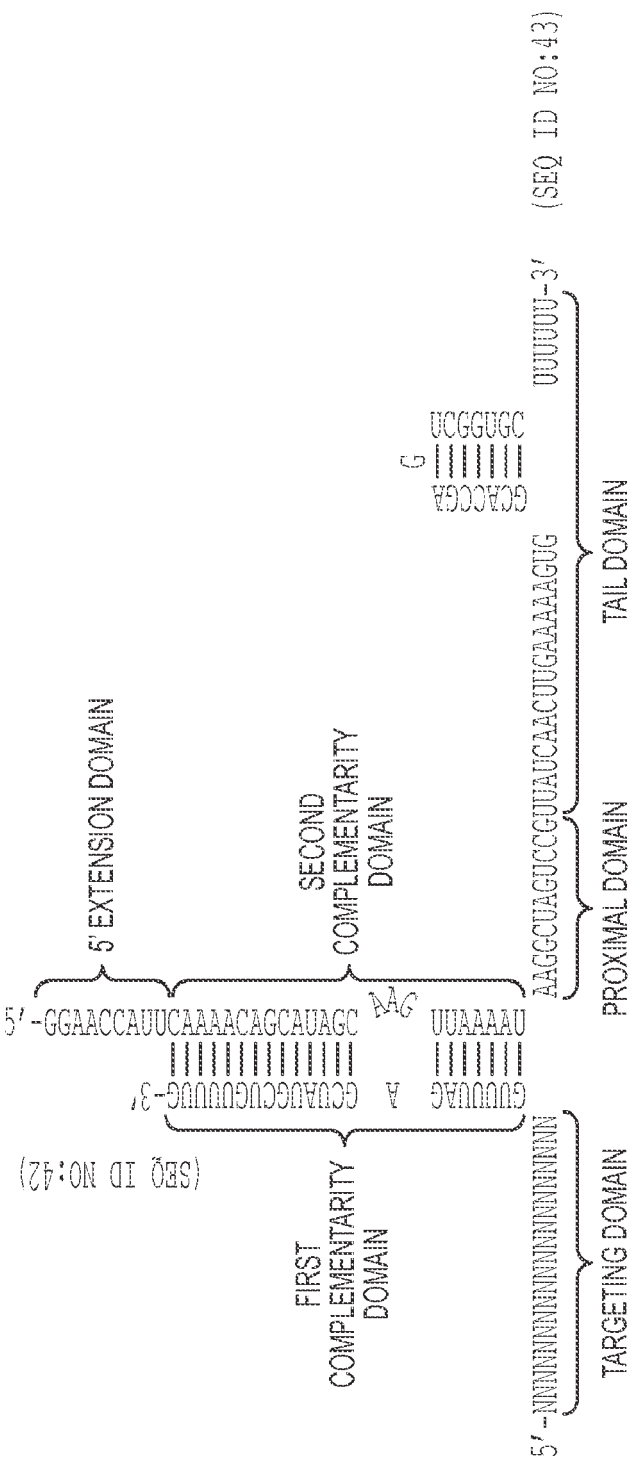

5' EXTENSION DOMAIN
SECOND COMPLEMENTARITY DOMAIN
5'-GGAACCAUUCAAAACAGCAUAGC
AAG
UUAAAAU
AAGGCUAGUCCGUUAUCAACUUGAAAAAGUG
UUUUUU-3'
(SEQ ID NO:43)
UCGGUGC
G
GCACCGA
GUUUUAG
A
GCUAUGCUGUUUUG-3'
(SEQ ID NO:42)
5'-NNNNNNNNNNNNNNNNNNNN
FIRST COMPLEMENTARITY DOMAIN
TARGETING DOMAIN
PROXIMAL DOMAIN
TAIL DOMAIN

FIG. 1A

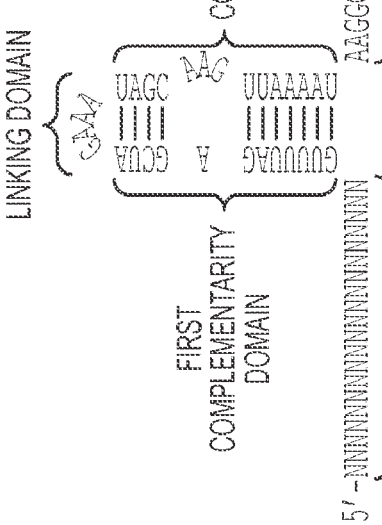

LINKING DOMAIN
GAA
UAGC
GCUA
A
GUUUAG
UUAAAAU
AAGGCUAGUCCG-3'
(SEQ ID NO:44)
FIRST COMPLEMENTARITY DOMAIN
SECOND COMPLEMENTARITY DOMAIN
5'-NNNNNNNNNNNNNNNNNNNN
TARGETING DOMAIN
PROXIMAL DOMAIN

FIG. 1B

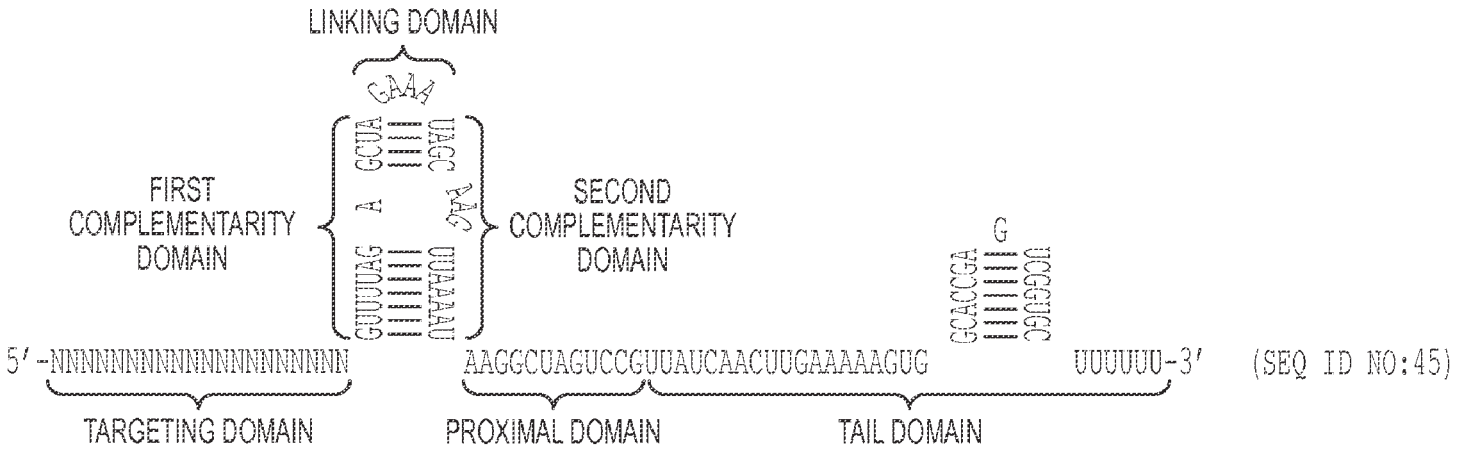

LINKING DOMAIN
GAAA
FIRST
COMPLEMENTARITY
DOMAIN
SECOND
COMPLEMENTARITY
DOMAIN
5′-NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCGUUAUCAACUUGAAAAAGUG
UUUUUU-3′
(SEQ ID NO:45)
TARGETING DOMAIN
PROXIMAL DOMAIN
TAIL DOMAIN

FIG. 1C

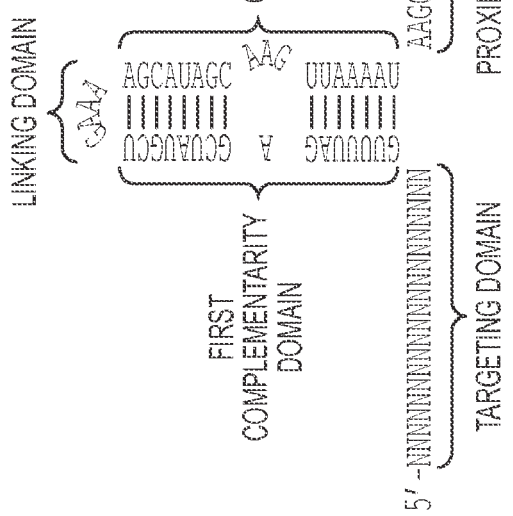

LINKING DOMAIN
GAA
AGCAUAGC
GCUAUGCU
A
GUUUUAG
UUAAAAU
AAG
FIRST COMPLEMENTARITY DOMAIN
SECOND COMPLEMENTARITY DOMAIN
5'-NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCGUUAUC-3'
(SEQ ID NO:46)
TARGETING DOMAIN
PROXIMAL DOMAIN
TAIL DOMAIN

FIG. 1D

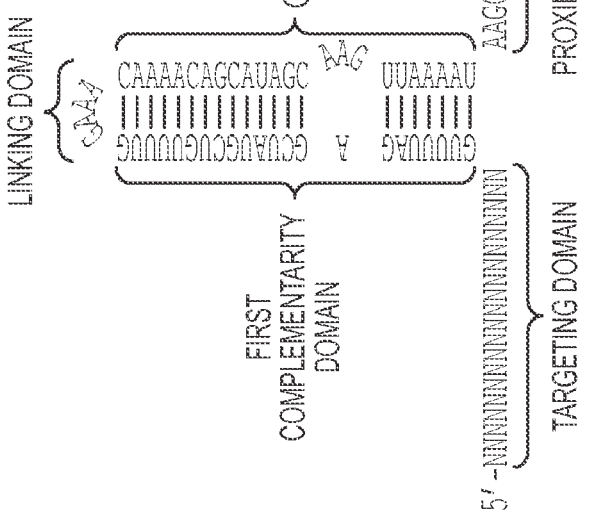

LINKING DOMAIN
GAA
CAAAACAGCAUAGC
GCUAUGCUGUUUUG
SECOND COMPLEMENTARITY DOMAIN
FIRST COMPLEMENTARITY DOMAIN
A
UUAAAAU
GUUUUAG
5'-NNNNNNNNNNNNNNNNNNNN
TARGETING DOMAIN
AAGGCUAGUCCGUUAUC-3'
PROXIMAL DOMAIN
TAIL DOMAIN
(SEQ ID NO:47)

FIG. 1E

STREPTOCOCCUS THERMOPHILUS

A) STRUCTURE (SEE, E.G., KARVELIS *ET AL*. RNA BIOLOGY. 2013; 10(5): 841-851)

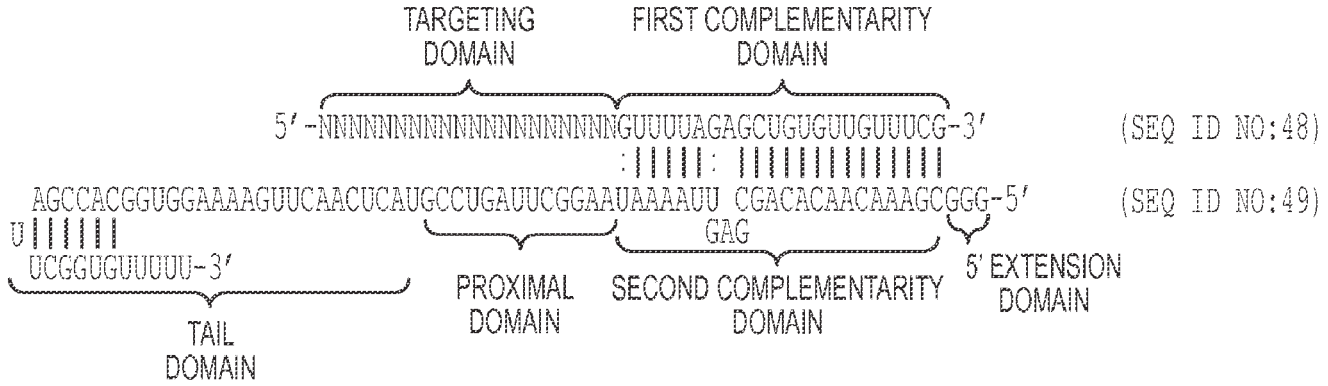


FIG. 1F

ALIGNMENT

```
S. PYOGENES          5′-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGUUUUG-3′          (SEQ ID NO:50)
S. THERMOPHILUS      5′-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUGUGUUGUUUCG-3′          (SEQ ID NO:51)
                                ********** ** ***** *

S. PYOGENES          5′-GAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGC-UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU-3′
S. THERMOPHILUS      5′------GGGCGAAACAACACAGCGAGUUAAAAUAAGGCUUAGUCCGUACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUUU----3′
                           * ***** ** *** ************** ********  ************ ********** ****** ****

S. PYOGENES - CONT       (SEQ ID NO:52)
S. THERMOPHILUS - CONT   (SEQ ID NO:53)
```

FIG. 1G


CLUSTAL FORMAT ALIGNMENT BY MAFFT (v7.058b)
Y
SM KKPYSIGLDIGTNSVGWAVVTDDYKVPAKKMKVLGNTDKSHIEKNLLGALLFDSGNTAED
SP DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
ST TKPYSIGLDIGTNSVGWAVITDNYKVPSKKMKVLGNTSKKYIKKNLLGVLLFDSGITAEG
LI KKPYTIGLDIGTNSVGWAVITDQYDLVKRKMKIAGDSEKKQIKKNFWGVRLFDEGQTAAD
MOTIF: -K-Y*IGLDIGTNSVGWAV-TD*Y-*--*K*K*-G**-*--I*KN*-G--LFD-G-TA--
SM RRLKRTARRRYTRRRNRILYLQEIFSEEMGKVDDSFFHRLEDSFLVTEDKRGERHPIFGN
SP TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN
ST RRLKRTARRRYTRRRNRILYLQEIFSTEMATLDDAFFQRLDDSFLVPDDKRDSKYPIFGN
LI RRMARTARRRIERRRNRISYLQGIFAEEMSKTDANFFCRLSDSFYVDNEKRNSRHPFFAT
MOTIF: -R*-RTARRR--RR*NRI-YLQ-IF*-EM---D--FF-RL-*SF-V-**K*--**P*F--
SM LEEEVKYHENFPTIYHLRQYLADNPEKVDLRLVYLALAHIIKFRGHFLIEGKFDTRNNDV
SP IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV
ST LVEEKAYHDEFPTIYHLRKYLADSTKKADLRLVYLALAHMIKYRGHFLIEGEFNSKNNDI
LI IEEEVEYHKNYPTIYHLREELVNSSEKADLRLVYLALAHIIKYRGNFLIEGALDTQNTSV
MOTIF: *-*E--YH-**PTIYHLR*-L-*---K-DLRL*YLALAH*IK*RGNFLIEG-**--N--*

FIG. 2A

```
SM          QRLFQEFLAVYDNTFENSS------LQEQNVQVEEILTDKISKSAKKDRVLKLFPNEKSN
SP          DKLFIQLVQTYNQLFEENP-------INASGVDAKAILSARLSKSRRLENLIAQLPGEKKN
ST          QKNFQDFLDTYNAIFESDL------SLENSKQLEEIVKDKISKLEKKDRILKLFPGEKNS
LI          DGIYKQFIQTYNQVFASGIEDGSLKKLEDNKDVAKILVEKVTRKEKLERILKLYPGEKSA
            :  : :::. .*:  * ..          .. :   *:  ::::  : :.::   *.**.
MOTIF:      *--*-***--y*--f----------------*---I*--****--*-*-**---P-EK--

SM          GRFAEFLKLIVGNQADFKKHFELEEKAPLQFSKDTYEEELEVLLAQIGDNYAELFLSAKK
SP          GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
ST          GIFSEFLKLIVGNQADFRKCFNLDEKASLHFSKESYDEDLETLLGYIGDDYSDVFLKAKK
LI          GMFAQFISLIVGSKGNFQKPFDLIEKSDIECAKDSYEEDLESLLALIGDEYAELFVAAKN
            * *.::: * :*   :*:. *:* *.: :. :*:.*:::*: **. ***:*::*: **:
MOTIF:      G-F-***-L-*G---*F*--F*L-E-*-*--*K**Y***L*-LL--IGD*Y***F*-AK*

SM          LYDSILLSGILTVTDVGTKAPLSASMIQRYNEHQMDLAQLKQFIRQKLSDKYNEVFSDVS
SP          LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS
ST          LYDAILLSGFLTVTDNETEAPLSSAMIKRYNEHKEDLALLKEYIRNISLKTYNEVFKDDT
LI          AYSAVVLSSIITVAETETNAKLSASMIERFDTHEEDLGELKAFIKLHLPKHYEEIFSNTE
              .:::**.:: *    *:* **::**:*:: *. **  **  ::    . *:*:* :
MOTIF:      ---***LS-**-V----T*A-LS**MI*R**-H--DL--LK--**------Y*E*F-*--

SM          KDGYAGYIDGKTNQEAFYKYLKGLLNKIEGSGYFLDKIEREDFLRKQRTFDNGSIPHQIH
SP          KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIH
ST          KNGYAGYIDGKTNQEDFYVYLKKLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIH
LI          KHGYAGYIDGKTKQADFYKYMKMTLENIEGADYFIAKIEKENFLRKQRTFDNGAIPHQLH
            *.******** :.*  ** ::*  * :::*:  :: *:::*::********** :*:*:*
MOTIF:      K-GYAGYIDG-*-Q--FY-**K--L-***G*--**-K***E**LRKQRTFDNG*IP*Q*H
```

FIG. 2B

```
SM          LQEMRAIIRRQAEFYPFLADNQDRIEKLLTFRIPYYVGPLARGKSDFAWLSRKSADKITP
SP          LGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP
ST          LQEMRAILDKQAKFYPFLAKNKERIEKILTFRIPYYVGPLARGNSDFAWSIRKRNEKITP
LI          LEELEAILHQQAKYYPFLKENYDKIKSLVTFRIPYFVGPLANGQSEFAWLTRKADGEIRP
            * *:.**: :* .:**** .* ::*:.::****** *****.*:* ***  **    * *
MOTIF:      L-E*-AI*-*Q--*YPFL--N-**I*-**TFRIPY*VGPLA-G*S-FAW--RK-----I-P

SM          WNFDEIVDKESSAEAFINRMTNYDLYLPNQKVLPKHSLLYEKFTVYNELTKVKYKTE-QG
SP          WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
ST          WNFEDVIDKESSAEAFINRMTSFDLYLPEEKVLPKHSLLYETFNVYNELTKVRFIAESMR
LI          WNIEEKVDFGKSAVDFIEKMTNKDTYLPKENVLPKHSLCYQKYLVYNELTKVRYIND-QG
            **::: :*   **  **.:**. *  **.::******** *: : ********::  :
MOTIF:      WN***-*D---SA--FI**MT--D--LP***VLPKHSL-Y*-*-VYNELTKV**--*---

SM          KTAFFDANMKQEIFDGVFKVYRKVTKDKLMDFLEKEFDEFRIVDLTGLDKENKVFNASYG
SP          KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR---FNASLG
ST          DYQFLDSKQKKDIVRLYFKDKRKVTDKDIIEYL-HAIYGYDGIELKGIEKQ---FNSSLS
LI          KTSYFSGQEKEQIFNDLFKQKRKVKKKDLELFL-RNMSHVESPTIEGLEDS---FNSSYS
            .  ::.: *: *.   **  ***. ..:    : :      : *:.    **:* .
MOTIF:      ---**--*-K*-I----FK--RKV----*-----*-*--------*-G**-----FN*S--

SM          TYHDLCKIL-DKDFLDNSKNEKILEDIVLTLTLFEDREMIRKRLENYSDLLTKEQVKKLE
SP          TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLK
ST          TYHDLLNIINDKEFLDDSSNEAIIEEIIHTLTIFEDREMIKQRLSKFENIFDKSVLKKLS
LI          TYHDLLKVGIKQEILDNPVNTEMLENIVKILTVFEDKRMIKEQLQQFSDVLDGVVLKKLE
            ***** ::  .:::***:  * ::*:*:   **:***:.** .::*. : .::    :*:*.
MOTIF:      TYHDL-**---***LD*--N--**E*I*--LT*FED*-MI-**L--*--**----*K*L-
```

FIG. 2C

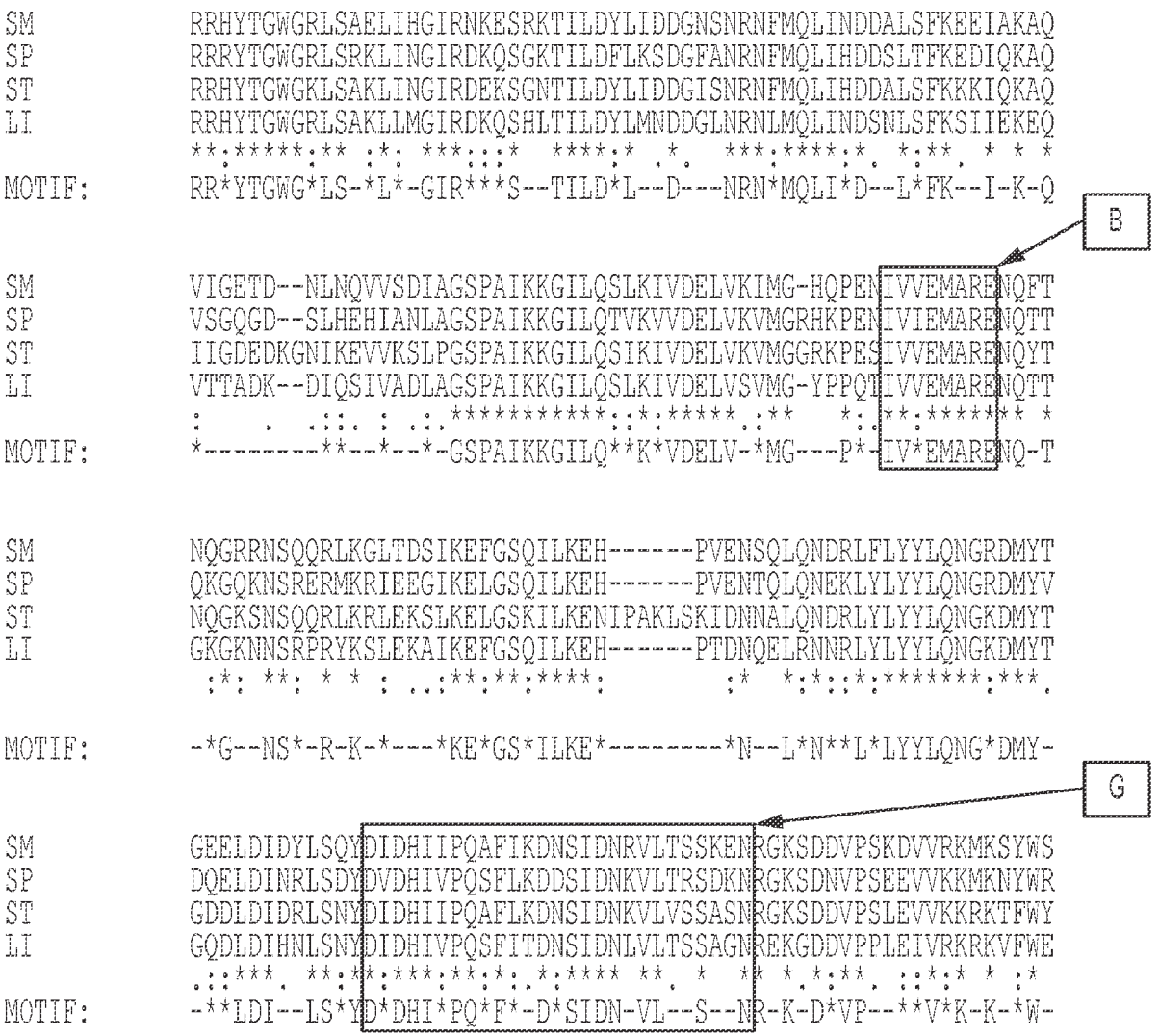

SM RRHYTGWGRLSAELIHGIRNKESRKTILDYLIDDGNSNRNFMQLINDDALSFKEEIAKAQ
SP RRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
ST RRHYTGWGKLSAKLINGIRDEKSGNTILDYLIDDGISNRNFMQLIHDDALSFKKKIQKAQ
LI RRHYTGWGRLSAKLLMGIRDKQSHLTILDYLMNDDGLNRNLMQLINDSNLSFKSIIEKEQ
MOTIF: RR*YTGWG*LS-*L*-GIR***S--TILD*L--D---NRN*MQLI*D--L*FK--I-K-Q
B
SM VIGETD--NLNQVVSDIAGSPAIKKGILQSLKIVDELVKIMG-HQPENIVVEMARENQFT
SP VSGQGD--SLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTT
ST IIGDEDKGNIKEVVKSLPGSPAIKKGILQSIKIVDELVKVMGGRKPESIVVEMARENQYT
LI VTTADK--DIQSIVADLAGSPAIKKGILQSLKIVDELVSVMG-YPPQTIVVEMARENQTT
MOTIF: *--------**--*--*-GSPAIKKGILQ**K*VDELV-*MG---P*-IV*EMARENQ-T
SM NQGRRNSQQRLKGLTDSIKEFGSQILKEH------PVENSQLQNDRLFLYYLQNGRDMYT
SP QKGQKNSRERMKRIEEGIKELGSQILKEH------PVENTQLQNEKLYLYYLQNGRDMYV
ST NQGKSNSQQRLKRLEKSLKELGSKILKENIPAKLSKIDNNALQNDRLYLYYLQNGKDMYT
LI GKGKNNSRPRYKSLEKAIKEFGSQILKEH-------PTDNQELRNNRLYLYYLQNGKDMYT
MOTIF: -*G--NS*-R-K-*---*KE*GS*ILKE*--------*N--L*N**L*LYYLQNG*DMY-
G
SM GEELDIDYLSQYDIDHIIPQAFIKDNSIDNRVLTSSKENRGKSDDVPSKDVVRKMKSYWS
SP DQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWR
ST GDDLDIDRLSNYDIDHIIPQAFLKDNSIDNKVLVSSASNRGKSDDVPSLEVVKKRKTFWY
LI GQDLDIHNLSNYDIDHIVPQSFITDNSIDNLVLTSSAGNREKGDDVPPLEIVRKRKVFWE
MOTIF: -**LDI--LS*YD*DHI*PQ*F*-D*SIDN-VL--S--NR-K-D*VP--**V*K-K-*W-

FIG. 2D


SM KLLSAKLITQRKFDNLTKAERGGLTDDDKAGFIKRQLVETRQITKHVARILDERFNTETD
SP QLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ST QLLKSKLISQRKFDNLTKAERGGLSPEDKAGFIQRQLVETRQITKHVARLLDEKFNNKKD
LI KLYQGNLMSKRKFDYLTKAERGGLTEADKARFIHRQLVETRQITKNVANILHQRFNYEKD
MOTIF: *L---*L***RKFD-LTKAERGGL*--DKA-FI*RQLVETRQITK*VA-*L--**N-*-D
B
SM ENNKKIRQVKIVTLKSNLVSNFRKEFELYKVREINDYHHAHDAYLNAVIGKALLGVYPQL
SP ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL
ST ENNRAVRTVKIITLKSTLVSQFRKDFELYKVREINDFHHAHDAYLNAVVASALLKKYPKL
LI DHGNTMKQVRIVTLKSALVSQFRKQFQLYKVRDVNDYHHAHDAYLNGVVANTLLKVYPQL
MOTIF: **---**-V***TLKS-LVS*FRK*F**LYKV**N**HHAHDAYLN-V*--*L*--YP*L
SM EPEFVYGDYPHFHGHKE--------NK-ATAKKFFYSNIMNFFKKDDVRTD---------
SP ESEFVYGDYKVYDVRKMIAKSEQEIGK-ATAKYFFYSNIMNFFKTEITLANGEIRKRPLI
ST EPEFVYGDYPKYNSFRE--------RKSATEKVYFYSNIMNIFKKSISLADGRVIERPLI
LI EPEFVYGDYHQFDWFKA--------NK-ATAKKQFYTNIMLFFAQKDRIID---------
MOTIF: E-EFVYGDY--*---*----------K-AT-K--FY*NIM-*F-------*---------
SM -----KNGEIIWKKDEHISNIKKVLSYPQVNIVKKVEEQTGGFSKE----------SILPK
SP ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKE----------SILPK
ST EVNEETGESVWNKESDLATVRRVLSYPQVNVVKKVEEQNHGLDRGKPKGLFNANLSSKPK
LI -----ENGEILWDK-KYLDTVKKVMSYRQMNIVKKTEIQKGEFSKA----------TIKPK
MOTIF: ----*-GE-*W-K---*--***V*M--Q*N*VKK-E-Q---*-*----------*--PK

FIG. 2E

```
SM          GNSDK-LIPRKTKKFYWDTKKYGGFDSPIVAYSILVIADIEKGKSKKLKTVKALVGVTIM
SP          RNSDK-LIARKKD---WDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM
ST          PNSNENLVGAKEY---LDPKKYGGYAGISNSFTVLVKGTIEKGAKKKITNVLEFQGISIL
LI          GNSSK-LIPRKTN---WDPMKYGGLDSPNMAYAVVI--EYAKGKN-KLVFEKKIIRVTIM
             **.: *:  *     *. ****  .  :::::::    ** . *:     :  ::*:
MOTIF:      -NS-*-L*--K------D--KYGG------******-----KG---K*-----*--**I*

SM          EKMTFERDPVAFLERKGYRNVQEENIIKLPKYSLFKLENGRKRLLAS-------ARELQK
SP          ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLAS-------AGELQK
ST          DRINYRKDKLNFLLEKGYKDI--ELIIELPKYSLFELSDGSRRMLASILSTNNKRGEIHK
LI          ERKAFEKDEKAFLEEQGYRQP--KVLAKLPKYTLYECEEGRRRMLAS-------ANEAQK
            ::  :.::   **  :**::   . : :****:*:: .:* :*:***         * :*
MOTIF:      **--*-**---FL--*GY**-----*-*LPKY*L**--*G-*R*LAS---------E-*K

SM          GNEIVLPNHLGTLLYHAKNIHKV------DEPKHLDYVDKHKDEFKELLDVVSNFSKKYT
SP          GNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL-FVEQHKHYLDEIIEQISEFSKRVI
ST          GNQIFLSQKFVKLLYHAKRISNT------INENHRKYVENHKKEFEELFYYILEFNENYV
LI          GNQQVLPNHLVTLLHHAANCEVS-------DGKSLDYIESNREMFAELLAHVSEFAKRYT
            **:  *..:  .:*: * .           : :   :::.::. : *::    : :* :.
MOTIF:      GN*--L--*---*L*-A-------------*-*---***-**--*-E**--*-*F-*---

SM          LAEGNLEKIKELYAQNNGEDLKELASSFI--------NLLTFTAIGAPATFKFFDKNIDR
SP          LADANLDKVLSAYNKHRDKPIREQAENII--------HLFTLTNLGAPAAFKYFDTTIDR
ST          GAKKNGKLLNSAFQSWQNHSIDELCSSFIGPTGSERKGLFELTSRGSAADFEFLGVKIPR
LI          LAEANLNKINQLFEQNKEGDIKAIAQSFV---------DLMAFNAMGAPASFKFFETTIER
             *. *  . : . : . .   :  ..::          *: :.  *:.* *:::  .* *
MOTIF:      -A--N---*---*--------*------**----------L*-*---G*-A-F***---I-R
```

FIG. 2F

```
SM          KR-YTSTTEILNATLIHQSITGLYETRIDLNKLGGD  (SEQ ID NO:1)
SP          KR-YTSTKEVLDATLIHQSITGLYETRIDLSQLGGD  (SEQ ID NO:2)
ST          YRDYTPSSLLKDATLIHQSVTGLYETRIDLAKLGEG  (SEQ ID NO:3)
LI          KR-YNNLKELLNSTIIYQSITGLYESRKRLD----D  (SEQ ID NO:4)
             * *.  . : ::*:*:**:*****:*  *     .
Motif:      -R-Y-----*-**T*I*QS*TGLYE*R--L------
```

FIG. 2G

ALIGNMENT OF THE N TERMINAL RucV-LIKE DOMAINS DISCLOSED IN CHYLINSKI *ET AL.* (EXCLUDING SEQUENCE OUTLIERS).
(CLUSTAL FORMAT ALIGNMENT BY MAFFT (v7.058b))

1	DIGTNSVGWAVT	(SEQ ID NO:54)
12	DIGTNSVGWAVT	(SEQ ID NO:55)
3	DVGTNSVGWAVT	(SEQ ID NO:56)
20	DVGTNSVGWAVT	(SEQ ID NO:57)
15	DMGTNSVGWAVT	(SEQ ID NO:58)
4	DVGTSSVGWAVT	(SEQ ID NO:59)
7	DIGTASVGWAVT	(SEQ ID NO:60)
6	DVGTGSVGWAVT	(SEQ ID NO:61)
9	DIGTNSVGWAVV	(SEQ ID NO:62)
10	DIGTNSVGWAVI	(SEQ ID NO:63)
11	DIGTNSVGWAVL	(SEQ ID NO:64)
42	DLGTNSIGWAVV	(SEQ ID NO:65)
48	DLGTNSIGWAI-	(SEQ ID NO:66)
43	DLGTNSIGWALV	(SEQ ID NO:67)
2	DIGTNSVGWCVT	(SEQ ID NO:68)
14	DIGTNSVGYAVT	(SEQ ID NO:69)
5	DMGTGSLGWAVT	(SEQ ID NO:70)
16	DIGTSSVGWAAI	(SEQ ID NO:71)
8	DLGTGSVGWAVV	(SEQ ID NO:72)
22	DLGVGSVGWAIV	(SEQ ID NO:73)
23	DLGIASIGWAII	(SEQ ID NO:74)
24	DLGIASVGWAIV	(SEQ ID NO:75)
25	DLGVASVGWSIV	(SEQ ID NO:76)
26	DIGIASVGWAIL	(SEQ ID NO:77)
28	DLGISSVGWSVI	(SEQ ID NO:78)
32	DIGIASVGWSVI	(SEQ ID NO:79)
33	DVGIGSIGWAVI	(SEQ ID NO:80)
39	DLGVGSIGFAIV	(SEQ ID NO:81)
34	DIGYASIGWAVI	(SEQ ID NO:82)
47	DTGTNSLGWAIV	(SEQ ID NO:83)
50	DLGTNSIGWCLL	(SEQ ID NO:84)
49	DIGTDSLGWAVF	(SEQ ID NO:85)
18	DIGSNSIGFAVV	(SEQ ID NO:86)
41	DLGVGSIGVAVA	(SEQ ID NO:87)
45	DLGIASCGWGVV	(SEQ ID NO:88)

FIG. 3A

21	DLGIASVGWCLT	(SEQ ID NO:89)
27	DIGIGSVGVGIL	(SEQ ID NO:90)
29	DIGITSVGYGLI	(SEQ ID NO:91)
30	DIGITSVGFGII	(SEQ ID NO:92)
31	DVGITSTGYAVL	(SEQ ID NO:93)
40	DLGITSFGYAIL	(SEQ ID NO:94)
17	DIGNASVGWSAF	(SEQ ID NO:95)
19	DVGTNSCGWVAM	(SEQ ID NO:96)
35	DVGERSIGLAAV	(SEQ ID NO:97)
36	DVGLNSVGLAAV	(SEQ ID NO:98)
37	DVGLMSVGLAAI	(SEQ ID NO:99)
38	DVGTFSVGLAAI	(SEQ ID NO:100)
13	DIGTGSVGYACM	(SEQ ID NO:101)
44	DLGTTSIGFAHI	(SEQ ID NO:102)
46	DLGTNSIGSSVR	(SEQ ID NO:103)
	* * * *	

FIG. 3B

ALIGNMENT OF THE N TERMINAL RucV-LIKE DOMAINS DISCLOSED IN CHYLINSKI *ET AL.*
(CLUSTAL FORMAT ALIGNMENT BY MAFFT (v7.058b))

1	D----IGTNSVGWAVT	(SEQ ID NO:104)
12	D----IGTNSVGWAVT	(SEQ ID NO:105)
3	D----VGTNSVGWAVT	(SEQ ID NO:106)
20	D----VGTNSVGWAVT	(SEQ ID NO:107)
15	D----MGTNSVGWAVT	(SEQ ID NO:108)
4	D----VGTSSVGWAVT	(SEQ ID NO:109)
7	D----IGTASVGWAVT	(SEQ ID NO:110)
6	D----VGTGSVGWAVT	(SEQ ID NO:111)
9	D----IGTNSVGWAVV	(SEQ ID NO:112)
10	D----IGTNSVGWAVI	(SEQ ID NO:113)
52	D----IGTNSIGWAVI	(SEQ ID NO:114)
11	D----IGTNSVGWAVL	(SEQ ID NO:115)
42	D----LGTNSIGWAVV	(SEQ ID NO:116)
48	D----LGTNSIGWAI-	(SEQ ID NO:117)
43	D----LGTNSIGWALV	(SEQ ID NO:118)
2	D----IGTNSVGWCVT	(SEQ ID NO:119)
14	D----IGTNSVGYAVT	(SEQ ID NO:120)
5	D----MGTGSLGWAVT	(SEQ ID NO:121)
16	D----IGTSSVGWAAI	(SEQ ID NO:122)
8	D----LGTGSVGWAVV	(SEQ ID NO:123)
22	D----LGVGSVGWAIV	(SEQ ID NO:124)
23	D----LGIASIGWAII	(SEQ ID NO:125)
24	D----LGIASVGWAIV	(SEQ ID NO:126)
68	D----LGIASVGWAVV	(SEQ ID NO:127)
25	D----LGVASVGWSIV	(SEQ ID NO:128)
26	D----IGIASVGWAIL	(SEQ ID NO:129)
66	D----IGIASVGWAVL	(SEQ ID NO:130)
59	D----IGIASIGWAVI	(SEQ ID NO:131)
61	D----IGIASVGWAII	(SEQ ID NO:132)
64	D----VGIASVGWAVI	(SEQ ID NO:133)
62	D----IGIASVGWAL-	(SEQ ID NO:134)
67	D----IGIASVGWAMV	(SEQ ID NO:135)
32	D----IGIASVGWSVI	(SEQ ID NO:136)
28	D----LGISSVGWSVI	(SEQ ID NO:137)
63	D----IGITSVGWAVI	(SEQ ID NO:138)

FIG. 4A

33	D----VGIGSIGWAVI	(SEQ ID NO:139)
57	D----LGISSLGWAIV	(SEQ ID NO:140)
39	D----LGVGSIGFAIV	(SEQ ID NO:141)
34	D----IGYASIGWAVI	(SEQ ID NO:142)
50	D----LGTNSIGWCLL	(SEQ ID NO:143)
54	D----LGTNSIGWGLL	(SEQ ID NO:144)
47	D----TGTNSLGWAIV	(SEQ ID NO:145)
49	D----IGTDSLGWAVF	(SEQ ID NO:146)
51	D----LGSTSLGWAIF	(SEQ ID NO:147)
58	D----IGISSIGWAFS	(SEQ ID NO:148)
21	D----LGIASVGWCLT	(SEQ ID NO:149)
45	D----LGIASCGWGVV	(SEQ ID NO:150)
18	D----IGSNSIGFAVV	(SEQ ID NO:151)
65	D----IGTTSIGFSVI	(SEQ ID NO:152)
29	D----IGITSVGYGLI	(SEQ ID NO:153)
30	D----IGITSVGFGII	(SEQ ID NO:154)
44	D----LGTTSIGFAHI	(SEQ ID NO:155)
27	D----IGIGSVGVGIL	(SEQ ID NO:156)
41	D----LGVGSIGVAVA	(SEQ ID NO:157)
31	D----VGITSTGYAVL	(SEQ ID NO:158)
40	D----LGITSFGYAIL	(SEQ ID NO:159)
53	D----IGTSSIGWWLY	(SEQ ID NO:160)
55	D----LGSNSLGWFVT	(SEQ ID NO:161)
56	D----LGANSLGWFVV	(SEQ ID NO:162)
17	D----IGNASVGWSAF	(SEQ ID NO:163)
19	D----VGTNSCGWVAM	(SEQ ID NO:164)
35	D----VGERSIGLAAV	(SEQ ID NO:165)
36	D----VGLNSVGLAAV	(SEQ ID NO:166)
37	D----VGLMSVGLAAI	(SEQ ID NO:167)
38	D----VGTFSVGLAAI	(SEQ ID NO:168)
13	D----IGTGSVGYACM	(SEQ ID NO:169)
46	D----LGTNSIGSSVR	(SEQ ID NO:170)
60	DIGLRIGITSCGWSI-	(SEQ ID NO:171)
69	D----MGAKYTGVFYA	(SEQ ID NO:172)
73	D----LGGKNTGFFSF	(SEQ ID NO:173)
74	D----LGVKNTGVFSA	(SEQ ID NO:174)
70	D----LGAKFTGVALY	(SEQ ID NO:175)
71	D----LGGKFTGVCLS	(SEQ ID NO:176)
72	D----LGGTYTGTFIT	(SEQ ID NO:177)
	* * *	

FIG. 4B

ALIGNMENT OF THE HNH-LIKE DOMAINS DISCLOSED IN CHYLINSKI *ET AL.*
(CLUSTAL FORMAT ALIGNMENT BY MAFFT (v7.058b))

```
1      YDIDHIYPRS-LTKD------DSF-DNLVLCERTAN   (SEQ ID NO:178)
2      -DIDHIYPRSKVIKD------DSF-DNLVLVLKNEN   (SEQ ID NO:179)
3      -DRDHIYPQS-KIKD------DSI-DNLVLVNKTYN   (SEQ ID NO:180)
4      -DIDHIYPRS-KIKD------DSI-TNRVLVEKDIN   (SEQ ID NO:181)
6      -DIDHIYPQS-KIKD------DSI-SNRVLVCSSCN   (SEQ ID NO:182)
5      -DIDHIYPQS-KTMD------DSL-NNRVLVKKNYN   (SEQ ID NO:183)
7      -DQDHIYPKS-KIYD------DSL-ENRVLVKKNLN   (SEQ ID NO:184)
8      -QIDHIVPQS-LVKD------DSF-DNRVLVVPSEN   (SEQ ID NO:185)
9      -DIDHIIPQA-FIKD------NSI-DNRVLTSSKEN   (SEQ ID NO:186)
12     -DIDHIIPQA-FLKD------NSI-DNKVLVSSASN   (SEQ ID NO:187)
16     -DIDHIIPQA-YTKD------NSL-DNRVLVSNITN   (SEQ ID NO:188)
11     -DIDHIVPQS-FITD------NSI-DNLVLTSSAGN   (SEQ ID NO:189)
10     -DVDHIVPQS-FLKD------DSI-DNKVLTRSDKN   (SEQ ID NO:190)
14     -NIDHIYPQS-MVKD------DSL-DNKVLVQSEIN   (SEQ ID NO:191)
18     -DIDHILPQS-LIKD------DSL-DNRVLVNATIN   (SEQ ID NO:192)
19     -DIDHILPQS-FIKD------DSL-ENRVLVKKAVN   (SEQ ID NO:193)
13     -EVDHIFPRS-FIKD------DSI-DNKVLVIKKMN   (SEQ ID NO:194)
15     -EVDHIIPRS-YIKD------DSF-ENKVLVYREEN   (SEQ ID NO:195)
17     -DIDHIIPQA-VTQN------DSI-DNRVLVARAEN   (SEQ ID NO:196)
22     -EIDHIIPYS-ISFD------DSS-SNKLLVLAESN   (SEQ ID NO:197)
24     -EIDHIIPYS-LCFD------DSS-ANKVLVHKQSN   (SEQ ID NO:198)
32     -DIDHIIPYS-RSMD------DSY-SNKVLVLSGEN   (SEQ ID NO:199)
63     -DIDHIIPYS-KSMD------DSF-NNKVLCLAEEN   (SEQ ID NO:200)
59     -EIDHIYPYS-RSFD------DSY-MNKVLVFTKQN   (SEQ ID NO:201)
65     -QIDHIYPYS-RSMD------DSY-MNKVLVLTDEN   (SEQ ID NO:202)
64     -EIDHIIPFS-RSFD------DSL-SNKILVLGSEN   (SEQ ID NO:203)
68     -EIDHALPFS-RTWD------DSF-NNKVLVLGSEN   (SEQ ID NO:204)
69     -EIDHALPFS-RTWD------DSF-NNKVLVLASEN   (SEQ ID NO:205)
28     -EIDHIIPIS-ISLD------DSI-NNKVLVLSKAN   (SEQ ID NO:206)
30     -EVDHIIPIS-ISLD------DSI-TNKVLVTHREN   (SEQ ID NO:207)
62     -QVDHALPYS-RSYD------DSK-NNKVLVLTHEN   (SEQ ID NO:208)
27     -EVDHILPLS-ITFD------DSL-ANKVLVYATAN   (SEQ ID NO:209)
```

FIG. 5A

26	-EIDHIIPRS-ISFD------DAR-SNKVLVYRSEN	(SEQ ID NO:210)
29	-EVDHIIPRS-VSFD------NSY-HNKVLVKQSEN	(SEQ ID NO:211)
67	-DIDHILPYS-ITFD------DSF-RNKVLVTSQEN	(SEQ ID NO:212)
58	-EIDHILPRS-RSAD------DSF-ANKVLCLARAN	(SEQ ID NO:213)
51	-EIEHLLPFS-LTLD------DSM-ANKTVCFRQAN	(SEQ ID NO:214)
55	-DIDHILPFS-VSLD------DSA-ANKVVCLREAN	(SEQ ID NO:215)
57	-DIDHLIPFS-ISWD------DSA-ANKVVCMRYAN	(SEQ ID NO:216)
56	-DIDHILPVA-MTLD------DSP-ANKIICMRYAN	(SEQ ID NO:217)
54	-DVDHILPYS-RTLD------DSF-PNRTLCLREAN	(SEQ ID NO:218)
52	-EIEHILPFS-RTLD------DSL-NNRTVAMRRAN	(SEQ ID NO:219)
31	-EVDHIIPYS-ISWD------DSY-TNKVLTSAKCN	(SEQ ID NO:220)
45	-QVDHILPWS-RFGD------DSY-LNKTLCTARSN	(SEQ ID NO:221)
53	-QVDHILPFS-KTLD------DSF-ANKVLAQHDAN	(SEQ ID NO:222)
60	-QIDHAFPLS-RSLD------DSQ-SNKVLCLTSSN	(SEQ ID NO:223)
21	-DIDHIVPRS-ISFD------DSF-SNLVIVNKLDN	(SEQ ID NO:224)
23	-EIEHIIPYS-MSYD------NSQ-ANKILTEKAEN	(SEQ ID NO:225)
25	-EIDHVIPYS-KSAD------DSW-FNKLLVKKSTN	(SEQ ID NO:226)
49	-EMDHILPYS-RSLD------NGW-HNRVLVHGKDN	(SEQ ID NO:227)
33	-EVDHIVPYS-LILD------NTI-NNKALVYAEEN	(SEQ ID NO:228)
42	-EIEHVIPQS-LYFD------DSF-SNKVICEAEVN	(SEQ ID NO:229)
43	-DIEHIIPQA-RLFD------DSF-SNKTLEARSVN	(SEQ ID NO:230)
44	-EIEHIVPKA-RVFD------DSF-SNKTLTFHRIN	(SEQ ID NO:231)
20	-DKDHIIPQS-MKKD------DSIINNLVLVNKNAN	(SEQ ID NO:232)
66	-EVEHIWPRS-RSFD------NSP-RNKTLCRKDVN	(SEQ ID NO:233)
61	-IVNHIIPYN-RSFD------DTY-HNRVLTLTETK	(SEQ ID NO:234)
46	-DMEHTIPKS-ISFD------NSD-QNLTLCESYYN	(SEQ ID NO:235)
47	-DIEHTIPRS-AGGD------STK-MNLTLCSSRFN	(SEQ ID NO:236)
48	-DIEHTIPRS-ISQD------NSQ-MNKTLCSLKFN	(SEQ ID NO:237)
50	-DIDHVIPLA-RGGR------DSL-DNMVLCQSDAN	(SEQ ID NO:238)
39	-DIEHLFPIA-ESED------NGR-NNLVISHSACN	(SEQ ID NO:239)
41	-DVDHIFPRD-DTAD------NSY-GNKVVAHRQCN	(SEQ ID NO:240)
40	-DIEHIVPQS-LGGL------STD-YNTIVTLKSVN	(SEQ ID NO:241)
35	-ELDHIVPRT-DGGS------NRH-ENLAITCGACN	(SEQ ID NO:242)
36	-EMDHIVPRKGVGST------NTR-TNFAAVCAECN	(SEQ ID NO:243)
37	-EMDHIVPRKGVGST------NTR-VNLAAACAACN	(SEQ ID NO:244)
38	-EMDHIVPRAGQGST------NTR-ENLVAVCHRCN	(SEQ ID NO:245)
70	-EIDHILPRS-LIKDARGIVFNAE-PNLIYASSRGN	(SEQ ID NO:246)
71	-EIDHIIPRS-LTGRTKKTVFNSE-ANLIYCSSKGN	(SEQ ID NO:247)
73	-EIDHIIPRS-LTLKKSESIYNSE-VNLIFVSAQGN	(SEQ ID NO:248)
72	-EIDHIYPRS-LSKKHFGVIFNSE-VNLIYCSSQGN	(SEQ ID NO:249)

FIG. 5B

```
74    -EIDHILPRS-HTLKIYGTVFNPE-GNLIYVHQKCN   (SEQ ID NO:250)
75    -ELDHIIPRS-HKKY---GTLNDE-ANLICVTRGDN   (SEQ ID NO:251)
34    -ELEHIVPHS-FRQS------NAL-SSLVLTWPGVN   (SEQ ID NO:252)
        :*  *             .   .       :
```

FIG. 5C

ALIGNMENT OF THE HNH-LIKE DOMAINS DISCLOSED IN CHYLINSKI *ET AL.* (EXCLUDING SEQUENCE OUTLIERS).
(CLUSTAL FORMAT ALIGNMENT BY MAFFT (v7.058b))

1	YDIDHIYPRS-LTKDDS-FDNLVLCERTAN	(SEQ ID NO:253)
2	-DIDHIYPRSKVIKDDS-FDNLVLVLKNEN	(SEQ ID NO:254)
3	-DRDHIYPQS-KIKDDS-IDNLVLVNKTYN	(SEQ ID NO:255)
4	-DIDHIYPRS-KIKDDS-ITNRVLVEKDIN	(SEQ ID NO:256)
6	-DIDHIYPQS-KIKDDS-ISNRVLVCSSCN	(SEQ ID NO:257)
5	-DIDHIYPQS-KTMDDS-LNNRVLVKKNYN	(SEQ ID NO:258)
7	-DQDHIYPKS-KIYDDS-LENRVLVKKNLN	(SEQ ID NO:259)
8	-QIDHIVPQS-LVKDDS-FDNRVLVVPSEN	(SEQ ID NO:260)
9	-DIDHIIPQA-FIKDNS-IDNRVLTSSKEN	(SEQ ID NO:261)
12	-DIDHIIPQA-FLKDNS-IDNKVLVSSASN	(SEQ ID NO:262)
16	-DIDHIIPQA-YTKDNS-LDNRVLVSNITN	(SEQ ID NO:263)
11	-DIDHIVPQS-FITDNS-IDNLVLTSSAGN	(SEQ ID NO:264)
10	-DVDHIVPQS-FLKDDS-IDNKVLTRSDKN	(SEQ ID NO:265)
14	-NIDHIYPQS-MVKDDS-LDNKVLVQSEIN	(SEQ ID NO:266)
18	-DIDHILPQS-LIKDDS-LDNRVLVNATIN	(SEQ ID NO:267)
19	-DIDHILPQS-FIKDDS-LENRVLVKKAVN	(SEQ ID NO:268)
13	-EVDHIFPRS-FIKDDS-IDNKVLVIKKMN	(SEQ ID NO:269)
15	-EVDHIIPRS-YIKDDS-FENKVLVYREEN	(SEQ ID NO:270)
17	-DIDHIIPQA-VTQNDS-IDNRVLVARAEN	(SEQ ID NO:271)
21	-DIDHIVPRS-ISFDDS-FSNLVIVNKLDN	(SEQ ID NO:272)
22	-EIDHIIPYS-ISFDDS-SSNKLLVLAESN	(SEQ ID NO:273)
24	-EIDHIIPYS-LCFDDS-SANKVLVHKQSN	(SEQ ID NO:274)
28	-EIDHIIPIS-ISLDDS-INNKVLVLSKAN	(SEQ ID NO:275)
30	-EVDHIIPIS-ISLDDS-ITNKVLVTHREN	(SEQ ID NO:276)
27	-EVDHILPLS-ITFDDS-LANKVLVYATAN	(SEQ ID NO:277)
26	-EIDHIIPRS-ISFDDA-RSNKVLVYRSEN	(SEQ ID NO:278)
29	-EVDHIIPRS-VSFDNS-YHNKVLVKQSEN	(SEQ ID NO:279)
31	-EVDHIIPYS-ISWDDS-YTNKVLTSAKCN	(SEQ ID NO:280)
32	-DIDHIIPYS-RSMDDS-YSNKVLVLSGEN	(SEQ ID NO:281)
23	-EIEHIIPYS-MSYDNS-QANKILTEKAEN	(SEQ ID NO:282)
33	-EVDHIVPYS-LILDNT-INNKALVYAEEN	(SEQ ID NO:283)
25	-EIDHVIPYS-KSADDS-WFNKLLVKKSTN	(SEQ ID NO:284)
49	-EMDHILPYS-RSLDNG-WHNRVLVHGKDN	(SEQ ID NO:285)
42	-EIEHVIPQS-LYFDDS-FSNKVICEAEVN	(SEQ ID NO:286)

FIG. 6A

```
43    -DIEHIIPQA-RLFDDS-FSNKTLEARSVN        (SEQ ID NO:287)
44    -EIEHIVPKA-RVFDDS-FSNKTLTFHRIN        (SEQ ID NO:288)
20    -DKDHIIPQS-MKKDDSIINNLVLVNKNAN        (SEQ ID NO:289)
45    -QVDHILPWS-RFGDDS-YLNKTLCTARSN        (SEQ ID NO:290)
50    -DIDHVIPLA-RGGRDS-LDNMVLCQSDAN        (SEQ ID NO:291)
46    -DMEHTIPKS-ISFDNS-DQNLTLCESYYN        (SEQ ID NO:292)
47    -DIEHTIPRS-AGGDST-KMNLTLCSSRFN        (SEQ ID NO:293)
48    -DIEHTIPRS-ISQDNS-QMNKTLCSLKFN        (SEQ ID NO:294)
39    -DIEHLFPIA-ESEDNG-RNNLVISHSACN        (SEQ ID NO:295)
41    -DVDHIFPRD-DTADNS-YGNKVVAHRQCN        (SEQ ID NO:296)
40    -DIEHIVPQS-LGGLST-DYNTIVTLKSVN        (SEQ ID NO:297)
35    -ELDHIVPRT-DGGSNR-HENLAITCGACN        (SEQ ID NO:298)
36    -EMDHIVPRKGVGSTNT-RTNFAAVCAECN        (SEQ ID NO:299)
37    -EMDHIVPRKGVGSTNT-RVNLAAACAACN        (SEQ ID NO:300)
38    -EMDHIVPRAGQGSTNT-RENLVAVCHRCN        (SEQ ID NO:301)
34    -ELEHIVPHS-FRQSNA-LSSLVLTWPGVN        (SEQ ID NO:302)
       : :*  *       .   .        *
```

FIG. 6B

SEQUENCE ALIGNMENT BETWEEN SpCas9 AND NmCas9

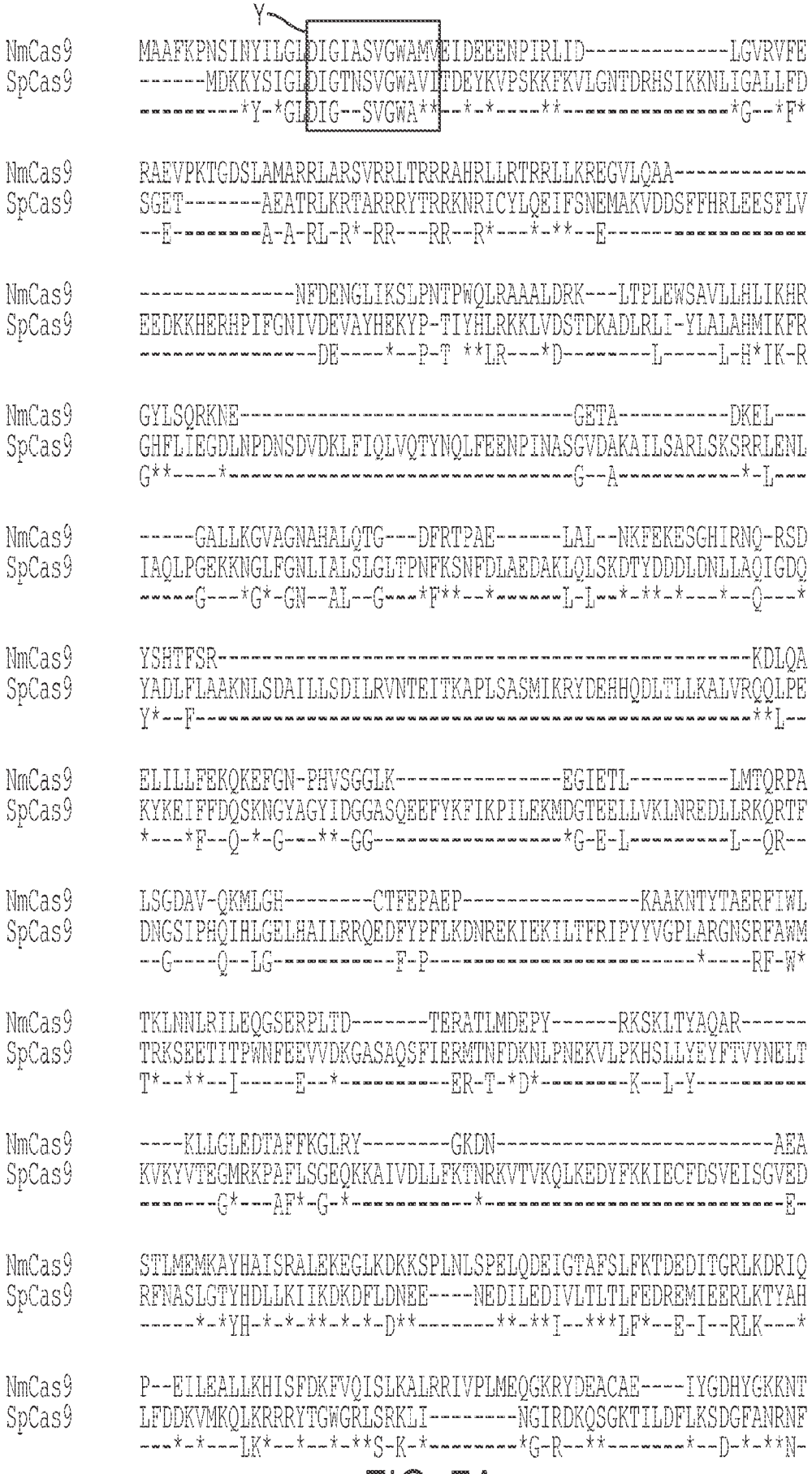


FIG. 7A

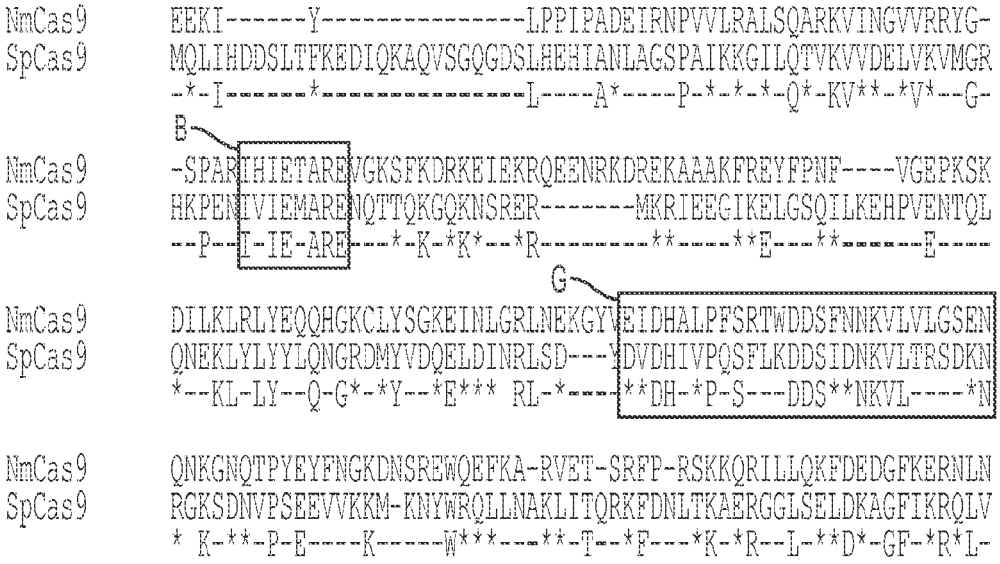


FIG. 7A

CONTINUED

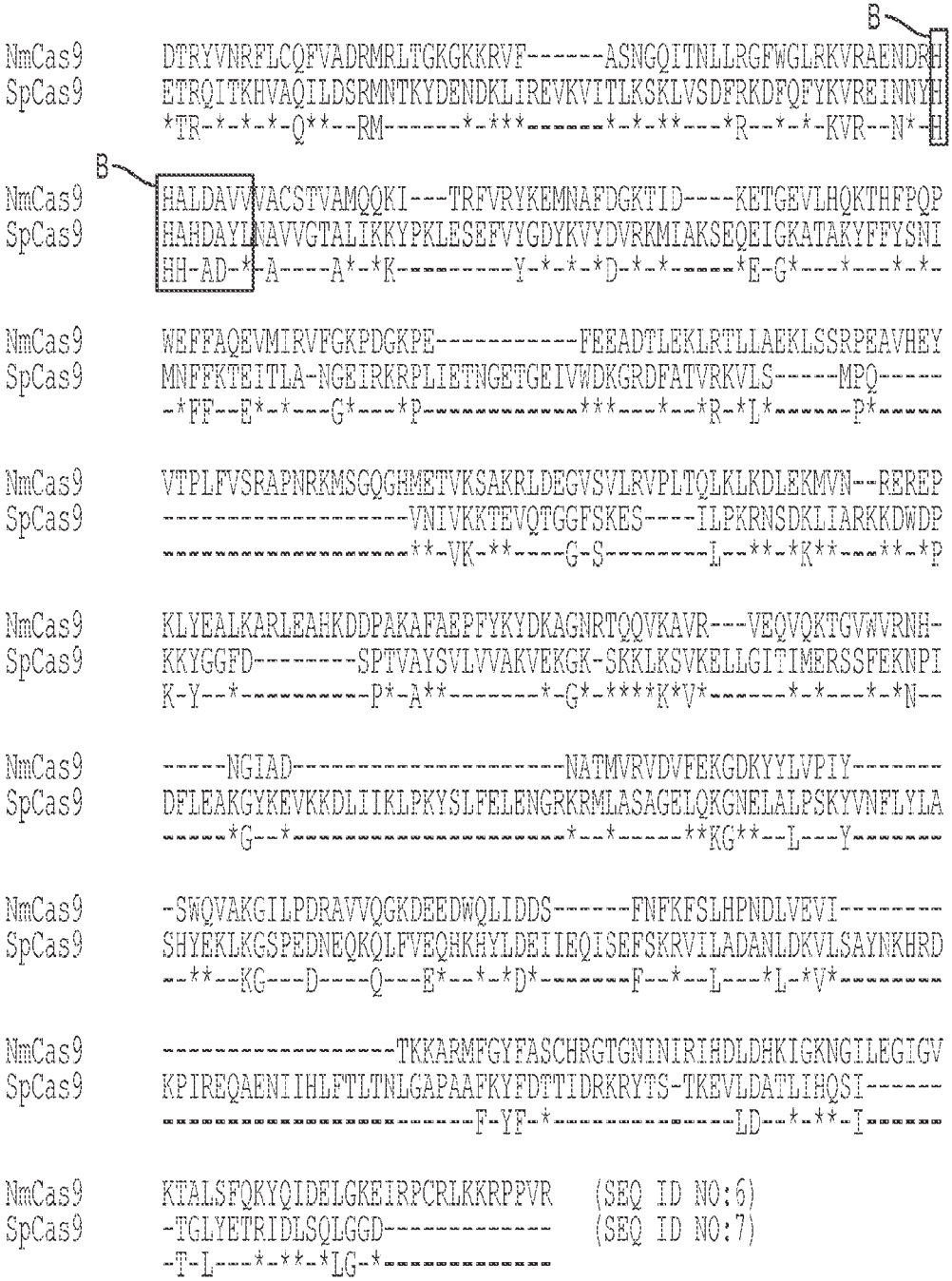

B
B
NmCas9 DTRYVNRFLCQFVADRMRLTGKGKKRVF------ASNGQITNLLRGFWGLRKVRAENDRH
SpCas9 ETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYH
TR--*-*-Q**--RM------*-***------*-*-**---*R--*-*-KVR--N*-H
NmCas9 HALDAVVACSTVAMQQKI---TRFVRYKEMNAFDGKTID----KETGEVLHQKTHFPQP
SpCas9 HAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
HH-AD-*-A----A*-*K---------Y-*-*-*D-*-*-----*E-G*---*---*-*-
NmCas9 WEFFAQEVMIRVFGKPDGKPE----------FEEADTLEKLRTLLAEKLSSRPEAVHEY
SpCas9 MNFFKTEITLA-NGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS-----MPQ-----
-*FF--E*-*---G*---*P----------***---*--*R-*L*------P*-----
NmCas9 VTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQLKLKDLEKMVN--REREP
SpCas9 ----------VNIVKKTEVQTGGFSKES----ILPKRNSDKLIARKKDWDP
----------**-VK-**----G-S--------L--**-*K**---**-*P
NmCas9 KLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQQVKAVR---VEQVQKTGVWVRNH-
SpCas9 KKYGGFD--------SPTVAYSVLVVAKVEKGK-SKKLKSVKELLGITIMERSSFEKNPI
K-Y--*----------P*-A**-------*-G*-****K*V*------*-*---*-*N--
NmCas9 ------NGIAD----------NATMVRVDVFEKGDKYYLVPIY--------
SpCas9 DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
------*G--*----------*--*------**KG**--L---Y--------
NmCas9 -SWQVAKGILPDRAVVQGKDEEDWQLIDDS------FNFKFSLHPNDLVEVI--------
SpCas9 SHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD
--**--KG---D----Q---E*--*-*D*-------F--*--L---*L-*V*---------
NmCas9 ----------TKKARMFGYFASCHRGTGNINIRIHDLDHKIGKNGILEGIGV
SpCas9 KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS-TKEVLDATLIHQSI------
----------F-YF-*----------LD--*-**-I-------
NmCas9 KTALSFQKYQIDELGKEIRPCRLKKRPPVR (SEQ ID NO:6)
SpCas9 -TGLYETRIDLSQLGGD---------- (SEQ ID NO:7)
-T-L---*-**-*LG-*----------
PERCENT IDENTITY MATRIX - CREATED BY CLUSTAL2.1

FIG. 7B

SEQUENCE OF THE NmCas9 ORF WITH DUAL NLS AND HA TAGS



R: SV40 NLS, G: HA TAG, O: SYNTHETIC NLS (1); ALL ELSE NmCas9

FIG. 8

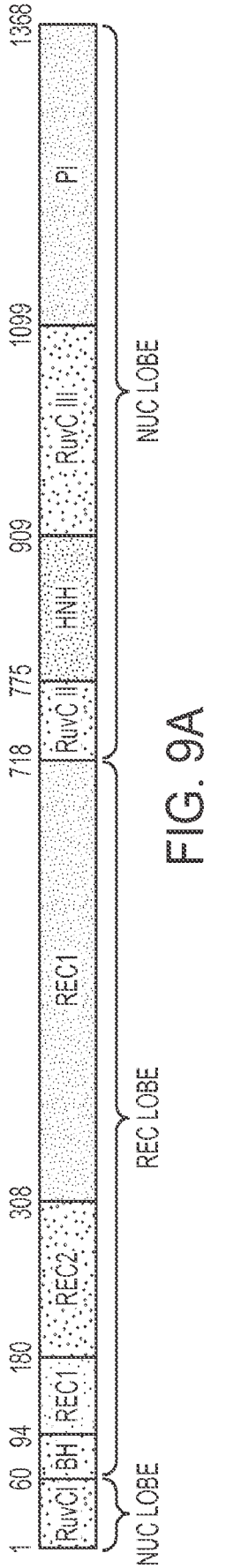

1
60
94
180
308
718
775
909
1099
1368
RuvCI
BH
REC1
REC2
REC1
RuvC II
HNH
RuvC III
PI
NUC LOBE
REC LOBE
NUC LOBE

FIG. 9A

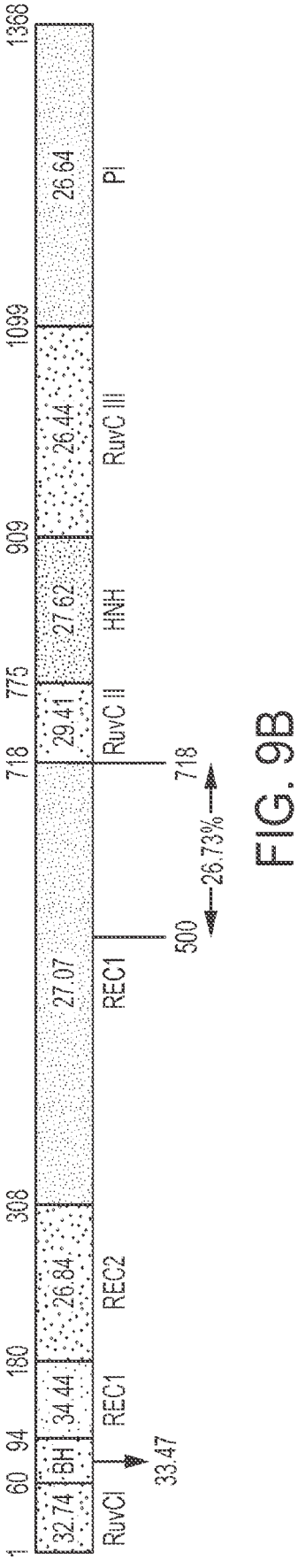

1
60
94
180
308
718
775
909
1099
1368
32.74
BH
34.44
26.84
27.07
29.41
RuvC II
27.62
HNH
26.44
RuvC III
26.64
PI
RuvCI
REC1
REC2
REC1
33.47
500
718
26.73%

FIG. 9B

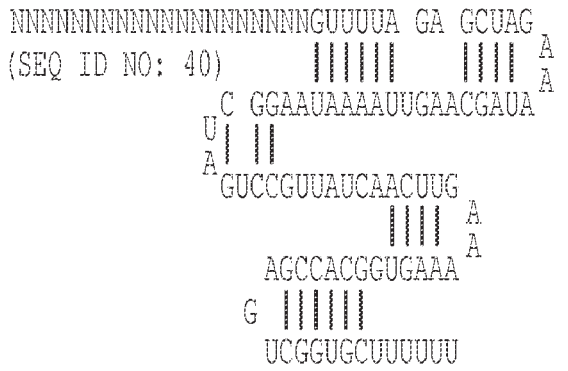


FIG. 10A

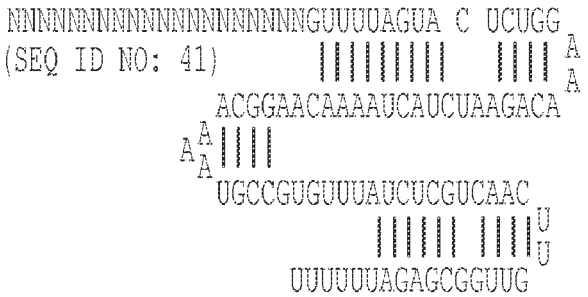


FIG. 10B

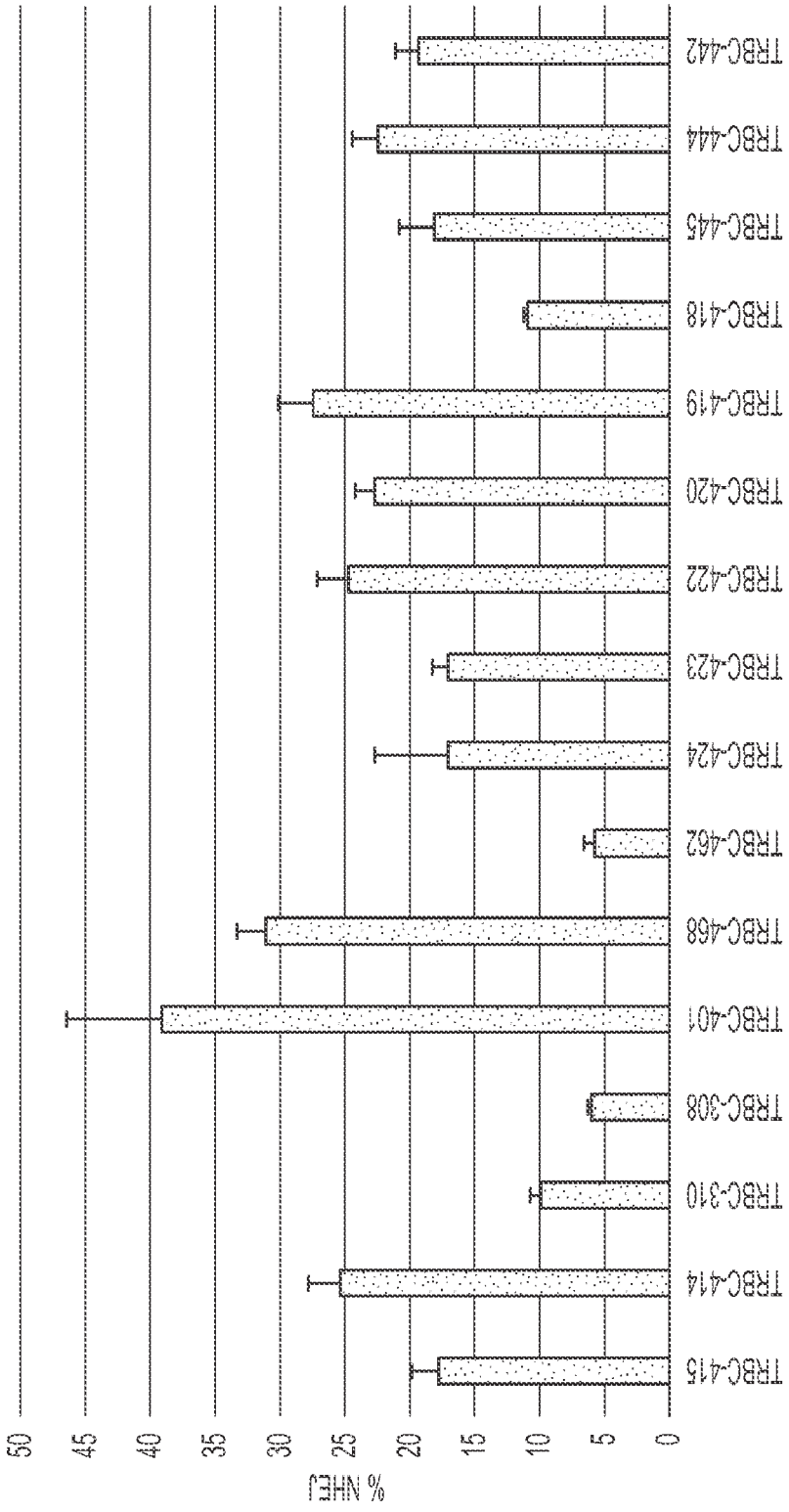

% NHEJ
0
5
10
15
20
25
30
35
40
45
50
TRBC-415
TRBC-414
TRBC-310
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TRBC-401
TRBC-468
TRBC-462
TRBC-424
TRBC-423
TRBC-422
TRBC-420
TRBC-419
TRBC-418
TRBC-445
TRBC-444
TRBC-442

FIG. 11

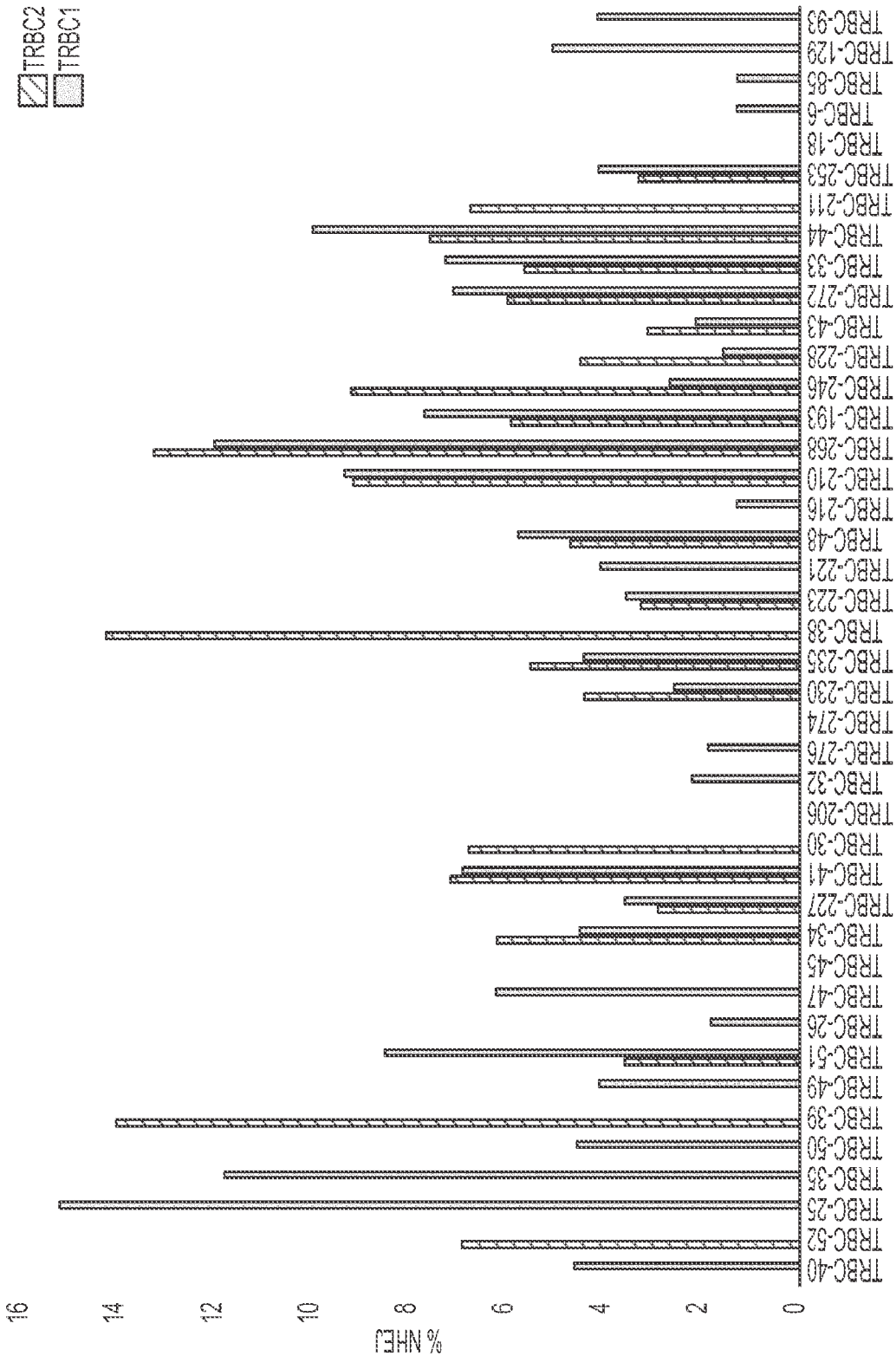

TRBC2
TRBC1
% NHEJ
0
2
4
6
8
10
12
14
16
TRBC-40
TRBC-52
TRBC-25
TRBC-35
TRBC-50
TRBC-39
TRBC-49
TRBC-51
TRBC-26
TRBC-47
TRBC-45
TRBC-34
TRBC-227
TRBC-41
TRBC-30
TRBC-206
TRBC-32
TRBC-276
TRBC-274
TRBC-230
TRBC-235
TRBC-38
TRBC-223
TRBC-221
TRBC-48
TRBC-216
TRBC-210
TRBC-268
TRBC-193
TRBC-246
TRBC-228
TRBC-43
TRBC-272
TRBC-33
TRBC-44
TRBC-211
TRBC-253
TRBC-18
TRBC-6
TRBC-85
TRBC-129
TRBC-93

FIG. 12

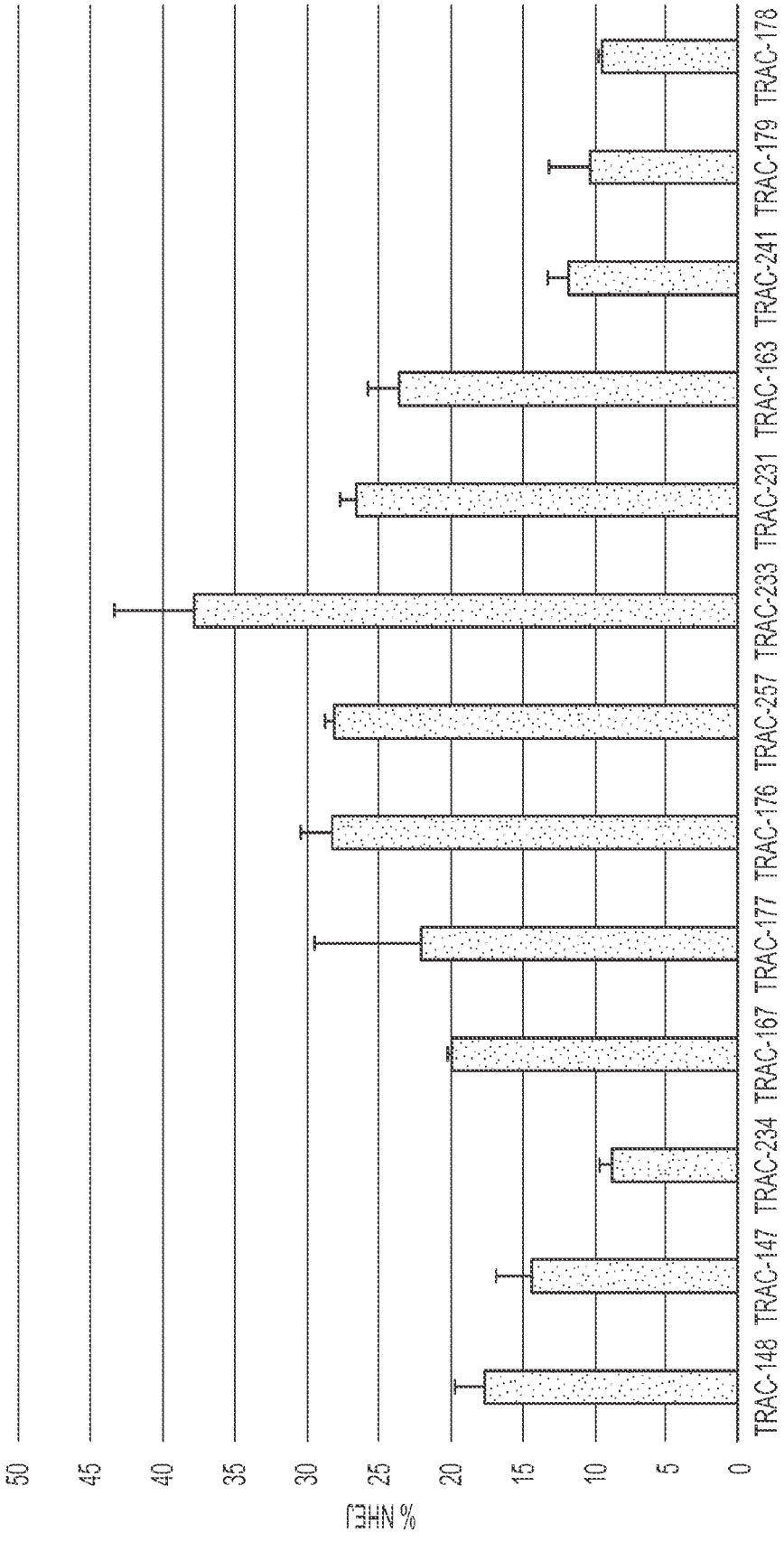

50
45
40
35
30
25
20
15
10
5
0
INDEL %
TRAC-148
TRAC-147
TRAC-234
TRAC-167
TRAC-177
TRAC-176
TRAC-257
TRAC-233
TRAC-231
TRAC-163
TRAC-241
TRAC-179
TRAC-178

FIG. 13

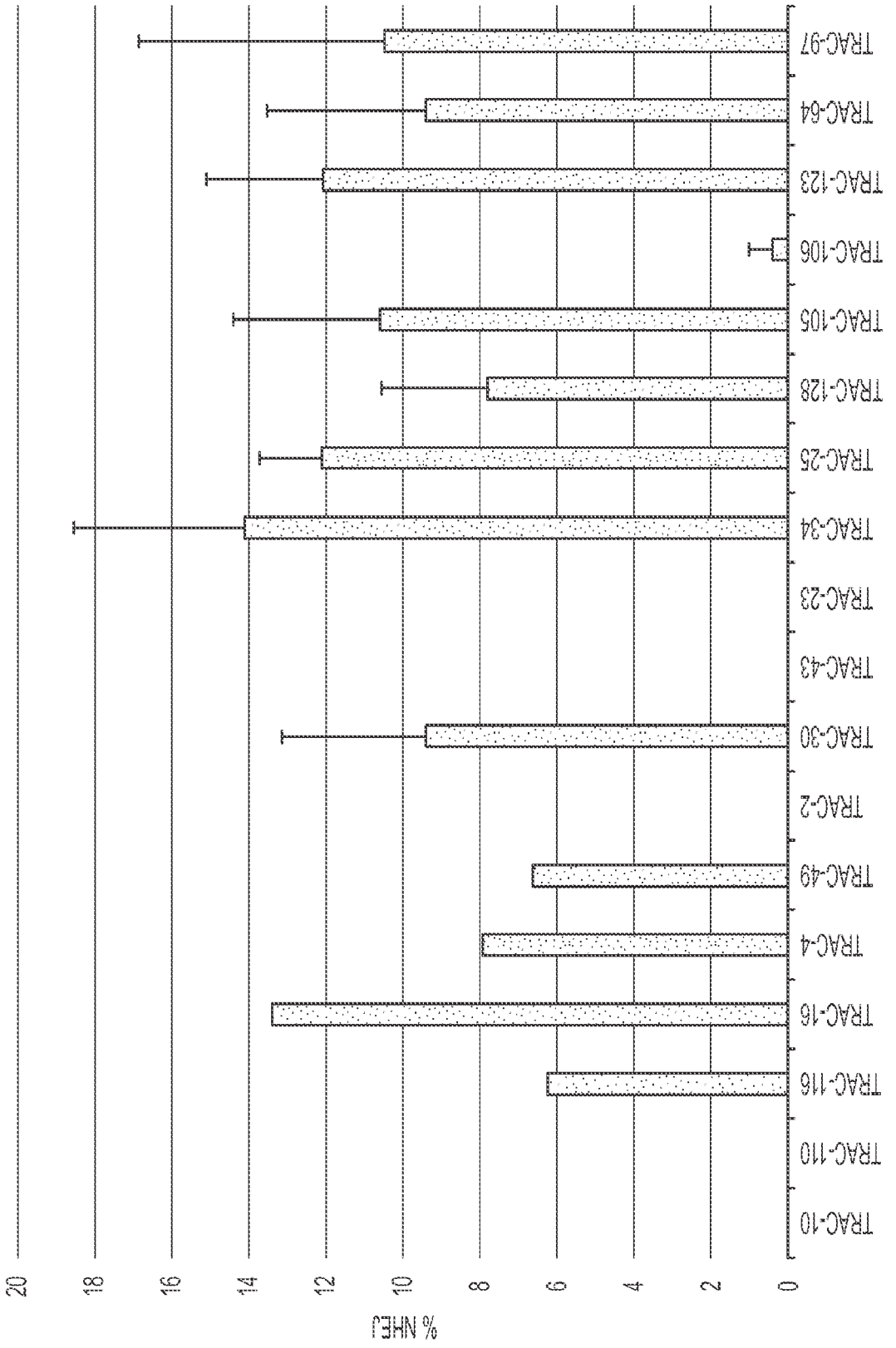

% NHEJ
0
2
4
6
8
10
12
14
16
18
20
TRAC-10
TRAC-110
TRAC-116
TRAC-16
TRAC-4
TRAC-49
TRAC-2
TRAC-30
TRAC-43
TRAC-23
TRAC-34
TRAC-25
TRAC-128
TRAC-105
TRAC-106
TRAC-123
TRAC-64
TRAC-97

FIG. 14

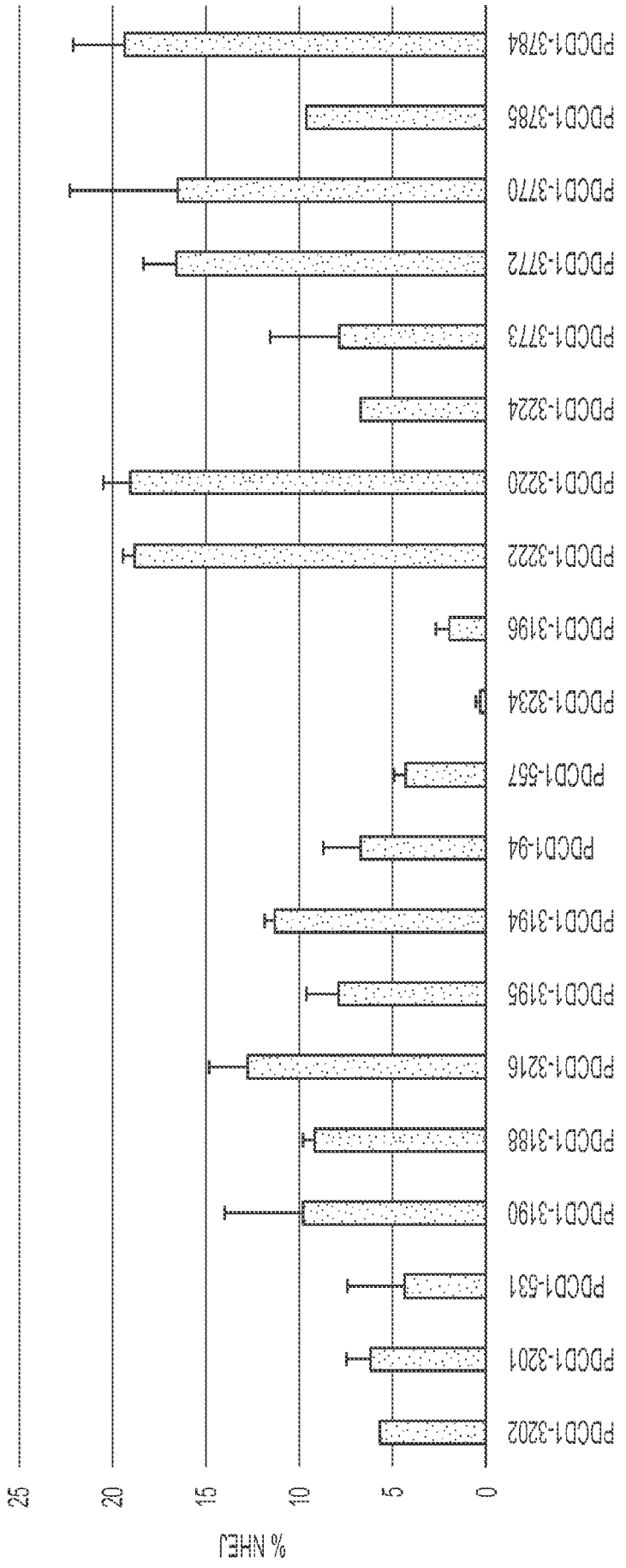

PDCD1-3784
PDCD1-3785
PDCD1-3770
PDCD1-3772
PDCD1-3773
PDCD1-3224
PDCD1-3220
PDCD1-3222
PDCD1-3196
PDCD1-3234
PDCD1-557
PDCD1-94
PDCD1-3194
PDCD1-3195
PDCD1-3216
PDCD1-3188
PDCD1-3190
PDCD1-531
PDCD1-3201
PDCD1-3202
gRNA
25
20
15
10
5
0
% NHEJ

FIG. 15

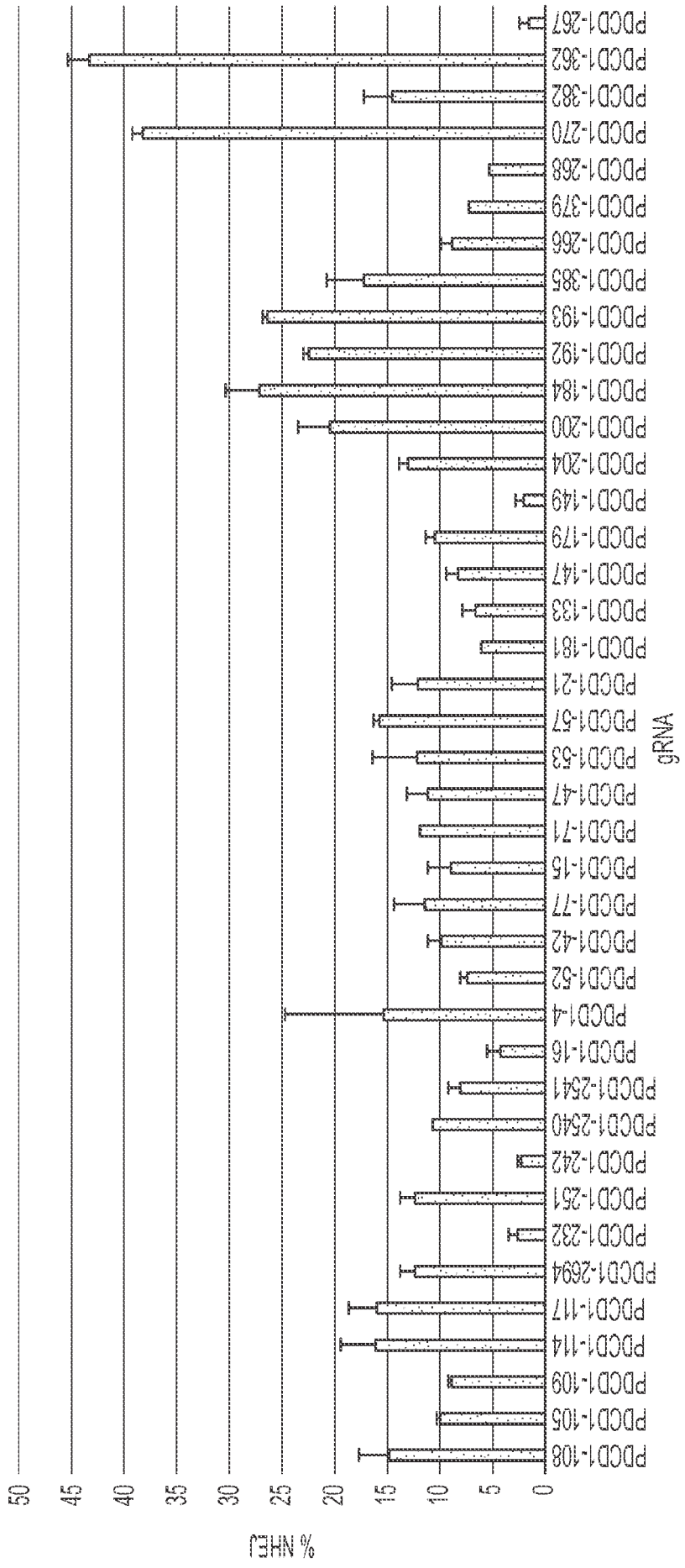

% NHEJ
0
5
10
15
20
25
30
35
40
45
50
gRNA
PDCD1-108
PDCD1-105
PDCD1-109
PDCD1-114
PDCD1-117
PDCD1-2694
PDCD1-232
PDCD1-251
PDCD1-242
PDCD1-2540
PDCD1-2541
PDCD1-16
PDCD1-4
PDCD1-52
PDCD1-42
PDCD1-77
PDCD1-15
PDCD1-71
PDCD1-47
PDCD1-53
PDCD1-57
PDCD1-21
PDCD1-181
PDCD1-133
PDCD1-147
PDCD1-179
PDCD1-149
PDCD1-204
PDCD1-200
PDCD1-184
PDCD1-192
PDCD1-193
PDCD1-385
PDCD1-266
PDCD1-379
PDCD1-268
PDCD1-270
PDCD1-382
PDCD1-362
PDCD1-267

FIG. 16

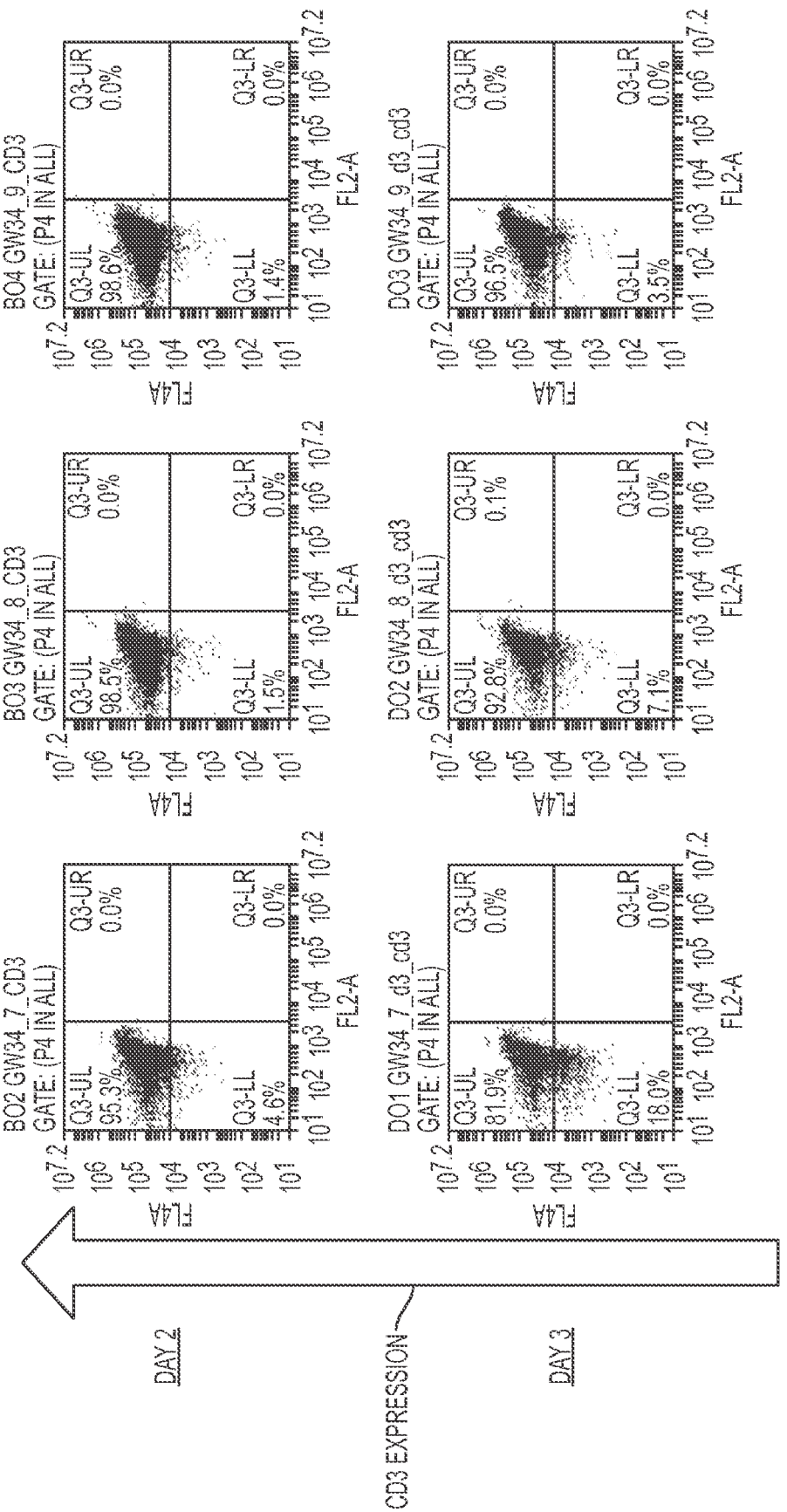

TRBC-210
TRAC-4
AAVS1
DAY 2
DAY 3
CD3 EXPRESSION
BO2 GW34_7_CD3
GATE: (P4 IN ALL)
Q3-UL 95.3%
Q3-UR 0.0%
Q3-LL 4.6%
Q3-LR 0.0%
BO3 GW34_8_CD3
GATE: (P4 IN ALL)
Q3-UL 98.5%
Q3-UR 0.0%
Q3-LL 1.5%
Q3-LR 0.0%
BO4 GW34_9_CD3
GATE: (P4 IN ALL)
Q3-UL 98.6%
Q3-UR 0.0%
Q3-LL 1.4%
Q3-LR 0.0%
DO1 GW34_7_d3_cd3
GATE: (P4 IN ALL)
Q3-UL 81.9%
Q3-UR 0.0%
Q3-LL 18.0%
Q3-LR 0.0%
DO2 GW34_8_d3_cd3
GATE: (P4 IN ALL)
Q3-UL 92.8%
Q3-UR 0.1%
Q3-LL 7.1%
Q3-LR 0.0%
DO3 GW34_9_d3_cd3
GATE: (P4 IN ALL)
Q3-UL 96.5%
Q3-UR 0.0%
Q3-LL 3.5%
Q3-LR 0.0%
FL4A
FL2-A

FIG. 17A

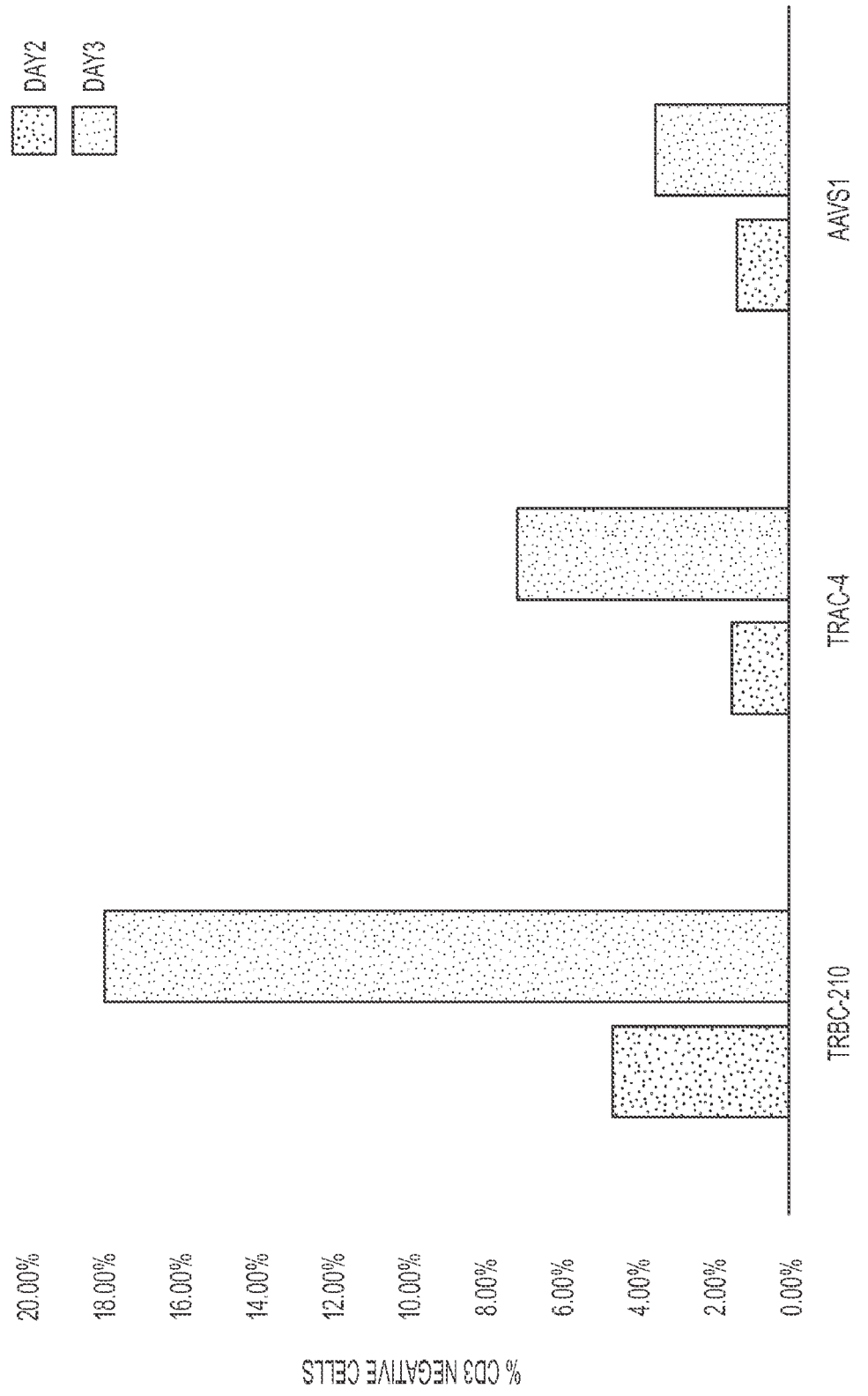

DAY2
DAY3
% CD3 NEGATIVE CELLS
20.00%
18.00%
16.00%
14.00%
12.00%
10.00%
8.00%
6.00%
4.00%
2.00%
0.00%
TRBC-210
TRAC-4
AAVS1

FIG. 17B


TRBC2 LOCUS ANALYSIS
% NHEJ
6.0
5.0
4.0
3.0
2.0
1.0
0.0
TRBC-210
TRAC-4
AAVS1
TRAC LOCUS ANALYSIS
% NHEJ
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TRBC-210
TRAC-4
AAVS1

FIG. 17C

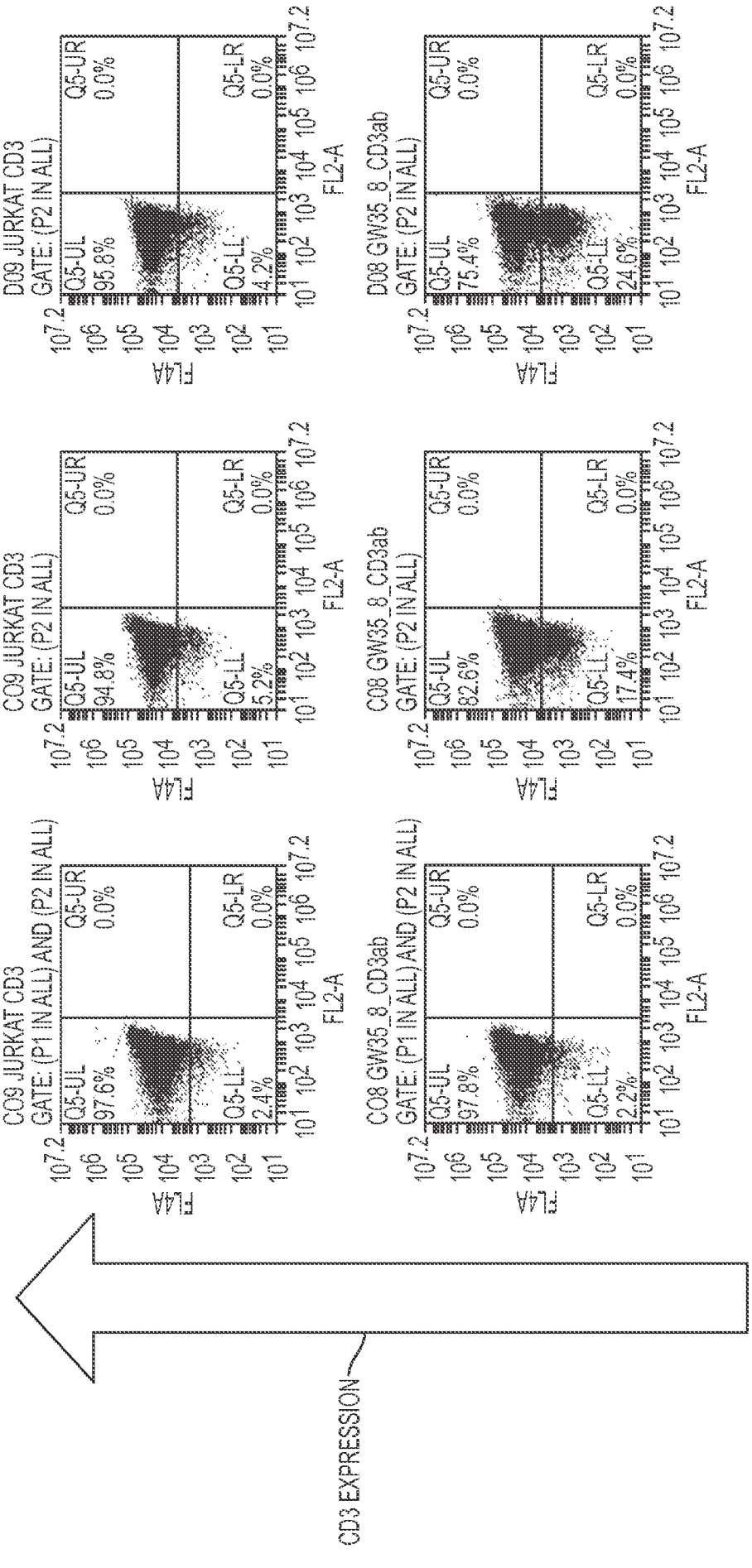

DAY 1
DAY 1
DAY 3
CO9 JURKAT CD3
GATE: (P1 IN ALL) AND (P2 IN ALL)
CO8 GW35_8_CD3ab
GATE: (P1 IN ALL) AND (P2 IN ALL)
CO9 JURKAT CD3
GATE: (P2 IN ALL)
CO8 GW35_8_CD3ab
GATE: (P2 IN ALL)
D09 JURKAT CD3
GATE: (P2 IN ALL)
D08 GW35_8_CD3ab
GATE: (P2 IN ALL)
Q5-UL
97.6%
Q5-LL
2.4%
Q5-UR
0.0%
Q5-LR
0.0%
Q5-UL
97.8%
Q5-LL
2.2%
Q5-UR
0.0%
Q5-LR
0.0%
Q5-UL
94.8%
Q5-LL
5.2%
Q5-UR
0.0%
Q5-LR
0.0%
Q5-UL
82.6%
Q5-LL
17.4%
Q5-UR
0.0%
Q5-LR
0.0%
Q5-UL
95.8%
Q5-LL
4.2%
Q5-UR
0.0%
Q5-LR
0.0%
Q5-UL
75.4%
Q5-LL
24.6%
Q5-UR
0.0%
Q5-LR
0.0%
FL4A
FL2-A
CD3 EXPRESSION

FIG. 18A

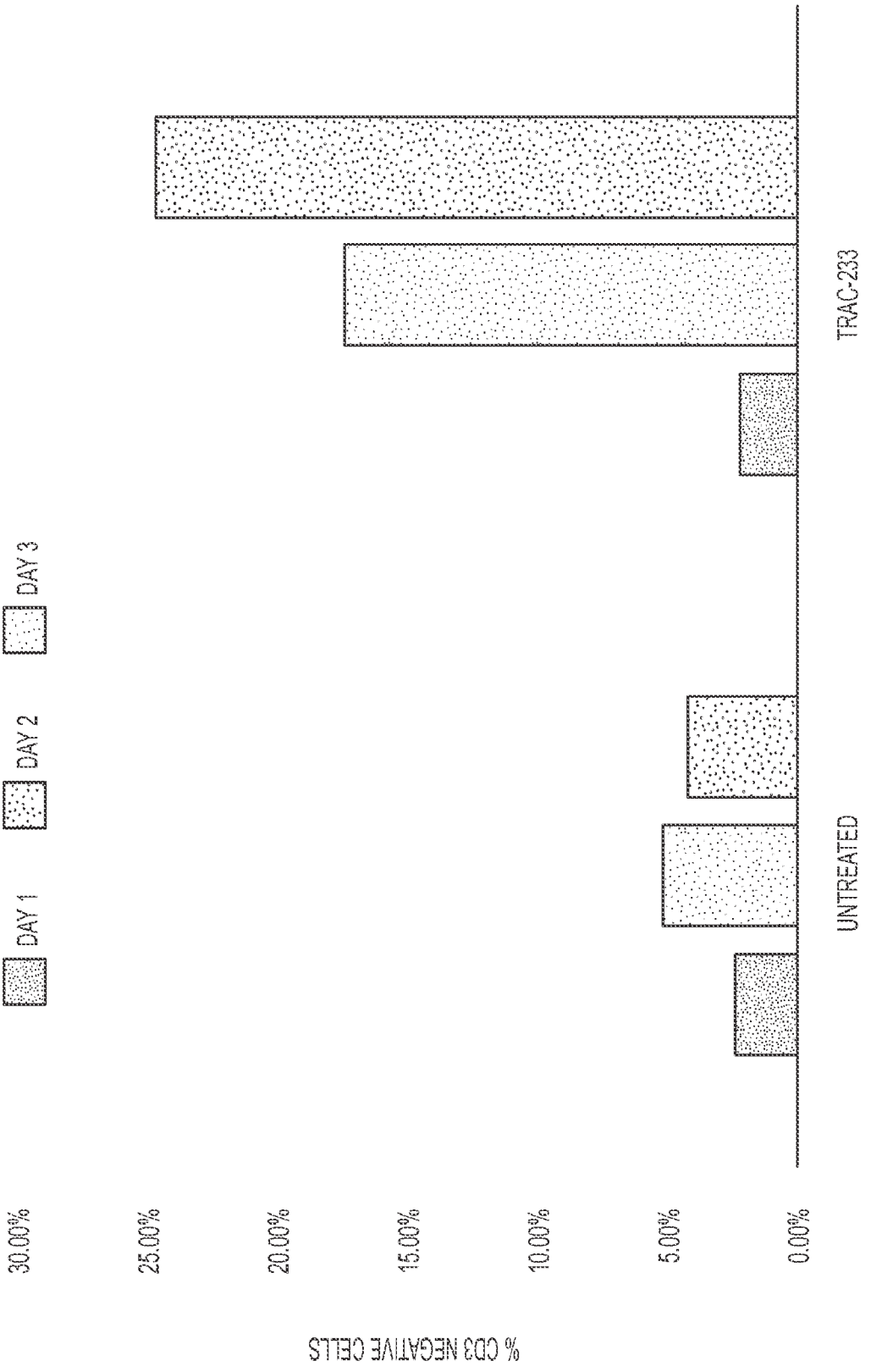

% CD3 NEGATIVE CELLS
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
DAY 1
DAY 2
DAY 3
UNTREATED
TRAC-233

FIG. 18B

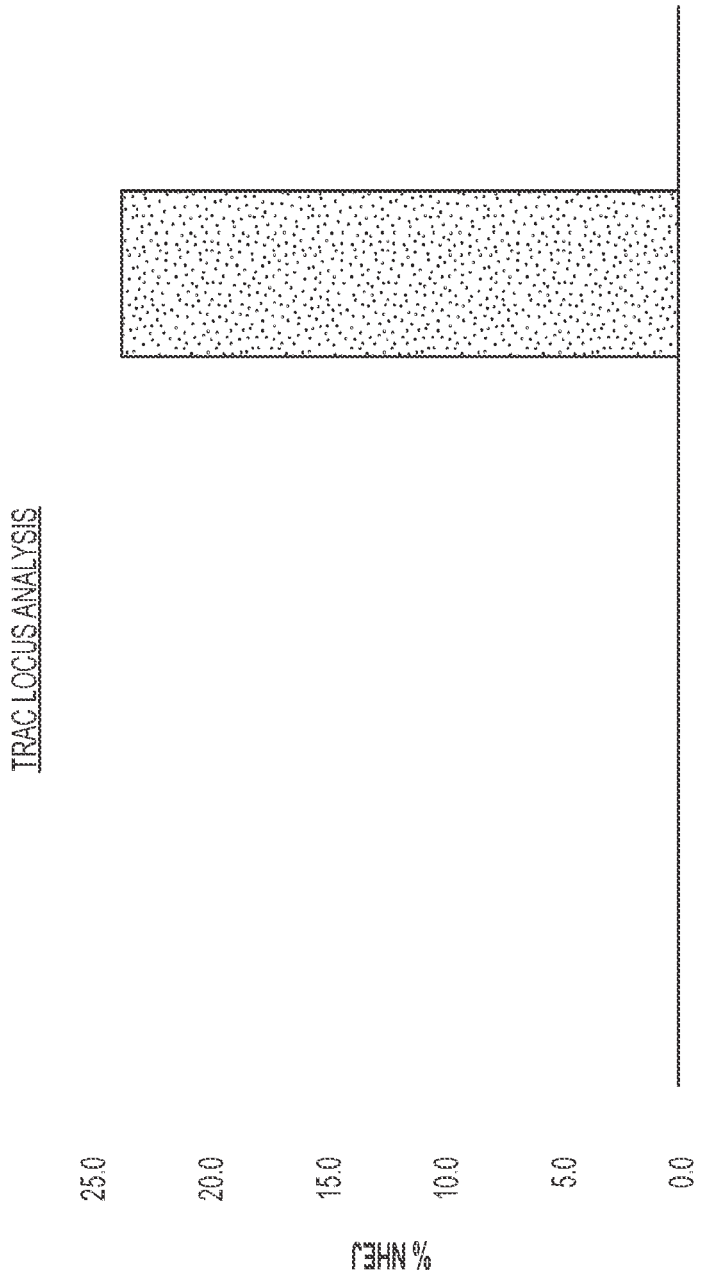

TRAC LOCUS ANALYSIS
INDEL %
25.0
20.0
15.0
10.0
5.0
0.0
UNTREATED
TRAC-233

FIG. 18C

ARCA STRUCTURE

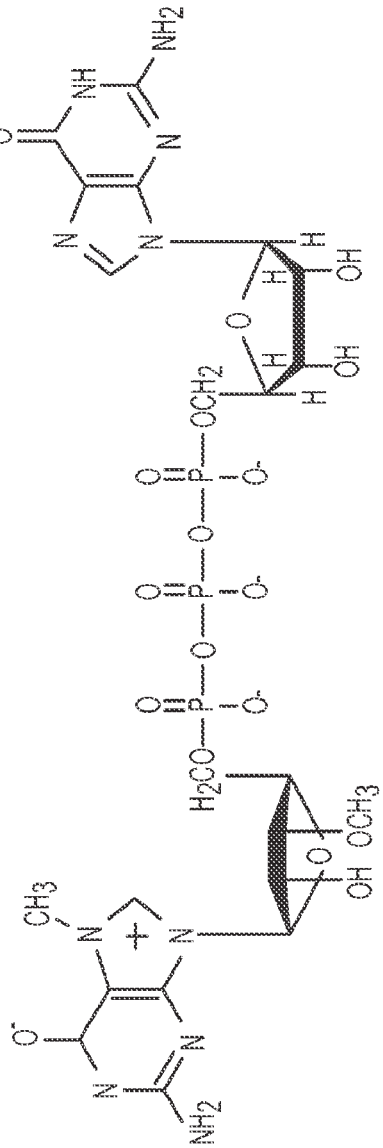


FIG. 19

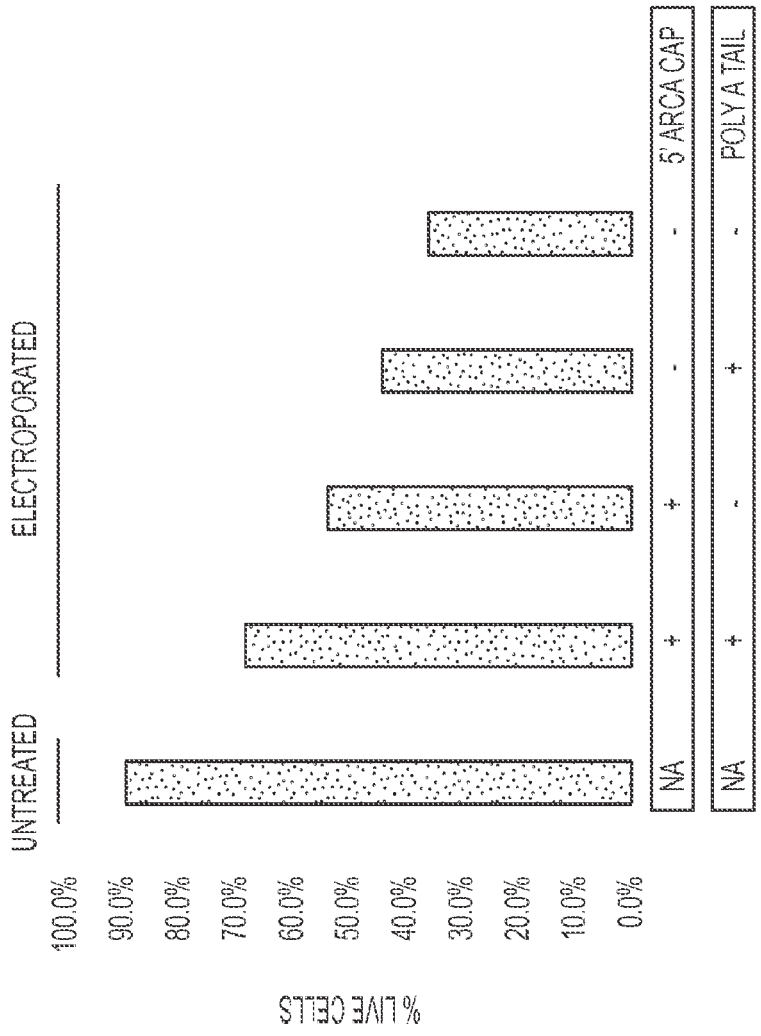

UNTREATED
ELECTROPORATED
% LIVE CELLS
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
NA
+
+
-
-
5' ARCA CAP
NA
+
-
+
-
POLY A TAIL

FIG. 20

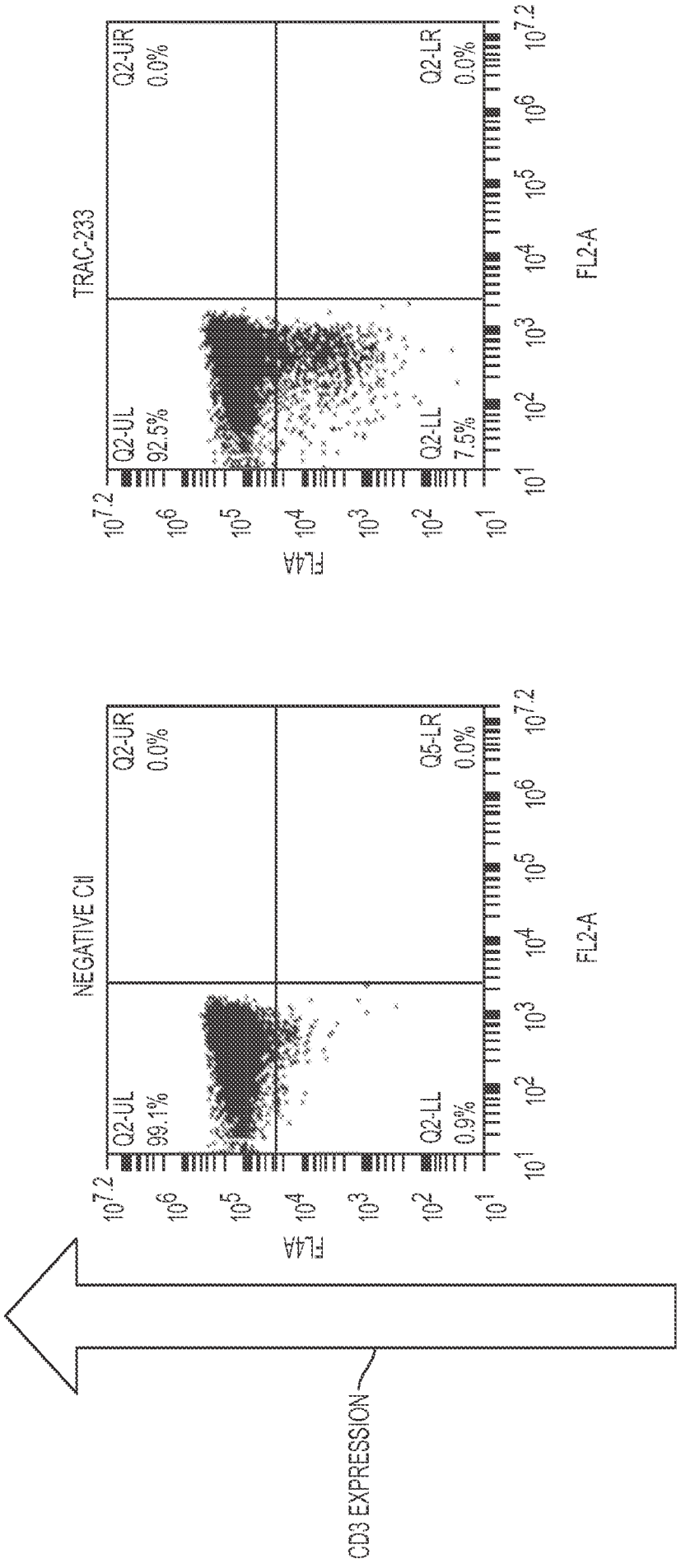

CD3 EXPRESSION
NEGATIVE CH
Q2-UL
99.1%
Q2-UR
0.0%
Q2-LL
0.9%
Q5-LR
0.0%
FL4A
FL2-A
TRAC-233
Q2-UL
92.5%
Q2-UR
0.0%
Q2-LL
7.5%
Q2-LR
0.0%
FL4A
FL2-A

FIG. 21A

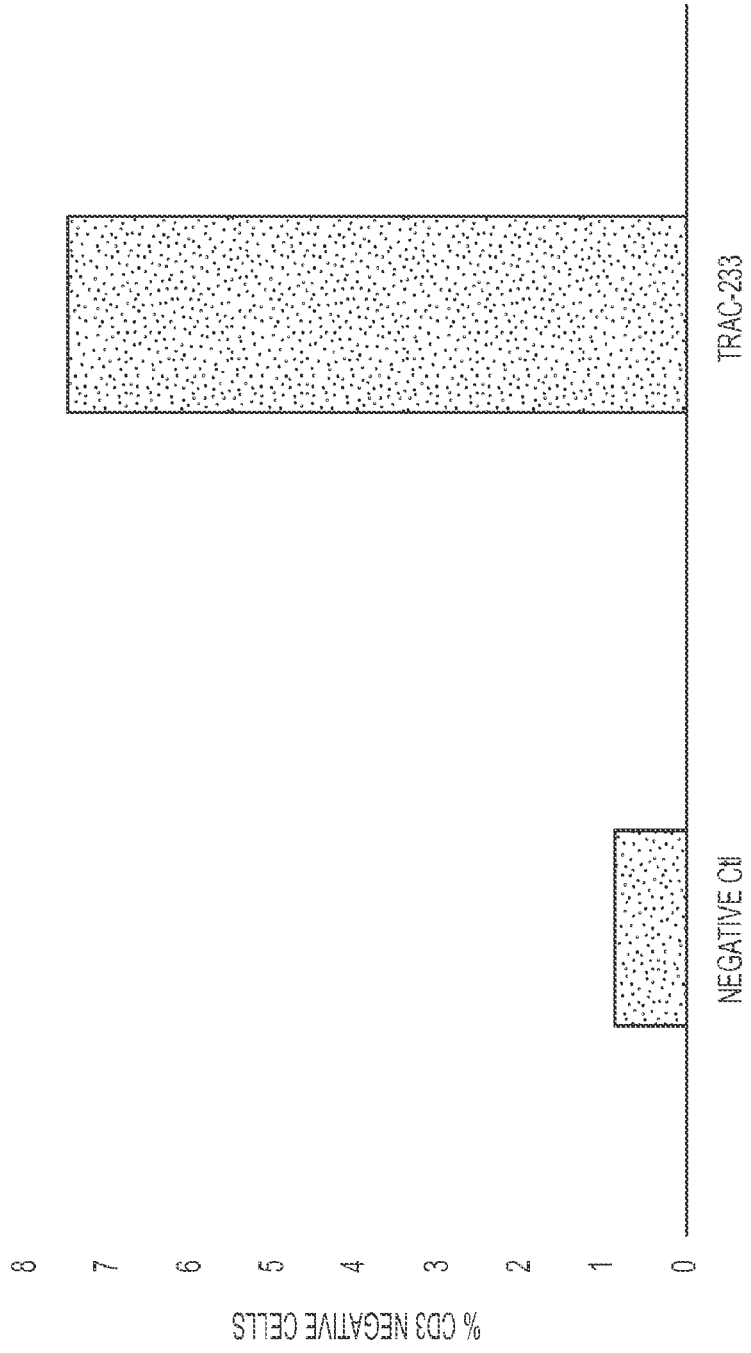


FIG. 21B

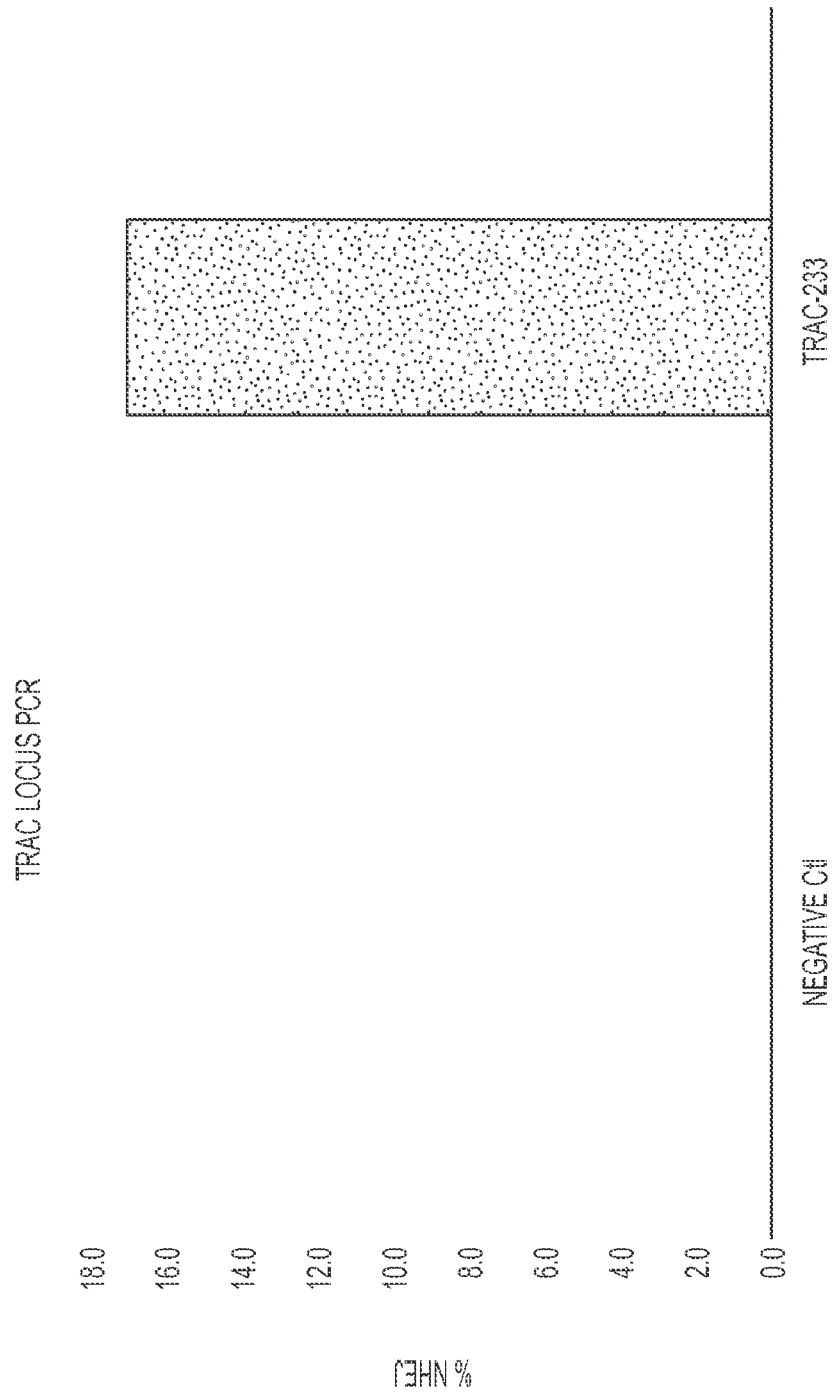

TRAC LOCUS PCR
% NHEJ
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
NEGATIVE CH
TRAC-233

FIG. 21C

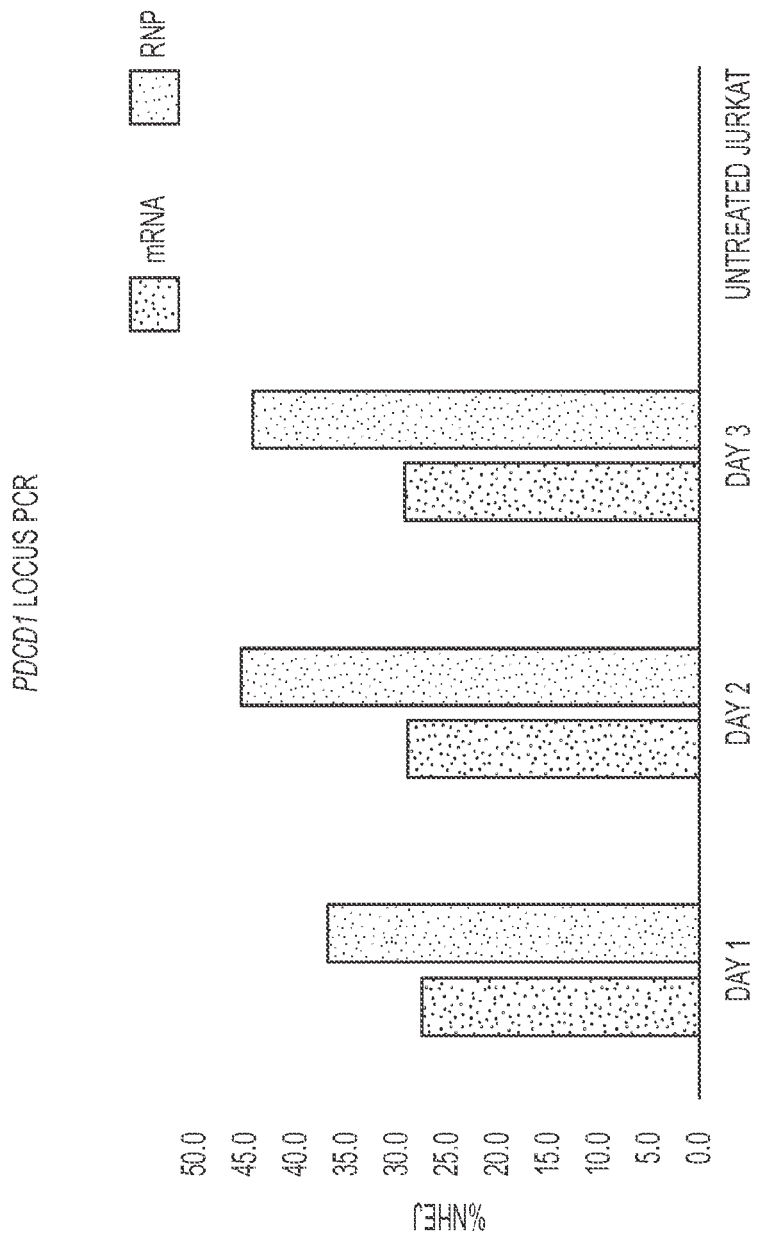

PDCD1 LOCUS PCR
mRNA
RNP
%NHEJ
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
DAY 1
DAY 2
DAY 3
UNTREATED JURKAT

FIG. 22