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Pulé et al.(10) **Pub. No.: US 2016/0296562 A1**(43) **Pub. Date: Oct. 13, 2016**(54) **CELL**(71) Applicant: **UCL BUSINESS PLC**, London (GB)(72) Inventors: **Martin Pulé**, London (GB); **Khai Kong**, London (GB); **Shaun Cordoba**, London (GB)(73) Assignee: **UCL BUSINESS PLC**, London (GB)(21) Appl. No.: **15/037,391**(22) PCT Filed: **Nov. 21, 2014**(86) PCT No.: **PCT/GB2014/053451**

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Jun. 19, 2014 (GB) 1410934.2**Publication Classification**(51) **Int. Cl.****A61K 35/17** (2006.01)**C12N 5/0783** (2006.01)**C07K 14/725** (2006.01)**C12N 9/16** (2006.01)**C07K 16/28** (2006.01)**C07K 14/705** (2006.01)(52) **U.S. Cl.**CPC **A61K 35/17** (2013.01); **C07K 16/2803** (2013.01); **C07K 14/70521** (2013.01); **C07K 14/7051** (2013.01); **C07K 14/70517** (2013.01); **C12N 9/16** (2013.01); **C07K 14/70589** (2013.01); **C12Y 301/03048** (2013.01); **C12N 5/0637** (2013.01); **C07K 2319/74** (2013.01); **C07K 2319/03** (2013.01); **C07K 2317/622** (2013.01); **A61K 38/00** (2013.01)

(57)

ABSTRACT

The present invention provides a cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR at the cell surface, each CAR comprising : (i) an antigen-binding domain; (ii) a spacer (iii) a trans-membrane domain; and (iv) an endodomain wherein the antigen binding domains of the first and second CARs bind to different antigens, and wherein each of the first and second CARs is an activating CAR comprising an activating endodomain.

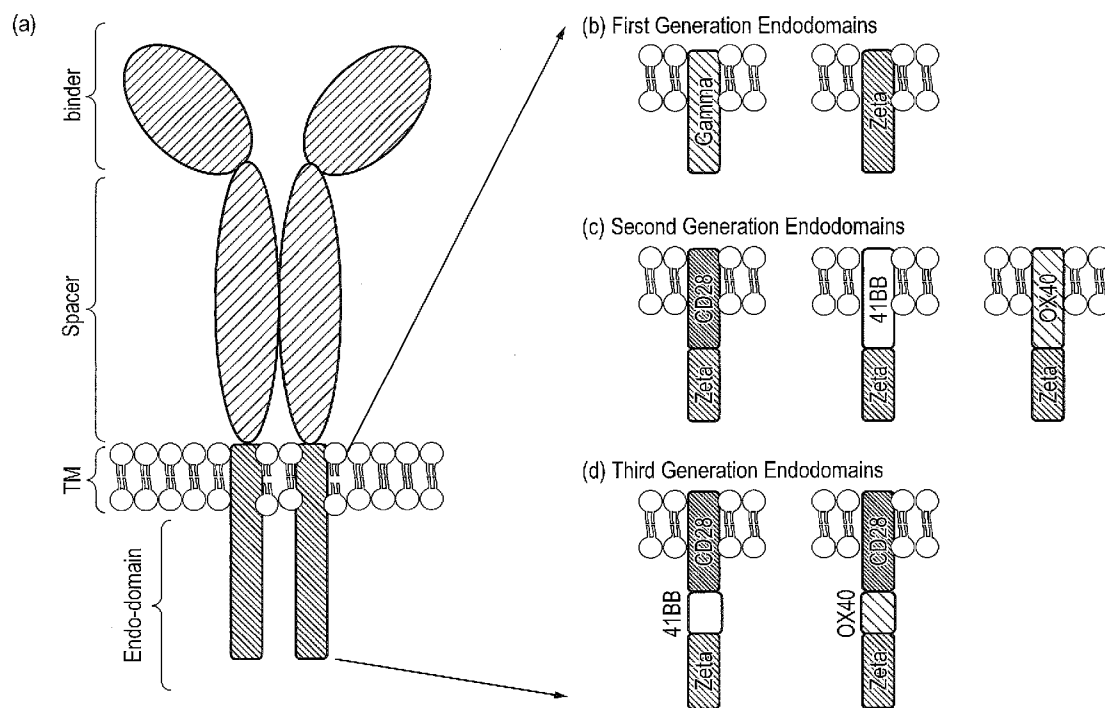


FIG. 1

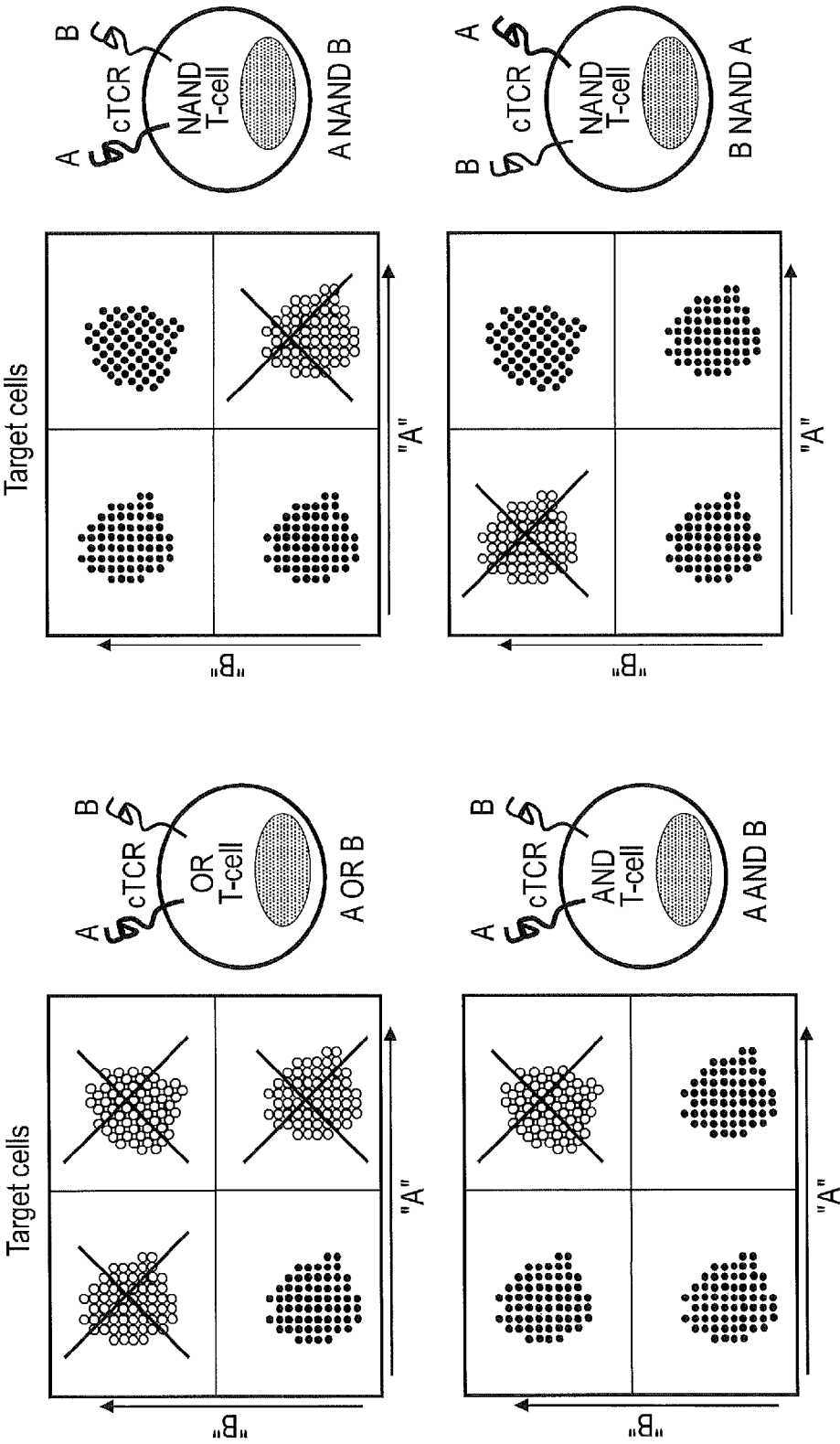


FIG. 2

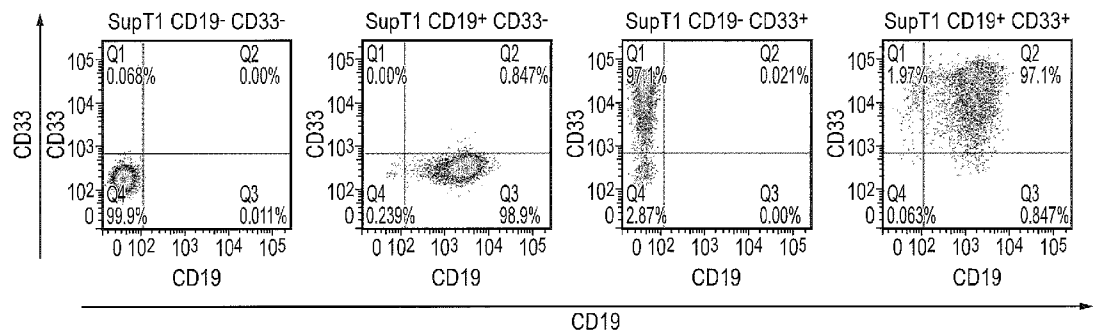


FIG. 3

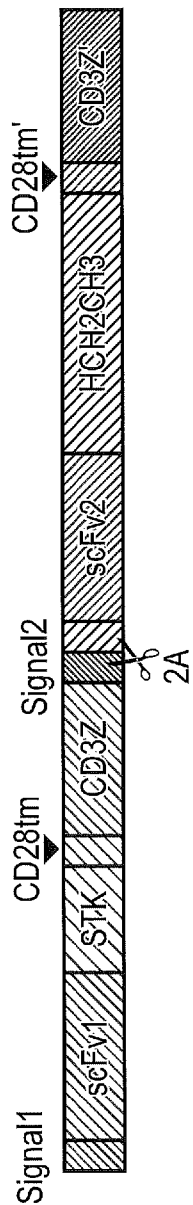


FIG. 4

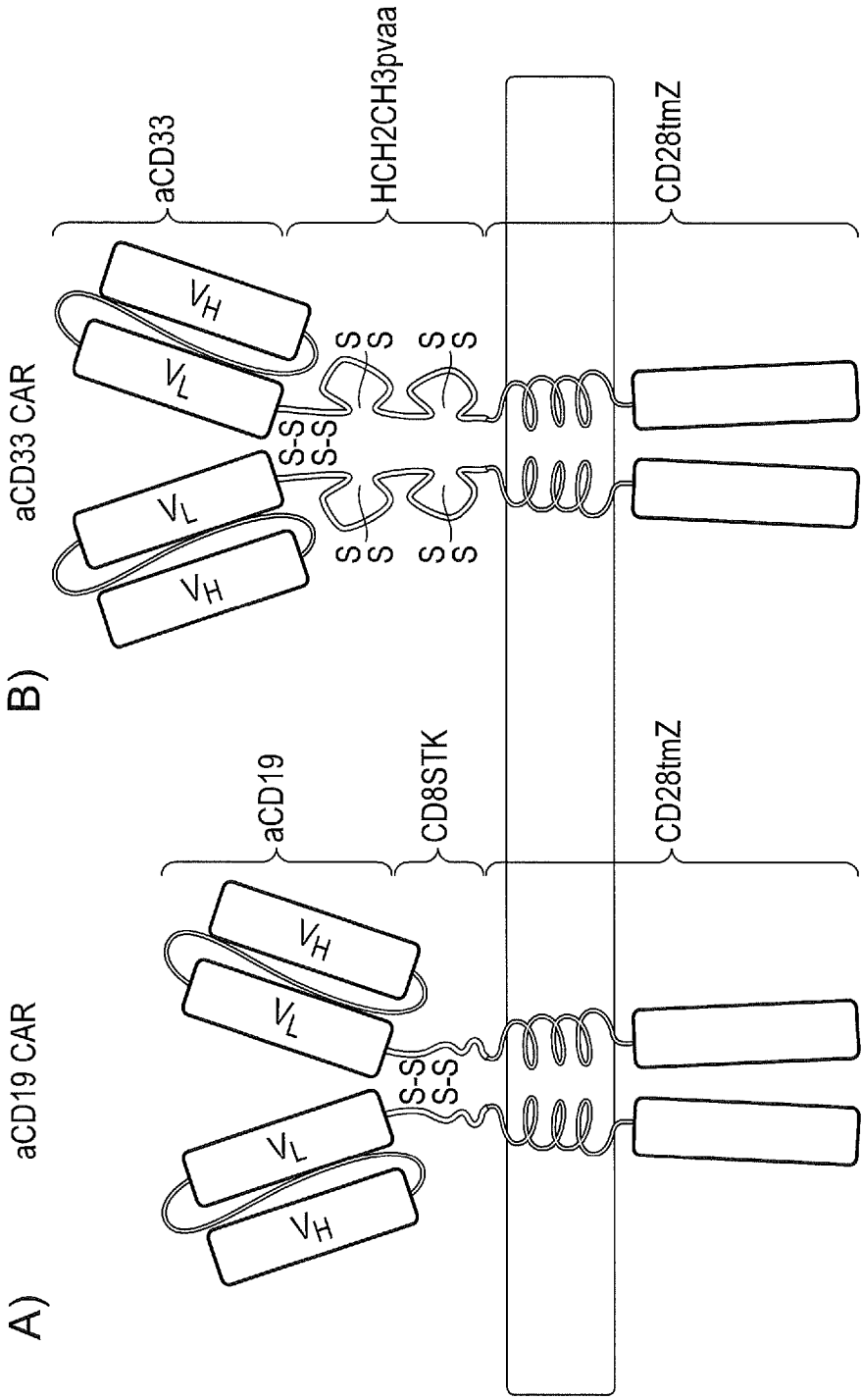


FIG. 5

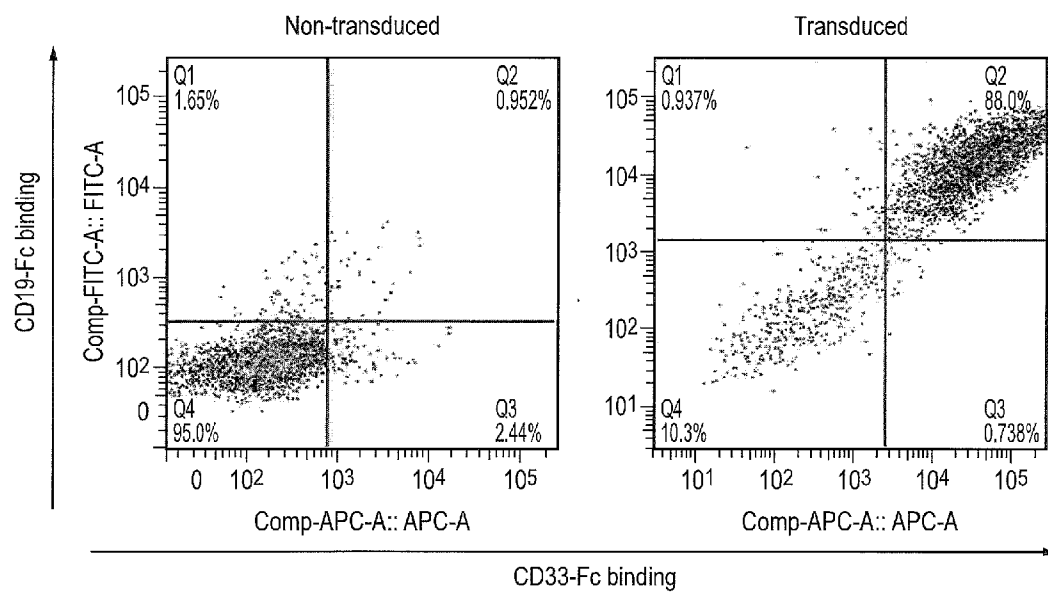


FIG. 6

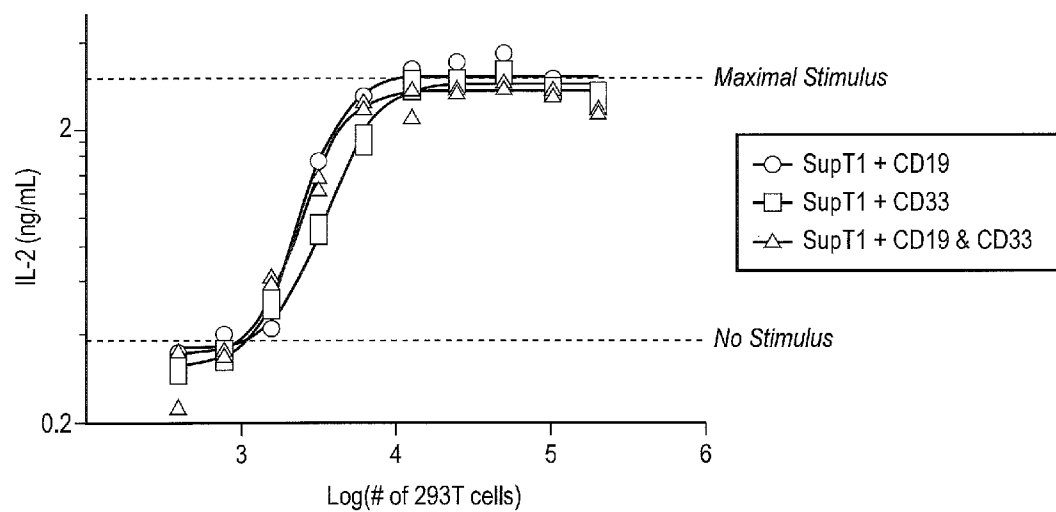


FIG. 7

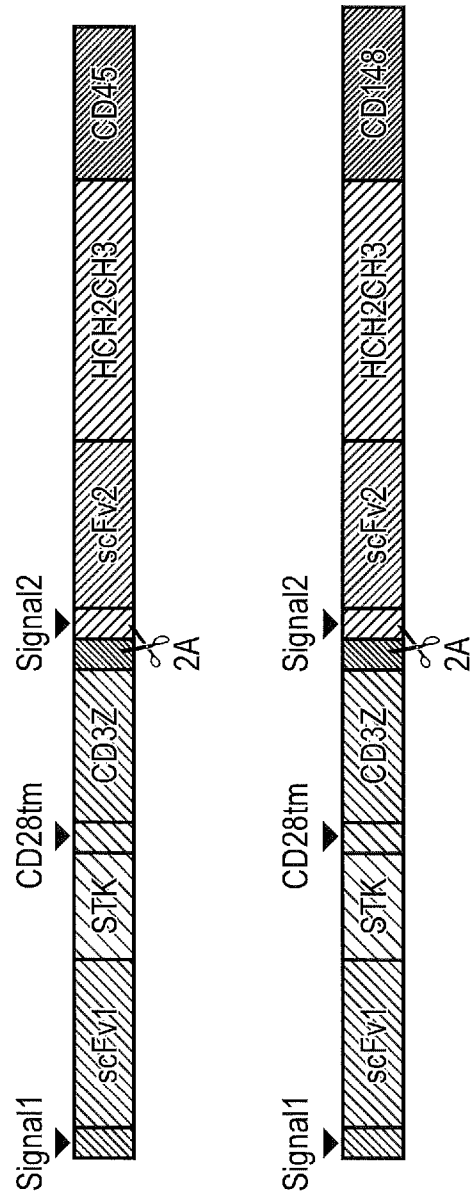


FIG. 8

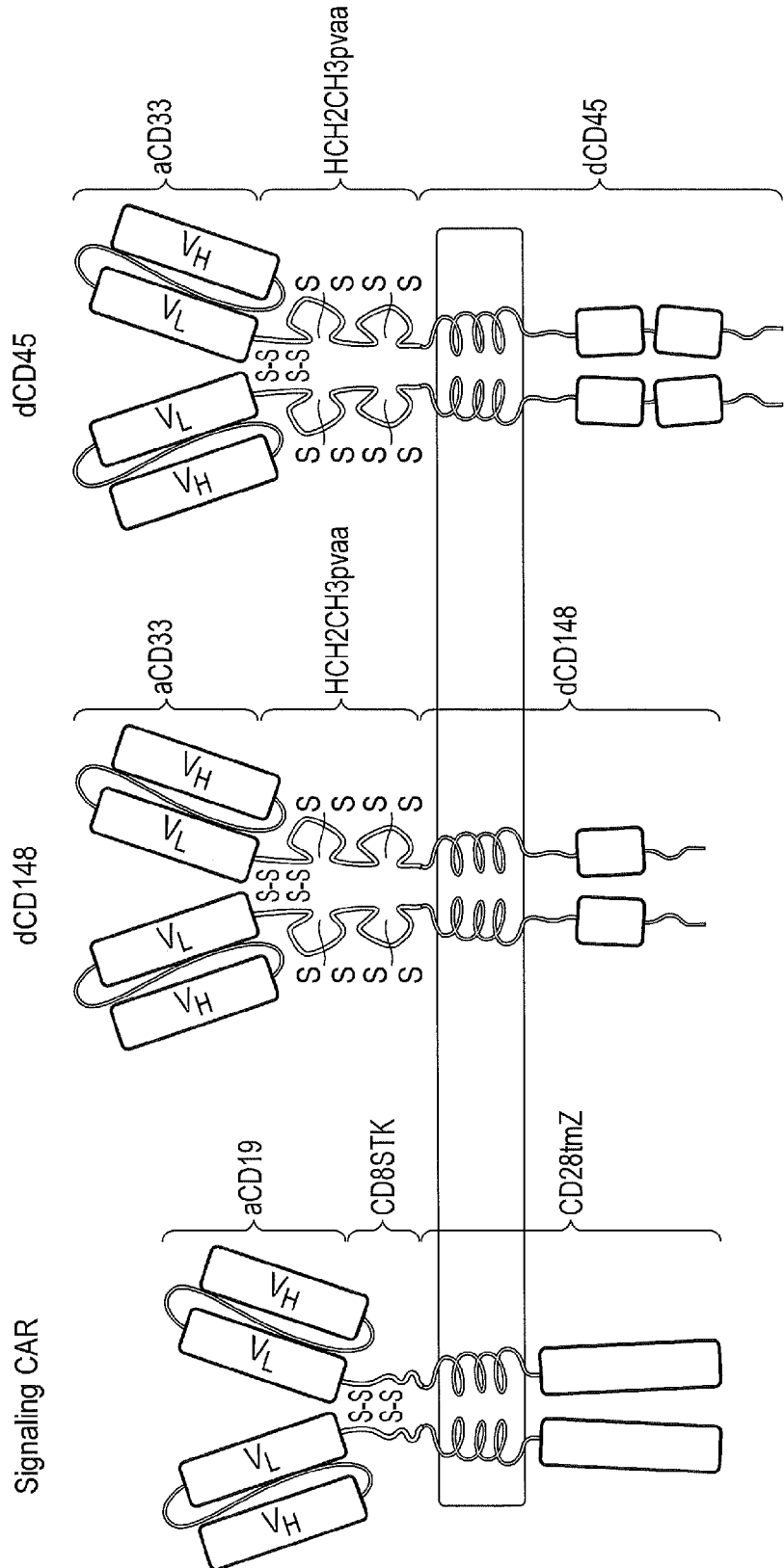


FIG. 9

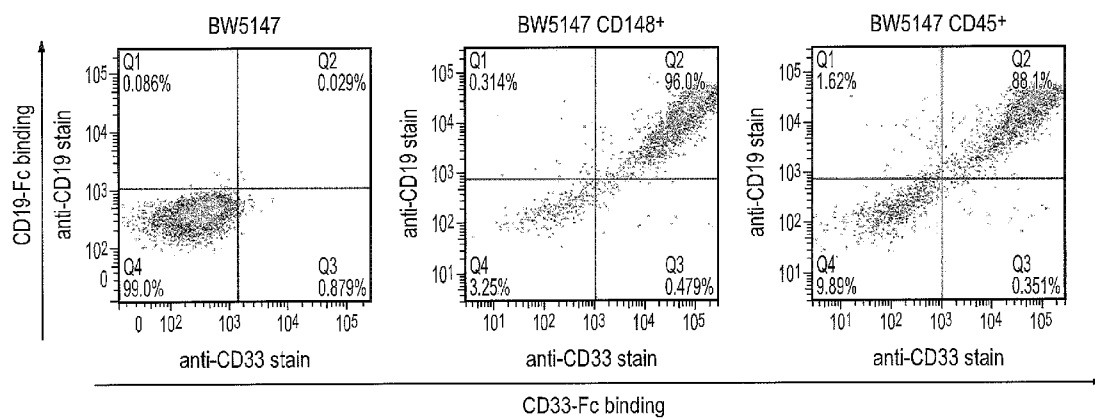


FIG. 10

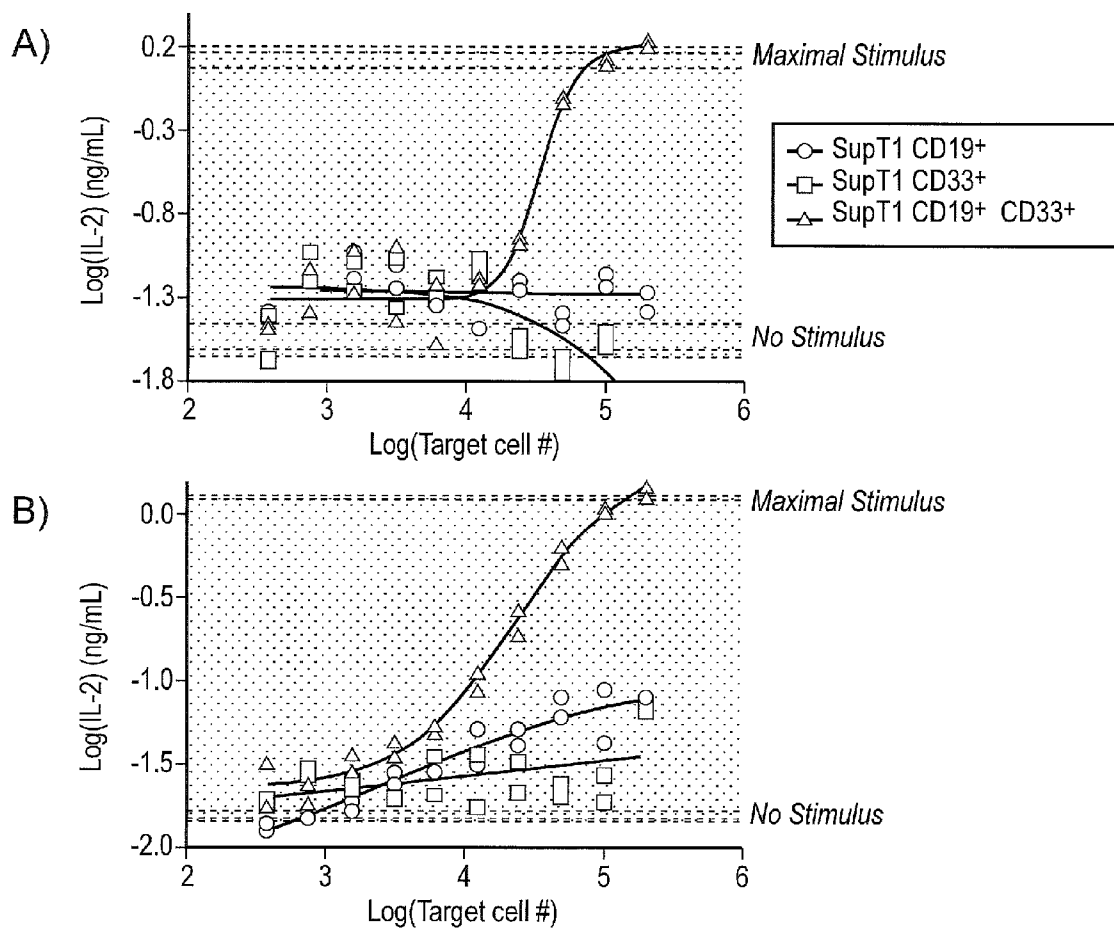


FIG. 11

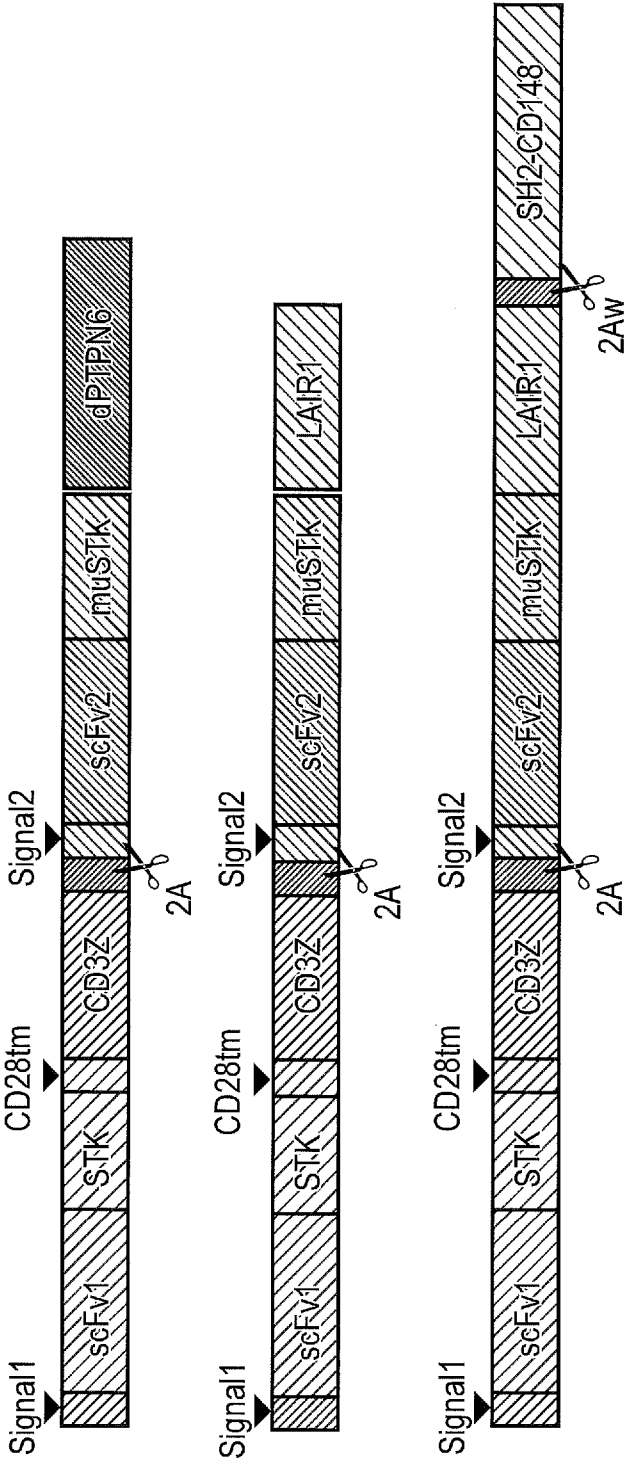


FIG. 12

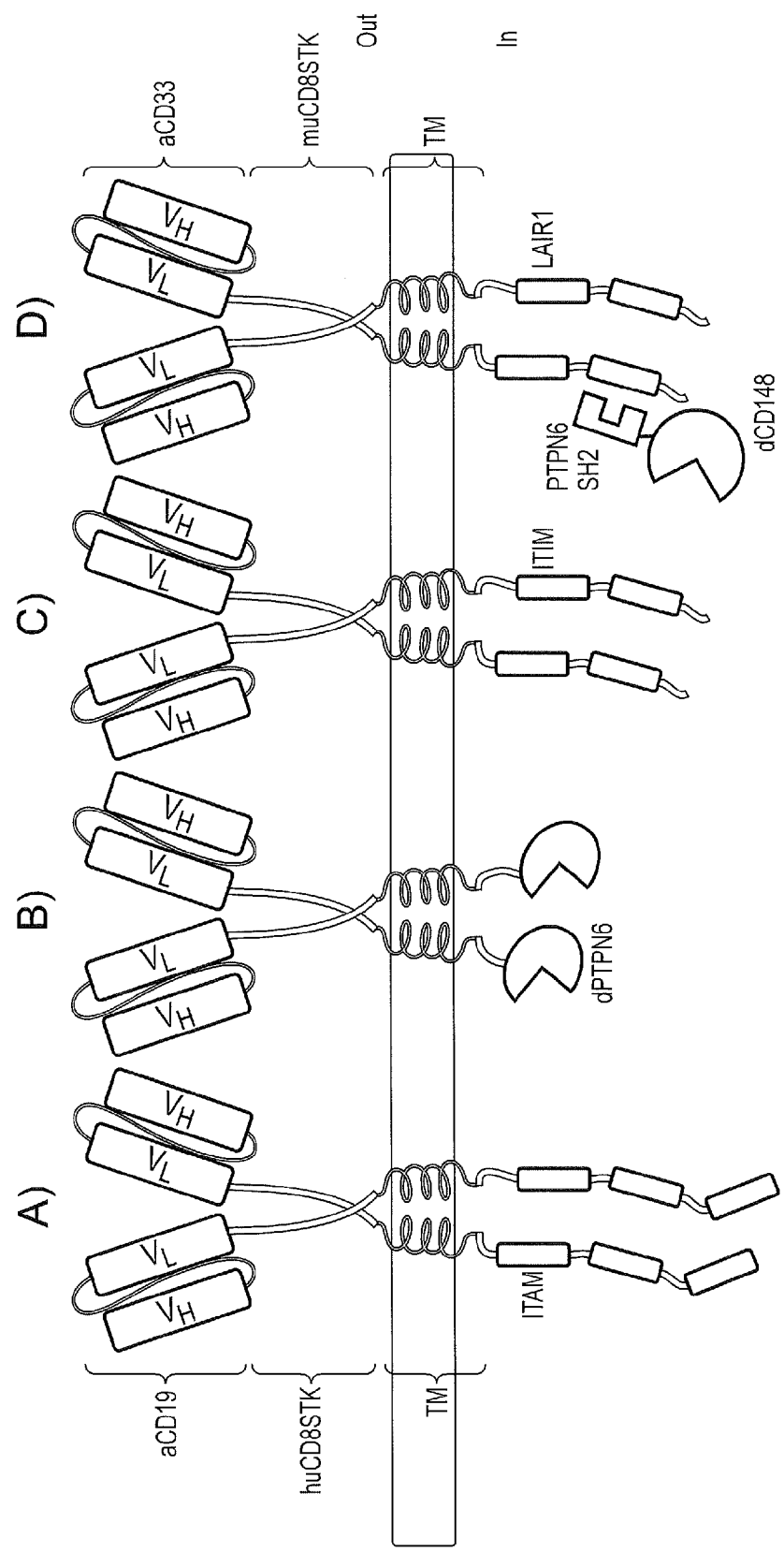


FIG. 13

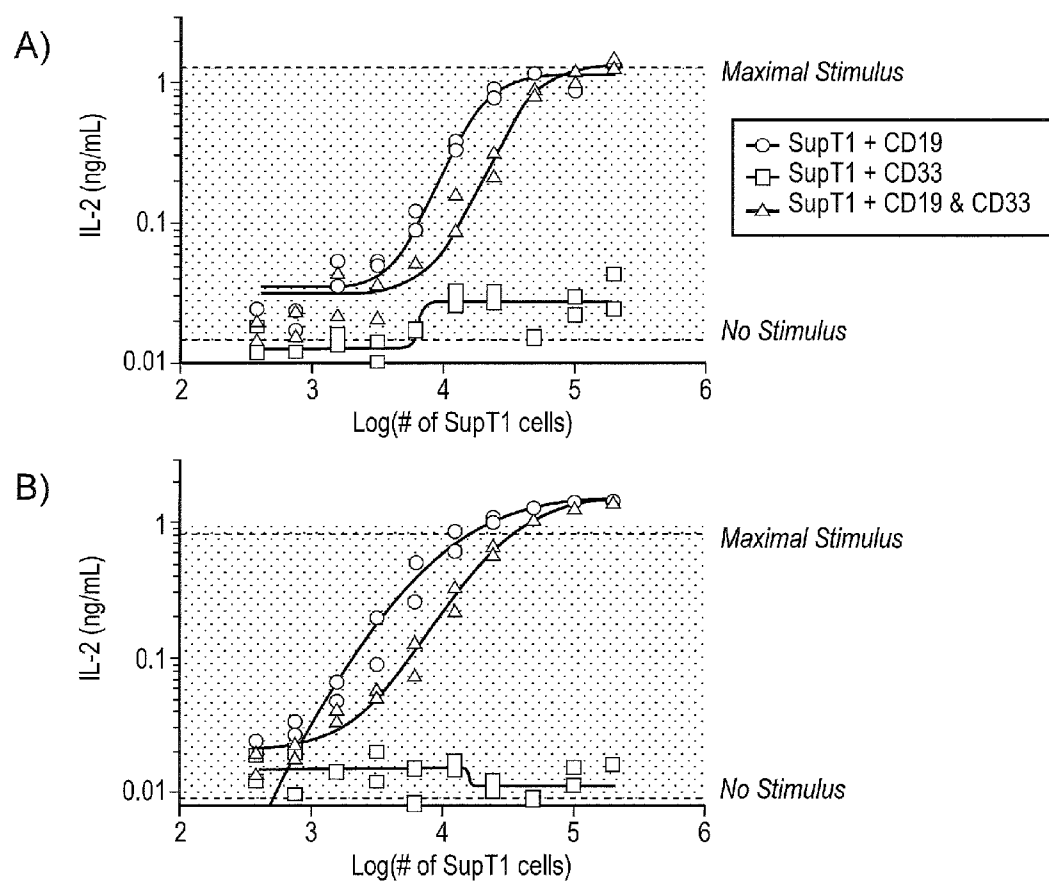


FIG. 14

>MP13974.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-CD28tmZw

MSLPVTALLPLALLLHAAREDIQMTQTTSSLSASIGDRVTISCRASQDISKYINWYQQKPDGTVKLLIYHTSRLHSGVPSRF
 SGGSGTDTSLTISNLEQEDATYFCQQGNTLPYTFGGGTKLEITKAGGGSGGGSGGGSGGGSEVKLQESGPGLVAPSC
 SLSVTCITVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRITIKDNSKSQVFLKMNSLQTDITAIYYCAKHY
 YYGGSYAMDYWGQTSVTVS~~SDP~~NTTPAPREFTPAINTASQPFSTFRLACQPPFEGGWHTRGDFHCDNFWVLVVVGVIACY
 SLLVTVAFTIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAE
 AYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRAEGRGSLLTCGDVEENPGPMVPTQVLGLLLLWTLDARC
 DIOMTQSPSSLSASVGDRTLTIRASELLYENWYQQKPKAPKLLIYDNLADGVPSRFSGGSGCTQYPLTLSSQPPDE
 ATYFCQHYKNYPLTEGQTKLELKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVQPGGSLRLSCAASGFTLISNMGH
 WIRQPPRKGLEWVSSISLNGGSTYYRESVKGRETTSDNAKSTLYLOMNSLRATEDTAWYVCAADANTSCYFDYWGQGTIAVW
 GSMDPAEPKSPDKTHTCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAATKPRE
 EQYNSTYRVSVLTIVLHQLDNLNGKEYCKVSKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
 IAVWEWSNGQEPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNYTQKSLSLSPGKKDKPKEFWVLVVG
 GVLACYSLLVTVAFTIFWVRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
 QDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

Region	Description
Signal1	Signal peptide 1
scFv1	scFv 1 – anti-CD19
SDP	Linker and chain break
STK	CD8alpha stalk
CD28tmZ	CD28 transmembrane domain and CD3 Zeta endodomain
FMD-2A	Foot-and-mouth disease 2A peptide
Signal2	Signal peptide 2
scFv2	scFv 2 – anti-CD33
MDP	Linker and chain break
HCH2CH3	Hinge, CH2 and CH3 of human IgG1
CD28tmZ	CD28 transmembrane domain and CD3 Zeta endodomain

FIG. 15

[illegible]

Region	Description
Signal1	Signal peptide 1
scFv1	scFv 1 – anti-CD19
SDP	Linker and chain break
STK	CD8alpha stalk
CD28tm2	CD28 transmembrane domain and CD3 Zeta endodomain
FMD-2A	Foot-and-mouth disease 2A peptide
Signal2	Signal peptide 2
scFv2	scFv 2 – anti-CD33
MDP	Linker and chain break
HCH2CH3	Hinge, CH2 and CH3 of human IgG1
dCD148 / dCD45	Trans-membrane and endo-domains of CD148 and CD45

FIG. 16

FIG. 17

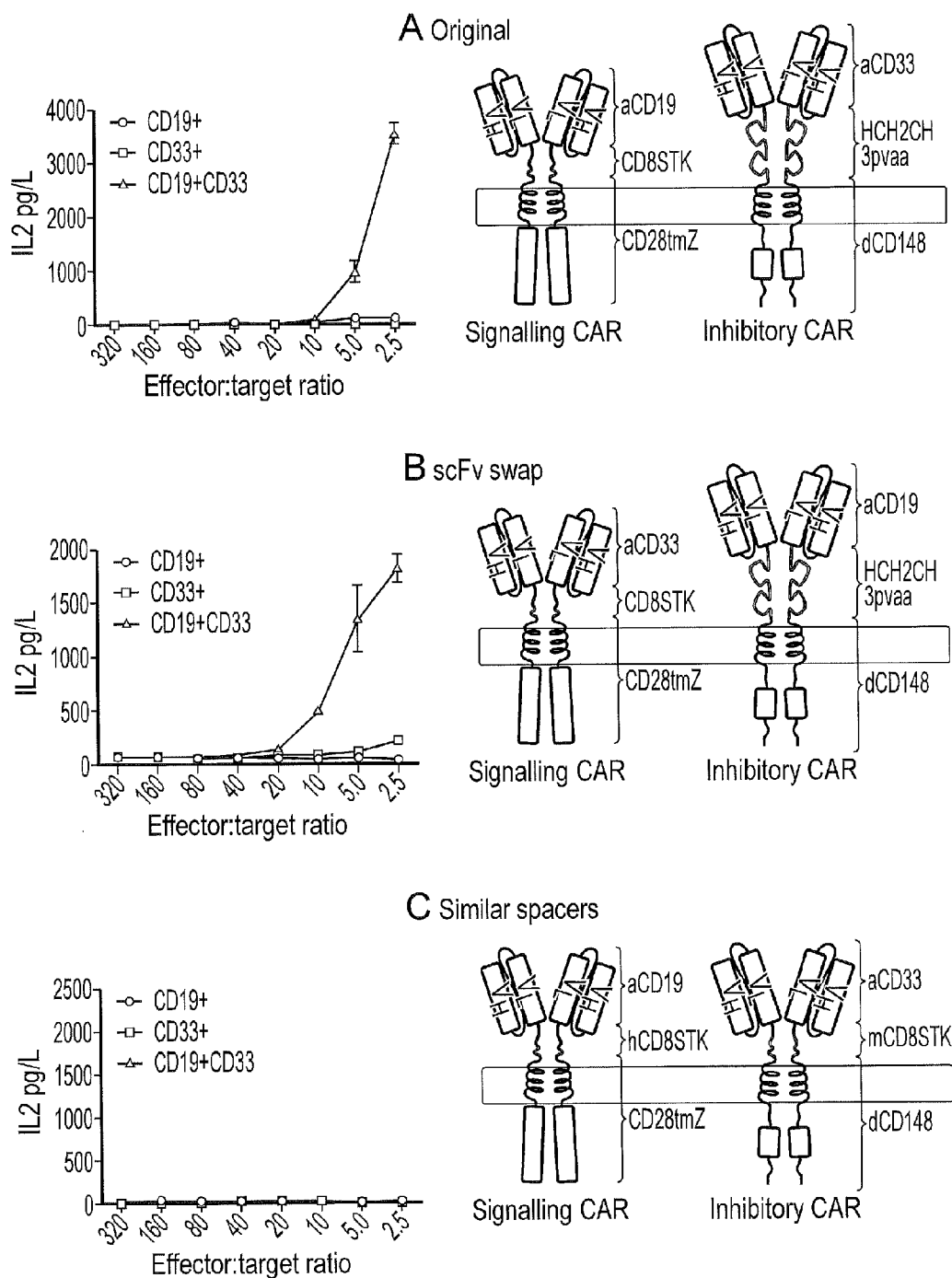


FIG. 18

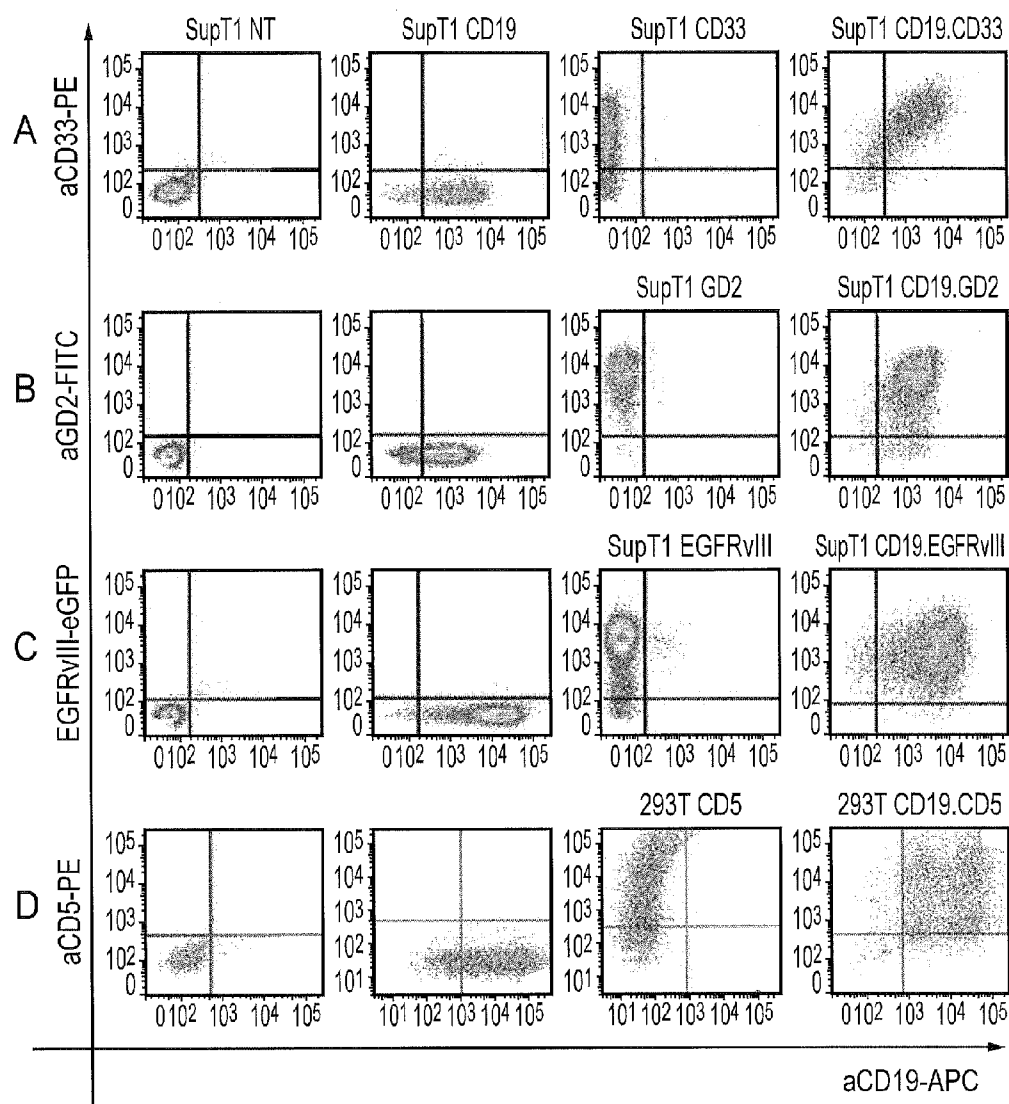


FIG. 19

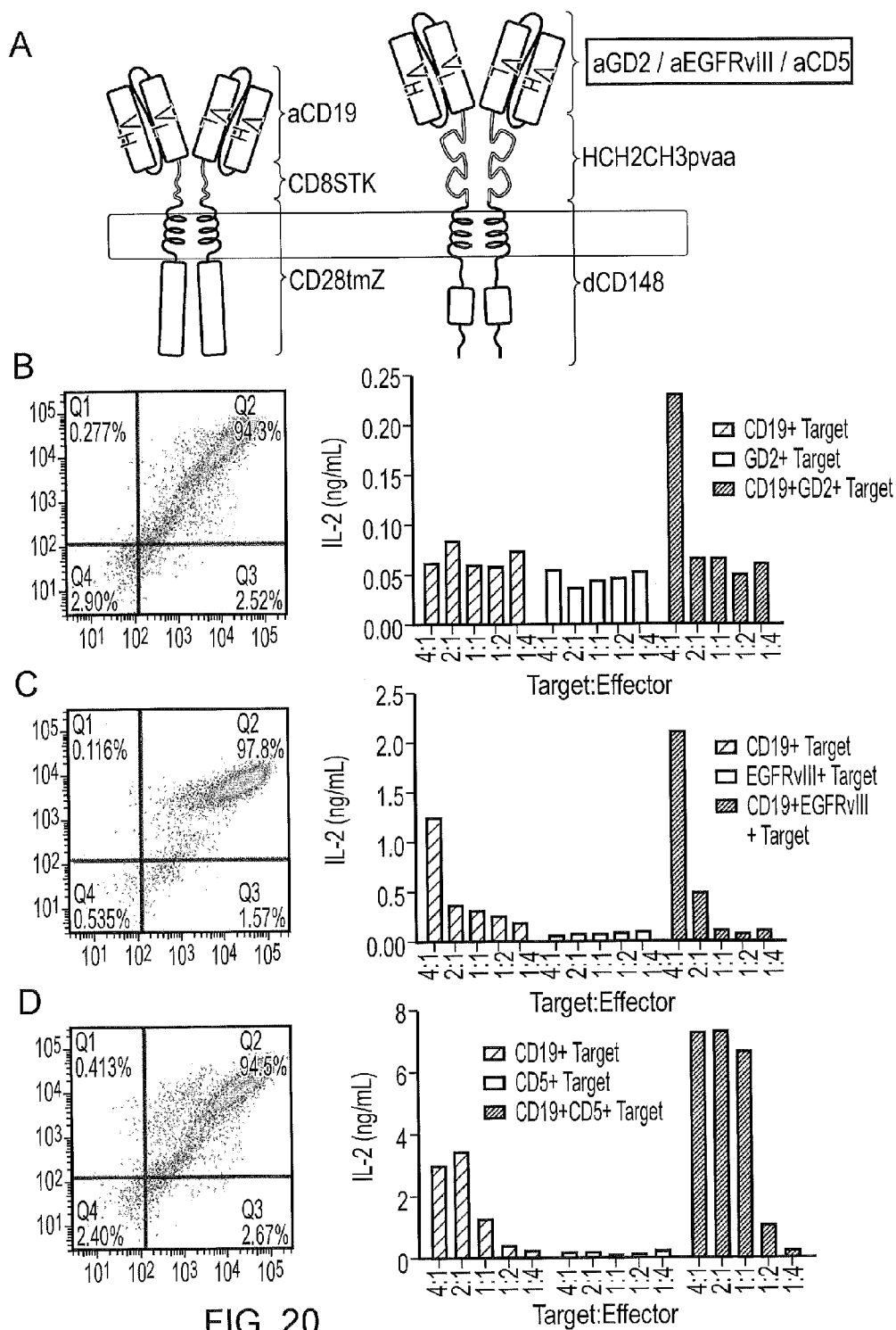


FIG. 20

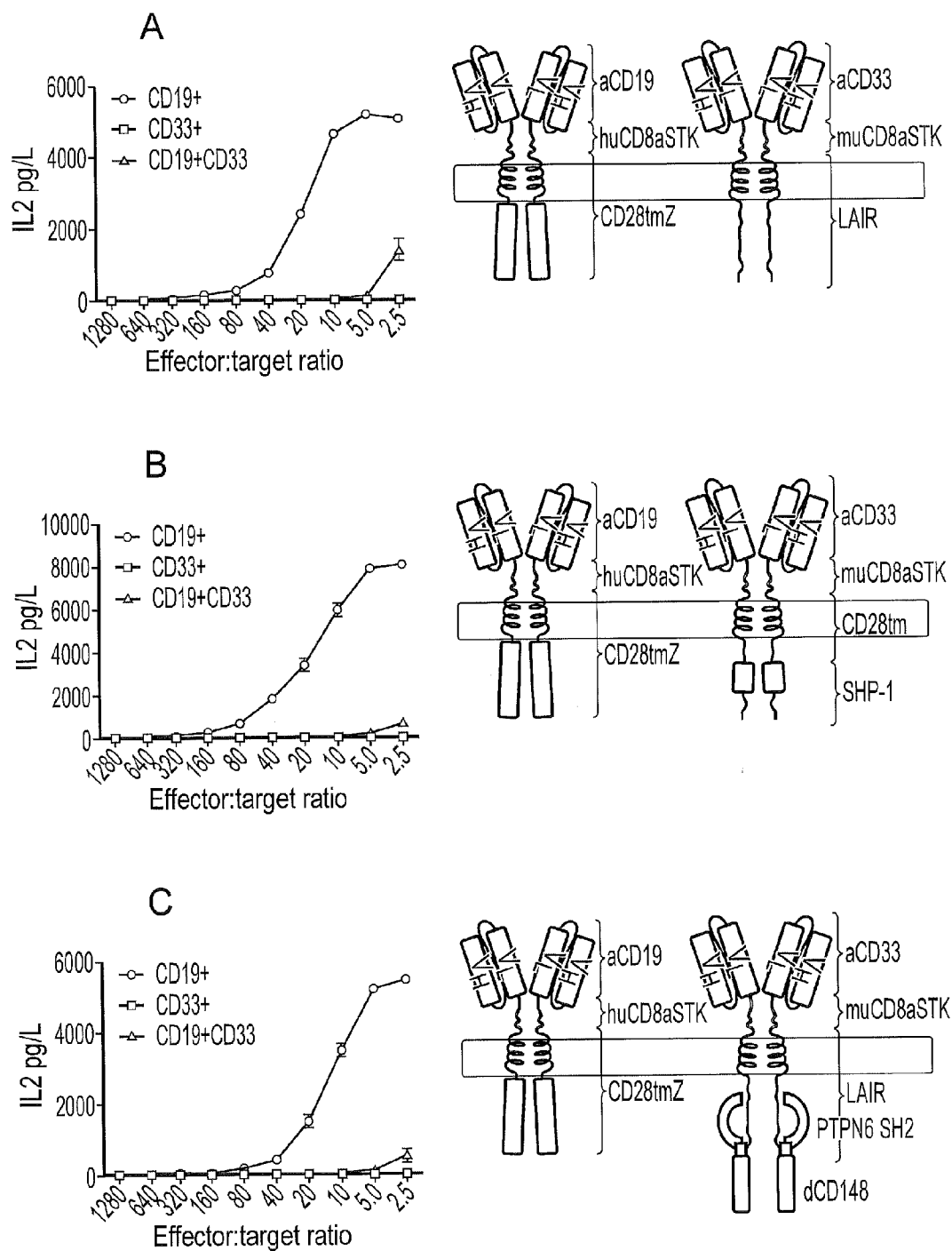


FIG. 21

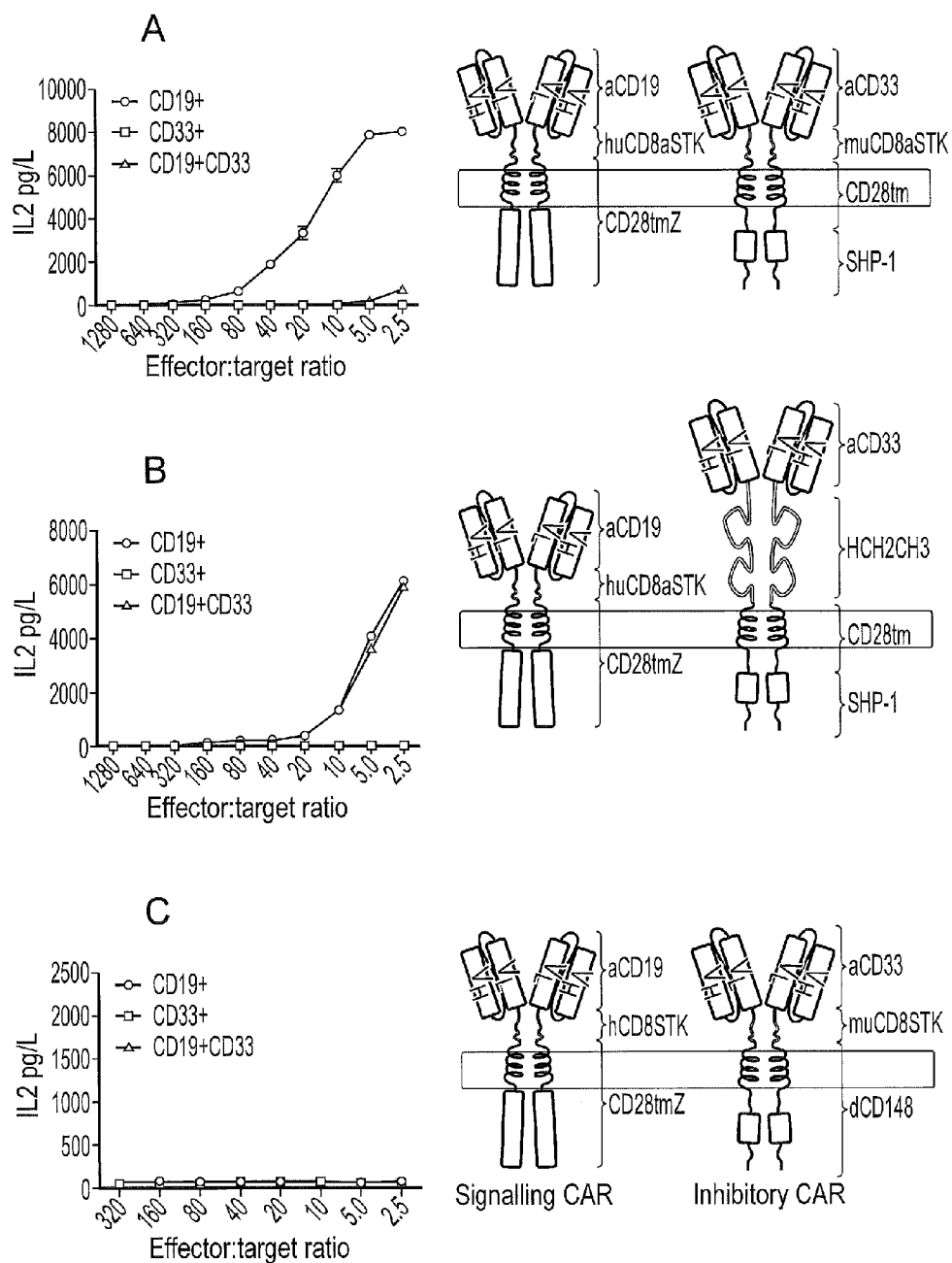


FIG. 22

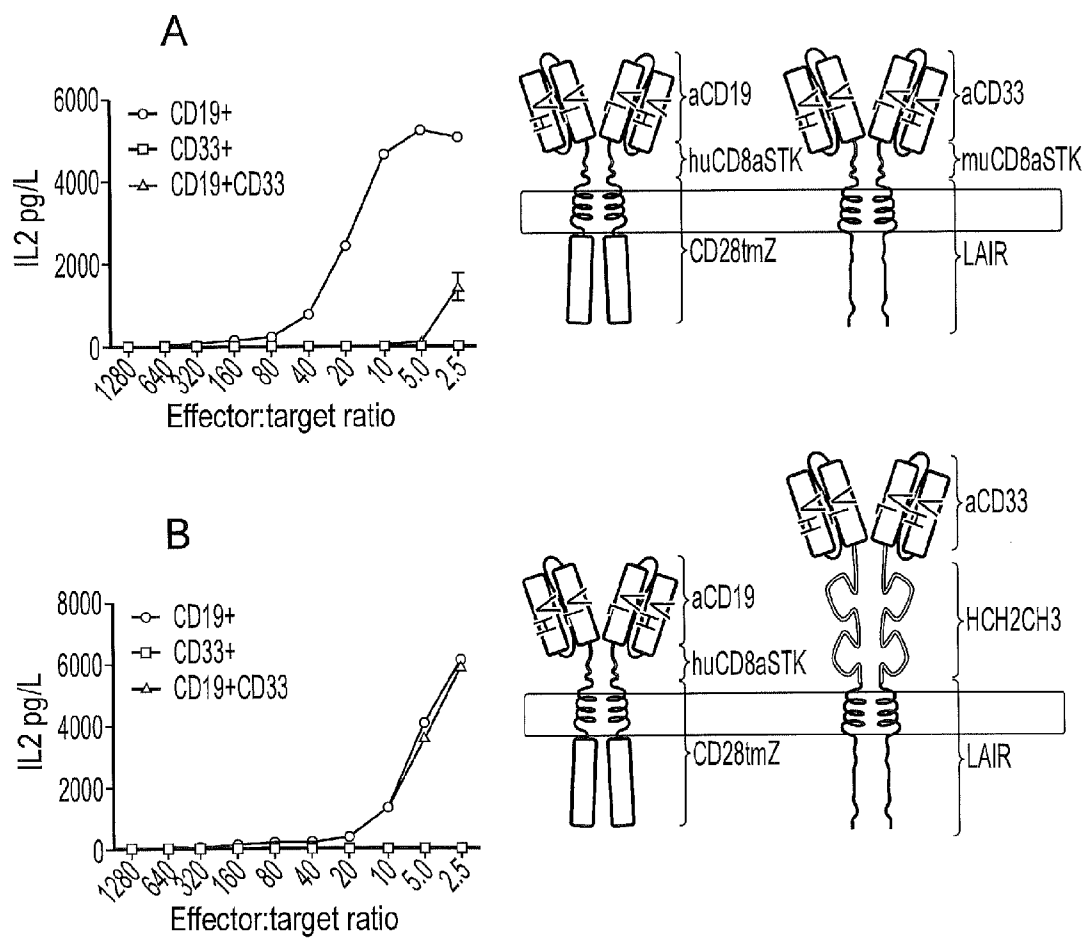


FIG. 23

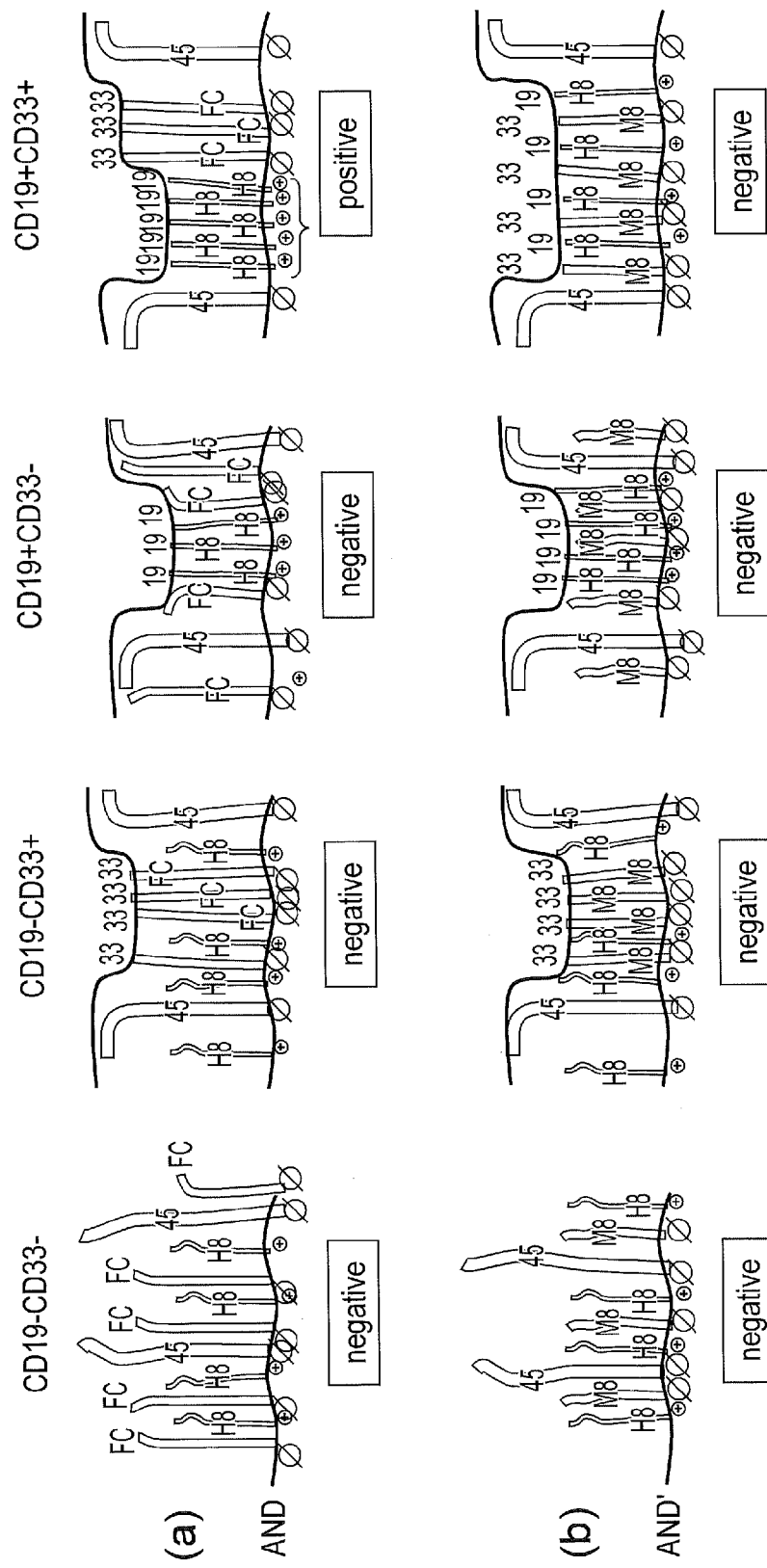


FIG. 24

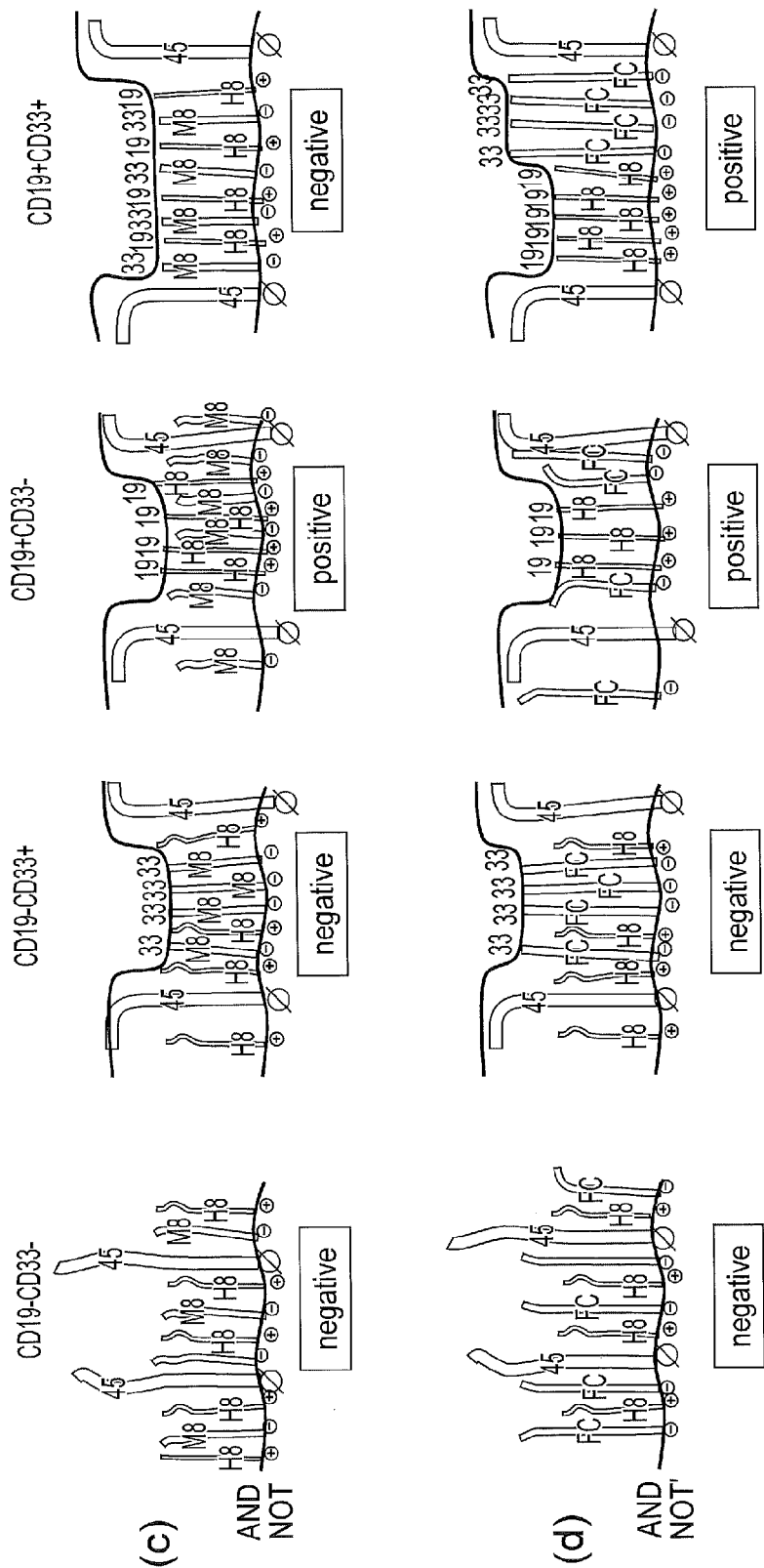


FIG. 24 (Continued)

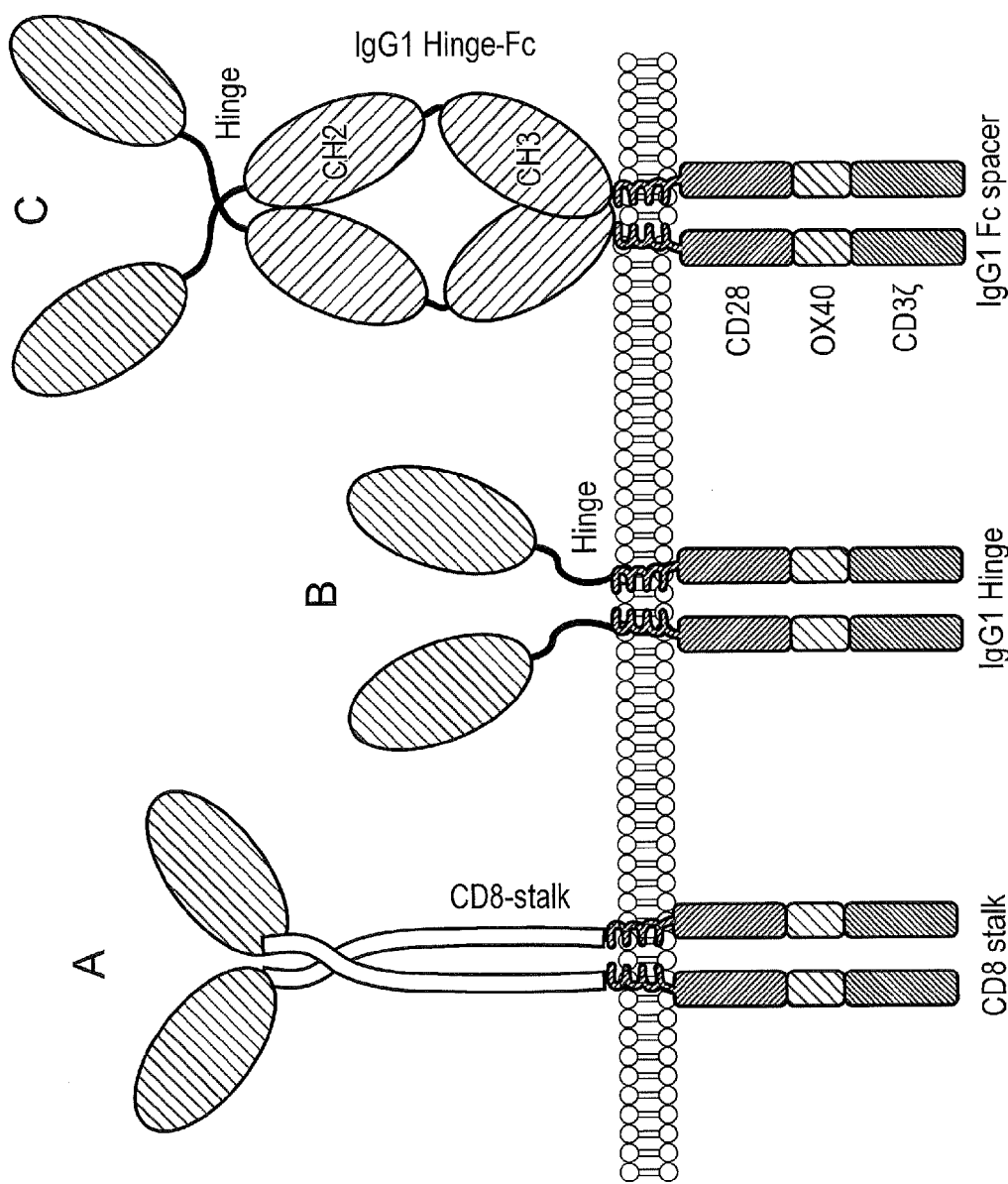


FIG. 25

A

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METDTLLLWVLLWVPGSTG SVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRICIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL SGGGSDPFTTEAPRPPTTPTTASOPLSLRPFACRPAAGGAVHTRGLDE
// FFWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA
AYRSRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGR
RREYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
TKDITYDALHMQALPPR
    
```

B

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METDTLLLWVLLWVPGSTG SVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRICIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL SGGGSDPFTTEAPRPPTTPTTASOPLSLRPFACRPAAGGAVHTRGLDE
// FFWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA
AYRSRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGR
RREYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
TKDITYDALHMQALPPR
    
```

C

```

METDTLLLWVLLWVPGSTG SVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRICIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL SGGGSDPFTTEAPRPPTTPTTASOPLSLRPFACRPAAGGAVHTRGLDE
// FFWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA
AYRSRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGR
RREYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
TKDITYDALHMQALPPR
    
```

Signal Peptide	Efficient signal peptide
dAPRIL	Truncated APRIL
Spacer	Either hinge-CH2CH3 of human IgG1, human CD8 stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 26

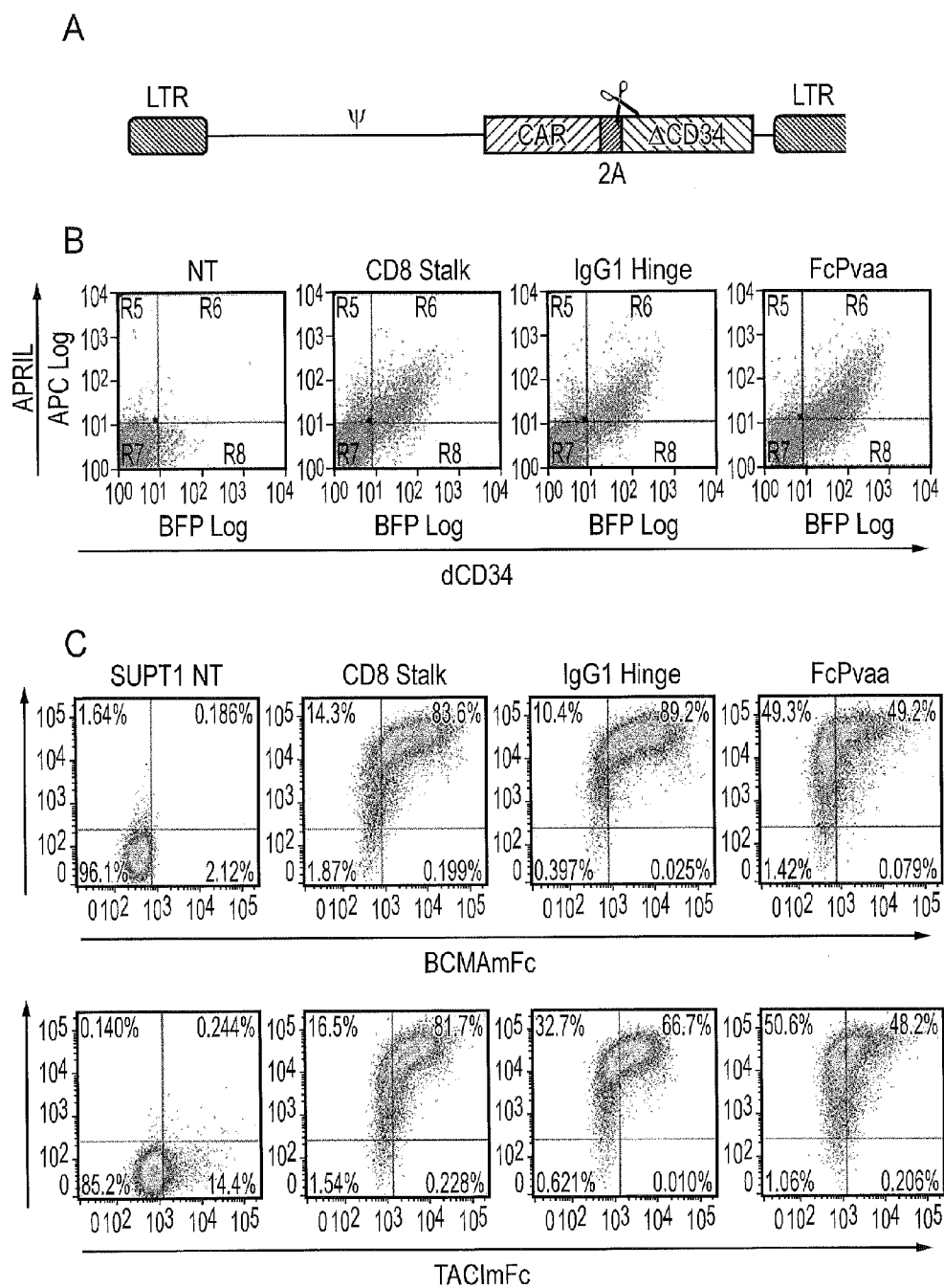


FIG. 27

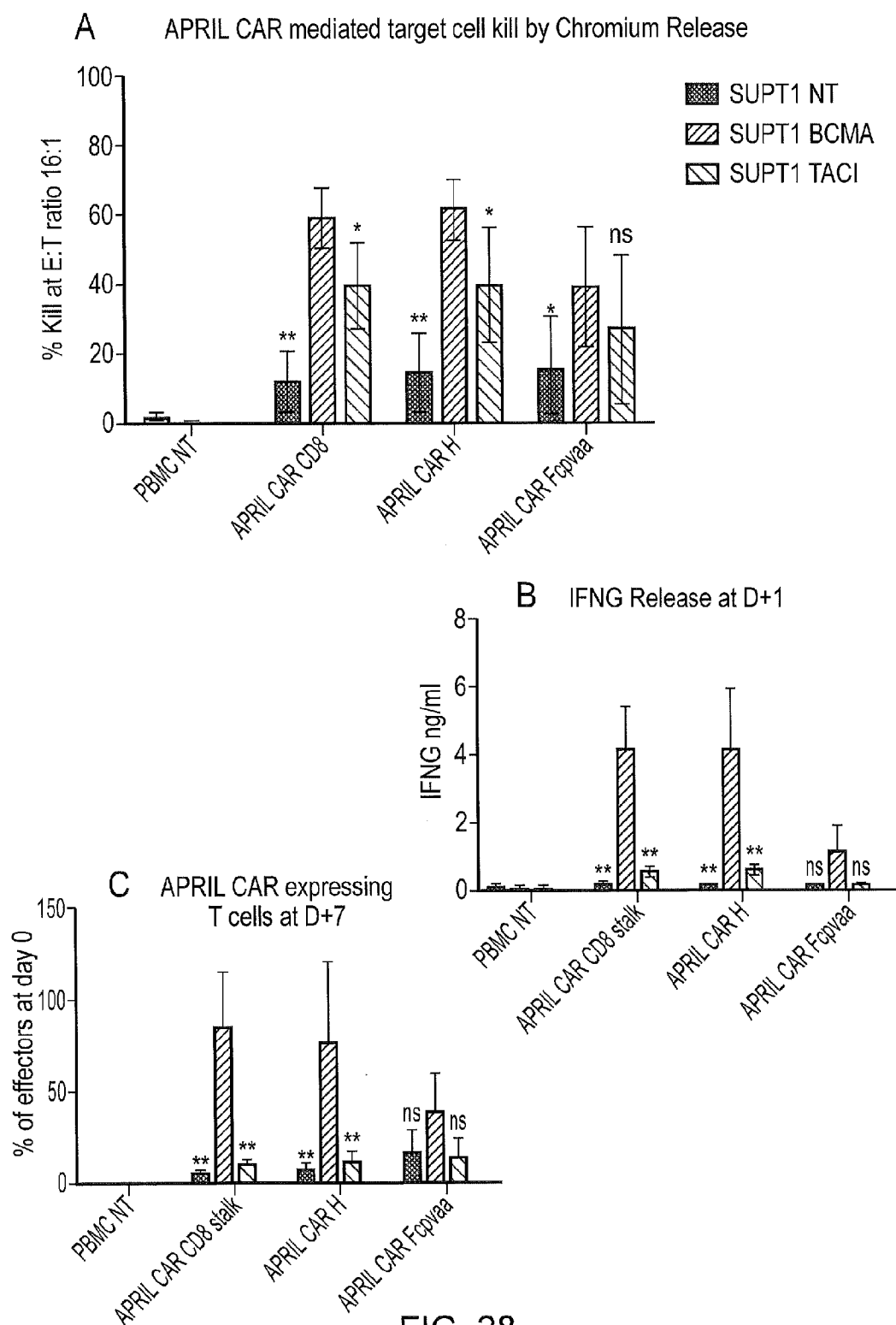


FIG. 28

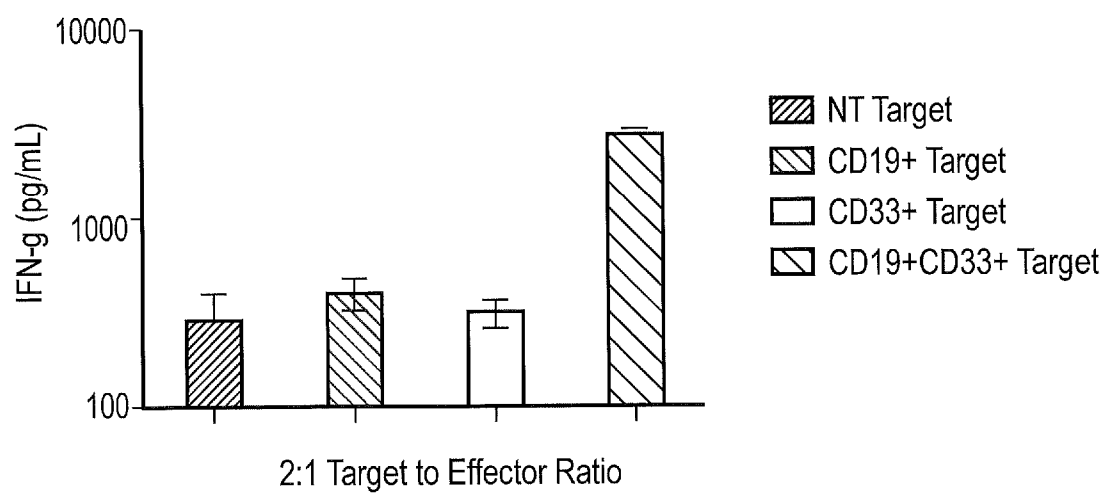


FIG. 29

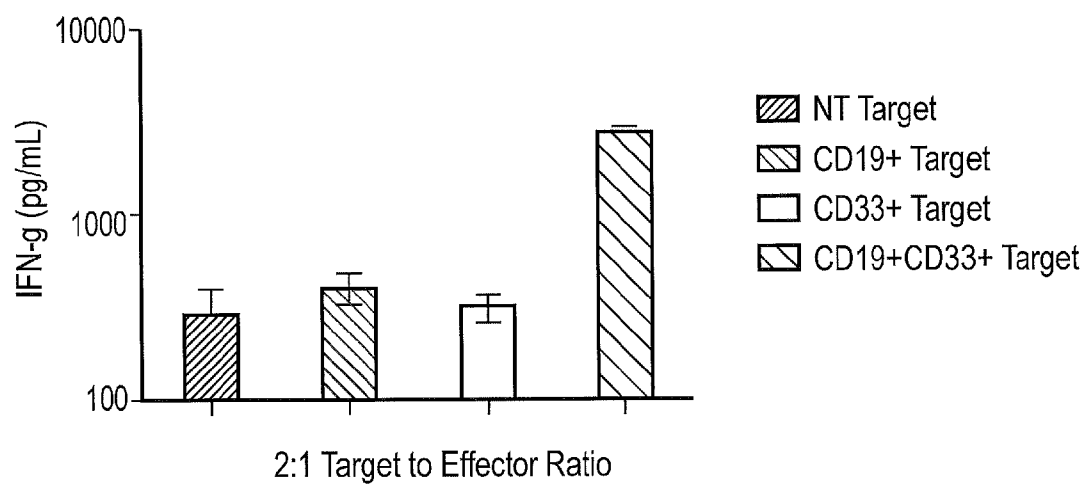


FIG. 30A

		Section 1				
	(1)	1	10	20	30	47
AntiCD19: CD28TM-TCRz	(1)	TTT	TGGGT	CTGGTGGTGGT	TGGTGGAGT	CCTGGCTTGCTATAGCTT
AntiCD33: CD28TM-TCRz	(1)	TTCTGGGT	CTGGTGGTGGT	GGAGGGCGT	GCTGGCTTGT	TACTCTCT
Consensus	(1)	TT	TGGGT	CTGGTGGTGGT	GG GG GT	CTGGC TG TA T
		Section 2				
	(48)	48	60	70	80	94
AntiCD19: CD28TM-TCRz	(48)	GCTAGTAACAGTGGCCTT	TATATTTCTGGGTGAGGAGAGTGAAGT			
AntiCD33: CD28TM-TCRz	(48)	CCTGGTGACC	GTGGCCTTCATCATCTTTGGGTGCGCGGTGAAGT			
Consensus	(48)	CT GT AC	GTGGCCTT AT AT TT	TGGGTG G	G	GTGAAGT
		Section 3				
	(95)	95	100	110	120	141
AntiCD19: CD28TM-TCRz	(95)	TCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAAC				
AntiCD33: CD28TM-TCRz	(95)	TTTCTCGCTCTGCCATGCCCCAGCCTATCAGCAGGGCCAGAA				
Consensus	(95)	T	G	GC GA	GCCCC GC TA	CAGCAGGGCCAGAA CAG
		Section 4				
	(142)	142	150	160	170	188
AntiCD19: CD28TM-TCRz	(142)	CTCTATAACGAGCTCAATCTAGGACGAAAGAGAGGAGTACGATGTTTT				
AntiCD33: CD28TM-TCRz	(142)	CTGTACCAATCAACTGAACCTGGGCAGGCGGAGGAGTACGACGTGCT				
Consensus	(142)	CT TA AA	GA CT AA CT GG	G	G	GACGAGTACGA GT T
		Section 5				
	(189)	189	200	210	220	235
AntiCD19: CD28TM-TCRz	(189)	GGACAAGAGACCTGGCCGGGACCCTGAGATGGGGGGAAGCCGAGAA				
AntiCD33: CD28TM-TCRz	(189)	GGATAAGCGGAGAGGCGAGAGACCCCGAGATGGGGCGCAAAACACGGC				
Consensus	(189)	GGA AAG G	G GGC G	GACCC	GAGATGGG	GG AA CC G
		Section 6				
	(236)	236	250	260	270	282
AntiCD19: CD28TM-TCRz	(236)	GGAGAAGCCCTCAGGAAGGCCTGTACAATGAAGTGCAGAAAGATAAG				
AntiCD33: CD28TM-TCRz	(236)	GCAAAAATCCCGAGGAGGCACTCTATAACGAGCTGCAGAAAGGACAAA				
Consensus	(236)	G AA AA CC	CAGGA GG CT TA AA	GA	CTGCAGAA	GA AA
		Section 7				
	(283)	283	290	300	310	329
AntiCD19: CD28TM-TCRz	(283)	ATGGCGGAGGCCTACAGT	GAGATTGGGATGAAAGGCGAGCGCCGGAG			
AntiCD33: CD28TM-TCRz	(283)	ATGGCGGAGGCCTATTCC	GAGATCGGCATGAAGGGAGAGAGAAAGACG			
Consensus	(283)	ATGGC	GAGGCCTA	GAGAT GG	ATGAA GG	GAG G G G
		Section 8				
	(330)	330	340	350	360	376
AntiCD19: CD28TM-TCRz	(330)	GGCAAGGGGCACGATGGCCTTTACAGGGTCTCAGTACAGCCACCA				
AntiCD33: CD28TM-TCRz	(330)	GGAAAGGGCCACGACGGCCTGTATCAGGGATTGTCCACCGCTACAA				
Consensus	(330)	GG	AAGGG CACGA	GGCCT TA	CAGGG T	AC GC AC A
		Section 9				
	(377)	377	390	400	410	420
AntiCD19: CD28TM-TCRz	(377)	AGGACACCTACGACGCCCTT	CACATGCAGGCCCTGCCCTCCTCC			
AntiCD33: CD28TM-TCRz	(377)	AAGATACATATGATGCCCTG	CACATGCAGGCCCTGCCACCCAGA			
Consensus	(377)	A	GA AC TA	GA GCCCT	CACATGCAGGCCCTGCC	CC G

Section 1							
	(1)	1	10	20	30	47	
AntiCD19: CD28TM-TCRz	(1)	FWVLVVVGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQ					
AntiCD33: CD28TM-TCRz	(1)	FWVLVVVGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQ					
Consensus	(1)	FWVLVVVGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQ					
Section 2							
	(48)	48	60	70	80	94	
AntiCD19: CD28TM-TCRz	(48)	LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK					
AntiCD33: CD28TM-TCRz	(48)	LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK					
Consensus	(48)	LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK					
Section 3							
	(95)	95	100	110	120	130	140
AntiCD19: CD28TM-TCRz	(95)	MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR					
AntiCD33: CD28TM-TCRz	(95)	MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR					
Consensus	(95)	MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR					

FIG. 30B

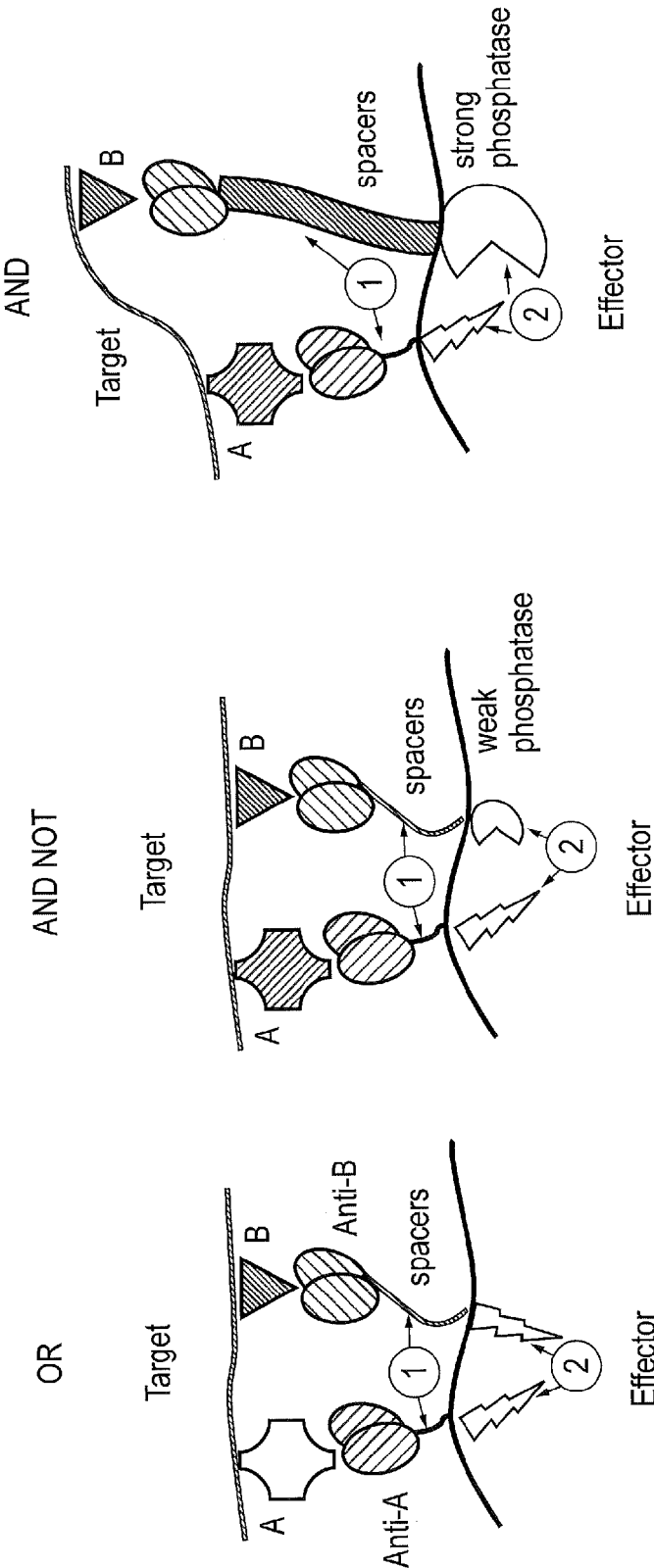


FIG. 31

CELL

FIELD OF THE INVENTION

[0001] The present invention relates to a cell which comprises more than one chimeric antigen receptor (CAR). The cell may be capable of specifically recognising a target cell, due to a differential pattern of expression (or non-expression) of two or more antigens by the target cell.

BACKGROUND TO THE INVENTION

[0002] A number of immunotherapeutic agents have been described for use in cancer treatment, including therapeutic monoclonal antibodies (mAbs), immunoconjugated mAbs, radioconjugated mAbs and bi-specific T-cell engagers.

[0003] Typically these immunotherapeutic agents target a single antigen: for instance, Rituximab targets CD20; Myelotarg targets CD33; and Alemtuzumab targets CD52.

[0004] However, it is relatively rare for the presence (or absence) of a single antigen effectively to describe a cancer.

[0005] A particular problem in the field of oncology is provided by the Goldie-Coldman hypothesis: which describes that the sole targeting of a single antigen may result in tumour escape by modulation of said antigen due to the high mutation rate inherent in most cancers. This modulation of antigen expression may reduce the efficacy of known immunotherapeutics.

[0006] Tumour heterogeneity describes the observation that different tumour cells can show distinct morphological and phenotypic profiles, including cellular morphology, gene expression, metabolism, proliferation and metastatic potential. This phenomenon occurs both between tumours and within tumours. Tumour heterogeneity has been observed in leukaemias, breast, prostate, colon, brain, head and neck, bladder and gynecological cancers, for example. Tumour heterogeneity may result in the expression of different antigens on the surface of cells within a tumour or between tumours. This heterogeneity of cancer cells introduces significant challenges in designing effective treatment strategies.

[0007] There is thus a need for immunotherapeutic agents which are capable of more targeting to reflect the complex pattern of marker expression that is associated with many cancers.

[0008] Chimeric Antigen Receptors (CARs)

[0009] Chimeric antigen receptors are proteins which graft the specificity of a monoclonal antibody (mAb) to the effector function of a T-cell. Their usual form is that of a type I transmembrane domain protein with an antigen recognizing amino terminus, a spacer, a transmembrane domain all connected to a compound endodomain which transmits T-cell survival and activation signals (see FIG. 1A).

[0010] The most common form of these molecules are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies which recognize a target antigen, fused via a spacer and a trans-membrane domain to a signaling endodomain. Such molecules result in activation of the T-cell in response to recognition by the scFv of its target. When T cells express such a CAR, they recognize and kill target cells that express the target antigen. Several CARs have been developed against tumour associated antigens, and adoptive transfer approaches using such CAR-expressing T cells are currently in clinical trial for the treatment of various cancers.

[0011] It has been observed that using a CAR approach for cancer treatment, tumour heterogeneity and immunoediting can cause escape from CAR treatment. For example, in the study described by Grupp et al (2013; New Eng. J. Med 368:1509-1518) CAR-modified T cell approach was used for the treatment of acute lymphoid leukemia. It was found that one patient of the anti-CD19 CAR trial relapsed with CD19-negative disease two months after treatment.

[0012] There is thus a need for alternative CAR treatment approaches which address the problems of cancer escape and tumour heterogeneity.

[0013] Expression of two CAR Binding Specificities

[0014] Bispecific CARs known as tandem CARs or TanCARs have been developed in an attempt to target multiple cancer specific markers simultaneously. In a TanCAR, the extracellular domain comprises two antigen binding specificities in tandem, joined by a linker. The two binding specificities (scFvs) are thus both linked to a single trans-membrane portion: one scFv being juxtaposed to the membrane and the other being in a distal position. Grada et al (2013, Mol Ther Nucleic Acids 2:e105 describes a TanCAR which includes a CD19-specific scFv, followed by a Gly-Ser linker and then a HER2-specific scFv. The HER2-scFv was in the juxta-membrane position, and the CD19-scFv in the distal position. The Tan CAR was shown to induce distinct T cell reactivity against each of the two tumour restricted antigens. This arrangement was chosen because the respective lengths of HER2 (632 aa/125A) and CD19 (280aa, 65A) lends itself to that particular spatial arrangement. It was also known that the HER2 scFv bound the distal-most 4 loops of HER2.

[0015] The problem with this approach is that the juxta-membrane scFv may be inaccessible due to the presence of the distal scFv, especially which it is bound to the antigen. In view of the need to choose the relative positions of the two scFvs in view of the spatial arrangement of the antigen on the target cell, it may not be possible to use this approach for all scFv binding pairs. Moreover, it is unlikely that the TanCAR approach could be used for more than two scFvs, a TanCAR with three or more scFvs would be a very large molecule and the scFvs may well fold back on each other, obscuring the antigen-binding sites. It is also doubtful that antigen-binding by the most distal scFv, which is separated from the transmembrane domain by two or more further scFvs, would be capable of triggering T cell activation.

[0016] There is thus a need for an alternative approach to express two CAR binding specificities on the surface of a cell such as a T cell.

DESCRIPTION OF THE FIGURES

[0017] FIG. 1: (a) Generalized architecture of a CAR: A binding domain recognizes antigen; the spacer elevates the binding domain from the cell surface; the trans-membrane domain anchors the protein to the membrane and the endodomain transmits signals. (b) to (d): Different generations and permutations of CAR endodomains: (b) initial designs transmitted ITAM signals alone through Fc ϵ R1- γ or CD3 ξ endodomain, while later designs transmitted additional (c) one or (d) two co-stimulatory signals in cis.

[0018] FIG. 2: Schematic diagram illustrating the invention

[0019] The invention relates to engineering T-cells to respond to logical rules of target cell antigen expression. This is best illustrated with an imaginary FACS scatter-plot.

Target cell populations express both, either or neither of antigens "A" and "B". Different target populations (marked in red) are killed by T-cells transduced with a pair of CARs connected by different gates. With OR gated receptors, both single-positive and double-positive cells will be killed. With AND gated receptors, only double-positive target cells are killed. With AND NOT gating, double-positive targets are preserved while single-positive targets

[0020] FIG. 3: Creation of target cell populations

[0021] SupT1 cells were used as target cells. These cells were transduced to express either CD19, CD33 or both CD19 and CD33. Target cells were stained with appropriate antibodies and analysed by flow cytometry.

[0022] FIG. 4: Cassette design for an OR gate

[0023] A single open reading frame provides both CARs with an in-frame FMD-2A sequence resulting in two proteins. Signal1 is a signal peptide derived from IgG1 (but can be any effective signal peptide). scFv1 is the single-chain variable segment which recognizes CD19 (but can be a scFv or peptide loop or ligand or in fact any domain which recognizes any desired arbitrary target). STK is the CD8 stalk but may be any suitable extracellular domain. CD28tm is the CD28 trans-membrane domain but can be any stable type I protein transmembrane domain and CD3Z is the CD3 Zeta endodomain but can be any endodomain which contains ITAMs. Signal2 is a signal peptide derived from CD8 but can be any effective signal peptide which is different in DNA sequence from signal1. scFv recognizes CD33 but as for scFv1 is arbitrary. HC2CH3 is the hinge-CH2-CH3 of human IgG1 but can be any bulky extracellular domain which does not cross-pair with the spacer used in the first CAR. CD28tm' and CD3Z' code for the same protein sequence as CD28tm and CD3Z but are codon-wobbled to prevent homologous recombination.

[0024] FIG. 5: Schematic representation of the chimeric antigen receptors (CARs) for an OR gate Stimulatory CARs were constructed consisting of either an N-terminal A) anti-CD19 scFv domain followed by the extracellular hinge region of human CD8 or B) anti-CD33 scFv domain followed by the extracellular hinge, CH2 and CH3 (containing a pva mutation to reduce FcR binding) region of human IgG1. Both receptors contain a human CD28 transmembrane domain and a human CD3 Zeta (CD247) intracellular domain. "S" depicts the presence of disulphide bonds.

[0025] FIG. 6: Expression data showing co-expression of both CARs on the surface of one T-cell.

[0026] FIG. 7: Functional analysis of the OR gate Effector cells (5×10^4 cells) expressing the OR gate construct were co-incubated with a varying number of target cells and IL-2 was analysed after 16 hours by ELISA. The graph displays the average maximum IL-2 secretion from a chemical stimulation (PMA and Ionomycin) of the effector cells alone and the average background IL-2 from effector cells without any stimulus from three replicates.

[0027] FIG. 8: Cartoon showing both versions of the cassette used to express both AND gates Activating and inhibiting CARs were co-expressed once again using a FMD-2A sequence. Signal1 is a signal peptide derived from IgG1 (but can be any effective signal peptide). scFv1 is the single-chain variable segment which recognizes CD19 (but can be a scFv or peptide loop or ligand or in fact any domain which recognizes any desired arbitrary target). STK is the CD8 stalk but may be any non-bulky extracellular domain. CD28tm is the CD28 trans-membrane domain but can be

any stable type I protein transmembrane domain and CD3Z is the CD3 Zeta endodomain but can be any endodomain which contains ITAMs. Signal2 is a signal peptide derived from CD8 but can be any effective signal peptide which is different in DNA sequence from signal1. scFv recognizes CD33 but as for scFv1 is arbitrary. HC2CH3 is the hinge-CH2-CH3 of human IgG1 but can be any bulky extracellular domain. CD45 and CD148 are the transmembrane and endodomains of CD45 and CD148 respectively but can be derived from any of this class of protein.

[0028] FIG. 9: Schematic representation of the protein structure of chimeric antigen receptors (CARs) for the AND gates

[0029] The stimulatory CAR consisting of an N-terminal anti-CD19 scFv domain followed by the extracellular stalk region of human CD8, human CD28 transmembrane domain and human CD3 Zeta (CD247) intracellular domain. Two inhibitory CARs were tested. These consist of an N-terminal anti-CD33 scFv domain followed by the extracellular hinge, CH2 and CH3 (containing a pva mutation to reduce FcR binding) region of human IgG1 followed by the transmembrane and intracellular domain of either human CD148 or CD45. "S" depicts the presence of disulphide bonds.

[0030] FIG. 10: Co-expression of activation and inhibitory CARs

[0031] BW5147 cells were used as effector cells and were transduced to express both the activation anti-CD19 CAR and one of the inhibitory anti-CD33 CARs. Effector cells were stained with CD19-mouse-Fc and CD33-rabbit-Fc and with appropriate secondary antibodies and analysed by flow cytometry.

[0032] FIG. 11: Functional analysis of the AND gates

[0033] Effector cells (5×10^4 cells) expressing activation anti-CD19 CAR and the inhibitory anti-CD33 CAR with the A) CD148 or B) CXD45 intracellular domain were co-incubated with a varying number of target cells and IL-2 was analysed after 16 hours by ELISA. The graph displays the maximum IL-2 secretion from a chemical stimulation (PMA and Ionomycin) of the effector cells alone and the background IL-2 from effector cells without any stimulus from three replicates.

[0034] FIG. 12: Cartoon showing three versions of the cassette used to generate the AND NOT gate

[0035] Activating and inhibiting CARs were co-expressed once again using a FMD-2A sequence. Signal1 is a signal peptide derived from IgG1 (but can be any effective signal peptide). scFv1 is the single-chain variable segment which recognizes CD19 (but can be a scFv or peptide loop or ligand or in fact any domain which recognizes any desired arbitrary target). STK is the human CD8 stalk but may be any non-bulky extracellular domain. CD28tm is the CD28 trans-membrane domain but can be any stable type I protein transmembrane domain and CD3Z is the CD3 Zeta endodomain but can be any endodomain which contains ITAMs. Signal2 is a signal peptide derived from CD8 but can be any effective signal peptide which is different in DNA sequence from signal1. scFv recognizes CD33 but as for scFv1 is arbitrary. muSTK is the mouse CD8 stalk but can be any spacer which co-localises but does not cross-pair with that of the activating CAR. dPTPN6 is the phosphatase domain of PTPN6. LAIR1 is the transmembrane and endodomain of LAIR1. 2Aw is a codon-wobbled version of the FMD-2A sequence. SH2-CD148 is the SH2 domain of PTPN6 fused with the phosphatase domain of CD148.

[0036] FIG. 13: Schematic representation of the chimeric antigen receptors (CARs) for the NOT AND gates

[0037] A) A stimulatory CAR consisting of an N-terminal anti-CD19 scFv domain followed by the stalk region of human CD8, human CD28 transmembrane domain and human CD247 intracellular domain. B) An inhibitory CAR consisting of an N-terminal anti-CD33 scFv domain followed by the stalk region of mouse CD8, transmembrane region of mouse CD8 and the phosphatase domain of PTPN6. C) an inhibitory CAR consisting of an N-terminal anti-CD33 scFv domain followed by the stalk region of mouse CD8 and the transmembrane and intracellular segments of LAIR1. D) An inhibitory CAR identical to previous CAR except it is co-expressed with a fusion protein of the PTPN6 SH2 domain and the CD148 phosphatase domain.

[0038] FIG. 14: Functional analysis of the NOT AND gate

[0039] Effector cells (5×10^4 cells) expressing the A) full length SHP-1 or B) truncated form of SHP-1 were co-cubated with a varying number of target cells and IL-2 was analysed after 16 hours by ELISA. The graph displays the average maximum IL-2 secretion from a chemical stimulation (PMA and Ionomycin) of the effector cells alone and the average background IL-2 from effector cells without any stimulus from three replicates.

[0040] FIG. 15: Amino acid sequence of an OR gate

[0041] FIG. 16: Amino acid sequence of a CD148 and a CD45 based AND gate

[0042] FIG. 17: Amino acid sequence of three AND NOT gates

[0043] FIG. 18: Dissection of AND gate function

[0044] A. The prototype AND gate is illustrated on the right and its function in response to CD19, CD33 single and CD19, CD33 double positive targets is shown on the left. B. The scFvs are swapped so the activating endodomain is triggered by CD33 and the inhibitory endodomain is activated by CD19. This AND gate remains functional despite this scFv swap. C. The CD8 mouse stalk replaced Fc in the spacer of the inhibitory CAR. With this modification, the gate fails to respond to either CD19 single positive or CD19, CD33 double positive targets.

[0045] FIG. 19: Expression of target antigens on artificial target cells A. Shows flow cytometry scatter plots CD19 vs CD33 of the original set of artificial target cells derived from SupT1 cells. From left to right: double negative SupT1 cells, SupT1 cells positive for CD19, positive for CD33 and positive for both CD19 and CD33. B. Shows flow cytometry scatter plots CD19 vs GD2 of the artificial target cells generated to test the CD19 AND GD2 gate: From left to right: negative SupT1 cells, SupT1 cells expressing CD19, SupT1 cells transduced with GD2 and GM3 synthase vectors which become GD2 positive and SupT1 cells transduced with CD19 as well as GD2 and GM3 synthase which are positive for both GD2 and CD19. C. Shows flow cytometry scatter plots of CD19 vs EGFRvIII of the artificial targets generated to test the CD19 AND EGFRvIII gate. From left to right: negative SupT1 cells, SupT1 cells expressing CD19, SupT1 cells transduced with EGFRvIII and SupT1 cells transduced with both CD19 and EGFRvIII. D. Shows flow cytometry scatter plots of CD19 vs CD5 of the artificial targets generated to test the CD19 AND CD5 gate. From left to right: negative 293T cells, 293T cells transduced with CD19, 293T cells transduced with CD5, 293T cells transduced with both CD5 and CD19 vectors.

[0046] FIG. 20: Generalizability of the AND gate

[0047] A. Cartoon of AND gate modified so the second CAR's specificity is changed from the original specificity of CD33, to generate 3 new CARs: CD19 AND GD2, CD19 AND EGFRvIII, CD19 AND CD5. B. CD19 AND GD2 AND gate: Left: expression of AND gate is shown recombinant CD19-Fc staining (x-axis) for the CD19 CAR, versus anti-human-Fc staining (Y-axis) for the GD2 CAR. Right: function in response to single positive and double positive targets. C. CD19 AND EGFRvIII AND gate: Left: expression of AND gate is shown recombinant CD19-Fc staining (x-axis) for the CD19 CAR, versus anti-human-Fc staining (Y-axis) for the EGFRvIII CAR. Right: function in response to single positive and double positive targets. D. CD19 AND CD5 AND gate: Left: expression of AND gate is shown recombinant CD19-Fc staining (x-axis) for the CD19 CAR, versus anti-human-Fc staining (Y-axis) for the CD5 CAR. Right: function in response to single positive and double positive targets.

[0048] FIG. 21: Function of the AND NOT gates

[0049] Function of the three implementations of an AND NOT gate is shown. A cartoon of the gates tested is shown to the right, and function in response to single positive and double positive targets is shown to the left. A. PTPN6 based AND NOT gate whereby the first CAR recognizes CD19, has a human CD8 stalk spacer and an ITAM containing activating endodomain; is co-expressed with a second CAR that recognizes CD33, has a mouse CD8 stalk spacer and has an endodomain comprising of a PTPN6 phosphatase domain. B. ITIM based AND NOT gate is identical to the PTPN6 gate, except the endodomain is replaced by the endodomain from LAIR1. C. CD148 boosted AND NOT gate is identical to the ITIM based gate except an additional fusion between the PTPN6 SH2 and the endodomain of CD148 is expressed. All three gates work as expected with activation in response to CD19 but not in response to CD19 and CD33 together.

[0050] FIG. 22: Dissection of PTPN6 based AND NOT gate function

[0051] The original PTPN6 based AND NOT gate is compared with several controls to demonstrate the model. A cartoon of the gates tested is shown to the right, and function in response to single positive and double positive targets is shown to the left. A. Original AND NOT gate whereby the first CAR recognizes CD19, has a human CD8 stalk spacer and an ITAM containing activating endodomain; is co-expressed with a second CAR recognizes CD33, has a mouse CD8 stalk spacer and has an endodomain comprising of a PTPN6 phosphatase domain. B. AND NOT gate modified so the mouse CD8 stalk spacer is replaced with an Fc spacer. C. AND NOT gate modified so that the PTPN6 phosphatase domain is replaced with the endodomain from CD148. Original AND NOT gate (A.) functions as expected triggering in response to CD19, but not in response to both CD19 and CD33. The gate in B. triggers both in response to CD19 along or CD19 and CD33 together. The gate in C. does not trigger in response to one or both targets.

[0052] FIG. 23: Dissection of LAIR1 based AND NOT gate

[0053] Functional activity against CD19 positive, CD33 positive and CD19, CD33 double-positive targets is shown. A. Structure and activity of the original ITIM based AND NOT gate. This gate is composed of two CARs: the first recognizes CD19, has a human CD8 stalk spacer and an ITAM containing endodomain; the second CAR recognizes

CD33, has a mouse CD8 stalk spacer and an ITIM containing endodomain. B Structure and activity of the control ITIM based gate where the mouse CD8 stalk spacer has been replaced by an Fc domain. This gate is composed of two CARs: the first recognizes CD19, has a human CD8 stalk spacer and an ITAM containing endodomain; the second CAR recognizes CD33, has an Fc spacer and an ITIM containing endodomain. Both gates respond to CD19 single positive targets, while only the original gate is inactive in response to CD19 and CD33 double positive targets.

[0054] FIG. 24: Kinetic segregation model of CAR logic gates

[0055] Model of kinetic segregation and behaviour of AND gate, NOT AND gate and controls. CARs recognize either CD19 or CD33. The immunological synapse can be imagined between the blue line, which represents the target cell membrane and the red line, which represents the T-cell membrane. '45' is the native CD45 protein present on T-cells. 'H8' is a CAR ectodomain with human CD8 stalk as the spacer. 'Fc' is a CAR ectodomain with human HCH2CH3 as the spacer. 'M8' is a CAR ectodomain with murine CD8 stalk as the spacer. '19' represents CD19 on the target cell surface. '33' represents CD33 on the target cell surface. The symbol '⊕' represents an activating endodomain containing ITAMS. The symbol '⊖' represents a phosphatase with slow kinetics—a 'ligation on' endodomain such as one comprising of the catalytic domain of PTPN6 or an ITIM. The symbol 'Ø' represents a phosphatase with fast kinetics—a 'ligation off' endodomain such as the endodomain of CD45 or CD148. This symbol is enlarged in the figure to emphasize its potent activity.

[0056] (a) Shows the postulated behaviour of the functional AND gate which comprises of a pair of CARs whereby the first CAR recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; and the second CAR recognizes CD33, has an Fc spacer and a CD148 endodomain;

[0057] (b) Shows the postulated behaviour of the control AND gate. Here, the first CAR recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; and the second CAR recognizes CD33, but has a mouse CD8 stalk spacer and a CD148 endodomain;

[0058] (c) Shows the behaviour of a functional AND NOT gate which comprises of a pair of CARs whereby the first CAR recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; and the second CAR recognizes CD33, has a mouse CD8 stalk spacer and a PTPN6 endodomain;

[0059] (d) Shows the postulated behaviour of the control AND NOT gate which comprises of a pair of CARs whereby the first CAR recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; and the second CAR recognizes CD33, but has an Fc spacer and a PTPN6 endodomain;

[0060] In the first column, target cells are both CD19 and CD33 negative. In the second column, targets are CD19 negative and CD33 positive. In the third column, target cells are CD19 positive and CD33 negative. In the fourth column, target cells are positive for both CD19 and CD33.

[0061] FIG. 25: Design of APRIL-based CARs.

[0062] The CAR design was modified so that the scFv was replaced with a modified form of A proliferation-inducing ligand (APRIL), which interacts with BCMA, TACI and proteoglycans, to act as an antigen binding

domain: APRIL was truncated so that the proteoglycan binding amino-terminus is absent. A signal peptide was then attached to truncated APRIL amino-terminus to direct the protein to the cell surface. Three CARs were generated with this APRIL based binding domain: A. In the first CAR, the human CD8 stalk domain was used as a spacer domain. B. In the second CAR, the hinge from IgG1 was used as a spacer domain. C. In the third CAR, the hinge, CH2 and CH3 domains of human IgG1 modified with the pva/a mutations described by Hombach et al (2010 Gene Ther. 17:1206-1213) to reduce Fc Receptor binding was used as a spacer (henceforth referred as Fc-pvaa). In all CARs, these spacers were connected to the CD28 transmembrane domain and then to a tripartite endodomain containing a fusion of the CD28, OX40 and the CD3-Zeta endodomain (Pule et al, Molecular therapy, 2005: Volume 12; Issue 5; Pages 933-41).

[0063] FIG. 26: Annotated Amino acid sequence of the above three APRIL-CARS

[0064] A: Shows the annotated amino acid sequence of the CD8 stalk APRIL CAR; B: Shows the annotated amino acid sequence of the APRIL IgG1 hinge based CAR; C: Shows the annotated amino acid sequence of the APRIL Fc-pvaa based CAR.

[0065] FIG. 27: Expression and ligand binding of different APRIL based CARs

[0066] A. The receptors were co-expressed with a marker gene truncated CD34 in a retroviral gene vector. Expression of the marker gene on transduced cells allows confirmation of transduction. B. T-cells were transduced with APRIL based CARs with either the CD8 stalk spacer, IgG1 hinge or Fc spacer. To test whether these receptors could be stably expressed on the cell surface, T-cells were then stained with anti-APRIL-biotin/Streptavidin APC and anti-CD34. Flow-cytometric analysis was performed. APRIL was equally detected on the cell surface in the three CARs suggesting they are equally stably expressed. C. Next, the capacity of the CARs to recognize TACI and BCMA was determined. The transduced T-cells were stained with either recombinant BCMA or TACI fused to mouse IgG2a Fc fusion along with an anti-mouse secondary and anti-CD34. All three receptor formats showed binding to both BCMA and TACI. A surprising finding was that binding to BCMA seemed greater than to TACI. A further surprising finding was that although all three CARs were equally expressed, the CD8 stalk and IgG1 hinge CARs appeared better at recognizing BCMA and TACI than that with the Fc spacer.

[0067] FIG. 28: Function of the different CAR constructs.

[0068] Functional assays were performed with the three different APRIL based CARs. Normal donor peripheral blood T-cells either non-transduced (NT), or transduced to express the different CARs. Transduction was performed using equal titer supernatant. These T-cells were then CD56 depleted to remove non-specific NK activity and used as effectors. SupT1 cells either non-transduced (NT), or transduced to express BCMA or TACI were used as targets. Data shown is mean and standard deviation from 5 independent experiments. A. Specific killing of BCMA and TACI expressing T-cells was determined using Chromium release. B. Interferon- μ release was also determined. Targets and effectors were co-cultured at a ratio of 1:1. After 24 hours, Interferon-p in the supernatant was assayed by ELISA. C. Proliferation/survival of CAR T-cells were also determined by counting number of CAR T-cells in the same co-culture

incubated for a further 6 days. All 3 CARs direct responses against BCMA and TACI expressing targets. The responses to BCMA were greater than for TACI.

[0069] FIG. 29: AND gate functionality in primary cells.

[0070] PBMCs were isolated from blood and stimulated using PHA and IL-2. Two days later the cells were transduced on retronectin coated plates with retro virus containing the CD19:CD33 AND gate construct. On day 5 the expression level of the two CARs translated by the AND gate construct was evaluated via flow cytometry and the cells were depleted of CD56+ cells (predominantly NK cells). On day 6 the PBMCs were placed in a co-culture with target cells at a 1:2 effector to target cell ratio. On day 8 the supernatant was collected and analysed for IFN-gamma secretion via ELISA

[0071] FIG. 30: A. DNA alignment of the CD28 transmembrane and cytosolic domain of TCRz in the OR gate platform B. Protein alignment of the CD28 transmembrane and cytosolic domain of TCRz in the OR gate platform.

[0072] FIG. 31: Design rules for building logic gated CAR T-cells. OR, AND NOT and AND gated CARs are shown in cartoon format with the target cell on top, and the T-cell at the bottom with the synapse in the middle. Target cells express arbitrary target antigens A, and B.

[0073] T-cells express two CARs which comprise of anti-A and anti-B recognition domains, spacers and endodomains. An OR gate requires (1) spacers simply which allow antigen recognition and CAR activation, and (2) both CARs to have activatory endodomains; An AND NOT gate requires (1) spacers which result in co-segregation of both CARs upon recognition of both antigens and (2) one CAR with an activatory endodomain, and the other whose endodomain comprises or recruits a weak phosphatase; An AND gate requires (1) spacers which result in segregation of both CARs into different parts of the immunological synapse upon recognition of both antigens and (2) one CAR with an activatory endodomain, and the other whose endodomain comprises of a potent phosphatase.

SUMMARY OF ASPECTS OF THE INVENTION

[0074] The present inventors have developed a panel of “logic-gated” chimeric antigen receptor pairs which, when expressed by a cell, such as a T cell, are capable of detecting a particular pattern of expression of at least two target antigens. If the at least two target antigens are arbitrarily denoted as antigen A and antigen B, the three possible options are as follows:

[0075] “OR GATE”—T cell triggers when either antigen A or antigen B is present on the target cell

[0076] “AND GATE”—T cell triggers only when both antigens A and B are present on the target cell

[0077] “AND NOT GATE”—T cell triggers if antigen A is present alone on the target cell, but not if both antigens A and B are present on the target cell

[0078] Engineered T cells expressing these CAR combinations can be tailored to be exquisitely specific for cancer cells, based on their particular expression (or lack of expression) of two or more markers.

[0079] Thus in a first aspect, the present invention provides a cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR at the cell surface, each CAR comprising:

[0080] (i) an antigen-binding domain;

[0081] (ii) a spacer

[0082] (iii) a trans-membrane domain; and

[0083] (iv) an intracellular T cell signaling domain (endodomain)

[0084] wherein the antigen binding domains of the first and second CARs bind to different antigens, and wherein each of the first or second CARs is an activating CAR comprising an activating intracellular T cell signaling domain.

[0085] The cell may be an immune effector cell, such as a T-cell or natural killer (NK) cell. Features mentioned herein in connection with a T cell apply equally to other immune effector cells, such as NK cells.

[0086] The spacer of the first CAR may be different to the spacer of the second CAR, such that the first and second CARs do not form heterodimers

[0087] The spacer of the first CAR may have a different length and/or size and/or configuration from the spacer of the second CAR, such that each CAR is tailored for recognition of its respective target antigen.

[0088] The spacer of the first CAR may have a different length and/or charge and/or shape and/or configuration and/or glycosylation to the spacer of the second CAR, such that when the first CAR and the second CAR bind their respective target antigens, the first CAR and second CAR become spatially separated on the T cell. Ligation of the first and second CARs to their respective antigens causes them to be compartmentalized together or separately in the immunological synapse resulting in control of activation. This may be understood when one considers the kinetic separation model of T-cell activation (see below).

[0089] The first spacer or the second spacer may comprise a CD8 stalk and the other spacer may comprise the hinge, CH2 and CH3 domain of an IgG1.

[0090] In the present invention, relating to the “OR” gate, both the first and second CAR are activating CARs. An activating CAR comprises an activating endodomain, such that it causes T cell activation upon antigen binding. Since the OR gate comprises first and second CARs which are both activating, T cell activation occurs when a target cell expresses either or both target antigens.

[0091] Either the first or the second CAR may bind CD19 and the other CAR may bind CD20. This is advantageous because some lymphomas and leukemias become CD19 negative after CD19 targeting, so it gives a “back-up” antigen, should this occur.

[0092] In a second aspect, the present invention provides a nucleic acid sequence encoding both the first and second chimeric antigen receptors (CARs) as defined in the first aspect of the invention.

[0093] The nucleic acid sequence according may have the following structure: AgB1-spacer1-TM1-endo1-coexpr-AgB2-spacer2-TM2-endo2 in which

[0094] AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

[0095] spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

[0096] TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CAR;

[0097] endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR;

[0098] coexpr is a nucleic acid sequence allowing co-expression of two CARs (e.g. a cleavage site);

[0099] AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

[0100] spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

[0101] TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CAR;

[0102] endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR;

which nucleic acid sequence, when expressed in a T cell, encodes a polypeptide which is cleaved at the cleavage site such that the first and second CARs are co-expressed at the T cell surface.

[0103] The nucleic acid sequence allowing co-expression of two CARs may encode a self-cleaving peptide or a sequence which allows alternative means of co-expressing two CARs such as an internal ribosome entry sequence or a 2nd promoter or other such means whereby one skilled in the art can express two proteins from the same vector.

[0104] Alternative codons may be used in regions of sequence encoding the same or similar amino acid sequences, such as the transmembrane and/or intracellular T cell signalling domain (endodomain) in order to avoid homologous recombination. An example of such "codon wobbling" is shown in FIG. 30.

[0105] In a third aspect, the present invention provides a kit which comprises

[0106] (i) a first nucleic acid sequence encoding the first chimeric antigen receptor (CAR) as defined in the first aspect of the invention, which nucleic acid sequence has the following structure:

[0107] AgB1-spacer1-TM1-endo1

in which

[0108] AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

[0109] spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

[0110] TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CAR;

[0111] endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR; and

[0112] (ii) a second nucleic acid sequence encoding the second chimeric antigen receptor (CAR) as defined in the first aspect of the invention, which nucleic acid sequence has the following structure:

[0113] AgB2-spacer2-TM2-endo2

[0114] AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

[0115] spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

[0116] TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CAR;

[0117] endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR.

[0118] In a fourth aspect, the present invention provides a kit comprising: a first vector which comprises the first nucleic acid sequence as defined above; and a second vector which comprises the first nucleic acid sequence as defined above.

[0119] The vectors may be plasmid vectors, retroviral vectors or transposon vectors. The vectors may be lentiviral vectors.

[0120] In a fifth aspect, the present invention provides a vector comprising a nucleic acid sequence according to the second aspect of the invention. The vector may be a lentiviral vector.

[0121] The vector may be a plasmid vector, a retroviral vector or a transposon vector.

[0122] In a sixth aspect, the present invention involves co-expressing more than two CARs in such a fashion that a complex pattern of more than two antigens can be recognized on the target cell.

[0123] In a seventh aspect, the present invention provides a method for making a T cell according to the first aspect of the invention, which comprises the step of introducing one or more nucleic acid sequence(s) encoding the first and second CARs; or one or more vector(s) as defined above into a T cell.

[0124] The T cell may be from a sample isolated from a patient, a related or unrelated haematopoietic transplant donor, a completely unconnected donor, from cord blood, differentiated from an embryonic cell line, differentiated from an inducible progenitor cell line, or derived from a transformed T cell line.

[0125] In an eighth aspect, the present invention provides a pharmaceutical composition comprising a plurality of T cells according to the first aspect of the invention.

[0126] In a ninth aspect, the present invention provides a method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to the eighth aspect of the invention to a subject.

[0127] The method may comprise the following steps:

[0128] (i) isolation of a T cell as listed above.

[0129] (ii) transduction or transfection of the T cells with one or more nucleic acid sequence(s) encoding the first and second CAR or one or more vector(s) comprising such nucleic acid sequence(s); and

[0130] (iii) administering the T cells from (ii) to the subject.

[0131] The disease may be a cancer.

[0132] In a tenth aspect, the present invention provides a pharmaceutical composition according to the eighth aspect of the invention for use in treating and/or preventing a disease.

[0133] The disease may be a cancer.

[0134] In an eleventh aspect, the present invention provides use of a T cell according to the first aspect of the invention in the manufacture of a medicament for treating and/or preventing a disease.

[0135] The disease may be a cancer.

[0136] The present invention also provides a nucleic acid sequence which comprises:

[0137] a) a first nucleotide sequence encoding a first chimeric antigen receptor (CAR);

[0138] b) a second nucleotide sequence encoding a second CAR;

[0139] c) a sequence encoding a self-cleaving peptide positioned between the first and second nucleotide sequences, such that the two CARs are expressed as separate entities.

[0140] Alternative codons may be used in one or more portion(s) of the first and second nucleotide sequences in regions which encode the same or similar amino acid sequence(s).

[0141] The present invention also provides a vector and a cell comprising such a nucleic acid.

[0142] By providing two or more CARs on the surface of the T cell, it is possible to target multiple cancer markers simultaneously, providing better therapeutic efficacy for heterogeneous tumours and avoiding the problem of cancer escape.

[0143] Because the CARs are expressed on the surface of the T cell as separate molecules, this approach overcomes the spatial and accessibility issues associated with TanCARs. T-cell activation efficiency is also improved. As each CAR has its own spacer, it is possible to tailor the spacer and therefore the distance that the binding domain projects from the T cell surface and its flexibility etc to the particular target antigen. This choice is unfettered by the design considerations associated with TanCARs, i.e. that one CAR needs to be juxtaposed to the T cell membrane and one CAR needs to be distal, positioned in tandem to the first CAR.

[0144] By providing a single nucleic acid which encodes the two CARs separated by a cleavage site, it is possible to engineer T cells to co-express the two CARs using a simple single transduction procedure. A double transfection procedure could be used with CAR-encoding sequences in separate constructs, but this would be more complex and expensive and requires more integration sites for the nucleic acids. A double transfection procedure would also be associated with uncertainty as to whether both CAR-encoding nucleic acids had been transduced and expressed effectively. This is especially true for a multiple CAR approach where three or more CARs are introduced to the cell.

[0145] The two or more CARs will have portions of high homology, for example the transmembrane and/or intracellular signalling domains are likely to be highly homologous. If the same or similar linkers are used for the two CARs, then they will also be highly homologous. This would suggest that an approach where both CARs are provided on a single nucleic acid sequence would be inappropriate, because of the likelihood of homologous recombination between the sequences. However, the present inventors have found that by "codon wobbling" the portions of sequence encoding areas of high homology, it is possible to express two CARs from a single construct with high efficiency. Codon wobbling involves using alternative codons in regions of sequence encoding the same or similar amino acid sequences.

FURTHER ASPECTS OF THE INVENTION

[0146] The present invention also relates to the aspects listed in the following numbered paragraphs:

[0147] 1. A T cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR at the cell surface, each CAR comprising:

[0148] (i) an antigen-binding domain;

[0149] (ii) a spacer

[0150] (iii) a trans-membrane domain; and

[0151] (iv) an endodomain

wherein the antigen binding domains of the first and second CARs bind to different antigens, wherein the spacer of the first CAR is different to the spacer of the second CAR and wherein one of the first or second CARs is an activating CAR comprising an activating endodomain and the other CAR is either an activating CAR comprising an activating endodomain or an inhibitory CAR comprising a ligation-on or ligation-off inhibitory endodomain.

[0152] 2. A T cell according to paragraph 1, wherein the spacer of the first CAR has a different length and/or charge and/or size and/or configuration and/or glycosylation of the spacer of the second CAR, such that when the first CAR and the second CAR bind their respective target antigens, the first CAR and second CAR become spatially separated on the T cell membrane.

[0153] 3. A T cell according to paragraph 2, wherein either the first spacer or the second spacer comprises a CD8 stalk and the other spacer comprises the hinge, CH2 and CH3 domain of IgG1.

[0154] 4. A T cell according to paragraph 1, wherein both the first and second CARs are activating CARs.

[0155] 5. A T cell according to paragraph 4, wherein one CAR binds CD19 and the other CAR binds CD20.

[0156] 6. A T cell according to paragraph 2 or 3, wherein one of the first or second CARs is an activating CAR comprising an activating endodomain, and the other CAR is an inhibitory CAR comprising a ligation-off inhibitory endodomain, which inhibitory CAR inhibits T-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but does not significantly inhibit T-cell activation by the activating CAR when the inhibitory CAR is ligated.

[0157] 7. A T cell according to paragraph 6, wherein the inhibitory endodomain comprises all or part of the endodomain from CD148 or CD45.

[0158] 8. A T cell according to paragraph 6 or 7, wherein the antigen-binding domain of the first CAR binds CD5 and the antigen-binding domain of the second CAR binds CD19.

[0159] 9. A T cell according to paragraph 1 wherein the first and second spacers are sufficiently different so as to prevent cross-pairing of the first and second CARs but are sufficiently similar to result in co-localisation of the first and second CARs following ligation.

[0160] 10. A T cell according to paragraph 9, wherein one of the first or second CARs is an activating CAR comprising an activating endodomain, and the other CAR is an inhibitory CAR comprising a ligation-on inhibitory endodomain, which inhibitory CAR does not significantly inhibit T-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but inhibits T-cell activation by the activating CAR when the inhibitory CAR is ligated.

[0161] 11. A T cell according to paragraph 10, wherein the ligation-on inhibitory endodomain comprises at least part of a phosphatase.

[0162] 12. A T cell according to paragraph 11, wherein the ligation-on inhibitory endodomain comprises all or part of PTPN6.

[0163] 13. A T cell according to paragraph 10, wherein the ligation-on inhibitory endodomain comprises at least one ITIM domain.

[0164] 14. A T cell according to paragraph 13, wherein activity of the ligation-on inhibitory endodomain is enhanced by co-expression of a PTPN6-CD45 or -CD148 fusion protein.

[0165] 15. A T cell according to any of paragraphs 10 to 14, wherein the CAR comprising the activating endodomain comprises an antigen-binding domain which binds CD33 and the CAR which comprises the ligation-on inhibitory endodomain comprises an antigen-binding domain which binds CD34.

[0166] 16. A T cell which comprises more than two CARs as defined in the preceding paragraphs such that it is

specifically stimulated by a cell, such as a T cell, bearing a distinct pattern of more than two antigens.

[0167] 17. A nucleic acid sequence encoding both the first and second chimeric antigen receptors (CARs) as defined in any of paragraphs 1 to 16.

[0168] 18. A nucleic acid sequence according to paragraph 17, which has the following structure:

[0169] AgB1-spacer1-TM1-endo1-coexpr-AbB2-spacer2-TM2-endo2

in which

[0170] AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

[0171] spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

[0172] TM1 is a a nucleic acid sequence encoding the transmembrane domain of the first CAR;

[0173] endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR;

[0174] coexpr is a nucleic acid sequence enabling co-expression of both CARs

[0175] AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

[0176] spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

[0177] TM2 is a a nucleic acid sequence encoding the transmembrane domain of the second CAR;

[0178] endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR; which nucleic acid sequence, when expressed in a T cell, encodes a polypeptide which is cleaved at the cleavage site such that the first and second CARs are co-expressed at the T cell surface.

[0179] 19. A nucleic acid sequence according to paragraph 18, wherein coexpr encodes a sequence comprising a self-cleaving peptide.

[0180] 20. A nucleic acid sequence according to paragraph 18 or 19, wherein alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

[0181] 21. A kit which comprises

[0182] (i) a first nucleic acid sequence encoding the first chimeric antigen receptor (CAR) as defined in any of paragraphs 1 to 16, which nucleic acid sequence has the following structure:

[0183] AgB1-spacer1-TM1-endo1

in which

[0184] AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

[0185] spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

[0186] TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CAR;

[0187] endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR; and

[0188] (ii) a second nucleic acid sequence encoding the second chimeric antigen receptor (CAR) as defined in any of paragraphs 1 to 16, which nucleic acid sequence has the following structure:

[0189] AgB2-spacer2-TM2-endo2

[0190] AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

[0191] spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

[0192] TM2 is a a nucleic acid sequence encoding the transmembrane domain of the second CAR;

[0193] endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR.

[0194] 22. A kit comprising: a first vector which comprises the first nucleic acid sequence as defined in paragraph 21; and a second vector which comprises the first nucleic acid sequence as defined in paragraph 21.

[0195] 23. A kit according to paragraph 22, wherein the vectors are integrating viral vectors or transposons.

[0196] 24. A vector comprising a nucleic acid sequence according to any of paragraphs 17 to 20.

[0197] 25. A retroviral vector or a lentiviral vector or a transposon according to paragraph 24.

[0198] 26. A method for making a T cell according to any of paragraphs 1 to 16, which comprises the step of introducing: a nucleic acid sequence according to any of paragraphs 17 to 20; a first nucleic acid sequence and a second nucleic acid sequence as defined in paragraph 21; and/or a first vector and a second vector as defined in paragraph 22 or a vector according to paragraph 24 or 25, into a T cell.

[0199] 27. A method according to paragraph 24, wherein the T cell is from a sample isolated from a subject.

[0200] 28. A pharmaceutical composition comprising a plurality of T cells according to any of paragraphs 1 to 16.

[0201] 29. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to paragraph 28 to a subject.

[0202] 30. A method according to paragraph 29, which comprises the following steps:

[0203] (i) isolation of a T cell-containing sample from a subject;

[0204] (ii) transduction or transfection of the T cells with: a nucleic acid sequence according to any of paragraphs 17 to 20; a first nucleic acid sequence and a second nucleic acid sequence as defined in paragraph 21; a first vector and a second vector as defined in paragraph 22 or 23 or a vector according to paragraph 24 or 25; and

[0205] (iii) administering the T cells from (ii) to a the subject.

[0206] 31. A method according to paragraph 29 or 30, wherein the disease is a cancer.

[0207] 32. A pharmaceutical composition according to paragraph 28 for use in treating and/or preventing a disease.

[0208] 33. The use of a T cell according to any of paragraphs 1 to 16 in the manufacture of a medicament for treating and/or preventing a disease.

DETAILED DESCRIPTION

[0209] Chimeric Antigen Receptors (CARs)

[0210] CARs, which are shown schematically in FIG. 1, are chimeric type I trans-membrane proteins which connect an extracellular antigen-recognizing domain (binder) to an intracellular signalling domain (endodomain). The binder is typically a single-chain variable fragment (scFv) derived from a monoclonal antibody (mAb), but it can be based on other formats which comprise an antibody-like antigen binding site. A spacer domain is usually necessary to isolate the binder from the membrane and to allow it a suitable orientation. A common spacer domain used is the Fc of IgG1. More compact spacers can suffice e.g. the stalk from CD8a and even just the IgG1 hinge alone, depending on the

antigen. A trans-membrane domain anchors the protein in the cell membrane and connects the spacer to the endodomain.

[0211] Early CAR designs had endodomains derived from the intracellular parts of either the γ chain of the Fc ϵ R1 or CD3 ξ . Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3 ξ results in second generation receptors which can transmit an activating and co-stimulatory signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most potent co-stimulatory signal—namely immunological signal 2, which triggers T-cell proliferation. Some receptors have also been described which include TNF receptor family endodomains, such as the closely related OX40 and 41BB which transmit survival signals. Even more potent third generation CARs have now been described which have endodomains capable of transmitting activation, proliferation and survival signals.

[0212] CAR-encoding nucleic acids may be transferred to T cells using, for example, retroviral vectors. Lentiviral vectors may be employed. In this way, a large number of cancer-specific T cells can be generated for adoptive cell transfer. When the CAR binds the target-antigen, this results in the transmission of an activating signal to the T-cell it is expressed on. Thus the CAR directs the specificity and cytotoxicity of the T cell towards tumour cells expressing the targeted antigen.

[0213] The first aspect of the invention relates to a T-cell which co-expresses a first CAR and a second CAR such that a T-cell can recognize a desired pattern of expression on target cells in the manner of a logic gate as detailed in the truth tables: table 1, 2 and 3.

[0214] Both the first and second (and optionally subsequent) CARs comprise:

[0215] (i) an antigen-binding domain;

[0216] (ii) a spacer;

[0217] (iii) a transmembrane domain; and

[0218] (iii) an intracellular domain.

TABLE 1

Truth Table for CAR OR GATE		
Antigen A	Antigen B	Response
Absent	Absent	No activation
Absent	Present	Activation
Present	Absent	Activation
Present	Present	Activation

TABLE 2

Truth Table for CAR AND GATE		
Antigen A	Antigen B	Response
Absent	Absent	No activation
Absent	Present	No Activation
Present	Absent	No Activation
Present	Present	Activation

TABLE 3

Truth Table for CAR AND NOT GATE		
Antigen A	Antigen B	Response
Absent	Absent	No activation
Absent	Present	No Activation
Present	Absent	Activation
Present	Present	No Activation

[0219] The first and second CAR of the T cell of the present invention may be produced as a polypeptide comprising both CARs, together with a cleavage site.

[0220] SEQ ID No. 1 to 5 give examples of such polypeptides, which each comprise two CARs. The CAR may therefore comprise one or other part of the following amino acid sequences, which corresponds to a single CAR.

[0221] SEQ ID No 1 is a CAR OR gate which recognizes CD19 OR CD33

[0222] SEQ ID No 2 Is a CAR AND gate which recognizes CD19 AND CD33 using a CD148 phosphatase

[0223] SEQ ID No 3 Is an alternative implementation of the CAR AND GATE which recognizes CD19 AND CD33 which uses a CD45 phosphatase

[0224] SEQ ID No 4 Is a CAR AND NOT GATE which recognizes CD19 AND NOT CD33 based on PTPN6 phosphatase

[0225] SEQ ID No 5 Is an alternative implementation of the CAR AND NOT gate which recognizes CD19 AND NOT CD33 and is based on an ITIM containing endodomain from LAIR1 SEQ ID No 6. Is a further alternative implementation of the CAR AND NOT gate which recognizes CD19 AND NOT CD33 and recruits a PTPN6-CD148 fusion protein to an ITIM containing endodomain.

SEQ ID No. 1

MSLPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDI
 SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDSLISNLE
 QEDIATYFCQQNTLPYTFGGGKLEITKAGGGSGGGSGGGSGGGGS
 EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
 IWGSETTYNSALKSRLTIKDNKSQVFLKMNSLQTDITAIYYCAKHYY
 YGGSYAMDYWGQTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
 PAAGGAVHTRGLDFACDIFWVLVVGGVLACYSLLVTVAFIIFWVRVVKF
 SRSADAPAYQQQNQLYNELNLGRREYDVLDKRRGRDPEMGKPKRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
 HMQALPPRAEGRGSLTTCGDVEENPGPMAVPTQVLGLLLLWLTARCDI
 QMTQSPSSLSASVGDRTITCRASEDIYFNLVWYQQKPKGAPKLLIYDTN
 RLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGT
 KLEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVQPGGSLRLS
 CAASGFTLSNYGMHWIRQAPGKLEWVSSIISLNGGSTYYRDSVKGRFTIS
 RDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVSSM
 DPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW

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LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSLKLTVD
KSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKKDPKFWLVVVGGVLA
CYSLLVTVAFIIFWVRSRVKFSRSDAPAYQQGQNQLYNELNLGRREYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 2

MSLPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE
QEDIATYFCQQGNTLPYTFGGGKLEITKAGGGSGGGSGGGSGGGGS
EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
IWGETTYNSALKSRLTIKDNSKSQVFLKIVINSLQTDDBTAIYYCAKH
YYYGGSYAMDYWGQGSTVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEA
CRPAAGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRV
KFSRSADAPAYQQGQNQLYNELNLGRREYDVLDRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPRAEGRGSLTTCGDVEENPGMAVPTQVLGLLLLLWLTARCDI
DIQMTQSPSSLSASVGRVTITCRASEDIYFNLVWYQQKPKGAPKLLIYD
TNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQ
GKLEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVPGGSLR
LSAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFT
ISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVS
SMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSLKLT
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKKDPKAVFGCIGAL
VIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKSKLIRVENFEAYFKKQQA
DSNCGFAEEYEDLKLGVISQPKYAAELAENRGKNRYNNVLPYDISRVKLS
VQTHSTDDYINANYMPGYHKKDFIATQGGLPNTLKDFWRMVWEKNVYAI
IMLTCKVEQGRTKCEEYWPQQAQDYGDITVAMTSEIVLPEWTIRDFTVK
NIQTSESHPLRQFHTSWPDHGVPTDITLLINFRYLVRDYMKGQSPPEPI
LVHCSAGVGRGTGTFIAIDRLIYQIENENTVDVYGVYDLRMRPLMVQTE
DQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTFGKTN
IA

SEQ ID No. 3

MSLPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGIVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE
QEDIATYFCQQGNTLPYTFGGGKLEITKAGGGSGGGSGGGSGGGGS
EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
IWGETTYNSALKSRLTIKDNSKSQVFLKMNLSLQTDDBTAIYYCAKH
YGGSYAMDYWGQGSTVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYLLVTVAFIIFWVRRVKFS

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IWGSETTYNSALKSRLTIKDNSKSQVFLKMNLSLQTDDBTAIYYCAKH
YGGSYAMDYWGQGSTVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKF
SRSADAPAYQQGQNQLYNELNLGRREYDVLDRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
HMQALPPRAEGRGSLTTCGDVEENPGMAVPTQVLGLLLLLWLTARCDI
QMTQSPSSLSASVGRVTITCRASEDIYFNLVWYQQKPKGAPKLLIYDTN
RLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGT
KLEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVPGGSLRLS
CAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTIS
RDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVSSM
DPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEVTCV
VDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSLKLTVD
KSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKKDPKALIAFLAFILIV
TSIALLVVLYKIYDLHKKRSCNLDQQLVERDDEKQLMNVEPIHADILL
ETYKRKIADEGRFLAEFQSI PRVFSKFPKIEARKPFQNKKNRYVDILPY
DYNRVELSEINGDAGSNYINASYIDGKFEPRKYIAAQGPRDETVDDFWRM
IWEQKATVIVMVRCEEGNRNKAIEYWPMSMEETRAFGDVVVKINQHKRC
PDYIIQKLNIVNKKKATGREVTHIQFTSWPDHGVPEPHLLKLRRRVN
AFSNFFSGPIVVHCSAGVGRGTGTIYIGIDAMLEGLEAENKVDVYGVVVKLR
RQRCLMVQVEAQYILIHQALVEYNQFGETEVLNSELHPYLHNMKKRDPPS
EPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVI PYDYNRVLPHK
ELEMSKESEHDSDESSDDSDSEEPSKYINASFIMSYWKEPMIAAQGGL
KETIGDFWQMIQKRVKIVMLTELKHGQDEICAQYWGEGKQTYGDI EVD
LKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQTNWSVEQLPAEPKELIS
MIQVVKQKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLESA
ETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKN
NHQEDKIEFDNEVDKVKQDANCNVNPLGAPEKLEPAKEQAEGSEPTSGTEG
PEHSVNGPASPALNQGS

SEQ ID No. 4

MSLPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDI
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EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
IWGETTYNSALKSRLTIKDNSKSQVFLKMNLSLQTDDBTAIYYCAKH
YGGSYAMDYWGQGSTVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYLLVTVAFIIFWVRRVKFS

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RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
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 MQALPPRAEGRGSLTTCGDVEENPGMAVPTQVLGLLLLLWLTARCDIQ
 MTQSPSSLSASVGDVRTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNR
 LADGVPSRFSGSGSGTQYTLTISSLPEDFATYYCQHYKNYPLTFGQGTK
 LEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVQPGGSLRLSC
 AASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISR
 DNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVSSMD
 PATTTKPVLRTPSPVHPTGTSGPQRPEDCRPRGSKVKTGLDFACDIYWAP
 LAGICVALLLSLIITLCYHRSRKRVCSSGGGSFWEFESLQKQEVKNLH
 QRLEGQRPENKGNRYKNILPFDHSRVLQGRDSNIPGSDYINANYIKNQ
 LLGPDENAKTYIASQGCLEATVNDWFQMAWQENSRIVMTTREVKEGRNK
 CVPYWPVEGMQRAYGPYSVTNCGEHDTEYKLRQLQVSPLDNGDLIREIW
 HYQYLSWPDHGVPEPGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRI
 GTIIVIDMLMENISTKGLDCDIDIKTIQMVRAQRSGMVQTEAQYKFIYV
 AIAQFIETTKKKL

SEQ ID No. 5

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 QEDIATYFCQQGNTLPYTFGGGKLEITKAGGGSGGGSGGGSGGGSGGS
 EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
 IWGETTYNSALKSRLTIKDNSKSVFLKMNSLQTDATAIYYCAKHYY
 YGGSYAMDYWGQGSTVTVSSDPITTPAPRPPTPAPTIASQPLSLRPEACR
 PAAGGAVHTRGLDFACDIFWVLVVGGLVACYSLLVTVAFIIFWVRRVKF
 SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDAL
 HMQALPPRAEGRGSLTTCGDVEENPGMAVPTQVLGLLLLLWLTARCDI
 QMTQSPSSLSASVGDVRTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTN
 RLADGVPSRFSGSGSGTQYTLTISSLPEDFATYYCQHYKNYPLTFGQGT
 KLEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVQPGGSLRLS
 CAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTIS
 RDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVSSM
 DPATTTKPVLRTPSPVHPTGTSGPQRPEDCRPRGSKVKTGLDFACDILIG
 VSVVFLFCLLLLVLFLHRQNQIKQGPGRSKDEEQKQQRPD LAVDVLER
 TADKATVNLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQTARAVSP
 QSTKPAESITYAAVARH

SEQ ID No. 6

MSLPVTALLPLALLLHAARPDIMTQTSSLSASLGDRVTISCRASQDI
 SKYLNWYQQKPDGTVKLLIYHTRSRLHSGVPSRFSGSGSGTDYSLTISNLE
 QEDIATYFCQQGNTLPYTFGGGKLEITKAGGGSGGGSGGGSGGGSGGS
 EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG

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IWGSETTYNSALKSRLTIKDNSKSVFLKMNSLQTDATAIYYCAKHYY
 YGGSYAMDYWGQGSTVTVSSDPITTPAPRPPTPAPTIASQPLSLRPEACR
 PAAGGAVHTRGLDFACDIFWVLVVGGLVACYSLLVTVAFIIFWVRRVKF
 SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDAL
 HMQALPPRAEGRGSLTTCGDVEENPGMAVPTQVLGULLLWLTARCDIQ
 MTQSPSSLSASVGDVRTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNR
 LADGVPSRFSGSGSGTQYTLTISSLPEDFATYYCQHYKNYPLTFGQGTK
 LEIKRSGGGSGGGSGGGSGGGSGRSEVQLVESGGGLVQPGGSLRLSC
 AASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISR
 DNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGLTVTVSSMD
 PATTTKPVLRTPSPVHPTGTSGPQRPEDCRPRGSKVKTGLDFACDILIG
 SVVFLFCLLLLVLFLHRQNQIKQGPGRSKDEEQKQQRPD LAVDVLERT
 ADKATVNLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQTARAVSPQ
 STKPAESITYAAVARHRAEGRGSLTTCGDVEENPGPWYHGHMSGGQAET
 LLQAKGEPWTFVLVRESLSQPGDFVLSVLSQPKAGPGSPLRVTHIKVMCE
 GGRYTVGGLTDFSLTDLVEHFKKTGIEEASGAFVYLRQPYSGGGGSFEA
 YFKKQADNSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYD
 ISRVKLSVQTHSTDDYINANYMPGYHKKDFIATQGPLPNTLKDFWRMVW
 EKNVYAIIMLTCKVEQGRTKCEEYWP SKQAQDYGDITVAMTSEIVLPEWT
 IRDFTVKNIQTSSEHPLRQFHFTSWPDHGVPTD TDLINFRYLVRDYMKQ
 SPPEPILVHCSAGVGRGTGTFIAIDRLIYQIENENTVDYGVIVYDLRMHR
 PLMVQTEDQYVFLNQCVLDIVRSQKSKVDLIYQNTTAMTIYENLAPVTT
 FGKTYNGIASGS

[0226] The CAR may comprise a variant of the CAR-encoding part of the sequence shown as SEQ ID No. 1, 2, 3, 4, 5 or 6 having at least 80, 85, 90, 95, 98 or 99% sequence identity, provided that the variant sequence is a CAR having the required properties.

[0227] Methods of sequence alignment are well known in the art and are accomplished using suitable alignment programs. The % sequence identity refers to the percentage of amino acid or nucleotide residues that are identical in the two sequences when they are optimally aligned. Nucleotide and protein sequence homology or identity may be determined using standard algorithms such as a BLAST program (Basic Local Alignment Search Tool at the National Center for Biotechnology Information) using default parameters, which is publicly available at <http://blast.ncbi.nlm.nih.gov>. Other algorithms for determining sequence identity or homology include: LALIGN (<http://www.ebi.ac.uk/Tools/psa/lalign/>) and <http://www.ebi.ac.uk/Tools/psa/lalign/nucleotide.html>), AMAS (Analysis of Multiply Aligned Sequences, at <http://www.compbio.dundee.ac.uk/Software/Amas/amas.html>), FASTA (<http://www.ebi.ac.uk/Tools/sss/fasta/>) Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>), SIM (<http://web.expasy.org/sim/>), and EMBOSS Needle (http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html).

[0228] CAR Logical or Gate

[0229] In this embodiment, the antigen binding domains of the first and second CARs of the present invention bind to different antigens and both CARs comprise an activating endodomain. The spacer domains may be the same, or sufficiently different to prevent cross-pairing of the two different receptors. A T cell can hence be engineered to activate upon recognition of either or both antigens. This is useful in the field of oncology as indicated by the Goldie-Coldman hypothesis: sole targeting of a single antigen may result in tumour escape by modulation of said antigen due to the high mutation rate inherent in most cancers. By simultaneously targeting two antigens, the probability of such escape is exponentially reduced.

[0230] Various tumour associated antigens are known as shown in the following Table 4. For a given disease, the first CAR and second CAR may bind to two different TAAs associated with that disease. In this way, tumour escape by modulating a single antigen is prevented, since a second antigen is also targeted. For example, when targeting a B-cell malignancy, both CD19 and CD20 can be simultaneously targeted. In this embodiment, it is important that the two CARs do not heterodimerize.

TABLE 4

Cancer type	TAA
Diffuse Large B-cell Lymphoma	CD19, CD20
Breast cancer	ErbB2, MUC1
AML	CD13, CD33
Neuroblastoma	GD2, NCAM
B-CLL	CD19, CD52
Colorectal cancer	Folate binding protein, CA-125

Kinetic Segregation Model

[0231] Subsequent pairing of CARs to generate the AND gate and the AND NOT gate are based on the kinetic segregation model (KS) of T-cell activation. This is a functional model, backed by experimental data, which explains how antigen recognition by a T-cell receptor is converted into down-stream activation signals. Briefly: at the ground state, the signalling components on the T-cell membrane are in dynamic homeostasis whereby dephosphorylated ITAMs are favoured over phosphorylated ITAMs. This is due to greater activity of the transmembrane CD45/CD148 phosphatases over membrane-tethered kinases such as Lck. When a T-cell engages a target cell through a T-cell receptor (or CAR) recognition of cognate antigen, tight immunological synapses form. This close juxtapositioning of the T-cell and target membranes excludes CD45/CD148 due to their large ectodomains which cannot fit into the synapse. Segregation of a high concentration of T-cell receptor associated ITAMs and kinases in the synapse, in the absence of phosphatases, leads to a state whereby phosphorylated ITAMs are favoured. ZAP70 recognizes a threshold of phosphorylated ITAMs and propagates a T-cell activation signal. This advanced understanding of T-cell activation is exploited by the present invention. In particular, the invention is based on this understanding of how ectodomains of different length and/or bulk and/or charge and/or configuration and/or glycosylation result in differential segregation upon synapse formation.

[0232] The CAR Logical and Gate

[0233] In this embodiment, one CAR comprises an activating endodomain and one CAR comprises an inhibitory endodomain whereby the inhibitory CAR constitutively inhibits the first activating CAR, but upon recognition of its cognate antigen releases its inhibition of the activating CAR. In this manner, a T-cell can be engineered to trigger only if a target cell expresses both cognate antigens. This behaviour is achieved by the activating CAR comprising an activating endodomain containing ITAM domains for example the endodomain of CD3 Zeta, and the inhibitory CAR comprising the endodomain from a phosphatase able to dephosphorylate an ITAM (e.g. CD45 or CD148). Crucially, the spacer domains of both CARs are significantly different in size and/or shape and/or charge etc. When only the activating CAR is ligated, the inhibitory CAR is in solution on the T-cell surface and can diffuse in and out of the synapse inhibiting the activating CAR. When both CARs are ligated, due to differences in spacer properties, the activating and inhibiting CAR are differentially segregated allowing the activating CAR to trigger T-cell activation unhindered by the inhibiting CAR.

[0234] This is of considerable utility in the field of cancer therapy. Currently, immunotherapies typically target a single antigen. Most cancers cannot be differentiated from normal tissues on the basis of a single antigen. Hence, considerable “on-target off-tumour” toxicity occurs whereby normal tissues are damaged by the therapy. For instance, whilst targeting CD20 to treat B-cell lymphomas with Rituximab, the entire normal B-cell compartment is depleted. For instance, whilst targeting CD52 to treat chronic lymphocytic leukaemia, the entire lymphoid compartment is depleted. For instance, whilst targeting CD33 to treat acute myeloid leukaemia, the entire myeloid compartment is damaged etc. By restricting activity to a pair of antigens, much more refined targeting, and hence less toxic therapy can be developed. A practical example is targeting of CLL which expresses both CD5 and CD19. Only a small proportion of normal B-cells express both antigens, so the off-target toxicity of targeting both antigens with a logical AND gate is substantially less than targeting each antigen individually.

[0235] The design of the present invention is a considerable improvement on previous implementation as described by Wilkie et al. ((2012). *J. Clin. Immunol.* 32, 1059-1070) and then tested in vivo (Kloss et al (2013) *Nat. Biotechnol.* 31, 71-75). In this implementation, the first CAR comprises of an activating endodomain, and the second a co-stimulatory domain. This way, a T-cell only receives an activating and co-stimulatory signal when both antigens are present. However, the T-cell still will activate in the sole presence of the first antigen resulting in the potential for off-target toxicity. Further, the implementation of the present invention allows for multiple compound linked gates whereby a cell can interpret a complex pattern of antigens.

TABLE 5

Cancer Type	Antigens
Chronic Lymphocytic Leukaemia	CD5, CD19
Neuroblastoma	ALK, GD2
Glioma	EGFR, Vimentin
Multiple myeloma	BCMA, CD138

TABLE 5-continued

Cancer Type	Antigens
Renal Cell Carcinoma	Carbonic anhydrase IX, G250
T-ALL	CD2, N-Cadherin
Prostate Cancer	PSMA, hepsin (or others)

[0236] The CAR Logical AND NOT Gate

[0237] In this embodiment, one CAR comprises an activating endodomain and one CAR comprises an inhibitory endodomain such that this inhibitory CAR is only active when it recognizes its cognate antigen. Hence a T-cell engineered in this manner is activated in response to the sole presence of the first antigen but is not activated when both antigens are present. This invention is implemented by inhibitory CARs with a spacer that co-localise with the first CAR but either the phosphatase activity of the inhibitory CAR should not be so potent that it inhibits in solution, or the inhibitory endodomain in fact recruits a phosphatase solely when the inhibitory CAR recognizes its cognate target. Such endodomains are termed “ligation-on” or semi-inhibitory herein.

[0238] This invention is of use in refining targeting when a tumour can be distinguished from normal tissue by the presence of tumour associated antigen and the loss of an antigen expressed on normal tissue. The AND NOT gate is of considerable utility in the field of oncology as it allows targeting of an antigen which is expressed by a normal cell, which normal cell also expresses the antigen recognised by the CAR comprising the activating endodomain. An example of such an antigen is CD33 which is expressed by normal stem cells and acute myeloid leukaemia (AML) cells. CD34 is expressed on stem cells but not typically expressed on AML cells. A T-cell recognizing CD33 AND NOT CD34 would result in destruction of leukaemia cells but sparing of normal stem cells.

[0239] Potential antigen pairs for use with AND NOT gates are shown in Table 6.

TABLE 6

Disease	TAA	Normal cell which expresses TAA	Antigen expressed by normal cell but not cancer cell
AML	CD33	stem cells	CD34
Myeloma	BCMA	Dendritic cells	CD1c
B-CLL	CD160	Natural Killer cells	CD56
Prostate cancer	PSMA	Neural Tissue	NCAM
Bowel cancer	A33	Normal bowel epithelium	HLA class I

[0240] Compound Gates

[0241] The kinetic segregation model with the above components allows compound gates to be made e.g. a T-cell which triggers in response to patterns of more than two target antigens. For example, it is possible to make a T cell which only triggers when three antigens are present (A AND B AND C). Here, a cell expresses three CARs, each recognizing antigens A, B and C. One CAR is excitatory and two are inhibitory, which each CAR having spacer domains which result in differential segregation. Only when all three are ligated, will the T-cell activate. A further example: (A OR B) AND C: here, CARs recognizing antigens A and B are activating and have spacers which co-localise, while CAR

recognizing antigen C is inhibitory and has a spacer which results in different co-segregation. A further example (A AND NOT B) AND C: Here CAR against antigen A has an activating endodomain and co-localises with CAR against antigen B which has a conditionally inhibiting endodomain. CAR against antigen C has a spacer who segregates differently from A or B and is inhibitory. In fact, ever more complex boolean logic can be programmed with these simple components of the invention with any number of CARs and spacers.

[0242] Signal Peptide

[0243] The CARs of the T cell of the present invention may comprise a signal peptide so that when the CAR is expressed inside a cell, such as a T-cell, the nascent protein is directed to the endoplasmic reticulum and subsequently to the cell surface, where it is expressed.

[0244] The core of the signal peptide may contain a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognized and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

[0245] The signal peptide may be at the amino terminus of the molecule.

[0246] The signal peptide may comprise the SEQ ID No. 7, 8 or 9 or a variant thereof having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that the signal peptide still functions to cause cell surface expression of the CAR.

SEQ ID No. 7:
MGTSLLCWMALCLLGADHADG

[0247] The signal peptide of SEQ ID No. 7 is compact and highly efficient. It is predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

SEQ ID No. 8:
MSLPVTALLPLALLLHAARP

[0248] The signal peptide of SEQ ID No. 8 is derived from IgG1.

SEQ ID No. 9:
MAVPTQVLGLLLLLWLTAR

[0249] The signal peptide of SEQ ID No. 9 is derived from CD8.

[0250] The signal peptide for the first CAR may have a different sequence from the signal peptide of the second CAR (and from the 3rd CAR and 4th CAR etc).

[0251] Antigen Binding Domain

[0252] The antigen binding domain is the portion of the CAR which recognizes antigen. Numerous antigen-binding domains are known in the art, including those based on the antigen binding site of an antibody, antibody mimetics, and T-cell receptors. For example, the antigen-binding domain may comprise: a single-chain variable fragment (scFv)

derived from a monoclonal antibody; a natural ligand of the target antigen; a peptide with sufficient affinity for the target; a single domain antibody; an artificial single binder such as a Darpin (designed ankyrin repeat protein); or a single-chain derived from a T-cell receptor.

[0253] The antigen binding domain may comprise a domain which is not based on the antigen binding site of an antibody. For example the antigen binding domain may comprise a domain based on a protein/peptide which is a soluble ligand for a tumour cell surface receptor (e.g. a soluble peptide such as a cytokine or a chemokine); or an extracellular domain of a membrane anchored ligand or a receptor for which the binding pair counterpart is expressed on the tumour cell.

[0254] Examples 11 to 13 relate to a CAR which binds BCMA, in which the antigen binding domain comprises APRIL, a ligand for BCMA.

[0255] The antigen binding domain may be based on a natural ligand of the antigen.

[0256] The antigen binding domain may comprise an affinity peptide from a combinatorial library or a de novo designed affinity protein/peptide.

[0257] Spacer Domain

[0258] CARs comprise a spacer sequence to connect the antigen-binding domain with the transmembrane domain and spatially separate the antigen-binding domain from the endodomain. A flexible spacer allows the antigen-binding domain to orient in different directions to facilitate binding.

[0259] In the T cell of the present invention, the first and second CARs comprise different spacer molecules. For example, the spacer sequence may, for example, comprise an IgG1 Fc region, an IgG1 hinge or a human CD8 stalk or the mouse CD8 stalk. The spacer may alternatively comprise an alternative linker sequence which has similar length and/or domain spacing properties as an IgG1 Fc region, an IgG1 hinge or a CD8 stalk. A human IgG1 spacer may be altered to remove Fc binding motifs.

[0260] Examples of amino acid sequences for these spacers are given below:

(hinge-CH2CH3 of human IgG1) SEQ ID No. 10
 AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVV
 DVSHDEPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVUTVLHQD
 WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL
 TVDKSRWQGNVFSQVMHEALHNHYTQKSLSLSPGKKD
 SEQ ID No. 11 (human CD8 stalk):
 TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
 SEQ ID No. 12 (human IgG1 hinge):
 AEPKSPDKTHTCPPCPKDPK
 SEQ ID No. 13 (CD2 ectodomain)
 KEITNALETWALGQDINLDIPSPQMSDDIDDIKWEKTSDDKKIAQFR
 KEKETPFKEKDYTLKFNGLTKIKHLKTDDQDIYKVSIIYDTKGKNVLEKI
 FDLKIQERVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQ
 RVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCEPKGLD

-continued

(CD34 ectodomain) SEQ ID no. 14
 SLDNNGTATPELPTQGTFSNVSTNVSYQETTTSTLGLSTSLHPVSQHG
 EATTNITETTIVKFTSTSVITSVYGNINSSVQSQTSVISTVFTTPANVST
 PETTLKPSLSPGNVSDSLTSTSLATSPKPYTSSSPILSDIKAEIKCS
 GIREVKLTQGICLEQNKTSSCAEFKKDRGEGLARVLCGEEQADADAGAQ
 VCSLLLAQSEVRPQCCLLVLANRTEISSKLQLMKKHQSDLKKLGILDF
 EQDVASHQSYSQKT

[0261] Since CARs are typically homodimers (see FIG. 1a), cross-pairing may result in a heterodimeric chimeric antigen receptor. This is undesirable for various reasons, for example: (1) the epitope may not be at the same "level" on the target cell so that a cross-paired CAR may only be able to bind to one antigen; (2) the VH and VL from the two different scFv could swap over and either fail to recognize target or worse recognize an unexpected and unpredicted antigen. For the "OR" gate and the "AND NOT" gate, the spacer of the first CAR may be sufficiently different from the spacer of the second CAR in order to avoid cross-pairing. The amino acid sequence of the first spacer may share less than 50%, 40%, 30% or 20% identity at the amino acid level with the second spacer.

[0262] In other aspects of the invention (for example the AND gate) it is important that the spacer of the first CAR has a different length, and/or charge and/or shape and/or configuration and/or glycosylation, such that when both first and second CARs bind their target antigen, the difference in spacer charge or dimensions results in spatial separation of the two types of CAR to different parts of the membrane to result in activation as predicted by the kinetic separation model. In these aspects, the different length, shape and/or configuration of the spacers is carefully chosen bearing in mind the size and binding epitope on the target antigen to allow differential segregation upon cognate target recognition. For example the IgG1 Hinge, CD8 stalk, IgG1 Fc, ectodomain of CD34, ectodomain of CD45 are expected to differentially segregate.

[0263] Examples of spacer pairs which differentially segregate and are therefore suitable for use with the AND gate are shown in the following Table:

Stimulatory CAR spacer	Inhibitory CAR spacer
Human-CD8STK	Human-IgG-Hinge-CH2CH3
Human-CD3z ectodomain	Human-IgG-Hinge-CH2CH3
Human-IgG-Hinge	Human-IgG-Hinge-CH2CH3
Human-CD28STK	Human-IgG-Hinge-CH2CH3
Human-CD8STK	Human-IgM-Hinge-CH2CH3CD4
Human-CD3z ectodomain	Human-IgM-Hinge-CH2CH3CD4
Human-IgG-Hinge	Human-IgM-Hinge-CH2CH3CD4
Human-CD28STK	Human-IgM-Hinge-CH2CH3CD4

[0264] In other aspects of the invention (for example the AND NOT gate), it is important that the spacer be sufficiently different as to prevent cross-pairing, but to be sufficiently similar to co-localise. Pairs of orthologous spacer sequences may be employed. Examples are murine and human CD8 stalks, or alternatively spacer domains which are monomeric—for instance the ectodomain of CD2.

[0265] Examples of spacer pairs which co-localise and are therefore suitable for use with the AND NOT gate are shown in the following Table:

Stimulatory CAR spacer	Inhibitory CAR spacer
Human-CD8aSTK	Mouse CD8aSTK
Human-CD28STK	Mouse CD8aSTK
Human-IgG-Hinge	Human-CD3z ectodomain
Human-CD8aSTK	Mouse CD28STK
Human-CD28STK	Mouse CD28STK
Human-IgG-Hinge-CH2CH3	Human-IgM-Hinge-CH2CH3CD4

[0266] All the spacer domains mentioned above form homodimers. However the mechanism is not limited to using homodimeric receptors and should work with monomeric receptors as long as the spacer is sufficiently rigid. An example of such a spacer is CD2 or truncated CD22.

[0267] Transmembrane Domain

[0268] The transmembrane domain is the sequence of the CAR that spans the membrane.

[0269] A transmembrane domain may be any protein structure which is thermodynamically stable in a membrane. This is typically an alpha helix comprising of several hydrophobic residues. The transmembrane domain of any transmembrane protein can be used to supply the transmembrane portion of the invention. The presence and span of a transmembrane domain of a protein can be determined by those skilled in the art using the TMHMM algorithm (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>). Further, given that the transmembrane domain of a protein is a relatively simple structure, i.e. a polypeptide sequence predicted to form a hydrophobic alpha helix of sufficient length to span the membrane, an artificially designed TM domain may also be used (U.S. Pat. No. 7,052,906 B1 describes synthetic transmembrane components).

[0270] The transmembrane domain may be derived from CD28, which gives good receptor stability.

[0271] Activating Endodomain

[0272] The endodomain is the signal-transmission portion of the CAR. After antigen recognition, receptors cluster, native CD45 and CD148 are excluded from the synapse and a signal is transmitted to the cell. The most commonly used endodomain component is that of CD3-zeta which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling may be needed. For example, chimeric CD28 and OX40 can be used with CD3-Zeta to transmit a proliferative/survival signal, or all three can be used together.

[0273] In the “OR gate”, the cell of the present invention comprises two CARs, each with an activating endodomain. The activating endodomain is capable of transmitting both immunological signal 1 and immunological signal 2. An endodomain is not considered “activating” if it just comprises CD3 ξ , which is sufficient to trigger T-cell killing of cognate target cells does not fully activate the T-cell to proliferate and survive. An “activating endodomain” is a compound endodomains: in which the intracellular part of a T-cell co-stimulatory molecule is fused to that of CD3 ξ resulting in a “second generation” receptor which can transmit an activating and co-stimulatory signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most

potent co-stimulatory signal—namely immunological signal 2, which triggers T-cell proliferation. In addition to a co-stimulatory domain, the activating endodomain may also include TNF receptor family endodomains, such as the closely related OX40 and 41BB which transmit survival signals.

[0274] An “activating” endodomain may therefore not comprise the CD3-Zeta endodomain or the CD28 endodomain alone. An activating endodomain may, for example, comprise the CD3-Zeta endodomain with that of either CD28 or OX40 or the CD28 endodomain and OX40 and CD3-Zeta endodomain.

[0275] Each of the CARs in the OR gate is independently capable of activating the T cell. The T cell is thus activated by the presence of either antigen alone. The two CARs are not “complementary” in the sense that activation of both CARs is necessary to provide activation and co-stimulatory signals.

[0276] A endodomain which contains an ITAM motif can act as an activation endodomain in this invention. Several proteins are known to contain endodomains with one or more ITAM motifs. Examples of such proteins include the CD3 epsilon chain, the CD3 gamma chain and the CD3 delta chain to name a few. The ITAM motif can be easily recognized as a tyrosine separated from a leucine or isoleucine by any two other amino acids, giving the signature YxxL/I. Typically, but not always, two of these motifs are separated by between 6 and 8 amino acids in the tail of the molecule (YxxL/Ix(6-8)YxxL/I). Hence, one skilled in the art can readily find existing proteins which contain one or more ITAM to transmit an activation signal. Further, given the motif is simple and a complex secondary structure is not required, one skilled in the art can design polypeptides containing artificial ITAMs to transmit an activation signal (see WO 2000063372, which relates to synthetic signalling molecules).

[0277] The transmembrane and intracellular T-cell signaling domain (endodomain) of a CAR with an activating endodomain may comprise the sequence shown as SEQ ID No. 16 or 17 or a variant thereof having at least 80% sequence identity.

comprising CD28 transmembrane domain and CD3 Z endodomain

SEQ ID No. 15

FWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQDKMAEA
YSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR

comprising CD28 transmembrane domain and CD28 and CD3 Zeta endodomains

SEQ ID No. 16

FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSLHSDYNNMTPRRPGP
TRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGE
RRRGKGDGLYQGLSTATKDTYDALHMQALPPR

comprising CD28 transmembrane domain and CD28, OX40 and CD3 Zeta endodomains.

SEQ ID No. 17

FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSLHSDYNNMTPRRPGP
TRKHYQPYAPPRDFAAYRSRDQRLPDPDAHKPPGGGSFRTPIQEEQADAH

-continued

STLAKIRVKFSRSADAPAYQQGQNLNELNLGRREYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQUALPPR

[0278] A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID No. 16 or 17, provided that the sequence provides an effective trans-membrane domain and an effective intracellular T cell signaling domain.

[0279] “Ligation-Off” Inhibitory Endodomain

[0280] In the embodiment referred above as the AND gate, one of the CARs comprises an inhibitory endodomain such that the inhibitory CAR inhibits T-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but does not significantly inhibit T-cell activation by the activating CAR when the inhibitory CAR is ligated. This is termed a “ligation-off” inhibitory endodomain.

[0281] In this case, the spacer of the inhibitory CAR is of a different length, charge, shape and/or configuration and/or glycosylation from the spacer of the activating CAR, such that when both receptors are ligated, the difference in spacer dimensions results in isolation of the activating CARs and the inhibitory CARs in different membrane compartments of the immunological synapse, so that the activating endodomain is released from inhibition by the inhibitory endodomain.

[0282] The inhibitory endodomains for use in a ligation-off inhibitory CAR may therefore comprise any sequence which inhibits T-cell signaling by the activating CAR when it is in the same membrane compartment (i.e. in the absence of the antigen for the inhibitory CAR) but which does not significantly inhibit T cell signaling when it is isolated in a separate part of the membrane from the inhibitory CAR.

[0283] The ligation-off inhibitory endodomain may be or comprise a tyrosine phosphatase, such as a receptor-like tyrosine phosphatase. An inhibitory endodomain may be or comprise any tyrosine phosphatase that is capable of inhibiting the TCR signalling when only the stimulatory receptor is ligated. An inhibitory endodomain may be or comprise any tyrosine phosphatase with a sufficiently fast catalytic rate for phosphorylated ITAMs that is capable of inhibiting the TCR signalling when only the stimulatory receptor is ligated.

[0284] For example, the inhibitory endodomain of an AND gate may comprise the endodomain of CD148 or CD45. CD148 and CD45 have been shown to act naturally on the phosphorylated tyrosines up-stream of TCR signalling.

[0285] CD148 is a receptor-like protein tyrosine phosphatase which negatively regulates TCR signaling by interfering with the phosphorylation and function of PLC γ 1 and LAT.

[0286] CD45 present on all hematopoietic cells, is a protein tyrosine phosphatase which is capable of regulating signal transduction and functional responses, again by phosphorylating PLC γ 1.

[0287] An inhibitory endodomain may comprise all of part of a receptor-like tyrosine phosphatase. The phosphatase may interfere with the phosphorylation and/or function of elements involved in T-cell signalling, such as PLC γ 1 and/or LAT.

[0288] The transmembrane and endodomain of CD45 and CD148 is shown as SEQ ID No. 18 and No. 19 respectively.

CD45 trans-membrane and endodomain sequence

SEQ ID 18

ALIAFLAFLIIVTSIALLVLYKIYDLHKRSCNLDEQQELVERDDEKQL

MNVEPIHADILLETYKRKIADEGRFLAEFQSPRVFSKFPKEARKPFPN

QNKRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKPRKYIAAQG

PRDETVDDFWRMIWEQKATVIVMVTCEEGNRNKAEBYWPSEEGTAFG

DVVVKINQHKRCPDYIIQKLNIVNKEKATGREVTHIQFTSWPDHGVPE

PHLLLKLRRRVNAFNFSGPIWHCSAGVGRGTGTIYGIDAMLEGLEANK

VDVYGVVVKLRQRCLMVQVEAQYILIHQALVEYNQFGETEVNLSLHPY

LHNMKRDPPESEPSPLAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVI

PYDYNRVPLKHELEMSKESEHDSDESDSDSEEPSKYINASFIMSYWK

PEVMIAAQGPLKETIGDFWQMIFQKVKVIVMLTELKHGDQEI CAQYWGE

GKQTYGDI EVDLKD TDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVE

QLPAEPKELISMIQWKQLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIF

CALLNLLESAETEEWDIFQWKALRKARPGMVSTFEQYQFLYDVIASTYPA

QNGQVKKNHQEDKIEFDNEVDKVKQDANCNVNPLGAPEKLPEAKEQAEGS

EPTSGTEGPEHVSNGPASPALNQGS

CD148 trans-membrane and endodomain sequence

SEQ ID 19

AVFGCIFGALVIVTVGGFI FWRKKRKDAKNNEVSFSQIKPKKSKLIRVEN

FEAYPKKQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVL

PYDISRVKLSVQTHSTDDYINANYMPGYHKKDFIATQGPLNTLKD FWR

MVWEKNVYAIIMLT KCVEQGR TKCEEYWP SKQAQDYGDITVAMTSEIVLP

EVVTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVDPDTDLLINFRYLVRD

YMKQSPPEPILVHCSAGVGRGTGTIAIDRLIYQIENENTVDVYGIVYDL

RMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLA

PVTTFGKTNGYIA

[0289] An inhibitory CAR may comprise all or part of SEQ ID No 18 or 19 (for example, it may comprise the phosphatase function of the endodomain). It may comprise a variant of the sequence or part thereof having at least 80% sequence identity, as long as the variant retains the capacity to basally inhibit T cell signalling by the activating CAR.

[0290] Other spacers and endodomains may be tested for example using the model system exemplified herein. Target cell populations can be created by transducing a suitable cell line such as a SupT1 cell line either singly or doubly to establish cells negative for both antigens (the wild-type), positive for either and positive for both (e.g. CD19–CD33–, CD19+CD33–, CD19–CD33+ and CD19+CD33+). T cells such as the mouse T cell line BW5147 which releases IL-2 upon activation may be transduced with pairs of CARs and their ability to function in a logic gate measured through measurement of IL-2 release (for example by ELISA). For example, it is shown in Example 4 that both CD148 and CD45 endodomains can function as inhibitory CARs in combination with an activating CAR containing a CD3 Zeta

endodomain. These CARs rely upon a short/non-bulky CD8 stalk spacer on one CAR and a bulky Fc spacer on the other CAR to achieve AND gating. When both receptors are ligated, the difference in spacer dimensions results in isolation of the different receptors in different membrane compartments, releasing the CD3 Zeta receptor from inhibition by the CD148 or CD45 endodomains. In this way, activation only occurs once both receptors are activated. It can be readily seen that this modular system can be used to test alternative spacer pairs and inhibitory endodomains. If the spacers do not achieve isolation following ligation of both receptors, the inhibition would not be released and so no activation would occur. If the inhibitory endodomain under test is ineffective, activation would be expected in the presence of ligation of the activating CAR irrespective of the ligation status of the inhibitory CAR.

[0291] “Ligation-On” Endodomain

[0292] In the embodiment referred above as the AND NOT gate, one of the CARs comprises a “ligation-on” inhibitory endodomain such that the inhibitory CAR does not significantly inhibit T-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but inhibits T-cell activation by the activating CAR when the inhibitory CAR is ligated.

[0293] The “ligation-on” inhibitory endodomain may be or comprise a tyrosine phosphatase that is incapable of inhibiting the TCR signalling when only the stimulatory receptor is ligated.

[0294] The “ligation-on” inhibitory endodomain may be or comprise a tyrosine phosphatase with a sufficiently slow catalytic rate for phosphorylated ITAMs that is incapable of inhibiting the TCR signalling when only the stimulatory receptor is ligated but it is capable of inhibiting the TCR signalling response when concentrated at the synapse. Concentration at the synapse is achieved through inhibitory receptor ligation.

[0295] If a tyrosine phosphatase has a catalytic rate which is too fast for a “ligation-on” inhibitory endodomain, then it is possible to tune-down the catalytic rates of phosphatase through modification such as point mutations and short linkers (which cause steric hindrance) to make it suitable for a “ligation-on” inhibitory endodomain.

[0296] In this first embodiment the endodomain may be or comprise a phosphatase which is considerably less active than CD45 or CD148, such that significant dephosphorylation of ITAMS only occurs when activating and inhibitory endodomains are co-localised. Many suitable sequences are known in the art. For example, the inhibitory endodomain of a NOT AND gate may comprise all or part of a protein-tyrosine phosphatase such as PTPN6.

[0297] Protein tyrosine phosphatases (PTPs) are signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. The N-terminal part of this PTP contains two tandem Src homolog (SH2) domains, which act as protein phospho-tyrosine binding domains, and mediate the interaction of this PTP with its substrates. This PTP is expressed primarily in hematopoietic cells, and functions as an important regulator of multiple signaling pathways in hematopoietic cells.

[0298] The inhibitor domain may comprise all of PTPN6 (SEQ ID No. 20) or just the phosphatase domain (SEQ ID No. 21).

sequence of PTPN6

SEQ ID 20

MVRWFHRDLSGLDAETLLKGRGVHGSFLARPSRKNQGDFFSLSVRVGDQVT
HIRIQNSGDFYDLYGGEKFATLTTELVEYYTQQQGVLDQRDGTIHLKYPL
NCSDP TSE R V V Y H G H M S G G Q A E T L L Q A K G E P W T F L V R E S L S Q P G D F V L S V
L S D Q P K A G P G S P L R V T H I K V M C E G G R Y T V G G L E T F D S L T D L V E H F K K T G I
E E A S G A F V Y L R Q P Y Y A T R V N A A D I E N R V L E L N K K Q E S E D T A K A G F W E E F E
S L Q K Q E V K N L H Q R L E G Q R P E N K G K N R Y K N I L P F D H S R V I L Q G R D S N I P G S
D Y I N A N Y I K N Q L L G P D E N A K T Y I A S Q G C L E A T V N D F W Q M A W Q E N S R V I V M
T T R E V E K G R N K C V P Y V V P E V G M Q R A Y G P Y S V T N C G E H D T T E Y K L R T L Q V S
P L D N G D L I R E I W H Y Q Y L S W P D H G V P S E P G G V L S F L D Q I N Q R Q E S L P H A G P
I I V H C S A G I G R T G T I I V I D M L M E N I S T K G L D C D I D I Q K T I Q M V R A Q R S G M
V Q T E A Q Y K F I Y V A I A Q F I E T T K K K L E V L Q S Q K G Q E S E Y G N I T Y P P A M K N A
H A K A S R T S S K H K E D V Y E N L H T K N K R E E K V K Q R S A D K E K S K G S L K R K

sequence of phosphatase domain of PTPN6

SEQ ID 21

F W E E F E S L Q K Q E V K N L H Q R L E G Q R P E N K G K N R Y K N I L P F D H S R V I L Q G R D
S N I P G S D Y I N A N Y I K N Q L L G P D E N A K T Y I A S Q G C L E A T V N D F W Q M A W Q E N
S R V I V M T T R E V E K G R N K C V P Y W P E V G M Q R A Y G P Y S V T N C G E H D T T E Y K L R
T L Q V S P L D N G D L I R E I W H Y Q Y L S W P D H G V P S E P G G V L S F L D Q I N Q R Q E S L
P H A G P I I V H C S A G I G R T G T I I V I D M L M E N I S T K G L D C D I D I Q K T I Q M V R A
Q R S G M V Q T E A Q Y K F I Y V A I A Q F

[0299] A second embodiment of a ligation-on inhibitory endodomain is an ITIM (Immunoreceptor Tyrosine-based Inhibition motif) containing endodomain such as that from CD22, LAIR-1, the Killer inhibitory receptor family (KIR), LILRB1, CTLA4, PD-1, BTLA etc. When phosphorylated, ITIMs recruits endogenous PTPN6 through its SH2 domain. If co-localised with an ITAM containing endodomain, dephosphorylation occurs and the activating CAR is inhibited.

[0300] An ITIM is a conserved sequence of amino acids (S/I/N/LxYxxIN/L) that is found in the cytoplasmic tails of many inhibitory receptors of the immune system. One skilled in the art can easily find protein domains containing an ITIM. A list of human candidate ITIM-containing proteins has been generated by proteome-wide scans (Staub, et al (2004) Cell. Signal. 16, 435-456). Further, since the consensus sequence is well known and little secondary structure appears to be required, one skilled in the art could generate an artificial ITIM.

[0301] ITIM endodomains from PDCD1, BTLA4, LILRB1, LAIR1, CTLA4, KIR2DL1, KIR2DL4, KIR2DL5, KIR3DL1 and KIR3DL3 are shown in SEQ ID 22 to 31 respectively

PDCD1 endodomain

SEQ ID 22

C S R A A R G T I G A R R T G Q P L K E D P S A V P V F S V D Y G E L D F Q W R E K T P E P P V P C
V P E Q T E Y A T I V F P S G M G T S S P A R R G S A D G P R S A Q P L R P E D G H C S W P L

-continued

BTLA4

SEQ ID 23

KLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPLHSLGCYNPMM
EDGISYTTLRFPENIPRTGDAESSEMQRPPDCDDTVTYSALHKRQVGD
YENVIPDFPEDEGIHYSELIQFGVGERPQAQENVYVILKH

LILRB1

SEQ ID 24

LRHRRQGHWTSTQRKADFOHPAGAVGPEPTDRGLQWRSSPAADAQEENL

YAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLS

GEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPS

QEGPSPAVPSIYATLAIH

LAIR1

SEQ ID 25

HRQNQIKQGPSPKDEEQKPQQRPD LAVDLERTADKATVNGLEPKDRET
DTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMASITYAAVAR
H

CTLA4

SEQ ID 26

FLWLILAAVSSGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPPEPEC
EKQFQPYFIPIN

KIR2DL1

SEQ ID 27

GNSRHLHVLIGTSVVIIPFAILLFLLHRWCANKKNNAVMDQEPAGNRTV
NREDSDEQDPQEVITYQLNHCVFTQRKITRPSQRPKTPPTDIIIVYTELPN
AESRSKWSCP

KIR2DL4

SEQ ID 28

GIARHLHAVIRYSVAIILFTILPFFLLHRWSCKKKENAAVMNQEPAGHRT
VNREDSDEQDPQENTTYAQLDHCIFTQRKITGPSQRKRPSTDTSVCIEL
PNAEPRLSPAHEHHSQALMGSSRETTALSQTQLASSNVPAAGI

KIR2DL5

SEQ ID 29

TGIRRLHLILIGTSVAIILFIILFFLLHCCSNKKNAAVMDQEPAGDRT
VNRESDDDQDPQEVITYAQLDHCVFTQTKITSPSQRPKTPPTDTTMYMELP
NAKPRSLSPAHHKHSQALRGSSRETTALSQNRVASSHVPAAGI

KIR3DL1

SEQ ID 30

KDPRHLHLILIGTSVVIILFIILFFLLHLWCANKKNAAVMDQEPAGNRTA
NREDSDEQDPQEVITYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPN
AKPRSKVSCP

KIR3DL3

SEQ ID 31

KDPGNSRHLHLIGTSVVIIPFAILLFLLHRWCANKKNNAVMDQEPAGN
RTVNREDSDEQDPQEVITYAQLNHCVFTQRKITRPSQRPKTPPTDTSV

[0302] A third embodiment of a ligation-on inhibitory endodomain is an ITIM containing endodomain co-expressed with a fusion protein. The fusion protein may comprise at least part of a protein-tyrosine phosphatase and at least part of a receptor-like tyrosine phosphatase. The fusion may comprise one or more SH2 domains from the protein-tyrosine phosphatase. For example, the fusion may be between a PTPN6 SH2 domain and CD45 endodomain or between a PTPN6 SH2 domain and CD148 endodomain. When phosphorylated, the ITIM domains recruit the fusion protein bring the highly potent CD45 or CD148 phosphatase to proximity to the activating endodomain blocking activation.

[0303] SEQUENCES of fusion proteins are listed 32 and 33

PTPN6-CD45 fusion protein

SEQ ID 32

WYHGHSMSGQAETLLQAKGEPVVTFLVRESLSQPGDFVLSVLSQPKAGP

GSPLRVTHIKVIVICEGGRYTVGGLETFDLSLTDLVEHFKKTGIEEASGAF

VYLRQPYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETY

KRKIADEGRFLAEFQSIPIRVFSKFPIKEARKPFNNQKNRYVDILPYDYN

RVELSEINGDAGSNYINASYIDGFKPEPRKYIAAQGRDETVDDFWRMIWE

QKATVIVMVIRCEEGNRNKAIEYWPMSMEEGTRAFGDVVVKINQHKRCPDY

IIQKLNIVNKKKATGREVTHIQFTSWPDHGVPEPDLHLKLRNRVNAFS

NFFSGPIVHVCSAGVGRGTGYIGIDAMLEGLEAENKVDVYGVVKKLRQR

CLMVQVEAQYILIHQALVEYNQFGETEVLNSELHPYLHNMKKRDPPSEPS

PLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPIYDYNVLKHELEM

SKSEHSDSDSDSDSEEPSKYINASFIMSYVVKPEVMIAAQGPLKET

IGDFMIQRKVKVIVMLTELKHGQDEICAQYWGEGKQTYGDI EVDLKD TDK

SSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVK

QKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGFICALLNLESAETE EVV

DIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDK

IEFDNEVDKVKQDANCNVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVN

GPASPALNQQS

PTPN6-CD148 fusion

SEQ ID 33

ETLLQAKGEPWTFVLVRESLSQPGDFVLSVLSQPKAGPGSPPLRVTHIKVM

CEGGRYTVGGLETFDLSLTDLVEHFKKTGIEEASGAFVYLRQPYRKKRKA

KNNEVSFSQIKPKKSKLIRVENFEAYFKKQQAADSNCGFAEEYEDLKLVGI

SQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGY

HSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTCKVEQGRTKCEEYW

PSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSSEHPLRQPHFTSW

PDHGVPTD TDLINFRYLRVDMYKQSPPEPILVHCSAGVGRGTGTFIAID

RLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQK

DSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0304] A ligation-on inhibitory CAR may comprise all or part of SEQ ID No 20 or 21. It may comprise all or part of SEQ ID 22 to 31. It may comprise all or part of SEQ ID 22 to 31 co-expressed with either SEQ ID 32 or 33. It may comprise a variant of the sequence or part thereof having at least 80% sequence identity, as long as the variant retains the capacity to inhibit T cell signaling by the activating CAR upon ligation of the inhibitory CAR.

[0305] As above, alternative spacers and endodomains may be tested for example using the model system exemplified herein. It is shown in Example 5 that the PTPN6 endodomain can function as a semi-inhibitory CAR in combination with an activating CAR containing a CD3 Zeta endodomain. These CARs rely upon a human CD8 stalk spacer on one CAR and a mouse CD8 stalk spacer on the other CAR. The orthologous sequences prevent cross pair-

ing. However, when both receptors are ligated, the similarity between the spacers results in co-segregation of the different receptors in the same membrane compartments.

[0306] This results in inhibition of the CD3 Zeta receptor by the PTPN6 endodomain. If only the activating CAR is ligated the PTPN6 endodomain is not sufficiently active to prevent T cell activation. In this way, activation only occurs if the activating CAR is ligated and the inhibitory CAR is not ligated (AND NOT gating). It can be readily seen that this modular system can be used to test alternative spacer pairs and inhibitory domains. If the spacers do not achieve co-segregation following ligation of both receptors, the inhibition would not be effective and so activation would occur. If the semi-inhibitory endodomain under test is ineffective, activation would be expected in the presence of ligation of the activating CAR irrespective of the ligation status of the semi-inhibitory CAR.

[0307] Co-Expression Site

[0308] The second aspect of the invention relates to a nucleic acid which encodes the first and second CARs.

[0309] The nucleic acid may produce a polypeptide which comprises the two CAR molecules joined by a cleavage site. The cleavage site may be self-cleaving, such that when the polypeptide is produced, it is immediately cleaved into the first and second CARs without the need for any external cleavage activity.

[0310] Various self-cleaving sites are known, including the Foot-and-Mouth disease virus (FMDV) 2a self-cleaving peptide, which has the sequence shown as SEQ ID No. 34:

SEQ ID No. 34
RAEGRGSLLTGCDVEENPGP.

[0311] The co-expressing sequence may be an internal ribosome entry sequence (IRES). The co-expressing sequence may be an internal promoter.

[0312] Cell

[0313] The first aspect of the invention relates to a cell which co-expresses a first CAR and a second CAR at the cell surface.

[0314] The cell may be any eukaryotic cell capable of expressing a CAR at the cell surface, such as an immunological cell.

[0315] In particular the cell may be an immune effector cell such as a T cell or a natural killer (NK) cell.

[0316] T cells or T lymphocytes are a type of lymphocyte that play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. There are various types of T cell, as summarised below.

[0317] Helper T helper cells (TH cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. TH cells express CD4 on their surface. TH cells become activated when they are presented with peptide antigens by MHC class II molecules on the surface of antigen presenting cells (APCs). These cells can differentiate into one of several subtypes, including TH1, TH2, TH3, TH17, Th9, or TFH, which secrete different cytokines to facilitate different types of immune responses.

[0318] Cytotoxic T cells (TC cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated

in transplant rejection. CTLs express the CD8 at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of all nucleated cells. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis.

[0319] Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with “memory” against past infections. Memory T cells comprise three subtypes: central memory T cells (TCM cells) and two types of effector memory T cells (TEM cells and TEMRA cells). Memory cells may be either CD4+ or CD8+. Memory T cells typically express the cell surface protein CD45RO.

[0320] Regulatory T cells (Treg cells), formerly known as suppressor T cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus.

[0321] Two major classes of CD4+ Treg cells have been described—naturally occurring Treg cells and adaptive Treg cells.

[0322] Naturally occurring Treg cells (also known as CD4+CD25+FoxP3+ Treg cells) arise in the thymus and have been linked to interactions between developing T cells with both myeloid (CD11c+) and plasmacytoid (CD123+) dendritic cells that have been activated with TSLP. Naturally occurring Treg cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP3. Mutations of the FOXP3 gene can prevent regulatory T cell development, causing the fatal autoimmune disease IPEX.

[0323] Adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response.

[0324] The T cell of the invention may be any of the T cell types mentioned above, in particular a CTL.

[0325] Natural killer (NK) cells are a type of cytolytic cell which forms part of the innate immune system. NK cells provide rapid responses to innate signals from virally infected cells in an MHC independent manner

[0326] NK cells (belonging to the group of innate lymphoid cells) are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils and thymus where they then enter into the circulation.

[0327] The CAR cells of the invention may be any of the cell types mentioned above.

[0328] CAR-expressing cells, such as CAR-expressing T or NK cells may either be created ex vivo either from a patient's own peripheral blood (1st party), or in the setting of a haematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party).

[0329] The present invention also provide a cell composition comprising CAR expressing T cells and/or CAR expressing NK cells according to the present invention. The

cell composition may be made by transducing a blood-sample ex vivo with a nucleic acid according to the present invention.

[0330] Alternatively, CAR-expressing cells may be derived from ex vivo differentiation of inducible progenitor cells or embryonic progenitor cells to the relevant cell type, such as T cells. Alternatively, an immortalized cell line such as a T-cell line which retains its lytic function and could act as a therapeutic may be used.

[0331] In all these embodiments, CAR cells are generated by introducing DNA or RNA coding for the CARs by one of many means including transduction with a viral vector, transfection with DNA or RNA.

[0332] A CAR T cell of the invention may be an ex vivo T cell from a subject. The T cell may be from a peripheral blood mononuclear cell (PBMC) sample. T cells may be activated and/or expanded prior to being transduced with CAR-encoding nucleic acid, for example by treatment with an anti-CD3 monoclonal antibody.

[0333] A CAR T cell of the invention may be made by:

[0334] (i) isolation of a T cell-containing sample from a subject or other sources listed above; and

[0335] (ii) transduction or transfection of the T cells with one or more nucleic acid sequence(s) encoding the first and second CAR.

[0336] The T cells may then be purified, for example, selected on the basis of co-expression of the first and second CAR.

[0337] Nucleic Acid Sequences

[0338] The second aspect of the invention relates to one or more nucleic acid sequence(s) which codes for a first CAR and a second CAR as defined in the first aspect of the invention.

[0339] The nucleic acid sequence may comprise one of the following sequences, or a variant thereof

[0340] SEQ ID 35 OR gate

[0341] SEQ ID 36 AND gate using CD45

[0342] SEQ ID 37 AND gate using CD148

[0343] SEQ ID 38 AND NOT gate using PTPN6 as endodomain

[0344] SEQ ID 39 AND NOT gate using LAIR1 endodomain

[0345] SEQ ID 40 AND NOT gate using LAIR1 and PTPN6 SH2 fusion with CD148 phosphatase

SEQ ID No. 35:

>MP13974.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33g1x-HCH2CH3pvaa-CD28tmZw

ATGAGCCTGCCCCGTGACCGCCCTGCTGCTGCCCTGGCCCTGCTGCTGCACGCCGCC

AGACCAGACATCCAGATGACCCAGACCACCAGCAGCCTGAGCGCCAGCCTGGGCGAC

CGGGTGACCATCAGCTGCAGAGCCAGCCAGGACATCAGCAAGTACCTGAACCTGGTACC

AGCAGAAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGCCGGCTGCACA

GCGGCGTGCCAGCCGGTTTTCAGCGGCAGCGGCAGCGGCACCGACTACAGCCTGACC

ATCAGCAACCTGGAGCAGGAGGACATCGCCACCTACTTCTGCCAGCAGGGCAACACCC

TGCCCTACACCTTCGGAGGCGGCACCAAGCTGGAGATCACCAAGCCGGAGGCGGAG

GCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGGT

GAAGCTGCAGGAGTCTGGCCAGGCTGGTGGCCCCAAGCCAGAGCCTGAGCGTGAC

CTGCACCGTGAGCGCGTGAGCCTGCCGACTACGGCGTGAGCTGGATCAGGCAGCC

CCACGGAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTA

CAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAGGT

GTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGCGCCAAG

CACTACTACTATGGCGGCAGCTACGCTATGGACTACTGGGGCCAGGGCACCAGCGTG

ACCGTGAGCTCAGATCCACACGACGCCAGCGCCGCGACCACCAACACCGGCGCCC

ACCATCGCGTCGACGCCCCGTGTCCTGCGCCAGAGGCGTGCCCGCCAGCGGCGGG

GGGCGCAGTGACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTGGT

GGTGGTTGGTGGAGTCTGGCTTGCTATAGCTTGCTAGTAACAGTGCGCTTTATATTT

TCTGGGTGAGGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCTACCAGCAGG

GCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTT

GGACAAGAGACGTGGCCGGGACCTTGAGATGGGGGGAAGCCGAGAAGGAAGAAC

CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGA

GATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGG

GTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTACATGCAGGCCCTGCCTCC

-continued

TCGCAGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCC
CGGGCCCATGGCCGTGCCACTCAGGTCTGGGGTTGTTGCTACTGTGGCTTACAGAT
GCCAGATGTGACATCCAGATGACACAGTCTCCATCTTCCCTGTCTGCATCTGTCGGAGA
TCGCGTCACCATCACCTGTGAGCAAGTGAGGACATTATTTTAATTTAGTGTGGTATCA
GCAGAAACCAGGAAAGGCCCTAAGCTCCTGATCTATGATACAAATCGCTTGGCAGAT
GGGGTCCCATCACGGTTCAGTGGCTCTGGATCTGGCACACAGTATACTCTAACCATAA
GTAGCCTGCAACCCGAAGATTTCGCAACCTATTATTGTCAACACTATAAGAATTATCCGC
TCACGTTTCGGTCAGGGGACCAAGCTGGAAATCAAAGATCTGGTGGCGGAGGGTCAG
GAGCGGAGGCAGCGAGGCGGTGGCTCGGAGGCGGAGGCTCGAGATCTGAGGTG
CAGTTGGTGGAGTCTGGGGGCGGCTGGTGCAGCCTGGAGGGTCCCTGAGGCTCTCC
TGTGCAGCCTCAGGATTCACCTCTCAGTAATTATGGCATGCACTGGATCAGGCAGGCTC
CAGGGAAGGGTCTGGAGTGGGTCTCGTCTATTAGTCTTAATGGTGGTAGCACTTACTAT
CGAGACTCCGTGAAGGCGGATTCACTATCTCCAGGGACAATGCAAAAAGCACCCCTCT
ACCTTCAAATGAATAGTCTGAGGGCCGAGGACACGGCCGTCTATTACTGTGCAGCACA
GGACGCTTATACGGGAGGTACTTTGATTACTGGGGCCAAGGAACGCTGGTCACAGTC
TCGTCTATGGATCCCGCCGAGCCCAAATCTCTGACAAAACACACATGCCACCCT
GCCCAGCACCTCCCGTGGCCGGCCCGTCAGTCTTCTCTTCCCCCAAACCCAGGA
CACCTCATGATCGCCCGACCCCTGAGGTCAATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCTGGAGGTGCATAATGCC
AAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTC
ACCGTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACA
AAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGA
ACCACAGGTGTACACCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTCAG
CCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGAACCGGAGAACAACACTACAAGACCACGCCTCCCGTGTGGACTCCGACGG
CTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC
GTCTTCTCATGCTCCGTGATGCATGAGGCCCTGCACAATCACTATACCCAGAAATCTCT
GAGTCTGAGCCAGGCAAGAAGGACCCCAAGTTCTGGGTCTGGTGGTGGTGGGAGG
CGTGCTGGCCTGTTACTCTCTCTGGTGACCGTGGCCTTCATCATCTTTTGGGTGCGCT
CCCGGTGAAGTTTCTCGCTCTGCCGATGCCCCAGCCTATCAGCAGGGCCAGAATCA
GCTGTACAATGAACTGAACCTGGGCAGGCGGAGGAGTACGACGTGCTGGATAAGCG
GAGAGGCAGAGACCCGAGATGGGCGGCAAACCACGGCGCAAAAATCCCAGGAGG
GACTCTATAACGAGCTGCAGAAGGACAAAATGGCCGAGGCCTATTCGAGATCGGCAT
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TCCTCTTCTGTCTCCTCCTCCTGGTCTCTCTGCTCCATCGCCAGAATCAGATAAAG
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 CTTTGTCTACCTGCGGCAGCCGTACAGCGGTGGCGGTGGCAGCTTTGAGGCCTACTTC
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 TCAACGGATGACTACATCAATGCCAACTACATGCCTGGCTACCACTCCAAGAAAGATT
 TATTGCCACACAAGGACCTTTACCGAACACTTTGAAAGATTTTGGCGTATGGTTGGG
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 GTGAGGAGTATTGGCCCTCCAAGCAGGCTCAGGACTATGGAGACATAACTGTGGCAAT
 GACATCAGAAATTGTTCTTCCGAATGGACCATCAGAGATTTACAGTGAAAAATATCC
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 TGTTCCCGACACCACTGACCTGCTCATCAACTCCGGTACCTCGTTCTGCTACTACATGA
 AGCAGAGTCCTCCGAATCGCCGATTCTGGTGCATTGCAGTGTGGGTCGGAAGGA
 CGGGCACTTTTATTGCCATTGATCGTCTCATCTACCAGATAGAGAATGAGAACACCGTG
 GATGTGTATGGGATTGTGTATGACCTTCGAATGCATAGGCCTTTAATGGTGCAGACAGA
 GGACCAATATGTTTCTCAATCAGTGTGTTTGGATATTGTCAGATCCAGAAAGACTC
 AAAAGTAGATCTTATCTACCAGAACACAACGTGCAATGACAATCTATGAAAACCTTGCGCC
 CGTGACCACATTGGAAGACCAATGGTTACATCGCCAGCGGTAGCTAA

[0346] The nucleic acid sequence may encode the same amino acid sequence as that encoded by SEQ ID No. 35, 36, 37, 38, 39 or 40, but may have a different nucleic acid sequence, due to the degeneracy of the genetic code. The nucleic acid sequence may have at least 80, 85, 90, 95, 98 or 99% identity to the sequence shown as SEQ ID No. 35, 36, 37, 38, 39 or 40, provided that it encodes a first CAR and a second CAR as defined in the first aspect of the invention.

[0347] Vector

[0348] The present invention also provides a vector, or kit of vectors which comprises one or more CAR-encoding nucleic acid sequence(s). Such a vector may be used to introduce the nucleic acid sequence(s) into a host cell so that it expresses the first and second CARs.

[0349] The vector may, for example, be a plasmid or a viral vector, such as a retroviral vector or a lentiviral vector, or a transposon based vector or synthetic mRNA.

[0350] The vector may be capable of transfecting or transducing a T cell.

[0351] Pharmaceutical Composition

[0352] The present invention also relates to a pharmaceutical composition containing a plurality of CAR-expressing T cells or NK cells according to the first aspect of the invention. The pharmaceutical composition may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical composition may optionally comprise one or more further pharmaceutically active polypeptides and/or compounds. Such a formulation may, for example, be in a form suitable for intravenous infusion.

[0353] Method Of Treatment

[0354] The T cells of the present invention may be capable of killing target cells, such as cancer cells. The target cell may be recognisable by a defined pattern of antigen expres-

sion, for example the expression of antigen A AND antigen B; the expression of antigen A OR antigen B; or the expression of antigen A AND NOT antigen B or complex iterations of these gates.

[0355] T cells of the present invention may be used for the treatment of an infection, such as a viral infection.

[0356] T cells of the invention may also be used for the control of pathogenic immune responses, for example in autoimmune diseases, allergies and graft-vs-host rejection.

[0357] T cells of the invention may be used for the treatment of a cancerous disease, such as bladder cancer, breast cancer, colon cancer, endometrial cancer, kidney cancer (renal cell), leukemia, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer and thyroid cancer.

[0358] It is particularly suited for treatment of solid tumours where the availability of good selective single targets is limited.

[0359] T cells of the invention may be used to treat: cancers of the oral cavity and pharynx which includes cancer of the tongue, mouth and pharynx; cancers of the digestive system which includes oesophageal, gastric and colorectal cancers; cancers of the liver and biliary tree which includes hepatocellular carcinomas and cholangiocarcinomas; cancers of the respiratory system which includes bronchogenic cancers and cancers of the larynx; cancers of bone and joints which includes osteosarcoma; cancers of the skin which includes melanoma; breast cancer; cancers of the genital tract which include uterine, ovarian and cervical cancer in women, prostate and testicular cancer in men; cancers of the renal tract which include renal cell carcinoma and transitional cell carcinomas of the uterers or bladder; brain cancers including gliomas, glioblastoma multiforme and medulloblastomas; cancers of the endocrine system including thyroid cancer, adrenal carcinoma and cancers associated with multiple endocrine neoplasm syndromes; lymphomas including Hodgkin's lymphoma and non-Hodgkin lymphoma; Multiple Myeloma and plasmacytomas; leukemias both acute and chronic, myeloid or lymphoid; and cancers of other and unspecified sites including neuroblastoma.

[0360] Treatment with the T cells of the invention may help prevent the escape or release of tumour cells which often occurs with standard approaches.

[0361] The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1

Creation of Target Cell Populations

[0362] For the purposes of proving the principle of the invention, receptors based on anti-CD19 and anti-CD33 were arbitrarily chosen. Using retroviral vectors, CD19 and CD33 were cloned. These proteins were truncated so that they do not signal and could be stably expressed for prolonged periods. Next, these vectors were used to transduce the SupT1 cell line either singly or doubly to establish cells negative for both antigen (the wild-type), positive for either and positive for both. The expression data are shown in FIG. 3.

Example 2

Design and Function of the OR Gate

[0363] To construct the OR gate, a pair of receptors recognizing CD19 and CD33 were co-expressed. Different spacers were used to prevent cross-pairing. Both receptors had a trans-membrane domain derived from CD28 to improve surface stability and an endodomain derived from that of CD3 Zeta to provide a simple activating signal. In this way, a pair of independent 1st generation CARs were co-expressed. The retroviral vector cassette used to co-express the sequences utilizes a foot-and-mouth 2A self-cleaving peptide to allow co-expression 1:1 of both receptors. The cassette design is shown in FIG. 4, and the protein structures in FIG. 5. The nucleotide sequence of homologous regions was codon-wobbled to prevent recombination during retroviral vector reverse transcription.

Example 3

Testing the OR Gate

[0364] Expression of both CARs was tested on the T-cell surface by staining with cognate antigen fused to Fc. By using different species of Fc domains (mouse for CD19 and rabbit for CD33), co-expression of both CARs was determined on the cell surface by staining with different secondary antibodies conjugated with different fluorophores. This is shown in FIG. 6.

[0365] Functional testing was then carried out using the mouse T-cell line BW5147. This cell line releases IL2 upon activation allowing a simple quantitative readout. These T-cells were co-cultured with increasing amounts of the artificial target cells described above. T-cells responded to target cells expressing either antigen, as shown by IL2 release measured by ELISA. Both CARs were shown to be expressed on the cell surfaces and the T-cells were shown to respond to either or both antigens. These data are shown in FIG. 7.

Example 4

Design and function of the AND Gate

[0366] The AND gate combines a simple activating receptor with a receptor which basally inhibits activity, but whose inhibition is turned off once the receptor is ligated. This was achieved by combining a standard 1st generation CAR with a short/non-bulky CD8 stalk spacer and a CD3 Zeta endodomain with a second receptor with a bulky Fc spacer whose endodomain contained either CD148 or CD45 endodomains. When both receptors are ligated, the difference in spacer dimensions results in isolation of the different receptors in different membrane compartments, releasing the CD3 Zeta receptor from inhibition by the CD148 or CD45 endodomains. In this way, activation only occurs once both receptors are activated. CD148 and CD45 were chosen for this as they function in this manner natively: for instance, the very bulky CD45 ectodomain excludes the entire receptor from the immunological synapse. The expression cassette is depicted in FIG. 8 and the subsequent proteins in FIG. 9.

[0367] Surface staining for the different specificity showed that both receptor pairs could be effectively expressed on the cell surface shown in FIG. 10. Function in

BW5147 shows that the T-cell is only activated in the presence of both antigens (FIG. 11).

Example 5

Demonstration of Generalizability of the AND Gate

[0368] To ensure that the observations were not a manifestation of some specific characteristic of CD19/CD33 and their binders which had been used, the two targeting scFvs were swapped such that now, the activation (ITAM) signal was transmitted upon recognition of CD33, rather than CD19; and the inhibitory (CD148) signal was transmitted upon recognition of CD19, rather than of CD33. Since CD45 and CD148 endodomains are considered to be functionally similar, experimentation was restricted to AND gates with CD148 endodomain. This should still result in a functional AND gate. T-cells expressing the new logic gate were challenged with targets bearing either CD19 or CD33 alone, or both. The T-cells responded to targets expressing both CD19 and CD33, but not to targets expressing only one or none of these antigens. This shows that the AND gate is still functional in this format (FIG. 18B).

[0369] On the same lines, it was sought to establish how generalizable our AND gate is: the AND gate should be generalizable across different targets. While there may be lesser or greater fidelity of the gate given relative antigen density, cognate scFv binding kinetics and precise distance of the scFv binding epitope, one would expect to see some AND gate manifestations with a wide set of targets and binders. To test this, three additional AND gates were generated. Once again, experimentation was restricted to the CD148 version of the AND gate. The second scFv from the original CD148 AND gate was replaced with the anti-GD2 scFv huK666 (SEQ ID 41 and SEQ ID 42), or with the anti-CD5 scFv (SEQ ID 43 and SEQ ID 44), or the anti-EGFRvIII scFv MR1.1 (SEQ ID 45 AND SEQ ID 46) to generate the following CAR AND gates: CD19 AND GD2; CD19 AND CD5; CD19 AND EGFRvIII. The following artificial antigen expressing cell lines were also generated: by transducing SupT1, and our SupT1.CD19 with GM3 and GD2 synthases SupT1.GD2 and SupT1.CD19.GD2 were generated. By transducing SupT1 and SupT1.CD19 with a retroviral vector coding for EGFRvIII SupT1.EGFRvIII and SupT1.CD19.EGFRvIII were generated. Since CD5 is expressed on SupT1 cells, a different cell line was used to generate the target cells: 293T cells were generated which express CD19 alone, CD5 alone and both CD5 and CD19 together. Expression was confirmed by flow-cytometry (FIG. 19). T-cells expressing the three new CAR AND gates were challenged with SupT1.CD19 and respective cognate double positive and single positive target cells. All three AND gates demonstrated reduced activation by the double positive cell lines in comparison with the single positive targets (FIG. 20). This demonstrates generalizability of the AND gate design to arbitrary targets and cognate binders.

Example 6

Experimental Proof of Kinetic Segregation Model of CAR AND Gate

[0370] The aim was to prove the model that differential segregation caused by different spacers is the central mechanism behind the ability to generate these logic CAR gates. The model is that if only the activating CAR is ligated, the

potent inhibiting ‘ligation off’ type CAR is in solution in the membrane and can inhibit the activating CAR. Once both CARs are ligated, if both CAR spacers are sufficiently different, they will segregate within the synapse and not co-localize. Hence, a key requirement is that the spacers are sufficiently different. If the model is correct, if both spacers are sufficiently similar so they co-localize when both receptors are ligated, the gate will fail to function. To test this, the “bulky” Fc spacer in the original CAR we replaced with a murine CD8 spacer. It was predicted that this has the similar length, bulk and charge as human CD8 but so should not cross-pair with it. Hence, the new gate had a first CAR which recognizes CD19, a human CD8 stalk spacer and an activatory endodomain; while the second CAR recognizes CD33, has a mouse CD8 stalk spacer and a CD148 endodomain (FIG. 18C). T-cells were transduced to express this new CAR gate. These T-cells were then challenged with SupT1 cells expressing CD19 alone, CD33 alone or CD19 and CD33 together. T-cells did not respond to SupT1 cells expressing either antigen alone as per the original AND gate. However, CAR T-cells failed to respond to SupT1 cells expressing both antigens, thereby confirming the model (FIG. 18C). A functional AND gate requires both CARs to have spacers sufficiently different so that they do not co-localize within an immunological synapse (FIGS. 23A and B).

Example 7

Design and Function of an AND NOT Gate

[0371] Phosphatases such as CD45 and CD148 are so potent that even a small amount entering an immunological synapse can inhibit ITAM activation. This is the basis of inhibition of the logical AND gate. Other classes of phosphatases are not as potent e.g. PTPN6 and related phosphatases. It was predicted that a small amount of PTPN6 entering a synapse by diffusion would not inhibit activation. In addition, it was predicted that if an inhibitory CAR had a sufficiently similar spacer to an activating CAR, it could co-localize within a synapse if both CARs were ligated. In this case, large amounts of the inhibitory endodomain would be sufficient to stop the ITAMS from activating when both antigens were present. In this way, an AND NOT gate could be created.

[0372] For the NOT AND gate, the second signal needs to “veto” activation. This is done by bringing an inhibitory signal into the immunological synapse, for example by bringing in the phosphatase of an enzyme such as PTPN6. We hence generated an initial AND NOT gate as follows: two CARs co-expressed whereby the first recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; co-expressed with an anti-CD33 CAR with a mouse CD8 stalk spacer and an endodomain comprising of the catalytic domain of PTPN6 (SEQ ID 38, FIGS. 13A with B). A suitable cassette is shown in FIG. 12 and preliminary functional data are shown in FIG. 14.

[0373] In addition, an alternative strategy was developed for generating an AND NOT gate. Immune Tyrosinase Inhibitory Motifs (ITIMs) are activated in a similar manner to ITAMS, in that they become phosphorylated by Ick upon clustering and exclusion of phosphatases. Instead of triggering activation by binding ZAP70, phosphorylated ITIMs recruit phosphatases like PTPN6 through their cognate SH2 domains. An ITIM can function as an inhibitory endodo-

main, as long as the spacers on the activating and inhibiting CARs can co-localize. To generate this construct, an AND NOT gate was generated as follows: two CARs co-expressed—the first recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; co-expressed with an anti-CD33 CAR with a mouse CD8 stalk spacer and an ITIM containing endodomain derived from that of LAIR1 (SEQ ID 39, FIGS. 13A with C).

[0374] A further, more complex AND NOT gate was also developed, whereby an ITIM is enhanced by the presence of an additional chimeric protein: an intracellular fusion of the SH2 domain of PTPN6 and the endodomain of CD148. In this design three proteins are expressed—the first recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; co-expressed with an anti-CD33 CAR with a mouse CD8 stalk spacer and an ITIM containing endodomain derived from that of LAIR1. A further 2A peptide, allows co-expression of the PTPN6-CD148 fusion (SEQ ID 40, FIGS. 13A and D). It was predicted that these AND NOT gates would have a different range of inhibition: PTPN6-CD148 >PTPN6>>ITIM.

[0375] T-cells were transduced with these gates and challenged with targets expressing either CD19 or CD33 alone, or both CD19 and CD33 together. All three gates responded to targets expressing only CD19, but not targets expressing both CD19 and CD33 together (FIG. 21), confirming that all three of the AND NOT gates were functional.

Example 8

Experimental Proof of Kinetic Segregation Model of PTPN6 Based AND NOT Gate

[0376] The model of the AND NOT gate centres around the fact that the nature of the spacers used in both CARs is pivotal for the correct function of the gate. In the functional AND NOT gate with PTPN6, both CAR spacers are sufficiently similar that when both CARs are ligated, both co-localize within the synapse so the high concentration even the weak PTPN6 is sufficient to inhibit activation. If the spacers were different, segregation in the synapse will isolate the PTPN6 from the ITAM allowing activation disrupting the AND NOT gate. To test this, a control was generated replacing the murine CD8 stalk spacer with that of Fc. In this case, the test gate consisted of two CARs, the first recognizes CD19, has a human CD8 stalk spacer and an ITAM endodomain; while the second CAR recognizes CD33, has an Fc spacer and an endodomain comprising of the phosphatase from PTPN6. This gate activates in response to CD19, but also activates in response to CD19 and CD33 together (FIG. 22B, where function of this gate is compared with that of the original AND NOT, and the control AND gate variant described in Example 6). This experimental data proves the model that for a functional AND NOT gate with PTPN6, co-localizing spacers are needed.

Example 9

Experimental Proof of Kinetic Segregation Model of ITIM Based AND NOT Gate

[0377] Similar to the PTPN6 based AND NOT gate, the ITIM based gate also requires co-localization in an immunological synapse to function as an AND NOT gate. To prove this hypothesis, a control ITIM based gate was gen-

erated as follows: two CARs co-expressed—the first recognizes CD19, has a human CD8 stalk spacer and an activating endodomain; co-expressed with an anti-CD33 CAR with an Fc spacer and an ITIM containing endodomain derived from that of LAIR1. The activity of this gate was compared with that of the original ITIM based AND NOT gate. In this case, the modified gate activated in response to targets expressing CD19, but also activated in response to cells expressing both CD19 and CD33. These data indicate that ITIM based AND NOT gates follow the kinetic segregation based model and a correct spacer must be selected to create a functional gate (FIG. 23B).

Example 10

Summary of Model of CAR Logic Gates Generated by Kinetic Segregation

[0378] Based on current understanding of the kinetic-segregation model and the experimental data described herein, a summary of the model for a two-CAR gate is presented in FIG. 24. The Figure shows a cell expressing two CARs, each recognizing a different antigen. When either or both CARs recognize a target antigen on a cell, a synapse forms and native CD45 and CD148 are excluded from the synapse due to the bulk of their ectodomain. This sets the stage for T-cell activation. In the case that the target cell bears only one cognate antigen, the cognate CAR is ligated and the cognate CAR segregates into the synapse. The unligated CAR remains in solution on the T-cell membrane and can diffuse in and out of the synapse so that an area of high local concentration of ligated CAR with low concentration of unligated CAR forms. In this case, if the ligated CAR has an ITAM and the non-ligated CAR has 'ligation off' type inhibitory endodomain such as that of CD148, the amount of non-ligated CAR is sufficient to inhibit activation and the gate is off. In contrast, in this case, if the ligated CAR has an ITAM and the non-ligated CAR has a 'ligation on' type inhibitory endodomain such as PTPN6, the amount of non-ligated CAR is insufficient to inhibit and the gate is on. When challenged by a target cell bearing both cognate antigens, both cognate CARs are ligated and form part of an immunological synapse. Importantly, if the CAR spacers are sufficiently similar, the CARs co-localize in the synapse but if the CAR spacers are sufficiently different the CARs segregate within the synapse. In this latter case, areas of membrane form whereby high concentrations of one CAR are present but the other CAR is absent. In this case since segregation is complete, even if the inhibitory endodomain is a 'ligation off' type, the gate is on. In the former case, areas of membrane form with high concentrations of both CARs mixed together. In this case, since both endodomains are concentrated, even if the inhibitory endodomain is 'ligation on' type, the gate is off. By selecting the correct combination of spacer and endodomain logic can be programmed into a CAR T-cell.

[0379] Based on our work above, we have established a series of design rules to allow generation of logic-gated CARs (illustrated in FIG. 31). To generate an "antigen A OR antigen B" gated CAR T-cell, anti-A and anti-B CARs must be generated such that (1) each CAR has a spacer which simply allows antigen access and synapse formation such that the CAR functions, and (2) Each CAR has an activating endodomain; To generate an "antigen A AND NOT B" gated

CAR T-cell, anti-A and anti-B CARs must be generated such that (1) both CARs have spacers which do not cross-pair, but which will allow the CARs to co-segregate upon recognition of both cognate antigens on the target cell, (2) and one CAR has an activating endodomain, while the other CAR has an endodomain which comprises or recruits a weak phosphatase (e.g. PTPN6); (3) To generate an “antigen A AND antigen B” gated CAR T-cell, anti-A and anti-B CARs must be generated such that (1) one CAR has a spacer sufficiently

different from the other CAR such that both CARs will not co-segregate upon recognition of both cognate antigens on the target cell, (2) one CAR has an activating endodomain, while the other car has an endodomain which comprises of a potent phosphatase (e.g. that of CD45 or CD148). The correct spacers to achieve the desired effect can be selected from a set of spacers with known size/shape etc as well as taking into consideration size/shape etc of the target antigen and the location of the cognate epitope on the target antigen.

SEQ ID No 41: SFG.aCD19-CD8STK-CD28tmZ-2A-aGD2-HCH2CH3pvaa-dCD148
MSLPVTALLPLALLHAARPDIQMTQTSSLSASLGDRVTISCRASQDI SKYLNWYQQKPDGTVKLL
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WLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAA DTAVYYCAKRSDDYSWFAWYGQGLV
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YSTSNLASGVPSRPSGSGSGTDYTLTISSLQPEDFATYYCQYSGYPI TEGQGTKEIKRSDPAEPKS
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CSVMHEALHNHYTQKSLSLSPGKKDPKAVFGCIFGALVIVTVGGFIFWRKKRKDAKNNVFSFSQIKPK
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SVQTHSTDDYINANYMPGYHKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLT KCVEQGRTKCEEY
WPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGV PDTDLLINFRY
LVRDYMKSPPESPILVHCSAGVGRGTGTFIAIDRLIYQIENENTVDVYGI VYDLRMHRPLMVQTEDQY
VFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTEGKTNGYIA

SEQ ID No. 42: SFG.aCD19-CD8STK-CD28tmZ-2A-aGD2-HCH2CH3pvaa-dCD148
ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCTGGCCCTGCTGCTGCACGCCCGCAGACAGACAT
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GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTG
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Example 11

Design and Construction of APRIL Based CARs

[0380] APRIL in its natural form is a secreted type II protein. The use of APRIL as a BCMA binding domain for a CAR requires conversion of this type II secreted protein to a type I membrane bound protein and for this protein to be stable and to retain binding to BCMA in this form. To generate candidate molecules, the extreme amino-terminus of APRIL was deleted to remove binding to proteoglycans. Next, a signal peptide was added to direct the nascent protein to the endoplasmic reticulum and hence the cell surface. Also, because the nature of spacer used can alter the function of a CAR, three different spacer domains were tested: an APRIL based CAR was generated comprising (i) a human IgG1 spacer altered to remove Fc binding motifs; (ii) a CD8 stalk; and (iii) the IgG1 hinge alone (cartoon in FIG. 25 and amino acid sequences in FIG. 26). These CARs were expressed in a bicistronic retroviral vector (FIG. 27A) so that a marker protein—truncated CD34 could be co-expressed as a convenient marker gene.

Example 12

Expression and Function of APRIL Based CARs

[0381] The aim of this study was to test whether the APRIL based CARs which had been constructed were expressed on the cell surface and whether APRIL had folded to form the native protein. T-cells were transduced with these different CAR constructs and stained using a commercially available anti-APRIL mAb, along with staining for the marker gene and analysed by flow-cytometry. The results of this experiment are shown in FIG. 27B where APRIL binding is plotted against marker gene fluorescence. These data show that in this format, the APRIL based CARs are

expressed on the cell surface and APRIL folds sufficiently to be recognized by an anti-APRIL mAb.

[0382] Next, it was determined whether APRIL in this format could recognize BCMA and TACI. Recombinant BCMA and TACI were generated as fusions with mouse IgG2a-Fc. These recombinant proteins were incubated with the transduced T-cells. After this, the cells were washed and stained with an anti-mouse fluorophore conjugated antibody and an antibody to detect the marker gene conjugated to a different fluorophore. The cells were analysed by flow cytometry and the results are presented in FIG. 27C. The different CARs were able to bind both BCMA and TACI. Surprisingly, the CARs were better able to bind BCMA than TACI. Also, surprisingly CARs with a CD8 stalk or IgG1 hinge spacer were better able to bind BCMA and TACI than CAR with an Fc spacer.

Example 13

APRIL Based Chimeric Antigen Receptors are Active Against BCMA Expressing Cells

[0383] T-cells from normal donors were transduced with the different APRIL CARs and tested against SupT1 cells either wild-type, or engineered to express BCMA and TACI. Several different assays were used to determine function. A classical chromium release assay was performed. Here, the target cells (the SupT1 cells) were labelled with ⁵¹Cr and mixed with effectors (the transduced T-cells) at different ratio. Lysis of target cells was determined by counting ⁵¹Cr in the co-culture supernatant (FIG. 28A shows the cumulative data).

[0384] In addition, supernatant from T-cells cultured 1:1 with SupT1 cells was assayed by ELISA for Interferon-gamma (FIG. 28B shows cumulative data). Measurement of T-cell expansion after one week of co-culture with SupT1 cells was also performed (FIG. 28C). T-cells were counted

by flow-cytometry calibrated with counting beads. These experimental data show that APRIL based CARs can kill BCMA expressing targets. Further, these data show that CARs based on the CD8 stalk or IgG1 hinge performed better than the Fc-pvaa based CAR.

Example 14

Functional Analysis of the AND Gate in Primary Cells

[0385] PBMCs were isolated from blood and stimulated using PHA and IL-2. Two days later the cells were transduced on retronectin coated plates with retro virus containing the CD19:CD33 AND gate construct. On day 5 the expression level of the two CARs translated by the AND gate construct was evaluated via flow cytometry and the cells were depleted of CD56+ cells (predominantly NK cells). On day 6 the PBMCs were placed in a co-culture with

target cells at a 1:2 effector to target cell ratio. On day 8 the supernatant was collected and analysed for IFN-gamma secretion via ELISA (FIG. 29).

[0386] These data demonstrate that the AND gate functions in primary cells.

[0387] All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology, cell biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 55

<210> SEQ ID NO 1

<211> LENGTH: 1129

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric antigen receptor (CAR)

<400> SEQUENCE: 1

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Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20          25          30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35          40          45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50          55          60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65          70          75          80

Ser Arg Phe Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85          90          95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100         105         110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115         120         125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130         135         140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145         150         155         160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165         170         175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180         185         190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195         200         205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser

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210					215					220					
Lys 225	Ser	Gln	Val	Phe	Leu 230	Lys	Met	Asn	Ser	Leu 235	Gln	Thr	Asp	Asp	Thr 240
Ala	Ile	Tyr	Tyr	Cys 245	Ala	Lys	His	Tyr	Tyr 250	Tyr	Gly	Gly	Ser	Tyr	Ala 255
Met	Asp	Tyr	Trp 260	Gly	Gln	Gly	Thr	Ser 265	Val	Thr	Val	Ser	Ser 270	Asp	Pro
Thr	Thr	Thr	Pro 275	Ala	Pro	Arg	Pro 280	Pro	Thr	Pro	Ala	Pro 285	Thr	Ile	Ala
Ser	Gln 290	Pro	Leu	Ser	Leu	Arg 295	Pro	Glu	Ala	Cys 300	Arg	Pro	Ala	Ala	Gly
Gly 305	Ala	Val	His	Thr	Arg 310	Gly	Leu	Asp	Phe	Ala 315	Cys	Asp	Ile	Phe	Trp 320
Val	Leu	Val	Val 325	Val	Gly	Gly	Val	Leu	Ala 330	Cys	Tyr	Ser	Leu 335	Leu	Val
Thr	Val	Ala	Phe 340	Ile	Ile	Phe	Trp	Val 345	Arg	Arg	Val	Lys	Phe 350	Ser	Arg
Ser	Ala 355	Asp	Ala	Pro	Ala	Tyr	Gln 360	Gln	Gly	Gln	Asn	Gln 365	Leu	Tyr	Asn
Glu 370	Leu	Asn	Leu	Gly	Arg	Arg 375	Glu	Glu	Tyr	Asp 380	Val	Leu	Asp	Lys	Arg
Arg 385	Gly	Arg	Asp	Pro	Glu 390	Met	Gly	Gly	Lys	Pro 395	Arg	Arg	Lys	Asn	Pro 400
Gln	Glu	Gly	Leu 405	Tyr	Asn	Glu	Leu	Gln	Lys 410	Asp	Lys	Met	Ala 415	Glu	Ala
Tyr	Ser	Glu	Ile 420	Gly	Met	Lys	Gly	Glu 425	Arg	Arg	Arg	Gly	Lys 430	Gly	His
Asp	Gly	Leu 435	Tyr	Gln	Gly	Leu	Ser 440	Thr	Ala	Thr	Lys	Asp 445	Thr	Tyr	Asp
Ala 450	Leu	His	Met	Gln	Ala	Leu 455	Pro	Pro	Arg	Arg	Ala 460	Glu	Gly	Arg	Gly
Ser 465	Leu	Leu	Thr	Cys	Gly 470	Asp	Val	Glu	Glu	Asn 475	Pro	Gly	Pro	Met	Ala 480
Val	Pro	Thr	Gln 485	Val	Leu	Gly	Leu	Leu 490	Leu	Leu	Trp	Leu	Thr	Asp 495	Ala
Arg	Cys	Asp	Ile 500	Gln	Met	Thr	Gln	Ser 505	Pro	Ser	Ser	Leu 510	Ser	Ala	Ser
Val	Gly	Asp 515	Arg	Val	Thr	Ile	Thr 520	Cys	Arg	Ala	Ser	Glu 525	Asp	Ile	Tyr
Phe 530	Asn	Leu	Val	Trp	Tyr	Gln 535	Gln	Lys	Pro	Gly	Lys 540	Ala	Pro	Lys	Leu
Leu 545	Ile	Tyr	Asp	Thr	Asn 550	Arg	Leu	Ala	Asp	Gly 555	Val	Pro	Ser	Arg	Phe 560
Ser	Gly	Ser	Gly 565	Ser	Gly	Thr	Gln	Tyr	Thr 570	Leu	Thr	Ile	Ser	Ser	Leu 575
Gln	Pro	Glu	Asp 580	Phe	Ala	Thr	Tyr	Tyr 585	Cys	Gln	His	Tyr	Lys 590	Asn	Tyr
Pro	Leu	Thr	Phe 595	Gly	Gln	Gly	Thr 600	Lys	Leu	Glu	Ile	Lys 605	Arg	Ser	Gly
Gly 610	Gly	Gly	Ser	Gly	Gly 615	Gly	Gly	Ser	Gly	Gly 620	Gly	Gly	Ser	Gly	Gly

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Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	625	630	635	640
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	645	650	655	
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	660	665	670	
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	675	680	685	
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	690	695	700	
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	705	710	715	720
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	725	730	735	
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	740	745	750	
Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	755	760	765	
Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	770	775	780	
Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	785	790	795	800
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	805	810	815	
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	820	825	830	
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	835	840	845	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	850	855	860	
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	865	870	875	880
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	885	890	895	
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	900	905	910	
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	915	920	925	
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	930	935	940	
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	945	950	955	960
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	965	970	975	
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Asp	Pro	Lys	Phe	Trp	Val	Leu	980	985	990	
Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val	Thr	Val	995	1000	1005	
Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Arg	Val	Lys	Phe	Ser	Arg		1010	1015	1020	

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Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr
1025						1030					1035			
Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp
1040						1045					1050			
Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg
1055						1060					1065			
Lys	Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys
1070						1075					1080			
Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg
1085						1090					1095			
Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala
1100						1105					1110			
Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro
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Arg

<210> SEQ ID NO 2
 <211> LENGTH: 1350
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric antigen receptor (CAR)

<400> SEQUENCE: 2

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His	Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu
			20					25					30		
Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln
			35				40					45			
Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr
	50					55					60				
Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro
	65				70					75				80	
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile
			85						90					95	
Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly
			100					105					110		
Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr
	115					120					125				
Lys	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	130					135					140				
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly
	145				150					155				160	
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly
			165						170					175	
Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg
			180					185					190		
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr
	195					200					205				
Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser
	210					215					220				
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr

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225					230					235					240
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala
				245					250					255	
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Asp	Pro
			260					265					270		
Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala
		275					280					285			
Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly
	290					295				300					
Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Phe	Trp
305					310					315					320
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val
			325					330						335	
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg
			340					345					350		
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn
		355					360					365			
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg
	370					375					380				
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro
385					390					395					400
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala
			405					410						415	
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His
		420						425					430		
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp
		435					440					445			
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly
	450					455					460				
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala
465					470					475					480
Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala
			485					490						495	
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
		500						505					510		
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr
		515					520					525			
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu
	530					535					540				
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe
545					550					555					560
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu
			565					570					575		
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr
			580					585					590		
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly
		595					600					605			
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly
	610					615					620				
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu
625					630					635					640

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Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	645	650	655
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	660	665	670
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	675	680	685
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	690	695	700
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	705	710	715
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	725	730	735
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	740	745	750
Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	755	760	765
Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	770	775	780
Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	785	790	795
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	805	810	815
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	820	825	830
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	835	840	845
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	850	855	860
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	865	870	875
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	885	890	895
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	900	905	910
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	915	920	925
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	930	935	940
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	945	950	955
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	965	970	975
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Asp	Pro	Lys	Ala	Val	Phe	Gly	980	985	990
Cys	Ile	Phe	Gly	Ala	Leu	Val	Ile	Val	Thr	Val	Gly	Gly	Phe	Ile	Phe	995	1000	1005
Trp	Arg	Lys	Lys	Arg	Lys	Asp	Ala	Lys	Asn	Asn	Glu	Val	Ser	Phe		1010	1015	1020
Ser	Gln	Ile	Lys	Pro	Lys	Lys	Ser	Lys	Leu	Ile	Arg	Val	Glu	Asn		1025	1030	1035

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Phe	Glu	Ala	Tyr	Phe	Lys	Lys	Gln	Gln	Ala	Asp	Ser	Asn	Cys	Gly
1040						1045					1050			
Phe	Ala	Glu	Glu	Tyr	Glu	Asp	Leu	Lys	Leu	Val	Gly	Ile	Ser	Gln
1055						1060					1065			
Pro	Lys	Tyr	Ala	Ala	Glu	Leu	Ala	Glu	Asn	Arg	Gly	Lys	Asn	Arg
1070						1075					1080			
Tyr	Asn	Asn	Val	Leu	Pro	Tyr	Asp	Ile	Ser	Arg	Val	Lys	Leu	Ser
1085						1090					1095			
Val	Gln	Thr	His	Ser	Thr	Asp	Asp	Tyr	Ile	Asn	Ala	Asn	Tyr	Met
1100						1105					1110			
Pro	Gly	Tyr	His	Ser	Lys	Lys	Asp	Phe	Ile	Ala	Thr	Gln	Gly	Pro
1115						1120					1125			
Leu	Pro	Asn	Thr	Leu	Lys	Asp	Phe	Trp	Arg	Met	Val	Trp	Glu	Lys
1130						1135					1140			
Asn	Val	Tyr	Ala	Ile	Ile	Met	Leu	Thr	Lys	Cys	Val	Glu	Gln	Gly
1145						1150					1155			
Arg	Thr	Lys	Cys	Glu	Glu	Tyr	Trp	Pro	Ser	Lys	Gln	Ala	Gln	Asp
1160						1165					1170			
Tyr	Gly	Asp	Ile	Thr	Val	Ala	Met	Thr	Ser	Glu	Ile	Val	Leu	Pro
1175						1180					1185			
Glu	Trp	Thr	Ile	Arg	Asp	Phe	Thr	Val	Lys	Asn	Ile	Gln	Thr	Ser
1190						1195					1200			
Glu	Ser	His	Pro	Leu	Arg	Gln	Phe	His	Phe	Thr	Ser	Trp	Pro	Asp
1205						1210					1215			
His	Gly	Val	Pro	Asp	Thr	Thr	Asp	Leu	Leu	Ile	Asn	Phe	Arg	Tyr
1220						1225					1230			
Leu	Val	Arg	Asp	Tyr	Met	Lys	Gln	Ser	Pro	Pro	Glu	Ser	Pro	Ile
1235						1240					1245			
Leu	Val	His	Cys	Ser	Ala	Gly	Val	Gly	Arg	Thr	Gly	Thr	Phe	Ile
1250						1255					1260			
Ala	Ile	Asp	Arg	Leu	Ile	Tyr	Gln	Ile	Glu	Asn	Glu	Asn	Thr	Val
1265						1270					1275			
Asp	Val	Tyr	Gly	Ile	Val	Tyr	Asp	Leu	Arg	Met	His	Arg	Pro	Leu
1280						1285					1290			
Met	Val	Gln	Thr	Glu	Asp	Gln	Tyr	Val	Phe	Leu	Asn	Gln	Cys	Val
1295						1300					1305			
Leu	Asp	Ile	Val	Arg	Ser	Gln	Lys	Asp	Ser	Lys	Val	Asp	Leu	Ile
1310						1315					1320			
Tyr	Gln	Asn	Thr	Thr	Ala	Met	Thr	Ile	Tyr	Glu	Asn	Leu	Ala	Pro
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Val	Thr	Thr	Phe	Gly	Lys	Thr	Asn	Gly	Tyr	Ile	Ala			
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<210> SEQ ID NO 3

<211> LENGTH: 1717

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric antigen receptor (CAR)

<400> SEQUENCE: 3

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
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His	Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu
			20					25					30		
Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln
		35					40				45				
Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr
	50					55					60				
Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro
	65				70					75					80
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile
			85						90					95	
Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly
		100						105					110		
Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr
	115						120					125			
Lys	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	130					135					140				
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly
	145				150					155					160
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly
			165						170					175	
Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg
		180						185					190		
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr
	195						200					205			
Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser
	210					215						220			
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr
	225				230					235					240
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala
				245					250					255	
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Asp	Pro
		260						265					270		
Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala
		275					280					285			
Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly
	290					295					300				
Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Phe	Trp
	305				310					315					320
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val
				325					330					335	
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg
		340					345						350		
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn
		355					360					365			
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg
	370					375					380				
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro
	385				390					395					400
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala
			405						410					415	
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His

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420							425					430				
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	
435							440					445				
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly	
450							455					460				
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala	
465	470					475					480					
Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala	
485					490					495						
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	
500			505					510								
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr	
515							520					525				
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	
530							535					540				
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe	
545	550					555					560					
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	
565					570					575						
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr	
580			585					590								
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly	
595		600					605									
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
610		615					620									
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	
625	630					635					640					
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	
645			650					655								
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	
660			665					670								
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	
675		680					685									
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
690		695					700									
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	
705	710					715					720					
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	
725			730					735								
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	
740		745					750									
Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	
755		760					765									
Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	
770		775					780									
Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	
785	790					795					800					
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	
805			810					815								
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	
820		825					830									

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Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
	835						840					845			
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
	850					855					860				
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
865					870					875				880	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr
			885						890					895	
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
		900						905					910		
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
	915						920					925			
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr
	930					935					940				
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
945					950					955					960
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
			965						970					975	
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Asp	Pro	Lys	Ala	Leu	Ile	Ala
	980						985						990		
Phe	Leu	Ala	Phe	Leu	Ile	Ile	Val	Thr	Ser	Ile	Ala	Leu	Leu	Val	Val
	995						1000						1005		
Leu	Tyr	Lys	Ile	Tyr	Asp	Leu	His	Lys	Lys	Arg	Ser	Cys	Asn	Leu	
	1010					1015						1020			
Asp	Glu	Gln	Gln	Glu	Leu	Val	Glu	Arg	Asp	Asp	Glu	Lys	Gln	Leu	
	1025					1030					1035				
Met	Asn	Val	Glu	Pro	Ile	His	Ala	Asp	Ile	Leu	Leu	Glu	Thr	Tyr	
	1040					1045						1050			
Lys	Arg	Lys	Ile	Ala	Asp	Glu	Gly	Arg	Leu	Phe	Leu	Ala	Glu	Phe	
	1055					1060						1065			
Gln	Ser	Ile	Pro	Arg	Val	Phe	Ser	Lys	Phe	Pro	Ile	Lys	Glu	Ala	
	1070					1075						1080			
Arg	Lys	Pro	Phe	Asn	Gln	Asn	Lys	Asn	Arg	Tyr	Val	Asp	Ile	Leu	
	1085					1090						1095			
Pro	Tyr	Asp	Tyr	Asn	Arg	Val	Glu	Leu	Ser	Glu	Ile	Asn	Gly	Asp	
	1100					1105						1110			
Ala	Gly	Ser	Asn	Tyr	Ile	Asn	Ala	Ser	Tyr	Ile	Asp	Gly	Phe	Lys	
	1115					1120						1125			
Glu	Pro	Arg	Lys	Tyr	Ile	Ala	Ala	Gln	Gly	Pro	Arg	Asp	Glu	Thr	
	1130					1135						1140			
Val	Asp	Asp	Phe	Trp	Arg	Met	Ile	Trp	Glu	Gln	Lys	Ala	Thr	Val	
	1145					1150						1155			
Ile	Val	Met	Val	Thr	Arg	Cys	Glu	Glu	Gly	Asn	Arg	Asn	Lys	Cys	
	1160					1165						1170			
Ala	Glu	Tyr	Trp	Pro	Ser	Met	Glu	Glu	Gly	Thr	Arg	Ala	Phe	Gly	
	1175					1180						1185			
Asp	Val	Val	Val	Lys	Ile	Asn	Gln	His	Lys	Arg	Cys	Pro	Asp	Tyr	
	1190					1195						1200			
Ile	Ile	Gln	Lys	Leu	Asn	Ile	Val	Asn	Lys	Lys	Glu	Lys	Ala	Thr	
	1205					1210						1215			

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Gly Arg 1220	Glu Val Thr His 1225	Ile Gln Phe Thr Ser 1230	Trp Pro Asp His 1235
Gly Val 1235	Pro Glu Asp Pro His 1240	Leu Leu Leu Lys 1245	Leu Arg Arg Arg 1250
Val Asn 1250	Ala Phe Ser Asn Phe 1255	Phe Ser Gly Pro 1260	Ile Val Val His 1265
Cys Ser 1265	Ala Gly Val Gly Arg 1270	Thr Gly Thr Tyr 1275	Ile Gly Ile Asp 1280
Ala Met 1280	Leu Glu Gly Leu Glu 1285	Ala Glu Asn Lys 1290	Val Asp Val Tyr 1295
Gly Tyr 1295	Val Val Lys Leu Arg 1300	Arg Gln Arg Cys 1305	Leu Met Val Gln 1310
Val Glu 1310	Ala Gln Tyr Ile Leu 1315	Ile His Gln Ala 1320	Leu Val Glu Tyr 1325
Asn Gln 1325	Phe Gly Glu Thr Glu 1330	Val Asn Leu Ser 1335	Glu Leu His Pro 1340
Tyr Leu 1340	His Asn Met Lys Lys 1345	Arg Asp Pro Pro 1350	Ser Glu Pro Ser 1355
Pro Leu 1355	Glu Ala Glu Phe Gln 1360	Arg Leu Pro Ser 1365	Tyr Arg Ser Trp 1370
Arg Thr 1370	Gln His Ile Gly Asn 1375	Gln Glu Glu Asn 1380	Lys Ser Lys Asn 1385
Arg Asn 1385	Ser Asn Val Ile Pro 1390	Tyr Asp Tyr Asn 1395	Arg Val Pro Leu 1400
Lys His 1400	Glu Leu Glu Met Ser 1405	Lys Glu Ser Glu 1410	His Asp Ser Asp 1415
Glu Ser 1415	Ser Asp Asp Asp Ser 1420	Asp Ser Glu Glu 1425	Pro Ser Lys Tyr 1430
Ile Asn 1430	Ala Ser Phe Ile Met 1435	Ser Tyr Trp Lys 1440	Pro Glu Val Met 1445
Ile Ala 1445	Ala Gln Gly Pro Leu 1450	Lys Glu Thr Ile 1455	Gly Asp Phe Trp 1460
Gln Met 1460	Ile Phe Gln Arg Lys 1465	Val Lys Val Ile 1470	Val Met Leu Thr 1475
Glu Leu 1475	Lys His Gly Asp Gln 1480	Glu Ile Cys Ala 1485	Gln Tyr Trp Gly 1490
Glu Gly 1490	Lys Gln Thr Tyr Gly 1495	Asp Ile Glu Val 1500	Asp Leu Lys Asp 1505
Thr Asp 1505	Lys Ser Ser Thr Tyr 1510	Thr Leu Arg Val 1515	Phe Glu Leu Arg 1520
His Ser 1520	Lys Arg Lys Asp Ser 1525	Arg Thr Val Tyr 1530	Gln Tyr Gln Tyr 1535
Thr Asn 1535	Trp Ser Val Glu Gln 1540	Leu Pro Ala Glu 1545	Pro Lys Glu Leu 1550
Ile Ser 1550	Met Ile Gln Val Val 1555	Lys Gln Lys Leu 1560	Pro Gln Lys Asn 1565
Ser Ser 1565	Glu Gly Asn Lys His 1570	His Lys Ser Thr 1575	Pro Leu Leu Ile 1580
His Cys 1580	Arg Asp Gly Ser Gln 1585	Gln Thr Gly Ile 1590	Phe Cys Ala Leu 1595
Leu Asn 1595	Leu Leu Glu Ser Ala 1600	Glu Thr Glu Glu 1605	Val Val Asp Ile 1610

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1595	1600	1605
Phe Gln Val Val Lys Ala Leu Arg Lys Ala Arg Pro Gly Met Val		
1610	1615	1620
Ser Thr Phe Glu Gln Tyr Gln Phe Leu Tyr Asp Val Ile Ala Ser		
1625	1630	1635
Thr Tyr Pro Ala Gln Asn Gly Gln Val Lys Lys Asn Asn His Gln		
1640	1645	1650
Glu Asp Lys Ile Glu Phe Asp Asn Glu Val Asp Lys Val Lys Gln		
1655	1660	1665
Asp Ala Asn Cys Val Asn Pro Leu Gly Ala Pro Glu Lys Leu Pro		
1670	1675	1680
Glu Ala Lys Glu Gln Ala Glu Gly Ser Glu Pro Thr Ser Gly Thr		
1685	1690	1695
Glu Gly Pro Glu His Ser Val Asn Gly Pro Ala Ser Pro Ala Leu		
1700	1705	1710
Asn Gln Gly Ser		
1715		

<210> SEQ ID NO 4
 <211> LENGTH: 1114
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric antigen receptor (CAR)

<400> SEQUENCE: 4

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu		
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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu		
20	25	30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln		
35	40	45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr		
50	55	60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro		
65	70	75
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile		
85	90	95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly		
100	105	110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr		
115	120	125
Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly		
130	135	140
Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly		
145	150	155
Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly		
165	170	175
Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg		
180	185	190
Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr		
195	200	205
Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser		

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210					215					220					
Lys 225	Ser	Gln	Val	Phe	Leu 230	Lys	Met	Asn	Ser	Leu 235	Gln	Thr	Asp	Asp	Thr 240
Ala	Ile	Tyr	Tyr	Cys 245	Ala	Lys	His	Tyr	Tyr 250	Tyr	Gly	Gly	Ser	Tyr	Ala 255
Met	Asp	Tyr	Trp 260	Gly	Gln	Gly	Thr	Ser 265	Val	Thr	Val	Ser	Ser 270	Asp	Pro
Thr	Thr	Thr	Pro 275	Ala	Pro	Arg	Pro 280	Pro	Thr	Pro	Ala	Pro 285	Thr	Ile	Ala
Ser	Gln 290	Pro	Leu	Ser	Leu	Arg 295	Pro	Glu	Ala	Cys 300	Arg	Pro	Ala	Ala	Gly
Gly 305	Ala	Val	His	Thr	Arg 310	Gly	Leu	Asp	Phe	Ala 315	Cys	Asp	Ile	Phe	Trp 320
Val	Leu	Val	Val 325	Val	Gly	Gly	Val	Leu	Ala 330	Cys	Tyr	Ser	Leu 335	Leu	Val
Thr	Val	Ala	Phe 340	Ile	Ile	Phe	Trp	Val 345	Arg	Arg	Val	Lys	Phe 350	Ser	Arg
Ser	Ala 355	Asp	Ala	Pro	Ala	Tyr	Gln 360	Gln	Gly	Gln	Asn	Gln 365	Leu	Tyr	Asn
Glu 370	Leu	Asn	Leu	Gly	Arg	Arg 375	Glu	Glu	Tyr	Asp 380	Val	Leu	Asp	Lys	Arg
Arg 385	Gly	Arg	Asp	Pro	Glu 390	Met	Gly	Gly	Lys	Pro 395	Arg	Arg	Lys	Asn	Pro 400
Gln	Glu	Gly	Leu 405	Tyr	Asn	Glu	Leu	Gln	Lys 410	Asp	Lys	Met	Ala 415	Glu	Ala
Tyr	Ser	Glu	Ile 420	Gly	Met	Lys	Gly	Glu 425	Arg	Arg	Arg	Gly	Lys 430	Gly	His
Asp	Gly	Leu 435	Tyr	Gln	Gly	Leu	Ser 440	Thr	Ala	Thr	Lys	Asp 445	Thr	Tyr	Asp
Ala 450	Leu	His	Met	Gln	Ala	Leu 455	Pro	Pro	Arg	Arg	Ala 460	Glu	Gly	Arg	Gly
Ser 465	Leu	Leu	Thr	Cys	Gly 470	Asp	Val	Glu	Glu	Asn 475	Pro	Gly	Pro	Met	Ala 480
Val	Pro	Thr	Gln 485	Val	Leu	Gly	Leu	Leu	Leu 490	Leu	Trp	Leu	Thr	Asp 495	Ala
Arg	Cys	Asp	Ile 500	Gln	Met	Thr	Gln	Ser 505	Pro	Ser	Ser	Leu 510	Ser	Ala	Ser
Val	Gly	Asp 515	Arg	Val	Thr	Ile	Thr 520	Cys	Arg	Ala	Ser	Glu 525	Asp	Ile	Tyr
Phe 530	Asn	Leu	Val	Trp	Tyr	Gln 535	Gln	Lys	Pro	Gly	Lys 540	Ala	Pro	Lys	Leu
Leu 545	Ile	Tyr	Asp	Thr	Asn 550	Arg	Leu	Ala	Asp	Gly 555	Val	Pro	Ser	Arg	Phe 560
Ser	Gly	Ser	Gly	Ser 565	Gly	Thr	Gln	Tyr	Thr 570	Leu	Thr	Ile	Ser	Ser	Leu
Gln	Pro	Glu	Asp 580	Phe	Ala	Thr	Tyr	Tyr 585	Cys	Gln	His	Tyr	Lys 590	Asn	Tyr
Pro	Leu	Thr	Phe 595	Gly	Gln	Gly	Thr 600	Lys	Leu	Glu	Ile	Lys 605	Arg	Ser	Gly
Gly 610	Gly	Gly	Ser	Gly	Gly 615	Gly	Gly	Ser	Gly	Gly 620	Gly	Gly	Ser	Gly	Gly

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Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	625	630	635	640
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	645	650	655	
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	660	665	670	
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	675	680	685	
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	690	695	700	
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	705	710	715	720
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	725	730	735	
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	740	745	750	
Ala	Thr	Thr	Thr	Lys	Pro	Val	Leu	Arg	Thr	Pro	Ser	Pro	Val	His	Pro	755	760	765	
Thr	Gly	Thr	Ser	Gln	Pro	Gln	Arg	Pro	Glu	Asp	Cys	Arg	Pro	Arg	Gly	770	775	780	
Ser	Val	Lys	Gly	Thr	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Trp	Ala	785	790	795	800
Pro	Leu	Ala	Gly	Ile	Cys	Val	Ala	Leu	Leu	Leu	Ser	Leu	Ile	Ile	Thr	805	810	815	
Leu	Ile	Cys	Tyr	His	Arg	Ser	Arg	Lys	Arg	Val	Cys	Lys	Ser	Gly	Gly	820	825	830	
Gly	Ser	Phe	Trp	Glu	Glu	Phe	Glu	Ser	Leu	Gln	Lys	Gln	Glu	Val	Lys	835	840	845	
Asn	Leu	His	Gln	Arg	Leu	Glu	Gly	Gln	Arg	Pro	Glu	Asn	Lys	Gly	Lys	850	855	860	
Asn	Arg	Tyr	Lys	Asn	Ile	Leu	Pro	Phe	Asp	His	Ser	Arg	Val	Ile	Leu	865	870	875	880
Gln	Gly	Arg	Asp	Ser	Asn	Ile	Pro	Gly	Ser	Asp	Tyr	Ile	Asn	Ala	Asn	885	890	895	
Tyr	Ile	Lys	Asn	Gln	Leu	Leu	Gly	Pro	Asp	Glu	Asn	Ala	Lys	Thr	Tyr	900	905	910	
Ile	Ala	Ser	Gln	Gly	Cys	Leu	Glu	Ala	Thr	Val	Asn	Asp	Phe	Trp	Gln	915	920	925	
Met	Ala	Trp	Gln	Glu	Asn	Ser	Arg	Val	Ile	Val	Met	Thr	Thr	Arg	Glu	930	935	940	
Val	Glu	Lys	Gly	Arg	Asn	Lys	Cys	Val	Pro	Tyr	Trp	Pro	Glu	Val	Gly	945	950	955	960
Met	Gln	Arg	Ala	Tyr	Gly	Pro	Tyr	Ser	Val	Thr	Asn	Cys	Gly	Glu	His	965	970	975	
Asp	Thr	Thr	Glu	Tyr	Lys	Leu	Arg	Thr	Leu	Gln	Val	Ser	Pro	Leu	Asp	980	985	990	
Asn	Gly	Asp	Leu	Ile	Arg	Glu	Ile	Trp	His	Tyr	Gln	Tyr	Leu	Ser	Trp	995	1000	1005	
Pro	Asp	His	Gly	Val	Pro	Ser	Glu	Pro	Gly	Gly	Val	Leu	Ser	Phe		1010	1015	1020	

Leu

<400> SEQUENCE: 5

Met 1	Ser	Leu	Pro 5	Val	Thr	Ala	Leu	Leu 10	Pro	Leu	Ala	Leu	Leu 15	Leu	
His	Ala	Ala	Arg 20	Pro	Asp	Ile	Gln	Met 25	Thr	Gln	Thr	Thr	Ser 30	Ser	Leu
Ser	Ala	Ser 35	Leu	Gly	Asp	Arg	Val 40	Thr	Ile	Ser	Cys 45	Arg	Ala	Ser	Gln
Asp	Ile 50	Ser	Lys	Tyr	Leu	Asn 55	Trp	Tyr	Gln	Gln	Lys 60	Pro	Asp	Gly	Thr
Val 65	Lys	Leu	Leu	Ile	Tyr 70	His	Thr	Ser	Arg	Leu 75	His	Ser	Gly	Val	Pro 80
Ser	Arg	Phe	Ser 85	Gly	Ser	Gly	Ser	Gly	Thr 90	Asp	Tyr	Ser	Leu	Thr 95	Ile
Ser	Asn	Leu	Glu 100	Gln	Glu	Asp	Ile	Ala 105	Thr	Tyr	Phe	Cys 110	Gln	Gln	Gly
Asn	Thr 115	Leu	Pro	Tyr	Thr	Phe	Gly 120	Gly	Gly	Thr	Lys	Leu 125	Glu	Ile	Thr
Lys 130	Ala	Gly	Gly	Gly	Gly	Ser 135	Gly	Gly	Gly	Gly	Ser 140	Gly	Gly	Gly	Gly
Ser 145	Gly	Gly	Gly	Gly	Ser 150	Glu	Val	Lys	Leu	Gln 155	Glu	Ser	Gly	Pro	Gly 160
Leu	Val	Ala	Pro 165	Ser	Gln	Ser	Leu	Ser	Val 170	Thr	Cys	Thr	Val	Ser 175	Gly
Val	Ser	Leu 180	Pro	Asp	Tyr	Gly	Val	Ser 185	Trp	Ile	Arg	Gln	Pro 190	Pro	Arg
Lys	Gly 195	Leu	Glu	Trp	Leu	Gly	Val 200	Ile	Trp	Gly	Ser	Glu 205	Thr	Thr	Tyr
Tyr 210	Asn	Ser	Ala	Leu	Lys 215	Ser	Arg	Leu	Thr	Ile	Ile	Lys 220	Asp	Asn	Ser
Lys 225	Ser	Gln	Val	Phe	Leu 230	Lys	Met	Asn	Ser	Leu 235	Gln	Thr	Asp	Asp	Thr 240
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala

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245								250					255				
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Asp	Pro		
			260				265						270				
Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala		
			275				280						285				
Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly		
			290	295						300							
Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Phe	Trp		
305				310						315			320				
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val		
			325						330			335					
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg		
			340			345						350					
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn		
			355			360						365					
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg		
			370			375						380					
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro		
385				390						395			400				
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala		
			405						410			415					
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His		
			420			425						430					
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp		
			435			440						445					
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly		
			450			455						460					
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala		
465				470						475			480				
Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala		
			485						490			495					
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser		
			500			505						510					
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr		
			515			520						525					
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu		
			530			535						540					
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe		
545				550						555			560				
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu		
			565						570			575					
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr		
			580			585						590					
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly		
			595			600						605					
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly		
			610			615						620					
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu		
625				630						635			640				
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe		
			645			650						655					

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Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
 660 665 670
 Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
 675 680 685
 Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
 690 695 700
 Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
 705 710 715 720
 Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
 725 730 735
 Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
 740 745 750
 Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
 755 760 765
 Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
 770 775 780
 Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Leu Ile Gly
 785 790 795 800
 Val Ser Val Val Phe Leu Phe Cys Leu Leu Leu Leu Val Leu Phe Cys
 805 810 815
 Leu His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp
 820 825 830
 Glu Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu
 835 840 845
 Glu Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp
 850 855 860
 Arg Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val
 865 870 875 880
 Thr Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg
 885 890 895
 Ala Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr
 900 905 910
 Ala Ala Val Ala Arg His
 915

<210> SEQ ID NO 6
 <211> LENGTH: 1363
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric antigen receptor (CAR)

<400> SEQUENCE: 6

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
 1 5 10 15
 His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
 20 25 30
 Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
 35 40 45
 Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
 50 55 60
 Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
 65 70 75 80

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Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	85	90	95
Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	100	105	110
Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr	115	120	125
Lys	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	130	135	140
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	145	150	155
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	165	170	175
Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	180	185	190
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	195	200	205
Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	210	215	220
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	225	230	235
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	245	250	255
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Asp	Pro	260	265	270
Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	275	280	285
Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	290	295	300
Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Phe	Trp	305	310	315
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val	325	330	335
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg	340	345	350
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	355	360	365
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	370	375	380
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	385	390	395
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	405	410	415
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	420	425	430
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	435	440	445
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly	450	455	460
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala	465	470	475
																		480

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Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala	
			485						490					495		
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	
			500					505					510			
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr	
		515					520					525				
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	
	530					535					540					
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe	
545					550					555					560	
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	
				565					570					575		
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr	
			580					585					590			
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly	
		595					600					605				
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
	610					615					620					
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	
625					630					635					640	
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	
				645					650					655		
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	
			660					665					670			
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	
	675						680					685				
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
	690					695					700					
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	
705					710					715					720	
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	
				725					730					735		
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	
			740					745					750			
Ala	Thr	Thr	Thr	Lys	Pro	Val	Leu	Arg	Thr	Pro	Ser	Pro	Val	His	Pro	
		755					760					765				
Thr	Gly	Thr	Ser	Gln	Pro	Gln	Arg	Pro	Glu	Asp	Cys	Arg	Pro	Arg	Gly	
	770					775					780					
Ser	Val	Lys	Gly	Thr	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Leu	Ile	Gly	
785					790					795					800	
Val	Ser	Val	Val	Phe	Leu	Phe	Cys	Leu	Leu	Leu	Val	Leu	Phe	Cys		
				805					810				815			
Leu	His	Arg	Gln	Asn	Gln	Ile	Lys	Gln	Gly	Pro	Pro	Arg	Ser	Lys	Asp	
			820					825					830			
Glu	Glu	Gln	Lys	Pro	Gln	Gln	Arg	Pro	Asp	Leu	Ala	Val	Asp	Val	Leu	
		835					840					845				
Glu	Arg	Thr	Ala	Asp	Lys	Ala	Thr	Val	Asn	Gly	Leu	Pro	Glu	Lys	Asp	
	850					855					860					
Arg	Glu	Thr	Asp	Thr	Ser	Ala	Leu	Ala	Ala	Gly	Ser	Ser	Gln	Glu	Val	
865					870					875					880	
Thr	Tyr	Ala	Gln	Leu	Asp	His	Trp	Ala	Leu	Thr	Gln	Arg	Thr	Ala	Arg	

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885							890							895						
Ala	Val	Ser	Pro	Gln	Ser	Thr	Lys	Pro	Met	Ala	Glu	Ser	Ile	Thr	Tyr					
900							905							910						
Ala	Ala	Val	Ala	Arg	His	Arg	Ala	Glu	Gly	Arg	Gly	Ser	Leu	Leu	Thr					
915							920							925						
Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Trp	Tyr	His	Gly	His	Met					
930							935							940						
Ser	Gly	Gly	Gln	Ala	Glu	Thr	Leu	Leu	Gln	Ala	Lys	Gly	Glu	Pro	Trp					
945							950							955						
Thr	Phe	Leu	Val	Arg	Glu	Ser	Leu	Ser	Gln	Pro	Gly	Asp	Phe	Val	Leu					
965							970							975						
Ser	Val	Leu	Ser	Asp	Gln	Pro	Lys	Ala	Gly	Pro	Gly	Ser	Pro	Leu	Arg					
980							985							990						
Val	Thr	His	Ile	Lys	Val	Met	Cys	Glu	Gly	Gly	Arg	Tyr	Thr	Val	Gly					
995							1000							1005						
Gly	Leu	Glu	Thr	Phe	Asp	Ser	Leu	Thr	Asp	Leu	Val	Glu	His	Phe						
1010							1015							1020						
Lys	Lys	Thr	Gly	Ile	Glu	Glu	Ala	Ser	Gly	Ala	Phe	Val	Tyr	Leu						
1025							1030							1035						
Arg	Gln	Pro	Tyr	Ser	Gly	Gly	Gly	Gly	Ser	Phe	Glu	Ala	Tyr	Phe						
1040							1045							1050						
Lys	Lys	Gln	Gln	Ala	Asp	Ser	Asn	Cys	Gly	Phe	Ala	Glu	Glu	Tyr						
1055							1060							1065						
Glu	Asp	Leu	Lys	Leu	Val	Gly	Ile	Ser	Gln	Pro	Lys	Tyr	Ala	Ala						
1070							1075							1080						
Glu	Leu	Ala	Glu	Asn	Arg	Gly	Lys	Asn	Arg	Tyr	Asn	Asn	Val	Leu						
1085							1090							1095						
Pro	Tyr	Asp	Ile	Ser	Arg	Val	Lys	Leu	Ser	Val	Gln	Thr	His	Ser						
1100							1105							1110						
Thr	Asp	Asp	Tyr	Ile	Asn	Ala	Asn	Tyr	Met	Pro	Gly	Tyr	His	Ser						
1115							1120							1125						
Lys	Lys	Asp	Phe	Ile	Ala	Thr	Gln	Gly	Pro	Leu	Pro	Asn	Thr	Leu						
1130							1135							1140						
Lys	Asp	Phe	Trp	Arg	Met	Val	Trp	Glu	Lys	Asn	Val	Tyr	Ala	Ile						
1145							1150							1155						
Ile	Met	Leu	Thr	Lys	Cys	Val	Glu	Gln	Gly	Arg	Thr	Lys	Cys	Glu						
1160							1165							1170						
Glu	Tyr	Trp	Pro	Ser	Lys	Gln	Ala	Gln	Asp	Tyr	Gly	Asp	Ile	Thr						
1175							1180							1185						
Val	Ala	Met	Thr	Ser	Glu	Ile	Val	Leu	Pro	Glu	Trp	Thr	Ile	Arg						
1190							1195							1200						
Asp	Phe	Thr	Val	Lys	Asn	Ile	Gln	Thr	Ser	Glu	Ser	His	Pro	Leu						
1205							1210							1215						
Arg	Gln	Phe	His	Phe	Thr	Ser	Trp	Pro	Asp	His	Gly	Val	Pro	Asp						
1220							1225							1230						
Thr	Thr	Asp	Leu	Leu	Ile	Asn	Phe	Arg	Tyr	Leu	Val	Arg	Asp	Tyr						
1235							1240							1245						
Met	Lys	Gln	Ser	Pro	Pro	Glu	Ser	Pro	Ile	Leu	Val	His	Cys	Ser						
1250							1255							1260						
Ala	Gly	Val	Gly	Arg	Thr	Gly	Thr	Phe	Ile	Ala	Ile	Asp	Arg	Leu						
1265							1270							1275						

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Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile
1280 1285 1290

Val Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu
1295 1300 1305

Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg
1310 1315 1320

Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr
1325 1330 1335

Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly
1340 1345 1350

Lys Thr Asn Gly Tyr Ile Ala Ser Gly Ser
1355 1360

<210> SEQ ID NO 7
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Signal peptide

<400> SEQUENCE: 7

Met Gly Thr Ser Leu Leu Cys Trp Met Ala Leu Cys Leu Leu Gly Ala
1 5 10 15

Asp His Ala Asp Gly
20

<210> SEQ ID NO 8
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Signal peptide

<400> SEQUENCE: 8

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro
20

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Signal peptide

<400> SEQUENCE: 9

Met Ala Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1 5 10 15

Asp Ala Arg Cys
20

<210> SEQ ID NO 10
<211> LENGTH: 234
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Spacer (hinge-CH2CH3 of human IgG1)

<400> SEQUENCE: 10

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Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
 1 5 10 15

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 20 25 30

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp
 225 230

<210> SEQ ID NO 11
 <211> LENGTH: 46
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Spacer (human CD8 stalk)

<400> SEQUENCE: 11

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
 1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
 20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
 35 40 45

<210> SEQ ID NO 12
 <211> LENGTH: 20
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Spacer (human IgG1 hinge)

<400> SEQUENCE: 12

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro

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1	5	10	15
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Lys Asp Pro Lys
20

<210> SEQ ID NO 13
 <211> LENGTH: 185
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Spacer (CD2 ectodomain)

<400> SEQUENCE: 13

Lys	Glu	Ile	Thr	Asn	Ala	Leu	Glu	Thr	Trp	Gly	Ala	Leu	Gly	Gln	Asp
1				5					10					15	
Ile	Asn	Leu	Asp	Ile	Pro	Ser	Phe	Gln	Met	Ser	Asp	Asp	Ile	Asp	Asp
		20					25						30		
Ile	Lys	Trp	Glu	Lys	Thr	Ser	Asp	Lys	Lys	Lys	Ile	Ala	Gln	Phe	Arg
		35					40					45			
Lys	Glu	Lys	Glu	Thr	Phe	Lys	Glu	Lys	Asp	Thr	Tyr	Lys	Leu	Phe	Lys
	50					55				60					
Asn	Gly	Thr	Leu	Lys	Ile	Lys	His	Leu	Lys	Thr	Asp	Asp	Gln	Asp	Ile
65				70					75					80	
Tyr	Lys	Val	Ser	Ile	Tyr	Asp	Thr	Lys	Gly	Lys	Asn	Val	Leu	Glu	Lys
			85						90					95	
Ile	Phe	Asp	Leu	Lys	Ile	Gln	Glu	Arg	Val	Ser	Lys	Pro	Lys	Ile	Ser
		100					105						110		
Trp	Thr	Cys	Ile	Asn	Thr	Thr	Leu	Thr	Cys	Glu	Val	Met	Asn	Gly	Thr
		115					120					125			
Asp	Pro	Glu	Leu	Asn	Leu	Tyr	Gln	Asp	Gly	Lys	His	Leu	Lys	Leu	Ser
	130				135						140				
Gln	Arg	Val	Ile	Thr	His	Lys	Trp	Thr	Thr	Ser	Leu	Ser	Ala	Lys	Phe
145					150				155					160	
Lys	Cys	Thr	Ala	Gly	Asn	Lys	Val	Ser	Lys	Glu	Ser	Ser	Val	Glu	Pro
			165						170					175	
Val	Ser	Cys	Pro	Glu	Lys	Gly	Leu	Asp							
		180					185								

<210> SEQ ID NO 14
 <211> LENGTH: 259
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Spacer (CD34 ectodomain)

<400> SEQUENCE: 14

Ser	Leu	Asp	Asn	Asn	Gly	Thr	Ala	Thr	Pro	Glu	Leu	Pro	Thr	Gln	Gly
1				5					10					15	
Thr	Phe	Ser	Asn	Val	Ser	Thr	Asn	Val	Ser	Tyr	Gln	Glu	Thr	Thr	Thr
			20					25					30		
Pro	Ser	Thr	Leu	Gly	Ser	Thr	Ser	Leu	His	Pro	Val	Ser	Gln	His	Gly
		35					40					45			
Asn	Glu	Ala	Thr	Thr	Asn	Ile	Thr	Glu	Thr	Thr	Val	Lys	Phe	Thr	Ser
	50					55					60				
Thr	Ser	Val	Ile	Thr	Ser	Val	Tyr	Gly	Asn	Thr	Asn	Ser	Ser	Val	Gln
65				70					75					80	

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Ser Gln Thr Ser Val Ile Ser Thr Val Phe Thr Thr Pro Ala Asn Val
 85 90 95
 Ser Thr Pro Glu Thr Thr Leu Lys Pro Ser Leu Ser Pro Gly Asn Val
 100 105 110
 Ser Asp Leu Ser Thr Thr Ser Thr Ser Leu Ala Thr Ser Pro Thr Lys
 115 120 125
 Pro Tyr Thr Ser Ser Ser Pro Ile Leu Ser Asp Ile Lys Ala Glu Ile
 130 135 140
 Lys Cys Ser Gly Ile Arg Glu Val Lys Leu Thr Gln Gly Ile Cys Leu
 145 150 155 160
 Glu Gln Asn Lys Thr Ser Ser Cys Ala Glu Phe Lys Lys Asp Arg Gly
 165 170 175
 Glu Gly Leu Ala Arg Val Leu Cys Gly Glu Glu Gln Ala Asp Ala Asp
 180 185 190
 Ala Gly Ala Gln Val Cys Ser Leu Leu Leu Ala Gln Ser Glu Val Arg
 195 200 205
 Pro Gln Cys Leu Leu Leu Val Leu Ala Asn Arg Thr Glu Ile Ser Ser
 210 215 220
 Lys Leu Gln Leu Met Lys Lys His Gln Ser Asp Leu Lys Lys Leu Gly
 225 230 235 240
 Ile Leu Asp Phe Thr Glu Gln Asp Val Ala Ser His Gln Ser Tyr Ser
 245 250 255
 Gln Lys Thr

<210> SEQ ID NO 15
 <211> LENGTH: 140
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CD28 transmembrane domain and CD3 Z endodomains

<400> SEQUENCE: 15

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
 1 5 10 15
 Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe
 20 25 30
 Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
 35 40 45
 Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
 50 55 60
 Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
 65 70 75 80
 Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
 85 90 95
 Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
 100 105 110
 Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
 115 120 125
 Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
 130 135 140

<210> SEQ ID NO 16
 <211> LENGTH: 180
 <212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD28 transmembrane domain and CD28 and CD3 Zeta endodomains

<400> SEQUENCE: 16

```

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1           5           10           15
Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
20           25           30
Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
35           40           45
Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
50           55           60
Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
65           70           75           80
Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
85           90           95
Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
100          105          110
Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
115          120          125
Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
130          135          140
Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
145          150          155          160
Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
165          170          175
Leu Pro Pro Arg
180

```

<210> SEQ ID NO 17

<211> LENGTH: 216

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD28 transmembrane domain and CD28, OX40 and CD3 Zeta endodomains

<400> SEQUENCE: 17

```

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1           5           10           15
Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
20           25           30
Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
35           40           45
Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
50           55           60
Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro
65           70           75           80
Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp
85           90           95
Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala
100          105          110
Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu

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115	120	125
Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly		
130	135	140
Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu		
145	150	155
Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser		
165	170	175
Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly		
180	185	190
Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu		
195	200	205
His Met Gln Ala Leu Pro Pro Arg		
210	215	
<210> SEQ ID NO 18		
<211> LENGTH: 729		
<212> TYPE: PRT		
<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: CD45 transmembrane and endodomain		
<400> SEQUENCE: 18		
Ala Leu Ile Ala Phe Leu Ala Phe Leu Ile Ile Val Thr Ser Ile Ala		
1	5	10
Leu Leu Val Val Leu Tyr Lys Ile Tyr Asp Leu His Lys Lys Arg Ser		
20	25	30
Cys Asn Leu Asp Glu Gln Gln Glu Leu Val Glu Arg Asp Asp Glu Lys		
35	40	45
Gln Leu Met Asn Val Glu Pro Ile His Ala Asp Ile Leu Leu Glu Thr		
50	55	60
Tyr Lys Arg Lys Ile Ala Asp Glu Gly Arg Leu Phe Leu Ala Glu Phe		
65	70	75
Gln Ser Ile Pro Arg Val Phe Ser Lys Phe Pro Ile Lys Glu Ala Arg		
85	90	95
Lys Pro Phe Asn Gln Asn Lys Asn Arg Tyr Val Asp Ile Leu Pro Tyr		
100	105	110
Asp Tyr Asn Arg Val Glu Leu Ser Glu Ile Asn Gly Asp Ala Gly Ser		
115	120	125
Asn Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Phe Lys Glu Pro Arg Lys		
130	135	140
Tyr Ile Ala Ala Gln Gly Pro Arg Asp Glu Thr Val Asp Asp Phe Trp		
145	150	155
Arg Met Ile Trp Glu Gln Lys Ala Thr Val Ile Val Met Val Thr Arg		
165	170	175
Cys Glu Glu Gly Asn Arg Asn Lys Cys Ala Glu Tyr Trp Pro Ser Met		
180	185	190
Glu Glu Gly Thr Arg Ala Phe Gly Asp Val Val Val Lys Ile Asn Gln		
195	200	205
His Lys Arg Cys Pro Asp Tyr Ile Ile Gln Lys Leu Asn Ile Val Asn		
210	215	220
Lys Lys Glu Lys Ala Thr Gly Arg Glu Val Thr His Ile Gln Phe Thr		
225	230	235
Ser Trp Pro Asp His Gly Val Pro Glu Asp Pro His Leu Leu Leu Lys		

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245																250						255			
Leu	Arg	Arg	Arg	Val	Asn	Ala	Phe	Ser	Asn	Phe	Phe	Ser	Gly	Pro	Ile										
260																265						270			
Val	Val	His	Cys	Ser	Ala	Gly	Val	Gly	Arg	Thr	Gly	Thr	Tyr	Ile	Gly										
275																280						285			
Ile	Asp	Ala	Met	Leu	Glu	Gly	Leu	Glu	Ala	Glu	Asn	Lys	Val	Asp	Val										
290																295						300			
Tyr	Gly	Tyr	Val	Val	Lys	Leu	Arg	Arg	Gln	Arg	Cys	Leu	Met	Val	Gln										
305																310						315			
Val	Glu	Ala	Gln	Tyr	Ile	Leu	Ile	His	Gln	Ala	Leu	Val	Glu	Tyr	Asn										
320																325						330			
Gln	Phe	Gly	Glu	Thr	Glu	Val	Asn	Leu	Ser	Glu	Leu	His	Pro	Tyr	Leu										
335																340						345			
His	Asn	Met	Lys	Lys	Arg	Asp	Pro	Pro	Ser	Glu	Pro	Ser	Pro	Leu	Glu										
350																355						360			
Ala	Glu	Phe	Gln	Arg	Leu	Pro	Ser	Tyr	Arg	Ser	Trp	Arg	Thr	Gln	His										
365																370						375			
Ile	Gly	Asn	Gln	Glu	Glu	Asn	Lys	Ser	Lys	Asn	Arg	Asn	Ser	Asn	Val										
380																385						390			
Ile	Pro	Tyr	Asp	Tyr	Asn	Arg	Val	Pro	Leu	Lys	His	Glu	Leu	Glu	Met										
395																400						405			
Ser	Lys	Glu	Ser	Glu	His	Asp	Ser	Asp	Glu	Ser	Ser	Asp	Asp	Asp	Ser										
410																415						420			
Asp	Ser	Glu	Glu	Pro	Ser	Lys	Tyr	Ile	Asn	Ala	Ser	Phe	Ile	Met	Ser										
425																430						435			
Tyr	Trp	Lys	Pro	Glu	Val	Met	Ile	Ala	Ala	Gln	Gly	Pro	Leu	Lys	Glu										
440																445						450			
Thr	Ile	Gly	Asp	Phe	Trp	Gln	Met	Ile	Phe	Gln	Arg	Lys	Val	Lys	Val										
455																460						465			
Ile	Val	Met	Leu	Thr	Glu	Leu	Lys	His	Gly	Asp	Gln	Glu	Ile	Cys	Ala										
470																475						480			
Gln	Tyr	Trp	Gly	Glu	Gly	Lys	Gln	Thr	Tyr	Gly	Asp	Ile	Glu	Val	Asp										
485																490						495			
Leu	Lys	Asp	Thr	Asp	Lys	Ser	Ser	Thr	Tyr	Thr	Leu	Arg	Val	Phe	Glu										
500																505						510			
Leu	Arg	His	Ser	Lys	Arg	Lys	Asp	Ser	Arg	Thr	Val	Tyr	Gln	Tyr	Gln										
515																520						525			
Leu	Thr	Asn	Trp	Ser	Val	Glu	Gln	Leu	Pro	Ala	Glu	Pro	Lys	Glu	Leu										
530																535						540			
Tyr	Thr	Asn	Trp	Ser	Val	Glu	Gln	Leu	Pro	Ala	Glu	Pro	Lys	Glu	Leu										
545																550						555			
Ile	Ser	Met	Ile	Gln	Val	Val	Lys	Gln	Lys	Leu	Pro	Gln	Lys	Asn	Ser										
560																565						570			
Ser	Glu	Gly	Asn	Lys	His	His	Lys	Ser	Thr	Pro	Leu	Leu	Ile	His	Cys										
575																580						585			
Arg	Asp	Gly	Ser	Gln	Gln	Thr	Gly	Ile	Phe	Cys	Ala	Leu	Leu	Asn	Leu										
590																595						600			
Leu	Glu	Ser	Ala	Glu	Thr	Glu	Glu	Val	Val	Asp	Ile	Phe	Gln	Val	Val										
605																610						615			
Lys	Ala	Leu	Arg	Lys	Ala	Arg	Pro	Gly	Met	Val	Ser	Thr	Phe	Glu	Gln										
620																625						630			
Tyr	Gln	Phe	Leu	Tyr	Asp	Val	Ile	Ala	Ser	Thr	Tyr	Pro	Ala	Gln	Asn										
635																640						645			

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Gly Gln Val Lys Lys Asn Asn His Gln Glu Asp Lys Ile Glu Phe Asp
660 665 670

Asn Glu Val Asp Lys Val Lys Gln Asp Ala Asn Cys Val Asn Pro Leu
675 680 685

Gly Ala Pro Glu Lys Leu Pro Glu Ala Lys Glu Gln Ala Glu Gly Ser
690 695 700

Glu Pro Thr Ser Gly Thr Glu Gly Pro Glu His Ser Val Asn Gly Pro
705 710 715 720

Ala Ser Pro Ala Leu Asn Gln Gly Ser
725

<210> SEQ ID NO 19
<211> LENGTH: 362
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD148 transmembrane and endodomain

<400> SEQUENCE: 19

Ala Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly
1 5 10 15

Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu
20 25 30

Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val
35 40 45

Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys
50 55 60

Gly Phe Ala Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln
65 70 75 80

Pro Lys Tyr Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr
85 90 95

Asn Asn Val Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln
100 105 110

Thr His Ser Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr
115 120 125

His Ser Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr
130 135 140

Leu Lys Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile
145 150 155 160

Ile Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu
165 170 175

Tyr Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala
180 185 190

Met Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr
195 200 205

Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His
210 215 220

Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu
225 230 235 240

Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro
245 250 255

Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly
260 265 270

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Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn
 275 280 285
 Thr Val Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro
 290 295 300
 Leu Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val
 305 310 315 320
 Leu Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr
 325 330 335
 Gln Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr
 340 345 350
 Thr Phe Gly Lys Thr Asn Gly Tyr Ile Ala
 355 360

<210> SEQ ID NO 20

<211> LENGTH: 595

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: sequence of PTPN6

<400> SEQUENCE: 20

Met Val Arg Trp Phe His Arg Asp Leu Ser Gly Leu Asp Ala Glu Thr
 1 5 10 15
 Leu Leu Lys Gly Arg Gly Val His Gly Ser Phe Leu Ala Arg Pro Ser
 20 25 30
 Arg Lys Asn Gln Gly Asp Phe Ser Leu Ser Val Arg Val Gly Asp Gln
 35 40 45
 Val Thr His Ile Arg Ile Gln Asn Ser Gly Asp Phe Tyr Asp Leu Tyr
 50 55 60
 Gly Gly Glu Lys Phe Ala Thr Leu Thr Glu Leu Val Glu Tyr Tyr Thr
 65 70 75 80
 Gln Gln Gln Gly Val Leu Gln Asp Arg Asp Gly Thr Ile Ile His Leu
 85 90 95
 Lys Tyr Pro Leu Asn Cys Ser Asp Pro Thr Ser Glu Arg Trp Tyr His
 100 105 110
 Gly His Met Ser Gly Gly Gln Ala Glu Thr Leu Leu Gln Ala Lys Gly
 115 120 125
 Glu Pro Trp Thr Phe Leu Val Arg Glu Ser Leu Ser Gln Pro Gly Asp
 130 135 140
 Phe Val Leu Ser Val Leu Ser Asp Gln Pro Lys Ala Gly Pro Gly Ser
 145 150 155 160
 Pro Leu Arg Val Thr His Ile Lys Val Met Cys Glu Gly Gly Arg Tyr
 165 170 175
 Thr Val Gly Gly Leu Glu Thr Phe Asp Ser Leu Thr Asp Leu Val Glu
 180 185 190
 His Phe Lys Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala Phe Val Tyr
 195 200 205
 Leu Arg Gln Pro Tyr Tyr Ala Thr Arg Val Asn Ala Ala Asp Ile Glu
 210 215 220
 Asn Arg Val Leu Glu Leu Asn Lys Lys Gln Glu Ser Glu Asp Thr Ala
 225 230 235 240
 Lys Ala Gly Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val
 245 250 255

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Lys Asn Leu His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly
      260                      265                      270

Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile
      275                      280                      285

Leu Gln Gly Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala
      290                      295                      300

Asn Tyr Ile Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr
      305                      310                      315                      320

Tyr Ile Ala Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp
      325                      330                      335

Gln Met Ala Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg
      340                      345                      350

Glu Val Glu Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val
      355                      360                      365

Gly Met Gln Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu
      370                      375                      380

His Asp Thr Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu
      385                      390                      395                      400

Asp Asn Gly Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser
      405                      410                      415

Trp Pro Asp His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe
      420                      425                      430

Leu Asp Gln Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly Pro
      435                      440                      445

Ile Ile Val His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr Ile Ile
      450                      455                      460

Val Ile Asp Met Leu Met Glu Asn Ile Ser Thr Lys Gly Leu Asp Cys
      465                      470                      475                      480

Asp Ile Asp Ile Gln Lys Thr Ile Gln Met Val Arg Ala Gln Arg Ser
      485                      490                      495

Gly Met Val Gln Thr Glu Ala Gln Tyr Lys Phe Ile Tyr Val Ala Ile
      500                      505                      510

Ala Gln Phe Ile Glu Thr Thr Lys Lys Lys Leu Glu Val Leu Gln Ser
      515                      520                      525

Gln Lys Gly Gln Glu Ser Glu Tyr Gly Asn Ile Thr Tyr Pro Pro Ala
      530                      535                      540

Met Lys Asn Ala His Ala Lys Ala Ser Arg Thr Ser Ser Lys His Lys
      545                      550                      555                      560

Glu Asp Val Tyr Glu Asn Leu His Thr Lys Asn Lys Arg Glu Glu Lys
      565                      570                      575

Val Lys Lys Gln Arg Ser Ala Asp Lys Glu Lys Ser Lys Gly Ser Leu
      580                      585                      590

Lys Arg Lys
      595

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<210> SEQ ID NO 21

<211> LENGTH: 272

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: sequence of phosphatase domain of PTPN6

<400> SEQUENCE: 21

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Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val Lys Asn Leu
1      5      10      15
His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly Lys Asn Arg
20      25      30
Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile Leu Gln Gly
35      40      45
Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala Asn Tyr Ile
50      55      60
Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr Tyr Ile Ala
65      70      75      80
Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp Gln Met Ala
85      90      95
Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg Glu Val Glu
100     105     110
Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val Gly Met Gln
115     120     125
Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu His Asp Thr
130     135     140
Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu Asp Asn Gly
145     150     155     160
Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser Trp Pro Asp
165     170     175
His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe Leu Asp Gln
180     185     190
Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly Pro Ile Ile Val
195     200     205
His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr Ile Ile Val Ile Asp
210     215     220
Met Leu Met Glu Asn Ile Ser Thr Lys Gly Leu Asp Cys Asp Ile Asp
225     230     235     240
Ile Gln Lys Thr Ile Gln Met Val Arg Ala Gln Arg Ser Gly Met Val
245     250     255
Gln Thr Glu Ala Gln Tyr Lys Phe Ile Tyr Val Ala Ile Ala Gln Phe
260     265     270

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<210> SEQ ID NO 22

<211> LENGTH: 97

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PDCD1 endodomain

<400> SEQUENCE: 22

```

Cys Ser Arg Ala Ala Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln
1      5      10      15
Pro Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr
20      25      30
Gly Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val
35      40      45
Pro Cys Val Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser
50      55      60
Gly Met Gly Thr Ser Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro
65      70      75      80

```

Leu

<400> SEQUENCE: 23

Gln Ala Gln Glu Asn Val Asp Tyr Val Ile Leu Lys His
130 135 140

<400> SEQUENCE: 24

Glu Asp Arg Gln Met Asp Thr Glu Ala Ala Ala Ser Glu Ala Pro Gln
115 120 125

-continued

Asp Val Thr Tyr Ala Gln Leu His Ser Leu Thr Leu Arg Arg Glu Ala
130 135 140

Thr Glu Pro Pro Pro Ser Gln Glu Gly Pro Ser Pro Ala Val Pro Ser
145 150 155 160

Ile Tyr Ala Thr Leu Ala Ile His
165

<210> SEQ ID NO 25
<211> LENGTH: 101
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LAIR1 endodomain

<400> SEQUENCE: 25

His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp Glu
1 5 10 15

Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu Glu
20 25 30

Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp Arg
35 40 45

Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val Thr
50 55 60

Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg Ala
65 70 75 80

Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr Ala
85 90 95

Ala Val Ala Arg His
100

<210> SEQ ID NO 26
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CTLA4 endodomain

<400> SEQUENCE: 26

Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe Tyr
1 5 10 15

Ser Phe Leu Leu Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys Arg
20 25 30

Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu Pro
35 40 45

Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
50 55 60

<210> SEQ ID NO 27
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KIR2DL1 endodomain

<400> SEQUENCE: 27

Gly Asn Ser Arg His Leu His Val Leu Ile Gly Thr Ser Val Val Ile
1 5 10 15

-continued

```

Ile Pro Phe Ala Ile Leu Leu Phe Phe Leu Leu His Arg Trp Cys Ala
      20                25                30
Asn Lys Lys Asn Ala Val Val Met Asp Gln Glu Pro Ala Gly Asn Arg
      35                40                45
Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln Glu Val Thr
      50                55                60
Tyr Thr Gln Leu Asn His Cys Val Phe Thr Gln Arg Lys Ile Thr Arg
      65                70                75                80
Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Ile Ile Val Tyr Thr
      85                90                95
Glu Leu Pro Asn Ala Glu Ser Arg Ser Lys Val Val Ser Cys Pro
      100               105               110

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<210> SEQ ID NO 28
<211> LENGTH: 143
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KIR2DL4 endodomain

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<400> SEQUENCE: 28

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```

Gly Ile Ala Arg His Leu His Ala Val Ile Arg Tyr Ser Val Ala Ile
1      5      10      15
Ile Leu Phe Thr Ile Leu Pro Phe Phe Leu Leu His Arg Trp Cys Ser
      20                25                30
Lys Lys Lys Glu Asn Ala Ala Val Met Asn Gln Glu Pro Ala Gly His
      35                40                45
Arg Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln Glu Val
      50                55                60
Thr Tyr Ala Gln Leu Asp His Cys Ile Phe Thr Gln Arg Lys Ile Thr
      65                70                75                80
Gly Pro Ser Gln Arg Ser Lys Arg Pro Ser Thr Asp Thr Ser Val Cys
      85                90                95
Ile Glu Leu Pro Asn Ala Glu Pro Arg Ala Leu Ser Pro Ala His Glu
      100               105               110
His His Ser Gln Ala Leu Met Gly Ser Ser Arg Glu Thr Thr Ala Leu
      115               120               125
Ser Gln Thr Gln Leu Ala Ser Ser Asn Val Pro Ala Ala Gly Ile
      130               135               140

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<210> SEQ ID NO 29
<211> LENGTH: 143
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KIR2DL5 endodomain

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<400> SEQUENCE: 29

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```

Thr Gly Ile Arg Arg His Leu His Ile Leu Ile Gly Thr Ser Val Ala
1      5      10      15
Ile Ile Leu Phe Ile Ile Leu Phe Phe Phe Leu Leu His Cys Cys Cys
      20                25                30
Ser Asn Lys Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asp
      35                40                45
Arg Thr Val Asn Arg Glu Asp Ser Asp Asp Gln Asp Pro Gln Glu Val
      50                55                60

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Thr Tyr Ala Gln Leu Asp His Cys Val Phe Thr Gln Thr Lys Ile Thr
65              70              75              80

Ser Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Thr Met Tyr
              85              90              95

Met Glu Leu Pro Asn Ala Lys Pro Arg Ser Leu Ser Pro Ala His Lys
              100             105             110

His His Ser Gln Ala Leu Arg Gly Ser Ser Arg Glu Thr Thr Ala Leu
              115             120             125

Ser Gln Asn Arg Val Ala Ser Ser His Val Pro Ala Ala Gly Ile
              130             135             140

```

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<210> SEQ ID NO 30
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KIR3DL1 endodomain

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<400> SEQUENCE: 30

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

Ile Leu Phe Ile Leu Leu Leu Phe Phe Leu Leu His Leu Trp Cys Ser
20         25         30

Asn Lys Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asn Arg
35         40         45

Thr Ala Asn Ser Glu Asp Ser Asp Glu Gln Asp Pro Glu Glu Val Thr
50         55         60

Tyr Ala Gln Leu Asp His Cys Val Phe Thr Gln Arg Lys Ile Thr Arg
65         70         75         80

Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Ile Leu Tyr Thr
85         90         95

Glu Leu Pro Asn Ala Lys Pro Arg Ser Lys Val Val Ser Cys Pro
100        105        110

```

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<210> SEQ ID NO 31
<211> LENGTH: 97
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KIR3DL3 endodomain

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```

<400> SEQUENCE: 31

```

```

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

Val Val Ile Ile Pro Phe Ala Ile Leu Leu Phe Phe Leu Leu His Arg
20         25         30

Trp Cys Ala Asn Lys Lys Asn Ala Val Val Met Asp Gln Glu Pro Ala
35         40         45

Gly Asn Arg Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln
50         55         60

Glu Val Thr Tyr Ala Gln Leu Asn His Cys Val Phe Thr Gln Arg Lys
65         70         75         80

Ile Thr Arg Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Ser
85         90         95

```

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Val

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<210> SEQ ID NO 32
<211> LENGTH: 807
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PTPN6-CD45 fusion protein

<400> SEQUENCE: 32
Trp Tyr His Gly His Met Ser Gly Gly Gln Ala Glu Thr Leu Leu Gln
1           5           10           15
Ala Lys Gly Glu Pro Trp Thr Phe Leu Val Arg Glu Ser Leu Ser Gln
20           25           30
Pro Gly Asp Phe Val Leu Ser Val Leu Ser Asp Gln Pro Lys Ala Gly
35           40           45
Pro Gly Ser Pro Leu Arg Val Thr His Ile Lys Val Met Cys Glu Gly
50           55           60
Gly Arg Tyr Thr Val Gly Gly Leu Glu Thr Phe Asp Ser Leu Thr Asp
65           70           75           80
Leu Val Glu His Phe Lys Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala
85           90           95
Phe Val Tyr Leu Arg Gln Pro Tyr Lys Ile Tyr Asp Leu His Lys Lys
100          105          110
Arg Ser Cys Asn Leu Asp Glu Gln Gln Glu Leu Val Glu Arg Asp Asp
115          120          125
Glu Lys Gln Leu Met Asn Val Glu Pro Ile His Ala Asp Ile Leu Leu
130          135          140
Glu Thr Tyr Lys Arg Lys Ile Ala Asp Glu Gly Arg Leu Phe Leu Ala
145          150          155          160
Glu Phe Gln Ser Ile Pro Arg Val Phe Ser Lys Phe Pro Ile Lys Glu
165          170          175
Ala Arg Lys Pro Phe Asn Gln Asn Lys Asn Arg Tyr Val Asp Ile Leu
180          185          190
Pro Tyr Asp Tyr Asn Arg Val Glu Leu Ser Glu Ile Asn Gly Asp Ala
195          200          205
Gly Ser Asn Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Phe Lys Glu Pro
210          215          220
Arg Lys Tyr Ile Ala Ala Gln Gly Pro Arg Asp Glu Thr Val Asp Asp
225          230          235          240
Phe Trp Arg Met Ile Trp Glu Gln Lys Ala Thr Val Ile Val Met Val
245          250          255
Thr Arg Cys Glu Glu Gly Asn Arg Asn Lys Cys Ala Glu Tyr Trp Pro
260          265          270
Ser Met Glu Glu Gly Thr Arg Ala Phe Gly Asp Val Val Val Lys Ile
275          280          285
Asn Gln His Lys Arg Cys Pro Asp Tyr Ile Ile Gln Lys Leu Asn Ile
290          295          300
Val Asn Lys Lys Glu Lys Ala Thr Gly Arg Glu Val Thr His Ile Gln
305          310          315          320
Phe Thr Ser Trp Pro Asp His Gly Val Pro Glu Asp Pro His Leu Leu
325          330          335
Leu Lys Leu Arg Arg Arg Val Asn Ala Phe Ser Asn Phe Phe Ser Gly
340          345          350

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Pro	Ile	Val	Val	His	Cys	Ser	Ala	Gly	Val	Gly	Arg	Thr	Gly	Thr	Tyr	355	360	365	
Ile	Gly	Ile	Asp	Ala	Met	Leu	Glu	Gly	Leu	Glu	Ala	Glu	Asn	Lys	Val	370	375	380	
Asp	Val	Tyr	Gly	Tyr	Val	Val	Lys	Leu	Arg	Arg	Gln	Arg	Cys	Leu	Met	385	390	395	400
Val	Gln	Val	Glu	Ala	Gln	Tyr	Ile	Leu	Ile	His	Gln	Ala	Leu	Val	Glu	405	410	415	
Tyr	Asn	Gln	Phe	Gly	Glu	Thr	Glu	Val	Asn	Leu	Ser	Glu	Leu	His	Pro	420	425	430	
Tyr	Leu	His	Asn	Met	Lys	Lys	Arg	Asp	Pro	Pro	Ser	Glu	Pro	Ser	Pro	435	440	445	
Leu	Glu	Ala	Glu	Phe	Gln	Arg	Leu	Pro	Ser	Tyr	Arg	Ser	Trp	Arg	Thr	450	455	460	
Gln	His	Ile	Gly	Asn	Gln	Glu	Glu	Asn	Lys	Ser	Lys	Asn	Arg	Asn	Ser	465	470	475	480
Asn	Val	Ile	Pro	Tyr	Asp	Tyr	Asn	Arg	Val	Leu	Lys	His	Glu	Leu	Glu	485	490	495	
Met	Ser	Lys	Glu	Ser	Glu	His	Asp	Ser	Asp	Glu	Ser	Ser	Asp	Asp	Asp	500	505	510	
Ser	Asp	Ser	Glu	Glu	Pro	Ser	Lys	Tyr	Ile	Asn	Ala	Ser	Phe	Ile	Met	515	520	525	
Ser	Tyr	Trp	Lys	Pro	Glu	Val	Met	Ile	Ala	Ala	Gln	Gly	Pro	Leu	Lys	530	535	540	
Glu	Thr	Ile	Gly	Asp	Phe	Met	Ile	Gln	Arg	Lys	Val	Lys	Val	Ile	Val	545	550	555	560
Met	Leu	Thr	Glu	Leu	Lys	His	Gly	Asp	Gln	Glu	Ile	Cys	Ala	Gln	Tyr	565	570	575	
Trp	Gly	Glu	Gly	Lys	Gln	Thr	Tyr	Gly	Asp	Ile	Glu	Val	Asp	Leu	Lys	580	585	590	
Asp	Thr	Asp	Lys	Ser	Ser	Thr	Tyr	Thr	Leu	Arg	Val	Phe	Glu	Leu	Arg	595	600	605	
His	Ser	Lys	Arg	Lys	Asp	Ser	Arg	Thr	Val	Tyr	Gln	Tyr	Gln	Tyr	Thr	610	615	620	
Asn	Trp	Ser	Val	Glu	Gln	Leu	Pro	Ala	Glu	Pro	Lys	Glu	Leu	Ile	Ser	625	630	635	640
Met	Ile	Gln	Val	Val	Lys	Gln	Lys	Leu	Pro	Gln	Lys	Asn	Ser	Ser	Glu	645	650	655	
Gly	Asn	Lys	His	His	Lys	Ser	Thr	Pro	Leu	Leu	Ile	His	Cys	Arg	Asp	660	665	670	
Gly	Ser	Gln	Gln	Thr	Gly	Ile	Phe	Cys	Ala	Leu	Leu	Asn	Leu	Leu	Glu	675	680	685	
Ser	Ala	Glu	Thr	Glu	Glu	Val	Val	Asp	Ile	Phe	Gln	Val	Val	Lys	Ala	690	695	700	
Leu	Arg	Lys	Ala	Arg	Pro	Gly	Met	Val	Ser	Thr	Phe	Glu	Gln	Tyr	Gln	705	710	715	720
Phe	Leu	Tyr	Asp	Val	Ile	Ala	Ser	Thr	Tyr	Pro	Ala	Gln	Asn	Gly	Gln	725	730	735	
Val	Lys	Lys	Asn	Asn	His	Gln	Glu	Asp	Lys	Ile	Glu	Phe	Asp	Asn	Glu	740	745	750	

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Val Asp Lys Val Lys Gln Asp Ala Asn Cys Val Asn Pro Leu Gly Ala
755 760 765

Pro Glu Lys Leu Pro Glu Ala Lys Glu Gln Ala Glu Gly Ser Glu Pro
770 775 780

Thr Ser Gly Thr Glu Gly Pro Glu His Ser Val Asn Gly Pro Ala Ser
785 790 795 800

Pro Ala Leu Asn Gln Gly Ser
805

<210> SEQ ID NO 33
 <211> LENGTH: 434
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PTPN6-CD148 fusion protein

<400> SEQUENCE: 33

Glu Thr Leu Leu Gln Ala Lys Gly Glu Pro Trp Thr Phe Leu Val Arg
1 5 10 15

Glu Ser Leu Ser Gln Pro Gly Asp Phe Val Leu Ser Val Leu Ser Asp
20 25 30

Gln Pro Lys Ala Gly Pro Gly Ser Pro Leu Arg Val Thr His Ile Lys
35 40 45

Val Met Cys Glu Gly Gly Arg Tyr Thr Val Gly Gly Leu Glu Thr Phe
50 55 60

Asp Ser Leu Thr Asp Leu Val Glu His Phe Lys Lys Thr Gly Ile Glu
65 70 75 80

Glu Ala Ser Gly Ala Phe Val Tyr Leu Arg Gln Pro Tyr Arg Lys Lys
85 90 95

Arg Lys Asp Ala Lys Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro
100 105 110

Lys Lys Ser Lys Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys
115 120 125

Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp
130 135 140

Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala
145 150 155 160

Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp Ile
165 170 175

Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp Tyr Ile
180 185 190

Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp Phe Ile Ala
195 200 205

Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe Trp Arg Met Val
210 215 220

Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu Thr Lys Cys Val Glu
225 230 235 240

Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro Ser Lys Gln Ala Gln
245 250 255

Asp Tyr Gly Asp Ile Thr Val Ala Met Thr Ser Glu Ile Val Leu Pro
260 265 270

Glu Trp Thr Ile Arg Asp Phe Thr Val Lys Asn Ile Gln Thr Ser Glu
275 280 285

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Ser His Pro Leu Arg Gln Phe His Phe Thr Ser Trp Pro Asp His Gly
 290 295 300

Val Pro Asp Thr Thr Asp Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg
 305 310 315 320

Asp Tyr Met Lys Gln Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys
 325 330 335

Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu
 340 345 350

Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val
 355 360 365

Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln
 370 375 380

Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys
 385 390 395 400

Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr Ile
 405 410 415

Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys Thr Asn Gly Tyr
 420 425 430

Ile Ala

<210> SEQ ID NO 34
 <211> LENGTH: 20
 <212> TYPE: PRT
 <213> ORGANISM: Foot-and-mouth disease virus

<400> SEQUENCE: 34

Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu
 1 5 10 15

Asn Pro Gly Pro
 20

<210> SEQ ID NO 35
 <211> LENGTH: 3390
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Nucleic acid sequences coding for CARs
 (MP13974.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33g1x-HCH2CH3pvaa-CD
 28tmZw)

<400> SEQUENCE: 35

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ccagacatcc agatgaccca gaccaccagc agcctgagcg ccagcctggg cgaccgggtg 120

accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180

cccgcaggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc 240

agccggttca gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag 300

caggaggaca tcgccaccta cttctgccag cagggcaaca cctgccccta caccttcgga 360

ggcggcacca agctggagat caccaaggcc ggaggcggag gctctggcgg aggcggctct 420

ggcggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc 480

ctggtggccc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc 540

gactacggcg tgagctggat caggcagccc ccacggaagg gcctggagtg gctgggcgtg 600

atctggggca gcgagaccac ctactacaac agcgccctga agagccggct gaccatcatc 660

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aaggacaaca gcaagagcca ggtgttcctg aagatgaaca gcctgcagac cgacgacacc	720
gccatctact actgcgcaa gcactactac tatggcgga gctacgctat ggactactgg	780
ggccaggga ccagcgtgac cgtgagctca gatcccacca cgacgccagc gccgcgacca	840
ccaacaccgg cgcaccat cgcgtgcag cccctgtccc tgcgccaga ggcgtgccgg	900
ccagcggcgg ggggcgcagt gcacacgagg gggctggact tcgcctgtga tatcttttgg	960
gtgctgggtg tggttgggtg agtcctggct tgctatagct tgctagtaac agtggccttt	1020
attattttct ggggtaggag agtgaagtcc agcaggagcg cagacgcccc cgcgtaccag	1080
cagggccaga accagctcta taacgagctc aatctaggac gaagagagga gtacgatgtt	1140
ttggacaaga gacgtggcgg ggaccctgag atggggggaa agccgagaag gaagaaccct	1200
caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgaatt	1260
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acagccacca aggacaccta cgacgccctt cacatgcagg ccctgcctcc tcgcagagcc	1380
gagggcaggg gaagtcttct aacatgcggg gacgtggagg aaaatcccgg gcccatggcc	1440
gtgccactc aggtcctggg gttgttgcta ctgtggctta cagatgccag atgtgacatc	1500
cagatgacac agtctccatc ttcctgtct gcactctgct gagatcgctg caccatcacc	1560
tgctgagcaa gtgaggacat ttattttaat ttagtgtggg atcagcagaa accaggaaag	1620
gcccctaagc tcctgatcta tgatacaaat cgcttggcag atgggggtccc atcacggttc	1680
agtggctctg gatctggcac acagtatact ctaaccataa gtacccctga acccgaagat	1740
ttcgcaacct attattgtca acactataag aattatccgc tcacgttcgg tcaggggacc	1800
aagctggaaa tcaaaagatc tgggtggcga gggtcaggag gcggaggcag cgaggcggt	1860
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gtgcagcctg gagggtccct gaggtctctc tgtgcagcct caggattcac tctcagtaat	1980
tatggcatgc actggatcag gcaggctcca gggaagggtc tggagtgggt ctctctatt	2040
agtcttaatg gtggtagcac ttactatcga gactccgtga agggccgatt cactatctcc	2100
agggacaatg caaaaagcac cctctacctt caaatgaata gtctgagggc cgaggacacg	2160
gccgtctatt actgtgcagc acaggacgct tatacgggag gttactttga ttactggggc	2220
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agcctgacct gcctggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc	2760
aatgggcaac cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc	2820
ttcttctct acagcaagct caccgtggac aagagcaggg ggcagcaggg gaacgtcttc	2880
tcatgctccg tgatgcata ggcctgcac aatcactata ccagaaaac tctgagtctg	2940

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agcccaggca agaaggaccc caagttctgg gtccctgggtg tgggtgggagg cgtgctggcc 3000
tggtactctc tcctgggtgac cgtggccttc atcatctttt ggggtgcgctc ccgggtgaag 3060
ttttctcgct ctgccgatgc ccagccctat cagcagggcc agaatacagct gtacaatgaa 3120
ctgaacctgg gcaggcgagg ggagtagcag gtgctggata agcggagagg cagagacccc 3180
gagatgggag gcaaaccacg gcgcaaaaat cccaggagg gactctataa cgagctgcag 3240
aaggacaaaa tggccgaggc ctattccgag atcggcatga agggagagag aagacgcgga 3300
aagggccacg acggcctgta tcagggattg tccaccgcta caaaagatac atatgatgcc 3360
ctgcacatgc aggccctgcc acccagatga 3390

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<210> SEQ ID NO 36

<211> LENGTH: 5154

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleic acid sequences coding for CARs

(MP14802.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3p
vaa-dCD45)

<400> SEQUENCE: 36

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atgagcctgc ccgtgaccgc cctgtgtctg cccctggccc tgctgtctgca cgccgccaga 60
ccagacatcc agatgaccca gaccaccagc agcctgagcg ccagcctggg cgaccgggtg 120
accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180
cccgcaggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc 240
agccgggttc gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag 300
caggaggaca tcgccaccta cttctgccag cagggaaca ccttgcctta caccttcgga 360
ggcggaacca agctggagat caccaaggcc ggaggcggag gctctggcgg aggcggctct 420
ggcgaggcgg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc 480
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gactacggcg tagctggat caggcagccc ccacggaagg gcctggagtg gctgggcgtg 600
atctggggca gcgagaccac ctactacaac agcgcctga agagccggct gaccatcatc 660
aaggacaaca gcaagagcca ggtgttcctg aagatgaaca gcctgcagac cgacgacacc 720
gccatctact actgcgcaa gcactactac tatggcgga gctacgctat ggactactgg 780
ggccagggca ccagcgtgac cgtgagctca gatccacca cgacgccagc gccgcgacca 840
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caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgaagatt 1260
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acagccacca aggacaccta cgacgccctt cacatgcagg ccttgcctcc tcgcagagcc 1380
gagggcaggg gaagtcttct aacatgcggg gacgtggagg aaaatcccgg gcccatggcc 1440

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gtgcccactc aggtcctggg gttgttgcta ctgtggctta cagatgccag atgtgacatc	1500
cagatgacac agtctccatc ttccctgtct gcactctgtc gagatcgcgt caccatcacc	1560
tgtcgagcaa gtgaggacat ttattttaat ttagtgtggg atcagcagaa accaggaaaag	1620
gcccctaagc tcctgatcta tgatacaaat cgcttggcag atgggggtccc atcacggttc	1680
agtggctctg gatctggcac acagtatact ctaaccataa gtagcctgca acccgaagat	1740
ttcgcaacct attattgtca acactataag aattatccgc tcacgttcgg tcaggggacc	1800
aaagtggaaa tcaaaagatc tgggtggcga gggtcaggag gcggaggcag cggaggcggt	1860
ggctcgggag gcggaggctc gagatctgag gtgcagttgg tggagtctgg gggcggcttg	1920
gtgcagcctg gaggggtccc gaggtctctc tgtgcagcct caggattcac tctcagtaat	1980
tatggcatgc actggatcag gcaggctcca gggaagggtc tggagtgggt ctcgctctatt	2040
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gccgtctatt actgtgcagc acaggacgct tatacgggag gttactttga ttactggggc	2220
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ccccaaaa ccaaggacac cctcatgac gcccggaacc ctgaggtcac atgcgtgggtg	2400
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag	2460
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	2520
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc	2580
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	2640
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc	2700
agcctgacct gcctggtcaa aggtctctat cccagcgaca tcgccgtgga gtgggagagc	2760
aatgggcaac cggagaacaa ctacaagacc acgcctcccg tgcaggactc cgaacggctc	2820
ttcttctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc	2880
tcctgctccg tgatgcataa ggcctgcac aatcactata cccagaaatc tctgagcttg	2940
agcccaggca agaaggaccc caaggcactg atagcatttc tggcatttct gattattgtg	3000
acatcaatag ccctgcttgt tgttctctac aaaatctatg atctacataa gaaaagatcc	3060
tgcaatttag atgaacagca ggagcttggt gaaagggatg atgaaaaaca actgatgaat	3120
gtggagccaa tccatgcaga tattttgttg gaaacttata agaggaagat tgctgatgaa	3180
ggaagacttt ttctggctga atttcagagc atcccgcggg tgttcagcaa gtttcctata	3240
aaggaagctc gaaagccctt taaccagaat aaaaaccgtt atgttgacat tcttccttat	3300
gattataacc gtgttgaaat ctctgagata aacggagatg cagggtcaaa ctacataaat	3360
gccagctata ttgatggttt caaagaaccc aggaataaca ttgctgcaca aggtcccagg	3420
gatgaaactg ttgatgattt ctggaggatg atttgggaac agaaagccac agttattgtc	3480
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gaagagggca ctcgggcttt tggagatggt gttgtaaaga tcaaccagca caaaagatgt	3600
ccagattaca tcattcagaa attgaacatt gtaaataaaa aagaaaaagc aactggaaga	3660
gaggtgactc acattcagtt caccagctgg ccagaccacg gggtgctga ggatcctcac	3720

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<210> SEQ ID NO 37

<211> LENGTH: 4053

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleic acid sequences coding for CARs

(MP14801.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3p
vaa-dCD148)

<400> SEQUENCE: 37

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ccagacatcc agatgaccca gaccaccagc agcctgagcg ccagcctggg cgaccgggtg 120
accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180
cccgaaggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc 240
agccggttca gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag 300
caggaggaca tcgccacctt cttctgccag cagggaaca ccctgcccta caccttcgga 360
ggcggcacca agctggagat caccaaggcc ggaggcggag gctctggcgg aggcggctct 420
ggcggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc 480

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atctggggca	gagagaccac	ctactacaac	agcgccctga	agagccggct	gaccatcatc	660
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<210> SEQ ID NO 38

<211> LENGTH: 3345

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleic acid sequences coding for CARs
 (16076.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-tm-dPTP
 N6)

<400> SEQUENCE: 38

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accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180
cccagcggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc 240
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ggcggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc 480
ctggtggccc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc 540
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ccagcggcgg ggggcgcagt gcacacgagg gggctggact tcgcctgtga tatcttttgg	960
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<210> SEQ ID NO 39

<211> LENGTH: 2757

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleic acid sequences coding for CARs

(MP16091.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33g1x-muCD8STK-LAIR1
tm-endo)

<400> SEQUENCE: 39

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<210> SEQ ID NO 40

<211> LENGTH: 4092

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Nucleic acid sequences coding for CARs
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tm-endo-2A-PTPN6_SH2-dCD148)

<400> SEQUENCE: 40

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accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180
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ctggtgggcc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc 540
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<210> SEQ ID NO 41

<211> LENGTH: 1341

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Single-chain variable fragment (scFv)
 SFG.acD19-CD8STK-CD28tmZ-2A-agD2-HCH2CH3pvaa-dCD148

<400> SEQUENCE: 41

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20          25          30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35          40          45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50          55          60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65          70          75          80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85          90          95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100         105         110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr

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Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly		
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Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly		
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Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly		
	165	170 175
Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg		
	180	185 190
Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr		
	195	200 205
Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser		
	210	215 220
Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr		
	225	230 235 240
Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala		
	245	250 255
Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro		
	260	265 270
Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala		
	275	280 285
Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly		
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Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp		
	305	310 315 320
Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val		
	325	330 335
Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg		
	340	345 350
Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn		
	355	360 365
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg		
	370	375 380
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro		
	385	390 395 400
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala		
	405	410 415
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His		
	420	425 430
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp		
	435	440 445
Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly		
	450	455 460
Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu		
	465	470 475 480
Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser		
	485	490 495
Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro		
	500	505 510
Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala		
	515	520 525

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Ser	Tyr	Asn	Ile	His	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu
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Trp	Leu	Gly	Val	Ile	Trp	Ala	Gly	Gly	Ser	Thr	Asn	Tyr	Asn	Ser	Ala
545					550					555					560
Leu	Met	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Gln	Val
				565					570					575	
Phe	Leu	Lys	Met	Ser	Ser	Leu	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Tyr
			580					585					590		
Cys	Ala	Lys	Arg	Ser	Asp	Asp	Tyr	Ser	Trp	Phe	Ala	Tyr	Trp	Gly	Gln
		595					600					605			
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
	610					615					620				
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Asn	Gln	Met	Thr	Gln	Ser	Pro	Ser
625					630					635					640
Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Met	Thr	Cys	Arg	Ala
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Ser	Ser	Ser	Val	Ser	Ser	Ser	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser
			660					665					670		
Gly	Lys	Ala	Pro	Lys	Val	Trp	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser
		675					680					685			
Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr
	690					695					700				
Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys
705					710					715					720
Gln	Gln	Tyr	Ser	Gly	Tyr	Pro	Ile	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val
			725						730					735	
Glu	Ile	Lys	Arg	Ser	Asp	Pro	Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr
			740					745					750		
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val
		755					760					765			
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr
	770					775					780				
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu
785					790					795					800
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
			805						810					815	
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
			820					825					830		
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
		835					840					845			
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile
		850				855					860				
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
865					870					875					880
Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu
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Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
			900					905					910		
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
		915					920						925		

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Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	930	935	940
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His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	965	970	975
Asp	Pro	Lys	Ala	Val	Phe	Gly	Cys	Ile	Phe	Gly	Ala	Leu	Val	Ile	Val	980	985	990
Thr	Val	Gly	Gly	Phe	Ile	Phe	Trp	Arg	Lys	Lys	Arg	Lys	Asp	Ala	Lys	995	1000	1005
Asn	Asn	Glu	Val	Ser	Phe	Ser	Gln	Ile	Lys	Pro	Lys	Lys	Ser	Lys		1010	1015	1020
Leu	Ile	Arg	Val	Glu	Asn	Phe	Glu	Ala	Tyr	Phe	Lys	Lys	Gln	Gln		1025	1030	1035
Ala	Asp	Ser	Asn	Cys	Gly	Phe	Ala	Glu	Glu	Tyr	Glu	Asp	Leu	Lys		1040	1045	1050
Leu	Val	Gly	Ile	Ser	Gln	Pro	Lys	Tyr	Ala	Ala	Glu	Leu	Ala	Glu		1055	1060	1065
Asn	Arg	Gly	Lys	Asn	Arg	Tyr	Asn	Asn	Val	Leu	Pro	Tyr	Asp	Ile		1070	1075	1080
Ser	Arg	Val	Lys	Leu	Ser	Val	Gln	Thr	His	Ser	Thr	Asp	Asp	Tyr		1085	1090	1095
Ile	Asn	Ala	Asn	Tyr	Met	Pro	Gly	Tyr	His	Ser	Lys	Lys	Asp	Phe		1100	1105	1110
Ile	Ala	Thr	Gln	Gly	Pro	Leu	Pro	Asn	Thr	Leu	Lys	Asp	Phe	Trp		1115	1120	1125
Arg	Met	Val	Trp	Glu	Lys	Asn	Val	Tyr	Ala	Ile	Ile	Met	Leu	Thr		1130	1135	1140
Lys	Cys	Val	Glu	Gln	Gly	Arg	Thr	Lys	Cys	Glu	Glu	Tyr	Trp	Pro		1145	1150	1155
Ser	Lys	Gln	Ala	Gln	Asp	Tyr	Gly	Asp	Ile	Thr	Val	Ala	Met	Thr		1160	1165	1170
Ser	Glu	Ile	Val	Leu	Pro	Glu	Trp	Thr	Ile	Arg	Asp	Phe	Thr	Val		1175	1180	1185
Lys	Asn	Ile	Gln	Thr	Ser	Glu	Ser	His	Pro	Leu	Arg	Gln	Phe	His		1190	1195	1200
Phe	Thr	Ser	Trp	Pro	Asp	His	Gly	Val	Pro	Asp	Thr	Thr	Asp	Leu		1205	1210	1215
Leu	Ile	Asn	Phe	Arg	Tyr	Leu	Val	Arg	Asp	Tyr	Met	Lys	Gln	Ser		1220	1225	1230
Pro	Pro	Glu	Ser	Pro	Ile	Leu	Val	His	Cys	Ser	Ala	Gly	Val	Gly		1235	1240	1245
Arg	Thr	Gly	Thr	Phe	Ile	Ala	Ile	Asp	Arg	Leu	Ile	Tyr	Gln	Ile		1250	1255	1260
Glu	Asn	Glu	Asn	Thr	Val	Asp	Val	Tyr	Gly	Ile	Val	Tyr	Asp	Leu		1265	1270	1275
Arg	Met	His	Arg	Pro	Leu	Met	Val	Gln	Thr	Glu	Asp	Gln	Tyr	Val		1280	1285	1290
Phe	Leu	Asn	Gln	Cys	Val	Leu	Asp	Ile	Val	Arg	Ser	Gln	Lys	Asp		1295	1300	1305
Ser	Lys	Val	Asp	Leu	Ile	Tyr	Gln	Asn	Thr	Thr	Ala	Met	Thr	Ile				

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Tyr Ile Ala
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<210> SEQ ID NO 42
 <211> LENGTH: 4026
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Single-chain variable fragment (scFv)
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<400> SEQUENCE: 42

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tatgatattt cccgtgtcaa actttcggtc cagaccatt caacggatga ctacatcaat	3300
gccaaactaca tgcctggcta ccaactcaag aaagatttta ttgccacaca aggaccttta	3360
ccgaacactt tgaaagatht ttggcgtatg gtttgggaga aaaatgtata tgccatcatt	3420
atgttgacta aatgtgttga acaggaaga accaaatgtg aggagtattg gccctccaag	3480
caggctcagg actatggaga cataactgtg gcaatgacat cagaaattgt tcttccggaa	3540
tggaccatca gagatttcac agtgaaaaat atccagacaa gtgagagtca cctctgaga	3600
cagttccatt tcacctctg gccagaccac ggtgttcccg acaccactga cctgctcatc	3660
aacttccggt acctcgttcg tgactacatg aagcagagtc ctcccgaatc gccgattctg	3720
gtgcattgca gtgctggggt cggaaggacg ggcactttca ttgccattga tcgtctcatc	3780
taccagatag agaatgagaa caccgtggat gtgtatggga ttgtgtatga ccttcgaatg	3840
cataggcctt taatggtgca gacagaggac cagtatgttt tcctcaatca gtgtgttttg	3900
gatattgtca gatcccagaa agactcaaaa gtagatctta tctaccagaa cacaactgca	3960
atgacaatct atgaaaacct tgcgcccgtg accacatttg gaaagaccaa tggttacatc	4020

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gcctaa

4026

<210> SEQ ID NO 43
 <211> LENGTH: 1341
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Single-chain variable fragment (scFv)
 SFG.aCD19-CD8STK-CD28tmZ-2A-aCD5-HCH2CH3pvaa-dCD148

 <400> SEQUENCE: 43

 Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
 1 5 10 15
 His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
 20 25 30
 Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
 35 40 45
 Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
 50 55 60
 Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
 65 70 75 80
 Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
 85 90 95
 Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
 100 105 110
 Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
 115 120 125
 Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 130 135 140
 Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
 145 150 155 160
 Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
 165 170 175
 Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
 180 185 190
 Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
 195 200 205
 Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
 210 215 220
 Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
 225 230 235 240
 Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
 245 250 255
 Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
 260 265 270
 Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
 275 280 285
 Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
 290 295 300
 Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
 305 310 315 320
 Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
 325 330 335

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Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg	
		340						345					350			
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	
		355					360					365				
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	
	370				375						380					
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	
385					390					395					400	
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	
			405						410					415		
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	
		420						425					430			
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	
	435						440					445				
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly	
	450					455					460					
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Glu	
465					470					475					480	
Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	
			485					490						495		
Thr	Gly	Gln	Val	Thr	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Ile	Leu	Lys	Pro	
		500						505					510			
Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Ser	Phe	Ser	Gly	Phe	Ser	Leu	Ser	
		515					520					525				
Thr	Ser	Gly	Met	Gly	Val	Gly	Trp	Ile	Arg	Gln	Pro	Ser	Gly	Lys	Gly	
	530					535					540					
Leu	Glu	Trp	Leu	Ala	His	Ile	Trp	Trp	Asp	Asp	Asp	Val	Tyr	Tyr	Asn	
545					550					555					560	
Pro	Ser	Leu	Lys	Asn	Gln	Leu	Thr	Ile	Ser	Lys	Asp	Ala	Ser	Arg	Asp	
			565						570					575		
Gln	Val	Phe	Leu	Lys	Ile	Thr	Asn	Leu	Asp	Thr	Ala	Asp	Thr	Ala	Thr	
		580					585						590			
Tyr	Tyr	Cys	Val	Arg	Arg	Arg	Ala	Thr	Gly	Thr	Gly	Phe	Asp	Tyr	Trp	
		595					600					605				
Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
	610					615					620					
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asn	Ile	Val	Met	Thr	Gln	Ser	
625					630					635					640	
His	Lys	Phe	Met	Ser	Thr	Ser	Val	Gly	Asp	Arg	Val	Ser	Ile	Ala	Cys	
			645						650					655		
Lys	Ala	Ser	Gln	Asp	Val	Gly	Thr	Ala	Val	Ala	Trp	Tyr	Gln	Gln	Lys	
			660				665						670			
Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Thr	Ser	Thr	Arg	His	
		675				680						685				
Thr	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
		690				695					700					
Thr	Leu	Thr	Ile	Thr	Asn	Val	Gln	Ser	Glu	Asp	Leu	Ala	Asp	Tyr	Phe	
705					710					715					720	
Cys	His	Gln	Tyr	Asn	Ser	Tyr	Asn	Thr	Phe	Gly	Ser	Gly	Thr	Arg	Leu	
				725					730						735	

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Glu	Leu	Lys	Arg	Ser	Asp	Pro	Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	740	745	750
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	755	760	765
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	770	775	780
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	785	790	800
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	805	810	815
Thr	Lys	Pro	Arg	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser		820	825	830
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	835	840	845
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	850	855	860
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	865	870	875
Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	885	890	895
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	900	905	910
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	915	920	925
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	930	935	940
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	945	950	955
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	965	970	975
Asp	Pro	Lys	Ala	Val	Phe	Gly	Cys	Ile	Phe	Gly	Ala	Leu	Val	Ile	Val	980	985	990
Thr	Val	Gly	Gly	Phe	Ile	Phe	Trp	Arg	Lys	Lys	Arg	Lys	Asp	Ala	Lys	995	1000	1005
Asn	Asn	Glu	Val	Ser	Phe	Ser	Gln	Ile	Lys	Pro	Lys	Lys	Ser	Lys		1010	1015	1020
Leu	Ile	Arg	Val	Glu	Asn	Phe	Glu	Ala	Tyr	Phe	Lys	Lys	Gln	Gln		1025	1030	1035
Ala	Asp	Ser	Asn	Cys	Gly	Phe	Ala	Glu	Glu	Tyr	Glu	Asp	Leu	Lys		1040	1045	1050
Leu	Val	Gly	Ile	Ser	Gln	Pro	Lys	Tyr	Ala	Ala	Glu	Leu	Ala	Glu		1055	1060	1065
Asn	Arg	Gly	Lys	Asn	Arg	Tyr	Asn	Asn	Val	Leu	Pro	Tyr	Asp	Ile		1070	1075	1080
Ser	Arg	Val	Lys	Leu	Ser	Val	Gln	Thr	His	Ser	Thr	Asp	Asp	Tyr		1085	1090	1095
Ile	Asn	Ala	Asn	Tyr	Met	Pro	Gly	Tyr	His	Ser	Lys	Lys	Asp	Phe		1100	1105	1110
Ile	Ala	Thr	Gln	Gly	Pro	Leu	Pro	Asn	Thr	Leu	Lys	Asp	Phe	Trp		1115	1120	1125
Arg	Met	Val	Trp	Glu	Lys	Asn	Val	Tyr	Ala	Ile	Ile	Met	Leu	Thr				

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1130	1135	1140
Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro		
1145	1150	1155
Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met Thr		
1160	1165	1170
Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val		
1175	1180	1185
Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His		
1190	1195	1200
Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu		
1205	1210	1215
Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser		
1220	1225	1230
Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly		
1235	1240	1245
Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile		
1250	1255	1260
Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val Tyr Asp Leu		
1265	1270	1275
Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln Tyr Val		
1280	1285	1290
Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys Asp		
1295	1300	1305
Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr Ile		
1310	1315	1320
Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys Thr Asn Gly		
1325	1330	1335
Tyr Ile Ala		
1340		

<210> SEQ ID NO 44

<211> LENGTH: 4026

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Single-chain variable fragment (scFv)

SFG.aCD19-CD8STK-CD28tmZ-2A-aCD5-HCH2CH3pvaa-dCD148

<400> SEQUENCE: 44

```

atgagcctgc ccgtgaccgc cctgctgctg cccctggccc tgctgctgca cgccgccaga    60
ccagacatcc agatgaccca gaccaccagc agcctgagcg ccagcctggg cgaccgggtg    120
accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag    180
cccgcaggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc    240
agccggttca gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag    300
caggaggaca tcgccaccta cttctgccag cagggcaaca ccctgcccta caccttcgga    360
ggcggcacca agctggagat caccaaggcc ggaggcggag gctctggcgg aggcggctct    420
ggcggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc    480
ctggtggccc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc    540
gactacggcg tgagctggat caggcagccc ccacggaagg gcctggagtg gctgggcgtg    600
atctggggca gcgagaccac ctactacaac agcgccctga agagccggct gaccatcatc    660

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aaggacaaca gcaagagcca ggtgttcctg aagatgaaca gcctgcagac cgacgacacc	720
gccatctact actgcgcaa gcactactac tatggcgga gctacgctat ggactactgg	780
ggccaggga ccagcgtgac cgtgagctca gatccccca cgacgccagc gccgcgacca	840
ccaacaccgg cgcaccat cgcgtgcag cccctgtccc tgcgccaga ggcgtgcgg	900
ccagcggcgg ggggcgcagt gcacacgagg gggctggact tcgcctgtga tatcttttgg	960
gtgctggtgg tggttggtgg agtcctggct tgctatagct tgctagtaac agtggccttt	1020
attattttct gggtagggag agtgaagtcc agcaggagcg cagacgcccc cgcgtaccag	1080
cagggccaga accagctcta taacgagctc aatctaggac gaagagagga gtacgatgtt	1140
ttggacaaga gacgtggcgg ggaccctgag atggggggaa agccgagaag gaagaaccct	1200
caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgaatt	1260
gggatgaaag gcgagcgccg gaggggcaag gggcacgatg gcctttacca gggctctcagt	1320
acagccacca aggacaccta cgacgccctt cacatgcagg cctgcctcc tcgcagagcc	1380
gagggcaggg gaagtcttct aacatgcggg gacgtggagg aaaatcccgg gcccatggag	1440
accgacccc tgctgctgtg ggtgctgtg ctgtgggtgc ccggcagcac cggccaggtg	1500
accctgaagg agagcggctc cggcctctg aagcccagcc agaccctgag cctgacctgc	1560
agcttcacgg gcttcagcct gagcaccagc ggcatgggcg tgggctggat tcggcagccc	1620
agcggcaagg gcctggagtg gctggccac atctgggtgg acgacgacgt gtactacaac	1680
cccagcctga agaaccagct gaccatcagc aaggacgcca gccgggacca ggtgttcctg	1740
aagatcacca acctggacac cgcgcacacc gccacctact actgcgtgcg gcgcggggcc	1800
accggcaccg gcttcgacta ctggggccag ggcaccaccc tgaccgtgag cagcgggtggc	1860
ggtggcagcg gcggcgccgg aagcggaggt ggtggcagca acatcgtgat gaccagagc	1920
cacaagttca tgagcaccag cgtggggcag cgggtgagca tcgcctgcaa ggcagccag	1980
gacgtgggca ccgccgtggc ctggtaccag cagaagcctg gccagagccc caagctgctg	2040
atctactgga ccagcaccgg gcacaccggc gtgcccagcc ggttcaccgg cagcggcagc	2100
ggcaccgact tcacctgac catcaccaac gtgcagagcg aggacctggc cgactacttc	2160
tgccaccagt acaacagcta caaaccttc ggcagcgga cccggctgga gctgaagcgg	2220
tcggatcccc ccgagcccaa atctcctgac aaaactcaca catgcccacc gtgcccagca	2280
cctcccgtgg ccggcccgtc agtcttcttc tccccccaa aacccaagga caccctcatg	2340
atcggccgga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	2400
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	2460
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	2520
tggctgaatg gcaaggagta caagtgaag gtctccaaca aagccctccc agcccccatc	2580
gagaaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc	2640
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggcttc	2700
tatcccagcg acatcgccgt ggagtgggag agcaatgggc aaccggagaa caactacaag	2760
accagcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcaa gctcacgtg	2820
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggccctg	2880
cacaatcact ataccagaa atctctgagt ctgagcccag gcaagaagga cccaaggcg	2940

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gtttttggct gtatctttgg tgccctgggt attgtgactg tgggaggctt catcttctgg 3000
agaaagaaga ggaaagatgc aaagaataat gaagtgtcct tttctcaaat taaacctaaa 3060
aaatctaagt taatcagagt ggagaathtt gaggcctact tcaagaagca gcaagctgac 3120
tccaactgtg ggttcgcaga ggaatacgaa gatctgaagc ttgttggaat tagtcaacct 3180
aaatatgcag cagaactggc tgagaataga ggaaagaatc gctataataa tgttctgccc 3240
tatgatattt cccgtgtcaa actttcggtc cagaccatt caacggatga ctacatcaat 3300
gccaaactaca tgccctggcta ccaactccaag aaagatttta ttgccacaca aggaccttta 3360
ccgaacactt tgaaagattt ttggcgtatg gtttgggaga aaaatgtata tgccatcatt 3420
atgttgacta aatgtgttga acagggaaga accaaatgtg aggagtattg gccctccaag 3480
caggctcagg actatggaga cataactgtg gcaatgacat cagaaattgt tcttcgggaa 3540
tggaccatca gagatttcac agtgaaaaat atccagacaa gtgagagtca ccctctgaga 3600
cagttccatt tcacctcctg gccagaccac ggtgttcccg acaccactga cctgctcatc 3660
aacttcgggt acctcgttcg tgactacatg aagcagagtc ctcccgaatc gccgattctg 3720
gtgcattgca gtgctggggg cggaaggacg ggcactttca ttgccattga tcgtctcatc 3780
taccagatag agaatgagaa caccgtggat gtgtatggga ttgtgtatga ccttcgaatg 3840
cataggcctt taatggtgca gacagaggac cagtatgttt tctcaatca gtgtgttttg 3900
gatattgtca gatcccagaa agactcaaaa gtagatctta tctaccagaa cacaactgca 3960
atgacaatct atgaaaacct tgcgcccgtg accacatttg gaaagaccaa tggttacatc 4020
gcctaa 4026

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<210> SEQ ID NO 45
<211> LENGTH: 1342
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Single-chain variable fragment (scFv)
SFG.acd19-CD8STK-CD28tmZ-2A-aEGFRvIII-HCH2CH3pvaa-dCD148

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<400> SEQUENCE: 45

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Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1      5      10      15
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20     25     30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35     40     45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50     55     60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65     70     75     80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85     90     95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100    105    110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115    120    125
Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130    135    140

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Ser Gly Gly Gly Gly	Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145	150 155 160
Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly	
	165 170 175
Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg	
	180 185 190
Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr	
	195 200 205
Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser	
	210 215 220
Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr	
	225 230 235 240
Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala	
	245 250 255
Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro	
	260 265 270
Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala	
	275 280 285
Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly	
	290 295 300
Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp	
	305 310 315 320
Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val	
	325 330 335
Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg	
	340 345 350
Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn	
	355 360 365
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg	
	370 375 380
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro	
	385 390 395 400
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala	
	405 410 415
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His	
	420 425 430
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp	
	435 440 445
Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly	
	450 455 460
Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu	
	465 470 475 480
Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser	
	485 490 495
Thr Gly Gln Val Lys Leu Gln Gln Ser Gly Gly Gly Leu Val Lys Pro	
	500 505 510
Gly Ala Ser Leu Lys Leu Ser Cys Val Thr Ser Gly Phe Thr Phe Arg	
	515 520 525
Lys Phe Gly Met Ser Trp Val Arg Gln Thr Ser Asp Lys Arg Leu Glu	
	530 535 540

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Trp	Val	Ala	Ser	Ile	Ser	Thr	Gly	Gly	Tyr	Asn	Thr	Tyr	Tyr	Ser	Asp
545					550					555					560
Asn	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Glu	Asn	Ala	Lys	Asn	Thr
				565					570					575	
Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Lys	Ser	Glu	Asp	Thr	Ala	Leu	Tyr
			580					585					590		
Tyr	Cys	Thr	Arg	Gly	Tyr	Ser	Ser	Thr	Ser	Tyr	Ala	Met	Asp	Tyr	Trp
		595					600					605			
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly
	610					615					620				
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Glu	Leu	Thr	Gln	Ser
625					630					635					640
Pro	Ala	Ser	Leu	Ser	Val	Ala	Thr	Gly	Glu	Lys	Val	Thr	Ile	Arg	Cys
			645						650					655	
Met	Thr	Ser	Thr	Asp	Ile	Asp	Asp	Asp	Met	Asn	Trp	Tyr	Gln	Gln	Lys
			660				665						670		
Pro	Gly	Glu	Pro	Pro	Lys	Phe	Leu	Ile	Ser	Glu	Gly	Asn	Thr	Leu	Arg
	675						680					685			
Pro	Gly	Val	Pro	Ser	Arg	Phe	Ser	Ser	Ser	Gly	Thr	Gly	Thr	Asp	Phe
	690					695					700				
Val	Phe	Thr	Ile	Glu	Asn	Thr	Leu	Ser	Glu	Asp	Val	Gly	Asp	Tyr	Tyr
705					710					715					720
Cys	Leu	Gln	Ser	Phe	Asn	Val	Pro	Leu	Thr	Phe	Gly	Asp	Gly	Thr	Lys
			725						730					735	
Leu	Glu	Ile	Lys	Arg	Ser	Asp	Pro	Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys
			740				745						750		
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser
	755						760					765			
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg
	770					775					780				
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
785					790					795					800
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
			805						810					815	
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
			820					825					830		
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
	835						840					845			
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
	850					855					860				
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
865					870					875					880
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
			885						890					895	
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
			900					905					910		
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
	915						920					925			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
	930					935					940				
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala

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945	950	955	960
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	965	970	975
Lys Asp Pro Lys Ala Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile	980	985	990
Val Thr Val Gly Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala	995	1000	1005
Lys Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser	1010	1015	1020
Lys Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln	1025	1030	1035
Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp Leu	1040	1045	1050
Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala	1055	1060	1065
Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp	1070	1075	1080
Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp	1085	1090	1095
Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp	1100	1105	1110
Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe	1115	1120	1125
Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu	1130	1135	1140
Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp	1145	1150	1155
Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met	1160	1165	1170
Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr	1175	1180	1185
Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe	1190	1195	1200
His Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp	1205	1210	1215
Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln	1220	1225	1230
Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val	1235	1240	1245
Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln	1250	1255	1260
Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val Tyr Asp	1265	1270	1275
Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln Tyr	1280	1285	1290
Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys	1295	1300	1305
Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr	1310	1315	1320
Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys Thr Asn	1325	1330	1335

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Gly Tyr Ile Ala
1340

<210> SEQ ID NO 46
<211> LENGTH: 4029
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Single-chain variable fragment (scFv)
SFG.aCD19-CD8STK-CD28tmZ-2A-aEGFRvIII-HCH2CH3pvaa-dCD148

<400> SEQUENCE: 46

```
atgagcctgc ccgtgaccgc cctgctgctg cccctggccc tgctgctgca cgccgccaga    60
ccagacatcc agatgaccca gaccaccagc agcctgagcg ccagcctggg cgaccgggtg    120
accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag    180
cccgacggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc    240
agccggttca gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag    300
caggaggaca tcgccaccta cttctgccag cagggcaaca ccttgccta caccttcgga    360
ggcggcacca agctggagat caccaaggcc ggaggcggag gctctggcgg aggcggctct    420
ggcggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc    480
ctggtggccc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc    540
gactacggcg tgagctggat caggcagccc ccacggaagg gcctggagtg gctgggcgtg    600
atctggggca gcgagaccac ctactacaac agcgcctga agagccggct gaccatcatc    660
aaggacaaca gcaagagcca ggtgttctcg aagatgaaca gcctgcagac cgacgacacc    720
gccatctact actgcgcaa gcactactac tatggcggca gctacgctat ggactactgg    780
ggccagggca ccagcgtgac cgtgagctca gatcccacca cgacgccagc gccgcgacca    840
ccaacaccgg cgcaccatc cgcgtgcag cccctgtccc tgcgccaga ggcgtgccgg    900
ccagcggcgg ggggcgcagt gcacacgagg gggctggact tcgcctgtga tatcttttgg    960
gtgctggtgg tggttggtgg agtcctggct tgctatagct tgctagtaac agtggccttt   1020
attatcttct gggtgaggag agtgaagttc agcaggagcg cagacgcccc cgcgtaccag   1080
cagggccaga accagctcta taacgagctc aatctaggac gaagagagga gtacgatgtt   1140
ttggacaaga gacgtggcgg ggaccctgag atggggggaa agccgagaag gaagaaccct   1200
caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgagatt   1260
gggatgaaag gcgagcgccg gaggggcaag gggcacgatg gcctttacca gggctctcagt   1320
acagccacca aggacaccta cgacgccctt cacatgcagg ccctgcctcc tcgcagagcc   1380
gagggcaggg gaagtcttct aacatgcggg gacgtggagg aaaatcccgg gcccatggag   1440
accgaccccc tgctgctgtg ggtgctgctg ctgtgggtgc ccggcagcac cggccagggtg   1500
aagctgcagc agagcggcgg aggcctgtgt aagcccggcg ccagcctgaa gctgagctgc   1560
gtgaccagcg gcttcacctt ccggaagttc ggcatgagct gggtgccgca gaccagcgac   1620
aagcggctgg agtgggtggc cagcatcagc accggcggct acaacaccta ctacagcgac   1680
aacgtgaagg gccggttcac catcagccgg gagaacgcca agaacaccct gtacctgcag   1740
atgagcagcc tgaagagcga ggacaccgcc ctgtactact gcacccgggg ctacagcagc   1800
accagctacg ctatggacta ctggggccag ggcaccaccg tgacagttag cagcggcgga   1860
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ggaggcagtg gtgggggtgg atctggcgga ggtggcagcg acatcgagct gaccagagc	1920
cccgcagcc tgagcgtggc caccggcgag aaggtgacca tccggtgcat gaccagcacc	1980
gacatcgagc acgacatgaa ctggtaccag cagaagcccg gcgagccccc aaagttcctg	2040
atcagcgagg gcaacaccct gcggcccggc gtgccagacc ggttcagcag cagcggcacc	2100
ggcaccgact tcgtgttcac catcgagaac accctgagcg aggacgtggg cgactactac	2160
tgctgcaga gcttcaacgt gcccctgacc ttcggcgagc gcaccaagct ggagatcaag	2220
cggtcggatc ccgcgagcc caaatctcct gacaaaactc acacatgccc accgtgccc	2280
gcacctccc tgcccgccc gtcagtcttc ctcttcccc caaaaccca ggacacctc	2340
atgatcgccc ggacctctga ggtcacatgc gtggtggtgg acgtgagcca cgaagacct	2400
gaggtaagt tcaactggta cgtggacggc gtggaggtgc ataatgcca gacaaagccg	2460
cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcacctg cctgcaccag	2520
gactggtga atggcaagga gtacaagtgc aaggtctcca acaagacct cccagcccc	2580
atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacacctg	2640
ccccatccc gggatgagct gaccaagaac caggtcagcc tgacctgctt ggtcaaaggc	2700
ttctatccca gcgacatgc cgtggagtgg gagagcaatg ggcaaccgga gaacaactac	2760
aagaccagc ctcccgctg ggactcgcac ggtccttctt tcctctacag caagctcacc	2820
gtggacaaga gcagggtggc gcagggggaa gtcttctcat gctccgtgat gcatgaggcc	2880
ctgcacaatc actataccca gaaatctctg agtctgagcc caggcaagaa ggacccaag	2940
gcggtttttg gctgtatctt tgggtgacct gttattgtga ctgtgggagg ctctcatctt	3000
tggagaaaga agaggaaaga tgcaagaat aatgaagtgt ccttttctca aattaaacct	3060
aaaaaatcta agttaatcag agtgagaaat tttgaggcct acttcaagaa gcagcaagct	3120
gactccaact gtgggttcgc agaggaatac gaagatctga agcttgttgg aattagtcaa	3180
cctaaatatg cagcagaact ggctgagaat agaggaaaga atcgctataa taatgttctg	3240
ccctatgata ttcccgctg caaactttcg gtccagacct attcaacgga tgactacatc	3300
aatgccaaact acatgcctgg ctaccactcc aagaaagatt ttattgccac acaaggacct	3360
ttaccgaaca ctttgaaaga tttttggcgt atggtttggg agaaaaatgt atatgccatc	3420
attatgttga ctaaatgtgt tgaacaggga agaaccaat gtgaggagta ttggccctcc	3480
aagcaggctc aggactatgg agacataact gtggcaatga catcagaaat tgttcttcg	3540
gaatggacca tcagagattt cacagtgaat aatatccaga caagtgagag tcacctctg	3600
agacagttcc atttcacctc ctggccagac cagggtgttc ccgacaccac tgacctgctc	3660
atcaacttcc ggtacctcgt tcgtgactac atgaagcaga gtccctccga atcgccgatt	3720
ctggtgcatt gcagtgtcgg ggtcggaagg acgggcactt tcattgccat tgatcgtctc	3780
atctaccaga tagagaatga gaacaccgtg gatgtgtatg ggattgtgta tgaccttcga	3840
atgcataggc ctttaatggt gcagacagag gaccagtatg ttttcctcaa tcagtgtgtt	3900
ttggatattg tcagatccca gaaagactca aaagtagatc ttatctacca gaacacaact	3960
gcaatgacaa tctatgaaaa ccttgcgccc gtgaccacat ttggaaagac caatggttac	4020
atcgctaa	4029

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<210> SEQ ID NO 47
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Immunoreceptor tyrosine-based inhibition motif
      (ITIM)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa may be Ser, Ile, Val or Leu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (4)..(5)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa may be Ile, Val or Leu

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<400> SEQUENCE: 47

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Xaa Xaa Tyr Xaa Xaa Xaa
1             5

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<210> SEQ ID NO 48
<211> LENGTH: 1114
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence of a AND NOT gate
      (16076.SFG.acd19fmc63-CD8STK-CD28tmZ-2A-acd33glx-muCD8STK-tm-dPTP
      N6)

```

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<400> SEQUENCE: 48

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```

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1             5             10             15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
      20             25             30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
      35             40             45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
      50             55             60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
      65             70             75             80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
      85             90             95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
      100            105            110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
      115            120            125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
      130            135            140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
      145            150            155            160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
      165            170            175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
      180            185            190

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Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
 195 200 205
 Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
 210 215 220
 Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
 225 230 235 240
 Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
 245 250 255
 Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
 260 265 270
 Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
 275 280 285
 Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
 290 295 300
 Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
 305 310 315 320
 Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
 325 330 335
 Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
 340 345 350
 Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
 355 360 365
 Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
 370 375 380
 Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
 385 390 395 400
 Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
 405 410 415
 Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
 420 425 430
 Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
 435 440 445
 Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
 450 455 460
 Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
 465 470 475 480
 Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
 485 490 495
 Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
 500 505 510
 Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
 515 520 525
 Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
 530 535 540
 Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
 545 550 555 560
 Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
 565 570 575
 Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
 580 585 590

Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly	
		595					600					605				
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
	610					615					620					
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	
					630					635					640	
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	
				645					650					655		
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	
			660					665					670			
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr	
	675						680					685				
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
	690					695						700				
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	
					710					715					720	
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe	
				725					730					735		
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro	
			740					745					750			
Ala	Thr	Thr	Thr	Lys	Pro	Val	Leu	Arg	Thr	Pro	Ser	Pro	Val	His	Pro	
		755					760					765				
Thr	Gly	Thr	Ser	Gln	Pro	Gln	Arg	Pro	Glu	Asp	Cys	Arg	Pro	Arg	Gly	
	770					775					780					
Ser	Val	Lys	Gly	Thr	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Trp	Ala	
					790					795					800	
Pro	Leu	Ala	Gly	Ile	Cys	Val	Ala	Leu	Leu	Leu	Ser	Leu	Ile	Ile	Thr	
				805					810					815		
Leu	Ile	Cys	Tyr	His	Arg	Ser	Arg	Lys	Arg	Val	Cys	Lys	Ser	Gly	Gly	
			820					825					830			
Gly	Ser	Phe	Trp	Glu	Glu	Phe	Glu	Ser	Leu	Gln	Lys	Gln	Glu	Val	Lys	
		835					840					845				
Asn	Leu	His	Gln	Arg	Leu	Glu	Gly	Gln	Arg	Pro	Glu	Asn	Lys	Gly	Lys	
	850					855					860					
Asn	Arg	Tyr	Lys	Asn	Ile	Leu	Pro	Phe	Asp	His	Ser	Arg	Val	Ile	Leu	
					870					875					880	
Gln	Gly	Arg	Asp	Ser	Asn	Ile	Pro	Gly	Ser	Asp	Tyr	Ile	Asn	Ala	Asn	
				885					890					895		
Tyr	Ile	Lys	Asn	Gln	Leu	Leu	Gly	Pro	Asp	Glu	Asn	Ala	Lys	Thr	Tyr	
			900					905					910			
Ile	Ala	Ser	Gln	Gly	Cys	Leu	Glu	Ala	Thr	Val	Asn	Asp	Phe	Trp	Gln	
		915					920					925				
Met	Ala	Trp	Gln	Glu	Asn	Ser	Arg	Val	Ile							

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995					1000					1005				
Pro	Asp	His	Gly	Val	Pro	Ser	Glu	Pro	Gly	Gly	Val	Leu	Ser	Phe
1010					1015							1020		
Leu	Asp	Gln	Ile	Asn	Gln	Arg	Gln	Glu	Ser	Leu	Pro	His	Ala	Gly
1025					1030							1035		
Pro	Ile	Ile	Val	His	Cys	Ser	Ala	Gly	Ile	Gly	Arg	Thr	Gly	Thr
1040					1045							1050		
Ile	Ile	Val	Ile	Asp	Met	Leu	Met	Glu	Asn	Ile	Ser	Thr	Lys	Gly
1055					1060							1065		
Leu	Asp	Cys	Asp	Ile	Asp	Ile	Gln	Lys	Thr	Ile	Gln	Met	Val	Arg
1070					1075							1080		
Ala	Gln	Arg	Ser	Gly	Met	Val	Gln	Thr	Glu	Ala	Gln	Tyr	Lys	Phe
1085					1090							1095		
Ile	Tyr	Val	Ala	Ile	Ala	Gln	Phe	Ile	Glu	Thr	Thr	Lys	Lys	Lys
1100					1105							1110		

Leu

<210> SEQ ID NO 49

<211> LENGTH: 918

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Amino acid sequence of a AND NOT gate
 (MP16091.SFG.acD19fmc63-CD8STK-CD28tmZ-2A-acD33glx-muCD8STK-LAIR1
 tm-endo)

<400> SEQUENCE: 49

Met	Ser	Leu	Pro	Val	Thr	Ala	Leu	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu
1				5					10					15	
His	Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu
			20					25					30		
Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln
			35				40					45			
Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr
			50			55					60				
Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro
			65		70					75				80	
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile
			85						90				95		
Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly
			100				105						110		
Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr
			115				120					125			
Lys	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
			130			135						140			
Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly
			145		150					155				160	
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly
			165						170				175		
Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg
			180					185					190		
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr
			195			200						205			

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Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser
210						215					220				
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr
225					230					235					240
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala
				245					250					255	
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Asp	Pro
			260					265					270		
Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala
			275				280					285			
Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly
	290					295					300				
Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Phe	Trp
305					310					315					320
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val
				325					330					335	
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Arg	Val	Lys	Phe	Ser	Arg
			340					345					350		
Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn
		355					360					365			
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg
	370					375					380				
Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro
385					390					395					400
Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala
			405						410					415	
Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His
			420					425					430		
Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp
		435					440					445			
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly
	450					455					460				
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala
465					470					475					480
Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala
			485						490					495	
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
			500					505					510		
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr
		515					520					525			
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu
	530					535					540				
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe
545					550					555					560
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu
				565					570					575	
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr
			580				585						590		
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly
		595					600					605			
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly

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610					615					620					
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu
625					630					635					640
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe
			645						650					655	
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys
			660					665					670		
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr
		675					680					685			
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala
		690					695					700			
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr
705					710					715					720
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe
				725					730					735	
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro
			740					745					750		
Ala	Thr	Thr	Thr	Lys	Pro	Val	Leu	Arg	Thr	Pro	Ser	Pro	Val	His	Pro
			755					760					765		
Thr	Gly	Thr	Ser	Gln	Pro	Gln	Arg	Pro	Glu	Asp	Cys	Arg	Pro	Arg	Gly
			770				775					780			
Ser	Val	Lys	Gly	Thr	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Leu	Ile	Gly
785					790					795					800
Val	Ser	Val	Val	Phe	Leu	Phe	Cys	Leu	Leu	Leu	Leu	Val	Leu	Phe	Cys
				805					810					815	
Leu	His	Arg	Gln	Asn	Gln	Ile	Lys	Gln	Gly	Pro	Pro	Arg	Ser	Lys	Asp
			820					825					830		
Glu	Glu	Gln	Lys	Pro	Gln	Gln	Arg	Pro	Asp	Leu	Ala	Val	Asp	Val	Leu
		835					840					845			
Glu	Arg	Thr	Ala	Asp	Lys	Ala	Thr	Val	Asn	Gly	Leu	Pro	Glu	Lys	Asp
		850					855					860			
Arg	Glu	Thr	Asp	Thr	Ser	Ala	Leu	Ala	Ala	Gly	Ser	Ser	Gln	Glu	Val
865					870					875					880
Thr	Tyr	Ala	Gln	Leu	Asp	His	Trp	Ala	Leu	Thr	Gln	Arg	Thr	Ala	Arg
			885					890					895		
Ala	Val	Ser	Pro	Gln	Ser	Thr	Lys	Pro	Met	Ala	Glu	Ser	Ile	Thr	Tyr
			900					905					910		
Ala	Ala	Val	Ala	Arg	His										
			915												

<210> SEQ ID NO 50
 <211> LENGTH: 1362
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of a AND NOT gate
 (MP16092.SFG.acD19fmc63-CD8STK-CD28tmZ-2A-acD33glx-muCD8STK-
 LAIR1 tm-endo-2A-PTPN6_SH2-dCD148)
 <400> SEQUENCE: 50

Met	Ser	Leu	Pro	Val	Thr	Ala	Leu	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu
1				5						10				15	
His	Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu
			20					25					30		

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Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
 35 40 45
 Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
 50 55 60
 Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
 65 70 75 80
 Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
 85 90 95
 Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
 100 105 110
 Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
 115 120 125
 Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 130 135 140
 Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
 145 150 155 160
 Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
 165 170 175
 Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
 180 185 190
 Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
 195 200 205
 Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
 210 215 220
 Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
 225 230 235 240
 Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
 245 250 255
 Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
 260 265 270
 Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
 275 280 285
 Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
 290 295 300
 Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
 305 310 315 320
 Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
 325 330 335
 Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
 340 345 350
 Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
 355 360 365
 Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
 370 375 380
 Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
 385 390 395 400
 Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
 405 410 415
 Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
 420 425 430

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Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp
435							440					445			
Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Glu	Gly	Arg	Gly
450						455					460				
Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala
465					470					475					480
Val	Pro	Thr	Gln	Val	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Thr	Asp	Ala
			485						490					495	
Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
			500					505					510		
Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asp	Ile	Tyr
		515					520					525			
Phe	Asn	Leu	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu
530						535					540				
Leu	Ile	Tyr	Asp	Thr	Asn	Arg	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe
545					550					555					560
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu
				565					570					575	
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Tyr	Lys	Asn	Tyr
			580					585					590		
Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ser	Gly
		595					600					605			
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly
610					615						620				
Gly	Gly	Ser	Arg	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu
625					630					635					640
Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe
			645						650					655	
Thr	Leu	Ser	Asn	Tyr	Gly	Met	His	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys
			660					665					670		
Gly	Leu	Glu	Trp	Val	Ser	Ser	Ile	Ser	Leu	Asn	Gly	Gly	Ser	Thr	Tyr
		675					680					685			
Tyr	Arg	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala
690					695						700				
Lys	Ser	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr
705					710					715					720
Ala	Val	Tyr	Tyr	Cys	Ala	Ala	Gln	Asp	Ala	Tyr	Thr	Gly	Gly	Tyr	Phe
			725						730					735	
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Met	Asp	Pro
			740					745					750		
Thr	Thr	Thr	Lys	Pro	Val	Leu	Arg	Thr	Pro	Ser	Pro	Val	His	Pro	Thr
		755					760					765			
Gly	Thr	Ser	Gln	Pro	Gln	Arg	Pro	Glu	Asp	Cys	Arg	Pro	Arg	Gly	Ser
770					775						780				
Val	Lys	Gly	Thr	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Leu	Ile	Gly	Val
785					790					795					800
Ser	Val	Val	Phe	Leu	Phe	Cys	Leu	Leu	Leu	Val	Leu	Phe	Cys	Leu	
			805						810					815	
His	Arg	Gln	Asn	Gln	Ile	Lys	Gln	Gly	Pro	Pro	Arg	Ser	Lys	Asp	Glu
			820					825					830		
Glu	Gln	Lys	Pro	Gln	Gln	Arg	Pro	Asp	Leu	Ala	Val	Asp	Val	Leu	Glu

835					840					845						
Arg	Thr	Ala	Asp	Lys	Ala	Thr	Val	Asn	Gly	Leu	Pro	Glu	Lys	Asp	Arg	
850					855					860						
Glu	Thr	Asp	Thr	Ser	Ala	Leu	Ala	Ala	Gly	Ser	Ser	Gln	Glu	Val	Thr	
865					870					875					880	
Tyr	Ala	Gln	Leu	Asp	His	Trp	Ala	Leu	Thr	Gln	Arg	Thr	Ala	Arg	Ala	
885					890					895						
Val	Ser	Pro	Gln	Ser	Thr	Lys	Pro	Met	Ala	Glu	Ser	Ile	Thr	Tyr	Ala	
900					905					910						
Ala	Val	Ala	Arg	His	Arg	Ala	Glu	Gly	Arg	Gly	Ser	Leu	Leu	Thr	Cys	
915					920					925						
Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Trp	Tyr	His	Gly	His	Met	Ser	
930					935					940						
Gly	Gly	Gln	Ala	Glu	Thr	Leu	Leu	Gln	Ala	Lys	Gly	Glu	Pro	Trp	Thr	
945					950					955					960	
Phe	Leu	Val	Arg	Glu	Ser	Leu	Ser	Gln	Pro	Gly	Asp	Phe	Val	Leu	Ser	
965					970					975						
Val	Leu	Ser	Asp	Gln	Pro	Lys	Ala	Gly	Pro	Gly	Ser	Pro	Leu	Arg	Val	
980					985					990						
Thr	His	Ile	Lys	Val	Met	Cys	Glu	Gly	Gly	Arg	Tyr	Thr	Val	Gly	Gly	
995					1000					1005						
Leu	Glu	Thr	Phe	Asp	Ser	Leu	Thr	Asp	Leu	Val	Glu	His	Phe	Lys		
1010					1015					1020						
Lys	Thr	Gly	Ile	Glu	Glu	Ala	Ser	Gly	Ala	Phe	Val	Tyr	Leu	Arg		
1025					1030					1035						
Gln	Pro	Tyr	Ser	Gly	Gly	Gly	Gly	Ser	Phe	Glu	Ala	Tyr	Phe	Lys		
1040					1045					1050						
Lys	Gln	Gln	Ala	Asp	Ser	Asn	Cys	Gly	Phe	Ala	Glu	Glu	Tyr	Glu		
1055					1060					1065						
Asp	Leu	Lys	Leu	Val	Gly	Ile	Ser	Gln	Pro	Lys	Tyr	Ala	Ala	Glu		
1070					1075					1080						
Leu	Ala	Glu	Asn	Arg	Gly	Lys	Asn	Arg	Tyr	Asn	Asn	Val	Leu	Pro		
1085					1090					1095						
Tyr	Asp	Ile	Ser	Arg	Val	Lys	Leu	Ser	Val	Gln	Thr	His	Ser	Thr		
1100					1105					1110						
Asp	Asp	Tyr	Ile	Asn	Ala	Asn	Tyr	Met	Pro	Gly	Tyr	His	Ser	Lys		
1115					1120					1125						
Lys	Asp	Phe	Ile	Ala	Thr	Gln	Gly	Pro	Leu	Pro	Asn	Thr	Leu	Lys		
1130					1135					1140						
Asp	Phe	Trp	Arg	Met	Val	Trp	Glu	Lys	Asn	Val	Tyr	Ala	Ile	Ile		
1145					1150					1155						
Met	Leu	Thr	Lys	Cys	Val	Glu	Gln	Gly	Arg	Thr	Lys	Cys	Glu	Glu		
1160					1165					1170						
Tyr	Trp	Pro	Ser	Lys	Gln	Ala	Gln	Asp	Tyr	Gly	Asp	Ile	Thr	Val		
1175					1180					1185						
Ala	Met	Thr	Ser	Glu	Ile	Val	Leu	Pro	Glu	Trp	Thr	Ile	Arg	Asp		
1190					1195					1200						
Phe	Thr	Val	Lys	Asn	Ile	Gln	Thr	Ser	Glu	Ser	His	Pro	Leu	Arg		
1205					1210					1215						
Gln	Phe	His	Phe	Thr	Ser	Trp	Pro	Asp	His	Gly	Val	Pro	Asp	Thr		
1220					1225					1230						

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Thr Asp Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met
 1235 1240 1245
 Lys Gln Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala
 1250 1255 1260
 Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile
 1265 1270 1275
 Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val
 1280 1285 1290
 Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp
 1295 1300 1305
 Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser
 1310 1315 1320
 Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala
 1325 1330 1335
 Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys
 1340 1345 1350
 Thr Asn Gly Tyr Ile Ala Ser Gly Ser
 1355 1360

<210> SEQ ID NO 51
 <211> LENGTH: 424
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: APRIL-based (A proliferation-inducing
 ligand-based) CAR, CD8 stalk APRIL CAR
 <400> SEQUENCE: 51

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser
 20 25 30
 Lys Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg
 35 40 45
 Arg Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp
 50 55 60
 Ala Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr
 65 70 75 80
 Phe Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu
 85 90 95
 Thr Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala
 100 105 110
 Tyr Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp
 115 120 125
 Ile Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser
 130 135 140
 Pro His Gly Thr Phe Leu Gly Phe Val Lys Leu Ser Gly Gly Gly Ser
 145 150 155 160
 Asp Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
 165 170 175
 Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
 180 185 190
 Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile

-continued

195					200					205					
Phe	Trp	Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu
210						215					220				
Leu	Val	Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Lys	Arg	Ser
225					230					235					240
Arg	Leu	Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro	Arg	Arg	Pro	Gly
				245					250					255	
Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro	Arg	Asp	Phe	Ala
			260					265					270		
Ala	Tyr	Arg	Ser	Arg	Asp	Gln	Arg	Leu	Pro	Pro	Asp	Ala	His	Lys	Pro
		275					280					285			
Pro	Gly	Gly	Gly	Ser	Phe	Arg	Thr	Pro	Ile	Gln	Glu	Glu	Gln	Ala	Asp
	290					295					300				
Ala	His	Ser	Thr	Leu	Ala	Lys	Ile	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala
305					310					315					320
Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu
				325					330					335	
Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly
			340					345					350		
Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu
		355					360					365			
Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser
	370					375					380				
Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly
385					390					395					400
Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu
				405					410					415	
His	Met	Gln	Ala	Leu	Pro	Pro	Arg								
				420											

<210> SEQ ID NO 52

<211> LENGTH: 398

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: APRIL-based (A proliferation-inducing ligand-based) CAR, APRIL IgG1 hinge based CAR

<400> SEQUENCE: 52

Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
1				5					10					15	
Gly	Ser	Thr	Gly	Ser	Val	Leu	His	Leu	Val	Pro	Ile	Asn	Ala	Thr	Ser
			20					25					30		
Lys	Asp	Asp	Ser	Asp	Val	Thr	Glu	Val	Met	Trp	Gln	Pro	Ala	Leu	Arg
		35					40					45			
Arg	Gly	Arg	Gly	Leu	Gln	Ala	Gln	Gly	Tyr	Gly	Val	Arg	Ile	Gln	Asp
		50				55					60				
Ala	Gly	Val	Tyr	Leu	Leu	Tyr	Ser	Gln	Val	Leu	Phe	Gln	Asp	Val	Thr
		65				70				75					80
Phe	Thr	Met	Gly	Gln	Val	Val	Ser	Arg	Glu	Gly	Gln	Gly	Arg	Gln	Glu
				85					90					95	
Thr	Leu	Phe	Arg	Cys	Ile	Arg	Ser	Met	Pro	Ser	His	Pro	Asp	Arg	Ala
				100				105						110	

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<210> SEQ ID NO 53
<211> LENGTH: 614
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: APRIL-based (A proliferation-inducing ligand-
      based) CAR, APRIL Fc-pvaa based CAR

<400> SEQUENCE: 53
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Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
1				5					10					15	
Gly	Ser	Thr	Gly	Ser	Val	Leu	His	Leu	Val	Pro	Ile	Asn	Ala	Thr	Ser
			20					25					30		
Lys	Asp	Asp	Ser	Asp	Val	Thr	Glu	Val	Met	Trp	Gln	Pro	Ala	Leu	Arg
		35					40					45			
Arg	Gly	Arg	Gly	Leu	Gln	Ala	Gln	Gly	Tyr	Gly	Val	Arg	Ile	Gln	Asp
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Ala	Gly	Val	Tyr	Leu	Leu	Tyr	Ser	Gln	Val	Leu	Phe	Gln	Asp	Val	Thr	65	70	75	80
Phe	Thr	Met	Gly	Gln	Val	Val	Ser	Arg	Glu	Gly	Gln	Gly	Arg	Gln	Glu	85	90	95	
Thr	Leu	Phe	Arg	Cys	Ile	Arg	Ser	Met	Pro	Ser	His	Pro	Asp	Arg	Ala	100	105	110	
Tyr	Asn	Ser	Cys	Tyr	Ser	Ala	Gly	Val	Phe	His	Leu	His	Gln	Gly	Asp	115	120	125	
Ile	Leu	Ser	Val	Ile	Ile	Pro	Arg	Ala	Arg	Ala	Lys	Leu	Asn	Leu	Ser	130	135	140	
Pro	His	Gly	Thr	Phe	Leu	Gly	Phe	Val	Lys	Leu	Ser	Gly	Gly	Gly	Ser	145	150	155	160
Asp	Pro	Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	165	170	175	
Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	180	185	190	
Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	195	200	205	
Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	210	215	220	
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	225	230	235	240
Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	245	250	255	
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	260	265	270	
Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	275	280	285	
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	290	295	300	
Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	305	310	315	320
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	325	330	335	
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	340	345	350	
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	355	360	365	
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	370	375	380	
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Asp	Pro	Lys	Phe	Trp	385	390	395	400
Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val	405	410	415	
Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Lys	Arg	Ser	Arg	Leu	420	425	430	
Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro	Arg	Arg	Pro	Gly	Pro	Thr	435	440	445	
Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro	Arg	Asp	Phe	Ala	Ala	Tyr	450	455	460	

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Arg	Ser	Arg	Asp	Gln	Arg	Leu	Pro	Pro	Asp	Ala	His	Lys	Pro	Pro	Gly
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Gly	Gly	Ser	Phe	Arg	Thr	Pro	Ile	Gln	Glu	Gln	Ala	Asp	Ala	His	
				485				490					495		
Ser	Thr	Leu	Ala	Lys	Ile	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
			500					505					510		
Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
		515					520					525			
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
	530					535					540				
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
545					550					555					560
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
			565					570						575	
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
		580						585					590		
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
	595						600					605			
Gln	Ala	Leu	Pro	Pro	Arg										
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<210> SEQ ID NO 54

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: AntiCD19: CD28TM-TCRz

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taccagcagg gccagaacca gctctataac gagctcaatc taggacgaag agaggagtac      180
gatgttttgg acaagagacg tggccgggac cctgagatgg ggggaaagcc gagaaggaag      240
aaccctcagg aaggcctgta caatgaactg cagaaagata agatggcgga ggcctacagt      300
gagattggga tgaaaggcga gcgccggagg ggcaaggggc acgatggcct ttaccagggt      360
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<210> SEQ ID NO 55

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<400> SEQUENCE: 55

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tatcagcagg gccagaatca gctgtacaat gaactgaacc tgggcaggcg ggaggagtac      180

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gacgtgctgg ataagcggag aggcagagac cccgagatgg gcggcaaacc acggcgcaaa	240
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gagatcggca tgaagggaga gagaagacgc ggaaggggcc acgacggcct gtatcaggga	360
ttgtccaccg ctacaaaaga tacatatgat gccctgcaca tgcaggccct gccaccacaga	420

1-28. (canceled)

29. A nucleic acid sequence which comprises:

- a) a first nucleotide sequence encoding a first chimeric antigen receptor (CAR);
- b) a second nucleotide sequence encoding a second CAR;
- c) a sequence encoding a self-cleaving peptide positioned between the first and second nucleotide sequences, such that the two CARs are expressed as separate entities.

30. The nucleic acid sequence according to claim 1, wherein alternative codons are used in one or more portion(s) of the first and second nucleotide sequences in regions which encode the same or similar amino acid sequence(s).

31. A vector comprising a nucleic acid according to claim 29 or 30.

32. The vector according to claim 31 which is a retroviral vector, a lentiviral vector or a transposon.

33. A cell which comprises a nucleic acid sequence according to claim 29 or 30.

34. The cell according to claim 33, which is a T-cell or NK cell.

35. A method for making a cell, which comprises the step of introducing into the cell ex vivo a nucleic acid sequence or a vector comprising:

- a) a first nucleotide sequence encoding a first chimeric antigen receptor (CAR),
- b) a second nucleotide sequence encoding a second CAR, and
- c) a sequence encoding a self-cleaving peptide positioned between the first and second nucleotide sequences, such that the two CARs are expressed as separate entities.

36. The method of claim 35 wherein alternative codons are used in one or more portion(s) of the first and second nucleotide sequences in regions which encode the same or similar amino acid sequence(s).

37. The method of claim 35 wherein the vector is a retroviral vector, a lentiviral vector or a transposon.

38. The method of claim 35 wherein the cell is a T-cell or NK cell.

39. A method according to claim 35, wherein the cell is from a sample isolated from a subject.

40. A pharmaceutical composition comprising a plurality of cells according to claim 33.

41. A pharmaceutical composition comprising a plurality of cells according to claim 34.

42. A method for treating a disease, which comprises the step of administering a pharmaceutical composition according to claim 40 to a subject.

43. A method for treating a disease, which comprises the step of administering a pharmaceutical composition according to claim 41 to a subject.

44. A method for treating a disease, which comprises the following steps:

- a) isolation of a T cell-containing sample from a subject;
- b) transduction or transfection of the T cells with a nucleic acid sequence or a vector comprising:
 - i) a first nucleotide sequence encoding a first chimeric antigen receptor (CAR),
 - ii) a second nucleotide sequence encoding a second CAR, and
 - iii) a sequence encoding a self-cleaving peptide positioned between the first and second nucleotide sequences, such that the two CARs are expressed as separate entities; and
- c) administering the T cells from b) to a the subject.

45. The method according to claim 43, wherein the disease is a cancer.

46. The method according to claim 44, wherein the disease is a cancer.

* * * * *