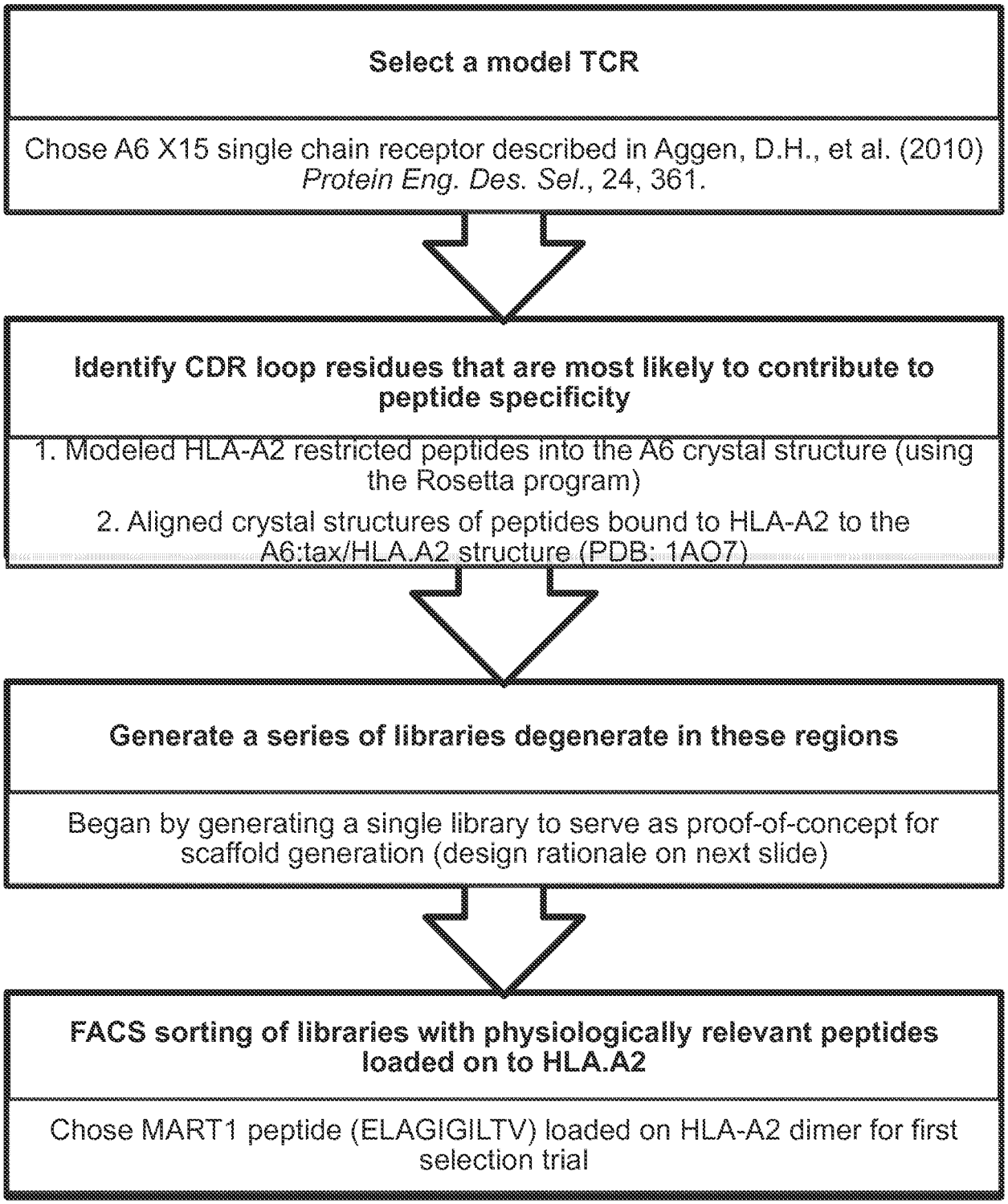

Select a model TCR
Chose A6 X15 single chain receptor described in Aggen, D.H., et al. (2010) *Protein Eng. Des. Sel.*, 24, 361.
Identify CDR loop residues that are most likely to contribute to peptide specificity
1. Modeled HLA-A2 restricted peptides into the A6 crystal structure (using the Rosetta program)
2. Aligned crystal structures of peptides bound to HLA-A2 to the A6:tax/HLA.A2 structure (PDB: 1AO7)
Generate a series of libraries degenerate in these regions
Began by generating a single library to serve as proof-of-concept for scaffold generation (design rationale on next slide)
FACS sorting of libraries with physiologically relevant peptides loaded on to HLA.A2
Chose MART1 peptide (ELAGIGILTV) loaded on HLA-A2 dimer for first selection trial

FIG. 1

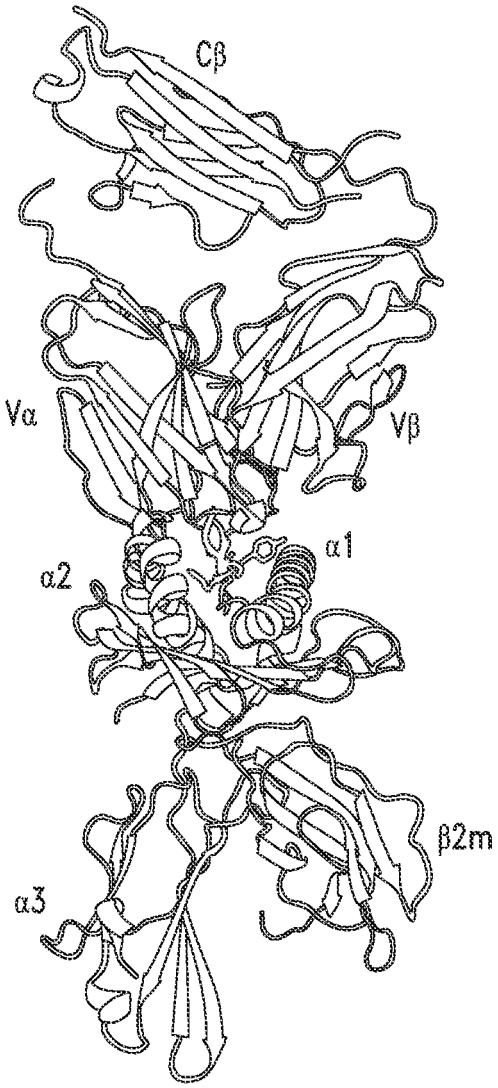


FIG. 2A

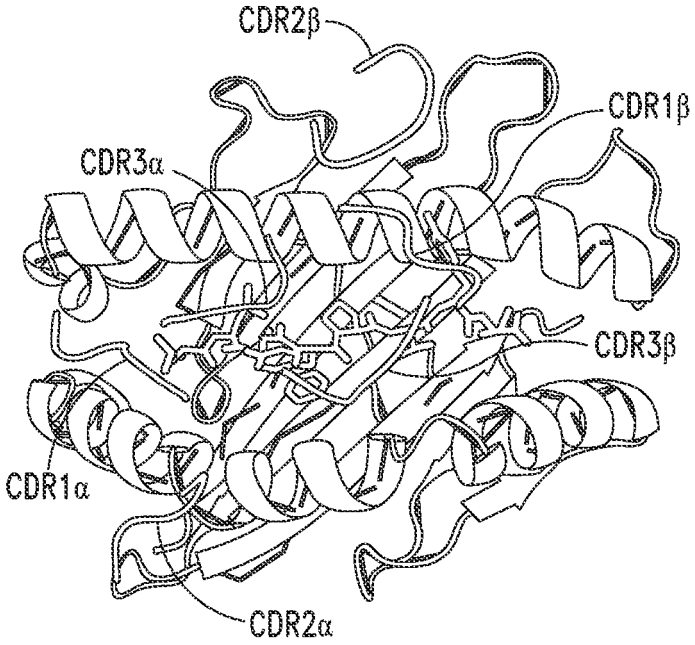


FIG. 2B

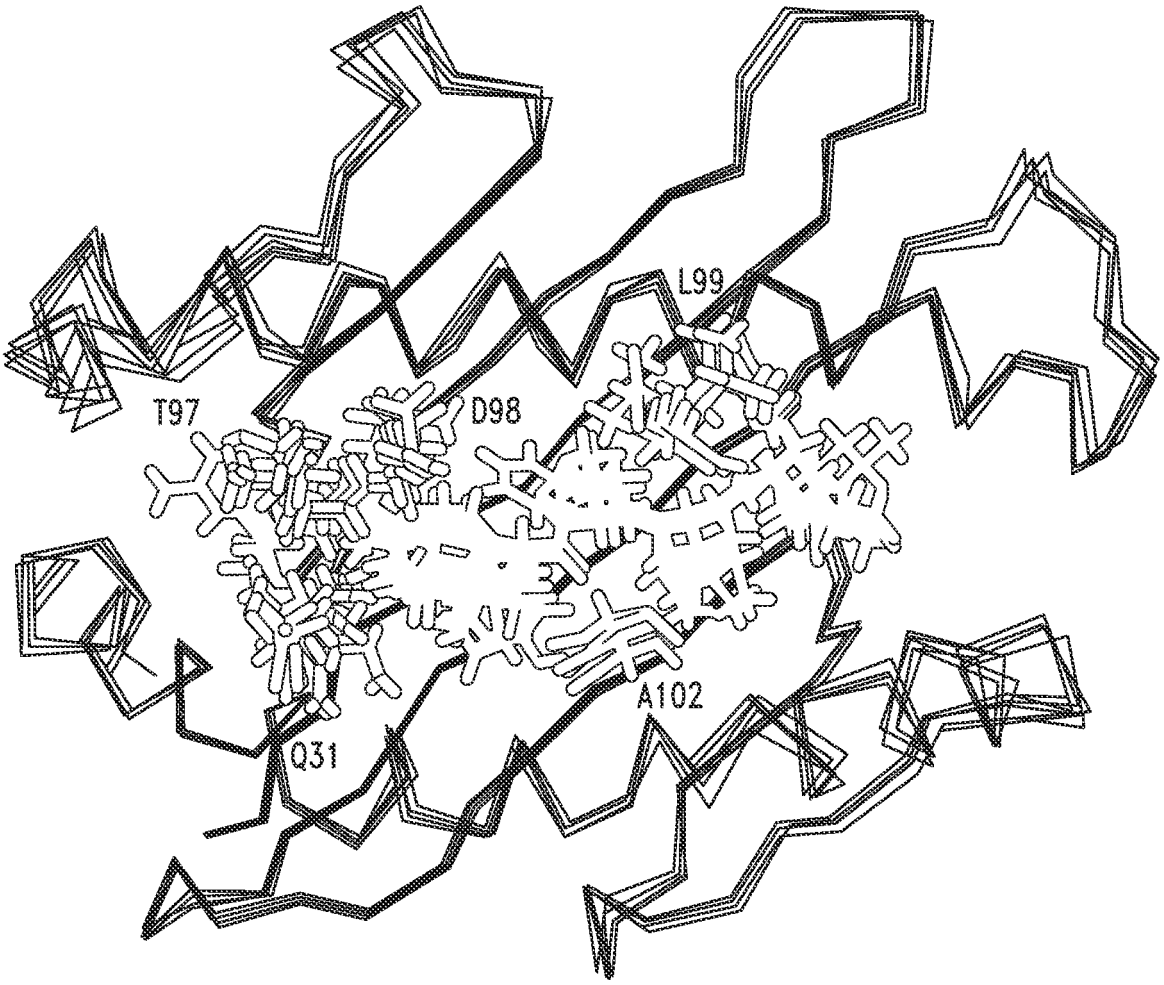


FIG. 3

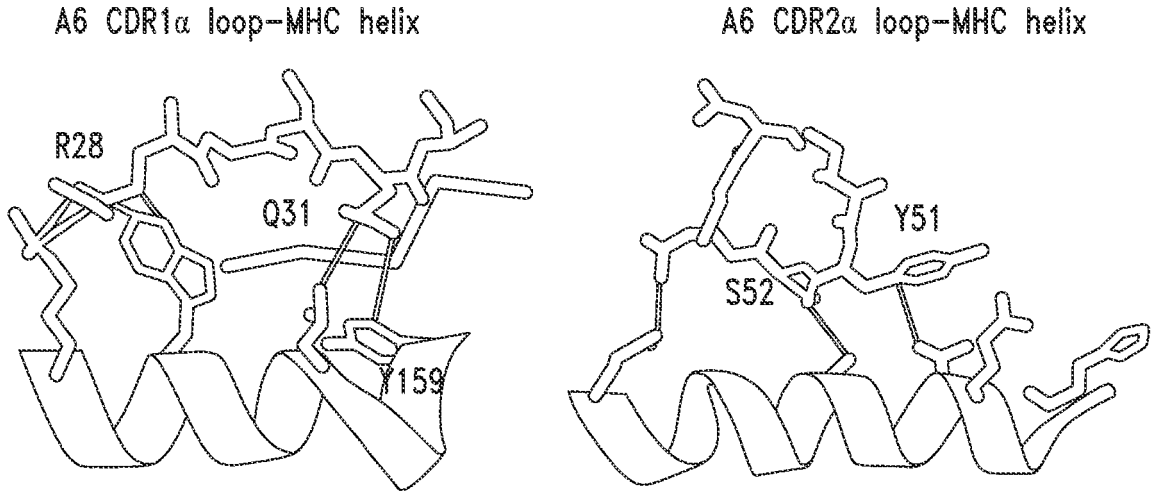

A6 CDR1α loop-MHC helix
A6 CDR2α loop-MHC helix
R28
Q31
Y159
Y51
S52

FIG. 4A

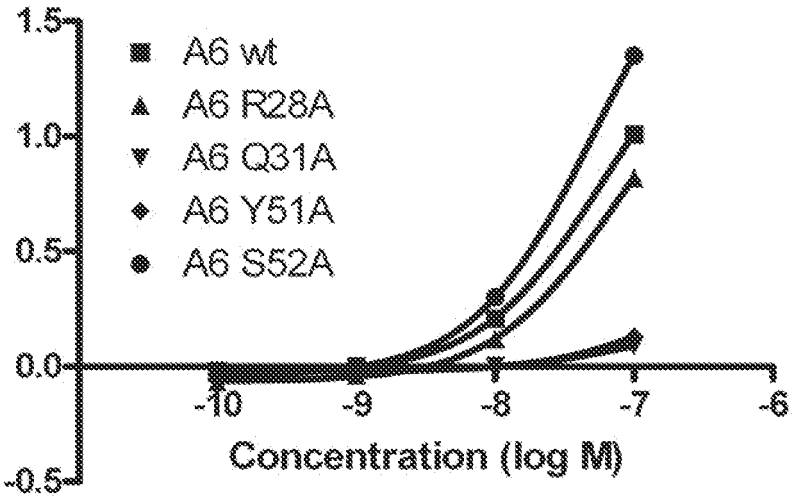

1.5
1.0
0.5
0.0
-0.5
A6 wt
A6 R28A
A6 Q31A
A6 Y51A
A6 S52A
-10
-9
-8
-7
-6
Concentration (log M)

FIG. 4B

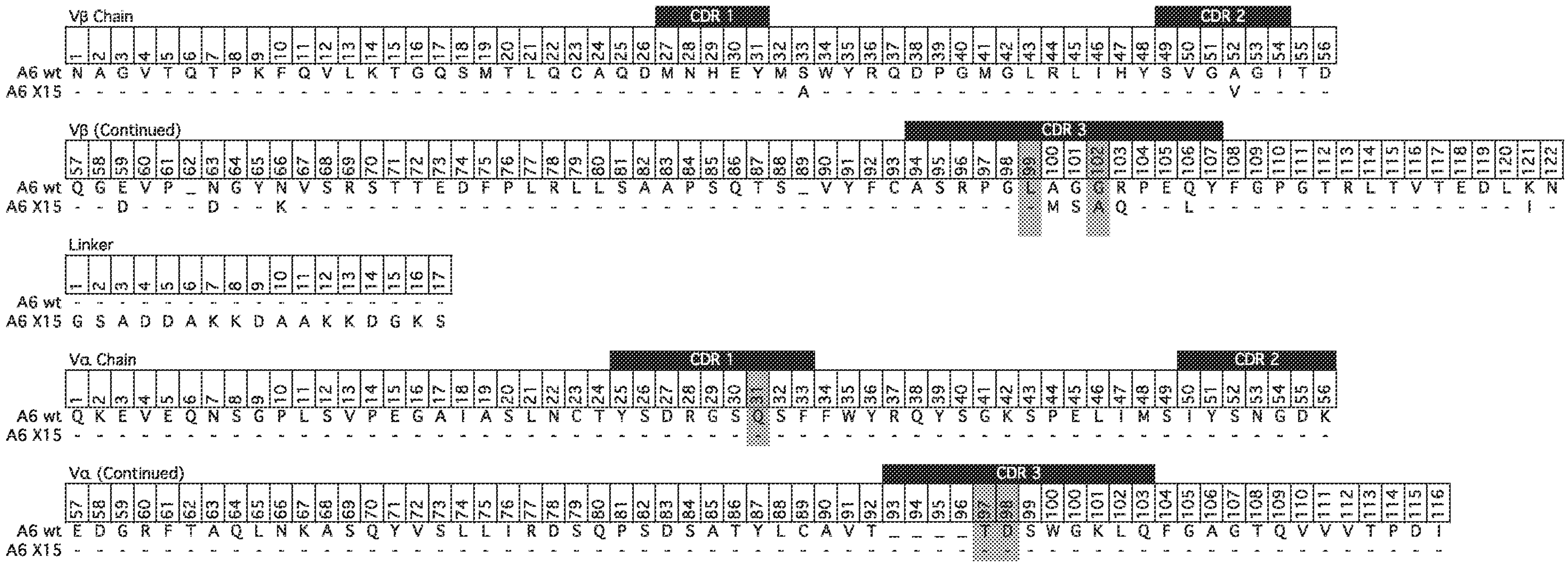

Vβ Chain
CDR 1
CDR 2
A6 wt N A G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q D P G M G L R L I H Y S V G A G I T D
A6 X15 A V
Vβ (Continued)
CDR 3
A6 wt Q G E V P _ N G Y N V S R S T T E D F P L R L L S A A P S Q T S _ V Y F C A S R P G L A G G R P E Q Y F G P G T R L T V T E D L K N
A6 X15 D D K M S A Q L I
Linker
A6 wt
A6 X15 G S A D D A K K D A A K K D G K S
Vα Chain
CDR 1
CDR 2
A6 wt Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R G S Q S F F W Y R Q Y S G K S P E L I M S I Y S N G D K
A6 X15
Vα (Continued)
CDR 3
A6 wt E D G R F T A Q L N K A S Q Y V S L L I R D S Q P S D S A T Y L C A V T _ _ _ _ T D S W G K L Q F G A G T Q V V V T P D I
A6 X15

FIG. 5

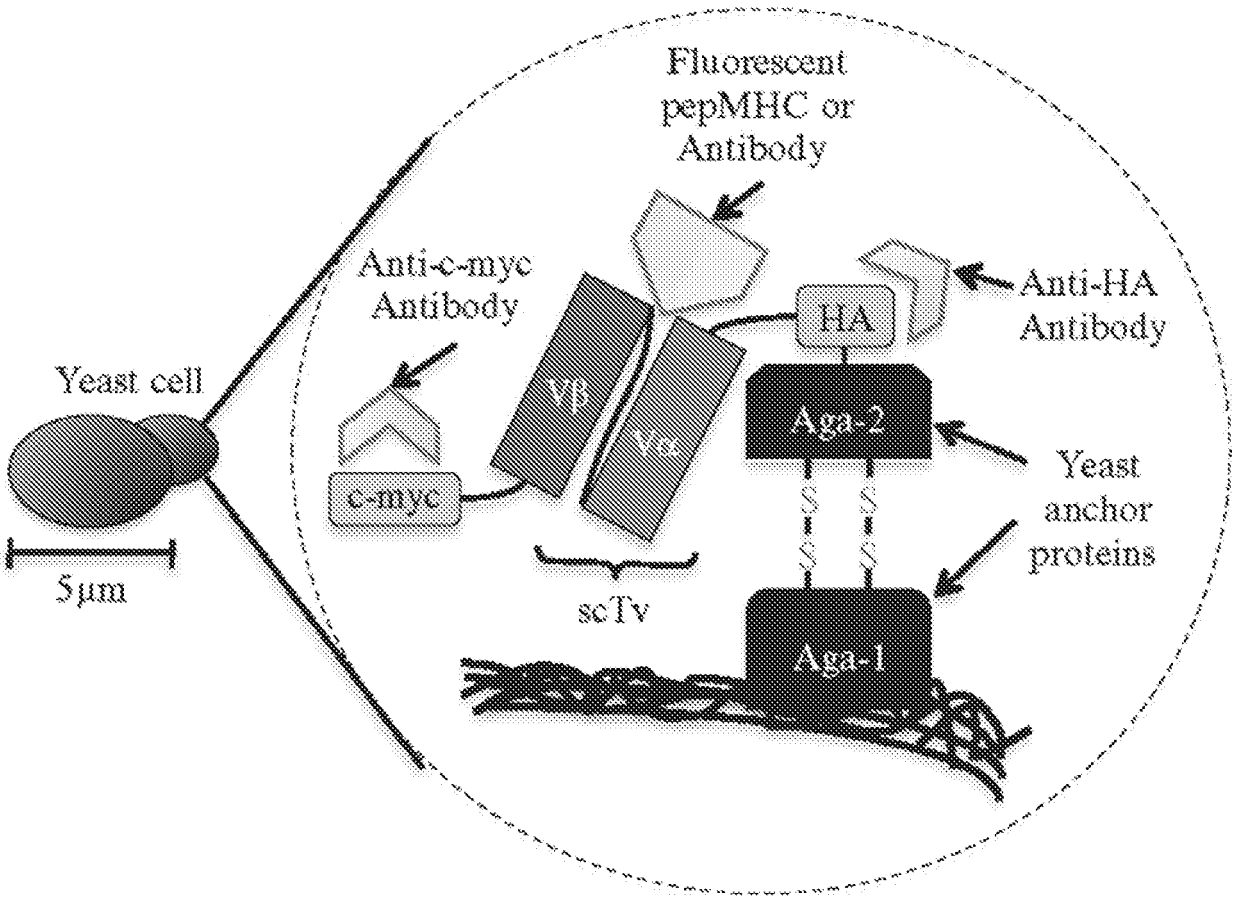

Fluorescent
pepMHC or
Antibody
Anti-c-myc
Antibody
Anti-HA
Antibody
HA
Yeast cell
Vβ
Vα
Aga-2
c-myc
Yeast
anchor
proteins
5μm
scTv
Aga-1

FIG. 6

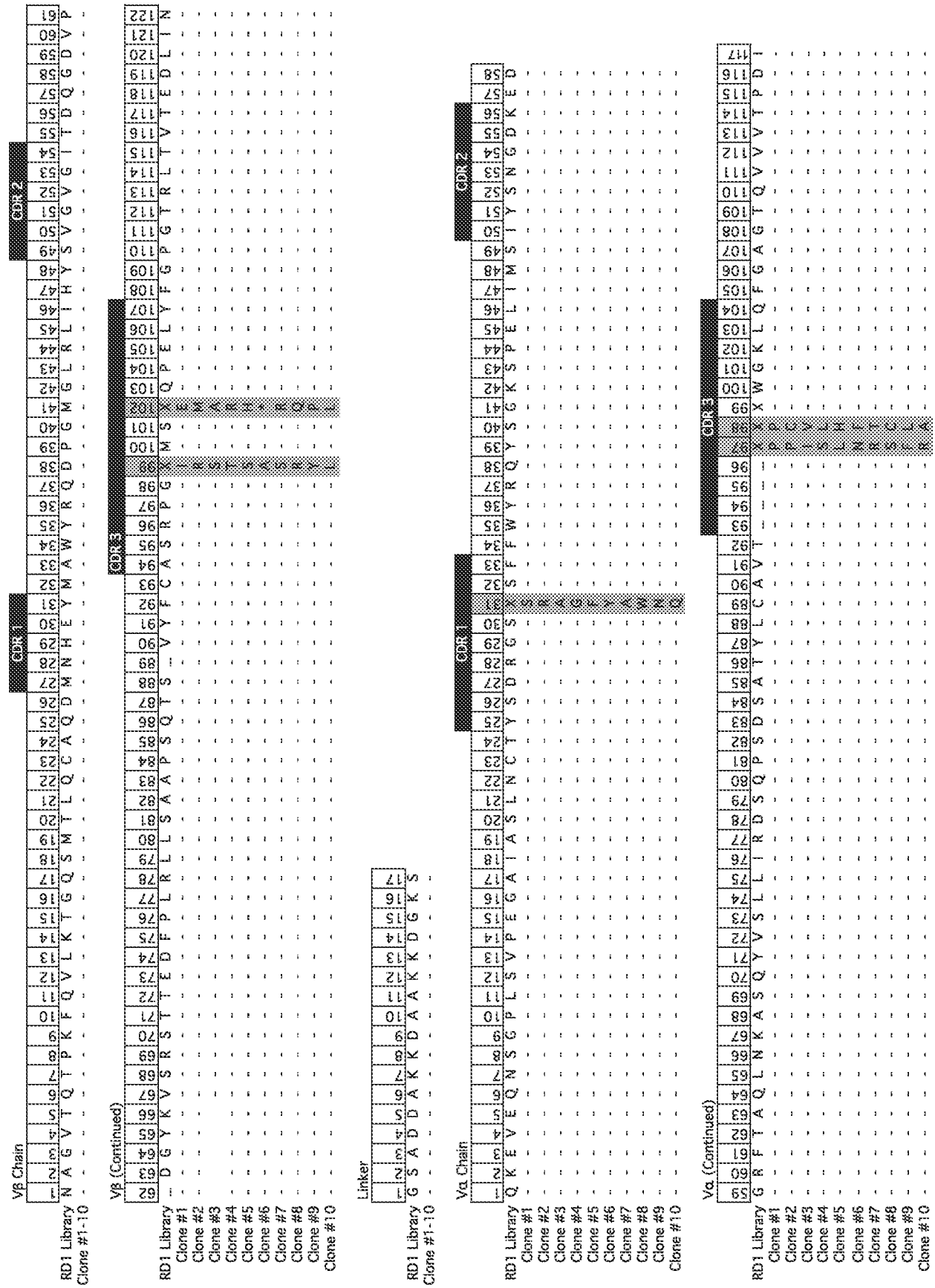

Vβ Chain
CDR 1
CDR 2
CDR 3
Vβ (Continued)
Linker
Vα Chain
Vα (Continued)
RD1 Library
Clone #1
Clone #2
Clone #3
Clone #4
Clone #5
Clone #6
Clone #7
Clone #8
Clone #9
Clone #10-10

FIG. 7

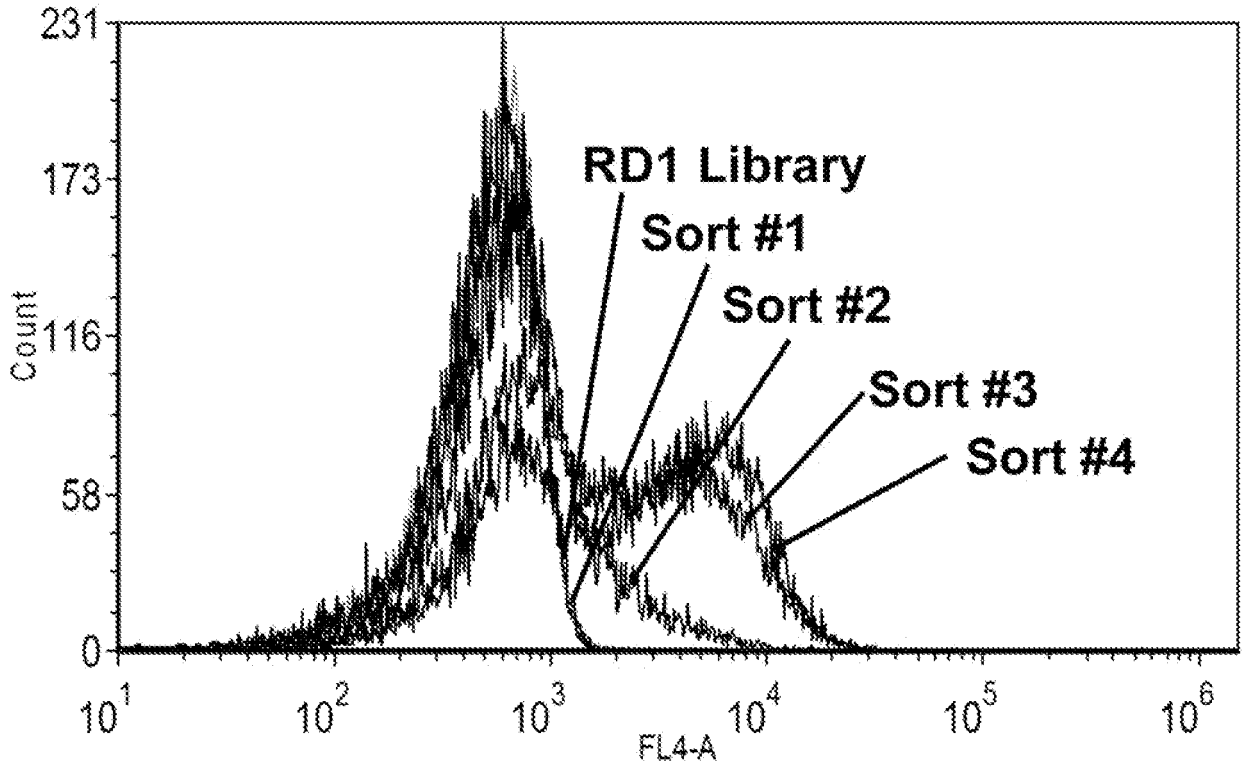

231
173
116
58
0
Count
RD1 Library
Sort #1
Sort #2
Sort #3
Sort #4
10^1
10^2
10^3
10^4
10^5
10^6
FL4-A

FIG. 8

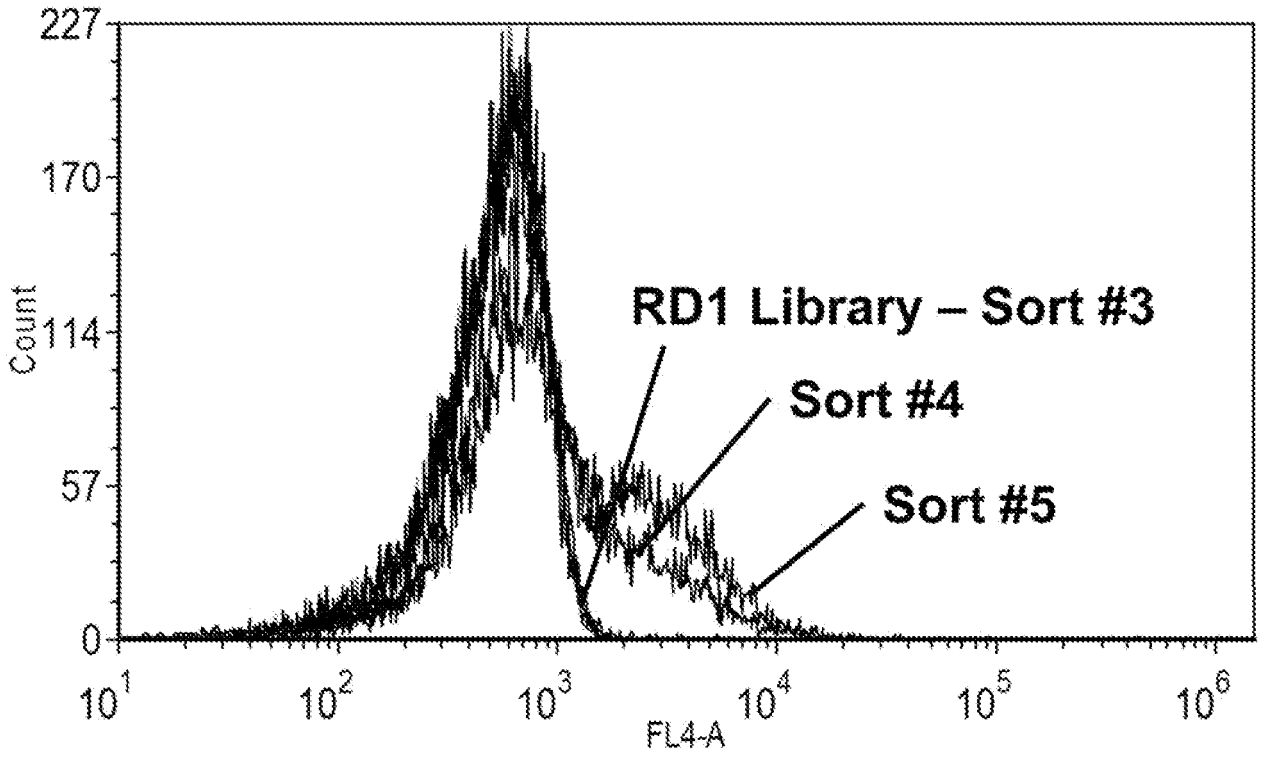

227
170
114
57
0
Count
RD1 Library – Sort #3
Sort #4
Sort #5
10^1
10^2
10^3
10^4
10^5
10^6
FL4-A

FIG. 9

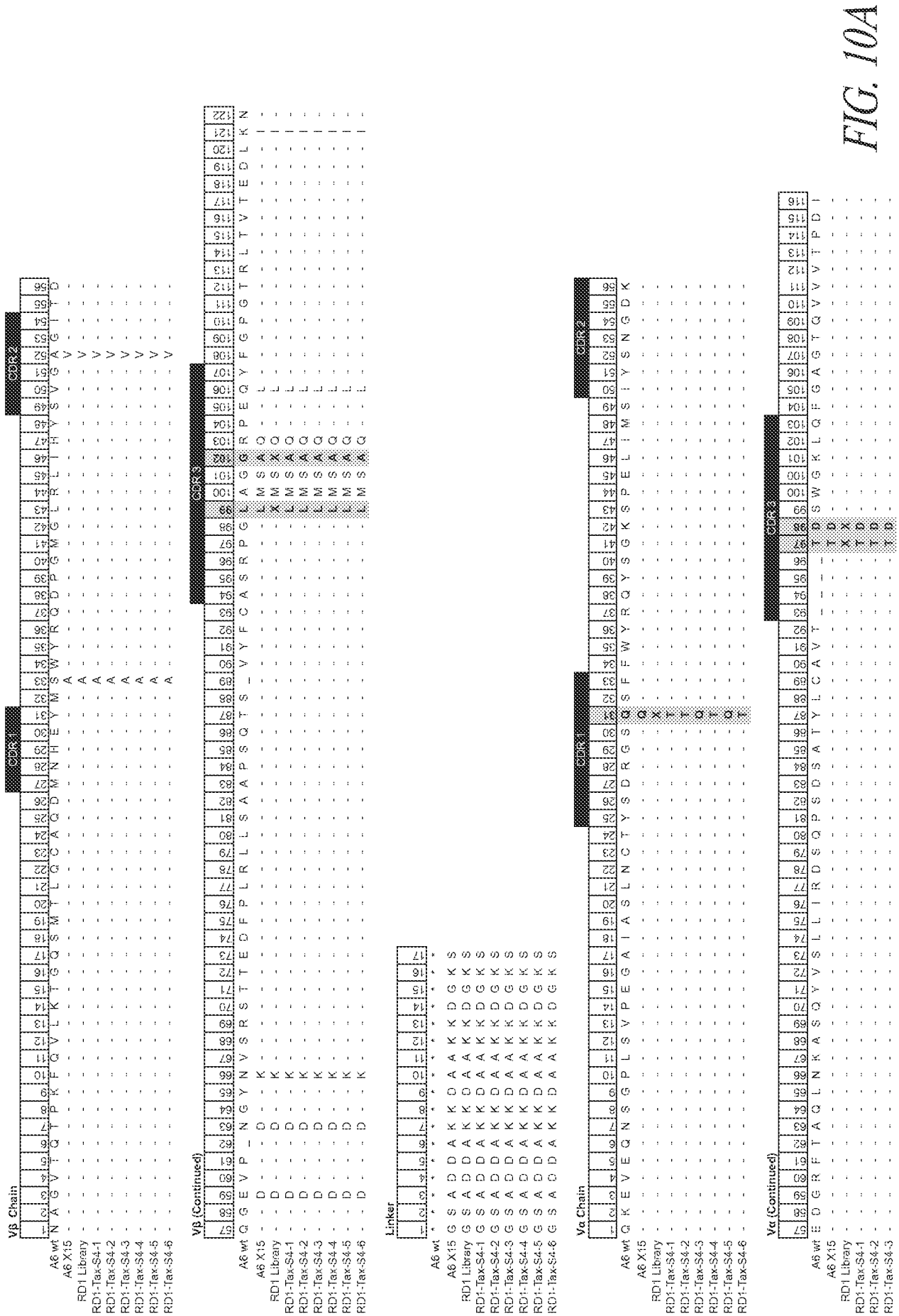

Vβ Chain
CDR1
CDR2
Vβ (Continued)
CDR3
Linker
Vα Chain
CDR1
CDR2
Vα (Continued)
CDR3
A6 wt
A6 X15
RD1 Library
RD1-Tax-S4-1
RD1-Tax-S4-2
RD1-Tax-S4-3
RD1-Tax-S4-4
RD1-Tax-S4-5
RD1-Tax-S4-6

FIG. 10A

Position	1			2			3			4			5		
RD1 Library	N	N	S	N	N	S	N	N	S	N	N	N	N	N	S
	X			X			X			X			X		
RD1-Tax-S4-1	T	T	G	G	C	C	A	C	C	A	C	G	G	A	C
	L (3)			A (2)			T (2)			T (2)			D (1)		
RD1-Tax-S4-2	T	T	G	G	C	G	A	C	C	A	C	C	G	A	C
	L (3)			A (2)			T (2)			T (2)			D (1)		
RD1-Tax-S4-3	C	T	C	G	C	C	C	A	G	A	C	C	G	A	C
	L (3)			A (2)			Q (1)			T (2)			D (1)		
RD1-Tax-S4-4	T	T	G	G	C	G	A	C	C	A	C	C	G	A	C
	L (3)			A (2)			T (2)			T (2)			D (1)		
RD1-Tax-S4-5	C	T	C	G	C	C	C	A	G	A	C	C	G	A	C
	L (3)			A (2)			Q (1)			T (2)			D (1)		
RD1-Tax-S4-6	T	T	G	G	C	G	A	C	C	A	C	C	G	A	C
	L (3)			A (2)			T (2)			T (2)			D (1)		

FIG. 10B

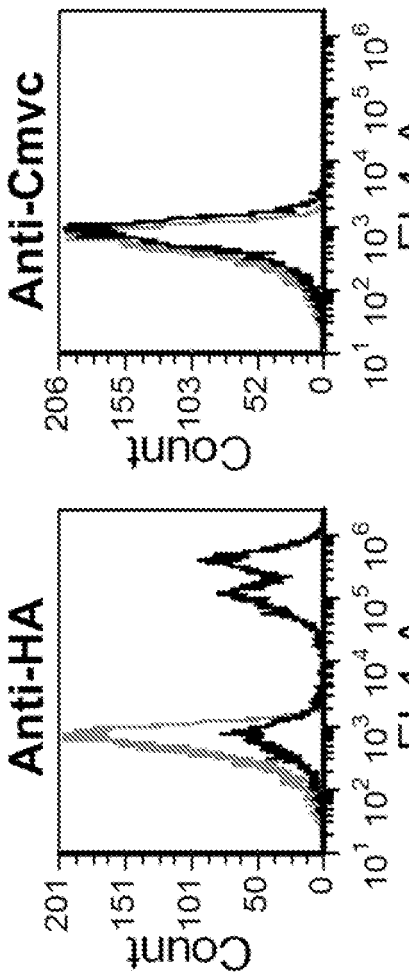


FIG. 11A

FIG. 11B

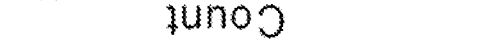


FIG. 11C

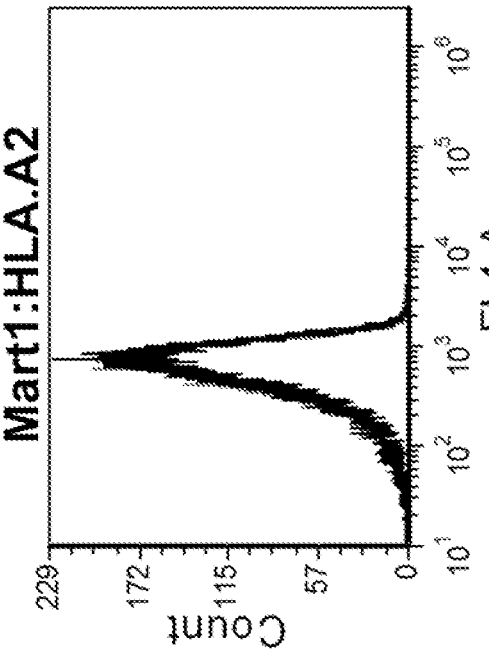


FIG. 11D

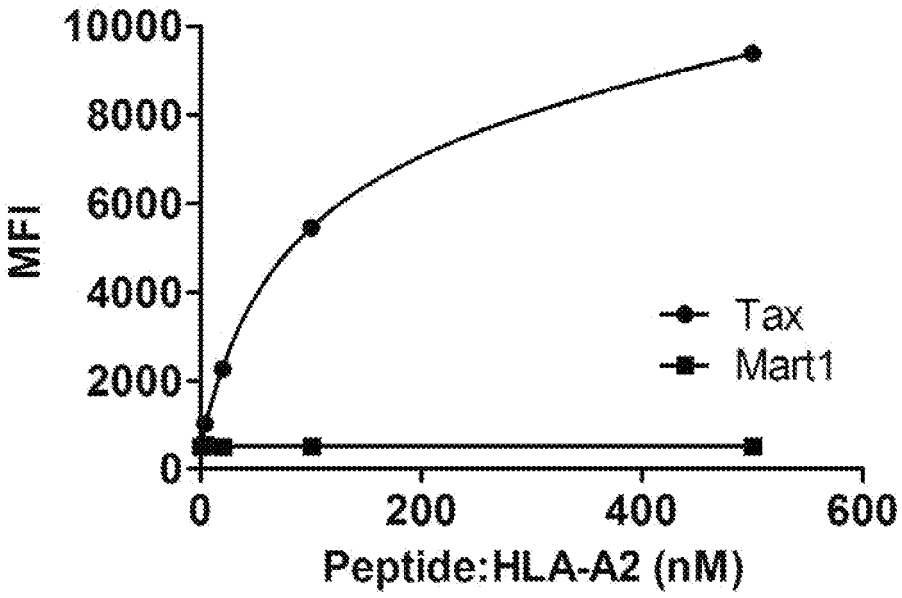

10000
8000
6000
4000
2000
0
MFI
0
200
400
600
Peptide:HLA-A2 (nM)
Tax
Mart1

FIG. 11E

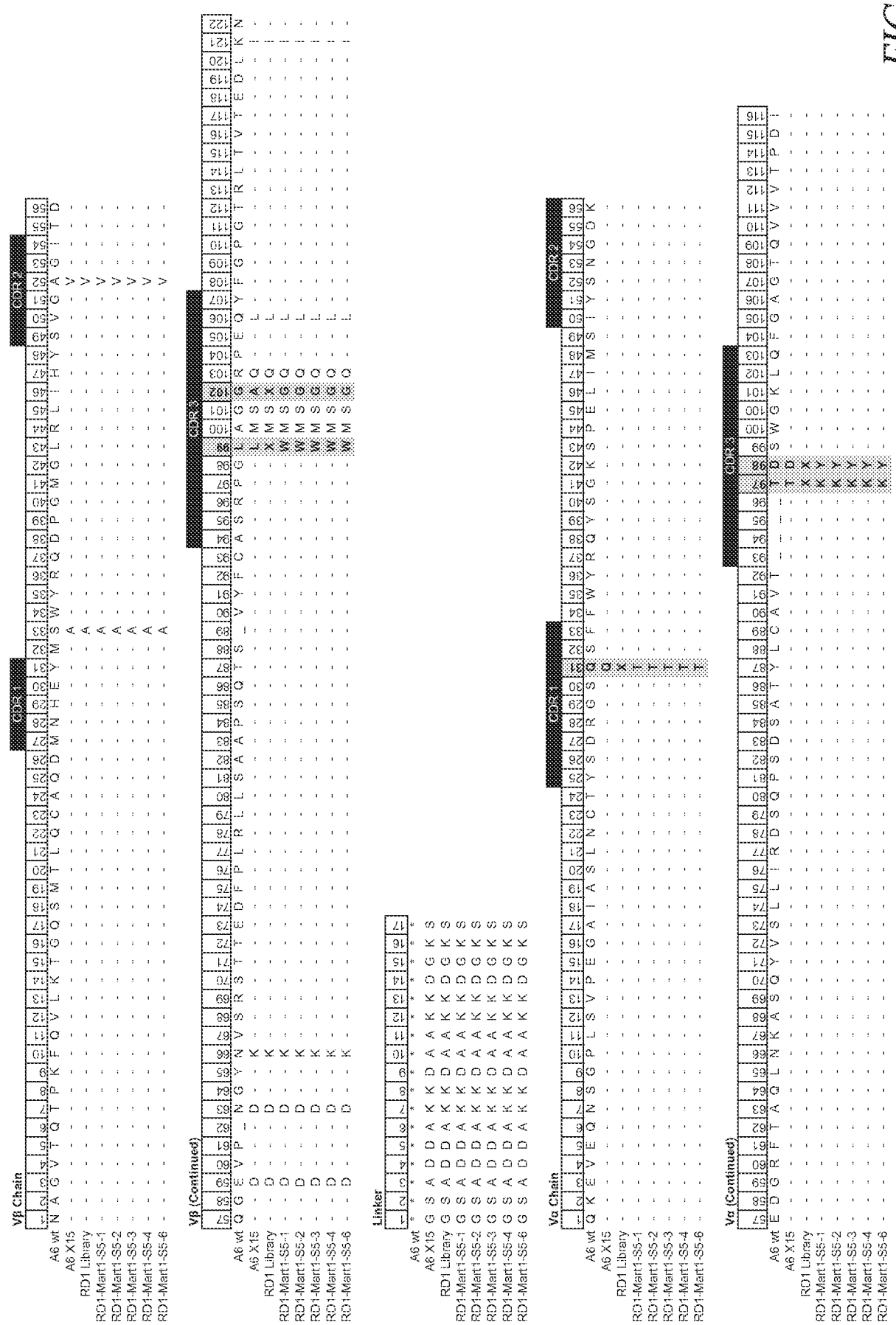


FIG. 12A

Position	1			2			3			4			5		
RD1 Library	N	N	S	N	N	S	N	N	S	N	N	N	N	N	S
	X			X			X			X			X		
RD1-Mart1-S5-1	T	G	G	G	G	C	A	C	G	A	A	G	T	A	C
	W (1)			G (2)			T (2)			K (1)			Y (1)		
RD1-Mart1-S5-2	T	G	G	G	G	C	A	C	G	A	A	G	T	A	C
	W (1)			G (2)			T (2)			K (1)			Y (1)		
RD1-Mart1-S5-3	T	G	G	G	G	C	A	C	G	A	A	G	T	A	C
	W (1)			G (2)			T (2)			K (1)			Y (1)		
RD1-Mart1-S5-4	T	G	G	G	G	C	A	C	G	A	A	G	T	A	C
	W (1)			G (2)			T (2)			K (1)			Y (1)		
RD1-Mart1-S5-6	T	G	G	G	G	C	A	C	G	A	A	G	T	A	C
	W (1)			G (2)			T (2)			K (1)			Y (1)		

FIG. 12B

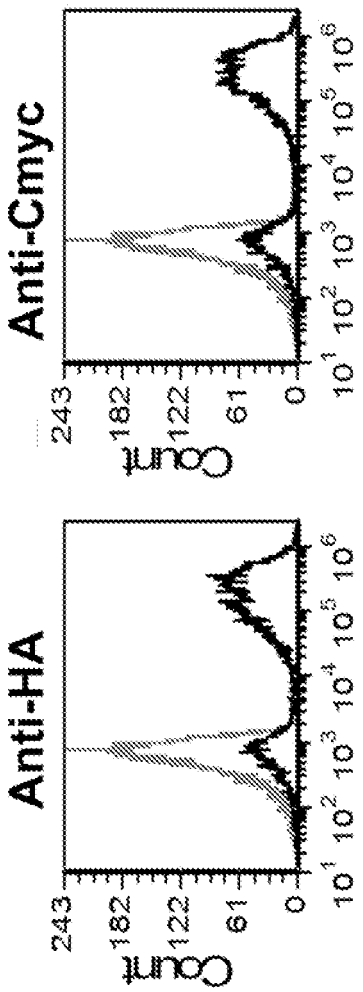


FIG. 13A

FIG. 13B



FIG. 13C

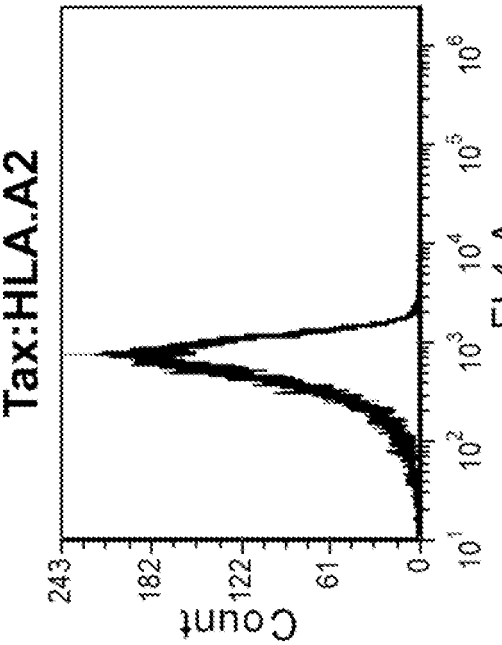


FIG. 13D

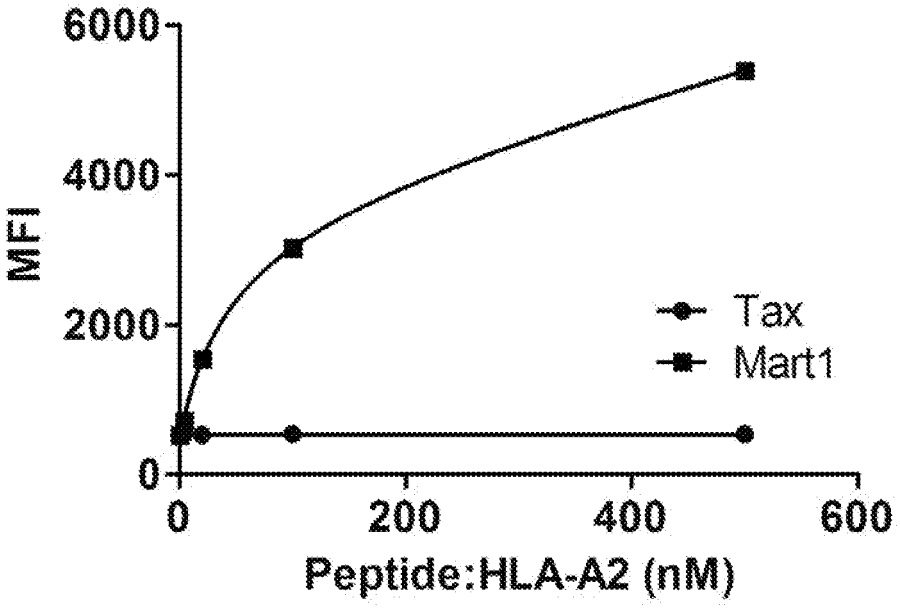


FIG. 13E

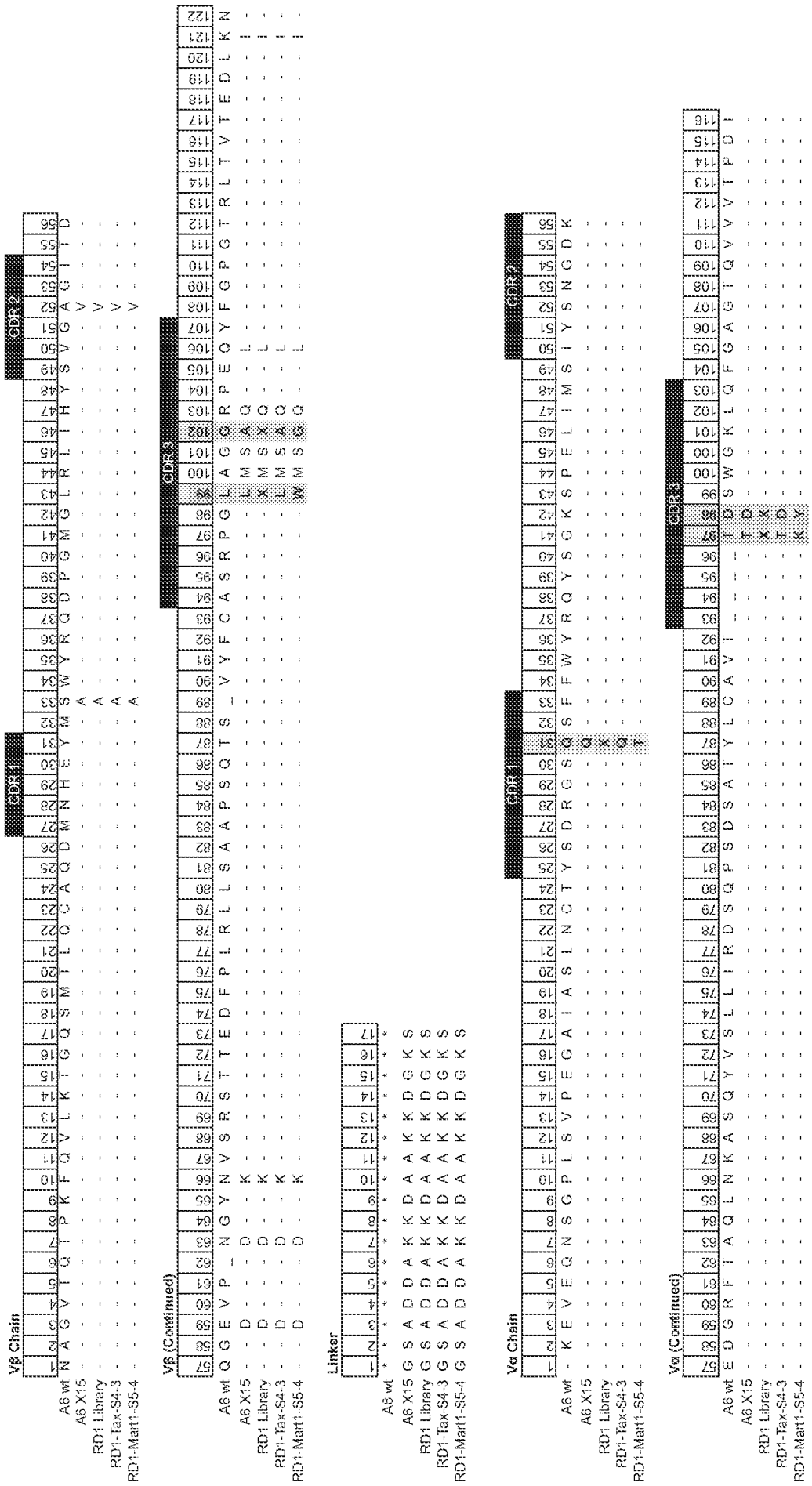

Vβ Chain
CDR1
CDR2
Vβ (Continued)
CDR3
Linker
Vα Chain
CDR1
CDR2
Vα (Continued)
CDR3
A6 wt
A6 X15
RD1 Library
RD1-Tax-S4-3
RD1-Mart1-S5-4

FIG. 14

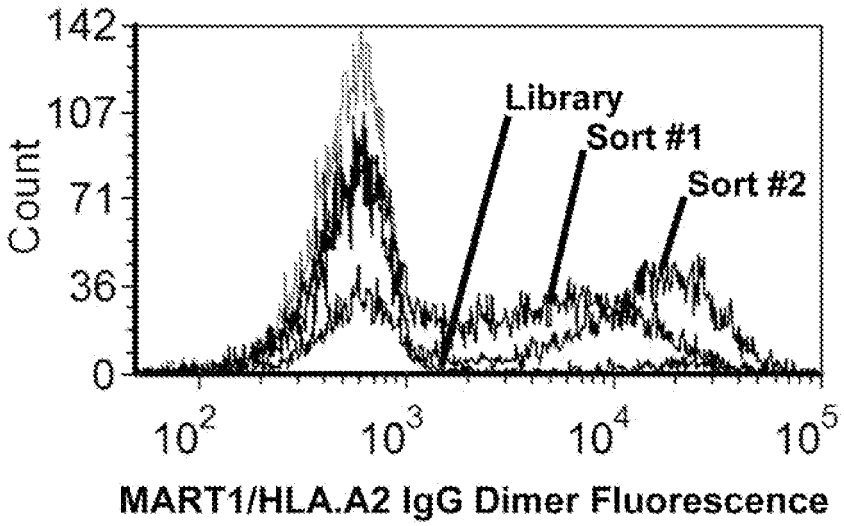


FIG. 15A

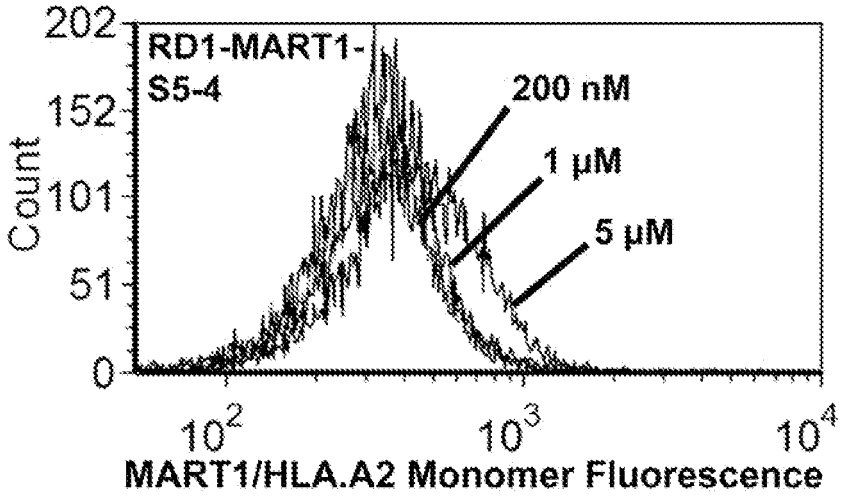


FIG. 15B

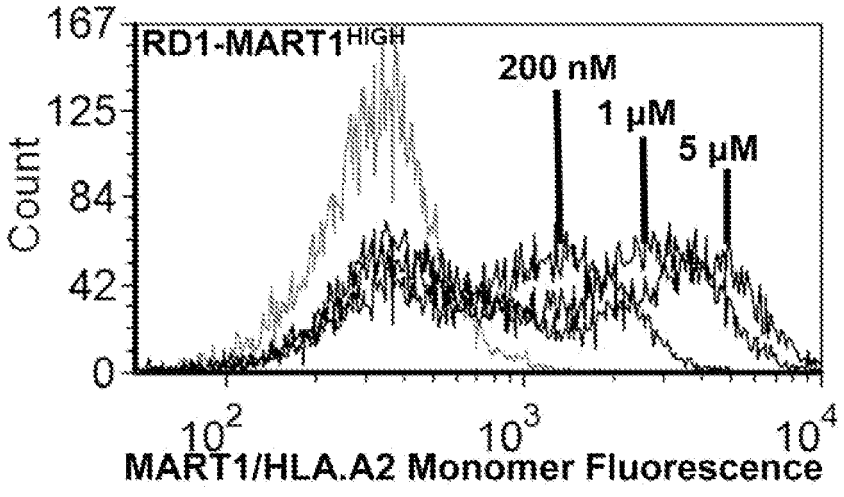


FIG. 15C

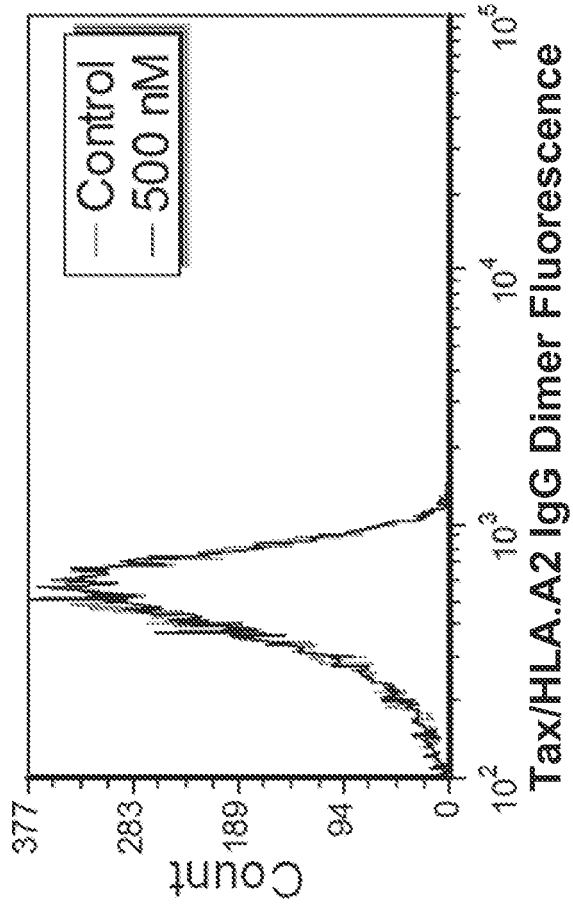


FIG. 16B

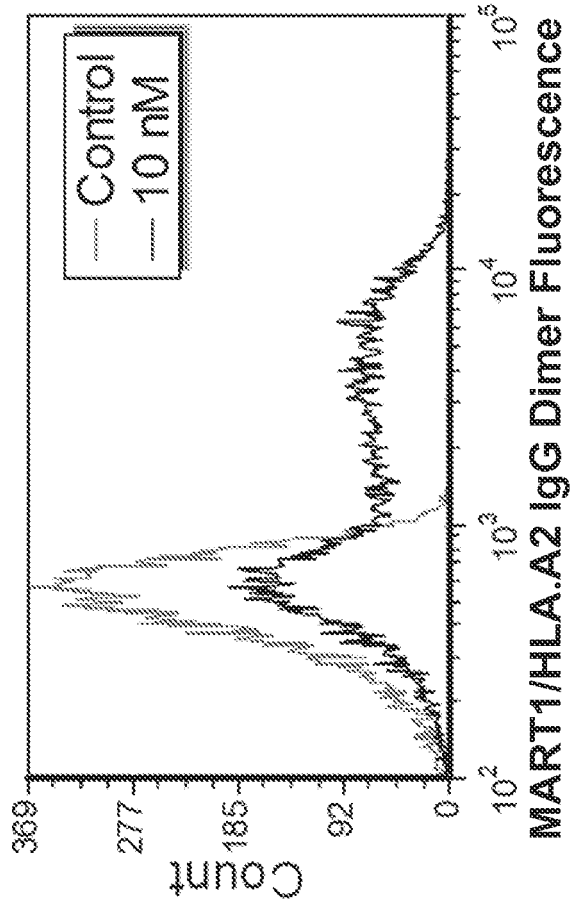


FIG. 16A

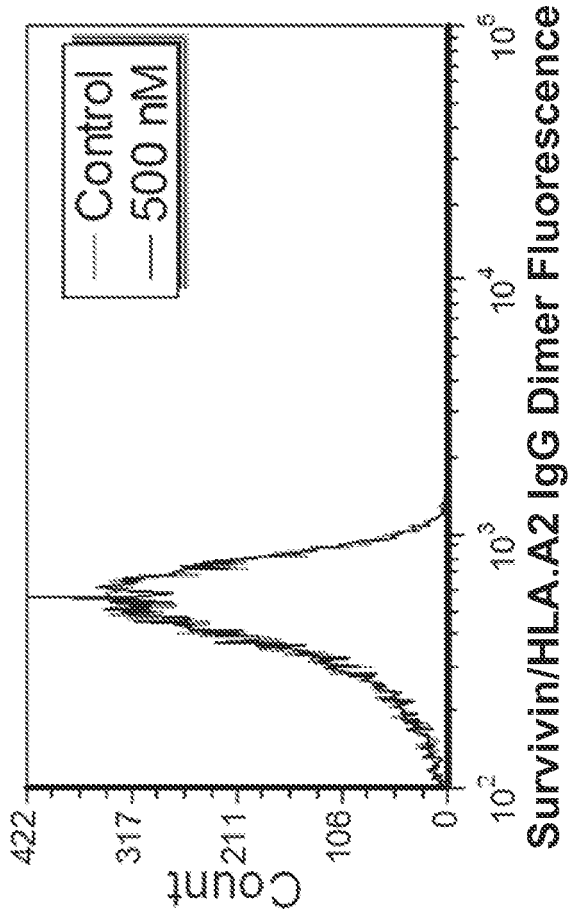


FIG. 16D

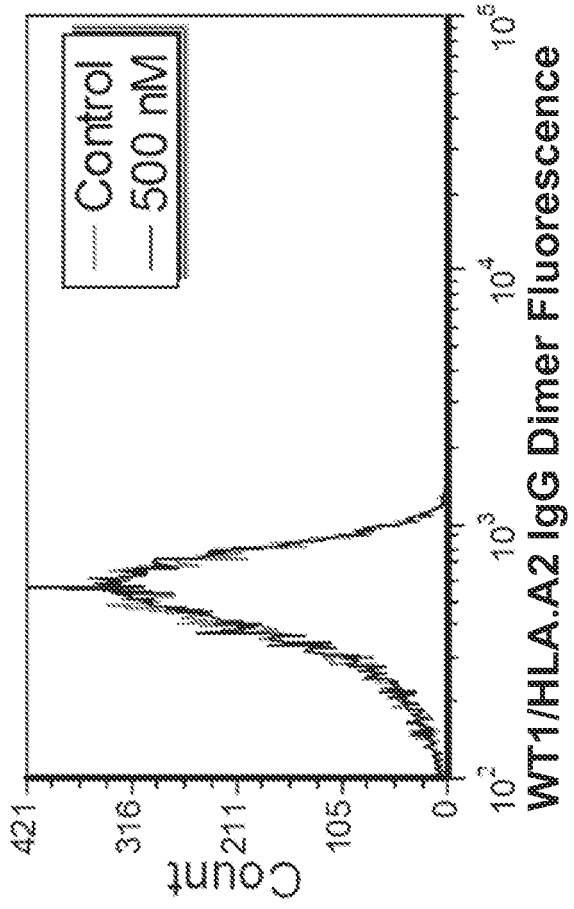


FIG. 16C

Binding and specificity of soluble RD1-MART1^{HIGH} with peptide pulsed T2 cells

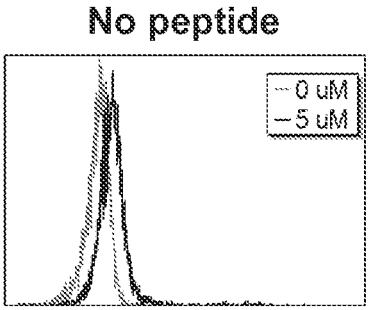


FIG. 17A

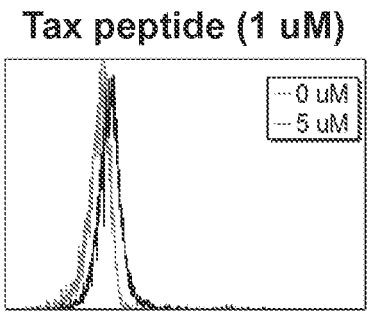


FIG. 17B

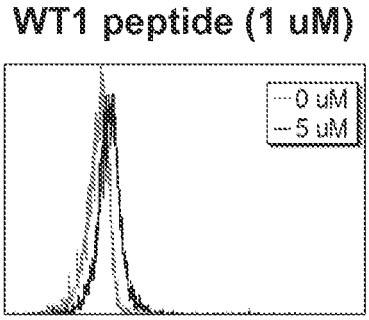


FIG. 17C

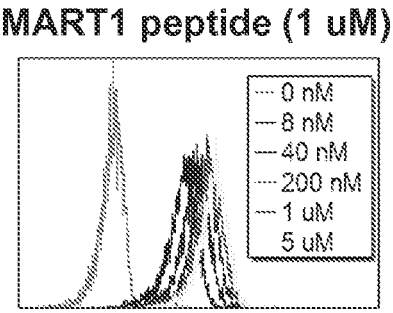


FIG. 17D

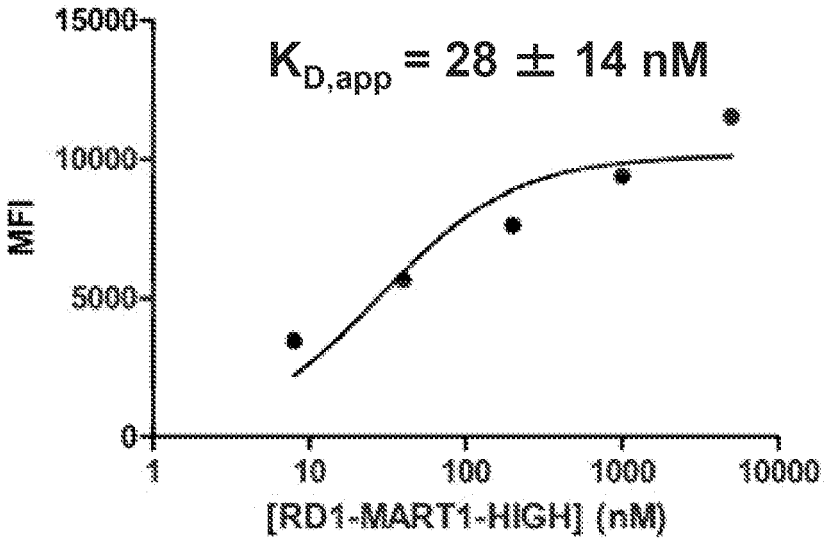


FIG. 17E

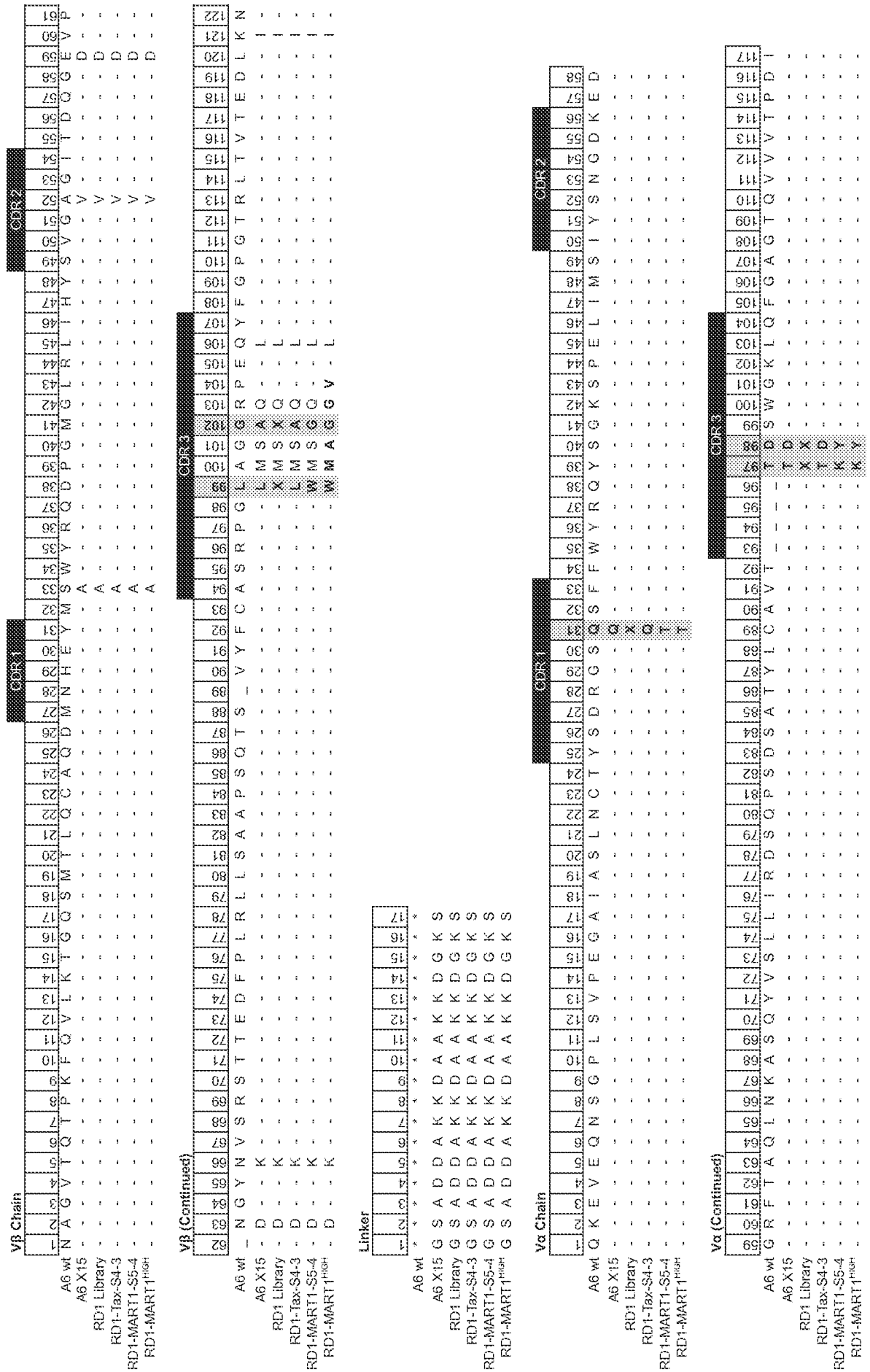

Vβ Chain
CDR1
CDR2
A6 wt
A6 X15
RD1 Library
RD1-Tax-S4-3
RD1-MART1-S5-4
RD1-MART1HIGH
Vβ (Continued)
CDR3
Linker
Vα Chain
Vα (Continued)

FIG. 18

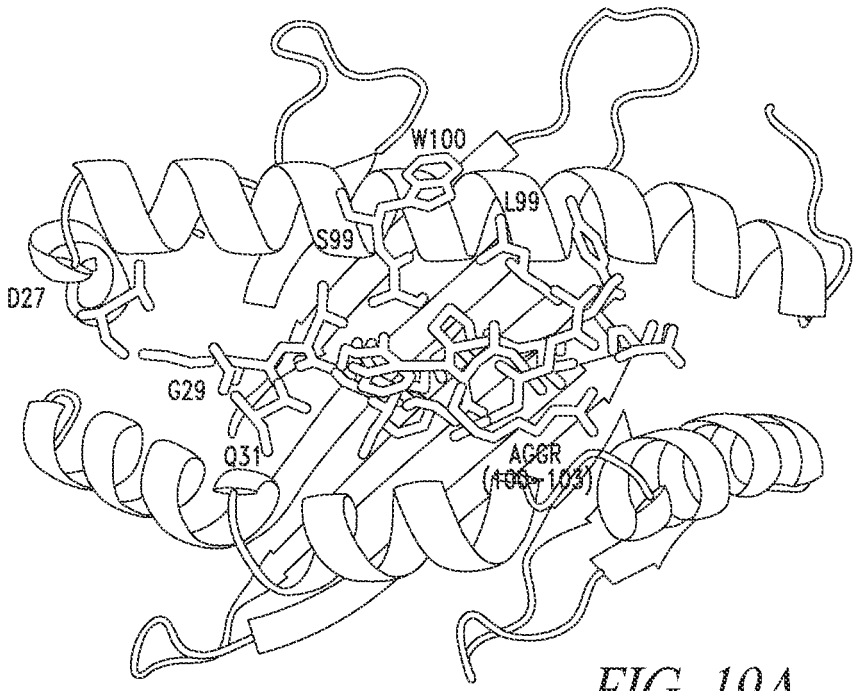


FIG. 19A

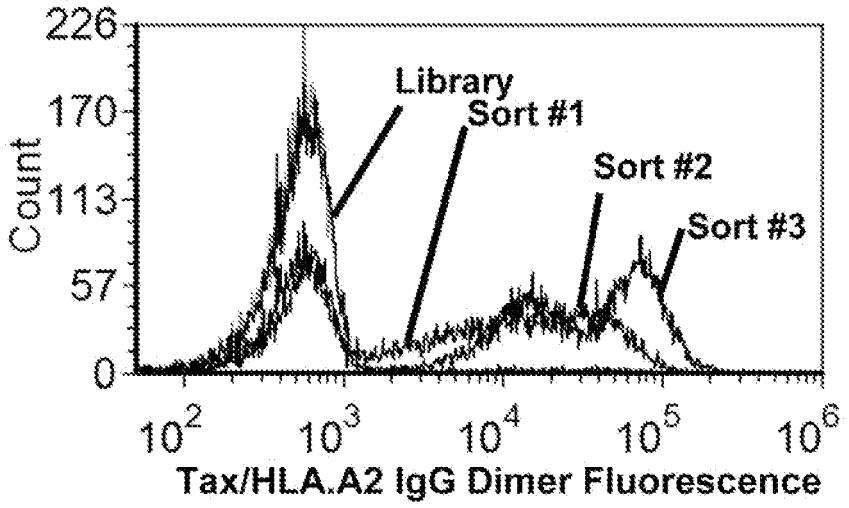


FIG. 19B

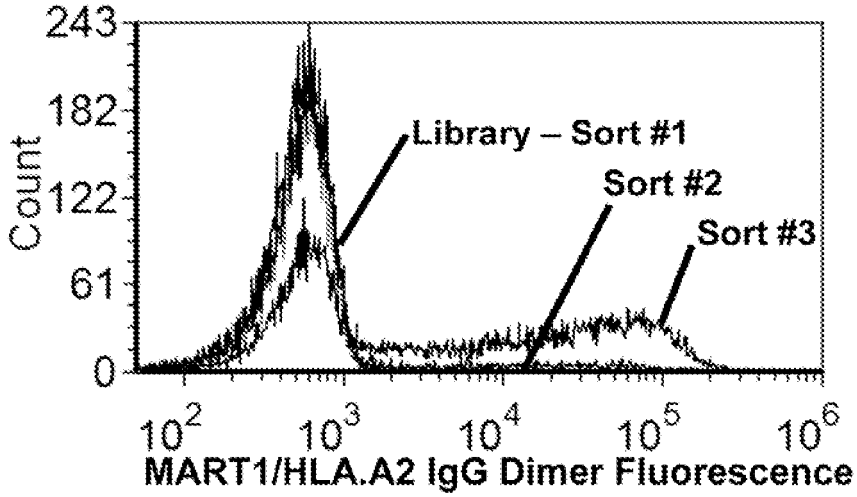


FIG. 19C

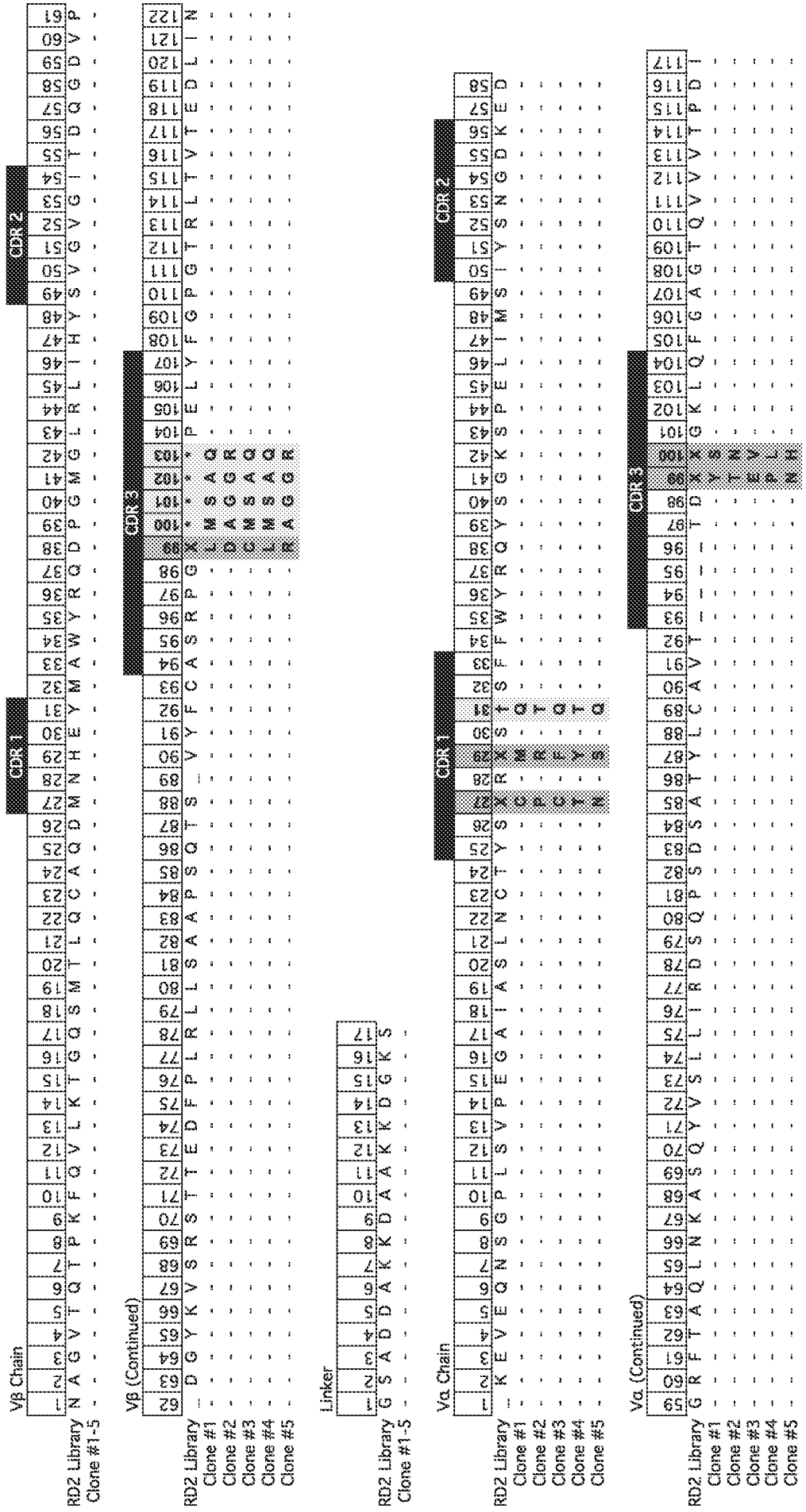

Vβ Chain
CDR1
CDR2
RD2 Library
Clone #1-5
Vβ (Continued)
CDR3
Linker
Vα Chain
Vα (Continued)
Clone #1
Clone #2
Clone #3
Clone #4
Clone #5

FIG. 20

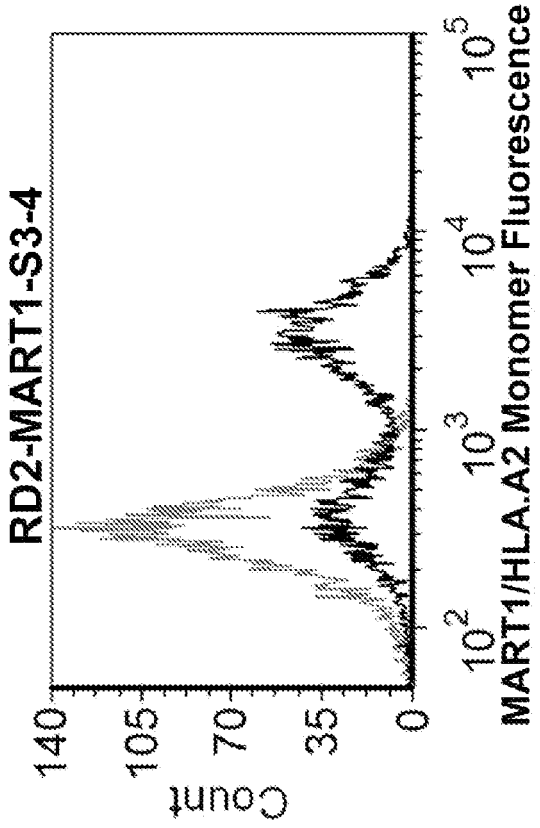


FIG. 21B

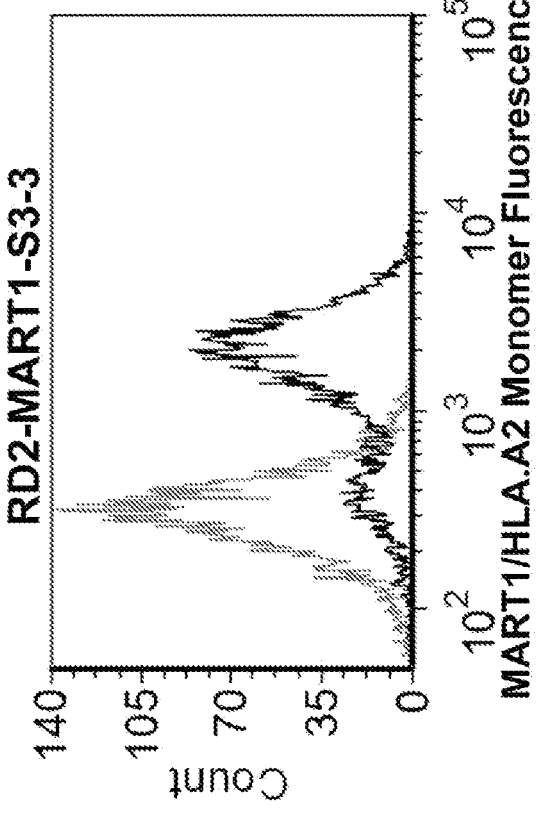


FIG. 21A

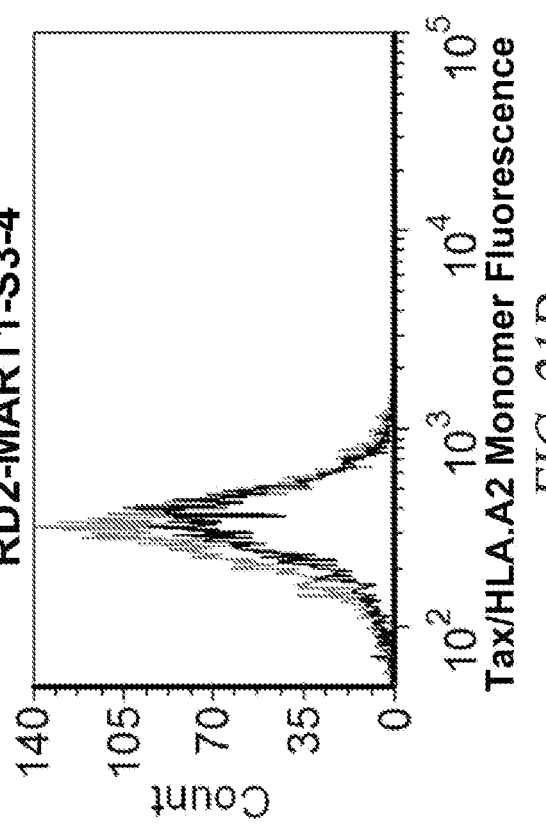


FIG. 21D

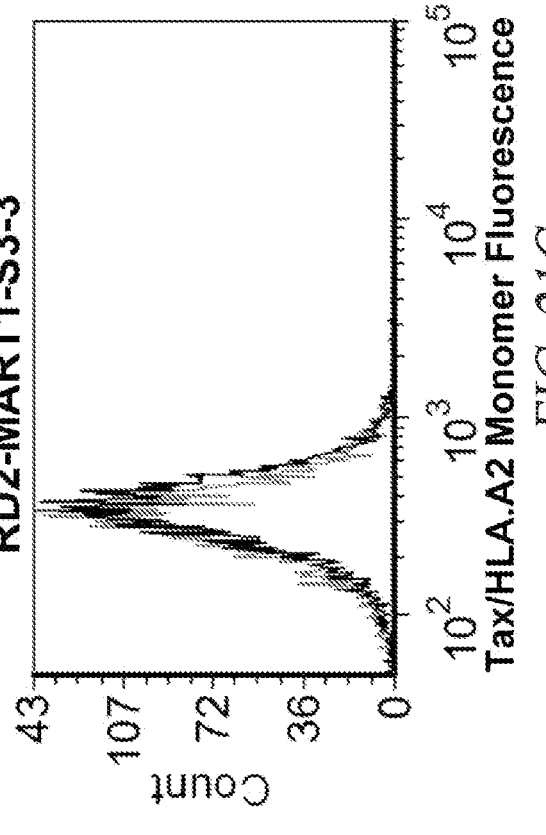


FIG. 21C

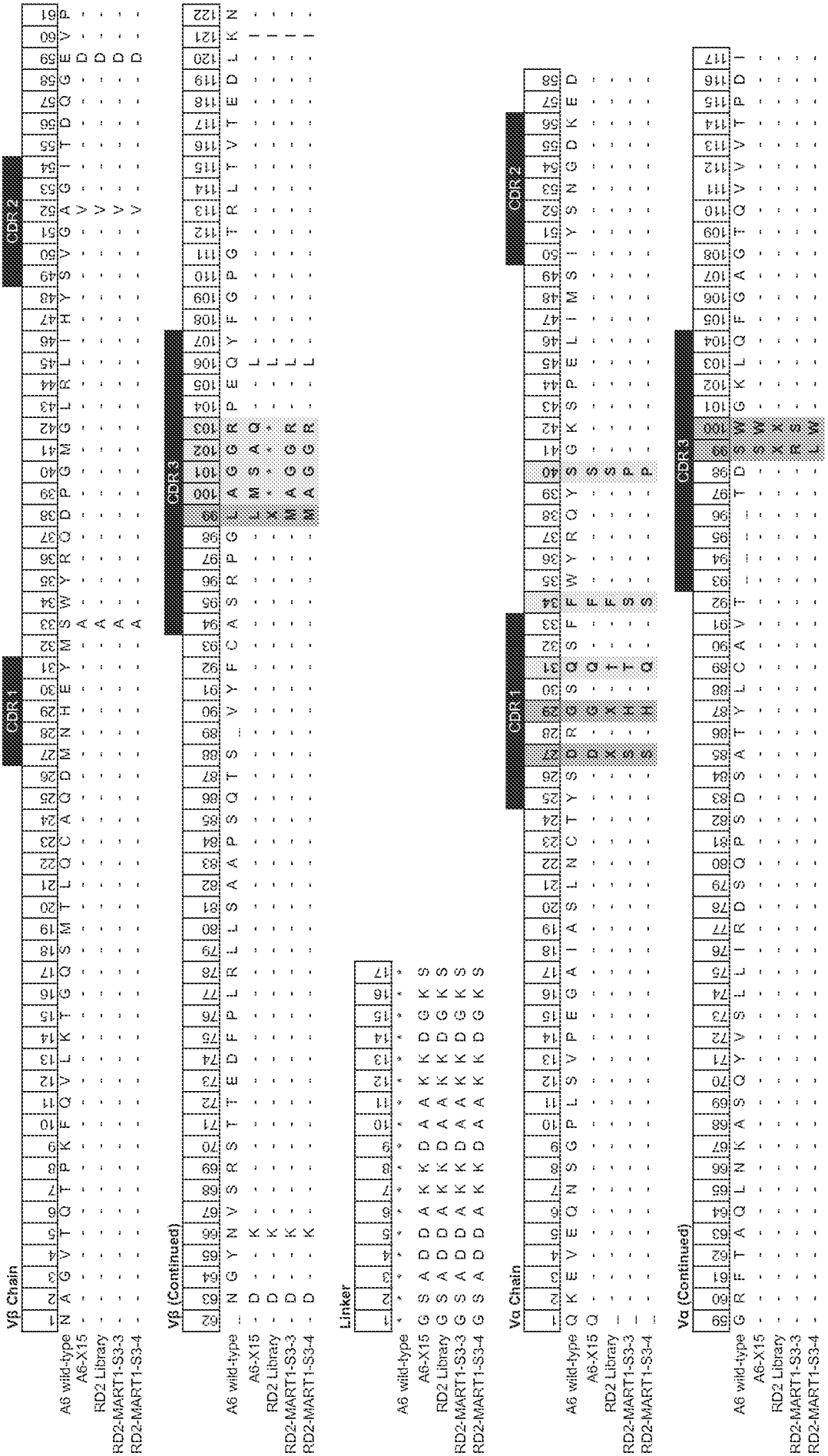

Vβ Chain
CDR1
CDR2
Vβ (Continued)
CDR3
Linker
Vα Chain
CDR1
CDR2
Vα (Continued)
CDR3
A6 wild-type
A6-X15
RD2 Library
RD2-MART1-S3-3
RD2-MART1-S3-4

FIG. 21E

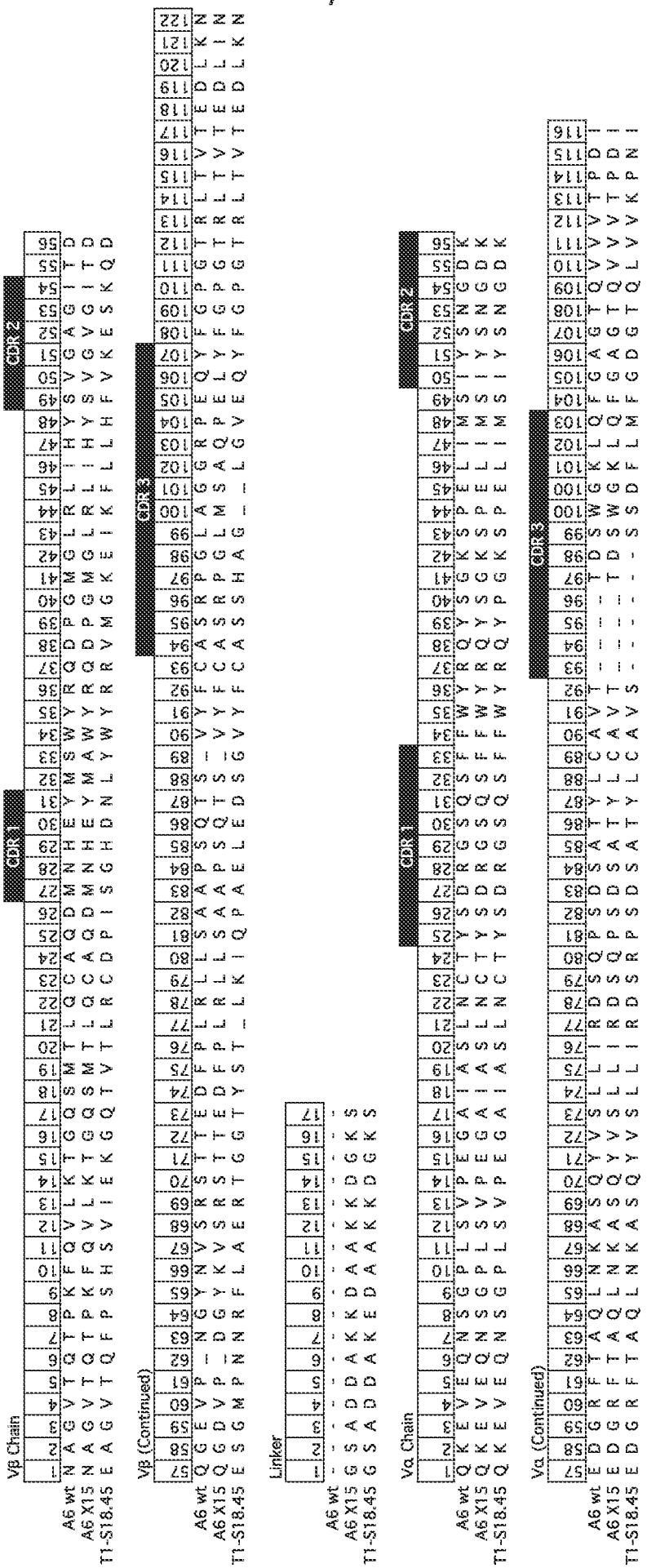

Vβ Chain
CDR1
CDR2
A6 wt N A G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q D P G M G L R L I H Y S V G A G I T D
A6 X15 N A G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M A W Y R Q D P G M G L K L I H Y S V G V G I T D
T1-S18.45 E A G V T Q F P S H S V I E K G Q T V T L R C D P I S G H D N L Y W Y R R V M G K E I K F L L H F V K E S K Q D
Vβ (Continued)
CDR3
A6 wt Q G E V P N G Y N V S R S T T E D F P L R L L S A A P S Q T S V Y F C A S S R P G L A G G R P E Q Y F G P G T R L T V T E D L K N
A6 X15 Q G D V P D G Y K V S R S T T E D F P L R L L S A A P S Q T S V Y F C A S S R P G L M S A Q P E L Y F G P G T R L T V T E D L I N
T1-S18.45 E S G M P N N R F L A E R T G G T Y S T L K I Q P A E L E D S G V Y F C A S S H A G G L G V E Q Y F G P G T R L T V L E D L K N
Linker
A6 wt - - - - - - - - - - - - - - - -
A6 X15 G S A D D A K K D A A K K D D G K S
T1-S18.45 G S A D D A K E D A A K K D D G K S
Vα Chain
CDR1
CDR2
A6 wt Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R G S Q S F F W Y R Q Y P G K G P E F L I M S I Y S N G D K E D G R F T A Q L N K A S Q Y V S L L I R D S Q P S D S A T Y L C A V T - - - - - - T D S W G K L Q F G A G T Q V V V T P D I Q N P D P A V Y Q L R D S K S S D K S V C L F T D F D S Q T N V S Q S K D S D V Y I T D K C V L D M R S M D F K S N S A V A W S N K S E F A C A N A F N N S I I P E D T F F P S P E S S C D V K L V E K S F E T D T N L N F Q N L S V I G F R I L L L K V A G F N L L M T L R L W S S
Vα (Continued)
CDR3
A6 wt E D G R F T A Q L N K A S Q Y V S L L I R D S Q P S D S A T Y L C A V T - - - - - - T D S W G K L Q F G A G T Q V V V T P D I
A6 X15 E D G R F T A Q L N K A S Q Y V S L L I R D S R P S D S A T Y L C A V S - - - - - - S S D F L M F G D G T Q L V V K P N I

FIG. 22

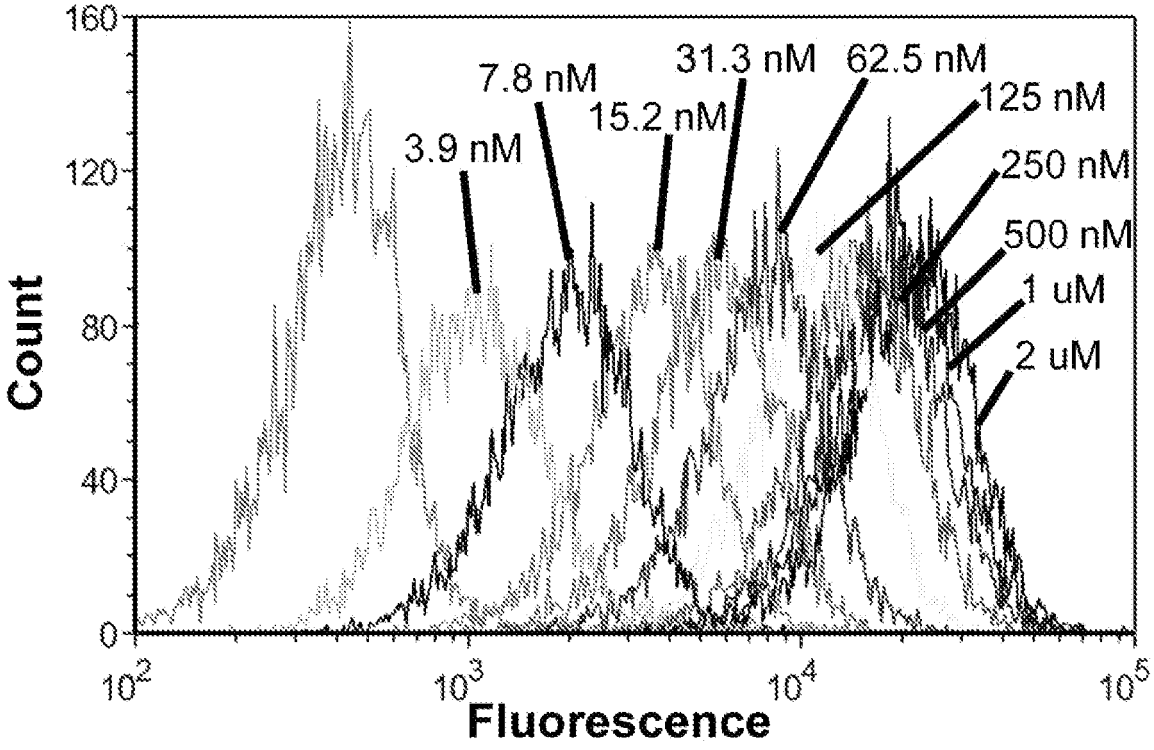


FIG. 23A

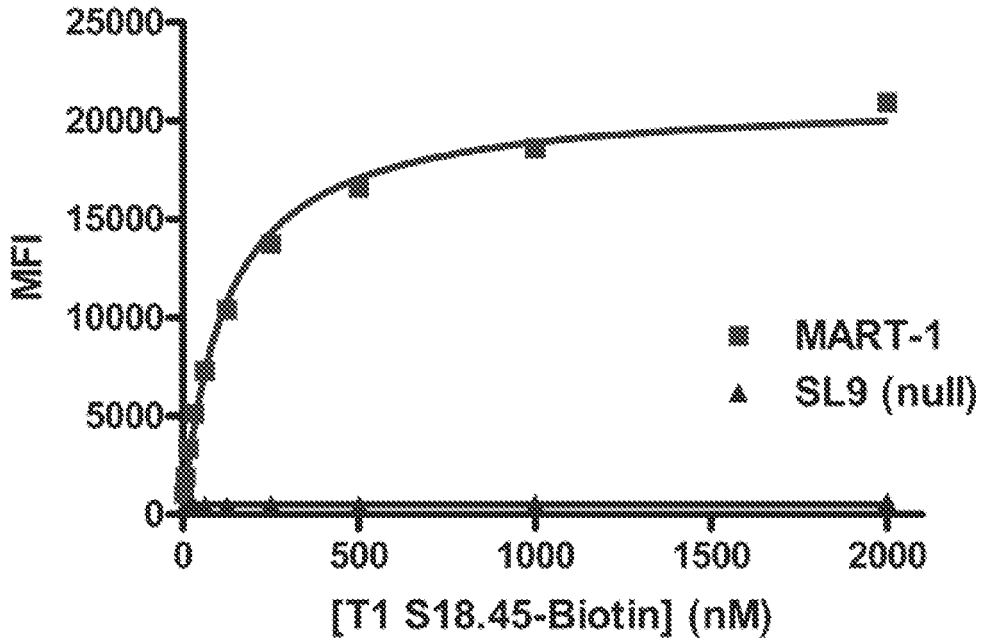


FIG. 23B

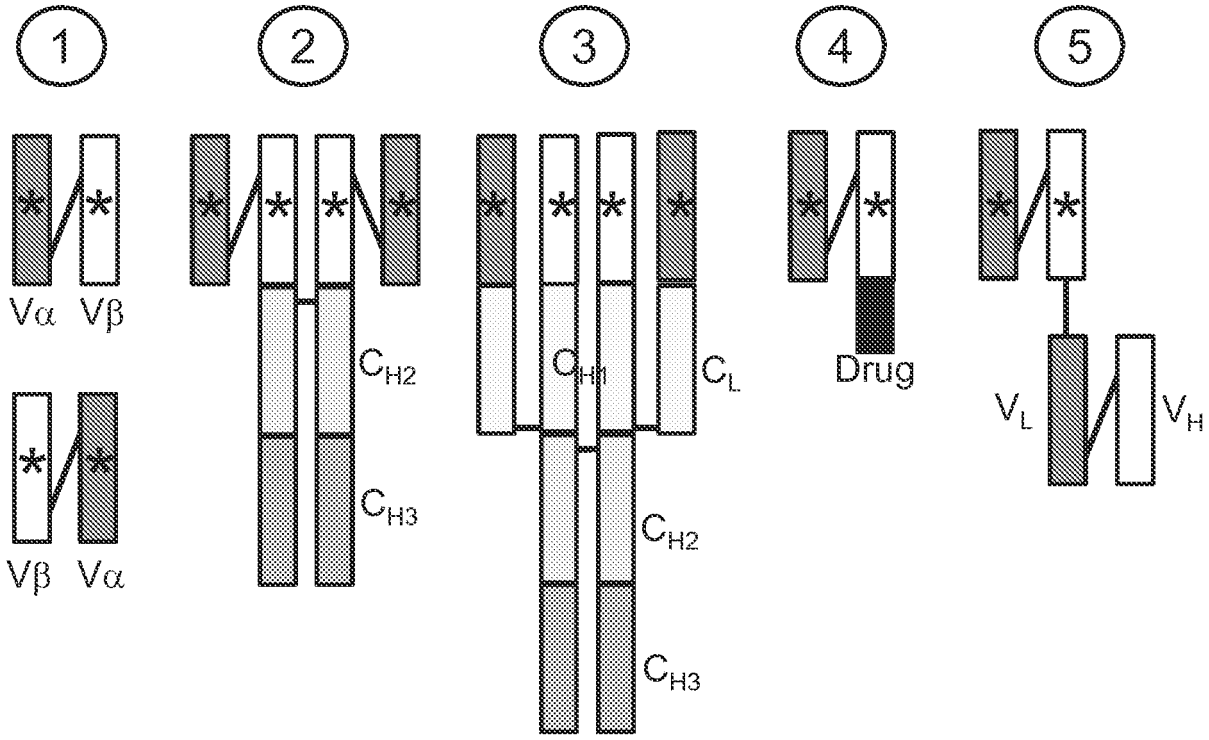


FIG. 24A

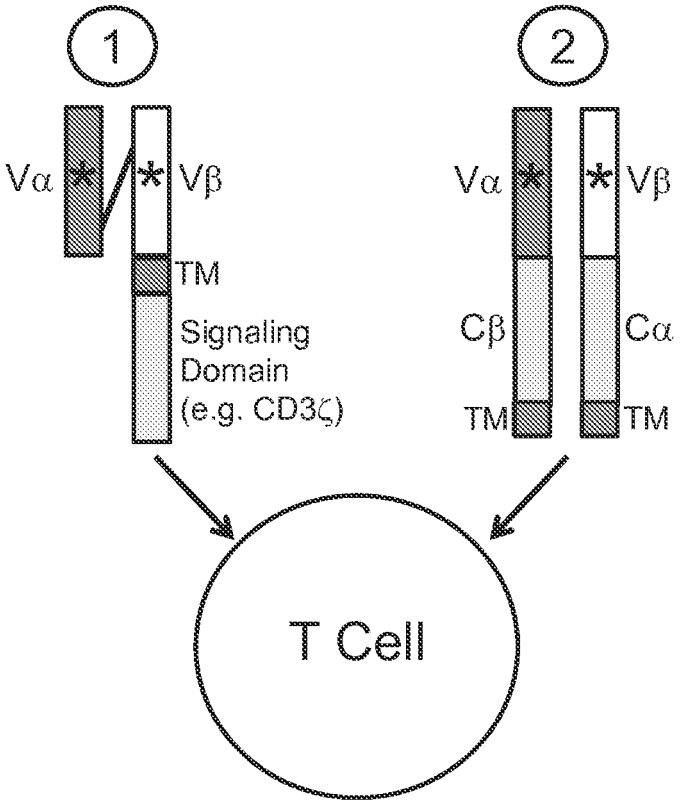


FIG. 24B