB) transcripts were sequenced, revealing almost complete population identity with the TCRs expressed in clones A3 and A20 (Table 1). The expression of ITAM and ITIM receptors in TCR-identical FL8-specific CD8⁺ T cells was then quantified at the single-cell level using Fluidigm (Fig. 5A). Remarkably, a population of single cells expressed FCRL3, whereas another population of single cells expressed LRRN3 (Fig. 5B).

CLAIMS

1. A genetically engineered cell comprising a receptor that comprises an antigen binding domain and a T-cell activating function and at least one co-receptor which modulates the activity of the receptor, wherein the cell overexpresses the co-receptor and in which the co-receptor is selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3).
2. A cell as claimed in claim 1, in which the receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).
3. A nucleic acid sequence encoding a chimeric antigen receptor (CAR) and a co-receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3).
4. A vector comprising a nucleic acid sequence as claimed in claim 3.
5. A cell comprising a vector as claimed in claim 4.
6. A cell as claimed in claim 1 or claim 2, a nucleic acid sequence as claimed in claim 3, a vector as claimed in claim 4 or a cell as claimed in claim 5 for use in medicine.
7. A method of treatment of a disease comprising administration of an effective amount of a cell as claimed in claim 1 or claim 2, a nucleic acid sequence as claimed in claim 3, a vector as claimed in claim 4 or a cell comprising a vector as claimed in claim 5 to a subject in need thereof.
8. A kit comprising a cell as claimed in claim 1 or claim 2, a nucleic acid sequence as claimed in claim 3, a vector as claimed in claim 4 or a cell comprising a vector as claimed in claim 5, and a physiologically acceptable diluent or adjuvant.
9. A specific binding molecule which binds to a receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) for use in medicine.
10. A method of treatment of a disease comprising administration of an effective amount of the specific binding molecule which binds to a receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) to a subject in need thereof.

11. A kit comprising a specific binding molecule which binds to a receptor selected from the group consisting of Fc-Receptor-Like-3 (FCRL3), Fc-Receptor-Like-6 (FCRL6), and Leucine-Rich-Repeat-Neuronal-3 (LRRN3) and a physiologically acceptable diluent or adjuvant.

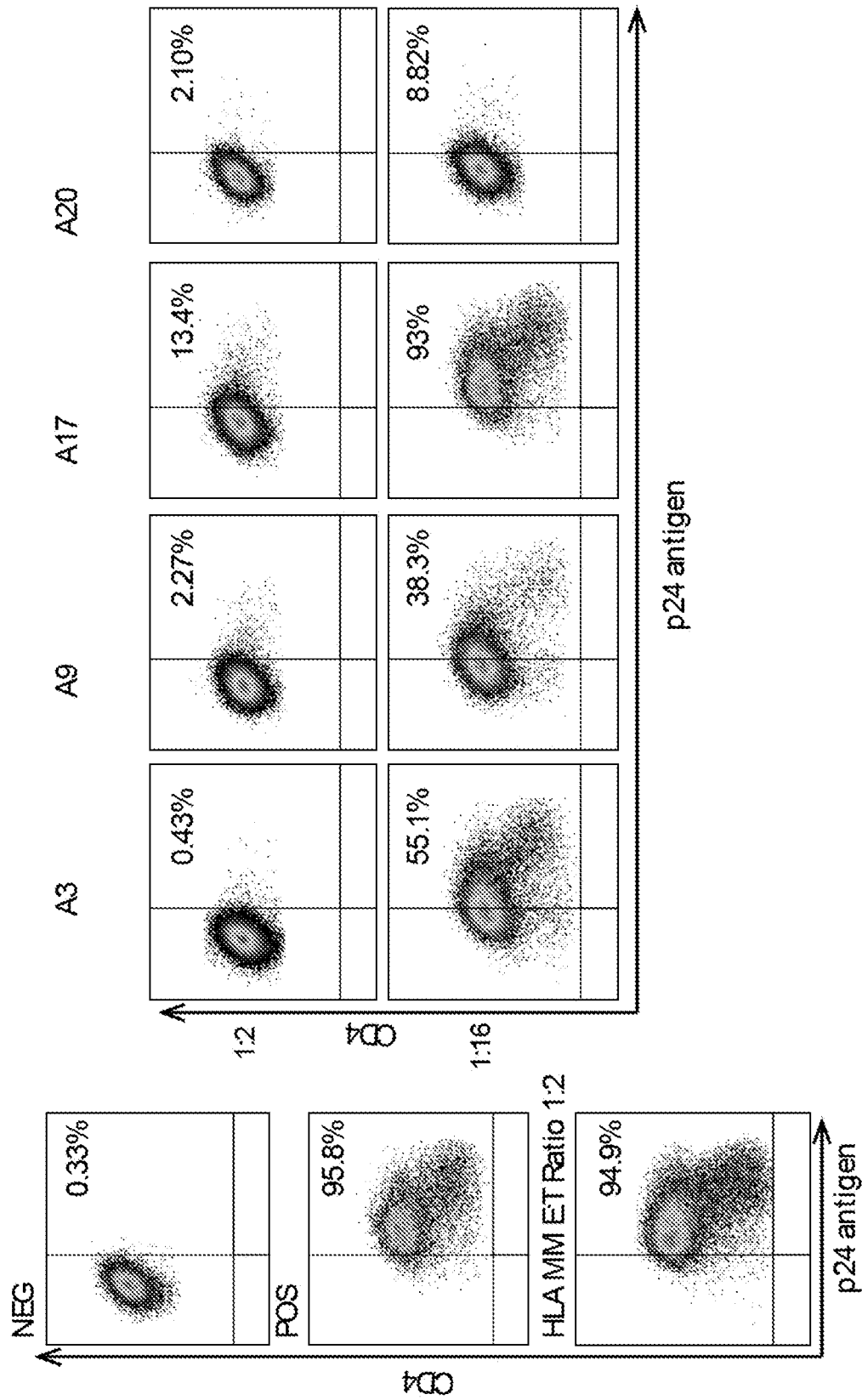


Fig. 1A

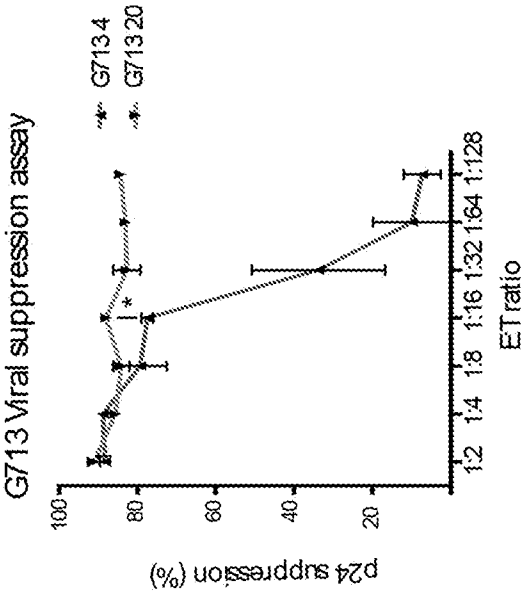


Fig. 1C

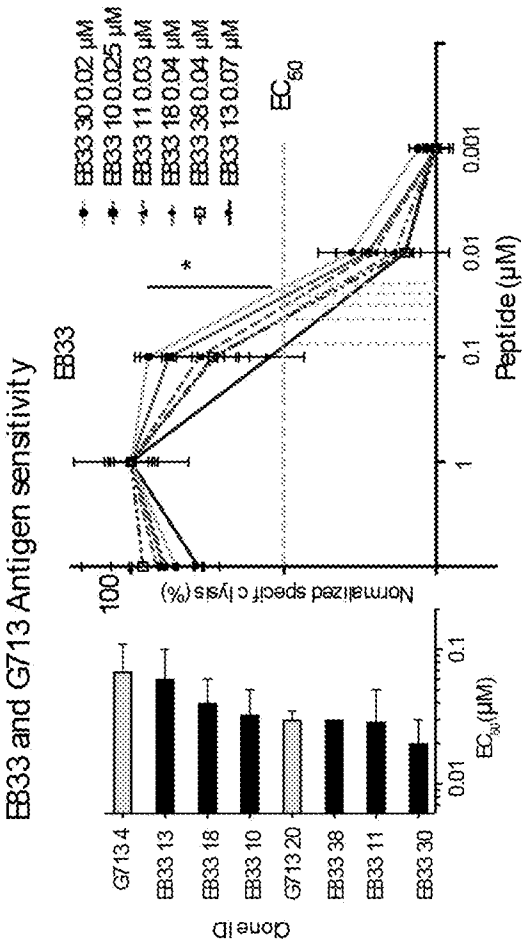


Fig. 1E

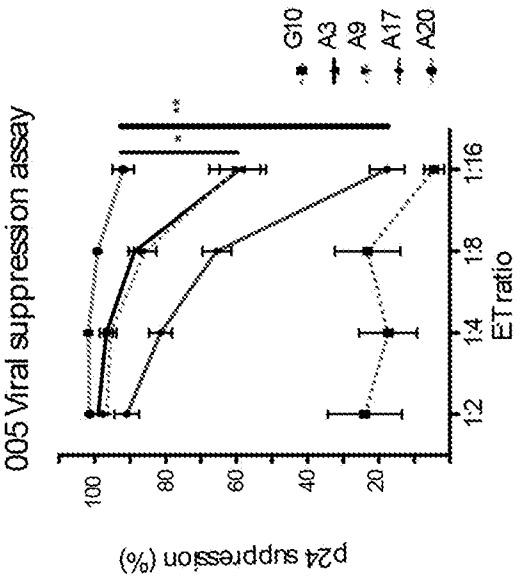


Fig. 1B

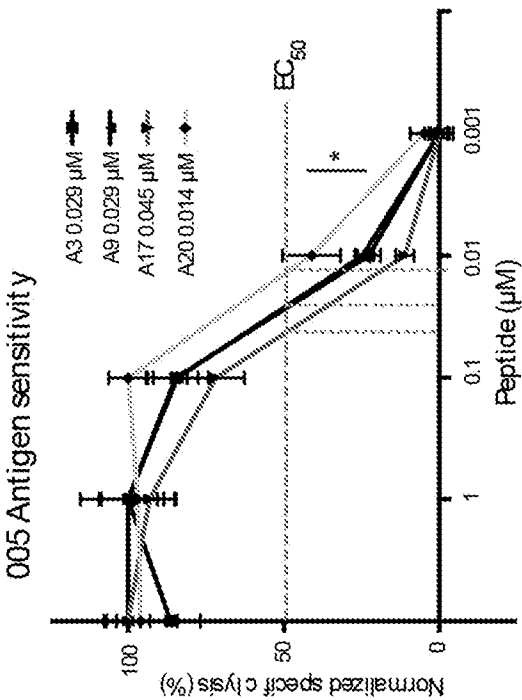


Fig. 1D

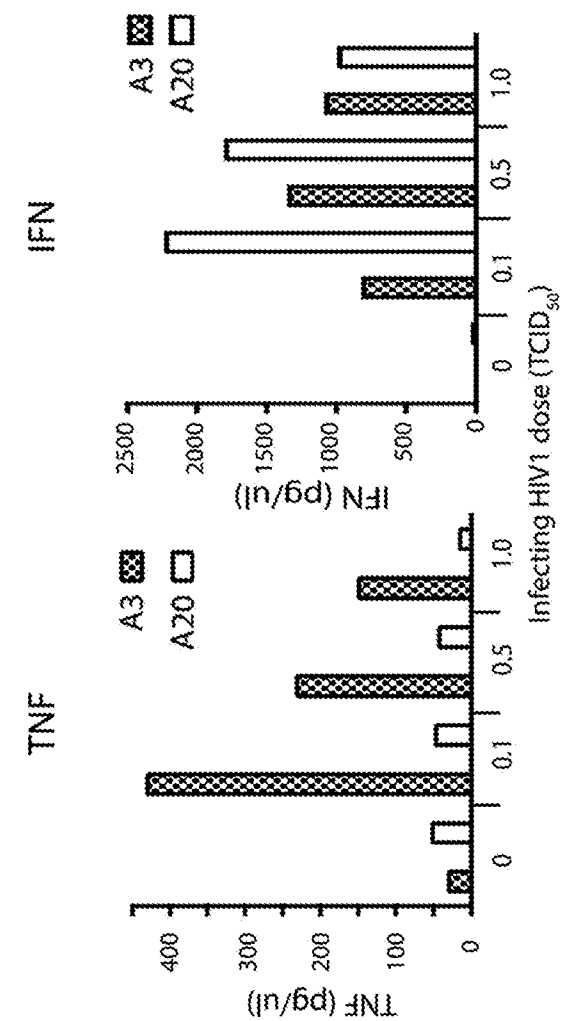


Fig. 2B

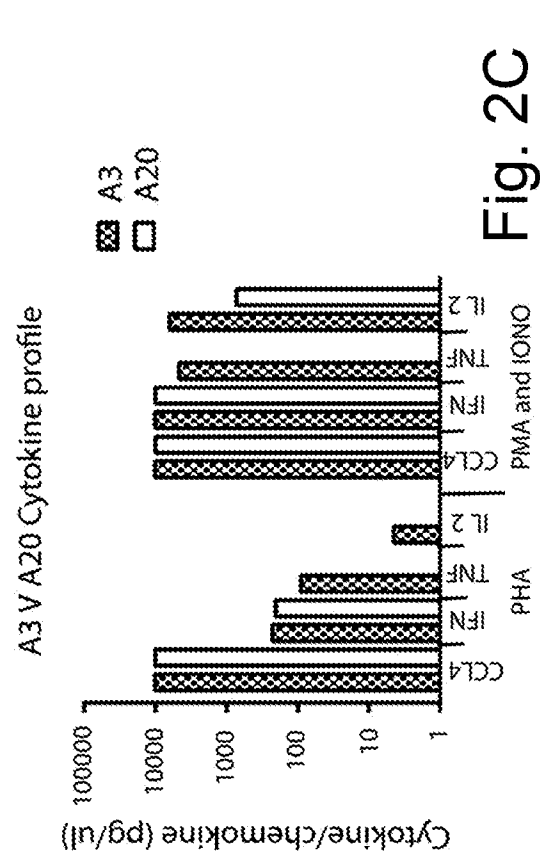


Fig. 2C

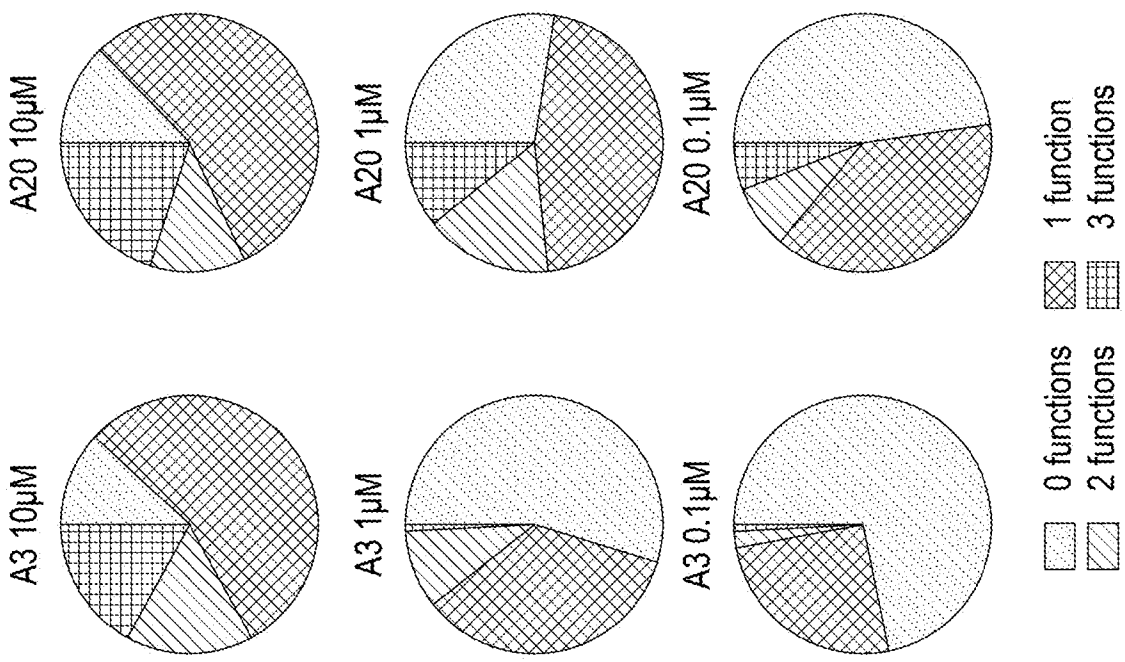
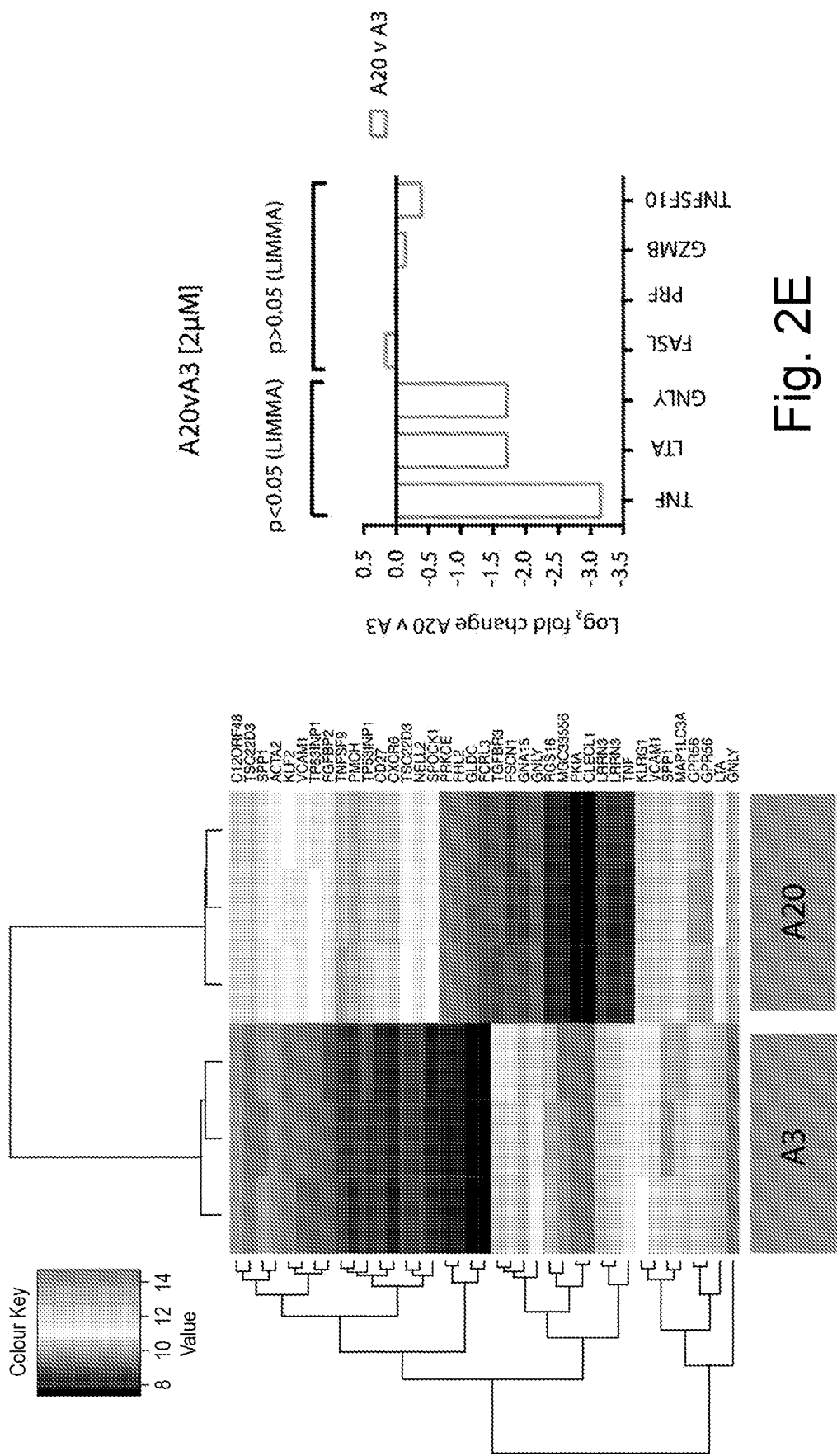


Fig. 2A



5/26

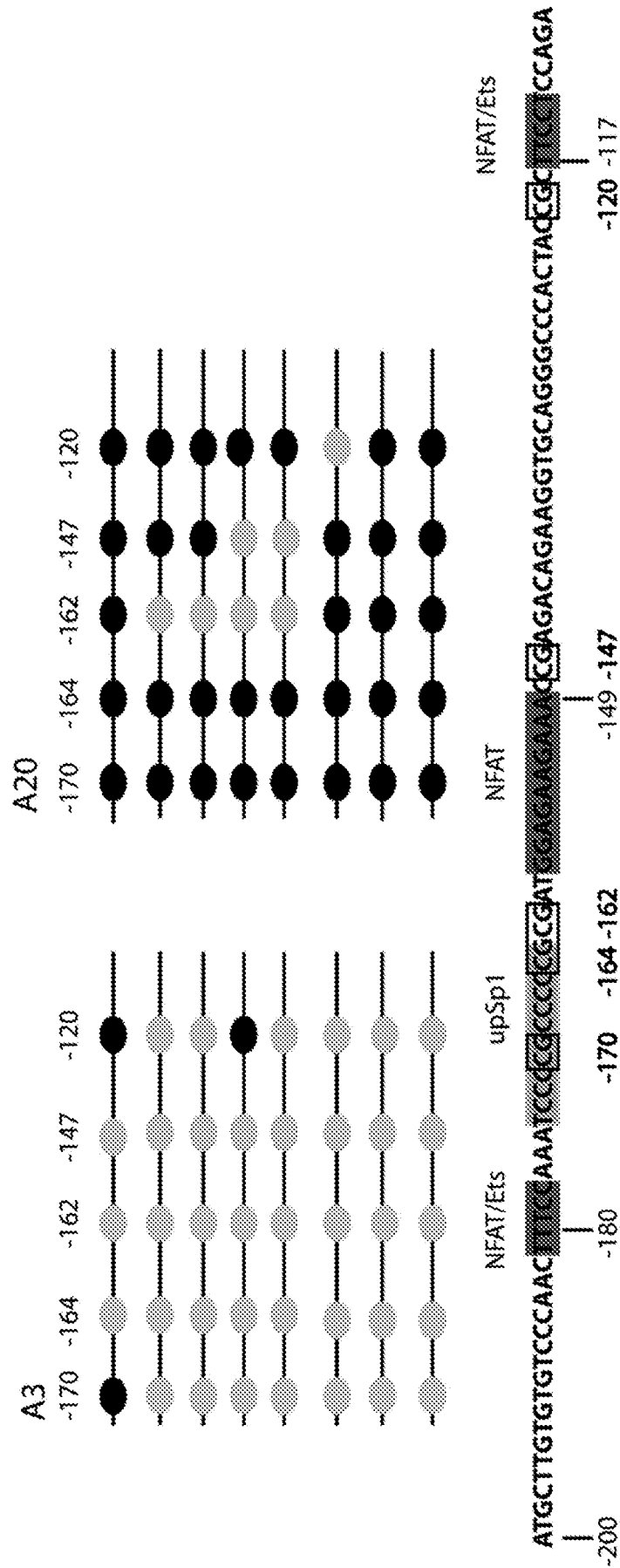


Fig. 2F

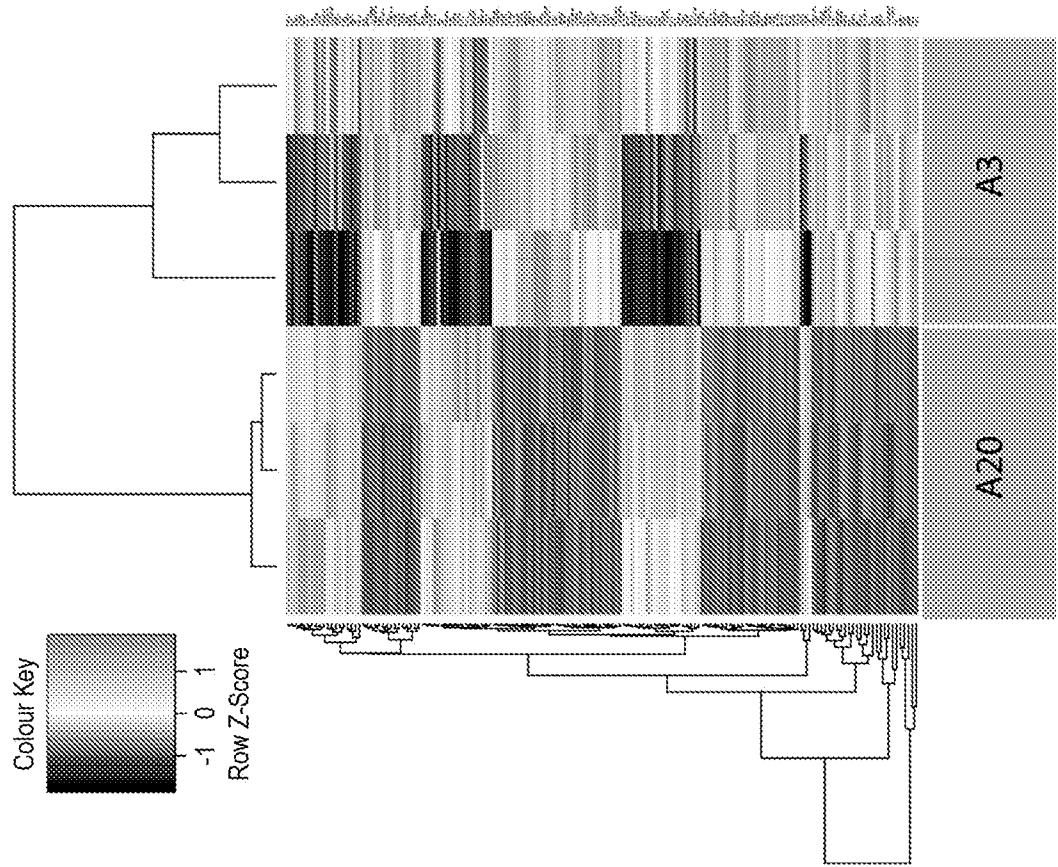


Fig. 3B

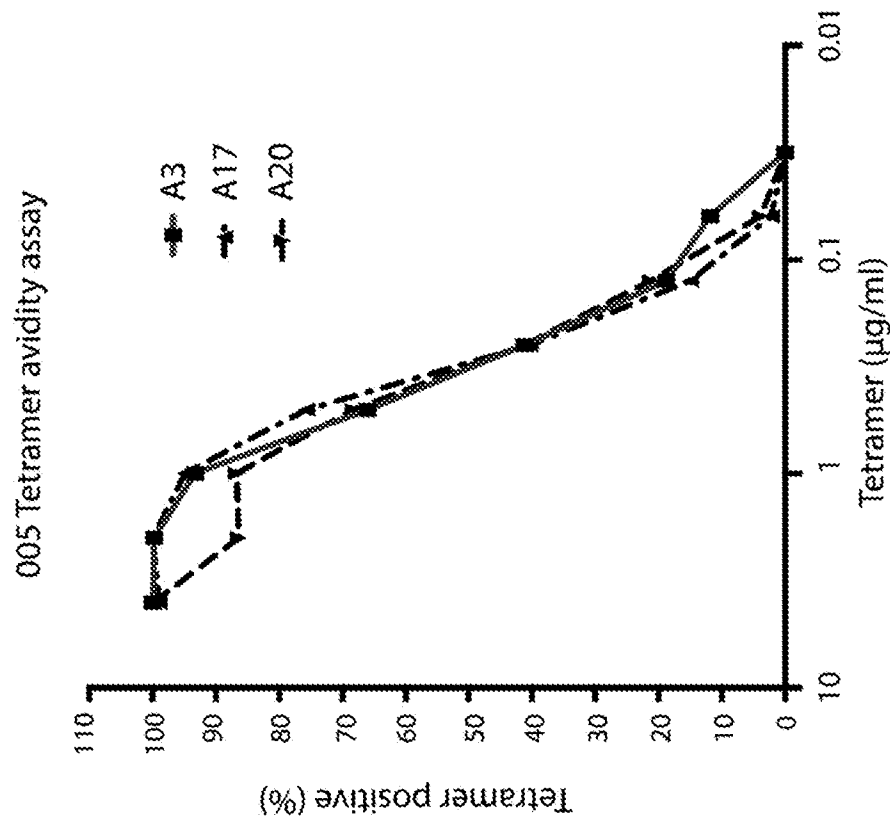


Fig. 3A

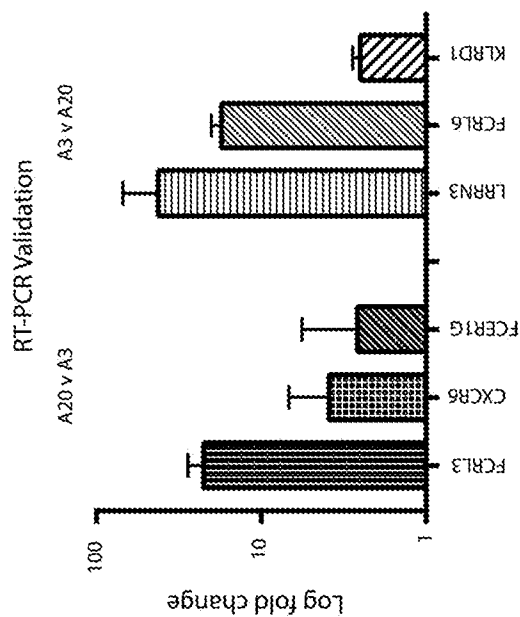


Fig. 3D

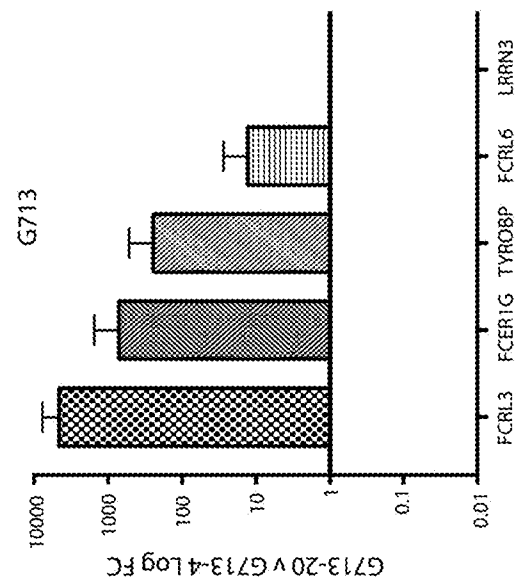


Fig. 3F

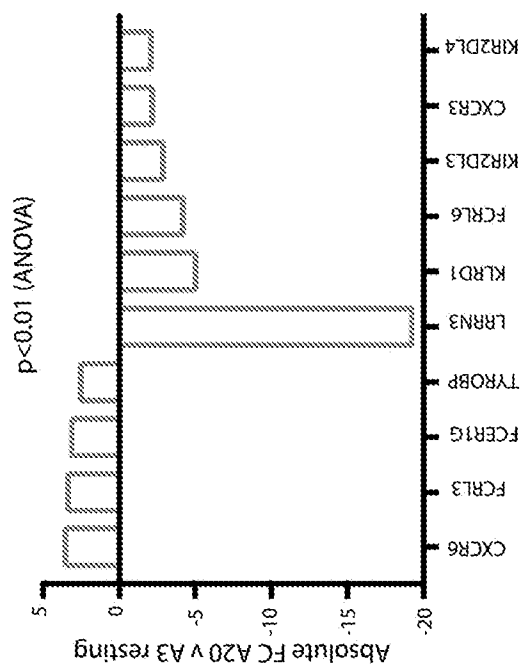


Fig. 3C

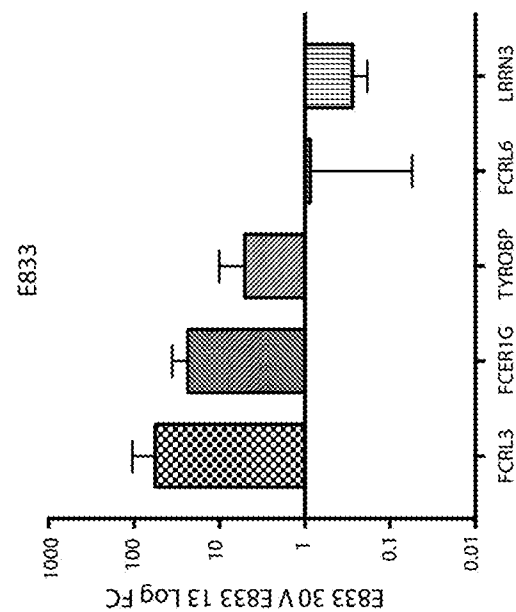


Fig. 3E

8/26

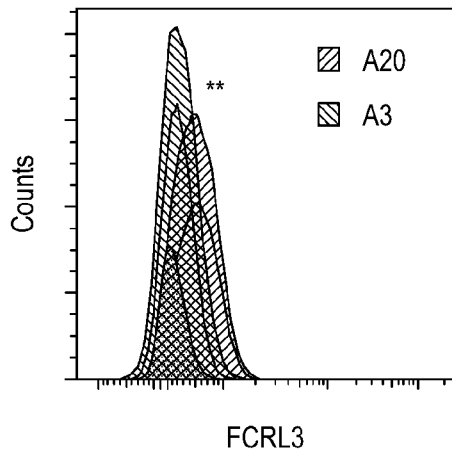
FCRL3 Expression TCR-identical CD8⁺ T-cell clones

Fig. 4A

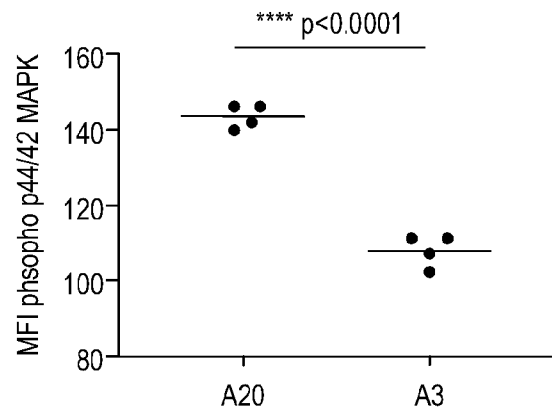


Fig. 4B

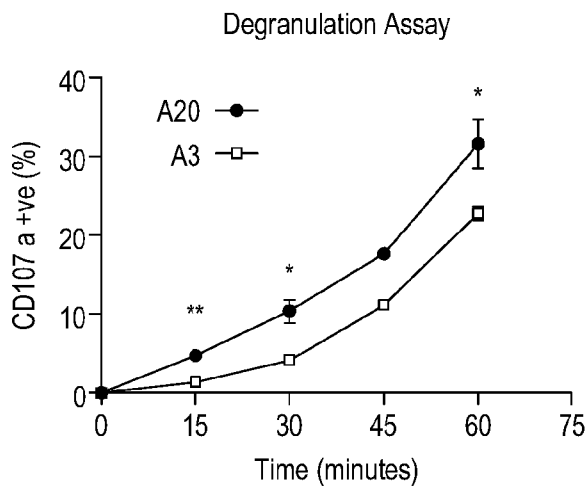


Fig. 4C

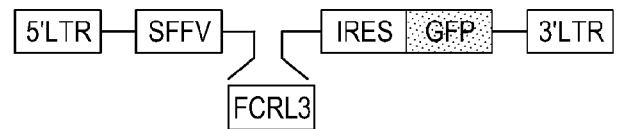


Fig. 4D

JURKAT FCRL3 Transduction: OKT3 Activation

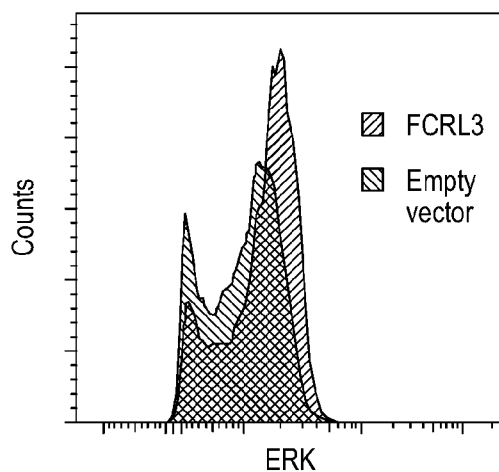


Fig. 4E

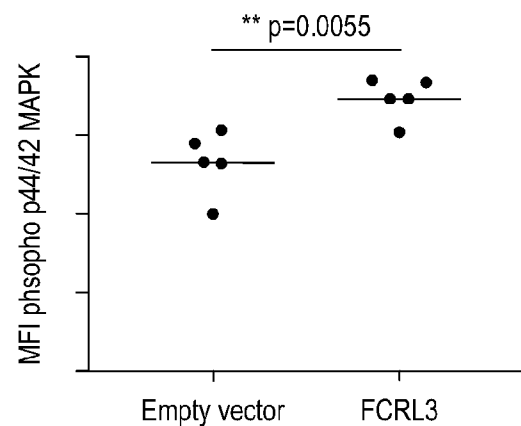


Fig. 4F

9/26

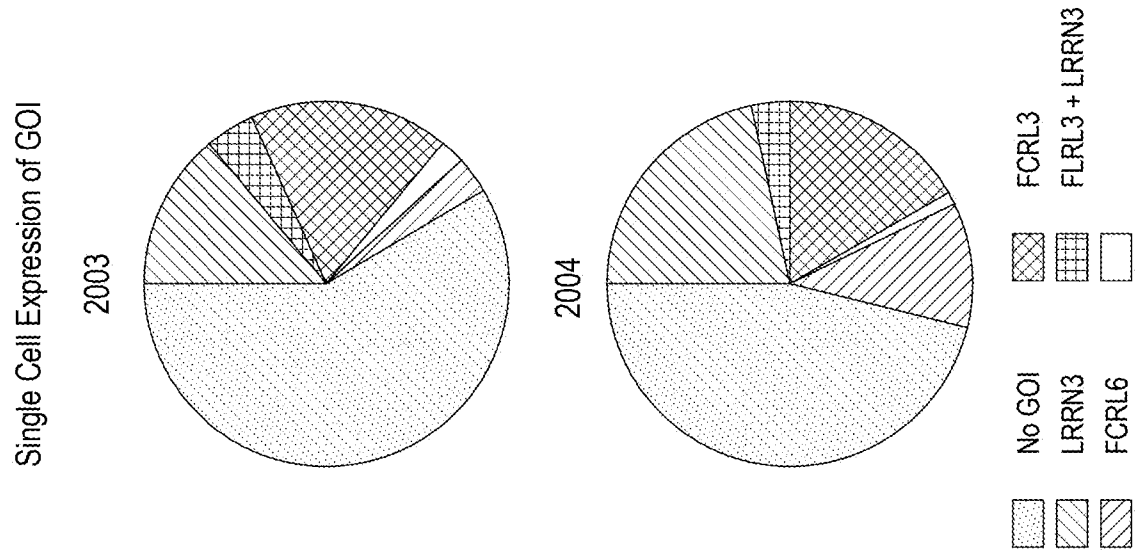


Fig. 5B

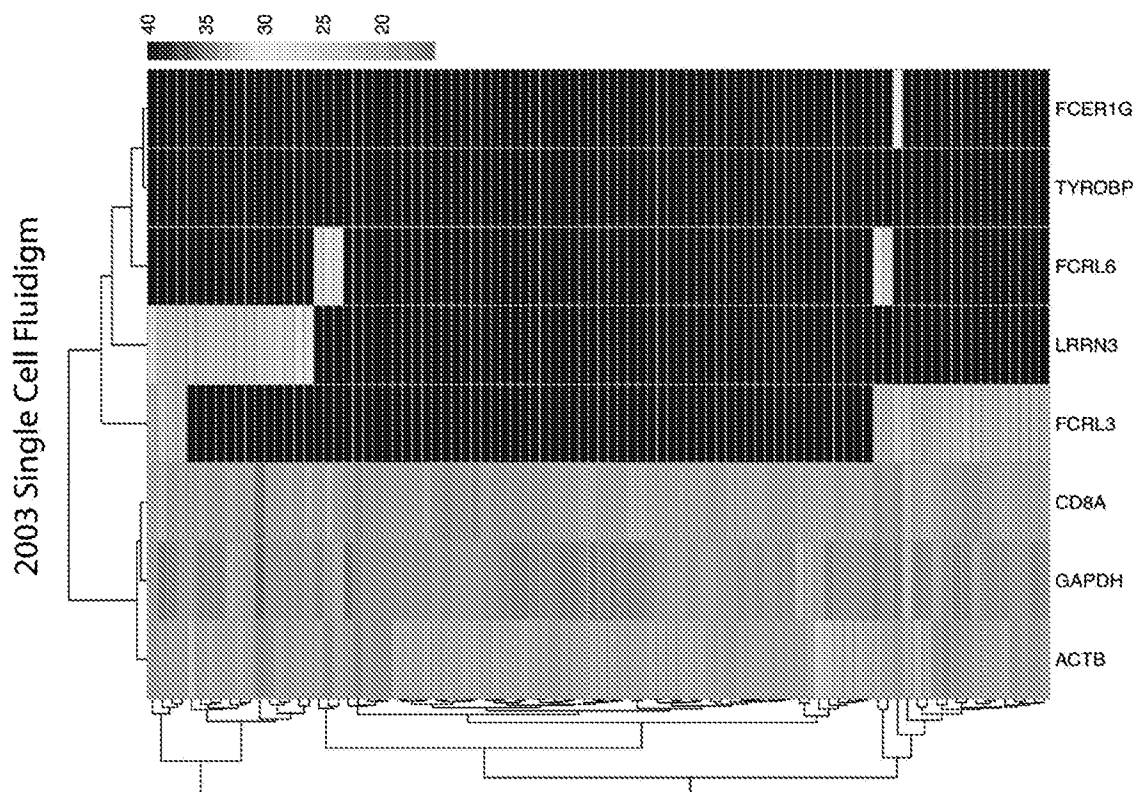


Fig. 5A

[illegible]

MLLWLLLLILTPGREQSGVAPKAVLLLLNPPWSTAFKGEKVALICSSISHSLAQGDYWYHDEKLLKIKHD
KIQITEPGNYQCKTRGSSLSDAVHVEFSPDWLILQALHPVFEGDNVILRCQGKDNKNTHQKVYYKDQKQL
PNSYNLEKITVNSVSRDNSKYHCTAYRKFYILDIEVTSKPLNIQVQELFLHPVLRASSSTPIEGSPMTLT
CETQLSPQRPDVQLQFSLFRDSQTLGLGWSRSPRLQIPAMWTEDSGSYWCEVETVTHSIKKRSLRSQIRV
QRPVPSNVNLEIRPTGGQLIEGENMVLICSAQSGTFTFSWHKEGRVRSRSLGRKTQRSLLAELHVLTVKE
SDAGRYYYCAADNVHSPILSTWIRVTVRIPVSHPVLTFRAPRAHTVVGDILLELHCESLRGSPPIRYFYHE
DVTLGNSSAPSGGGASFNLSLTAEHSGNYS CDADNGLGAQHS HGVS LRVTVPVSRPVLT LRAPGAQAVVG
DLELHCHCESLRGSFPILYWIFYHEDDTLGNISAHSGGGASFNLSL TTEHSGNYSCEADNGLGAQHSKVTL
NVTGTSRNTGLTAAGITGLVLSILVAAAAALLHYARARRKPGGLSATGTSSHSPSECQEPSSSRPSRI
DPQEPTHSKPLAMELEPMYSNVNPGDSSNPIYSIQWISQHTKENSANCPMMHQEHEELTVLYSELKKTTP
DDSAGEASSRGRAHEEDDEENYENVPRVLLASDH

SUBSTITUTE SHEET (RULE 26)

11/26

- (a) MLLWLLLLILTPGREQSGVAPKAVLLLLNPPWSTAFKGEKVALICSSISHSLAQGDTYWH
DEKLLKIKHDKIQITEPGNYQCKTRGSSSLSDAVHVEFSPDWLILQALHPVFEGDNVILRC
QGKDNKNTHQKVYYKDGKQLPNSYNLEKITVNSVSRDNSKYHCTAYRKFYILDIEVTSKP
LNIQVQELFLHPVLRASSSTPIEGSPMTLTCETQLSPQRPDVQLQFSLFRDSQTLGLGWS
RSPRLQIPAMWTEDSGSYWCEVETVTHSIKKRSLRSQIRVQRPVPSNVNLEIRPTGGQLI
EGENMVLICSV AQSGGT VTF SWHKEGRVRS LGRKTQ RSLLAELHVLTVKESDAGRYCAA
DNVHSPILSTWIRVTVRI PVSHPVLTFRAPRAHTVVGDLLELHCESLRGSFPILYRFYHE
DVT LGNSSAPSGGGASFNLSLTAHSGNYSCDADNGLGAQHSHGVSLRVTVPVSRPVLT
RAPGAQAVVGDLLELHCESLRGSFPILYWFYHEDDTLGNISAHSGGGASFNLSLTTEHSG
NYSCEADNGLGAQH SKVVT LNV TGT SRNRTGLTAAGITGLVLSILVLA AAAALLHYARAR
RKPGGLSATGTSSHSPSECQEPSSSRPSRIDPQEPTH SKPLAPMELEPMYSNVNPGDSNP
IYSQIWSIQHTKENSANC PMMQEHEELTVLYSELKKTHPDDSAGEASSRGRAHEEDDEE
NYENVPRVLLASDH
- (b) MLLWLLLLILTPGREQSGVAPKAVLLLLNPPWSTAFKGEKVALICSSISHSLAQGDTYWH
DEKLLKIKHDKIQITEPGNYQCKTRGSSSLSDAVHVEFSPDWLILQALHPVFEGDNVILRC
QGKDNKNTHQKVYYKDGKQLPNSYNLEKITVNSVSRDNSKYHCTAYRKFYILDIEVTSKP
LNIQVQELFLHPVLRASSSTPIEGSPMTLTCETQLSPQRPDVQLQFSLFRDSQTLGLGWS
RSPRLQIPAMWTEDSGSYWCEVETVTHSIKKRSLRSQIRVQRPVPSNVNLEIRPTGGQLI
EGENMVLICSV AQSGGT VTF SWHKEGRVRS LGRKTQ RSLLAELHVLTVKESDAGRYCAA
DNVHSPILSTWIRVTVRTLLSPSPVSHPVLTFRAPRAHTVVGDLLELHCESLRGSFPIL
YRFYHEDVT LGNSSAPSGGGASFNLSLTAHSGNYSCDADNGLGAQHSHGVSLRVTVPV
RPVLT LRAPGAQAVVGDLLELHCESLRGSFPILYWFYHEDDTLGNISAHSGGGASFNLSL
TTEHSGNYSCEADNGLGAQH SKVVT LNV TGT SRNRTGLTAAGITGLVLSILVLA AAAALL
HYARARRKPGGLSATGTSSHSPSECQEPSSSRPSRIDPQEPTH SKPLAPMELEPMYSNVN
PGDSNP IYSQIWSIQHTKENSANC PMMQEHEELTVLYSELKKTHPDDSAGEASSRGRAH
EEDDEENYENVPRVLLASDH
- (c) MLLWLLLLILTPGREQSGVAPKAVLLLLNPPWSTAFKGEKVALICSSISHSLAQGDTYWH
DEKLLKIKHDKIQITEPGNYQCKTRGSSSLSDAVHVEFSPDWLILQALHPVFEGDNVILRC
QGKDNKNTHQKVYYKDGKQLPNSYNLEKITVNSVSRDNSKYHCTAYRKFYILDIEVTSKP
LNIQVQELFLHPVLRASSSTPIEGSPMTLTCETQLSPQRPDVQLQFSLFRDSQTLGLGWS
RSPRLQIPAMWTEDSGSYWCEVETVTHSIKKRSLRSQIRVQRPVPSNVNLEIRPTGGQLI
EGENMVLICSV AQSGGT VTF SWHKEGRVRS LGRKTQ RSLLAELHVLTVKESDAGRYCAA
DNVHSPILSTWIRVTVRI PVSHPVLTFRAPRAHTVVGDLLELHCESLRGSFPILYRFYHE
DVT LGNSSAPSGGGASFNLSLTAHSGNYSCDADNGLGAQHSHGVSLRVTVPVSRPVLT
RAPGAQAVVGDLLELHCESLRGSFPILYWFYHEDDTLGNISAHSGGGASFNLSLTTEHSG
NYSCEADNGLGAQH SKVVT LNV TGT SRNRTGLTAAGITGLVLSILVLA AAAALLHYARAR
RKPGGLSATGTSSHSPSECQEPSSSRPSRIDPQEPTH SKPLAPMELEPMYSNVNPGDSNP
IYSQIWSIQHTKENSANC PMMQEHEELTVLYSELKKTHPDDSAGEASSRGRAHEEDDEE
NYENILNPRKNKVQDFPCLCNT
- (d) MLLWLLLLILTPGREQSGVAPKAVLLLLNPPWSTAFKGEKVALICSSISHSLAQGDTYWH
DEKLLKIKHDKIQITEPGNYQCKTRGSSSLSDAVHVEFSPDWLILQALHPVFEGDNVILRC
QGKDNKNTHQKVYYKDGKQLPNSYNLEKITVNSVSRDNSKYHCTAYRKFYILDIEVTSKP
LNIQVQELFLHPVLRASSSTPIEGSPMTLTCETQLSPQRPDVQLQFSLFRDSQTLGLGWS
RSPRLQIPAMWTEDSGSYWCEVETVTHSIKKRSLRSQIRVQRPVPSNVNLEIRPTGGQLI
EGENMVLICSV AQSGGT VTF SWHKEGRVRS LGRKTQ RSLLAELHVLTVKESDAGRYCAA
DNVHSPILSTWIRVTVRI PVSHPVLTFRAPRAHTVVGDLLELHCESLRGSFPILYRFYHE
DVT LGNSSAPSGGGASFNLSLTAHSGNYSCDADNGLGAQHSHGVSLRVTVPVSRPVLT
RAPGAQAVVGDLLELHCESLRGSFPILYWFYHEDDTLGNISAHSGGGASFNLSLTTEHSG
NYSCEADNGLGAQH SKVVT LNV TGT SRNRTGLTAAGITGLVLSILVLA AAAALLHYARAR
RKPGGLSATGTSSHSPSECQEPSSSRPSRIDPQEPTH SKPLAPMELEPMYSNVNPGDSNP
IYSQIWSIQHTKENSANC PMMQEHEELTVLYSELKKEQSSRFSMSL

Fig. 7

12/26

GTCGACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAG
CCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCA
AGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAG
ATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATA
TGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC
AATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA
ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGC
CCGCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCT
ATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGT
CTCCACCCCATGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGCGCGTTTTGCTGTACTG
GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATA
AAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCGCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCT
TTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGC
TCTCTGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCA
AAAATTTTACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTA
GATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGAAAGAAAAAATATAAATTAACATATAGTATGGGCA
AGCAGGGAGCTAGAACGATTGCGAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGA
CAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCCTCTATTGTG
GCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAG
ACCACCGCACAGCAAGCGGCCGCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAA
GTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGG
TGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCTTGGGTCTTGGGAGCAGCAGGAAGCACTATG
GGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG
CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATC
CTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACCTATTTGCACC
ACTGCTGTGCCTTGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGACCTGGATGGAG
TGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAGAATCGAAAACCAGCAAGAAAAG
AATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGG
TATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTAAGAATAGTTTTTGTGTACTTTCTATAGTGAA
TAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCCCGA
AGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACGGATCGGCACTGCGT
GCGCCAATTCTGCAGACAAATGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
AGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACAAATTACAAAAATTCA
AAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGTTAGTACCGGGCCCGCTCTAGTCGAGGTCGAC
GGTATCGATAAGCTCGCTTACGAGATTCCAGCAGGTCGAGGGACCTAATAACTTCGTATAGCATACATTATACGA
AGTTATATTAAGGGTTCCAAGCTTAAGCGGCCGCGCTGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTGC

Fig. 8

[illegible]

SUBSTITUTE SHEET (RULE 26)

14/26

ACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCC
ACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGC
TGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGG
TCCTGCTGGAGTTCGTGACCGCCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGGCCGC
CAGCACAGTGGTCGAAATTCGTGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATACATGTTTA
AGGGTCCGGTCCACTAGGTACAATTCGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAA
GATTGACTGGTATTCTTAAGTATGTTGCTCTTTACGCTATGTGGATACGCTGCTTAATGCCTTTGTATCATGCTA
TTGCTCCCGTATGGCTTTCAATTTCTCTCTGTATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGTGGCCCG
TTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTG
TCAGCTCTTTCCGGGACTTTGCTTTCCCTCCCTATTGCCACGGCGGAACCTCATCGCCGCTGCCTTCCCGCTG
CTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTCGGGAAATCATCGTCTTTCTTGGCTG
CTCGCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCCTTGGGCCCTCAATCCAGCGGACCT
TCCTTCCCGCGGCTGCTGCCGCTCTGCGGCTCTCCGCGTCTTGCCTTCGCCCTCAGACGAGTCGGATCTCCC
TTTGGGCCGCTCCCCGCATCGATACCGTCGACCTCGATCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGC
AATACAGCAGCTACCAATGCTGATTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCAC
ACCTCAGGTACCTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAGGGGGGA
CTGGAAGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
CTGATTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTAC
CAGTTGAGCAAGAGAAGGTAGAAGAAGCCAATGAAGGAGAGAACACCCGCTTGTACACCCTGTGAGCCTGCAT
GGGATGGATGACCCGGAGAGAGAAGTATTAGAGTGGAGGTTTGACAGCCGCTAGCATTTATCATCATGGCCCG
AGAGCTGCATCCGGACTGTACTGGGTCTCTGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG
GAACCCACTGCTTAAGCCTCAATAAAGCTTGCTTGAGTGCTTCAAGTAGTGTGTGCCGCTGTGTTGTGTGACTCTG
GTAAGTACAGATCCCTCAGACCTTTTAGTCAGTGTGGAAAATCTCTAGCAGCATGTGAGCAAAAGGCCAGCAAA
AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAA
ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC
TCGTGCGCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTCGGGAAGCGTGGCGCTT
TCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCC
CCGTTACAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCC
ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGT
GGCCTAACTACGGCTACACTAGAAGAAGTATTTGGTATCTGCGCTGCTGAAGCCAGTTACCTTCGGAAAAAG
AGTTGGTAGCTCTTGATCCGGCAAACAAACCCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACG
CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCAC
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTAAATTAATAAATGAAGTTTTAA
TCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT

Fig. 8
(continued)

15/26

CTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTG
GCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCG
GAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG
AGTAAGTAGTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCG
TTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAG
CGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGC
ACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG
AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAAC
TTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGT
TCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAAC
AGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTCA
ATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAA
TAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAC

Fig. 8
(continued)

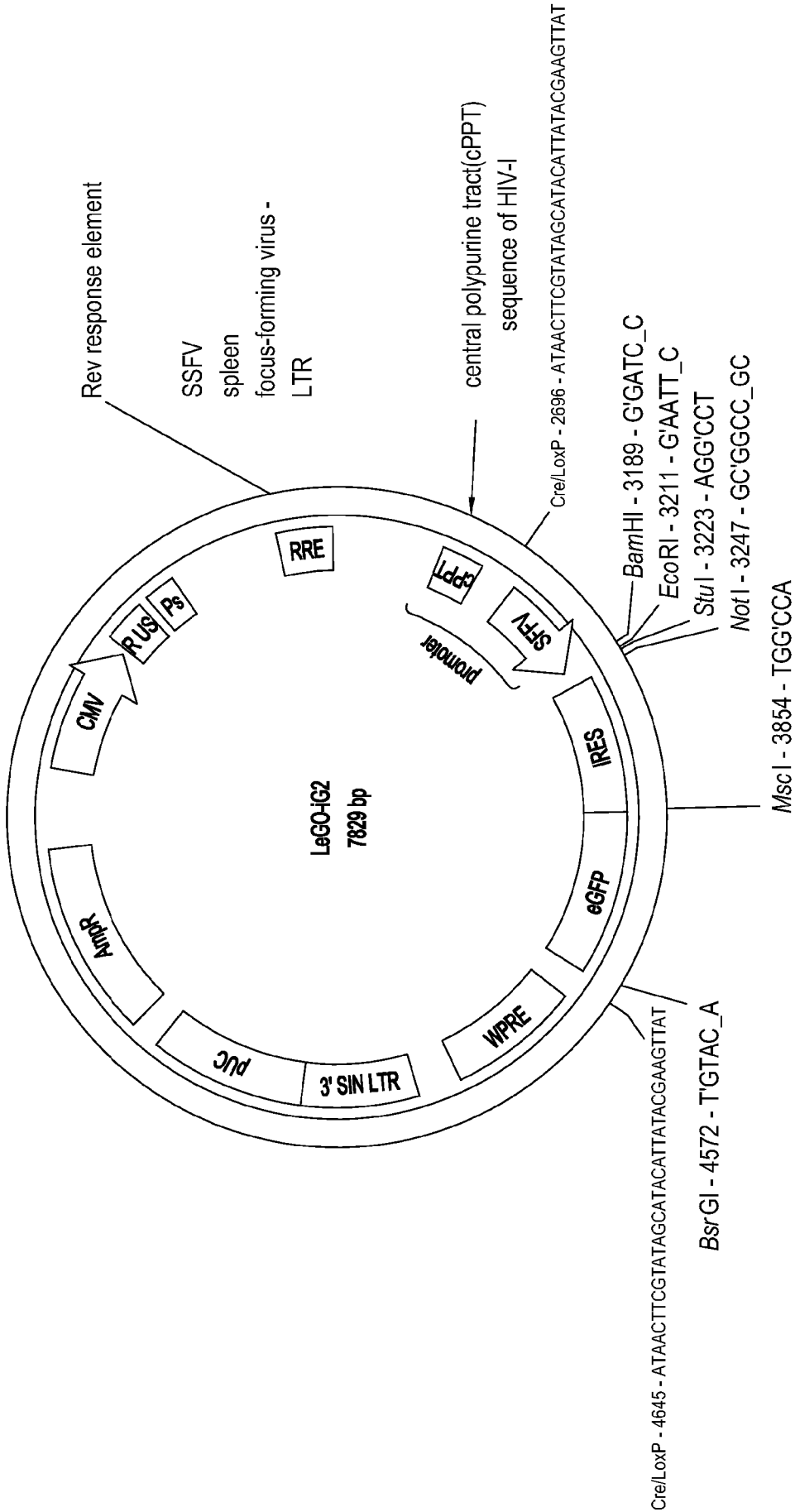


Fig. 9

17/26

TTTCAGCCAACCAGACTGGAGCAGCTGCGAGTGCTACATCTTGGCTGTCTGAAGCGATTGGCTCCTCTCT
GGGGAGTGGAGGGTGTTCAGTTATTAATGACCGCTGAGCAGGCAGCACCATGTCAAGTGTGACAACTGATC
GGGTGAACGATGCACCACTAACCACCATGGAACAAGGAAAAATAAGCCAGCTCACAGGATCTCTCTTC
ACTGGATTGAGAGCCTCAGCCTGCCGACTGAGAAAAAGAGTTCCAGGAAAAAGAAGGAATCCCGGCTGCA
GCCTCCTGCCTTCTTTATATTTTAAAAATAGAGAGATAAGATTGCGTGCATGTGTGCATATCTATAGTAT
ATATTTTGTACACTTTGTTACACAGACACACAAATGCACCTATTTATACCGGGCAAGAACACAACCATGT
GATTATCTCAACCAAGGAACTGAGGAATCCAGCACGCAAGGACATCGGAGGTGGGCTAGCACTGAAACTG
CTTTTCAAGGAGGTGAAGCATTCCACAGATAATCAGCTACTCTATTTCAGGGAAGGAGTGTAAACATCACC
ACTTTTTGCATGTTTGCATAAAAAAGGCCCACTGAATCAGTTAAAGACGAGGAAGAGGAGGAGAAAGAGA
AAGAAGAGGAAGATGTTGGGCAACATTTATTTAACATGCTCCACAGCCCGGACCCTGGCATCATGCTGCT
ATTCTGCAAATACTGAAGAAGCATGGGATTTAAATATTTTACTTCTAAATAAATGAATTACTCAATCTC
CTATGACCATCTATACATACTCCACCTTCAAAAAAGTACATCAATATTATATCATTAAAGGAAATAGTAACC
TTCTCTTCTCCAATATGCATGACATTTTTGGACAATGCAATTGTGGCACTGGCACTTATTTCACTGAAGA
AAAACCTTTGTGGTTCTATGGCATTTCATCTTTGACAAATGCAAGCATCTTCTTATCAATCAGCTCCTAT
TGAACCTACTAGCACTGACTGTGGAATCCTTAAGGGCCCATTAATTTCTGAAGAAGAAAGCTAAGATGA
AGGACATGCCACTCCGAATTCATGTGCTACTTGGCCTAGCTATCACTACACTAGTACAAGCTGTAGATAA
AAAAGTGGATTGTCCACGGTTATGTACGTGTGAAATCAGGCCTTGGTTTACACCCAGATCCATTTATATG
GAAGCATCTACAGTGGATTGTAATGATTTAGGTCTTTTAACTTTCCAGCCAGATTGCCAGCTAACACAC
AGATTCTTCTCTACAGACTAACAATATTGCAAAAAATTGAATACTCCACAGACTTTCCAGTAAACCTTAC
TGGCCTGGATTTATCTCAAAACAATTTATCTTCAGTCACCAATATTAATGTAAAAAAGATGCCTCAGCTC
CTTTCTGTGTACCTAGAGGAAAAACAACTTACTGAAGTGCCTGAAAAATGTCTGTCCGAAGTGAAGCACT
TACAAGAAGCTCTATATTAATCACAACCTTGTCTTCTACAATTTTACCTGGAGCCTTTATTGGCCTACATAA
TCTTCTTCGACTTCATCTCAATTCAAATAGATTGCAGATGATCAACAGTAAGTGGTTTGATGCTCTTCCA
AATCTAGAGATTCTGATGATTGGGGAAAATCCAATTATCAGAATCAAAGACATGAACCTTAAAGCCTCTTA
TCAATCTTCGCAGCCTGGTTATAGCTGGTATAAACCTCACAGAAATACCAGATAACGCCTTGGTTGGACT
GGAAAACTTAGAAAGCATCTCTTTTTACGATAACAGGCTTATTAAGTACCCCATGTTGCTCTTCAAAAA
GTTGTAAATCTCAAATTTTTGGATCTAAATAAAAAATCCTATTAATAGAATACGAAGGGGTGATTTTAGCA
ATATGCTACACTTAAAAGAGTTGGGGATAAATAATATGCCTGAGCTGATTTCCATCGATAGTCTTGCTGT
GGATAACCTGCCAGATTTAAGAAAAATAGAAGCTACTAACAACCTTAGATTGTCTTACATTCACCCCAAT
GCATTTTTTCAGACTCCCCAAGCTGGAATCACTCATGCTGAACAGCAATGCTCTCAGTGGCCTGTACCATG
GTACCATTGAGTCTCTGCCAAACCTCAAGGAAATCAGCATACACAGTAACCCCATCAGGTGTGACTGTGT
CATCCGTTGGATGAACATGAACAAAACCAACATTGATTGATGAGCCAGATTCACTGTTTTGCGTGGAC
CCACCTGAATTCCAAGGTCAGAATGTTCCGCAAGTGCATTTCAAGGACATGATGGAAATTTGTCTCCCTC
TTATAGCTCCTGAGAGCTTTCTTCTAATCTAAATGTAGAAGCTGGGAGCTATGTTTCTTTCACTGTAG
AGCTACTGCAGAACCACAGCCTGAAATCTACTGGATAACACCTTCTGGTCAAAAACTCTTGCCTAATACC
CTGACAGACAAGTTCTATGTCCATTCTGAGGGAACACTAGATATAAATGGCGTAACTCCCAAGAAAGGGG
GTTTATATACTTGTATAGCAACTAACCTAGTTGGCGCTGACTTGAAGTCTGTTATGATCAAAAGTGGATGG
ATCTTTTCCACAAGATAACAATGGCTCTTTGAATATTAATAAGAGATATTCAGGCCAATTCAGTTTTG
GTGTCCTGGAAAGCAAGTTCTAAATTTCTCAAATCTAGTGTTAAATGGACAGCCTTTGTCAAGACTGAAA
ATTCTCATGCTGCGCAAAGTGCTCGAATACCATCTGATGTCAAGGTATATAATCTTACTCATCTGAATCC
ATCAACTGAGTATAAAATTTGTATTGATTTCCACCATCTATCAGAAAAACAGAAAAAATGTGTAAAT
GTCACCACCAAAGGTTTGCACCCTGATCAAAAAAGAGTATGAAAAGAATAATACCACAACACTTATGGCCT
GTCTTGGAGGCCTTCTGGGGATTATTGGTGTGATATGTCTTATCAGCTGCCTCTCTCCAGAAATGAACTG
TGATGGTGGACACAGCTATGTGAGGAATTACTTACAGAAACCAACCTTTGCATTAGGTGAGCTTTATCCT
CCTCTGATAAATCTCTGGGAAGCAGGAAAAAGAAAAAGTACATCACTGAAAGTAAAAGCAACTGTTATAG
GTTTACCAACAAATATGTCCTAAAAACCAAGGAAACCTACTCCAAAAATGAACAAAAAAGGAAAG
CGAAAGACTGCAGTTGTGCTAAAAACAAAACAAAACAAAACAAAACAAAAGTAAAAAAGATTACT
TTCGAGAGAGAAGTTTAAAGCTTCAACCAATGCTGCTCCTGACCAATGGAAATATGTACAACCTTCAGCATTT
TAAGTAACTGGCTTCAAGGGGTACTGTGGCAACCAATAAAATAACTCCATTTTCTAAAACCTTCATGTA
ACTTTTATGTCTGGACTACAGTTCAAGTGGACAAAACATTTCTGTATTTTTTTAAGTAAATAAGAGTA
GTTGAACTGAGCAATACCTCCTCTGTGTTGATTACACATATTAGCCACGAGTTTTTGCAGTGACCAGA
TAACTTGAATTGACACGTGGTGTAAATAAATGGACAAATCTGTAGAGTAGACACAGTGAGTATGTGGA
CCTCTTTTATAAGGAAAAATACATTTTGGATTAAATCAATTGCTTCTGTCTTGTGTTTGTCTAAATAA
AGAATAATTTCTGGGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAG

Fig. 10(a)

18/26

MKDMPLRIHVLLGLAITTLVQAVDKKVDPCRLCTCEIRPWFTPRSIYMEASTVDCNDLGLLTFPARLPAN
 TQILLQTNNAKIEYSTDFPVNLTGLDLSQNNLSSVTNINVKKMPQLLSVYLEENKLTELPEKCLSELS
 NLQELYINHNLLSTISPGAFIHLNLLRLHLNSNRLQMINSKWFDALPNLEILMIGENPIIRIKDMNFKP
 LINLRSLVIAGINLTEIPDNALVGLENLESISFYDNRLIKVPHVALQKVNLKFLDLNKNPINRIRRGDF
 SNMLHLKELGINNMPELISIDSLAVDNLPLDKIEATNNPRLSYIHPNAFFRLPKLESLMLNSNALSALY
 HGTIESLPNLKEISIHSPNIRCD CVIRWMNMNKTNI RFMEPDSLFCVDPPEFQGNVRQVHFRDMMEICL
 PLIAPESFPSNLNVEAGSYVSFHCRAAEQPEIYWITPSGQKLLPNTLTDKFYVHSEGTL DINGVTPKE
 GGLYTCTIATNLVGADLKSVMIKVDGSFPQDNNGSLNIKIRDIQANSVLVSWKASSKILKSSVKWTA FVKT
 ENSHAAQSARIPSDVKVYNLTHLNPSTEYKICIDIPTIYQKNRKKCVNVTTKGLHPDQKEYEKNNTTLM
 ACLGGLLGIIGVICLISCLSPENMCDGGHSYVRNYLQKPTFALGELYPPLINLWEAGKEKSTSLKVKATV
 IGLPTNMS

Fig. 10(b)

GGATCCGCCACCATGAAAGATATGCCCCCTGCGAATCCACGTGCTCCTCGGTCTTGCCATAACTACCCTGGTTCAG
 GCCGTCGATAAGAAGGTCGATTGTCCCAGACTCTGTACATGCGAAATCCGCCCTGGTTCCTCCCGCAGCATT
 TACATGGAAGCGTCCACTGTTGACTGTAACGACTTGGGCCTGCTTACTTTTCTGCAAGACTGCCAGCTAATACCC
 AAATCCTGCTGCTCCAGACCAATAACATAGCGAAAATTGAATATTCTACCGACTTCCCAGTAACTTGACAGGGCT
 GGATTTGTCTCAAAACAACCTGAGCTCCGTGACCAATATCAATGTAAAAAGATGCCCAACTGCTCAGCGTGTAT
 CTCGAAGAAAAATAAATTGACAGAACTCCCCGAAAAATGTCTGTCCGAGCTGTCAAACCTCCAGGAACCTCTATATTA
 ATCACAACCTGCTTTCCACAATCAGCCCCGGCGCTTTTATCGGTCTGCACAATCTGCTTAGACTTCACCTCAATAG
 CAATAGGTTGCAGATGATCAACAGTAAGTGGTTCGATGCCCTCCCTAACCTGGAAATCCTTATGATTGGCGAAAAT
 CCTATCATCAGGATCAAGGACATGAACCTTTAAACCTCTGATTAACCTTCGGTCACTGGTGATTGCTGGCATTATCT
 GACAGAGATACCTGATAATGCTCTGGTCGGCCTTGAGAACCTGGAATCTATTAGTTTTATGACAATAGGCTTATC
 AAAGTGCCCCACGTCGCTCTGCAGAAGGTTGTCAACCTGAAGTTCCTCGACCTCAATAAGAATCCGATCAACCGC
 ATACGCCGAGGAGATTTTTCTAATATGCTTCACTTGAAAGAGTTGGGAATCAACAACATGCCCGAACTCATCTCCA
 TTGACTCTCTCGCTGTTGACAACTTGCCCCGACCTCAGAAAAATCGAGGCCACTAACAATCCACGCCTTAGCTATAT
 TCATCCAAATGCATTTTTCCGATTGCCTAACTTGAGAGCCTCATGCTGAACTCAAATGCACTGAGCGCCCTGTAT
 CATGGCACAATTGAATCTCTGCCTAATTTGAAAGAAATCAGCATTCACTTAACCTATCCGATGCGACTGCGTGAT
 CCGATGGATGAACATGAACAAGACGAACATCCGATTTATGGAACCCGATAGTCTCTTCTGCGTGGACCCCTCCCGA
 GTTTCAAGGTCAGAATGTGAGGCAGGTACACTTTAGGGATATGATGGAGATCTGCCTTCCCTGATCGCACCCGA
 ATCTTTTCTAGCAATCTGAACGTTGAGGCCGGAAGCTATGTGTCATTCCATTGCAGGGCCACAGCTGAACCCCA
 GCCAGAAATCTACTGGATTACCCCGTCCGGACAGAACTGCTGCCGAACACGCTGACGGATAAATTCTACGTGCA
 TTCTGAAGGAACTCTCGATATAAACGGTGTGACTCCAAAAGAAGGCGGATTGTACACCTGTATCGCCACCAACCTC
 GTAGGCGCCGATCTGAAGAGCGTAATGATCAAAGTAGATGGCTCTTTCCCTCAAGATAATAACGGGAGCCTGAAC
 ATCAAATTCGGGATATTCAGGCTAACTCAGTGCTGGTTAGTTGGAAAGCCTCCTCAAAGATTCTGAAATCATCAG
 TTAAATGGACCGCATTCTGTCAAACCGAGAACAGTCACGCAGCCCAATCAGCTCGAATCCCTCCGACGTGAAAG
 TCTACAACCTGACACATCTTAATCCCTCTACCGAGTATAAAATCTGTATCGATATTCACAATTTACCAGAAGAAC
 AGGAAAAAATGTGTGAACGTCCTACCAAAGGACTTCATCCTGACCAGAAAGAATACGAAAAAACAATACAACAA
 CACTGATGGCTTGCCCTGGGAGGACTGCTGGGCATCATTGGAGTGATATGTTTGATCTCATGCCTGTCTCCGGAGA
 TGAAGTGATGGCGGCCATTCTTATGTGAGAACTATCTGCAAAAACCTACTTTCGCTCTTGGAGAAGTGTACCC
 ACCATTGATCAACTTGTGGGAGGCCGGAAGGAAAAATCCACATCATTGAAAGTGAAAGCCACCGTTATCGGACT
 CCCAACCAATATGTCTTGAGCGGCCGC

Fig. 10(c)

19/26

GTTTCTGACCTGTGTTCCCTCCGCTGTGCCAGAACAGGCCCATGCTGCTCTGGACGGCTGTGCTGCTCT
TTGTTCCCTGTGTTGGGAAAACCTGTCTGGCTGTACCTCCAAGCCTGGCCAAACCCTGTGTTTGAAGGAGA
TGCCCTGACTCTGCGATGTCAGGGATGGAAGAATACACCACTGTCTCAGGTGAAGTTCTACAGAGATGGA
AAATTCCTTCATTTCTCTAAGGAAAACCAGACTCTGTCCATGGGAGCAGCAACAGTGCAGAGCCGTGGCC
AGTACAGCTGCTCTGGGCAGGTGATGTATATCCACAGACATTACACAAAACCTTCAGAGACTGCCATGGT
TCAAGTCCAAGAGCTGTTTCCACCTCCTGTGCTGAGTGCCATCCCCCTCCTGAGCCCCGAGAGGGTAGC
CTGGTGACCCTGAGATGTCAGACAAAGCTGCACCCCTGAGGTGAGCCTTGAGGCTCCTTTTCTCCTTCC
ACAAGGACGGCCACACCTTGCAAGACAGGGGCCCTCACCCAGAACTCTGCATCCCGGGAGCCAAGGAGGGAG
ACTCTGGGCTTTACTGGTGTGAGGTGGCCCTGAGGGTGGCCAGGTCCAGAAGCAGAGCCCCCAGCTG
GAGGTGAGAGTGCAGGCTCCTGTATCCCGTCTGTGCTCACTCTGCACCACGGGCCTGCTGACCCTGCTG
TGGGGGACATGGTGCAGCTCCTCTGTGAGGCACAGAGGGGCTCCCTCCGATCCTGTATTCTTTCTACCT
TGATGAGAAGATTGTGGGGAACCACTCAGCTCCCTGTGGTGAACCACTCCCTCCTTCCCAGTGAAG
TCAGAACAGGATGCTGGGAACACTCCTGCGAGGCTGAGAACAGTGTCTCCAGAGAGAGGAGTGAGCCCA
AGAAGCTGTCTCTGAAGGGTTCTCAAGTCTTGTTCACTCCCGCCAGCAACTGGCTGGTTCTTTGGCTTCC
TGCGAGCCTGCTTGGCCTGATGGTTATTGCTGCTGCACTTCTGGTTTATGTGAGATCCTGGAGAAAAGCT
GGGCCCCCTTCCATCCCAGATACCAACCCACAGCTCCAGGTGGAGAGCAGTGCCCACTATATGCCAACGTGC
ATCACCAAGAAAGGAAAGATGAAGGTGTTGTCTACTCTGTGGTGCATAGAACCCTCAAAGAGGAGTGAAGC
CAGGTCTGCTGAGTTCACCGTGGGGAGAAAGGACAGTTCTATCATCTGTGCGGAGGTGAGATGCCTGCAG
CCCAGTGAGGTTTCATCCACGGAGGTGAATATGAGAAGCAGGACTCTCCAAGAACCCCTTAGCGACTGTG
AGGAGGTTCTCTGCTAGTGATGGTGTCTCCTATCAACACACGCCCCACCCCCAGTCTCCAGTGCTCCTCA
GGAAGACAGTGGGGTCTCAACTCTTTCTGTGGGTCTTCAGTTCCCAAGCCCAGCATCACAGAGCCCCC
TGAGCCCTTGTCTGGTCAGGAGCACCTGAACCTGGGTTCTTTTCTTAGCAGAAGACCAACCAATGGAA
TGGGAAGGGAGATGCTCCCAACACACACACTTAGGTTCAATCAGTGACACTGGACACATAAGCCACA
GATGTCTTCTTTCCATACAAGCATGTTAGTTCGCCCCAATATACATATATATGAAATAGTCATGTGCC
GCATAACAACATTTCACTCAGTGATAGACTGCATACACAACAGTGGTCCCATAAGACTGTAATGGAGTTT
AAAAATTCCTACTGCCTAGTGATATCATAGTTGCCTTAACATCATAACACAACACATTTCTCACGCGTTT
GTGGTGATGCTGGTACAAACAAGCTACAGCGCCGCTAGTCATATACAAATATAGCACATACAATTATGTA
CAGTACACTATACTTGATAATGATAATAAACAACTATGTTACTGGTTTATGTA

Fig. 11(a)

20/26

MLLWTAVLLFVPCVGKTVWLYLQAWPNPVFEGDALTLRCQGWKNTPLSQVKFYRDGKFLHFSKENQTL
 SMGAATVQSRGQYSCSGQVMYIPQTFTQTSETAMVQVQELFPPPVLSAIPSPPEPREGSLVTLRCQTKLHPLR
 SALRLLFSFHKDGH TLQDRGPHPELCIPGAKEGDSGLYWCEVAPEGGQVQKQSPQLEVRVQAPVSRPVL
 TLHHGPADPAVGDMVQLLCEAQRGSPPILYSFYLDEKIVGNHSAPCGGTTSLFPVKSEQDAGNYSCEAEN
 SVSRERSEPKKLSLKGSQVLF TPASNWLVPWLPASLLGLMVIAAALLVYVRSWRKAGPLPSQIPPTAPGG
 EQCPLYANVHHQKGKDEGVVYSVVHRTSKRSEARSAEFTVGRKDSSIICAEVRCLQPSEVSSTEVMRSR
 TLQEPLSDCEEVLC

Fig. 11(b)

GGATCCGCCACCATGCTGCTCTGGACAGCCGTA CTCTTGTTCTGACCTTGTGTTGGAAG
 ACTGTCTGGTTGTATCTGCAGGCCTGGCCTAATCCAGTTTTCGAGGGGGATGCTCTGACA
 CTCAGATGCCAAGGTTGGAAGAACACACCATTGTCTCAAGTGAAGTTTTACCGCGACGGC
 AAATTCCTCCACTTCAGTAAAGAAAATCAGACACTGTCTATGGGGGCCGCCACAGTTCAG
 TCCAGAGGTCAGTATAGTTGTAGTGGACAGGTCATGTACATTCTCAGACCTTTACACAG
 ACTAGCGAAACTGCGATGGTTCAAGTTCAAGAATTGTTTCTCCCCCGTCTGTCTGCA
 ATCCATCACCCGAGCCTCGAGAAGGCTCTCTCGTCACCCCTGCGGTGCCAGACAAAGCTC
 CATCCACTTAGATCTGCTCTCCGGCTGCTCTTCTCCTTCCACAAGGATGGACATACTCTT
 CAAGATCGCGGCCACATCCCGAACTGTGCATACCCGGCGCTAAGGAGGGCGACAGTGGC
 CTTTATTGGTGCGAAGTTGCGCCCGAAGGCGGACAAGTGCAGAAACAGTCCCCCAGCTC
 GAAGTCCGGGTTCCAGGCCCTGTCTCTAGGCCAGTGTGACCCTGCACCATGGGCCTGCC
 GACCCAGCCGTAGGGGATATGGTCCAGCTGCTGTGTGAGGCCCAACGAGGTTACCCCCC
 ATCCTGTACTCCTTCTACCTTGACGAAAAGATCGTCGGCAACCATTAGCTCCTTGCGGT
 GGGACTACTAGCCTGTTGTTTCCAGTTAAATCCGAACAGGATGCAGGTAATTACTCTTGC
 GAAGCAGAGAACTCCGTGAGTAGAGAGAGGTCTGAACCAAAAAAGCTGTCTCTGAAAGGC
 TCACAGGTATTGTTTACTCCAGCATCAAACCTGGTTGGTCCCTTGGCTCCCCGCGTCATTG
 CTCGGCCTTATGGTGATCGCAGCCGCCCTGCTGGTCTACGTGCGGT CATGGCGGAAAGCG
 GGGCCTCTTCCATCTCAAATACCACCAACAGCACCAGGAGGGGAACAATGTCTCTTTAC
 GCAAATGTCCATCATCAAAAAGGGAAAGATGAAGGCGTGTTTACAGTGTGGTACACCGG
 ACATCCAAACGGAGCGAAGCCAGAAGTGCAGAGTTTACAGTTGGACGGAAGGACTCCTCC
 ATTATTTGTGCCGAGGTGCGATGTCTGCAACCCAGCGAAGTTAGCAGCACTGAAGTAAAT
 ATGAGGTCCCGGACCCTGCAGGAGCCACTTAGTGATTGCGAAGAAGTACTTTGTTAGGCG
 GCCGC

Fig. 11(c)

lentiviral vector comprising FCRL6

Fig. 12

22/26

aaaaagctgtctcgaaggctcacagggtattgttaccagcatcaaaactgggtgggtccctgggtcccccgggtcattgctcggccttatgggtgacgcagccgccc
gtgtgtctacgtgcgggtcatggcgaaagggggccctctccatctcaaatccacccacagcaaccaggaggggaacaaatgtctctttatgcgaatgtccatcatc
aaaaaggggaaagatgaaggcgtgtttacagtggtgacacccggacatccaaacggagcgaagccagaagtgcagagttacagttggaoggaagggaactccctcc
attattgtgcggagggtgcgatgtctgcaaccacagcgaagttagcagcacgaagtaaatatgagggtcccggaacctgcaggagccacttagttagtgcgaagaagi
actttgtaggcggcggcTACGTAAATTCCGCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGC
CGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGCAATGTGAGG
GCCCCGAAACCTGGCCCTGTCTTTGACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAGGAATGCAAGG
TCTGTTGAATGTCTGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCTTG
CAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCA
AAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGT
ATCAACAAGGGGCTGAAGGATGCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACAT
GCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCTTTGAA
AAACACGATGATAATATGGCCACAACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCC
TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT
ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCAC
CCTGACCTACGGCGTGCACTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC
ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG
TGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACAT
CCTGGGGCACAAGCTGGAGTACAACATAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC
ATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA
ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAA
AGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATG
GACGAGCTGTACAAGTAAAGCGGCCGGCCGCCAGCACAGTGGTCGAAATTCGTGAGGGACCTAATAACTTCG
TATAGCATACATTATACGAAGTTATACATGTTAAGGGTCCGGTTCCTACTAGGTACAATTCGATATCAAGCTTAT
CGATAATCAACCTCTGGATTACAAAATTTGTAAAGATTGACTGGTATTCTTAAGTATGTTGCTCCTTTTACGCTAT
GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGATAAA
TCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAACGTGGCGTGGTGTGCACTGTGTTTGC
TGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTCCGGGACTTTCGCTTTCCCCCTCC
CTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGA
CAATCCGTGGTGTGTCGGGGAAATCATCGTCTTTCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGC
GCGGGACGTCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCCTTCCCGCGGCCCTGCTGCCGGC
TCTGCCGGCTCTTCCGCGTCTTCGCCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCGGCCCTCCCCGCATC
GATACCGTCGACCTCGATCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGC
TGATTGTGCTTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGAC
CAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATTCCT
CCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACA
CACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGTGCTACAAGCTAGTACCAGTTGAGCAAGAGAAG
GTAGAAGAAGCCAATGAAGGAGAGAACACCCGCTTGTACACCTGTGAGCCTGCATGGGATGGATGACCCGG
AGAGAGAAGTATTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACATGGCCCGAGAGCTGCATCCGGAC
TGACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAA
GCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATC
CCTCAGACCCCTTTTAGTCAGTGTGGAAATCTCTAGCAGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACC
GTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCA
AGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCTTGAAGCTCCCTCGTGCGCT
CTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT
AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGT
TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG

Fig. 12
(continued)

23/26

GCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAG
AGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTAC
GCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTC
ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTA
AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGC
GATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC
ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCCAGC
CAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
GAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCA
CGCTCGTCGTTTGGTATGGCTTCATTAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTG
TGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATG
GTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAA
CCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCG
CCACATAGCAGAACTTTAAAAGTGCTCATCATTGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCG
CTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTT
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTC
ATACTCTTCCTTTTTCAATATTATTGAAGCATTATTCAGGGTTATTGTCTCATGACGGATACATATTTGAATGTAT
TTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAC

Fig. 12
(continued)

24/26

lentiviral vector comprising LRRN3

GTCGACGGATCGGGAGATCTCCCGATCCCGTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTA
 AGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTGCTGAGTAGTGC GCGAGCAAAATTTAAGCTACAACAAG
 GCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGG
 CCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCC
 ATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCCAT
 TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATT
 TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
 GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTAT
 TAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGATAGCGGTTTTGACTCACGG
 GGATTTCCAAGTCTCCACCCCAATTGACGTCAATGGGAGTTTGTTTTGGCACC AAAATCAACGGGACTTTCCAAAA
 TGTCGTAACAACCTCGCCCCATTGACGCAAAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATAAGCAGCG
 CGTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCC
 ACTGCTTAAGCCTCAATAAAGCTTGCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAA
 CTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGC
 GAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGG
 GCGGCGACTGGTGAGTACGCCAAAAATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTC
 AGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAA
 ATTA AACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTGCGAGTTAATCCTGGCCTGTTAGAAACATCAGA
 AGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAA
 TACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGA
 GGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAAGCGGCCGCGCGCTGATCTTCAGACCTGGAGGAG
 GAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCAC
 CCACCAAGGC AAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCTTGGGTT
 CTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT
 GGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTG
 GGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATT
 GGGGTTGCTCTGGAAAACCTATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAAC
 AGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAA
 TTGAAGAATCGCAAACCAGCAAGAAAAAGAAATGAACAAGAAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGA
 ATTGTTTAACATAACAAATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAG
 AATAGTTTTTCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTC
 CCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAGGTGGAGAGAGAGACAGAGACAGATCC
 ATTCGATTAGTGAACGGATCGGCACTGCGTGCCCAATTCTGCAGACAAATGGCAGTATTCATCCACAATTTTAA
 AAGAAAAGGGGGGATTGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTA
 AAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGTT
 AGTACCGGGCCCGCTCTAGTCGAGGTCGACGGTATCGATAAGCTCGCTTCACGAGATTCCAGCAGGTCGAGGG
 ACCTAATAACTTCGTATAGCATACATTATACGAAGTTATATTAAGGGTTCCAAGCTTAAGCGGCCGCGCGCTGAA
 AGACCCACCTGTAGGTTTGGCAAGCTAGCTGCAGTAACGCCATTTTGAAGGCATGGAAAAATACCAAAACCA
 GAATAGAGAAGTTCAGATCAAGGGCGGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCGGTGA
 GCAGTTTCGGCCCCCGGCCGGGCCAAGAACAGATGGTCACCGCAGTTTCGGCCCCGGCCGAGGCCAAGAA
 CAGATGGTCCCCAGATATGGCCCAACCCTCAGCAGTTTCTTAAGACCCATCAGATGTTTCCAGGCTCCCCCAAG
 GACCTGAAATGACCCTGCGCCTTATTTGAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTGCGCGCTTCTGCT
 TCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAGTCCCTCGATTGACTGAGTCGCCCGgga
 cgcacacatgaagatagccctgcgaatccacgctgctctcgtgcttgcataacacacccctggcagggccgtgataaagaggctgattgcccagactctgtacat
 ggcgaalccgccccctggcactccccgcagcatctacaggaagcgtccactgttgactgtaacgactgggcccgtctactttccgtcaagactgcccagcctaacc
 aaatcctgctgctccagaccataacatagcgaataatgaatctacacgactccagtaaaccttgacagggctggtattgtctcaaaacacactgagctccgtgac
 caataatcaatgtaaaaaagatgccccactgctcagcggtatctcgaagaaataaatgacagaactccccgaataatgctgtccgagctgtcaaacctccagg
 aactctatataatcacacactgcttccacacacagccccggcgcttctcaggtctgcacacactgcttagactcacctcaalagcaatagggtgcagatgatcaacag
 taagtgttgcagctccctccataacctggaatccttatgattggcgaaatcctatcatcaggatcaaggacatgaactttaaactctgtatgaacttgggtcactggig
 attgctggcattatctgacagagataacctgataatgctcgtggcgccctgagaacctggaatcattatgattatgacaataggcttatcaagtgccccacgtcgtct
 gcagaagggtgcaacctgaagttcctgacctcaataagaatccgatcaacgcgcatagccgaggagattttctaataatgcttcaactgaagagtgggaatcaac

Fig. 13

25/26

aacatgccgaactcaicccattgactctctcgtgtgacaacttgcccgacccagaaaaaafcgaggccactaacaatccacgocctagctatattcaacaaatgc
 attttccgattgcctaaactgagagccctcatgctgaacicaaagcagcagagcccgatcatggcacaattgaatctctgocctaatfagaagaaatcagcaatcatt
 ctaacccctatccgattgocgactgctgatalccgaltggaacatgaacaagaogaaacalcgatttatggaacccgatalgicctctctgctgggacccctccgagttca
 aggtcagaatgtgaggcaggtacactttagggaatgtagtgagatctgcttccctgatgcacccgaatctttccctagcaatcgaacgttgaggccgggaagctatg
 tglcattccattgcagggccacagctgaacccagccagaaatctactggaaccccgctcgggacagaaactgctgcgaacacgctgaacggatataaattciaogtg
 cattctgaagggaactcgcatalataaacggigtgactccaaaaagaaggcggatgtacacctgatatgcacccaacccloglagggccgcatctgaagagcgtaatgat
 caaagtagatggcctcttccccaagalaataacggggagccctgaacatcaaaaattcgggatalccaggctaaactcagtgctggtagttggaaagccctcccaagait
 ctgaatcaicagttaaatggacccgcatlogicaaaaacggagaacagtcacgcagcccaacagclogaalcctcccgacgtgaaagctacaaactgacacalc
 ftaacccctaccgagatataaastctgtatgatattccaaacttaaccagagaaacagggaataatgtgtgaacgtcactaaccaggacttcatctgacccagaa
 agaatacgaataaacaafacaacaacactgaltggttgcctggggaggactgctgggcacattggagtgatgtttgatctcagcctgtctcgggagatgaactgt
 gatggggccattcttatgtgagaactaictgcaaaaacctacttgcctctgggagaactgaccacccattgatcaactgtgtggaggccgggaagggaataatcca
 catcattgaagagagacccggtatcggactcccaaccasatgtcttgagggccgcTACGTAAATTCCGCCCCCCCCCCCCCTCTCCC
 TCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCC
 ACCATATTGCCGCTTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGG
 TCTTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTG
 AAGACAAACAACGTCTGTAGCGACCCTTTCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGC
 CAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCACTGCCACGTTGTGAGTTGGATAGTTGT
 GGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTA
 TGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCC
 CCGAACACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCACAACCATGGTGAGCAAGGGCG
 AGGAGCTGTTACCGGGGTGGTGCCCATCTGGTCGAGCTGGACGGCGACGTAACGGCCACAAGTTTCAGCGT
 GTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTTCATCTGCACCACCGGCAAGCT
 GCCCGTGCCCTGGCCACCCCTCGTGACCACCCTGACCTACGGCGTGACGTGCTTCAGCCGCTACCCCGACCAC
 ATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGA
 CGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAA
 GGGCATCGACTTCAAGGAGGACGGCAACATCTGGGGACAAGCTGGAGTACAACACTACAACAGCCACAACGTC
 TATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAGTTCAAGATCCGCCACAACATCGAGGACGGCAG
 CGTGACGCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCCACAACCA
 CTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC
 GTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGCCGACACAGTGG
 TCGAAATTCGTGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATACATGTTTAAGGGTTCCGGT
 TCCACTAGGTACAATTGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGG
 TATTCTTAATATGTTGCTCCTTTACGCTATGTGGATACGCTGCTTAATGCCTTTGTATCATGCTATTGCTTCCC
 GTATGGCTTTTCAATTTCTCCTCTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCGTTGTGAG
 GCAACGTGGCGTGGTGTGCACTGTGTTTGTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTGAG
 CTCTTTCCGGGACTTTGCTTTCCCTCTCCTATTGCCACGGCGGAACCTCATCGCCGCTGCTTGGCCGCTG
 CTGGACAGGGGCTCGGCTGTTGGGCACTGACAATCCGTGGTGTGTCGGGGAAATCATCGTCCTTTCTTGGC
 TGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCG
 GACCTTCTTCCCGCGGCTGCTGCGGGCTCTGCGGCTCTTCCGCGTCTTCGCTTCGCCCTCAGACGAGTC
 GGATCTCCCTTTGGGCCGCTCCCCGCATCGATACCGTCGACCTCGATCGAGACCTAGAAAAACATGGAGCAAT
 CACAAGTAGCAATACAGCAGCTACCAATGCTGATTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGT
 TTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAG
 AAAAGGGGGGACTGGAAGGGCTAATCACTCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA

Fig. 13
(continued)

26/26

CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTG
CTACAAGCTAGTACCAAGTTGAGCAAGAGAAGGTAGAAGAAGCCAATGAAGGAGAGAAACACCCGCTTGTTACACC
CTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTATTAGAGTGGAGGTTTGACAGCCGCCTAGCATT
TCATCACATGGCCCCGAGAGCTGCATCCGGACTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC
TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCC
GTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCCTTTAGTCAGTGTGGAAAATCTCTAGCAGCATGT
GAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC
CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAAAGATACCAGG
CGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTT
CTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTC
CAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGT
CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT
AGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG
CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGC
GGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTA
CGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCA
CCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTC
GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCT
CACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTT
ATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCAAGTTAAGTTTGCGCAA
CGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA
ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT
CAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATC
CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTG
CTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAAC
GTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCC
AACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAA
AAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGG
GTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCC
CCGAAAAGTGCCACCTGAC

Fig. 13
(continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/053514

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N5/0783 C07K14/725 C07K14/735 C07K14/705 A61K35/17
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/097231 A2 (CELLECTIS [FR]) 23 June 2016 (2016-06-23) -----	3-8
X	US 2015/004176 A1 (GARCIA SANTANA CARLOS A [US]) 1 January 2015 (2015-01-01) claims 13-14 paragraph [0051] paragraph [0204] -----	9-11
X	KULEMZIN S V ET AL: "FCRL6 receptor: Expression and associated proteins", IMMUNOLOGY LETTERS, ELSEVIER BV, NL, vol. 134, no. 2, 30 January 2011 (2011-01-30), pages 174-182, XP027566468, ISSN: 0165-2478 [retrieved on 2010-12-20] page 178 ----- -/-	9-11

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

5 March 2020

Date of mailing of the international search report

19/03/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Paresce, Donata

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/053514

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/103905 A2 (US GOV HEALTH & HUMAN SERV [US]; PASTAN IRA [US] ET AL.) 28 August 2008 (2008-08-28) page 62 -----	9-11
A	WO 2016/193696 A1 (UCL BUSINESS PLC [GB]) 8 December 2016 (2016-12-08) page 1 - page 3 -----	1-11
A	WO 2016/174407 A1 (UCL BUSINESS PLC [GB]) 3 November 2016 (2016-11-03) page 10 -----	1-11
A,P	WO 2019/068007 A1 (IMPACT BIO LTD [IL]; GROSS GIDEON [IL] ET AL.) 4 April 2019 (2019-04-04) -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2019/053514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016097231 A2	23-06-2016	AU 2015367317 A1 CA 2969384 A1 EP 3233095 A2 JP 2018504104 A US 2017296623 A1 WO 2016097231 A2	01-06-2017 23-06-2016 25-10-2017 15-02-2018 19-10-2017 23-06-2016
US 2015004176 A1	01-01-2015	AU 2012279225 A1 AU 2017204850 A1 AU 2019210584 A1 BR 112013033988 A2 CA 2840424 A1 CN 103782173 A EP 2726879 A2 JP 6173310 B2 JP 2014528697 A KR 20140061374 A US 2015004176 A1 US 2018259522 A1 WO 2013006474 A2	16-01-2014 03-08-2017 22-08-2019 14-02-2017 10-01-2013 07-05-2014 07-05-2014 02-08-2017 30-10-2014 21-05-2014 01-01-2015 13-09-2018 10-01-2013
WO 2008103905 A2	28-08-2008	NONE	
WO 2016193696 A1	08-12-2016	AU 2016272457 A1 BR 112017023190 A2 CA 2986956 A1 CL 2017003057 A1 CN 107667170 A EP 3303568 A1 JP 2018517410 A KR 20180012762 A RU 2017145074 A US 2019023761 A1 WO 2016193696 A1 ZA 201707334 B	14-12-2017 17-07-2018 08-12-2016 16-03-2018 06-02-2018 11-04-2018 05-07-2018 06-02-2018 09-07-2019 24-01-2019 08-12-2016 26-02-2020
WO 2016174407 A1	03-11-2016	EP 3288583 A1 US 2018111993 A1 WO 2016174407 A1	07-03-2018 26-04-2018 03-11-2016
WO 2019068007 A1	04-04-2019	NONE	