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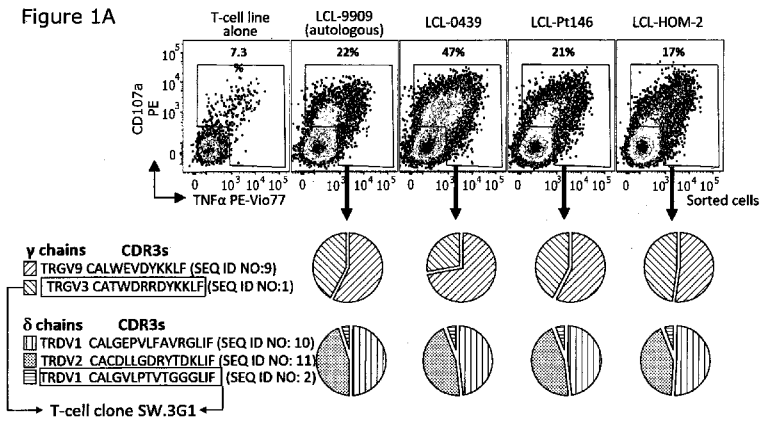
(54) Title: NOVEL GAMMA DELTA T-CELL RECEPTOR AND ITS LIGAND

Figure 1A



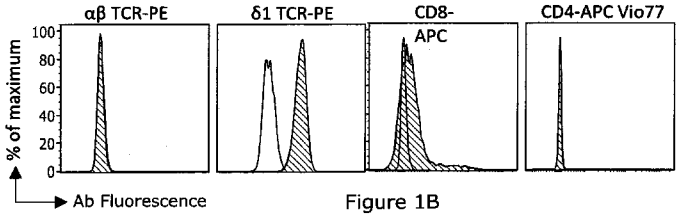


Figure 1B

(57) Abstract: The present disclosure relates to a new T-cell receptor (TCR), in particular at least one complementarity-determining region (CDR) thereof; a T-cell expressing said TCR; a clone expressing said TCR; a vector encoding said TCR; a soluble version of said TCR; a pharmaceutical composition or immunogenic agent or bispecific or vaccine comprising said TCR, said cell, said clone or said vector; said TCR or said cell or said clone or said vector or said pharmaceutical composition or immunogenic agent or bispecific or vaccine for use in the treatment of cancer; a method of treating cancer using said TCR, said cell, said clone, said vector, said pharmaceutical composition, immunogenic agent, bispecific or vaccine comprising said TCR; and a ligand with which said TCR binds.

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NOVEL GAMMA DELTA T-CELL RECEPTOR AND ITS LIGAND

Technical field

The present disclosure relates to a new T-cell receptor (TCR), in particular at least one complementarity-determining region (CDR) thereof; a T-cell expressing said TCR; a clone expressing said TCR; a vector encoding said TCR; a soluble version of said TCR; a pharmaceutical composition or immunogenic agent or bispecific or vaccine comprising said TCR, said cell, said clone or said vector; use of said TCR or said cell or said clone or said vector or said pharmaceutical composition or immunogenic agent or bispecific or vaccine to treat cancer; a method of treating cancer using said TCR, said cell, said clone, said vector, said pharmaceutical composition, immunogenic agent, bispecific or vaccine comprising said TCR; and a ligand with which said TCR binds.

Background

We have discovered a new class of γδ T-cell effective for treating cancer, which require that the target cell expresses an intact SCNN1A gene for recognition. This T-cell does not follow the convention of requiring a specific Human Leukocyte Antigen (HLA) for target recognition and is therefore said to be 'unconventional'. The HLA locus is highly variable with over 17,000 different alleles having been described today. As such, any therapeutic approach that works via an HLA can only be effective in a minority of patients. In contrast, the entire human population expresses SCNN1A, the gene required for recognition of cancer cells via our γδ TCR and its corresponding new T-cell clone, termed hereinafter SW.3G1. This clone was discovered during a screen for γδ T-cells that could recognize Lymphoblastoid Cell Lines (LCLs) created by infecting healthy B-cells with Epstein-Barr virus (EBV) also called human herpesvirus 4 (HHV-4). Advantageously, the SW.3G1 γδ T-cell clone does not respond to healthy B-cells or other healthy cell lines.

Further studies have shown that the SW.3G1 γδ T-cell clone can recognize most, if not all, cancer cells. The SCNN1A gene is required for this recognition and so is the binding ligand for the SW.3G1 TCR.

As is known, and as shown in Figure 2, the TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable gamma and delta chains that associate with the invariant CD3 chain molecules to form a complete functioning TCR. T cells expressing this receptor are referred to as γ:δ (or γδ) T cells.

The γ and δ chains are composed of extracellular domains comprising a Constant (C) region and a Variable (V) region. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the Variable region binds to the ligand. The ligand for most γδ T cells remains unknown.

The variable domain of both the TCR γ-chain and δ-chain each has three variable regions called complementarity determining regions (CDRs). In general, the antigen-binding site is formed by the CDR loops of the TCR γ-chain and δ-chain. CDR1γ and CDR2γ are encoded by the individual Vγ genes whereas CDR1δ and CDR2δ are encoded by the individual Vδ genes. The CDR3 of the TCR γ-chain is hypervariable due to the potential for nucleotide addition and removal around the joining of the V region and a Joining region. The TCR δ-chain CDR3 has even more capacity for variation as it can also include a diversity (D) gene after VDJ recombination has occurred.

In 2015 about 90.5 million people had cancer. About 14.1 million new cases occur a year (not including skin cancer other than melanoma). It causes about 8.8 million deaths (15.7%) of human deaths. The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. In females, the most common types of cancer are breast cancer, colorectal cancer, lung cancer and cervical cancer. If skin cancer, other than melanoma, were included in total new cancers each year it would account for around 40% of cases. In children, acute lymphoblastic leukaemia and brain tumours are most common except in Africa where non-Hodgkin lymphoma occurs more often. In 2012, about 165,000 children under 15 years of age were diagnosed with cancer. The risk of cancer increases significantly with age and many cancers occur more commonly in developed countries. Rates are increasing as more people live to an old age and as lifestyle changes occur in the developing world. The financial costs of cancer were estimated at $1.16 trillion USD per year as of 2010. It follows that there is a need to provide better and safer ways of treating or eradicating this disease. An immunotherapy

that uses the body's natural defence systems to kill aberrant tissue is acknowledged to be safer than chemical intervention but, to be effective, the immunotherapy must be cancer specific. Moreover, the discovery of an immunotherapy that is effective against any type of cancer would be extremely beneficial as not only could it be administered to individuals suffering from many different types of cancer (i.e. it would have pan-population application) but it could also be administered to a single individual suffering from more than one type of cancer. Additionally, the identification of an immunotherapy that was not MHC-restricted would also be extremely advantageous as it means it could be administered to any individual regardless of MHC tissue type.

The T-cells we have identified herein have the afore advantageous characteristics in that they are effective against any type of cancer and they are not MHC-restricted and so have pan-population application due to the ubiquitous expression of the SCNN1A gene product that is required for recognition.

Statements of invention

According to a first aspect of the invention there is provided a tumour specific T-cell receptor (TCR), or a fragment thereof, characterised by at least one complementarity-determining region (CDR) comprising or consisting of CATWDRRDYKKLF (SEQ ID NO: 1) and/or CALGVLPTVTGGGLIF (SEQ ID No: 2).

In a preferred embodiment of the invention said CDR comprises or consists of (CDR) CATWDRRDYKKLF (SEQ ID NO: 1) and/or CALGVLPTVTGGGLIF (SEQ ID No: 2) or a CDR that shares at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

The CDRs described herein represent the CDR3s of said TCR and so are the main CDRs responsible for recognizing processed antigen or ligand. The other CDRs (CDR1gamma, CDR2gamma, CDR1delta and CDR2delta) are encoded by the germline. Therefore, the invention further concerns a TCR also including one or more of these other CDRs i.e. CDR1gamma, CDR2gamma, CDR1delta and/or CDR2delta in combination with the said one or more CDR3 sequences.

Accordingly, in a preferred embodiment said TCR comprises or consists of one or more, including any combination, of the following complementarity-determining regions:

VTNTFY (CDR1γ) SEQ ID NO:3

YDVSTARD (CDR2γ) SEQ ID NO:4

TSWWSYY (CDR1δ) SEQ ID NO:5

QGS (CDR2δ) SEQ ID NO:6

Reference herein to a tumour specific TCR is to a TCR that specifically recognises a tumour cell or a tumour cell ligand, in the context of SCNN1A gene expression, and is activated by same but is not activated by a non-tumour cell or a non-tumour cell ligand.

In a preferred embodiment of the invention said TCR is an γδ TCR having a γ chain and a δ chain and said CDR of said γ chain comprises or consists of the CDR: CATWDRRDYKKLF (SEQ ID NO: 1) or a CDR that shares at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%; and said CDR of said δ chain comprises or consists of the CDR: CALGVLPTVTGGGLIF (SEQ ID No: 2) or a CDR that shares at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Accordingly, said TCR may comprise one or both of the afore CDRs and in a preferred embodiment comprises both of said CDRs.

In a further preferred embodiment of the invention said CDR of said TCR additionally or alternatively comprises or consists of a gamma chain sequence that is CALWEVDYKKLF (SEQ ID NO: 9) and/or a delta chain sequence that is CALGEPVLFAVRGLIF (SEQ ID NO: 10) and/or CACDLLGDRYTDKLIF (SEQ ID NO: 11) or a CDR that shares at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

In yet a further preferred embodiment said TCR is unconventional in that it is not MHC-restricted, rather it binds to a tumour specific ligand in the context of SCNN1A gene expression. The fact that these T-cells and their TCRs are not MHC-restricted means they

have pan-population therapy potential and so represent an extremely important new cancer therapy.

In a further preferred embodiment of the invention said TCR γ chain comprises or consists of:

SSNLEGRTKSVTRQTGSSAEITCDLT**VTNTFY**IHWYLHQEGKAPQRLLY**YDVSTA RD**VLESGLSPGKYYTHTPRRWSWILRLQNLIENDSGVYY**CATWDRRDYKKLF**G SGTTLVVTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWQE KKSNTILGSQEGNTMKTNDTYMKFSWLTVPEKSLDKEHRCIVRHENNKNGVDQ EIIFPPIKT (SEQ ID NO:7)

or a sequence that has at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

In a further preferred embodiment of the invention said TCR δ chain comprises or consists of:

AQKVTQAQSSVSMPVRKAVTLNCLYE**TSWWSYY**IFWYKQLPSKEMIFLIR**QGS**DEQN AKSGRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGVLPTVTGGGLIF**GKGTRVTVEP NSQPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDPAIVISPSGKYNAVK LGKYEDSNSVTCSVQHDNKTVHSTDFEVKTDST (SEQ ID NO:8) or a sequence that has at least 88% identity therewith, such as 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

(In the above paragraphs, the bold and underlined text represents the CDRs i.e. 1, 2, and 3 in that order).

In yet a further preferred embodiment of the invention said TCR comprises said afore TCR γ chain and said afore TCR δ chain.

In yet a further preferred embodiment, said TCR is a soluble TCR, or sTCR, and so lacks the transmembrane and, ideally also, intracellular domains.

In yet another preferred embodiment of the invention said TCR is part of a chimeric receptor having the functionality described herein. Ideally, said TCR is fused to an αβ TCR constant domain.

In the alternative, there is provided a fragment of said TCR such as a monomeric part thereof, ideally a single chain form of the TCR.

According to a further aspect of the invention there is provided a T-cell expressing said TCR of the invention, ideally, in either a soluble or membrane compatible form i.e. having a transmembrane region and intracellular region.

According to a yet further aspect of the invention there is provided a T-cell clone expressing said TCR of the invention, ideally, in either a soluble or membrane compatible form i.e. having a transmembrane region and intracellular region. Preferably said clone is a SW.3G1 clone as described herein.

According to a yet further aspect of the invention there is provided a vector encoding said TCR of the invention.

According to a yet further aspect of the invention there is provided a pharmaceutical composition or immunogenic agent or bispecific or vaccine comprising said TCR or cell or clone or vector of the invention.

In a preferred embodiment said pharmaceutical composition or immunogenic agent or bispecific or vaccine is used to treat any cancer, ideally colorectal cancer, lung, kidney, prostrate, bladder, cervical, melanoma (skin), bone, breast, blood cancer, brain, pancreas, testicle, ovary, head/neck, liver, bladder, thyroid, and uterine.

According to a yet further aspect of the invention there is provided the TCR or cell or clone or vector as herein described for use in the treatment of cancer.

According to a yet further aspect of the invention there is provided a method of treating cancer comprising administering said TCR or cell or clone or vector to an individual to be treated.

Ideally said cancer is of any type but in particular colorectal cancer, lung, kidney, prostrate, bladder, cervical, melanoma (skin), bone, breast, blood cancer, brain, pancreas, testicle, ovary, head/neck, liver, bladder, thyroid, and uterine.

In a preferred method of the invention said TCR, cell, clone or vector is administered in combination with an anti-tumour agent such as, but not limited to, a bispecific.

Reference herein to a bispecific is reference to a bispecific monoclonal antibody (BsMAb, BsAb) which is an artificial protein that can simultaneously bind to two different types of antigen.

Alternatively still, said TCR may form part of a Bispecific wherein said bispecific includes said TCR, for the purpose of binding to its ligand on a cancer cell, and also an immune cell activating component or ligand that binds and so activates an immune cell such as a Killer T-cell.

According to a yet further aspect of the invention there is provided the use of said TCR or cell or clone or vector in the manufacture of a medicament to treat cancer.

According to a yet further aspect of the invention there is provided a combination therapeutic for the treatment of cancer comprising:

a) said TCR or cell or clone or vector or immunogenic agent or bispecific or vaccine in combination with
b) a further cancer therapeutic agent.

According to a yet further aspect of the invention there is provided a TCR or polypeptide or bispecific or antibody, or a fragment of said antibody, that binds to at least one of the SCNNA1 gene product isoforms shown in Figure 9 and in particular the extracellular domain thereof.

In a preferred embodiment of the invention said polypeptide, antibody or fragment inhibits the activity of said SCNNA1 gene product and, in the instance of said antibody is most ideally monoclonal.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprises", or variations such as "comprises" or "comprising" is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

All references, including any patent or patent application, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. Further, no admission is made that any of the prior art constitutes part of the common general knowledge in the art.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking, the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including the accompanying claims and drawings). Thus, features, integers, characteristics, compounds or chemical moieties described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein, unless incompatible therewith.

Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

An embodiment of the present invention will now be described by way of example only with reference to the following wherein:

Figure 1 shows how a γδ T-cell line reactive to autologous and non-autologous lymphoblastoid cell lines (LCLs) was clonotyped and found to express a TCR comprised of the TRGV3 and TRDV1 genes with the CDR3s CATWDRRDYKKLF (SEQ ID NO: 1) and CALGVLPTVTGGGLIF (SEQ ID NO: 2), respectively. A clone was grown by limited dilution that expressed this TCR and named SW.3G1. (A) Purified γδ T-cells from a healthy donor, 9909, were primed (day 0) and re-stimulated (day 14) with a pool of LCLs from three donors (0439, pt146 and Hom-2). On day 28 the T-cell line was incubated with the LCLs used for priming and also autologous LCL-9909 for 4 h with activation assessed by inclusion of TAPI-0, anti-CD107a and anti-TNFα antibodies. The activated cells were sorted by flow cytometry and the T-cell receptors (TCRs) analysed by next generation sequencing. Pie charts depict the proportion of the displayed TCR chains and CDR3s (complementarity determining regions) that were present in the sorted cells. The percentage of activated cells for the flow cytometry plots is shown above each gate. T-cell clone SW.3G1 obtained from the lines expresses the highlighted TCR chains, with the full TCR sequence shown in Figure 2. (B) Clone SW.3G1 was phenotyped with the antibodies and confirmed to express a TCRδ1 chain. SW.3G1 did not express an αβTCR or CD8 or CD4 glycoproteins associated with recognition of conventional peptide-HLA antigens.

Figure 2 shows the T-cell receptor sequence of the γ and δ TCR chains of clone SW.3G1. The mRNA structures (top) show that for each chain CDR1 and CDR2 are encoded in the germline. CDR3 is the product of junctional diversity at V–J joins of T cell receptor (TCR)- chain and V–D–J joins in TCR- chain. CDR3 is consequently hypervariable. The colour code adopted for the CDR loops is maintained throughout the figure. The areas coloured in grey represent the constant and variable domains of the TCRs (not including the hypervariable CDR loops). The panel on the right shows the expected protein fold. TCRs adopt similar tertiary structures that position the complementarity-determining regions (CDR) loops at the membrane distal end of the molecules. Together the six CDR loops form the antigen binding site.

Figure 3 shows SW.3G1 can recognize and kill autologous and non-autologous LCLs, but not healthy cells of various tissue origins. (A) Co-incubation of SW.3G1 with LCLs for 4 h, with activation assessed by inclusion of TAPI-0, anti-CD107a and anti-TNFα antibodies. (B) 6.5 h chromium release cytotoxicity assay using the same LCLs as in (A). (C) Autologous healthy B-cells magnetically purified directly *ex-vivo* from donor 9909 were used in activations assays as in A, with LCL-9909 used as a positive control for activation. (D) Activation assays as in A, using LCL-9909 and the healthy cell lines, CIL-1 (non-pigmented ciliary epithelium) and Hep2 (hepatocyte). Percentage of gated cells is shown.

Figure 4 shows SW.3G1 mediated lysis of LCLs from multiple donors that share no common HLA and an array of cancer cell lines from different tissues. SW.3G1 was used in 6.5 h chromium release cytotoxicity assays. (A) A panel of lymphoblastic cell lines (LCLs) from 24 donors (named on the x-axis). Red bars for LCLs that were used to generate the T-cell lines from donor 9909 from which SW.3G1 was cloned. T-cell to LCL ratio of 1:1. (B) SW.3G1-mediated killing of panel of cancer cell lines (named on the x-axis) of different tissue origin (key) at a T-cell to cancer cell ratio of 10:1.

Figure 5 shows that SW.3G1 does not recognize target cells by known mechanisms. (A) γδ SW.3G1 clone was co-incubated for 4 h with HMB-PP, the lymphoblastic cell line (LCL)-9909 and phytohaemagluttinin (PHA). T-cells were also incubated alone. T-cell activation was assessed by inclusion of TAPI-0, anti-CD107a and anti-TNFα antibodies, with the percentage of activated cells shown above the gated cells. (B) Using the same activation assay as in (A), SW.3G1 was incubated with LCLs that had been pre-labelled with antibodies (Abs) that bind the proteins named on the x-axis. SW.3G1 was also incubated with the LCLs without Ab (no Ab control). The percentage of reactivity is shown graphically (y-axis). MICA/B (Major Histocompatibility Complex (MHC) Class-I related chain A/B) and EPCR (Endothelial protein C receptor). Anti-MHC class I and II Abs were also included.

Figure 6 shows the whole genome CRISPR/Cas9 approach used to identify candidate genes/proteins involved in target cell recognition by SW.3G1.

Figure 7 shows the results of the whole genome CRISPR/Cas9 approach that identified multiple candidate genes for target cell recognition by SW.3G1. (A) Autologous LCL-9909 and cancer cell line KBM7s were transduced with a whole genome CRISPR/Cas9 library. The libraries were put though several selections using the SW.3G1 T-cell clone to generate a target cell line that was resistant to lysis. The surviving target cells (post-selection) were tested alongside the pre-selected cell lines in activation assays with SW.3G1. Activation assessed by inclusion of TAPI-0, anti-CD107a and anti-TNFα antibodies. (B) Sequencing of the post-selection libraries revealed enriched guide RNAs corresponding to the genes shown in (C). (C) Candidate genes seen in both the LCL-9909 and KBM7 libraries, or seen only for LCL-9909 or KBM7s. Gene and (protein) names are shown with website links for further information.

Figure 8 shows information about the candidate gene/protein SCNN1A, which was identified by the whole genome CRISPR/cas9 library approach.

Figure 9 shows the canonical protein sequence of SCNN1A which aligned with five expressed splice variants. Isoform 1 is the canonical sequence (Isoform 1 UniProt P37088-1). Highlighted amino acids in BLACK: region used to generate the polyclonal Ab used in this study. RED: Sites of protein variants due to different amino acid residues to the ones shown. The amino acid residues of the protein variants and are not displayed here, but can be found at http://www.uniprot.org/uniprot/P37088. BLUE: amino acid differences between splice isoforms.

Figure 10 shows the results of experiments to validate the role of SCNN1A in target cell recognition by SW.3G1. (A) Schematic of the SCNN1A gene and protein, with guide RNA (gRNA) sites from the whole genome GeCKO library (number 1) and a different validation gRNA SCNN1A sequence we designed (number 2). Figure adapted from Chen 2014. (B) Long term killing assay using SW.3G1 with LCL .174 wild-type, GeCKO gRNA-1 and gRNA-2 knock-out LCL.174 cells. (C) Western blot analysis of the breast cancer cell line MDA-MB-231 that had received the gRNA-2 for SCNN1A. Wild-type cells used for comparison, with red arrow indicating the 76 kDa SCNN1A protein. (D) MDA-MB-231 cells from (C) and melanoma MM909.24, wild-type and SCNN1A knock-out (KO) cell lines

used in long term killing assays with SW.3G1. Cancer cells were used as lines and selected by puromycin treatment for those expressing the gRNAs, with no subsequent cell cloning.

Figure 11 shows that transfer of the TCR from SW.3G1 confers target cell reactivity to αβ T-cells from three healthy donors. (A) Purified CD8+ T-cells from three donors were co-transduced with the SW.3G1 T-cell receptor chains (marker and purification via rat(r)CD2) and a gRNA to render the recipient T-cells TCRβ chain negative (selected by puromyicn treatment) (Legut et al 2017). Purity of the cells was checked with rCD2 antibody (Ab) and anti-γδ TCR Ab. (B) The cells from one donor were tested in long-term killing assays (lower graph). LCL .174 cell lines were used: wildtype, SCNN1A knockout (using gRNA-1 and -2) and SCNN1A knock-in (KO cells that had received a codon optimized SCNN1A transgene) cells.

Detailed description

Methods and Materials

T-cell line generation and clonotyping

Peripheral blood mononuclear cells (PBMCs) were purified from the blood of a healthy donor (code 9909) by standard density gradient separation. The dominant population of γδ T-cells in peripheral blood express a Vγ9Vδ2 TCR and typically respond to antigens derived from bacteria. In order to enrich γδTCR+/Vδ2- T-cells thereby increasing the likelihood of finding cancer reactive T-cells, we modified a magnetic based purification protocol. The first adaptation was to stain the PBMCs with a PE conjugated anti-Vδ2 antibody (Ab) (clone B6, BioLegend, San Diego, CA). Next, γδ TCR+ T-cells were negatively enriched by positively removing γδ TCR- cells according the manufacturer's instructions (Miltheyi Biotec, Bergish Gladbach, Germany). The second adaptation involved adding anti-PE microbeads (Miltneyi Biotec) to the beads of the γδ TCR purification kit, thereby removing δ2+ cells at the same time as the γδ TCR- cells. The purified cells were co-incubated with irradiated (3000-3100 rad) LCLs from three donors that had been generated from PBMC by immortalizing B-cells with Epstein-Barr Virus (EBV). All LCLs were grown in R10 media (RPMI-1640, 10% heat-inactivated fetal calf

serum, 2 mM L-glutamine, 100 U/mL Penicillin and 100 µg/mL Streptomycin, all Life Technologies, Carlsbad, CA) as suspension cells. After 14 days the T-cells were restimulated with irradiated LCLs from the same donors. On day 28 the T-cells were harvested and used in activation assays to assess reactivity towards LCLs. T-cells (30,000) were incubated for 4 h in 96 U well plates with an equivalent number of LCLs. 30 mM of the TNFα Processing Inhibitor-0 (TAPI-0 from Sigma Aldrich) (Haney et al., 2011), anti-CD107a Ab (H4A2, Becton Dickinson (BD), Franklin Lakes, NJ) and anti-TNFα Ab (cA2, Miltenyi Biotec) were added to the assay media at the start of the assay, with the cells subsequently stained with the cell viability dye, Vivid (Life Technologies, 1:40 dilution in PBS then 2 µL per stain in 50 µL) and anti-CD3 antibody (Ab) (BW264/56, Miltenyi Biotec). Activated cells were sorted on a BD FACS Aria in to RLT Plus buffer (supplemented with 40 mM DTT) (Qiagen) ready for sequencing of the TCR chains. RNA was extracted using the RNEasy Micro kit (Qiagen, Hilden, Germany). cDNA was synthesized using the 5'/3' SMARTer kit (Clontech, Paris, France) according to the manufacturer's instructions. The SMARTer approach used a Murine Moloney Leukaemia Virus (MMLV) reverse transcriptase, a 3' oligo-dT primer and a 5' oligonucleotide to generate cDNA templates, which were flanked by a known, universal anchor sequence. PCRs were performed using anchor-specific forward primers and reverse primers of the constant regions of the γ or δ TCR chains. The final PCR products were gel purified and prepared for next generation sequencing (Donia et al., 2017).

Clone SW.3G1 procurement and phenotyping

T-cells were cloned directly from the T-cell line by limiting dilution (Theaker et al., 2016). After 4 weeks of culture, 50% of each clone by culture volume was harvested and used for the activation assays with LCLs as above. Prior to performing activation assays, T-cell clones were washed and incubated for 24 h in reduced serum media. Clones that exhibited reactivity towards the LCLs were grown to sufficient numbers for TCR sequencing (below). Clone SW.3G1 was stained with Abs for surface expression of CD3 (Miltenyi Biotec), CD8 (BW135/80, Miltenyi Biotec), CD4 (M-T466, Miltenyi Biotec), αβ TCR (BW242/412, Miltenyi Biotec) and TCR Vδ1 chain (REA173, Miltenyi Biotec).

Sequencing of the SW.3G1 TCR

As above for sequencing the T-cell lines with the purified PCR products after the final PCR being cloned into Zero-Blunt TOPO and transformed into One Shot Chemically Competent *E.coli* cells for standard sequencing (both from Life Technologies).

SW.3G1 recognized LCLs but not healthy cells

To confirm SW.3G1 reactivity towards LCLs, activation assays as above, and chromium release cytotoxicity assays were performed. Healthy B-cells were purified from donor 9909 using a PE conjugated anti-CD19 Ab (HIB19, Miltenyi Biotec) and positive capture with anti-PE microbeads (Miltenyi Biotec) and used immediately in assays. Other healthy cell lines and their proprietary culture media were obtained from Sciencell (Carlsbad, CA): CIL-1 (human non-pigmented ciliary epithelium) and Hep2 (human hepatocyte) were used in activation as above.

SW.3G1 killed all immortalized and cancer cell lines tested

LCLs and tumour cells were labelled with chromium 51 for cytotoxicity assays (Ekeruche-Makinde et al., 2012), with T-cell to target cell ratios of 1:1 (LCLs) or 10:1 (cancer cells). LCLs were maintained as above. Cancer cells lines (ATCC® reference for background and culture information) / tissue of origin: SiHa (HTB-35) and MS751 (HTB-34) / cervical; MCF7 (HTB-22), MDA-MB-231 (CRM-HTB-26) and SKBR3 (HTB-30) / breast; TK143 (CRL-8303) and U20S (HTB-96) / bone; HCT-116 (CCL-247) and Colo205 (CCL-222) / colon; Jurkat (TIB-152), K562 (CCL-243), THP-1 (TIB-202), U266 (TIB-196) and Molt-3 (CRL-1552) / blood; Caki-1 (HTB-46) / kidney; A549 (CCL-185) and H69 (HTB-119) / lung. MM909.11, MM909.12, MM909.15, MM909.46 and MM909.24 are skin melanomas obtained from cancer patients treated at the Center for Cancer Immune Therapy (CCIT, Herlev Hospital, Copenhagen, Denmark). The 'MM' cell lines and melanomas Mel 526 and Mel 624 were maintained as adherent cells in R10, passaged once weekly or when required, aiming for 20-80% confluence. Cells were detached from tissue culture flasks

by rinsing with D-PBS followed by incubation with D-PBS and 2 mM EDTA at 37 °C until detached.

SW.3G1 did not recognize target cells by known mechanisms

The Vγ9Vδ2 T-cell activator *(E)*-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) (Sigma Aldrich) was reconstituted in DMSO and added directly to assay wells. The following monoclonal Abs were used for blocking assays: anti-HLA, -B, -C (clone W6/32, Biolegend), anti-HLA-DR, -DP, -DQ (clone Tu39, Bioloegend), anti-EPCR (polyclonal, R&D systems), anti-MICA/MICB (clone 6D4, BioLegend) and anti-CD1d (clone 51.1, Miltenyi Biotech) were used at a final concentration of 10 µg/mL.

Gene trapping by whole genome CRISPR

A whole genome CRISPR/Cas9 library approach was used (**Figure 5** for an overview). Whole genome targeted LCL-9909 and KBM7s using the GeCKO v2 sub-libraries A and B (Adgene plasmid, #1000000048, kindly provided by Dr. Feng Zhang (Patel et al., 2017)) were used for selection by SW.3G1. Briefly, successfully transduced target cells selected with puromycin were co-incubated with SW.3G1 at a predefined ratio for 2-3 weeks in 96 U well plates. Activation assays (as above) were performed with pre- and post-selected target cells to confirm loss of SW.3G1 activity towards the selected cells. Genomic DNA from the target cells that had survived two rounds of selection with SW.3G1 was used for next generation sequencing to reveal inserted guide RNAs and candidate genes.

Confirming SCNN1A role in target cell recognition

Lentiviral particles were generated by calcium chloride transfection of HEK 293T cells and concentrated by ultracentrifugation prior to transduction of target cells using 8 µg/mL of polybrene and spinfection. gRNAs were cloned into the pLentiCRISPR v2 plasmid (kindly provided by Dr. Feng Zhang, Addgene plasmid 52961), which encodes the SpCas9

protein and a puromycin resistance marker gene (pac, puromycin N-acetyltransferase), and co-transfected with packaging and envelope plasmids pMD2.G and psPAX2 (all from Addgene). Full-length codon optimized SCNN1A transgene (Isoform 1, UniProt P37088-1) was cloned in to a 3rd generation lentiviral transfer vector pELNS (kindly provided by Dr. James Riley, University of Pennsylvania, PA). The pELNS vector contains rat CD2 (rCD2) gene for selection of cells using an anti-rCD2 PE Ab (OX-34, BioLegend). SCNN1A expression in target cells was assessed using the rabbit anti-SCNN1A polyclonal antibody (PA1-902A, ThermoFisher Scientific) for flow cytometry (data not sown) and western blot analysis according to the manufacturer's instructions.

Transduction of polyclonal T-cells with the SW.3G1 TCR confers target cell recognition

Codon optimized, full length TCR chains, separated by a self-cleaving 2A sequence, were synthesized (Genewiz) and cloned into the 3rd generation lentiviral transfer vector pELNS (kindly provided by Dr. James Riley, University of Pennsylvania, PA). The pELNS vector contains a rat CD2 (rCD2) marker gene separated from the TCR by another self-cleaving 2A sequence. Additionally, cells were co-transduced with a gRNA to ablate TCRβ chain expression in recipient cells by targeting both TCR-β constant domains (manuscript currently at *Blood* for publication). Lentiviral particles were generated by calcium chloride transfection of HEK293T cells. TCR transfer vectors were co-transfected with packaging and envelope plasmids pMD2.G, pRSV-Rev and pMDLg/pRRE. Lentiviral particles were concentrated by ultracentrifugation prior to transduction of CD8+ T-cells using 5 μg/ml of polybrene, with the CD8+ T-cells purified by magnetic separation (Miltenyi Biotec) from three healthy donors 24 h in advance and activated overnight with CD3/CD28 beads (Dynabeads, Life Technologies) at 3:1 bead:T-cell ratio. T-cells that had taken up the virus were selected by incubation with 2 μg/ml puromycin (TCRβ chain knock-out) and enriched with anti-rCD2 PE Ab (OX-34, BioLegend) followed by anti-PE magnetic beads (Miltenyi Biotec). 14 d post transduction T-cells were expanded with allogeneic feeders and PHA. TCR transduced cells were used in longterm killing assays whereby LCL.174 targets were plated in duplicate at the density of 50,000 cells/well in 96 U well plates.

SW.3G1 was added to the target and incubated for 7 days. Target cells were also plated without T-cells, to serve as a 100% survival control. Cells were harvested, washed with PBS, and stained with Vivid and anti-CD3 antibody (to exclude T-cells). As an internal control, CountBright™ Absolute Counting Beads (Life Technologies) were added to each well prior to harvesting/washing (approximately 10,000 beads/well). The samples were the acquired on FACS Canto II, and at least 1,000 bead events were acquired per sample. The survival of target cells was calculated according to the following formula:

Results

CLONE CHARACTERISATION

1. Purified γδ T-cells from a healthy donor (9909) primed and re-stimulated with a pool of three non-autologous lymphoblastoid cell lines (-0439, -pt146 and -HOM-2). Reactivity towards each of the cells lines was tested at day 28 (Figure 1). The T-cell line also recognized autologous LCL-9909 (Figure 1).

2. T-cells from the aforementioned line were flow cytometry sorted based on reactivity to each of the LCLs and their TCRs analysed by next generation sequencing (Figure 1). For the γ-chain sequencing, two unique CDR3s were present with variable chains TRGV9 and TRGV3. For the δ-chains, three CDR3s were present with variable chains TRDV1 and TRDV2.

3. T-cells clones procured from the donor 9909 T-cell line expressed a γ3δ1 TCR and CDR3s CATWDRRDYKKL and CALGVLPTVTGGGLIF for each respective chain (Figure 1 and Figure 2). All the clones that grew expressed the same TCR. This clone was named SW.3G1.

Ab staining of SW.3G1 confirmed expression of the Vδ1 chain, and αβ TCR-/CD8 low/CD4- (Figure 1B).

4. Activation assays using TNFα and CD107a as the readouts confirmed SW.3G1 reactivity towards autologous LCL-9909 and non-autologous LCL-0439 (Figure 3A). Donors 9909 and 0439 are completely HLA mismatched for both MHC class I and class II alleles, therefore SW.3G1 is recognizing target cells in an HLA-independent manner. SW.3G1 was also able to lyse LCL-9909 and -0439 and is therefore cytotoxic (Figure 3B). The recognition of the LCLs was dependent on the immortalization process when EBV infects a B-cell, as autologous healthy B-cells purified directly ex-vivo from 9909 did not act as targets for SW.3G1 (Figure 3C). Similarly, the healthy cells CIL-1 (epithelial cell) and Hep2 (hepatocyte) did not elicit SW.3G1 activation (Figure 3D).

5. SW.3G1 was able to lyse LCLs from all 24 donors tested (Figure 3B for the autologous LCL and Figure 4A for 23 non-autologous LCLs) providing further confirmation that SW.3G1 is acting in a HLA independent manner. Furthermore, LCL .174 (Figure 4A, 4th bar from the left) only expresses one copy of chromosome 6, the human chromosome that carries the MHC locus. The chromosome 6 in cell LCL .174 contains a large deletion and does not carry genes for MHC class II and many components involved in MHC class I antigen processing.

6. SW.3G1 killed 23 cancer cell lines that originate from 8 different tissues: skin/melanoma, kidney, colon, breast, blood/leukemia, lung, cervix and bone. (Figure 4B).

7. SW.3G1 did not respond to the known γδ T-cell antigen, HMB-PP (Figure 5A), which leads to recognition of pathogen infected cells by the Vγ9Vδ2 TCR T-cell subset in a similar manner to recognition of the self pyrophosphate . This pathway requires target cells to express Butyrophillin 3A1. SW.3G1 reactivity towards LCL-9909 (autologous) -0439 and –pt146 was not hindered by inclusion of blocking antibodies that bind known γδ T-cell ligands: Major Histocompatibility Complex (MHC) Class-I related chain A and B (MICA/MICB), EPCR Endothelial Protein C Receptor (EPCR) and CD1d (Figure 5B).

MHC class I and class II also failed to block SW.3G1 activation. Although not an extensive exclusion process, these data suggested that SW.3G1 might recognize an unknown γδ TCR ligand at the surface of cancer cells. Therefore, a whole genome CRISPR/Cas9 library approach was adopted to find candidate genes/proteins involved in SW.3G1 recognition of target cells (Figure 6).

8. Whole genome CRISPR/Cas9 libraries were used to create gene knockouts in autologous LCL-9909 and the haploid myeloid leukaemia cell line KBM7. Both libraries were co-incubated with SW.3G1 for successive rounds of selection to enrich for target cells containing gRNAs that allowed escape from SW.3G1-mediated lysis (Figure 7A). SW.3G1 reactivity dropped from 59% for pre-selected LCL-9909 to 12% post-selection. For KMB7s reactivity went from 12% to 4.2%. The post-selected LCL-9909s and KBM7s were used for next generation sequencing to identify gRNAs that had been enriched. Key genes were identified with 4 of the total 7 genes shared between the LCL-9909 and KBM7 libraries (Figures 7B and 7C). Guides specific for the gene SCNN1A (also used here to describe the encoded protein), which encodes for the protein Sodium Channel Epithelial 1 Alpha Subunit, were highly enriched present in both libraries (Figure 7B&C). SCNN1A gene and protein aliases are shown in Figure 8. The protein is cell surface expressed and therefore a good candidate for further exploration. SCNN1A has 6 splice variant isoforms and various naturally occurring mutations (Figures 8 and 9).

9. LCL.174 transduced with SCNN1A gRNA from the whole genome library (GeCKO, gRNA-1) or a different guide designed in-house (gRNA-2) (Figure 10A) were no longer targets of SW.3G1, thereby confirming SCNN1A's role in target cell recognition (Figure 10B), with lysis falling to below 5% for the knockout cell lines compared to 100% killing for the wildtype cells. SCNN1A gene knockout lines were created in two cancer cells, which either partially or completely escaped lysis by SW.3G1 (Figure 10C). It is noteworthy that the SCNN1A knockout cells created throughout this study were used as lines, and not cloned before performing assays. This may account for the residual reactivity seen for some of the 'knockout' cell lines as a minority proportion of the cells within a knockout line probably still express SCNN1A, due to escape from puromycin

selection and/or unsuccessful ablation of the SCNN1A gene. Western blot analysis of the SCNN1A knockout MDA-MB-231 cells used for SW.3G1 activation assays revealed a substantial reduction of the SCNN1A protein in the knockout cell line compared to the wildtype cells (Figure 10C) confirming the gene knockout. The Ab used can recognise all SCNN1A isoforms (Figure 9). We also noted that the SCNN1A knockout cells became less viable with extensive culture (3+ weeks) and in some cases cell division halted completely. This observation was unique to the SCNN1A gRNA as the same cell lines transduced with gRNAs for many other genes did not exhibit the same change in cell growth and vitality. This result suggested that the SCNN1A gene is essential for long-term growth of cells in culture.

10. Transfer of the SW.3G1 TCR in to polyclonal CD8+ T-cells from three healthy donors conferred reactivity to target cell LCL-pt146 (Figure 11A). TCR transduced cells exhibited the same functional profile to SCNN1A knockout cells as described above for the SW.3G1 clone (Figure 11B). To compliment the SCNN1A knockout data, and to further confirm the role of SCNN1A in target cell recognition, we transduced the knockout cells with the SCNN1A gene. The introduction of a native SCNN1A gene to knockout cells expressing the SCNN1A gRNAs would lead to gene ablation of the transgene. Therefore, a codon-optimized gene was introduced, different to the DNA sequence of the native gene (Isoform 1 UniProt P37088-1, Figure 9), but expressing the same protein. Killing of the gRNA-1 or gRNA-2 transduced cells was ablated but could be restored by expressing the SCNN1A gene in the knockout cells (Figure 11B).

Conclusion

The SW.3G1 TCR enables T-cells to recognise a wide range of tumours. Recognition occurs via the SCNN1A gene product. SW.3G1 T-cell clone recognises a cancer-cell specific SCNN1A ligand in the absence of MHC restriction.

This invention centres around the TCR identified in T-cell clone SW.3G1. This TCR recognises a wide range of cancer cells through the expression of SCNN1A. This TCR does not recognise non-tumour cells. CRISPR/Cas9 knockout of SCNN1A from tumour

lines or antibody blocking confirmed there TCR requires the SCNN1A gene product for recognition of tumour cells. The SW.3G1 TCR can be used in a variety of different cancer immunotherapy strategies. The broad tumour recognition and human leukocyte antigen (HLA)-independence of recognition unlocks exciting possibilities for pan-cancer, pan-population immunotherapies using this TCR.

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Claims

1. A tumour specific T-cell receptor (TCR), or a fragment thereof, characterised by at least one complementarity-determining region comprising or consisting of (CDR) CATWDRRDYKKLF (SEQ ID NO: 1) and/or CALGVLPTVTGGGLIF (SEQ ID No: 2) or a CDR that shares at least 88% identity with either CDR (i.e. SEQ ID NO: 1 or 2).
2. The tumour specific T-cell receptor (TCR) according to claim 1 wherein said wherein said TCR comprises both of the said CDRs.
3. The tumour specific T-cell receptor (TCR) according to claim 1 or claim 2 wherein said TCR comprises or consists of one or more, including any combination, of the following complementarity-determining regions:
   VTNTFY (CDR1γ) SEQ ID NO:3

   YDVSTARD (CDR2γ) SEQ ID NO:4

   TSWWSYY (CDR1δ) SEQ ID NO:5 or

   QGS (CDR2δ) SEQ ID NO:6.

4. The tumour specific T-cell receptor (TCR) according to any one of claims 1-3 wherein said TCR is a gamma chain comprising or consisting of:
   SSNLEGRTKSVTRQTGSSAEITCDLTVTNTFYIHWYLHQEGKAPQRLLYYDVST
   ARDVLESGLSPGKYYTHTPRRWSWILRLQNLIENDSGVYYCATWDRRDYKKLF
   GSGTTLVVTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWQ
   EKKSNTILGSQEGNTMKTNDTYMKFSWLTVPEKSLDKEHRCIVRHENNKNGVD
   QEIIFPPIKT (SEQ ID NO:7) or a gamma chain that shares at least 88% identity therewith.
5. The tumour specific T-cell receptor (TCR) according to any one of claims 1-3 wherein said TCR is a delta chain comprising or consisting of:
   AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGS
   DEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGVLPTVTGGGLIFG
   KGTRVTVEPNSQPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDP

AIVISPSGKYNAVKLGKYEDSNSVTCSVQHDNKTVHSTDFEVKTDST (SEQ ID NO:8) or a delta chain that shares at least 88% identity therewith.

6. The tumour specific T-cell receptor (TCR) according to any one of claims 1-5 wherein said CDR of said TCR additionally or alternatively comprises or consists of a gamma chain sequence that is CALWEVDYKKLF (SEQ ID NO: 9); and/or a delta chain sequence that is CALGEPVLFAVRGLIF (SEQ ID NO: 10) and/or CACDLLGDRYTDKLIF (SEQ ID NO: 11); or a CDR that shares at least 88% identity with said gamma chain sequence or said delta chain sequence.
7. The tumour specific T-cell receptor (TCR) according to any one of claims 1-6 wherein said TCR is soluble.
8. A T-cell expressing said TCR according to any one of claims 1-7.
9. A T-cell clone expressing said TCR according to any one of claims 1-7.
10. The T-cell clone according to claim 9 wherein said clone is a SW.3G1 clone.
11. A vector encoding the TCR according to anyone of claims 1-7.
12. A pharmaceutical composition or immunogenic agent or bispecific or vaccine comprising said TCR according to any one of claims 1-7 or said cell according to claim 8 or said clone according to claims 9 or 10 or said vector according to claim 11.
13. The TCR according to any one of claims 1-7 or the said cell according to claim 8 or the said clone according to claims 9 or 10 or the said vector according to claim 11 or the said pharmaceutical composition or immunogenic agent or bispecific or vaccine according to claim 12 for use in the treatment of cancer.
14. The TCR, the cell, the clone, the vector, the pharmaceutical composition or immunogenic agent or bispecific or vaccine according to claim 13 wherein said cancer is selected from the group comprising or consisting of colorectal cancer, lung, kidney, prostrate, bladder, cervical, melanoma (skin), bone, breast, blood cancer, brain, pancreas, testicle, ovary, head/neck, liver, bladder, thyroid, and uterine.
15. A method of treating cancer comprising administering the said TCR according to any one of claims 1-7 or the said cell according to claim 8 or the said clone according to claims 9 or 10 or the said vector according to claim 11 or the said

pharmaceutical composition or immunogenic agent or bispecific or vaccine according to claim 12 to an individual to be treated.

16. The method according to claim 15 wherein said cancer is selected from the group comprising or consisting of colorectal cancer, lung, kidney, prostrate, bladder, cervical, melanoma (skin), bone, breast, blood cancer, brain, pancreas, testicle, ovary, head/neck, liver, bladder, thyroid, and uterine.
17. The method according to claim 15 or 16 wherein said TCR, cell, clone, vector, pharmaceutical composition, immunogenic agent, bispecific or vaccine is administered in combination with an anti-tumour agent.
18. Use of said TCR according to claims 1-7 or the said cell according to claim 8 or the said clone according to claims 9 or 10 or the said vector according to claim 11 in the manufacture of a medicament to treat cancer.
19. A combination therapeutic for the treatment of cancer comprising:
    a) the said TCR according to claims 1-7 or the said cell according to claim 8 or the said clone according to claims 9 or 10 or the said vector according to claim 11 or the said pharmaceutical composition or immunogenic agent or bispecific or vaccine according to claim 12 in combination with;
    b) a further cancer therapeutic agent.
20. A TCR or polypeptide or bispecific or antibody, or a fragment of said antibody, that binds to at least one of the SCNNA1 gene product isoforms shown in Figure 9.
21. The TCR or polypeptide or bispecific or antibody, or a fragment of said antibody, according to claim 20 that binds to the extracellular domain of said isoform.
22. The TCR or polypeptide or bispecific or antibody, or a fragment of said antibody, according to claim 20 or claim 21 wherein said antibody is monoclonal.
23. At least one SCNNA1 gene product isoform shown in Figure 9, or a fragment thereof, that binds to a TCR according to any one of claims 1-7.
24. A TCR, cell, clone, vector, pharmaceutical composition, immunogenic agent, bispecific or vaccine or polypeptide or antibody, or a fragment of said antibody, as substantially here in described.

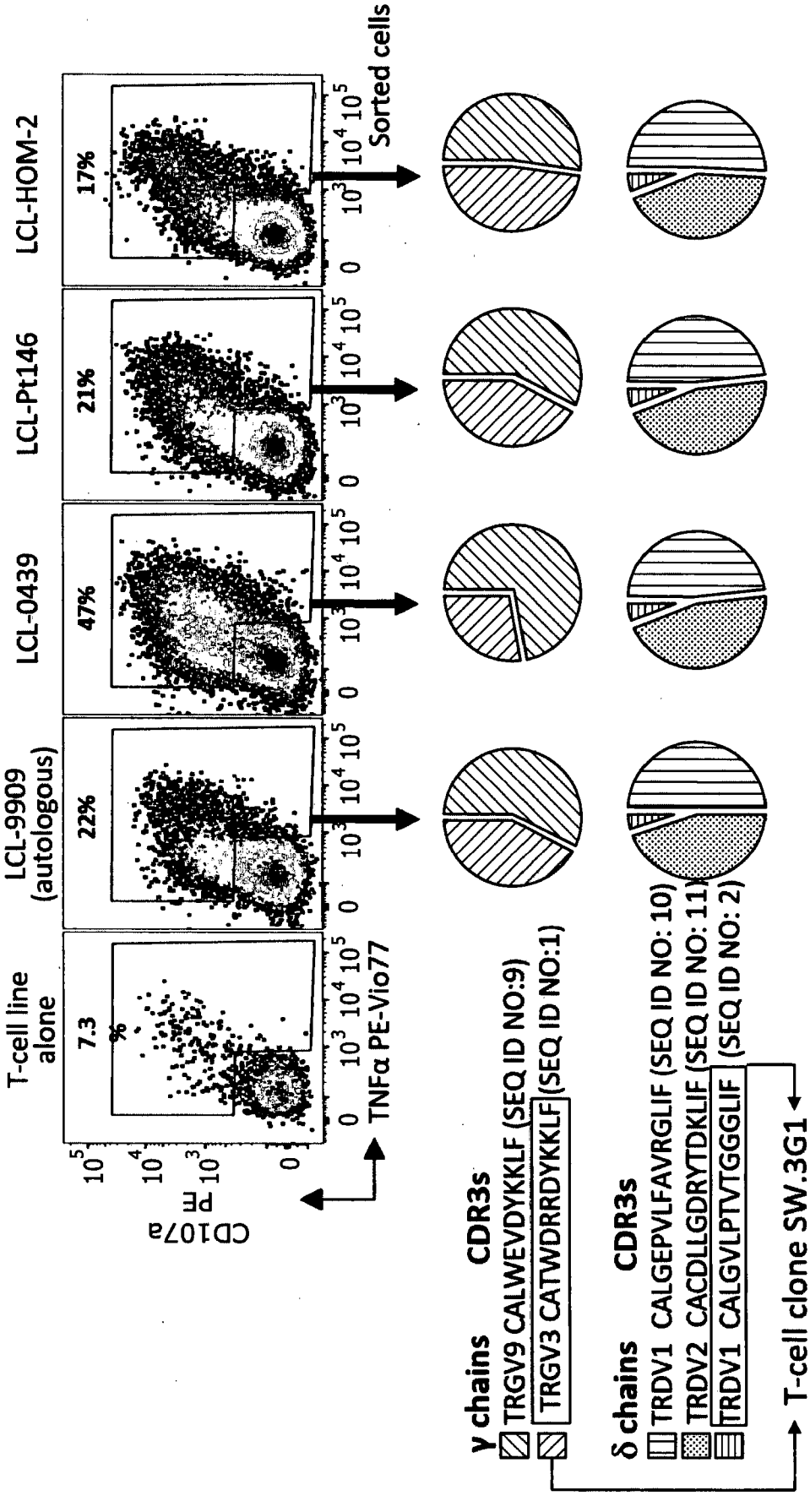
T-cell line alone
LCL-9909 (autologous)
LCL-0439
LCL-Pt146
LCL-HOM-2
7.3 %
22%
47%
21%
17%
CD107a PE
TNFα PE-Vio77
Sorted cells
γ chains CDR3s
TRGV9 CALWEVDYKKLF (SEQ ID NO:9)
TRGV3 CATWDRRDYKKLF (SEQ ID NO:1)
δ chains CDR3s
TRDV1 CALGEPVLFAVRGLIF (SEQ ID NO: 10)
TRDV2 CACDLLGDRYTDKLIF (SEQ ID NO: 11)
TRDV1 CALGVLPTVTGGGLIF (SEQ ID NO: 2)
T-cell clone SW.3G1

Figure 1A

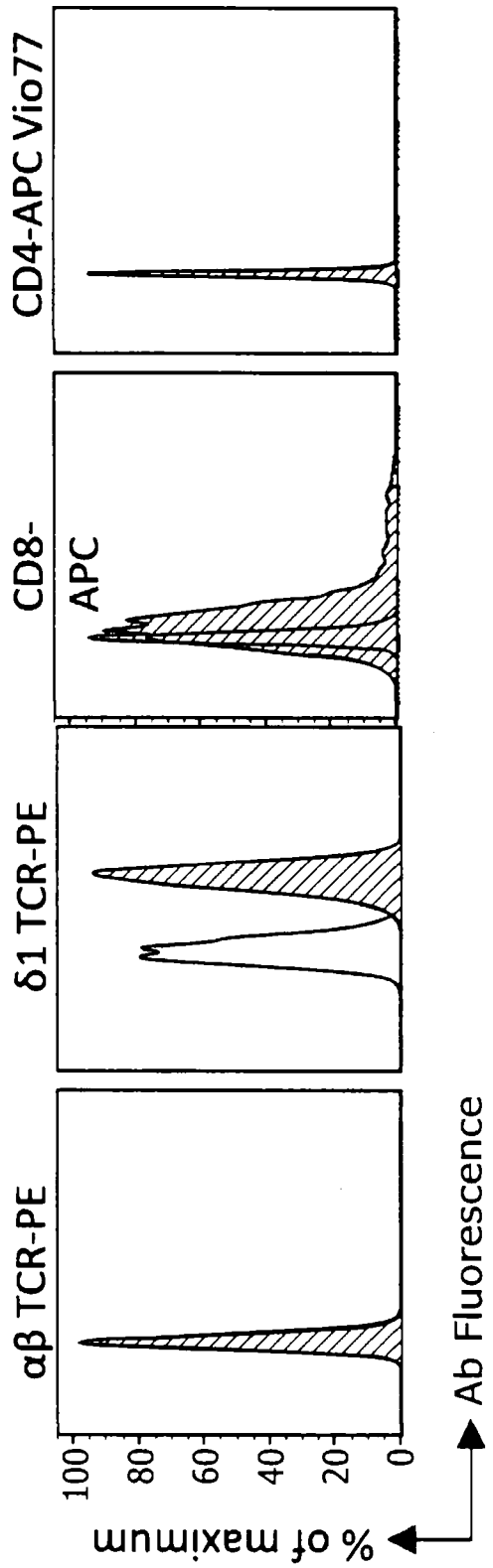
αβ TCR-PE
δ1 TCR-PE
CD8-APC
CD4-APC Vio77
% of maximum
100
80
60
40
20
0
Ab Fluorescence

Figure 1B

SW.3G1 clone Vγ3 chain protein sequence

CDR regions are shown in bold underlined text and colour coded throughout the figure

SSNLEGRTKSVTRQTGSSAEITCDLTVTNTFYIHWYLHQEGKAPQRLLYYDVSTARDVLESGLSPGKYYTHT
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GTYLCLLEKFFPDVIKIHWQEKKSNTILGSQEGNTMKTNDTYMKFSWLTVPEKSLDKEHRCIVRHENNKNG
VDQEIIFPPIKT (SEQ IN NO: 7)

3G1 clone Vδ1 chain protein sequence

CDR regions are shown in bold underlined text and colour coded throughout the figure

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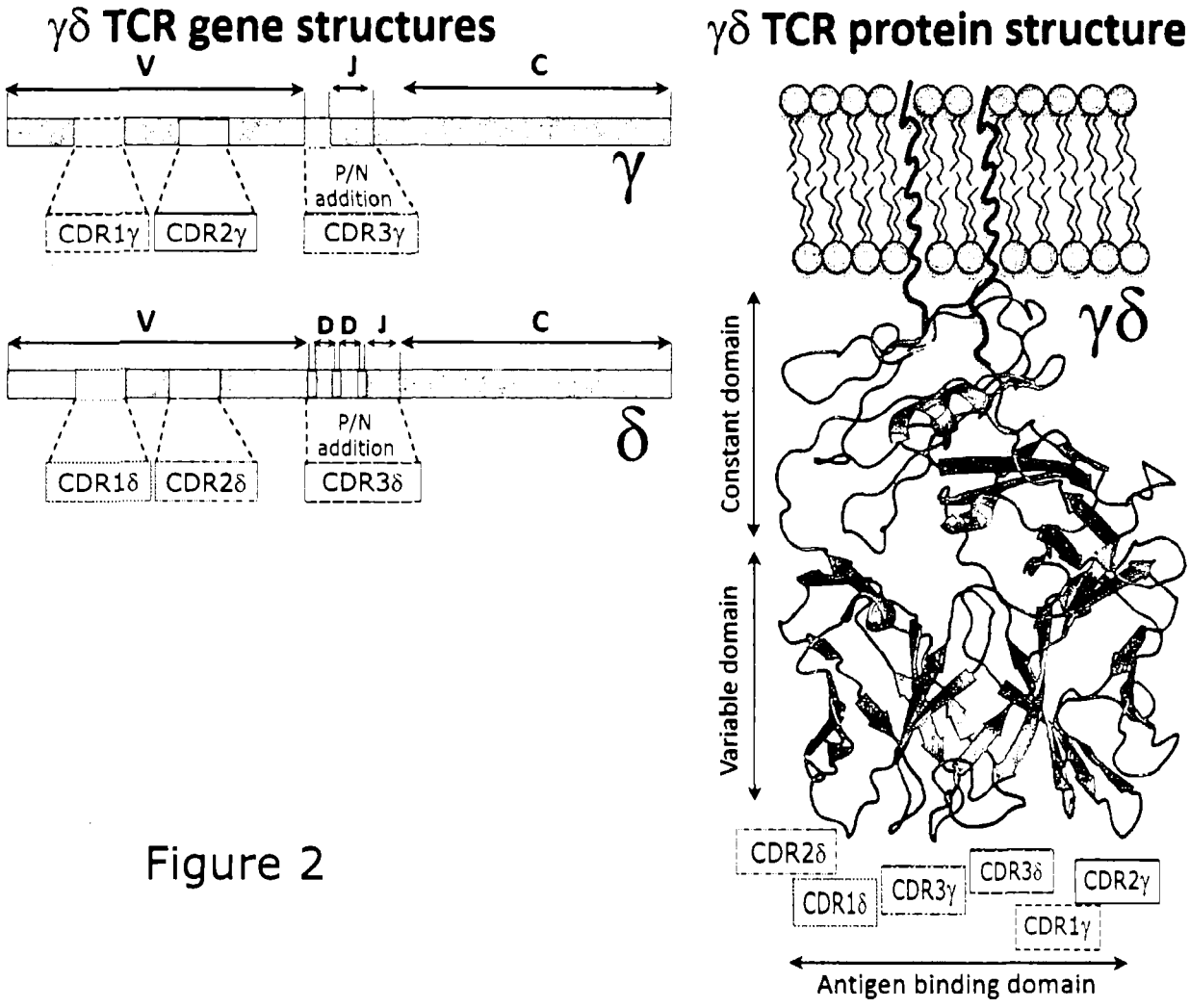


Figure 2

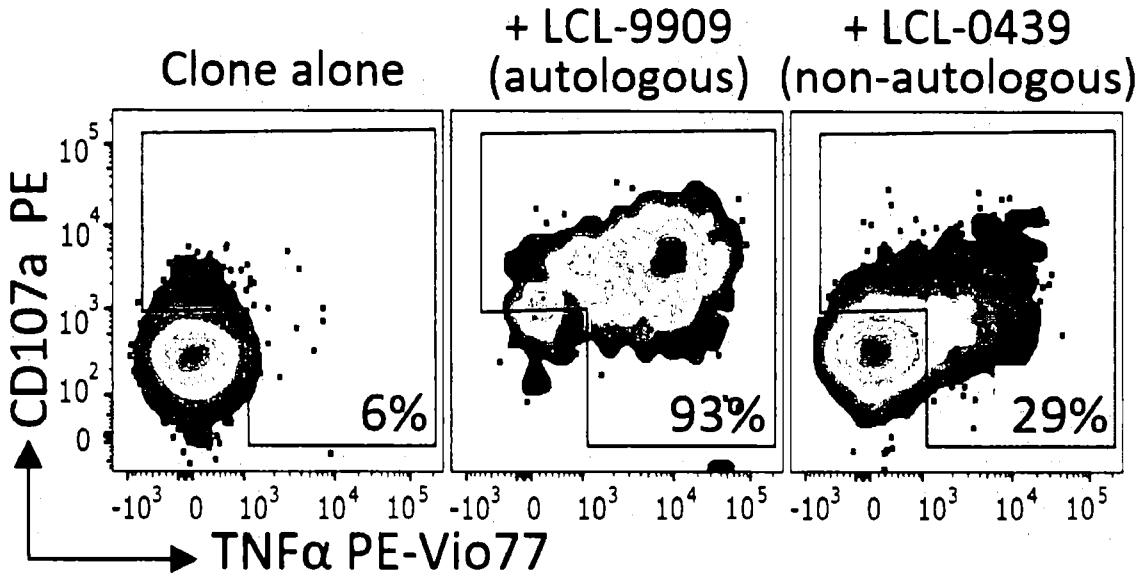


Figure 3A

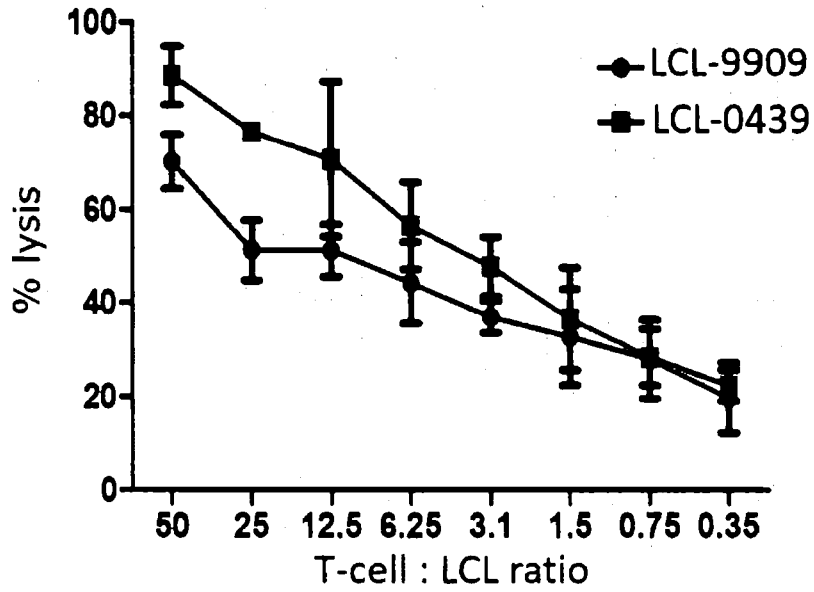


Figure 3B

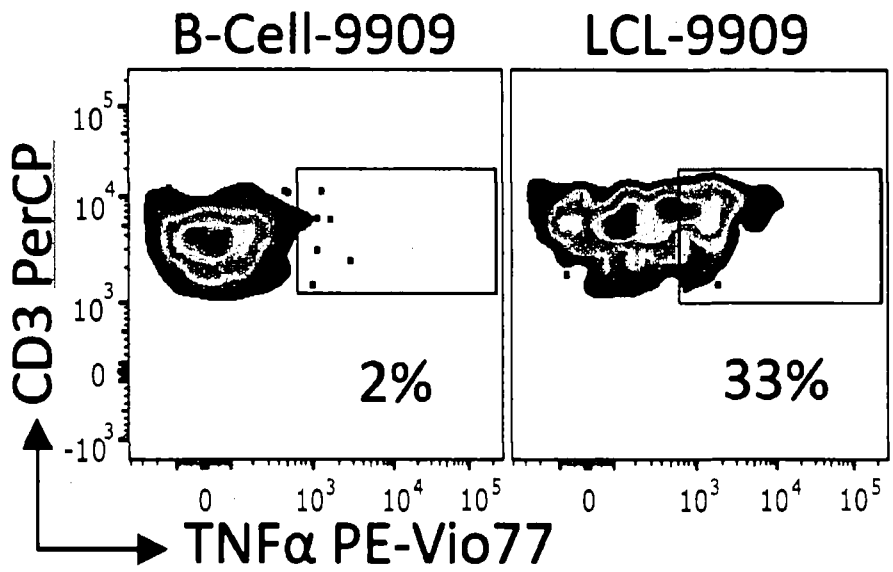


Figure 3C

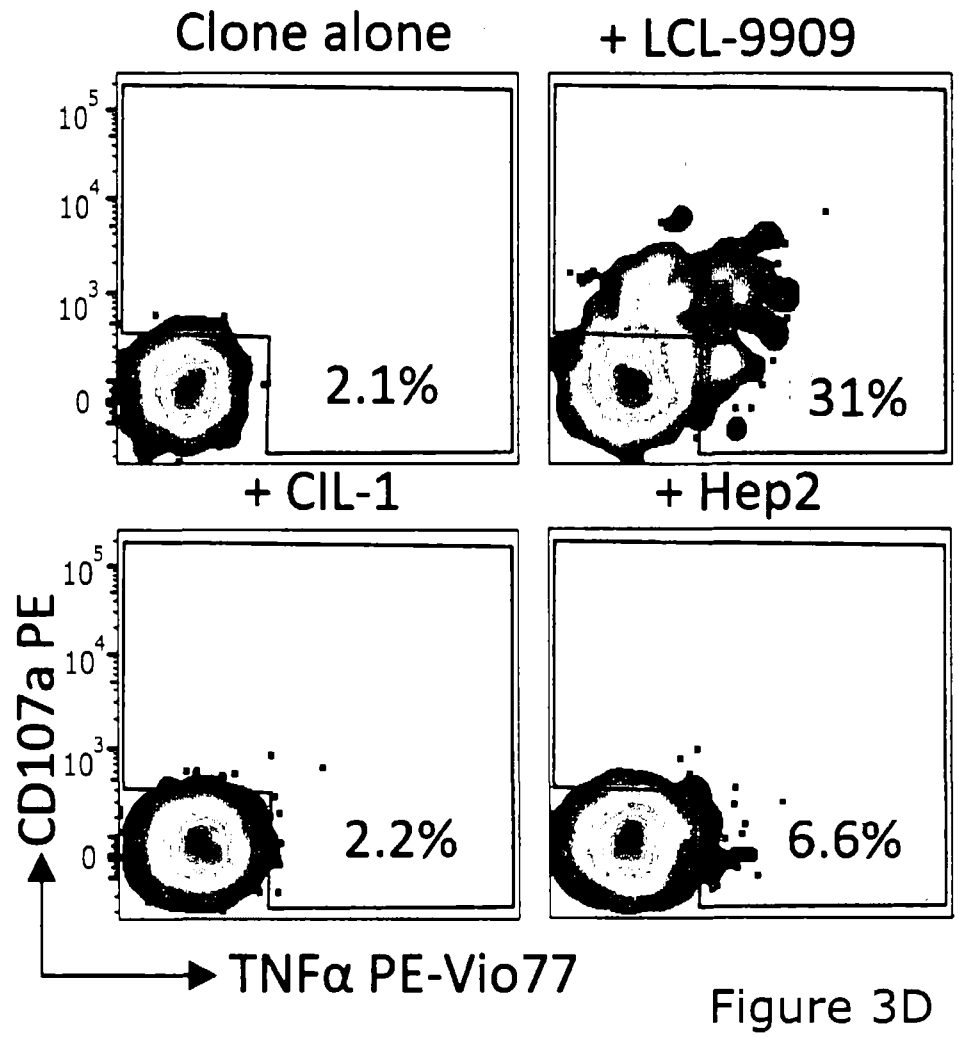


Figure 3D

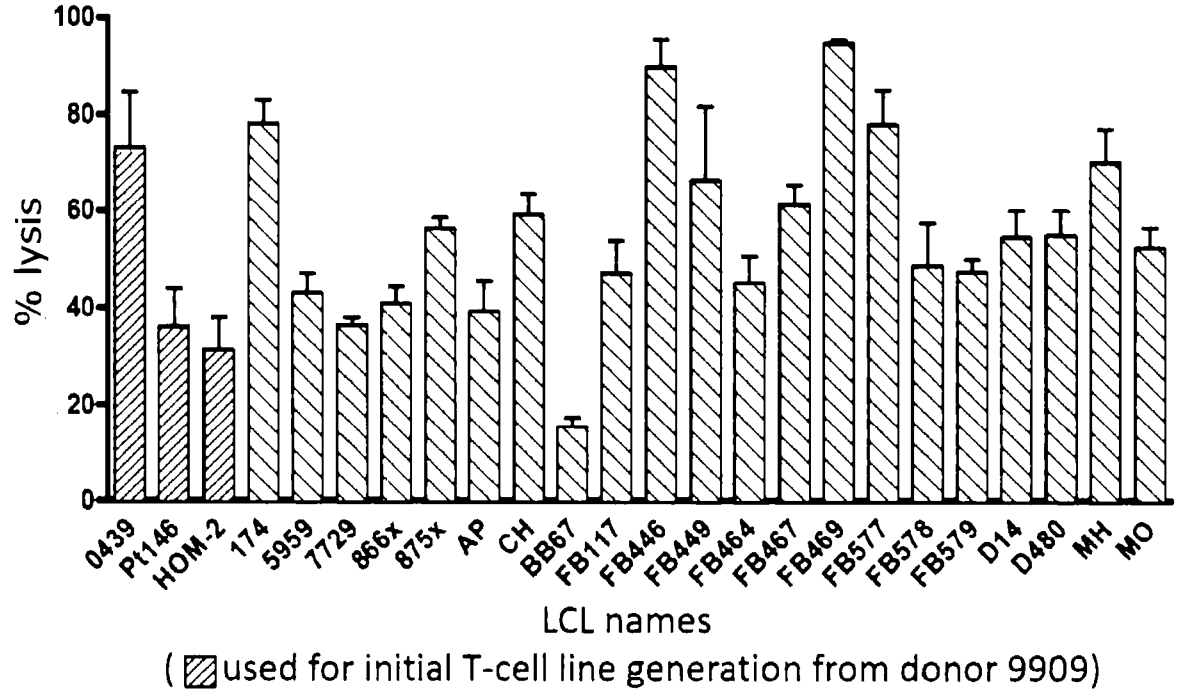


Figure 4A

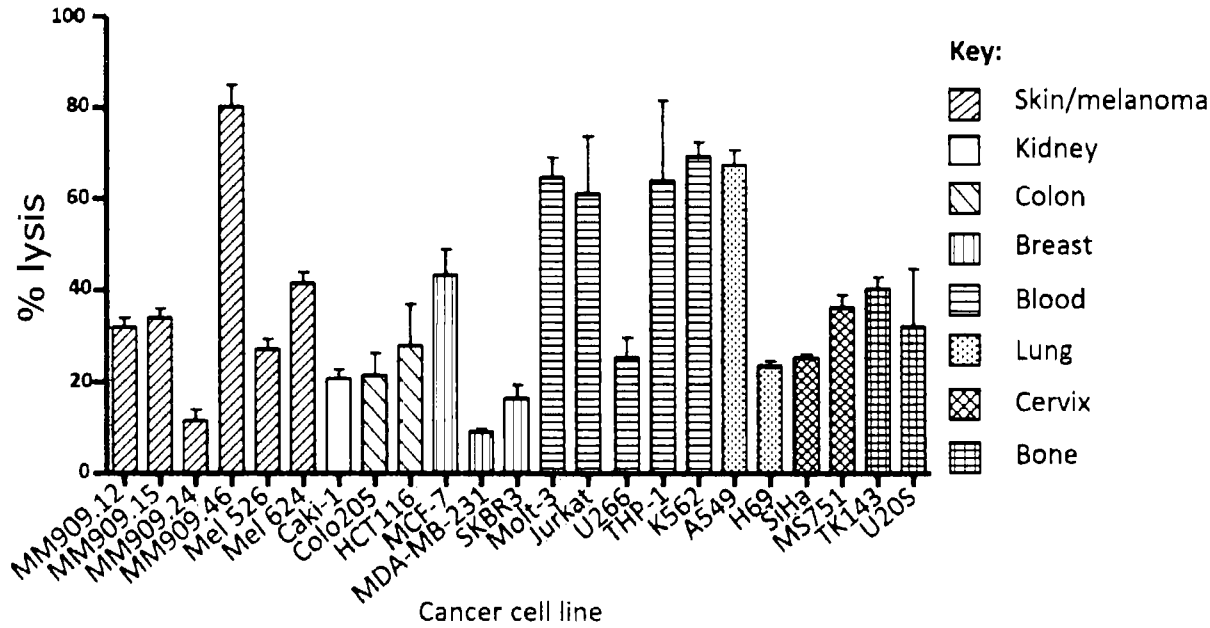


Figure 4B

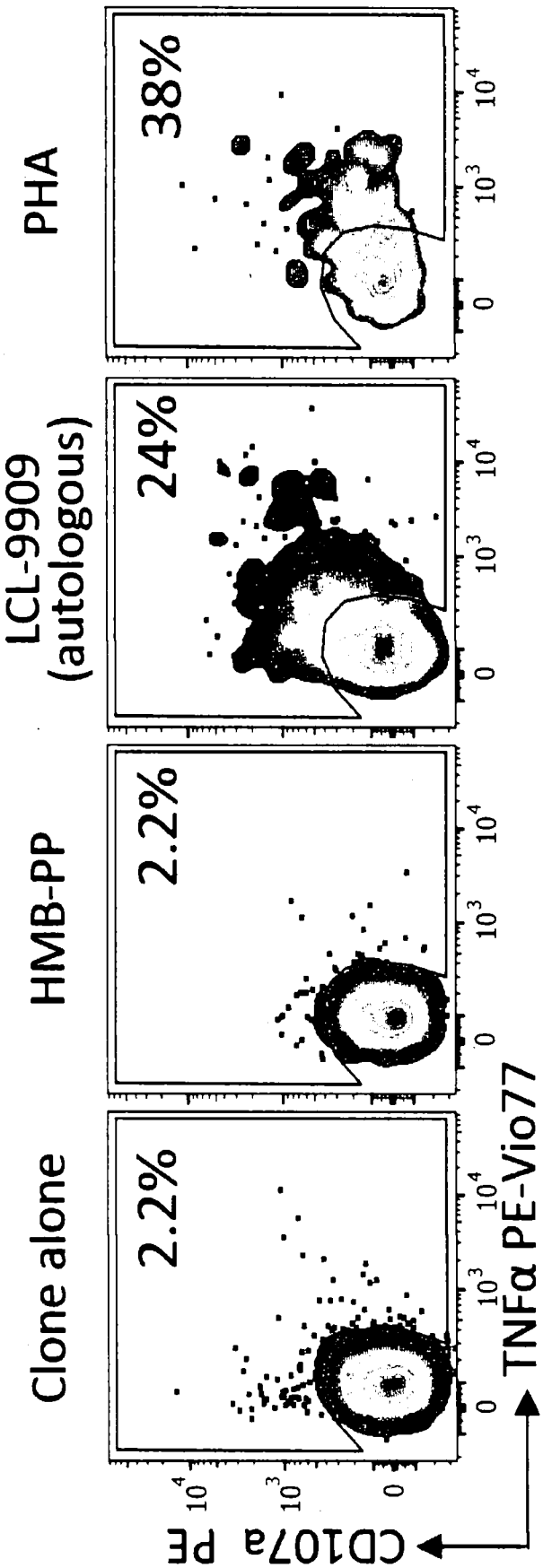

Clone alone
HMB-PP
LCL-9909 (autologous)
PHA
2.2%
2.2%
24%
38%
CD107a PE
TNFα PE-Vio77
0
10^3
10^4

Figure 5A

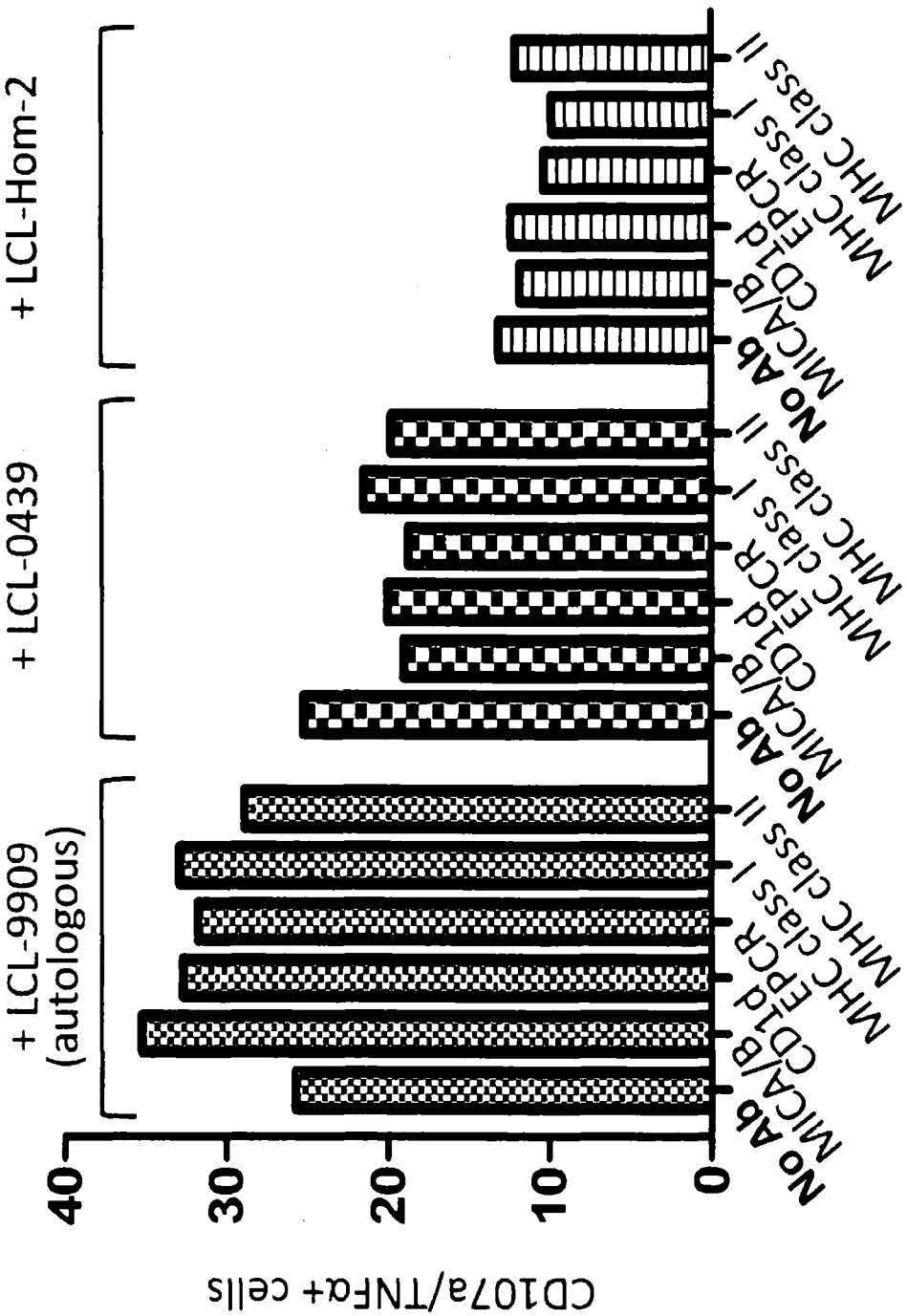

CD107a/TNFα+ cells
40
30
20
10
0
+ LCL-9909 (autologous)
+ LCL-0439
+ LCL-Hom-2
No Ab
MICA/B
CD1d
EPCR
MHC class I
MHC class II

Figure 5B

The Power of Whole Genome CRISPR
Whole genome CRISPR lentiviral libraries allow knockout of all genes in any cell line
Simple selection strategies then allow discovery of all genes connected to a given process
GENE TRAPPING METHODOLOGY
~5x10^7 of any human or mouse cell line
Transduce with lentiviral CRISPR library
1
Cell population knocked out for all genes
Killing assay or cell selection
2
3
Bar-coded PCR of CRISPR guide RNAs
4
NGS sequencing of guides from multiple experiments
5
Ranked gene list
6
Confirmation of phenotype by targeted CRISPR knockout with other guide RNAs
7
8
OUTPUTS
New targets
Intellectual Property
New Therapies
Web tool deconvolution
POTENTIAL SCREENS
Can be set up as live/die selection or to allow sorting of +ve cells by flow cytometry etc.
Gain of marker expression
Loss of marker expression
Loss of reporter expression
Gain of reporter expression
Drug Resistance or susceptibility
Resistance to pathogen (e.g. loss of entry receptor)
Permissive to pathogen (e.g. expresses entry receptor)
Resistance to immune attack (e.g. lack ligand)

Figure 6

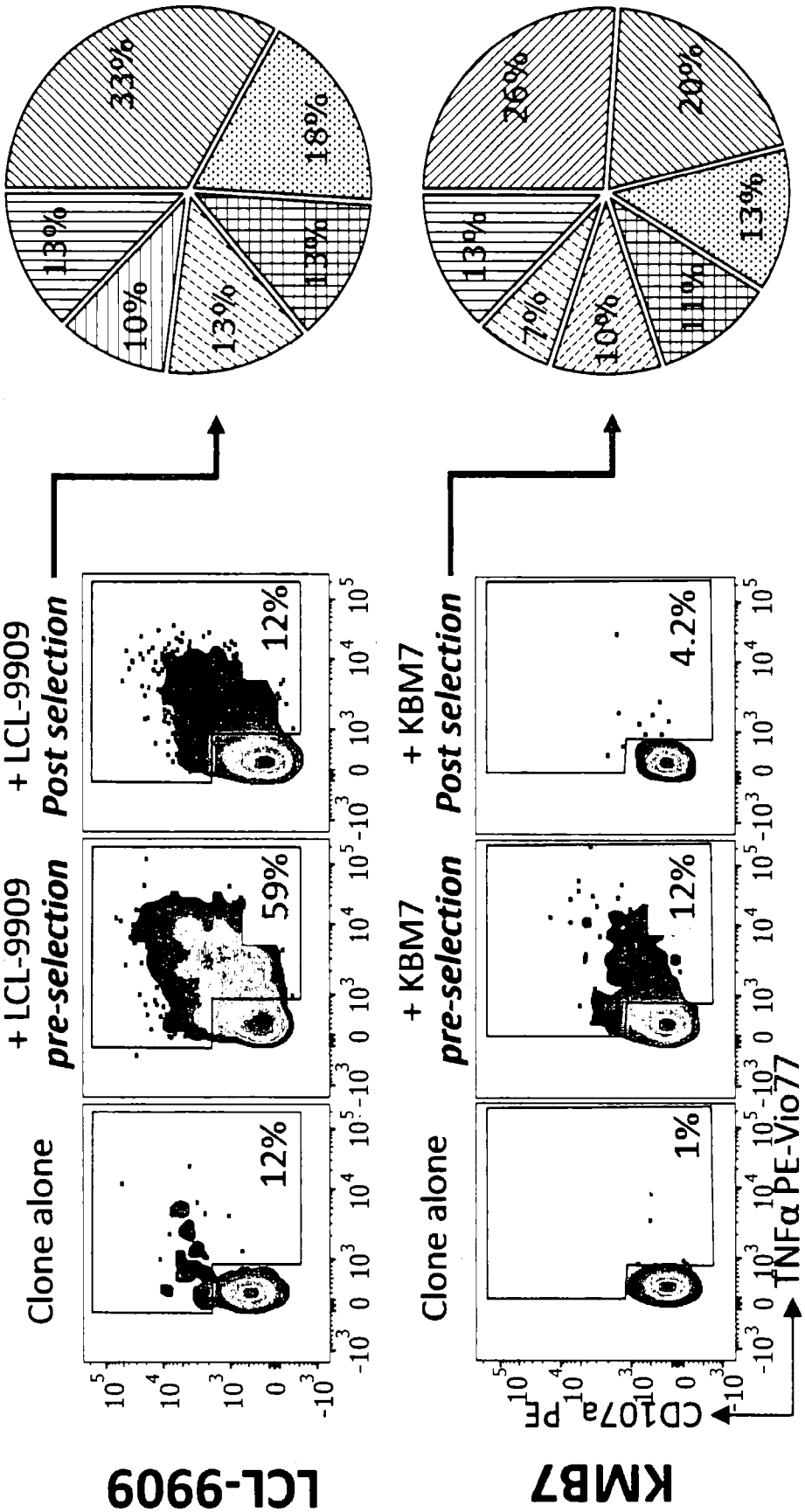


Figure 7A

Figure 7B

LCL-9909 and KMB7:

Gene: **SCNN1A** (Sodium Channel Epithelial 1 Alpha Subunit)
http://www.uniprot.org/uniprot/P37088

Gene: **DTNB** (Dystrobrevin Beta)
http://www.uniprot.org/uniprot/O60941

Gene: **NDUFV3** (NADH: Ubiquinone Oxidoreductase Subunit V3)
http://www.uniprot.org/uniprot/P56181

Gene: **LRRIQ3** (Leucine Rich Repeats and IQ Motif Containing 3)
http://www.uniprot.org/uniprot/A6PVS8

Other genes

LCL-9909:

Gene: **Homo sapiens (Hsa) MIR610** (microRNA 610)
http://www.genecards.org/cgi-bin/carddisp.pl?gene=MIR610

KBM7:

Gene: **ZYX** (Zyxin)
http://www.uniprot.org/uniprot/Q15942

Gene: **NSUN5** (Probable 28S rRNA (cytosine-C(5))-methyltransferase)
http://www.uniprot.org/uniprot/Q96P11

Figure 7C

Gene Aliases: SCNN1A; BESC2; ENaCa; ENaCalpha; SCNEA; SCNN1.

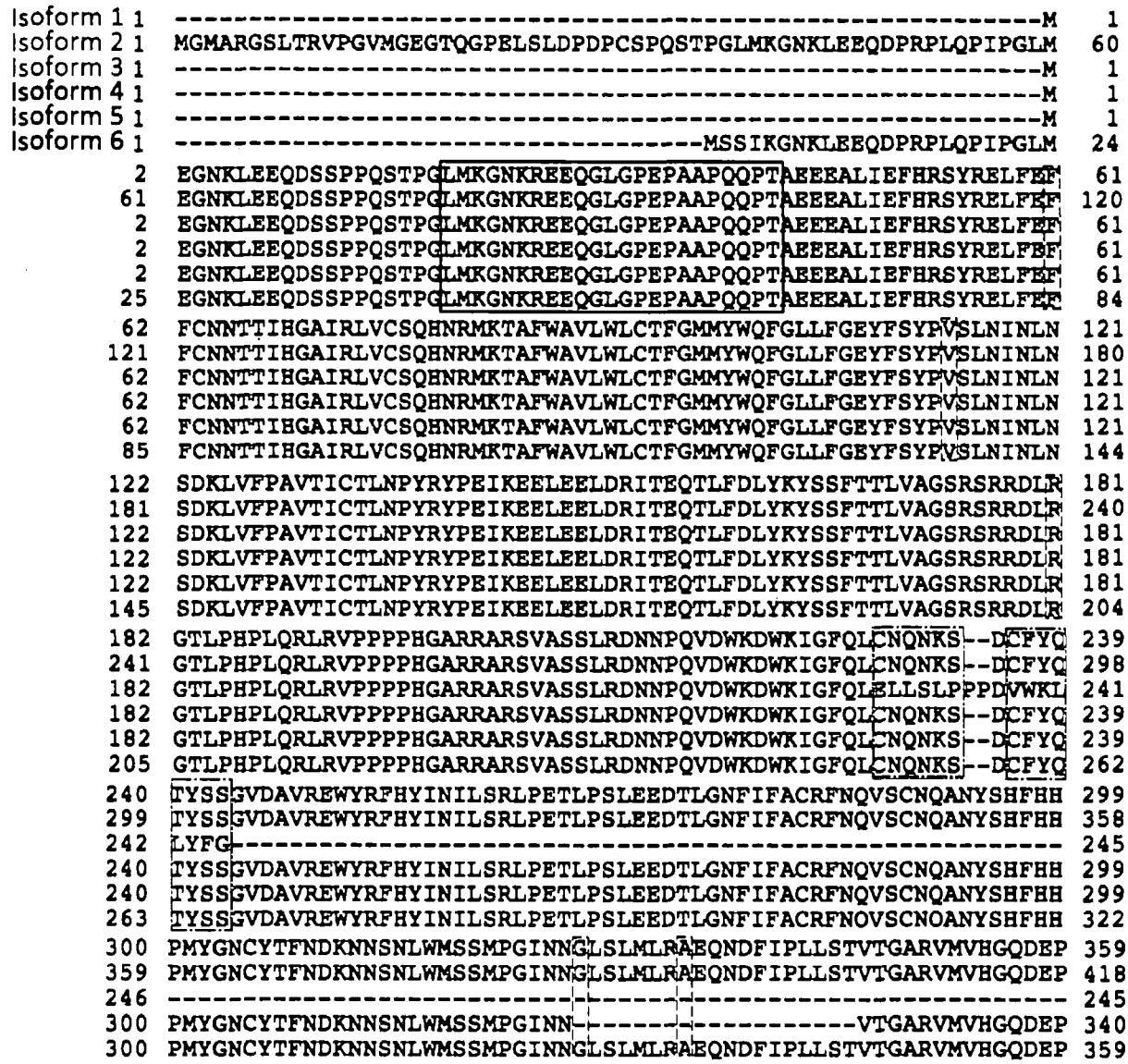
Protein Aliases: alpha ENaC-2; Alpha-ENaC; Alpha-NaCH; amiloride-sensitive epithelial sodium channel alpha subunit; Amiloride-sensitive sodium channel subunit alpha; amiloride-sensitive sodium channel subunit alpha 2; ENaCalpha; Epithelial a (+) channel subunit alpha; Epithelial Na(+) channel subunit alpha; FLJ21883; nasal epithelial sodium channel alpha subunit; Nonvoltage-gated sodium channel 1 subunit alpha; SCNEA; SCNN1; sodium channel, non voltage gated 1 alpha subunit; sodium channel, non-voltage-gated 1 alpha subunit; sodium channel, nonvoltage-gated 1 alpha.

Isoforms: Six splice variant isoforms described (**Figure 9**). Universal Protein Source (UniProt) identification: P37088-1, -2, -3, -4, -5 and -6. Isoform 1 is considered the canonical sequence and was ***used for this study***. Amino acid length and size (kDa) for each respective isoform: 669/75,704; 728/81.856; 245/28,328; 650/73,603; 691/77,980; 692/78,234. Natural variants have been described.

Antibodies: *PA5-29136*: Rabbit polyclonal anti-human against residues 272-555. *PA5-35364*: Rabbit polyclonal against residues 365-391. *PA1-920A*: Rabbit polyclonal anti-human (crossreacts with mouse and rat) against residues 20-42, and was ***used for this study***. *OSR00124W*: Rabbit polyclonal anti-human against the extracellular domain.

Homology with other species: 90% with other primates and 50% with mouse.

Figure 8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Isoform 2 1 MGMARGSLTRVPGVMGEGTQGPELSLDPDPCSPQSTPGLMKGNKLEEQDPRPLQPIPGLM 60
Isoform 3 1 ----------M 1
Isoform 4 1 ----------M 1
Isoform 5 1 ----------M 1
Isoform 6 1 ----------MSSIKGNKLEEQDPRPLQPIPGLM 24
2 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 61
61 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 120
2 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 61
2 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 61
2 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 61
25 EGNKLEEQDSSPPQSTPGLMKGNKREEQGLGPEPAAPQQPTAEEEALIEFHRSYRELFEF 84
62 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 121
121 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 180
62 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 121
62 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 121
62 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 121
85 FCNNTTIHGAIRLVCSQHNRMKTAFWAVLWLCTFGMMYWQFGLLFGEYFSYPVSLNINLN 144
122 SDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLYKYSSFTTLVAGSRSRRDLR 181
181 SDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLYKYSSFTTLVAGSRSRRDLR 240
122 SDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLYKYSSFTTLVAGSRSRRDLR 181
122 SDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLYKYSSFTTLVAGSRSRRDLR 181
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145 SDKLVFPAVTICTLNPYRYPEIKEELEELDRITEQTLFDLYKYSSFTTLVAGSRSRRDLR 204
182 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLCNQNKS--DCFYQ 239
241 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLCNQNKS--DCFYQ 298
182 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLELLSLPPPDVWKL 241
182 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLCNQNKS--DCFYQ 239
182 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLCNQNKS--DCFYQ 239
205 GTLPHPLQRLRVPPPPHGARRARSVASSLRDNNPQVDWKDWKIGFQLCNQNKS--DCFYQ 262
240 TYSSGVDAVREWYRFHYINILSRLPETLPSLEEDTLGNFIFACRFNQVSCNQANYSHFHH 299
299 TYSSGVDAVREWYRFHYINILSRLPETLPSLEEDTLGNFIFACRFNQVSCNQANYSHFHH 358
242 LYFG---------- 245
240 TYSSGVDAVREWYRFHYINILSRLPETLPSLEEDTLGNFIFACRFNQVSCNQANYSHFHH 299
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263 TYSSGVDAVREWYRFHYINILSRLPETLPSLEEDTLGNFIFACRFNQVSCNQANYSHFHH 322
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359 PMYGNCYTFNDKNNSNLWMSSMPGINNGLSLMLRAEQNDFIPLLSTVTGARVMVHGQDEP 418
246 ---------- 245
300 PMYGNCYTFNDKNNSNLWMSSMPGINN----------VTGARVMVHGQDEP 340
300 PMYGNCYTFNDKNNSNLWMSSMPGINNGLSLMLRAEQNDFIPLLSTVTGARVMVHGQDEP 359

Figure 9

```
323 PMYGNCYTFNDKNNSNLWMSSMPGINNGLSLMLRAEQNDFIPLLSTVTGARVMVHGQDEP 382
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419 AFMDDGGFNLRPGVETSISMRKETLDRLGGDYGDCTKNGSDVPVENLYPSKYTQQVCIHS 478
246 ------------------------------------------------------------ 245
341 AFMDDGGFNLRPGVETSISMRKETLDRLGGDYGDCTKNGSDVPVENLYPSKYTQQVCIHS 400
360 AFMDDGGFNLRPGVETSISMRKETLDRLGGDYGDCTKNGSDVPVENLYPSKYTQQVCIHS 419
383 AFMDDGGFNLRPGVETSISMRKETLDRLGGDYGDCTKNGSDVPVENLYPSKYTQQVCIHS 442
420 CFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWG----------------------YCY 457
479 CFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWG----------------------YCY 516
246 ------------------------------------------------------------ 245
401 CFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWG----------------------YCY 438
420 CFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWGQVRSLTPVIPALWEAEAGGSRGYCY 479
443 CFQESMIKECGCAYIFYPRPQNVEYCDYRKHSSWG----------------------YCY 480
458 YKLQVDFSSDHLGCFTKCRKPCSVTSYQLSAGYSRWPSVTSQEWVFQMLSRQNNYTVNNK 517
517 YKLQVDFSSDHLGCFTKCRKPCSVTSYQLSAGYSRWPSVTSQEWVFQMLSRQNNYTVNNK 576
246 ------------------------------------------------------------ 245
439 YKLQVDFSSDHLGCFTKCRKPCSVTSYQLSAGYSRWPSVTSQEWVFQMLSRQNNYTVNNK 498
480 YKLQVDFSSDHLGCFTKCRKPCSVTSYQLSAGYSRWPSVTSQEWVFQMLSRQNNYTVNNK 539
481 YKLQVDFSSDHLGCFTKCRKPCSVTSYQLSAGYSRWPSVTSQEWVFQMLSRQNNYTVNNK 540
518 RNGVAKVNIFFKELNYKTNSESPSVTMVTLLSNLGSQWSLWFGSSVLSVVEMAELVFDLL 577
577 RNGVAKVNIFFKELNYKTNSESPSVTMVTLLSNLGSQWSLWFGSSVLSVVEMAELVFDLL 636
246 ------------------------------------------------------------ 245
499 RNGVAKVNIFFKELNYKTNSESPSVTMVTLLSNLGSQWSLWFGSSVLSVVEMAELVFDLL 558
540 RNGVAKVNIFFKELNYKTNSESPSVTMVTLLSNLGSQWSLWFGSSVLSVVEMAELVFDLL 599
541 RNGVAKVNIFFKELNYKTNSESPSVTMVTLLSNLGSQWSLWFGSSVLSVVEMAELVFDLL 600
578 VIMFLMLLRRFRSRYWSPGRGGRGAQEVASTLASSPPSHFCPHPMSLSLSQPGPAPSPAL 637
637 VIMFLMLLRRFRSRYWSPGRGGRGAQEVASTLASSPPSHFCPHPMSLSLSQPGPAPSPAL 696
246 ------------------------------------------------------------ 245
559 VIMFLMLLRRFRSRYWSPGRGGRGAQEVASTLASSPPSHFCPHPMSLSLSQPGPAPSPAL 618
600 VIMFLMLLRRFRSRYWSPGRGGRGAQEVASTLASSPPSHFCPHPMSLSLSQPGPAPSPAL 659
601 VIMFLMLLRRFRSRYWSPGRGGRGAQEVASTLASSPPSHFCPHPMSLSLSQPGPAPSPAL 660
638 TAPPPAYATLGPRPSPGGSAGASSSTCPLGGP 669
697 TAPPPAYATLGPRPSPGGSAGASSSTCPLGGP 728
246 ------------------------------ 245
619 TAPPPAYATLGPRPSPGGSAGASSSTCPLGGP 650
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661 TAPPPAYATLGPRPSPGGSAGASSSTCPLGGP 692
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Figure 9 (Continued)

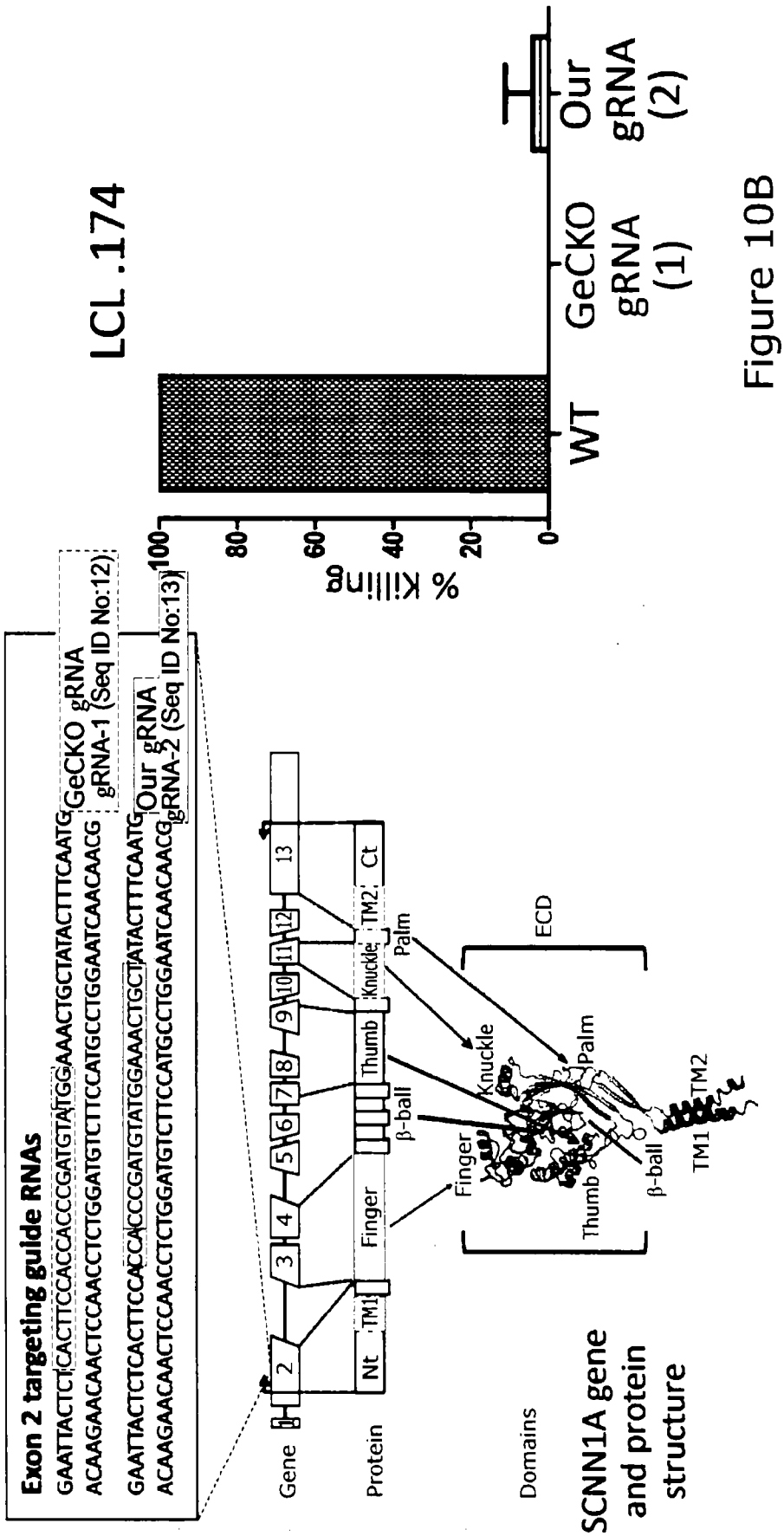

Figure 10B

Figure 10A

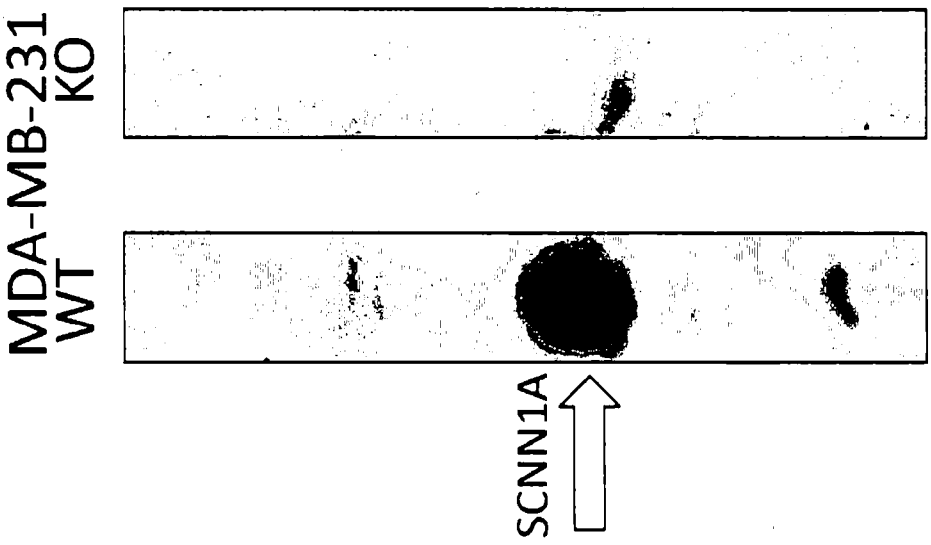

Figure 10C

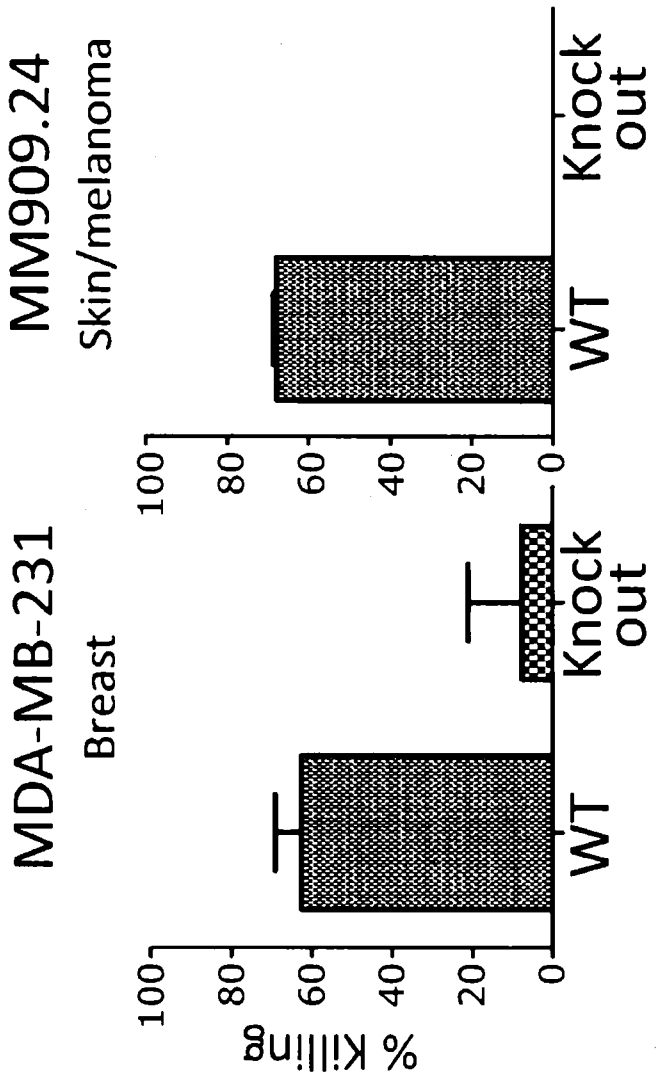

Figure 10D

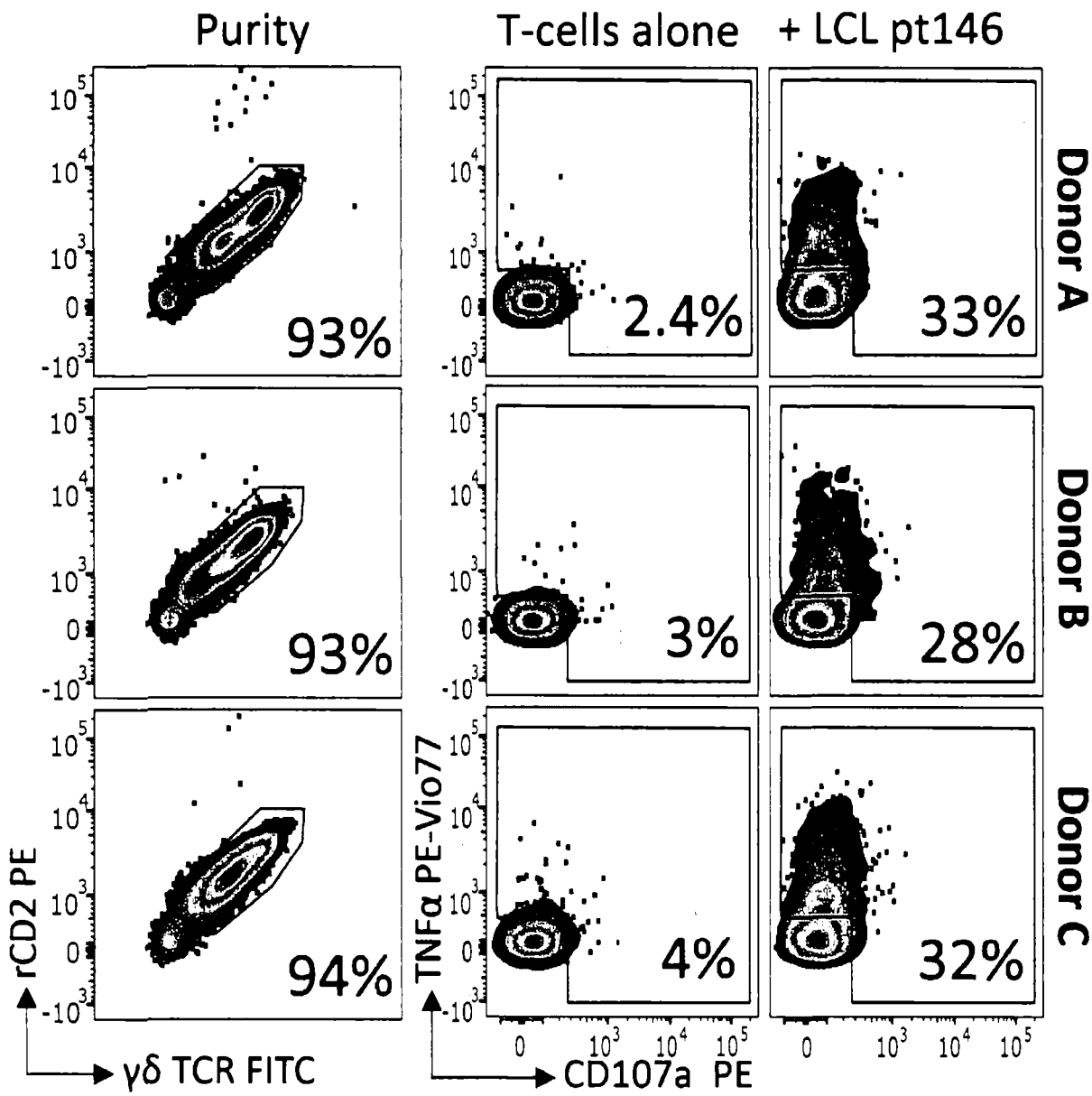
Purity
T-cells alone
+ LCL pt146
Donor A
Donor B
Donor C
93%
93%
94%
2.4%
3%
4%
33%
28%
32%
rCD2 PE
γδ TCR FITC
TNFα PE-Vio77
CD107a PE

Figure 11A

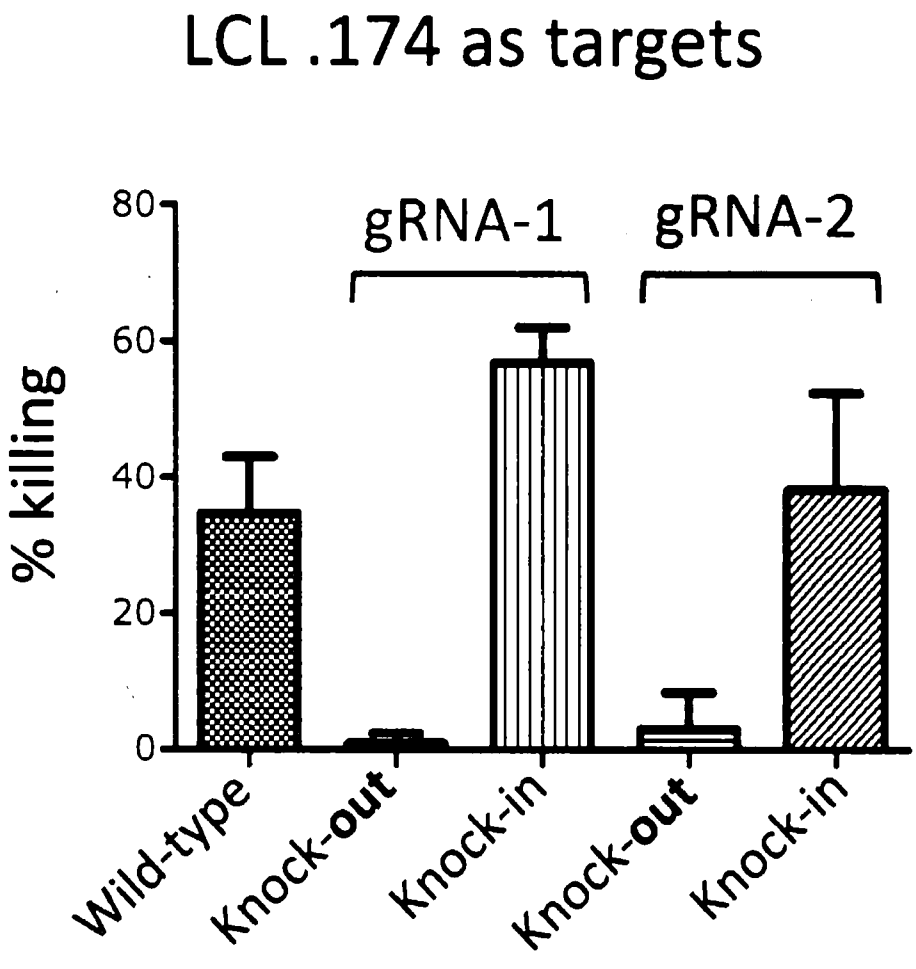
LCL .174 as targets
gRNA-1
gRNA-2
% killing
80
60
40
20
0
Wild-type
Knock-out
Knock-in
Knock-out
Knock-in

Figure 11B

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/053321

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47
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)
C07K

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)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA, 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	MATEUSZ LEGUT ET AL: "The promise of [gamma][delta] T cells and the [gamma][delta] T cell receptor for cancer immunotherapy", CELLULAR & MOLECULAR IMMUNOLOGY, vol. 12, no. 6, 13 April 2015 (2015-04-13) , pages 656-668, XP055373398, CH ISSN: 1672-7681, DOI: 10.1038/cmi.2015.28	24
A	the whole document	1-23

X	US 5 260 223 A (BRENNER MICHAEL B [US] ET AL) 9 November 1993 (1993-11-09)	24
A	column 6, line 62 - line 64; sequence 4	1-23

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
21 December 2018	30/01/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Strobel, Andreas

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2015/121454 A1 (CELLECTIS [FR]) 20 August 2015 (2015-08-20) page 39, line 18 - line 19; claims 31,33,35; table 11	24 1-23
A	MOUNIA S. BRAZA ET AL: "Anti-tumour immunotherapy with V[gamma]9V[delta]2 T lymphocytes: from the bench to the bedside", BRITISH JOURNAL OF HAEMATOLOGY, vol. 160, no. 2, 15 October 2012 (2012-10-15), pages 123-132, XP055536828, GB ISSN: 0007-1048, DOI: 10.1111/bjh.12090 page 132, left-hand column, paragraph 2	1,23
A,P	MATEUSZ LEGUT ET AL: "CRISPR-mediated TCR replacement generates superior anticancer transgenic T cells", BLOOD, vol. 131, no. 3, 18 January 2018 (2018-01-18), pages 311-322, XP055536727, US ISSN: 0006-4971, DOI: 10.1182/blood-2017-05-787598 abstract Discussion section	1-24
A,P	ANDRÉ E. SIMÕES ET AL: "Molecular Determinants of Target Cell Recognition by Human [gamma][delta] T Cells", FRONTIERS IN IMMUNOLOGY, vol. 9, 27 April 2018 (2018-04-27), XP055536993, DOI: 10.3389/fimmu.2018.00929 abstract; table 1	1-24
X	DATABASE UniProt [Online] 1 October 1994 (1994-10-01), "RecName: Full=Amiloride-sensitive sodium channel subunit alpha; AltName: Full=Alpha-NaCH; AltName: Full=Epithelial Na(+) channel subunit alpha; Short=Alpha-ENaC; Short=ENaCA; AltName: Full=Nonvoltage-gated sodium channel 1 subunit alpha; AltName: Full=SCNEA;", XP002787613, retrieved from EBI accession no. UNIPROT:P37088 Database accession no. P37088 the whole document	24

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2018/053321

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5260223 A	09-11-1993	AT 122148 T	15-05-1995
		AU 631780 B2	10-12-1992
		CA 1340140 C	24-11-1998
		DE 3853718 D1	08-06-1995
		DE 3853718 T2	07-09-1995
		EP 0345318 A1	13-12-1989
		HK 1005893 A1	29-01-1999
		JP 3176049 B2	11-06-2001
		JP 3176338 B2	18-06-2001
		JP H1169975 A	16-03-1999
		JP H03503956 A	05-09-1991
		KR 0132680 B1	11-04-1998
		US 5260223 A	09-11-1993
		WO 8903996 A1	05-05-1989
WO 2015121454 A1	20-08-2015	AU 2015216875 A1	28-07-2016
		BR 112016017806 A2	10-10-2017
		CA 2937711 A1	22-07-2016
		CN 106029875 A	12-10-2016
		EP 3105317 A1	21-12-2016
		JP 2017506636 A	09-03-2017
		KR 20160138404 A	05-12-2016
		RU 2016136702 A	19-03-2018
		US 2018201901 A1	19-07-2018
		WO 2015121454 A1	20-08-2015