Figura 1A

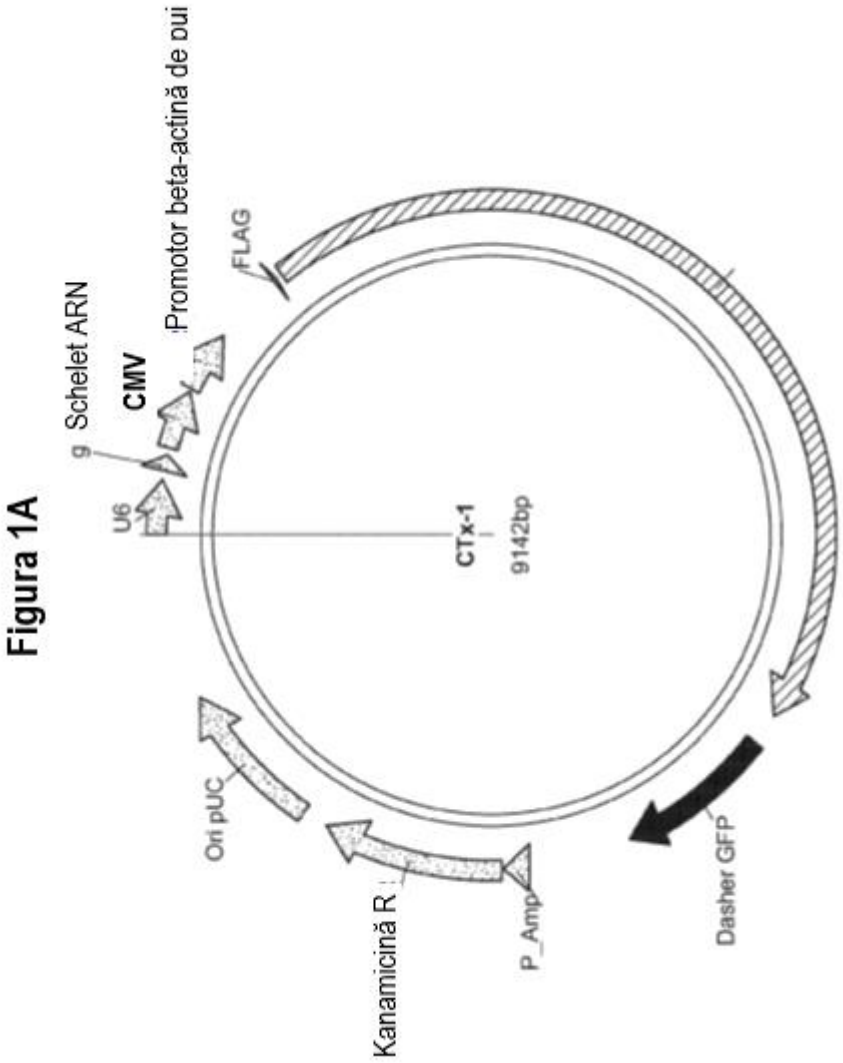

Schelet ARN
CMV
Promotor beta-actină de pui
FLAG
U6
CTx-1
9142bp
Ori pUC
Kanamicină R
P_Amp
Dasher GFP

Figura 1B

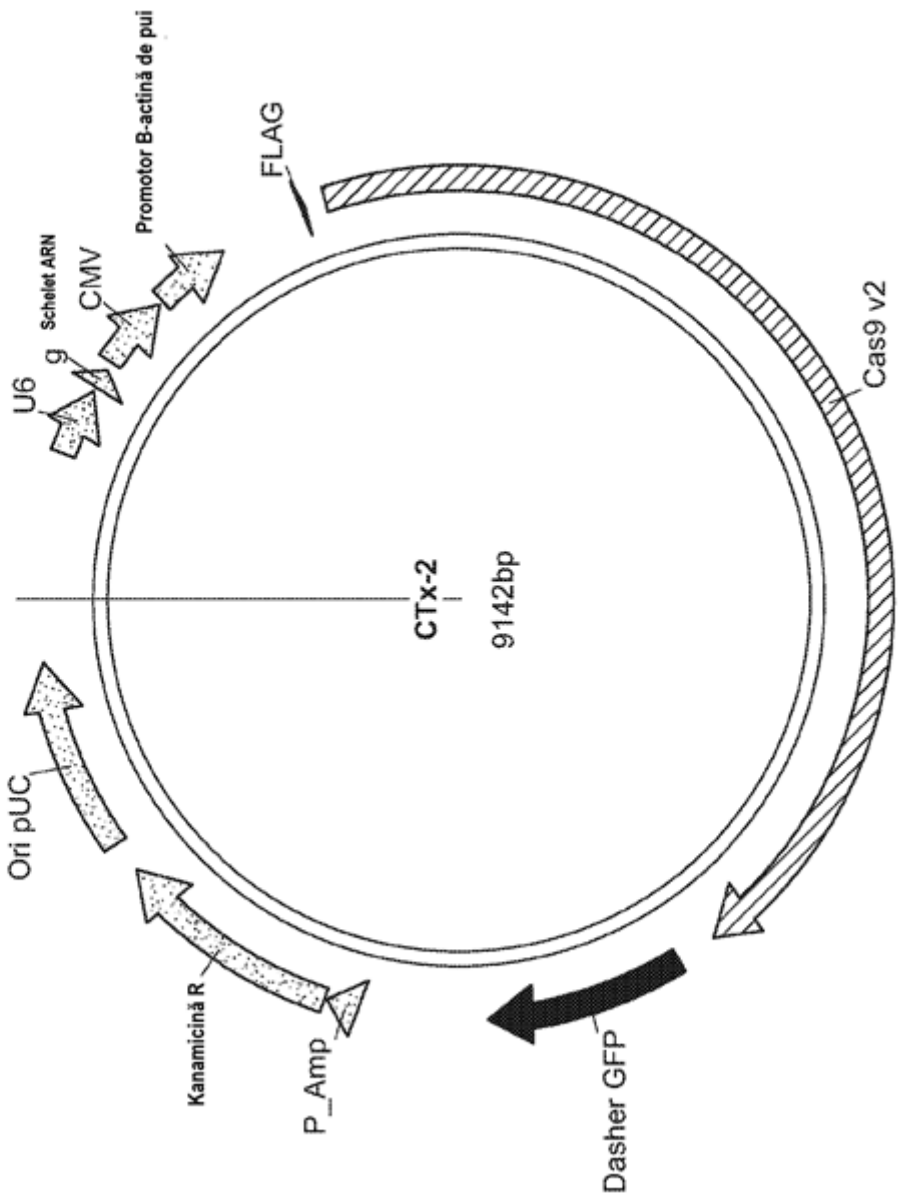

Promotor B-actină de pui
FLAG
Schelet ARN
CMV
g
U6
Cas9 v2
CTx-2
9142bp
Ori pUC
Kanamicină R
P_Amp
Dasher GFP

Figura 1C

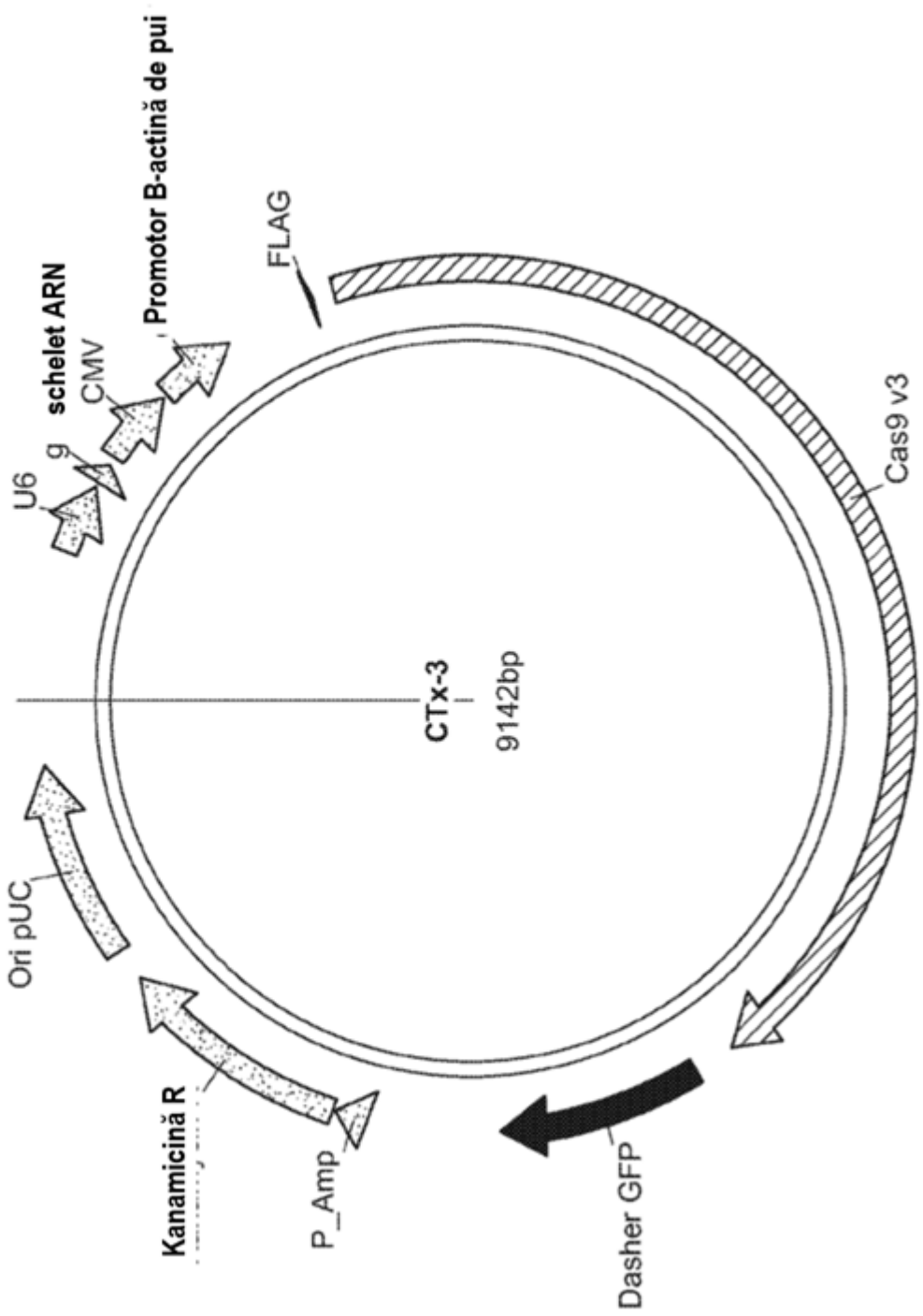

Promotor B-actină de pui
CMV
schelet ARN
g
U6
FLAG
Cas9 v3
CTx-3
9142bp
Ori pUC
Kanamicină R
P_Amp
Dasher GFP

Figura 2A

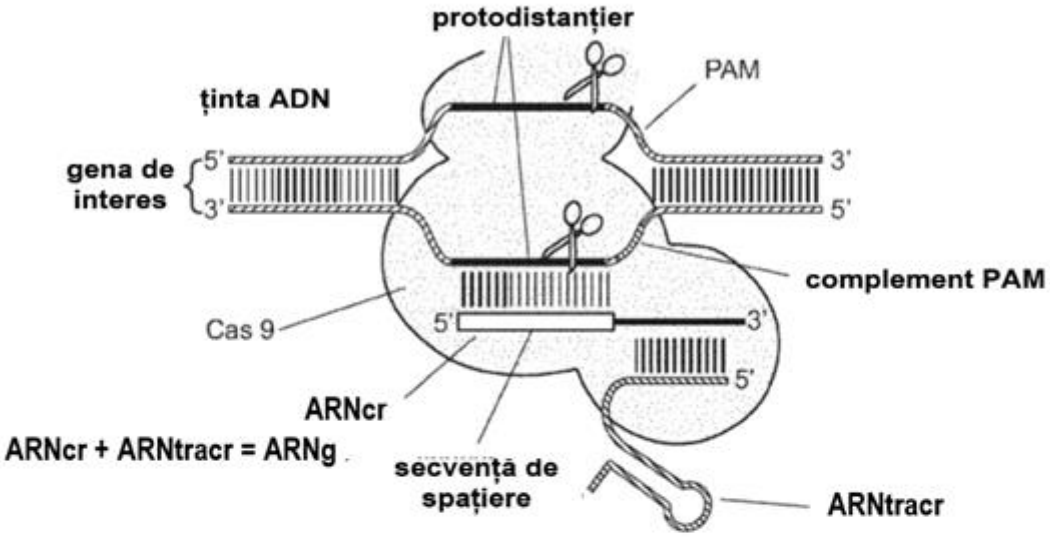


Figura 2B

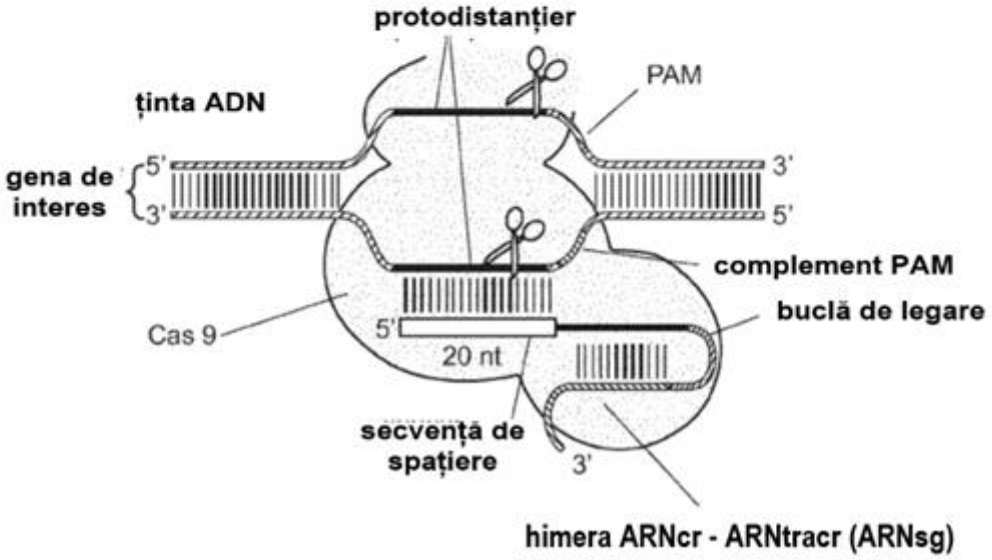


FIGURA 3

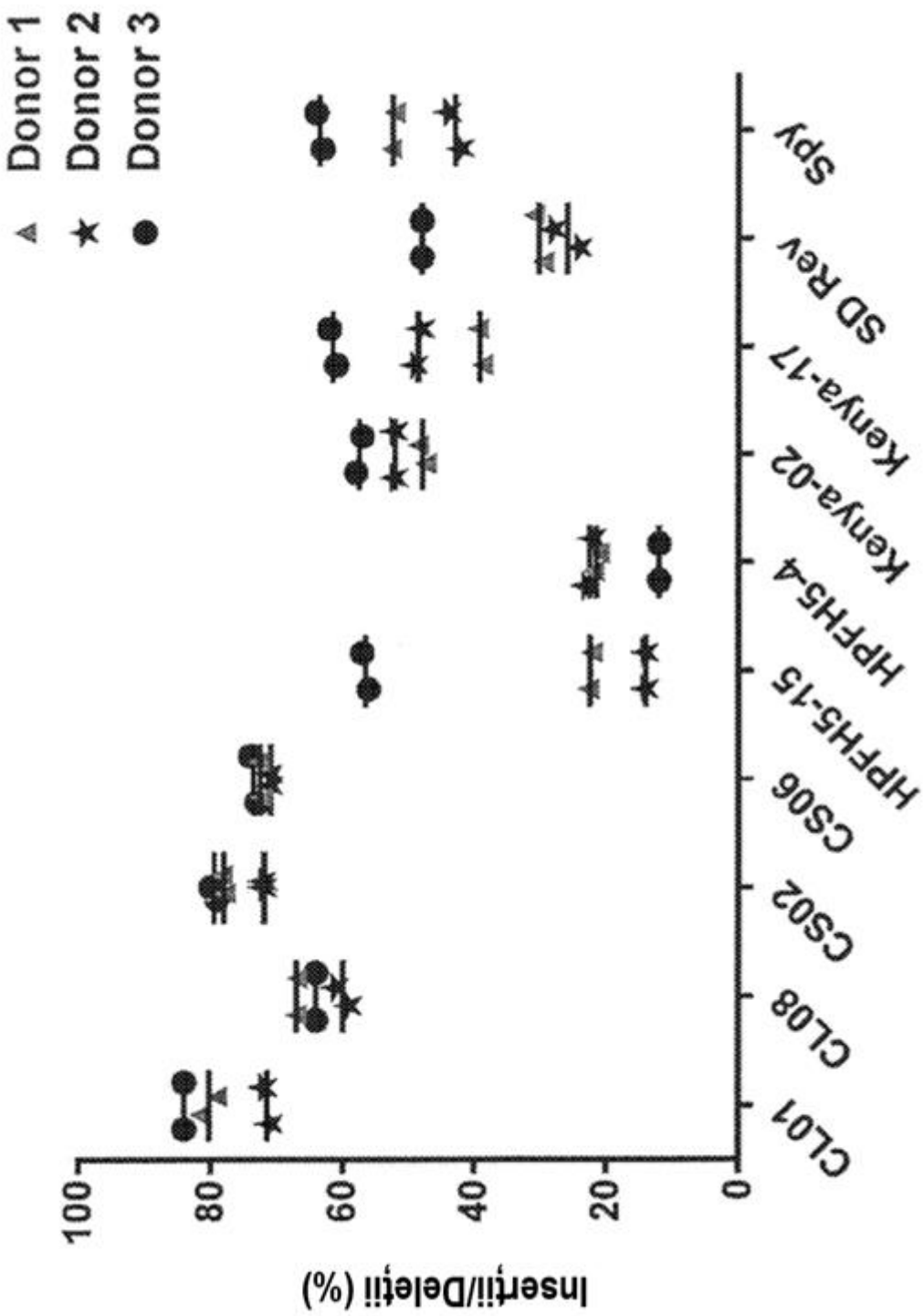

Donor 1
Donor 2
Donor 3
Insertii/Deletii (%)
100
80
60
40
20
0
CL01
CL08
CS02
CS06
HPFH5-15
HPFH5-4
Kenya-02
Kenya-17
SD Rev
Spy

FIGURA 4A

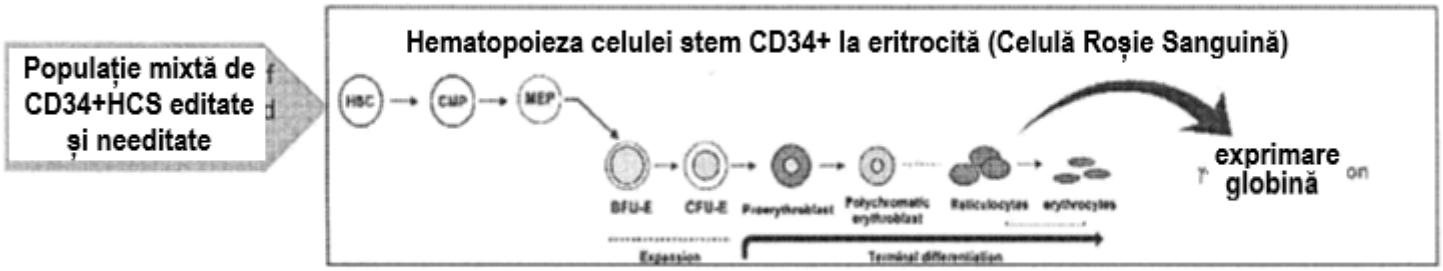


FIGURA 4B

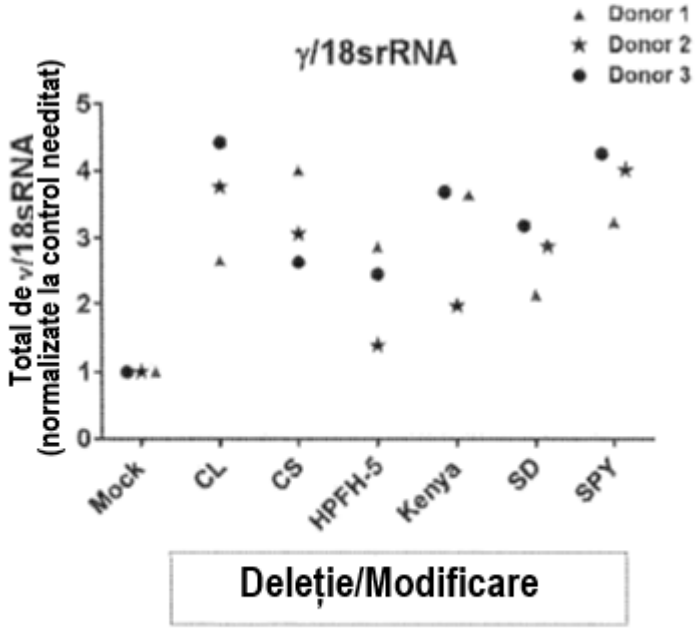
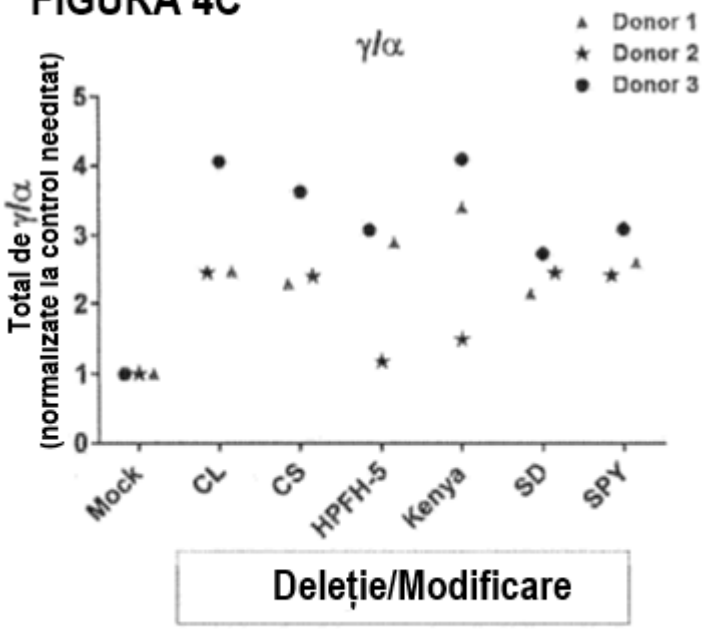


FIGURA 4C



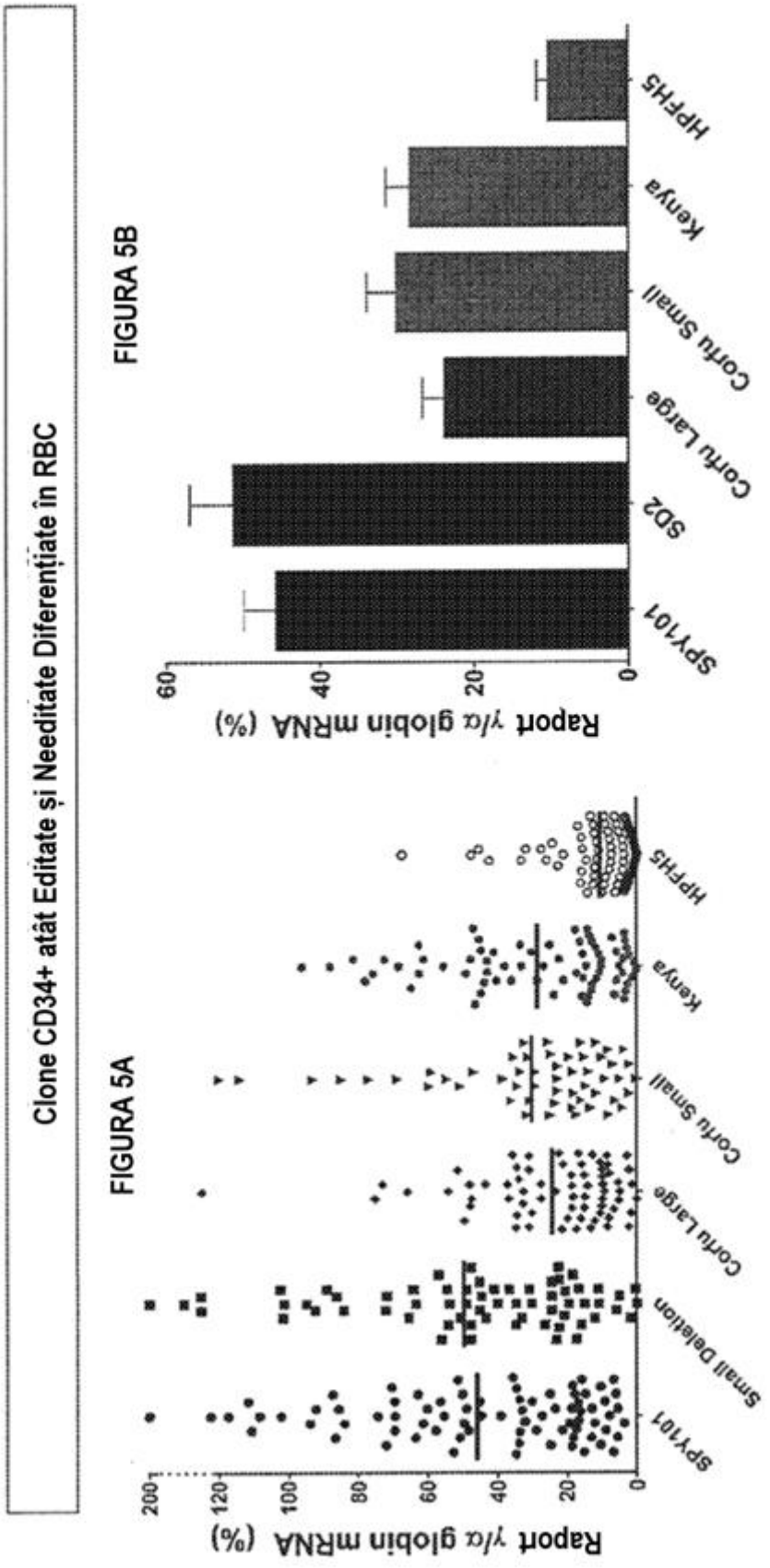
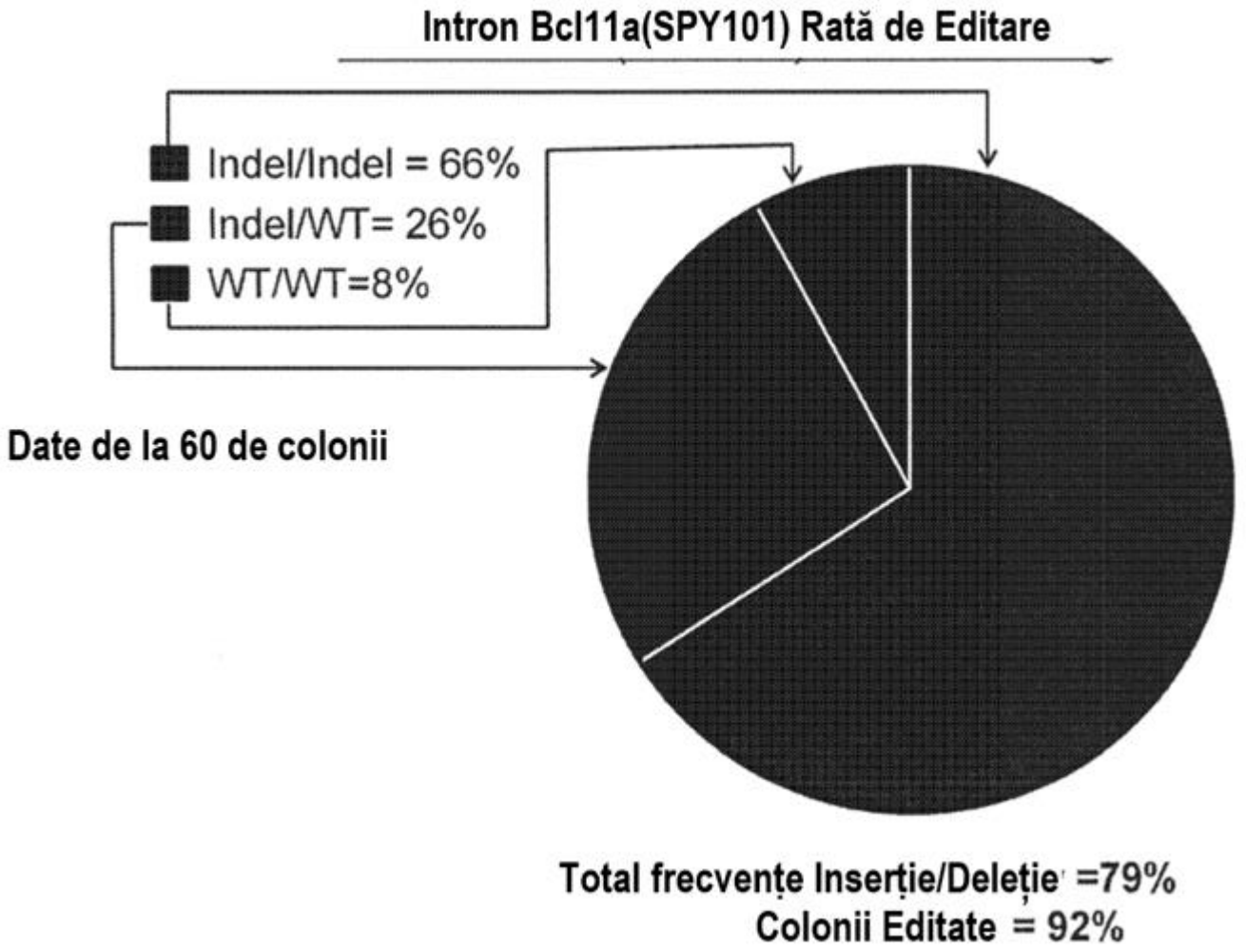

Clone CD34+ atât Editate și Needitate Diferențiate în RBC
FIGURA 5A
Raport γ/α globin mRNA (%)
200
120
100
80
60
40
20
0
SPY101
Small Deletion
Corfu Large
Corfu Small
Kenya
HPFH5
FIGURA 5B
Raport γ/α globin mRNA (%)
60
40
20
0
SPY101
SD2
Corfu Large
Corfu Small
Kenya
HPFH5

FIGURA 6



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Editare Intron Bcl11a (SPY101)
Figura 7A
Procentul de HBG în raport cu HBA (HBG+HBA)
200
120
100
80
60
40
20
0
Indel/Indel
Indel/WT
WT/WT
HBG/HBA
Figura 7B
Procentul de globine beta asemănătoare HBG/(HBB+HBG)
100
80
60
40
20
0
Indel/Indel
Indel/WT
WT/WT
HBG/(HBB+HBG)

Figura 8

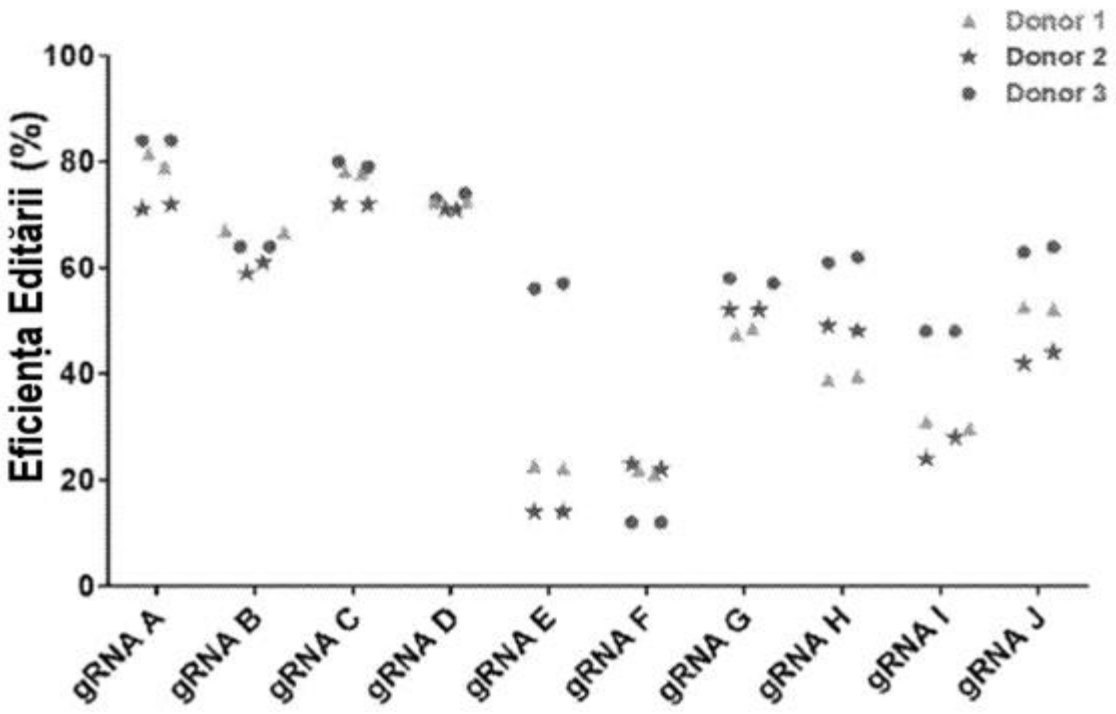

Donor 1
Donor 2
Donor 3
Eficiența Editării (%)
100
80
60
40
20
0
gRNA A
gRNA B
gRNA C
gRNA D
gRNA E
gRNA F
gRNA G
gRNA H
gRNA I
gRNA J

Figura 9A

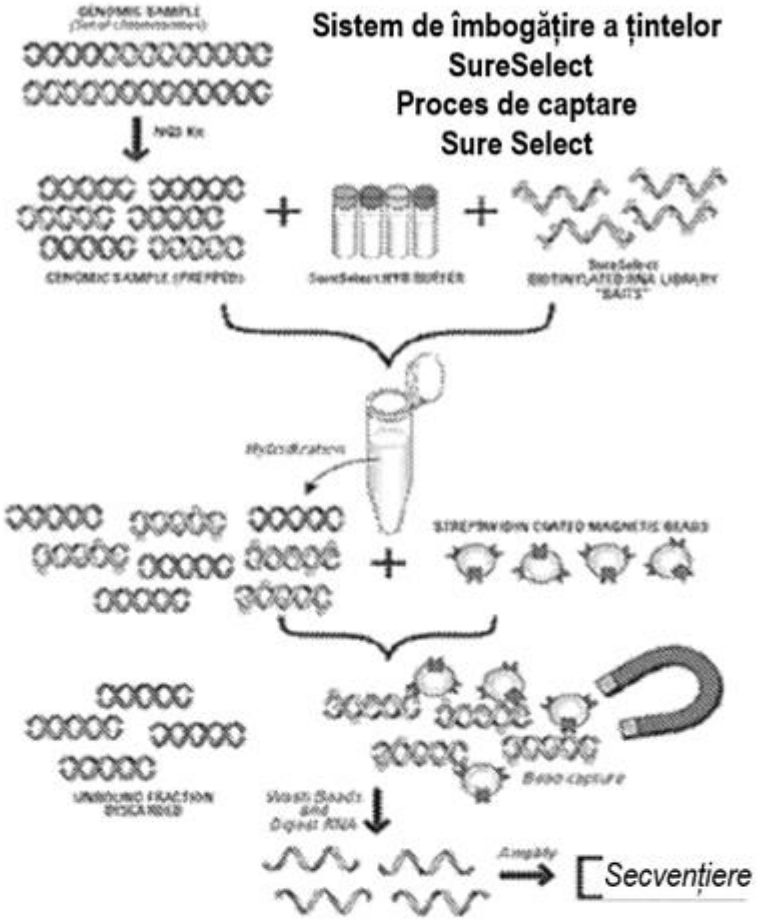


Figura 9B

gRNA	# de situri de tăiere în afara țintei examinate	# de situri tăiate în afara țintei detectate	Eficiența maximă de clivaj în afara țintei
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%

Figura 10A

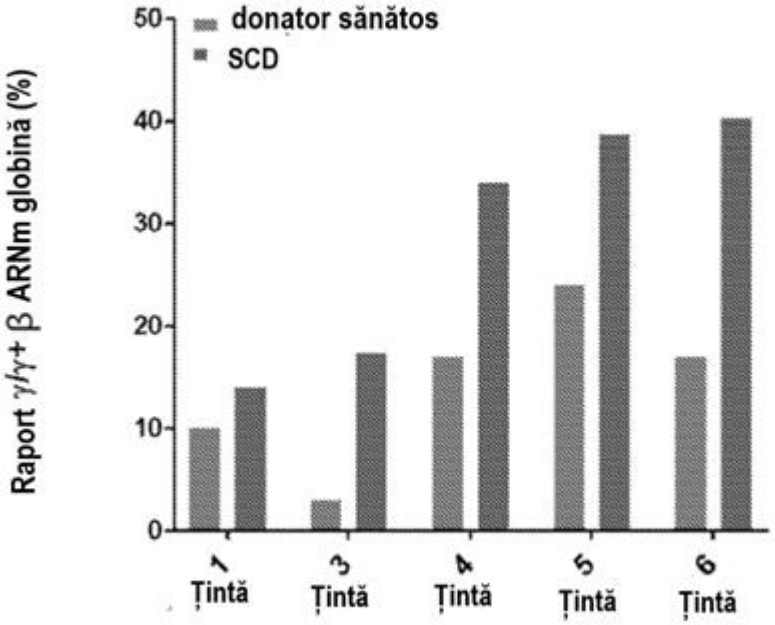


Figura 10B

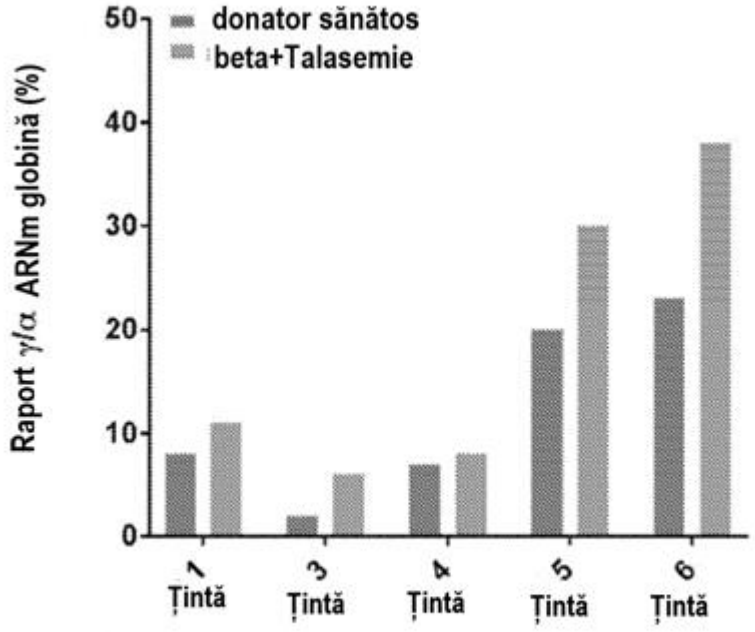


Figura 11A

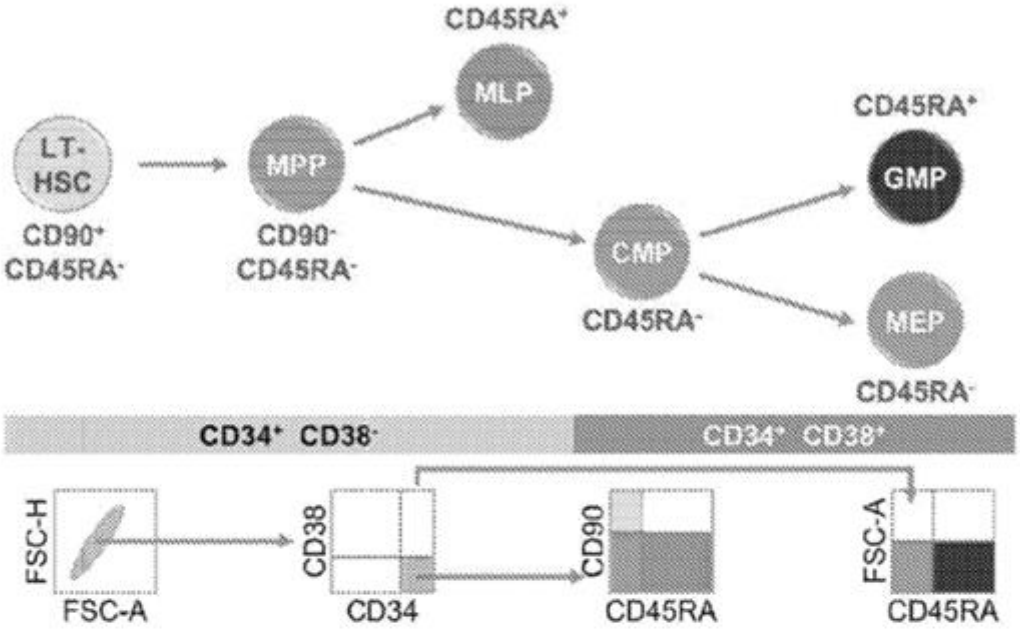


Figura 11B

Distribuția tipurilor de celule rămase nemodificate după editare

Populație	Frecvență	
	Simul	*Editat*
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

Figura 11C

Eficiențe ridicate ale editării în toate tipurile de celule

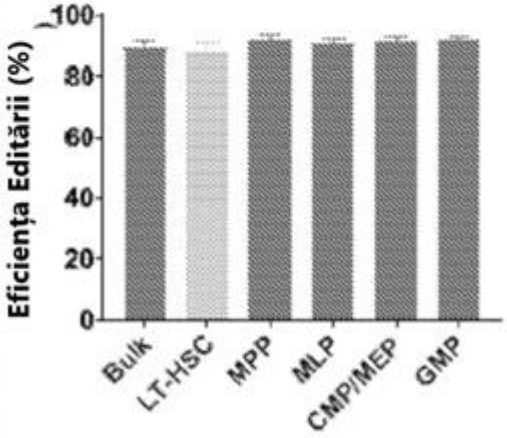


Figura 12

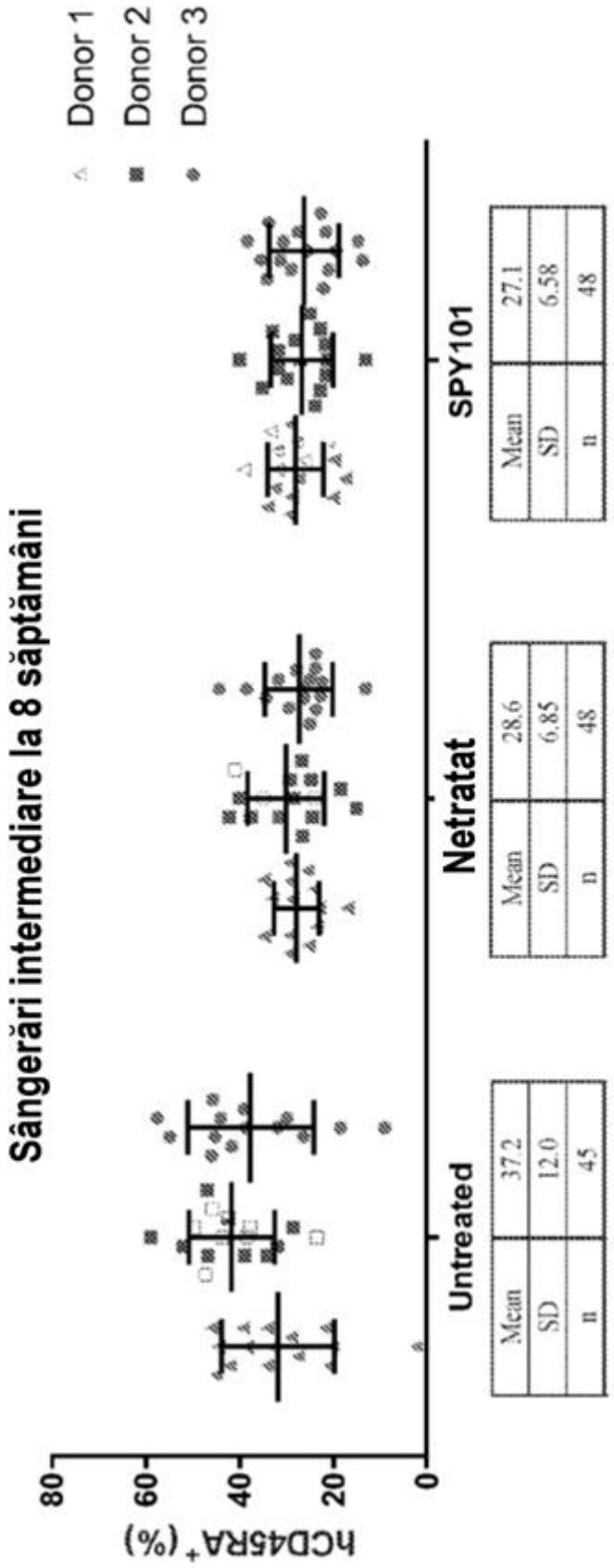

Donor 1
Donor 2
Donor 3
Sângerări intermediare la 8 săptămâni
hCD45RA+ (%)
80
60
40
20
0
Untreated
Netratat
SPY101
Mean 37.2
SD 12.0
n 45
Mean 28.6
SD 6.85
n 48
Mean 27.1
SD 6.58
n 48

Figura 13

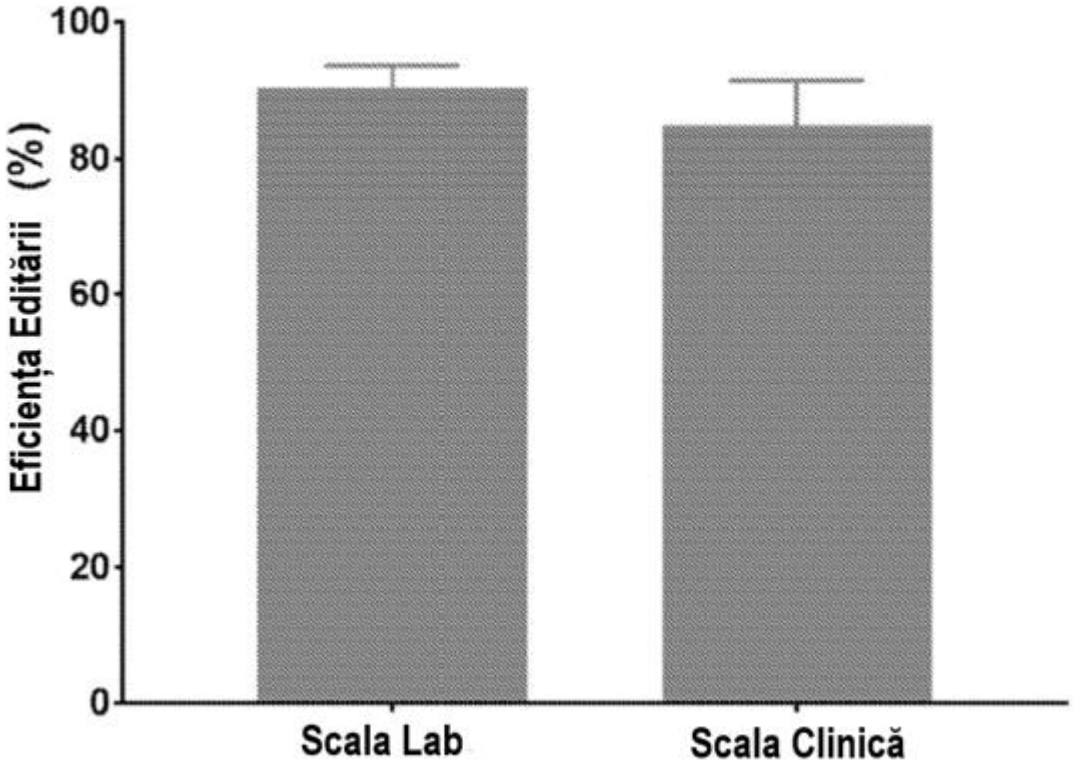


Figura 14

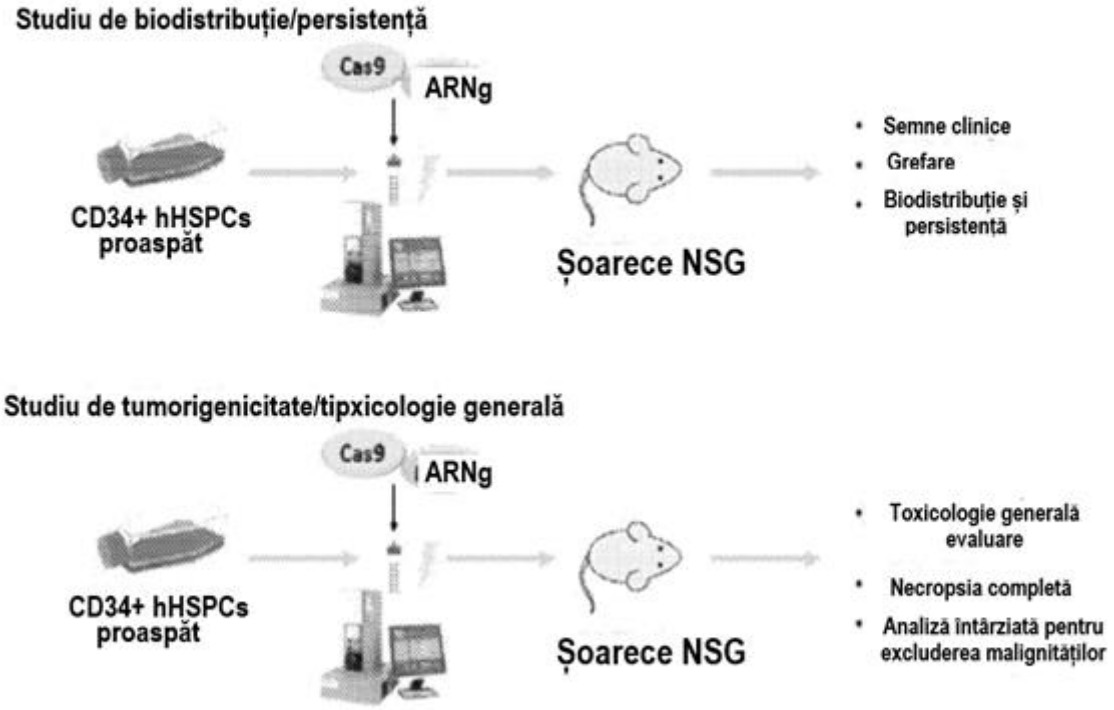


Figura 15

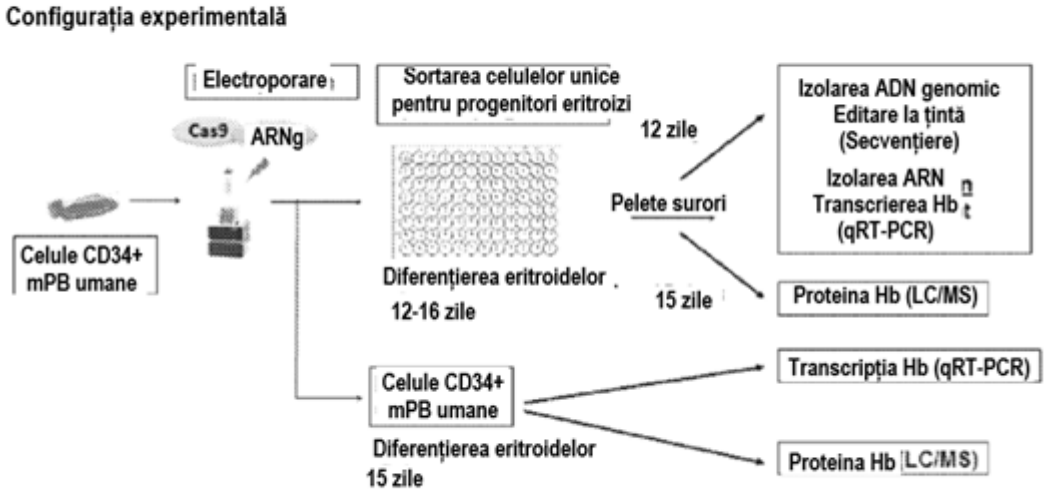


Figura 16A

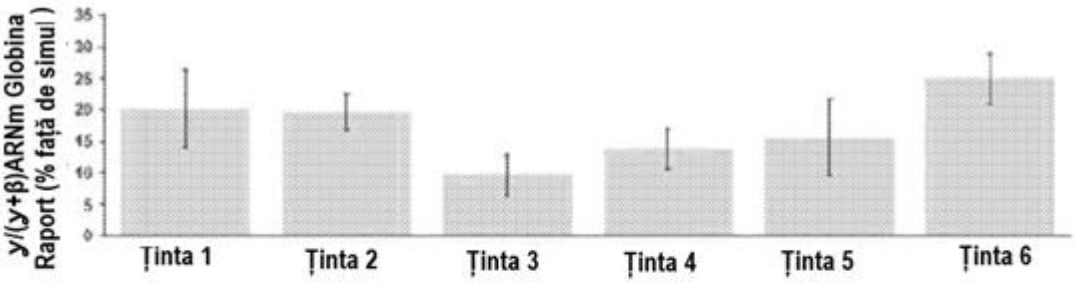


Figura 16B

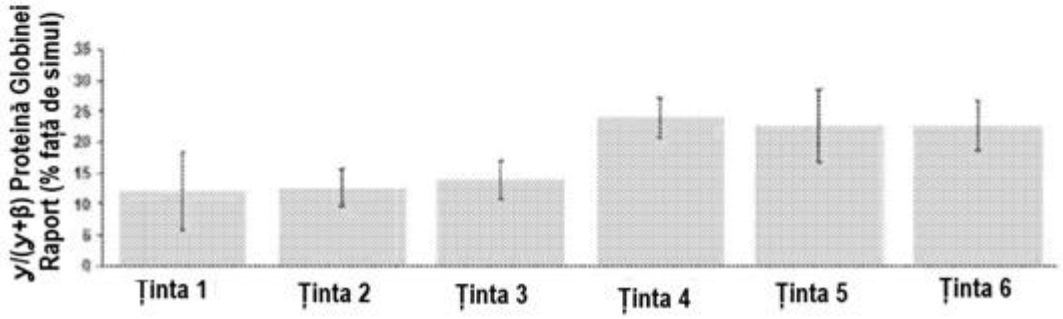


Figura 17

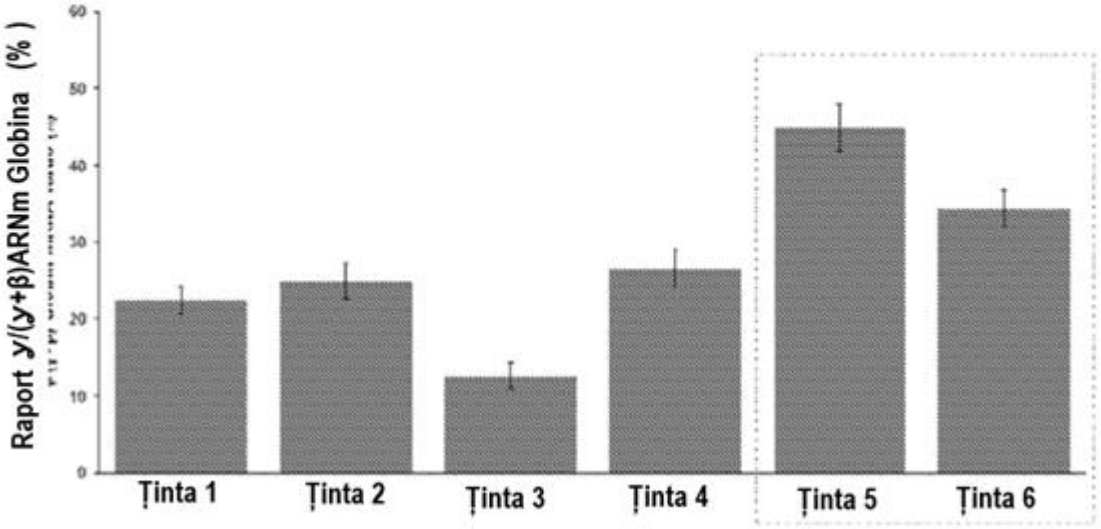

Raport γ/(γ+β)ARNm Globina (%)
60
50
40
30
20
10
0
Ținta 1
Ținta 2
Ținta 3
Ținta 4
Ținta 5
Ținta 6

Figura 18A

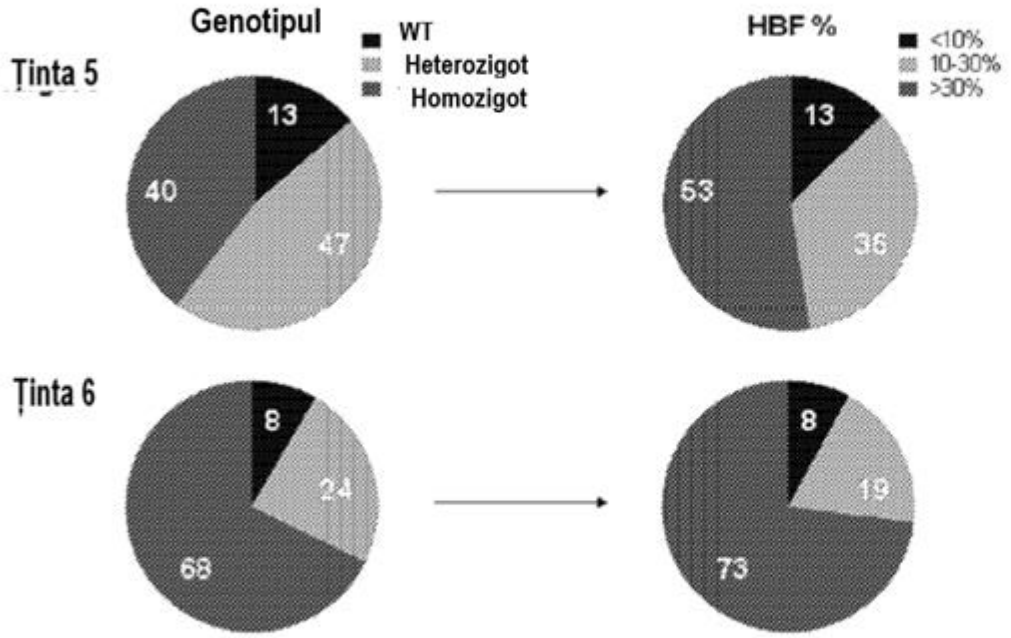


Figura 18B

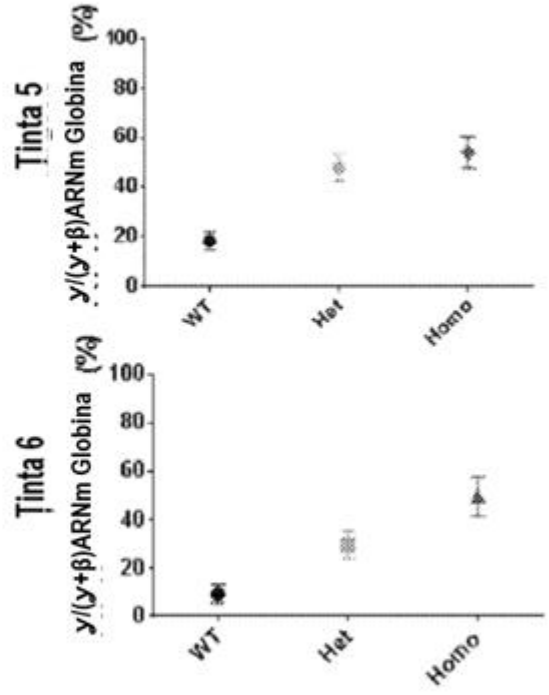
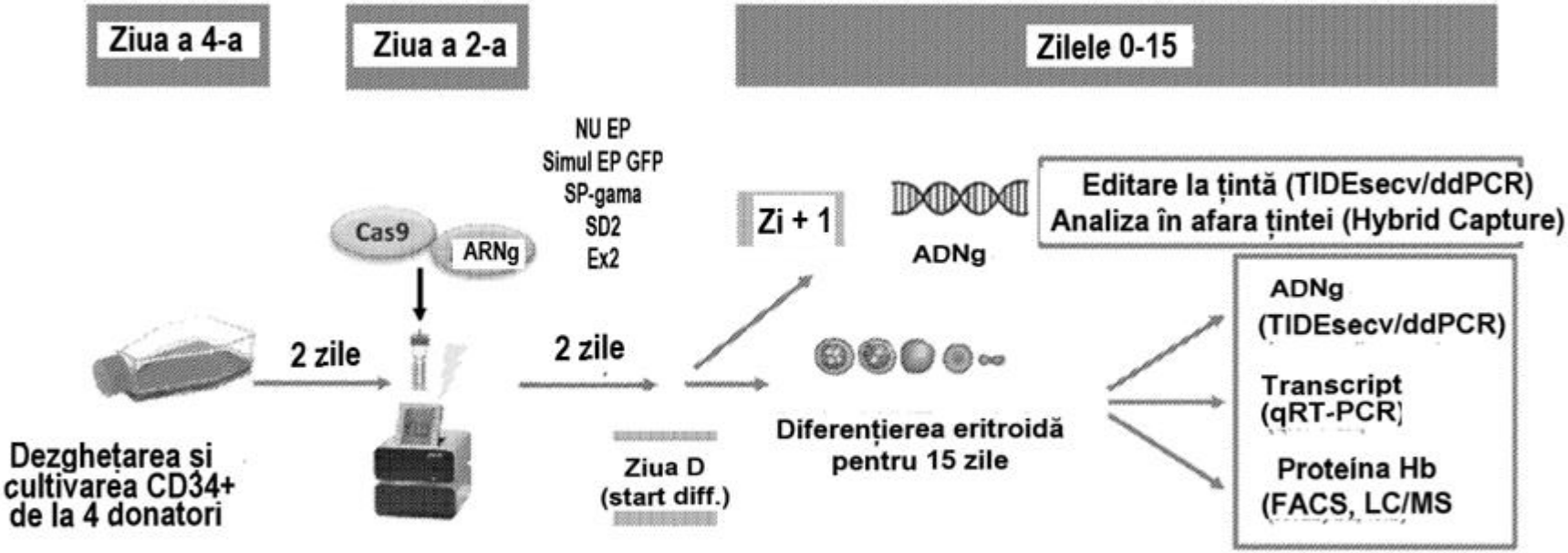


Figura 19

Fluxul de Lucru în Bloc și Lista de Date în Bloc



Editare % menținută pe toată durata diferențierii eritroidei ex vivo a celulelor mPBCD34+.

Figura 20A

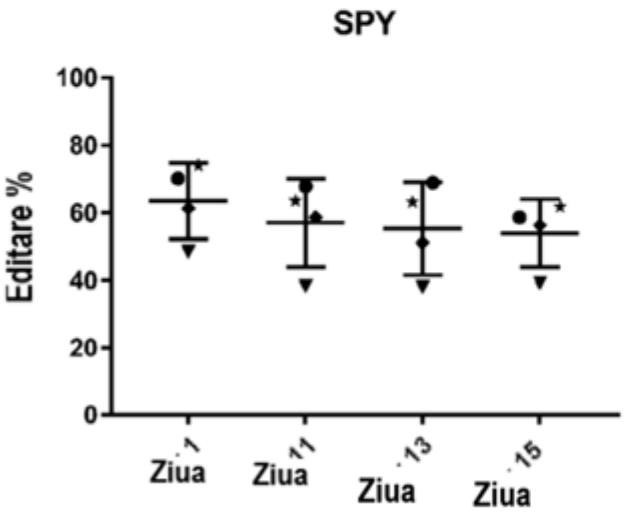
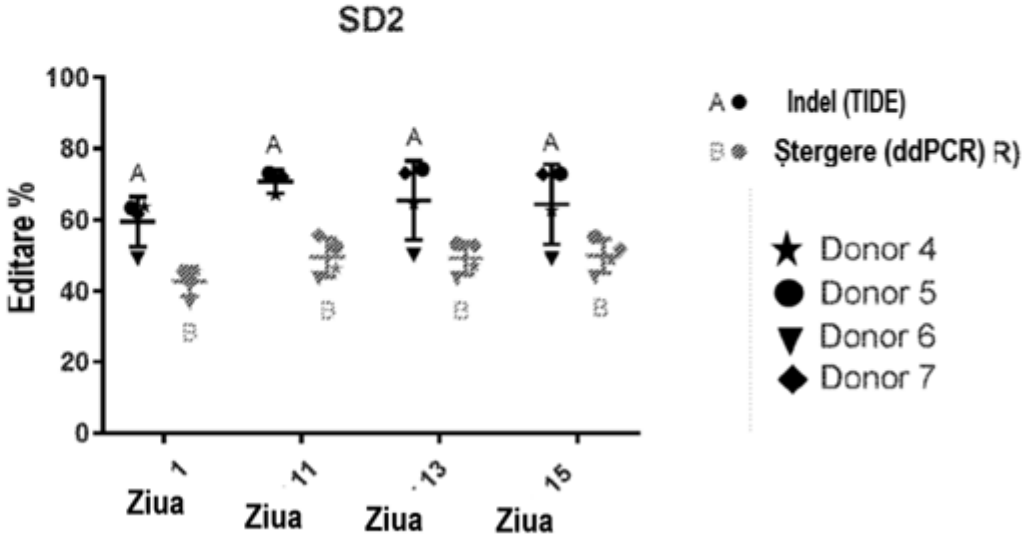


Figura 20B



Creșterea prin post editarea trascriptului ɣ-globinei

Figura 21A

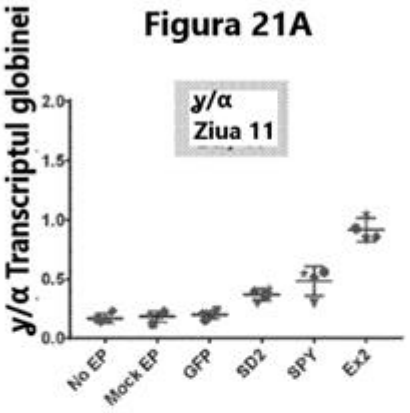


Figura 21B

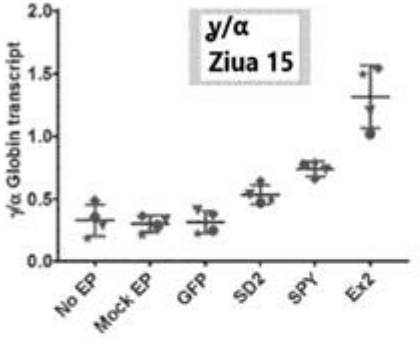


Figura 21C

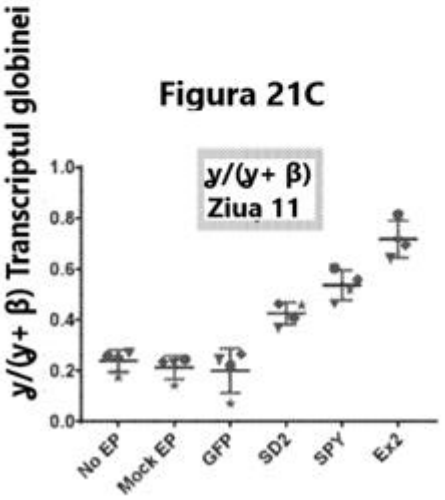
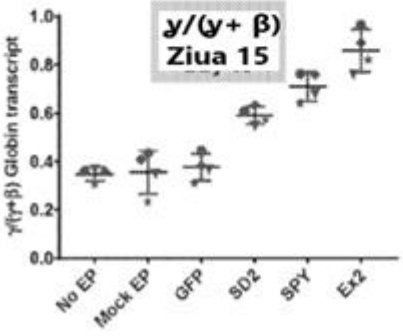


Figura 21D



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Analiza FACS arată o creștere a γ-globinei în celulele editate.

Figura 22A

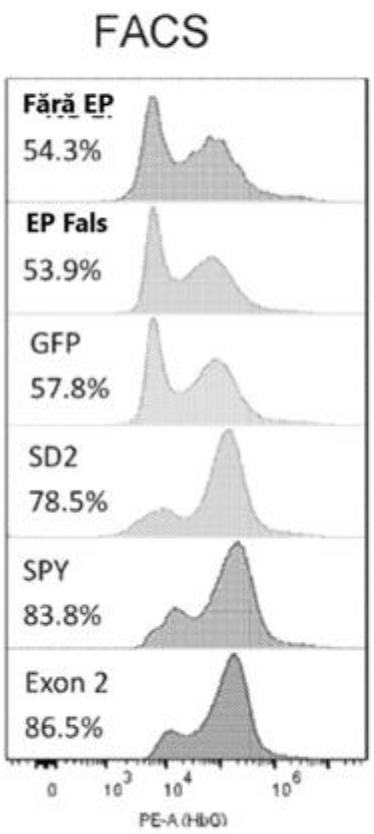
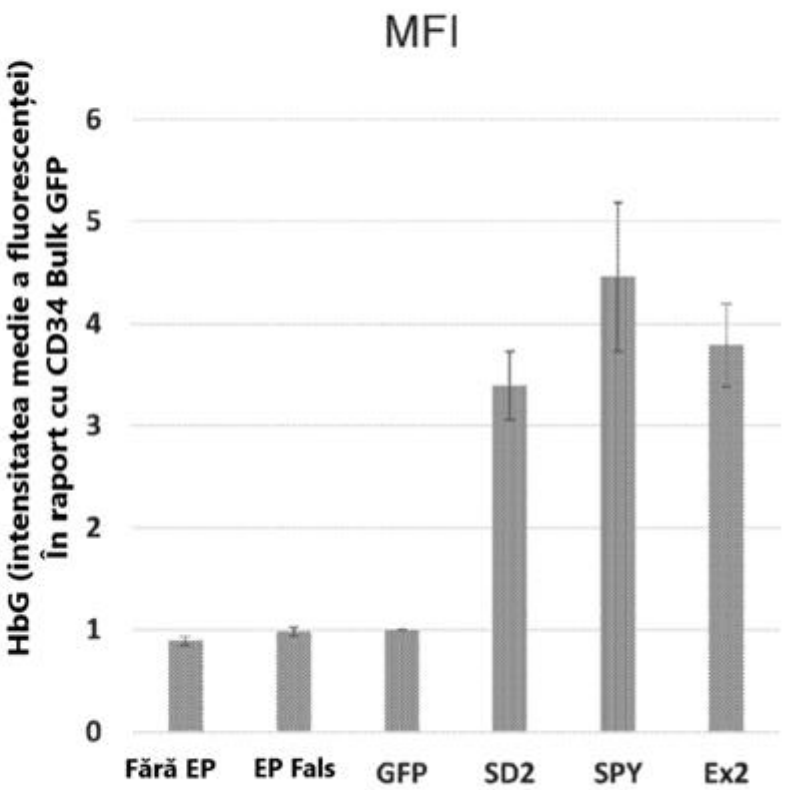


Figura 22B



LC-MS în vrac validează în continuare creșterea γ-globinei după editare

Figura 23A

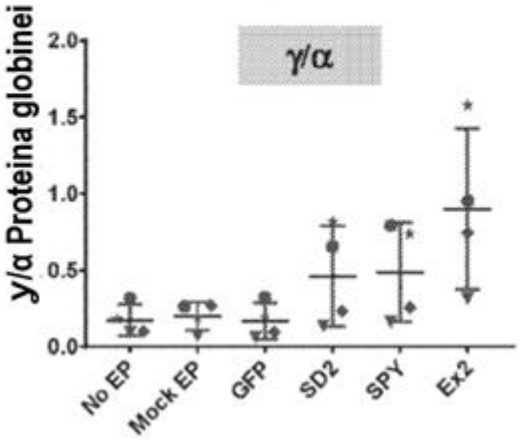
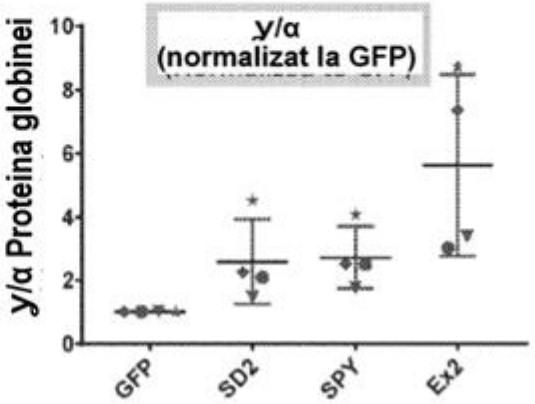


Figura 23B



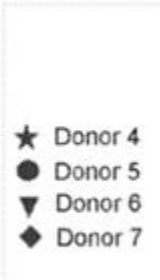


Figura 23C

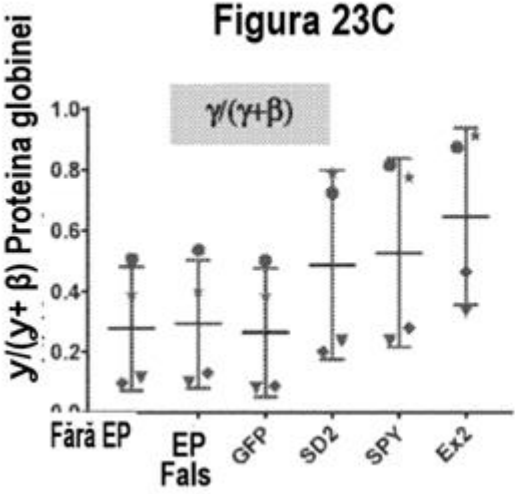


Figura 23D

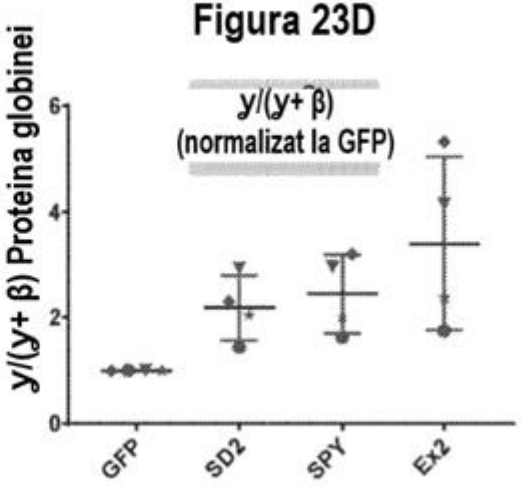


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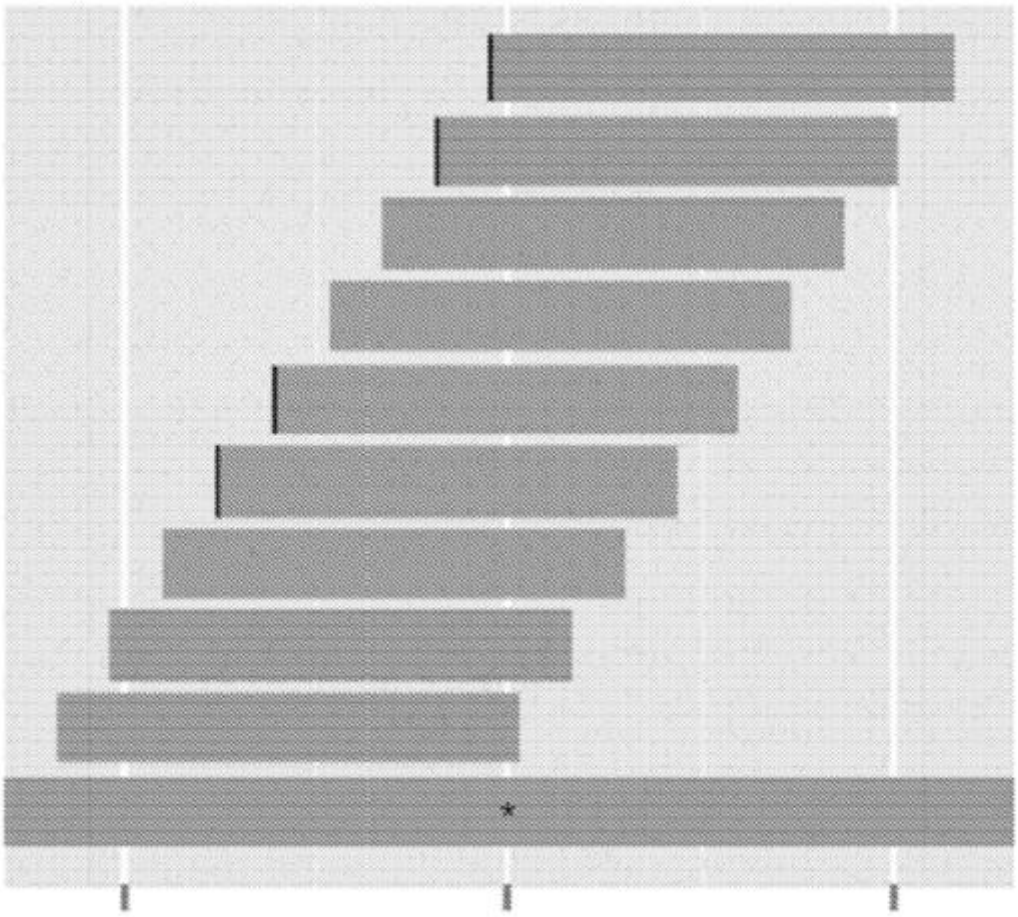
Figura 24



Design hibrid de captare momeală

Figura 25

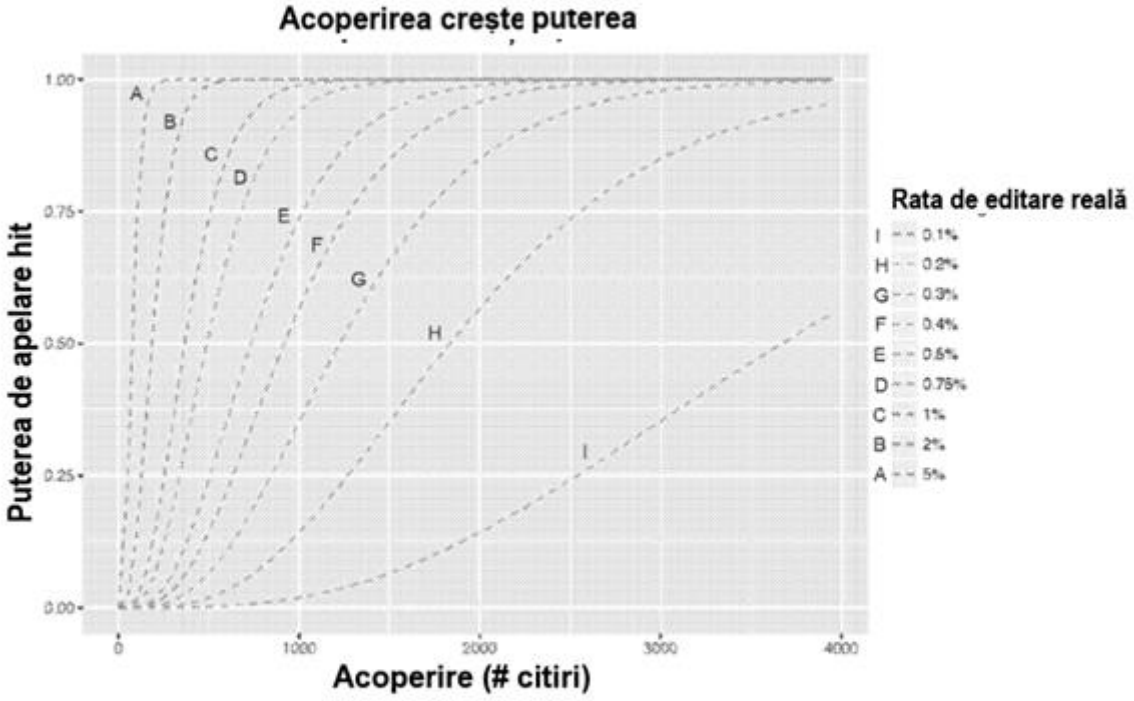

Acoperirea crește puterea
Puterea de apelare hit
1.00
0.75
0.50
0.25
0.00
0
1000
2000
3000
4000
Acoperire (# citiri)
A
B
C
D
E
F
G
H
I
Rata de editare reală
I 0.1%
H 0.2%
G 0.3%
F 0.4%
E 0.5%
D 0.75%
C 1%
B 2%
A 5%

Figura 26

Rezumatul capturii hibride (SPY1010)

SPY101	Runda 1	Runda 2
Număr de donori	3	4
Format Cas9	mRNA	RNP
Acoperirea medie a secvențierii	~800	2492
Numărul de situs-uri	840	5003
Număr de neconcor dante și tipuri de PAMS	4: NGG și NAG	5: NGG, NAG 4: NGA, NAA, NCG, NGC, NTG, NGT
# Numărul de situs-uri în afara țintei cu dovezi de tăiere	0* Frecv. indel >0.5%	0$^{\Delta}$ $^{\Delta}$ Frecv. indel >0.2%

Figura 27

Rezumatul capturii hibride (SD2)

SD2	Runda 1	Runda 2
Număr de donori	3	4
Format Cas9	mRNA	RNP
Acoperirea medie a secvențierii	~800	2775
Numărul de situs-uri	677	3272
Număr de neconcordanțe și tipuri de PAMS	4: NGG și NAG[$]	5: NGG, NAG 4: NGA, NAA, NCG, NGC, NTG, NGT
# Numărul de situs-uri în afara țintei cu dovezi de tăiere	0* *Frecv. indel >0.5%	0[Δ] [Δ]Frecv. indel>0.2%

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Figura 28

Plan de studiu pilot de grefare

Grup	Tratament	Doza de radiații (cGy)	Rata Dozei de Radiație (cGy/min)	Number of Female Animals		
				Cohorta 1 origine celulară: Donator#1	Cohorta 2 origine celulară: Donator#2	Cohorta 3 origine celulară: Donator#3
1	Celule netratate	200	160	16	16	16
2	"electroporare simulată" de celule fără nicio componentă de editare CRISPR/Cas9	200		16	16	16
3	Eletroporarea celulelor cu ARN GFPGuide și proteină Cas9	200		16	16	16
4	Eletroporarea celulelor cu ARNg SPY101 și proteina Cas9	200		16	16	16
5	Eletroporare de celule cu SD2 gRNA și proteina Cas9	200		16	16	16
6	Control netratat	200		10	10	10

Figura 29A

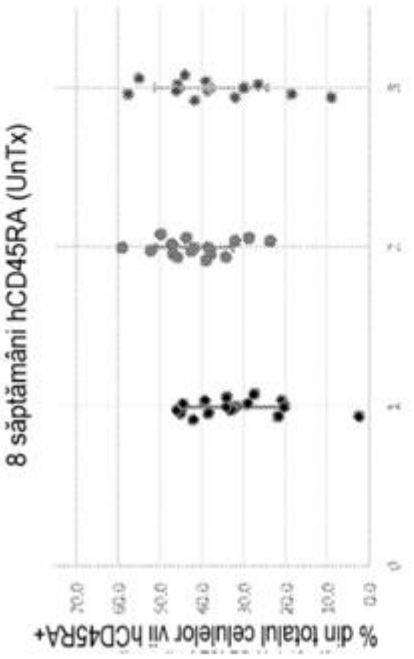


Figura 29B

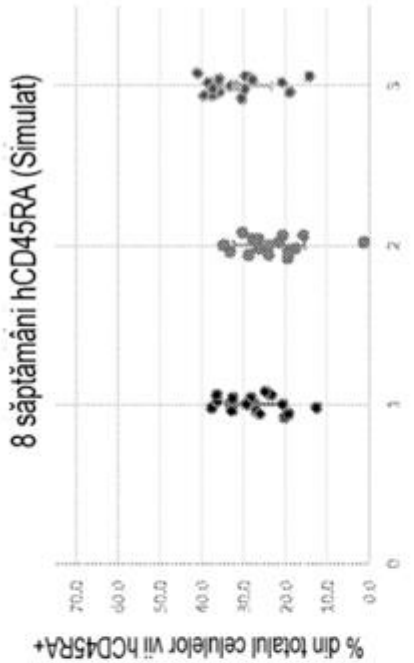


Figura 29C

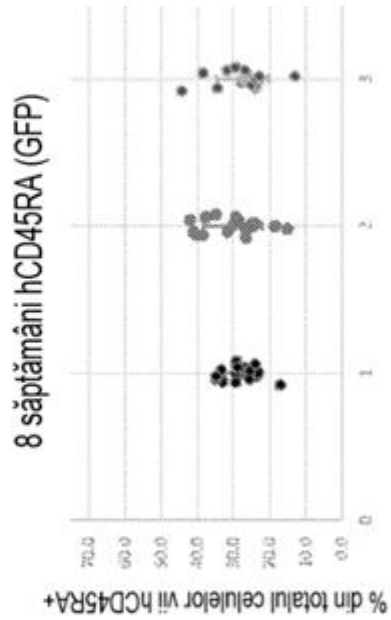


Figura 29D

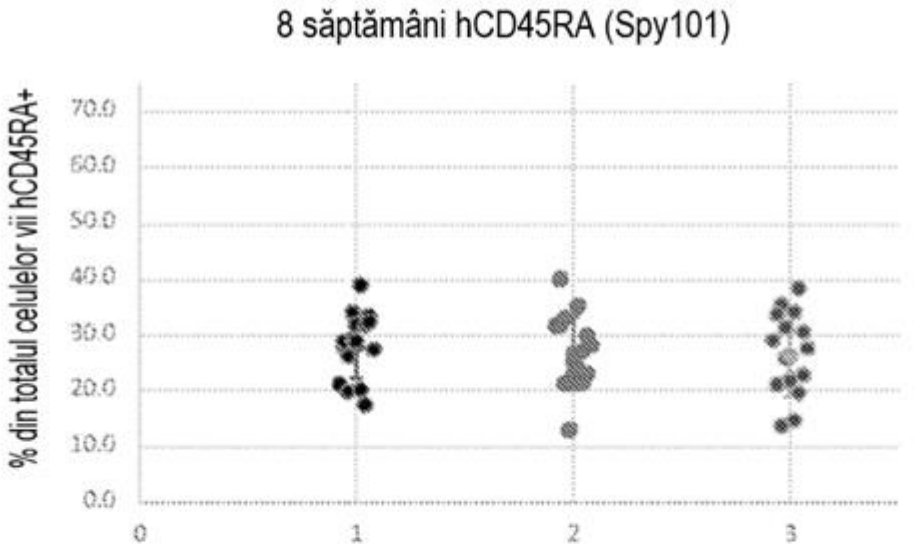


Figura 29E

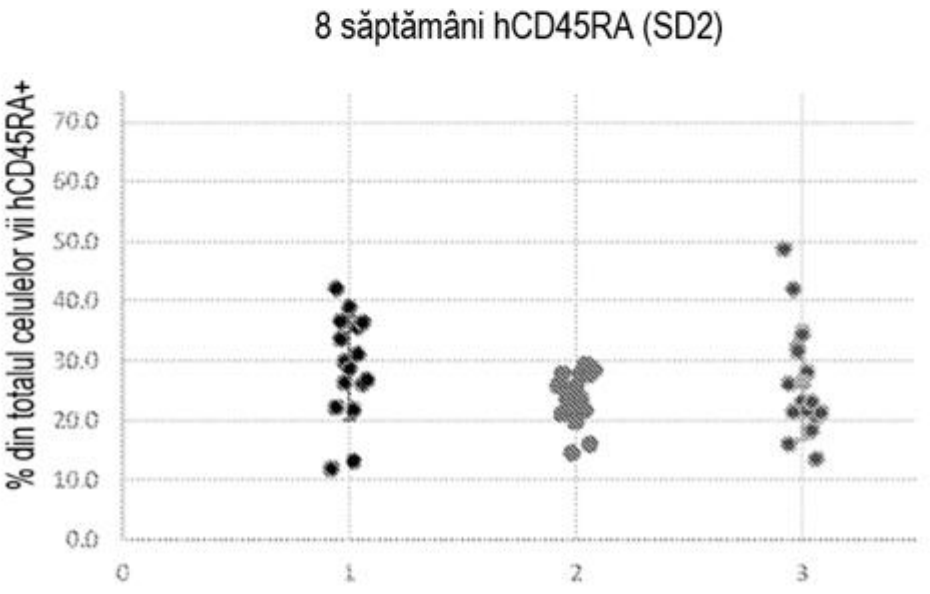


Figura 30

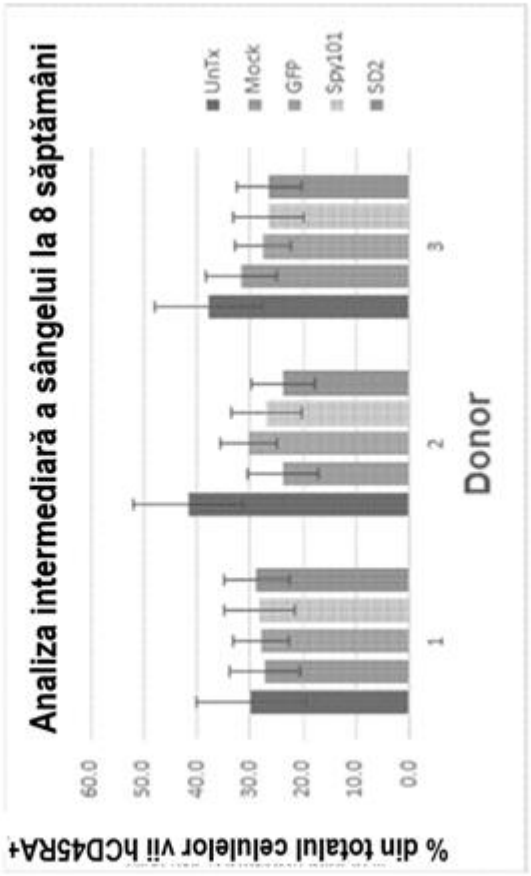
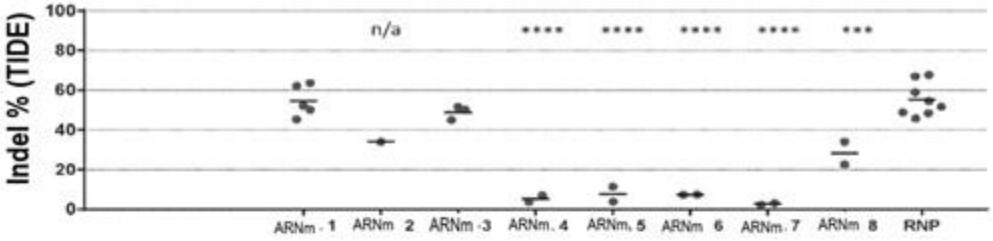

Analiza intermediară a sângelui la 8 săptămâni
% din totalul celulelor vii hCD45RA+
60.0
50.0
40.0
30.0
20.0
10.0
0.0
UnTx
Mock
GFP
Spy101
SD2
1
2
3
Donor

Figura 31

Cas9RNP este la fel de eficient în editare ca și ARNm Cas9, dar superior în ceea ce privește viabilitatea post- electroporare

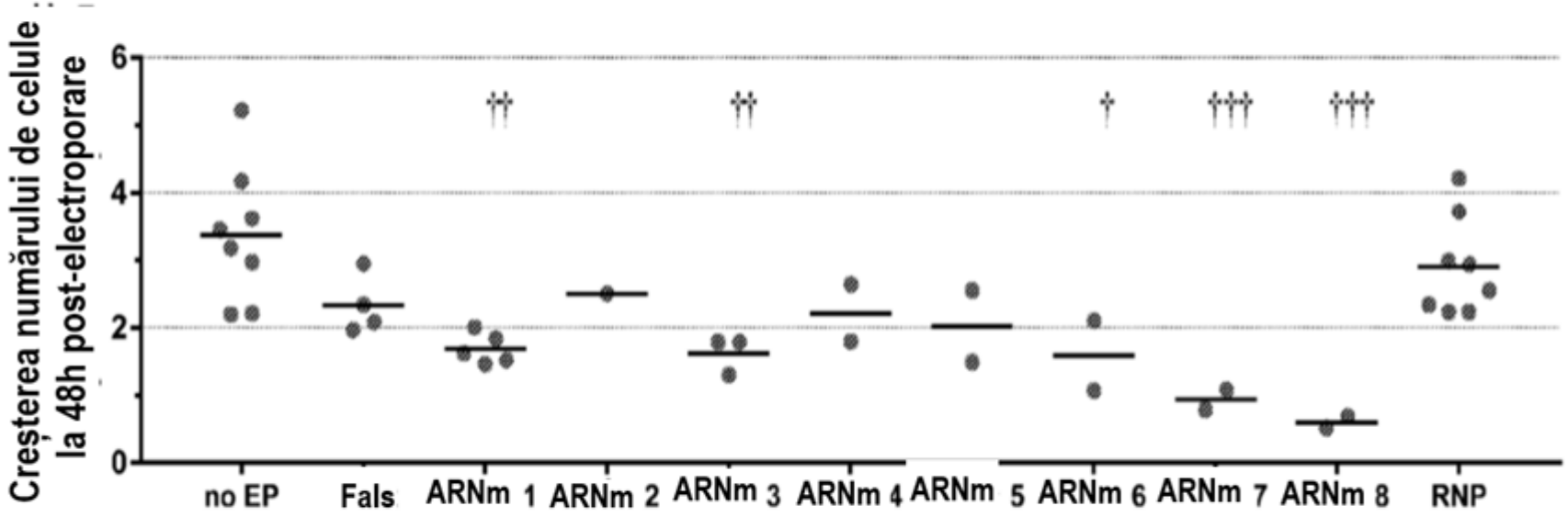


*** p<0.001 comparativ cu RNP prin ANOVA unidirecțională
**** p<0.0001 comparativ cu RNP prin ANOVA unidirecțională

e 2019 0261

Cas9RNP este superioară față de ARNm Cas9 în ceea ce privește viabilitatea celulelor în post- electroporare

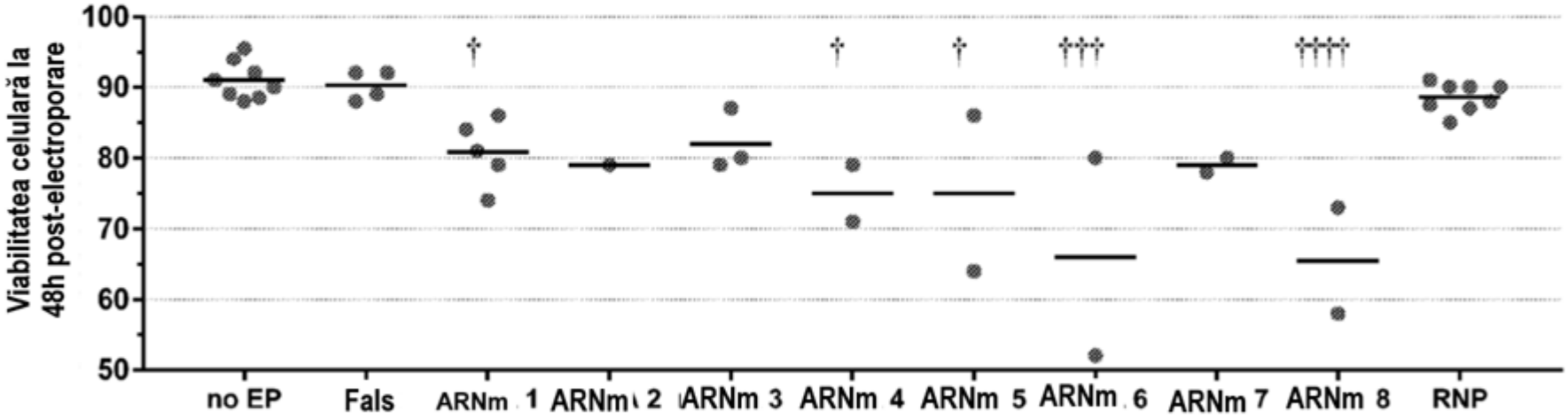
Figura 32A



† p<0.05, †† p<0.01, ††† p<0.001, †††† p<0.0001 în comparație cu cea fără EP prin ANOVA unidirecțională

Cas9RNP este superioară față de ARNm Cas9 în ceea ce privește viabilitatea celulelor în post- electroporare

Figura 32B



† p<0.05, †† p<0.01, ††† p<0.001, †††† p<0.0001 în comparație cu cea fără EP prin ANOVA unidirecțională

Figura 33A

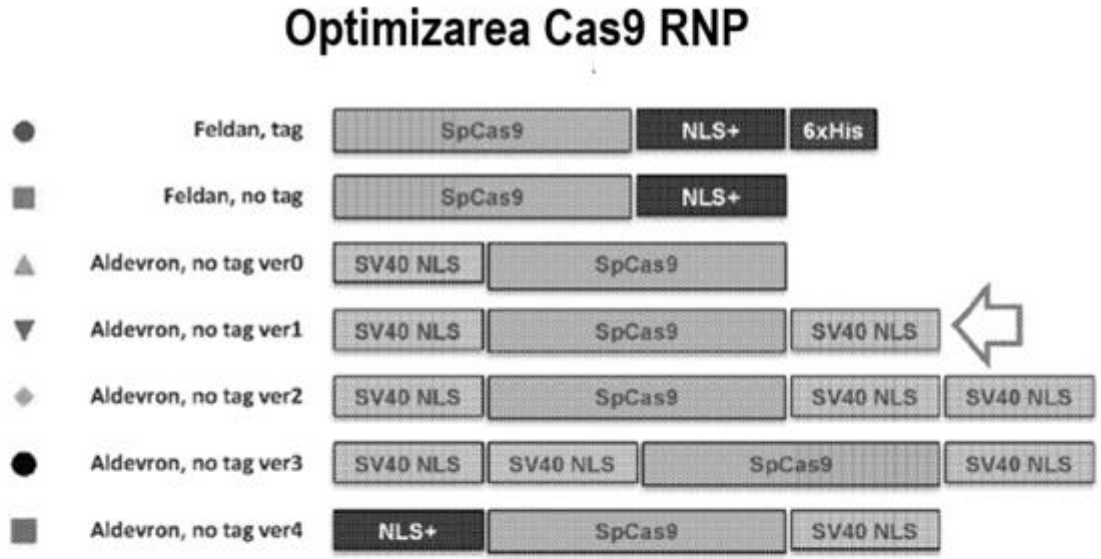


Figura 33B

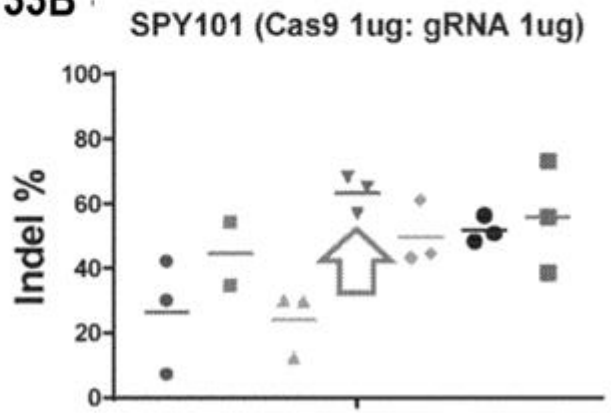


Figura 33 C

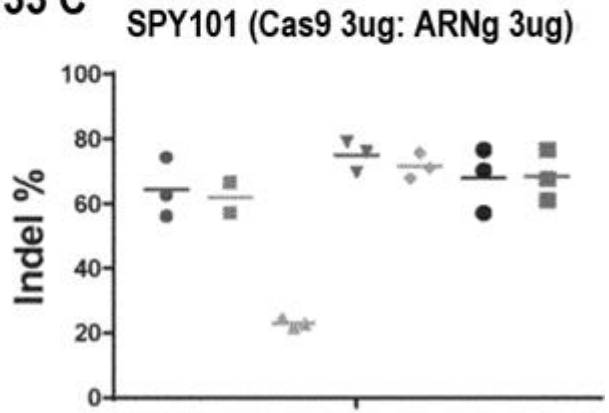


Figura 34A

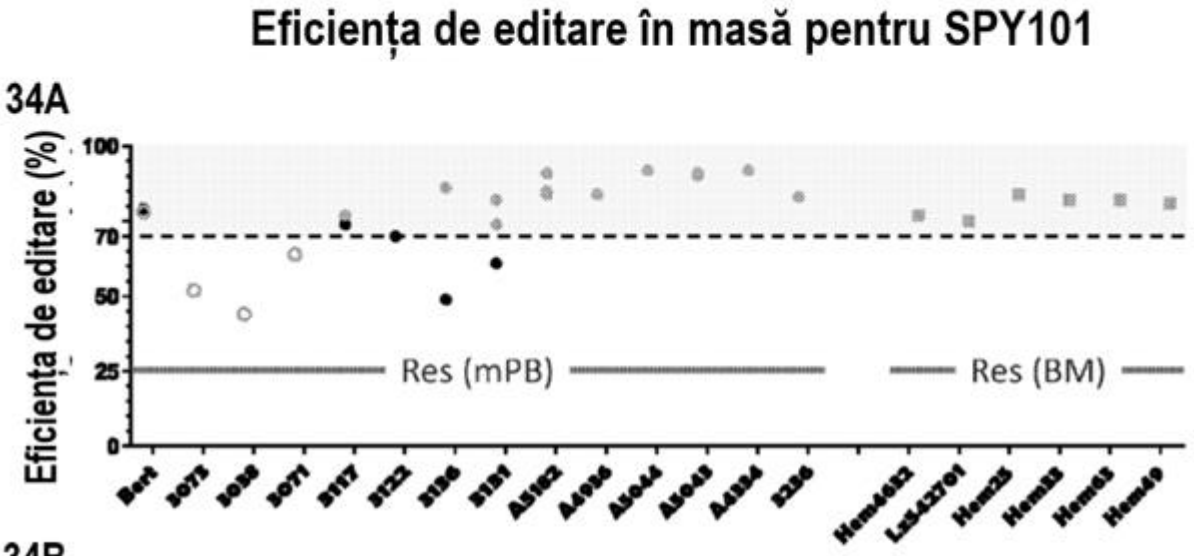
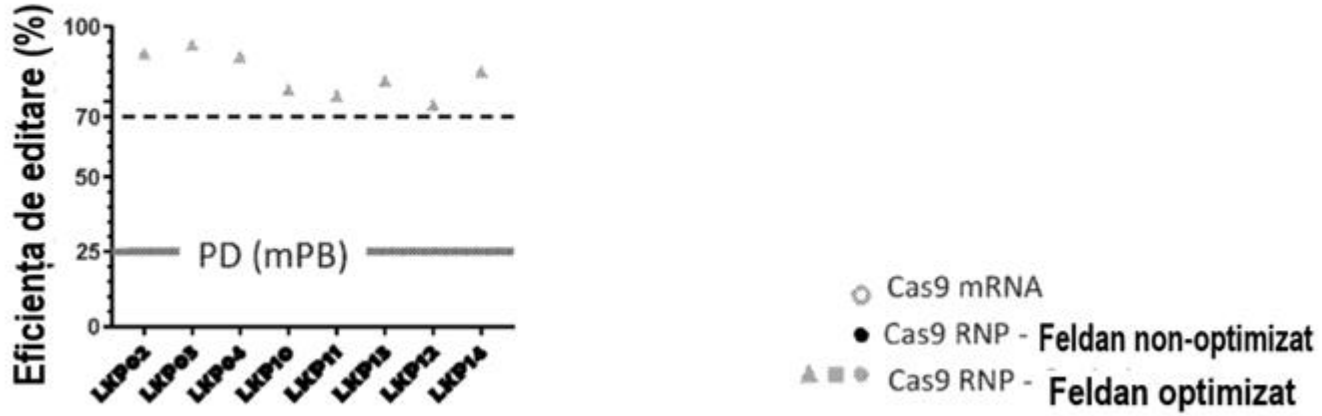


Figura 34B



Eficacitatea SPY 101 în celulele CD34+ mPB

Figura 35A

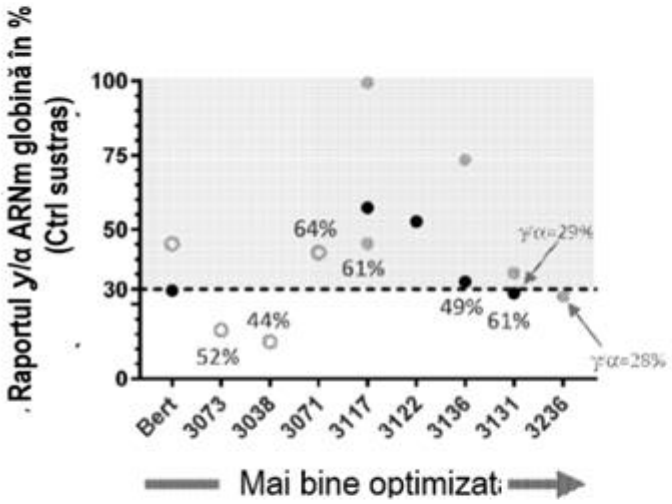
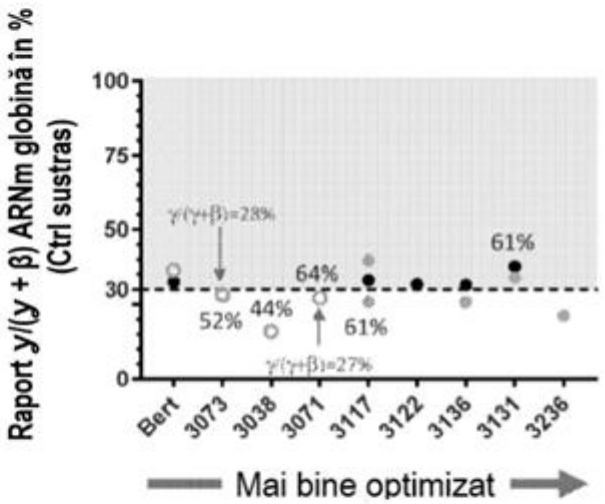


Figura 35B



% = Eficiența de editare

Toate punctele de date neetichetate au avut
> 70% eficiență de editare

○ Cas9 mRNA - Diferențiere non-optimizată
● Cas9 RNP - Diferențiere non-optimizată
● Cas9 RNP - Diferențiere optimizată

Eficacitatea SPY 101 în celulele CD34+ BM

Figura 36A

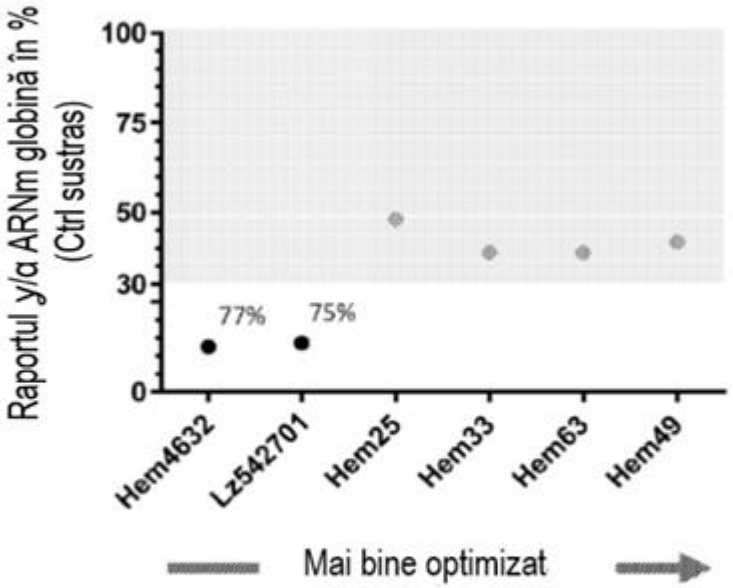
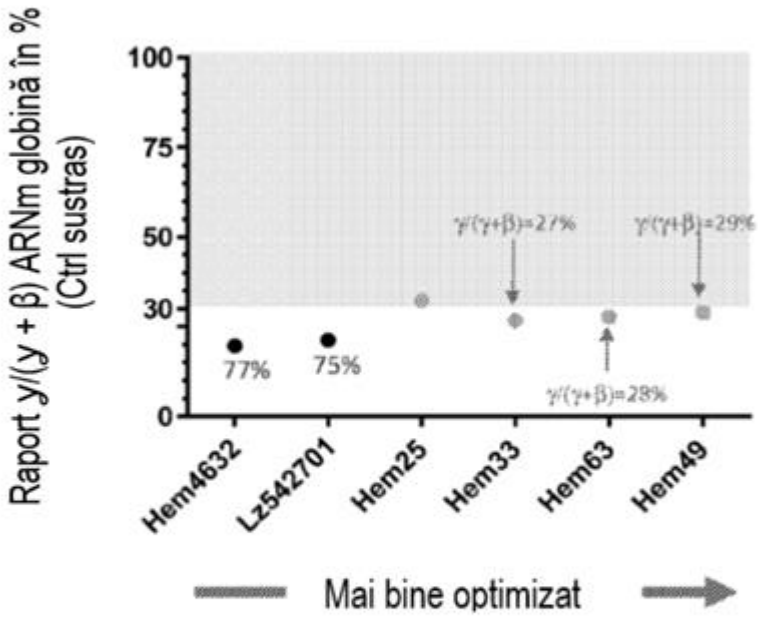


Figura 36B

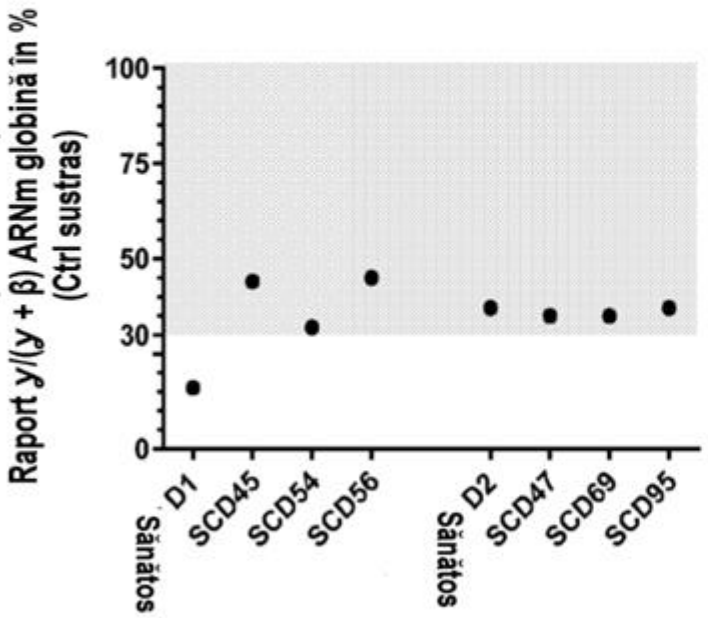


% = Eficiența de editare
Toate punctele de date neetichetate au avut
> 80% eficiență de editare

● Cas9 RNP - Non-optimizat
● Cas9 RNP - Optimizat

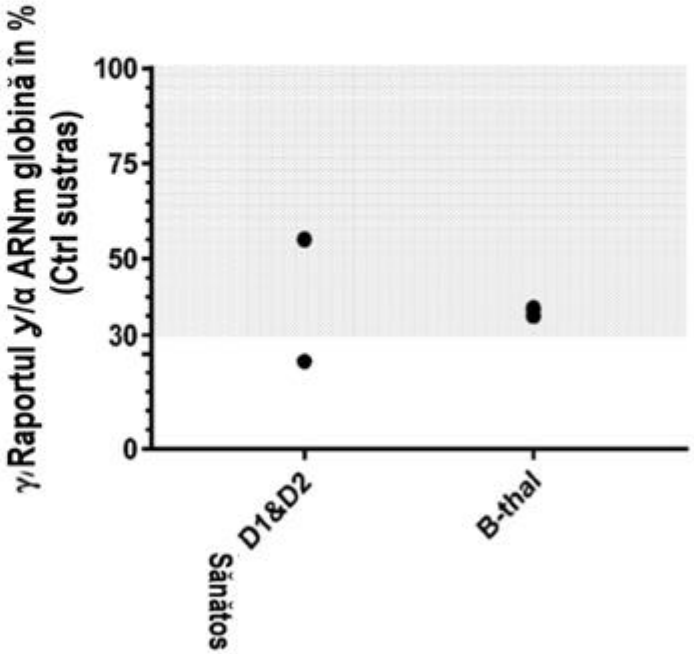
Eficacitatea SPY 101 în eșantioanele pacienților

Figura 37A



De la pacienții cu SCD

Figura 37B

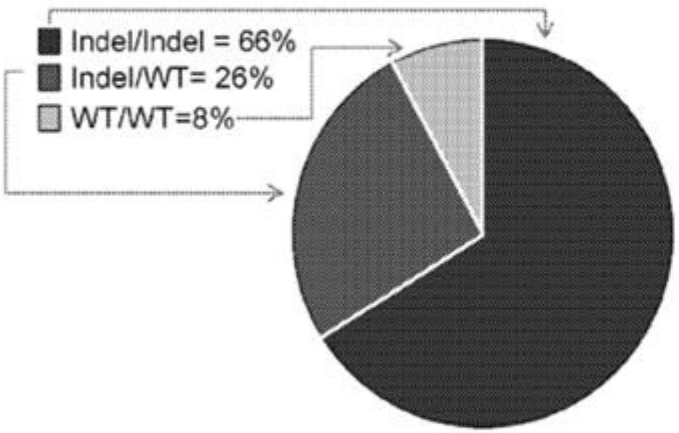


De la pacienti β-talasemici

Degradarea genotipului editat SPY 101

Figura 38A

Cas9 ARNm



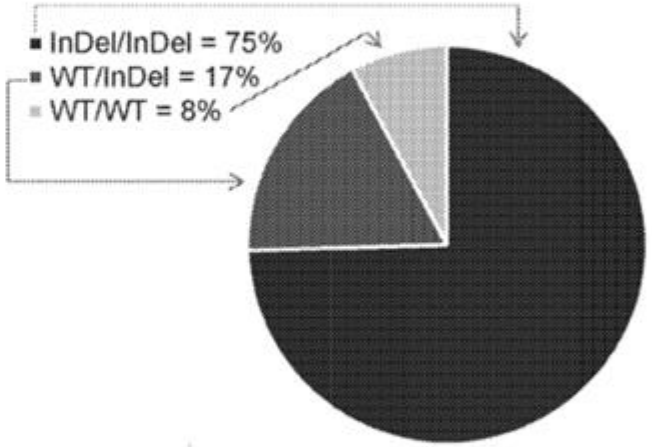
Frecvența Indel = 79%.
Colonii editate = 92%.

Date de la 60 de colonii

Data from 60 colonies

Figura 38B

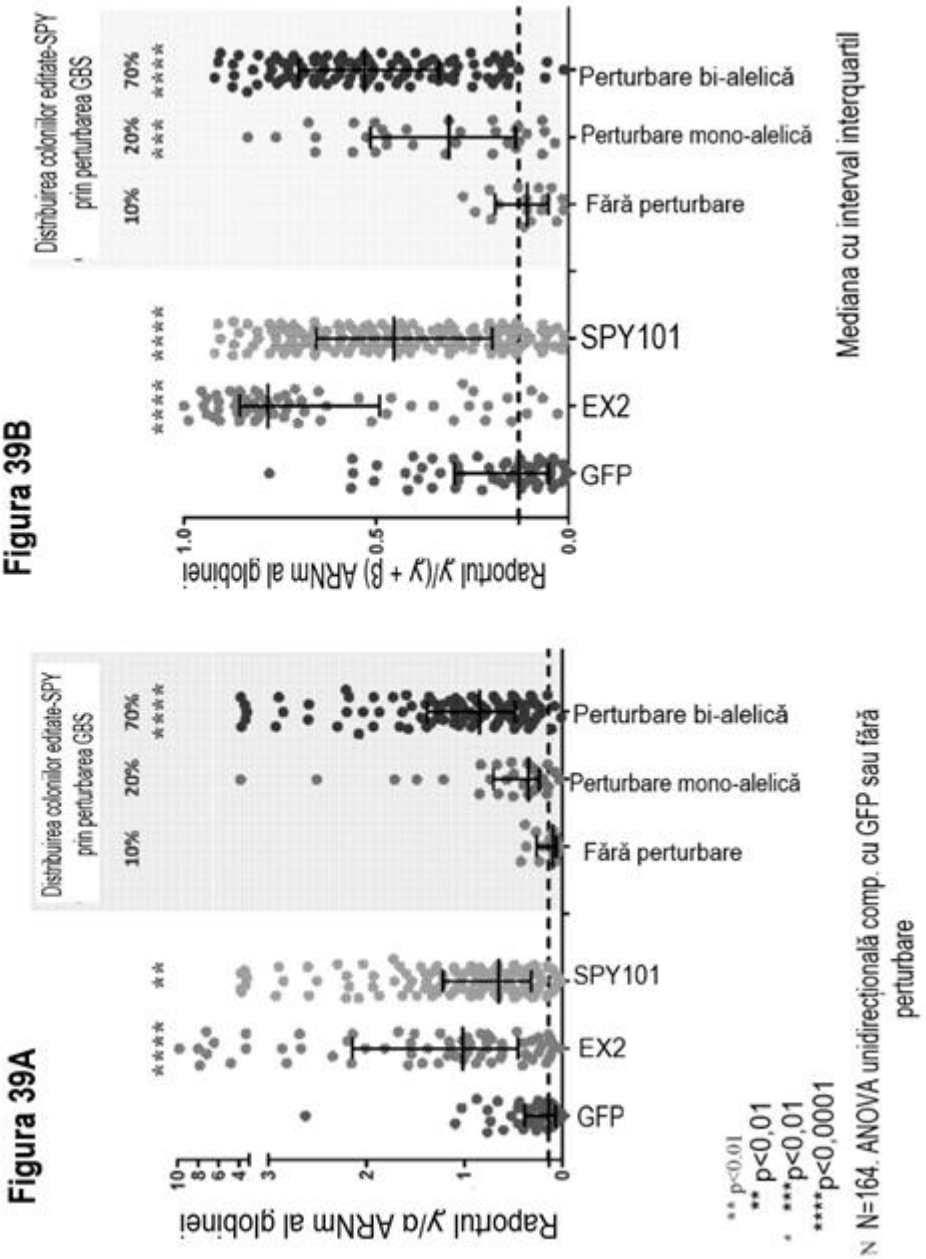
Cas9 RNP



Frecvența Indel = 83%
Colonii editate = 92%

Date de la 164 de colonii

Perturbarea situsului de legare a Gata 1 de către SPY 101 față de exprimarea γ-globinei crescută



SPY101 a crescut exprimarea γ-globinei

Figura 40A

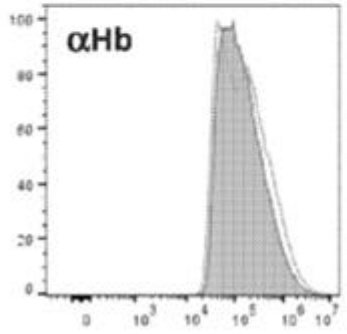


Figura 40B

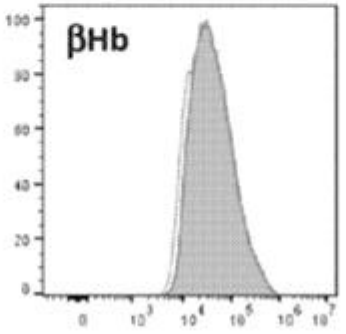


Figura 40C

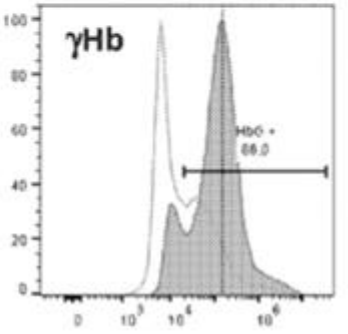


Figura 40D

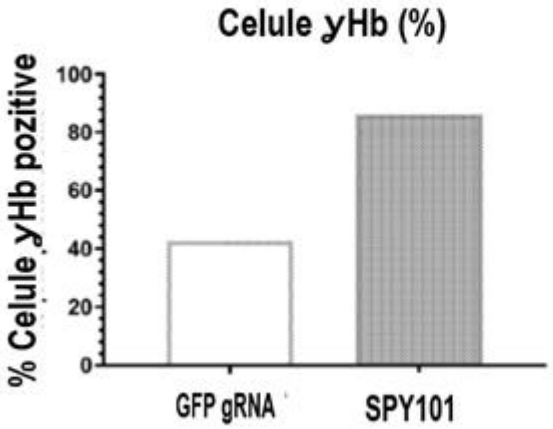


Figura 40E