

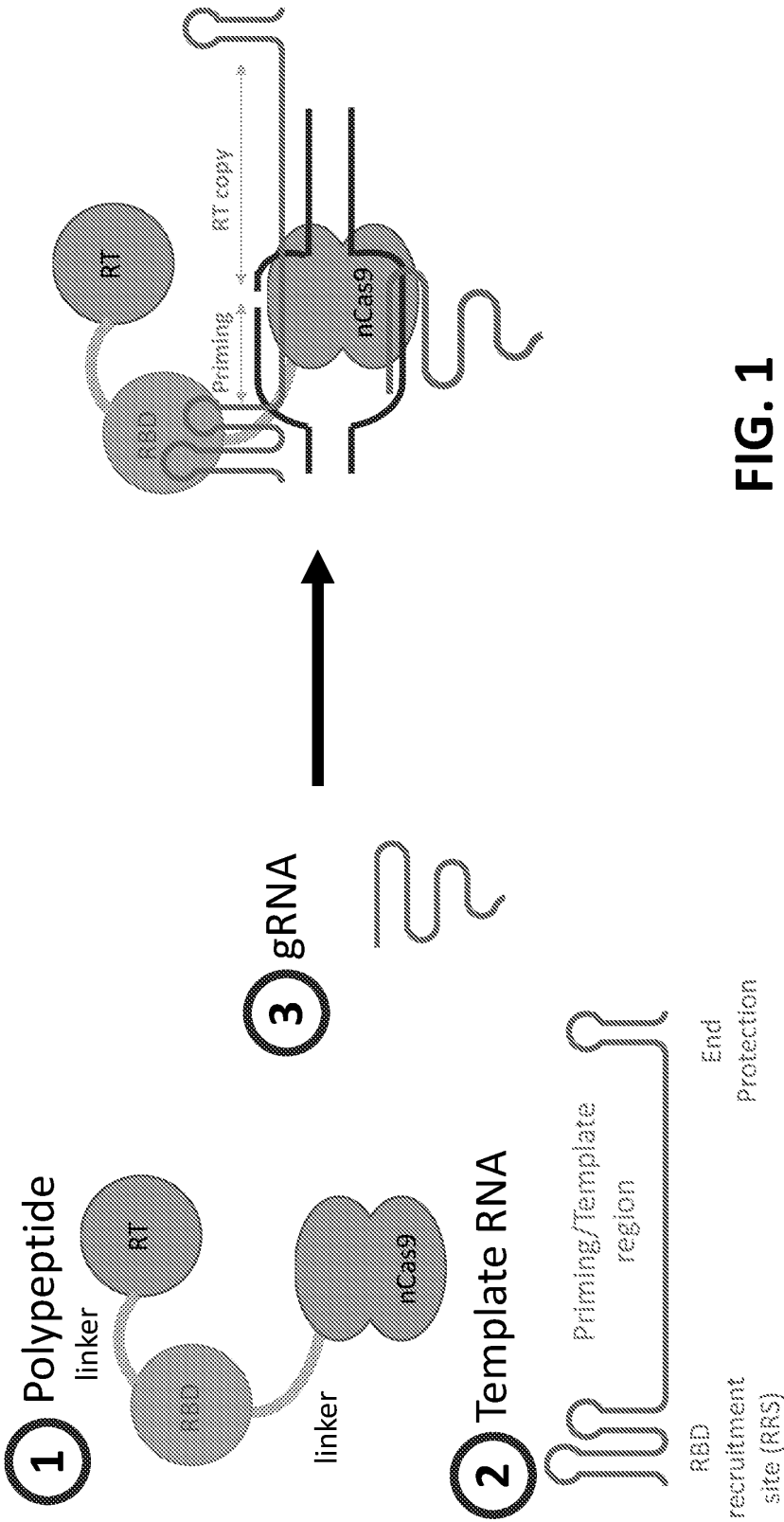
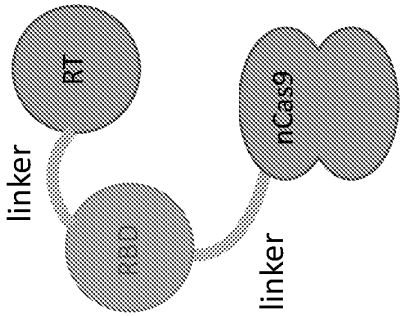


FIG. 1

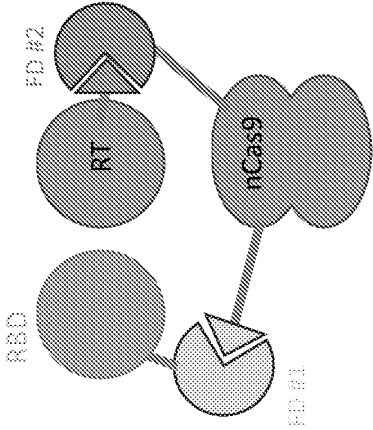
FIG. 2A



Possible Configurations

- Cas9 – {linker} – RT – {linker} – {RBD}n
- RT – {linker} – Cas9 – {linker} – {RBD}n
- {RBD}n – {linker} – Cas9 {linker} – RT
- {RBD}n – {linker} – RT {linker} – Cas9
- Cas9 – {linker} – {RBD}n – {linker} – RT
- RT – {linker} – {RBD}n – {linker} – Cas9

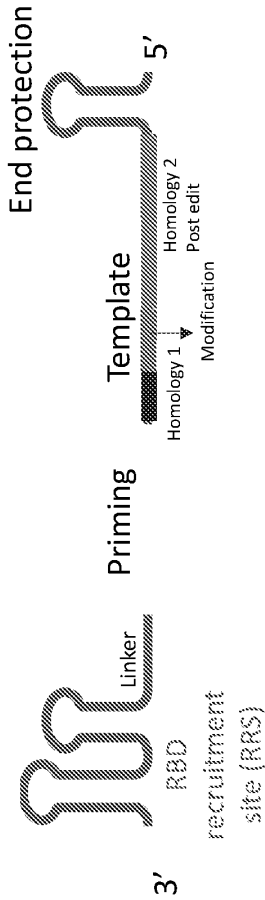
FIG. 2B



Possible Configurations

- Dimerization domains can be present multiple times as tandem repeats (n=1-30) for each domain

FIG. 3A



Possible Configurations

$\{RRS\}n - \{linker\} - \{RRS\}n$
 $\{RRS\}n - \{linker\} - \{RRS\}n - \{linker\} - \{RRS\}n$
 $\{linker\} - \{RRS\}n - \{linker\} - \{RRS\}n$
 $\{linker\} - \{RRS\}n - \{linker\} - \{RRS\}n$

FIG. 3B

- RBD recruitment site (RRS)
 - Of different origins (see seq table)
 - can be present n times: 1-10
- Linker: 0-20 nts -> see sequence table
- Priming: 8-17 nts -> see sequence table
- Template:
 - Homology region pre edit 1: 0-20 nts
 - Homology region post edit: 5-500 nts
 - Modification: Long and short insertions, deletions, point mutations
- End block (see sequence table)

FIG. 3C

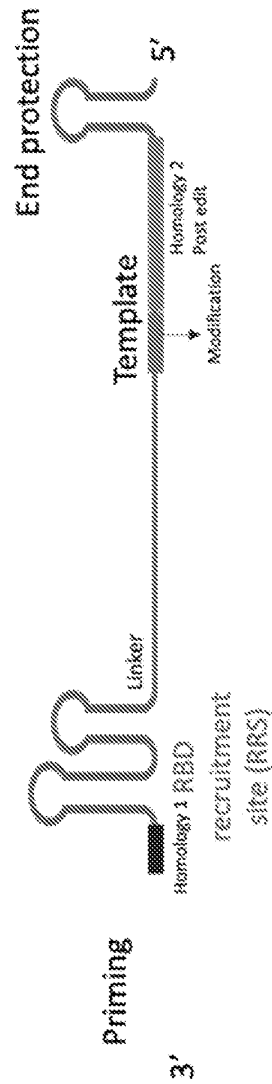


FIG. 4B

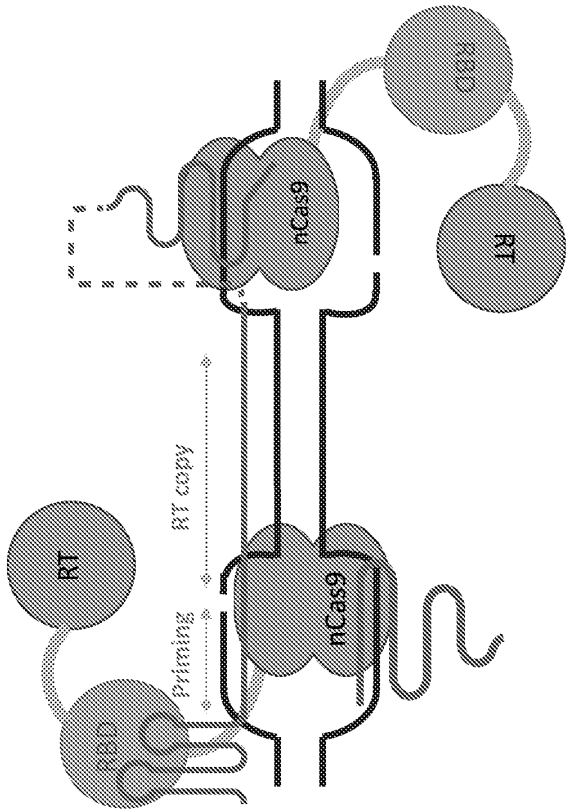
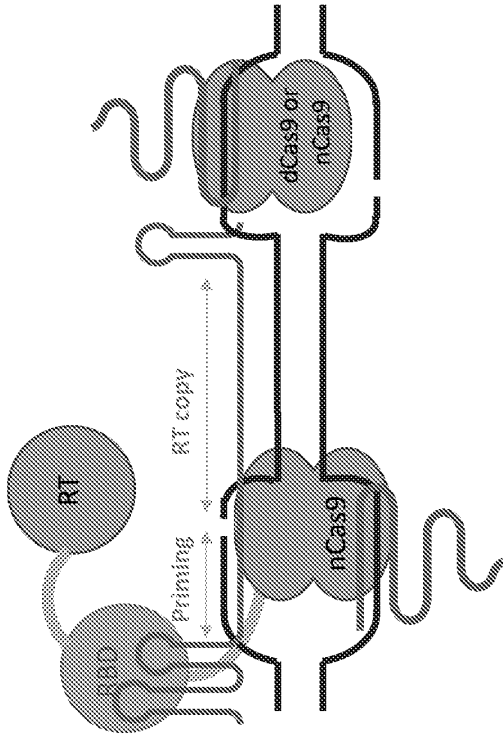


FIG. 4A



gRNA (scaffold/spacer) to direct
unwinding/second nicking to second strand

FIG. 5B

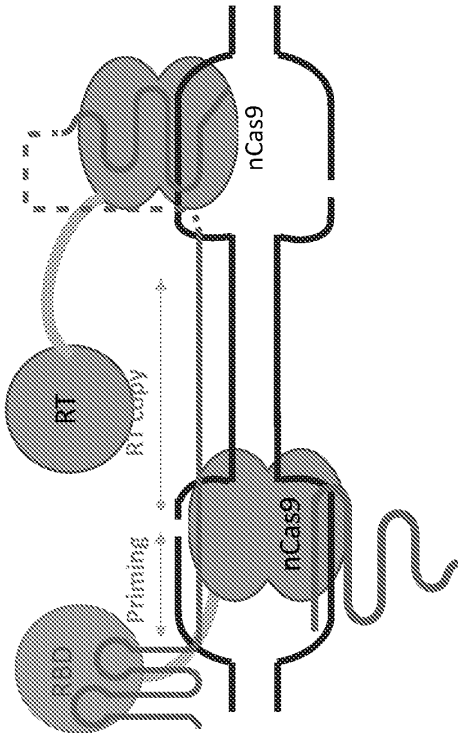
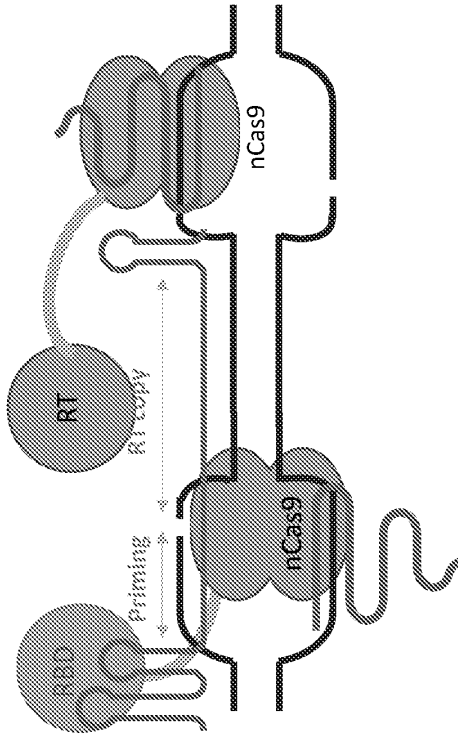
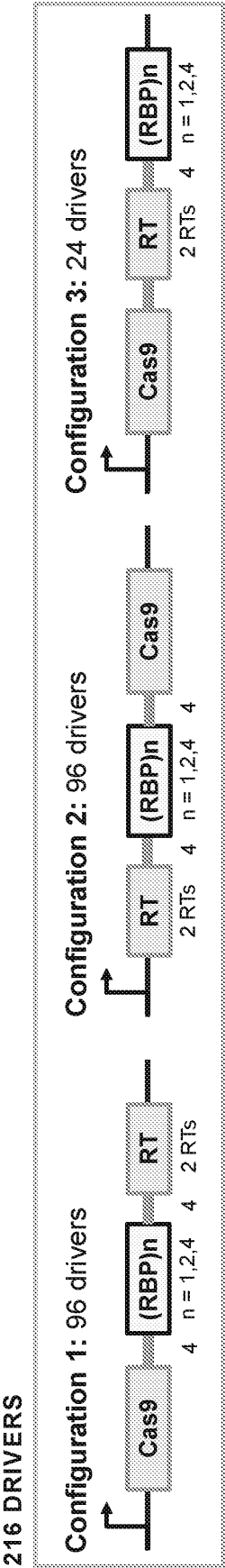


FIG. 5A



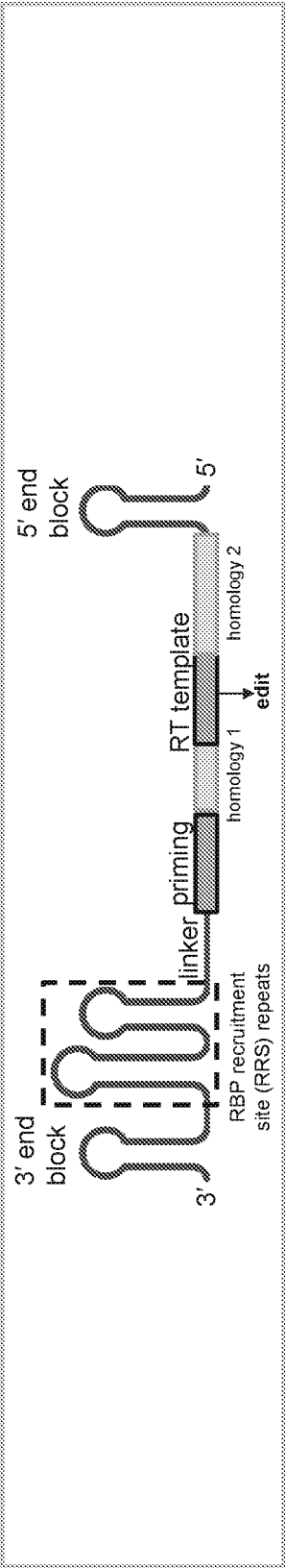


Driver variables:

- Configurations (3)
- RT domains (2)
- Number of RBP repeats (3)
- Linker combinations (16)
(4 linker options for config3)

FIG. 6A

384 TEMPLATES (288 ACTIVE CONFIGURATIONS + 96 NEGATIVE CONTROLS)



Template variables:

- 3' end blocks (2)
 - no end block
 - control ePEG end-block
- Number of RRS repeats (3)
 - 1x MS2
 - 2x MS2
 - 4x MS2
- Linker lengths (2)
 - 8 nt
 - 16 nt
- Priming (PBS) length (3+1)
 - 8 nt
 - 13 nt
 - 17 nt
 - (non-complementary PBS)
- Homology 2 length (2)
 - 12 nt
 - 63 nt with 4 SNPS at regular intervals
- 5' end blocks (4)
 - no end block
 - control ePEG end-block
 - Cas9 scaffold (no spacer)
 - Cas9 scaffold with 16 nt spacer

FIG. 6B

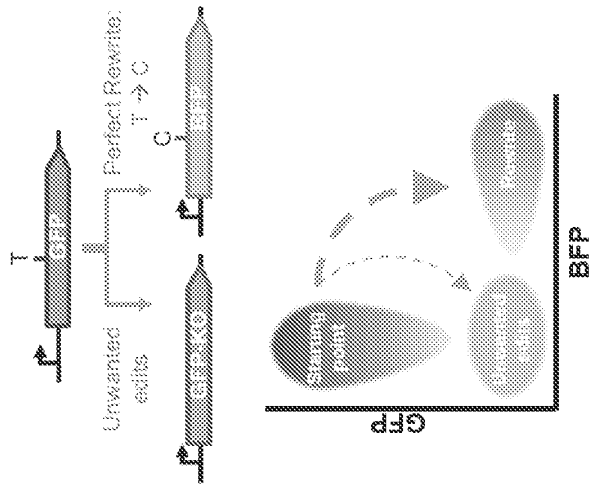


FIG. 7A

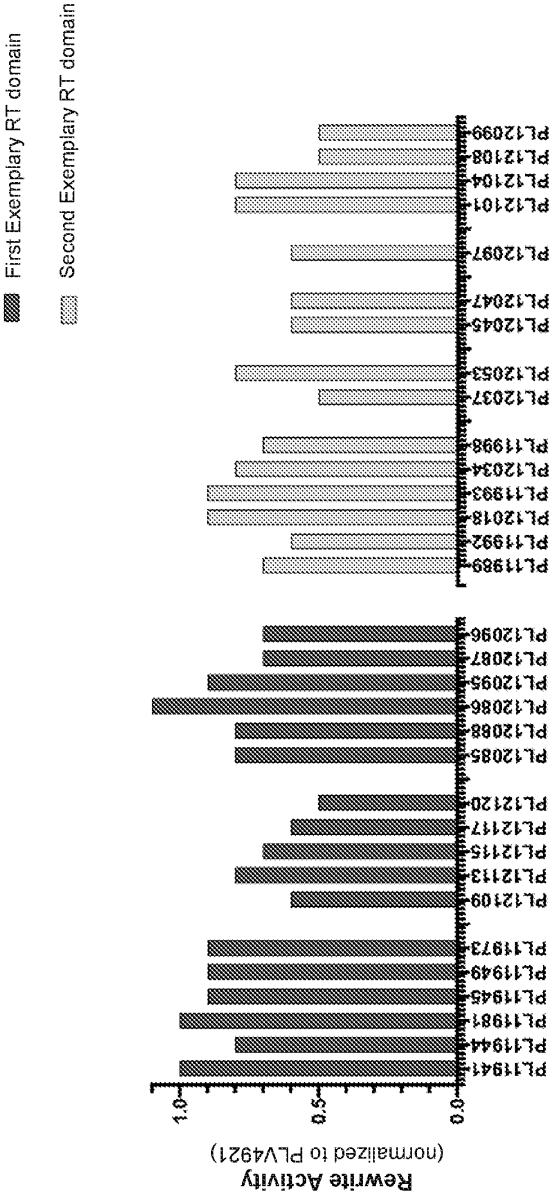
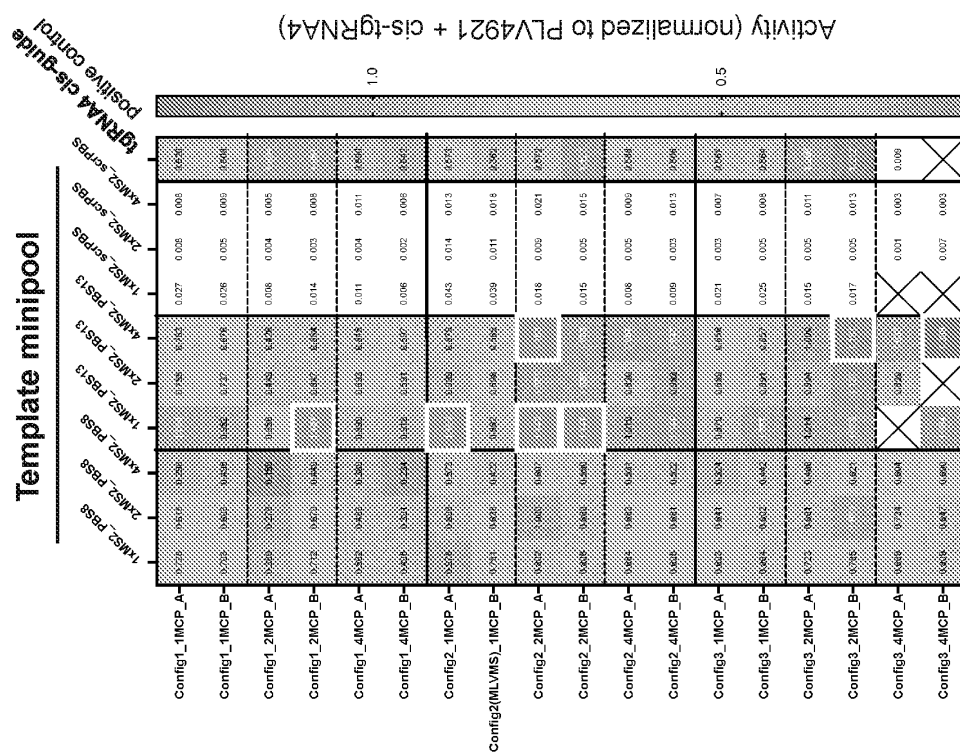


FIG. 7B



8
G²
L



9
G²
E

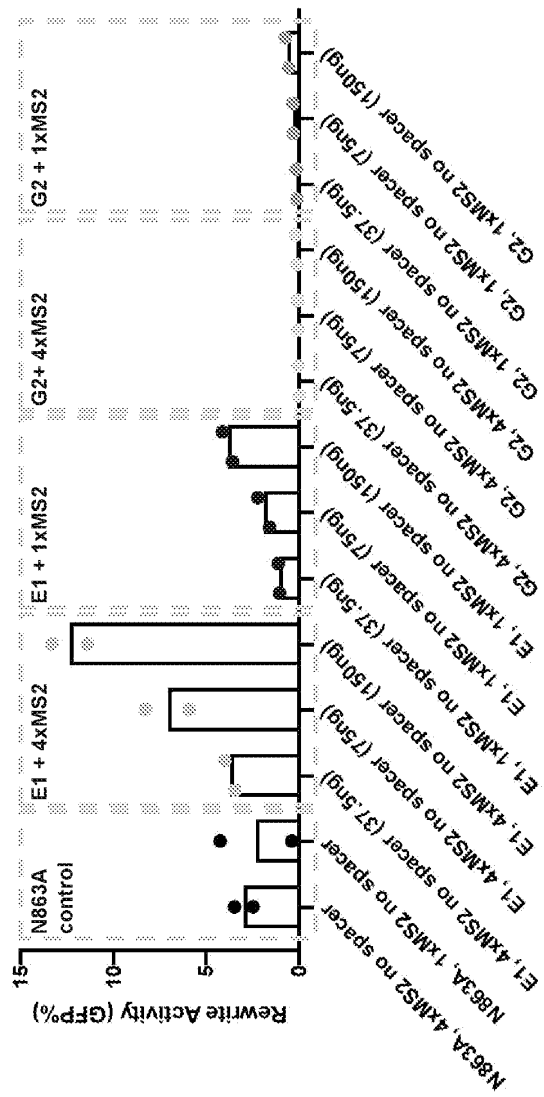


FIG. 10

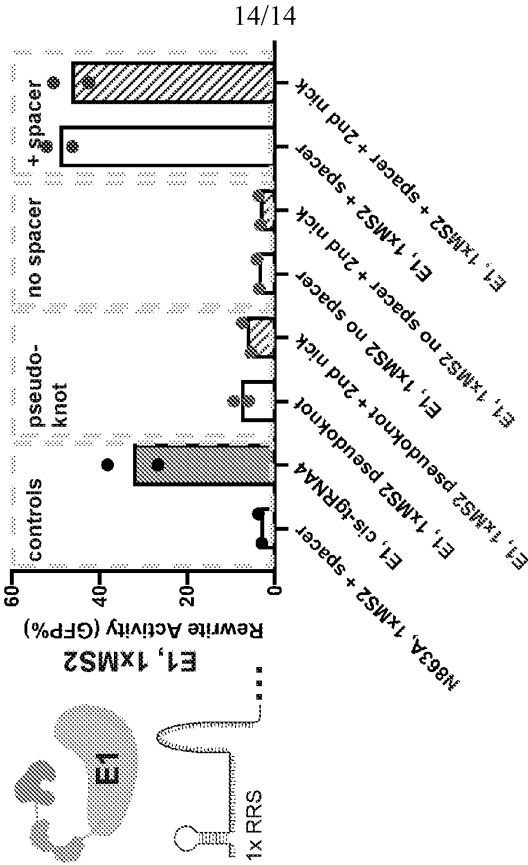


FIG. 11B

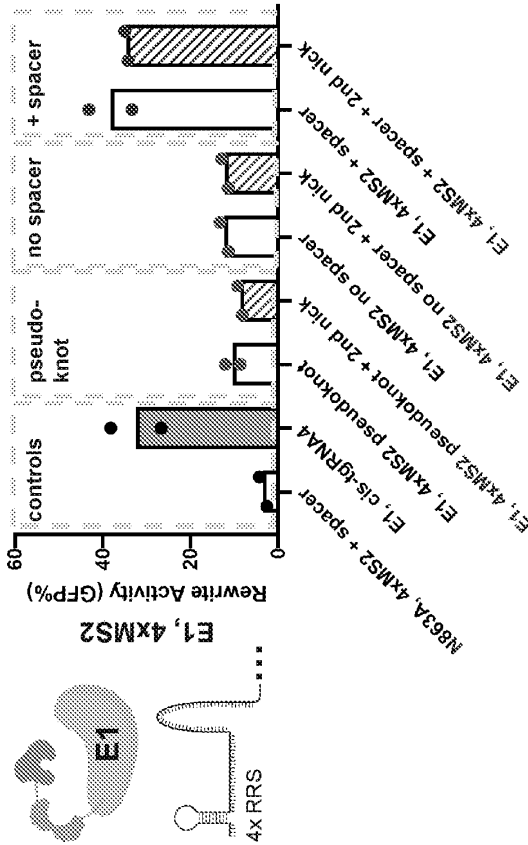


FIG. 11A