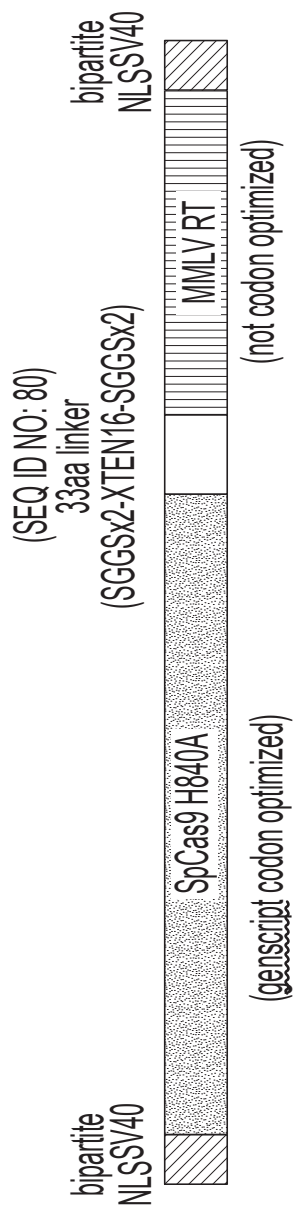
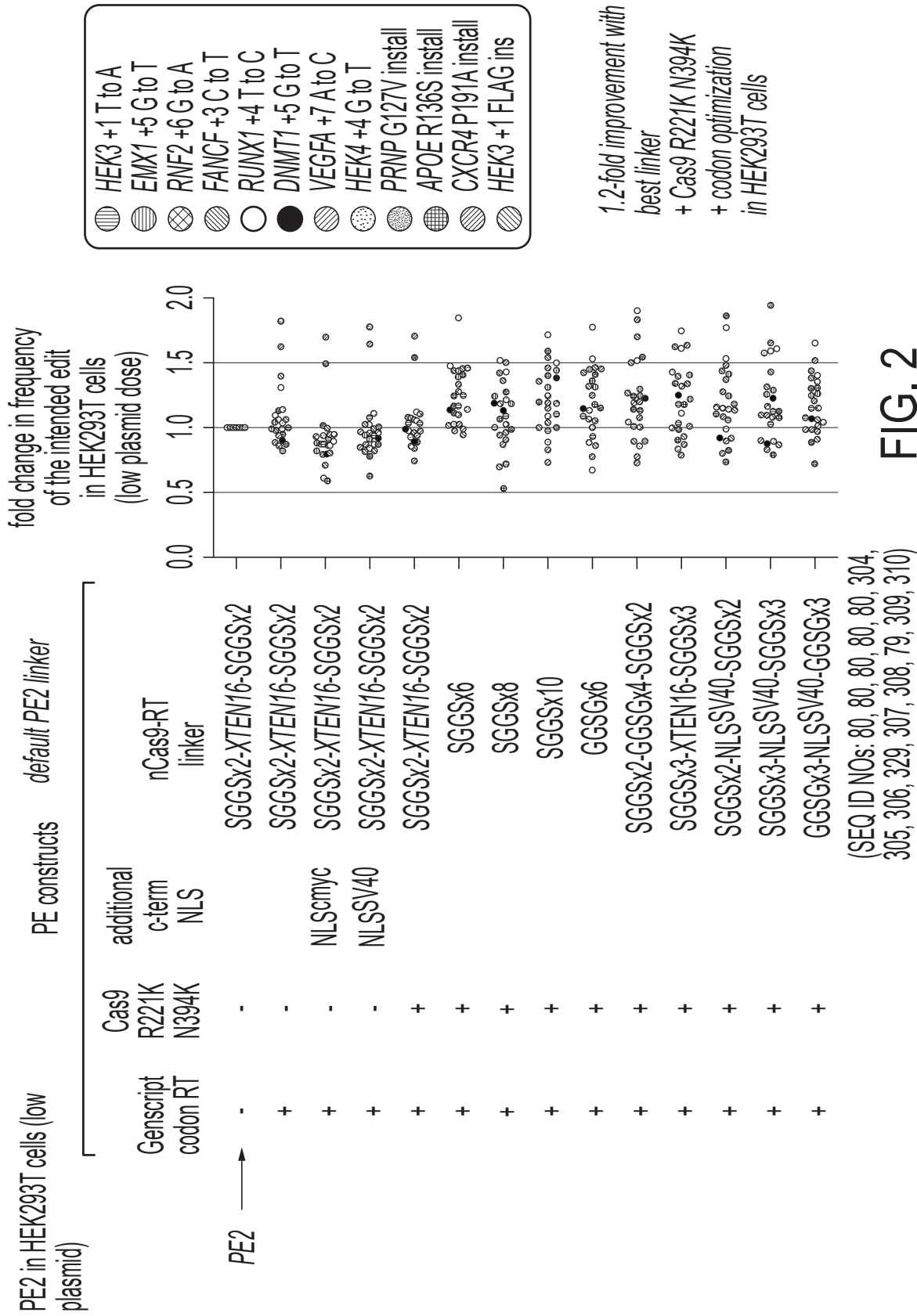


Optimization of PE2 protein



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

FIG. 1



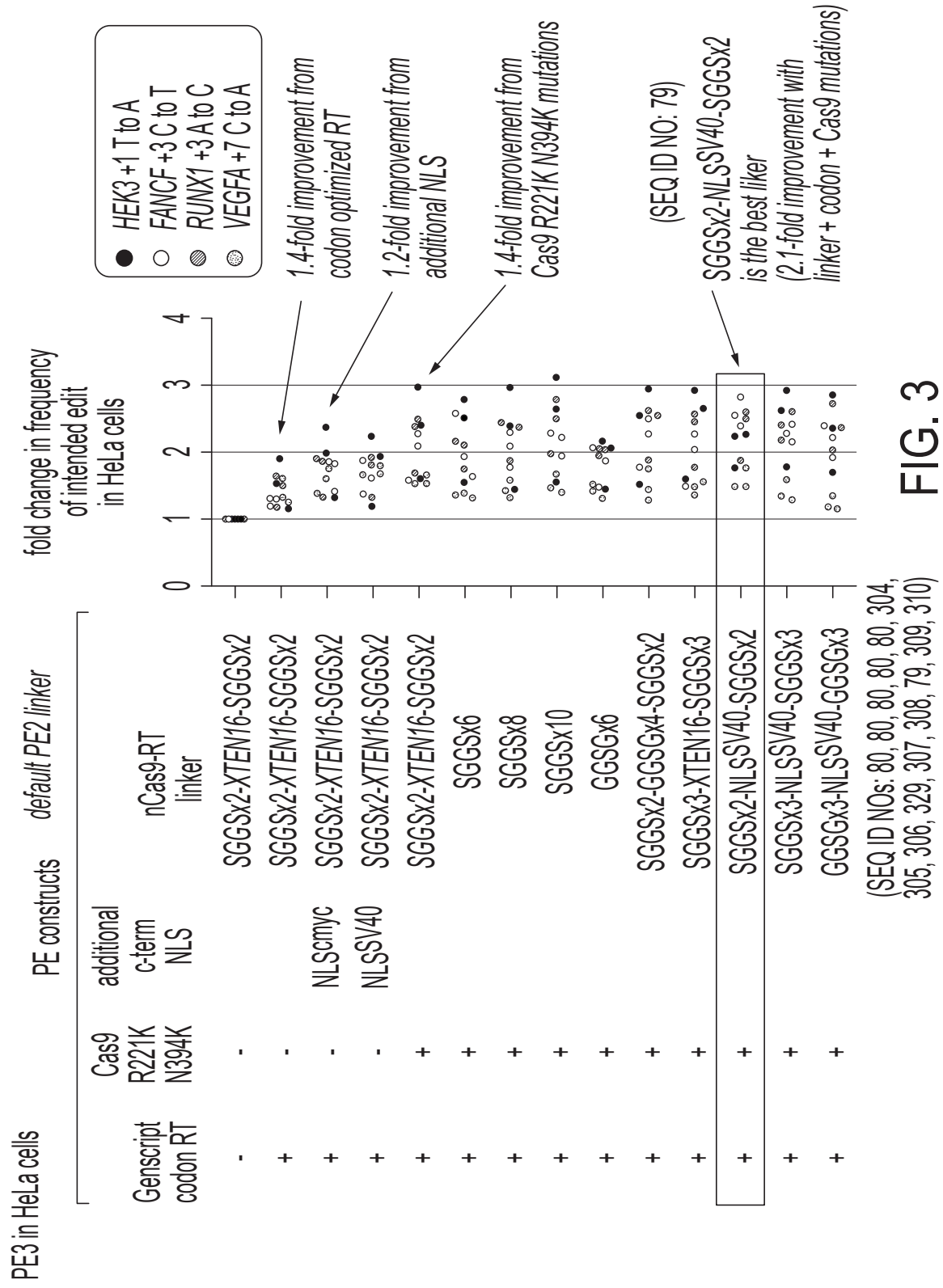
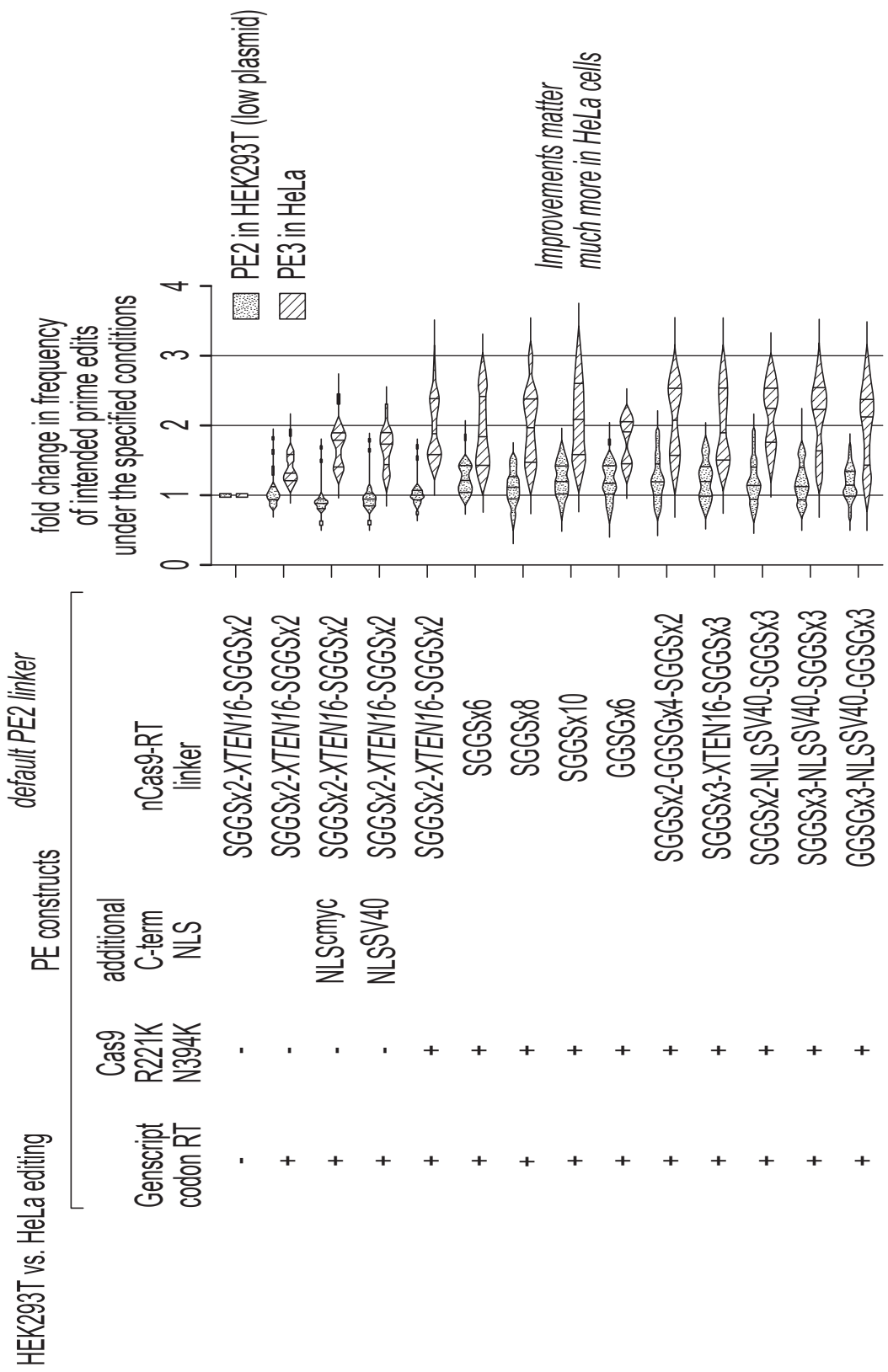


FIG. 3



(SEQ ID NOs: 80, 80, 80, 80, 80, 304, 305, 306, 329, 307, 308, 79, 309, 310)

FIG. 4

PE3 in HeLa cells, NLS architecture optimization

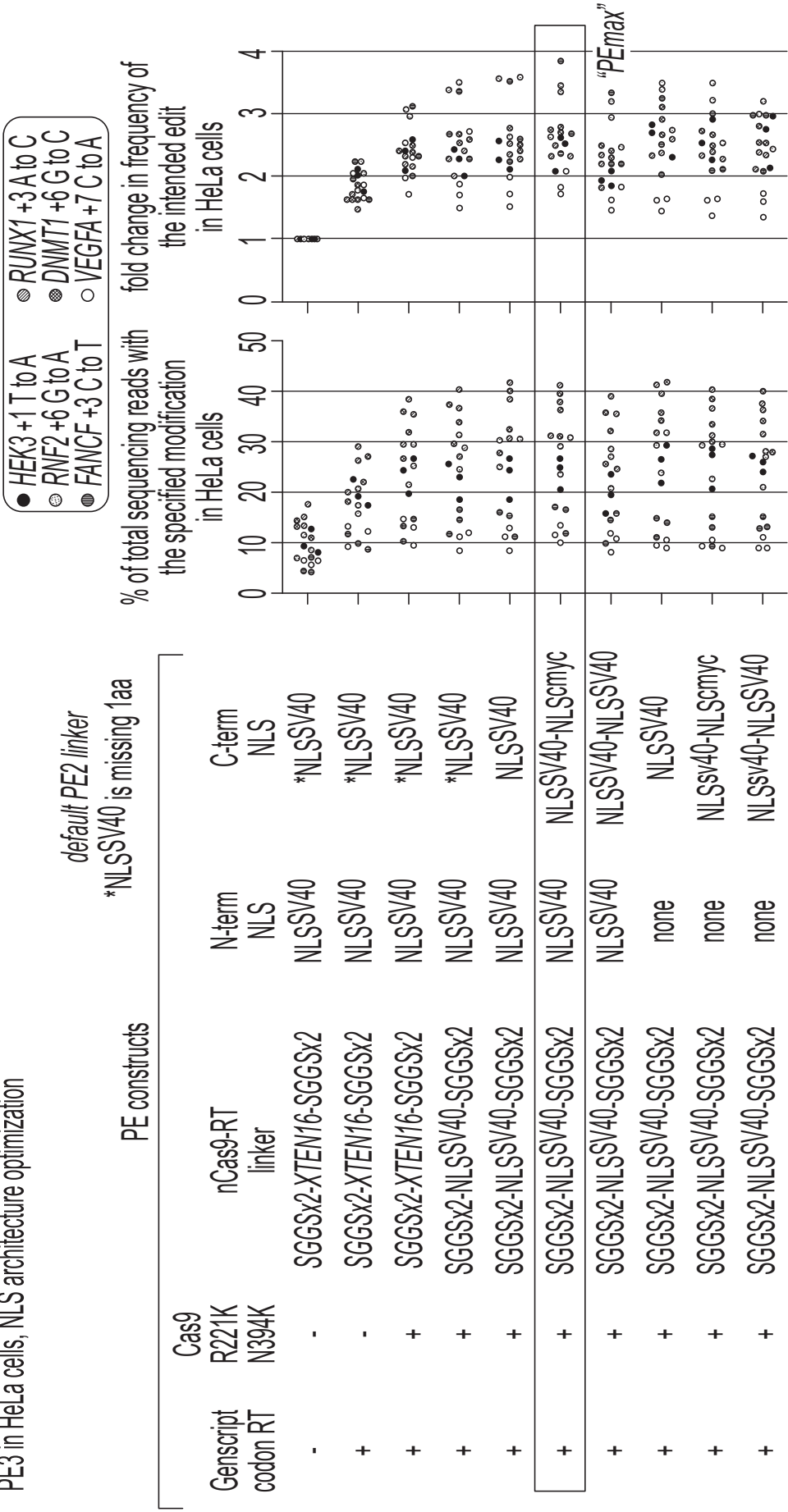


FIG. 5

Final PEmax construct

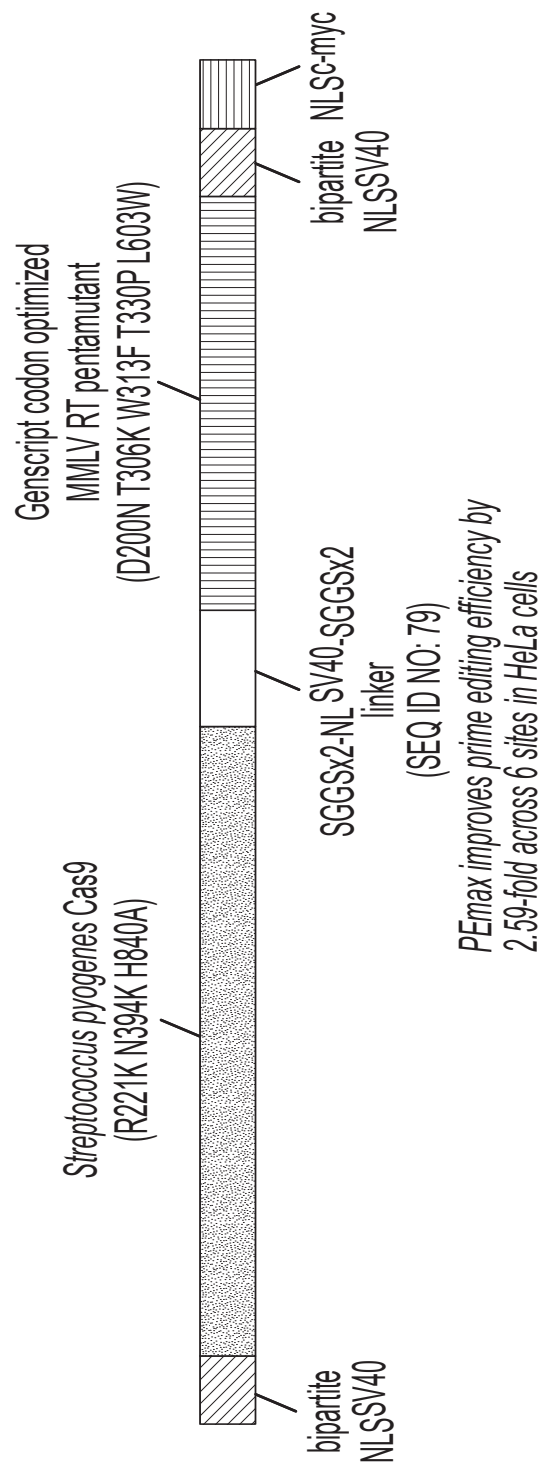


FIG. 6

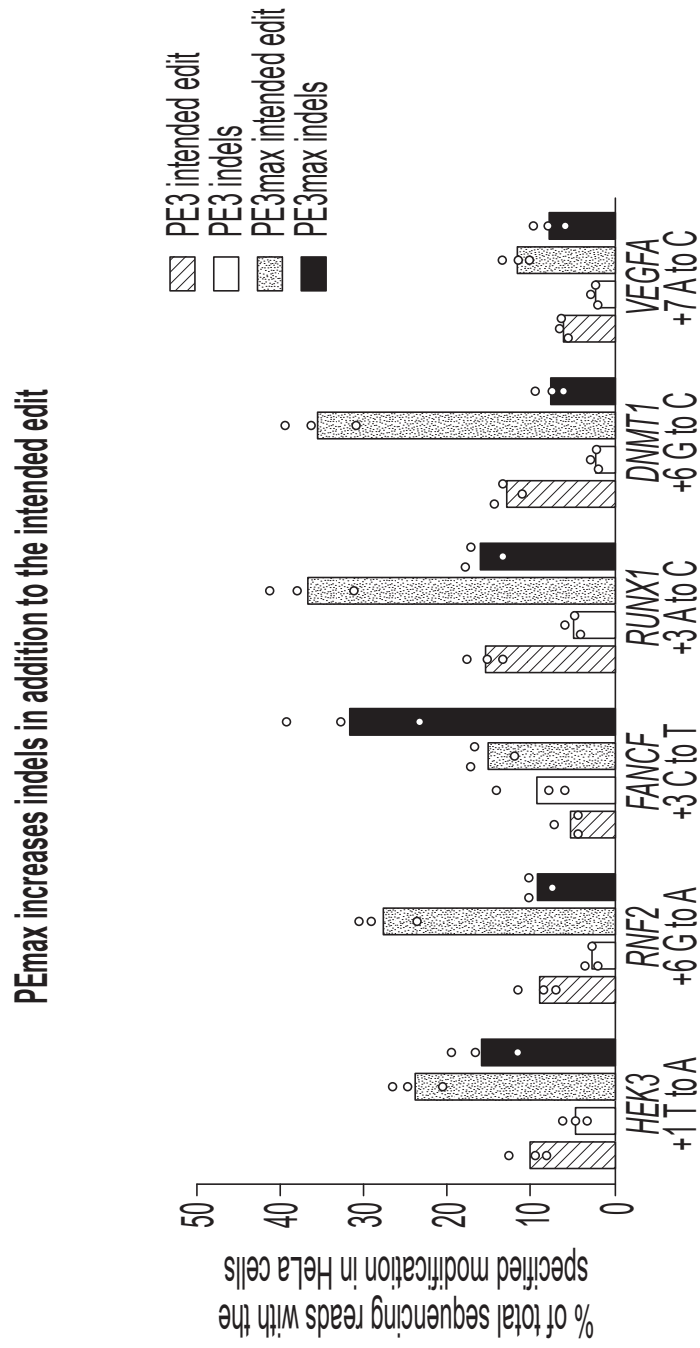
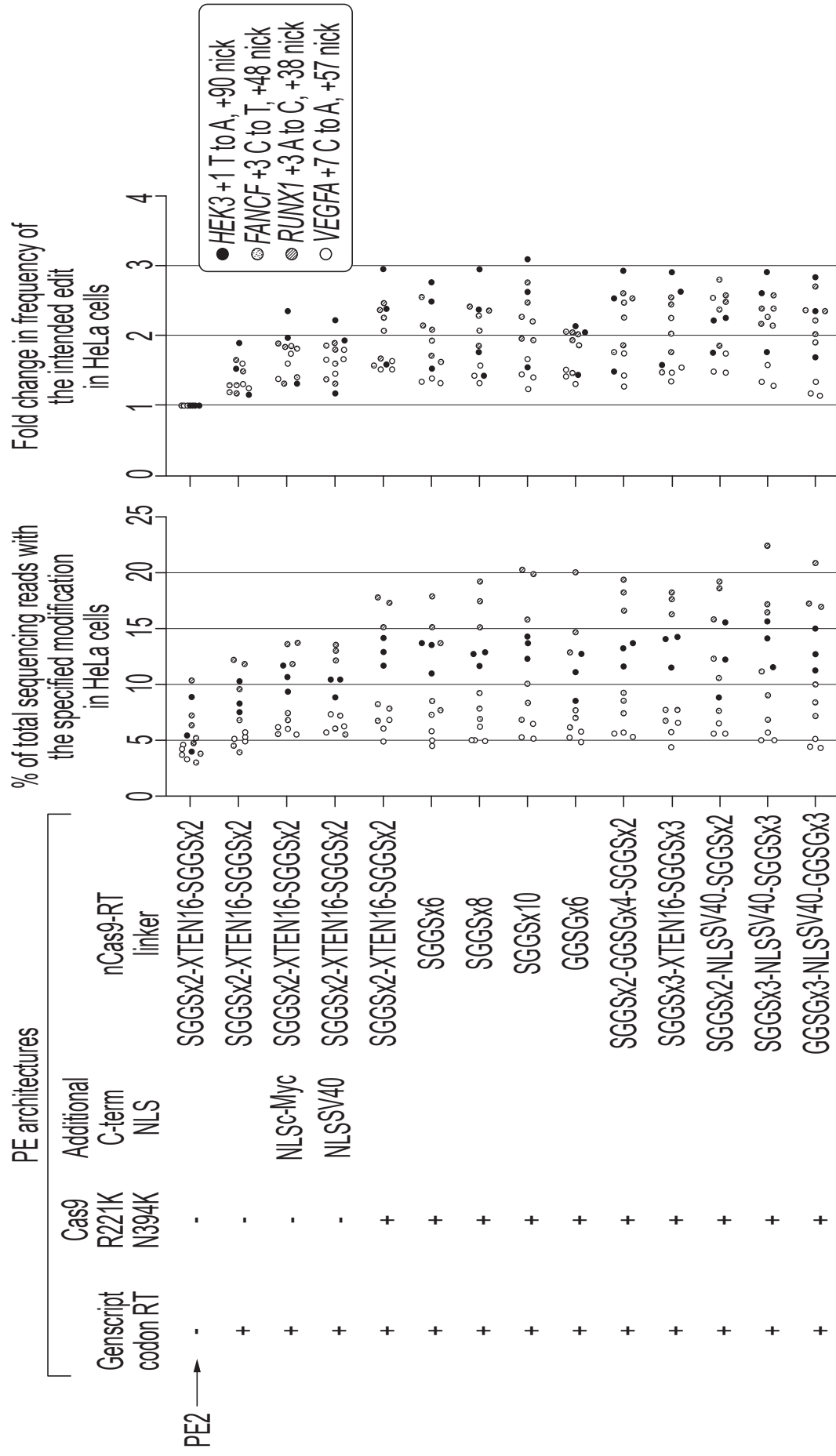


FIG. 7



(SEQ ID NOs: 80, 80, 80, 80, 304,
305, 306, 329, 307, 308, 79, 309, 310)

FIG. 8A

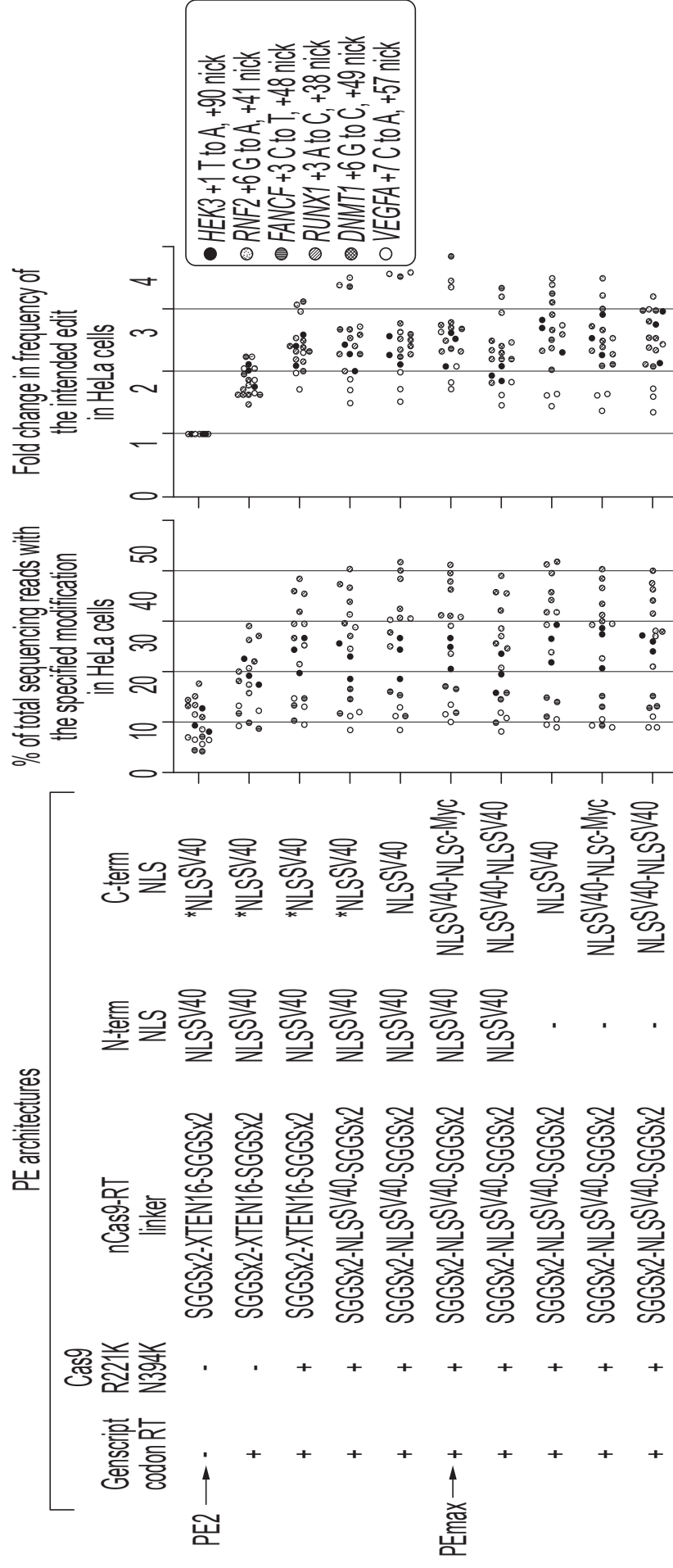


FIG. 8B

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

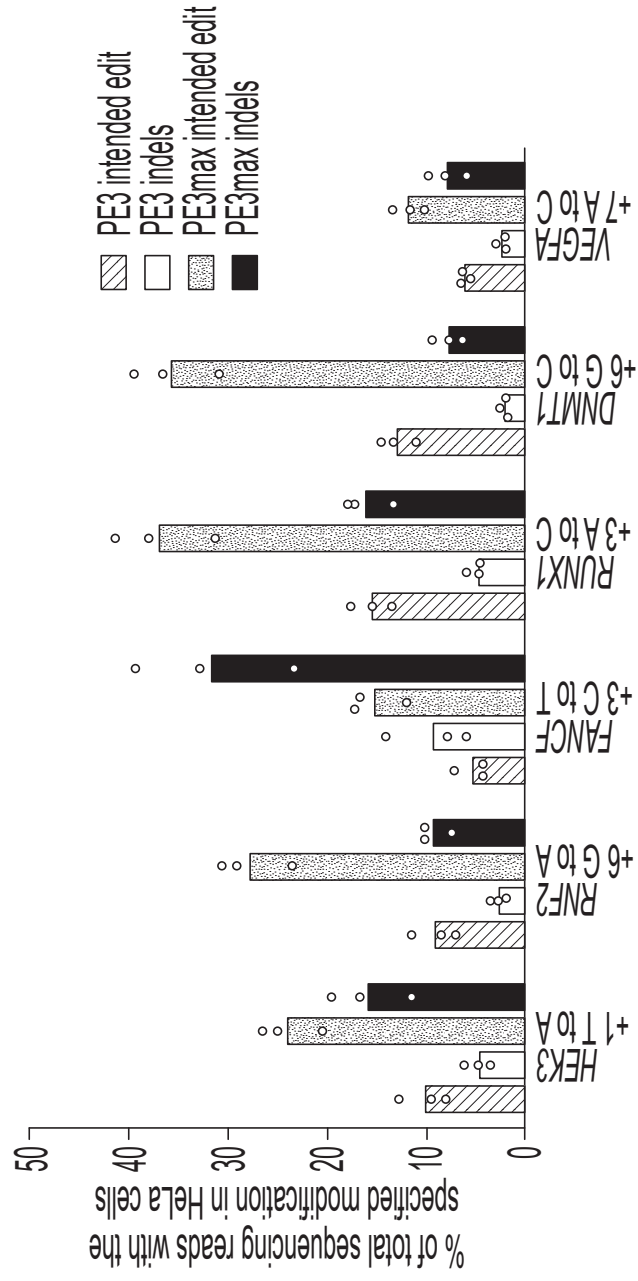


FIG. 8C

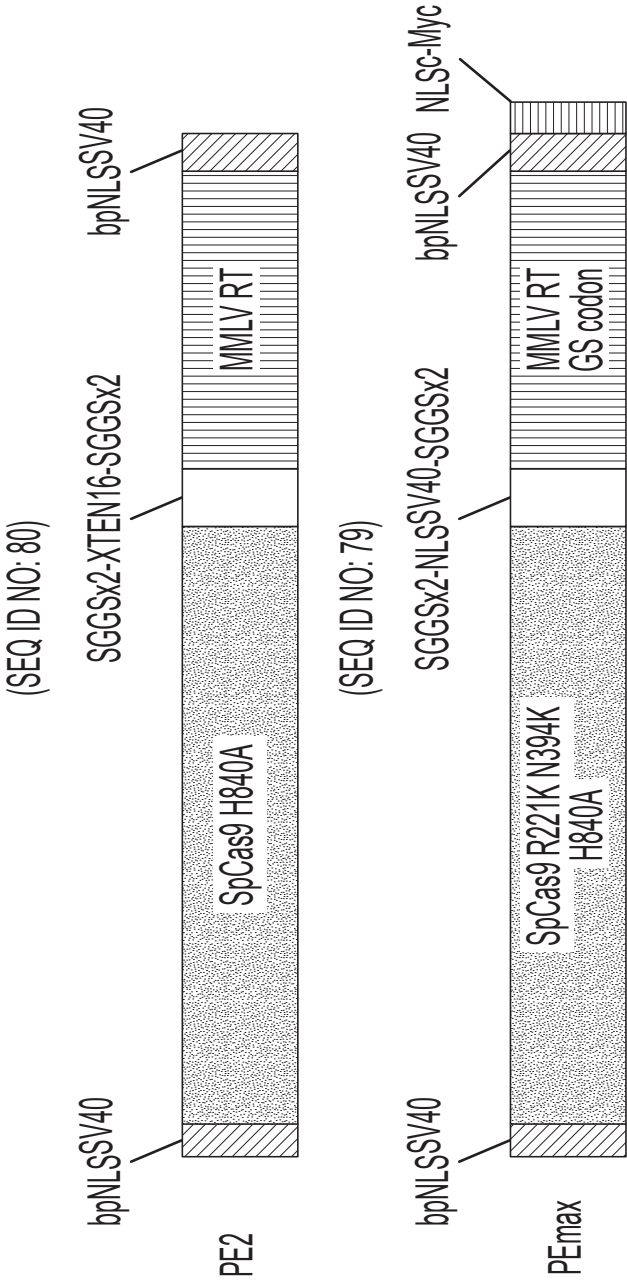


FIG. 9

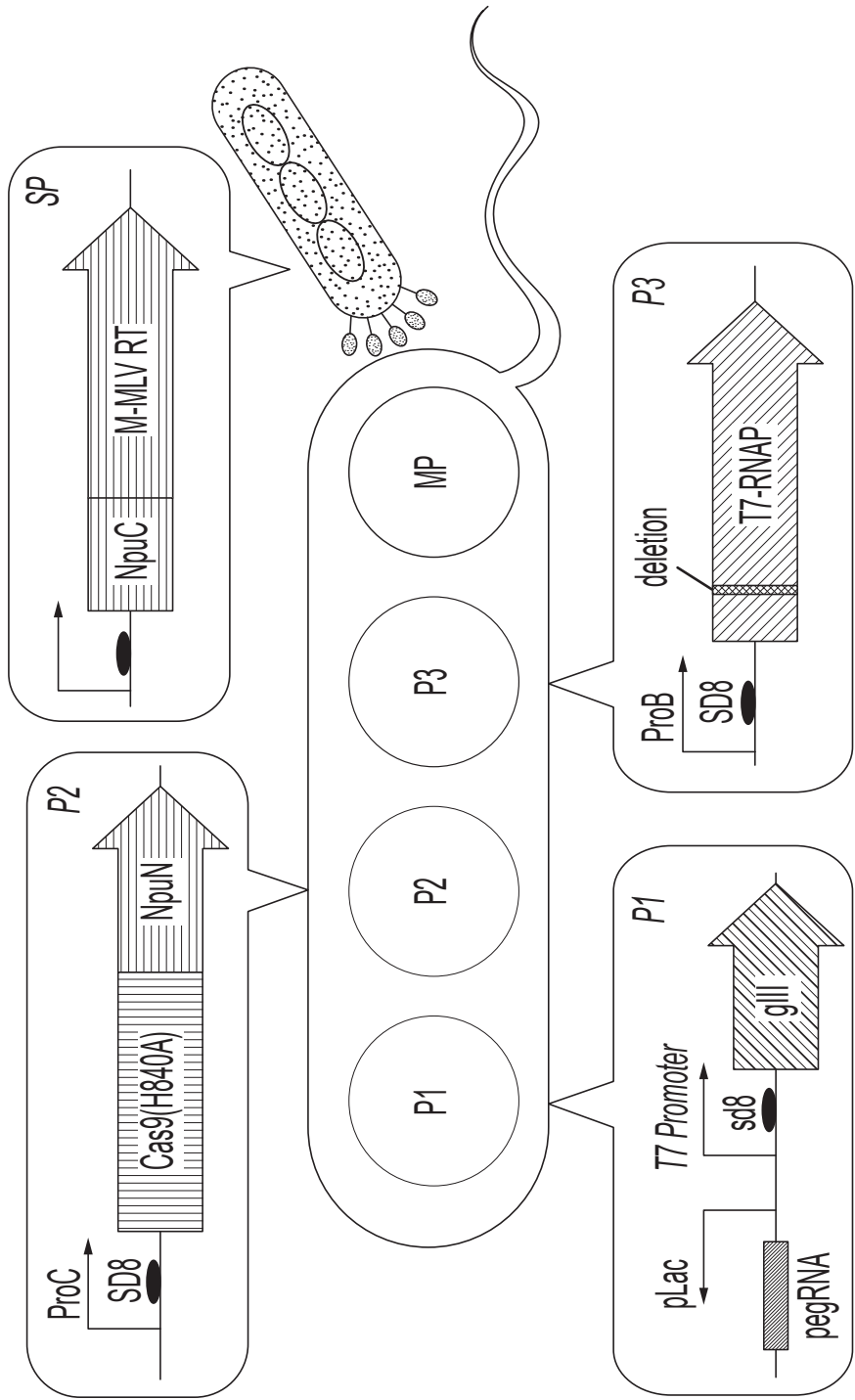


FIG. 10

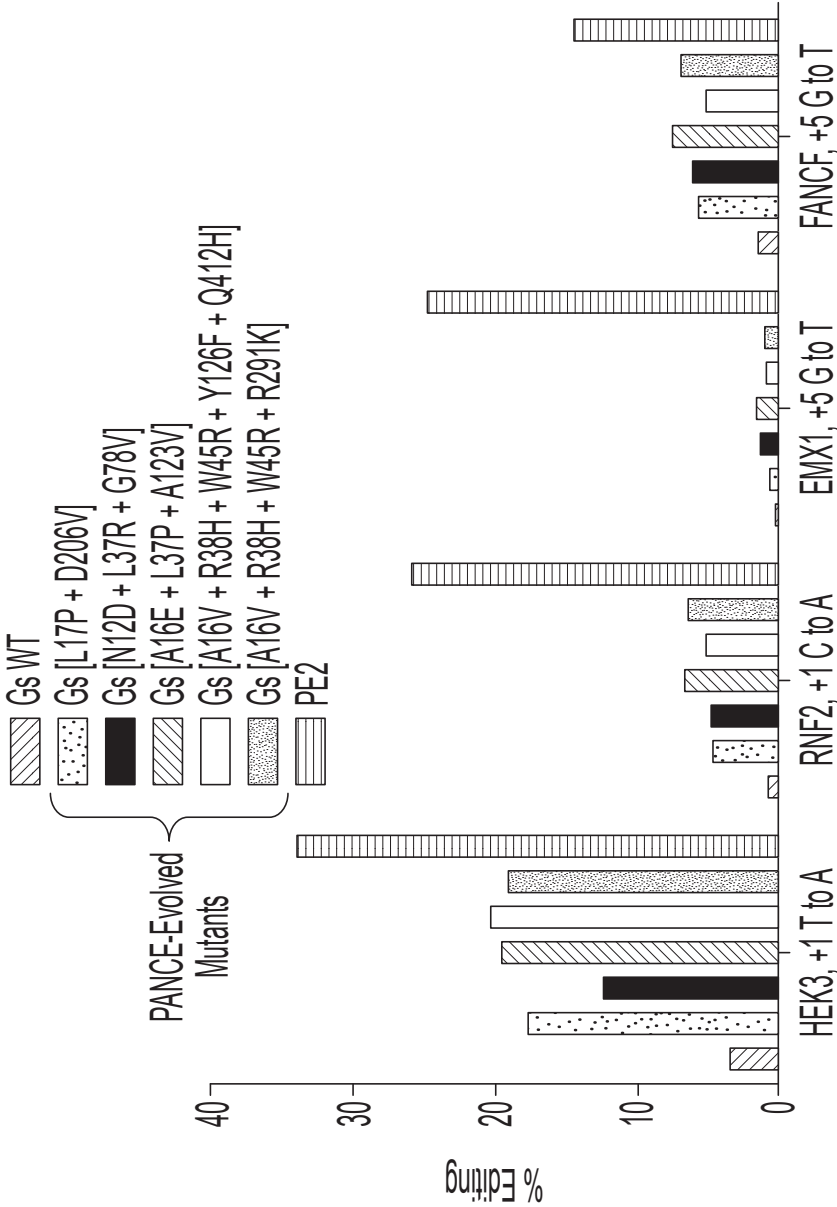


FIG. 11

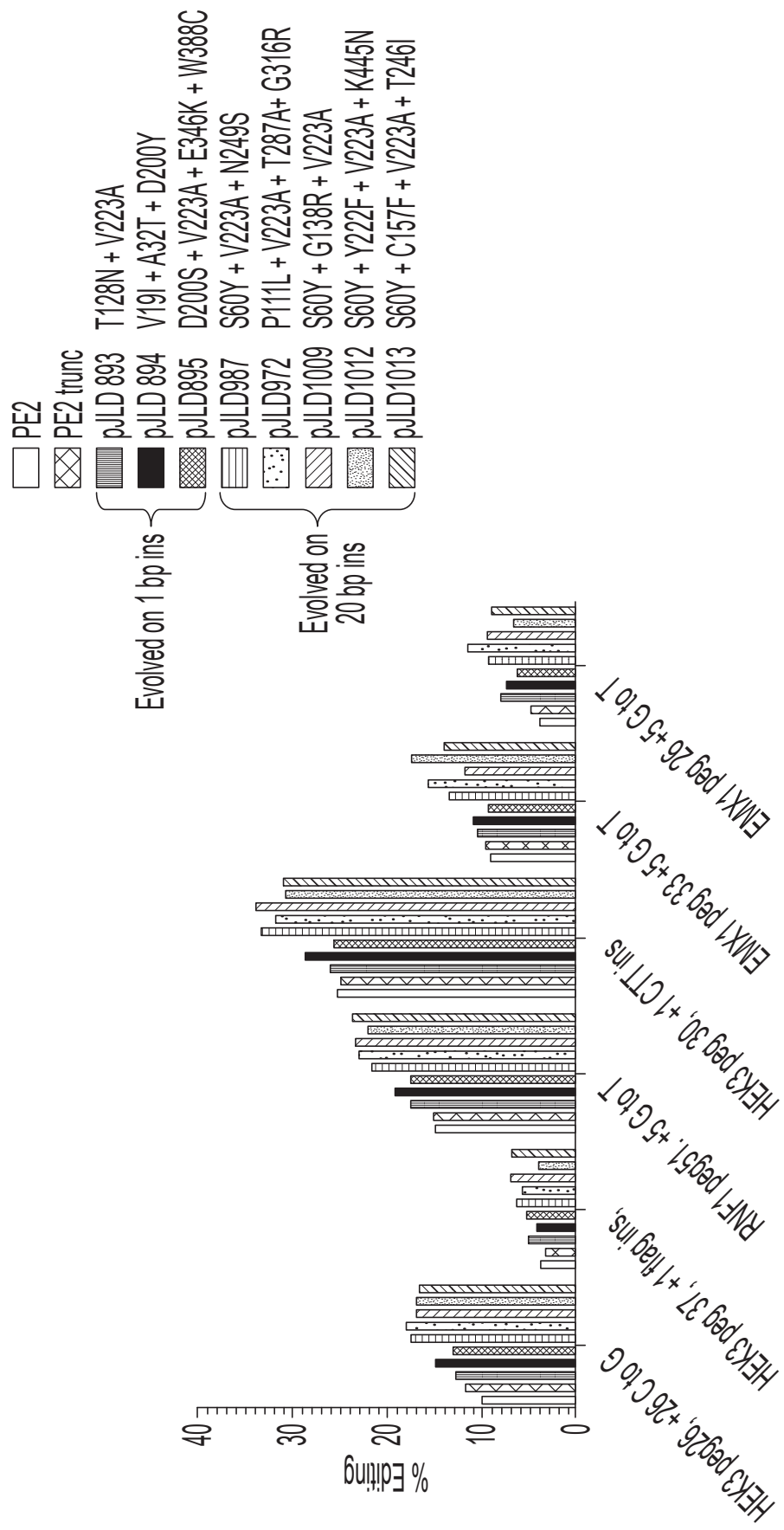


FIG. 12

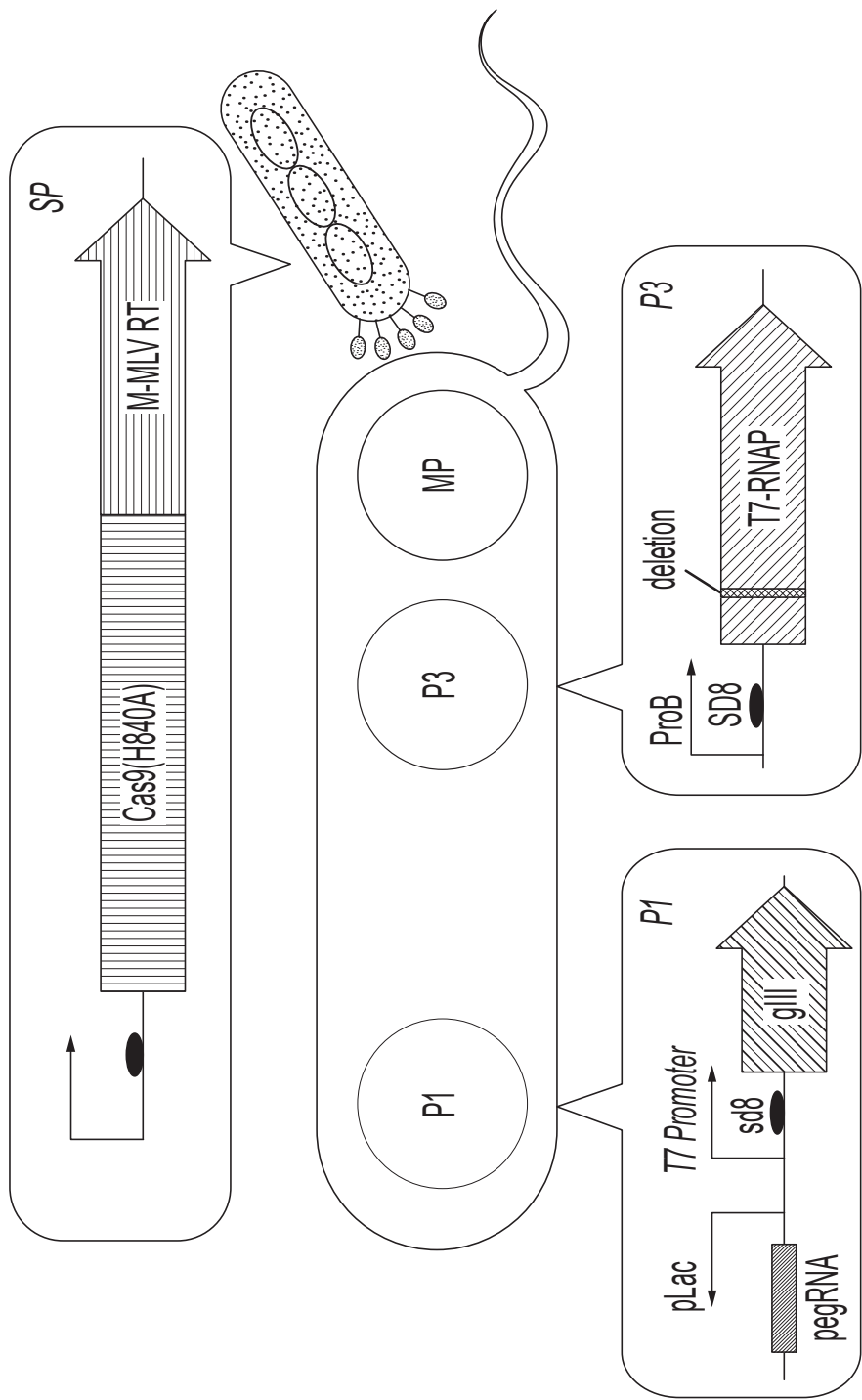


FIG. 13

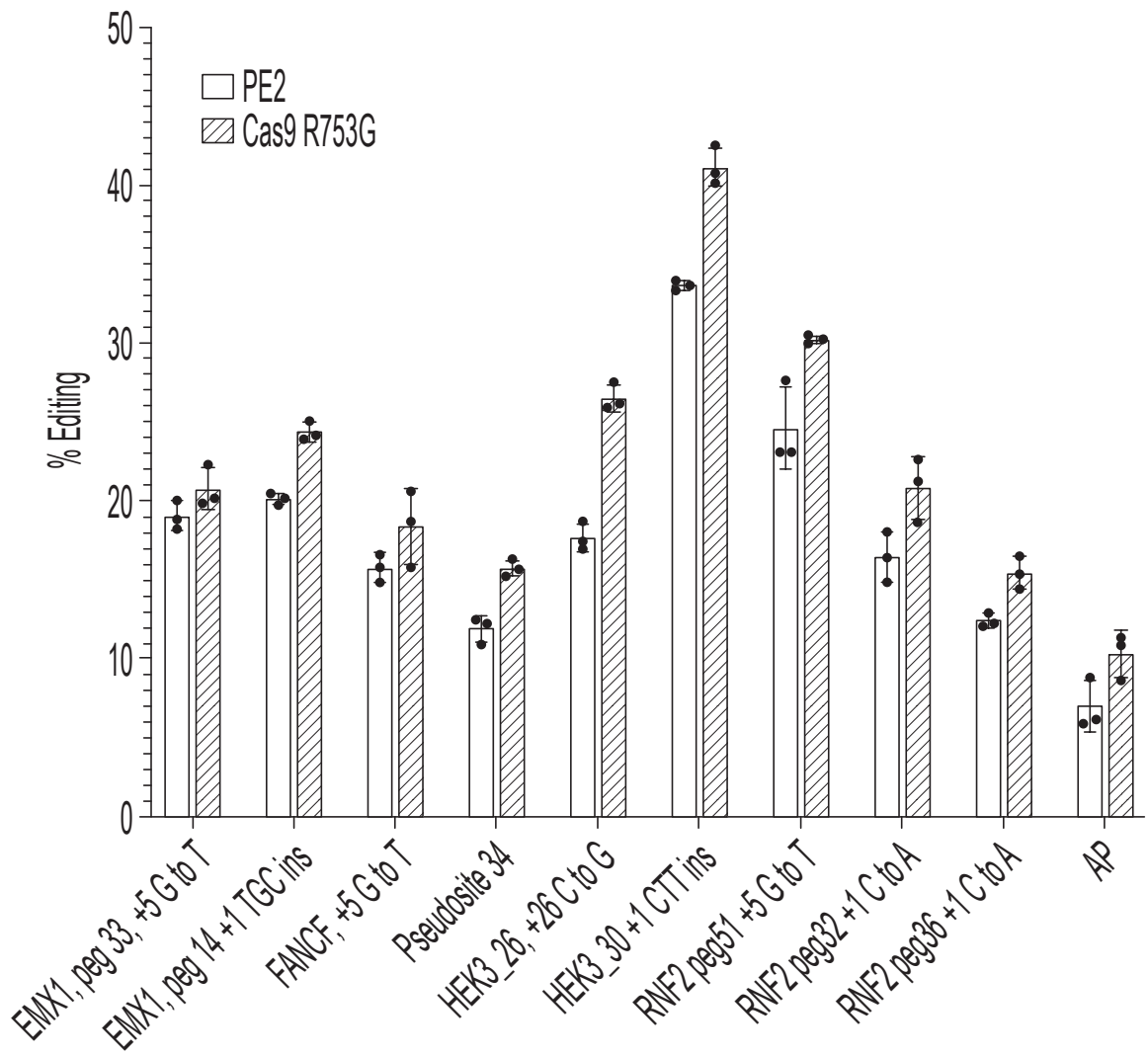


FIG. 14

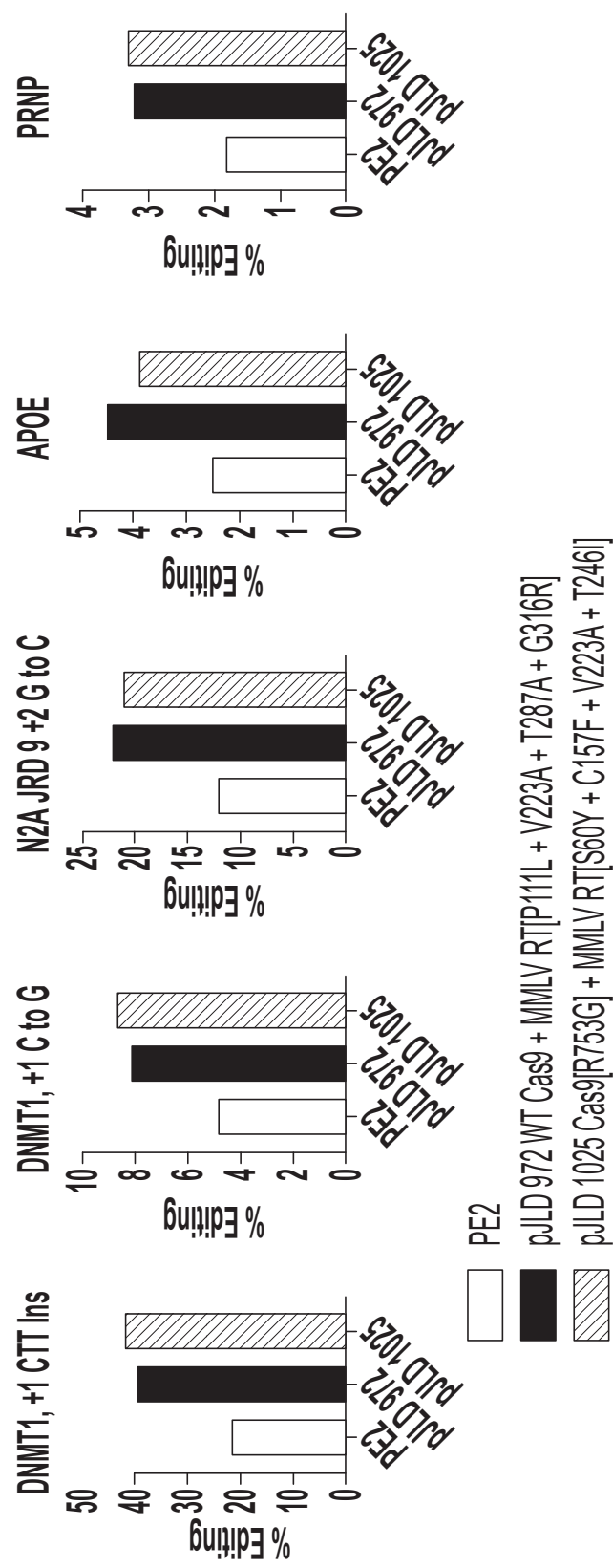


FIG. 15

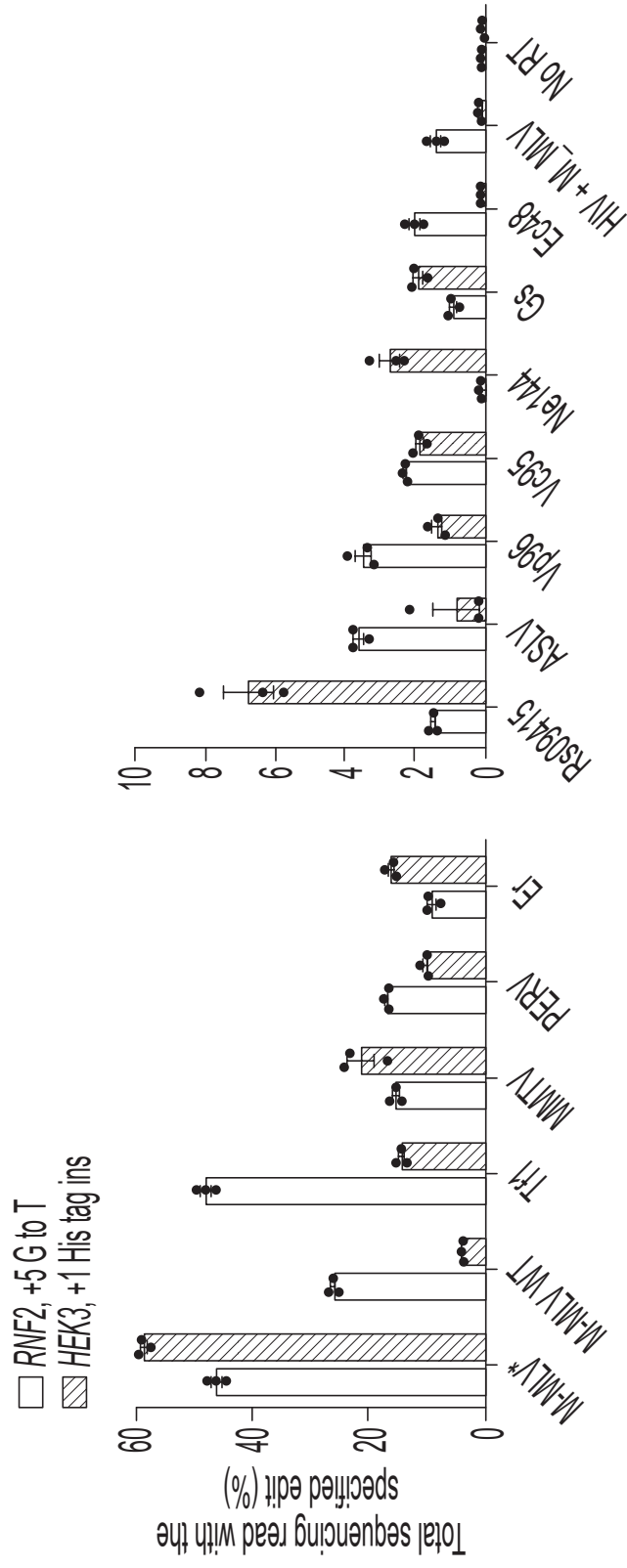


FIG. 16

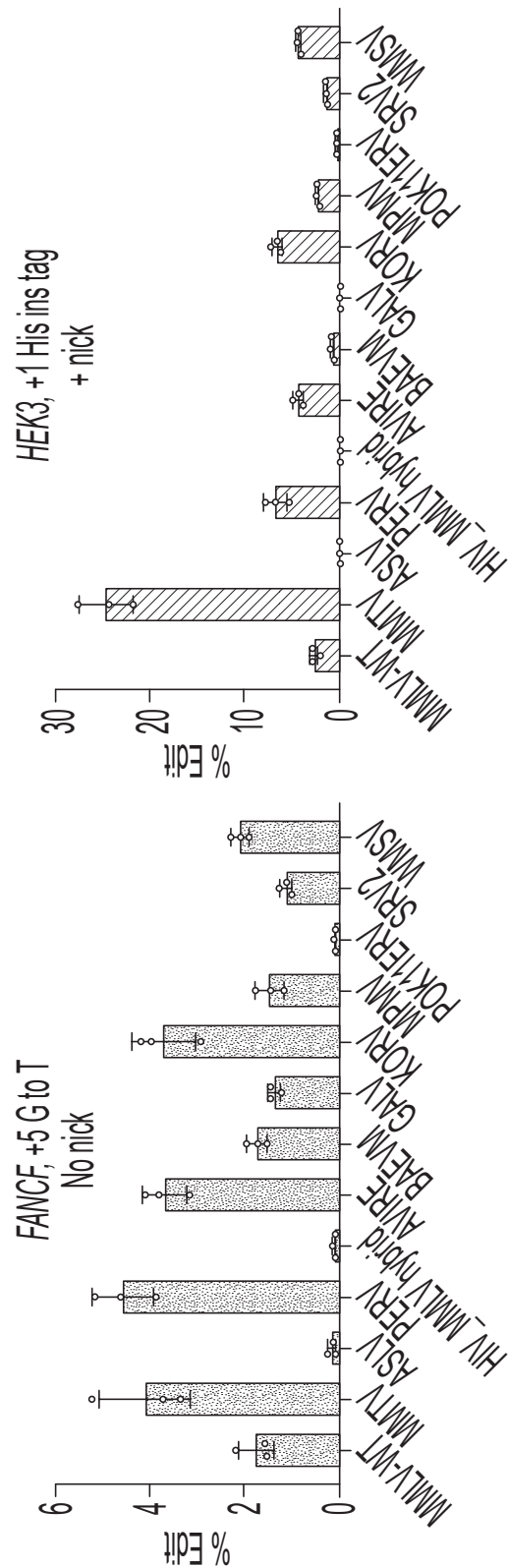


FIG. 17

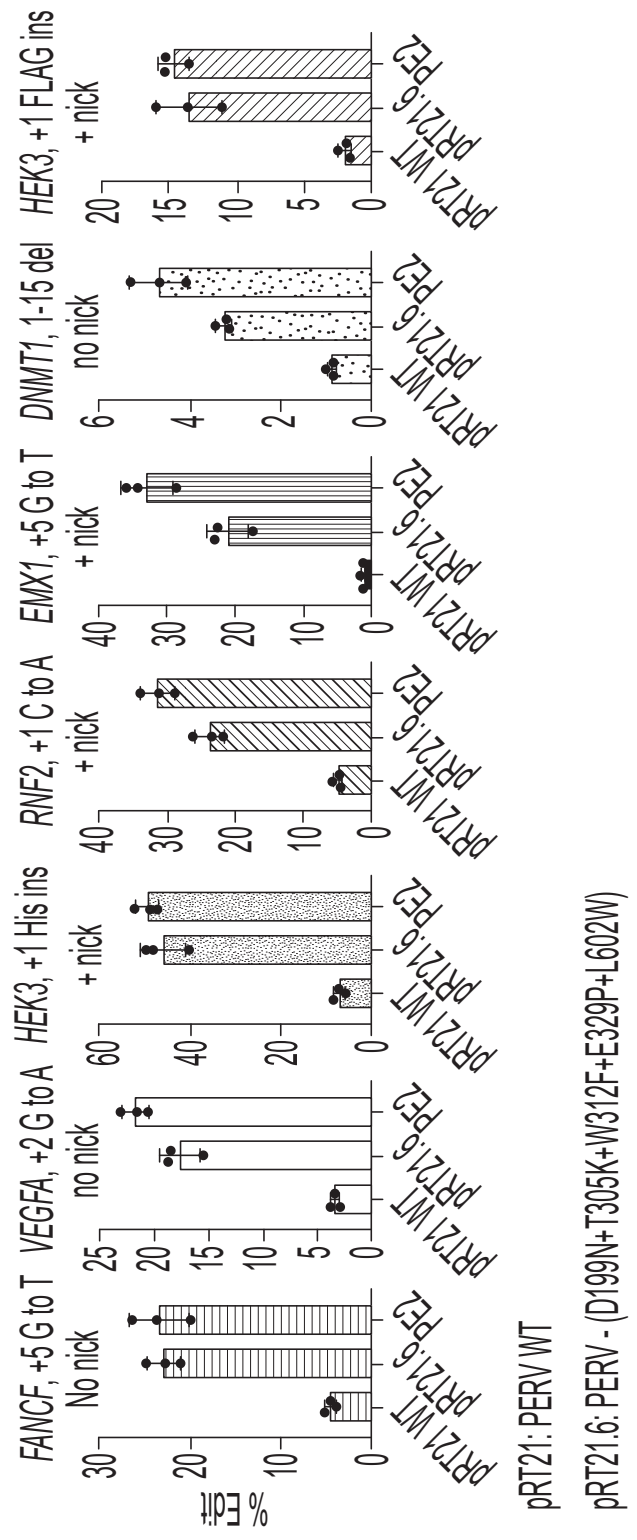


FIG. 18

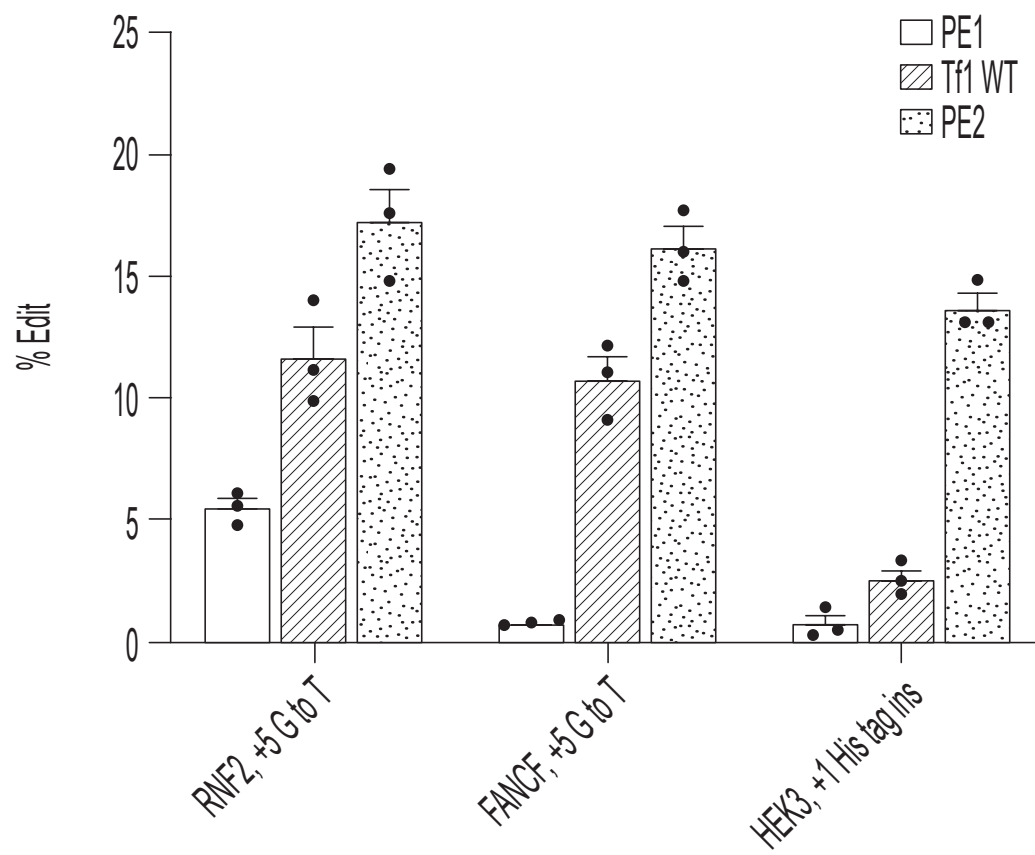


FIG. 19

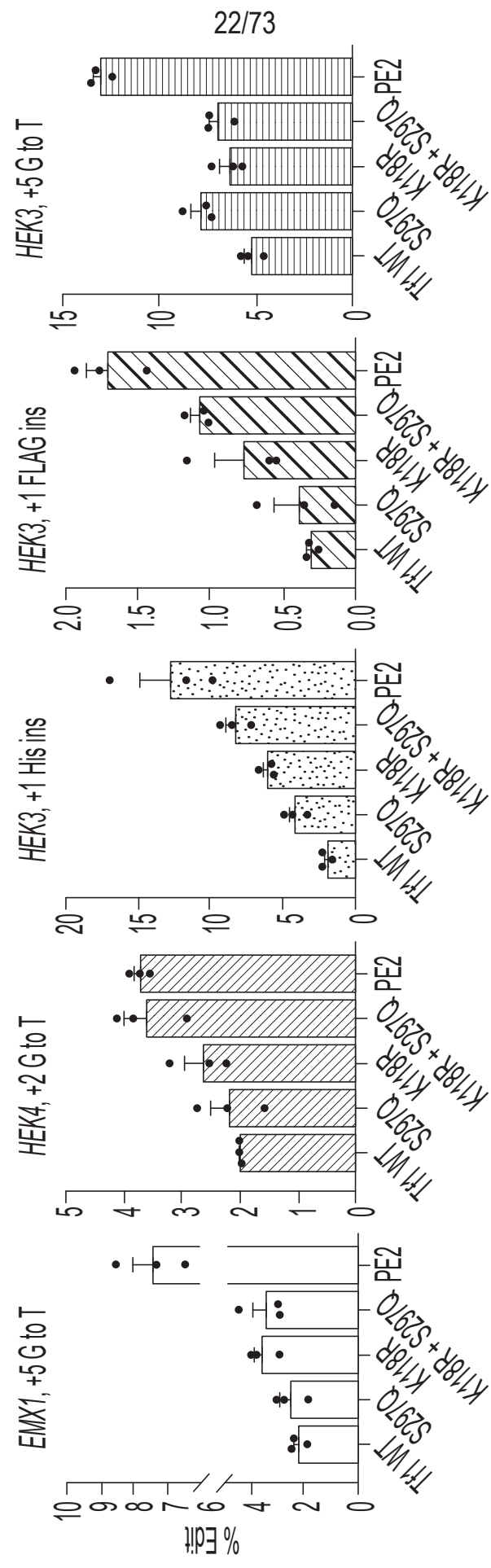
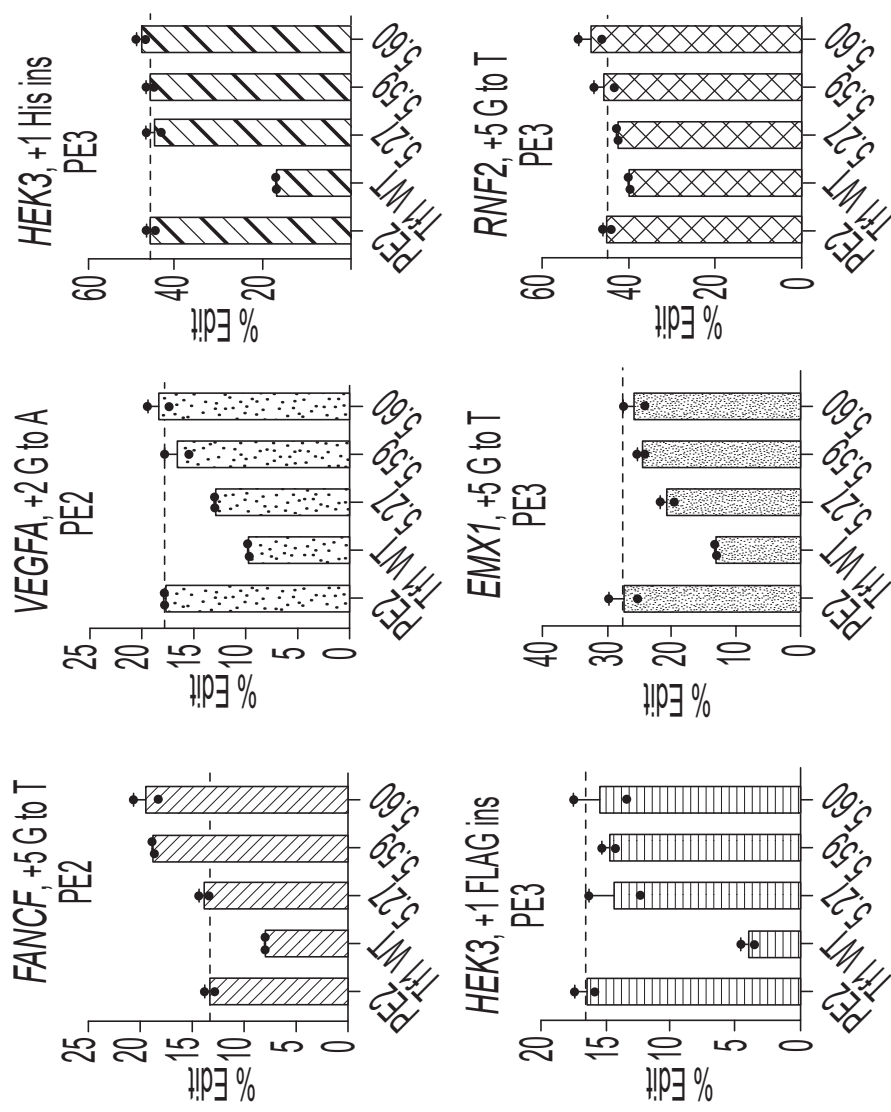


FIG. 20



- 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

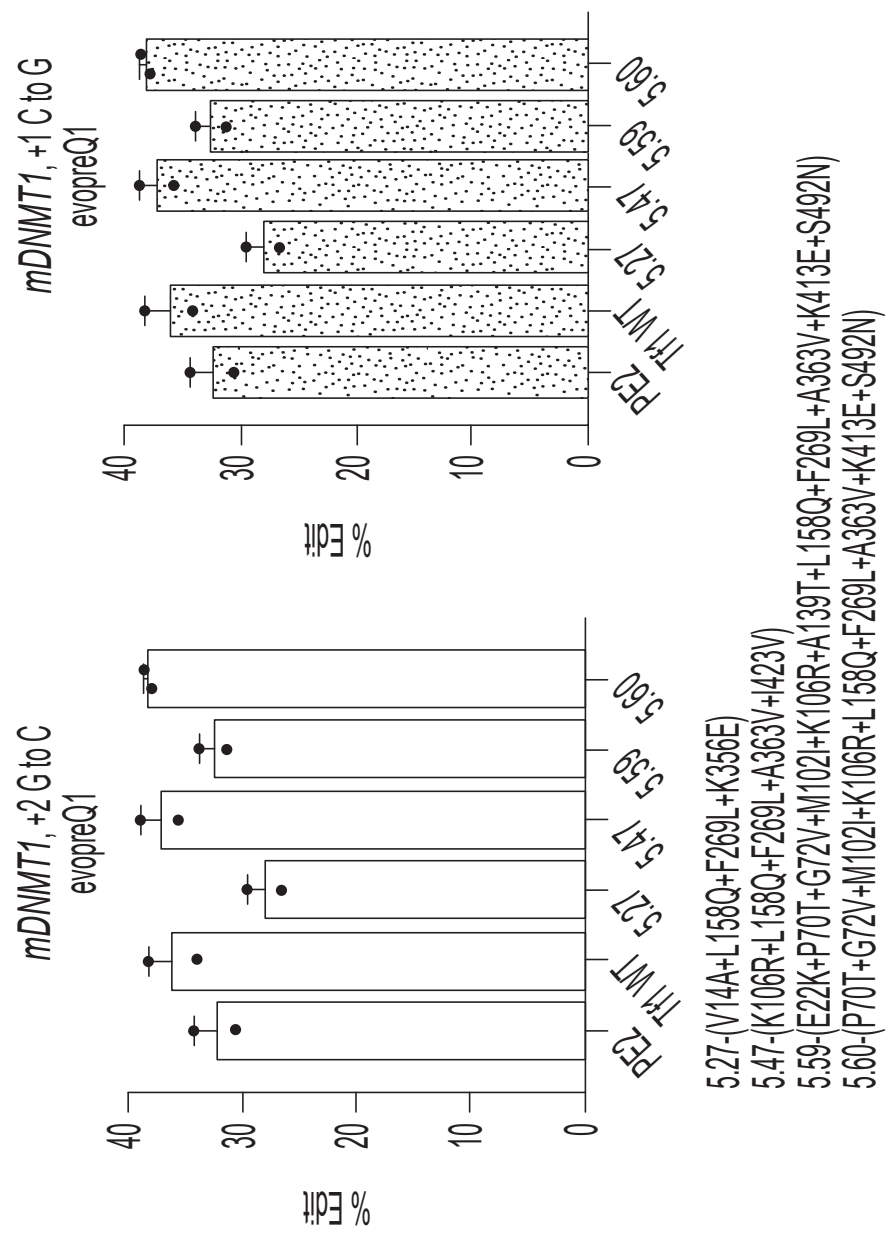


FIG. 22

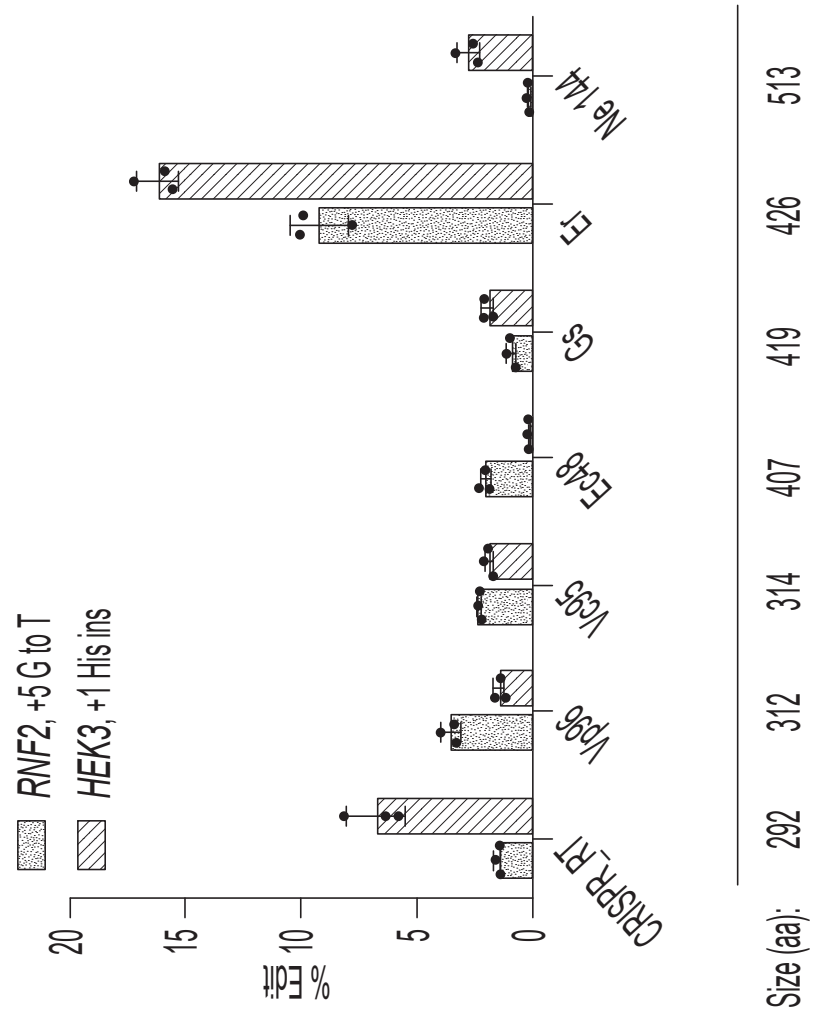


FIG. 23

Size (aa): 292 312 314 407 419 426 513

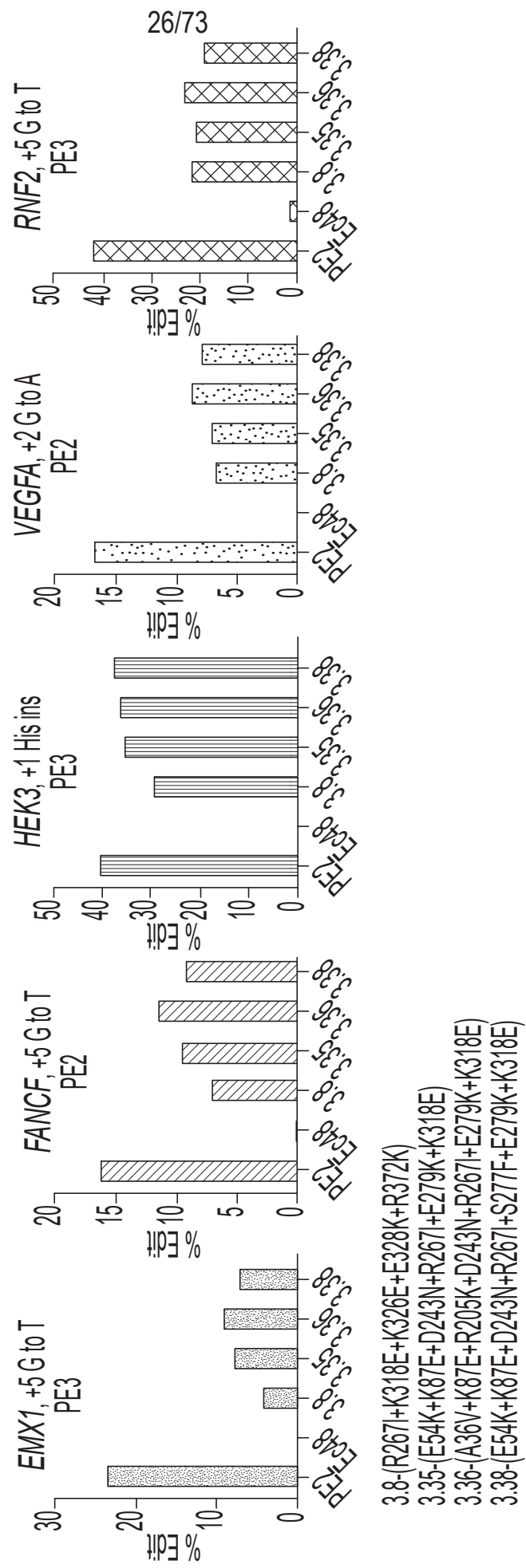


FIG. 24

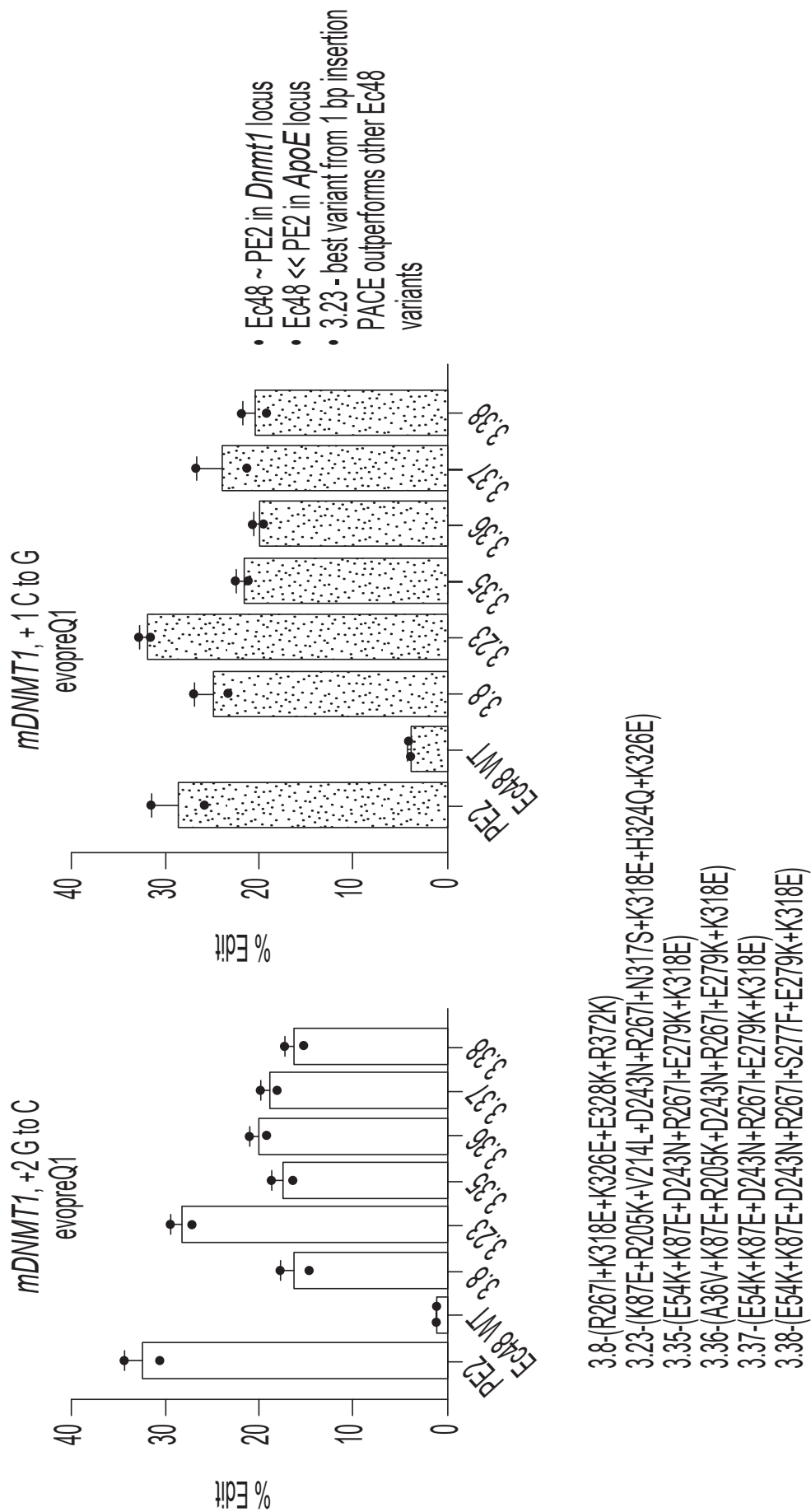
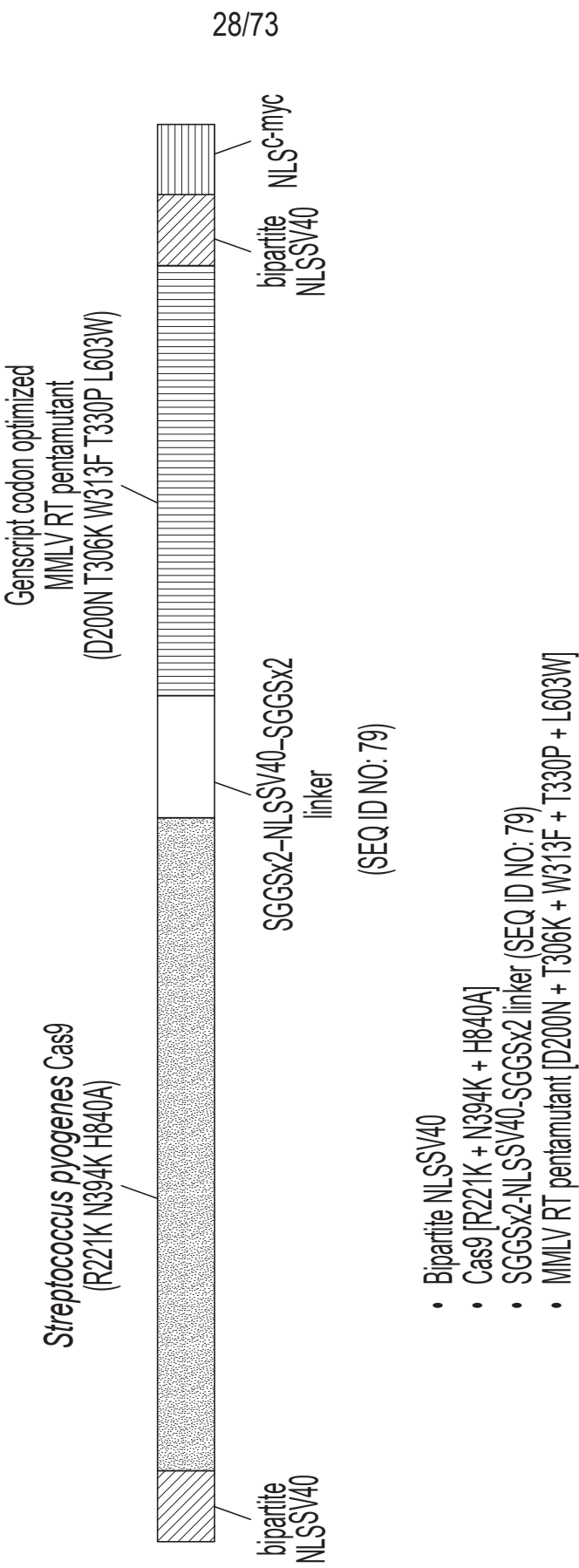


FIG. 25

PEmax



- Bipartite NLSV40
- Cas9 [R221K + N394K + H840A]
- SGGsx2-NLSV40-SGGsx2 linker (SEQ ID NO: 79)
- MMLV RT pentamutant [D200N + T306K + W313F + T330P + L603W]

FIG. 26

Engineering Strategies to Generation PE Variants

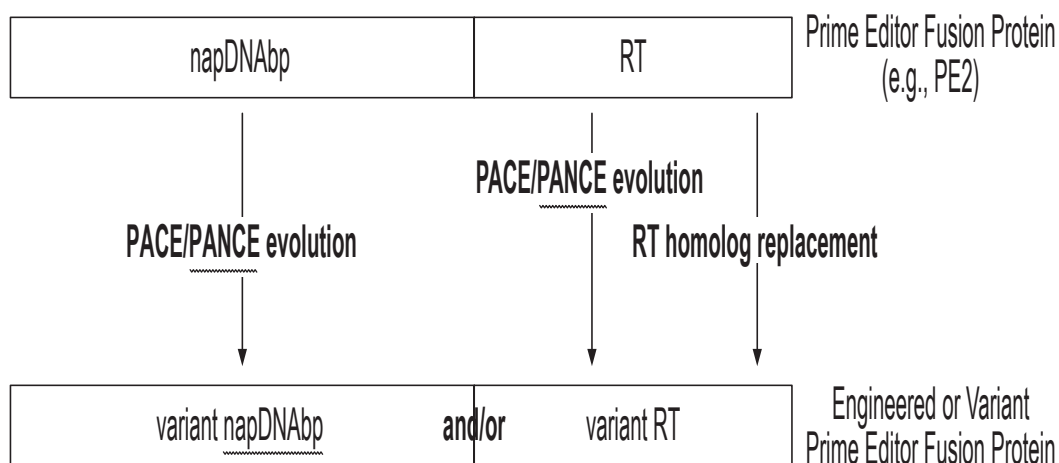


FIG. 27A

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

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

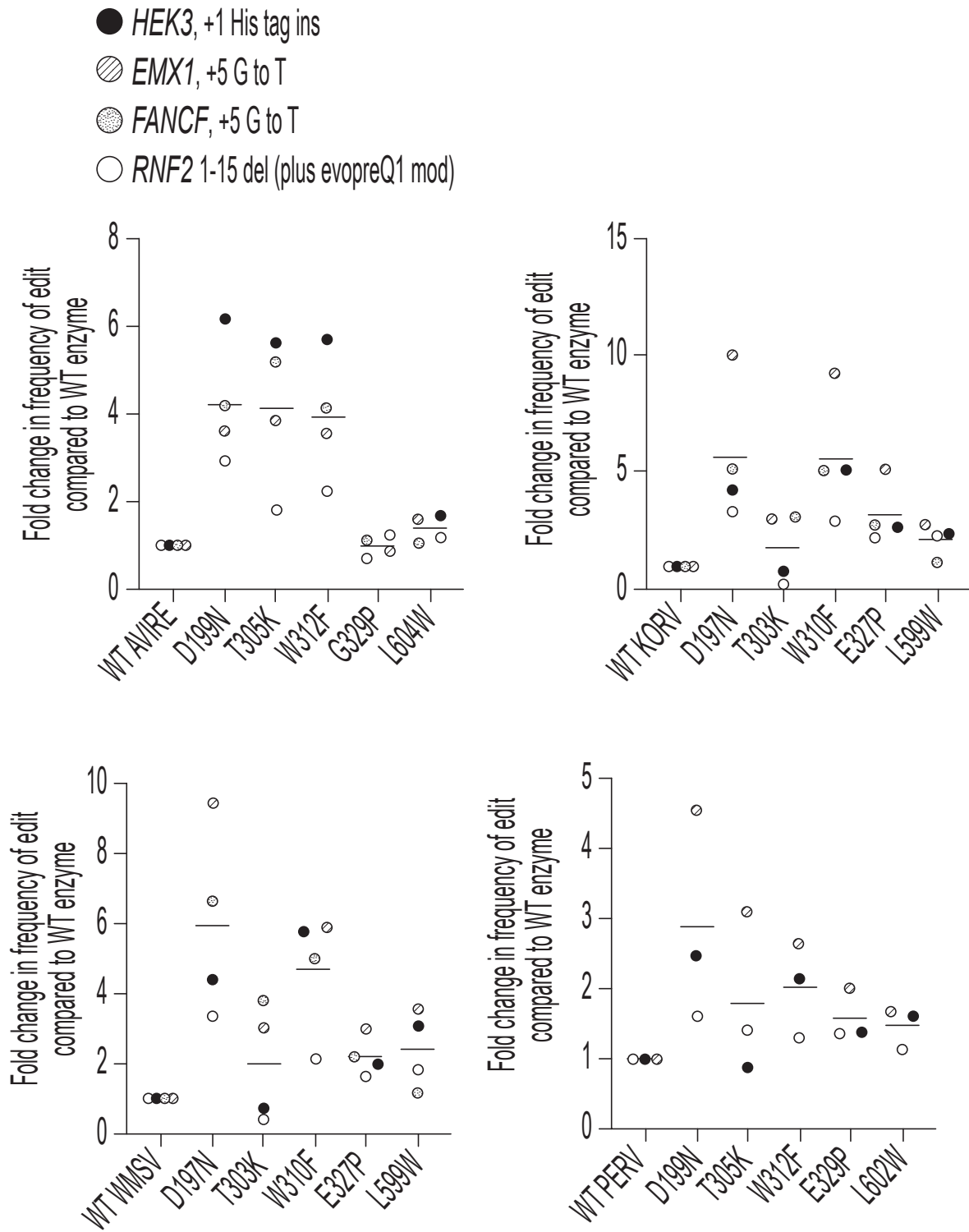


FIG. 29

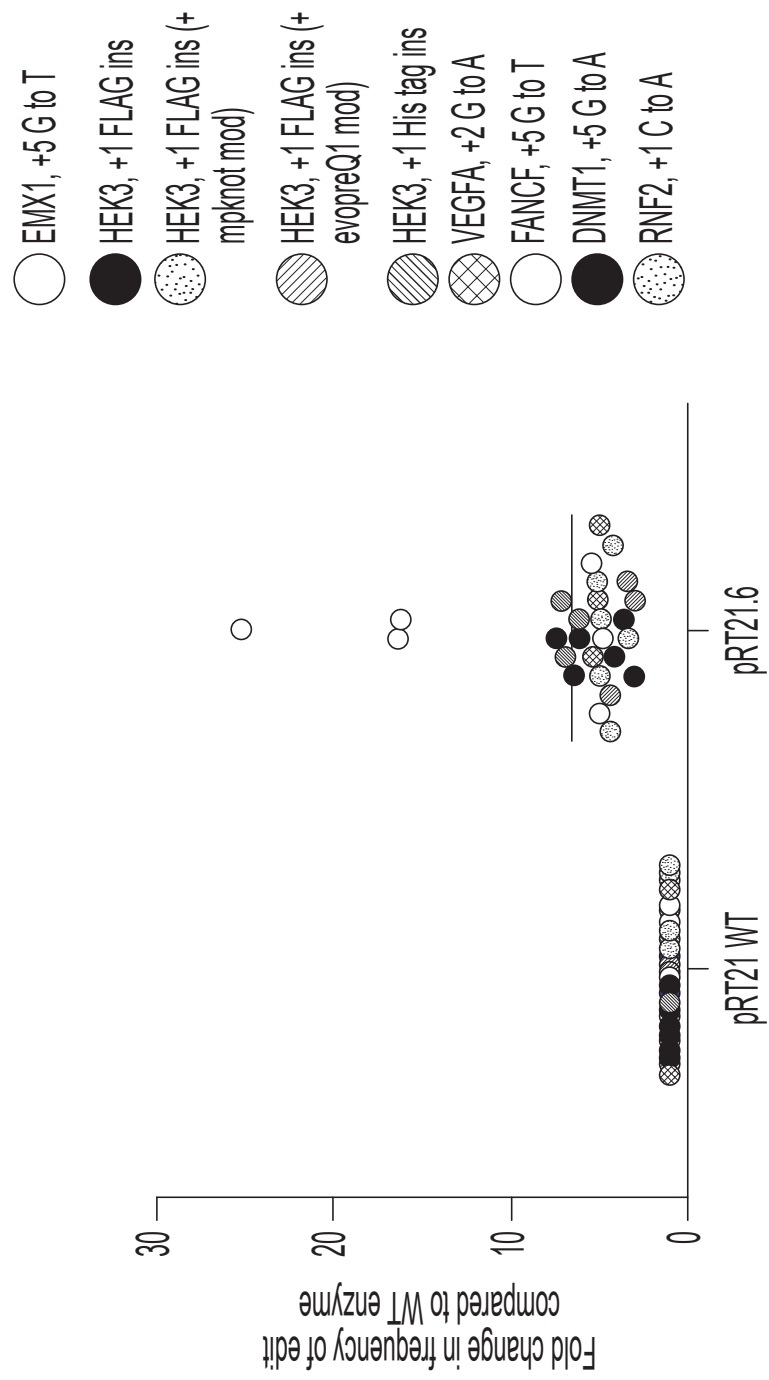


FIG. 30

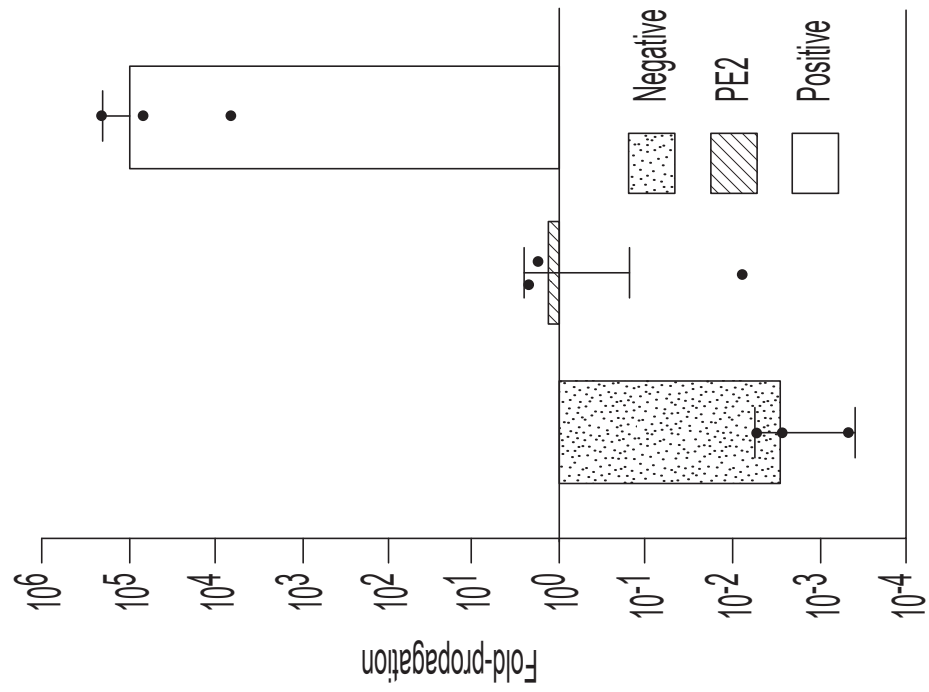


FIG. 31A

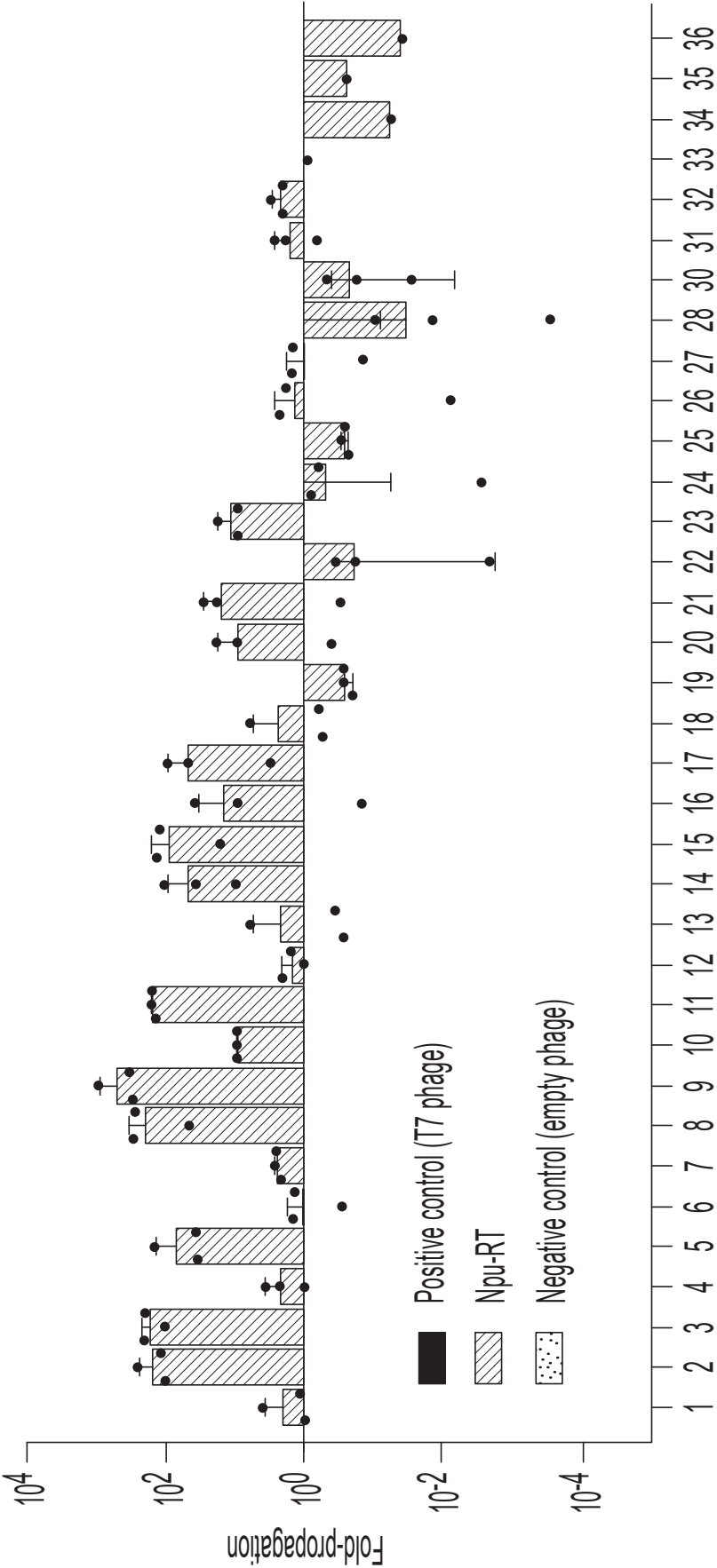


FIG. 31B

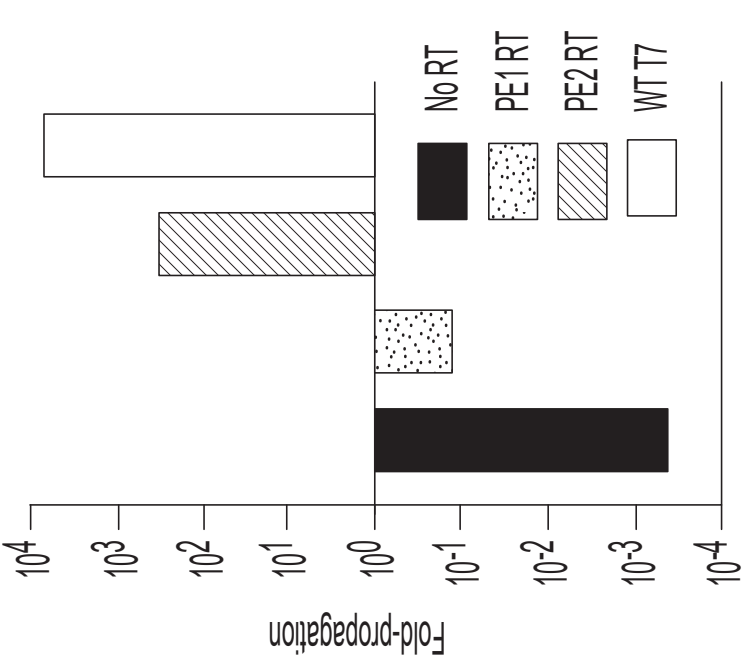


FIG. 31C

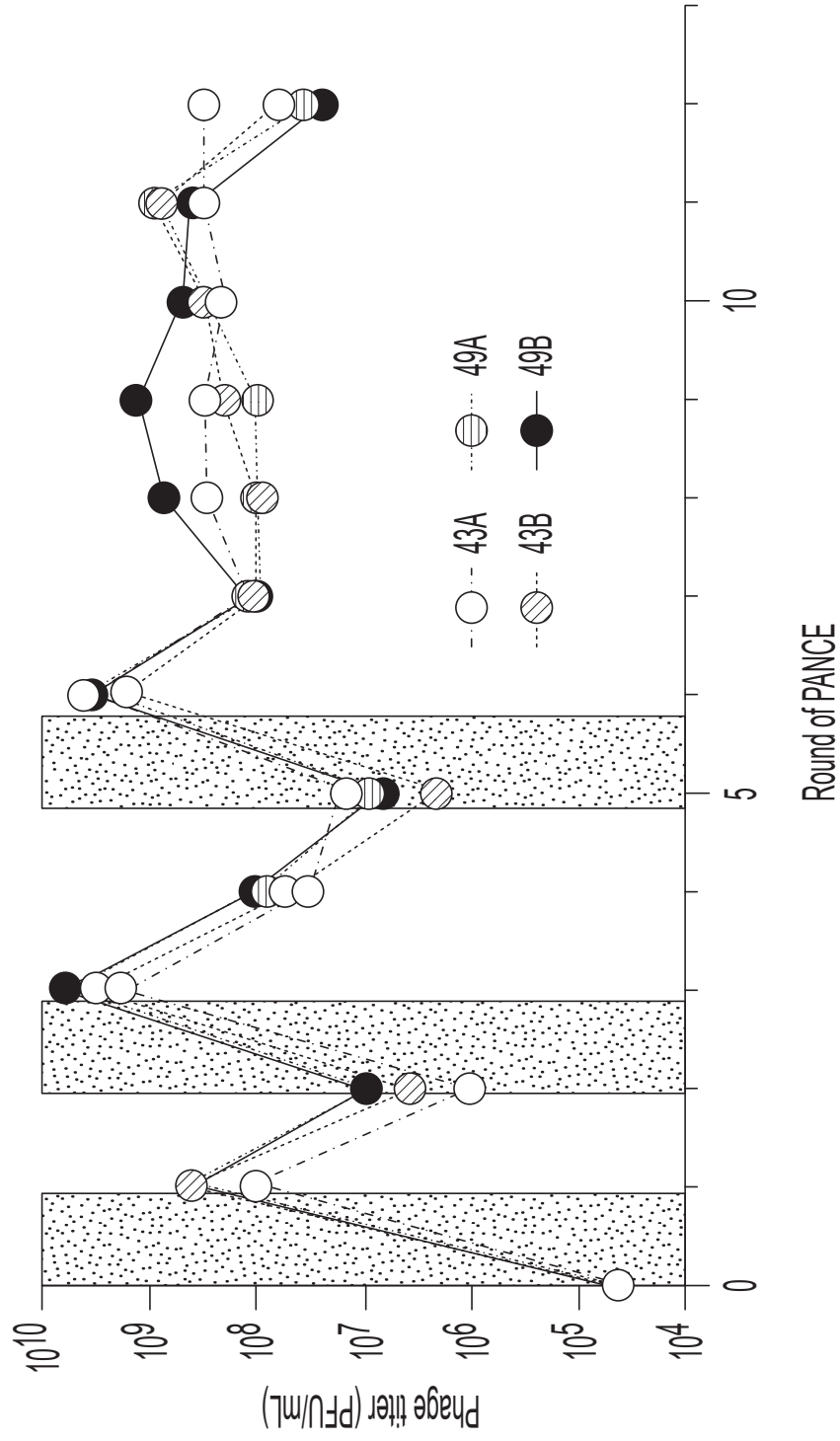


FIG. 31D

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

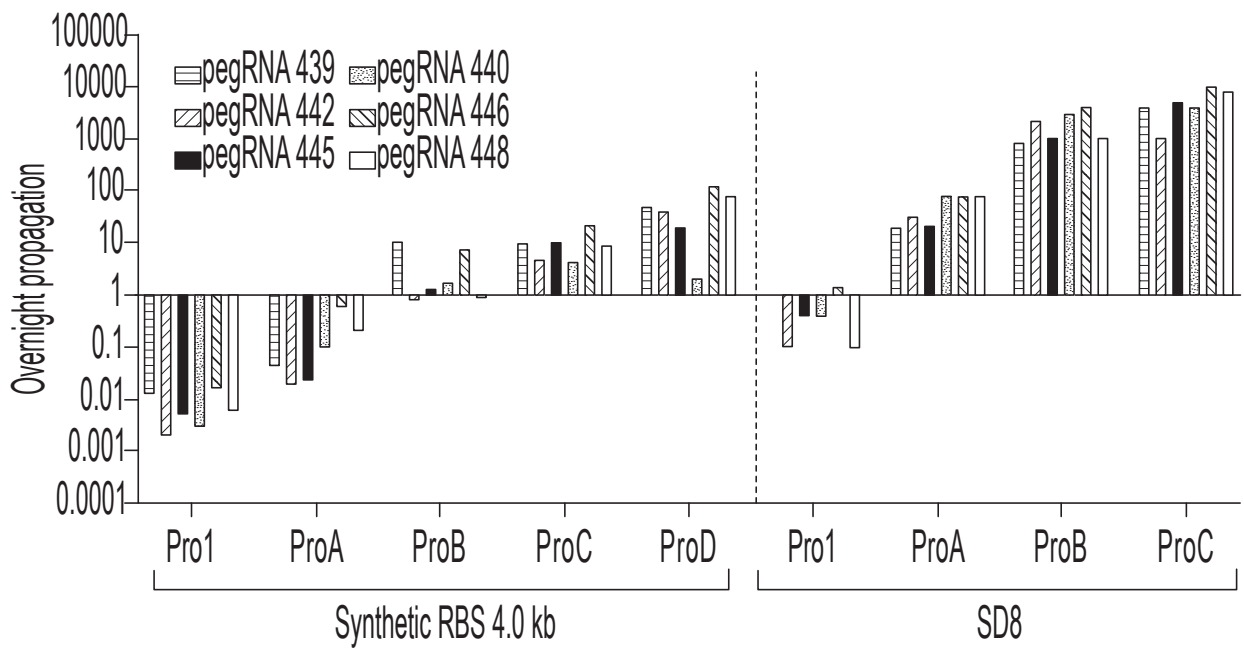


FIG. 33A

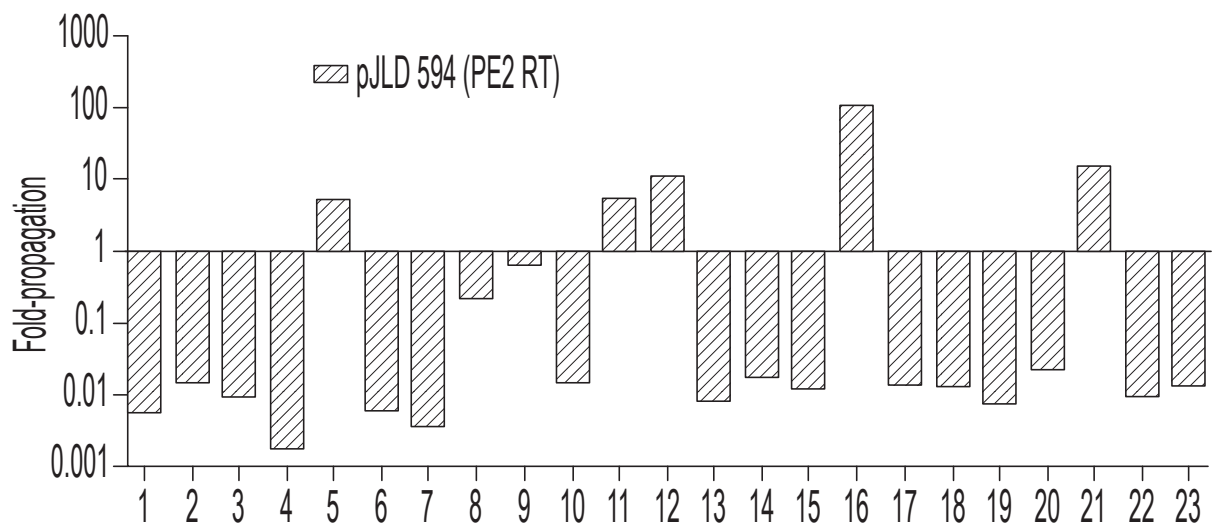


FIG. 33B

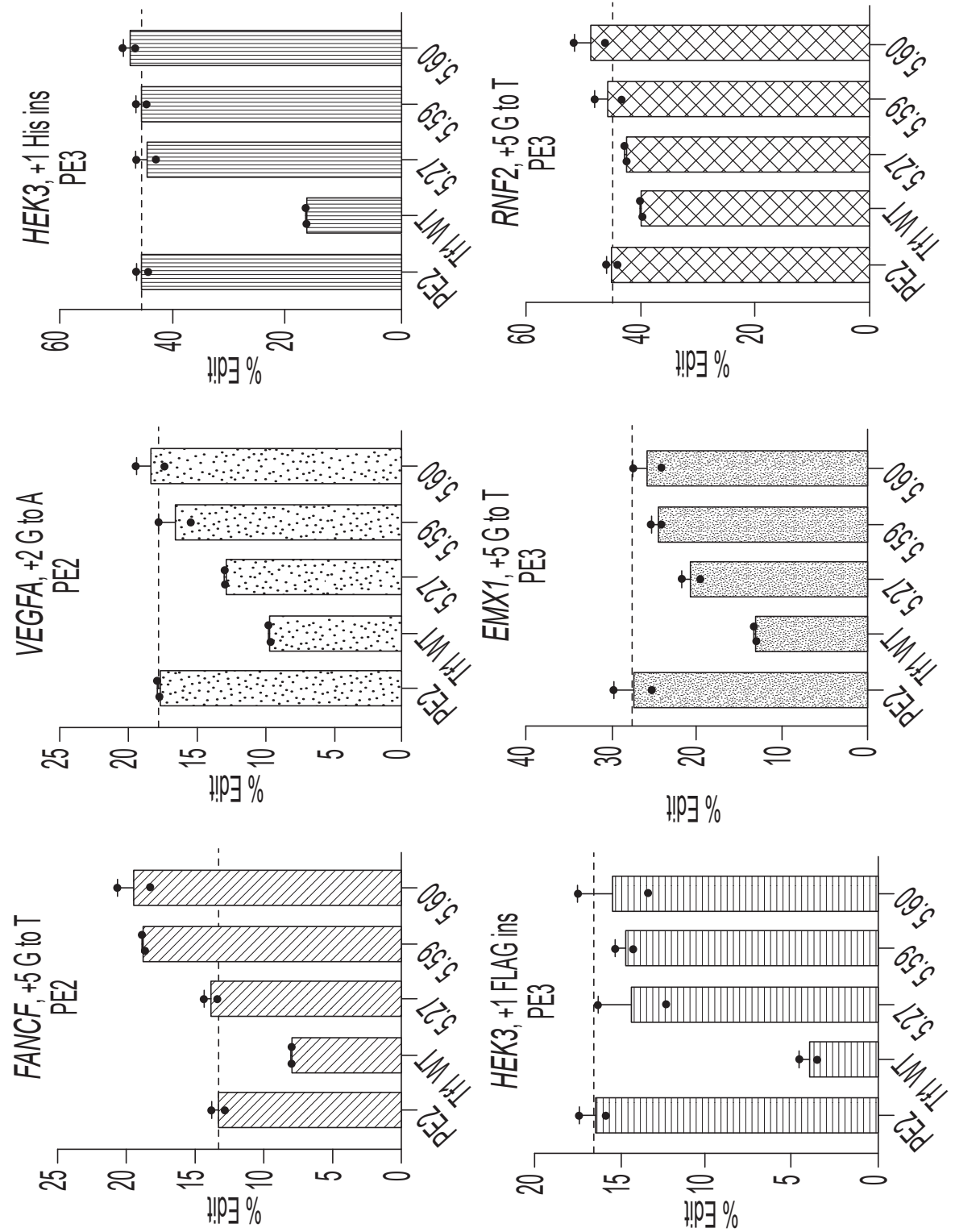


FIG. 34

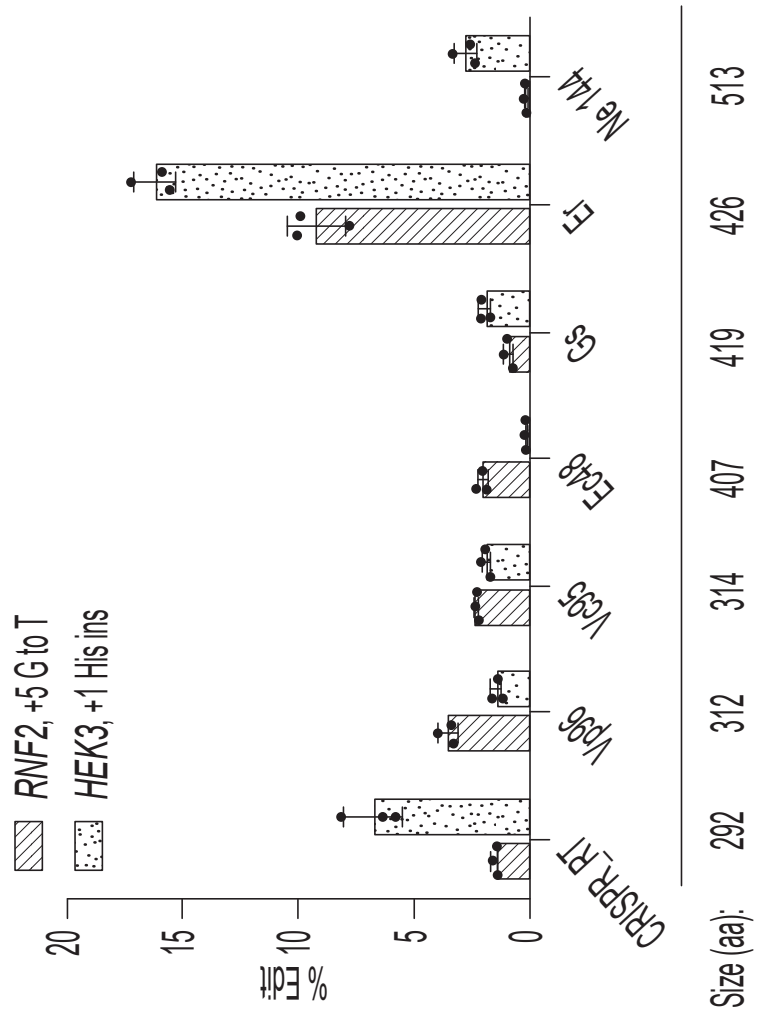


FIG. 35

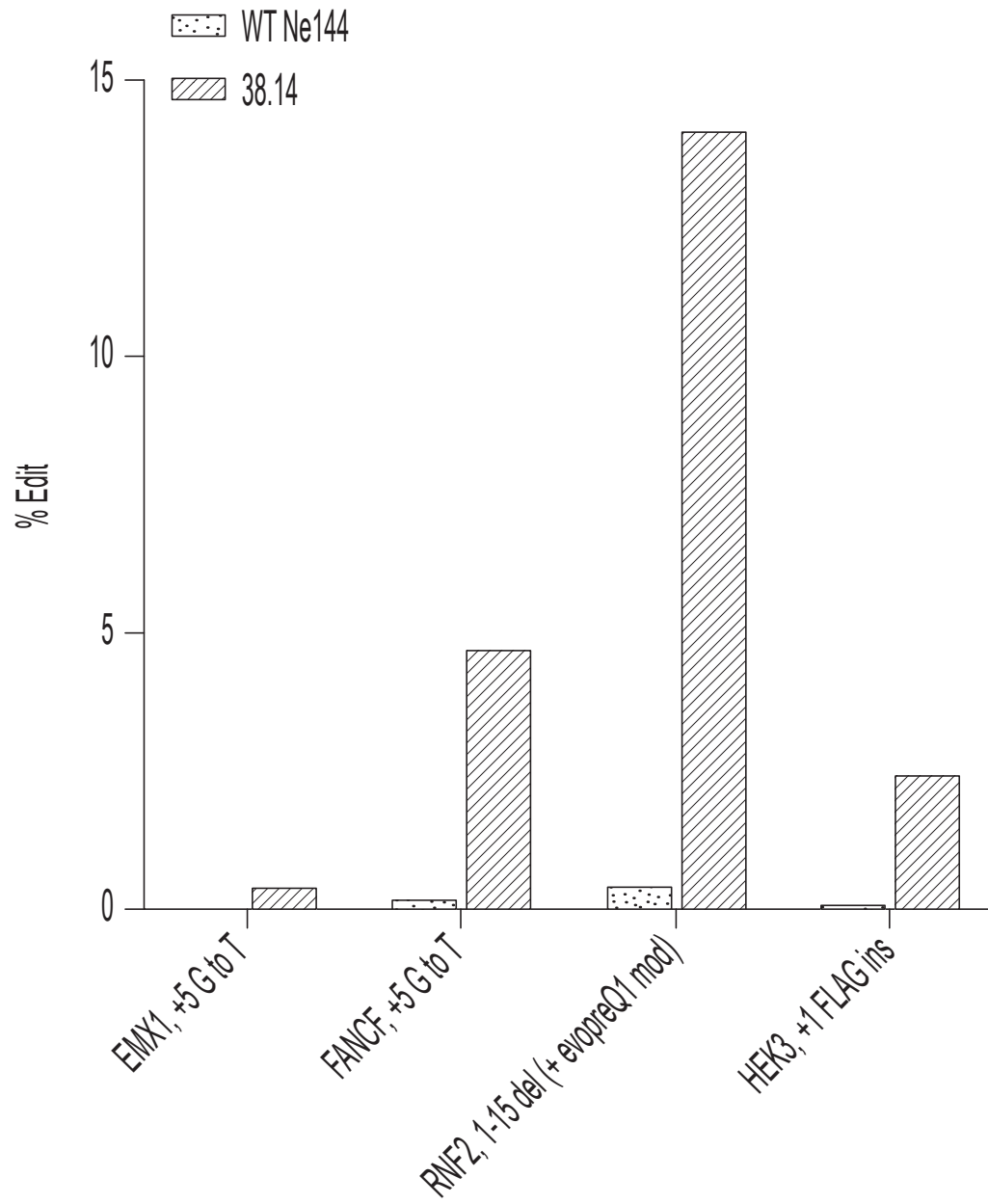


FIG. 36

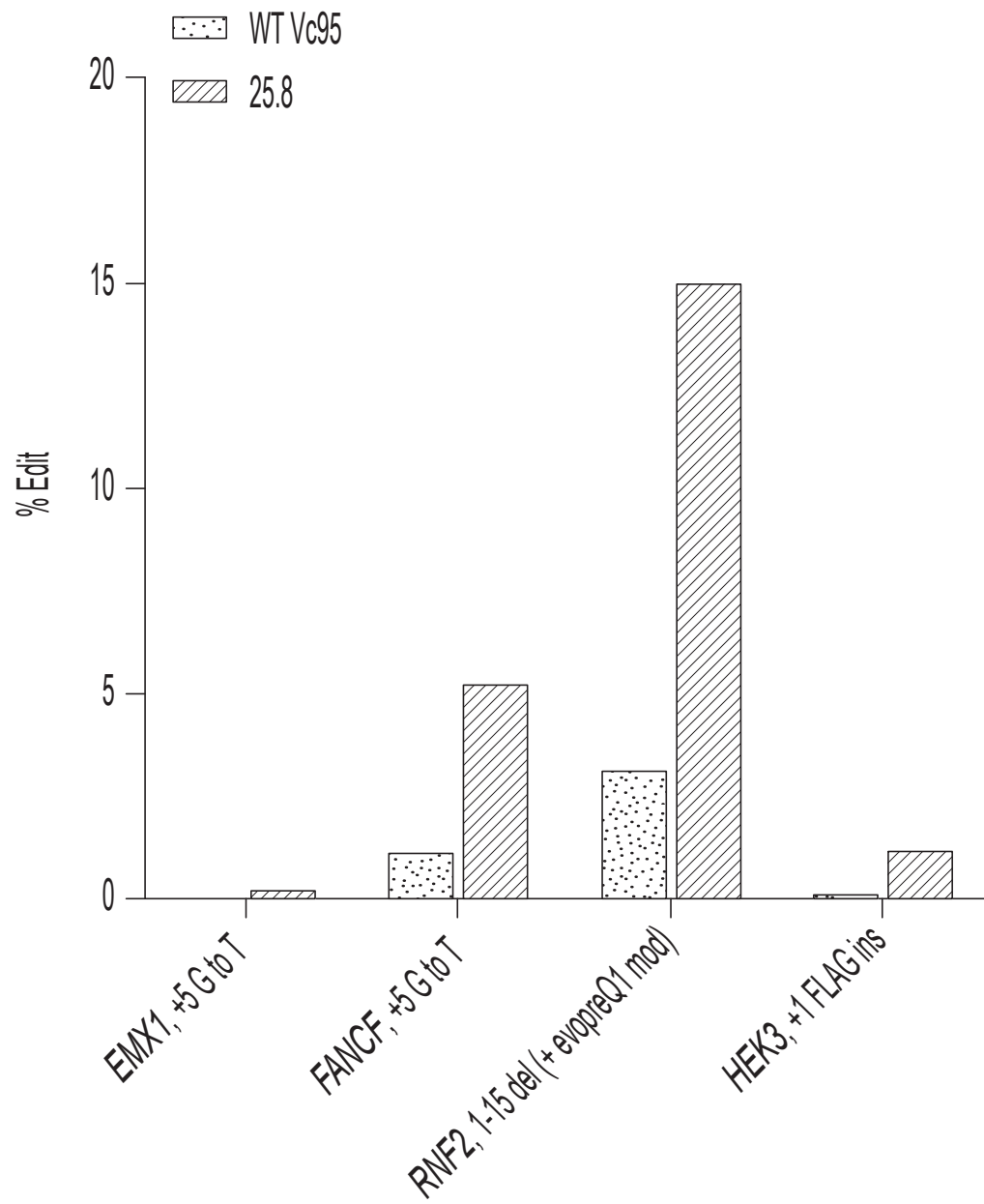


FIG. 37

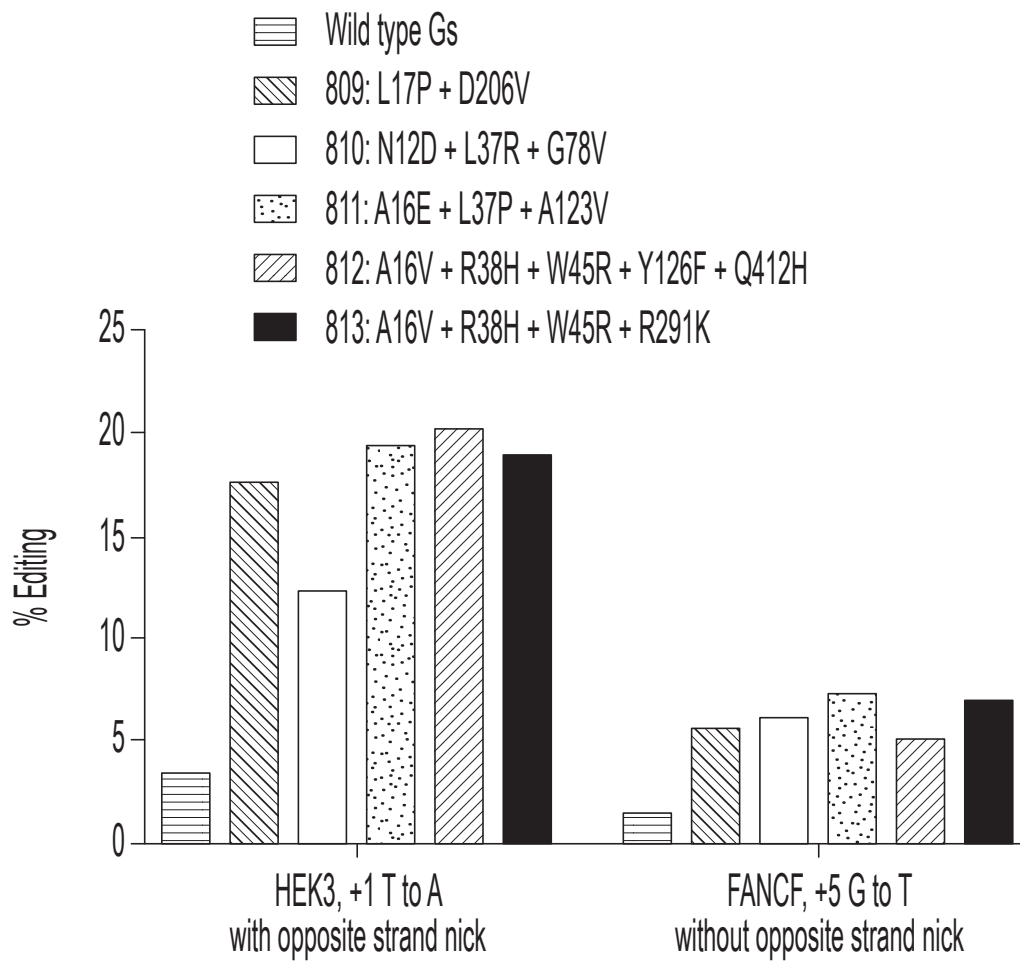


FIG. 38A

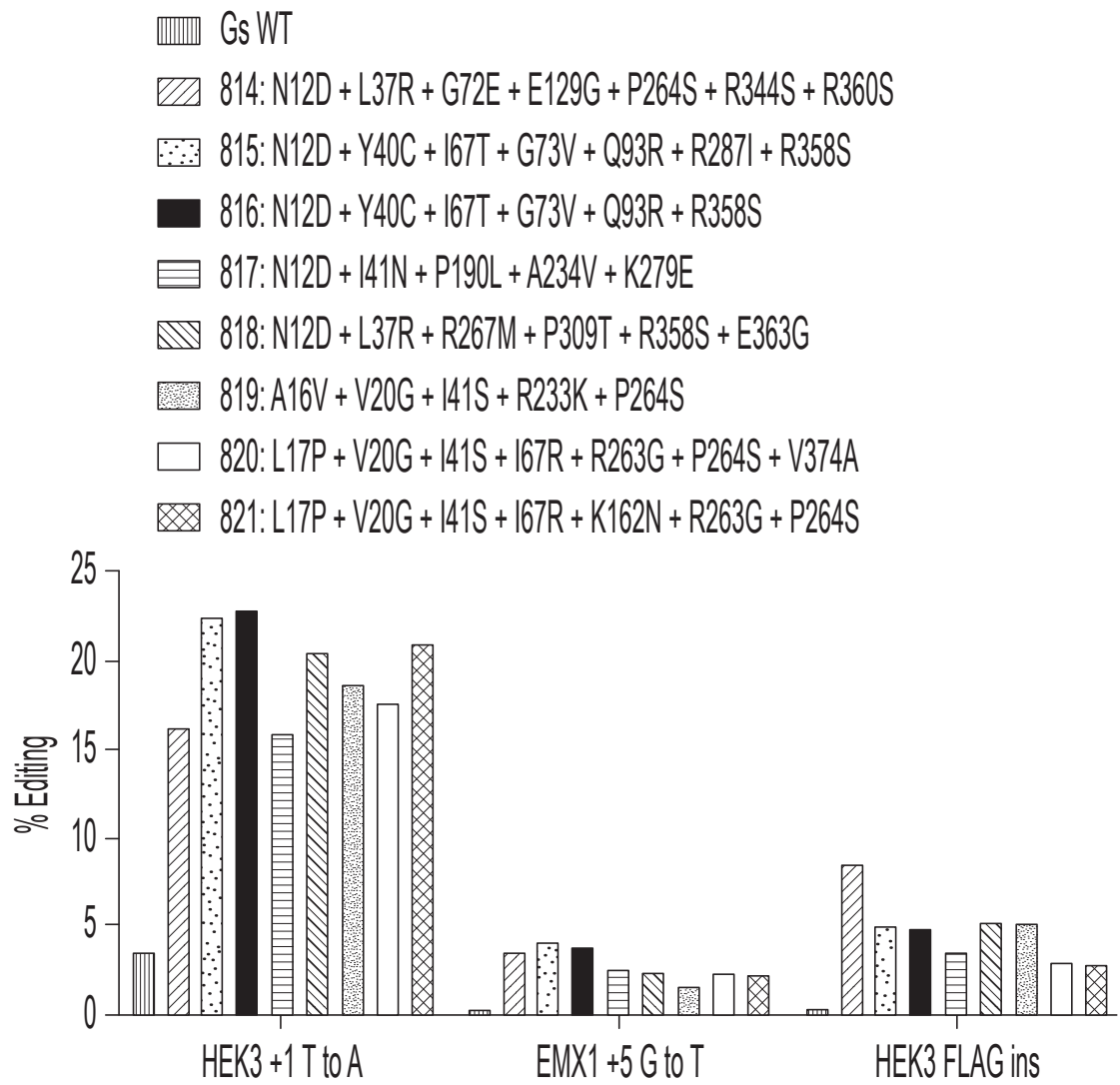


FIG. 38B

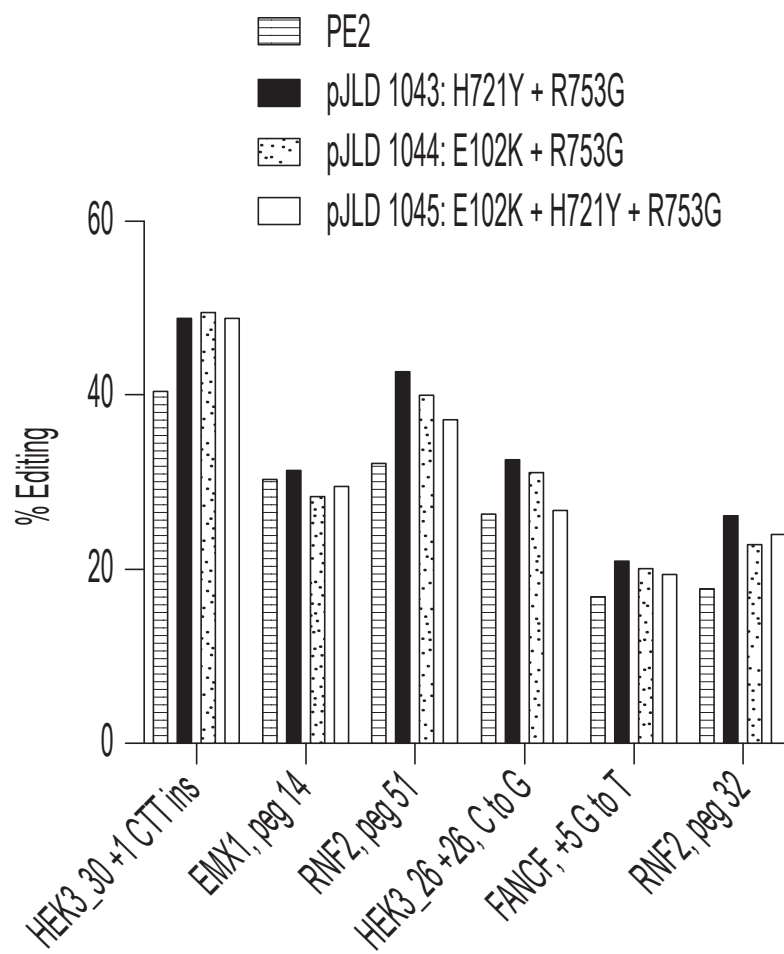


FIG. 39

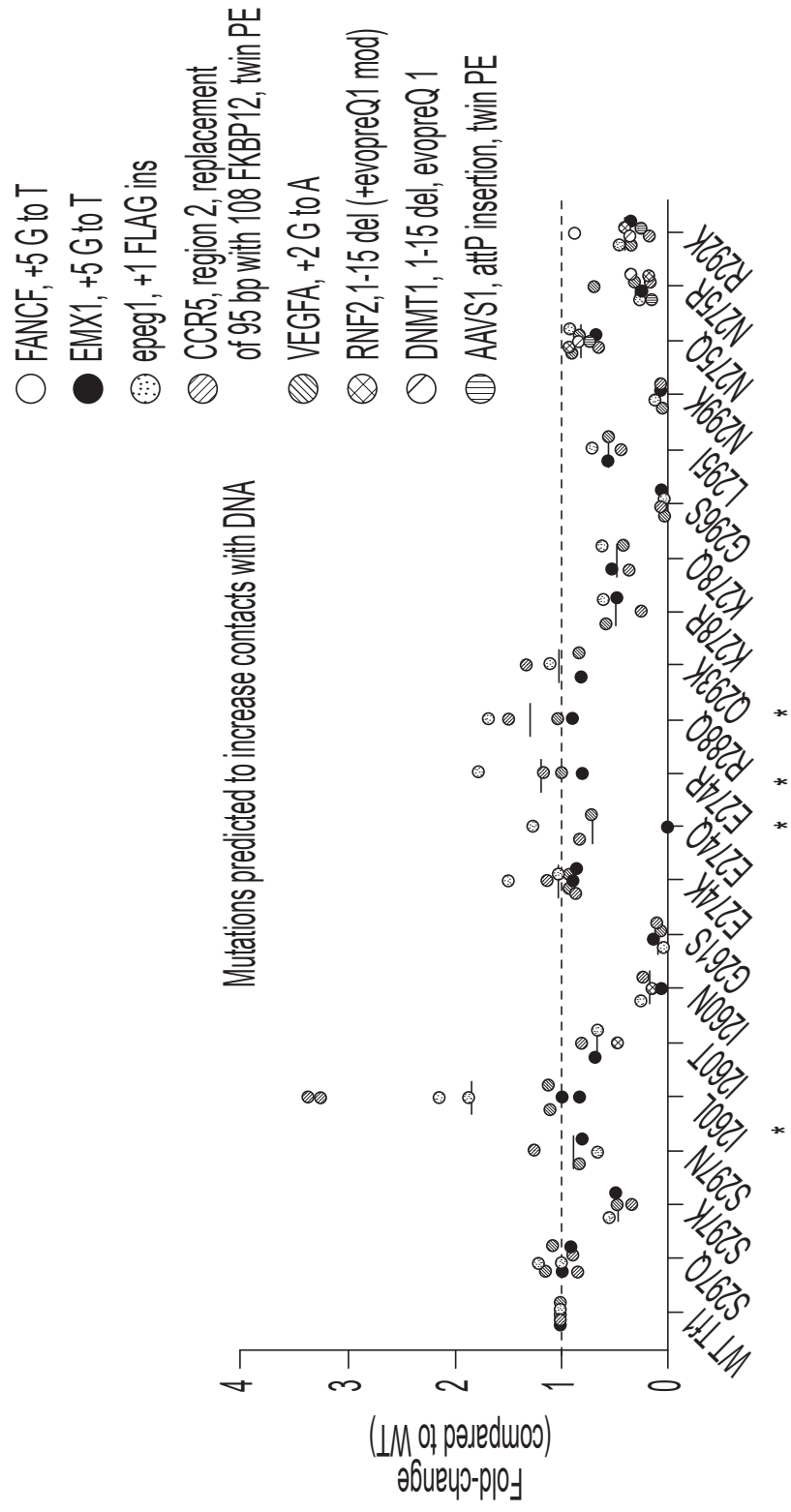


FIG. 40

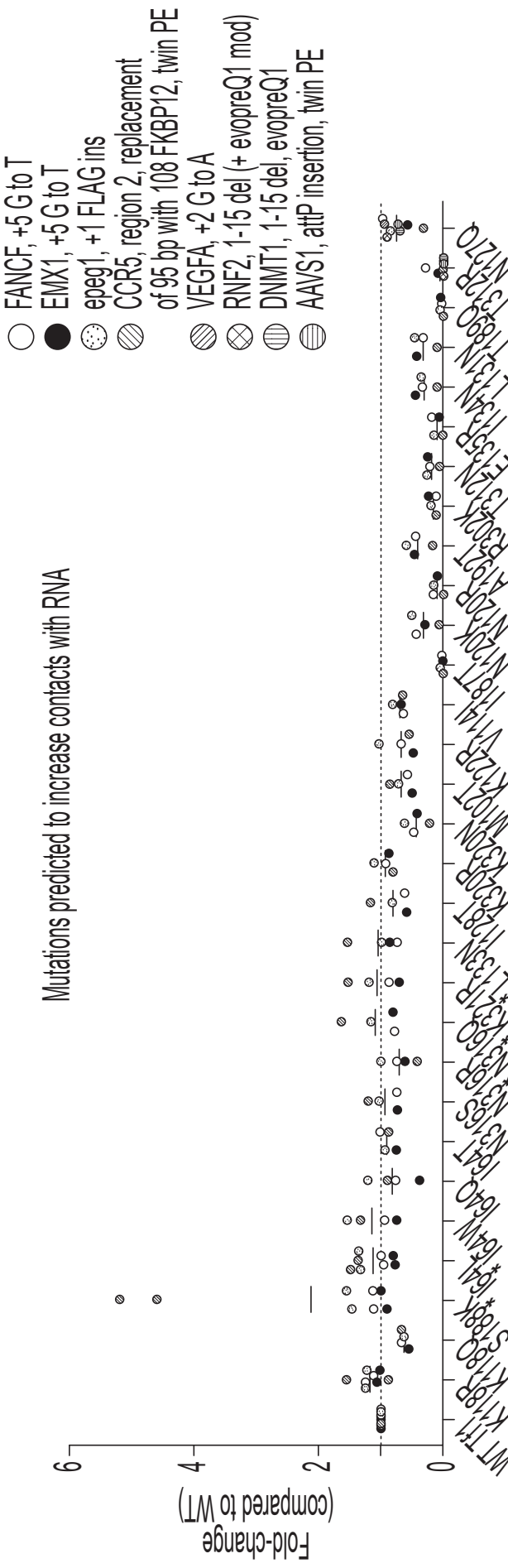


FIG. 41

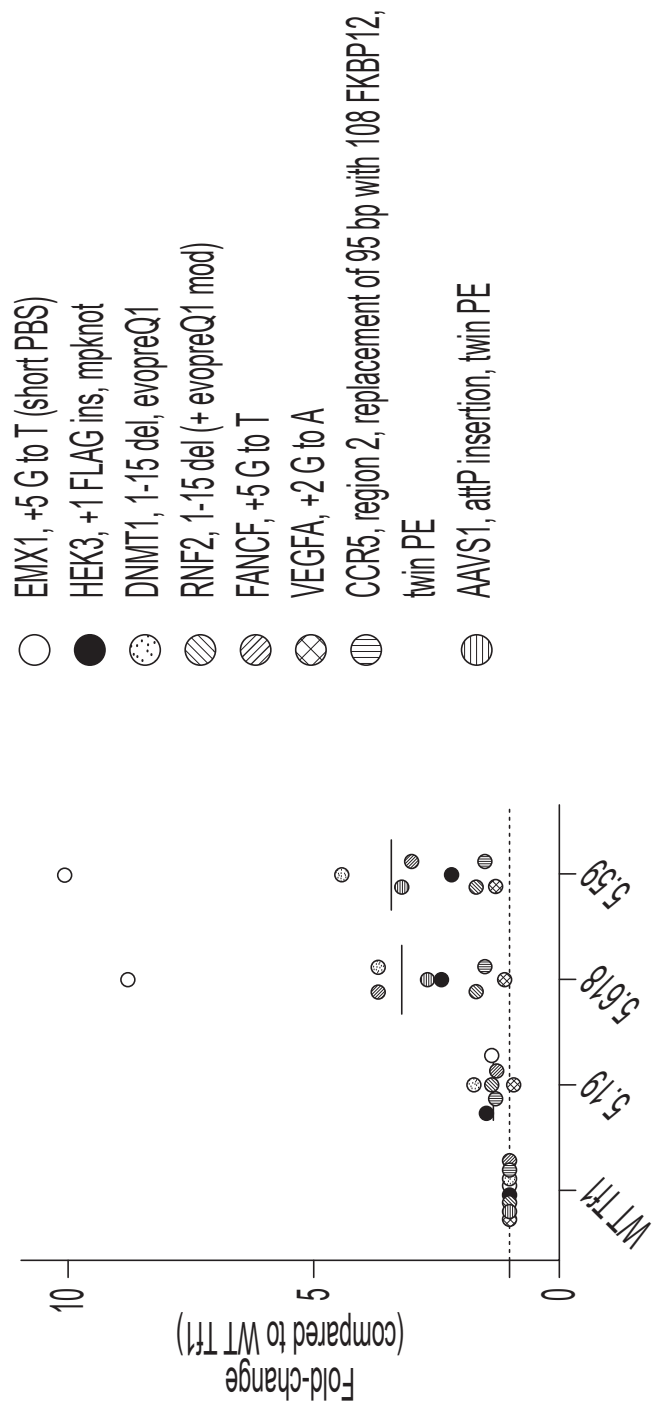


FIG. 42

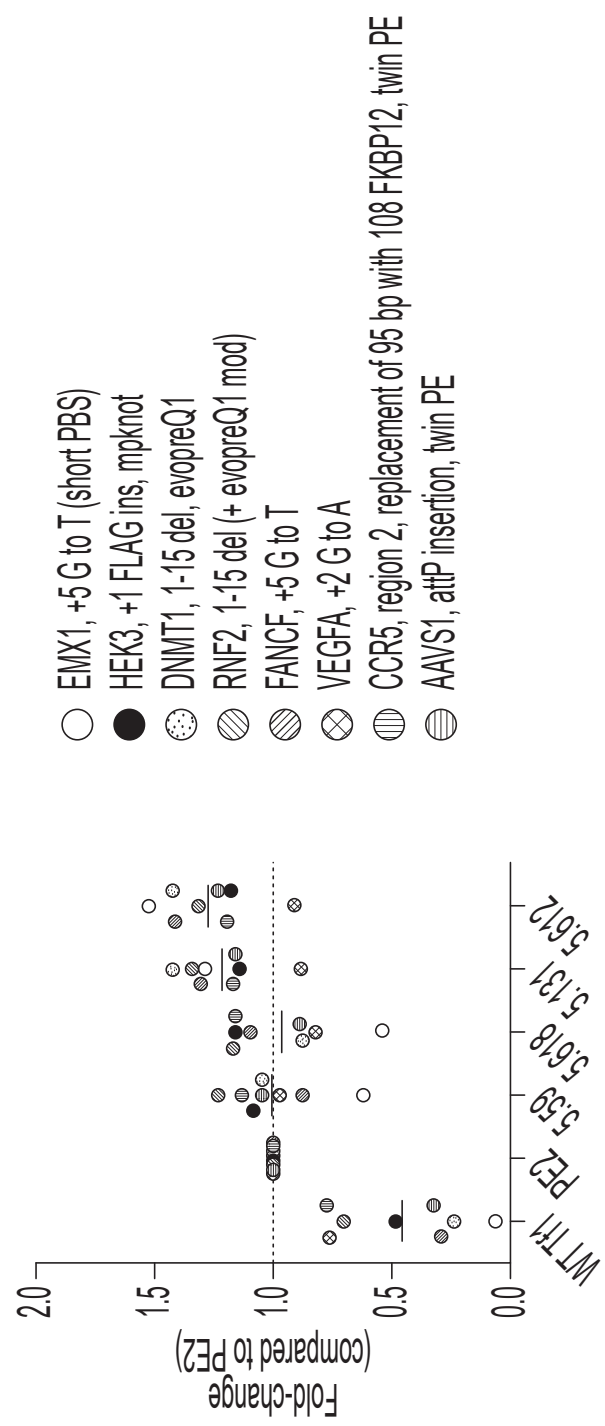


FIG. 43

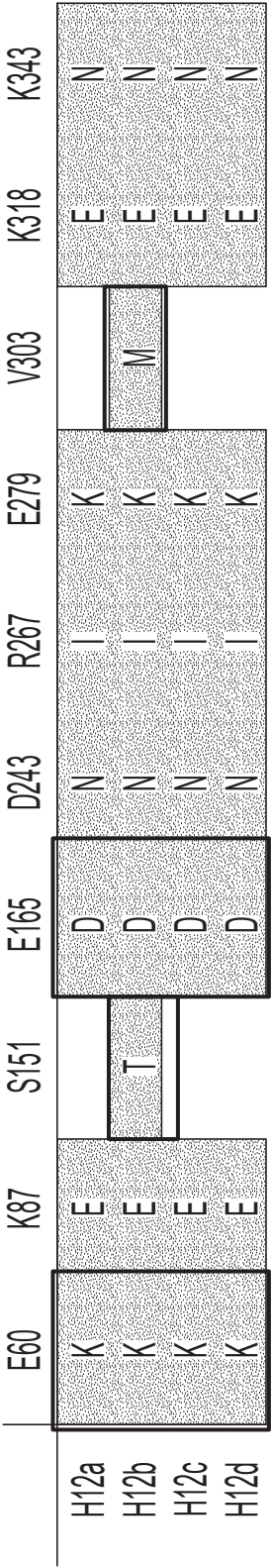


FIG. 44A

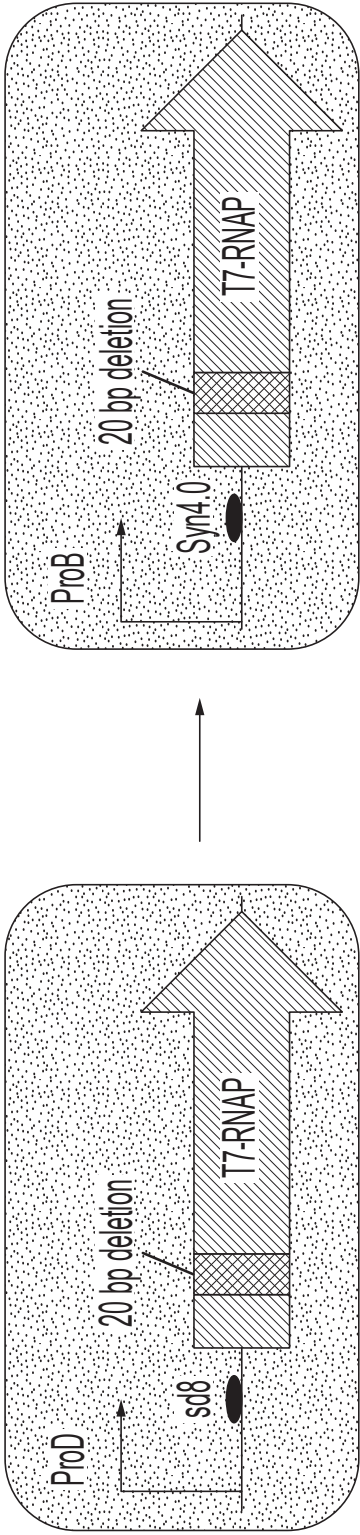


FIG. 44B

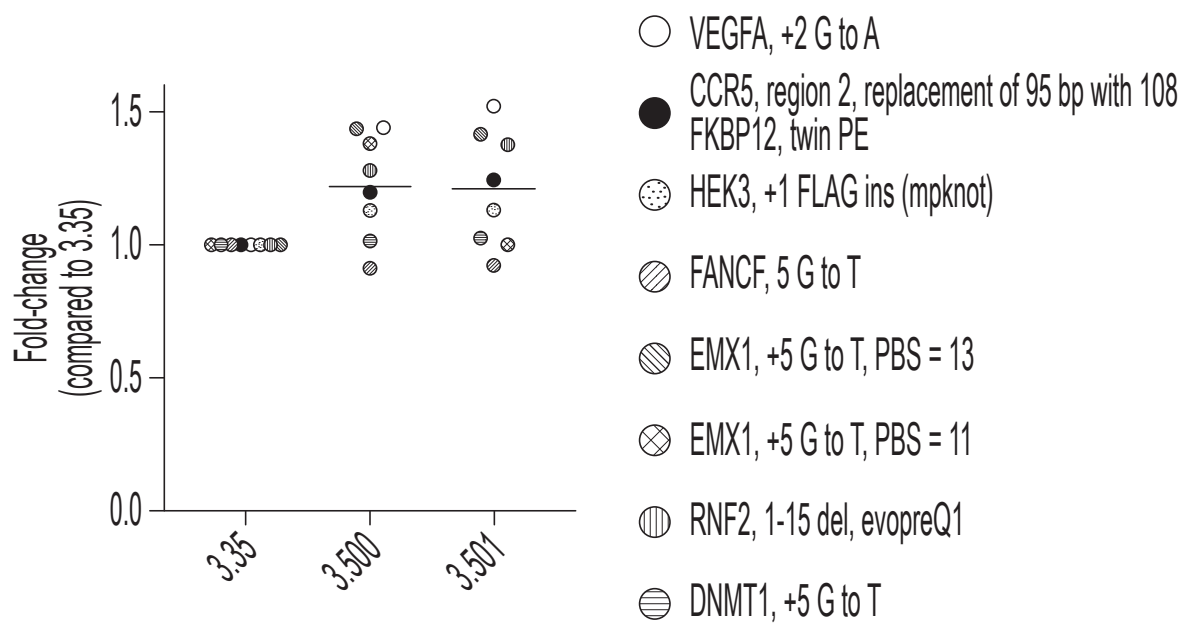


FIG. 45

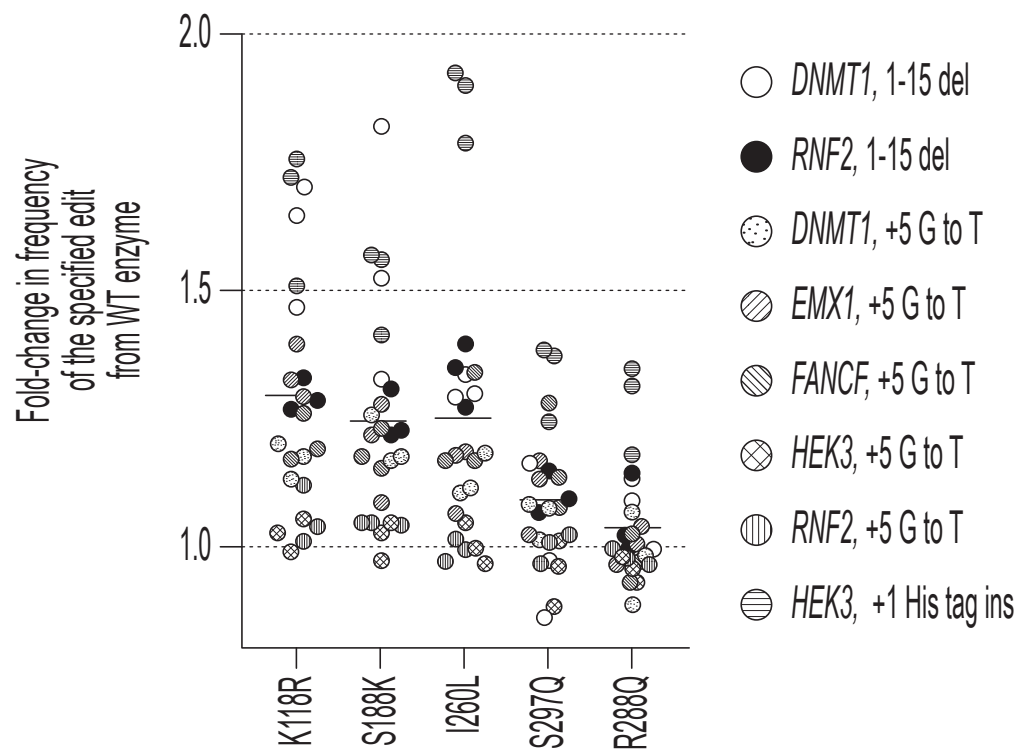


FIG. 46

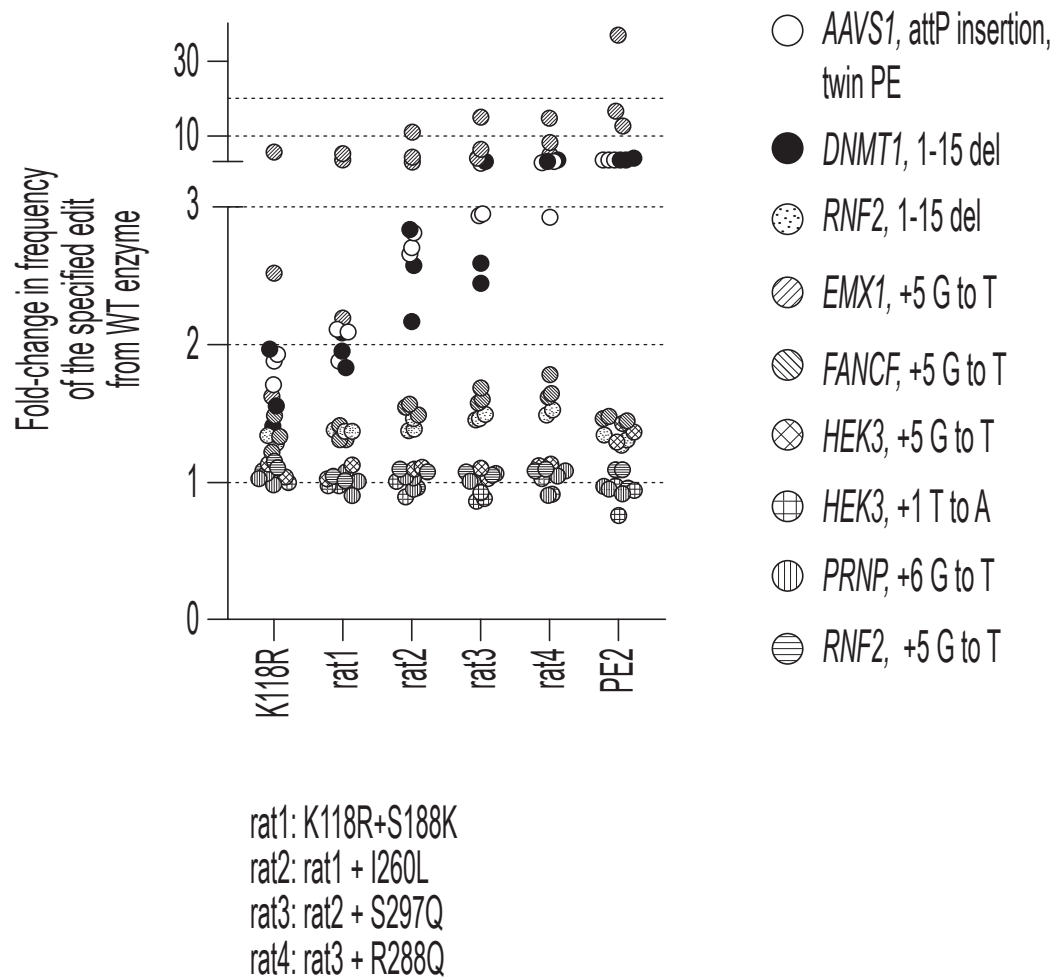


FIG. 47

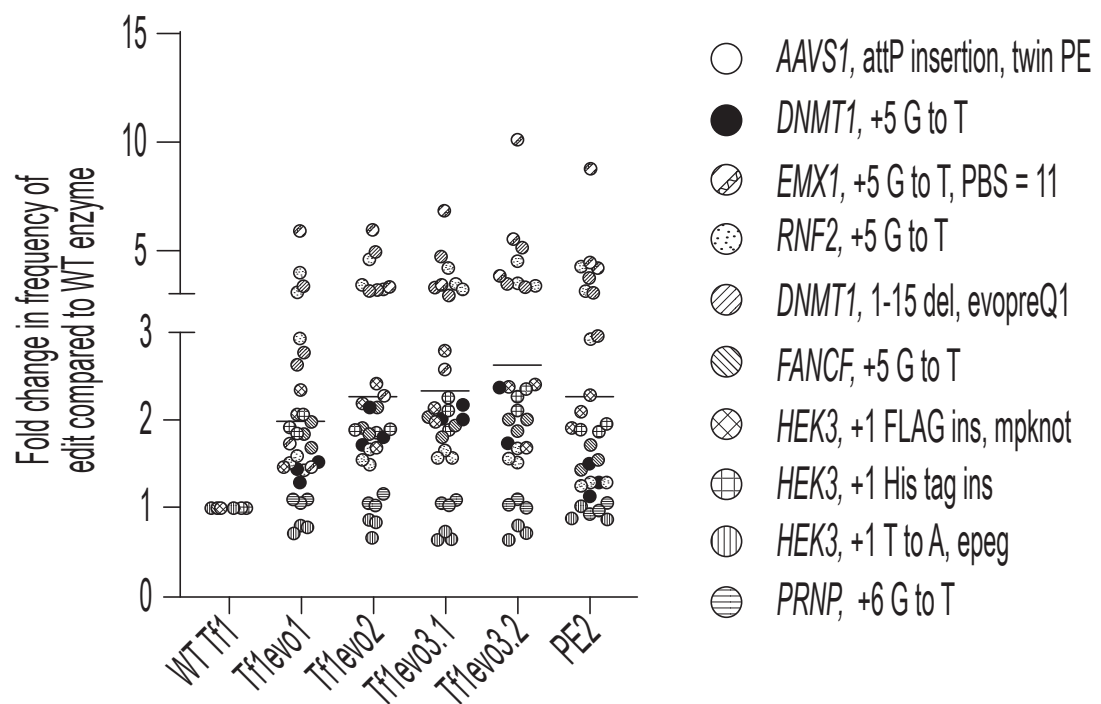


FIG. 48

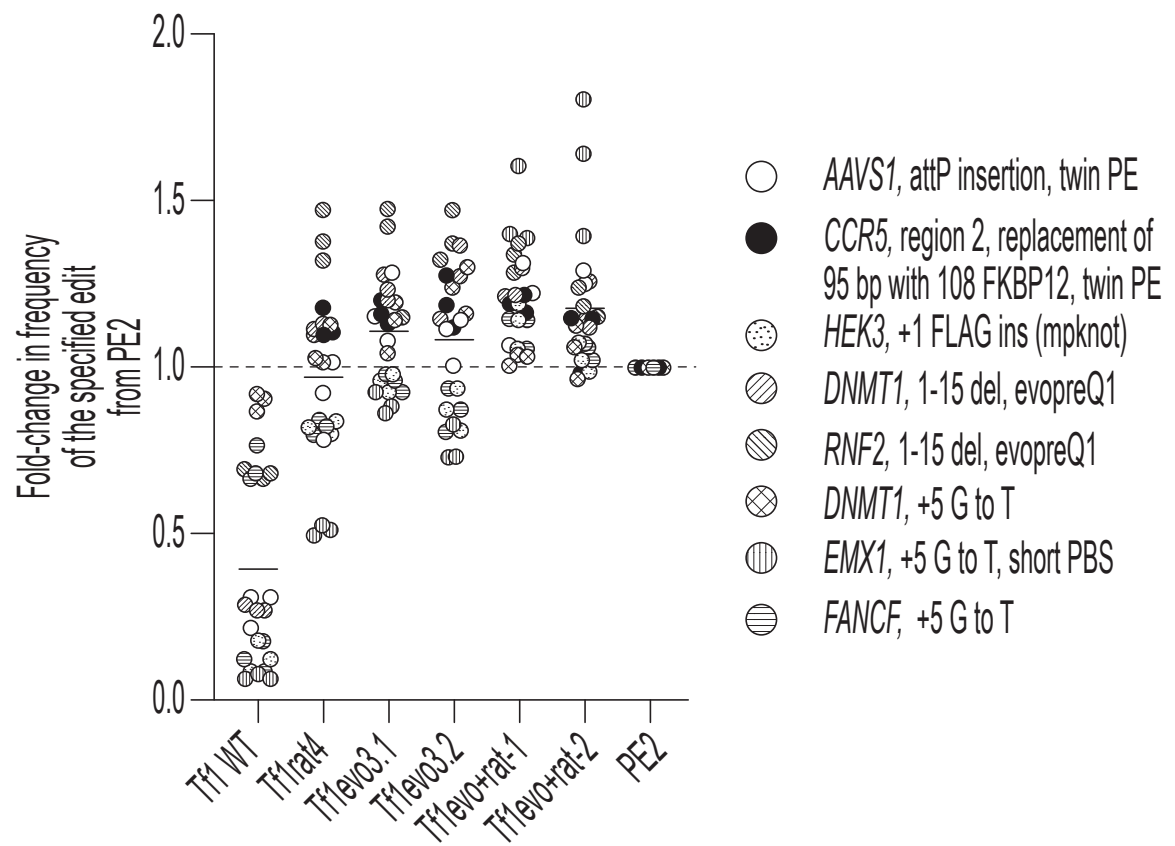


FIG. 49

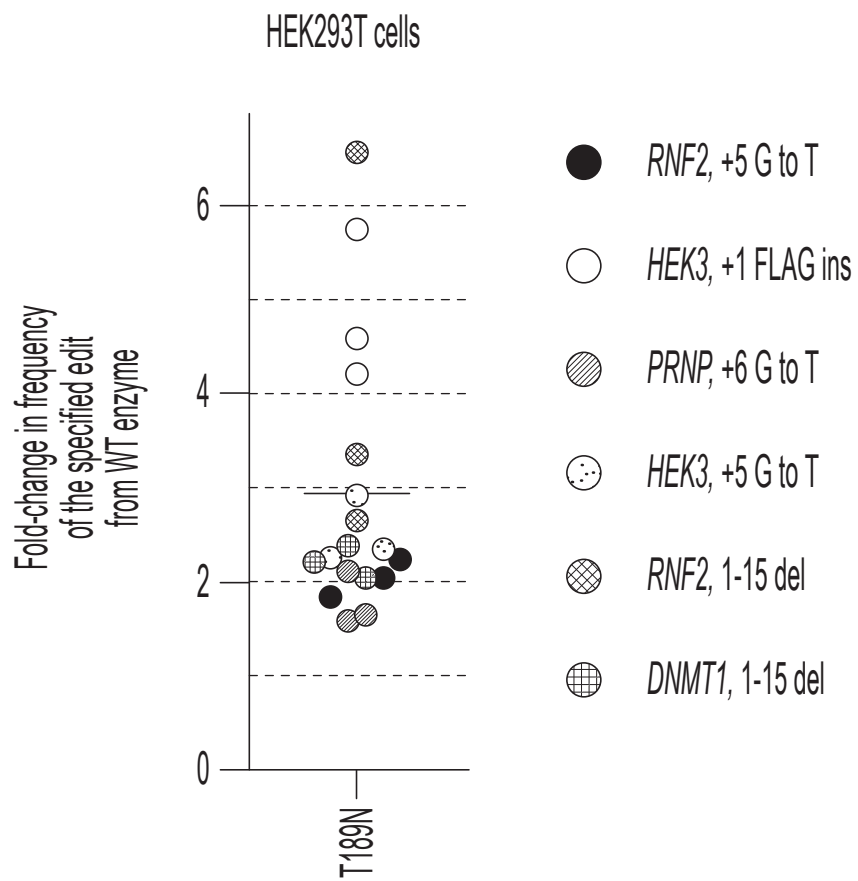


FIG. 50A

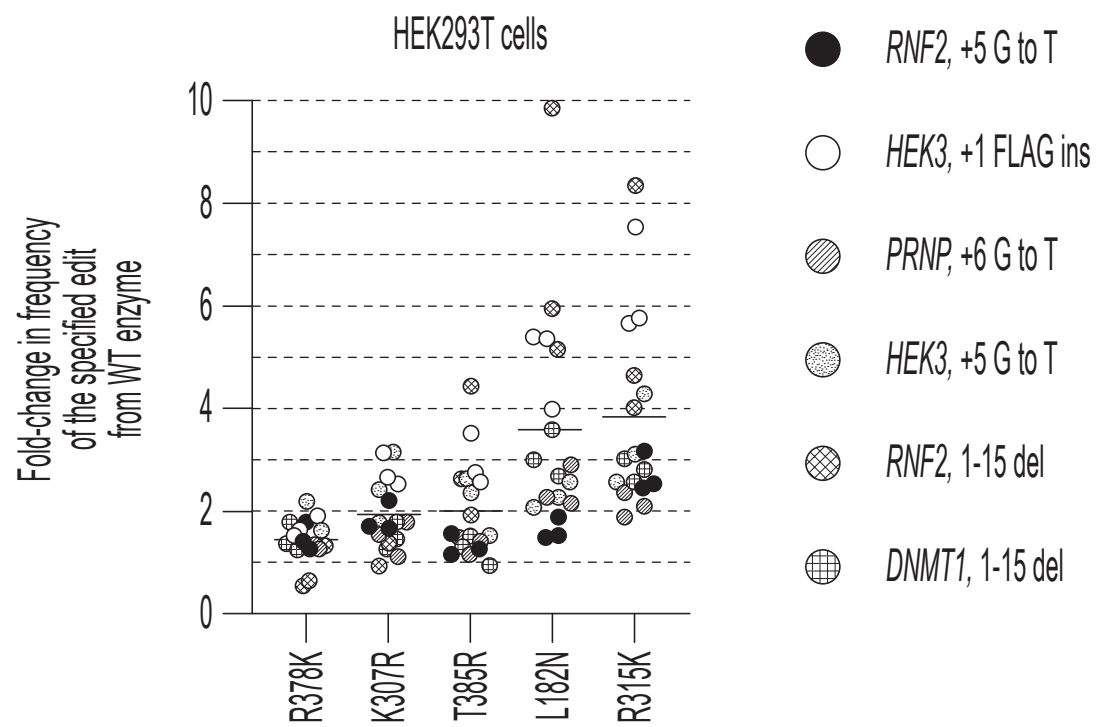


FIG. 50B

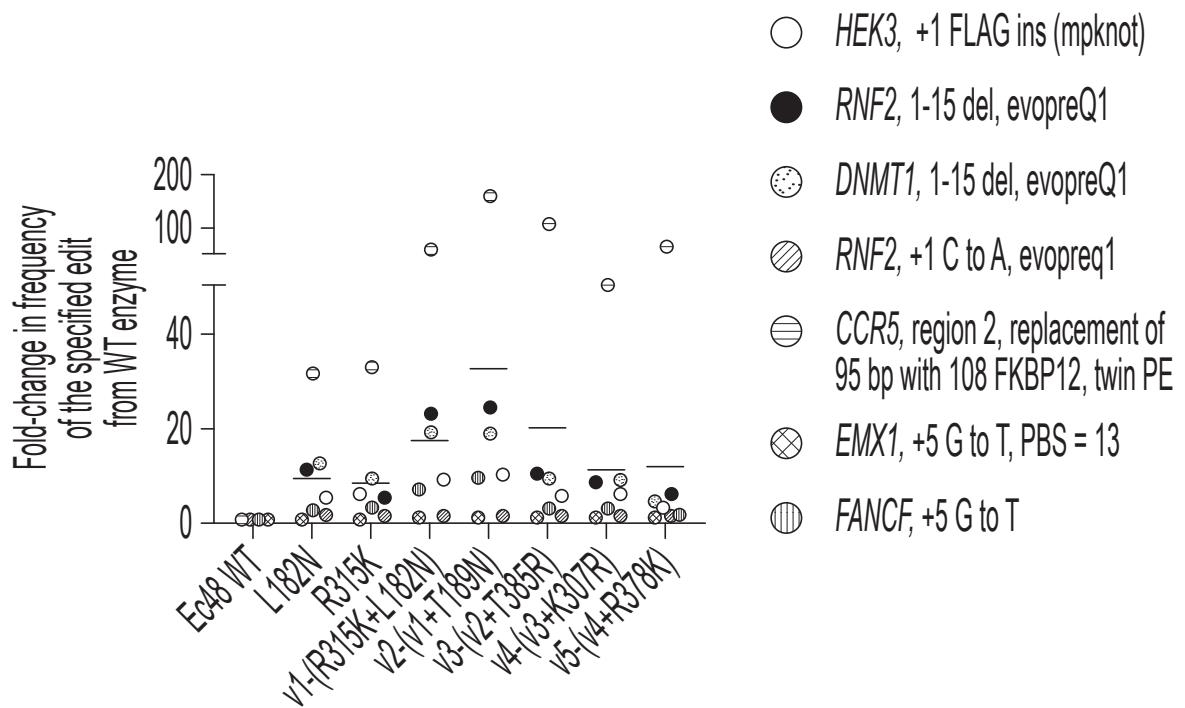


FIG. 51

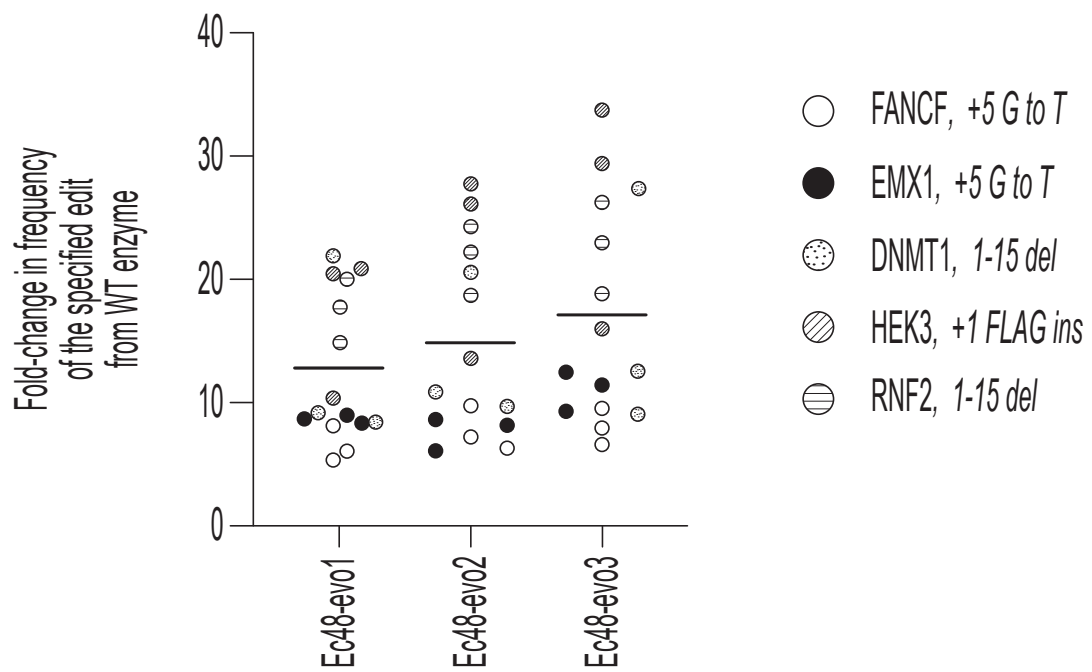


FIG. 52

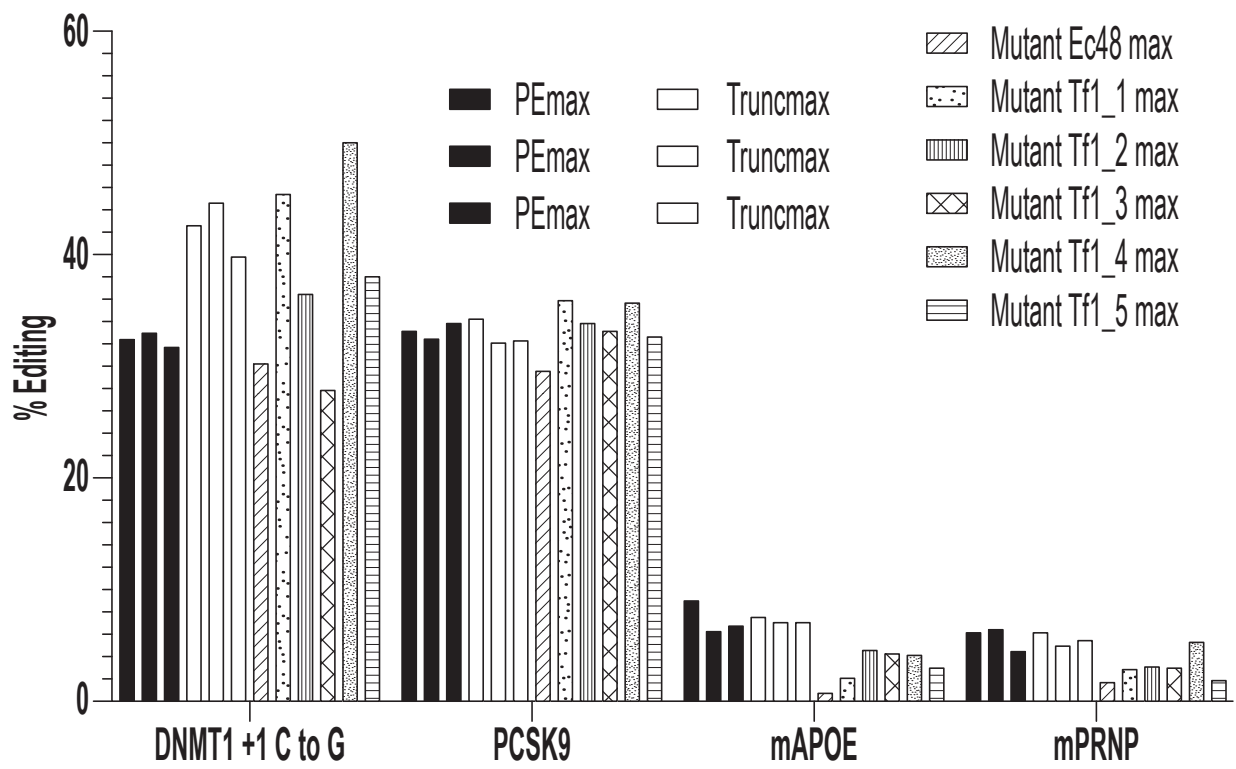


FIG. 53

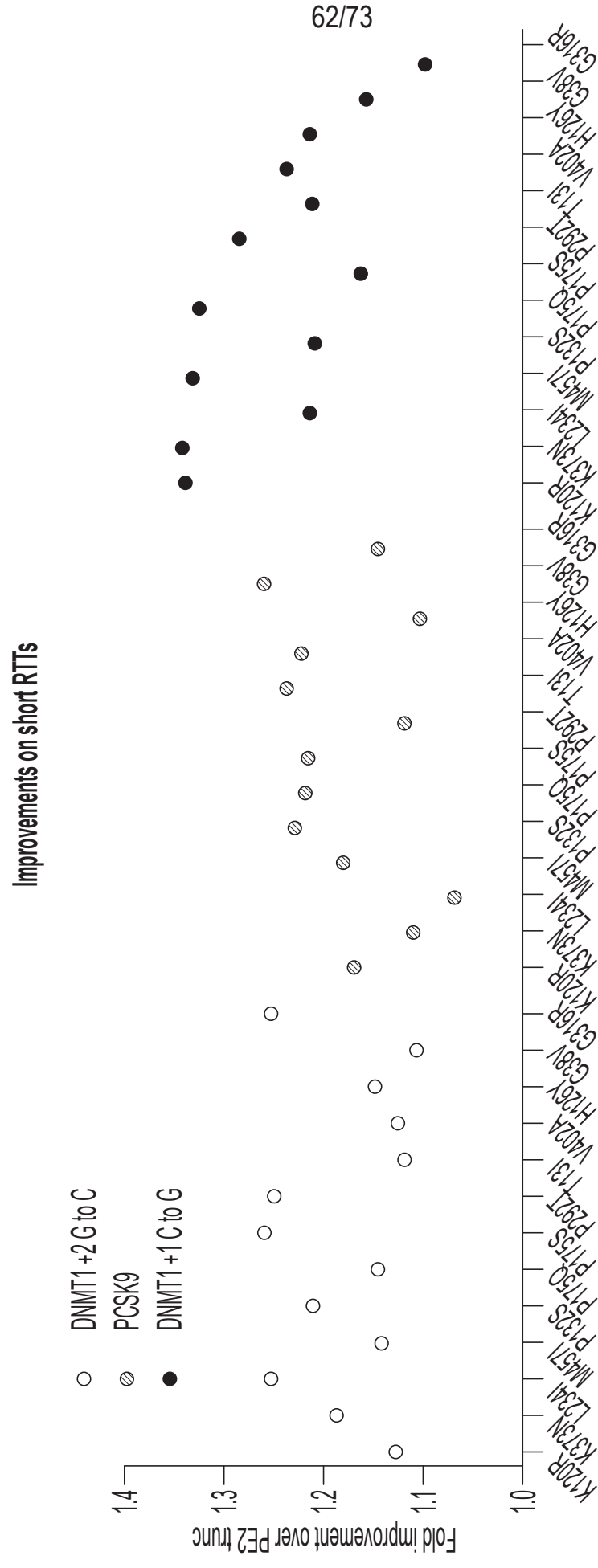


FIG. 54

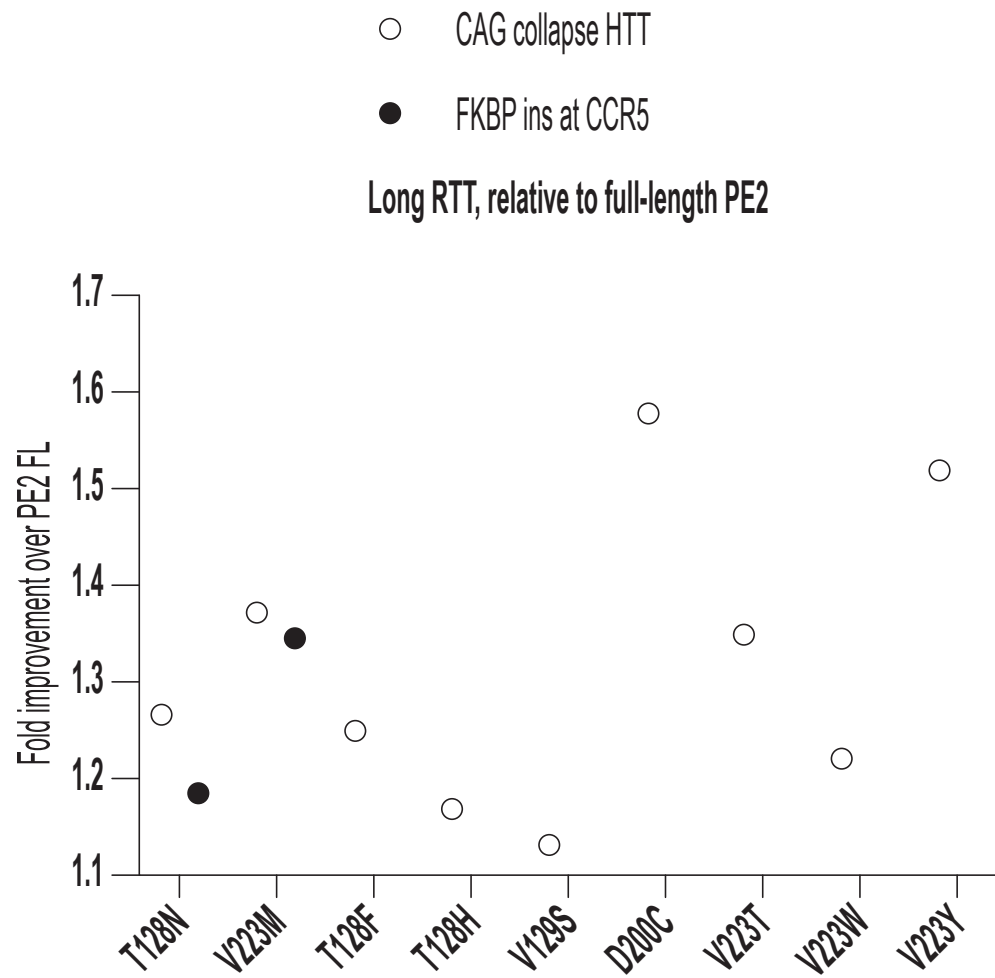


FIG. 55A



FIG. 55B

Fold-improvement over WT at PCSK9 edit in N2A cells

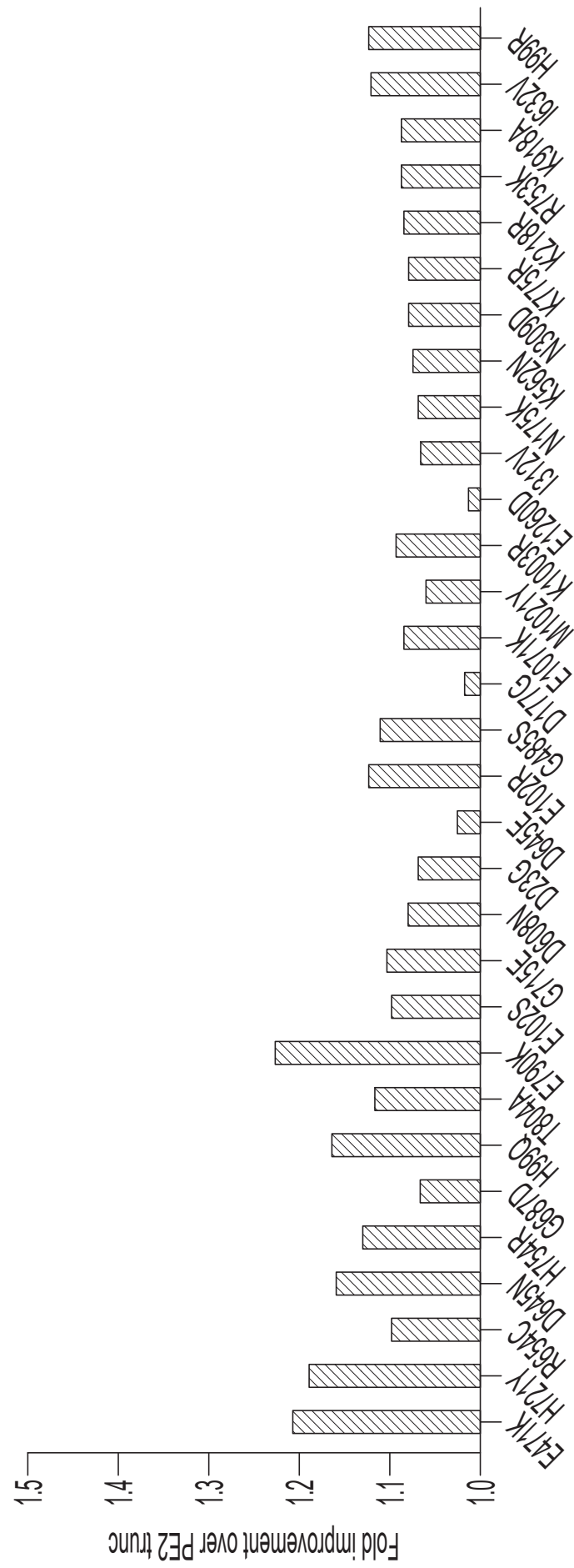


FIG. 56

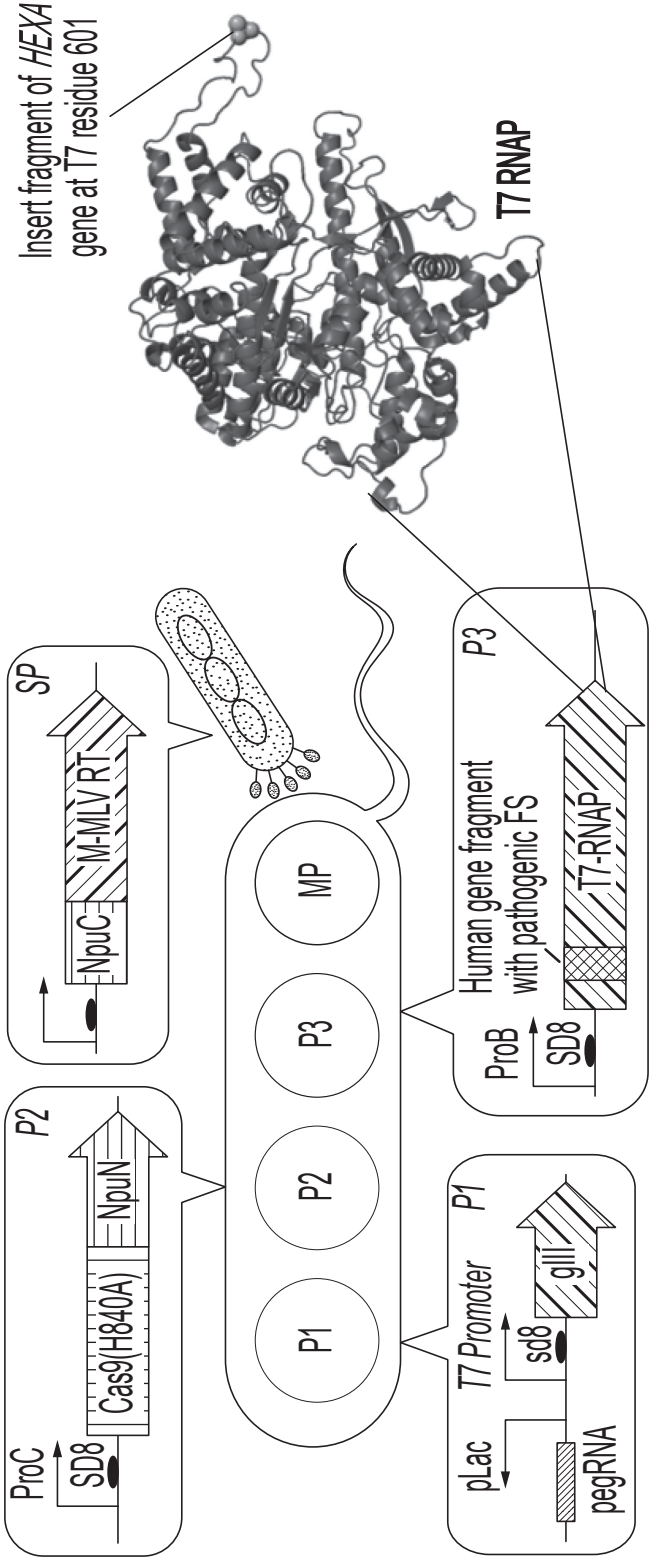


FIG. 57A

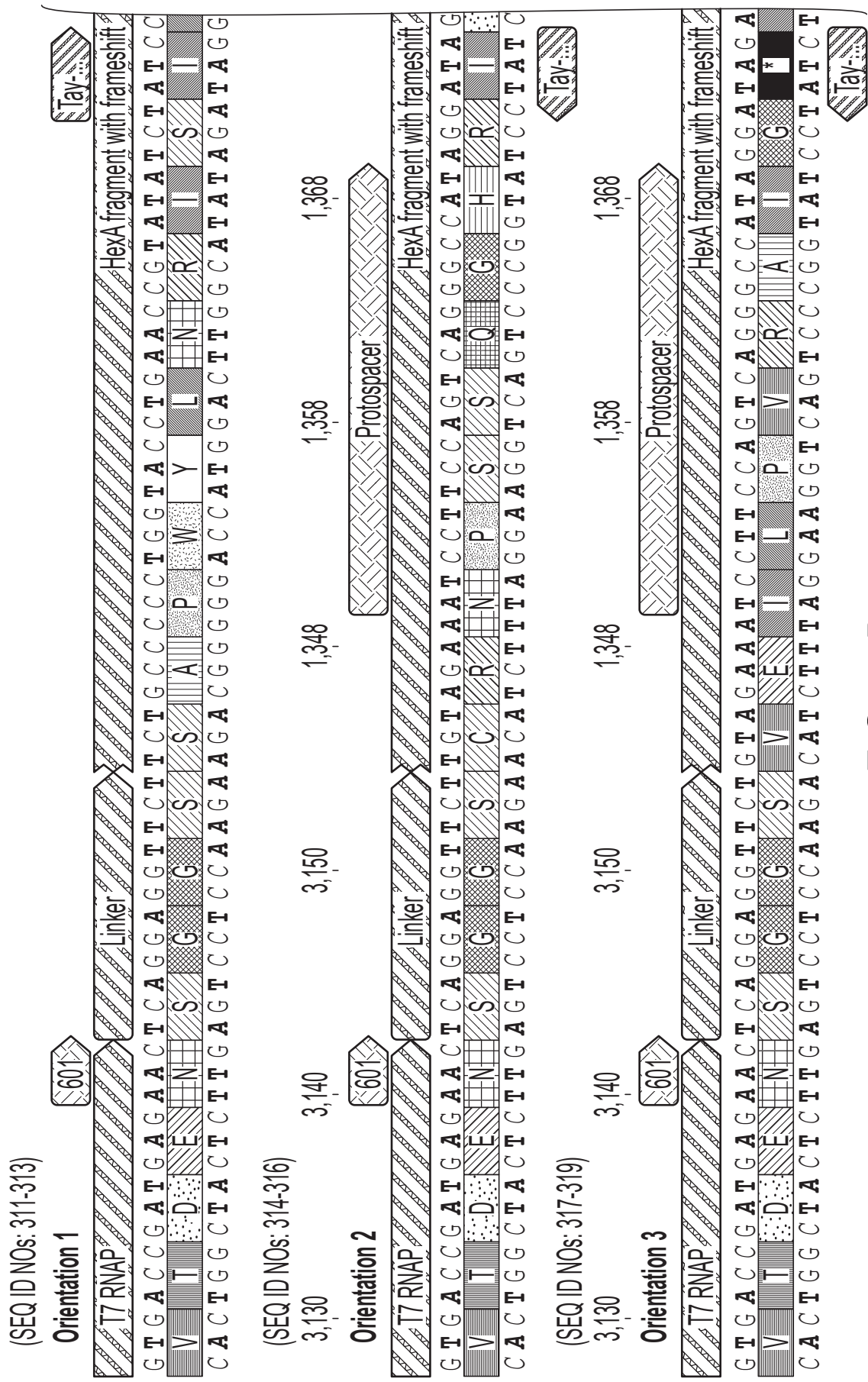


FIG. 57B

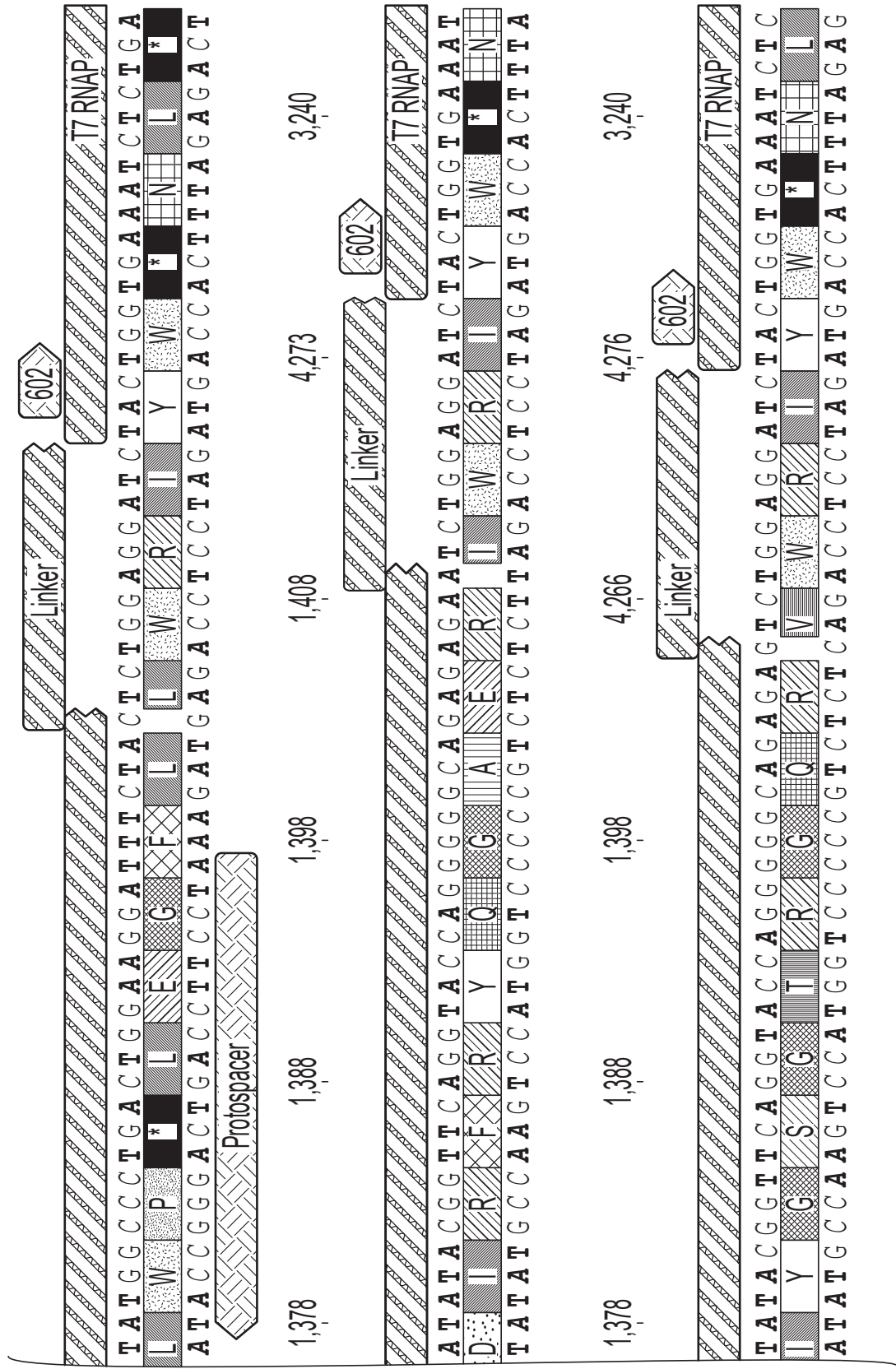
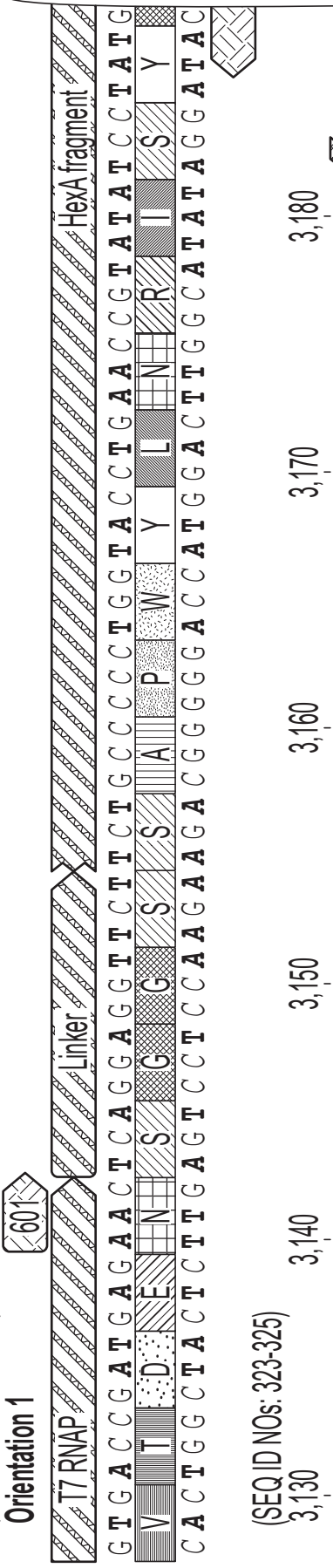
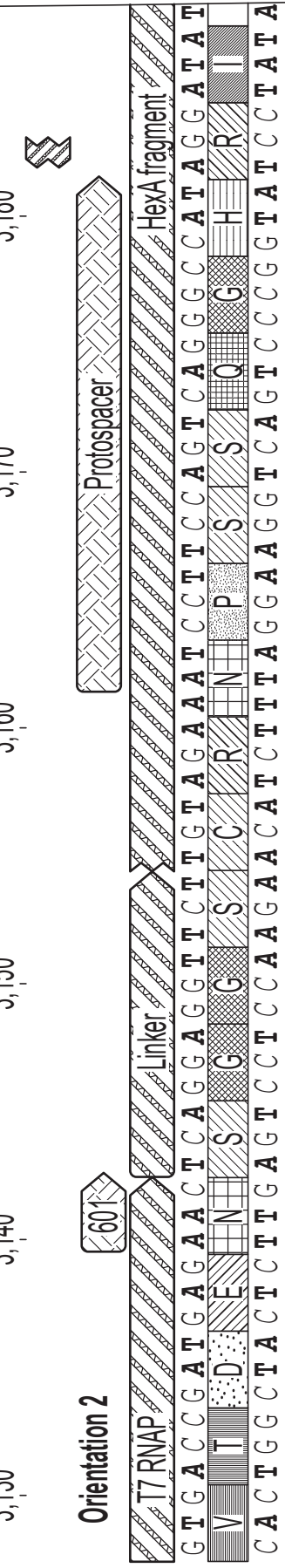


FIG. 57B
CONTINUED

(SEQ ID NOS: 320-322)
Orientation 1



(SEQ ID NOS: 323-325)
Orientation 2



(SEQ ID NOS: 326-328)
Orientation 3

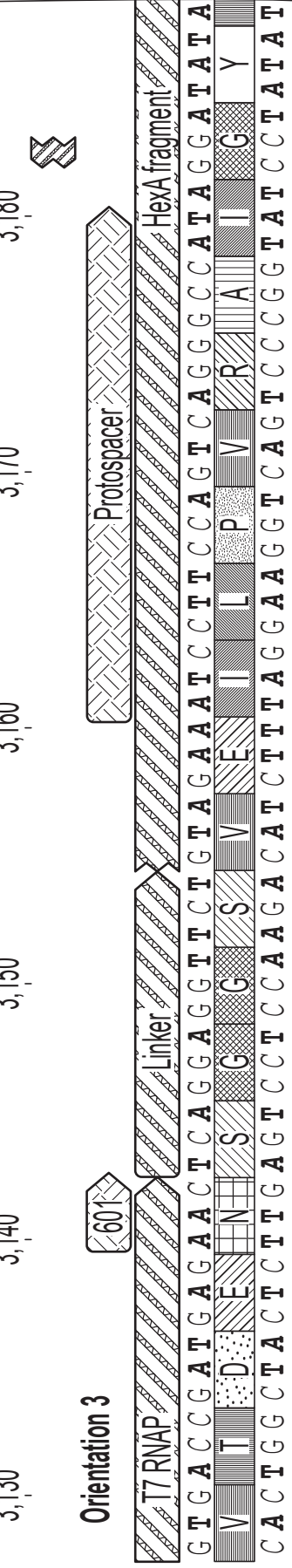


FIG. 57C

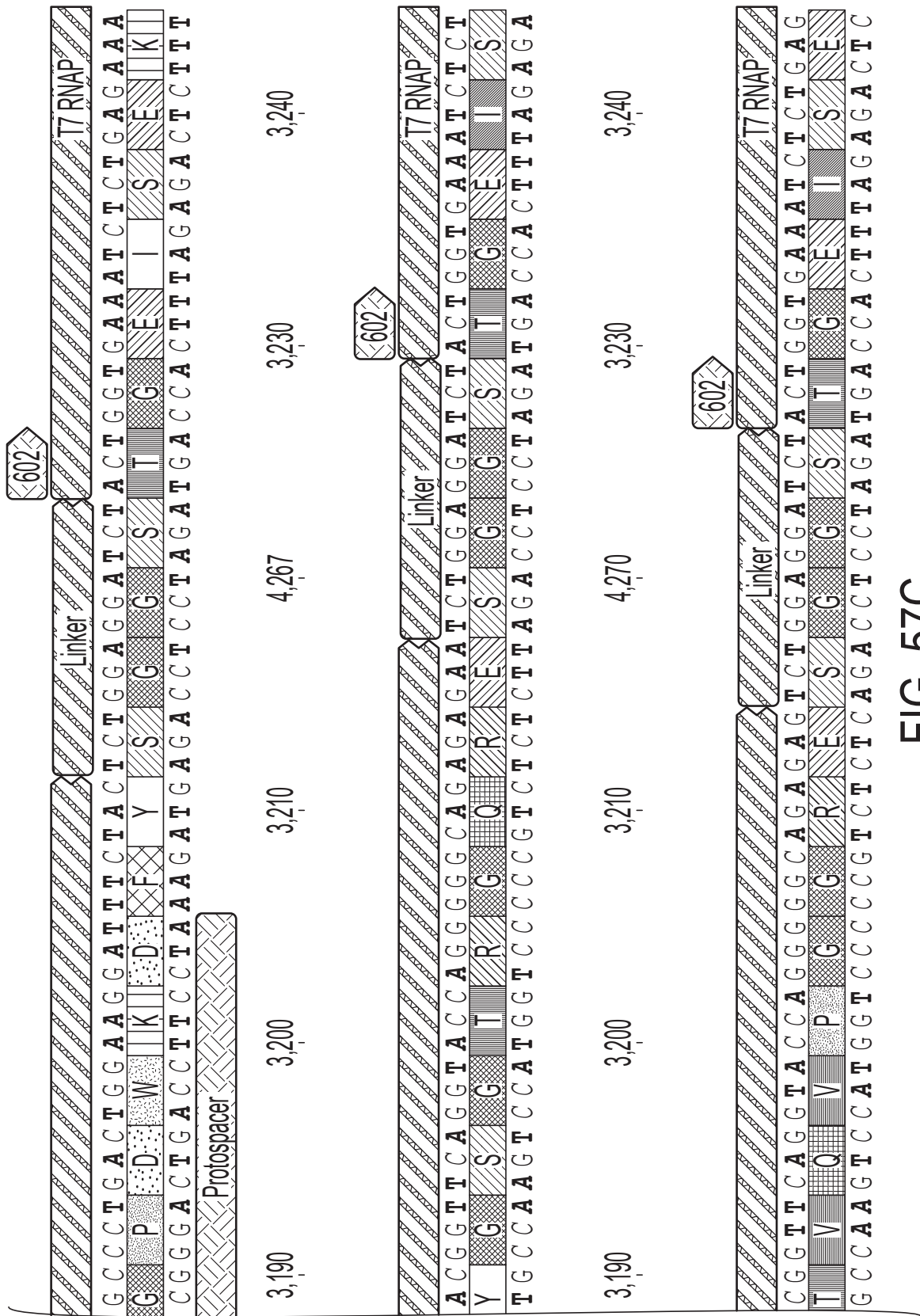


FIG. 57C
CONTINUED

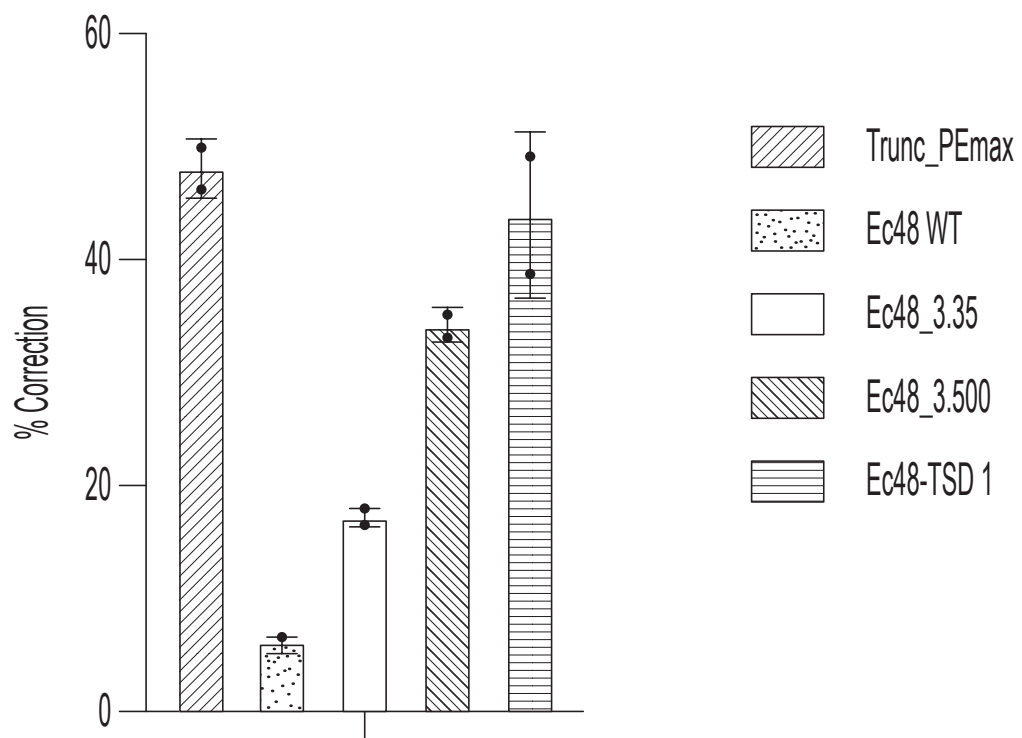


FIG. 58A

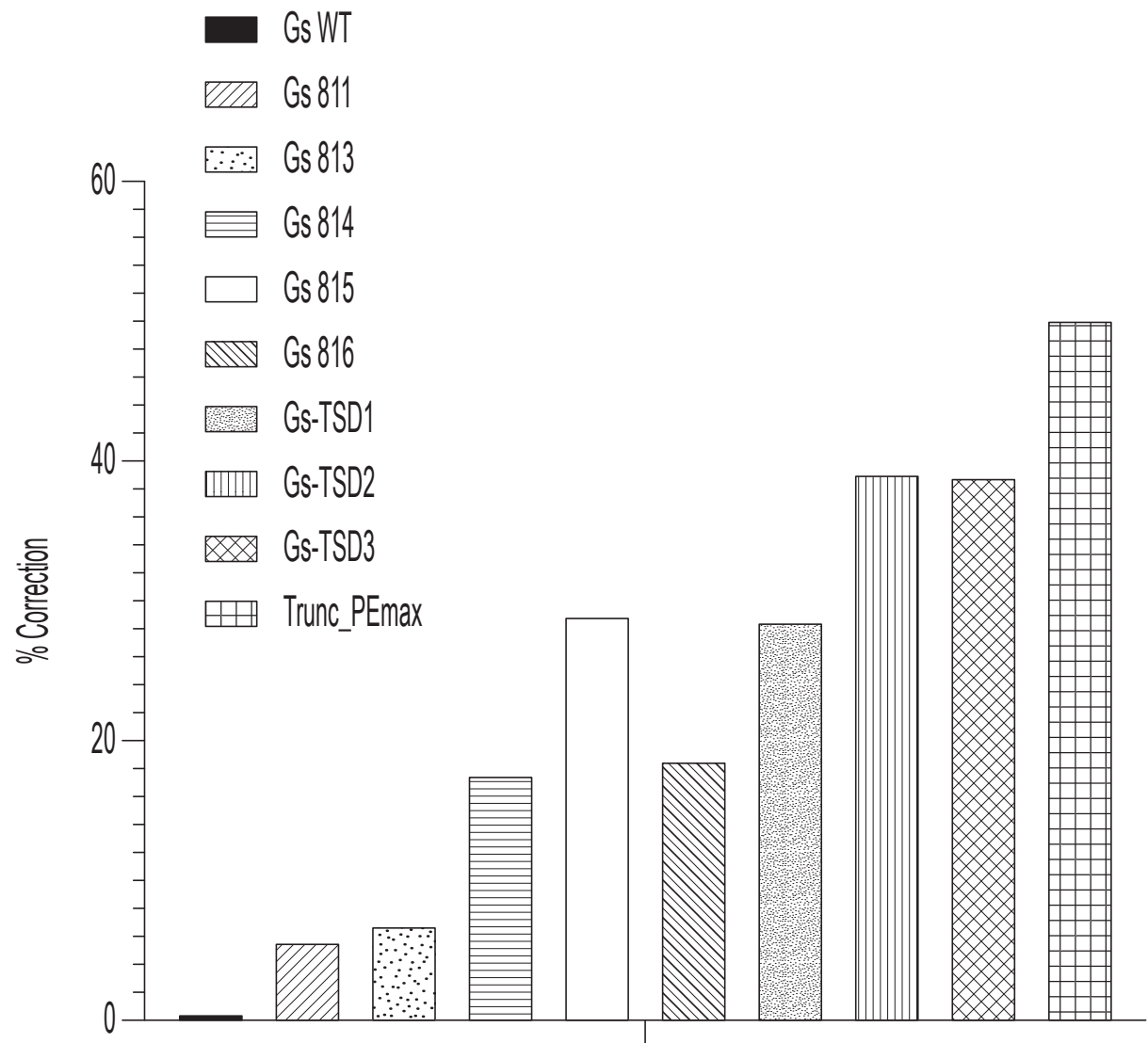


FIG. 58B

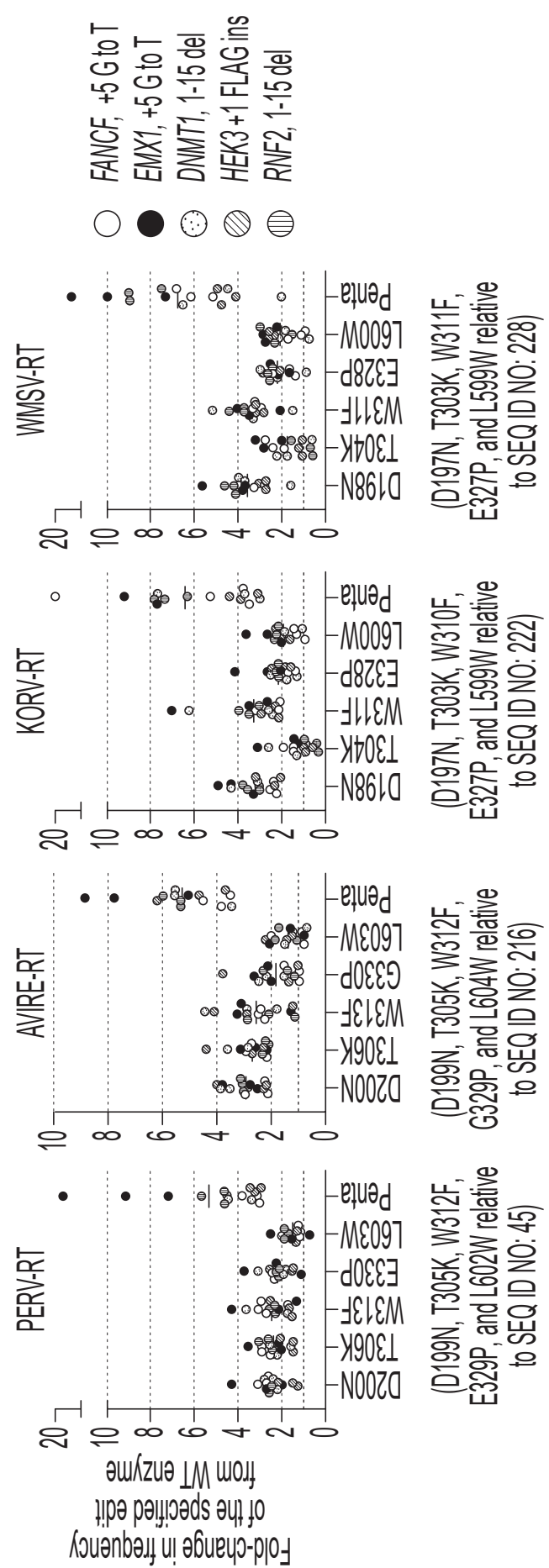


FIG. 59