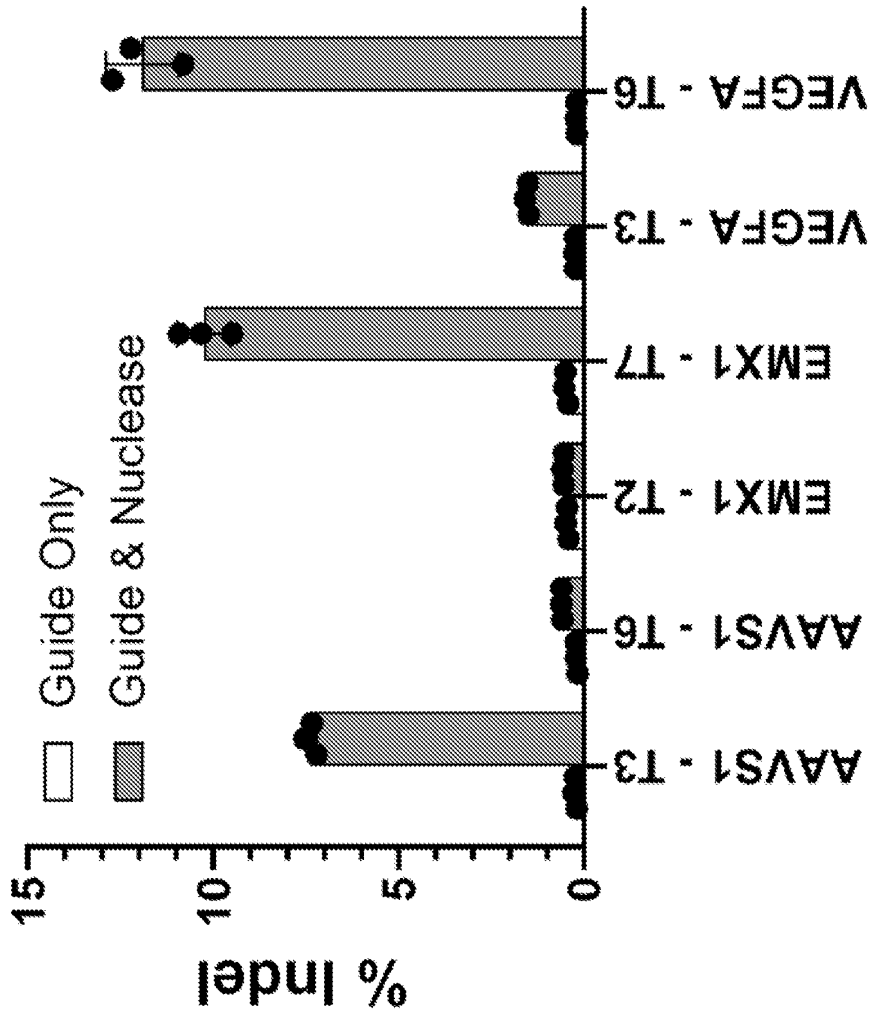
FIG. 1


% Indel
15
10
5
0
Guide Only
Guide & Nuclease
AAVS1 - T3
AAVS1 - T6
EMX1 - T2
EMX1 - T7
VEGFA - T3
VEGFA - T6

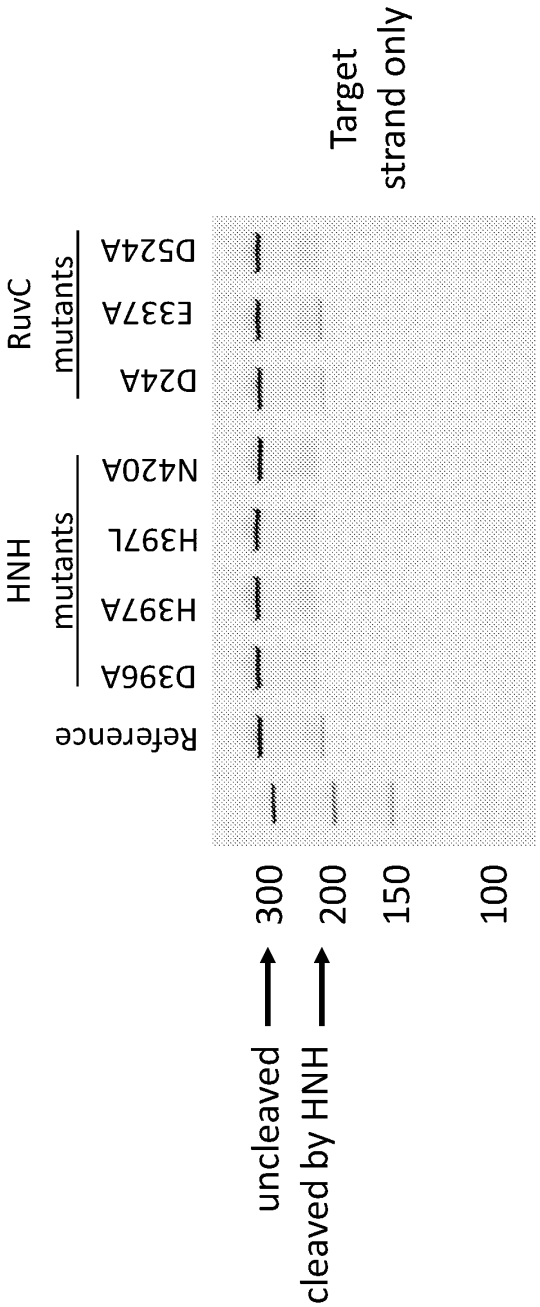

Target strand only
RuvC mutants
D524A
E337A
D24A
HNH mutants
N420A
H397L
H397A
D396A
Reference
300
200
150
100
uncleaved
cleaved by HNH

FIG. 2A

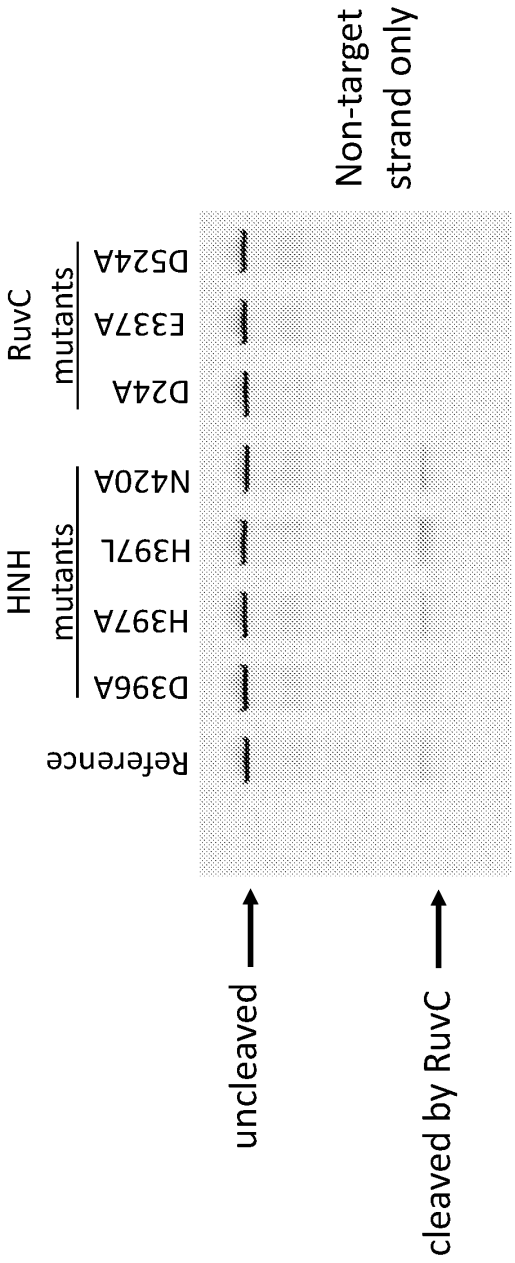

Non-target strand only
RuvC mutants
D524A
E337A
D24A
HNH mutants
N420A
H397L
H397A
D396A
Reference
uncleaved
cleaved by RuvC

FIG. 2B

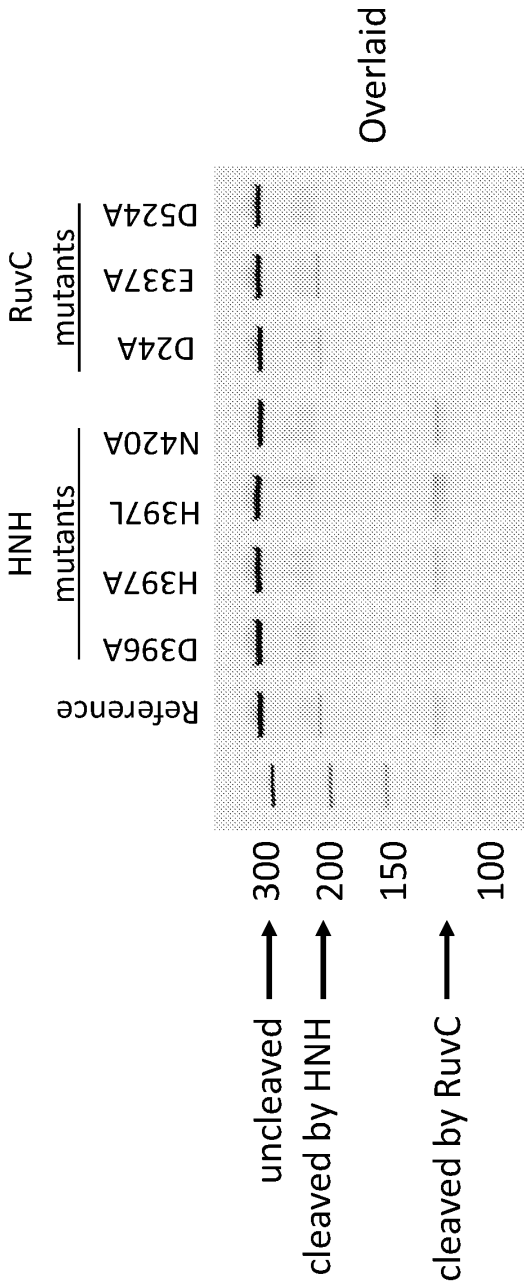

Overlaid
RuvC mutants
HNH mutants
Reference
D396A
H397A
H397L
N420A
D24A
E337A
D524A
300
200
150
100
uncleaved
cleaved by HNH
cleaved by RuvC

FIG. 2C

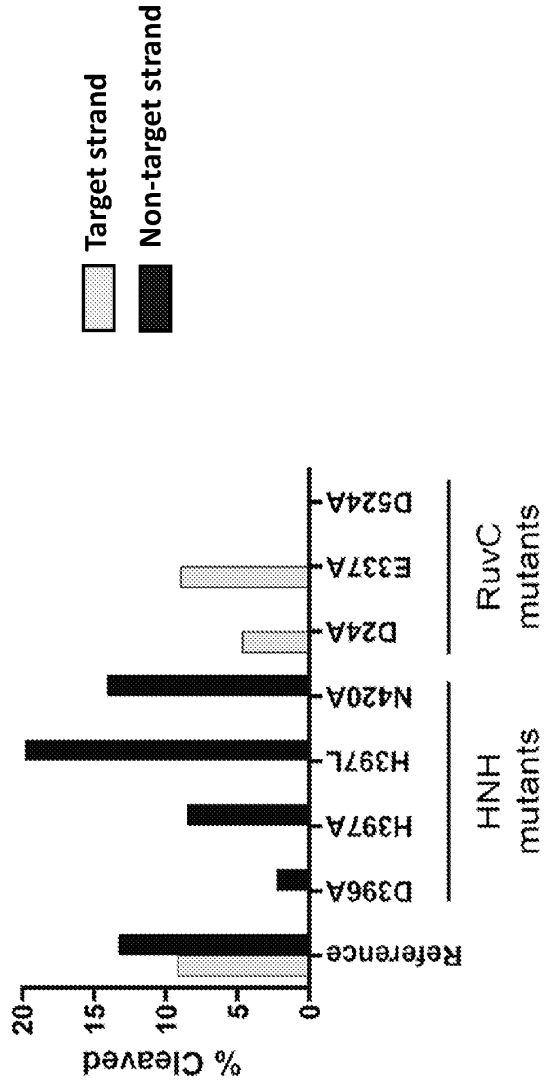

Target strand
Non-target strand
% Cleaved
0
5
10
15
20
Reference
D396A
H397A
H397L
N420A
D24A
E337A
D524A
HNH mutants
RuvC mutants

FIG. 2D

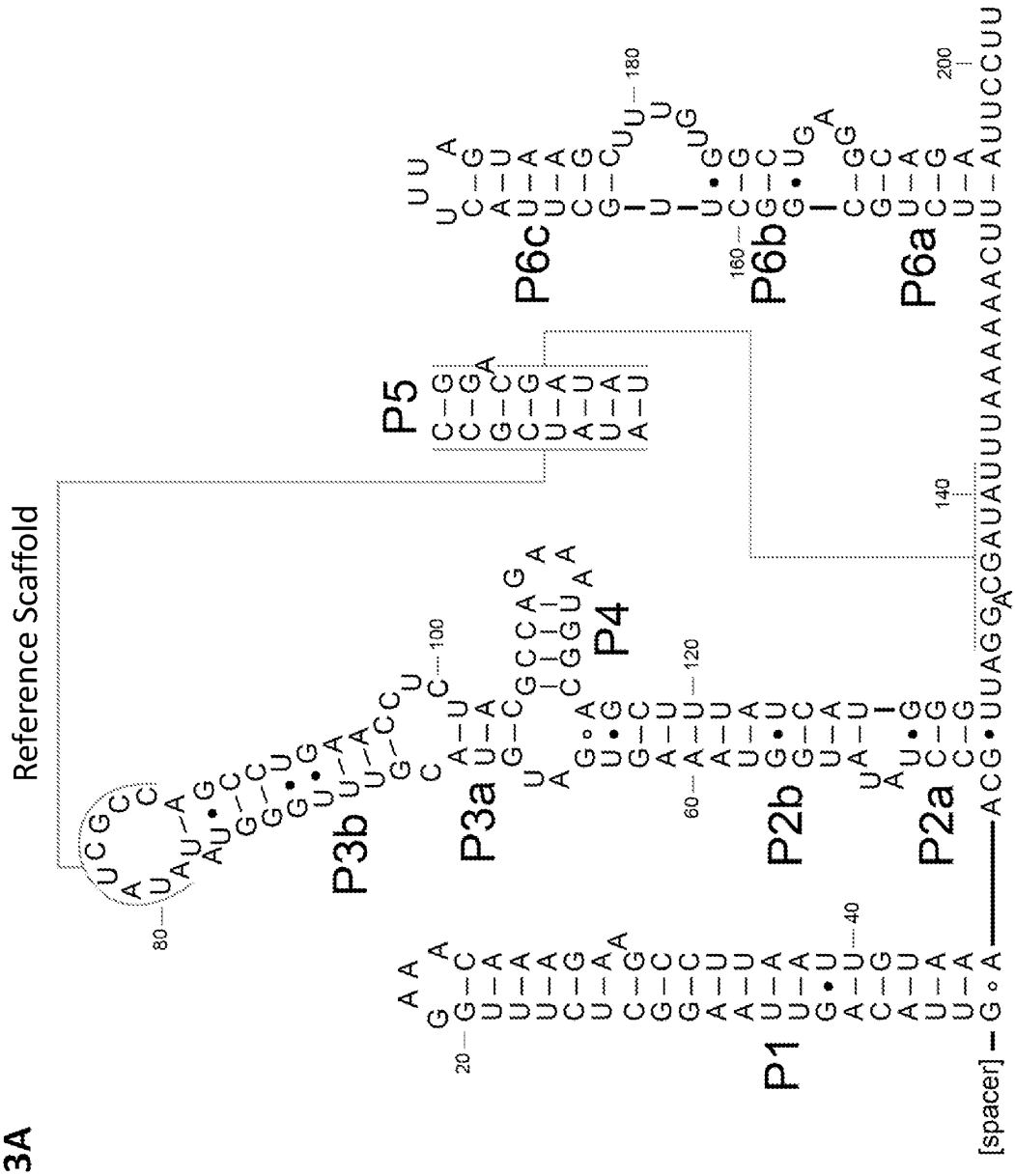

Reference Scaffold
P1
P2a
P2b
P3a
P3b
P4
P5
P6a
P6b
P6c
[spacer]
20
40
60
80
100
120
140
160
180
200

FIG. 3A

FIG. 3C

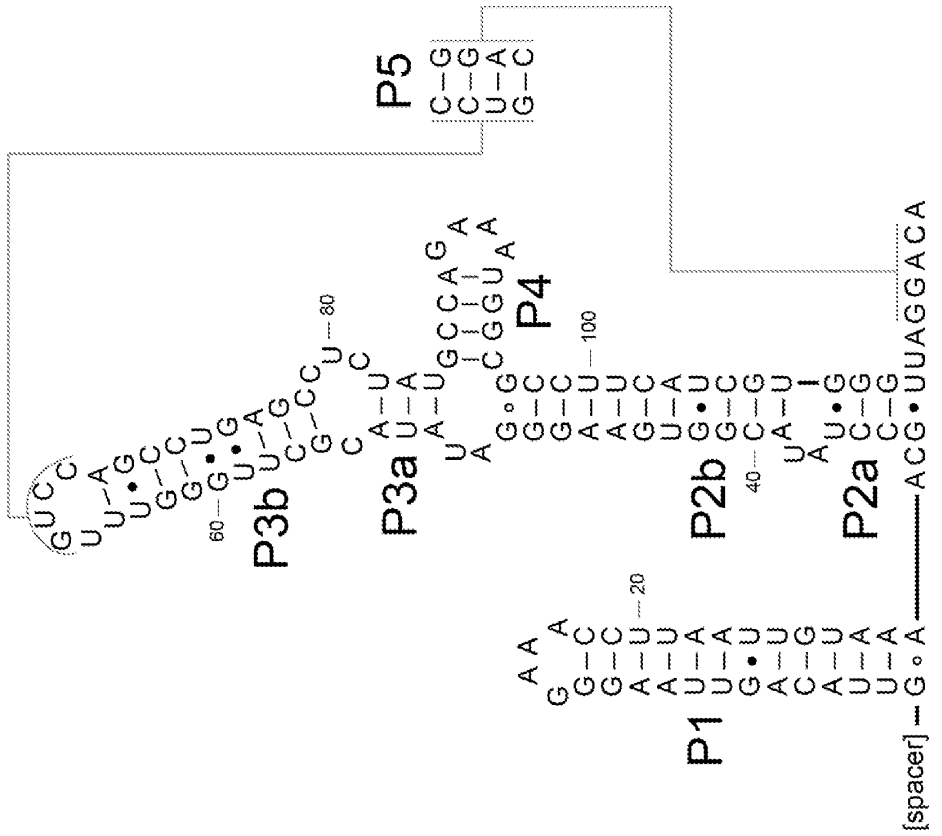
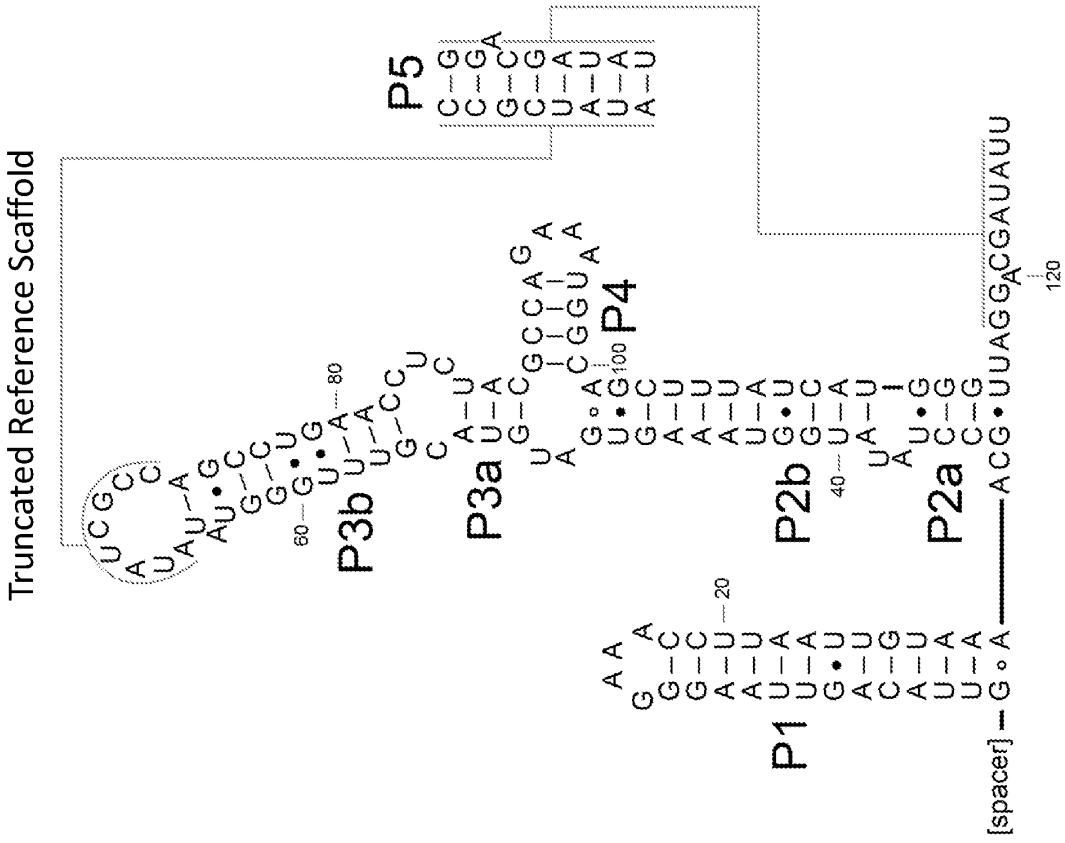

Scaffold 2
P5
P3b
P3a
P4
P2b
P2a
P1
[spacer]

FIG. 3B


Truncated Reference Scaffold
P5
P3b
P3a
P4
P2b
P2a
P1
[spacer]