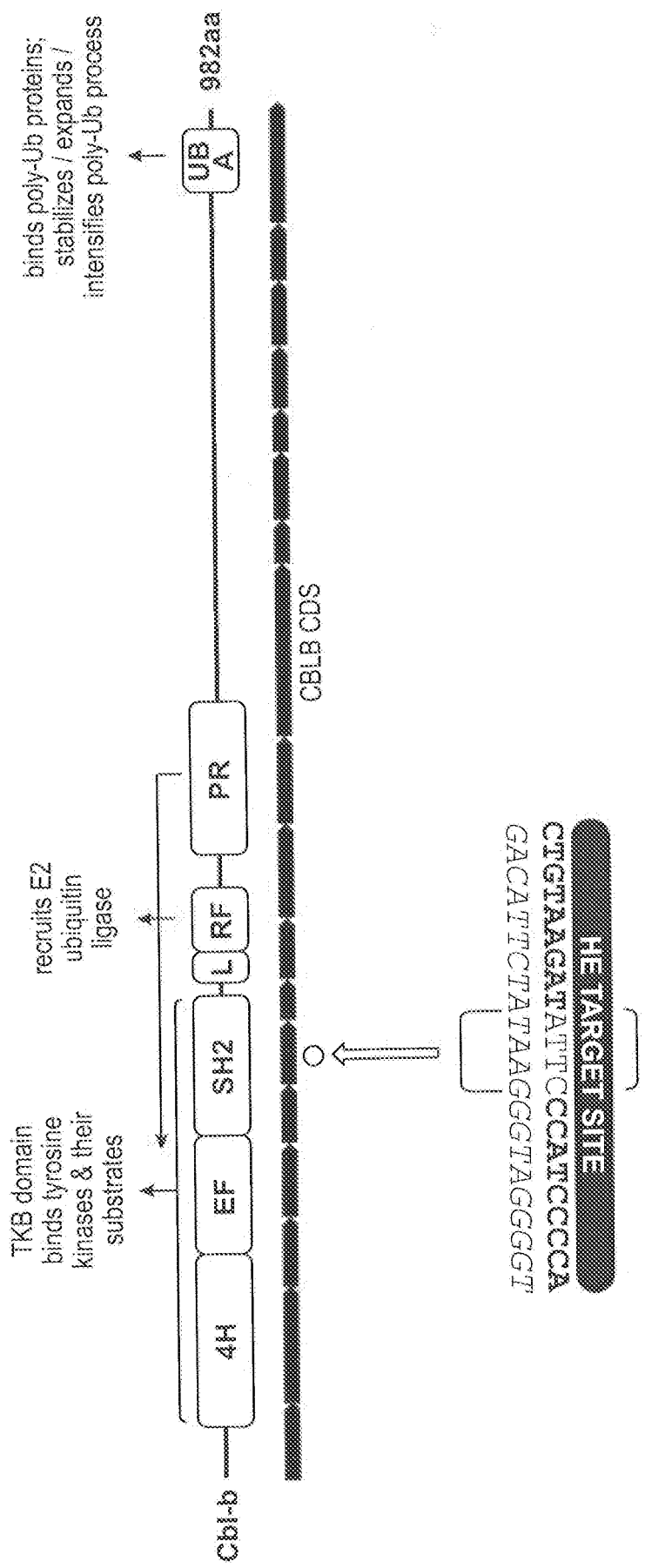
FIGURE 1


binds poly-Ub proteins;
stabilizes / expands /
intensifies poly-Ub process
UB
A
982aa
recruits E2
ubiquitin
ligase
PR
RF
L
SH2
EF
4H
TKB domain
binds tyrosine
kinases & their
substrates
Cbl-b
CBLB CDS
HE TARGET SITE
CTGTAAGATATTCCCATCCCCA
GACATTCTATAAGGGTAGGGGT

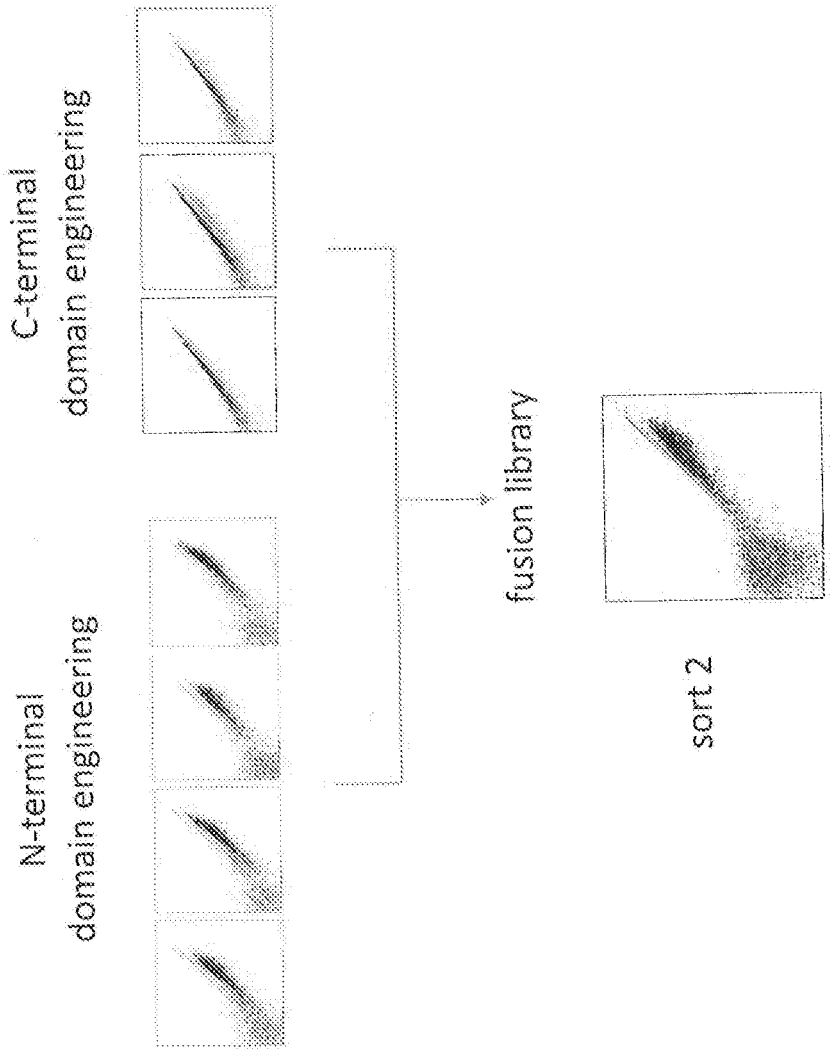
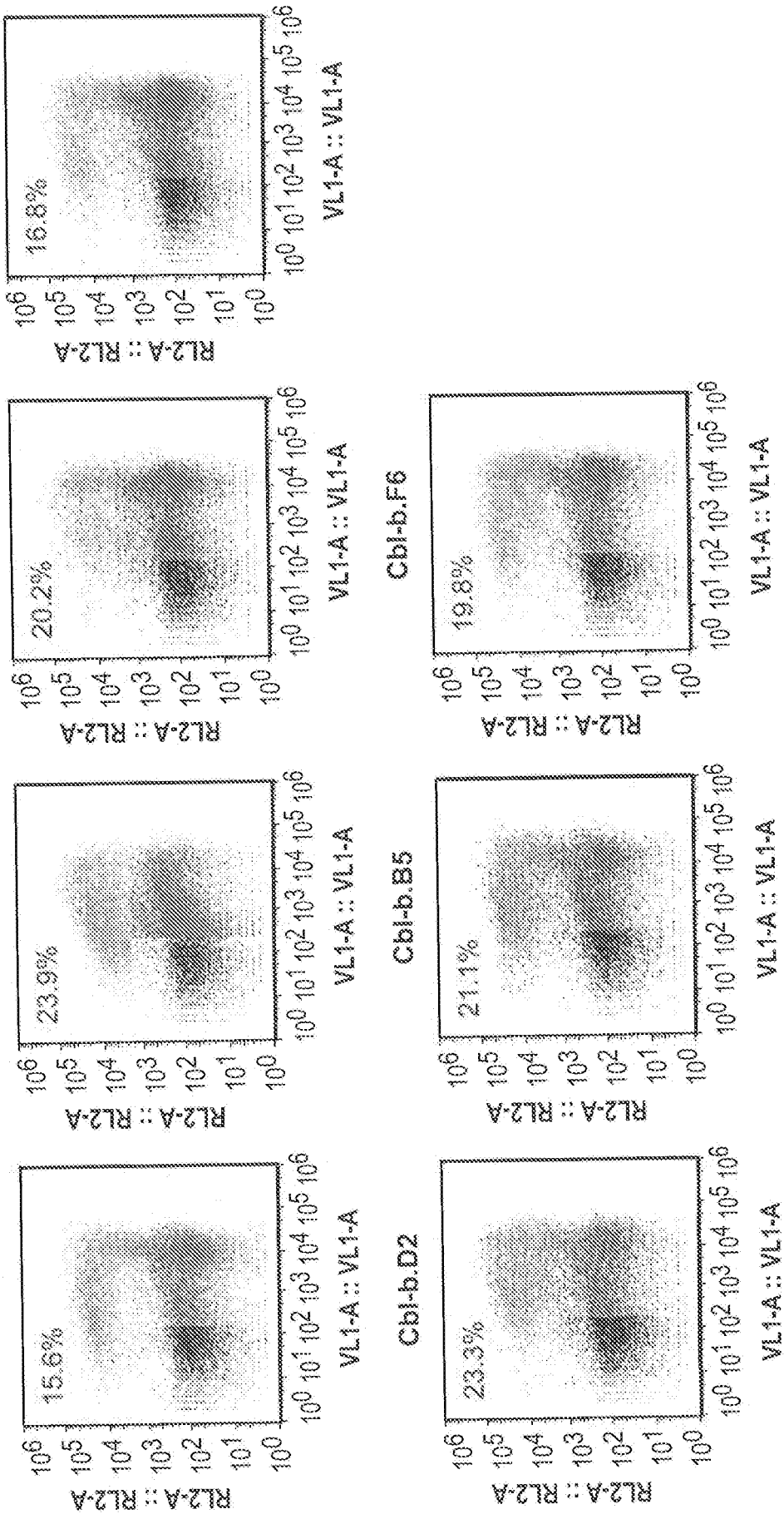

C-terminal
domain engineering
N-terminal
domain engineering
fusion library
sort 2

FIGURE 2

FIGURE 3


Cbl-b.E3
15.6%
Cbl-b.A8
23.9%
Cbl-b.C2
20.2%
Cbl-b.E6
16.8%
Cbl-b.D2
23.3%
Cbl-b.B5
21.1%
Cbl-b.F6
19.8%
RL2-A :: RL2-A
VL1-A :: VL1-A

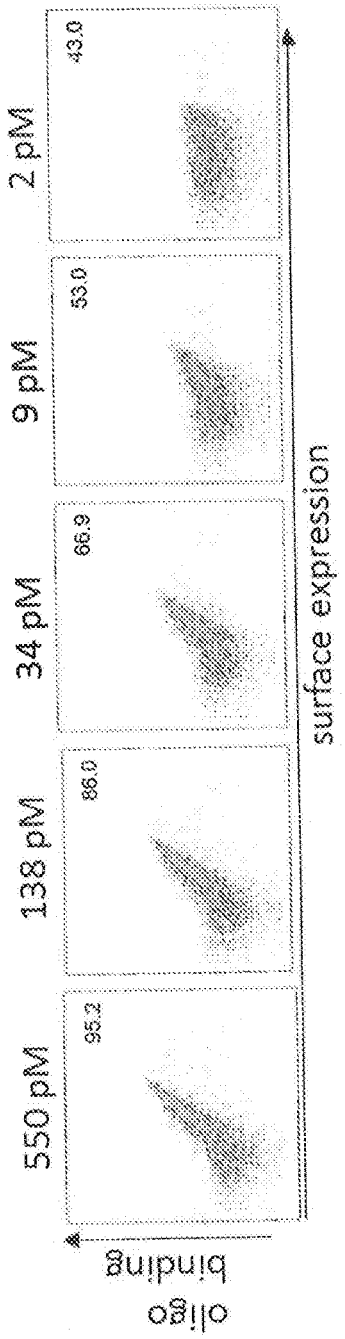

550 pM
138 pM
34 pM
9 pM
2 pM
95.2
86.0
66.9
53.0
43.0
oligo binding
surface expression

FIGURE 4

FIGURE 5

	1–70
(consensus)	SINPWILTGFADAEGCFRLDIRNANDLRAGYRTRLSFEIVLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
1. I-OnuI	SINPWILTGFADAEGSFLLRIRNNNKSSVGYSTELGFQITLHNKDKSILENIQSTWKVGVIANSGDNAVSL
2. Cblb.E3	SINPWILTGFADAEGCFRLDIRNANDLRAGYRTRLAFEIVLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
3. Cblb.F6	SINPWILTGFADAEGCFRLDIRNANDLRAGYRTRLAFEIVLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
4. Cblb.E6	SINPWILTGFADAEGCFRLDIRNANDLRAGYRTRLSFEISLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
5. Cblb.D2	SINPWILTGFADAEGCFRLDIHNANVLRSGYRTRLSFEIVLHNKDKSILENIQSTWKVGKIYNAGDNAVRL
6. Cblb.C2	SINPWILTGFADAEGCFGLYIHNSNVLRSGYRTRLSFEISLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
7. Cblb.B5	SINPWILTGFADAEGCFRLDIHNANVLRSGYRTRLSFEIVLHNKDKSILENIQSTWKVGTIYNAGDNAVRL
8. Cblb.A8	SINPWILTGFADAEGCFRLDIRNANDLRAGYRTRLAFEIVLHNKDKSILENIQSTWKVGTIYNAGDNAVRL

	80–140
(consensus)	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
1. I-OnuI	KVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKLNWGLTDELKKAF
2. Cblb.E3	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
3. Cblb.F6	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
4. Cblb.E6	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
5. Cblb.D2	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSLMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
6. Cblb.C2	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSLMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
7. Cblb.B5	QVTRFEDLKVIIDHFEKYPLITQKLGDYKLFKQAFSLMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF
8. Cblb.A8	QVTRFEDLKVIIGHFEKYPLITQKLGDYKLFKQAFSVMENKEHLKENGIKELVRIKAKMNWGLNDELKKAF

FIGURE 5 (cont.)

	150 ... 210
	PENISKERPLINKMIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC
1. I-OnuI	PENISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSKSKLGVQVQLVFSITQHIKDKNLMNSLITYLGC
2. Cblb.E3	PENISKERSLINKNIPNLKWLAGFTSGDGSFVVELKKRRSPVKVGVRLRFSITQHIRDKNLMNSLITYLGC
3. Cblb.F6	PENISKERPLINKNIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC
4. Cblb.E6	PENISKERPLINKNIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC
5. Cblb.D2	PENISKERPLINKNIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC
6. Cblb.C2	PENISKERPLINKNIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC
7. Cblb.B5	PENISKERPLINKNIPNLKWLAGFTSGDGSFVVELKKRRSPVKVGVRLRFGITQHIRDKNLMNSLITYLGC
8. Cblb.A8	PENISKERPLINKNIPNLKWLAGFTSGDGSFMVELMKNKNNVIVRVRLRFSISQHIRDKNLMNSLITYLGC

	220 ... 280
	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
1. I-OnuI	GYIKEKNKSEFSWLDFVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
2. Cblb.E3	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
3. Cblb.F6	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
4. Cblb.E6	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
5. Cblb.D2	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
6. Cblb.C2	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
7. Cblb.B5	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK
8. Cblb.A8	GRIVENNKSEHSWLEFIVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKKHLTESGLDEIKK

FIGURE 5 (cont.)

```
                                290       300
                                 |         |
                    IKLNMNKGRVFSGR**
                                290 293
                                 |   |
1. I-Onul    IKLNMNKGR
2. Cbib.E3   IKLNMNKGRVFSGR**
3. Cbib.F6   IKLNMNKGRVFSGR**
4. Cbib.E6   IKLNMNKGRVFSGR**
5. Cbib.D2   IKLNMNKGRVFSGR**
6. Cbib.C2   IKLNMNKGRVFSGR**
7. Cbib.B5   IKLNMNKGRVFSGR**
8. Cbib.A8   IKLNMNKGRVFSGR**
```

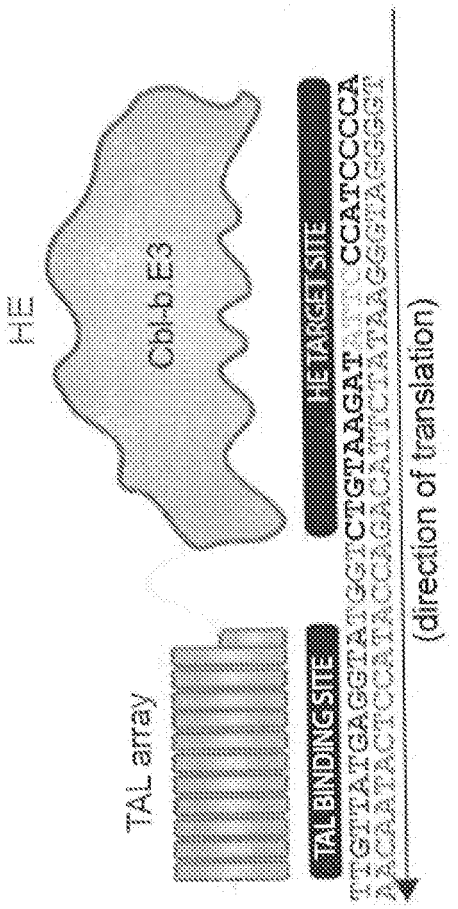

HE
Cbl-b E3
TAL array
TAL BINDING SITE
HETEROSITE
TTGTTATGAGGTATGGTCTGTAAGATTTCCATCCCCA
AACAATACTCCATACCAGACATTCTATAAGGGTAGGGT
(direction of translation)

FIGURE 6

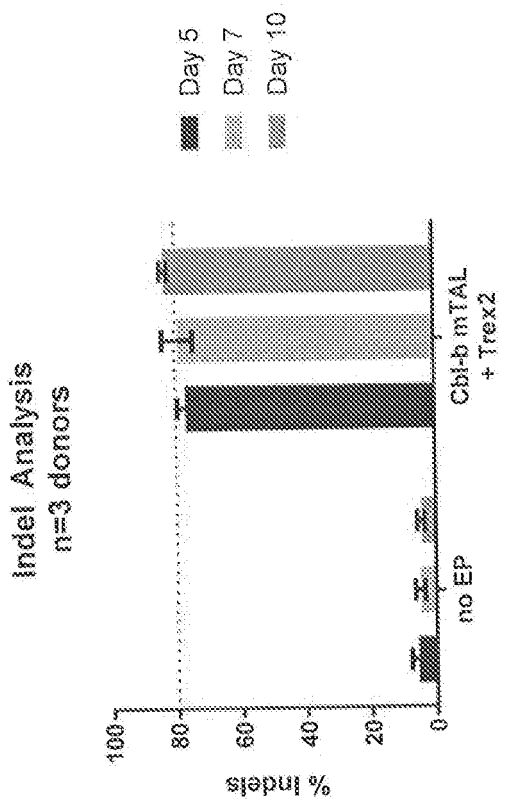

Indel Analysis
n=3 donors
Day 5
Day 7
Day 10
% Indels
100
80
60
40
20
0
no EP
Cbl-b mTAL
+ Trex2

FIGURE 7

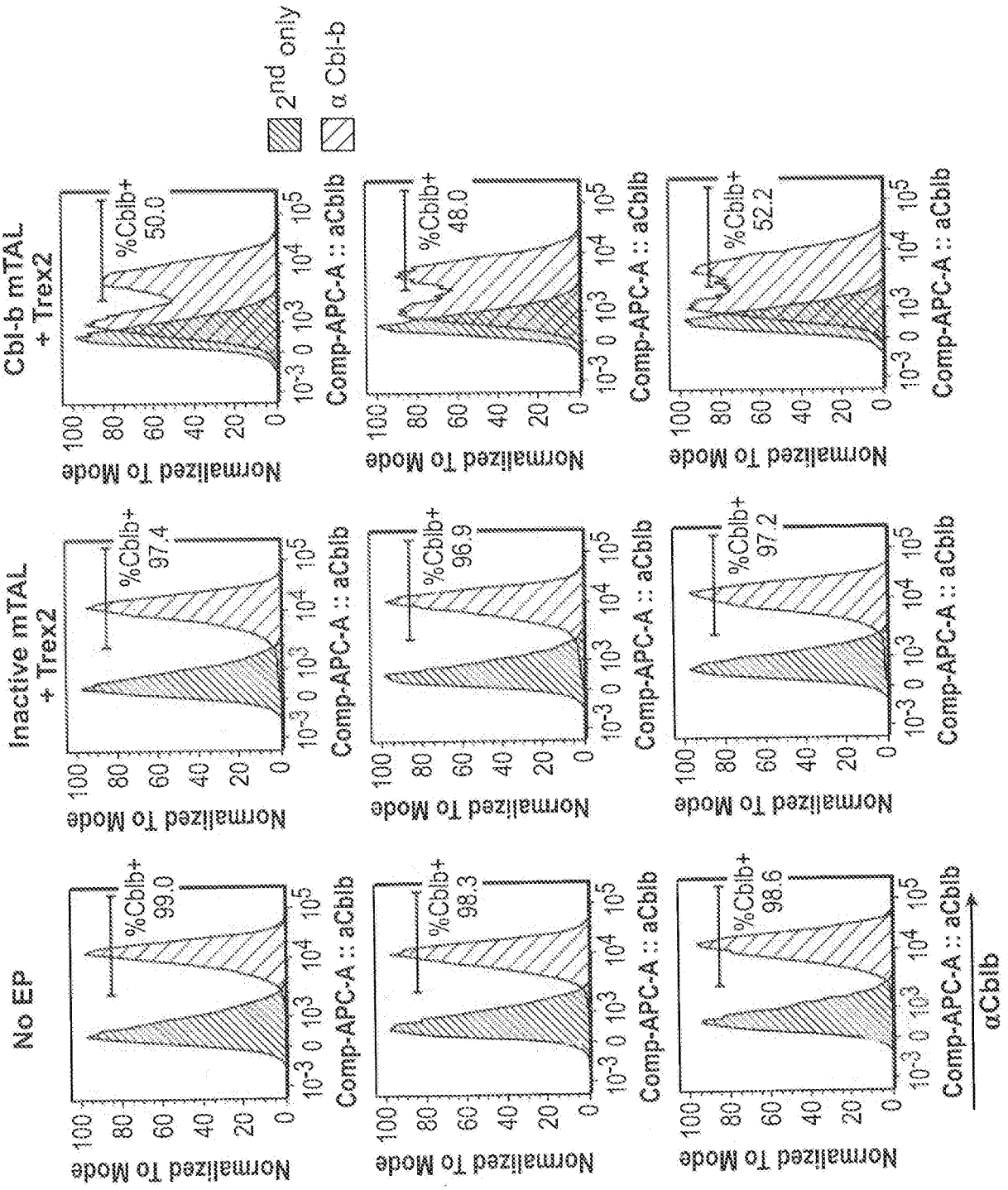
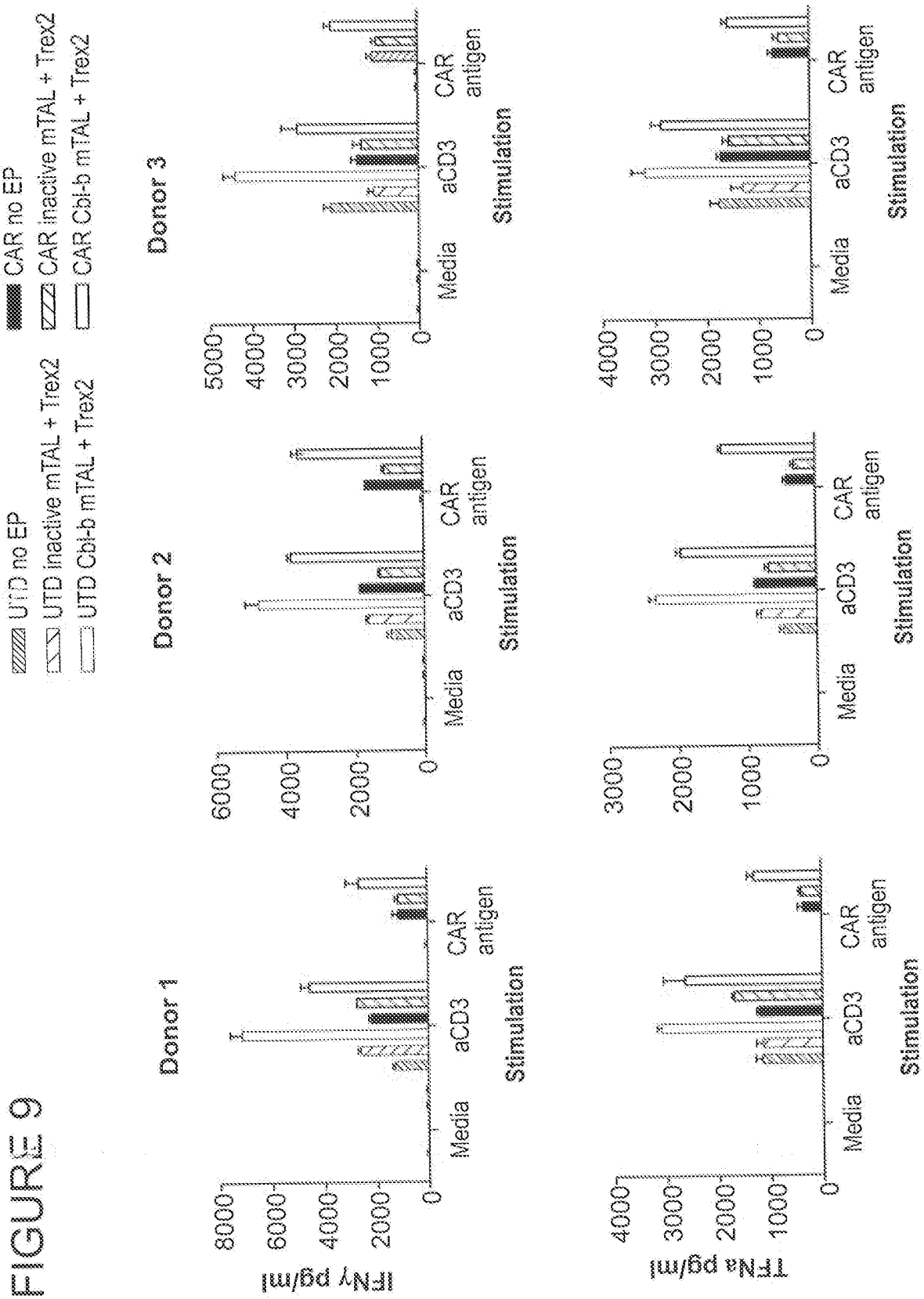

Donor
No EP
Inactive mTAL + Trex2
Cbl-b mTAL + Trex2
1
2
3
Normalized To Mode
Comp-APC-A :: aCblb
%Cblb+ 99.0
%Cblb+ 98.3
%Cblb+ 98.6
%Cblb+ 97.4
%Cblb+ 96.9
%Cblb+ 97.2
%Cblb+ 50.0
%Cblb+ 48.0
%Cblb+ 52.2
2nd only
α Cbl-b
αCblb

FIGURE 8

FIGURE 9


UTD no EP
UTD inactive mTAL + Trex2
UTD Cbl-b mTAL + Trex2
CAR no EP
CAR inactive mTAL + Trex2
CAR Cbl-b mTAL + Trex2
Donor 1
Donor 2
Donor 3
IFNγ pg/ml
TFNa pg/ml
Media
aCD3
CAR antigen
Stimulation

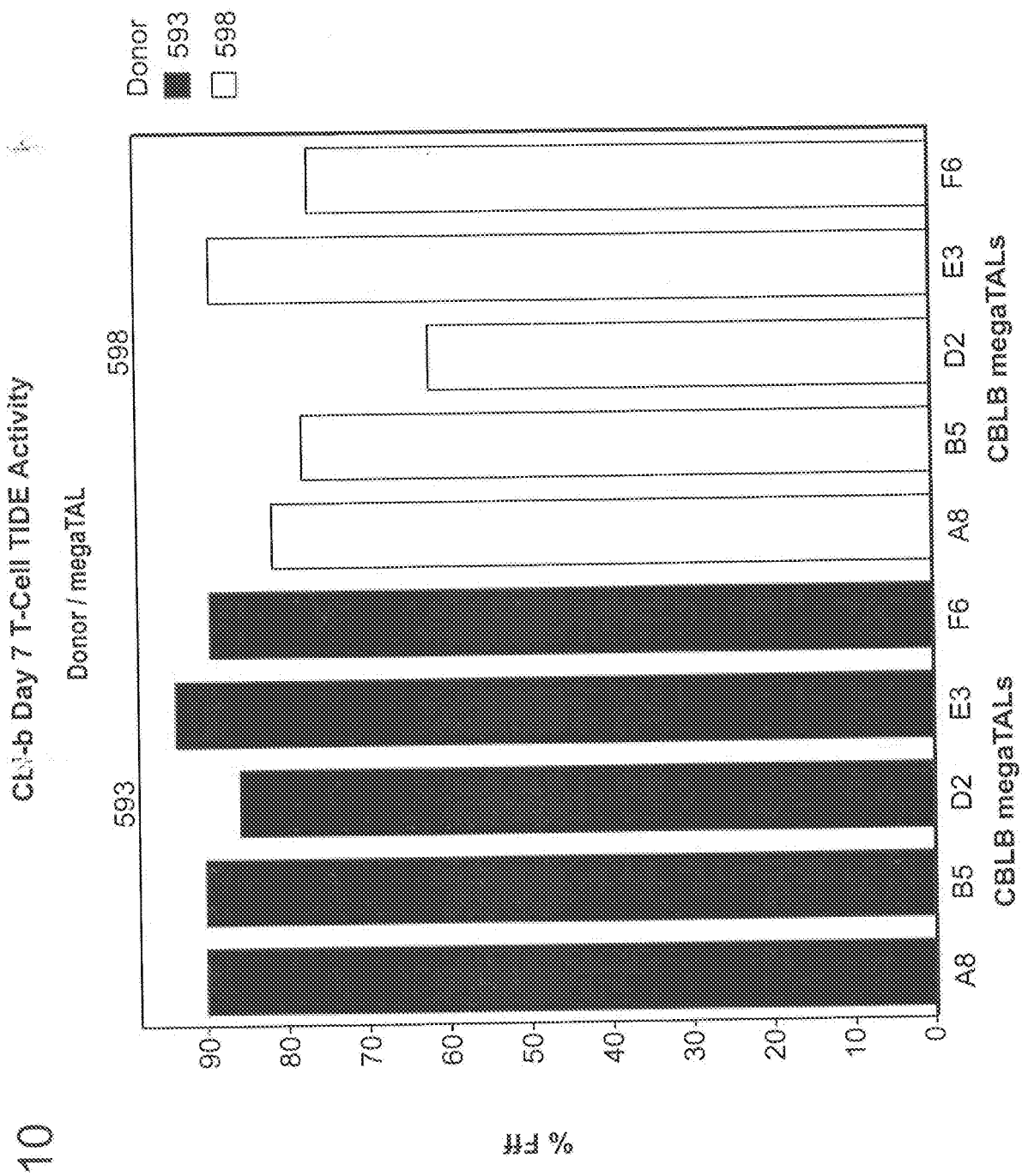

CBLB Day 7 T-Cell TIDE Activity
Donor / megaTAL
Donor
593
598
593
598
A8
B5
D2
E3
F6
A8
B5
D2
E3
F6
CBLB megaTALs
CBLB megaTALs
% Indels
0
10
20
30
40
50
60
70
80
90

FIGURE 10

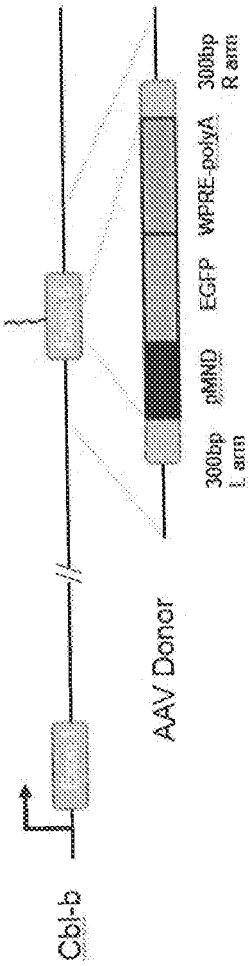


FIGURE 11

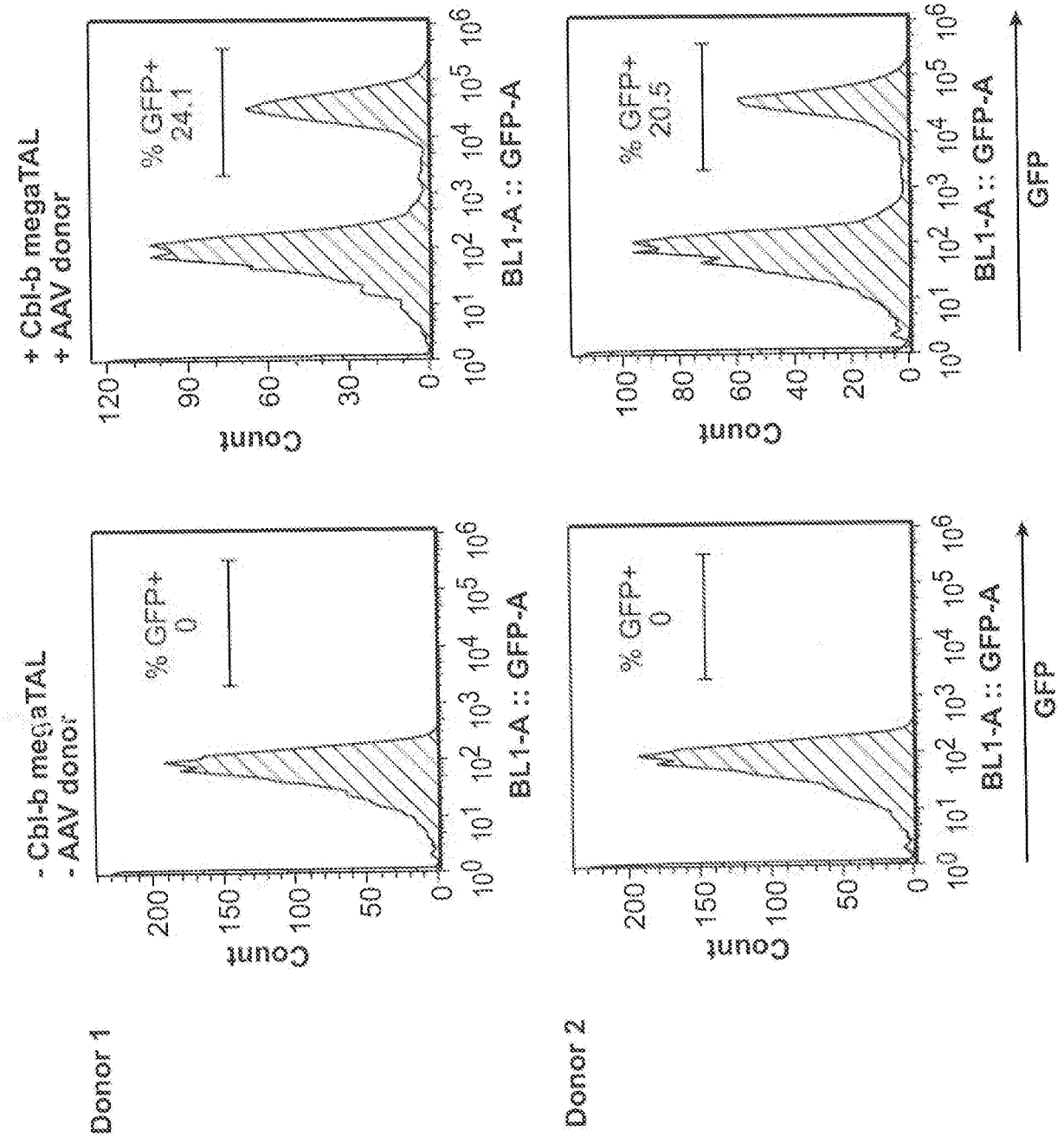

- Cbl-b megaTAL
- AAV donor
+ Cbl-b megaTAL
+ AAV donor
% GFP+
0
% GFP+
24.1
% GFP+
0
% GFP+
20.5
Count
BL1-A :: GFP-A
GFP
Donor 1
Donor 2

FIGURE 12

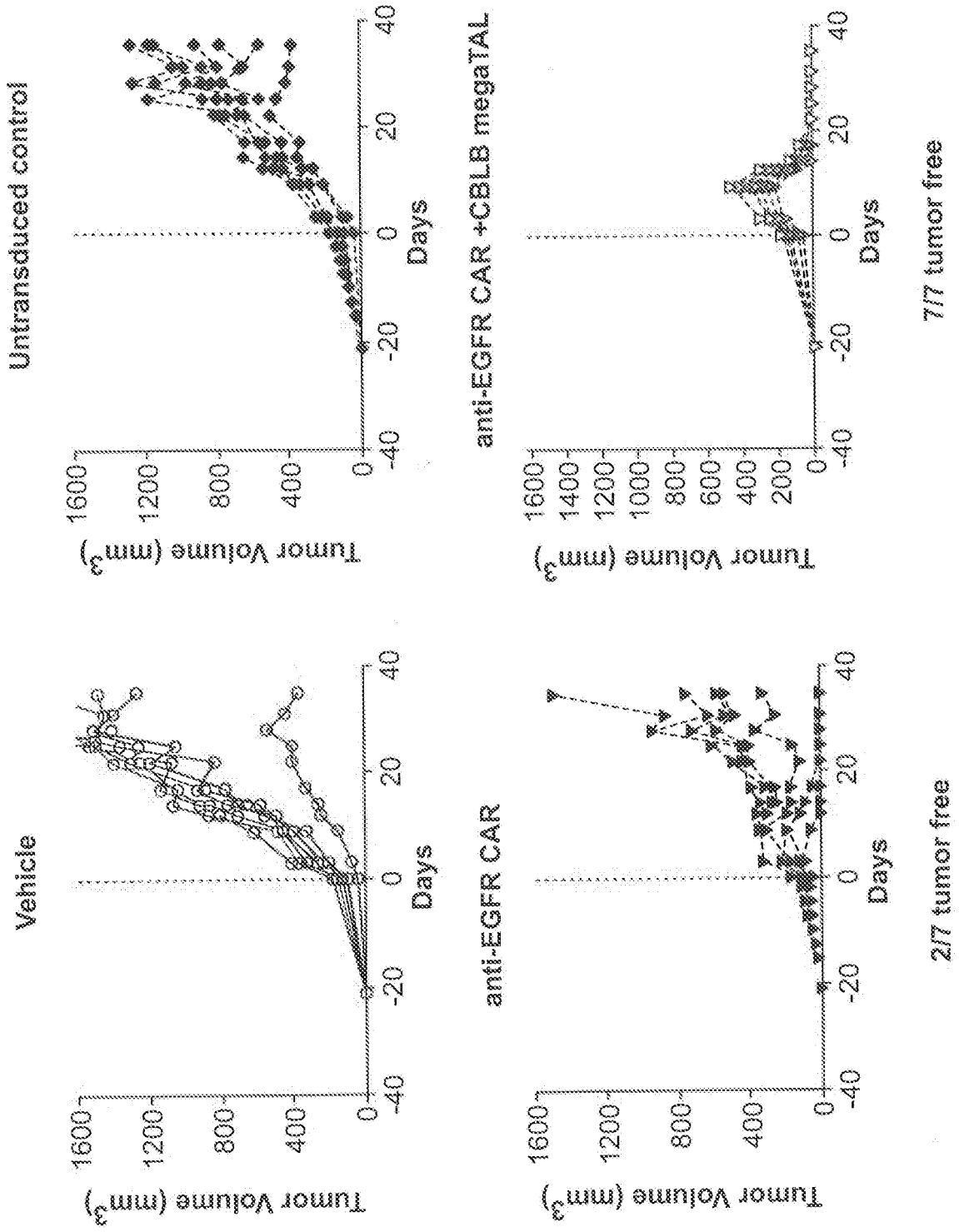

Untransduced control
Vehicle
anti-EGFR CAR +CBLB megaTAL
anti-EGFR CAR
Tumor Volume (mm3)
Days
7/7 tumor free
2/7 tumor free

FIGURE 13