
639
635
613
TM
389
Proline rich repeats
263
160
C
123
99
AB
60
54
32
A
29
SS
1

FIG. 1

SUBSTITUTE SHEET (RULE 26)

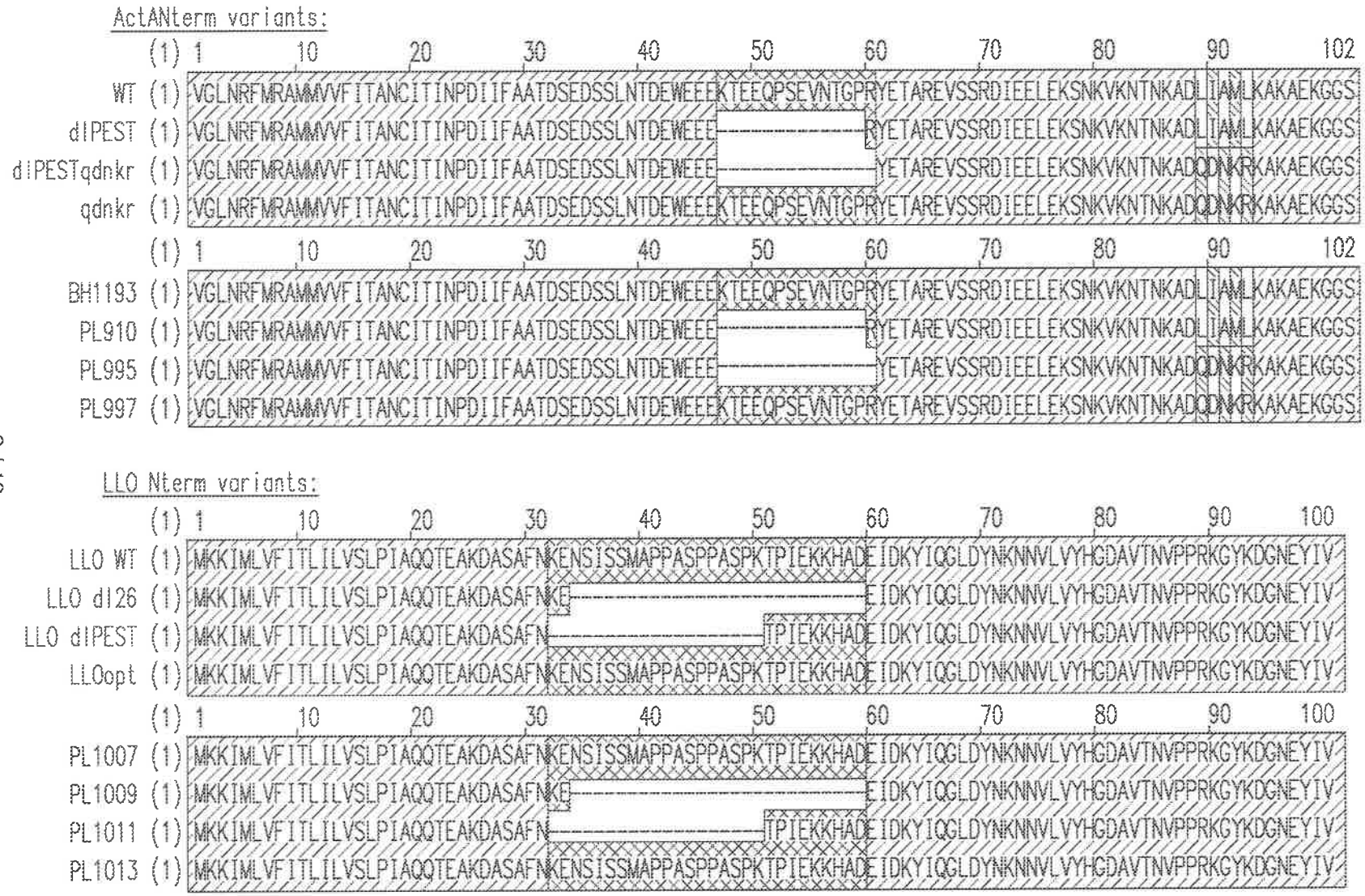


FIG.2

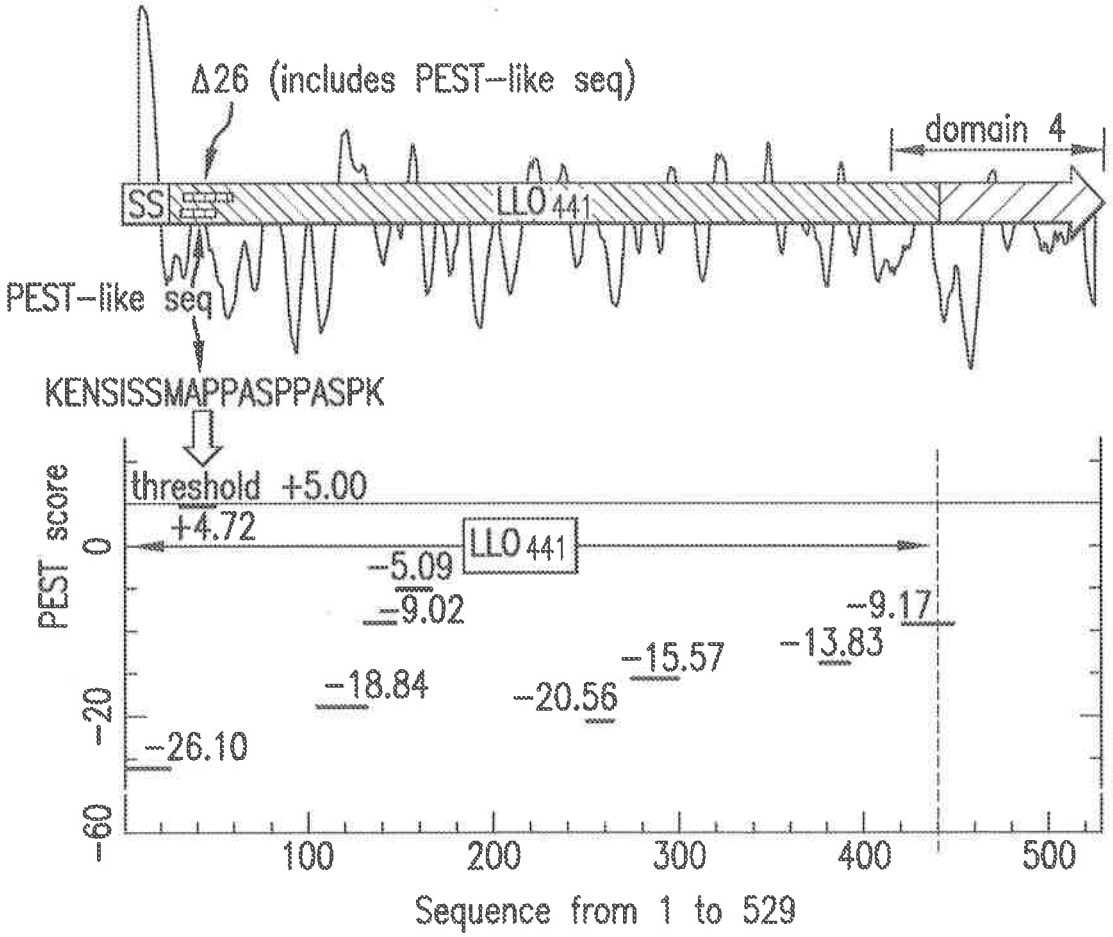

Δ26 (includes PEST-like seq)
domain 4
SS
LLO 441
PEST-like seq
KENSISSMAPPASPPASPK
threshold +5.00
+4.72
LLO 441
-5.09
-9.02
-9.17
-13.83
-15.57
-18.84
-20.56
-26.10
PEST score
0
-20
-60
100
200
300
400
500
Sequence from 1 to 529

FIG.3

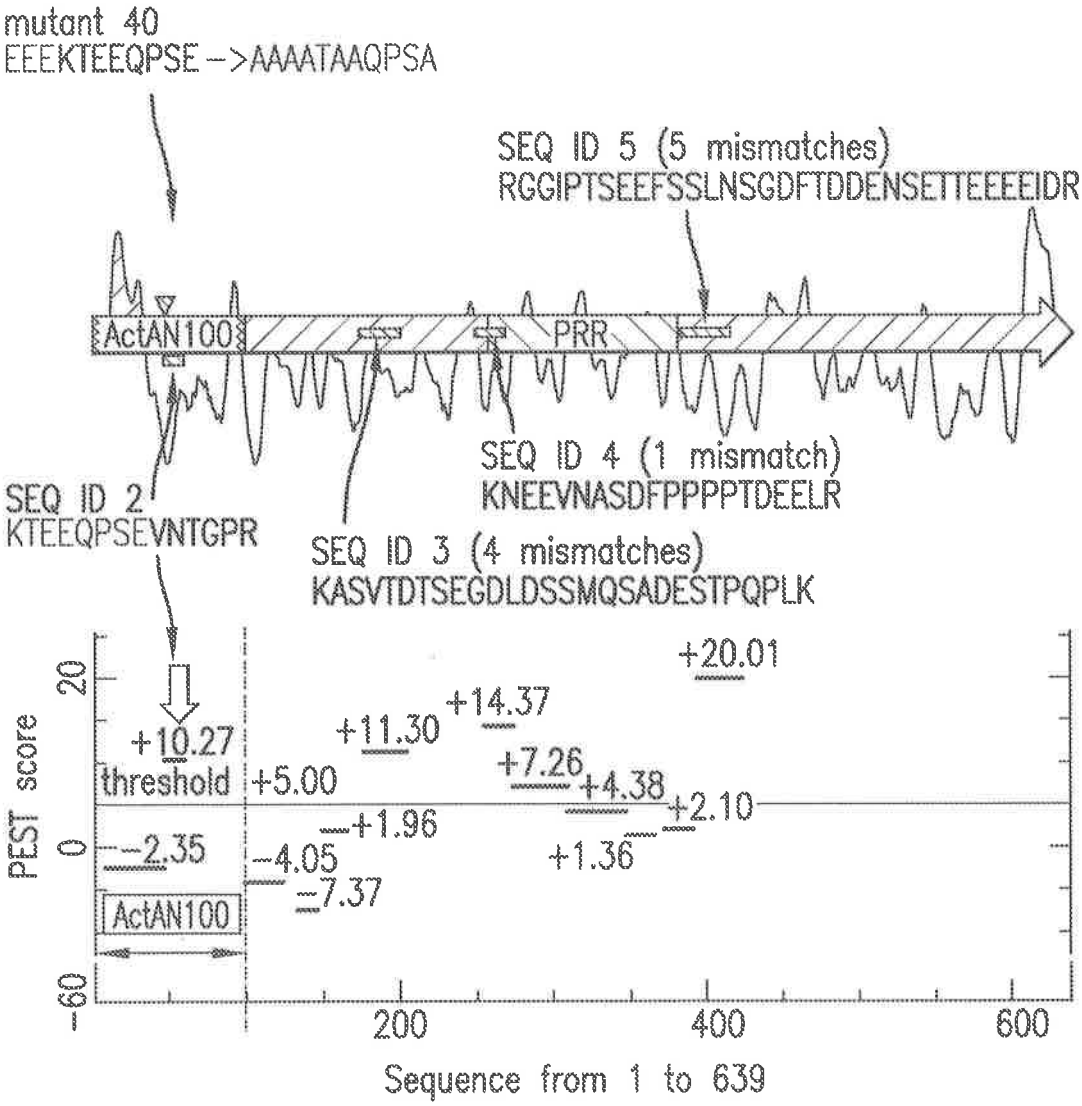
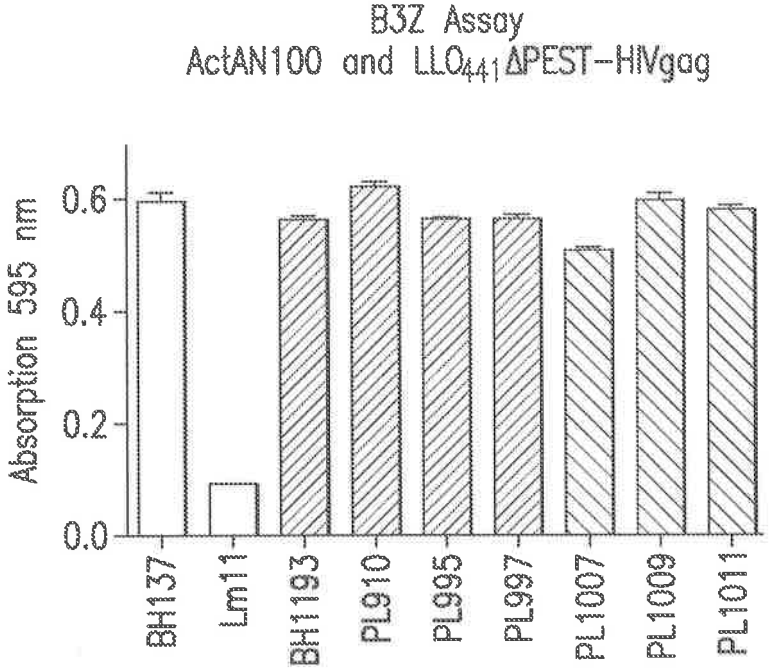

mutant 40
EEEKTEEQPSE->AAAATAAQPSA
SEQ ID 5 (5 mismatches)
RGGIPTSEEFSSLNSGDFTDDENSETTEEEEIDR
ActAN100
PRR
SEQ ID 4 (1 mismatch)
KNEEVNASDFPPPPTDEELR
SEQ ID 2
KTEEQPSEVNTGPR
SEQ ID 3 (4 mismatches)
KASVTDTSEGDLDSSMQSADESTPQPLK
+20.01
+14.37
+11.30
+10.27
+7.26
+4.38
threshold
+5.00
+2.10
+1.96
-2.35
-4.05
+1.36
-7.37
ActAN100
PEST score
20
0
-60
200
400
600
Sequence from 1 to 639

FIG.4



Strain	Construct
BH137	ActAN100–OVA positive control
Lm11	negative control
BH1193	ActAN100–HIVgagB–SL8
PL910	ActAN100ΔP–HIVgagB–SL8
PL995	ActAN100ΔP–QDNKR–HIVgagB–SL8
PL997	ActAN100–QDNKR–HIVgagB–SL8
PL1007	hlyp–LLO_{441}WT–A18K–HIVgagB–SL8
PL1009	hlyp–LLO_{441}Δ26–A18K–HIVgagB–SL8
PL1011	hlyp–LLO_{441}ΔPEST–A18K–HIVgagB–SL8

FIG.5

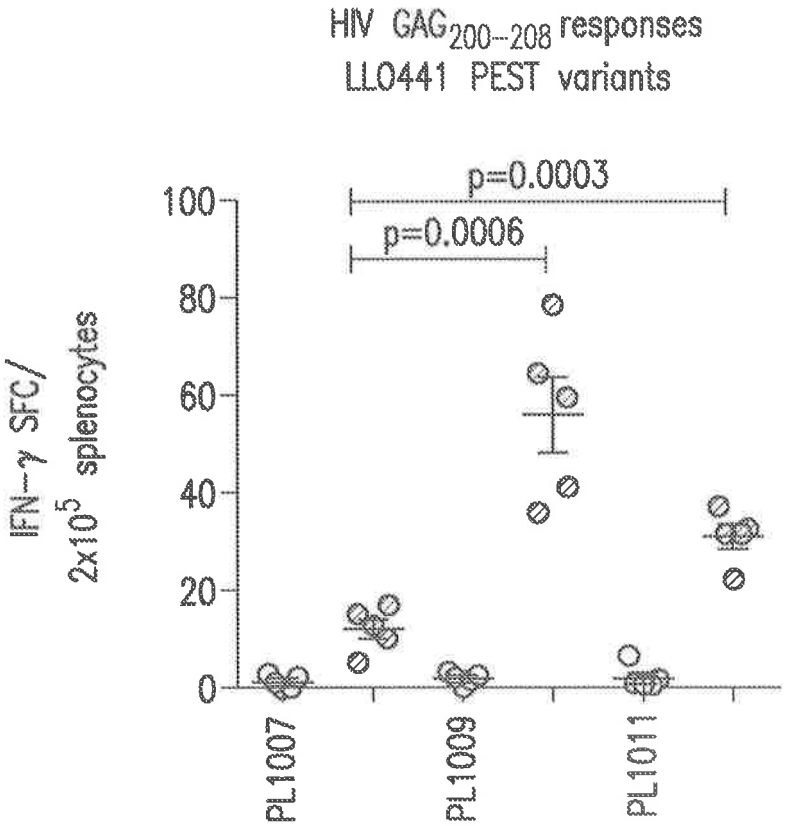

HIV GAG200–208 responses
LLO441 PEST variants
p=0.0003
p=0.0006
IFN-γ SFC/
2x10^5 splenocytes
100
80
60
40
20
0
PL1007
PL1009
PL1011

FIG.6A

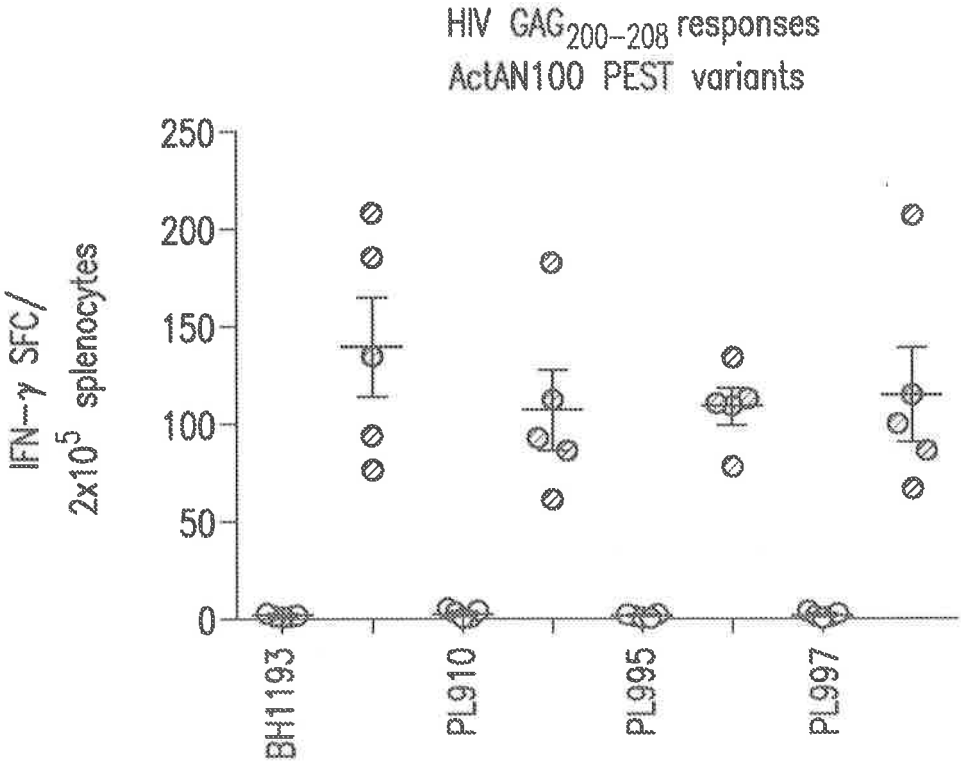

HIV GAG200–208 responses
ActAN100 PEST variants
IFN-γ SFC/
2x10^5 splenocytes
250
200
150
100
50
0
BH1193
PL910
PL995
PL997

FIG.6B

WT ActA:	LNTDEWEEEKTEEQPSEVNTGPRYETAREVSSR
Mutant 40:	LNTDEWAAAATAAQPSAVNTGPRYETAREVSSR
ΔP:	LNTDEWEEE------Δ-------YETAREVSSR
"RKR":	LNTDEWEEEKRKRQRKRVNRGKRYETAREVSSR
"KRK":	LNTDEWEEEKRKRQRKRVNRGKRYETAREVSSR

FIG.7

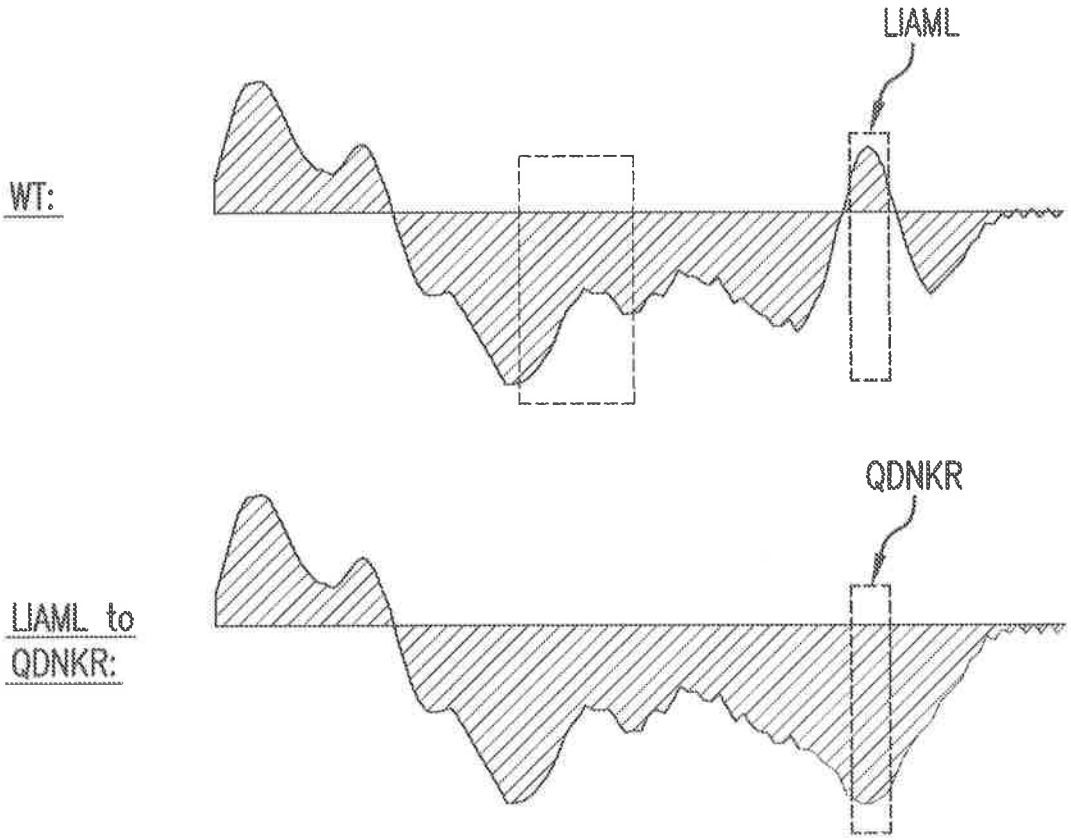


FIG.8

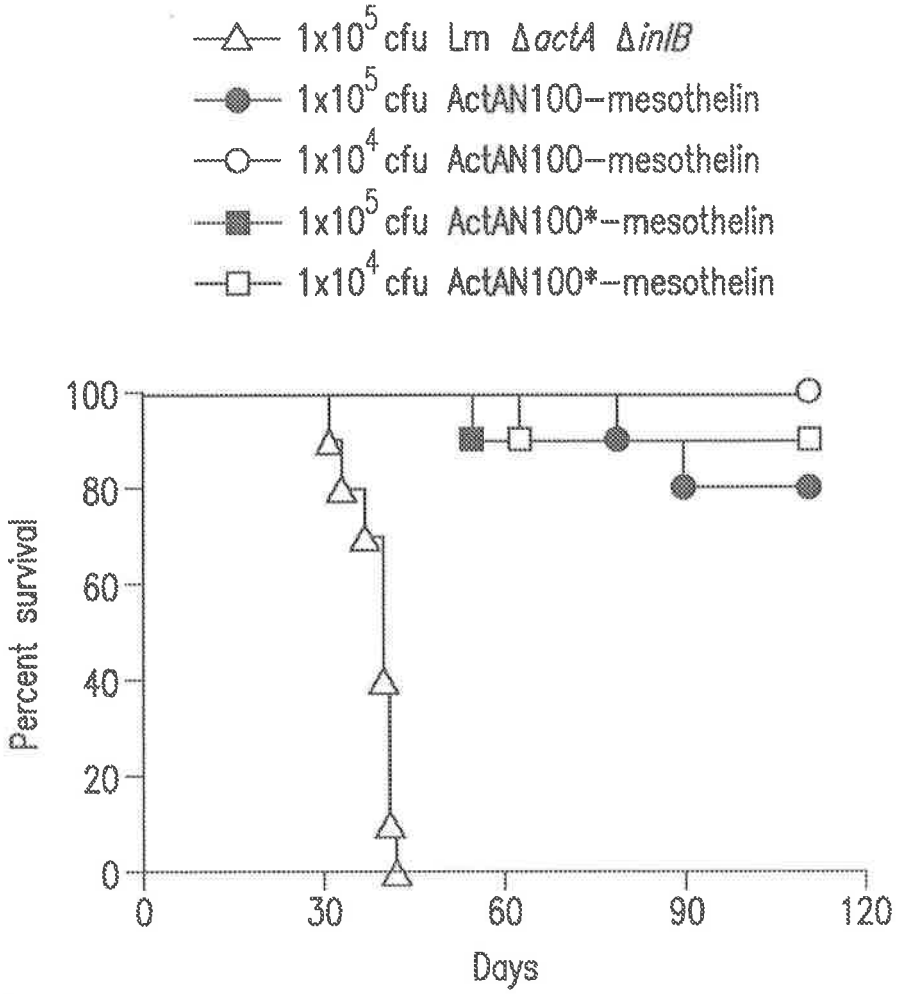
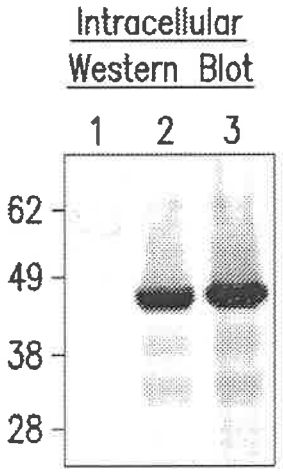

1x10^5 cfu Lm ΔactA ΔinlB
1x10^5 cfu ActAN100-mesothelin
1x10^4 cfu ActAN100-mesothelin
1x10^5 cfu ActAN100*-mesothelin
1x10^4 cfu ActAN100*-mesothelin
Percent survival
100
80
60
40
20
0
0
30
60
90
120
Days

FIG.9


actA promoter
tRNA^Arg:
ActAN100*
EGFRvIIIx5
NY-ESO-1(1-165)

FIG.10


Intracellular
Western Blot
1
2
3
62
49
38
28

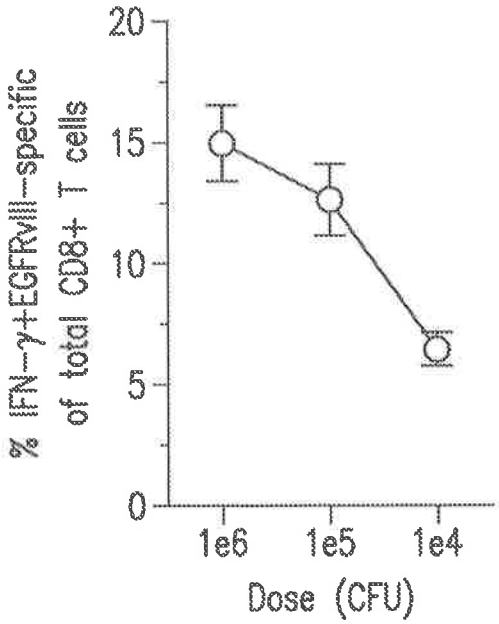


FIG.11A

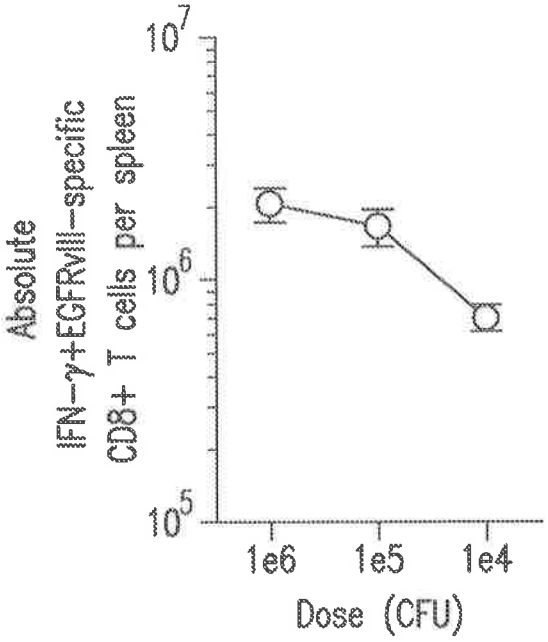


FIG.11B

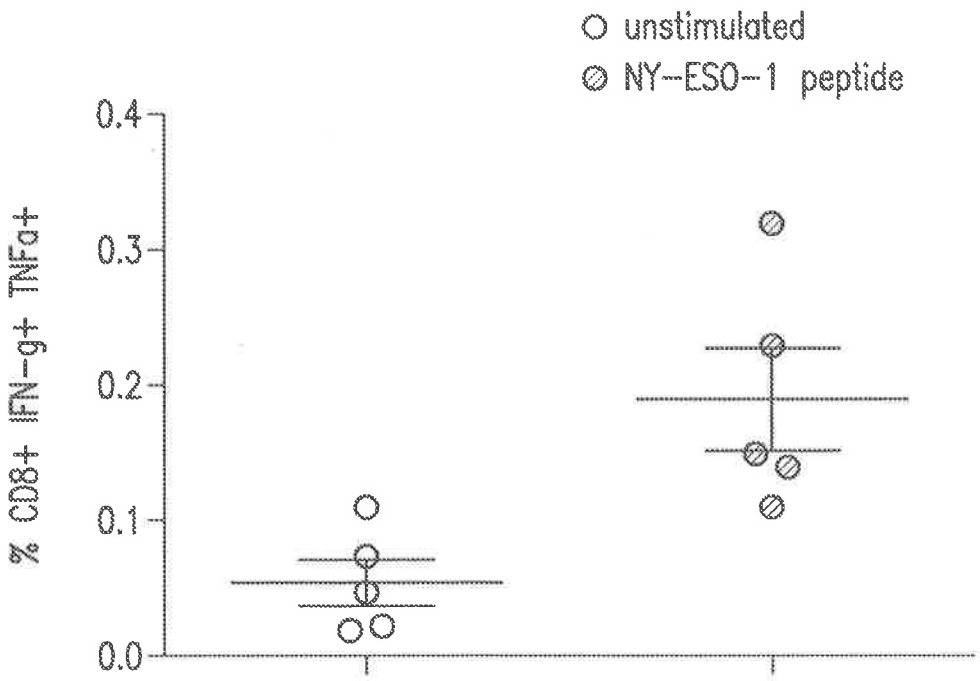


FIG. 12