
~20% of individuals (SNP)
G
C
47898
ATTGAATTCATAACTATCCCAAAGACCTATCCATTGCACTATGCTTTATTT
TAACTTAAGTATTGATAGGGTTTCTGGATAGGTAACGTGATACGAAATAAA
47171
Albumin

FIG. 1A

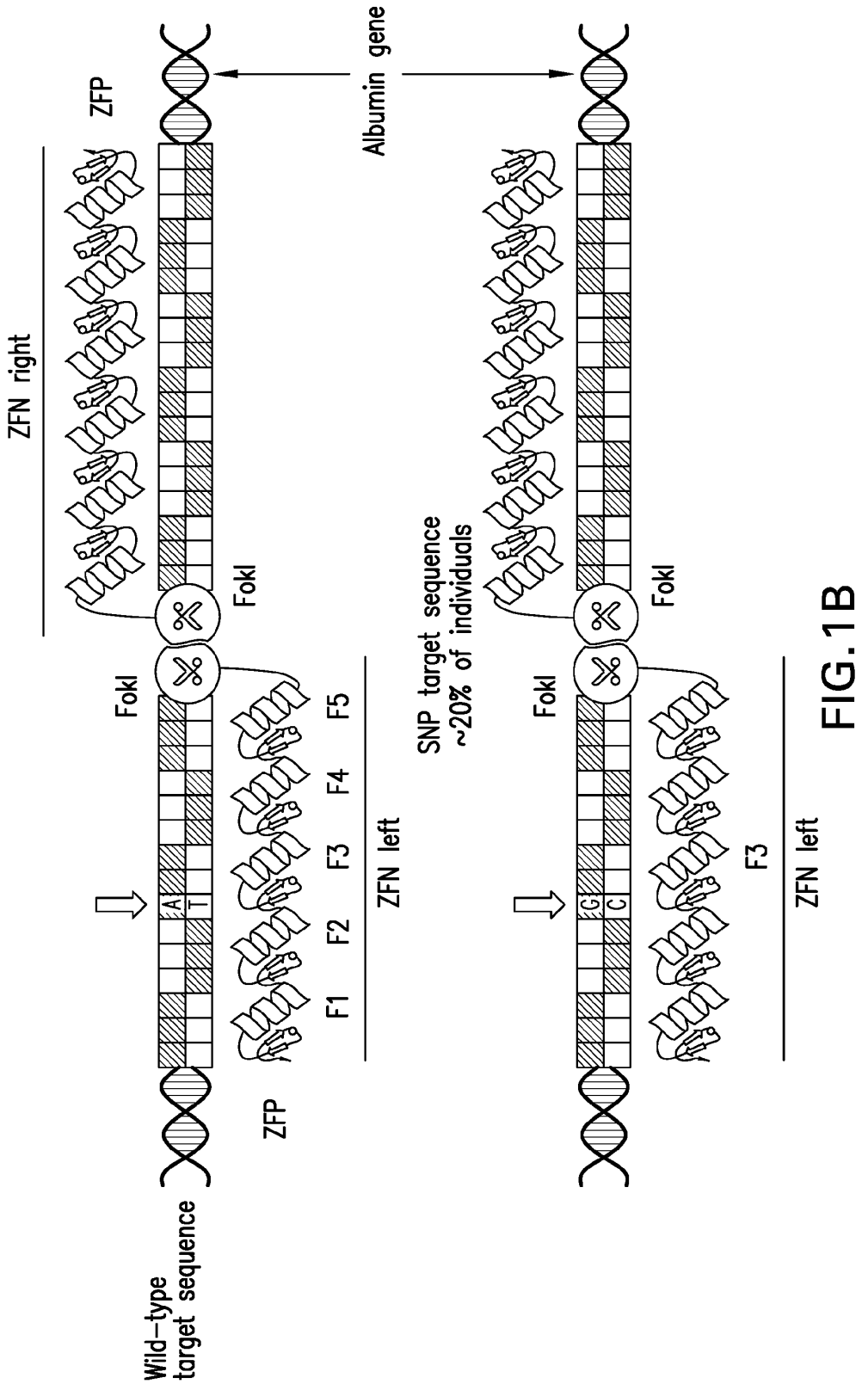

ZFN right
ZFP
FokI
FokI
F1 F2 F3 F4 F5
ZFP
ZFN left
A
T
Wild-type target sequence
Albumin gene
SNP target sequence ~20% of individuals
FokI
FokI
F3
ZFN left
G
C

FIG. 1B

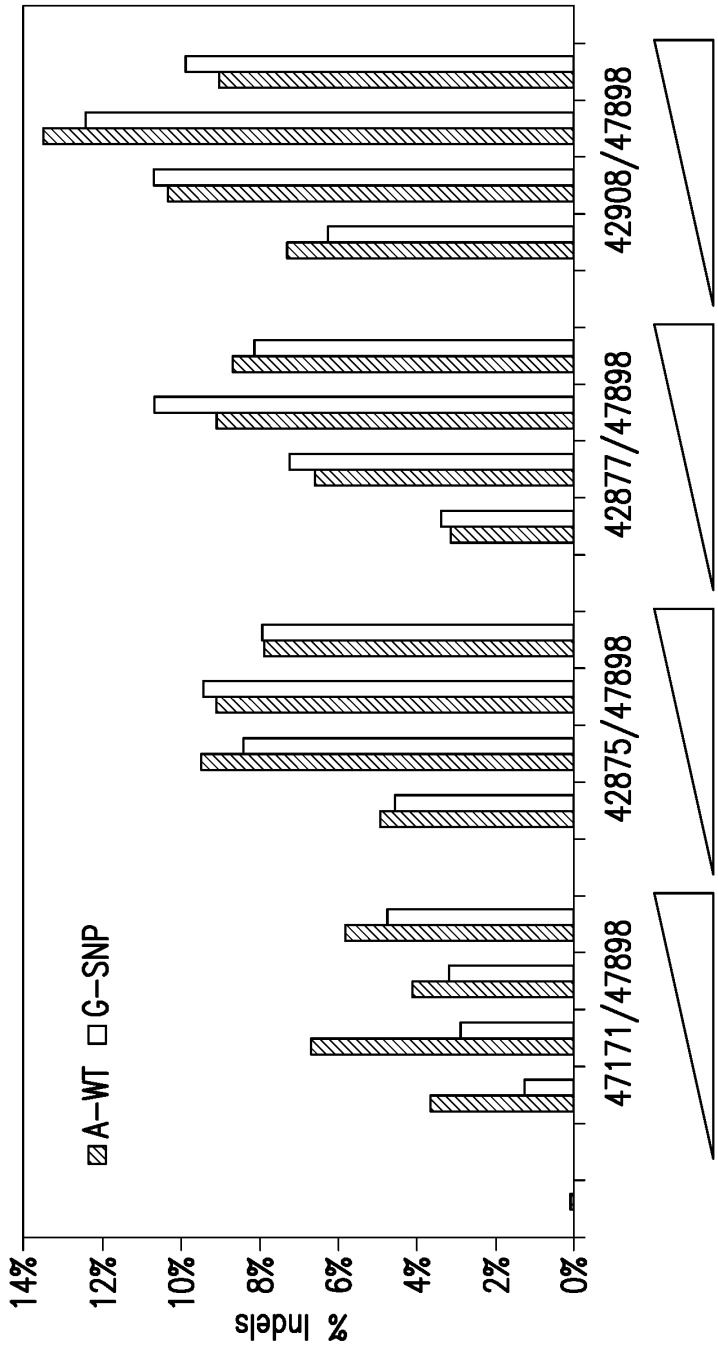

14%
12%
10%
8%
6%
4%
2%
0%
% Indels
A-WT
G-SNP
47171/47898
42875/47898
42877/47898
42908/47898
10, 25, 50, 75 ng

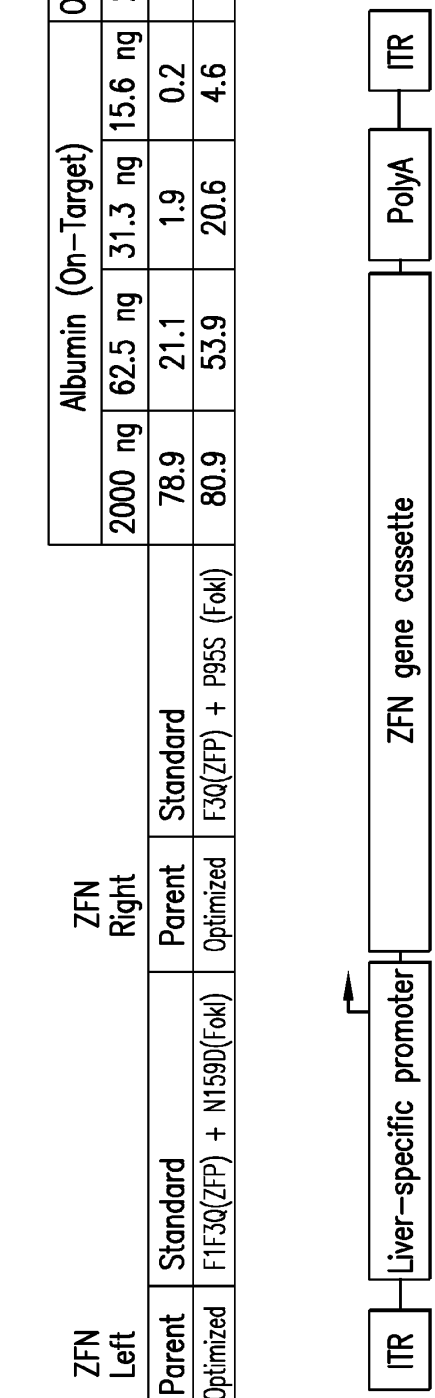
FIG.2

ZFN1		ZFN2		Albumin %indels				SMCHD1
				2000 ng	62.5 ng	31.25 ng	15.6 ng	2000 ng
71545	42875-F1F3Q-R416H	71673	47898-F3Q-R416H	82.4	39.8	6.1	0.8	0.017
71545	42875-F1F3Q-R416H	71721	47874-F3Q-R416H	82.7	44.5	9.2	2.0	0.018
71545	42875-F1F3Q-R416H	71739	47874-F1F3Q-S418P	84.2	63.3	19.6	2.8	0.011
71545	42875-F1F3Q-R416H	71741	47874-F1F3Q-L424F	85.1	61.1	23.9	4.5	0.017
71557	42875-F1F3Q-N542D	71721	47874-F3Q-R416H	80.8	46.8	8.9	1.8	0.018
71557	42875-F1F3Q-N542D	71728	47874-F3Q-P478S	80.9	53.9	20.6	4.6	0.012
71639	42908-F1F3Q-WT	71696	47898-F1F3Q-P478S	70.3	16.6	1.0	0.1	0.012
71641	42908-F1F3Q-R416H	71673	47898-F3Q-R416H	72.6	12.9	0.8	0.1	0.008
71641	42908-F1F3Q-R416H	71741	47874-F1F3Q-L424F	69.3	38.3	6.5	0.5	0.009
71653	42908-F1F3Q-N542D	71673	47898-F3Q-R416H	67.4	11.5	0.8	0.0	0.014
71653	42908-F1F3Q-N542D	71721	47874-F3Q-R416H	86.4	19.9	2.5	0.2	0.018
71653	42908-F1F3Q-N542D	71741	47874-F1F3Q-L424F	87.7	37.9	4.8	0.3	0.011
47171	Parent	47898	Parent	78.9	21.1	1.9	0.2	1.225

GFP	
0.098	Albumin Plate 1
0.033	Albumin Plate 2
0.008	SMCHD1

FIG.3A

ZFN Left		ZFN Right		Albumin (On-Target)				Off-Target
				2000 ng	62.5 ng	31.3 ng	15.6 ng	2000 ng
Parent	Standard	Parent	Standard	78.9	21.1	1.9	0.2	1.23
Optimized	F1F3Q(ZFP) + N159D(FokI)	Optimized	F3Q(ZFP) + P95S (FokI)	80.9	53.9	20.6	4.6	0.01



Transgene Modifications

- Addition of a 5'UTR increases ZFN expression
- Addition of an N-terminal peptide increases cutting efficiency
- Addition of a WPRE increases ZFN mRNA stability and translation

FIG.3B

	Gene/ Location	Capture events	K562 % indels	HepG2 % indels
	ALB (on-target)	3498	87.7%	98.2%
1	SKA2	18	ns	ns
2	Chr5 (intergenic)	12	ns	ns
3	Chr9 (intergenic)	11	ns	ns
4	AC079807.4	10	ns	ns
5	Chr1 (intergenic)	9	ns	ns
6	CSMD3	9	ns	ns
7	Chr18 (intergenic)	9	ns	ns
8	Chr19 (intergenic)	9	ns	ns
9	Chr1 (intergenic)	8	ns	0.08^
10	HECTD4	8	ns*	ns
11	Chr17 (intergenic)	8	ns	ns
12	Chr4 (intergenic)	8	ND	ND
13	CSMD3	8	ns	ns
14	Chr11 (intergenic)	8	ns	ns
15	Chr1 (intergenic)	7	ns	ns
16	AKAP6	7	ND	ND
17	Chr19 (intergenic)	7	ns	ns
18	OSBPL6	7	ns	ns
19	QPCT	7	ns	ns
20	RP11-269F21.3	7	ns	ns
21	LPHN3	7	ns	ns
22	CYFIP2	7	ns	ns
23	AUTS2	7	ns	ns
24	COL4A6	7	ns	ns
25	ChrX (intergenic)	7	ns	ns
26	ACAN	7	ns	ns

FIG.3C

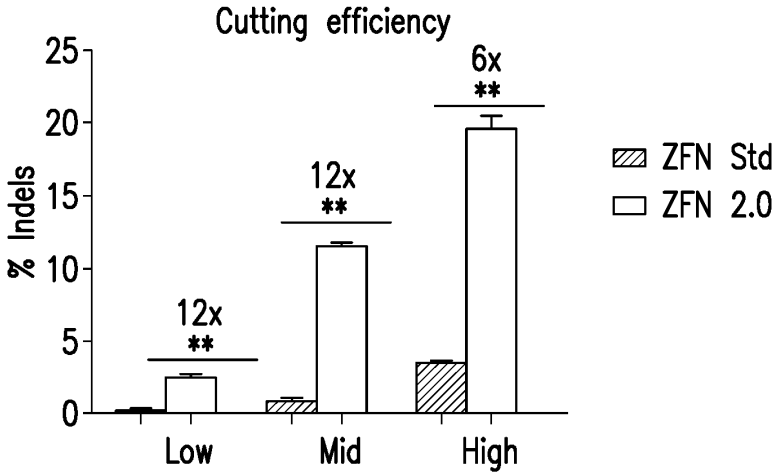


FIG.3D

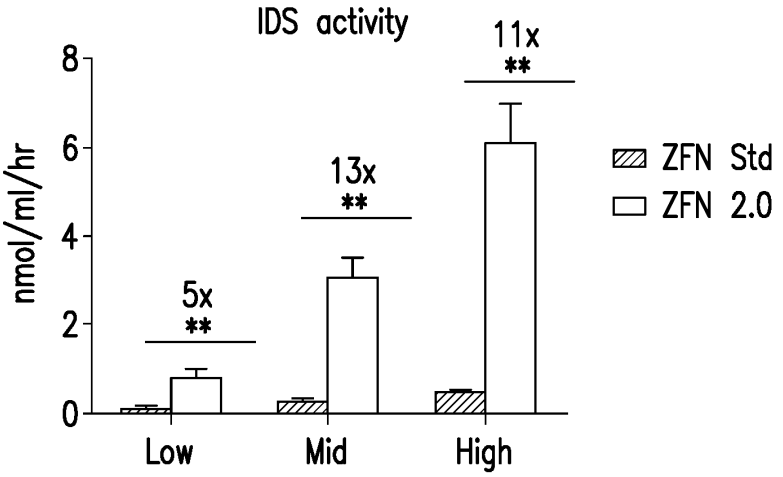


FIG.3E(i)

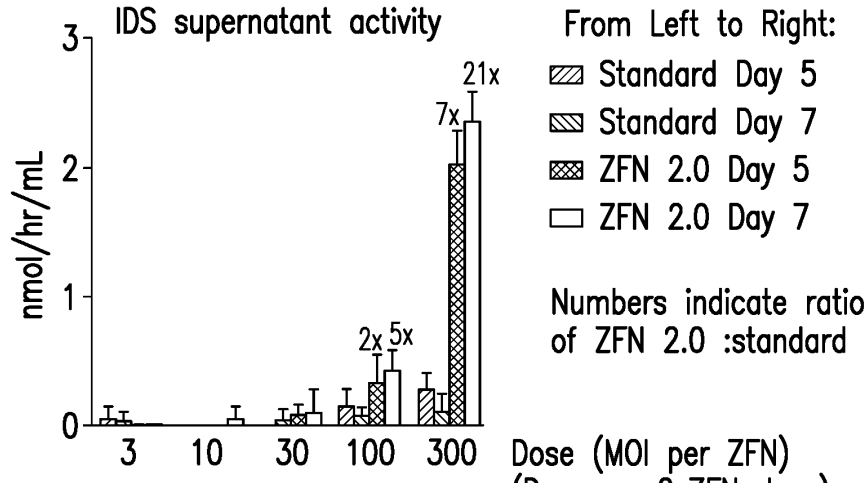


FIG.3E(ii)

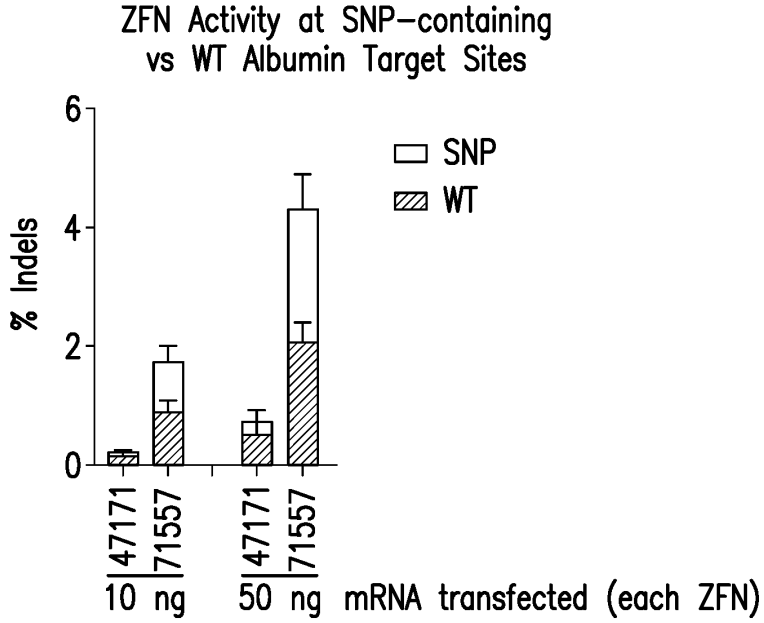


FIG.3F(i)

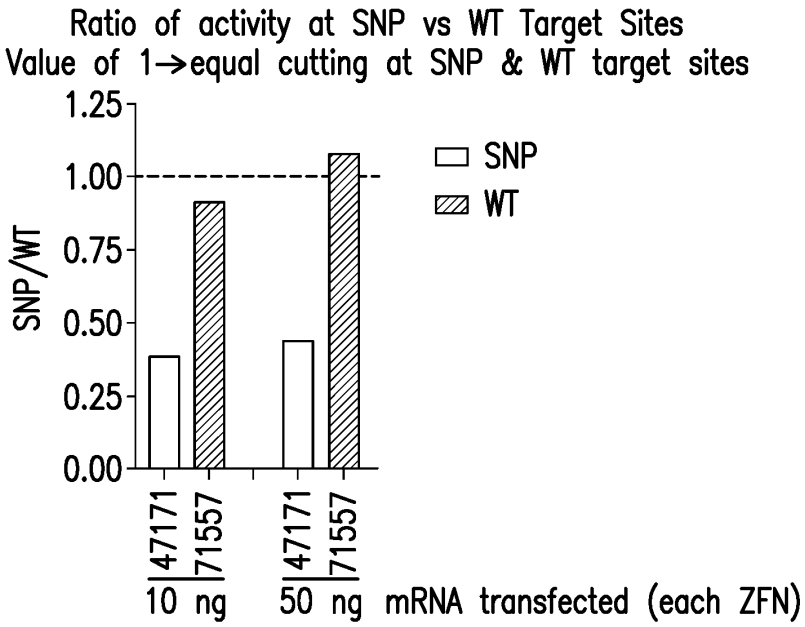


FIG.3F(ii)

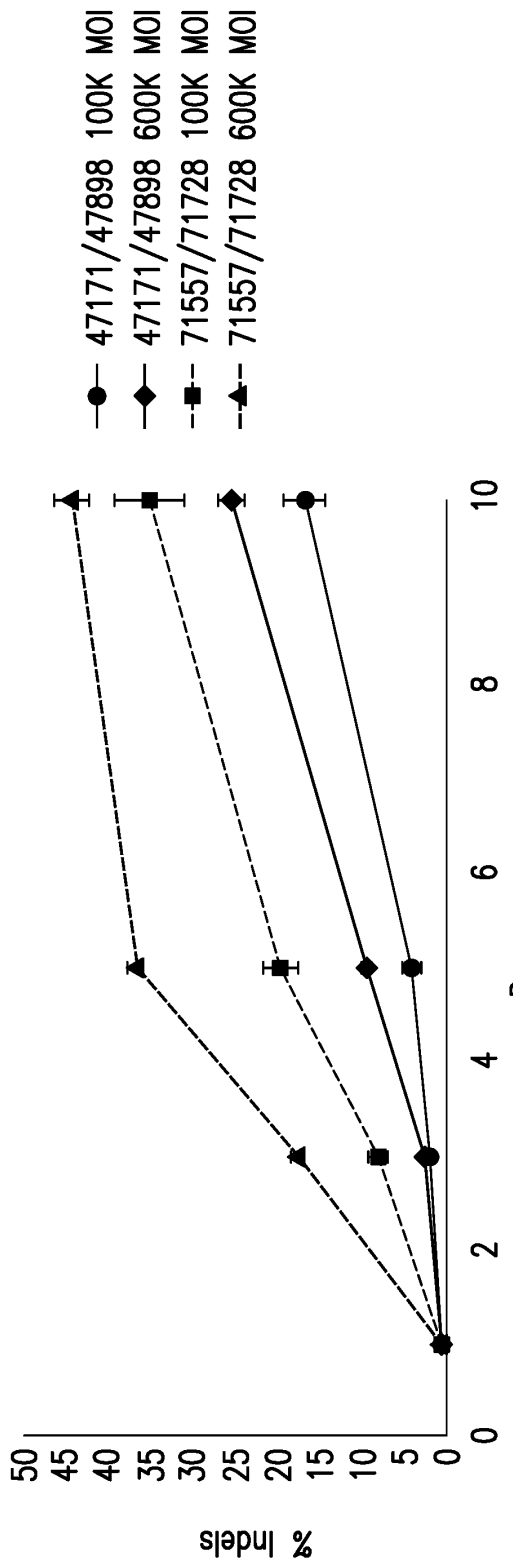

47171/47898 100K MOI
47171/47898 600K MOI
71557/71728 100K MOI
71557/71728 600K MOI
% Indels
0
5
10
15
20
25
30
35
40
45
50
0
2
4
6
8
10
Days

FIG.3G

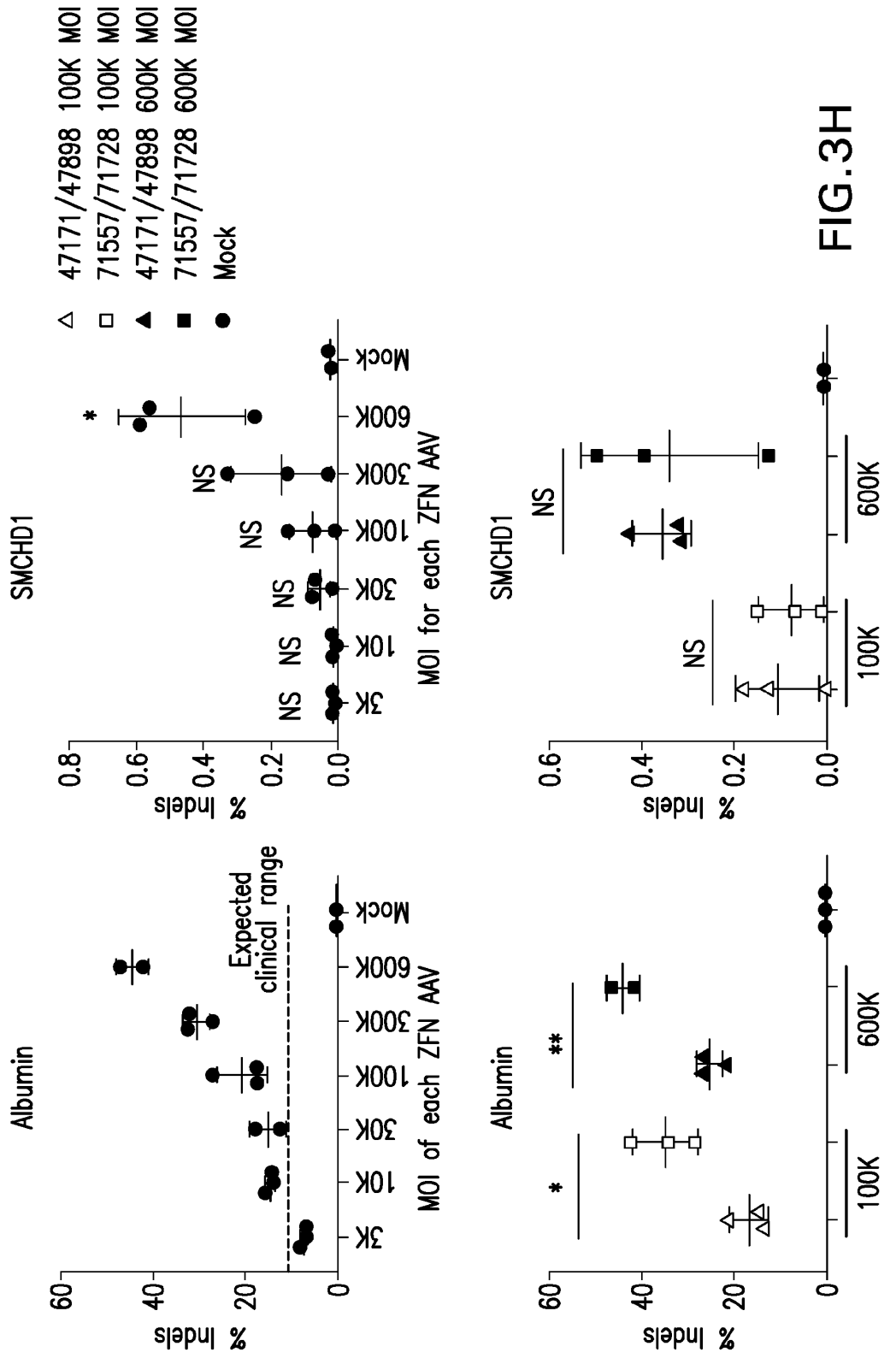

47171/47898 100K MOI
71557/71728 100K MOI
47171/47898 600K MOI
71557/71728 600K MOI
Mock
SMCHD1
% Indels
MOI for each ZFN AAV
3K
10K
30K
100K
300K
600K
Mock
NS
*
Albumin
Expected clinical range
**
100K
600K

FIG.3H

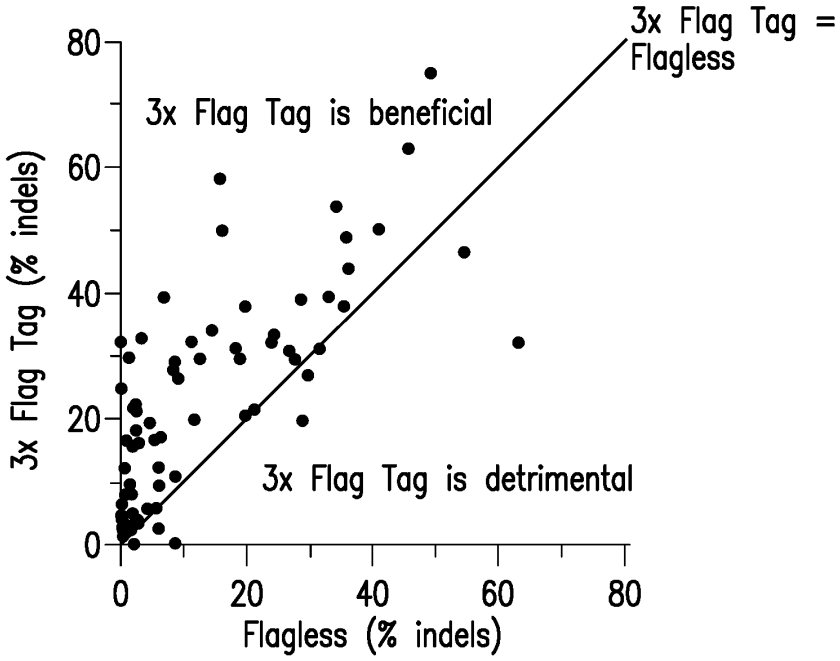


FIG.4A

	3xFlag/Flagless
Positive effect >10%	61
Neutral (within 10%)	7
Negative effect >10%	6
	n=74
Average	4.1x

FIG.4B

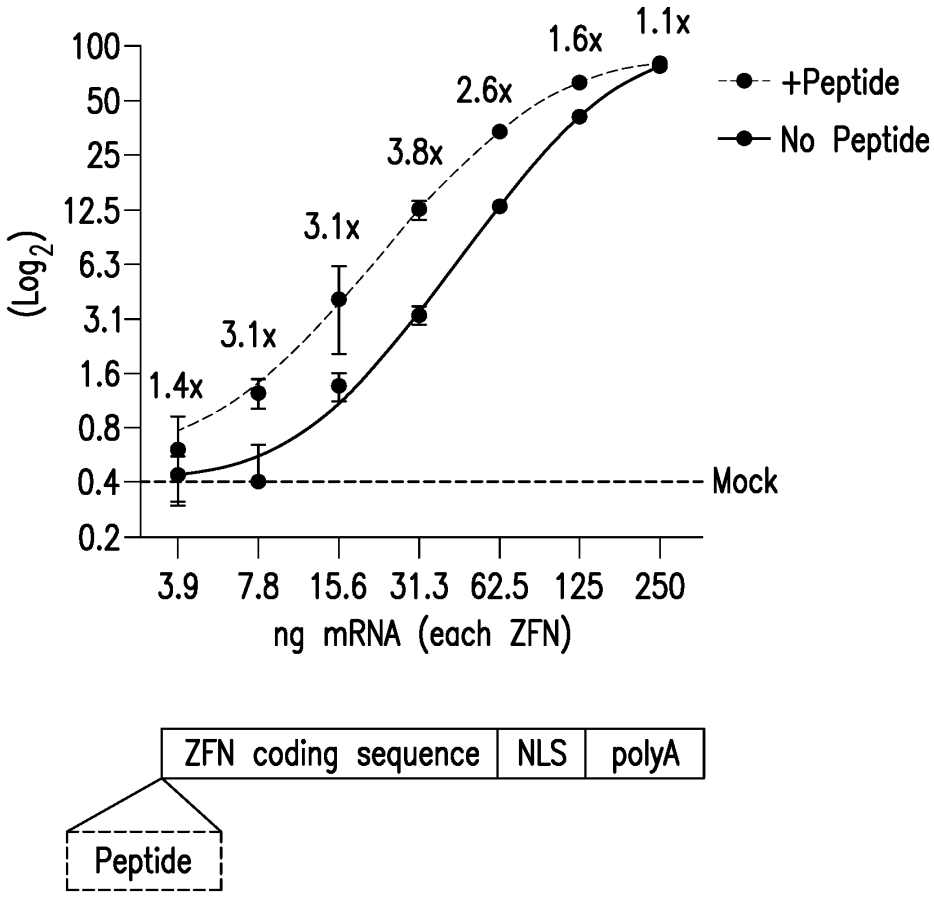

100
50
25
12.5
6.3
3.1
1.6
0.8
0.4
0.2
(Log2)
1.4x
3.1x
3.1x
3.8x
2.6x
1.6x
1.1x
+Peptide
No Peptide
Mock
3.9
7.8
15.6
31.3
62.5
125
250
ng mRNA (each ZFN)
ZFN coding sequence
NLS
polyA
Peptide

FIG.4C

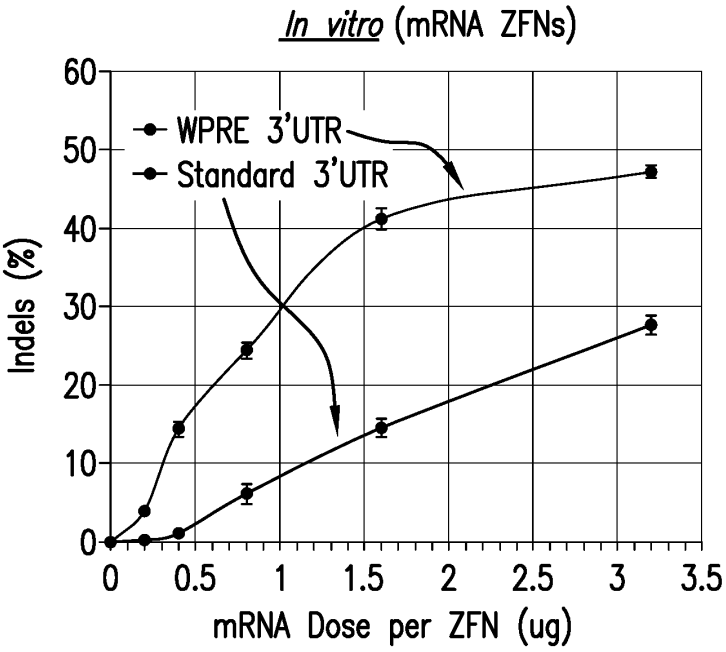

In vitro (mRNA ZFNs)
WPRE 3'UTR
Standard 3'UTR
Indels (%)
mRNA Dose per ZFN (ug)
0
10
20
30
40
50
60
0
0.5
1
1.5
2
2.5
3
3.5

FIG.5A

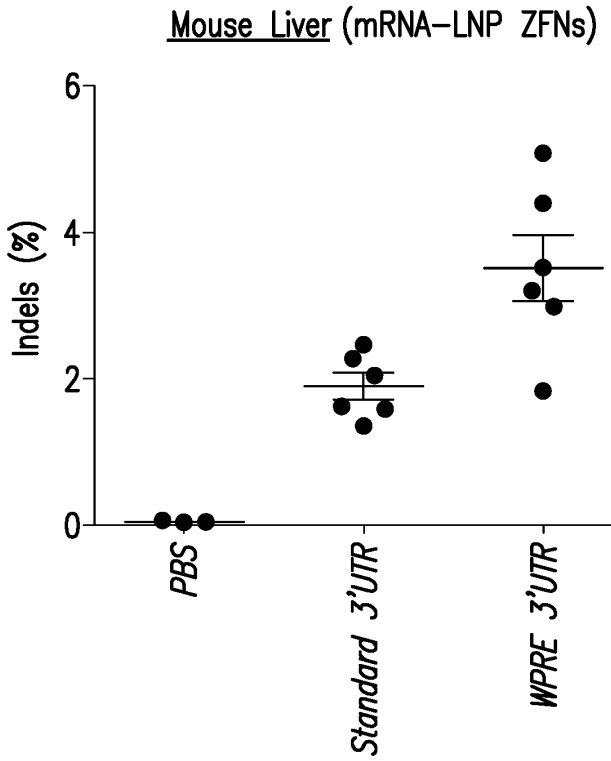

Mouse Liver (mRNA–LNP ZFNs)
Indels (%)
0
2
4
6
PBS
Standard 3'UTR
WPRE 3'UTR

FIG.5B

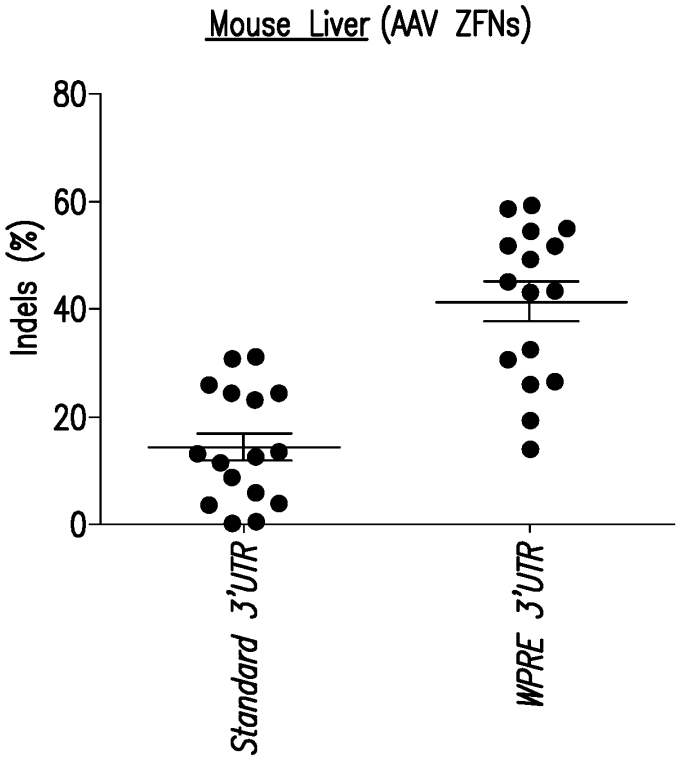

Mouse Liver (AAV ZFNs)
Indels (%)
80
60
40
20
0
Standard 3'UTR
WPRE 3'UTR

FIG.5C

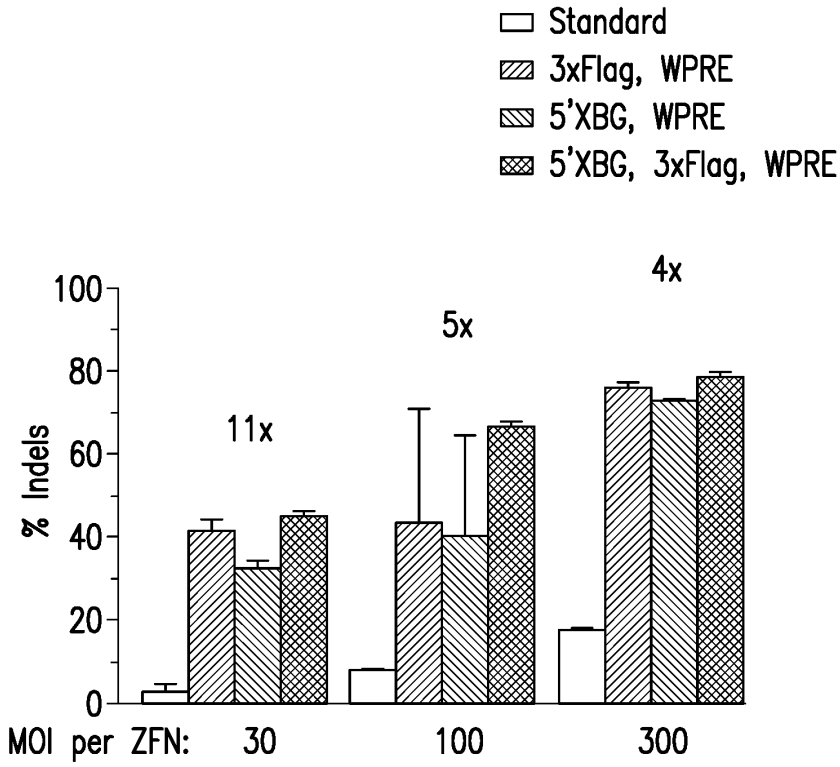

Standard
3xFlag, WPRE
5'XBG, WPRE
5'XBG, 3xFlag, WPRE
% Indels
100
80
60
40
20
0
11x
5x
4x
MOI per ZFN:
30
100
300

FIG.6

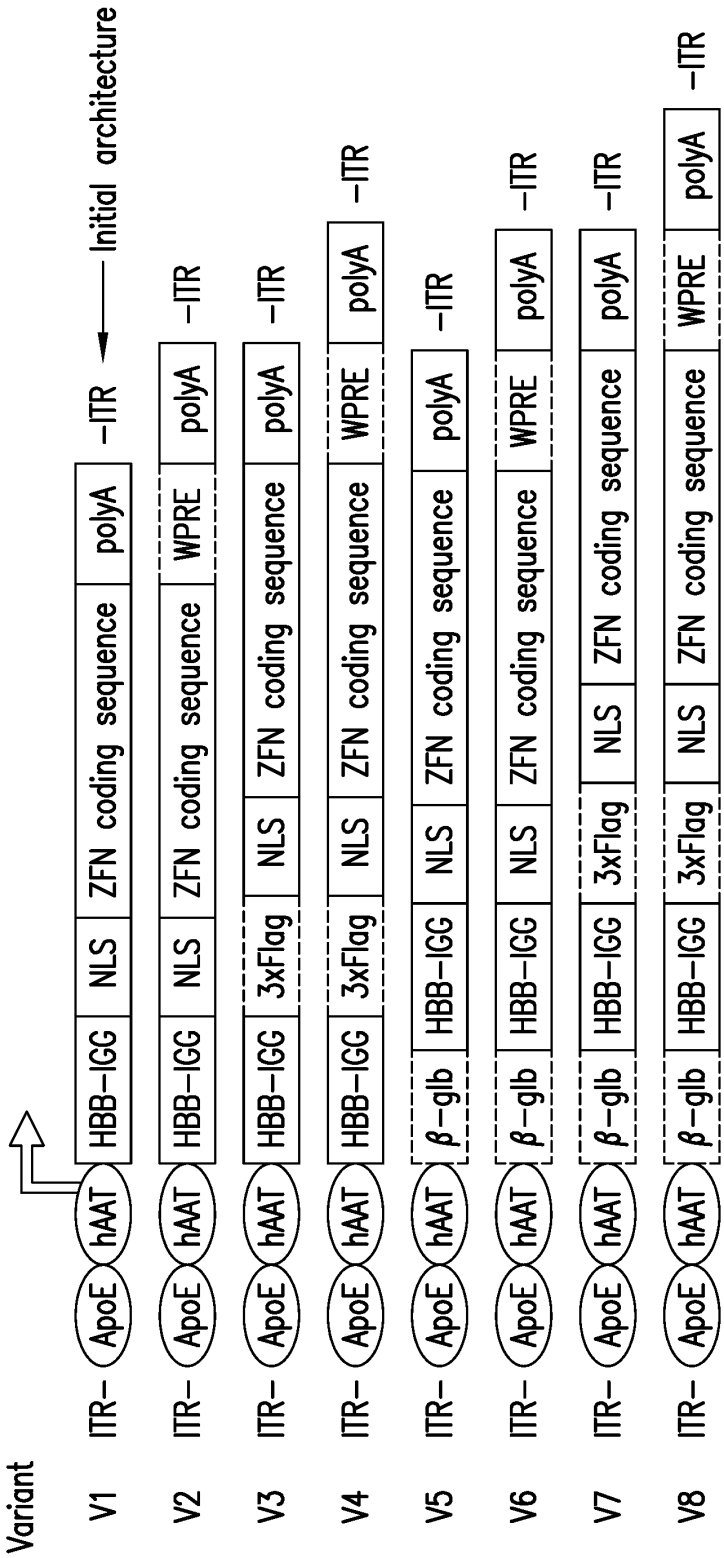

Variant
V1
V2
V3
V4
V5
V6
V7
V8
ITR–
ApoE
hAAT
HBB–IGG
β–glb
NLS
3xFlag
ZFN coding sequence
WPRE
polyA
–ITR
Initial architecture

FIG.7A

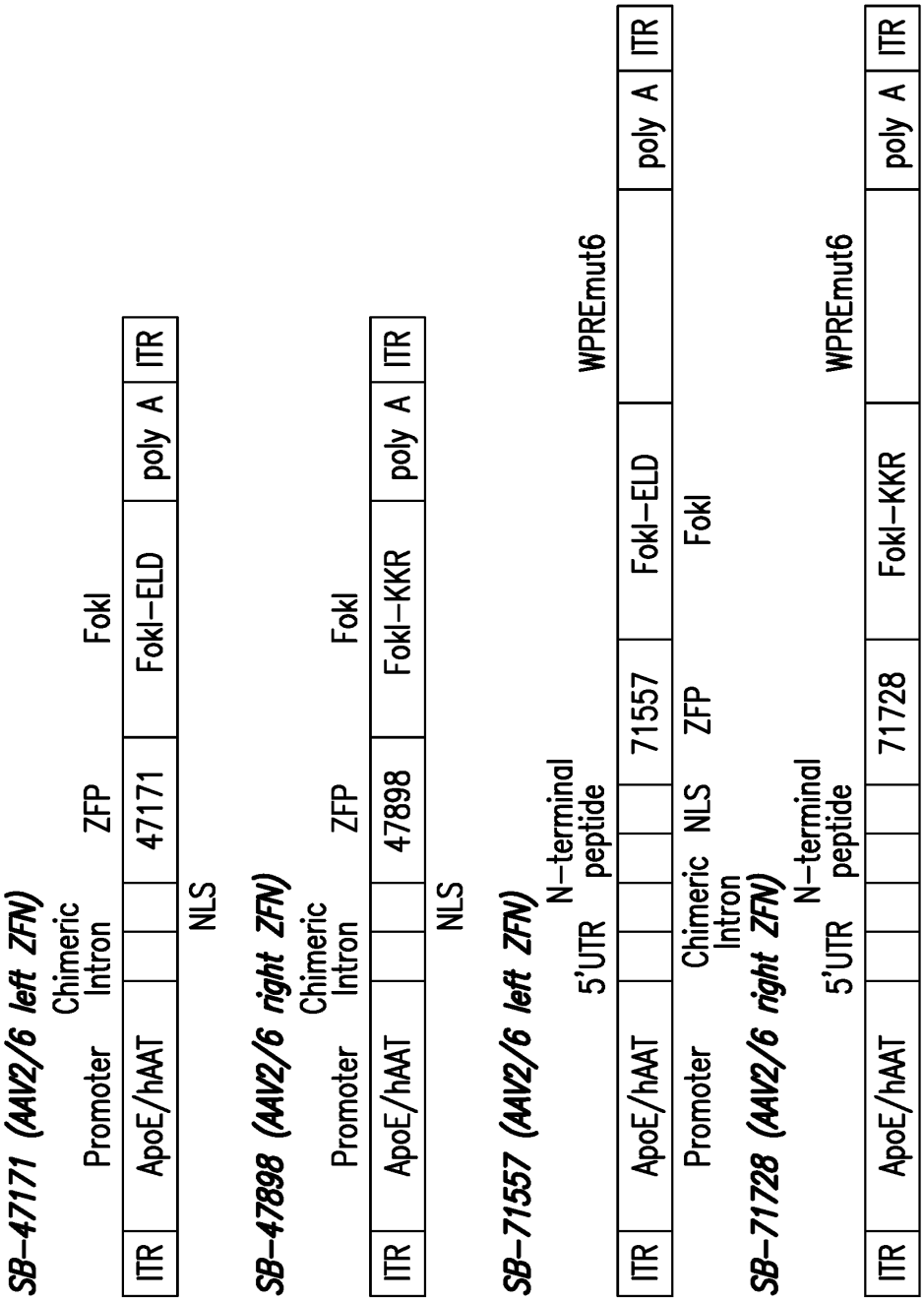

SB-47171 (AAV2/6 left ZFN)
Promoter
Chimeric Intron
ZFP
FokI
ITR
ApoE/hAAT
47171
FokI-ELD
poly A
ITR
NLS
SB-47898 (AAV2/6 right ZFN)
Promoter
Chimeric Intron
ZFP
FokI
ITR
ApoE/hAAT
47898
FokI-KKR
poly A
ITR
NLS
SB-71557 (AAV2/6 left ZFN)
5'UTR
N-terminal peptide
WPREmut6
ITR
ApoE/hAAT
71557
FokI-ELD
poly A
ITR
Promoter
Chimeric Intron
NLS
ZFP
FokI
SB-71728 (AAV2/6 right ZFN)
5'UTR
N-terminal peptide
WPREmut6
ITR
ApoE/hAAT
71728
FokI-KKR
poly A
ITR
Promoter
Chimeric Intron
NLS
ZFP
FokI

FIG.7B

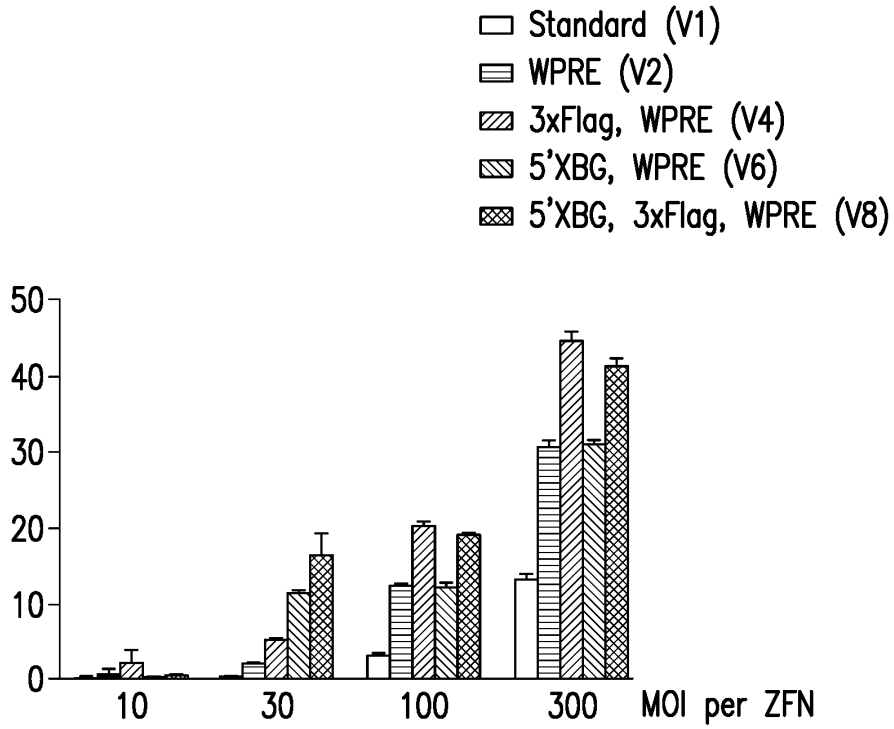

Standard (V1)
WPRE (V2)
3xFlag, WPRE (V4)
5'XBG, WPRE (V6)
5'XBG, 3xFlag, WPRE (V8)
50
40
30
20
10
0
10
30
100
300
MOI per ZFN

FIG.8

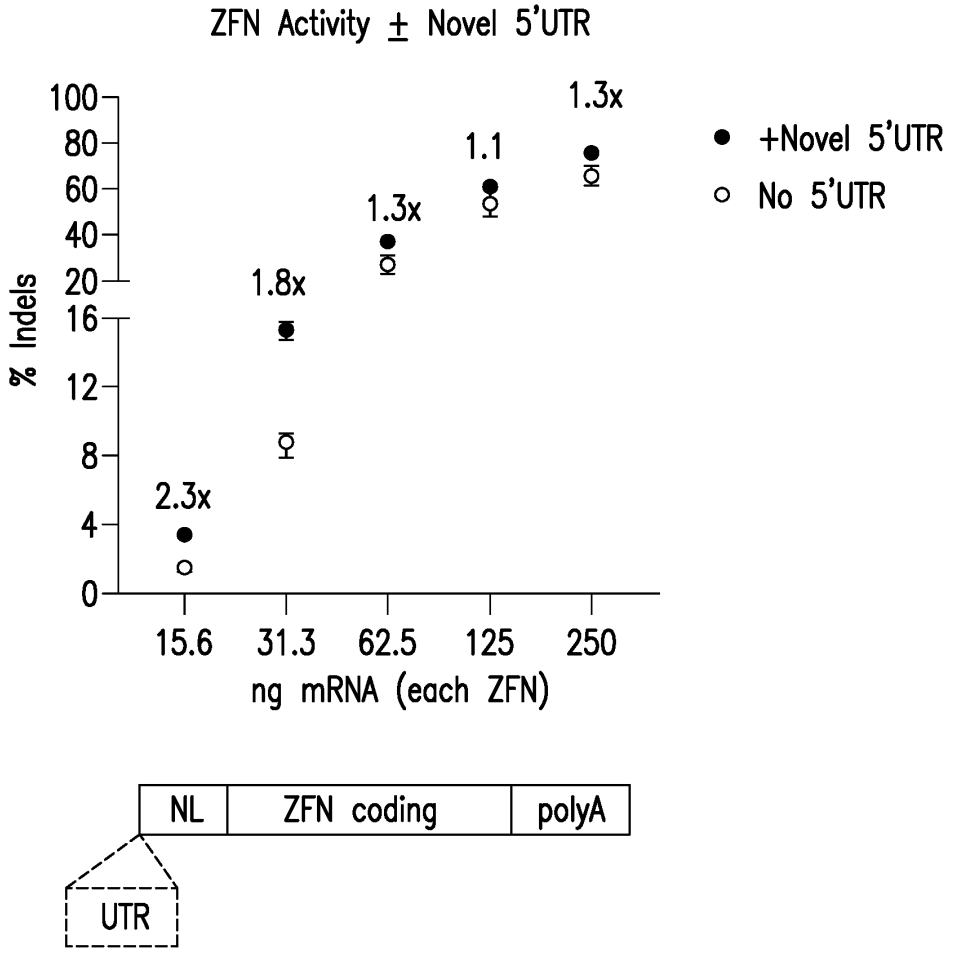

ZFN Activity ± Novel 5'UTR
% Indels
100
80
60
40
20
16
12
8
4
0
2.3x
1.8x
1.3x
1.1
1.3x
15.6
31.3
62.5
125
250
ng mRNA (each ZFN)
+Novel 5'UTR
No 5'UTR
NL
ZFN coding
polyA
UTR

FIG.9

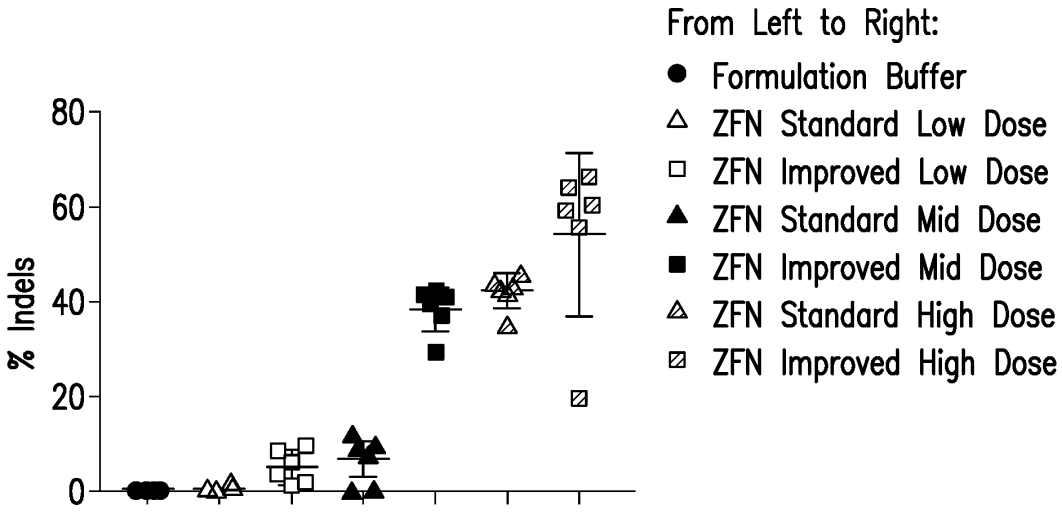


FIG.10A

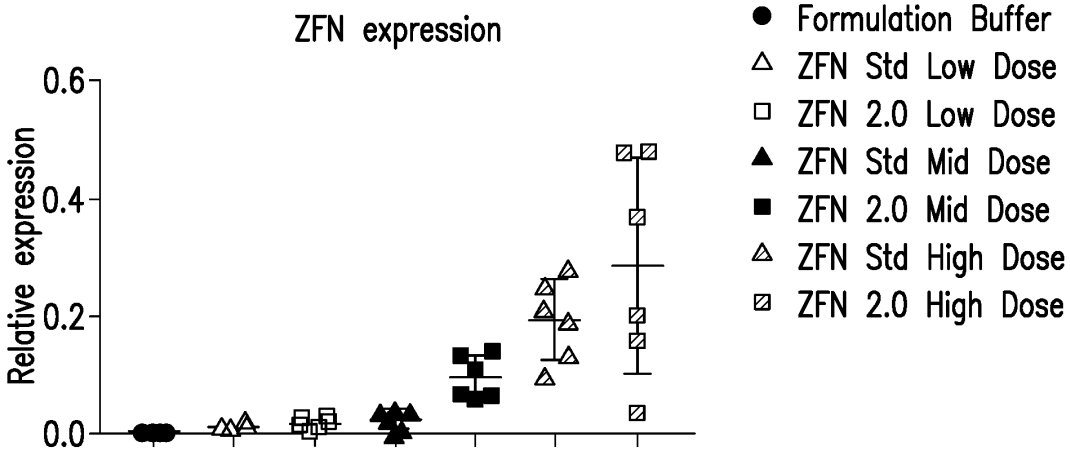


FIG.10B

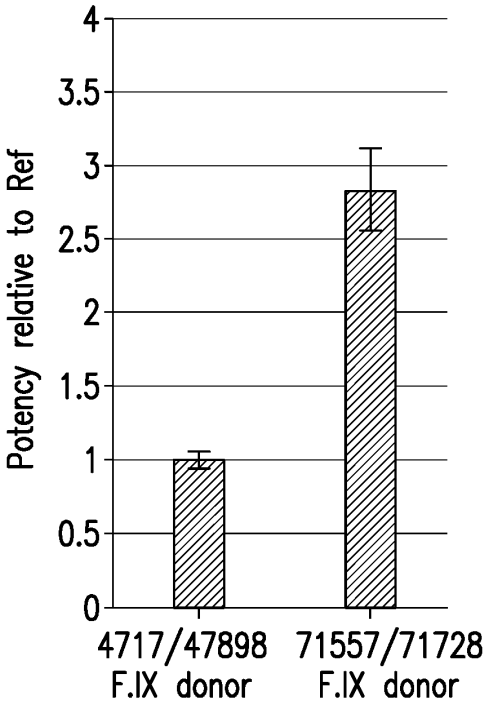
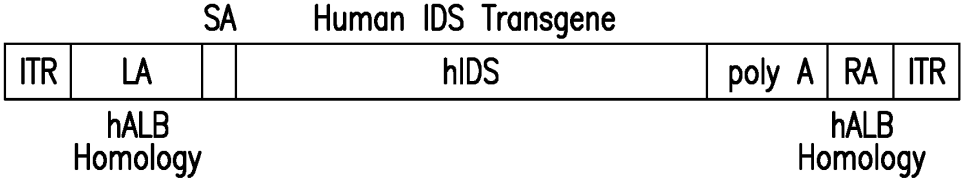
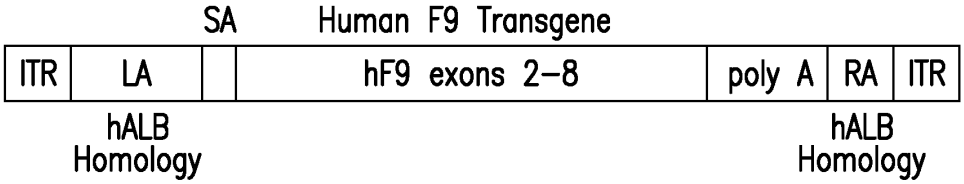

Potency relative to Ref
4
3.5
3
2.5
2
1.5
1
0.5
0
4717/47898
F.IX donor
71557/71728
F.IX donor

FIG.10C

SB-IDS transgene donor (AAV2/6 donor)



SB-F9 transgene donor (rAAV2/6 donor)



SB-IDUA donor (hIDUA donor – AAV2/6)

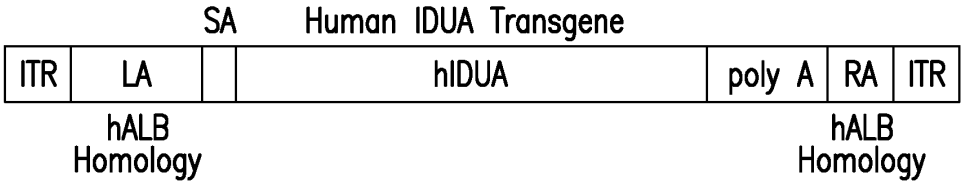


FIG.11A

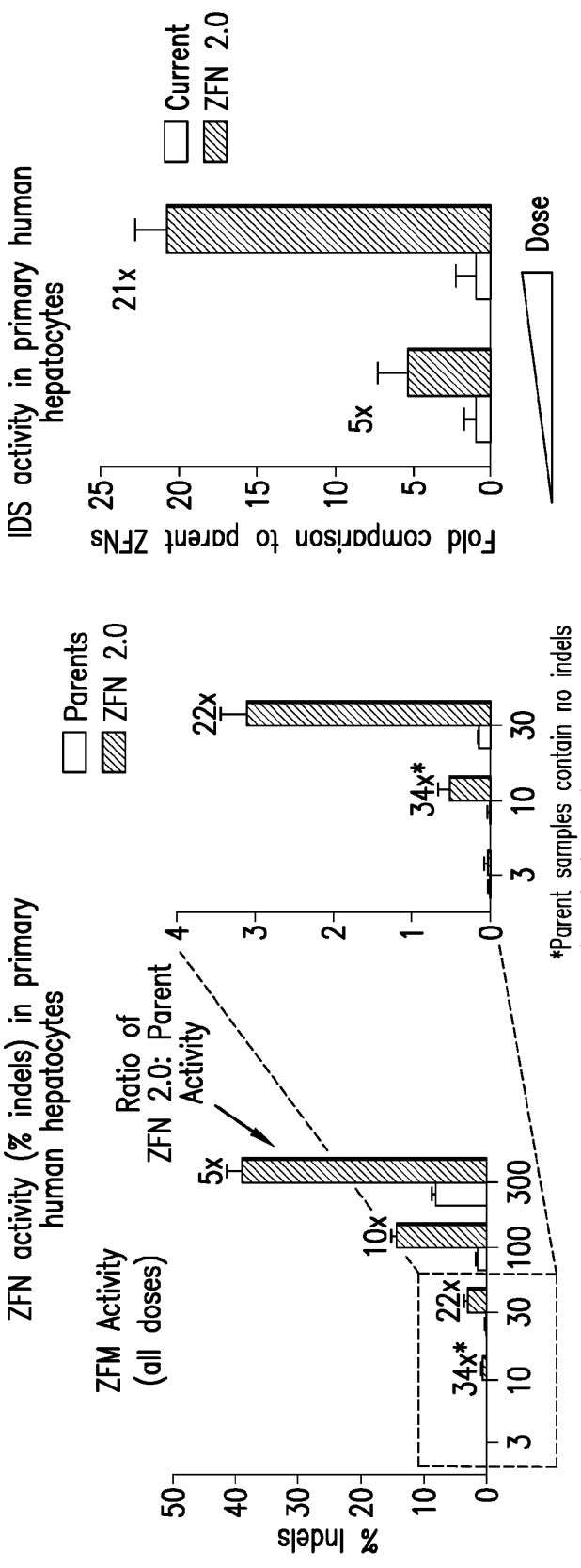

IDS activity in primary human hepatocytes
Fold comparison to parent ZFNs
25
20
15
10
5
0
21x
5x
Current
ZFN 2.0
Dose
ZFN activity (% indels) in primary human hepatocytes
% Indels
50
40
30
20
10
0
ZFM Activity (all doses)
3
10
30
100
300
34x*
22x
10x
5x
Ratio of ZFN 2.0: Parent Activity
4
3
2
1
0
3
10
30
34x*
22x
Parents
ZFN 2.0
*Parent samples contain no indels above background

FIG.11B

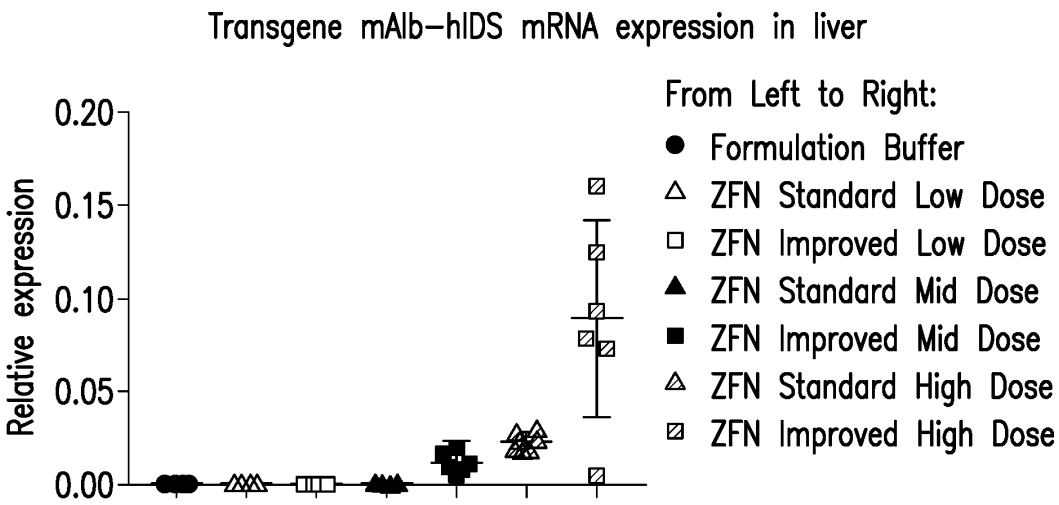

Transgene mAlb–hIDS mRNA expression in liver
Relative expression
0.20
0.15
0.10
0.05
0.00
From Left to Right:
Formulation Buffer
ZFN Standard Low Dose
ZFN Improved Low Dose
ZFN Standard Mid Dose
ZFN Improved Mid Dose
ZFN Standard High Dose
ZFN Improved High Dose

FIG.11C

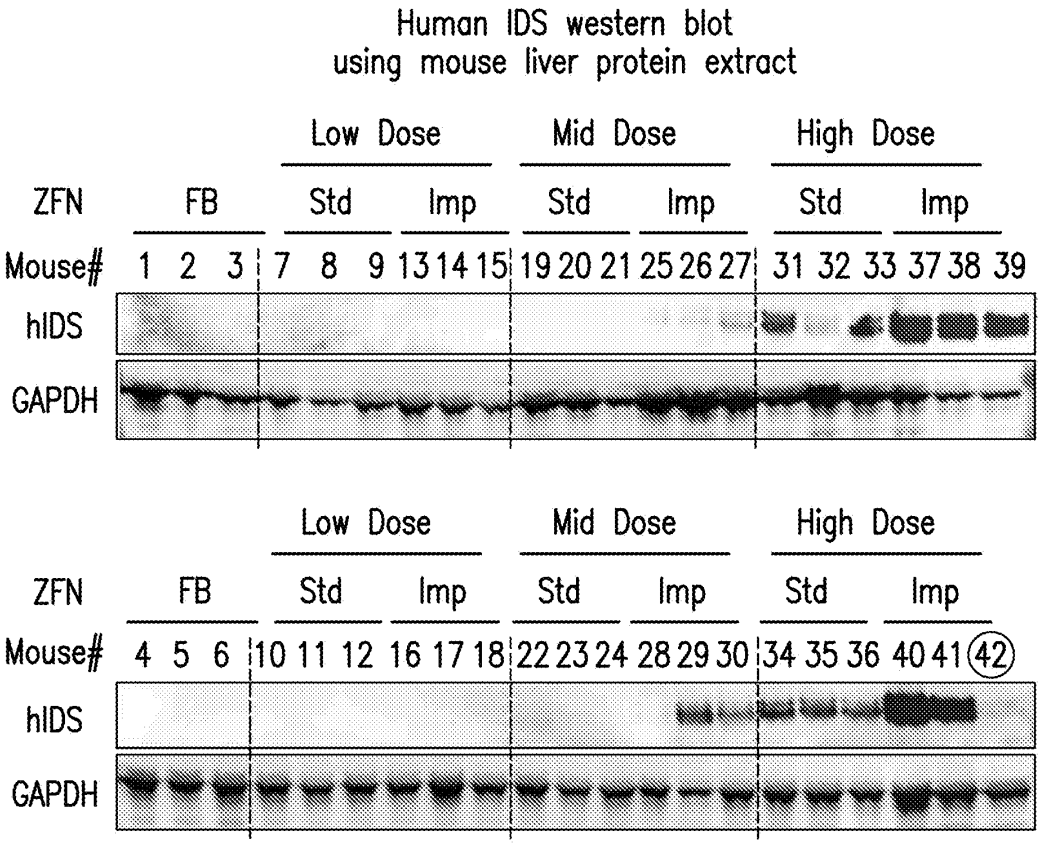

Human IDS western blot
using mouse liver protein extract
Low Dose
Mid Dose
High Dose
ZFN
FB
Std
Imp
Std
Imp
Std
Imp
Mouse#
1 2 3 7 8 9 13 14 15 19 20 21 25 26 27 31 32 33 37 38 39
hIDS
GAPDH
Low Dose
Mid Dose
High Dose
ZFN
FB
Std
Imp
Std
Imp
Std
Imp
Mouse#
4 5 6 10 11 12 16 17 18 22 23 24 28 29 30 34 35 36 40 41 42
hIDS
GAPDH

FIG.12

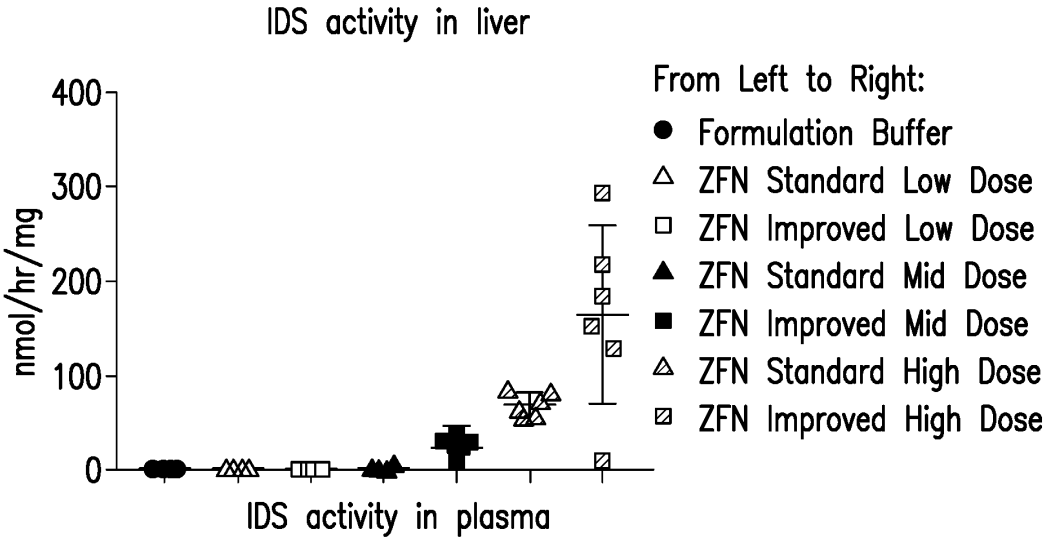

IDS activity in liver
nmol/hr/mg
400
300
200
100
0
IDS activity in plasma
From Left to Right:
Formulation Buffer
ZFN Standard Low Dose
ZFN Improved Low Dose
ZFN Standard Mid Dose
ZFN Improved Mid Dose
ZFN Standard High Dose
ZFN Improved High Dose

FIG.13

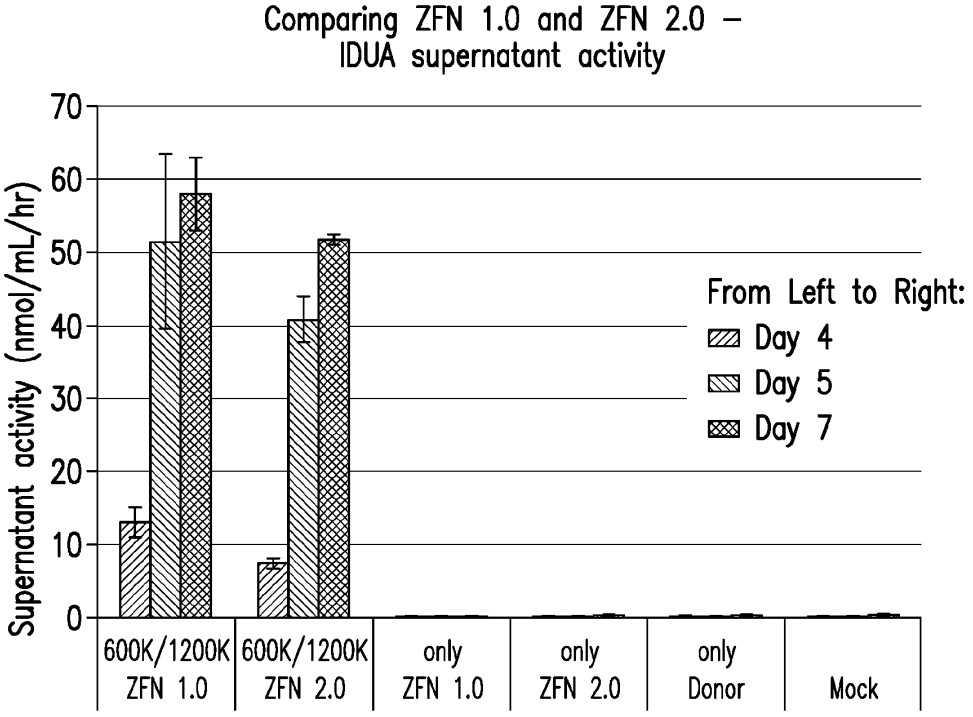


FIG.14A

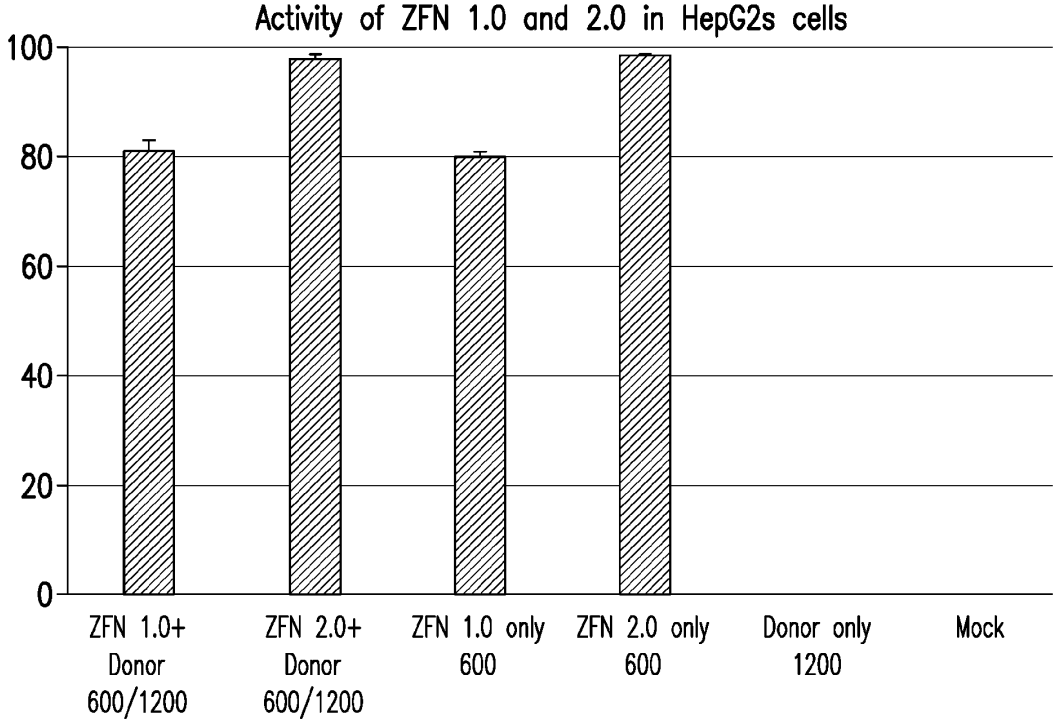


FIG.14B