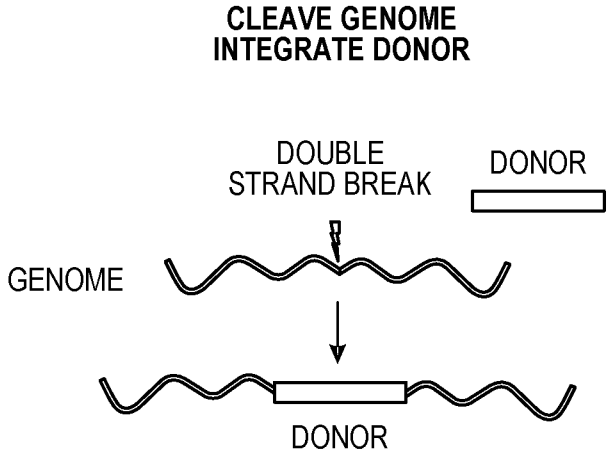

CLEAVE GENOME
INTEGRATE DONOR
DOUBLE
STRAND BREAK
DONOR
GENOME
DONOR

FIG. 1A

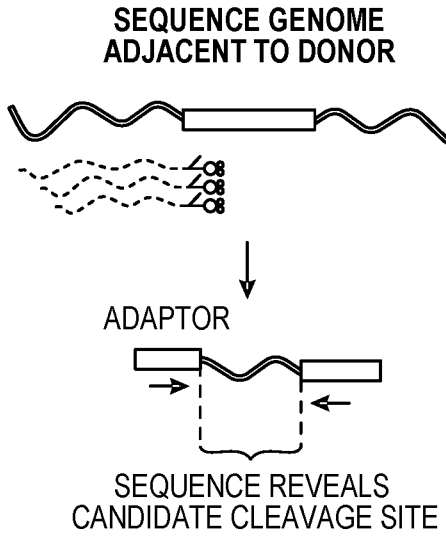

SEQUENCE GENOME
ADJACENT TO DONOR
ADAPTOR
SEQUENCE REVEALS
CANDIDATE CLEAVAGE SITE

FIG. 1B

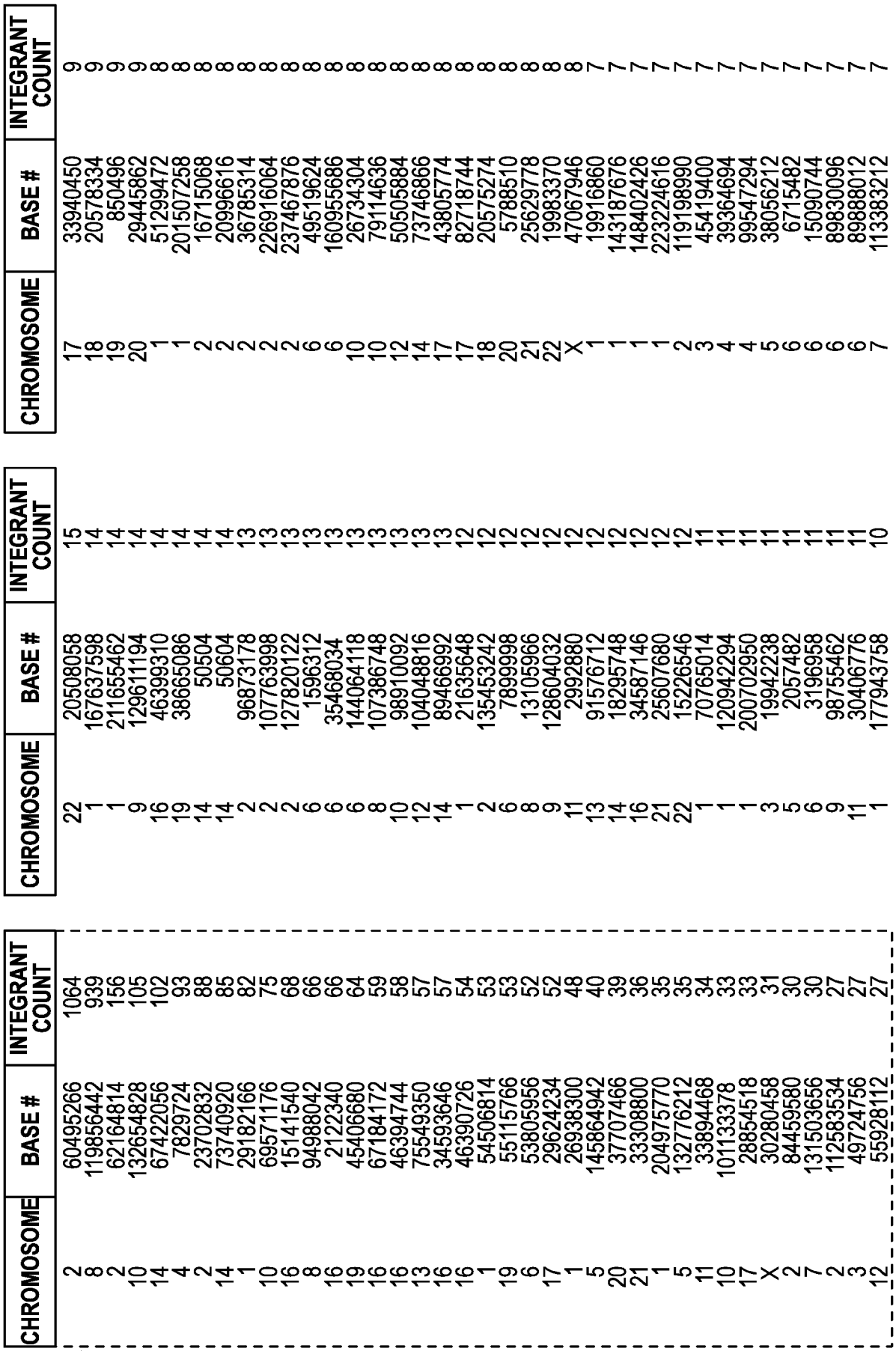
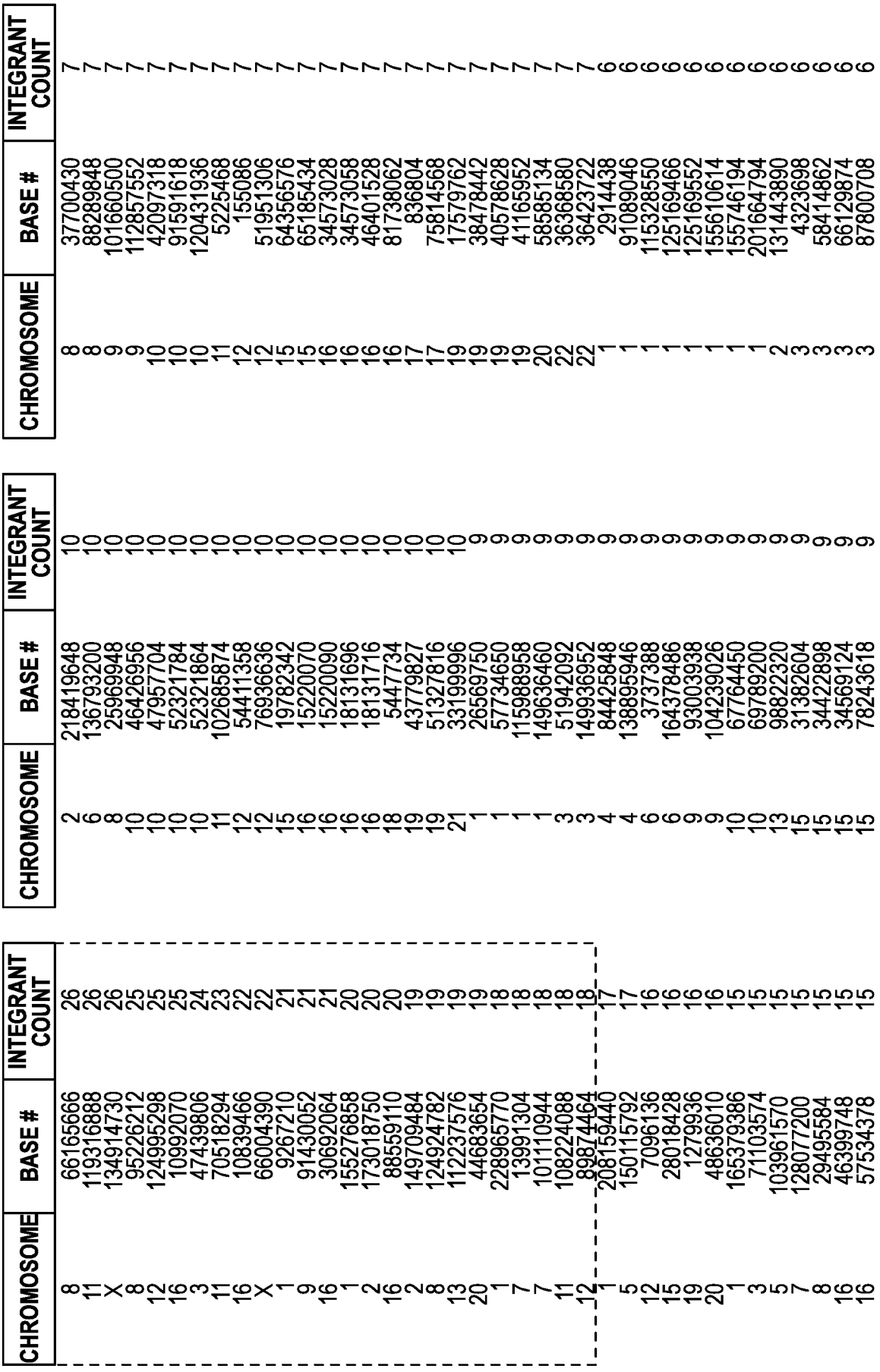

BCL11A
CHROMOSOME
BASE #
INTEGRANT COUNT
2 60495266 1064
8 119856442 939
2 62164814 156
10 132654828 105
14 67422056 102
4 7829724 93
2 23702832 88
14 73740920 85
1 29182166 82
10 69571176 75
16 15141540 68
8 94988042 66
16 2122340 66
19 45406680 64
16 67184172 59
16 46394744 58
13 75549350 57
16 34593646 57
16 46390726 54
1 54506814 53
19 55115766 53
6 53805956 52
17 29624234 52
1 26938300 48
5 145864942 40
20 37707466 39
21 33308800 36
1 20497570 35
5 132776212 35
11 33894468 34
10 101133378 33
17 28854518 33
X 30280458 31
2 84459580 30
7 131503656 30
2 112583534 27
3 49724756 27
12 55928112 27
22 20508058 15
1 167637598 14
1 211655462 14
9 129611194 14
16 46399310 14
19 38665086 14
14 50504 14
14 50604 14
2 96873178 13
2 107763998 13
2 127820122 13
6 1596312 13
6 35468034 13
6 144064118 13
8 107386748 13
10 98910092 13
12 104048816 13
14 89466992 13
1 21635648 12
2 135453242 12
6 7899998 12
8 13105966 12
9 128604032 12
11 2992880 12
13 91576712 12
14 18295748 12
16 34587146 12
21 25607680 12
22 15226546 12
1 70765014 11
1 120942294 11
1 200702950 11
3 19942238 11
5 2057482 11
6 3196958 11
9 98755462 11
11 30406776 11
1 177943758 10
17 33940450 9
18 20578334 9
19 850496 9
20 29445862 9
1 51299472 8
1 20150725 8
2 16715068 8
2 20996616 8
2 36785314 8
2 226916064 8
2 237467876 8
6 49519624 8
6 160955686 8
10 26734304 8
10 79114636 8
12 50505884 8
14 73746866 8
17 43805774 8
17 82718744 8
18 20575274 8
20 5788510 8
21 25629778 8
22 19983370 8
X 47067946 8
1 19916860 7
1 143187676 7
1 148402426 7
1 22322461 7
2 119198990 7
3 45419400 7
4 39364694 7
4 99547294 7
5 38056212 7
6 6715482 7
6 15090744 7
6 89830096 7
6 89888012 7
7 113383212 7

FIG. 2



CHROMOSOME	BASE #	INTEGRANT COUNT
8	66165666	26
11	119316888	26
X	134914730	26
8	95226212	25
12	124995298	25
16	10992070	25
3	47439806	24
11	70518294	23
16	10839466	22
X	66004390	22
1	9267210	21
9	91430052	21
16	30692064	21
1	155276858	20
2	173018750	20
16	88559110	20
2	149709484	19
8	124924782	19
13	112237576	19
20	44683654	19
1	228965770	18
7	13991304	18
7	101110944	18
11	108224088	18
12	89874464	18
1	208159440	17
5	150115792	17
12	7096136	16
15	28018428	16
19	1279936	16
20	48636010	16
1	165379386	15
3	71103574	15
5	103961570	15
7	128077200	15
8	29495584	15
16	46399748	15
16	57534378	15

CHROMOSOME	BASE #	INTEGRANT COUNT
2	218419648	10
6	136793200	10
8	25969948	10
10	46426956	10
10	47957704	10
10	52321784	10
10	52321864	10
11	102685874	10
12	54411358	10
12	76936636	10
15	19782342	10
16	15220070	10
16	15220090	10
16	18131696	10
16	18131716	10
18	5447734	10
19	43779827	10
19	51327816	10
21	33199996	10
1	26569750	9
1	57734650	9
1	115988958	9
1	149636460	9
3	51942092	9
3	149936952	9
4	84425848	9
4	138895946	9
6	3737388	9
6	164378486	9
9	93003938	9
9	104239026	9
10	67764450	9
10	69789200	9
13	98822320	9
15	31382604	9
15	34422898	9
15	34569124	9
15	78243618	9

CHROMOSOME	BASE #	INTEGRANT COUNT
8	37700430	7
8	88289848	7
9	101660500	7
9	112857552	7
10	42097318	7
10	91591618	7
10	120431936	7
11	5225468	7
12	155086	7
12	51951306	7
15	64356576	7
15	65185434	7
16	34573028	7
16	34573058	7
16	46401528	7
16	81738062	7
17	836804	7
17	75814568	7
19	17579762	7
19	38478442	7
19	40578628	7
19	41165952	7
20	58585134	7
22	36368580	7
22	36423722	7
1	2914438	6
1	91089046	6
1	115328550	6
1	125169466	6
1	125169552	6
1	155610614	6
1	155746194	6
1	201664794	6
2	131443890	6
3	4323698	6
3	58414862	6
3	66129874	6
3	87800708	6

FIG. 2 (cont.)

CHROMOSOME	BASE #	INTEGRANT COUNT
3	141228736	6
4	151031930	6
5	178121148	6
6	28931180	6
6	108893514	6
6	136088656	6
6	163725808	6
7	162658	6
7	35016604	6
8	30408876	6
8	91876290	6
8	119945566	6
9	40907722	6
9	104968230	6
10	72293352	6
10	79866034	6
11	118997014	6
11	121069264	6
12	3073480	6
12	26186106	6
13	43878548	6
15	41284346	6
15	58555840	6
15	60478340	6
16	3106742	6
16	4518046	6
16	29292606	6
16	77989246	6
16	88266652	6
16	88732066	6
17	36938710	6
19	267174	6
22	39690408	6
X	40259106	6
X	40835932	6
1	1724636	5
1	6161778	5
1	17419988	5
13	79775976	5
15	40114388	5
15	68307028	5
15	82539916	5
15	86572492	5
16	21645824	5
16	53042972	5
16	58873960	5
16	67841642	5
16	78808108	5
17	17025784	5
17	26884866	5
17	72902448	5
18	11456592	5
18	33538826	5
19	7159860	5
19	11922082	5
19	18462114	5
19	45686048	5
20	29881056	5
20	43558622	5
20	47575112	5
22	19545504	5
22	23284226	5
14	33684	5
1	6359676	4
1	18556502	4
1	24862574	4
1	25442520	4
1	30801948	4
1	51631166	4
1	72774624	4
1	109213944	4
1	153609522	4
1	175878760	4
1	201154009	4
1	224687886	4
2	137774808	4
9	9227906	4
9	97934932	4
9	121566378	4
9	124392210	4
9	126591182	4
9	138212048	4
10	25357460	4
10	41847448	4
10	41847504	4
10	42103514	4
10	64508254	4
10	76825618	4
10	109893420	4
11	35944902	4
11	72431102	4
12	6232920	4
12	7242432	4
12	49404046	4
12	75511410	4
12	88031364	4
12	95073610	4
12	113153372	4
13	18964478	4
13	34779902	4
13	113600728	4
14	92616966	4
15	33229354	4
15	64142544	4
15	67498752	4
15	75356538	4
15	76355710	4
15	684294	4
15	14285910	4
15	85317250	4
17	9710280	4
17	21372082	4
17	21906776	4
17	26603948	4

FIG. 2 (cont.)

CHROMOSOME	BASE #	INTEGRANT COUNT
1	36401004	5
1	143208542	5
1	150528982	5
1	154750274	5
2	86563402	5
2	105038552	5
2	117954094	5
2	130230372	5
2	176188548	5
3	2041446	5
3	16078092	5
3	75669968	5
3	178998880	5
3	184689238	5
4	49119414	5
4	73573948	5
4	78700116	5
5	7057496	5
5	11767140	5
5	56314802	5
5	132314286	5
6	58116958	5
6	60870558	5
7	128792974	5
7	133081754	5
9	92835866	5
9	131373156	5
10	50701042	5
10	87382350	5
11	49652236	5
11	61612468	5
11	62795536	5
11	67277754	5
11	82032970	5
11	88966978	5
12	6326346	5
12	104138422	5
12	121500124	5

CHROMOSOME	BASE #	INTEGRANT COUNT
2	166910124	4
2	234668446	4
3	49802584	4
3	195856500	4
3	198148214	4
4	1686620	4
4	3480800	4
4	99869180	4
4	138090616	4
4	158876704	4
5	10727038	4
5	36318878	4
5	36322678	4
5	65562414	4
5	100709408	4
5	142193948	4
5	149564992	4
5	164896712	4
6	176740968	4
6	2643624	4
6	27782516	4
6	45156450	4
6	146750040	4
6	169090710	4
7	1214740	4
7	37357350	4
7	37357420	4
7	62355868	4
7	71679460	4
7	106778616	4
7	133077880	4
8	5753270	4
8	5753290	4
8	8244554	4
8	8371950	4
8	101955394	4
8	119000446	4
9	38068006	4

CHROMOSOME	BASE #	INTEGRANT COUNT
17	26603992	4
17	40557500	4
17	43796376	4
17	49883740	4
18	13272880	4
18	30136646	4
18	30845084	4
18	74019902	4
19	40266218	4
19	42741976	4
19	42767202	4
19	42881190	4
19	49683046	4
20	4673782	4
20	24683868	4
20	44911634	4
20	48621130	4
20	49279476	4
20	49699412	4
20	56914278	4
20	62819166	4
21	10442640	4
22	22533154	4
22	22648222	4
22	22956152	4
22	23111094	4
22	23162790	4
22	24629320	4
22	32136074	4
22	45216526	4
X	61346666	4
X	39688532	4
X	52934730	4
X	71283192	4
X	90323678	4
X	106863278	4
Y	3900438	4

FIG. 2 (cont.)

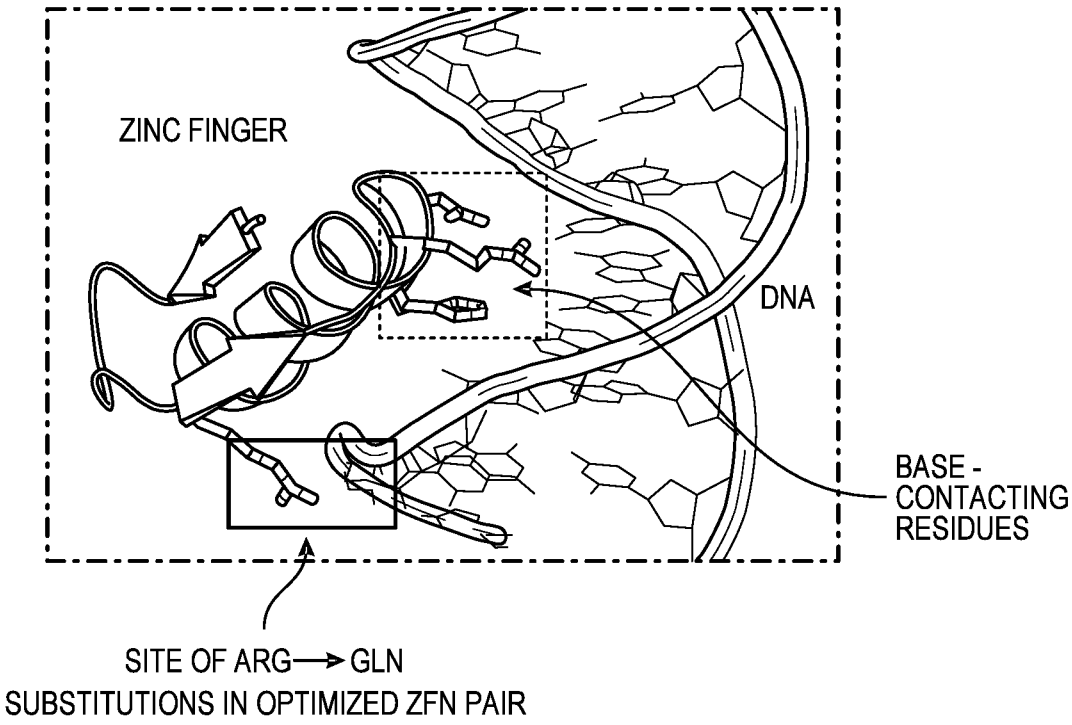

ZINC FINGER
DNA
BASE - CONTACTING RESIDUES
SITE OF ARG → GLN
SUBSTITUTIONS IN OPTIMIZED ZFN PAIR

FIG. 3A

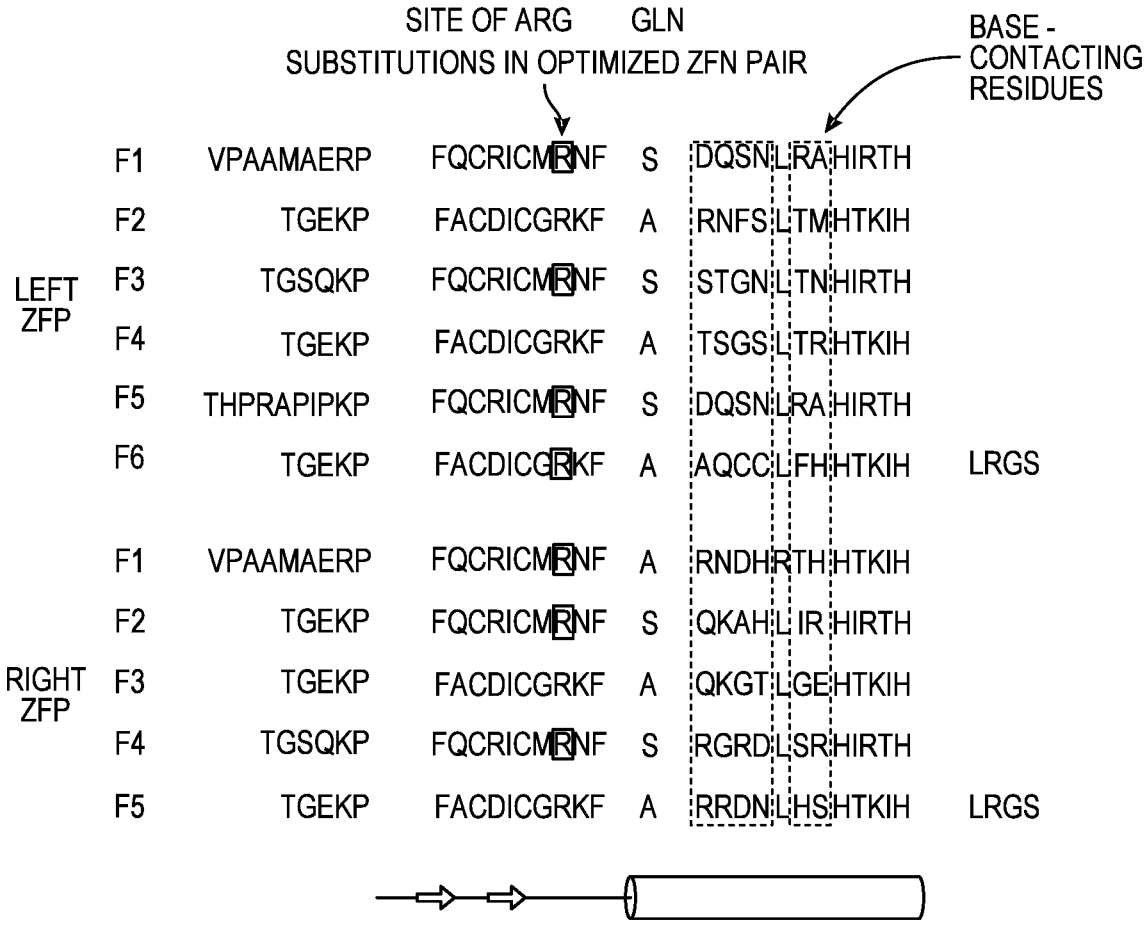

SITE OF ARG GLN
SUBSTITUTIONS IN OPTIMIZED ZFN PAIR
BASE - CONTACTING RESIDUES
LEFT ZFP
F1 VPAAMAERP FQCRICMRNF S DQSNLRAHIRTH
F2 TGEKP FACDICGRKF A RNFSLTMHTKIH
F3 TGSQKP FQCRICMRNF S STGNLTNHIRTH
F4 TGEKP FACDICGRKF A TSGSLTRHTKIH
F5 THPRAPIPKP FQCRICMRNF S DQSNLRAHIRTH
F6 TGEKP FACDICGRKF A AQCCLFHHTKIH LRGS
RIGHT ZFP
F1 VPAAMAERP FQCRICMRNF A RNDHRTHHTKIH
F2 TGEKP FQCRICMRNF S QKAHLIRHIRTH
F3 TGEKP FACDICGRKF A QKGTLGEHTKIH
F4 TGSQKP FQCRICMRNF S RGRDLSRHIRTH
F5 TGEKP FACDICGRKF A RRDNLHSHTKIH LRGS

FIG. 3B

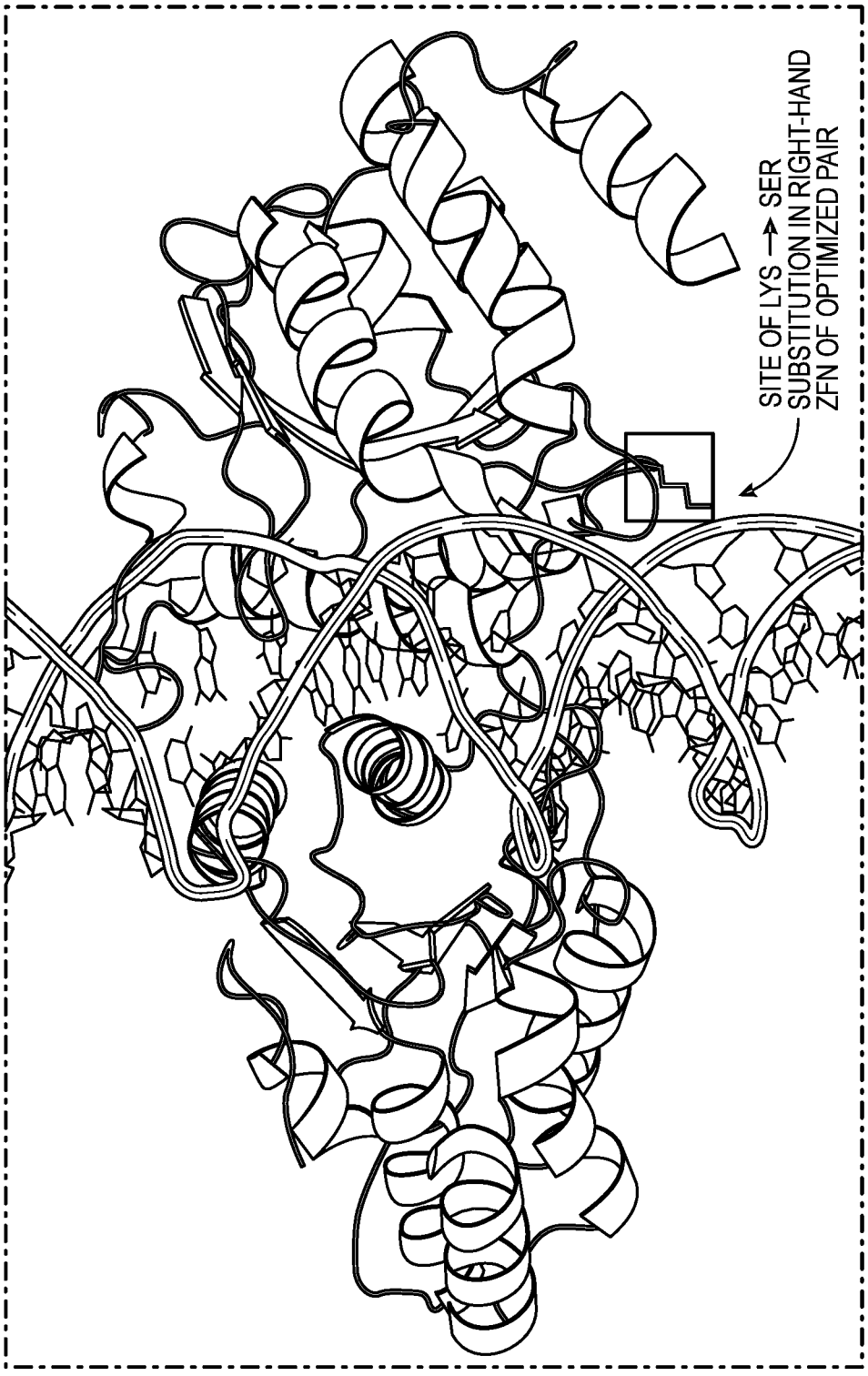

SITE OF LYS → SER
SUBSTITUTION IN RIGHT-HAND
ZFN OF OPTIMIZED PAIR

FIG. 3C

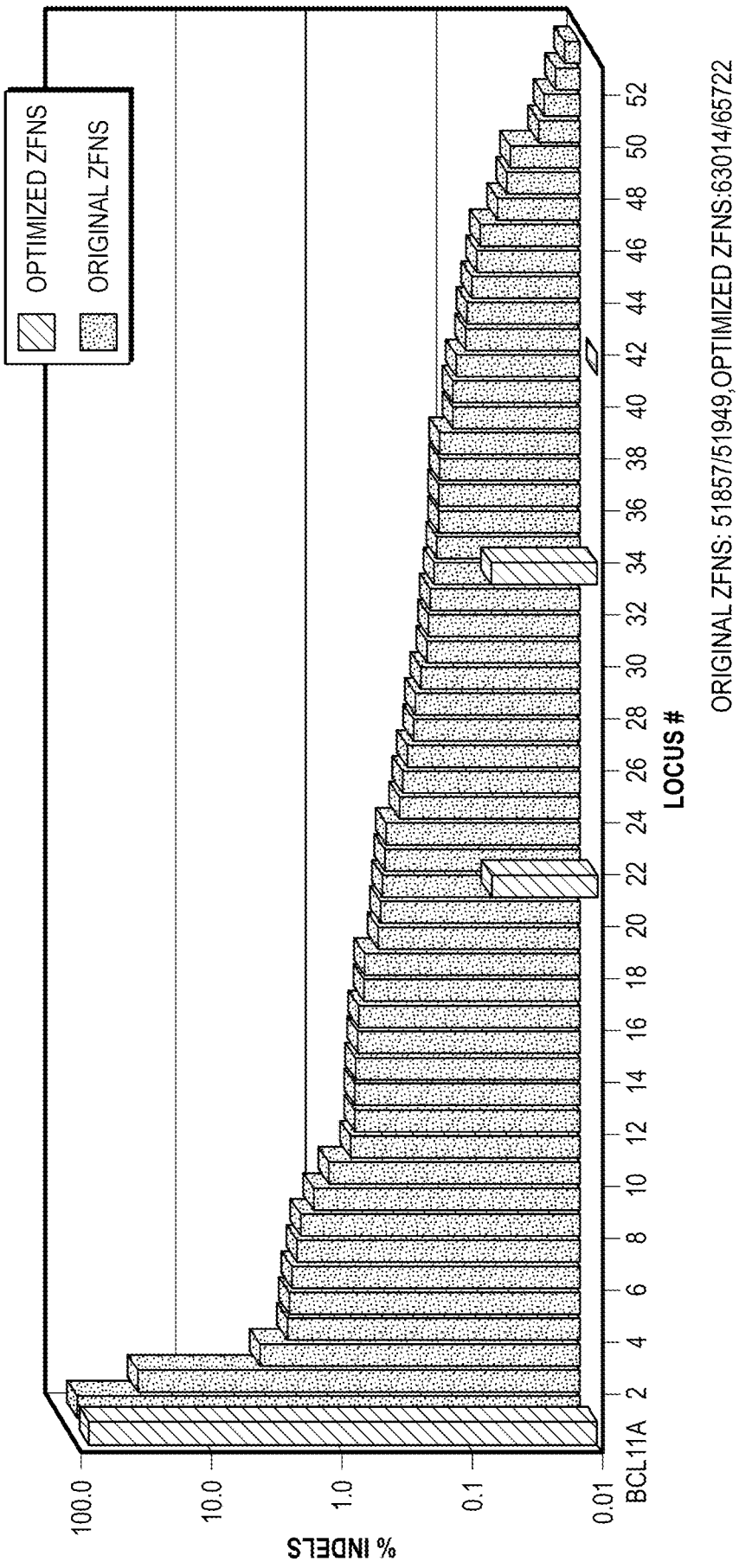

OPTIMIZED ZFNS
ORIGINAL ZFNS
% INDELS
100.0
10.0
1.0
0.1
0.01
BCL11A
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
LOCUS #
ORIGINAL ZFNS: 51857/51949,OPTIMIZED ZFNS:63014/65722

FIG. 4

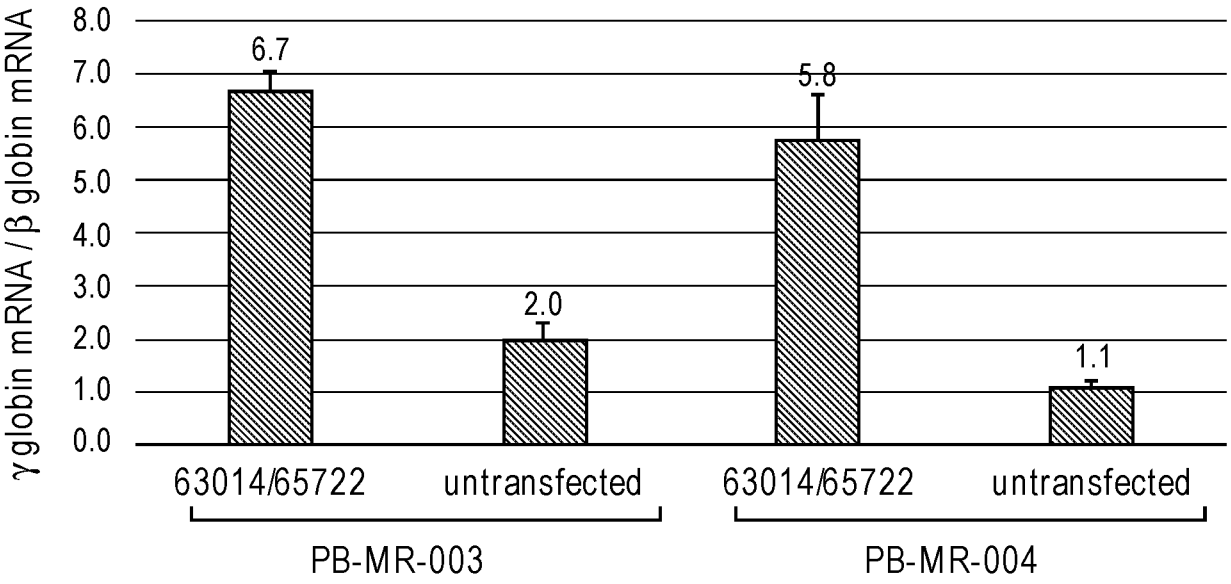

γ globin mRNA / β globin mRNA
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
6.7
2.0
5.8
1.1
63014/65722
untransfected
63014/65722
untransfected
PB-MR-003
PB-MR-004

FIG. 5A

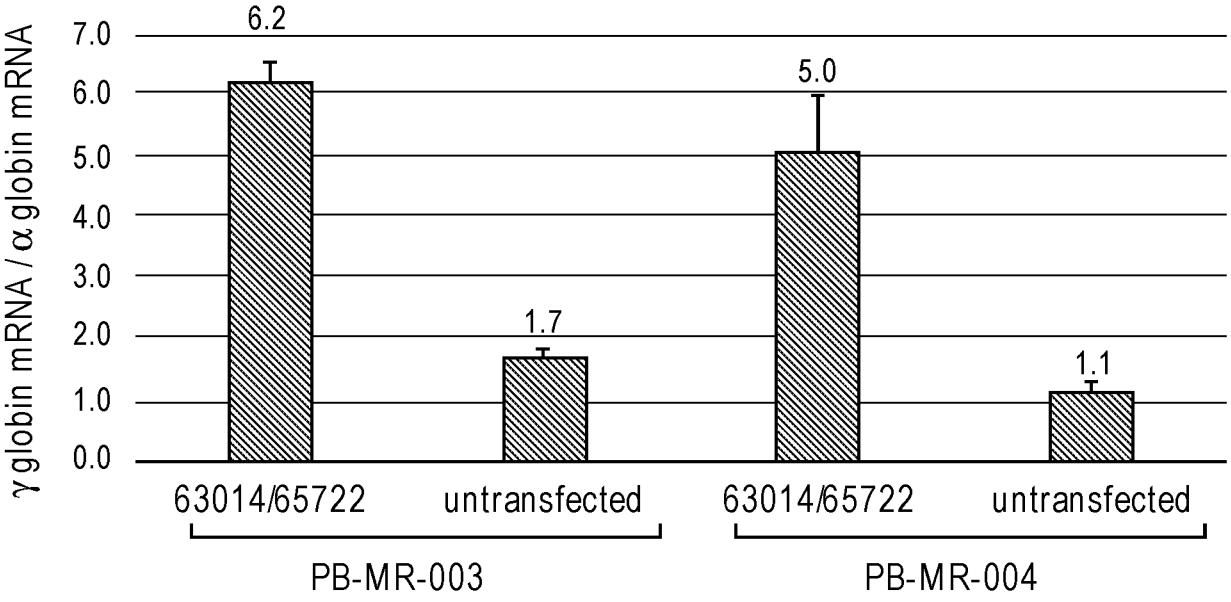

γ globin mRNA / α globin mRNA
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
6.2
1.7
5.0
1.1
63014/65722
untransfected
63014/65722
untransfected
PB-MR-003
PB-MR-004

FIG. 5B

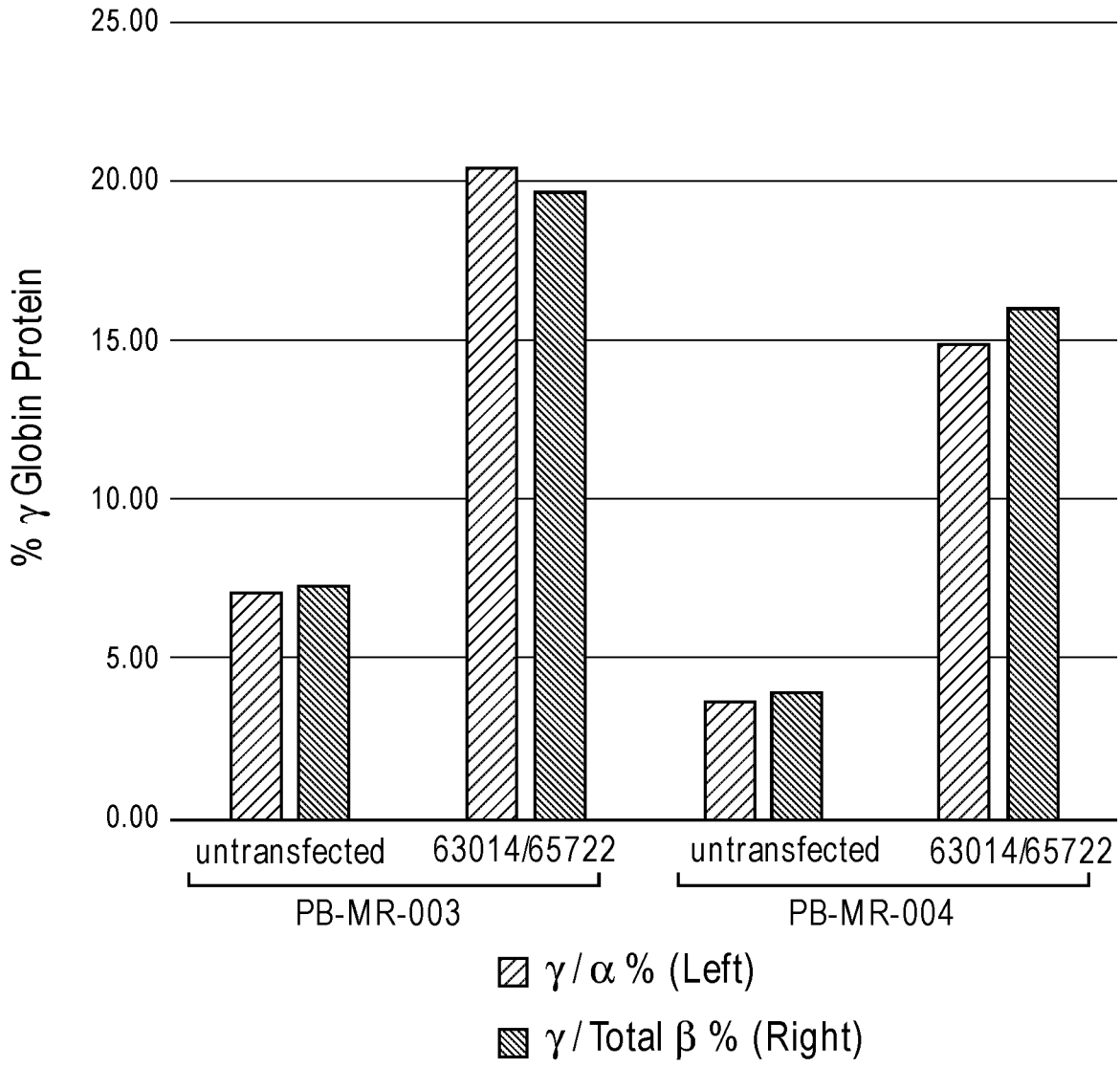

25.00
20.00
15.00
10.00
5.00
0.00
% γ Globin Protein
untransfected
63014/65722
untransfected
63014/65722
PB-MR-003
PB-MR-004
γ / α % (Left)
γ / Total β % (Right)

FIG. 6

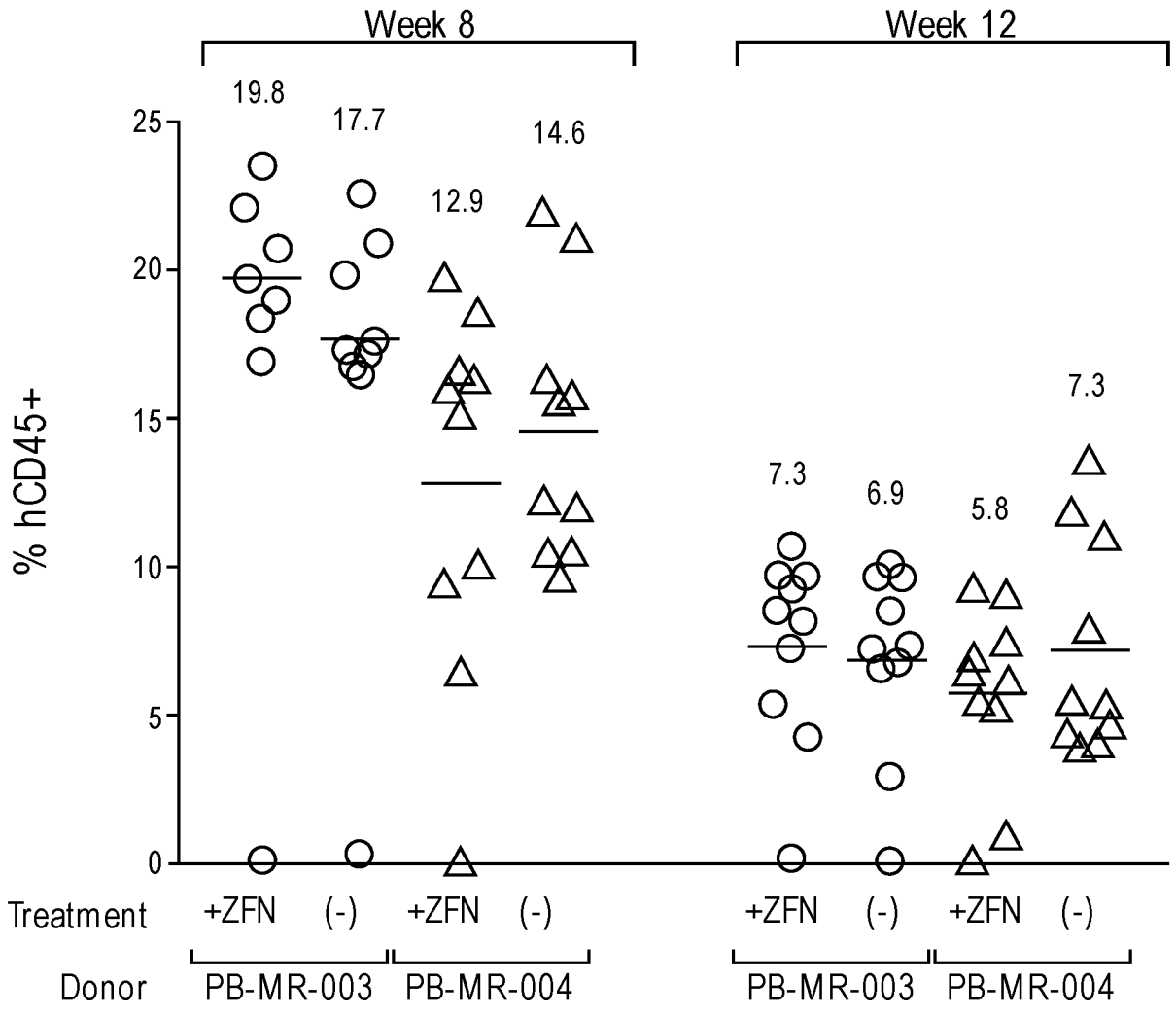

Week 8
Week 12
19.8
17.7
14.6
12.9
7.3
6.9
5.8
7.3
25
20
15
10
5
0
% hCD45+
Treatment
+ZFN
(-)
+ZFN
(-)
+ZFN
(-)
+ZFN
(-)
Donor
PB-MR-003
PB-MR-004
PB-MR-003
PB-MR-004

FIG. 7

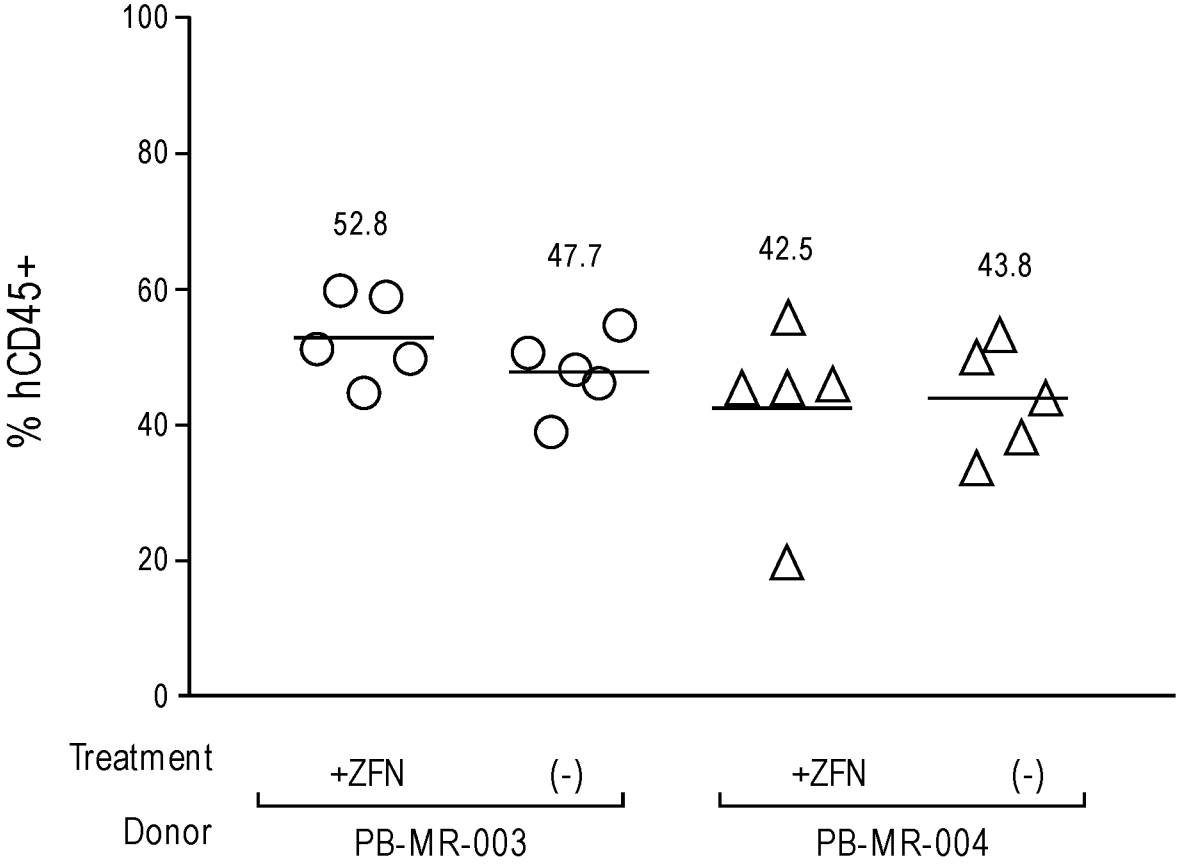

100
80
60
40
20
0
% hCD45+
52.8
47.7
42.5
43.8
Treatment
+ZFN
(-)
+ZFN
(-)
Donor
PB-MR-003
PB-MR-004

FIG. 8

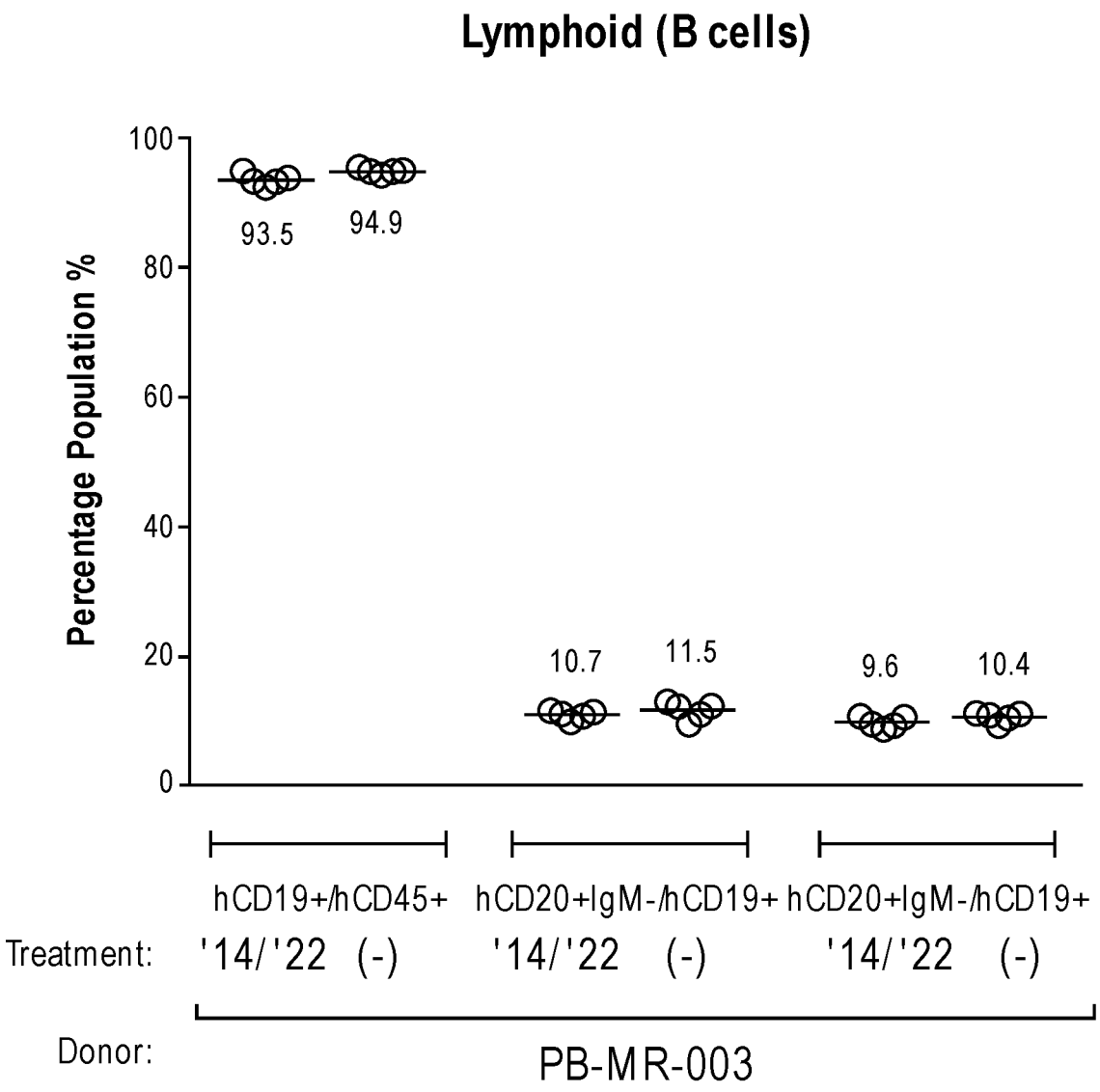

Lymphoid (B cells)
Percentage Population %
100
80
60
40
20
0
93.5
94.9
10.7
11.5
9.6
10.4
hCD19+/hCD45+
hCD20+IgM-/hCD19+
hCD20+IgM-/hCD19+
Treatment:
'14/'22 (-)
'14/'22 (-)
'14/'22 (-)
Donor:
PB-MR-003

FIG. 9A

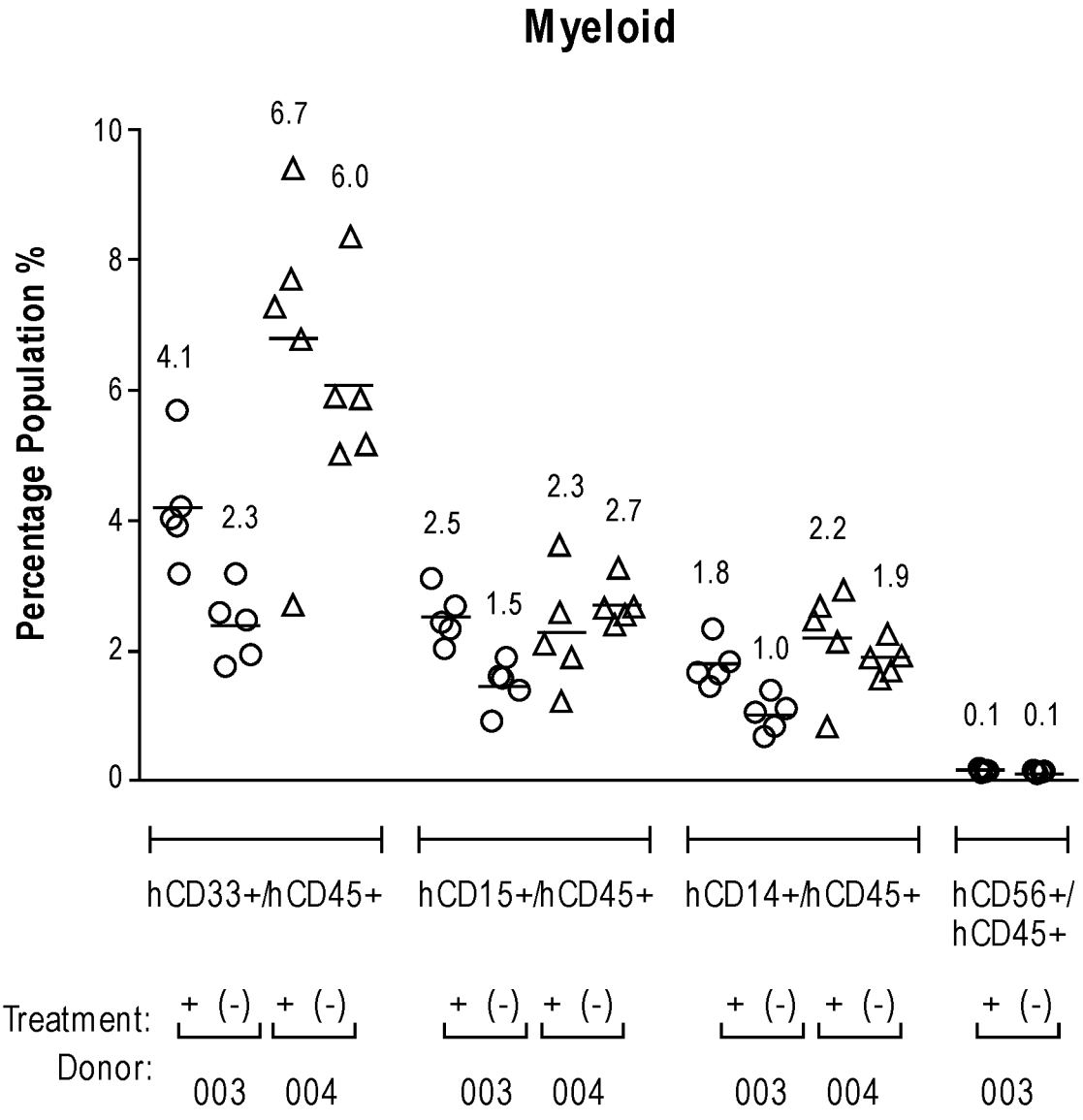

Myeloid
Percentage Population %
10
8
6
4
2
0
6.7
6.0
4.1
2.3
2.5
1.5
2.3
2.7
1.8
1.0
2.2
1.9
0.1
0.1
hCD33+/hCD45+
hCD15+/hCD45+
hCD14+/hCD45+
hCD56+/
hCD45+
Treatment:
+ (-) + (-)
+ (-) + (-)
+ (-) + (-)
+ (-)
Donor:
003 004
003 004
003 004
003

FIG. 9B

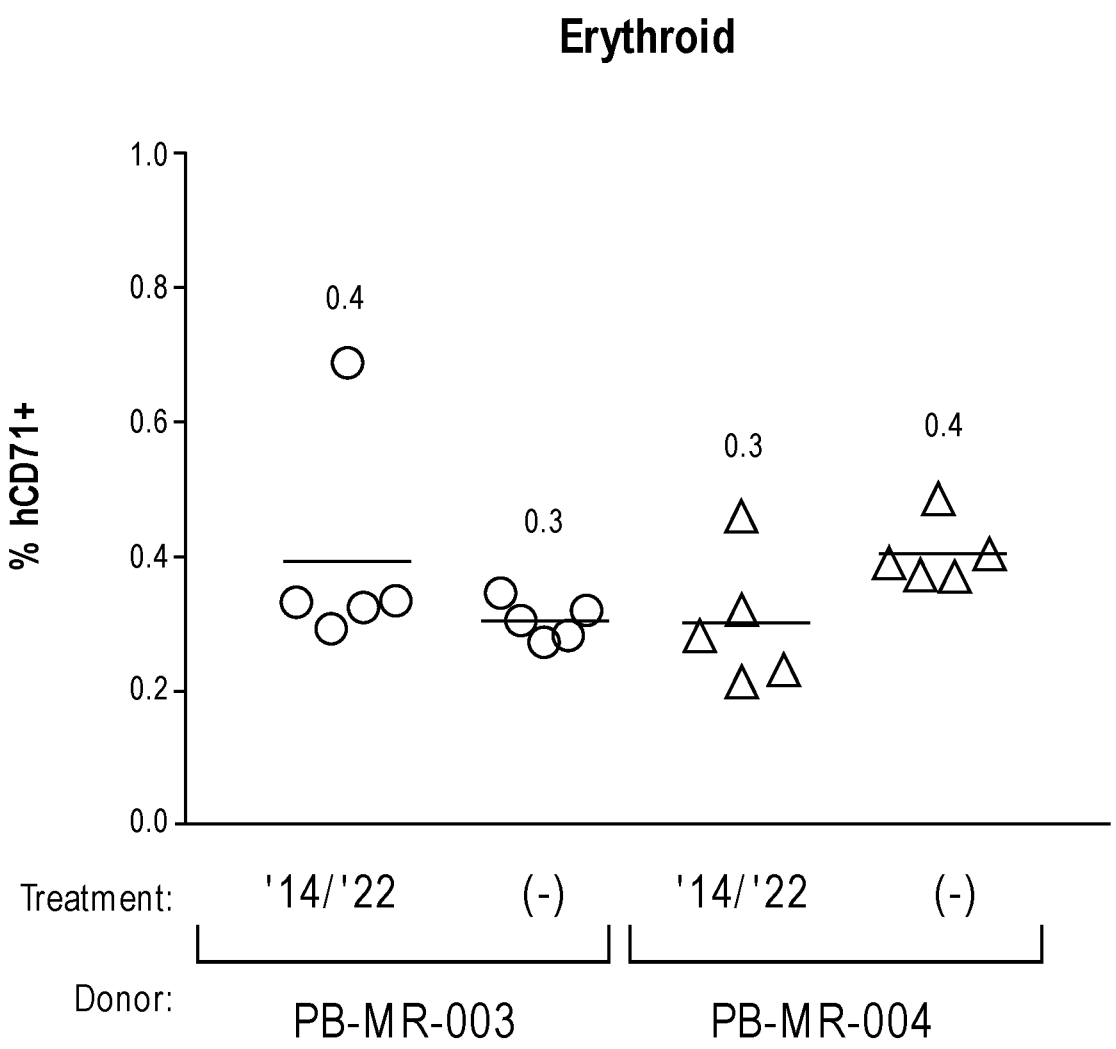

Erythroid
% hCD71+
1.0
0.8
0.6
0.4
0.2
0.0
0.4
0.3
0.3
0.4
Treatment:
'14/'22
(-)
'14/'22
(-)
Donor:
PB-MR-003
PB-MR-004

FIG. 9C

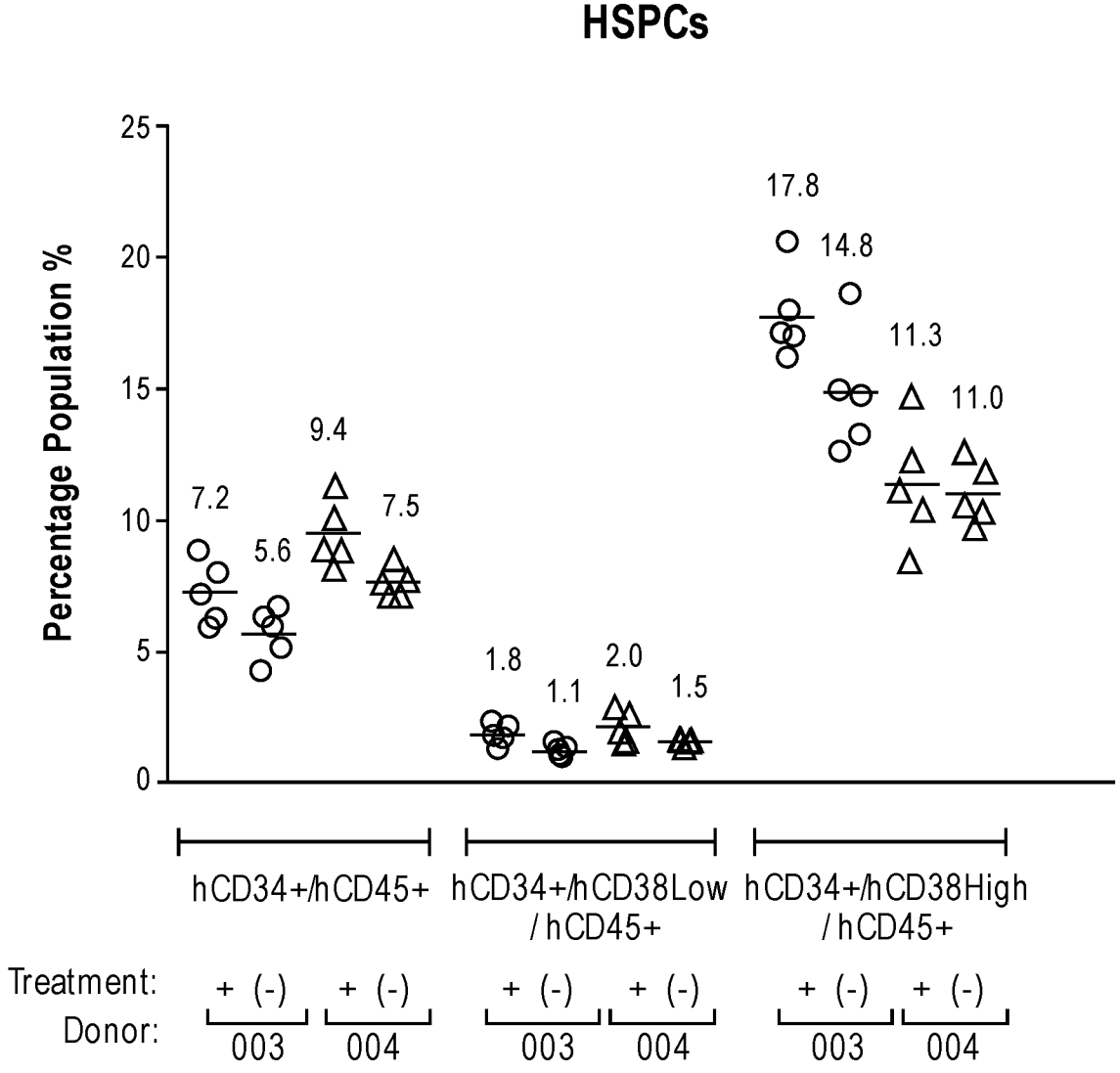

HSPCs
Percentage Population %
25
20
15
10
5
0
7.2
5.6
9.4
7.5
1.8
1.1
2.0
1.5
17.8
14.8
11.3
11.0
hCD34+/hCD45+
hCD34+/hCD38Low /hCD45+
hCD34+/hCD38High /hCD45+
Treatment:
+ (-) + (-) + (-) + (-) + (-) + (-)
Donor:
003 004 003 004 003 004

FIG. 9D

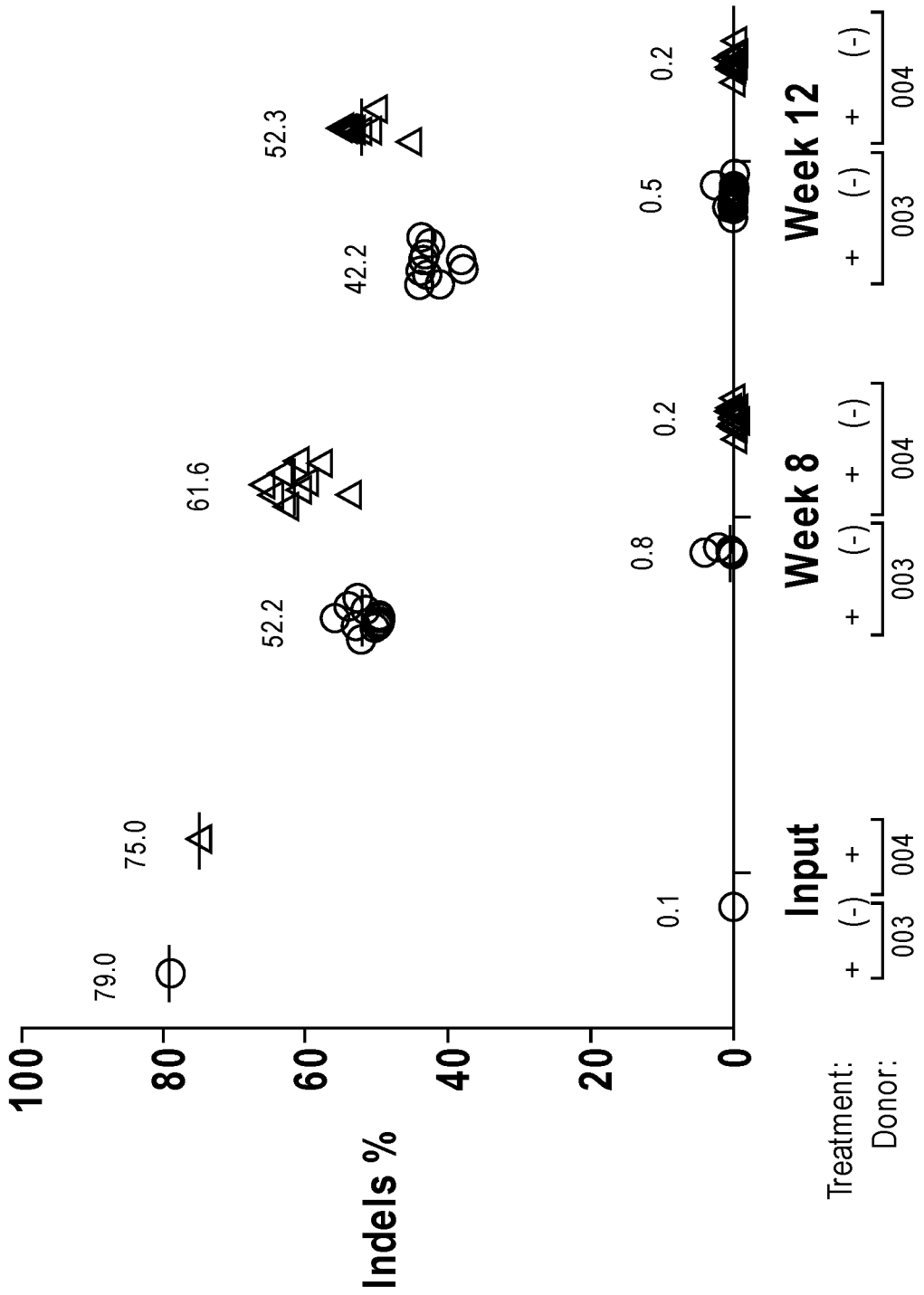

Indels %
100
80
60
40
20
0
79.0
75.0
61.6
52.2
52.3
42.2
0.1
0.8
0.2
0.5
0.2
Input
Week 8
Week 12
Treatment:
Donor:
+ (-) +
003 004
+ (-) + (-)
003 004
+ (-) + (-)
003 004

FIG. 10

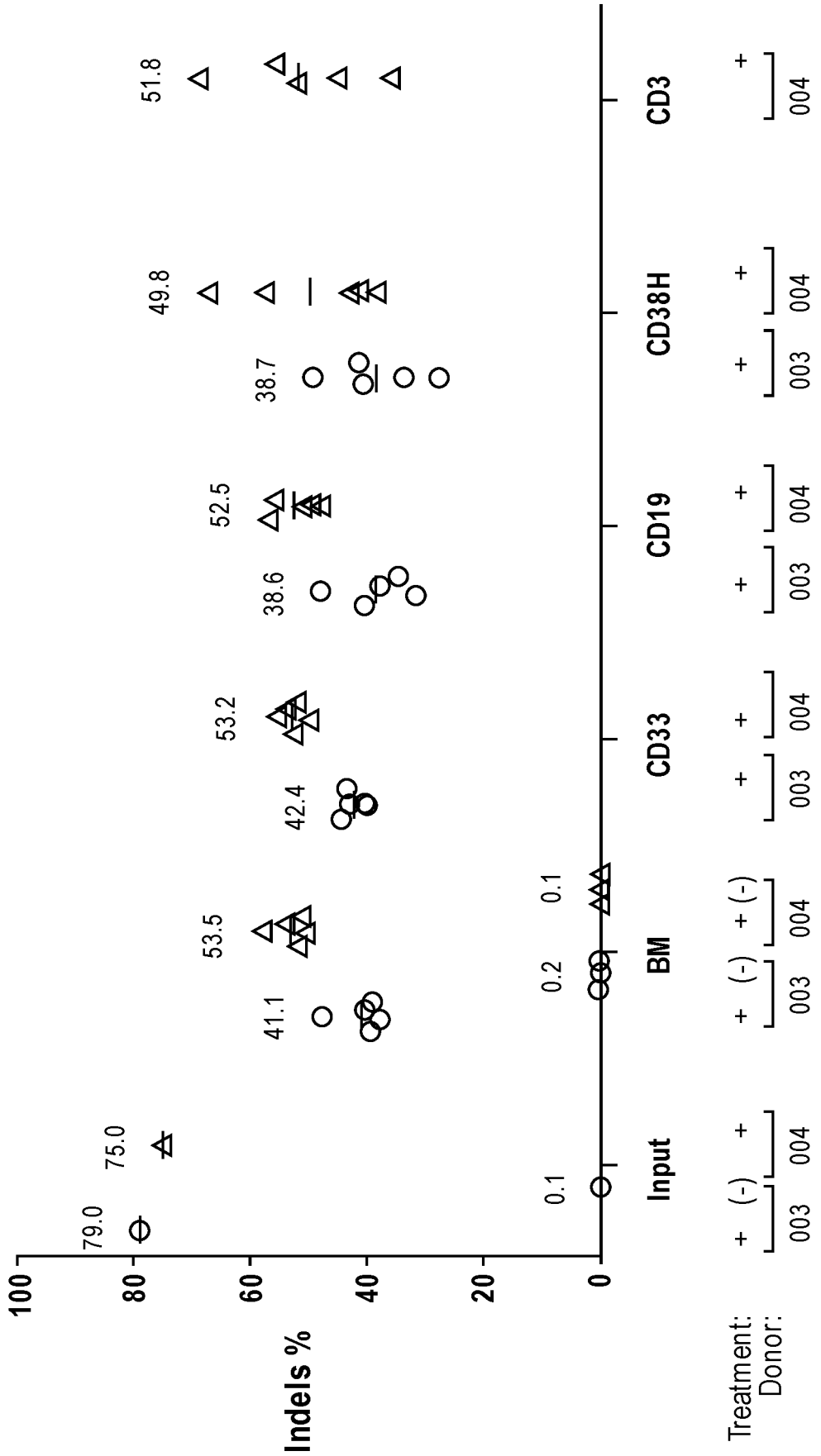

Indels %
100
80
60
40
20
0
79.0
75.0
0.1
53.5
41.1
0.2
0.1
53.2
42.4
52.5
38.6
49.8
38.7
51.8
Input
BM
CD33
CD19
CD38H
CD3
Treatment:
Donor:
+ (-) +
003 004
+ (-) + (-)
003 004
+ +
003 004
+ +
003 004
+ +
003 004
+
004

FIG. 11

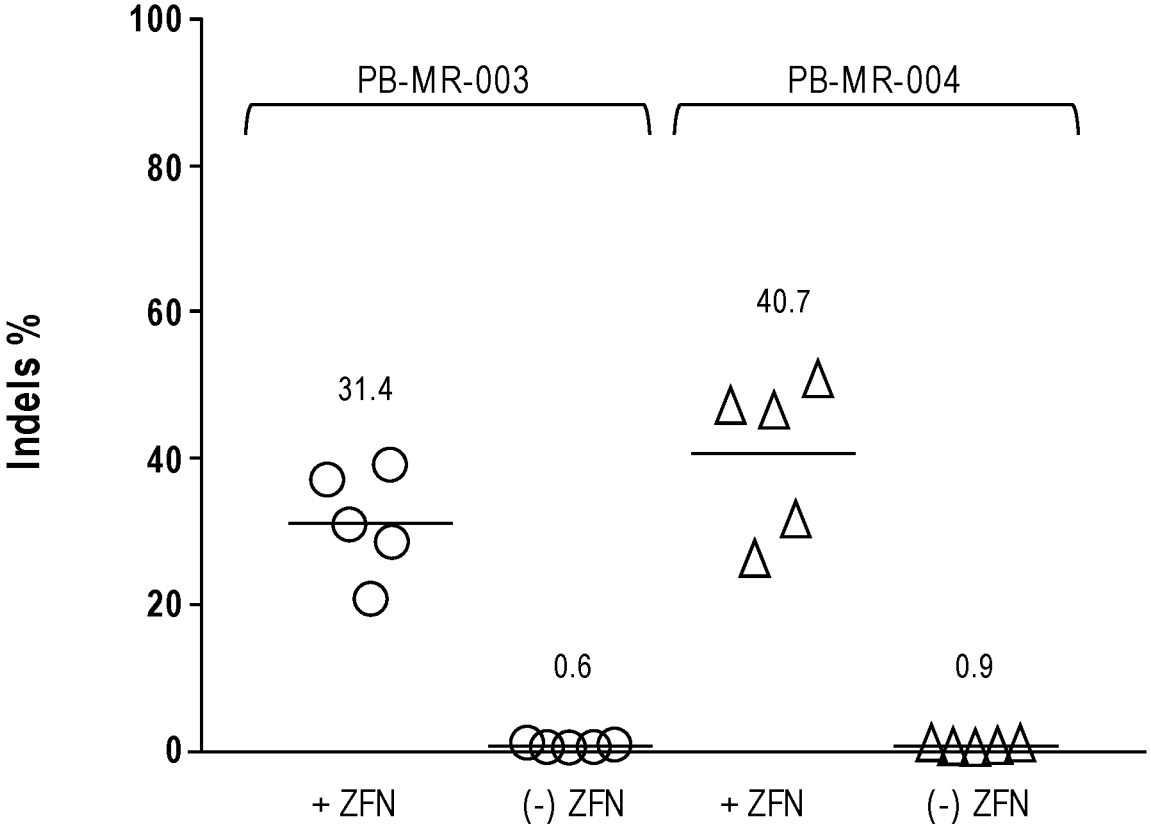

100
80
60
40
20
0
Indels %
PB-MR-003
PB-MR-004
31.4
40.7
0.6
0.9
+ ZFN
(-) ZFN
+ ZFN
(-) ZFN

FIG. 12

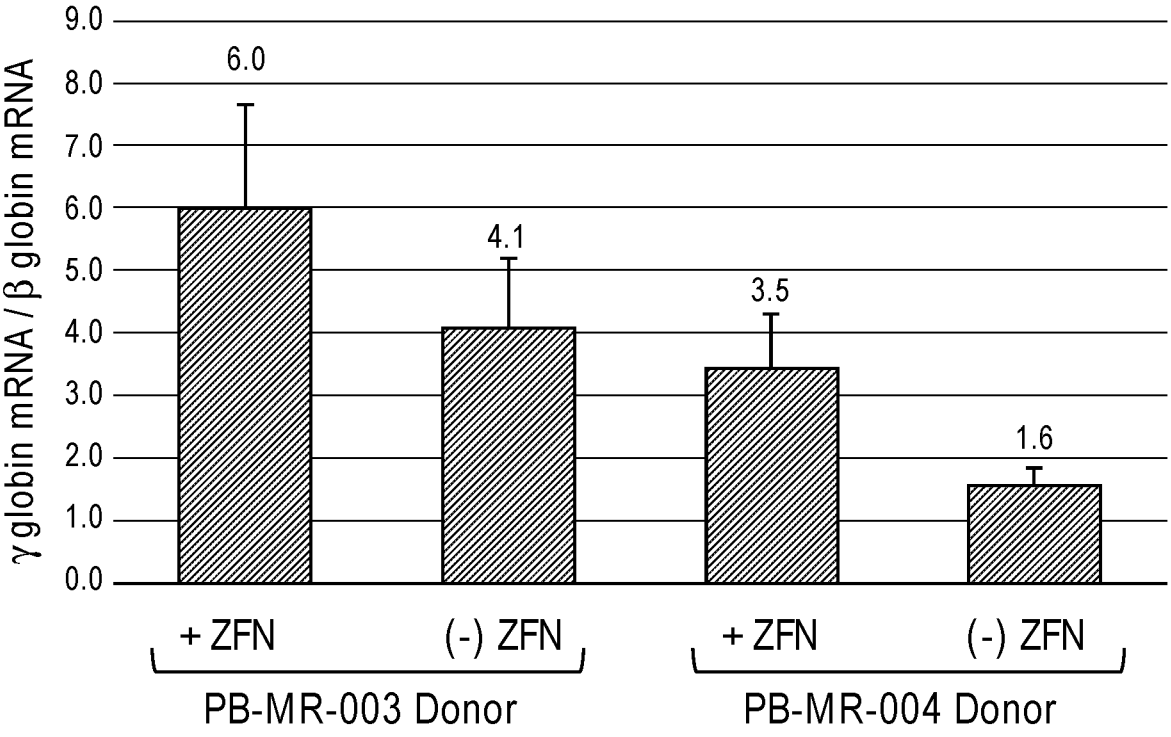


FIG. 13A

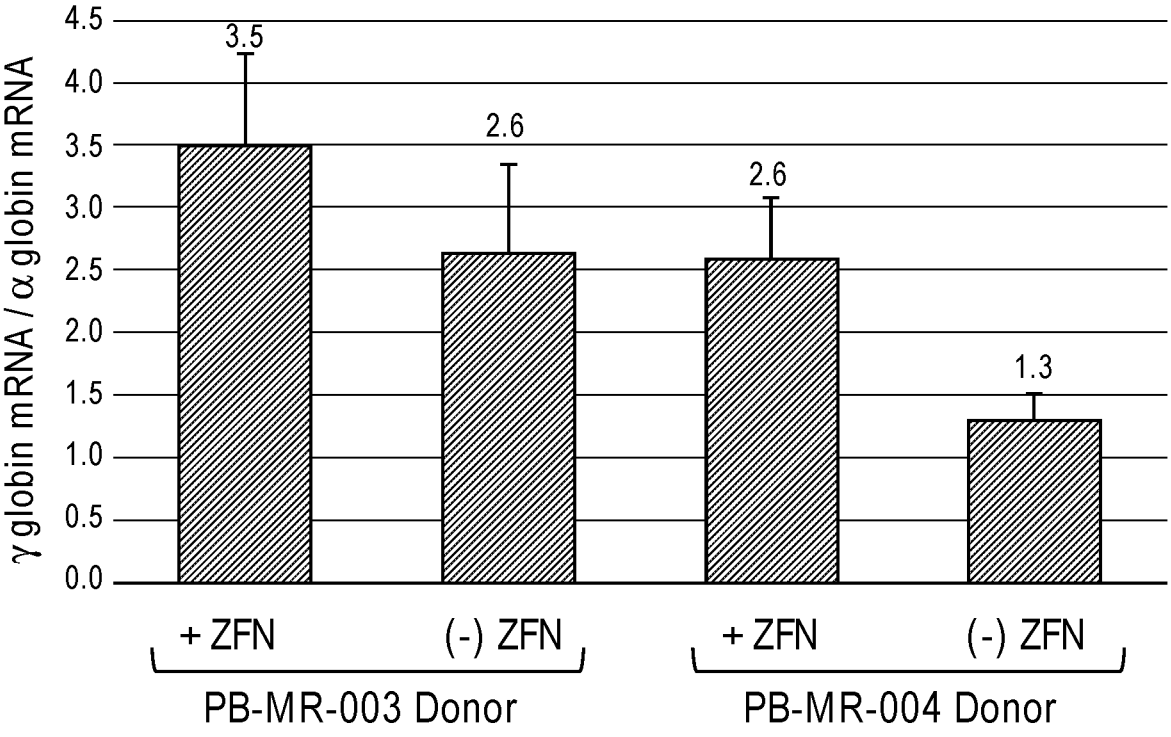


FIG. 13B

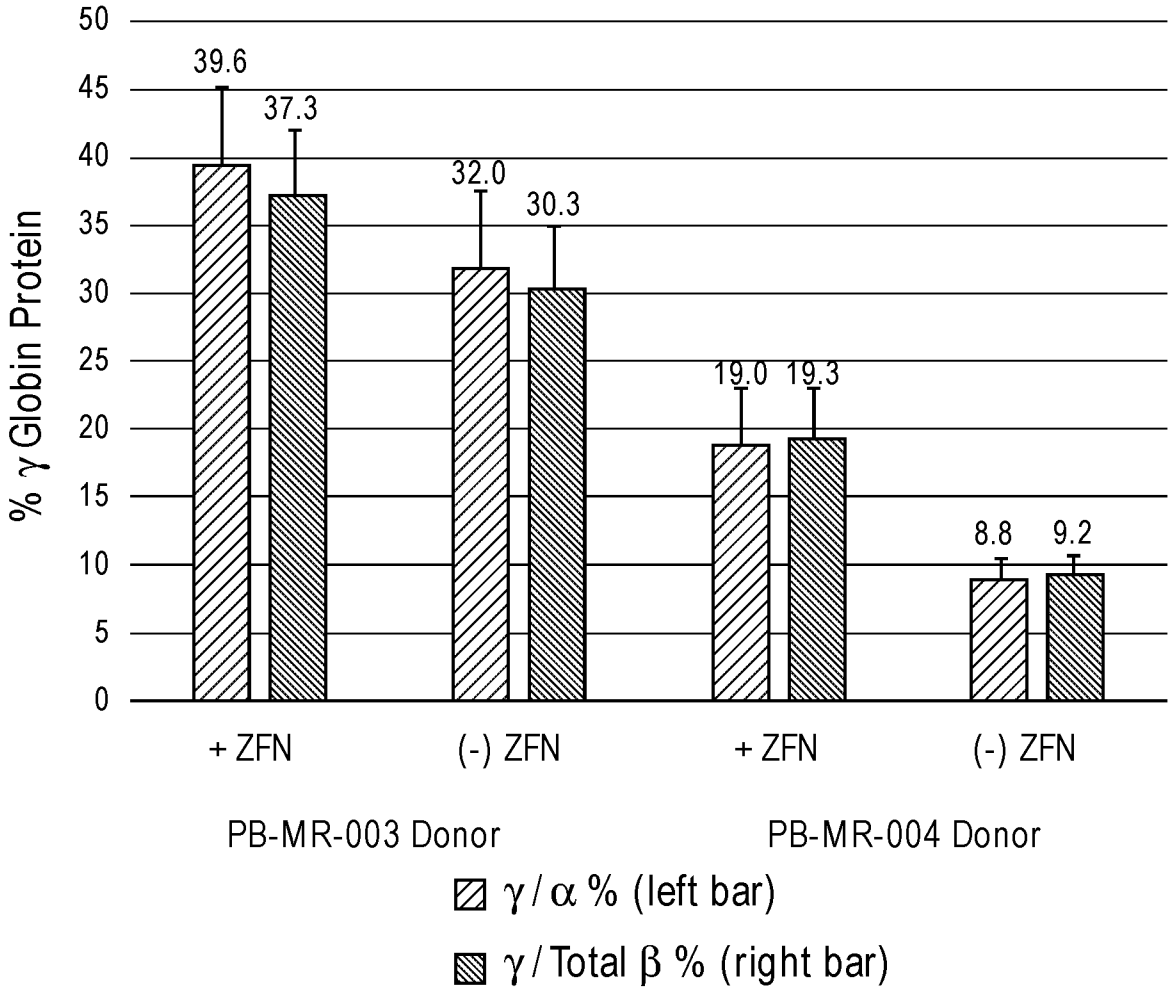

50
45
40
35
30
25
20
15
10
5
0
% γ Globin Protein
39.6
37.3
32.0
30.3
19.0
19.3
8.8
9.2
+ ZFN
(-) ZFN
+ ZFN
(-) ZFN
PB-MR-003 Donor
PB-MR-004 Donor
γ / α % (left bar)
γ / Total β % (right bar)

FIG. 14