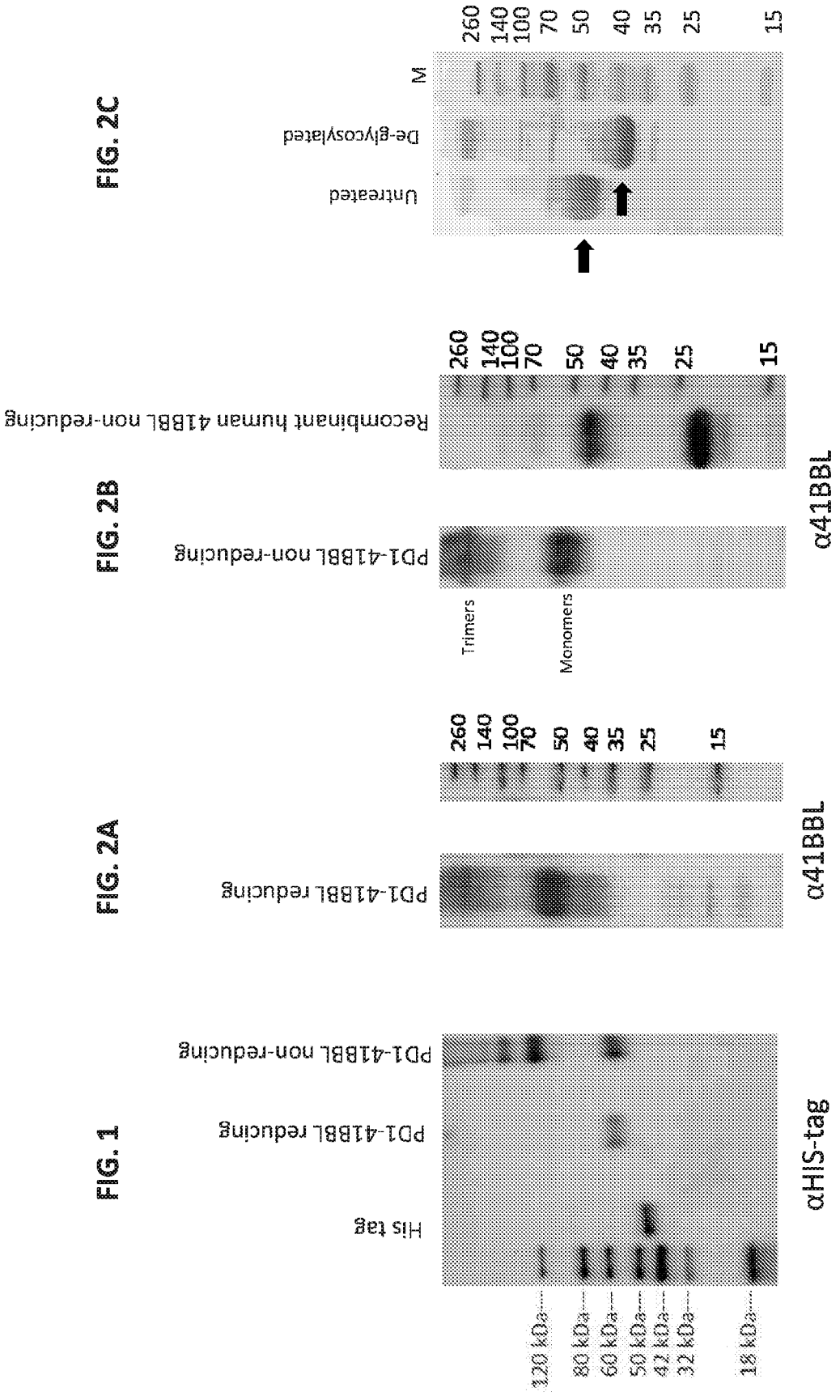


FIG. 1
His tag
PD1-41BBL reducing
PD1-41BBL non-reducing
αHIS-tag
120 kDa
80 kDa
60 kDa
50 kDa
42 kDa
32 kDa
18 kDa
FIG. 2A
PD1-41BBL reducing
α41BBL
260
140
100
70
50
40
35
25
15
FIG. 2B
PD1-41BBL non-reducing
Recombinant human 41BBL non-reducing
Trimers
Monomers
α41BBL
260
140
100
70
50
40
35
25
15
FIG. 2C
M
De-glycosylated
Untreated
260
140
100
70
50
40
35
25
15

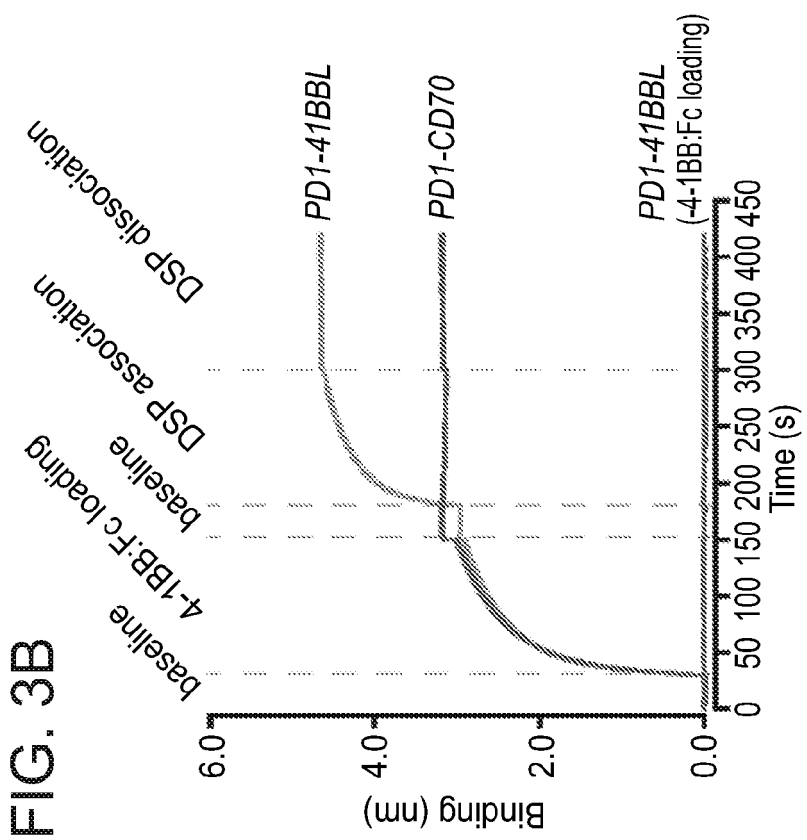


FIG. 3B
Binding (nm)
6.0
4.0
2.0
0.0
0 50 100 150 200 250 300 350 400 450
Time (s)
baseline
4-1BB:Fc loading
baseline
DSP association
DSP dissociation
PD1-41BBL
PD1-CD70
PD1-41BBL
(-4-1BB:Fc loading)

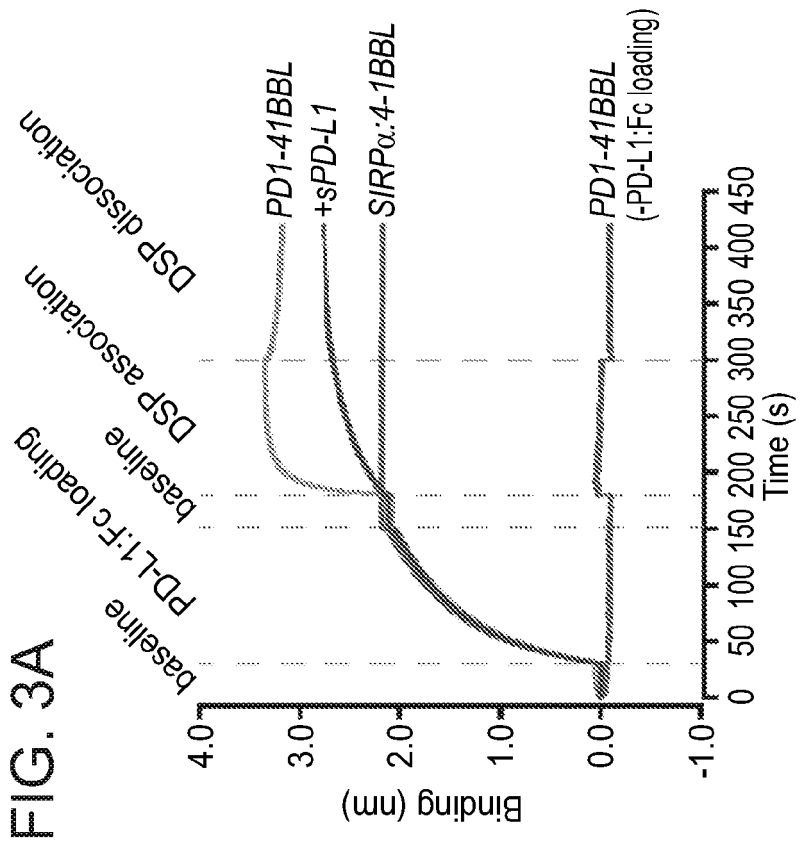


FIG. 3A
Binding (nm)
4.0
3.0
2.0
1.0
0.0
-1.0
0 50 100 150 200 250 300 350 400 450
Time (s)
baseline
PD-L1:Fc loading
baseline
DSP association
DSP dissociation
PD1-41BBL
+sPD-L1
SIRPα:4-1BBL
PD1-41BBL
(-PD-L1:Fc loading)

FIG. 4A

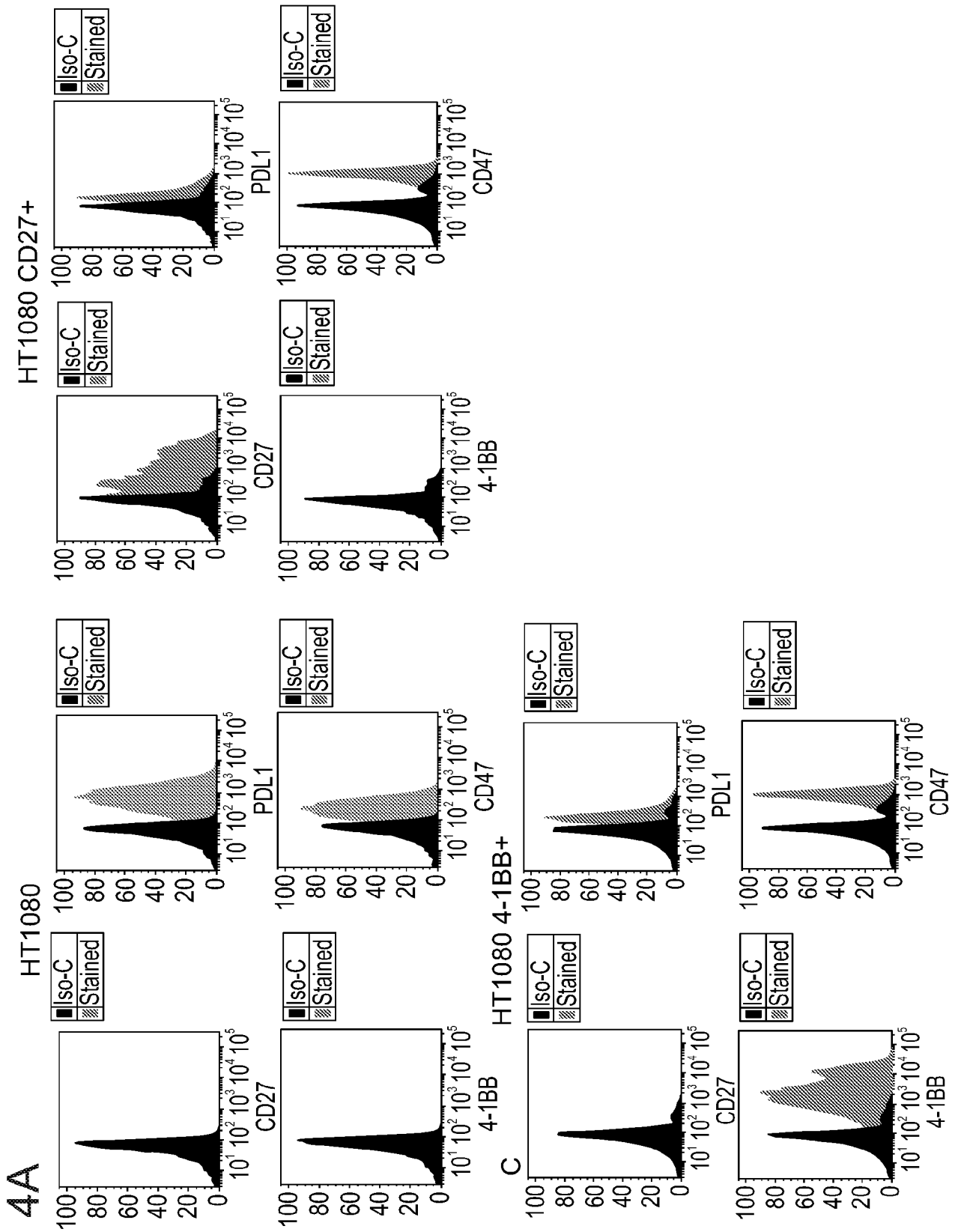

HT1080
HT1080 4-1BB+
HT1080 CD27+
C
CD27
4-1BB
PDL1
CD47
Iso-C
Stained

FIG. 4A (Continued)

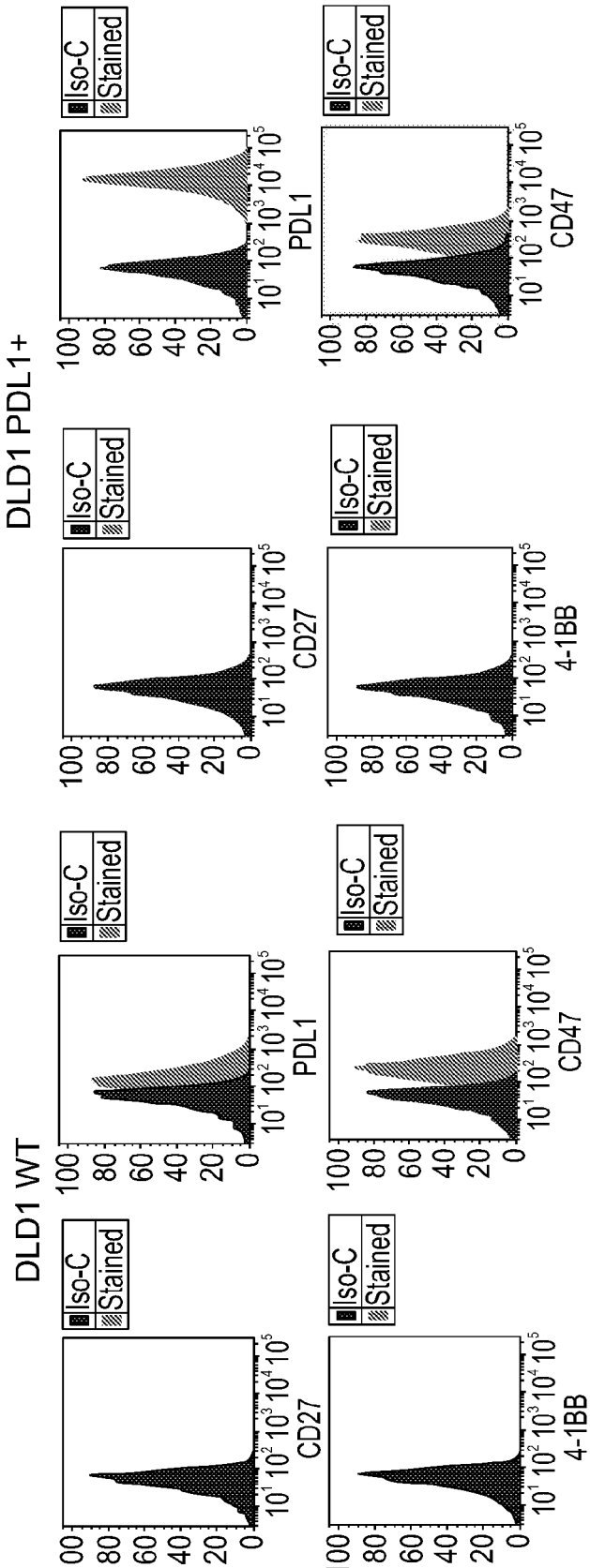
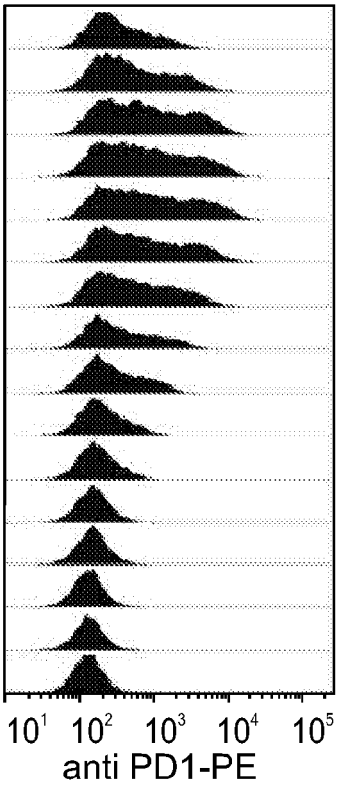


FIG. 4B

	CD27	41BB	PDL1	CD47
HT1080-WT	0	0	540	139
HT1080-CD27+	208	0	92	731
HT1080-41BB+	0	1404	130	791
DLD1-WT	0	0	93	170
DLD1-PDL1+	0	0	10098	215

FIG. 5A



50 ug/ml	340
25 ug/ml	522
12.5 ug/ml	740
6.25 ug/ml	753
3.13 ug/ml	825
1.56 ug/ml	672
0.78 ug/ml	532
0.39 mg/ml	366
0.2 ug/ml	277
0.1 ug/ml	205
0.05 ug/ml	174
0.02 ug/ml	152
0.01 ug/ml	147
Isc-C+protein	131
Isc-C	132
Unstained	129

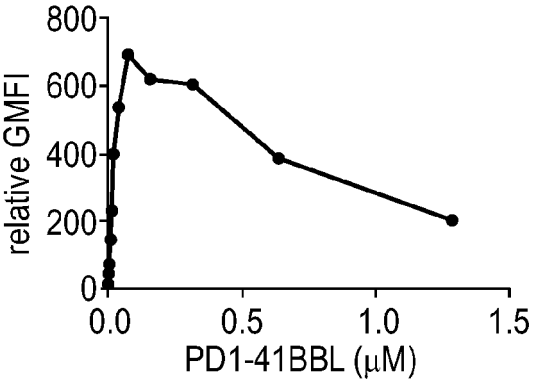
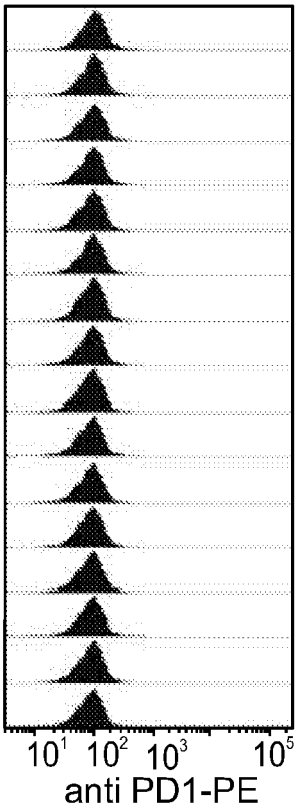


FIG. 5B



50 ug/ml	96.0
25 ug/ml	92.1
12.5 ug/ml	89.4
6.25 ug/ml	86.8
3.13 ug/ml	84.9
1.56 ug/ml	86.5
0.78 ug/ml	85.1
0.39 mg/ml	82.7
0.2 ug/ml	80.3
0.1 ug/ml	80.6
0.05 ug/ml	80.4
0.02 ug/ml	83.1
0.01 ug/ml	81.0
Isc-C+protein	83.0
Isc-C	83.2
Unstained	81.5

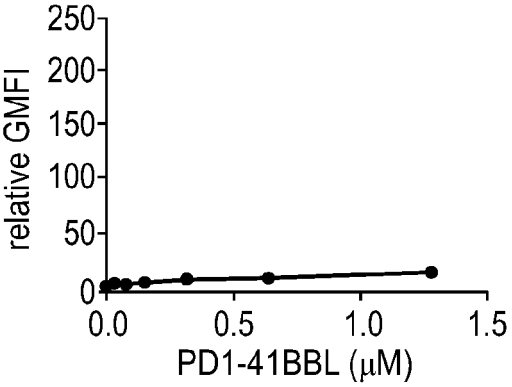
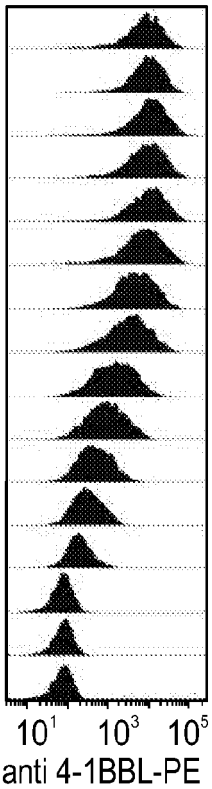


FIG. 6A



50 ug/ml	6887
25 ug/ml	7829
12.5 ug/ml	9042
6.25 ug/ml	7527
3.13 ug/ml	8305
1.56 ug/ml	6424
0.78 ug/ml	4173
0.39 ug/ml	2974
0.2 ug/ml	1414
0.1 ug/ml	887
0.05 ug/ml	480
0.02 ug/ml	316
0.01 ug/ml	203
Isc-C+protein	70.8
Isc-C	76.1
Unstained	73.4

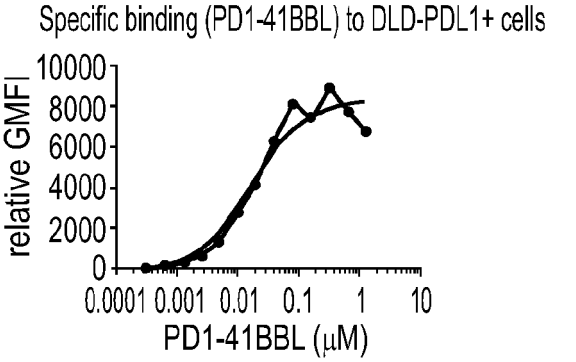
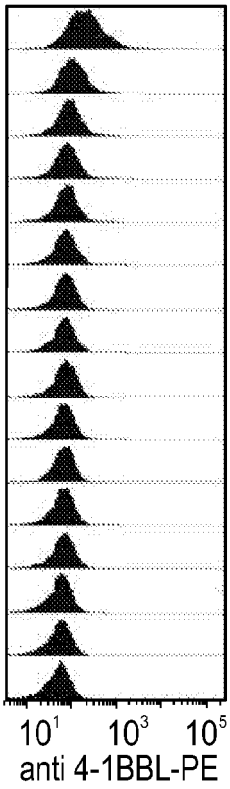


FIG. 6B



50 ug/ml	204
25 ug/ml	105
12.5 ug/ml	82.4
6.25 ug/ml	71.3
3.13 ug/ml	67.8
1.56 ug/ml	64.3
0.78 ug/ml	62.2
0.39 ug/ml	58.8
0.2 ug/ml	63.8
0.1 ug/ml	55.6
0.05 ug/ml	57.4
0.02 ug/ml	56.0
0.01 ug/ml	57.4
Isc-C+protein	43.2
Isc-C	43.0
Unstained	40.5

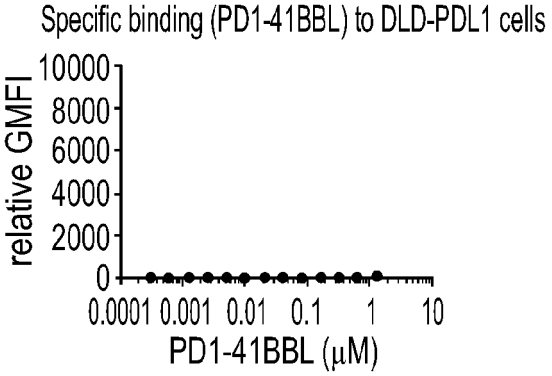


FIG. 7

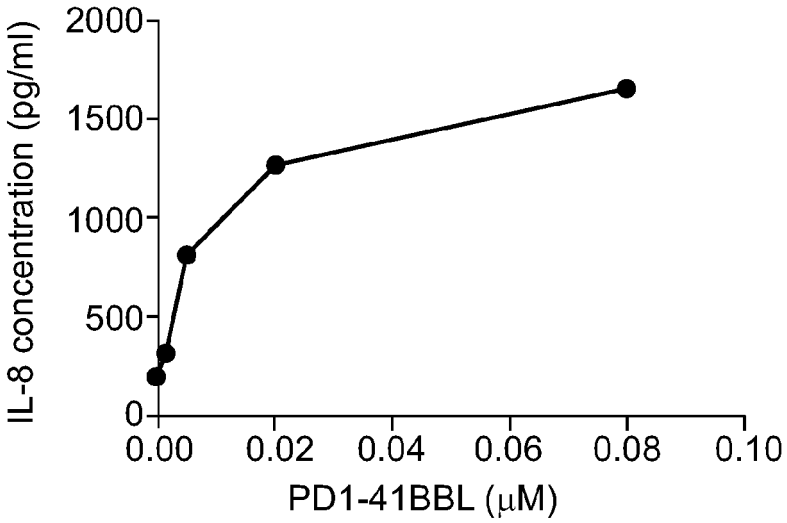

IL-8 concentration (pg/ml)
2000
1500
1000
500
0
0.00
0.02
0.04
0.06
0.08
0.10
PD1-41BBL (μM)

FIG. 8

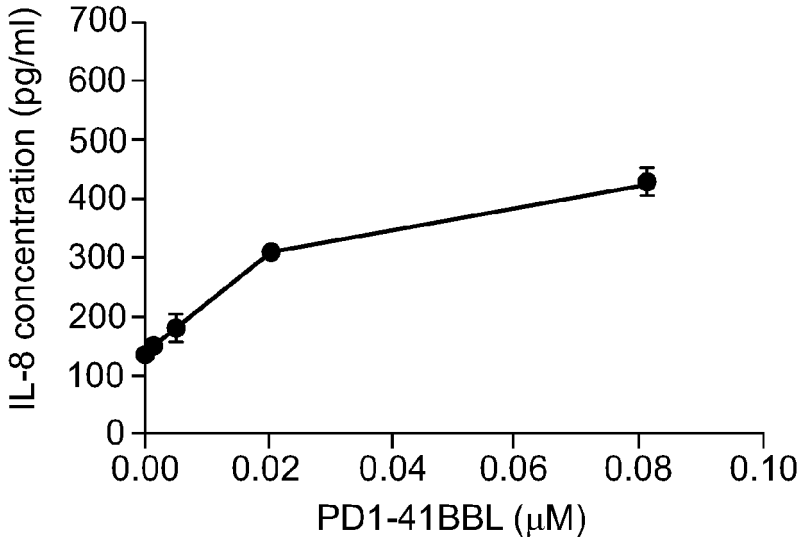

IL-8 concentration (pg/ml)
700
600
500
400
300
200
100
0
0.00
0.02
0.04
0.06
0.08
0.10
PD1-41BBL (μM)

FIG. 9A

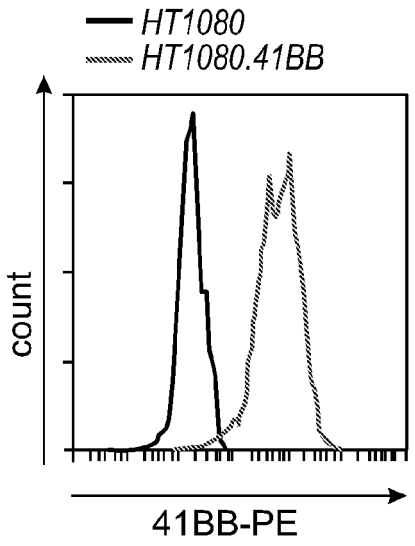


FIG. 9B

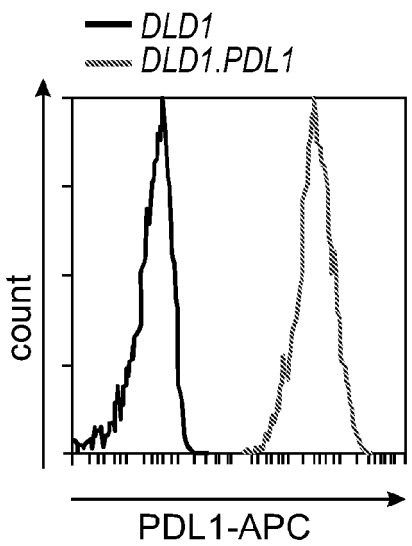


FIG. 9C

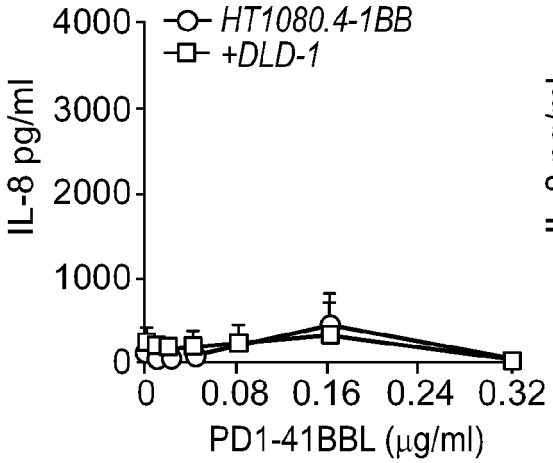


FIG. 9D

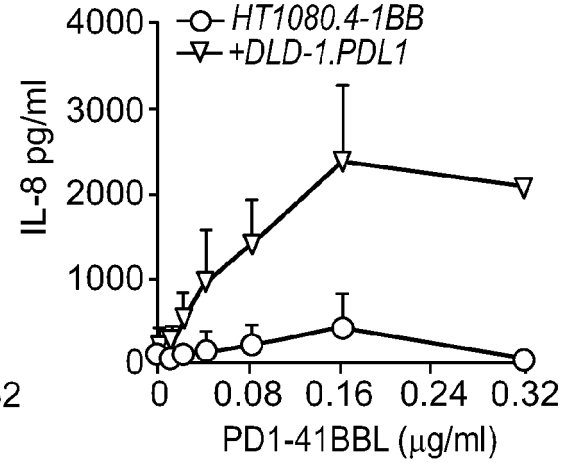
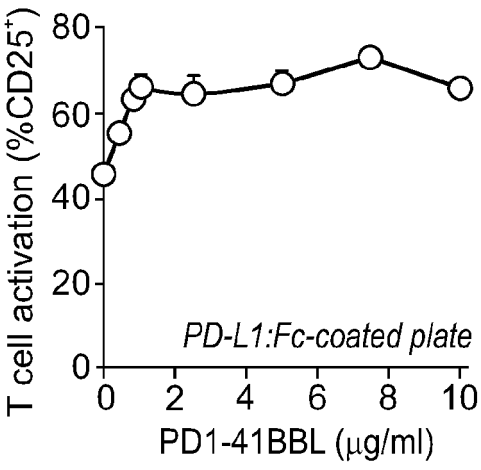


FIG. 10



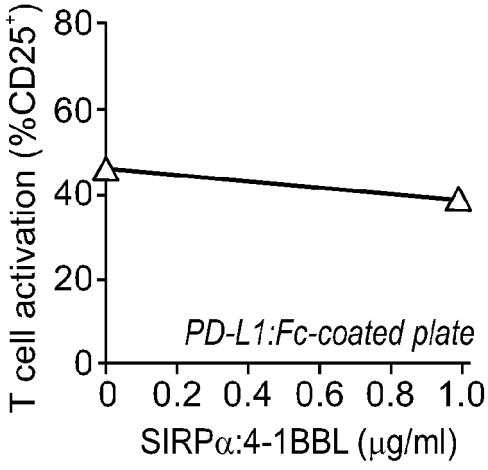


FIG. 11A

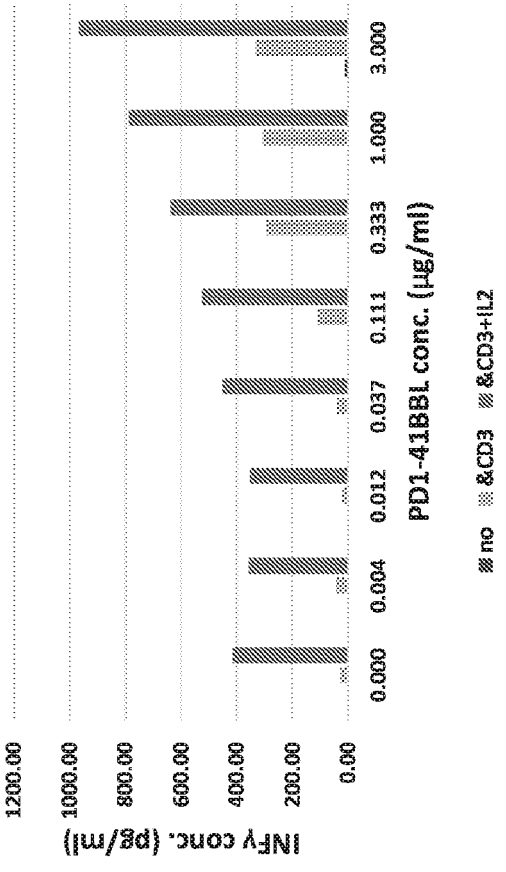
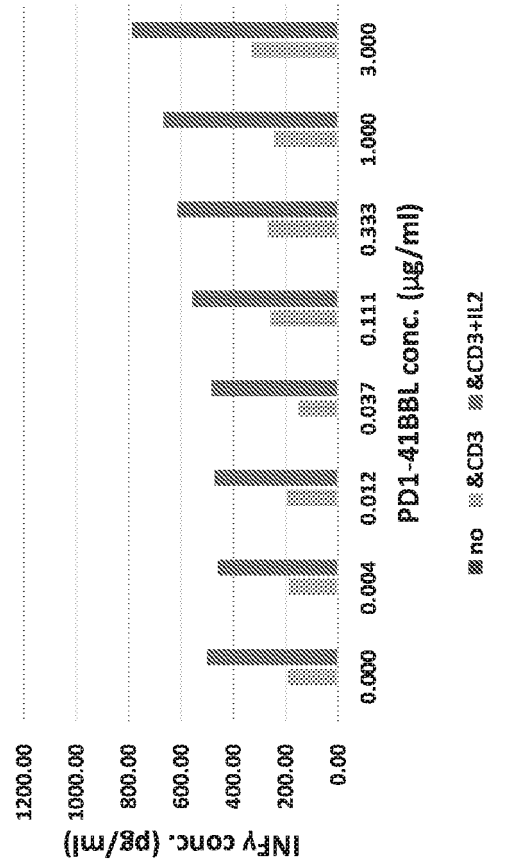


FIG. 11B



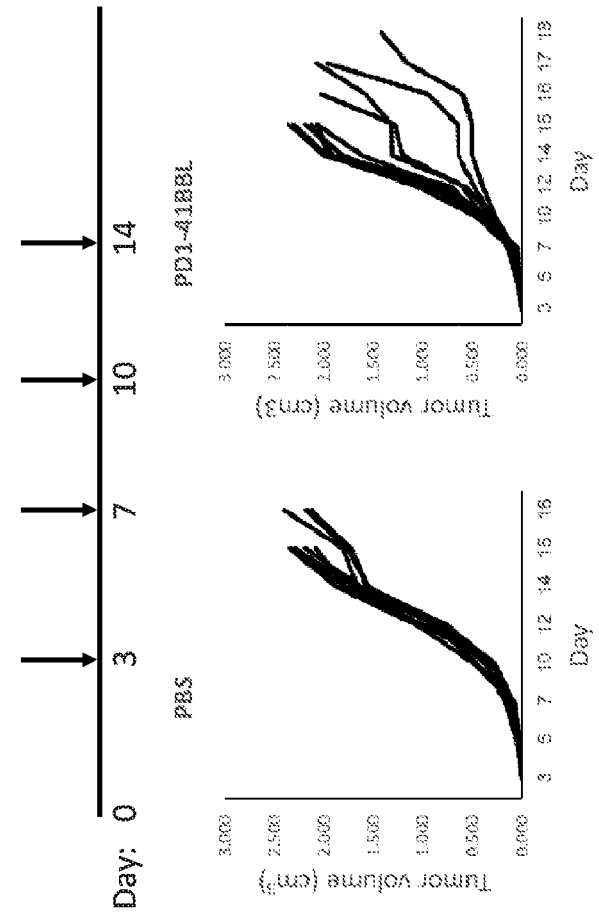


FIG. 12A

FIG. 12B

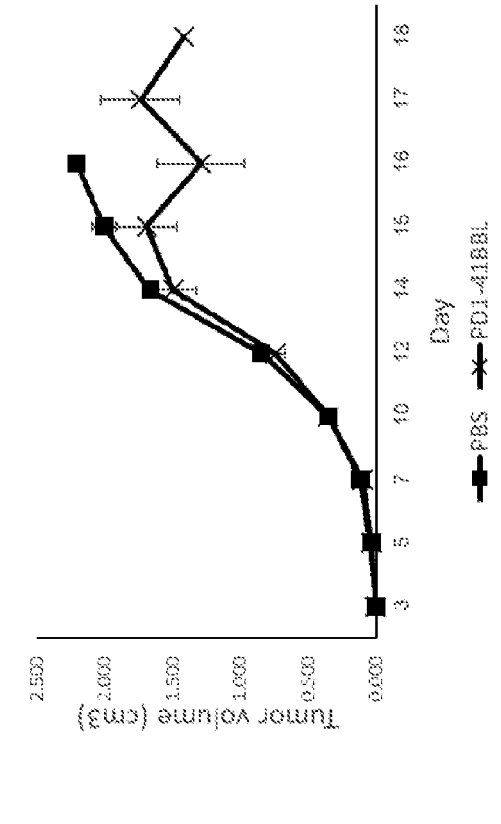


FIG. 12C

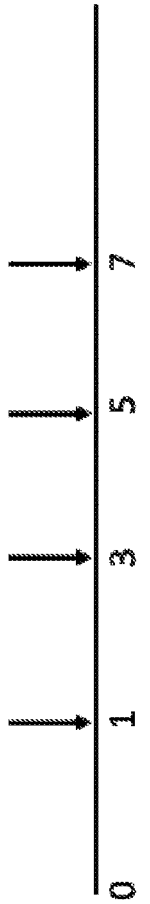


FIG. 13A

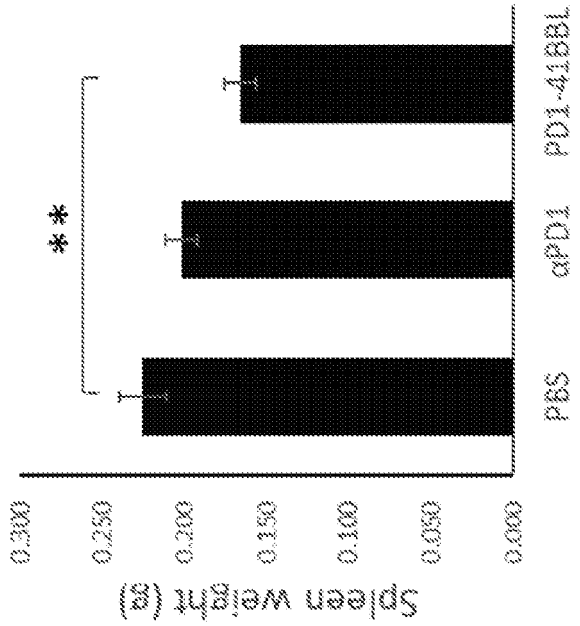


FIG. 13B