Streptococcus pyogenes Cas9
GenBank AKP81606

```
   1 mdkkysigld igtnsvgwav itddykvpsk kfkvlgntdr hsikknliga llfdsgetae
  61 atrlkrtarr rytrrknric ylqeifsnem akvddsffhr leesflveed kkherhpifg
 121 nivdevayhe kyptiyhlrk klvdstdkad lrliylalah mikfrghfli egdlnpdnsd
 181 vdklfiqlvq tynqlfeenp inasgvdaka ilsarlsksr rlenliaqlp gekknslfgn
 241 lialslgltp nfksnfdlae daklqlskdt ydddldnlla qigdqyadlf laaknlsdai
 301 llsdilrvnt eitkaplsas mikrydehhq dltllkalvr qqlpekykei ffdqskngya
 361 gyidggasqe efykfikpil ekmdgteell vklnredllr kqrtfdngsi phqihlgelh
 421 ailrrqedfy pflkdnreki ekiltfripy yvgplargns rfawmtrkse etitpwnfee
 481 vvdkgasaqs fiermtnfdk nlpnekvlpk hsllyeyftv yneltkvkyv tegmrkpafl
 541 sgeqkkaivd llfktnrkvt vkqlkedyfk kiecfdsvei sgvedrfnas lgtyhdllki
 601 ikdkdfldne enedilediv ltltlfedre mieerlktya hlfddkvmkq lkrrrytgwg
 661 rlsrklingi rdkqsgktil dflksdgfan rnfmqlihdd sltfkediqk aqvsgqgdsl
 721 hehianlags paikkgilqt vkvvdelvkv mgrhkpeniv iemarenqtt qkgqknsrer
 781 mkrieegike lgsqilkehp ventqlqnek lylyylqngr dmyvdqeldi nrlsdydvdh
 841 ivpqsflkdd sidnkvltrs dknrgksdnv pseevvkkmk nywrqllnak litqrkfdnl
 901 tkaerggLse ldkagfikrq lvetrqitkh vaqildsrmn tkydendkli revkvitlks
 961 klvsdfrkdf qfykvreinn yhhahdaylh avvgtalikk ypklesefvy gdykvydvrk
1021 miakseqeig katakyffys nimnffktei tlangeirkr plietngetg eivwdkgrdf
1081 atvrkvlsmp qvnivkktev qtggfskesi lpkrnsdkli arkkdwdpkk yggfdsptva
1141 ysvlvvakve kgkskklksv kellgitime rssfeknpid fleakgykev kkdliiklpk
1201 yslfelengr krmlasagel qkgnelalps kyvnflylas hyeklkgspe dneqkqlfve
1261 qhkhyldeii eqisefskrv iladanldkv lsaynkhrdk pireqaenii hlftltnlga
1321 paafkyfdtt idrkrytstk evldatlihq sitglyetri dlsqlggd
```

Motif 1
Domain 1
Motif 2
Motif 3
Domain 2
Motif 4

FIG. 1

Francisella tularensis subsp. novicida U112 Cpf1

```
   1 msiyqefvnk yslsktlrfe lipqgktlen ikarglildd ekrakdykka kqiidkyhqf
  61 fieeilssvc isedllqnys dvyfklkksd ddnlqkdfks akdtikkqis eyikdsekfk
 121 nlfnqnlida kkgqesdlil wlkqskdngi elfkansdit dideallqik sfkgwttyfk
 181 gfhenrknvy ssndiptsii yrivddnlpk flenkakyes lkdkapeain yeqikkdlae
 241 eltfdidykt sevngrvfsl devfeianfn nylnqsgitk fntiiggkfv ngentkrkgi
 301 neyinlyscq indktlkkyk msvlfkqils dtesksfvid kleddsdvvt tmqsfyeqia
 361 afktveeksi ketlslifdd lkaqkldlsk iyfkndkslt dlsqqvfddy svigtavley
 421 itqqiapknl dnpskkeqel iakktekaky lsletiklal eefnkhrdid kqcrfeeila
 481 nfaaipmifd eiaqnkdnla qisikyqnqg kkdllqasae ddvkaikdll dqtnnllhkl
 541 kifhisqsed kanildkdeh fylvfeecyf elanivplyn kirnyitqkp ysdekfklnf
 601 enstlangwd knkepdntai lfikddkyyl gvmnkknnki fddkaikenk gegykkivyk
 661 llpgankmlp kvffsaksik fynpsedilr irnhsthtkn gspqkgyekf efniedcrkf
 721 idfykqsisk hpewkdfgfr fsdtqrynsi defyrevenq gykltfenis esyidsvvnq
 781 gklylfqiyn kdfsayskgr pnlhtlywka lfdernlqdv vyklngeael fyrkqsipkk
 841 ithpakeaia nknkdnpkke svfeydlikd krftedkfff hcpitinfks sgankfndei
 901 nlllkekand vhilsidrge rhlayytlvd gkgniikqdt fniignderak tnyhdklaai
 961 ekdrdsarkd wkkinnikem kegylsqvvh eiaklvieyn aivvfedlnf gfkrgrfkve
1021 kqvyqklekm lieklnylvf kdnefdktgg vlrayqltap fetfkkmgkq tgiiyyvpag
1081 ftskicpvtg fvnqlypkye svsksqeffs kfdkicynld kgyfefsfdy knfgdkaakg
1141 kwtiasfgsr linfrnsdkn hnwdtrevyp tkelekllkd ysieyghgec ikaaicgesd
1201 kkffakltsv lntillqmrns ktgteldyli spvadvngnf fdsrqapknm pqdadangay
1261 higlkglmll grikknqegk klnlvikneе yfefvqnrnn
```

FIG. 2

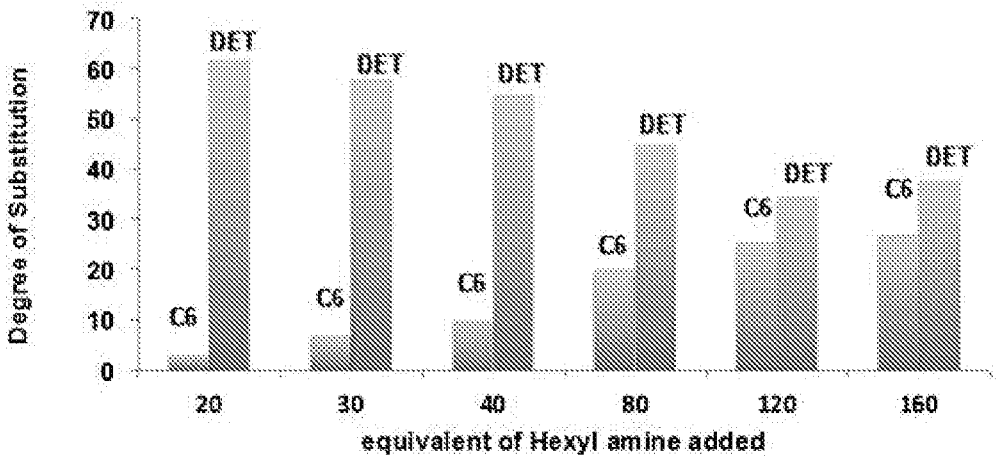

Degree of Substitution
70
60
50
40
30
20
10
0
DET
C6
20
30
40
80
120
160
equivalent of Hexyl amine added

FIG. 3

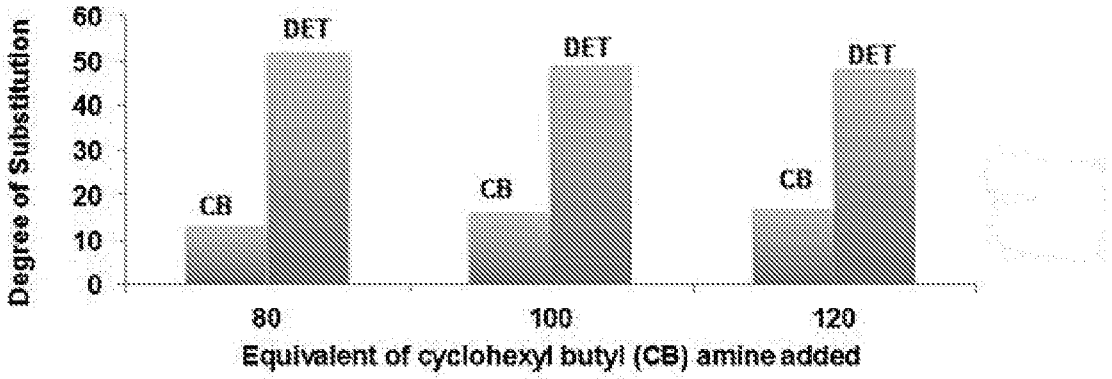

Degree of Substitution
60
50
40
30
20
10
0
DET
CB
80
100
120
Equivalent of cyclohexyl butyl (CB) amine added

FIG. 4

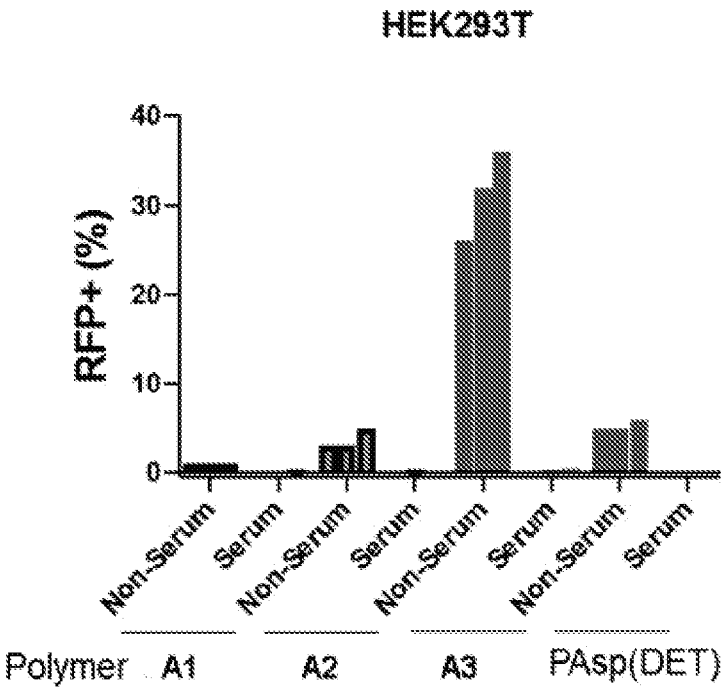

HEK293T
RFP+ (%)
40
30
20
10
0
Non-Serum
Serum
Non-Serum
Serum
Non-Serum
Serum
Non-Serum
Serum
Polymer
A1
A2
A3
PAsp(DET)

FIG. 5

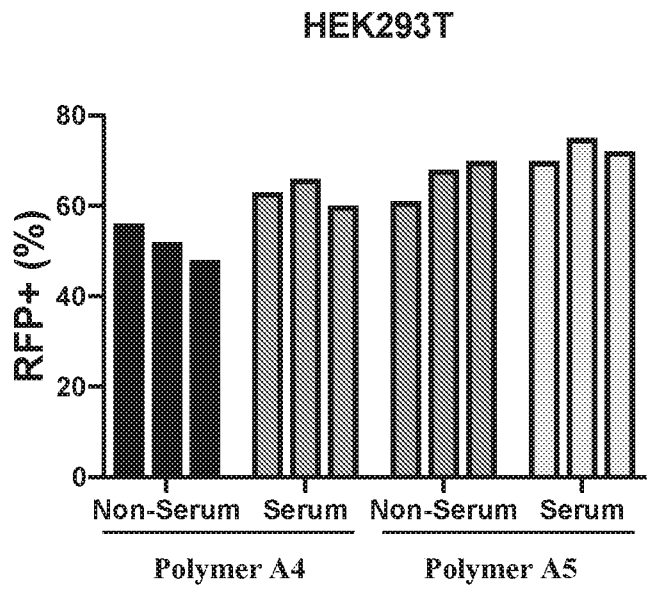

HEK293T
RFP+ (%)
80
60
40
20
0
Non-Serum
Serum
Non-Serum
Serum
Polymer A4
Polymer A5

FIG. 6

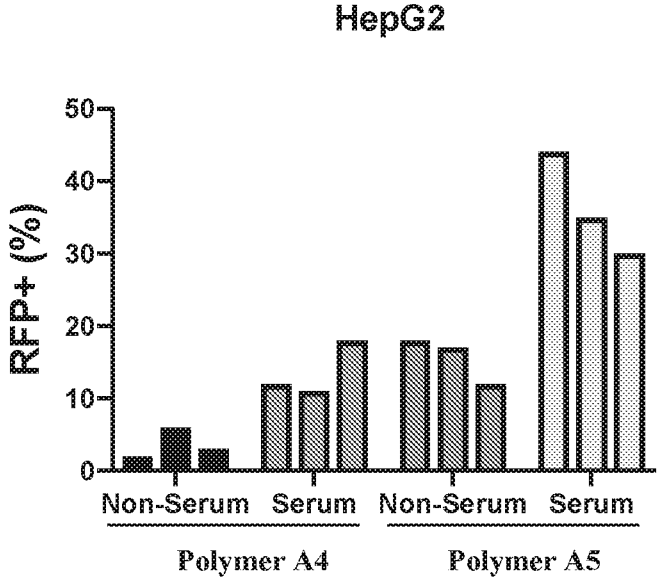

HepG2
RFP+ (%)
50
40
30
20
10
0
Non-Serum
Serum
Non-Serum
Serum
Polymer A4
Polymer A5

FIG. 7

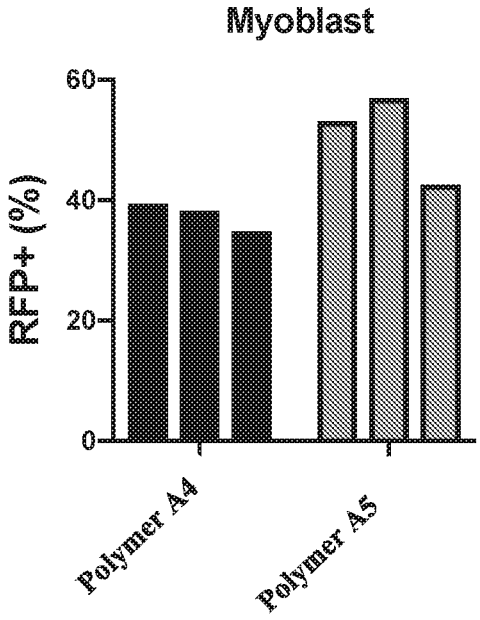

Myoblast
RFP+ (%)
60
40
20
0
Polymer A4
Polymer A5

FIG. 8

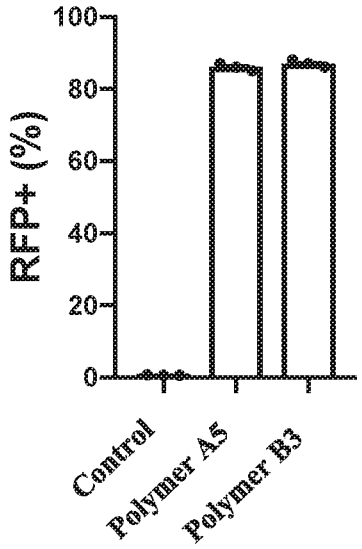

RFP+ (%)
100
80
60
40
20
0
Control
Polymer A5
Polymer B3

FIG. 9

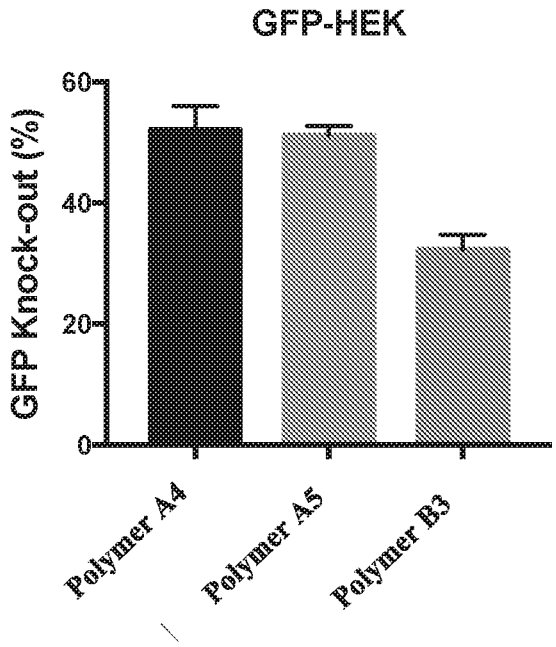

GFP-HEK
GFP Knock-out (%)
60
40
20
0
Polymer A4
Polymer A5
Polymer B3

FIG. 10

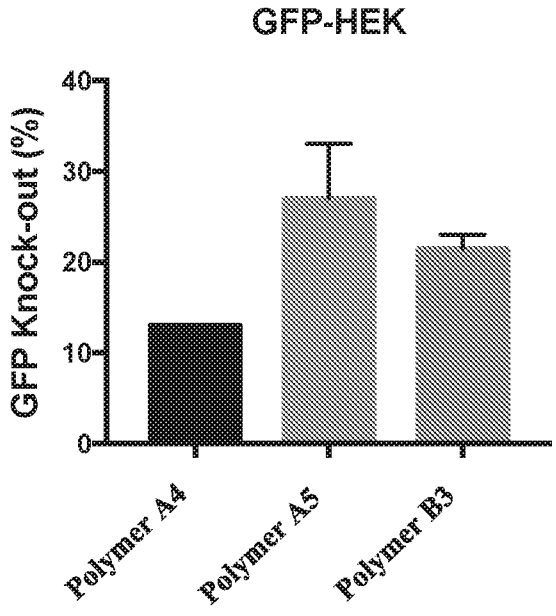

GFP-HEK
GFP Knock-out (%)
40
30
20
10
0
Polymer A4
Polymer A5
Polymer B3

FIG. 11

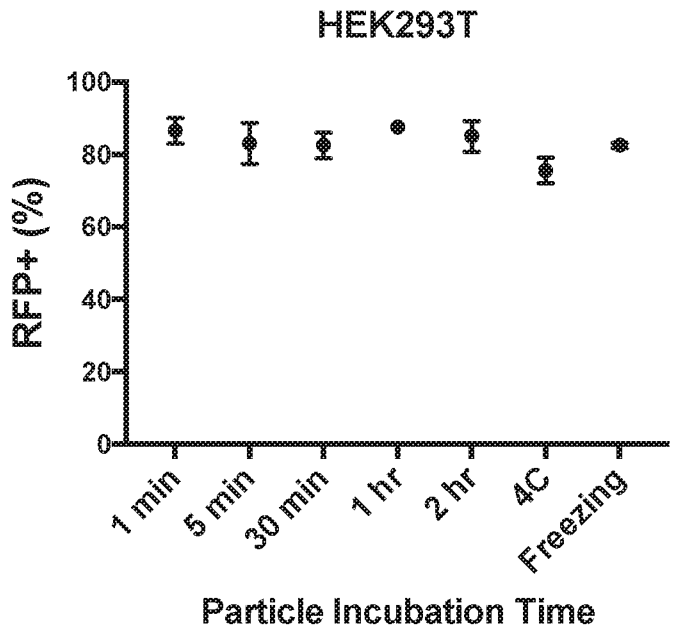

HEK293T
RFP+ (%)
100
80
60
40
20
0
1 min
5 min
30 min
1 hr
2 hr
4C
Freezing
Particle Incubation Time

FIG. 12

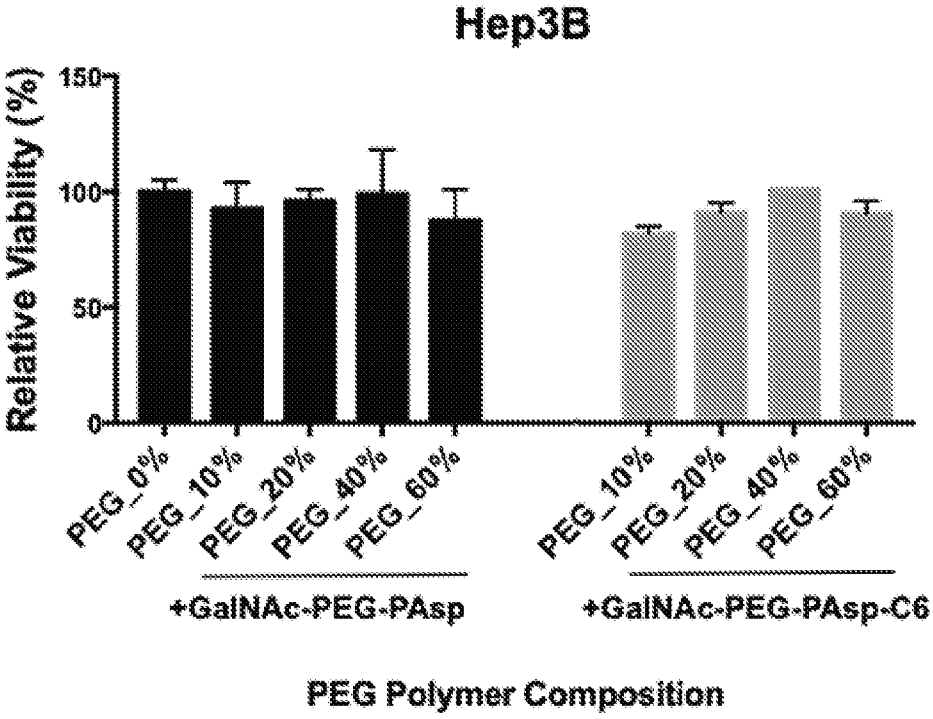

Hep3B
Relative Viability (%)
150
100
50
0
PEG_0%
PEG_10%
PEG_20%
PEG_40%
PEG_60%
PEG_10%
PEG_20%
PEG_40%
PEG_60%
+GalNAc-PEG-PAsp
+GalNAc-PEG-PAsp-C6
PEG Polymer Composition

FIG. 13

AsCpf1

```
   1 mtqfegftnl yqvsktlrfe lipqgktlkh iqeqgfieed karndhykel kpiidriykt
  61 yadqclqlvq ldwenlsaai dsyrkektee trnalieeqa tyrnaihdyf igrtdnltda
 121 inkrhaeiyk glfkaelfng kvlkqlgtvt ttehenallr sfdkfttyfs gfyenrknvf
 181 saedistaip hrivqdnfpk fkenchiftr litavpslre hfenvkkaig ifvstsieev
 241 fsfpfynqll tqtqidlynq llggisreag tekikglnev lnlaiqknde tahiiaslph
 301 rfiplfkqil sdrntlsfil eefksdeevi qsfckyktll rnenvletae alfnelnsid
 361 lthifishkk letissalcd hwdtlrnaly erriseltgk itksakekvq rslkhedinl
 421 qeiisaaqke lseafkqkts eilshahaal dqplpttlkk qeekeilksq ldsliglyhl
 481 ldwfavdesn evdpefsarl tgiklemeps lsfynkarny atkkpysvek fklnfqmptl
 541 asgwdvnkek nngailfvkn glyylgimpk qkgrykalsf eptektsegf dkmyydyfpd
 601 aakmipkcst qlkavtahfq thttpillsn nfiepleitk eiydlnnpek epkkfqtaya
 661 kktgdqkgyr ealckwidft rdflskytkt tsidlsslrp ssqykdlgey yaelnpllyh
 721 isfqriaeke imdavetgkl ylfqiynkdf akghhgkpnl htlywtglfs penlaktsik
 781 lngqaelfyr pksrmkrmah rlgekmlnkk lkdqktpipd tlyqelydyv nhrlshdlsd
 841 earallpnvi tkevsheiik drrftsdkff fhvpitlnyq aanspskfnq rvnaylkehp
 901 etpiigidrg ernliyitvi dstgkileqr slntiqqfdy qkkldnreke rvaarqawsv
 961 vgtikdlkqg ylsqviheiv dlmihyqavv vlenlnfgfk skrtgiaeka vyqqfekmli
1021 dklnclvlkd ypaekvggvl npyqltdqft sfakmgtqsg flfyvpapyt skidpltgfv
1081 dpfvwktikn hesrkhfleg fdflhydvkt gdfilhfkmn rnlsfqrglp gfmpawdivf
1141 eknetqfdak gtpfiagkri vpvienhrft gryrdlypan elialleekg ivfrdgsnil
1201 pkllenddsh aidtmvalir svlqmrnsna atgedyinsp vrdlngvcfd srfqnpewpm
1261 dadangayhi alkgqlllnh lkeskdlklq ngisnqdwla yiqelrn
```

FIG. 14

LbCpf1

AASKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLDRYYL
SFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLRKEIAKAFKGAAGYKSLF
KKDIIETILPEAADDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKSTSIAFRCINEN
LTRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNA
IIGGFVTESGEKIKGLNEYINLYNAKTKQALPKFKPLYKQVLSDRESLSFYGEGYTSDEE
VLEVFRNTLNKNSEIFSSIKKLEKLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNLIR
DKWNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQEYADADLSVVEKLKEIII
QKVDEIYKVYGSSEKLFDADFVLEKSLKKNDAVVAIMKDLLDSVKSFENYIKAFFGEGKE
TNRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKE
TDYRATILRYGSKYYLAIMDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFS
KKWMAYYNPSEDIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSE
TEKYKDIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDKSHGTPNL
HTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPDNPKKTTTL
SYDVYKDKRFSEDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGIDRGERNLL
YIVVVDGKGNIVEQYSLNEIINNFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKEL
KAGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKMLIDKLNYMVD
KKSNPCATGGALKGYQITNKFESFKSMSTQNGFIFYIPAWLTSKIDPSTGFVNLLKTKYT
SIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYGNRIRIFAAAK
KNNVFAWEEVCLTSAYKELFNKYGINYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRN
SITGRTDVDFLISPVKNSDGIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFK
KAEDEKLDKVKIAISNKEWLEYAQTSVK

FIG. 15

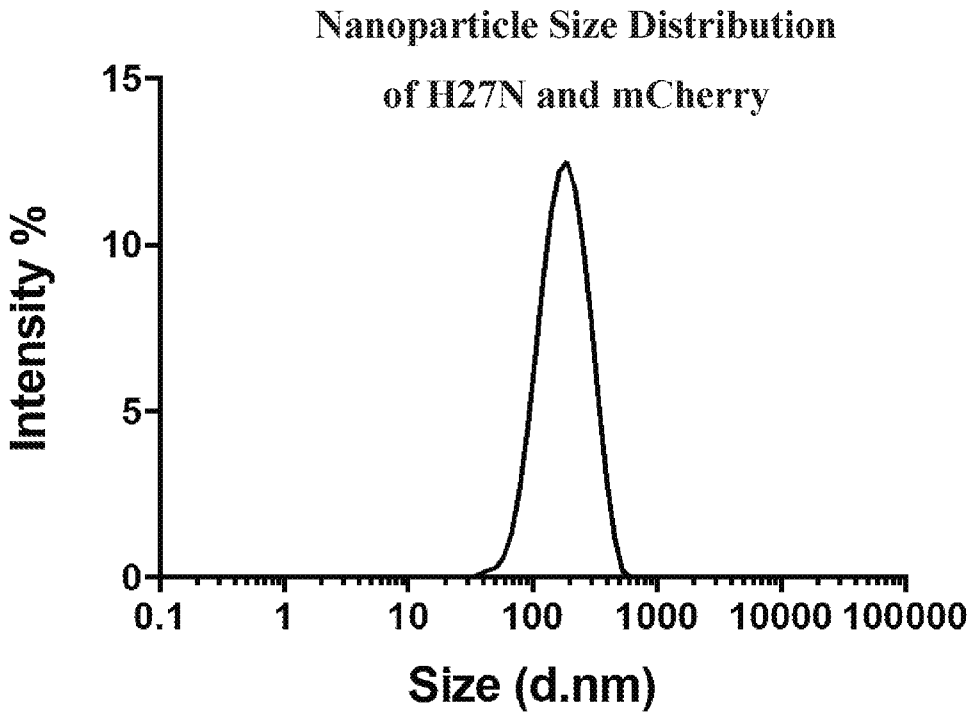

Nanoparticle Size Distribution
of H27N and mCherry
Intensity %
15
10
5
0
0.1
1
10
100
1000
10000
100000
Size (d.nm)

FIG. 16

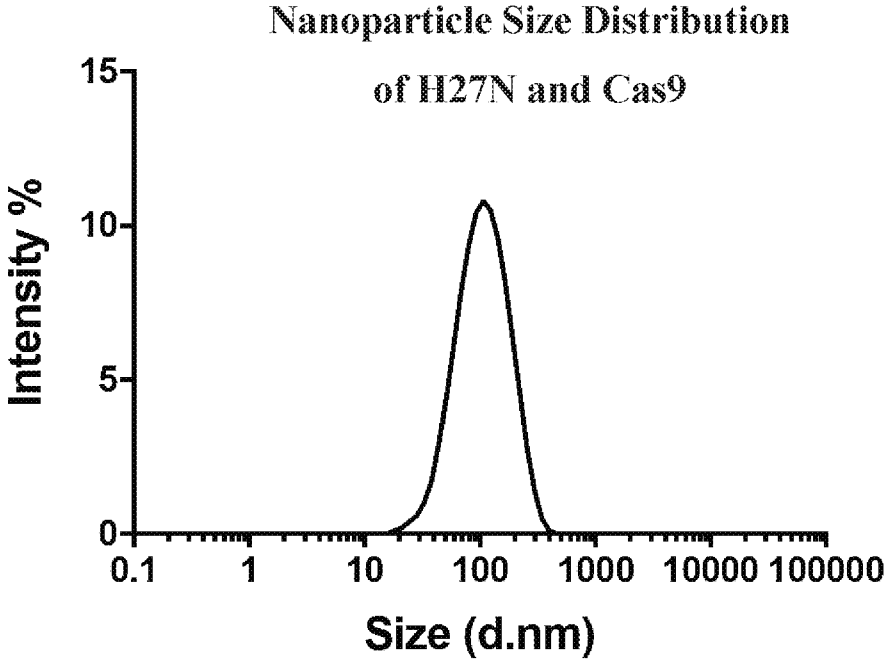

Nanoparticle Size Distribution
of H27N and Cas9
Intensity %
15
10
5
0
0.1
1
10
100
1000
10000
100000
Size (d.nm)

FIG. 17

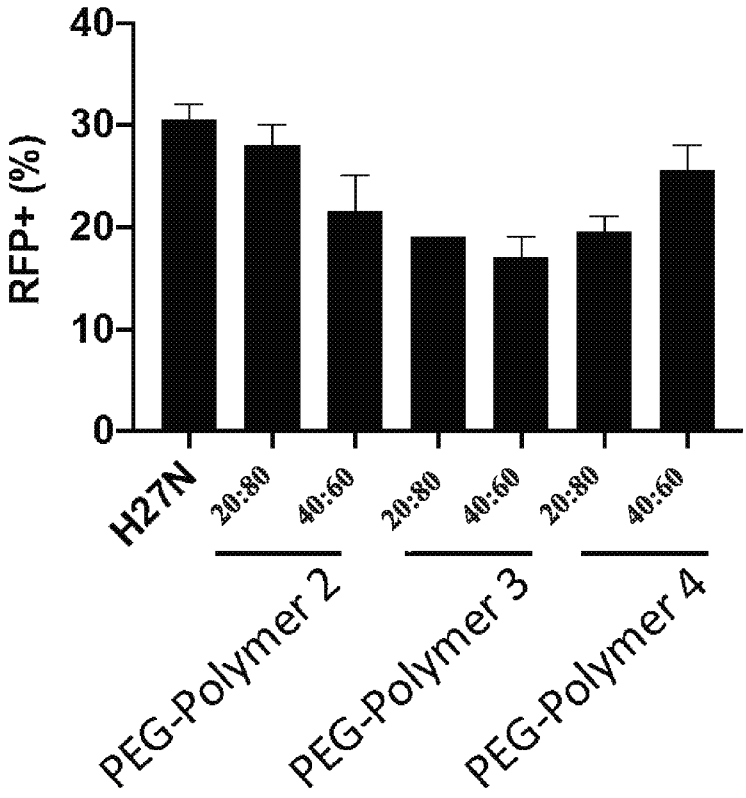

40
30
20
10
0
RFP+ (%)
H27N
20:80
40:60
20:80
40:60
20:80
40:60
PEG-Polymer 2
PEG-Polymer 3
PEG-Polymer 4

FIG. 18

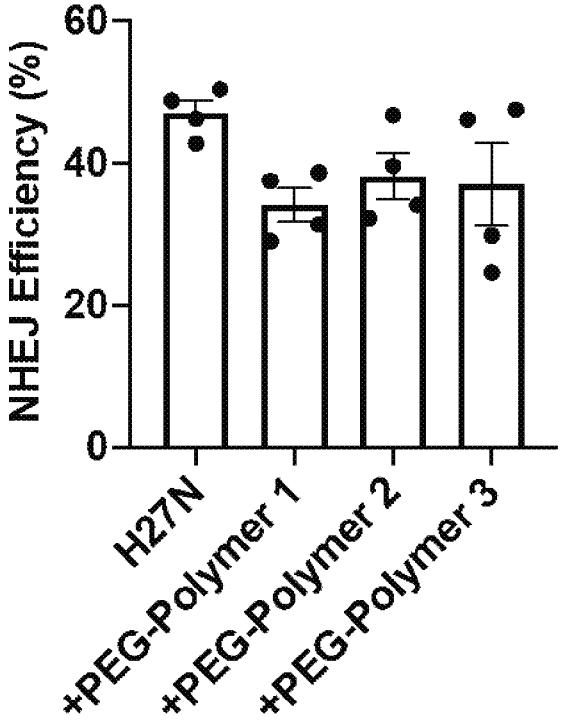

NHEJ Efficiency (%)
60
40
20
0
H27N
+PEG-Polymer 1
+PEG-Polymer 2
+PEG-Polymer 3

FIG. 19

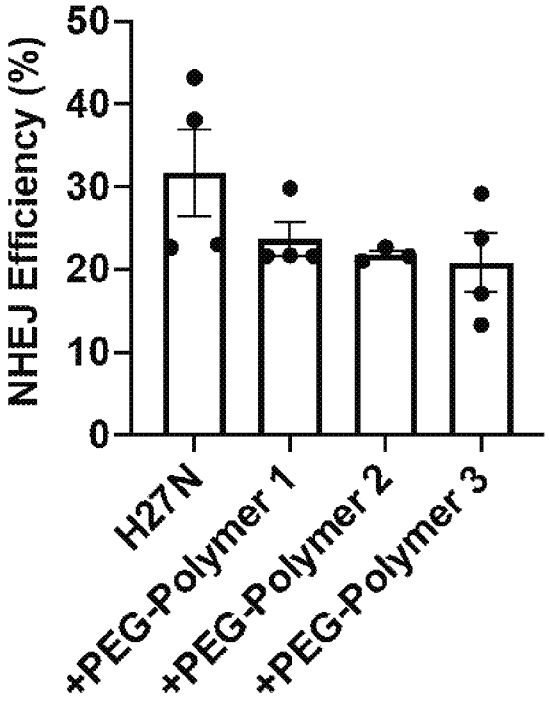
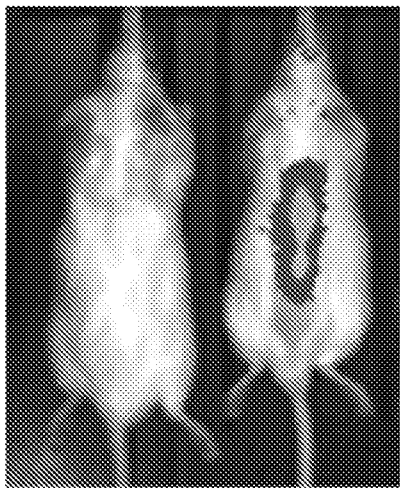

NHEJ Efficiency (%)
50
40
30
20
10
0
H27N
+PEG-Polymer 1
+PEG-Polymer 2
+PEG-Polymer 3

FIG. 20

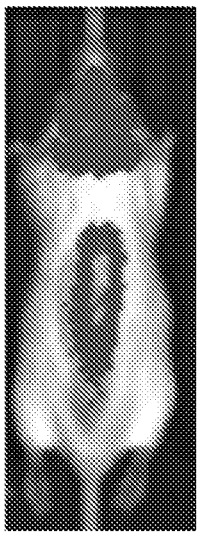


FIG. 21



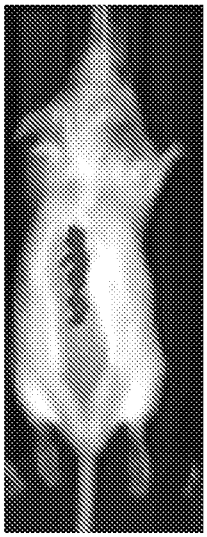
Control H27N and Cre mRNA

FIG. 22A



H27N and Cre mRNA

FIG. 22B



H27N and Cre mRNA

FIG. 22C

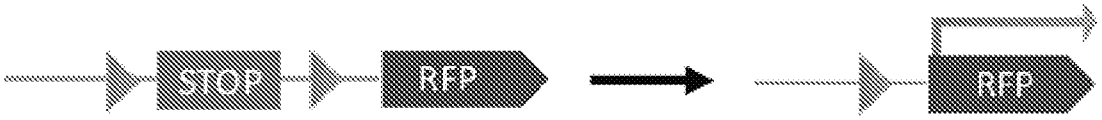

STOP
RFP
RFP

FIG. 23

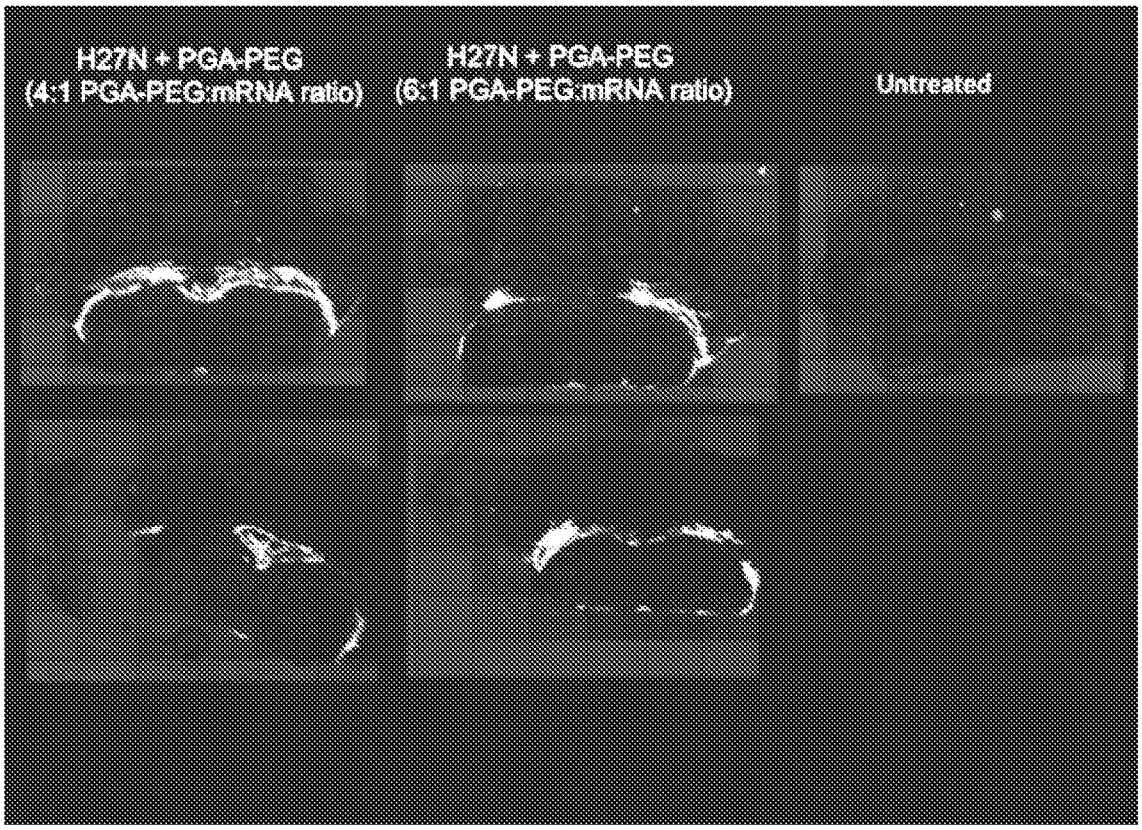

H27N + PGA-PEG
(4:1 PGA-PEG:mRNA ratio)
H27N + PGA-PEG
(6:1 PGA-PEG:mRNA ratio)
Untreated

FIG. 24

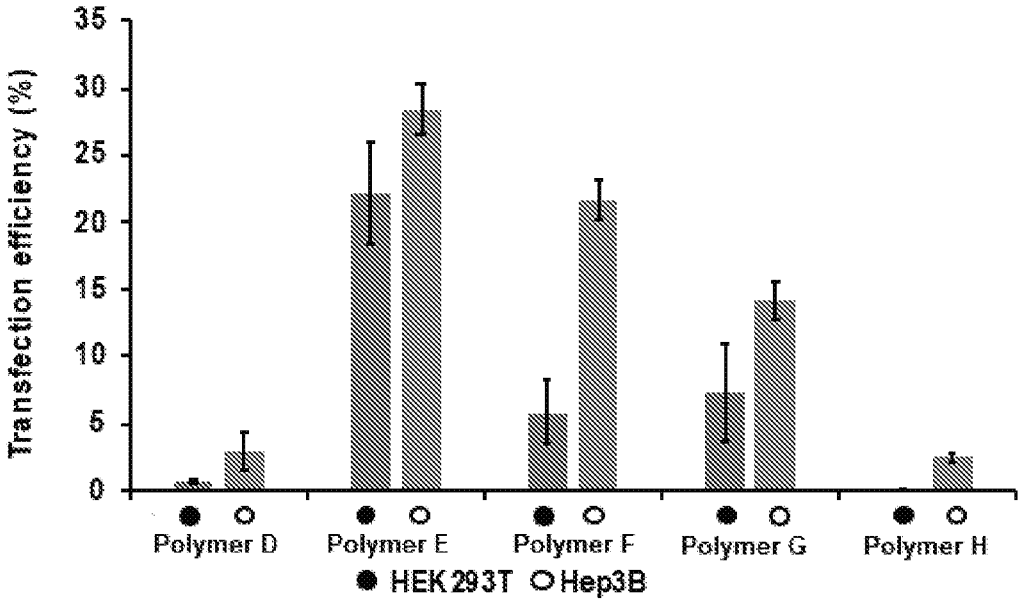

Transfection efficiency (%)
35
30
25
20
15
10
5
0
Polymer D
Polymer E
Polymer F
Polymer G
Polymer H
HEK293T
Hep3B

FIG. 25