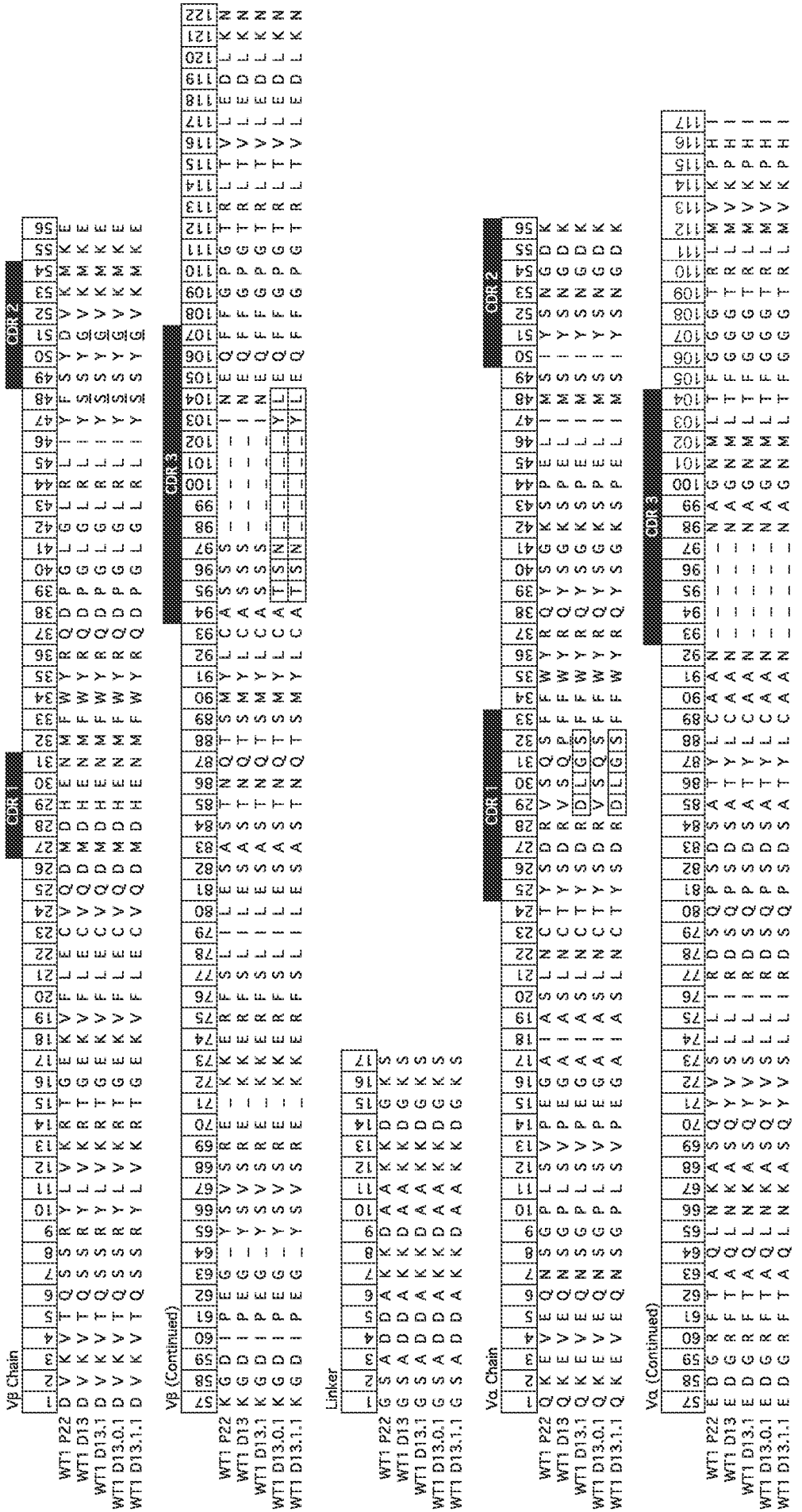

Vβ Chain
CDR1
CDR2
Vβ (Continued)
CDR3
Linker
Vα Chain
CDR1
CDR2
Vα (Continued)
CDR3
WT1 P22
WT1 D13
WT1 D13.1
WT1 D13.0.1
WT1 D13.1.1

FIG. 1

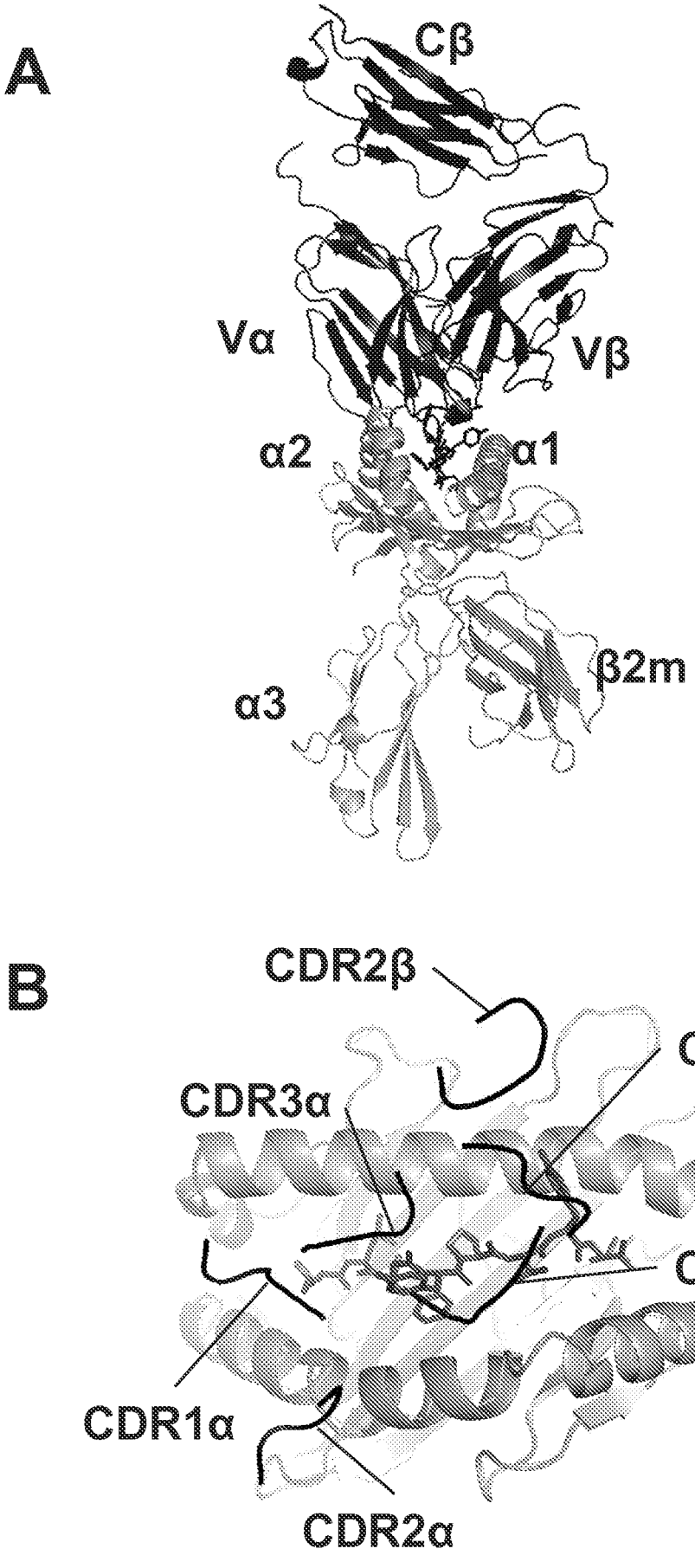


FIG. 2

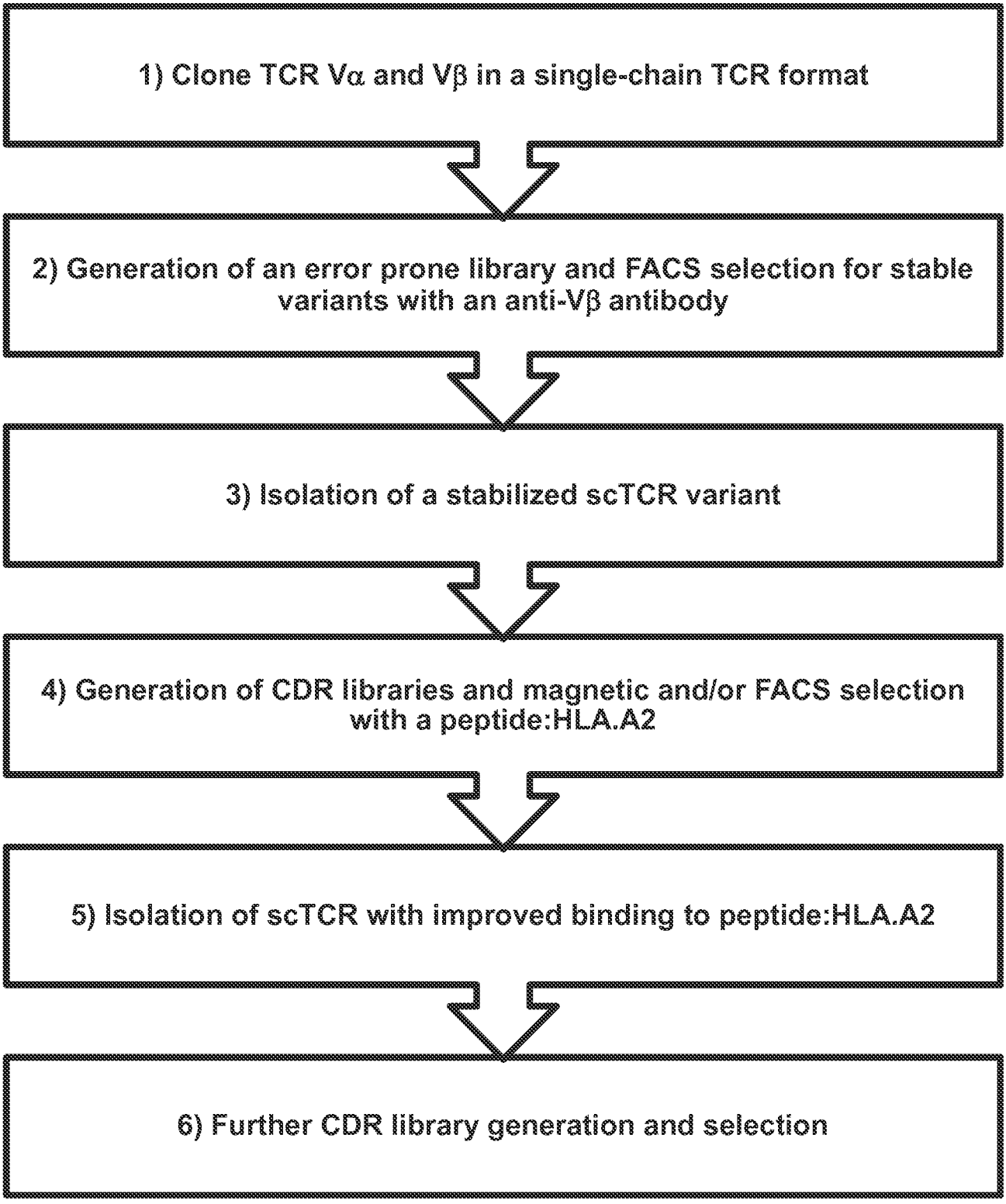

1) Clone TCR Vα and Vβ in a single-chain TCR format
2) Generation of an error prone library and FACS selection for stable variants with an anti-Vβ antibody
3) Isolation of a stabilized scTCR variant
4) Generation of CDR libraries and magnetic and/or FACS selection with a peptide:HLA.A2
5) Isolation of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

FIG. 3

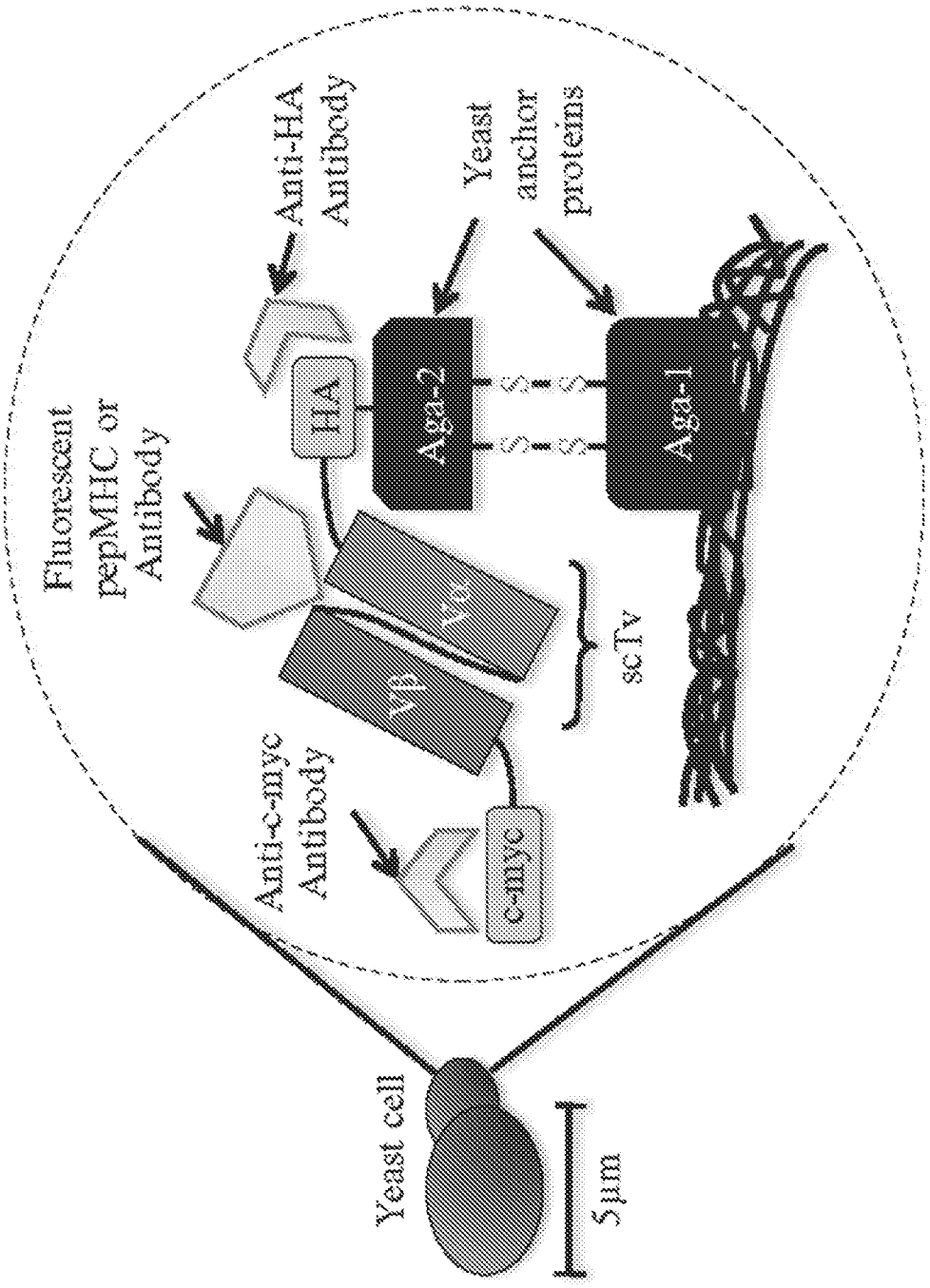

Yeast cell
5 µm
Anti-c-myc Antibody
c-myc
Vβ
Vα
scTv
Fluorescent pepMHC or Antibody
Anti-HA Antibody
HA
Aga-2
Yeast anchor proteins
Aga-1

FIG. 4

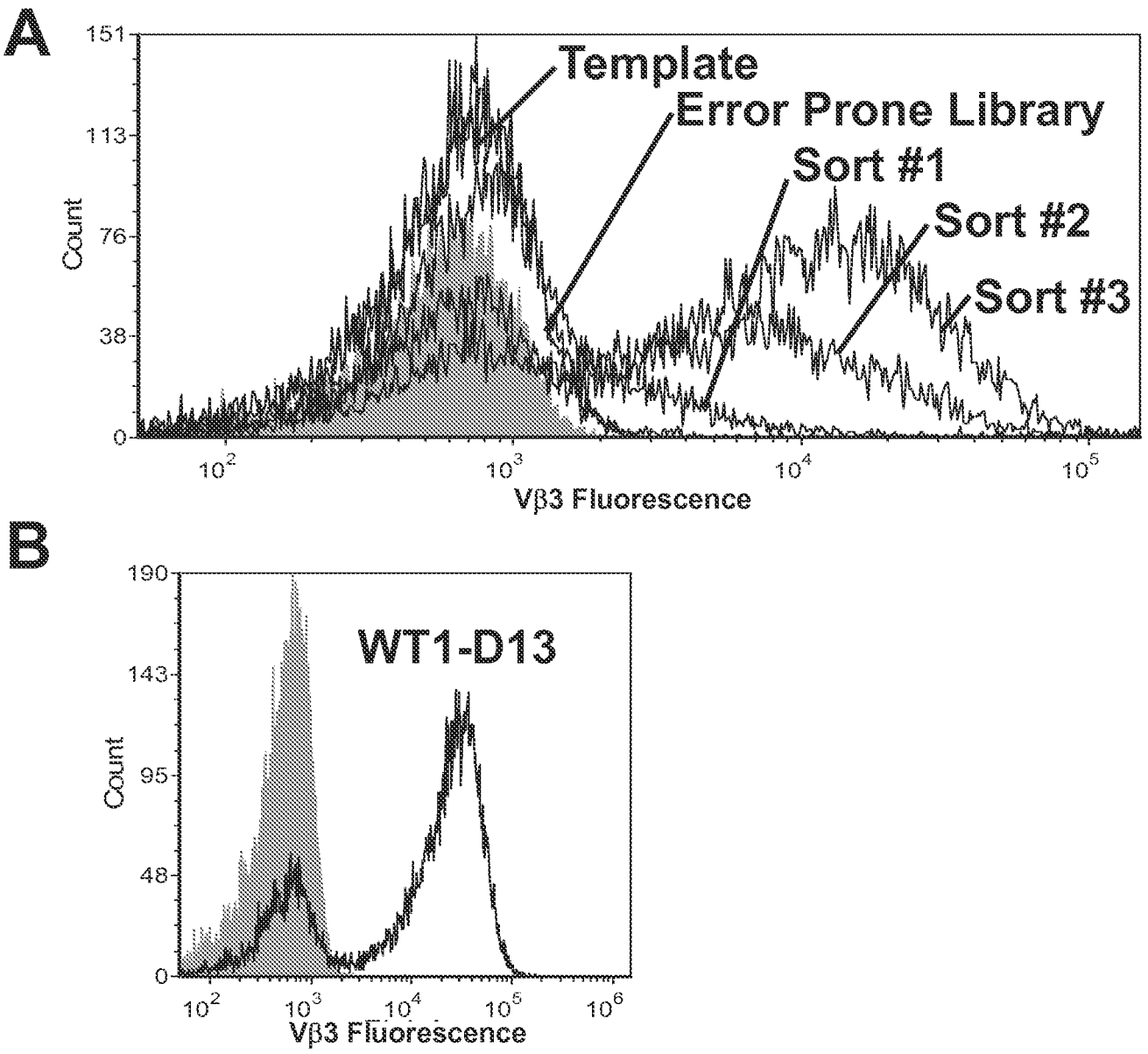

A
151
113
76
38
0
Count
Template
Error Prone Library
Sort #1
Sort #2
Sort #3
10^2
10^3
10^4
10^5
Vβ3 Fluorescence
B
190
143
95
48
0
Count
WT1-D13
10^2
10^3
10^4
10^5
10^6
Vβ3 Fluorescence

FIG. 5

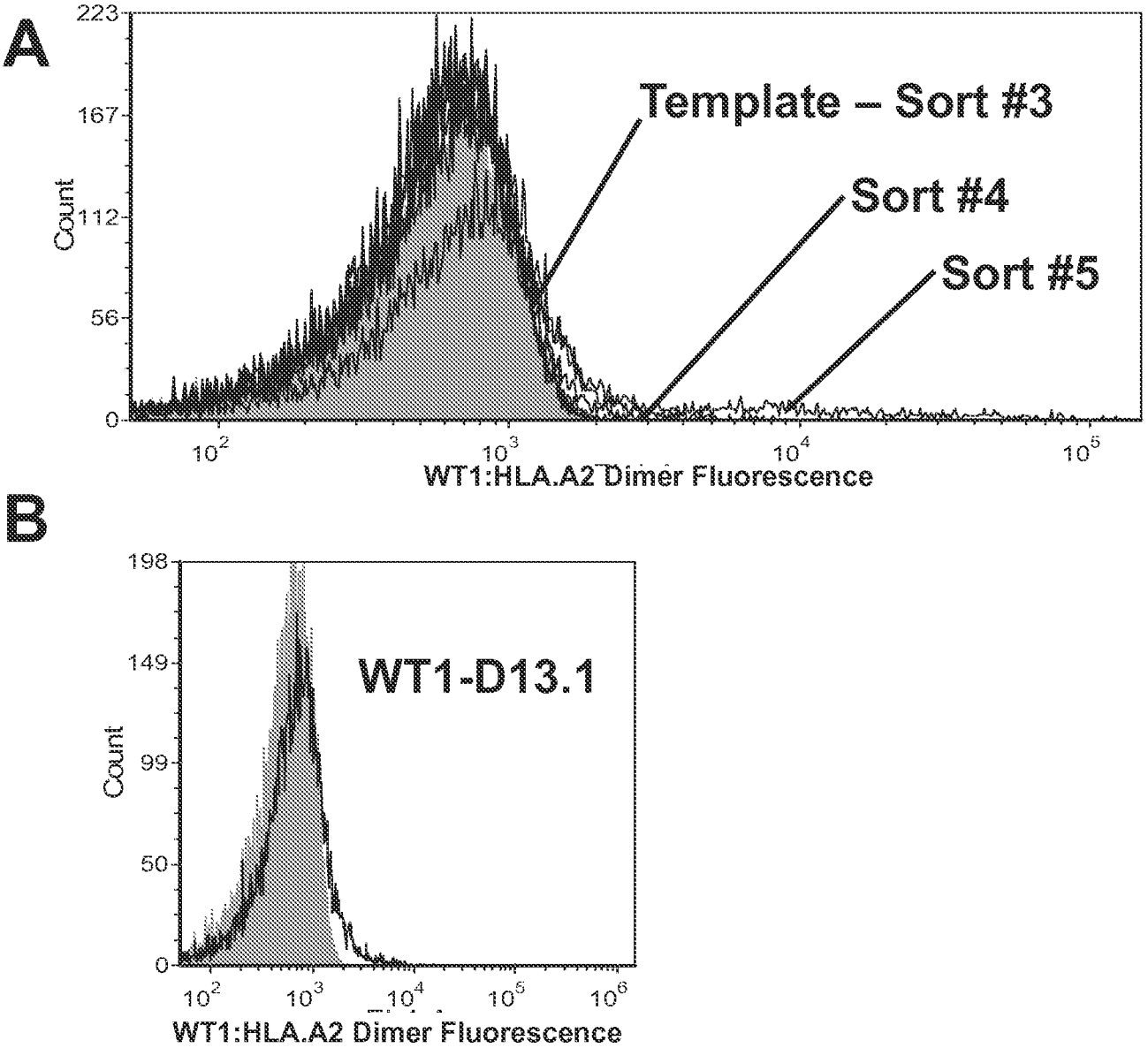

A
223
167
112
56
0
Count
Template – Sort #3
Sort #4
Sort #5
10^2
10^3
10^4
10^5
WT1:HLA.A2 Dimer Fluorescence
B
198
149
99
50
0
Count
WT1-D13.1
10^2
10^3
10^4
10^5
10^6
WT1:HLA.A2 Dimer Fluorescence

FIG. 6

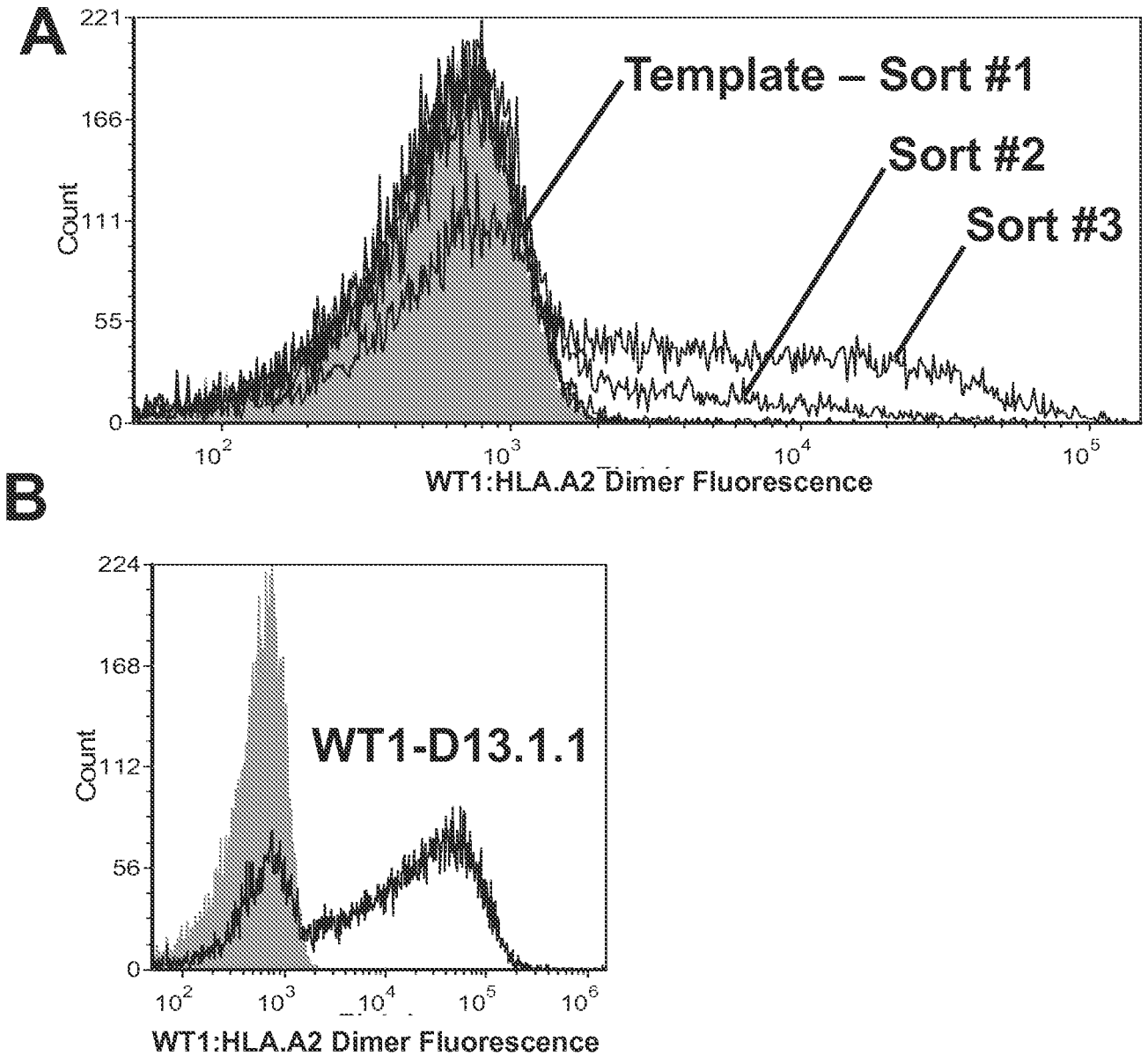

A
221
166
111
55
0
Count
Template – Sort #1
Sort #2
Sort #3
10^2
10^3
10^4
10^5
WT1:HLA.A2 Dimer Fluorescence
B
224
168
112
56
0
Count
WT1-D13.1.1
10^2
10^3
10^4
10^5
10^6
WT1:HLA.A2 Dimer Fluorescence

FIG. 7

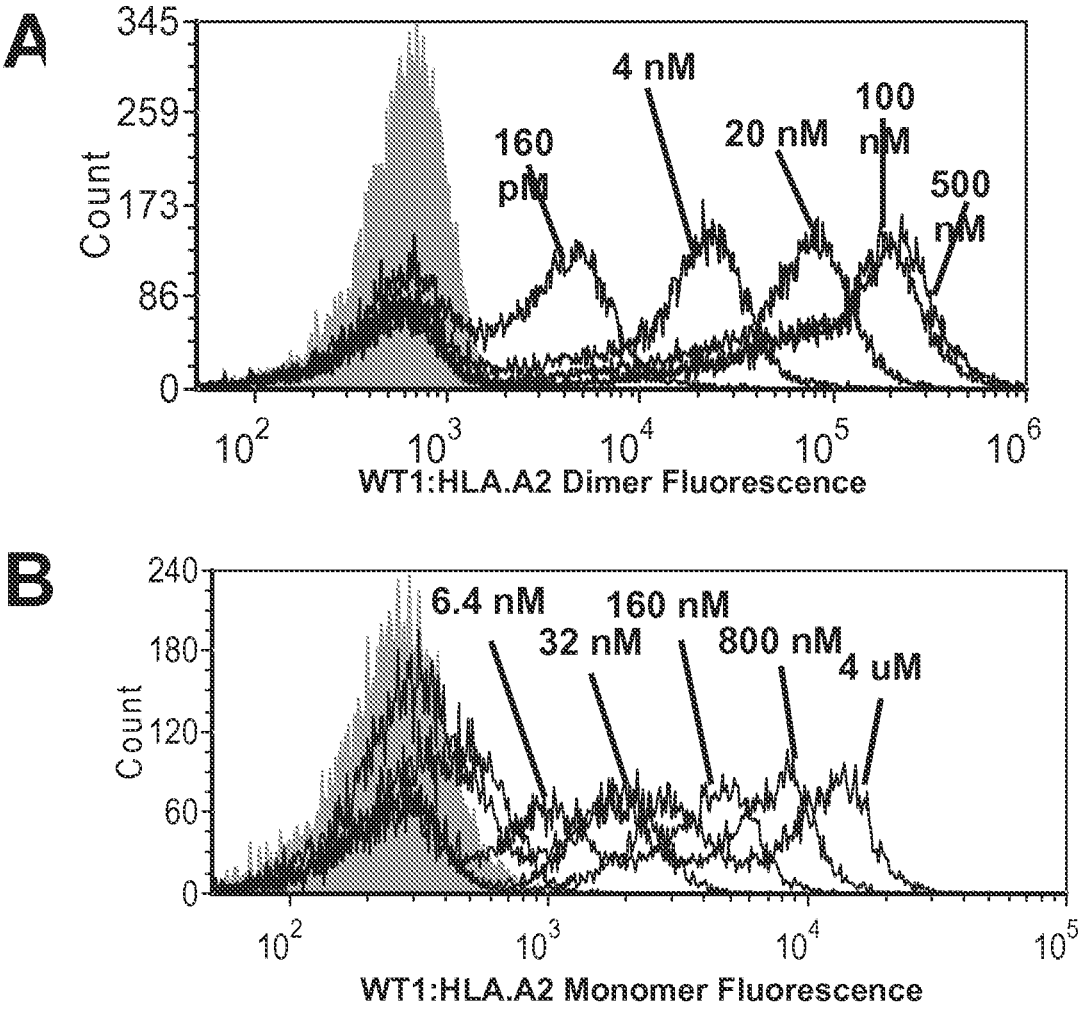

A
345
259
173
86
0
Count
160 pM
4 nM
20 nM
100 nM
500 nM
10^2
10^3
10^4
10^5
10^6
WT1:HLA.A2 Dimer Fluorescence
B
240
180
120
60
0
Count
6.4 nM
32 nM
160 nM
800 nM
4 uM
10^2
10^3
10^4
10^5
WT1:HLA.A2 Monomer Fluorescence

FIG. 8

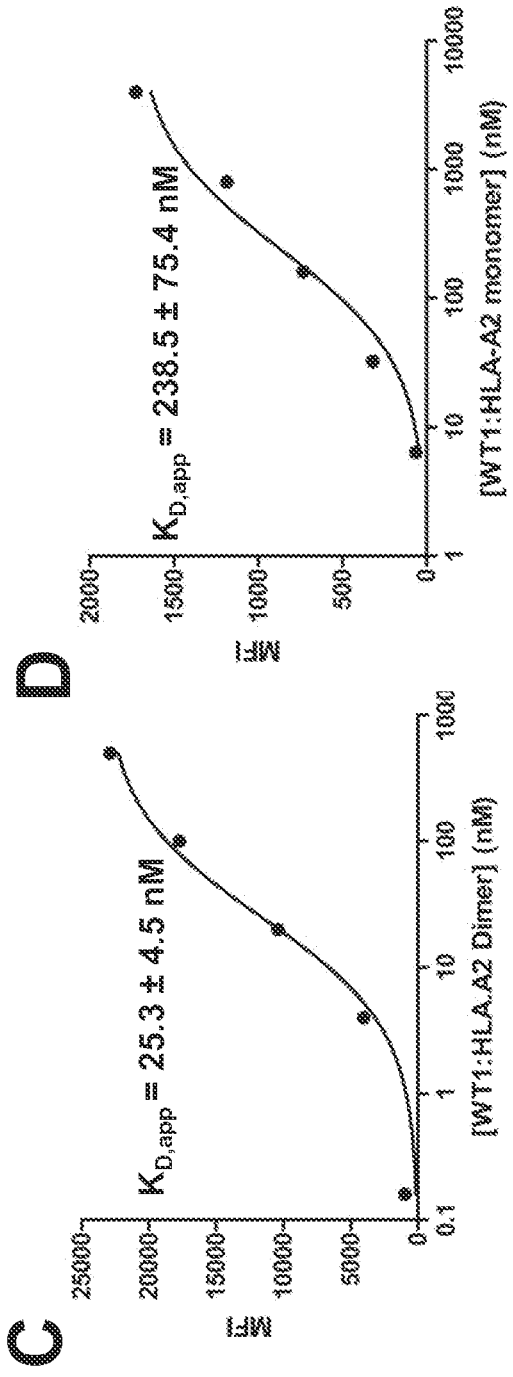

C
D
MFI
$K_{D,app}$ = 25.3 ± 4.5 nM
$K_{D,app}$ = 238.5 ± 75.4 nM
[WT1:HLA-A2 Dimer] (nM)
[WT1:HLA-A2 monomer] (nM)

FIG. 8

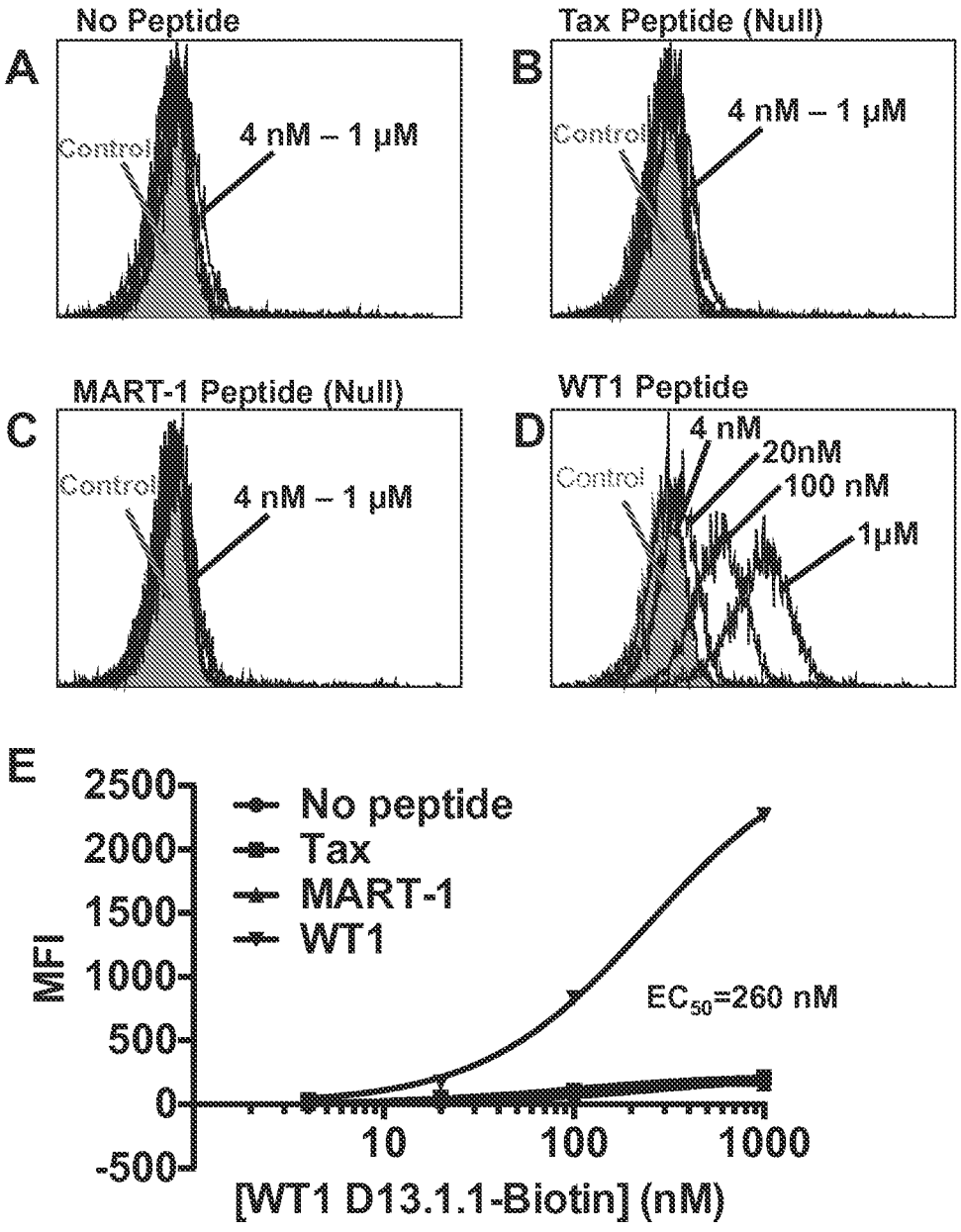

A
No Peptide
Control
4 nM – 1 μM
B
Tax Peptide (Null)
Control
4 nM – 1 μM
C
MART-1 Peptide (Null)
Control
4 nM – 1 μM
D
WT1 Peptide
Control
4 nM
20nM
100 nM
1μM
E
MFI
2500
2000
1500
1000
500
0
-500
No peptide
Tax
MART-1
WT1
EC50=260 nM
10
100
1000
[WT1 D13.1.1-Biotin] (nM)

FIG. 9

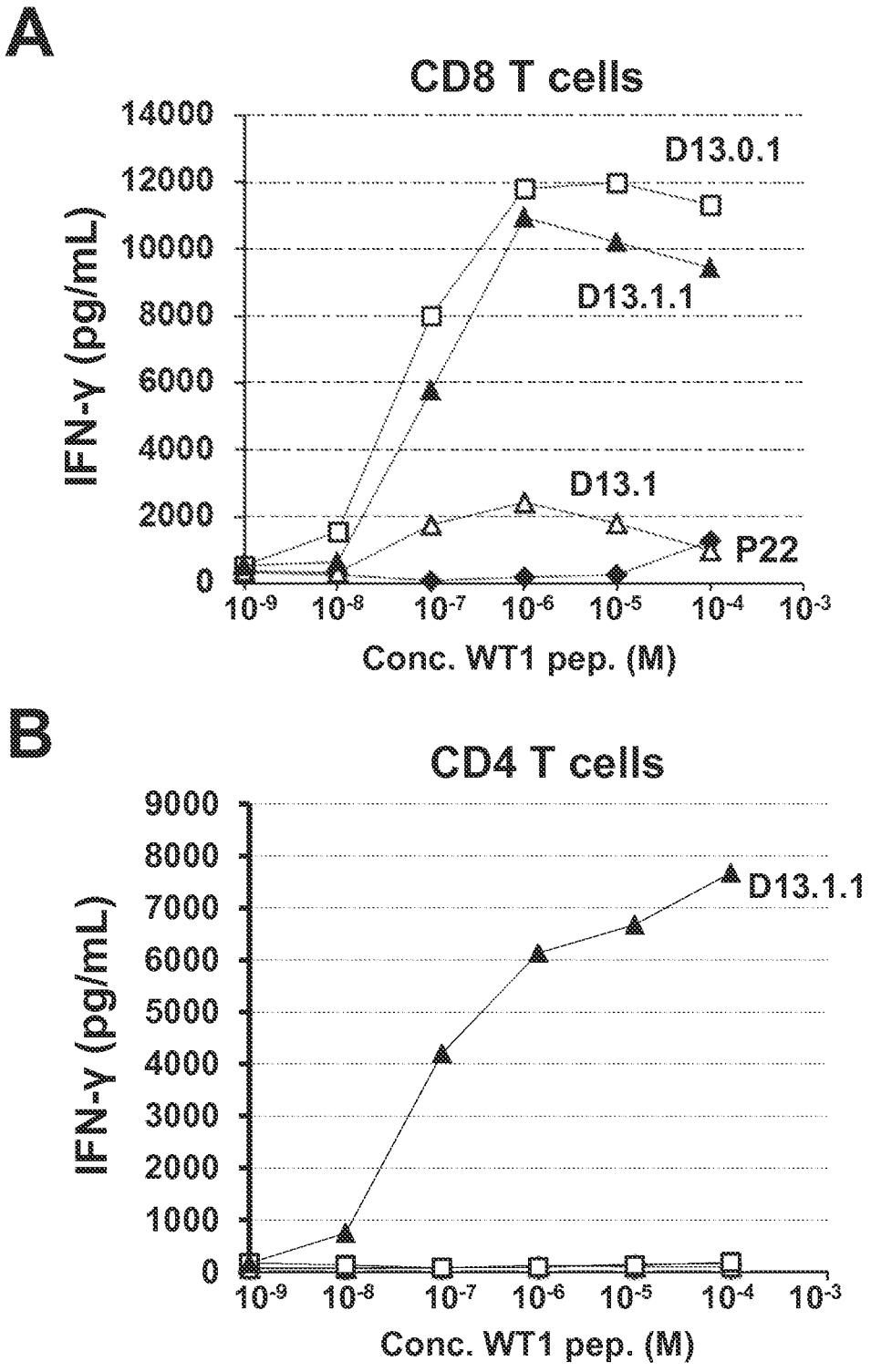

A
CD8 T cells
IFN-γ (pg/mL)
14000
12000
10000
8000
6000
4000
2000
0
D13.0.1
D13.1.1
D13.1
P22
10-9
10-8
10-7
10-6
10-5
10-4
10-3
Conc. WT1 pep. (M)
B
CD4 T cells
IFN-γ (pg/mL)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
D13.1.1
10-9
10-8
10-7
10-6
10-5
10-4
10-3
Conc. WT1 pep. (M)

FIG. 10

Number	Peptide
WT1	RMFPNAPYL
#1	KLFSQLSYL
#2	KLFSTAMFL
#3	HMWGQSLYI
#4	KIFGSLAFL
#5	KIFPSCLFV
#6	KLWPSVAFA
#7	HLWGQSLYI
#8	RIWLSVVFV
#9	HLFSSLPFA
#10	RIFSTALFV

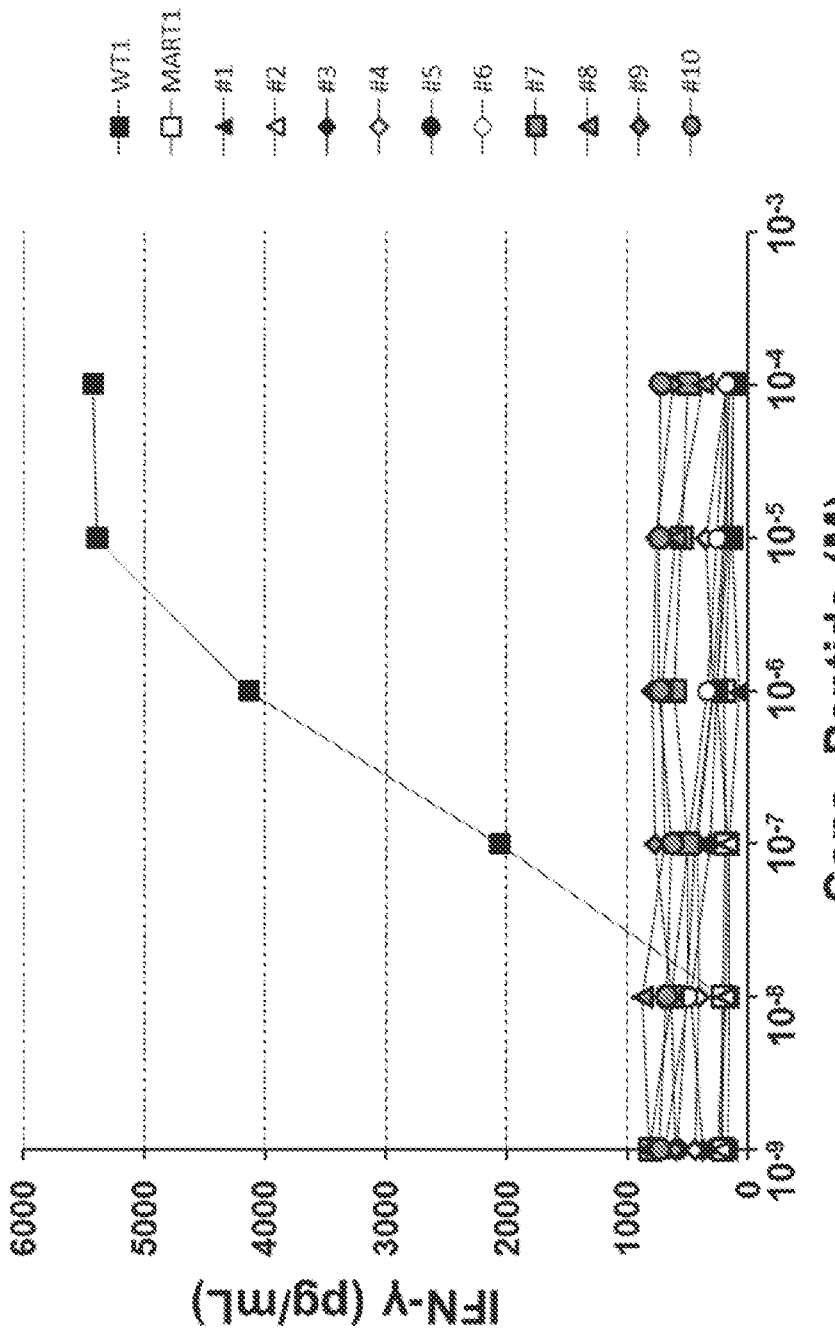


FIG. 11

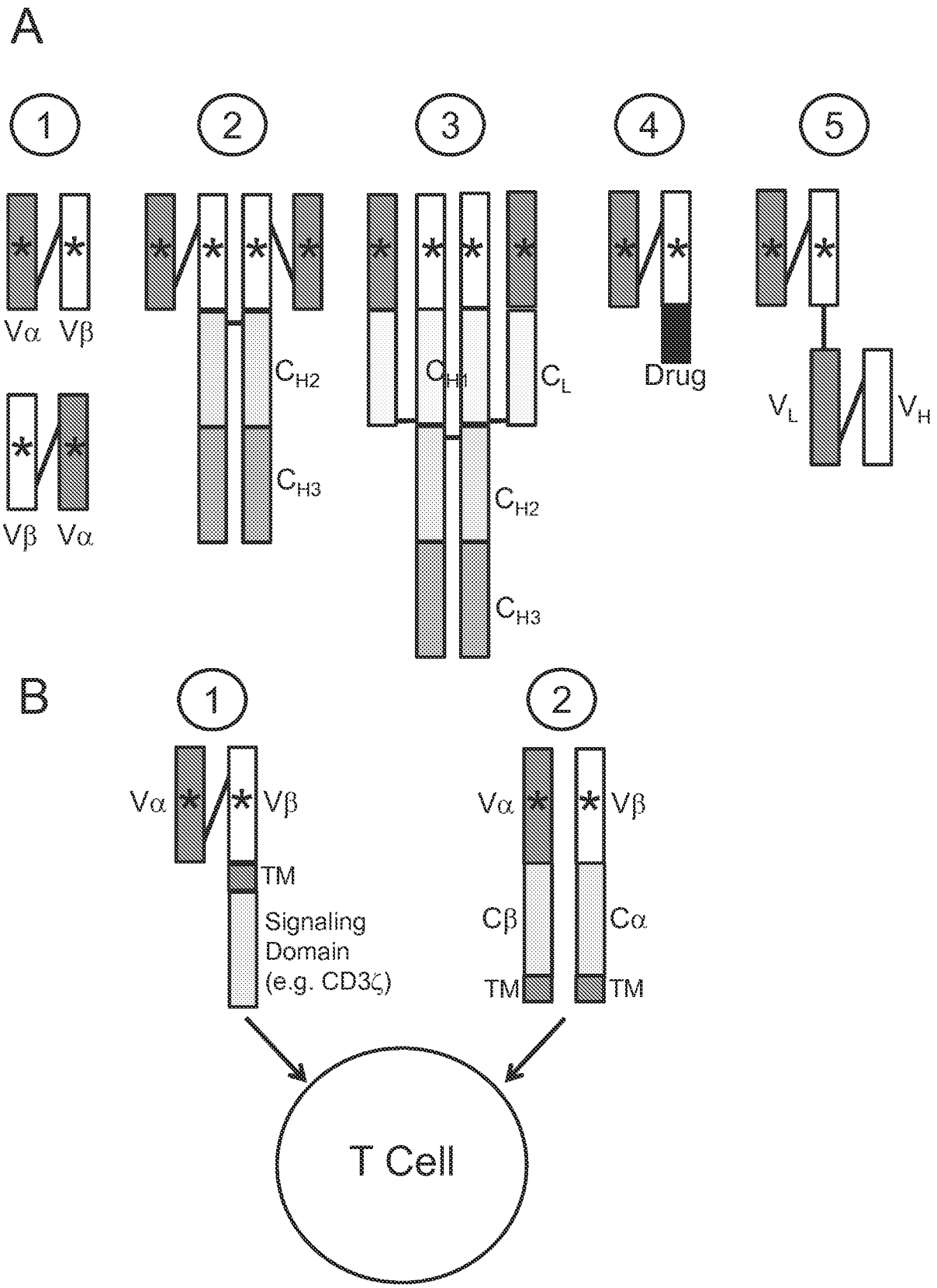

A
1
2
3
4
5
Vα
Vβ
Vβ
Vα
C_{H2}
C_{H3}
C_{H1}
C_L
C_{H2}
C_{H3}
Drug
V_L
V_H
B
1
2
Vα
Vβ
TM
Signaling
Domain
(e.g. CD3ζ)
Vα
Vβ
Cβ
Cα
TM
TM
T Cell

FIG. 12