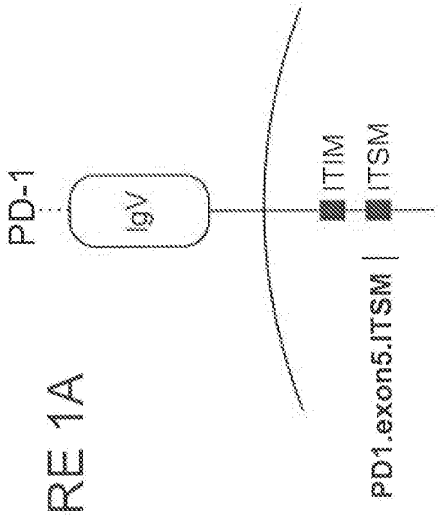


FIGURE 1A

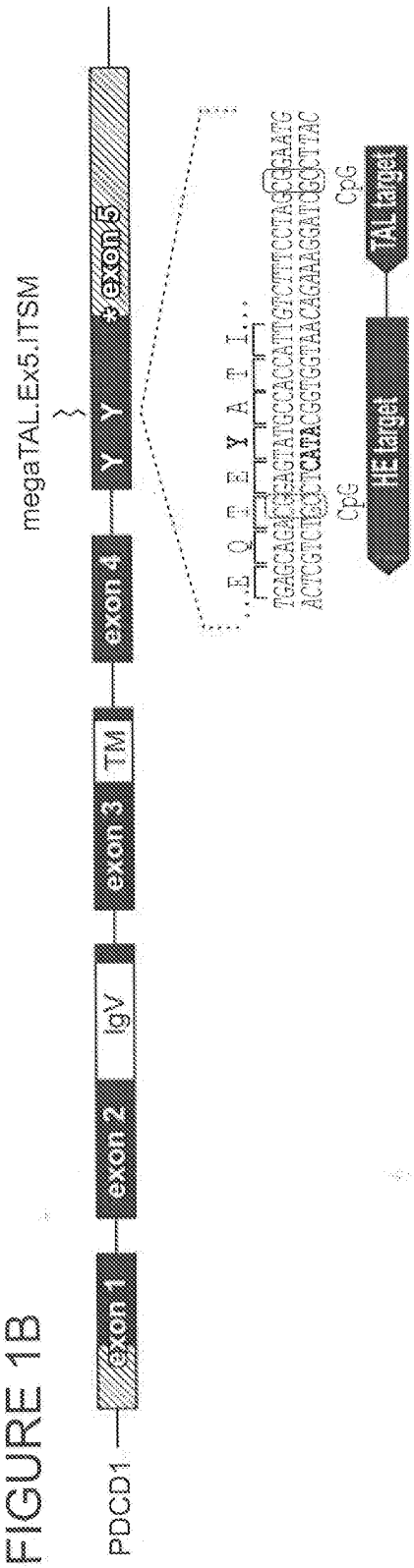


FIGURE 1B

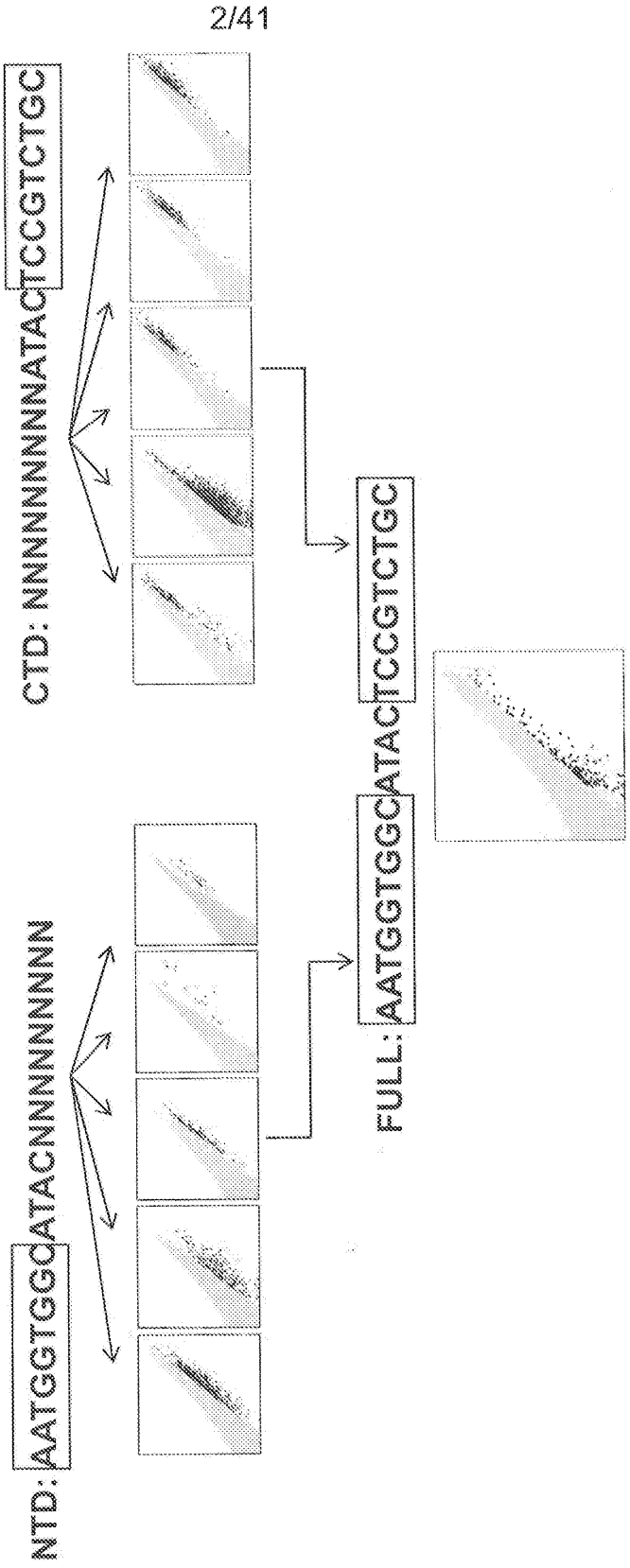

NTD: AATGGTGGCATACNNNNNNNNN
CTD: NNNNNNNNNNATACTCCGTCTGC
FULL: AATGGTGGCATACTCCGTCTGC

FIGURE 2

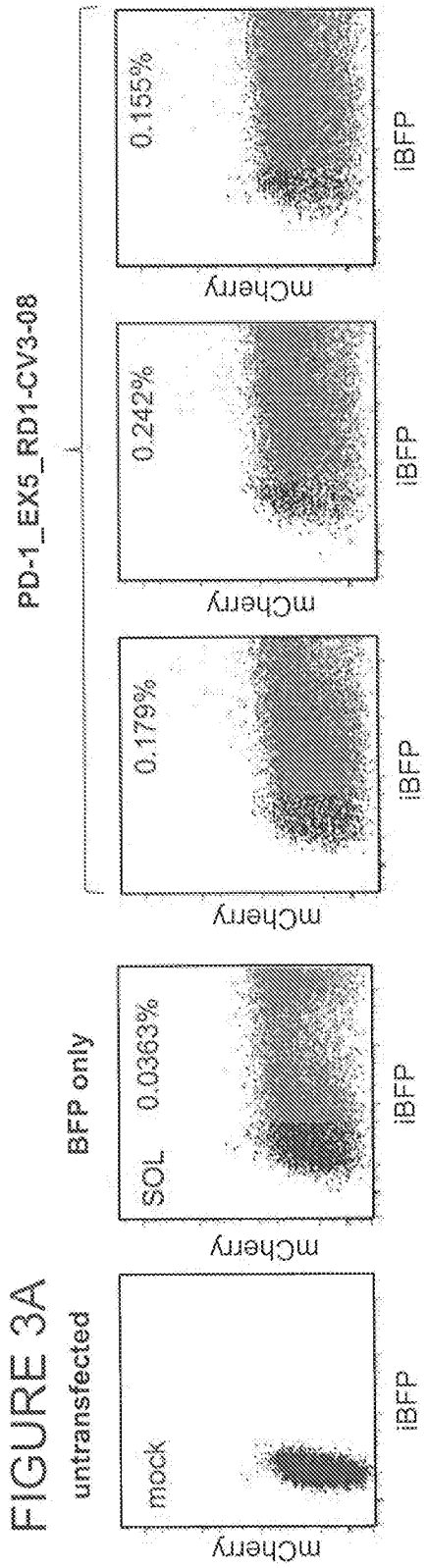


FIGURE 3A
untransfected
mock
mCherry
iBFP
BFP only
SOL 0.0363%
PD-1_EX5_RD1-CV3-08
0.179%
0.242%
0.155%

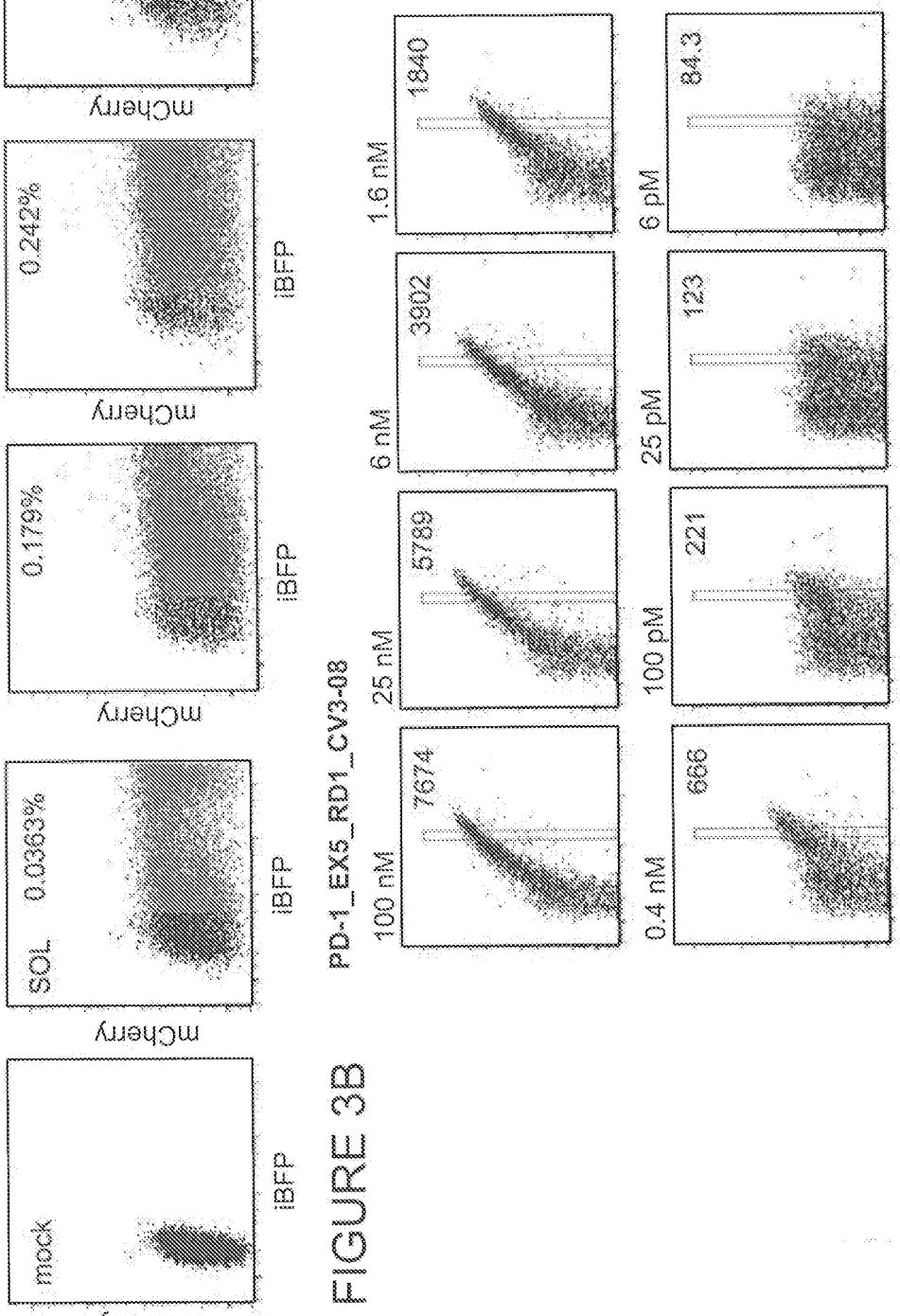


FIGURE 3B
PD-1_EX5_RD1_CV3-08
100 nM
25 nM
6 nM
1.6 nM
0.4 nM
100 pM
25 pM
6 pM
7674
5789
3902
1840
666
221
123
84.3

FIGURE 4

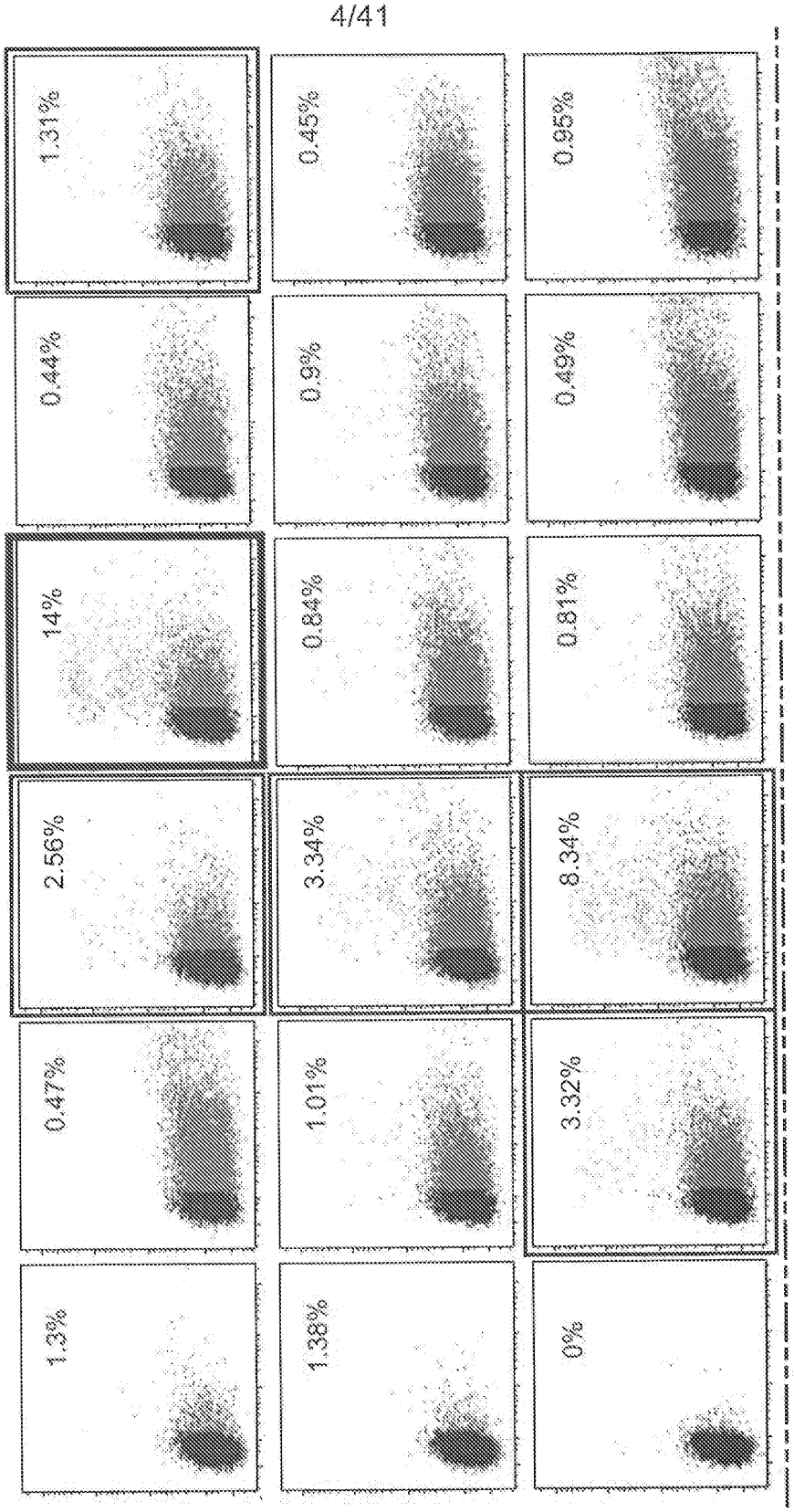
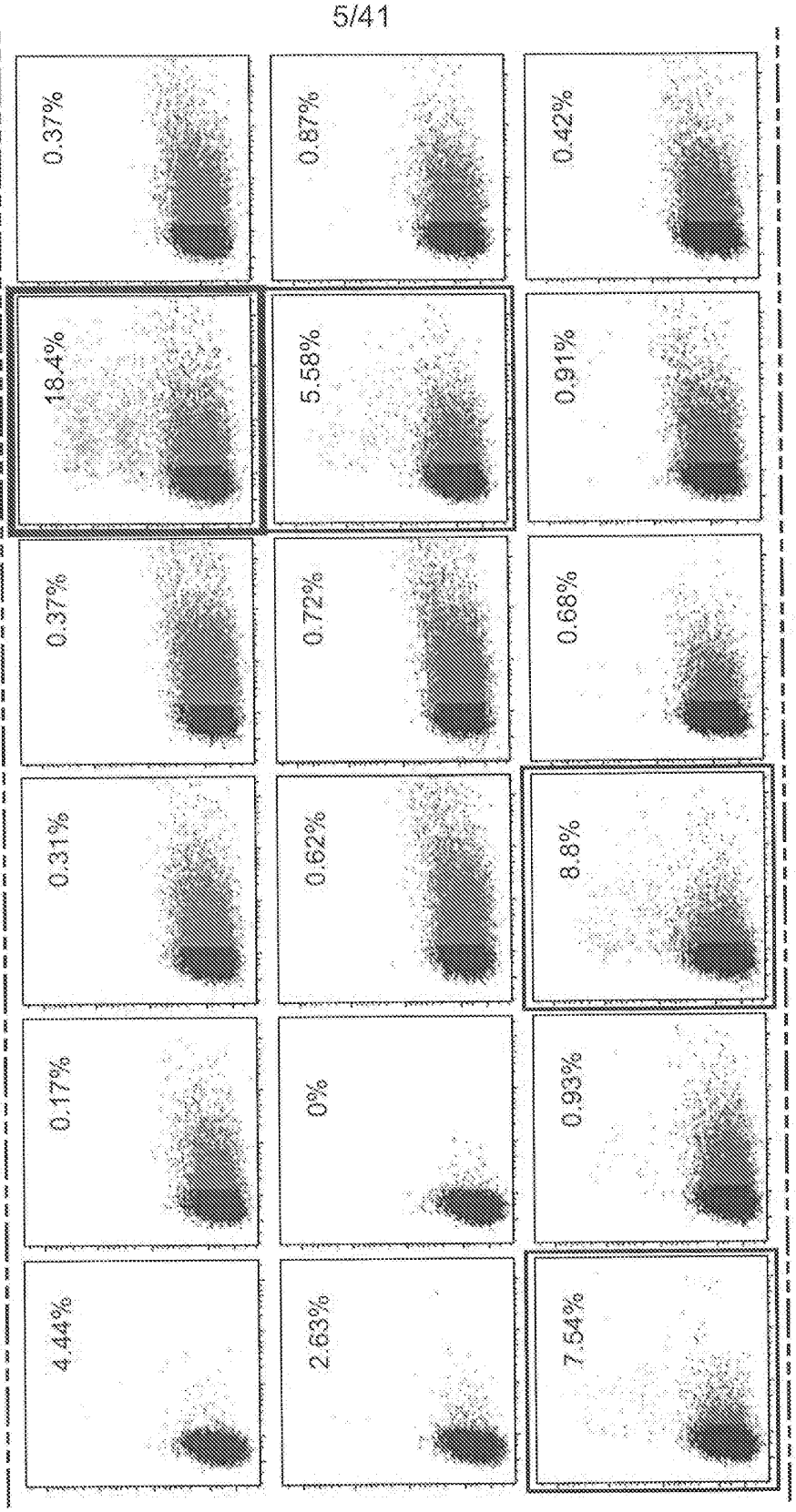


FIGURE 4 (cont.)



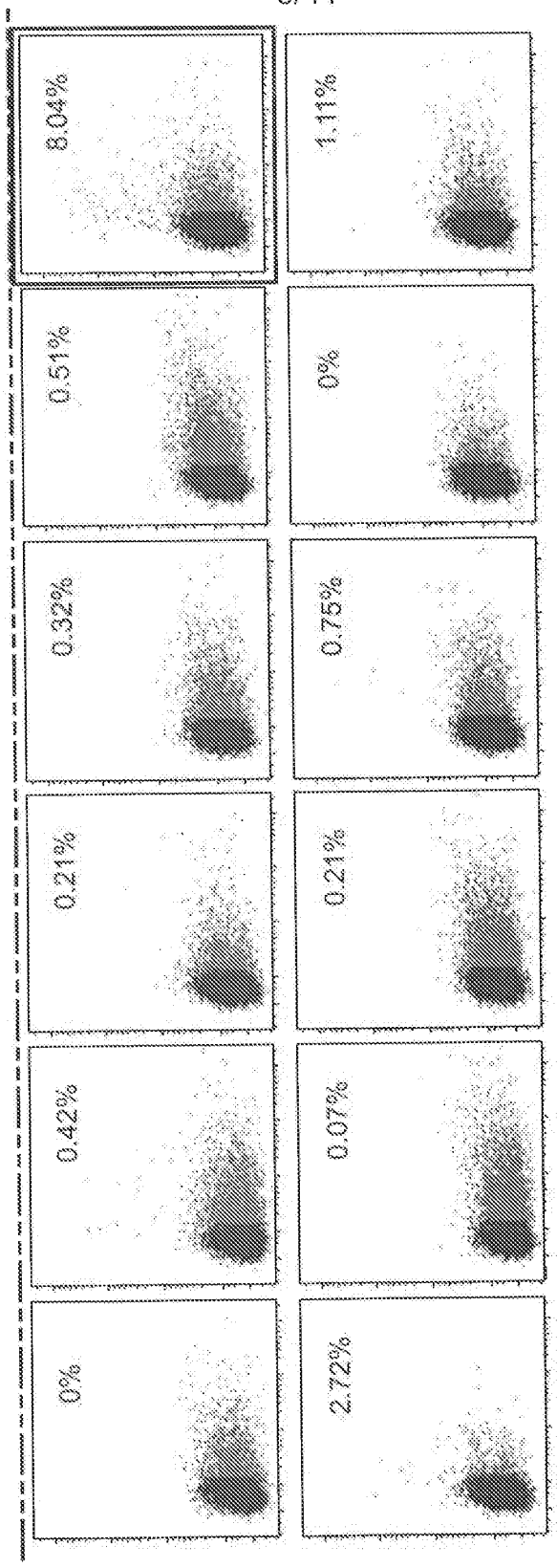
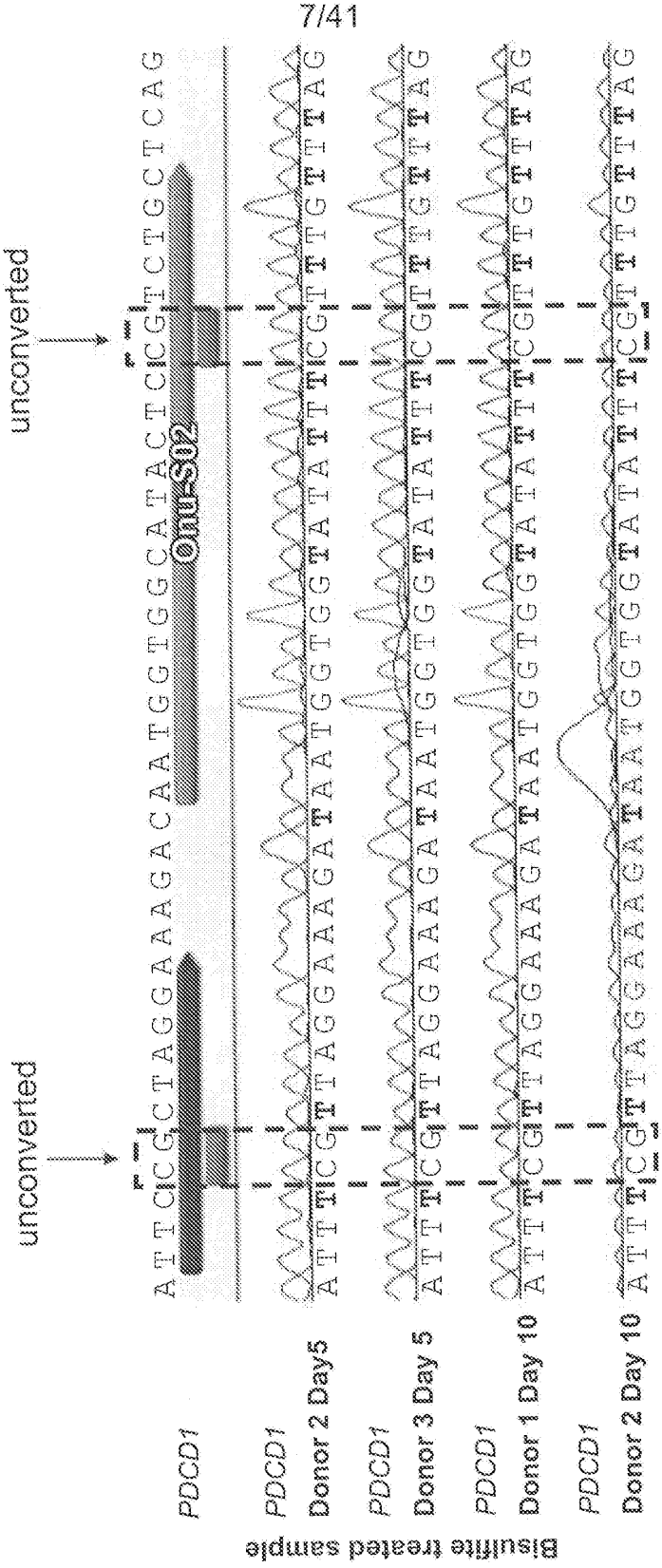


FIGURE 4 (cont.)

FIGURE 5


unconverted
unconverted
Omu-S02
Bisulfite treated sample
PDCD1
PDCD1 Donor 2 Day5
PDCD1 Donor 3 Day 5
PDCD1 Donor 1 Day 10
PDCD1 Donor 2 Day 10

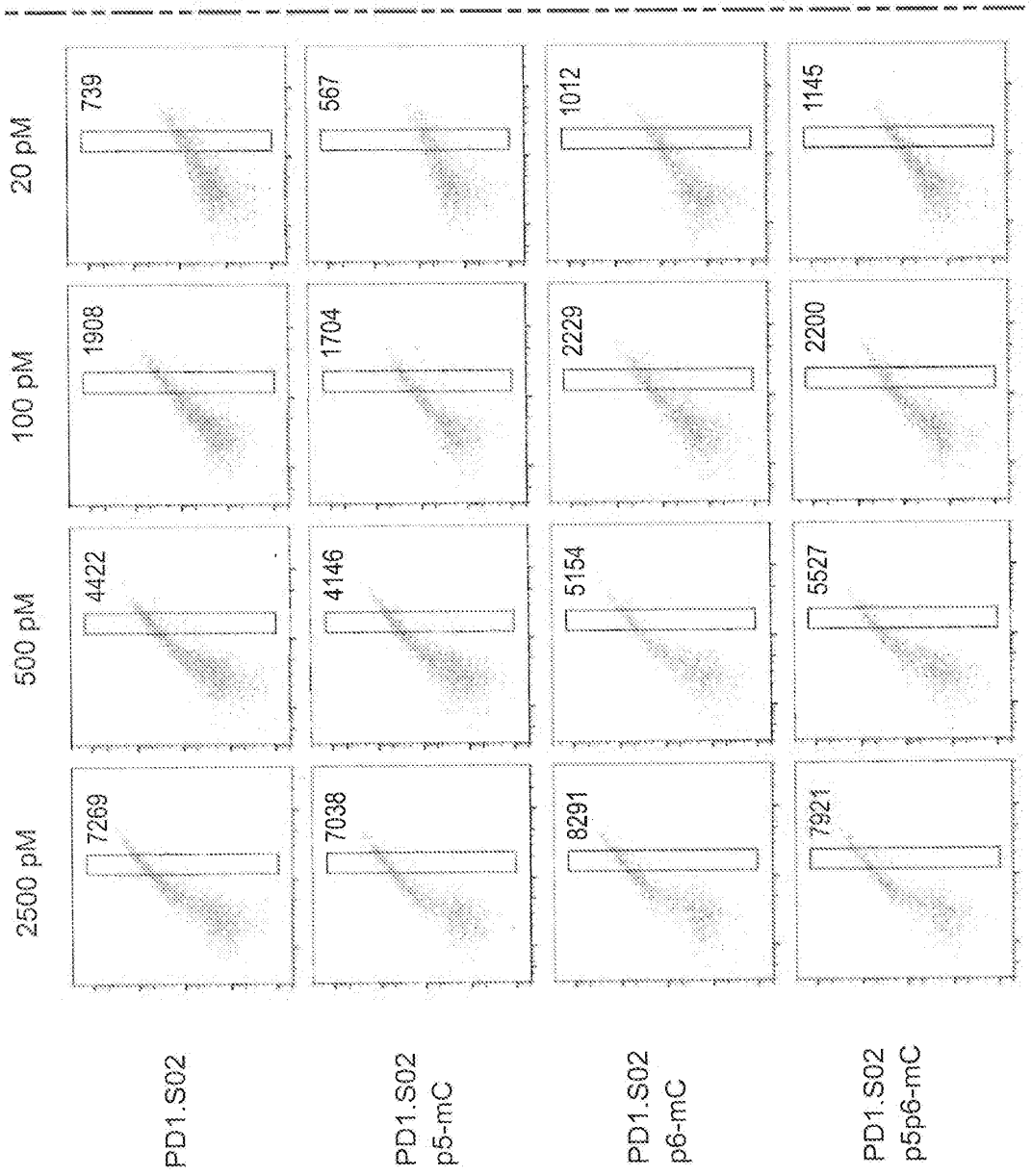

2500 pM
500 pM
100 pM
20 pM
7269
4422
1908
739
7038
4146
1704
567
8291
5154
2229
1012
7921
5527
2200
1145
PD1.S02
PD1.S02 p5-mC
PD1.S02 p6-mC
PD1.S02 p5p6-mC

FIGURE 6

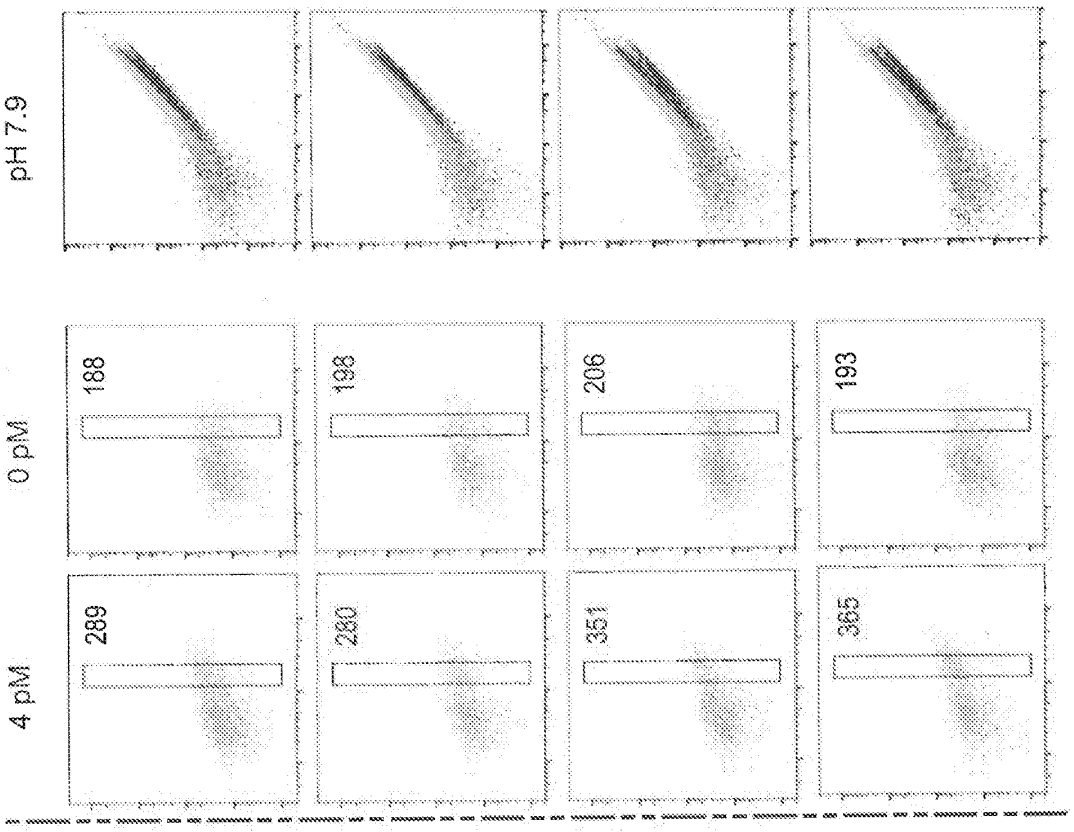


FIGURE 6 (cont.)

FIGURE 7A

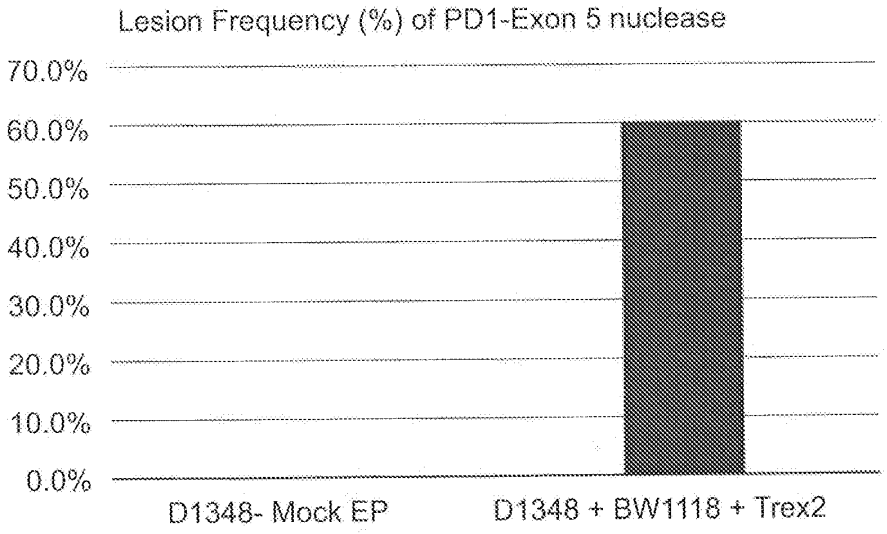


FIGURE 7B

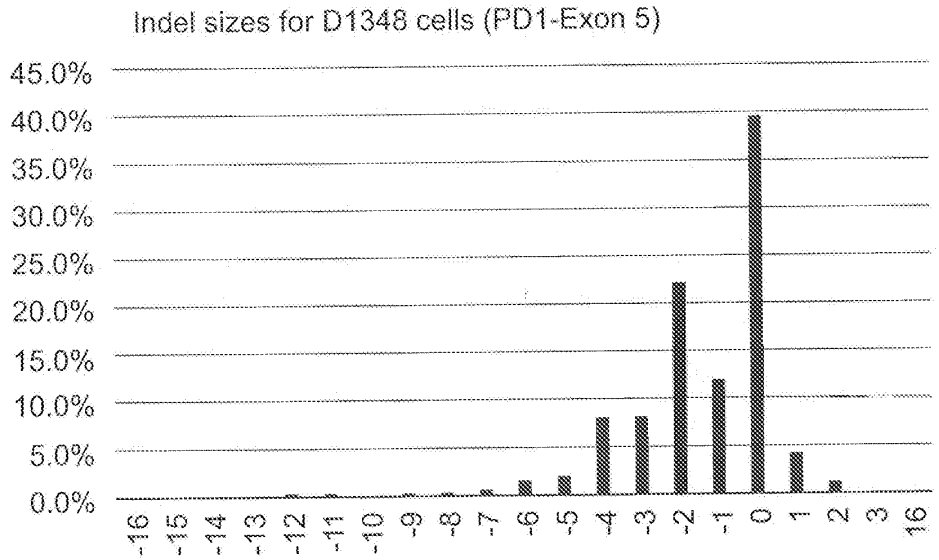


FIGURE 8

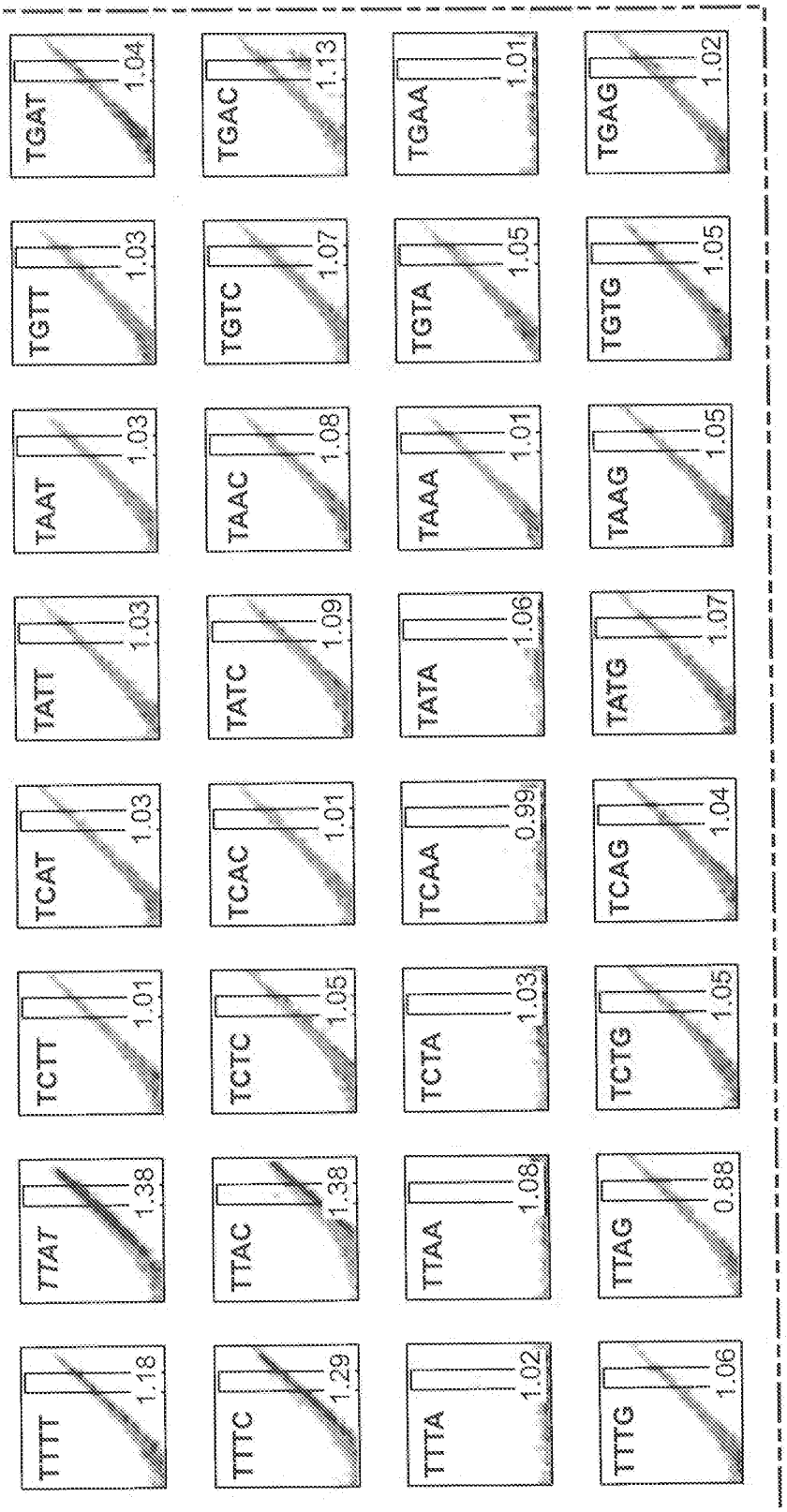

TTTT 1.18
TTAT 1.38
TCTT 1.01
TCAT 1.03
TATT 1.03
TAAT 1.03
TGTT 1.03
TGAT 1.04
TTTC 1.29
TTAC 1.38
TCTC 1.05
TCAC 1.01
TATC 1.09
TAAC 1.08
TGTC 1.07
TGAC 1.13
TTTA 1.02
TTAA 1.08
TCTA 1.03
TCAA 0.99
TATA 1.06
TAAA 1.01
TGTA 1.05
TGAA 1.01
TTTG 1.06
TTAG 0.88
TCTG 1.05
TCAG 1.04
TATG 1.07
TAAG 1.05
TGTG 1.05
TGAG 1.02

FIGURE 8 (cont.)

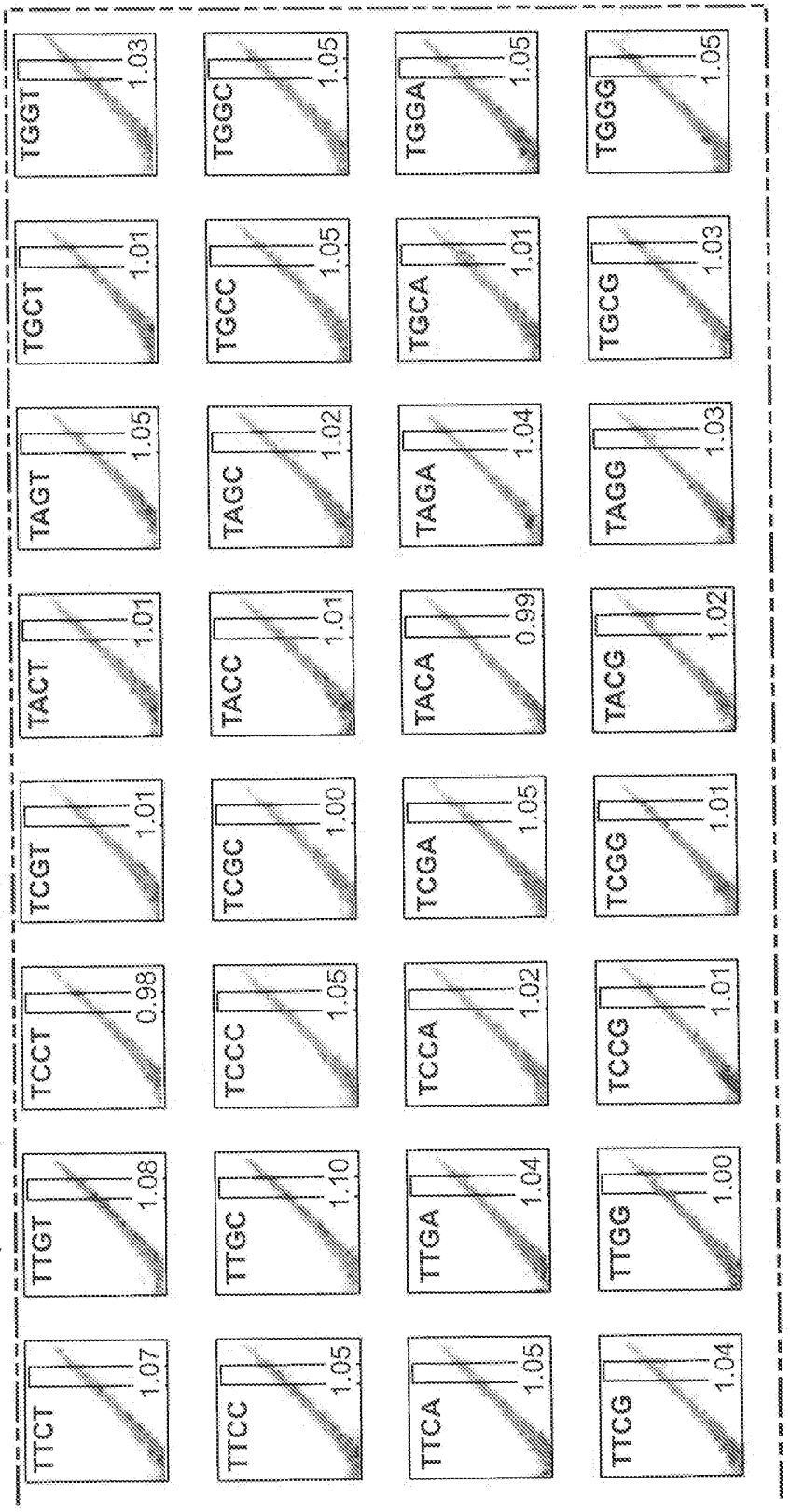


FIGURE 8 (cont.)

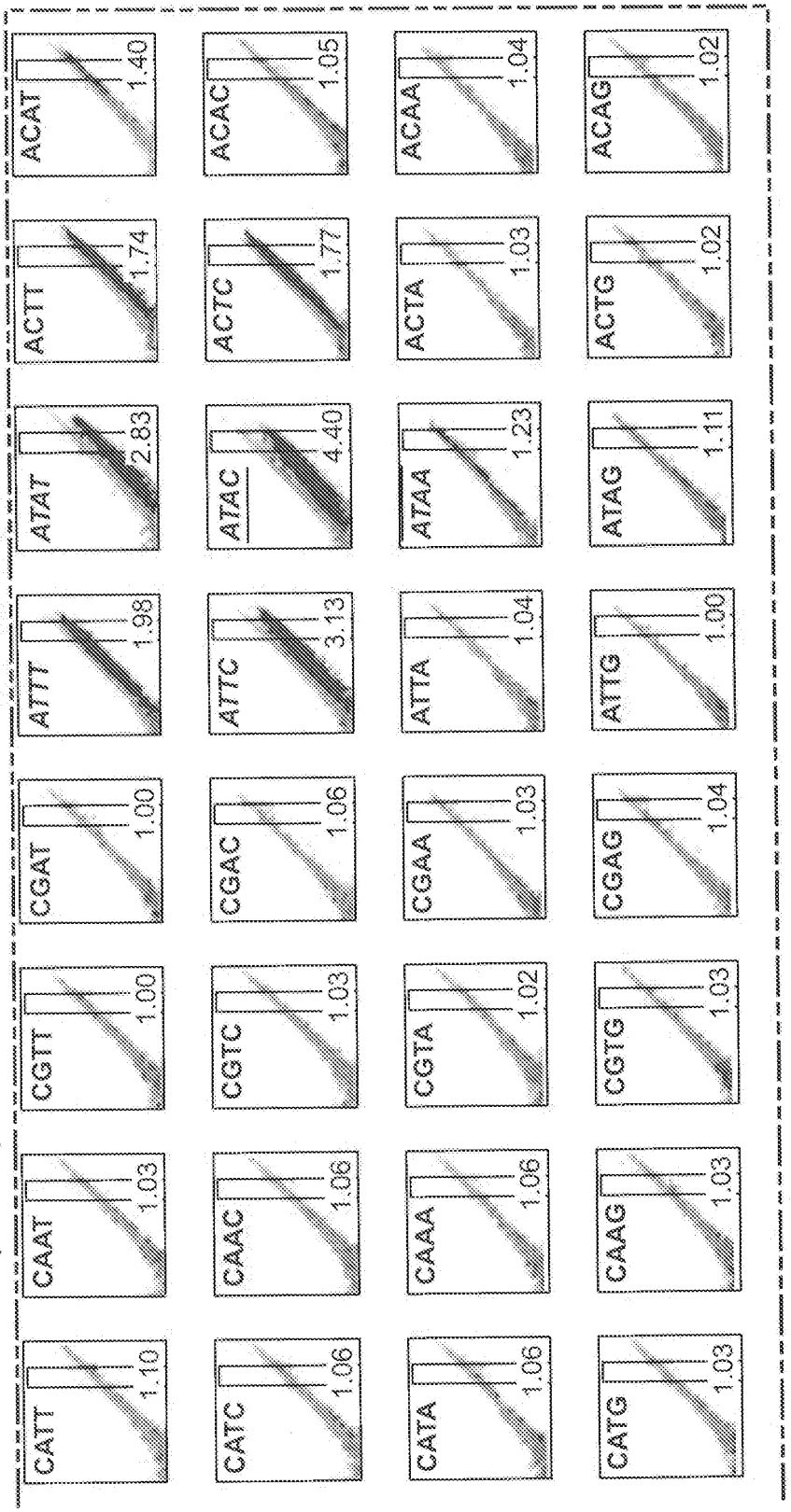


FIGURE 8 (cont.)

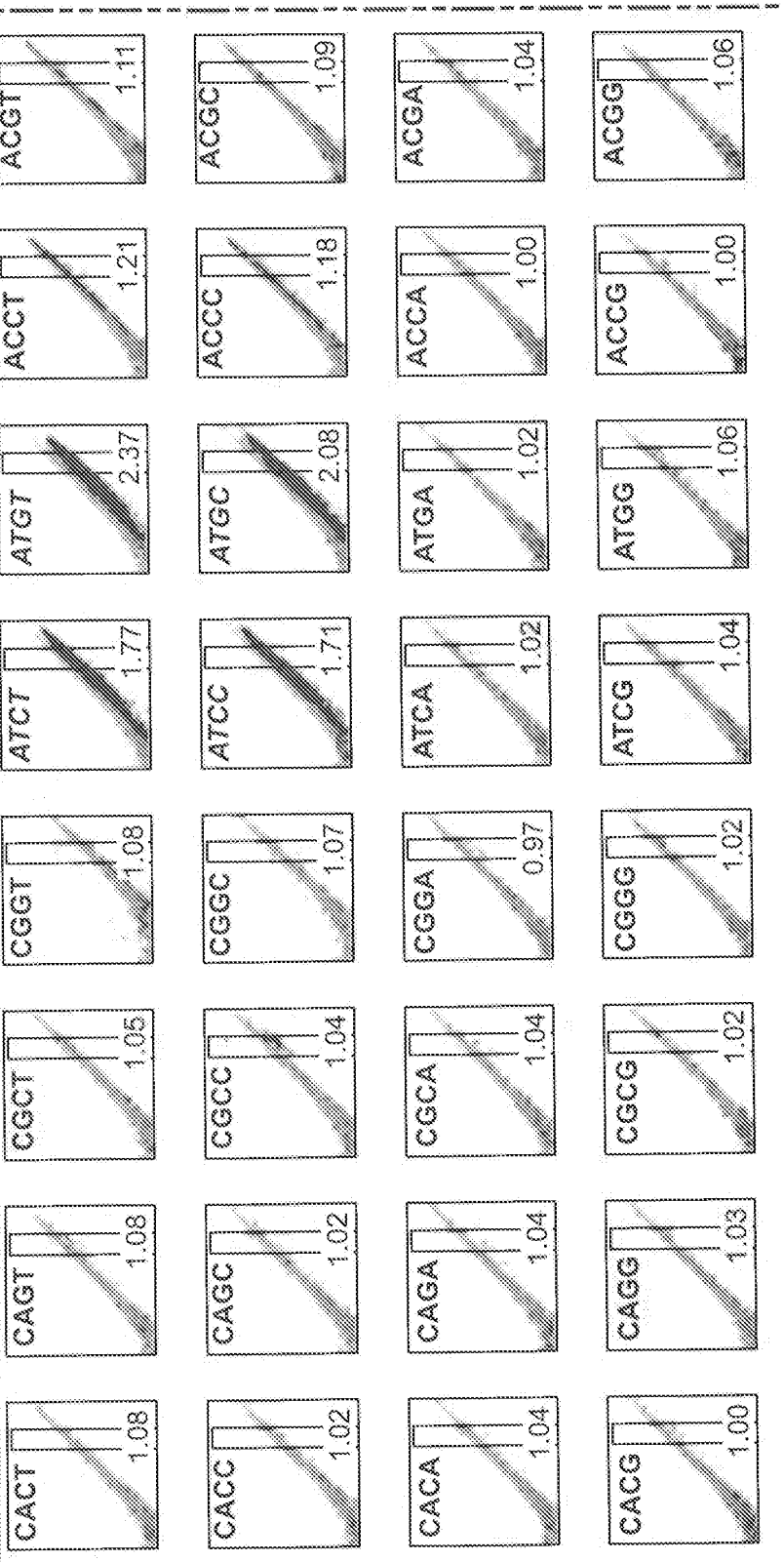


FIGURE 8 (cont.)

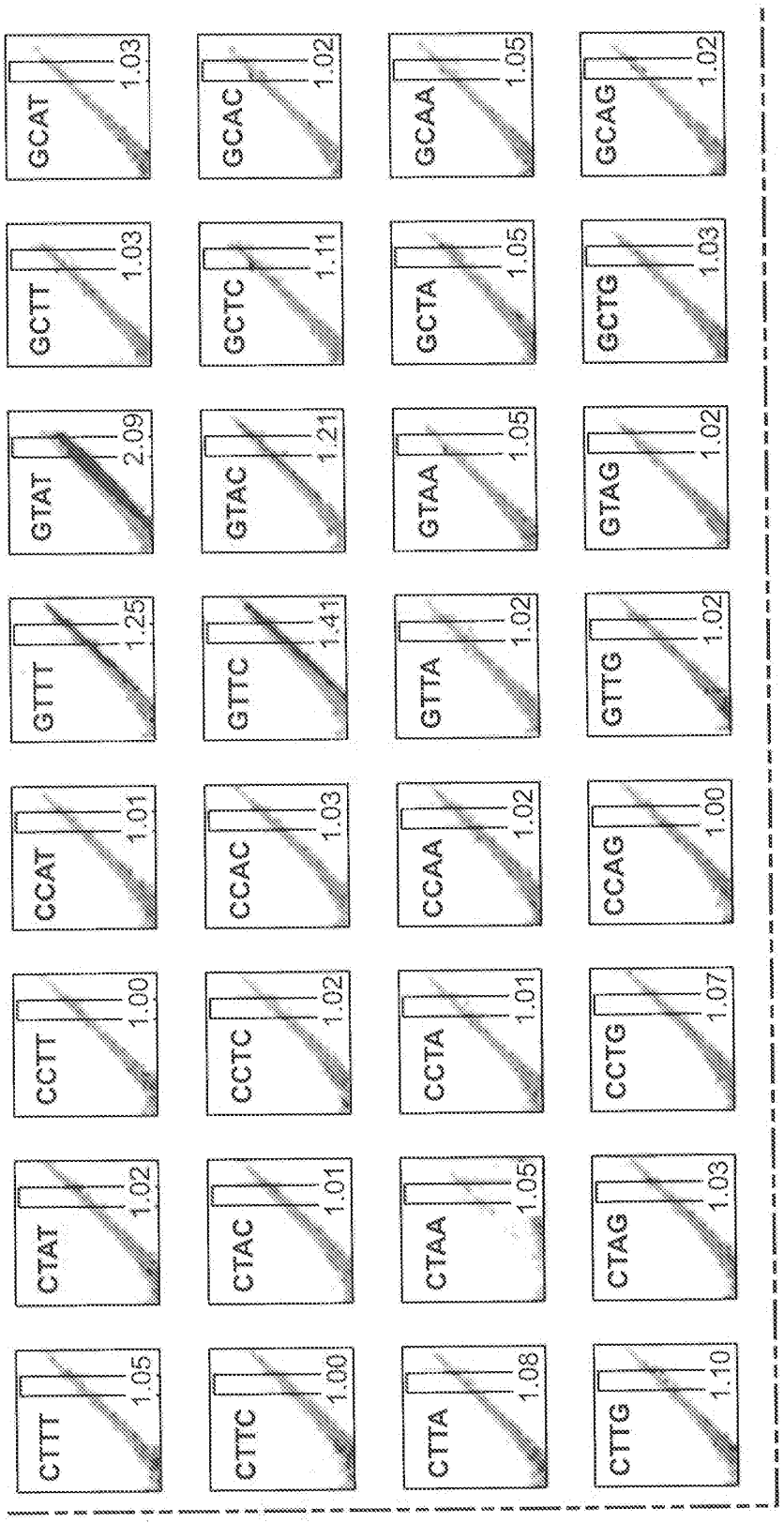


FIGURE 8 (cont.)

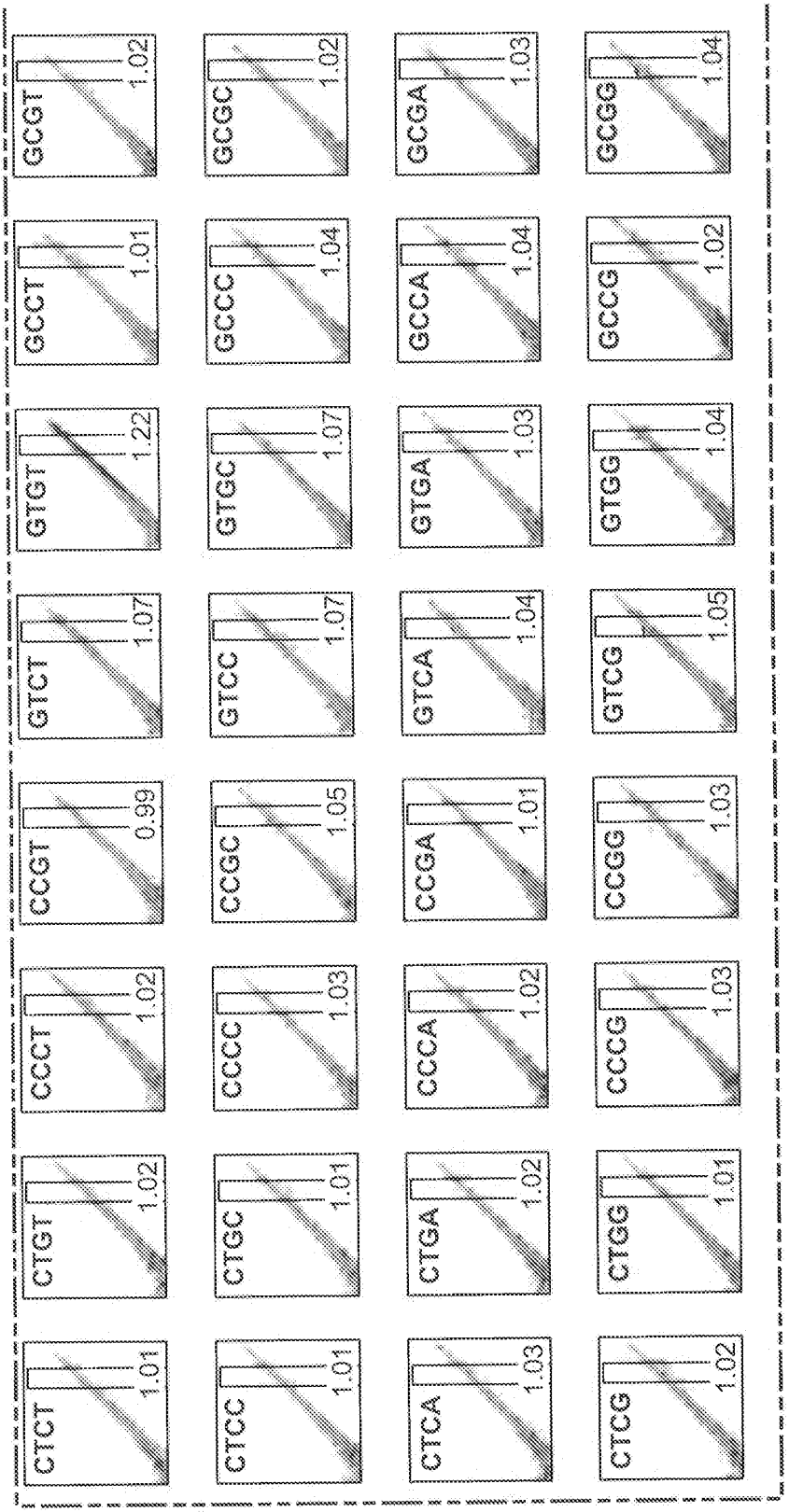


FIGURE 8 (cont.)

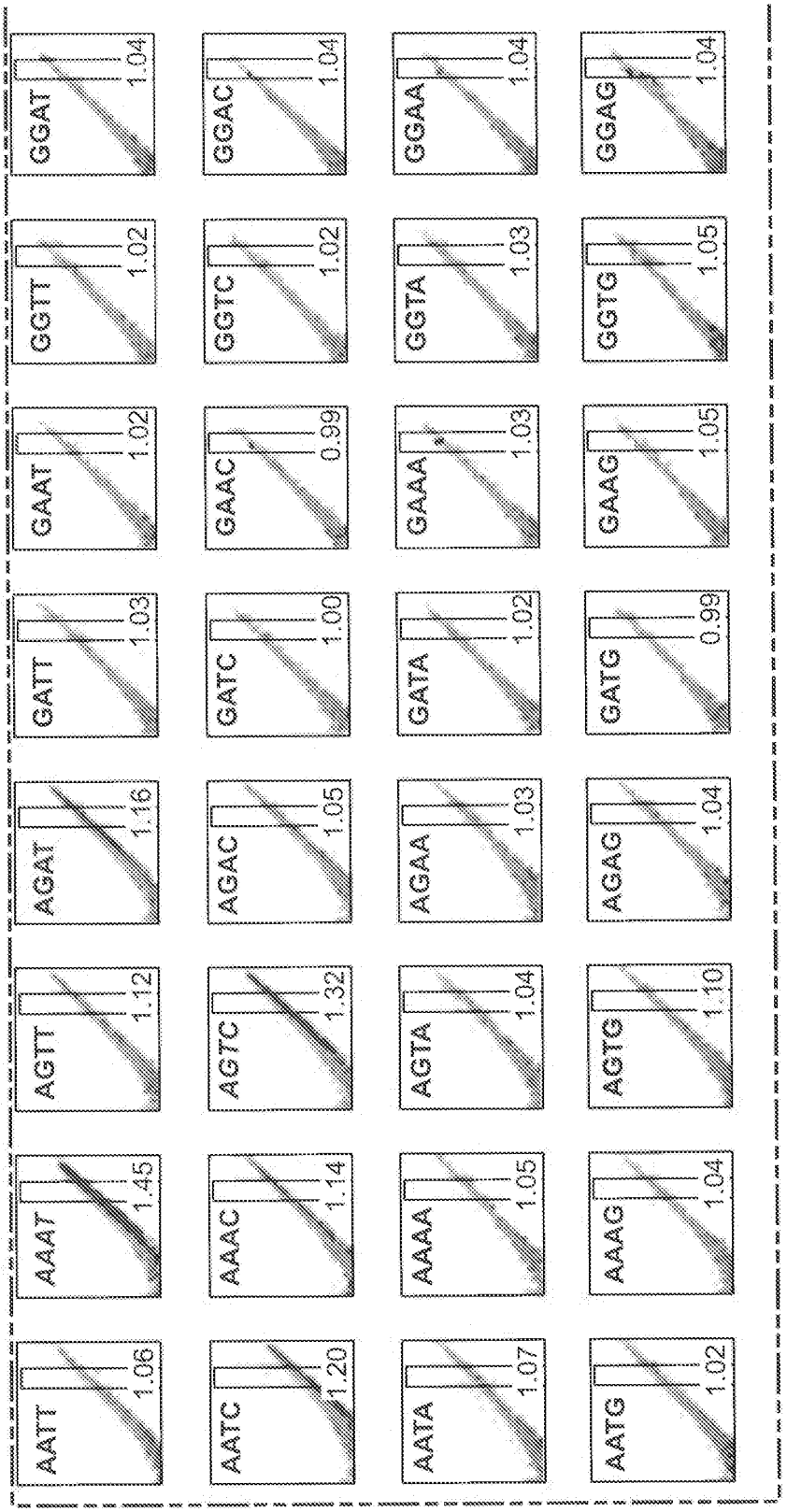


FIGURE 8 (cont.)

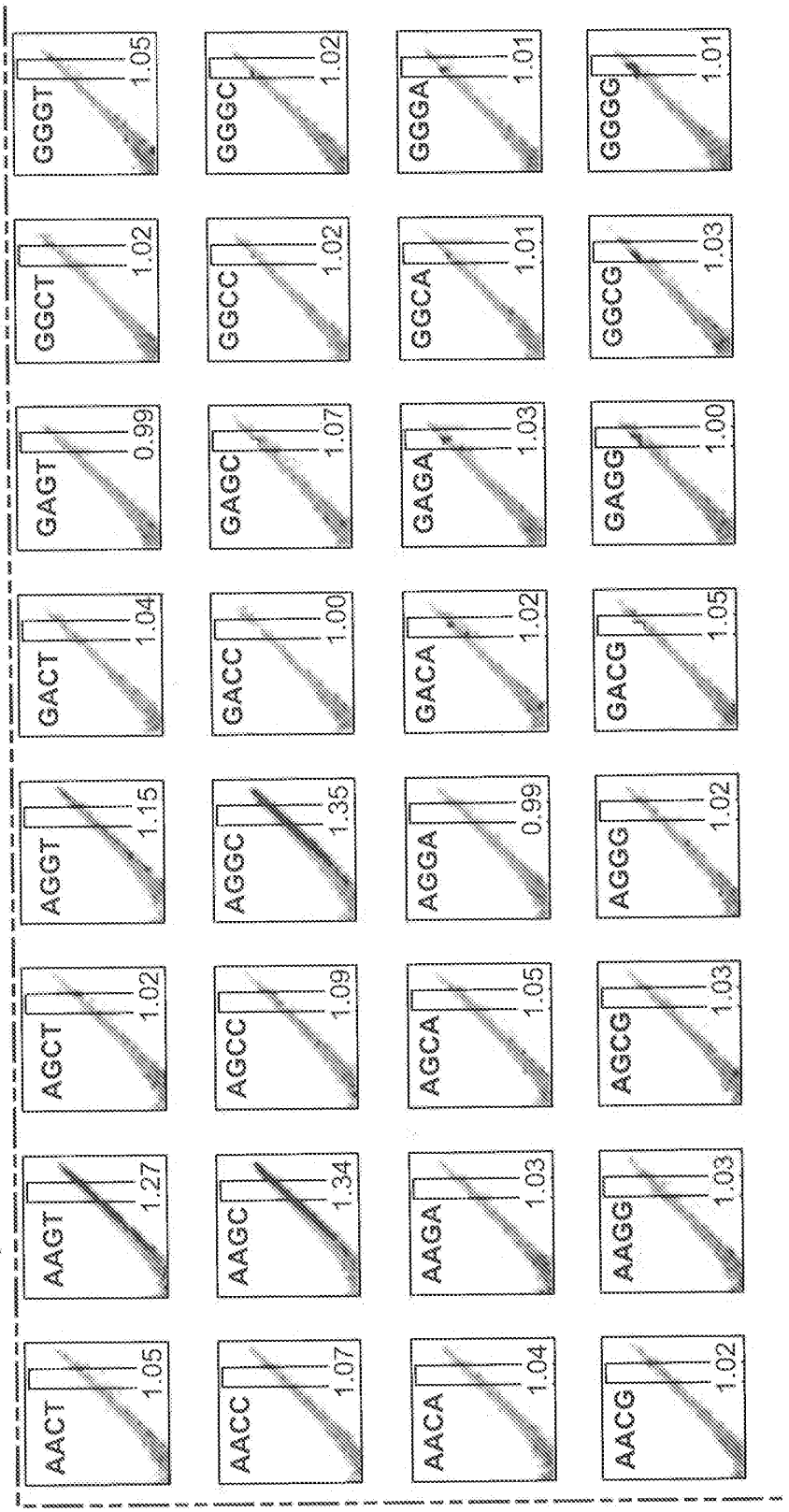


FIGURE 9A

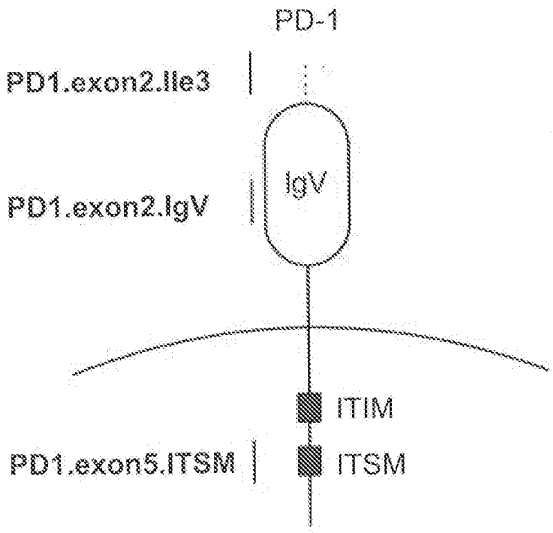


FIGURE 9B

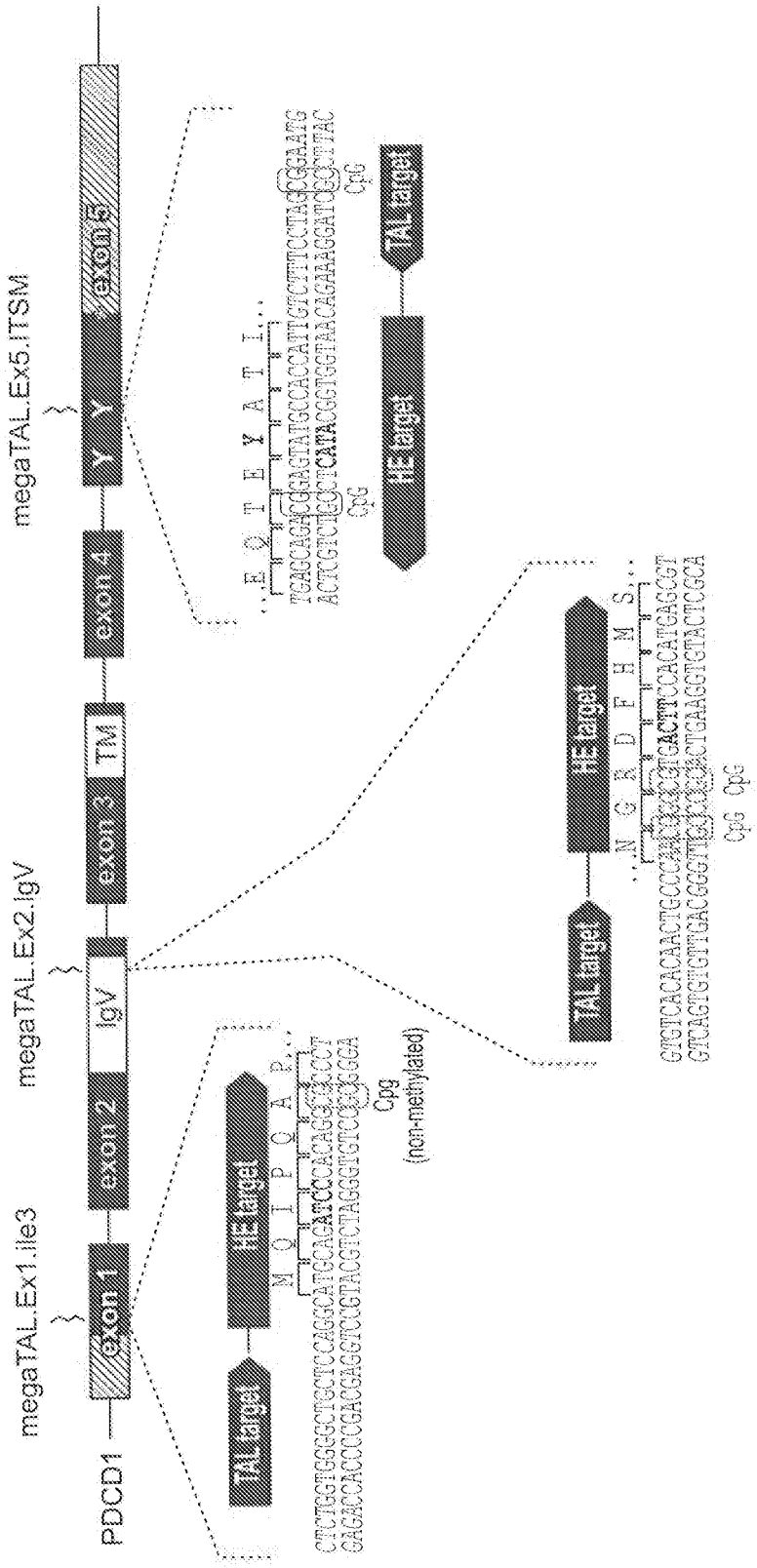

PDCD1
megaTAL.Ex1.ile3
exon 1
exon 2
megaTAL.Ex2.IgV
IgV
exon 3
TM
exon 4
megaTAL.Ex5.ITSM
Y Y
exon 5
TAL target
HE target
M Q I P Q A P...
Cpg
(non-methylated)
TAL target
HE target
...N G R D F H M S...
CpG CpG
...E Q T E Y A T I...
CpG
HE target
TAL target

FIGURE 10

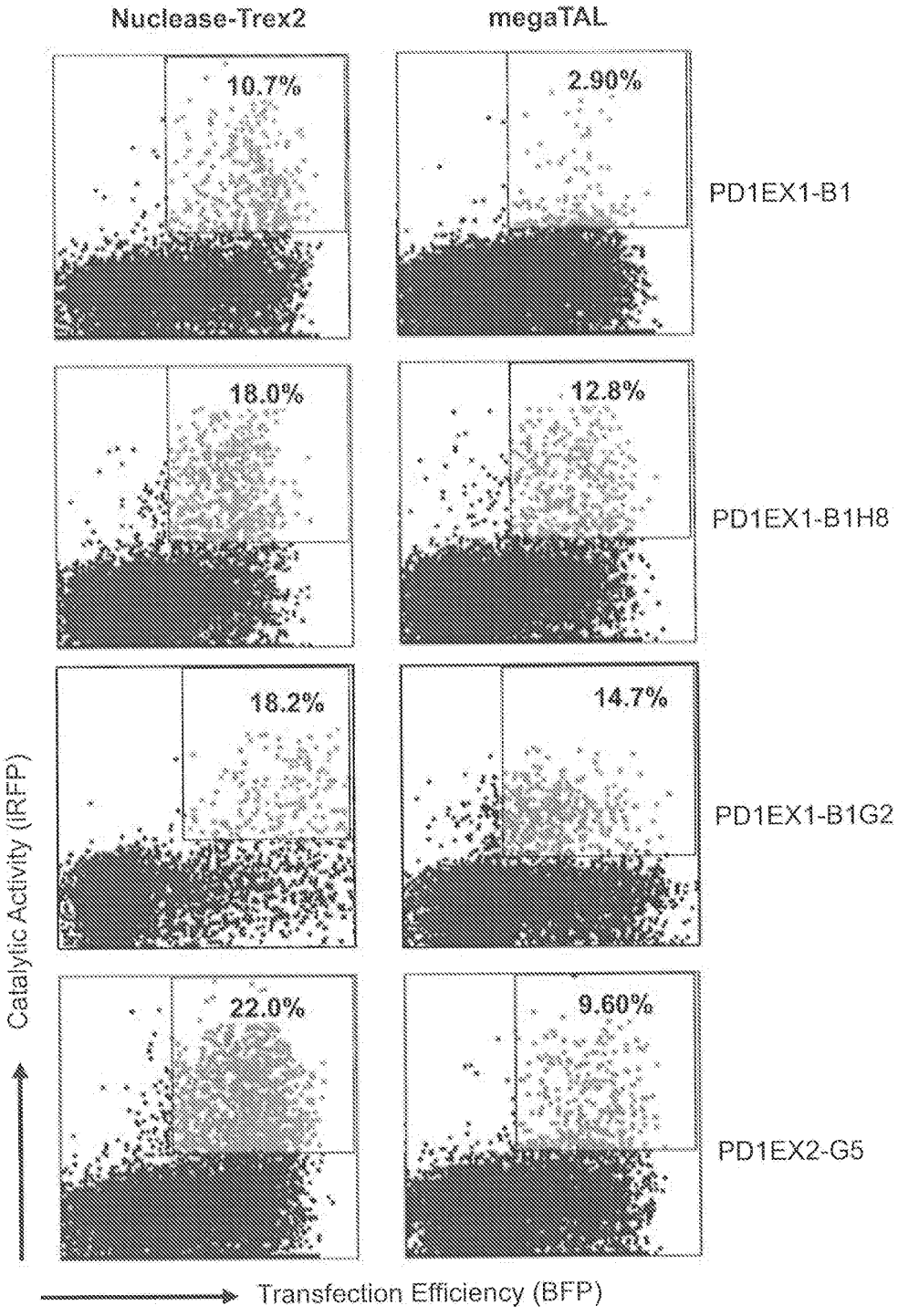
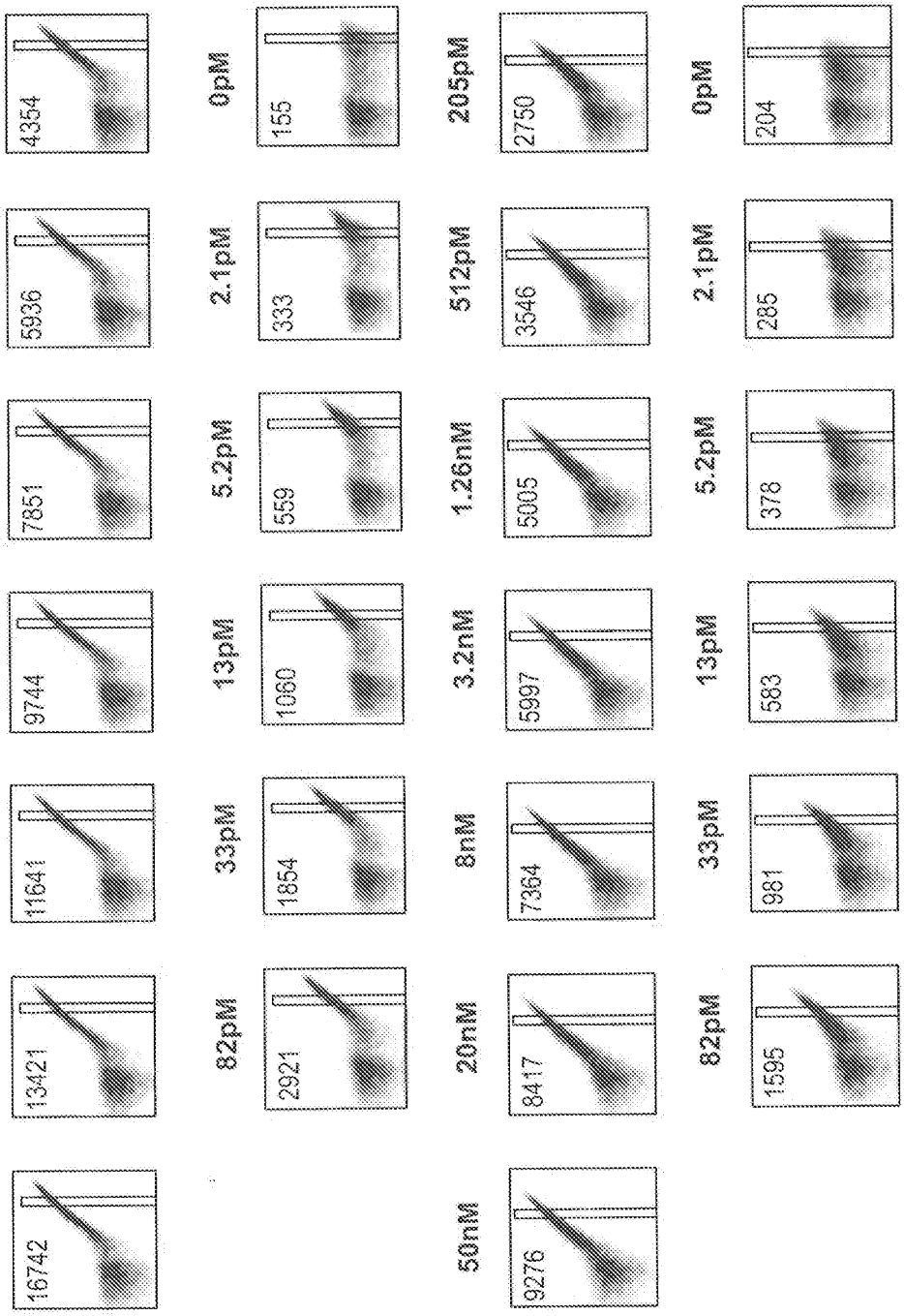

Nuclease-Trex2
megaTAL
10.7%
2.90%
PD1EX1-B1
18.0%
12.8%
PD1EX1-B1H8
18.2%
14.7%
PD1EX1-B1G2
22.0%
9.60%
PD1EX2-G5
Catalytic Activity (iRFP)
Transfection Efficiency (BFP)

FIGURE 11A


PD1EX1-B1H8
50nM
16742
20nM
13421
8nM
11641
3.2nM
9744
1.28nM
7851
512pM
5936
205pM
4354
82pM
2921
33pM
1854
13pM
1060
5.2pM
559
2.1pM
333
0pM
155
PD1EX2-G5
50nM
9276
20nM
8417
8nM
7364
3.2nM
5997
1.26nM
5005
512pM
3546
205pM
2750
82pM
1595
33pM
981
13pM
583
5.2pM
378
2.1pM
285
0pM
204

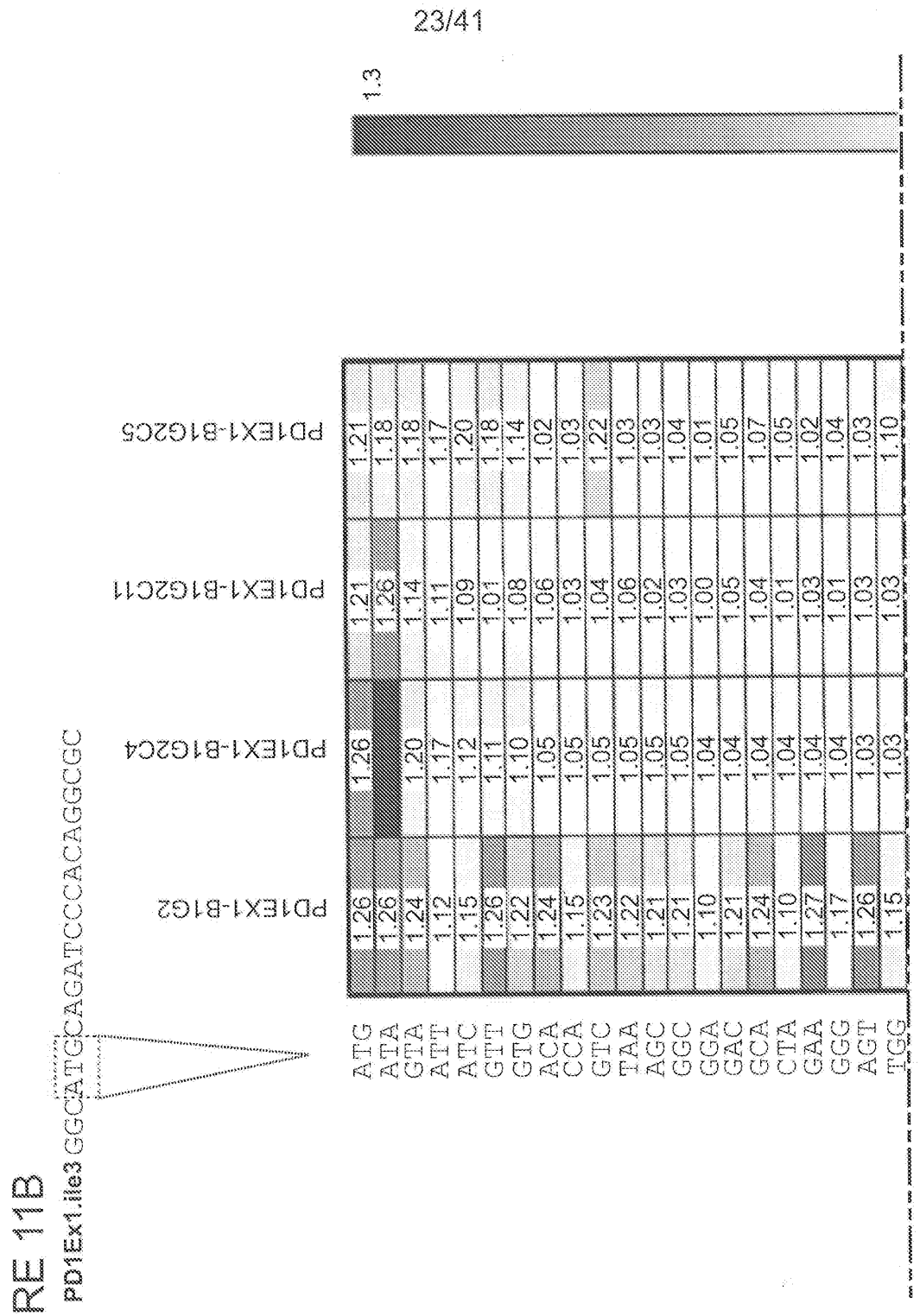


FIGURE 11B

PD1Ex1.Ile3 GGCATGCAGATCCCACAGGCGC

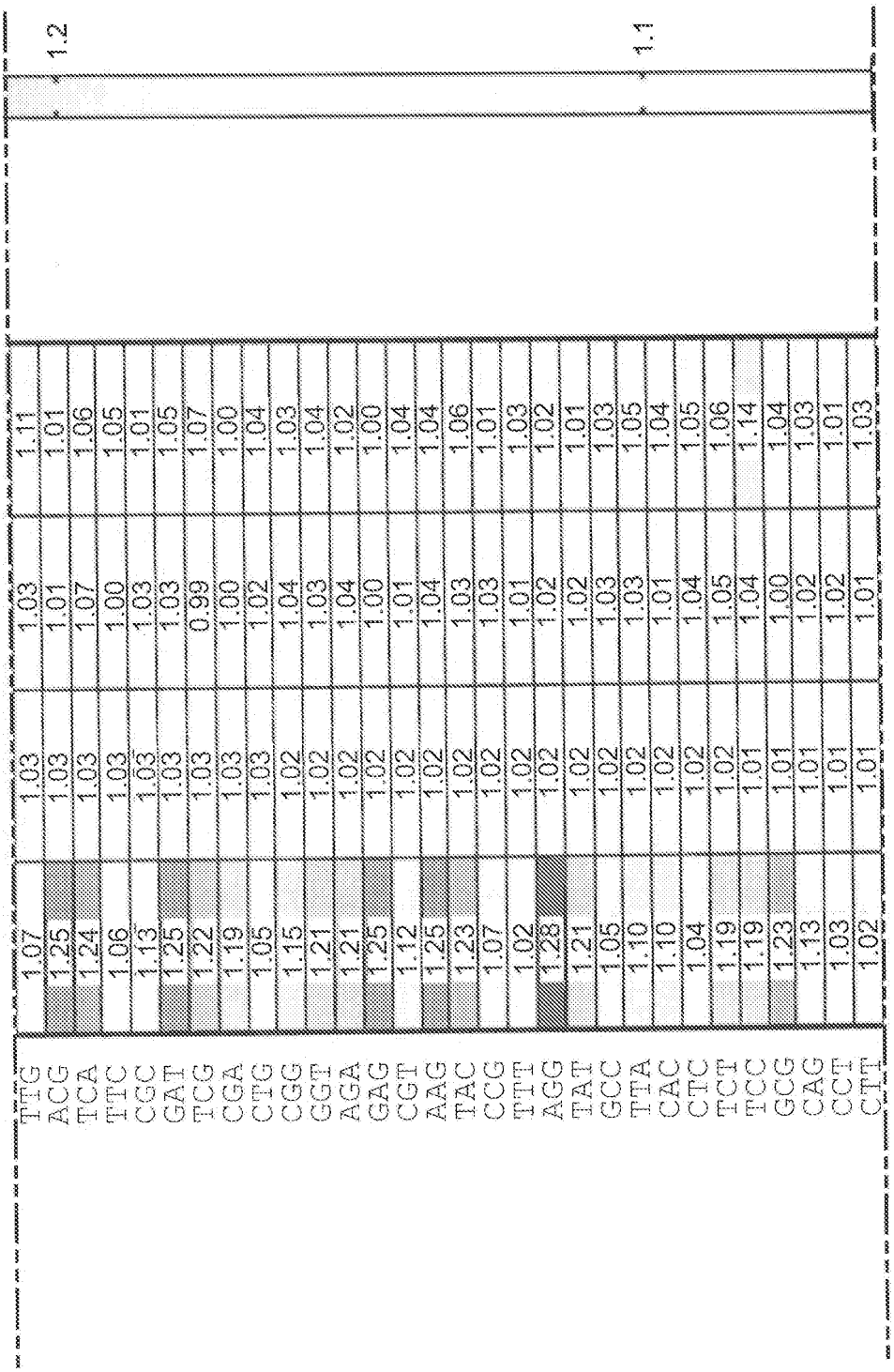


FIGURE 11B (cont.)

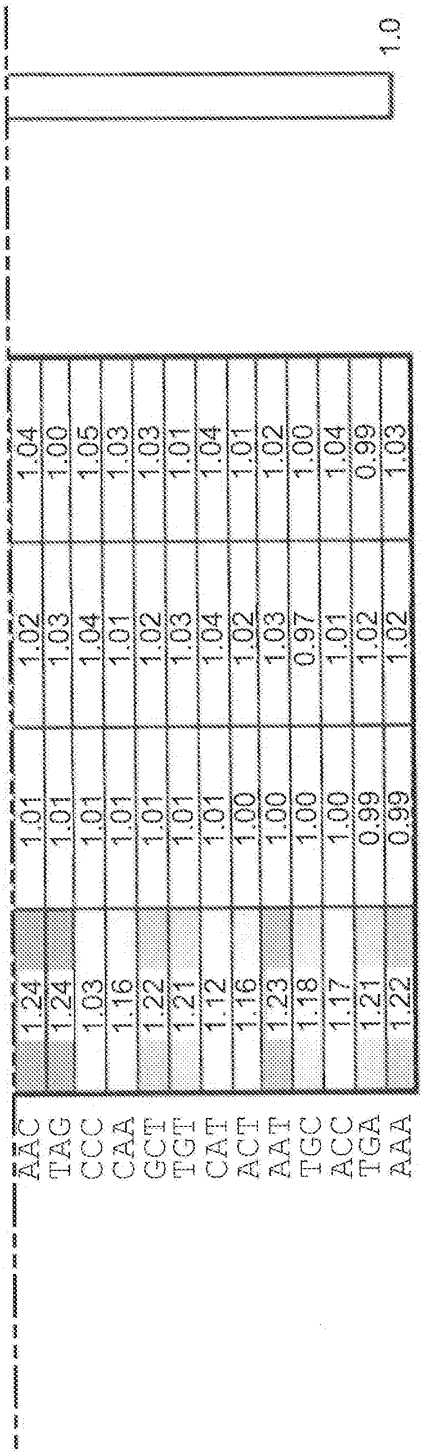


FIGURE 11B (cont.)

FIGURE 12

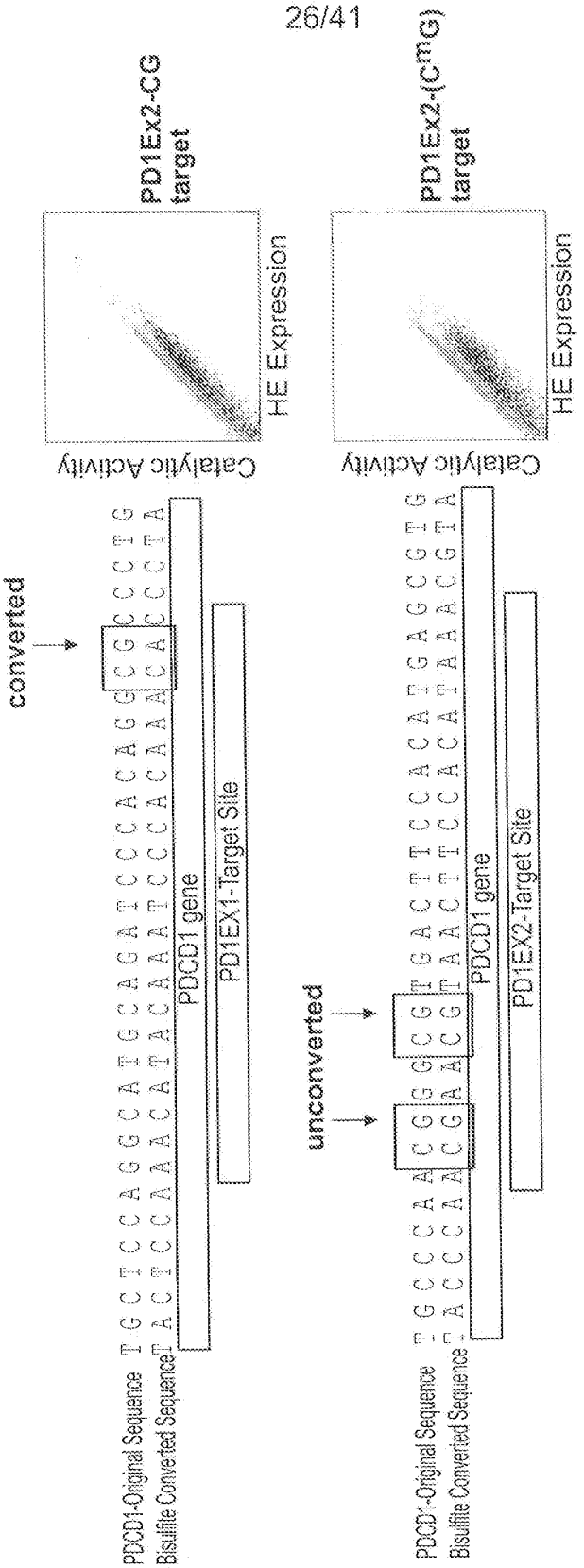

PD1Ex2-CG target
HE Expression
Catalytic Activity
PD1Ex2-(CmG) target
HE Expression
Catalytic Activity
converted
unconverted
PDCD1 gene
PD1EX1-Target Site
PDCD1 gene
PD1EX2-Target Site
PDCD1-Original Sequence
Bisulfite Converted Sequence
PDCD1-Original Sequence
Bisulfite Converted Sequence

FIGURE 13A

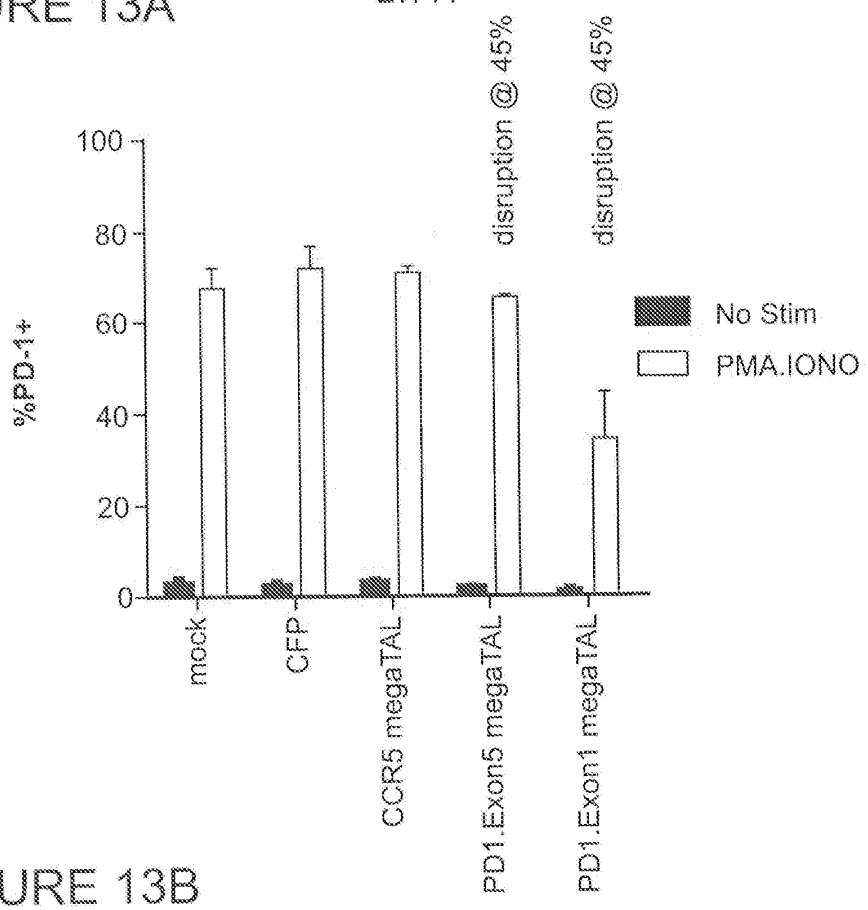


FIGURE 13B

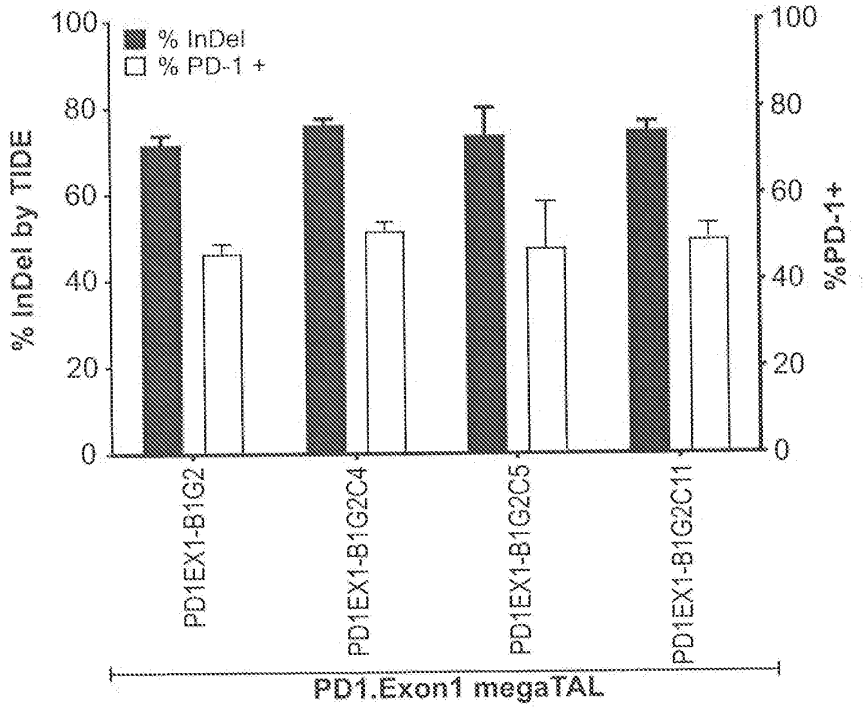


FIGURE 14

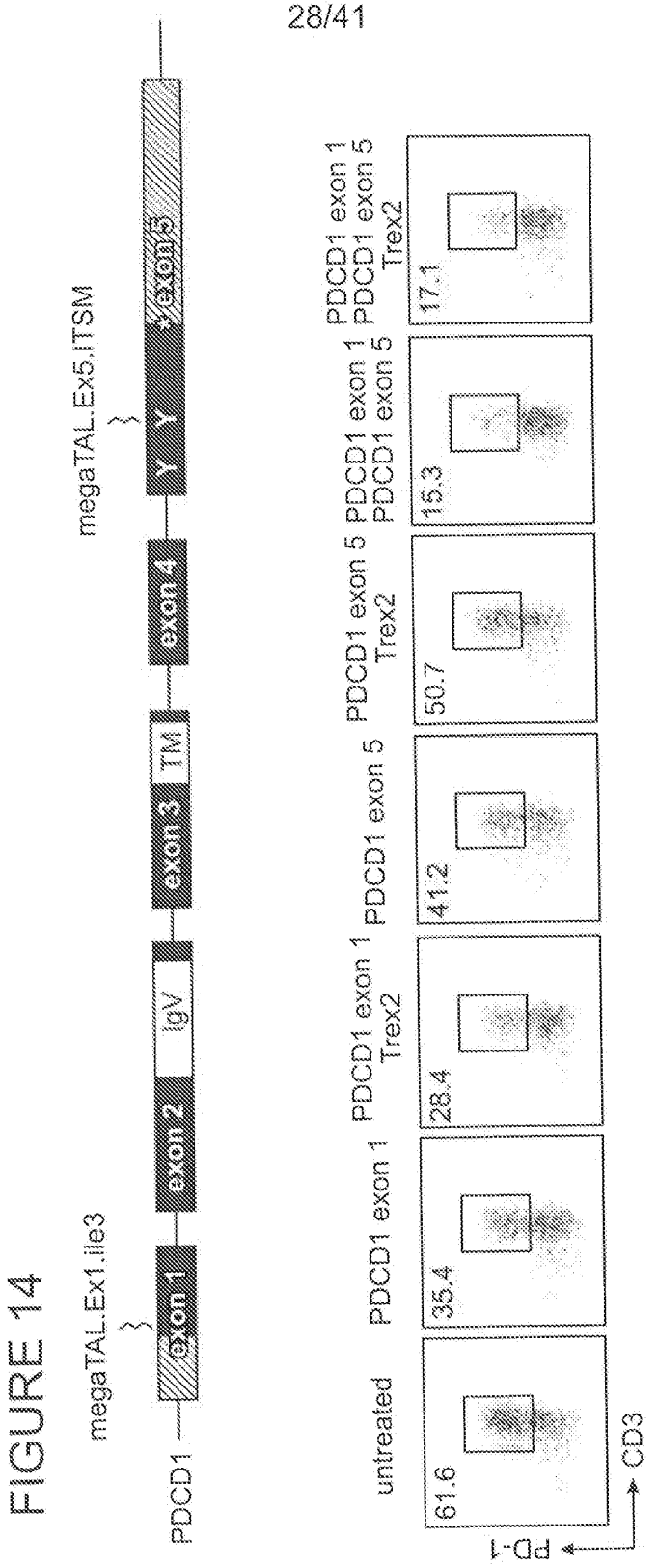

megaTAL.Ex1.Ile3
megaTAL.Ex5.ITSM
PDCD1
exon 1
exon 2
IgV
exon 3
TM
exon 4
Y
Y
exon 5
untreated
PDCD1 exon 1
PDCD1 exon 1 Trex2
PDCD1 exon 5
PDCD1 exon 5 Trex2
PDCD1 exon 1 PDCD1 exon 5
PDCD1 exon 1 PDCD1 exon 5 Trex2
61.6
35.4
28.4
41.2
50.7
15.3
17.1
PD-1
CD3

FIGURE 15A

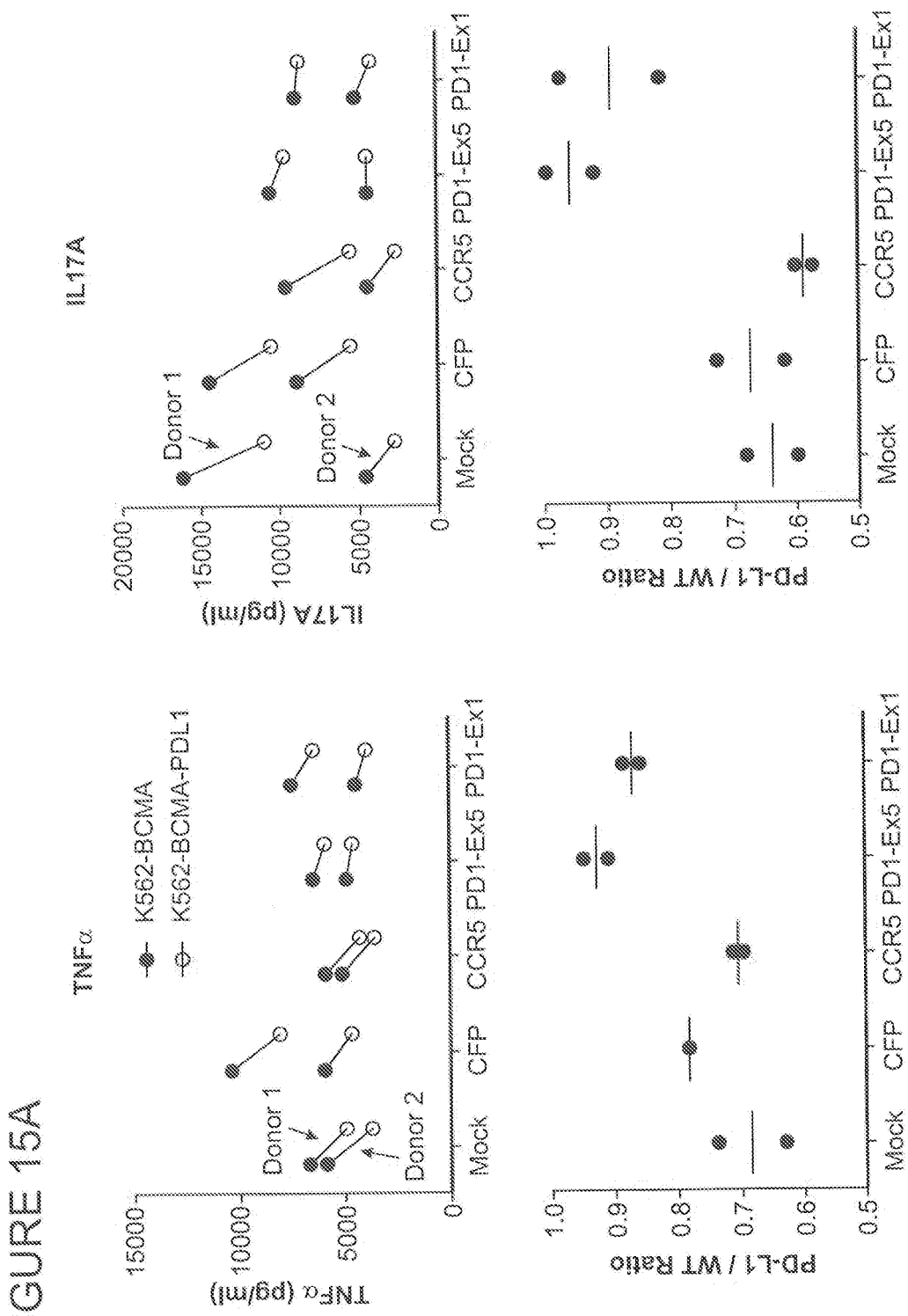

TNFα
K562-BCMA
K562-BCMA-PDL1
TNFα (pg/ml)
15000
10000
5000
0
Donor 1
Donor 2
Mock
CFP
CCR5 PD1-Ex5 PD1-Ex1
IL17A
IL17A (pg/ml)
20000
15000
10000
5000
0
Donor 1
Donor 2
Mock
CFP
CCR5 PD1-Ex5 PD1-Ex1
PD-L1 / WT Ratio
1.0
0.9
0.8
0.7
0.6
0.5
Mock
CFP
CCR5 PD1-Ex5 PD1-Ex1
PD-L1 / WT Ratio
1.0
0.9
0.8
0.7
0.6
0.5
Mock
CFP
CCR5 PD1-Ex5 PD1-Ex1

FIGURE 15B

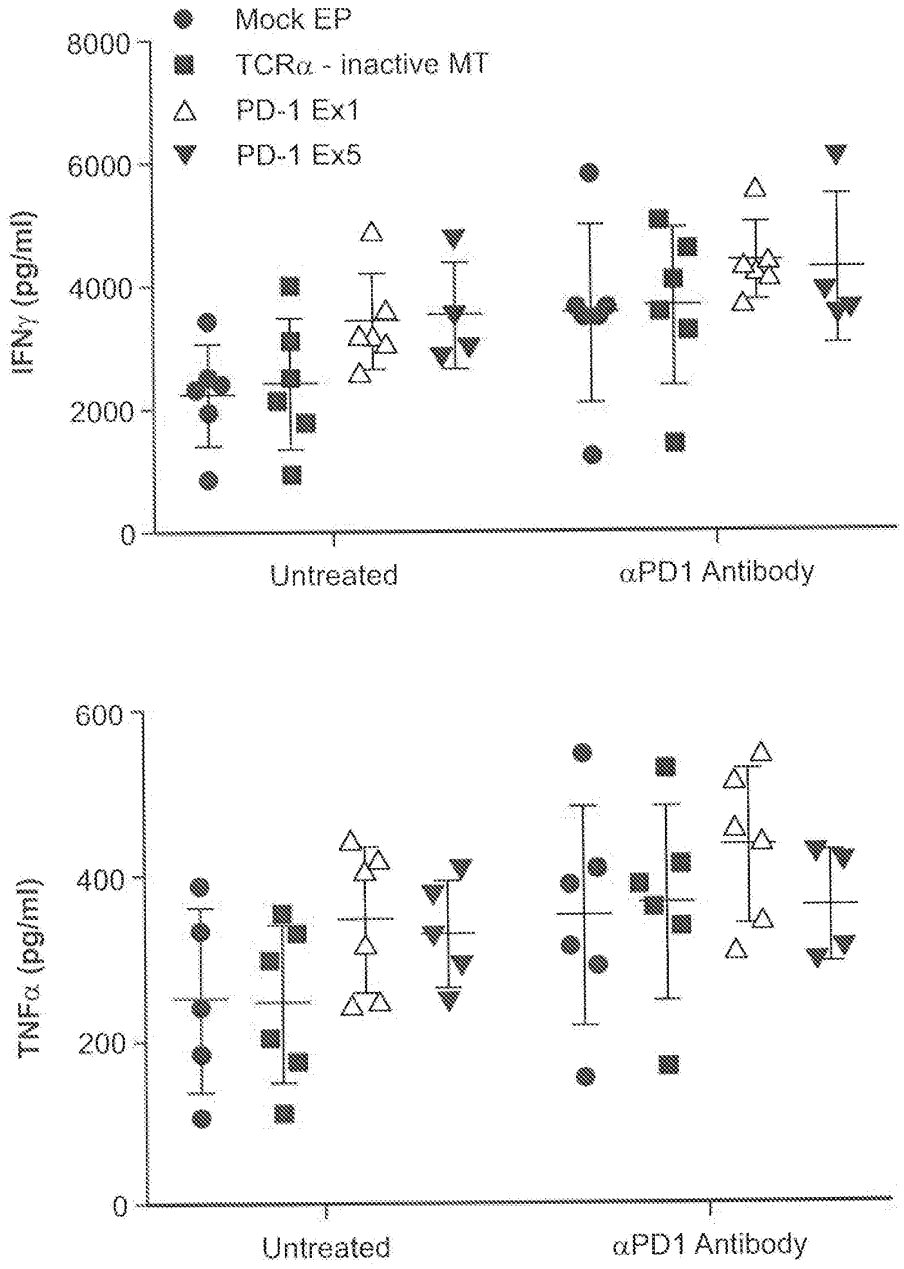

Mock EP
TCRα - inactive MT
PD-1 Ex1
PD-1 Ex5
IFNγ (pg/ml)
8000
6000
4000
2000
0
Untreated
αPD1 Antibody
TNFα (pg/ml)
600
400
200
0
Untreated
αPD1 Antibody

FIGURE 16A

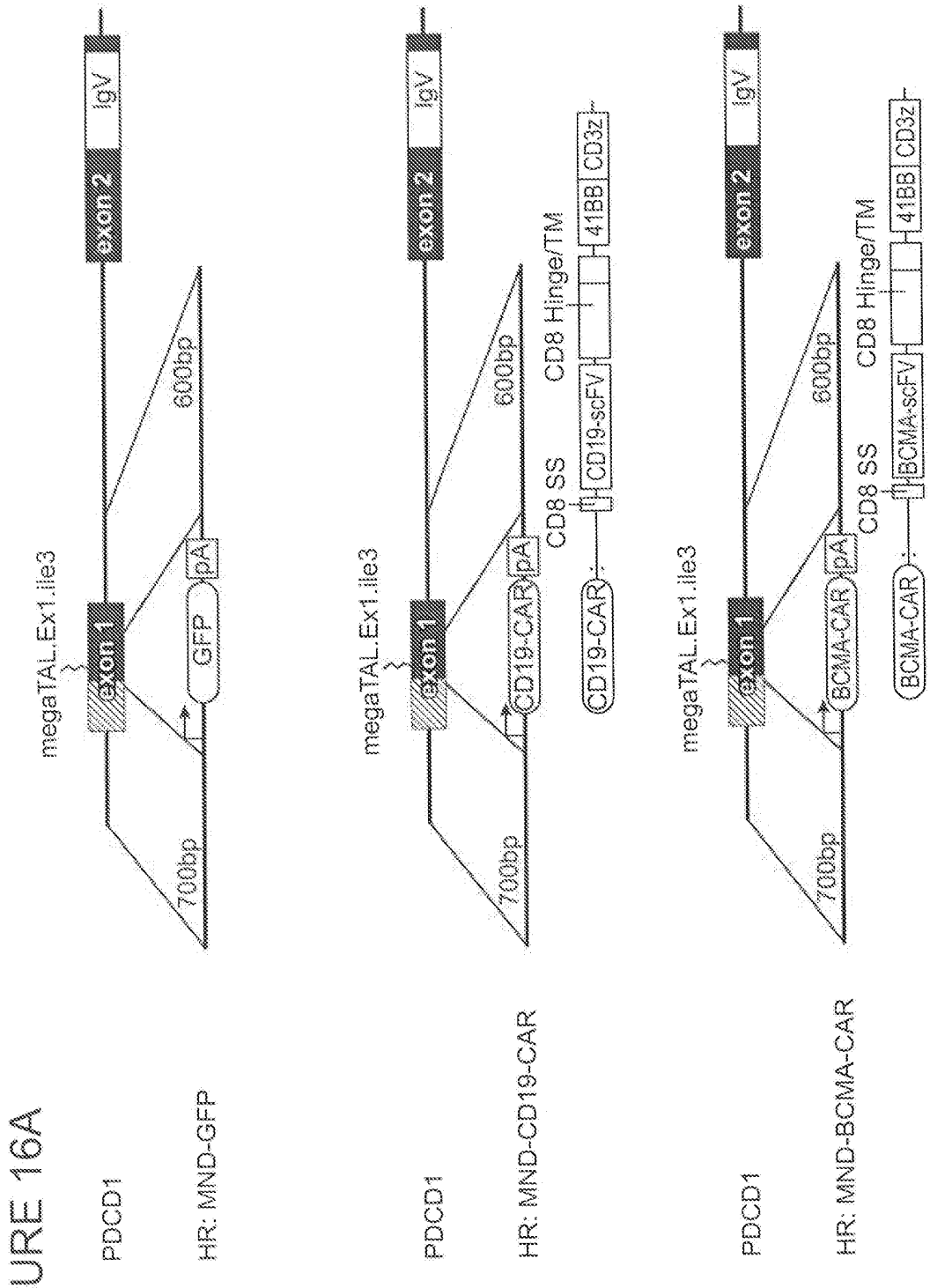
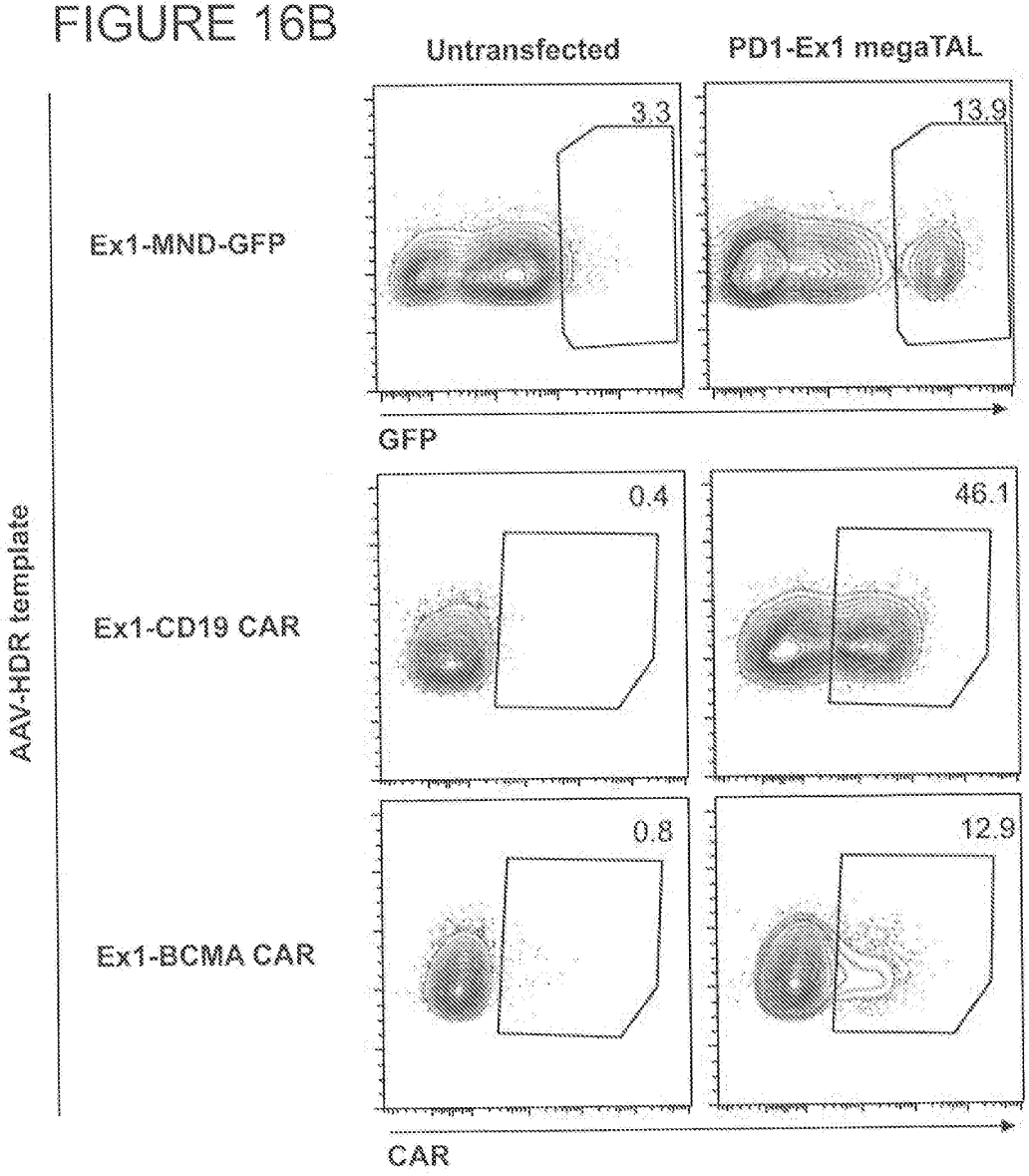

megaTAL.Ex1.ile3
exon 1
exon 2
IgV
700bp
600bp
GFP
pA
PDCD1
HR: MND-GFP
megaTAL.Ex1.ile3
exon 1
exon 2
IgV
700bp
600bp
CD19-CAR
pA
CD8 SS
CD19-CAR
CD19-scFV
CD8 Hinge/TM
41BB
CD3z
PDCD1
HR: MND-CD19-CAR
megaTAL.Ex1.ile3
exon 1
exon 2
IgV
700bp
600bp
BCMA-CAR
pA
CD8 SS
BCMA-CAR
BCMA-scFV
CD8 Hinge/TM
41BB
CD3z
PDCD1
HR: MND-BCMA-CAR

FIGURE 16B


Untransfected
PD1-Ex1 megaTAL
AAV-HDR template
Ex1-MND-GFP
3.3
13.9
GFP
Ex1-CD19 CAR
0.4
46.1
Ex1-BCMA CAR
0.8
12.9
CAR

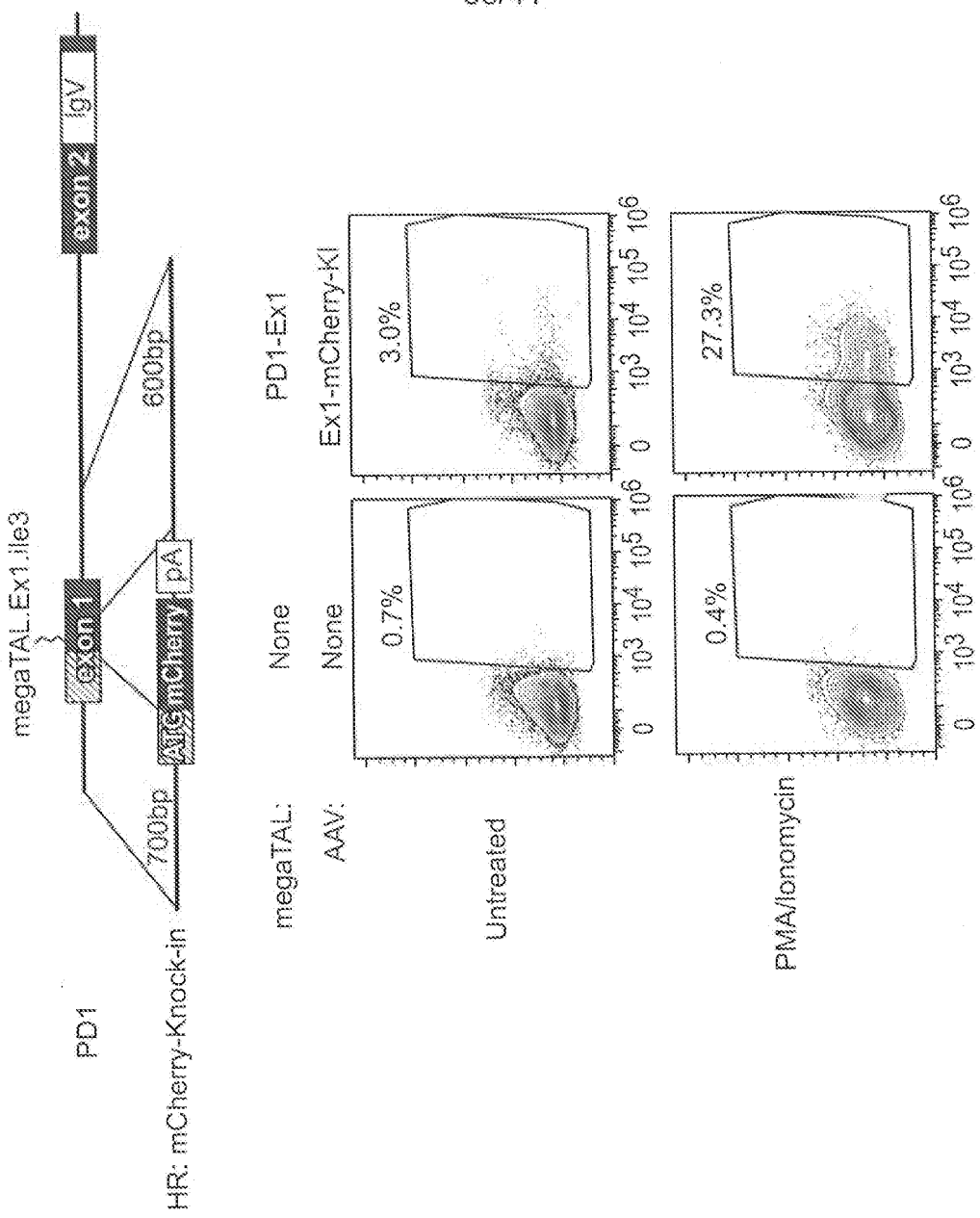
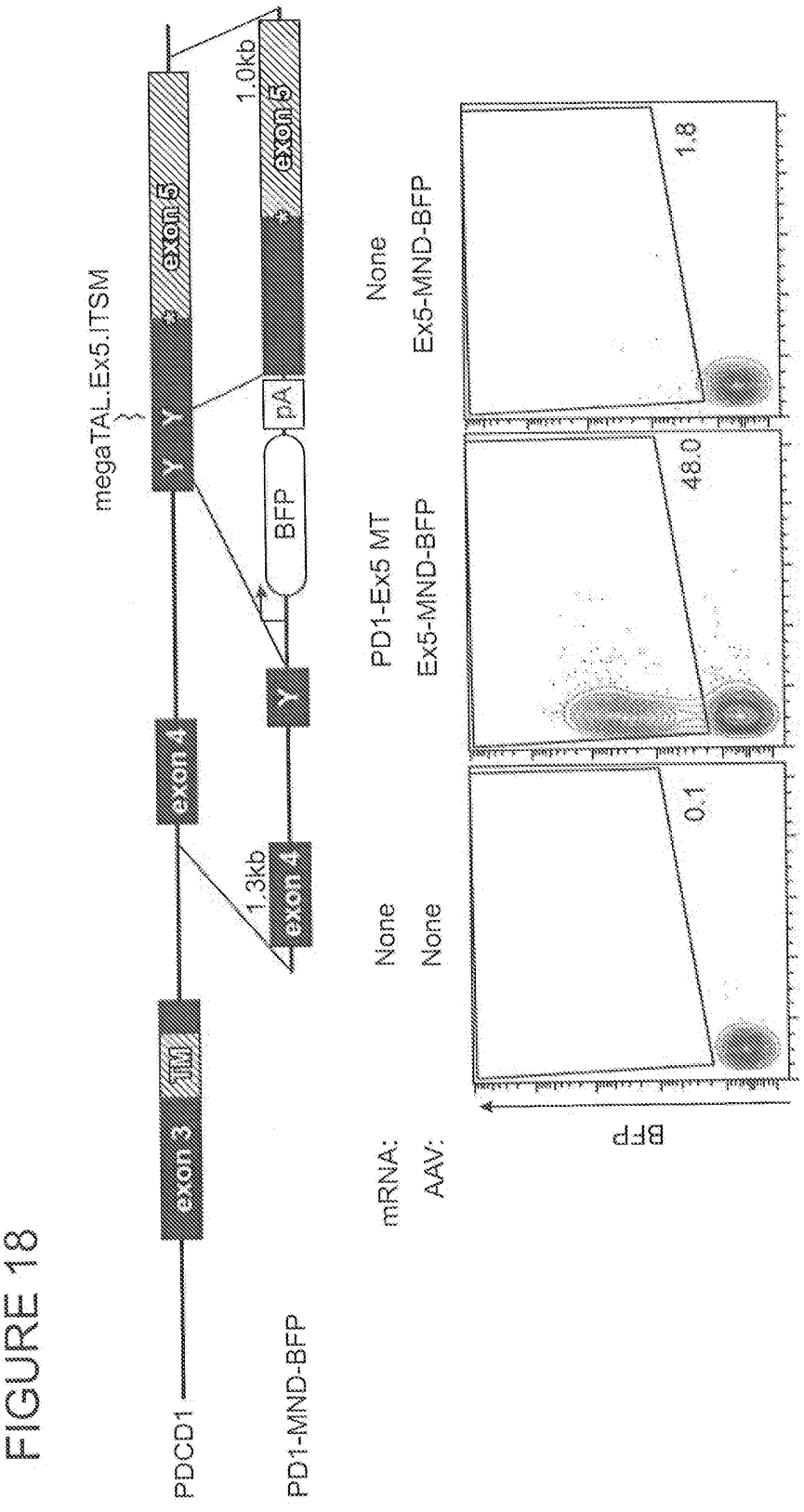

megaTAL.Ex1.Ile3
exon 1
exon 2 IgV
PD1
HR: mCherry-Knock-in
700bp
mCherry
pA
600bp
megaTAL:
AAV:
None
None
PD1-Ex1
Ex1-mCherry-KI
0.7%
3.0%
0.4%
27.3%
Untreated
PMA/Ionomycin

FIGURE 17

FIGURE 18


megaTAL.Ex5.ITSM
exon 5
exon 5
1.0kb
pA
BFP
exon 1
1.3kb
exon 4
exon 3
TM
PDCD1
PD1-MND-BFP
mRNA:
AAV:
None
None
PD1-Ex5 MT
Ex5-MND-BFP
None
Ex5-MND-BFP
0.1
48.0
1.8
BFP

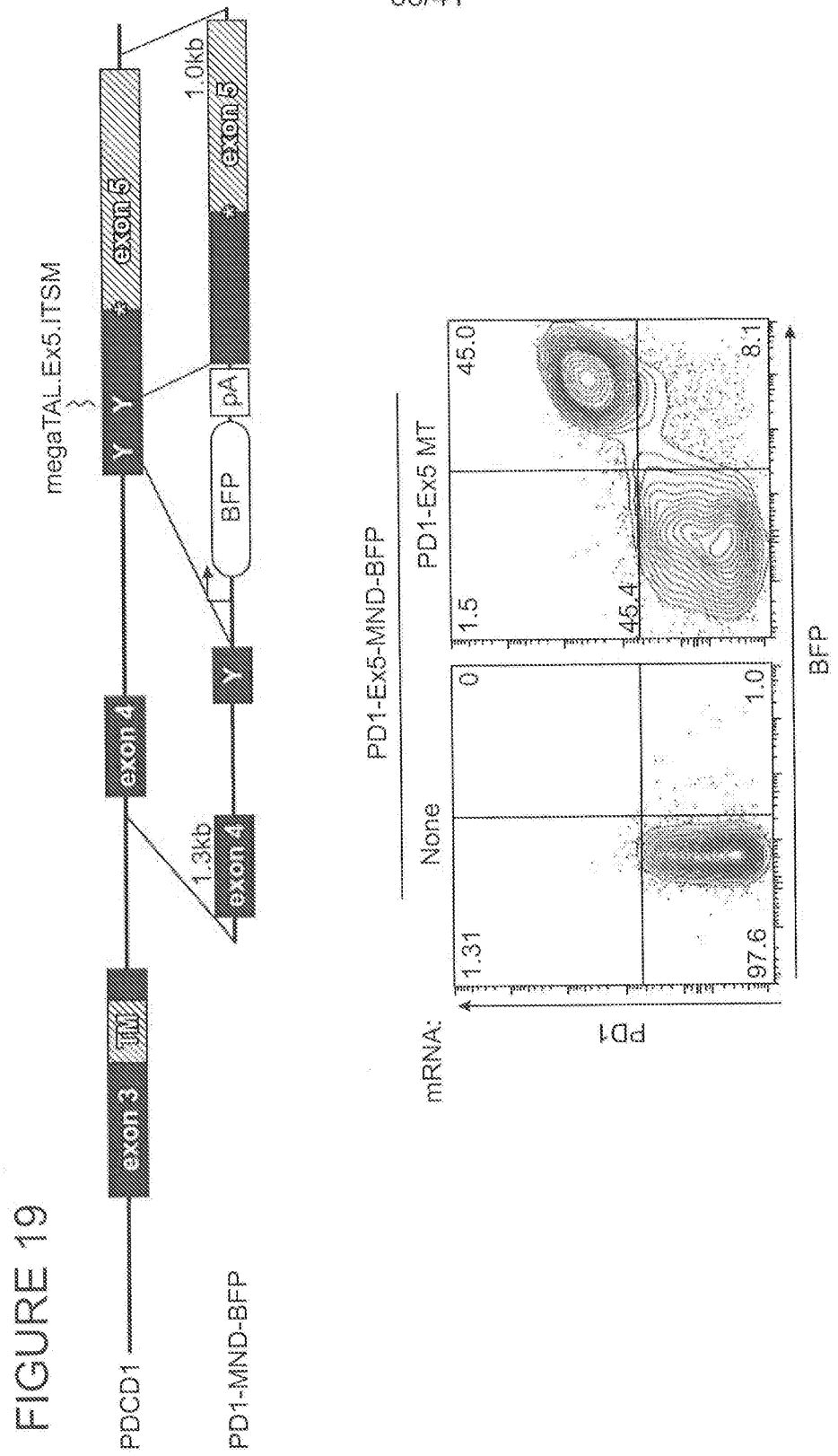

megaTAL.Ex5.ITSM
exon 5
1.0kb
exon 5
pA
BFP
exon 4
1.3kb
exon 4
exon 3
PDCD1
PD1-MND-BFP
PD1-Ex5-MND-BFP
PD1-Ex5 MT
None
mRNA:
PD1
BFP
1.31
0
1.0
97.6
1.5
45.4
45.0
8.1

FIGURE 19

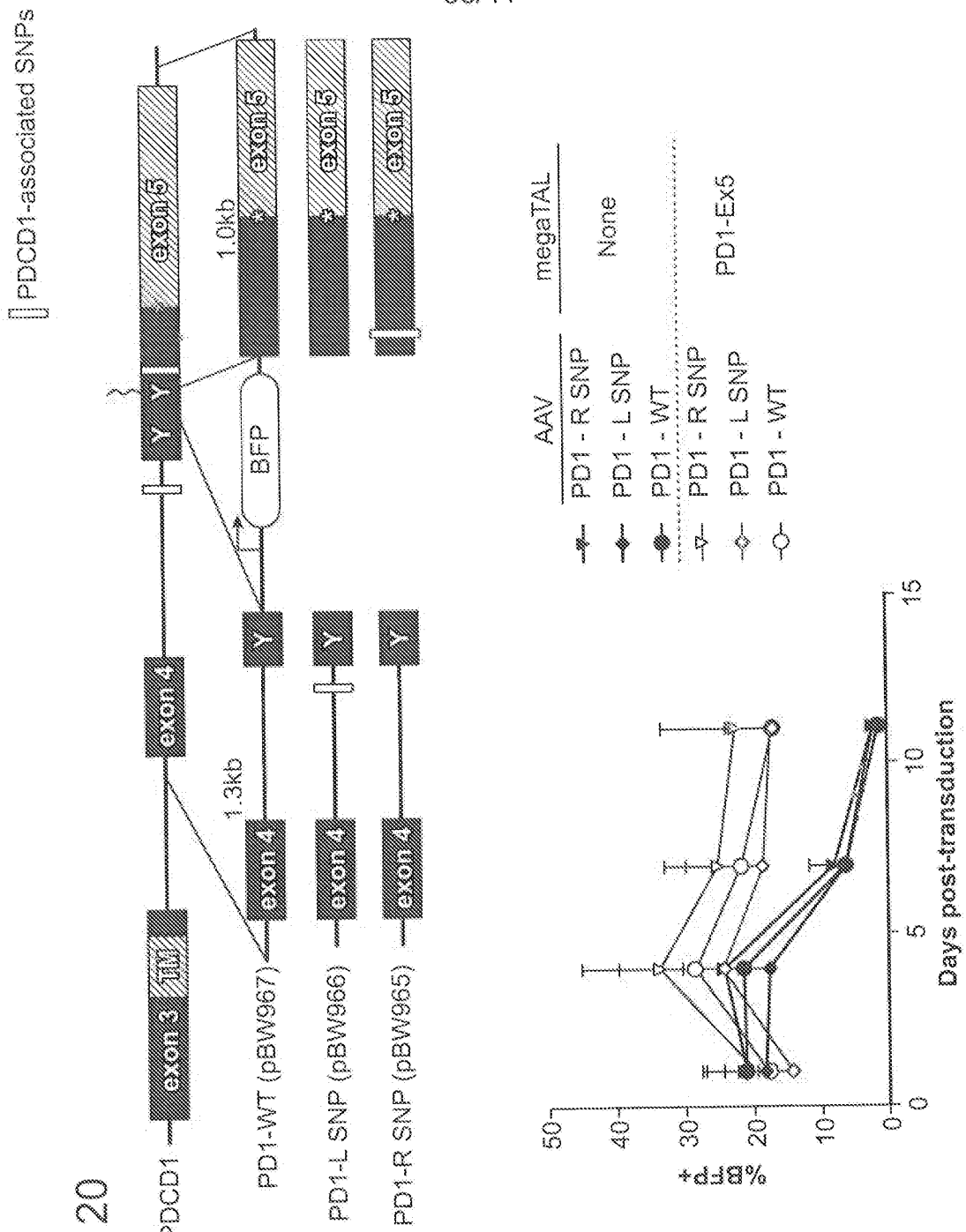
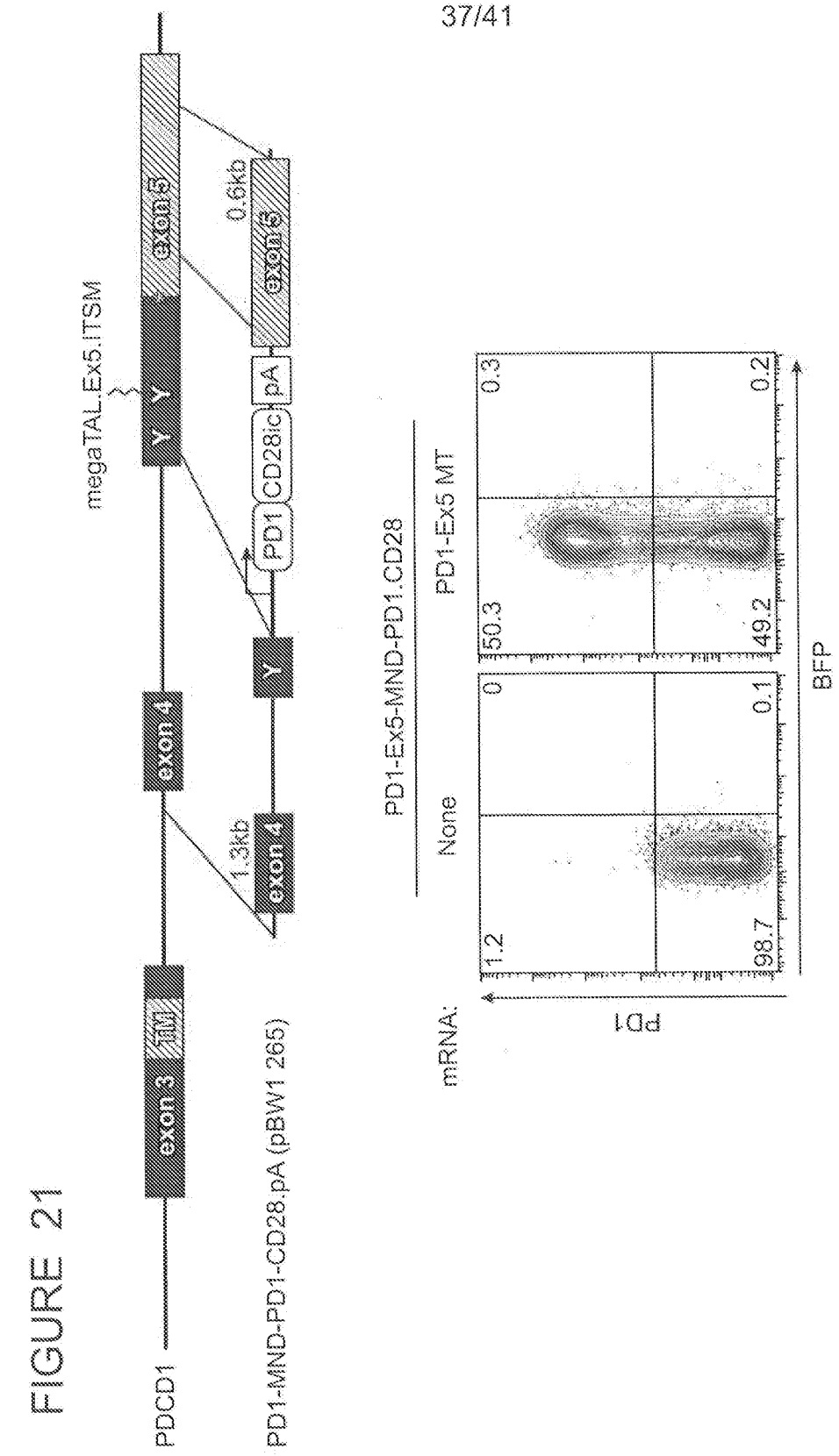

PDCD1-associated SNPs
exon 5
1.0kb
BFP
exon 3
exon 4
1.3kb
PDCD1
PD1-WT (pBW967)
PD1-L SNP (pBW966)
PD1-R SNP (pBW965)
AAV
megaTAL
PD1 - R SNP
PD1 - L SNP
PD1 - WT
None
PD1-Ex5
%BFP+
Days post-transduction

FIGURE 20

FIGURE 21


PDCD1
exon 3
TM
exon 4
mega TAL.Ex5.ITSM
exon 5
Y Y
1.3kb
exon 4
0.6kb
exon 5
Y
PD1
CD28ic
pA
PD1-MND-PD1-CD28.pA (pBW1 265)
PD1-Ex5-MND-PD1.CD28
None
PD1-Ex5 MT
mRNA:
PD1
BFP
1.2
98.7
0
0.1
50.3
49.2
0.3
0.2

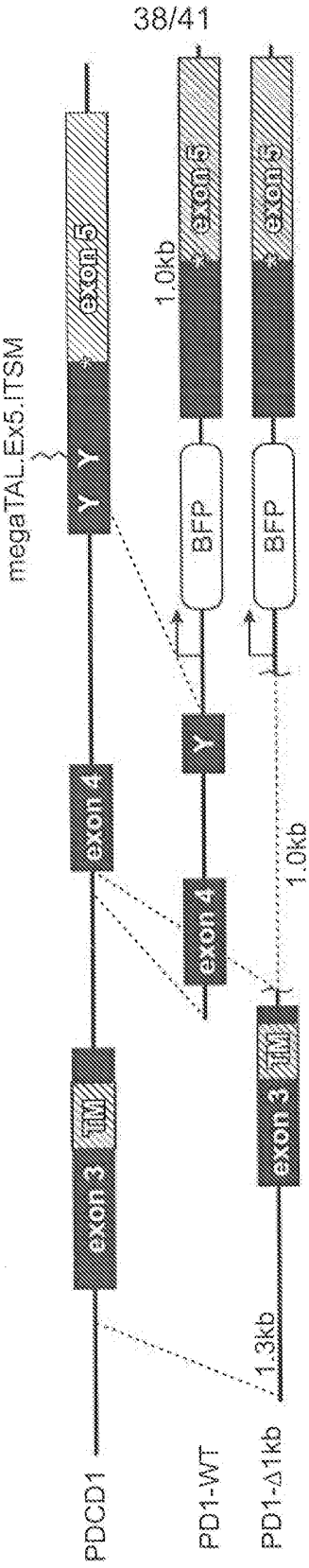

PDCD1
PD1-WT
PD1-Δ1kb
1.3kb
exon 3
TM
exon 4
Y
megaTAL.Ex5.ITSM
exon 5
BFP
1.0kb

FIGURE 22

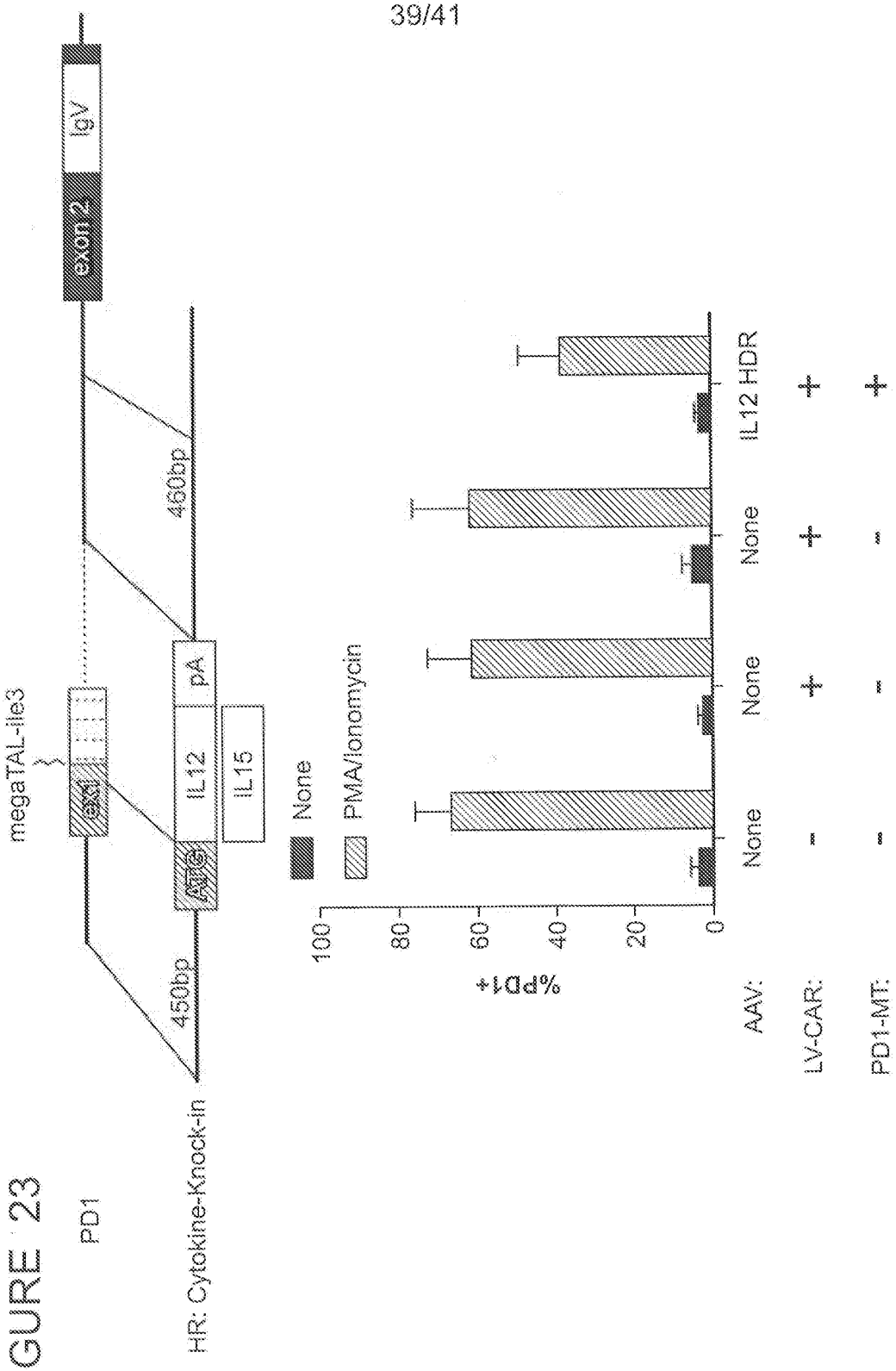
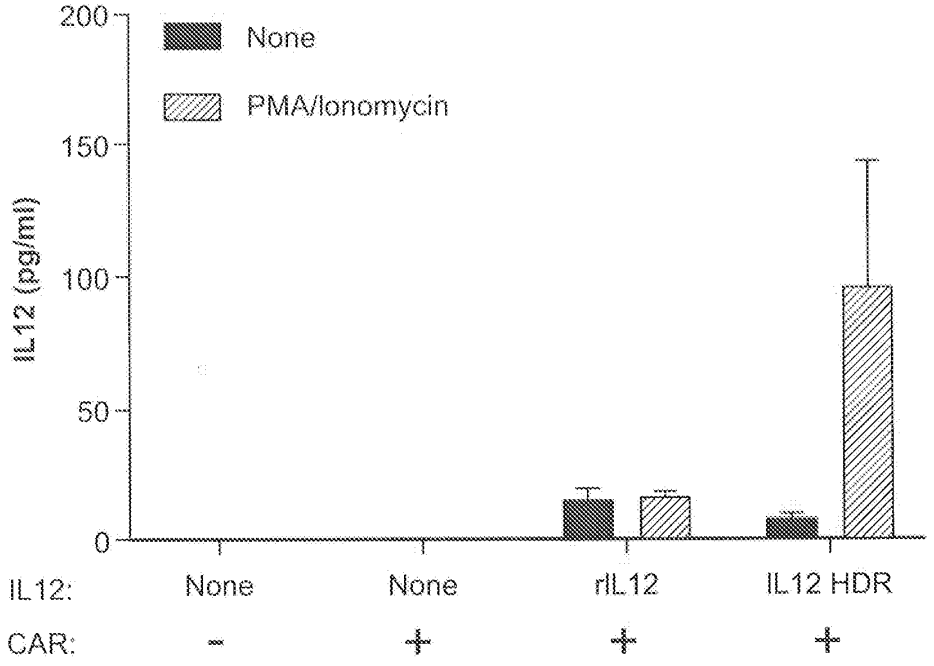

exon 2
IgV
460bp
megaTAL-ile3
ex1
IL12
pA
IL15
ATG
450bp
PD1
HR: Cytokine-Knock-in
None
PMA/Ionomycin
%PD1+
100
80
60
40
20
0
AAV:
LV-CAR:
PD1-MT:
None
None
None
IL12 HDR
-
+
-
+
-
+
-
+
+
+

FIGURE 23

FIGURE 24A


200
150
100
50
0
IL12 (pg/ml)
None
PMA/Ionomycin
IL12:
None
None
rIL12
IL12 HDR
CAR:
-
+
+
+

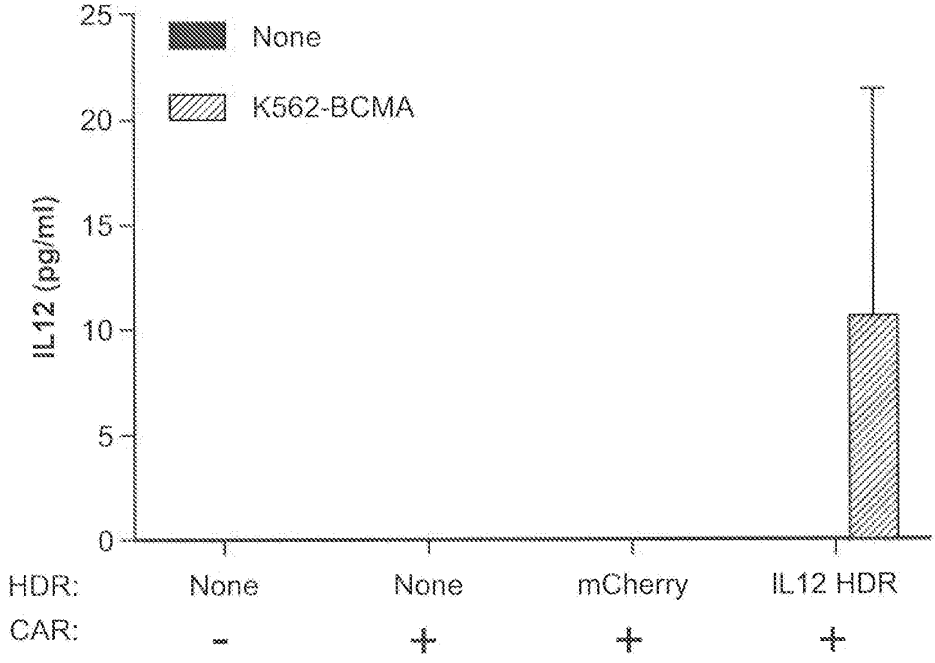
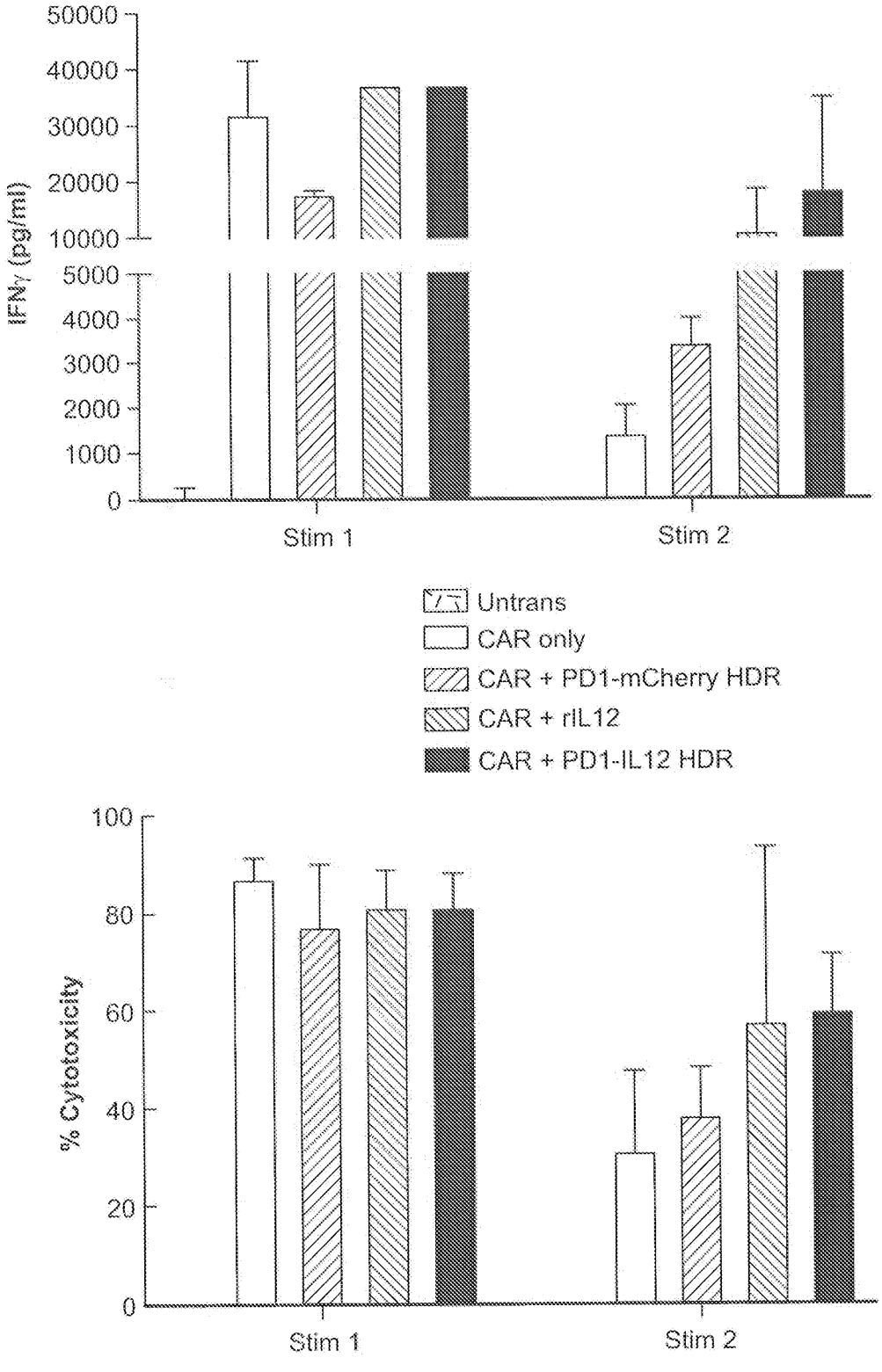

25
20
15
10
5
0
IL12 (pg/ml)
None
K562-BCMA
HDR:
None
None
mCherry
IL12 HDR
CAR:
-
+
+
+

FIGURE 24B


50000
40000
30000
20000
10000
5000
4000
3000
2000
1000
0
IFNγ (pg/ml)
Stim 1
Stim 2
Untrans
CAR only
CAR + PD1-mCherry HDR
CAR + rIL12
CAR + PD1-IL12 HDR
100
80
60
40
20
0
% Cytotoxicity
Stim 1
Stim 2