FIGURE 1A

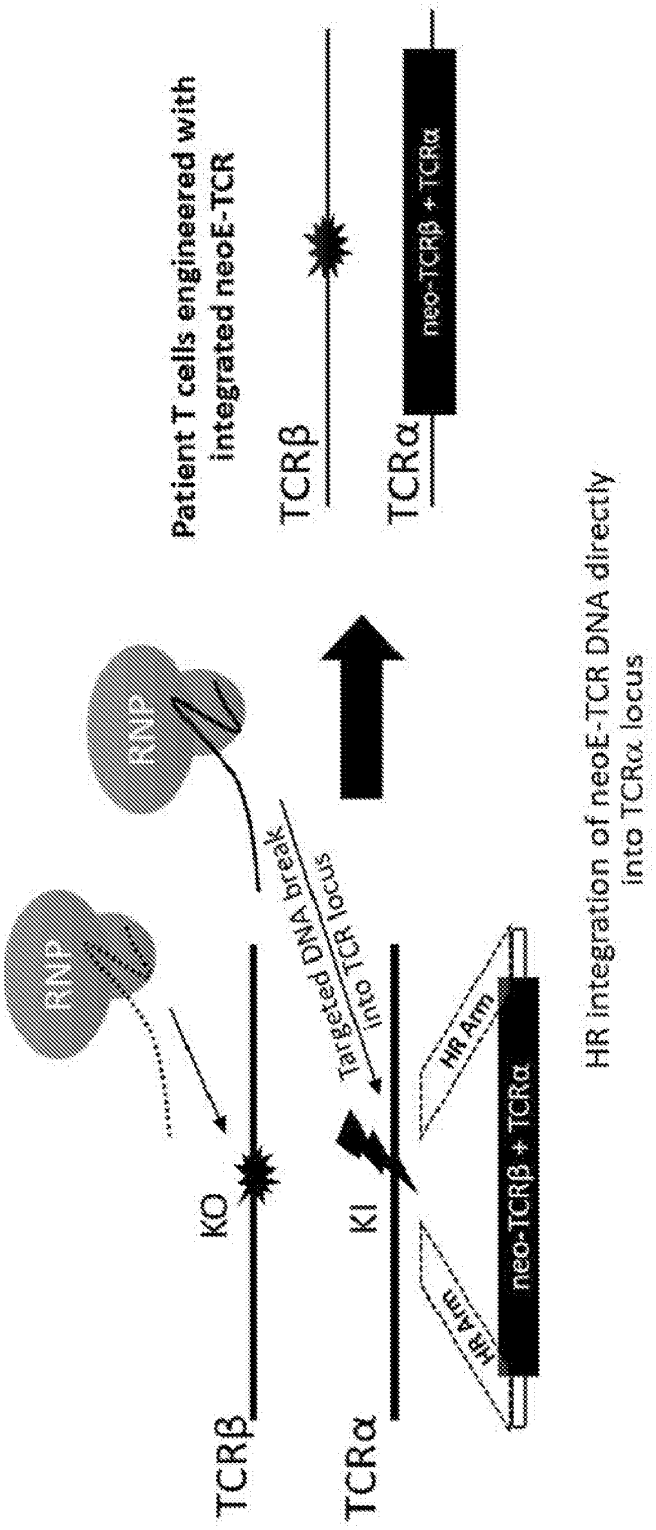

Patient T cells engineered with integrated neoE-TCR
TCRβ
TCRα
neo-TCRβ + TCRα
RNP
RNP
Targeted DNA break into TCR locus
KO
KI
HR Arm
HR Arm
neo-TCRβ + TCRα
TCRβ
TCRα
HR integration of neoE-TCR DNA directly into TCRα locus

FIGURE 1B

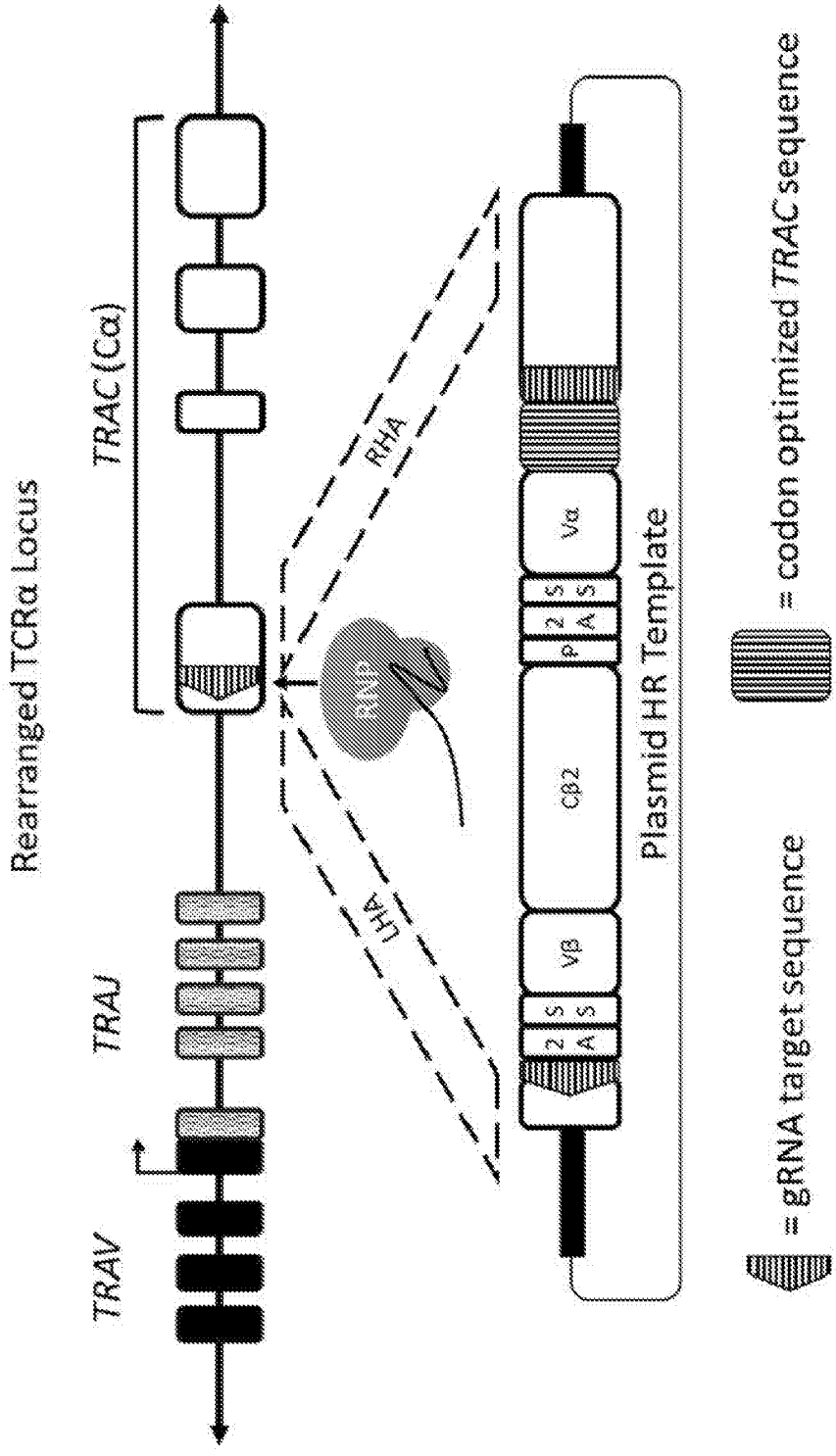

Rearranged TCRα Locus
TRAV
TRAJ
TRAC (Cα)
LHA
RHA
CRISPR
Plasmid HR Template
Cβ2
Vβ
Vα
= gRNA target sequence
= codon optimized TRAC sequence

FIGURE 1C

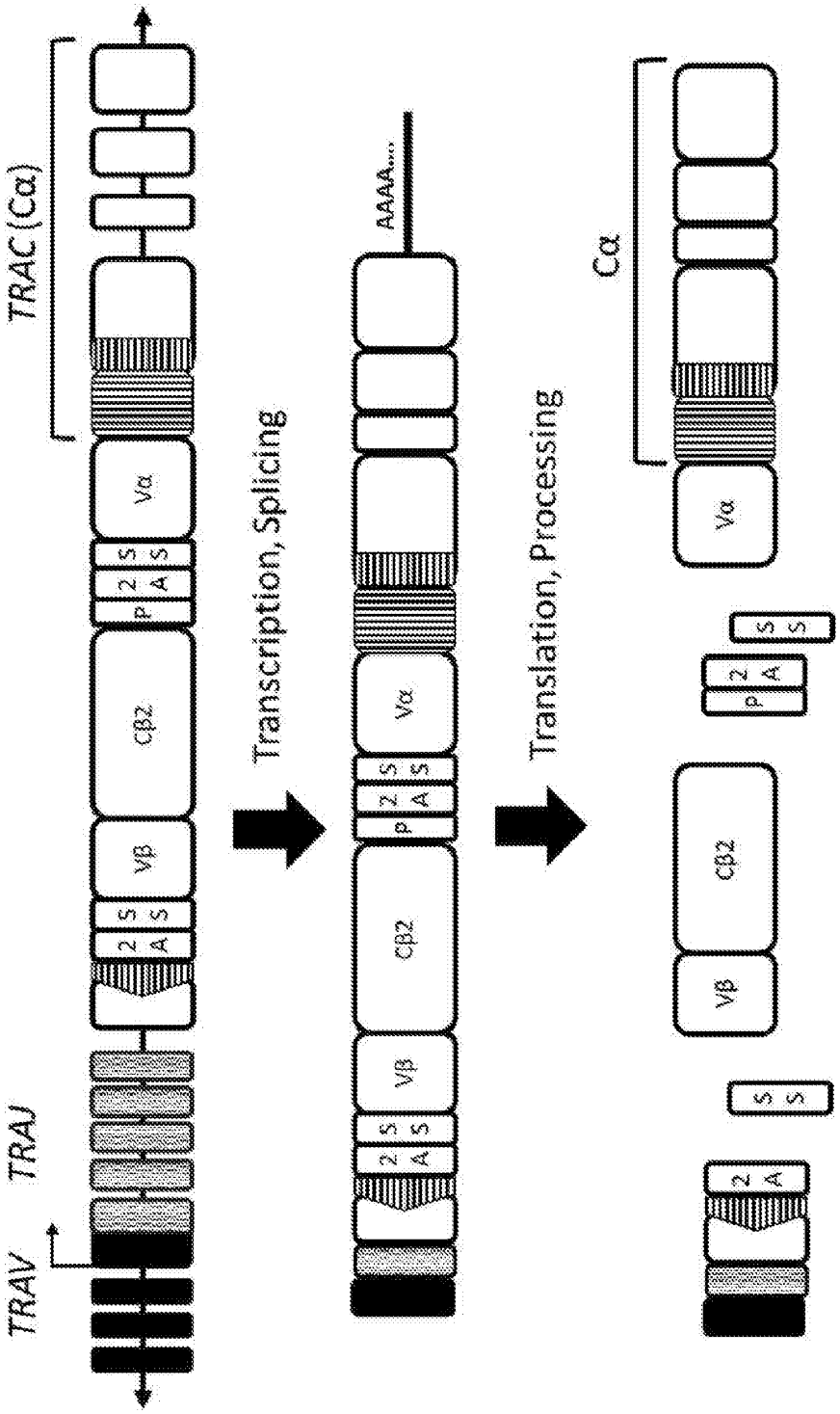

TRAC (Cα)
TRAV
TRAJ
Vα
P
2
A
S
S
Cβ2
Vβ
2
A
S
S
Transcription, Splicing
AAAA...
Translation, Processing
Cα

FIGURE 2A

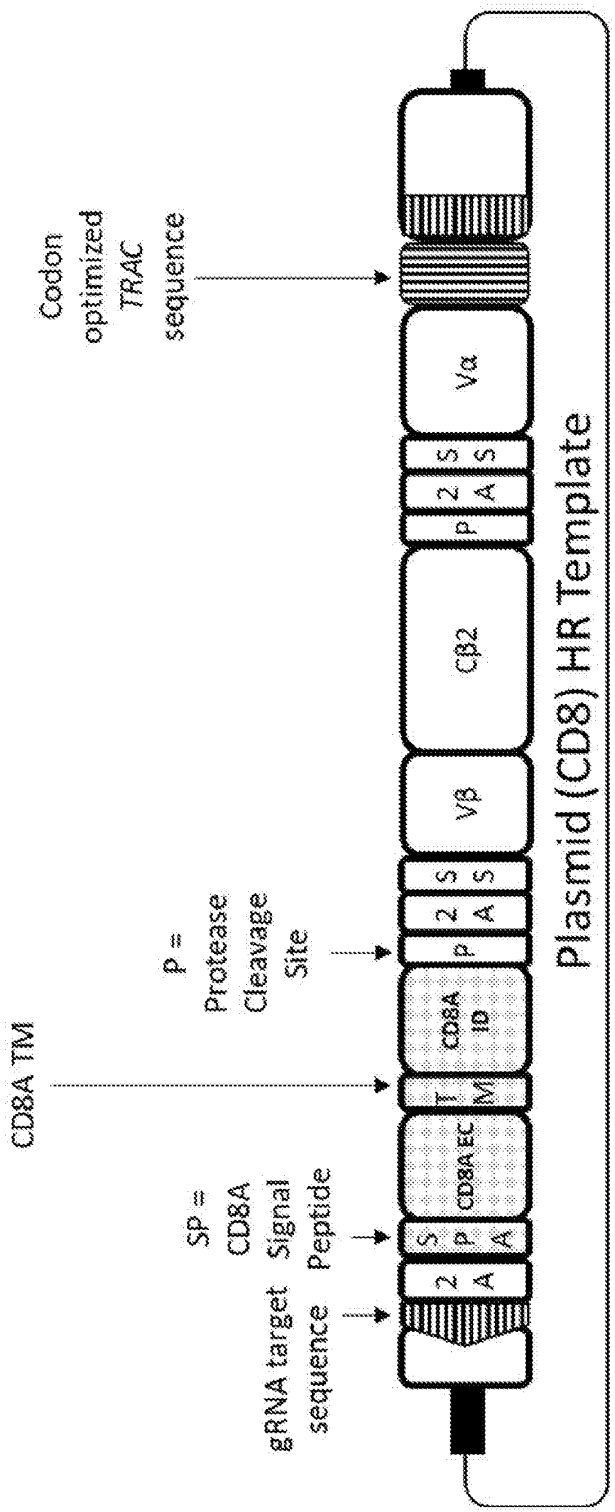

Codon optimized TRAC sequence
Vα
S
S
2
A
P
Cβ2
Vβ
S
S
2
A
P
CD8A TM
P = Protease Cleavage Site
CD8A TD
T
M
CD8A EC
S
P
A
2
A
SP = CD8A Signal Peptide
gRNA target sequence
Plasmid (CD8) HR Template
CD8 Product 1

FIGURE 2B

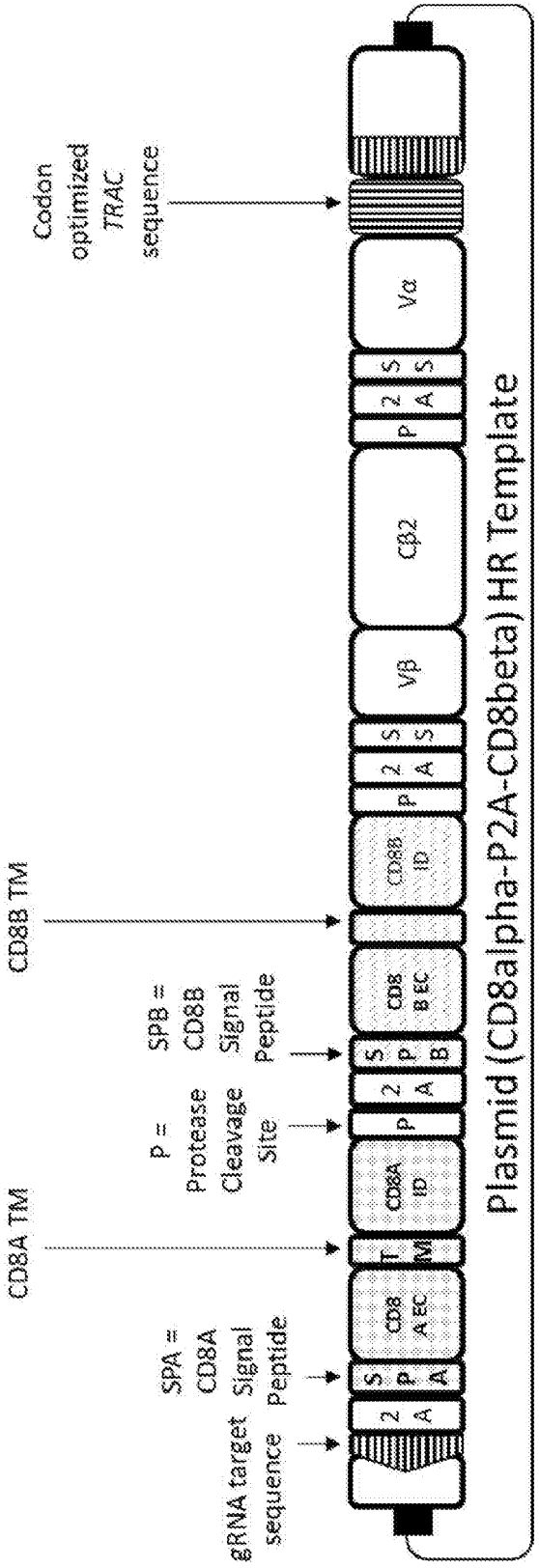

Codon optimized TRAC sequence
CD8B TM
CD8A TM
SPB = CD8B Signal Peptide
P = Protease Cleavage Site
SPA = CD8A Signal Peptide
gRNA target sequence
Vα
Cβ2
Vβ
Plasmid (CD8alpha-P2A-CD8beta) HR Template
CD8 Product 2

FIGURE 2C

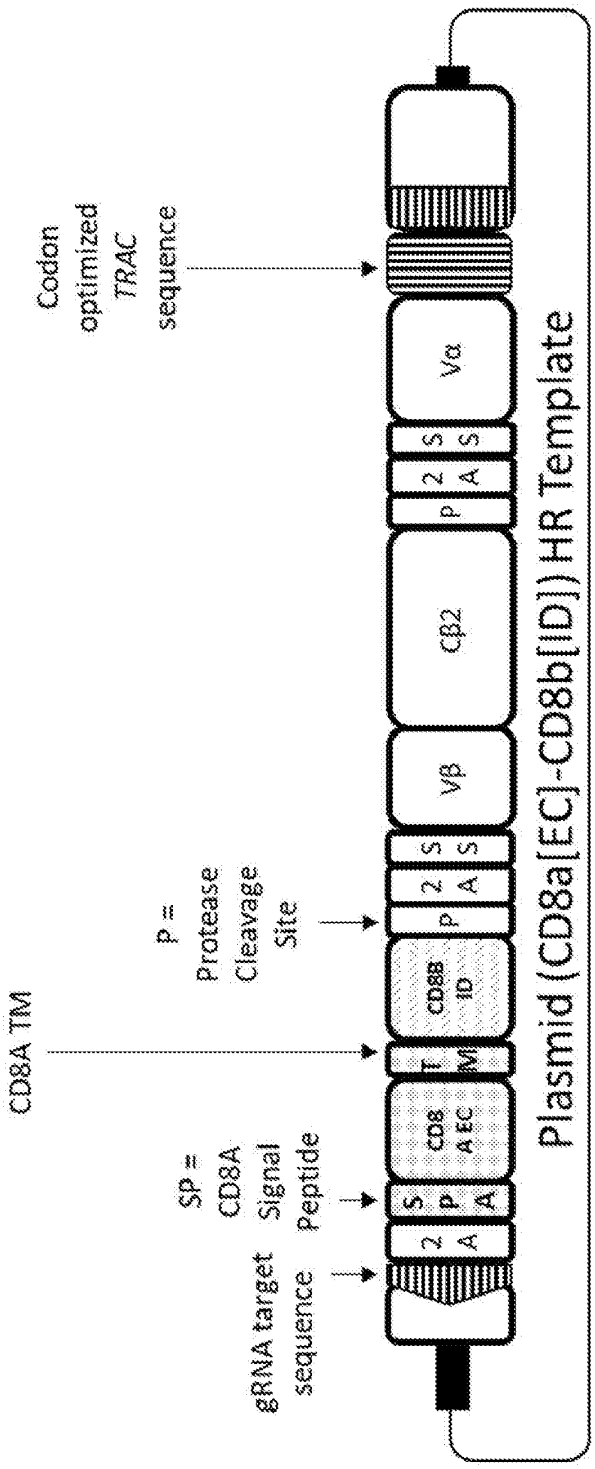

Codon optimized TRAC sequence
Vα
S S
P 2 A
Cβ2
Vβ
S S
P 2 A
P = Protease Cleavage Site
CD8B 1D
T M
CD8A TM
CD8A EC
S P A
2 A
SP = CD8A Signal Peptide
gRNA target sequence
Plasmid (CD8a[EC]-CD8b[ID]) HR Template
CD8 Product 3

FIGURE 2D

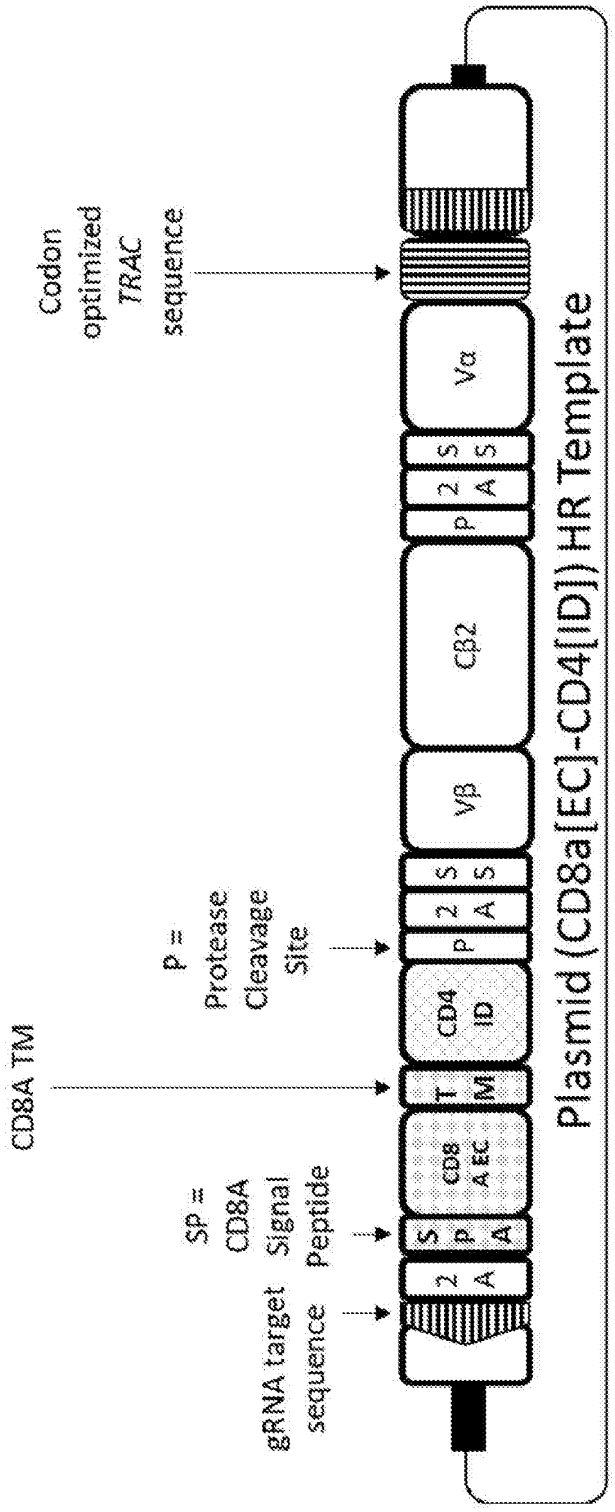

Codon optimized TRAC sequence
Vα
S
S
2
A
P
CB2
Vβ
S
S
2
A
P
CD4 ID
T
M
CD8 A EC
S
P
A
2
A
gRNA target sequence
SP = CD8A Signal Peptide
CD8A TM
P = Protease Cleavage Site
Plasmid (CD8a[EC]-CD4[ID]) HR Template
CD8 Product 4

FIGURE 3A

(CD8A_CD8 Product 1)

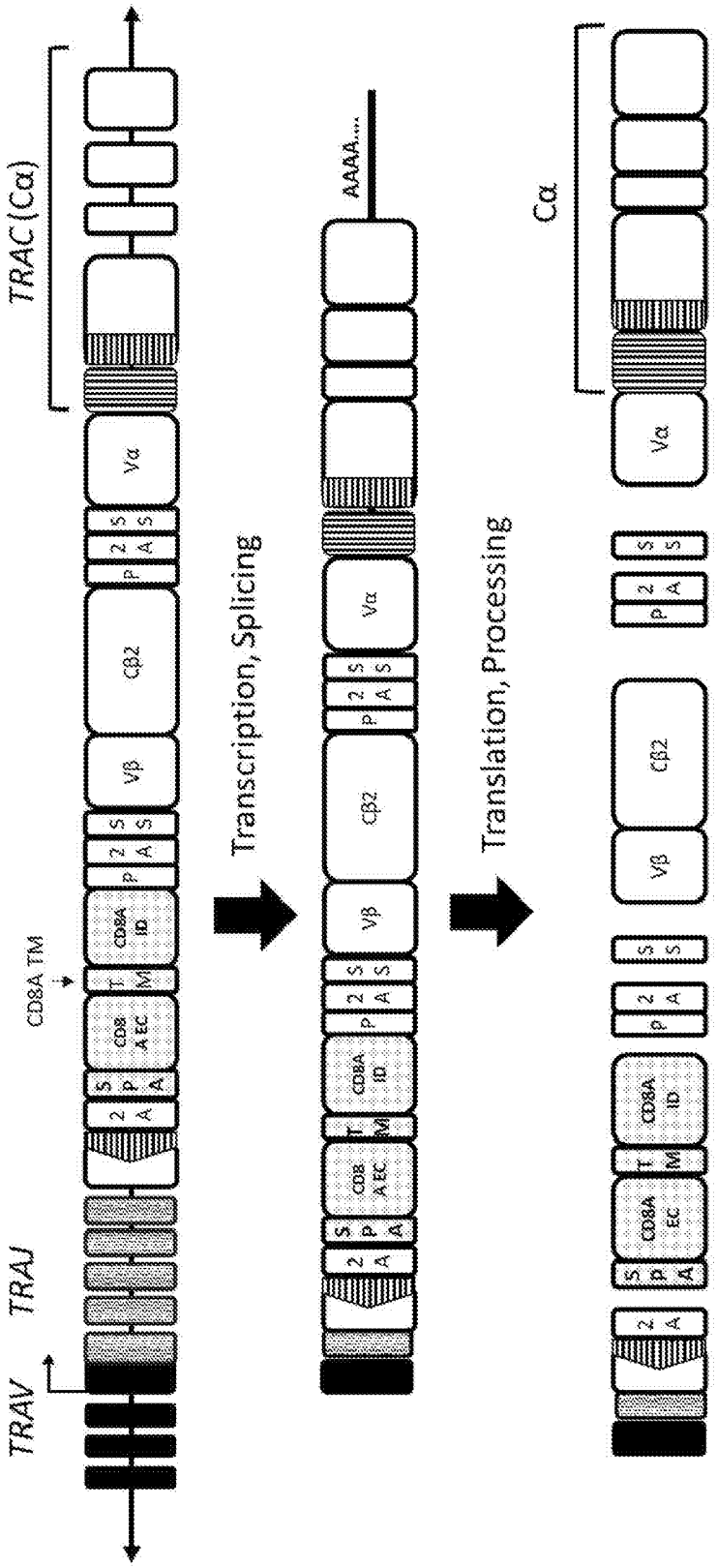


FIGURE 3B

(CD8alpha-P2A-CD8beta_CD8 Product 2)

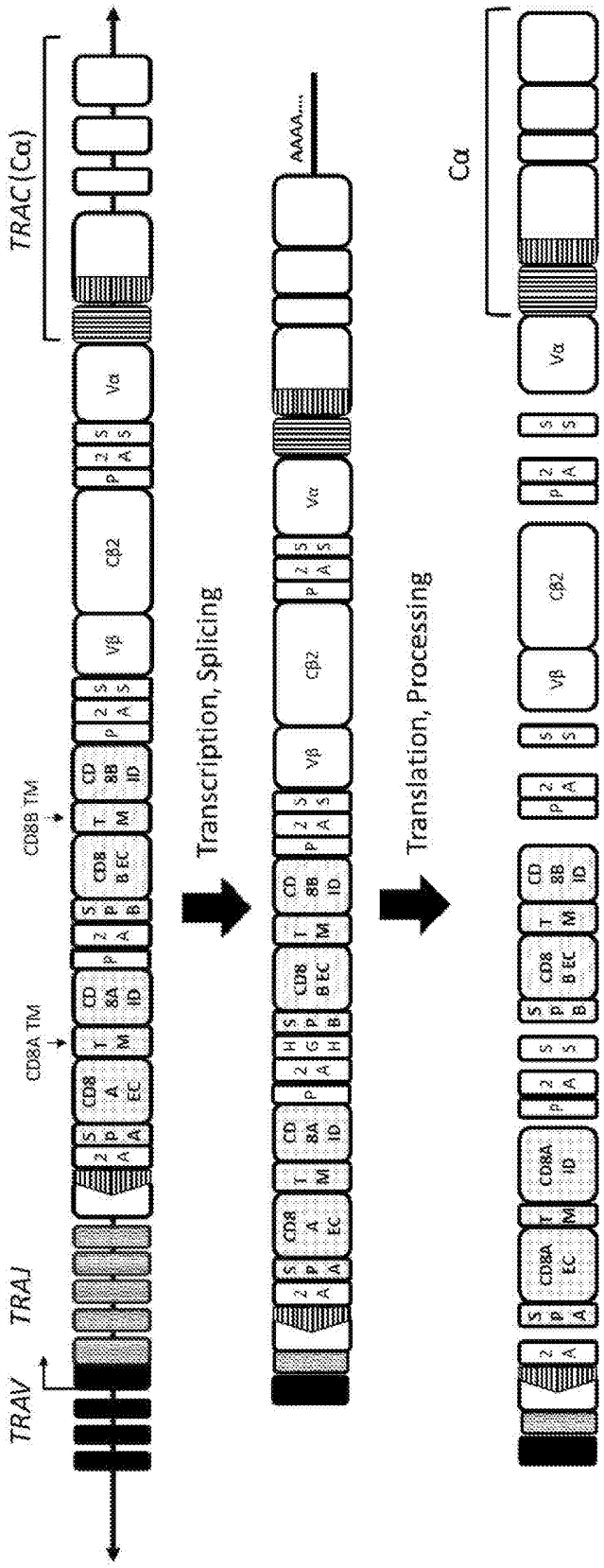


FIGURE 3C

(CD8A[EC]-CD8B[ID]_CD8 Product 3)

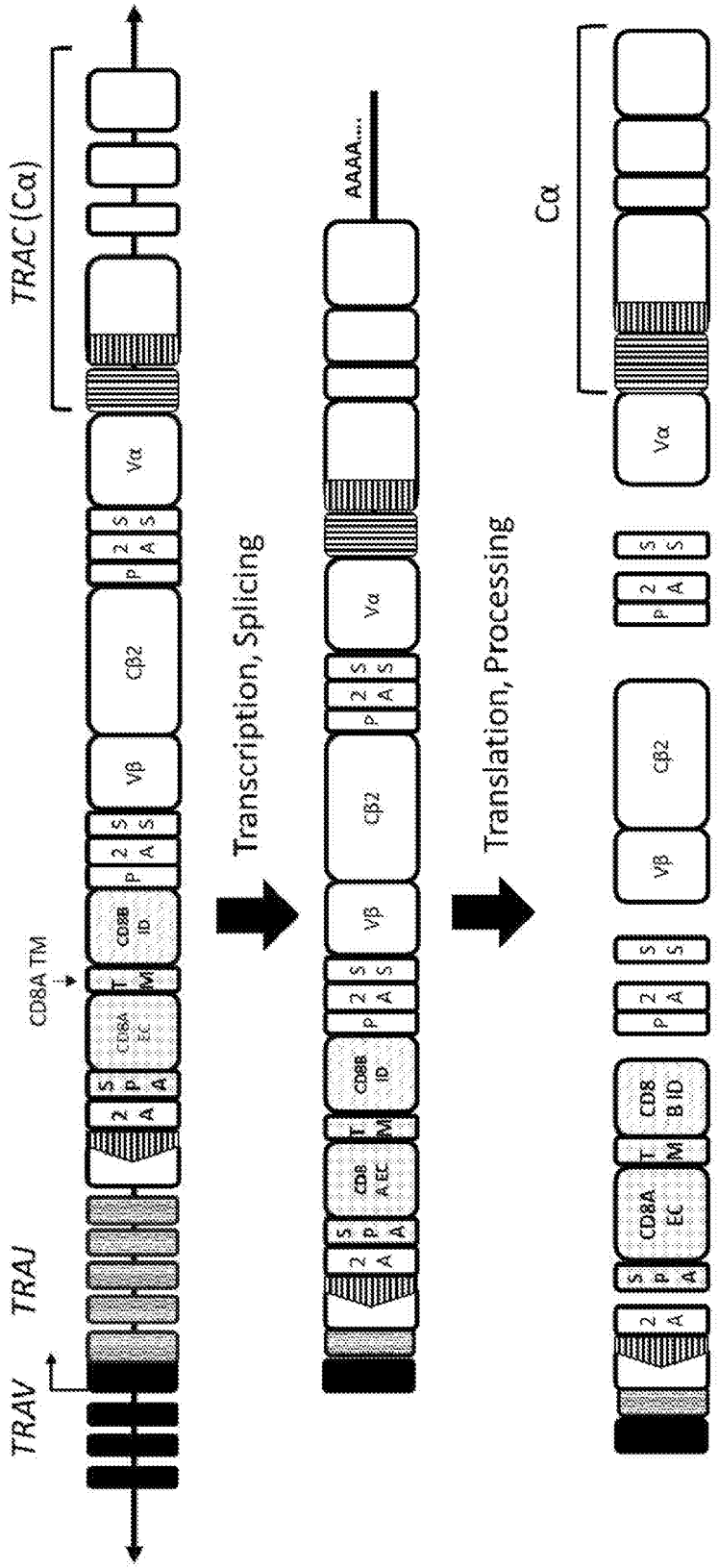


FIGURE 3D

(CD8a[EC]-CD4[ID]_CD8 Product 4)

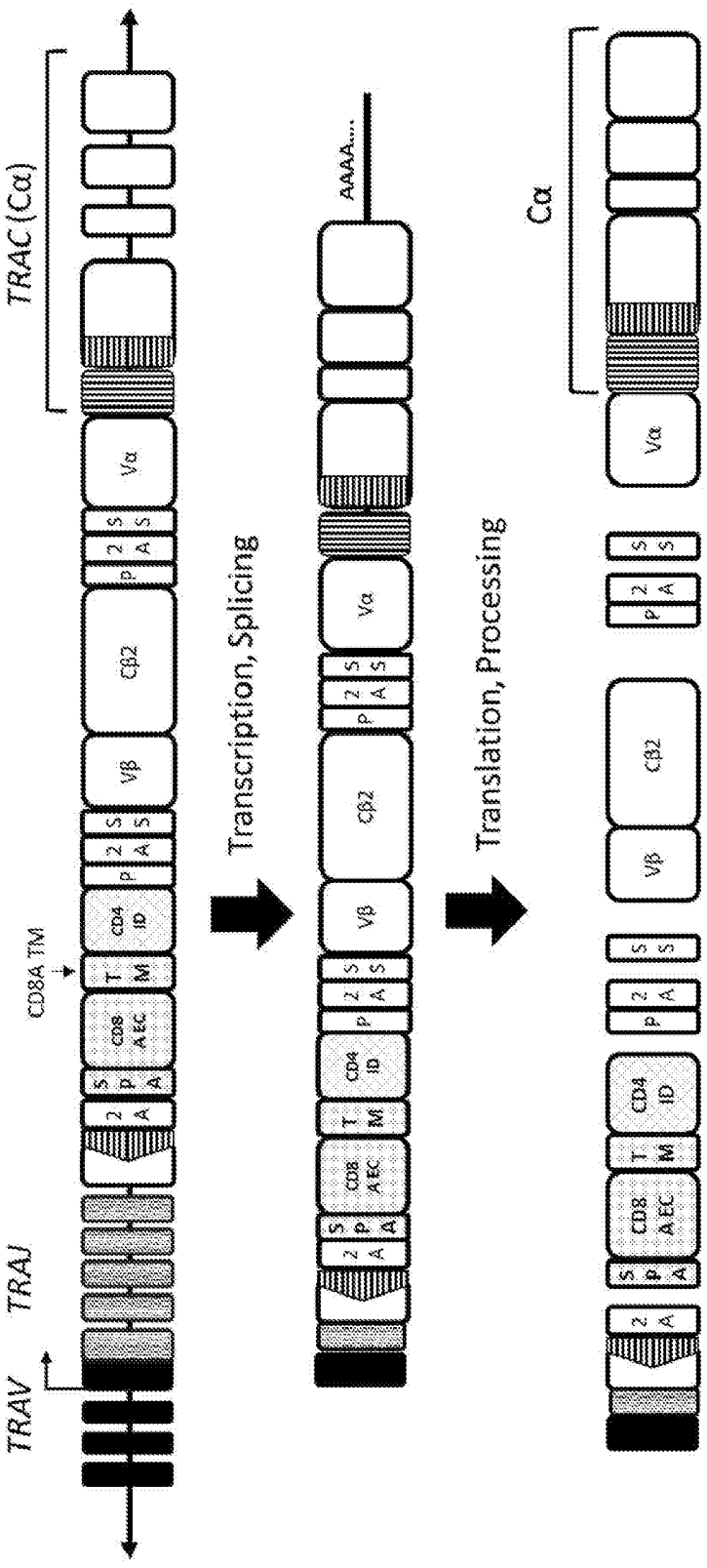


FIGURE 4A

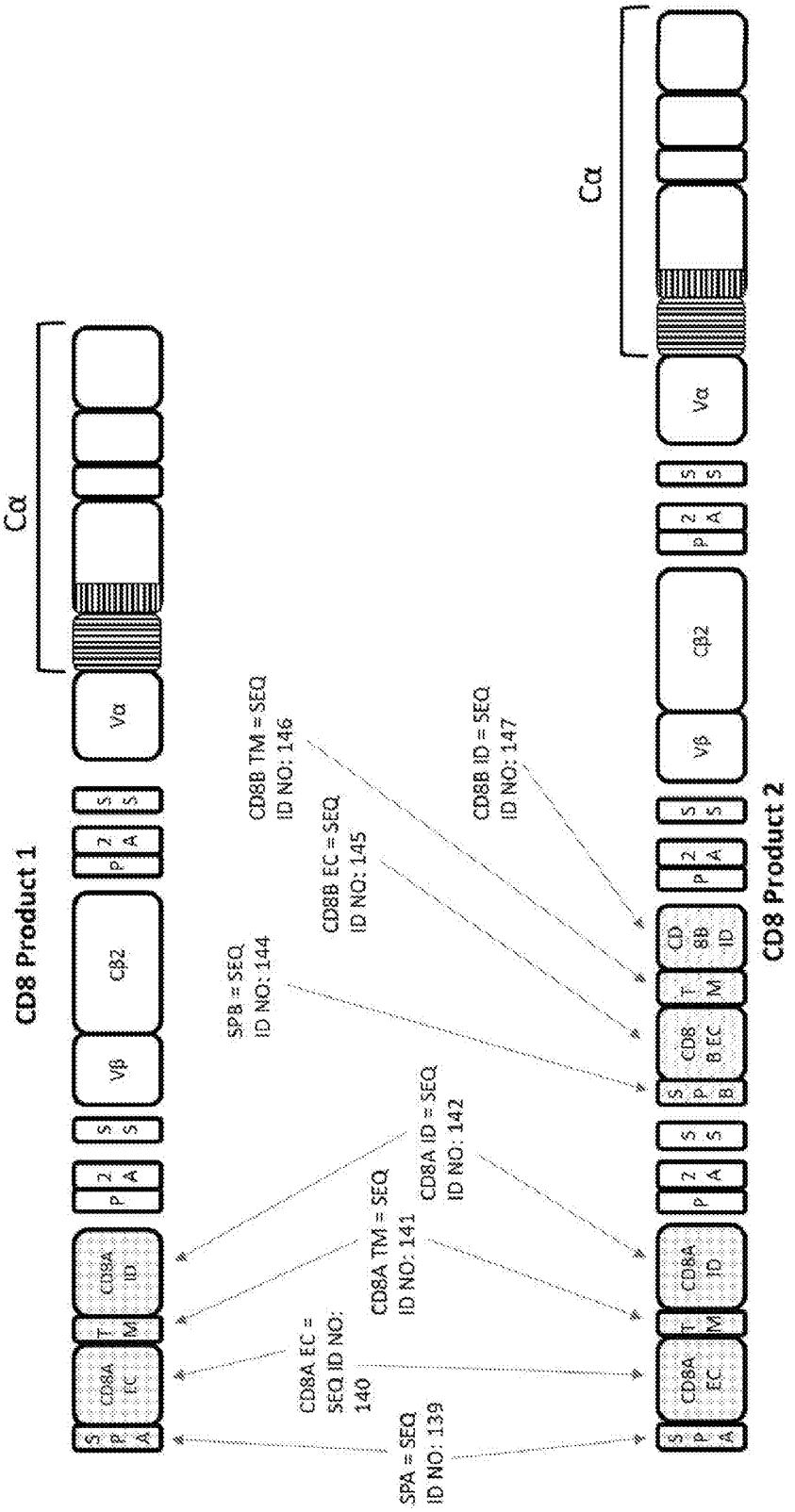

CD8 Product 1
Cα
Vα
Cβ2
Vβ
CD8A EC
CD8A ID
SPA = SEQ ID NO: 139
CD8A EC = SEQ ID NO: 140
CD8A TM = SEQ ID NO: 141
CD8A ID = SEQ ID NO: 142
SPB = SEQ ID NO: 144
CD8B EC = SEQ ID NO: 145
CD8B TM = SEQ ID NO: 146
CD8B ID = SEQ ID NO: 147
CD8 Product 2
Cα
Vα
Cβ2
Vβ
CD8A EC
CD8A ID
CD8 B EC
CD 8B ID

FIGURE 4B

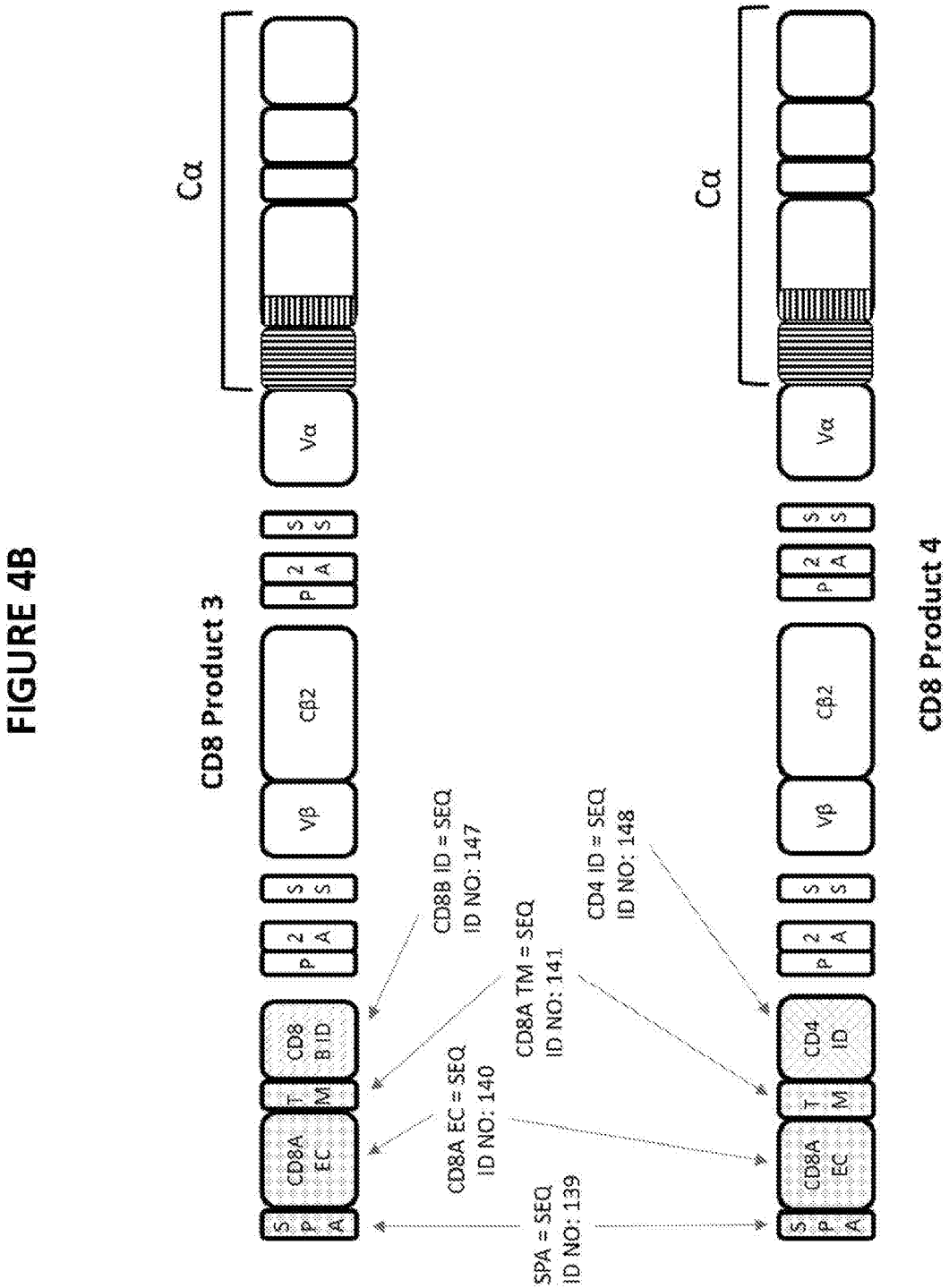

CD8 Product 3
Cα
Vα
Cβ2
Vβ
CD8 B ID
CD8A EC
CD8B ID = SEQ ID NO: 147
CD8A EC = SEQ ID NO: 140
CD8A TM = SEQ ID NO: 141
SPA = SEQ ID NO: 139
CD4 ID = SEQ ID NO: 148
CD4 ID
CD8A EC
CD8 Product 4

FIGURE 5

NeoTCR Product Nucleic Acid Sequence

Left HR Arm (SEQ ID NO:1)

ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGC
CTCCAGCAGCTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTA
GCACTCTATCACGGCCATATTCTGGCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTT
TACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTCTTTCTCAGAAGAGCCTGGCT
AGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGGAGCT
GCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTG
TTCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTT
CCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGA
GACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTA
CTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGT
ATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAACG
TTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTC
CATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCC
AGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCA
AAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAG

TRAC CDS (SEQ ID NO:2)

ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTG
ACAAGTCTGTCTGCCTATTC

GSG Linker 1 (SEQ ID NO:3)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:4)

GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGG
TCCT

FIGURE 5 Cont.

HGH/SS/2 (SEQ ID NO:5)

ATGGCCACCGGCTCTAGAACAAGCCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTC
CAAGAAGGATCTGCT

Insertion site for neoTCR TRB_VDJ sequence

[[TRB_VDJ Sequence, specific for the NeoTCR]]

TCR-beta/constant (SEQ ID NO:7)

CTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCC
ACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACTG
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGA
AAGAACAGCCCGCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGC
CACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCG
AGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCTGCCGAAG
CCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGC
CACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCT
GGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCC

Furin Cleavage Site (SEQ ID NO: 8)

CGGGCCAAGCGG

GSG Linker (SEQ ID NO:9)

GGCAGCGGC

P2A (SEQ ID NO:10)

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT

HGH SS (SEQ ID NO:11)

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCT
GCAAGAGGGTTCCGCC

FIGURE 5 Cont.

[[TRA_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRA_VDJ sequence

ATATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACAAGAGC
GTGTGTTTGTTC

TCR-alpha/constant (SEQ ID NO:13)

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC
AAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCA
ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGG

TRAC CDS/right HR arm(SEQ ID NO: 14)

TAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC
ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC
GGGAAAAAAGCAGATGAAGAAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCT
CTCCAACTGAGTTCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTTCTAGG
CCTCATTCTAAGCCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT
CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGG
CACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA
GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTT
CAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAG
GGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAG
GACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
GTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG

Right HR arm (SEQ ID NO:15)

GCTAGC

(SEQ ID NO:16)

FIGURE 5 Cont.

CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG
CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGAT
GGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC
CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGA
GAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCCAGACGAAA
TACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTG
ATGGTCGGAAGAGGCATAAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCA
TTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAG
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAG
CATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCAT

AACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATC
TTGTGCAATGTAACATCAGAGATTTTGAGACAC

pBR322_origin (SEQ ID NO:17)

Kanamycin resistant gene (KanR2) (SEQ ID NO:18)

Kanamycin Promoter (SEQ ID NO:19)

FIGURE 6

CD8 Product 1 Product Nucleic Acid Sequence

Left HR Arm (SEQ ID NO:22)

ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGC
CTCCAGCAGCTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTA
GCACTCTATCACGGCCATATTCTGGCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTT
TACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTCTTTCTCAGAAGAGCCTGGCT
AGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGGAGCT
GCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTG
TTCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTT
CCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGA
GACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGGCCTTTTTTCCCATGCCTGCCTTTA
CTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGT
ATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAACG
TTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTC
CATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCC
AGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCA
AAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAG

TRAC CDS (SEQ ID NO:23)

ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG
TCTGCCTATTC

GSG Linker 1 (SEQ ID NO:24)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:25)

GCCACTAACTTCAGCCTGTTGAAGCAGGCCGGCGACGTTGAGGAAAACCCCGGTCCT

FIGURE 6 Cont.

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCC
G

CD8A Signal peptide (SEQ ID NO:26)

AGCCAGTTCCGGGTGTCGCCGCTGGATCGGACCTGGAACCTGGGCGAGACAGTGGAGCTG
AAGTGCCAGGTGCTGCTGTCCAACCCGACGTCGGGCTGCTCGTGGCTCTTCCAGCCGCGCG
GCGCCGCCGCCAGTCCCACCTTCCTCCTATACCTCTCCCAAAACAAGCCCAAGGCGGCCGAG
GGGCTGGACACCCAGCGGTTCTCGGGCAAGAGGTTGGGGGACACCTTCGTCCTCACCCTG
AGCGACTTCCGCCGAGAGAACGAGGGCTACTATTTCTGCTCGGCCCTGAGCAACTCCATCAT
GTACTTCAGCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGC
GACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTG
CCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGAT

CD8A Extracellular domain (SEQ ID NO:27)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACC

CD8A transmembrane domain (SEQ ID NO:28)

CTTTACTGCAACCACAGGAACCGAAGACGTGTTTGCAAATGTCCCCGGCCTGTGGTCAAATC
GGGAGACAAGCCCAGCCTTTCGGCGAGATACGTC

CD8A Intracellular domain (SEQ ID NO:29)

AGGGCTAAACGG

Furin cleavage site (SEQ ID NO:30)

GAATTCGGCTCCGGA

GSG Linker (SEQ ID NO:31)

GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT

P2A (SEQ ID NO:32)

ATGGCCACCGGCTCTAGAACAAGCCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTC
CAAGAAGGATCTGCT

HGH/SS/2 (SEQ ID NO:33)

FIGURE 6 Cont.

[[TRB_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRB_VDJ sequence

CTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCC
ACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACTG
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGA
AAGAACAGCCCGCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGC
CACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCG
AGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCTGCCGAAG
CCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGC
CACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCT
GGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCC

TCR-beta/constant (SEQ ID NO:35)

CGGGCCAAGCGG

Furin cleavage site (SEQ ID NO:36)

GGCAGCGGC

GSG linker (SEQ ID NO:37)

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT

P2A (SEQ ID NO:38)

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCT
GCAAGAGGGTTCCGCC

HGH/SS (SEQ ID NO:39)

[[TRA_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRA_VDJ sequence

ATATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACACAAGAGC
GTGTGTTTGTTC

TCR-alpha/constant (SEQ ID NO:41)

FIGURE 6 Cont.

TRAC CDS/right HR arm (SEQ ID NO:42)

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC
AAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCA
ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGG

Right HR arm (SEQ ID NO:43)

TAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC
ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC
GGGAAAAAAGCAGATGAAGAAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCT
CTCCAACTGAGTTCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGG
CCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT
CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGG
CACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA
GAGGAAGCACCATTCTAGTTGGGGGAGCCCCATCTGTCAGCTGGGAAAAAGTCCAAATAACTT
CAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAAACAGCTACCTTCAGGACAAAAGTCAG
GGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAG
GACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
GTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG

(SEQ ID NO:44)

GCTAGC

FIGURE 6 Cont.

pBR322_origin (SEQ ID NO:45)

CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG
CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGAT
GGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC
CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGA
GAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCCAGACGAAA
TACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTG
ATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCA
TTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAG
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAG
CATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCAT

AACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATC
TTGTGCAATGTAACATCAGAGATTTTGAGACAC

Kanamycin resistance (KanR2) (SEQ ID NO:46)

Kanamycin promoter (SEQ ID NO:47)

FIGURE 7

CD8 Product 2 Product Nucleic Acid Sequence

Left HR Arm (SEQ ID NO:49)

ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGC
CTCCAGCAGCTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTA
GCACTCTATCACGGCCATATTCTGGCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTT
TACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTCTTTCTCAGAAGAGCCTGGCT
AGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGGAGCT
GCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTG
TTCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTT
CCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGA
GACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTTCCCATGCCTGCCTTTA
CTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTTAATAAAAGAATAAGCAGT
ATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAACG
TTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTC
CATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCC
AGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCA
AAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAG

TRAC CDS (SEQ ID NO:50)

ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG
TCTGCCTATTC

GSG Linker 1 (SEQ ID NO:51)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:52)

GCCACTAACTTCAGCCTGTTGAAGCAGGCCGGCGACGTTGAGGAAAACCCCGGTCCT

FIGURE 7 Cont.

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCG	**CD8A Signal peptide (SEQ ID NO:53)**
AGCCAGTTCCGGGTGTCGCCGCTGGATCGGACCTGGAACCTGGGCGAGACAGTGGAGCTG	
AAGTGCCAGGTGCTGCTGTCCAACCCGACGTCGGGCTGCTCGTGGCTCTTCCAGCCGCGCG	
GCGCCGCCGCCAGTCCCACCTTCCTCCTATACCTCTCCCAAAACAAGCCCAAGGCGGCCGAG	**CD8A Extracellular domain (SEQ ID NO:54)**
GGGCTGGACACCCAGCGGTTCTCGGGCAAGAGGTTGGGGGACACCTTCGTCCTCACCCTG	
AGCGACTTCCGCCGAGAGAACGAGGGCTACTATTTCTGCTCGGCCCTGAGCAACTCCATCAT	
GTACTTCAGCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGC	
GACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTG	
CCGGCCAGCGGCGGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGAT	
ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACC	**CD8A transmembrane domain (SEQ ID NO:55)**
CTTTACTGCAACCACAGGAACCGAAGACGTGTTTGCAAATGTCCCCGGCCTGTGGTCAAATC	**CD8A Intracellular domain (SEQ ID NO:56)**
GGGAGACAAGCCCAGCCTTTCGGCGAGATACGTC	
AGAGCAAAGCGG	**Furin cleavage site (SEQ ID NO:57)**
GGCTCCGGA	**GSG Linker (SEQ ID NO:58)**
GCTACCAATTTTAGCCTCCTGAAGCAGGCTGGCGATGTTGAGGAAAACCCTGGTCCC	**P2A (SEQ ID NO:59)**
ATGCGGCCGCGGCTGTGGCTCCTCTTGGCCGCGCAGCTGACAGTTCTCCATGGCAACTCAGT C	**CD8B Signal Peptide (SEQ ID NO:60)**

FIGURE 7 Cont.

CTCCAGCAGACCCCTGCATACATAAAGGTGCAAACCAACAAGATGGTGATGCTGTCCTGCGA
GGCTAAAATCTCCCTCAGTAACATGCGCATCTACTGGCTGAGACAGCGCCAGGCACCGAGCA
GTGACAGTCACCACGAGTTCCTGGCCCTCTGGGATTCCGCAAAAGGGACTATCCACGGTGA
AGAGGTGGAACAGGAGAAGATAGCTGTGTTTCGGGATGCAAGCCGGTTCATTCTCAATCTC
ACAAGCGTGAAGCCGGAAGACAGTGGCATCTACTTCTGCATGATCGTCGGGAGCCCCGAGC
TGACCTTCGGGAAGGGAACTCAGCTGAGTGTGGTTGATTTCCTTCCCACCACTGCCCAGCCC
ACCAAGAAGTCCACCCTCAAGAAGAGAGTGTGCCGGTTACCCAGGCCAGAGACCCAGAAG
GGCCCACTTTGTAGCCCC

CD8B Extracellular domain (SEQ ID NO:61)

ATCACCCTTGGCCTGCTGGTGGCTGGCGTCCTGGTTCTGCTGGTTTCCCTGGGAGTGGCCAT
C

CD8B Transmembrane domain (SEQ ID NO:62)

CACCTGTGCTGCCGGCGGAGGAGAGCCCGGCTTCGTTTCATGAAACAATTTTACAAA

CD8B Intracellular domain (SEQ ID NO:63)

AGGGCTAAACGG

Furin cleavage site (SEQ ID NO:64)

GAATTCGGCTCCGGA

GSG Linker (SEQ ID NO:65)

GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT

P2A (SEQ ID NO:66)

ATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTC
CAAGAAGGATCTGCT

HGH/SS/2 (SEQ ID NO:67)

FIGURE 7 Cont.

[[TRB_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRB_VDJ sequence

CTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCC
ACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACTG
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACACAGATCCCCAGCCTCTGA

TCR-beta/constant (SEQ ID NO:69)

AAGAACAGCCCCGCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGC
CACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCG
AGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCTGCCGAAG
CCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGC
CACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCT
GGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCC

Furin cleavage site (SEQ ID NO:70)

CGGGCCAAGCGG

GSG Linker (SEQ ID NO:71)

GGCAGCGGC

P2A (SEQ ID NO:72)

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT

HGH/SS/2 (SEQ ID NO:73)

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCT
GCAAGAGGGTTCCGCC

[[TRA_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRA_VDJ sequence

ATATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACAAGAGC
GTGTGTTTGTTC

TCR-alpha/constant (SEQ ID NO:75)

FIGURE 7 Cont.

TRAC CDS/right HR arm (SEQ ID NO:76)

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC
AAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCA
ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGG

TAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC
ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC
GGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCT
CTCCAACTGAGTTCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGG
CCTCATTCTAAGCCCCCTTCTCCAAGTTGCCTCTCCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT
CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGG
CACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA
GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTT
CAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAG
GGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAG
GACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
GTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG

GCTAGC

Right HR arm (SEQ ID NO:77)

(SEQ ID NO:78)

FIGURE 7 Cont.

pBR322_origin (SEQ ID NO:79)

CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG
CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGAT
GGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC
CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGA
GAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCCAGACGAAA
TACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTG
ATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCA
TTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAG
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAG
CATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCAT

AACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATC
TTGTGCAATGTAACATCAGAGATTTTGAGACAC

Kanamycin resistance (KanR2) (SEQ ID NO:80)

Kanamycin promoter (SEQ ID NO:81)

FIGURE 8

CD8 Product 3 Product Nucleic Acid Sequence

Left HR Arm (SEQ ID NO:83)

ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGC
CTCCAGCAGCTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTA
GCACTCTATCACGGCCATATTCTGGCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTT
TACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTCTTTCTCAGAAGAGCCTGGCT
AGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGGAGCT
GCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTG
TTCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTT
CCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGA
GACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTTCCCATGCCTGCCTTTA
CTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTTAATAAAAGAATAAGCAGT
ATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAACG
TTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTC
CATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCC
AGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCA
AAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAG

TRAC CDS (SEQ ID NO:84)

ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG
TCTGCCTATTC

GSG Linker 1 (SEQ ID NO:85)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:86)

GCCACTAACTTCAGCCTGTTGAAGCAGGCCGGCGACGTTGAGGAAAACCCCGGTCCT

FIGURE 8 Cont.

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCC
G

CD8A Signal peptide (SEQ ID NO:87)

AGCCAGTTCCGGGTGTCGCCGCTGGATCGGACCTGGAACCTGGGCGAGACAGTGGAGCTG
AAGTGCCAGGTGCTGCTGTCCAACCCGACGTCGGGCTGCTCGTGGCTCTTCCAGCCGCGCG
GCGCCGCCGCCAGTCCCACCTTCCTCCTATACCTCTCCCAAAACAAGCCCAAGGCGGCCGAG
GGGCTGGACACCCAGCGGTTCTCGGGCAAGAGGTTGGGGGACACCTTCGTCCTCACCCTG
AGCGACTTCCGCCGAGAGAACGAGGGCTACTATTTCTGCTCGGCCCTGAGCAACTCCATCAT
GTACTTCAGCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGC
GACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCCTGTCCCTGCGCCCAGAGGCGTG
CCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGAT

CD8A Extracellular domain (SEQ ID NO:88)

CD8A transmembrane domain (SEQ ID NO:89)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACC

CD8B Intracellular domain (SEQ ID NO:90)

CACCTGTGCTGCCGGCGGAGGAGAGCCCGGCTTCGTTTCATGAAACAATTTTACAAA

Furin cleavage site (SEQ ID NO:91)

AGGGCTAAACGG

GSG Linker (SEQ ID NO:92)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:93)

GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT

HGH/SS/2 (SEQ ID NO:94)

ATGGCCACCGGCTCTAGAACAAGCCTGCTCGCTTTTTGGCCTGCTCTGCCTCCCATGGCTC
CAAGAAGGATCTGCT

FIGURE 8 Cont.

[[TRB_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRB_VDJ sequence

CTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCC
ACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACTG
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGA
AAGAACAGCCCGCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGC
CACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCG
AGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCTGCCGAAG
CCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGC
CACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCT
GGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCC

TCR-beta/constant (SEQ ID NO:96)

CGGGCCAAGCGG

Furin cleavage site (SEQ ID NO:97)

GGCAGCGGC

GSG Linker (SEQ ID NO:98)

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT

P2A (SEQ ID NO:99)

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCT
GCAAGAGGGTTCCGCC

HGH/SS/2 (SEQ ID NO:100)

[[TRA_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRA_VDJ sequence

ATATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACAAGAGC
GTGTGTTTGTTC

TCR-alpha/constant (SEQ ID NO:102)

FIGURE 8 Cont.

TRAC CDS/right HR arm (SEQ ID NO:103)

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC
AAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCA
ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGG

Right HR arm (SEQ ID NO:104)

TAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC
ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC
GGGAAAAAAGCAGATGAAGAAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCT
CTCCAACTGAGTTCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGG
CCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT
CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGG
CACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA
GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTT
CAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAG
GGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAG
GACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
GTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG

(SEQ ID NO:105)

GCTAGC

FIGURE 8 Cont.

pBR322_origin (SEQ ID NO:106)

CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG
CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGAT
GGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC
CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGA
GAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCCAGACGAAA
TACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTG
ATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCA
TTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAG
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAG
CATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCAT

AACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATC
TTGTGCAATGTAACATCAGAGATTTTGAGACAC

Kanamycin resistance (KanR2) (SEQ ID NO:107)

Kanamycin promoter (SEQ ID NO:108)

FIGURE 9

CD8 Product 4 Product Nucleic Acid Sequence

Left HR Arm (SEQ ID NO:110)

ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGC
CTCCAGCAGCTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTA
GCACTCTATCACGGCCATATTCTGGCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTT
TACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTCTTTCTCAGAAGAGCCTGGCT
AGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGGAGCT
GCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTG
TTCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTT
CCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGA
GACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTA
CTCTGCCAGAGTTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGT
ATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAACG
TTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTC
CATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCC
AGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCA
AAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAG

TRAC CDS (SEQ ID NO:111)

ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG
TCTGCCTATTC

GSG Linker 1 (SEQ ID NO:112)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:113)

GCCACTAACTTCAGCCTGTTGAAGCAGGCCGGCGACGTTGAGGAAAACCCCGGTCCT

FIGURE 9 Cont.

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCC
G

CD8A Signal peptide (SEQ ID NO:114)

AGCCAGTTCCGGGTGTCGCCGCTGGATCGGACCTGGAACCTGGGCGAGACAGTGGAGCTG
AAGTGCCAGGTGCTGCTGTCCAACCCGACGTCGGGCTGCTCGTGGCTCTTCCAGCCGCGCG
GCGCCGCCGCCAGTCCCACCTTCCTCCTATACCTCTCCCAAAACAAGCCCAAGGCGGCCGAG
GGGCTGGACACCCAGCGGTTCTCGGGCAAGAGGTTGGGGGACACCTTCGTCCTCACCCTG
AGCGACTTCCGCCGAGAGAACGAGGGCTACTATTTCTGCTCGGCCCTGAGCAACTCCATCAT
GTACTTCAGCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGC
GACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTG
CCGGCCAGCGGCGGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGAT

CD8A Extracellular domain (SEQ ID NO:115)

CD8A transmembrane domain (SEQ ID NO:116)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACC

CD4 Intracellular domain (SEQ ID NO:117)

TGTGTCAGGTGCCGGCACCGAAGGCGCCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCC
TCAGTGAGAAGAAGACCTGCCAGTGTCCTCACCGGTTTCAGAAGACATGTAGCCCCATT

Furin cleavage site (SEQ ID NO:118)

AGGGCTAAACGG

GSG Linker (SEQ ID NO:119)

GAATTCGGCTCCGGA

P2A (SEQ ID NO:120)

GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT

HGH/SS/2 (SEQ ID NO:121)

ATGGCCACCGGCTCTAGAACAAGCCTGCTCGCTTTTTGGCCTGCTCTGCCTCCCATGGCTC
CAAGAAGGATCTGCT

FIGURE 9 Cont.

[[TRB_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRB_VDJ sequence

CTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTCGAGCCTTCTGAGGCCGAGATCAGCC
ACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACTG
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGA
AAGAACAGCCCGCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGC
CACCTTCTGGCAGAACCCCAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCG
AGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCTGCCGAAG
CCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGC
CACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCT
GGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCC

TCR-beta/constant (SEQ ID NO:123)

CGGGCCAAGCGG

Furin cleavage site (SEQ ID NO:124)

GGCAGCGGC

GSG Linker (SEQ ID NO:125)

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT

P2A (SEQ ID NO:126)

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCT
GCAAGAGGGTTCCGCC

HGH/SS/2 (SEQ ID NO:127)

[[TRA_VDJ Sequence, specific for the NeoTCR]]

Insertion site for neoTCR TRA_VDJ sequence

ATATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACAAGAGC
GTGTGTTTGTTC

TCR-alpha/constant (SEQ ID NO:129)

FIGURE 9 Cont.

TRAC CDS/right HR arm (SEQ ID NO:130)

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC
AAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCA
ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGG

Right HR arm (SEQ ID NO:131)

TAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC
ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC
GGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCT
CTCCAACTGAGTTCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGG
CCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT
CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGG
CACATGAATGCACCAGGTGTTGAAGTGGAGGAATTATAAAAAGTCAGATGAGGGGTGTGCCCA
GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTT
CAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAG
GGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAG
GACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
GTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG

(SEQ ID NO:132)

GCTAGC

FIGURE 9 Cont.

pBR322_origin (SEQ ID NO:133)

CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG
CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGAT
GGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC
CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGA
GAATGGCAAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCCAGACGAAA
TACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTG
ATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCA
TTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAG
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAG
CATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCAT

Kanamycin resistance (KanR2) (SEQ ID NO:134)

AACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATC
TTGTGCAATGTAACATCAGAGATTTTGAGACAC

Kanamycin promoter (SEQ ID NO:135)

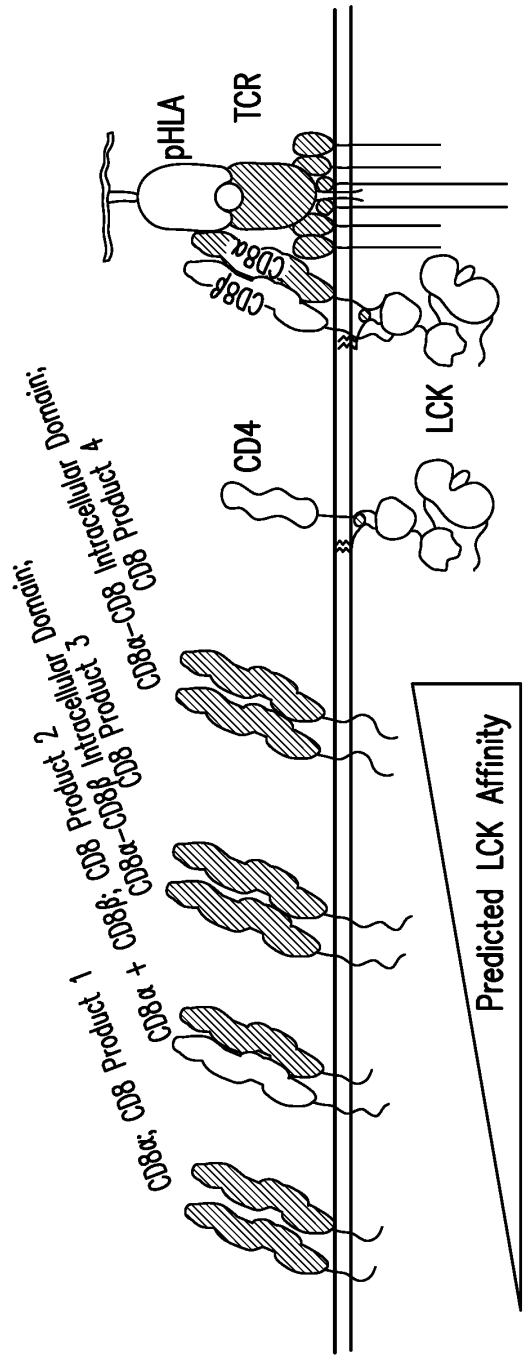

pHLA
TCR
CD8α
CD8β
CD4
LCK
CD8α; CD8 Product 1
CD8α + CD8β; CD8 Product 2
CD8α–CD8β Intracellular Domain; CD8 Product 3
CD8α–CD8 Intracellular Domain; CD8 Product 4
Predicted LCK Affinity

FIGURE 10

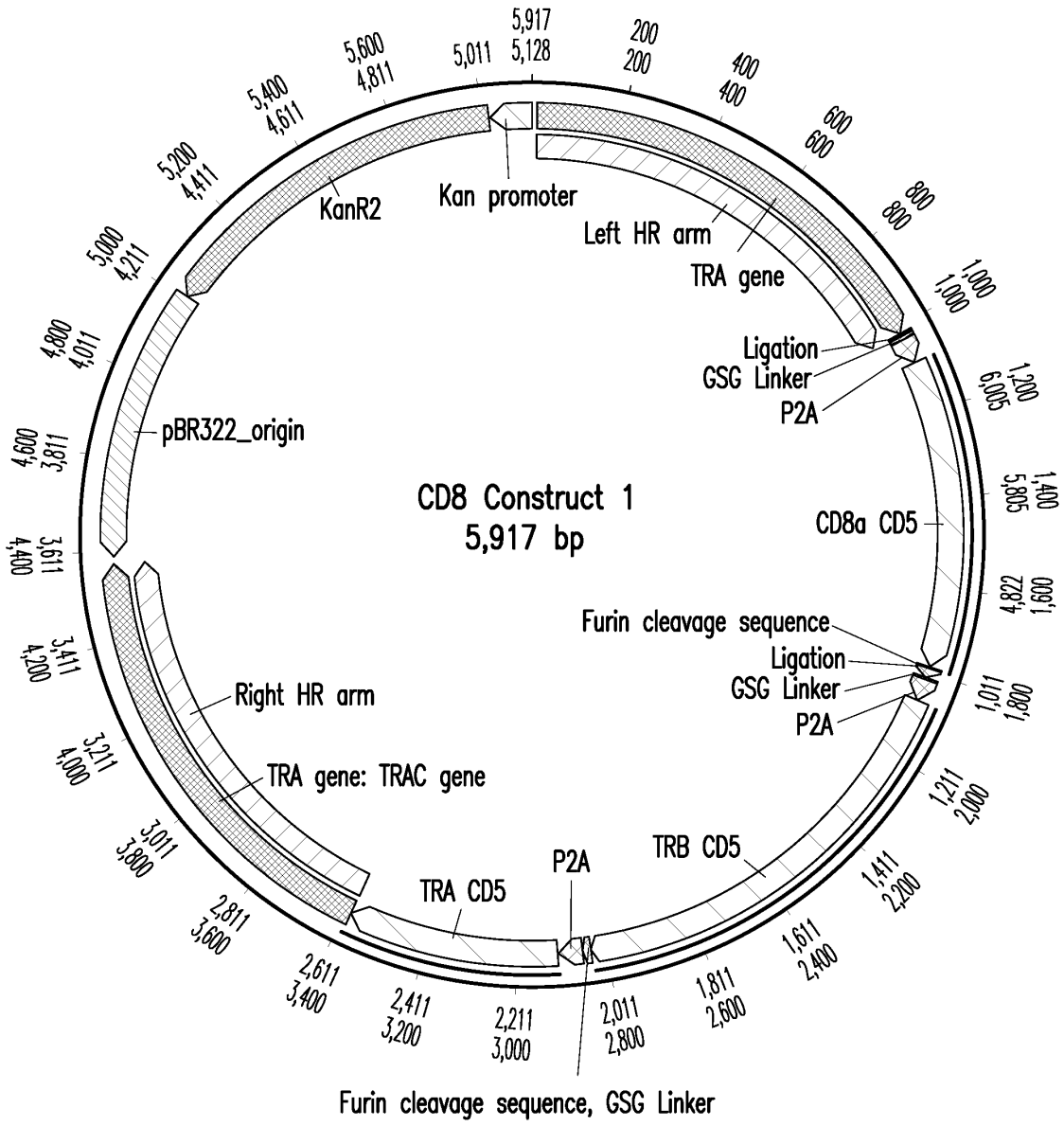

CD8 Construct 1
5,917 bp
Kan promoter
KanR2
Left HR arm
TRA gene
Ligation
GSG Linker
P2A
CD8a CD5
Furin cleavage sequence
Ligation
GSG Linker
P2A
TRB CD5
P2A
TRA CD5
TRA gene: TRAC gene
Right HR arm
pBR322_origin
Furin cleavage sequence, GSG Linker

FIGURE 11A

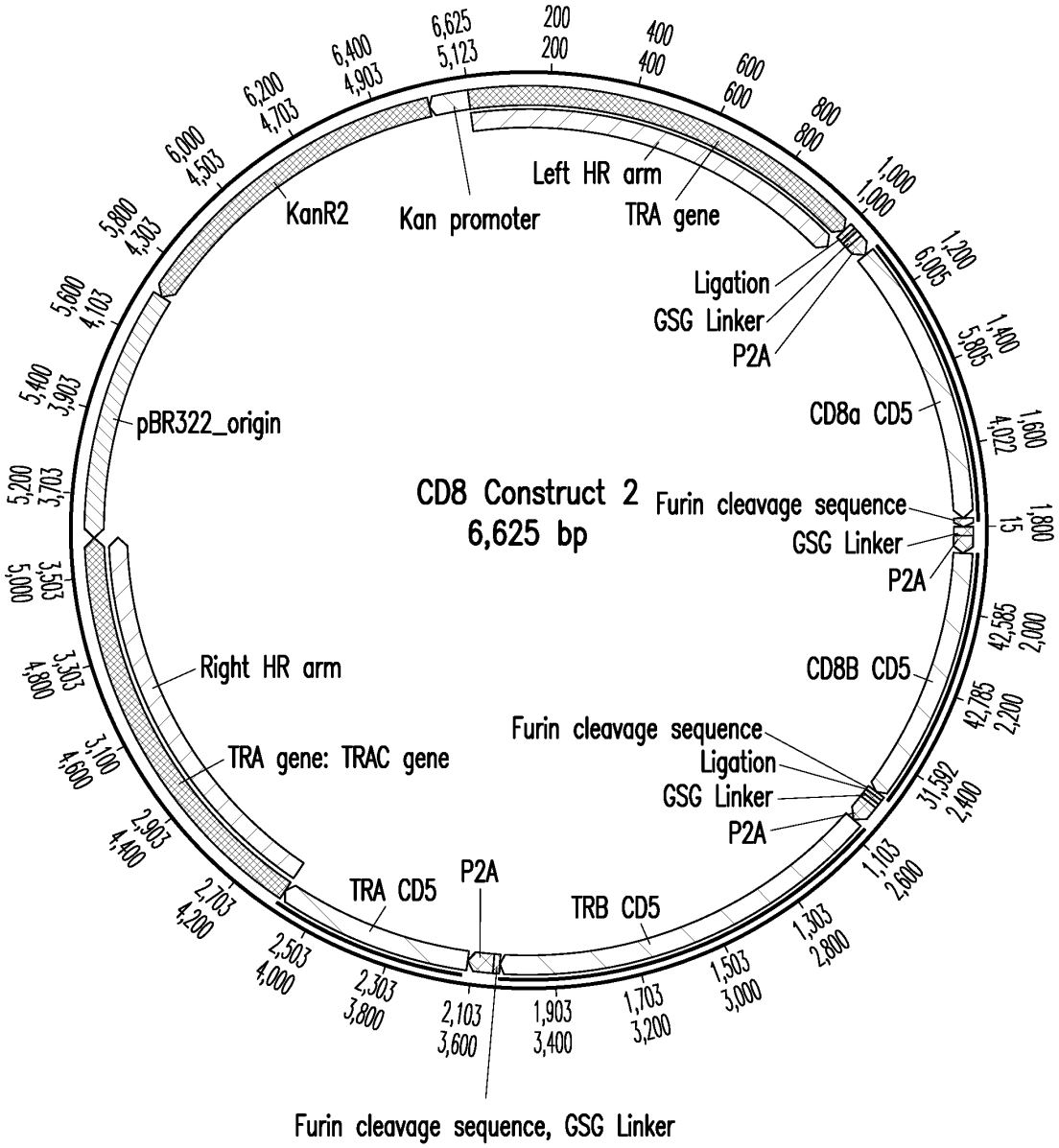

CD8 Construct 2
6,625 bp
Left HR arm
TRA gene
Ligation
GSG Linker
P2A
CD8a CD5
Furin cleavage sequence
GSG Linker
P2A
CD8B CD5
Furin cleavage sequence
Ligation
GSG Linker
P2A
TRB CD5
P2A
TRA CD5
Right HR arm
TRA gene: TRAC gene
pBR322_origin
KanR2
Kan promoter
Furin cleavage sequence, GSG Linker

FIGURE 11B

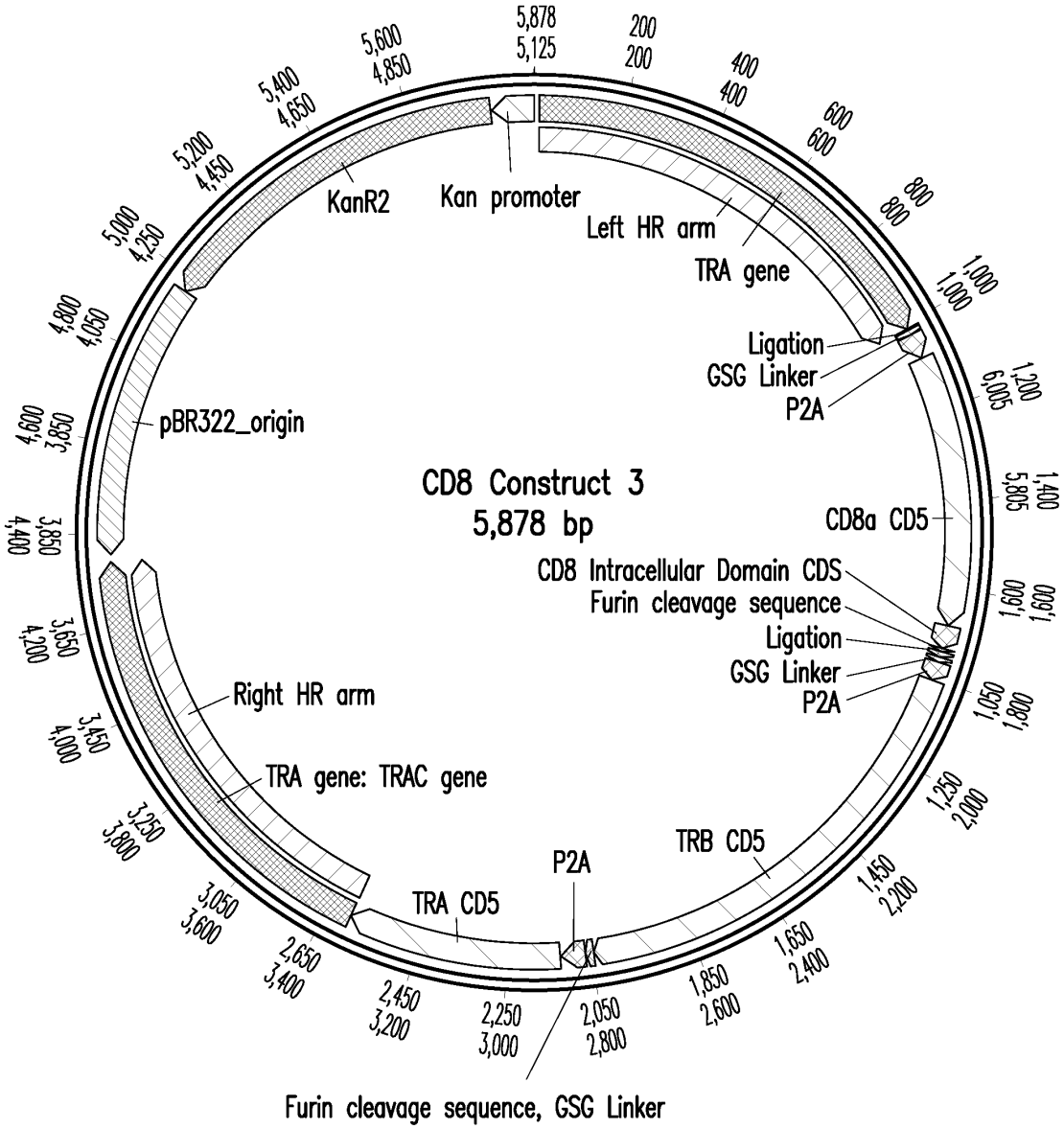

CD8 Construct 3
5,878 bp
KanR2
Kan promoter
Left HR arm
TRA gene
Ligation
GSG Linker
P2A
CD8a CD5
CD8 Intracellular Domain CDS
Furin cleavage sequence
Ligation
GSG Linker
P2A
TRB CD5
P2A
TRA CD5
TRA gene: TRAC gene
Right HR arm
pBR322_origin
Furin cleavage sequence, GSG Linker

FIGURE 11C

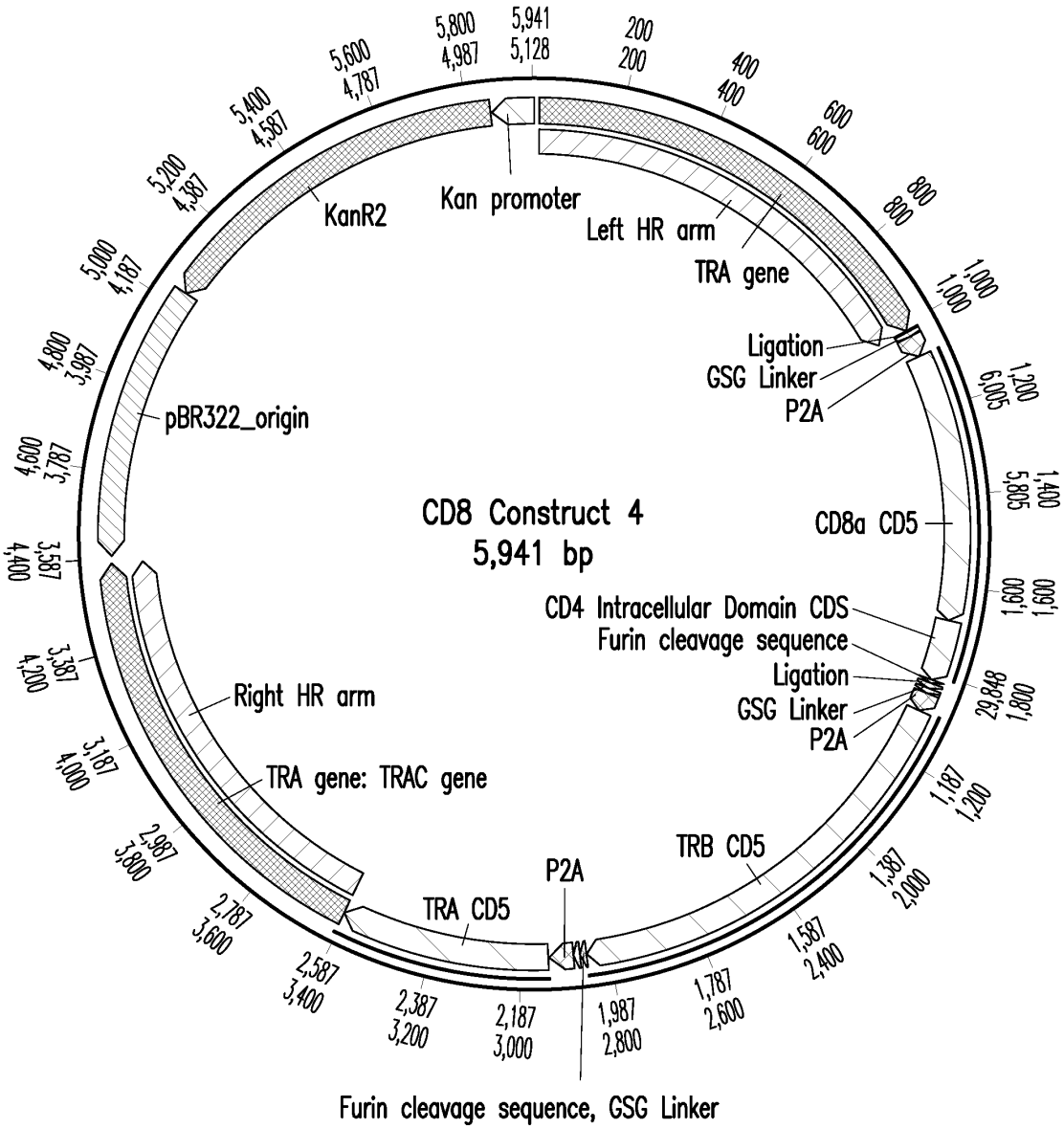

CD8 Construct 4
5,941 bp
Kan promoter
KanR2
Left HR arm
TRA gene
Ligation
GSG Linker
P2A
pBR322_origin
CD8a CD5
CD4 Intracellular Domain CDS
Furin cleavage sequence
Ligation
GSG Linker
P2A
Right HR arm
TRA gene: TRAC gene
TRB CD5
P2A
TRA CD5
Furin cleavage sequence, GSG Linker

FIGURE 11D

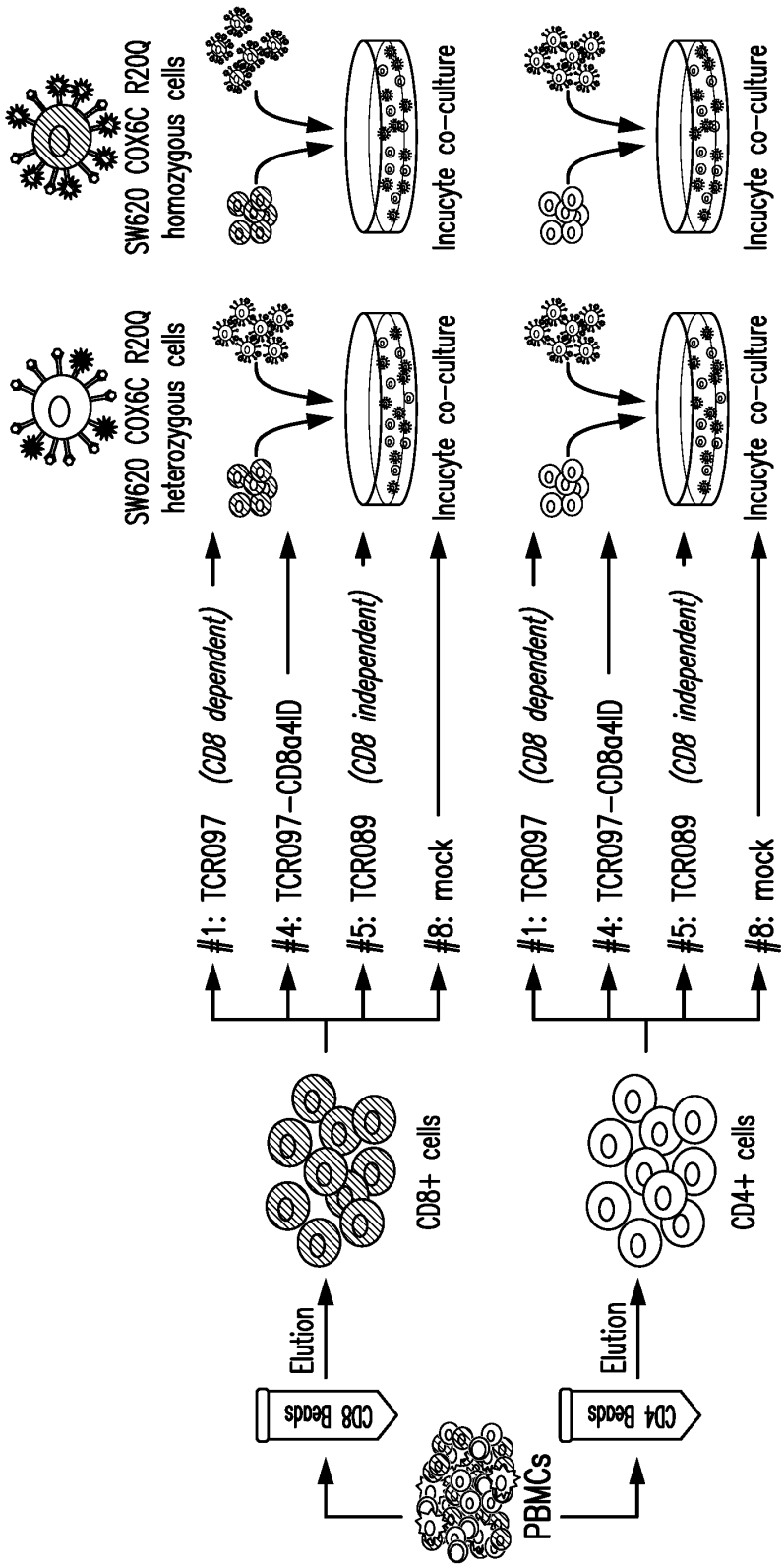

PBMCs
CD8 Beads
CD4 Beads
Elution
Elution
CD8+ cells
CD4+ cells
#1: TCR097 (CD8 dependent)
#4: TCR097-CD8a4ID
#5: TCR089 (CD8 independent)
#8: mock
#1: TCR097 (CD8 dependent)
#4: TCR097-CD8a4ID
#5: TCR089 (CD8 independent)
#8: mock
SW620 COX6C R20Q heterozygous cells
SW620 COX6C R20Q homozygous cells
Incucyte co-culture
Incucyte co-culture
Incucyte co-culture
Incucyte co-culture

FIGURE 12

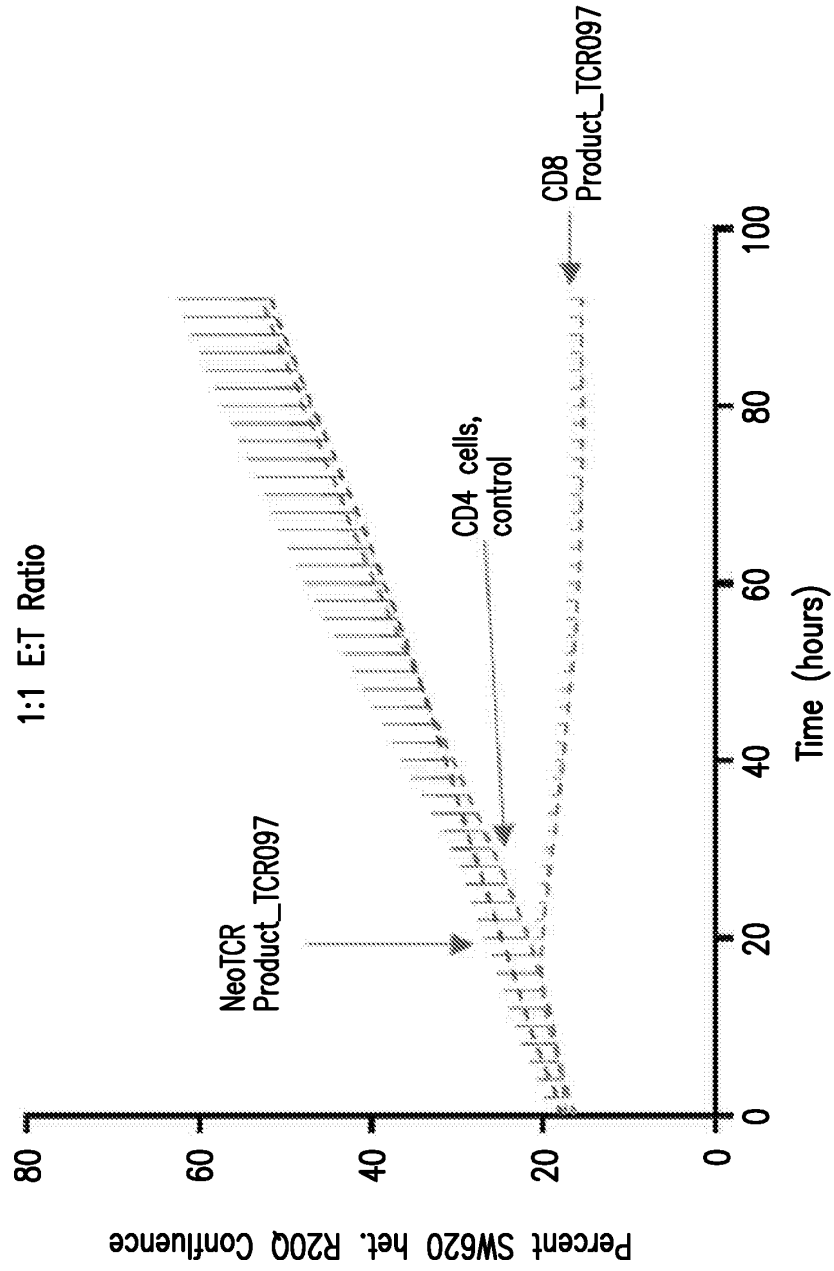

Percent SW620 het. R20Q Confluence
80
60
40
20
0
0
20
40
60
80
100
Time (hours)
1:1 E:T Ratio
NeoTCR Product_TCR097
CD4 cells, control
CD8 Product_TCR097

FIGURE 13A

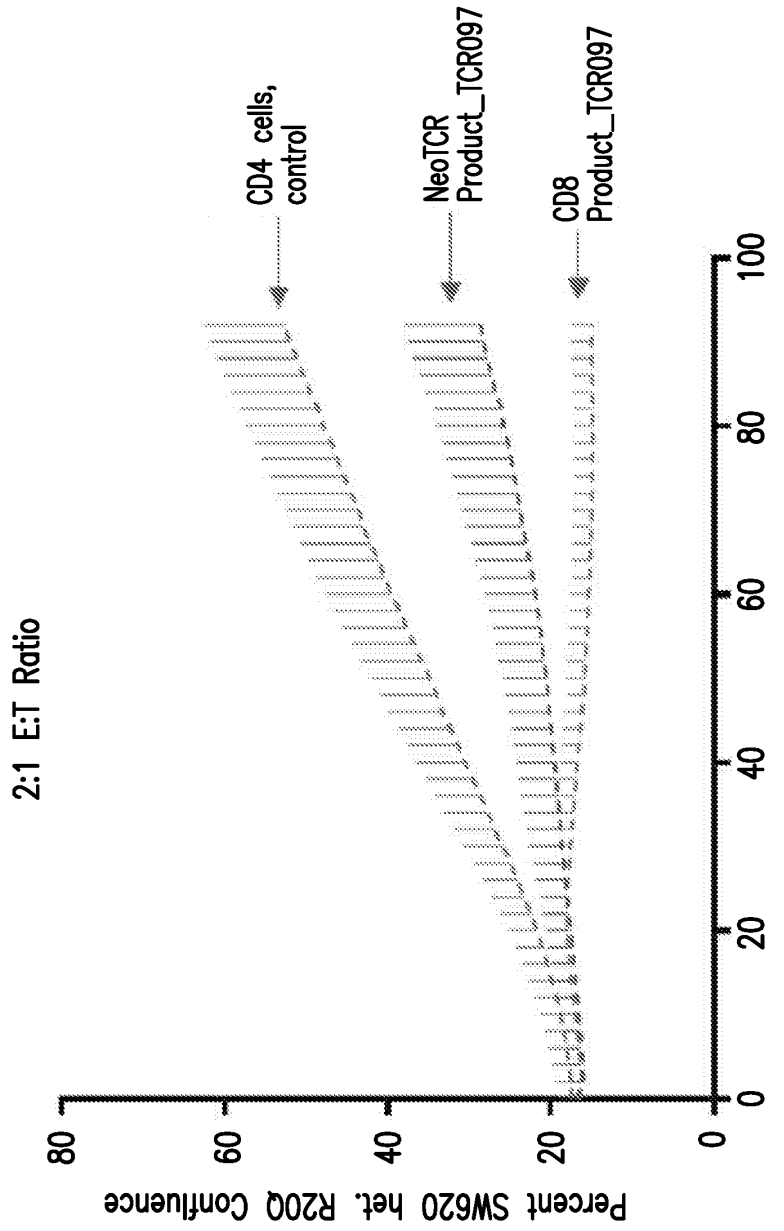

CD4 cells, control
NeoTCR Product_TCR097
CD8 Product_TCR097
2:1 E:T Ratio
Percent SW620 het. R200Q Confluence
0
20
40
60
80
0
20
40
60
80
100
Time (hours)

FIGURE 13B

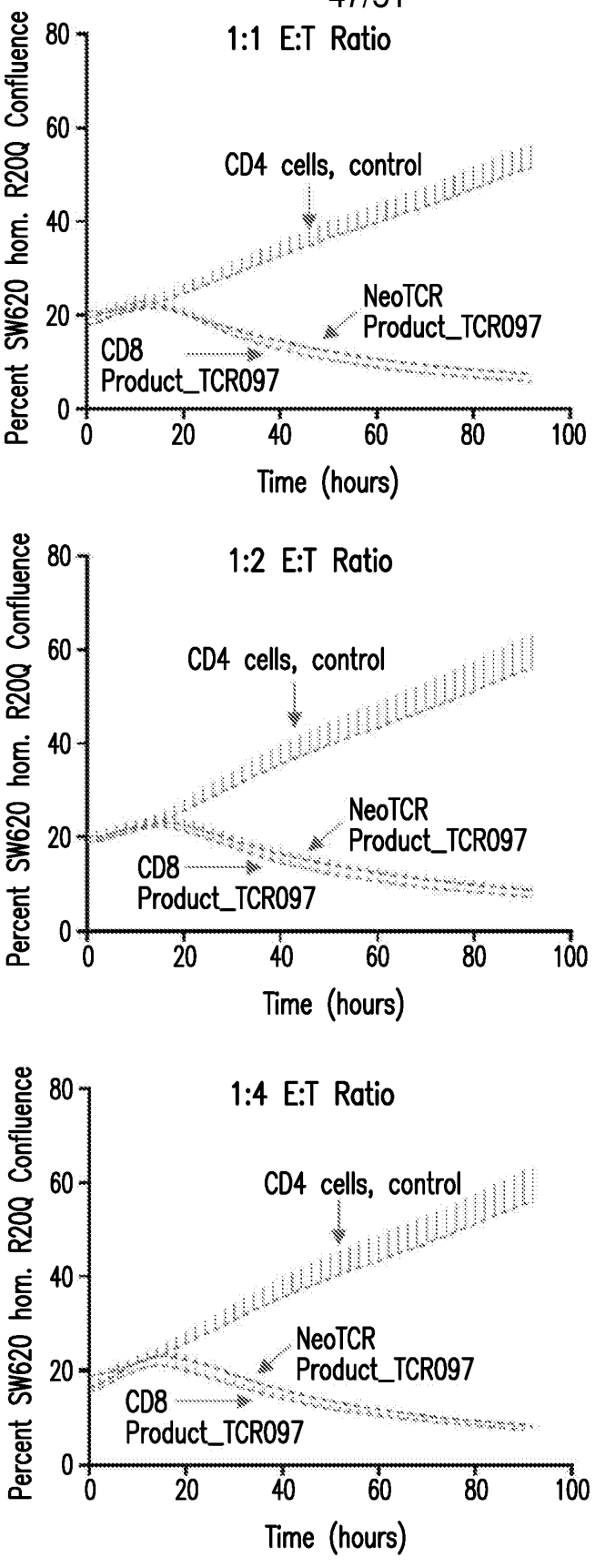
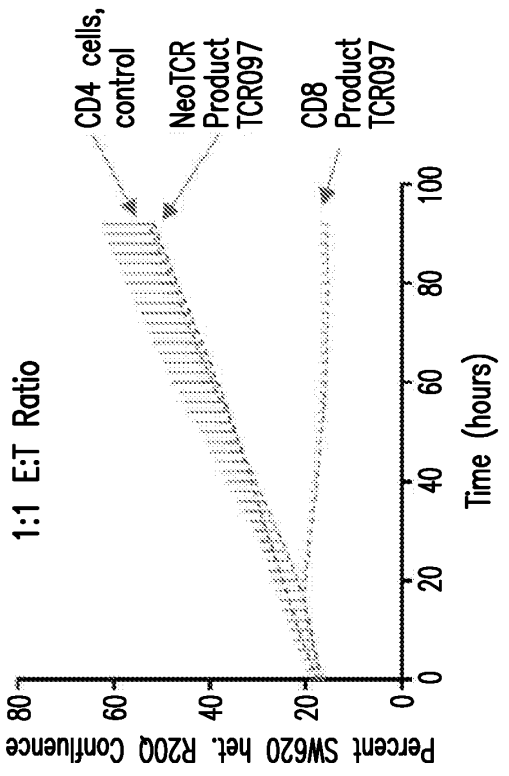

1:1 E:T Ratio
Percent SW620 hom. R20Q Confluence
CD4 cells, control
NeoTCR
Product_TCR097
CD8
Product_TCR097
Time (hours)
1:2 E:T Ratio
Percent SW620 hom. R20Q Confluence
CD4 cells, control
NeoTCR
Product_TCR097
CD8
Product_TCR097
Time (hours)
1:4 E:T Ratio
Percent SW620 hom. R20Q Confluence
CD4 cells, control
NeoTCR
Product_TCR097
CD8
Product_TCR097
Time (hours)

FIGURE 14



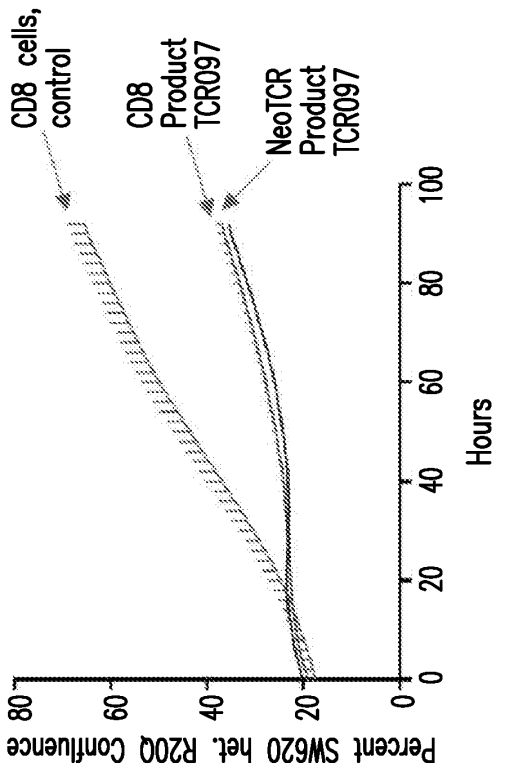
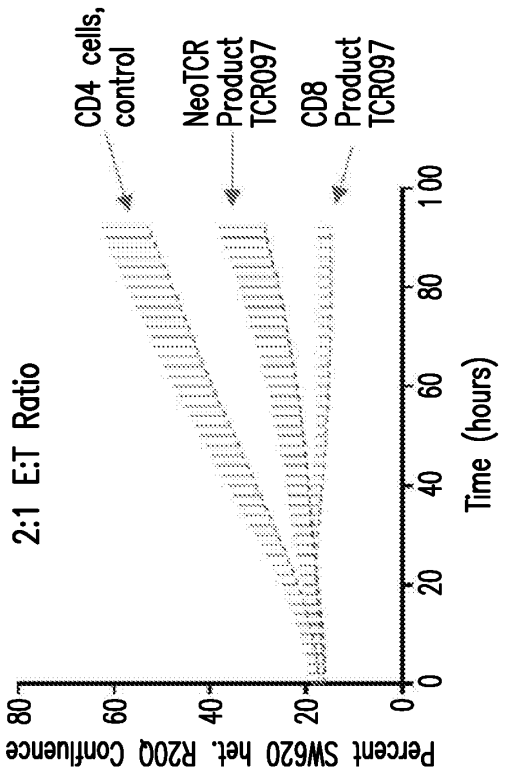


FIGURE 15B



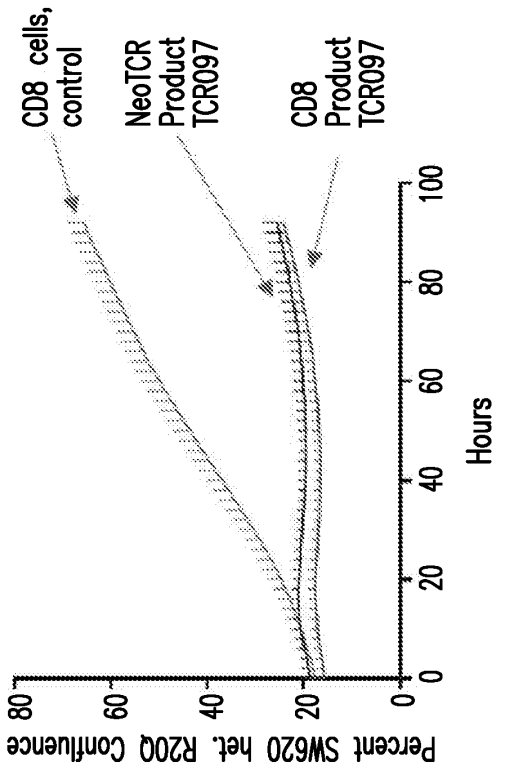


FIGURE 15A

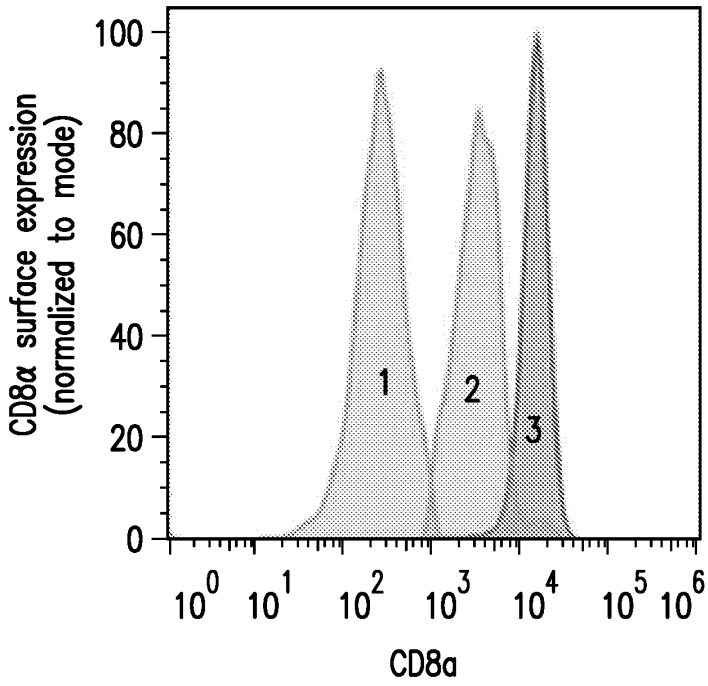

CD8α surface expression
(normalized to mode)
100
80
60
40
20
0
1
2
3
10^0 10^1 10^2 10^3 10^4 10^5 10^6
CD8α

FIGURE 16

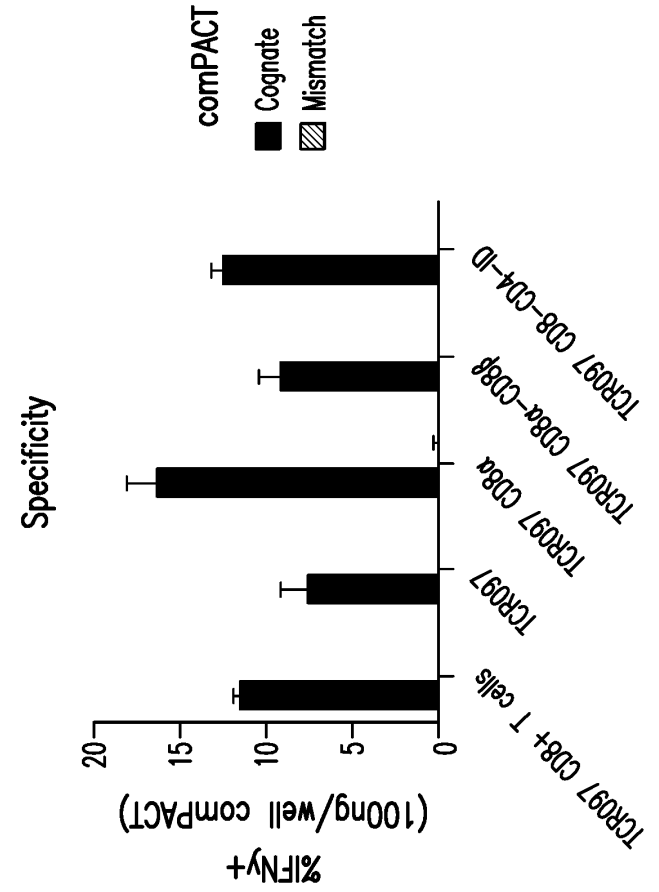


FIGURE 17B

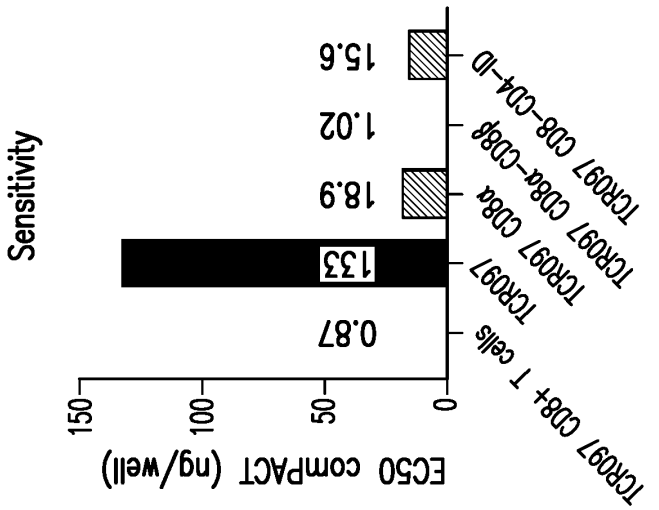


FIGURE 17A

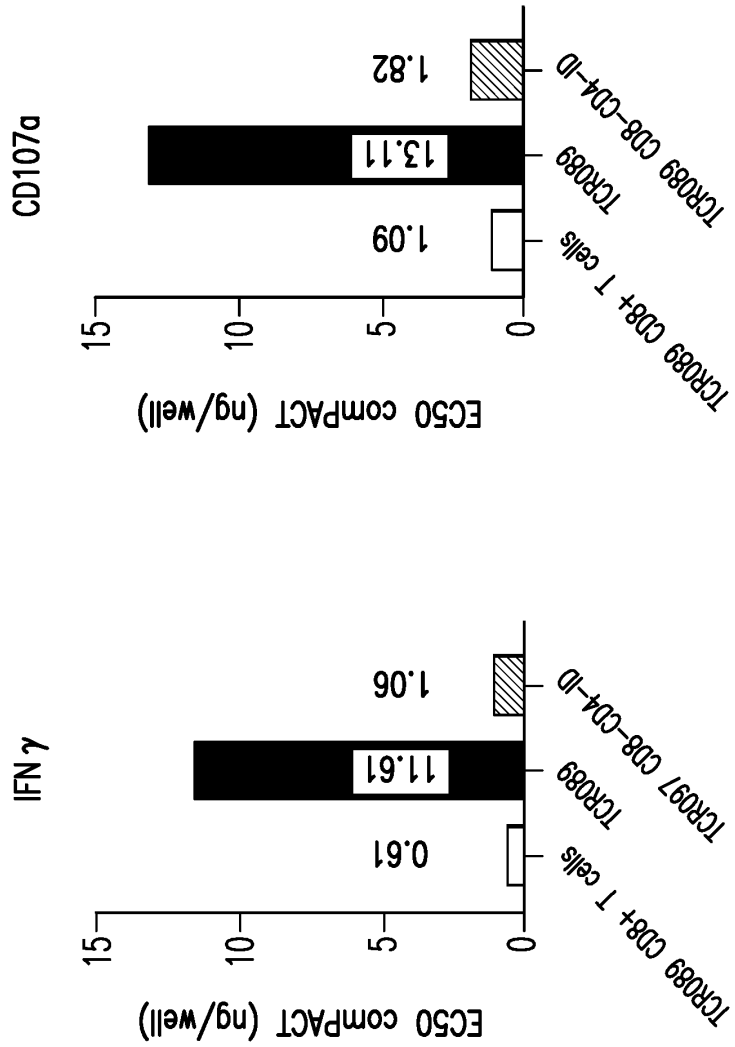

CD107a
EC50 comPACT (ng/well)
15
10
5
0
1.09
13.11
1.82
TCR089 CD8+ T cells
TCR089
TCR089 CD8-CD4-ID
IFN γ
EC50 comPACT (ng/well)
15
10
5
0
0.61
11.61
1.06
TCR089 CD8+ T cells
TCR089
TCR097 CD8-CD4-ID

FIGURE 18