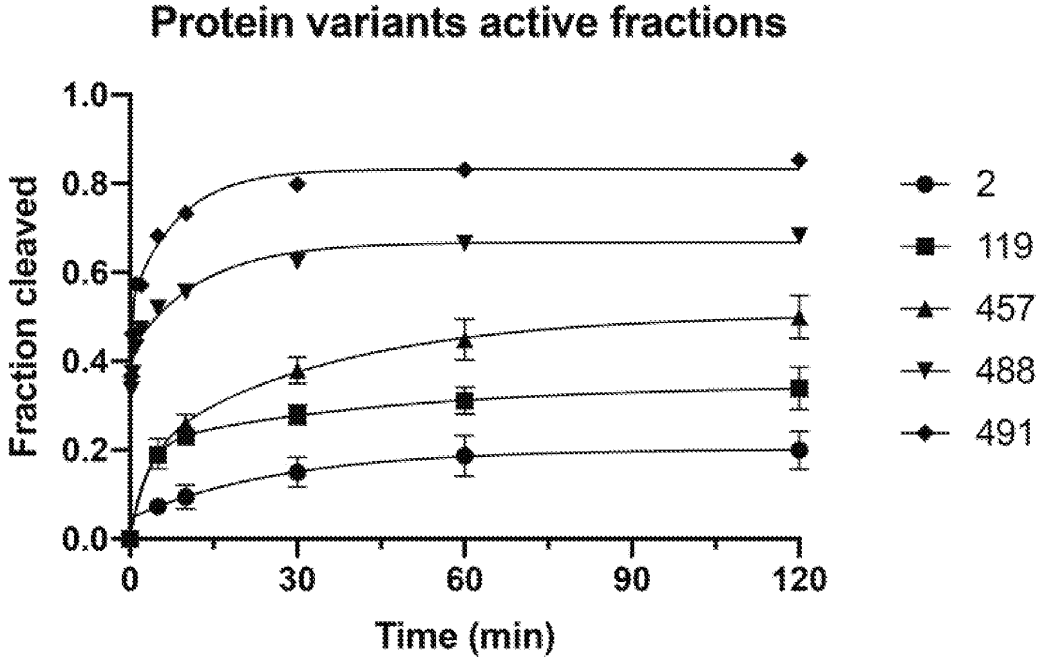

Protein variants active fractions
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
Time (min)
2
119
457
488
491

FIG. 1

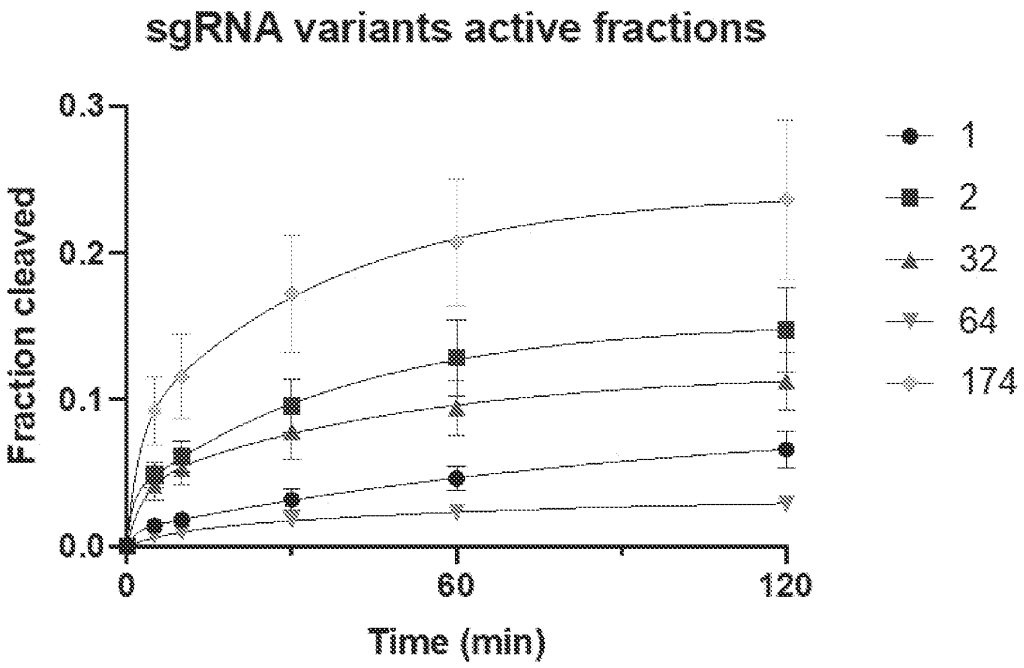

sgRNA variants active fractions
Fraction cleaved
0.3
0.2
0.1
0.0
0
60
120
Time (min)
1
2
32
64
174

FIG. 2

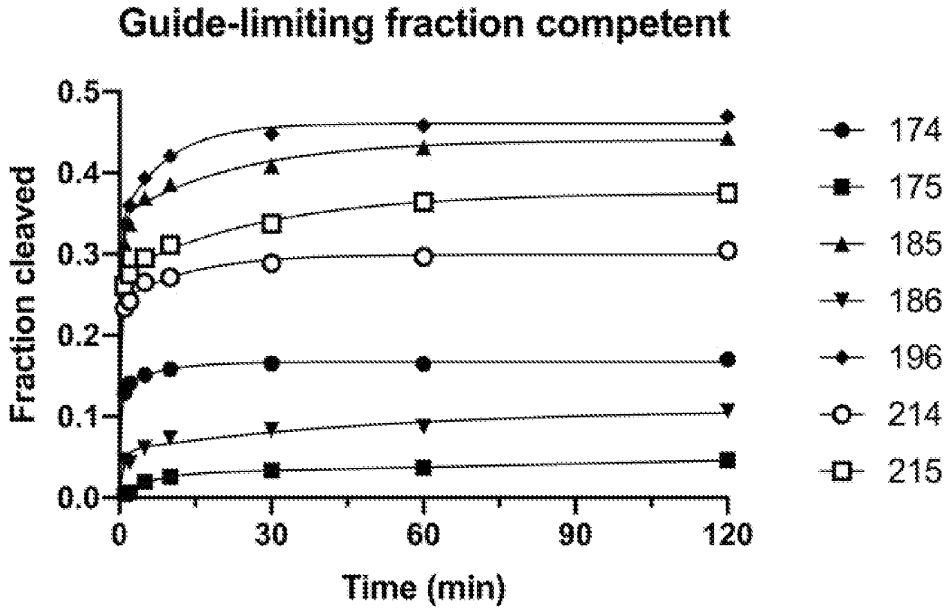

Guide-limiting fraction competent
Fraction cleaved
0.5
0.4
0.3
0.2
0.1
0.0
0
30
60
90
120
Time (min)
174
175
185
186
196
214
215

FIG. 3

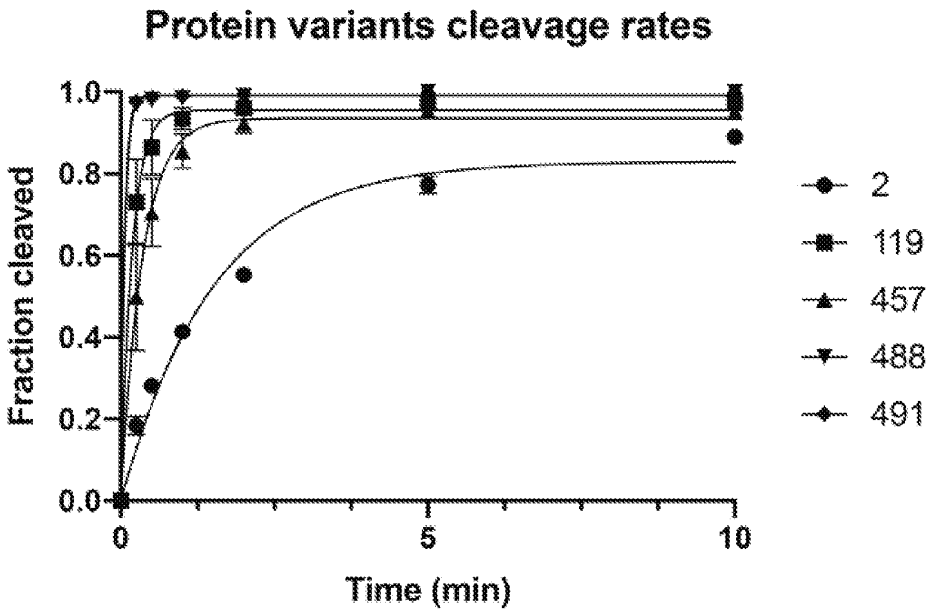

Protein variants cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
2
119
457
488
491

FIG. 4

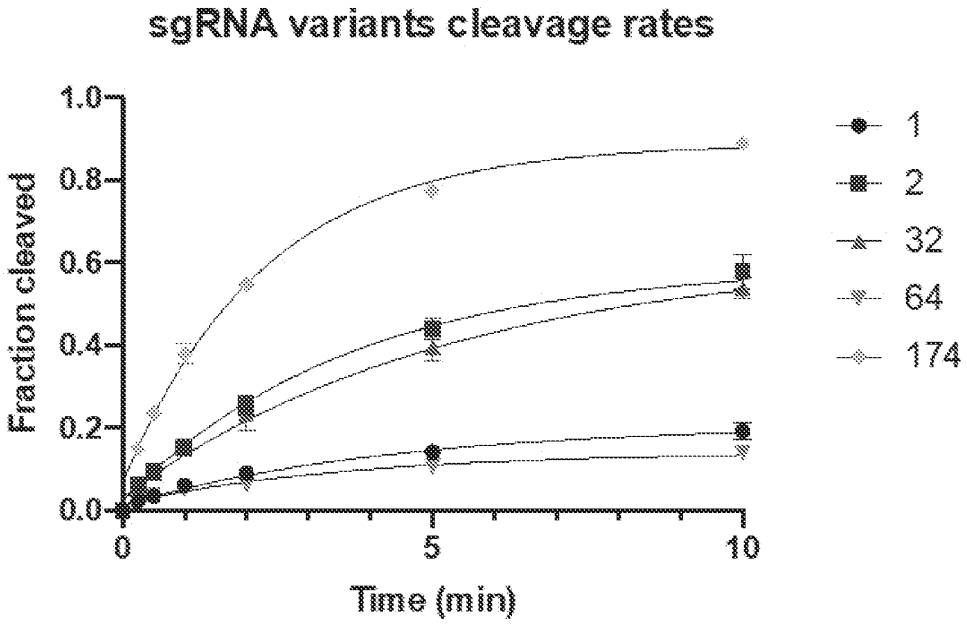

sgRNA variants cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
1
2
32
64
174

FIG. 5

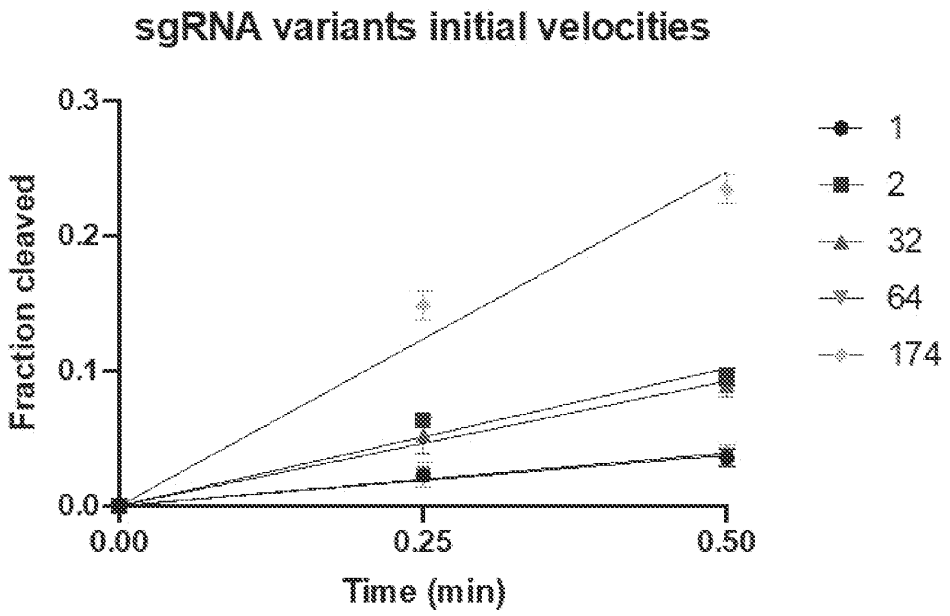

sgRNA variants initial velocities
Fraction cleaved
0.3
0.2
0.1
0.0
0.00
0.25
0.50
Time (min)
1
2
32
64
174

FIG. 6

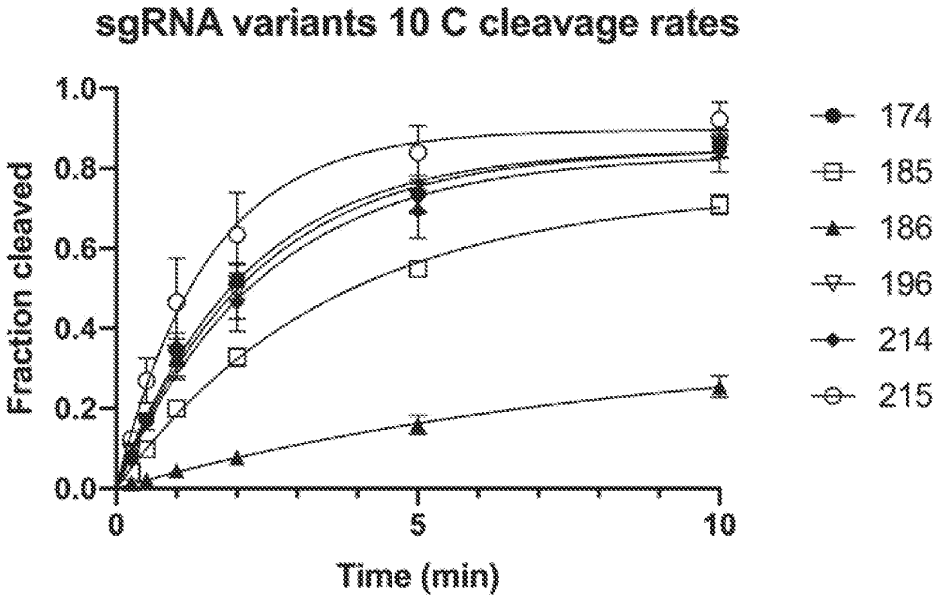

sgRNA variants 10 C cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
174
185
186
196
214
215

FIG. 7

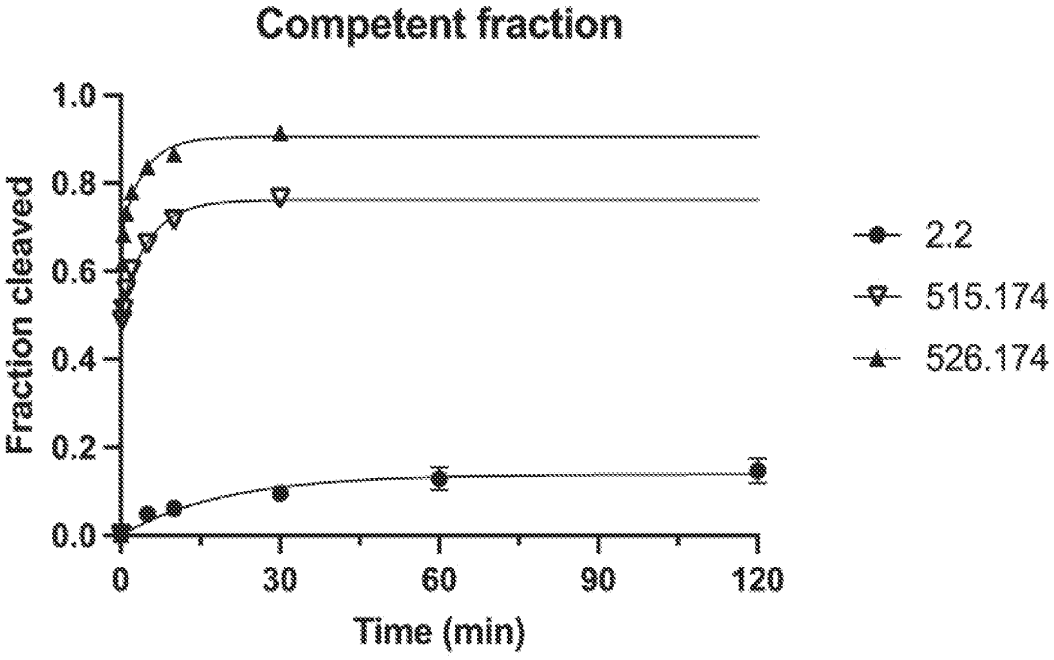

Competent fraction
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
Time (min)
2.2
515.174
526.174

FIG. 8

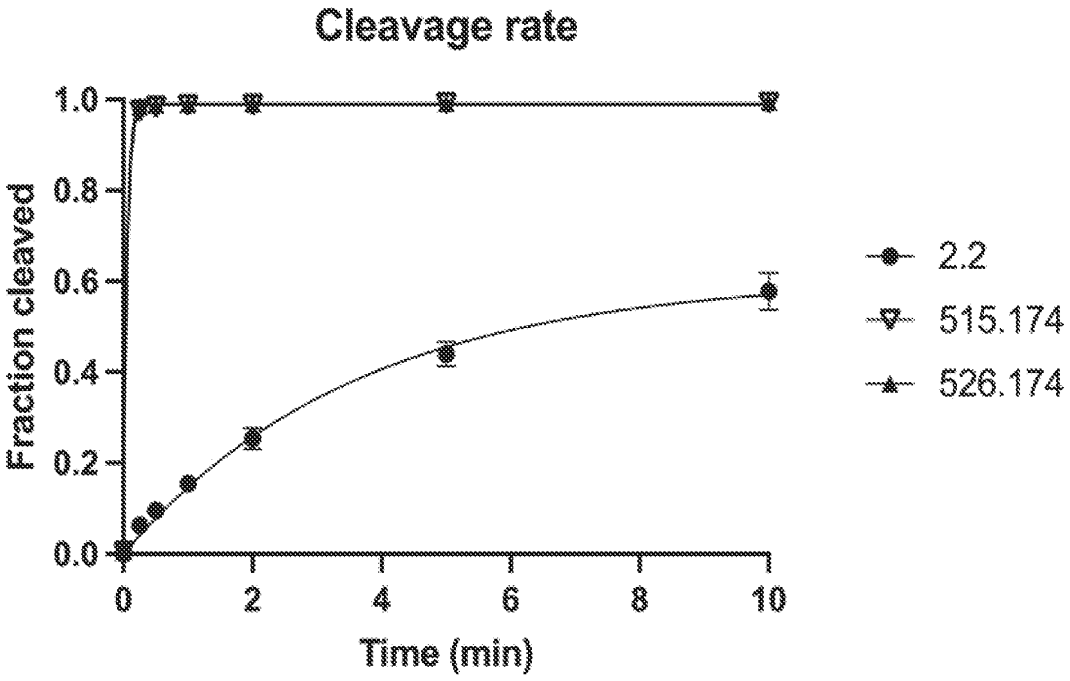

Cleavage rate
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Time (min)
2.2
515.174
526.174

FIG. 9

Cleavage rate on NTC PAMs

FIG. 10A

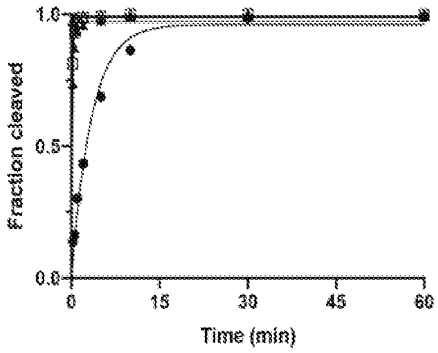


FIG. 10B

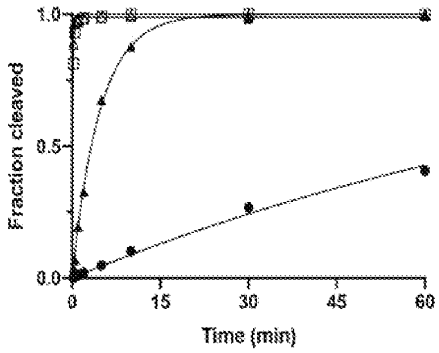


FIG. 10C

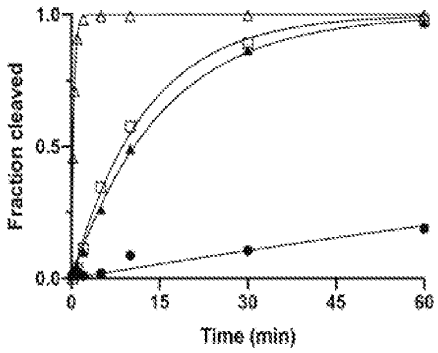
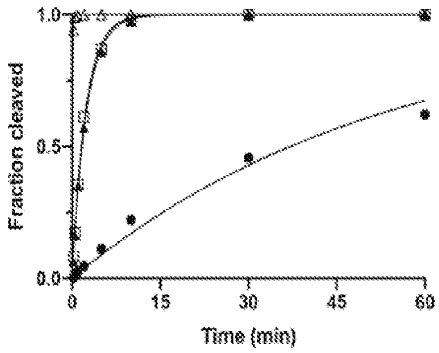


FIG. 10D



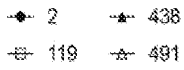


FIG. 11A

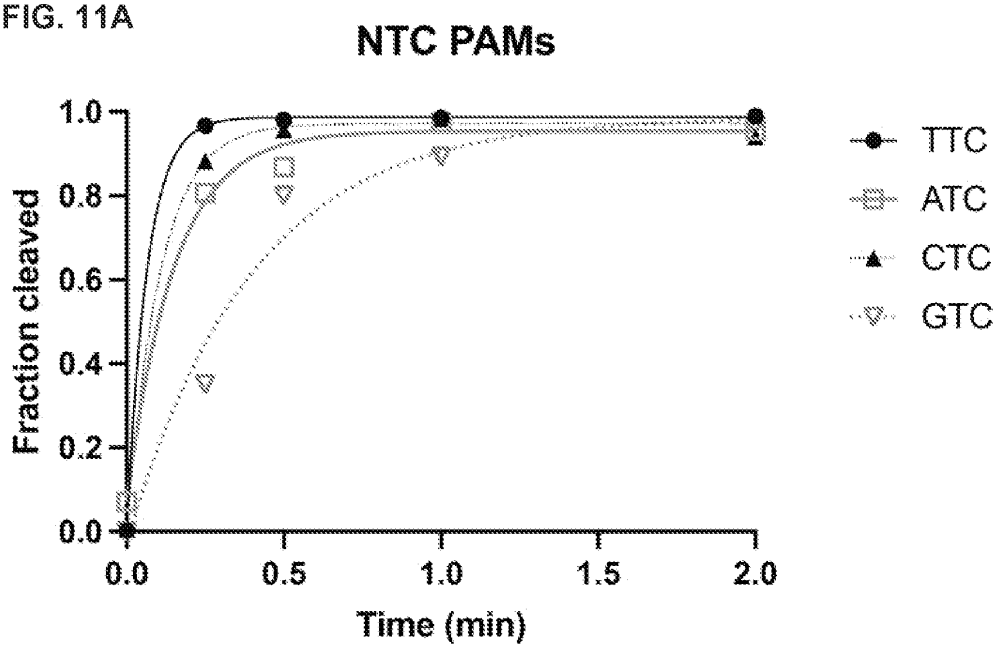


FIG. 11B

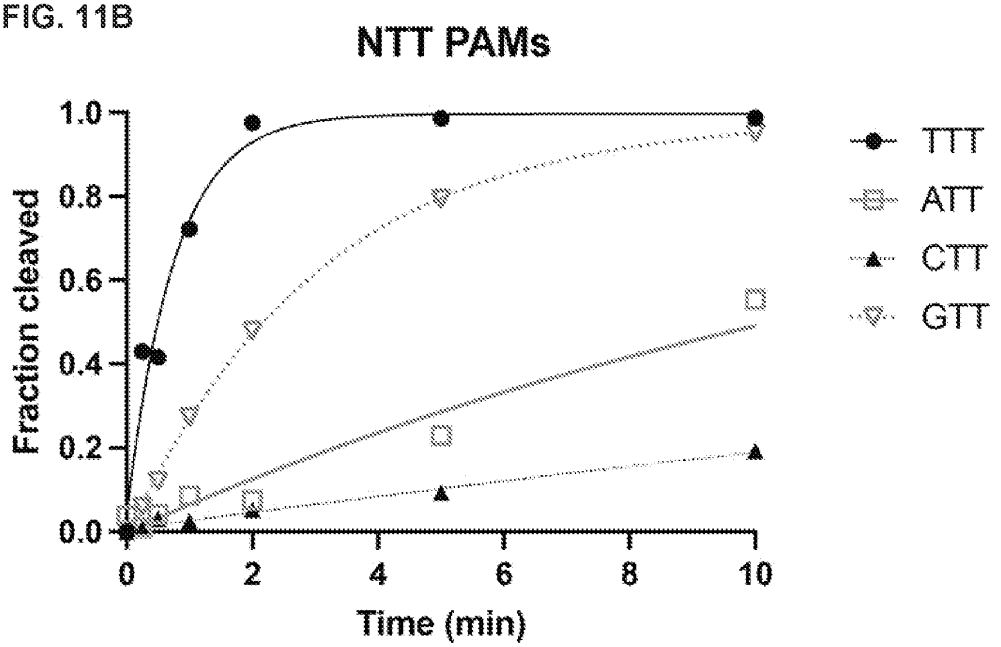


FIG. 12A

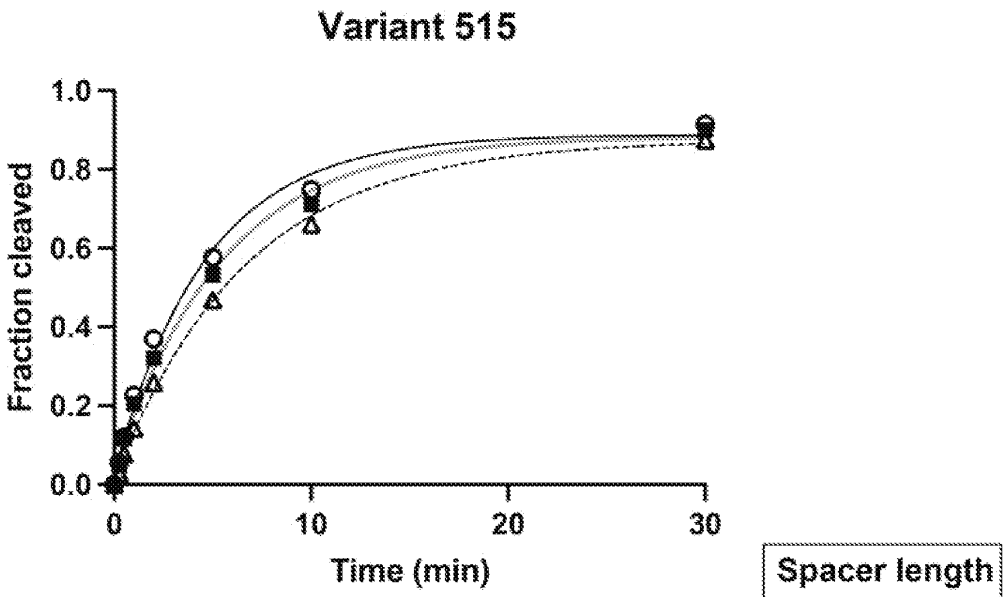
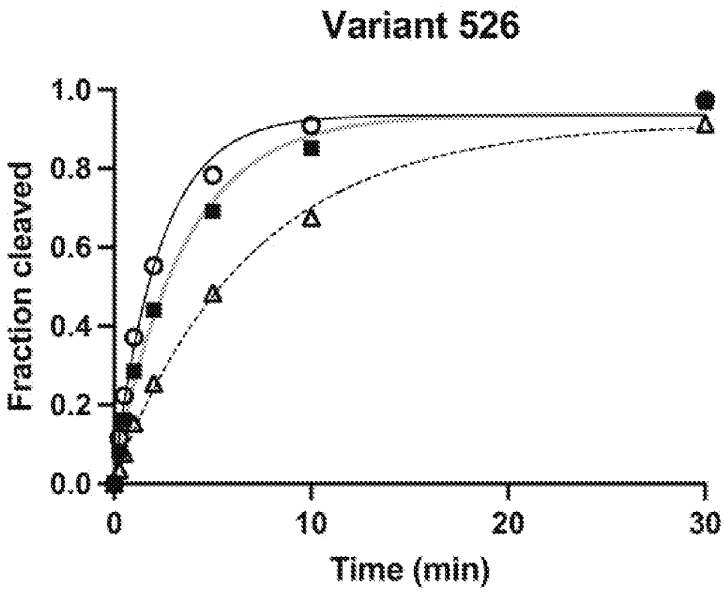


FIG. 12B



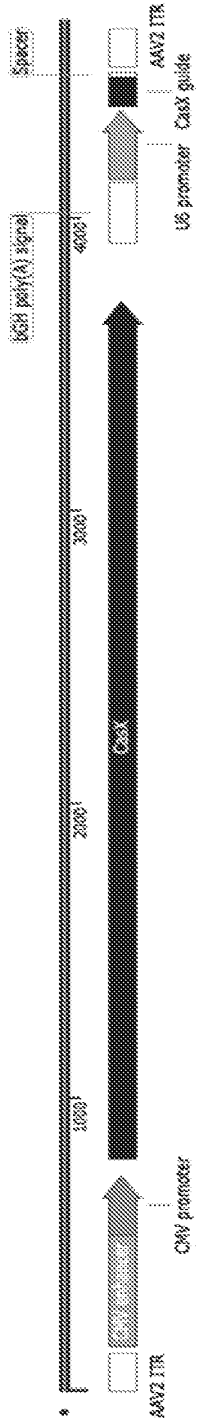

AAV2 ITR
CNV promoter
Cas
U6 promoter
Cas9 guide
AAV2 ITR
bGH poly(A) signal
Spacer
1000
2000
3000
4000

FIG. 13

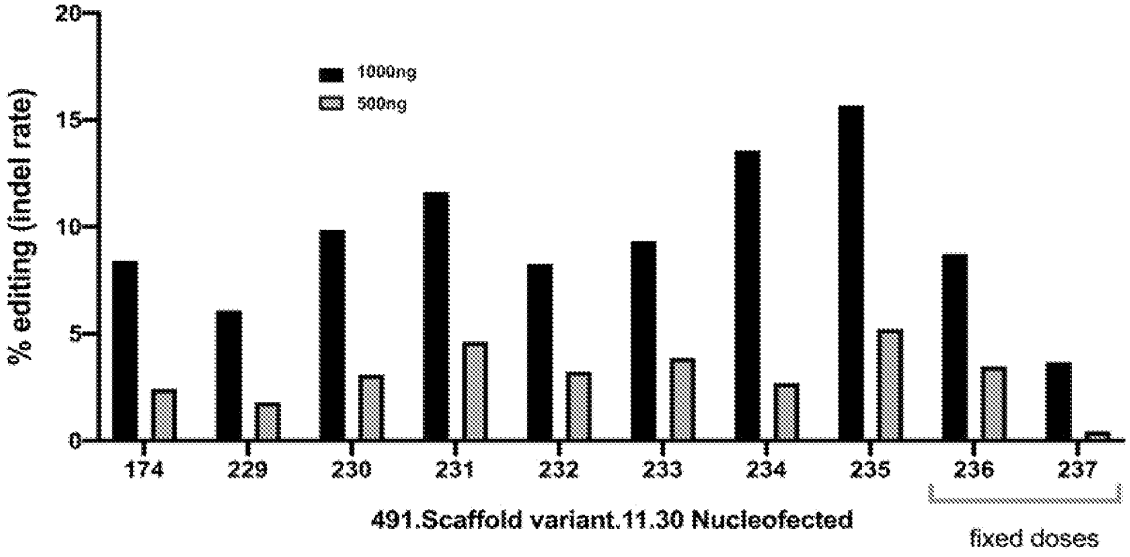

20
15
10
5
0
% editing (indel rate)
1000ng
500ng
174
229
230
231
232
233
234
235
236
237
491.Scaffold variant.11.30 Nucleofected
fixed doses

FIG. 14

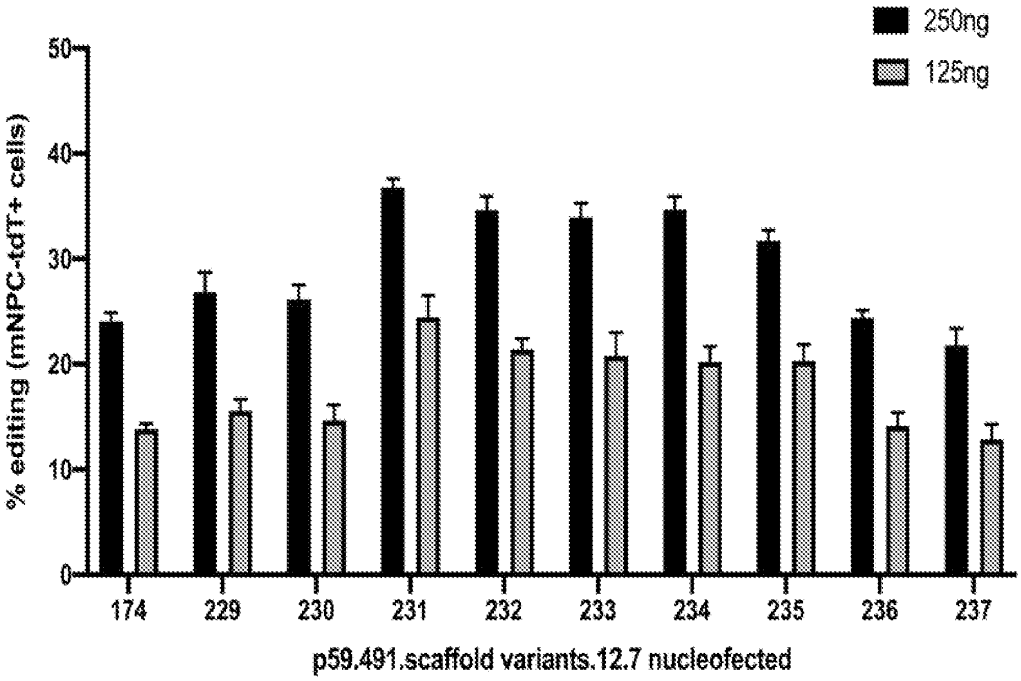

250ng
125ng
% editing (mNPC-tdT+ cells)
50
40
30
20
10
0
174
229
230
231
232
233
234
235
236
237
p59.491.scaffold variants.12.7 nucleofected

FIG. 15

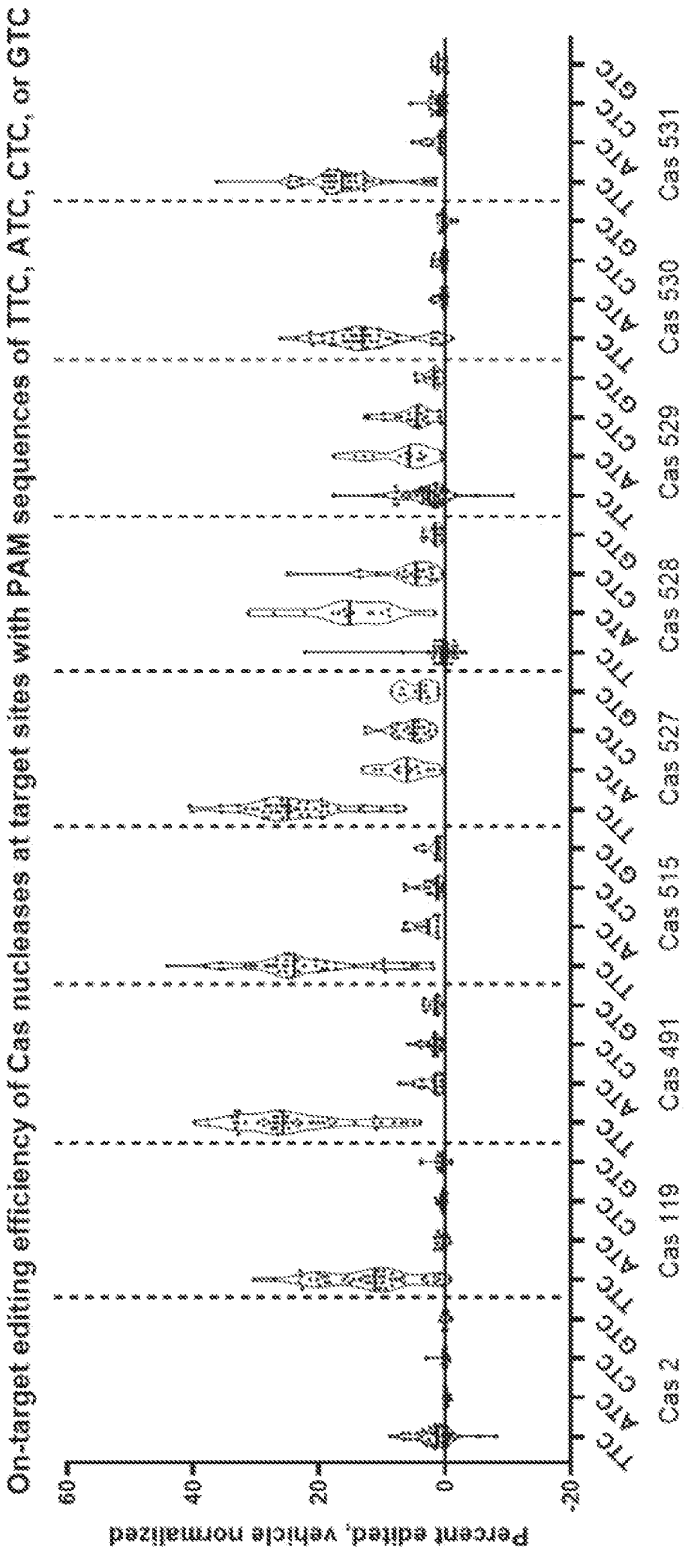

On-target editing efficiency of Cas nucleases at target sites with PAM sequences of TTC, ATC, CTC, or GTC
Percent edited, vehicle normalized
60
40
20
0
-20
Cas 2
Cas 119
Cas 491
Cas 515
Cas 527
Cas 528
Cas 529
Cas 530
Cas 531
Cas Protein

FIG. 16

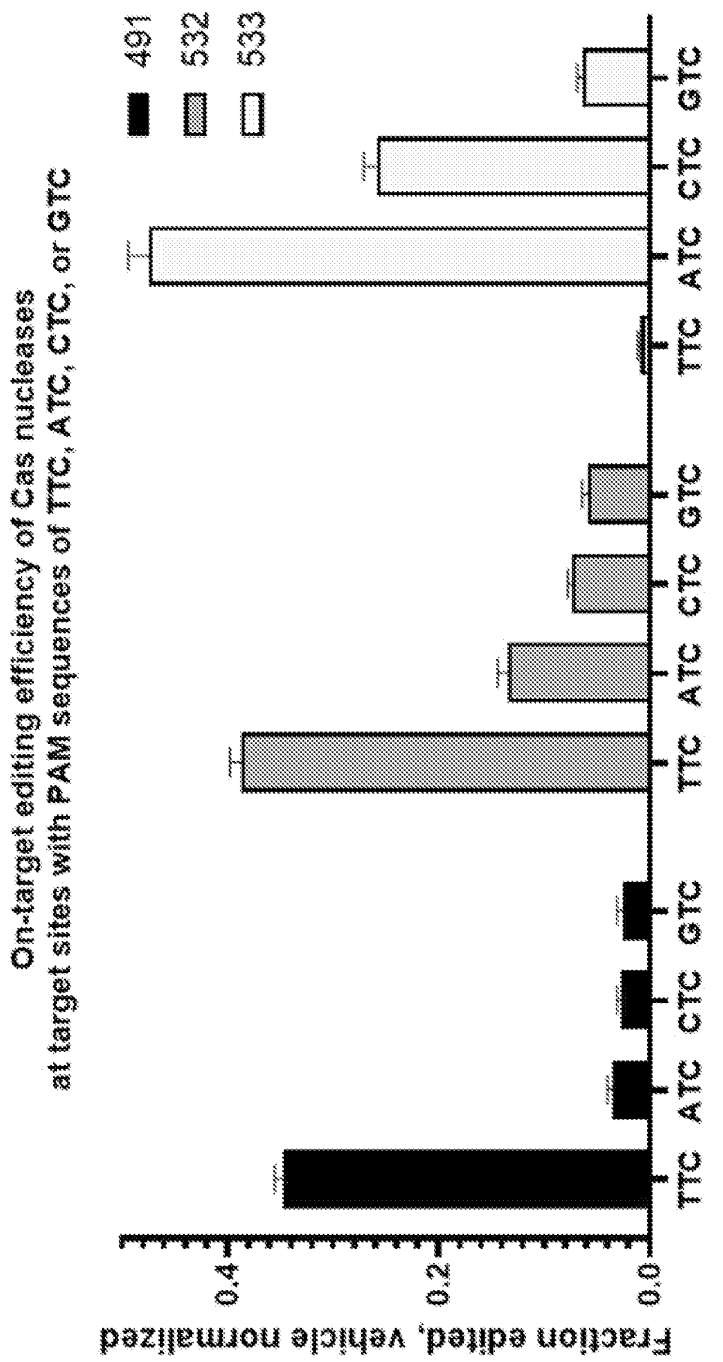

On-target editing efficiency of Cas nucleases
at target sites with PAM sequences of TTC, ATC, CTC, or GTC
491
532
533
Fraction edited, vehicle normalized
0.0
0.2
0.4
TTC
ATC
CTC
GTC
Cas 491
Cas 532
Cas 533
Cas Protein

FIG. 17

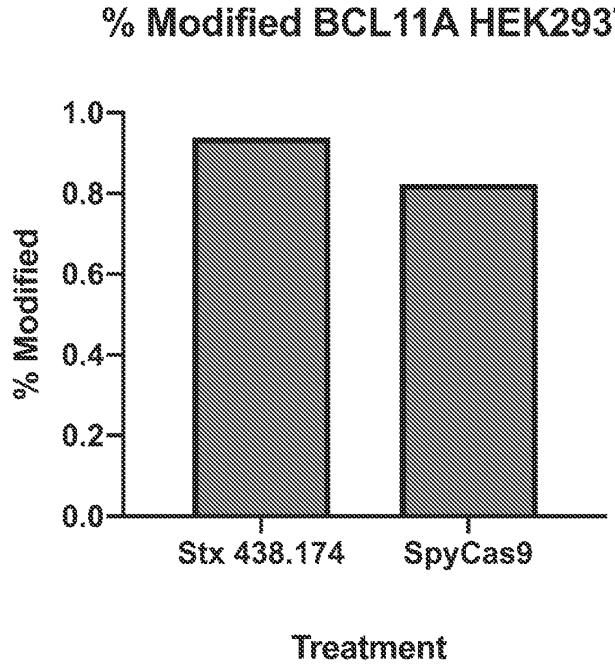

% Modified BCL11A HEK293T
1.0
0.8
0.6
0.4
0.2
0.0
% Modified
Stx 438.174
SpyCas9
Treatment

FIG. 18

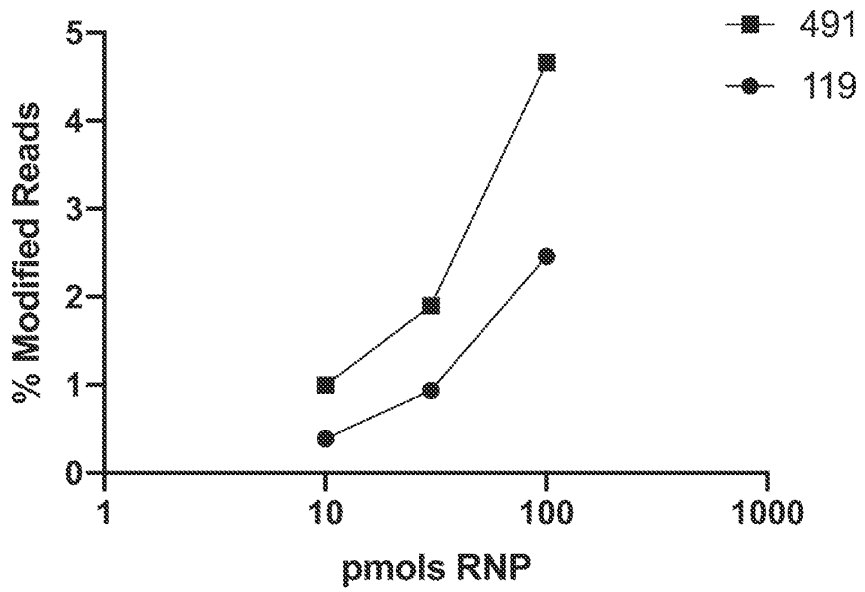


FIG. 19

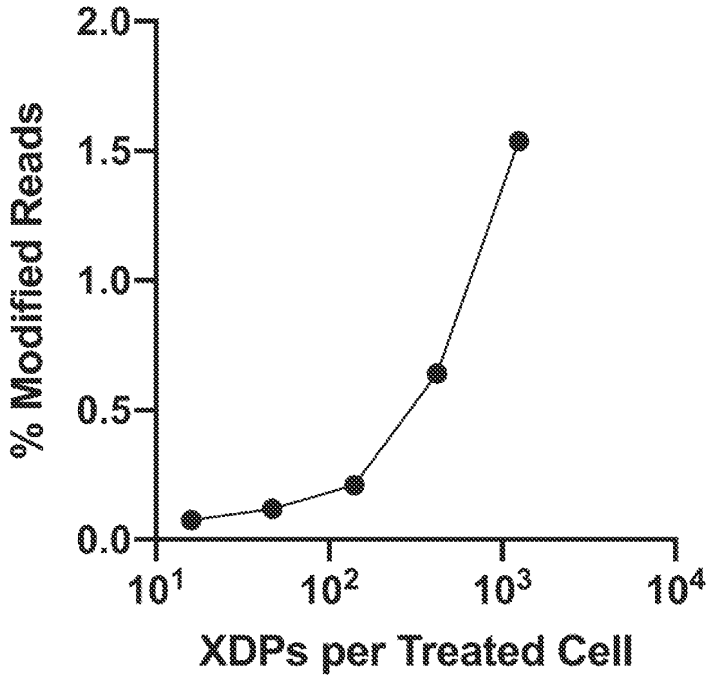

2.0
1.5
1.0
0.5
0.0
% Modified Reads
10^1
10^2
10^3
10^4
XDPs per Treated Cell

FIG. 20

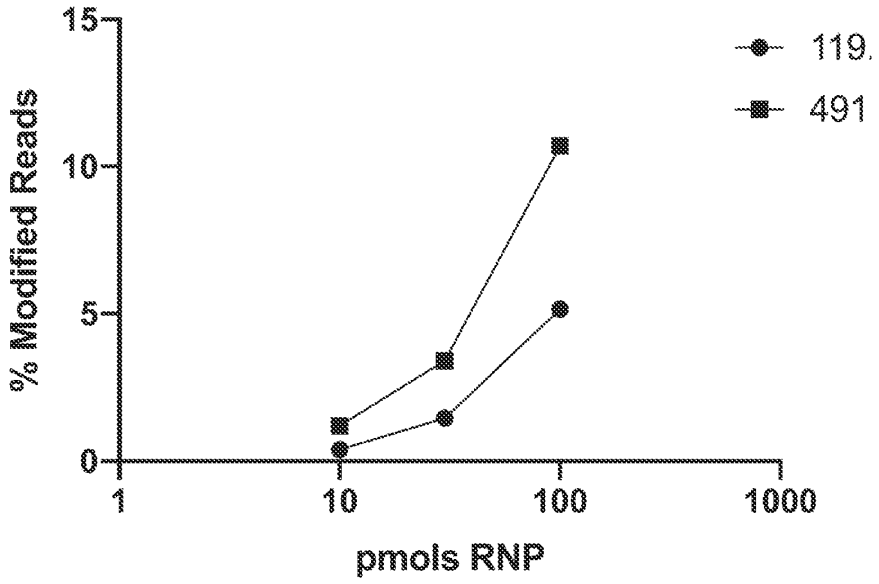


FIG. 21

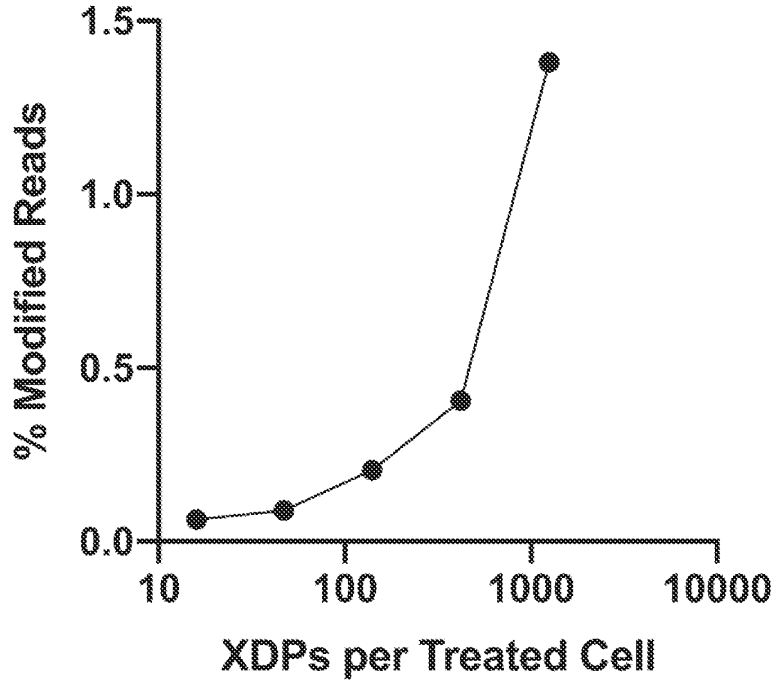

1.5
1.0
0.5
0.0
% Modified Reads
10
100
1000
10000
XDPs per Treated Cell

FIG. 22

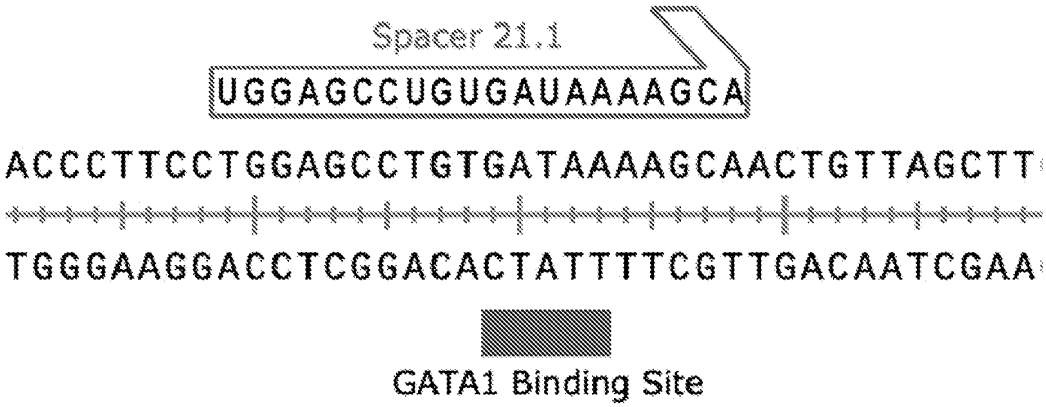

Spacer 21.1
UGGAGCCUGUGAUAAAAGCA
ACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTT
TGGGAAGGACCTCGGACACTATTTTCGTTGACAATCGAA
GATA1 Binding Site

FIG. 23