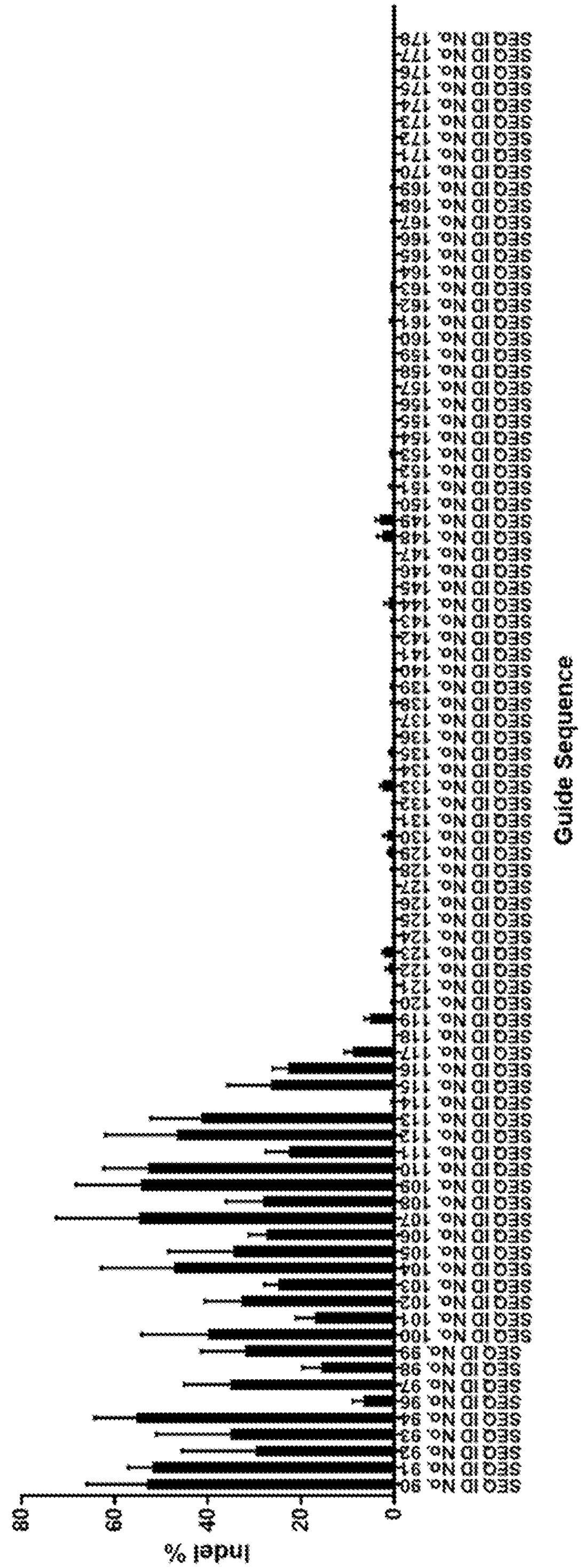

Indel %
0
20
40
60
80
SEQ ID No. 90
SEQ ID No. 91
SEQ ID No. 92
SEQ ID No. 93
SEQ ID No. 94
SEQ ID No. 95
SEQ ID No. 96
SEQ ID No. 97
SEQ ID No. 98
SEQ ID No. 99
SEQ ID No. 100
SEQ ID No. 101
SEQ ID No. 102
SEQ ID No. 103
SEQ ID No. 104
SEQ ID No. 105
SEQ ID No. 106
SEQ ID No. 107
SEQ ID No. 108
SEQ ID No. 109
SEQ ID No. 110
SEQ ID No. 111
SEQ ID No. 112
SEQ ID No. 113
SEQ ID No. 114
SEQ ID No. 115
SEQ ID No. 116
SEQ ID No. 117
SEQ ID No. 118
SEQ ID No. 119
SEQ ID No. 120
SEQ ID No. 121
SEQ ID No. 122
SEQ ID No. 123
SEQ ID No. 124
SEQ ID No. 125
SEQ ID No. 126
SEQ ID No. 127
SEQ ID No. 128
SEQ ID No. 129
SEQ ID No. 130
SEQ ID No. 131
SEQ ID No. 132
SEQ ID No. 133
SEQ ID No. 134
SEQ ID No. 135
SEQ ID No. 136
SEQ ID No. 137
SEQ ID No. 138
SEQ ID No. 139
SEQ ID No. 140
SEQ ID No. 141
SEQ ID No. 142
SEQ ID No. 143
SEQ ID No. 144
SEQ ID No. 145
SEQ ID No. 146
SEQ ID No. 147
SEQ ID No. 148
SEQ ID No. 149
SEQ ID No. 150
SEQ ID No. 151
SEQ ID No. 152
SEQ ID No. 153
SEQ ID No. 154
SEQ ID No. 155
SEQ ID No. 156
SEQ ID No. 157
SEQ ID No. 158
SEQ ID No. 159
SEQ ID No. 160
SEQ ID No. 161
SEQ ID No. 162
SEQ ID No. 163
SEQ ID No. 164
SEQ ID No. 165
SEQ ID No. 166
SEQ ID No. 167
SEQ ID No. 168
SEQ ID No. 169
SEQ ID No. 170
SEQ ID No. 171
SEQ ID No. 172
SEQ ID No. 173
SEQ ID No. 174
SEQ ID No. 175
SEQ ID No. 176
SEQ ID No. 177
SEQ ID No. 178
Guide Sequence

FIG 1

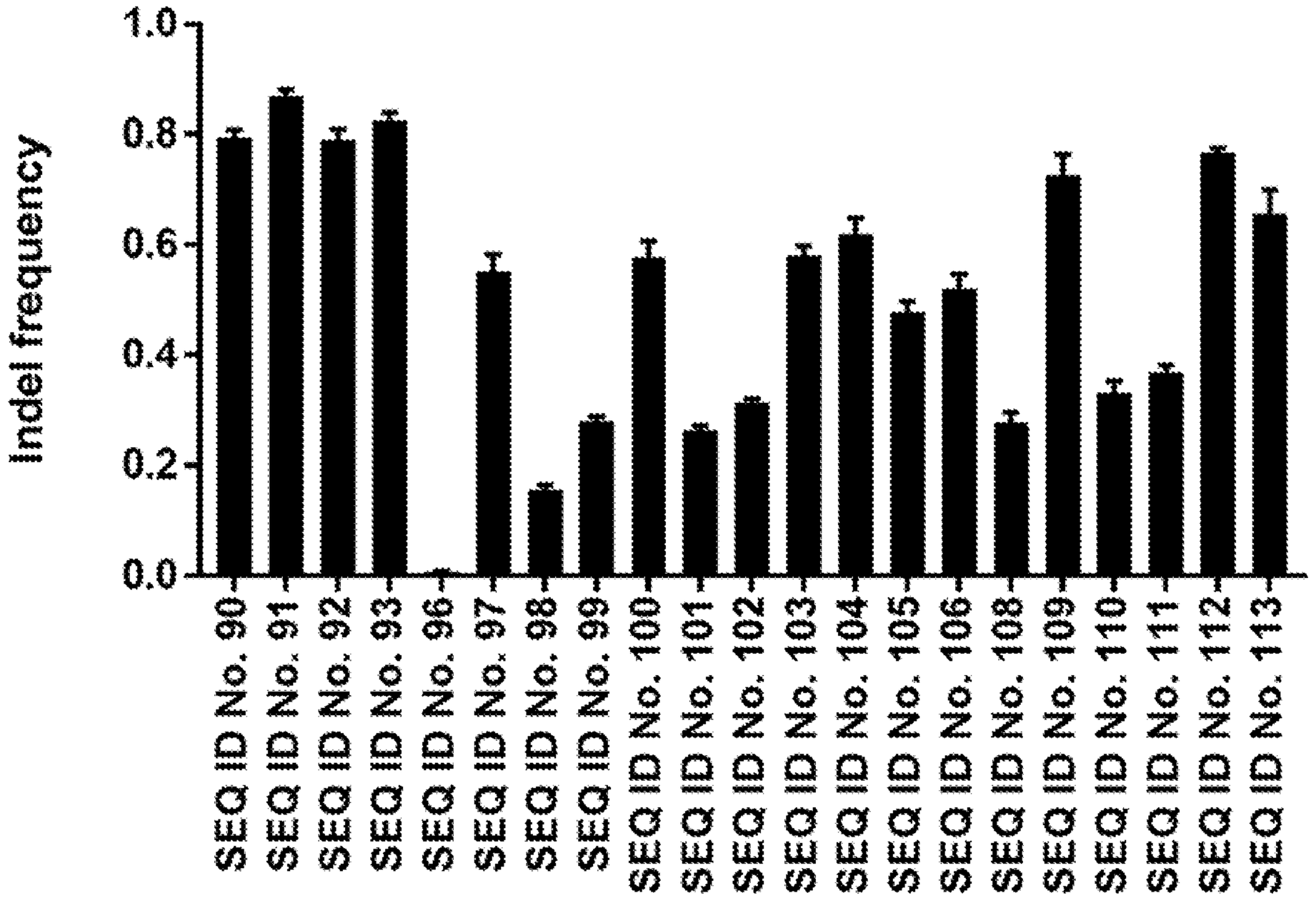

Indel frequency
1.0
0.8
0.6
0.4
0.2
0.0
SEQ ID No. 90
SEQ ID No. 91
SEQ ID No. 92
SEQ ID No. 93
SEQ ID No. 96
SEQ ID No. 97
SEQ ID No. 98
SEQ ID No. 99
SEQ ID No. 100
SEQ ID No. 101
SEQ ID No. 102
SEQ ID No. 103
SEQ ID No. 104
SEQ ID No. 105
SEQ ID No. 106
SEQ ID No. 108
SEQ ID No. 109
SEQ ID No. 110
SEQ ID No. 111
SEQ ID No. 112
SEQ ID No. 113

FIG 2

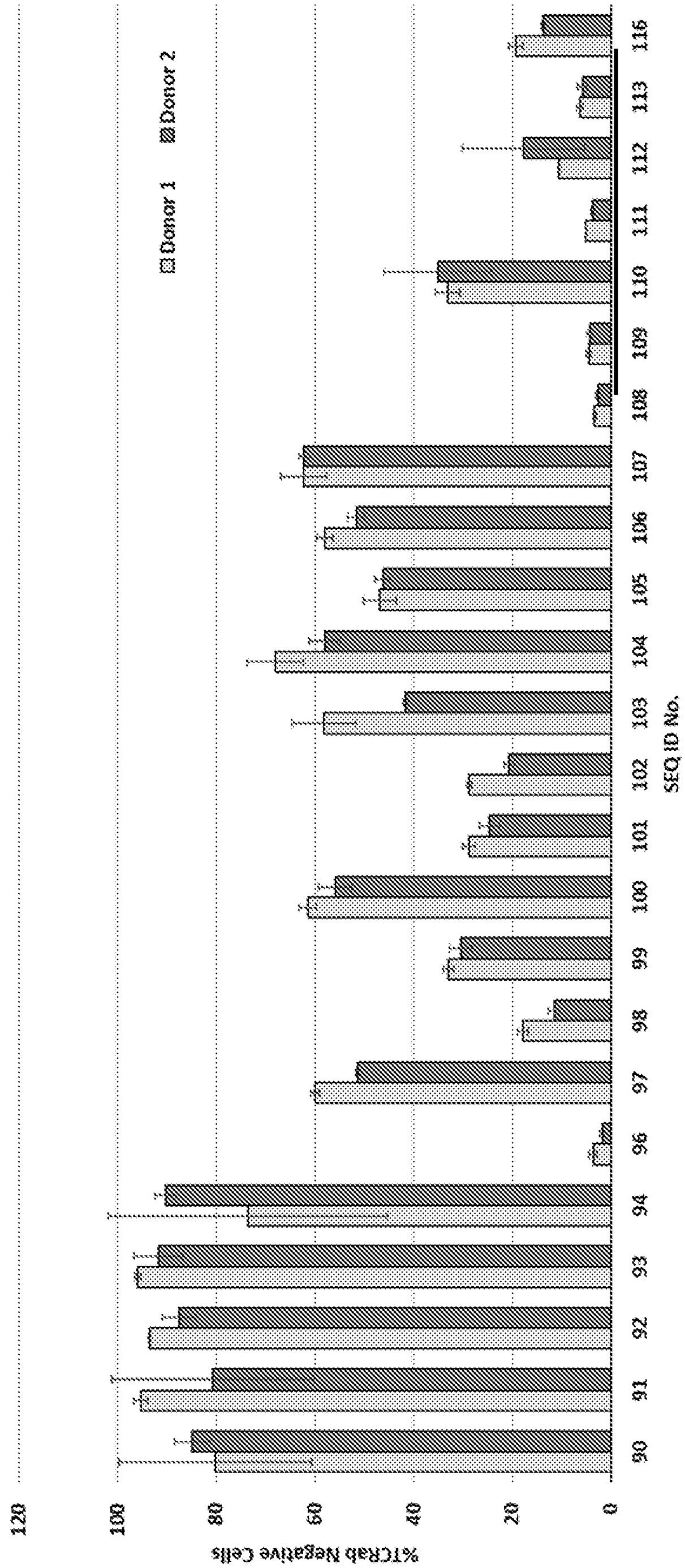
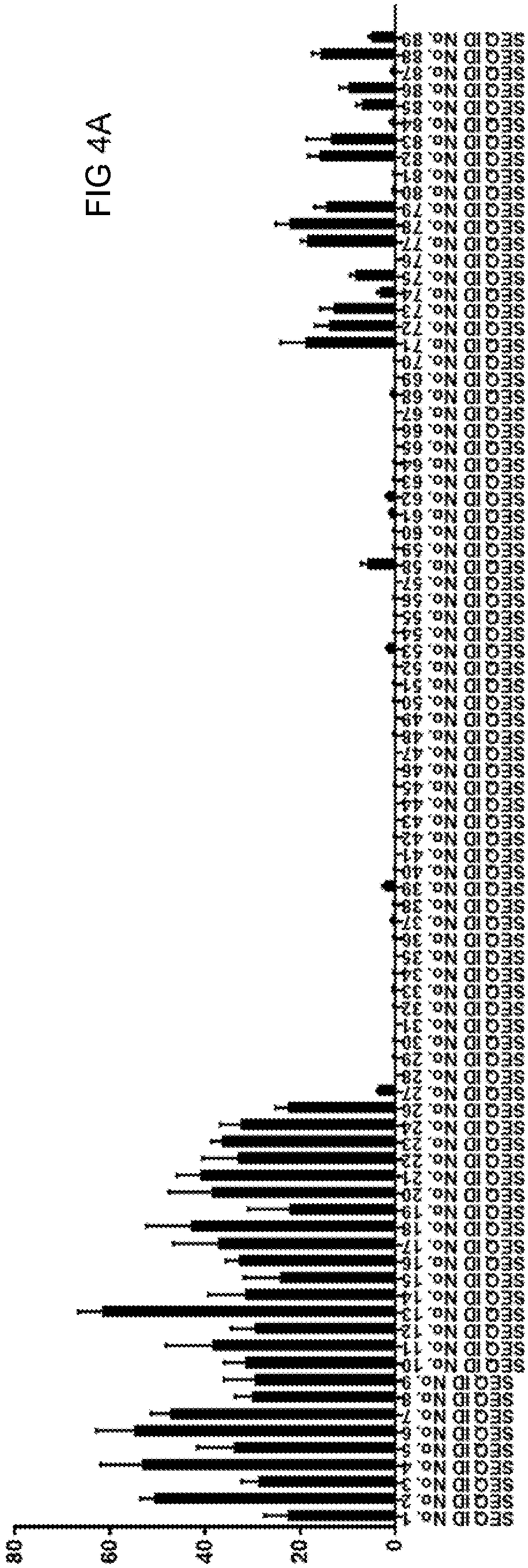

Donor 1
Donor 2
%TCRab Negative Cells
120
100
80
60
40
20
0
SEQ ID No.
90
91
92
93
94
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
116

FIG 3

FIG 4A


SEQ ID No. 89
SEQ ID No. 88
SEQ ID No. 87
SEQ ID No. 86
SEQ ID No. 85
SEQ ID No. 84
SEQ ID No. 83
SEQ ID No. 82
SEQ ID No. 81
SEQ ID No. 80
SEQ ID No. 79
SEQ ID No. 78
SEQ ID No. 77
SEQ ID No. 76
SEQ ID No. 75
SEQ ID No. 74
SEQ ID No. 73
SEQ ID No. 72
SEQ ID No. 71
SEQ ID No. 70
SEQ ID No. 69
SEQ ID No. 68
SEQ ID No. 67
SEQ ID No. 66
SEQ ID No. 65
SEQ ID No. 64
SEQ ID No. 63
SEQ ID No. 62
SEQ ID No. 61
SEQ ID No. 60
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SEQ ID No. 56
SEQ ID No. 55
SEQ ID No. 54
SEQ ID No. 53
SEQ ID No. 52
SEQ ID No. 51
SEQ ID No. 50
SEQ ID No. 49
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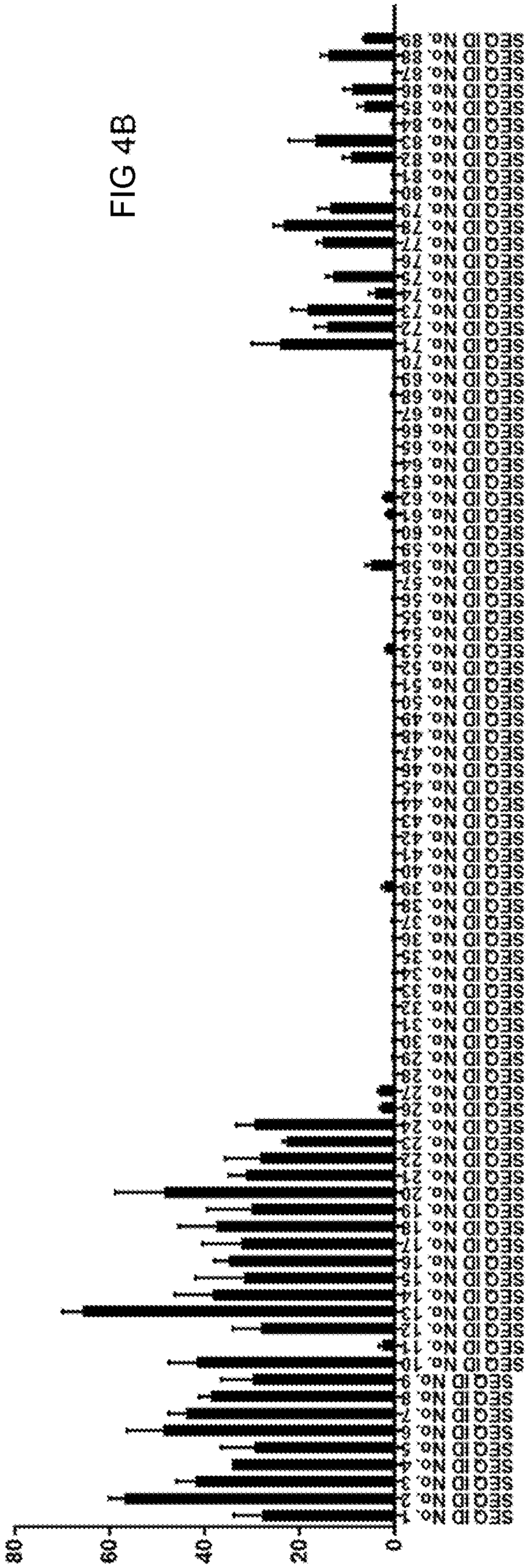
SEQ ID No. 47
SEQ ID No. 46
SEQ ID No. 45
SEQ ID No. 44
SEQ ID No. 43
SEQ ID No. 42
SEQ ID No. 41
SEQ ID No. 40
SEQ ID No. 39
SEQ ID No. 38
SEQ ID No. 37
SEQ ID No. 36
SEQ ID No. 35
SEQ ID No. 34
SEQ ID No. 33
SEQ ID No. 32
SEQ ID No. 31
SEQ ID No. 30
SEQ ID No. 29
SEQ ID No. 28
SEQ ID No. 27
SEQ ID No. 26
SEQ ID No. 25
SEQ ID No. 24
SEQ ID No. 23
SEQ ID No. 22
SEQ ID No. 21
SEQ ID No. 20
SEQ ID No. 19
SEQ ID No. 18
SEQ ID No. 17
SEQ ID No. 16
SEQ ID No. 15
SEQ ID No. 14
SEQ ID No. 13
SEQ ID No. 12
SEQ ID No. 11
SEQ ID No. 10
SEQ ID No. 9
SEQ ID No. 8
SEQ ID No. 7
SEQ ID No. 6
SEQ ID No. 5
SEQ ID No. 4
SEQ ID No. 3
SEQ ID No. 2
SEQ ID No. 1
0
20
40
60
80
Indel %

FIG 4B


SEQ ID No. 89
SEQ ID No. 88
SEQ ID No. 87
SEQ ID No. 86
SEQ ID No. 85
SEQ ID No. 84
SEQ ID No. 83
SEQ ID No. 82
SEQ ID No. 81
SEQ ID No. 80
SEQ ID No. 79
SEQ ID No. 78
SEQ ID No. 77
SEQ ID No. 76
SEQ ID No. 75
SEQ ID No. 74
SEQ ID No. 73
SEQ ID No. 72
SEQ ID No. 71
SEQ ID No. 70
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SEQ ID No. 67
SEQ ID No. 66
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SEQ ID No. 64
SEQ ID No. 63
SEQ ID No. 62
SEQ ID No. 61
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SEQ ID No. 59
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SEQ ID No. 34
SEQ ID No. 33
SEQ ID No. 32
SEQ ID No. 31
SEQ ID No. 30
SEQ ID No. 29
SEQ ID No. 28
SEQ ID No. 27
SEQ ID No. 26
SEQ ID No. 25
SEQ ID No. 24
SEQ ID No. 23
SEQ ID No. 22
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SEQ ID No. 18
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SEQ ID No. 16
SEQ ID No. 15
SEQ ID No. 14
SEQ ID No. 13
SEQ ID No. 12
SEQ ID No. 11
SEQ ID No. 10
SEQ ID No. 9
SEQ ID No. 8
SEQ ID No. 7
SEQ ID No. 6
SEQ ID No. 5
SEQ ID No. 4
SEQ ID No. 3
SEQ ID No. 2
SEQ ID No. 1
0
20
40
60
80
Indel %

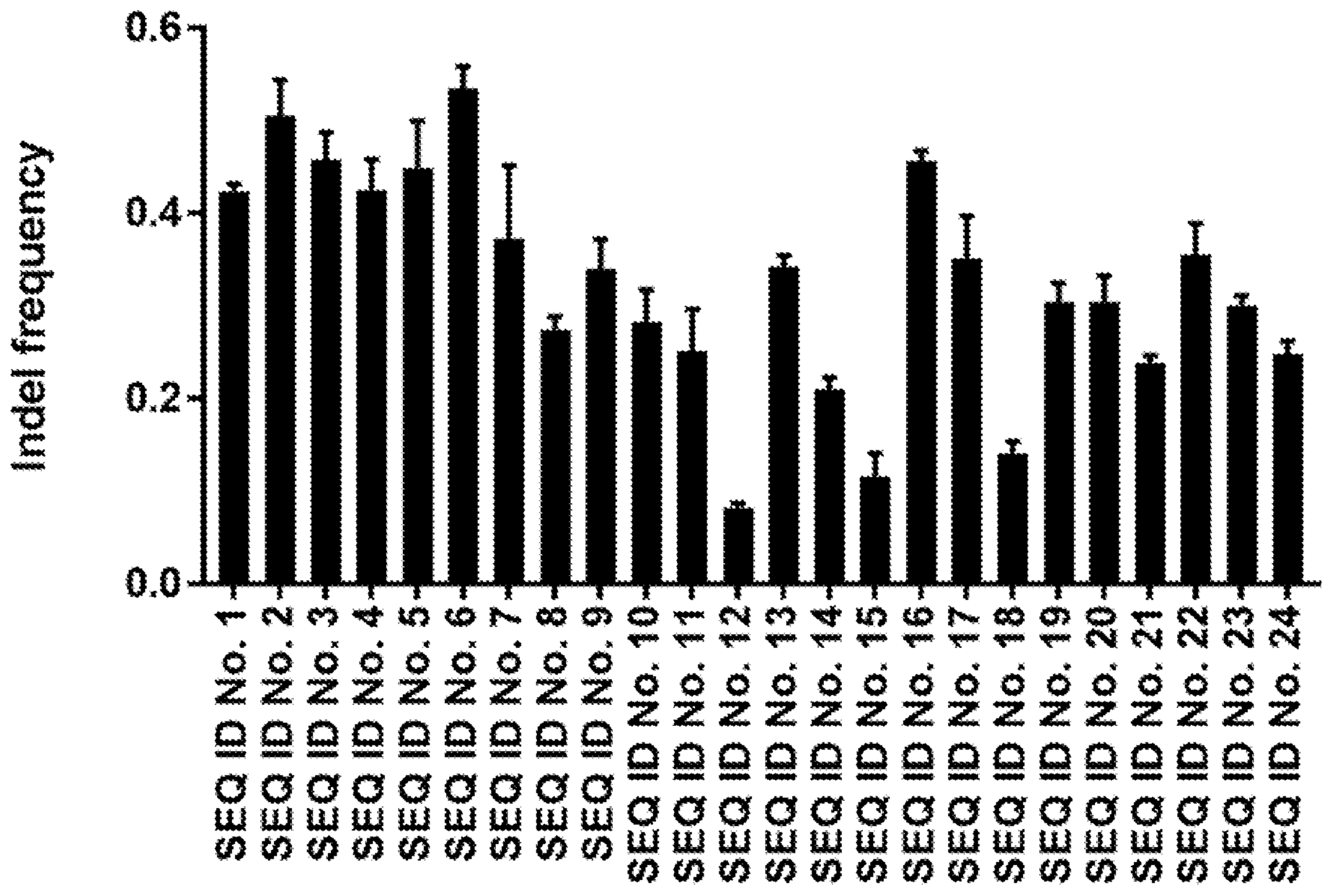
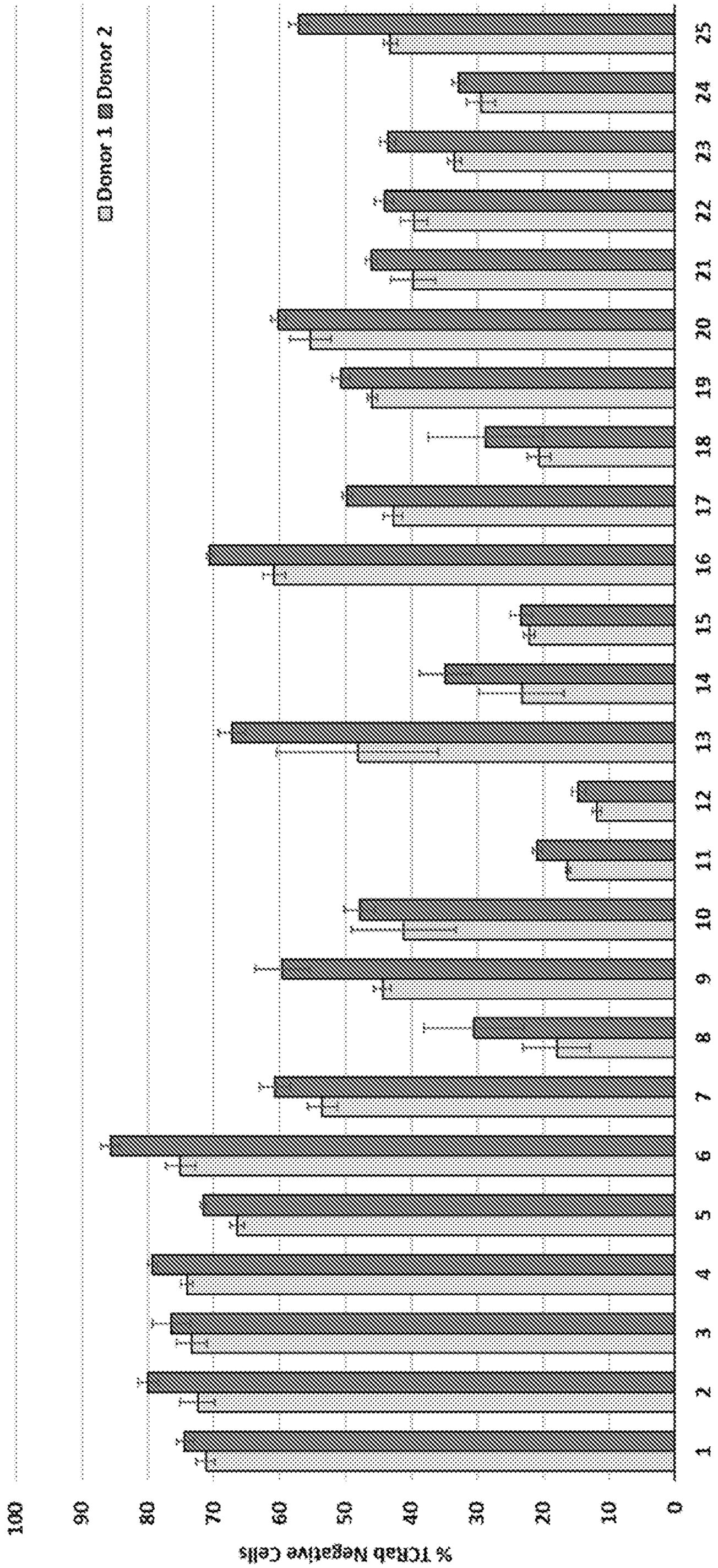

Indel frequency
0.6
0.4
0.2
0.0
SEQ ID No. 1
SEQ ID No. 2
SEQ ID No. 3
SEQ ID No. 4
SEQ ID No. 5
SEQ ID No. 6
SEQ ID No. 7
SEQ ID No. 8
SEQ ID No. 9
SEQ ID No. 10
SEQ ID No. 11
SEQ ID No. 12
SEQ ID No. 13
SEQ ID No. 14
SEQ ID No. 15
SEQ ID No. 16
SEQ ID No. 17
SEQ ID No. 18
SEQ ID No. 19
SEQ ID No. 20
SEQ ID No. 21
SEQ ID No. 22
SEQ ID No. 23
SEQ ID No. 24

FIG 5


Donor 1
Donor 2
% TCRab Negative cells
0
10
20
30
40
50
60
70
80
90
100
SEQ ID No.
1
2
3
4
5
6
7
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11
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16
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18
19
20
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22
23
24
25

FIG 6

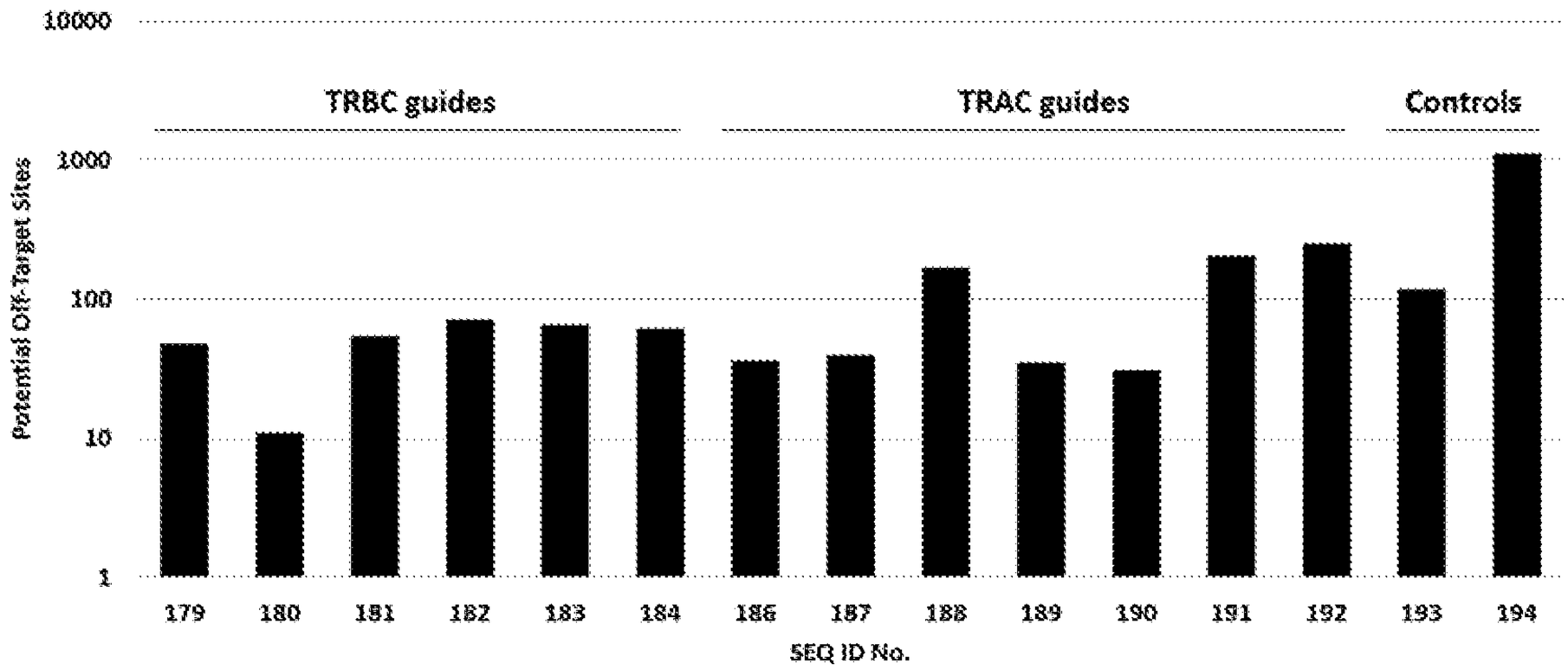
10000
1000
100
10
1
Potential Off-Target Sites
TRBC guides
TRAC guides
Controls
179
180
181
182
183
184
186
187
188
189
190
191
192
193
194
SEQ ID No.

FIG 7A

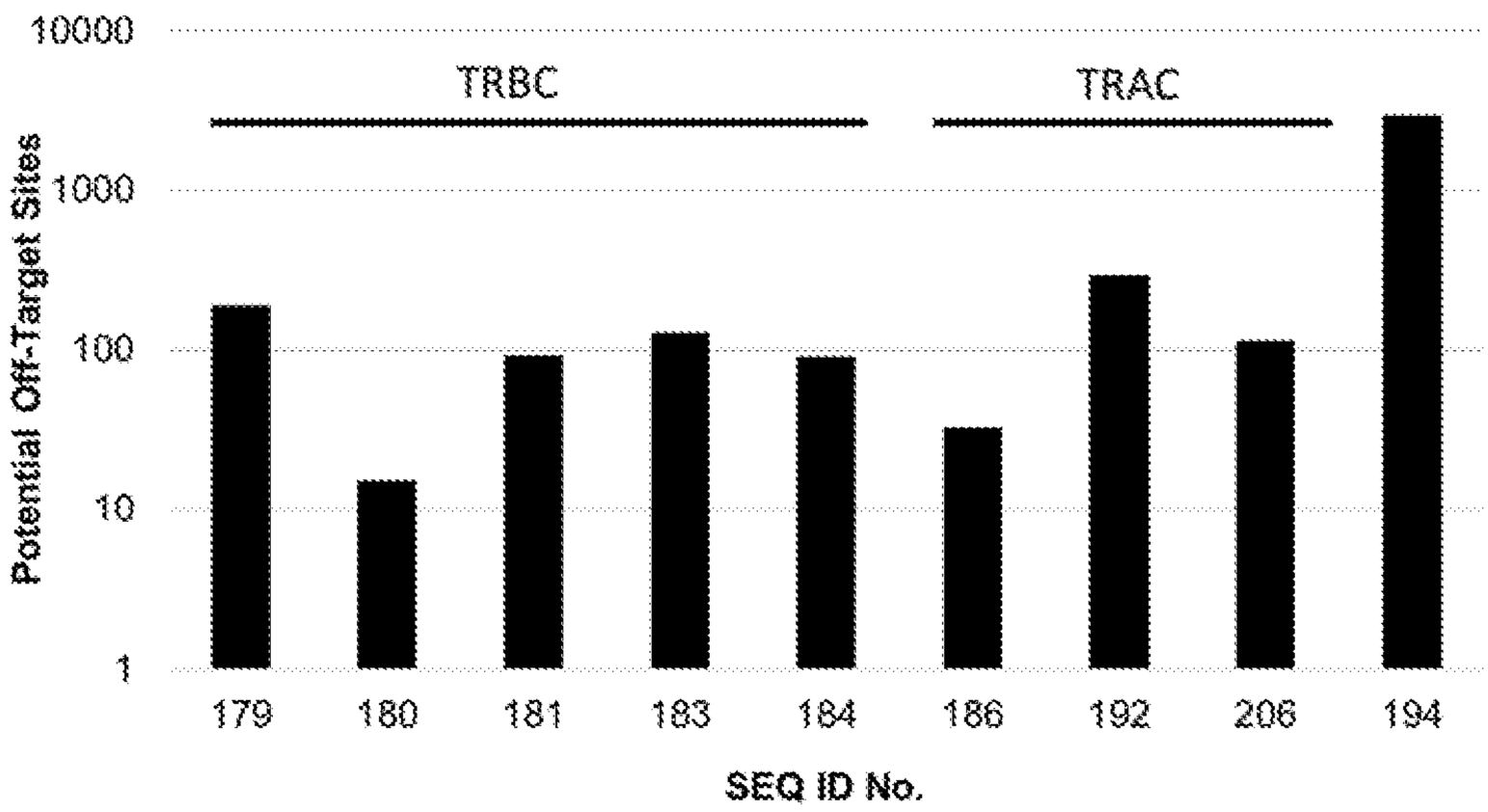
10000
1000
100
10
1
Potential Off-Target Sites
TRBC
TRAC
179
180
181
183
184
186
192
206
194
SEQ ID No.

FIG 7B

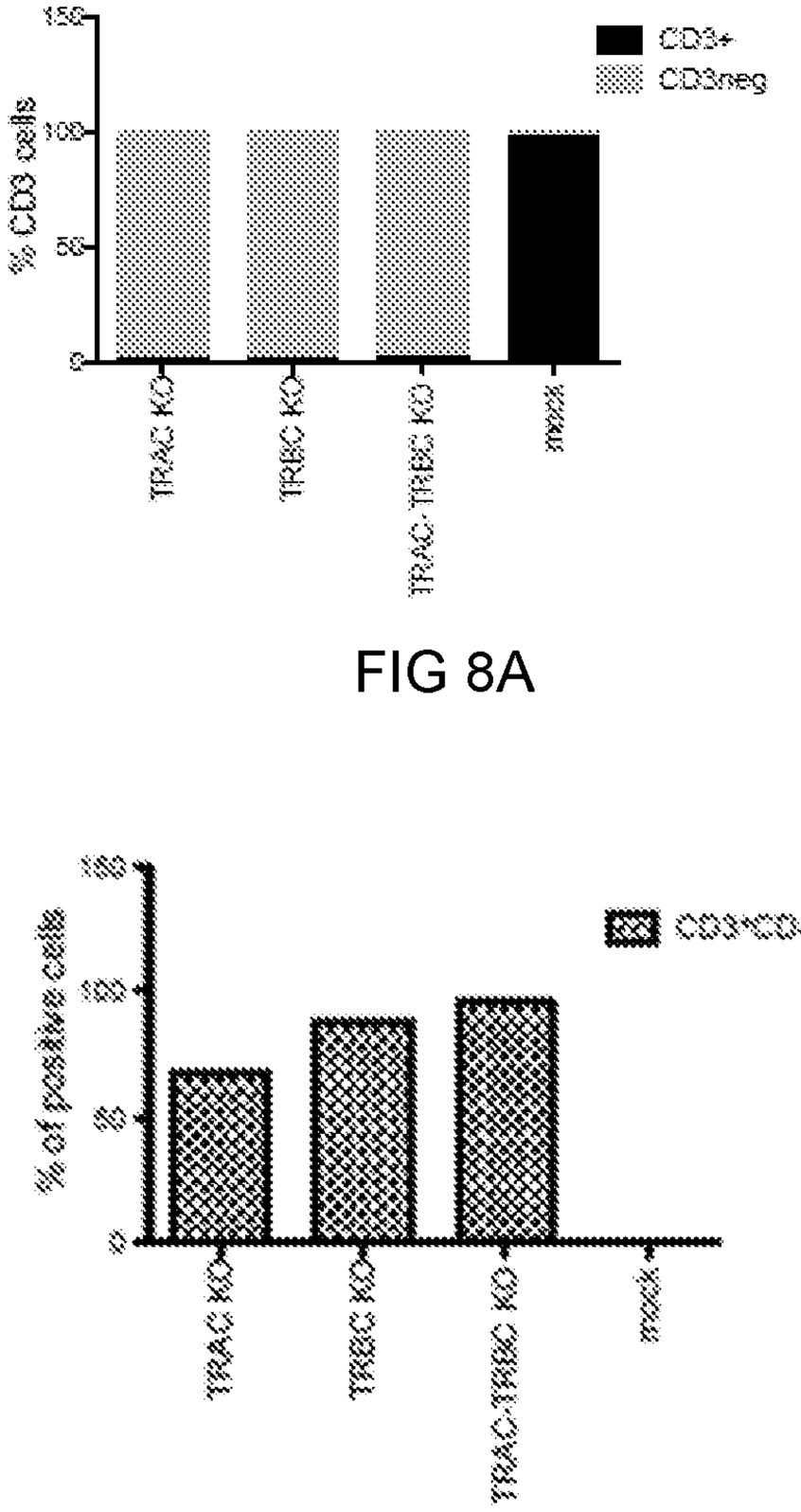

FIG 8A

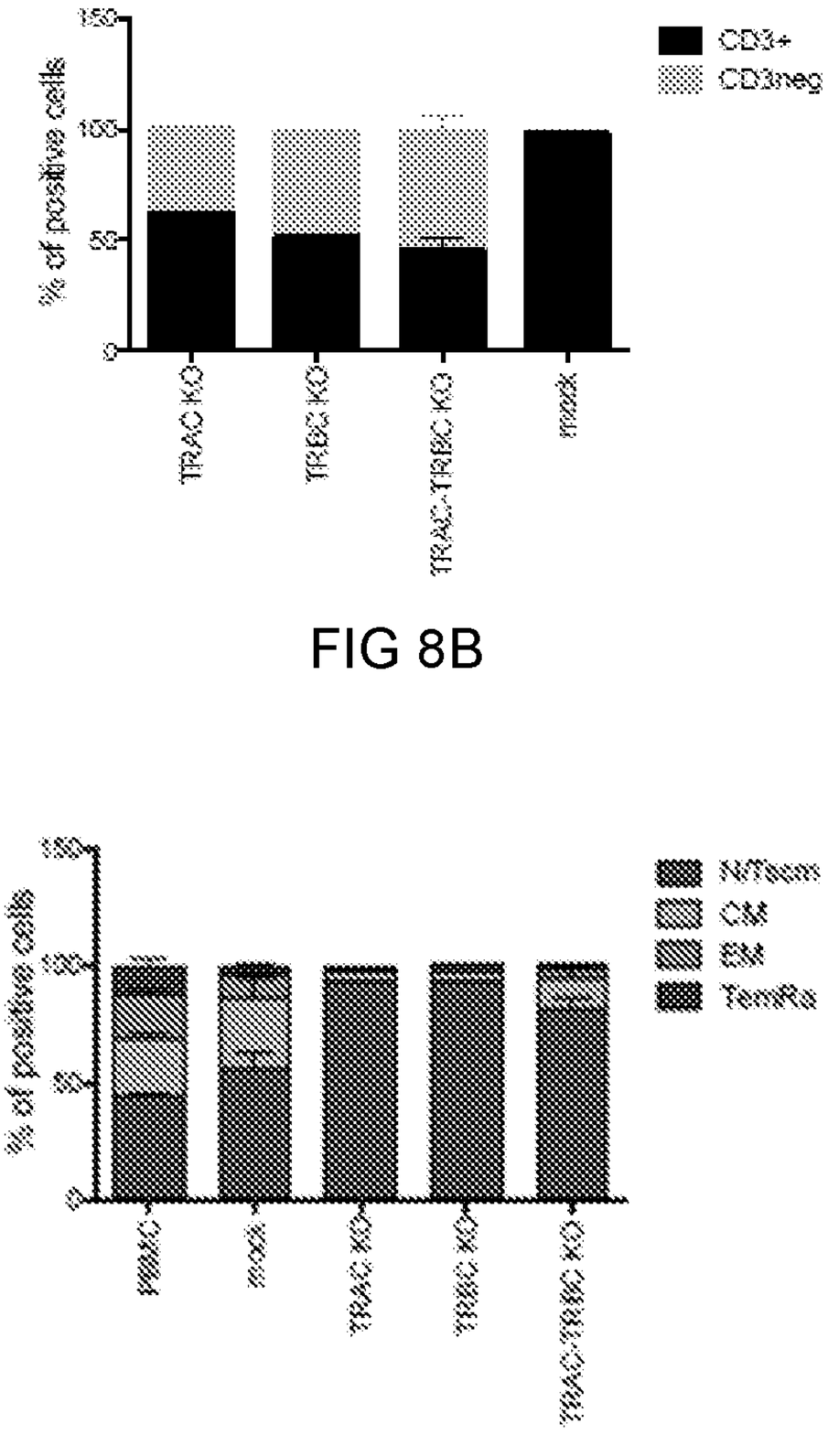

FIG 8B

FIG 8C

FIG 8D

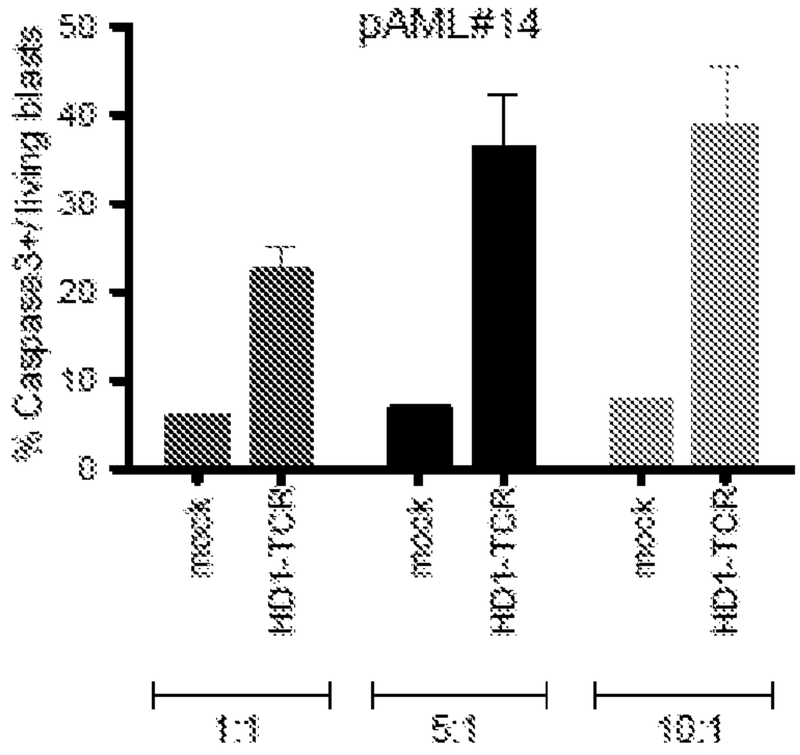

FIG 9A

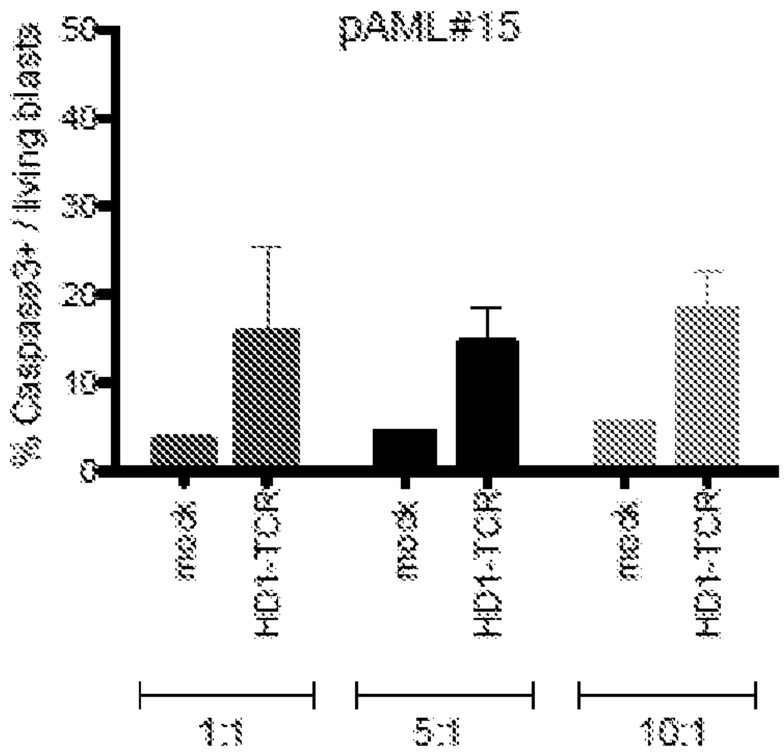

FIG 9B

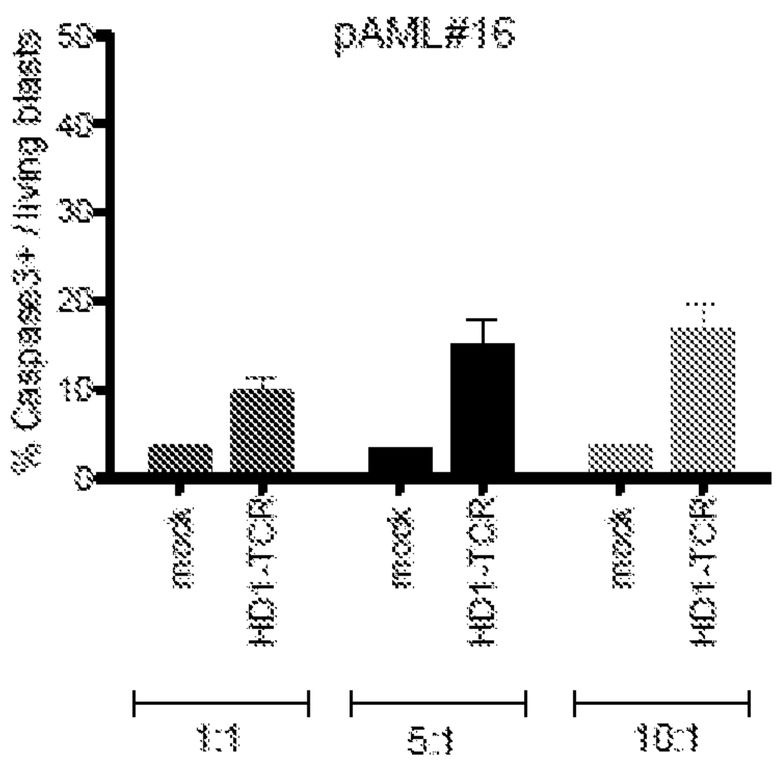

FIG 9C

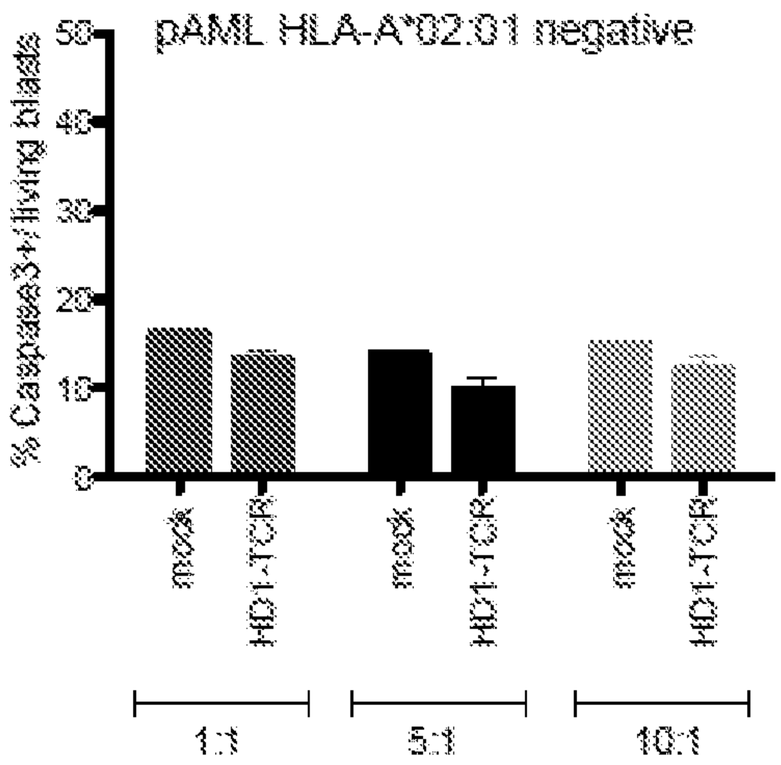

FIG 9D

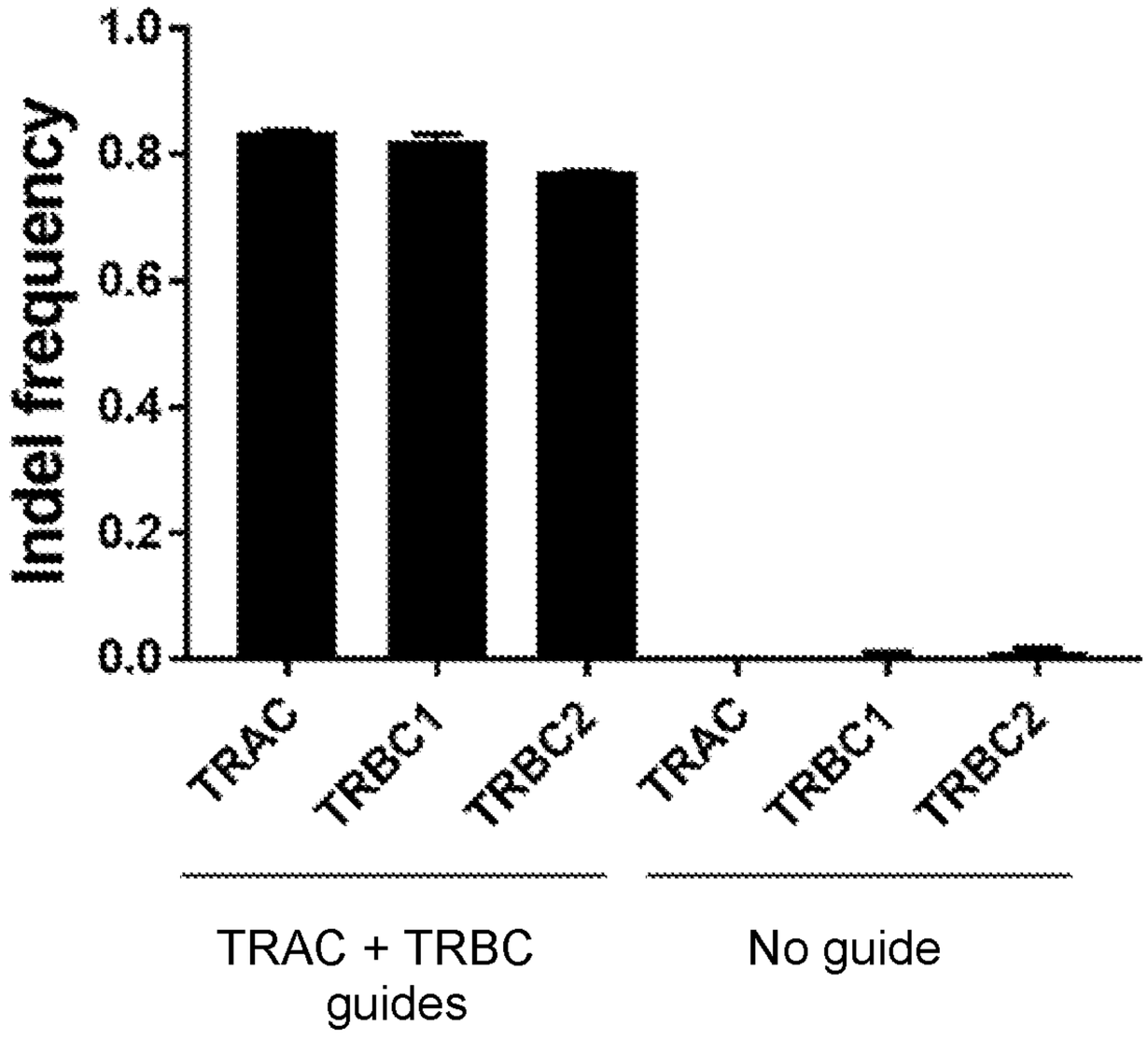
Indel frequency
1.0
0.8
0.6
0.4
0.2
0.0
TRAC
TRBC1
TRBC2
TRAC
TRBC1
TRBC2
TRAC + TRBC guides
No guide

FIG 10

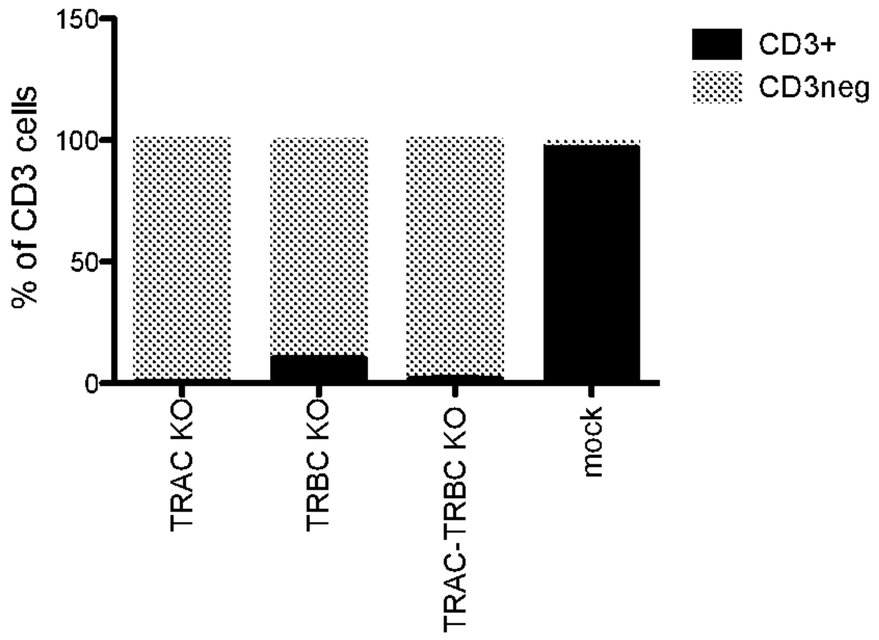

FIG 11A

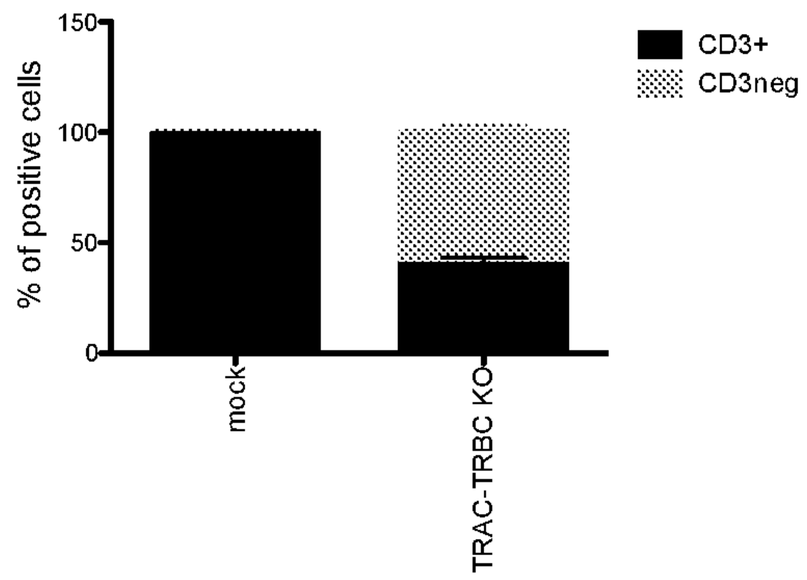

FIG 11B

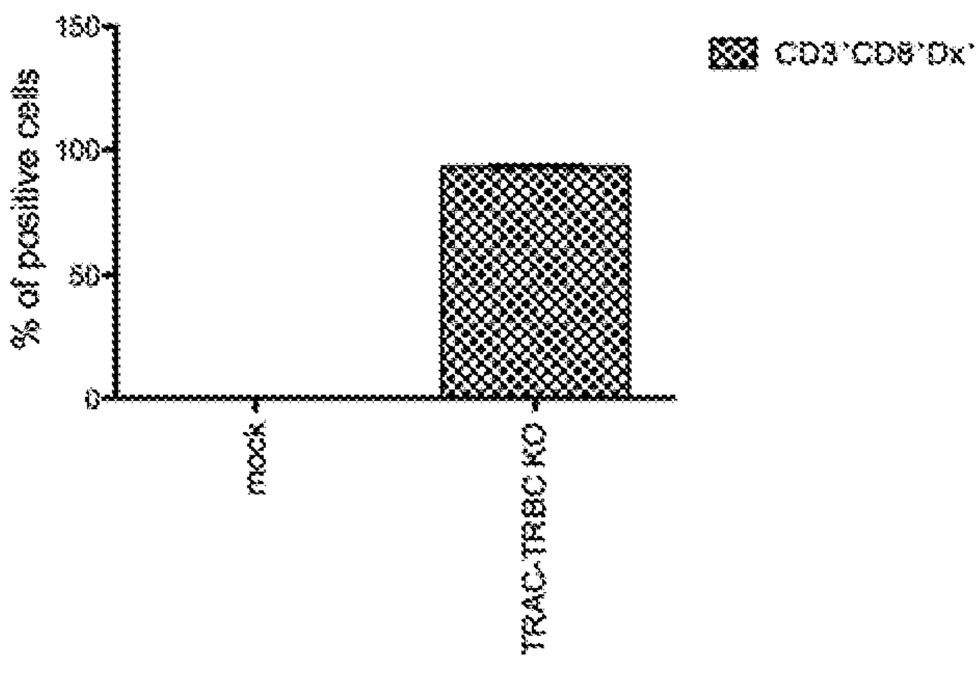

FIG 11C

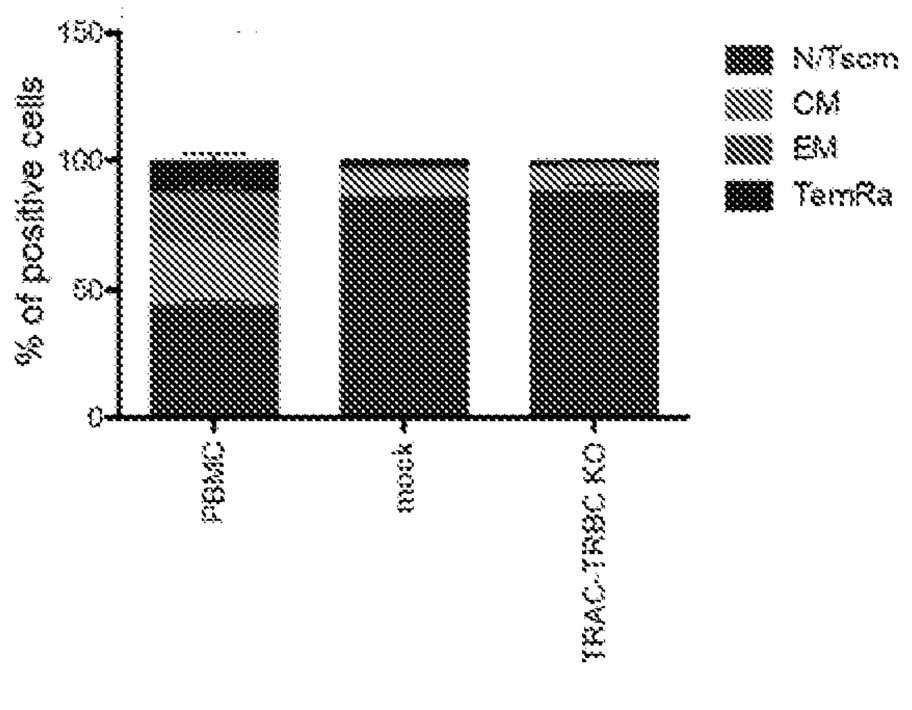

FIG 11D

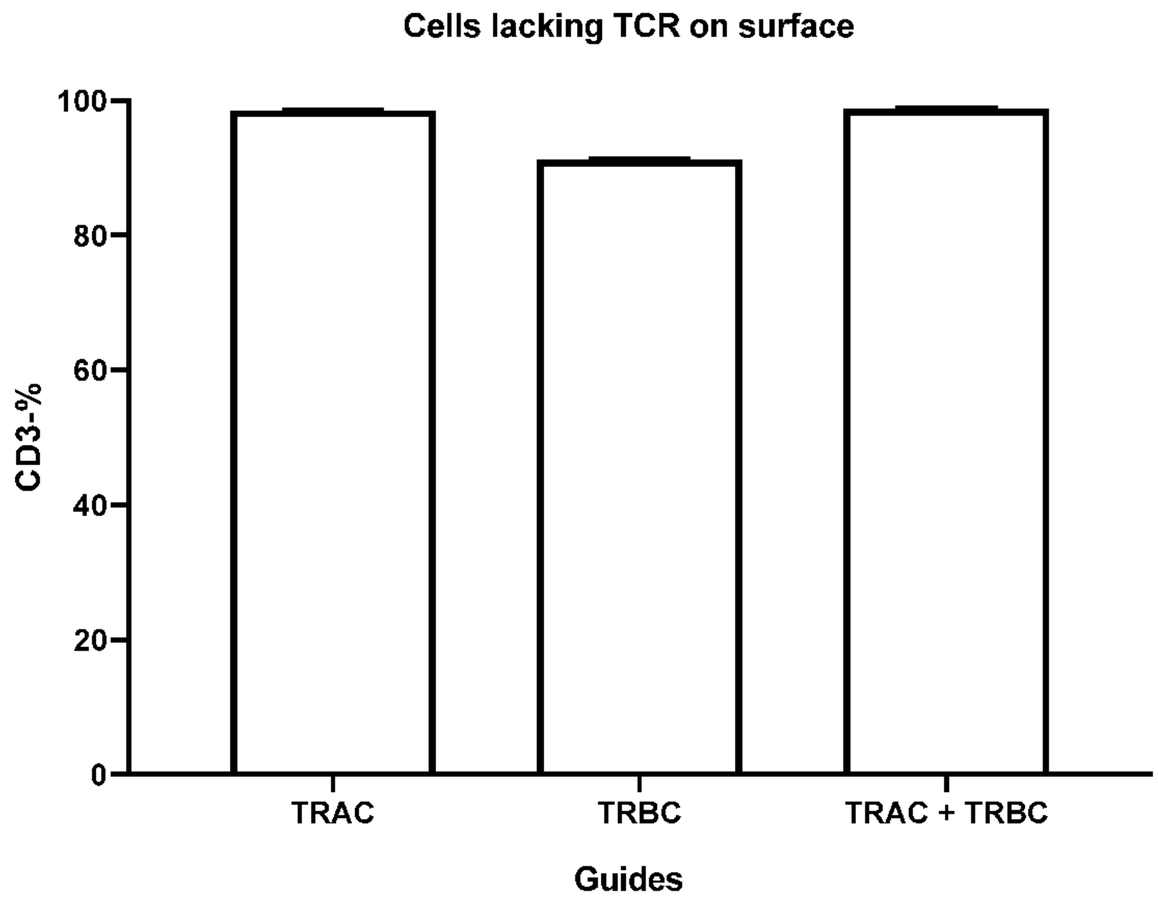

FIG 12A

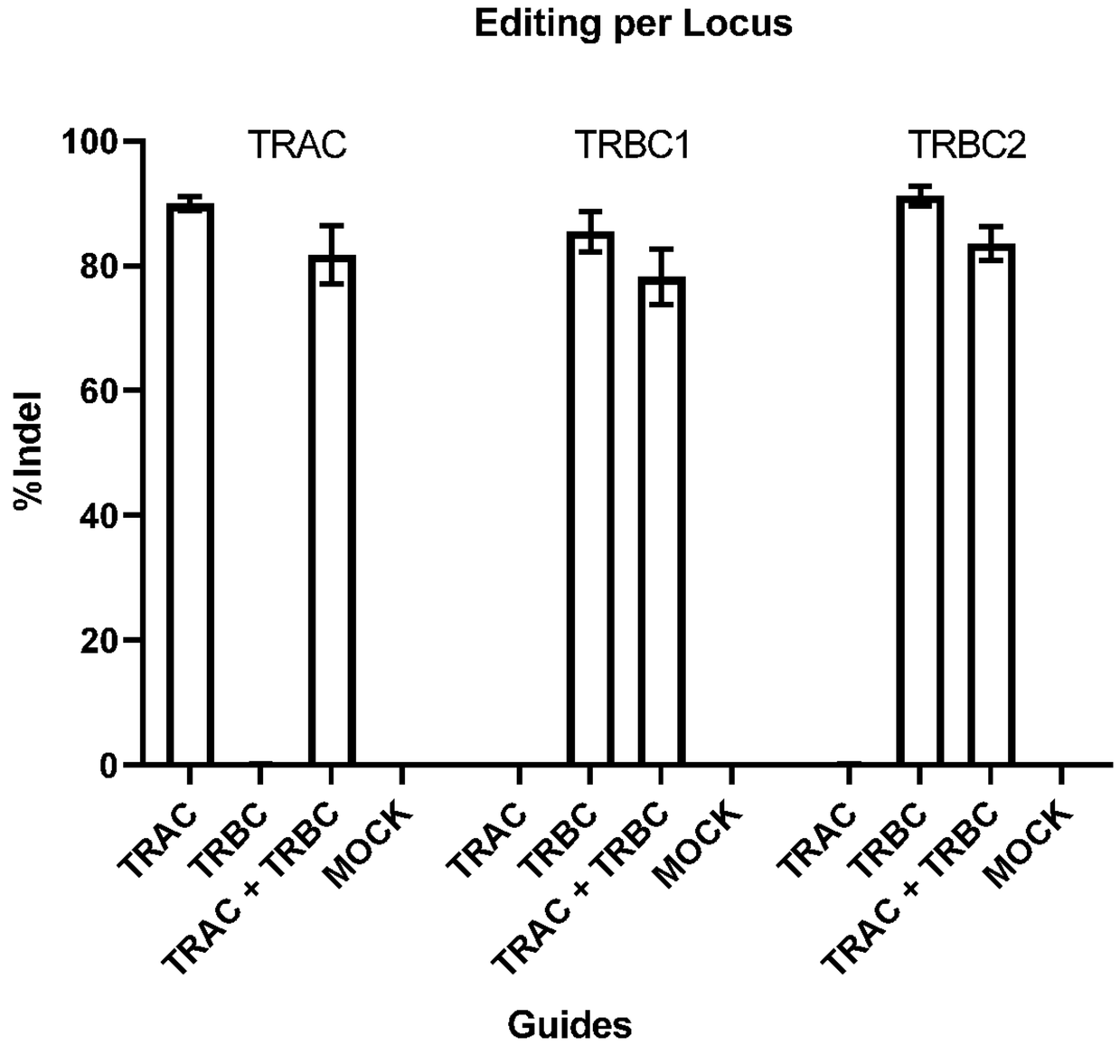

FIG 12B

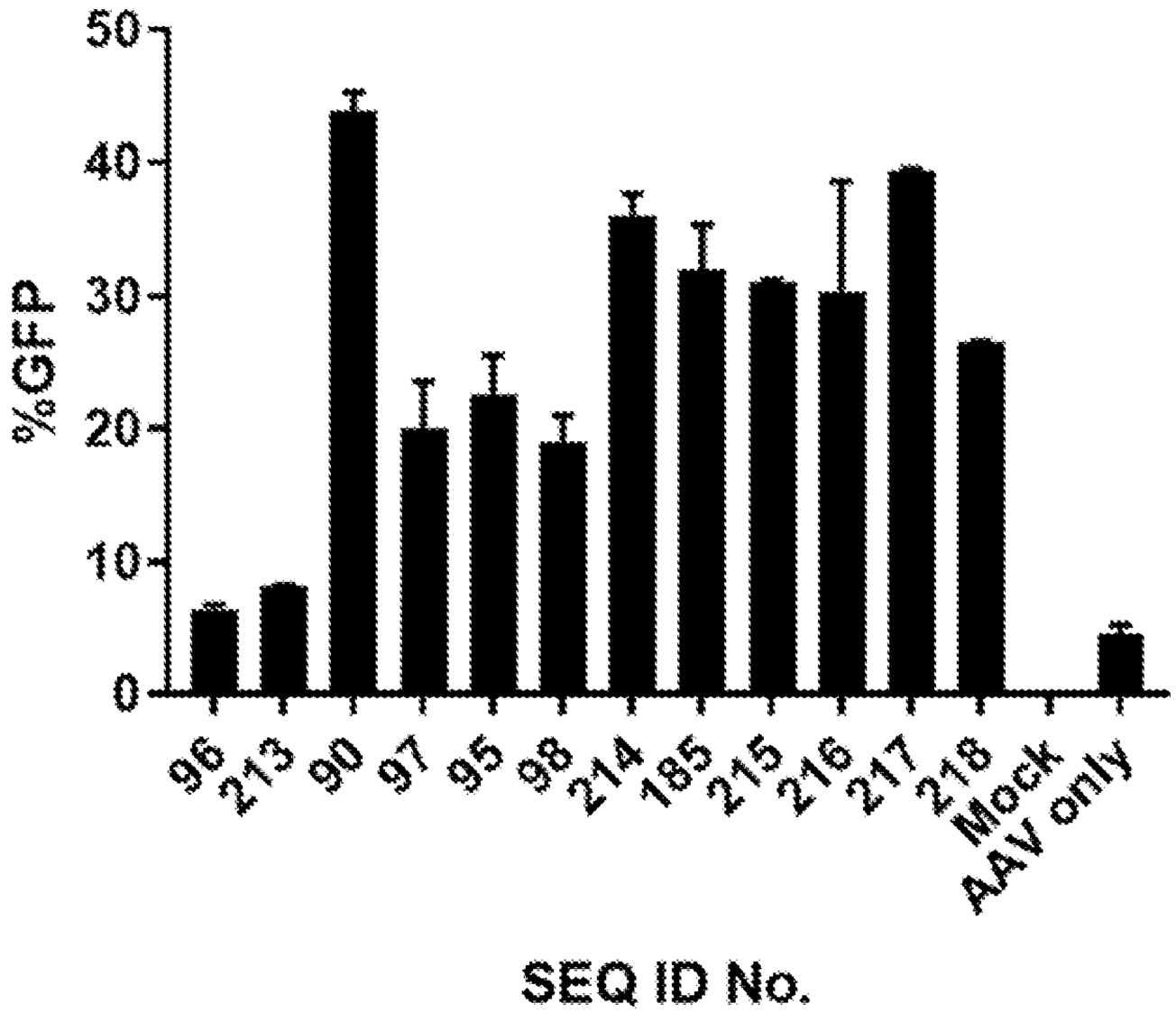

FIG 13A

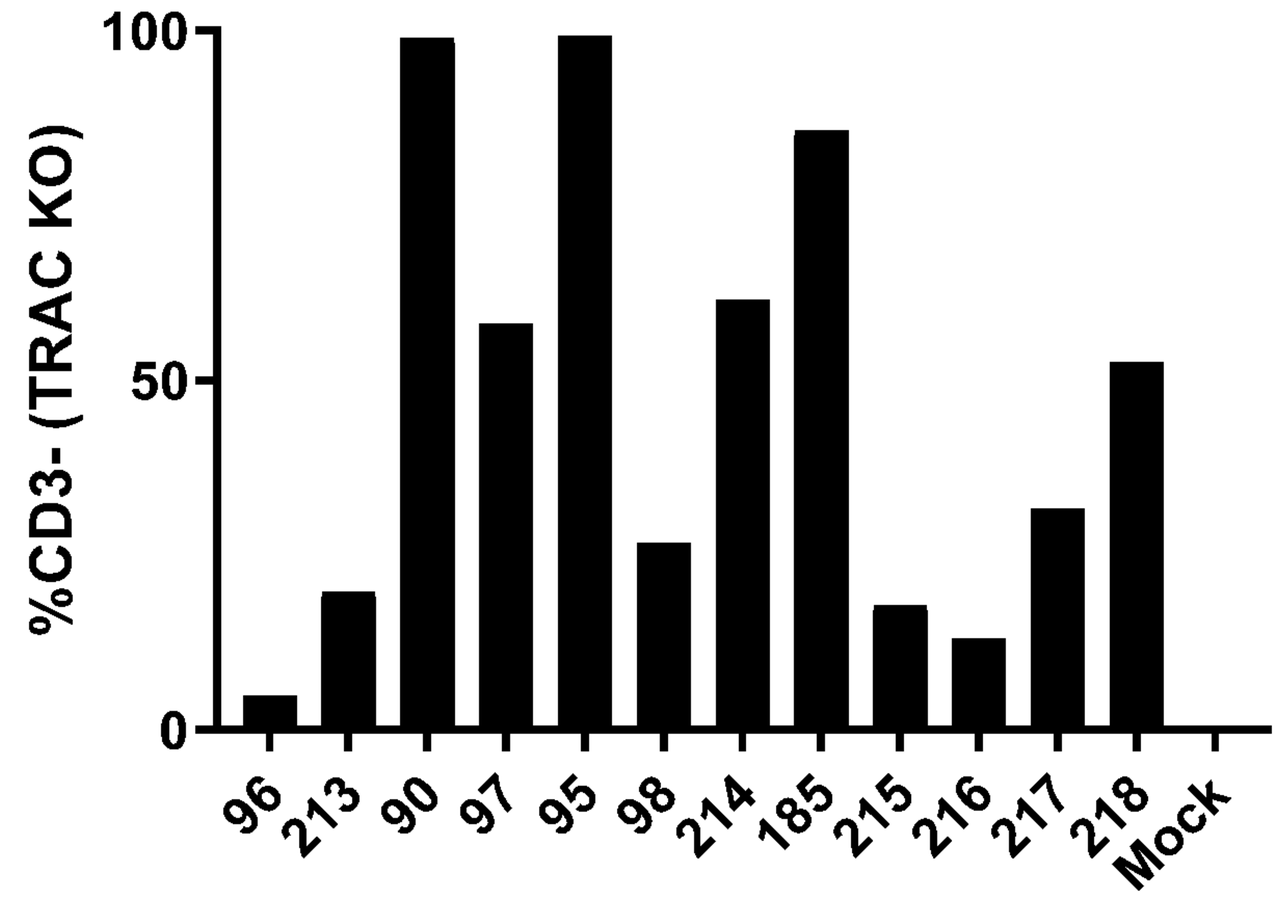

FIG 13B

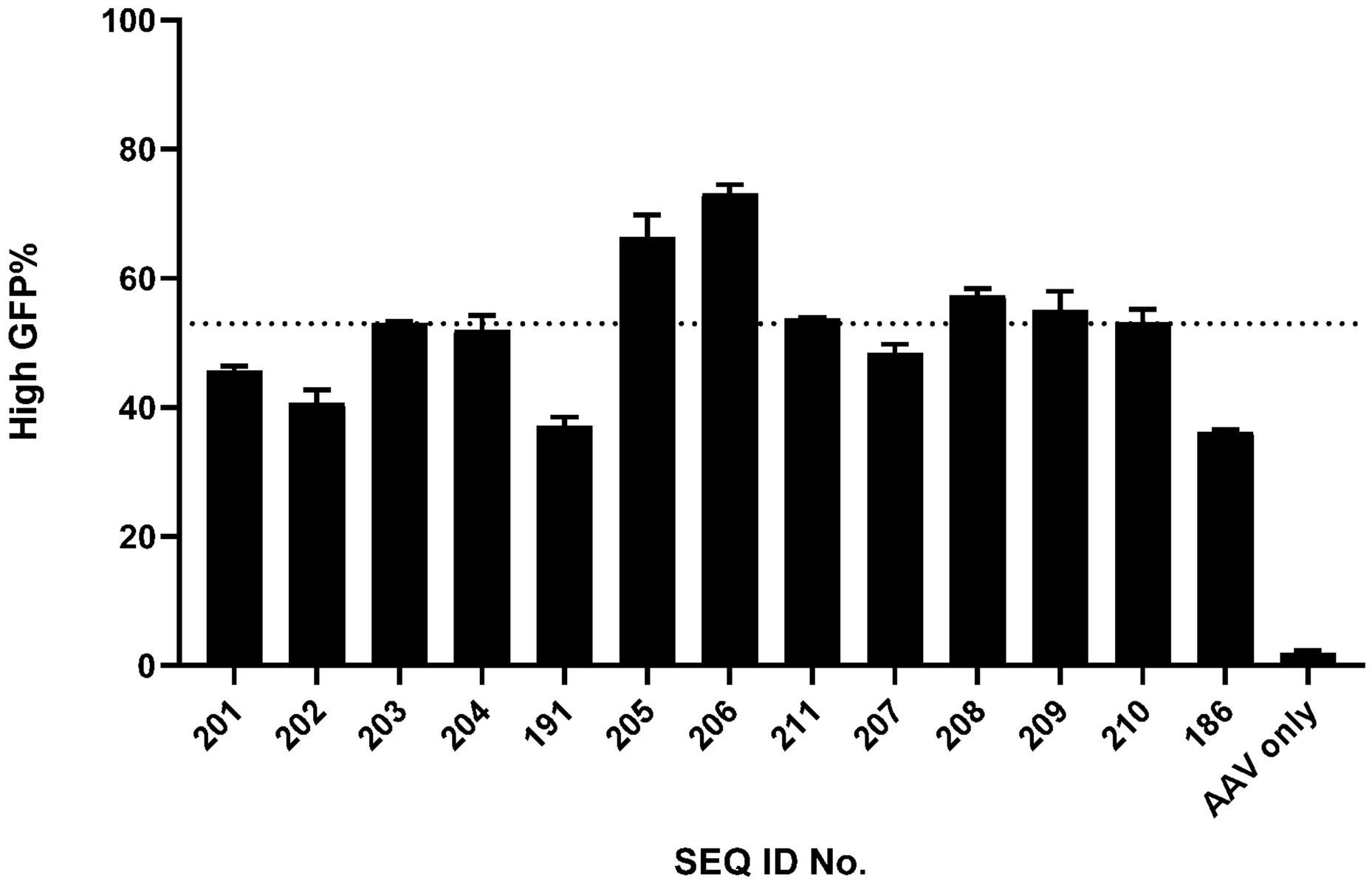

FIG 14A

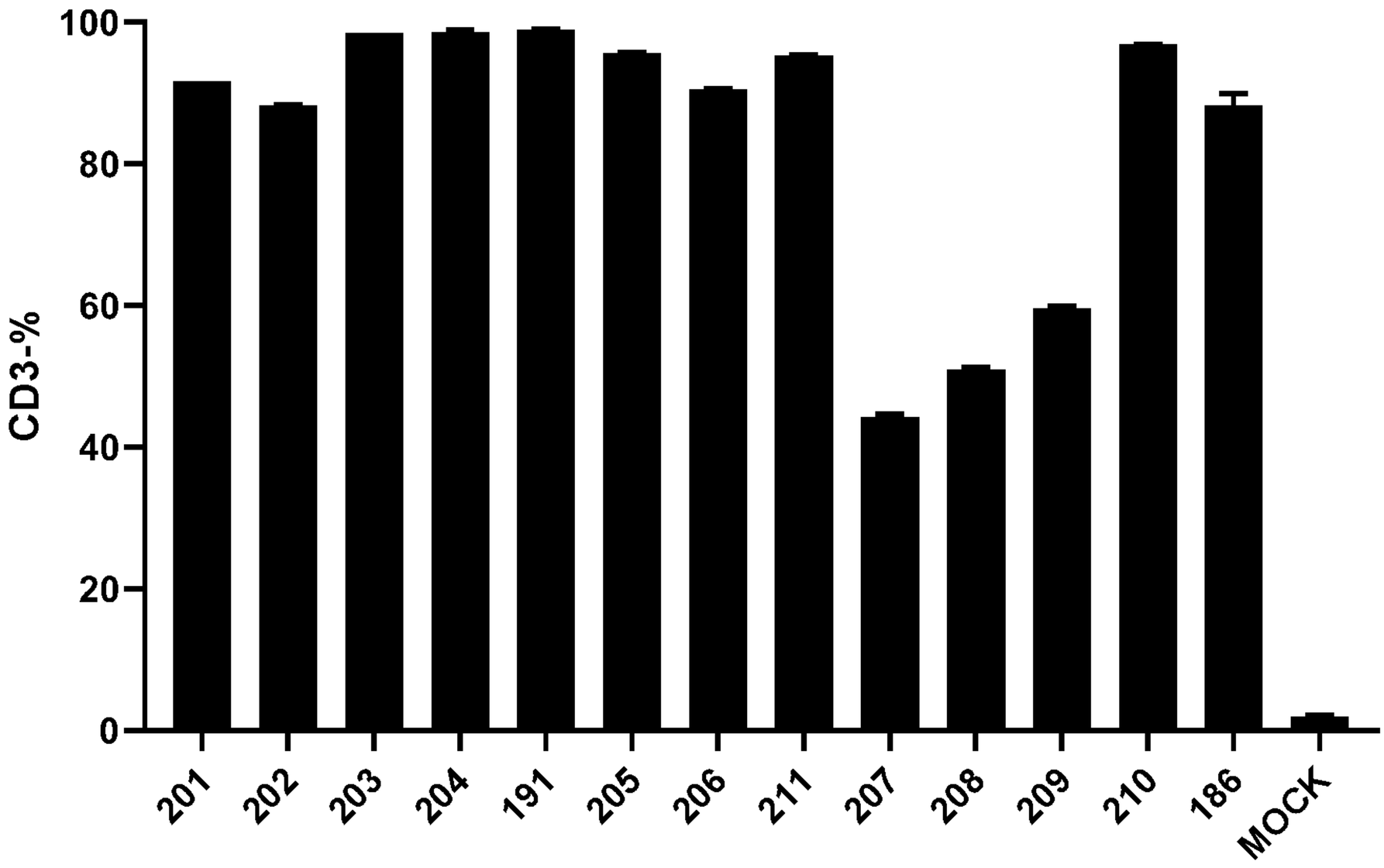

FIG 14B

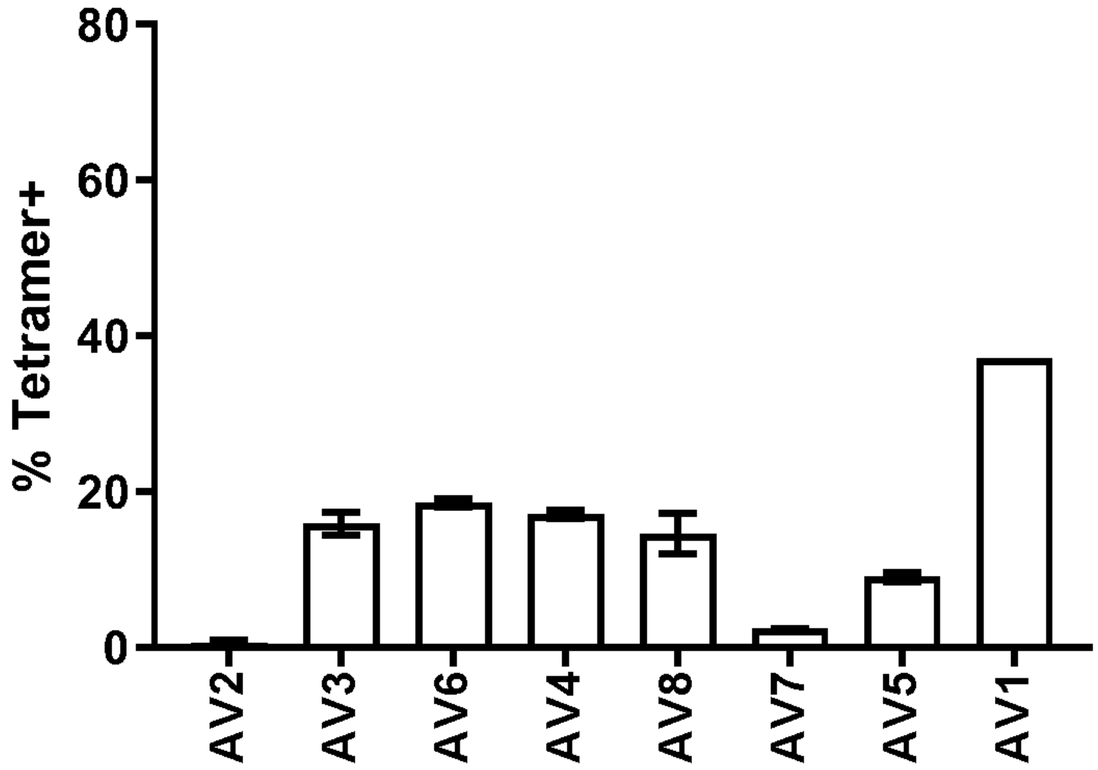

FIG 15A

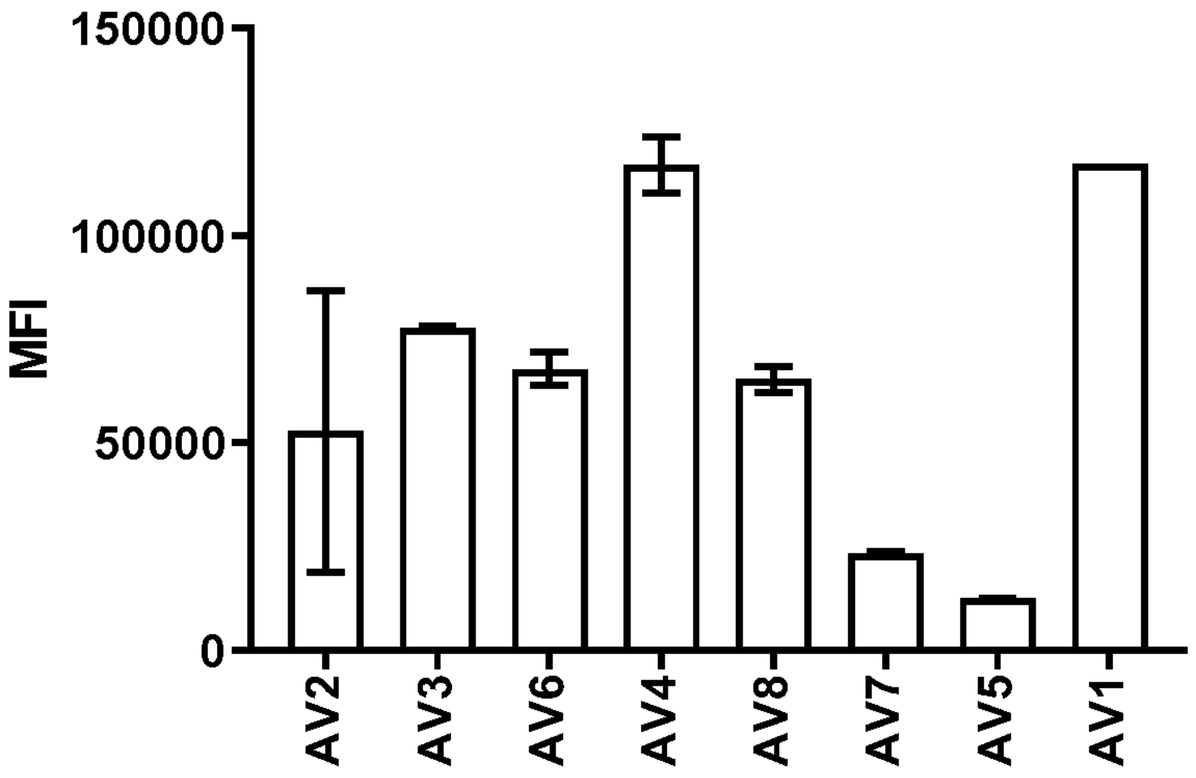

FIG 15B

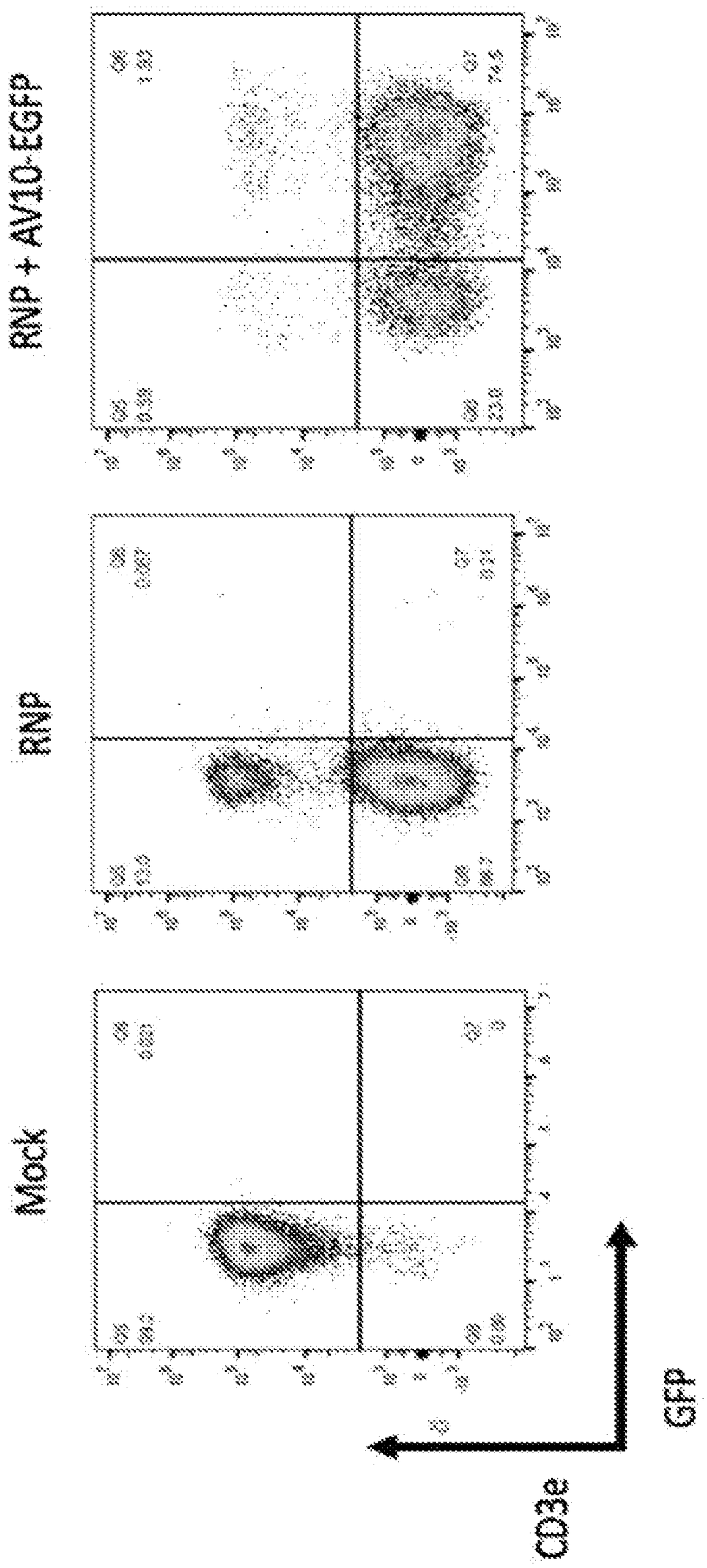

FIG 16B

FIG 16C

FIG 16A

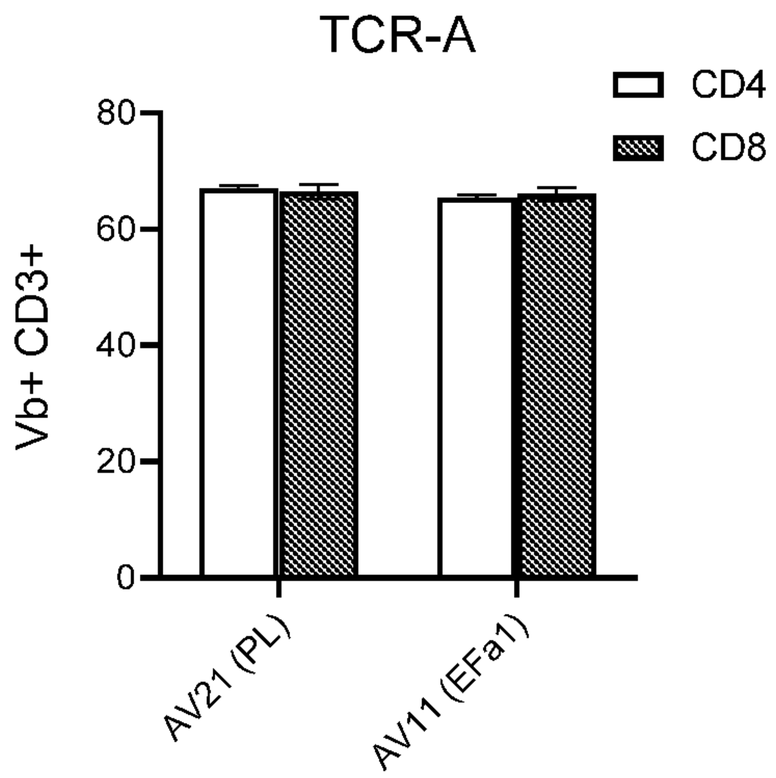

FIG 17A

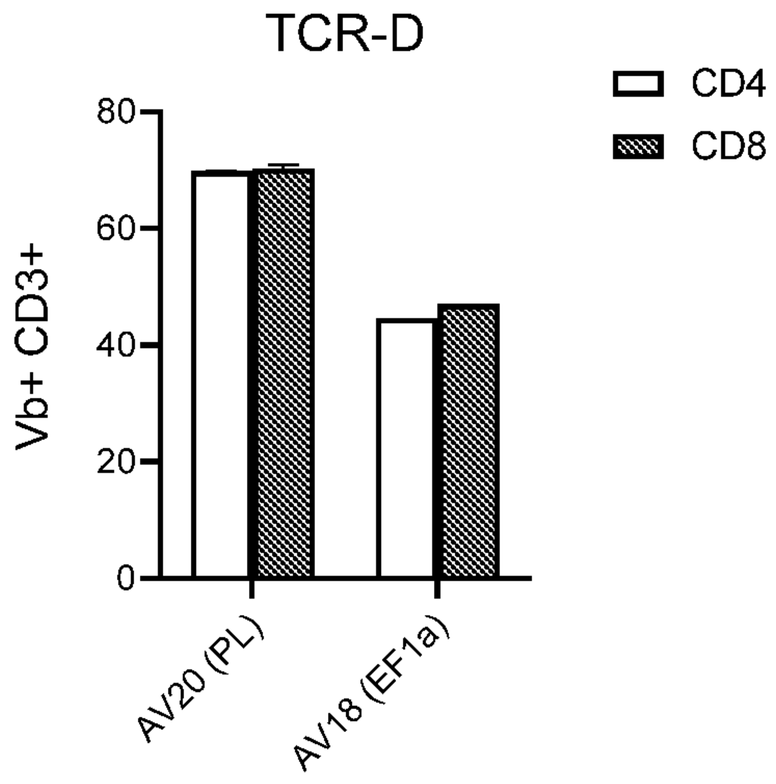

FIG 17B

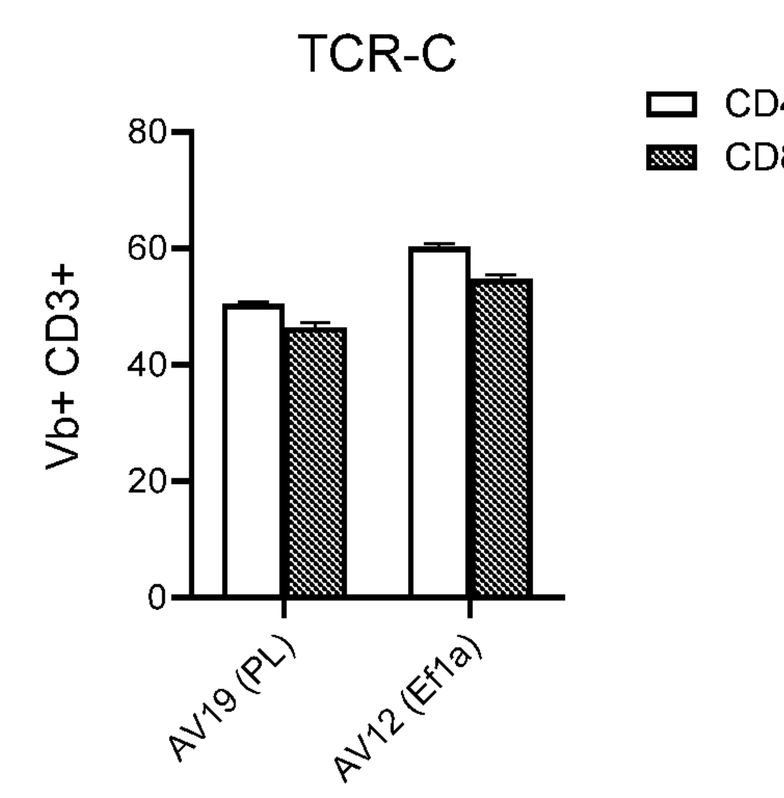

FIG 17C

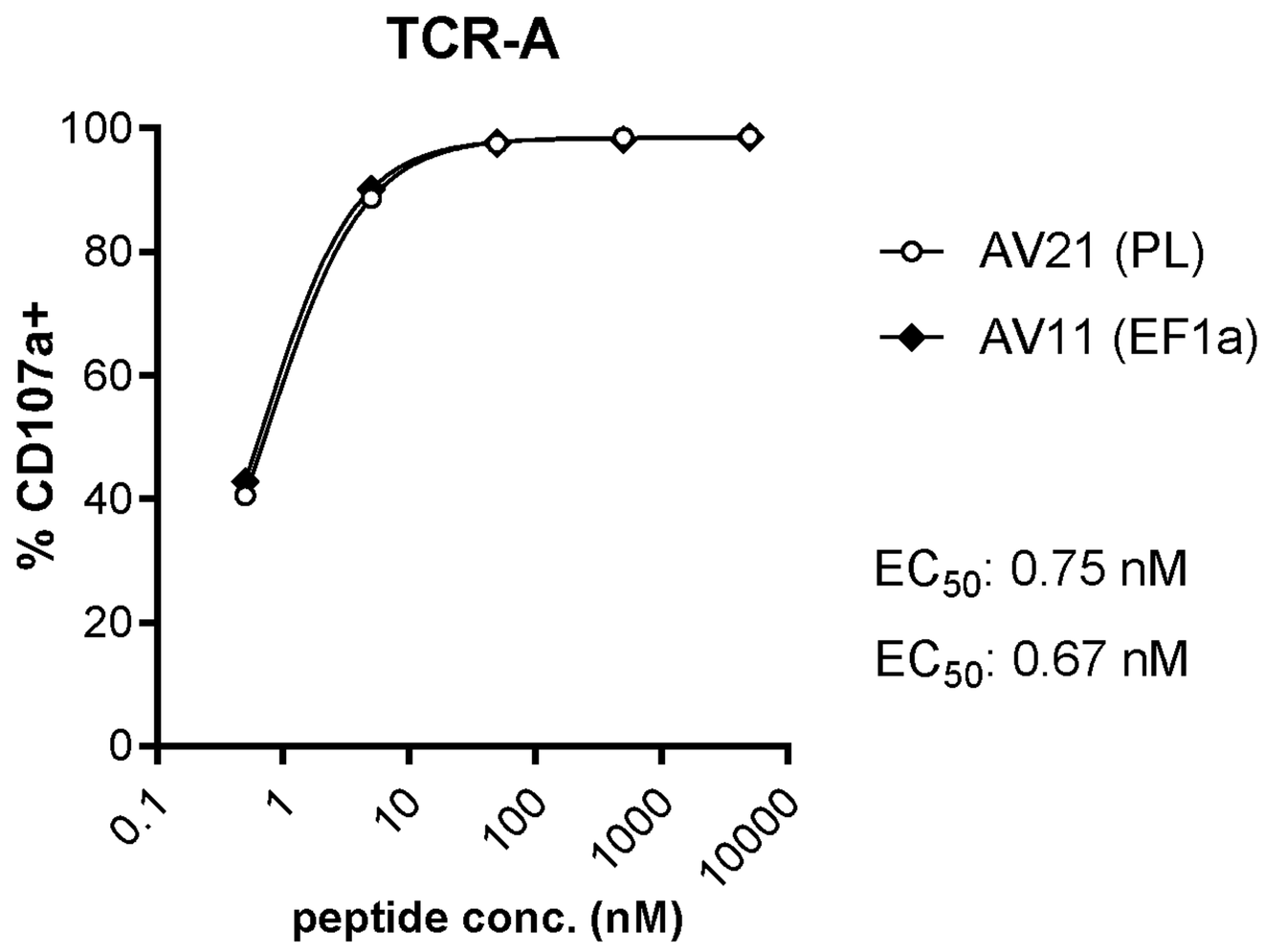

FIG 18A

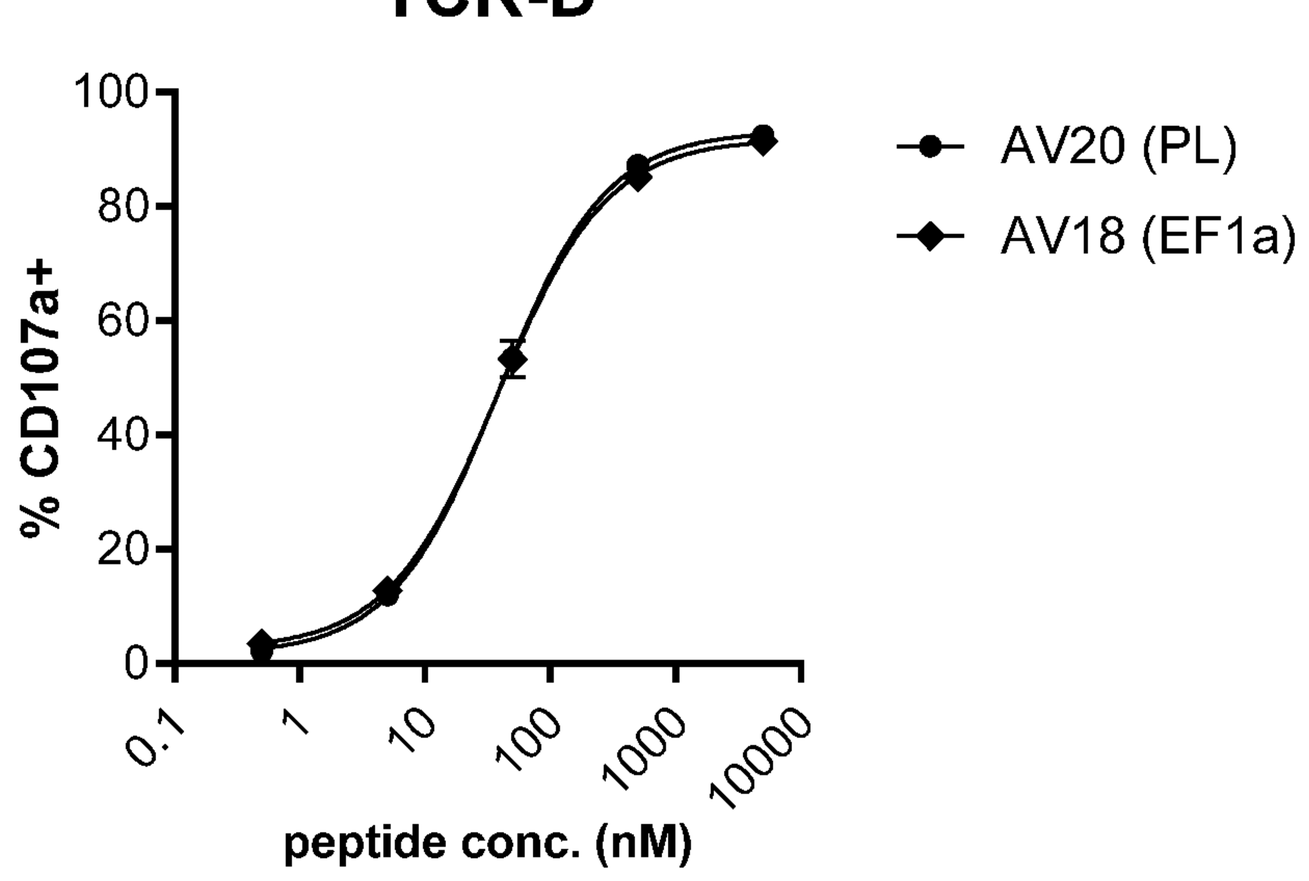

FIG 18B

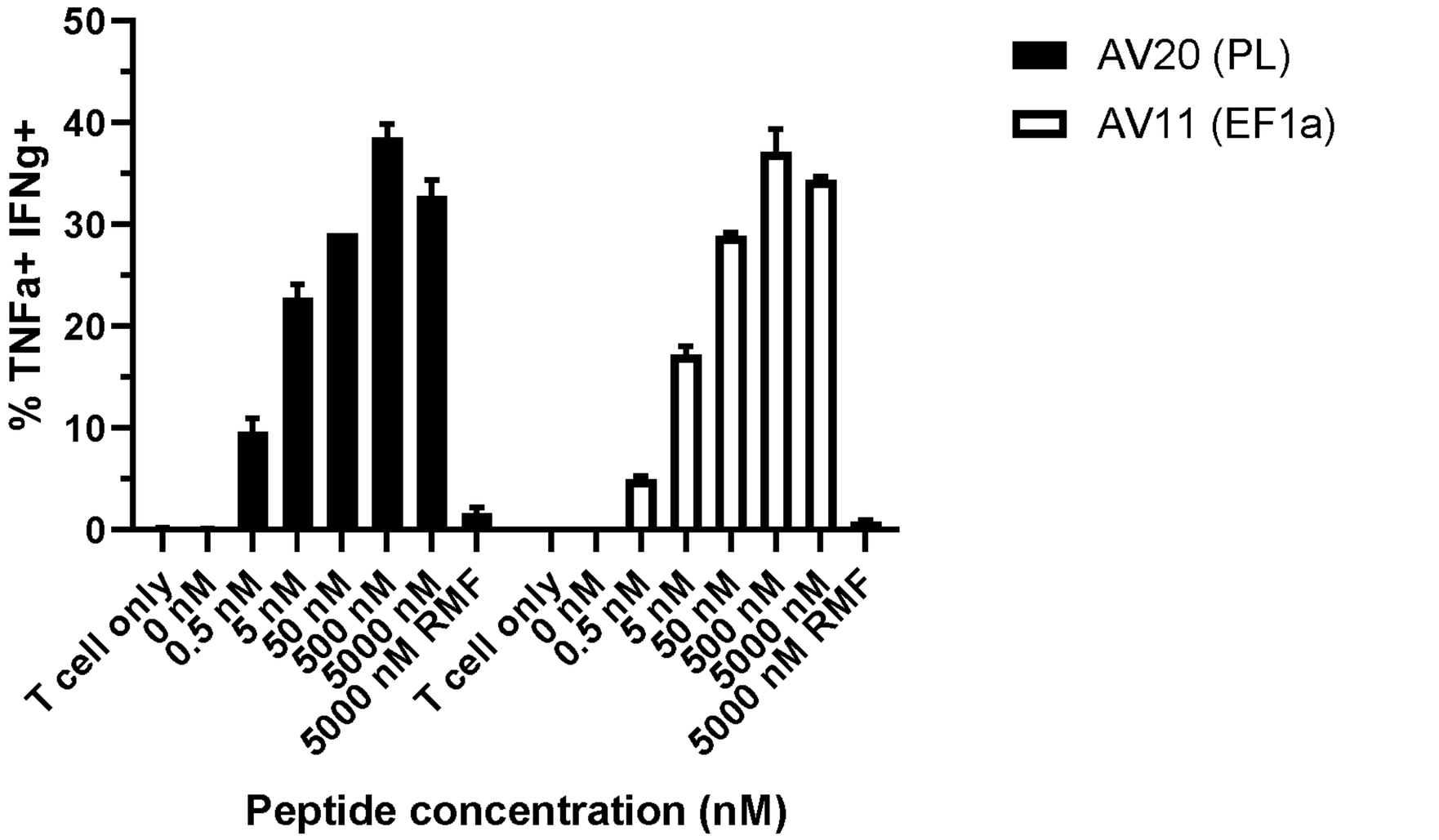

FIG 19A

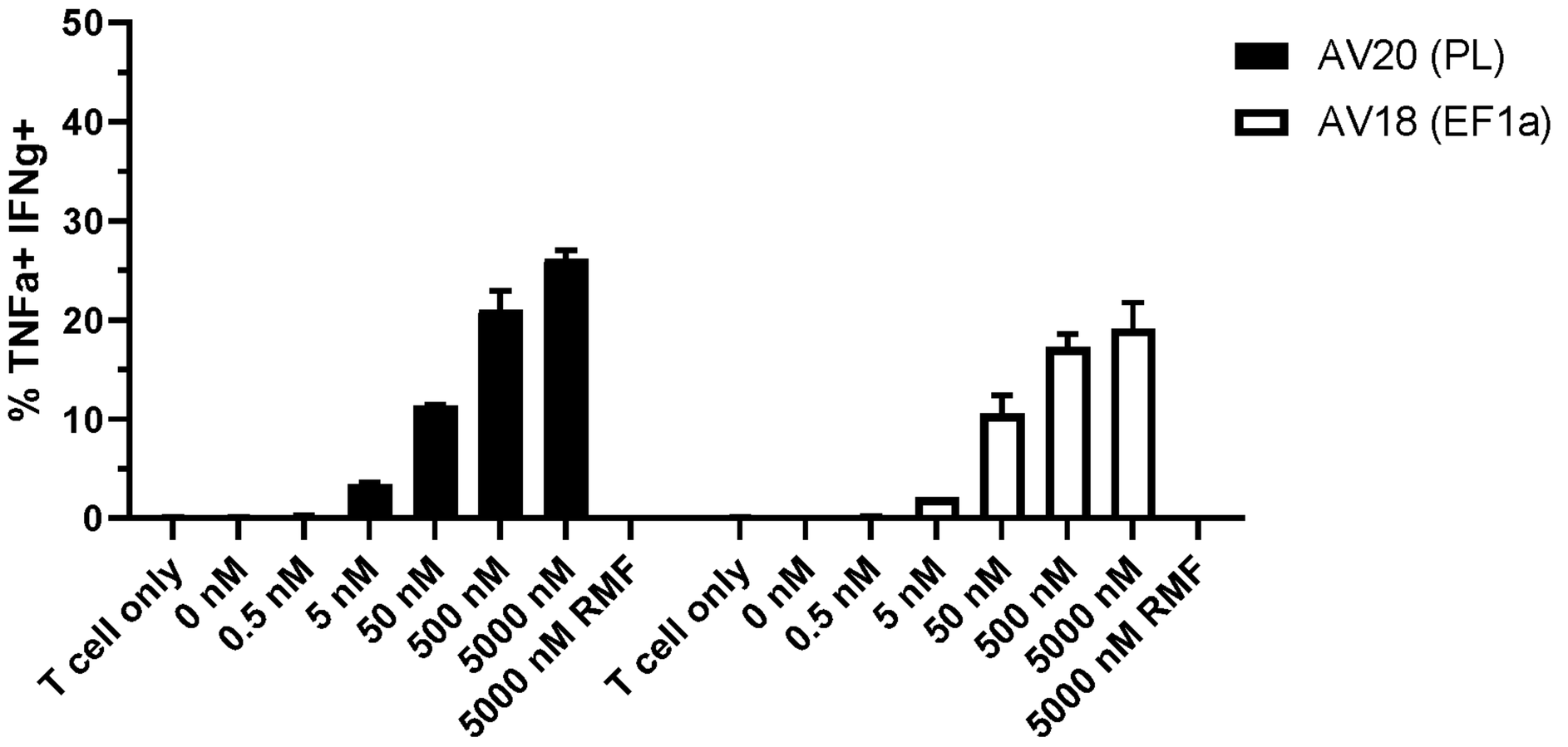

FIG 19B

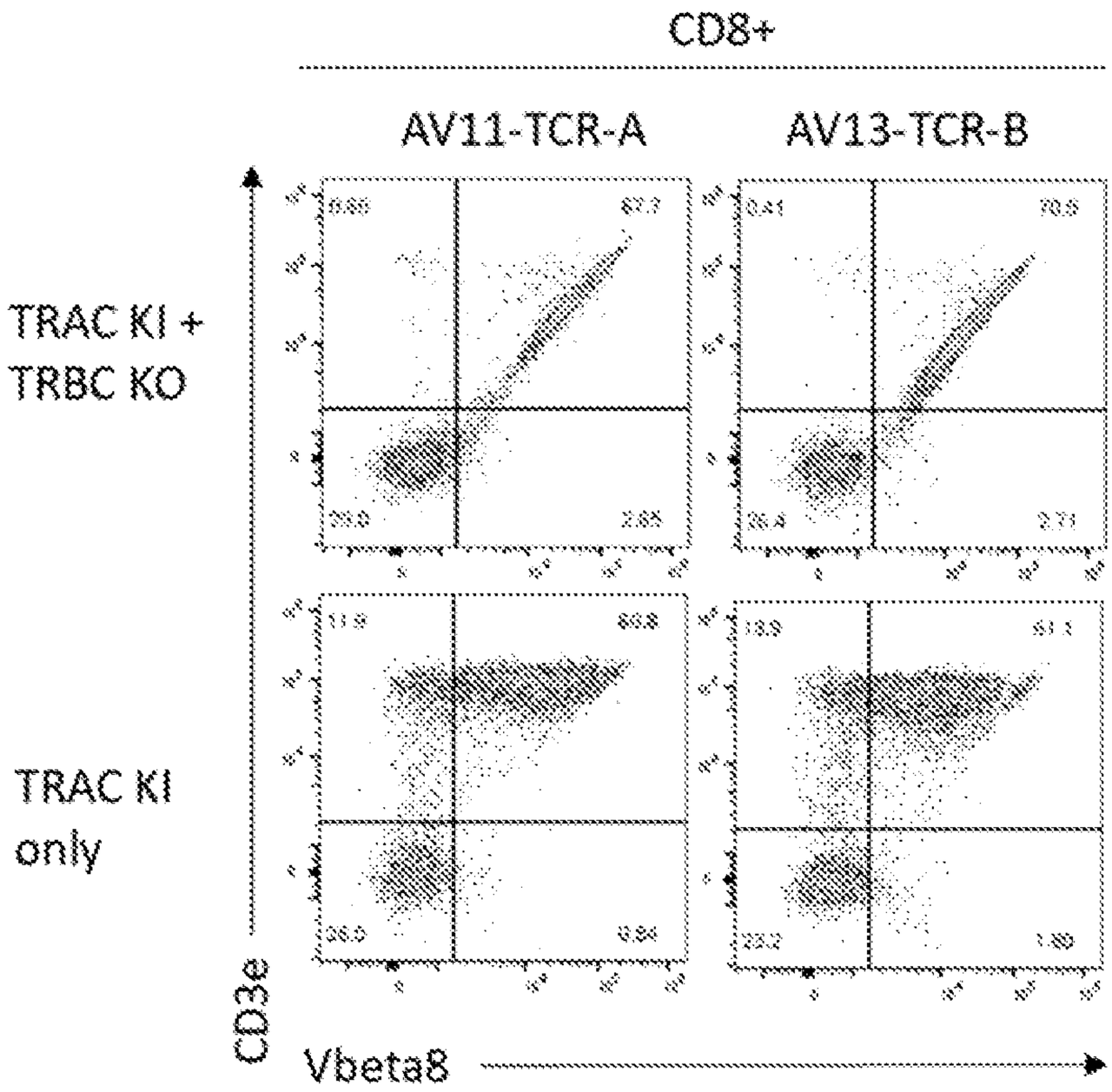

FIG 20A

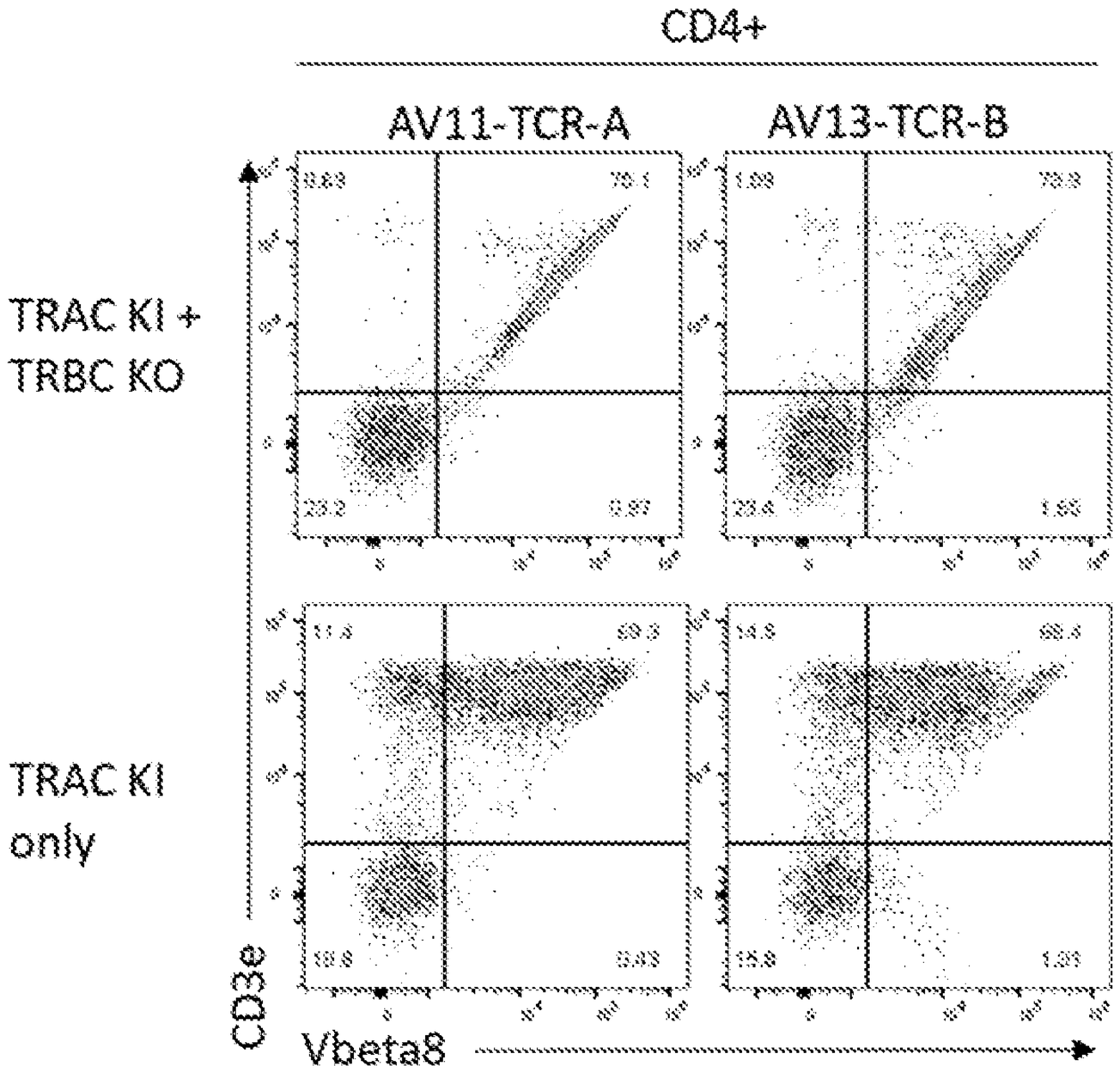

FIG 20B

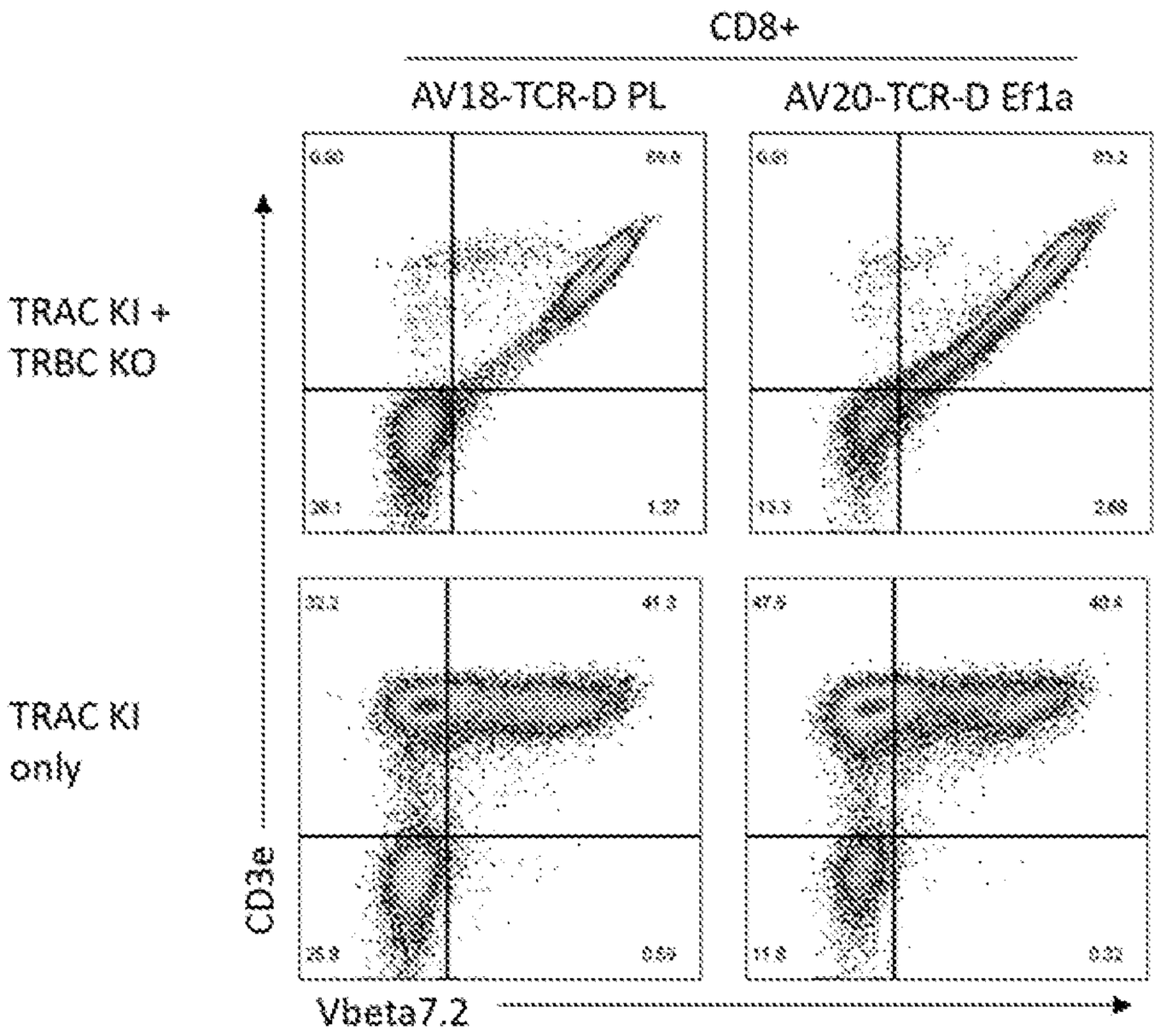

FIG 21A

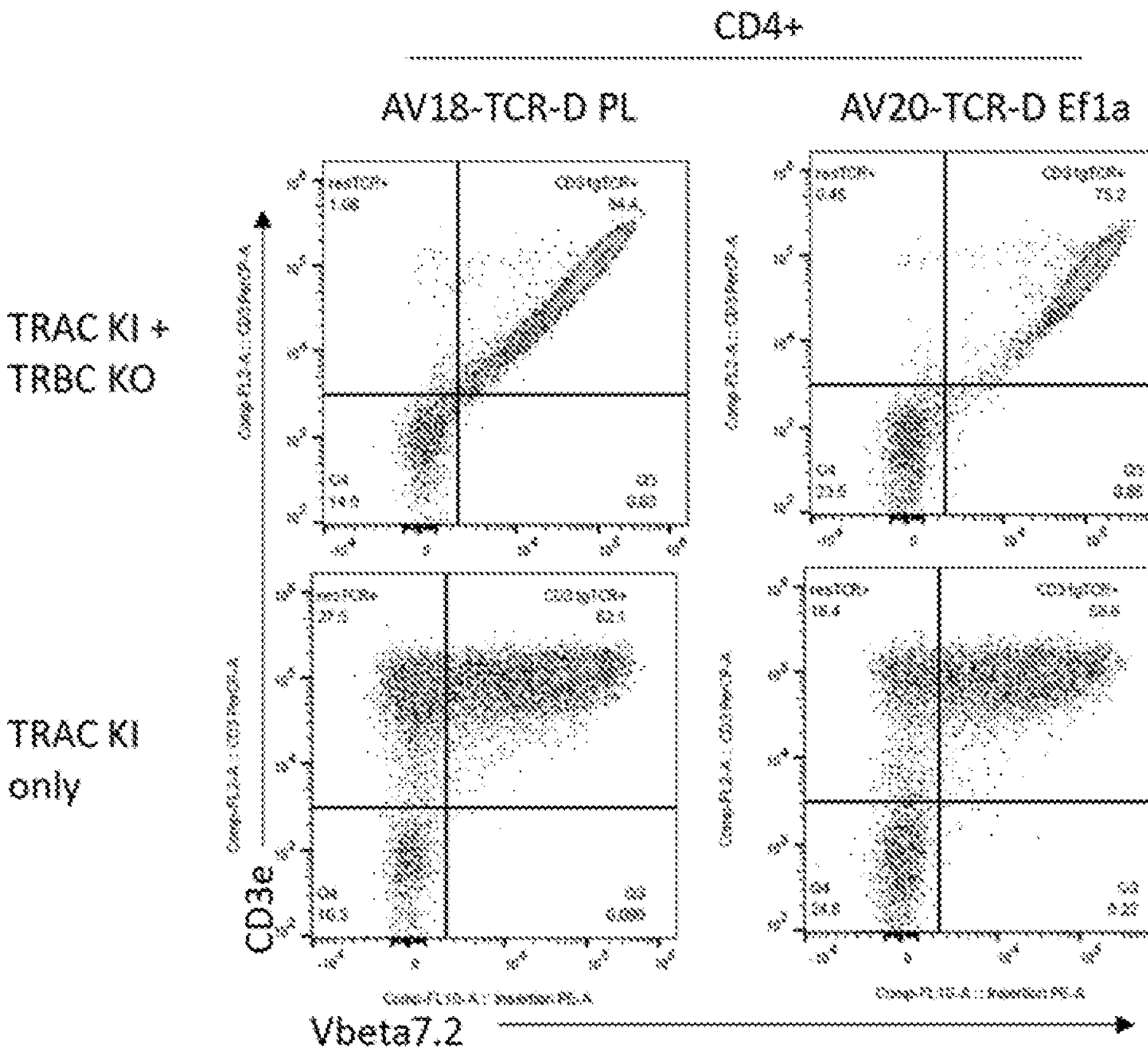

FIG 21B

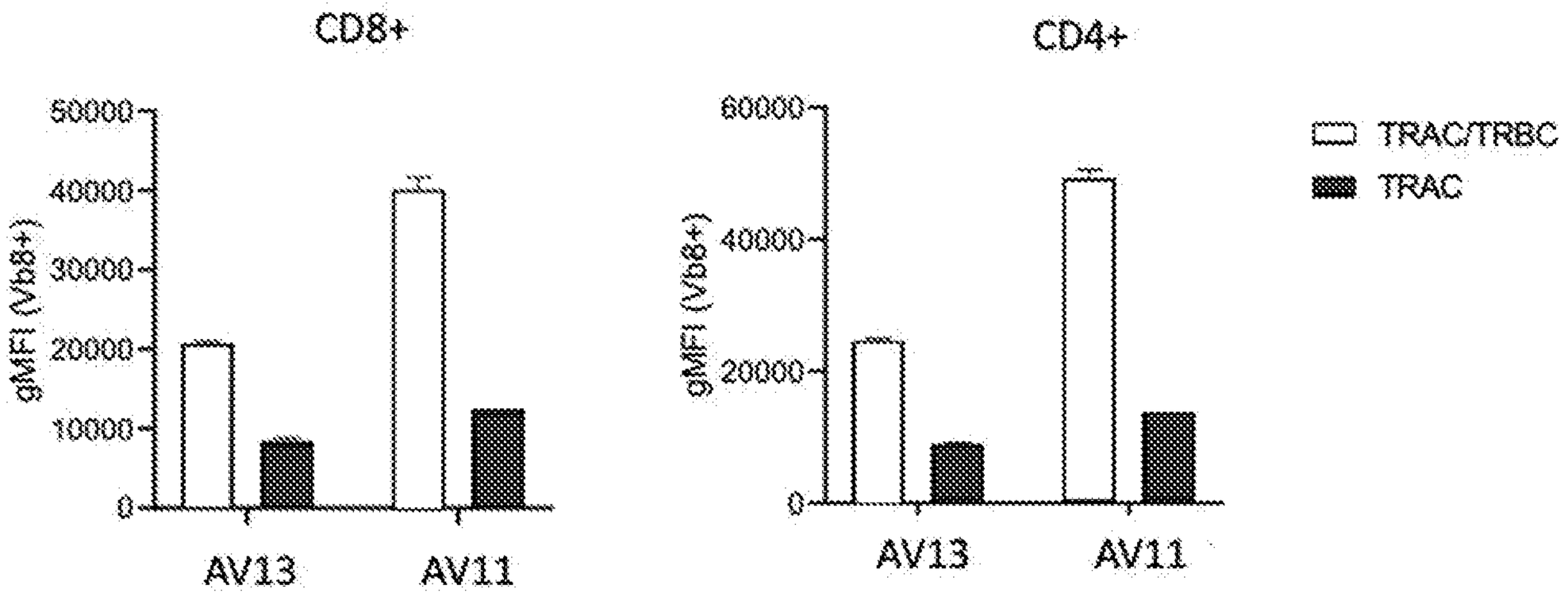

FIG 21C

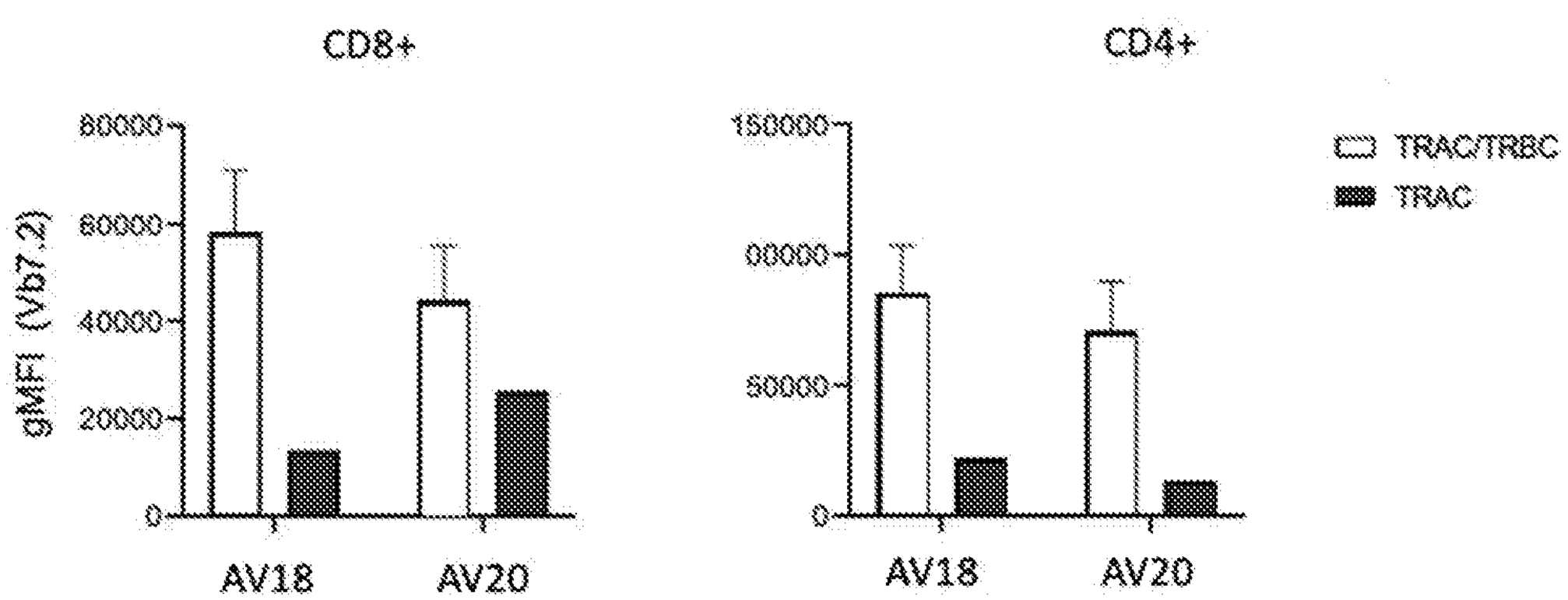

FIG 21D

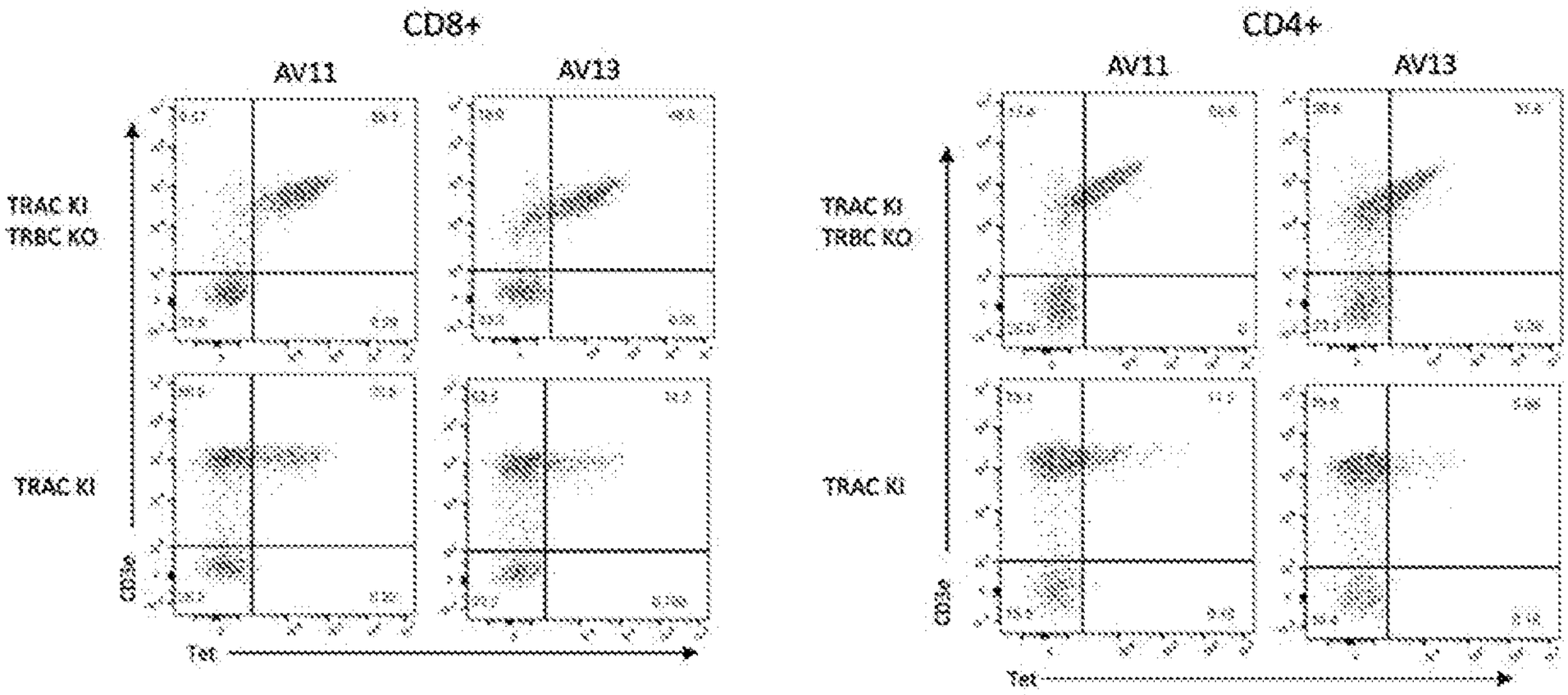
CD8+
AV11
AV13
TRAC KI
TRBC KO
TRAC KI
CD3ε
Tet
CD4+
AV11
AV13
TRAC KI
TRBC KO
TRAC KI
CD3ε
Tet

FIG 22

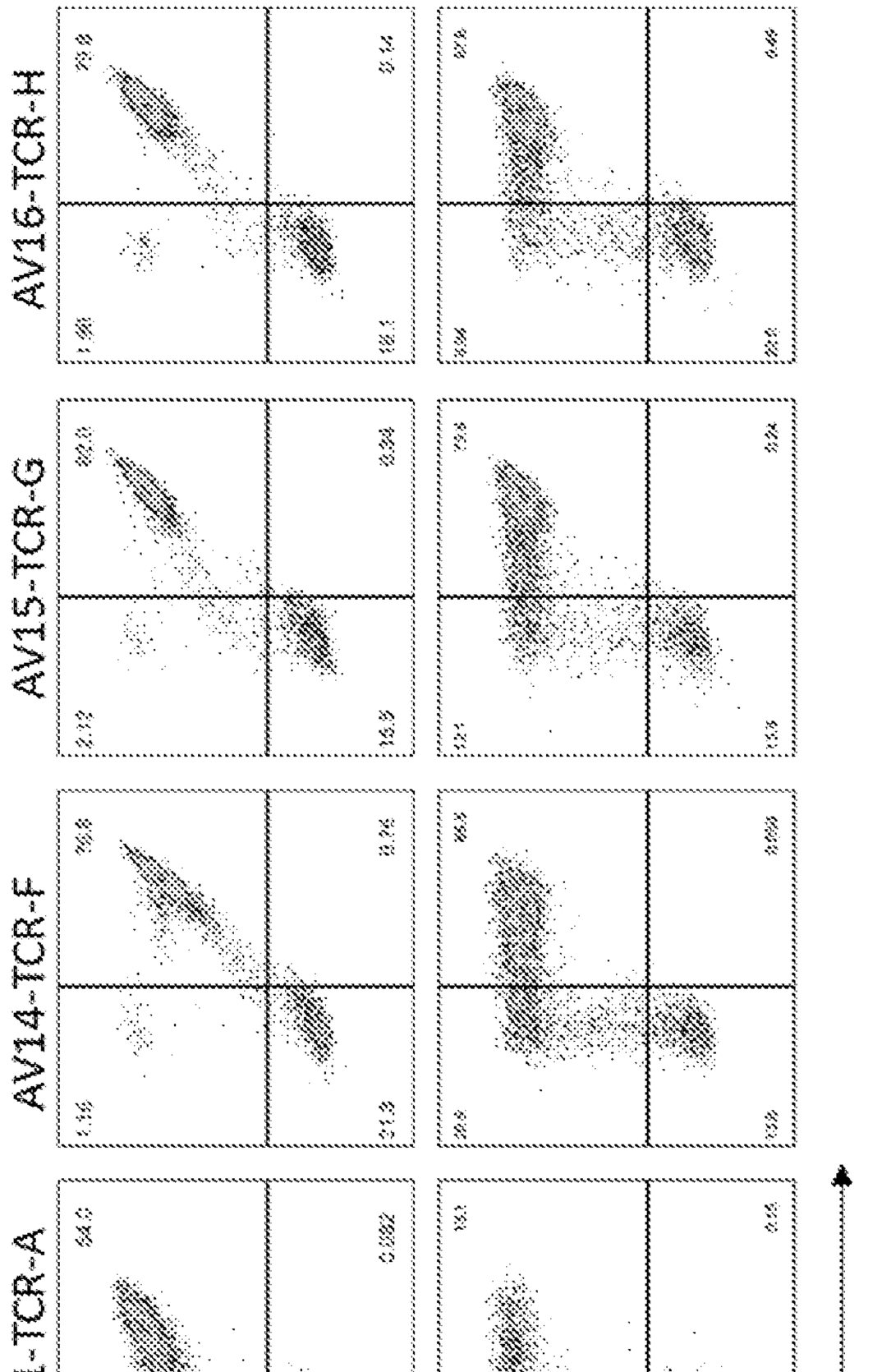
TRAC KI
TRBC KO
TRAC KI
No AAV
AV11-TCR-A
AV14-TCR-F
AV15-TCR-G
AV16-TCR-H
CD8+
CD3ε
V LD Tetramer

Fig. 23

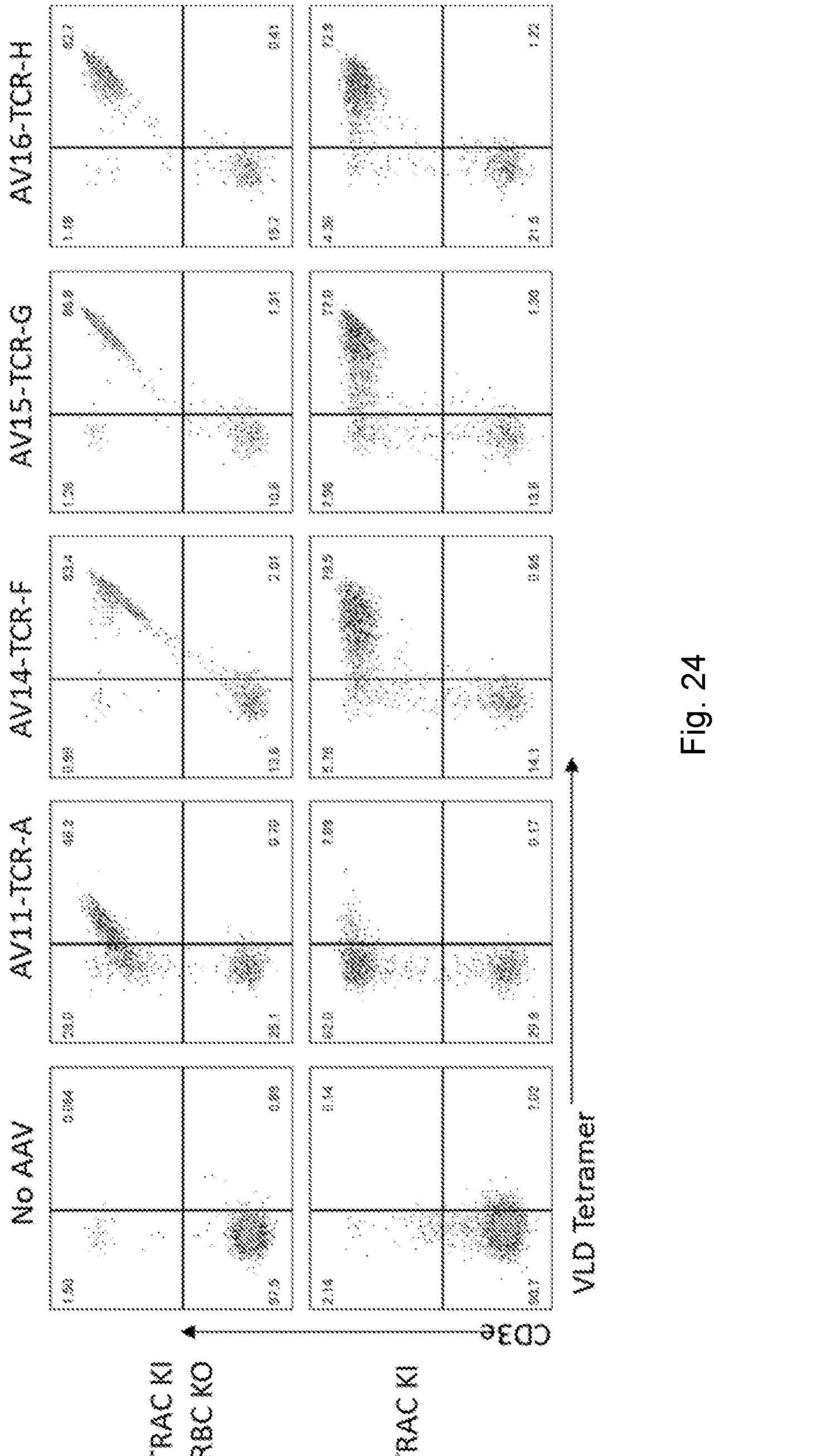
CD4+
AV16-TCR-H
AV14-TCR-F
AV15-TCR-G
AV11-TCR-A
No AAV
CD3e
VLD Tetramer
TRAC KI
TRBC KO
TRAC KI

Fig. 24

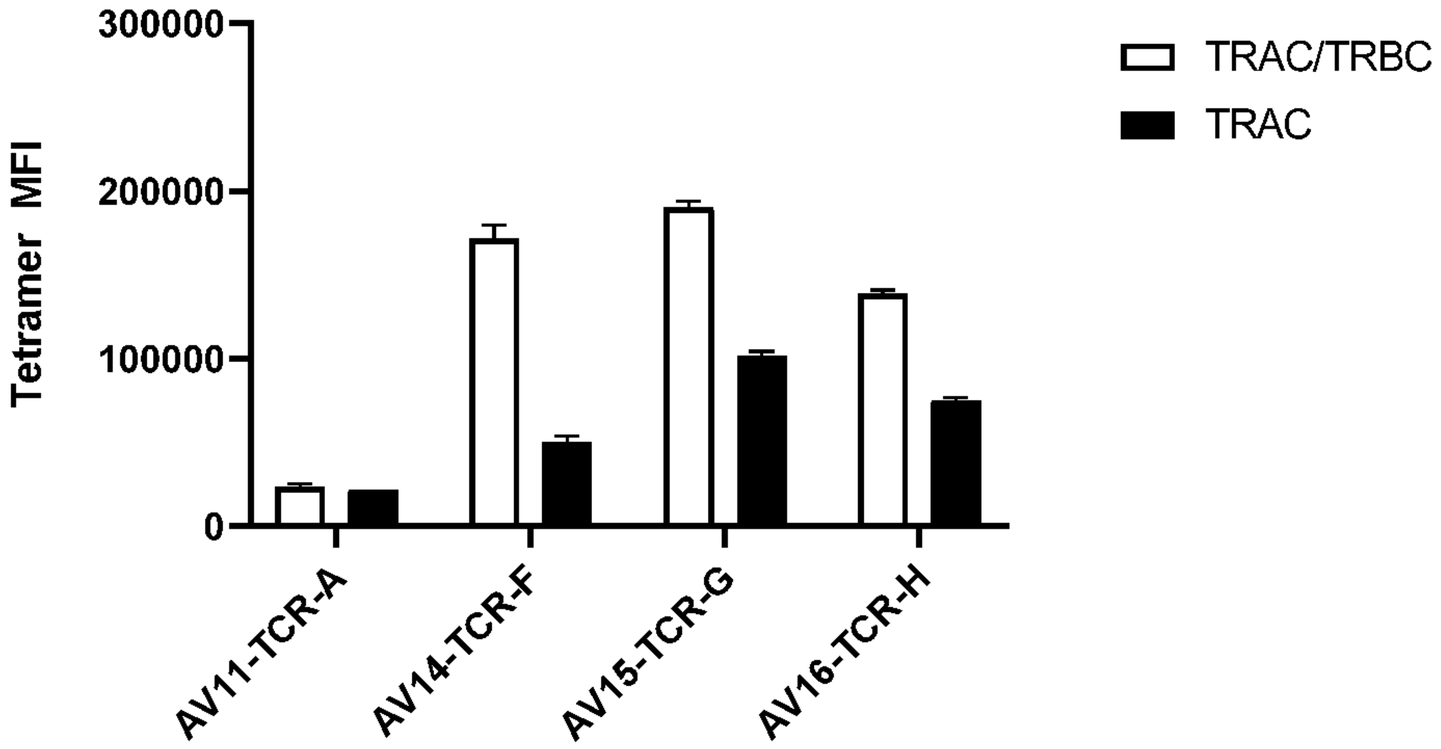
CD8+ VLD Tetramer+
Tetramer MFI
300000
200000
100000
0
TRAC/TRBC
TRAC
AV11-TCR-A
AV14-TCR-F
AV15-TCR-G
AV16-TCR-H

FIG 25

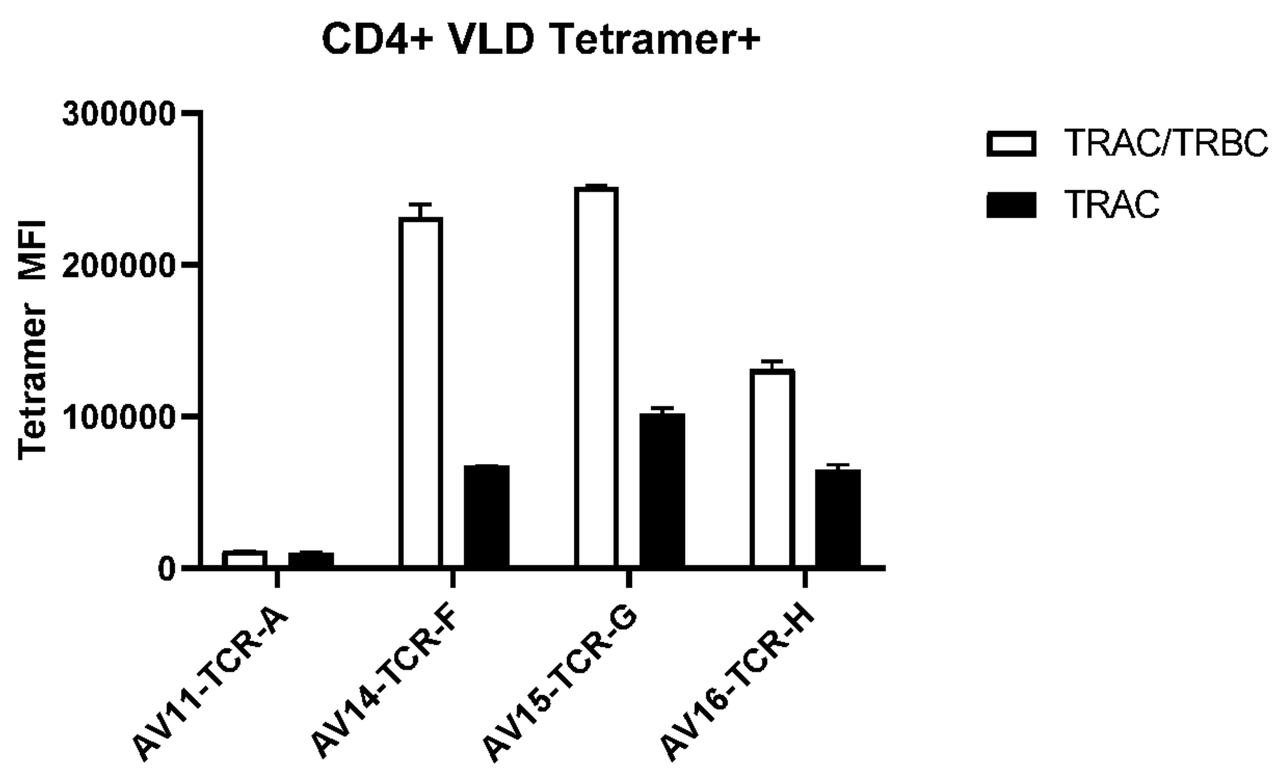
CD4+ VLD Tetramer+
Tetramer MFI
300000
200000
100000
0
TRAC/TRBC
TRAC
AV11-TCR-A
AV14-TCR-F
AV15-TCR-G
AV16-TCR-H

FIG 26

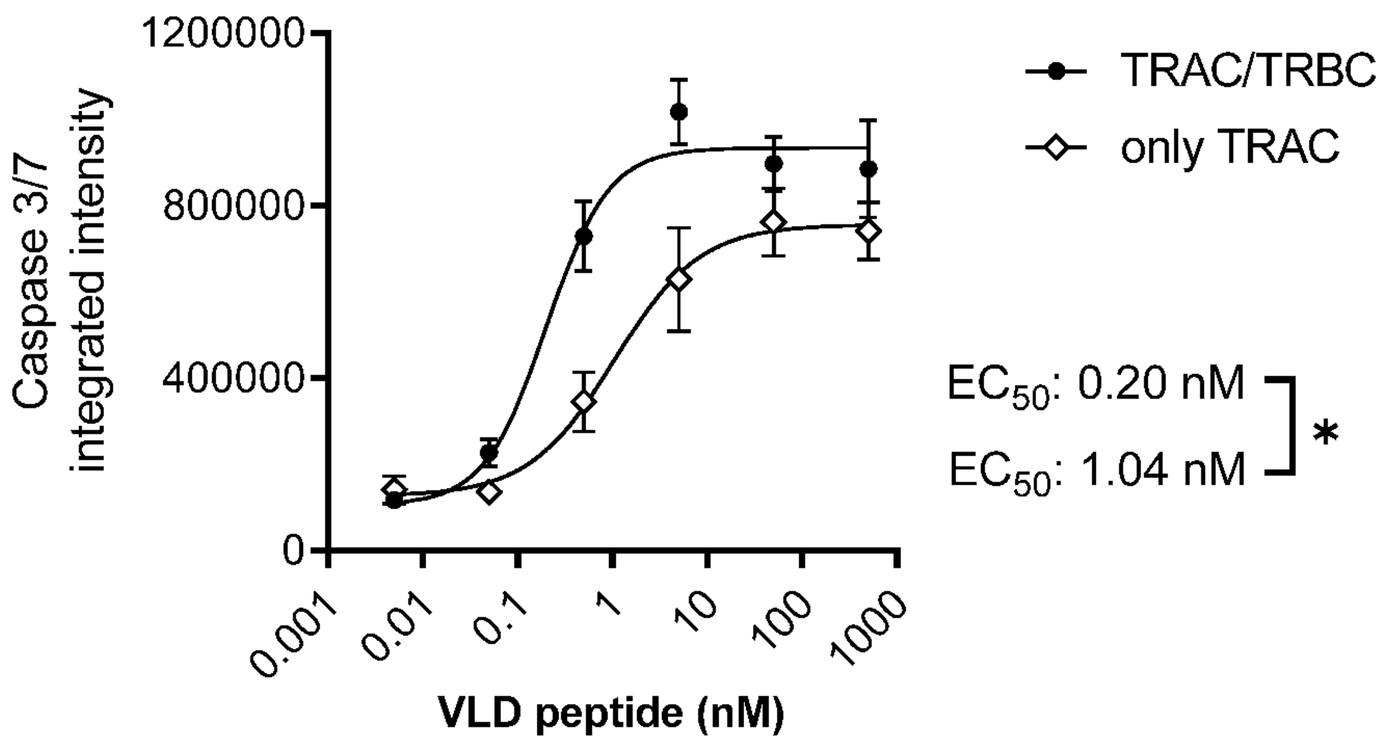

FIG 27A

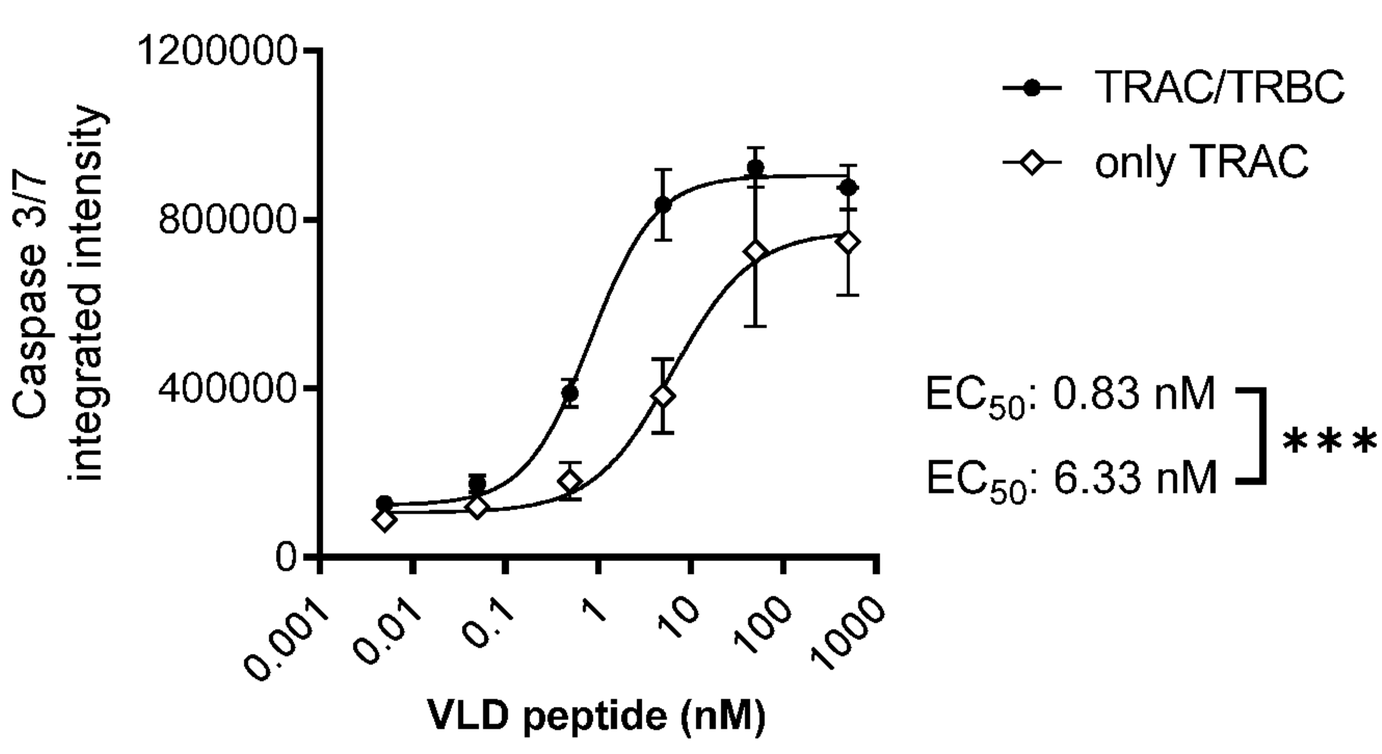

FIG 27B

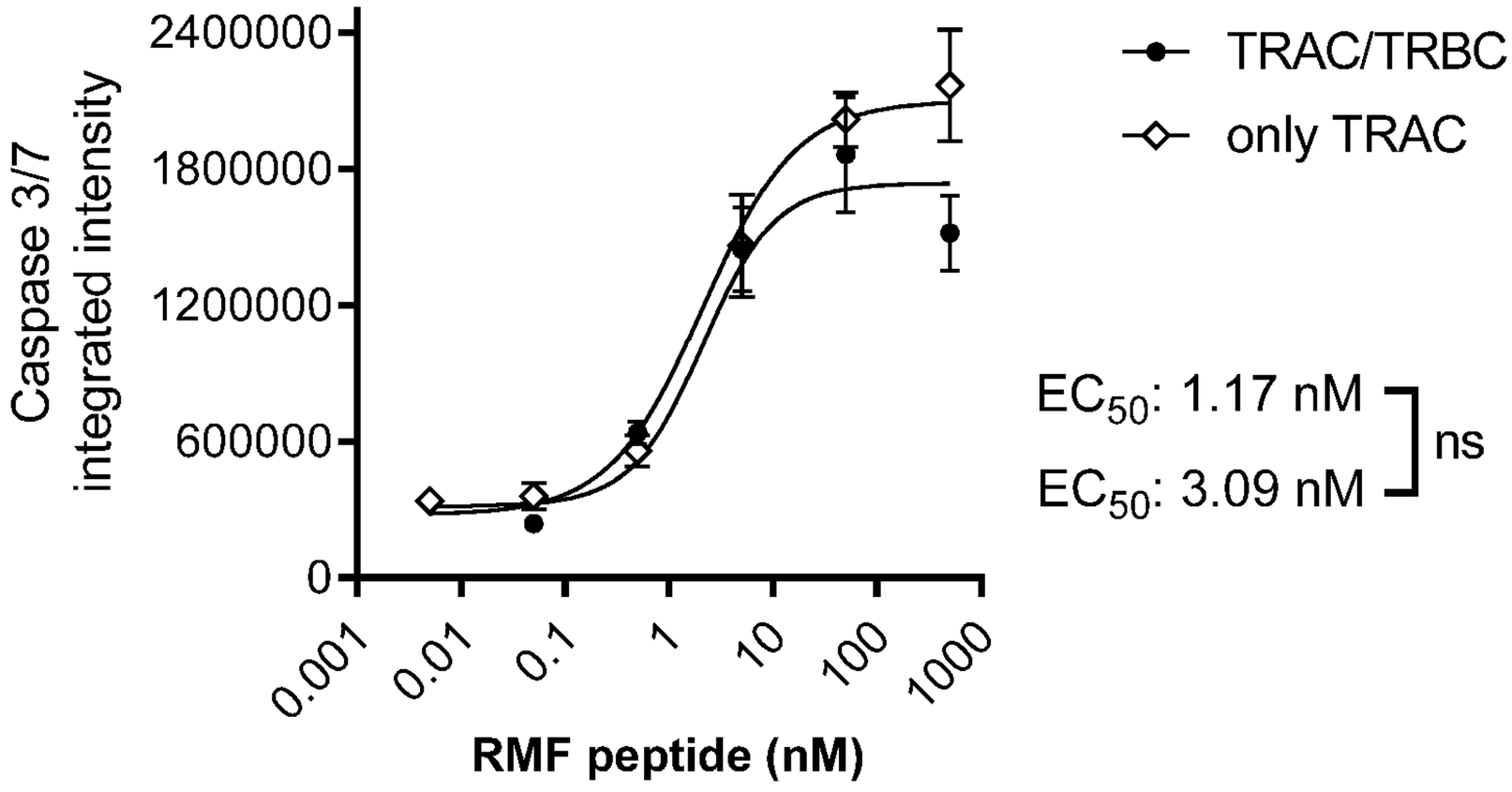
AV12-TCR-C
Caspase 3/7 integrated intensity
2400000
1800000
1200000
600000
0
0.001
0.01
0.1
1
10
100
1000
RMF peptide (nM)
TRAC/TRBC
only TRAC
EC50: 1.17 nM
EC50: 3.09 nM
ns

FIG 27C

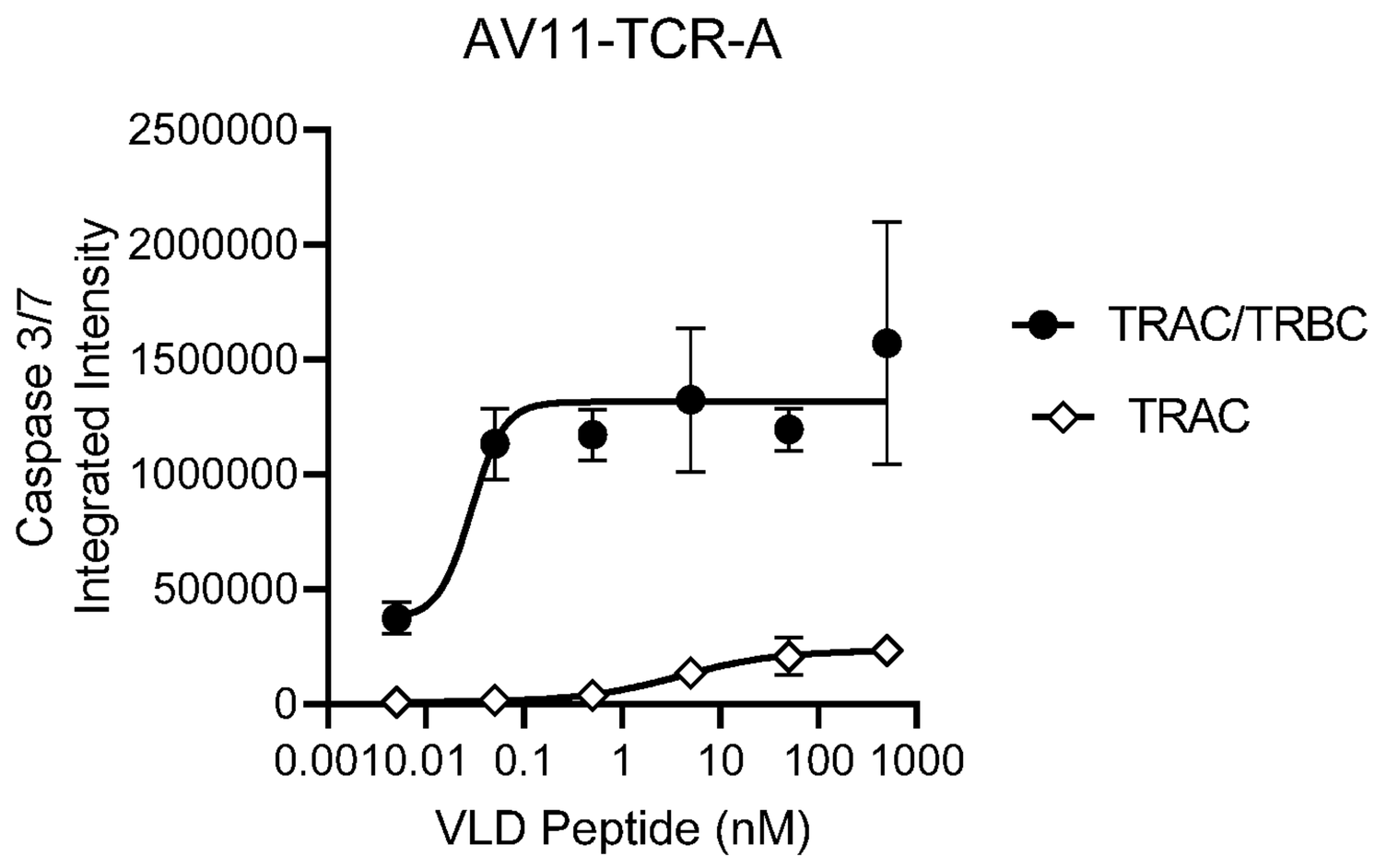

FIG 28A

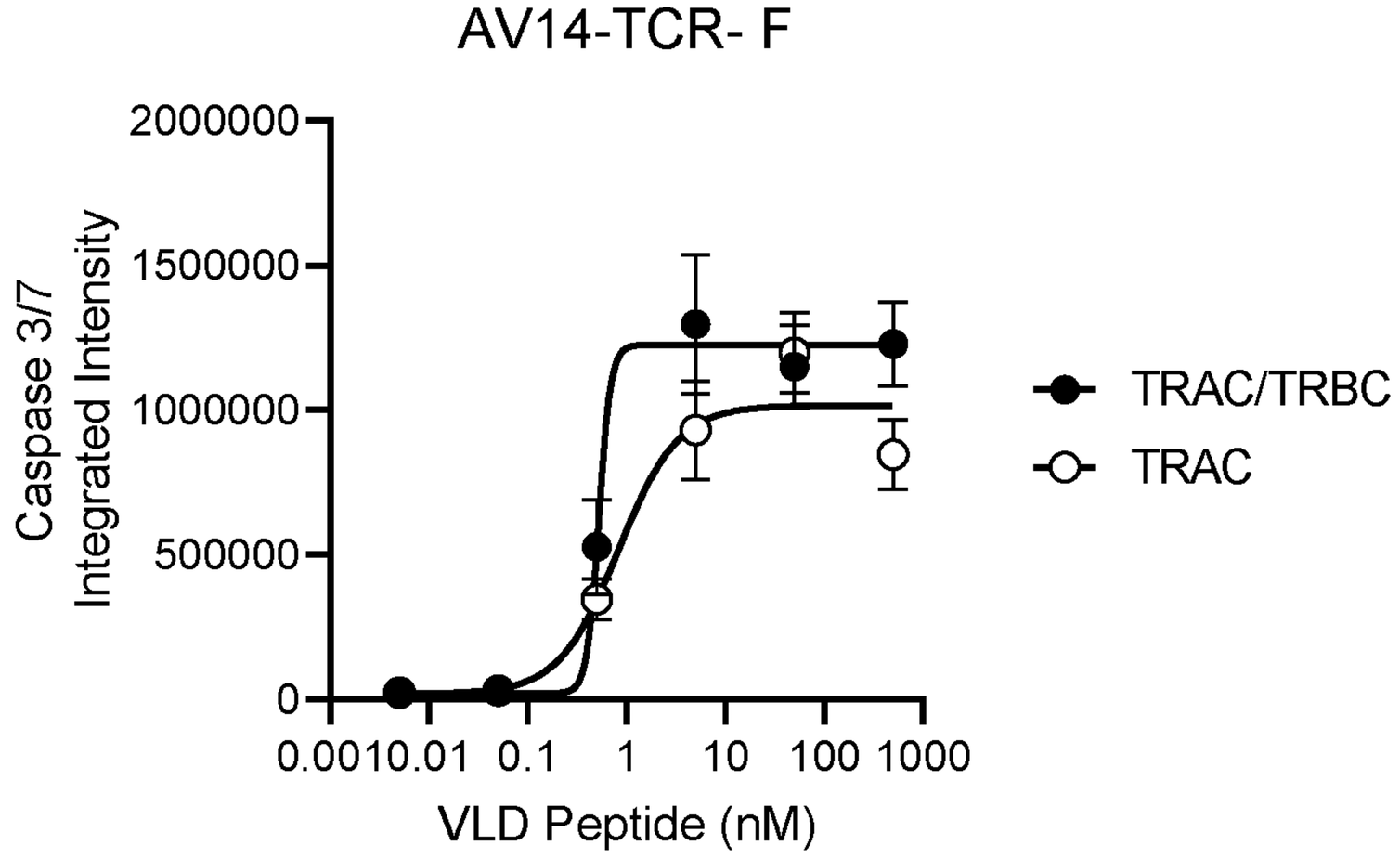

FIG 28B

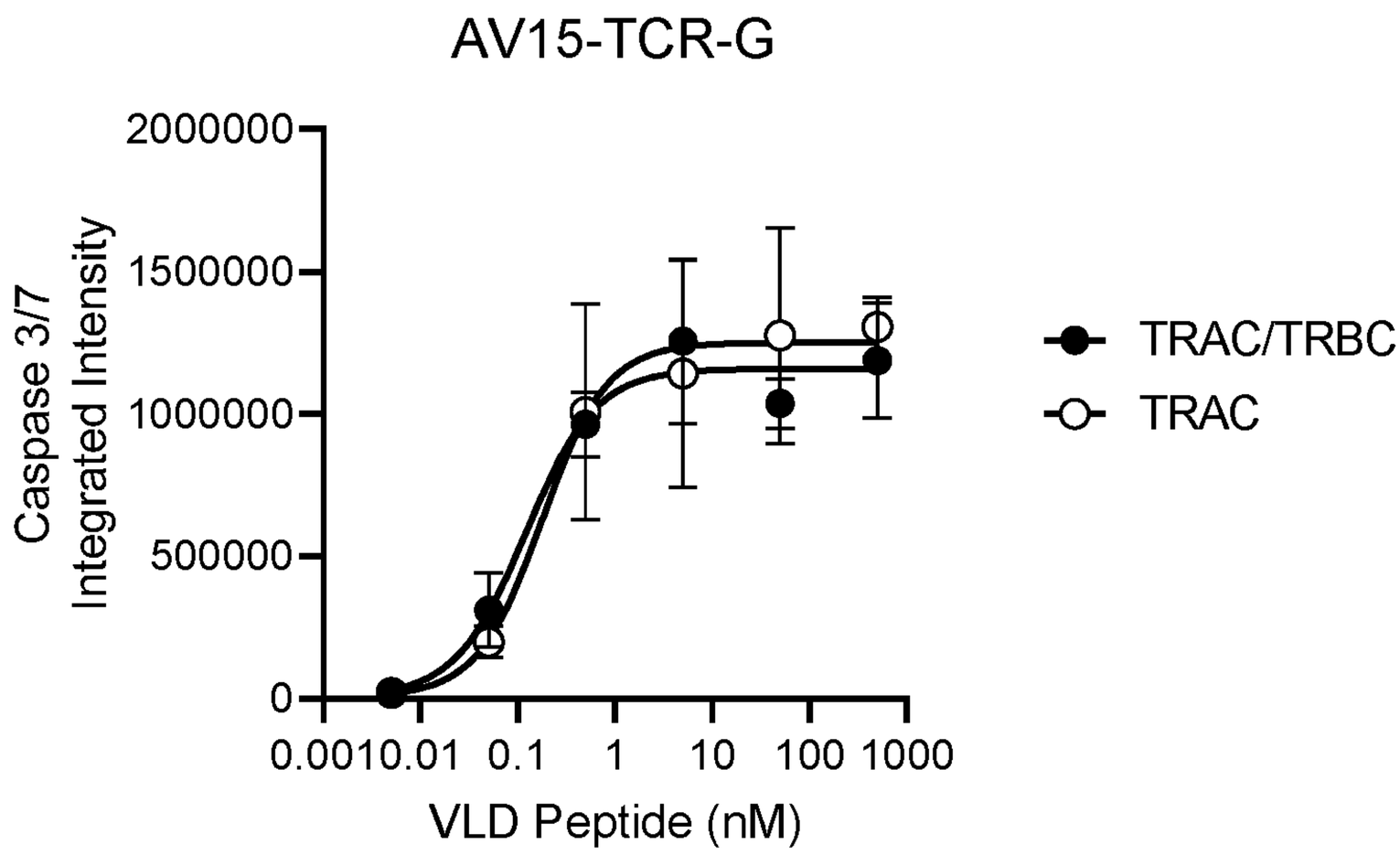

FIG 28C

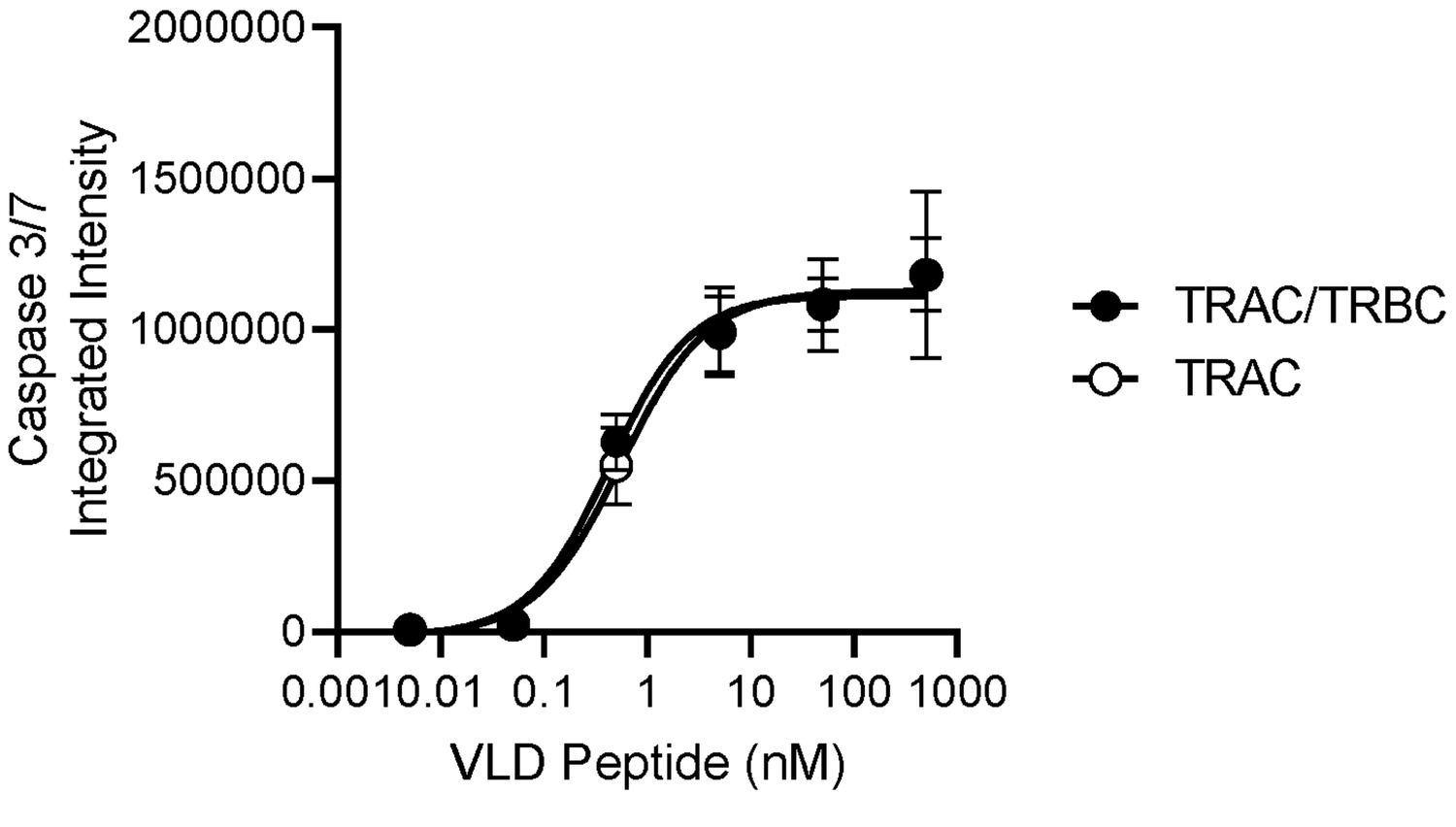

FIG 28D

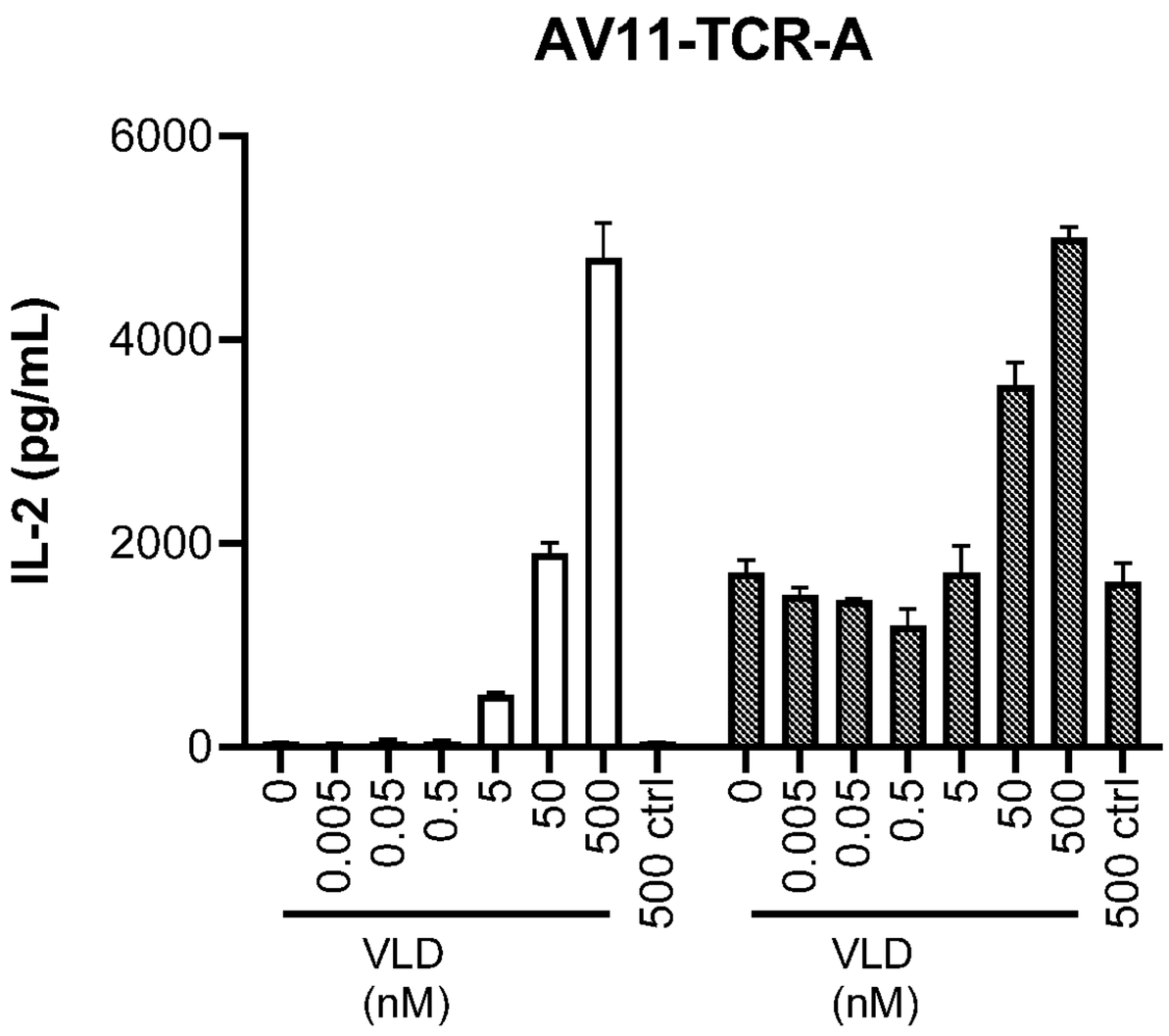

FIG 29A

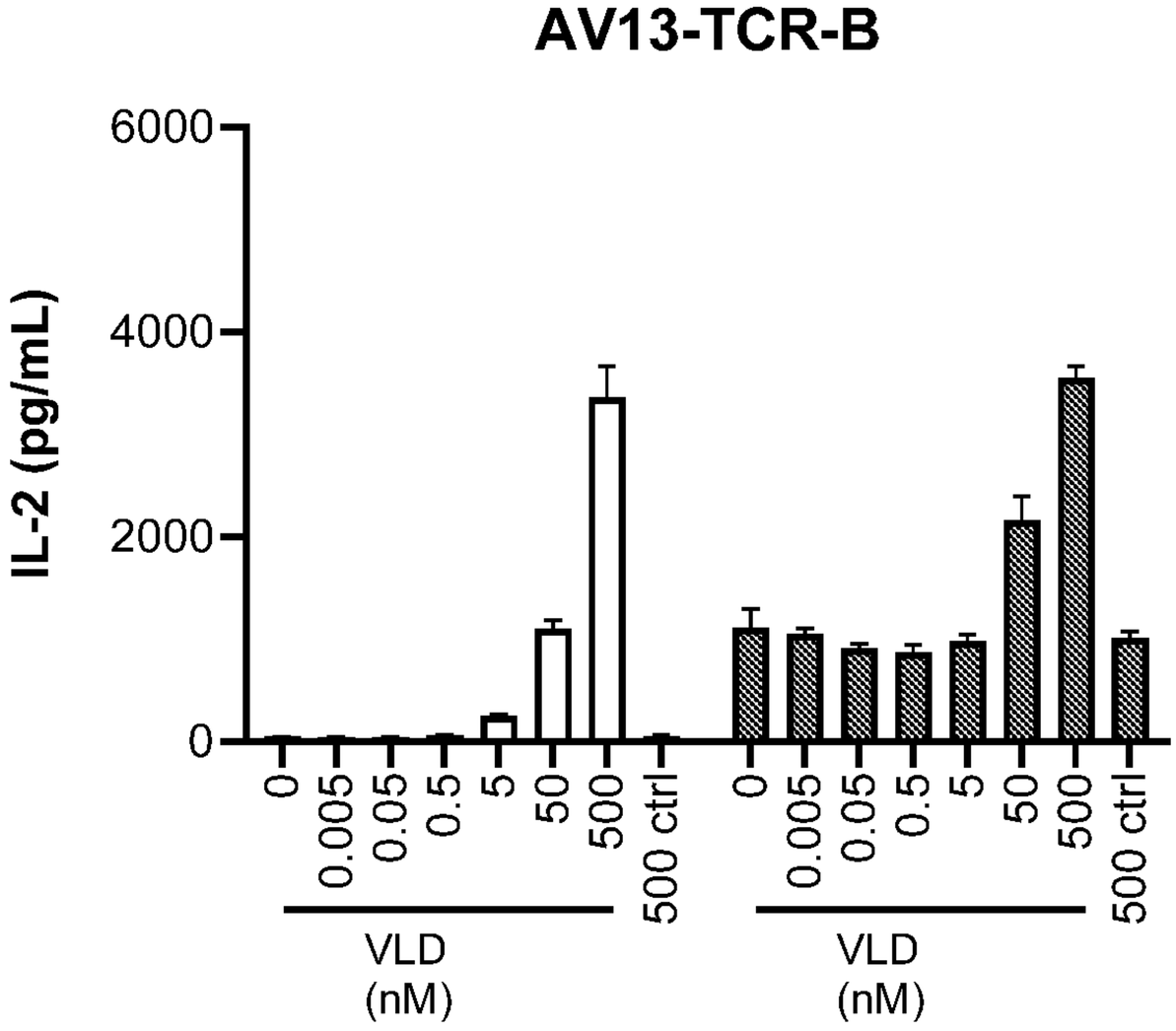

Fig 29B

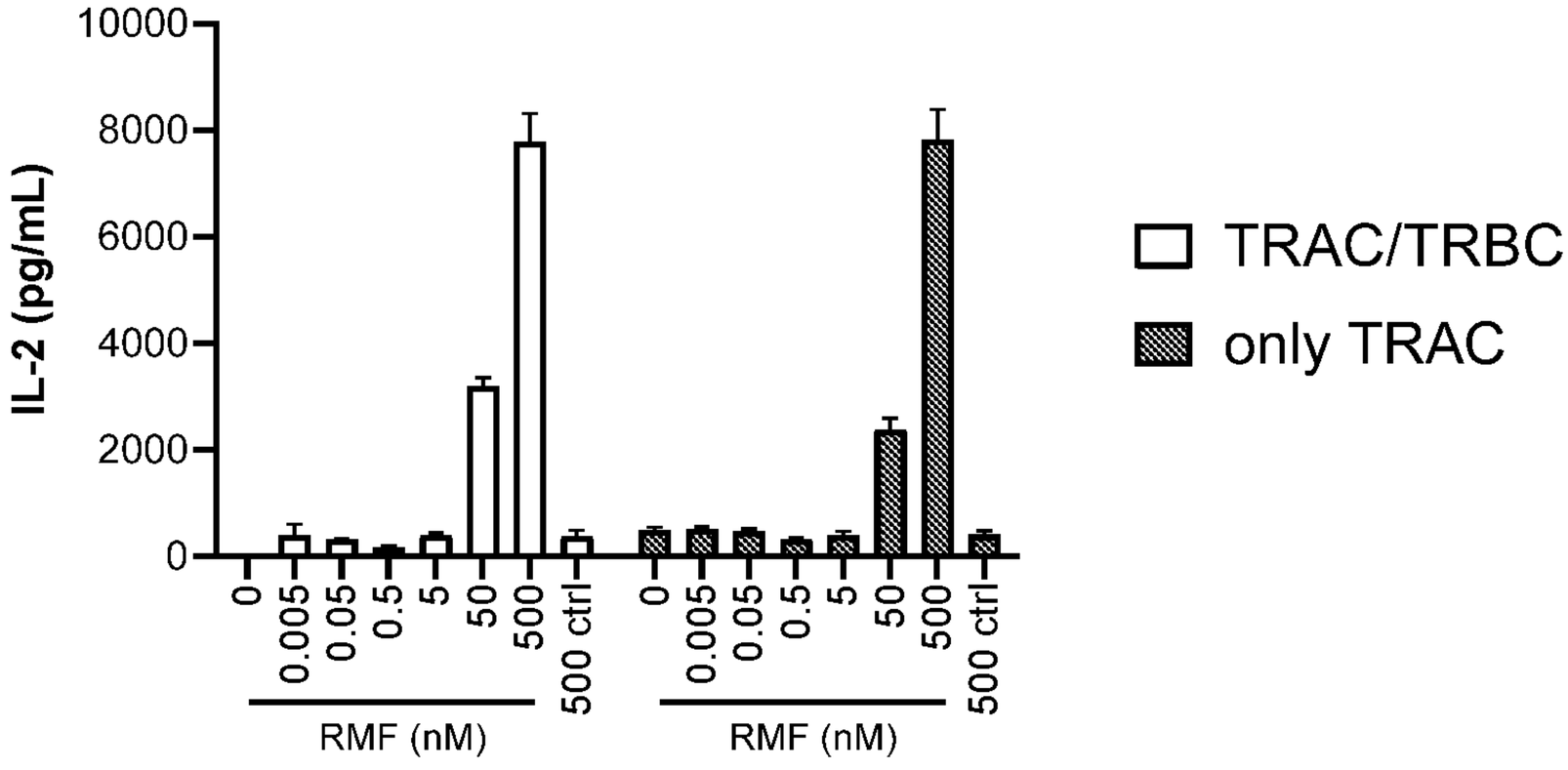

Fig 29C

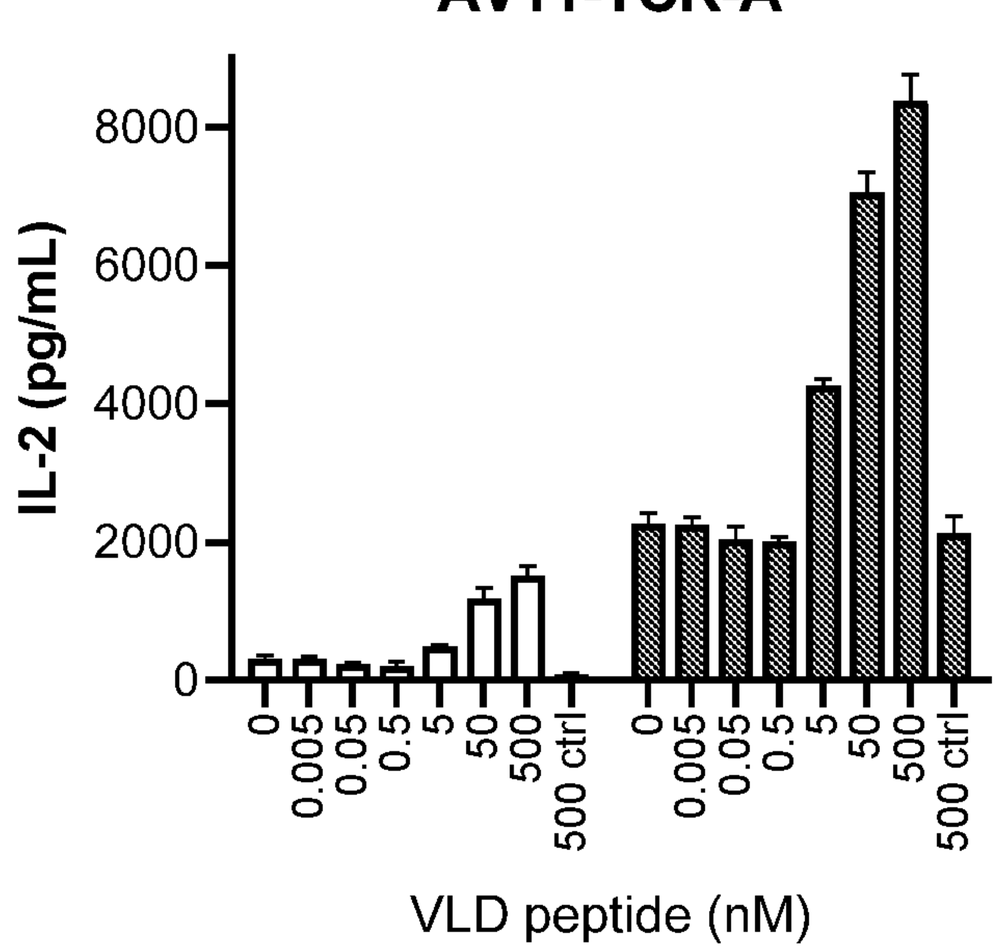

FIG 29D

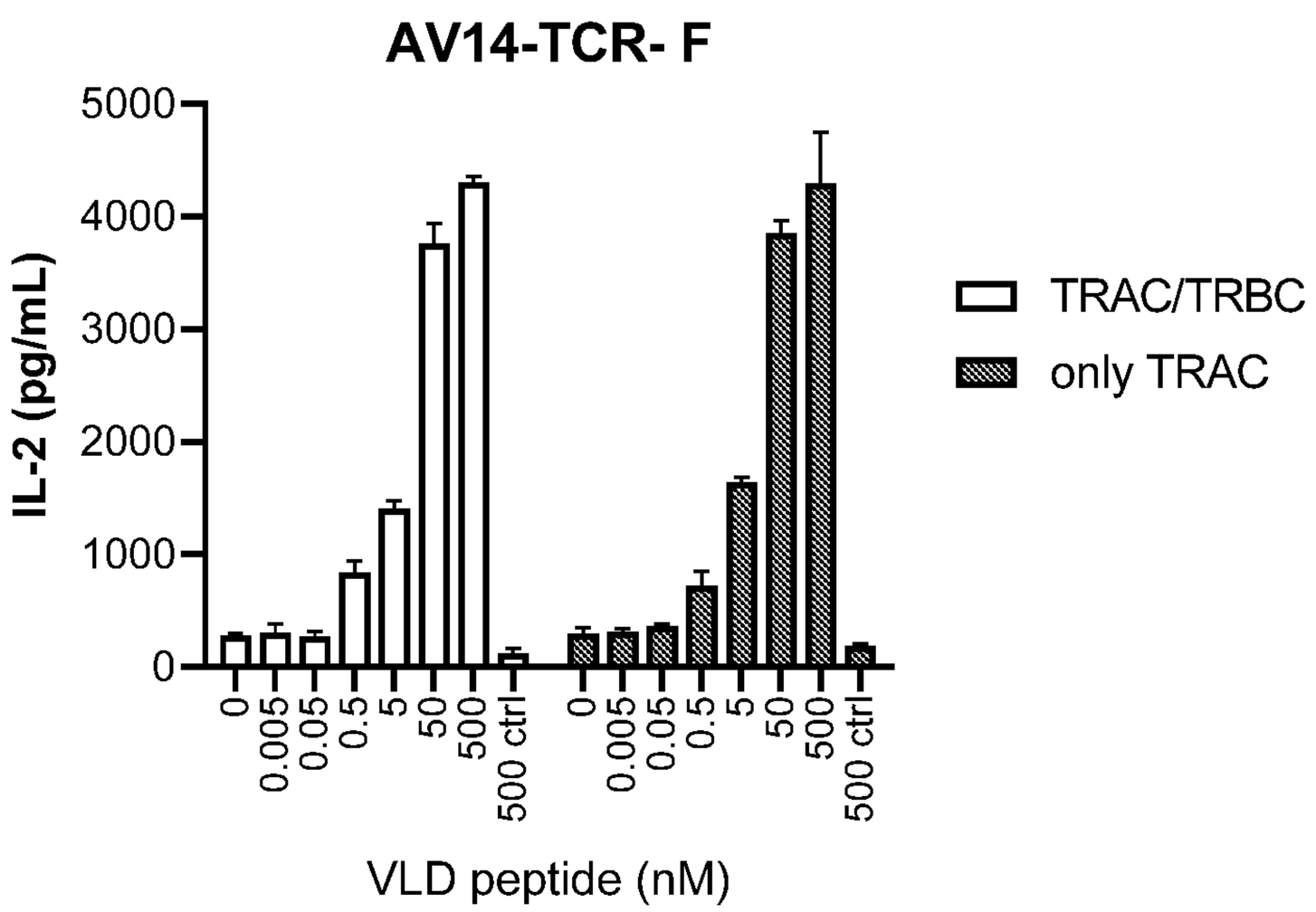

FIG 29E

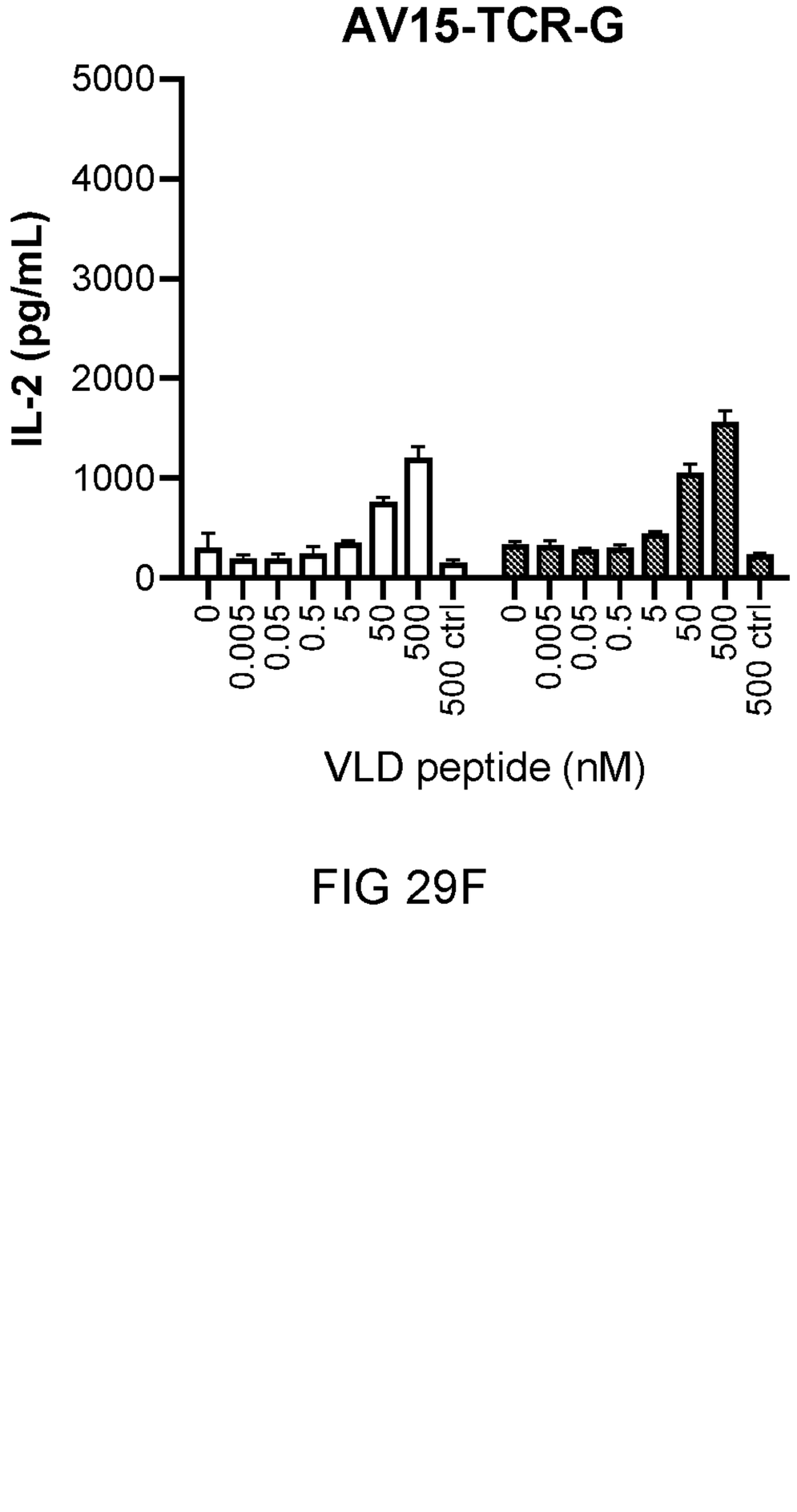

FIG 29F

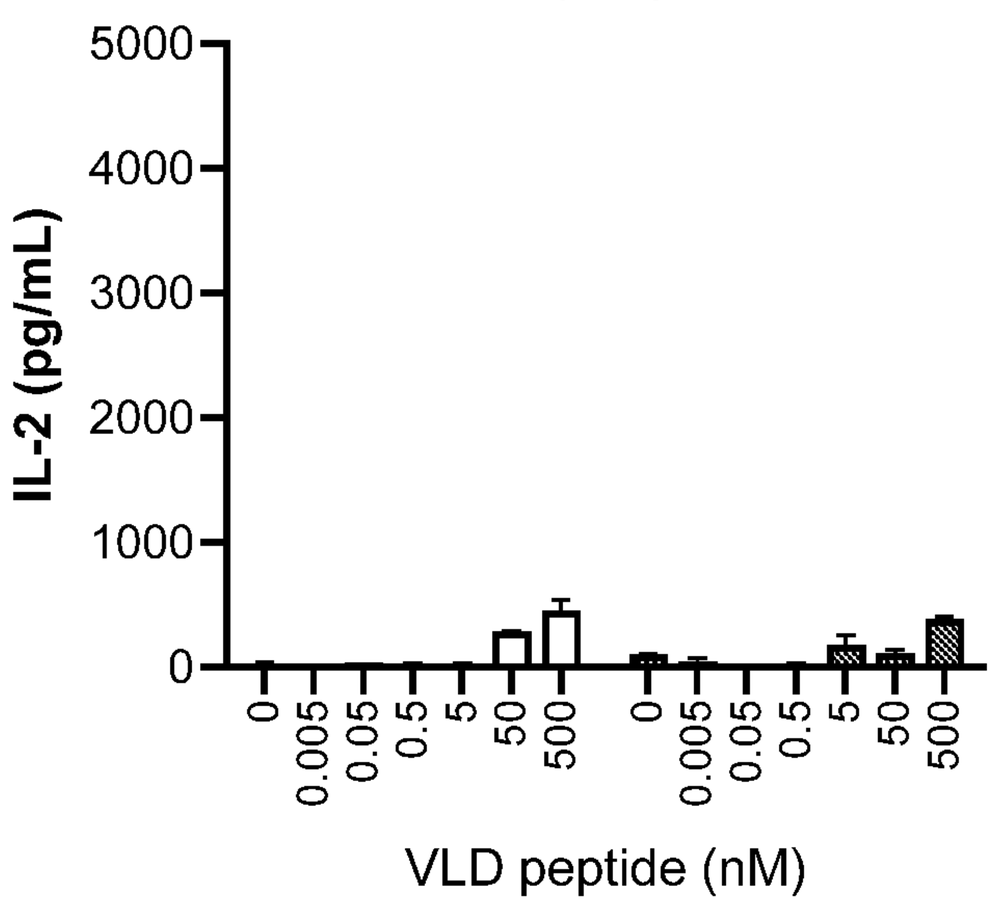
AV16-TCR-H
IL-2 (pg/mL)
5000
4000
3000
2000
1000
0
0
0.005
0.05
0.5
5
50
500
0
0.005
0.05
0.5
5
50
500
VLD peptide (nM)

FIG 29G

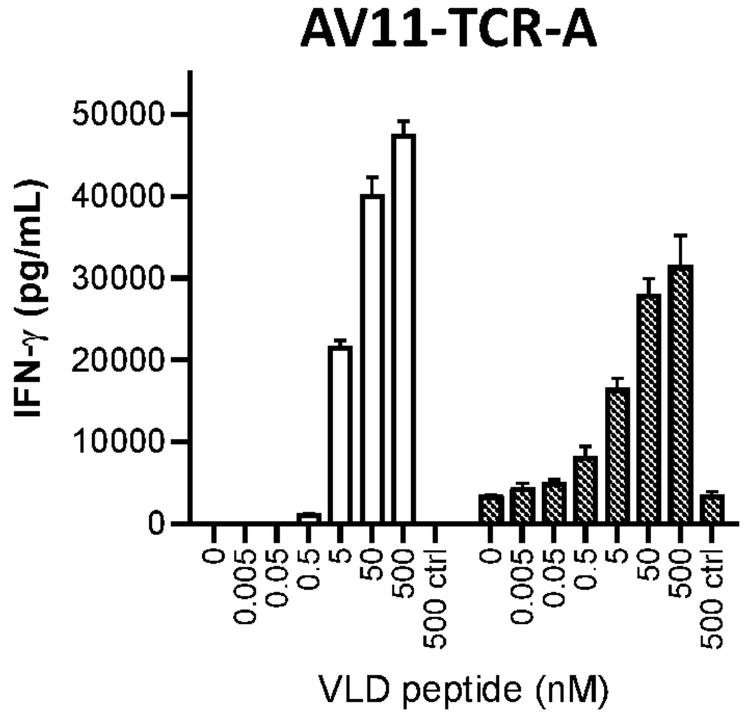

FIG 30A

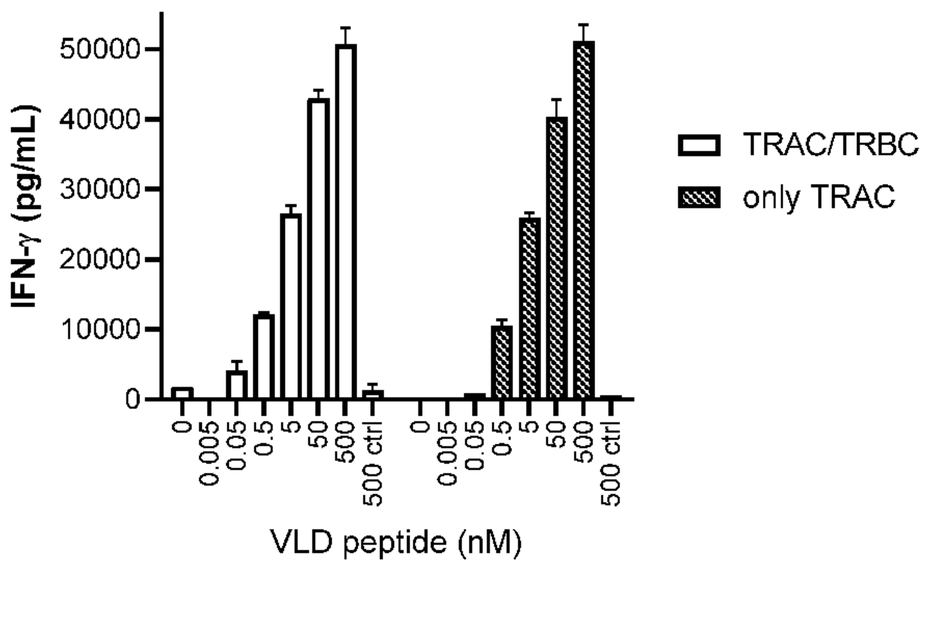

FIG 30B

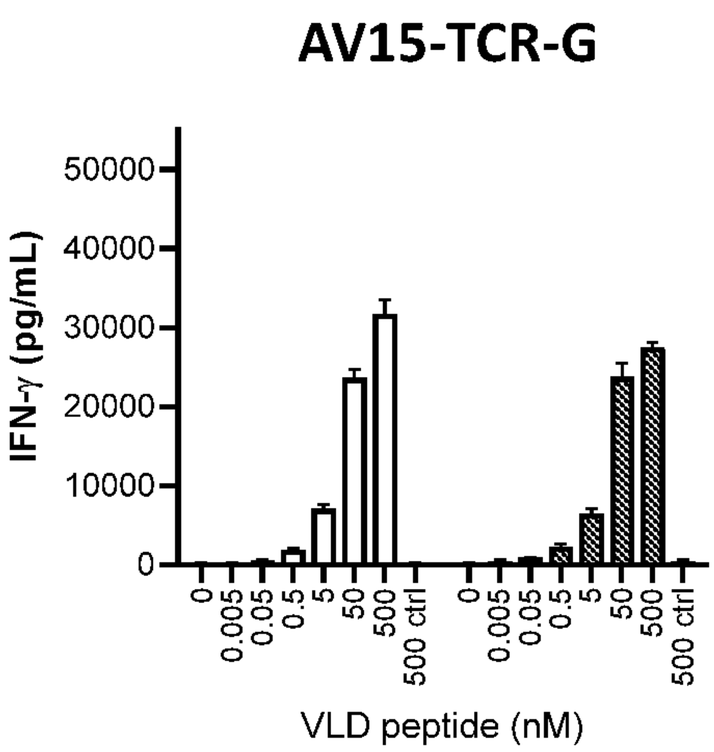

FIG 30C

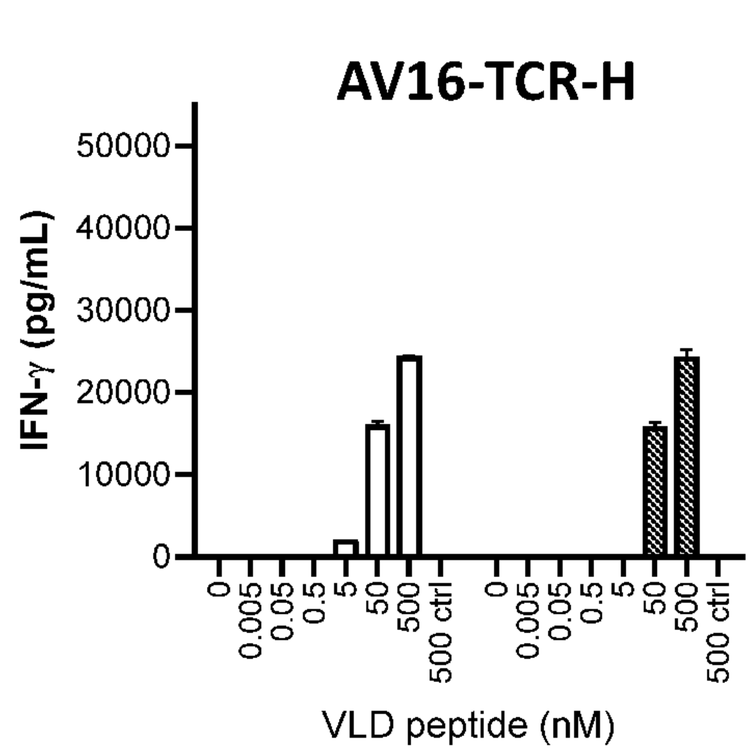

FIG 30D

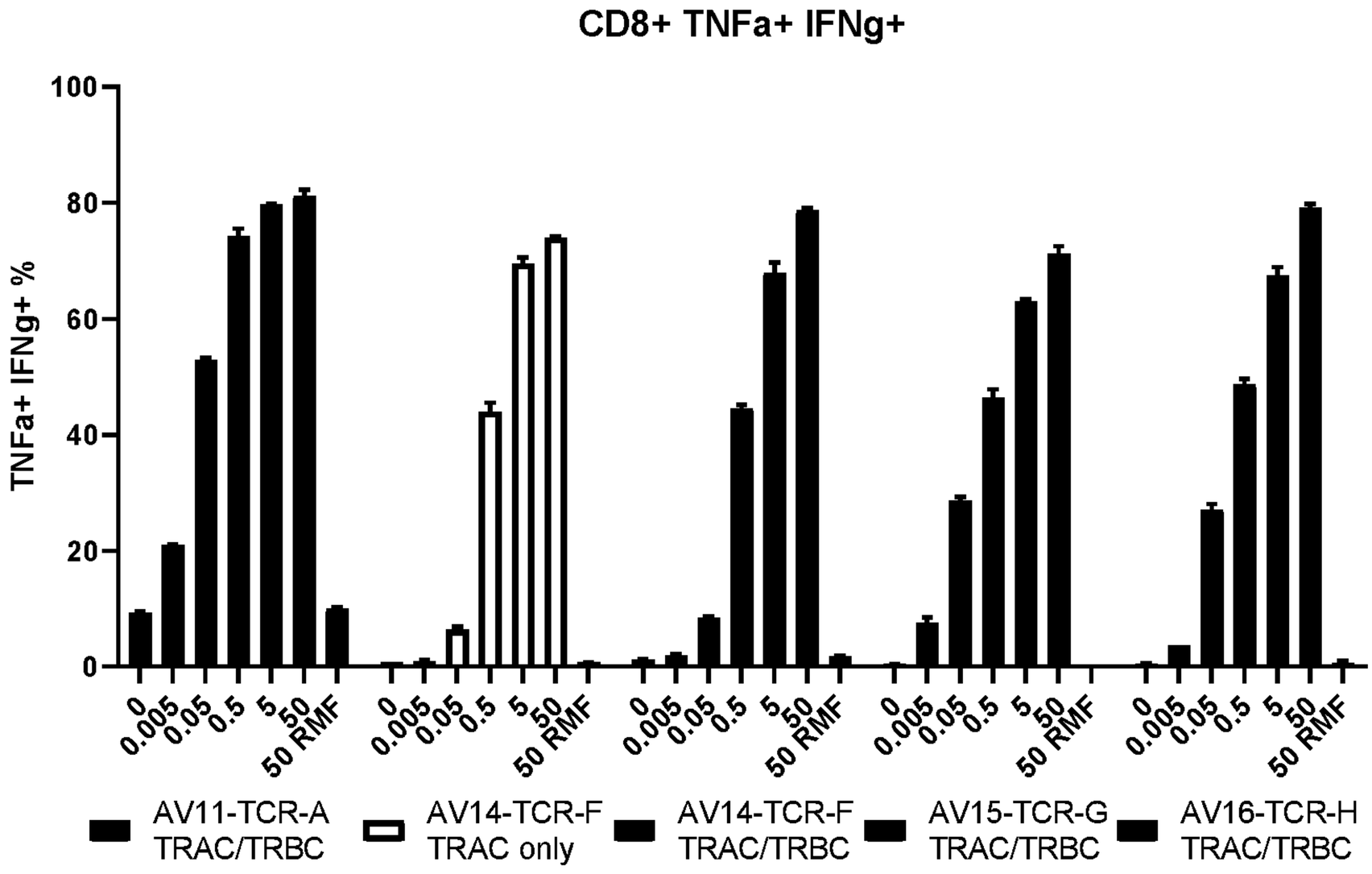

FIG 31A

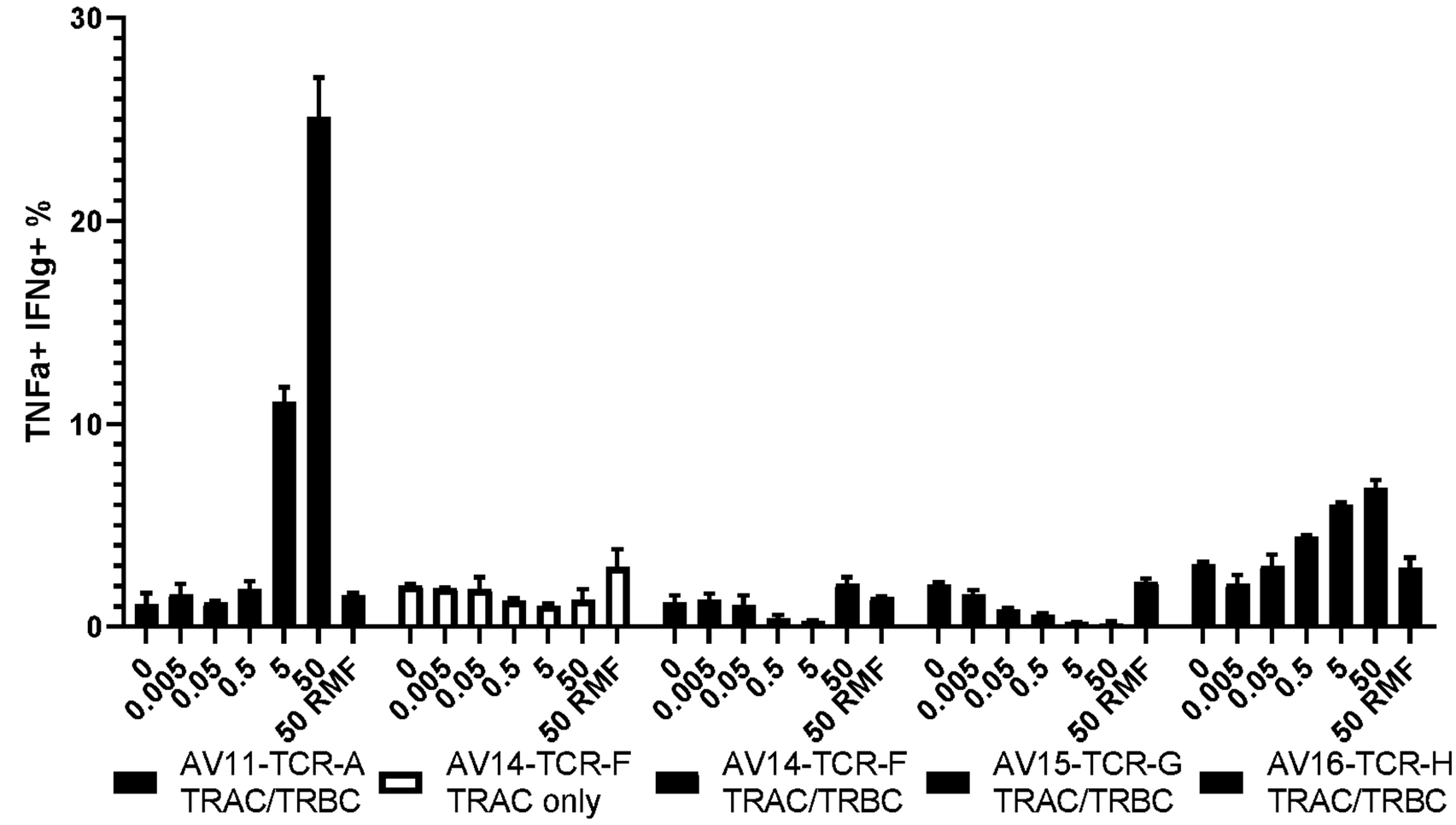

FIG 31B

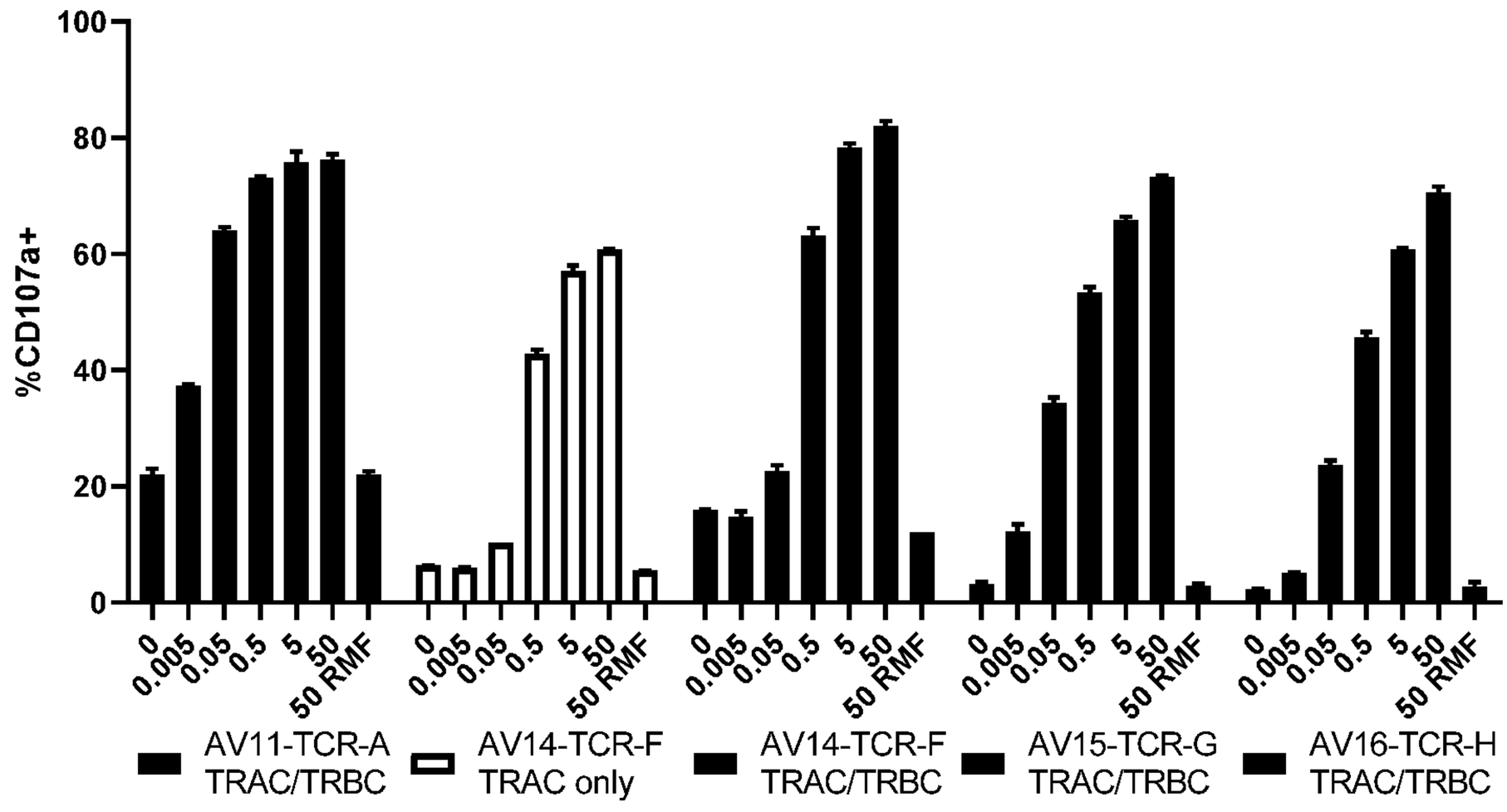

FIG 32A

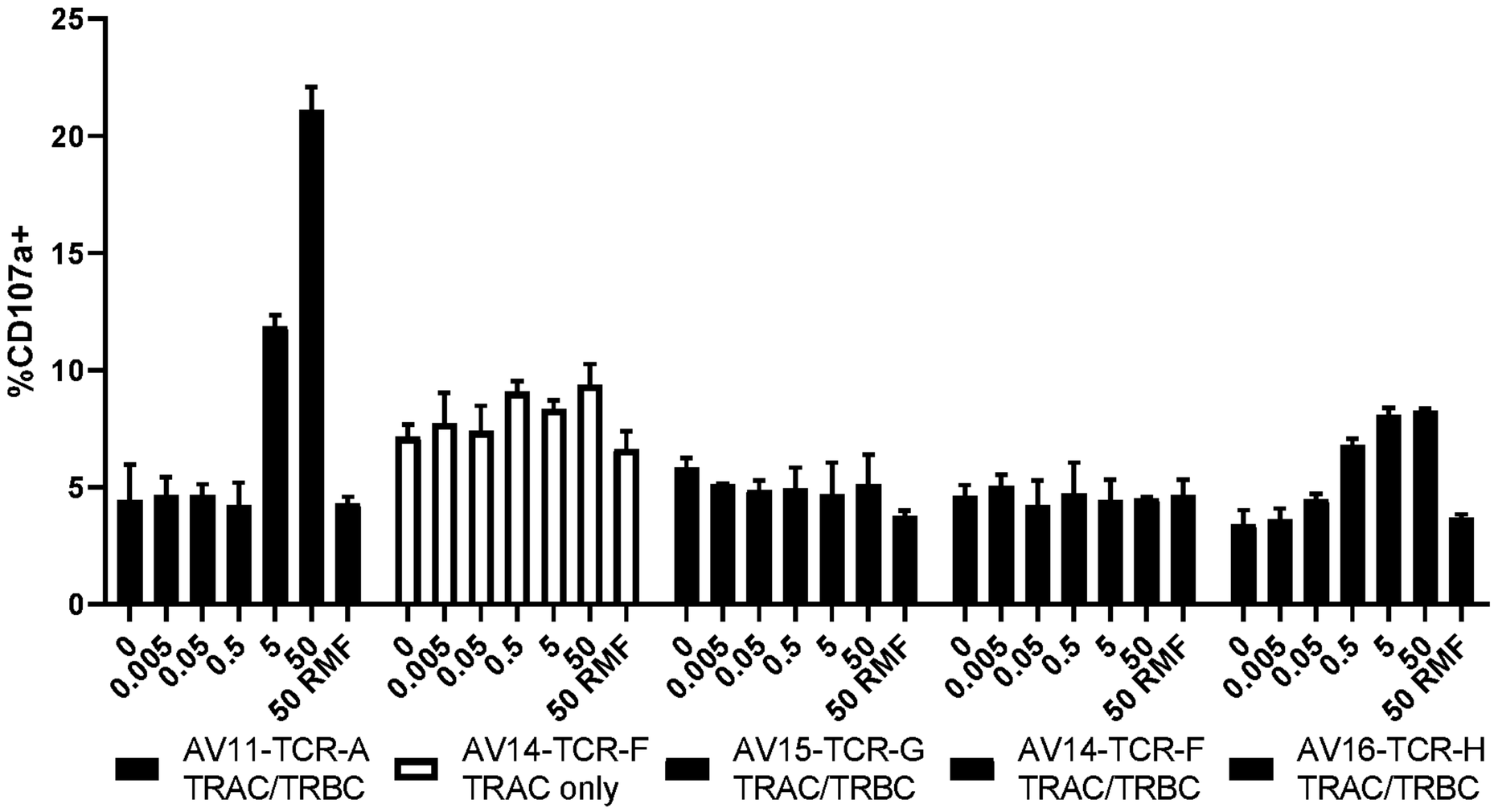

FIG 32B

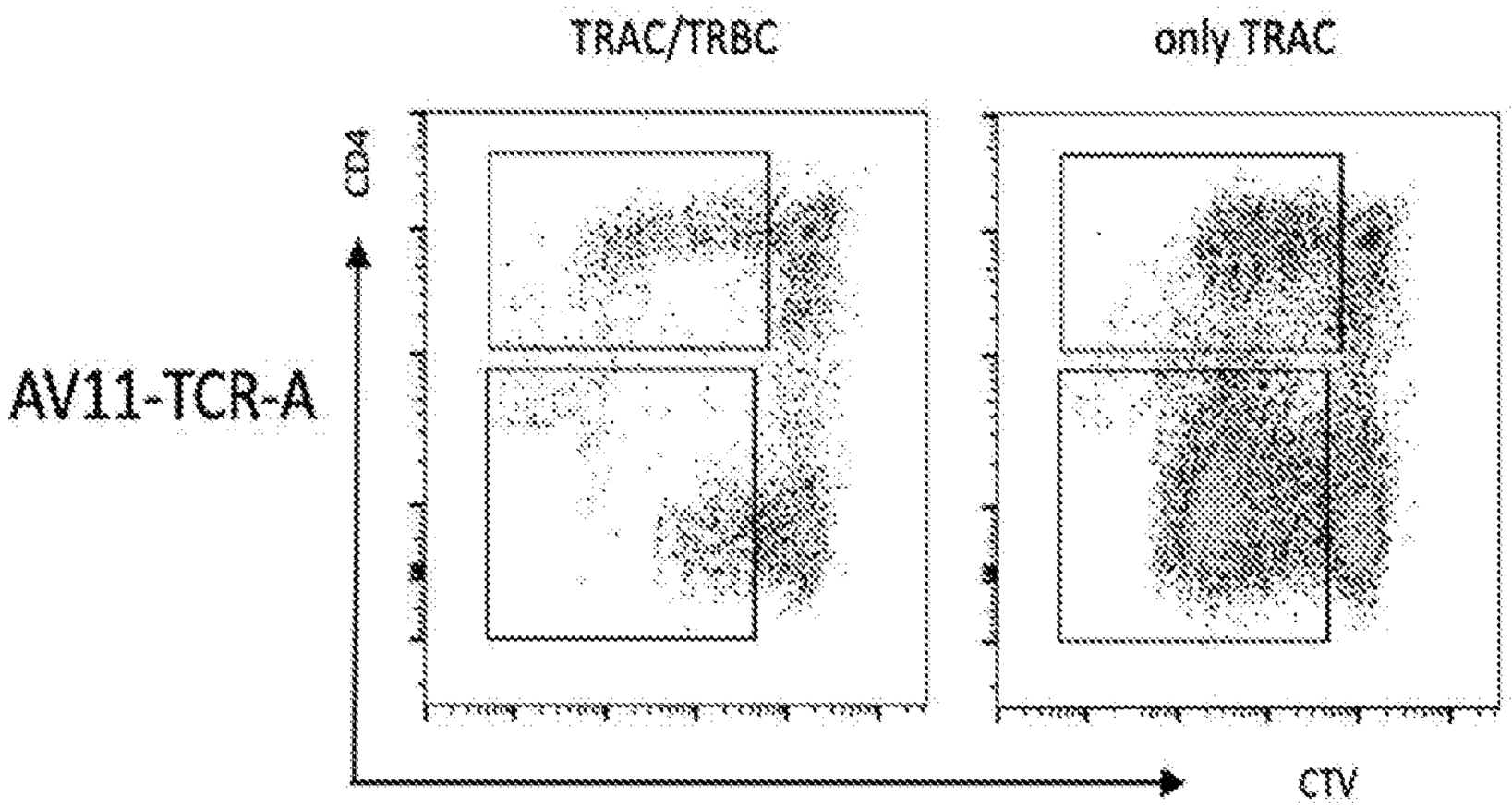

FIG 33A FIG 33B

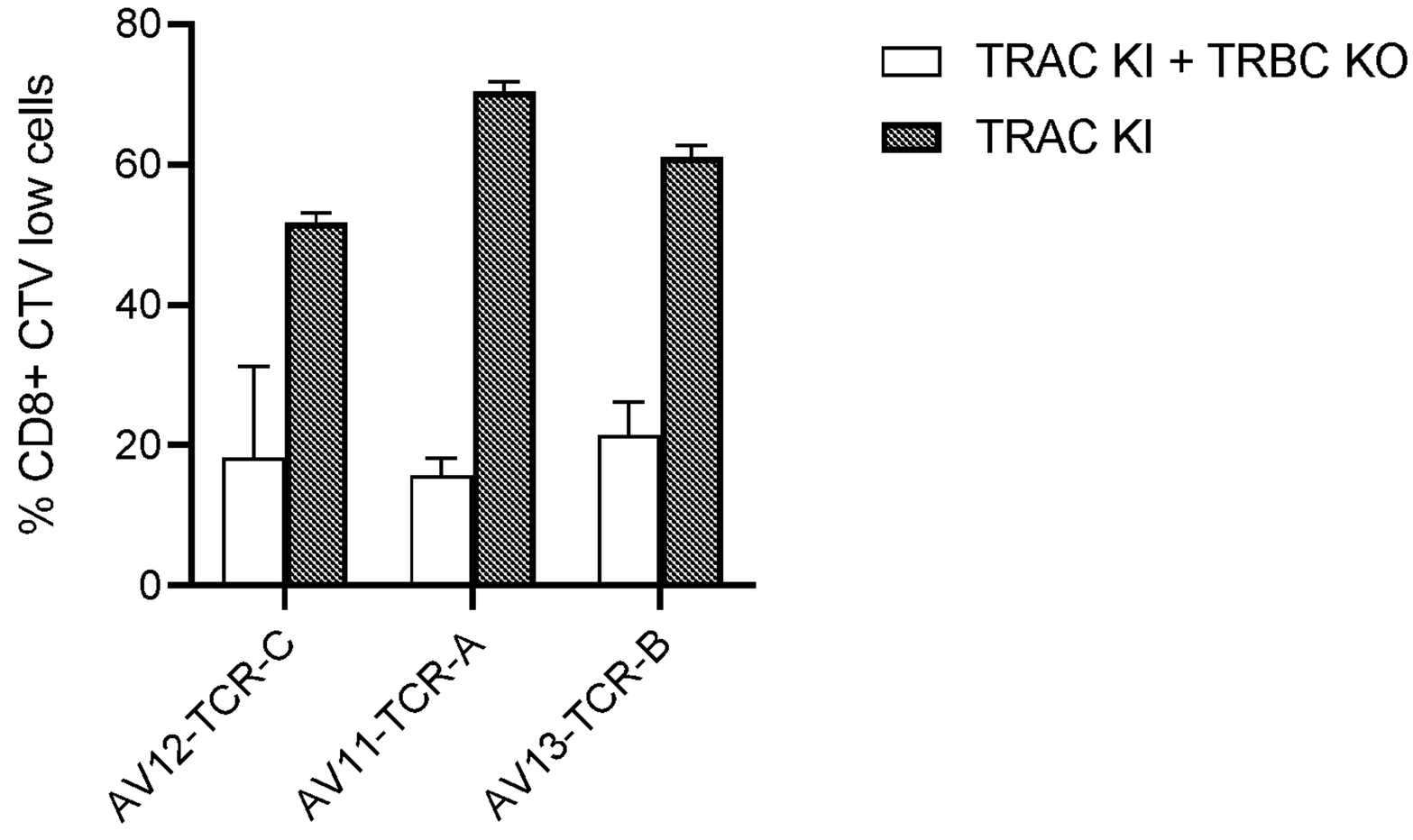

FIG 33C

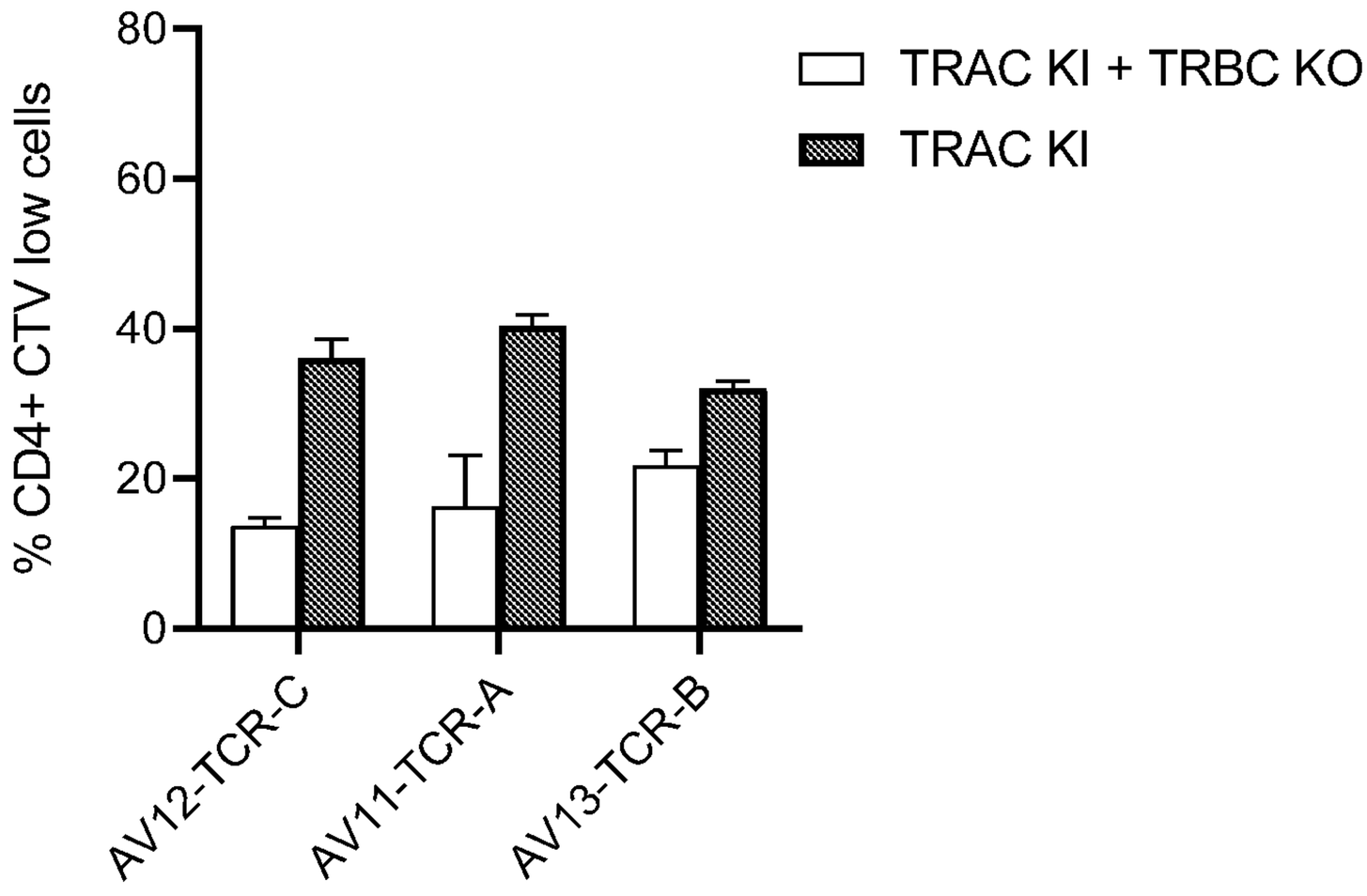
TRAC KI + TRBC KO
TRAC KI
% CD4+ CTV low cells
80
60
40
20
0
AV12-TCR-C
AV11-TCR-A
AV13-TCR-B

FIG 33D