FIG. 1A

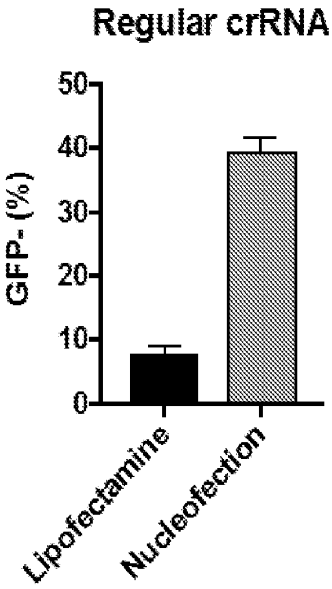

Regular crRNA
GFP- (%)
50
40
30
20
10
0
Lipofectamine
Nucleofection

FIG. 1B

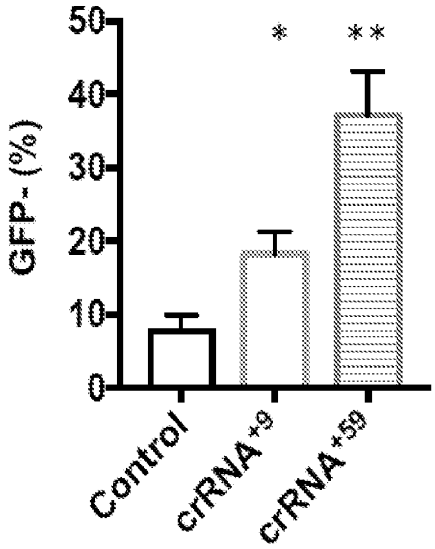

GFP- (%)
50
40
30
20
10
0
*
**
Control
crRNA+9
crRNA+59

FIG. 1C

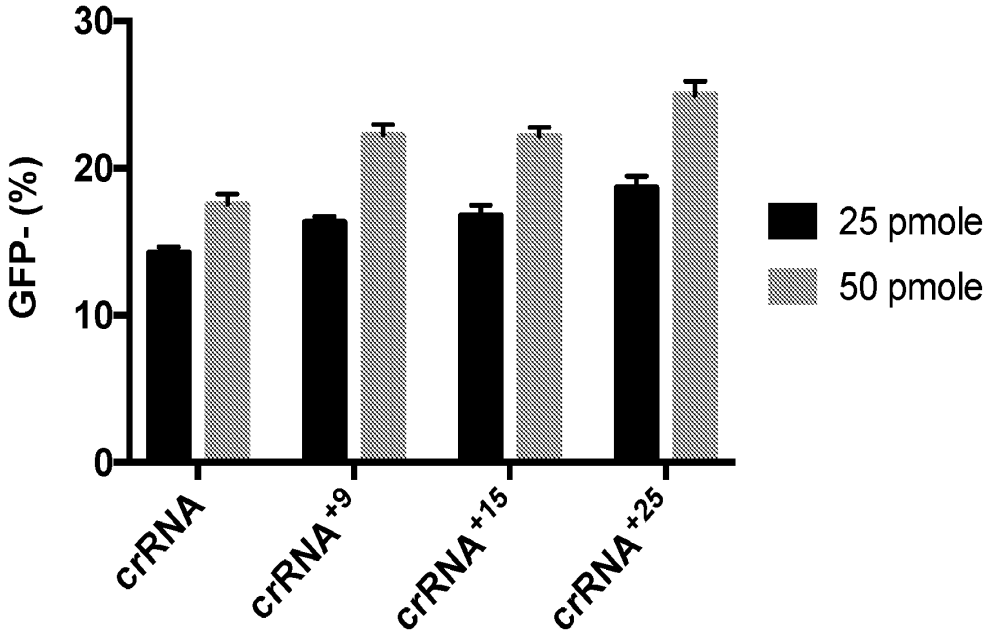


FIG. 1D

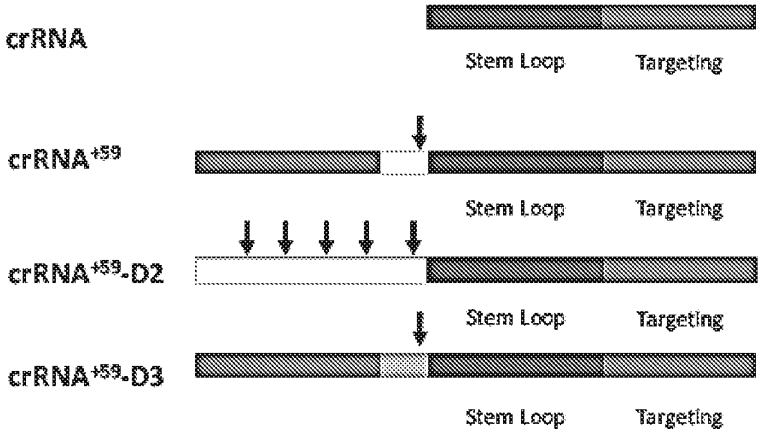

crRNA
Stem Loop
Targeting
crRNA+59
Stem Loop
Targeting
crRNA+59-D2
Stem Loop
Targeting
crRNA+59-D3
Stem Loop
Targeting

FIG. 1E

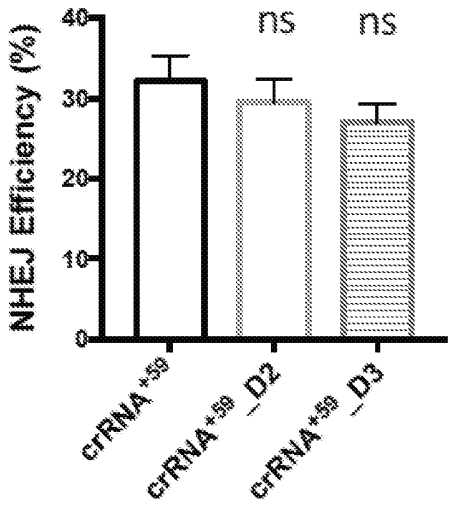

NHEJ Efficiency (%)
40
30
20
10
0
ns
ns
crRNA+59
crRNA+59_D2
crRNA+59_D3

FIG. 1F

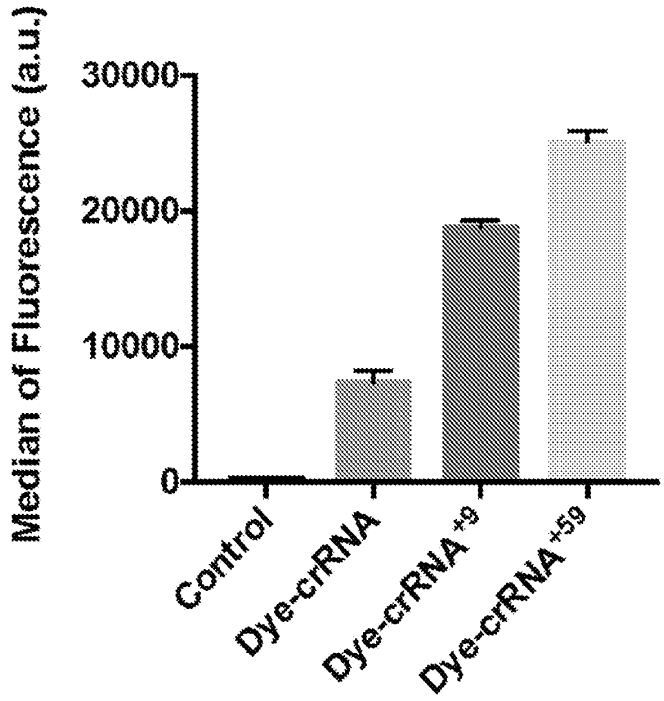

Median of Fluorescence (a.u.)
30000
20000
10000
0
Control
Dye-crRNA
Dye-crRNA +9
Dye-crRNA +59

FIG. 2

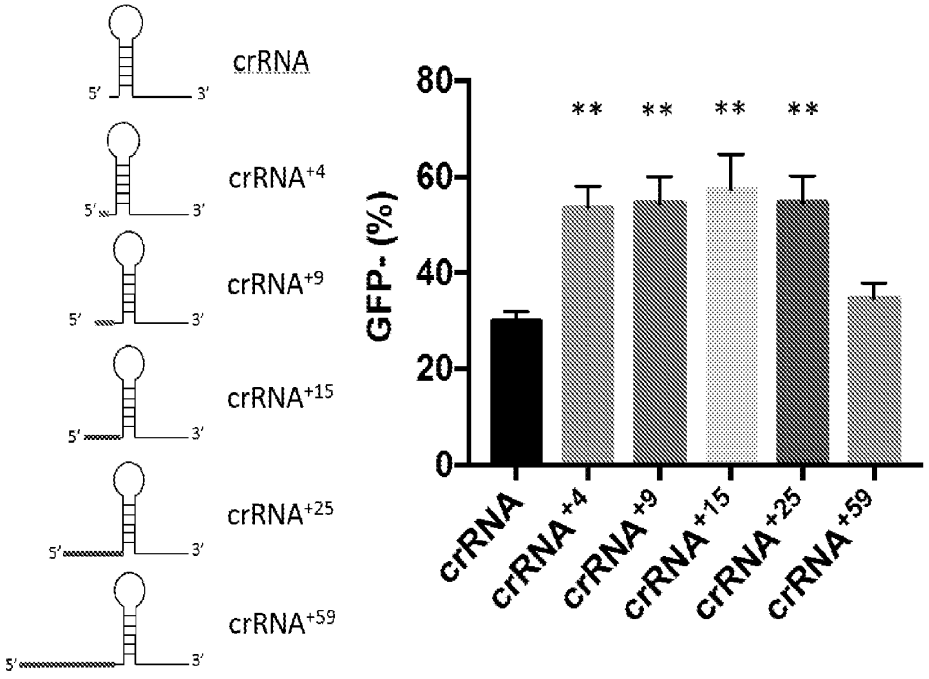

crRNA
crRNA+4
crRNA+9
crRNA+15
crRNA+25
crRNA+59
5′
3′
GFP- (%)
80
60
40
20
0
**
**
**
**
crRNA
crRNA+4
crRNA+9
crRNA+15
crRNA+25
crRNA+59

FIG. 3A

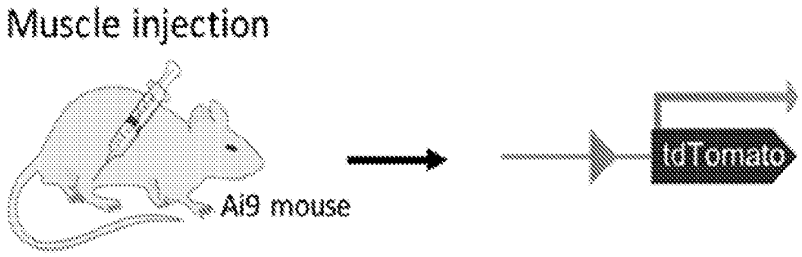


FIG. 3B

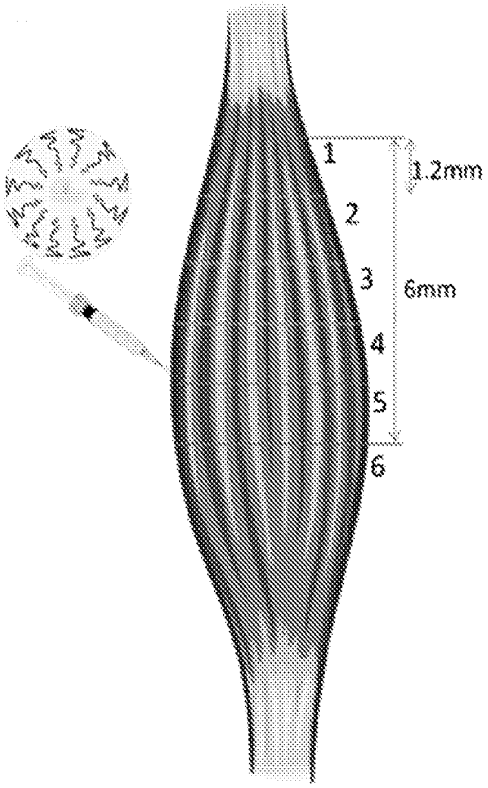


FIG. 4A

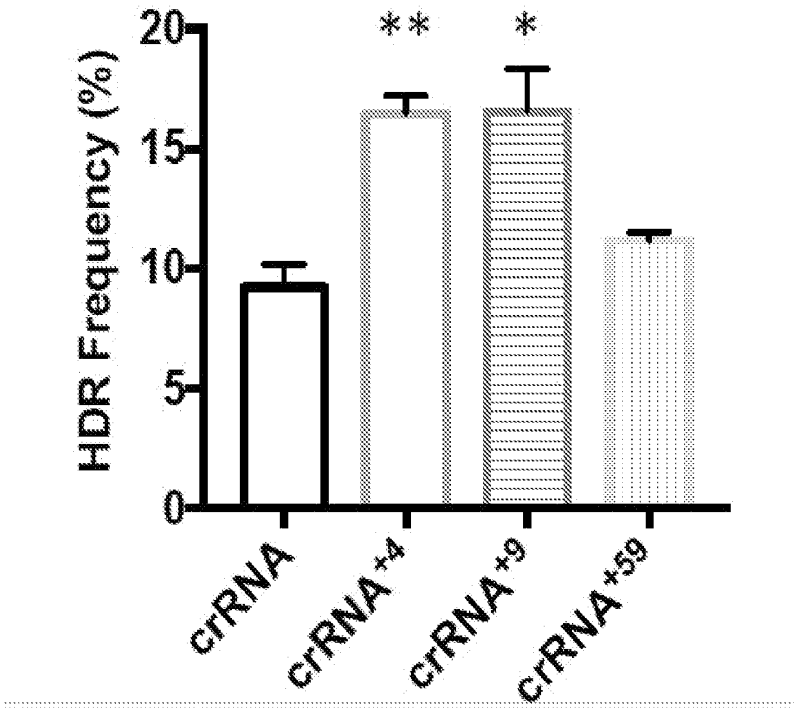


FIG. 4B

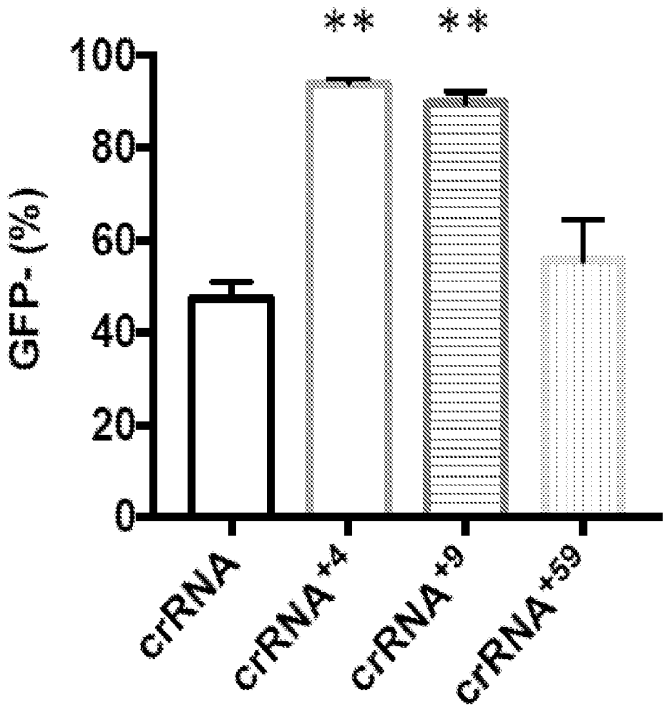


FIG. 4C

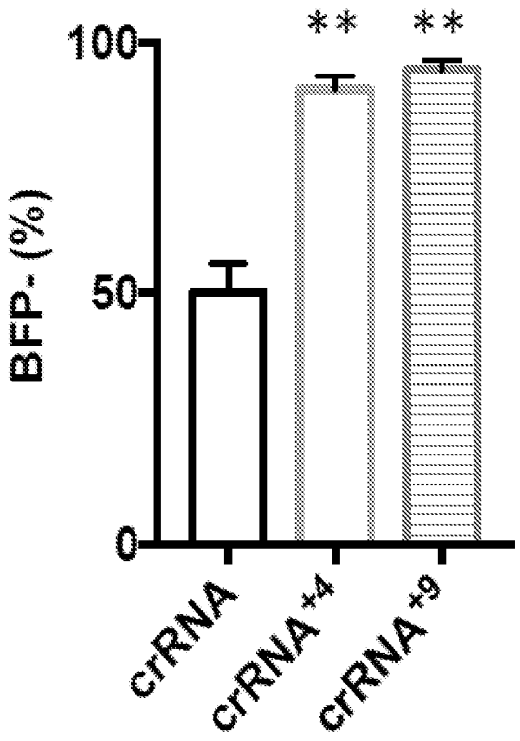


FIG. 4D

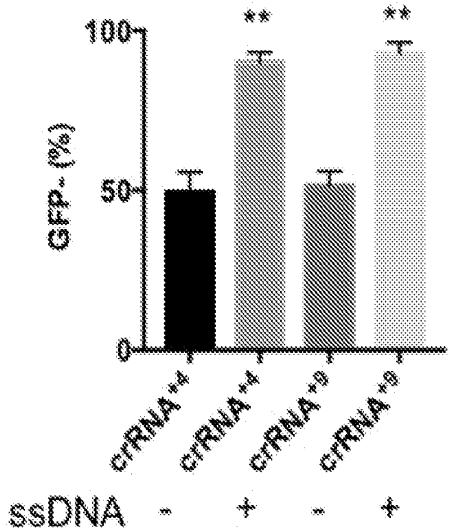

100
50
0
GFP- (%)
**
**
crRNA+4
crRNA+4
crRNA+9
crRNA+9
ssDNA - + - +

FIG. 4E

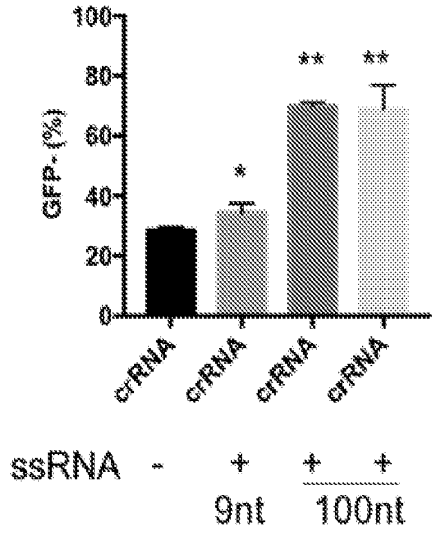

100
80
60
40
20
0
GFP- (%)
*
**
**
crRNA
crRNA
crRNA
crRNA
ssRNA - + + +
9nt 100nt

FIG. 4F

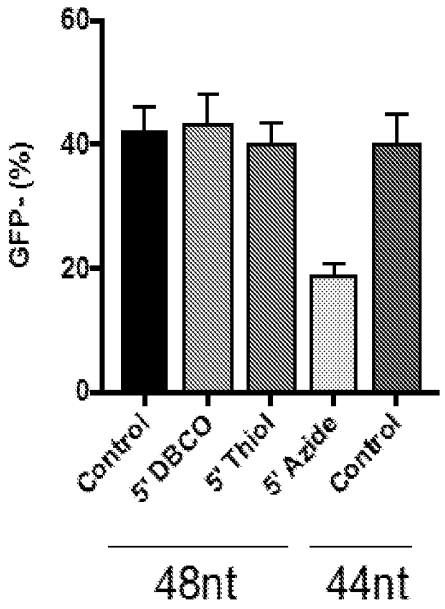


FIG. 5A

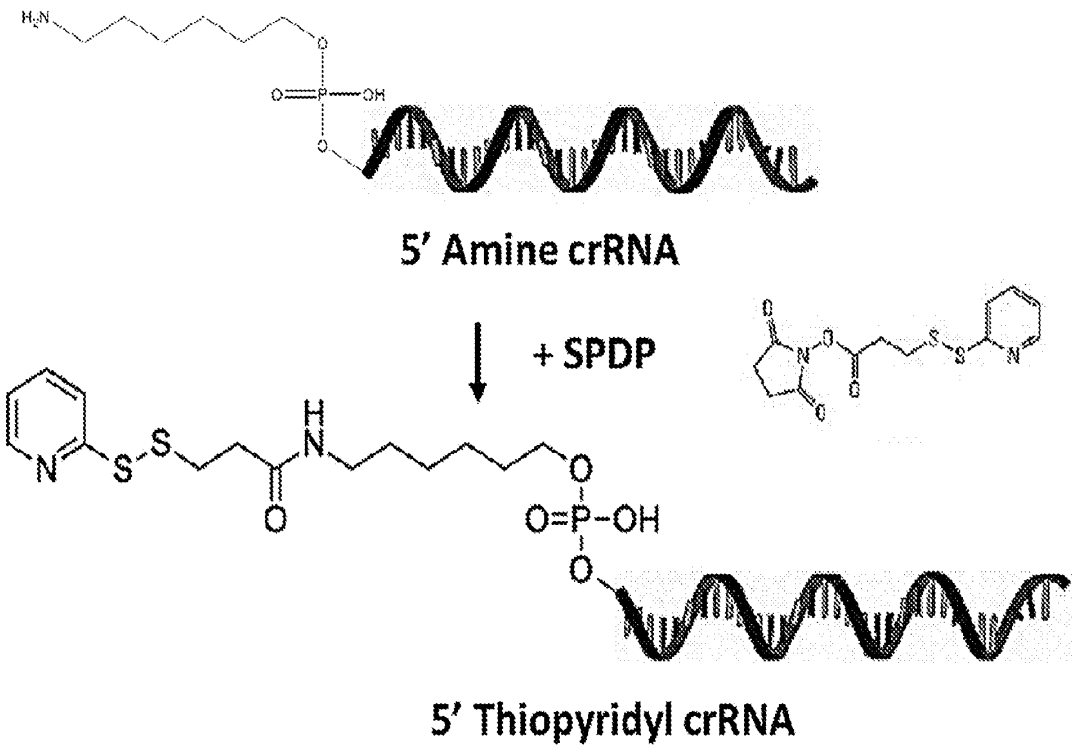

5' Amine crRNA
+ SPDP
5' Thiopyridyl crRNA
3' Disulfide Donor DNA
3' Thiol Donor DNA

FIG. 5B

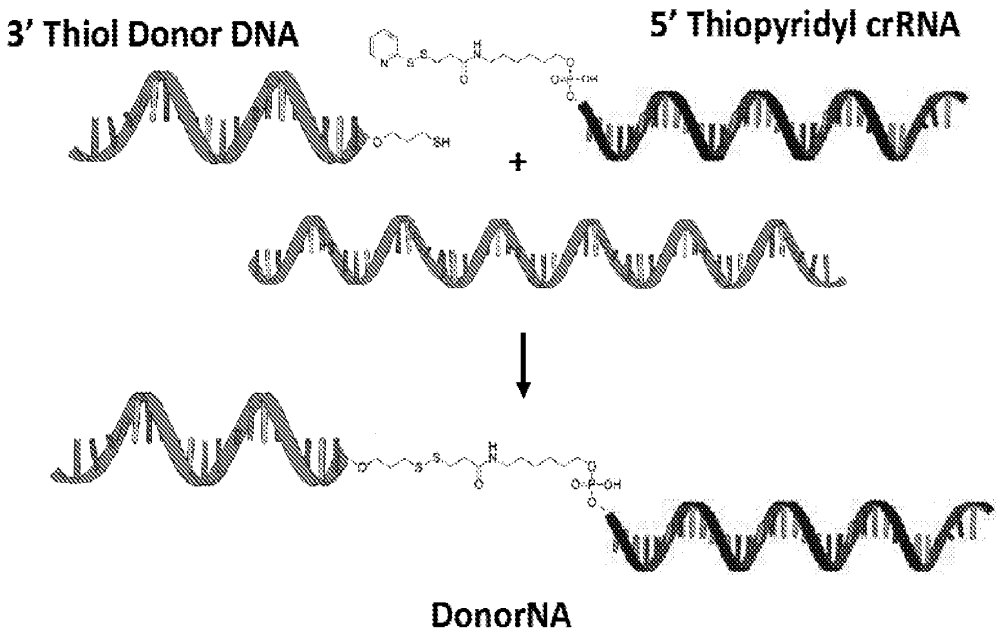

3' Thiol Donor DNA
5' Thiopyridyl crRNA
+
DonorNA

FIG. 5C

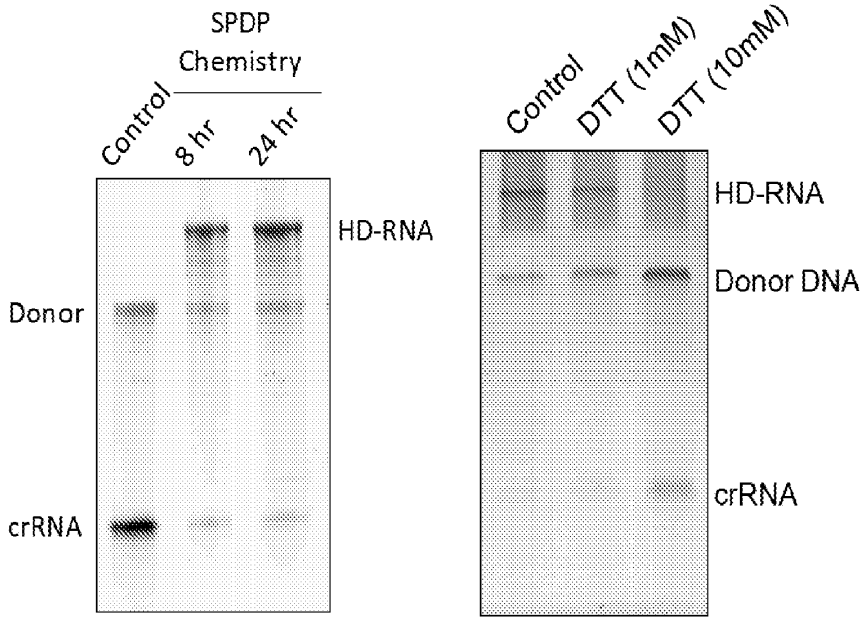

SPDP
Chemistry
Control
8 hr
24 hr
HD-RNA
Donor
crRNA
Control
DTT (1mM)
DTT (10mM)
HD-RNA
Donor DNA
crRNA

FIG. 6

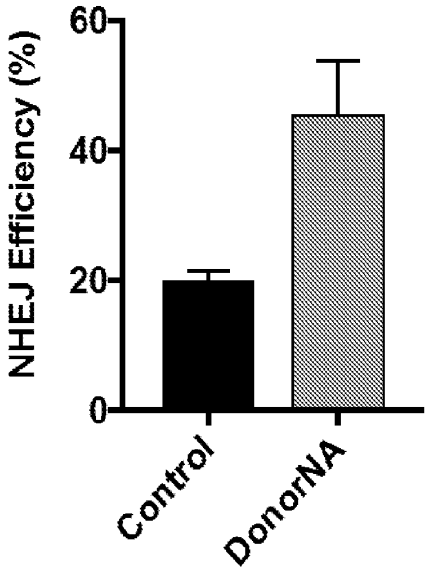

NHEJ Efficiency (%)
60
40
20
0
Control
DonorNA

FIG. 7

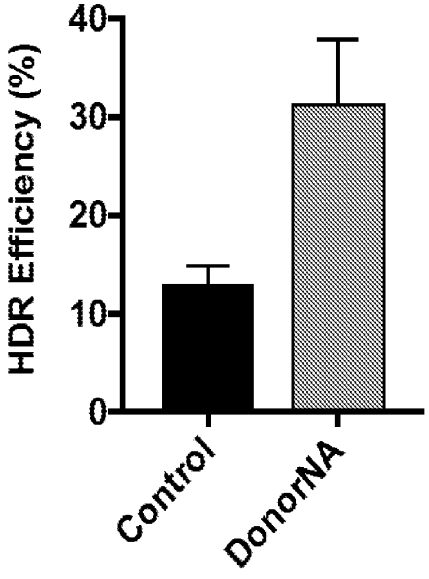

HDR Efficiency (%)
40
30
20
10
0
Control
DonorNA

Figure 8

Francisella tularensis subsp. novicida U112 Cpf1

```
   1 msiyqefvnk yslsktlrfe lipqgktlen ikarglildd ekrakdykka kqiidkyhqf
  61 fieeilssvc isedllqnys dvyfklkksd ddnlqkdfks akdtikkqis eyikdsekfk
 121 nlfnqnlida kkgqesdlil wlkqskdngi elfkansdit didealeiik sfkgwttyfk
 181 gfhenrknvy ssndiptsii yrivddnlpk flenkakyes lkdkapeain yeqikkdlae
 241 eltfdidykt sevnqrvfsl devfeianfn nylnqsgitk fntiiggkfv ngentkrkgi
 301 neyinlysqq indktlkkyk msvlfkqils dtesksfvid kleddsdvvt tmqsfyeqia
 361 afktveeksi ketlslldfdd lkaqkldlsk iyfkndkslt dlsqqvfddy svigtavley
 421 itqqiapknl dnpskkeqel iakktekaky lsletiklal eefnkhrdid kqcrfeeila
 481 nfaaipmifd eiaqnkdnla qisikyqnqg kkdllqasae ddvkaikdll dqtnnllhkl
 541 kifhisqsed kanildkdeh fylvfeecyf elanivplyn kirnyitqkp ysdekfklnf
 601 enstlangwd knkepdntai lfikddkyyl gvmnkknnki fddkaikenk gegykkivyk
 661 llpgankmlp kvffsaksik fynpsedilr irnhsthtkn gspqkgyekf efniedcrkf
 721 idfykqsisk hpewkdfgfr fsdtqrynsi defyrevenq gykltfenis esyidsvvnq
 781 gklylfqiyn kdfsayskgr pnlhtlywka lfdernlqdv vyklngeael fyrkqsipkk
 841 ithpakeaia nknkdnpkke svfeydlikd krftedkfff hcpitinfks sgankfndei
 901 nlllkekand vhilsidrge rhlayytlvd gkgniikqdt fniignd rmk tnyhdklaai
 961 ekdrdsarkd wkkinnikem kegylsqvvh eiaklvieyn aivvfedlnf gfkrgrfkve
1021 kqvyqklekm lieklnylvf kdnefdktgg vlrayqltap fetfkkmgkq tgiiyyvpag
1081 ftskicpvtg fvnqlypkye svsksqeffs kfdkicynld kgyfefsfdy knfgdkaakg
1141 kwtiasfgsr linfrnsdkn hnwdtrevyp tkelekllkd ysieyghgec ikaaicgesd
1201 kkffakltsv lntilqmrns ktgteldyli spvadvngnf fdsrqapknm pqdadangay
1261 higlkglmll griknnqegk klnlviknee yfefvqnrnn (SEQ ID NO: 1)
```

FIGURE 9

Type/ Species	Direct Repeat Sequences of Cpf1 Orthologs (5' – 3')	
	Processing Sequence	Cpf1 crRNA Sequence
FnCpf1	GUCUAAGAACUUUAAAU (SEQ ID NO: 2)	AAUUUCUACU-GUUGUAGAU (SEQ ID NO: 21)
AsCpf1	GUCAAAAGACCUUUUU (SEQ ID NO: 3)	AAUUUCUACUC-UUGUAGAU (SEQ ID NO: 22)
LbCpf1	GUUUCAAAGAUUAAAU (SEQ ID NO: 4)	AAUUUCUACUAAGUGUAGAU (SEQ ID NO: 23)
TsCpf1	CUCUAGCAGGCCUGGCA (SEQ ID NO: 5)	AAUUUCUACU-GUUGUAGAU (SEQ ID NO: 24)
SaCpf1	AUUUGAAAGCAUCUUUU (SEQ ID NO: 6)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 25)
Pb2Cpf1	GGCUAUAAAGCUUAUUU (SEQ ID NO: 7)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 26)
PgCpf1	GCCAAAUACCUCUAUAA (SEQ ID NO: 8)	AAUUUCUACU-UUUGUAGAU (SEQ ID NO: 27)
MlCpf1	GUCUAACUACCUUUU (SEQ ID NO: 9)	AAUUUCUACUGUUUGUAGAU (SEQ ID NO: 28)
Mb2Cpf1	GUCUAACUACCUUUU (SEQ ID NO: 10)	AAUUUCUACUGUUUGUAGAU (SEQ ID NO: 29)
Mb3Cpf1	GUCUAACUACCUUUU (SEQ ID NO: 11)	AAUUUCUACUGUUUGUAGAU (SEQ ID NO: 30)
Lb4Cpf1	CUCUAAUAAGAGAUAUG (SEQ ID NO: 12)	AAUUUCUACU-GUUGUAGAU (SEQ ID NO: 31)
Lb5Cpf1	UGCUUAGAACAUUUAAAG (SEQ ID NO: 13)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 32)
FbCpf1	GUUUAAAACCACUUUAA (SEQ ID NO: 14)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 33)
CRbCpf1	CUCUACAACUGAUAAAG (SEQ ID NO: 15)	AAUUUCUACU-UUUGUAGAU (SEQ ID NO: 34)
CPbCpf1	GUUUAAAAGUCCUAUUG (SEQ ID NO: 16)	GAUUUCUACU-UUUGUAGAU (SEQ ID NO: 35)
CMaCpf1	GUCUAAAACUCAUUCAG (SEQ ID NO: 17)	AAUUUCUACU-AGUGUAGAU (SEQ ID NO: 36)
BsCpf1	UGCUUAGUACUUAUAAAG (SEQ ID NO: 18)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 37)
BfCpf1	GCCAAGAACCUAUAGAU (SEQ ID NO: 19)	AAUUUCUACU-GUUGUAGAU (SEQ ID NO: 38)
BoCpf1	GUCUAUAAGACAUUUAU (SEQ ID NO: 20)	AAUUUCUACU-AUUGUAGAU (SEQ ID NO: 39)

FIGURE 10A

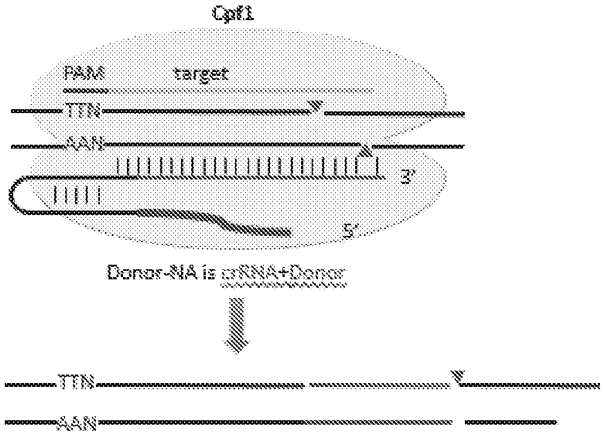


FIGURE 10B



FIGURE 10C

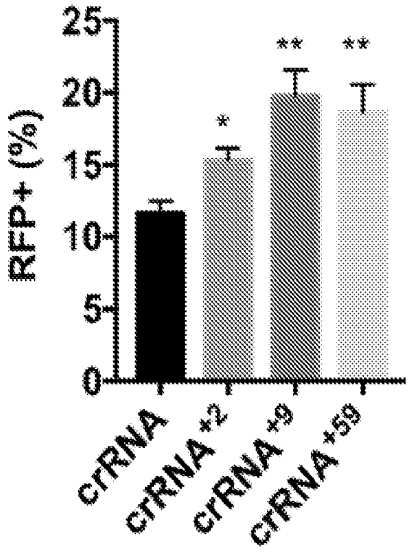


FIGURE 10D

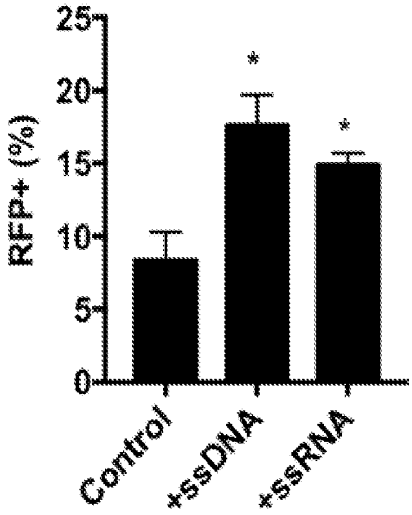


FIGURE 10E

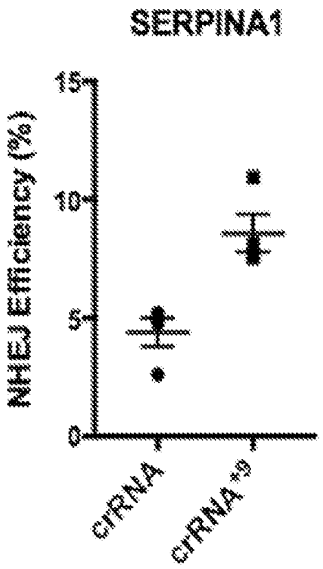
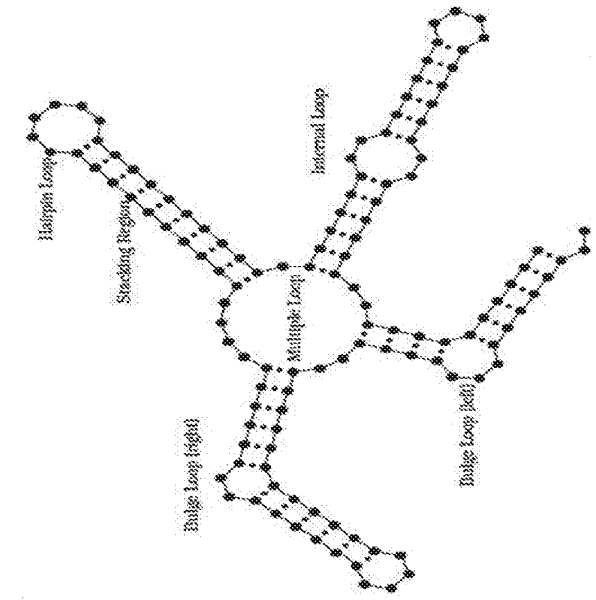


FIGURE 11A


Hairpin Loop
Stacking Region
Internal Loop
Multiple Loop
Bulge Loop (right)
Bulge Loop (left)

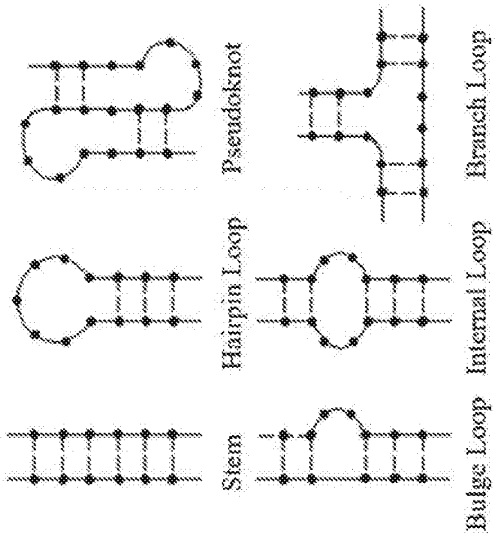

Stem
Hairpin Loop
Pseudoknot
Bulge Loop
Internal Loop
Branch Loop

FIGURE 11B

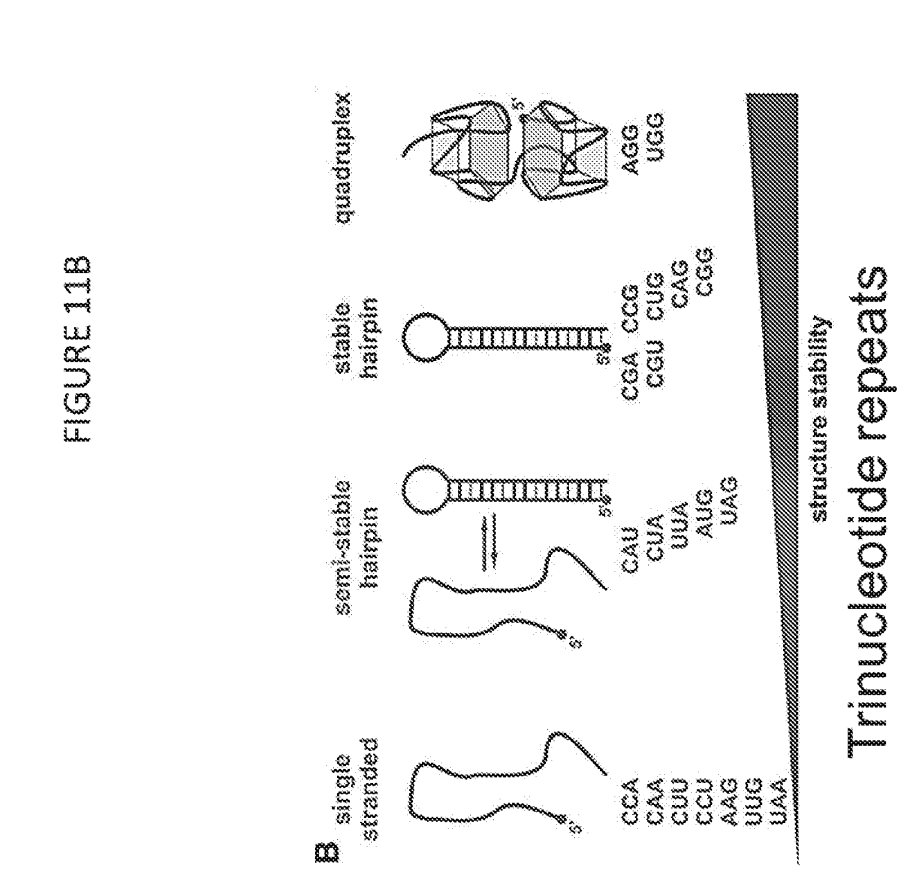

R: random
S1: pseudoknot
S2: quadraplex
S3: stable hairpin
B
single stranded
semi-stable hairpin
stable hairpin
quadruplex
CCA
CAA
CUU
CCU
AAG
UUG
UAA
CAU
CUA
UUA
AUG
UAG
CGA CCG
CGU CUG
CAG
CGG
AGG
UGG
structure stability
Trinucleotide repeats

FIGURE 11C

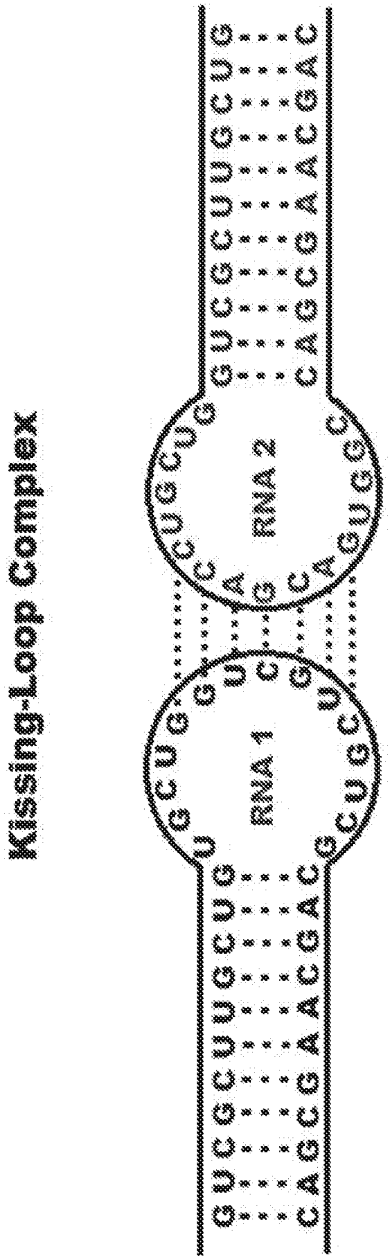


FIGURE 11D

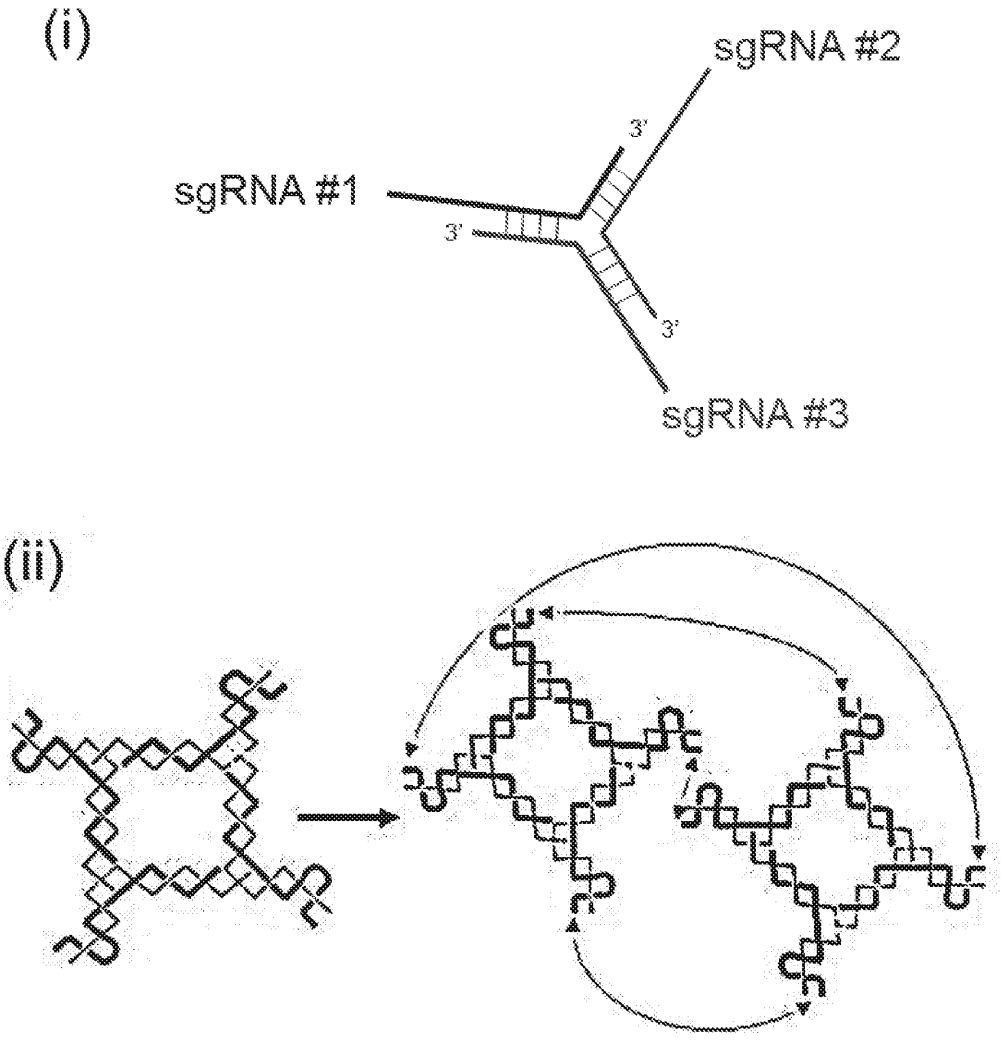


FIGURE 12

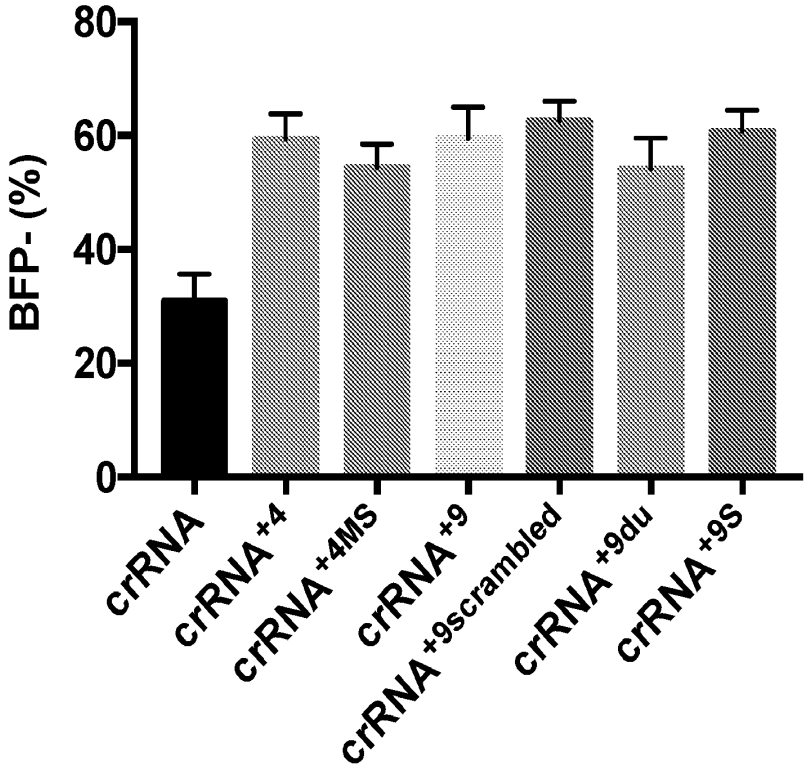

80
60
40
20
0
BFP- (%)
crRNA
crRNA+4
crRNA+4MS
crRNA+9
crRNA+9scrambled
crRNA+9du
crRNA+9S

FIGURE 13A

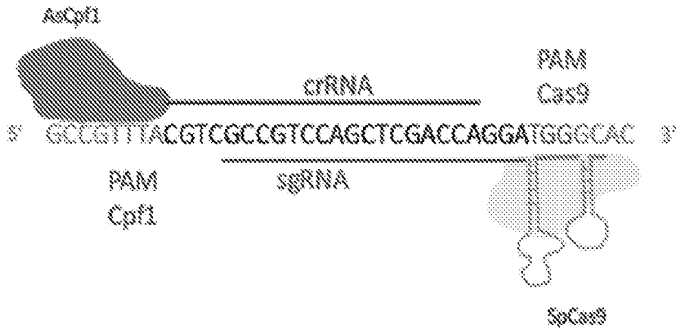


FIGURE 13B

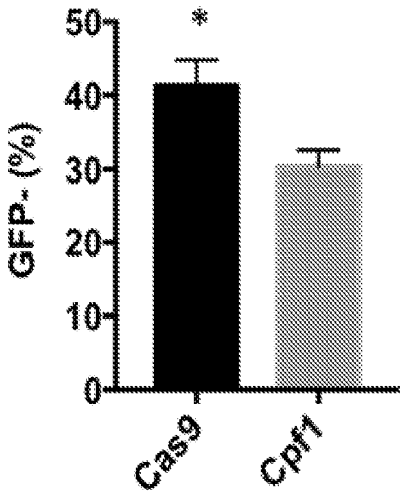


FIGURE 13C

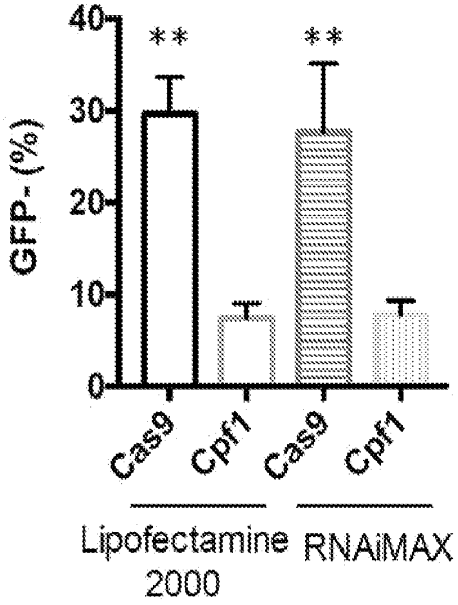

40
30
20
10
0
GFP- (%)
**
**
Cas9
Cpf1
Cas9
Cpf1
Lipofectamine 2000
RNAiMAX

FIGURE 14

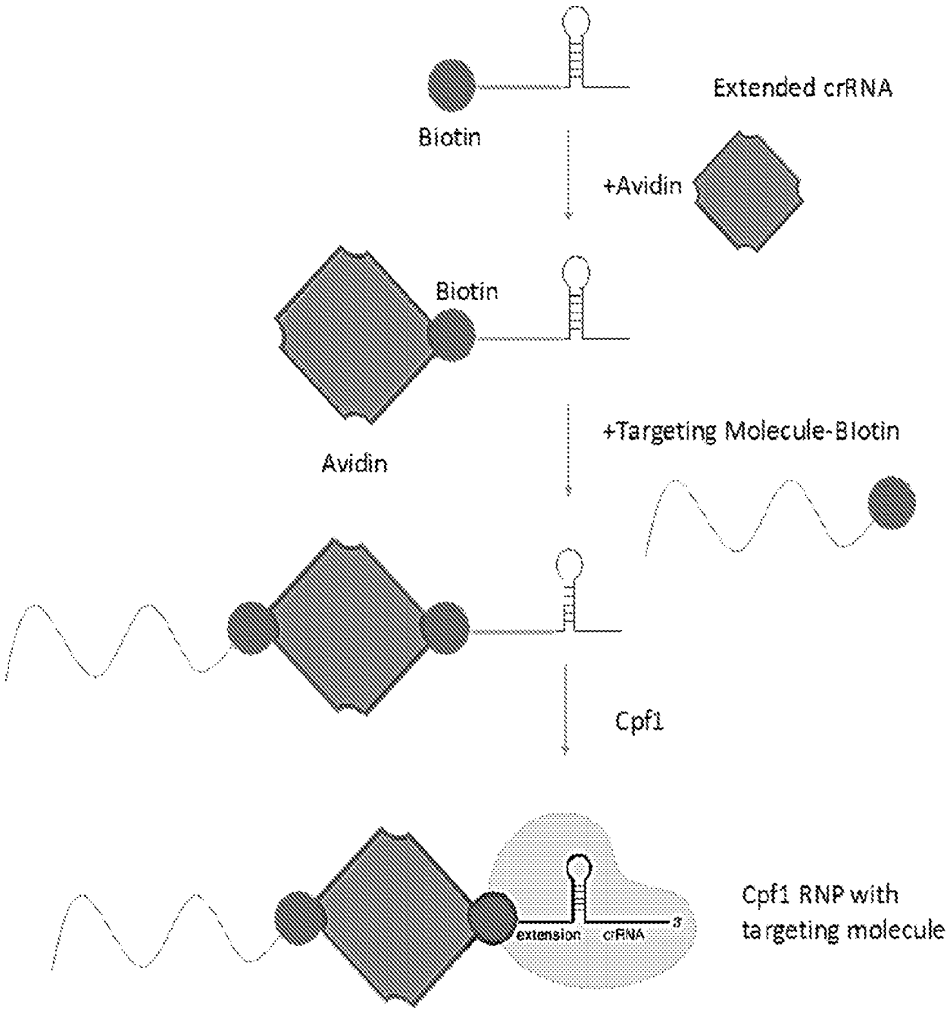

Extended crRNA
Biotin
+Avidin
Biotin
Avidin
+Targeting Molecule-Biotin
Cpf1
extension
crRNA
3'
Cpf1 RNP with
targeting molecule

FIG. 15A

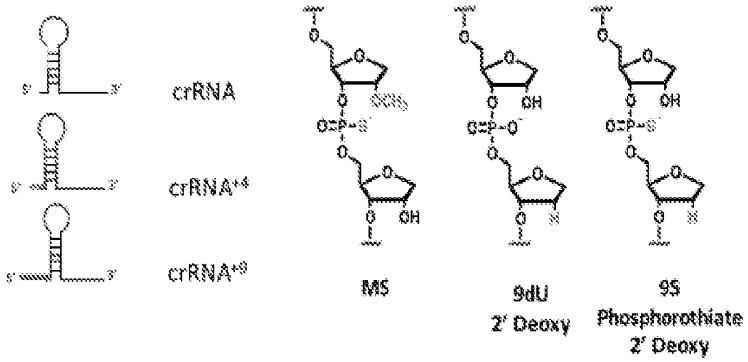


FIG. 15B

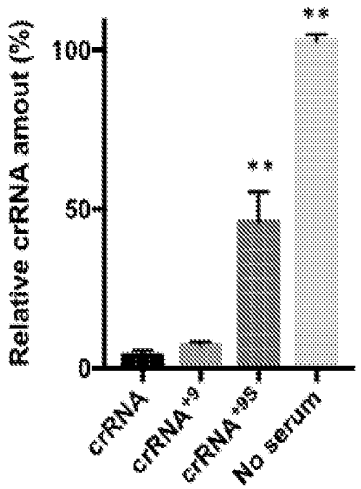


FIG. 15C

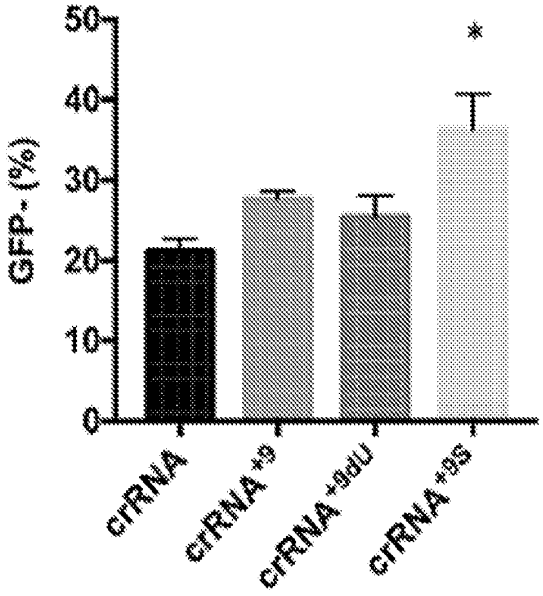


FIG. 15D

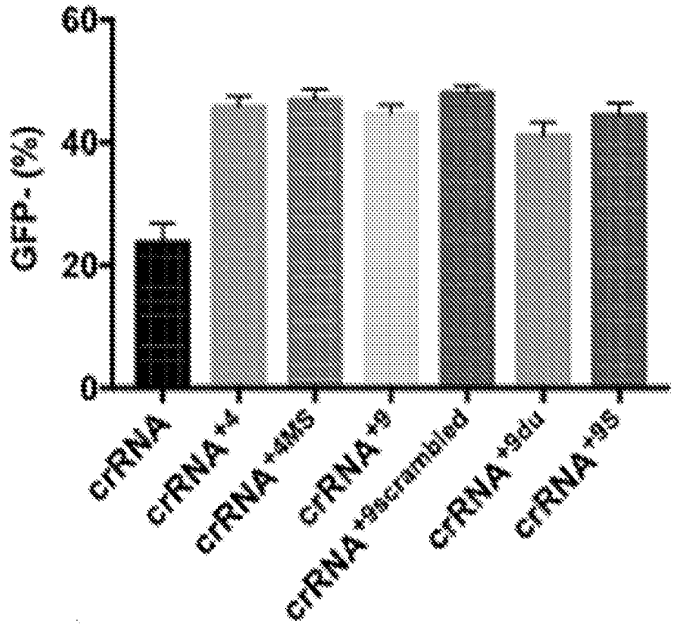


FIG. 16

