Bcl11a miR1 oligonucleotides:

Sense (SEQ. ID. NO: 1) ACGCT**CGCACAGAACACTCATGGATT***ctccatgtggtagag***AATCCATGAGTGTTCTGTGCG**AG

Anti-sense (SEQ. ID. NO: 2) CGCACT**CGCACAGAACACTCATGGATT***ctctaccacatggag***AATCCATGAGTGTTCTGTGCG**A

Bcl11a miR2 oligonucleotides:

Sense (SEQ. ID. NO: 3) ACGCT**CCAGAGGATGACGATTGTTTA***ctccatgtggtagag***TAAACAATCGTCATCCTCTGG**ag

Anti-sense (SEQ. ID. NO: 4) CGCAct**CCAGAGGATGACGATTGTTTA***ctctaccacatggag***TAAACAATCGTCATCCTCTGG**a

Italicized lower bases = shRNA loop
Bold and upper case bases = BCL11A targeting sequence for forming a shRNA
Underlined bases = Cloning ends (synthetic)

FIG. 1

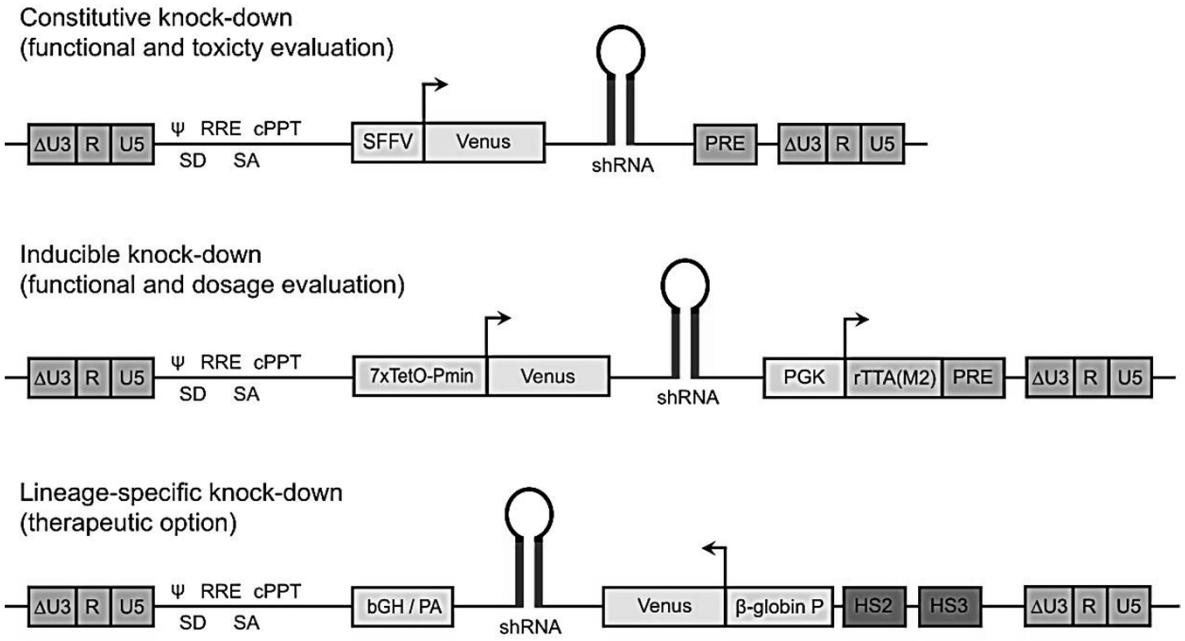
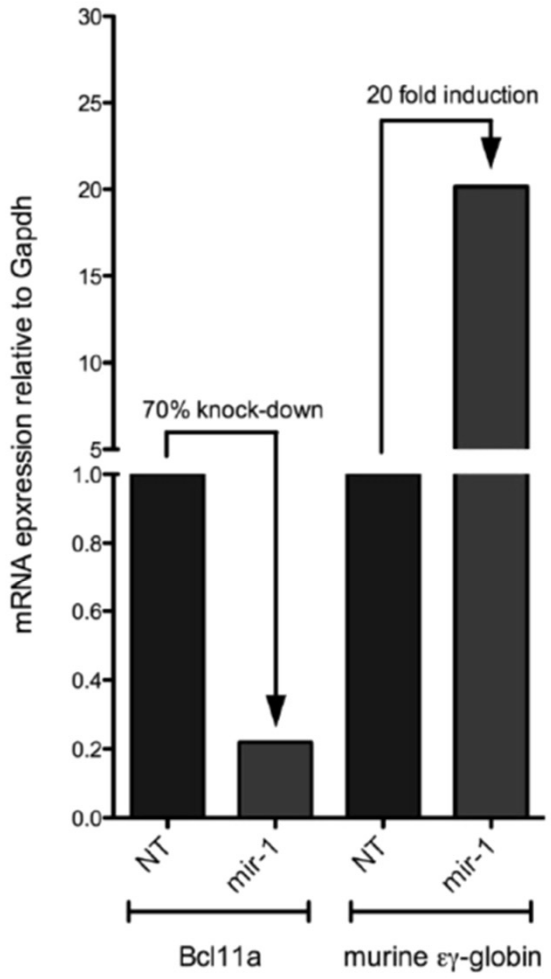


FIG. 2

SFFV-LV efficiently knocks-down Bcl11a and induces εγ-globin expression

in vitro: transduced murine erythroleukemia cells



in vivo: transplanted transduced LSK-HSC

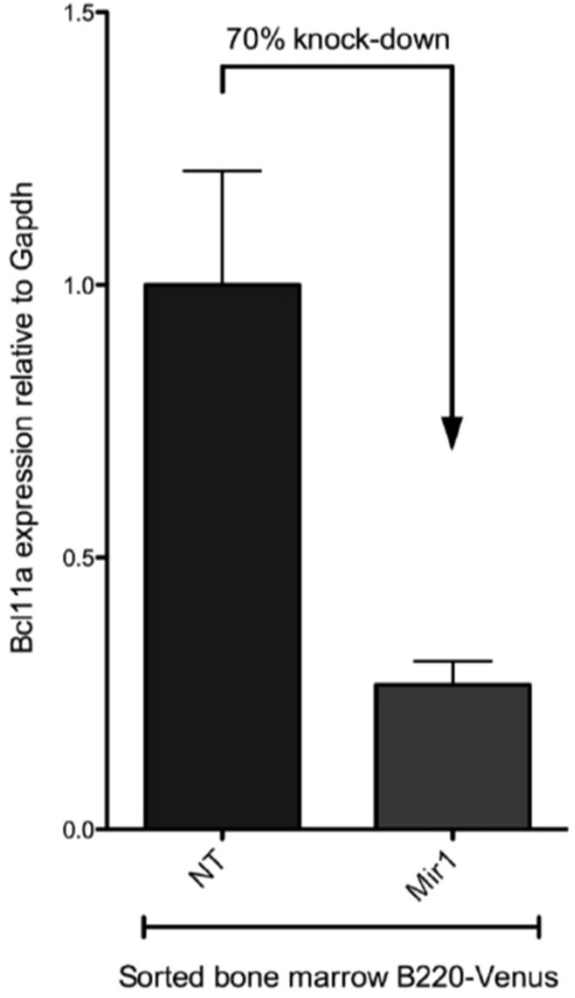
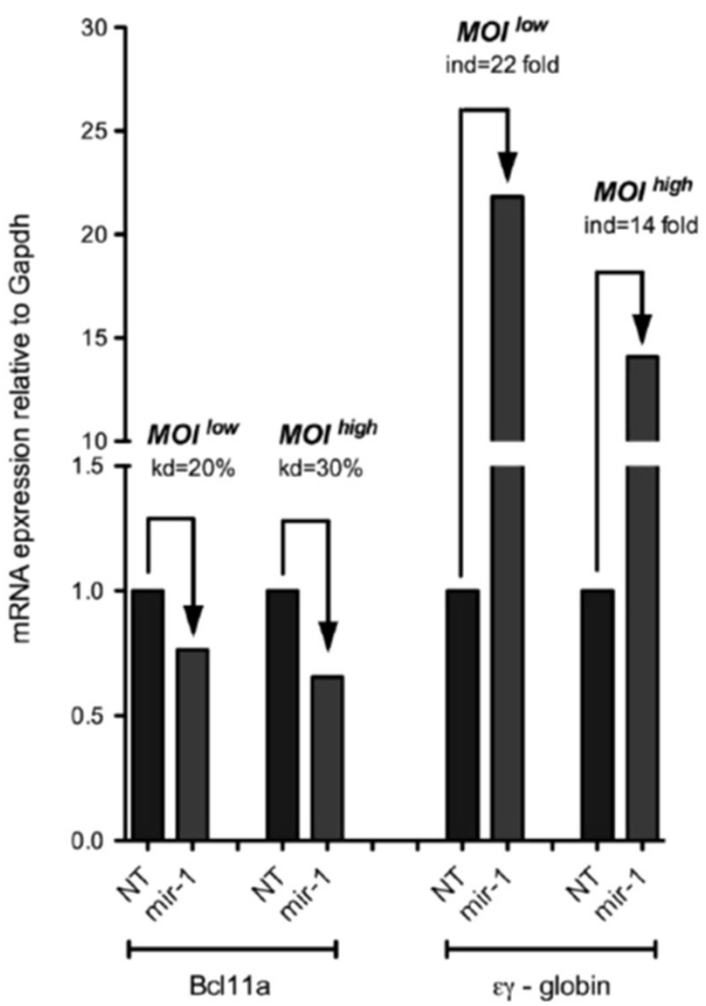


FIG. 3

LCR/TET-LV efficiently knock-down Bcl11a and induce εγ-globin expression

LCR-LV : effects of higher MOI transduction



TET-LV : doxycycline-induction dose-dependence

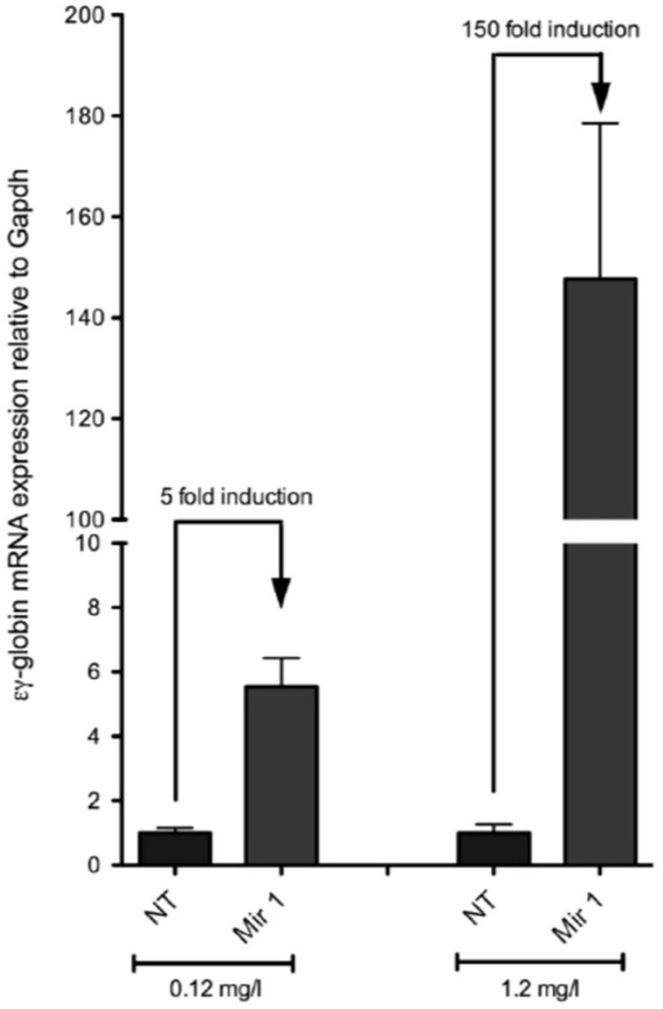
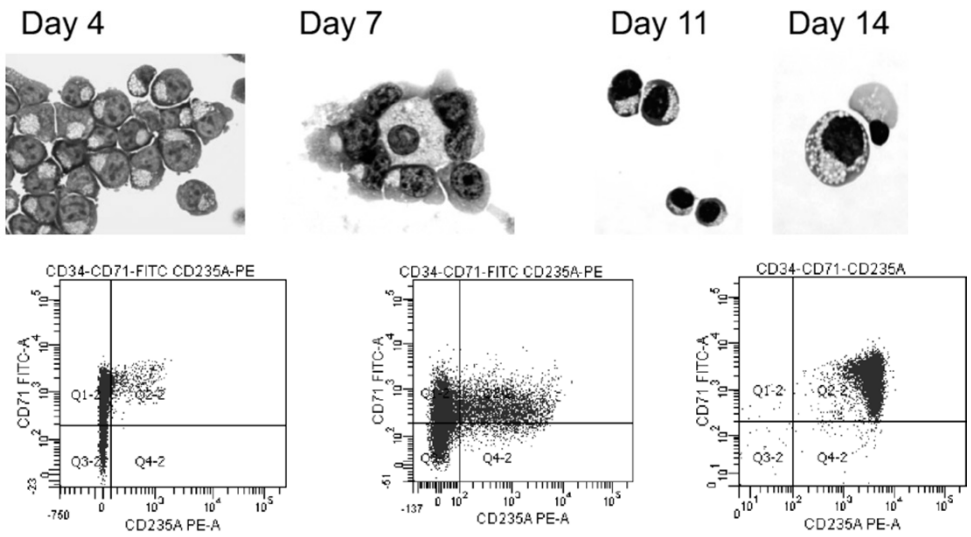
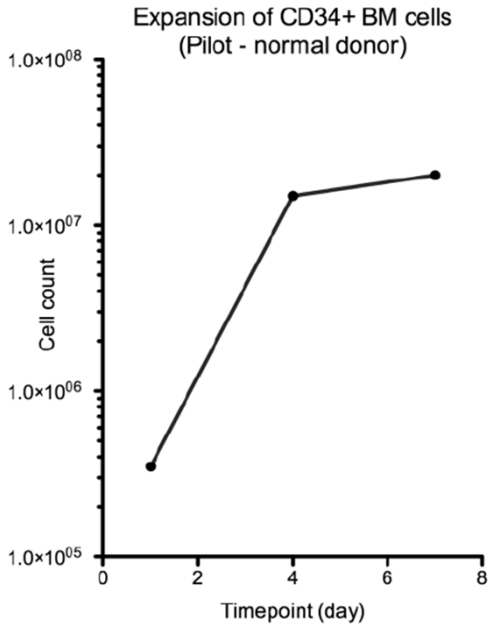
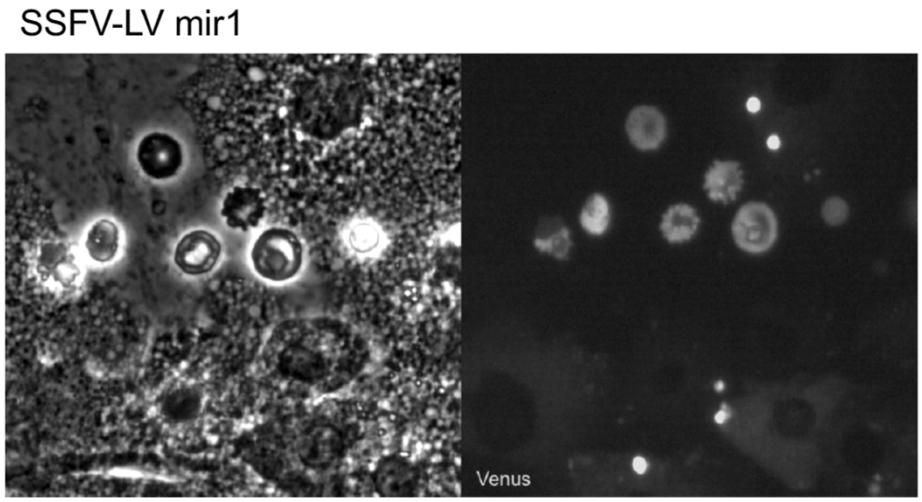


FIG. 4

Transduced CD34+ HSC differentiate ex-vivo into erythrocytes and express HbF







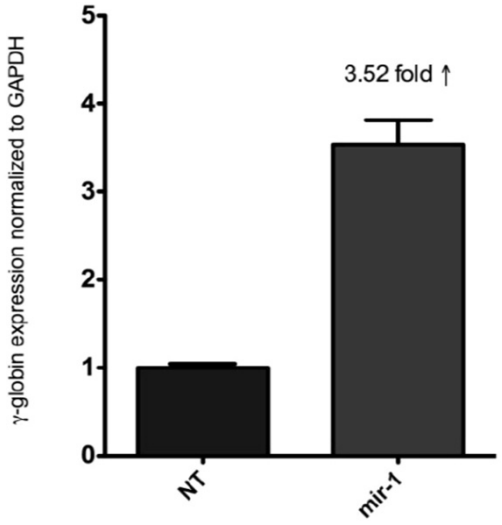


FIG. 5

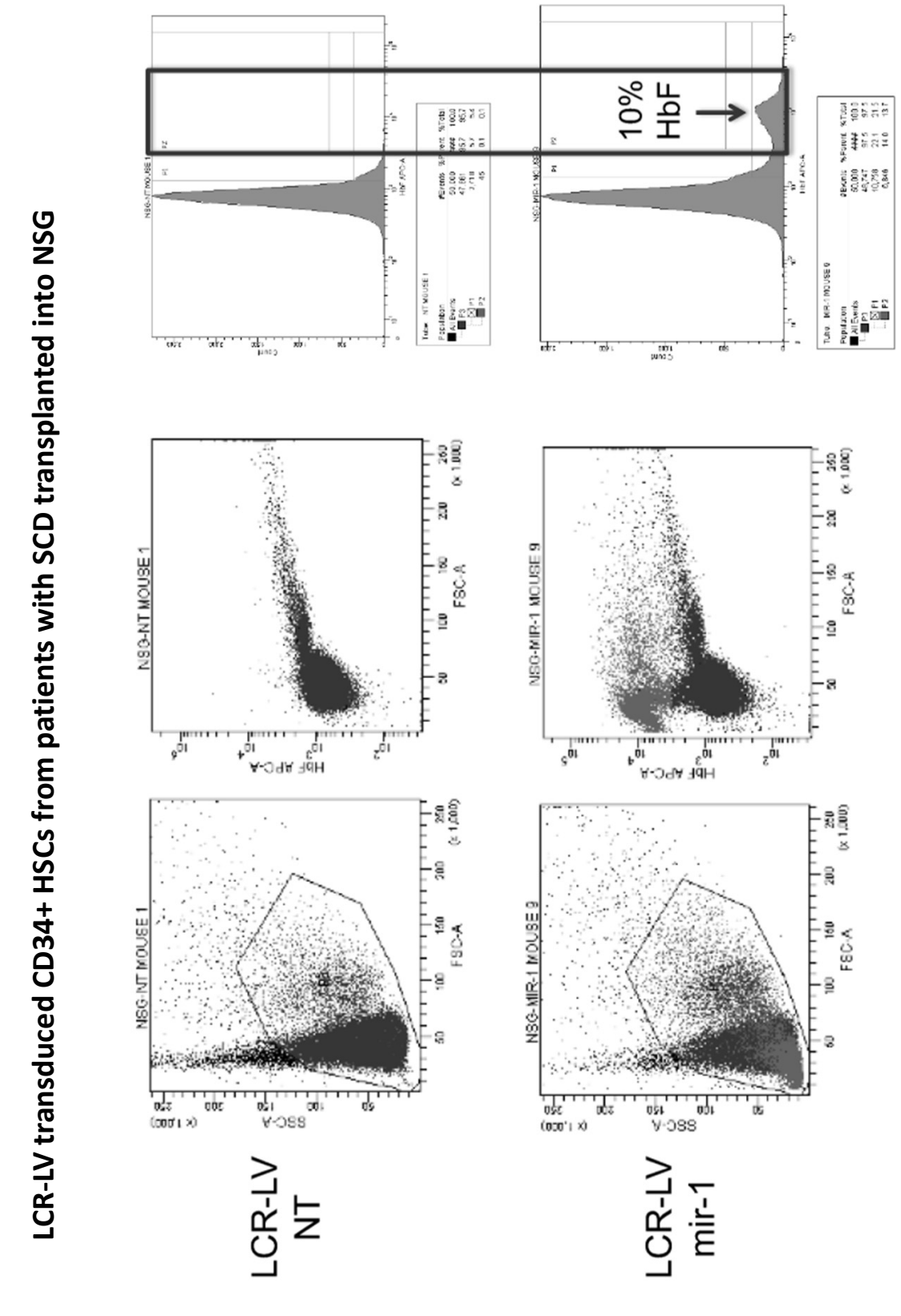

LCR-LV transduced CD34+ HSCs from patients with SCD transplanted into NSG
LCR-LV NT
LCR-LV mir-1
NSG-NT MOUSE 1
NSG-MIR-1 MOUSE 9
SSC-A
FSC-A
HbF APC-A
10% HbF

FIG. 6

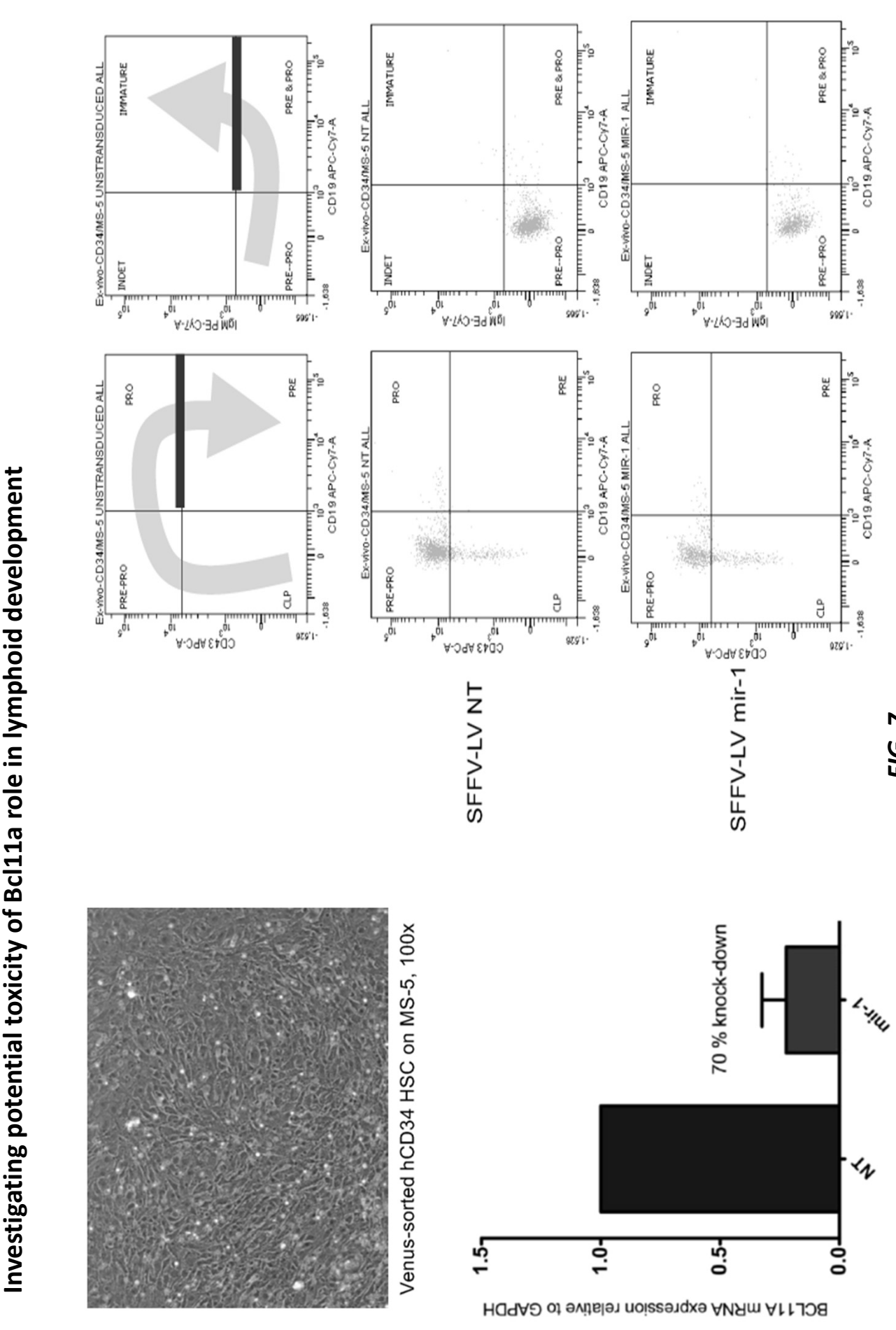

Investigating potential toxicity of Bcl11a role in lymphoid development
Venus-sorted hCD34 HSC on MS-5, 100x
BCL11A mRNA expression relative to GAPDH
1.5
1.0
0.5
0.0
NT
mir-1
70 % knock-down
SFFV-LV NT
SFFV-LV mir-1
Ex-vivo-CD34/MS-5 UNSTRANSDUCED ALL
Ex-vivo-CD34/MS-5 NT ALL
Ex-vivo-CD34/MS-5 MIR-1 ALL
INDET
IMMATURE
PRE-PRO
PRE--PRO
PRE & PRO
PRO
PRE
CLP
IgM PE-CY7-A
CD19 APC-Cy7-A
CD43 APC-A

FIG. 7

shRNAs vs microRNA adapted shRNAs

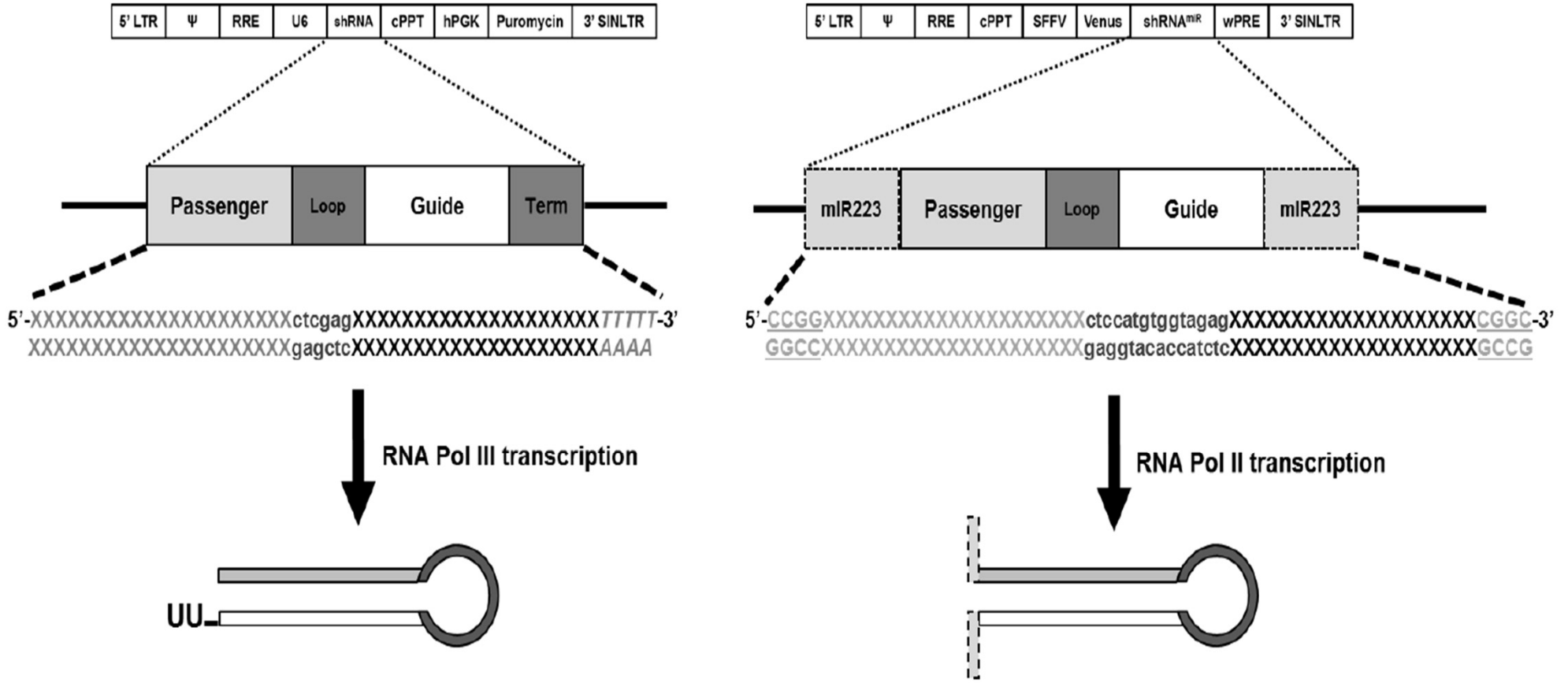


FIG. 8A

shRNA screen targeting Bcl11A using pLKO vector

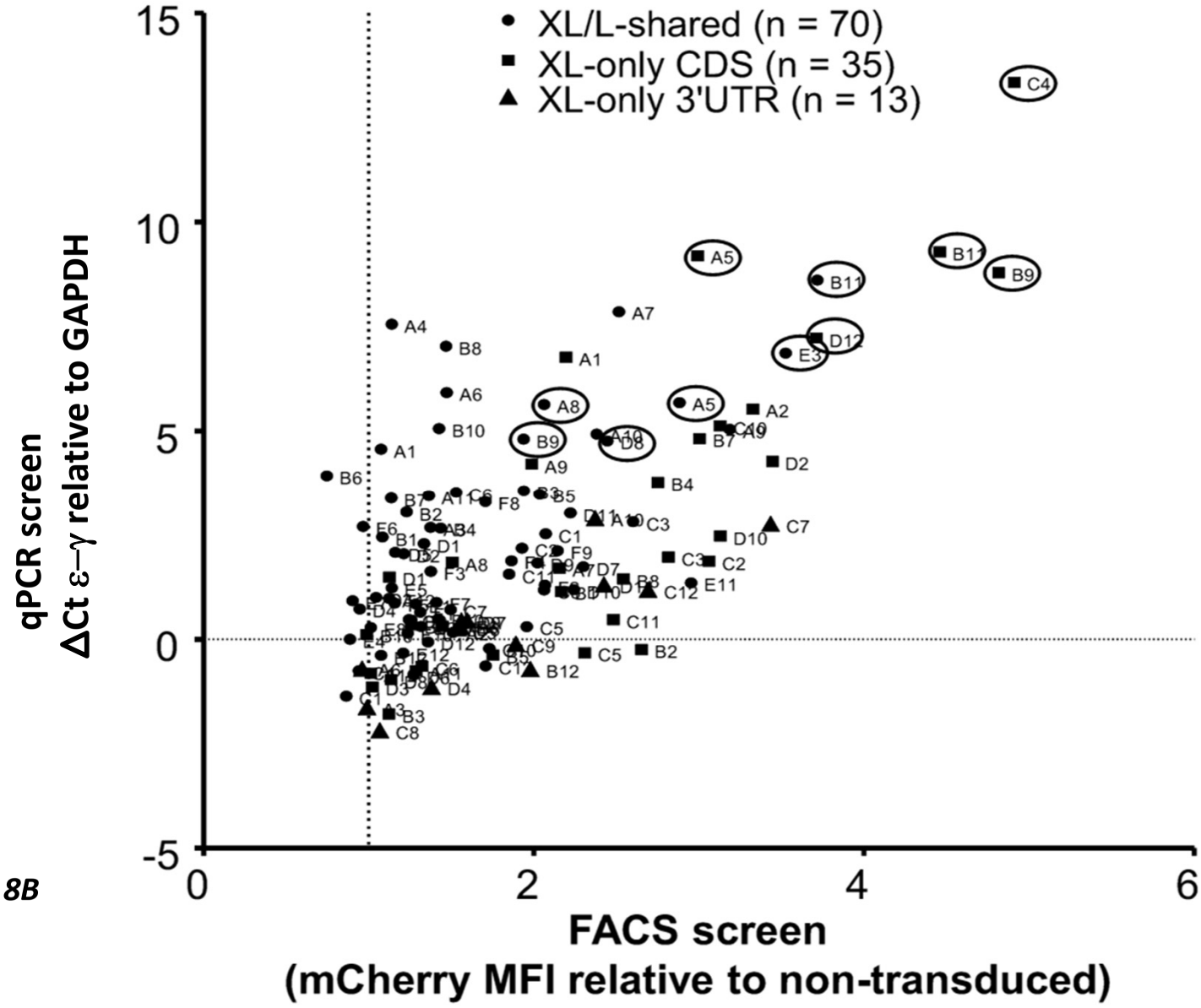


FIG. 8B

BCL11A knockdown and ε-γ induction in pLKO and LEGO back bones

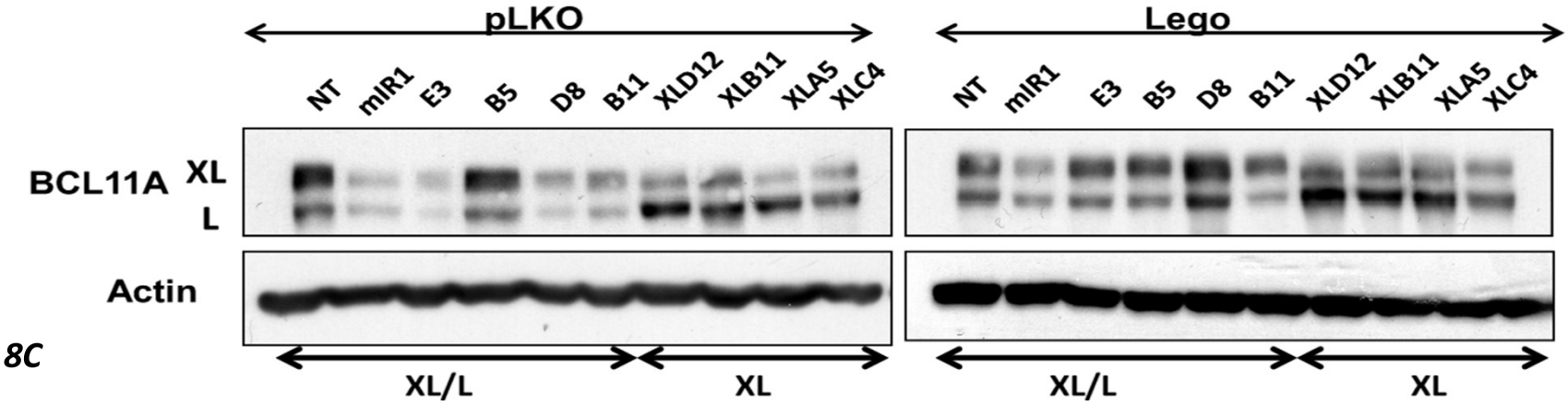


FIG. 8C

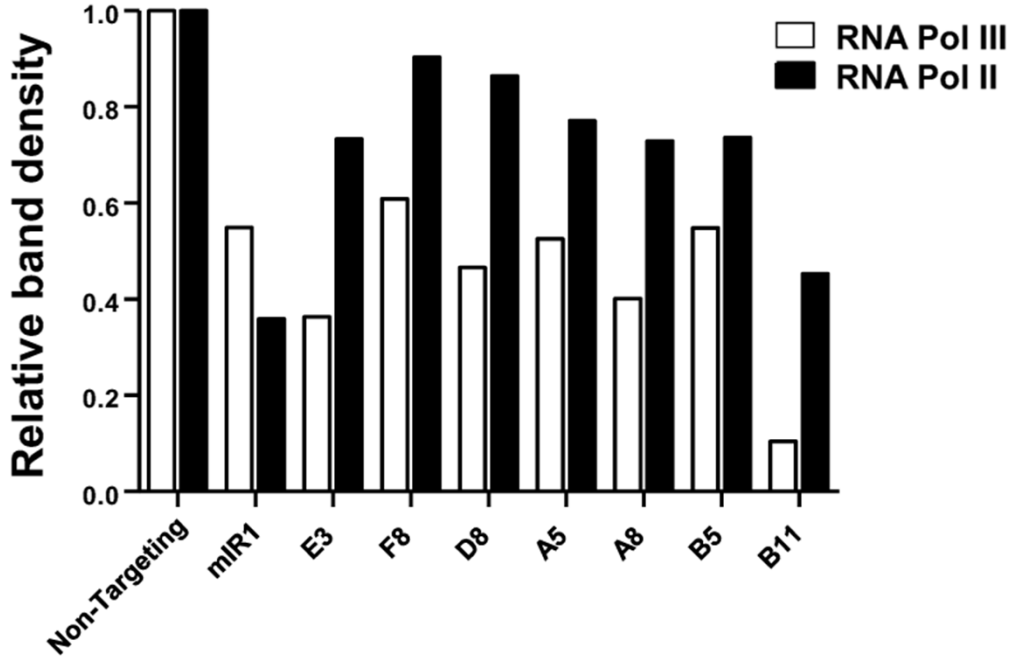


FIG. 8D

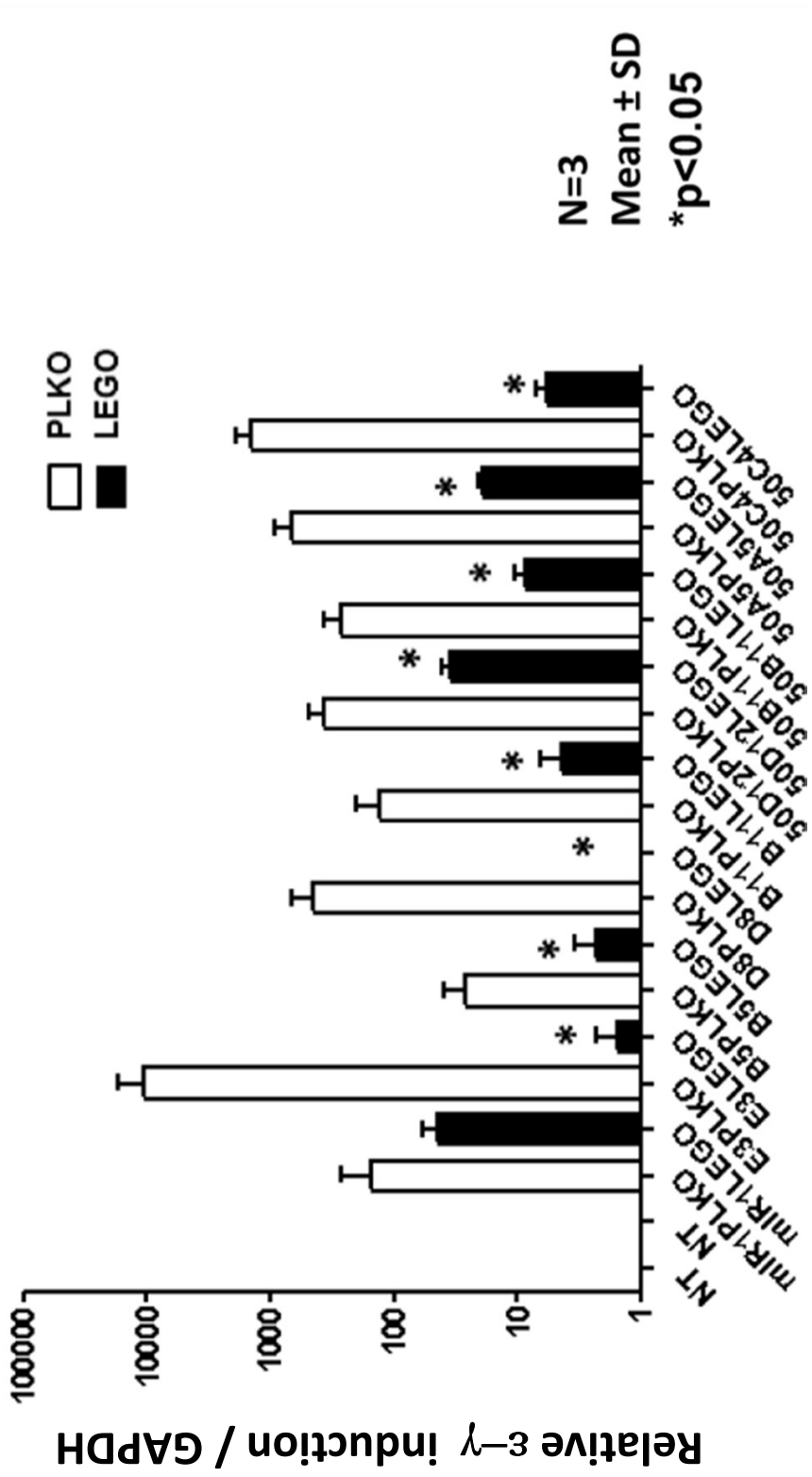

Relative ε–γ induction / GAPDH
100000
10000
1000
100
10
1
PLKO
LEGO
N=3
Mean ± SD
*p<0.05
NT
miR-1PLKO
miR-1LEGO
E3PLKO
E3LEGO
B5PLKO
B5LEGO
D8PLKO
D8LEGO
B11PLKO
B11LEGO
50D12PLKO
50D12LEGO
50B11PLKO
50B11LEGO
50A5PLKO
50A5LEGO
50C4PLKO
50C4LEGO

FIG. 8E

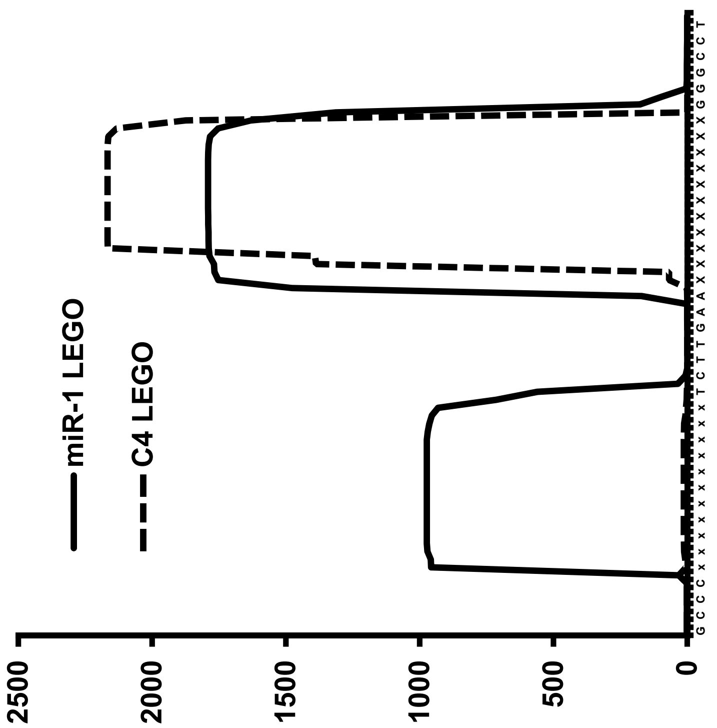

miR-1 LEGO
C4 LEGO
2500
2000
1500
1000
500
0

FIG. 9B

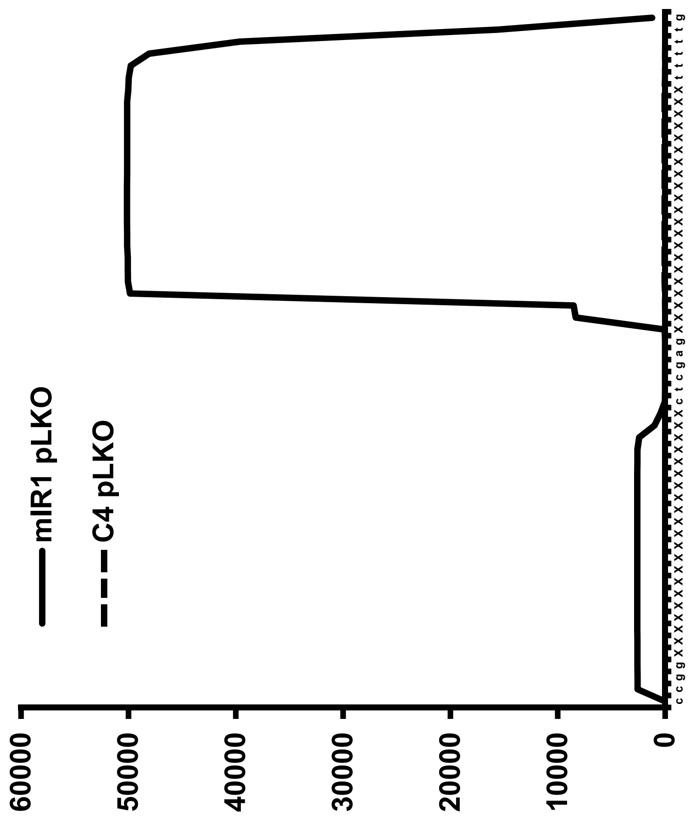

mIR1 pLKO
C4 pLKO
60000
50000
40000
30000
20000
10000
0

FIG. 9A

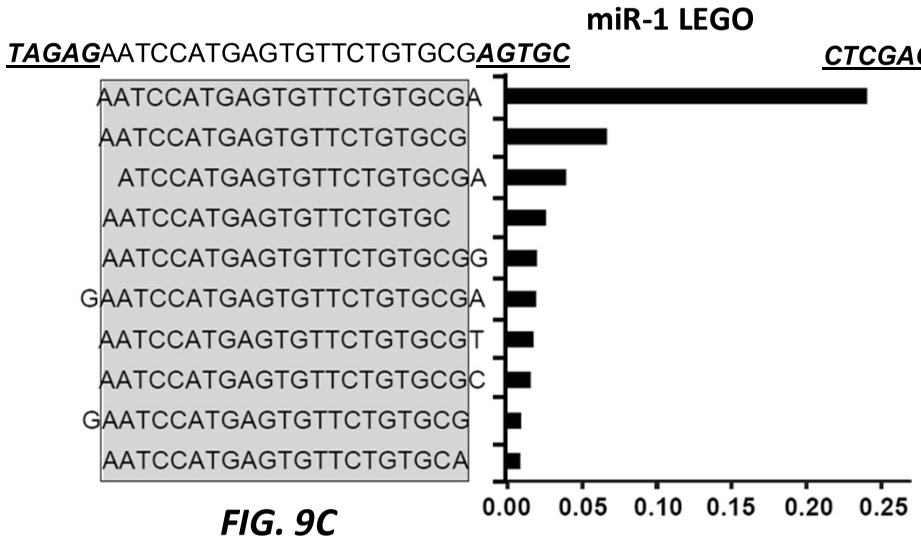


FIG. 9C

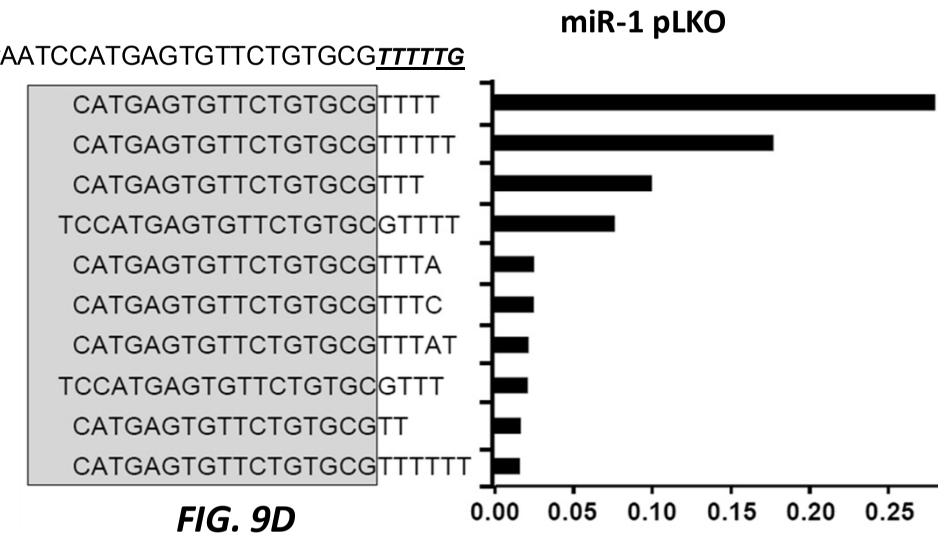


FIG. 9D

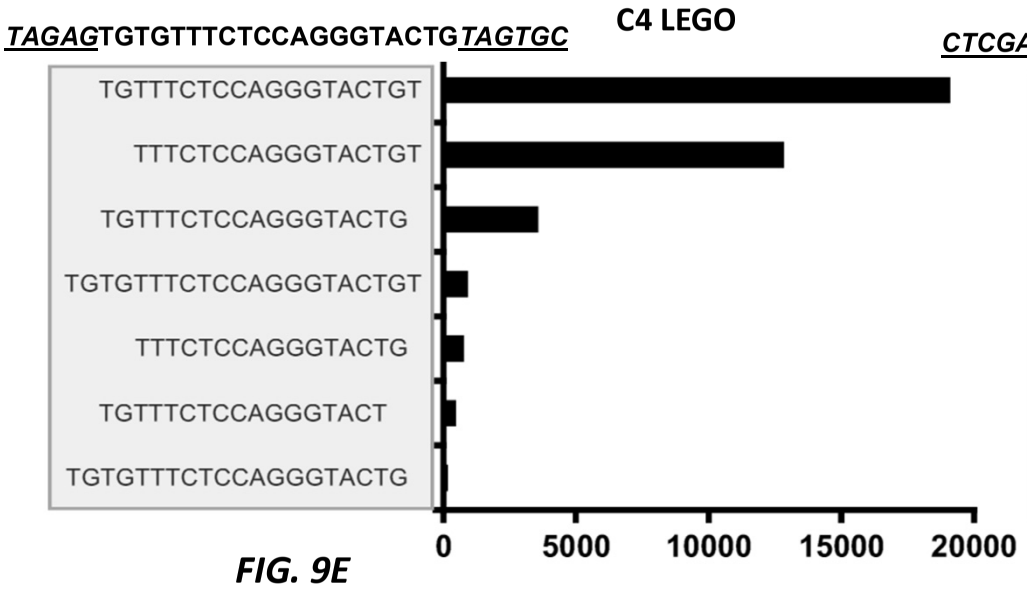


FIG. 9E

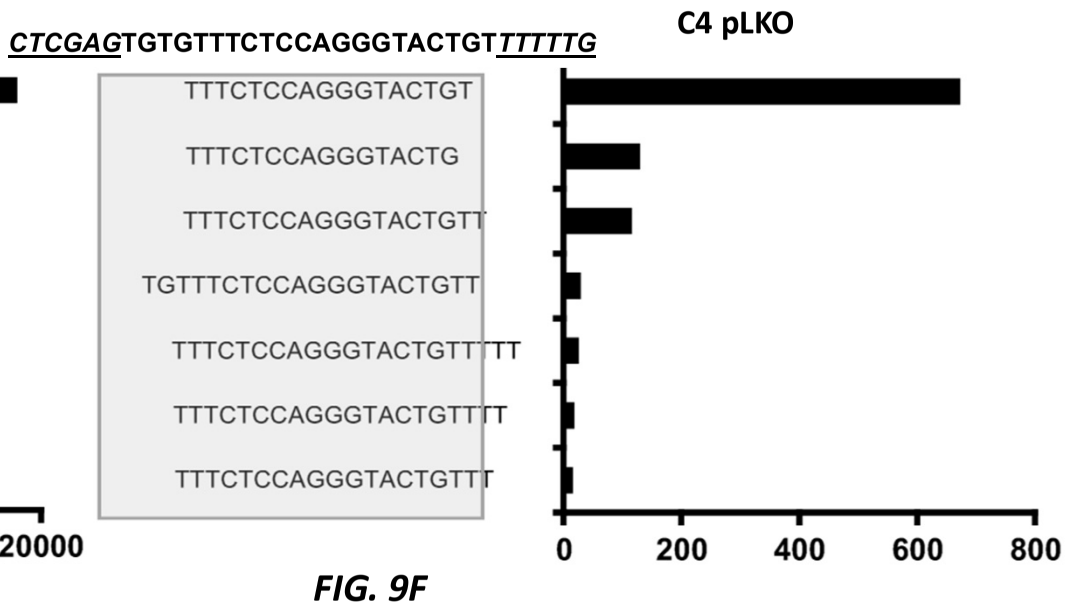


FIG. 9F

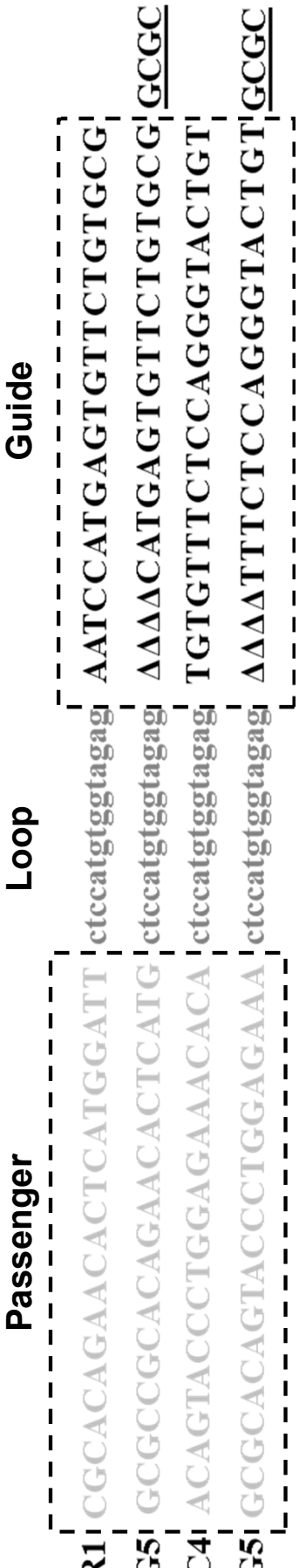


FIG. 10A

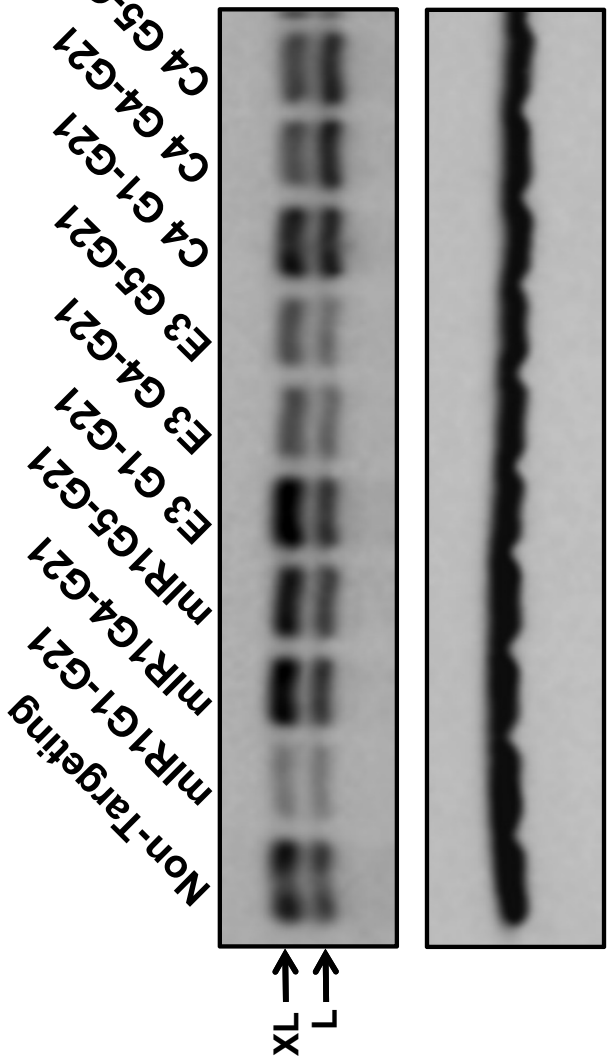


FIG. 10B

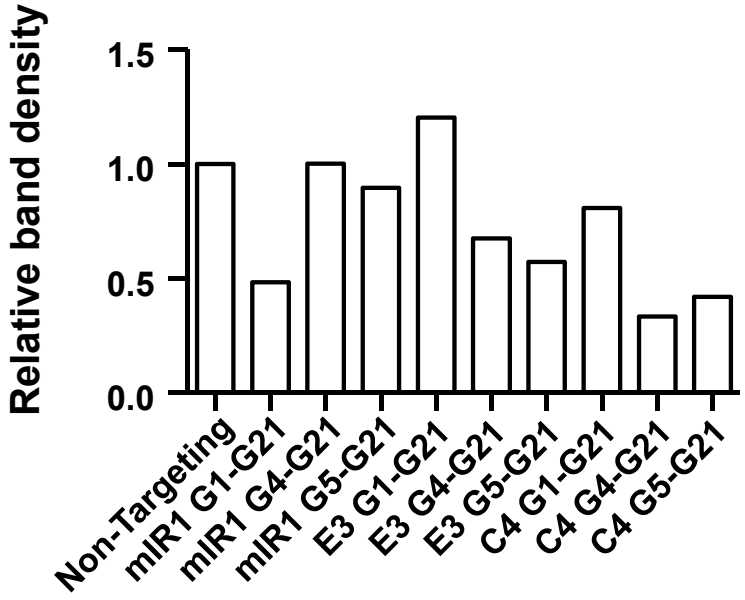


FIG. 10C

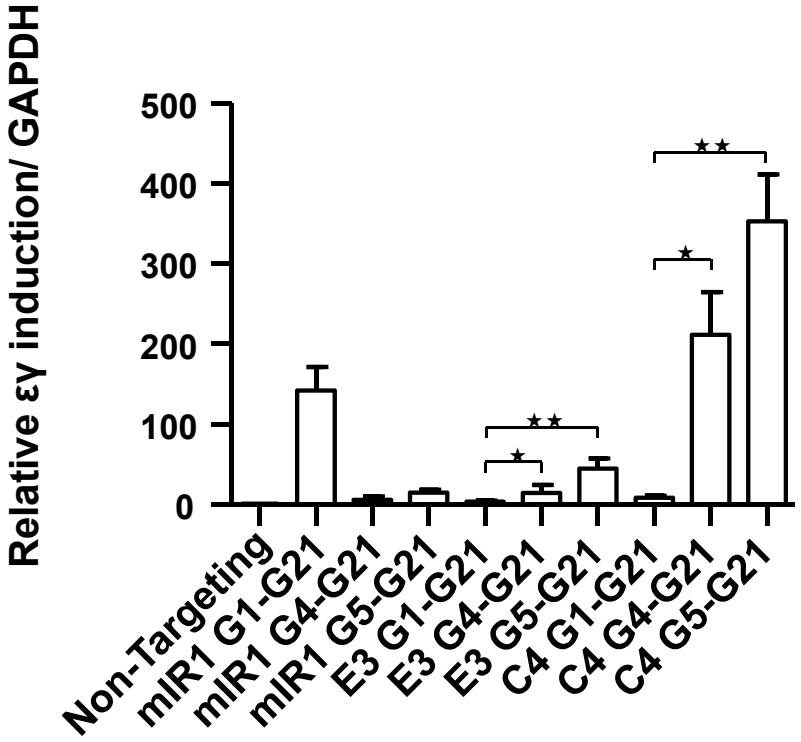


FIG. 10D

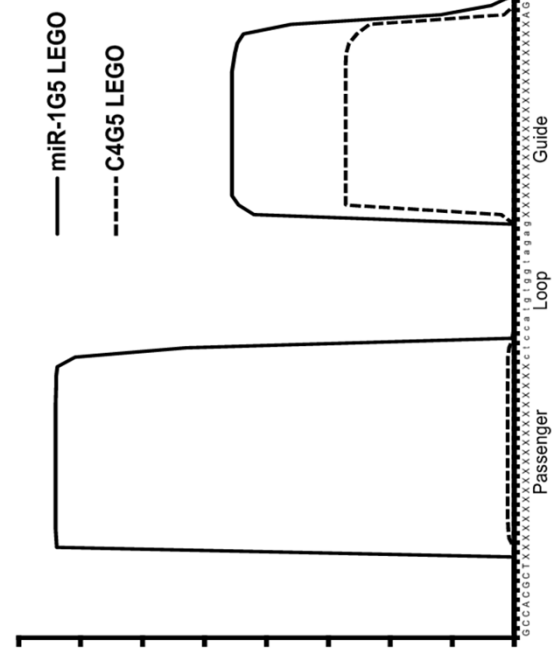


FIG. 11A

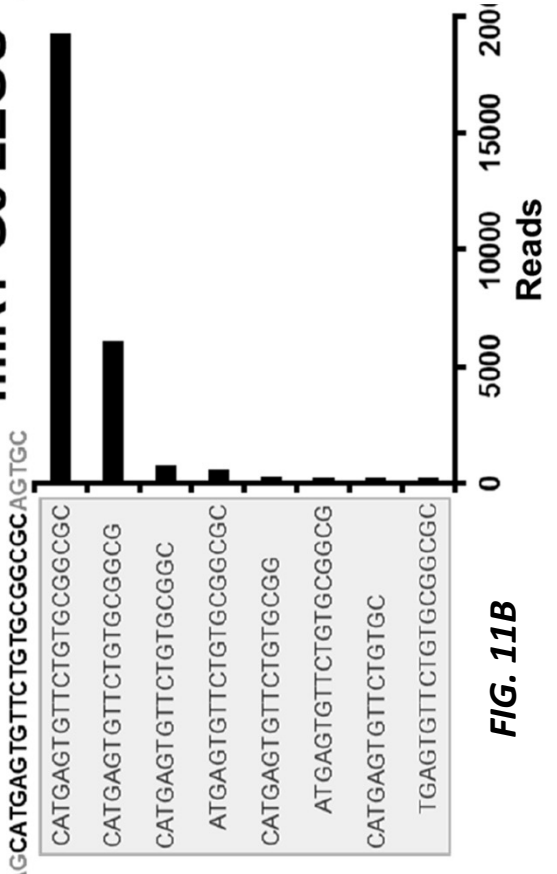


FIG. 11B

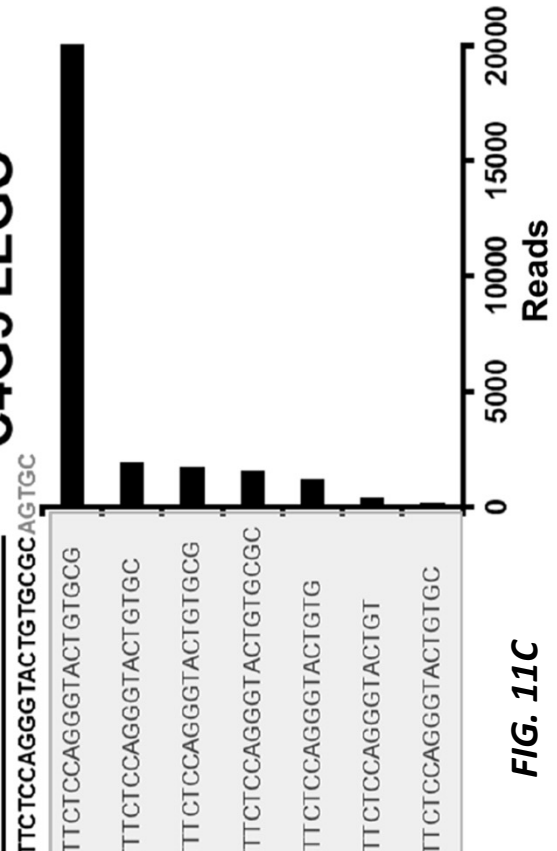


FIG. 11C

Candidates from the shRNA screen targeting Bcl11A using pLKO vector

	Passenger	Loop	Guide	
mIR1	CGCACAGAACACTCATGGATT	ctccatgtggtagag	AATCCATGAGTGTTCTGTGCG	SEQ. ID. NO: 25
E3	TCGGAGACTCCAGACAATCGC	ctccatgtggtagag	GCGATTGTCTGGAGTCTCCGA	SEQ. ID. NO: 26
B5	CCTCCAGGCAGCTCAAAGATC	ctccatgtggtagag	GATCTTTGAGCTGCCTGGAGG	SEQ. ID. NO: 27
D8	TTCTCTTGCAACACGCACAGA	ctccatgtggtagag	TCTGTGCGTGTTGCAAGAGAA	SEQ. ID. NO: 28
B11	TCAGGACTAGGTGCAGAATGT	ctccatgtggtagag	ACATTCTGCACCTAGTCCTGA	SEQ. ID. NO: 29
50D12	GATCGAGTGTTGAATAATGAT	ctccatgtggtagag	ATCATTATTCAACACTCGATC	SEQ. ID. NO: 30
50B11	CACTGTCCACAGGAGAAGCCA	ctccatgtggtagag	TGGCTTCTCCTGTGGACAGTG	SEQ. ID. NO: 32
50A5	CAGTACCCTGGAGAAACACAT	ctccatgtggtagag	ATGTGTTTCTCCAGGGTACTG	SEQ. ID. NO: 31
50C4	ACAGTACCCTGGAGAAACACA	ctccatgtggtagag	TGTGTTTCTCCAGGGTACTGT	SEQ. ID. NO: 33

FIG. 12A

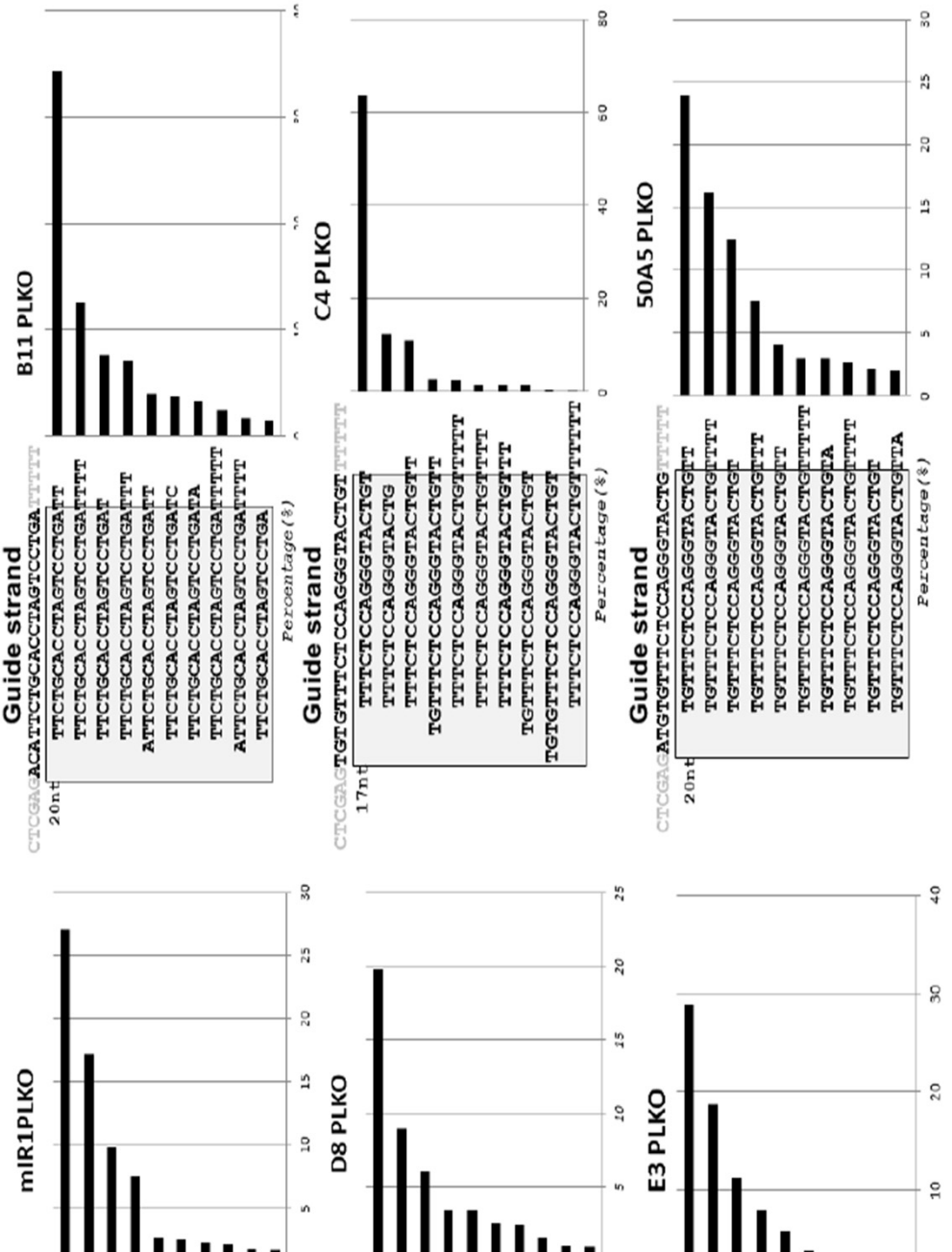

Guide strand sequence composition and distribution in PLKO
B11 PLKO
C4 PLKO
50A5 PLKO
mIR1PLKO
D8 PLKO
E3 PLKO
B5 PLKO
Guide strand
Percentage(%)

FIG. 12B

Guide strand sequence composition and distribution in LEGO

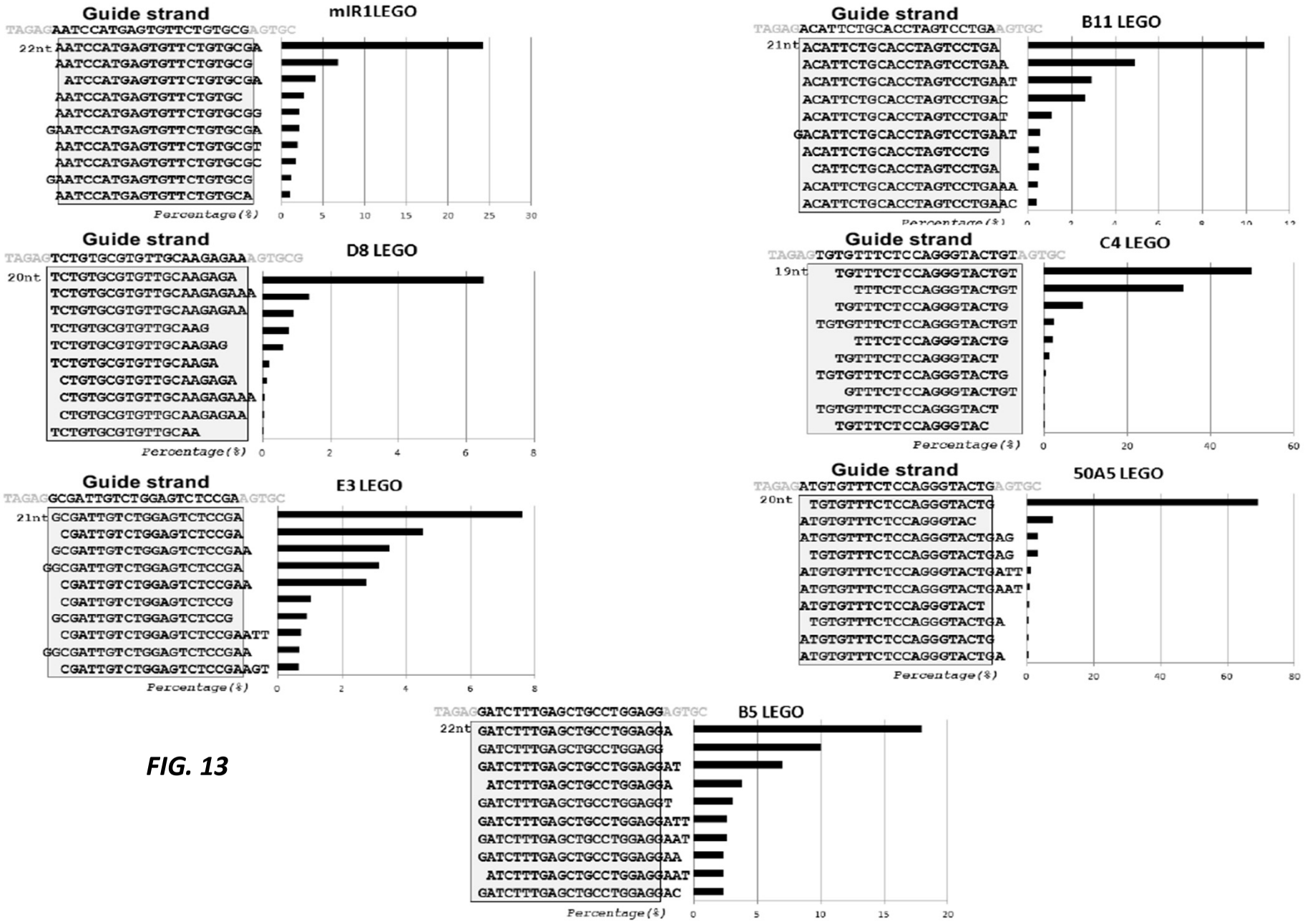


FIG. 13

Design of new shRNAs to mimic mature guide strands produced in pLKO vector

	Passenger	Loop	Guide	
mIR1G5	gcgcCGCACAGAACACTCATGxxxx	ctccatgtggtagag	xxxxCATGAGTGTTCTGTGCGgcgc	SEQ. ID. NO: 34
E3G5	gcgcTCGGAGACTCCAGACAAxxxx	ctccatgtggtagag	xxxxTTGTCTGGAGTCTCCGAgcgc	SEQ. ID. NO: 35
B5G5	gcgcCCTCCAGGCAGCTCAAAxxxx	ctccatgtggtagag	xxxxTTTGAGCTGCCTGGAGGgcgc	SEQ. ID. NO: 36
D8G5	gcgcTTCTCTTGCAACACGCAxxxx	ctccatgtggtagag	xxxxTGCGTGTTGCAAGAGAAgcgc	SEQ. ID. NO: 37
B11G5	gcgcTCAGGACTAGGTGCAGAxxxx	ctccatgtggtagag	xxxxTCTGCACCTAGTCCTGAgcgc	SEQ. ID. NO: 38
50D12G5	gcgcGATCGAGTGTTGAATAAxxxx	ctccatgtggtagag	xxxxTTATTCAACACTCGATCgcgc	SEQ. ID. NO: 39
50B11G5	gcgcCACTGTCCACAGGAGAAxxxx	ctccatgtggtagag	xxxxTTCTCCTGTGGACAGTGgcgc	SEQ. ID. NO: 41
50A5G5	GCGCCAGTACCCTGGAGAAACxxxx	ctccatgtggtagag	xxxxGTTTCTCCAGGGTACTGgcgc	SEQ. ID. NO: 40
50C4G5	gcgcACAGTACCCTGGAGAAAxxxx	ctccatgtggtagag	xxxxTTTCTCCAGGGTACTGTgcgc	SEQ. ID. NO: 42

FIG. 14A

Guide strand sequence composition and distribution in MODIFIED LEGO

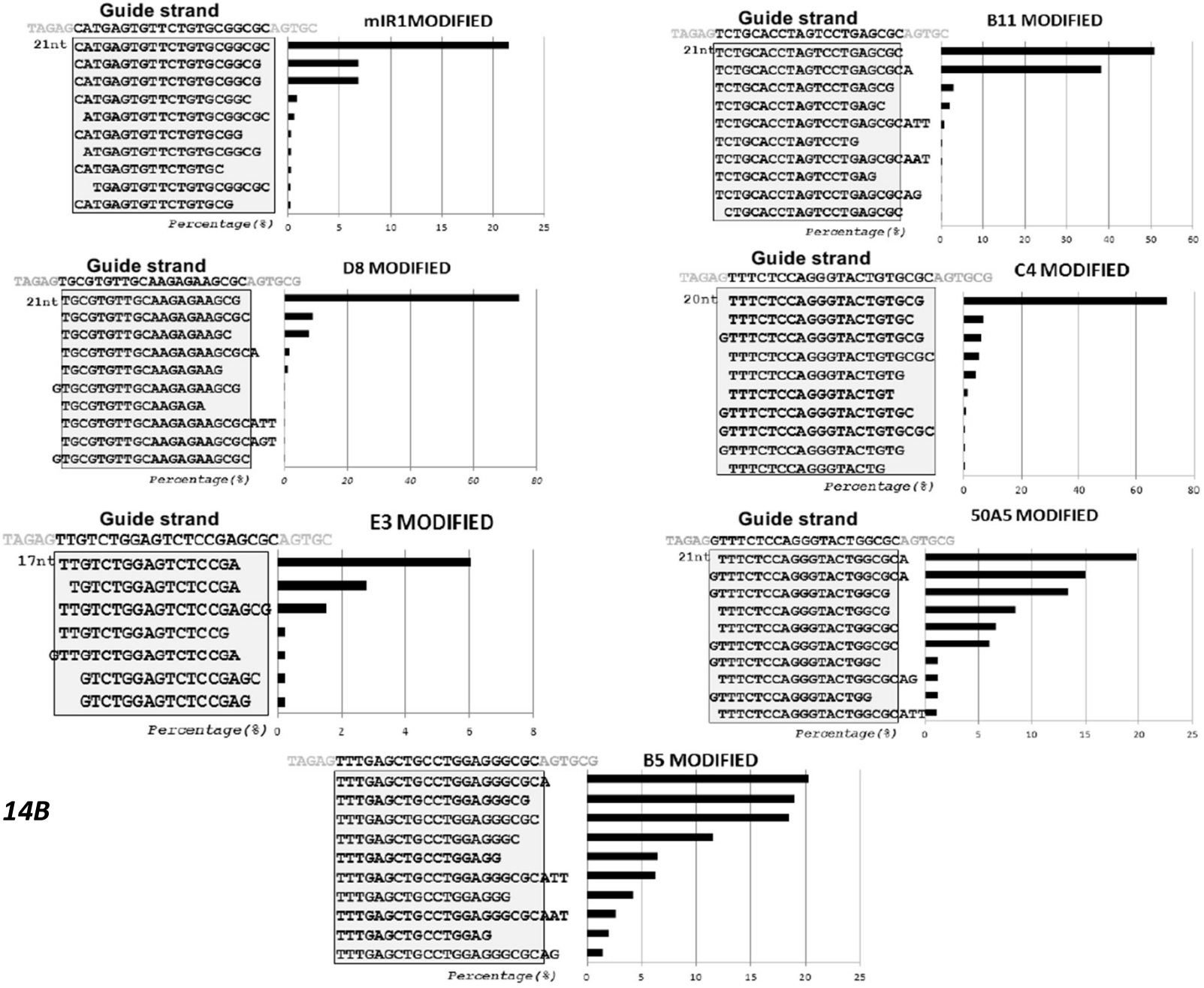


FIG.14B

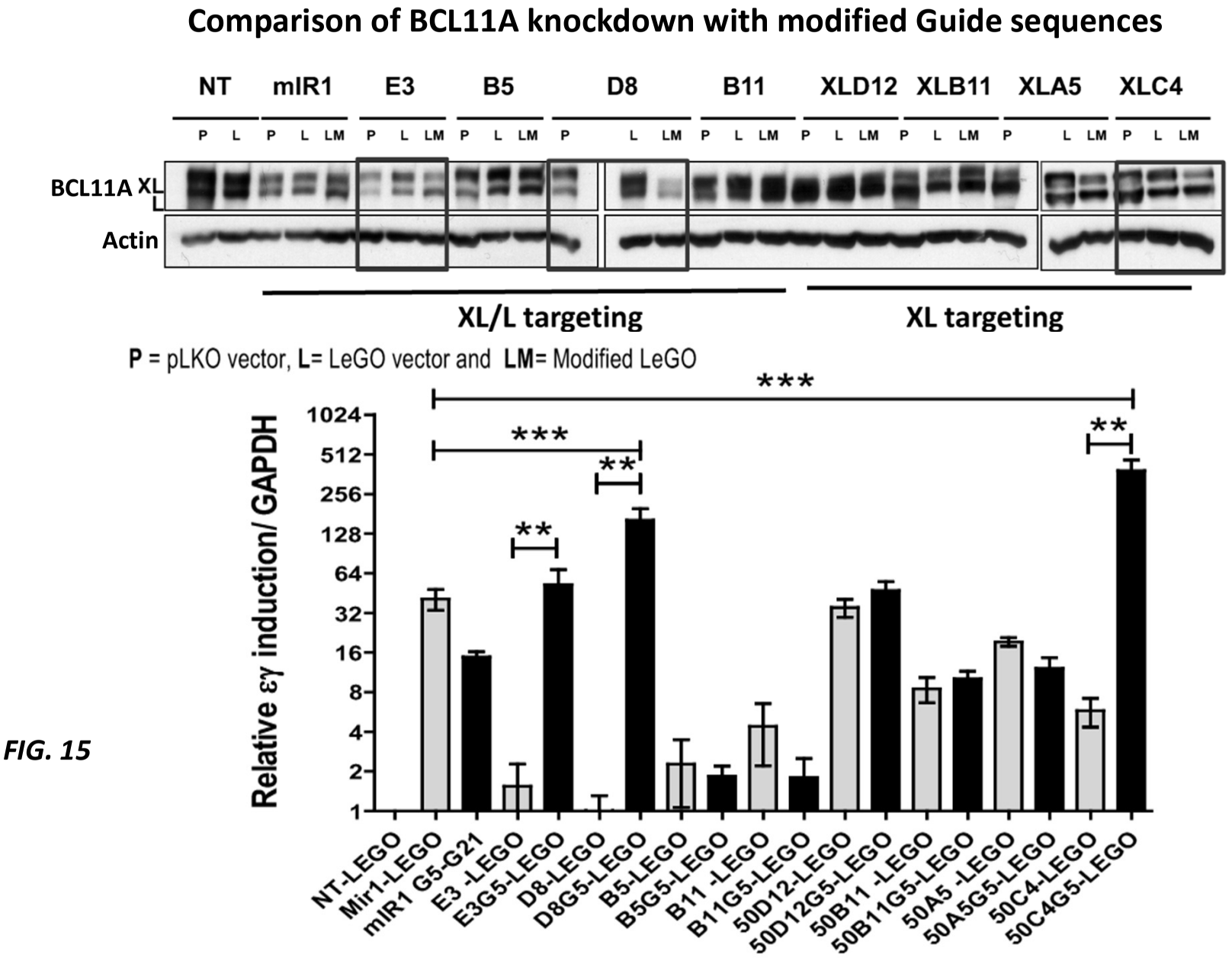

Comparison of BCL11A knockdown with modified Guide sequences
NT
mIR1
E3
B5
D8
B11
XLD12
XLB11
XLA5
XLC4
P L P L LM P L LM P L LM P L LM P L LM P L LM P L LM P L LM P L LM
BCL11A XL
Actin
XL/L targeting
XL targeting
P = pLKO vector, L= LeGO vector and LM= Modified LeGO
Relative εγ induction/ GAPDH
1024
512
256
128
64
32
16
8
4
2
1
***
***
**
**
**
NT-LEGO
Mir1-LEGO
mIR1 G5-G21
E3 -LEGO
E3G5-LEGO
D8-LEGO
D8G5-LEGO
B5-LEGO
B5G5-LEGO
B11 -LEGO
B11G5-LEGO
50D12-LEGO
50D12G5-LEGO
50B11 -LEGO
50B11G5-LEGO
50A5 -LEGO
50A5G5-LEGO
50C4-LEGO
50C4G5-LEGO

FIG. 15

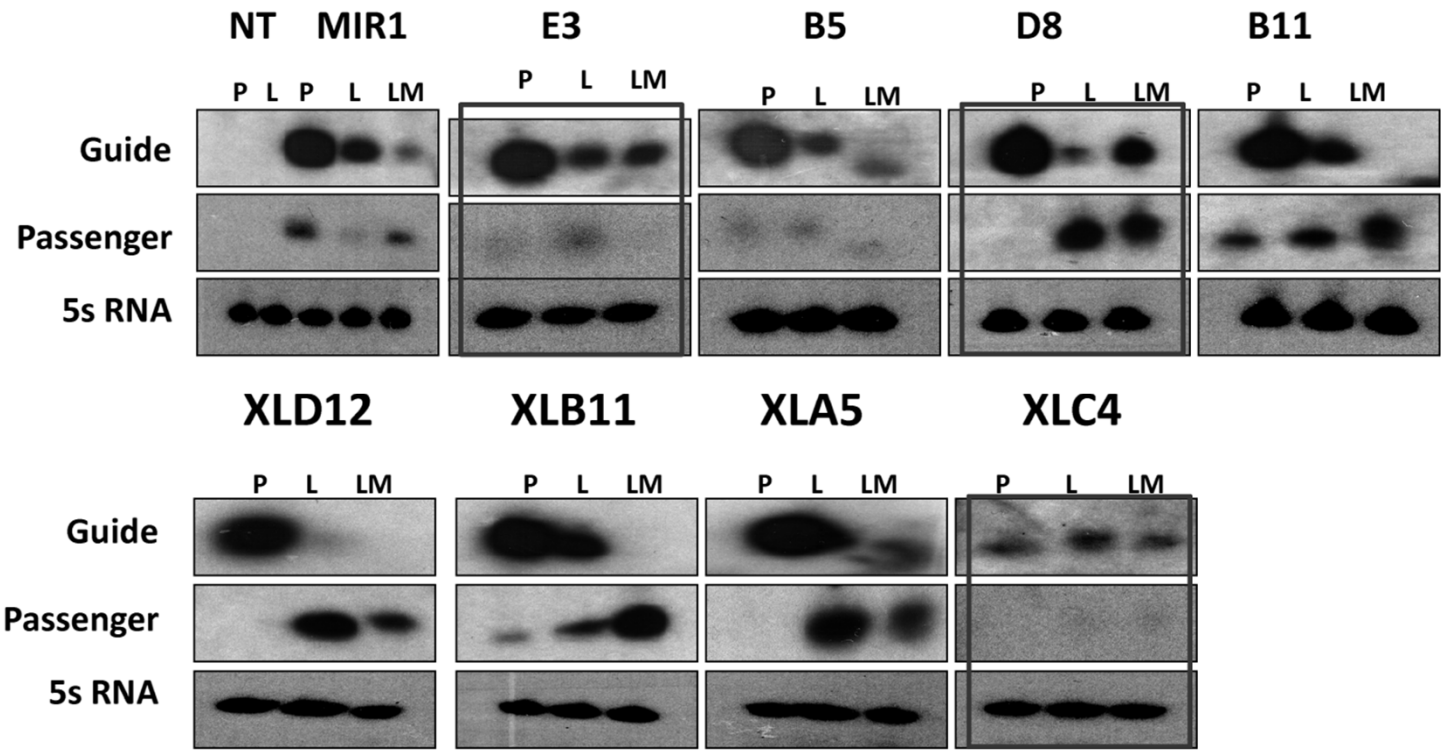

Comparison of miR expression with modified Guide sequences
NT
MIR1
E3
B5
D8
B11
P L P L LM
P L LM
P L LM
P L LM
P L LM
Guide
Passenger
5s RNA
XLD12
XLB11
XLA5
XLC4
P L LM
P L LM
P L LM
P L LM
Guide
Passenger
5s RNA
P – pLKO, L- pLEGO, LM- pLEGO modifed

FIG. 16

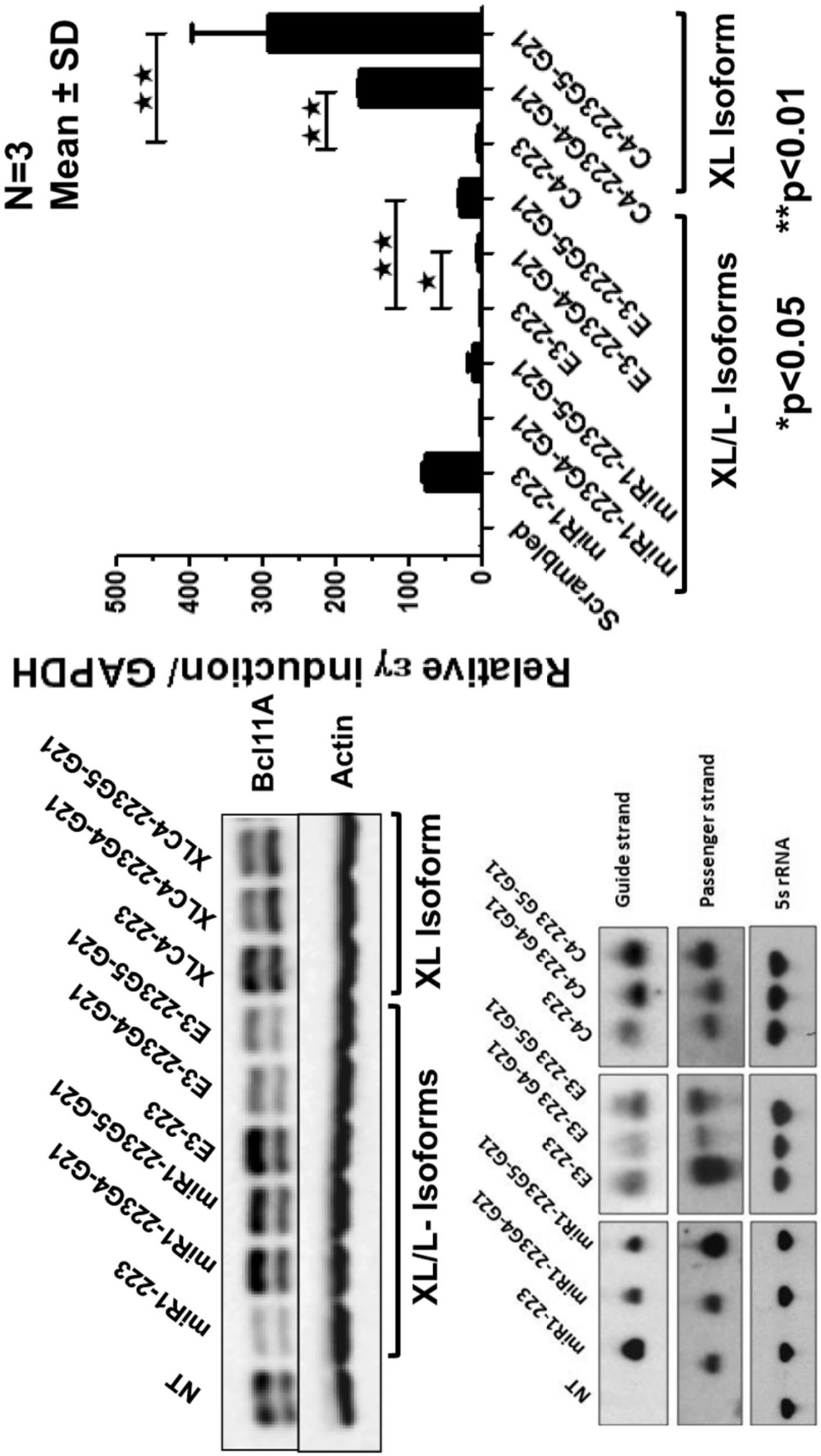

BCL11a knockdown efficiency and εY induction with LEGO vectors
Guide strand
Passenger strand
5s rRNA
NT
miR1-223
miR1-223GA-G21
miR1-223G5-G21
E3-223
E3-223 GA-G21
E3-223 G5-G21
C4-223
C4-223 GA-G21
C4-223 G5-G21
Bcl11A
Actin
XL/L- Isoforms
XL Isoform
Relative εγ induction/ GAPDH
N=3
Mean ± SD
Scrambled
miR1-223
miR1-223GA-G21
miR1-223G5-G21
E3-223
E3-223GA-G21
E3-223G5-G21
C4-223
C4-223GA-G21
C4-223G5-G21
*p<0.05
**p<0.01

FIG. 17

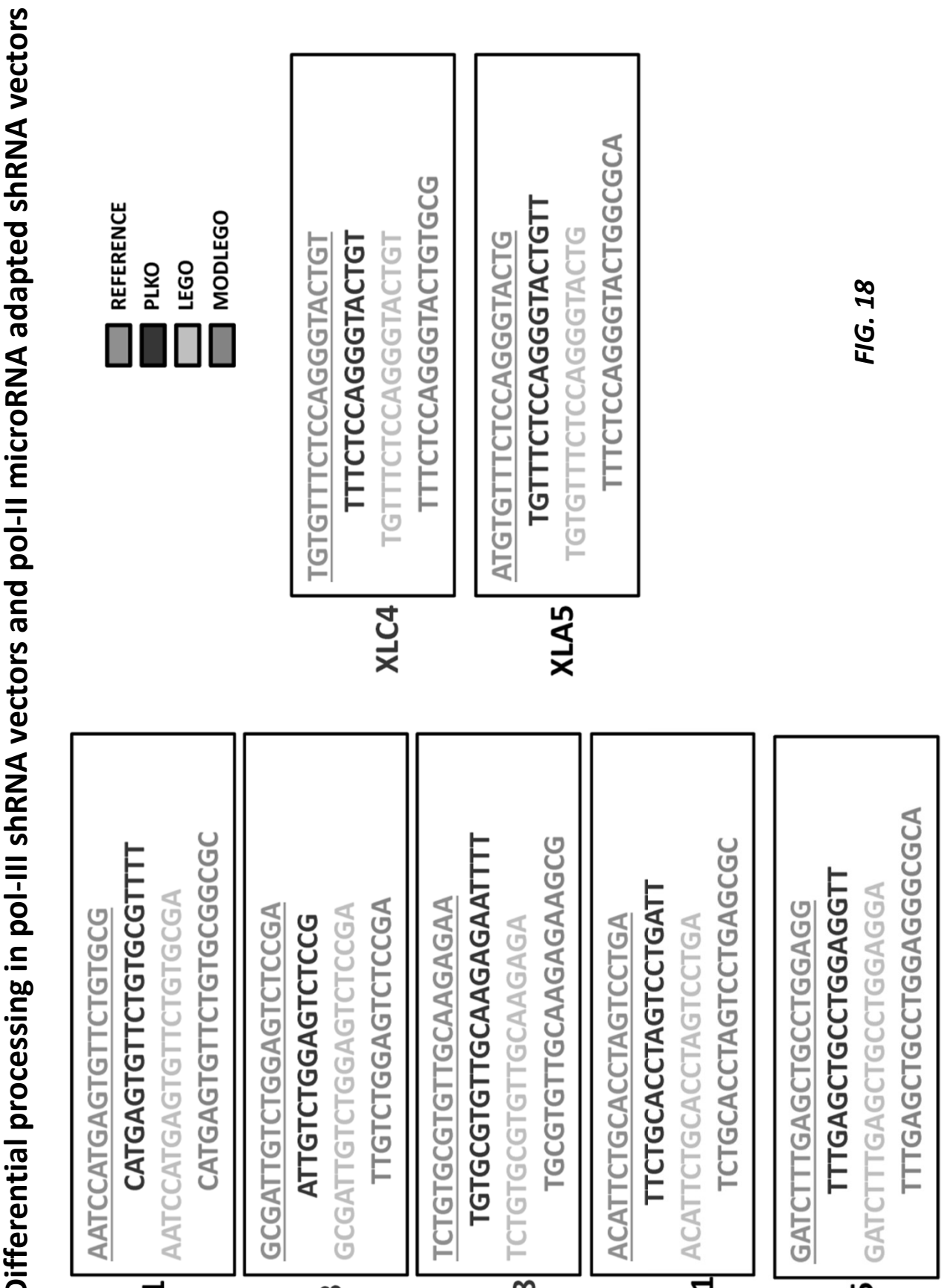

Differential processing in pol-III shRNA vectors and pol-II microRNA adapted shRNA vectors
REFERENCE
PLKO
LEGO
MODLEGO
miR1
AATCCATGAGTGTTCTGTGCG
CATGAGTGTTCTGTGCGTTTT
AATCCATGAGTGTTCTGTGCGA
CATGAGTGTTCTGTGCGGCGC
E3
GCGATTGTCTGGAGTCTCCGA
ATTGTCTGGAGTCTCCG
GCGATTGTCTGGAGTCTCCGA
TTGTCTGGAGTCTCCGA
XLC4
TGTGTTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTGT
TGTTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTGTGCG
D8
TCTGTGCGTGTTGCAAGAGAA
TGTGCGTGTTGCAAGAGAATTTT
TCTGTGCGTGTTGCAAGAGA
TGCGTGTTGCAAGAGAAGCG
XLA5
ATGTGTTTCTCCAGGGTACTG
TGTTTCTCCAGGGTACTGTT
TGTGTTTCTCCAGGGTACTG
TTTCTCCAGGGTACTGGCGCA
B11
ACATTCTGCACCTAGTCCTGA
TTCTGCACCTAGTCCTGATT
ACATTCTGCACCTAGTCCTGA
TCTGCACCTAGTCCTGAGCGC
B5
GATCTTTGAGCTGCCTGGAGG
TTTGAGCTGCCTGGAGGTT
GATCTTTGAGCTGCCTGGAGGA
TTTGAGCTGCCTGGAGGGCGCA

FIG. 18

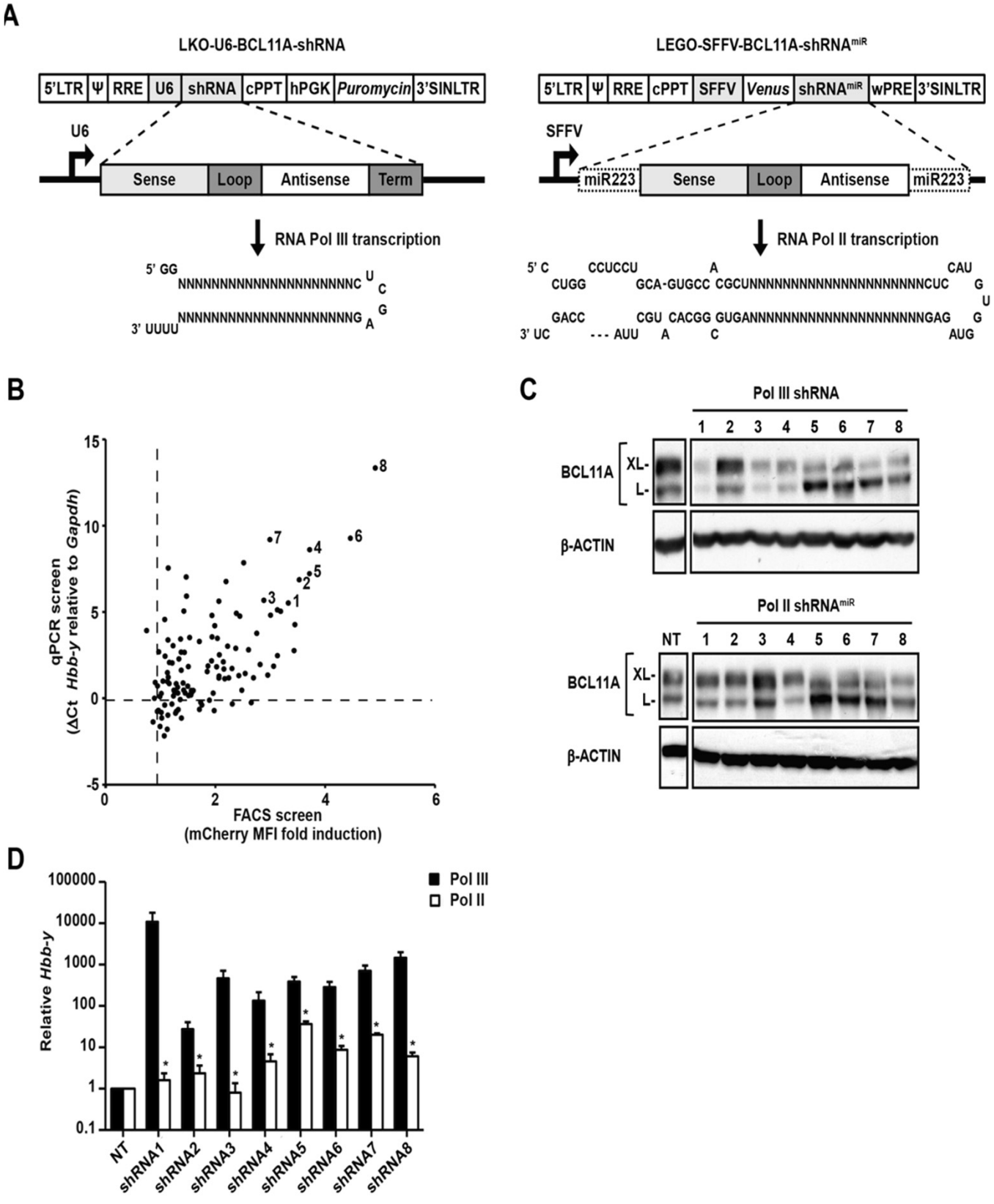

A
LKO-U6-BCL11A-shRNA
5'LTR
Ψ
RRE
U6
shRNA
cPPT
hPGK
Puromycin
3'SINLTR
U6
Sense
Loop
Antisense
Term
RNA Pol III transcription
5' GG
NNNNNNNNNNNNNNNNNNNNC
U
C
3' UUUU
NNNNNNNNNNNNNNNNNNNNG
A
G
LEGO-SFFV-BCL11A-shRNAmiR
5'LTR
Ψ
RRE
cPPT
SFFV
Venus
shRNAmiR
wPRE
3'SINLTR
SFFV
miR223
Sense
Loop
Antisense
miR223
RNA Pol II transcription
5' C
CCUCCU
A
CAU
CUGG
GCA-GUGCC CGCUNNNNNNNNNNNNNNNNNNNNNCUC
G
U
GACC
CGU CACGG GUGANNNNNNNNNNNNNNNNNNNNNGAG
G
3' UC
--- AUU
A
C
AUG
B
qPCR screen
(ΔCt Hbb-y relative to Gapdh)
FACS screen
(mCherry MFI fold induction)
15
10
5
0
-5
0
2
4
6
8
7
6
4
5
2
3
1
C
Pol III shRNA
1 2 3 4 5 6 7 8
BCL11A
XL-
L-
β-ACTIN
Pol II shRNAmiR
NT 1 2 3 4 5 6 7 8
BCL11A
XL-
L-
β-ACTIN
D
Pol III
Pol II
Relative Hbb-y
100000
10000
1000
100
10
1
0.1
NT
shRNA1
shRNA2
shRNA3
shRNA4
shRNA5
shRNA6
shRNA7
shRNA8

FIG. 19

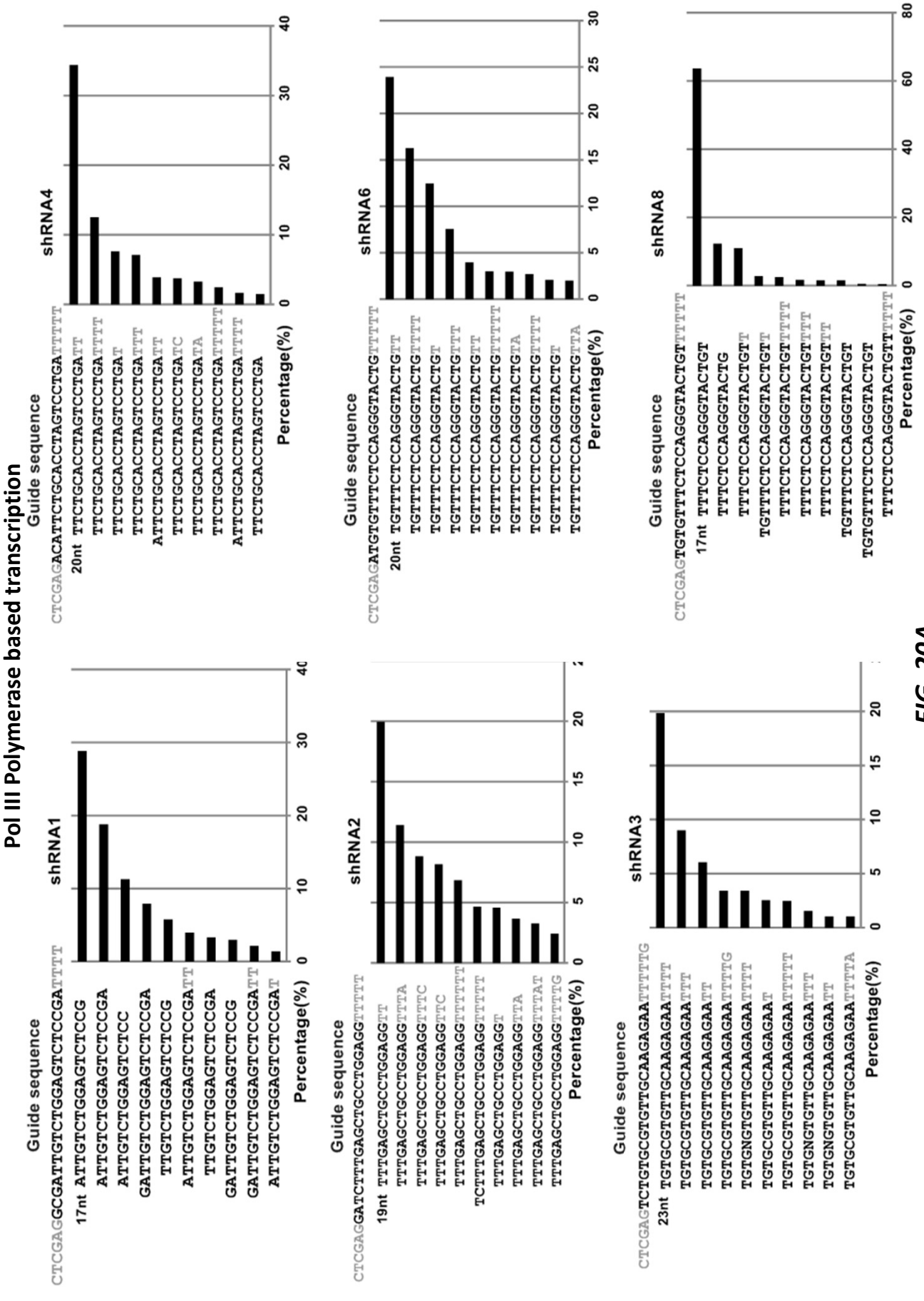

Pol III Polymerase based transcription
shRNA1
Guide sequence
CTCGAGGCGATTGTCTGGAGTCTCCGATTTT
17nt ATTGTCTGGAGTCTCCG
ATTGTCTGGAGTCTCCGA
ATTGTCTGGAGTCTCC
GATTGTCTGGAGTCTCCGA
TTGTCTGGAGTCTCCG
ATTGTCTGGAGTCTCCGATT
TTGTCTGGAGTCTCCGA
GATTGTCTGGAGTCTCCG
GATTGTCTGGAGTCTCCGATT
ATTGTCTGGAGTCTCCGAT
Percentage(%)
0 10 20 30 40
shRNA4
Guide sequence
CTCGAGACATTCTGCACCTAGTCCTGATTTTT
20nt TTCTGCACCTAGTCCTGATT
TTCTGCACCTAGTCCTGATTTT
TTCTGCACCTAGTCCTGAT
TTCTGCACCTAGTCCTGATTT
ATTCTGCACCTAGTCCTGATT
TTCTGCACCTAGTCCTGATC
TTCTGCACCTAGTCCTGATA
TTCTGCACCTAGTCCTGATTTT
ATTCTGCACCTAGTCCTGATTT
TTCTGCACCTAGTCCTGA
Percentage(%)
0 10 20 30 40
shRNA2
Guide sequence
CTCGAGGATCTTTGAGCTGCCTGGAGGTTTTT
19nt TTTGAGCTGCCTGGAGGTT
TTTGAGCTGCCTGGAGGTTTA
TTTGAGCTGCCTGGAGGTTTC
TTTGAGCTGCCTGGAGGTTC
TTTGAGCTGCCTGGAGGTTTTT
TCTTTGAGCTGCCTGGAGGTTTTT
TTTGAGCTGCCTGGAGGT
TTTGAGCTGCCTGGAGGTTA
TTTGAGCTGCCTGGAGGTTTAT
TTTGAGCTGCCTGGAGGTTTTG
Percentage(%)
0 5 10 15 20
shRNA6
Guide sequence
CTCGAGATGTGTTTCTCCAGGGTACTGTTTTTT
20nt TGTTTCTCCAGGGTACTGTT
TGTTTCTCCAGGGTACTGTTTT
TGTTTCTCCAGGGTACTGT
TGTTTCTCCAGGGTACTGTTT
TGTTTCTCCAGGGTACTGTT
TGTTTCTCCAGGGTACTGTTTTT
TGTTTCTCCAGGGTACTGTA
TGTTTCTCCAGGGTACTGTTTT
TGTTTCTCCAGGGTACTGT
TGTTTCTCCAGGGTACTGTTA
Percentage(%)
0 5 10 15 20 25 30
shRNA8
Guide sequence
CTCGAGTGTGTTTCTCCAGGGTACTGTTTTTT
17nt TTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTG
TTTCTCCAGGGTACTGTT
TGTTTCTCCAGGGTACTGTT
TTTCTCCAGGGTACTGTTTTT
TTTCTCCAGGGTACTGTTTT
TTTCTCCAGGGTACTGTTT
TGTTTCTCCAGGGTACTGT
TGTGTTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTGTTTTTT
Percentage(%)
0 20 40 60 80
shRNA3
Guide sequence
CTCGAGTCTGTGCGTGTTGCAAGAGAAATTTTTG
23nt TGTGCGTGTTGCAAGAGAAATTTT
TGTGCGTGTTGCAAGAGAAATTT
TGTGCGTGTTGCAAGAGAAATT
TGTGCGTGTTGCAAGAGAAATTTTTG
TGTGNGTGTTGCAAGAGAAATTTT
TGTGCGTGTTGCAAGAGAAAT
TGTGCGTGTTGCAAGAGAAATTTTTT
TGTGNGTGTTGCAAGAGAAATTT
TGTGNGTGTTGCAAGAGAAATT
TGTGCGTGTTGCAAGAGAAATTTTA
Percentage(%)
0 5 10 15 20

FIG. 20A

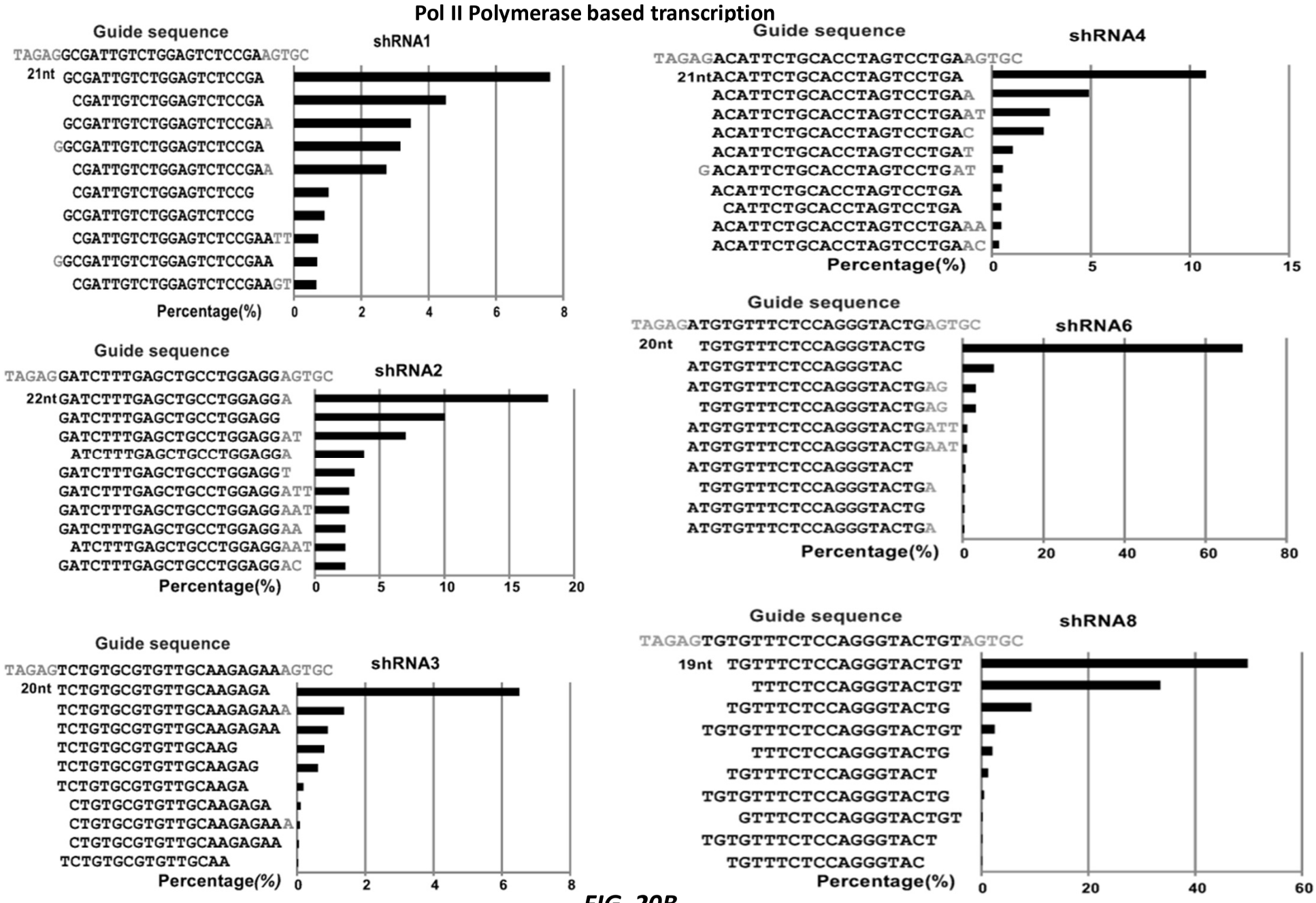

Pol II Polymerase based transcription
Guide sequence
shRNA1
TAGAGGCGATTGTCTGGAGTCTCCGAAGTGC
21nt GCGATTGTCTGGAGTCTCCGA
CGATTGTCTGGAGTCTCCGA
GCGATTGTCTGGAGTCTCCGAA
GGCGATTGTCTGGAGTCTCCGA
CGATTGTCTGGAGTCTCCGAA
CGATTGTCTGGAGTCTCCG
GCGATTGTCTGGAGTCTCCG
CGATTGTCTGGAGTCTCCGAATT
GGCGATTGTCTGGAGTCTCCGAA
CGATTGTCTGGAGTCTCCGAAGT
Percentage(%)
0 2 4 6 8
Guide sequence
shRNA2
TAGAGGATCTTTGAGCTGCCTGGAGGAGTGC
22nt GATCTTTGAGCTGCCTGGAGGA
GATCTTTGAGCTGCCTGGAGG
GATCTTTGAGCTGCCTGGAGGAT
ATCTTTGAGCTGCCTGGAGGA
GATCTTTGAGCTGCCTGGAGGT
GATCTTTGAGCTGCCTGGAGGATT
GATCTTTGAGCTGCCTGGAGGAAT
GATCTTTGAGCTGCCTGGAGGAA
ATCTTTGAGCTGCCTGGAGGAAT
GATCTTTGAGCTGCCTGGAGGAC
Percentage(%)
0 5 10 15 20
Guide sequence
shRNA3
TAGAGTCTGTGCGTGTTGCAAGAGAAAGTGC
20nt TCTGTGCGTGTTGCAAGAGA
TCTGTGCGTGTTGCAAGAGAAA
TCTGTGCGTGTTGCAAGAGAA
TCTGTGCGTGTTGCAAG
TCTGTGCGTGTTGCAAGAG
TCTGTGCGTGTTGCAAGA
CTGTGCGTGTTGCAAGAGA
CTGTGCGTGTTGCAAGAGAAA
CTGTGCGTGTTGCAAGAGAA
TCTGTGCGTGTTGCAA
Percentage(%)
0 2 4 6 8
Guide sequence
shRNA4
TAGAGACATTCTGCACCTAGTCCTGAAGTGC
21ntACATTCTGCACCTAGTCCTGA
ACATTCTGCACCTAGTCCTGAA
ACATTCTGCACCTAGTCCTGAAT
ACATTCTGCACCTAGTCCTGAC
ACATTCTGCACCTAGTCCTGAT
GACATTCTGCACCTAGTCCTGAT
ACATTCTGCACCTAGTCCTGA
CATTCTGCACCTAGTCCTGA
ACATTCTGCACCTAGTCCTGAAA
ACATTCTGCACCTAGTCCTGAAC
Percentage(%)
0 5 10 15
Guide sequence
shRNA6
TAGAGATGTGTTTCTCCAGGGTACTGAGTGC
20nt TGTGTTTCTCCAGGGTACTG
ATGTGTTTCTCCAGGGTAC
ATGTGTTTCTCCAGGGTACTGAG
TGTGTTTCTCCAGGGTACTGAG
ATGTGTTTCTCCAGGGTACTGATT
ATGTGTTTCTCCAGGGTACTGAAT
ATGTGTTTCTCCAGGGTACT
TGTGTTTCTCCAGGGTACTGA
ATGTGTTTCTCCAGGGTACTG
ATGTGTTTCTCCAGGGTACTGA
Percentage(%)
0 20 40 60 80
Guide sequence
shRNA8
TAGAGTGTGTTTCTCCAGGGTACTGTAGTGC
19nt TGTTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTGT
TGTTTCTCCAGGGTACTG
TGTGTTTCTCCAGGGTACTGT
TTTCTCCAGGGTACTG
TGTTTCTCCAGGGTACT
TGTGTTTCTCCAGGGTACTG
GTTTCTCCAGGGTACTGT
TGTGTTTCTCCAGGGTACT
TGTTTCTCCAGGGTAC
Percentage(%)
0 20 40 60

FIG. 20B

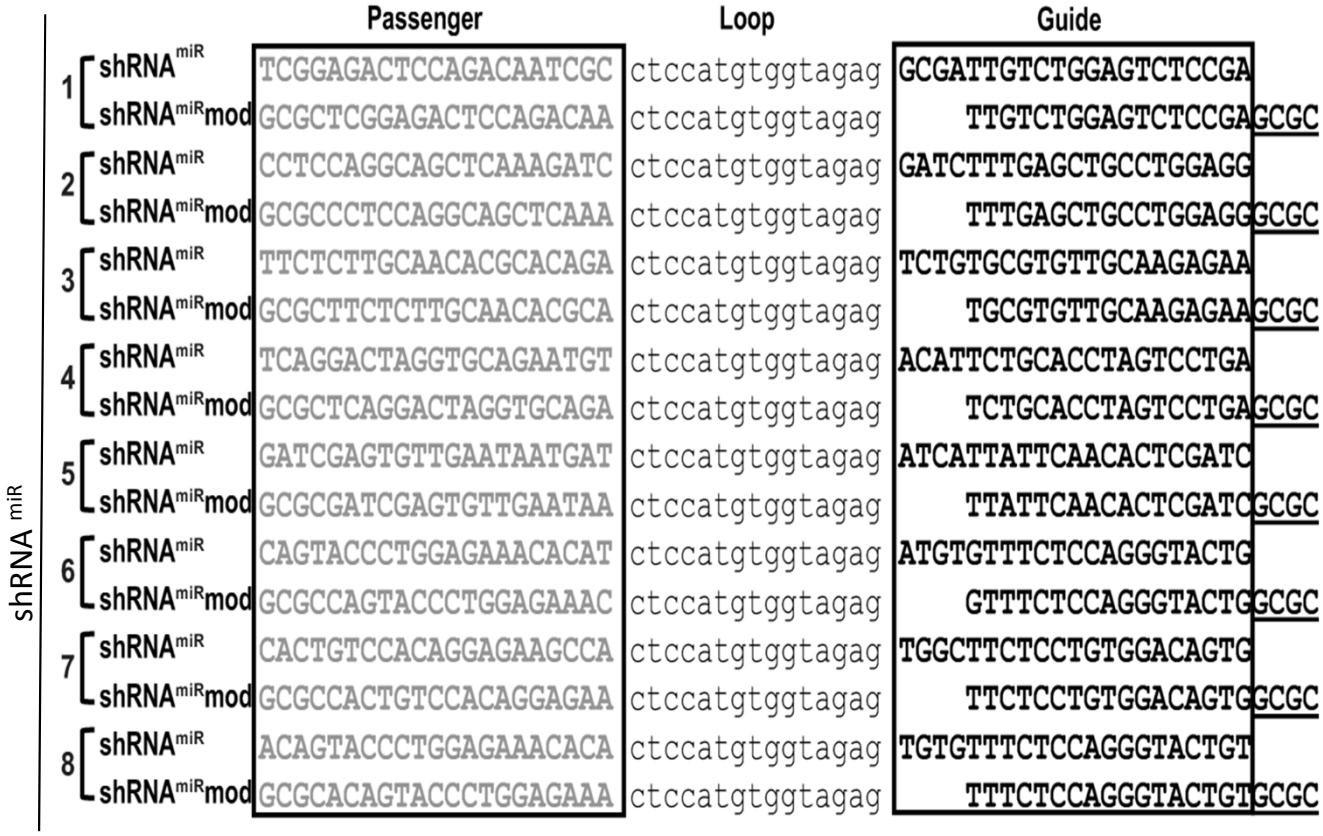


FIG. 21A

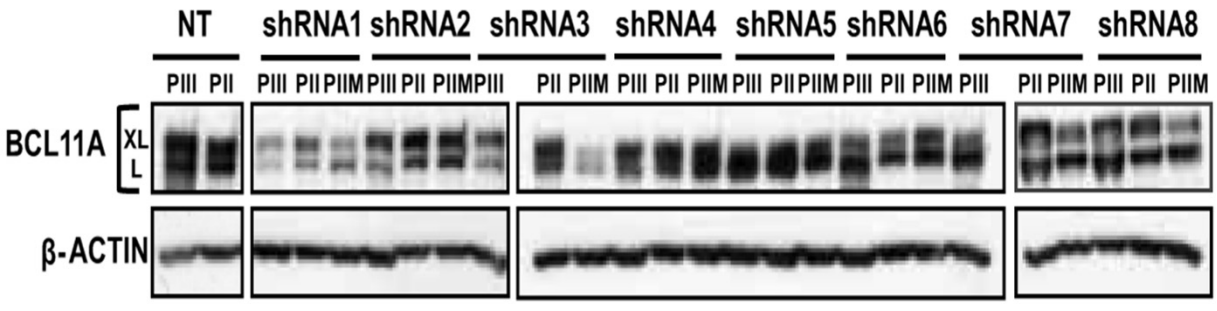


FIG. 21B

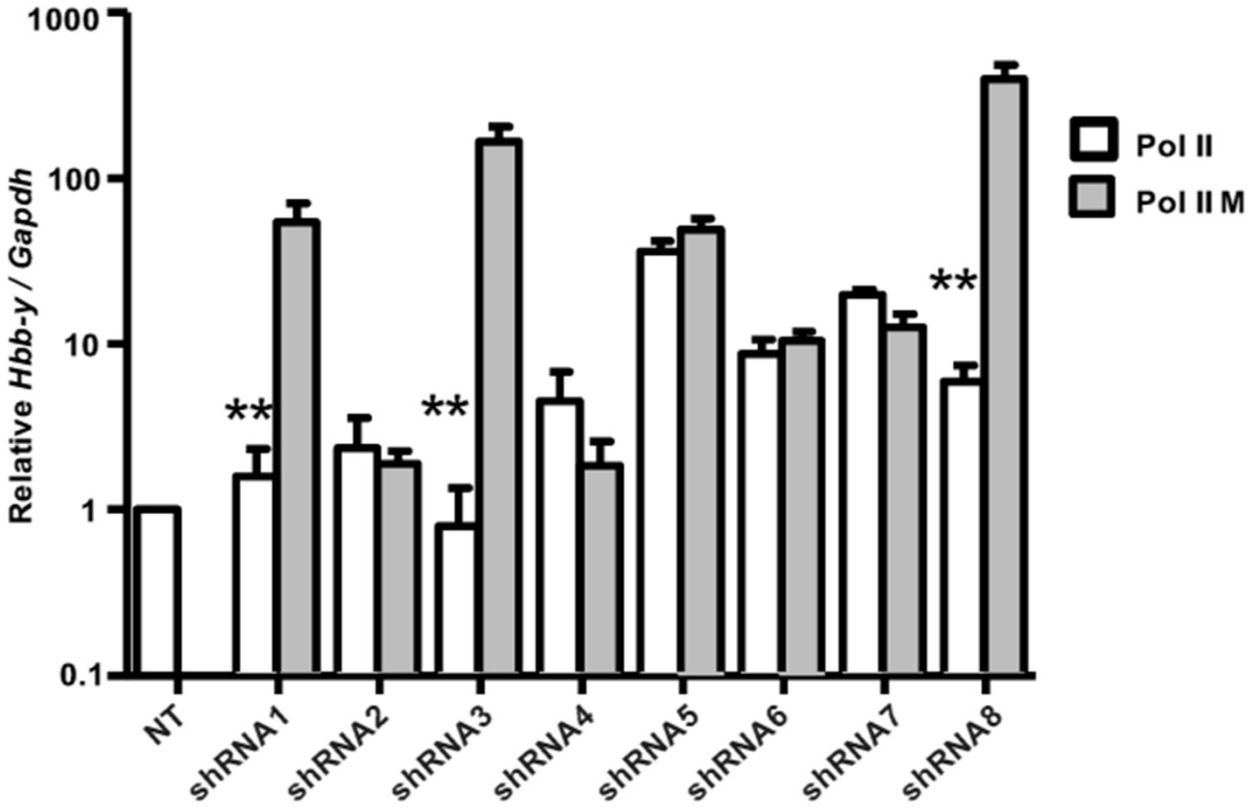


FIG. 21C

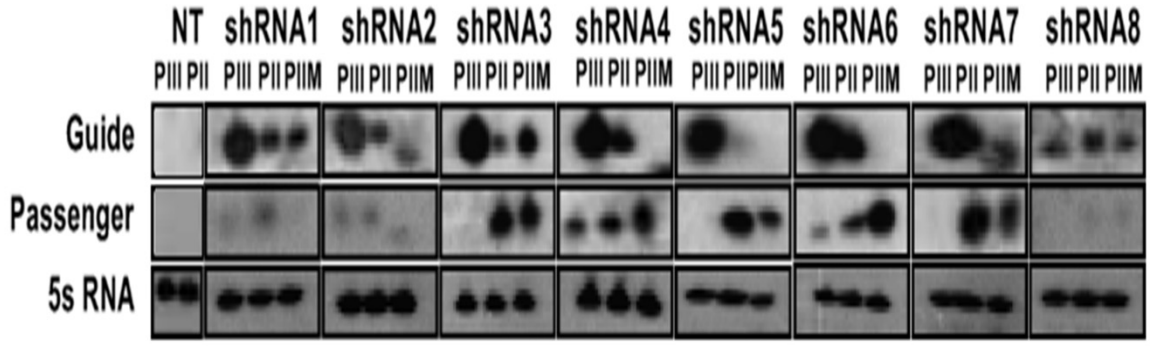


FIG. 21D

Modified for Pol II Polymerase based transcription

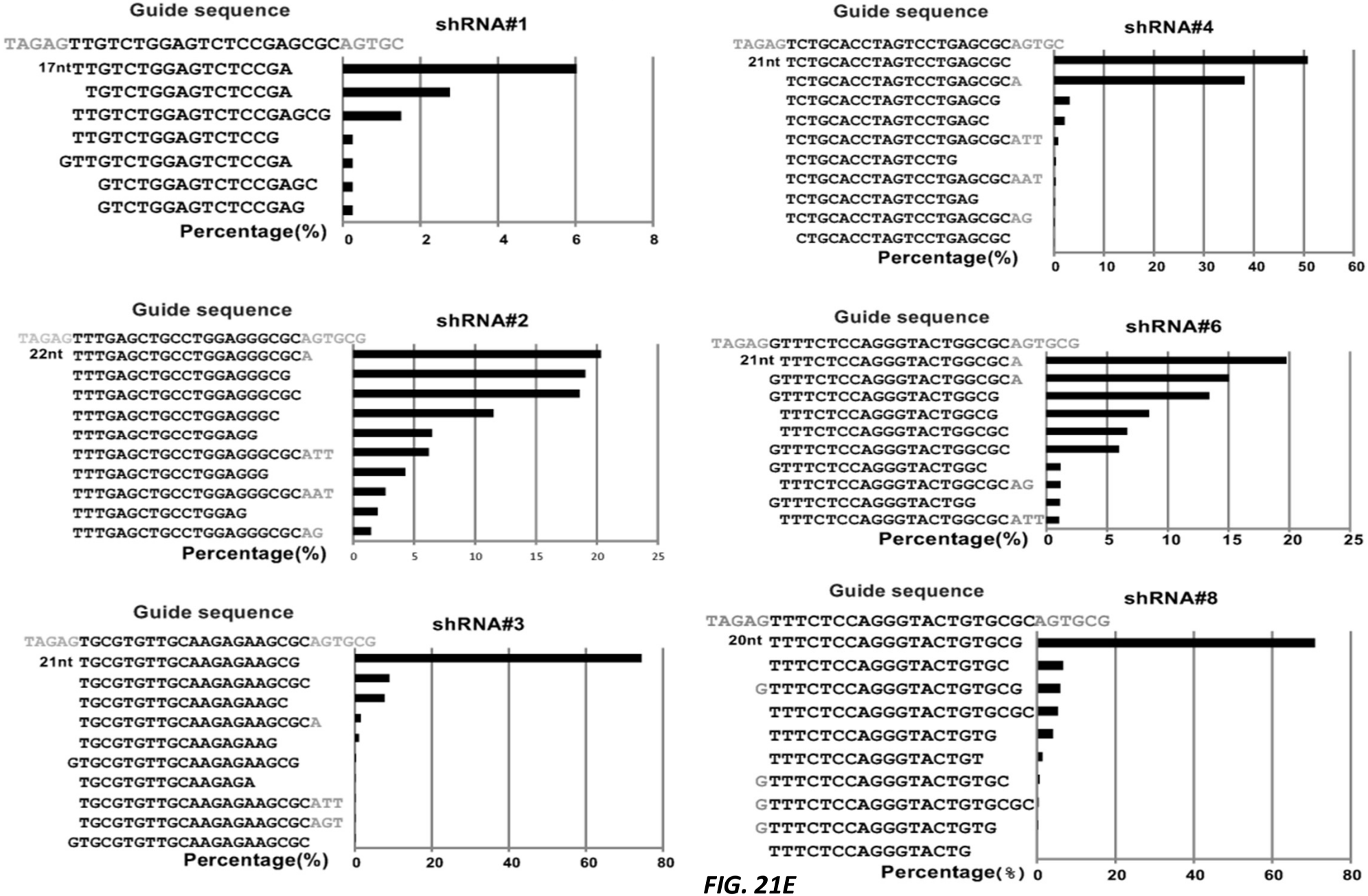


FIG. 21E

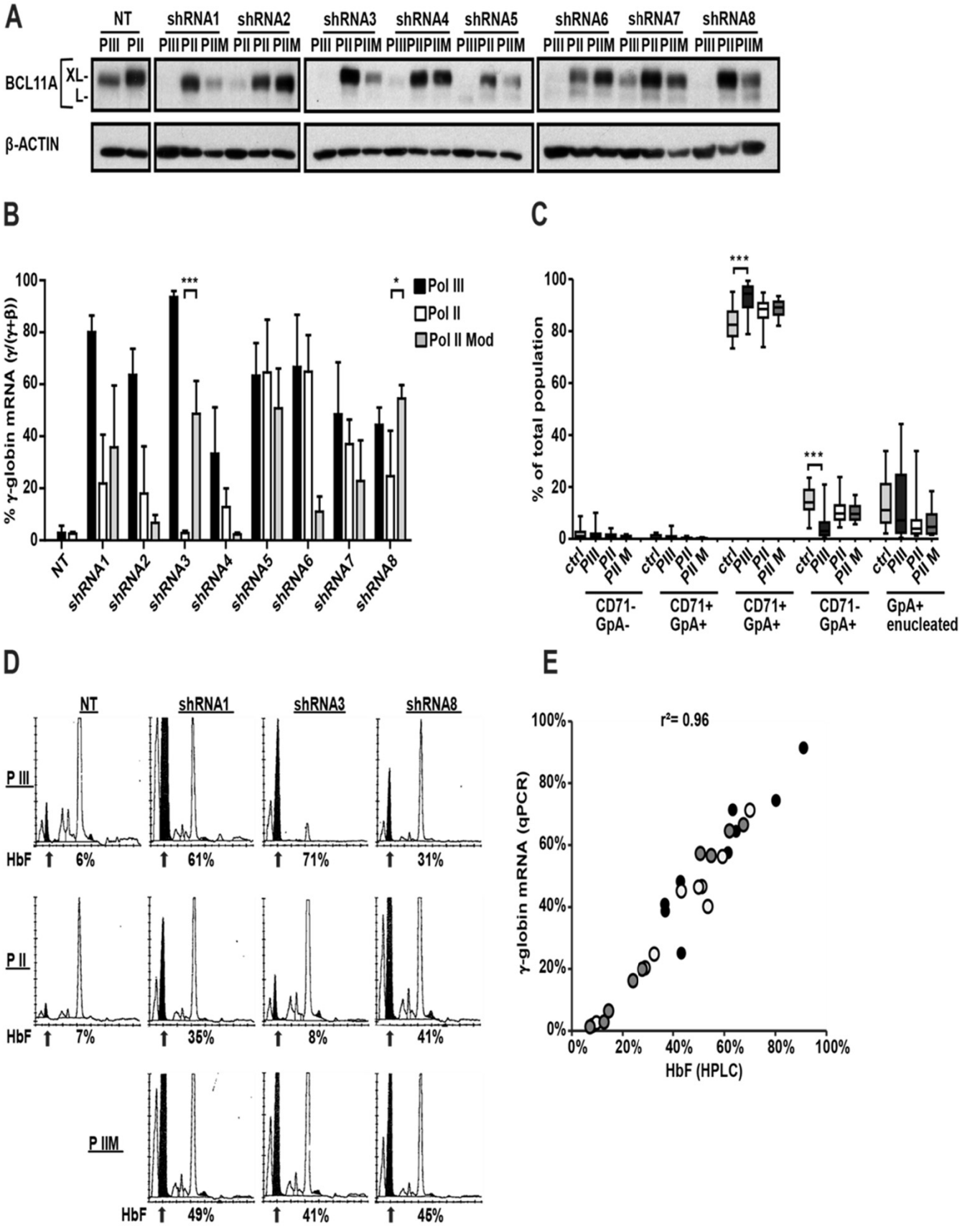

A
NT
shRNA1
shRNA2
shRNA3
shRNA4
shRNA5
shRNA6
shRNA7
shRNA8
PIII PII PIIM
BCL11A
XL-
L-
β-ACTIN
B
% γ-globin mRNA (γ/(γ+β))
Pol III
Pol II
Pol II Mod
C
% of total population
ctrl
CD71- GpA-
CD71+ GpA+
CD71+ GpA+
CD71- GpA+
GpA+ enucleated
D
NT
shRNA1
shRNA3
shRNA8
P III
P II
P IIM
HbF
6%
61%
71%
31%
7%
35%
8%
41%
49%
41%
45%
E
r²= 0.96
γ-globin mRNA (qPCR)
HbF (HPLC)

FIG. 22

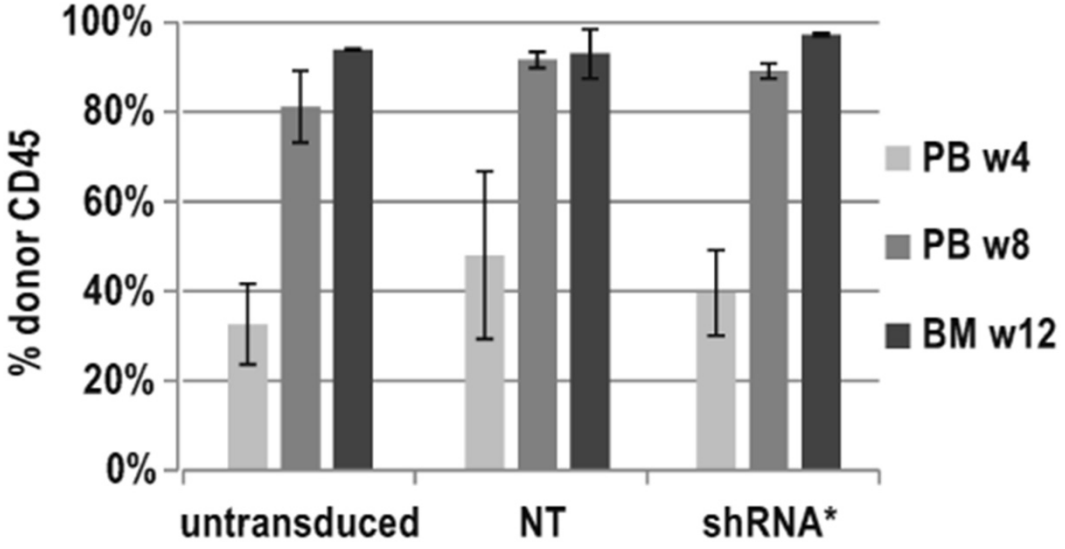


FIG. 23A

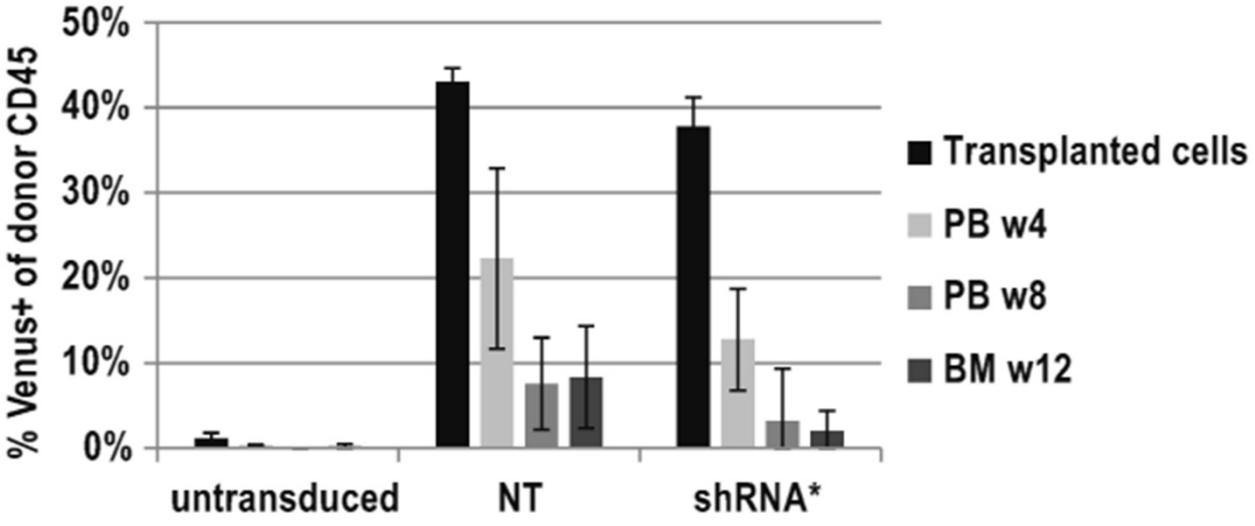


FIG. 23B

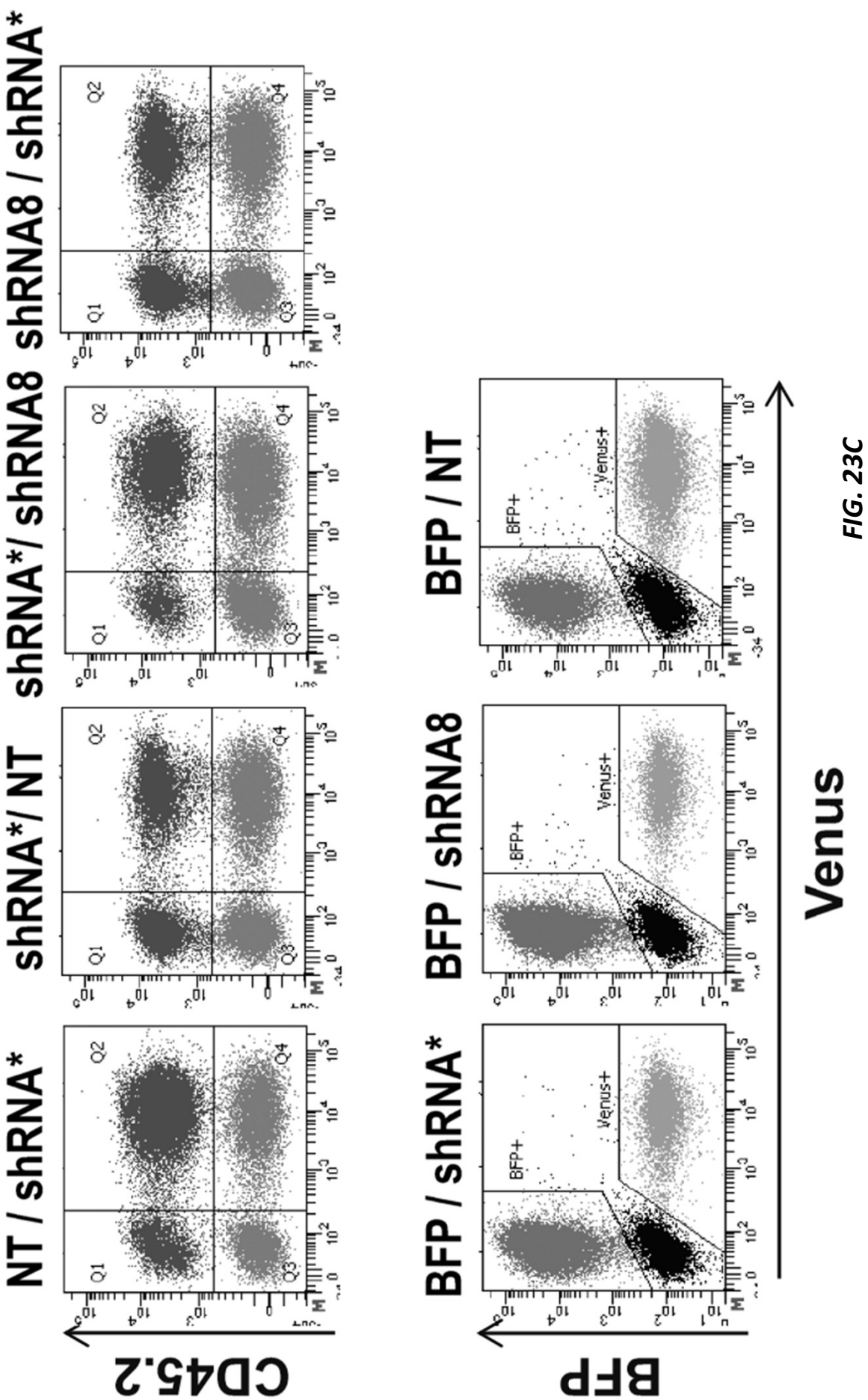

NT / shRNA*
shRNA*/ NT
shRNA*/ shRNA8
shRNA8 / shRNA*
BFP / shRNA*
BFP / shRNA8
BFP / NT
CD45.2
BFP
Venus
Q1
Q2
Q3
Q4
BFP+
Venus+

FIG. 23C

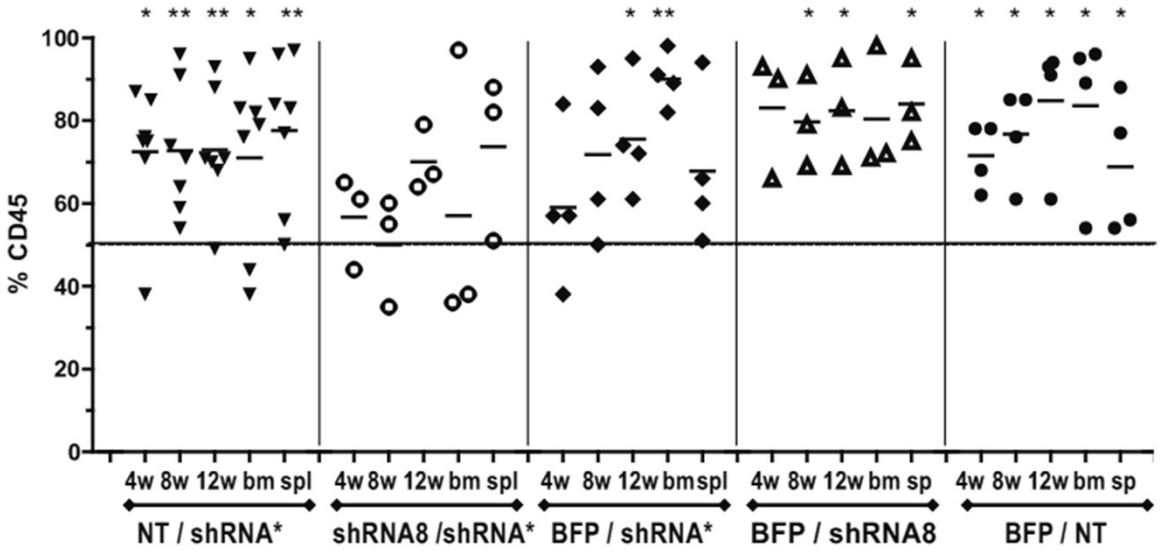


FIG. 23D

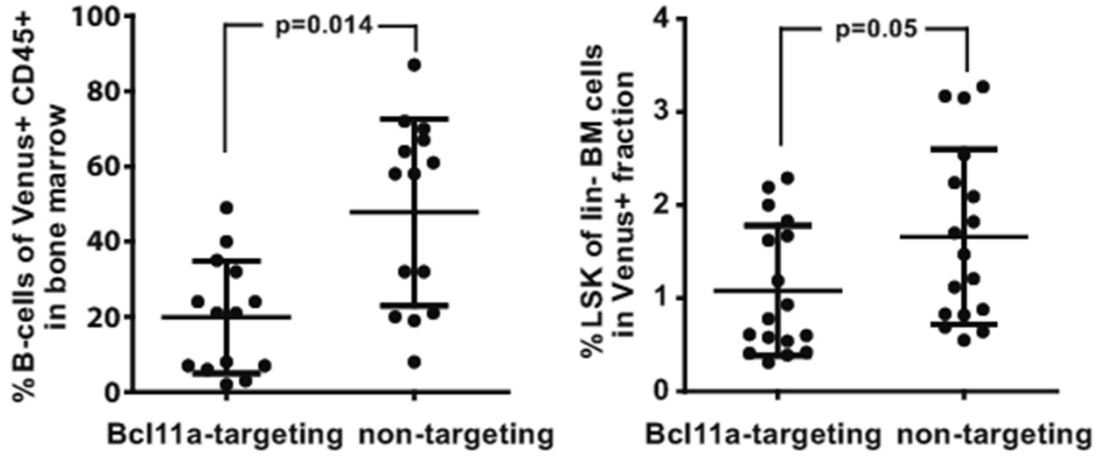


FIG. 23E



FIG. 23F

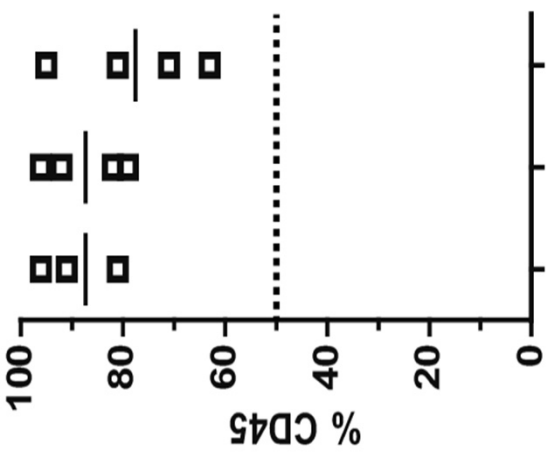


FIG. 23H

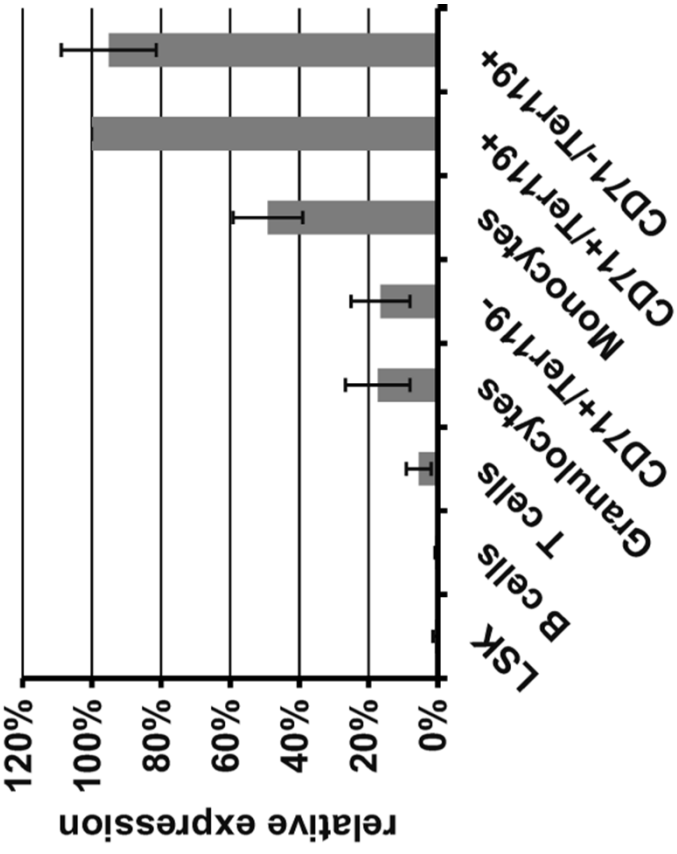


FIG. 23G

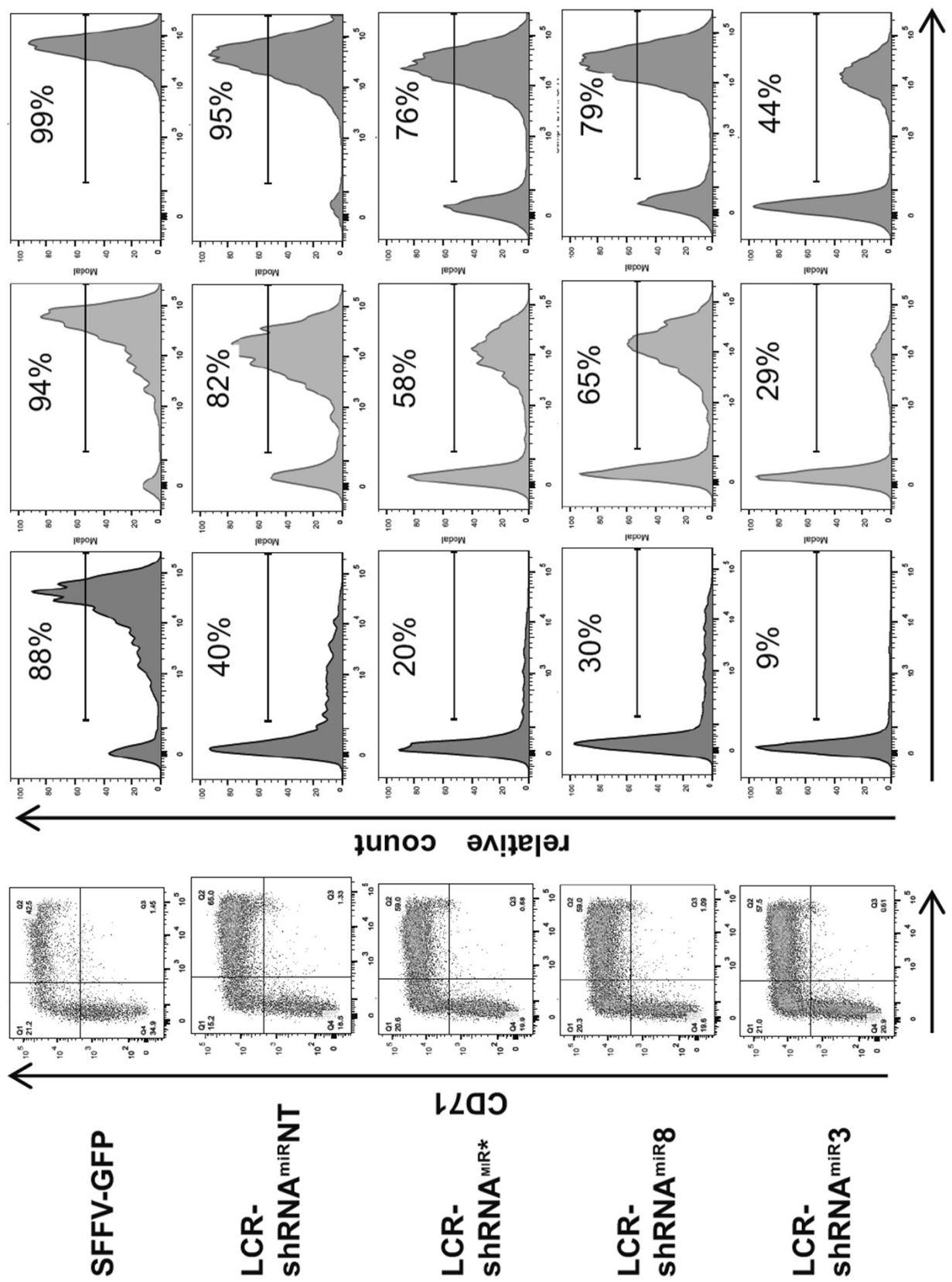

CD71- / GpA-
CD71+ / GpA-
CD71+/GpA+
88%
94%
99%
40%
82%
95%
20%
58%
76%
30%
65%
79%
9%
29%
44%
relative count
Venus
SFFV-GFP
LCR-shRNA^miRNT
LCR-shRNA^miR*
LCR-shRNA^miR8
LCR-shRNA^miR3
CD71
GpA

FIG. 23I

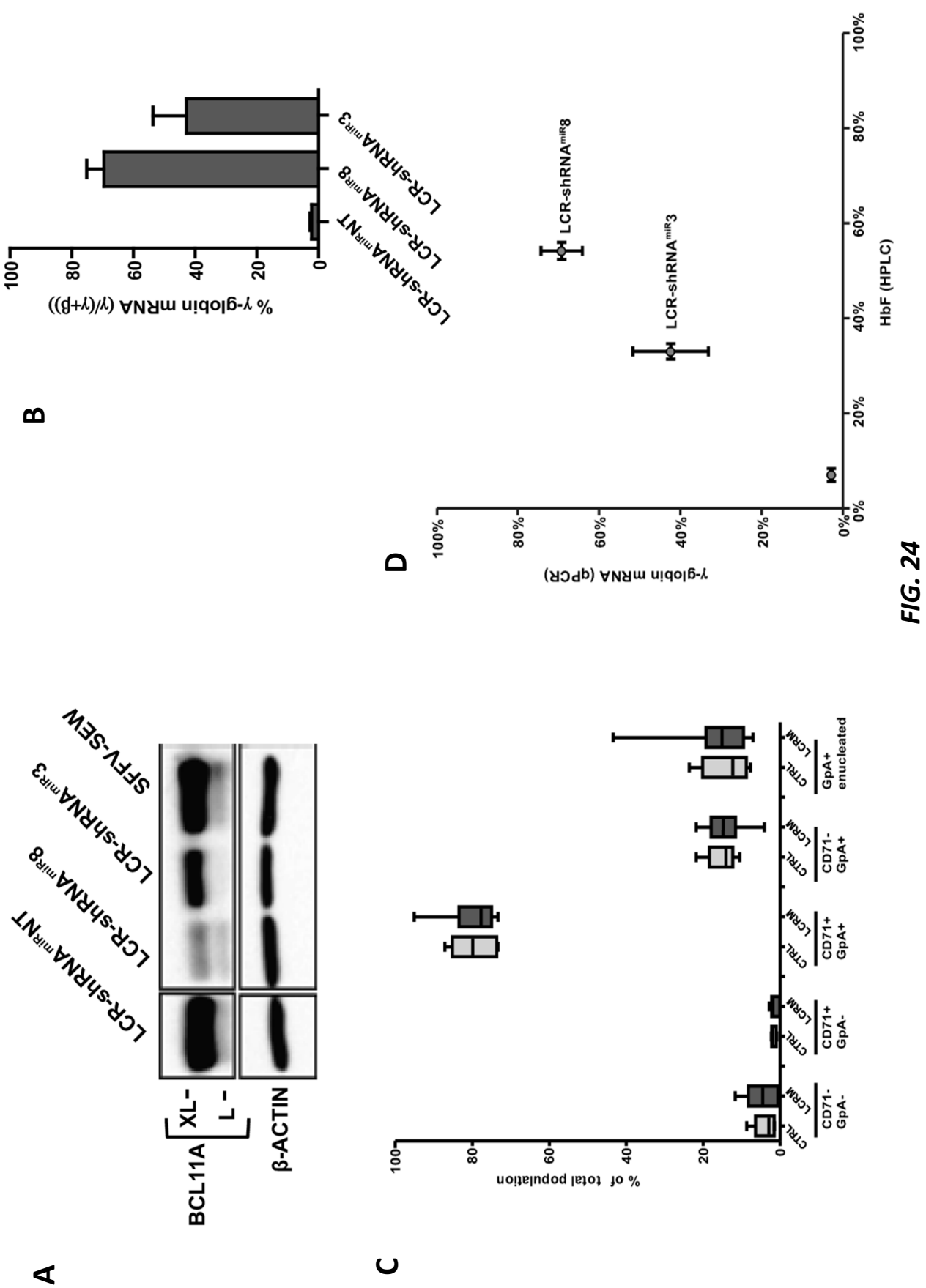

A
BCL11A
XL-
L-
β-ACTIN
LCR-shRNA miR NT
LCR-shRNA miR8
LCR-shRNA miR3
SFFV-SEW
B
% γ-globin mRNA (γ/(γ+β))
LCR-shRNA miR NT
LCR-shRNA miR8
LCR-shRNA miR3
C
% of total population
CD71- GpA-
CD71+ GpA-
CD71+ GpA+
CD71- GpA+
GpA+ enucleated
CTRL
LCRM
D
γ-globin mRNA (qPCR)
LCR-shRNA miR8
LCR-shRNA miR3
HbF (HPLC)

FIG. 24

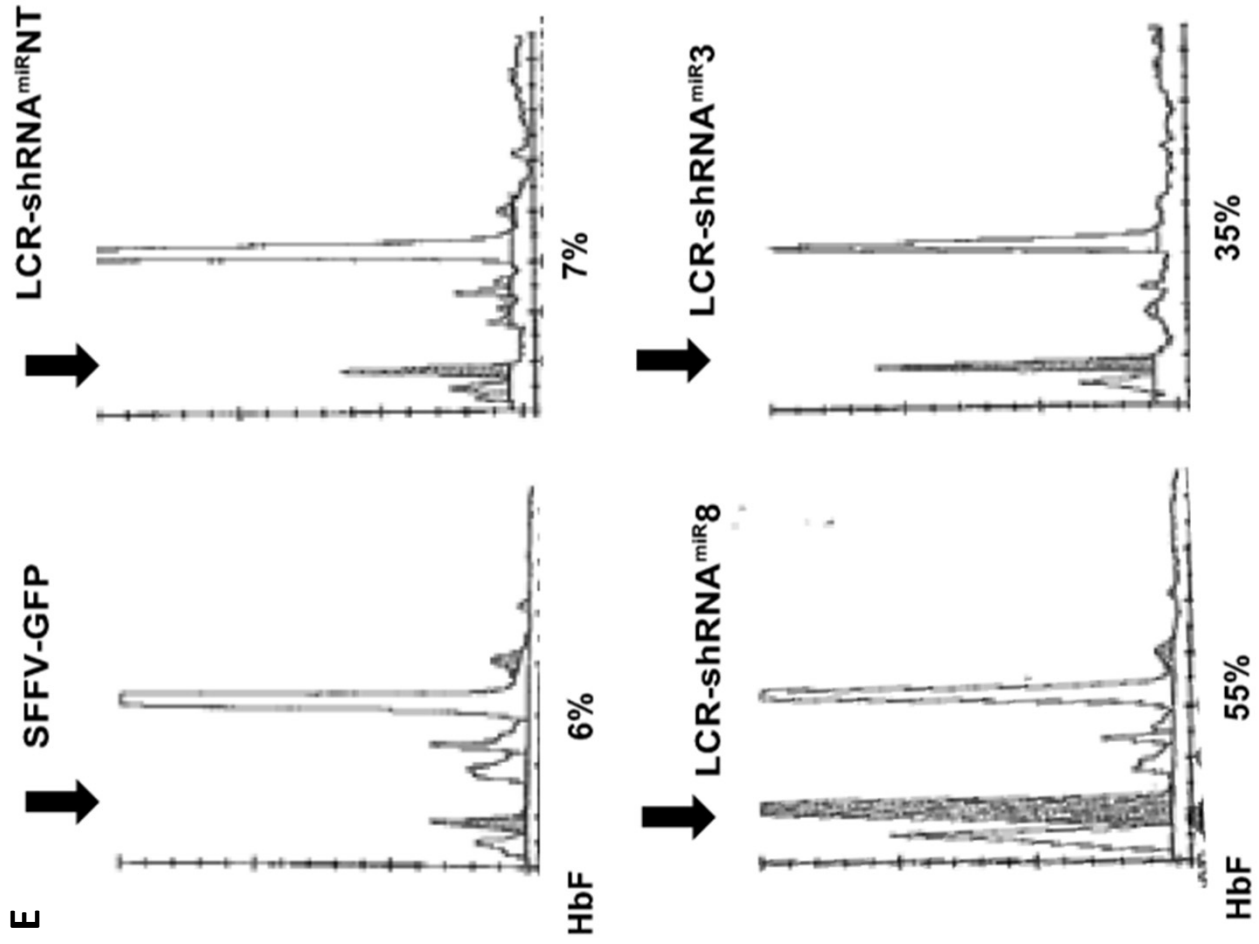

E
SFFV-GFP
LCR-shRNA^{miR}NT
LCR-shRNA^{miR}3
LCR-shRNA^{miR}8
HbF
6%
7%
35%
55%
F
% γ-globin mRNA (γ/(γ+β))
0
10
20
30
40
50
60
untrans-duced ctrl
NT
3
LCR-shRNA^{miR}

FIG. 24

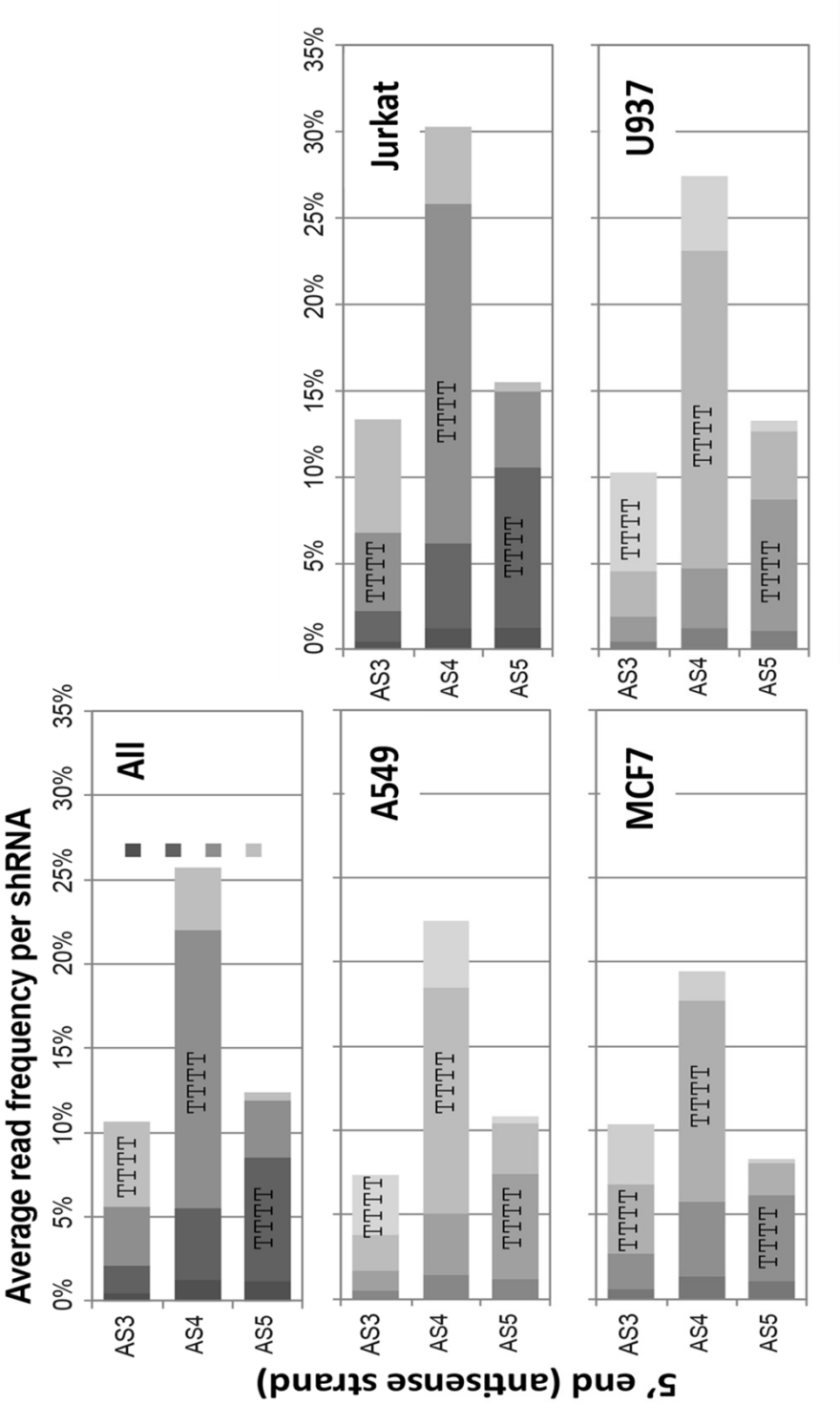

Average read frequency per shRNA
0%
5%
10%
15%
20%
25%
30%
35%
All
A549
MCF7
Jurkat
U937
TTTT
AS3
AS4
AS5
5' end (antisense strand)

FIG. 25

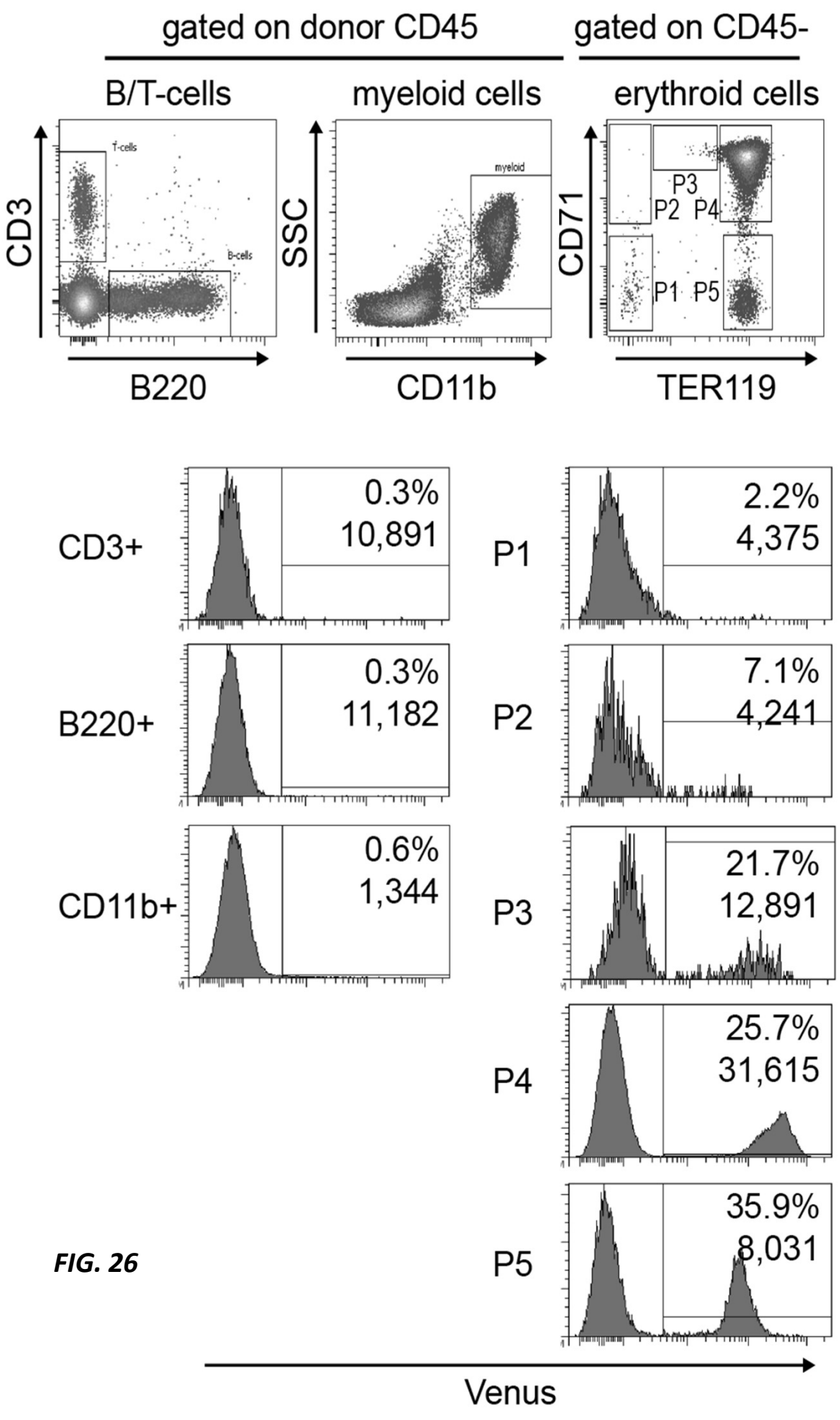

gated on donor CD45
gated on CD45-
B/T-cells
myeloid cells
erythroid cells
CD3
T-cells
B-cells
B220
SSC
myeloid
CD11b
CD71
P3
P2
P4
P1
P5
TER119
CD3+
0.3%
10,891
B220+
0.3%
11,182
CD11b+
0.6%
1,344
P1
2.2%
4,375
P2
7.1%
4,241
P3
21.7%
12,891
P4
25.7%
31,615
P5
35.9%
8,031
Venus

FIG. 26

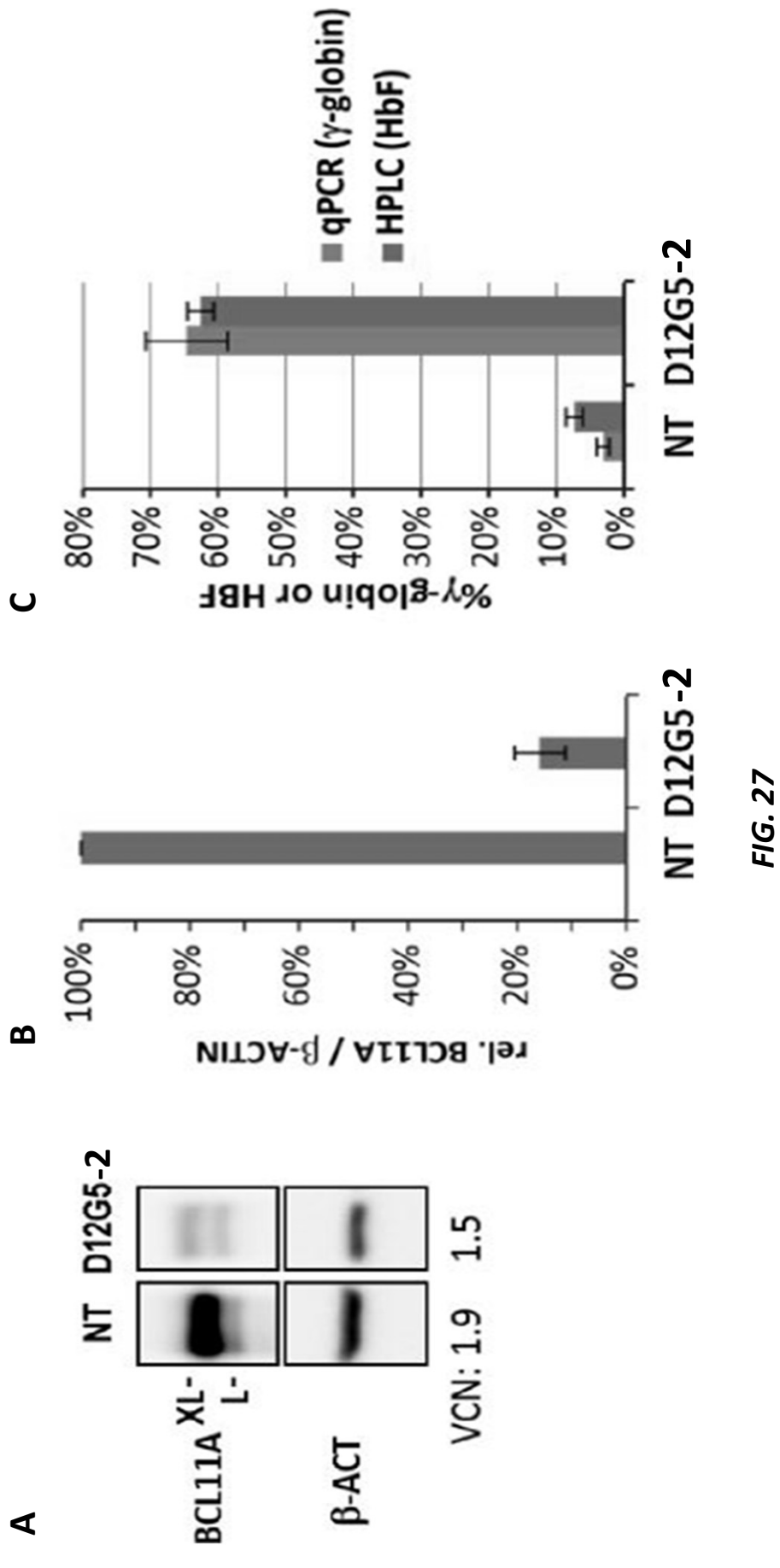

A
NT
D12G5-2
BCL11A XL-
L-
β-ACT
VCN: 1.9
1.5
B
rel. BCL11A / β-ACTIN
100%
80%
60%
40%
20%
0%
NT
D12G5-2
C
qPCR (γ-globin)
HPLC (HbF)
%γ-globin or HBF
80%
70%
60%
50%
40%
30%
20%
10%
0%
NT
D12G5-2

FIG. 27

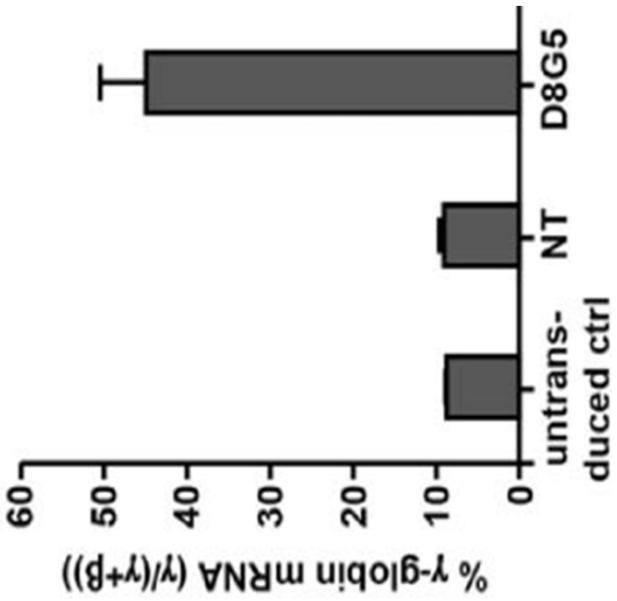


FIG. 28

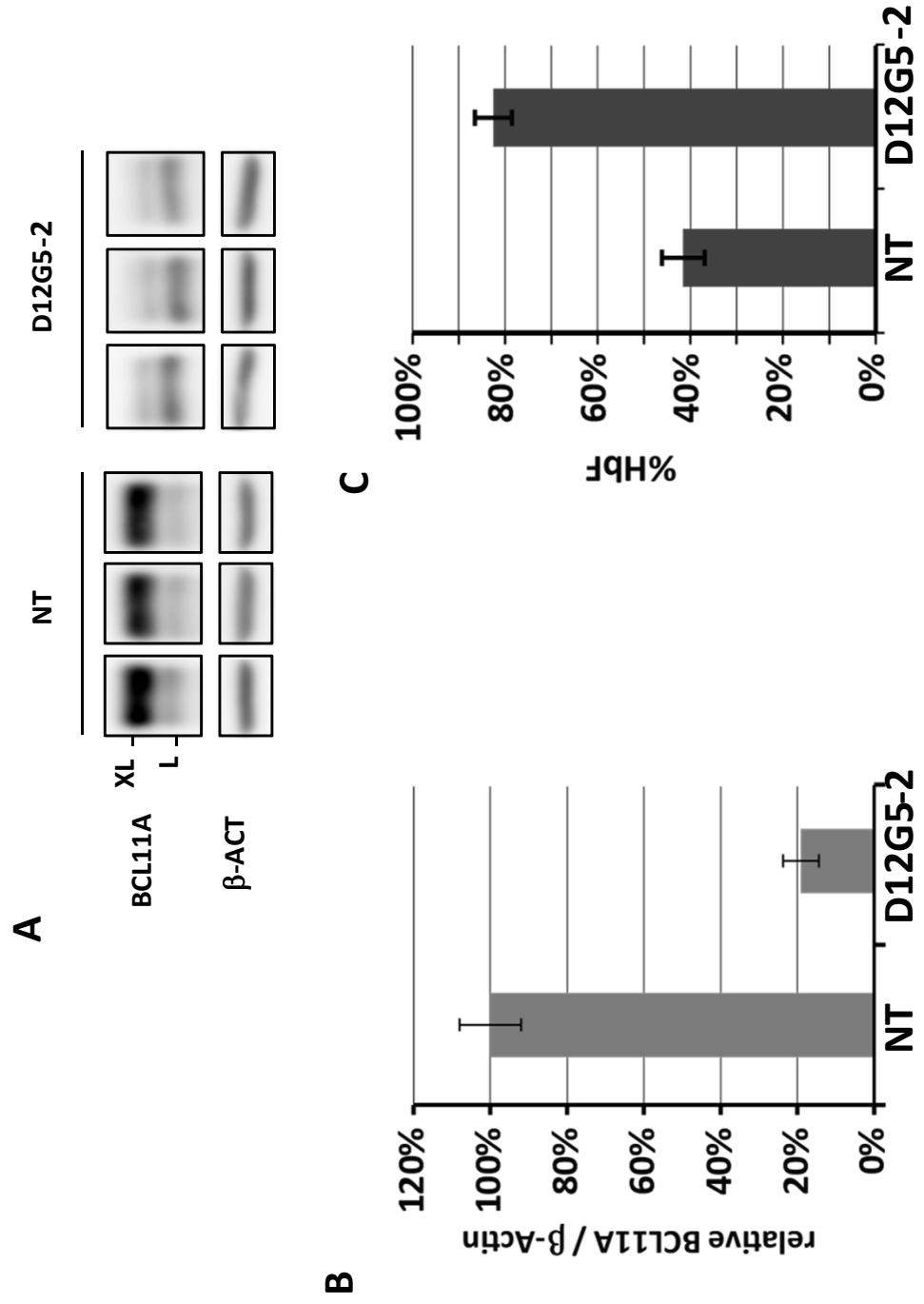

A
NT
D12G5-2
XL
L
BCL11A
β-ACT
B
relative BCL11A / β-Actin
120%
100%
80%
60%
40%
20%
0%
NT
D12G5-2
C
%HbF
100%
80%
60%
40%
20%
0%
NT
D12G5-2

FIG. 29

BCL11A knockdown with sequences in both SFFV and LCR backbones in CD34 differentiated erythroid cells.

ShRNA	SEQUENCE	
Mir1	CGCACAGAACACTCATGGATTctccatgtggtagagAATCCATGAGTGTTCTGTGCG	SEQ. ID. NO: 25
E3G5	GCGCTCGGAGACTCCAGACAActccatgtggtagagTTGTCTGGAGTCTCCGAgcgc	SEQ. ID. NO: 35
D8G5	GCGCTTCTCTTGCAACACGCActccatgtggtagagTGCGTGTTGCAAGAGAAgcgc	SEQ. ID. NO: 37
C4G5	GCGCACAGTACCCTGGAGAAActccatgtggtagagTTTCTCCAGGGTACTGTgcgc	SEQ. ID. NO: 42
D12G5	GCGCGATCGAGTGTTGAATAActccatgtggtagagTTATTCAACACTCGATCgcgc	SEQ. ID. NO: 39
D12	GATCGAGTGTTGAATAATGATctccatgtggtagagATCATTATTCAACACTCGATC	SEQ. ID. NO: 30
A5	CAGTACCCTGGAGAAACACATctccatgtggtagagATGTGTTTCTCCAGGGTACTG	SEQ. ID. NO: 31
NT	CAACAAGATGAAGAGCACCAActccatgtggtagagTTGGTGCTCTTCATCTTGTTG	SEQ. ID. NO: 45

FIG. 30

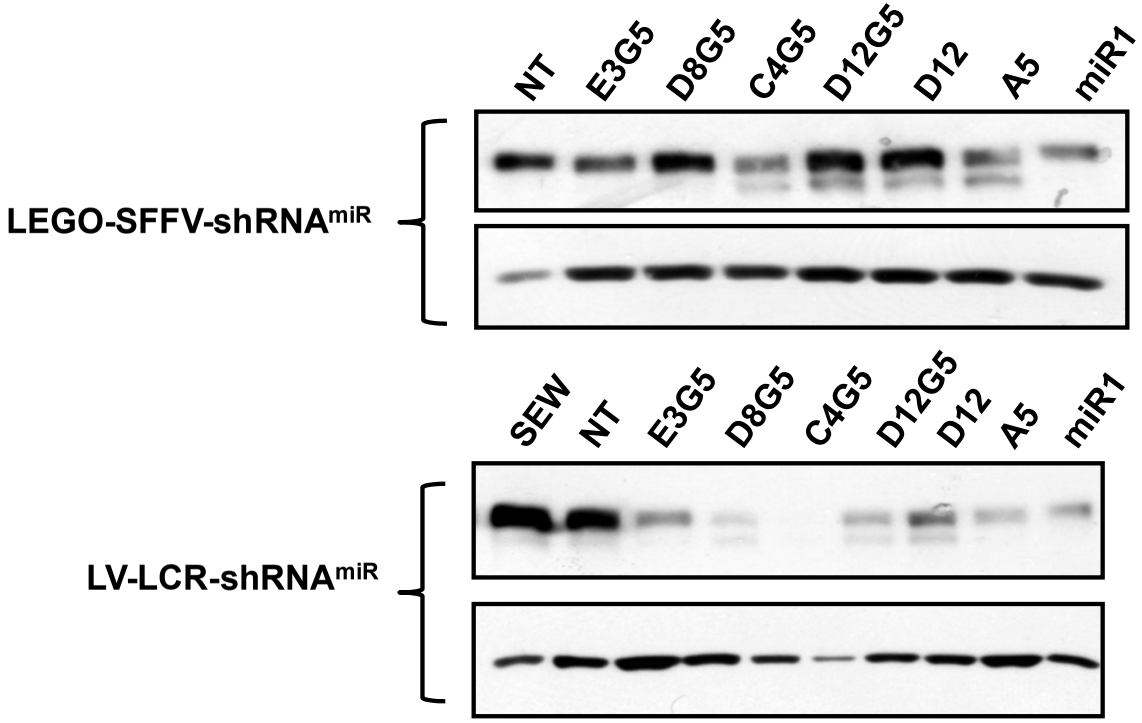


FIG. 31