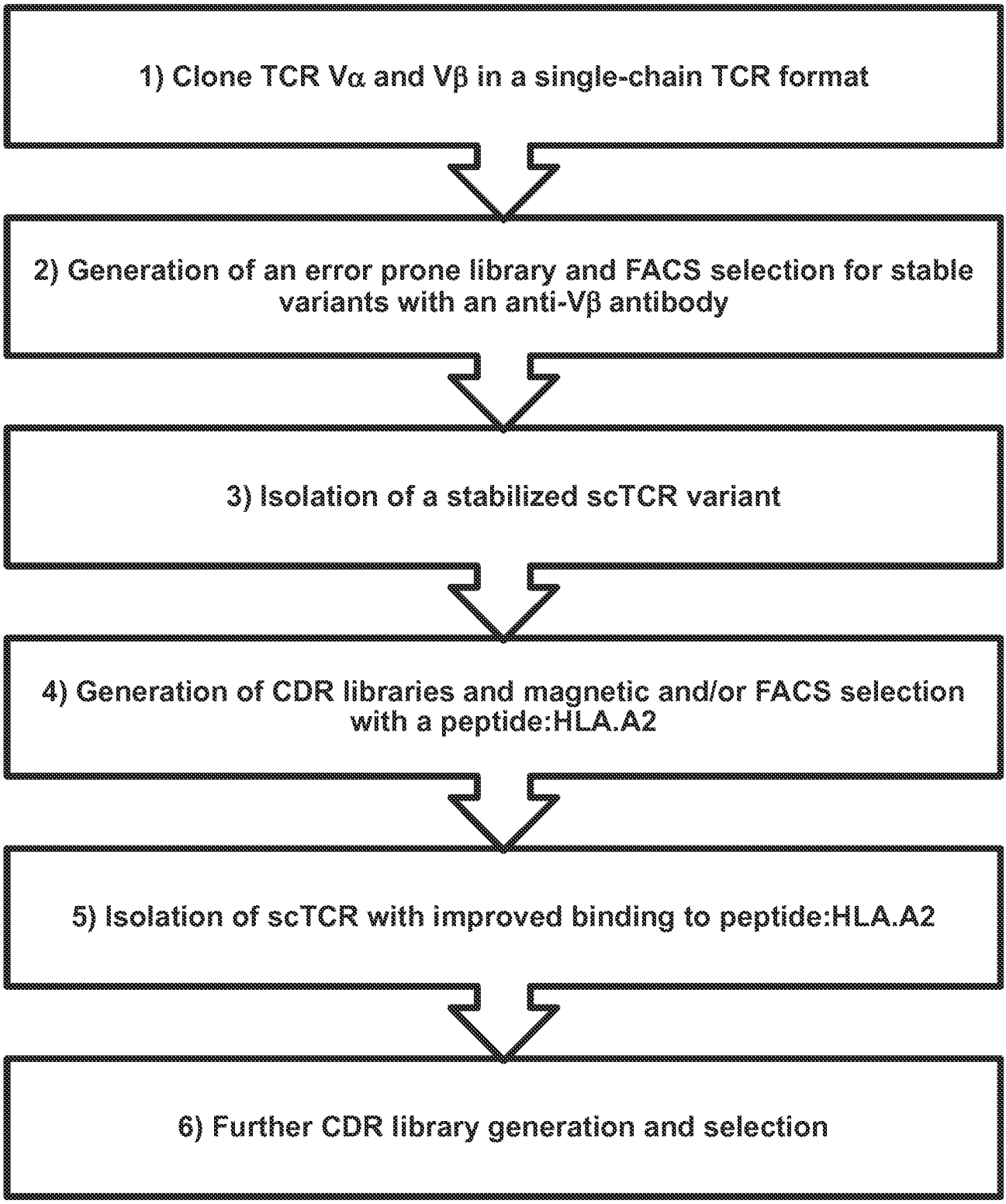

1) Clone TCR Vα and Vβ in a single-chain TCR format
2) Generation of an error prone library and FACS selection for stable variants with an anti-Vβ antibody
3) Isolation of a stabilized scTCR variant
4) Generation of CDR libraries and magnetic and/or FACS selection with a peptide:HLA.A2
5) Isolation of scTCR with improved binding to peptide:HLA.A2
6) Further CDR library generation and selection

FIG. 1

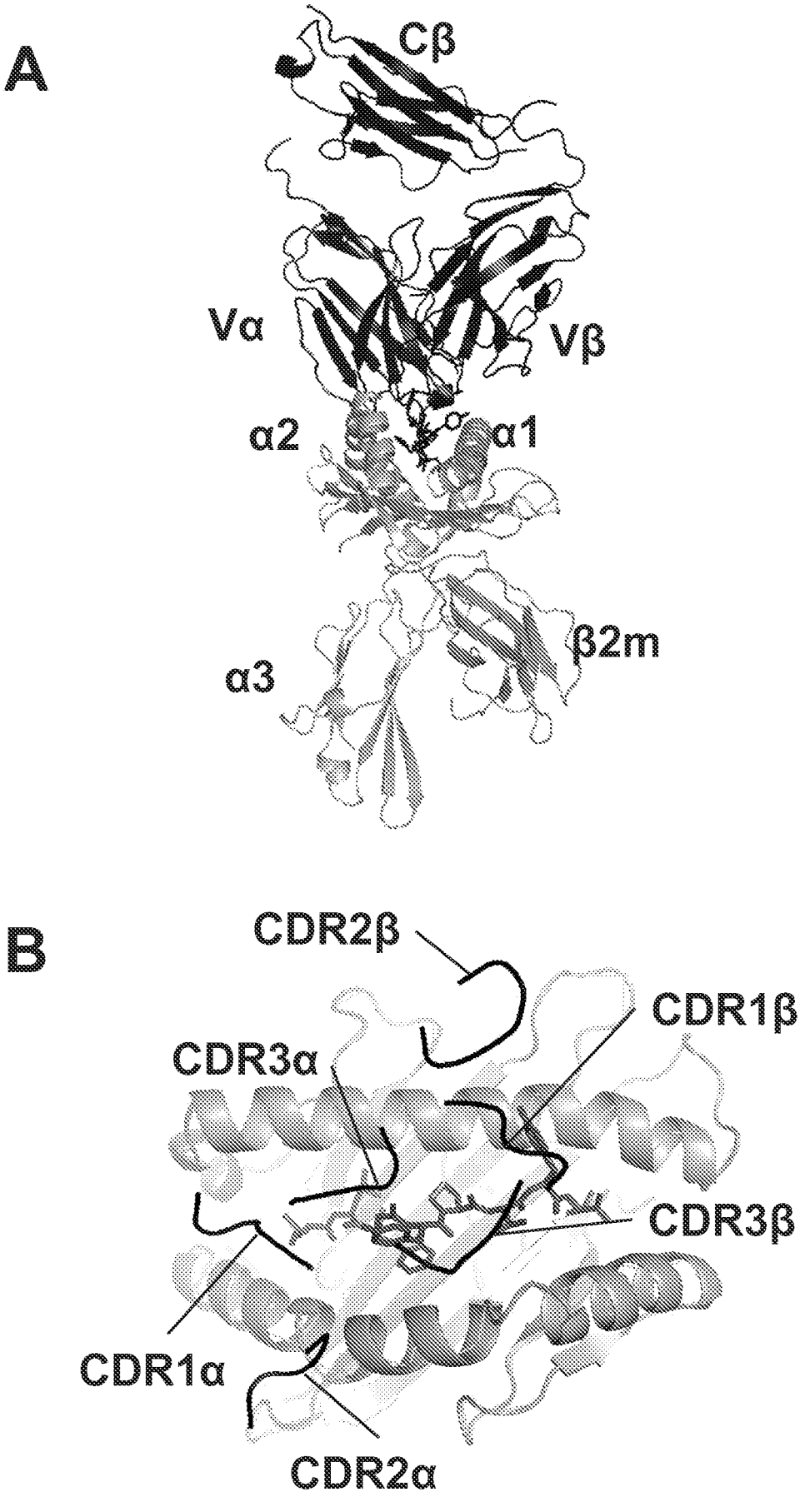


FIG. 2

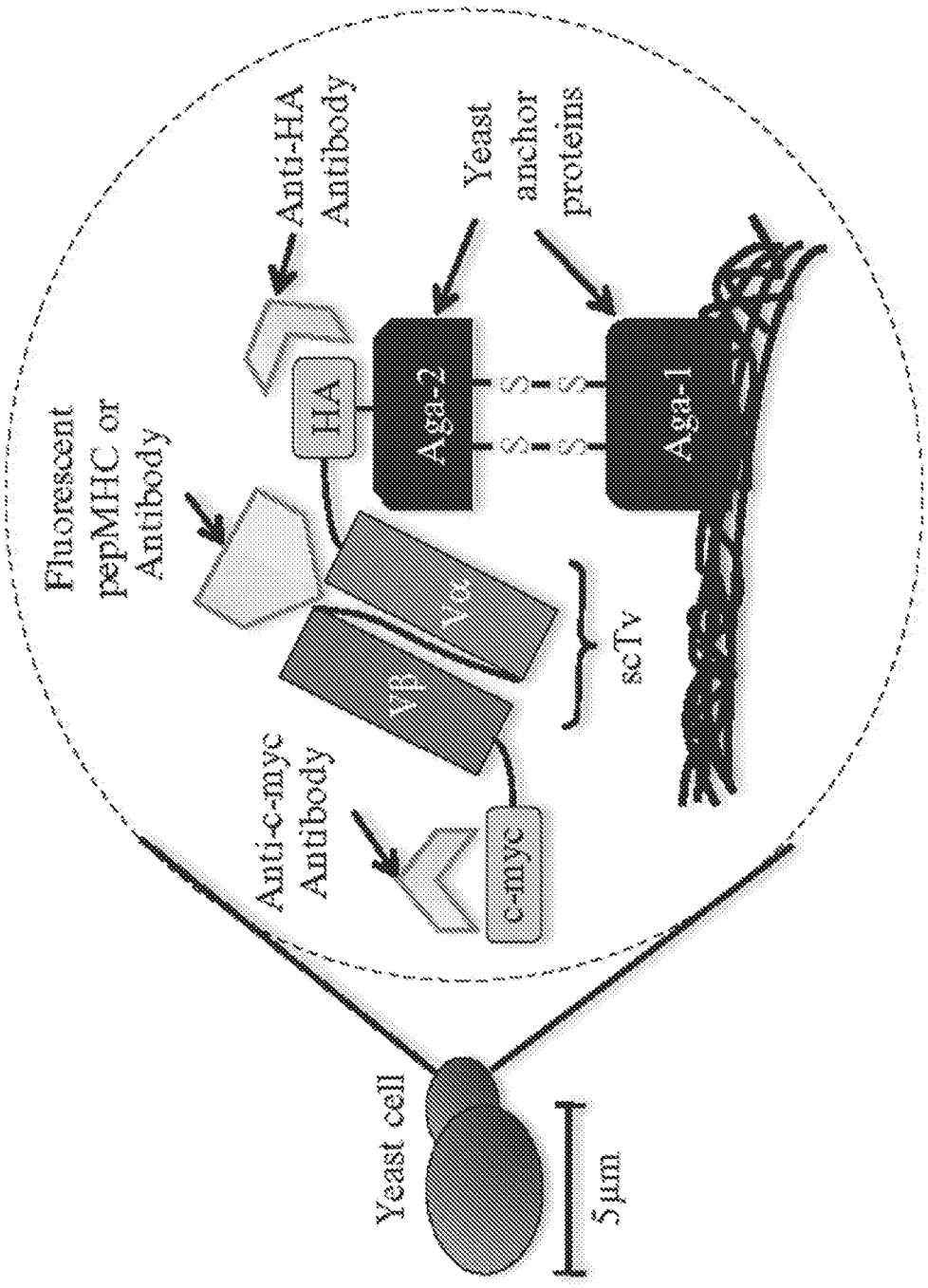

Fluorescent pepMHC or Antibody
Anti-HA Antibody
Yeast anchor proteins
HA
Aga-2
Aga-1
Vβ
Vα
scTv
Anti-c-myc Antibody
c-myc
Yeast cell
5 μm

FIG. 3

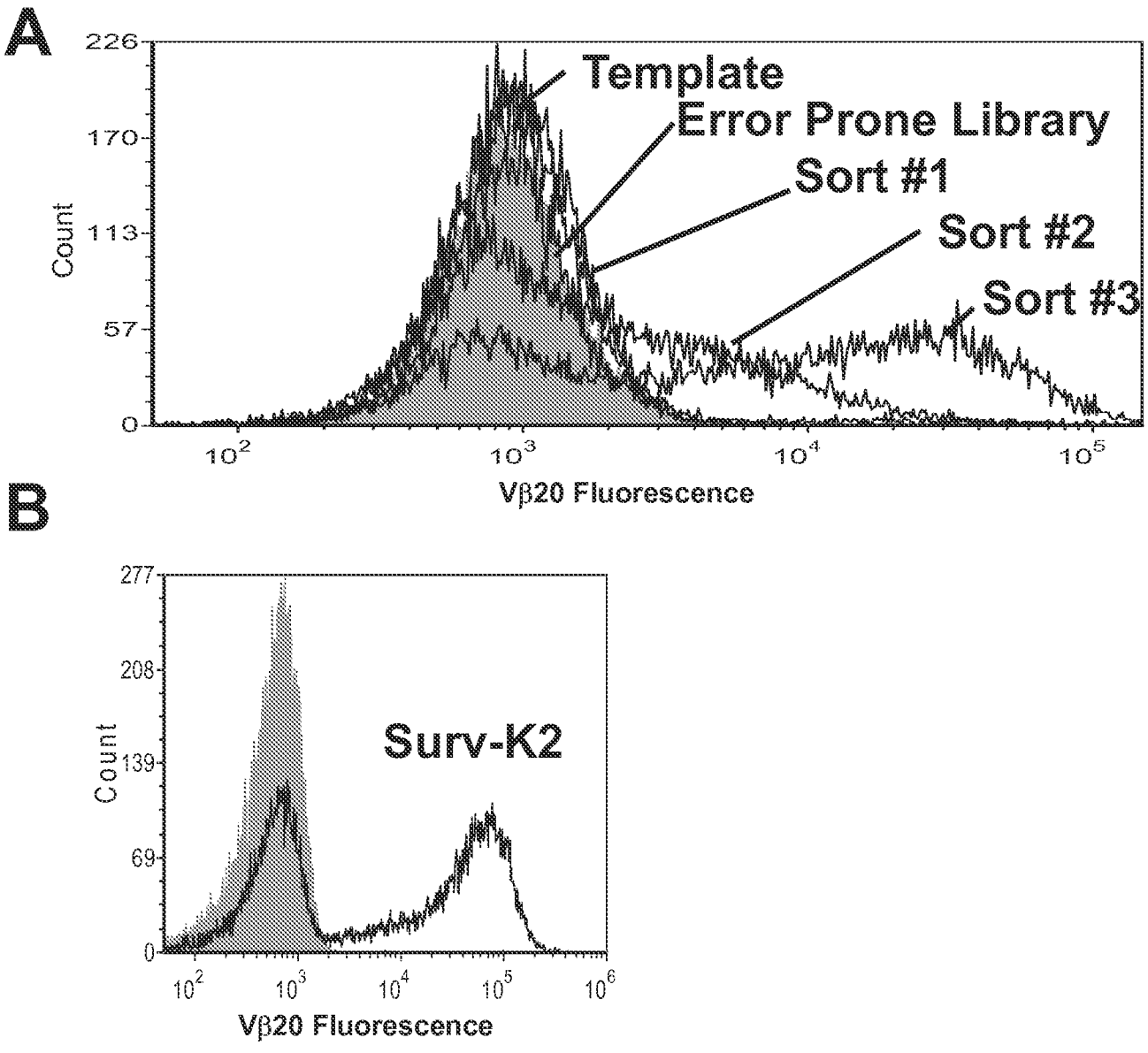

A
226
170
113
57
0
Count
Template
Error Prone Library
Sort #1
Sort #2
Sort #3
10^2
10^3
10^4
10^5
Vβ20 Fluorescence
B
277
208
139
69
0
Count
Surv-K2
10^2
10^3
10^4
10^5
10^6
Vβ20 Fluorescence

FIG. 4

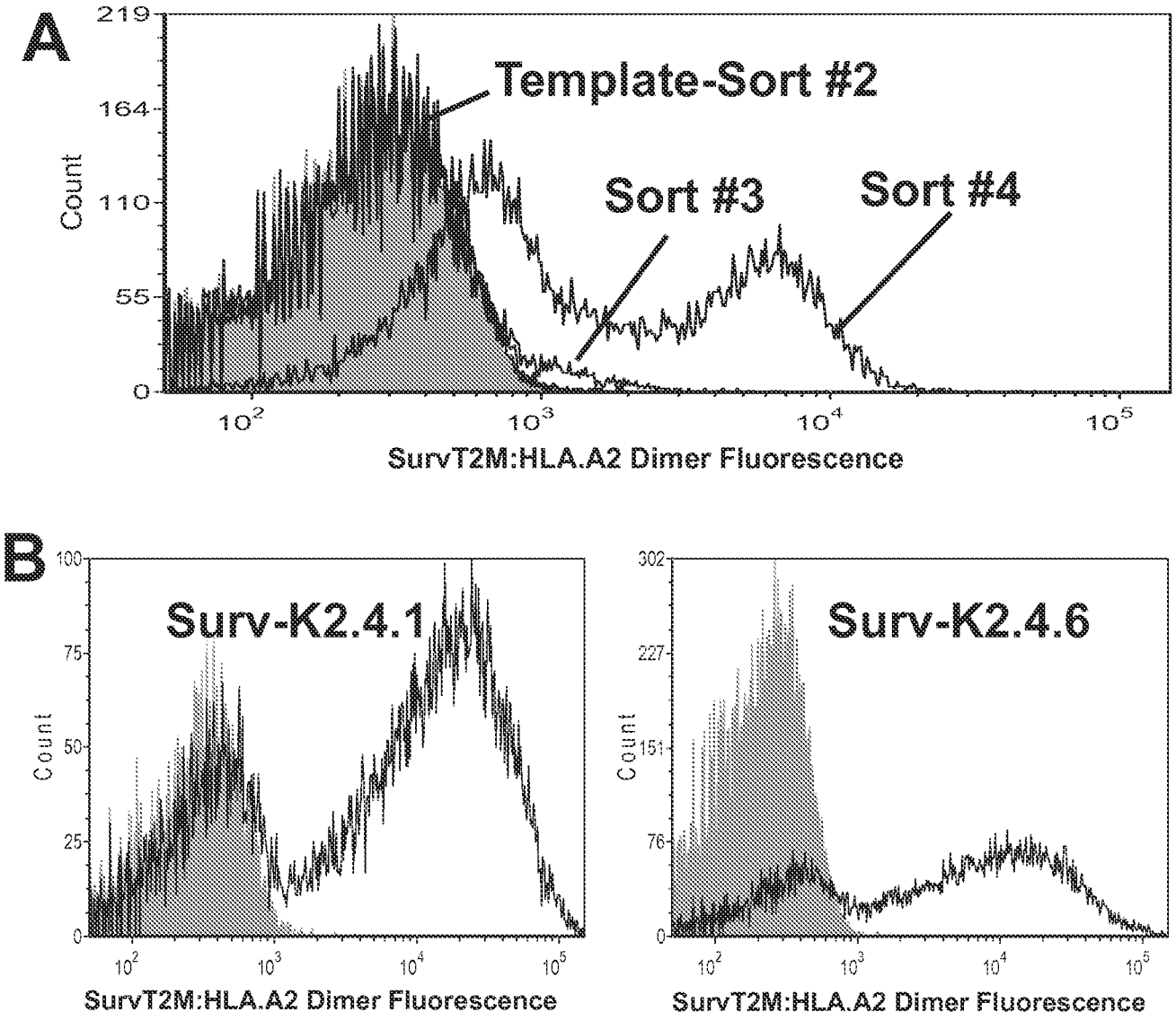
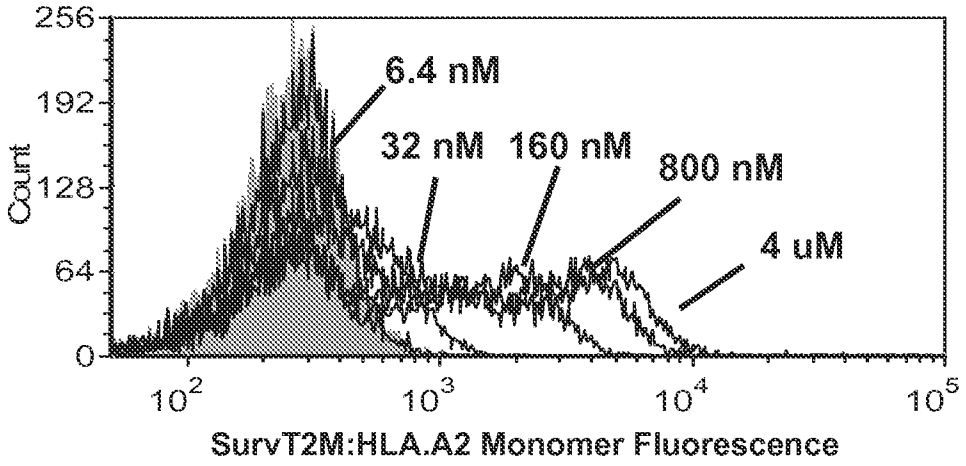

A
219
164
110
55
0
Count
Template-Sort #2
Sort #3
Sort #4
10^2
10^3
10^4
10^5
SurvT2M:HLA.A2 Dimer Fluorescence
B
100
75
50
25
0
Count
Surv-K2.4.1
10^2
10^3
10^4
10^5
SurvT2M:HLA.A2 Dimer Fluorescence
302
227
151
76
0
Count
Surv-K2.4.6
10^2
10^3
10^4
10^5
SurvT2M:HLA.A2 Dimer Fluorescence

FIG. 5

A


256
192
128
64
0
Count
6.4 nM
32 nM
160 nM
800 nM
4 uM
10^2
10^3
10^4
10^5
SurvT2M:HLA.A2 Monomer Fluorescence

B

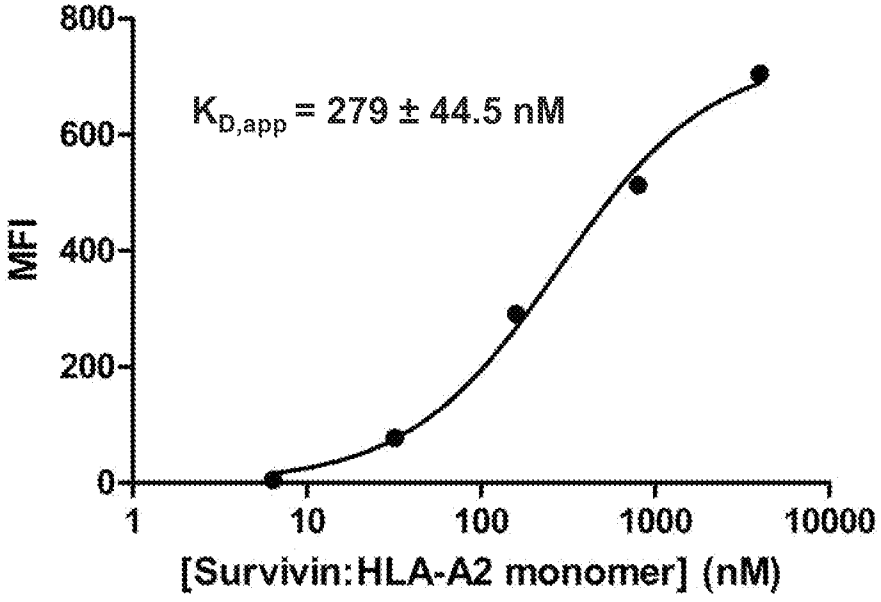

800
600
400
200
0
MFI
$K_{D,app}$ = 279 ± 44.5 nM
1
10
100
1000
10000
[Survivin:HLA-A2 monomer] (nM)

FIG. 6

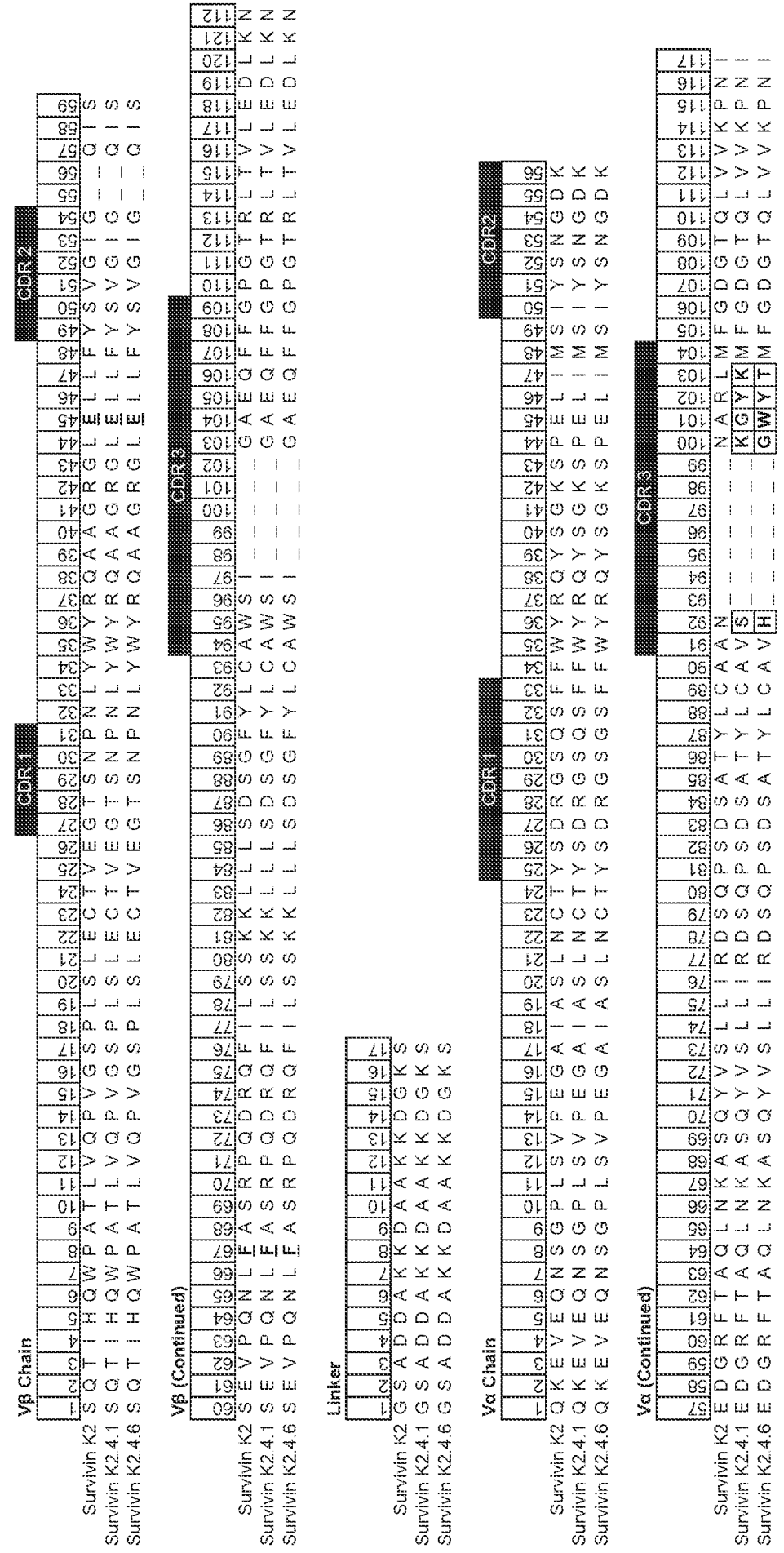
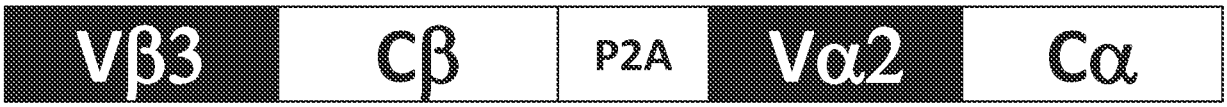
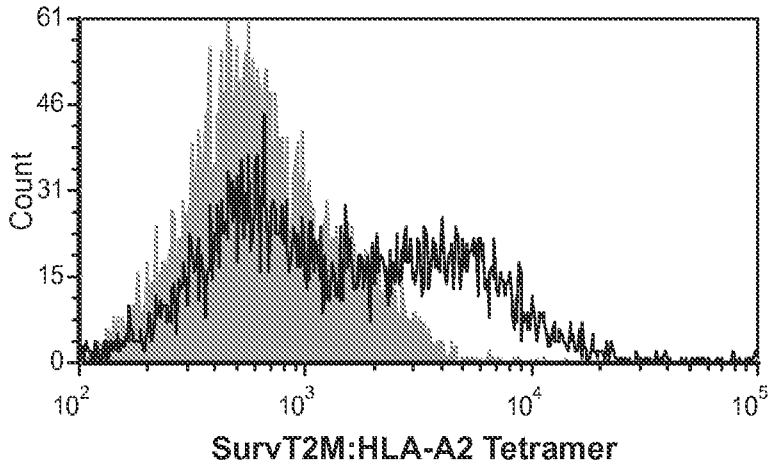

Vβ Chain
CDR1
CDR2
Survivin K2 SQTIHQWPATLVQPVGSPLSLECTVEGTSNPNLYWYRQAAGRGLELLFYSVGIG--QIS
Survivin K2.4.1 SQTIHQWPATLVQPVGSPLSLECTVEGTSNPNLYWYRQAAGRGLELLFYSVGIG--QIS
Survivin K2.4.6 SQTIHQWPATLVQPVGSPLSLECTVEGTSNPNLYWYRQAAGRGLELLFYSVGIG--QIS
Vβ (Continued)
CDR3
Survivin K2 SEVPQNLEASRPQDRQFILSSKKLLLSDSGFYLCAWSI-----GAEQFFGPGTRLTVLEDLKN
Survivin K2.4.1 SEVPQNLEASRPQDRQFILSSKKLLLSDSGFYLCAWSI-----GAEQFFGPGTRLTVLEDLKN
Survivin K2.4.6 SEVPQNLEASRPQDRQFILSSKKLLLSDSGFYLCAWSI-----GAEQFFGPGTRLTVLEDLKN
Linker
Survivin K2 GSADDAKKDAAKKDGKS
Survivin K2.4.1 GSADDAKKDAAKKDGKS
Survivin K2.4.6 GSADDAKKDAAKKDGKS
Vα Chain
CDR1
CDR2
Survivin K2 QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIYSNGDK
Survivin K2.4.1 QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIYSNGDK
Survivin K2.4.6 QKEVEQNSGPLSVPEGAIASLNCTYSDRGSSGFFWYRQYSGKSPELIMSIYSNGDK
Vα (Continued)
CDR3
Survivin K2 EDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVSNARLMFGDGTQLVVKPNI
Survivin K2.4.1 EDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVSKGYKMFGDGTQLVVKPNI
Survivin K2.4.6 EDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVHGWYTMFGDGTQLVVKPNI

FIG. 7

A


Vβ3
Cβ
P2A
Vα2
Cα

B


61
46
31
15
0
Count
10^2
10^3
10^4
10^5
SurvT2M:HLA-A2 Tetramer

C

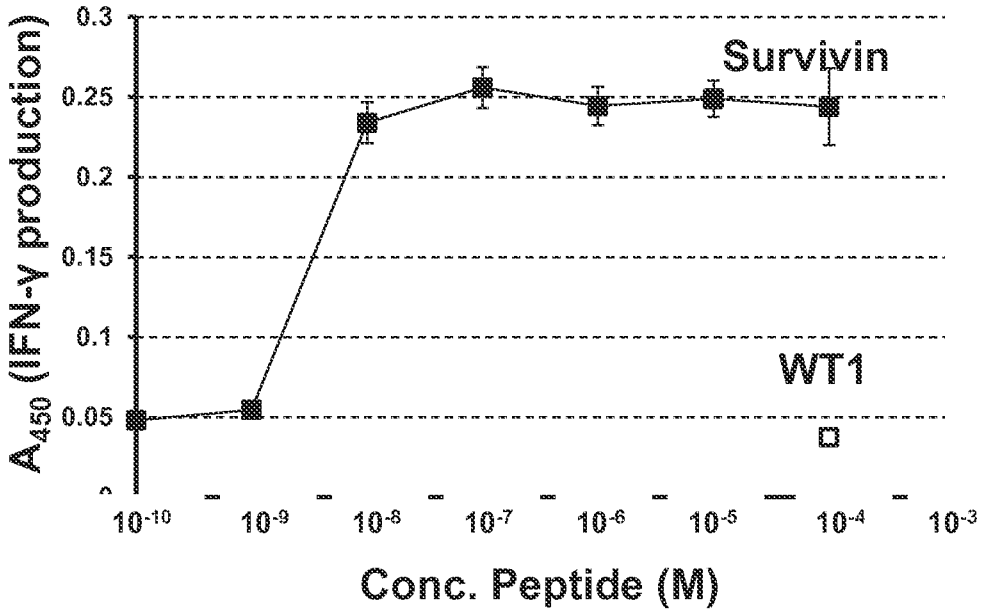

0.3
0.25
0.2
0.15
0.1
0.05
A450 (IFN-γ production)
Survivin
WT1
10-10
10-9
10-8
10-7
10-6
10-5
10-4
10-3
Conc. Peptide (M)

FIG. 8

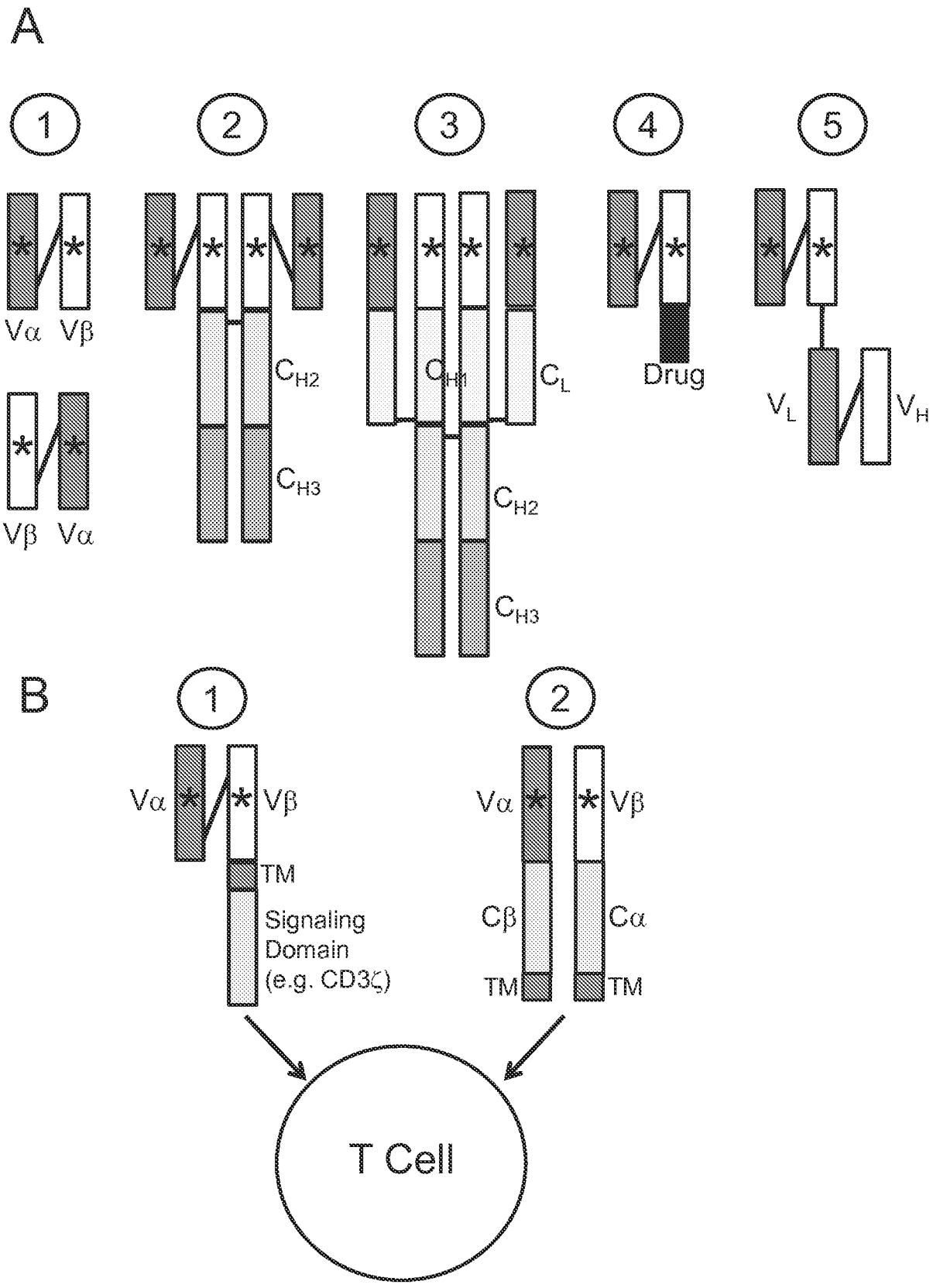

A
1
2
3
4
5
Vα
Vβ
Vβ
Vα
C_{H2}
C_{H3}
C_{H1}
C_L
C_{H2}
C_{H3}
Drug
V_L
V_H
B
1
2
Vα
Vβ
TM
Signaling
Domain
(e.g. CD3ζ)
Vα
Vβ
Cβ
Cα
TM
TM
T Cell

FIG. 9