
639
635
613
TM
389
Proline rich repeats
263
160
C
123
99
AB
60
54
52
A
29
SS
1

FIG. 1

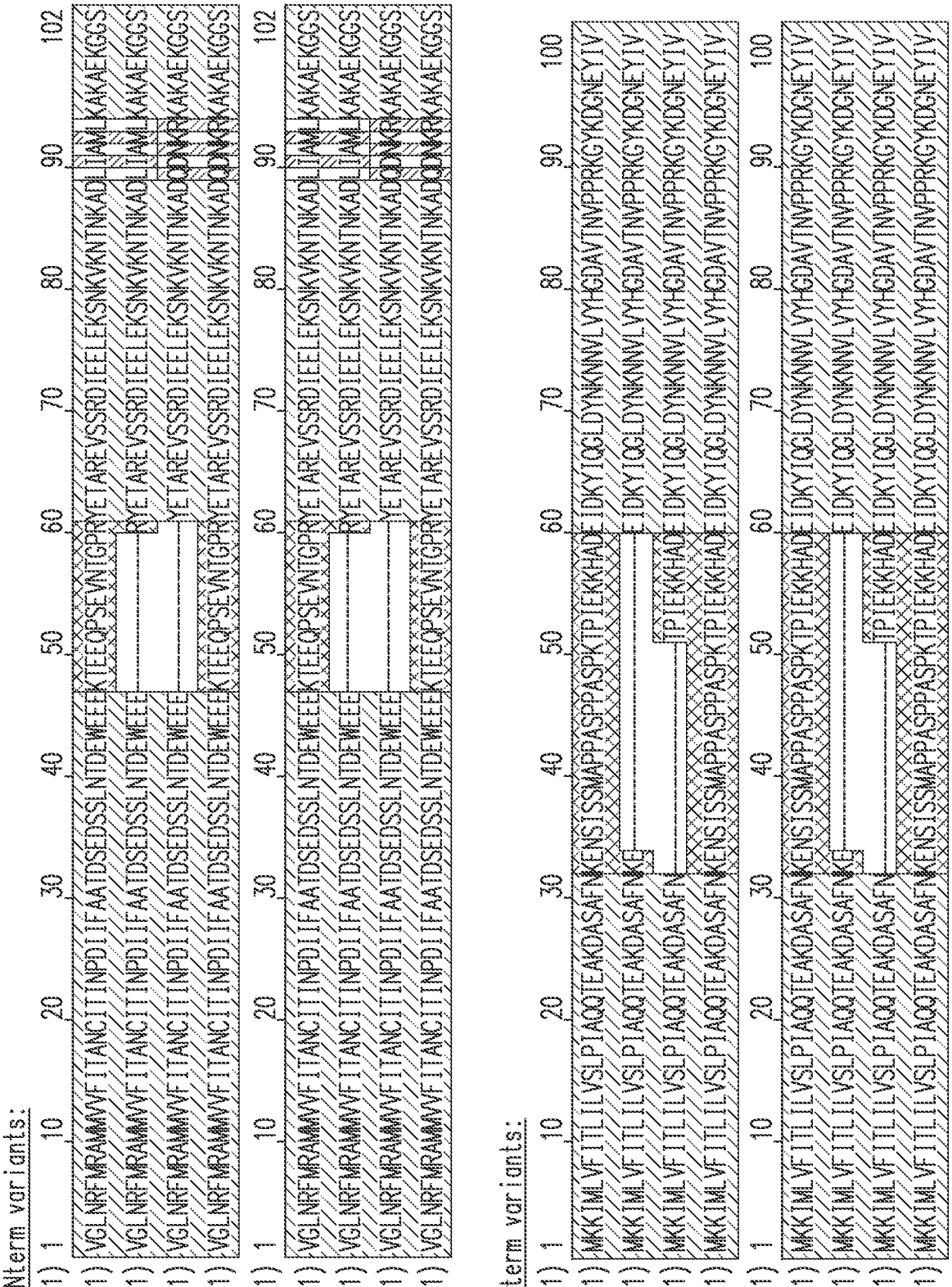

ActANterm variants:
WT
dIPEST
dIPESTqdnkr
qdnkr
BH1193
PL910
PL995
PL997
LLO Nterm variants:
LLO WT
LLO dI26
LLO dIPEST
LLOopt
PL1007
PL1009
PL1011
PL1013

FIG. 2

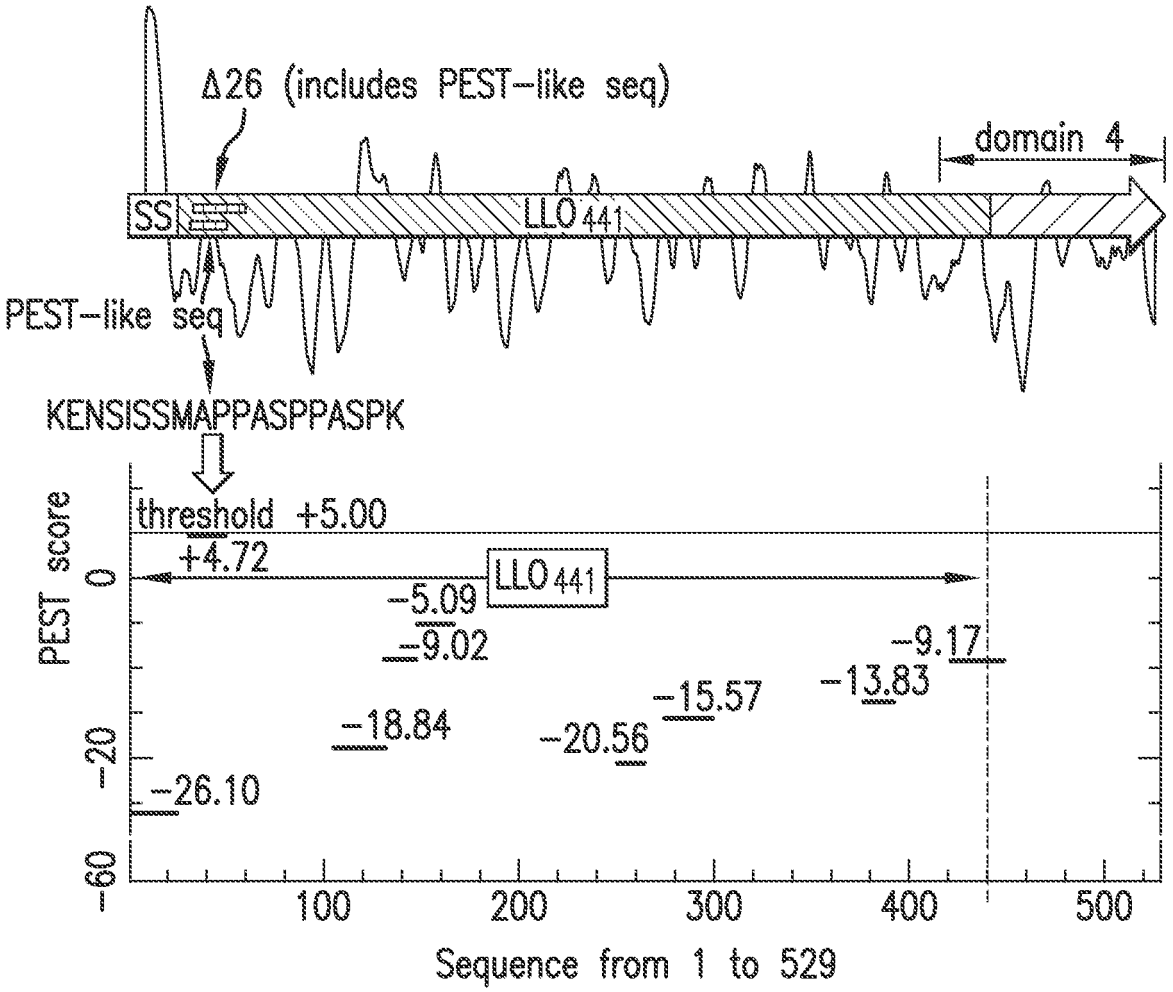

Δ26 (includes PEST-like seq)
domain 4
SS
LLO441
PEST-like seq
KENSISSMAPPASPPASPK
threshold +5.00
+4.72
LLO441
-5.09
-9.02
-9.17
-13.83
-15.57
-18.84
-20.56
-26.10
PEST score
0
-20
-60
100
200
300
400
500
Sequence from 1 to 529

FIG. 3

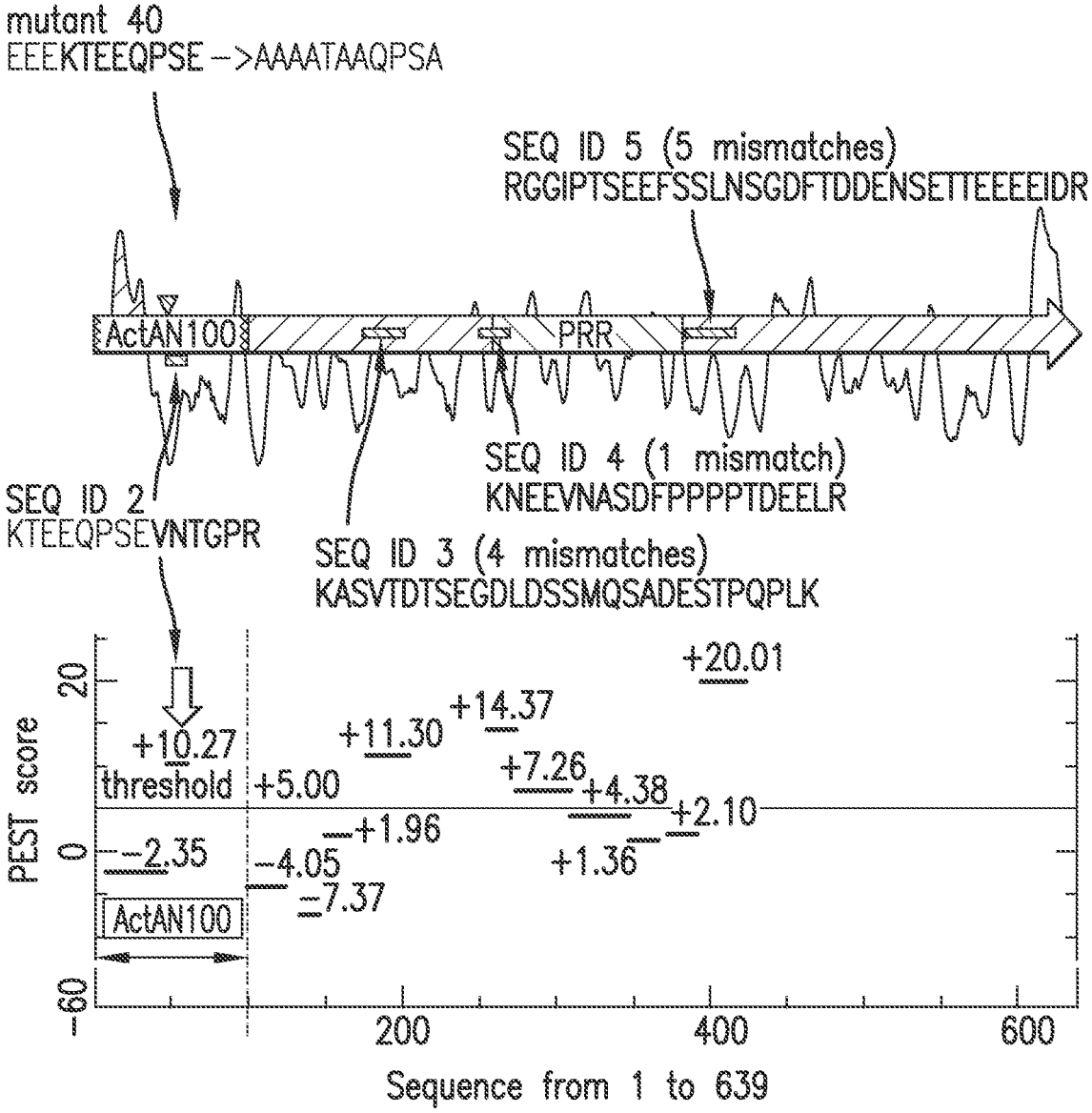
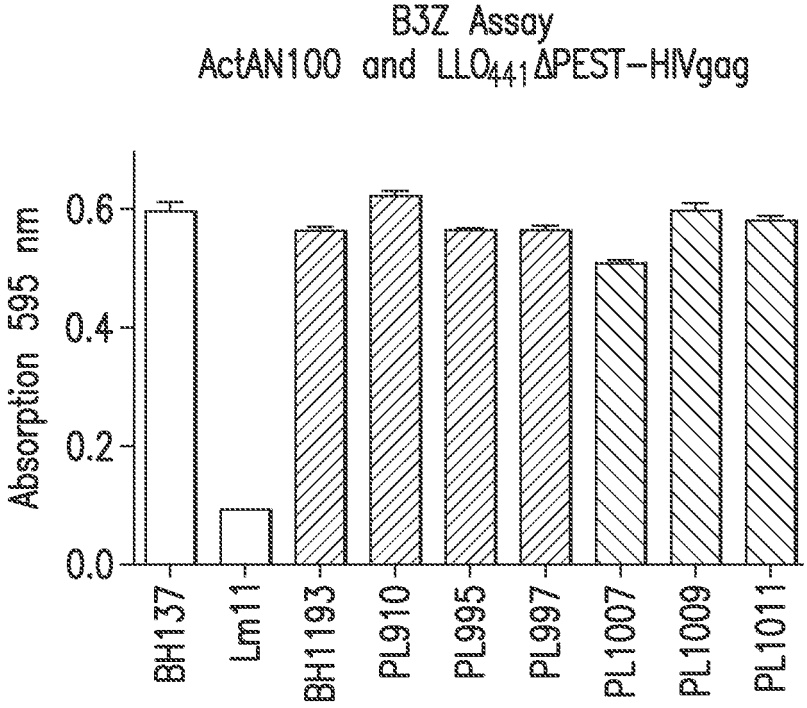

mutant 40
EEEKTEEQPSE->AAAATAAQPSA
SEQ ID 5 (5 mismatches)
RGGIPTSEEFSSLNSGDFTDDENSETTEEEEIDR
ActAN100
PRR
SEQ ID 4 (1 mismatch)
KNEEVNASDFPPPPTDEELR
SEQ ID 2
KTEEQPSEVNTGPR
SEQ ID 3 (4 mismatches)
KASVTDTSEGDLDSSMQSADESTPQPLK
+20.01
+14.37
+11.30
+10.27
+7.26
threshold
+5.00
+4.38
+2.10
+1.96
-2.35
-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

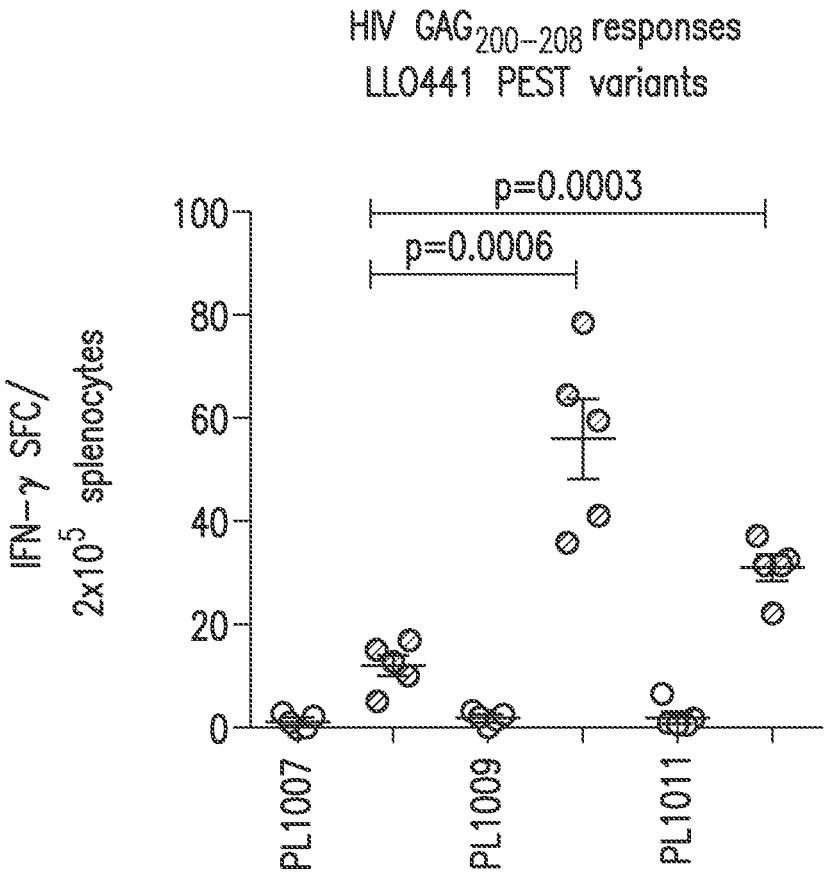

HIV GAG200-208 responses
LLO441 PEST variants
p=0.0003
p=0.0006
IFN-γ SFC/
2x10^5 splenocytes
100
80
60
40
20
0
PL1007
PL1009
PL1011

FIG.6A

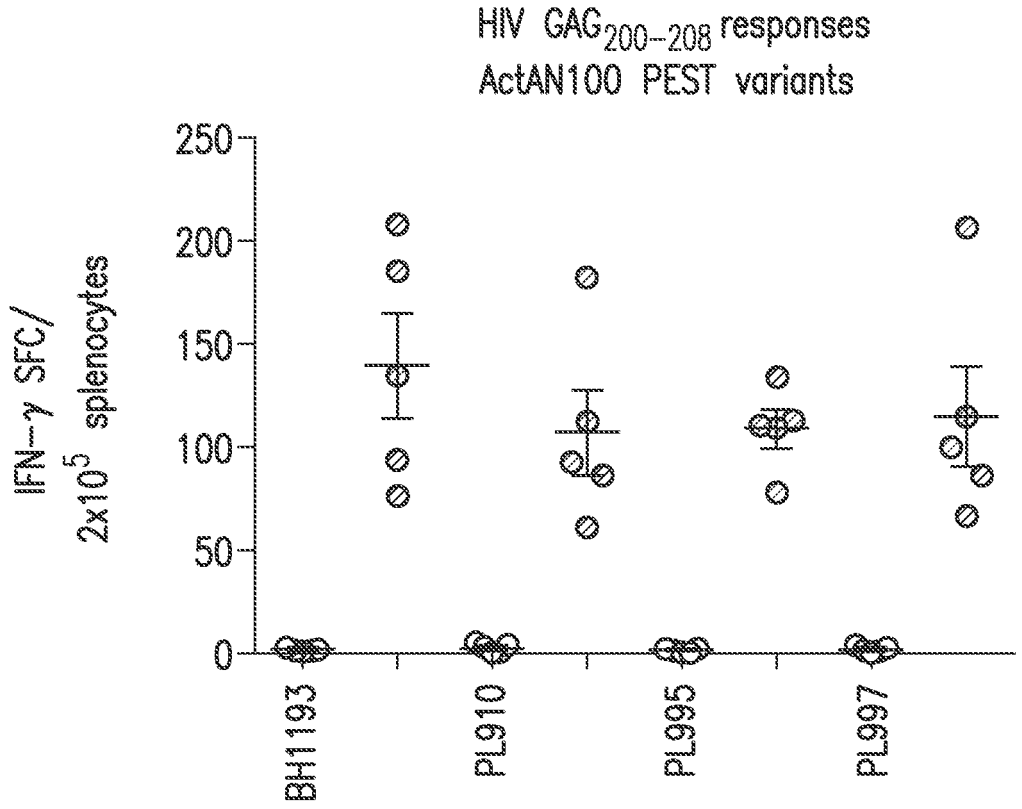

HIV GAG200–208 responses
ActAN100 PEST variants
IFN-γ SFC/
2x10^5 splenocytes
250
200
150
100
50
0
BH1193
PL910
PL995
PL997

FIG.6B

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

FIG.7

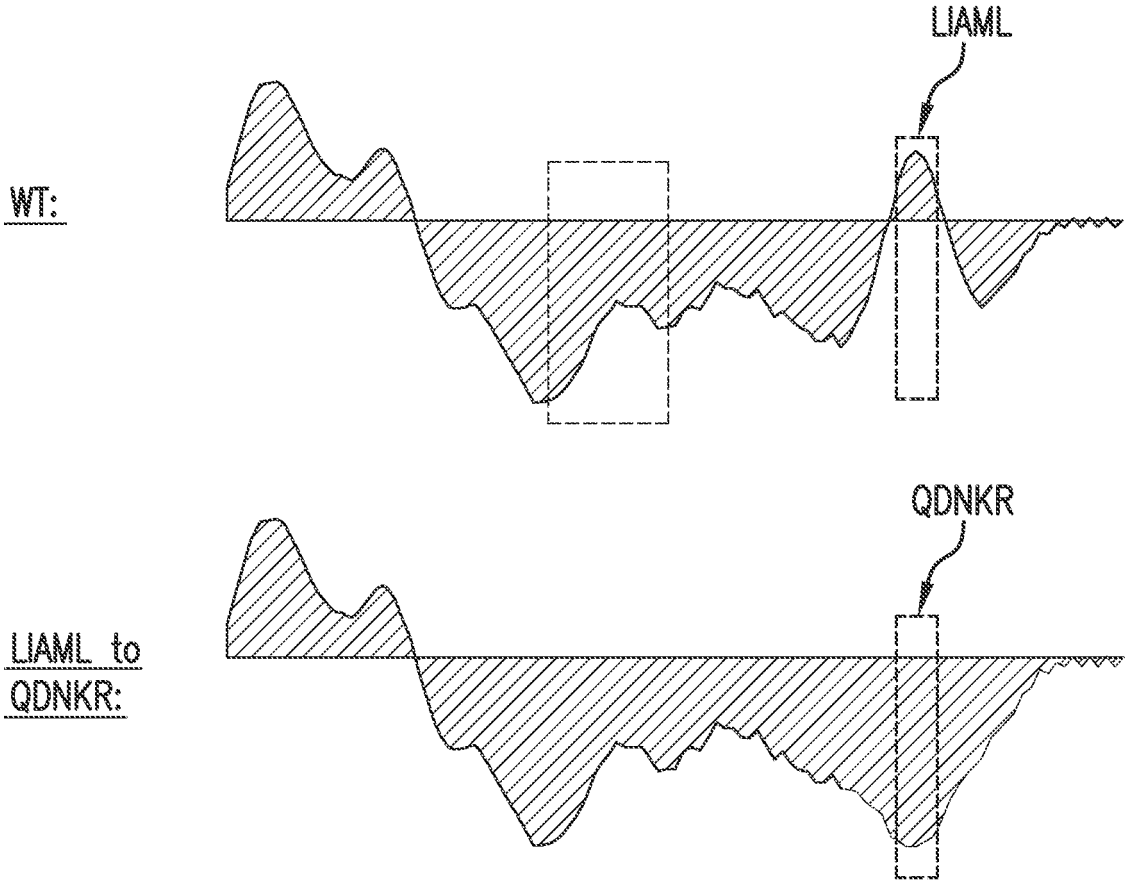


FIG.8

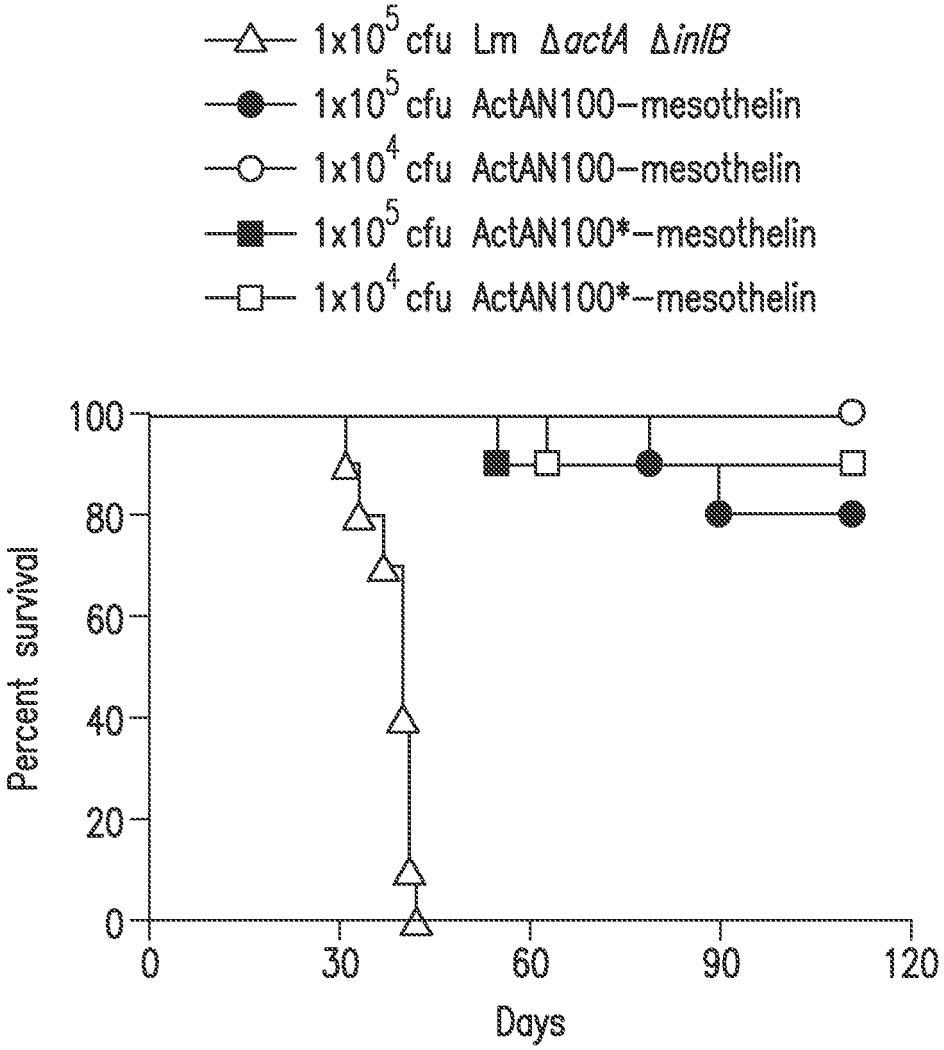
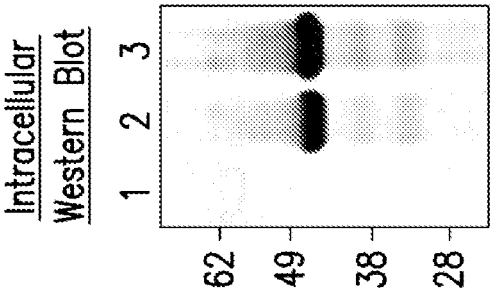

1x10^5 cfu Lm ΔactA ΔinlB
1x10^5 cfu ActAN100–mesothelin
1x10^4 cfu ActAN100–mesothelin
1x10^5 cfu ActAN100*–mesothelin
1x10^4 cfu ActAN100*–mesothelin
Percent survival
100
80
60
40
20
0
0
30
60
90
120
Days

FIG.9


Intracellular
Western Blot
1 2 3
62
49
38
28

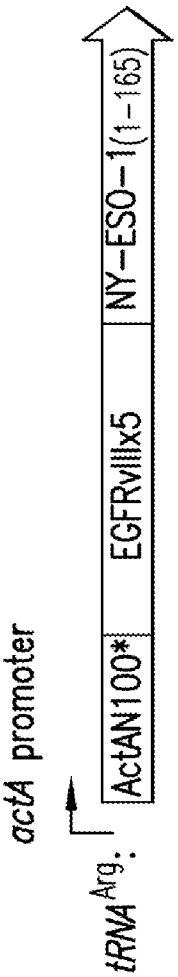

actA promoter
tRNA^Arg.
ActAN100*
EGFRvIIIx5
NY-ESO-1(1-165)

FIG. 10

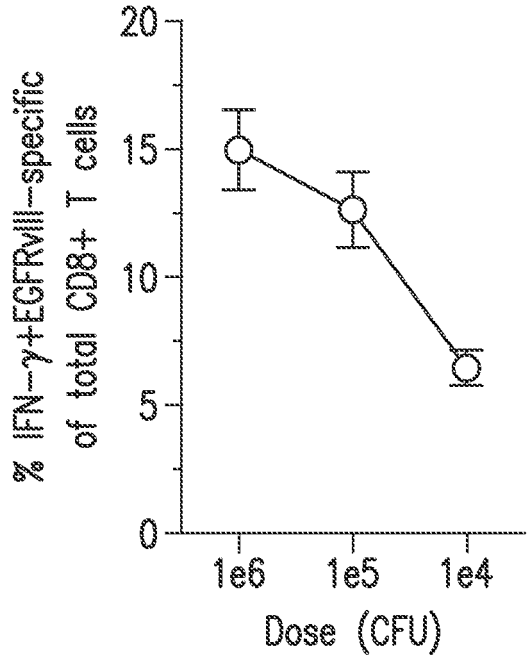


FIG.11A

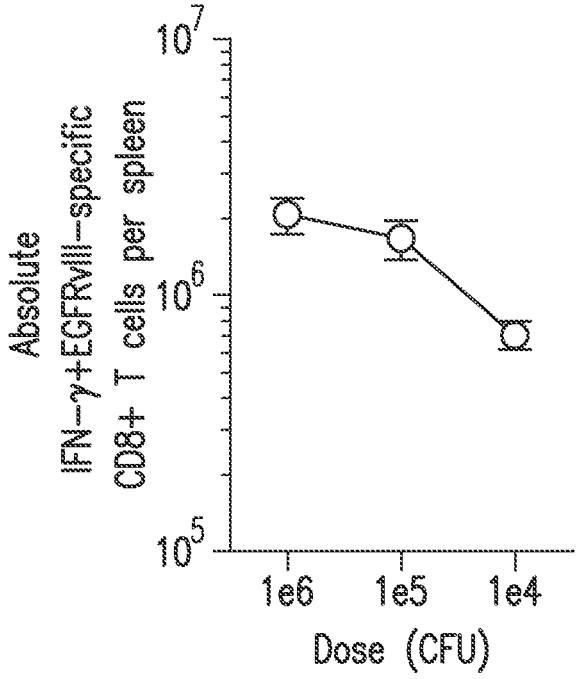


FIG.11B

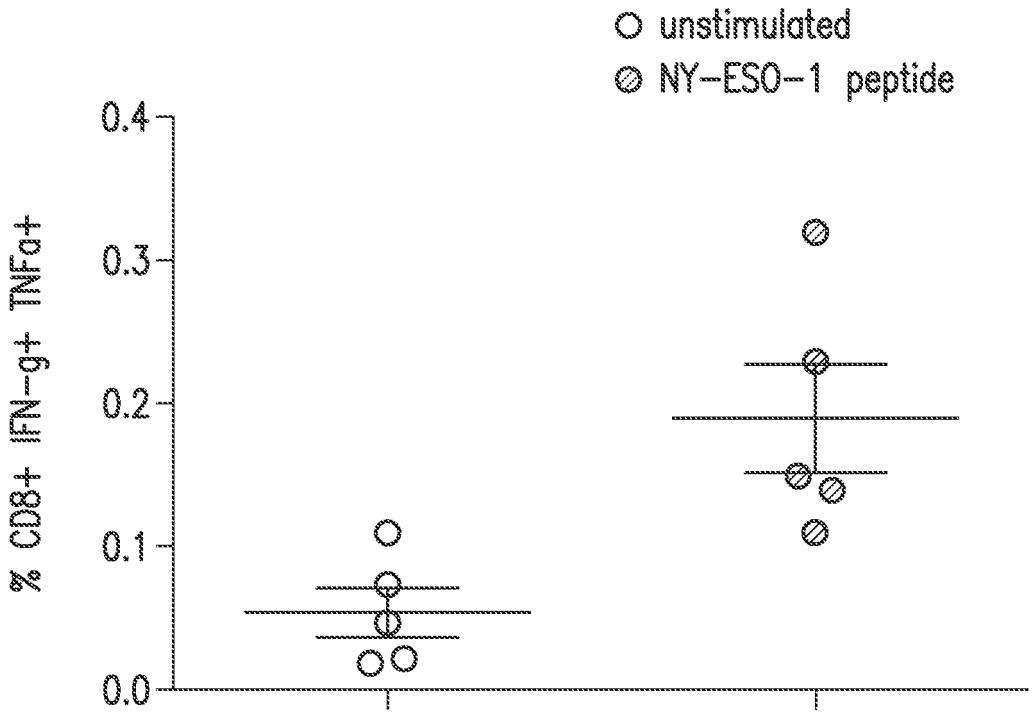


FIG.12