

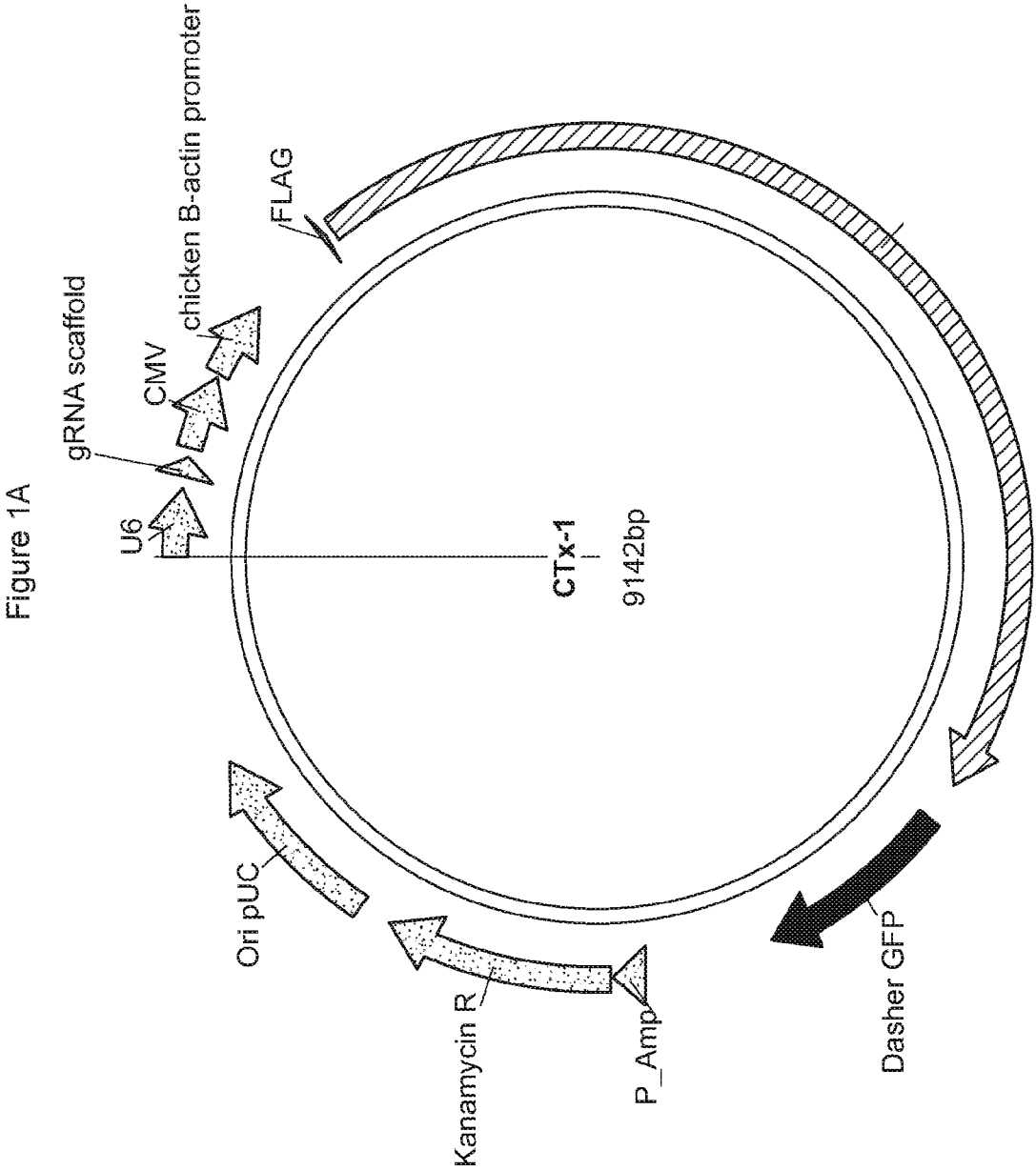


Figure 1B

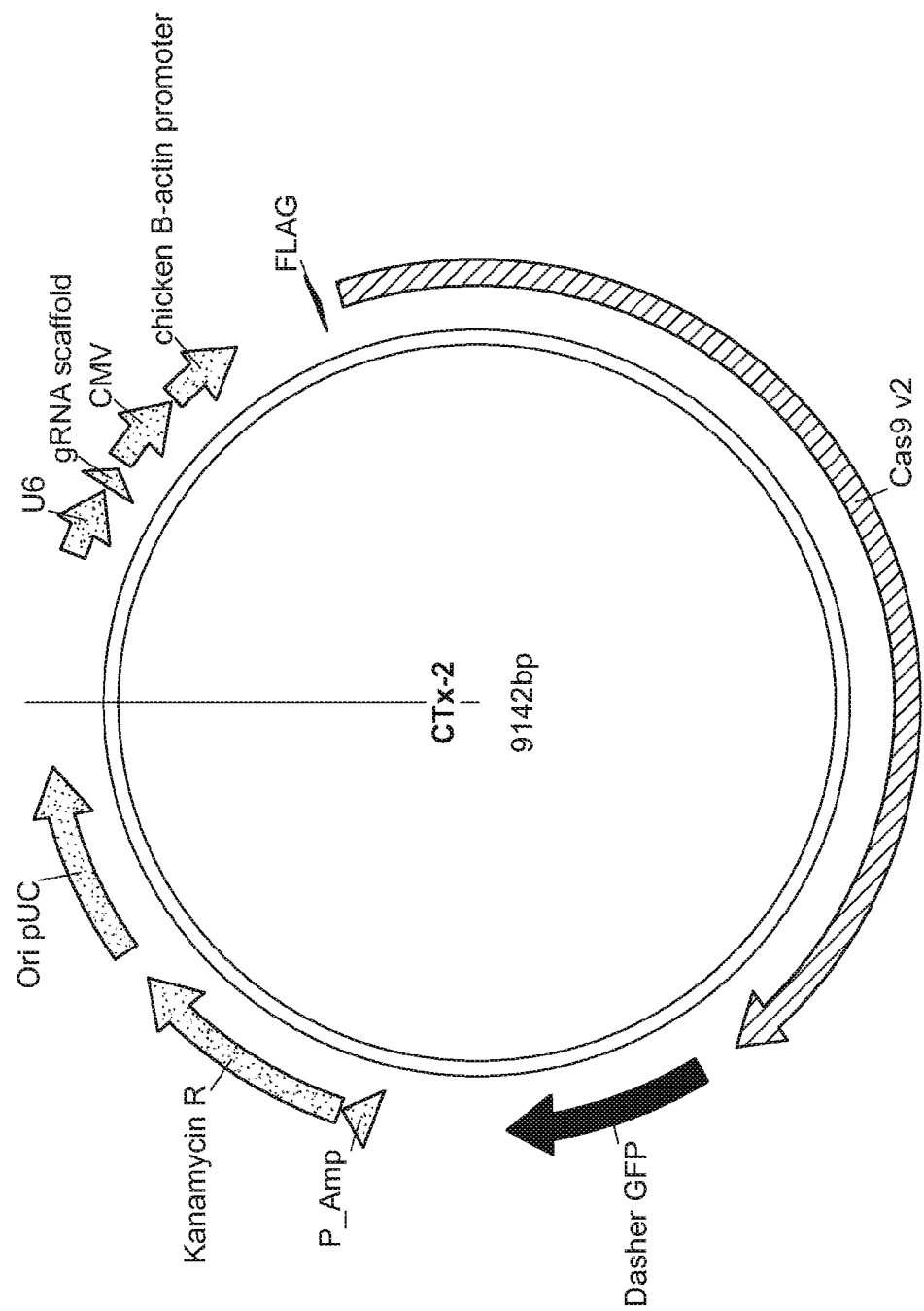


Figure 1C

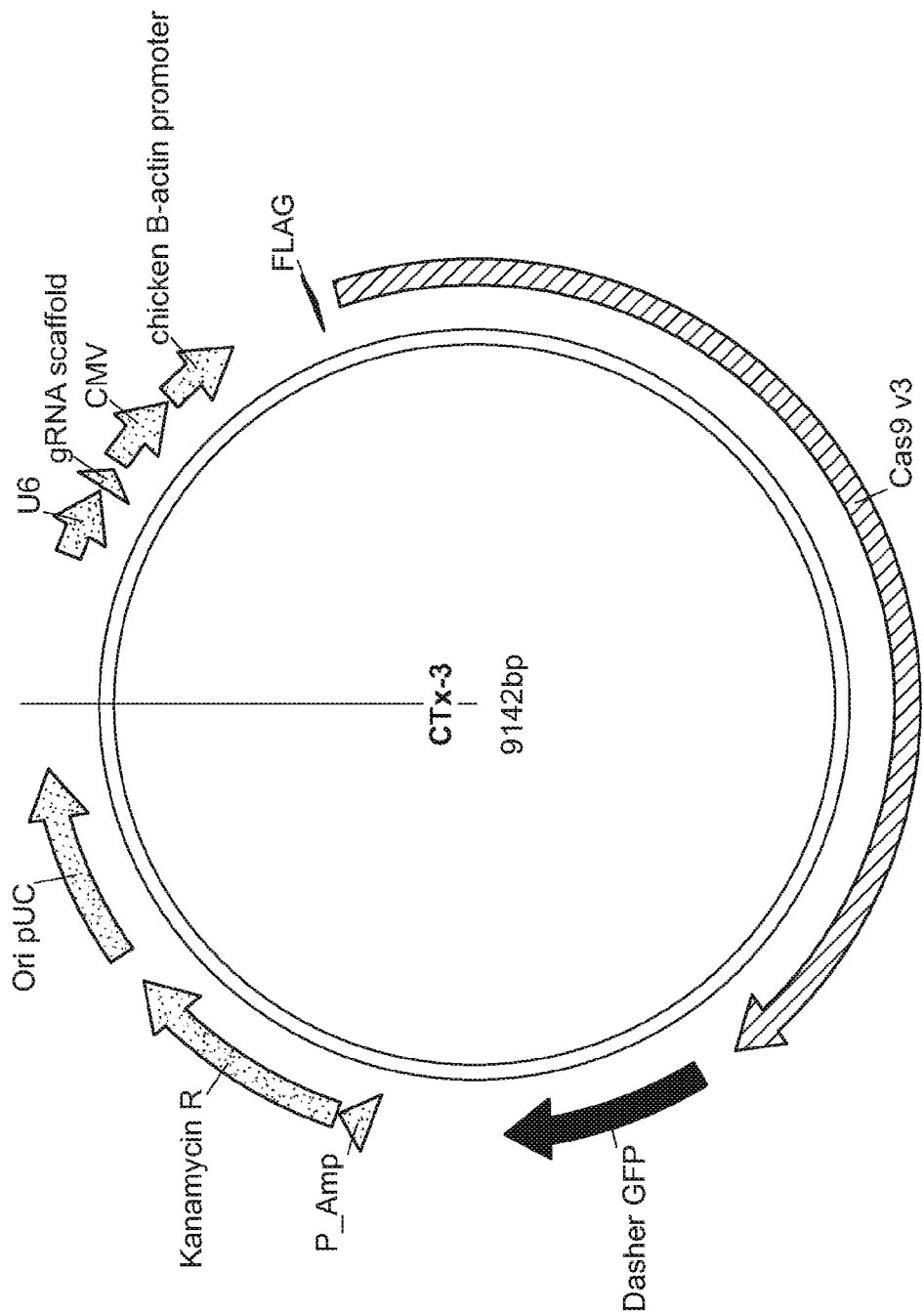


Figure 2A

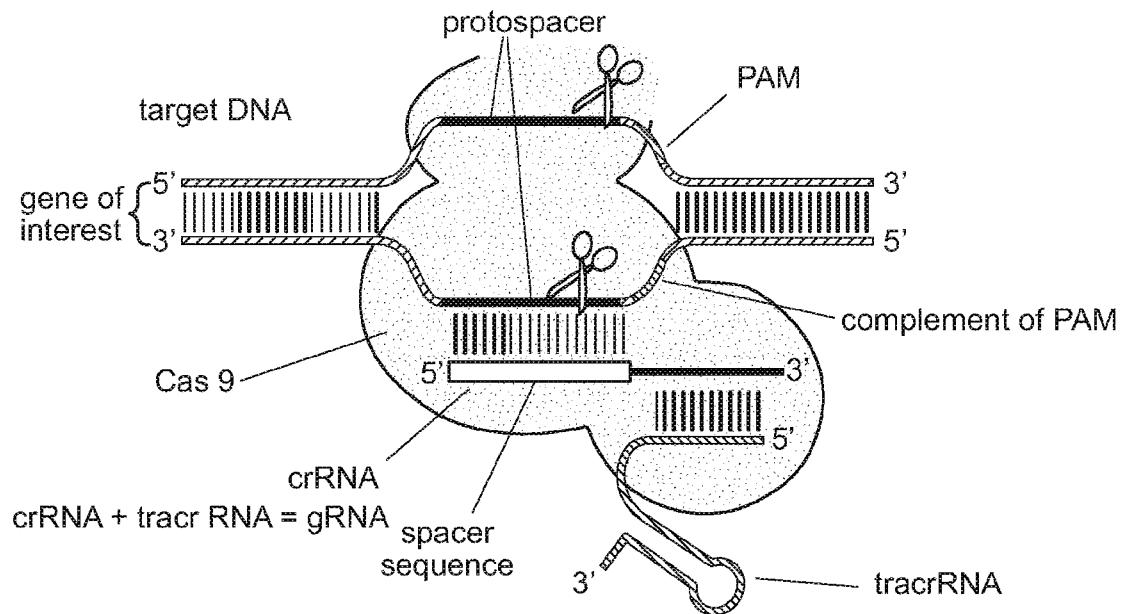


Figure 2B

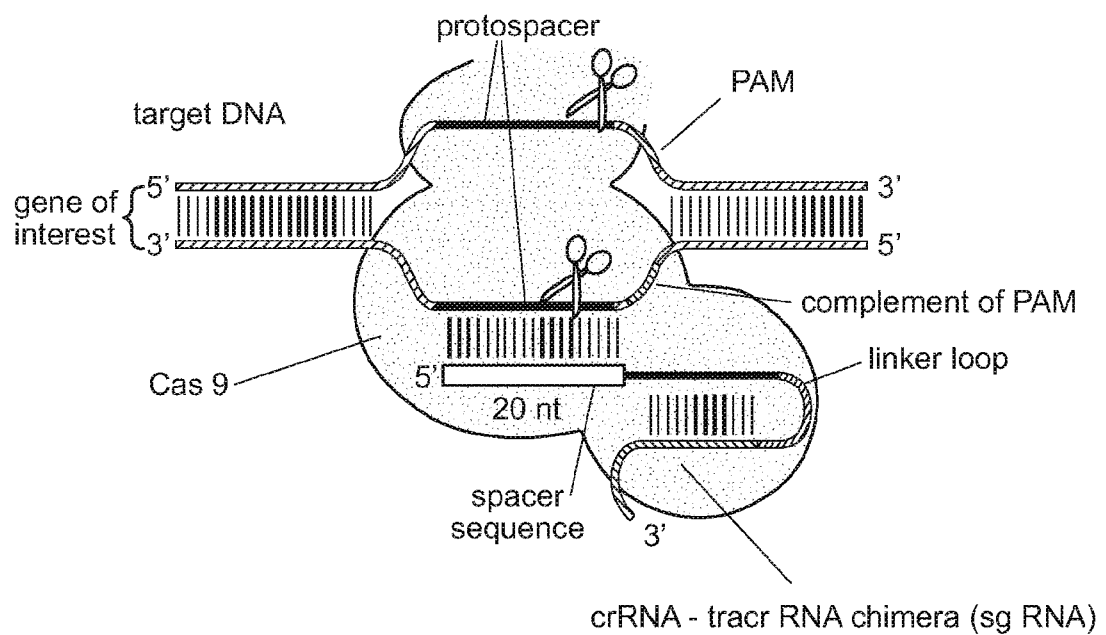


FIGURE 3

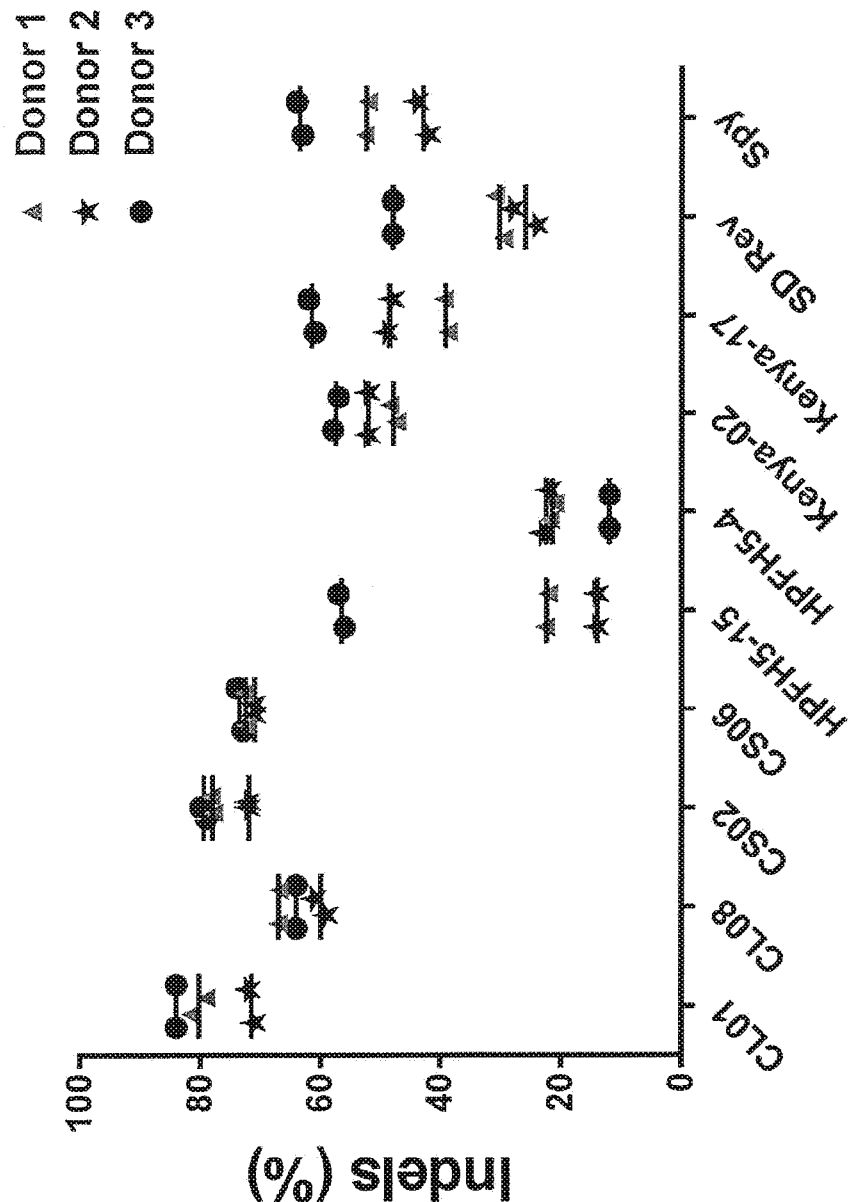


Figure 4A

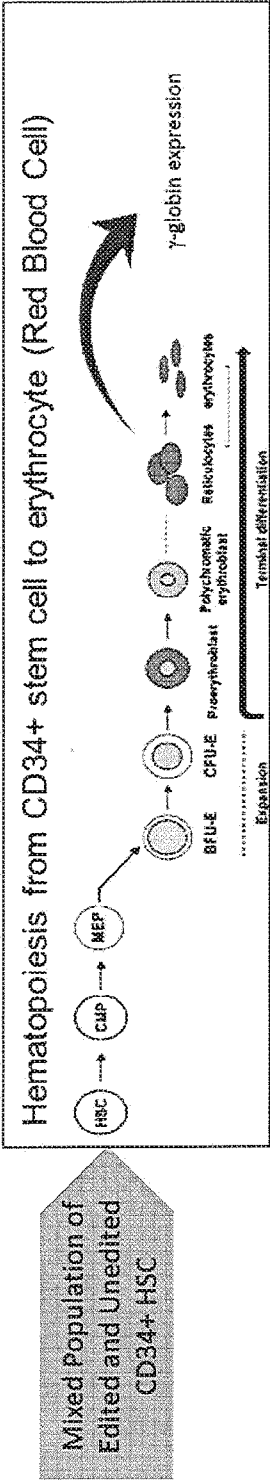


Figure 4B

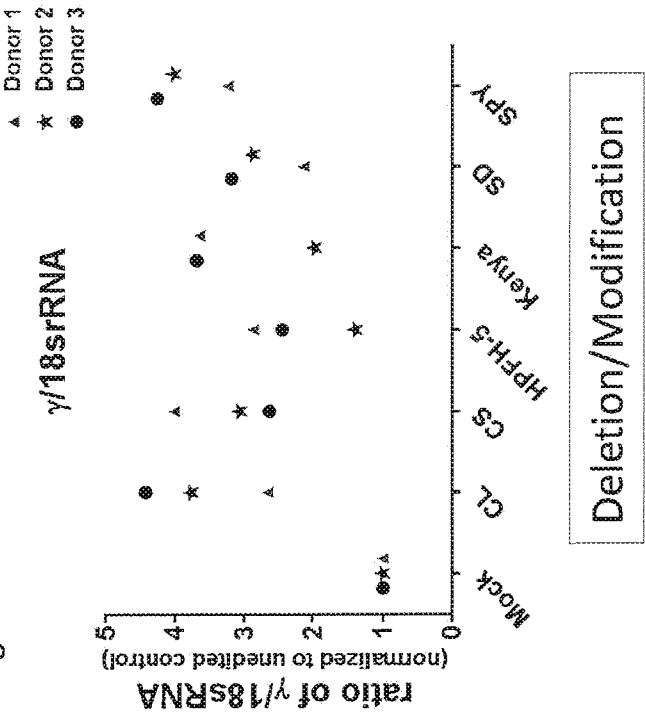
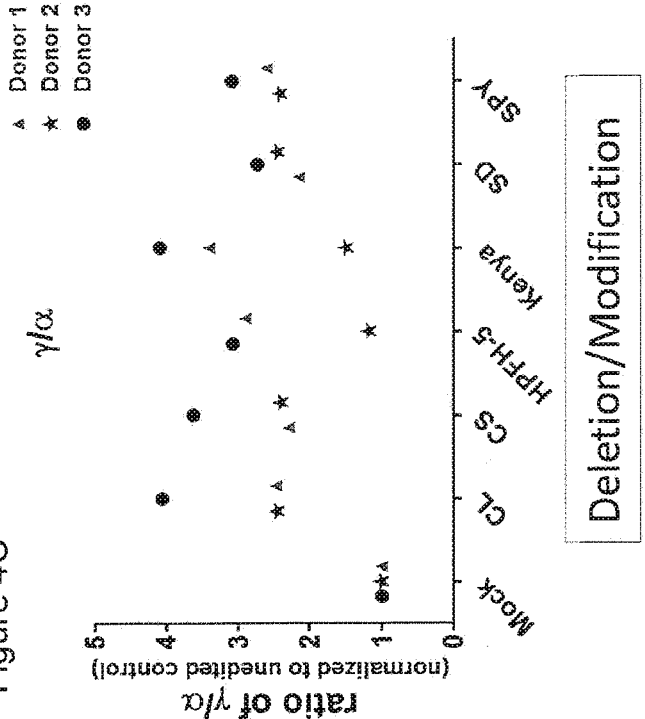


Figure 4C



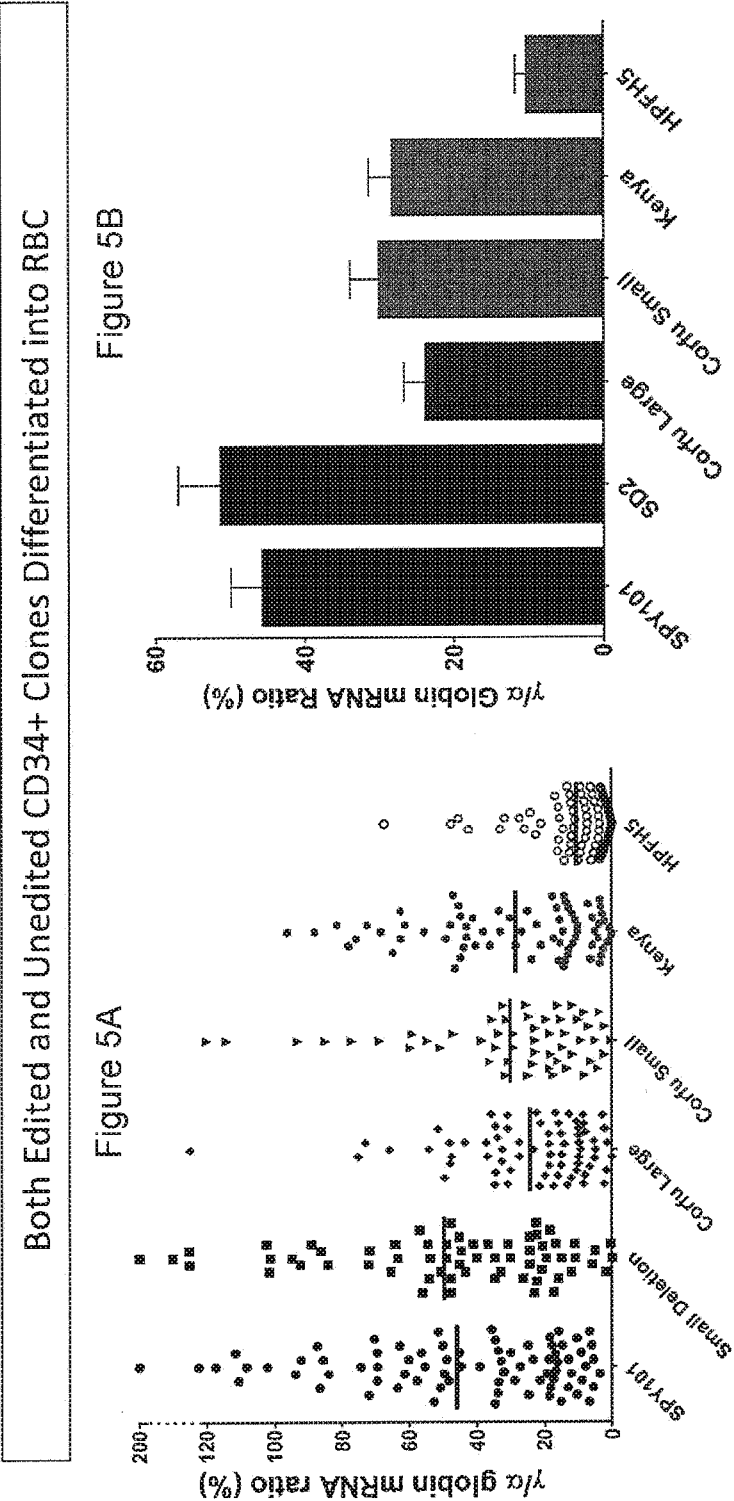
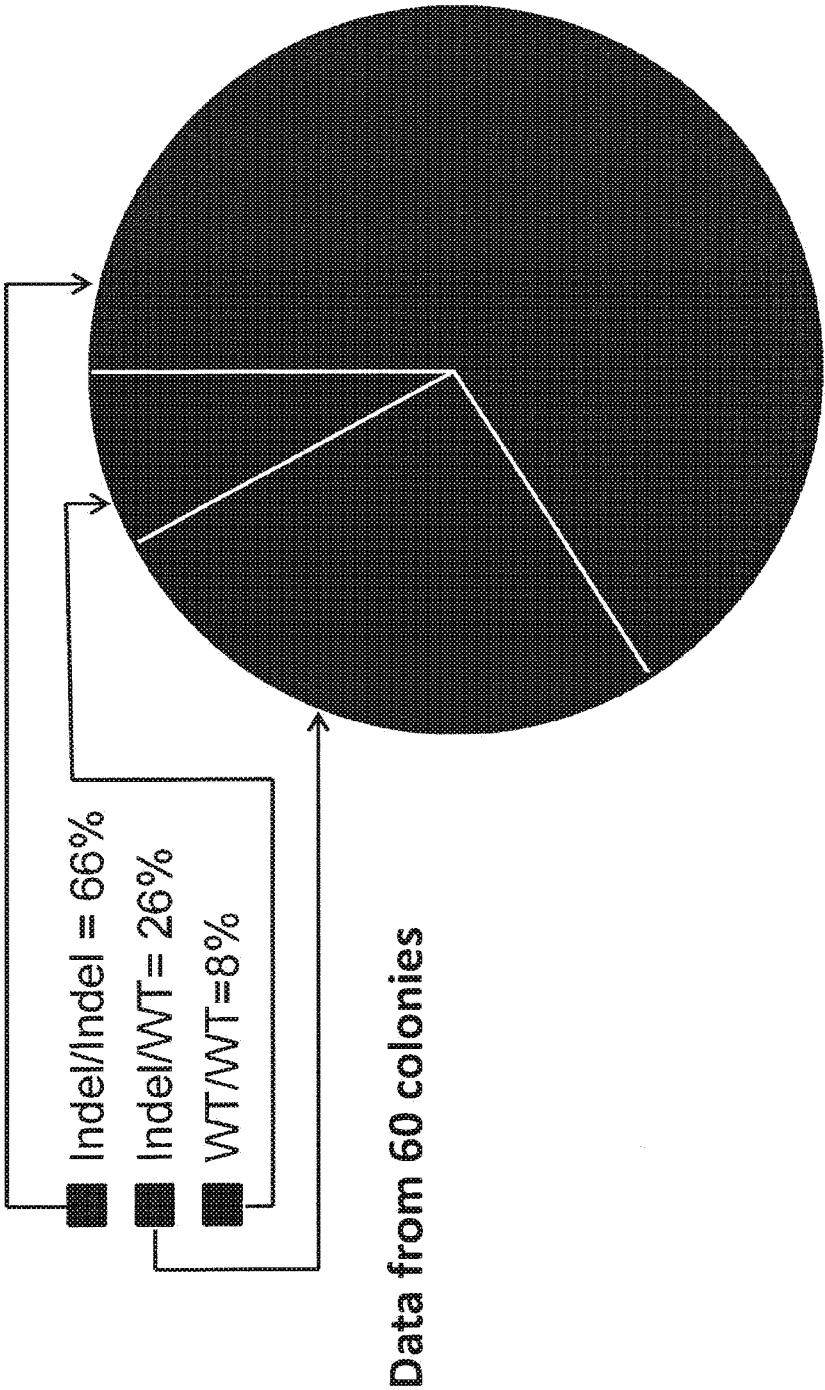


FIGURE 6

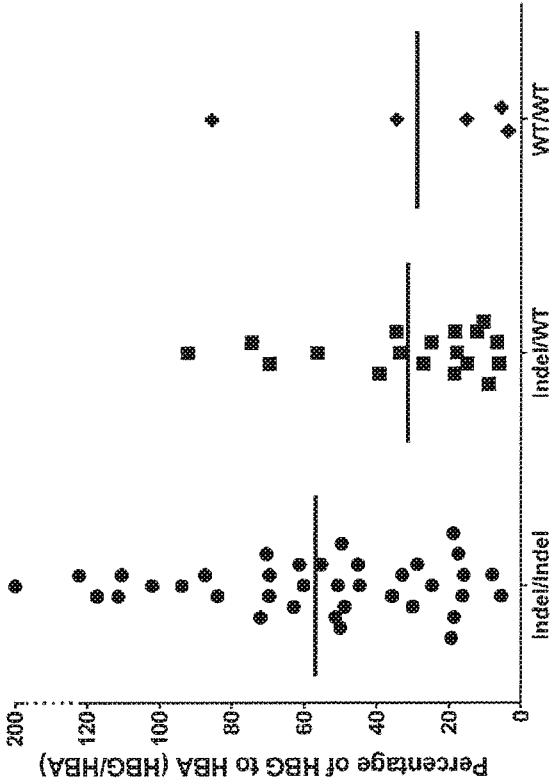
Bcl11a Intron (SPY101) Rate of Editing



Total Indel Frequency = 79%
Edited colonies = 92%

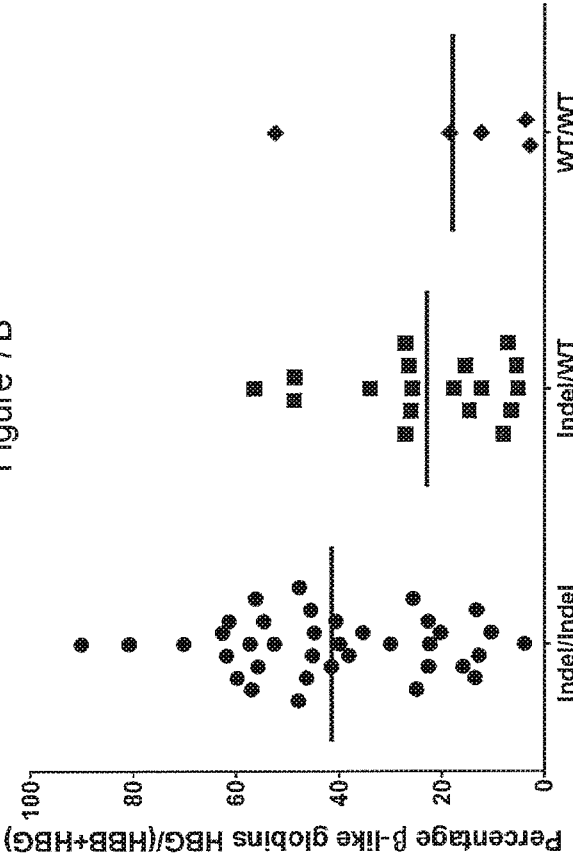
Bcl11a Intron (SPY101) Edit

Figure 7A



HBG/HBA

Figure 7B



HBG/(HBB+HBG)

FIGURE 8

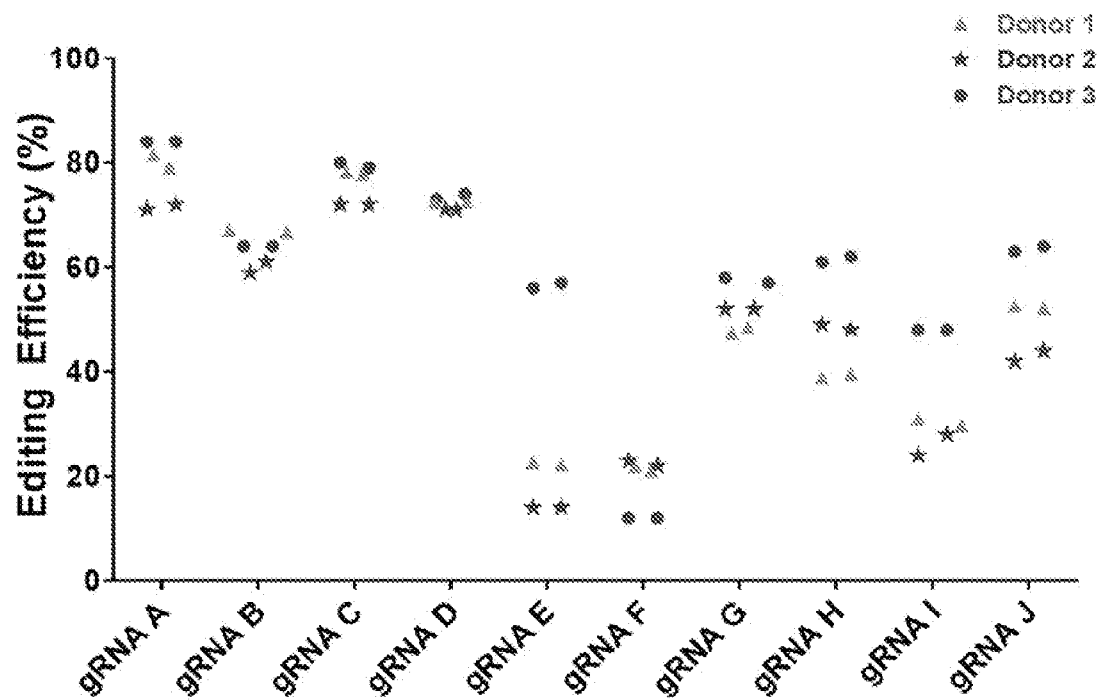


Figure 9A

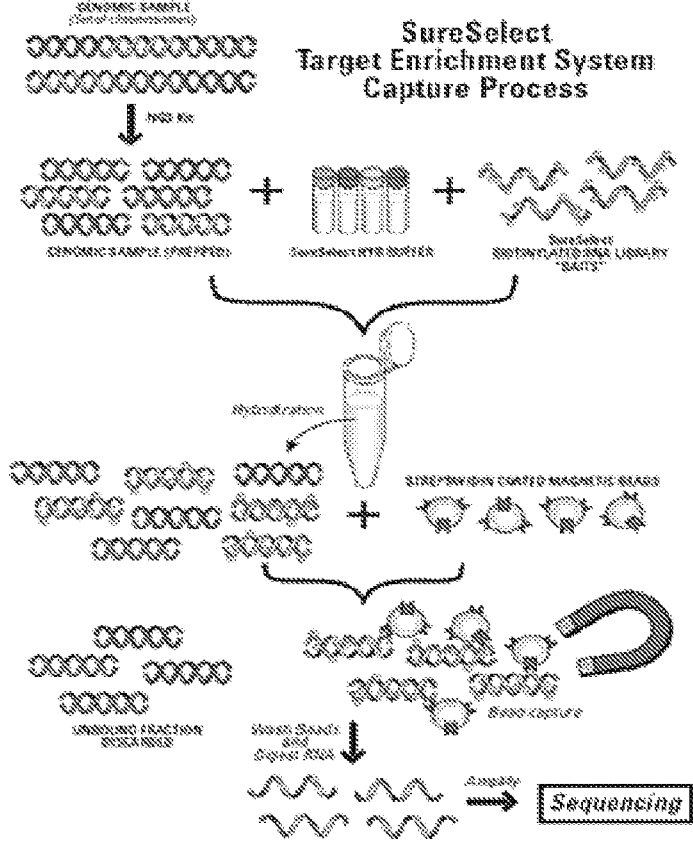


Figure 9B

gRNA	# of Off Target cut sites examined	# of Off Target cut sites detected	Max Off Target cleavage efficiency
A	365	0	-
B	576	0	-
D	330	0	-
E	696	0	-
F	483	0	-
H	676	0	-
I	677	0	-
J	840	0	-
C	419	8	3.6%
G	530	1	0.6%

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Figure 10A

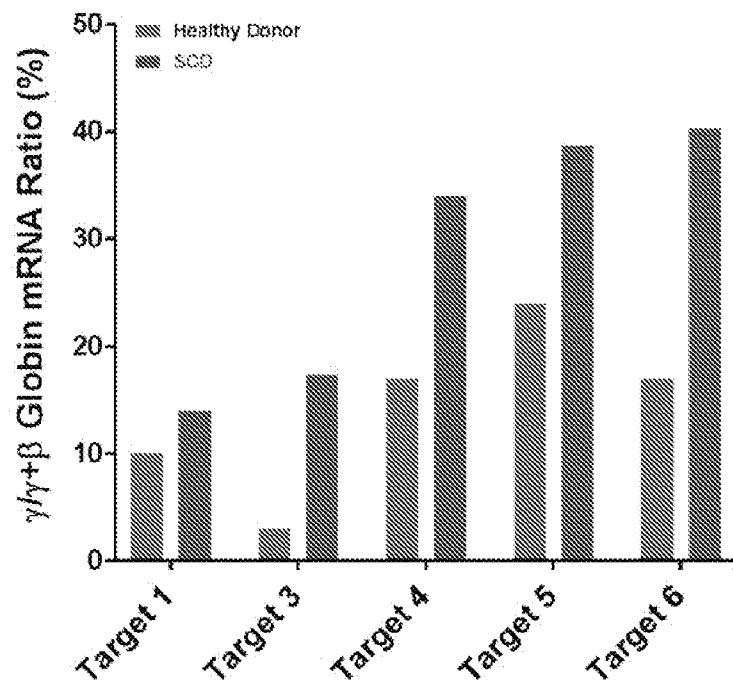


Figure 10B

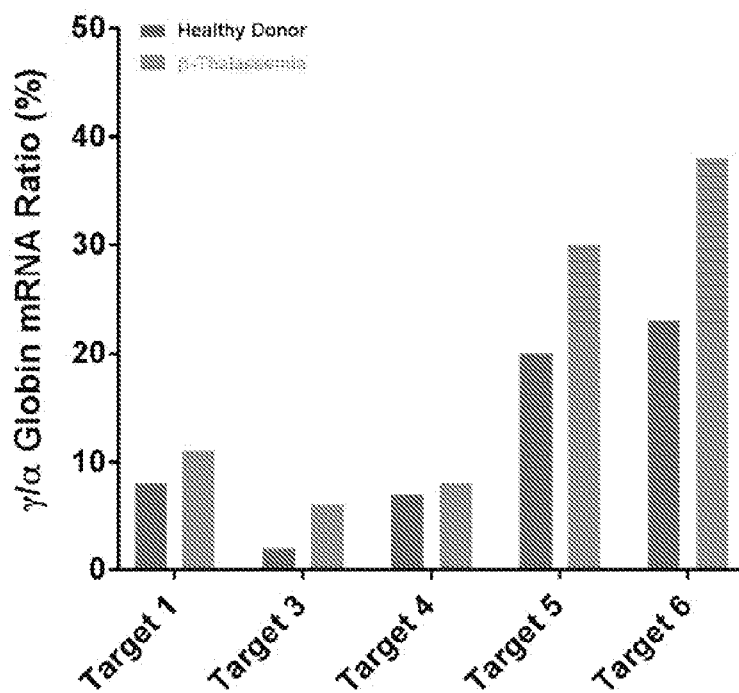


Figure 11A

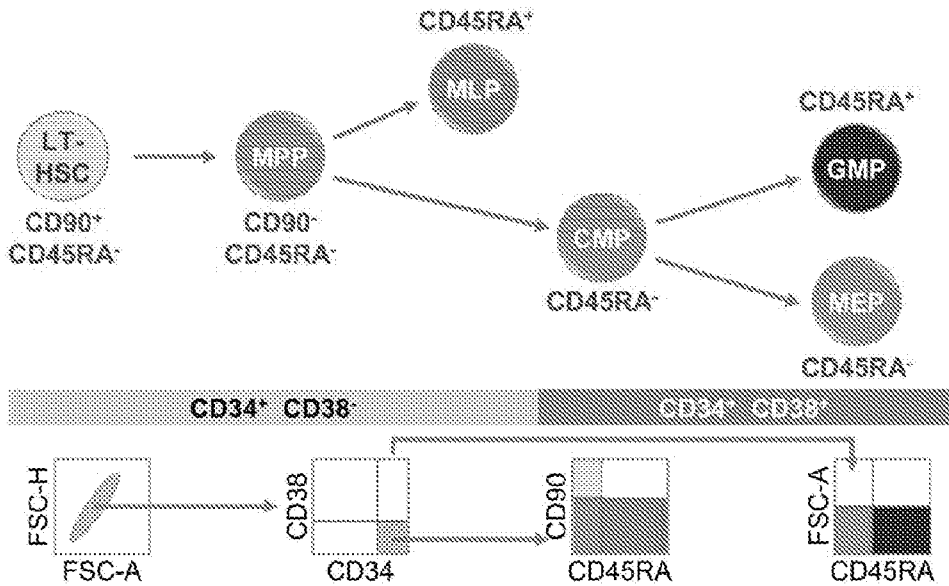


Figure 11B

Distribution of cell types remains unchanged after editing

Population	Frequency	
	<i>Mock</i>	<i>Edited</i>
Bulk	94.83	94.30
LT-HSC	7.82	7.98
MPP	18.97	16.38
MLP	13.23	8.47
CMP/MEP	11.23	11.32
GMP	8.39	13.26

Figure 11C

High editing efficiencies in all cell types

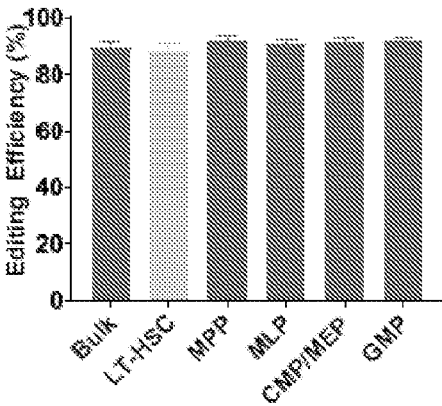
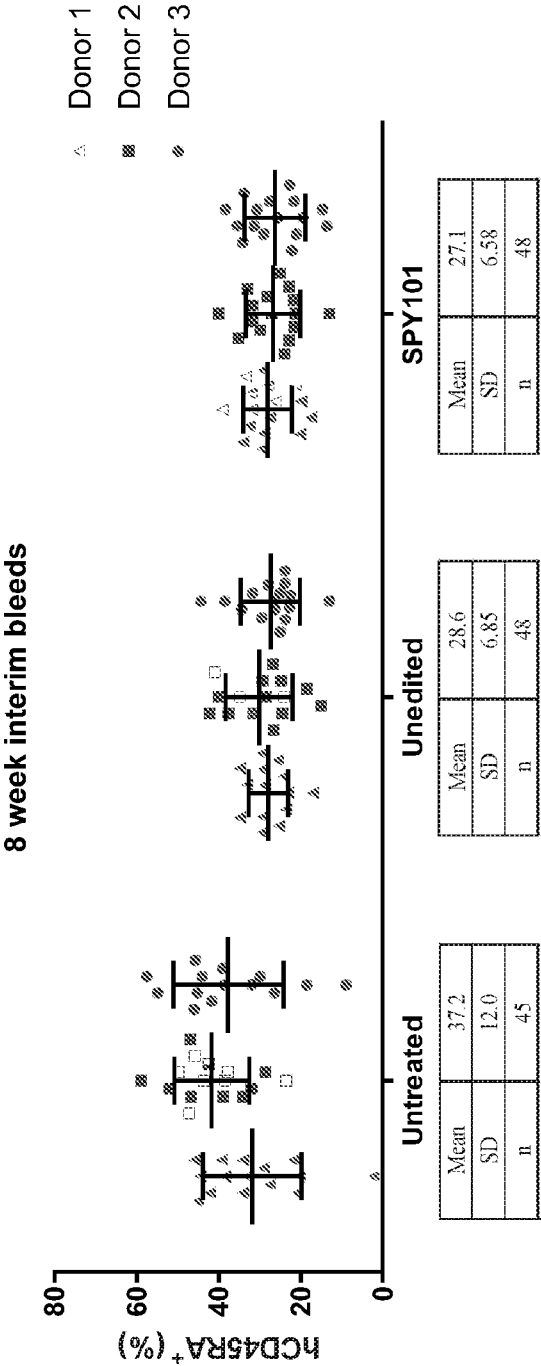


Figure 12



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Figure 13

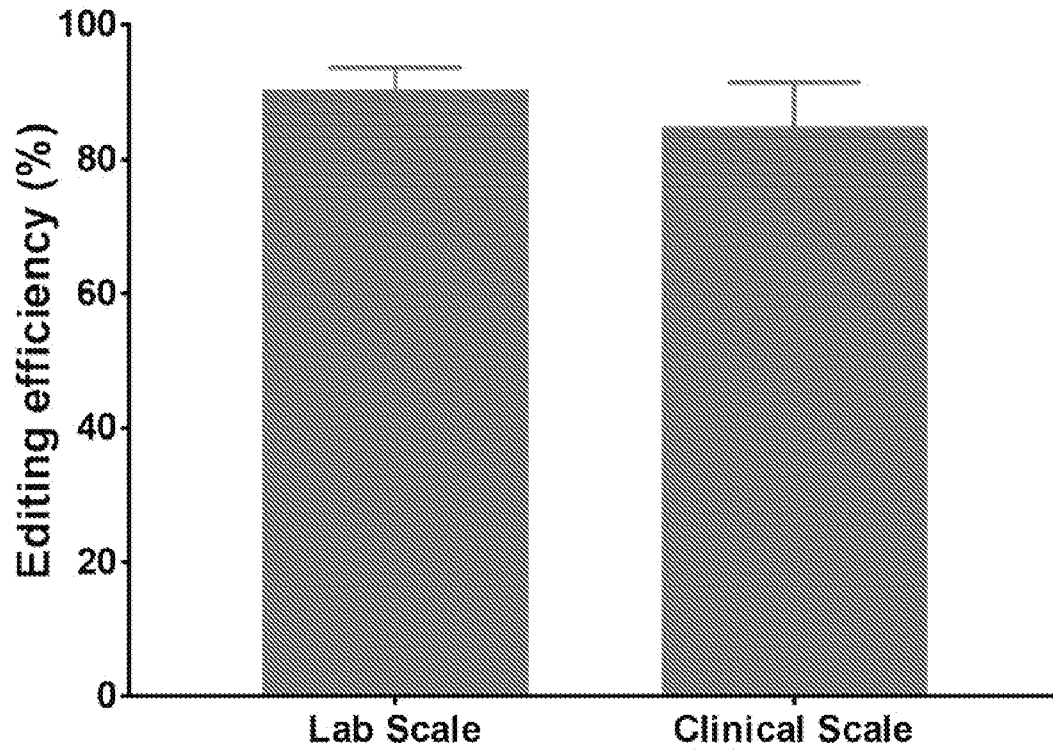


Figure 14

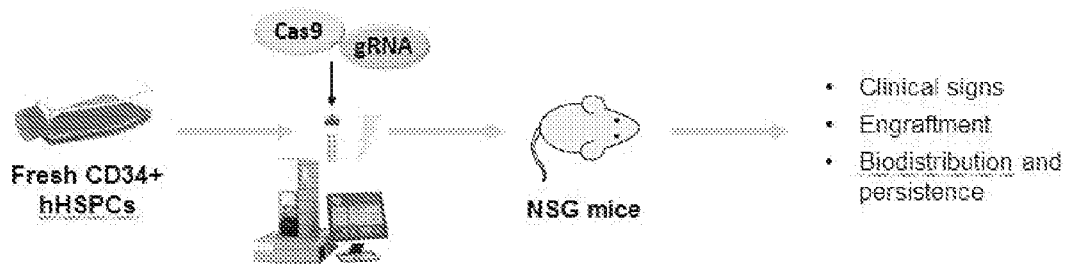
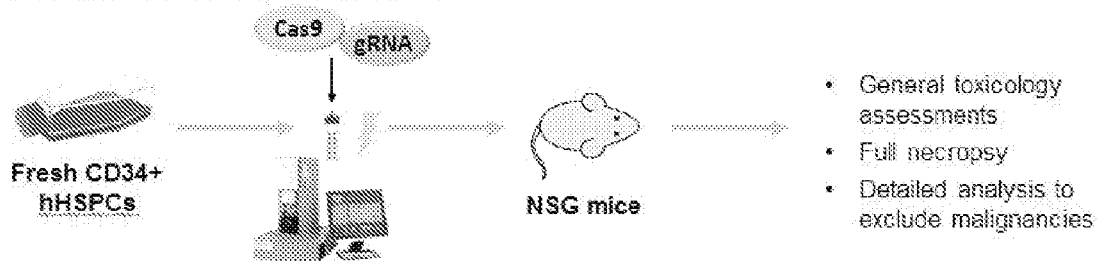
Biodistribution / Persistence Study**Tumorigenicity / General Toxicology Study**

Figure 15

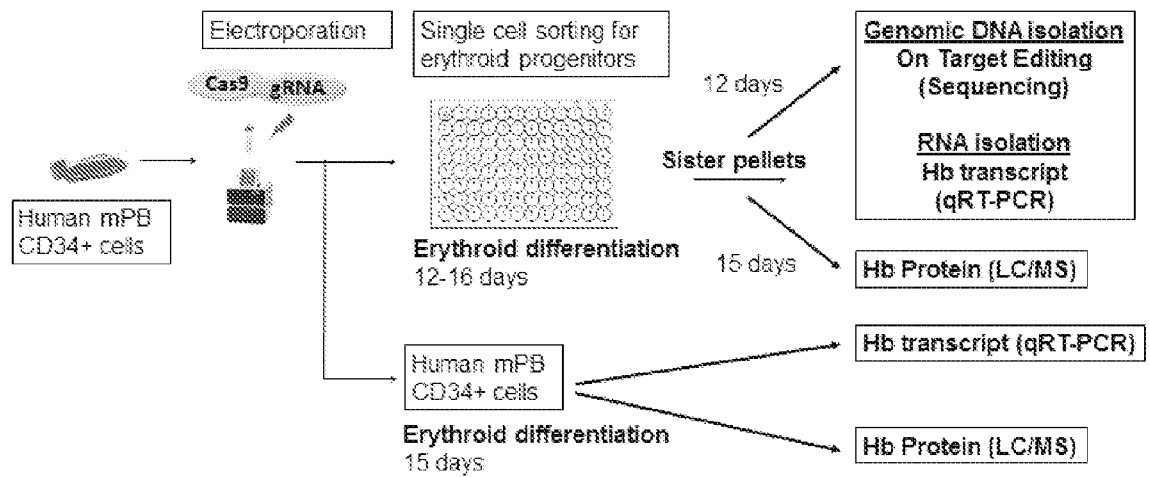
Experimental setup

Figure 16A

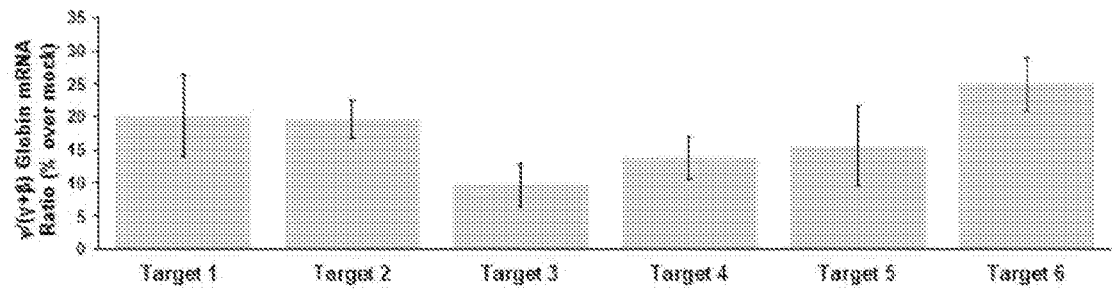
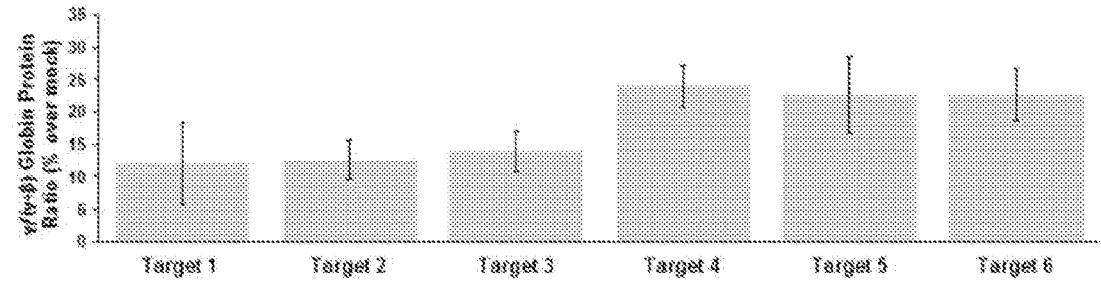


Figure 16B



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Figure 17

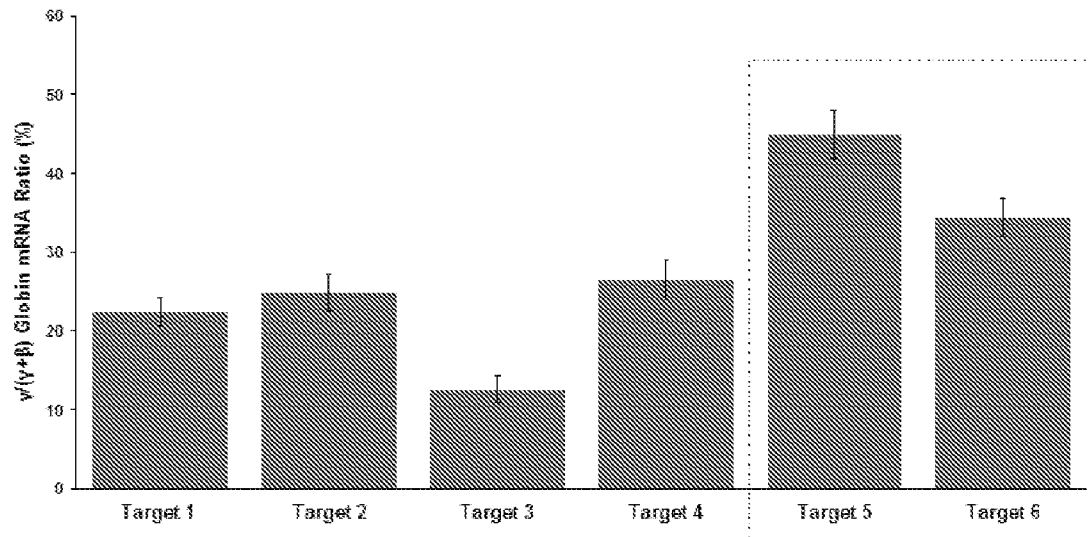


Figure 18B

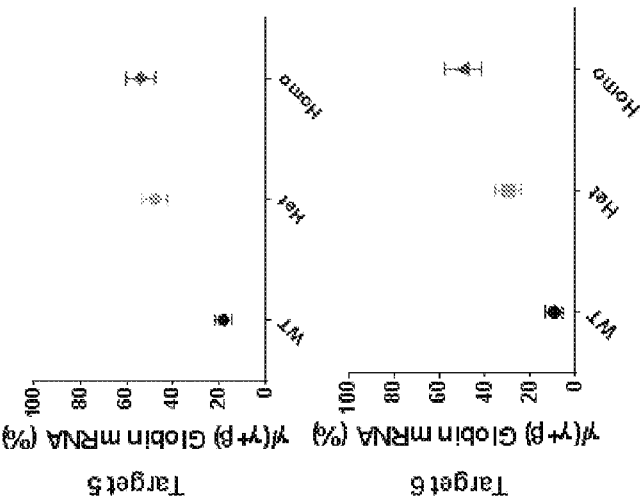


Figure 18A

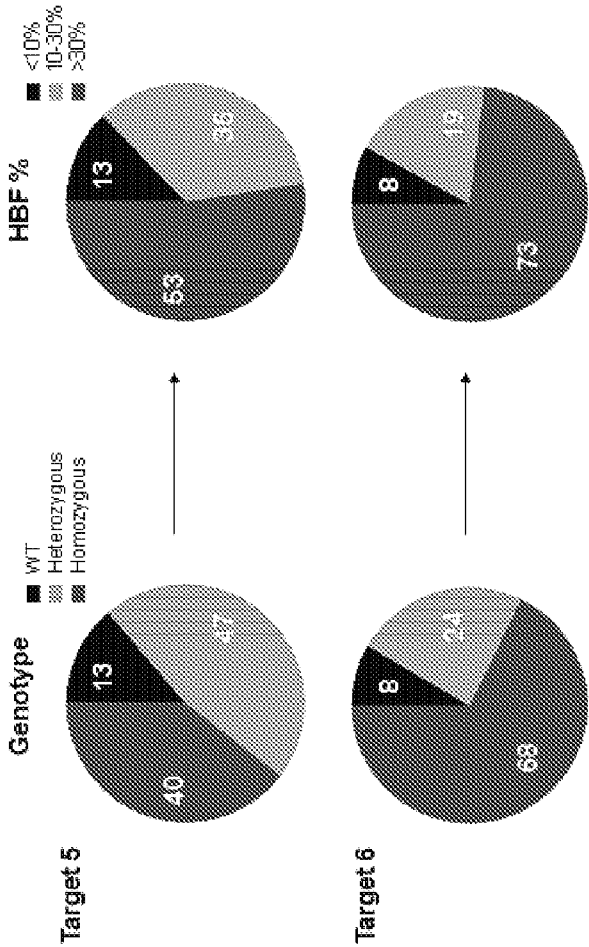
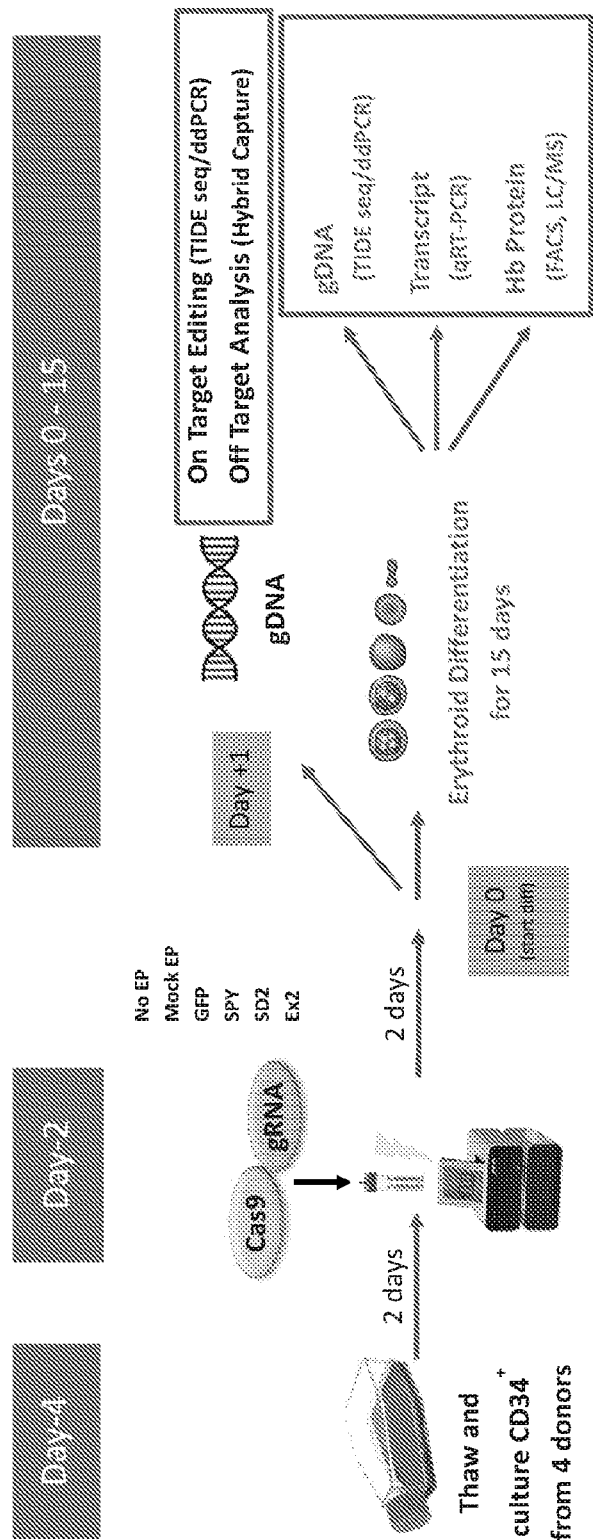


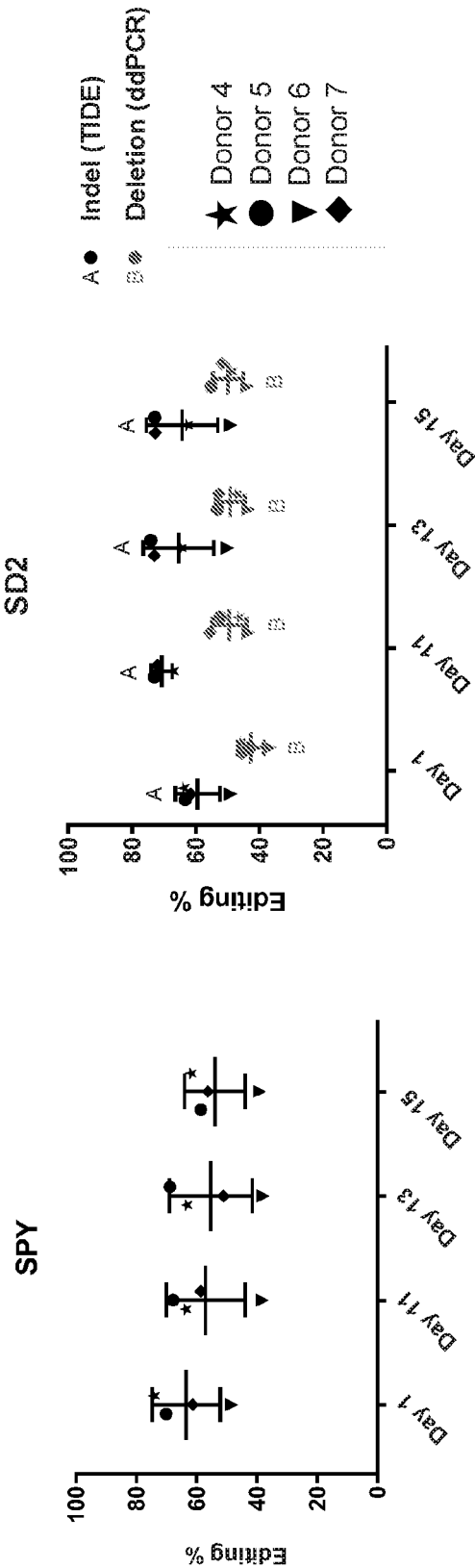
Figure 19
Bulk Workflow and the List of Bulk Data



Editing % maintained throughout ex vivo erythroid differentiation of mPB CD34+ cells

Figure 20A

Figure 20B



Increase in γ -globin transcript post editing

Figure 21A

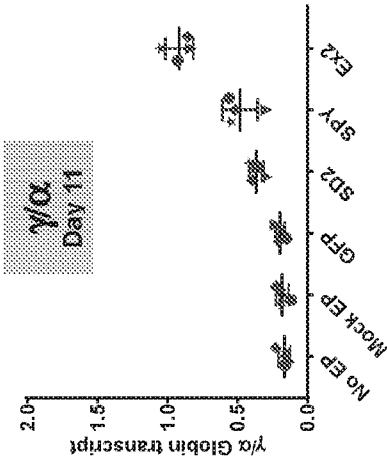


Figure 21B

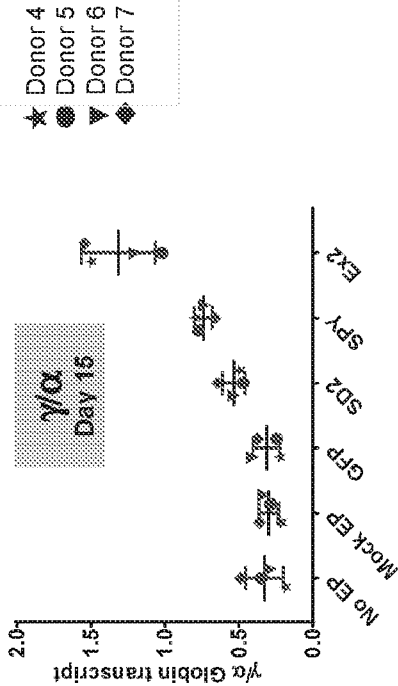


Figure 21C

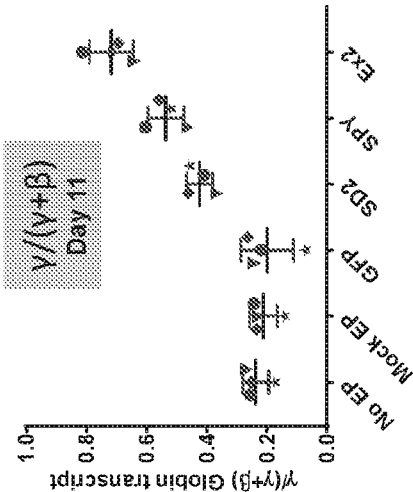
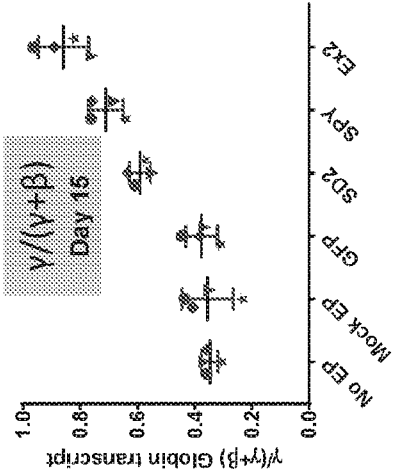


Figure 21D



FACS analysis shows upregulation of γ -globin in edited cells

Figure 22A

FACS

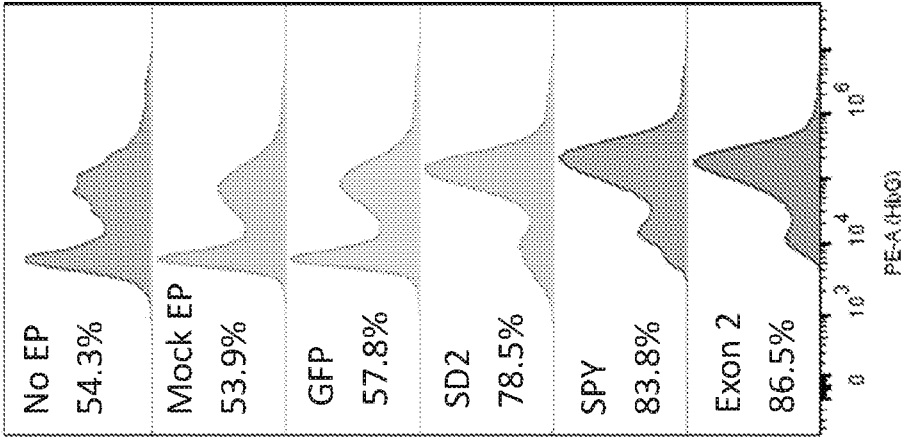
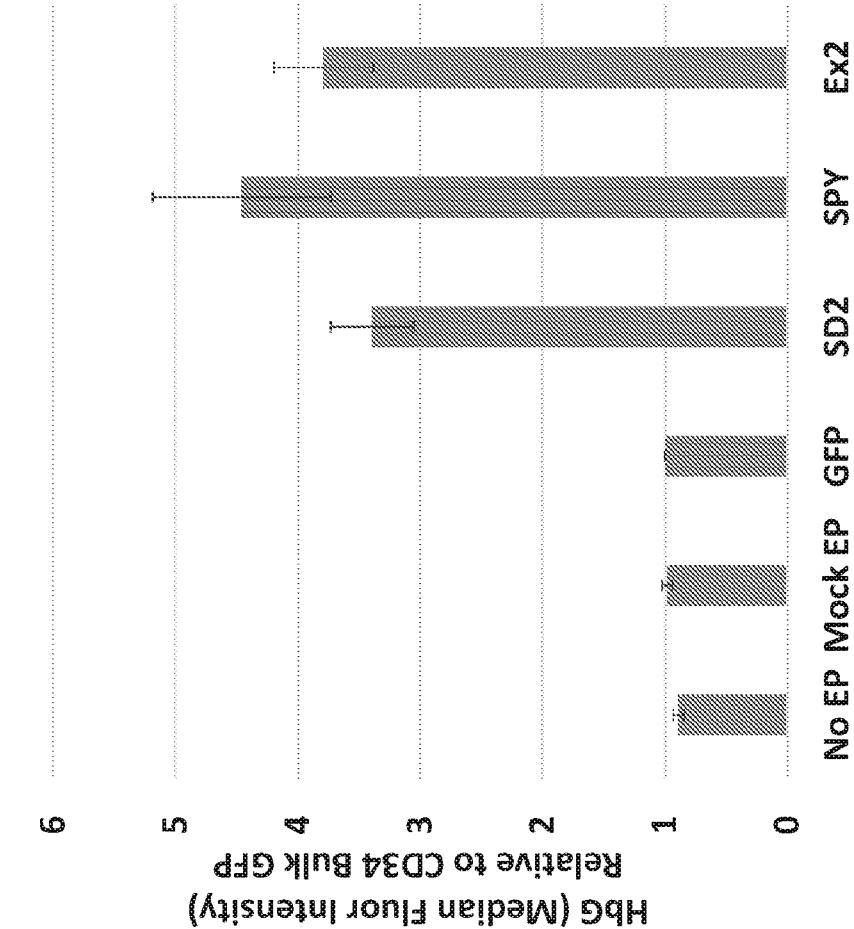


Figure 22B

MFI



Bulk LC-MS data further validates upregulation of γ -globin post editing

Figure 23A

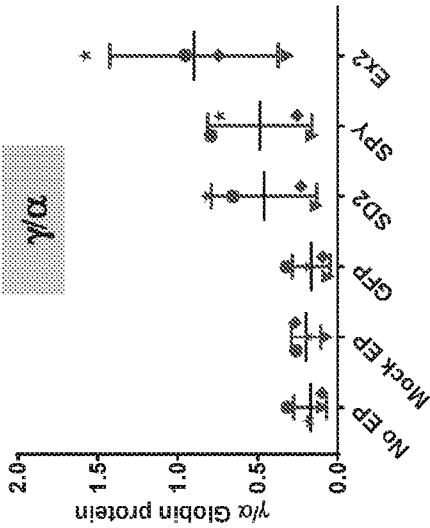


Figure 23B

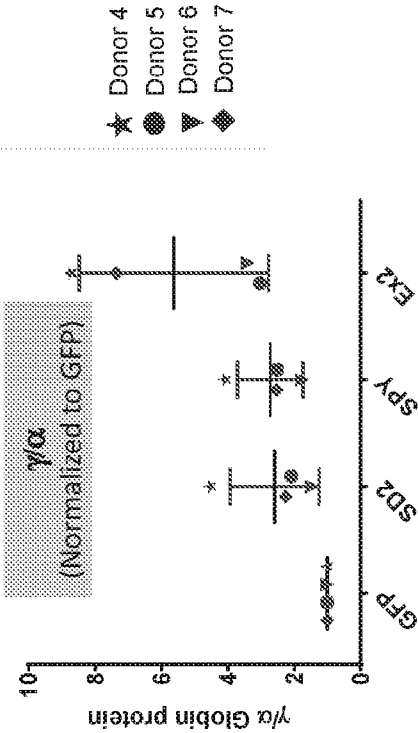


Figure 23C

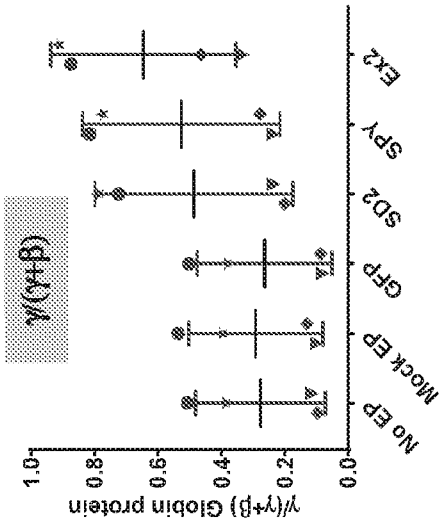


Figure 23D

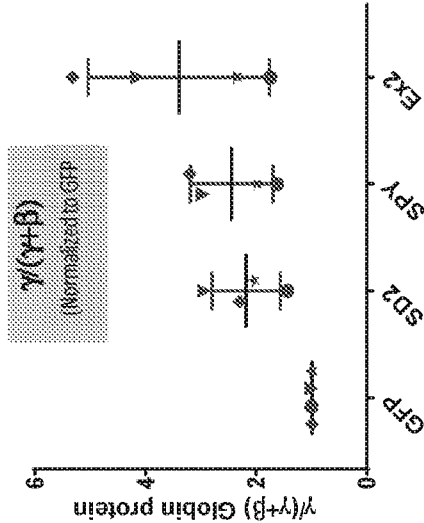
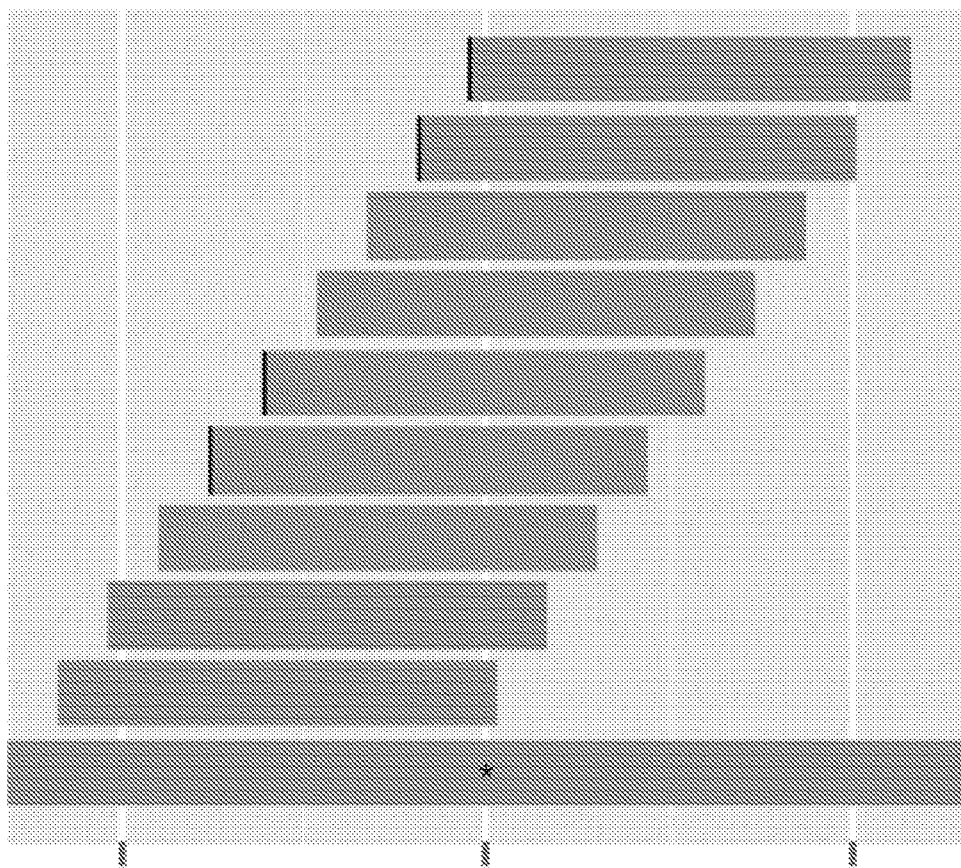


Figure 24



Hybrid Capture Bait Design

Figure 25

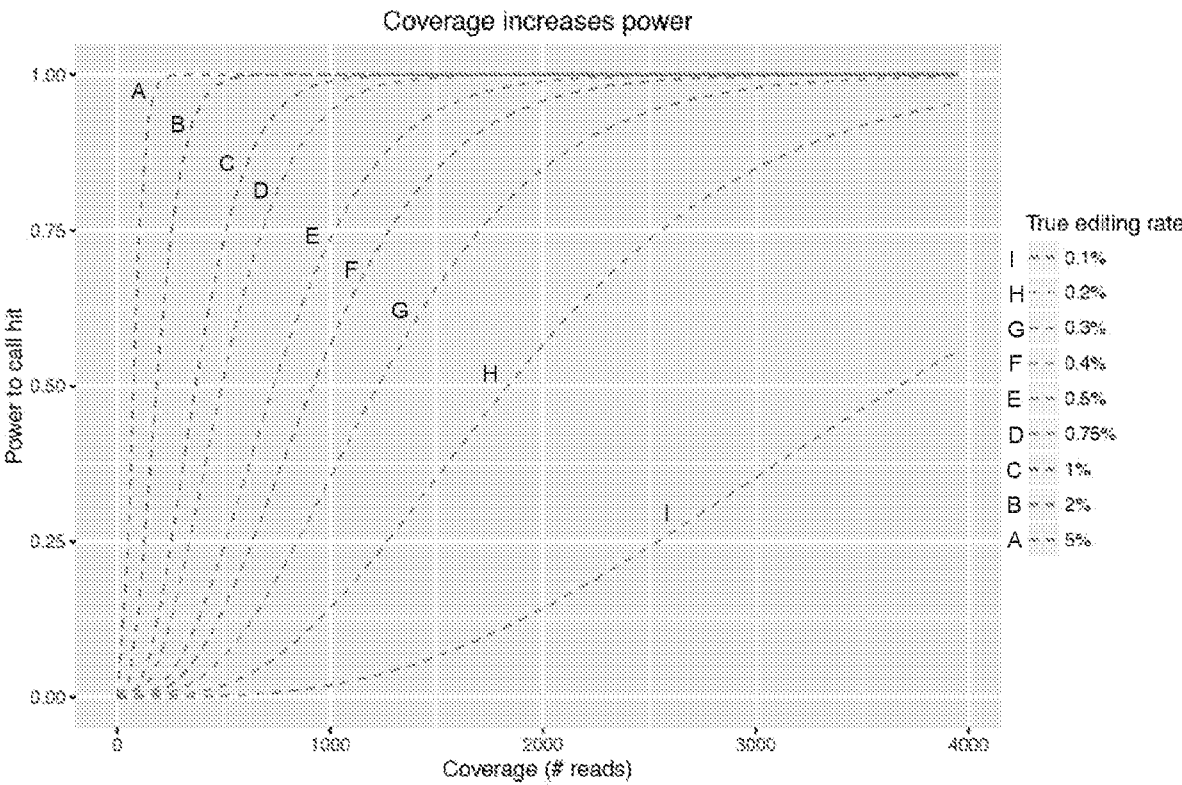


Figure 26
Hybrid capture summary (SPY101)

SPY101	Round 1	Round 2
Number of donors	3	4
Cas9 format	mRNA	RNP
Median sequencing coverage	~800	2492
Number of sites	840	5003
Number of mismatches and types of PAMs	4: NGG and NAG	5: NGG, NAG 4: NGA, NAA, NCG, NGC, NTG, NGT
# of off-target sites with evidence of cutting	0* *Indel freq >0.5%	0 ^Δ ^Δ Indel freq >0.2%

Figure 27
Hybrid capture summary (SD2)

SD2	Round 1	Round 2
Number of donors	3	4
Cas9 format	mRNA	RNP
Median sequencing coverage	~800	2775
Number of sites	677	3272
Number of mismatches and types of PAMs	4: NGG and NAG	5: NGG, NAG 4: NGA, NAA, NCG, NGC, NTG, NGT
# of off-target sites with evidence of cutting*	0* *Indel freq >0.5%	0 ^Δ ^Δ Indel freq >0.2%

Figure 28
Pilot Engraftment Study Plan

Group	Treatment	Radiation dose (cGy)	Radiation Dose Rate (cGy/min)	Number of Female Animals		
				Cohort 1 cell origin: Donor #1	Cohort 2 cell origin: Donor #2	Cohort 3 cell origin: Donor #3
1	Untreated cells	200		16	16	16
2	"mock electroporation" of cells without any CRISPR/Cas9 editing components	200		16	16	16
3	Electroporation of cells with GFP Guide RNA and Cas9 protein	200	160	16	16	16
4	Electroporation of cells with SPY101 gRNA and Cas9 protein	200		16	16	16
5	Electroporation of cells with SD2 gRNA and Cas9 protein	200		16	16	16
6	Control Untreated	200		10	10	10

Figure 29A

8 Week hCD45RA (Untx)

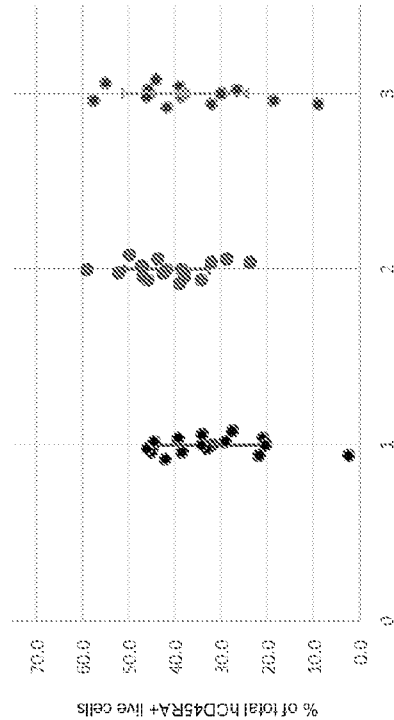


Figure 29B

8 Week hCD45RA (Mock)

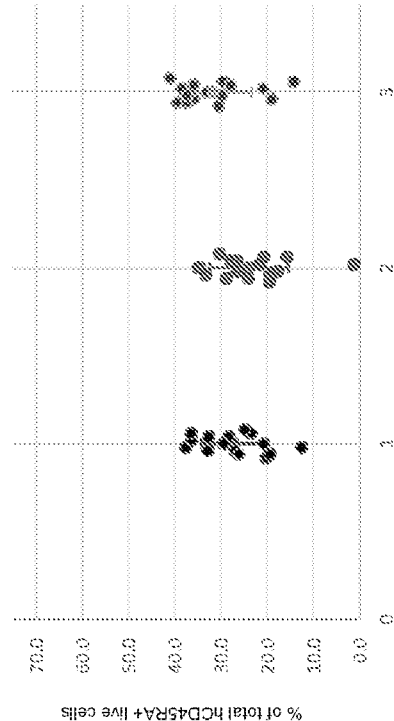
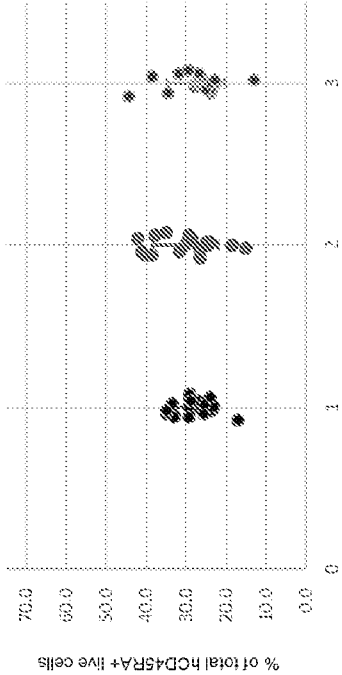


Figure 29C

8 Week hCD45RA (GFP)



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Figure 29D

8 Week hCD45RA (Spy101)

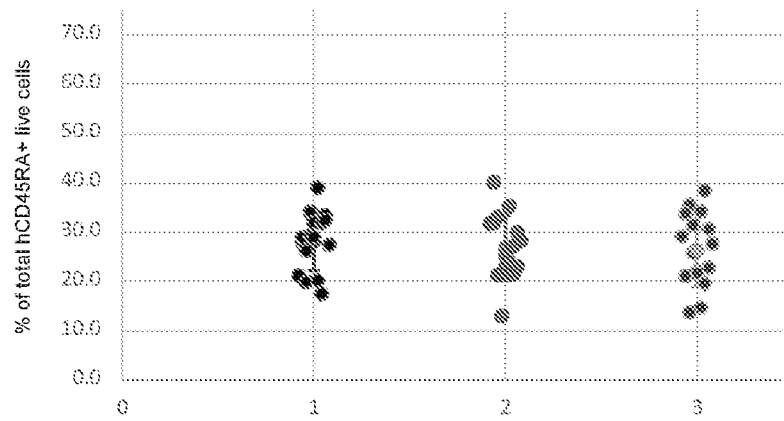


Figure 29E

8 Week hCD45RA (SD2)

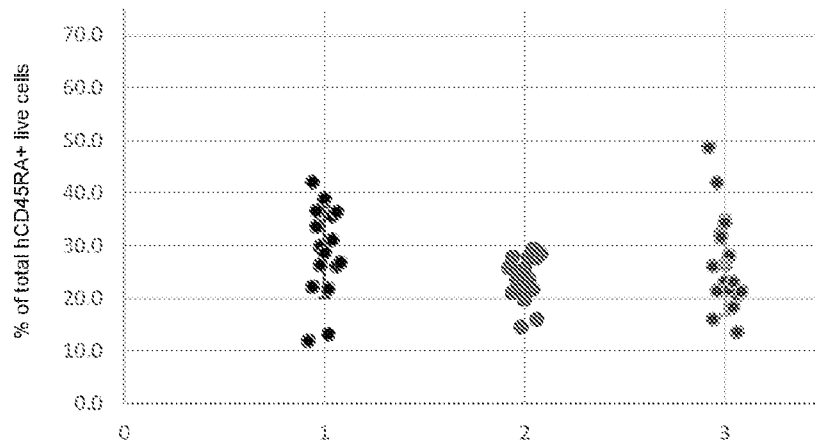


Figure 30

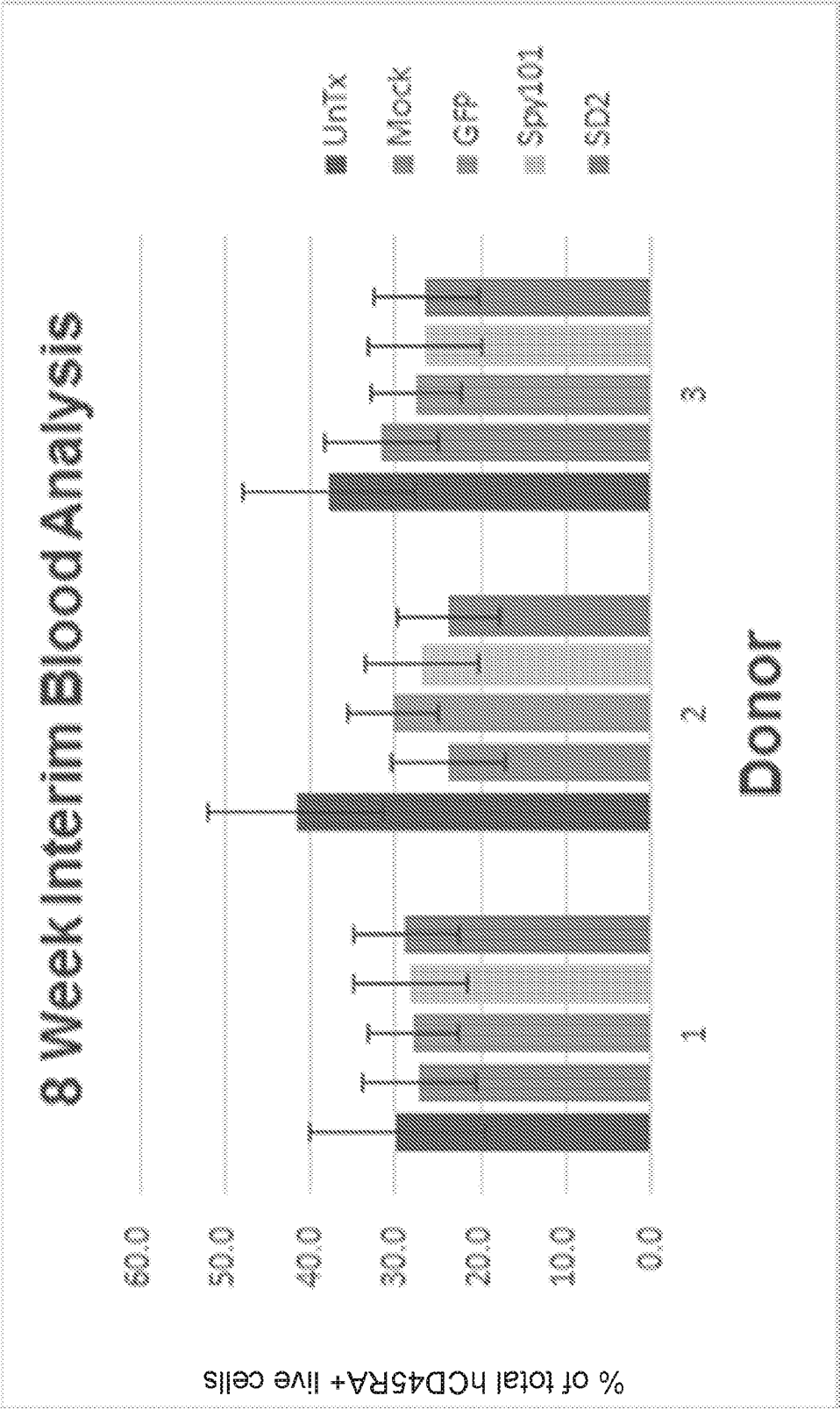
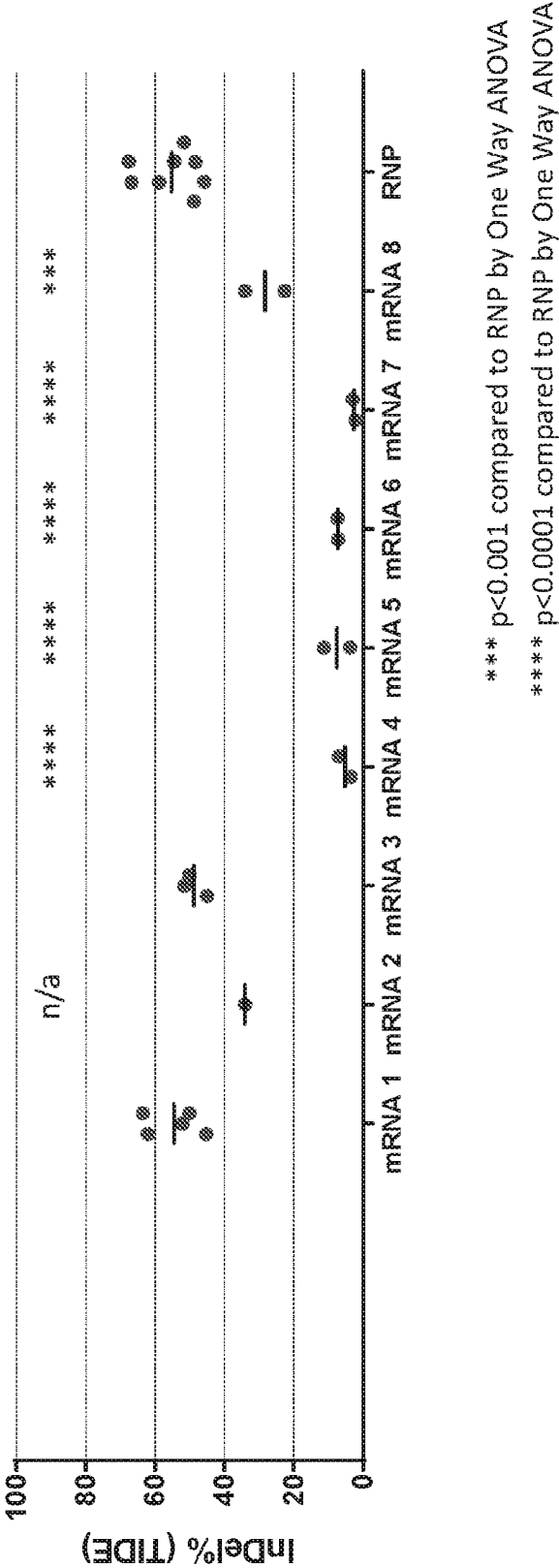


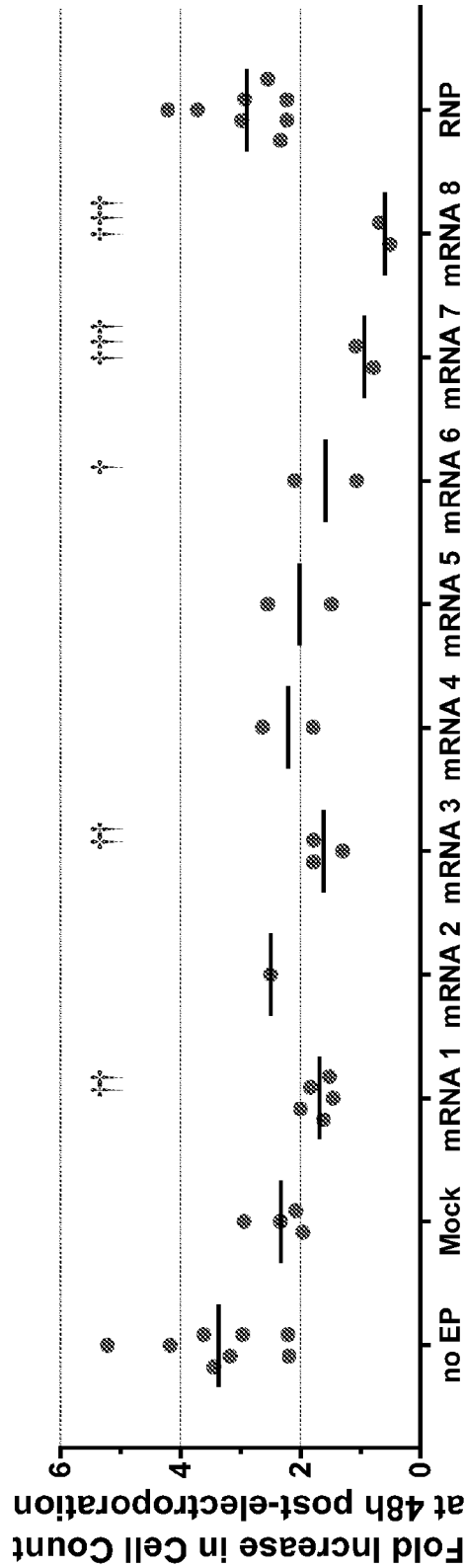
Figure 31

Cas9 RNP is as efficient in editing as Cas9 mRNA but superior in post-electroporation viability



Cas9 RNP is superior to Cas9 mRNA in post-electroporation cell viability

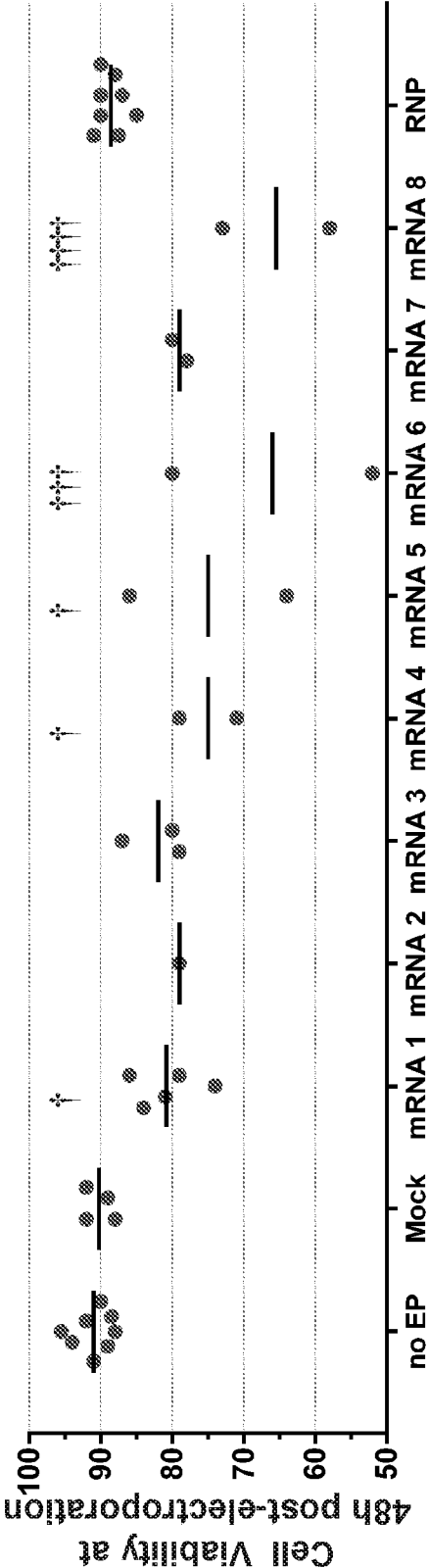
Figure 32A



† p<0.05, †† p<0.01, ††† p<0.001, †††† p<0.0001 compared to No EP by One Way ANOVA

Cas9 RNP is superior to Cas9 mRNA in post-electroporation cell viability

Figure 32B



+ p<0.05, ++ p<0.01, +++ p<0.001, ++++ p<0.0001 compared to No EP by One Way ANOVA

Figure 33A Cas9 RNP optimization

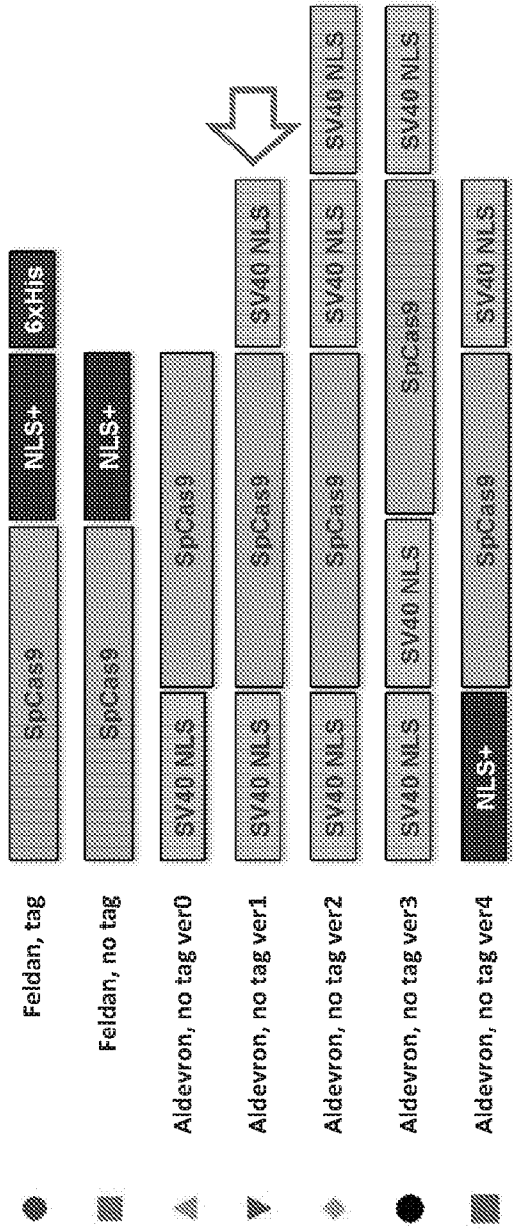


Figure 33B SPY101 (Cas9 1ug: gRNA 1ug)

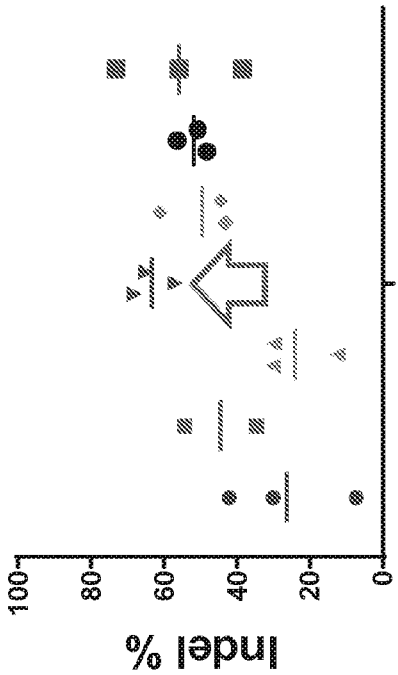
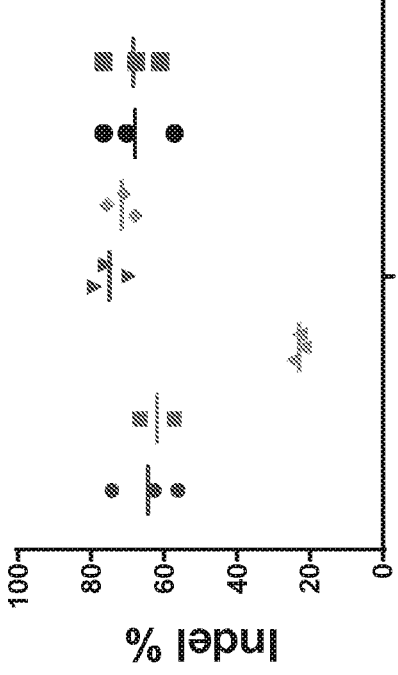


Figure 33C SPY101 Cas9 3ug: gRNA 3ug)



Bulk Editing Efficiency for SPY101

Figure 34A

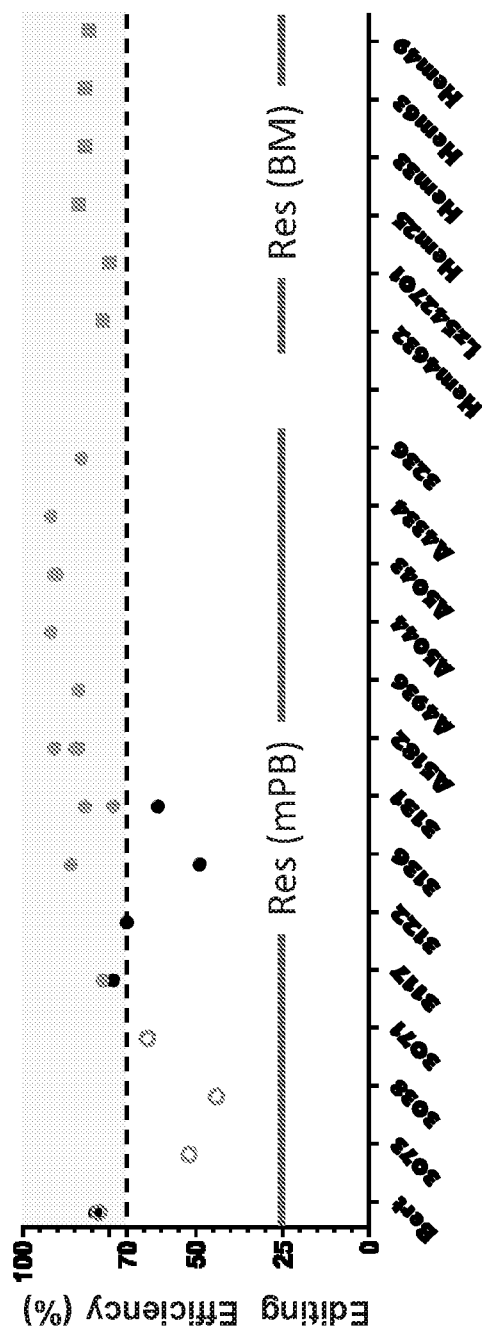
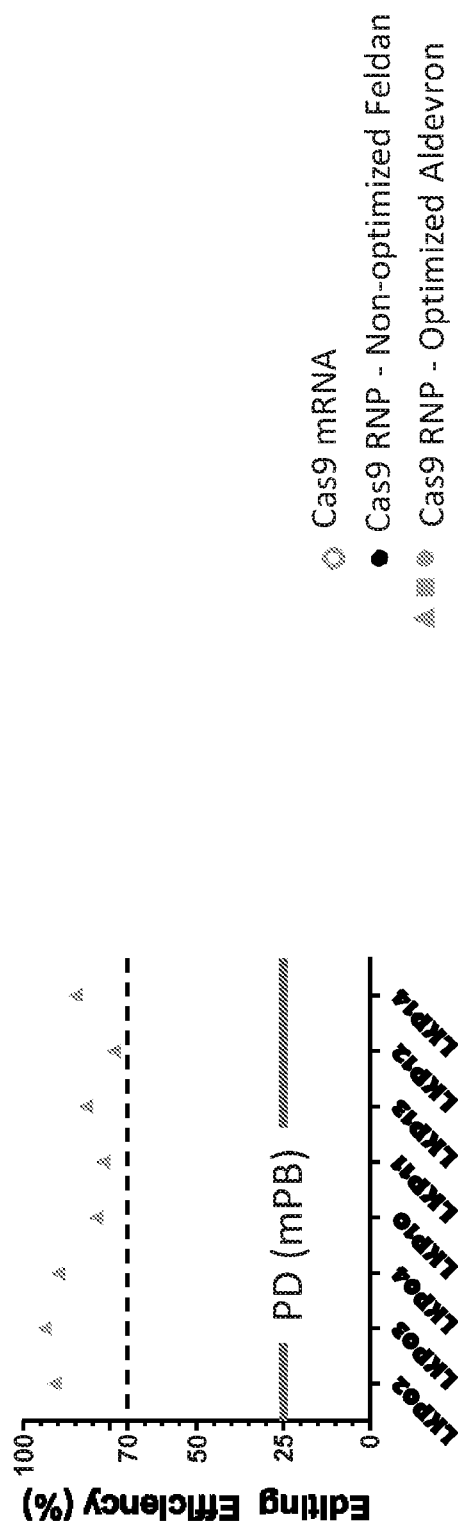
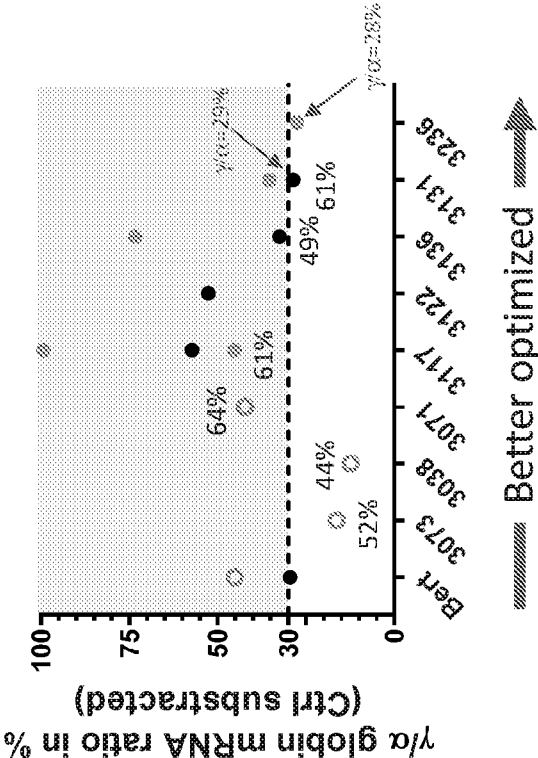


Figure 34B



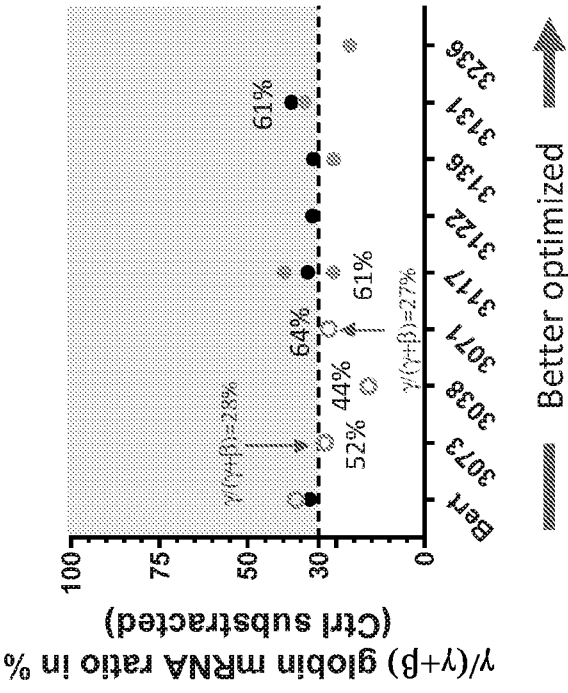
Efficacy of SPY101 in mPB CD34+ cells

Figure 35A



% = Editing efficiency
All unlabeled data points had
> 70% editing efficiency

Figure 35B



- Cas9 mRNA - Non optimized Differentiation
- Cas9 RNP - Non-optimized Differentiation
- ⊗ Cas9 RNP - Optimized Differentiation

Efficacy of SPY101 in BM CD34+ cells

Figure 36A

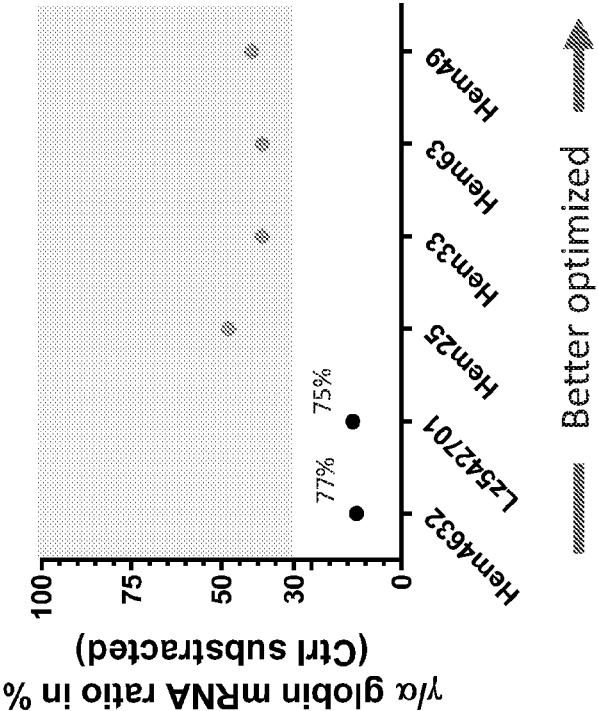
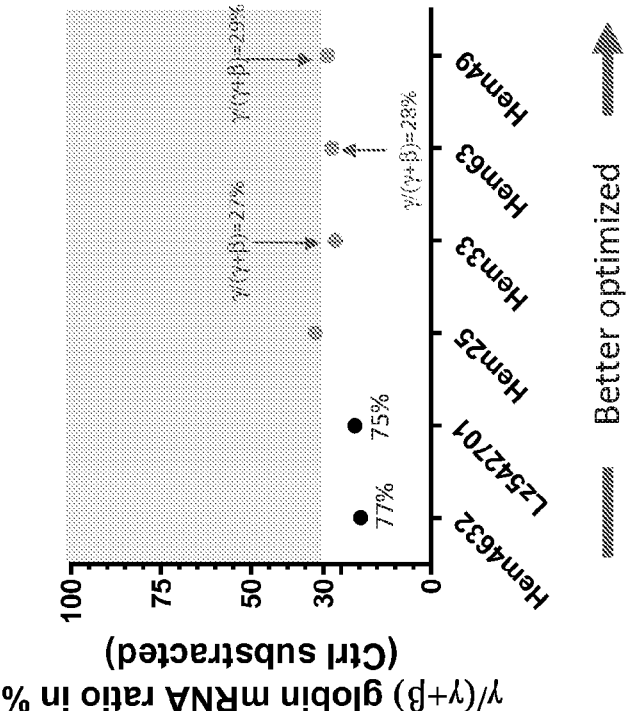


Figure 36B



% = Editing efficiency
All unlabeled data points had
> 80% editing efficiency

● Cas9 RNP - Non-optimized
◆ Cas9 RNP - Optimized

Efficacy of SPY101 in patient samples

Figure 37A

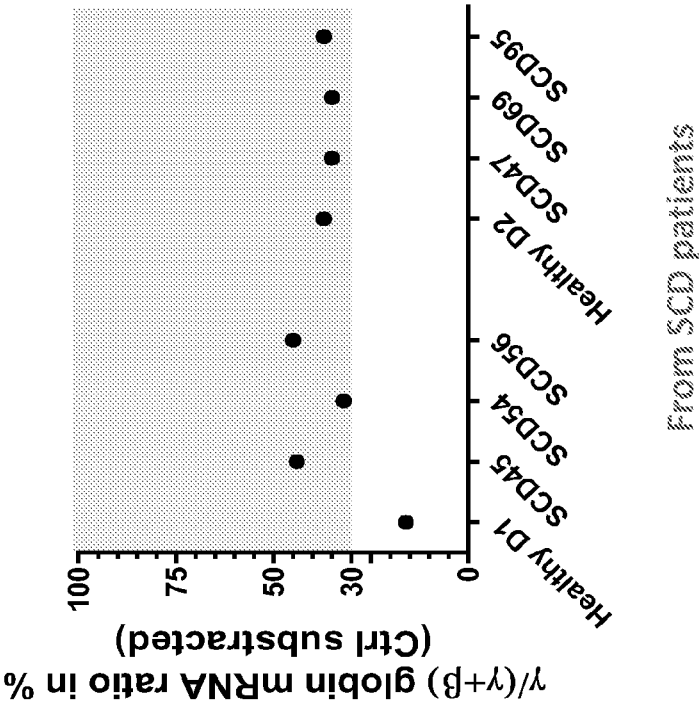
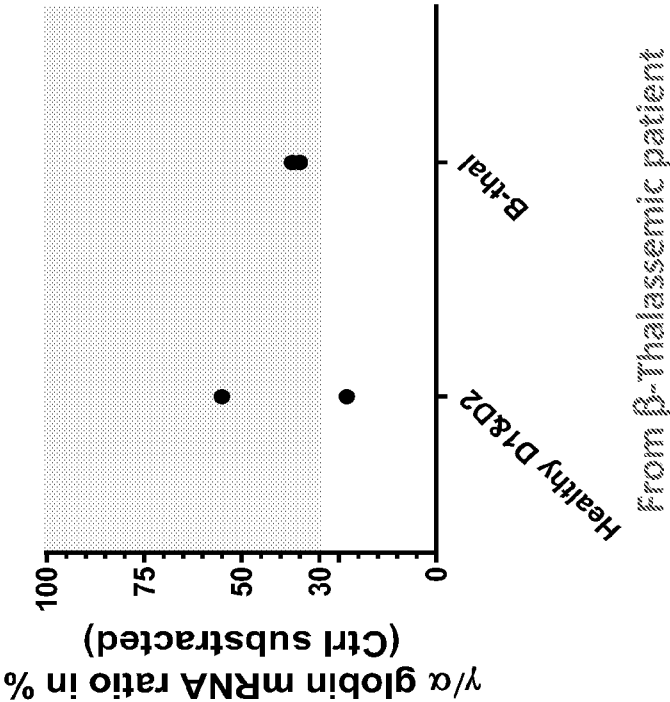


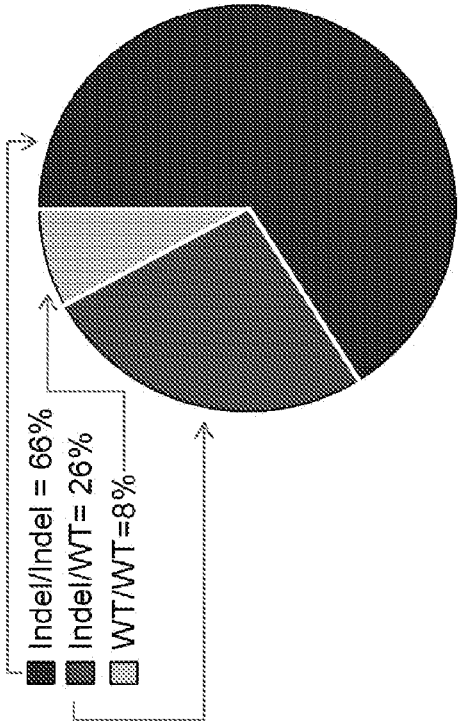
Figure 37B



SPY101 edited genotype breakdown

Figure 38A

Cas9 mRNA

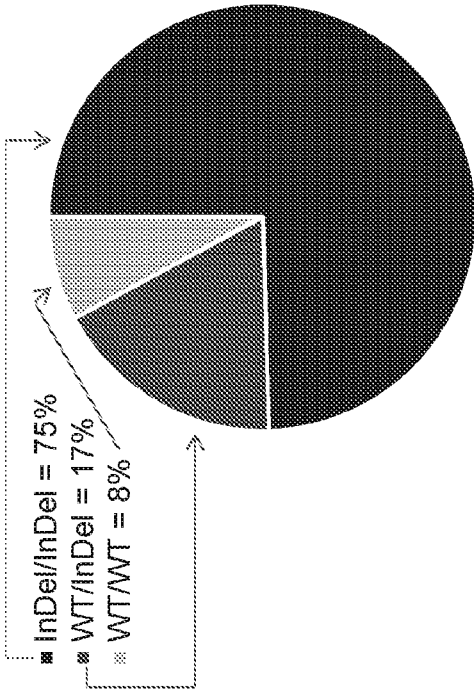


Indel Frequency = 79%
Edited colonies = 92%

Data from 60 colonies

Figure 38B

Cas9 RNP



Indel Frequency = 83%
Edited colonies = 92%

Data from 164 colonies

Gata1 Binding Site disruption by SPY101 linked to increased γ -globin expression

Figure 39A

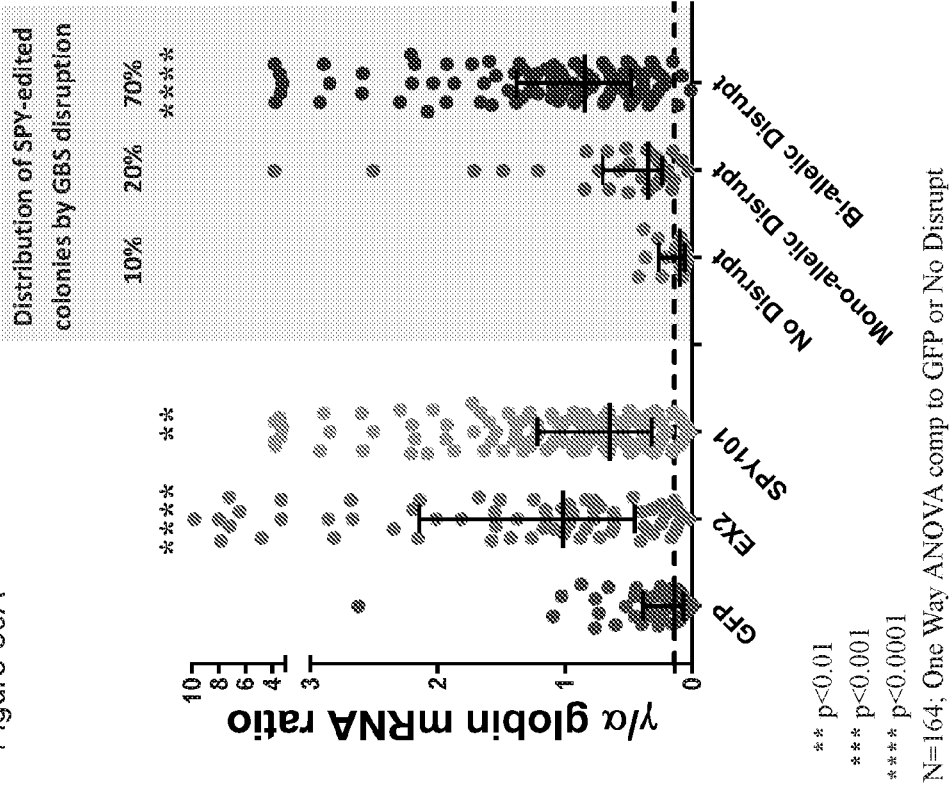
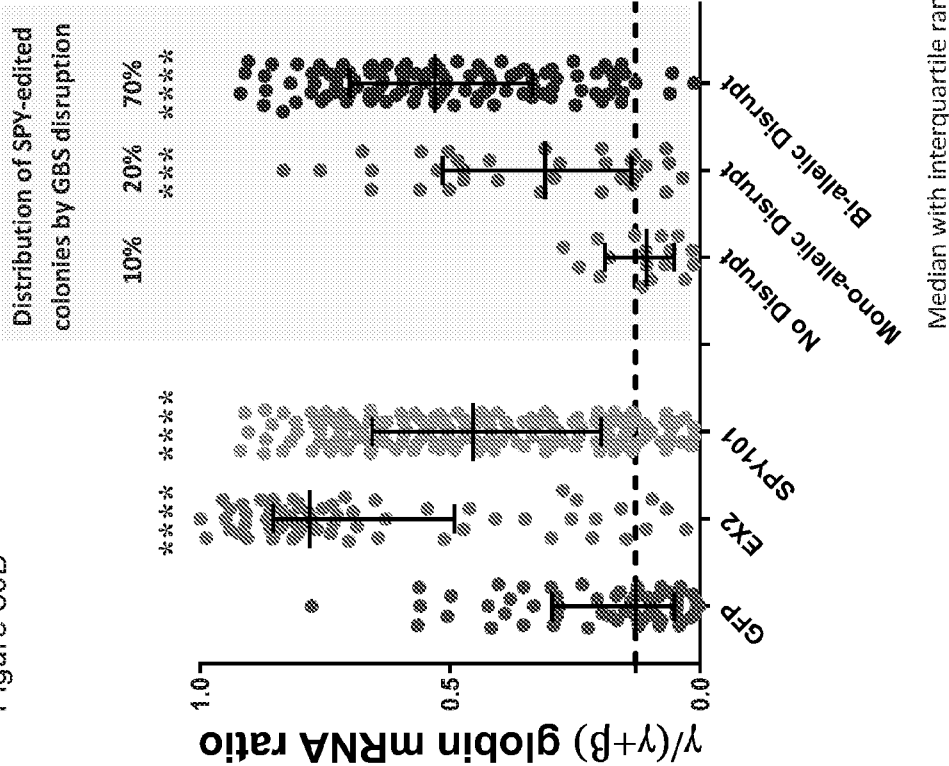


Figure 39B



SPY101 increased γ -globin expression

Figure 40A

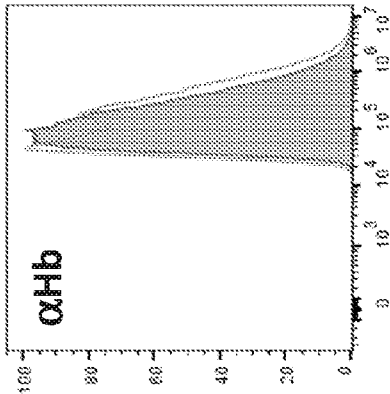


Figure 40B

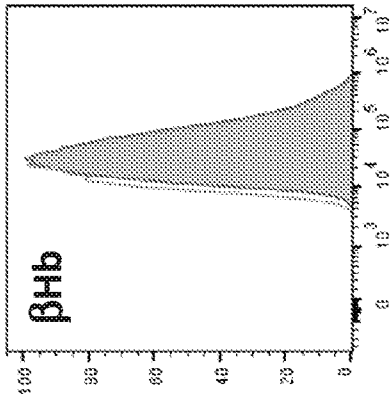


Figure 40C

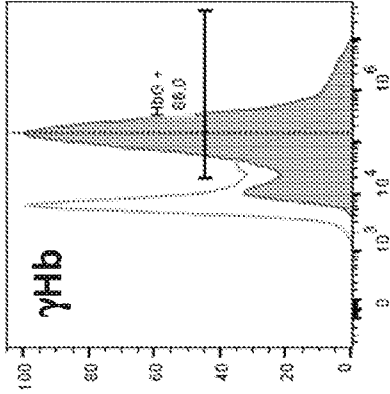


Figure 40D

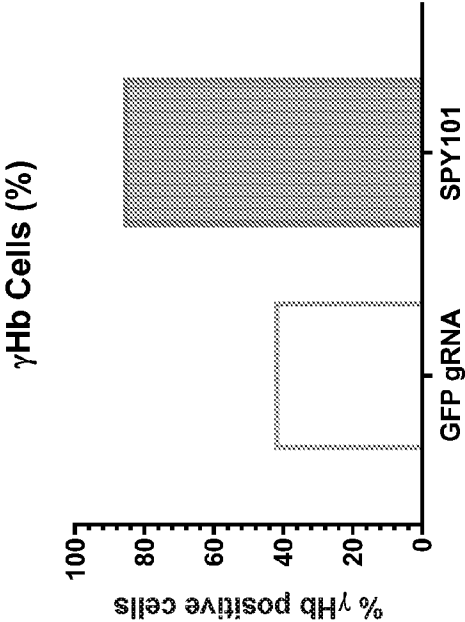


Figure 40E

