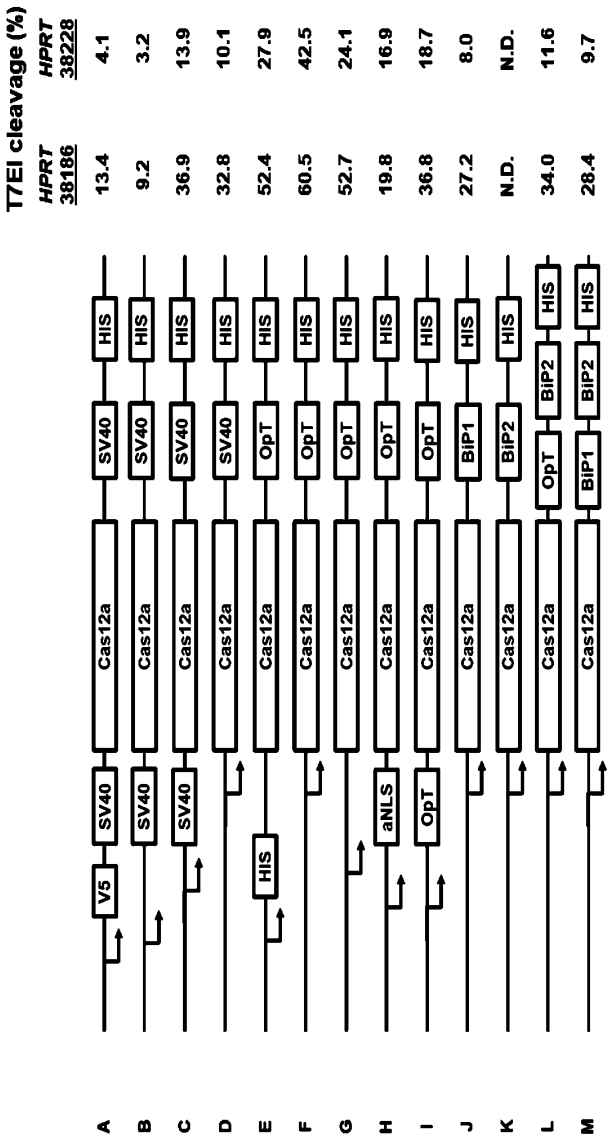

T7EI cleavage (%)
HPRT 38186
HPRT 38228
A 13.4 4.1
B 9.2 3.2
C 36.9 13.9
D 32.8 10.1
E 52.4 27.9
F 60.5 42.5
G 52.7 24.1
H 19.8 16.9
I 36.8 18.7
J 27.2 8.0
K N.D. N.D.
L 34.0 11.6
M 28.4 9.7
V5
SV40
HIS
aNLS
OpT
BiP1
BiP2
Cas12a

FIG. 1

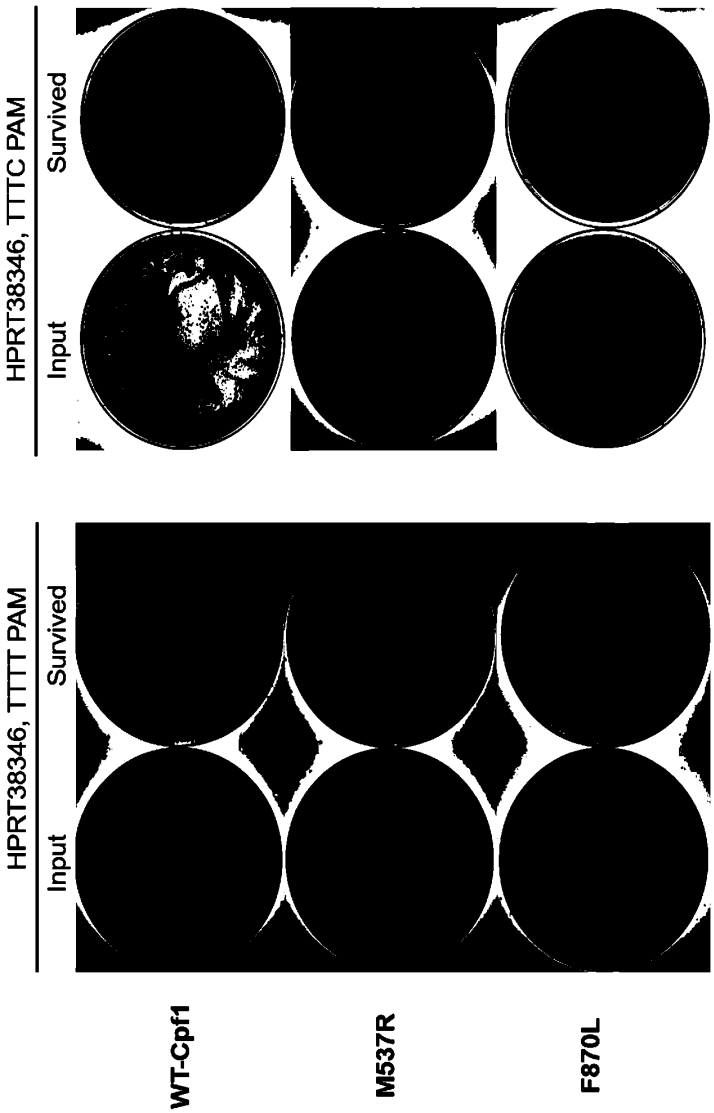

HPRT38346, TTTC PAM
Input
Survived
HPRT38346, TTTT PAM
Input
Survived
WT-Cpf1
M537R
F870L

FIG. 2

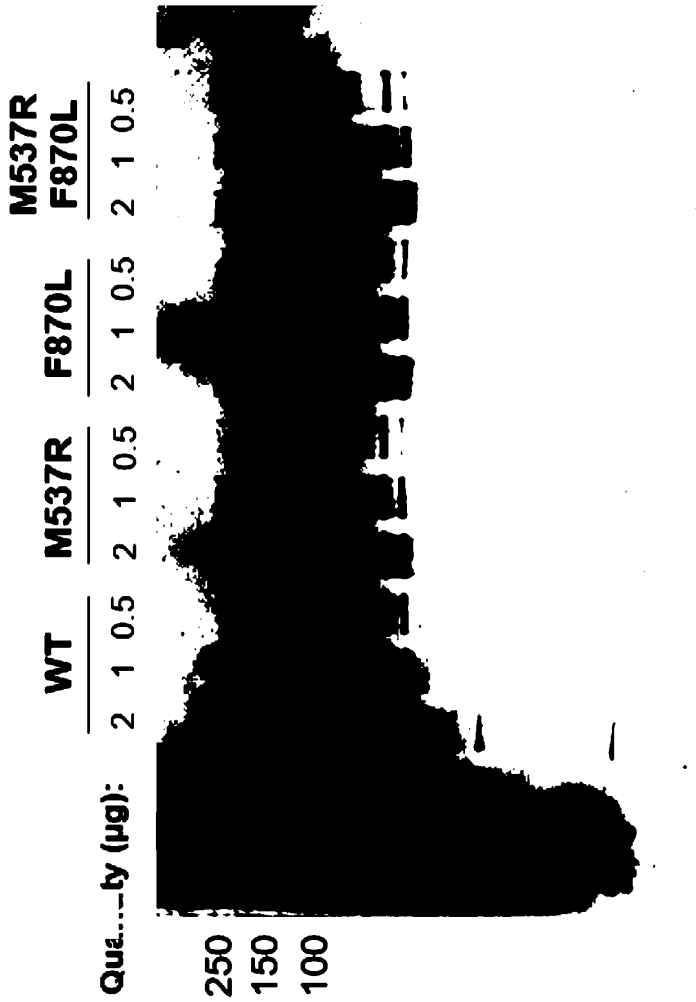

Qua...ty (µg):
WT
2 1 0.5
M537R
2 1 0.5
F870L
2 1 0.5
M537R F870L
2 1 0.5
250
150
100

FIG. 3A

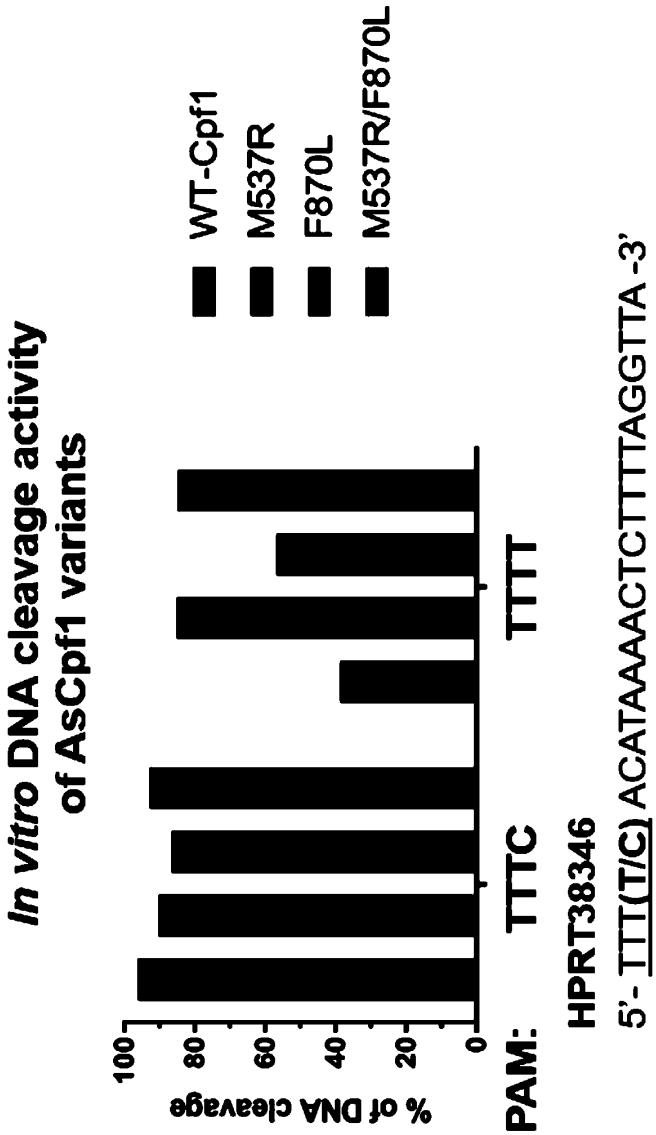

In vitro DNA cleavage activity
of AsCpf1 variants
WT-Cpf1
M537R
F870L
M537R/F870L
% of DNA cleavage
100
80
60
40
20
0
PAM:
TTTC
TTTT
HPRT38346
5'- TTT(T/C) ACATAAAACTCTTTTAGGTTA -3'

FIG. 3B

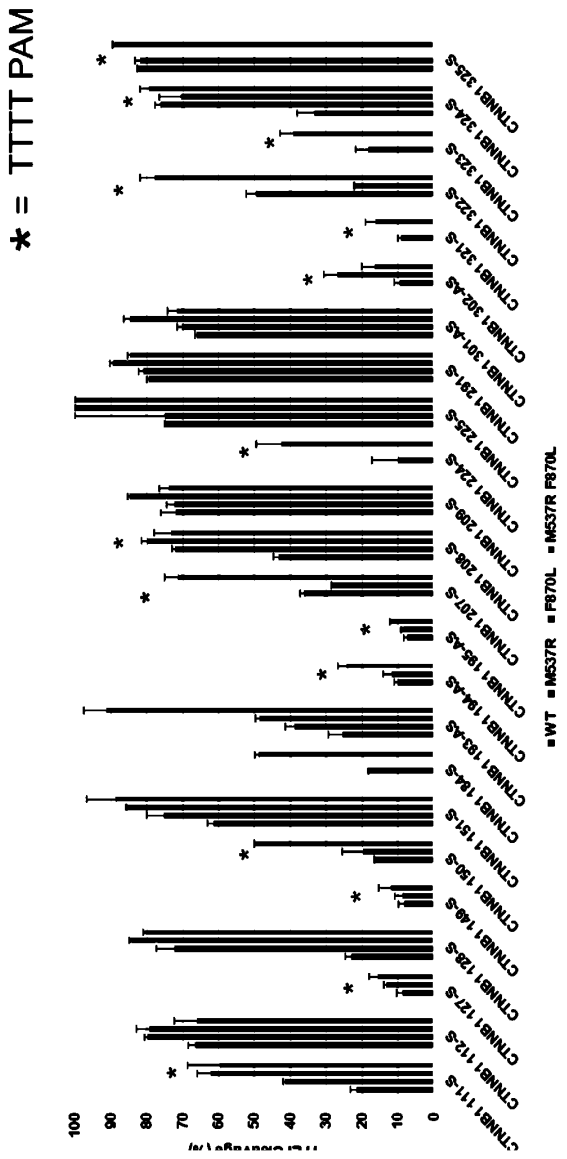

* = TTTT PAM
WT
M537R
F870L
M537R F870L
CTNNB1 325-S
CTNNB1 324-S
CTNNB1 323-S
CTNNB1 322-S
CTNNB1 321-S
CTNNB1 302-AS
CTNNB1 301-AS
CTNNB1 291-S
CTNNB1 225-S
CTNNB1 224-S
CTNNB1 209-S
CTNNB1 208-S
CTNNB1 207-S
CTNNB1 195-AS
CTNNB1 194-AS
CTNNB1 193-AS
CTNNB1 184-S
CTNNB1 151-S
CTNNB1 130-S
CTNNB1 149-S
CTNNB1 128-S
CTNNB1 127-S
CTNNB1 112-S
CTNNB1 111-S
100
90
80
70
60
50
40
30
20
10
0

FIG. 4

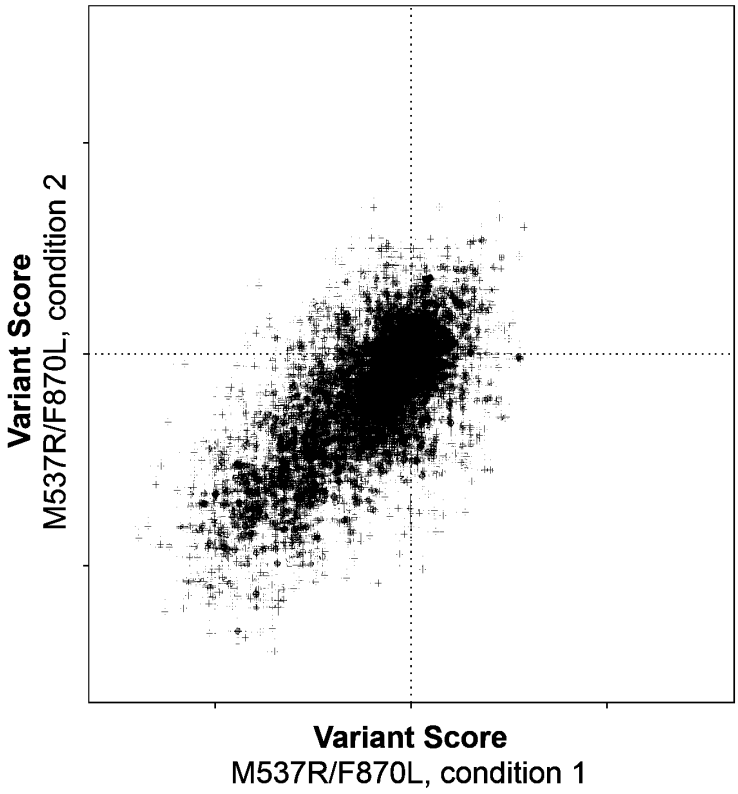

Variant Score
M537R/F870L, condition 2
Variant Score
M537R/F870L, condition 1

FIG. 5A

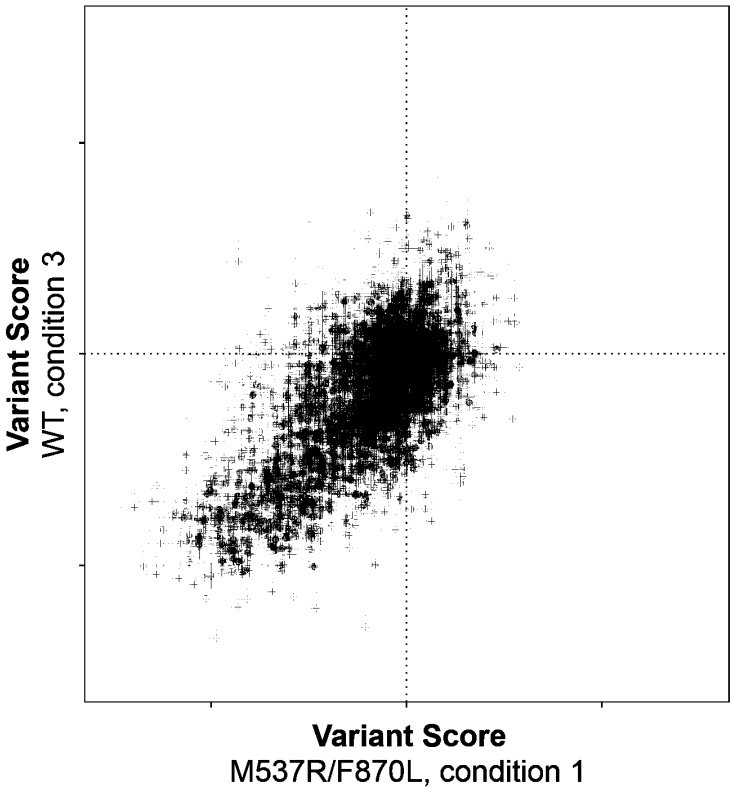

Variant Score
WT, condition 3
Variant Score
M537R/F870L, condition 1

FIG. 5B

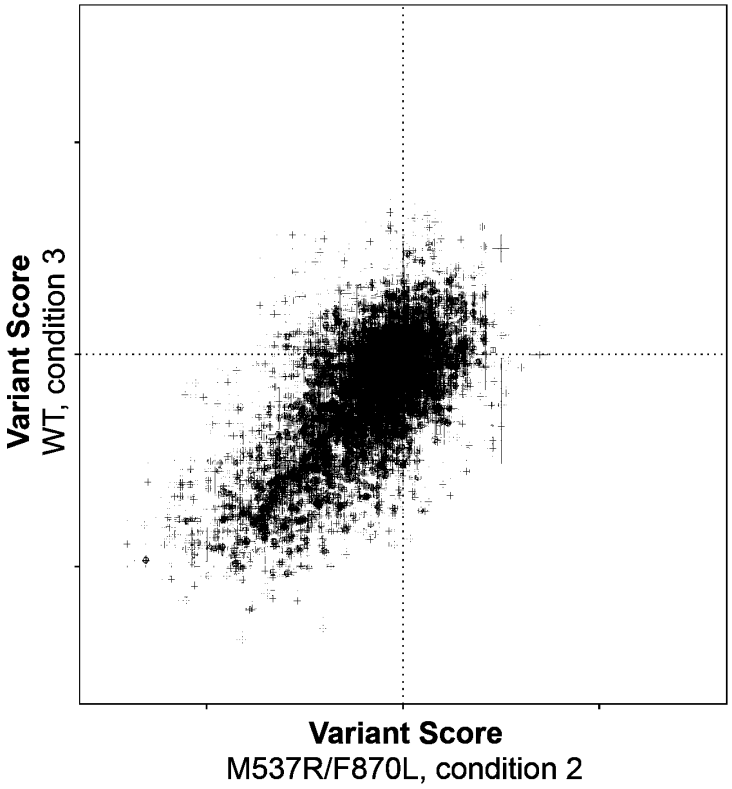

Variant Score
WT, condition 3
Variant Score
M537R/F870L, condition 2

FIG. 5C

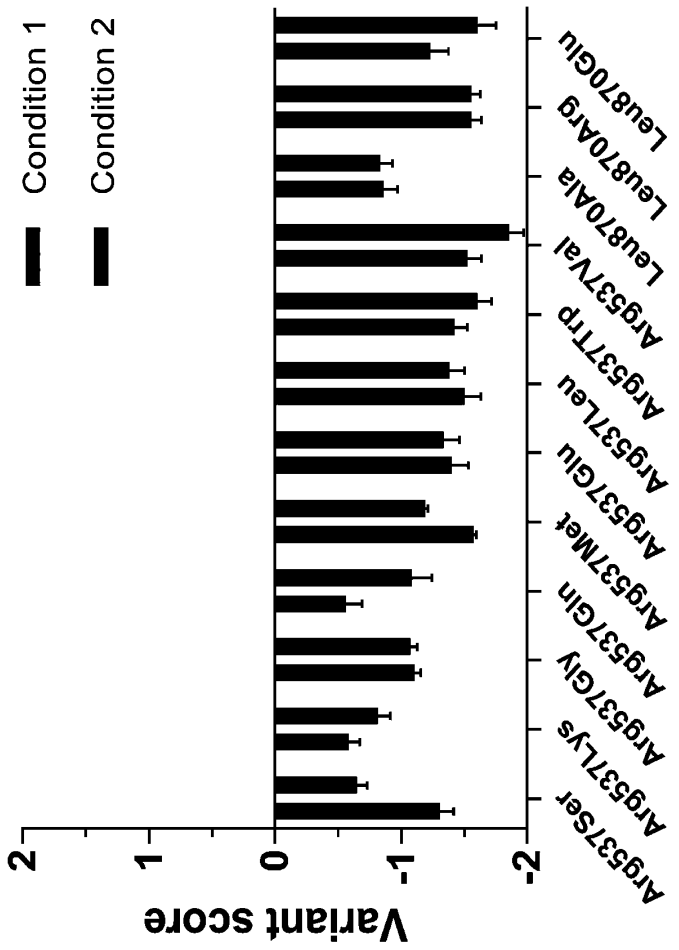

Condition 1
Condition 2
Variant score
2
1
0
-1
-2
Arg537Ser
Arg537Lys
Arg537Gly
Arg537Gln
Arg537Met
Arg537Glu
Arg537Leu
Arg537Trp
Arg537Val
Leu870Ala
Leu870Arg
Leu870Glu

FIG. 5D

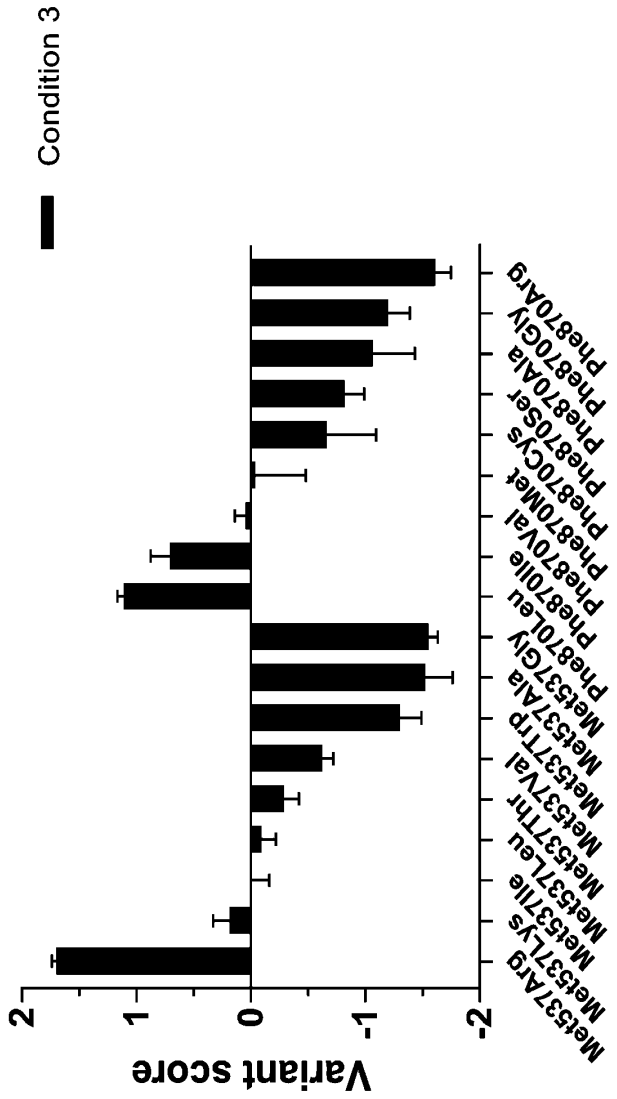

Condition 3
Variant score
2
1
0
-1
-2
Met537Arg
Met537Lys
Met537Ile
Met537Leu
Met537Thr
Met537Val
Met537Trp
Met537Ala
Met537Gly
Phe870Leu
Phe870Ile
Phe870Val
Phe870Met
Phe870Cys
Phe870Ser
Phe870Ala
Phe870Gly
Phe870Arg

FIG. 5E

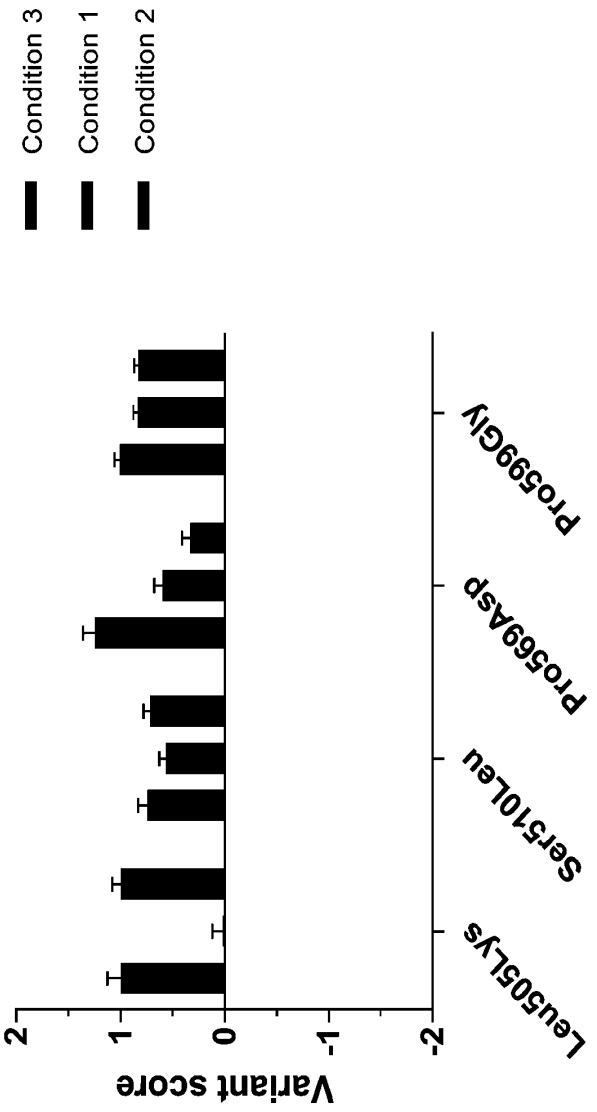

Condition 3
Condition 1
Condition 2
Variant score
2
1
0
-1
-2
Leu505Lys
Ser510Leu
Pro569Asp
Pro599Gly

FIG. 5F

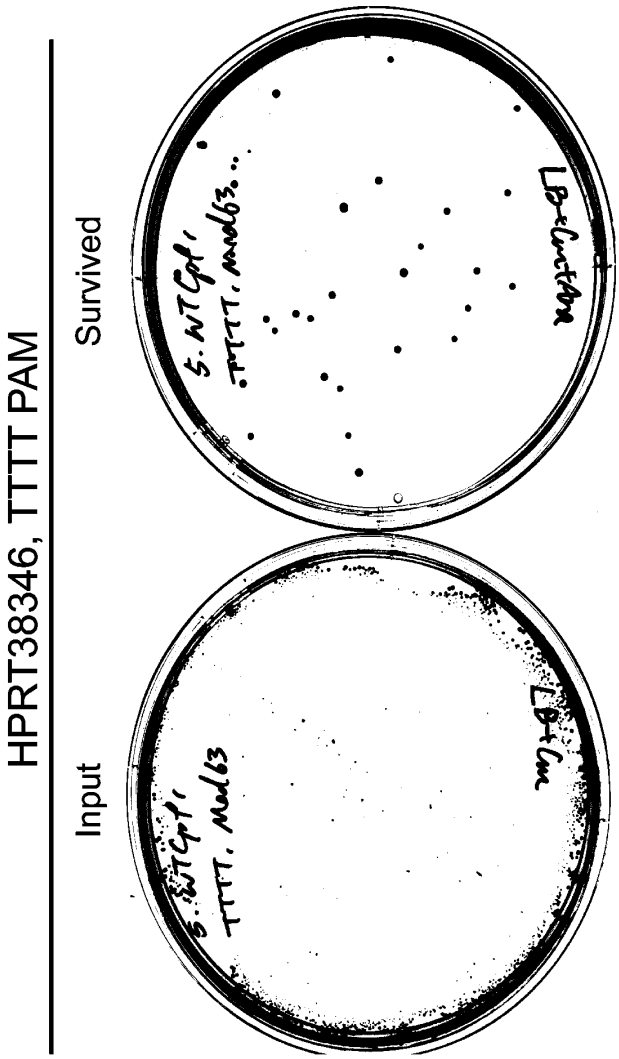


FIG. 6A



FIG. 6B

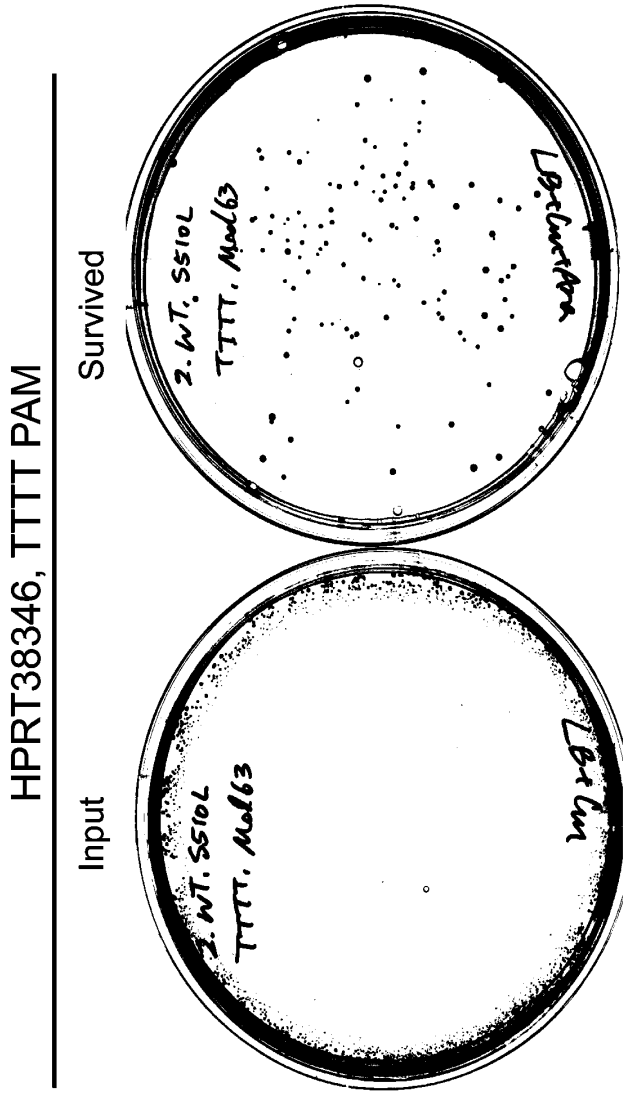

HPRT38346, TTTT PAM
Input
Survived
2. WT. SS10L
TTTT. Mod63
LB+Carb
2. WT. SS10L
TTTT. Mod63
LB+Carb

FIG. 6C



FIG. 6D

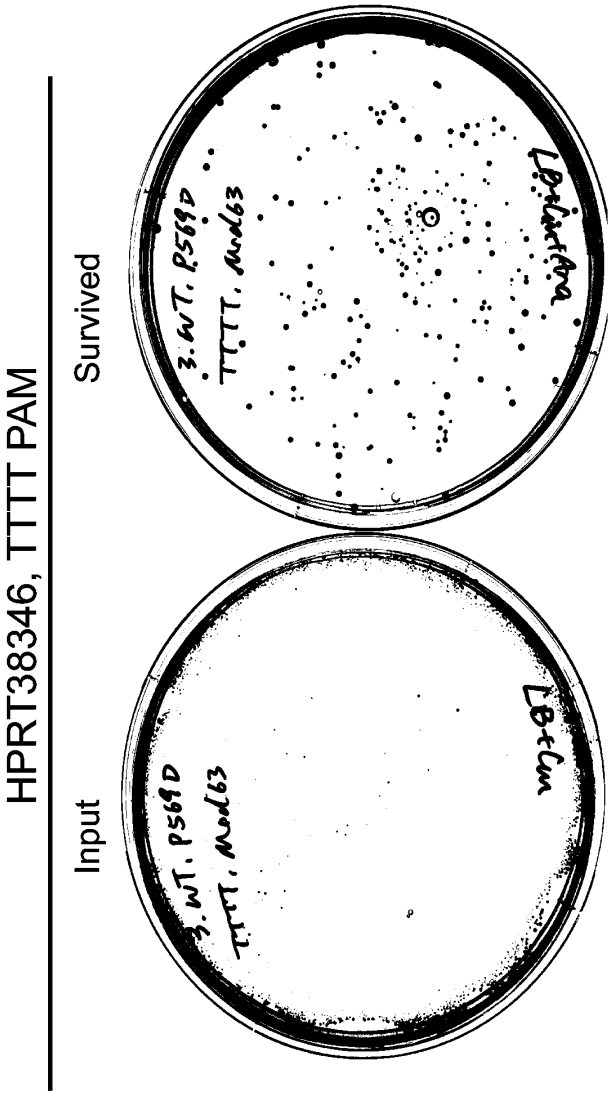

HPRT38346, TTTT PAM
Input
Survived
LB+Cm
3. wT. PS69D
TTTT, Mod63
LB+Cm+Ara
3. wT. PS69D
TTTT, Mod63

FIG. 6E



FIG. 6F



FIG. 7A

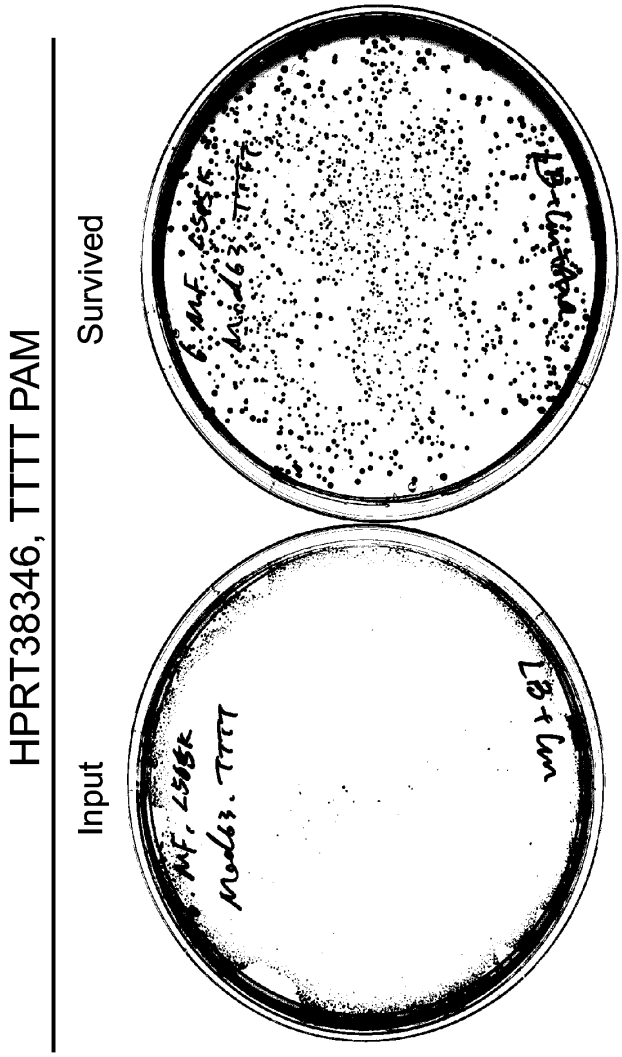


FIG. 7B

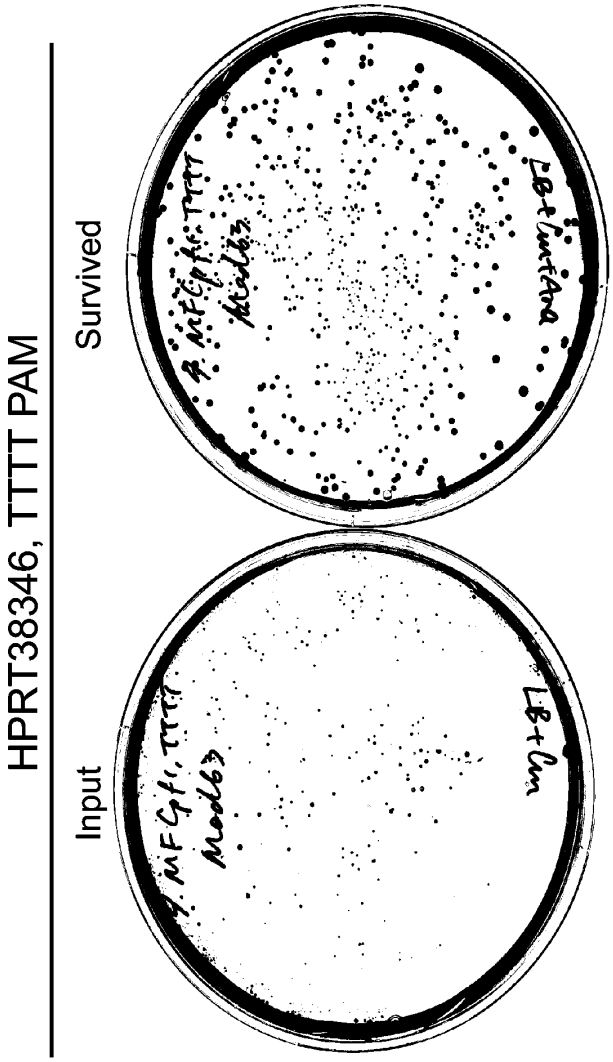


FIG. 7C

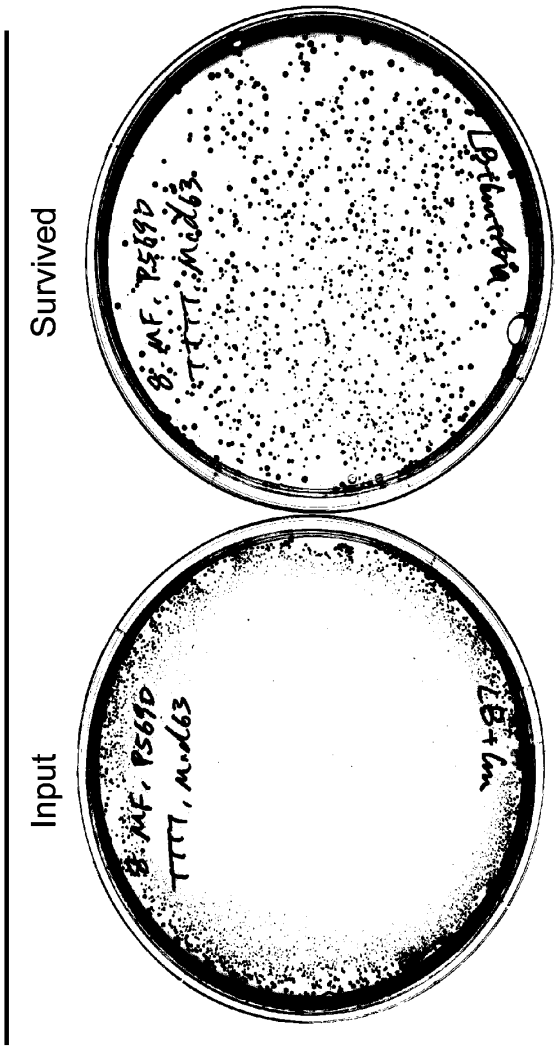

HPRT38346, TTTT PAM
Survived
Input
B. inf. PS69D
TTTT, mut63
LB+Lm
B. inf. PS69D
TTTT, mut63
LB+Lm

FIG. 7D



FIG. 7E

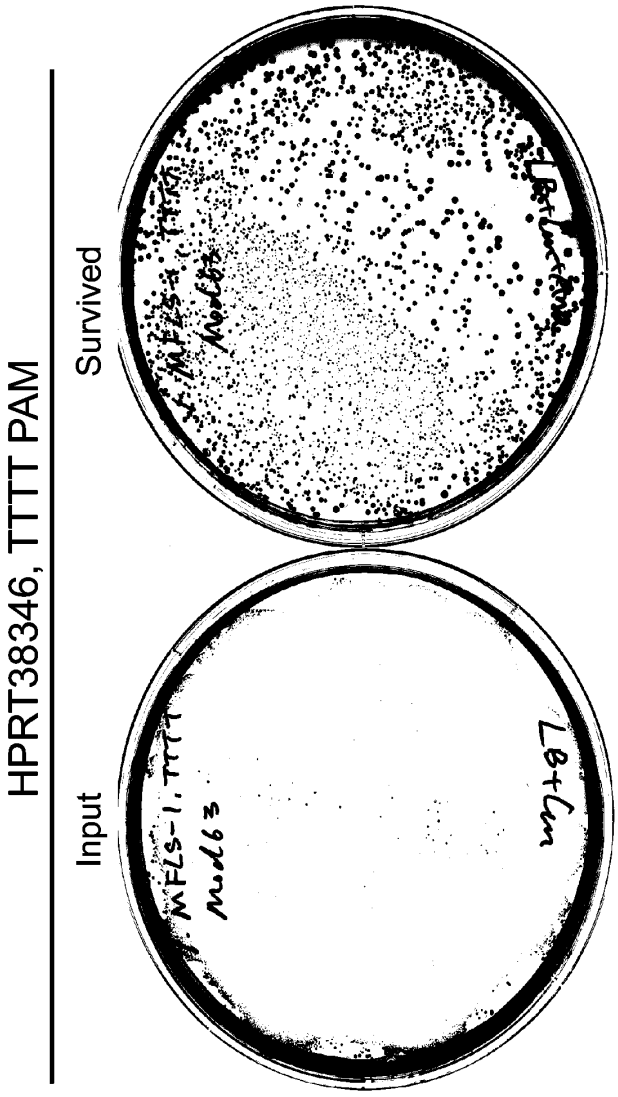


FIG. 7F