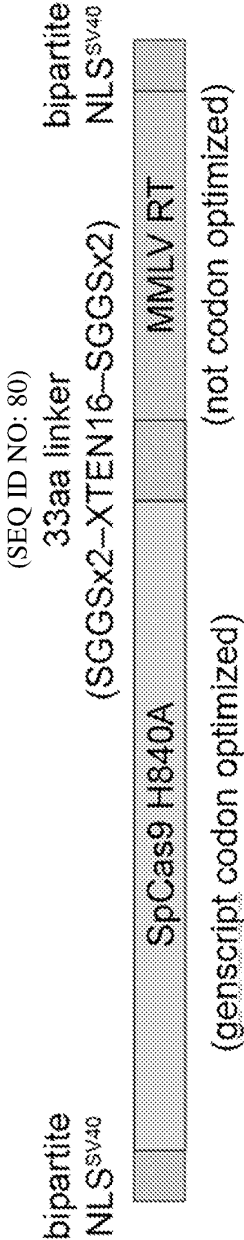


Optimization of PE2 protein



1. Codon optimize MMLV RT
2. Mutations to SpCas9 to improve activity
3. Linker optimization
4. Improve nuclear localization

FIG. 1

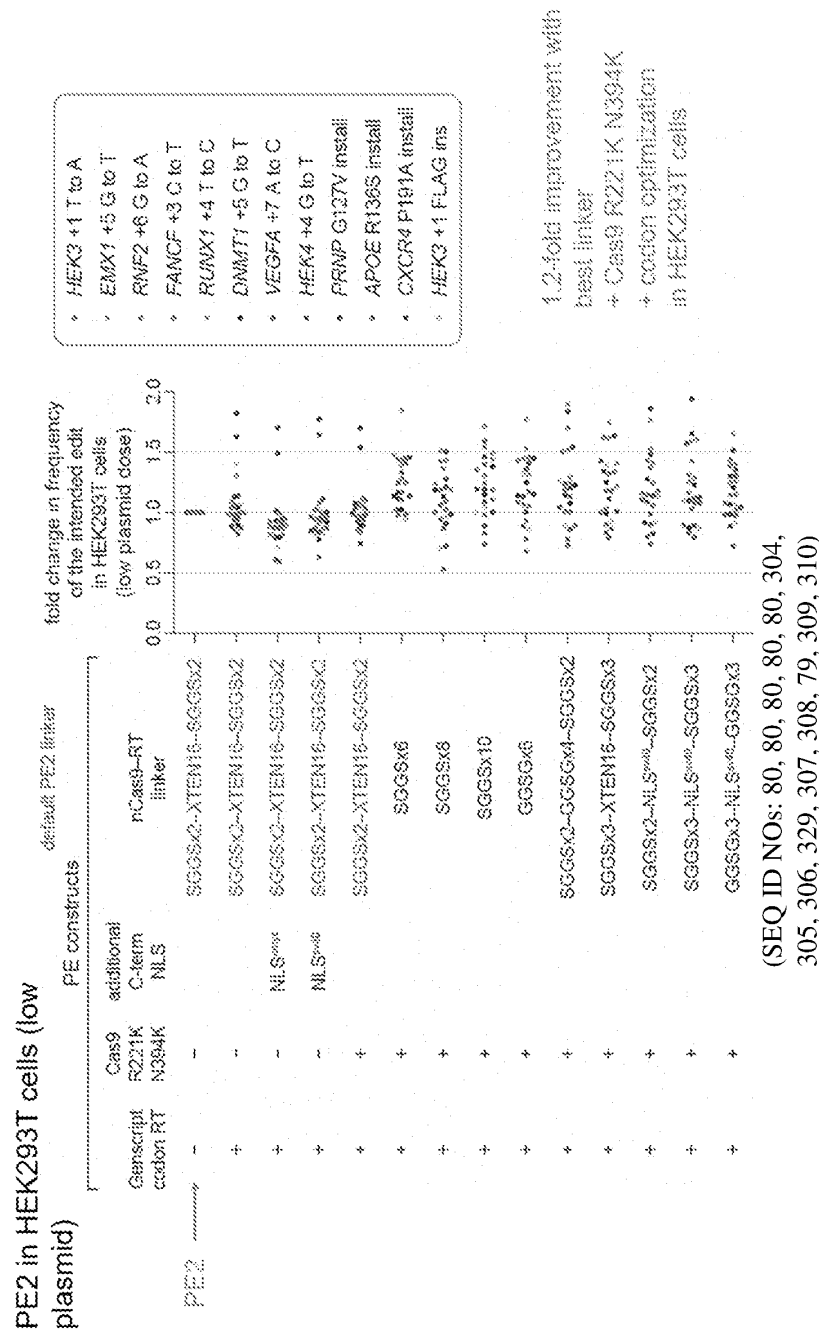


FIG. 2

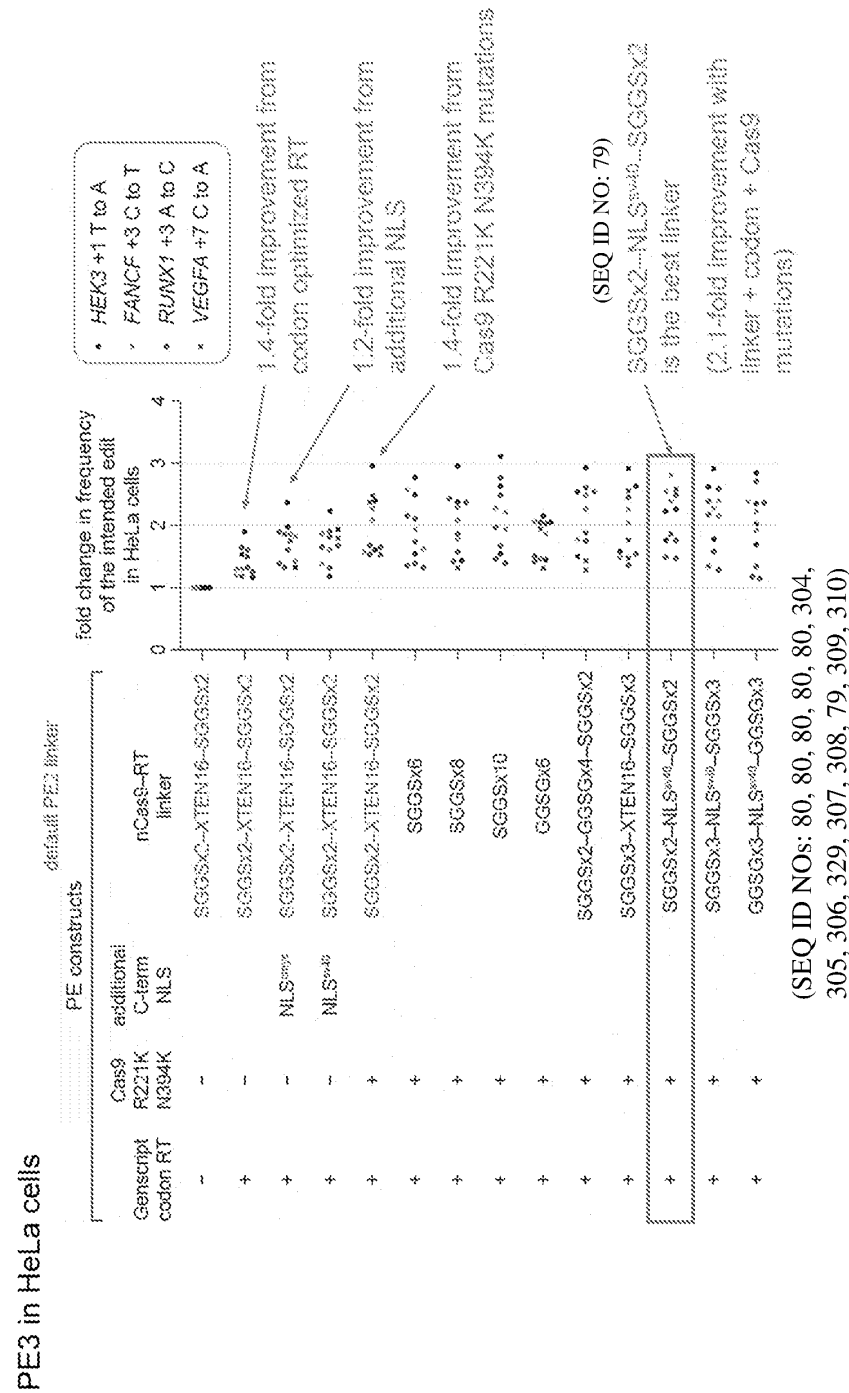


FIG. 3

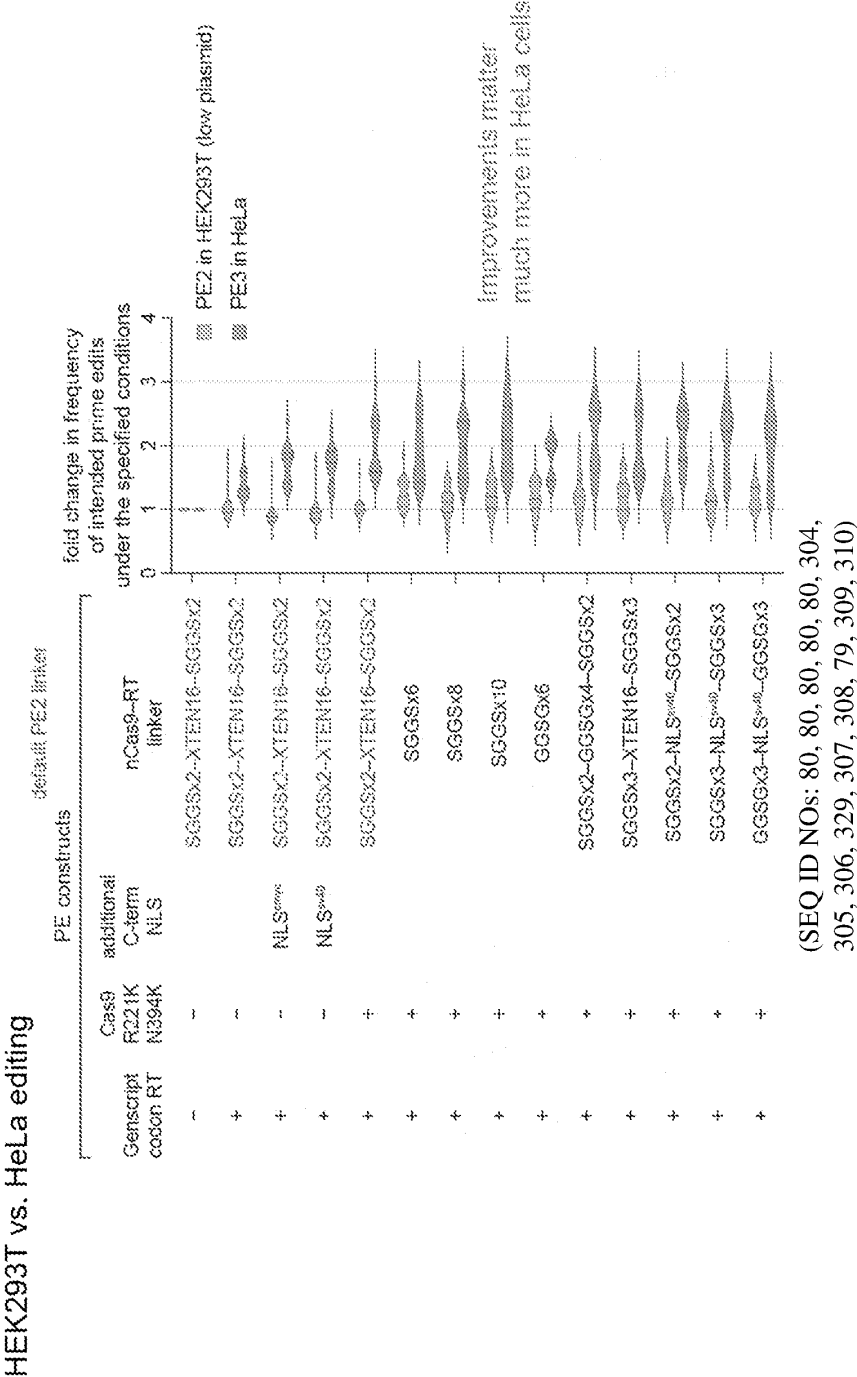


FIG. 4

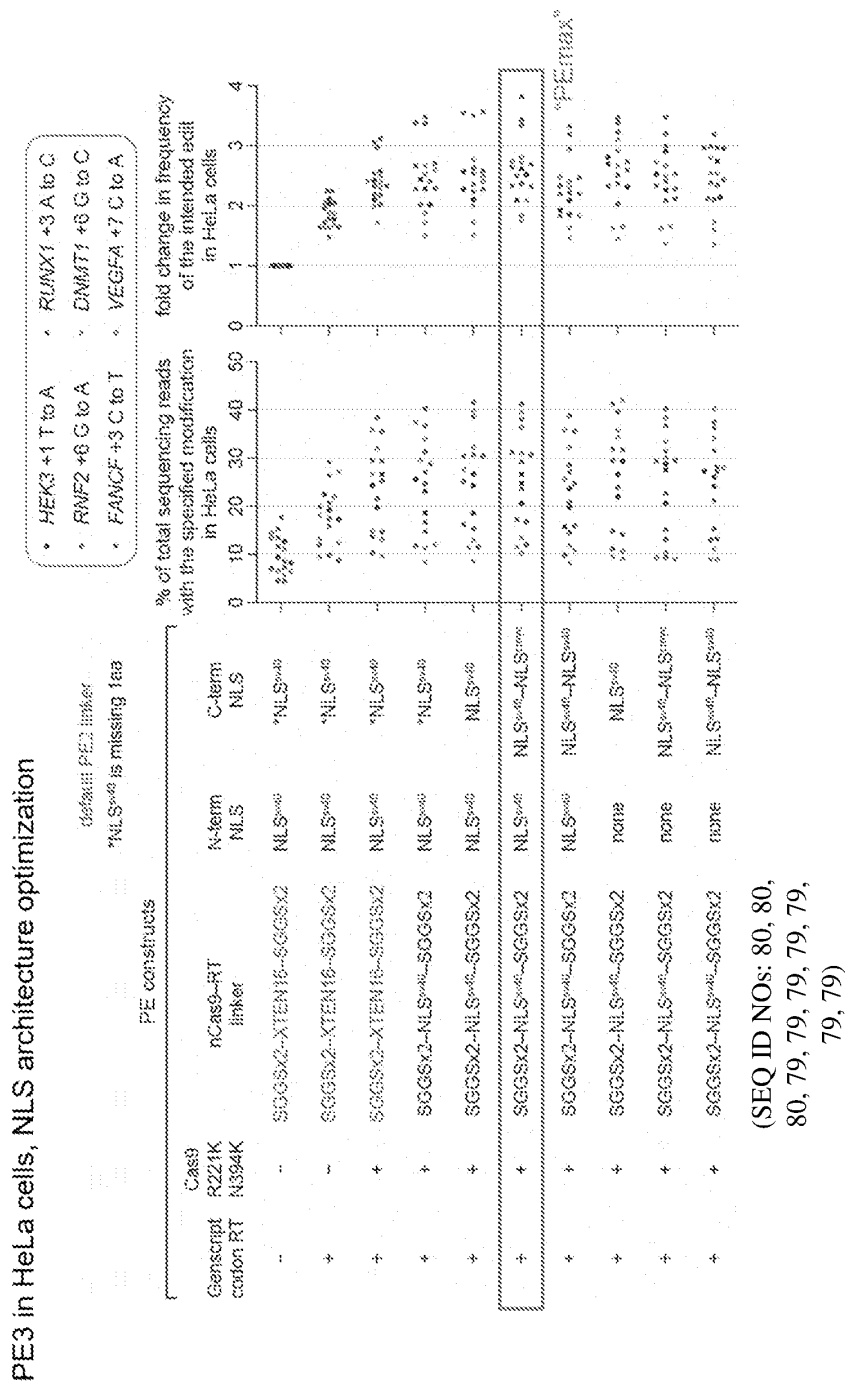
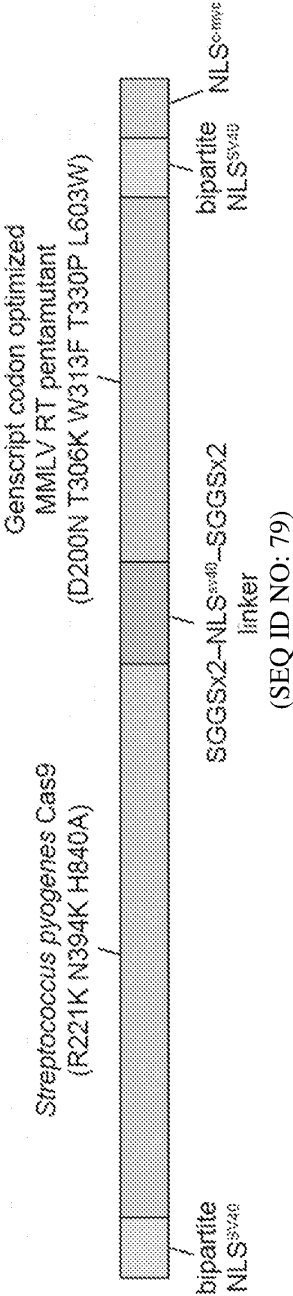


FIG. 5

Final PEmax construct



PEmax improves prime editing efficiency by
2.59-fold across 6 sites in HeLa cells

FIG. 6

PEmax increases indels in addition to the intended edit

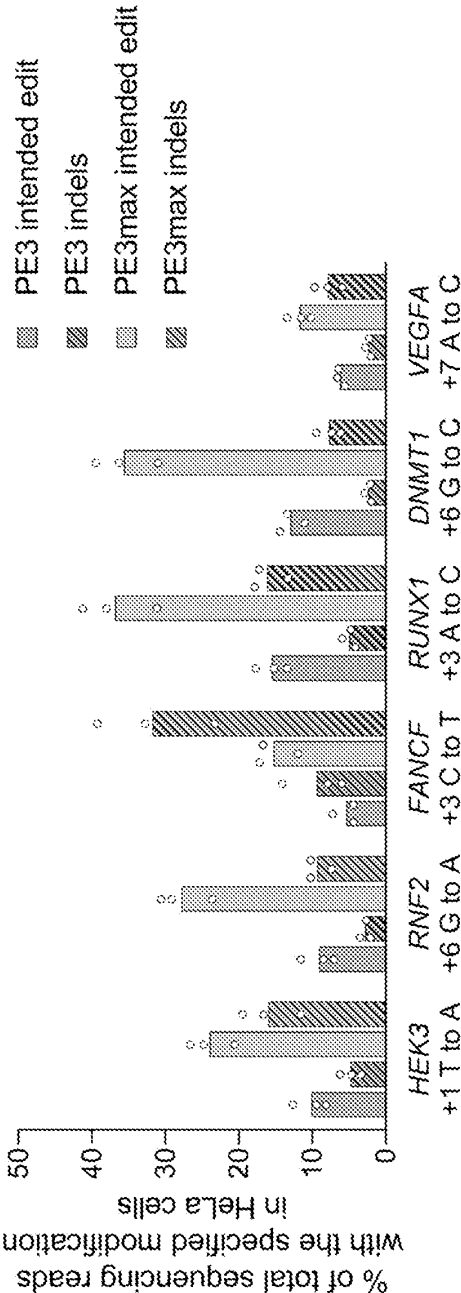


FIG. 7

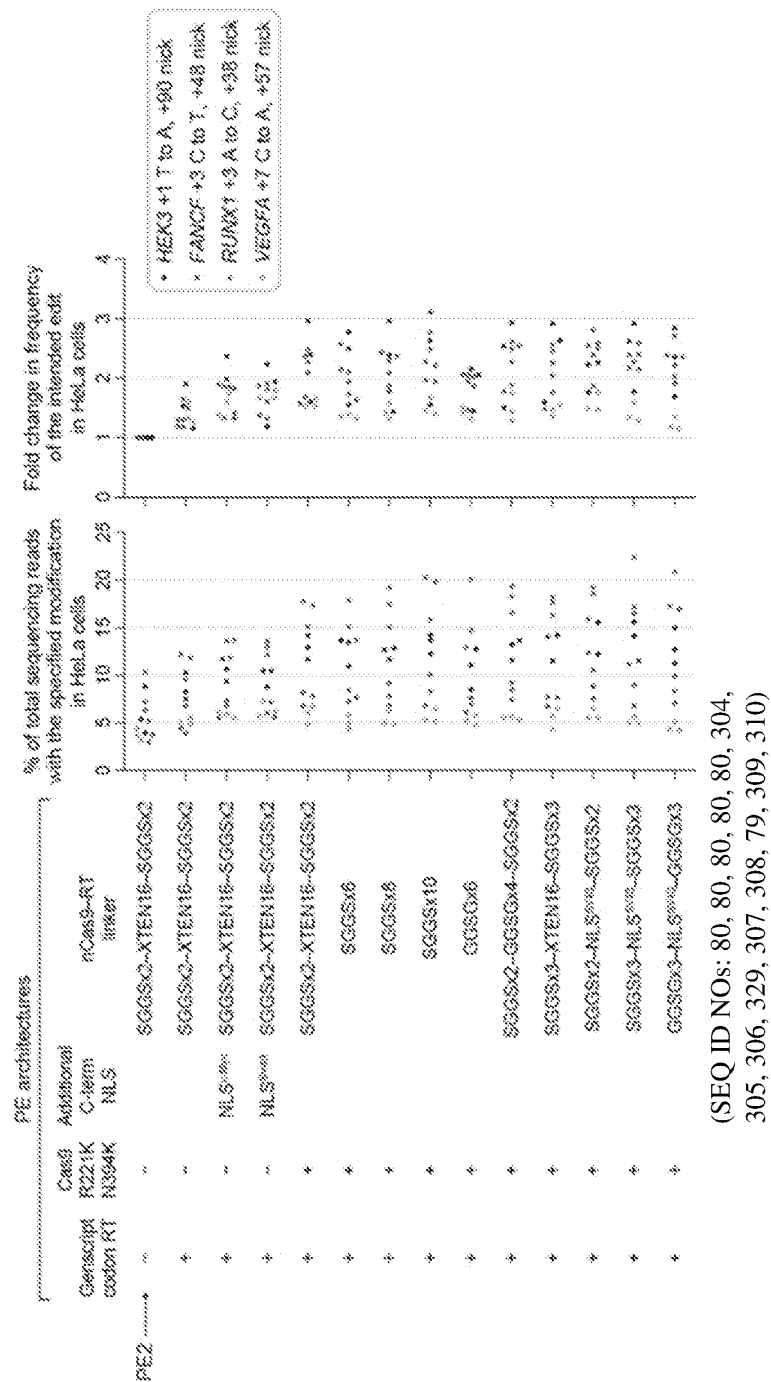
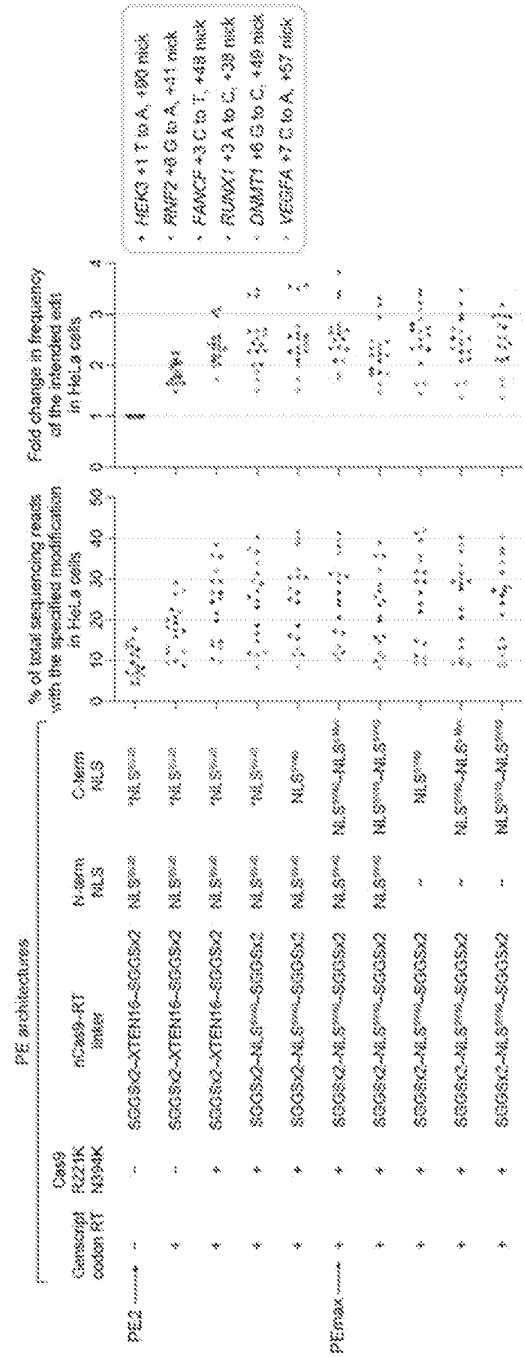


FIG. 8A



(SEQ ID NOS: 80, 80, 80,
79, 79, 79, 79, 79, 79, 79)

FIG. 8B

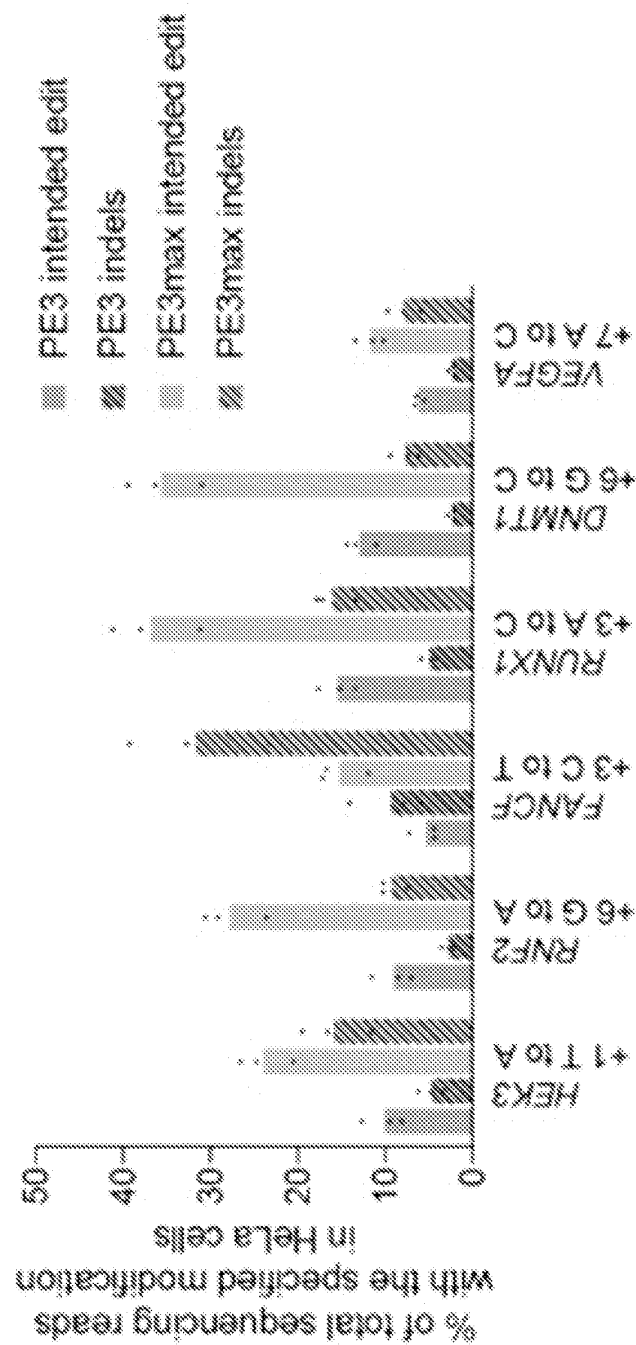


FIG. 8C

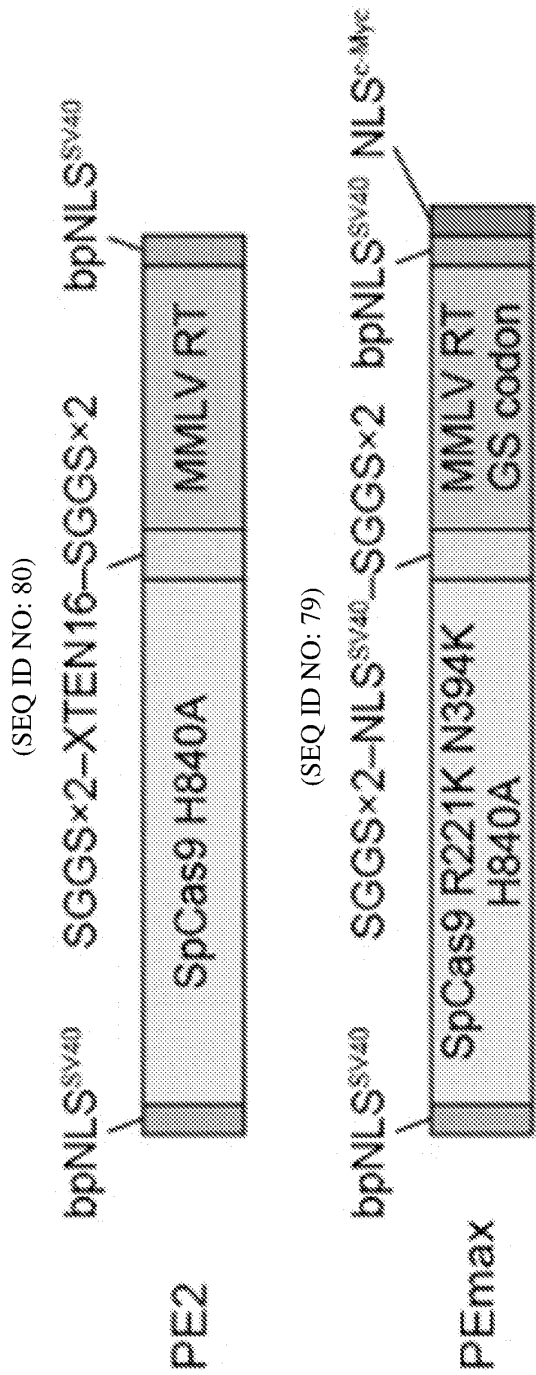


FIG. 9

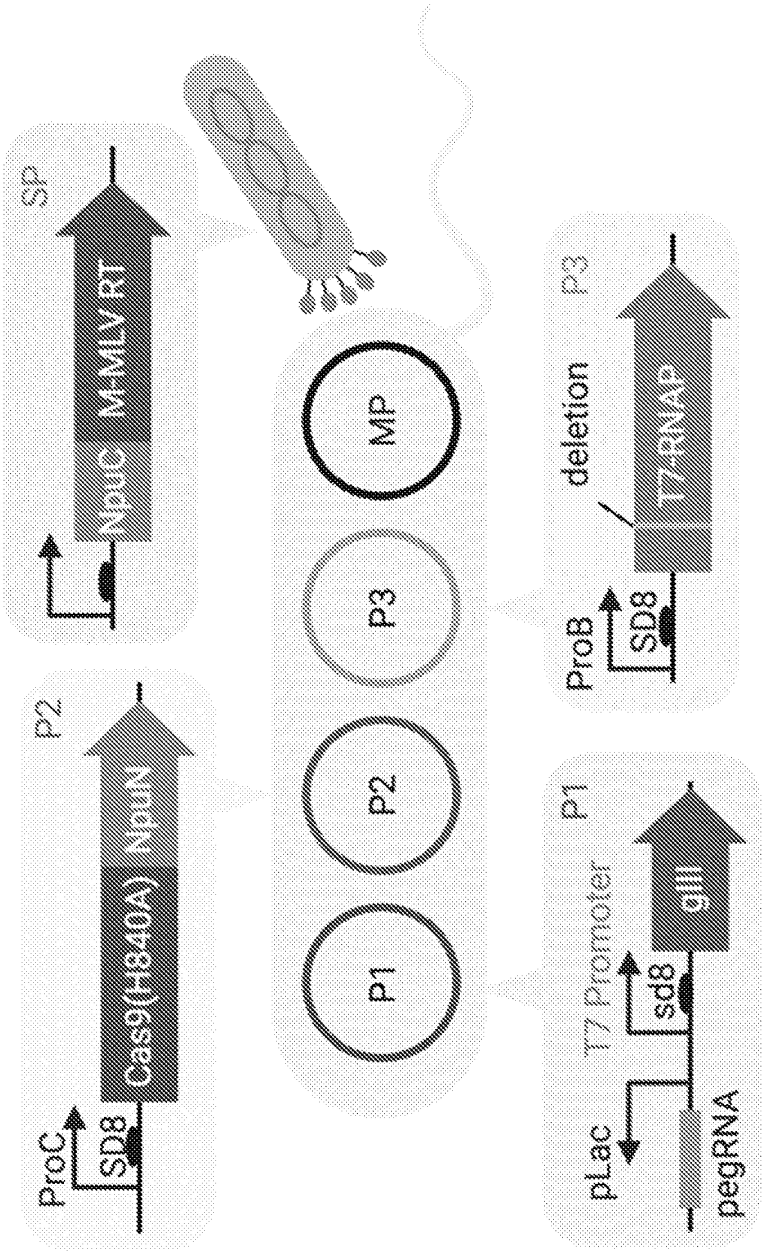


FIG. 10

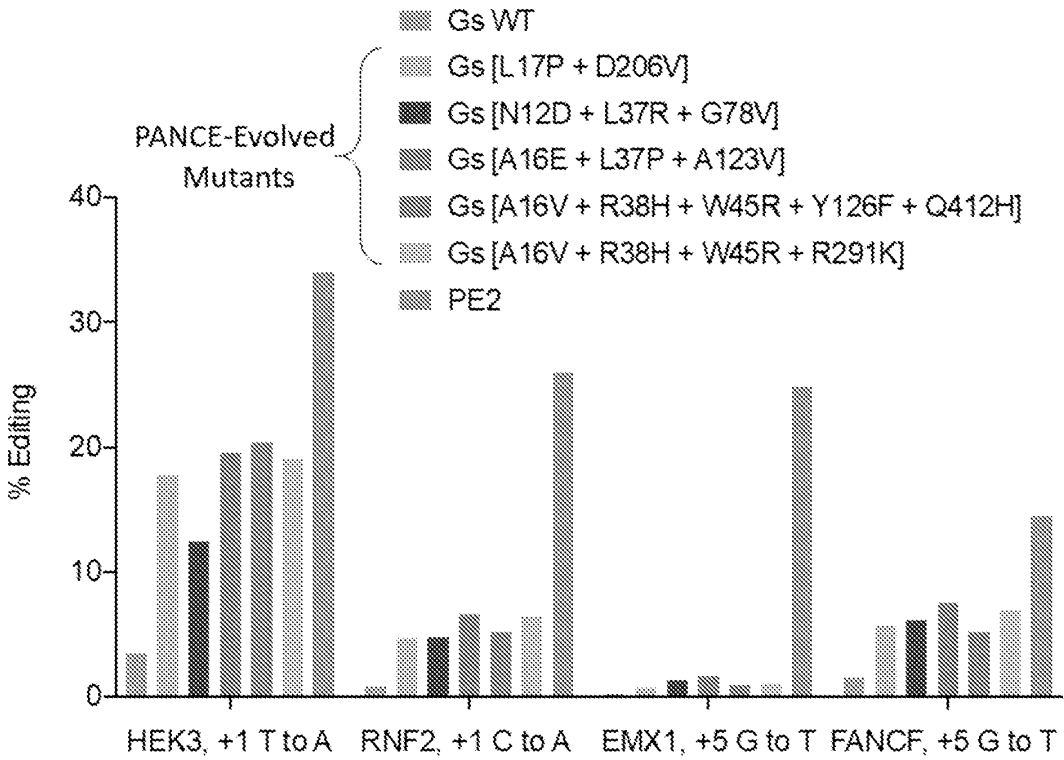


FIG. 11

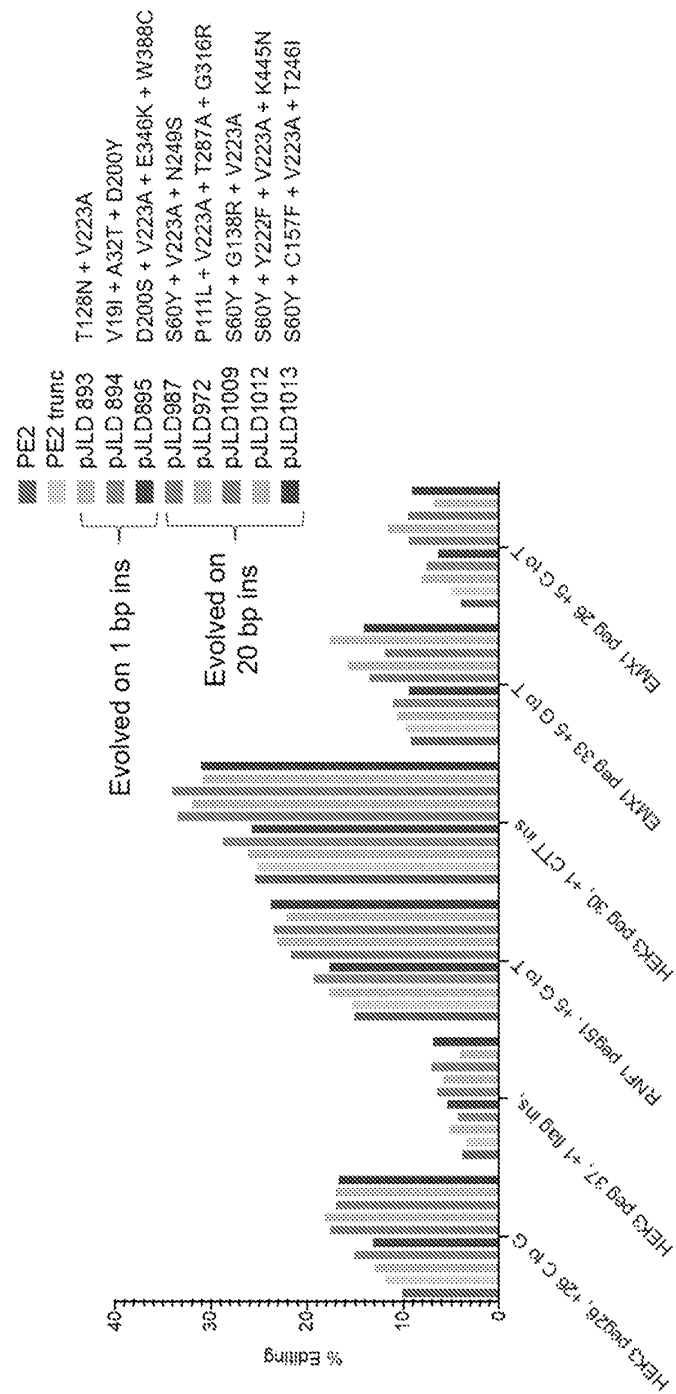


FIG. 12

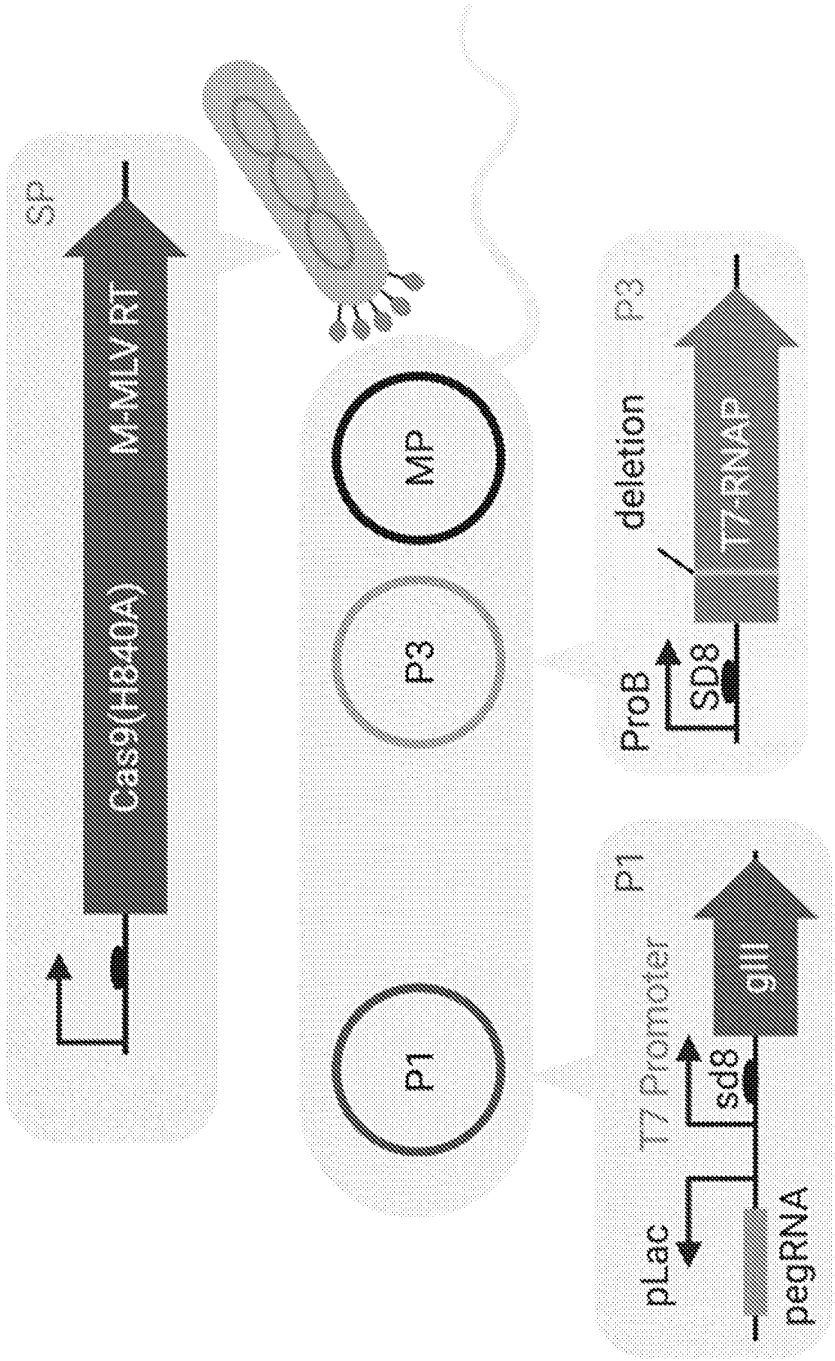


FIG. 13

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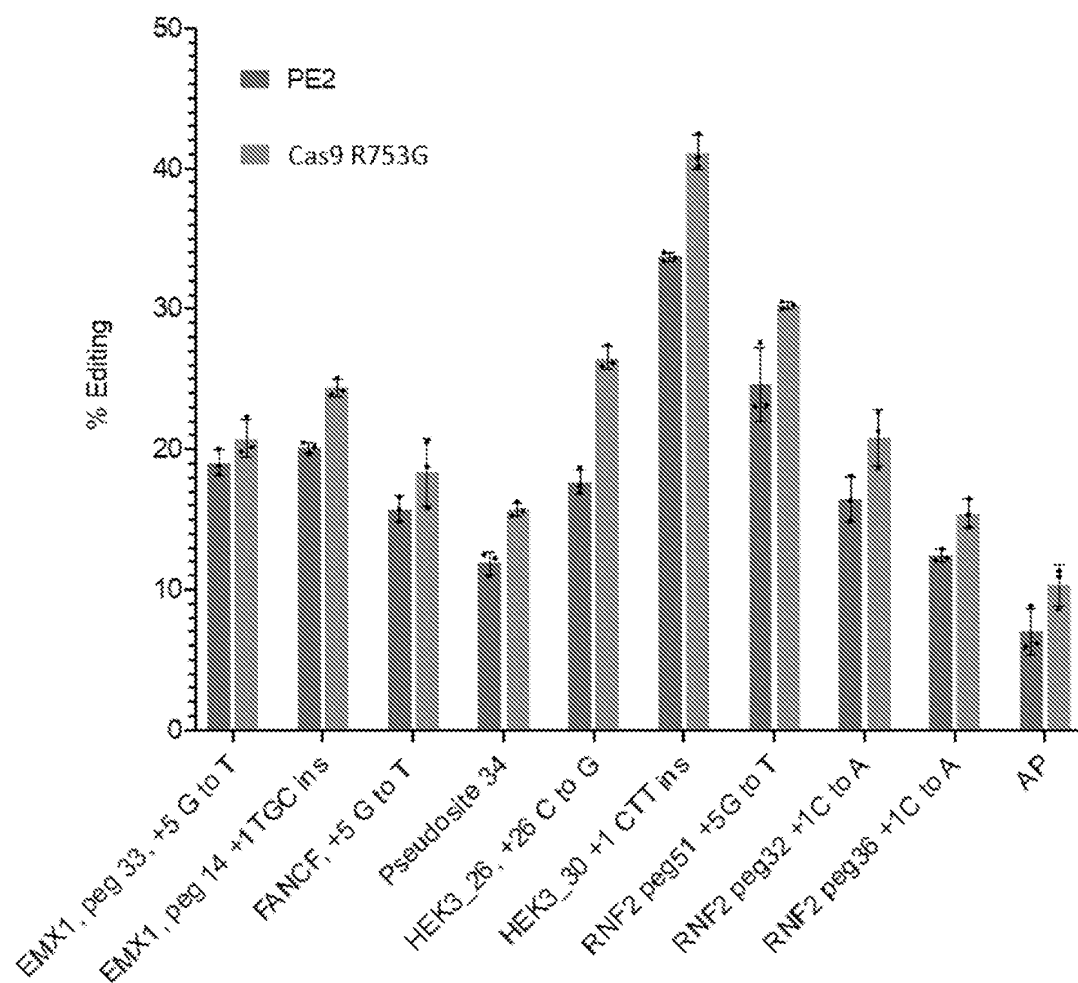


FIG. 14

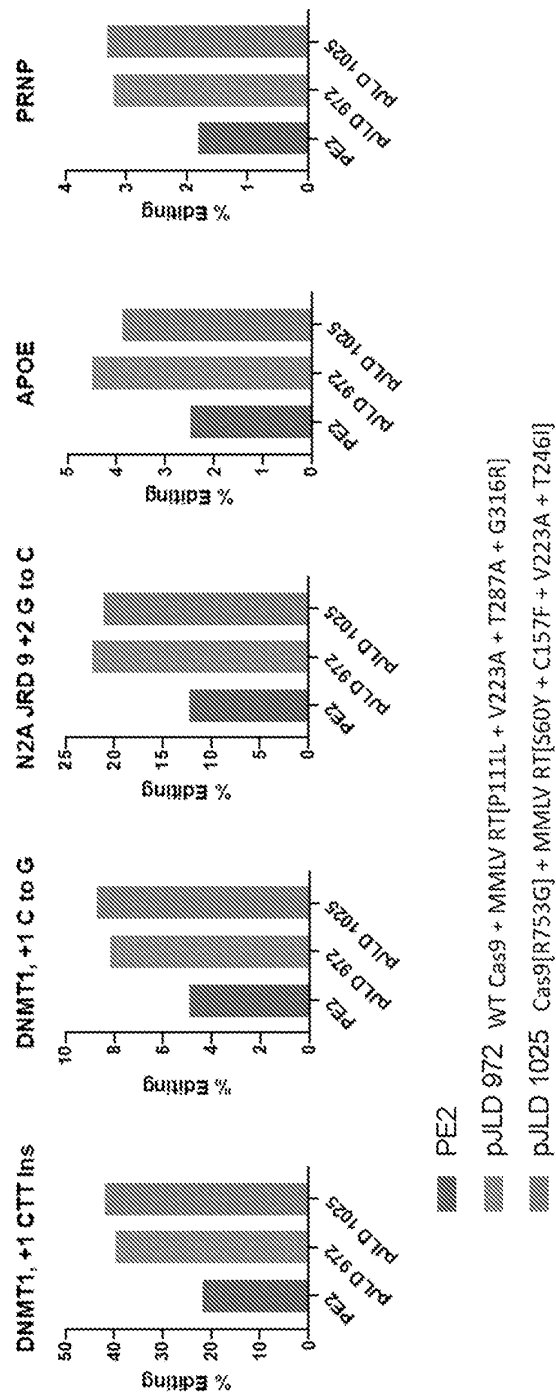


FIG. 15

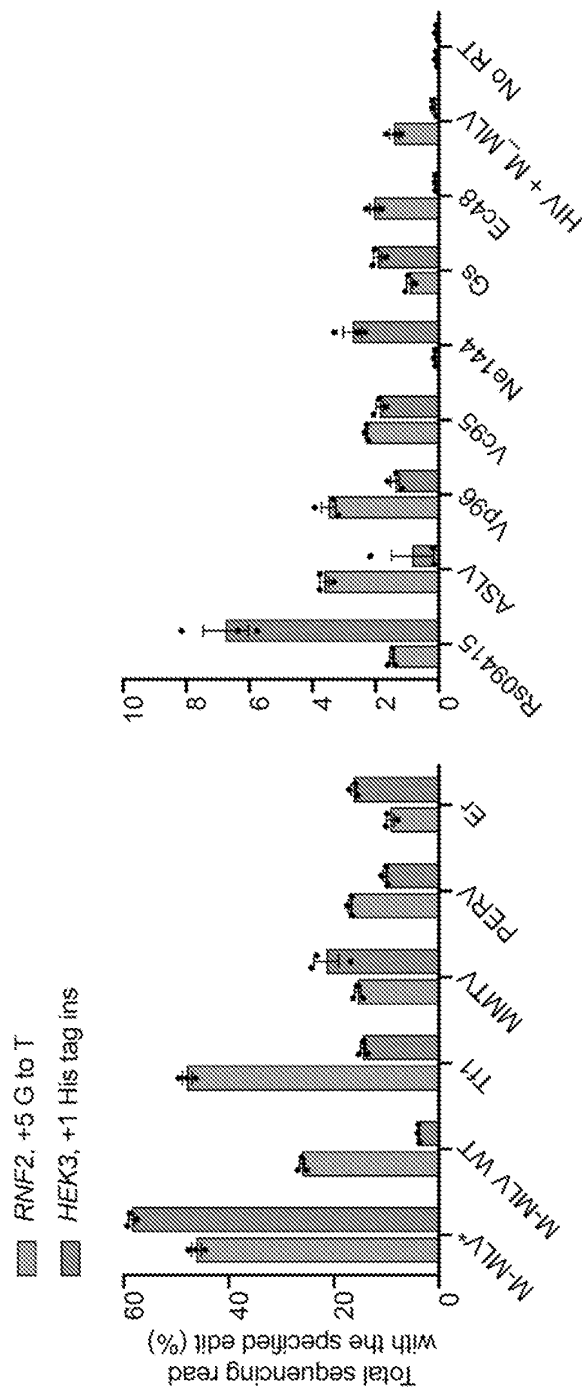


FIG. 16

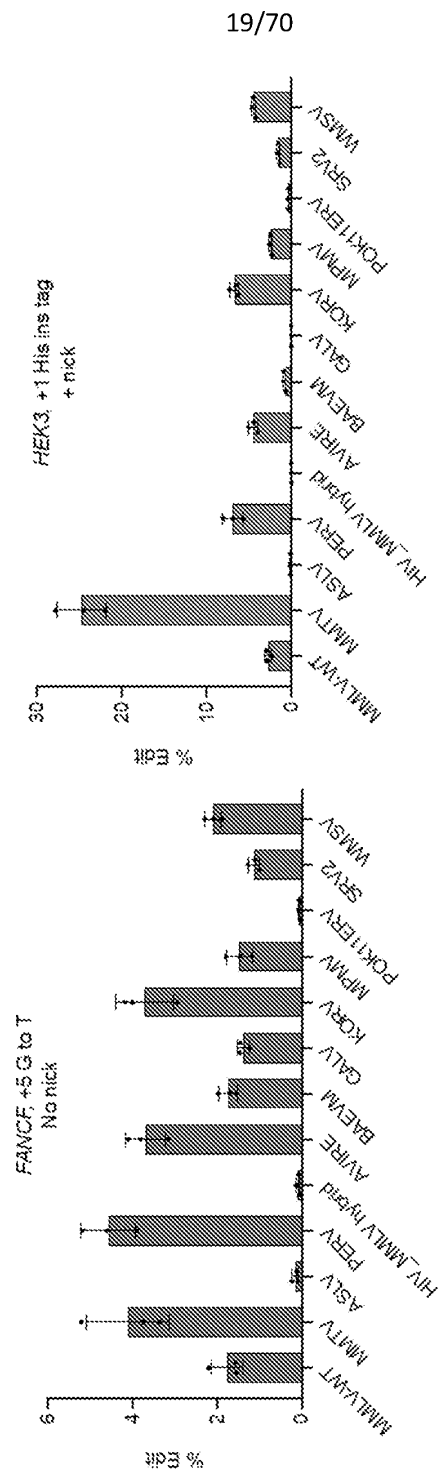
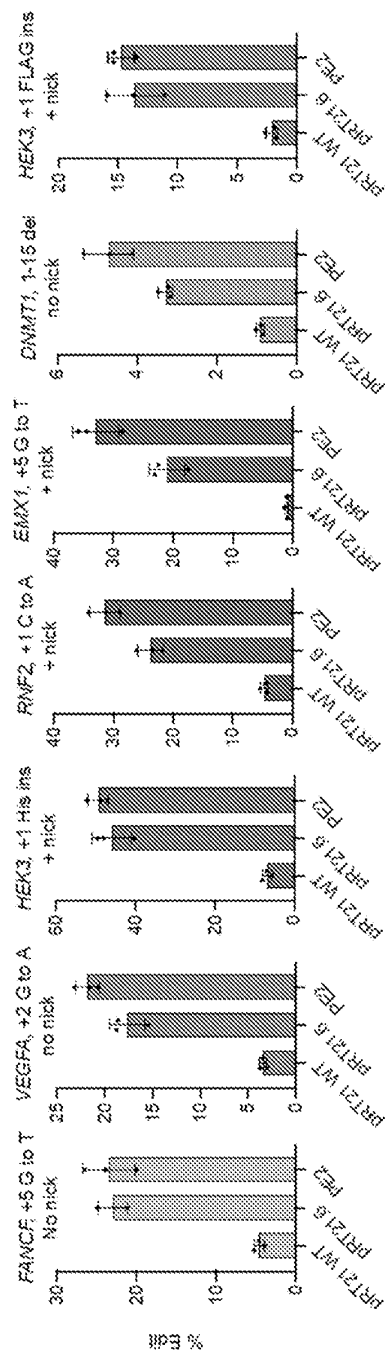


FIG. 17



pRT21: PERV WT
pRT21.6: PERV - (D199N+T305K+W312F+E329P+L602W)

FIG. 18

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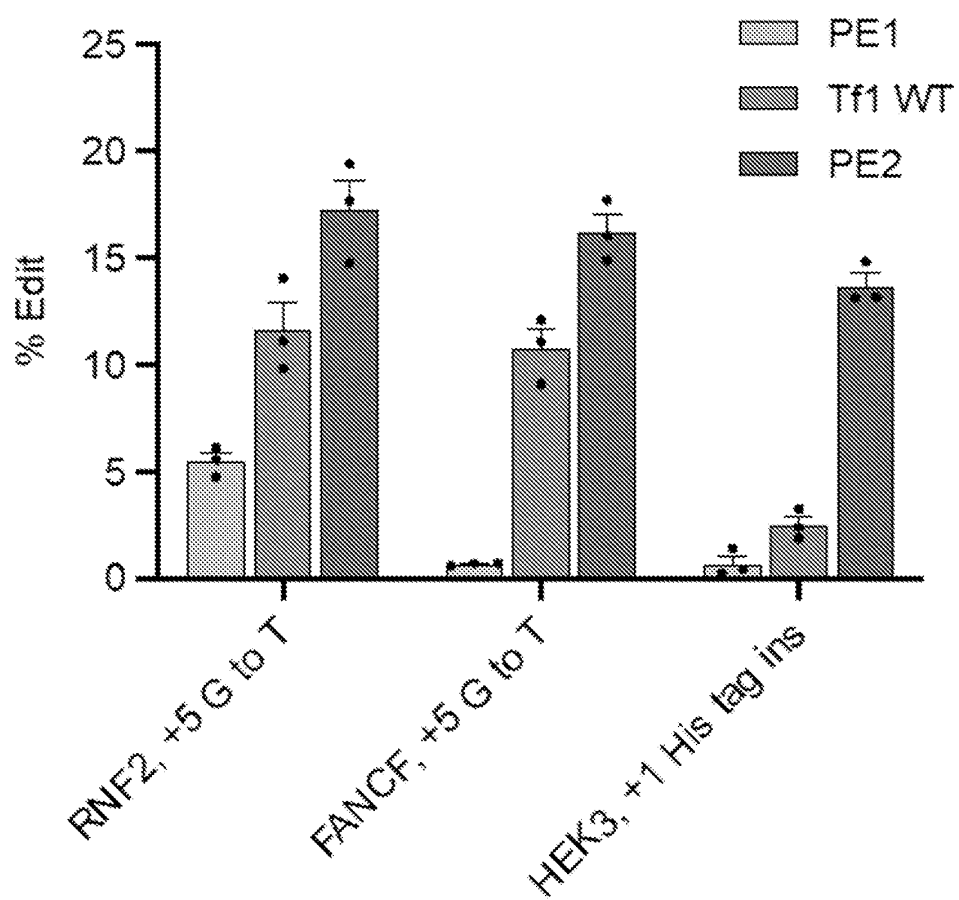


FIG. 19

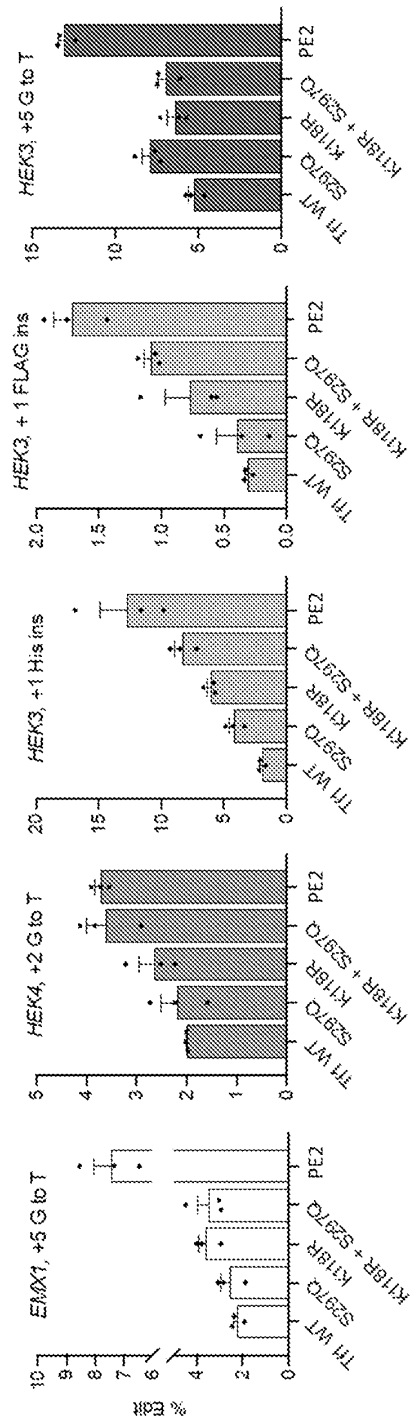
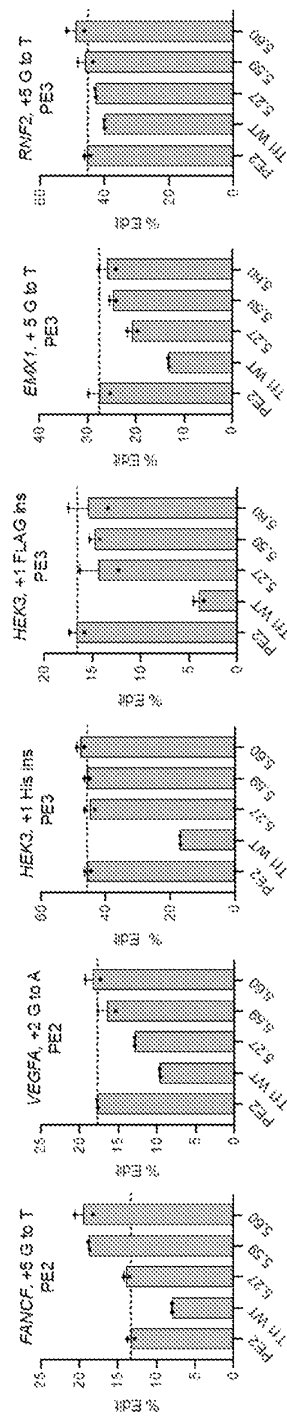


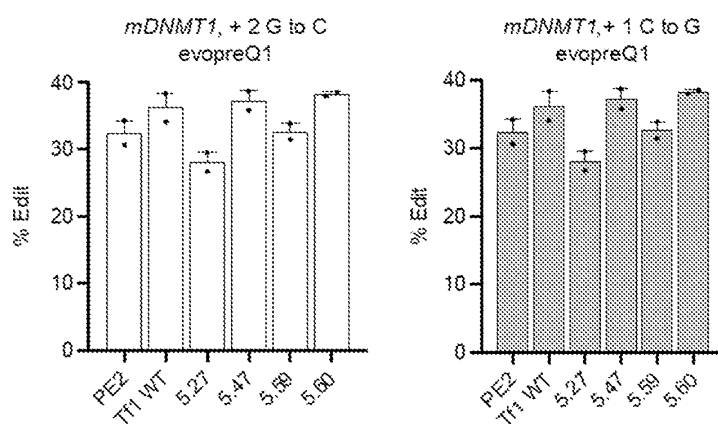
FIG. 20



5.27-(V14A+L158Q+F269L+K356E)
5.59-(E22K+P70T+G72V+M102I+K106R+A139T+L158Q+F269L+A363V+K413E+S492N)
5.60-(P70T+G72V+M102I+K106R+L158Q+F269L+A363V+K413E+S492N)

FIG. 21

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5.27-(V14A+L158Q+F269L+K356E)

5.47-(K106R+L158Q+F269L+A363V+I423V)

5.59-(E22K+P70T+G72V+M102I+K106R+A139T+L158Q+F269L+A363V+K413E+S492N)

5.60-(P70T+G72V+M102I+K106R+L158Q+F269L+A363V+K413E+S492N)

FIG. 22

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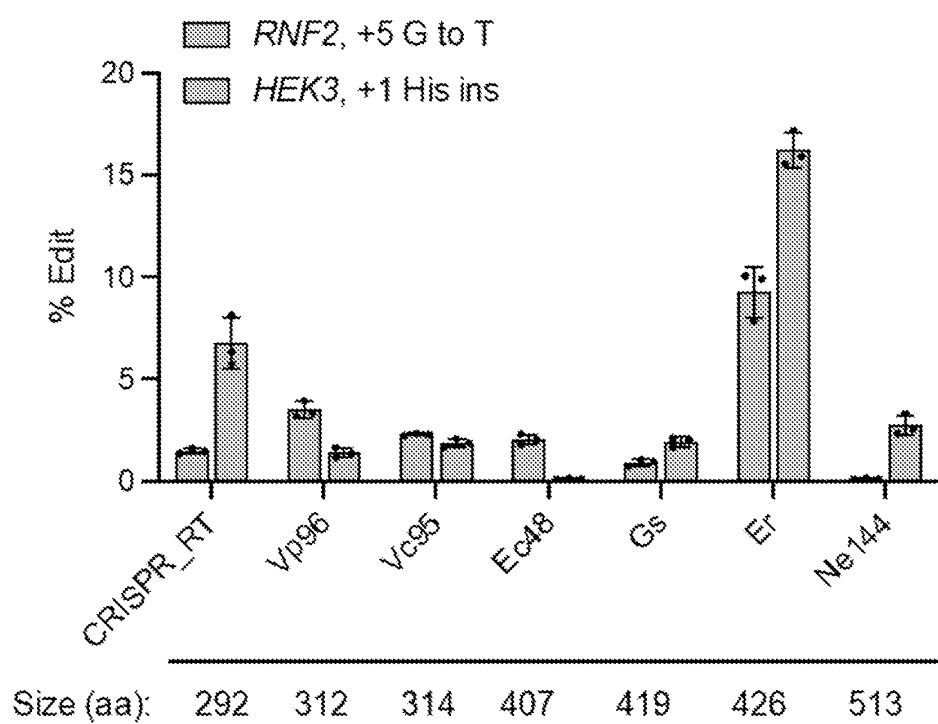


FIG. 23

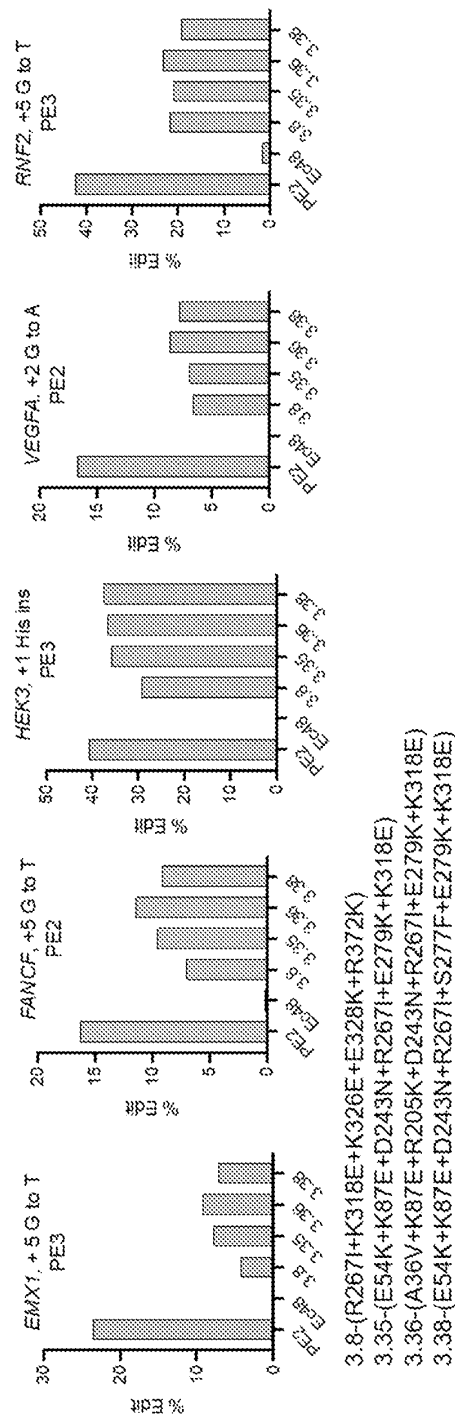


FIG. 24

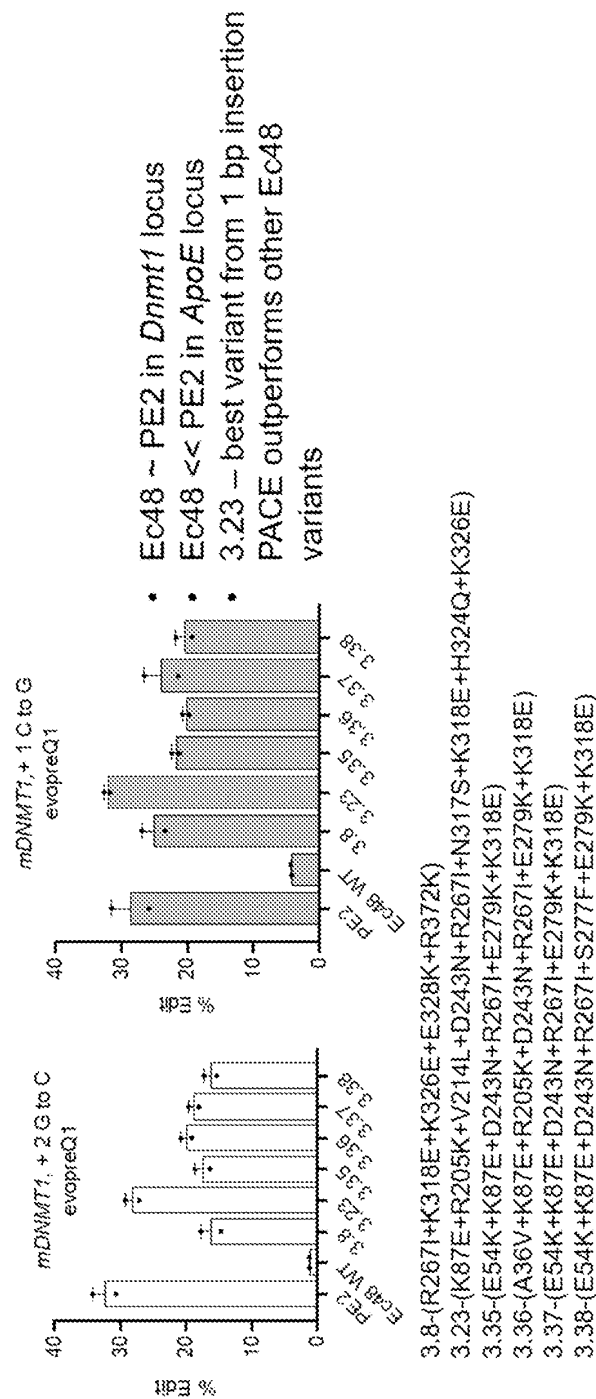


FIG. 25

PEmax

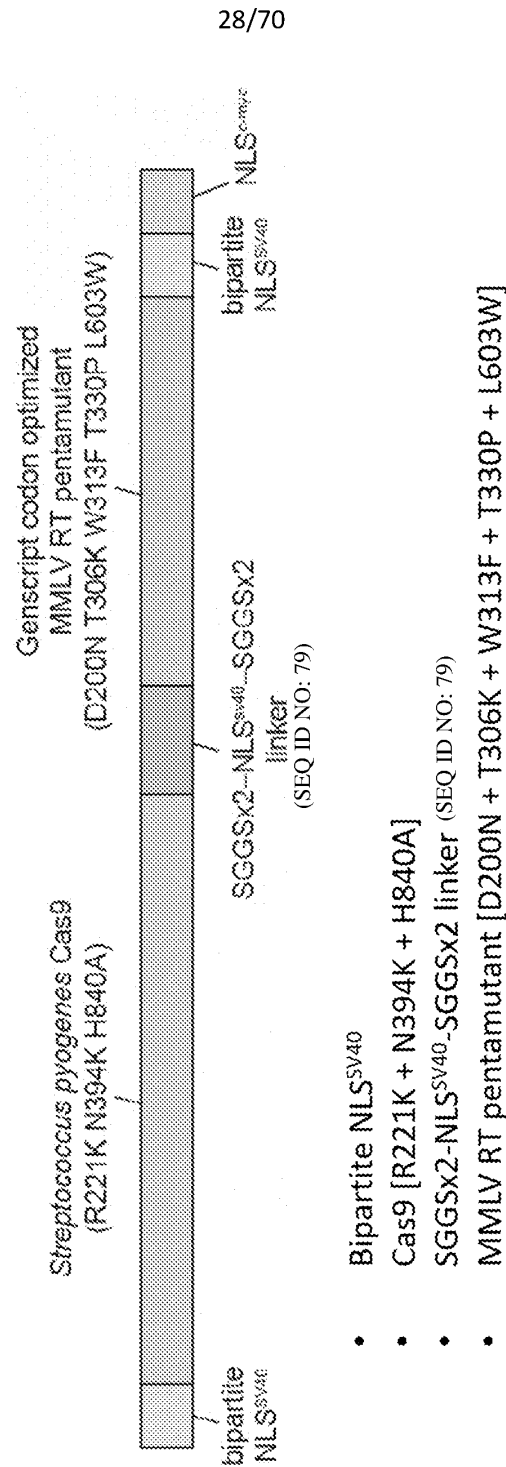
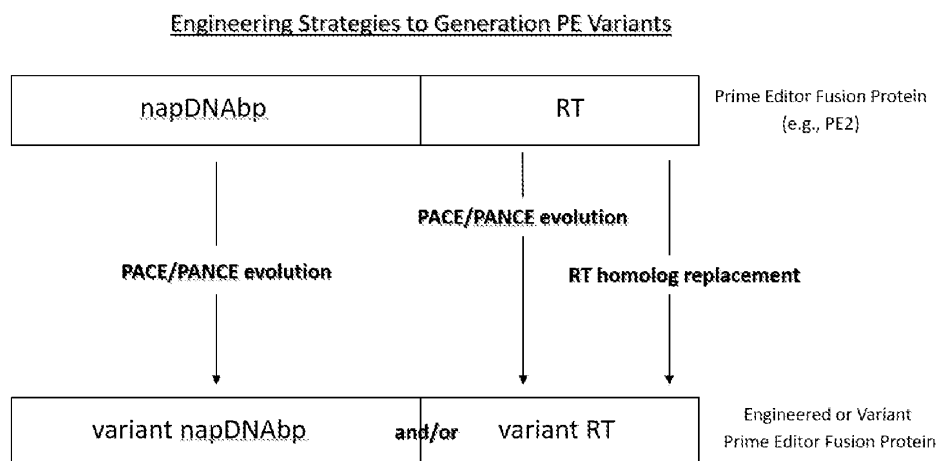


FIG. 26

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**FIG. 27A**

PACE-evolved Cas9 + RT

- WT Cas9 + MMLV RT[T128N + V223A]
- WT Cas9 + MMLV RT[V19I + A32T + D200Y]
- WT Cas9 + MMLV RT[D200S + V223A + E346K + W388C]
- WT Cas9 + MMLV RT[S60Y + V223A + N249S]
- WT Cas9 + MMLV RT[P111L + V223A + T287A + G316R]
- WT Cas9 + MMLV RT[S60Y + G138R + V223A]
- WT Cas9 + MMLV RT[S60Y + Y222F + V223A + K445N]
- WT Cas9 + MMLV RT[S60Y + C157F + V223A + T246I]
- Cas9[R753G] + WT MMLV RT
- Cas9[R753G] + MMLV RT[S60Y + C157F + V223A + T246I]

FIG. 27B

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PE2 replaced with alternate RT domains

- PE2 using Tf1 RT
- PE2 using MMLV-WT RT
- PE2 using AVIRE RT
- PE2 using BAEVM RT
- PE2 using GALV RT
- PE2 using KORV RT
- PE2 using MPMV
- PE2 using POK11ERV RT
- PE2 using SRV2 RT
- PE2 using MMTV RT
- PE2 using PERV RT
- PE2 using Er RT
- PE2 using Rs09415 RT
- PE2 using ASLV RT
- PE2 using Vp96 RT
- PE2 using Vc95 RT
- PE2 using Ne144 RT
- PE2 using Gs RT
- PE using Ec48 RT
- PE2 using HIV + MMLV RT

FIG. 28

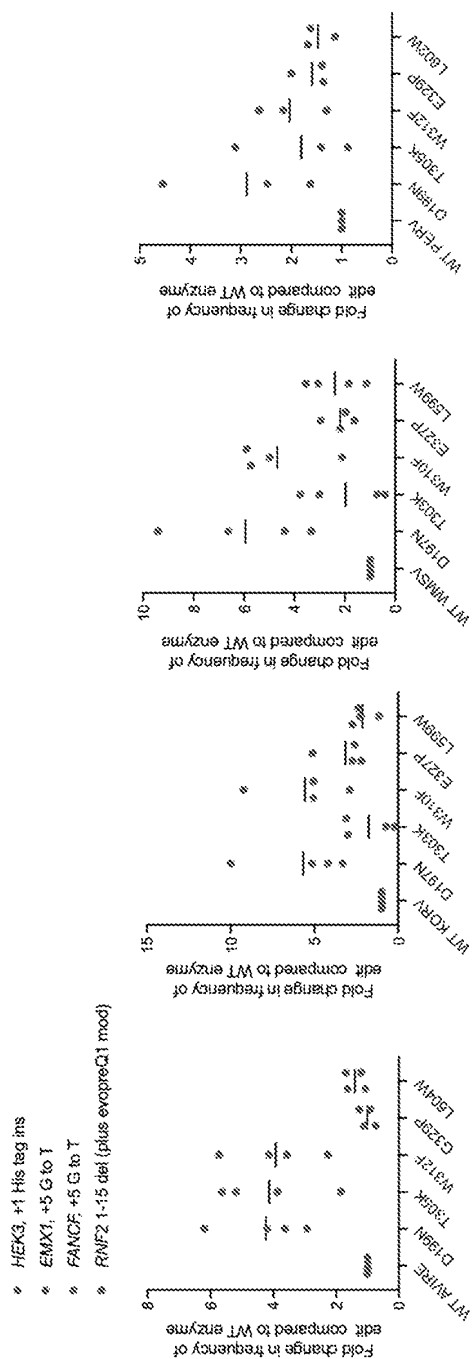
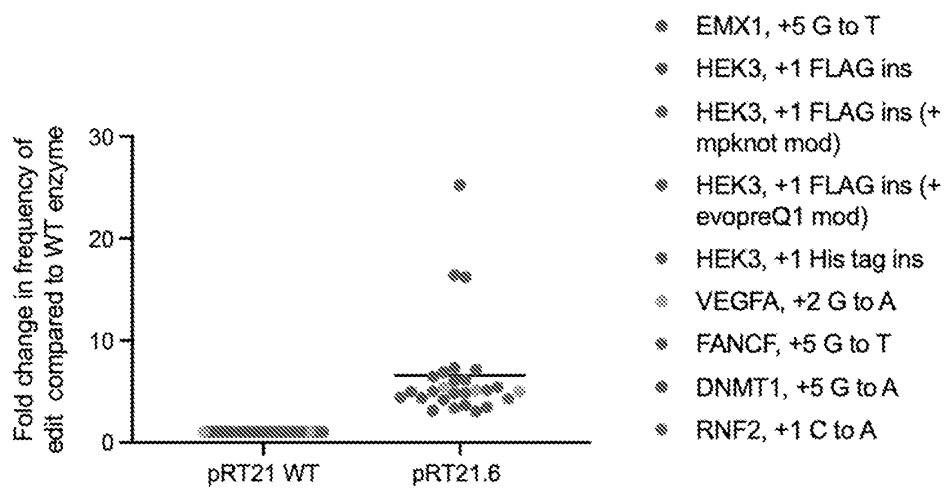


FIG. 29

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**FIG. 30**

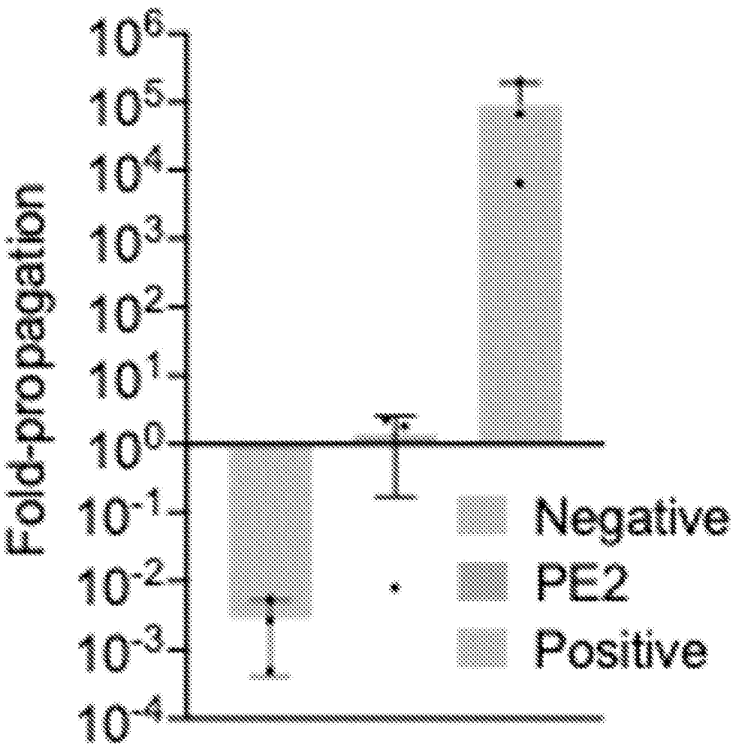


FIG. 31A

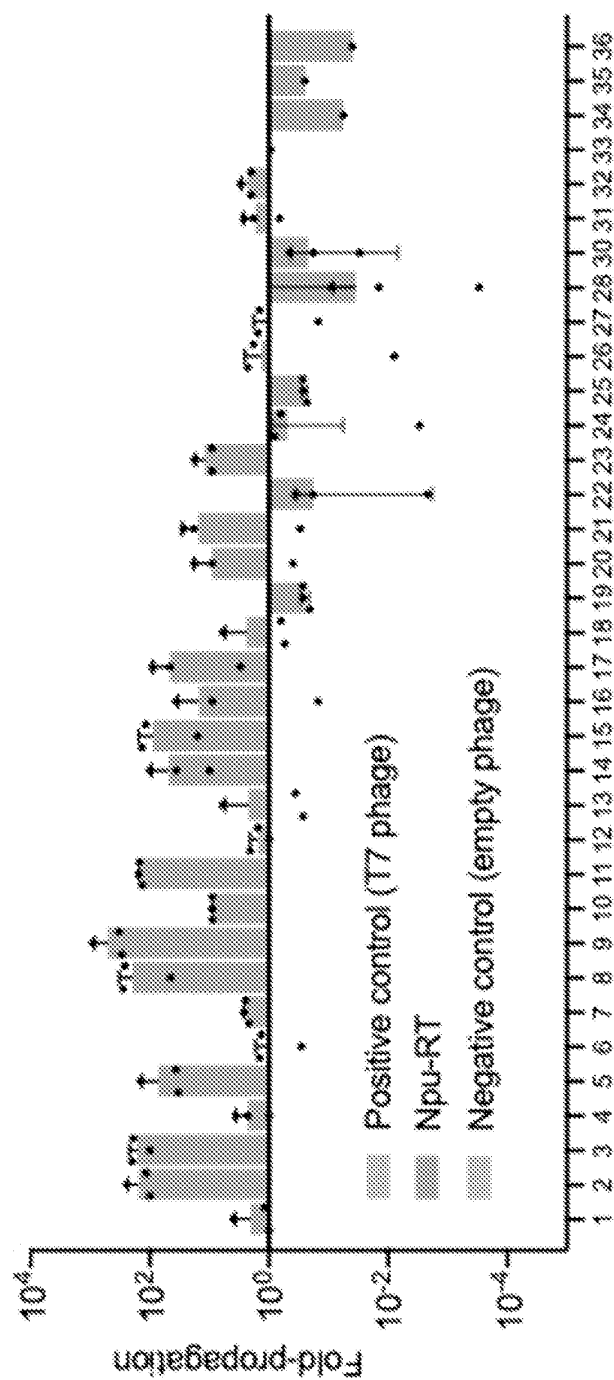


FIG. 31B

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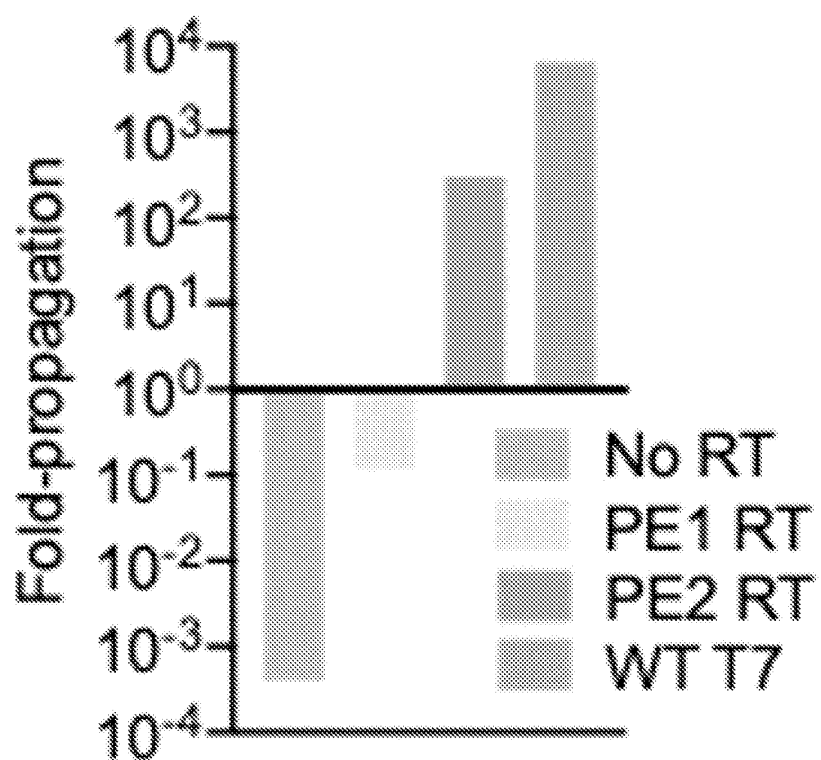


FIG. 31C

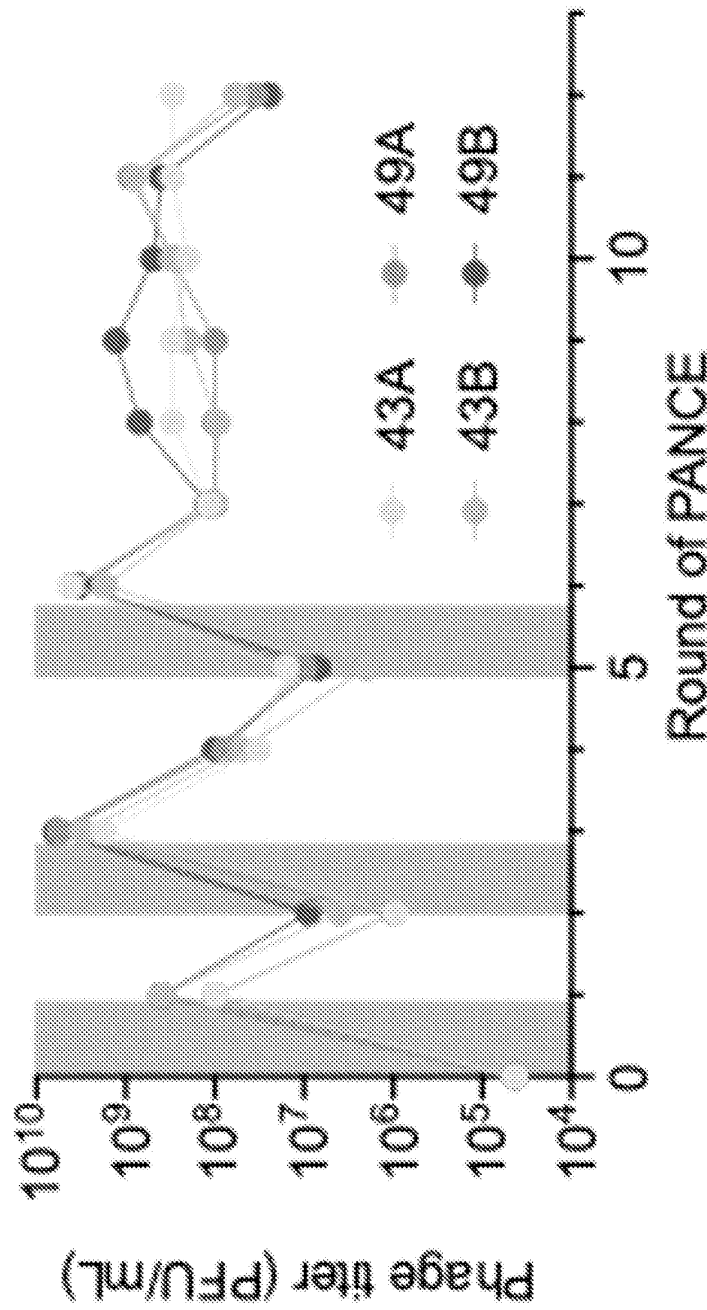


FIG. 31D

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Clone	D200	V223	E302	T306	M457	A462
1	Y		A			
2	Y		A			
3	Y	A			I	
4	Y	A			I	
5		M		K		S
6		M		K		S
7		M		K		S
8		M		K		S
9	N		K			
10	N		K			
11	Y		K			
12	Y			K		
13	Y					
14	Y		K			
15		M				
16	Y					

FIG. 32

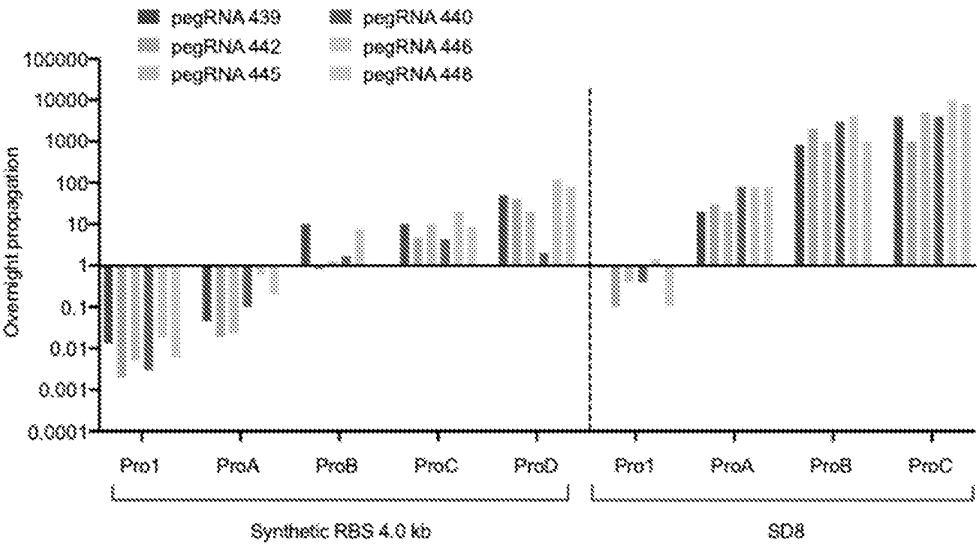


FIG. 33A

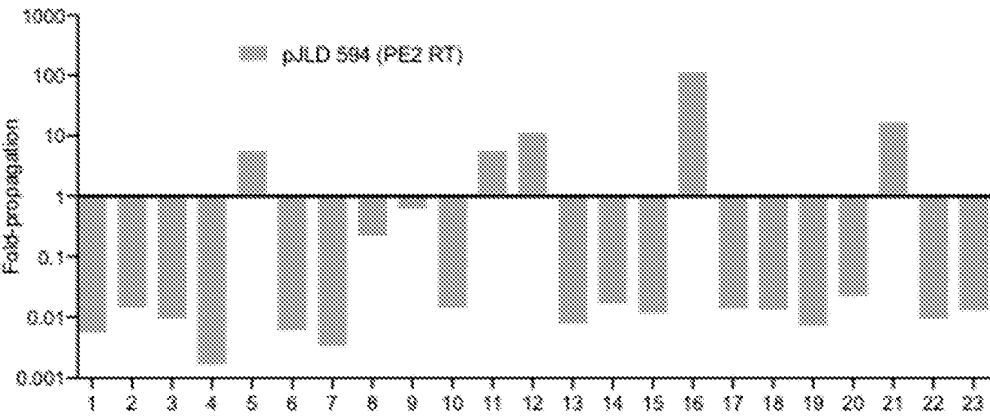


FIG. 33B

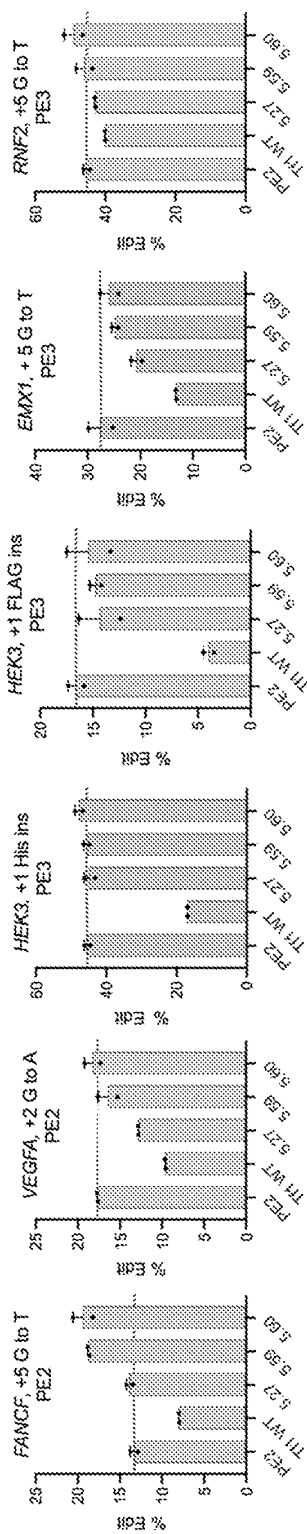


FIG. 34

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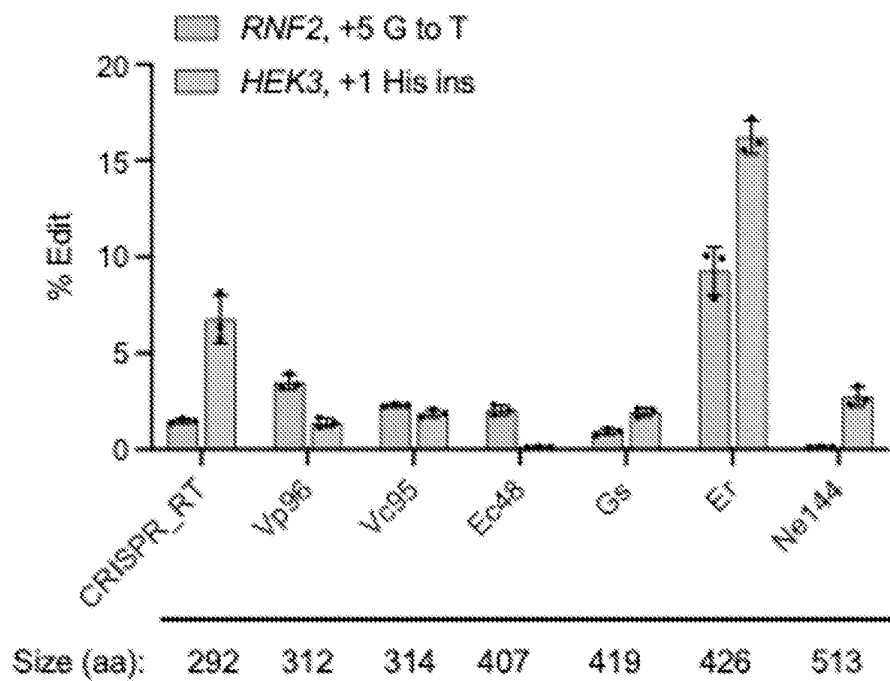


FIG. 35

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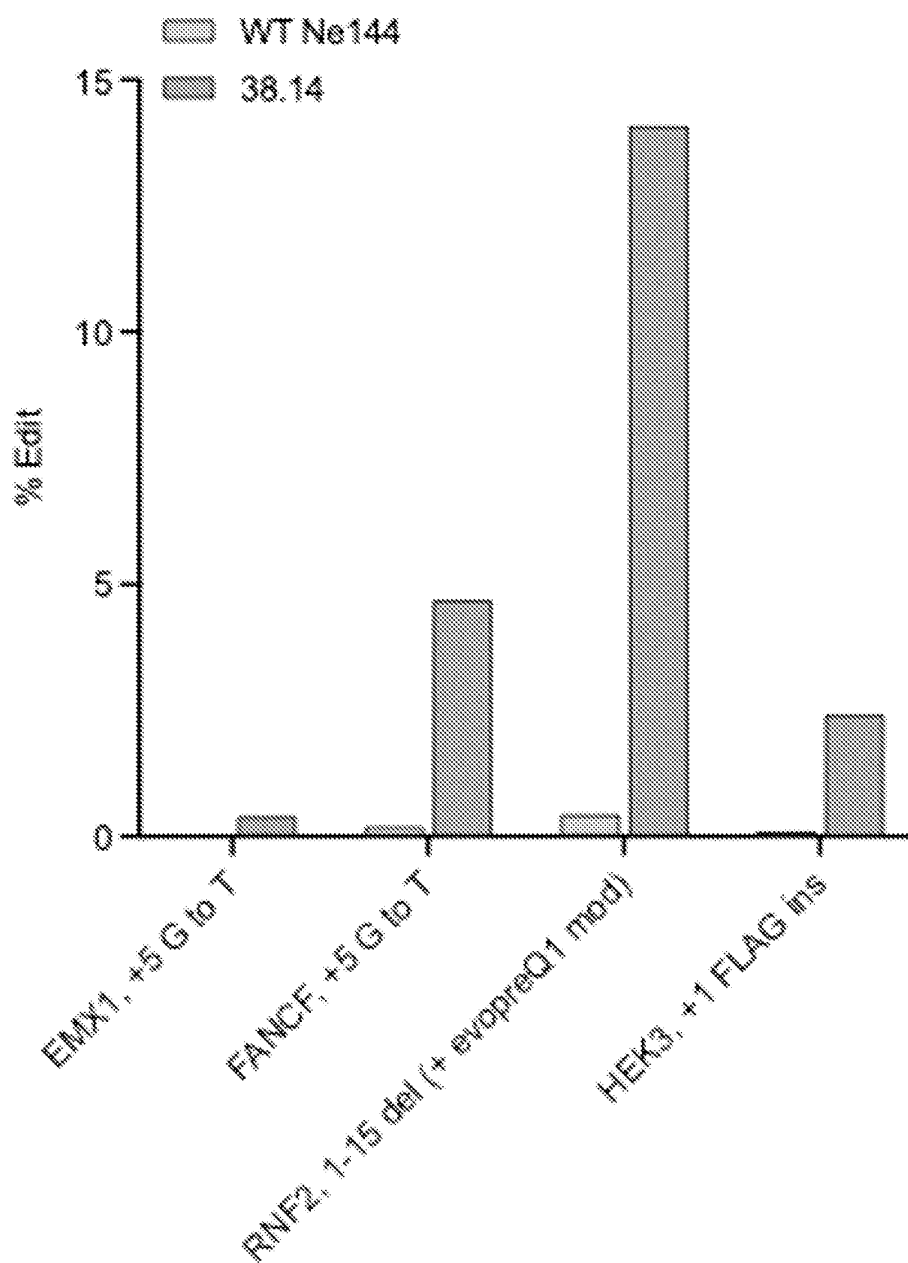


FIG. 36

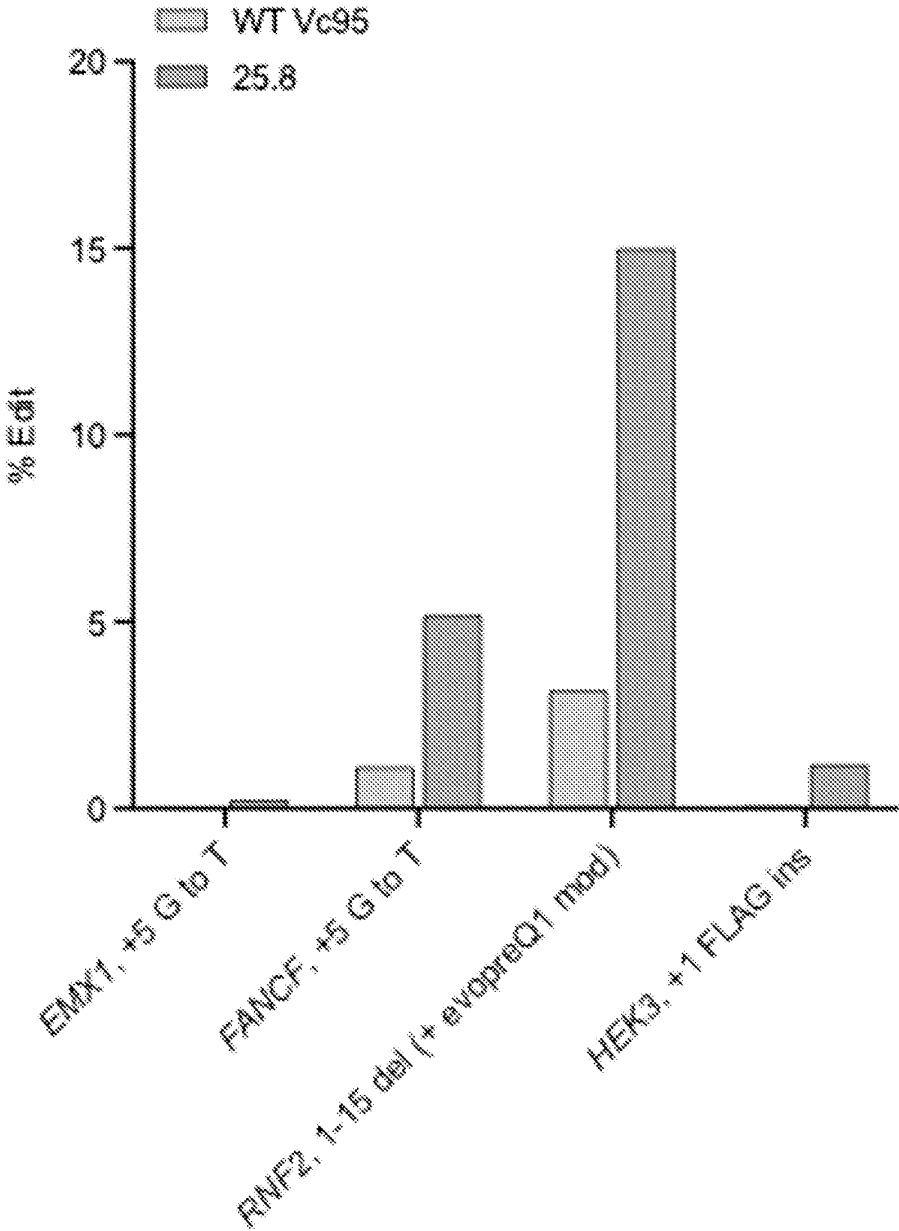


FIG. 37

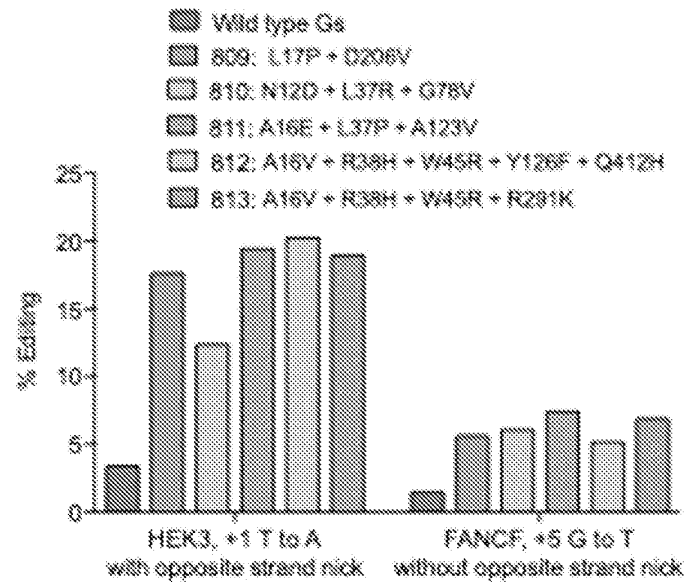


FIG. 38A

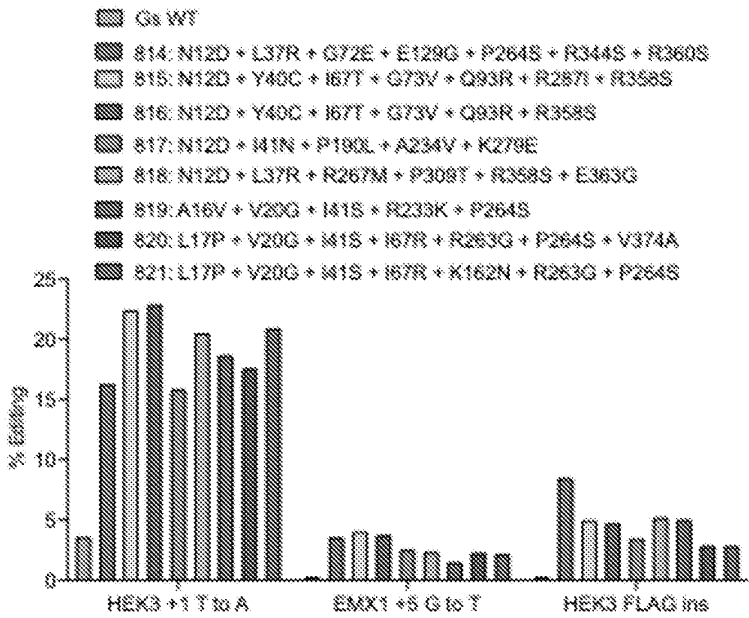


FIG. 38B

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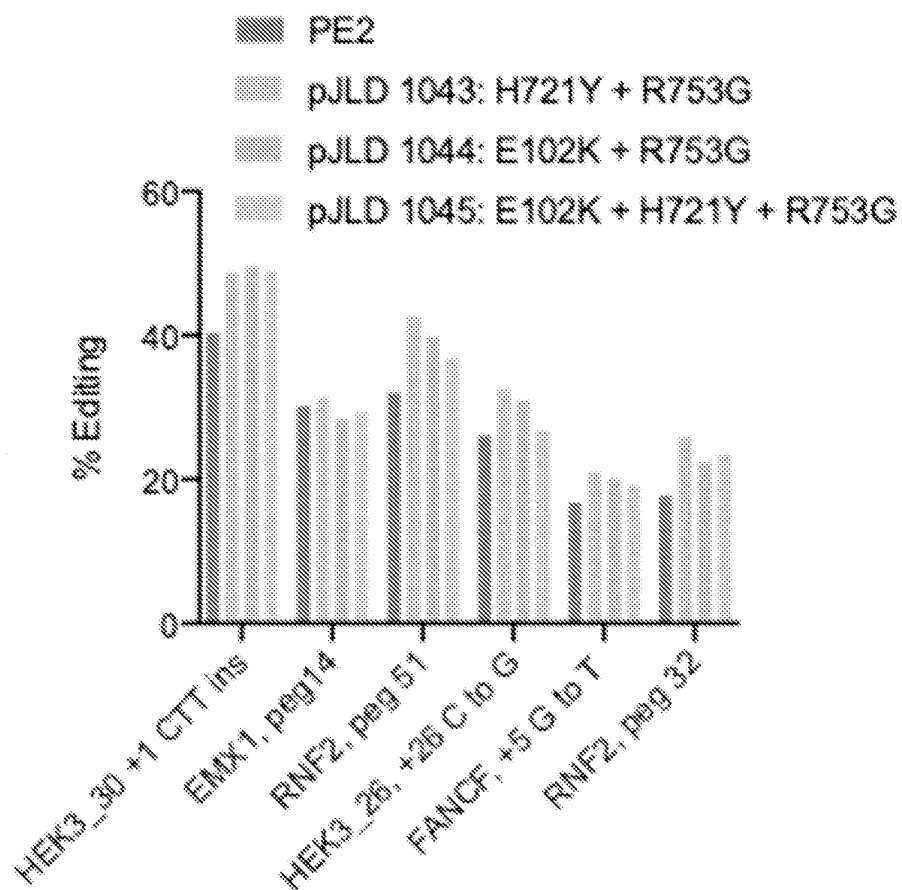


FIG. 39

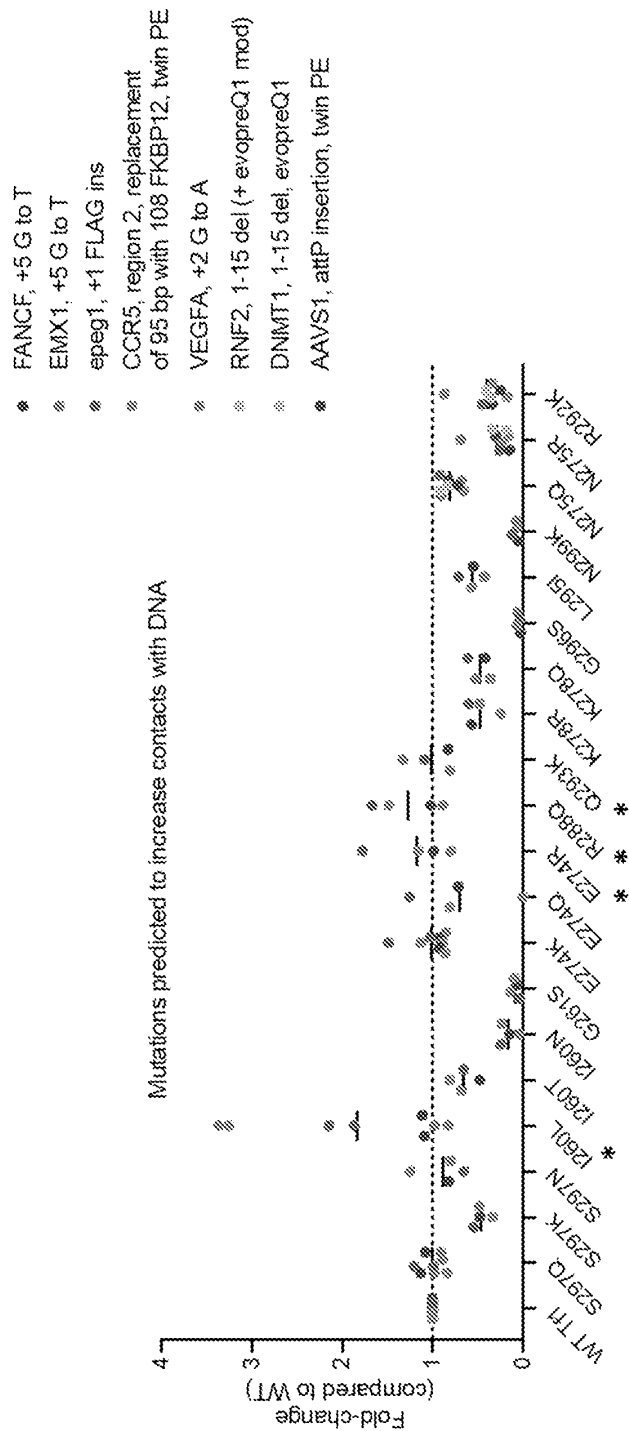


FIG. 40

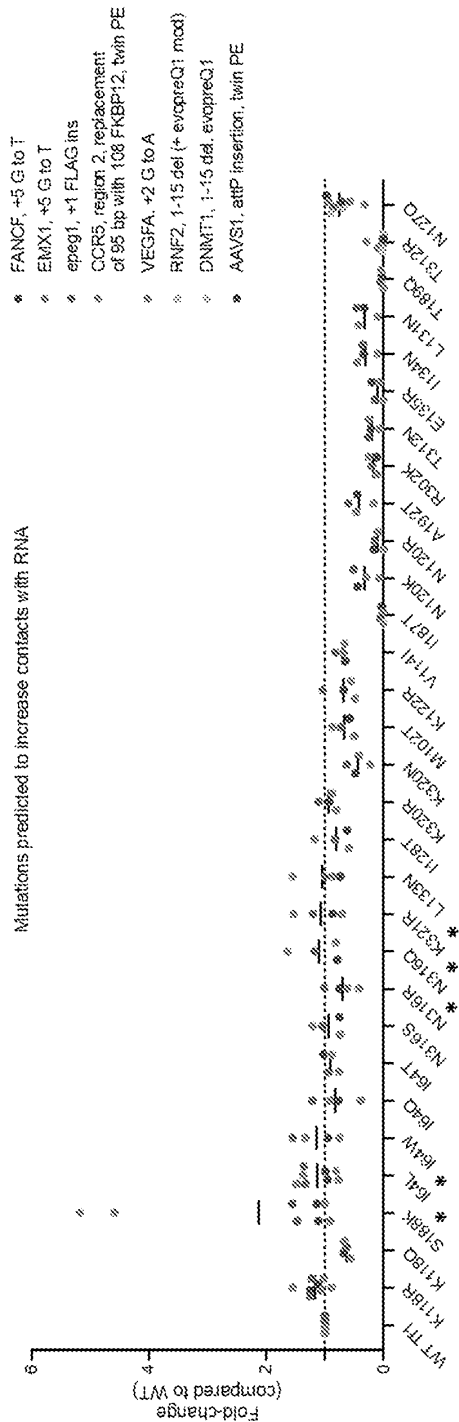


FIG. 41

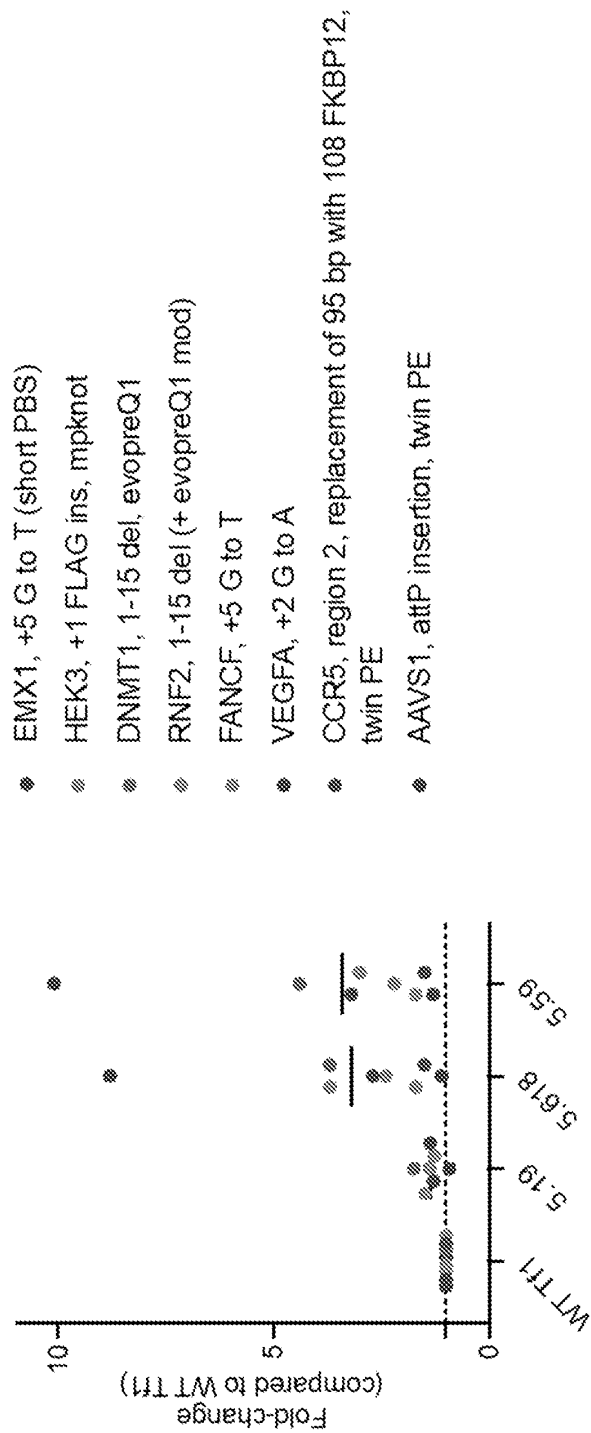


FIG. 42

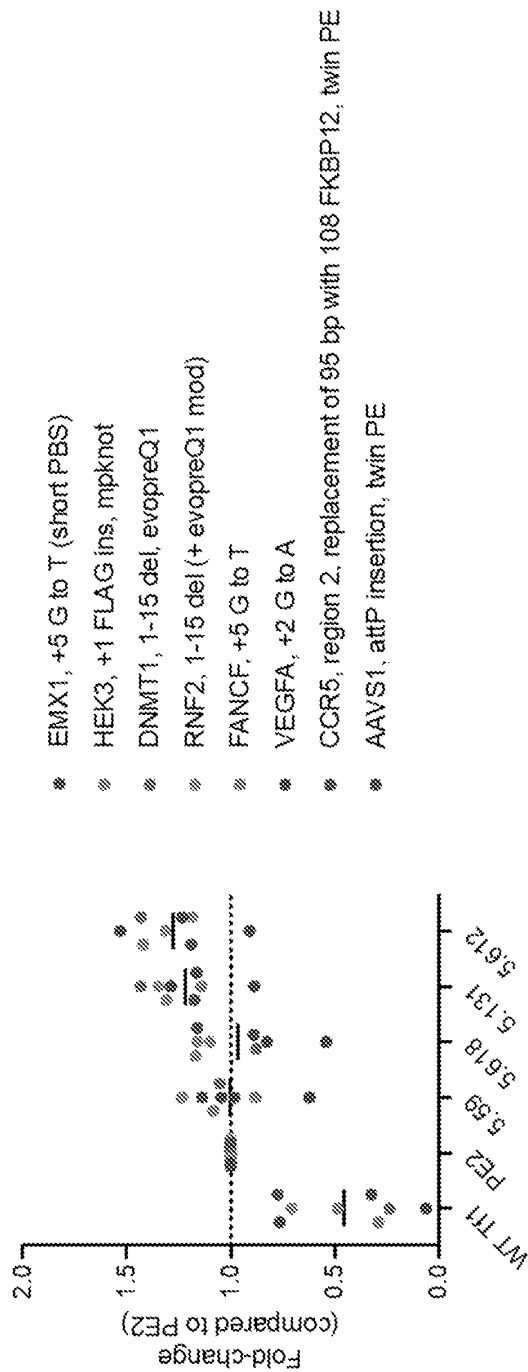


FIG. 43

	E60	K87	S151	E165	D243	R267	E279	V303	K318	K343
H12a	K	E		D	N	I	K		E	N
H12b	K	E	T	D	N	I	K	M	E	N
H12c	K	E		D	N	I	K		E	N
H12d	K	E		D	N	I	K		E	N

FIG. 44A

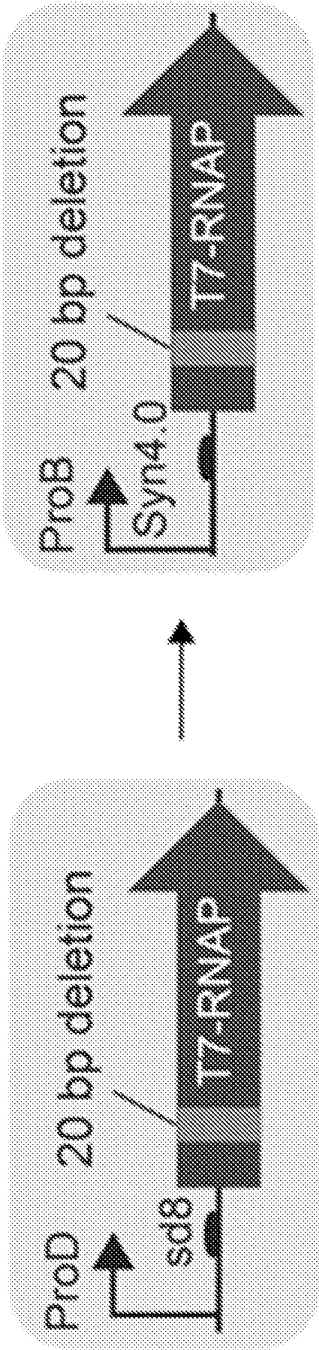


FIG. 44B

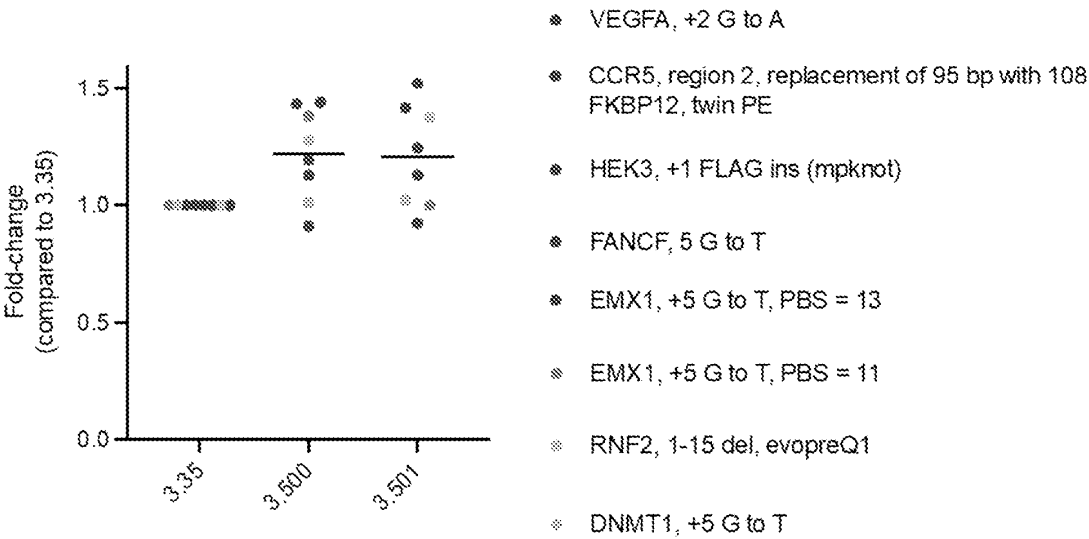


FIG. 45

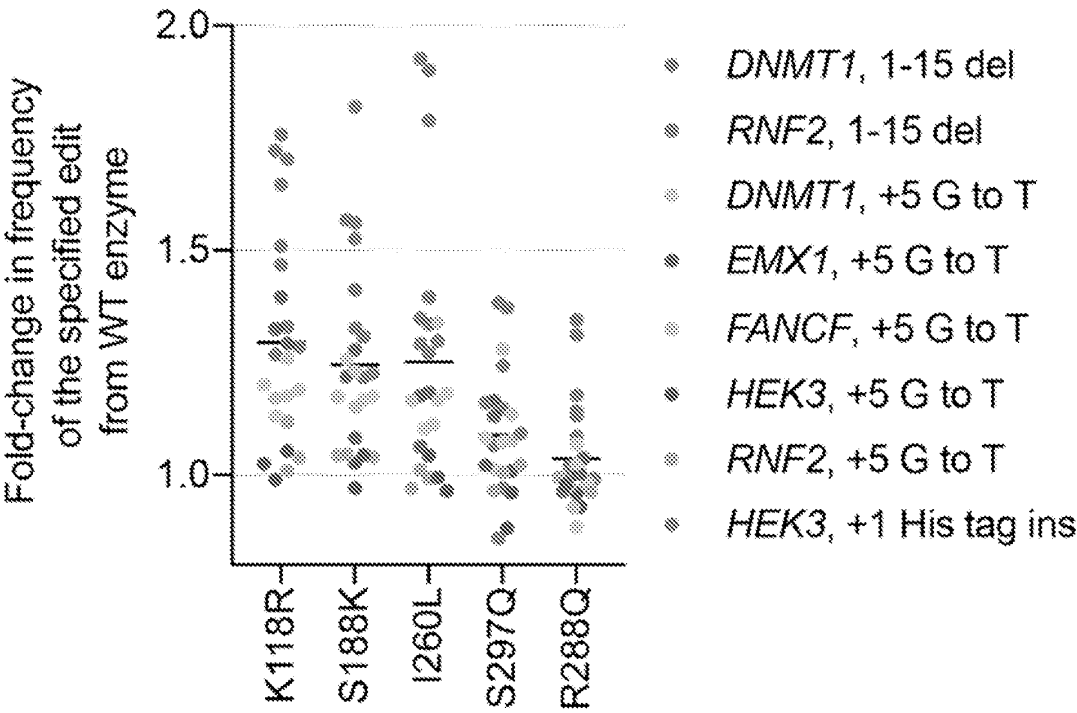


FIG. 46

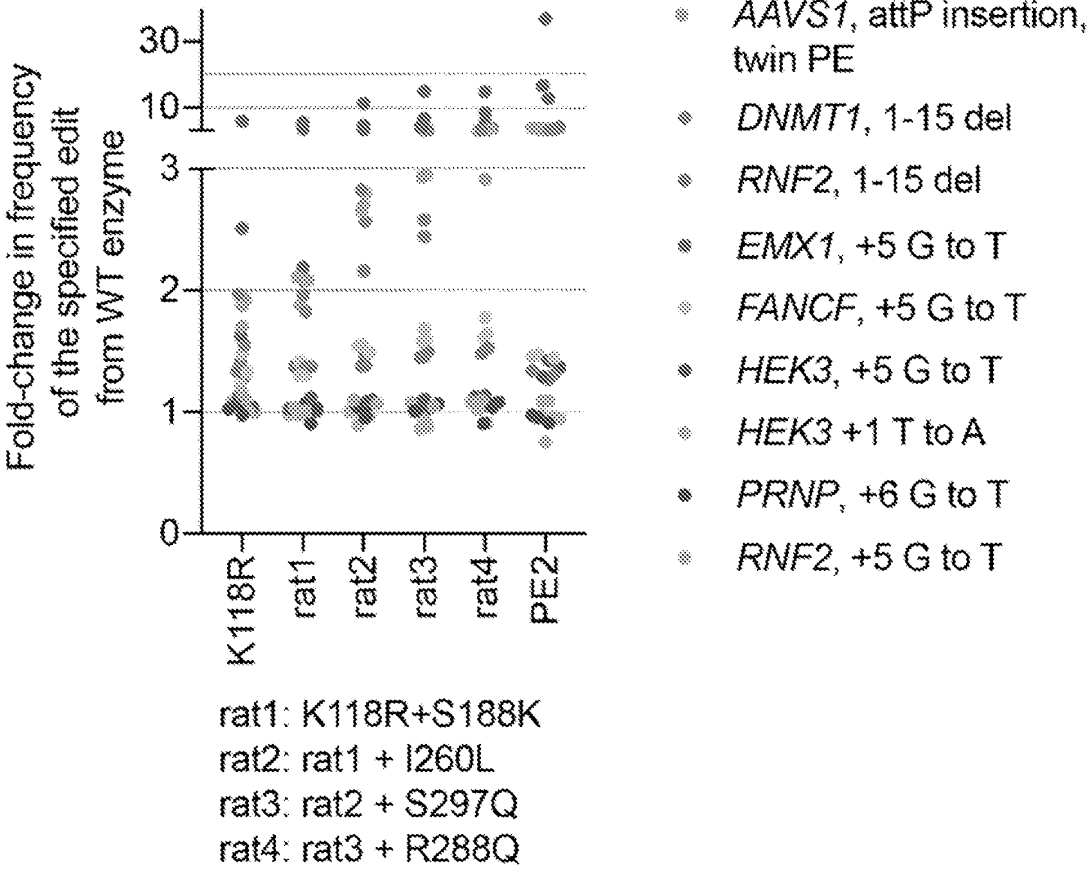


FIG. 47

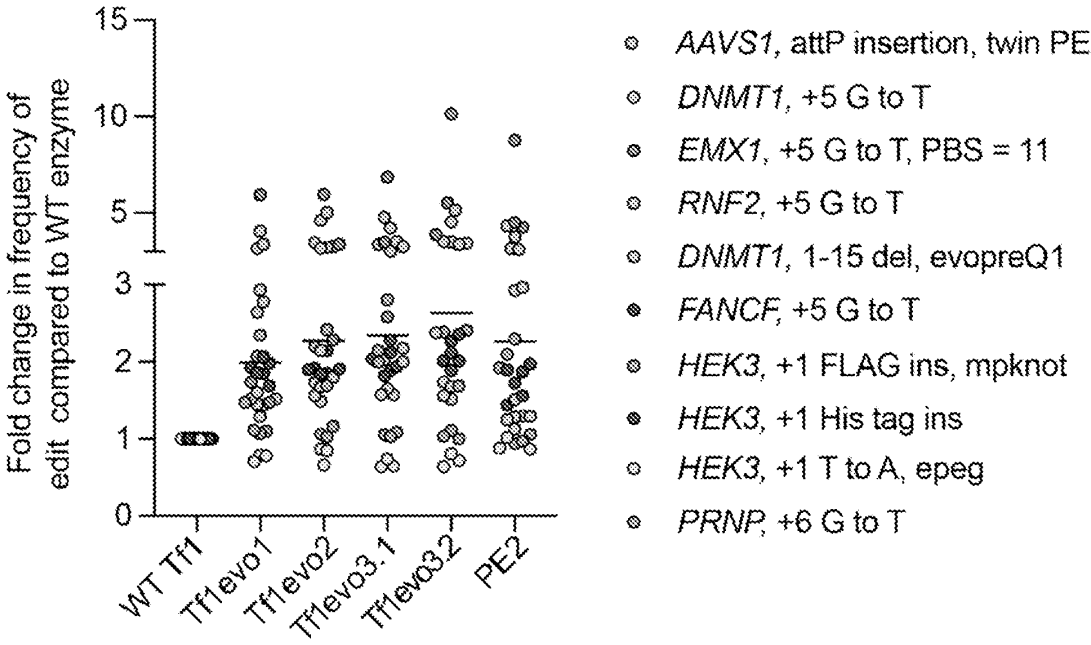


FIG. 48

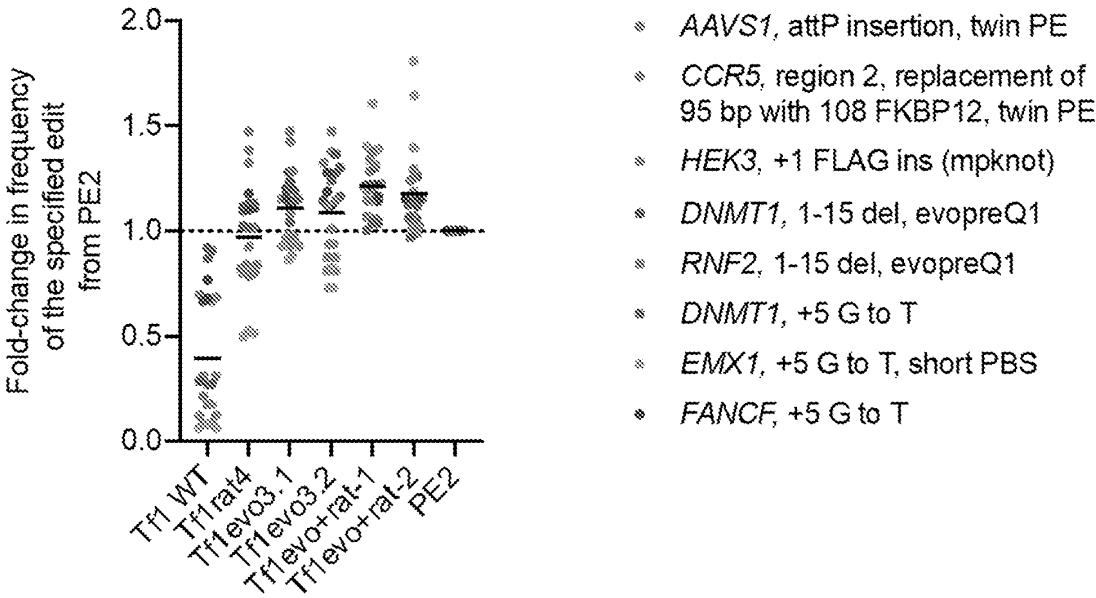


FIG. 49

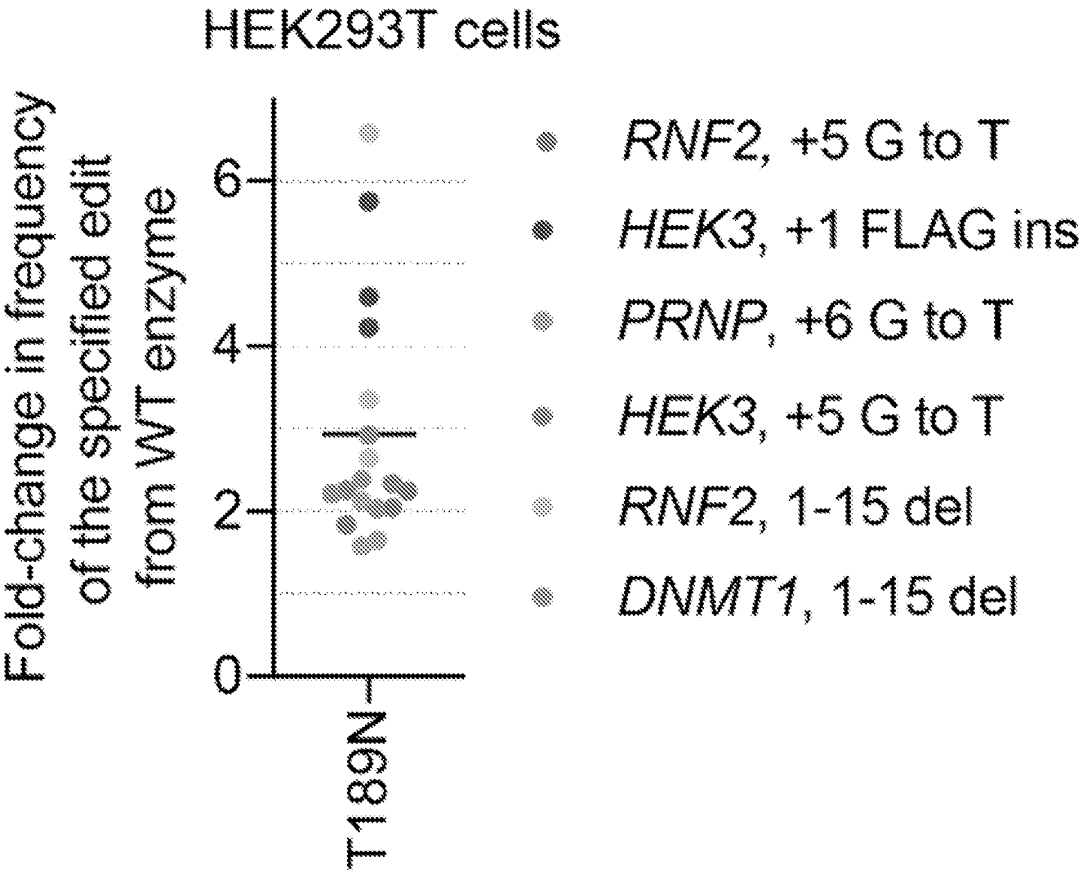


FIG. 50A

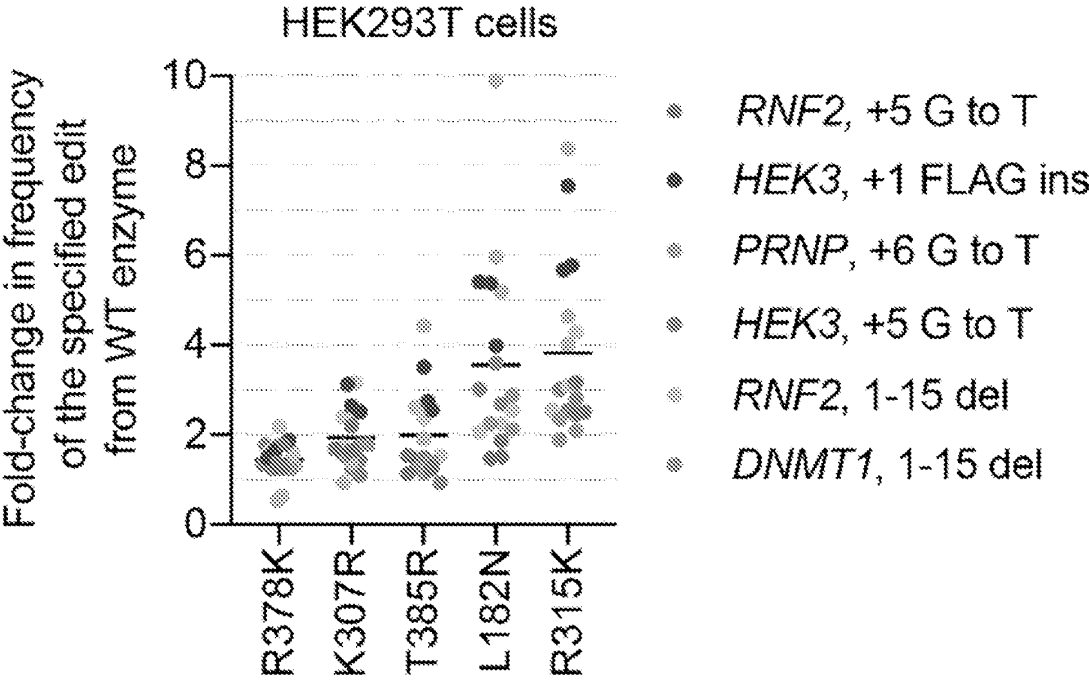


FIG. 50B

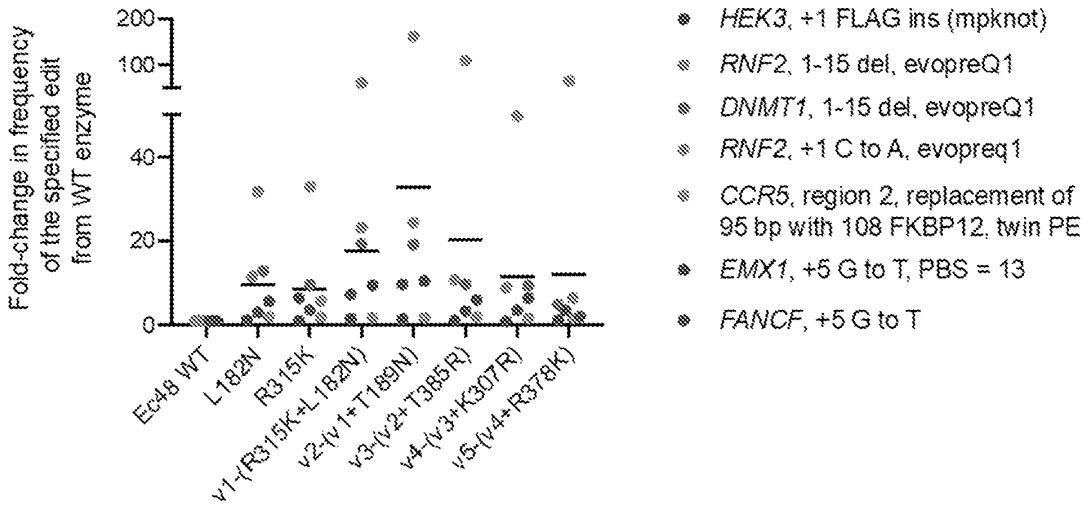


FIG. 51

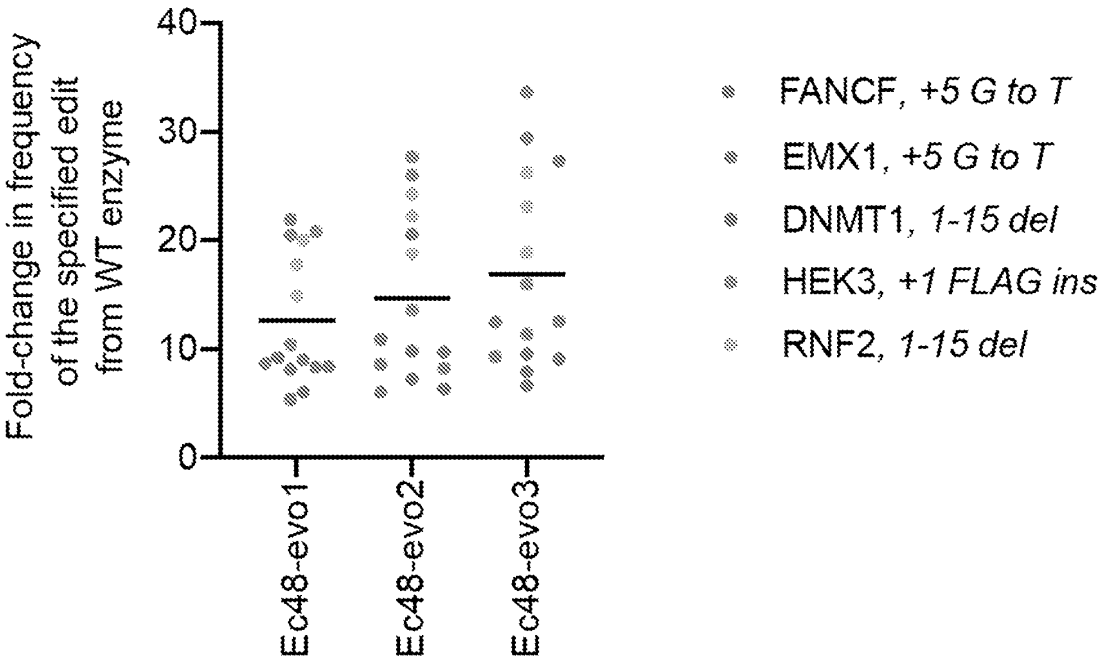


FIG. 52

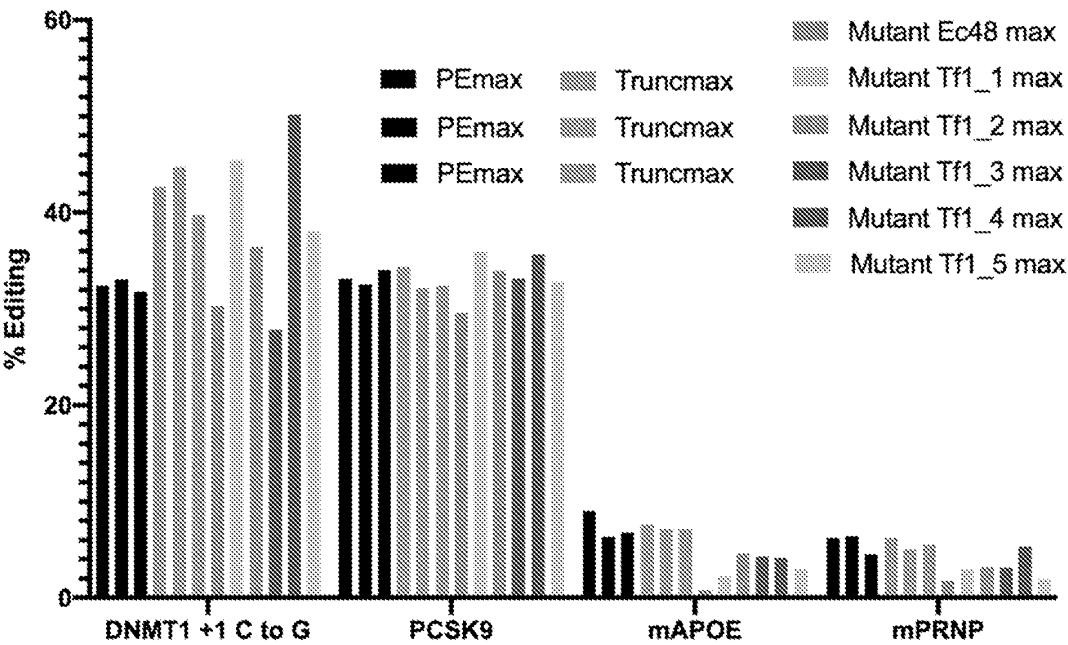


FIG. 53

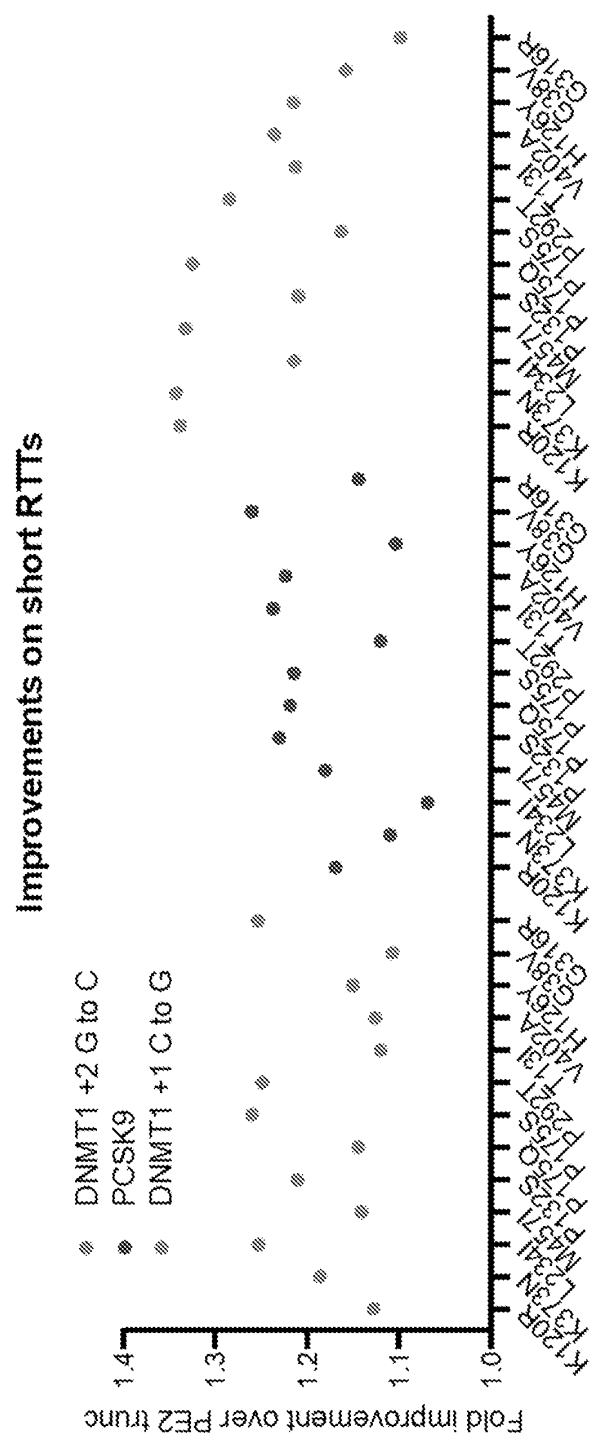


FIG. 54

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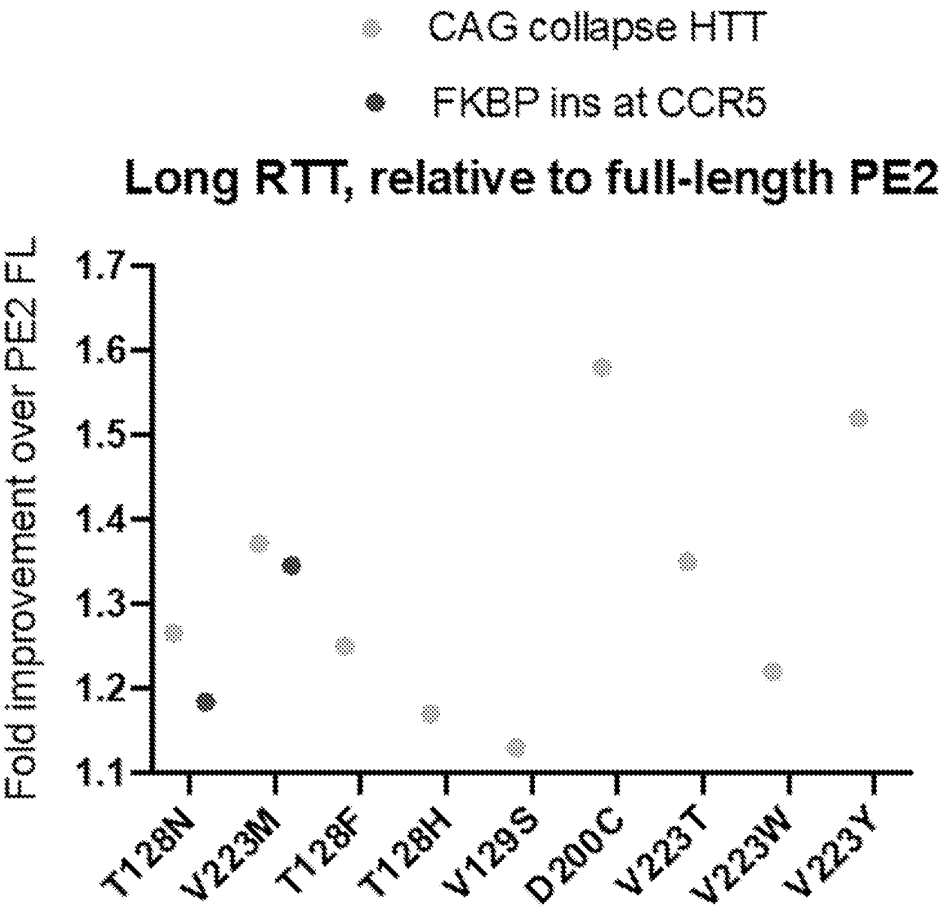
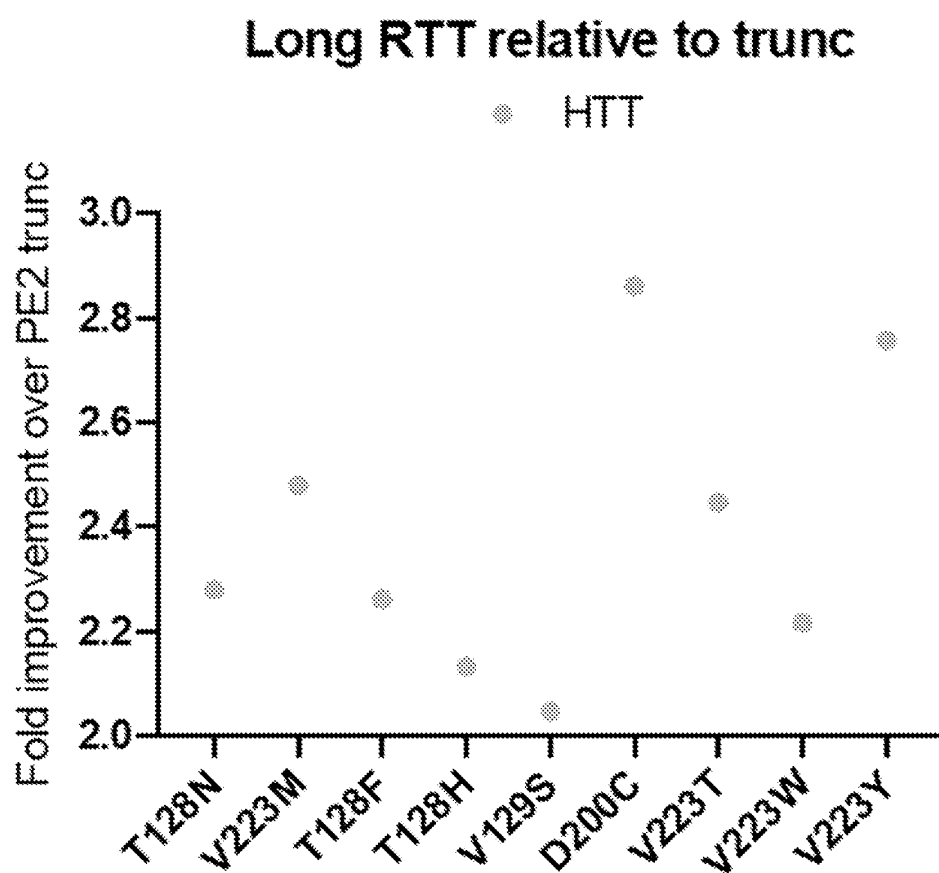


FIG. 55A

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**FIG. 55B**

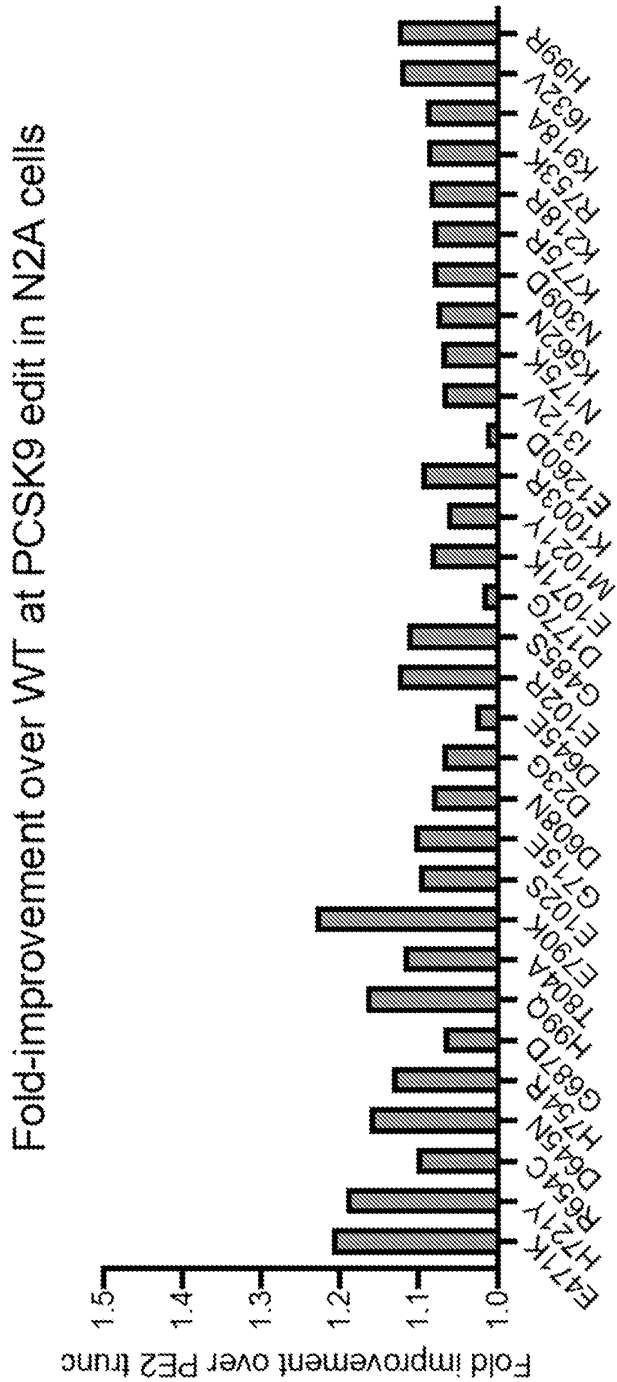


FIG. 56

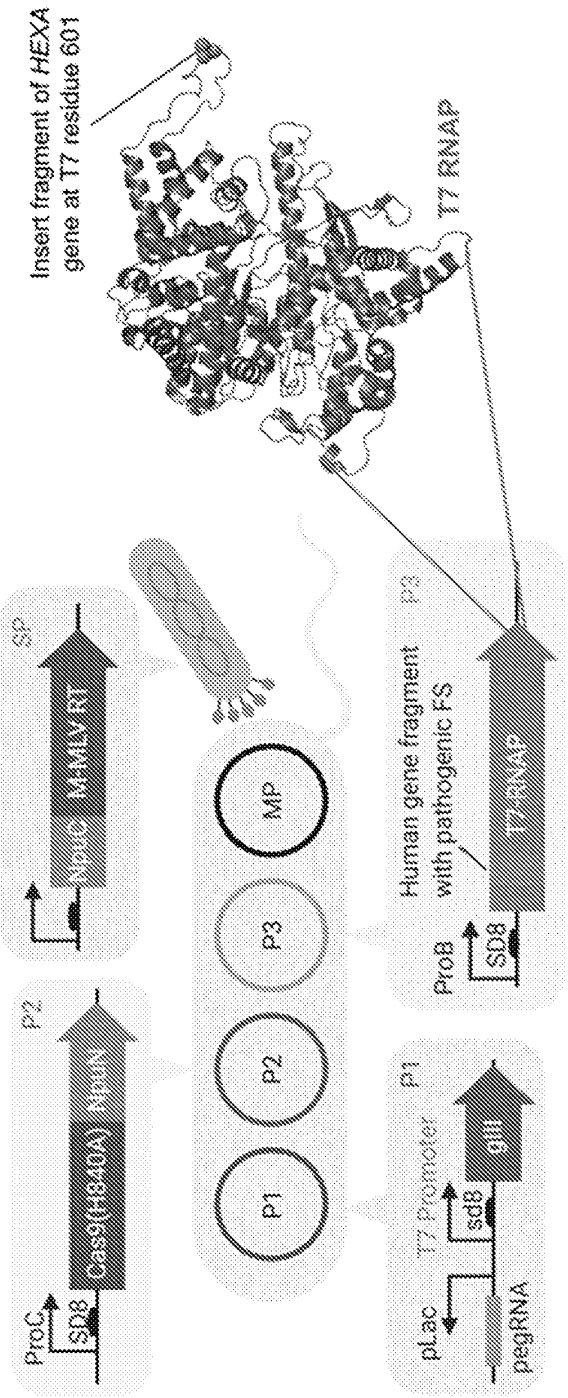


FIG. 57A

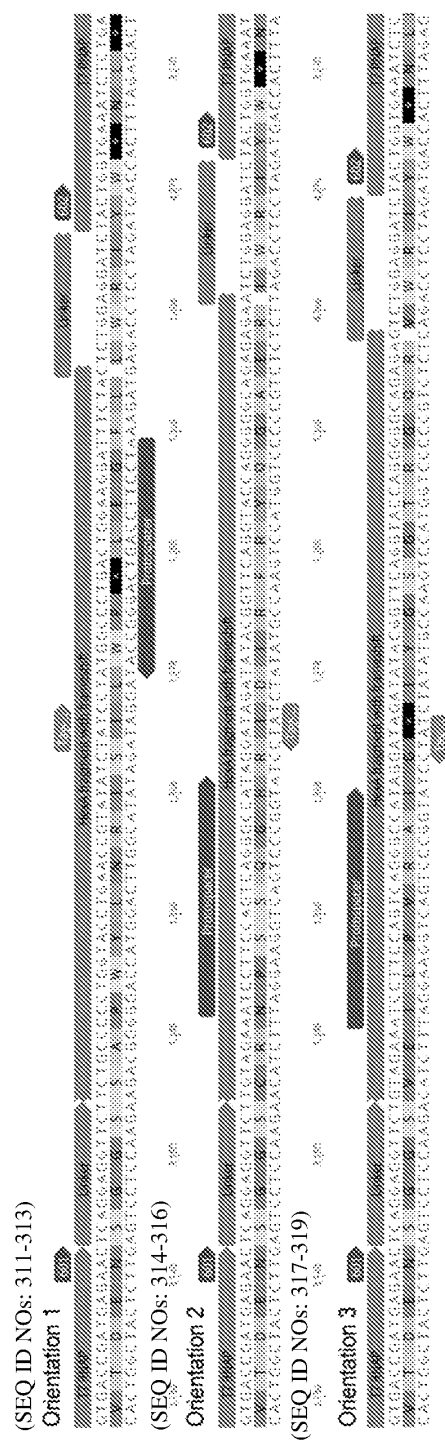


FIG. 57B

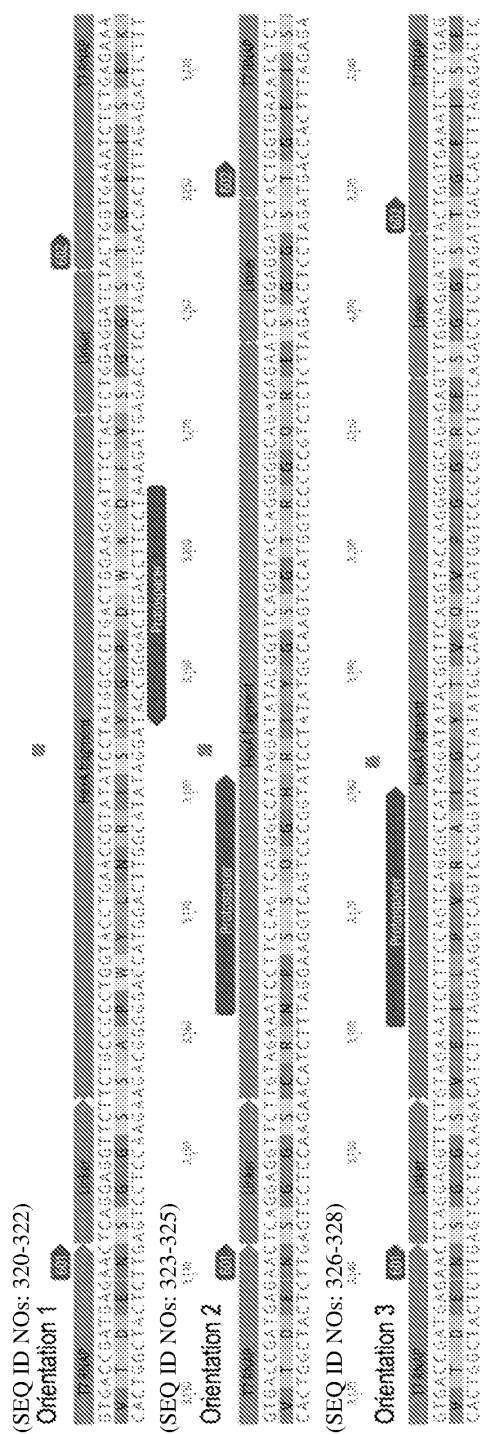


FIG. 57C

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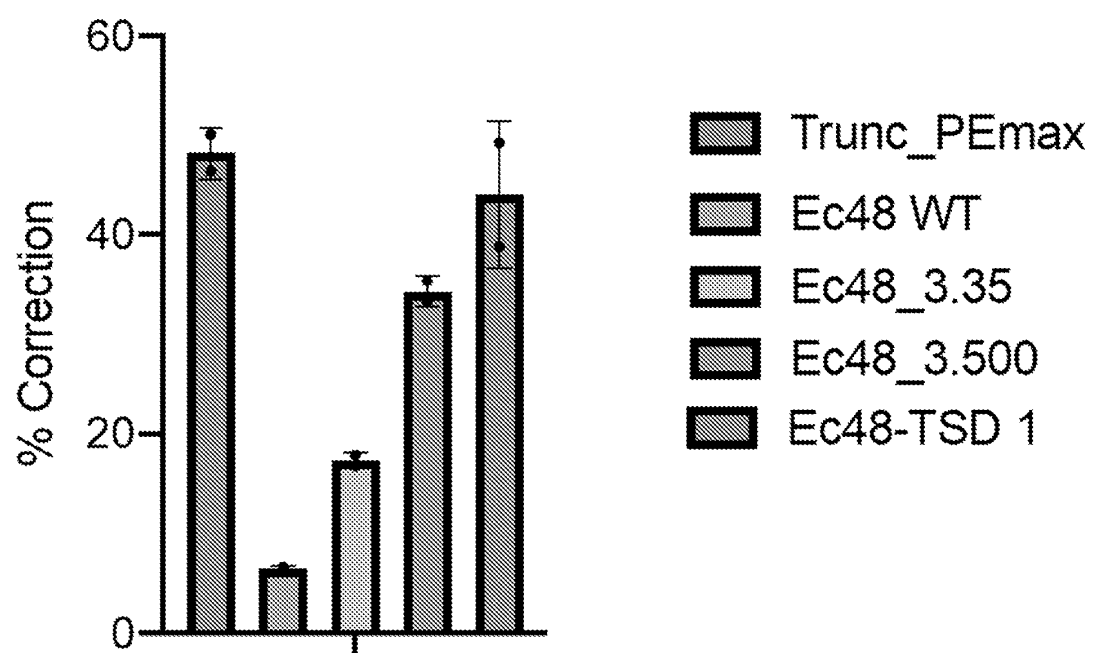


FIG. 58A

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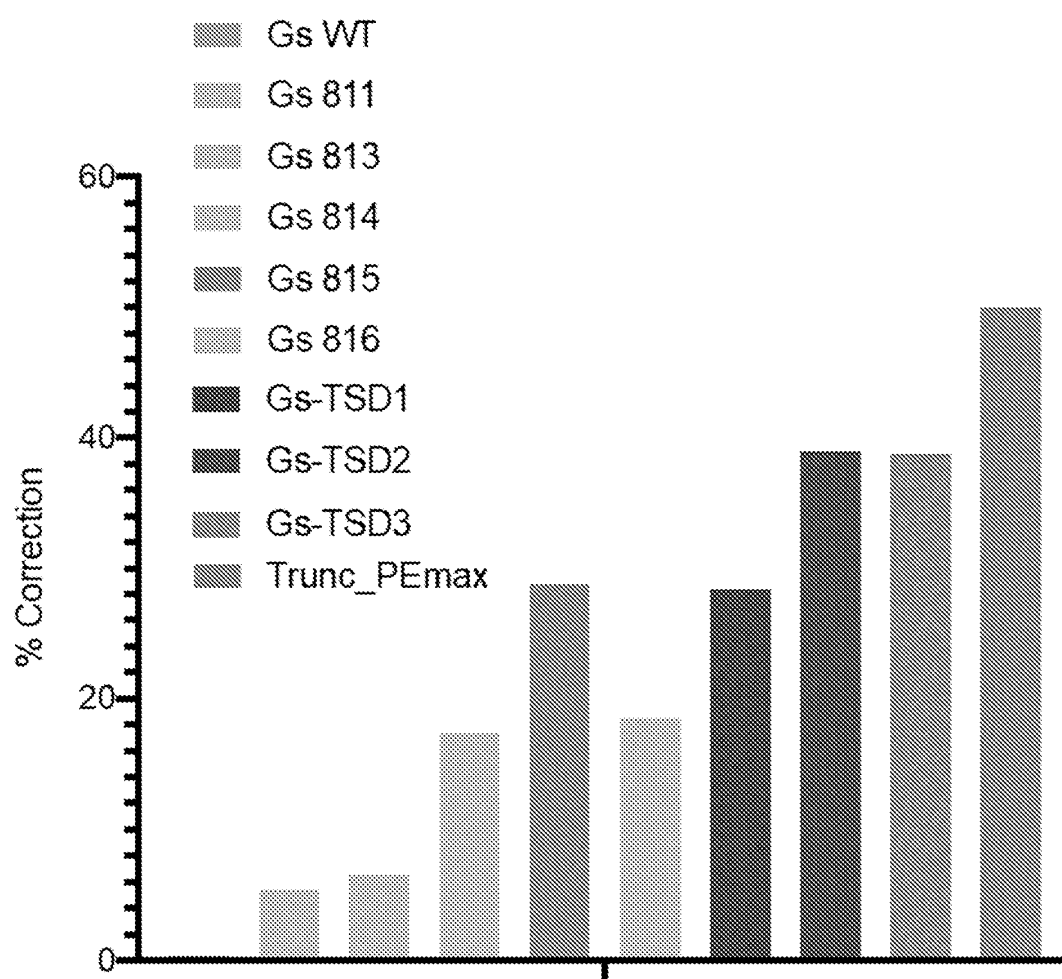


FIG. 58B

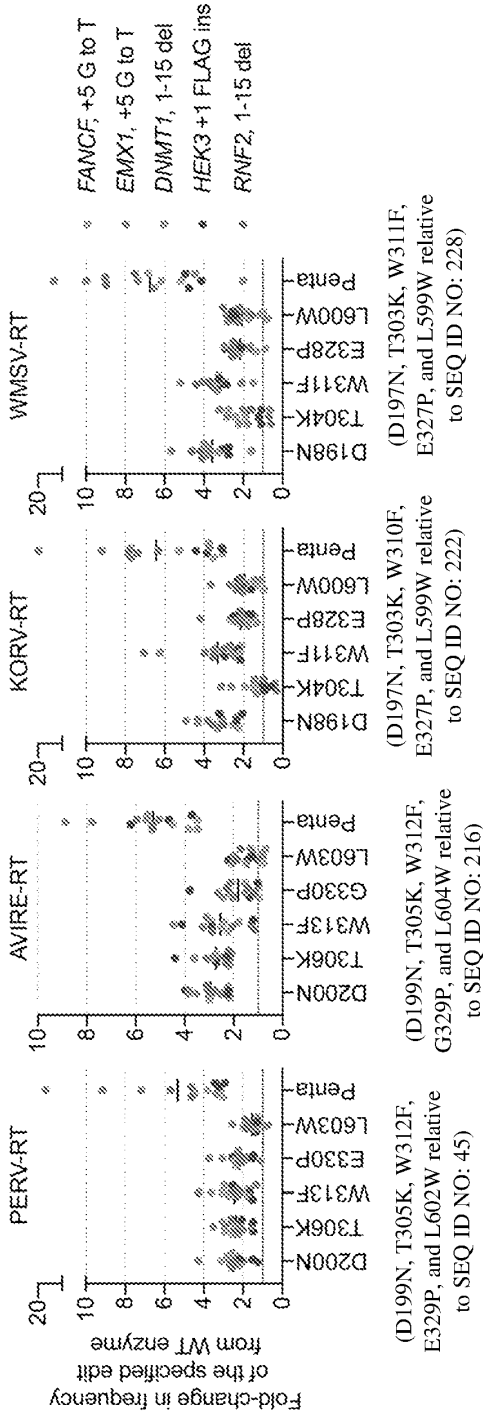


FIG. 59